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

Abstract #5638

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De novo spatial proteomics

Abstract

High-sensitivity hypothesis-free subcellular proteomics is challenging due to the limited sensitivity of mass spectrometry and the lack of amplification tools for proteins. Without such technology, it is not possible to discover proteins at specific locations of interest in cells or tissue samples. Here, we introduce a total-sync ultra-content microscopic system termed Microscoop™ that integrates microscopy, optics, FPGA-based mechatronics, photochemistry, and deep learning or computer vision to enable high-content in situ photolabeling. Microscoop™ photolabels proteins at user defined regions of interests (ROIs) under a microscope utilizing directed photochemistry in one field of view (FOV) at a time for tens of thousands of FOVs with similar morphological features. With this platform, we are able to photolabel proteins with biotin probes in cellular organelles, granules or cell-cell contact surfaces with a high precision at nanoscale resolution, and obtain sufficient amount of biotinylated proteins for mass spectrometry. We made a robust demonstration in the proteome mapping of human cellular nucleus from single-shot experiment to >1000 nuclear protein identification with > 90% specificity. Further data analysis revealed identification of a hundred of low protein copy number proteins and a high coverage of nuclear complexes. In proteome mapping of the nucleolus, we ranked proteins by order of abundance and revealed that 97 out of the top 100 proteins were annotated as nucleolar proteins. Unexpectedly, in mapping the stress granule (SG) proteome, a relatively low SG specificity (74%) were found in the top 50 abundant proteins, therefore we further characterize the proteins that have no prior SG annotation by immunostaining. 11 out of the 13 unexplored proteins including PDLIM7, EIF3CL, YWHAE, RPSA, MTA2, UGDH, DDX17, ANLN, PSMD3, PSMA6, and MCM2 were found to have SG patterns and co-localized with SG marker (G3BP1), raising our top 50 SG specificity to up to 96%. Together, our total-sync ultra-content microscopic platform enables hypothesis-free, de novo subcellular proteome mapping at user defined ROIs with high sensitivity and specificity, thereby broadly benefits the cell biology field in finding novel proteins or biomarkers.

Microscoop™: hypothesis-free subcellular protein discovery platform

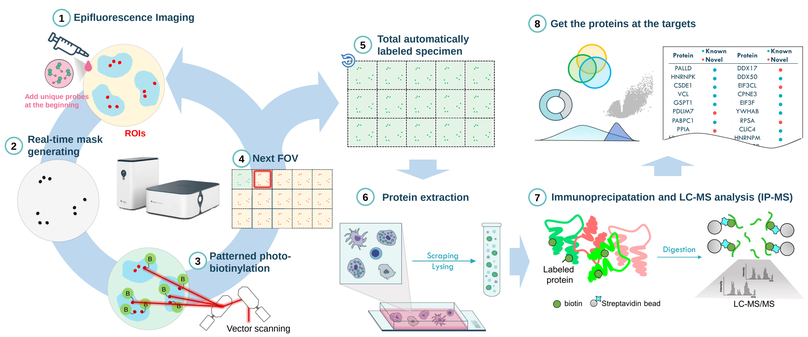


Fig. 1 | Schematic workflow of SYNCELL Microscoop™ . A total-sync ultra-content microscopic platform that integrates image acquisition, photochemistry, microscopy, optics, and mechatronics enable high-content *in situ* photolabeling followed by mass spectrometry analysis.

Microscoop™: synchronized high-content system control to a high-speed and accurate image masking and labeling

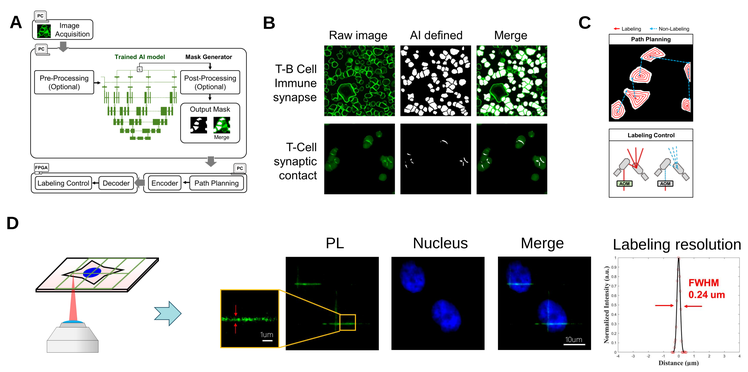


Fig. 2 | **A-B**, Deep learning image segmentation and recognition of complex models from raw images. **C**, Algorithm generates the labeling path and the non-labeling path of an input mask, and labeling control of the galvanometer system and the AOM. **D**, PFA fixed U-2 OS cells were photo-biotinlylated with a thin "cross" pattern, and photolabeling resolution was measured by super-resolution structured illumination microscopy.

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

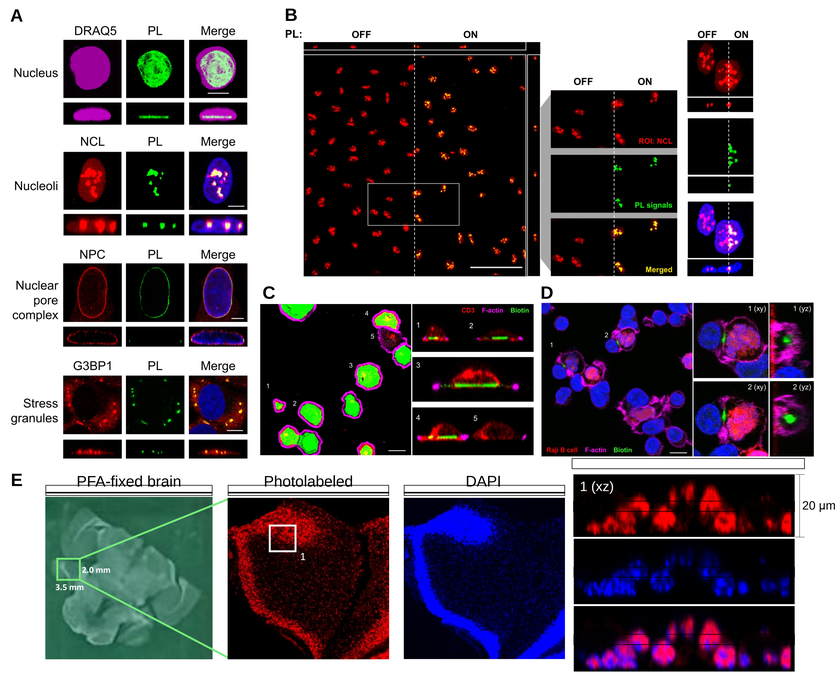


Fig. 3 | **A**, Top- (xy) and side- (z) views of photolabeled subcellular compartments. **B**, Photolabeled (ON) and non-labeled (OFF) ROIs within nucleoli. **C**, Top- and side views of each labeled synapse (C1-4) in spreading assay. The side view of photolabeling region is co-localized with CD3, immune 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. **E**, Microscoop™ allows 3D-biotinlyation of nuclei on/in mouse brain. Red: Neutravidin-550 fluorescent.

High sensitivity and specificity of photolabeled nuclear and nucleolar proteomes

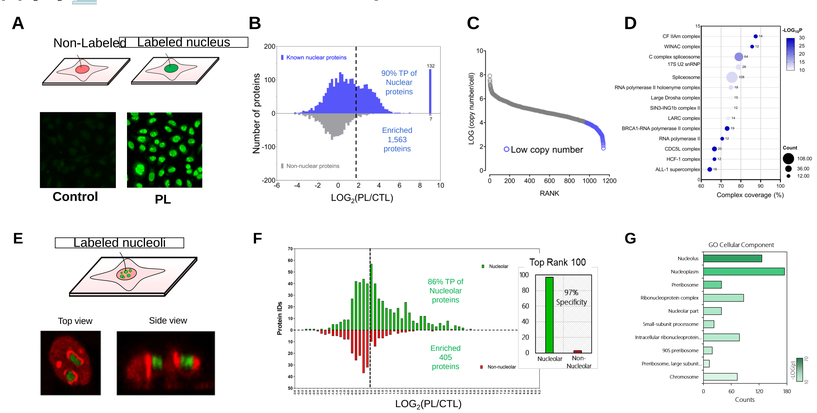
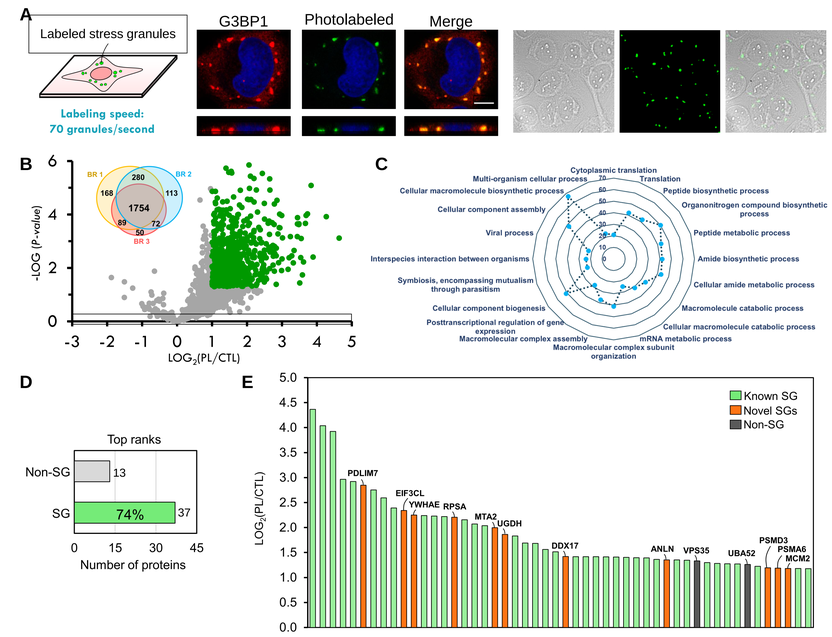


Fig. 4 | **A**, Precisely labeled nuclei were harvested for LC-MS/MS analysis. **B**, Protein distribution of true positive of three biological replicates of nuclear proteome. **C**, The distribution of protein copy number (Blue: < 10,000 protein copy number per cell). **D**, CORUM analyses of protein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

Mapping the proteome of stress granules and discovery of novel biomarkers



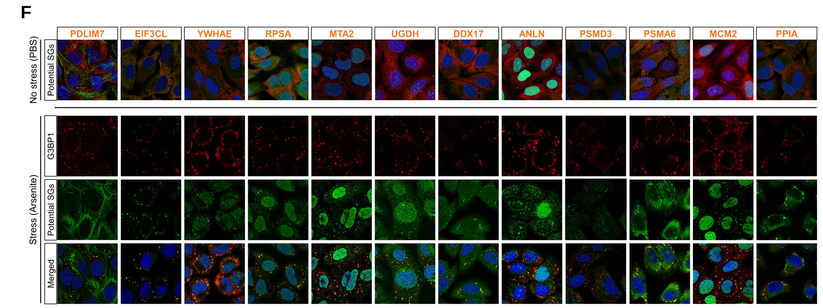


Fig. 5 | **A**, Photolabled stress granules were harvested for LC-MS/MS analysis. Confocal micrographs depicting precise and accurate photolabeling of SGs. **B**, Three biological replicates of stress granule proteomics. 1754 proteins were subjected to a volcano plot of relative protein levels in photolabeled samples to control samples (PL/CTL ratio). Over-represented (enriched) proteins are shown in green. **C**, 124 enriched proteins were subjected to Gene ontology to reveal SG related biological process. **D**, 74% of true positive SGs are found in the top 50 proteins ranked by PL/CTL ratio. **E**, Proteins have no prior annotation as stress granule (orange and gray) from the top 50 ranked proteins. Novel SG proteins (orange) discovered by Microscoop™. **F**, Confocal micrographs depicting SG formation of potential stress granule proteins in U-2 OS cells with or without arsenite stress. Twelve potential SG proteins (green) are highly co-localized with G3BP1 SG markers. lens: 63x oil. Red: G3BP1, Blue: DAPI.

Summary

- **SYNCELL Microscoop™ is a novel platform to enable hypothesis-free subcellular spatial proteomics**
- **The platform integrates microscopy, deep learning, two-photon illumination, and mechatronics to facilitate high-content image-guided photo-labeling**
- **Spatially specific proteins from hundreds of thousands of individual cells can be rapidly and precisely labeled by Microscoop™ to achieve sensitivity of mass spectrometry**
- **In mapping the proteome of stress granules, 48 of top 50 ranked proteins (48/50, 96%) identified with Microscoop™ were stress granule proteins**