

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 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]

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

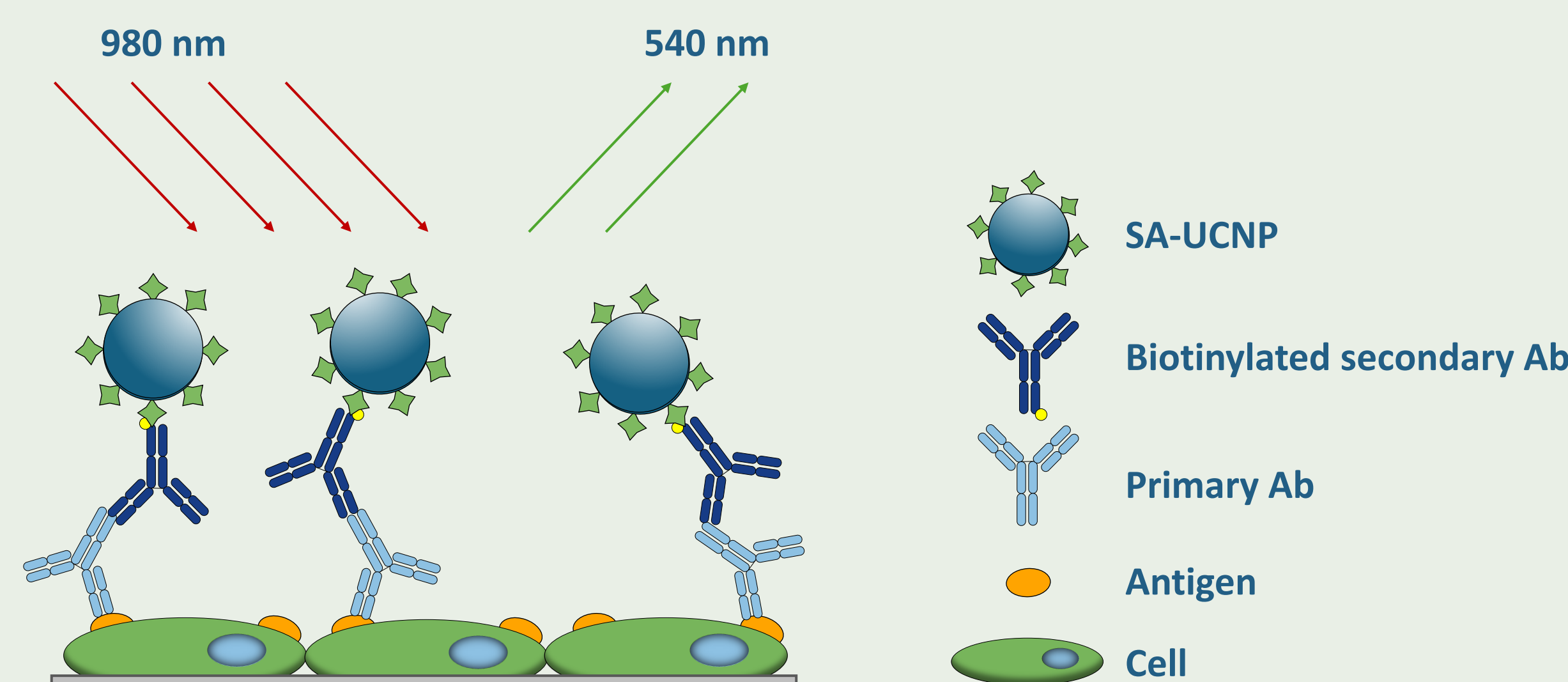
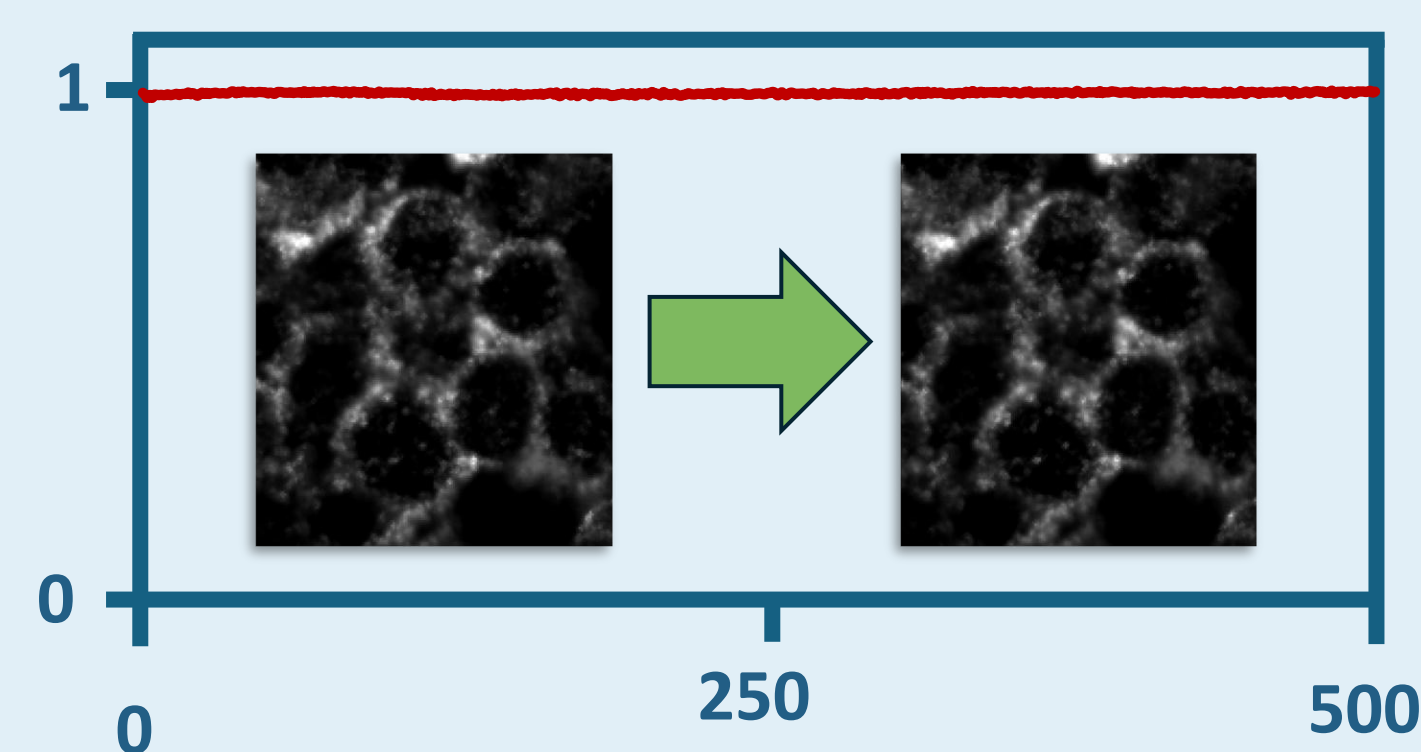


Figure 2: IHC assay scheme.

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 3: 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.



HER2 Dynamic Range Control

UCNP labelling of a HER2 control pellet array (Figure 4).

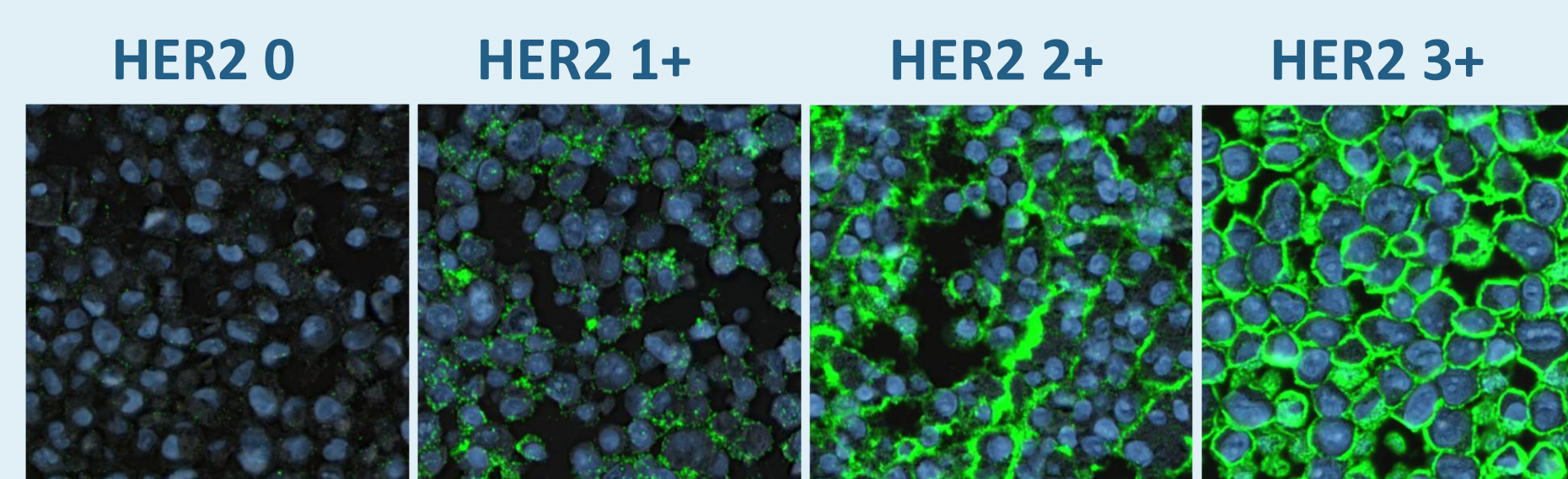


Figure 4: UCNPs were used to label HER2 on a control pellet array (AMS). First, a brightfield image of the haematoxylin counterstain was acquired, followed by 980 nm excitation of the UCNPs at the exact same position. The brightfield image was converted to pseudo fluorescence and the UCNP image superimposed.

HER2 Primary Antibody Titration

Serial dilutions of an anti-HER2 antibody (A0485, Dako) between 0–3 $\mu\text{g}/\text{mL}$ 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.

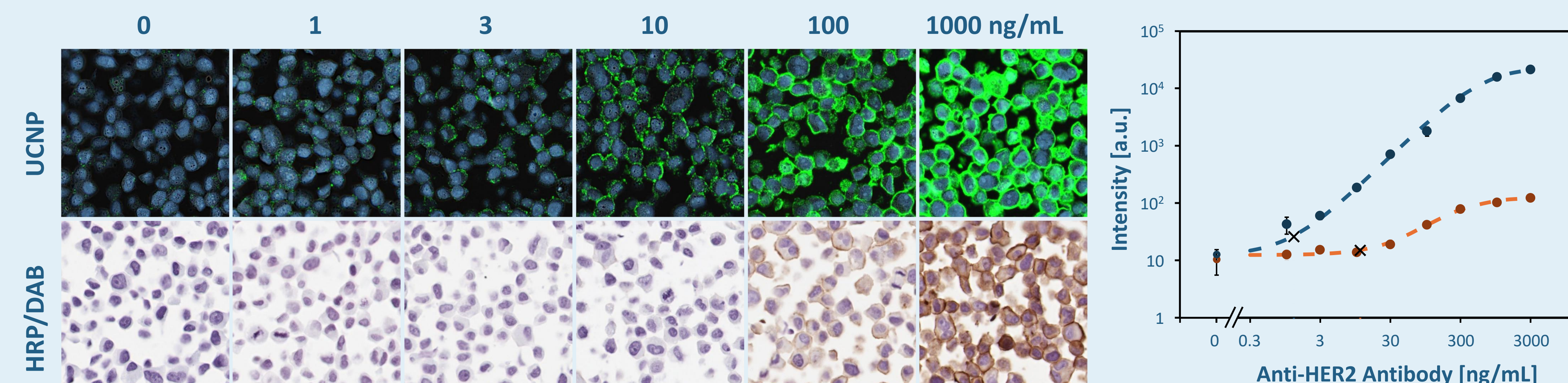


Figure 5: HER2 primary antibody (A0485, Dako) titration on BT474 cell pellets. Left: Image sections of dilution series labelled with UCNP (Lumito) and HRP/DAB. Numbers on top indicate the corresponding primary antibody concentration. Right: Average cell intensities plotted against primary antibody concentration. 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

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.

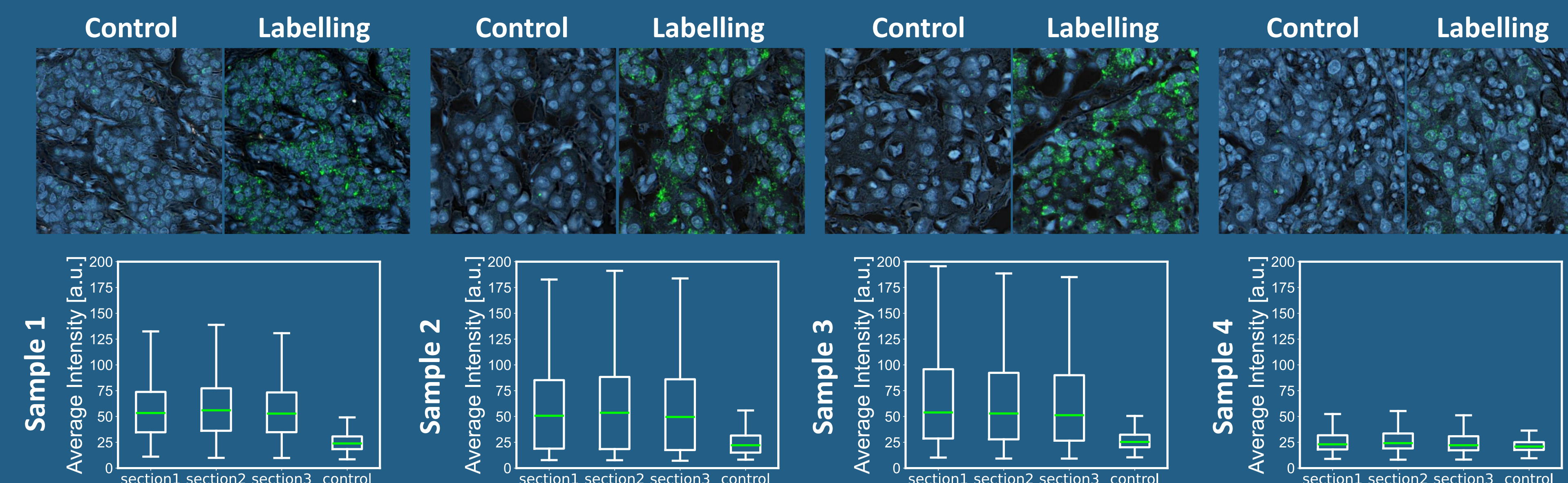


Figure 6: HER2 labelling of tumour tissue samples using A0485 (Dako) in triplicates. Top: Image sections of negative control and corresponding tissue area labelled with SCIZYS UCNPs (Lumito). Bottom: Boxplots of mean intensity around cell nuclei for triplicate labelling and negative control.

Conclusions

We introduced UCNPs as novel labels for IHC applications. Bright UCNP particles and autofluorescence-free imaging provides enhanced sensitivity and could lead to more accurate diagnoses as well as improving the development of targeted therapies for patients with HER2-low breast cancer. Exceptional photostability facilitates working under ambient light and offers the possibility to rescan samples numerous times.

