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High contrast breast cancer biomarker imaging using upconverting nanoparticles

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Abstract: Breast cancer is a leading cause of death in women and has scope for improvement in treatment stratification. We report the use of high contrast UCNP staining to distinguish different levels of HER2 expression in HER2 analyte control and HER2-positive breast cancer tissue biopsies. A contrast of 40 was found as compared to the negative control and 25 as compared to conventional DAB staining. © 2022 The Author(s)

1. Introduction

Breast cancer is the most common cancer in women in the United States, constituting about 30% of reported malignancies every year. It is the second leading cause of death in women with the current trend of increasing the incident rate by 0.5 % annually [1]. The current gold standard diagnostics and prognostics of tumours are based on immunoenzyme-based immunohistochemical (IHC). Standard IHC stains molecular targets on breast cancer tissue (oestrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2)) to create molecular maps of the cancer biomarkers. This enables oncologists to create optimal treatment strategies tailored to individual patient requirements. However, the prediction of patient response to treatment remains a challenge. Some of this can be explained in part by heterogeneity in tumour–genomic, epigenomic and micro-environmental variations. These complex tumour growth dynamics create demand for development of tumour specific tools and their integration into standard breast cancer diagnostics and treatment stratification.

Despite its widespread use and incorporation into clinical practice guidelines, standard IHC has inherent limitations including low dynamic range, difficulties in quantification, subjectivity, multiplexing and co-localisation. Upconverting nanoparticles (UCNPs) have recently emerged as a versatile fluorescence imaging platform with unique photophysical properties desirable in a variety of applications [2, 3]. In comparison, UCNP can provide higher sensitivity, anti-Stokes shifted emission enabling zero tissue autofluorescence background, no photobleaching, and a wider dynamic range of signals for accurate biomarker quantification and prediction of therapeutic response.

The majority of work based on UCNP for breast cancer biomarker imaging is performed on cell lines and lacks biomarker quantification [4]. In this work, we present for the first time the use of UCNP to distinguish different levels of HER2 expression in HER2 analyte control. In addition, we extend the results to image the UCNP and DAPI stained HER2 positive breast cancer tissue.

2. Material and methods

HER2 analyte control DR (HistoCyte Laboratories Ltd, HCL028) with different HER2 expressions were incubated with anti-HER2 rabbit antibody, biotinylated anti-rabbit antibody, and finally labelled with streptavidin-PEG-UCNPs (NaYF4, 40 – 50 nm diameter with 2% Tm3+ and 18% Yb3+ doping). The samples were counterstained with DAPI. Samples were imaged using specially designed multimodal microscope with UCNP excitation at 976 nm and emission at 800 nm. The microscope includes additional excitation lines to perform H&E, DAPI, and DAB imaging. The images were processed with standard background correction and evaluated using spatial Fourier transform filter to allow quantification of the HER2 expression level. In addition, breast cancer slides with DAB staining was used to calculate contrast enhancement as compared to UCNP staining.

3. Results

The results of UCNP imaging of different levels of HER2 expression in HER2 analyte control are shown in Fig. 1. The completeness of cell wall staining at UCNP emission (800 nm) is steadily diminishing with decreasing HER2

levels (3+, 2+, 1+, 0). This indicates less UCNP bound to cells in HER2 analyte control due to lower number of HER2 receptors thus the lower level of HER2 expression. The UCNP images were found to have 40 times higher contrast for HER2 (3+) versus HER2 negative control breast cancer slides. A contrast of 25 was found when compared to classical DAB imaging. All images were processed to remove background obtained from negative control images and transformed to Fourier domain to extract sharp features of cell boundary to quantify HER2 expression. The evaluation showed that we could distinguish different levels of HER2 expression in HER2 analyte control.

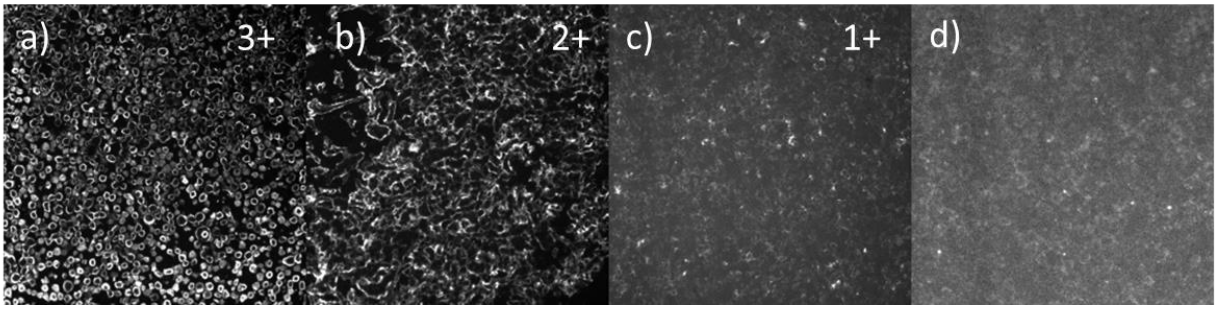


Fig. 1. Microscope images of UCNP stained HER2 analyte control with different levels of HER2 expression a) 3+, b) 2+, c) 1+, d) 0

The staining and image processing methods were extended to real HER2 positive breast cancer tissue to evaluate the effectiveness of the methods developed for HER2 analyte control. Fig. 2 shows the microscope image of UCNP stained HER2 positive breast cancer tissue. The effect of non-specific binding was evaluated. The UCNP staining was found to have superior contrast, 20 times as compared to negative control breast cancer tissue.

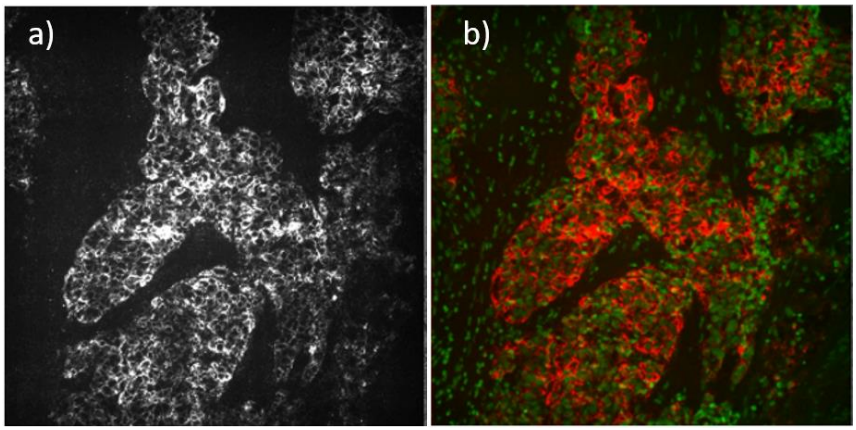


Fig. 2. UCNP Microscope image of a) HER2 positive breast cancer tissue stained with UCNP b) overlay of breast cancer tissue stained with UCNP staining HER2 receptors (red) and DAPI staining the nuclei (green).

4. Conclusion

For the first time, we have reported the use of high contrast UCNP staining to distinguish different levels of HER2 expression in HER2 analyte control and HER2 positive breast cancer biopsies. A Fourier transform-based tool was developed to quantify HER2 expression levels. A contrast 40 times higher was found as compared to negative control breast cancer biopsy which was 25 times higher than the conventional DAB staining. With a great need for better biomarker quantification and multiplexing for treatment stratification, the development of UCNP imaging and advancements in staining processes, we foresee the use of UCNP-based tools in clinical practice for high contrast IHC imaging to improve treatment outcomes for patients.

3. References

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