WHITE PAPER

A Comparative Study on Direct vs Indirect Labelling of Biomarkers using SCIZYS Erbium

Achieving Quantitative Biomarker Detection with Reduced Assay Complexity

LUMITO

ATLAS ANTIBODIES

Achieving Quantitative Biomarker Detection with Reduced Assay Complexity

This white paper compares direct and indirect immunohistochemistry workflows using validated antibodies from Atlas Antibodies in combination with Lumito's SCIZYS system, showing that simplified direct labelling can match the quantitative performance of traditional indirect methods while reducing assay complexity.

Background & Aim

Reliable detection of protein biomarkers is fundamental to surgical pathology and translational research, where assay performance directly impacts biological interpretation and diagnostic confidence. Traditional immunohistochemistry (IHC) typically relies on indirect detection, in which a primary antibody binds the target antigen and is subsequently recognized by a secondary antibody conjugated to a signal generating label.[1] While this approach enables signal amplification, it can also introduce assay complexity and increased background staining, particularly when the primary antibody and the tissue share the same host species. Direct labelling strategies, using conjugated primary antibodies, offer a simplified workflow and a reduced processing time. However, concerns around reduced sensitivity have limited broader adoption.[2]

Photon upconverting nanoparticles (UCNPs) are a novel class of luminescent labels with photophysical properties particularly well suited for IHC applications. UCNPs are excited in the Near-infrared (NIR) region at 976 nm. Upon absorbing two or more NIR photons, UCNPs convert this low energy excitation into visible emission. Because excitation occurs in the NIR window and the emitted signal arises from an upconversion process, autofluorescence is eliminated, enabling highly sensitive detection of UCNPs.[3] Building on these advantages, Lumito has translated the UCNP technology into a dedicated reagent kit (SCIZYS Erbium-SA) and wholeslide scanner (SCIZYS S1), collectively marketed as SCIZYS (for research use only).

In this study, we evaluated direct and indirect labelling approaches using validated primary antibodies from Atlas Antibodies together with Lumito's SCIZYS platform with the aim of assessing assay performance, quantitative robustness, and practical applicability in tissue-based biomarker analysis.

Study Design & Experimental Approach

To ensure a robust and unbiased comparison, the same primary antibodies from Atlas Antibodies were used across both direct and indirect labelling strategies. A schematic representation of the labelling is displayed in **Figure 1.**

Primary antibodies targeting progesterone receptor (PR) and DNA mismatch repair proteins MSH2 and MSH6 (AMAb91529, AMAb91892 and AMAb91903, respectively) were extensively validated using conventional IHC-DAB in panels of cell line controls (including PR Dynamic Range Analyte Control HCL034 and MMR Dynamic Range Analyte Control HCL043, Histocyte Laboratories, Newcastle upon Tyne, UK) and control formalin-fixed paraffin-embedded (FFPE) tissues (purchased from commercial biobanks, Asterand®, BioIVT, West Sussex, UK; Tissue Solutions Ltd, Scotland, UK; and Indivumed GmbH, Hamburg, Germany) prior to UCNP-IHC analysis. Brightfield IHC images were captured using automated scanning microscopy (MetaSystems, Altlussheim, Germany).

For direct labelling, primary antibodies were biotinylated using a site-specific conjugation method and detected using the Lumito UCNP-based system. In the indirect approach, unconjugated primary antibodies were detected via biotinylated secondary antibodies. Multiple tissue types were analysed using both strategies.

Digital whole-slide images were acquired using the Lumito SCIZYS-S1 imaging platform, followed by automated cell segmentation and quantitative analysis. Average cell intensities were measured, positive cells were identified based on predefined thresholds, and agreement between labelling strategies was evaluated using Bland–Altman analysis.

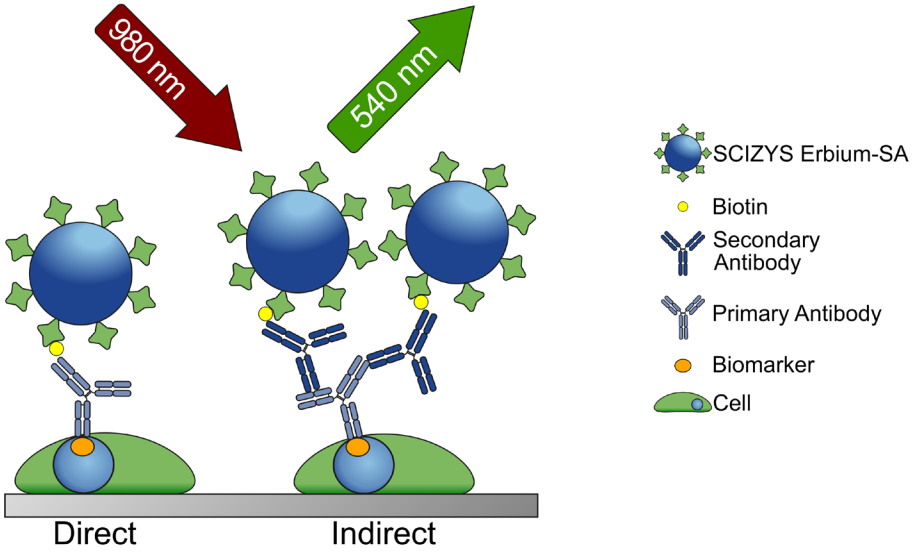

Figure 1
Assay scheme for direct and indirect immunolabelling using SCIZYS Erbium-SA. In the direct format *(left)*, tissue sections are incubated with a biotinylated primary antibody, followed by SCIZYS Erbium-SA. In the indirect format *(right)*, a biotinylated secondary antibody binds to the unlabelled primary antibody before incubation with SCIZYS Erbium-SA. During image acquisition, the SCIZYS Erbium-SA particles are excited with NIR light and emit visible light, which is subsequently detected.

Results

Primary antibody specificity confirmed by multiple cell line and tissue controls

Anti-PR, Anti-MSH2 and Anti-MSH6 monoclonal antibodies were validated by IHC-DAB using dynamic range cell line controls with predetermined protein expression, as well as in normal and cancerous FFPE tissues.

Anti-PR monoclonal antibody AMAb91892 displayed expected staining pattern in the dynamic range cell line controls with PR expression levels ranging from negative to high (**Figure 2A-D**). In control FFPE human tissues, antibody could successfully detect PR positivity in the PR-positive breast cancer (**Figure 2E**) and was negative in the PR-negative sample (**Figure 2F**). Furthermore, normal tissues including fallopian tube and pancreas, used as high-expressing positive control and low-expressing control, exhibited expected immunoreactivity in glandular cells of fallopian tube and in a subset of cells in pancreatic islets (**Figure 2 G,H**).

Both Anti-MSH2 AMAb91892 (**Figure 3**) and Anti-MSH6 AMAb91903 (not shown) antibodies exhibited expected expression pattern in the MMR cell line controls and FFPE human tissues.
Figure 3 shows expected MSH2 expression pattern in cell line controls with intact expression in cell lines A, B and D, and loss of expression in cell line C. Expected nuclear expression was also detected in non-mutant colorectal cancer sample (**Figure 3E**), as well as positive control tonsil and testis tissues (**Figure 3F,G**), while absence of positivity was observed in liver used as a negative control (**Figure 3H**).

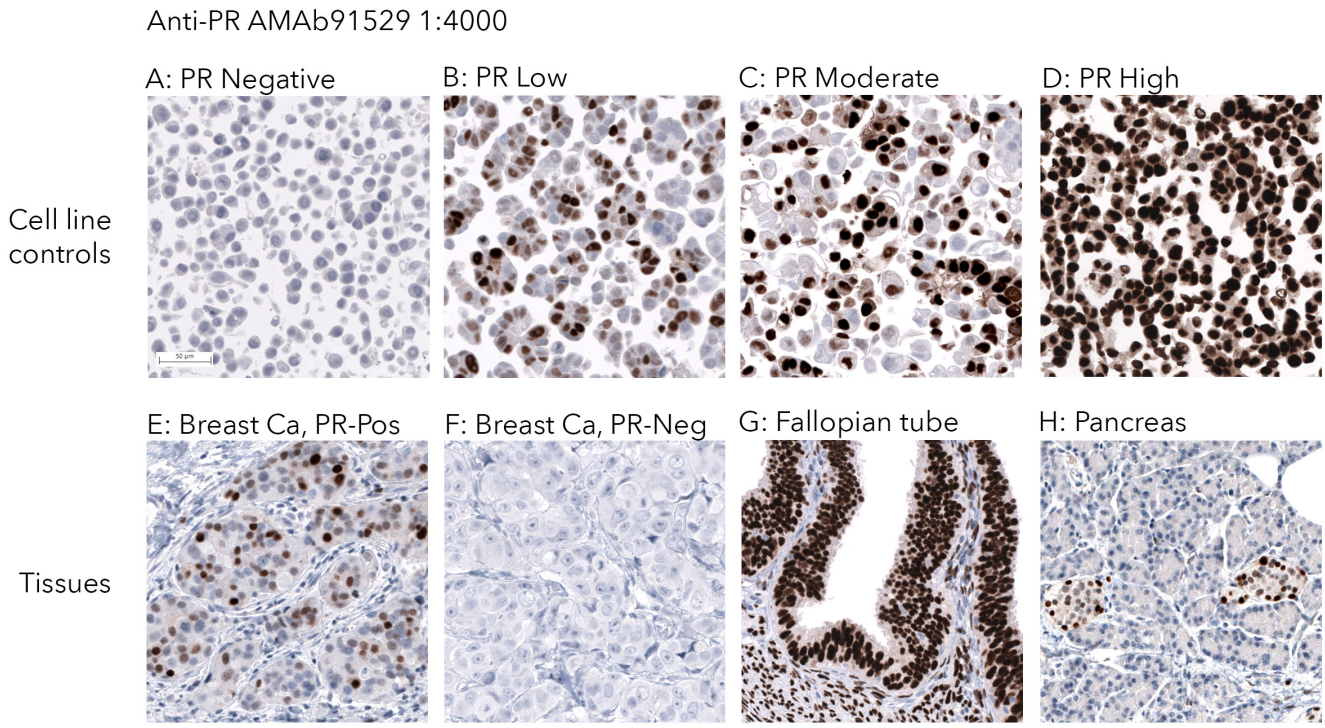

Figure 2
IHC-DAB validation of specificity and sensitivity of the Anti-PR monoclonal antibody AMAb91529 using dynamic range cell line controls (**A-D**), and FFPE tissues with predefined PR expression, including moderate (breast cancer, **E**), negative (breast cancer, **F**), high (fallopian tube, **G**) and low (pancreas, **H**) protein expression levels.

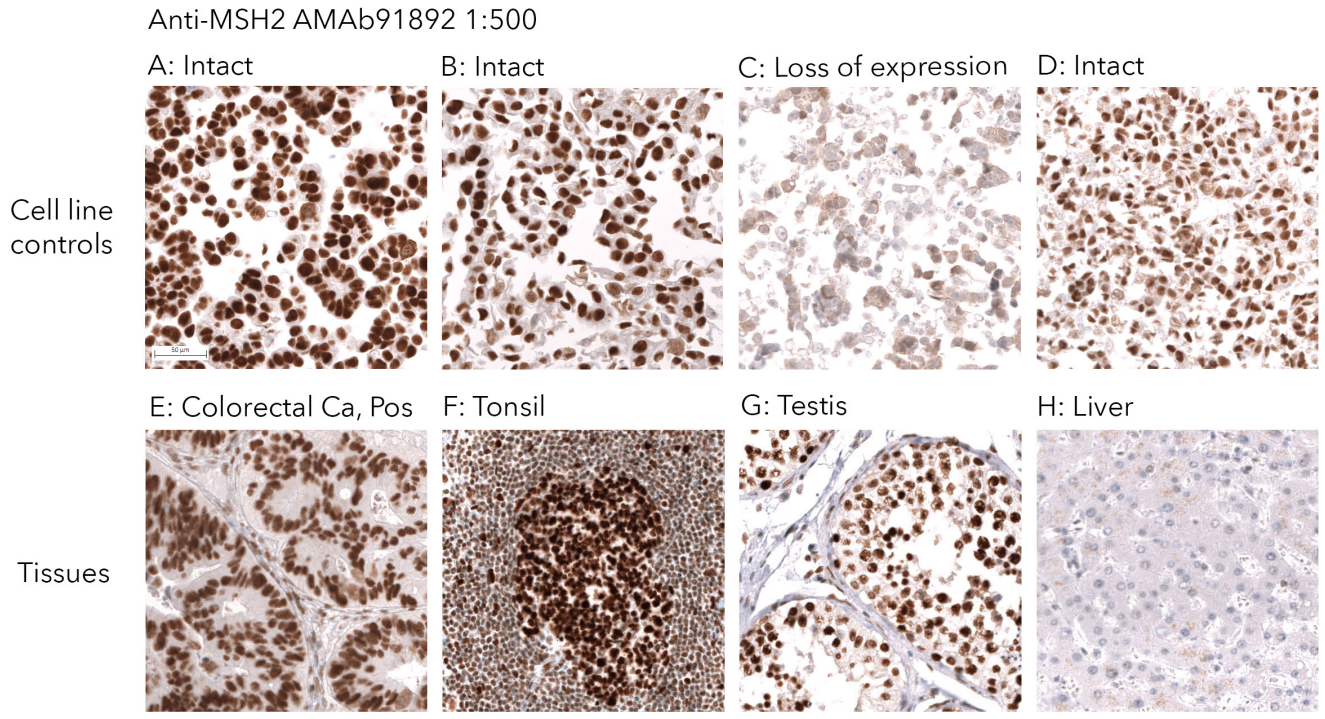

Figure 3
IHC-DAB validation of specificity and sensitivity of the Anti-MSH2 monoclonal antibody AMAb91892 using cell line controls with predefined MSH2 expression (**A-D**), and FFPE tissues with expected high (colorectal cancer, tonsil and testis, **E-G**) and negative (liver, **H**) expression.

Direct vs Indirect Labelling of Biomarkers using SCIZYS Erbium-SA: comparable detection with simplified workflow

To ensure comparability, the same primary antibodies from Atlas Antibodies were used for both approaches.

For direct labelling, the primary antibodies (Anti-PR AMAb91892; Anti-MSH2 AMAb91892; Anti-MSH6 AMAb91903) were biotinylated and subsequently incubated with SCIZYS Erbium-SA, the indirect method employed biotinylated secondary antibodies and the unconjugated version of the primary antibodies.

Exemplary for the dynamic range analyte controls, Figure 4 displays the direct (**Figure 4A–D**) and indirect labelling (**Figure 4E–H**) of PR using SCIZYS Erbium-SA. Both techniques resulted in a similar number of positive cell nuclei, but overall intensities for the direct labelling were lower (not shown, brightness increased for direct labelling). Also on tissue samples, the direct labelling (**Figure 5A,C,E**) produced staining patterns similar to the indirect labelling with lower intensity (**Figure 5B,D,F**).

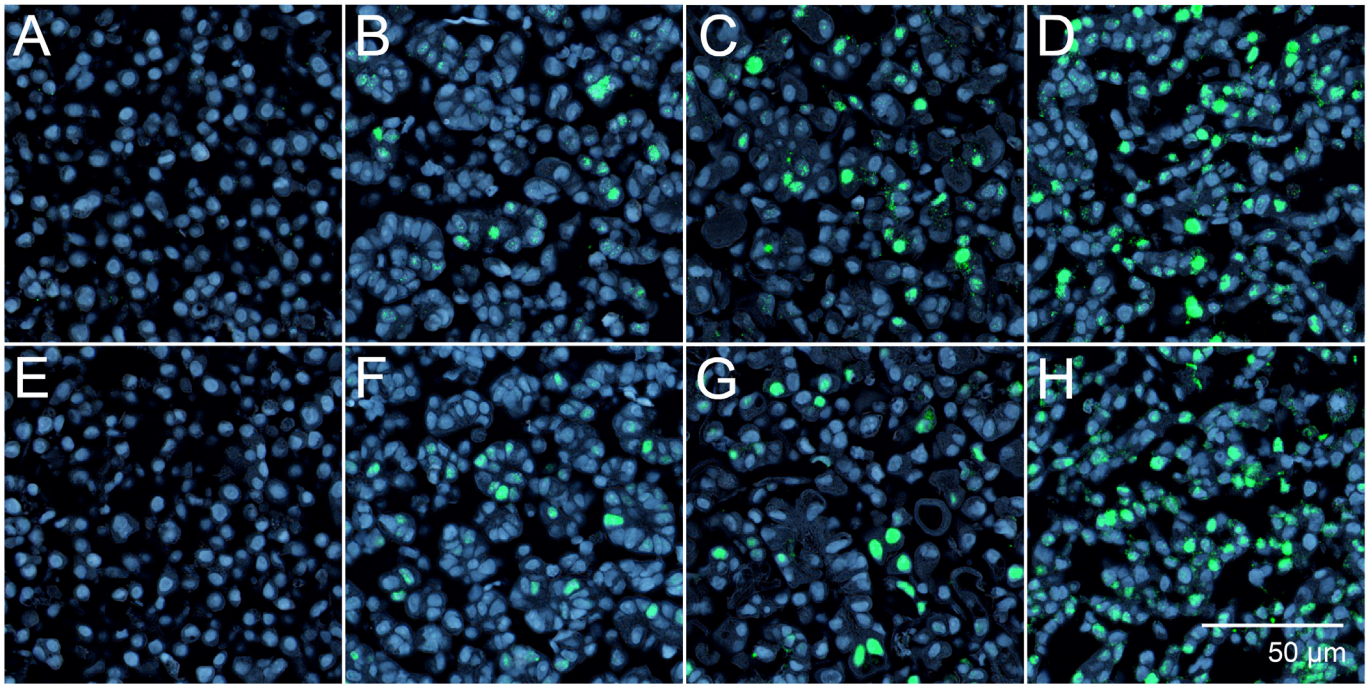

Figure 4
Direct (**A–D**) and indirect (**E–H**) immunolabelling of a PR dynamic range analyte control using SCIZYS Erbium-SA. **A,E:** PR negative ductal carcinoma; **B,F:** PR low breast adenocarcinoma; **C,G:** PR intermediate ductal carcinoma; **D,H:** PR high ductal carcinoma cell lines.

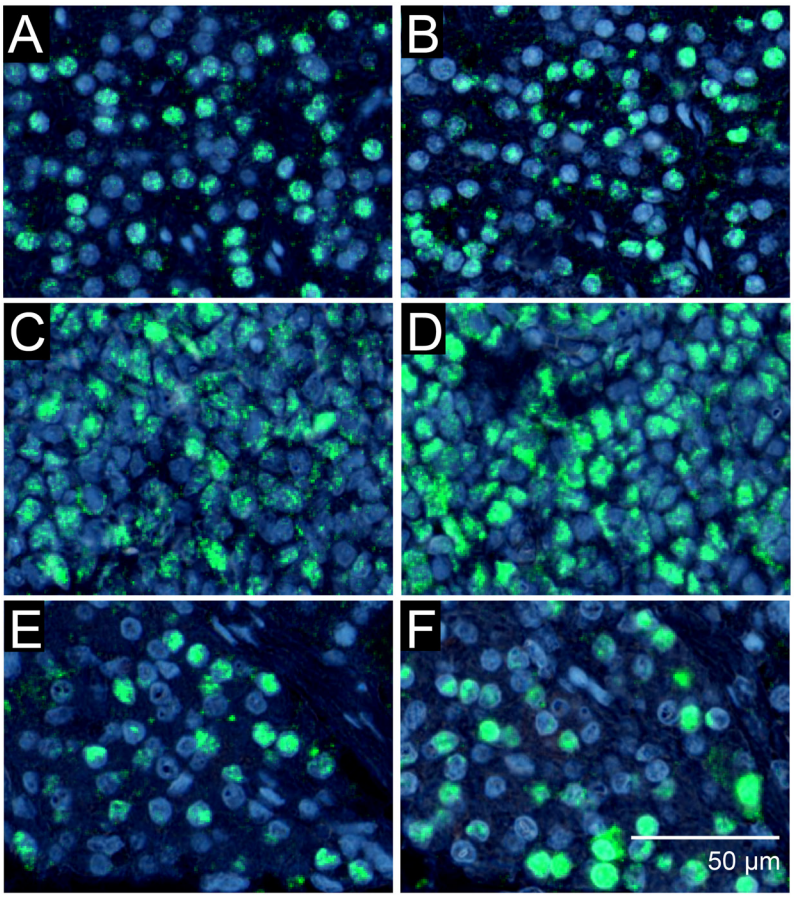

Figure 5
Direct *(left)* and indirect *(right)* labelling of biomarkers in tissue sections using SCIZYS Erbium-SA (green). Cell nuclei (blue) were counterstained with haematoxylin. The brightfield image was converted to pseudo fluorescence. **A,B:** Labelling of PR in pancreatic islets. **C,D:** Labelling of MSH2 in tonsil. **E,F:** Labelling of MSH6 in testis. The brightness was increased for images A, C, E.

Several tissue types were labelled using both techniques, and cell segmentation was performed using the digital whole slides scans obtained from the SCIZYS S1 scanner. Average cell intensities were measured and cells above a set threshold were counted. Plotting the difference of the indirect and direct measurement against the average number of positive cells of both methods gives a Bland-Altman plot (**Figure 6**).

The Bland–Altman analysis showed excellent agreement between direct and indirect labelling, with an average sigma of 10.4%. The intraclass correlation coefficient (ICC) was 0.89, further confirming the strong agreement between the two methods.

The indirect labelling produced higher signal intensities due to amplification by multiple secondary antibodies binding to a single primary antibody.
To compare relative signal strength, average intensities of positive cells were normalised to negative controls (no primary antibody) and visualised in a spider plot (**Figure 7**).

Signal to background ratios showed similar trends for both direct and indirect labelling. Although indirect labelling produced brighter signals, the secondary antibody step also introduced higher non-specific binding.

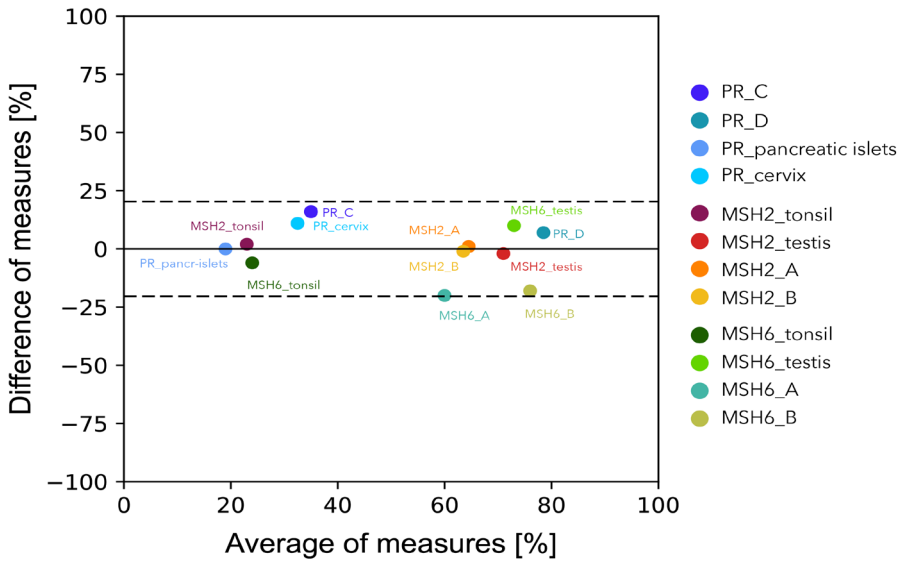

Figure 6
Bland-Altman plot of indirect/direct labelling on different tissue types. Points laying on the zero-line indicate perfect agreement of direct/indirect labelling. Points above the line represent labellings where the indirect labelling resulted in more positive cells than the direct labelling. Points below the zero-line represent labellings where the direct labelling gave more positive cells that the indirect labelling.

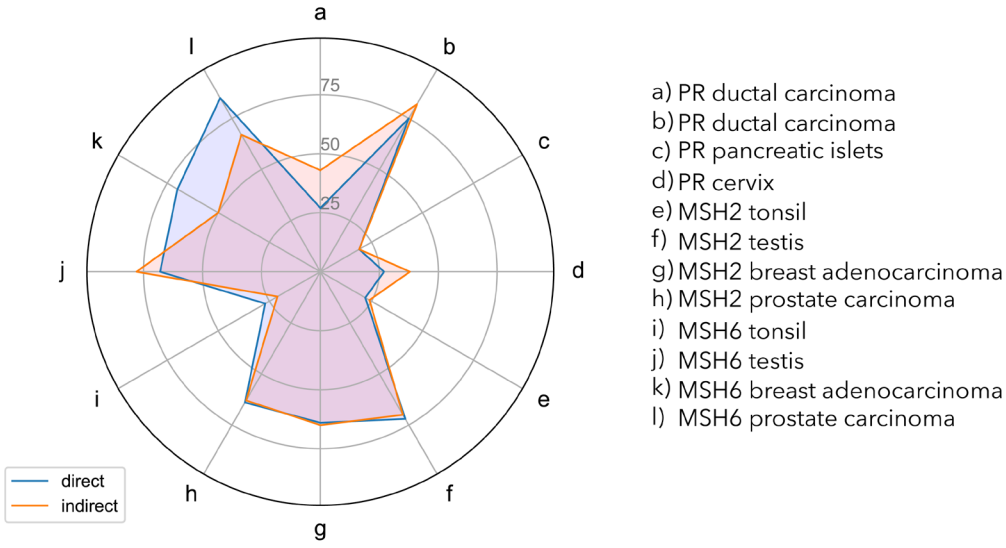

Figure 7
Spider plot of signal-to-background ratios for direct and indirect labelling of PR, MSH2, MSH6 in different tissues. Background was obtained from corresponding negative controls (no primary antibody added).

Conclusions

Accurate and reproducible antibody-based assays are essential in tissue-based biomarker analysis, where false-positive or false-negative results can have significant downstream consequences.

This study demonstrates that the SCIZYS system, combined with well validated antibodies from Atlas Antibodies, enables reliable biomarker detection with high sensitivity and quantitative reliability. By reducing assay complexity while maintaining high performance, direct labelling represents an attractive alternative to traditional indirect workflows.

Together, these complementary technologies provide a flexible and scalable solution for research and pathology applications, supporting confident biomarker assessment across research and clinical pathology applications.

References

[1] I. D. Odell, D. Cook, J. Invest. Dermatol., 2013, 133(1), 1–4.
[2] J.-M. Fritschy, Eur. J. Neurosci., 2008, 28(12), 2365–2370.
[3] Z. Farka, M. J. Mickert, M. Pastucha, Z. Mikušová, P. Skládal, H.-H. Gorris, Angew. Ch. Int. Ed., 2020, 29(27), 10746–10773.

Acknowledgements

The results presented in this white paper are the outcome of a collaborative effort between Lumito AB and Atlas Antibodies AB.
We gratefully acknowledge **Lumito AB (Lund, Sweden)** for providing the SCIZYS Erbium-SA reagent kit and SCIZYS S1 whole-slide scanner, as well as for their expertise in photon upconverting nanoparticle technology and digital image acquisition and analysis.
We thank **Atlas Antibodies AB (Stockholm, Sweden)** for supplying the validated monoclonal primary antibodies used throughout this study, including Anti-PR (AMAb91529), Anti-MSH2 (AMAb91892), and Anti-MSH6 (AMAb91903), and for their expertise in immunohistochemistry and antibody validation.
We also thank **Histocyte Laboratories (Newcastle upon Tyne, UK)** for providing the PR (HCL034) and MMR (HCL043) Dynamic Range Analyte Controls used for IHC validation, which were instrumental in confirming antibody specificity and sensitivity prior to UCNP-IHC analysis.

LUMITO

ATLAS ANTIBODIES

HistoCyte Laboratories