A Novel Tool for Immunohistochemistry: SCIZYS by Lumito

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Background

SCIZYS Erbium-SA is a new label type based on photon-upconversion nanoparticles (UCNPs). UCNPs are near-infrared (NIR) absorbing luminescent nanoparticles with an excitation 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] This photon upconversion process completely removes tissue autofluorescence. It greatly enhances the detection sensitivity of the system, enabling the visualisation of individual UCNPs. In addition, UCNPs possess extreme photostability and can thus be handled under ambient light and maintain a constant emission over hundreds of scan cycles.

Here, we show some of the outstanding properties of UCNP-based immunohistochemistry (IHC) labels in the SCIZYS system.

Results

Autofluorescence

For studying tissue autofluorescence, formalin-fixed paraffin-embedded human liver tissue sections were dewaxed, rehydrated and antigen retrieval was performed.

For SCIZYS, sections were counterstained with haematoxylin only (no UCNPs) and imaged under brightfield illumination (Fig. 1A) and in the UCNP channel under 976 nm NIR illumination (Fig. 1B).

For comparison, similar sections were imaged without any counterstain in the Alexa Fluor (AF) 488 (Fig. 1C) and AF 647 (Fig. 1D) channel using a conventional fluorescence microscope.

Results demonstrate that SCIZYS is free from autofluorescence (Fig. 1B), with only camera noise visible at high brightness settings.

Significant autofluorescence was found upon excitation using an AF 488 filter set (Fig. 1C). Lower but still clearly visible autofluorescence was present if images were acquired using a filter set for AF 647 (Fig. 1D).

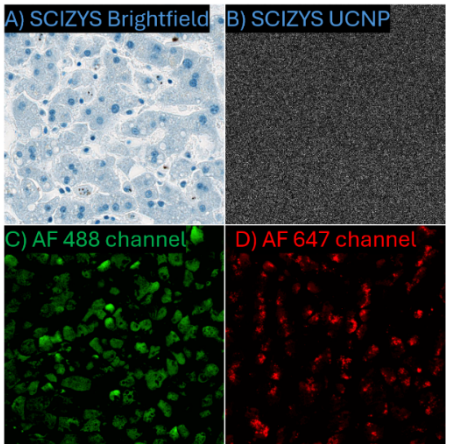

Figure 1: SCIZYS autofluorescence comparison. A) Brightfield image of haematoxylin-counterstained human liver tissue. B) Same region as A) under 976 nm irradiation. Autofluorescence of unstained human liver tissue at C) 488 nm excitation and D) 647 nm excitation. Images A) and B) were captured with the SCIZYS scanner, C), and D) using a conventional epifluorescence microscope.

SCIZYS Scanner Sensitivity

The low optical background provided by the UCNP technology enables the SCIZYS scanner to detect individual UCNPs as diffraction limited spots (Fig. 2).

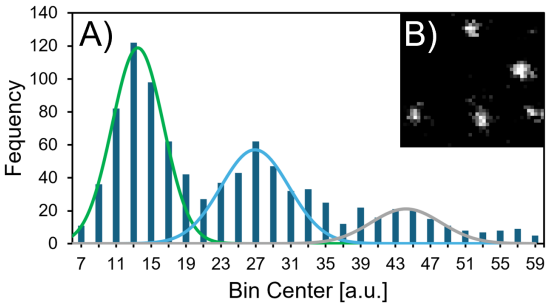

Figure 2: Statistical analysis of the UCNP intensity distribution.[3] A) Intensities of 1000 individual diffraction-limited spots were measured and arranged in a histogram (blue bars). Three peaks were identified and fitted with a Gaussian function. Distribution of individual UCNPs (green). Two, or more overlapping UCNPs (blue, grey). B) Image of five diffraction-limited spots from the analysed image.

The SCIZYS scanner's ability to detect individual luminescent labels using 20× magnification and an exposure time of 150 ms makes it extremely sensitive. The device scans large areas in a short time, while providing single-particle sensitivity. SCIZYS Erbium-SA particles have a typical diameter of approximately 35 nm, but they appear larger due to the diffraction limit of the optical system. Detecting individual UCNPs provides a strong foundation for biomarker quantification.

[1] Sedlmeier, A., Hlaváček, A., Birner, L., Mickert, M. J., Muhr, V., Hirsch, T., Corstjens, P. L. A. M., Tanke, H. J., Soukka, T., Gorris, H. H. (**2016**). Highly sensitive laser scanning of photon-upconverting nanoparticles on a macroscopic scale. *Anal. Chem.*, *88*(3), 1835-1841.

[2] Gorris, H. H., & Wolfbeis, O. S. (**2013**). Photon-upconverting nanoparticles for optical encoding and multiplexing of cells, biomolecules, and microspheres. *Angew. Chem. Int. Ed.*, *52*(13), 3584-3600.

[3] Hlaváček, A., Mickert, M. J., Soukka, T., Lahtinen, S., Tallgren, T., Pizúrová, N., Król, A., Gorris, H. H. (**2018**). Large-scale purification of photon-upconversion nanoparticles by gel electrophoresis for analogue and digital bioassays. *Anal. Chem.*, *91*(2), 1241-1246.

SCIZYS Photostability

For assessing the photostability of SCIZYS Erbium-SA, an area with low biomarker expression was selected within a cell pellet section. Over 500 images were acquired at the maximum exposure time of the system (500 ms). Fig. 3A displays a small section of the analysed field of view at the beginning of the experiment and Fig. 3B shows the same area after over 500 exposures.

Plotting the average intensities for consecutive scans against the scan number revealed consistent emission levels throughout the entire experiment. (Fig. 3C). This demonstrates the superior photostability of SCIZYS Erbium-SA.

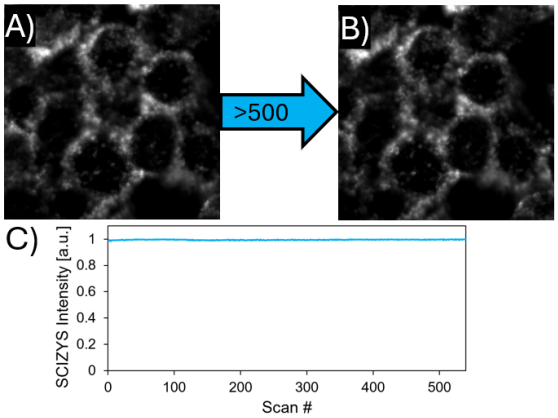

Figure 3: A) Low HER2 expressing cells labelled with SCIZYS Erbium-SA scanned using the SCIZYS scanner. B) Same area after >500 scans. C) Normalised average intensity of SCIZYS Erbium-SA.

SCIZYS Assay Sensitivity

For assessing the assay sensitivity of the SCIZYS system, a HER2 primary antibody titration was performed (Fig. 4) and compared with the HRP/DAB gold standard.

The polymeric HRP/DAB secondary antibody label showed faint brownish membranous labeling on BT474 cells at 20 ng/mL of primary anti-HER2 antibody and clear membranous labelling at 60 ng/mL of primary antibody (Fig. 4A).

SCIZYS Erbium-SA was bound to the primary antibody via a biotinylated secondary antibody. The assay showed noticeable membranous labelling at 6 ng/mL of primary antibody. Strong membranous labelling was observed at 20 ng/mL (Fig. 4B).

Additionally, the biomarker PD-L1 was labelled on a PD-L1 dynamic range control (Fig. 5). No significant particle binding was observed on PD-L1 negative breast ductal carcinoma cells (Fig. 5A). Low PD-L1 expressing osteosarcoma showed membranous binding of SCIZYS Erbium-SA (Fig. 5B). Medium PD-L1 expressing fibrosarcoma showed strong binding (Fig. 5C) and the strongest signals were observed on high PD-L1 expressing T-cell non-Hodgkin lymphoma (Fig. 5D).

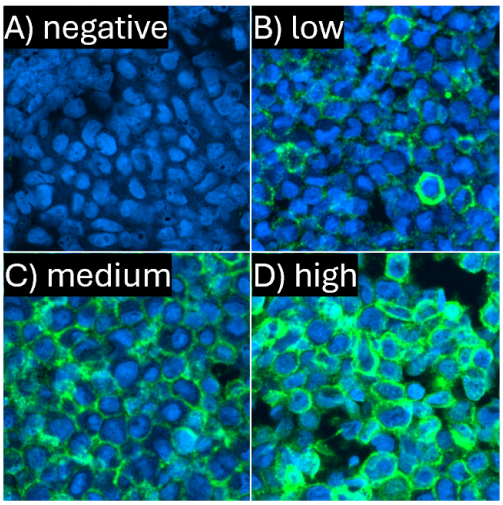

Figure 5: Labelling of PD-L1 using SCIZYS Erbium-SA (green) on A) PD-L1 negative breast ductal carcinoma, B) low PD-L1 expressing osteosarcoma, C) medium PD-L1 expressing fibrosarcoma, and D) high expression T-cell non-Hodgkin lymphoma. Haematoxylin counterstain (blue) displayed in pseudo fluorescence. Image brightness lowered in C) and D) for better visibility.

Conclusions

We demonstrated several advantages unique to the SCIZYS UCNP-based system making it a powerful novel tool for IHC applications. Autofluorescence-free imaging greatly enhances the system sensitivity down to the single particle level, opening the possibility for highly sensitive biomarker quantification. Exceptional photostability facilitates working under ambient light and the possibility to rescan samples numerous times. In IHC applications the SCIZYS system is proven to be efficient in detecting minute amounts of analytes.

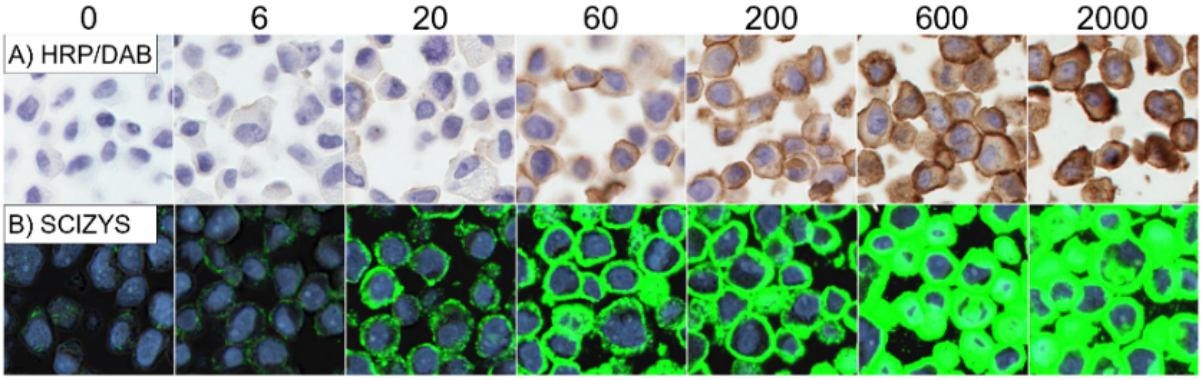

Figure 4: Anti-HER2 primary antibody titration on BT474 cells using A) HRP/DAB, and B) SCIZYS Erbium-SA (green) all image sections are shown with the same brightness settings. The haematoxylin counterstain in B) was converted to pseudo fluorescence for better visibility. Numbers above the panels indicate the primary antibody concentrations in ng/mL.