Subcellular spatial proteomics by microscopy-guided photo-biotinylation reveals novel protein constituents in primary cilia

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Abstract

Image-guided protein extraction at organelle-scale resolution holds significant promise for discovering novel protein constituents within disease- or function-related subcellular regions like primary cilia. Our firmware-integrated microscopy platform facilitates spatial protein purification through in situ subcellular photo-biotinylation at user-defined regions of interest (ROIs) one field of view (FOV) at a time, automatically processing thousands of FOVs. Illumination patterns of the ROI for each FOV are calculated in real-time using machine learning or traditional image processing. Light activation of amino acid crosslinkers is achieved by a two-photon laser in the platform, rendering precise protein biotinylation with 300-nanometer precision. A high-speed mechatronic control is implemented to coordinate imaging, pattern generation, targeted illumination, and FOV movement, allowing for the rapid biotinylation of millions of ROI spots within hours in cell or tissue samples. Once enough proteins are biotinylated, subsequent cell lysis, avidin pulldown and LC-MS/MS analysis unveil the subcellular proteome with exceptional sensitivity, specificity, and resolution. Using this technology, termed optoproteomics, we investigated the proteome of primary cilia in RPE-1 cells, identifying the proteome including 524 known ciliary proteins notably enriched. The top identified proteins encompassed key ciliary trafficking components and those involved in structural support and cellular organization. Gene ontology (GO) enrichment analysis highlighted the significant association of high-ranking proteins with critical ciliary processes such as assembly, transportation, and signaling, particularly includingproteins involved in intraciliary transport. A group of novel protein constituents were identified, providing testable hypotheses for their roles in primary cilia. These findings underscore the efficacy of targeted photolabeling and proteomic analysis in unraveling the network of proteins essential for ciliary function and structure, showcasing optoproteomics' potential for comprehensive subcellular spatial proteome discovery and its broad utility in cell biology for discovering novel protein compositions or biomarkers.

Microscoop®: Ultrahigh-content microscopy-guided photo-biotinlyation platform

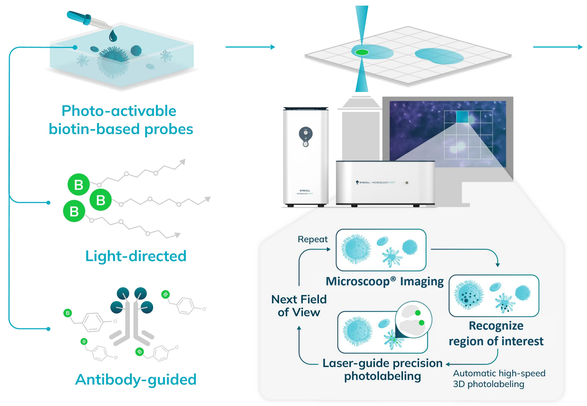

Fig. 1 | Schematic workflow of SYNCELL Microscoop®. A total-sync ultra-content microscopic platform that integrates image acquisition, photochemistry, microscopy, optics, and FPGA-based mechatronics enable high-content in situ photolabeling followed by mass spectrometry analysis.

Microscoop® : Synchronized high-content system control at nanoscale resolution

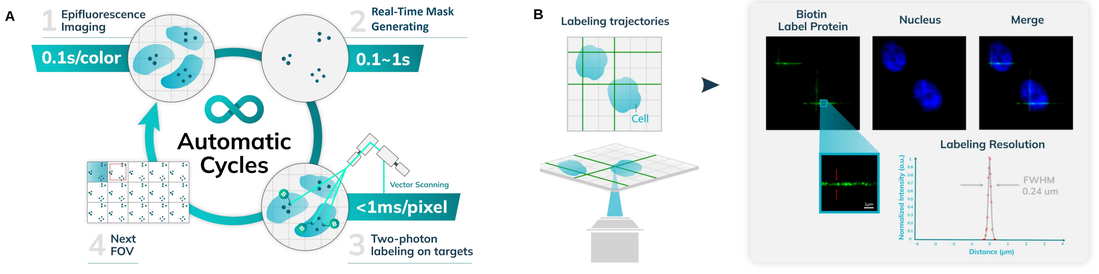

Fig. 2 | A, Workflow for ultrahigh-content targeted pohoto-biotinylation includes: (1) identifying and acquiring images of regions of interest by light microscope; (2) generating real-time patterns of ROIs; (3) illuminating the selected region within ROIs for protein photo-biotinylation; (4) moving the stage to the next FOV; and repeating steps 1-4 for each FOV until all FOVs have been processed. **B,** Resolution of photo-biotinylation. A line “cross” pattern is photolabeled on fixed U-2OS cells, and the biotinylated molecules are shown in green. DAPI: Blue, scale bar: 10 μm. 40x/0.95 NA objective.

Microscoop® : Photo-induced biotinylation within primary cilia

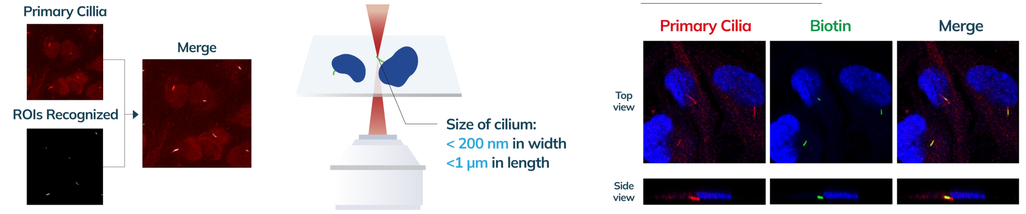

Fig. 3 | Primary cilia are processed by filtering and segmentation by image processing (left), Confocal micrographs depicting precise and accurate photolabeled primary cilia at lateral (xy)- and axial (z) directions (right). Red: GT335, Green: NeutrAvidin-488, Blue: DAPI.

Unveiling spatial proteomics of primary cilia and their functional insights

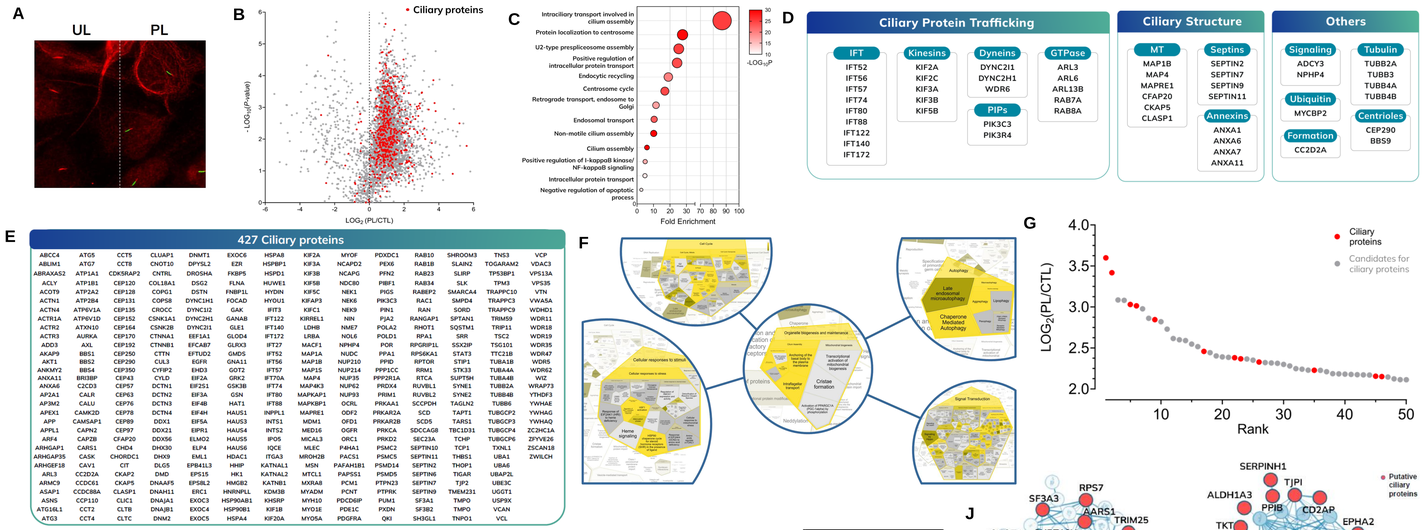

Fig. 4 | A, Confocal micrographs of unphotolabeled (UL) and photolabeled (PL) at user defined primary cilia. **B,** A distribution of overall protein abundances is binned by the ratio of copies in a photolabeled (PL) sample to those in a control (CTL) sample annotated as PL/CTL ratio. Ciliary proteins (red) are enriched in the PL group compared to the CTL sample. **C,** The top 100 enriched proteins were subjected to Gene ontology to reveal cilia related biological process. **D,** Well-known ciliary proteins identified by Microscoop®. **E,** 427 ciliary proteins significantly enriched by Microscoop®. **F,** The 427 enriched ciliary proteins were subjected to Reactome to reveal cilia related pathways. **G,** The ranking of the top 50 protein abundances (PL/CTL), where ciliary proteins are indicated in red and nonciliary proteins are indicated in grey. **H,** The list of the top 30 non-ciliary proteins (putative ciliary proteins) enriched by Microscoop®. **I,** The top 30 putative ciliary proteins (H) were subjected to Gene ontology to reveal cilia related biological process. **J,** Top 100 ranked proteins were subjected to STRING to reveal protein-protein interaction networks, where the 30 putative ciliary proteins (H) are indicated in red.

References:
Microscopy-guided subcellular proteomic discovery by high-speed ultra-content photo-biotinylation. Chen et al. bioRxiv 2023.12.27.573388.

Summary

- An innovative platform that combines microscopy, deep learning, two-photon illumination, and mechatronics for advanced image-guided photo-biotinylation hypothesis-free proteomics.
- Fast and precise photo-biotinylation of spatially specific proteins from hundreds of thousands of cells enhances the sensitivity of mass spectrometry.
- In mapping the ciliary proteome, 427 known ciliary proteins were enriched, and the validation of previously unreported proteins in primary cilia is underway.