SYNCELL APPLICATION NOTE

Unlocking Submicron Proteomes: Spatial Proteomics Analysis with Microscoop™ on Tissue Biospecimen

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Introduction

In conventional spatial biology research on tissue biospecimens, researchers typically use co-staining with multiple known markers to characterize the regional distribution of proteins through imaging. Microscoop™ introduces a paradigm shift in tissue analysis and enables hypothesis-free identification of proteomes within submicron structures in tissue biospecimens, regardless of whether they are formalin-fixed, paraffin-embedded (FFPE) or fresh frozen tissue sections. With just one preliminary marker required to indicate the protein of interest (POI), Microscoop™ coupled with LC-MS/MS allows targeted biotin-tagging and subsequent identification of the proteome in proximity to the POI. This enriches researchers with an additional layer of biologically significant insights. In this application note, we showcase the versatile application of Microscoop™ in tissue sections, using brain, intestine, and lung tissues as illustrative examples.

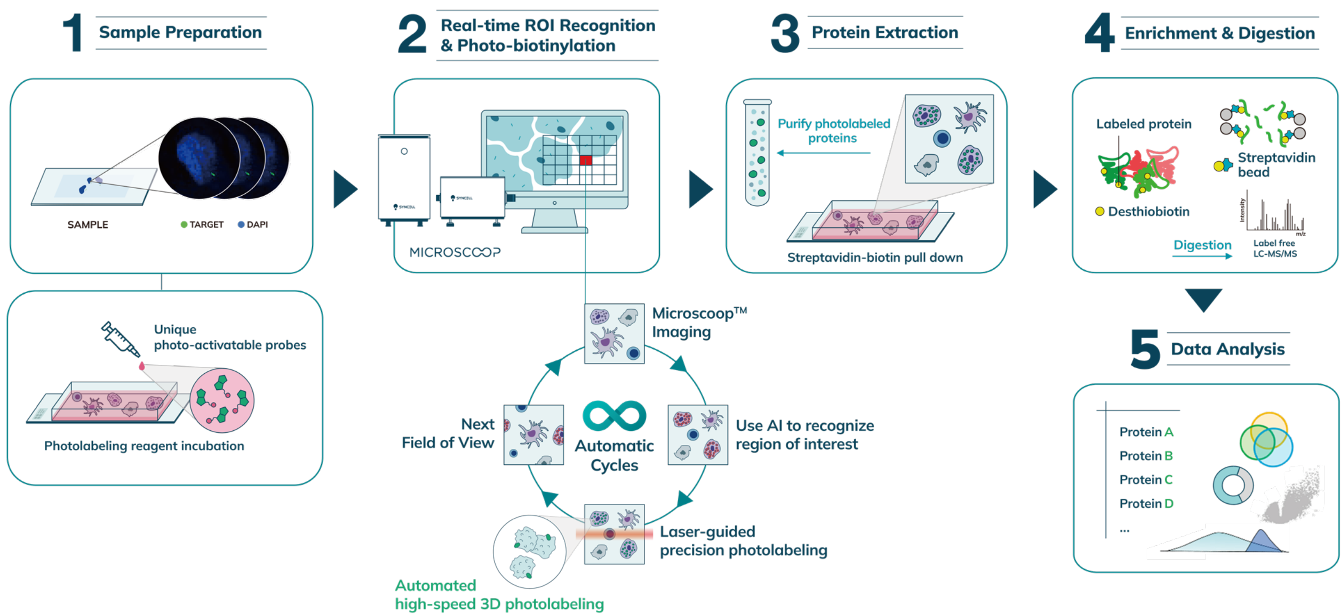

Fig.1 | Illustrates how the advanced platform, Microscoop™ is used for automated image-guided protein biotinylation. With AI or traditional image processing, Microscoop™ recognizes the specified regions of interest (ROIs) within cellular or tissue samples and induces precise photo-biotinylation at organelle-scale resolution via its femtosecond pulsed two-photon illumination. The real-time and automatic "ROI recognition to photo-biotinylation" cycles renders biotin-tagging of sufficient proteins for subsequent pulldown and protein identification via LC-MS/MS.

Application in intestine and lung tissues

IF staining of nuclei with H2AX (H2A histone family member X) as marker was conducted on sections from FFPE mouse small intestine (Fig. 2A) and lung tissues (Fig. 2D). Nuclei was targeted for image-guided biotinylation. Confocal images (Fig. 2C, Fig. 2F) with co-staining of nuclear labeling dye (DRAQ5, indicated in magenta) and Biotin (stained by NeutrAvidin Dy488, indicated in green) showed that the biotinylation

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is within the nucleus in all xyz directions, demonstrating that photo-activable biotin-tagging of nuclear proteins is achievable in FFPE sections.

Fig. 2 | Immunofluorescence staining of mouse FFPE tissue sections. Panels A, B, and C depict small intestine tissue, while panels D, E, and F show lung tissue. Panels B and E highlight the un-labeled (UL) and photolabeled (PL) regions within dashed rectangles from panels A and D, respectively. Panels C and F provide enlarged views and Z-sections of panels B and E, respectively, detailing the structures of the photolabeled regions. Colors represent DAPI (Blue) for the nucleus, Neutravidin 488 (Green) for biotinylation, H2AX for histone, and DRAQ5 for the nucleus (Purple).

Application in Brain Tissue

Immunostaining of Calbindin D28K was performed on the cerebellum cortex of mouse brain FFPE tissue to indicate the Purkinje cells (PKJ cells). The image processing function equipped in the Microscoop™ software (Autoscoop™) enables segmentation of the PKJ cell only at the cell bodies (Fig. 3). With the aid of this image-process algorithm, targeted biotinylation was performed on the Purkinje cell bodies for proteomic analysis (Fig. 4A), which resulted in a proteome of 1,674 IDs (Fig. 4B). Among these IDs, 1,494 (89%) were associated with the cytoplasm, and 679 (41%) were related to nuclear proteins. At least 15 markers mapped to the known PKJ database (48%) were identified. They include well-known PKJ-related proteins such as Fabp7, Prkcg, ATP2A3, and Dner (Fig. 4C). Further analysis of the proteome may yield crucial insights into PKJ protein composition and key pathways, contributing to neuroscience by revealing novel contributors to brain function.

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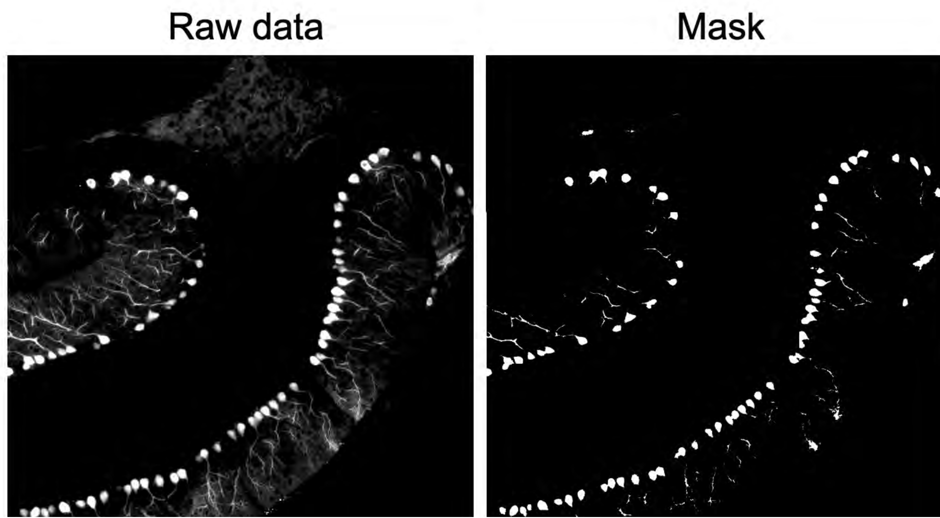

Fig. 3 | Immunofluorescence images of mouse brain FFPE tissue stained with Calbindin D28K, a marker for Purkinje cells. (A) Raw image showing the staining pattern. (B) Cell bodies of PKJ cells is masked for photolabeling.

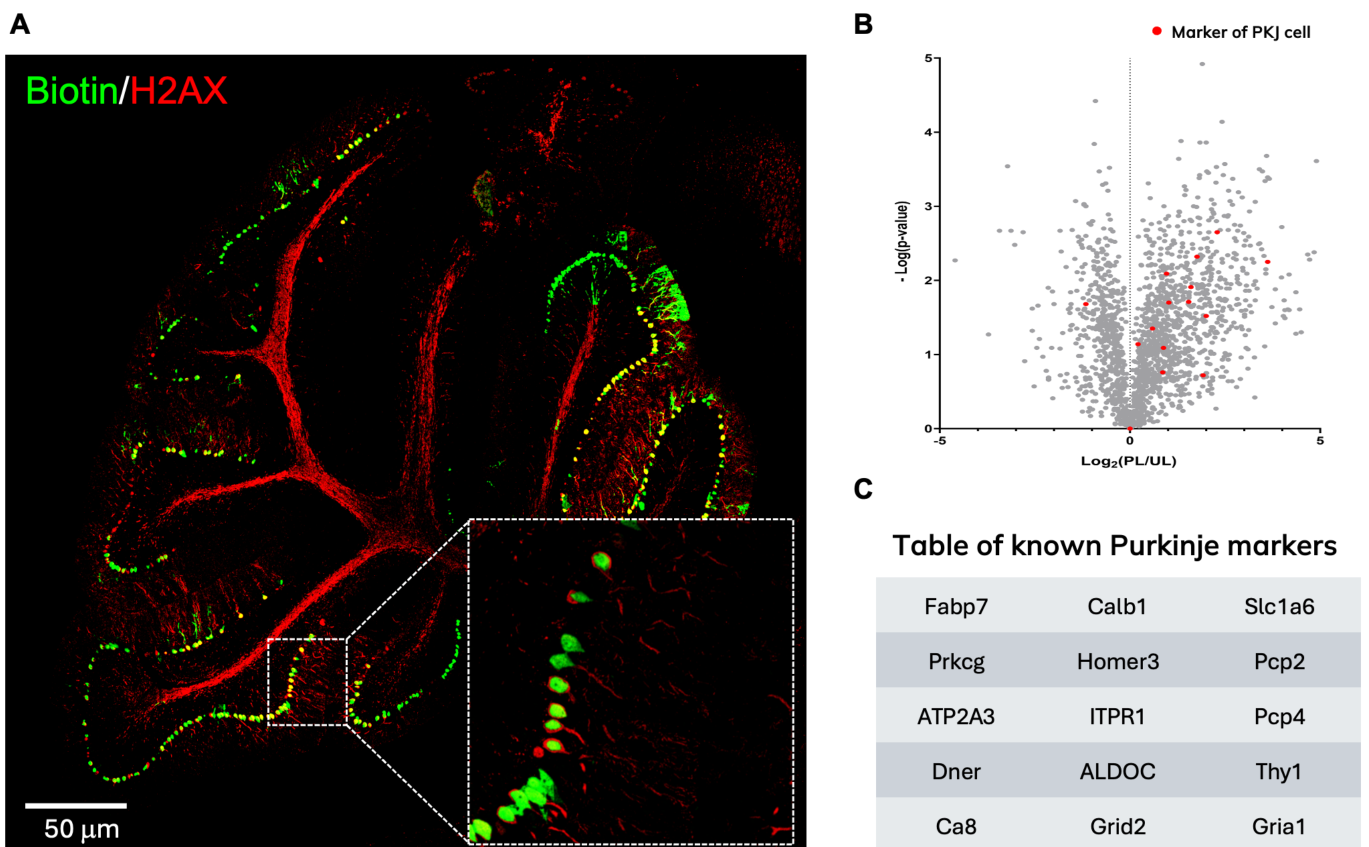

Table of known Purkinje markers

Fabp7	Calb1	Slc1a6
Prkcg	Homer3	Pcp2
ATP2A3	ITPR1	Pcp4
Dner	ALDOC	Thy1
Ca8	Grid2	Gria1

Fig. 4 | Photolabeling and proteomics data for Purkinje cells. (A) Immunofluorescence images of mouse brain FFPE tissue showing the photolabeling pattern (green region). (B) Volcano plots displaying the comparison of labeled and unlabeled proteins. Red dots represent Purkinje-related proteins with good enrichment ratios. (C) List of Purkinje markers (i.e. the red dots in B) identified by Microscoop™.

Conclusion

Microscoop™ enables researchers to delve deeper into tissue biology, empowering comprehensive exploration of subcellular proteomes. Its precision for targeted proteomic analysis accelerates biomarker discovery, aiding diagnostics and therapeutics development.

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