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Diffuse reflectance spectroscopy in the identification of oral potentially malignant disorders

Thesis presented by

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For the degree of:

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Table of Contents

Table of contents.....ii

Index of figures.....v

Index of figures.....vi

Index of tables.....viii

Terms and Abbreviations.....ix

Declaration.....xi

Acknowledgements.....xii

Abstract.....xiii

1 Introduction.....1

2 Literature Review.....5

2.1 Overview and Search Strategy.....5

2.2 Oral Potentially Malignant Disorder – a clinical and histological diagnosis.....6

2.2.1 Epidemiology.....8
2.2.2 Leukoplakia.....11
2.2.3 Proliferative Verrucous Leukoplakia (PVL).....18
2.2.4 Oral Lichen Planus.....22
2.2.5 Erythroplakia.....29
2.2.6 Oral Lichenoid Lesion.....32
2.2.7 Actinic keratosis/actinic cheilitis.....34
2.2.8 Other premalignant conditions.....37
2.2.9 Dysplasia and squamous cell carcinoma.....38
2.2.10 Oral Epithelial Dysplasia.....38
2.2.11 Squamous cell carcinoma.....41
2.2.12 Management of OSCC and OED.....42

2.3 Existing tools for the detection of OPMD and OSCC.....47

2.3.1 Screening tools.....47
2.3.2 Adjunct Screening Tools.....49
2.3.3 Adjuncts not suitable for screening.....54
2.3.4 Optical techniques.....55
2.3.5 The use of spectroscopic techniques in the detection of OPMD and OSCC.....65
2.3.6 Raman Spectroscopy.....70
2.3.7 Infrared Spectroscopy.....71
2.3.8 Fluorescence spectroscopy.....71
2.3.9 Diffuse Reflectance Spectroscopy.....72
2.3.10 Multimodal spectroscopy.....75
2.3.11 Development of DRS.....75
2.3.12 Resolving issues around DR probe design.....92
2.3.13 Summary and knowledge gap.....93

2.4 Aims and objectives.....94

2.4.1 Project aims.....94

3 Materials and methods.....95

3.1 Overview.....95

3.1.1 Graphic representation of study flow....96
3.1.2 Ethical considerations....97

3.2 Preclinical phase....97
3.2.1 Development of protocol....97
3.2.2 Building of protocol....97
3.2.3 Protocol development....98

3.3 Phase 0....99
3.3.1 Overview....99

3.4 Phase 1 recruitment....100
3.4.1 Overview....100
3.4.2 Sample size calculation....101

3.5 Recruitment....101
3.5.1 OPMD and SCC groups....101
3.5.2 Control group....102

3.6 Participants and recruitment....102
3.6.1 Inclusion criteria (study group):....102
3.6.2 Exclusion criteria (study group):....102
3.6.3 Control group....102

3.7 Data Collection....103
3.7.1 Demographics....103
3.7.2 Saliva collection....103
3.7.3 Imaging....104
3.7.4 Collecting DRS and Raman data....104
3.7.5 Data collection protocol....108
3.7.6 Variance data....110

3.8 Analysis of in vivo data....110
3.8.1 Reflectance data preprocessing and analysis....110
3.8.2 Statistical analysis....110

3.9 Assessment of protocol recommendations....113
3.9.1 Testing of the guideline....117

4 Results....118

4.1 Overview....118

4.2 Preclinical phase results....118

4.3 Phase 0 results....120
4.3.1 Overview....120
4.3.2 Familiarisation with DRS equipment and clinical procedure....120
4.3.3 Use of the protocol....120
4.3.4 Test reflectance spectra acquisition....120

4.4 Phase 1 results....121
4.4.1 Overview....121
4.4.2 Demographics....121
4.4.3 Summary statistics....122
4.4.4 Saliva data....126
4.4.5 Imaging data....128
4.4.6 Raman Data....128

4.4.7 DRS Data 128
4.4.8 Inter-examiner variance figures 137

4.5 Phase 2 results 140
4.5.1 Perceptions of DRS and protocol for clinical use 140
4.5.2 Readability 144

5 Discussion 145

5.1 Overview 145

5.2 Accuracy of DRS 145

5.3 Barriers to implementation of DRS and protocol to clinical practice 150

5.4 Reproducibility and repeatability 156

5.5 Study design 159
5.5.1 Overview 159
5.5.2 Initial pilot stage 160
5.5.3 Control group 160
5.5.4 Population 162
5.5.5 Inclusion/exclusion criteria 163

5.6 Study strengths 163
5.6.1 Study population 163
5.6.2 Study design 164
5.6.3 Multiple observers 164
5.6.4 Multiple opinions 165

5.7 Study limitations 165
5.7.1 Comparison with gold standard 165
5.7.2 Point-wise nature of comparisons 165
5.7.3 Probe design 166
5.7.4 Unsuspected sources of variation 167
5.7.5 Difficulties in translational research 168

5.8 Suggestions for future research 169

6 Conclusions 171

7 References 173

8 Appendices 199

8.1 Appendix A: User protocol for use of DRS 199

User protocol for use of diffuse reflectance spectroscopy (DRS) in the detection of oral potentially malignant disease (OPMD) and squamous cell carcinoma (SCC) 200

Stakeholder group 200

Scope of the guidance 200

Why this guidance is needed 200

Introduction 200

Innovation 201

Current care pathway 201

Indications **201**

Prior to investigation **201**

Clinical evidence ***202***

Procedure and clinical guideline **202**

Published evidence 202
Summary of published studies 202
Preparation 202
Calibration 203
Probe Cleaning/Sterilizing 203
Data Collection 203

Flow diagram of user protocol **209**

References ***210***

Index of figures

Figure 1. An example of an oral lichen planus (buccal mucosa) 25
Figure 2. An example of actinic keratosis..... 36
Figure 3. Histologic presentation of oral squamous cell carcinoma. 42
Figure 4. The Liverpool algorithm for management of dysplastic lesions..... 44
Figure 5. An example of autofluorescence of submucous fibrosis of buccal mucosa. Characteristic loss of fluorescence is seen for the high-risk part..... 58
Figure 6. GOCCLES used for visualizing intraoral OPMD 59
Figure 7. Endoscopy of hard palate erythroplakia on left with NBI on right..... 63
Figure 8. Using laser confocal microscopy on the lower anterior gingiva..... 64
Figure 9. The chance of photon interaction with deoxy and oxyhaemoglobin as against its wavelength..... 67
Figure 10. Example of a spectrum produced from oral tissue. 73
Figure 11. Varying source-detector distance to preferentially get data from superficial layers .. 77
Figure 12. (top) An example of a multi-detector array with fixed fibre diameters with the possibility to vary the source-detector distance. 80
Figure 13. (bottom) Another example of a multi-channel detector array with detector fibres labelled to allow collection from deeper and superficial layers. 80
Figure 14. An example of DRS spectra obtained from different mammary tissue. 82
Figure 15. This shows diffuse reflectance imaging of an ulcer on the right lateral tongue. (a) clinical photograph, (b) histopathology, (c) monochrome 545/575nm reflectance image, (d) colourised reflectance at 545 and 575nm 84
Figure 16. The roles of the multidisciplinary team on the clinic 100
Figure 17. Representation of study groups..... 101
Figure 18. Schema used to map mouth 104
Figure 19. Common end configuration 106
Figure 20. Linear end configuration 106
Figure 21. This is our DRS and Raman configuration.. 107
Figure 22. Customised reflectance stand holder 109
Figure 23. Using the probe. Room lights are turned on for the purposes of the photo. 109
Figure 24. Extraction of spectral features using LASSO for dorsal surface of the tongue..... 111
Figure 25. Smoking and alcohol habits compared across groups. 123
Figure 26. Habits of processed meat consumption and use of alcohol-containing mouthwash across groups 123
Figure 27. Sites of OPMD included in the study..... 125
Figure 28. Number of participants with each diagnosis. Control is included as a comparison... 126
Figure 29. A spectrum obtained from a saliva analyte..... 127
Figure 30. Number of data files suitable for analysis, divided by site and study group..... 129
Figure 31. Patient 55. Oral Lichen Planus on the buccal mucosa. 130
Figure 32. Patient 58. Oral leukoplakia on the ventral tongue.. 131
Figure 33. (a) above and (b) below. DRS spectra with standard deviation. Floor of mouth (left) and dorsum of tongue (right)..... 131
Figure 34. Mean and standard deviation of collected spectra for dorsum of the tongue. 132
Figure 35. LASSO selected features from the dorsum of the tongue..... 133
Figure 36. Mean spectra (solid line) for buccal mucosa on all patients with standard deviation shown..... 134

Figure 37. LASSO – identified wavelengths for statistical comparison from the buccal mucosa. 134
Figure 38. Mean spectra for ventral tongue with standard deviation. 136
Figure 39. LASSO selected classifiers plotted with OPMD in blue and normal in red 136
Figure 40.(a) and (b). This shows the inter-observer variation for healthy buccal mucosa for one patient. Significant variability is inferred from the wide standard deviation for each observer. 138
Figure 41. Significant differences at shorter wavelengths 138
Figure 42.The curve for the buccal mucosa (left) and gingiva (right) are set side-by-side. The increased variance for gingival tissue is clear compared with buccal mucosa. 139
Figure 43.Types of clinical study.(308) 161
Figure 44. Multi fibre probe design capable of receiving photons in 360 degrees compared with linear array (right) 167
Figure 45.IDEAL framework. (322] 169

Index of tables

Table 1. Search strategy. 6
Table 2. Terms used in characterising premalignant conditions 7
Table 3. Important OPMD identified by the WHO's working group on cancer 8
Table 4. Summary table of OPMD 10
Table 5. Factors predictive of malignant transformation 15
Table 6. Diagnostic criteria for PVL. All criteria must be met in order to reach a diagnosis 22
Table 7. Associations between inflammation and cancer risk 26
Table 8. Histopathological features seen in dysplasia 40
Table 9. Advantages of optical spectroscopy 65
Table 10. Advantages and disadvantages of various optical systems 69
Table 11. Summary of DRS studies. 89
Table 12. Quality criteria of diagnostic accuracy studies derived from QUADAS-2.. 116
Table 13. Gender of the study participants 121
Table 14. Gender distribution between study arms. 122
Table 15. Co-morbidities by numbers of chronic illnesses reported per control group. 124
Table 16. Numbers of saliva samples usable for analysis. 127
Table 17. Number of data files obtained per patient group for DRS. 129
Table 18. Discriminant analysis for dorsal surface of the tongue. 133
Table 19. Discriminant analysis for the buccal mucosa. 135
Table 20. Discriminant analysis for ventral surface of the tongue. 137
Table 21. Professional background of clinicians interviewed. 140
Table 22. Biomarkers and their absorption peaks. 149

Terms and Abbreviations

AI	Artificial intelligence
AK/AC	Actinic keratosis/actinic cheilitis
AUC	Area Under the Curve
BMI	Body Mass Index
CI	Confidence Interval
CNN	Convolutional Neural Networks
COE	Clinical Oral Examination
COX	Cyclo-oxygenase pathway
DRS	Diffuse reflectance spectroscopy
ESS	Elastic scattering spectroscopy
FS	Fluorescence spectroscopy
FT-IR	Fourier-transform Infrared spectroscopy
HbO_2	Haemoglobin
IFN	Interferon
IL	Interleukin
LOH	Loss of heterozgosity
MHC	Major Histocompatibility Complex
ML	Machine Learning
MMP	Matrix Metalloproteinase
MT	Malignant Transformation
NK	Natural Killer
OE	Oral Erythroplakia
OED	Oral Epithelial Dysplasia

OL	Oral Leukoplakia
OLCR	Oral Lichenoid Contact Reaction
OLL	Oral Lichenoid Lesion
OLP	Oral Lichen Planus
OLR	Oral Lichenoid Reaction
OPMD	Oral Potentially Malignant Disorder
OPML	Oral Potentially Malignant Lesion
OR	Odds Ratio
OSCC	Oral Squamous Cell Carcinoma
P16, P53	May refer to tumour suppressor protein, or gene coding for same
PVL	Proliferative Verrucous Leukoplakia
RAS	Signalling protein
ROC	Receiver Operator Curve
RS	Raman Spectroscopy
Se	Sensitivity
SERS	Surface-Enhanced Raman Spectroscopy
Spe	Specificity
TAMS	Tumour Associated Macrophages
MIF	Macrophage Inhibitory Factor
TB	Toluidine blue
TGF	Transforming Growth Factor
TNF	Tumour Necrosis Factor
WHO	World Health Organisation

Declaration

This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism and intellectual property.

- *Edward Fahy, 15th April 2024*

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Abstract

Aims: Diffuse reflectance spectroscopy (DRS) examines the composition of tissue by analysing light reflected from inside the tissue. DRS has been applied to cancer diagnostics in liver, brain, breast and others. The aims of this study were to (i) to formulate a clinical protocol for use of DRS in diagnosis of oral potentially malignant disorder (OPMD), (ii) to explore the clinical utility for this probe in the mouth and (iii) to review the protocol and assess its potential use in clinical practice.

Materials and Methods: A clinical protocol for use of the DRS probe was formulated based on literature review and clinical experience. A translational clinical research study with two groups, one with histologically confirmed OPMD (n=53) and a control group (n=27) were enrolled. All participants received DRS of mucosal surfaces, including areas of OPMD, in the mouth. The readings were then investigated to find reliable biomarkers and their accuracy to differentiate the two groups. Repeatability and reproducibility with two users were examined. Interviews were carried out with oral surgery clinicians after reading the protocol.

Results: The protocol produced good results, which were grouped based on the site of acquisition. Our analysis found accuracy figures of 89% and 87% respectively for distinguishing OPMD and normal mucosa in the buccal mucosa and ventral tongue. This OPMD group was made up of mostly oral lichen planus (OLP), with small numbers of other diagnoses. (OLP n=41, oral leukoplakia n=5, others n=5) Accuracy figures for the dorsum of the tongue were poor. DRS was easy to use, quick and acceptable to patients. Repeatability was shown to be good, however reproducibility was fair. This may be due

to inter-user pressure differences in the mouth. Clinicians were sceptical of this new technology, in part due to lack of evidence of clinical utility compared with biopsy.

Conclusion: DRS has shown a new role in identifying oral lichen planus in the buccal mucosa and ventral tongue. This device may have poor accuracy in identifying oral lichen planus on the dorsal surface of the tongue. It has potential as a replacement for biopsy as a screening tool, however observed difficulties in clinical use preclude widespread implementation. Observed inter-user variability in the mouth has not been thus far explored in the DRS literature. Further studies are needed to confirm this observation.

1 Introduction

Oral potentially malignant disorders (OPMD) are a set of heterogenous conditions with a variable risk of progression to oral cancer. Easy, non-invasive methods of diagnosis and monitoring of these OPMDs are needed and useful for ongoing surveillance and risk stratification. Many patients follow routine surveillance regimes aimed at detecting malignant transformation of oral potentially malignant disorders. Risk is stratified modified based on published evidence.(1) Currently, the gold standard tool to assess neoplastic development of a potentially malignant lesion is biopsy and histopathological diagnosis. A point-of-care, accurate, non-invasive and reliable tool with quick results and high accuracy is the goal of research.(2–4)

The progression of OPMD into the development of oral squamous cell carcinoma (OSCC) has been well-recognised. Biopsy and histopathological diagnosis have been the standard of care for OPMD for many years. It has drawbacks including lack of predictability of malignant transformation, difficulty of selecting optimum site for sampling, fallibility of grading of risk of malignant transformation, invasive nature, expense and time taken for results. Histopathological grading of dysplasia in particular has been shown to have poor agreement in predicting the transformation of OPMD into OSCC.(13)

Oral squamous cell carcinoma (OSCC) comprises the vast majority of malignant oral tumours. In Ireland, oral and pharyngeal cancer is the 10th most common cancer.(6,7) Incidence rates in Ireland of head and neck cancer (HNC) are forecast

to increase by 66% between 2020 and 2045.(8) This is in part due to the emergence of new causative factors as well as demographic changes. Major risk factors are alcohol and tobacco, with smoking and tobacco having a combined synergistic effect making the risk of OSCC 38 times higher in a heavy smoker and drinker compared to abstainer.(9,10) Across the developed world, the incidence of oral cancer in men aged in their 40s and 50s is steadily rising.(11) Published data provides risk factors for malignant transformation of OPMD, with size, heterogeneous appearance, location, duration of presence, inability to cease causative habits, age, female gender and lack of causative habits all predictive to greater or lesser degrees.(12,13) Thus, there is an accepted malignant tendency and risk profile to OPMD, while there is similarly a need to detect OSCC in a timely fashion.

The identification of inflammatory environmental factors that promote the development of clonal cells such as smoking, air pollution and others has helped to reduce the disease-specific burden related to cancer throughout the 20th and 21st centuries. This public health effort has worked in concert with new medical advances to increase disease-specific survival and aid sooner diagnoses. Early detection of HNC is key. Survival at 5 years post-diagnosis is directly related to stage. If lesions are detected when they are small, localised, and treated expeditiously, survival rates of 70-90% can be achieved.(14) However, many oral cancers are currently detected at stage III/IV.(15) This has implications far beyond survival, with later detection necessitating extensive treatment with devastating impacts on feeding, speech and appearance.(16) The most significant factor behind this is diagnostic delay, with smaller tumours and first presentation to a general

dental practitioner (GDP) drivers of delay.(17,18) The rate of second primary OSCC tumours is also high at 3-7% (9,10) and more often the cause of death.(19) This necessitates ongoing monitoring, risk stratification and repeat biopsy in case of new or suspicious lesions in the mouth.

Modern molecular techniques have produced vast datasets which have enabled the identification of biomarkers whose presence signal the development of a tumour. Alliance with 'big data' analysis to investigate these cellular mechanisms has opened up a new frontier in the 'war on cancer'.(20) It is now recognised that tumourigenesis starts with immunologic alterations to tumour suppressor genes facilitating activation of oncogenes and clonal expansion of somatic cells. While early inflammatory alterations in the tumour microenvironment (TME) still defy time-sensitive identification by current technology, known precursors of oral cancer are recognised and visible in the mouth. This represents a change compared with the inaccessibility of other body tissue for screening, while identifiable precancerous areas are not always present in other cancers. Thus, early identification and
monitoring of precancerous areas offers a way to downstage oral cancer diagnosis.

Use of adjuncts, including fluorescence imaging and/or toluidine blue staining may increase the number of OPMD detected compared to oral examination. Furthermore, these adjuncts may outline the lesion borders more clearly.(12) These may be performed in primary care, unlike histopathological diagnosis which has historically been performed in secondary care, with corresponding wait times and travel times for patients. Novel immediate and primary care-based diagnostic tools

have attracted interest in clinical and academic circles.(21) A new approach to the diagnosis of OPMD has been the use of optical techniques. These aim to obtain data on the subsurface composition of tissues using non-invasive technology. Light passed through tissue is captured and analysed for patterns suggestive of the diagnosis. While interesting and promising, not least for their non-invasive nature, no optical device is widely accepted for diagnosis of OPMD or OSCC. This study will examine such a device in the context of diagnosis of OPMD and OSCC.

2 Literature Review

2.1 Overview and Search Strategy

The literature review is divided into three sections:

1) the prevalence and aetiopathogenesis of OPMD

2) the existing tools for detection of OSCC

3) the use of spectroscopic techniques for detection of OSCC.

An electronic search of Cochrane Library, PubMed and Medline EBSCO databases was undertaken for the terms outlined in the search strategy below. The PICOS framework was followed while undertaking the search. The reference lists of included studies were also searched for any trials of relevance. Inclusion and exclusion criteria were applied to the relevant articles and any duplicates were discarded. This was updated in April 2024 to take into account any new emerging evidence.

Table 1. Search strategy.

Population	#1; "Oral potentially malignant disorder" OR "oral potentially malignant condition" OR "oral lesion, precancerous" OR "oral lesion, premalignant" OR "oral potentially malignant disease" OR "oral pre-malignant lesion" OR "oral precancerous condition" OR "oral precancerous lesion" OR "oral premalignant lesion" OR "potentially malignant oral disease" OR "potentially malignant oral disorder" OR "precancerous oral lesion" OR "precarcinoma, mouth" OR "premalignant oral lesion" OR "oral cancer" OR "mouth cancer" OR "oral SCC" OR "oral squamous cell carcinoma"	34,874
Intervention	#2; "spectroscopy" OR "optical spectrometry" OR "VIS spectrometry" OR "VIS spectroscopy" OR "visible light spectrometry" OR "visible light spectroscopy" OR "visible spectrometry" OR "visible spectroscopy"	612,129
Conclusion	#3; "detection" OR "diagnosis" OR "screening"	5,271,824
Outcome	#4; "carcinoma" OR "cancer" OR "dysplasia" OR "dysplastic lesion" OR "precancer" OR "precancerous lesion"	3,252,987
Search combination	#1 AND #2 AND #3 AND #4	223
Database search	Cochrane library, Pubmed, EBSCO	

2.2 Oral Potentially Malignant Disorder – a clinical and histological diagnosis

OSCC can arise de novo or be preceded by oral potentially malignant disorders (OPMD). According to Warnakulasuriya et al (2020), OPMDs are defined as 'any oral mucosal abnormality that is associated with a statistically increased risk of developing oral cancer.' This was historically described by terms such as "pre-cancer", "precancerous/premalignant lesion" or "intra – epithelial neoplasia".(22,23) The initial concept of "precancer" was introduced in the early

19th century.(24) In the mid – 20th century, efforts were made to distinguish premalignant conditions into leukoplakia (white patch) or erythroplakia (red patch).(6,25) Warnakulasuriya further elucidated the term as applying to a distinct area distinguishable by clinical appearance that may have a premalignant potential.(26) The WHO Collaborating Centre (2020) introduced the term OPMD, which is now preferred as it implies that not all lesions will become malignant, but have the potential to do so.(23,27) This body of expert opinion recommended the use of the term OPMD, which replaced the previously used terms '*precancer, epithelial precursor lesions, premalignant, precancerous and intra-epithelial lesions'*.

Table 2. Terms used in characterising premalignant conditions(30)

Terms	***Definition***
Oral precancerous lesion	A morphologically altered tissue that has greater potential to cause malignancy than the corresponding normal counterpart does.(28,29)
Oral precancerous conditions	A generalized condition that does not necessarily show clinical alteration of oral mucosa but is associated with a significantly increased risk of cancer development.(28,29)
OPMDs	Any clinical presentations that carry a high risk of cancer development in the oral cavity, whether in a clinically definable precursor lesion or in clinically normal mucosa.(26)

The classification of OPMDs is reproduced in Table 3. There is a general consensus that in the majority of cancers, there is a detectable precancerous phase.(31) Since

the introduction of the OPMD term, there has been an increase in interest in this heterogenous group of conditions, leading to better reporting.(26)

Table 3. Important OPMD identified by the WHO's working group on cancer(22)

Leukoplakia
Proliferative verrucous leukoplakia
Erythroplakia
Oral submucous fibrosis
Oral lichen planus
Actinic keratosis (actinic cheilitis) (AK/AC)
Palatal lesions in reverse smokers
Oral lupus erythematosus
Dyskeratosis congenita
Oral lichenoid lesion

2.2.1 Epidemiology

The estimated overall global prevalence of OPMDs is about 4.5%, although this varies by geographical area.(32) Most authorities maintain that the prevalence rate among populations worldwide is 1-5%, It may vary depending on country, people surveyed, the pattern of tobacco used and the definition of leukoplakia employed.(31) One systematic review noted the highest incidence of OPMD in Asian populations with a prevalence of 10.54% for all OPMD.(32) This review only included those with a histopathological diagnosis, implicitly excluding population-based studies and including only those who have been treated by dental or medical practitioners. The largest population-based studies have been conducted in the

developing world. A study of 50915 persons in four different regions of India found a degree of regional variation from 0.2% in Bihar state in the north to 4.9% in Andhra Pradesh in the east, apparently related to patterns of tobacco use.(33) Van der Waal, in his review of the topic, provides a compelling argument for the true incidence of OPMD being 0.5% or less extrapolating reported rates of malignant change and reported hospitalisations for oral cancer to a population wide figure.(23)

This review will focus on the following conditions as they are the conditions being recruited to this study.

Table 4. Summary table of OPMD

Condition	Incidence	Aetiology	Malignancy risk	Management
Leukoplakia	2.6-3.32% (32,34)	Smoking, male gender confers increased risk (35,36)	1-36%	Monitoring, surgical excision, others
Proliferative verrucous leukoplakia	Uncommon	Older age higher risk, otherwise unknown(37–39)	30-70% (38–40)	Monitoring, surgical excision (37)
Oral lichen planus	1% (32)	T cell mediated, multifactorial	<1% (41-44)	Monitoring, topical corticosteroids
Erythroplakia	0.02-0.83% (32,45)	Tobacco, alcohol	14-50% (46)	Surgical removal
Oral lichenoid lesion	Uncommon	Drug-related or contact reaction with dental material (47)	2.5%(44)	Removal of causative factor
Actinic keratosis	0.45-2.4% (48)	Sun exposure, age, fair skin, immunosuppression, male gender(49)	Possibly 3% (50)	Sun protection, excision

2.2.2 Leukoplakia

2.2.2.1 Summary

The most common form of OPMD is leukoplakia. The varying clinical and histologic diagnoses that have been applied to the term leukoplakia over the course of the years has hindered collection of data on this disease entity, which is now defined as: "*predominantly white plaques of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer'*.(22,27) Currently, leukoplakia is a term used for a clinical diagnosis and should not be used once there is a histological diagnosis.

2.2.2.2 Epidemiology

Major studies into the incidence of leukoplakia have mainly been carried out in the Indian subcontinent. A study of 57518 mill and textile workers aged over 35 in Gujarat, India found 6718 leukoplakia as well as 35 lesions showing epithelial dysplasia, an overall prevalence of 11.7%.(33,51)

In industrialised countries, most studies have been conducted on referral and hospital populations as opposed to community surveys.(31) Axell's community-based survey of 20333 Swedes aged 15 years and over found white lesions in 24.8% overall.(52)

Petti summarised the worldwide prevalence of leukoplakia in a systematic review of 23 studies from 17 countries published in 2003.(34) Strengths of this analysis include using standardised worldwide definitions of leukoplakia and only including studies where the numbers of those presenting with oral leukoplakia (OL) and those without disease could be accurately calculated. Studies conducted at secondary or

tertiary care facilities were excluded. Petti found an overall prevalence of 2.6% (95% CI 1.72–2.74%), although the researchers found a high degree of heterogeneity. This is similar to the figures produced by Mello et al., who published a pooled prevalence figure of 3.32% for oral leukoplakia and 0.32% for oral erythroplakia, the two OPMDs the authors found they could reliably estimate the prevalence of.(32) Petti found an unequal distribution of OL, with the highest incidence being in the Asian population (7.77%).(32)

2.2.2.3 Aetiology

Tobacco use is the commonest pre-disposing factor for leukoplakia. Leukoplakia is six times more likely to appear in smokers compared to non-smokers.(53)

In developed countries, leukoplakia normally develops in middle-aged and elderly people, the majority occurring between the fourth and seventh decades of life, while in the developing world, they typically occur 5-10 years earlier.(31)

Further evidence for tobacco's causative role in the development of oral leukoplakia is provided by the Californian study, where leukoplakia disappeared in 44% of the 50 patients who stopped smoking following leukoplakia diagnosis.(35)

No cause could be found in 17.3% of the leukoplakia identified in Axell's study.(52) This proportion (roughly 15-30%) is echoed in other studies.(31) Alcohol's role in the development of OSCC is well known, however it does not have a well-established role in the development of leukoplakia. An Indian case-control study with 927 leukoplakia and 47,773 controls found a link between any consumption of alcohol and leukoplakia with a risk ratio of 1.5.(54) This study also found a correlation between BMI and risk of leukoplakia, possibly due to malnutrition in

slimmer people. Other potential risk factors for oral leukoplakia or oral erythroplakia have been inferred from OSCC cohorts and include factors such as use of an alcohol- containing mouthwash, poor oral hygiene, periodontal disease, missing teeth, role of chronic mucosal trauma, co-infection with candida and oral dysbiosis.(55)

2.2.2.4 Pathogenesis

The pathogenesis of leukoplakia is unknown; however some theories are presented below. Chronic irritation from an exogenous agent leads to tissue adaptation. This results in an increase in cell proliferation, shrinking cytosolic volume and size of organelles in response to the trauma. In the oral cavity, this results in cell proliferation and hyperplasia (growth of the progenitor compartment). If the irritant persists, the epithelium may display mucosal atrophy (thinning) and degeneration. As the stage of adaptation and cell damage continues, an irrevocable cell damage ensues, manifesting as malignant transformation or apoptosis.(56)

2.2.2.5 Clinical presentation

It has a similar appearance to other, benign oral presentations, including cheek biting, aka frictional keratosis, leukoedema.(22) Axell showed that the prevalence of leukoplakia can vary from 0.7% and 24.8% with a change in clinical criteria for defining white patches.(57) Leukoplakia can be divided into homogenous and non-homogenous types. Homogenous leukoplakia have a smooth surface, well-demarcated borders and often have cracks or fissuring on the surface. Non-homogenous leukoplakia have a more varied clinical presentation, and may display features such as speckling (erythroplakia), nodular appearance (polypoid or

rounded projections), or verrucous (exophytic, warty, wrinkled surface). A red component to leukoplakia, termed erythroplakia or erythroleukoplakia, is indicative of candida colonisation and implies a higher risk of malignant transformation.(22)

Table 5. Certainty (c-factor) of a leukoplakia diagnosis.(59)

C1	Evidence from a single visit, using inspection and palpation as only diagnostic means (initial clinical diagnosis)
C2	Evidence obtained by a negative result of elimination of other aetiological factors e.g. mechanical irritation, during a follow-up period of 2-4 weeks or in the absence of any suspected aetiological factor
C3	As C2, but complemented by incisional biopsy (provisional histopathological diagnosis)
C4	Evidence following excision and pathological examination of the resected specimen (definitive histopathological diagnosis)

2.2.2.6 Management

Visual inspection and palpation are recommended for all suspected leukoplakia, including the area suspected and the neck region. The gold standard for diagnosis is biopsy and histopathological diagnosis. This should be done to exclude any other pathologies and to evaluate and grade the presence of any epithelial dysplasia within the patch. This is graded into mild, moderate and severe (carcinoma in situ) and is the best predictor of malignant transformation.(22) Additional testing for candida can help to establish the risk of malignant change. Management of leukoplakia and OPMD in general involves establishing the correct diagnosis, assessing the risk, counselling on risk factors and selecting an appropriate intervention.(12) The objective is to reduce alcohol and smoking and/or use of

chewing tobacco or smokeless tobacco. High risk lesions such as leukoplakia with moderate or severe dysplasia should be excised.(12)

2.2.2.7 Malignant transformation

While leukoplakia can progress to OSCC, the literature demonstrates many spontaneously regress. (31,58) In the Bombay study, 42.5% of untreated leukoplakia spontaneously disappeared within 5 years, especially in those who chewed tobacco. In the Californian study, lesions became smaller or disappeared in 37% of the 133 who were smokers and continued to smoke, in 42% of smokers who quit when diagnosed with leukoplakia, but in only 3% of 72 non- smokers.(35)

Estimates of malignant transformation vary with population; in the large scale population-based surveys from India that form much of the seminal literature, the rate varies between 0.13-2.2%(31)

Table 5. Factors predictive of malignant transformation.(13)

	Feature	Parameter	Association
Clinical features	Size of lesion	>200mm^2	Strong
	Texture	Nonhomogenous	Strong
	Colour	Red/speckled	Strong
	Site	Tongue and floor of mouth	Strong
		Multifocal	Strong
	Sex	Female	Medium
	Age	>50 years	Medium
	Habits	Non-smoker	Weak

Histological features	Dysplasia	Severe	Strong
		High-risk	Strong
	HPV	HPV-16	Medium
	DNA content	Aneuploidy	Medium
	LOH	Many genes involved	Medium

Petti's systematic review found a pooled incidence of malignant transformation of 1.36% per year (95% CI 0.69-2.03%).(34) However, estimates vary from 1.1% to 17.5%.(31) A Californian study of 257 patients with oral leukoplakia found OSCC in 45 patients, a ratio of 17.5% transformation. Silverman noted a persisting high chance of malignant transformation even after cessation of the smoking habit.(59) He noted an initial transformation rate elevated in year 1, and a subsequent rate of 1% malignant transformation per year. Chaturvedi, in a large study of 4886 Californians, found a progression risk of 2.2% from leukoplakia without dysplasia, 11.9% for mild dysplasia, 8.7% for moderate dysplasia and 32.2% for severe dysplasia. He found that <5% of oral SCC had a detected premalignant stage, due to lack of screening tools. 39.6% cancers arose from biopsied leukoplakia without dysplasia.(60)

Most studies conclude that smoking or cessation thereof will confer an influence on the rate of transformation.

Red and or/erosive appearance, a verrucoid hyperplastic pattern and microscopic dysplasia all confer increased risk.(35) Roed-Petersen found an increased risk of malignant transformation in erythroplakia with 9.1% (1/11) of speckled leukoplakias

and 1.3% (8/598) among other types showed malignant transformation.(61) Female sex, larger surface area, and no history of smoking were all correlated with malignancy.

In another UK-based study, lesions on the floor of the mouth displayed high transformation rates. Of 63 leukoplakia of the floor of mouth referred to a secondary care facility, 17 had malignancy either at presentation or within three months, and a further seven patients from the remaining 46 went on to develop OSCC at a rate of 15.2%.(62) Waldron, in a large study of 3256 oral leukoplakia biopsied and sent to one lab for histopathological analysis, found that leukoplakia from the floor of mouth and tongue were more likely to have severe dysplasia/CIS on histological exam.(63) In Warnakulasuriya and Ariyawardana's systematic review, the buccal mucosa was the most common site overall for leukoplakia but had the lowest rate of transformation. The tongue accounted for 16.14% of lesions but was the most common site for transformation, with a rate of 24.22%, while the second most common site for transformation proved to be the tongue/floor of mouth site.(64) Advancing age, female gender, size of lesion >200mm^2, presence of red or verrucous/exophytic areas on the lesion, grade of dysplasia were all found to be important risk factors for malignant transformation.(64)

Candida infection has been cited as a risk factor for malignant transformation, with candida superinfection in an existing leukoplakia carrying a higher risk for malignant transformation. However, there is conflicting evidence whether candida infection is the cause of leukoplakia or an infection superimposed on a pre-existing lesion.

Homogeneity or heterogeneity of lesion has been correlated to risk of transformation. Homogenous lesions are uniformly flat and thin, have a smooth surface and may exhibit shallow cracks.(22) Non-homogenous lesions have a higher risk of malignant transformation.

Loss of heterozygosity at chromosomal sites 9p21 or 3p14 has been found to be a predictive biomarker of sites at high risk of OSCC development and also predictive of second primary tumour development.(65–67).(66,67) Aneuploidy also demonstrates a relationship with degree of differentiation from the norm and degree of DNA ploidy may be viewed as a surrogate for malignant change.(30) Other tissue markers, such as increased telomerase activity, keratins, integrin, TGF-α, cyclin D1, MMP9 and MMP1 are also upgraded in development of OPMD and carcinogenesis.(30) These tissue markers have all shown promise, however may prove difficult and expensive to extract.

Many indicators of malignant change are known, however there is no currently no reliable method of telling when a lesion is going to transform.

2.2.3 Proliferative Verrucous Leukoplakia (PVL)

2.2.3.1 Summary

Any leukoplakic lesion that becomes warty, widespread and that has recurred after treatment should raise suspicion of PVL. This condition may affect multiple sites and as such may be difficult to treat. The World Health Organization (WHO) (2017) described PVL as "*a distinct and aggressive form of oral leukoplakia that potentially*

is a malignant disorder, exhibiting high recurrence and malignant transformation rates".(68)

Thirty years after its recognition, it is a challenging disease entity in recognition in its early phase, recognition of aetiology and optimum mode of treatment. Diagnosis is based on a combination of clinical appearance and histological picture, which can be complicated by its similarity to other conditions. (69,71)

2.2.3.2 Epidemiology

PVL occurs more commonly in females with no gender predilection. The mean age at diagnosis is in the seventh decade.(39) Approximately two-thirds occur in never smokers and there is no substantial association with alcohol consumption.(71) Otherwise, the aetiology is unknown. Villa notes in his review that prevalence data does not currently exist as it is a rare condition with several competing sets of diagnostic criteria.(37)

2.2.3.3 Aetiopathogenesis

Epigenetic studies have shown a role for hypermethylation of genes, especially PCLB1, CD8A, SPOCK2, ARTN and OSR2 among others. PLCB1 and OSR2 products have been described as tumour-associated proteins and may have a role in carcinogenesis. ARTN has a role as an oral cancer oncogene and pain mediator. In head and neck cancer, SPOCK2 has been identified as a potential diagnostic biomarker.(72) Other studies have found a role for oral microbial dysbiosis in conferring an increased risk of the disease – however, the authors caution that many factors may play a role in changing the composition of the oral microbiota. Other genetic changes found have been changes in level of ploidy, LOH and allelic

loss, especially at 9p21.(73) Deletion of p14 and p16 have also been noted, along with an increase in expression of p53.(74)

2.2.3.4 Clinical presentation

It displays an especially aggressive behaviour, especially in relation to other leukoplakia.(23,31) It is slow-growing, has a tendency towards multifocality, is extremely persistent and tends to recur after removal. Lesions are characterised by an initial keratotic appearance, frequently display an erythematous portion and develop warty exophytic components with time. The most common site for PVL to arise is the mucosa, gingiva, palate and alveolar processes.(40,75) The most common site overall is the buccal mucosa and gingiva.(38) Villa sets down criteria for diagnosis in his article, which offer a simplified version of the diagnostic criteria set down by Bagan et al. (see below).

2.2.3.5 Management

Excision is usually the treatment of choice. Due to persistent recurrence, resections may be repeated and may end up being very large. Recurrence rates are high (>70%) even if more radical resections are undertaken.(13) Close follow-up and repeat biopsies are essential to ensure early diagnosis and appropriate management.

2.2.3.6 Malignant transformation

It is generally accepted that is has a malignant transformation rate of at least 40%, which can rise to 70% with prolonged observation, and can reach 100% in cases of erythroplakia.(37,40,70,76) De Mendoza found a MT rate of 65.8% in her systematic review, while Ramos – Garcia found a pooled MT rate of 43.8% in their

recent systematic review and Abadie found a rate of 63.9%.(38,39) Malignant transformation can occur over a long period of time, and thus require consistent monitoring.(38)

Malignant transformation can arise at any site in the mouth, as there is a 'field cancerisation' effect described in the literature, as patients may develop several cancers in different parts of the mouth.(38) Studies show the rates observed over periods of 4.4 to 11.6 years.(38,40,70) Systematic reviews show a time to malignancy between 6 and 7.4 years.(39,71) Neither smoking nor alcohol has not been noted to confer an increased risk of malignant change.(71)

Okutoro concluded that viruses or candida, although detected by immunohistochemistry in many samples of PVL, could not universally explain the incidence of the disease. This was supported by a further systematic review in 2019.(77) Similarly, he found no evidence for overexpression of p53 or COX-2 indicating high risk of MT. Overexpression of p53 is found in PVL, while COX-2 overexpression is a marker of non-PVL oral epithelial dysplastic change.(37,78,79) This makes its pathway of carcinogenic change somewhat mysterious.(78) An interesting paper from McParland and Warnakulasuriya reported on the appearance of histological lichenoid changes in early PVL before the progression to full-blown multifocal disease. Lichenoid dysplasia is known to have a high risk of transformation.(80)

Table 6. Diagnostic criteria for PVL(38). All criteria must be met in order to reach a diagnosis

White/keratotic lesions that may be smooth, fissured, verrucous, or erythematous with or without ulcer
Multifocal non-contiguous lesions or a single large lesion >4.0 cm involving one site or a single large lesion >3 cm involving contiguous sites
Lesions that progress/expand in size and/or develop multifocality over time.
Histopathology that, if not overtly exhibiting dysplasia or carcinoma, shows hyperkeratosis, parakeratosis, atrophy, or acanthosis with minimal to no cytologic atypia (KUS), with or without a lymphocytic band, or verrucous hyperplasia; these features must not support a diagnosis of frictional or reactive keratoses

2.2.4 Oral Lichen Planus

2.2.4.1 Summary

Oral lichen planus (OLP) is an mucocutaneous autoimmune T-cell mediated disease of unknown aetiology that usually affects those aged 40 and above and affects females more than males in a ratio of 1.4:1.

2.2.4.2 Epidemiology

OLP is estimated to affect 1.01% of the population worldwide, with a higher rate in Europe, Southern Central America and Africa (1.43%, 1.74%, 1.32% respectively).(81) It is considered a potentially malignant disease with a transformation risk of 1.14%.(42) Prevalence figures may be skewed by the likelihood that the most common, reticular, type is most likely to be asymptomatic and not to cause presentation at a physician. González-Moles identified an age of

onset of 40 years, from which the chances of having OLP increase. No gender predilection was identified in this systematic review.(81)

2.2.4.3 Aetiology

Even though OLP aetiology is still unknown, many different causes have been advocated in its aetiopathogenesis, the majors being microbial agents, psychological stress, local and systemic cell-mediated hypersensitivity, and immune response.(82)

2.2.4.4 Pathogenesis

During OLP lesion formation, CD8 T cells and CD4 T cells respond to a hitherto unknown antigen with interaction from MHC Class 1 and Class 2 on basal keratinocytes. CD8+ T-cells trigger keratinocyte apoptosis by release of TNF-alpha, while CD 4+ T helper cells also have a role in OLP pathogenesis. Subsequent release of IL-2 and IFN-gamma induces CD8+ T calls to initiate target cell lysis. Other cytokines implicated in the pathogenesis of OLP include TGF-beta-1, IL-12, IL-4, IL-10, IL-6, GM-CSF, IL-1-beta. IL-6, which is a NF-κβ-associated cytokine, is critical in perpetuating chronic inflammation and autoimmunity and is recognised as a key mediator in the development of chronic inflammation into cancer.(83)

2.2.4.5 Clinical presentation

The diagnosis of OLP is based on clinical and histopathological characteristics. OLP can be divided into several subtypes based on clinical appearance – reticular, bullous, papular, atrophic, erosive and plaque-like. Patients may often display more than one clinical subtype simultaneously. The usual presentation of OLP is reticular lace-like striations on the buccal mucosa and on other areas of the mouth, like

tongue and gingiva. These may be symptomatic or asymptomatic. Reticular OLP can also be found on the lips, floor of mouth, labial mucosa and rarely on the palate. Atrophic LP is usually found on the gingiva and may be described as desquamative gingivitis.

The classic histological features are hyperkeratosis, a dense band-like lymphocytic inflammatory infiltrate, normal maturation epithelium, liquefactive degeneration of basal cell layer, formation of Civatte bodies and saw-tooth rete ridges.(84) Features associated with dysplasia such as nuclear enlargement and increased mitotic figures may be found with features of lichen planus. This lesion should be classified as a dysplastic lesion rather than OLP, as this lesion has a greater risk of transformation. In OLP frozen sections, a linear pattern and intense positive fluorescence is observed at the basement membrane zone, with deposition of IgM, and less often IgA, IgG and complement C3 are found on the colloid bodies.

2.2.4.6 Management

Biopsy is recommended in cases of uncertainty about the diagnosis, or in an atypical presentation.(47). Maintenance of good oral hygiene can lessen symptoms and improve healing. First line treatment is with topical corticosteroids, however many drugs have been tried for symptomatic OLP, including corticosteroids, griseofulvin, topical retinoids, hydroxychloroquine, cyclosporin, clobetasol, tacrolimus, photodynamic therapy and others with variable success.(84) Systemic corticosteroids are used in cases of severe, widespread OLP or LP recalcitrant to treatment. Hydroxychloroquine and azathioprine are useful as “steroid-sparing” agents in systemic corticosteroid responsive OLP.(47). Many patients with OLP can

be asymptomatic, however atrophic-erosive forms can be painful. Although the risk of malignant transformation is low, long-term follow up is necessary.



Figure 1. An example of an oral lichen planus (buccal mucosa)

2.2.4.7 Malignant transformation

The notion that malignant change occurs from OLP is debated. Rudolf Virchow, physician, scientist and politician and "Father of modern pathology" was the first to notice the proliferation of leucocytes in malignant tissue as far back as 1863 and surmised the role of inflammation in carcinogenesis.(85) Mignogna notes the link between chronic inflammation and epithelial malignancy, and supports his argument by citing examples of chronically inflamed areas undergoing malignant change such as oesophageal carcinoma secondary to chronic reflux and oesophageal adenocarcinoma.(86)

Table 7. Associations between inflammation and cancer risk (87)

Malignancy	Inflammatory stimulus/condition
Bladder	Schistosomiasis
Cervical	Papillomavirus
Ovarian	Pelvic inflammatory disease
Gastric	*H. Pylori*-induced gastritis
MALT lymphoma	*H. pylori*
Oesophageal	Barrett's metaplasia
Colorectal	Inflammatory bowel disease
Hepatocellular	Hepatitis virus (B and C)
Bronchial	Silica, asbestos, cigarette smoke
Mesothelioma	Asbestos
Kaposi's sarcoma	Human herpesvirus type 8

Production of the free radical nitric oxide as a by-product of chronic inflammation is thought to be one factor driving carcinogenesis. High levels of circulating COX-2 enzyme secondary to the inflammatory process is also hypothesised to drive carcinogenesis. Further production of inflammatory cytokines leads to secretion of toxic malondialdehyde, which is known to cause DNA damage. Changes in the tumour microenvironment influence the development and induce carcinogenesis.

Tumour associated macrophages (TAMS) are able to stimulate tumour development and invasion through the production and growth of angiogenic factors as well as protease enzymes dissolving extracellular matrix.(86) CD34 has been

found to be overexpressed, especially in erosive and ulcerative forms. Macrophage inhibitory factor (MIF) has been shown to have an anti-apoptotic effect, repressing transcription of p53 and stimulates tumour cell proliferation.(86) p53 has been found to be overexpressed on basal and parabasal keratinocytes, correlating with increased dysplasia.(30) TAMs are also associated with excess production of MMPs, leading to tissue destruction and are a major source of TNF (tumour necrosis factor), a leading feature of carcinogenesis. TNF induces angiogenesis, and in high doses can destroy tumour cells, but when produced chronically, like in chronic inflammation, may act as a tumour promoter, influencing both malignant cells growth and remodelling tumour stroma.(85,86) On the other hand, another school of thought maintains that the low rates of OLP transformation are evidence in themselves, and this is evidence that the traditional risk factors of smoking and drinking are the reasons for development of tumours in oral lichen planus.(86). Epidemiological evidence is found for the inflammatory hypothesis that patients who are at higher risk of malignant transformation are Hepatitis C sufferers.(44) Another lab study showed that mice deficient in TNF gene are not likely to develop second primary tumours where they have received genetic engineering to make them susceptible to a particular genetic blood cancer.(86)

Systematic reviews of the evidence have established a malignant transformation risk. While there is currently a debate raging about what the exact measure and what the exact figure is, there is a consensus that older people with red or ulcerated lichen planus are more at risk.

Fitzpatrick conducted a systematic review in 2014 including 7806 patients with OLP and 125 patients with oral lichenoid lesions (OLL). From the patients with OLP, 85 developed SCC, giving a transformation rate of 1.09%. In the only study of those with OLL, four developed SCC, with a rate of 3.2%.(41) Giuliani found in 2017 that both OLP and OLL have a malignant transformation risk, with OLP having a risk of MT of 1.4% and an annual risk of 0.2%. He found that female gender, location of OLP on tongue and red lesions favoured transformation, and that OLL had a slightly increased risk of transformation compared with OLP.(82) Aghbari's review of 2017 established a risk of 1.1%.(44) Predisposing factors include smoking (OR=2, 95% CI 1.25-3.22), alcoholism (OR=3.52, 95% CI 1.54-8.03)and Hepatitis C co-infection (OR=5, 95% CI 1.56-16.07).(44) The site at highest risk of malignant transformation was the tongue, while female sex and age in the 60s were important predictive factors.(41)

In González – Mole's systematic review and meta – analysis of 2020, factors that favour progression of OLL/OLP to malignancy included smoking and/or drinking habits, Hepatitis C co-infection, presence of epithelial dysplasia.(42) This review also found that male gender increased likelihood of progression, although this was not significant, and counselled that many lesions clinically described as oral lichen planus have a higher than predicted chance of progression and therefore should be biopsied.(42) Furthermore, lesions that are atrophic or erosive showed the highest risk of transformation compared to reticular, which showed the lowest. This was described as a red versus white scenario. He found an overall risk of MT of 1.14% for OLP (95% CI = 0.84-1.49), 1.88% for OLL (95% CI = 0.15-4.95) and 1.71% for LRs (95% CI = 0.00-5.46).

Another systematic review of 2020 disagreed with the above, and using much more stringent criteria – for example, SCC has to be documented to arise from the site of an OLP/lichenoid lesion, there has to be a time delay between diagnosis of OLP and OSCC, no histopathological diagnosis of OLP, found a much lower transformation rate of 0.44%. Idrees, in this systematic review, criticised González-Moles and Aghbari for, among other things, including cases with proven epithelial dysplasia, which should exclude OLP as a diagnosis. However, he repeated their conclusions that MT is more likely in smokers, alcohol consumers and Hepatitis C positive people.(43)

2.2.5 Erythroplakia

2.2.5.1 Summary

Erythroplakia is defined by the WHO Collaborating Centre for Oral Cancer as '*a fiery red patch that cannot be characterised clinically or pathologically as any other defined disease'*.(45) Oral erythroplakia (OE) is usually quite well-defined, irregular in outline and have a bright red, velvety surface. Erythroplakia may occur in close combination with leukoplakic lesions.

2.2.5.2 Epidemiology

It has a prevalence of between 0.02-0.83%, making it a rare diagnosis.(87) The buccal mucosa, tongue, floor of mouth and gingiva are the most commonly involved subsites identified in the most recent meta-analysis.(45,87) It is more likely to be observed in males and in those over the age 50.(45) In 58 cases of OE

reported by Shafer and Waldron, 37 cases occurred in the sixth and seventh decades.(88)

2.2.5.3 Aetiology

Case control studies from India found that tobacco use (smoked and chewed), alcohol use, low vegetable intake, low BMI all contributed to development of this condition. These studies also found that chewing betel quid in particular raised the risk of OE, with a linear dose-response relationship after controlling for confounding factors.(89) Smoking was also associated with erythroplakia, however the relationship was not as strong as for chewing tobacco.(89) Alcohol consumption raised the risk of erythroplakia by 3 times, and gave rise to a multiplicative combined effect with tobacco use.(89)

2.2.5.4 Pathogenesis

The pathogenesis of erythroplakia is poorly understood. It is widely accepted that alcohol and tobacco play a role in the initiation of the condition and its progression.

2.2.5.5 Clinical presentation

The WHO 'Histological typing of cancer and precancer of the oral mucosa' described the clinical features of OE as follows: "Some erythroplakias are smooth and some are granular or nodular. Often there is a well-defined margin adjacent to mucosa of normal appearance".(91) The red colour, or occasionally speckling, may be due to mucosal atrophy and epithelial thinning and show through of the underlying microvasculature.(91)

Erythema migrans, atrophic candidiasis, gingivitis, pemphigoid, discoid lupus, erosive lichen planus and other inflammatory/infective conditions can also be mistaken for erythroplakia.(22)

2.2.5.6 Management

Surgical excision is the treatment of choice, in the absence of any contraindications to surgery.(12)

2.2.5.7 Malignant transformation

Erythroplakia and its near cousin erythroleukoplakia are associated with severe dysplasia, carcinoma in situ or frank carcinoma at biopsy.(87,88,92) Shafer found that 91% were dysplasia, carcinoma in situ or cancer.(88) Lorenzo – Pouso's meta-analysis found a malignant transformation rate of 19.9%.(45) This is lower than quoted by many authors, and he provides a reason, stating that erythroplakia should only be diagnosed as such if an initial biopsy fails to show SCC or carcinoma in situ and if the lesion is followed up. He also observed differences in location of OE corresponding with differences in risk of MT – for example, red lesions on the lip or buccal mucosa have a lower risk of MT, however, lesions on the floor of mouth or retromolar areas were found to be highest risk.(45) He concluded that the level of epithelial dysplasia observed in the initial biopsy is the most important prognostic factor in predicting MT.

2.2.6 Oral Lichenoid Lesion

2.2.6.1 Summary

Oral lichenoid lesion (OLL) are a group of intra-oral, reticulated, red and white lesions similar to oral lichen planus, however, this group of lesions have an underlying causative factor, as opposed to OLP, which is of unknown aetiology.

2.2.6.2 Epidemiology

Warnakulasuriya states that OLL can include atypical OLP and unilateral lichenoid lesions.(26) Oral lichenoid contact lesions (OLCR) may occur more often in older people – this may be due to the time involved in eliciting an immune response. One study showed an average age of 54.6 years.(95) In one study from Finland, all types of oral lichenoid reactions were more common in males and in current smokers.(96)

2.2.6.3 Pathogenesis

Holmstrup describes three different mechanisms for OLCR to occur – type IV sensitivity, toxic reactions and the much rarer acute or generalised sensitivity. Type IV hypersensitivity reaction is a cell-mediated, delayed response. Mercury salts from dental amalgam restorations accumulating in damaged mucosa will cause this contact reaction.(97) Drug-mediated oral lichenoid reactions are also type IV and associated with systemic medications, which include antihypertensives, oral hypoglycaemic agents, nonsteroidal anti-inflammatory drugs, beta-blockers, and HIV antiretrovirals.(98) Nonspecific reactions can also occur as lichenoid lesions with a similar clinical appearance.

2.2.6.4 Clinical presentation

Clinically, the appearance can be similar to OLP and shows a range of clinical presentations – striated, reticular, plaque-like, erythematous, vesicular and erosive forms. OLL caused by hypersensitivity reaction to dental materials are often localised to the site of the dental material, usually on the buccal mucosa, gingiva and tongue with a reticular or plaque - like appearance while OLP has a widespread, often bilateral appearance. OLL that are drug-induced have certain clinical features, with a predisposition to being unilateral and erosive. The diagnosis of OLP/OLL is based on history, clinical presentation, patch test if indicated and histopathology.

Oral lichenoid lesions show histologically similar signs to oral lichen planus – liquefaction of basal layer and lymphocytic infiltrate. Cutaneous patch tests are recognised and accepted as ways of identifying allergens responsible for Type I and Type IV allergic reactions.

2.2.6.5 Management

Resolution usually follows removal of the causative agent. OLR associated medications include antihypertensives, antimalarial or NSAID medications. A change of medication should be considered after consultation with the patient's GP. Thornhill found that 70% of amalgam contact hypersensitivity lesions were patch tested positive for mercury or amalgam compared with only 3.9% of OLP cases. Replacement of amalgam has resulted in lesion resolution of 93% of cases. (84)

2.2.6.6 Malignant transformation

Malignant transformation of oral lichenoid lesions follows a similar course to that of oral lichen planus, with inflammatory mediators playing a role. Malignant transformation has been estimated as 2.5%.(44). Compared to oral lichen planus (OLP), oral lichenoid lesion (OLL) exhibit much more loss of heterozygosity (LOH) and expression of Ki-67 proteins. LOH at tumour suppressor loci may favour changes in cell proliferation and apoptosis.(99) Other biomarkers of increased risk progression include upregulation of p53, p16, MCL-1, MMPs, TNF-α, IL-6 and COX-2.

2.2.7 Actinic keratosis/actinic cheilitis

2.2.7.1 Summary

This oral potentially malignant disorder can be defined as: "*A disorder that results from Sun damage and affects exposed areas of the lips, most commonly the vermilion border of the lower lip with a variable presentation of atrophic and erosive areas and white plaques*".(100) It is produced by the effect of sunlight on exposed tissues. First described by Ayres in 1923, it is predominantly a disease of light - skinned men who work in outdoor occupations.(50,101)

2.2.7.2 Epidemiology

Estimates of prevalence vary widely, with ranges from 0.45-2.4% of the population(48) , while in studies looking at outdoor populations, prevalence is more like 4-43%.(48)

2.2.7.3 Aetiology

Sun exposure is the most common risk factor, with people in the tropics at particular risk. Those with paler skin are more prone to the condition, with lack of

melanin making fair-skinned people more prone to UV damage. Males show a predisposition compared to females(48). The lower lip is more prone to sun damage than the upper lip, and is more delicate with less keratin and more sebaceous glands than the upper lip.(50) Although people of any age are at risk, those >50 years of age are at higher risk.(48) This can be for any number of reasons, including diagnostic delay, slow growth of the lesion and accumulation of UV damage in the skin over time. Other factors implicated are unhealthy lifestyles – smoking, poor diet and genetic predisposition.

2.2.7.4 Pathogenesis

UV radiation may be divided into three categories – UV-A, UV-B and UV-C. UV-C is mostly filtered out by the Earth's atmosphere and has little effect on human health. UV-B and UV-A have both been implicated in sun damage, skin cancer and AK. UV radiation directly affects human DNA, causing genetic changes. Oxidative stress, in common with other premalignant lesions is seen. Damage to DNA results in formation of cyclobutane pyrimidine dimers and starts a process of inflammation and carcinogenesis. UV radiation also causes immunosuppression, leading to reduced tumour capture.(102)

2.2.7.5 Clinical presentation

Clinical presentation is usually along the lines of flaky, keratotic, white lesion with signs of dryness.(50) Ulcerative lesions are common with inflammation, atrophy and loss of epithelium. Scaly red areas are also common. White plaques are seen after atrophy. Other clinical features include scaly sites, swelling, erythema, ulceration, indistinct vermilion border, transverse fissures, crusting, tissue pallor

and blotching.(30) Histologically, the epithelium may show hyperplasia or atrophy, disordered maturation, degrees of keratinisation or parakeratinisation, cytological atypia and increased mitotic activity.(26) Lichenoid inflammation is also present, and a diagnosis of lichenoid actinic keratosis is then appropriate.



Figure 2. An example of actinic keratosis.

2.2.7.6 Management

As with all OPMDs, assessment of risk and reduction of risk factors is a key goal. For actinic cheilitis, this involves counselling about the risk of sunlight and prescribing effective sun protection. Usually a lip balm with a an appropriate level of UV protection is adequate. Depending on clinical signs and symptoms and the result of a biopsy, treatment normally consists of surgical excision "lip shave" or CO_2 laser excision. The advantage of surgical excision is a specimen for histopathological analysis. If using laser, a pre-treatment biopsy should be taken. Other treatments, such as photodynamic therapy do not have a solid evidence base.(12,23)

2.2.7.7 Malignant transformation

Warnakulasuriya states that the risk of malignant transformation cannot be predicted due to degree of dysplasia being difficult to assess clinically.(26,49) Dancyger found a rate of 3%, from one study.(50) A study obtaining cells from the lower lip by smear showed an incidence of malignancy of 3.2% among Brazilian fishermen.(103) The rate of malignant transformation can be very slow, with studies reporting ranges from 1 – 30 years.(50)

Up to 89 genes have been implicated in malignant transformation.(104) Loss of tumour suppressor p53 is important. (50) 90% of SCC cases and 80% of AK have UV-specific mutations in p53. Other proteins implicated include anti-apoptotic bcl-2 and Ki-67 and Murine Double Minute 2 tumour suppressor protein. MDM2 proteins act to suppress action of p53, thus a lower level of MDM2 may mean that defective p53 proteins are not taken out of circulation, leading to carcinogenesis.(105) CD8+ and NK cells are also decreased in AK lesions. Regulatory T cells (Tregs) are involved in immunosuppression in oral cancer. Expression of Foxp3+Tregs cells inhibits T-cell proliferation, generating an immunosuppressive environment with high levels of circulating cytokines, such as IL-10 and lower levels of IFN-γ, the former of which has immunosuppressive properties, and the latter antitumourogenic properties.(106)

2.2.8 Other premalignant conditions

Other premalignant conditions will not be discussed, as they do not form a part of the clinical investigation.

2.2.8.1 Graft vs. host disease
2.2.8.2 Discoid Lupus Erythematosus
2.2.8.3 Oral Submucous Fibrosis
2.2.8.4 Palatal lesions in reverse smokers
2.2.8.5 Dyskeratosis congenita
2.2.8.6 Other OPMDs

2.2.9 Dysplasia and squamous cell carcinoma

2.2.10 Oral Epithelial Dysplasia

Oral Epithelial Dysplasia (OED) is a spectrum of architectural and cytological epithelial changes caused by accumulation of genetic changes, associated with an increased risk of progression to oral squamous cell carcinoma. It may be classified as low grade, high grade or by other systems.(68)

2.2.10.1 Histopathology

2.2.10.1.1 Oral Epithelial dysplasia

A minority of white lesions will display OED, however high-risk red or speckled lesions usually has some form of dysplasia. There is abnormal proliferation, maturation and differentiation of epithelial cells. Cytological and histological features determine the diagnosis and the grade of dysplasia. This process is operator-specific and shows poor agreement between interpreters. Consensus grading after review by more than one pathologist may enhance diagnostic reliability. There is no good evidence to show how the presence of specific features can be translated into a grade of dysplasia. No specific combination of features reliably distinguishes mild dysplasia from hyperplasia. Traditionally, OED is divided into three grades of severity. Mild dysplasia can be defined as cellular atypia limited to the basal third, moderate dysplasia as extension into the middle third, and

severe dysplasia by extension into the upper third of the epithelial depth. Further grade changes can be arrived at by the degree of cellular atypia and architectural changes. Marked atypia in the basal third of the epithelium may be sufficient for a diagnosis of severe dysplasia. Carcinoma in situ of the oral cavity is considered synonymous with severe dysplasia.(107)

2.2.10.2 Prognosis and predictive factors

Many dysplastic lesions will never transform, despite the fact that dysplasia is correlated with the emergence of oral squamous cell carcinoma. A meta-analysis of OED-related lesions revealed a transformation rate of 10.3–12% over a mean period of 4.3 years.(114) Oral squamous cell carcinoma is typically more likely to occur the more advanced the dysplasia is. According to the conventional three-grade system, the 15-year transformation rates of mild, moderate, and severe dysplasia are roughly 6%, 18%, and 36%, respectively, and the presence of dysplasia signals long-term risk.(108) Research on specific transformation risk is constrained by the poor inter-rater reliability of current measurements of dysplasia.(5)

Table 8. Histopathological features seen in dysplasia (111)

Architectural changes	***Cytological changes***
Irregular epithelial stratification	Abnormal variation in nuclear size
Loss of polarity of basal cells	Abnormal variation in nuclear shape
Drop-shaped rete ridges	Abnormal variation in cell size
Increased number of mitotic figures	Abnormal variation in cell shape
Abnormally superficial mitotic figures	Increased N:C ratio
Premature keratinization in single cells	Atypical mitotic figures
Keratin pearls within rete ridges	Increased number and size of nucleoli
Loss of epithelial cell cohesion	Hyperchromasia

2.2.10.3 Molecular progression of head and neck cancer

Neoplasms arise clonally from transformed cells that have undergone specific genetic alterations in proto-oncogenes or tumour suppressor genes.(109,110) Critical changes in gene expression at each step allows for identification of molecules associated with each stage. The deletion of chromosomal region 9p21, which is a putative tumour suppressor gene, occurs early in carcinogenesis and is the most common of genetic changes.(109,111) This results in inactivation of the p16 gene, which is an inhibitor of cyclin-dependent kinase, which regulates the cell cycle.(109,112)

Cellular atypia is typically the first histological sign, and the appearance of oral epithelial dysplasia provides further evidence of malignant change and offers a measure of the progression.(30)

Tumours can also evade tumour suppressor pathways such as Rb and p53 by unknown methods.(113) Over 50% of all cancers contain a mutation of p53 gene located at 17p13.(114)

2.2.11 Squamous cell carcinoma

Oral squamous cell carcinoma is a carcinoma with squamous differentiation arising from the mucosal epithelium.(68)

2.2.11.1 Macroscopy

SCC is notoriously variable in appearance, however major clinical signs included erythematous, indurated, nodular and ulcerated change. When present, ulcerative changes may have features such as rolled borders and raised margins. Although ulceration may be present in fewer than 50% of cases, non-healing ulcers are a classic sign of malignancy (18) Verrucous carcinoma typically has papillary projections that are extensively keratinized and has a more hyperplastic, fungating appearance. (107)

2.2.11.2 Histopathology

The majority of oral SCC are well to moderately differentiated, with poorly differentiated tumours less frequent. Well-differentiated SCC is characterized by nests, cords and islands of large cells with pink cytoplasm, prominent intracellular bridges and round nuclei, which may not be obviously hyperchromatic. Dysplasia features as previously described, including nuclear pleomorphism, nuclear

hyperchromasia, and mitotic figures (including atypical forms) increase with tumour grade.

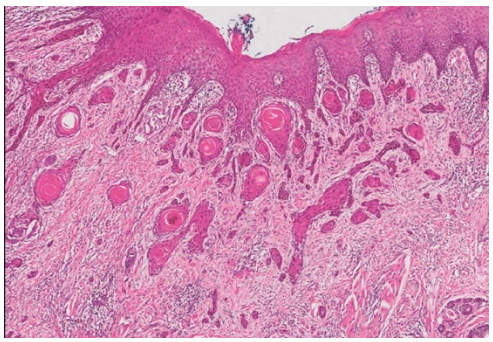


Figure 3. Histologic presentation of oral squamous cell carcinoma.(118)

The role of the basement membrane is as a barrier which provides a provides a conceptual and physiological Rubicon-like frontier which separates in-situ changes from invasive SCC.(115)

2.2.11.3 Prognosis and predictive factors

Conventional OSCC is an aggressive tumour. Tumour size, nodal status, and distant metastasis are the three most important prognostic variables. Non-cohesive pattern of invasion, perineural and lymphovascular invasion, bone invasion, and tumour thickness >4mm are all histological risk markers linked to a worse prognosis.*(115)*

2.2.12 Management of OSCC and OED

2.2.12.1 OSCC Management

Site and stage at diagnosis determines the management strategy. For lesions of the oral cavity and mobile tongue, surgery is the method of obtaining local control, with the use of adjuvant therapies such as radiotherapy and chemotherapy. If one or both of these treatment modalities fail and recurrence at a local or distant site

occurs, it is more likely that radiotherapy or chemotherapy will be used as a primary modality of treatment.(107)

2.2.12.2 Management of Oral Epithelial Dysplasia

Histopathological grading of oral epithelial dysplasia remains the main tool for assessment of the malignant transformation risk of a particular site and thus the management, surgical or otherwise. Despite documented difficulties in grading samples of epithelial dysplasia according to accepted criteria, systematic reviews have proven the utility of this system in predicting malignant transformation.(116) Some studies have found that the binary classification system, as originally set out by Kuffer and Lombardi, provides better prediction of which lesions will progress to malignancy, however there is no clear consensus.(119) A recent systematic review of the evidence found that many samples diagnosed as 'moderate dysplasia' under the WHO criteria could be reclassified as high-risk according to the binary classification.

Thus, this binary or trimodal approach to risk has its limitations, particularly in lesions like oral lichen planus which may not demonstrate dysplastic change prior to malignancy.

Surgical excision is the treatment of choice for high-risk OPMDs, with close surveillance afterwards. Up to 35% of lesions recur.(108) Mehanna's landmark study outlined the risk of transformation of oral dysplastic lesions and a strategy for management. This simple schema can be complicated further by patient and pattern of tobacco consumption. Lesions on the buccal mucosa in tobacco-chewers and palatal lesion of reverse smoking are also of just as much risk as the lesion on

the lateral tongue or floor of mouth. Females have been noted to be at higher risk of progression of PVL.(122) As previously noted, the lack of evident clarity and agreement with classification of dysplastic lesions makes the decision on management contingent on several factors.

It is generally agreed that all red or speckled lesions have a high risk of malignant change and should therefore be excised.(122)

The Liverpool algorithm provides a useful method of stratifying lesions by risk, planning biopsy and subsequent follow-up or excision.(1) Biopsy is an integral part of this pathway, initially establishing the diagnosis, then a major part of the risk stratification is the level of dysplasia. Multiple biopsies may be needed in larger areas.

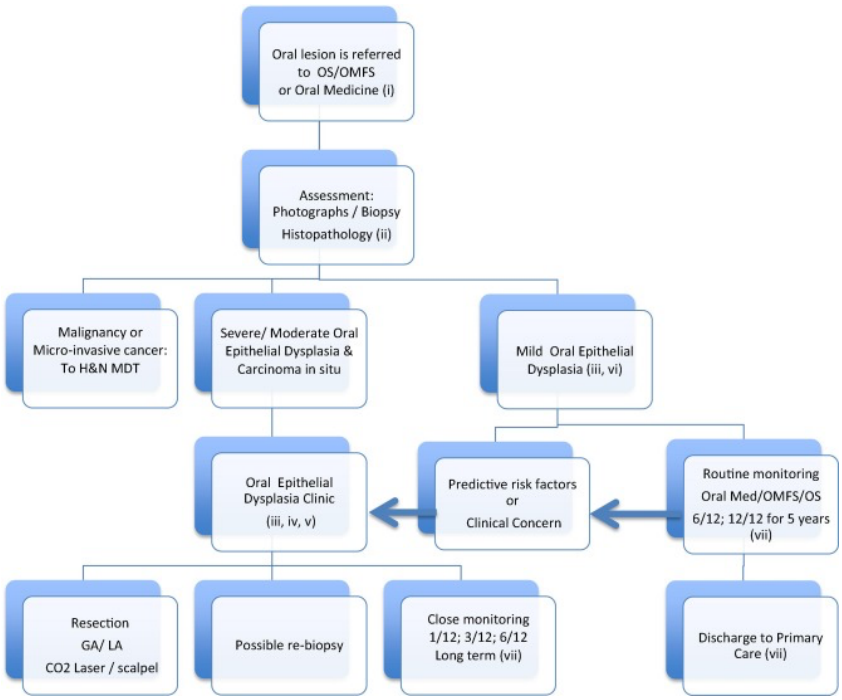


Figure 4. The Liverpool algorithm for management of dysplastic lesions(1)

Those with severe dysplasia are offered excision, while those with moderate dysplasia may be offered excision if the lesion is not too large, with a pre- or post-

excision period of clinical monitoring. This is usually one, three, six or twelve months. This period may be continued for a minimum of 5 years, or may be extended indefinitely, with clinical and histological monitoring.(1)

Lodi et al., in a Cochrane review, explored medical and surgical management options for oral leukoplakia. They noted that a reduction in size correlated a decreased risk of oral cancer. Other auxiliary methods of treating leukoplakia – systemic Vitamin A and systemic beta carotene or carotenoids were evaluated in the same trial – and found to be of benefit. NSAID therapy, herbal extracts, topical bleomycin and use of Bowman-Birk inhibitor were all mentioned in this study and no benefit was found in terms of resolving or reducing size of leukoplakia. Surgical intervention was not evaluated due to a lack of appropriate studies(123).

Oral cancer is frequently associated with the development of multiple primary tumours. This occurs at a rate higher than any other malignancy.(10) This led to Slaughter's concept of field cancerization, (unexpected multifocal early OSCC/synchronous primary tumours) where multiple primary tumours develop in the upper aerodigestive tract in response to long-term exposure to carcinogens.(21) Widespread neoplastic change makes identification of the borders of clinically healthy and diseased tissue extremely challenging. This theory, although not universally accepted, explains why some patients have recurrences – 35% of patients who are treated for a primary oral SCC have a new tumour develop within 5 years.(19) This is more likely to develop at the original site – in 10-30% cases.(124)

Optical techniques make it possible to detect biochemical changes that go with field cancerisation, however false positives are possible due to subtle tissue changes in buccal mucosa in distant cancers such as lung and oesophageal cancer.(125)

Innovative research using IR spectroscopy techniques with PCA – LDA component analysis has identified a role for spectroscopic techniques in predicting which lesions are at most risk of malignant transformation.(126)

This is the crucial role that optical techniques can fill – enabling the instantaneous, real time accurate detection of oral malignancy in a non-invasive manner. Repeated biopsies can be hard for patients to tolerate, thus extended periods of monitoring with clinical photographs are necessary. An accurate, real-time detector as offered by optical techniques offers great promise.

2.3 Existing tools for the detection of OPMD and OSCC

2.3.1 Screening tools

Screening tools for detection of premalignant and malignant conditions have been shown to significantly reduce morbidity and mortality from a range of conditions, such as the Pap test for cervical cancer and mammography for breast cancer screening.(21)

Screening tools for oral potentially malignant lesions most commonly used include visual examination with incandescent light. Carried out by a suitably qualified and experienced personnel with the availability of histopathological confirmation of diagnosis, this is the gold standard for detection and treatment of OPMD.

Prevention is tied into the World Health Organisation's (WHO) resolution to include oral cancer into national cancer control programmes.(127) Primary screening programmes for cancers such as breast, bowel and cervical cancer have improved mortality rates and helped to decrease the incidence of these cancers.(128,129)

Screening represents the possibility to identify suspicious lesions early and decrease the burden of disease on the patient by early identification of suspicious lesions. There is currently no NHS approved screening programme for oral cancer, as no current screening tool reaches the criteria for cost-effectiveness.

2.3.1.1 Screening for oral cancer and oral premalignancy

2.3.1.2 Clinical Oral Examination

Clinical oral examination (COE) using an incandescent light has long been the standard of care for oral cancer screening. It has been demonstrated that this is cost-effective.(130,131) COE has demonstrated an overall specificity of 0.97 and

sensitivity of 0.85 in a systematic review.(132) The authors of this systematic review concluded that this showed good results in comparison with cervical screening and mammography, which showed sensitivities of 0.8 and 0.98 respectively.(~~94~~) Another systematic review with stringent criteria found a sensitivity of 0.91 and specificity of 0.31.(133) An interesting study from Taiwan, a country with a strong centralised municipal medical system and cancer registry found a sensitivity and specificity 98.9 and 98.7% with experienced examiners and 13878 participants, using the Taiwan Cancer Registry as a surrogate for undetected cancers. It has been shown that regular clinical oral examination can increase overall long-term survival for high-risk populations, such as those who smoke and drink.(134,135) A Cuban study screening the entire 10 million population over a 7 year period downstaged oral cancer diagnoses nationwide, despite poor logistics hampering biopsy procedures.(136) It is logical that COE will serve as an efficient and adequate means of detecting oral abnormalities, while the adjuncts serve as risk stratifiers and means to identify high-risk areas not obvious to initial exam. An Indian trial demonstrated reductions in oral cancer deaths and increase in detection of oral cancer in a subgroup of tobacco users in a population-based screening programme, with reductions first noted at 9 years into the study maintained at 15 years.(137,138) A Cochrane review found that screening could possibly help reduce oral cancer death or to downstage cancers at diagnosis in a high-risk population.[9]

While oral examination may be effective as a screening tool, problems remain. Approximately 5-15% of the population may have oral mucosal abnormalities.(139) Of these, only a small percentage will be malignant or of clinical concern. And with any oral malignancy, it can also arise in areas of normal-appearing mucosa.

Thomson's study found that of 9 out of 26 consecutive patients with a newly diagnosed oral SCC, the contralateral site yielded a histologic result of dysplasia or in situ SCC on biopsy.(140)

Lesions discovered during routine screening or by other means may be difficult to distinguish in primary care and also suffer from underdiagnosis in secondary care. For this reason, adjuncts providing quicker, or more thorough, or easier, or cheaper, or more accurate findings hold promise and deserved further investigation as per the Cochrane review[11].

2.3.1.3 Biopsy and histopathological diagnosis

This is the gold standard for diagnosis of oral SCC and oral premalignant lesions. Has high sensitivity and specificity, however may have false negatives due to sampling of inappropriate area in larger lesions. Incisional biopsy has traditionally been favoured. There is a well-documented lack of inter-observer reliability between pathologists examining oral SCC, and Lee at al reported underdiagnosis of SCC in 12% of incisional biopsies quickly followed by complete excision in an OMFS unit in Taiwan.(5,141) She recommended multiple biopsies for nonhomogenous oral leukoplakia.

2.3.2 Adjunct Screening Tools

2.3.2.1 Oral cytology

Cytopathology involves laboratory analysis of cell samples collected from mucosal surfaces by brush biopsy. These samples are fixed onto a glass slide and stained using a Papanicolau stain, scanned and analysed microscopically by a

cytopathologist. Brush-based cytology offers the advantage over exfoliative cytology of being able to collect cells from one specific area. (143)

2.3.2.1.1 Brush Biopsy

The Brush Biopsy (CDx Laboratories, Suffren, NY) was introduced as a potential oral cancer case-finding device in 1999. It was designed for the interrogation of clinical lesions that would otherwise not be subjected to biopsy because the level of suspicion for carcinoma, based upon clinical features, was low.(21) It involves rotating a bristled brush over oral mucosa, to obtain cells for cytological analysis. While it has shown good results in identifying mouths and areas that would typically be biopsied due to their suspicious nature on visual examination, estimates of sensitivity and specificity are influenced higher by the lack of biopsies in cases ruled to not be suspicious by brush biopsy.(21) It may be useful in non-compliant patients or those with several clinical areas of concern. Recent meta-analysis has revealed good pooled sensitivity of 86% and specificity of 81% in use of brush biopsy, however sensitivity of 71% and specificity of 32% was reported in one trial.(144,145)

2.3.2.1.2 Advantages and disadvantages of oral brush cytology

Recent American Dental Association guidelines advocate brush cytology “may be used to initiate the diagnostic process until a biopsy can be performed (conditional recommendation, low-quality evidence)”.(146) This organization promotes its use particularly in cases suspect of malignancy where biopsy facilities are inaccessible or biopsy is declined by the patient. Its reported good sensitivity offers the clinician a chance to rule out a suspect lesion being malignant, however it may be difficult to

sample from deeper layers or in thickened hyperkeratotic lesions, making a severe dysplasia less likely to be detected. Indeed, it may be argued that the use of such a tool can be harmful to the patient as it may delay biopsy and eventual diagnosis.

2.3.2.1.3 DNA image cytometry

DNA-image cytometry was introduced for detection of malignant change. It detects DNA aneuploidy by microscopic analysis of saliva samples. DNA ploidy has been well-studied as a marker of malignant change. Ye's meta-analysis found sensitivity of 89% and specificity of 99% in detecting dysplastic and neoplastic mucosa from normal oral mucosa.(144)

2.3.2.2 Liquid-based cytology

Identification of tumour markers such as DNA hypermethylation and loss of heterozygosity in saliva samples has been made by spectroscopic techniques This has applications in recurrence monitoring and also in the detection of distant neoplasms.(147) The literature contains many systematic reviews with uneven standard. Many have small sample size and heterogeneity of the study groups is a recurring theme. (148, 150, 153) Salivary metabolomes that are identified to be upregulated in OPMDs and OSCC include lactic acid, amino acids (i.e., alanine, valine, and proline), and glucose (i.e., N-acetylglucosamine), while metabolomes that are downregulated are from the fatty acid family, such as propanoic acid and ethanedioic acid.(149) IL-6 and -8 show promise for detection of OSCC arising from OPMD.(150) Collections of salivary immunoglobulins are consistently lower in smokers and cohorts of PVL and OLL patients, however this in itself is not helpful. Matrix metalloproteinases (MMPs), in particular MMP-1 have shown risk-related

increases in salivary concentration with OPMD and again with SCC development, however are not accurate enough for use.(151)

One Japanese study found poor results for the identification of SCC, dysplasia and normal tissue. It found saliva gives fewer inadequate results compared with brush biopsy and the researchers supported the inclusion of LBC with oral cytology, presumably to increase the number of cells harvested and thus the overall predictive value.(158) Lack of understanding of how and when biomolecules are secreted into saliva as well as heterogeneity of sampling and analytic techniques hampers comparison.(148, 149) In the literature, heterogeneity of groups (primary site, parameter investigated etc), lack of consensus on what levels and what biomarker to investigate was noted in 2016 and still seems relevant.(148,159) It may be used as an adjunct, however lack of understanding of these factors hinders it as a standalone tool. Raman spectroscopy currently is favoured in the analysis of salivary metabolites, as it can detect low levels of target compounds and the weak confounding Raman activity of water.

2.3.2.3 Lab-on-a-chip

This is the adaptation, miniaturization, integration, and automation of analytical laboratory procedures into a single device or "chip." It uses liquid cytology and machine learning with information including age, gender, smoking history to predict likelihood of malignant change in an OPMD. It, however, needs more research before it can be routinely recommended.(161)

2.3.2.4 Contrast agents/vital dyes

2.3.2.4.1 Tolonium chloride/toluidine blue

Tolonium chloride / toluidine blue (TB) has been used for more than 40 years to aid in visual diagnosis of mucosal abnormalities of the mouth and of the cervix. It preferentially binds to rapidly dividing cells such as healing tissue or neoplastic tissue, to sites associated with DNA change such as OPML or both. Rosenberg's meta-analysis found sensitivity of 93.5% and specificity of 73.3%.(162) It has been advocated as a way of marking the outlines of a cancerous lesion prior to excision or as a way of identifying areas of dysplasia in otherwise normal areas of mucosa. It seems most sensible to use as an adjunct to screening in high-risk populations and has shown high sensitivity but specificity remains an issue, with almost 60% false positives identified in one study.(21,163,164) It is sensitive to carcinomas but cannot identify dysplasia reliably.(21) A systematic review of this product from 1989, supported by current literature noted that it had not reached acceptance as a screening tool in the United States as of yet because it tended to adhere to areas of inflammation and trauma as well as areas of suspected SCC.(132,162)

2.3.2.5 Artificial intelligence

Interesting studies from India have demonstrated a smartphone-based image and autofluorescence system where images and AF are sent to a specialist or analysed using convolutional neural networks (CNN) deep learning. Sensitivity and specificity of the algorithm and specialist are similar.(165,166) New techniques using deep neural networks to identify oral dysplastic lesions and SCC from photographs have had good results, with sensitivity and specificity exceeding that of general

practitioners in identifying dysplastic and cancerous oral mucosa.(167) This interesting application of AI seems suited to the task, with a wide variety of unpredictable factors coming into play.

2.3.3 Adjuncts not suitable for screening

2.3.3.1 Immunohistochemistry

Systemic circulating antibodies have been investigated as potential tumour biomarkers. Serum p53 antibodies and p53 immunoreactivity have also been examined as potential biomarkers.(169)

2.3.3.2 Bioimpedance

This novel technique aims to combine machine learning with readings of bioimpedance obtained from passing a weak electric current through bodily tissue. It is synonymous with electrical impedance, however the prefix bio- signifies its use in biological tissues. It has the advantage of immediate results, being cost-effective, and involving little guidance.(170) It has shown promise in differentiating low- and high-risk oral cavity lesions.(171) There is significant disparity in the method of use. Multiple variables in the cell volume make finding a predictable algorithm difficult. There are few studies published in this area.(170) Zedscan (Zilico, Manchester, UK) is a handheld electrical impedance spectroscopy device used in colposcopy for lesions of the cervix.

2.3.3.3 Nanotechnology

Use of nanoparticles that preferentially bind to cancer markers such as TNF-alpha and that can be detected by small sensors placed intraorally are an avenue of research. No such detectors are currently on the market, but may become available

in future. These offer rapid detection, simplicity of action and cost-effectiveness but need more research. Nanoparticles are currently used to enhance detection of molecules with low concentrations – for example to enhance Raman signal in surface-enhanced Raman spectroscopy.

2.3.3.4 Fine needle aspiration (FNA)

This technique is used in the evaluation of masses – for example solid tumours. A fine-bore needle is inserted into the lesion of interest and some cells are aspirated into a syringe. Cytological analysis can then identify malignant cells. However, it has the risk of false-negatives and spread of malignant cells.

2.3.4 Optical techniques

These may be broadly divided into techniques that produce a visible change and spectral techniques. A first category includes techniques that measure the light absorbed or scattered, detected by a user. Examples include vital staining, tissue autofluorescence and chemiluminescence. A common feature is that the tissue needs either dye or incident light to produce visible results. Imaging techniques produce a visible result – either on a screen or in the operator's field of view. These typically use a high-powered light to illuminate the tissue. Examples include narrow band imaging, optical coherence tomography, diffuse reflectance imaging or laser endomicroscopy. Spectral spectroscopic techniques include diffuse reflectance spectroscopy (DRS); autofluorescence spectroscopy (FS); elastic scattering spectroscopy (ESS); and Raman Spectroscopy (RS). The principle is the same: tissue characterization is performed by measuring the spectrum produced after the tissue

is illuminated with a selected spectral band of light. Any of the above may be combined to produce a result with features common to one or more systems.

2.3.4.1 Tissue autofluorescence tools

2.3.4.1.1 Overview

Tissue autofluorescence diagnosis is based on the differing composition of tissues undergoing carcinogenesis. Biological compounds present in all tissues, e.g. collagen, elastin absorb light and can emit it again at a different wavelength (fluorescence). The levels of these compounds (known as fluorophores) vary in normal and dysplastic tissue, typically resulting in loss of fluorescence through absorption and scattering of light. The main fluorophores in the mouth are nicotinamide adenine dinucleotide, flavin adenine dinucleotide and collagen crosslinks in the stroma of oral tissues.(172)

Changes in fluorescence reflect changed levels of fluorophores in tissue and structural changes in tissue morphology. The endogenous fluorophores that are most relevant for optical screening and the diagnosis of precancer and cancer are those that excite in the visible spectrum from visible violet/blue (400-450nm) to UV-A (315-400nm) and which have properties that have been spectroscopically correlated with oral cancer. Healthy tissues autofluoresce at 515 nm and produce green light.(173) Gillenwater, in a keystone paper in the 90s, produced sensitivity of 88% and specificity of 100% for differentiation of normal and dysplastic tissue.(174) Autofluorescence studies demonstrated sensitivities and specificities in the 90s for differentiating normal from malignant tissue in the mouth, however more recent research has contradicted this.(175) Moffa's recent systematic review found a

pooled sensitivity of 81.3% and specificity of 52.1%.(176) Mendonca's 2022 systematic review found pooled sensitivity of 0.75 (CI 0.72–0.78) and specificity 0.50(0.47 to 0.53). Many of these studies had drawbacks which preclude widespread long-term clinical use. These include use of ultraviolet light intra-orally, causing harm, or time-consuming protocol disqualifying full-mouth scanning.(177) It has consistently demonstrated poor specificity in modern data.

2.3.4.1.2 Velscope

A direct tissue fluorescence probe (Velscope, LED Dental, Vancouver, BC, Canada) has been used in detection of oral premalignant lesions in combination with visual examination. This device is convenient, non-invasive and effective at identifying areas of abnormal tissue, however there was poor data on its ability to distinguish pre-cancerous and non-cancerous areas.(178) Benign inflammatory conditions can cause increased blood flow to a lesion.The increased haemoglobin can cause light absorbance and mimic neoplasia. Reported sensitivity of VELscope in showing fluorescence visualization loss in OPMD and OSCC ranges from 30-100%, and specificity from 15.3-100%. The literature reports ability of this system to identify occult lesions.(179) The most likely utility of this system lies as an adjunct for diagnosis, in combination with another possibly spectrum-based modality due to its low specificity.(180) Disadvantages include high set up costs and necessity for a dark environment.(4) Mendonca found pooled sensitivity and specificity of 0.72(CI) (0.67–0.76), 0.38 (0.34–0.42).(181) There is much data published relating to Velscope.



Figure 5. An example of autofluorescence of submucous fibrosis of buccal mucosa. Characteristic loss of fluorescence is seen for the high-risk part(186).

Neither toluidine blue, brush biopsy nor fluorescence imaging was recommended by the authors of a recent Cochrane review.(9)

2.3.4.1.3 Advantages and disadvantages of autofluorescence

It offers realtime analysis with operator friendly simple controls. However, it cannot be recommended as an alternative to biopsy. One study found it can see areas that are invisible to the naked eye. This seems to be its main advantage, with the Chinese Association of Stomatology issuing a weak recommendation for its use in the identification of leukoplakia along with DNA ploidy and toluidine blue.(182) However, areas of loss of autofluorescence may have many causes, and thus has low specificity.

2.3.4.1.4 GOCCLES (Glasses for Oral Cancer – Curing Light Exposed – Screening)

This Italian - developed tool uses autofluorescence reflection from oral tissue illuminated by dental curing light to identify areas of dysplasia and OSCC. Moro found similar results to COE, however it has a high rate of false positives.(183)



Figure 6. GOCCLES used for visualizing intraoral OPMD(188)

2.3.4.2 Chemiluminescent tools

2.3.4.3 Overview

Chemiluminescence describes the emission of light from a chemical reaction. It has a history of use in the cervix, due to a preferential light emitting reaction with dysplastic or cancerous mucosa in this area. A 2021 systematic review found pooled sensitivity of 84.9% but low specificity of 51.8% for all forms of chemiluminescence in detecting OSCC and OPMD.(176) Another recent meta-analysis and systematic review found no benefit for chemiluminescence over clinical oral examination.(184) Mendonca's systematic review found sensitivity of 0.74(CI: 0.68–0.79) and specificity of 0.47 (0.40–0.53).

2.3.4.3.1 Vizilite and VizilitePlus

The Vizilite system was designed to detect cervical cancer. An acetic acid rinse removes surface contaminants and may increase the visibility of epithelial cell nuclei. Normal mucosal cells absorb a low-energy blue-white light and appear blue while abnormal cells with abnormal nuclei and high nuclear/cytoplasmic ratios

reflect light and display an ‘acetowhite’ change. Again, it has good sensitivity but low specificity, identifying many areas of benign oral change as areas of concern.(177) Scepticism about the actual utility of this tool is related to dysplastic progression occurring from deeper mucosal levels, reducing the diagnostic utility of reflected light from superficial levels. Hyperkeratotic leukoplakic areas will, however, preferentially reflect light.(179) This was borne out by the results of a systematic review by Rashid and Warnakulasuriya (2015) which found sensitivity of 77.3-100% for ViziLite and consistently low specificity of 0-27.8%.(179) Vizilite frequently identified conditions with a benign or inflammatory aetiology such as frictional keratosis as malignant or potentially malignant in this literature review. Additive use of Toluidine blue (TB) also known as Vizilite Plus system reduced sensitivity of this tool to a range of 0-59%, while specificity was improved to 75.5 – 78% in two studies. Severe dysplasia was more likely to be identified, however a considerable amount (1/3) of benign lesions were positive to ViziLite.(179) This chemiluminescence system has the advantage of positively identifying occult lesions undetectable to the naked eye, and is non-invasive and non-toxic. Epstein et al reported that ViziLite identified two lesions not identifiable on visual examination, with one of these lesions being an occult SCC(185). However, it must be noted that it also identified several lesions incorrectly as SCC.

High false-negatives make it hard to recommend ViziLite as a screening tool as it may lead to worry for patients, unnecessary biopsies with accompanying morbidity, or lead-time bias, where unnecessary biopsies of benign lesions may necessitate monitoring of low-risk lesions. It also suffers from high running costs.(4)

2.3.4.3.2 MicroLux DL

The MicroLux DL system is a multiuse system developed from a blue-white light emitting diode and a diffused fibre-optic light guide that generates a low-energy blue light.(142) Rashid and Warnakulasuriya reported a sensitivity of 77.8% and specificity of 70.7% in detecting oral cancer and OPMDs from one study.(179)

2.3.4.3.3 Orascoptic DK

The Orascoptic DK system is a LED light which is used with an acetic acid rinse to improve visualization of oral lesions.(142)

2.3.4.3.4 Advantages and disadvantages of chemiluminescence

Similar to autofluorescence, quick results and ease of use. Limited by poor specificity and sensitivity.

2.3.4.4 Optical Coherence Tomography (OCT)

OCT uses subepithelial reflection to build a cross-sectional image of tissue structure. It is analogous to ultrasound. It can detect architectural changes but not cellular atypia, limiting its use.(186) It is used in surveying the oropharyngeal region in cases of unknown primary tumours. Distortions were noted in tissue samples obtained for histopathological purposes versus the in vivo images taken by OCT, suggesting that in vivo OCT imaging of living tissues may represent a truer biological picture than conventional histopathological analysis. Systematic review has given sensitivity (93–96%) and specificity (74–49%) in groups of OSCC, leukoplakia with hyperplasia and healthy simple tissue(187).] No study has yet demonstrated an ability to assess grades of dysplasia, while it may be used to identify cancers breaching the basement membrane, it is not accurate at diagnosing OPMD.(188)

There is currently no classification to guide results.(189) Interpretation of scans is complicated by volume of data and heterogeneity of oral mucosa by anatomic subsite. AI has potential to help.

2.3.4.5 Narrow band imaging

NBI (Olympus Medical System Corporation, Tokyo, Japan) involves acquisition of images and analysis of intraepithelial capillary loops. It can be used to detect unknown primary tumours and to determine the severity of leukoplakia.(186) The pattern and behaviour of the capillary loops gives the diagnosis. It is possible to detect abnormally large vessels with destruction of surrounding tissue and angiogenesis. Types II/III-IV are generally accepted to be high-risk. It is necessary to be skilled in analysis of the bands. It has been shown to be superior to white light endoscopy. Systematic reviews have found good results in diagnosing high grade dysplasia and SCC(190). A high-quality review found high specificity with low pooled sensitivity (0.9 and 0.31)(181), while Saraniti found high sensitivity and specificity in diagnosing cancer.(191) It has reported potential in detecting early-stage malignancy in oral lichen planus.(192) Zhang et al. in a meta-analysis reported it to have relatively high overall diagnostic accuracy for the malignant transformation of OPMD.(195)

This technique requires a high setup cost, restricting its use. It also has a shallow depth of penetration. This can limit its ability to penetrate for example thick leukoplakia. Furthermore, the vascular pattern in ulcers and the vascular proliferation in inflammation of severe erosive lichen planus can confuse diagnosis.(190,194,195) The literature recommends the introduction of clinical

guidelines for use to encourage use of this modality.(196) A 2024 systematic review of systematic reviews concluded that NBI findings could be used to decide whether to biopsy.(197)

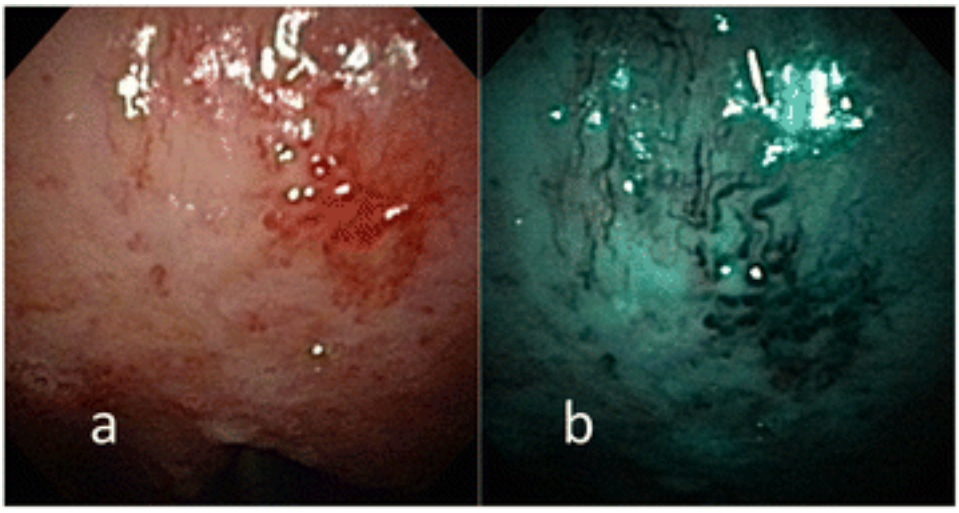


Figure 7. Endoscopy of hard palate erythroplakia on left with NBI on right. (195)

2.3.4.6 Fluorescence microscopy

Laser confocal microscopy relies on the infusion of fluorescein dye to obtain a magnified cross-sectional image. it is not applicable to keratinised mucosa, limiting its use on the mouth.(198) SCC was detected in the floor of mouth using this technique, however it is difficult to get images without motion artefact and also the bulk of the microscope is an issue.(199)

Reflectance confocal microscopy uses focused laser illumination and detects the back-scattered light. It does not use fluorescent mediums. It is thus possible to visualise nuclear density and morphology of the tissue. The Vivascope microscopes are used to perform RCM and can visualize 3- to 5-µm thin optical sections at a 0.5 to 1 µm resolution and up to depths of 200 to 300 µm (Caliber I.D., Rochester, New York). It has been in use in dermatology, particularly in identification of basal cell

carcinoma without a biopsy. It can be used in conjunction with 5-ALA to improve contrast or delineate surgical margins.(200) A systematic review from 2016 found that it offers promise, however more research is needed as to its accuracy and best way to use.(201) The large size of the microscope makes oral examination difficult. Motion artefacts is also an issue. Maitland et al. produced a miniaturised microscope capable of looking at posterior parts of the mouth.(202)

2.3.4.6.1 Multi-modality imaging using autofluorescence and high-resolution microendoscopy

A combined approach with autofluorescence and microscopy gives higher accuracy than either modality alone, with 91% sensitivity in diagnosing OPMD and SCC. (203) Autofluorescence is used to scan the mucosa for areas with loss of fluorescence, and the topical agent proflavine is used to stain the mucosa, followed by laser microscopic examination. It has shown ability to identify areas of moderate and higher dysplasia not obvious to examiners, however the converse is also true.(203) It warrants more research, in view of its high reported sensitivity.



Figure 8. Using laser confocal microscopy on the lower anterior gingiva

2.3.5 The use of spectroscopic techniques in the detection of OPMD and OSCC

Table 9. Advantages of optical spectroscopy

Real time
Enables optimal biopsy examination without removal of excess tissue
Guide to effective diagnosis
Guide to consent
Guide to effective sampling
Guide to treatment during and after operation
Potential to guide other methods of treatment such as photodynamic therapy

Spectroscopy relies on evaluating the physical properties of a given molecule based on the scattering of incident light. Fluorescence (FS), infrared (IS), and Raman (RS) spectroscopy evaluate the fluorescent, vibrational, and rotational properties of molecules, respectively. Optical spectroscopy offers potential to distinguish normal tissue, OPMD and malignancy in a non-invasive manner free from artefacts that may arise during excision, cutting, freezing and staining tissue.

2.3.5.1 Behaviour of light in living tissue

The optical properties of the medium are referred to as the refractive index, the absorption coefficient, the scattering coefficient and scattering anisotropy, each of which are given a mathematical value. For a given molecule, several possibilities can occur in an interaction with a photon.(204)

Absorption and attenuation, or scattering, are the relevant interactions here. The other interactions are not relevant as they involve high energy transfers. The

absorption of a photon can elevate an electron or electrons and it can radiate the energy of the photon as heat or light. If another photon is emitted, this event is known as fluorescence or phosphorescence, depending on the lifespan of the emitted electron. Reflection should be distinguished from scattering, which occurs in diffuse reflectance spectroscopy. In reflection, the light travels in straight lines, while in scattering, the light travels in different directions.(205)
Raman scattering can give information on the chemical composition and molecular structure of the molecule, while elastic scattering can reveal information on the size distribution of the scatterers.(205)

Predicting the behaviour of light in biological tissue is complex. In the physics literature it is generally referred to as 'the behaviour of light in a turbid medium'. This refers to the high number of scattering events that a photon of light is expected to undergo in a tissue. Optical absorption is weak in the 400-1350 nm spectral region. This is the spectrum in which most optical techniques, including DRS operate. The absorption coefficient μ_a is the probability of a photon being absorbed in a medium per unit path length. In biological tissue, it has a value of $0.1cm^{-1}$.(205,206)

In biological tissue, the main absorbers are haemoglobin, melanin, water and deoxyhaemoglobin. Fig. 9 shows the molar extinction coefficient of oxy- and deoxyhaemoglobin as a function of wavelength, where the extinction coefficient is described as the chance of photon interaction with matter per unit path length.

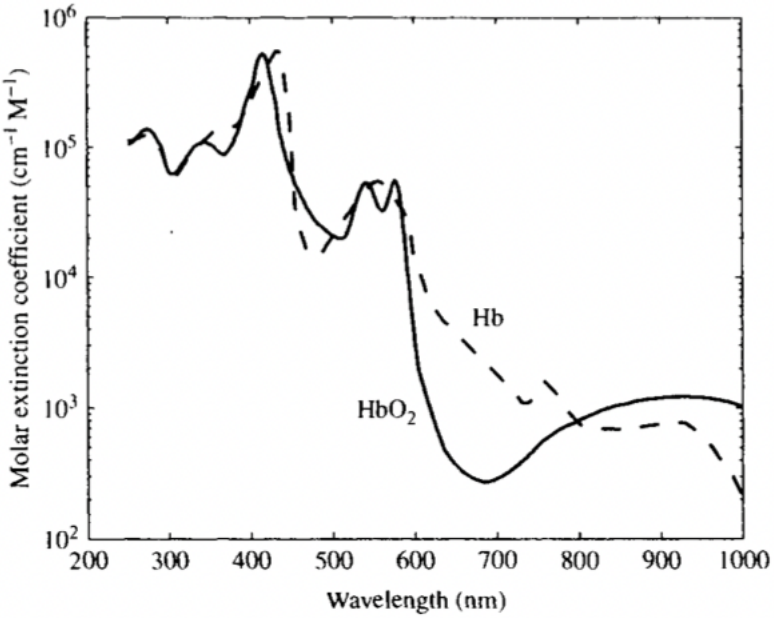


Figure 9. The chance of photon interaction with deoxy and oxyhaemoglobin as against its wavelength.(208)

As wavelength increases, the chance of a photon interaction with the compound in solution changes. This shows that, for a given wavelength and with prior knowledge of the optical properties of the molecule, the concentration can be estimated. However, in a biological tissue, other absorbers complicate the picture and create their own peaks.(207)

Knowledge of the extinction coefficients of oxy and deoxyhaemoglobin means that the concentration of oxyhaemoglobin and oxygen saturation in a tissue can be estimated by using light of two wavelengths and a formula. This is the principle on which pulse oximetry is based.

Traditional spectroscopic techniques measure the intensity of the light emitted (photons emitted on the y axis) with incident light of a fixed wavelength (x axis), and plot the received light, forming a curve where as wavelength increases, fewer photons are received.(208)

2.3.5.2 Monte Carlo simulation of light propagation in tissue

The Monte Carlo simulation can be used to estimate the concentration of absorbers in a tissue. The path of photons through a tissue can be described as a persistent random walk. The Monte Carlo method resists easy definition, however it may be defined as:

> *"In all applications of the Monte Carlo method, a stochastic model is constructed in which the expected value of a certain random variable (or of a combination of several variables) is equivalent to the value of a physical quantity to be determined. This expected value is then estimated by averaging multiple independent samples representing the random variable introduced above. For the construction of the series of independent samples, random numbers following the distribution of the variable to be estimated are used."(*205)

The Monte Carlo method involves, for a given set of photons, estimating the microscopic composition of sample that may be macroscopically homogenous by the angles of scattering and step sizes. The sheer amount of computations involved has been simplified by the advent of modern computing. The Monte Carlo method relies on the sampling of random variables from their probability distributions.

Table 10. Advantages and disadvantages of various optical systems

Optical diagnostic technique	Modus operandi	Advantages	Disadvantages
Diffuse reflectance spectroscopy	Capture of reflected light from tissue is analysed	In situ, almost real time, can be used endoscopically	Needs specialist equipment, technique sensitive, sampling strategy is subjective
Raman spectroscopy	Reflection of light from molecular bonds	Real time, in situ, can guide resection	Sampling strategy is subjective, high capital outlay, may not detect smaller changes
Chemiluminescence	Tissues fluoresce under green/purple light	Real time, in situ	Low specificity, needs specialist equipment, traditional techniques may cause harm
Microendoscopy		Cheap, in situ, real time	Depends on use, can only be used in specialist centres
Narrow band imaging	Inspection of capillary loops	Real time and in situ	Expensive
Optical coherence tomography	Infrared light reflected from subepithelial layers shows structure	Real time, in situ	Requires pathologist, images can be difficult to interpret, requires special probe for oral examination, images are expensive to process

2.3.6 Raman Spectroscopy

Raman spectroscopy provides vibrational molecular information based on light scattering from molecular species, including amino acids, lipids, proteins and nucleic acid.(211) Since each Raman-active functional group of nucleic acids, proteins, lipids and carbohydrates has a different emission wavelength, this allows for identification and characterization of tissue type.(208)

An increase in proteins and nucleic acids and a decrease in lipids has been reported to be a Raman indicator of carcinogenesis.(212) It has been utilized in detection of molecular differences in diseases such as breast, bladder, cervical, brain and gastrointestinal cancer during endoscopy.(211)

Recent systematic review has found accuracy of 98% in cancer detection, with accuracy of 97% in grading of differentiation of tumour and sensitivity of 95% and specificity of 88% in identifying involved margins on resected specimens. In vivo tumour detection reached 94%.(212) This is the highest figure of any technique studied in this review.

Although these results seem promising, analysing an entire specimen may take up to several hours, in vivo accessibility is difficult as only rigid scopes can be used, and interpretation of results is more mathematical than visual. This technique has the highest accuracy, however the need for rigorous protocols to obtain an accurate Raman signal makes it hard to recommend for clinical practice. The limitation of RS has been its intrinsically weak signals. Only 1 in approximately 10^7 photons is Raman shifted. This necessitates a larger setup that may be heavier and more sensitive to external contaminants.

Another issue includes the need to exclude all other sources of electromagnetic energy. However, if these problems can be attenuated, it has great potential.

2.3.7 Infrared Spectroscopy

IR spectroscopy combined with Fourier transform and machine learning (FT-IR) has also been studied for its ability to diagnose OSCC and OPMD.(213) Recent studies into FT-IR have demonstrated an ability to predict transformation of dysplastic lesions with a sensitivity of 79% and specificity of 76%.(126) Near-infrared spectroscopy has the purported advantage of deeper penetration into tissue. However, it is hampered by low specificity and is difficult to use in the mouth due to high water content.(214)

2.3.8 Fluorescence spectroscopy

Laser-induced fluorescence and xenon arc lamp are used as an excitation source and spectra were recorded with varying excitation wavelengths and spectroscopic devices. Pooled sensitivity and specificity of 0.96(CI) (0.91–0.98), 0.74(0.60–0.85) were reported by Mendonca.(181) It is hampered by the fact that compounds in the mouth have strong and overlapping fluorescent signals, and fluorescence from porphyrin-producing bacteria needs to be excluded. Furthermore, it is not applicable on keratinized surfaces, implicitly excluding the dorsal surface of the tongue.

2.3.8.1 5-amino levunic acid induced protoporphyrin IX fluorescence based imaging (5-ALA induced PPIX)

Application of a solution of 5-ALA to the mucosa via oral rinse and fluorescence imaging after a period of 1 to 2.5 hours gave a pooled sensitivity of 0.91 and

specificity of 0.78.(181) 5-ALA, when induced with protoporphyrin IX (PPIX) fluoresces in tissue. Topical administration of 5-ALA results in selective accumulation of PPIX in neoplastic tissue, differentiating it from surrounding tissue by high contrast.

2.3.8.2 Fluorescence and microendoscopy

This may be used with 5-ALA or fluorescein dye. It has been described as a way to optically section tissue, showing cellular atypia and dysplasia. A new application combining autofluorescence to identify suspect areas, and laser microscopy to visualize, has produced good results for sensitivity and specificity.(203)

2.3.8.3 Nanoparticles and spectroscopic techniques

Gold and silver colloid have been used in Surface Enhanced Raman Spectroscopy (SERS) to enhance the Raman signal. These nanoparticles create large electrical fields when they interact with photons undergoing Raman scattering, amplifying Raman signal. They have also been used to enhance DR signal, in pinpointing tumours by accumulating at the most acidic spot, and to guide therapy.(215)

2.3.9 Diffuse Reflectance Spectroscopy

DRS is based on light reflectance from light absorbers, including deoxygenated and oxygenated haemoglobin, lipids, water, cytochromes and melanin.(204,211,216–219) This technology is based on delivering white light in the frequency range of 400-720nm using a conglomerate source-detector pair. The reflected light can be collected as a single wavelength, a group of wavelengths, or in a spectrally resolved manner. The shape (in the case of spectrally resolved acquisition) and the

magnitude of the reflected light is indicative of light scattering (related to the tissue microstructure) and absorption (associated with its biomolecular content).

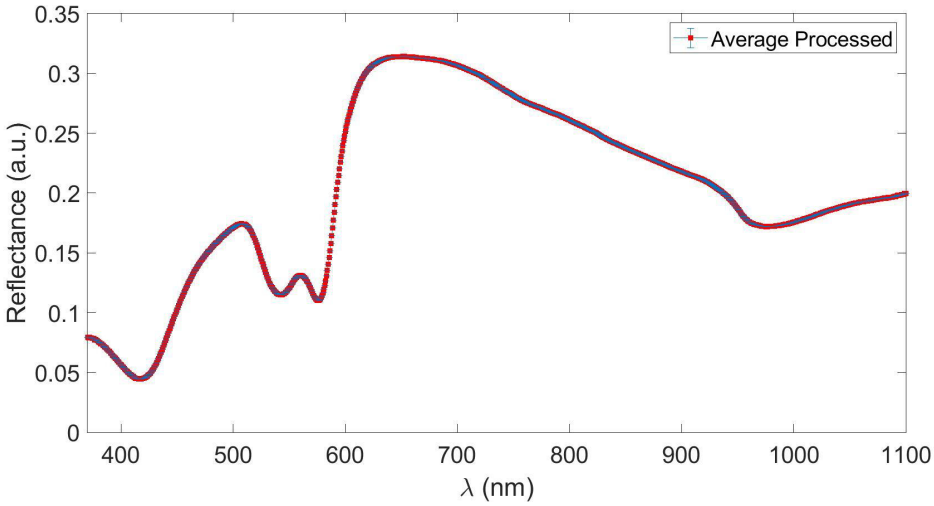


Figure 10. Example of a spectrum produced from oral tissue. Characteristic dips are noted in the oxy and deoxyhaemoglobin region as light is absorbed preferentially by the higher concentrations of this molecule in human tissue

2.3.9.1 Theory of DRS

The Beer-Lambert law states that there is a linear relationship between the concentration and the absorbance of the solution. It is commonly applied to chemical analysis measurements to determine the concentration of chemical species that absorb light. This law is widely applied in biomedical optics, for example, to calculate oxygen saturation in human tissues to determine the molar absorbance of bilirubin in blood plasma sample, and to determine the concentration of haemoglobin components or optical pathlength through a tissue.(205)

The reflectance spectrum can be analysed using a model of photon propagation in tissue to extract the absorption and reduced scattering coefficients. Analysis of the

combination of scattering and absorption (optical properties), as well as the orientation of the light source and detector can then be used for tissue identification based on the biochemical composition, oxygenation and microstructure of the tissue. (216,220,221) However, this can be time-consuming to run these models, therefore most studies have used the variation in the spectrum produced by varying concentrations of different biomarkers such as deoxy- and oxyhaemoglobin to characterise tissues, with discriminant analysis or other forms of statistical analysis to obtain results.(175) This is a cruder form of analysis, with patterns in the data associated with particular diagnoses identified with discriminant analysis or other forms of statistical analysis.(175) This may produce inaccuracies due to the inherent limitations of the statistical technique used. Nevertheless, a systematic review has given a figure of 98.6% sensitivity and 83% specificity in detecting OPMD and oral SCC, and recommended this as the most accurate biopsy adjunct.(223)

It is quick, with tissue interrogations taking less than a minute, and can accurately localize OPMD or SCC to one small area for biopsy or resection.

However, drawbacks include small area of examination, shallow depth of penetration, and tissue autofluorescence, raising the possibility of false negatives.(114)

Because optical techniques identify molecular pathologies that may be common to broader array of cancers, it is possible to identify cancers no matter their histological diagnosis, and potentially many other disorders based on their molecular signatures.(211)

2.3.10 Multimodal spectroscopy

Multimodality of an optical system implies the use of one or more optical techniques to improve the system's overall performance and maximum utility. An example is the approach taken by Greening et al. in a proof-of-concept study. He used fluorescence imaging to delineate areas of interest and then used sub-DRS to quantify the tissue optical properties.(224) Good results were obtained by Muller et al. and Mallia. Muller, in 2003, used trimodal spectroscopy (DRS, intrinsic fluorescence and light scattering spectroscopy) to distinguish dysplasia and cancer with sensitivity of 64% and specificity 90%.(225) Mallia used autofluorescence corrected by DRS to obtain good results in 2010.(226) Complementary techniques help to negate drawbacks.

2.3.11 Development of DRS

Keystone laboratory work underpinning the concept of diffuse reflectance includes research demonstrating mathematically the path of a detected photon from its last incident surface and important work by Sokolov identifying the role of diffuse reflectance in investigating changes in cell nucleoli due to dysplastic processes.(209,227) The lack of vascularity and necrosis at the centre of larger tumours makes identification of an advanced cancer easier using a DRS probe, which is especially good at distinguishing low oxygen and haemoglobin content tissues.(228,229) On the other hand, the identification of smaller tumours at an earlier stage is more difficult for a DRS probe.(220)

Early researchers scored success with the use of DRS probes in distinguishing SCC and normal tissue in the bowel.(230). Initial reports on DRS in the oral cavity found

poor differentiation between cancerous and benign tissue.(217) However, further research in the area has given much more promising results. The ultimate goal of research is to identify oral squamous cell carcinoma at a stage when it is very early and not when it is obvious.

2.3.11.1 Technological advances in the lab and clinically – finding the right configuration

Much research on the field of biophotonics pre-2015 focused on the technique of autofluorescence, however this technology was and is hampered by persistent poor specificity. The eventual decline in interest in autofluorescence affected the entire optical spectroscopy industry. While poor specificity was initially an issue with DRS, various methods have been used to increase efficiency and accuracy. Mallia's 2008 paper demonstrated excellent sensitivities (between 96-100%) in distinguishing neoplasia from dysplasia, dysplasia from hyperplastic tissue and hyperplastic tissue from normal, however specificity did not exceed 86%.(175) Resolution of the depth of investigation is a theme throughout the literature from 2007 onwards. In DR spectroscopy, incident light is directed perpendicular to the tissue surface and thus is aimed directly through the superficial epithelium, where carcinoma arises. Poor results were as a result of high scattering from the stroma lying deep to the epithelium, which is not of interest to researchers. Approaches to solving this problem included angulation of probe and source to allow an oblique beam of light to pass through the epithelium or in a similar fashion including a lens in the source array in order to change the angulation of the incident photon beam.(231–233)

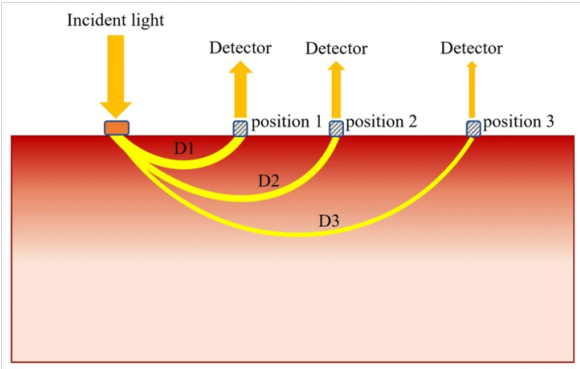


Figure 11. Varying source-detector distance to preferentially get data from superficial layers

Schwarz built on the work of Mallia and produced sensitivity of 82% and specificity of 87% in an in vivo study using a similar protocol while differentiating between keratinized and non-keratinised sites.(234) While an angled approach either with or without a lens is in use today and can produce excellent results (235–237), Amelink's work showed that it is unnecessary and adds to the size and complexity of the detector(238–240). Meglinski proposed varying the source-detector distance to interrogate superficial layers in an elegant 1999 paper, and this was supported by the work of Pfefer in 2003.(241,242)

Changing the probe-detector geometry to allow the incident light to penetrate deeper layers, a process known as multi-channel or depth-sensitive DRS, has also been used to provide good results, most notably by Schwarz and Amelink.(231,234,239) Single fibres can be used to propagate a beam of light and the same fibre can be used to collect the reflected signal, a process known as single-fibre DRS.(243) Multi-fibre systems typically have one, two, four or more collection fibres around a central incident fibre. See Figs. 11,12,13 for examples of geometry. Time-gated spectroscopy means allowing a set period of time to elapse when measuring DRS signal. This can be varied to allow only light from more

superficial layers time to return to the surface, preferentially excluding the deeper photons.

Use of anatomy- or tissue-specific algorithms to allow grouping of DRS readings taken from different body sites has also improved sensitivity and specificity. McGee found sensitivity and specificity of 85% and 97% in distinguishing healthy from dysplastic/malignant and 81% and 96% in differentiating healthy from benign in a cohort of 64 using autofluorescence and DRS in 2009.(244) This was achieved using a perpendicular source with six detector fibres. Further studies on the use of anatomy- or tissue based algorithms give good sensitivity and specificity. (228,239,245)

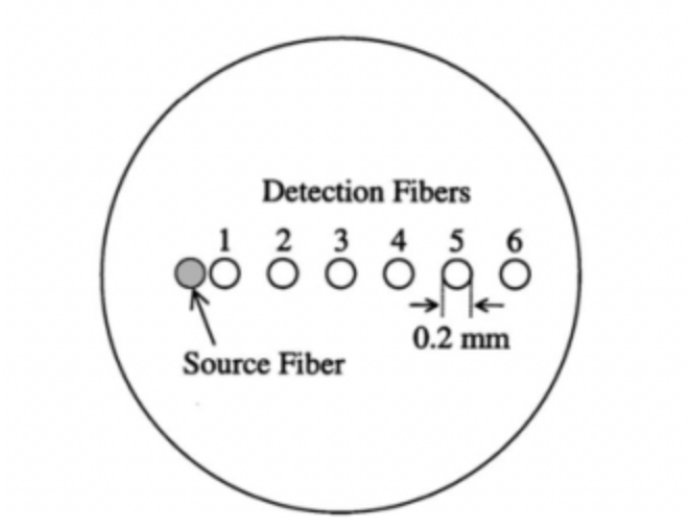
Altering the geometry of the source and detector has also been a controversy in the literature, with, as described previously, alterations in the source-detector distance allowing for changes in the depth of signal penetration. However, altering the spatial relationship of probe and detector or narrowing the radius of the collection fibre can change the depth of penetration(246). Using polarized light has been advocated to demarcate between detected light that has been reflected from deeper layers and light that has been reflected from more superficial layers(237,246). The theory is that the light which has undergone more scattering events (i.e. been reflected from deeper layers) will no longer be polarized and thus it can be excluded from detector array, while light that retains its polarization will have undergone fewer scattering events in the upper epithelium.

Experimental advances in computational algorithmic simulations (Monte Carlo methods) used to model phenomena with significant uncertainty and complicated

conditions allow for estimation of the path of photons on their path from light source to detector.(206) Hafez established wavelengths of visible light for which different types of mucosa – masticatory, lining and specialized mucosa can display measurable reflectance.(247)

2.3.11.2 Resolving issues around DR probe design

Farrell described in his 1992 paper the steady state diffusion theory, which provides one method of ascertaining the standard deviation of the molecules in a sample from the incident light.(248) Monte Carlo based theory of diffusion can extract the scattering coefficient of turbid media, including human epithelial tissue. Palmer et al. ingeniously demonstrated its applicability to human tissue in this experiment using haemoglobin dissolved in liquid.(249) Researchers have applied the use of Monte Carlo to produce look-up tables (LUT) which aim to reduce the complexity and time factor involved in the MC simulation.(250) Machine learning offers further developments in this area of resolving the DRS readings into useful data about the subsurface tissues.(250) Monte Carlo simulations have determined the optimal SDD for photon detection in a multilayered tissue.(242,251)



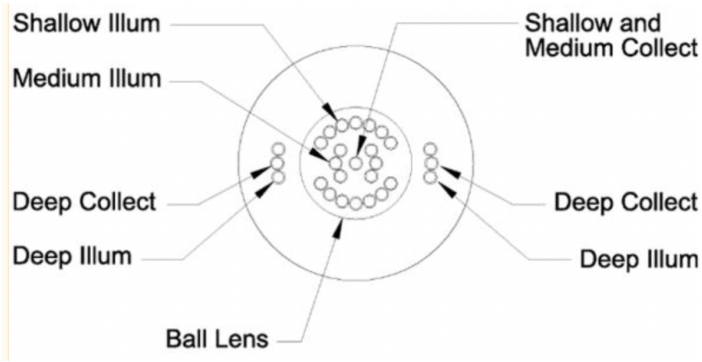


Figure 12. (top) An example of a multi-detector array with fixed fibre diameters with the possibility to vary the source-detector distance.(241)

Figure 13. (bottom) Another example of a multi-channel detector array with detector fibres labelled to allow collection from deeper and superficial layers.(234)

2.3.11.3 Confounding factors in oral DRS

Elevation of probe contact pressure produces major profile alterations in diffuse reflectance spectra between 400-650nm, making the standardization of operator pressure of high importance.(252–254) Local changes in pressure can alter blood volume, blood oxygenation and tissue morphology – all major constituents of a DRS probe's spectra. This can remain for some time after the pressure is removed, as the pressure causes the microvasculature to collapse, leading to a reduction in haemoglobin and a disappearance of small capillaries. Palmer incorporated a pressure sensor in his DRS probe and found an inverse relationship between

applied pressure and haemoglobin levels.(255) He advocated a gentle pressure less than 25mm Hg. Another study found significant differences in Hb readings when the pressure varies by 1psi. This is evidence of the significant role of pressure in using a DRS probe.(256) Further evidence of the long and short term effects of pressure on DRS readings is evident throughout the literature, with two of the most-cited papers coming in the early 2010s.(257,258) Conversely, stretching the mucosa alters its optical properties, with compression and stretching of blood vessels leading to an alteration in haemoglobin levels and a distortion of macro architecture.(205) The heterogeneity of oral mucosa also offers a challenge to DRS. It has been shown that tissue types can cause differences in DRS spectra. Most authors have used an anatomically based algorithm, and this is supported by Hafez, who found differences between keratinized and non-keratinised mucosa optical properties.(247) On the other hand, Hu et al organized the oral and upper airway mucosa by tissue type (muscle, lymphoid etc.) and found better accuracy than by using anatomical classifiers.(228)

Bugter et al demonstrated field cancerization in the buccal mucosa of patients who had been diagnosed with upper airway tract squamous cell carcinoma.(125)

2.3.11.4 Clinical application

Challenges to the implementation of this tool as a standard screening and diagnostic instrument include heterogeneity of biological specimens. Contaminants on mucosal surface can also make reading difficult. Water has high absorbance and thus can give a false signal. Blood, similarly, absorbs light preferentially along the 545nm/560nm part of the visible light spectrum and can influence results.

DRS technology has been used in *in vivo* in detection of oesophageal, liver, bowel, breast cancers and in resection of malignant metastases to the brain and has shown sensitivity and specificity in the 90th percentile in distinguishing normal tissue from cancerous structures.(207,220,259) In the Netherlands, a DRS probe demonstrated sensitivity of 95% and specificity of 92% in differentiating tumour and normal liver tissue. This was conducted in vivo in clinically neoplastic tissue and tissue at least 2cm distant from the nearest clinically obvious metastatic deposit.(221) Fluorescence spectroscopy provided no additional benefit over and above DRS in this study. The measurements in this case were complicated by the evident blood in the surgical field. It has also shown good results in breast cancer. Breast tissue is not homogenous, varying depending on age and hormonal status, while breast cancers display varied histology.(260) Ex vivo (lab) studies initially promised good results.(260,261) However, as early as 2008, investigators were able to reach sensitivity and specificity more than 95% in differentiating malignancy from other conditions.(261)

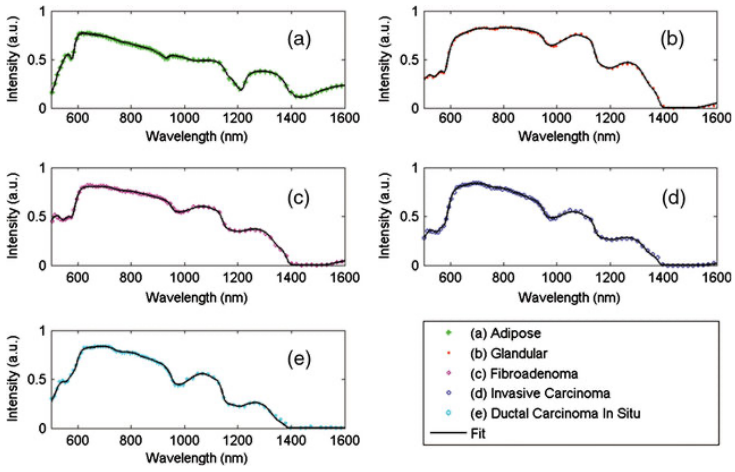


Figure 14. An example of DRS spectra obtained from different mammary tissue.(267)

However, it must be noted that the oral cavity itself and lesions presenting in the oral cavity are much more complex than those in other organs. Initial reports on DRS in the oral cavity found poor differentiation between cancerous and benign tissue.(195) Early investigations focused on lab studies of resected specimens.(199) Research into the technical aspects of DRS combined with theoretical advances led to improvements throughout the 2010s.(229)

2.3.11.5 Diffuse Optical Imaging

This is a whole field of view technique compared with the point view given by spectroscopy. The underlying basis is similar to diffuse reflectance spectroscopy. The interaction of light with haemoglobin is processed to produce spatially-resolved images. Narayanan's group in Trivandrum have produced several papers in this field.(245) Generally they have used light of the 545/575nm wavelength and measured the reflected and absorbed light to capture the haemoglobin content of tissue. In breast cancer, the standard of care is now x-ray imaging to detect microcalcifications suggestive of early cancer. Diffuse optical imaging is capable of capturing these subsurface areas without the use of radiation and with increased sensitivity and specificity.(263)

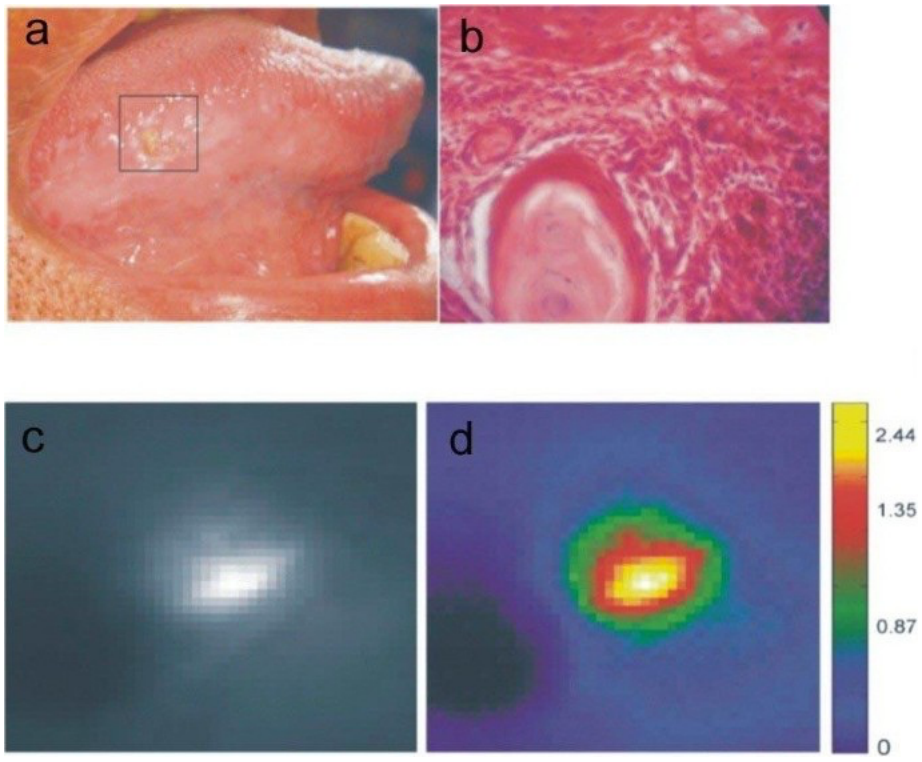


Figure 15. This shows diffuse reflectance imaging of an ulcer on the right lateral tongue. (a) clinical photograph, (b) histopathology, (c) monochrome 545/575nm reflectance image, (d) colourised reflectance at 545 and 575nm.(264)

2.3.11.5.1 IdentaFi

A tool combining DRS and fluorescence spectroscopy to screen for OPMD and SCC (Identafi, Dental EZ) is on the market. It emits light on a wavelength of 405 and 575nm with two wavelengths of light one green-amber and one white light for capturing tissue autofluorescence and diffuse reflectance. In one paper from Australia, the authors reported good ease of use and similar results in detection of OPMD and SCC to clinical oral examination.(172) Other authors have questioned the utility of Identafi and had good results from DRS alone.(245,265)

2.3.11.5.2 OralScan

Oral Scan is a multimodal device to detect oral cancer and loss of fluorescence from PpIX. The diffuse optical imaging system is set for DR intensity at the green (R545) and red (R610) wavelengths are used to observe the changes in concentration of

HbO_2 and deoxyhemoglobin and the extent of malignant transformation is assessed from the increase in the DR image ratio (R610/R545). A multicentric clinical study was conducted with OralScan and the findings were published.(245) In that study, a sensitivity of 82% and specificity of 96.6% was obtained for discrimination of oral premalignant and malignant tissues from healthy tissues.(245)

2.3.11.5.3 Intraoral DRS studies

Muller in 2003 advocated trimodal spectroscopy using reflectance, light scattering spectroscopy and diffuse spectroscopy to achieve sensitivity of 96% and specificity of 96% in distinguishing normal from cancerous tissue. However, low sensitivity of 64% in differentiating cancerous from dysplastic tissue was the major drawback of this study. (225) Multiple authors have combined different modalities of spectroscopic examination, usually giving improved results. (266)

Narayanan's Indian group produced several papers that set the direction for DR spectroscopy in oral cancer detection. The first paper in 2008 established the haemoglobin/deoxyhaemoglobin ratio as a surrogate marker for tumourigenesis.(229) This group produced sensitivity and specificity in 100% and 86% in distinguishing hyperplastic buccal tissue from dysplastic. This was in stark contrast to previous reports, such as De Veld's report in 2005 where sensitivity and specificity in distinguishing malignant from healthy tissue was only 80% and 89%. This led the author to conclude that DRS could not be recommended clinically.(217) This paper came from the Dutch group, which as detailed below, has carried out many important works in the field under the aegis of the Netherlands Cancer Institute. Another researcher from Narayanan's group, Mallia, produced a 2008

paper that demonstrated excellent sensitivities (between 96-100%) in distinguishing neoplasia from dysplasia, dysplasia from hyperplastic tissue and hyperplastic tissue from normal. However specificity did not exceed 86%.(175) Schwarz, in Texas, working under Dr. Ann Gillenwater, built on this work and with a rigorous protocol produced sensitivity of 82% and specificity of 87% in distinguishing normal, benign, mild, moderate or severe dysplasia and cancer in an in vivo study.(267) This study was characterized by the use of a validation set of measurements, where operator and patient were blinded to the histopathological diagnosis. It is well established that DRS is better at distinguishing malignant and normal tissue, however areas of benign and dysplastic tissue show more subtle changes that are more difficult to identify reliably. Contemporaneously, Nieman found sensitivity and specificity of 100% and 85% in differentiating normal and severely dysplastic tissue, however benign tissue and mild dysplasia were identified with only 92% sensitivity and 69% specificity.(235) Again, Amelink found in a 2008 paper statistically significant optical differences between normal tissue and SCC.(239)

Mallia in 2010 published a new study which found sensitivity and specificity of 100% each in differentiating severe dysplasia from SCC.(226) This study, however, used autofluorescence to guide the DRS probe. In 2011, the Dutch researcher Amelink published a major study attempting to solve what was becoming a difficult problem in oral cavity DRS research – distinguishing between dysplastic and non-dysplastic. Her DRS probe gave a sensitivity of 91% and specificity of 80% using a 2mm source-detector distance.(240) The next major report on DRS came from Duke University in the USA, where a team of researchers found their DRS probe could

distinguish node-positive tumours from node-negative tumours by scanning the tumour directly. They achieved sensitivity of 71% and 88% overall in identifying lesions by histopathological diagnosis with a wide range of anatomical subsites examined (larynx, pharynx, oral cavity). Their results were improved by using a tissue-specific (muscle, lymphoid, mucosal) rather than based on the organ sampled (anatomy-based algorithm).(228)

Narayanan et al achieved sensitivity of 96% and specificity of 100% in distinguishing cancerous and premalignant lesions from healthy mucosa in a different patient using DR imaging.(245) This study used site-specific algorithms to each area of the mouth and cloud-based machine learning. The authors credited grouping DR readings based on their originating sites in the mouth with achieving such good results. Hu also noted the role of differentiation between areas of the mouth when grouping the results of their in vivo DRS and benefit to using linear discriminant analysis.(228) Hafez advocated creation of a bank of DRS measurements of healthy mucosa to help in differentiating healthy and cancerous or pre-malignant mucosa.(247) Narayanan's research also supports this conclusion, with a finding from their study that sensitivity and specificity was increased by comparing malignant sites with a site in a similar anatomical location from a healthy volunteer, instead of a contralateral seemingly healthy site from the same patient. The effect of field cancerization may be the reason for this. De Koning obtained best results using a 1mm source – detector distance in separating healthy and cancerous tissue(268). Amelink experimentally found that a 1mm source-detector distance

interrogated preferentially the first 350μm of tissue depth, while another study with rigorous protocol from the same author found significant differences between dysplastic and non-dysplastic leukoplakia.(239,240) Use of spectral fitting and Monte Carlo simulations estimate the tissue depth interrogated.

Mendonca found pooled sensitivity of 0.79(CI) (0.67–0.89), specificity 0.86(0.80–0.91) for DRS in oral malignant and potentially malignant lesions.(181) This recent literature review conveyed a sense of anticipation about the prospects for this technology when it states: "studies indicated DRS to be a potential diagnostic, screening, biopsy and surgical guidance tool along with the added advantage of being able to discriminate all types of tissues of the oral cavity as compared to [fluorescence spectroscopy]. DRS was the most sensitive technique in the meta-analysis by Fuller et al (Se: 98.6%, Spe: 83.2%) in the diagnosis of dysplasia and malignancy.(223)

2.3.11.6 Advantages and disadvantages of DRS

2.3.11.6.1 Problems with DRS

DRS is sensitive to changes in pressure. Operator pressure is extremely important, as even the smallest changes in pressure can influence readings. Although invalid scans can be discarded, the perturbation to tissue physiology takes time to settle down and may affect the following valid scans.

DRS is sensitive to outside light. Readings must exclude as much electromagnetic energy as possible.

Temperature fluctuations, which occurs when the probe is brought in contact with live tissue or the probe–tissue coupling changes due to handshaking, can cause up

to a measurement drift up to ±0.5 psi. This measurement uncertainty reduces the accuracy in probe pressure control.

2.3.11.7 Advantages of DRS

Multiple studies have advocated this as a diagnostic tool in low and middle income countries which are disproportionately affected by oral cancer and do not have extensive histopathologic services.(256) Recent studies in the pursuit of this goal have succeeded in making devices portable and low-cost.(269) DRS readings are accurate, instantaneous and non-invasive. They do not require specialist input or interpretation. DRS is easy to use in comparison with other optical techniques, for example narrow band imaging or Raman spectroscopy.

Table 11. Summary of DRS studies. (Key: Se: sensitivity, Spe: specificity, MiD: mild dysplasia, MoD:moderate dysplasia, SD: severe dysplasia)

Year	Lead author	N = subjects (lesions)	Mode	Type of diagnoses	Findings	Comments
2003	Müller	23(91)	Trimodal spectroscopy with DRS, intrinsic fluorescence and light scattering spectroscopy	Normal, mild dysplasia, moderate dysplasia, severe dysplasia, SCC	Se96 Sp 96 in distinguishing normal from cancer/dysplastic. Se 64 Sp 90 in dysplasia from cancer	Used logistic regression and binary plot
2005	De Veld	225	Autofluorescence and DRS	Normal, dysplasia, benign, cancer (poorly described)	Excellent in distinguishing cancer from normal, neither system capable of distinguishing benign, premalignant.	Anatomical-based algorithm restricted to 3 areas – dorsal tongue, vermilion lip, other

					Se67% Sp77% in all malignant and dysplastic vs healthy	
2008	De Veld	155	DRS		Se69% Spe77% in differentiating all premalignant and malignant lesions from healthy	
2008	Nieman	27	oblique polarized reflectance spectroscopy	Benign, mild, moderate, severe dysplasia	Se100% Spe85% benign vs SD. Se75% Spe73% Benign vs MiD	No stratification according to tissue type
2008	Mallia	29	DRS	Healthy, hyperplasia, dysplasia, SCC	Se100% Spe86% in hyperplasia vs dysplasia	Healthy sites all from buccal mucosa only
2008	Amelink	31	Differential path length spectroscopy	SCC and normal mucosa. Confirmed by biopsy after measurements	Statistically significant differences in optical parameters between normal and SCC.	Used 1mm source-detector distance; good reporting of method
2009	Schwarz	124	depth-resolved DRS spectroscopy	Normal, cancerous and dysplastic sites in the mouth	Se 82% Spe87%	Several depths of interrogation employed, shallow and medium best
2009	McGee	64	DRS and autofluorescence	Malignant, dysplastic and benign	Se85 Spe97 in healthy vs premalignant	9 anatomical regions used in effort to improve accuracy
2010	Mallia	72	Autofluorescence and DRS	Healthy, hyperplastic, dysplastic, malignant	DRS gave Se 90% Spe 86% in hyperplastic vs dysplastic Se86% Spe94% dysplasia v malignant	Small sample size limiting quality of results. Superiority of DRS over autofluorescence noted
2010	Mallia	56	Autofluorescence with DRS readings	Healthy, hyperplastic, MiD, MoD, SD, well-, moderate-	Se 100 Spe 100 in distinguishing SD and well-diff SCC Se100% Spe 86% in	Used distribution scatterplots to improve analysis

			used to correct	and poorly-differentiated SCC	differentiating MiD from benign	
2011	Amelink	18 (74)	DRS	Leukoplakias – hyperkeratosis, hyperplasia, lichen planus, mild, moderate, severe dysplasia and carcinoma in situ	91% sensitivity and 80% specificity in discriminating dysplastic and non-dysplastic leukoplakias	Increased source-detector distance to interrogate deeper layers of hyperkeratinised lesions
2011	Jayanthi	130	DRS	Normal, hyperplastic, mild, moderate, severe dysplasia, well, moderate and poorly differentiated SCC	Se Spe between 95 and 100 for normal/ hyperplastic/dysplastic. Se 98.5 Spe 96 dysplasia vs SCC	Limited to buccal mucosa. Biopsy only for those with malignant features on DRS. Used LDA and box plots. Misclassifications of lesser nature (i.e. dysplastic as SCC and vice versa. Excluded hx of cancer and any medication use
2013	Stephen	88(155)	DRS imaging	Hyperplasia, mild, moderate and severe dysplasia. Well, moderate and poorly differentiated SCC	AUC 0.84 to distinguish premalignant from malignant	Misclassified one SCC as normal
2014	Hu	57(252)	DRS	Normal, hyperplastic, SCC	Se 71% Spe 88%	Sites from larynx and pharynx included. Results classified by tissue location
2016	Ko	6(15)	DRS	Normal	Monte Carlo simulation used to determine depth of buccal mucosa in healthy volunteers	
2017	Bailey	28(93)	DRS	Benign, mild or severe dysplasia	Benign vs mild dysplasia Se74 Spe 80	Did not use anatomy specific algorithm,

						oblique fibre-collection with width 100µm. No control group, used contralateral mucosa
2021	Narayanan	65(89)	DRS imaging	Leukoplakia, erythroplakia, oral submucous fibrosis, dysplasia, moderately to well-differentiated SCC	Se82% Spe 96.6% OPMD/SCC vs normal	Grouped into keratinized and non-keratinised mucosa. Exclusions were those who had previous cancer treatment or used medication for last 4/52. Improvement in accuracy when healthy volunteer data used. Able to distinguish traumatic ulcers from SCC
2023	Kolpakov	119	DRS	OLP, OLL, traumatic erosion, glossitis, fibroma, other	75% accuracy in vivo	Used PCA and ML models with discriminant analyses. Reported results only for ventral tongue and buccal mucosa.

2.3.12 Resolving issues around DR probe design

There are multiple factors that complicate sensitive detection of malignancies in the oral cavity, including high levels of keratinisation and variable epithelial thickness due to benign inflammatory conditions. Furthermore, neoplastic changes of epithelia and the underlying stroma in the oral cavity are associated with concurrent alterations in optical signals from different depths.(216)

2.3.13 Summary and knowledge gap

- **Squamous cell carcinoma of the oral cavity is an established and long-standing public health concern, with no appreciable reduction in deaths or new cases in the last twenty years**
- **Oral Potentially Malignant Disorders (OPMD) have been established as precursor lesions in some cases**
- **Screening for oral cancer has been shown to downstage cancer at diagnosis**
- **Current methods of oral cancer screening have been deemed not cost-effective and cumbersome**
- **Optical spectroscopic techniques have shown promise in detecting oral cancer**
- **There is no current consensus on the use of optical techniques in diagnosis and screening for oral cancer**

2.4 Aims and objectives

2.4.1 Project aims

This project is part of an overall effort to investigate the effectiveness of multimodality spectroscopic techniques with the intention of employing same for a future national screening programme in oral cancer.

Aim:

To develop a multimodality optical spectroscopic screening tool for the detection of oral cancer and oral potentially malignant disorders.

With this aim in mind, we developed three objectives:

1. To formulate a clinical protocol for use of DRS in the mouth
2. To explore the clinical utility for this probe in the mouth
3. Review of the protocol and assessment of its potential use in clinical practice

To fulfil these objective, a multiphase clinical research study was designed as set forth overleaf.

3 Materials and methods

3.1 Overview

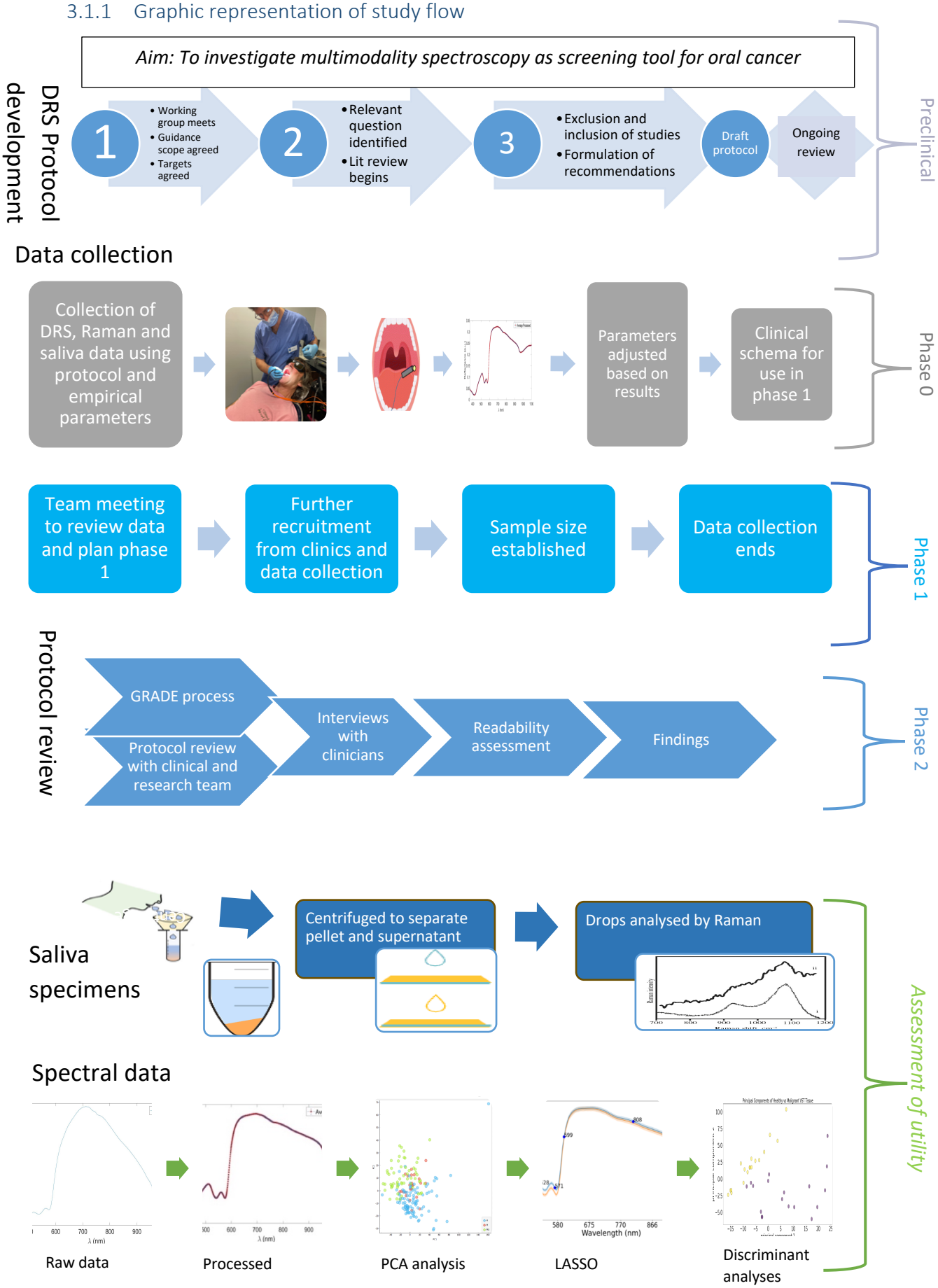
This study was divided into three main stages.

A preclinical phase involved development of an protocol for use of DRS in the mouth. The primary goal was to formulate a series of evidence-based steps to be followed in using DRS in the mouth. In Phase 0, the protocol was then tested in a pilot clinical study. It entailed use of DRS on oral lesions and normal oral mucosa in ten volunteers. The goals were to evaluate the protocol's applicability to a clinical setting, to identify optical parameters suitable for the oral mucosa and the familiarisation of the team with new procedures and materiel. Acquisition of saliva samples and Raman spectroscopy was carried out in both Phase 0 and Phase 1 to contribute to the larger goal of evaluating multimodality spectroscopy.

Phase 1 was the main clinical research element of this project. It involved recruitment of two groups (i) the study group, composed of patients with biopsy-proven OPMD, and (ii) a control group with acquisition of DRS, Raman and saliva data from all volunteers. The goals of this phase were (i) to evaluate the clinical utility of DRS to differentiate OPMD and normal using objective markers such as accuracy, repeatability and reproducibility and (ii) to evaluate the clinical effectiveness of the protocol.

Phase 2 involved collection of qualitative data on the protocol and DRS. The aim of this stage was to appraise the protocol and its potential use in clinical practice.

3.1.1 Graphic representation of study flow



3.1.2 Ethical considerations

Prior to commencement, ethical approval was sought and granted by the Clinical Research Ethics Committee of the Cork Teaching Hospitals (CREC), reference number: ECM 4 (y) 13/4/2021 & ECM 3 (permission to reopen study) 12/09/2023. All volunteers provided written informed consent before participation. Due to this project being a component part of a larger research project, ethical approval was obtained to include 500 patients with an oral mucosal abnormality for any type of oral spectroscopy.

3.2 Preclinical phase

3.2.1 Development of protocol

A protocol is defined as a method or procedure for carrying out an experiment, investigation, or course of medical treatment.(270) A guideline is defined as a rule, principle, or general statement which may be regarded as a guide to procedure, policy, interpretation.(271) This suggests that a protocol dictates actions which must be adhered to, whereas guidelines offer less rigid advice. This is the intention of the protocol - as a series of steps to be followed to optimise performance. It is envisaged to promote further development of DRS for oral mucosal conditions with standardised methods.

3.2.2 Building of protocol

Guidance on the development of clinical protocols was taken from National Institute of Health and Clinical Excellence (London, UK) guidelines on 'Interim process and methods statement for the production of Medtech Innovation Briefings

(MIBs)'. The processes described in this manual are consistent with international best practice.

The scope initially set out the target population, the target intervention, intended use and what the protocol will not consider. An initial scoping review was carried out to identify relevant literature and any other guidelines for use of the DRS probe in the mouth.

The target audience was decided to be oral medicine and oral surgery specialists, head and neck surgeons, doctors, both general practice and specialists, general dentists as well as auxiliaries such as dental therapists or dental hygienists who can use the probe. This was identified by a combination of literature review and expert consensus. The target population identified was anyone with a suspected diagnosis of oral potentially malignant disorders, oral cancer, those with a confirmed diagnosis of an oral potentially malignant disorder where there is a suspicion of transformation to cancer, and the general population where the clinician is carrying out an exam of the intra-oral soft tissues for oral disease or oral cancer.

3.2.3 Protocol development

3.2.3.1 Identifying question to be answered

A second stage of literature review identified the question to be answered and the population of relevance. A consensus was reached that certain populations, for example those with long-term premalignant conditions such as proliferative verrucous leukoplakia, oral submucous fibrosis or oral lichen planus that may require repeated biopsies would benefit from a non-invasive tool. This may also be of relevance to those with haematological disorders who need incisional biopsies.

3.2.3.2 Literature review process

Literature review searches were carried out on Pubmed, SPIES, EBSCO and guideline repositories such as Guidelines Index Network. No relevant guidelines were found. Further studies were identified from the references of included papers, from searching relevant journals and from grey literature. Studies were included based on the degree of explanation of their method, whether DRS was primarily used to obtain a result and clearly explained. Only studies which used a DRS probe in vivo were included. This was to reduce the risk of bias or incorrect method used in a lab or purely experimental setting. Proof of concept and preclinical studies were excluded.

3.3 Phase 0

3.3.1 Overview

Phase 0 was a pilot clinical study. We recruited ten volunteers with the goal of obtaining reflectance spectra reliably, quickly and with features capable of analysis while following the protocol outlined in the preclinical phase. Our DRS system had been used previously in a separate study looking at bowel cancer. Literature review found new settings for light intensity and acquisition time for obtaining signal from the full depth of oral mucosa. The time required for calibration and warm up of DRS and Raman system was found during this pre-clinical phase. Clinics were carried out weekly, with analysis of the data in the intervening time to determine success of experimental parameters.

Phase 0 entailed use of the protocol developed in the preclinical phase. It was anticipated that impracticalities evident in the protocol would be identified in this

phase before launching a full-scale research study. This was a translational clinical research study, with involvement of biophotonics researchers who had developed the DRS and Raman probes we used. The clinical team had no experience in using optical spectroscopy for oral mucosal diagnosis, so a period of familiarisation with the equipment was expected. Similarly, the biophotonics researchers had little experience in the clinical setting.

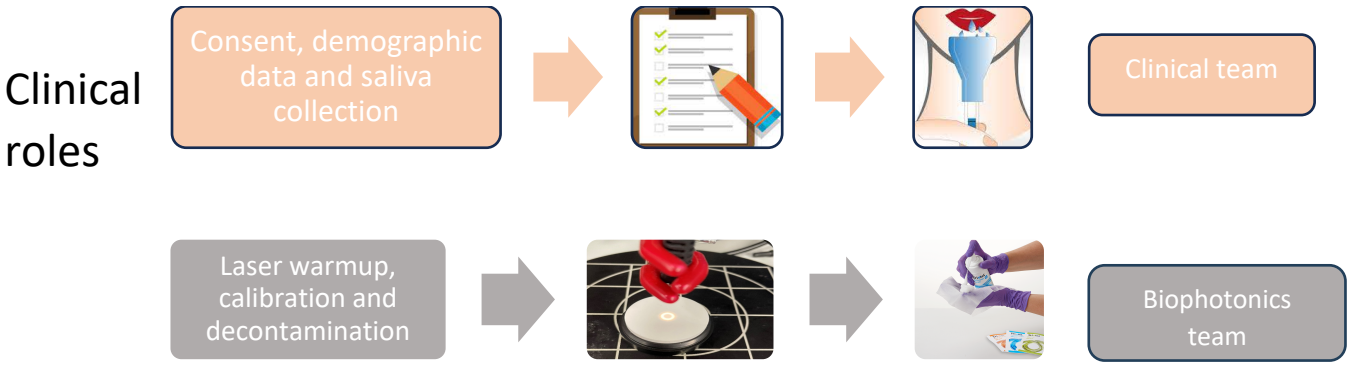


Figure 16. The roles of the multidisciplinary team on the clinic

3.4 Phase 1 recruitment

3.4.1 Overview

Phase 1 involved further recruitment of volunteers and data analysis. This was the main data gathering stage. The recruited participants were divided into two groups:

Group 1 with a histopathologically confirmed diagnosis of an OPMD

Group 2 of Control participants.

We used three different optical spectroscopy techniques – intra-oral mucosal DRS and Raman spectroscopy examination and also analysis of dried saliva by Raman Spectroscopy. This was an open-label controlled translational clinical research study.

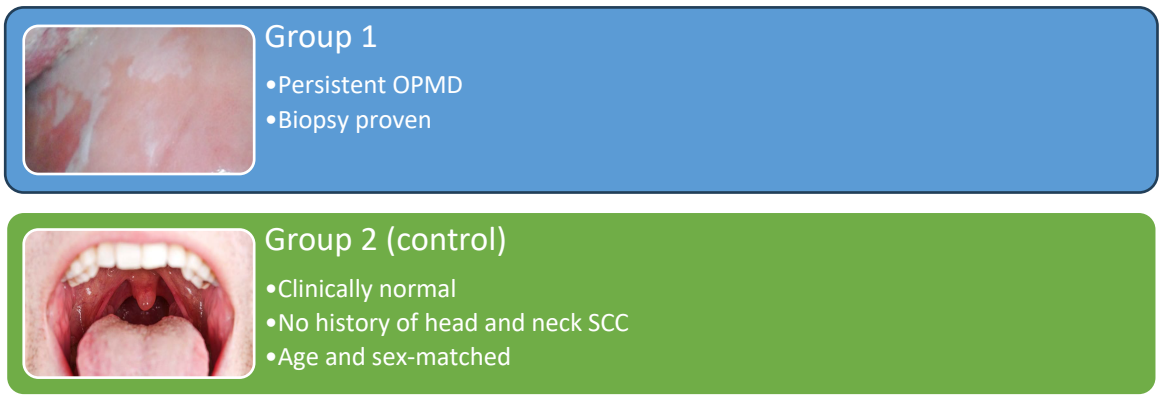


Figure 17. Representation of study groups

3.4.2 Sample size calculation

An initial target for data collection was set at 50 participants with a histological diagnosis of an oral potentially malignant disorder. A further control group of 20 age- and sex-matched controls was planned. The sample size was initially set from a literature review to be similar with other studies.(175,264,272) The target was reviewed based on interim analyses. Once the addition of further data did not alter the effect size, data collection was halted.

3.5 Recruitment

3.5.1 OPMD and SCC groups

Recruiting and data collection started in April 2022 and finished in Feb 2024, inclusive. A convenience sample of patients from Oral Surgery and Oral Medicine outpatient review and new patient clinics was recruited. These were all assessed by a specialist in Oral Medicine and had a biopsy-proven diagnosis of an OPMD. Clinical examination was carried out to determine the persistence of a lesion prior to inclusion. If there was no evidence of an OPMD, the patient was enrolled in the control group, with a note made of potential subclinical OPMD. One of these patients was excluded from the final analysis based on analysis of the spectral data.

3.5.2 Control group

Control participants were recruited from the Emergency and Dental Hygiene clinics. These patients had no oral mucosal abnormalities.

3.6 Participants and recruitment

3.6.1 Inclusion criteria (study group):

- Histopathological diagnosis of oral potentially malignant disorder or SCC
- Current visible intra-oral OPMD or SCC

It was considered essential to compare the results of spectroscopy with the gold-standard biopsy and histopathological diagnosis.

3.6.2 Exclusion criteria (study group):

The following criteria deemed patients ineligible for participation in the study

- Previous diagnosis of upper aerodigestive tract cancer

This was based on studies that had found spectroscopic changes in the buccal mucosa of patients with upper airway tract malignancy and found unsuspected malignancies in the contralateral normal mucosa in patients with oral cavity SCC.(125,140)

3.6.3 Control group

Inclusion criteria:

- no oral mucosal abnormality on clinical exam

Exclusion criteria:

- previous malignancy of the upper aerodigestive tract

The controls were age- and sex-matched to the participants in Groups 1 and 2. In vivo studies have shown that optical properties of skin and breast change with age. Although no studies have been published on changes in the mouth, it is likely that changes in collagen with ageing will have some effect in the mouth.(273,274)

3.7 Data Collection

Each prospective participant was first contacted by phone to confirm their eligibility for the study. All participants received written pre-trial instructions by post, which was signed and returned to clinic. One researcher (EF) went through the details of the trial on the day and obtained written informed consent for participation.

Each participant was assigned a pseudonymous number to act as their study identifier.

3.7.1 Demographics

Demographic details were recorded using a questionnaire. Age, gender, ethnicity, recent vigorous exercise (2 hours), oral hygiene, diabetes, hypertension, other co-morbidities and stress levels were all noted. The saliva flow rate (time taken to produce 1ml of saliva) was documented. Questions were chosen based on literature review which found factors that can affect skin autofluorescence as well as saliva flow rate and composition.(205,274) Other questions aimed to elucidate oral cancer risk, with questions on smoking, alcohol, use of alcohol-containing mouthwash and processed meat consumption, all factors that have been linked to oral cancer.(14)

3.7.2 Saliva collection

Saliva samples were also obtained from all participants as part of the project.

3.7.3 Imaging

An initial photograph was taken of any area to be inspected with the probe using an intraoral camera. The area was then marked on a schematic diagram.

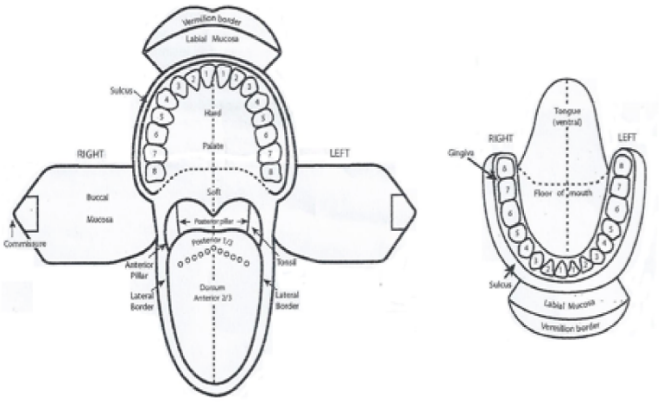


Figure 18. Schema used to map mouth

3.7.3.1 Microcamera development

It was envisaged the microcamera, which is a form of diffuse reflectance imaging, would act as a guide for DR and Raman spectroscopy. It was intended to find occult or highlight areas of atypical reflectance after processing. However, the integrated light source provided weak illumination of the site. We stopped using this early in data collection.

3.7.4 Collecting DRS and Raman data

A team of 2 worked in tandem to collect the DRS and Raman data, where one clinician held the probe in contact with the mucosa and a researcher operated the spectrometer detector. Two sets of readings were taken from each participant, with DRS being followed by Raman spectroscopy of the same areas. Five readings were taken from each area of OPMD or SCC, from a slightly different location each time. A further 5 readings were taken from the contralateral, clinically normal mucosa.

An anatomically based sequence was then followed, to take 5 readings from each area of the mouth, excluding the areas that had been already sampled. These area included the following:

a. buccal mucosa,
b. dorsal surface of the tongue
c. ventral surface of the tongue
d. floor of mouth
e. gingiva
f. lip

If the patient was included as a control, a similar process was followed.

Care was taken to place the probe in a way that the surface of the tissue to be measured provided an approximate angle of 90° toward the beam of the illumination light. The area of interest was also dried thoroughly using gauze and visually inspected for any saliva contamination. Multi-modality spectroscopic system and probes

3.7.4.1 DRS configuration

Our DRS system comprised a broadband light source with one spectrometer (QE-Pro, Ocean Optics, Edinburgh, United Kingdom). The source has a typical output power of 8.4mW, and a warm-up time of 10 minutes.

To obtain the reflectance spectrum preferentially from the superficial tissue, we used a quadrifurcated 400-µm-core Low-OH-Silica fibre optic probe (BF44LS01 1-to-4 Fan-Out Bundle, Thorlabs, Munich, Germany) with a minimum of 630 µm source-to-detector distance and a maximum of 1.2mm (SDD; fibre centre-to-centre

distance represented in Fig. 19. This was dual fibre DRS, with the light intensity detected by the spectrometer pre-processed to obtain the tissue reflectance spectra. SDD was formulated by first conducting a literature review to determine the depth of oral epithelium. Further literature review determined the optimal SDD to obtain signal preferentially from the epithelium, while minimizing signal from the underlying stroma.

One of the optical fibres was used for illumination, two other fibres were used for collection, and the remaining one was not used during the reflectance measurements.



Figure 19. Common end configuration

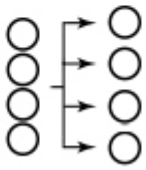


Figure 20. Linear end configuration

With 50ms of integration time, the reflection/backscattering probe acquired the diffuse reflectance spectra by means of two collection fibres. The collection probe with the shorter SDD obtained signal preferentially from the top layers, while the higher SDD obtained signal from the full thickness of epithelium and the stroma underneath.

3.7.4.2 Raman setup

The Raman laser was a 785 nm narrow line width laser with high suppression of side bands (>60 dB suppression at 0.3 nm from the laser peak, Cobolt 08-01 Series, Cobolt, Sweden). The spectrometer was the same as the one used for DRS. It is envisaged that this will be connected to an integrated probe that offers DRS, Raman and Raman microscopy. In our study, the optical fibre was changed when we wished to switch from DRS to Raman and vice versa. This takes a maximum of 5 minutes. The background and calibration are the same for all three configurations. The final system will be more automated, which will seamlessly switch between different configurations.

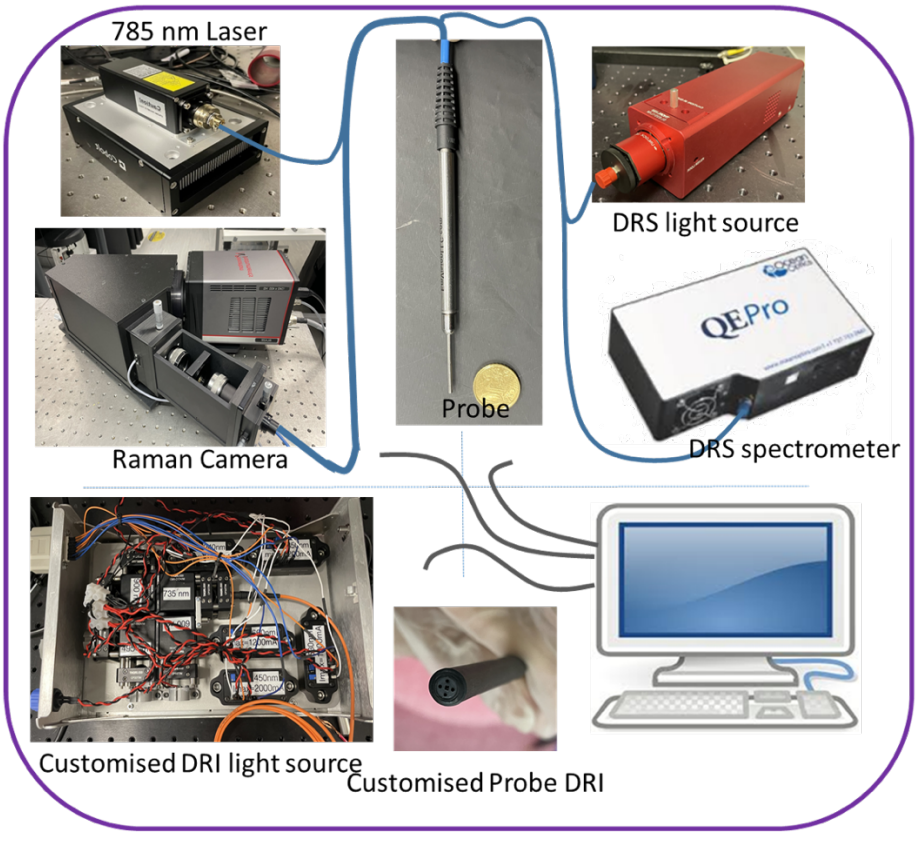
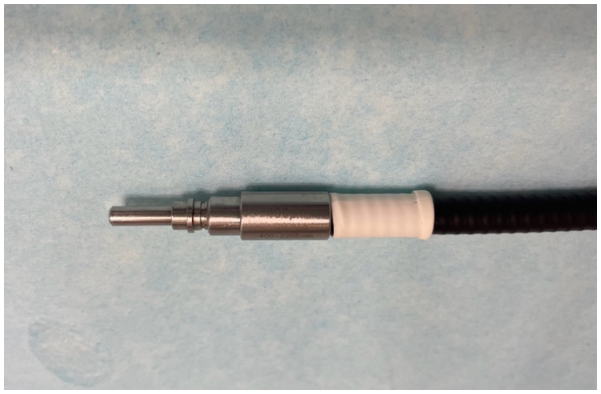


Figure 21. This is our DRS and Raman configuration. The central probe is envisaged to be a multi-modality probe, however in our study we used a similar probe for Raman and a smaller probe (below) for DRS.(281) The size of this DRS probe is optimised to the mouth.



3.7.5 Data collection protocol

All potential sources of extraneous light were excluded as much as possible, using blackout curtains and drapes. This created profound darkness in the room.

Prior to and after each session of clinical data collection, we collected the background and reference reflectance measurements. These were taken with the experimental conditions of darkness in force, before the patient sat in the chair. The objective was to find the natural variance in the lamp output and also the background light that must be subtracted from the readings. The reference measurements were taken on a specialized holder able to keep a fixed distance of 2 mm and 6 mm between the SDD probe and the reflectance standard (FWS-99-01c, Avian Technologies LLC, New London, USA), respectively. The fixed distance allows the comparison of the reflectance data across patients. The reflectance standard was WS-1-SL Diffuse Reflectance Standard (Ocean Insight, Orlando, Florida), composed of Spectralon. The reflectivity was 99% (400-1500 nm) and >96% (250-2000 nm) for s spectral range of 250-2000nm.



Figure 22. Customised reflectance stand holder

A period of time (usually more than 10 minutes and up to 30 minutes) was permitted for lamp warm-up. Measurement of the light output during this time was taken to estimate the periodic lamp intensity drift. After each set of tissue measurements, the probe was cleaned using Tristel chlorine dioxide wipes (Tristel, Newmarket, UK). The same probe was used for every clinical measurement throughout this study. Another researcher monitored the output readings and gave feedback to the clinical researcher holding the probe. Illumination of the mouth was provided when required by a third researcher.



Figure 23. Using the probe. Room lights are turned on for the purposes of the photo.

3.7.6 Variance data

Variance was estimated for two intervals. First, reproducibility (inter-examiner variance) was investigated. Two users conducted subsequent DRS exams with the same area being examined immediately after by examiner no. 2 with 5 measurements being taken of one area per examiner.

The second, repeatability (intra-examiner variance), was carried out by one examiner. This was probed on three patients, with a repeated exam following another. This was done as quickly as possible following the first DRS exam in order to avoid changes in clinical parameters due to time or movement by the participant. Paired t-test was used to analyse the values.

3.8 Analysis of in vivo data

3.8.1 Reflectance data preprocessing and analysis

The DRS data preprocessing comprised of the background subtraction and subsequent division of the captured signal (tissue reflected intensity) by the reflected intensity of the reference (reflectance standard):

$$Reflectance\ (\lambda) = \frac{tissue\ reflected\ intensity\ -\ background\ intensity}{reference\ reflected\ intensity - background\ intensity}$$

In order to prepare the reflectance data for classification, the data was centred and scaled/normalized between − 1 and + 1.

3.8.2 Statistical analysis

Quantitative and qualitative analysis established data related to pre-test questionnaires. Graphic analyses were created describing the research participants. Six sites had been identified as sites for analysis prior to start of the study. These

were gingiva, buccal mucosa, dorsal surface of tongue, ventral surface of the tongue, floor of mouth and lip.

Statistical analysis was conducted with the aid of a data scientist (SM) from Tyndall National Research Institute. All data analysis was coded for using Python version 3.12.2 on Windows 10. Functions for classification algorithms were obtained from open-source Python libraries such Pandas, pingouin, SKLearn, SciPy. Use of open source libraries helps to achieve uniformity across studies.

Outlying spectra with features not consistent with the overall data set were identified and removed if either created in error or to be not representative. These were done by looking at each spectrum individually. If a spectra was obviously aberrant, it was removed. Criteria for removal included areas of the spectrum below the x-axis and large reflectance spikes consistent with water contamination. Another operation removed all spectra with negative acquisitions on the x-axis.

Savitzky-Golay filters were applied to the DRS data. This is a least-squares fitting polynomial that reduces the signal-to-noise ratio by smoothing the curve.

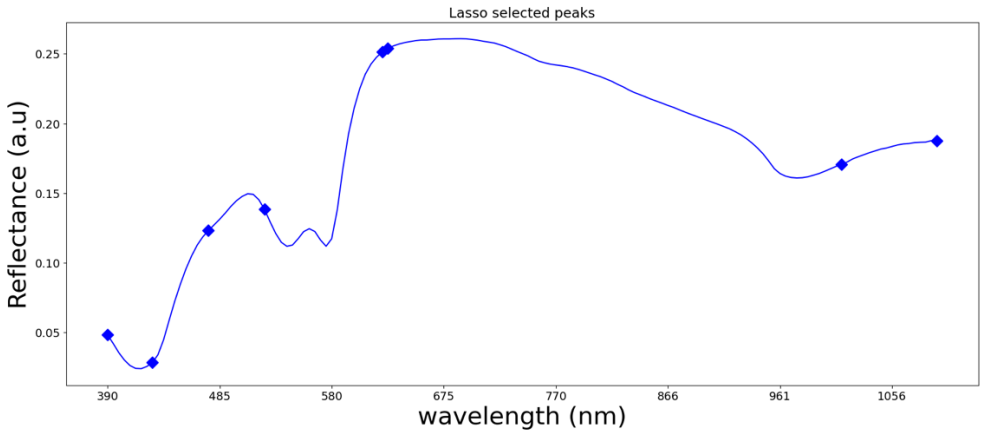


Figure 24. Extraction of spectral features using LASSO for dorsal surface of the tongue.

LASSO (Least Absolute Shrinkage and Selection Operator) was used to select identifiers on the spectra for DRS and Raman saliva data. LASSO is used in data analysis when there are a large number of independent variables or predictors (denoted by *p*) in a dataset and the data points are not all related to the outcome measure.

Using the extracted LASSO features for the three anatomical sites, we then carried out principal component analysis (PCA) on the DRS spectral data. Principal components were retained for further analysis. PCA aims to reduce the number of variables in a dataset while retaining as much information as possible. It identifies the dimensionality of the data by first centring the data around its mean and then finding the eigenvectors and eigenvalues of the covariance matrix. The eigenvectors represent the directions of maximum variance, while the eigenvalues represent the amount of variance explained by each eigenvector. The eigenvectors are then used to project the data onto a lower-dimensional space, so that spectral features common to all spectra are eliminated. This is well-studied in the literature and has applicability to the research question.(275)

Leave-one-out cross-validation was done to reduce the risk of overtraining and overfitting of saliva and DRS data. This is supported in the literature as a method of increasing accuracy of spectroscopy results.(237) Leave one out leaves out a single subject to generate the model, thus testing the external validity of the sample if a random sample is removed. Overtraining occurs when an algorithm, by being trained to be applicable to a small data set, becomes less accurate to another, as yet unknown data set.

Final assessment of accuracy was attempted by several methods. Previous approaches used in the literature and new approaches suggested by colleagues in the field at multi-disciplinary meetings were assessed and utilised. Support Vector Machines (SVM) are a set of supervised learning methods used for classification, regression and outliers detection. Linear Discriminant Analysis (LDA) and Quadratic Discriminant Analysis (QDA) were used in an attempt to classify the data. Discriminant analysis is well-studied in the literature and has produced good results.(175,276) It is often used for classification and feature selection.

Classification was attempted based on three approaches: First, the classifying features established using the LASSO tool were compared across the data set to establish accuracy. Then, classification was attempted without using the features. Finally, classification was attempted using all wavelengths.

3.9 Assessment of protocol recommendations

Rating the effectiveness of our clinical recommendations was needed in order to establish external validity. We chose to use the GRADE system in formulating our protocol, and to validate firmly the protocol in a clinical study. The GRADE approach has been endorsed by a large number of societies and institutions including The Cochrane Collaboration and has been used in various fields of medicine such as allergies, heart disease, oncology, endocrinology and respiratory and critical care medicine.(277) It is currently recommended by the BMJ in its “Instructions to Authors” that authors submitting to this journal should use the GRADE tool when submitting a clinical guidelines article. While this tool is described to be purposed for the formulation of guidelines as opposed to protocols, literature does show its

use in compiling clinical protocols.(278) Our project was aimed at formulating a consensus best-practice protocol from several other protocols in use in other studies. Thus it seemed logical to use this objective tool to influence the objectivity and effectiveness of the putative protocol.

GRADE assesses the following features for the evidence found for each outcome:

- study limitations (risk of bias) – the internal validity of the evidence.
- inconsistency – the heterogeneity or variability in the estimates of treatment effect across studies
- indirectness – the extent of differences between the population, intervention, comparator for the intervention and outcome of interest in the studies from that in the review protocol
- imprecision – the level of certainly in the effect estimate
- other considerations – publication bias, the degree of selective publication of studies.

In a standard GRADE approach, the certainty or confidence of evidence is classified as high, moderate, low or very low.

- High – further research is very unlikely to change our recommendation.
- Moderate – further research may have an important impact on our confidence in the estimate of effect and may change the strength of our recommendation.

- Low – further research is likely to have an important impact on our confidence in the estimate of effect and is likely to change the recommendation.
- Very low – any estimate of effect is very uncertain and further research will probably change the recommendation.

One author conducted the literature review and carried out the GRADE rating. We used the modified QUADAS-2 tool, as set down by Walsh et al. to assess for risk of bias.(4) This was used to assess the methodological quality of the studies over four key domains: patient selection, index test, reference standard, and flow and timing of participants.

The GRADE guidelines provide a systematic way of rating the quality of evidence in a reproducible manner. Opinions may differ between clinicians, however GRADE provides a way of objectively scoring the quality and difference in strength of recommendations, helping decision makers. Cross-sectional or cohort studies in the context of diagnostic uncertainty and in the framework of a new diagnostic test can be considered high-quality by the GRADE tool.(279)

Table 12. Quality criteria of diagnostic accuracy studies derived from QUADAS-2..(286)

Domain	Patient Selection	Index Test	Reference Standard	Flow and Timing
Description	Describe methods of patient selection Describe intended patients (previous testing, intended use of index test and setting)	Describe the index test and how it was conducted and interpreted	Describe the reference standard and how it was conducted and interpreted	Describe any patients who did not receive the index tests or reference standard or who were excluded from the 2 x 2 table. Describe the interval and any interventions between index tests and reference standard
Signalling questions (yes, no or unclear)	Was it a consecutive or random sample? Was a case-control design avoided? Did the study avoid inappropriate exclusions?	Were the index tests interpreted without knowledge of the reference standard? If a threshold was used, was it pre-specified?	Is the reference standard likely to correctly classify the target condition? Were the reference standard results interpreted without knowledge of the index test?	Was there an appropriate interval between index tests and reference standard? Did all patients receive a reference standard? Were all patients included in the analysis?
Risk of bias (yes, no or unclear)	Could the selection of patients have introduced bias?	Could the conduct or interpretation of the index test have introduced bias?	Could the reference standard in use or interpretation introduced bias?	Could the patient flow have introduced bias?

3.9.1 Testing of the guideline

In the next phase, we aimed to use the protocol in our study on the clinical utility of DRS, as described above. We also aimed to assess the guideline's readability and assess the potential impact of the new technology on clinicians. Six clinicians experienced at oral medicine and biopsy clinics but unfamiliar with the DRS technology were asked for their opinions on the technology and the protocol.

3.9.1.1 Clinician opinion

A questionnaire was formulated for distribution to clinicians based on a similar questionnaire used in the formulation of NICE guidance on an optical technique.(280) The results were synthesised and themes identified.

3.9.1.2 Readability

The Flesch reading ease score and Flesch-Kincaid Grade Level was used to assess readability of the guidance. This was assessed using an online tool <readability.io>. These are widely used, credible reading tools that give results based on the grade level that would be expected to read the document.(281) The protocol was then edited based on the issues identified from the software. This was continued until no further improvements in the score could be made without compromising the structure and content.

4 Results

4.1 Overview

In the reporting of the results, more weight will be given to the DRS findings as these were the focus of this project.

4.2 Preclinical phase results

The initial phase of the project was a preclinical phase. In this phase, a protocol for use of the DRS probe was formulated. A lab-based protocol for use of DRS was used a basis for formulation of recommendations.

A total of 7 studies with 439 participants with the subject matter of diffuse reflectance spectroscopy for detection of oral cancer or oral potentially malignant disorders were included in the development of the protocol (see Appendix A). Proof of concept and preclinical studies were excluded. No clinical guidelines or protocols were identified from searching the literature. Two systematic reviews with meta-analyses discussed DRS in the diagnosis of OSCC and OPMD.

An initial literature search found 97 studies, of which 10 fit the inclusion criteria.

Three in vivo studies were excluded. Schwarz 2009 was excluded on the basis they did not separate DR and fluorescence spectroscopy in the reporting of results. Liu 2023 was excluded on the basis of poor reporting of method. Kolpakov 2023 was excluded on the basis of no reporting of gold standard.(282–284)

The findings of literature review were synthesised and a value (strong, weak, moderate) was assigned to each recommendation.

The evidence found in the literature review on DRS was found to be of moderate quality. The evidence included five prospective clinical trials comparing DRS spectroscopy with biopsy and histopathology, a feasibility study that reported diagnostic accuracy and an experimental study demonstrating the effect of pressure on oral mucosa. Evaluation of the risk of bias was carried out using the QUADAS-2 tool. The most likely judgement of bias was moderate, due to the lack of multiple pathologist grading. Good consistency was noted across the studies, with lower effects most likely as a result of grouping heterogenous tissues together. The level of indirectness was most likely moderate-high, with variation in the type of DRS used and population variability.

The research was done in specialist centres. Clinical examination, biopsy and DRS were all done by highly trained surgeons and oral medicine specialists so may not be reproducible in all primary care settings. Multiple studies were carried out on similar referral groups with small numbers. The reporting of diagnoses included was generally good. The reporting of the gold standard used was in general good. The reporting of procedures used was adequate. The literature contains many reports with similar methods and similar results. A need was identified for larger studies with more diverse referral populations and in more clinical settings. Some studies contained few diagnoses from certain areas of the mouth, for example the gingiva and the palate.

4.3 Phase 0 results

4.3.1 Overview

A sample of 10 patients from Oral Medicine clinics were recruited for this preparatory study. Six were male and four were female. The mean age was 66.8. Five had no diagnosis of OPMD, three had diagnoses of OLP, one had PVL, one had mild dysplasia. The sites of lesion were ventral tongue (n=2), buccal mucosa (n=2) and lower lip (n=1). The whole cohort received DRS and Raman readings as laid out previously.

4.3.2 Familiarisation with DRS equipment and clinical procedure

This was found to be easy to use and intuitive. Few repeat readings were needed. Patients similarly found the experience to be straightforward.

4.3.3 Use of the protocol

The DRS protocol was used for all patients, was found to be repeatable and easy to follow. No issues were noted with its use and acquisition of data was uncomplicated. Only one modification was needed. This was to increase the number of acquisition spectra from three to five. This was to reduce the influence of aberrant spectra.

4.3.4 Test reflectance spectra acquisition

The objective of this part of the pilot study to find optical parameters such as light intensity and duration of acquisition.

Empirical optical parameters found from literature review were used in this phase. Analysis of the results gave more appropriate values. Areas such as the ventral tongue with increased microvessel density was noted to reflect more light, with

intensities overwhelming the sensor. In these areas, lower intensities and/or shorter exposure times were used. Raman settings were found by experiment to be 30mW intensity with an integration time of 0.43sec for 9 acquisitions.

DRS was 50msec integration time at 8.4mW output. DRS was noted to be easier to acquire and faster due to fewer unusable (redo) results. These settings were then used in the second phase of the study.

4.4 Phase 1 results

4.4.1 Overview

This phase involved use of the protocol to obtain DRS readings from the oral mucosa. A total of 53 participants were recruited to the study group, and 27 were recruited to the age- and sex-matched control group. One patient was excluded from the final control group due to spectral anomalies consistent with subclinical OPMD. This made the final total for the control group 26.

4.4.2 Demographics

The mean age of participants in the control group and the study/OPMD group was 56.5 and 62 years old respectively. The median age was 62 in the study group and 60 in the control group. 59% of the OPMD group were over 60, with 37% aged between 40-60. This is further shown in Table 13.

Table 13. Gender of the study participants.

Age (years)	Total	Control group	Study group
Mean	60.03 (SD: +/-10)	56.5 (SD: +/-13)	62.1 (SD: +/- 8.5)
Range	27 - 82	27 - 73	36 - 82

Almost 70% of the total participants (53/79) were female, with 26 being male. This imbalance was reflected in the composition of the control and study groups, with 68% of the study group being female, while 65% of the control group was female. Gender stratification was achieved between the two study arms. This is further shown in Table 14.

Table 14. Gender distribution between study arms.

Gender	Total	Control group	Study group
Male	26	9	17
Female	53	17	36
Total	79	26	53

4.4.3 Summary statistics

4.4.3.1 Tobacco and alcohol consumption

Comparatively few participants overall were current or former tobacco users, with 32% (17/53) in the study group and 50% (13/26) in the control group reporting current or former cigarette, pipe, vape or chewing tobacco use. Alcohol consumption patterns were closer to each other, however 84% (22/26) of the control group reported current or previous regular alcohol consumption, compared with 96% (52/54) of the study group. The majority reported current alcohol use, with 24 reporting consumption of more than 10 units alcohol weekly.

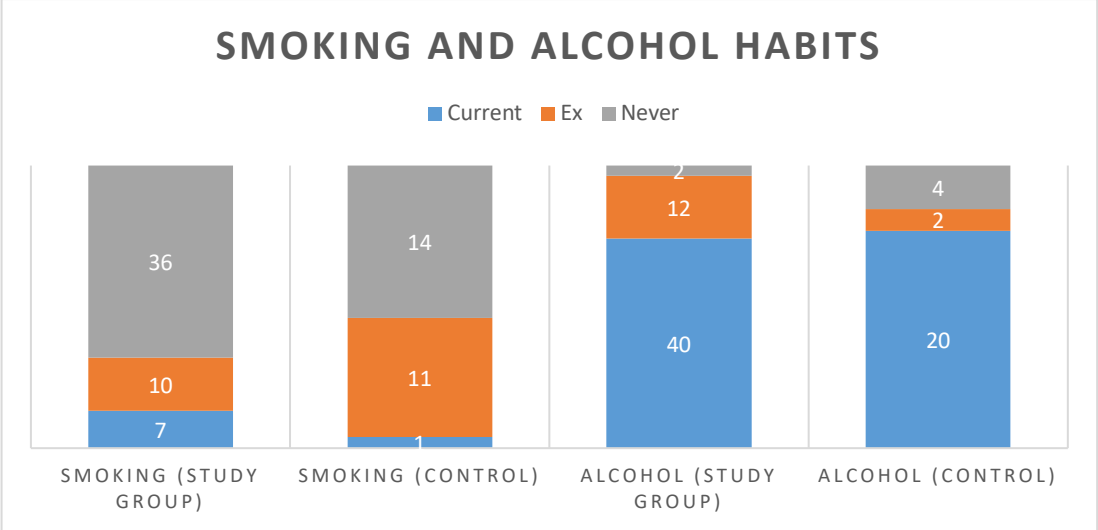


Figure 25. Smoking and alcohol habits compared across groups.

4.4.3.2 Dietary habits

The dietary habits of the two main study groups did not differ substantially, with the proportion of participants in the study group who consumed processed meat roughly similar across the categories of never, seldom, daily or weekly. Similarly, the use of alcohol-containing mouthwash was low, with a figure of 5% and 12% in the study and control groups, respectively, using a mouthwash frequently.

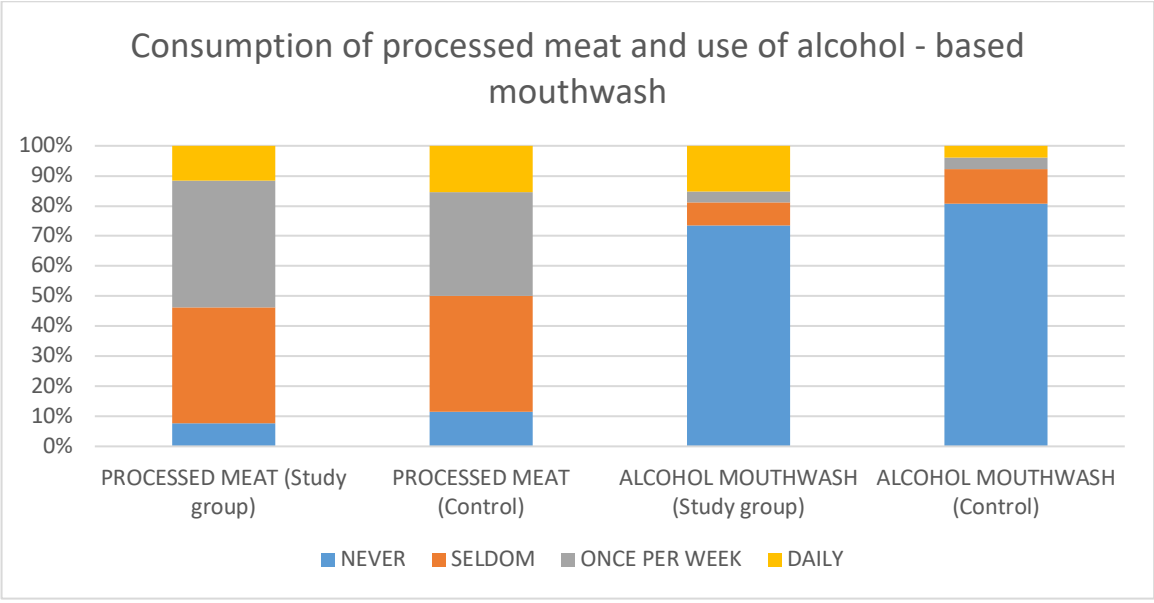


Figure 26. Habits of processed meat consumption and use of alcohol-containing mouthwash across groups

4.4.3.3 Ethnic background

The vast majority reported their ethnicity as White Irish, with 8 outliers reporting other ethnicities. Two were contained in the study group, with one of British ethnicity and another one of white Polish ethnicity. In the control group, five were white Europeans and one was of South Asian ethnicity.

4.4.3.4 Co-morbidities

The vast majority of all participants (48/53) and (25/26) reported good health, with most participants having one or two co-morbidities. In the control group, 8 reported no co-morbidities, 8 had one co-morbidity, 5 had 2, and 6 had more than 2 co-morbidities. In the study group, 9 had no co-morbidities, 18 had one co-morbidity, 11 had 2, and 14 had more than 2. The control group was less healthy than the study group, with more co-morbidities.

Table 15. Co-morbidities by numbers of chronic illnesses reported per control group.

	Total	Control	Study
0	17	8 (29%)	9 (17%)
1	26	8 (30%)	18 (34%)
2	16	5 (18.5%)	11 (21%)
>2	20	6 (22%)	14 (26%)

4.4.3.5 Body Mass Index

Many were unsure of their weight, and some of their height, therefore statistics for this group may be incomplete. In the study group, 12/44 (27%) were normal, 22/44 (50%) were overweight, 8/44 (18%) obese and 2/44 (5%) underweight according to

the NHS BMI calculator for adults. In the control group, 9/25 (36%) were normal, 13/25 (52%) were overweight, 3/25 (12%) were obese.(285)

4.4.3.6 Site-specific results

The results were grouped according to site and to histological diagnosis. There were comparatively few results of premalignant lesions from the floor of the mouth, lip and gingiva. The most common areas we found were the buccal mucosa, then ventral and dorsal surfaces of the tongue. Almost half of readings of premalignant sites (24/53) came from buccal mucosa. The next most common was ventral surface of the tongue, with 14/53 premalignant lesions we studied located on this surface. The dorsal surface of the tongue was next, with six total areas studied. Finally, the lip (4), gingiva (3), floor of mouth (1) comprised the remaining locations.

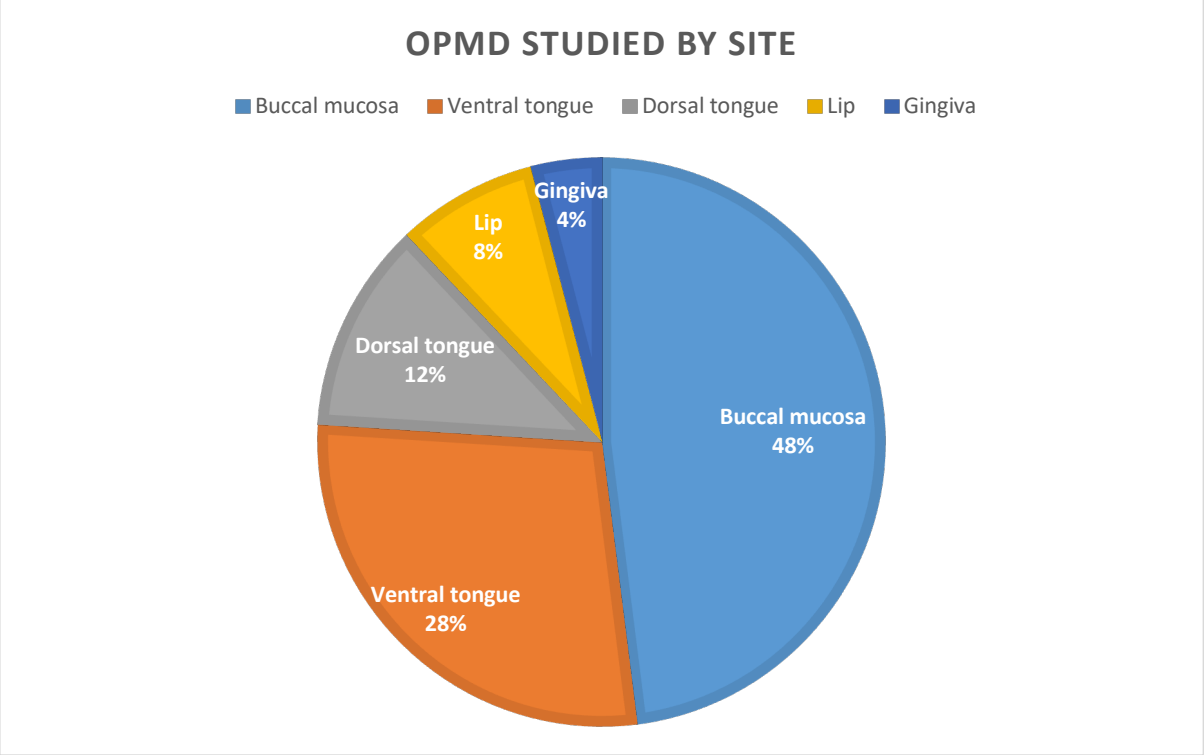


Figure 27. Sites of OPMD included in the study

4.4.3.7 *Oral premalignant conditions studied*

The most common diagnosis was oral lichen planus. Oral lichen planus patients comprised 41/53 of the oral diagnoses we included in this study. The less common diagnoses were leukoplakia (n=5), proliferative verrucous leukoplakia (n=1), actinic keratosis (n=4) and oral lichenoid lesion (n=1). Other diagnoses included geographic tongue (included in control group as not premalignant) and graft vs host disease (included in control group as completely resolved).

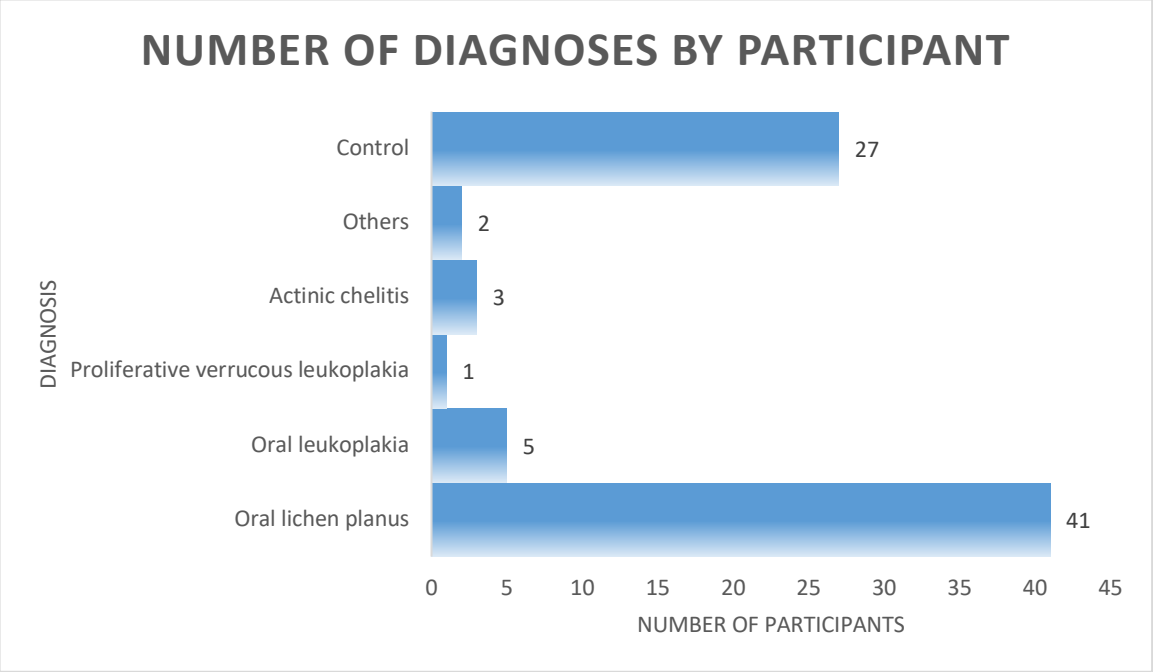


Figure 28. Number of participants with each diagnosis. Control is included as a comparison

4.4.4 Saliva data

Some saliva samples were unusable due to mucus contamination. The final amount of saliva samples that were used for analysis is given in the table below.

Table 16. Numbers of saliva samples usable for analysis.

Groups	No. of Patients	No. of data files or Locations
Control	14	215
OPMD	30	611

The Raman spectrum of saliva shows protein peaks that can be linked to known biomarkers. For instance, peaks around 1049 –1053 cm^{-1} are assignable to stretching and vibration of the C–C bond in lipids. Furthermore, a Raman band characteristic of amide vibration of salivary proteins at around 1639 – 1670 cm^{-1} are also discernible. Also, unique to the saliva specimen are two additional peaks at 877 cm^{-1} (polysaccharide) and 938 cm^{-1} (α-helix proteins).(286)

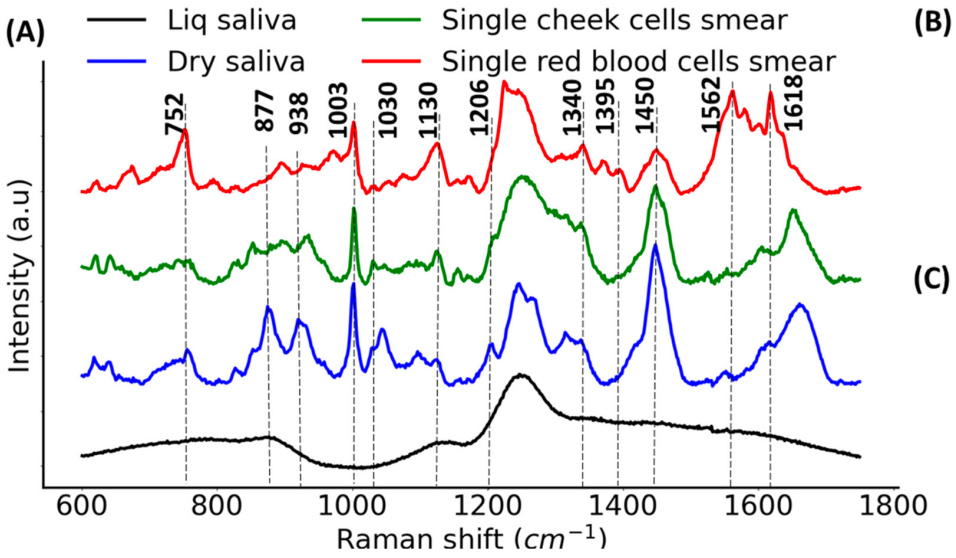


Figure 29. A spectrum obtained from a saliva analyte. Wavelengths of biomarkers are drawn in. Red blood cells and epithelial cells were isolated as part of the centrifuge process and analysed

This figure shows the difference between dry saliva, liquid saliva and the smear of the red blood cells and mucosal cells identified from the saliva analyte. Wet saliva is included for comparison and has few identifying features.

Classification was attempted using support vector machines (SVM). This gave a balanced accuracy of 81.6% and accuracy of 81.7% for healthy vs OPMD and SCC.

4.4.5 Imaging data

Two diffuse reflectance imaging microcameras with integrated light source were employed to guide sampling. Both proved unsatisfactory or offered little above what clinical oral examination with white light could achieve. Specular reflection was an issue at all wavelengths. In this case, the reflected light does not enter the tissues and is reflected at a surface level.

4.4.6 Raman Data

Analysis of the Raman data has shown differences between normal and premalignant sites in the mouth. This finding awaits further analysis and the production of accuracy figures. For now, the focus of this thesis is on solving problems with DRS.

4.4.7 DRS Data

4.4.7.1 Overview

Three anatomical areas were included for the purpose of statistical analysis: the dorsal surface of the tongue, the ventral surface of the tongue and the buccal mucosa. Due to differences in the tissue structure, an OPMD at one site cannot be grouped with a similar OPMD at another site. Only buccal mucosa, dorsal surface of tongue and ventral surface of tongue could be used for data analysis as we did not have sufficient numbers.

There were eight OPMDs found in the literature and generally recognised. We recruited as many patients as possible with each OPMD.

Table 17. Number of data files obtained per patient group for DRS.

Label	Description	No. of data files
Control	Readings taken from control group	1659
OPMD	Taken from OPMD lesion	795
Normal	Visibly healthy tissue from participant with OPMD	4926

We had notably many fewer OPMD in the lip, gingiva and dorsal tongue.

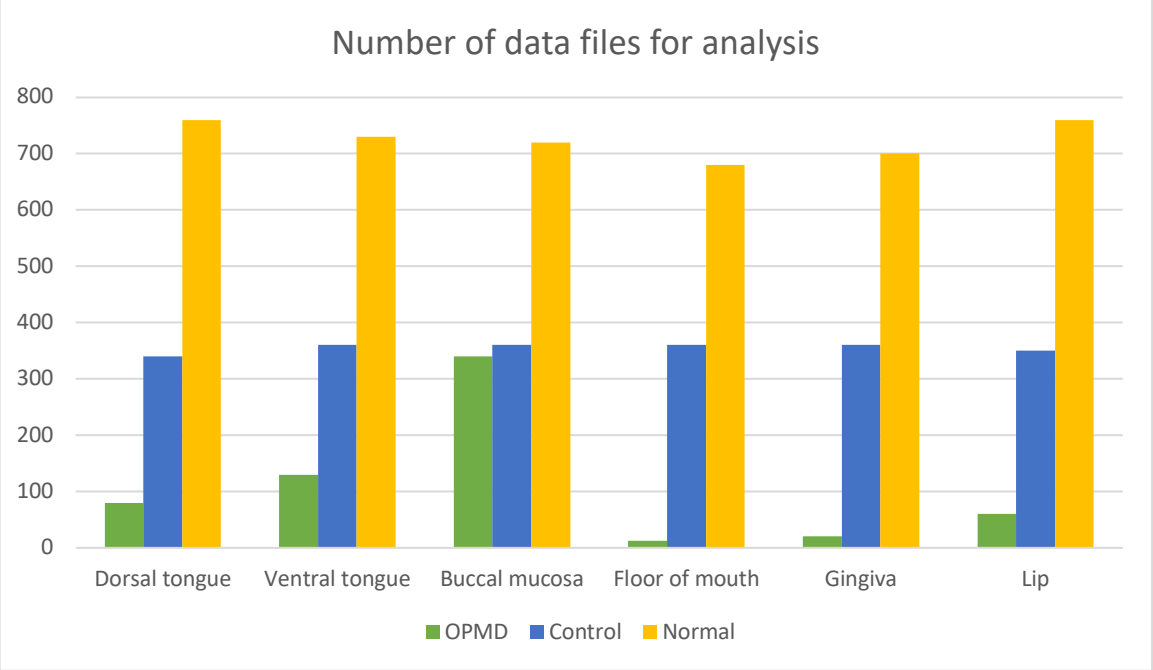


Figure 30. Number of data files suitable for analysis, divided by site and study group.

The buccal mucosa, ventral tongue and to a lesser extent the dorsal tongue have the most OPMD per site. Normal denotes a measurement taken from a site clinically normal in the mouth of a participant diagnosed with an OPMD.

4.4.7.2 Analytics of data collection

The time taken for data collection was remarkably uniform. The minimum time it took for a full mouth DRS readings with 5 points at each anatomical area including a control area was 4 minutes, the maximum was 13. Clustering around a central point of 8 minutes or so was consistent. This did not vary greatly from the first patient to the last, suggesting that the protocol is quick to become accustomed to.

4.4.7.3 Biomarkers and spectral shape

This spectrum (Fig. 27) shows the characteristic absorption pattern of OLP in the buccal mucosa. The dip between 400-500nm (referred to as an absorption peak) is due to the tissue presence of haemoglobin and deoxyhaemoglobin.

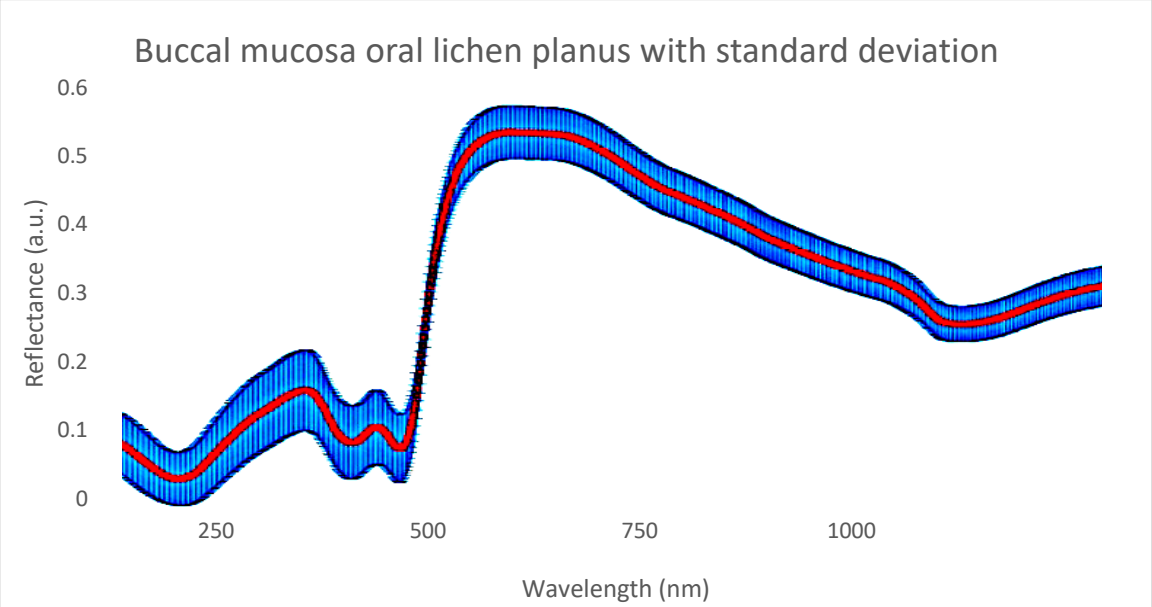


Figure 31. Patient 55. Oral Lichen Planus on the buccal mucosa. Mean of five readings in red and standard deviation in blue.

The reflectance curve for a patient who has a histological diagnosis of oral leukoplakia in the ventral surface of the tongue is shown. This is the average of 5 spectra collected, normalized to the mean. There is more overall reflectance (on the y-axis) noted in Fig. 27 (above) compared the leukoplakia below.

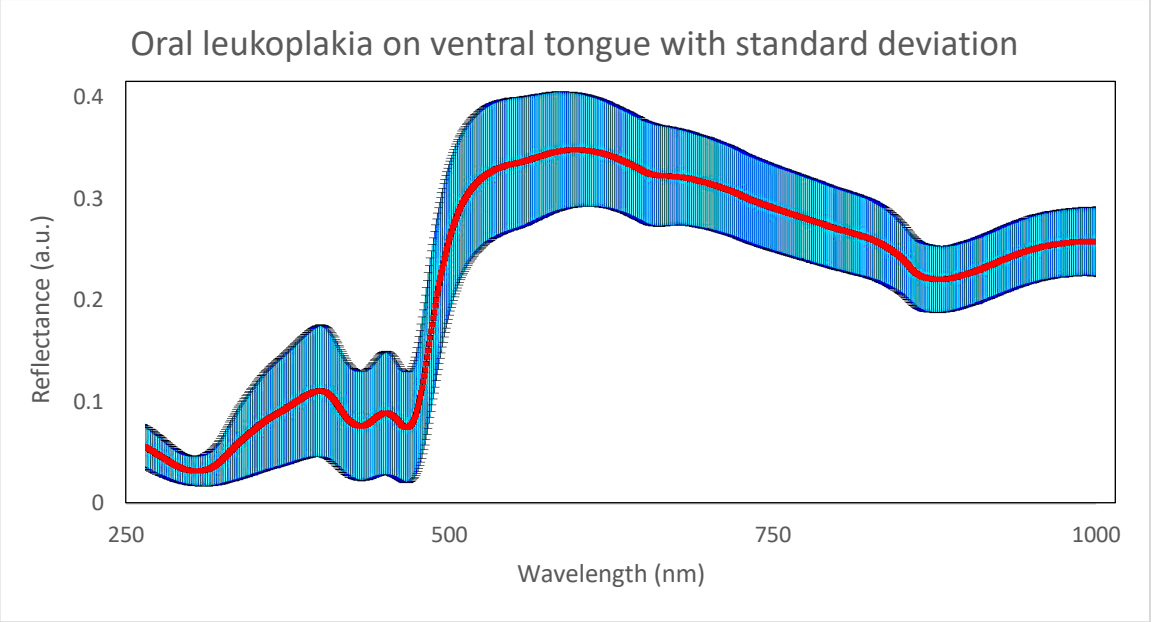


Figure 32. Patient 58. Oral leukoplakia on the ventral tongue. Mean of five readings in red and standard deviation in blue.

In the DRS spectra, the first segment (400–650 nm) is dominated by optical absorption of haemoglobin, myoglobin, and, to a lesser extent, beta-carotene. Water shows a characteristic local reduction in optical absorption at 1000 to 1160 nm.(287)

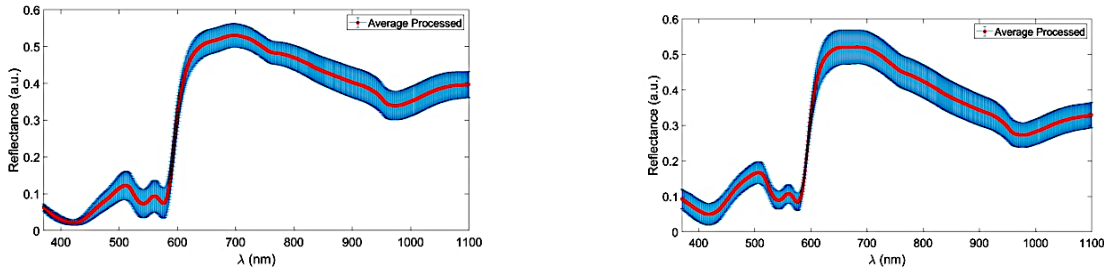


Figure 33. (a) above and (b) below. DRS spectra with standard deviation. Floor of mouth (left) and dorsum of tongue (right). The spectral shape is slightly different.

In Fig. 33 (a and b), two DRS spectra are set side by side from the same patient. The left is from the floor of mouth, the right from the dorsal surface of the tongue. The area in the spectrum from 500-600nm has a double dip appearance and is similar in both. The appearance of this area is due to haemoglobin levels, which preferentially

absorbs light in this wavelength region. The main differences in the spectrum are from 600nm onwards. These wavelengths have strong water absorption. If there is surface water, this will alter the spectral shape, making the removal of surface water of high priority.

4.4.7.4 Dorsal surface of the tongue

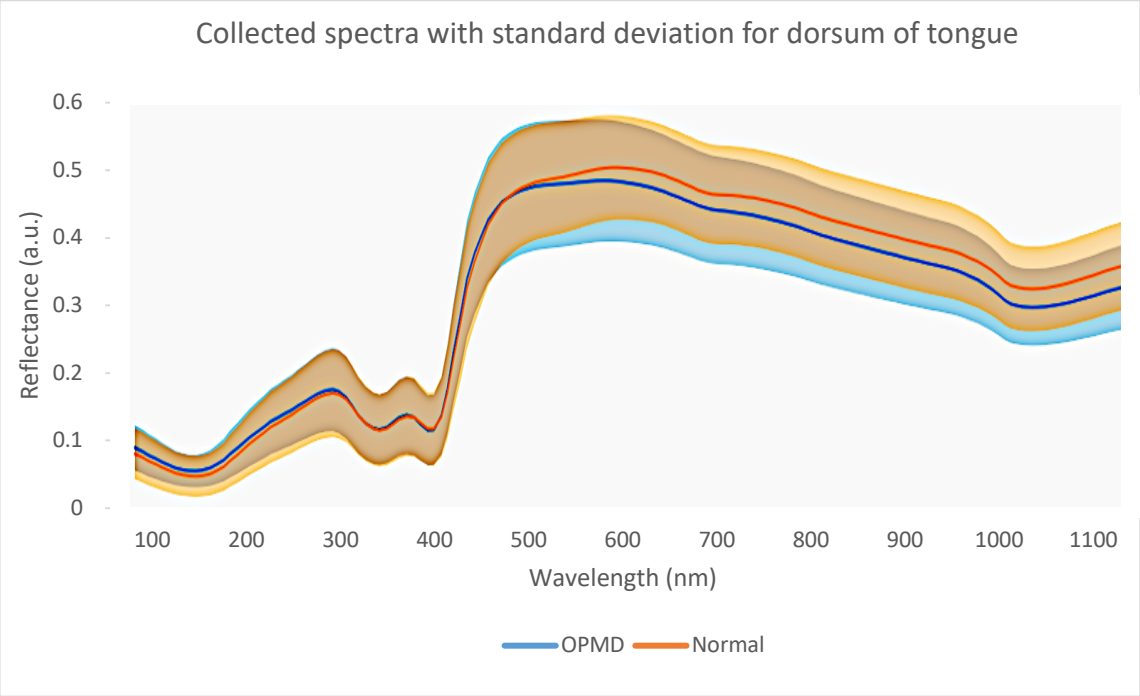


Figure 34. Mean and standard deviation of collected spectra for dorsum of the tongue.

This shows the mean for the collected spectra for the dorsal surface of the tongue (central, solid line). The lighter colours show the standard deviation around the normalised mean. Wide variance between patients is noted due to inter-patient differences however the spectral shape is retained.

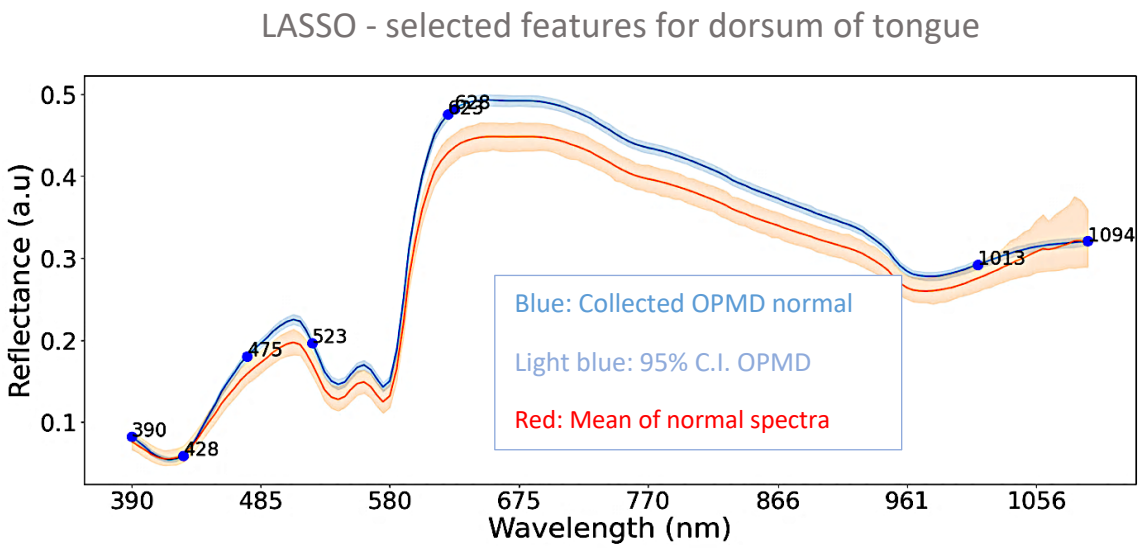


Figure 35. LASSO selected features from the dorsum of the tongue

LASSO selects those wavelengths with most reliable or greatest measurable variation in order to provide a point of differentiation. These wavelengths for the dorsal surface of the tongue were 390nm, 428nm, 475nm, 523nm, 623nm, 628nm, 1013nm and 1094nm. Classification of tissues was attempted based on two approaches: first, the classifiers identified using LASSO were used, and the mean of the result was taken as the true value. Second, the LASSO points were not used, and classification was attempted using all wavelengths. Classification on all wavelengths gave best results, however balanced accuracy was poor. Discriminant analyses gave poor results. Linear discriminant analysis gave superior results.

Table 18. Discriminant analysis for dorsal surface of the tongue.

Classification with features:

	LDA	QDA	SVM
Balanced Acc.	67 %	48 %	50 %

Classification on all wavelengths:

	LDA	QDA	SVM
Balanced Acc.	60 %	50 %	60 %

4.4.7.5 *Buccal mucosa*

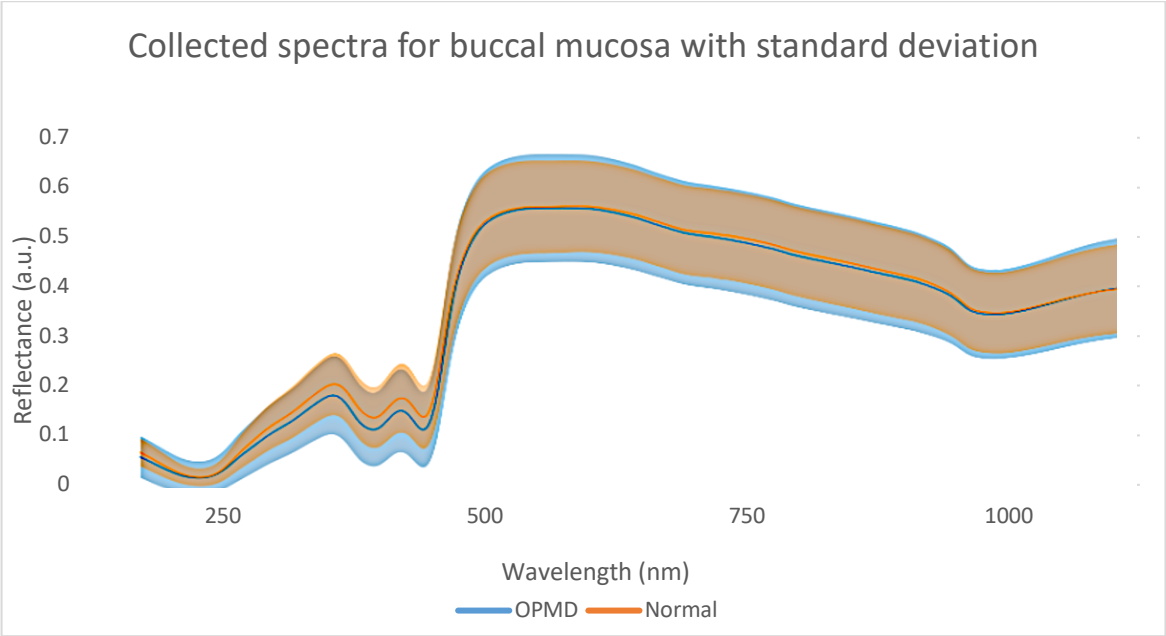


Figure 36. Mean spectra (solid line) for buccal mucosa on all patients with standard deviation shown.

The mean spectral curve for buccal mucosa with SD is shown above. Again, high levels of variance are noted, especially in the 300-500nm region, where oxy- and deoxyhaemoglobin are found and again from 500nm. This may be due to tissue variance.

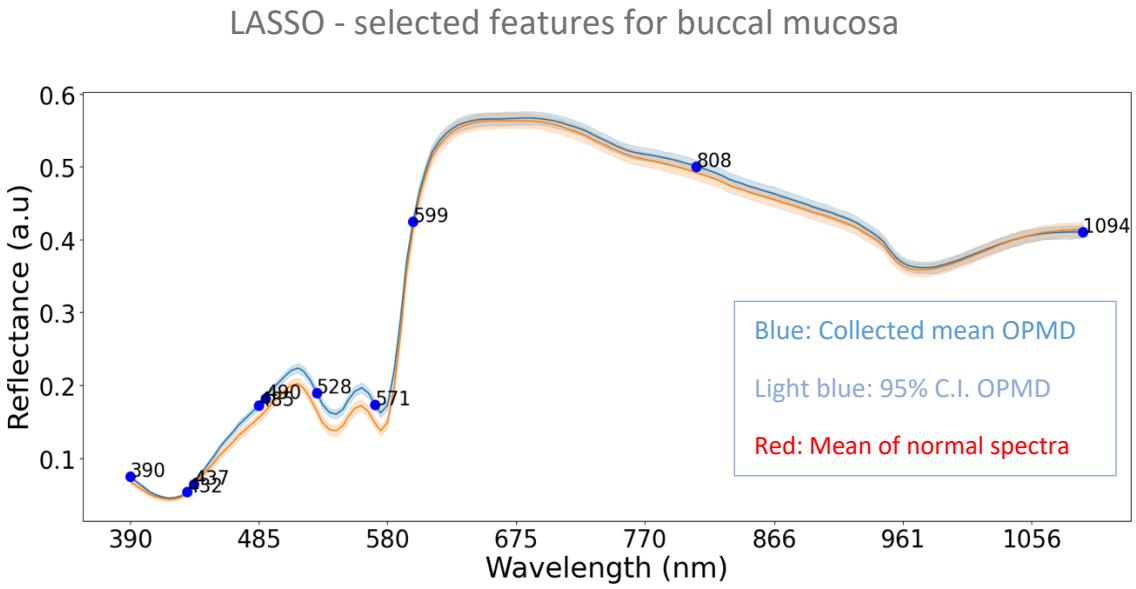


Figure 37. LASSO – identified wavelengths for statistical comparison from the buccal mucosa.

The wavelengths identified by LASSO in the buccal mucosa are 390nm, 432nm, 437nm, 485nm, 490nm, 528nm, 571nm, 599nm, 808nm and 1094nm.

Leave-one-out classification was performed to reduce the risk of overtraining and curve overfitting.

Table 19. Discriminant analysis for the buccal mucosa.

Classification with features:

	LDA	QDA	SVM
Balanced Acc.	85 %	85 %	89 %

Classification on all wavelengths:

	LDA	QDA	SVM
Balanced Acc.	81 %	73 %	71 %

The improvement and similarity of balanced accuracy figures with this more balanced dataset is striking.

4.4.7.6 *Ventral surface of the tongue*

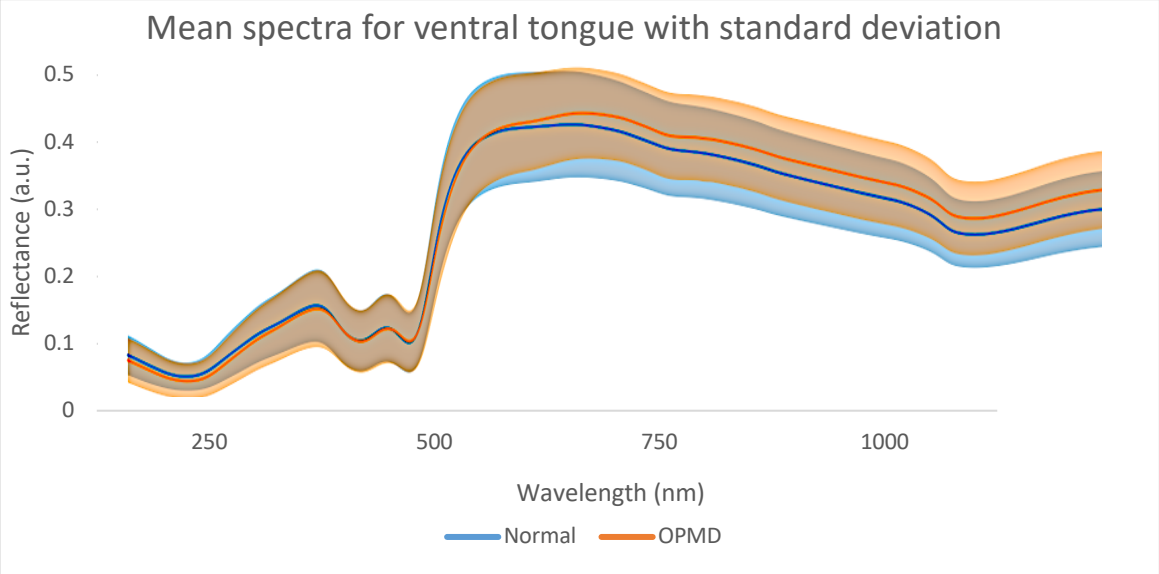


Figure 38. Mean spectra for ventral tongue with standard deviation.

There was less overall reflectance from the ventral tongue. This is most likely due to the increased haemoglobin and deoxyhaemoglobin in this vascular part of the tongue. These are light absorbers. Again, there is a large amount of variance due to inter-patient variation. More variation is noted in the 500-1000nm region. LASSO scores were highest for 713nm, 961nm, 718nm, 523nm, 437nm, 571nm, 723nm, 604nm wavelengths. These are more consistent with biomarkers from the literature. Leave-one-out cross-validation was performed in this case also.

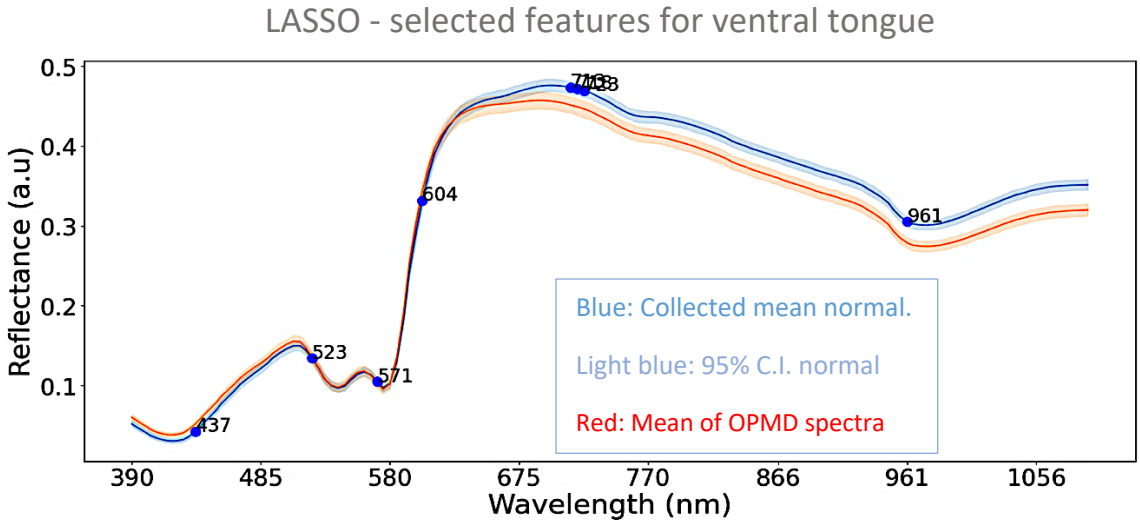


Figure 39. LASSO selected classifiers plotted with OPMD in blue and normal in red

Table 20. Discriminant analysis for ventral surface of the tongue.

Classification with features by majority voting:

	LDA	QDA	SVM
Balanced Acc.	86 %	78 %	68 %

Classification on all wavelengths

	LDA	QDA	SVM
Balanced Acc.	80 %	87 %	57 %

This dataset was more balanced and had more OPMD in its numbers than the dorsal tongue.

4.4.8 Inter-examiner variance figures

4.4.8.1.1 Reproducibility

Three patients (all OPMD) were used to test reproducibility of the DRS measurements – two different observers interrogated all tissue types at separate instances on the same day. The two datasets were analysed using two sample t-test to understand whether the means of both populations are significantly different. The data were analysed patient-wise and for the same tissue type.

The data shows mixed results. The example spectra below demonstrate the inter-observer variation, which can change depending on the observation at a given wavelength for the same patient. Five readings were taken for each site, from as close to the same area as the previous reading. These readings were normalized to the mean and analysed. The spectral shape was maintained, however significant inter – observer variation was noted, particularly in the lower wavelengths in the

haemoglobin region. This was observed throughout the sample at all anatomical sites.

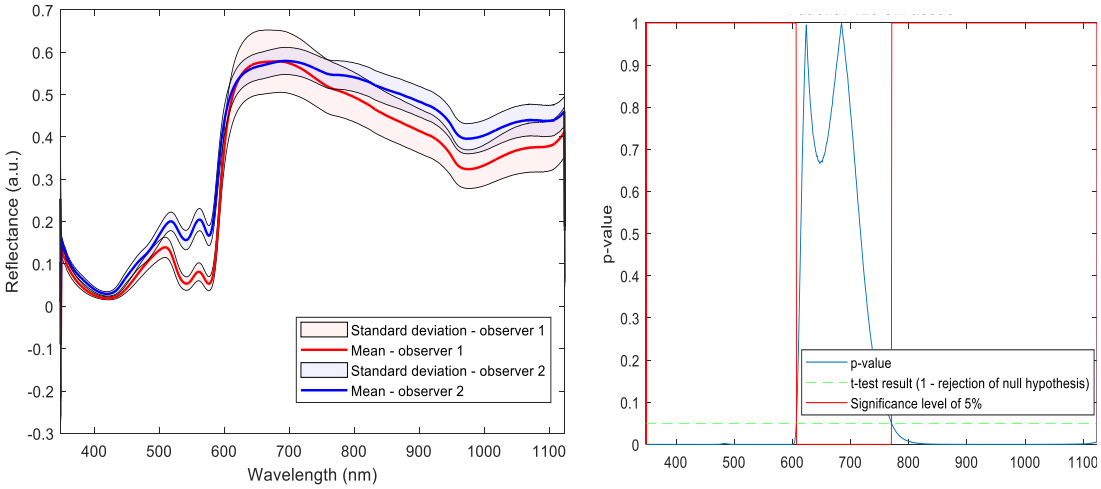


Figure 40.(a) and (b). This shows the inter-observer variation for healthy buccal mucosa for one patient. Significant variability is inferred from the wide standard deviation for each observer.

In Fig. 40, (above) differences are noted in the reflectance captured in the 500-600nm and from 800nm onwards. This is confirmed in the t-test graph shown on the right. Statistically significant inter-observer difference is achieved at all wavelengths excepting 600-800nm.

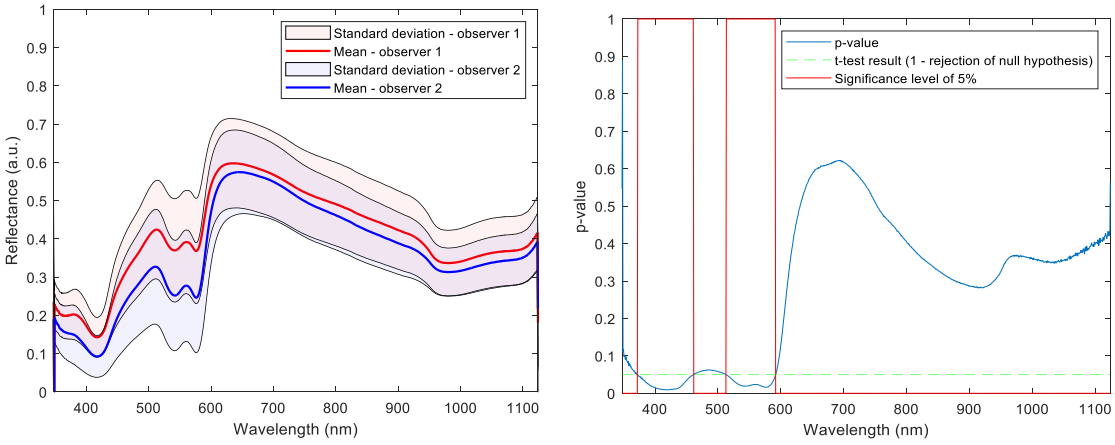


Figure 41. Significant differences at shorter wavelengths

While differences in the 500-600nm region were observed at all sites surveyed – buccal mucosa, dorsal ventral tongue, floor of mouth, gingiva and lip to a smaller

degree, other patterns were observed in the floor of the mouth. This showed significant variance at the 400-600nm region. This was expected as it is a vascular region, but also variance was observed in the 600-1000nm region. The lip was noted to have good reproducibility.

4.4.8.1.2 Repeatability (intra-observer variation)

Four patients (all OPMD) were used to test repeatability of the DRS measurements – same observer interrogated all tissue types twice at separate instances on the same day. The two datasets were analysed using paired t-test to understand whether the means of both populations are significantly different. The results for repeatability were improved compared to reproducibility. Differences were noted again in the 400-600nm region mostly. The buccal mucosa region showed some variability, mostly uniformly in the 500-900nm region. The dorsal surface of the tongue again showed good repeatability, however some variation was noted in the 400-600nm region. The anterior floor of mouth, similarly to the reproducibility tests, showed variance at longer wavelengths (600-1100nm). The lip was noted to have good repeatability.

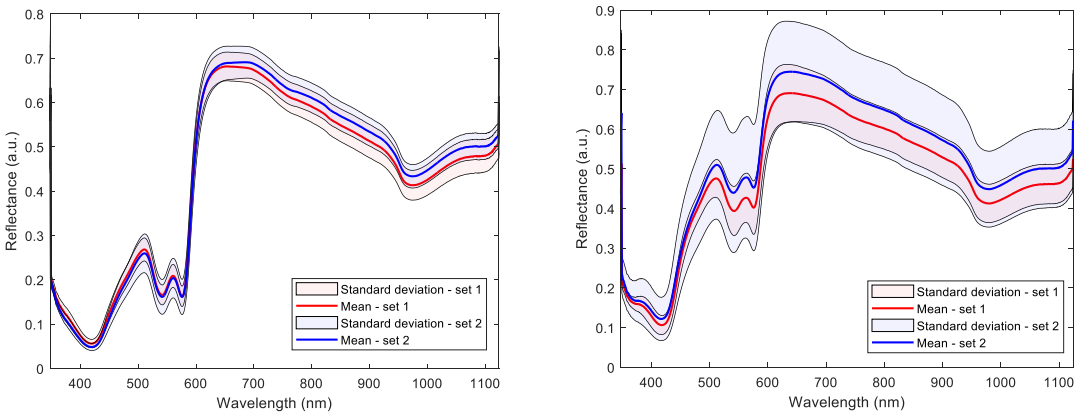


Figure 42.The curve for the buccal mucosa (left) and gingiva (right) are set side-by-side. The increased variance for gingival tissue is clear compared with buccal mucosa.

4.5 Phase 2 results

4.5.1 Perceptions of DRS and protocol for clinical use

4.5.1.1 Clinicians involved

We sought the opinion of six clinicians in the field after reading the protocol. All have practiced Oral Medicine for a minimum of one year and have carried out original research in the field. We also sought opinions from patients and from the researcher who had developed the DRS set-up (SM).

Table 21. Professional background of clinicians interviewed. (OS: Oral Surgery. OM: Oral Medicine)

	OS specialist	University and private practice	Consultant/senior lecturer	Practiced solely OM	Trainee
Participant 1	x	x	x	x	
Participant 2	x	x	x		
Participant 3	x	x			
Participant 4	x	x			
Participant 5	x				
Participant 6					x

The most experienced clinician had practiced solely in oral medicine for a period of five years, the next most experienced had practiced oral surgery for more than twenty years.

4.5.1.2 Clinical utility of DRS

All clinicians identified the utility of DRS techniques in treating anxious patients or those who needed multiple or repeat biopsies of larger areas. The dysplasia clinic was specifically mentioned by 2 participants (P2, P5) who talked about the invasiveness of repeated biopsies.

All participants thought that DRS was most likely to be of use to untrained staff, such as general practitioners, dentists or junior hospital staff. Another commenter saw its utility in diagnosing likely benign, or low risk lesions. The same consultant (P1) felt it would be of use in low-income countries, with high levels of oral cancer and poor access to advanced medical care. The time needed to train in the use of DRS and to become skilled at interpreting results would militate against its time-effectiveness, as per P3, and thus may not be useful in a hospital setting. Another issue raised was decontamination. While the probe can be wiped down using chlorine wipes and this is compliant with regulations for use in a dental practice, some clinicians felt uncomfortable with this. The current standard of care in dental practice is disinfection with an autoclave or to use disposable instruments. The probe cannot be autoclaved.

One clinician highlighted the issue of calibration and allowing for lamp fluctuations. In a hospital or clinic setting, a clinician would need extra training or would need to have someone trained present to carry out calibration and background measurements.

Another problem to be resolved was the issue of how to take measurements. The amount of pressure and how to orient the probe without deforming the mucosa needs some practice and instruction.

Participants in the study found the experience of being examined with the DRS probe to be an easy experience compared with conventional biopsy. Some found it uncomfortable with the probe touching a raw area of oral mucosa, such as erosive lichen planus. However, these were in the small minority, and were able to tolerate

without undue difficulty with a little care on the part of the clinician. The one person who was not satisfied with his participation in the study was disappointed as he thought we could provide an alternative diagnosis for his condition.

Some individuals found it difficult to produce enough saliva for the saliva sampling. In these cases, we took a smaller amount of saliva. This was sufficient for analysis. For similar reasons, those with a dry mouth found it difficult to hold the mouth open for long periods.

The research team found that the main problems with DRS were (i) the lack of ambient light to see which area is being sampled, and (ii) saliva contamination in the mouth had an impact on the spectral output. The difficulty in maintaining adequate pressure meant also that spectra had to be discarded or repeated. This was both personal experience, from working with the probe throughout the project, and also opinion taken from another clinician (RniR) who used the probe. I thought that the DRS probe worked very well and was easy to use. However, it was difficult to see where to put the probe and gauge the amount of pressure to be used in a dark room. Saliva contamination was also hard to judge in a dark room. A self-calibrating probe which would allow extraneous light during use of the probe would improve user experience. Clinician 2 (RniR) also commented on the difficulty in applying the correct amount of pressure and also the need for a finger rest to reduce involuntary movements or tiredness. The lack of an intuitive, visible result means that clinician trust in the device is likely to be guarded until definitive statements of utility are set forward.

4.5.1.3 Healthcare system impact

Most thought that DRS would not speed up patient flow and would have little impact on waiting lists. The participants generally assumed biopsy will still be needed in the future and could not be replaced by this technology. Concerns over accuracy would cast doubt on any outcome until DRS becomes more widespread in practice. Three interviewees (P1, P2, P3) stated that it may be helpful in triaging unknown lesions. One interviewee (P2) commented that the 2-week-wait (2WW) system means that likely cancers were already done on an expedited pathway, so the use of an instantaneous point-of-care diagnostic system would not add much speed to the referral pathway.

None of the group judged DRS as being clinically useful or likely to adopt it.

The most common judgment was that this would be better in the hands of high-throughput specialist units, however two interviewees identified a role in screening (P1, P2). The long calibration and warm-up time was highlighted by one commenter (P1). No one person thought the DRS device would be helpful for clinicians. One clinician raised the issue of clinical effectiveness versus cost-effectiveness (P2). While cost-effectiveness can be debated and studied, clinical effectiveness is currently poor, as patients must undergo biopsy as well as DRS to get a diagnosis.

The main issue identified was that the probe itself is unproven and thus cannot be recommended as a choice to biopsy.

The alternative solution to this is that the probe can be used as an adjunct to biopsy, in sampling some areas or as a guide to biopsy. However, this was not seen as favourable by any commenter, with the cost of the setup outweighing the

benefits. One interviewee recognized that studies in DRS have been ongoing since the early 2000s, without a firm answer on utility (P4).

The advantages proferred were (i) non-invasive, (ii) screening, (iii) as a guide to biopsy.

There was unanimous scepticism about the cost-benefit ratio to taking this up as an ongoing project.

The least experienced and the most experienced shared positive views on the new technology. One interviewee commented that it would be difficult to change people's minds on this and it would take some time before the newer generation coming through are trained in this new technology for things to change (P6).

4.5.1.4 Themes identified

1. High set up cost is an issue
2. Lack of clinical data to guide implementation
3. Need high patient throughput to justify inclusion of DRS in pathway
4. Current pathway for diagnosis and treatment seen as adequate

4.5.2 Readability

The readability of the protocol was scored as a 10th grade level, roughly equivalent to a 15 or 16 year old. The Flesch Reading Ease was 36.9, out of a maximum of 100.

5 Discussion

5.1 Overview

In the results of this project, certain key findings carry more importance. We will focus on these pre-eminent findings in discussing this thesis.

5.2 Accuracy of DRS

We were successful in recruiting 80 participants to this study. This large sample size is the second largest number of in vivo subjects in a trial of singular DRS spectroscopy for oral mucosal disease outside India. The protocol's accuracy in the ventral tongue and buccal mucosa reached 87 and 89% respectively. This shows the DRS probe can accurately identify OPMD, the vast majority of which is OLP, on the buccal mucosa and the ventral tongue. Due to insufficient numbers for other diagnoses, we grouped all OPMD together into one group. Almost 80% of diagnoses included were of OLP.

The high accuracy figures are further underlined by preclinical studies showing DRS' error range to vary from 1-10% in estimating the contents of a homogenous sample.(256) This shows the inherent limits of the technique and upper limits on its accuracy. Kolpakov et al in a 2023 report, found overall accuracy of 75% in an in vivo study with a wide range of oral mucosal diagnoses. This notable study reported results only from the ventral tongue and the buccal mucosa. This study is the most similar to ours in the literature in the ambition of its range of diagnoses and sample size, however the experimental protocol was not well described. Accuracy of 75% is poor compared with our findings. To improve our accuracy figures, we stratified and compared by site of acquisition (buccal mucosa, ventral and dorsal tongue, lip

etc.) and diagnosis (normal vs OPMD). This method is following that chosen by other studies, some of which have shown good results in examining OPMD on the buccal mucosa only.(175,288) Jayanthi et al 2011 produced sensitivities and specificities ranging from 90-100% in distinguishing a range of hyperplastic and dysplastic lesions from each other and SCC. However, the site reported was limited to the buccal mucosa, retromolar region and commissures.(288) Mallia similarly found improved sensitivity and specificity when analysing data from buccal mucosa vs. all other sites in the mouth.(175) No comment was offered by either author on the significance of DRS of the buccal mucosa giving more accurate results, however she noted more reflectance was observed in buccal mucosa and dorsal tongue compared with gingiva and alveolar mucosa.

McGee et al demonstrated that the microvascular and scattering properties of the tissue depend on the anatomical location.(244) She noted an increased accuracy gained by studies in spectroscopy literature when separating by anatomical subsite. This was especially noted for keratinised vs non-keratinised mucosa.

An advantage of these studies has been their prospective nature. In these studies, spectroscopic examination has been succeeded by biopsy of the same area measured.(239,240,276,282,288) Schwarz et al examined 124 people with oral cancer, OPMD and normal with biopsy succeeding DRS and fluorescence measurements. While this study noted a paucity of measurements of OPMD from keratinised sites to form training and validation sets, a lower accuracy of (AUC [area under the curve]: 0.76), sensitivity was 79% and specificity was 80%(282) was noted for keratinised tissue compared to non-keratinised tissue. For non-keratinised

tissue, AUC was 0.96 for training set and 0.93 for validation set, with sensitivity of 100% and specificity of 73% in the validation set. In this study, diagnoses were divided into quite empirical binary categories - abnormal/low risk (diagnoses including OLP – mild dysplasia, with hyperplasia included in this category) and high risk (moderate dysplasia – SCC).

Our protocol gave poor results in distinguishing OLP and normal on the dorsal surface of the tongue. Our sample size here was universally OLP. Difficulty in diagnosing on the dorsum of the tongue has not been reported thus far in the DRS literature. Autofluorescence studies from the 2000s found poor results on the dorsum of the tongue.(226,276) Mallia et al. excluded lesions from the dorsal and lateral tongue and vermilion border in a 2008 fluorescence study.(289) The same author found good accuracy later for DRS in a small data set for dysplastic lesions and SCC on the dorsal tongue.(175) One possible explanation for our result is the keratinised layer on the dorsal tongue. Our primary collected lesion, OLP, has a clinical appearance that is often keratotic. Reticular OLP is the most common form of OLP and was the type most often included in this study. This may be difficult to distinguish on the keratotic, linear architecture of the dorsal surface of the tongue. Some studies in the DRS literature have omitted to examine the tongue.(245,288,290) More research has been focused on the buccal mucosa. This imbalance should be corrected by future workers to get a truer picture of the utility of DRS. Recruitment of participants with OLP on the dorsal tongue would help to establish if this is an inherent weakness in DRS. The oral tissues are well recognised to be heterogenous and to contain high levels of haemoglobin and keratin.(244) Their heterogeneity is due to the number of tissue types present in the mouth.

Some investigators have separated readings by tissue type – muscle, lymphoid and other.(228) We chose to separate our readings by anatomical area, as set out in the paper by Mallia(226) and this is supported by up to date evidence.(247) There is no consensus in the literature what is the most appropriate approach to take, although the majority of studies have used our or similar pattern. External validation of our approach is provided by good accuracy figures. Our spectral analyses show wide variations between different tissues, thus grouping by tissue type may be too simplistic. McGee's study of spectra from different regions of the mouth grouped only gingiva and hard palate as keratinised.(244) Our data from this study showed poor accuracy figures in the 60s when all tissues are grouped together without separating by anatomical area.

Our results corroborate findings elsewhere that there is less reflectance from oral leukoplakia.(237) Although no single histologic variable specifically distinguishes OPMD, nuclear atypia seems directly related to the amount of light scattering.(291) SCC is denoted by increased absorption in the haemoglobin region of the spectrum, as angiogenesis increases local haemoglobin content.(256) Conversely, the epithelial layer displays greater reflectance due to increased nuclear size, increased DNA content and hyperchromasia.(256) Further tissue changes during development of OPMD are increased water and changes seen in the 800-1000nm region due to growth of the stratum corneum.(283) This may correspond with visual changes such as hyperkeratosis.(283) Increase in hyperkeratosis will also cause decrease in reflectance signal, as the effective distance from the haemoglobin-rich part of the lesion will increase.(240)

One of the themes in the literature is resolving the analyses to be used in order to make a judgement on accuracy. Feature selection models such as least absolute shrinkage selection operator (LASSO) have been used to enhance classification in oral cancer, lung and breast cancer studies among others.(292) The aim of this strategy is to identify markers on the spectrum common to OPMD and common to normal. This was expected to be water, lipids, deoxy- and oxyhaemoglobin prior to data collection. Using accepted biomarkers helps in verification and establishes external validity of results.

Table 22. Biomarkers and their absorption peaks.(219)

Biomarker	Absorption peak (nm)
Water	755, 972
Deoxyhaemoglobin	400-500
Oxyhaemoglobin	300-600
Lipid	760, 930

Across the literature, the wavelengths used for analysis are mainly in the 545, 565, 570nm region.(175,226,239,240) These correspond to deoxy- and oxyhaemoglobin. Bailey et al. investigated 450-469nm with equivocal results.(237) In contrast, the wavelengths selected by LASSO for the dorsum of the tongue were not as expected. Results were improved when the whole spectrum was used, and differences between peaks analysed. In contrast, LASSO for the ventral tongue and buccal mucosa regions displayed improved predictive ability. The tool selected biomarkers consistent with literature. Use of the correct models is crucial in generating robust datasets. A combination of linear discriminant and other discriminant analyses has

been used successfully in multiple similar trials before.(226,235,293) Review of previous studies validated this approach as a method for use in spectroscopy.(294) Leave one out analysis has also been validated across the literature as a means of improving accuracy by reducing the effect of outliers. (235)

Balanced accuracy was used to denote final results. In this dataset the populations are imbalanced. There are far more normal, or not diseased readings in this dataset compared to those who have an OPMD. Balanced accuracy in this case is more applicable than accuracy it applies equal weight to the OPMD and normal groups. Therefore, to have good balanced accuracy the algorithm must not only predict correctly that a reading is normal but must correctly diagnose OPMD. Discriminant analysis aims to separate classifiers by a simple separation plane.

5.3 Barriers to implementation of DRS and protocol to clinical practice

The explicit use of protocol over guideline was felt to be appropriate here for two reasons. First, this DRS probe may be unfamiliar to end-users who may not have domain expertise to interpret guidance correctly. Second, a deterministic approach implies standardisation of technique and simplifies systematic comparison of research findings.

The clinician reviewers identified one of the major barriers to implementation of the protocol is the long warm-up time. It is not uncommon for this to take 30 minutes or more before it is ready to start. Time is of the essence for the general practitioner, and the protocol would not then be useful for a handful of patients. On the other hand, time taken for acquisitions was rapid, with about 8 minutes

being required to obtain data from all areas of the mouth. In clinical practice, it is likely that less time than this will be required to conduct an exam as the focus will be on one area. If there is a large number of patients who need analysis on a particular day, then the investment in time would be worthwhile. This is unlikely in a general practice setting. DRS also requires considerable investment in training and financially. The long lead in time is partially due to warm-up time but also takes in time needed for calibration and background light measurements, which are then subtracted from the readings. The exclusion of all background light was a second requirement identified by one clinician as also being difficult in a clinic or hospital not set up for DRS. Our research team also identified the exclusion of background light as a significant drawback of DRS. This had caused difficulties in acquisition of data. One of the main reasons for spectra being defective or insufficient was either water contamination, excessive pressure or the probe not touching the mucosa. Checking these conditions are not fulfilled is more difficult in a darkened room. Water has a strong signal and can change the acquired spectrum. Therefore, saliva exclusion from the area being read was universally recommended by literature search. Minimal pressure was used during measurements and the mucosa was relaxed for measurements. This was to ensure the blood volume fraction (bvf), or amount of blood present in the tissue is constant. It is similarly universally observed to apply minimal pressure. The effect of excessive pressure is to deform the mucosa and to reduce blood flow to the area being studied. This will reduce the haemoglobin present in tissue, thus changing the reflectance.(255,274,295). However, measuring the mucosa in a relaxed condition may be difficult contingent

on the patient's mouth opening and the area being examined, with more posterior areas being more difficult.

It is envisaged that these difficulties in implementation can be addressed by future research. In a local research unit, Grygoryev produced a small mini-spectrometer that self-corrects for background light. In future, this can be used to replace the current iteration, as it does not require a measurement of background reflectance step and removes the requirement for darkness.(296) This could potentially speed up the process and make it easier to take accurate readings. A team in the US produced a portable DRS probe for screening of cervical cancer in a low-income country (Haiti). The results demonstrated successfully that a built-in supplementary channel in the DRS probe can be used to correct for the effects of fluctuations in the DRS intensity due to light source drifts and fibre-bending attenuation of the incident beam. The clinical study in Haiti also indicated that without a pressure sensor, the probe pressure could vary up to 3-5 psi.(256) This team included a pressure sensor capable of feeding back to the observer when too much pressure was being applied, potentially changing the haemoglobin content of tissue. This however, crucially, still needed conditions of darkness for measurements to be accurate. Nevertheless, this study indicates that a self-calibrating DRS setup can be successful in point-of-care diagnostics.

Saliva contamination was straightforward to manage, however if the mucosa was better illuminated during readings, then repeated leakage of saliva into the field would be easily detected.

Chang et al. used the self-calibrating system in a screening project for cervical cancer in Haiti.(258) Cervical cancer screening and oral cancer screening share some common features. First, the type and visibility of the precancerous lesions encountered in the cervix share a common history and grading system.(116) Second, some of the screening tools used in the mouth are applicable in the cervix.(185) Because of the accessibility of the cervix for screening and the tendency for late-stage presentations of cervical cancer, screening has been widely used in the field. One of the main drivers of cervical cancer is HPV infection.(116) Prior to collection of clinical data, Chang's team identified high-risk individuals. An initial smear test identified HPV, with DRS collected at colposcopically abnormal sites. The use of the self-calibrating channel eliminated the need for a calibration step, speeding up setup, while extraction of sample optical properties from a homogenous disc of uniform composition was more accurate using self-calibrating methods. The conclusion noted that calibration is necessary periodically in order to check the efficacy of the self-calibrating system, especially in the case of an untrained operator.(258)

If the lack of OPMD encountered in primary care may make the DRS protocol superfluous, OPMD are much more likely to be encountered in secondary care. The most referred oral medicine condition to our hospital is white lesions in females on the tongue in the age bracket 40-70.(297) This infers that the ability to identify correctly and quickly OLP and leukoplakia would be of benefit to secondary care clinicians. A large throughput of patients needing analysis would be needed to justify the cost and time sink of adapting to a new technology. An aim of the overall project of which this thesis is a part, is to investigate the potential role for multi-

modality spectroscopy in the detection of oral cancer as part of a national screening programme. National screening programmes for oral cancer have been successful, and it has been shown that screening downstages cancers at diagnosis and results in better health outcomes for some targeted groups.(136,298) A UK cost-benefit analysis from the early 2000s deemed a national screening programme for oral cancer not cost-effective.(130) However, this issue is ripe for re-exploration, with newer technologies available for screening,(146) a rise in cancers with different aetiologies,(299) renewed focus on prevention and screening,(300) and a fall in access to dental practitioners across both Ireland and UK.(301,302)

This preliminary study has shown this DRS protocol could be effective for a large-scale screening project, where a large cohort requiring triage of suspicious lesions is recruited. Biopsy can be carried out on high-risk lesions. At present, evidence for the protocol to diagnose high-risk dysplastic lesions and SCC is lacking. Further research is needed to identify biomarkers and levels typical of oral cancer, dysplasia and other OPMD. Technical work should meanwhile continue to mitigate technical difficulties we have encountered in this project. Amelink et al set out to provide a use for DRS in screening. This 2011 paper attempted to distinguish between dysplastic and non-dysplastic leukoplakia in a screening setting. This was aimed at finding a utility for DRS in risk stratification of a lesion of unknown malignant potential.(240)

Validity of our protocol recommendations was ensured by several methods. The readability of the protocol was scored by the Flesch-Kincaid Grade Level and the Flesch Reading Ease score. This was scored as a 10^{th} grade level, roughly equivalent

to a 15 or 16 year old's reading level. An evaluation of patient information leaflets gave an average reading difficulty of a 6th grade level.(281) It is reasonable that a technical bulletin should be higher.(303) The Flesch Reading Ease was 36.9, out of a maximum of 100. Over 60 is considered a good score. In order to improve the readability of the guidance, we removed longer words or rephrased them. Many sentences were also shortened in order to improve the score. However, this reached a certain limit where it became impossible to remove words or condense the document. Thus the document is as clearly written as it can be for a technical audience. Readability research has typically focused on consent forms and patient information leaflets. No clear guidance exists regarding readability of clinical guidelines in practice.

The protocol was easily understood by the clinician group. This provides external validation of readability. The steps were easy to follow, not numerous and easy to remember. This is important in a setting where there may be multiple demands on a clinician's time.

The six clinicians felt after reading the user protocol unanimously that it is inapplicable to clinical practice. This finding is a major barrier to implementation of DRS. The six clinicians interviewed here held a range of positions, all in secondary care and all with private practice careers also. All were experienced, with five out of six having more than five years' experience of managing OPMD. Thus, this cross-section is suited to judging clinical utility of the protocol.

In formulating the protocol, a paucity of published literature on DRS for detection of OPMD and OSCC was found. A literature search of Pubmed, SPIE and Embase

databases using the search terms “diffuse reflectance” OR “reflectance spectroscopy” AND “oral” OR “mouth” OR “oral cavity” AND “oral potentially malignant disorder” OR “OPMD” OR “squamous cell carcinoma” OR “SCC” OR “neoplasia” OR “cancer” OR “oral potentially malignant lesion” OR “OPML” OR “precancer” yielded only 10 clinical trials of DRS for this purpose. Only Kolpakov and de Veld et al included a significant number of OPMD other than leukoplakia and SCC.(217,283) De Veld, Amelink, Jayanthi, Mallia recorded the ages of participants, while only de Veld and Amelink supplied the gender of participants.(175,217,240,288) All studies were performed in secondary or tertiary institutions on referral populations. This draws the transferability of DRS research findings into question. Jayanthi 2011 focused solely on the buccal mucosa, and a similar study by the same author excluded any results from the tongue.(288,290) This may artificially inflate accuracy figures. No protocols were found in the literature.(240,276,290) The risk of imprecision was rated as high for all studies.

5.4 Reproducibility and repeatability

In this segment, we examined inter-operator variance (reproducibility) and intra-operator variance (repeatability). Both values were calculated on the same day. Reproducibility was estimated based on five measurements for each area of the mouth. Each was taken as a set by a different examiner one after the other from the same area, repeated for each area of the mouth. The spectra were then normalised to a mean, and t-test was used to examine for areas of the spectrum where variance was noted to be statistically significant ($p<0.05$). Wide values for reproducibility were noted on all tissues. The most common site for significant

inter-operator variability was the 400—600nm region, where haemoglobin and deoxyhaemoglobin display most absorbance. Thus, it is most likely that the observed variance was due to excess pressure being applied. If excess pressure is applied to the mucosa, this will collapse the microvasculature, thus causing measured haemoglobin levels to fall.(256) This can then give a misleading picture of the composition of the tissue. Haemoglobin levels are the primary method of differentiating malignant and normal tissue with DRS.(240)

In the floor of mouth, variance was noted was noted in the 600-1000nm region. Water has a strong signature in this region.(216) An explanation for this result would be saliva contamination, as the salivary ducts of Wharton and Bartholin are located in the floor of the mouth and it creates a natural reservoir, bounded by the mandibular alveolus and the base of the tongue. The lip was noted to have good reproducibility and repeatability.

From the literature, variance between different users is an unsuspected source of error. Few studies have examined repeatability and reproducibility, with fewer still looking at these values in oral tissue. Studies have used one operator, not stated the number of operators used or, if variance was addressed, tried to minimize this by a software preprocessing step.(290) Amelink et al used 3 – 5 measurements per site to reduce the effect of tissue heterogeneity.(239) Brooks found little variance between users in a pre-clinical study on human skin, and concluded that inter-operator variance is low in the mouth.(274) However, the dexterity required to use a probe in the mouth is more difficult than on skin. The mouth also presents unique challenges of visual access and variation in mucosa. Many groups have investigated

the effect of pressure on human tissue. Rylander found that contact pressure displaced interstitial water and blood underneath the contact region, induced zones of dehydration, reduced tissue thickness, and modified optical properties.(304) The change in tissue properties caused by excess pressure is dependent on the thickness of the tissue, the location (for example thin tissue overlying prominent bony outcrop), and may be affected by factors such as age, BMI, vascularity and lifestyle habits.(305). Li et al stated that the optical properties of the human earlobe are not affected with pressure less than 25kPa.(295) This conflicts with Yu's finding that 1 psi (roughly 4 times smaller) is the maximum pressure that can be applied without compromising spectral acquisition from buccal mucosa.(256) This shows that the oral mucosa and skin may have different requirements in respect of applied pressure. With that said, the oral mucosa is heterogenous and may display increased stiffness, while more subjective pressure may be applied to stiffer mucosa.(305) More research is now required to test this hypothesis that increased inter-operator pressure is a source of variance. There is no convincing evidence in the literature that inter-examiner variance for DRS is low. This has implications for its potential widespread use.

In our study, the area between the vermilion border and the wet-dry line gave the best results for reproducibility and repeatability. The most likely reason is that the lip has good visual and spatial access with little heterogeneity, compared with the floor of mouth or others, where our visual access is limited by darkness in the room.

Repeatability was good, with differences noted mainly on gingival tissue. Changes in the spectral shape and wide standard deviations were observed in gingival tissue.

This is most likely mainly due to tissue heterogeneity. Gingival tissue contains dentine, cementum, bone, attached and unattached mucosa, which can introduce variation. Lip repeatability was noted to be good. Buccal mucosa also was repeatable. Some differences noted in the haemoglobin region in the buccal mucosa may be due to pressure, tissue variation or saliva in the area. This area should not suffer from saliva contamination, so the most likely explanation is tissue heterogeneity or pressure. Bregar et al. found that intra-operator probe pressures are much more consistent than inter-operator deviations.(305) This is logical, as the idea of light pressure may be specific to one person.

The dorsal surface of the tongue showed poor repeatability in the haemoglobin region. This may be due to posturing of the tongue for analysis. This can be sometimes unavoidable, or it may be difficult for patients to consciously control their tongue. The floor of mouth, similarly to reproducibility tests, showed variance at longer wavelengths (600-1100nm). This is most likely due to saliva contamination of the area. It is much more difficult to confirm lack of saliva in the area when the room is in darkness. Nieman in 2008 examined repeatability and similarly noted little variance, however she remarked on the role that pressure can potentially exert on results, and called for further research in this area.(235)

5.5 Study design

5.5.1 Overview

This was a controlled, open-label clinical study. A clinical trial evaluates a CE-marked device for predetermined clinical outcomes e.g. success or failure or magnitude of effect. This study has elements of a technical report investigating a

new technology, however the outcome measures are not preset, e.g. magnitude of effect or success/failure.

In an open-label study, all participants receive the same intervention. This helps establish internal and external validity, as set out by Campbell et al.(306)

5.5.2 Initial pilot stage

Many other studies in the optical spectroscopy domain used an ex vivo stage to establish the efficacy of their probe.(224,229,239) In our case, we have already used this DRS probe in a study of bowel cancer specimens. This established a baseline set of optical parameters.

An initial pilot stage aimed at acquiring optical parameters bespoke to oral tissue. This also allowed for familiarisation of the research team with the new equipment, as consistent with good practice in trials of new techniques set out by Burke et al.(307). The optical parameters we used are robust and may be used by other studies. This reinforces external validity and demonstrates scientific rigour.

5.5.3 Control group

The selection of a control group is a critical decision in study design. We used a controlled parallel-group study design. This is consistent with more recent studies in the literature.(226,235,264)

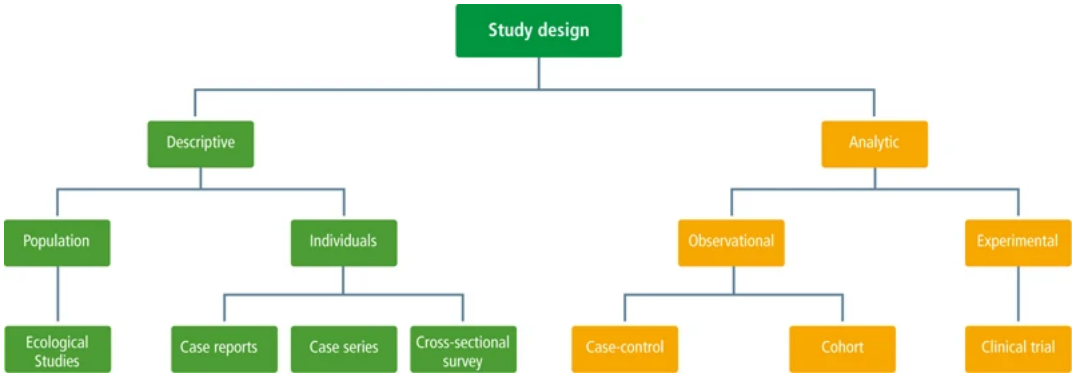


Figure 43.Types of clinical study.(308)

Brocklehurst and Hoare state the randomised controlled clinical trial is "*the workhorse of evidence-based healthcare and the only research design that can demonstrate causality"*.(308) Randomisation was not possible in this trial as the diagnostic accuracy of the device in question has not yet been attested. In order to achieve clinical relevance for widespread implementation of DRS, a (or multiple) well-designed RCT must be carried out comparing with gold standard. Split mouth design studies, where the other side is expected to act as the control, have limitations in this field. First, the healthy must be assumed to be indistinguishable from the general population. The field cancerisation effect has been recognised as applicable to oral spectroscopy, insofar as subjects with upper aerodigestive tract malignancy have been found to have spectroscopic changes in the oral mucosa.(125) Therefore, it is not safe to assume that one side will act as the other's control.

However, controlled clinical studies are not without their drawbacks. The first is selection bias, where only those who are the most affected by a disease are included. Others include blinding and attrition analysis. In this case, we controlled for these drawbacks by conducting an open-label trial design, while attrition was

minimised by sampling at one end point. Selection bias was diminished by recruiting similar demographics for the study and control group while recruiting as widely and as much as possible, introducing more variation into the matrix.

Thus, a multiphase controlled clinical study was the most appropriate design for this study.

5.5.4 Population

There was a female preponderance (68%) in our study group, and this imbalance was managed by stratifying for gender from the outset. This was in keeping with the overall preponderance of oral lichen planus patients. Oral lichen planus is predominantly to be found among females. Some of the highest incidence rates of OLP worldwide are to be found in Europe, and this disease has been shown to be underdiagnosed due to its latent asymptomatic nature.(32)

The participants were almost age-balanced, with a wider standard deviation in the control group compared with the study group. Fluorescence studies have shown a change in skin fluorescence with age, however the effect of this small discrepancy is unknown.(309)

Factors such as skin glucose content, hypertension and BMI have been known to affect spectroscopy.(216) Increased tissue perfusion with changes in capillary microstructures due to physiological changes in essential hypertension can lead to variation.(310,311) The BMI of the participants was similar, while the levels of concurrent co-morbidity were quantitatively similar, however uneven in terms of disease burden. Analysis is under way to determine the significance of these factors on results. The effect of this is so far unknown.

5.5.5 Inclusion/exclusion criteria

These were deliberately kept broad in order to include as many potential participants as possible. Those with a current or previous diagnosis of upper aerodigestive tract SCC were excluded due to the field cancerisation effect.(125) The final utility for this project is envisaged to cover a wide variety of presentations.

Our trial had adaptive features, insofar as the sample size was adjusted based on the results of interim analyses. This has scientific validity and can help to bring a quicker result while maintaining scientific rigour. However, this should be carefully planned in advance, and endpoints should be agreed with the involvement of statisticians.(312) This was instigated with the help of data scientists.

5.6 Study strengths

5.6.1 Study population

Our sample size is one of the largest in the literature for DRS in the oral cavity. This makes the results more robust and adds to a data bank that can be useful in further research. The population recruited is majority oral lichen planus. This sets our study apart in the literature, as only one other study has investigated the spectroscopic appearance of OLP in the mouth.(283) We have found excellent results in distinguishing OPMD, mainly OLP, on the buccal mucosa and ventral tongue. The finding that a DRS probe can distinguish normal and oral lichen planus on the buccal mucosa and ventral tongue has not been reported thus far in the literature, and thus is a major finding for this study. The large sample size also allows us to note trends in the data, which can then be used for future research projects.

Stratification in age and gender was also achieved. This helps ensure transferability of results.

5.6.2 Study design

We employed a multi-phase design with initial feasibility studies aimed at finding the clinical difficulties with DRS. This directly informed trial design, enabling us to find pragmatic methods of obtaining spectroscopic data on a large scale, and defining clinical parameters and outcomes. This ensures scientific rigour and helps to control for errors in experimental protocol and allows for familiarisation of the team with the equipment and protocol. This also allows for experimentation with design and removal of non-functional or not useful components of the study. An example is the DR imaging microcamera. This was trialled in an effort to delineate OPMD borders and find unsuspected areas but proved to be flawed.

We reported our method and how we arrived at our protocol rigorously and used multiple phases to ensure validity of the chosen experimental technique. One of the main criteria for judging the strength of a study is the quality of its reporting of method.

5.6.3 Multiple observers

The use of reproducibility and repeatability statistics helps to establish the validity of findings and opens up discussion in the research field. The finding that there is lack of reproducibility of DRS findings between different observers will inform the direction of future studies as well as the continuation of this project. Examination of the inter-operator differences can also inform continuing development of the protocol and is thus an essential step. File metadata showing time for acquisition in

a new examiner vs experienced examiner was also similar. This shows effectiveness of training and applicability of the clinical protocol.

5.6.4 Multiple opinions

We obtained opinions on our putative protocol from several experienced clinicians not involved with the project, from researchers and also from participants. Having a wide spectrum of views increases the transferability of the protocol. New technologies may fail to come to market due to lack of effective communication between developers of a product and end-users. Market research can help establish a gap in the market, while identifying gaps or failures in a current workflow may establish the space for a new innovation.(313)

5.7 Study limitations

5.7.1 Comparison with gold standard

We used one pathologist's opinion on the histopathology to guide our diagnosis. This was taken as our gold standard. The histopathology report was taken before we had conducted spectroscopy in the area. This may introduce uncertainty if the diagnosis was doubtful or if there had been changes in the interim. The use of a prospective trial design with multiple examiners in a prospective trial would help eliminate detection bias.

5.7.2 Point-wise nature of comparisons

A biopsy taken from one point may not adequately reflect the underlying physiology of a larger area. This is in keeping with what we now know as a more widespread, non-linear model of carcinogenesis. As an OPMD may affect a broad area of mucosa, this may introduce differences between the index test (DRS) and

the reference standard (biopsy and histopathology). The acquisition of multiple spectra with a calculated mean acting as the true value was intended to alleviate this shortcoming. It was not possible to identify the site of the biopsy. Evers et al. found minimal differences in excised biopsy specimens when examined with DRS. However, oral tissue is more heterogenous than breast.(260)

An early approach we took to identifying physiological differences was using diffuse optical imaging to detect haemoglobin variations in the tissue. Multi-modality spectroscopy works by adding complementary data to guide and correct the findings from one technique. This approach has potential, however in our case the imaging probe was not useful. Prospectively designed clinical trials with biopsy after DRS data acquisition would reduce the potential for this error. However, such trials are time-consuming and expensive.

5.7.3 Probe design

Our spectrometer contains a broadband light source with a narrow diffraction grating. Spatially offset detector fibres permit detection from deeper layers of the tissue (see Fig. 40 below). Our setup was a single-fibre light source, with an integrated detector array, without a lens and a small source-to-detector distance. Other, newer studies have used multi-fibre designs with multiple fibres in a detector bundle. This offers the advantage of receiving photons which have travelled in 360 degrees. Our DRS probe design can only receive photons which have travelled in the linear plane from the source to the detector.

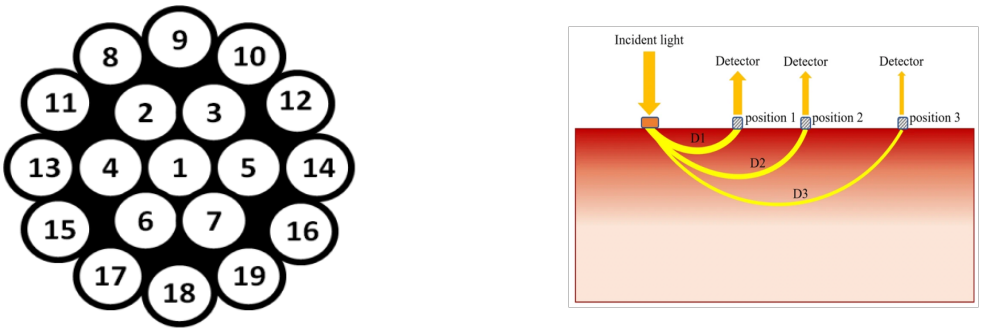


Figure 44. Multi fibre probe design capable of receiving photons in 360 degrees compared with linear array (right)

Studies in breast cancer DRS have shown that accuracy of the probe diminishes as the percentage of tumour cells in a particular slice of tissue decreases.(314) This makes a multiplanar accumulation of data in all directions, with consequent greater tissue volume acquisition most important for accuracy. Veluponnar found more accurate classification for tumour vs healthy in a DRS probe with three or more fibres compared with a single or two-fibre spectroscopy setup.(314) This study identified correctly three involved tumour margins using three or more fibres that was not identified by surgeon at time of operation.

We chose a design that involves placement of the probe at 90 degrees to the epithelium. This was to provide ergonomic comfort and to reduce the effect of pressure from a researcher trying to feel if an angled probe might be in contact with mucosa.

5.7.4 Unsuspected sources of variation

Inter-patient variation can make the measurements appear dissimilar. De Boer in her review of breast cancer DRS noted that inter-patient variation can play a role in

measurement variability, however no data has as yet been published in the mouth.(315)

If intra-operator and inter-operator differences are similar, then it is safe to assume that the difference is due to tissue heterogeneity.(274)

We found little difference in intra-operator readings, but large differences between operators. This finding needs further investigation, as it has not been reported thus far in the literature. We have a small sample size of three individuals. However the effect was noted for all sites and all spectra, so we feel the observed effect is genuine. One Russian study showed unsuspected variation between spectrometer readings by comparing readings of a homogenous medium with two spectrometers.(283) The readings of the same medium were significantly different for two different spectrometers.

5.7.5 Difficulties in translational research

Despite technological advancements and promising results, fibre-optic spectroscopy has not yet found clinical acceptance in practice. This gap between original research and final clinical implementation is not specific for these technologies. It is considered to be one of the key aspects of translational research in general, as it can take more than two decades before the findings of original research become part of routine clinical practice.[12, 13] However, awareness of the causes of the gap and potential strategies could help the field of DRS avoiding unnecessary delay in the evolution of this technology into clinical useful tools.

5.8 Suggestions for future research

We have conducted a robust literature review and delineated a set of evidence-based instructions for use of a DRS probe in the mouth. This protocol has been tested thoroughly with a large sample size and shown to be useful in the detection of OPMD in certain areas of the mouth. This protocol can be used as a template for researchers to carry out DRS in the mouth in the future.

Clinical research in general is hampered by lack of detailed protocols and poor documentation of results. Poor study design leads to waste of the work that has been put in by researchers and participants due to poor study design, flaws in reporting or incorrect method. For example, a recent Cochrane review excluded 46 studies on the basis of poor reporting or poor method and included only 23.(4,316)

As per the IDEAL framework for the development of a new surgical device, clinical trials demonstrating safety need to be followed up with multicentre studies, then with randomised controlled trials to determine superiority/inferiority.

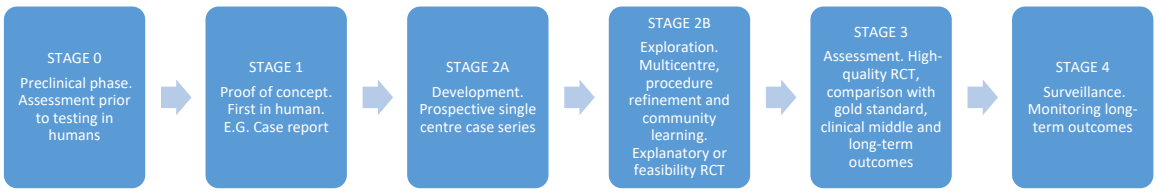


Figure 45.IDEAL framework. (322]

Further studies will include more participants with more diverse population of disease and with multiple users at multiple centres. While it is unlikely that data will be produced in the short-term to justify an RCT versus the current gold standard,

replication of positive findings of high sensitivity and specificity with good acceptability to patients will help to justify a role as a biopsy adjunct.

6 Conclusions

The study population is skewed towards oral lichen planus in its distribution, with 80% of its population OLP. The protocol shows over 87% accuracy in distinguishing between normal and our population of OPMD in the ventral tongue and buccal mucosa. Therefore it has high accuracy in detecting OLP in the ventral tongue and buccal mucosa. This is contrasted with the dorsal tongue, where accuracy does not exceed 70%. The use of this protocol to detect oral lichen planus may not be recommended on the dorsal surface of the tongue.

Fair reproducibility was noted, with good repeatability. This requires further investigation but may be due to applied pressure. This effect was noted across all tissues in a small sample size. There is a lack of similar studies comparing DRS measurements between observers. This observation calls into question the applicability of DRS across clinical settings and should be investigated further. The use of a unified protocol, if it is accepted generally, may help to standardise method and to systematically analyse any evidence produced.

The time taken to set up and warm up is a barrier to clinical implementation and to adoption generally among clinicians. The necessity for readings to be taken in darkness is also a barrier to implementation. Given these results, it would seem sensible to further develop the use of self-calibrating probes. These have the potential to offer more accurate DRS without darkness obscuring vision and with an inbuilt pressure sensor.

The overall aim to assess the utility of multimodality spectroscopy for a large-scale screening programme for oral cancer has been partially fulfilled. This is a preliminary study with a large sample size which has found a role for DRS in identifying OLP on the buccal mucosa and ventral tongue. This result shows promise, however further research is needed in diagnosis of other OPMD and oral cancer in the mouth. The probe is quick to take acquisitions, acceptable to patients and with excellent accuracy. It is best used in a specialist setting, where there are many patients to be tested.

1. The protocol has been formulated successfully
2. Our method of DRS can reliably distinguish between OLP and normal in the buccal mucosa and ventral tongue
3. Inter-observer variability may be high and needs further investigation
4. An application is identified for its use in a large-scale screening project, if further research can demonstrate acceptable sensitivity and specificity in distinguishing between other OPMD and oral cancer
5. Clinicians are sceptical about the protocol. The setup time and necessity for darkness while in use are major deterrents.

7 References

1. Field EA, McCarthy CE, Ho MW, Rajlawat BP, Holt D, Rogers SN, et al. The management of oral epithelial dysplasia: The Liverpool algorithm. Oral Oncol. 2015 Oct 1;51(10):883–7.

2. Sunny SP, Agarwal S, James BL, Heidari E, Muralidharan A, Yadav V, et al. Intra-operative point-of-procedure delineation of oral cancer margins using optical coherence tomography. Oral Oncol. 2019 May 1;92:12–9.

3. Mori K, Hamada T, Beppu M, Tsuchihashi H, Goto Y, Kume K, et al. Detecting Early-Stage Oral Cancer from Clinically Diagnosed Oral Potentially Malignant Disorders by DNA Methylation Profile. Cancers. 2022 May 26;14(11):2646.

4. Walsh T, Macey R, Kerr AR, Lingen MW, Ogden GR, Warnakulasuriya S. Diagnostic tests for oral cancer and potentially malignant disorders in patients presenting with clinically evident lesions. Cochrane Database Syst Rev. 2021 Jul 20;2021(7):CD010276.

5. Kujan O, Oliver RJ, Khattab A, Roberts SA, Thakker N, Sloan P. Evaluation of a new binary system of grading oral epithelial dysplasia for prediction of malignant transformation. Oral Oncol. 2006 Nov 1;42(10):987–93.

6. National Cancer Research Institute. Cancer in Ireland 1994-2019. Annual Report of the Cancer Registry [Internet]. Cork, Ireland; [cited 2022 Feb 23]. Available from: https://www.ncri.ie/publications/statistical-reports/cancer-ireland-1994-2019-annual-report-national-cancer-registry

7. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021 May;71(3):209–49.

8. National Cancer Research Institute. Cancer Incidence Projections for Ireland 2020 – 2045 [Internet]. Cork, Ireland; 2022 Feb. Available from: https://www.ncri.ie/sites/ncri/files/pubs/CancerIncidenceProjections_NCRI_fullreport_09042019_final.pdf

9. Brocklehurst P, Kujan O, Glenny AM, Oliver R, Sloan P, Ogden G, et al. Screening programmes for the early detection and prevention of oral cancer. Cochrane Database Syst Rev. 2010 Nov 10;(11):CD004150.

10. Day GL, Blot WJ. Second primary tumors in patients with oral cancer. Cancer. 1992;70(1):14–9.

11. SEER [Internet]. [cited 2022 Apr 20]. Cancer of the Oral Cavity and Pharynx - Cancer Stat Facts. Available from: https://seer.cancer.gov/statfacts/html/oralcav.html

12. Warnakulasuriya S. Oral potentially malignant disorders: A comprehensive review on clinical aspects and management. Oral Oncol. 2020 Mar;102:104550.

13. Speight PM, Khurram SA, Kujan O. Oral potentially malignant disorders: risk of progression to malignancy. Oral Surg Oral Med Oral Pathol Oral Radiol. 2018 Jun;125(6):612–27.

14. Silverman S. Oral cancer. Semin Dermatol. 1994 Jun;13(2):132–7.

15. Holmes JD, Dierks EJ, Homer LD, Potter BE. Is detection of oral and oropharyngeal squamous cancer by a dental health care provider associated with a lower stage at diagnosis? J Oral Maxillofac Surg. 2003 Mar;61(3):285–91.

16. Epstein JB, Robertson M, Emerton S, Phillips N, Stevenson-Moore P. Quality of life and oral function in patients treated with radiation therapy for head and neck cancer. Head Neck. 2001 May;23(5):389–98.

17. Mcleod N, Saeed N, Ali E. Oral Cancer: Delays in Referral and Diagnosis Persist. Br Dent J. 2005 Jul 1;198:681–4.

18. Onizawa K, Nishihara K, Yamagata K, Yusa H, Yanagawa T, Yoshida H. Factors associated with diagnostic delay of oral squamous cell carcinoma. Oral Oncol. 2003 Dec 1;39(8):781–8.

19. Lippman SM, Hong WK. Second malignant tumors in head and neck squamous cell carcinoma: The overshadowing threat for patients with early-stage disease. Int J Radiat Oncol. 1989 Sep 1;17(3):691–4.

20. Swanton C, Bernard E, Abbosh C, André F, Auwerx J, Balmain A, et al. Embracing cancer complexity: Hallmarks of systemic disease. Cell. 2024 Mar 28;187(7):1589–616.

21. Lingen MW, Kalmar JR, Karrison T, Speight PM. Critical Evaluation of Diagnostic Aids for the Detection of Oral Cancer. Oral Oncol. 2008 Jan;44(1):10–22.

22. Warnakulasuriya S. Clinical features and presentation of oral potentially malignant disorders. Oral Surg Oral Med Oral Pathol Oral Radiol. 2018 Jun;125(6):582–90.

23. van der Waal I. Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management. Oral Oncol. 2009 May;45(4–5):317–23.

24. Shimkin MB. Thirteen questions: some historical outlines for cancer research. J Natl Cancer Inst. 1957 Aug;19(2):295–328.

25. Behl I, Calado G, Vishwakarma A, Flint S, Galvin S, Healy CM, et al. Raman microspectroscopic study for the detection of oral field cancerisation using brush biopsy samples. J Biophotonics. 2020 Oct;13(10):e202000131.

26. Warnakulasuriya S, Kujan O, Aguirre-Urizar JM, Bagan JV, González-Moles MÁ, Kerr AR, et al. Oral potentially malignant disorders: A consensus report from an international seminar on nomenclature and classification, convened by the WHO Collaborating Centre for Oral Cancer. Oral Dis. 2021;27(8):1862–80.

27. Warnakulasuriya S, Johnson NW, van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med. 2007 Nov;36(10):575–80.

28. George A, Sreenivasan BS, Sunil S, Varghese SS, Thomas J, Gopakumar D, et al. Potentially malignant disorders of oral cavity. Oral Maxillofac Pathol J. 2011;2(1):95–100.

29. Sarode SC, Sarode GS, Tupkari JV. Oral potentially malignant disorders: A proposal for terminology and definition with review of literature. J Oral Maxillofac Pathol JOMFP. 2014 Sep;18(Suppl 1):S77–80.

30. Kumari P, Debta P, Dixit A. Oral Potentially Malignant Disorders: Etiology, Pathogenesis, and Transformation Into Oral Cancer. Front Pharmacol. 2022 Apr 20;13:825266.

31. Napier SS, Speight PM. Natural history of potentially malignant oral lesions and conditions: an overview of the literature. J Oral Pathol Med. 2008;37(1):1–10.

32. Mello FW, Miguel AFP, Dutra KL, Porporatti AL, Warnakulasuriya S, Guerra ENS, et al. Prevalence of oral potentially malignant disorders: A systematic review and meta-analysis. J Oral Pathol Med. 2018;47(7):633–40.

33. Mehta FS, Pindborg JJ, Gupta PC, Daftary DK. Epidemiologic and histologic study of oral cancer and leukoplakia among 50,915 villagers in India. Cancer. 1969 Oct;24(4):832–49.

34. Petti S. Pooled estimate of world leukoplakia prevalence: a systematic review. Oral Oncol. 2003 Dec;39(8):770–80.

35. Silverman S, Gorsky M, Lozada F. Oral leukoplakia and malignant transformation. A follow-up study of 257 patients. Cancer. 1984 Feb 1;53(3):563–8.

36. Roed-Petersen B. Effect on oral leukoplakia of reducing or ceasing tobacco smoking. Acta Derm Venereol. 1982;62(2):164–7.

37. Villa A, Menon R, Kerr A, De Abreu Alves F, Guollo A, Ojeda D, et al. Proliferative leukoplakia: Proposed new clinical diagnostic criteria. Oral Dis. 2018;24(5):749–60.

38. Ramos-García P, González-Moles MÁ, Mello FW, Bagan JV, Warnakulasuriya S. Malignant transformation of oral proliferative verrucous leukoplakia: A systematic review and meta-analysis. Oral Dis. 2021;27(8):1896–907.

39. Abadie WM, Partington EJ, Fowler CB, Schmalbach CE. Optimal Management of Proliferative Verrucous Leukoplakia: A Systematic Review of the Literature. Otolaryngol Head Neck Surg. 2015 Oct;153(4):504–11.

40. Cerero-Lapiedra R, Baladé-Martínez D, Moreno-López LA, Esparza-Gómez G, Bagán JV. Proliferative verrucous leukoplakia: a proposal for diagnostic criteria. Med Oral Patol Oral Cirugia Bucal. 2010 Nov 1;15(6):e839-845.

41. Fitzpatrick SG, Hirsch SA, Gordon SC. The malignant transformation of oral lichen planus and oral lichenoid lesions: a systematic review. J Am Dent Assoc 1939. 2014 Jan;145(1):45–56.

42. González-Moles MÁ, Ruiz-Ávila I, González-Ruiz L, Ayén Á, Gil-Montoya JA, Ramos-García P. Malignant transformation risk of oral lichen planus: A systematic review and comprehensive meta-analysis. Oral Oncol. 2019 Sep;96:121–30.

43. Idrees M, Kujan O, Shearston K, Farah CS. Oral lichen planus has a very low malignant transformation rate: A systematic review and meta-analysis using strict diagnostic and inclusion criteria. J Oral Pathol Med. 2021 Mar;50(3):287–98.

44. Aghbari SMH, Abushouk AI, Attia A, Elmaraezy A, Menshawy A, Ahmed MS, et al. Malignant transformation of oral lichen planus and oral lichenoid lesions: A meta-analysis of 20095 patient data. Oral Oncol. 2017 May;68:92–102.

45. Lorenzo-Pouso AI, Lafuente-Ibáñez de Mendoza I, Pérez-Sayáns M, Pérez-Jardón A, Chamorro-Petronacci CM, Blanco-Carrión A, et al. Critical update, systematic review, and meta-analysis of oral erythroplakia as an oral potentially malignant disorder. J Oral Pathol Med. 2022 Aug;51(7):585–93.

46. Bouquot JE, Ephros H. Erythroplakia: the dangerous red mucosa. Pract Periodontics Aesthetic Dent PPAD. 1995 Aug;7(6):59–67; quiz 68.

47. Al-Hashimi I, Schifter M, Lockhart PB, Wray D, Brennan M, Migliorati CA, et al. Oral lichen planus and oral lichenoid lesions: diagnostic and therapeutic considerations. Oral Surg Oral Med Oral Pathol Oral Radiol Endodontology. 2007 Mar 1;103:S25.e1-S25.e12.

48. de Santana Sarmento DJ, da Costa Miguel MC, Queiroz LMG, Godoy GP, da Silveira ÉJD. Actinic cheilitis: clinicopathologic profile and association with degree of dysplasia. Int J Dermatol. 2014;53(4):466–72.

49. Fernandez Figueras M t. From actinic keratosis to squamous cell carcinoma: pathophysiology revisited. J Eur Acad Dermatol Venereol. 2017;31(S2):5–7.

50. Dancyger A, Heard V, Huang B, Suley C, Tang D, Ariyawardana A. Malignant transformation of actinic cheilitis: A systematic review of observational studies. J Investig Clin Dent. 2018;9(4):e12343.

51. Smith LW, Bhargava K, Mani NJ, Malaowalla AM, Silverman S. Oral cancer and precancerous lesions in 57,518 industrial workers of Gujarat, India. Indian J Cancer. 1975 Jun;12(2):118–23.

52. Axéll T. Occurrence of leukoplakia and some other oral white lesions among 20,333 adult Swedish people. Community Dent Oral Epidemiol. 1987 Feb;15(1):46–51.

53. Baric JM, Alman JE, Feldman RS, Chauncey HH. Influence of cigarette, pipe, and cigar smoking, removable partial dentures, and age on oral leukoplakia. Oral Surg Oral Med Oral Pathol. 1982 Oct;54(4):424–9.

54. Hashibe M, Brennan P, Chuang SC, Boccia S, Castellsague X, Chen C, et al. Interaction between tobacco and alcohol use and the risk of head and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. Cancer Epidemiol Biomark Prev. 2009 Feb;18(2):541–50.

55. Herreros-Pomares A, Llorens C, Soriano B, Zhang F, Gallach S, Bagan L, et al. Oral microbiome in Proliferative Verrucous Leukoplakia exhibits loss of diversity and enrichment of pathogens. Oral Oncol. 2021 Sep 1;120:105404.

56. Mohammed F, Fairozekhan AT. Oral Leukoplakia. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 [cited 2022 Jul 3]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK442013/

57. Ikeda N, Ishii T, Iida S, Kawai T. Epidemiological study of oral leukoplakia based on mass screening for oral mucosal diseases in a selected Japanese population. Community Dent Oral Epidemiol. 1991;19(3):160–3.

58. Mehta FS, Shroff BC, Gupta PC, Daftary DK. Oral leukoplakia in relation to tobacco habits. A ten-year follow-up study of Bombay policemen. Oral Surg Oral Med Oral Pathol. 1972 Sep;34(3):426–33.

59. Einhorn J, Wersall J. Incidence of oral carcinoma in patients with leukoplakia of the oral mucosa. Cancer. 1967 Dec;20(12):2189–93.

60. Chaturvedi AK, Udaltsova N, Engels EA, Katzel JA, Yanik EL, Katki HA, et al. Oral Leukoplakia and Risk of Progression to Oral Cancer: A Population-Based Cohort Study. J Natl Cancer Inst. 2019 Dec 20;112(10):1047–54.

61. Scandinavian Division of the International Association for Dental Research—Abstracts of Papers Presented at the Annual Meeting:COPENHAGEN, DENMARK August 21-22, 1970. Vol. 50, Journal of Dental Research. 1971. p. 705–30.

62. Pogrel MA. Sublingual keratosis and malignant transformation. J Oral Pathol. 1979 Jun;8(3):176–8.

63. Waldron CA, Shafer WG. Leukoplakia revisited. A clinicopathologic study 3256 oral leukoplakias. Cancer. 1975 Oct;36(4):1386–92.

64. Warnakulasuriya S, Ariyawardana A. Malignant transformation of oral leukoplakia: a systematic review of observational studies. J Oral Pathol Med. 2016;45(3):155–66.

65. Mao L, Lee JS, Fan YH, Ro JY, Batsakis JG, Lippman S, et al. Frequent microsatellite alterations at chromosomes 9p21 and 3p14 in oral premalignant lesions and their value in cancer risk assessment. Nat Med. 1996 Jun;2(6):682–5.

66. Rosin MP, Lam WL, Poh C, Le ND, Li RJ, Zeng T, et al. 3p14 and 9p21 Loss Is a Simple Tool for Predicting Second Oral Malignancy at Previously Treated Oral Cancer Sites1. Cancer Res. 2002 Nov 15;62(22):6447–50.

67. Zhang L, Poh CF, Williams M, Laronde DM, Berean K, Gardner PJ, et al. Loss of Heterozygosity (LOH) Profiles – Validated Risk Predictors for Progression to Oral Cancer. Cancer Prev Res (Phila Pa). 2012 Sep;5(9):1081–9.

68. El-Naggar AK, Chan JK, Grandis JR, Takata T, Slootweg PJ. World Health Organization classification of head and neck tumours. IARC Lyon. 2017;

69. Aguirre-Urizar JM. Proliferative multifocal leukoplakia better name that proliferative verrucous leukoplakia. World J Surg Oncol. 2011 Oct 10;9:122.

70. Jacobson J, Van Dis M, Zakrzewska JM, Lopes V, Speight P, Hopper C. Proliferative verrucous leukoplakia. Oral Surg Oral Med Oral Pathol Oral Radiol Endodontology. 1996 Oct;82(4):396–401.

71. Pentenero M, Meleti M, Vescovi P, Gandolfo S. Oral proliferative verrucous leucoplakia: are there particular features for such an ambiguous entity? A systematic review. Br J Dermatol. 2014 May;170(5):1039–47.

72. Herreros-Pomares A, Llorens C, Soriano B, Bagan L, Moreno A, Calabuig-Fariñas S, et al. Differentially methylated genes in proliferative verrucous leukoplakia reveal potential malignant biomarkers for oral squamous cell carcinoma. Oral Oncol. 2021 May 1;116:105191.

73. Gouvêa AF, Santos Silva AR, Speight PM, Hunter K, Carlos R, Vargas PA, et al. High incidence of DNA ploidy abnormalities and increased Mcm2 expression may predict malignant change in oral proliferative verrucous leukoplakia. Histopathology. 2013 Mar;62(4):551–62.

74. Kresty LA, Mallery SR, Knobloch TJ, Li J, Lloyd M, Casto BC, et al. Frequent alterations of p16INK4a and p14ARF in oral proliferative verrucous leukoplakia. Cancer Epidemiol Biomark Prev. 2008 Nov;17(11):3179–87.

75. Hansen LS, Olson JA, Silverman S. Proliferative verrucous leukoplakia. A long-term study of thirty patients. Oral Surg Oral Med Oral Pathol. 1985 Sep;60(3):285–98.

76. Upadhyaya JD, Fitzpatrick SG, Islam MN, Bhattacharyya I, Cohen DM. A Retrospective 20-Year Analysis of Proliferative Verrucous Leukoplakia and Its

Progression to Malignancy and Association with High-risk Human Papillomavirus. Head Neck Pathol. 2018 Feb 9;12(4):500–10.

77. Rintala M, Vahlberg T, Salo T, Rautava J. Proliferative verrucous leukoplakia and its tumor markers: Systematic review and meta-analysis. Head Neck. 2019;41(5):1499–507.

78. Okoturo EM, Risk JM, Schache AG, Shaw RJ, Boyd MT. Molecular pathogenesis of proliferative verrucous leukoplakia: a systematic review. Br J Oral Maxillofac Surg. 2018 Nov;56(9):780–5.

79. Staines K, Rogers H. Oral leukoplakia and proliferative verrucous leukoplakia: a review for dental practitioners. Br Dent J. 2017 Nov;223(9):655–61.

80. McParland H, Warnakulasuriya S. Lichenoid morphology could be an early feature of oral proliferative verrucous leukoplakia. J Oral Pathol Med. 2021;50(2):229–35.

81. González-Moles MÁ, Warnakulasuriya S, González-Ruiz I, González-Ruiz L, Ayén Á, Lenouvel D, et al. Worldwide prevalence of oral lichen planus: A systematic review and meta-analysis. Oral Dis. 2021;27(4):813–28.

82. Giuliani M, Troiano G, Cordaro M, Corsalini M, Gioco G, Lo Muzio L, et al. Rate of malignant transformation of oral lichen planus: A systematic review. Oral Dis. 2019;25(3):693–709.

83. Zhu ZD, Ren XM, Zhou MM, Chen QM, Hua H, Li CL. Salivary cytokine profile in patients with oral lichen planus. J Dent Sci. 2022 Jan 1;17(1):100–5.

84. Ismail SB, Kumar SKS, Zain RB. Oral lichen planus and lichenoid reactions: etiopathogenesis, diagnosis, management and malignant transformation. J Oral Sci. 2007;49(2):89–106.

85. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? The Lancet. 2001 Feb 17;357(9255):539–45.

86. Mignogna MD, Fedele S, Lo Russo L, Lo Muzio L, Bucci E. Immune activation and chronic inflammation as the cause of malignancy in oral lichen planus: is there any evidence ? Oral Oncol. 2004 Feb 1;40(2):120–30.

87. Reichart PA, Philipsen HP. Oral erythroplakia—a review. Oral Oncol. 2005 Jul 1;41(6):551–61.

88. Shafer WG, Waldron CA. Erythroplakia of the oral cavity. Cancer. 1975;36(3):1021–8.

89. Hashibe M, Mathew B, Kuruvilla B, Thomas G, Sankaranarayanan R, Parkin DM, et al. Chewing tobacco, alcohol, and the risk of erythroplakia. Cancer Epidemiol Biomark Prev. 2000 Jul;9(7):639–45.

90. Qin GZ, Park JY, Chen SY, Lazarus P. A high prevalence of p53 mutations in pre-malignant oral erythroplakia. Int J Cancer. 1999;80(3):345–8.

91. Pindborg JJ. Histological Typing of Cancer and Precancer of the Oral Mucosa: In Collaboration with L.H.Sobin and Pathologists in 9 Countries. World Health Organization; 1997. 106 p.

92. Bouquot JE, Weiland LH, Kurland LT. Leukoplakia and carcinoma in situ synchronously associated with invasive oral/oropharyngeal carcinoma in Rochester, Minn., 1935–1984. Oral Surg Oral Med Oral Pathol. 1988 Feb;65(2):199–207.

93. Sanjaya PR, Gokul S, Gururaj Patil B, Raju R. Candida in oral pre-cancer and oral cancer. Med Hypotheses. 2011 Dec;77(6):1125–8.

94. Sitheeque M a. M, Samaranayake LP. Chronic hyperplastic candidosis/candidiasis (candidal leukoplakia). Crit Rev Oral Biol Med. 2003;14(4):253–67.

95. Thornhill MH, Pemberton MN, Simmons RK, Theaker ED. Amalgam-contact hypersensitivity lesions and oral lichen planus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003 Mar;95(3):291–9.

96. Oivio UM, Pesonen P, Ylipalosaari M, Kullaa A, Salo T. Prevalence of oral mucosal normal variations and lesions in a middle-aged population: a Northern Finland Birth Cohort 1966 study. BMC Oral Health. 2020 Dec 9;20(1):357.

97. McParland H, Warnakulasuriya S. Oral Lichenoid Contact Lesions to Mercury and Dental Amalgam—A Review. J Biomed Biotechnol. 2012;2012:1–8.

98. Güneş AT, Fetil E, Ilknur T, Birgin B, Ozkan S. Naproxen-induced lichen planus: report of 55 cases. Int J Dermatol. 2006 Jun;45(6):709–12.

99. Rodrigues L, Sousa S, Silva R, Abreu M, Pires F, Mesquita R, et al. Allelic loss in amalgam-associated oral lichenoid lesions compared to oral lichen planus and mucosa. Oral Dis. 2017;23(4):471–6.

100. Lugović-Mihić L, Pilipović K, Crnarić I, Šitum M, Duvančić T. Differential Diagnosis of Cheilitis - How to Classify Cheilitis? Acta Clin Croat. 2018 Jun;57(2):342–51.

101. AYRES S Jr. CHRONIC ACTINIC CHEILITIS. J Am Med Assoc. 1923 Oct 6;81(14):1183–6.

102. Narayanan DL, Saladi RN, Fox JL. Review: Ultraviolet radiation and skin cancer. Int J Dermatol. 2010;49(9):978–86.

103. Piñera-Marques K, Lorenço SV, da Silva LFF, Sotto MN, Carneiro PC. Actinic lesions in fishermen's lower lip: clinical, cytopathological and histopathologic analysis. Clinics. 2010 Apr;65(4):363–7.

104. Jadotte YT, Schwartz RA. Solar cheilosis: an ominous precursor: part I. Diagnostic insights. J Am Acad Dermatol. 2012 Feb;66(2):173–84; quiz 185–6.

105. Oliveira Alves MG, da Mota Delgado A, Balducci I, Carvalho YR, Cavalcante ASR, Almeida JD. Study of MDM2 and SUMO-1 expression in actinic cheilitis and lip cancer. Arch Dermatol Res. 2014 Nov 1;306(9):837–41.

106. Gasparoto TH, de Souza Malaspina TS, Damante JH, de Mello EF, Ikoma MRV, Garlet GP, et al. Regulatory T cells in the actinic cheilitis. J Oral Pathol Med. 2014 Nov;43(10):754–60.

107. Sciubba JJ. Oral Cancer. Am J Clin Dermatol. 2001 Aug 1;2(4):239–51.

108. Mehanna HM, Rattay T, Smith J, McConkey CC. Treatment and follow-up of oral dysplasia - a systematic review and meta-analysis. Head Neck. 2009 Dec;31(12):1600–9.

109. Forastiere A, Koch W, Trotti A, Sidransky D. Head and Neck Cancer. N Engl J Med. 2001 Dec 27;345(26):1890–900.

110. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell. 1990 Jun;61(5):759–67.

111. van der Riet P, Nawroz H, Hruban RH, Corio R, Tokino K, Koch W, et al. Frequent loss of chromosome 9p21-22 early in head and neck cancer progression. Cancer Res. 1994;54(5):1156–8.

112. Reed AL, Califano J, Cairns P, Westra WH, Jones RM, Koch W, et al. High frequency of p16 (CDKN2/MTS-1/INK4A) inactivation in head and neck squamous cell carcinoma. Cancer Res. 1996;56(16):3630–3.

113. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011 Mar 4;144(5):646–74.

114. Stepan KO, Li MM, Kang SY, Puram SV. Molecular margins in head and neck cancer: Current techniques and future directions. Oral Oncol. 2020 Nov 1;110:104893.

115. El-Naggar AK, Chan JKC, Grandis JR, Takata T, Slootweg PJ. WHO Classification of Head and Neck Tumours. International Agency for Research on Cancer; 2017. 347 p.

116. Odell E, Kujan O, Warnakulasuriya S, Sloan P. Oral epithelial dysplasia: Recognition, grading and clinical significance. Oral Dis. 2021 Nov;27(8):1947–76.

117. Yan F, Reddy PD, Nguyen SA, Chi AC, Neville BW, Day TA. Grading systems of oral cavity pre-malignancy: a systematic review and meta-analysis. Eur Arch Otorhinolaryngol. 2020 Nov;277(11):2967–76.

118. Iocca O, Sollecito TP, Alawi F, Weinstein GS, Newman JG, De Virgilio A, et al. Potentially malignant disorders of the oral cavity and oral dysplasia: A systematic

review and meta-analysis of malignant transformation rate by subtype. Head Neck. 2020;42(3):539–55.

119. de Freitas Silva BS, Batista DCR, de Souza Roriz CF, Silva LR, Normando AGC, dos Santos Silva AR, et al. Binary and WHO dysplasia grading systems for the prediction of malignant transformation of oral leukoplakia and erythroplakia: a systematic review and meta-analysis. Clin Oral Investig. 2021 Jul;25(7):4329–40.

120. Silverman S, Bhargava K, Smith LW, Malaowalla AM. Malignant transformation and natural history of oral leukoplakia in 57,518 industrial workers of Gujarat, India. Cancer. 1976 Oct;38(4):1790–5.

121. Pindborg JJ, Jølst O, Renstrup G, Roed-Petersen B. Studies in oral leukoplakia: A preliminary report on the period pervalence of malignant transformation in leukoplakia based on a follow-up study of 248 patients. J Am Dent Assoc. 1968 Apr;76(4):767–71.

122. Paglioni M de P, Khurram SA, Ruiz BII, Lauby-Secretan B, Normando AG, Ribeiro ACP, et al. Clinical predictors of malignant transformation and recurrence in oral potentially malignant disorders: A systematic review and meta-analysis. Oral Surg Oral Med Oral Pathol Oral Radiol. 2022 Nov 1;134(5):573–87.

123. Lodi G, Franchini R, Warnakulasuriya S, Varoni EM, Sardella A, Kerr AR, et al. Interventions for treating oral leukoplakia to prevent oral cancer. Cochrane Database Syst Rev. 2016 Jul 29;7:CD001829.

124. Poh CF, Zhang L, Anderson DW, Durham JS, Williams PM, Priddy RW, et al. Fluorescence Visualization Detection of Field Alterations in Tumor Margins of Oral Cancer Patients. Clin Cancer Res. 2006 Nov 22;12(22):6716–22.

125. Bugter O, Spaander MCW, Bruno MJ, Baatenburg de Jong RJ, Amelink A, Robinson DJ. Optical detection of field cancerization in the buccal mucosa of patients with esophageal cancer. Clin Transl Gastroenterol. 2018 Apr 30;9(4):152–152.

126. Ellis BG, Whitley CA, Triantafyllou A, Gunning PJ, Smith CI, Barrett SD, et al. Prediction of malignant transformation in oral epithelial dysplasia using infrared absorbance spectra. PLoS ONE. 2022 Mar 25;17(3):e0266043.

127. Petersen PE. Oral cancer prevention and control--the approach of the World Health Organization. Oral Oncol. 2009 May;45(4–5):454–60.

128. Hewitson P, Glasziou P, Watson E, Towler B, Irwig L. Cochrane systematic review of colorectal cancer screening using the fecal occult blood test (hemoccult): an update. Am J Gastroenterol. 2008 Jun;103(6):1541–9.

129. Gøtzsche PC, Jørgensen KJ. Screening for breast cancer with mammography. Cochrane Database Syst Rev. 2013 Jun 4;2013(6):CD001877.

130. Speight PM, Palmer S, Moles DR, Downer MC, Smith DH, Henriksson M, et al. The cost-effectiveness of screening for oral cancer in primary care. Health Technol Assess Winch Engl. 2006 Apr;10(14):1–144, iii–iv.

131. Speight PM, Zakrzewska J, Downer MC. Screening for oral cancer and precancer. Eur J Cancer B Oral Oncol. 1992 Jul;28B(1):45–8.

132. Downer MC, Moles DR, Palmer S, Speight PM. A systematic review of test performance in screening for oral cancer and precancer. Oral Oncol. 2004 Mar;40(3):264–73.

133. Epstein JB, Güneri P, Boyacioglu H, Abt E. The limitations of the clinical oral examination in detecting dysplastic oral lesions and oral squamous cell carcinoma. J Am Dent Assoc. 2012 Dec 1;143(12):1332–42.

134. Chang IH, Jiang RS, Wong YK, Wu SH, Chen FJ, Liu SA. Visual screening of oral cavity cancer in a male population: Experience from a medical center. J Chin Med Assoc. 2011 Dec;74(12):561.

135. Chuang SL, Su WWY, Chen SLS, Yen AMF, Wang CP, Fann JCY, et al. Population-based screening program for reducing oral cancer mortality in 2,334,299 Taiwanese cigarette smokers and/or betel quid chewers. Cancer. 2017;123(9):1597–609.

136. Santana JC, Delgado L, Miranda J, Sánchez M. Oral Cancer Case Finding Program (OCCFP). Oral Oncol. 1997 Jan;33(1):10–2.

137. Sankaranarayanan R, Ramadas K, Thomas G, Muwonge R, Thara S, Mathew B, et al. Effect of screening on oral cancer mortality in Kerala, India: a cluster-randomised controlled trial. Lancet. 2005 Jun 4;365(9475):1927–33.

138. Sankaranarayanan R, Ramadas K, Thara S, Muwonge R, Thomas G, Anju G, et al. Long term effect of visual screening on oral cancer incidence and mortality in a randomized trial in Kerala, India. Oral Oncol. 2013 Apr;49(4):314–21.

139. Bouquot JE. Common oral lesions found during a mass screening examination. J Am Dent Assoc. 1986 Jan 1;112(1):50–7.

140. Thomson PJ. Field change and oral cancer: new evidence for widespread carcinogenesis? Int J Oral Maxillofac Surg. 2002 Jun;31(3):262–6.

141. Lee JJ, Hung HC, Cheng SJ, Chiang CP, Liu BY, Yu CH, et al. Factors associated with underdiagnosis from incisional biopsy of oral leukoplakic lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007 Aug;104(2):217–25.

142. Patton LL, Epstein JB, Kerr AR. Adjunctive Techniques for Oral Cancer Examination and Lesion Diagnosis: A Systematic Review of the Literature. J Am Dent Assoc. 2008 Jul 1;139(7):896–905.

143. Sood A, Mishra D, Yadav R, Bhatt K, Priya H, Kaur H. Establishing the accuracy of a new and cheaper sample collection tool: Oral cytology versus oral histopathology. J Oral Maxillofac Pathol. 2020;24(1):52–6.

144. Ye X, Zhang J, Tan Y, Chen G, Zhou G. Meta-analysis of two computer-assisted screening methods for diagnosing oral precancer and cancer. Oral Oncol. 2015 Nov;51(11):966–75.

145. Poate TWJ, Buchanan J a. G, Hodgson TA, Speight PM, Barrett AW, Moles DR, et al. An audit of the efficacy of the oral brush biopsy technique in a specialist Oral Medicine unit. Oral Oncol. 2004 Sep;40(8):829–34.

146. Lingen MW, Abt E, Agrawal N, Chaturvedi AK, Cohen E, D'Souza G, et al. Evidence-based clinical practice guideline for the evaluation of potentially malignant disorders in the oral cavity: A report of the American Dental Association. J Am Dent Assoc. 2017 Oct 1;148(10):712-727.e10.

147. Nagler RM. Saliva as a tool for oral cancer diagnosis and prognosis. Oral Oncol. 2009 Dec 1;45(12):1006–10.

148. Araújo ALD, Santos-Silva AR, Kowalski LP. Diagnostic Accuracy of Liquid Biopsy for Oral Potentially Malignant Disorders and Head and Neck Cancer: an Overview of Systematic Reviews. Curr Oncol Rep. 2023 Apr 1;25(4):279–92.

149. Nazar NSBM, Ramanathan A, Ghani WMN, Rokhani FB, Jacob PS, Sabri NEB, et al. Salivary metabolomics in oral potentially malignant disorders and oral cancer patients—a systematic review with meta-analysis. Clin Oral Investig. 2024 Jan 16;28(1):98.

150. Piyarathne NS, Rasnayake RMSGK, Angammana R, Chandrasekera P, Ramachandra S, Weerasekera M, et al. Diagnostic salivary biomarkers in oral cancer and oral potentially malignant disorders and their relationships to risk factors – A systematic review. Expert Rev Mol Diagn. 2021 Aug 3;21(8):789–807.

151. Monea M, Pop AM. The Use of Salivary Levels of Matrix Metalloproteinases as an Adjuvant Method in the Early Diagnosis of Oral Squamous Cell Carcinoma: A Narrative Literature Review. Curr Issues Mol Biol. 2022 Dec 12;44(12):6306–22.

152. Supawat B, Aye KT, Ritpanja J, Nueangwong W, Kothan S, Pan J, et al. Differences in Spectroscopic Properties of Saliva Taken From Normal Subjects and Oral Cancer Patients: Comparison Studies. J Fluoresc. 2021 May 1;31(3):747–54.

153. Assad DX, Mascarenhas ECP, de Lima CL, de Toledo IP, Chardin H, Combes A, et al. Salivary metabolites to detect patients with cancer: a systematic review. Int J Clin Oncol. 2020 Jun 1;25(6):1016–36.

154. Su YF, Chen YJ, Tsai FT, Li WC, Hsu ML, Wang DH, et al. Current Insights into Oral Cancer Diagnostics. Diagnostics. 2021 Jul 16;11(7):1287.

155. Cristaldi M, Mauceri R, Di Fede O, Giuliana G, Campisi G, Panzarella V. Salivary Biomarkers for Oral Squamous Cell Carcinoma Diagnosis and Follow-Up: Current Status and Perspectives. Front Physiol. 2019 Dec 10;10:1476.

156. Liu D, Xin Z, Guo S, Li S, Cheng J, Jiang H. Blood and Salivary MicroRNAs for Diagnosis of Oral Squamous Cell Carcinoma: A Systematic Review and Meta-Analysis. J Oral Maxillofac Surg. 2021 May 1;79(5):1082.e1-1082.e13.

157. Chiamulera MMA, Zancan CB, Remor AP, Cordeiro MF, Gleber-Netto FO, Baptistella AR. Salivary cytokines as biomarkers of oral cancer: a systematic review and meta-analysis. BMC Cancer. 2021 Feb 27;21(1):205.

158. Kondo Y, Oya K, Sakai M, Fujiwara C, Tojo F, Usami Y, et al. Accuracy of liquid-based cytology (LBC) in the oral mucosa according to novel diagnostic guidelines in Japan: Classification of cytology for oral mucosal disease (JSCC, 2015). Oral Sci Int. 2020;17(1):22–8.

159. Stuani VT, Rubira CMF, Sant'Ana ACP, Santos PSS. Salivary biomarkers as tools for oral squamous cell carcinoma diagnosis: A systematic review. Head Neck. 2017;39(4):797–811.

160. Maryam S, Nogueira MS, Gautam R, Krishnamoorthy S, Venkata Sekar SK, Kho KW, et al. Label-Free Optical Spectroscopy for Early Detection of Oral Cancer. Diagnostics. 2022 Dec;12(12):2896.

161. McRae MP, Modak SS, Simmons GW, Trochesset DA, Kerr AR, Thornhill MH, et al. Point-of-care oral cytology tool for the screening and assessment of potentially malignant oral lesions. Cancer Cytopathol. 2020 Mar;128(3):207–20.

162. Rosenberg D, Cretin S. Use of meta-analysis to evaluate tolonium chloride in oral cancer screening. Oral Surg Oral Med Oral Pathol. 1989 May 1;67(5):621–7.

163. Barrellier P, Babin E, Louis MY, Meunier-Guttin A. [The use of toluidine blue in the diagnosis of neoplastic lesions of the oral cavity]. Rev Stomatol Chir Maxillofac. 1993;94(1):51–4.

164. Mills S. How effective is toluidine blue for screening and diagnosis of oral cancer and premalignant lesions? Evid Based Dent. 2022 Mar;23(1):34–5.

165. Song B, Sunny S, Li S, Gurushanth K, Mendonca P, Mukhia N, et al. Mobile-based oral cancer classification for point-of-care screening. J Biomed Opt. 2021 Jun;26(6):065003.

166. Uthoff R, Song B, Birur N P, Kuriakose M, Sunny S, Suresh A, et al. Development of a dual-modality, Dual-view smartphone-based imaging system for oral cancer detection Ross. 2018 Feb 4;

167. Warin K, Limprasert W, Suebnukarn S, Jinaporntham S, Jantana P, Vicharueang S. AI-based analysis of oral lesions using novel deep convolutional

neural networks for early detection of oral cancer. PLoS ONE. 2022 Aug 24;17(8):e0273508.

168. Khanagar SB, Naik S, Al Kheraif AA, Vishwanathaiah S, Maganur PC, Alhazmi Y, et al. Application and performance of artificial intelligence technology in oral cancer diagnosis and prediction of prognosis: A systematic review. Diagnostics. 2021;11(6):1004.

169. Khan AS, Ahmad S, Ullah Z, Sadiq N, Haq M, Sheikh AK. Predictive value of tissue p53 protein expression and serum p53 antibodies in oral potentially malignant disorders: Relative to oral squamous cell carcinoma. J Taibah Univ Med Sci. 2022 Jun;17(3):415–23.

170. Gupta V, Agrawal U, Goel P. Bioimpedance: A Tool for Screening Oral Cancer – A Systematic Review. Contemp Clin Dent. 2023;14(2):91–7.

171. Emran S, Hurskainen M, Tomppo L, Lappalainen R, Kullaa AM, Myllymaa S. Bioimpedance spectroscopy and spectral camera techniques in detection of oral mucosal diseases: a narrative review of the state-of-the-art. J Med Eng Technol. 2019 Nov 17;43(8):474–91.

172. Lalla Y, Matias MAT, Farah CS. Assessment of oral mucosal lesions with autofluorescence imaging and reflectance spectroscopy. J Am Dent Assoc. 2016 Aug;147(8):650–60.

173. López-Jornet P, De la Mano-Espinosa T. The efficacy of direct tissue fluorescence visualization in screening for oral premalignant lesions in general practice: an update. Int J Dent Hyg. 2011;9(2):97–100.

174. Gillenwater A, Jacob R, Ganeshappa R, Kemp B, El-Naggar AK, Palmer JL, et al. Noninvasive diagnosis of oral neoplasia based on fluorescence spectroscopy and native tissue autofluorescence. Arch Otolaryngol Head Neck Surg. 1998 Nov;124(11):1251–8.

175. Mallia R, Thomas SS, Mathews A, Kumar R, Sebastian P, Madhavan J, et al. Oxygenated hemoglobin diffuse reflectance ratio for in vivo detection of oral pre-cancer. J Biomed Opt. 2008;13(4):041306.

176. Moffa A, Giorgi L, Costantino A, De Benedetto L, Cassano M, Spriano G, et al. Accuracy of autofluorescence and chemiluminescence in the diagnosis of oral Dysplasia and Carcinoma: A systematic review and Meta-analysis. Oral Oncol. 2021 Oct;121:105482.

177. De Veld DCG, Witjes MJH, Sterenborg HJCM, Roodenburg JLN. The status of in vivo autofluorescence spectroscopy and imaging for oral oncology. Oral Oncol. 2005 Feb;41(2):117–31.

178. Balevi B. Evidence-Based Decision-Making: Should the General Dentist Adopt the Use of the VELscope for Routine Screening for Oral Cancer? J Can Dent Assoc. 2007;73(7):4.

179. Rashid A, Warnakulasuriya S. The use of light-based (optical) detection systems as adjuncts in the detection of oral cancer and oral potentially malignant disorders: a systematic review. J Oral Pathol Med. 2015;44(5):307–28.

180. Wang C, Qi X, Zhou X, Liu H, Li M. Diagnostic value of objective VELscope fluorescence methods in distinguishing oral cancer from oral potentially malignant disorders (OPMDs). Transl Cancer Res. 2022 Jun;11(6):1603–15.

181. Mendonca P, Sunny SP, Mohan U, Birur N P, Suresh A, Kuriakose MA. Non-invasive imaging of oral potentially malignant and malignant lesions: A systematic review and meta-analysis. Oral Oncol. 2022 Jul 1;130:105877.

182. Chen Q, Dan H, Pan W, Jiang L, Zhou Y, Luo X, et al. Management of oral leukoplakia: a position paper of the Society of Oral Medicine, Chinese Stomatological Association. Oral Surg Oral Med Oral Pathol Oral Radiol. 2021 Jul 1;132(1):32–43.

183. MORO A, DE WAURE C, DI NARDO F, SPADARI F, MIGNOGNA MD, GIULIANI M, et al. The GOCCLES® medical device is effective in detecting oral cancer and dysplasia in dental clinical setting. Results from a multicentre clinical trial. Acta Otorhinolaryngol Ital. 2015 Dec;35(6):449–54.

184. Kim DH, Lee J, Lee MH, Kim SW, Hwang SH. Efficacy of chemiluminescence in the diagnosis and screening of oral cancer and precancer: a systematic review and meta-analysis. Braz J Otorhinolaryngol. 2020 Jul 29;88(3):358–64.

185. Epstein JB, Gorsky M, Lonky S, Silverman S, Epstein JD, Bride M. The efficacy of oral lumenoscopy (ViziLite) in visualizing oral mucosal lesions. Spec Care Dent. 2006 Aug;26(4):171–4.

186. Green B, Cobb ARM, Brennan PA, Hopper C. Optical diagnostic techniques for use in lesions of the head and neck: review of the latest developments. Br J Oral Maxillofac Surg. 2014 Oct;52(8):675–80.

187. Kim DH, Kim SW, Hwang SH. Efficacy of optical coherence tomography in the diagnosing of oral cancerous lesion: systematic review and meta-analysis. Head Neck. 2023;45(2):473–81.

188. Obade AY, Pandarathodiyil AK, Oo AL, Warnakulasuriya S, Ramanathan A. Application of optical coherence tomography to study the structural features of oral mucosa in biopsy tissues of oral dysplasia and carcinomas. Clin Oral Investig. 2021 Sep 1;25(9):5411–9.

189. van Schaik JE, Halmos GB, Witjes MJH, Plaat BEC. An overview of the current clinical status of optical imaging in head and neck cancer with a focus on Narrow Band imaging and fluorescence optical imaging. Oral Oncol. 2021 Oct 1;121:105504.

190. Kim DH, Kim SW, Lee J, Hwang SH. Narrow-band imaging for screening of oral premalignant or cancerous lesions: A systematic review and meta-analysis. Clin Otolaryngol. 2021;46(3):501–7.

191. Saraniti C, Greco G, Verro B, Lazim NM, Chianetta E. Impact of Narrow Band Imaging in Pre-Operative Assessment of Suspicious Oral Cavity Lesions: A Systematic Review. Iran J Otorhinolaryngol. 2021 May;33(116):127–35.

192. Cozzani E, Russo R, Mazzola F, Garofolo S, Camerino M, Burlando M, et al. Narrow-band imaging: a useful tool for early recognition of oral lichen planus malignant transformation? Eur J Dermatol. 2019 Oct;29(5):500–6.

193. Mascharak S, Baird BJ, Holsinger FC. Detecting oropharyngeal carcinoma using multispectral, narrow-band imaging and machine learning. The Laryngoscope. 2018;128(11):2514–20.

194. Piazza C, Del Bon F, Paderno A, Grazioli P, Perotti P, Barbieri D, et al. The diagnostic value of narrow band imaging in different oral and oropharyngeal subsites. Eur Arch Otorhinolaryngol. 2016 Oct 1;273(10):3347–53.

195. Zhang Y, Wu Y, Pan D, Zhang Z, Jiang L, Feng X, et al. Accuracy of narrow band imaging for detecting the malignant transformation of oral potentially malignant disorders: A systematic review and meta-analysis. Front Surg. 2023 Jan 6;9:1068256.

196. Ansari UH, Wong E, Smith M, Singh N, Palme CE, Smith MC, et al. Validity of narrow band imaging in the detection of oral and oropharyngeal malignant lesions: A systematic review and meta-analysis. Head Neck. 2019;41(7):2430–40.

197. Lau J, Warnakulasuriya S, Balasubramaniam R, Frydrych A, Kujan O. Adjunctive aids for the detection of oral squamous cell carcinoma and oral potentially malignant disorders: A systematic review of systematic reviews. Jpn Dent Sci Rev. 2024 Dec 1;60:53–72.

198. Nathan CAO, Kaskas NM, Ma X, Chaudhery S, Lian T, Moore-Medlin T, et al. Confocal Laser Endomicroscopy in the Detection of Head and Neck Precancerous Lesions. Otolaryngol Neck Surg. 2014 Jul 1;151(1):73–80.

199. Jerjes W, Upile T, Conn B, Hamdoon Z, Betz CS, McKenzie G, et al. In vitro examination of suspicious oral lesions using optical coherence tomography. Br J Oral Maxillofac Surg. 2010 Jan 1;48(1):18–25.

200. Strome A, Kossatz S, Zanoni DK, Rajadhyaksha M, Patel S, Reiner T. Current Practice and Emerging Molecular Imaging Technologies in Oral Cancer Screening.

Mol Imaging [Internet]. 2018 Dec 3 [cited 2023 Jul 19];17. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6287312/

201. Lucchese A, Gentile E, Romano A, Maio C, Laino L, Serpico R. The potential role of in vivo reflectance confocal microscopy for evaluating oral cavity lesions: a systematic review. J Oral Pathol Med. 2016 Nov;45(10):723–9.

202. Maitland KC, Gillenwater AM, Williams MD, El-Naggar AK, Descour MR, Richards-Kortum RR. In Vivo Imaging of Oral Neoplasia Using a Miniaturized Fiber Optic Confocal Reflectance Microscope. Oral Oncol. 2008 Nov;44(11):1059–66.

203. Yang EC, Vohra IS, Badaoui H, Schwarz RA, Cherry KD, Jacob J, et al. Prospective evaluation of oral premalignant lesions using a multimodal imaging system: a pilot study. Head Neck. 2020;42(2):171–9.

204. Alerstam E, Andersson-Engels S, Svensson T. Improved accuracy in time-resolved diffuse reflectance spectroscopy. Opt Express. 2008 Jul 7;16(14):10440–54.

205. Tuchin VV. Tissue Optics: Light Scattering Methods and Instruments for Medical Diagnosis. SPIE Press; 2015. 988 p.

206. Alerstam E, Andersson-Engels S, Svensson T. White Monte Carlo for time-resolved photon migration. J Biomed Opt. 2008 Jul;13(4):041304.

207. Wang HW, Jiang JK, Lin CH, Lin JK, Huang GJ, Yu JS. Diffuse reflectance spectroscopy detects increased hemoglobin concentration and decreased oxygenation during colon carcinogenesis from normal to malignant tumors. Opt Express. 2009 Feb 16;17(4):2805–17.

208. Auner GW, Koya SK, Huang C, Broadbent B, Trexler M, Auner Z, et al. Applications of Raman spectroscopy in cancer diagnosis. Cancer Metastasis Rev. 2018 Dec 1;37(4):691–717.

209. Patterson MS, Andersson-Engels S, Wilson BC, Osei EK. Absorption spectroscopy in tissue-simulating materials: a theoretical and experimental study of photon paths. Appl Opt. 1995 Jan 1;34(1):22–30.

210. Martinelli M, Gardner A, Cuccia D, Hayakawa C, Spanier J, Venugopalan V. Analysis of single Monte Carlo methods for prediction of reflectance from turbid media. Opt Express. 2011 Sep 26;19(20):19627–42.

211. Jermyn M, Mercier J, Aubertin K, Desroches J, Urmey K, Karamchandiani J, et al. Highly Accurate Detection of Cancer *In Situ* with Intraoperative, Label-Free, Multimodal Optical Spectroscopy. Cancer Res. 2017 Jul 15;77(14):3942–50.

212. Faur CI, Falamas A, Chirila M, Roman RC, Rotaru H, Moldovan MA, et al. Raman spectroscopy in oral cavity and oropharyngeal cancer: a systematic review. Int J Oral Maxillofac Surg. 2022 Nov;51(11):1373–81.

213. Wang R, Naidu A, Wang Y. Oral Cancer Discrimination and Novel Oral Epithelial Dysplasia Stratification Using FTIR Imaging and Machine Learning. Diagnostics. 2021 Nov 17;11(11):2133.

214. Wang R, Wang Y. Fourier Transform Infrared Spectroscopy in Oral Cancer Diagnosis. Int J Mol Sci. 2021 Jan 26;22(3):1206.

215. Zhang Q, Hou D, Wen X, Xin M, Li Z, Wu L, et al. Gold nanomaterials for oral cancer diagnosis and therapy: Advances, challenges, and prospects. Mater Today Bio. 2022 Jun 1;15:100333.

216. Bydlon TM, Nachabé R, Ramanujam N, Sterenborg HJCM, Hendriks BHW. Chromophore based analyses of steady-state diffuse reflectance spectroscopy: current status and perspectives for clinical adoption. J Biophotonics. 2015;8(1–2):9–24.

217. Veld DCG de, Skurichina M, Witjes MJH, Duin RPW, Sterenborg HJCM, Roodenburg JLN. Autofluorescence and diffuse reflectance spectroscopy for oral oncology. Lasers Surg Med. 2005 Jun 1;36(5):356–64.

218. Utzinger U, Richards-Kortum RR. Fiber optic probes for biomedical optical spectroscopy. J Biomed Opt. 2003 Jan;8(1):121–47.

219. Hardy M, Kelleher L, de Carvalho Gomes P, Buchan E, Chu HOM, Goldberg Oppenheimer P. Methods in Raman spectroscopy for saliva studies – a review. Appl Spectrosc Rev. 2022 Mar 16;57(3):177–233.

220. Nogueira MS, Maryam S, Amissah M, Lu H, Lynch N, Killeen S, et al. Evaluation of wavelength ranges and tissue depth probed by diffuse reflectance spectroscopy for colorectal cancer detection. Sci Rep. 2021 Jan 12;11(1):798.

221. Tanis E, Evers DJ, Spliethoff JW, Pully VV, Kuhlmann K, van Coevorden F, et al. In vivo tumor identification of colorectal liver metastases with diffuse reflectance and fluorescence spectroscopy. Lasers Surg Med. 2016;48(9):820–7.

222. Valdés PA, Roberts DW, Lu FK, Golby A. Optical technologies for intraoperative neurosurgical guidance. Neurosurg Focus. 2016 Mar;40(3):E8.

223. Fuller C, Camilon R, Nguyen S, Jennings J, Day T, Gillespie MB. Adjunctive diagnostic techniques for oral lesions of unknown malignant potential: Systematic review with meta-analysis. Head Neck. 2015 May;37(5):755–62.

224. Greening GJ, James HM, Dierks MK, Vongkittiargorn N, Osterholm SM, Rajaram N, et al. Towards monitoring dysplastic progression in the oral cavity using a hybrid fiber-bundle imaging and spectroscopy probe. Sci Rep. 2016 May 25;6:26734.

225. Müller MG, Valdez TA, Georgakoudi I, Backman V, Fuentes C, Kabani S, et al. Spectroscopic detection and evaluation of morphologic and biochemical changes in early human oral carcinoma. Cancer. 2003;97(7):1681–92.

226. Mallia RJ, Subhash N, Mathews A, Kumar R, Thomas SS, Sebastian P, et al. Clinical grading of oral mucosa by curve-fitting of corrected autofluorescence using diffuse reflectance spectra. Head Neck. 2010;32(6):763–79.

227. Sokolov K, Drezek R, Gossage K, Richards-Kortum R. Reflectance spectroscopy with polarized light: is it sensitive to cellular and nuclear morphology. Opt Express. 1999 Dec 20;5(13):302–17.

228. Hu F, Vishwanath K, Beumer HW, Puscas L, Afshari HR, Esclamado RM, et al. Assessment of the sensitivity and specificity of tissue-specific-based and anatomical-based optical biomarkers for rapid detection of human head and neck squamous cell carcinoma. Oral Oncol. 2014 Sep;50(9):848–56.

229. Subhash N, Mallia JR, Thomas SS, Mathews A, Sebastian P, Madhavan J. Oral cancer detection using diffuse reflectance spectral ratio R540/R575 of oxygenated hemoglobin bands. J Biomed Opt. 2006 Feb;11(1):014018.

230. Zonios G, Perelman LT, Backman V, Manoharan R, Fitzmaurice M, Van Dam J, et al. Diffuse reflectance spectroscopy of human adenomatous colon polyps in vivo. Appl Opt. 1999 Nov 1;38(31):6628–37.

231. Fang C, Brokl D, Brand RE, Liu Y. Depth-selective fiber-optic probe for characterization of superficial tissue at a constant physical depth. Biomed Opt Express. 2011 Mar 14;2(4):838–49.

232. Schwarz RA, Arifler D, Chang SK, Pavlova I, Hussain IA, Mack V, et al. Ball lens coupled fiber-optic probe for depth-resolved spectroscopy of epithelial tissue. Opt Lett. 2005 May 15;30(10):1159–61.

233. Arifler D, Schwarz RA, Chang SK, Richards-Kortum R. Reflectance spectroscopy for diagnosis of epithelial precancer: model-based analysis of fiber-optic probe designs to resolve spectral information from epithelium and stroma. Appl Opt. 2005 Jul 10;44(20):4291–305.

234. Schwarz RA, Gao W, Daye D, Williams MD, Richards-Kortum R, Gillenwater AM. Autofluorescence and diffuse reflectance spectroscopy of oral epithelial tissue using a depth-sensitive fiber-optic probe. Appl Opt. 2008 Feb 20;47(6):825–34.

235. Nieman LT, Kan CW, Gillenwater A, Markey MK, Sokolov K. Probing local tissue changes in the oral cavity for early detection of cancer using oblique polarized reflectance spectroscopy: a pilot clinical trial. J Biomed Opt. 2008;13(2):024011-024011–11.

236. Shhadeh H, Bachir W, Karraz G. A Sensitive Fibre Optic Probe for Autofluorescence Spectroscopy of Oral Tongue Cancer: Monte Carlo Simulation Study. BioMed Res Int. 2020;2020:1936570.

237. Bailey MJ, Verma N, Fradkin L, Lam S, MacAulay CE, D.d.s CFP, et al. Detection of precancerous lesions in the oral cavity using oblique polarized

reflectance spectroscopy: a clinical feasibility study. J Biomed Opt. 2017 Jun;22(6):065002.

238. Amelink A, Sterenborg HJCM. Measurement of the local optical properties of turbid media by differential path-length spectroscopy. Appl Opt. 2004 May 20;43(15):3048–54.

239. Amelink A, Kaspers OP, Sterenborg HJCM, van der Wal JE, Roodenburg JLN, Witjes MJH. Non-invasive measurement of the morphology and physiology of oral mucosa by use of optical spectroscopy. Oral Oncol. 2008 Jan;44(1):65–71.

240. Amelink A, Sterenborg HJCM, Roodenburg JLN, Witjes MJH. Non-invasive measurement of the microvascular properties of non-dysplastic and dysplastic oral leukoplakias by use of optical spectroscopy. Oral Oncol. 2011 Dec;47(12):1165–70.

241. Pfefer TJ, Matchette LS, Bennett CL, Gall JA, Wilke JA, Durkin AJ, et al. Reflectance-based determination of optical properties in highly attenuating tissue. J Biomed Opt. 2003 Apr;8(2):206–15.

242. Meglinski IV, Matcher SD. Analysis of the spatial distribution of detector sensitivity in a multilayer randomly inhomogeneous medium with strong light scattering and absorption by the Monte Carlo method. Opt Spectrosc. 2001;91:654–9.

243. Alerstam E, Svensson T, Andersson-Engels S, Spinelli L, Contini D, Dalla Mora A, et al. Single-fiber diffuse optical time-of-flight spectroscopy. Opt Lett. 2012 Jul 15;37(14):2877–9.

244. McGee S, Mardirossian V, Elackattu A, Mirkovic J, Pistey R, Gallagher G, et al. Anatomy-Based Algorithms for Detecting Oral Cancer Using Reflectance and Fluorescence Spectroscopy. Ann Otol Rhinol Laryngol. 2009 Nov;118(11):817–26.

245. Narayanan S, Anand S, Prasanna R, Managoli S, Suvarnadas R, Shyamsundar V, et al. Bimodal multispectral imaging system with cloud-based machine learning algorithm for real-time screening and detection of oral potentially malignant lesions and biopsy guidance. J Biomed Opt. 2021 Aug;26(8).

246. Gomes AJ, Turzhitsky V, Ruderman S, Backman V. Monte Carlo model of the penetration depth for polarization gating spectroscopy: influence of illumination-collection geometry and sample optical properties. Appl Opt. 2012 Jul 10;51(20):4627–37.

247. Hafez R, Hamadah O, Bachir W. Mapping of healthy oral mucosal tissue using diffuse reflectance spectroscopy: ratiometric-based total hemoglobin comparative study. Lasers Med Sci. 2015 Nov 1;30(8):2135–41.

248. Farrell TJ, Patterson MS, Wilson B. A diffusion theory model of spatially resolved, steady-state diffuse reflectance for the noninvasive determination of tissue optical properties in vivo. Med Phys. 1992;19(4):879–88.

249. Palmer GM, Ramanujam N. Monte Carlo-based inverse model for calculating tissue optical properties. Part I: Theory and validation on synthetic phantoms. Appl Opt. 2006 Feb 10;45(5):1062–71.

250. Tsui SY, Wang CY, Huang TH, Sung KB. Modelling spatially-resolved diffuse reflectance spectra of a multi-layered skin model by artificial neural networks trained with Monte Carlo simulations. Biomed Opt Express. 2018 Mar 7;9(4):1531–44.

251. Pfefer TJ, Matchette LS, Ross AM, Ediger MN. Selective detection of fluorophore layers in turbid media: the role of fiber-optic probe design. Opt Lett. 2003 Jan 15;28(2):120–2.

252. Ti Y, Lin WC. Effects of probe contact pressure on in vivo optical spectroscopy. Opt Express. 2008 Mar 17;16(6):4250–62.

253. Yu B, Shah A, Nagarajan VK, Ferris DG. Diffuse reflectance spectroscopy of epithelial tissue with a smart fiber-optic probe. Biomed Opt Express. 2014 Feb 10;5(3):675–89.

254. Ruderman S, Gomes AJ, Stoyneva V, Rogers JD, Fought AJ, Jovanovic BD, et al. Analysis of pressure, angle and temporal effects on tissue optical properties from polarization-gated spectroscopic probe measurements. Biomed Opt Express. 2010 Aug 5;1(2):489–99.

255. Palmer GM, Zhang H, Lee CT, Mikati H, Herbert JA, Krieger M, et al. Assessing effects of pressure on tumor and normal tissue physiology using an automated self-calibrated, pressure-sensing probe for diffuse reflectance spectroscopy. J Biomed Opt. 2018 May;23(5):057004.

256. Yu B, Shah A, Nagarajan VK, Ferris DG. Diffuse reflectance spectroscopy of epithelial tissue with a smart fiber-optic probe. Biomed Opt Express. 2014 Feb 10;5(3):675–89.

257. Lim L, Nichols B, Rajaram N, Tunnell JW. Probe pressure effects on human skin diffuse reflectance and fluorescence spectroscopy measurements. J Biomed Opt. 2011 Jan;16(1):011012.

258. Chang VTC, Merisier D, Yu B, Walmer DK, Ramanujam N. Towards a field-compatible optical Spectroscopic device for cervical cancer screening in resource-limited settings: effects of calibration and pressure. Opt Express. 2011 Sep 12;19(19):17908–24.

259. Perelman LT. Optical diagnostic technology based on light scattering spectroscopy for early cancer detection. Expert Rev Med Devices. 2006 Nov;3(6):787–803.

260. Evers DJ, Nachabe R, Vranken Peeters M jeanne, van der Hage JA, Oldenburg HS, Rutgers EJ, et al. Diffuse reflectance spectroscopy: towards clinical application in breast cancer. Breast Cancer Res Treat. 2013 Jan;137(1):155–65.

261. Volynskaya Z, Haka AS, Bechtel KL, Fitzmaurice M, Shenk R, Wang N, et al. Diagnosing breast cancer using diffuse reflectance spectroscopy and intrinsic fluorescence spectroscopy. J Biomed Opt. 2008 Apr;13(2):024012.

262. Shaikh R, Prabitha VG, Dora TK, Chopra S, Maheshwari A, Deodhar K, et al. A comparative evaluation of diffuse reflectance and Raman spectroscopy in the detection of cervical cancer. J Biophotonics. 2017;10(2):242–52.

263. Herranz M, Ruibal A. Optical Imaging in Breast Cancer Diagnosis: The Next Evolution. J Oncol. 2012;2012:863747.

264. Stephen MM, Jayanthi JL, Unni NG, Kolady PE, Beena VT, Jeemon P, et al. Diagnostic accuracy of diffuse reflectance imaging for early detection of pre-malignant and malignant changes in the oral cavity: a feasibility study. BMC Cancer. 2013;13:278.

265. Messadi DV, Younai FS, Liu HH, Guo G, Wang CY. The clinical effectiveness of reflectance optical spectroscopy for the in vivo diagnosis of oral lesions. Int J Oral Sci. 2014 Sep;6(3):162–7.

266. Jeng MJ, Sharma M, Sharma L, Huang SF, Chang LB, Wu SL, et al. Novel Quantitative Analysis Using Optical Imaging (VELscope) and Spectroscopy (Raman) Techniques for Oral Cancer Detection. Cancers. 2020 Nov 13;12(11):E3364.

267. Schwarz RA, Gao W, Stepanek VMT, Le TT, Bhattar VS, Williams MD, et al. Prospective evaluation of a portable depth-sensitive optical spectroscopy device to identify oral neoplasia. Biomed Opt Express. 2010 Dec 8;2(1):89–99.

268. Brouwer de Koning SG, Baltussen EJM, Karakullukcu MB, Dashtbozorg B, Smit LA, Dirven R, et al. Toward complete oral cavity cancer resection using a handheld diffuse reflectance spectroscopy probe. J Biomed Opt. 2018 Oct;23(12):1–8.

269. Vishwanath K, Chang K, Klein D, Deng YF, Chang V, Phelps JE, et al. Portable, Fiber-Based, Diffuse Reflection Spectroscopy (DRS) Systems for Estimating Tissue Optical Properties. Appl Spectrosc. 2011 Feb 1;62(2):206–15.

270. protocol, n. meanings, etymology and more | Oxford English Dictionary [Internet]. [cited 2024 Mar 29]. Available from: https://www.oed.com/dictionary/protocol_n?tab=meaning_and_use&tl=true#27963133

271. guideline, n. meanings, etymology and more | Oxford English Dictionary [Internet]. [cited 2024 Mar 29]. Available from: https://www.oed.com/dictionary/guideline_n

272. Sah AN, Kumar P, Pradhan A. In-vivo Testing of Oral Mucosal Lesions with an In-house Developed Portable Imaging Device and Comparison with Spectroscopy Results. J Fluoresc. 2023 Jul 1;33(4):1375–83.

273. Calin MA, Parasca SV. In vivo study of age-related changes in the optical properties of the skin. Lasers Med Sci. 2010 Mar 1;25(2):269–74.

274. Brooks S, Hoy CL, Amelink A, Robinson DJ, Nijsten TE. Sources of variability in the quantification of tissue optical properties by multidiameter single-fiber reflectance and fluorescence spectroscopy. J Biomed Opt. 2015;20(5):057002–057002.

275. Nayak GS, Kamath S, Pai KM, Sarkar A, Ray S, Kurien J, et al. Principal component analysis and artificial neural network analysis of oral tissue fluorescence spectra: Classification of normal premalignant and malignant pathological conditions. Biopolymers. 2006;82(2):152–66.

276. Mallia RJ, Narayanan S, Madhavan J, Sebastian P, Kumar R, Mathews A, et al. Diffuse Reflection Spectroscopy: An Alternative to Autofluorescence Spectroscopy in Tongue Cancer Detection. Appl Spectrosc. 2010 Apr 1;64(4):409–18.

277. Pandis N, Fleming PS, Worthington H, Salanti G. The Quality of the Evidence According to GRADE Is Predominantly Low or Very Low in Oral Health Systematic Reviews. PLoS ONE. 2015 Jul 10;10(7):e0131644.

278. Lopes CB, Motta P de B, Campos TM, Leite DPV, Araki Yamamoto ÂT, Mota MS de A, et al. Protocol for the clinical practice of photodynamic therapy in endodontics: assessment of guideline quality using the AGREE II instrument. Photodiagnosis Photodyn Ther. 2022 Jun 1;38:102835.

279. GRADE handbook [Internet]. [cited 2024 Feb 13]. Available from: https://gdt.gradepro.org/app/handbook/handbook.html

280. The technology | Narrow band imaging for Barrett's oesophagus | Advice | NICE [Internet]. NICE; 2019 [cited 2024 Mar 29]. Available from: https://www.nice.org.uk/advice/mib179/chapter/The-technology

281. Alsoghier A, Ni Riordain R, Fedele S, Porter S. Web-based information on oral dysplasia and precancer of the mouth – Quality and readability. Oral Oncol. 2018 Jul 1;82:69–74.

282. Schwarz RA, Gao W, Weber CR, Kurachi C, Lee JJ, El-Naggar AK, et al. Noninvasive Evaluation of Oral Lesions Using Depth-Sensitive Optical Spectroscopy. Cancer. 2009 Apr 15;115(8):1669–79.

283. Kolpakov AV, Moshkova AA, Melikhova EV, Sokolova DYu, Muravskaya NP, Samorodov AV, et al. Diffuse Reflectance Spectroscopy of the Oral Mucosa: In Vivo Experimental Validation of the Precancerous Lesions Early Detection Possibility. Diagnostics. 2023 May 5;13(9):1633.

284. Liu D, Zheng J, Zhang Q, Zhang L, Gao F. A combined autofluorescence and diffuse reflectance spectroscopy for mucosa tissue diagnosis: Dual-distance system and data-driven decision. J Biophotonics. 2023;16(10):e202300086.

285. nhs.uk [Internet]. 2023 [cited 2024 Apr 7]. Calculate your body mass index (BMI) for adults - NHS. Available from: https://www.nhs.uk/health-assessment-tools/calculate-your-body-mass-index/calculate-bmi-for-adults

286. Maryam S, Sekar SKV, Ghauri MD, Fahy E, Nogueira MS, Lu H, et al. Mobile multi-configuration clinical translational Raman system for oral cancer application. Analyst. 2023 Mar 27;148(7):1514–23.

287. Langhout GC, Kuhlmann KFD, Schreuder P, Bydlon T, Smeele LE, van den Brekel MWM, et al. In vivo nerve identification in head and neck surgery using diffuse reflectance spectroscopy. Laryngoscope Investig Otolaryngol. 2018;3(5):349–55.

288. Jayanthi JL, Nisha GU, Manju S, Philip EK, Jeemon P, Baiju KV, et al. Diffuse reflectance spectroscopy: diagnostic accuracy of a non-invasive screening technique for early detection of malignant changes in the oral cavity. BMJ Open. 2011 Jan 1;1(1):e000071.

289. Mallia RJ, Thomas SS, Mathews A, Kumar R R, Sebastian P, Madhavan J, et al. Laser-induced autofluorescence spectral ratio reference standard for early discrimination of oral cancer. Cancer. 2008;112(7):1503–12.

290. Jayanthi JL, Subhash N, Stephen M, Philip EK, Beena VT. Comparative evaluation of the diagnostic performance of autofluorescence and diffuse reflectance in oral cancer detection: a clinical study. J Biophotonics. 2011;4(10):696–706.

291. Garcia-Uribe A, Zou J, Duvic M, Cho-Vega JH, Prieto VG, Wang LV. In Vivo Diagnosis of Melanoma and Nonmelanoma Skin Cancer Using Oblique Incidence Diffuse Reflectance Spectrometry. Cancer Res. 2012 May 31;72(11):2738–45.

292. McGregor HC, Short MA, McWilliams A, Shaipanich T, Ionescu DN, Zhao J, et al. Real-time endoscopic Raman spectroscopy for in vivo early lung cancer detection. J Biophotonics. 2017;10(1):98–110.

293. Lin D, Qiu S, Huang W, Pan J, Xu Z, Chen R, et al. Autofluorescence and white light imaging-guided endoscopic Raman and diffuse reflectance spectroscopy for in vivo nasopharyngeal cancer detection. J Biophotonics. 2018;11(4):e201700251.

294. Jayanthi J l., Mallia RJ, Shiny ST, Baiju KV, Mathews A, Kumar R, et al. Discriminant analysis of autofluorescence spectra for classification of oral lesions in vivo. Lasers Surg Med. 2009;41(5):345–52.

295. Li C, Xia H, Zhou Y, Li S, Liu R, Chen W, et al. Study on the influence of contact pressure on diffuse spectroscopy measurement of in vivo tissue. Infrared Phys Technol. 2021 May 1;114:103669.

296. Grygoryev K, Lu H, Li CL, Zhdanov AV, Burke R, Andersson-Engels S. Multi-Spectral Clinical Prototype for Fluorophore Detection. Front Phys. 2021:9

297. Ni Riordain R, O'Sullivan K, McCreary C. Retrospective evaluation of the referral pattern to an oral medicine unit in Ireland. Community Dent Health. 2011 Mar;28(1):107–10.

298. Brocklehurst P, Kujan O, O'Malley LA, Ogden G, Shepherd S, Glenny AM. Screening programmes for the early detection and prevention of oral cancer. Cochrane Database Syst Rev. 2013 Nov 19;2013(11):CD004150.

299. National Cancer Registry Ireland. Cancer Incidence Projections for Ireland 2020-2045 [Internet]. Cork, Ireland: National Cancer Registry Ireland; [cited 2022 Mar 9]. Available from: https://www.ncri.ie/publications/cancer-trends-and-projections/cancer-incidence-projections-ireland-2020-2045

300. Department of Health. National Cancer Strategy 2017-2026 [Internet]. Dublin, Ireland; 2017 Jul [cited 2024 Apr 12]. Available from: https://assets.gov.ie/9315/6f1592a09583421baa87de3a7e9cb619.pdf

301. Khan C. Pliers, abscesses and agonising pain: Britain's dental crisis – as seen from A&E. The Guardian [Internet]. 2024 Jan 30 [cited 2024 Apr 12]; Available from: https://www.theguardian.com/society/2024/jan/30/pliers-abscesses-and-agonising-pain-britains-dental-crisis-as-seen-from-ae

302. Irish Independent [Internet]. 2023 [cited 2024 Apr 12]. Dentist shortage leaves many patients waiting over three months for routine appointment. Available from: https://www.independent.ie/irish-news/dentist-shortage-leaves-many-patients-waiting-over-three-months-for-routine-appointment/a80452822.html

303. Lewis MA, Newton JT. An evaluation of the quality of commercially produced patient information leaflets. Br Dent J. 2006 Jul 22;201(2):114–7.

304. Rylander CG, Milner TE, Baranov SA, Nelson JS. Mechanical tissue optical clearing devices: Enhancement of light penetration in ex vivo porcine skin and adipose tissue. Lasers Surg Med. 2008;40(10):688–94.

305. Bregar M, Cugmas B, Naglic P, Hartmann D, Pernuš F, Likar B, et al. Properties of contact pressure induced by manually operated fiber-optic probes. J Biomed Opt. 2015 Dec;20(12):127002.

306. Juni P. Systematic reviews in health care: Assessing the quality of controlled clinical trials. BMJ. 2001 Jul 7;323(7303):42–6.

307. Crisp RJ, Burke FJT. Conducting trials. Br Dent J. 2005 Mar;198(5):282–3.

308. Brocklehurst P, Hoare Z. How to design a randomised controlled trial. Br Dent J. 2017 May 12;222(9):721–6.

309. Sokolov K, Follen M, Richards-Kortum R. Optical spectroscopy for detection of neoplasia. Curr Opin Chem Biol. 2002 Oct 1;6(5):651–8.

310. Malin SF, Ruchti TL, Blank TB, Thennadil SN, Monfre SL. Noninvasive Prediction of Glucose by Near-Infrared Diffuse Reflectance Spectroscopy. Clin Chem. 1999 Sep 1;45(9):1651–8.

311. Mulder DJ, Water TVD, Lutgers HL, Graaff R, Gans RO, Zijlstra F, et al. Skin Autofluorescence, a Novel Marker for Glycemic and Oxidative Stress-Derived Advanced Glycation Endproducts: An Overview of Current Clinical Studies, Evidence, and Limitations. Diabetes Technol Ther. 2006 Oct;8(5):523–35.

312. Thorlund K, Haggstrom J, Park JJ, Mills EJ. Key design considerations for adaptive clinical trials: a primer for clinicians. BMJ. 2018 Mar 8;360:k698.

313. Kanagal NB. Innovation and product innovation in marketing strategy. J Manag Mark Res. 2015;18.

314. Veluponnar D, Dashtbozorg B, Jong LJS, Geldof F, Guimaraes MDS, Peeters MJTFDV, et al. Diffuse reflectance spectroscopy for accurate margin assessment in breast-conserving surgeries: importance of an optimal number of fibers. Biomed Opt Express. 2023 Aug 1;14(8):4017–36.

315. Boer LL de, Hendriks BHW, Duijnhoven F van, Peeters-Baas MJTFDV, Vijver KV de, Loo CE, et al. Using DRS during breast conserving surgery: identifying robust optical parameters and influence of inter-patient variation. Biomed Opt Express. 2016 Dec 1;7(12):5188–200.

316. Ioannidis JPA, Greenland S, Hlatky MA, Khoury MJ, Macleod MR, Moher D, et al. Research: increasing value, reducing waste. Lancet. 2014 Jan 11;383(9912):166–75.

8 Appendices

8.1 Appendix A: User protocol for use of DRS

User protocol for use of diffuse reflectance spectroscopy (DRS) in the detection of oral potentially malignant disease (OPMD) and squamous cell carcinoma (SCC)

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Scope of the guidance

This guidance is intended for new users of diffuse reflectance spectroscopy (DRS) probes. It outlines evidence-based methods for its use to diagnose and differentiate between normal, oral premalignant disease (OPMD) and oral squamous cell carcinoma (OSCC). This guidance is intended to cover the clinical use of the probe. This will encourage future research in the field and provide standardised results.

Why this guidance is needed

There are multiple published studies on the use of diffuse reflectance probes for detection of oral OPMD and OSCC. There is currently no consensus or best practice on the use of DRS in the mouth.

Introduction

Diffuse reflectance spectroscopy is a light-based optical technique that aims to measure the light reflectance from light absorbers and reflectors. It requires the use of software and post-processing to produce a result. Using DRS, a spectrum is produced that can be analysed for of the ratio of deoxygenated haemoglobin to oxygenated haemoglobin in the tissue. It has been used to diagnose disease in the mouth, GI tract, liver, lungs, urinary tract, cervix, breast and brain.[1] This protocol is intended to be used only in the mouth. DRS shows greater sensitivity and specificity than other light-based optical techniques for recognising normal, benign, premalignant and malignancy in the mouth.[2] It requires the probe to be in contact with the mucosa in order to produce a result.

Innovation

DRS uses captured reflected white light in the 300-700nm region from an area of interest. Haemoglobin preferentially absorbs white light in this region. This can be measured and the ratio compared to other tissues to reach a diagnosis. Other systems have similar features, however DRS is simpler and provides information on composition and morphology of tissue.

Current care pathway

Current best practice dictates patients presenting with a new or changing oral mucosal abnormality will have a biopsy done by a surgeon or specialist dentist or doctor. Biopsies taken are examined under a microscope for OPMD or OSCC by a histopathologist. The extent of precancerous change, also termed as dysplasia, is graded. Once dysplasia is identified, the patient should be followed up with regular clinical exams and repeated surveillance biopsies in the event of changes or other worrying signs. This necessitates repeated biopsies in the mouth, with resulting increase in burden to the patient and healthcare system. In some cases, patients are recommended further treatment to remove or otherwise destroy the area. The literature recommends surgical excision of any area graded as moderate dysplasia or above.[3, 4] A new non-invasive, point-of-care solution would be of benefit to patients and to the healthcare system to reduce the number of biopsies and obtain quicker results.

Indications

Any patient with a suspected or proven OPMD or OSCC. DRS can be used for the first time in patients who have an undiagnosed oral lesion or can be used in patients to check for change in a previously diagnosed area. DRS can be used by any doctor or dentist with experience of diagnosing and treating oral premalignant conditions. Additional training may be needed to use the probe effectively.

Prior to investigation

Patients should be provided with the necessary information related to their health care in a format that enables them to make an informed decision. Consideration should be given to the use of written materials and diagrams. A written informed consent must be obtained for invasive diagnostic and therapeutic procedures.

Clinical evidence

Procedure and clinical guideline

Published evidence

7 studies are summarised in this guidance including 439 people who have oral potentially malignant disease or cancer.

There are two meta-analyses that are published with regard to DRS in the mouth. Fuller et al (2015) included 3 studies. This study found that DRS has the greatest accuracy of the techniques under investigation (sensitivity: 98.6% with 101 dysplasia and cancers sampled, with a p value of superiority vs: [Toluidine Blue (TB) <0.001], [clinical oral examination (COE) 0.014], [cytology 0.007], [light-induced autofluorescence (LIAF) 0.990]. Specificity: 83.2% with 21 benign lesions, p-value vs TB 0.013 and COE 0.098. Accuracy n= 122, 96.5% [2]

Mendonca (2022) included three studies with 63 dysplastic and 177 non-dysplastic lesions analysed. This showed high diagnostic index values (Sen: 0.79(CI) (0.67–0.89), Spe: 0.86(0.80–0.91), DOR: 40.47 (0.80, 2047.56) and SAUC of 0.911(SE = 0.034)[5]

Summary of published studies

The evidence for DRS is of moderate quality and provides some information as to how DRS could be used in the diagnosis of OPMD and SCC. The evidence includes five prospective clinical trials comparing DRS spectroscopy with biopsy and histopathology and a feasibility study that reports diagnostic accuracy. Evaluation of the risk of bias was carried out using the QUADAS-2 tool. The most likely judgement of bias was moderate, due to the lack of multiple pathologist grading. Good consistency was noted across the studies, with lower effects most likely as a result of grouping heterogenous tissues together. The level of indirectness was most likely moderate-high, with variation in the type of DRS used and population variability. The research was done in specialist centres. Clinical examination, biopsy and DRS all were done by highly trained surgeons and oral medicine specialists so may not be reproducible in all primary care settings. Further randomised trials are needed with standardised protocols and diagnostic criteria.

Preparation

- Background electromagnetic radiation needs to be excluded. This can be done by turning off the room lights and closing any curtains or blinds. (Strong recommendation – low risk of bias)[6–9]

- Patient and operating team should wear safety glasses when the laser is in operation. [6]

Calibration

System Warm up

- Prepare the system for measurements (start system, start software, warm up system etc.) This should be done at least 30 minutes before starting to eliminate fluctuations due to lamp intensity.[10] (Strong evidence, low risk of bias)

Data collection from Standard

- Background reflectance should be measured with the integrated light source switched off or covered.
- This background reflectance then be subtracted from the experimental reflectance readings.[7, 8, 11–13]
- Calibration using a reflectance standard is also carried out now. At least 10-15 minutes should be allocated for this.[10]

Probe Cleaning/Sterilizing

- Clean DRS probe prior to use. Tristel chlorine wipes to be used and rinsed at the end with deionized water.[14] (Conditional recommendation, high risk of bias)

Data Collection

- A pre-operative rinse for thirty seconds should be administered to reduce the effect of residual food.[6, 9, 15] (Moderate recommendation, weak evidence)
- The area assessed should be dried. Gauze is preferable (expert opinion)
- Photography helps to identify and cross-reference diagnosis. (expert opinion)
- The probe should be placed at a 90 degree angle to the dry mucosal surface to be analysed. (moderate recommendation, high risk of bias)
- A secure finger rest should be obtained at all times (expert opinion)
- The pressure applied should be minimal. [16–18](strong recommendation, low risk of bias)
- Move the probe slightly each time to ensure no deformation of the mucosa with loss of blood flow. At least 3 points, and preferably 5 samples are advised.[19] (conditional recommendation, high risk of bias)

Area of interest

The tissues in the oral cavity vary in their optical properties. There is currently no consensus on which tissues can be grouped together.[20] Most authors use a variation of the below to take readings.[14, 15, 19, 21]

1. Lesion.
2. Contralateral site.
3. Dorsal surface of tongue
4. Ventral surface of tongue.
5. Lip between vermilion border and wet-dry line.
6. Buccal mucosa.
7. Gingiva.
8. Floor of mouth.
9. Palate (hard/soft).

Precautions

1. Tap dry the area from which the data is being collected as water can change the signal.
2. The mucosa should be relaxed as much as possible. Stretching or dilating the mucosa will change the optical properties.

Summary of evidence

The below is a summary of the in vivo clinical trials included in this guidance. Studies were included based on the degree of explanation of their method, whether DRS was primarily used to obtain a result and clearly explained. Only studies which used a DRS probe in vivo were included. This was to reduce the risk of bias or incorrect method used in a lab or purely experimental setting. Studies were excluded if the reporting of their accuracy was not included or incomplete, or if the reporting of the gold standard diagnoses (i.e. histopathology) was either not carried out, not reported or was incomplete.

Yu 2017 [22]	
	DRS probe with integrated pressure sensor and automated pressure application. Applied to the buccal mucosa of 8 volunteers. Significant spectral differences observed above 1psi.
Bailey 2017[8]	
Study size, location and design	Clinical feasibility study with 28 patients, Canada. Included 93 measurements from 25 patients.
Intervention and comparison	Biopsy of all sites after spectroscopy. Reported by one pathologist. 2-3 normal site measurements
Key outcomes	Samples grouped by normal, benign, mild dysplasia, severe dysplasia. excellent performance in the discrimination of normal from benign and dysplasia. Spectra normalised, preprocessed and standardised. Maximum relevance minimum redundancy with leave-one-out cross-validation used to select most discriminatory spectral features – found by dividing spectra in 20nm blocks. No difference in depth-selective fibres in classification.
Strengths and limitations	Did not separate sites by anatomical area or by tissue type. Not blinded to histological diagnosis when calculating sensitivity/specificity. Low accuracy in calculating benign vs dysplasia. No control group – risk of confounding
Amelink 2011[19]	
Study size, location and design	Open – label clinical trial including 18 participants in the Netherlands. 27 leukoplakias, 75 DRS spectra. 45 dysplastic, 31 non-dysplastic lesions. 24 clinically normal sites taken as reference. The Netherlands
Intervention and comparison:	Diffuse reflectance spectroscopy of all lesions immediately followed by biopsy, blinded pathologist. Averaged results of 4-5 acquisitions from lesion
Key outcomes	Used linear discriminant analysis of blood volume fraction (bvf), vessel diameter and tissue oxygenation to discriminate dysplastic leukoplakia and non-dysplastic leukoplakia with a sensitivity and specificity of 91% and 80%, respectively. The area under the receiver-operating characteristic (ROC) curve was 0.87. Bvf borderline significant for difference between normal, non-dysplastic leukoplakia and dysplastic leukoplakia.
Strengths and limitations	One investigator part of industry. Did not use depth-sensitive spectroscope, therefore only obtained results from superior epithelium. Did not differentiate between grades of dysplasia, leading to imprecision in effect estimate. No control group, therefore risk of confounding

Jayanthi 2011[6]	
Study size, location and design	Prospective open-label clinical trial in outpatient department of dental school in India including 96 patients and 34 healthy volunteers.
Intervention and comparison	Incisional biopsy taken from all that showed malignant features on DRS spectra.
Key outcomes	Used training and validation sets. PCA-LDA performed on normalised spectra with PCA to extract spectral features and LDA used to generate discriminant function with leave-one-out cross-validation. Sensitivity 98.5% and specificity of 96.0% for distinguishing SCC from dysplasia. AUC 0.98 (95% CI 0.95 to 1.00) for distinguishing dysplasia from SCC
Strengths and limitations	Limited to buccal mucosa only which has a smaller risk for cancer, therefore results may not be applicable to all areas of the mouth. Not all participants received the gold standard for diagnosis of biopsy, this may have confounded results. Two pathologists confirmed histology. Controls not age- and sex-matched
Jayanthi 2011[23]	
Study size, location and design	Prospective clinical trial, India. 30 controls, 65 patients with oral mucosal disease
Intervention and comparison	Incisional biopsy taken from all patients with results interpreted by pathologist blinded to spectra
Key outcomes	Sensitivity 100% and specificity 96% in distinguishing dysplasia and SCC. AUC 0.991 for DRS. Used normalised spectral data and found best resulta with discriminant function analysis.
Strengths and limitations	Good variety of diagnoses. Excluded lateral tongue, vermilion border and dorsal surface of tongue which may confound results. Only one pathologist grading. Control group noted to have different habits to study group, which may confound results
Mallia 2010[7]	
Study size, location and design	Observational clinical trial, India. N=56. 36 healthy volunteers, 36 with high or low-risk lesions. 30 sites of 16 patients used to get algorithm for dorsal surface of tongue.
Intervention and comparison	DRS spectroscopy. Immediate biopsy and histopathology with visual inspection for healthy patients. Pathologist blinded to spectroscopy graded results.
Key outcomes	Sensitivity of 86% and specificity of 94% to discriminate dysplasia from SCC on dorsal of tongue. sensitivity of 90% and specificity of 86% in hyperplastic vs dysplasia. Used 545/575nm haemoglobin ratios and scatter plot.
Strengths and weaknesses	Limited to one area, Controls not age and sex matched, did not exclude cancer from control, little exclusions, only one pathologist

Mallia 2008[24]	
Study size, location and design	Observational clinical trial, India. 36 healthy volunteers and 29 patients with high or low risk oral abnormalities.
Intervention and comparison	Diffuse reflectance spectroscopy followed by immediate biopsy with pathologist blinded to results
Key outcomes	Use 545/575nm haemoglobin absorption ratio and scatter plot. 13 patients included in final analysis to obtain results for buccal mucosa only. Dysplasia and SCC distinguished with 96% sensitivity and 100% specificity
Strengths and limitations	Controls not age and sex matched. Volunteers had no tobacco habits. Divided by anatomical site. Buccal mucosa only. Diagnosed SCC that was undiagnosed by histology and SCC that was unsuspected by COE but confirmed by histology
Nieman 2008[25]	
Study size, location and design	Pilot clinical study, USA. n=24, 57 sites
Intervention and comparison	Biopsy of area taken after DRS measurements
Key outcomes	Able to differentiate mild dysplasia from severe dysplasia with sensitivity 80 and specificity 83%
Strengths and limitations	Examined repeatability, however pathologists not blinded to histology which may be a confounder, small sample size in this study, did not divide samples by anatomical location leading to imprecision
De Veld 2005	
Study size, location and design	Clinical trial, the Netherlands, 155 patients with oral mucosal abnormalities, 70 controls
Intervention and comparison	DRS measurements followed by biopsy
Key outcomes	Benign from dysplastic results: ROC-AUC 0.77, sensitivity 69%, specificity 77%). Author: “not good enough for clinical application”.
Strengths and limitations	Good reporting of dropouts and excluded readings. Imbalanced dataset, with few dysplastic lesions. Not all lesions biopsied, only those felt to be clearly premalignant or malignant. No description of histopathological examination. This early study’s results have not been replicated.

Excluded studies

Kolpakov 2023[26]

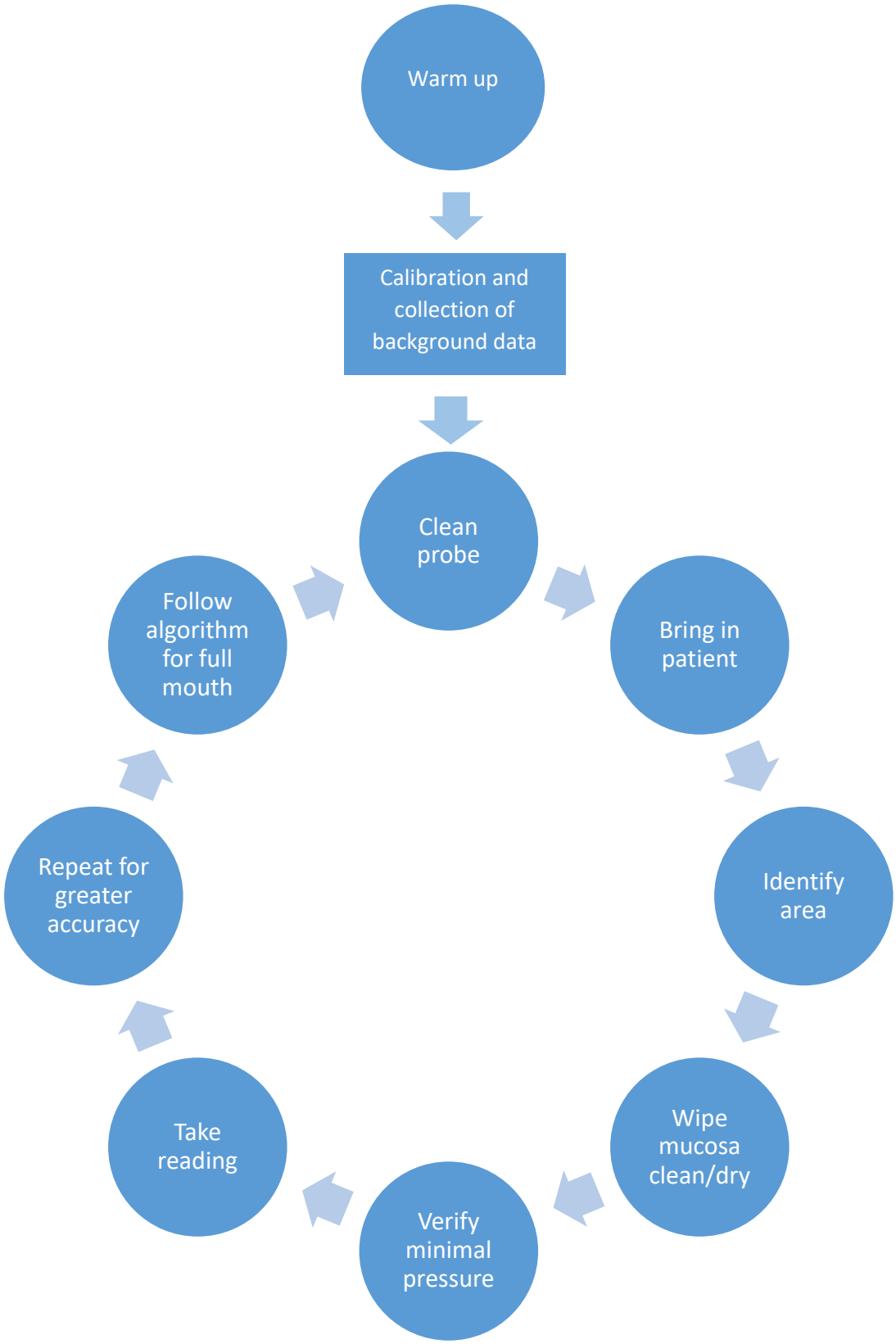
Schwarz 2009[21]

Liu 2023[27]

Recommendation for research/audit

This should be audited for clarity and for ease of reproducibility across healthcare settings and patient groups

Flow diagram of user protocol



References

1. Bydlon TM, Nachabé R, Ramanujam N, et al (2015) Chromophore based analyses of steady-state diffuse reflectance spectroscopy: current status and perspectives for clinical adoption. Journal of Biophotonics 8:9–24. https://doi.org/10.1002/jbio.201300198

2. Fuller C, Camilon R, Nguyen S, et al (2015) Adjunctive diagnostic techniques for oral lesions of unknown malignant potential: Systematic review with meta-analysis. Head Neck 37:755–762. https://doi.org/10.1002/hed.23667

3. Field EA, McCarthy CE, Ho MW, et al (2015) The management of oral epithelial dysplasia: The Liverpool algorithm. Oral Oncology 51:883–887. https://doi.org/10.1016/j.oraloncology.2015.06.015

4. Awadallah M, Idle M, Patel K, Kademani D (2018) Management update of potentially premalignant oral epithelial lesions. Oral Surg Oral Med Oral Pathol Oral Radiol 125:628–636. https://doi.org/10.1016/j.oooo.2018.03.010

5. Mendonca P, Sunny SP, Mohan U, et al (2022) Non-invasive imaging of oral potentially malignant and malignant lesions: A systematic review and meta-analysis. Oral Oncology 130:105877. https://doi.org/10.1016/j.oraloncology.2022.105877

6. Jayanthi JL, Nisha GU, Manju S, et al (2011) Diffuse reflectance spectroscopy: diagnostic accuracy of a non-invasive screening technique for early detection of malignant changes in the oral cavity. BMJ Open 1:e000071. https://doi.org/10.1136/bmjopen-2011-000071

7. Mallia RJ, Narayanan S, Madhavan J, et al (2010) Diffuse Reflection Spectroscopy: An Alternative to Autofluorescence Spectroscopy in Tongue Cancer Detection. Appl Spectrosc 64:409–418. https://doi.org/10.1366/000370210791114347

8. Bailey MJ, Verma N, Fradkin L, et al (2017) Detection of precancerous lesions in the oral cavity using oblique polarized reflectance spectroscopy: a clinical feasibility study. JBO 22:065002. https://doi.org/10.1117/1.JBO.22.6.065002

9. Veld DCG de, Skurichina M, Witjes MJH, et al (2005) Autofluorescence and diffuse reflectance spectroscopy for oral oncology. Lasers in Surgery and Medicine 36:356–364. https://doi.org/10.1002/lsm.20122

10. Yu B, Fu HL, Ramanujam N (2011) Instrument independent diffuse reflectance spectroscopy. J Biomed Opt 16:011010. https://doi.org/10.1117/1.3524303

11. Schwarz RA, Gao W, Daye D, et al (2008) Autofluorescence and diffuse reflectance spectroscopy of oral epithelial tissue using a depth-sensitive fiber-optic probe. Appl Opt 47:825–834

12. Arifler D, Schwarz RA, Chang SK, Richards-Kortum R (2005) Reflectance spectroscopy for diagnosis of epithelial precancer: model-based analysis of fiber-optic probe designs to resolve spectral information from epithelium and stroma. Appl Opt 44:4291–4305

13. Amelink A, Kaspers OP, Sterenborg HJCM, et al (2008) Non-invasive measurement of the morphology and physiology of oral mucosa by use of optical spectroscopy. Oral Oncol 44:65–71. https://doi.org/10.1016/j.oraloncology.2006.12.011

14. Bugter O, Spaander MCW, Bruno MJ, et al (2018) Optical detection of field cancerization in the buccal mucosa of patients with esophageal cancer. Clin Transl Gastroenterol 9:152–152. https://doi.org/10.1038/s41424-018-0023-6

15. Mallia RJ, Subhash N, Mathews A, et al (2010) Clinical grading of oral mucosa by curve-fitting of corrected autofluorescence using diffuse reflectance spectra. Head & Neck 32:763–779. https://doi.org/10.1002/hed.21251

16. Li C, Xia H, Zhou Y, et al (2021) Study on the influence of contact pressure on diffuse spectroscopy measurement of in vivo tissue. Infrared Physics & Technology 114:103669. https://doi.org/10.1016/j.infrared.2021.103669

17. Chan EK, Sorg B, Protsenko D, et al (1996) Effects of compression on soft tissue optical properties. IEEE Journal of Selected Topics in Quantum Electronics 2:943–950. https://doi.org/10.1109/2944.577320

18. Palmer GM, Zhang H, Lee C-T, et al (2018) Assessing effects of pressure on tumor and normal tissue physiology using an automated self-calibrated, pressure-sensing probe for diffuse reflectance spectroscopy. J Biomed Opt 23:057004. https://doi.org/10.1117/1.JBO.23.5.057004

19. Amelink A, Sterenborg HJCM, Roodenburg JLN, Witjes MJH (2011) Non-invasive measurement of the microvascular properties of non-dysplastic and dysplastic oral leukoplakias by use of optical spectroscopy. Oral Oncol 47:1165–1170. https://doi.org/10.1016/j.oraloncology.2011.08.014

20. Hu F, Vishwanath K, Beumer HW, et al (2014) Assessment of the sensitivity and specificity of tissue-specific-based and anatomical-based optical biomarkers for rapid detection of human head and neck squamous cell carcinoma. Oral Oncol 50:848–856. https://doi.org/10.1016/j.oraloncology.2014.06.015

21. Schwarz RA, Gao W, Weber CR, et al (2009) Noninvasive Evaluation of Oral Lesions Using Depth-Sensitive Optical Spectroscopy. Cancer 115:1669–1679. https://doi.org/10.1002/cncr.24177

22. Yu B, Shah A, Nagarajan VK, Ferris DG (2014) Diffuse reflectance spectroscopy of epithelial tissue with a smart fiber-optic probe. Biomed Opt Express 5:675–689. https://doi.org/10.1364/BOE.5.000675

23. Jayanthi JL, Subhash N, Stephen M, et al (2011) Comparative evaluation of the diagnostic performance of autofluorescence and diffuse reflectance in oral cancer detection: a clinical study. Journal of Biophotonics 4:696–706. https://doi.org/10.1002/jbio.201100037

24. Mallia R, Thomas SS, Mathews A, et al (2008) Oxygenated hemoglobin diffuse reflectance ratio for in vivo detection of oral pre-cancer. J Biomed Opt 13:041306. https://doi.org/10.1117/1.2952007

25. Nieman LT, Kan C-W, Gillenwater A, et al (2008) Probing local tissue changes in the oral cavity for early detection of cancer using oblique polarized reflectance spectroscopy: a pilot clinical trial. Journal of biomedical optics 13:024011-024011–11

26. Kolpakov AV, Moshkova AA, Melikhova EV, et al (2023) Diffuse Reflectance Spectroscopy of the Oral Mucosa: In Vivo Experimental Validation of the Precancerous Lesions Early Detection Possibility. Diagnostics (Basel) 13:1633. https://doi.org/10.3390/diagnostics13091633

27. Liu D, Zheng J, Zhang Q, et al (2023) A combined autofluorescence and diffuse reflectance spectroscopy for mucosa tissue diagnosis: Dual-distance system and data-driven decision. Journal of Biophotonics 16:e202300086. https://doi.org/10.1002/jbio.202300086