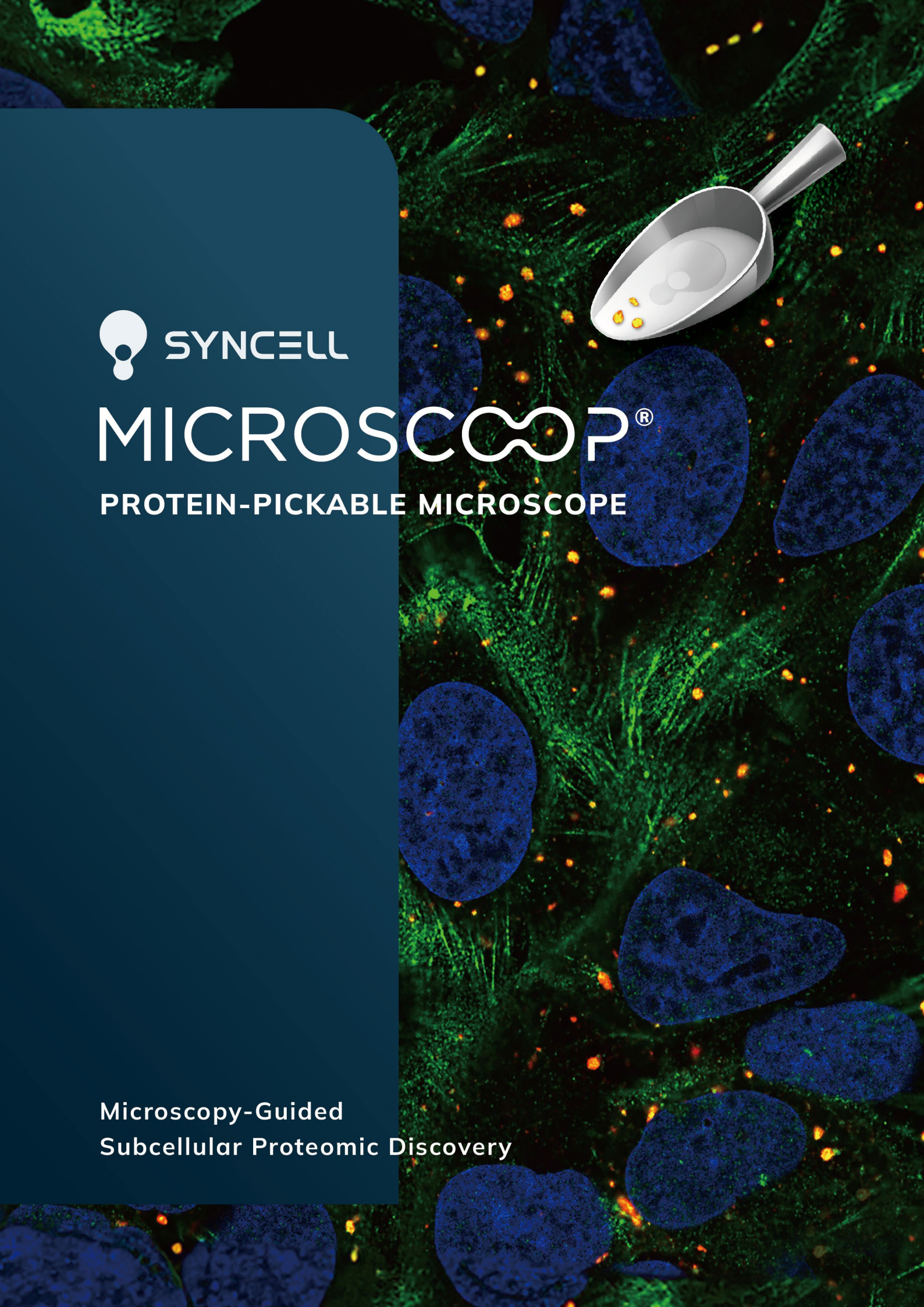


SYNCELL

MICROSCOOP®

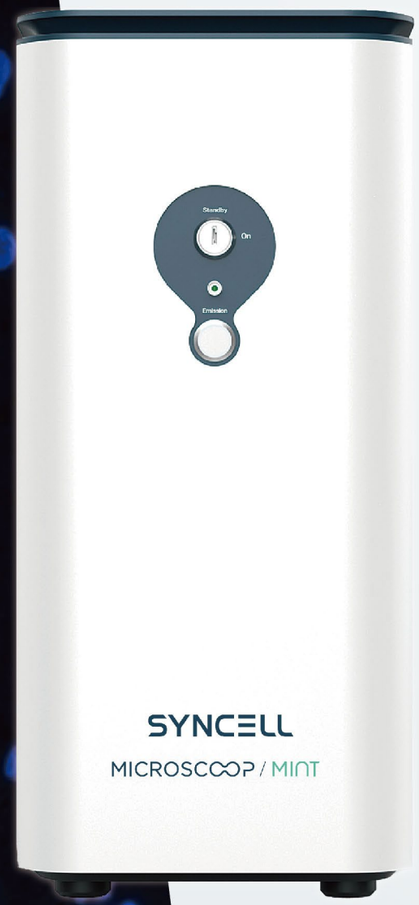
PROTEIN-PICKABLE MICROSCOPE

Microscopy-Guided
Subcellular Proteomic Discovery

AI MICROSCOPY-GUIDED PHOTO-BIOTINYLATION

TDP-43 aggregate

Revealing novel protein constituents at the TDP-43 aggregates of a postmortem FFPE brain section from an amyotrophic lateral sclerosis (ALS) patient. Sample provided by the Rossoll lab, Mayo Clinic.



TARGETED SCOOPING TO DISCOVER INVISIBLE PROTEINS

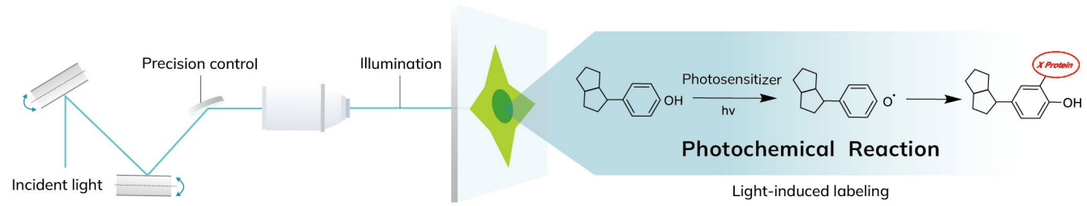
Microscoop® is a groundbreaking spatial proteomics platform that has been used to reveal novel protein constituents at specific subcellular regions of interest for many biological problems. Microscoop® performs microscopic scooping, i.e. automated ultra-content microscopy-guided photo-biotinylation to photolabel and isolate/pick enough subcellular proteins for mass spectrometry-based proteomic discovery. It is an unprecedented spatial pulldown technology that enables unbiased subcellular proteomic identification in high resolution, high sensitivity, and high specificity.

HOW MICROSCOOP® WORKS?

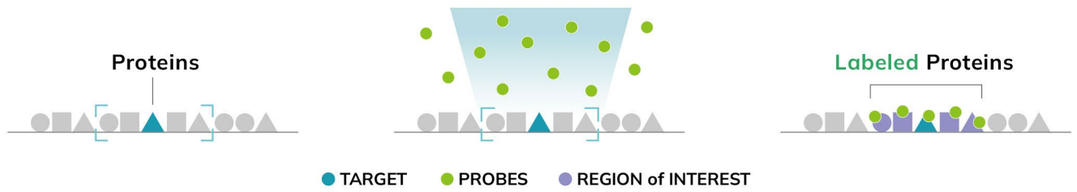
PHOTOCHEMISTRY

Submicron spatial photo-biotinylation

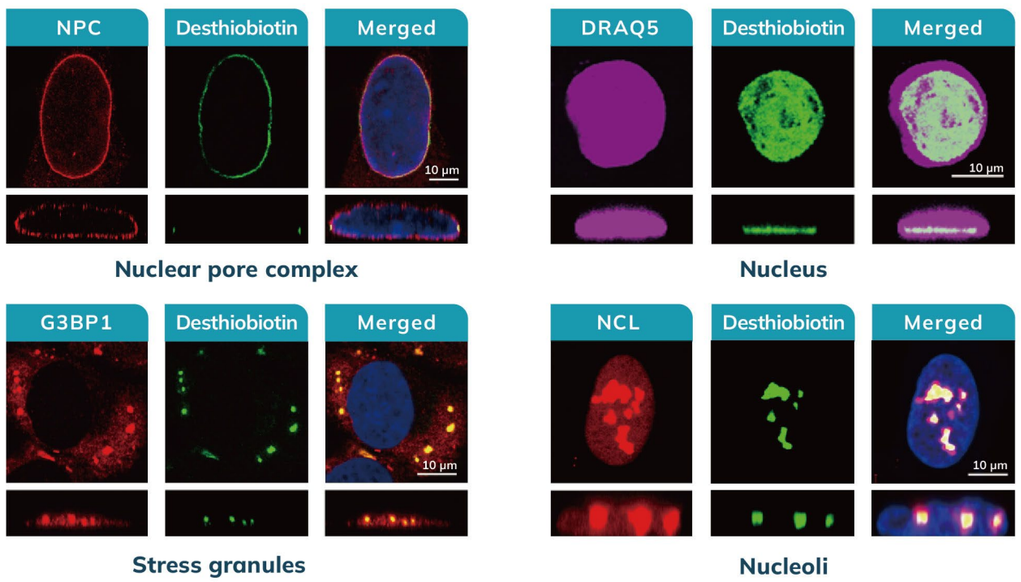
Photolabeling is achieved by utilizing two-photon illumination to trigger a photochemical reaction with a photocatalyst, which drives redox reactions of molecules that are composed of biotin and a photoactivable amino acid linker to form covalent bonds with, or biotinylate, amino acids within the illuminated focal spot at the submicron labeling resolution. Duration of each illumination spot is in the millisecond or sub-millisecond range to allow fast biotinylation for the entire sample.



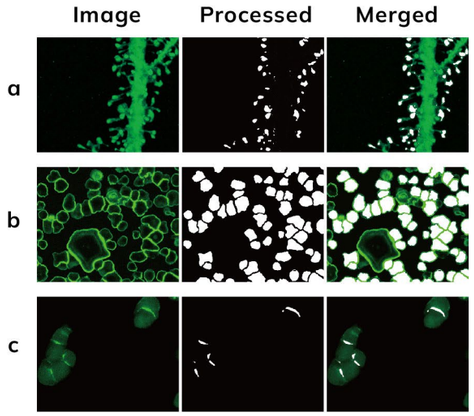
REGION of INTEREST ▶ ILLUMINATION ▶ LABELED



PHOTOLABELING IMAGING



ON-THE-FLY AI



AI-Guided Targeted Photolabeling

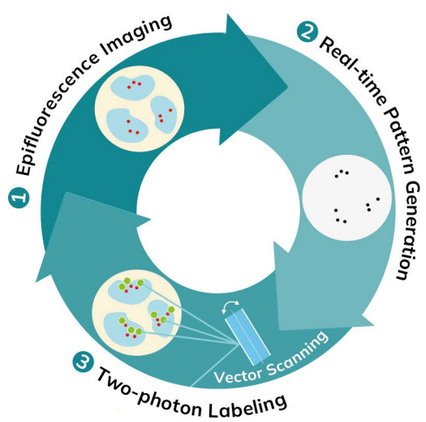
When traditional image processing is not precise enough to segment the region of interest, possibly due to the complexity of the images or image quality, one can use deep learning-based image segmentation to achieve proteomic discovery. Hundreds of annotated images are used to train the neural network for a specific biological problem. Microscoop's software Autoscoop™ calls the trained neural network so that the system can recognize the region of interest for each FOV on the fly. It is important to perform traditional image processing (a) or AI (b,c) on the fly to achieve high-speed photolabeling.

MECHATRONICS

Synchronized Automation

The hardware-firmware-software integrated mechatronic system enables accurate and fast control of scan systems, lasers, microscope, camera, epi-illumination light source, and peripheral devices in real time. The automated process was optimized by synchronizing steps from imaging to intelligent labeling with sub-millisecond temporal precision through this integrated system to allow high-speed, high-resolution spatial photolabeling.

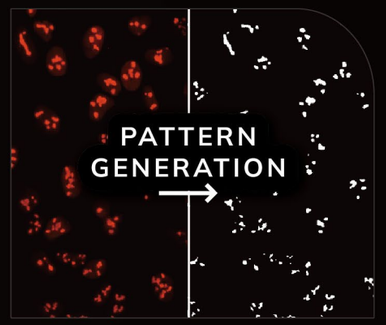
THOUSAND CYCLES OF REPEATS



Ultra-Content

Proteins collected from the regions of interest of one FOV are not enough for mass spectrometer's sensitivity to reveal low abundant proteins. To address the protein amplification problem, Microscoop® achieves protein accumulation by performing automated targeted photolabeling at ~10,000 or more FOVs to biotinylate enough proteins for mass spectrometry. The three steps of imaging-pattern generation-photolabeling are repeated for all FOVs. The speed of each step is optimized so that the entire photolabeling process can be finished overnight.

WORKFLOW



STEP 1

REAL-TIME IMAGE ANALYSIS

Photolabeling kit (i.e. Synlight-Pure™ Kit or Synlight-Rich™ Kit) is first added to a cell or tissue sample for a photochemical reaction. 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.

STEP 2

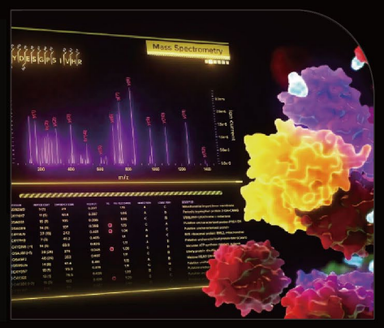
PATTERNED PHOTO-BIOTINYLATION

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.

STEP 3

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.



STEP 4

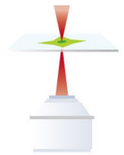
PROTEOMIC IDENTIFICATION

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.

REAGENT KITS FOR MICROSCOOP®

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.

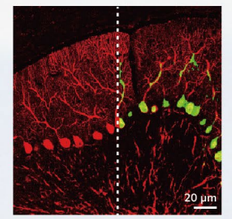
PHOTOLABELING KIT

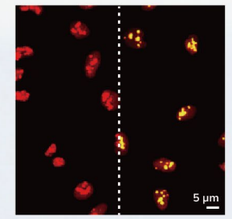


Targeted photolabeling by MICROSCOOP®

PRODUCT NO.	NAME	QUANTITY
SYN-RI0106* / SYN-RI0206	**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





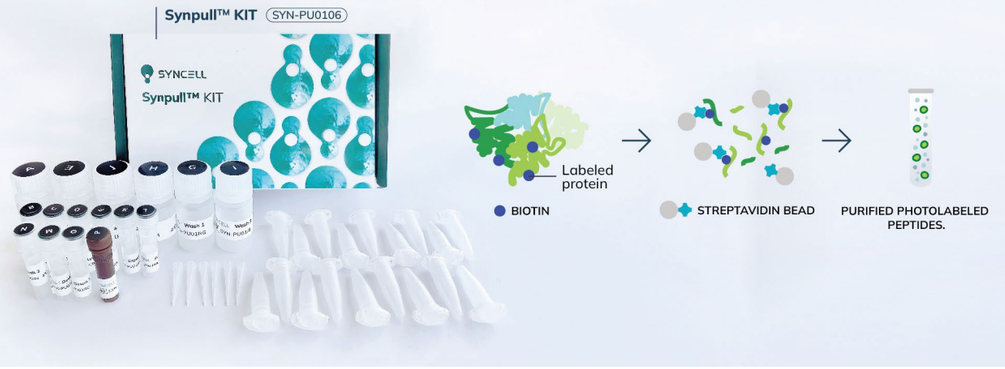
Synlight-Rich™ enables high efficiency photolabeling, suitable for sub-micron or micron size structures, such as the cell bodies (~10 μm) and axons (~2 μm) of Purkinje cells as well as nucleoli (~1 μm).

PROTEIN EXTRACTION KIT

Extraction and processing of photolabeled samples for MS-based proteomics

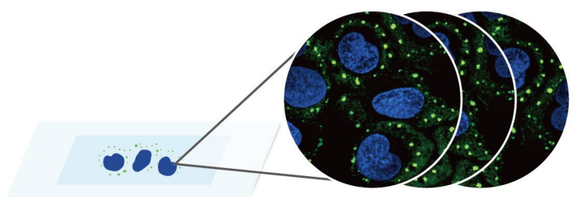
PRODUCT NO.	NAME	QUANTITY
SYN-PU0106	**Synpull™ KIT**	Up to 6 reactions (3 rounds** of LC-MS/MS)

**1 round=1 test group+1 control group



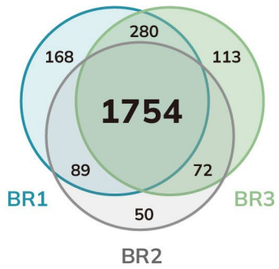
SUBCELLULAR SPATIAL PROTEOMIC DISCOVERY

INPUT

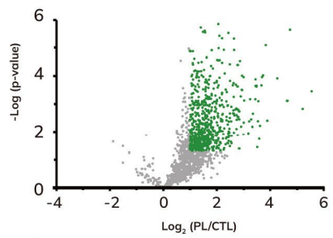


MICROSCOOP®
+
Mass Spectrometry

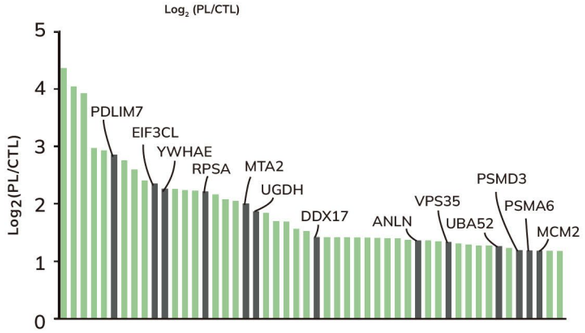
OUTPUT



Venn diagram of the stress granule proteins spatially isolated by Microscoop® and analyzed by mass spectrometry.



Volcano plot of relative protein levels in photolabeled (PL) samples to control (CTL) samples in log_2 scale. Over-represented (enriched) proteins are shown in green.



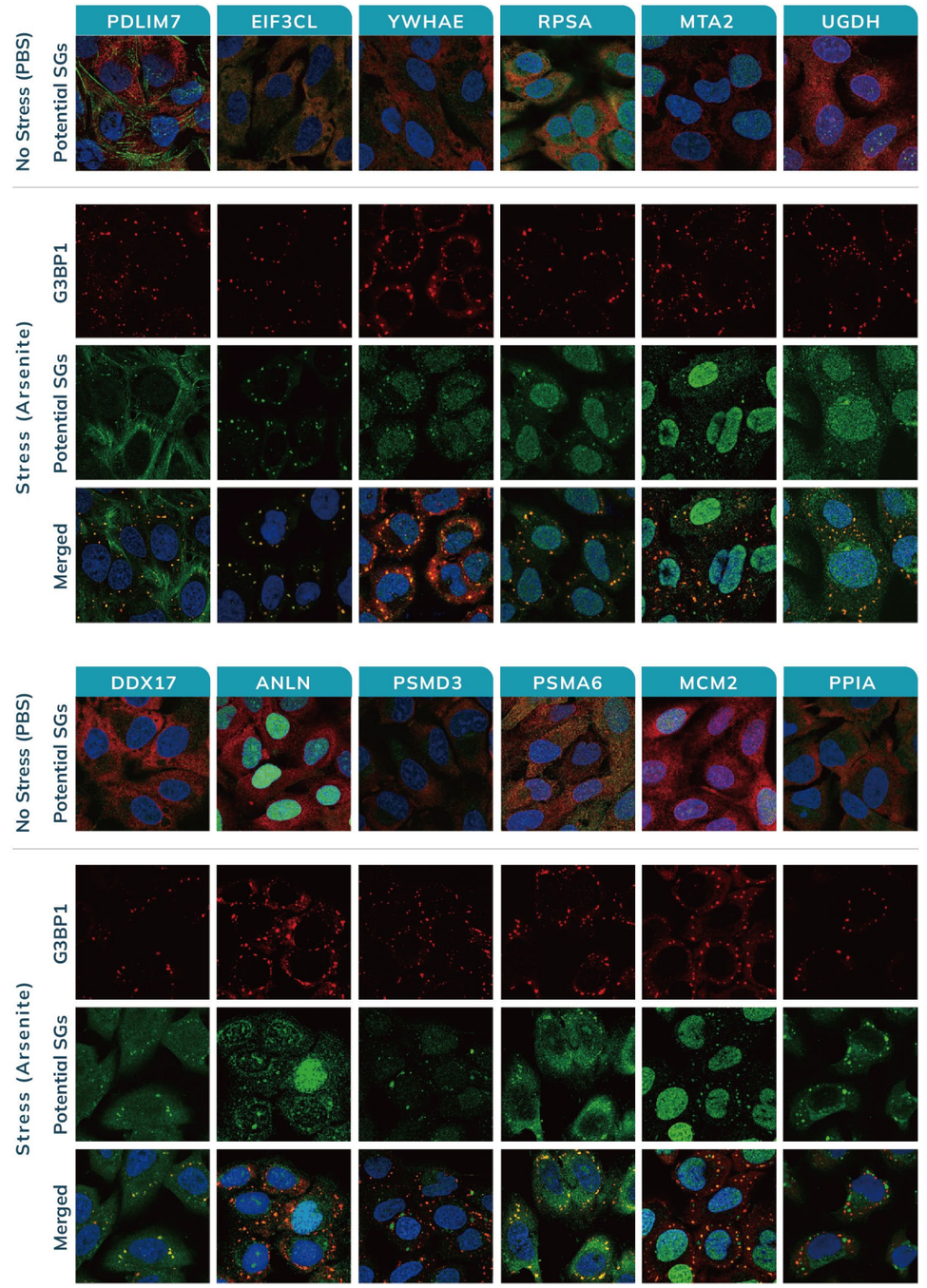
Top 50 spatially enriched proteins by Microscoop® include many known stress granule proteins (green) and others without clear prior annotation as stress granule proteins (gray).

Protein ● Known ● Novel	Log_2 (PL/CTL)
PALLD	4.37
HNRNPK	4.04
CSDE1	3.92
VCL	2.97
GSPT1	2.92
PDLIM7	2.83
PABPC1	2.74
GARS1	2.60
HNRNPH1	2.39
EIF3CL	2.34
YWHAE	2.25
TARDBP	2.24
FXR1	2.23
EIF3E	2.22
RPSA	2.21
MOV10	2.16
HDLBP	2.07
HNRNPL	2.04
MTA2	1.99
UGDH	1.86
PCBP1	1.83
SYNCRIP	1.69
VCP	1.68
PABPC4	1.56
DPYSL2	1.52
DDX17	1.42
DDX50	1.42
EEF1D	1.42
CPNE3	1.42

A List of Proteins at the Targets

COLOCALIZATION VALIDATION

Proteins without clear prior annotation as stress granule proteins were checked by co-immunostaining with stress granule marker G3BP1 one at a time. The colocalization result shows high specificity of the Microscoop® technology. Novel protein constituents of stress granules were identified in bulk.



Colocalization validation of novel protein components of stress granules identified by the Microscoop® technology. Confocal micrographs depict stress granule formation in U-2OS cells with or without an arsenite stress. Twelve proteins without clear prior annotation as stress granule proteins are highly colocalized with stress granule marker G3BP1. Green: proteins identified by Microscoop®; Red: G3BP1; Blue: DAPI.

BROAD DISCOVERY APPLICATIONS

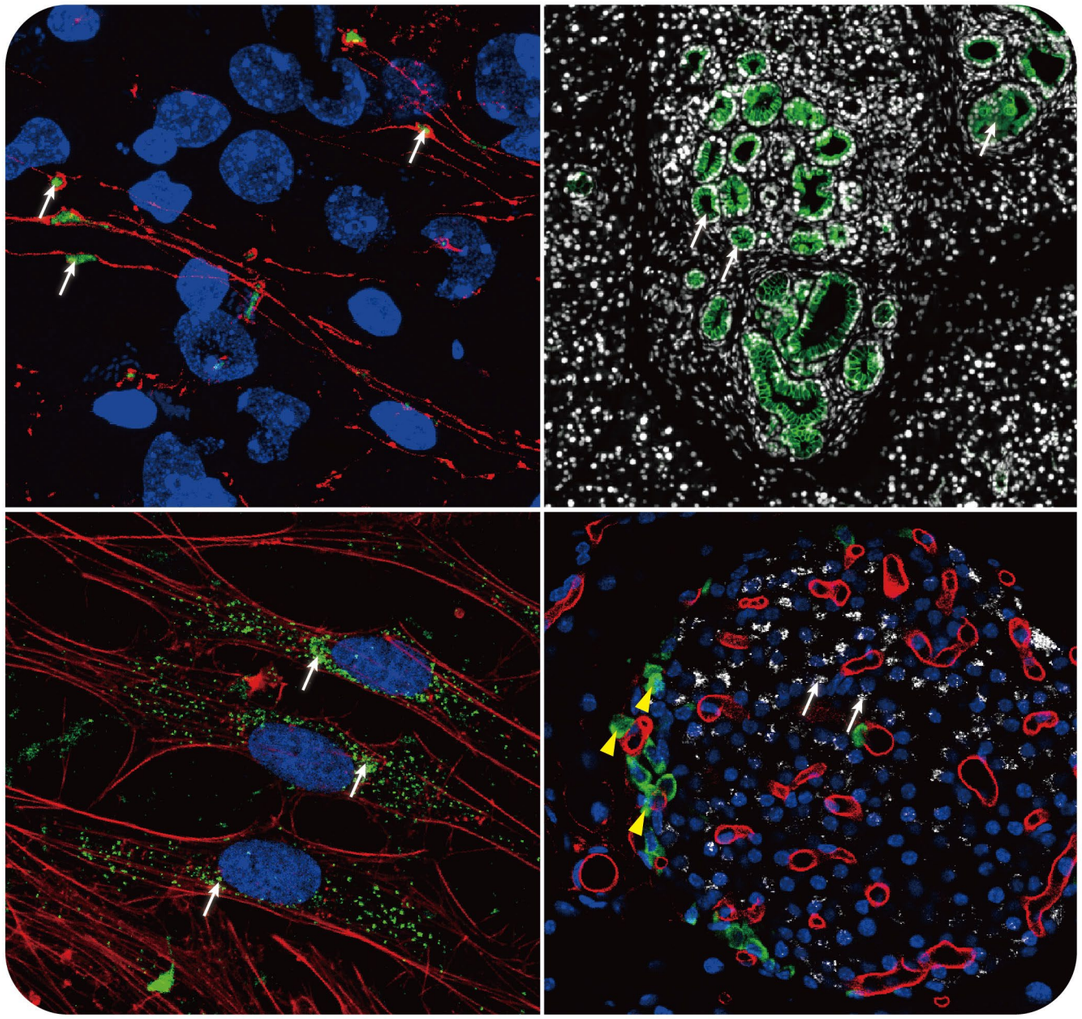
Neuroscience
Cancer Biology
Cell Biology
Immunology
Developmental Biology
Infectious Diseases
Aging
Metabolic Diseases
Inflammatory Diseases
Stem Cell Research
Immuno-Oncology
Pathology
Druggable Target Discovery
Biomarker Discovery
..... more

NEUROSCIENCE

Microscopy-recognizable structures such as β amyloids, reelin positive neurons at medial entorhinal layer II, or dendritic spines can be studied using Microscoop-enabled spatial enrichment.
Here is an example of hippocampal mossy fiber boutons photo-biotinylated by Microscoop® (Alexa488-neutravidin, green) for subsequent protein pulldown and proteome discovery.

CANCER BIOLOGY

Problems such as identifying E-cadherin-associated proteins of metastatic cells, proteins at the cancer cell-T cell interface, and the proteome of Ki67+ cells in triple negative breast cancer can be addressed by Microscoop-enabled spatial enrichment and the subsequent proteomics analysis. Here is an example of the early-stage cancer marker cytokeratin19 around lesions in mouse pancreatic cancer.



CELL BIOLOGY

Proteomes of subcellular features such as larger extracellular vesicles, stress granules, filopodia tips, focal adhesion, or ER-mitochondria interface can be identified using Microscoop® and mass spectrometry. Here shows an example of peroxisomes (PEX14, green) that can be photo-biotinylated using Microscoop® for subcellular spatial isolation and proteomics analysis.

METABOLIC BIOLOGY

Microscoop-enabled proteomic discovery can be performed on other biological problems in immunology, metabolic diseases, developmental biology, infectious diseases, etc. Here is an image of pancreatic islet, where one can isolate α cells (glucagon, green), β-cells (insulin, white), or blood vessels (WGA, red) and identify novel protein constituents.

About SYNCELL

Syncell is a life science technology company at the forefront of developing tools for next-generation proteomics. Our pioneering Microscoop® technology enables the discovery of spatial protein constituents, making de novo subcellular spatial proteomics feasible for the first time. This groundbreaking technology can be applied to a broad range of biological problems, aiding in the understanding of molecular mechanisms, identifying novel disease biomarkers, and revealing new drug targets.

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