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# Increasing Objectivity in Quantitative Analysis in Immune-Histochemistry Biomarker Mapping using Upconverting Nanoparticles

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**Abstract:** Today immunohistochemistry (IHC) stains molecular biomarkers for treatment stratification. IHC can become increasingly objective by improving sensitivity, quantification and multiplexing capabilities. We use upconverting nanoparticles to achieve this, potentially leading to improved treatment outcomes. © 2025 The Author(s)

## 1. Introduction - immunohistochemistry and the potential of upconverting nanoparticles staining

Immunohistochemistry (IHC) is a laboratory technique used to detect specific proteins in tissue samples. For clinical use it is combined with conventional histopathology to diagnose diseases like cancer by imaging a palette of protein expression patterns in cells and tissues. For research purposes IHC also plays an important role in mapping proteins within tissue. In neuroscience it is an essential tool to understand the brain by studying where specific proteins are located. IHC involves the use of antibodies that bind to target antigens (proteins), and these antibodies are linked to a reporter molecule, such as an enzyme that can catalyze a chromogenic reaction in the presence of a chromogenic substance, or a fluorescent dye. When the antibody binds to its target, the marker reveals the presence and location of the protein within the tissue. There is still potential for improving IHC, in particular in becoming increasingly objective, sensitive, quantifiable, and multiplexed in the same tissue sample to allow co-localisation analysis. A hypothesis is that the development of IHC in any of these aspects would facilitate a more accurate tool, clinically leading to improved understanding how to best treat patients, potentially leading to improved treatment outcomes.

Since several years, we have been studying whether we can replace the presently used reporter molecules with luminescent upconverting nanoparticles (UCNPs) [1]. Upconverting nanoparticles provide unique photophysical properties, including photostability and anti-Stokes shifted emission. UCNPs are neither photobleaching nor blinking, important properties for a staining agent. A photostable luminescent agent provides a background-free additive that has the potential to provide quantitative information with high signal-to-noise. The anti-Stokes emission shift makes it also possible to eliminate any autofluorescence background signal from the sample, yielding a uniquely low background signal level, yielding an increased sensitivity and thereby increased dynamic range in the detection. Also, the narrow emission lines open the possibility of biomarker multiplexing. Finally, UCNPs can be functionalized to specifically target specific protein biomarkers in the tissue. In this presentation we will mainly focus on IHC for breast cancer as an example.

## 2. UCNP-based immunohistochemistry results

Immunohistochemistry images were recorded using the LUMITO SCIZYS scanner employing SCIZYS Erbium-SA upconverting nanoparticles for staining. As a result of the low background signal obtained, we were able to detect individual UCNPs in a test sample using 20x objective lens and 150 ms exposure time (Fig. 2). This indicates that we have reached the ultimate sensitivity possible. For assessing the photostability of the UCNPs, an area within a pellet section of HER2 positive breast cancer cell with low biomarker expression was selected. Over 500 images were acquired at the system's maximum exposure time of 500 ms. Fig. 1C shows a high degree of photostability upon repeated scanning of the same area.

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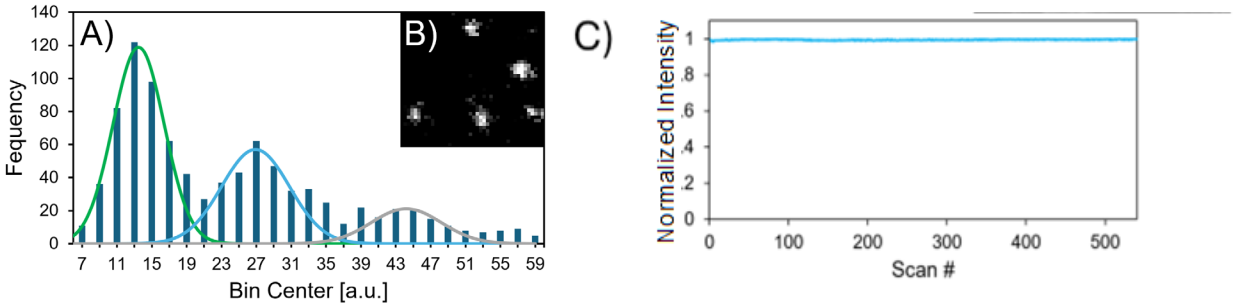



Figure 1: Statistical analysis of UCNP intensity distribution. A) Intensity of 1000 individual diffraction-limited spots analysed and arranged in a histogram (blue bars). Three peaks were identified and fitted with a Gaussian function. Green: Distribution of individual UCNPs. Blue, grey: Two, or more overlapping UCNPs. B) Image of five diffraction-limited spots from the analyzed image. C) Normalized average intensity (blue curve) of an area of low HER2 expressing cells scanned repeatedly. From [2].

Conventional IHC involves horse radish peroxidase/diaminobenzidine (HRP/DAB) staining causing a brownish pigmentation of the sample, visible in normal widefield microscopy. In the current immuno-histochemistry workflow in breast cancer diagnostics, the completeness of HER2 expression along the cell walls in a section of the tissue sample as well as the intensity is typically analyzed. Due to the limited sensitivity and dynamic range in analyzing this pigmentation, the grading can become rather subjective. In Fig. 2 we present anti-HER2 primary antibody (Dako A0485) titration on HER2-overexpressing BT474 cell pellet sections using UCNP-based imaging and compare it to the conventional HRP/DAB technique. We find that the limit of detection (LOD) is one order of magnitude better for the UCNP technique.

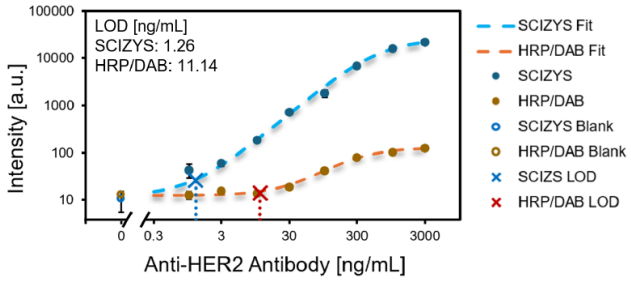



Figure 2: Mean intensities in the cytosol (including the cell membrane) of a 5000×5000 px2 region of BT474 cell pellet sections were calculated. The intensities were averaged and plotted against the primary antibody concentration (filled blue/brown circles). Error bars indicate the standard deviation between cell pellets (N=2–3). The datasets were shifted to start from the same y-value, allowing for better comparison. Four-parameter logistic regression (blue/orange dashed lines) was used to fit the data. Limits of detection (LOD, blue/red cross) were calculated by adding three times the standard deviation of the blanks to the minimum of the regression curve. Both axes in the graph are displayed in log scale.

## 3. Conclusion

We demonstrate several advantages unique to UCNP-based IHC. Autofluorescence-free imaging greatly enhances the sensitivity down to the single particle level, providing a wide linear dynamic detection range, opening the possibility for highly sensitive biomarker quantification. Exceptional photostability facilitates working under ambient light and the possibility to re-scan samples numerous times. In the presentation we further report the use of staining of functionalized high-contrast UCNP to distinguish different levels of HER2 expression in HER2 analyte controls. WE also show the technique working for other biomarkers. We predict that UCNP-stained tissue slide imaging will be introduced for histopathology to improve treatment outcomes for patients, as there is a great need for better biomarker quantification and multiplexing for treatment stratification.

## 4. Acknowlegements

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## 5. References

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