SYNCELL APPLICATION NOTE

Microscoop™: Two Photon-induced Biotinylation of Protein Constituents with Submicron Specificity

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Introduction

Spatial proteomic discovery at specific subcellular locations often encounters challenges due to the limitations of current technology. Microscoop™ has emerged as an advanced platform for automated, image-guided proteomic isolation. It targets specific regions of interest (ROIs) within cellular or tissue samples, enabling hypothesis-free protein identification through microscopic analysis (Fig.1). Microscoop™ employs precise photo-biotinylation with femtosecond pulses for two-photon illumination, achieving the scale of individual cellular organelles. Our technology enables a comprehensive exploration of subcellular protein locations and interactions, offering invaluable insights into the complex subcellar structures that are crucial for advancing biological research.

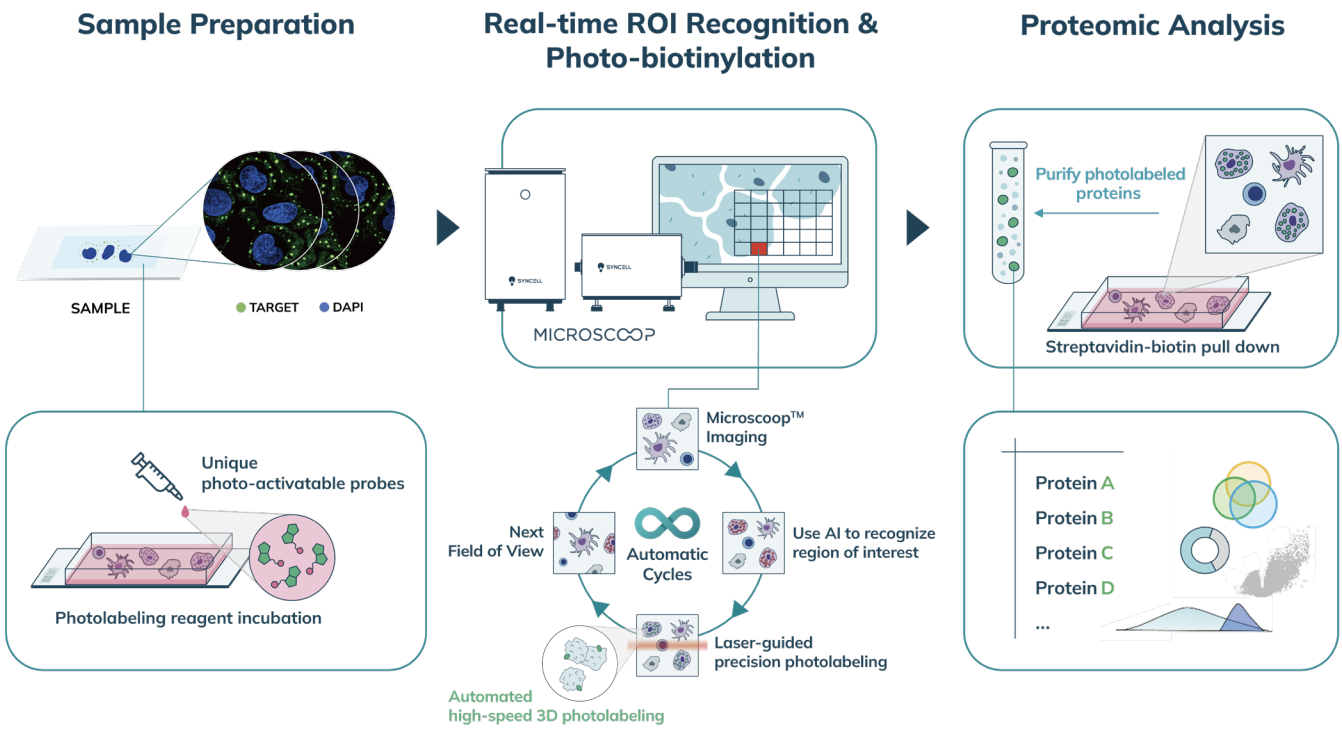

Figure 1 : Schematic Workflow of SYNCELL Microscoop™

Experimental workflow

1. Sample Preparation

Cells

Fix cells with suitable buffers (e.g., methanol or paraformaldehyde) and mount on single-well slides. Optimize cell seeding density for imaging ROIs, starting at 2.4×10^5 cells per well. Permeabilization is required for intracellular staining or internal ROIs.

Tissue sections

Utilize formalin-fixed, paraffin-embedded (FFPE) or frozen (OCT-embedded) sections, up to 20 µm thick. FFPE sections need antigen retrieval to reverse formalin-induced cross-links and expose epitopes for antibody access.

2. Visualization

Visualize cell/tissue samples under Microscoop™ to identify ROIs, either through immunostaining or endogenous expression above 470 nm wavelength.

3. ROI Recognition

Images were analyzed in real-time to segment the ROIs according to user specifications. Image processing involved combined methods such as thresholding, size exclusion, and morphology. Deep learning applications instantly segmented each Field of View image with a trained model to identify ROIs, facilitating efficient path planning and labeling control.

4. Photo-biotinylation

Two-photon illumination targets user-defined ROIs in real-time recognition, activating photochemical agents to biotinylate on proteins within submicron level. Microscoop™ automates photo-biotinylation at selected ROIs, one spot at a time through mechatronic position control across the entire samples.

5. Proteomic Analysis

Extracted proteins are subjected to streptavidin pull-down for hypothesis-free mass spectrometry (LC-MS/MS) analysis, or protein array for pathway analysis.

Results and Discussion

- ## The resolution of two-photon-induced biotinylation

  Photo-biotinylation on protein constituents was conducted by two-photon illumination on fixed U-2OS cells, shown as green fine lines (Fig. 2A). The resolution of the photo-biotinylation was measured by super-resolution structured illumination microscopy, achieving a width of 0.24 µm using a 40x/0.95 NA objective (Fig. 2B)

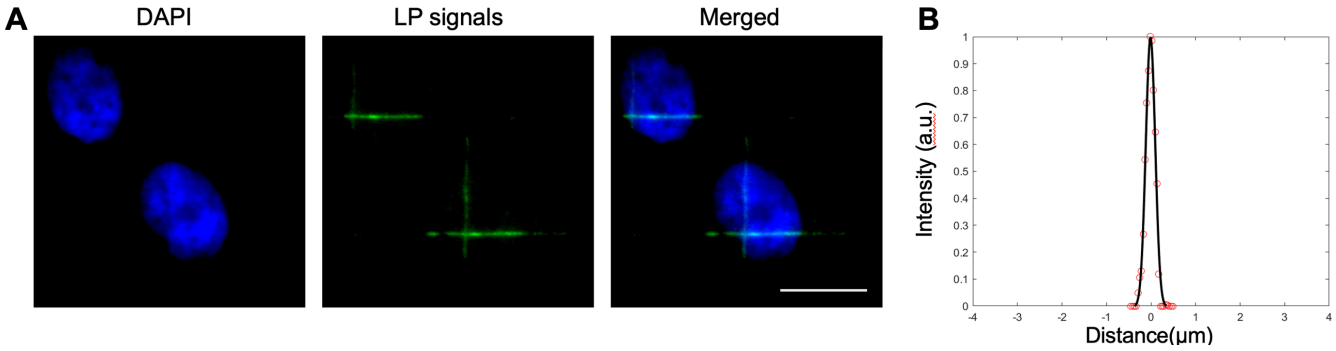

Fig 2. Resolution of photo-biotinylation. **A**, A line "cross" pattern is photolabeled on fixed U-2OS cells, and the biotinylated molecules are shown by Dy488-NeutrAvidin in green. DAPI: Blue, scale bar: 10 µm. **B**, Photolabeling resolution when using a 40x/0.95 NA objective, FWHM = 0.24 µm.

- ## Subcellular spatial biotinylation is applicable to cell and tissue samples

  Image segmentation was performed based on the characteristics of each structure, including the nucleus, nucleolus, nuclear membrane, and stress granules. The segmented regions were illuminated to induce targeted biotinylation. The *in situ* biotinylated regions matched well with the corresponding subcellular structures in both lateral (xy) and axial (z) directions, indicating high spatial labeling specificity (Fig. 3A).

Deep learning-based segmentation and targeted illumination were also applied to conjugates of Jurkat-Raji co-culture cells, achieving precise biotinylation of proteins at thin immune synapses (Fig. 3B). This demonstrates the capability of photolabeling in multi-color imaging context. Furthermore, the utility of photolabeling on tissue samples was shown by Calbindin-D28K staining of a FFPE mouse brain tissue section, which revealed Purkinje cells in cerebellum with axon and dendrite architecture and a distinctive neuron cell body layer. Deep learning-enabled segmentation and further targeted illumination resulted in specific biotinylation at the cell bodies of the Purkinje cells (Fig. 3C).

Photo-biotinylated cells or tissues are set for streptavidin pulldown, followed by LC-MS/MS or protein array analysis to discover novel proteins or pathway enrichment. By comparing unlabeled and photo-biotinylated proteomes, a location-specific proteome at the ROIs is ready for validation.

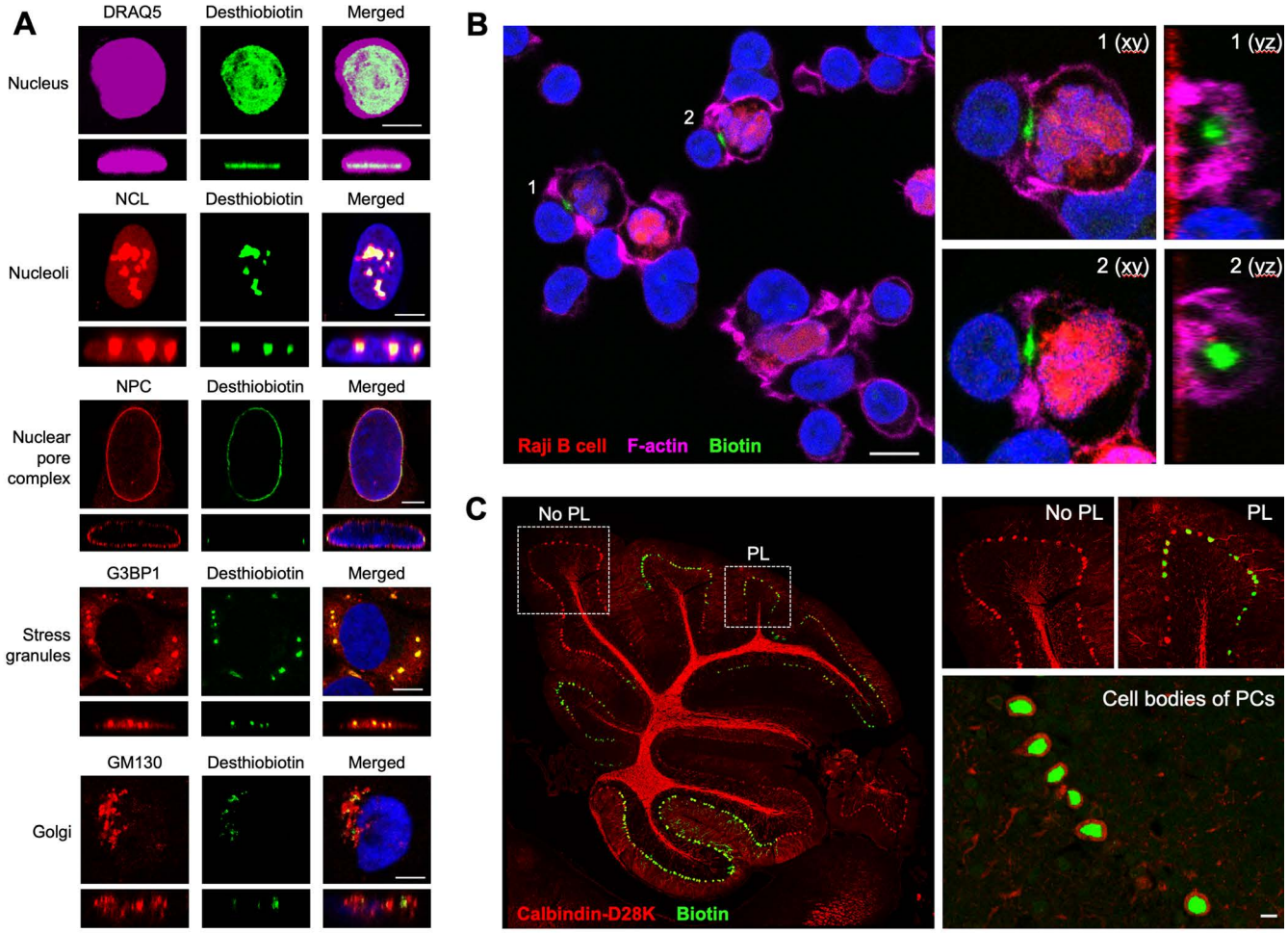

Fig. 3. **A**, Photo-biotinylation in xy (lateral) and z (axial) directions of various subcellular structures, visualized by confocal images. The ROIs are stained with Alexa Fluor 568 secondary antibody (red), the photolabeled signals are shown with Dy488-NeutrAvidin (green). **B**, Precision photo-biotinylation (Dy488-NeutrAvidin: green) of immune synapse of co-cultured Jurkat T cells (DAPI: blue) and Raji B cells (DAPI: blue and CellTracker Red CMTPX dye: red), and the periphery of immune synapses is visualized by F-actin (Phalladin-660: magenta), illustrating the capability for two-color image analysis. **C**, Photo-biotinylation (Dy488-NeutrAvidin: green) of proteins in an FFPE mouse brain tissue section. Cell bodies of Purkinje cells are selected for photolabeling. Scale bar: 10 µm.

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