A Novel Technique for Enhanced Detection of HER2-Low Using Photon Upconverting Nanoparticles

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Background

SCIZYS Erbium-SA is a label type based on photon upconverting nanoparticles (UCNPs). UCNPs are near-infrared (NIR) absorbing luminescent nanoparticles with an absorption maximum (976 nm) in the NIR optical window of tissue [1]. Unlike conventional fluorophores emitting light with lower energy/longer wavelength upon excitation (Stokes shift) UCNPs absorb more than one photon per excitation process and emit photons with higher energy/shorter wavelength (anti-Stokes shift) [2].

The NIR excitation and the large anti-Stokes shift removes tissue autofluorescence, enhancing the sensitivity of the system and gives a large dynamic range. UCNPs can be handled under ambient light and maintain constant emission over hundreds of scan cycles [3]. In addition, SCIZYS UCNPs are non-toxic, and compatible with autostainers.

Here we explore the advantages of UCNPs as labels in immunohistochemistry (IHC) compared to traditional chromogenic and fluorescence labelling methods. We demonstrate high sensitivity and wide dynamic range using the breast cancer biomarker HER2.

Results

Assay sensitivity

Boston Cell Standards HER2 IHCalibrators®* were labelled with UCNPs using anti-HER2 antibody CB11 (Leica Biosystems). Labelling was done according to the HER2 protocol published by Lumito [4].

Intensities of calibrator beads were measured, mean and standard deviations were calculated for each bead population. Because each slide contains five separate spots with control beads (no HER2), the average/standard deviation for the blank was calculated by averaging the mean bead intensities of the five control spots. The data was fitted using 4-parameter logistic regression. The LOD was calculated by adding 3-times the standard deviation of the blank into the curve function.

Limit of detection (LOD) of 2400 HER2 molecules/bead was calculated from the analysis of Boston Cell Standards IHCalibrators® labelled with UCNPs (Figure 1 and 2).

For comparison with immunofluorescence, Boston Cell Standards IHCalibrators® were labelled using Tyramide Signal Amplification and the fluorophore Cy5 (TSA-Cy5), using the same primary antibody as for the UCNP labelling. Slides were imaged using a 40x immunofluorescence microscope. LOD for the TSA-Cy5 assay was calculated to be about 16000 HER2 molecules.

Literature shows an LOD for DAB, using the same antibody as for UCNP and TSA-Cy5, to be about 29000 molecules [5].

In conclusion, these initial results demonstrate the LOD for UCNP compared to DAB labelling is about 12 times lower and compared to TSA-Cy5 about 6 times lower.

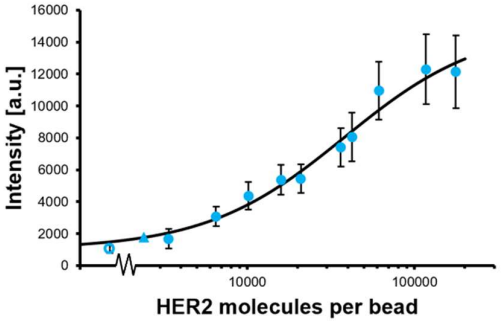

Figure 2. Intensities of UCNP labelled beads were measured, mean and standard deviations were calculated and plotted for each bead population.

Assay dynamic range

A Tissue Microarray (TMA) (AMSBIO Europe) was labelled for HER2 using UCNPs. The TMA was scanned using SCIZYS S1 scanner (Lumito) with standard settings for brightfield and UCNP modalities. The image was imported into the Paikon viewer (Katana Labs). The brightfield image was inverted for clarity and UCNP image superimposed (Figure 3). Two TMA pellets were selected for further analysis, one HER2 3+ and another HER2 0. By adjusting the range for the image both very weak labelling in the HER2 0 sample can be seen, as well as the HER2 3+ labelling without saturation, demonstrating the wide dynamic range of the UCNP labelling.

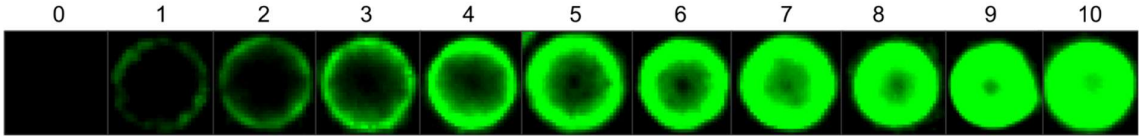

Figure 1. Boston Cell Standards HER2 IHCalibrators® were labelled with UCNPs using antibody CB11. The number above the image indicates the HER2 epitope level.

*Kindly provided by Boston Cell Standards

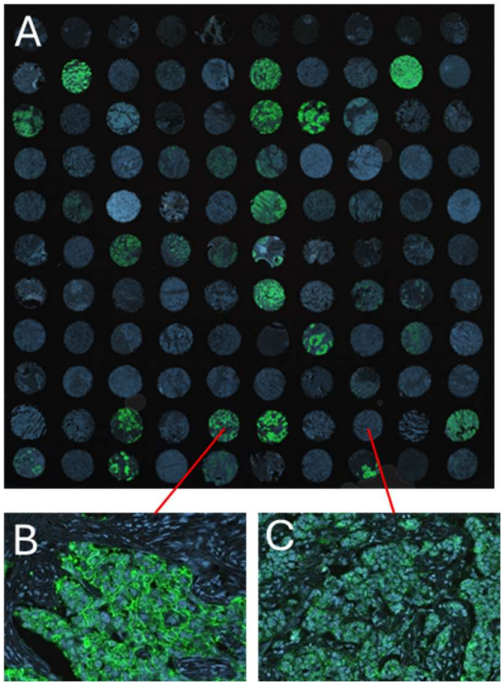

Figure 3. TMA with HER2 0 to 3+ samples in the same image. A) TMA labelled with UCNP. B) HER2 3+ pellet, image shown with range 0-12000 intensity for UCNP channel C) HER2 0 pellet, image shown with range 0-450 intensity for UCNP channel.

Quantitative detection of HER2-low

Four tumour tissue samples scored as HER2 0 by the tissue supplier (AMSBIO Europe) using HRP/DAB were labelled with UCNPs (Figure 4). Samples were scanned in the SCIZYS S1 scanner. Labelling intensities were analysed in QuPath [6] using Stardist [7] with nuclei dilation for cell measurements and were compared to negative control samples, without primary antibody.

Three out of the four samples showed significant HER2 expression compared to the negative control (samples 1-3 in Figure 4), while one sample does not show any significant expression of HER2.

Conclusions

We introduce UCNPs as a label for HER2-low IHC applications. High contrast and autofluorescence-free UCNP-imaging provide enhanced sensitivity and wide dynamic range which can assist in the development of new HER2 therapies as well as improving patient selection for emerging targeted therapies. In the future more accurate diagnostic tests for patients with HER2-low breast cancer could be implemented using UCNP labelling.

Exceptional photostability of UCNPs facilitates working under ambient light and offers the possibility to rescan samples numerous times. Non-toxic reagents and compatibility with autostainers result in methods that can easily be introduced in current IHC workflows.

References

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Contact and disclaimer

The authors may be contacted by sending an email to info@lumito.se.

The authors are employees of Lumito AB (www.lumito.se). The SCIZYS system is intended for research use only.

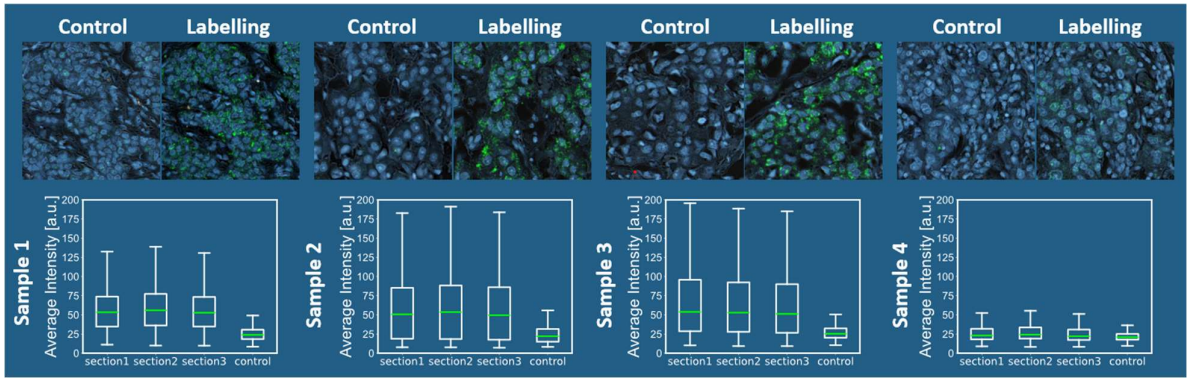

Figure 4 HER2 labelling of tumour tissue samples using A0485 (Dako) in triplicates. Top: Image sections of negative control and corresponding tissue area labelled with SCIZYS UCNPs (Lumito). Bottom: Boxplots of mean intensity around cell nuclei for triplicate labelling and negative control.