Total-sync ultra-content microscopic opto-biotinylation enables high-sensitivity hypothesis-free subcellular protein discovery

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Abstract

Studying proteomes within subcellular structures presents significant challenges, particularly for structures that are membrane-less or cannot be isolated from cells. Addressing this hurdle, Microscoop® emerges as a groundbreaking technology capable of precisely capturing proteins at specified subcellular regions of interest (ROIs) with nanoscale precision. This sophisticated system integrates microscopy, optics, mechatronics, photochemistry, and deep learning to enable high-content in situ photolabeling. With Microscoop®, proteins are precisely biotinylated within user-defined cellular organelles, granules, or cell-cell contact surfaces under a microscope, utilizing directed photochemistry within each field of view (FOV). This process is automatically repeated across thousands of FOVs to photo-label cellular structures sharing similar morphological features. Subsequently, ample biotinylated proteins are obtained for streptavidin pulldown and mass spectrometry analysis. The robustness of this approach is demonstrated through the successful mapping of the human cellular nucleus proteome, identifying over 1000 nuclear proteins with a specificity exceeding 90%. Further analysis reveals comprehensive coverage of nuclear complexes and identification of low-copy-number proteins. In one study using Microscoop® technology to explore the nucleolus proteome, 97 of the top 100 abundant proteins are confirmed to be originated from the nucleolus. In another investigation focusing on stress granules (SG) proteome, we identified 2,614 proteins, including 200 with low copy numbers. Although the specificity is moderate, with only 66% of the top 50 abundant proteins mapped with the known stress granules database, functional enrichment analysis highlights 13 non-SG associated proteins as high-confidence core interactors within the stress granule network. Among them, 11 proteins (PDLIM7, EIF3CL, YWHAE, RPSA, MTA2, UGDH, DDX17, ANLN, PSMD3, PSMA6, and MCM2) are confirmed to co-localize with G3BP1 through immunostaining, thus elevating the specificity eventually to 96% SG association. Overall, our study illustrates that Microscoop® enables hypothesis-free, comprehensive mapping of subcellular proteomes at user-defined regions of interest, significantly advancing cell biology by revealing new proteins or biomarkers.

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

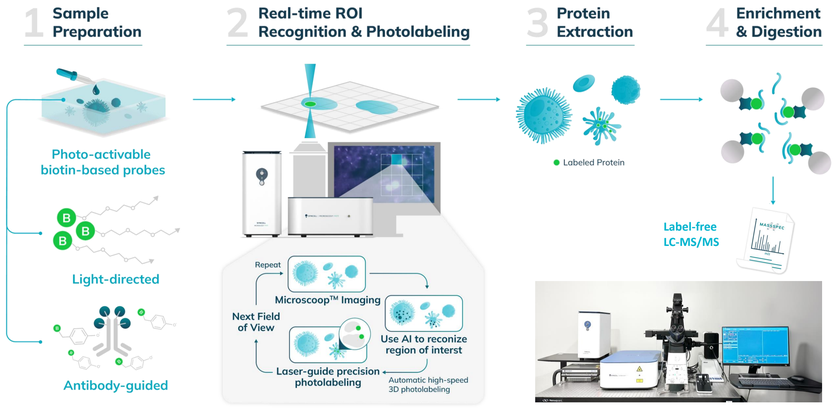

Fig. 1 | Schematic workflow of SYNCELL Microscoop®. A total-sync ultra-content microscopic platform that integrates image acquisition, photochemistry, microscopy, optics, and FPGA-based mechatronics enable high-content in situ photolabeling followed by mass spectrometry analysis.

Synchronized high-content system control to a high-speed and accurate image masking

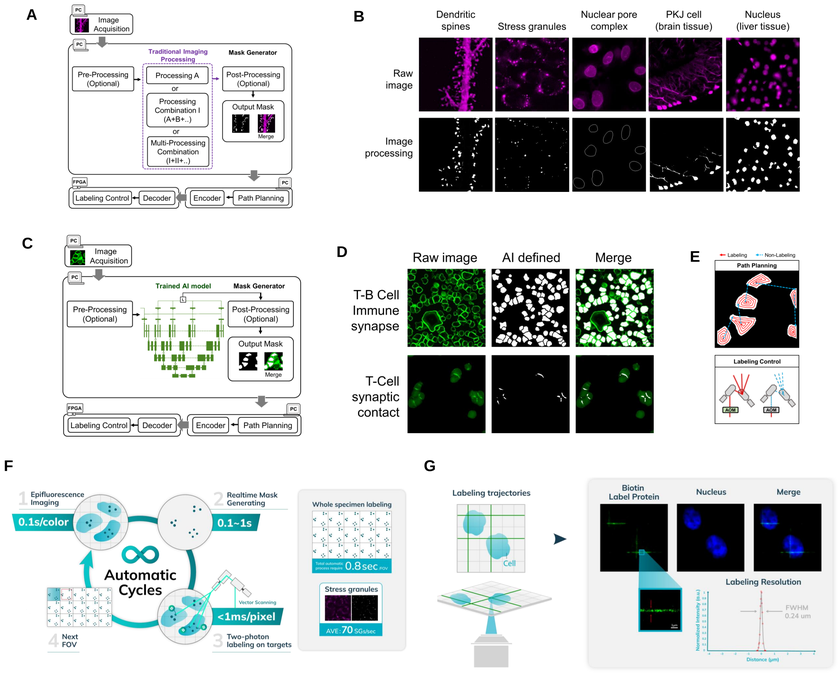

Fig. 2 | A-B, Image processing is applied to recognize the regions of interest of acquired images. **C-D,** Deep learning image segmentation and recognition of complex models from raw images. **E,** Algorithm generates the labeling path and the non-labeling path of an input mask, and labeling control of the galvanometer system and the AOM. **F,** Workflow for ultrahigh-content targeted pohoto-biotinylation includes: (1) identifying and acquiring images of regions of interest by light microscope; (2) generating realtime 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. **G,** 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.

Photo-induced spatial biotinlyation in subcellular compartments at a nanoscale resolution

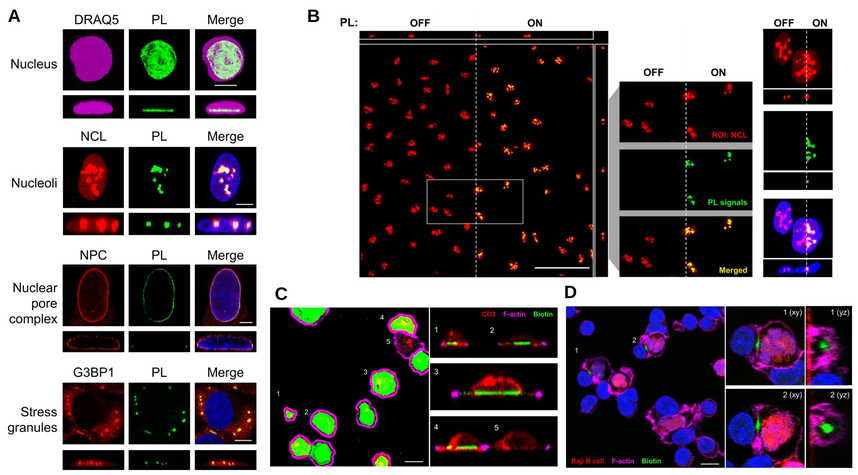

Fig. 3 | **A**, Top- (xy) and side- (z) views of photolabeled subcellular compartments. **B**, Photolabeled (ON) and unlabeled (OFF) ROIs within nucleoli. **C**, Top- and side views of each labeled synapse (C1-4) in spreading assay. The side view of photolabeling region is co-localized with CD3, immune synapse marker on the bottom of cells. No biotin signal is found in a non-photolabeled cell (C5). **D**, Photolabeled regions of immune synapse of Jurket T cells and Raji B cells are shown in green as a precise and thin labeled layer. Scale bar: 10 μm. Green: Neutravidin-488 fluorescent, Red: anti-primary antibody-568, Blue: DAPI.

High sensitivity and specificity of photolabeled nuclear and nucleolar proteomes

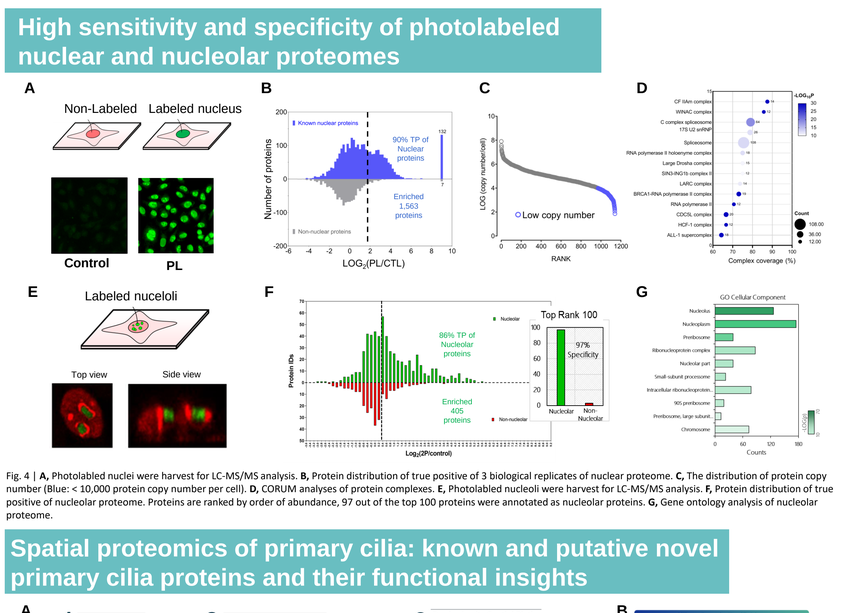

Fig. 4 | **A,** Photolabled nuclei were harvest for LC-MS/MS analysis. **B,** Protein distribution of true positive of 3 biological replicates of nuclear proteome. **C,** The distribution of protein copy number (Blue: < 10,000 protein copy number per cell). **D,** CORUM analyses of protein complexes. **E,** Photolabled nucleoli were harvest for LC-MS/MS analysis. **F,** Protein distribution of true positive of nucleolar proteome. Proteins are ranked by order of abundance, 97 out of the top 100 proteins were annotated as nucleolar proteins. **G,** Gene ontology analysis of nucleolar proteome.

Spatial proteomics of primary cilia: known and putative novel primary cilia proteins and their functional insights

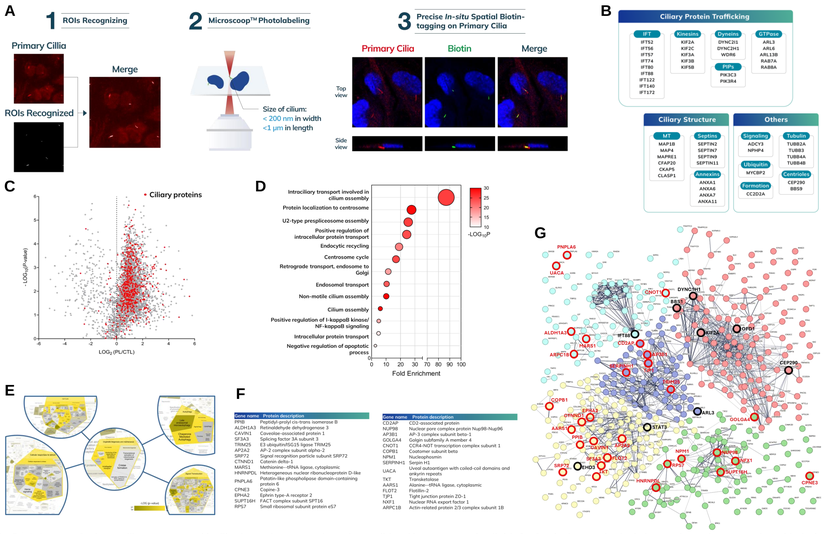

Fig. 5 | A, Images of primary cilia are processed by filtering and segmentation using traditional image processing (left), Confocal micrographs depicting precise and accurate photo-biotinylated primary cilia at lateral (xy)- and axial (z) directions (right). Red: GT335, Green: NeutrAvidin-488, Blue: DAPI. **B,** List of a few well-known ciliary proteins identified by Microscoop®. **C,** A distribution of overall protein abundances by the ratio of 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. **D,** The top 100 enriched proteins were subjected to Gene ontology to reveal cilia related biological process. **E,** 427 enriched ciliary proteins were subjected to Reactome to reveal cilia related pathways. **F,** The list of the top 30 non-ciliary proteins (putative ciliary proteins) enriched by Microscoop®. **G,** The 30 putative ciliary protein and 427 enriched ciliary proteins were subjected to STRING to reveal protein-protein interaction networks, where the 30 putative ciliary proteins (F) are indicated in red.

Mapping the proteome of stress granules and discovery of novel biomarkers

Fig. 6| **A,** Confocal micrographs of photolabeled (PL) and non-photolabeled (UL) at user defined stress granules (SG). **B,** A field of view of photolabeled SG with Microscoop®. **C-D,** Confocal micrographs depicting precise and accurate photolabeled SG at top- and side- view. **D,** Venn diagram of 3 biological replicates of SG proteomics. **E,** Volcano plot of relative protein levels in photolabeled samples to control samples (PL/CTL ratio) in $\log_2$ scale. Over-represented (enriched) proteins are shown in green. **F,** The SG percentage of the 124 enriched proteins. **G,** 74% of true positive SGs are found in the top 50 proteins ranked by PL/CTL ratio. Proteins have no prior annotation as SG (gray) and known SG (green) from the top 50 ranked proteins. **H,** List of novel SG proteins (red) discovered by Microscoop®. **I,** Confocal micrographs depicting SG formation of potential stress granule proteins in U-2OS cells with or without arsenite stress. Twelve potential SG proteins (green) are highly co-localized with G3BP1 SG markers. **J-K,** Side-view of the co-localization of the novel stress granules are shown in right panel. lens: 63× oil. PL: photolabed (biotinylated) proteins stained with NeutrAvidin-488, Red: G3BP1, Blue: DAPI. Scale bar: 10 μm.

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

Summary

- **SYNCELL Microscoop® :** A novel platform to enable hypothesis-free subcellular spatial proteomic discovery
- **Integration of Technologies:** Combines microscopy, deep learning, two-photon illumination, and mechatronics.
- **Purpose**: Facilitates high-content, image-guided photo-labeling at a nanoscale resolution
- **Capabilities:** Precisely labels spatially specific proteins from hundreds of thousands of individual cells, suitable for mass spectrometry analysis
- **Achievements:**
- Identified 608 known ciliary proteins, providing functional insights and listing putative proteins with high protein-protein interactions.
- Discovered dozen of novel stress granule proteins with high precision and accuracy