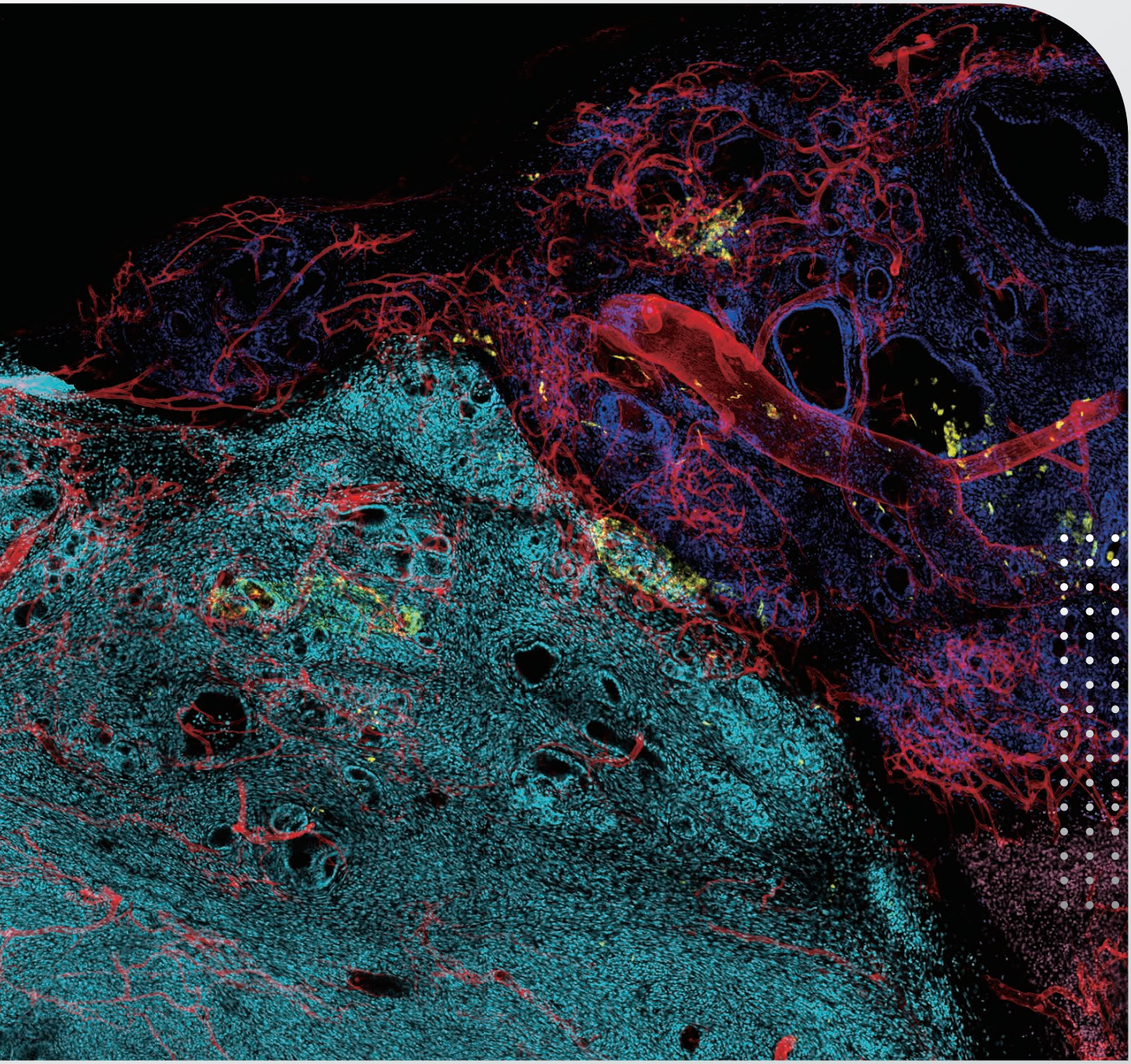
SYNCELL

Doc#GSD001 Version 1.1

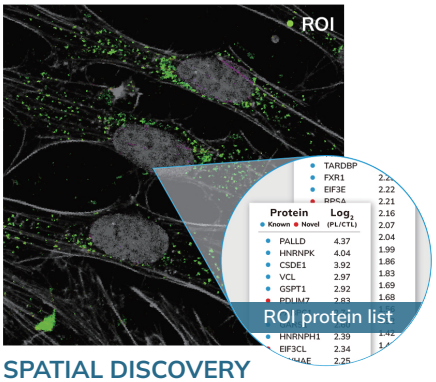
The MICROSCOOP® MINT System Grant Support Document



Why Spatial Discovery?

Spatial discovery represents a significant shift in proteomic research, focusing on the de novo identification of proteins in specific cell compartments and microenvironments. Unlike spatial targeted profiling that rely on predefined sets of known proteins that require antibodies or aptamers, spatial discovery starts with using Microscoop® to precisely extract the proteins localized to selected regions of cells, cellular compartments, or structures. These extracted proteins are then subjected to mass spectrometry analysis for identification and characterization.

The key advantage of spatial discovery lies in its ability to unveil previously unrecognized proteins that may play crucial roles in cellular processes within specific spatial contexts. By casting a wide net for protein identification, spatial discovery offers a comprehensive view of the protein landscape within selected cellular regions at subcellular precision, opening new avenues for understanding cellular function and signaling pathways.

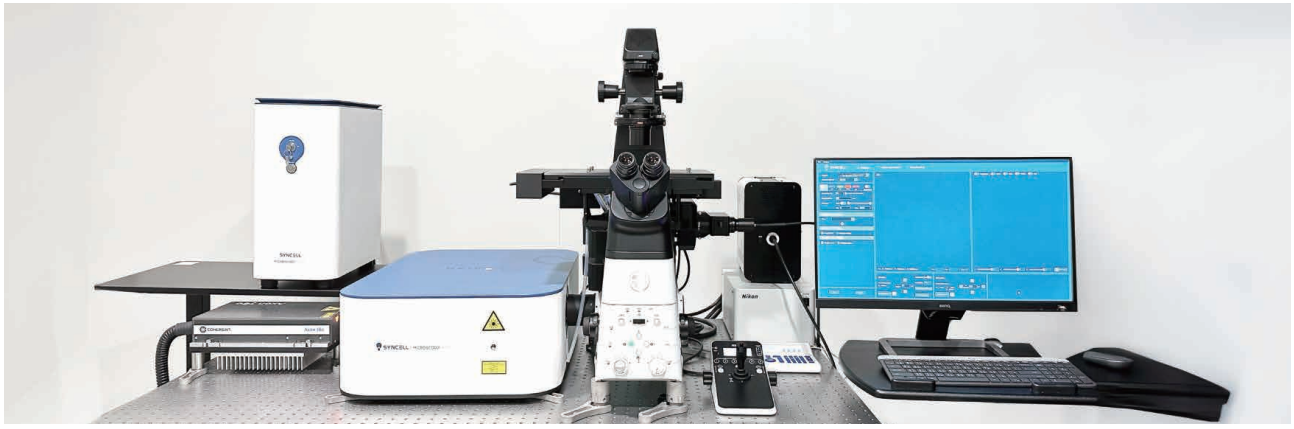


SPATIAL DISCOVERY
Identifying proteins in regions of interest (ROIs)

Platform Overview

Microscoop® Mint is the solution for spatial protein purification.

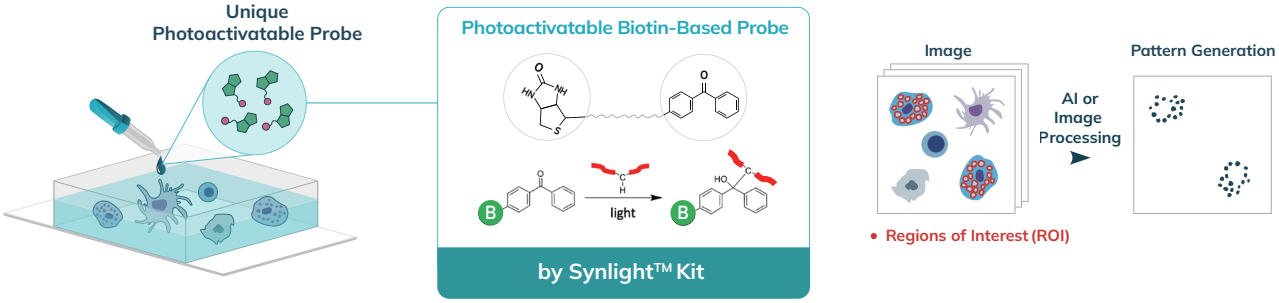
- Isolates proteins from specific subcellular locations with high precision.
- Enables unbiased discovery of new protein constituents at your region of interest (ROI).
- ROI's can be generated at the level of hundreds of nanometers.
- No protein candidate list is needed in advance.
- Automatically photo-biotinylates proteins in millions of similar spatial targets.
- Discovers novel biomarkers or therapeutic targets of disease-associated locations.
- Subsequent LC-MS/MS analysis to reveal novel spatial proteomes.



How Microscoop® Works

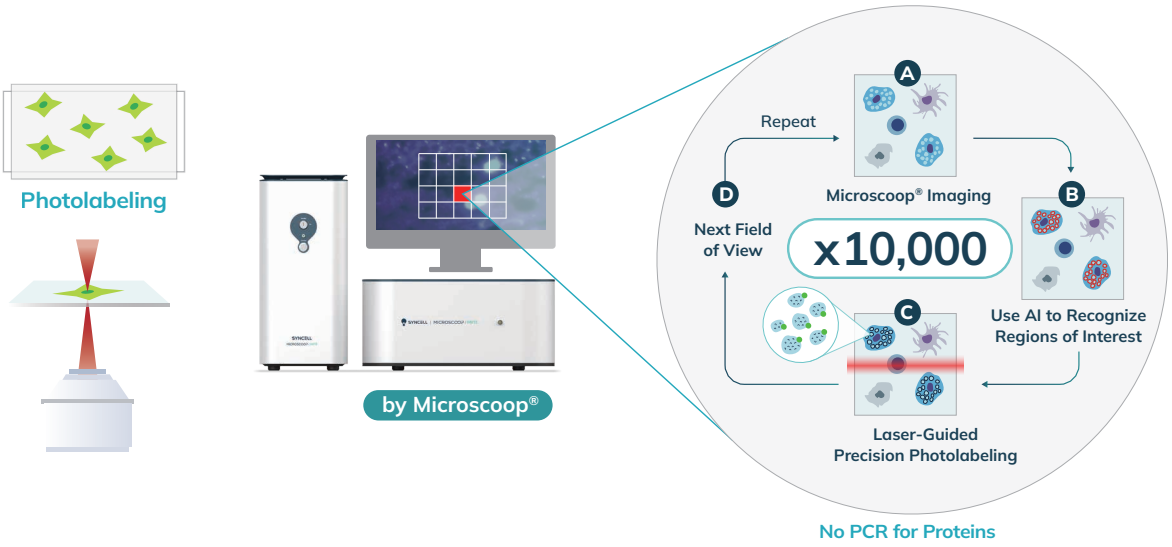
STEP1 Real-Time Image Analysis

Photolabeling kit (i.e. Synlight-Pure™ Kit or Synlight-Rich™ Kit) is first added to a cell or tissue (fixed, FFPE or frozen) sample for a photochemical reaction. An image mask is created from a user-defined stain (IF, brightfield, and more) for regions of interest (ROI's) that define the local area of interest for where proteins are extracted. After the sample is loaded onto the stage, Microscoop® takes an image (or images of multiple colors) of the sample at one field of view (FOV) at a time. The image or images are analyzed in real time by Microscoop's software Autoscoop™, which executes traditional image processing or AI deep learning to segment the user's region of interest. Pre- or post-processing can be included to enhance segmentation accuracy.



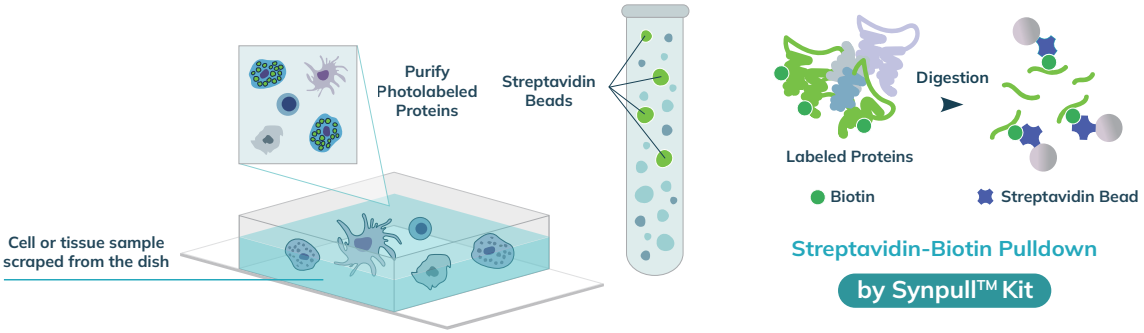
STEP2 Patterned Photo-Biotinylation

A femtosecond light source is controlled to illuminate the segmented region of interest one point at a time. This patterned illumination triggers targeted protein photo-biotinlyation in high spatial precision through the reactions of light-sensitive probes of Synlight-Pure™ Kit or Synlight-Rich™ Kit. This patterned photolabeling is repeated for thousands of FOVs automatically to assure that enough proteins are biotinlyated for later proteomics analysis using mass spectrometry.



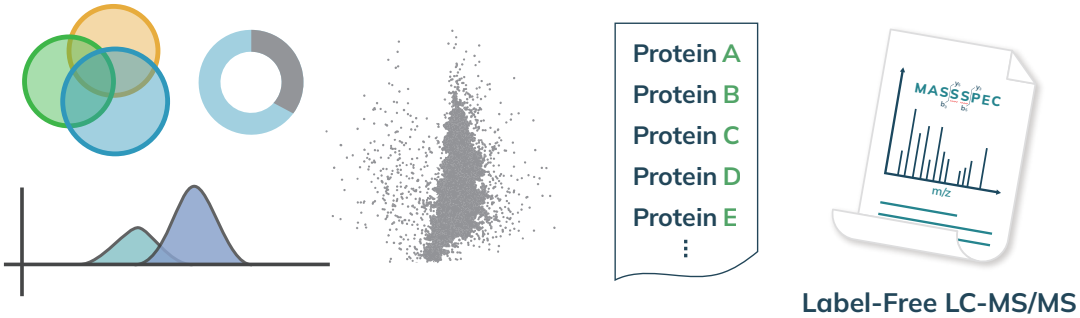
STEP3 Protein Extraction

Photolabeled cells or tissues are scraped from the slide or chamber. Materials from multiple slides or chambers can be pooled together to increase the total protein contents. The scraped sample is then treated with reagents of protein extraction kit (i.e. Synpull™) Kit to lyse the sample, enrich the proteins by immunoprecipitation, and digest them into peptides for proteomics analysis.



STEP4 Proteomic Identification

The collected peptides are sent to a mass spectrometer to perform LC-MS/MS analysis. Proteomes of both the photo-labeled and unlabeled (CTL) samples are obtained. By comparing the control and photolabeled proteomes, a location-specific proteome at the region of interest is obtained in high sensitivity, high specificity, and high spatial precision. Validation can be done by colocalization of immunostaining or additional functional assays.



The Microscoop® Mint Specifications

	DESCRIPTION	SPECIFICATIONS
	Function	Optoproteomics: Ultra-content high-speed microscopy-guided subcellular photoaffinity labeling for hypothesis-free high-precision proteomic discovery
	Workflow	Cyclic procedure of the following step: 1. Microscopy imaging: image acquisition 2. Pattern segmentation: selection of user-defined regions of interest 3. Patterned scanning illumination: point-by-point photochemical reactions 4. Stage movement: change of the field of view
	Components	❶ Microscoop® system (optical engine and electrical controller) ❷ Inverted epifluorescence microscope Filter sets for microscope ❸ Epifluorescence illumination light source ❹ Two-photon laser for Microscoop® photolabeling ❺ Camera ❻ Software package
❶	Dimensions (L x W x H)	Electrical controller : 44 cm × 22 cm × 47 cm Optical engine: 68 cm × 46 cm × 22 cm
❶	Power Source	100 - 240 VAC, 50/60 Hz
❷	Objectives	10x (up to NA 0.45) 20x (up to NA 0.80) 40x (up to NA 0.95)
❷	Stage	Motorized XY positioning stage (X: ±57 mm, Y: ±36.5 mm stroke) with a vessel holder, suitable for microscope slides, chamber slides, or micro-dishes
❸	Imagery Wavelength	Dyes: e.g. DAPI, FITC, Cy3, Cy5 Fluorescent proteins: e.g. EBFP2, EGFP, DsRed/mCherry
❺	Camera	sCMOS camera (resolution: 2048 × 2048, pixel size: 6.5 μm × 6.5 μm)
❺	Binning Options	Low resolution mode: 800 × 800 pixels High resolution mode: 1600 × 1600 pixels
❻	Operating System	Microsoft Windows 10
❻	Pattern Segmentation Options	Toolbox for traditional image processing Trained model using AI deep learning
	Labeling Resolution	300 nm+**
	Sample Format	Cells - fixed on a chambered coverslip Tissues - slide mounted FFPE (5 - 10 μm in thickness) or frozen tissue section (10 - 20 μm in thickness)
	Sample Size Requirement	Cell numbers: 4 x 10^5 to 1 x 10^6 cells for a single LC-MS/MS analysis* Tissue slides: 4 - 8 tissue section for a single LC-MS/MS analysis*

*Application dependent on and varying with the area and the number of ROIs. **Objective dependent

Supporting Data

Reproductivity of Microscoop®

Syncell Microscoop® demonstrates a robust identification and analysis of stress granules (SGs). Accurate photolabeling and efficient image processing techniques were developed, resulting in high reproducibility of SG image masking, photo-labeling, and proteomics experiments. Novel SG proteins were identified and validated, contributing to a better understanding of SG composition.

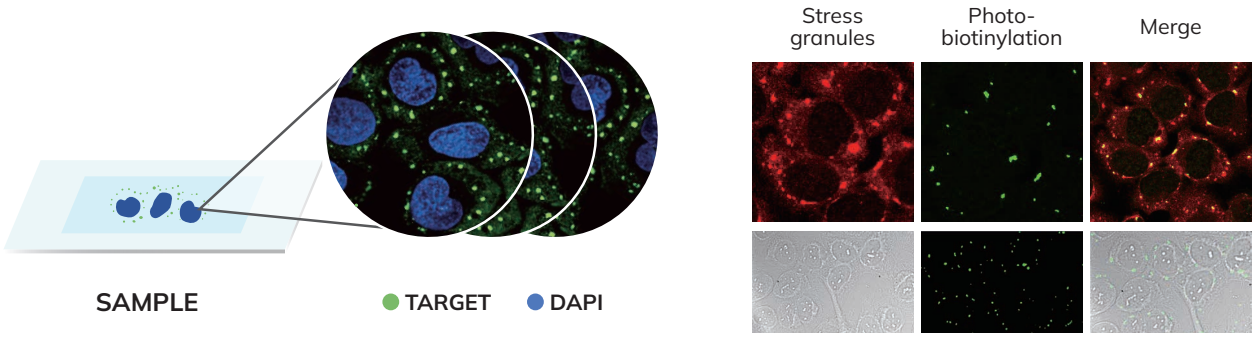


FIGURE 1 | Precise and accurate photolabeling for each stress granule in U-2OS cells.

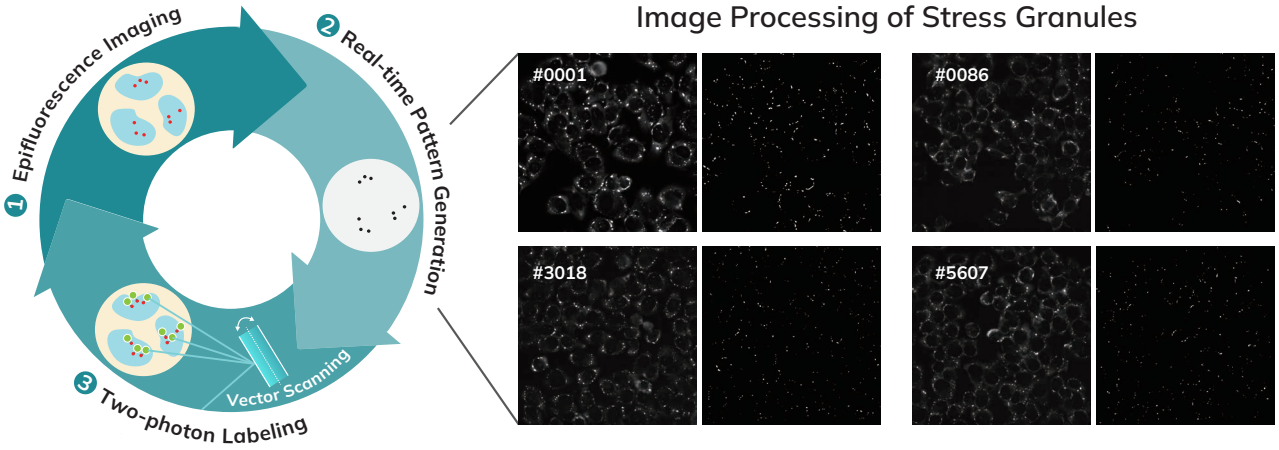


FIGURE 2 | Robust image processing isolates stress granules from background noise in each field of view (FOV), generating consistent patterns in all FOVs.

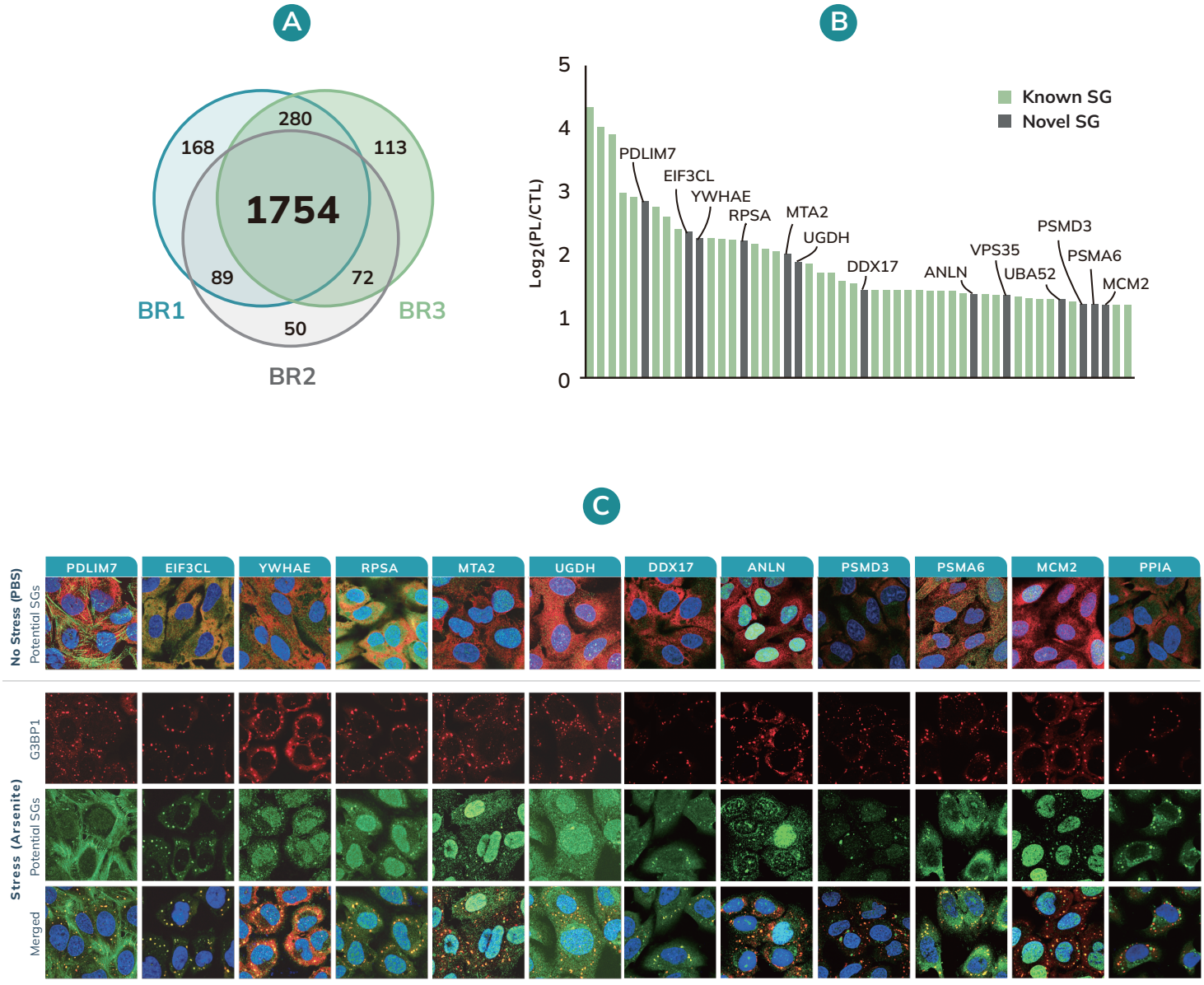
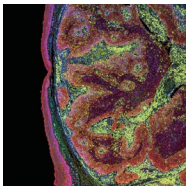


FIGURE 3 | A, High reproducibility was demonstrated between three biological replicates of spatial photolabeling SG proteomics. B, Identification of potentially novel SG protein among top-ranked spatially purified proteins. C, Validation of novel SG proteins by immunostaining in U-2OS cells, which form SGs under arsenite stress. Proteins identified as potential SG proteins (green) are highly co-localized with SG marker G3BP1 (red).

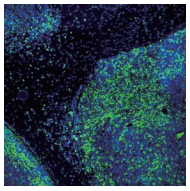
Applications

Microscoop® is a microscopy-guided protein-picking system designed for precise photobiotinylation at up to 240nm resolution. If you can clearly define your region of interest under a microscope using immunofluorescent imaging, Microscoop® can achieve targeted protein labeling. It supports a variety of dyes, including DAPI, FITC, Cy3, Cy5, and fluorescent proteins like EBFP2, EGFP, and DsRed/mCherry. Traditional image processing tools, such as adaptive thresholding and filtering, can be employed to define specific regions of interest. Applicable cellular patterns include subcellular structures, cell-cell contact sites, organelle contact sites, entire cells, and aggregates. Microscoop® has been successfully used with formalin-fixed, paraffin-embedded (FFPE), fresh frozen tissue samples, and fixed cultured and primary cell samples.

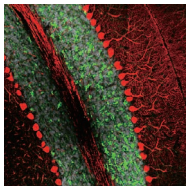
Broad Discover Applications



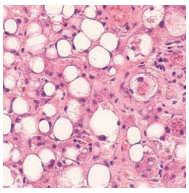
Oncology



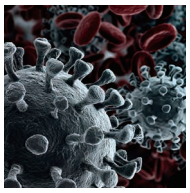
Cancer Immunotherapy



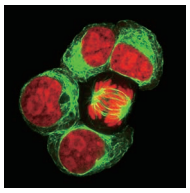
Neuroscience



Metabolic Disease

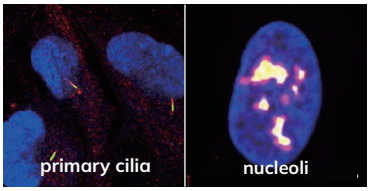


Cell Biology

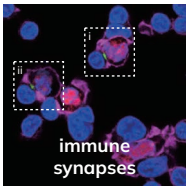


Developmental Biology

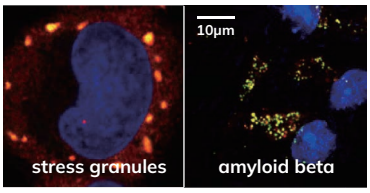
Applicable Cellular Patterns



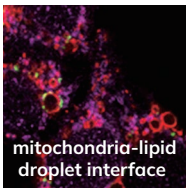
Subcellular structures



Cell-Cell contact sites



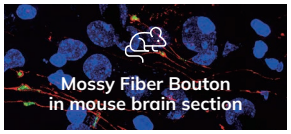
Aggregates



Organelles contact sites

Applicable Sample Types

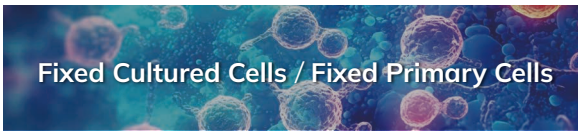
Tissue



FFPE | Formalin-fixed & Paraffin-embedded

Fresh Frozen sections

Cells



Applicable Areas

Microscoop® Consumables

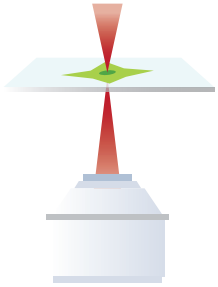
Syncell offers optimized reagent kits for Microscoop®. Synlight Kits are the proprietary kits that contain photochemical probes and other needed reagents for photolabeling. Synlight-Rich™ Kit is suitable for features larger than 300 nm with high labeling efficiency. Synpull™ Kit contains a large group of reagents optimized for low-volume pulldown and mass spectrometry-ready preparation suitable for both cell and tissue samples.

The following content is commercially available for precise photolabeling and pulldown:

Photolabeling Kit

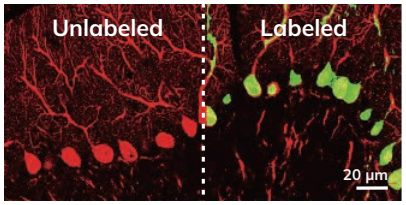
Synlight-Rich™ Kit

Up to 6 reactions (1-3 rounds** of LC-MS/MS)
*Positive control included **1 round=1 test group+1 control group

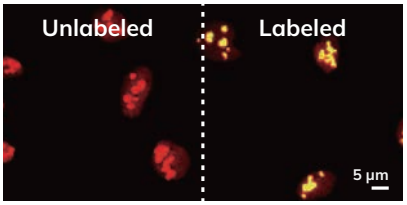


Targeted photolabeling by MICROSCOOP®

Synlight-Rich™ enables high efficiency photolabeling, suitable for sub-micron or micron size structures, such as the cell bodies (~10 μm) and axons (~2 μm) of Purkinje cells as well as nucleoli (~1 μm).



Purkinje cells
in the mouse cerebellum

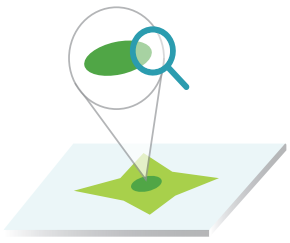


Nucleoli

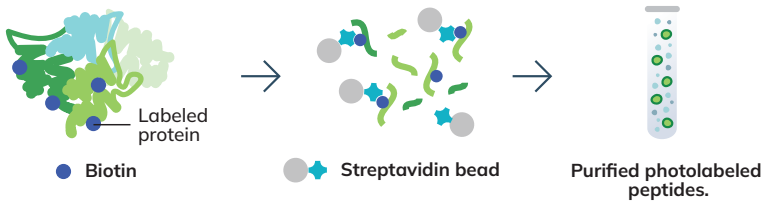
Protein Extraction Kit

Synpull ™ Kit

Up to 6 reactions (3 rounds** of LC-MS/MS)
**1 round=1 test group+1 control group



Extraction and processing of photolabeled samples for MS-based proteomics



Benefits to consider for your research

High Sensitivity
Microscoop® mechatronics controls the illumination of photoreactive reagents to accurately label all proteins in user specified locations.

High-Content
Users can perform multi-omics spatial labeling to identify and analyze tens and thousands of individual cells.

High Specificity
High Specificity-Unique combination of computer vision imaging and accurate photolabling provides unsurpassed high specificity for protein biomarker discover from a particular cellular or subcellular structure.

AI-Driven
Custom machine learning software solutions allow users to define different cellular and subcellular structures.

Photolabeling
Microscoop® utilizes two-photon excitation microcopy to provide accurate XYZ position labeling with subcellular resolution.

Speed
Imaging takes 0.1 seconds, followed by AI or image processing, which requires 0.1 to 0.5 seconds since the calculations are performed in real time.

Additional Resources

Training

The Microscoop® Mint support team offers expert guidance throughout the entire user experience, from installation to ongoing training. Users are invited to participate in virtual training sessions that cover a range of topics.
Additionally, Microscoop® users benefit from exclusive access to user-group meetings and workshops, where they can exchange best practices and success stories within the community, fostering a collaborative environment for maximizing the technology's potential.

Literature

For more details on Microscoop® platform, refer to our publications and supporting literature.
Publication
Product literature and research posters

Additional Support

General inquiries: info@syncell.com
Learn more about spatial protein purification solutions: contact a sales representative
Phone: +1 617-631-2746