Capturing the Full Spectrum: High Dynamic Range Biomarker Detection with Photon Upconverting Nanoparticles

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Background

Photon-upconverting nanoparticles (UCNP) are a novel class of luminescent nanomaterials with unique photophysical properties ideal for the detection of low abundant biomarkers. Here we explain the advantages of UCNPs as labels in immunohistochemistry (IHC) compared to traditional chromogenic labelling methods and demonstrate the high sensitivity and wide dynamic range on the breast cancer biomarker HER2.

UCNPs

In this work, we employed 35 nm spherical shaped $NaYF_4$ UCNPs doped with Yb^{3+} and Er^{3+} (Figure 1). Upon absorption of several low-energy near-infrared photons, UCNPs emit photons with higher energy (anti-Stokes emission). This process eliminates tissue autofluorescence, thus drastically decreasing the measurement background.[1]

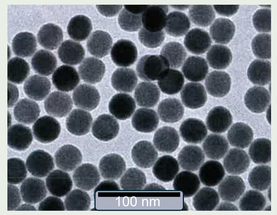

Figure 1: Transmission electron microscopy of UCNPs.

IHC Assay

Typical protocol steps for immunolabelling of biomarkers like HER2 with UCNPs (Figure 2).[2]

- Dewaxing and rehydration
- Antigen retrieval
- Haematoxylin counterstain
- Protein and biotin blocking
- Primary antibody
- Biotinylated secondary antibody
- Streptavidin-coated UCNPs
- Mounting
- Digital whole slide scanning

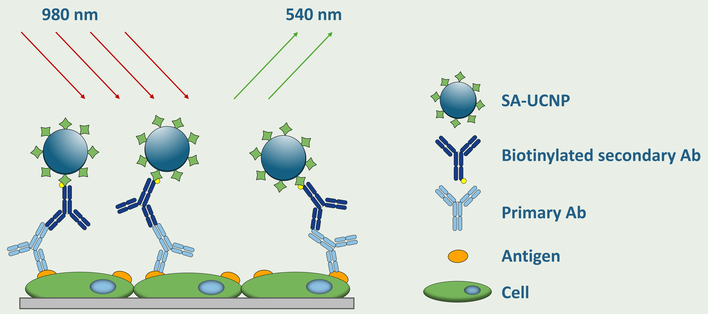

Figure 2: IHC assay scheme.

HER2 Primary Antibody Titration

Serial dilutions of an anti-HER2 antibody (A0485, Dako) between 0–3 µg/mL were applied onto BT474 cell pellets followed by visualization with either UCNP or HRP/DAB (Figure 5). The UCNP labelling was over 10x more sensitive compared to HRP/DAB under similar experimental conditions.

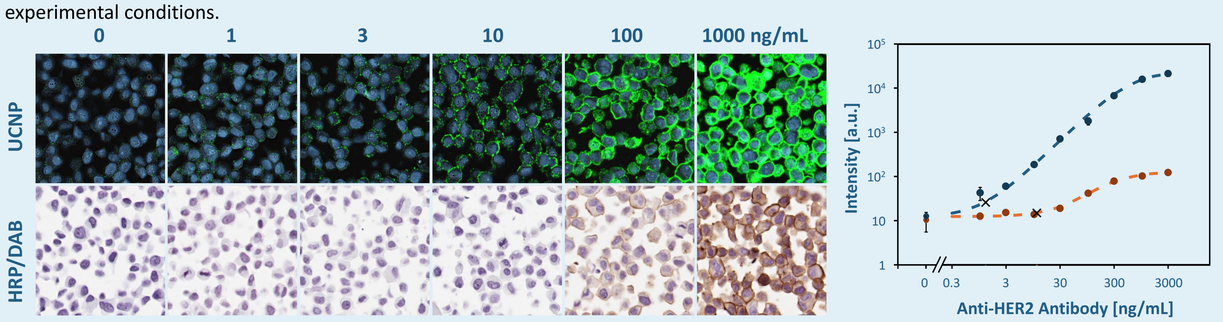

Figure 3: HER2 primary antibody (A0485, Dako) titration on BT474 cell pellets. Left: Image sections of dilution series labelled with UCNP (Lumito) and HRP/DAB. Numbers on top indicate the corresponding primary antibody concentration. Right: Average cell intensities plotted against primary antibody concentration. Curve fitting (dashed lines) was performed using 4-parameter logistic regression. LODs were calculated by adding three standard deviations of the control to the curve minimum and calculating the corresponding x-value.

Parameter	UCNP	HRP/DAB
Limit of Detection [ng/mL]	1.25	11.14
Lower Limit of Quantification [ng/mL]	3.4	31.9
Upper Limit of Quantification [ng/mL]	3900	1470
Dynamic Range [ng/mL]	3.4 - 3900	31.9 - 1470

Photostability

UCNPs are extremely photostable, showing constant emission over hundreds of excitation/emission cycles (Figure 3). Because of the high stability, UCNP labelling can be performed under ambient light.[3]

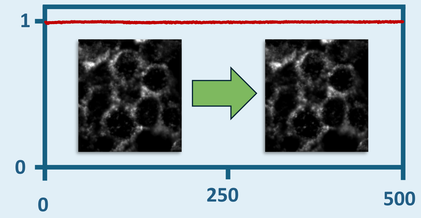

Figure 4: One field of view of a cell pellet with low HER2-expressing cells was scanned 500 times. The intensity in each image was measured, normalized against the highest intensity in the dataset, and plotted against the scan number.

Assay Sensitivity

Limit of detection (LOD) of 2400 HER2 molecules/bead was calculated from the analysis of Boston Cell Standards IHC calibrator beads.

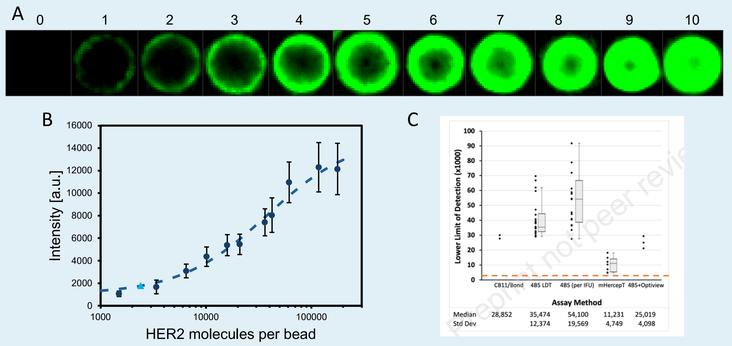

Figure 5: Boston Cell Standards HER2 Calibrator Beads were labelled with UCNPs using CB11 (Leica). A) Image section of HER2 calibrator beads labelled with UCNPs. The number above the image indicates the HER2 epitope level. B) Intensities of beads were measured, mean and standard deviations were calculated 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. C) Comparison with literature using HRP/DAB for visualization.[3]

Highly Sensitive Detection of HER2

Four tumour tissue samples scored as HER2 0 by the tissue supplier (AMS) using HRP/DAB were labelled with UCNPs (Figure 6). Three out of the four samples showed significant HER2 expression compared to the negative control.

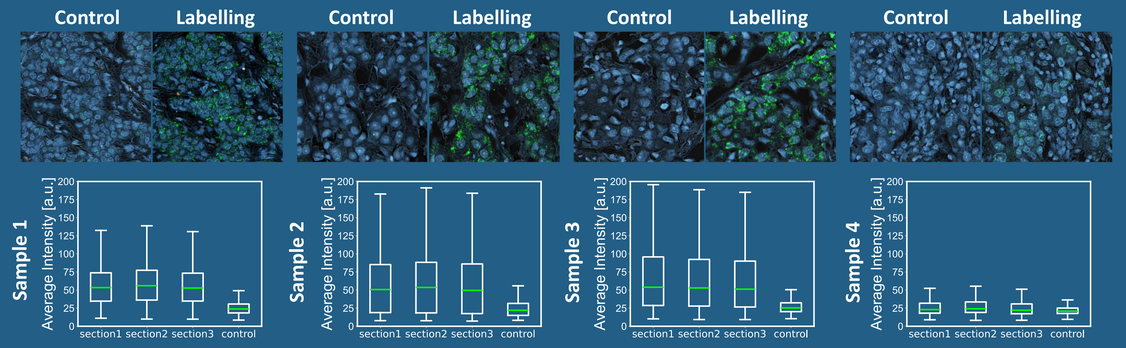

Figure 6: HER2 labelling of tumour tissue samples using A0485 (Dako) in triplicates. Top: Image sections of negative control and corresponding tissue area labelled with SCIZYS UCNPs (Lumito). Bottom: Boxplots of mean intensity around cell nuclei for triplicate labelling and negative control.

Conclusions

UCNP technology demonstrated high sensitivity and dynamic range compared to other traditional IHC methods.[4] Enhanced sensitivity and robustness could lead to more accurate diagnoses and improve the development of targeted therapies for patients with cancer where quantitation and/or ultra-low detection of biomarkers are important e.g., improvement of patient selection for emerging targeted therapies.

References

[1] Farka, Zdeněk, et al. "Surface design of photon-upconversion nanoparticles for high-contrast immunocytochemistry." Nanoscale 12.15 (2020): 8303-8313.
[2] HER2 labelling protocol, https://lumito.se/wp-content/uploads/2025/03/HER2.pdf (accessed: 2025-04-15).
[3] Dabbs, David, et al. "New Standards in Her2-Low Testing: The Casi-01 Comparative Methods Study." Preprint available at: https://ssrn.com/abstract=5087372
[4] Lumito white paper, https://lumito.se/wp-content/uploads/2024/07/White-Paper_A-Novel-Tool-for-Immunohistochemistry_SCIZYS_202406.pdf (accessed: 2025-04-15).

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