SYNCELL APPLICATION NOTE

# Pushing the Limits: Unveiling Proteome of Primary Cilia at Unprecedented Resolution Using Microscoop®

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## Introduction

Understanding the intricate structures of subcellular organelles demands cutting-edge technologies capable of unprecedented resolution. This application note highlights Microscoop®'s revolutionary capabilities, focusing on its ability to facilitate proteome extraction at tiny subcellular organelles like primary cilia with exceptional precision.

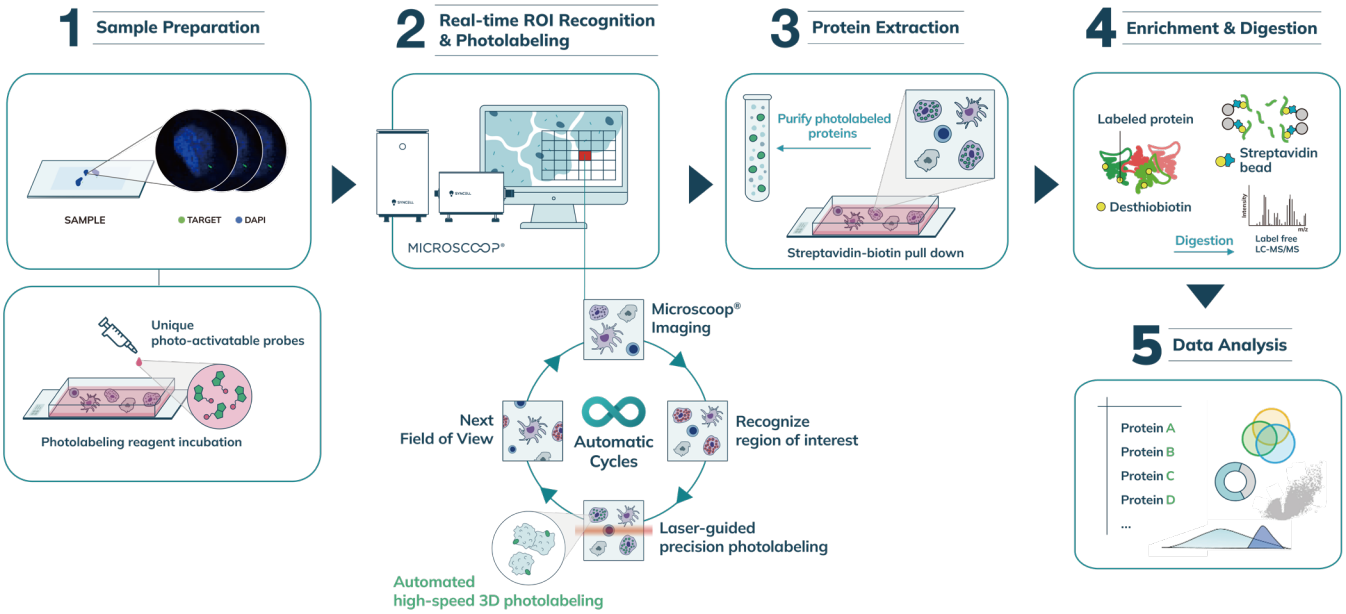


Fig. 1 | Schematic workflow for mapping the primary cilia proteome. A microscopy-guided protein discovery platform integrating image acquisition, photochemistry, microscopy, optics, and mechatronics enables ultrahigh-content in situ photolabeling followed by massspectrometry analysis.

## Biotinylating cilia proteins at high precision

Primary cilia in RPE-1 cells, which are tiny organelles as small as 0.2 µm wide and 1 µm long, are immunofluorescent stained for photo-biotinylation. Microscoop® identified primary cilia and precisely photolabeled cilia with its two-photon illumination, as evidenced by the overlap between biotin staining (green) and primary cilia (red) signal in both lateral (xy) (Fig. 2B) and axial (z) directions. (Fig. 2C).

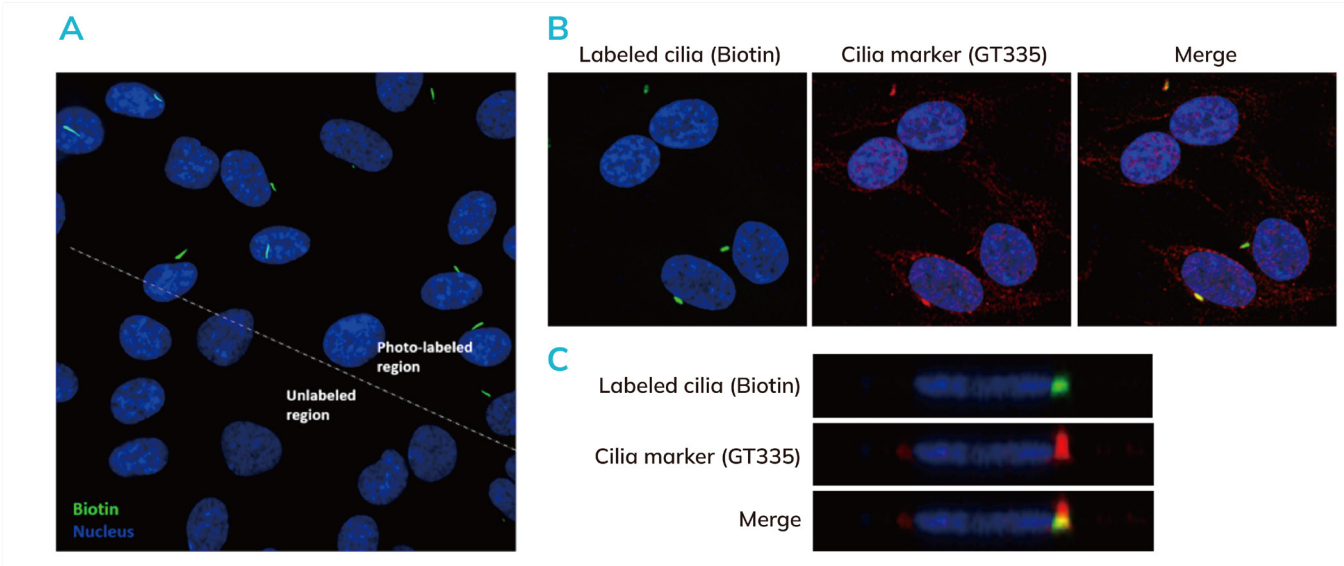


Fig. 2 | Validating Cilia Labeling with Microscoop® Technology. (A) Comparative analysis shows specificity and accuracy, with photolabeled proteins in green above the dashed line and unlabeled regions below. (B) Confocal microscopy images of photolabeled RPE-1 cells display precise protein localization within cilia, with ciliary structures stained in red (GT335) and photolabeled proteins in green. (C) Side-view analysis via confocal 3D imaging confirms unbiased photolabeling of specific ciliary regions.

## Exploring the Proteomic Landscape of Ciliary Components

Photolabeling experiments were meticulously carried out on PFA-fixed RPE-1 cells, with precise targeting of GT335-marked regions (Fig. 1, Fig. 3A). Subsequently, cells were lysed to extract proteins, which underwent enrichment via streptavidin pulldown, followed by trypsin digestion, and analysis through LC-MS/MS (Fig. 1). This comprehensive analysis revealed a proteome comprising 4,233 proteins, with a notable enrichment of 524 known ciliary proteins (Fig. 3B). Particularly notable among these identified proteins were key players in ciliary trafficking, such as intraflagellar transport proteins (IFTs), kinesins, dyneins, GTPases, and phosphatidylinositol phosphates (PIPs) (Fig. 3C). Additionally, proteins associated with structural support and cellular organization, including microtubules, septins, and annexins, showed significant enrichment.

Gene ontology (GO) enrichment analysis emphasized the significant association of high-ranking proteins with critical ciliary processes such as assembly, transportation, and signaling, particularly highlighting proteins involved in intraciliary transport (Fig. 3D). Furthermore, a group of novel protein constituents was identified, presenting testable hypotheses for their roles in primary cilia. These findings underscore the effectiveness of targeted photolabeling and proteomic analysis in elucidating the intricate network of proteins essential for ciliary structure and function.

## Conclusion

Microscoop® demonstrates its potential in unraveling intricate cellular mechanisms with unparalleled precision. The exceptional resolution offered by Microscoop® opens new avenues for studying small subcellular structures, paving the way for breakthroughs in biological research.

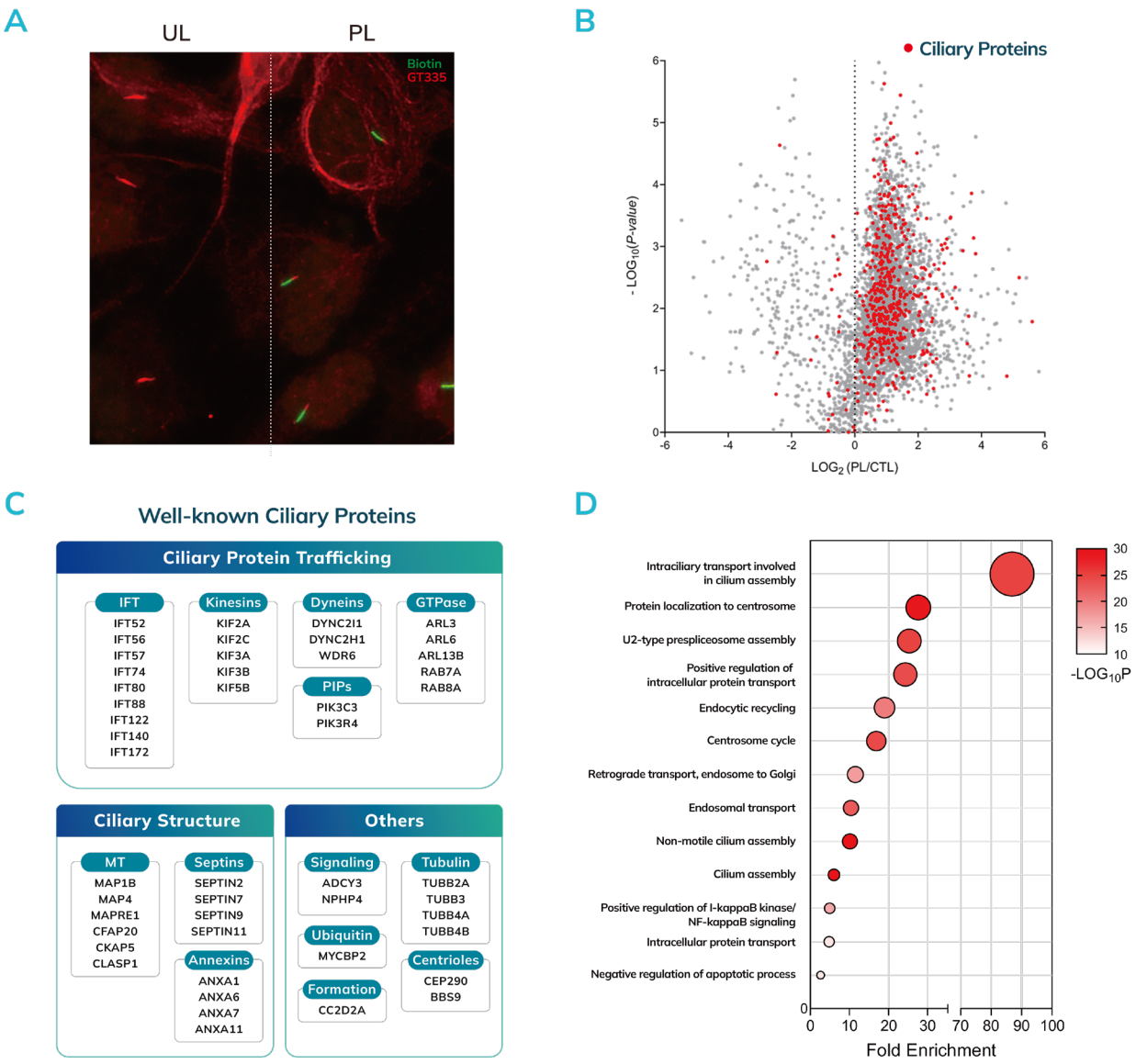


Fig. 3 | (A) Confocal micrographs of unphotolabeled (UL) and photolabeled (PL) user defined primary cilia. (B) A distribution of overall protein abundances is binned by the ratio of copies in a photolabeled (PL) sample to those in a control (CTL) sample annotated as PL/CTL ratio. Ciliary proteins (red) are enriched in the PL group compared to the CTL sample. (C) Well-known cilia proteins identified by Microscoop®. (D) The top 100 enriched proteins were subjected to Gene ontology to reveal cilia related biological process.

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