SYNCELL APPLICATION NOTE

Nucleus Spatial Purification with Microscoop^{TM}

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Introduction

Microscoop^{TM} is a novel microscopy-based platform designed for ultra-content microscope-guided photo-biotinylation within cellular structures at submicron level. This platform functions figuratively as a scoopable microscope, automatically collecting spatially specified proteins from numerous fields of view for subsequent LC-MS/MS-based proteome identification. This innovative microscope - MS pipeline, termed optoproteomics, enables high specificity, sensitivity, and resolution in subcellular proteomic discovery.

Figure 1 illustrates how Microscoop^{TM} is used for automated image-guided protein biotinylation. Using either AI or traditional image processing, Microscoop^{TM} identifies predefined regions of interest (ROIs) within cellular or tissue samples. It then employs femtosecond pulsed two-photon illumination to activate a proprietary biotin probe, leading to precise biotinylation of proteins at the organelle-scale resolution to approximately 300nm in size. 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.

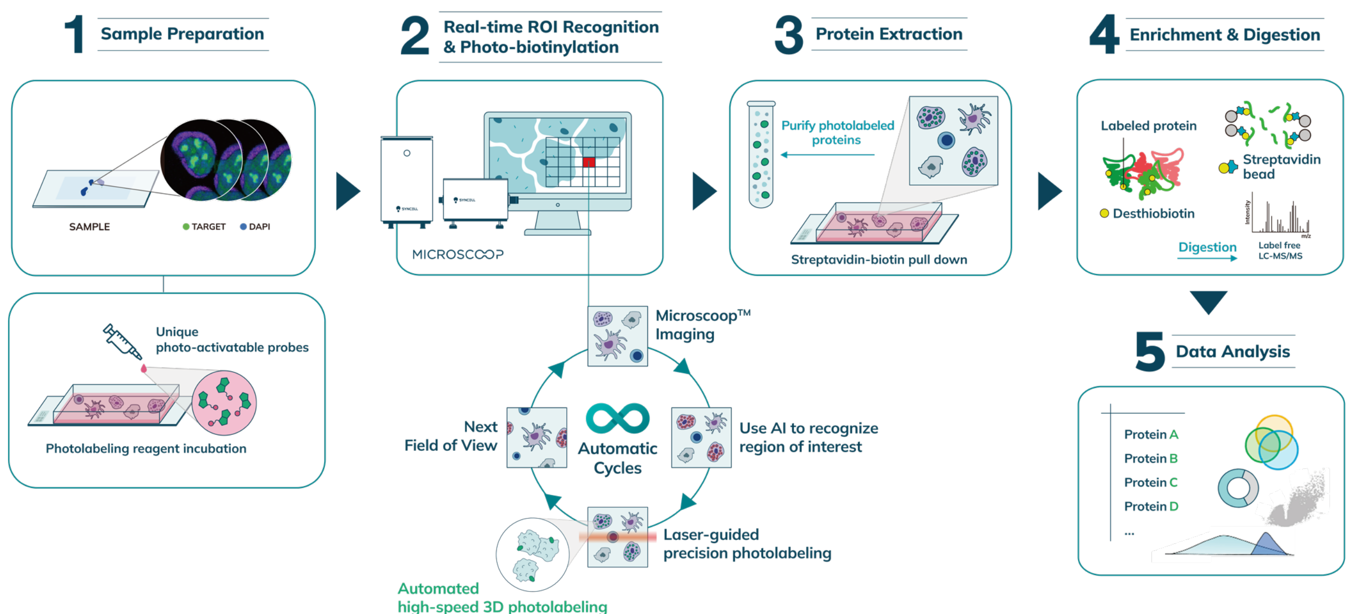

Fig. 1 | Schematic workflow of Syncell Microscoop^{TM}

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Microscoop-Optoproteomics showcases a remarkable capacity for nuclear proteomic identification with exceptional sensitivity and specificity

Nuclei within fixed U-2OS cells were labeled using DRAQ5, a far-red fluorescent DNA intercalating dye, to serve as a guide for Microscoop™'s region of interest (ROI) recognition. Other fluorescent dyes or immunostaining methods targeting the nucleus are also compatible. After 16 hours of photolabeling, biotinylated proteins were extracted using streptavidin beads, digested, and analyzed via LC-MS/MS (Fig. 2A, Fig. 2B). A total of 3 replicates were conducted, resulting in the identification of 4,820 proteins with high confidence (Fig. 2C). Out of these, 1,316 proteins exhibited differential enrichment compared to the unlabeled control group, with 1,207 annotated as nuclear proteins, indicating a 92% true positive rate. Notably, more than 10% of the identified nuclear proteins are considered low abundance (proteins known to be present at <10,000 copies per cell) (Fig. 2D), highlighting the effectiveness of optoproteomics in detecting proteins present in low quantities. Moreover, our platform revealed overrepresented complexes within the nucleus-targeted region, such as the spliceosome, histone complex, and RNA polymerase complex, showcasing its capability in uncovering protein complexes (Fig. 2E). This underscores the platform's ability to provide valuable insights into cellular processes and interactions through spatial proteomics.

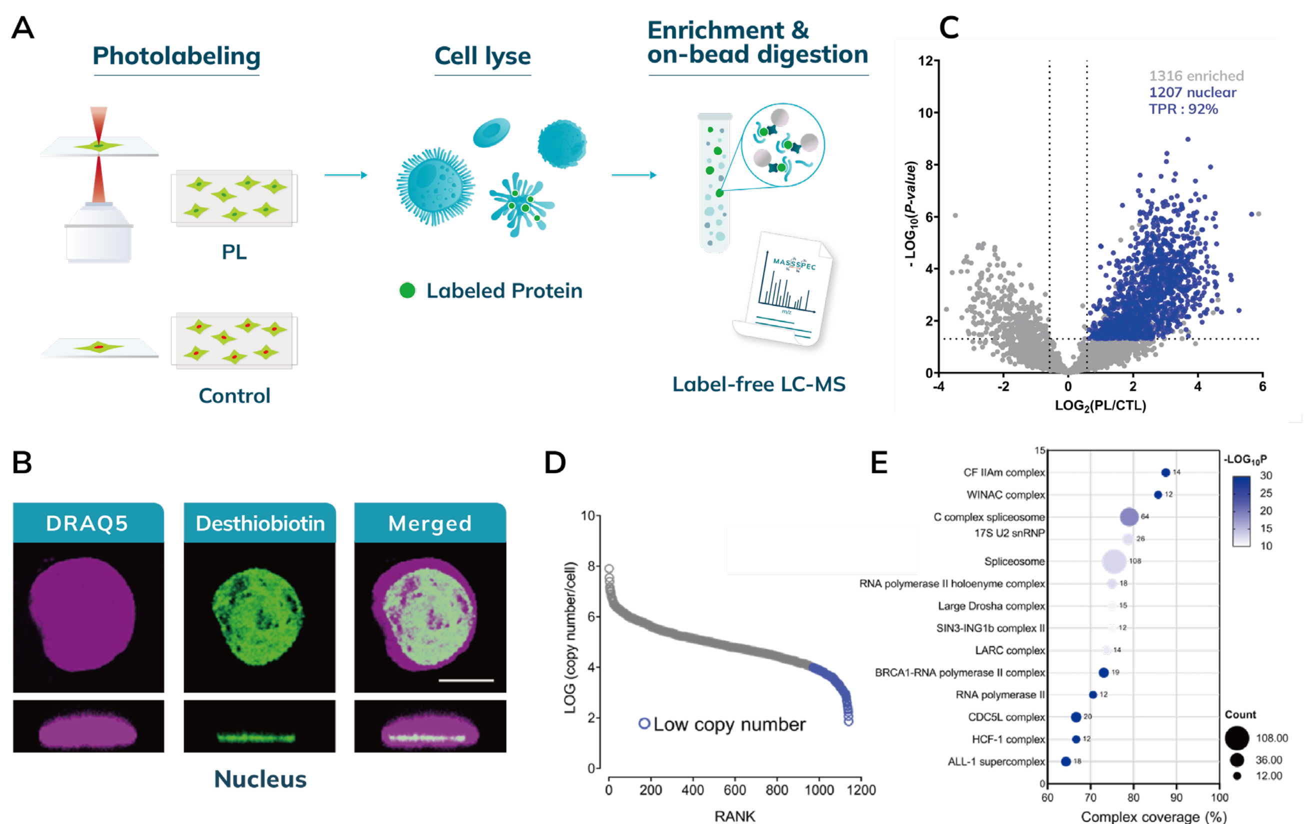

Fig. 2 | Subcellular proteomics by spatial purification. (A) Steps of proteomic profiling after targeted photo-biotinylation. Both photolabeled (PL) cells and control cells (without illumination) are lysed, enriched using streptavidin beads, and then digested with trypsin prior to LC-MS/MS analysis. (B) Photo-biotinylation in xy (top view) and z (side view) directions of various subcellular structures, visualized by confocal images. The ROIs are stained with Alexa Fluor 568 secondary antibody, and the photolabeled signals are shown with Dy488-NeutrAvidin. (C) High specificity of nuclear proteins obtained through spatial purification with targeted photo-biotinylation. 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. High true positive rate of nuclear proteins in the PL enriched group (blue) compared to the CTL sample. (D) Distribution of protein copy numbers, with the low copy number ones shown in blue (< 10,000 copy number per cell). (E) CORUM analysis of protein complexes, revealing major nuclear associated complexes. Scale bar: 10 μm.

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Conclusion

The Microscoop™'s high sensitivity and specificity as demonstrated in the nuclear study enhances research in subcellular spatial proteomics, offering valuable insights into cellular processes and interactions. This capability is particularly significant in detecting low-abundance proteins and revealing spatially related protein complexes, thereby advancing our understanding of cellular biology.

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