Microscoop, a Discovery-Based Image-Guided Proteomics Technology, Reveals Novel Factors on Amyloid-β Aggregates in Differentiated SH-SY5Y Cells

Chien-Chang Huang, Weng Man Chong, Chun-Kai Huang, Yi-De Chen, Chia-Wen Chung, Hsiao-Jen Chang, Jung-Chi Liao*

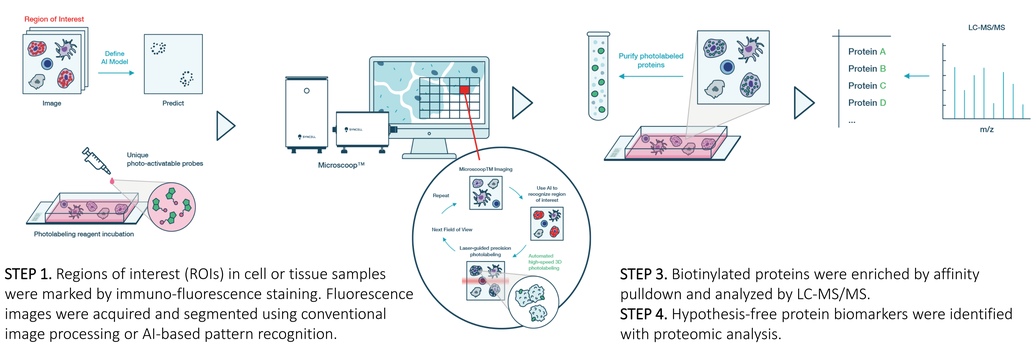
SYNCELL Inc., Taipei, Taiwan

*For more information, please visit https://syncell.com/ * corresponding author (Email: jcliao@syncell.com)*

Abstract :

Aggregation of amyloid-β peptides (Aβ) is a hallmark of Alzheimer's disease (AD). Subcellular distribution of Aβ is well recognized under a microscope, but its pathological and physiological functions remain unclear, partially due to our limited understanding of interacting proteins and corresponding signaling pathways associated with Aβ. Existing spatial proteomics technologies focus on mapping of known proteins using antibody panels/arrays, hindering hypothesis-free proteome discovery. In this study, we used Microscoop™, a fully-automated microscopy-guided subcellular photolabeling with a machine learning-based precision recognition to enable discovery-based image-guided proteomics. We applied Aβ1-42 deposition in human neuroblastoma, SH-SY5Y, differentiated cells as an AD model. Multiple images of Aβ1-42 aggregates were applied to segment to locations of specific Aβ1-42 aggregates of interest using convolutional neural networks (CNN)-based deep learning. Microscoop was used to illuminate these segmented regions to induce photochemical reactions of proprietary photosensitive probes and trigger spatial covalent labeling of proteins adjacent to Aβ1-42 aggregates. This spatial-specific photochemical labeling process was repeated automatically on thousands of microscopic fields of view to accumulate enough Aβ1-42-associated proteins for LC/MS-MS-based proteome identification. A series of novel factors were discovered to be associated with Aβ aggregation in SH-SY5Y differentiated cells. We further validated these newly identified proteins using antibody staining and found that these proteins indeed colocalized with Aβ1-42. The finding of these novel factors opens a door to reveal associated signaling pathways related to AD. They may also serve as new diagnostics biomarkers or new AD drug target. Our study not only reveals the Aβ-associated spatial proteome, but also demonstrates its possible broad applications on discovery-based spatial proteomics in neuroscience.

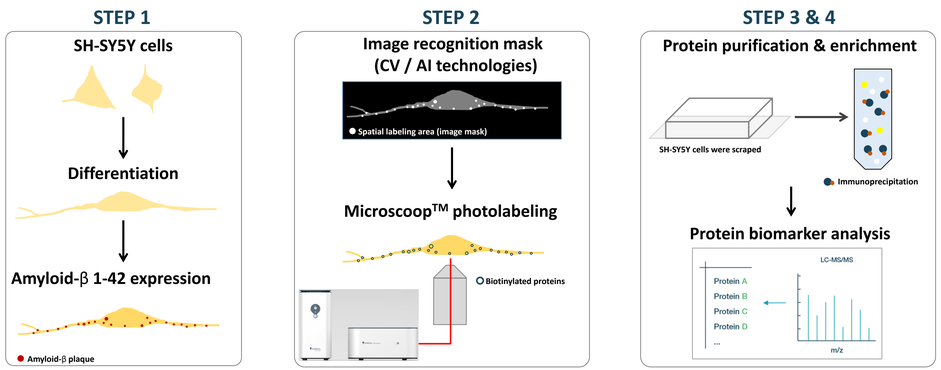
Microscoop™ : A novel platform for spatial proteomic profiling via machine learning-guided microscopic photolabeling



STEP 1. Regions of interest (ROIs) in cell or tissue samples were marked by immuno-fluorescence staining. Fluorescence images were acquired and segmented using conventional image processing or AI-based pattern recognition.
STEP 2. Samples were incubated with photoactivatable biotin probes. Photolabeling at subcellular ROIs were performed with two-photon illumination of the Microscoop system.

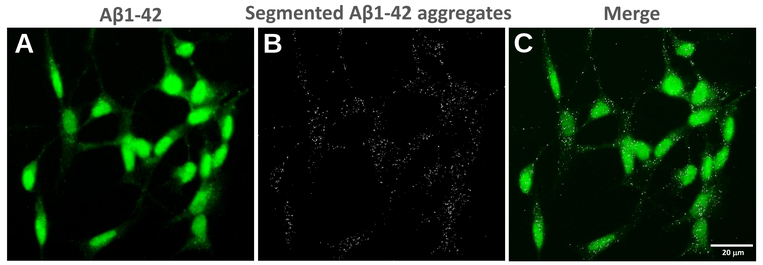
STEP 3. Biotinylated proteins were enriched by affinity pulldown and analyzed by LC-MS/MS.
STEP 4. Hypothesis-free protein biomarkers were identified with proteomic analysis.

Proteomic discovery assay for in vitro amyloid-β plagues



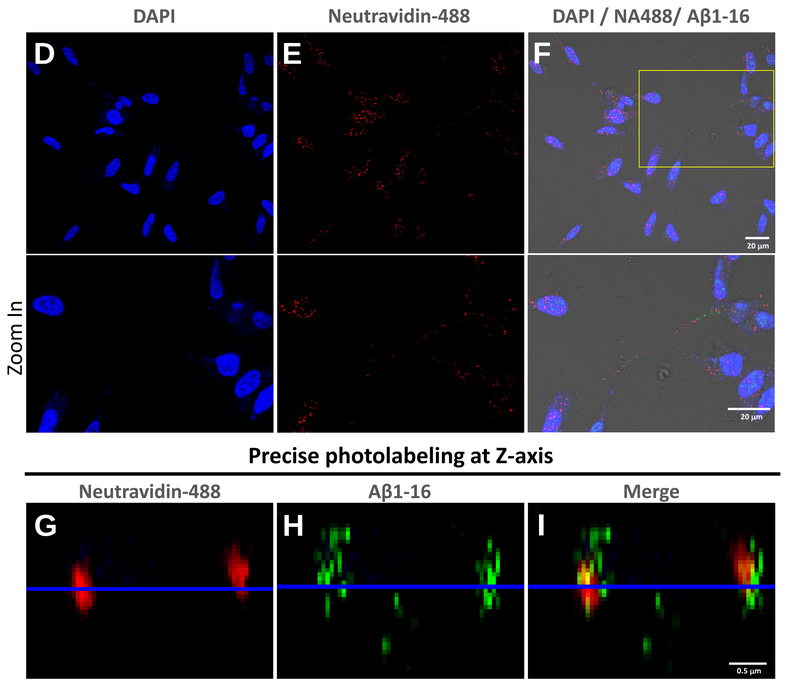
Locations of Aβ1-42 aggregates were segmented in real time using Microscoop

A modified otsu thresholding method was first applied to images after image pre-processing. A following white spot extraction method by logic operation was implemented to segment Aβ1-42 aggregates with high precision (Fig. A-C).



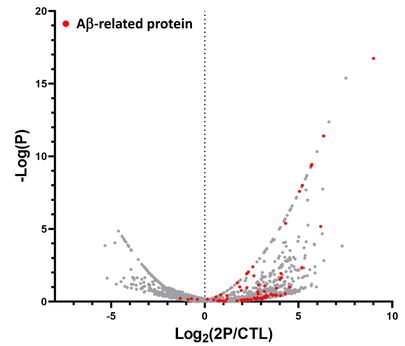
Targeted photo-induced biotinylation using Microscoop

SH-SY5Y cells were differentiated for 5 Days. Aβ1-42 were expressed by lentivirus infection for 72 hrs. The Aβ1-42 aggregated spots were indicated by immunostaining. The cells were then incubated with a photo-activatable probe, and the spatial photolabeling process was performed at the segmented locations for thousands of fields of view fully automated with Microscoop to biotinylate proteins in the regions of Aβ1-42 aggregates. Confocal imaging validated the precision of photolabeling (Fig. D-F). Fig. G-I indicates precise labeling viewed from the z direction. High-speed photolabeling for thousands of fields of view was necessary to assure collection of a large number of proteins of interest enough for mass spectrometry sensitivity.



Spatial protein composition of Aβ1-42 aggregates were identified by Microscoop

LC-MS/MS results of well-known Aβ-related proteins



The proteome of the photo-labeled sample by two-photon (2P) illumination was compared with that of the unlabeled sample (CTL). In total, 837 proteins were identified in the 2P enriched group. 24 proteins were found to map to the existing Aβ-related protein database in UniProt. Among them, 15 proteins are well-studied and highly related to Aβ aggregates. Further validation of high-ranked candidates by immunofluorescence staining will be performed to identify possible novel Aβ-associated proteins.

Drebrin	ADNP	VDAC1	3-hydroxyacyl-CoA dehydrogenase type-2
CDK5	Reticulon-3	Tmed10	Delta(24)-sterol reductase
TPK YES	Reticulon-4	Gelsolin	Ubiquitin-60S ribosomal protein L40
TPK LYN	hnRNP A2B1	Histone H2B type 2-E	Amyloid-β precursor protein
Rab11A	PARP1	Histone H2A type 2-C	Tubulin alpha-1B chain
Pin1	OSTC	Histone H4	Clathrin heavy chain 1
SynAz1*			
SynAz2*			

Conclusion

SYNCELL Microscoop is a new technology platform for hypothesis-free spatial protein biomarker discovery. It has been used to identify novel stress granule proteins in bulk in another study. Here we have precisely biotinylated proteins in Aβ1-42 aggregates of the SH-SY5Y differentiated cell model in high content using Microscoop. We have further performed LC-MS/MS and identified the proteome of these Aβ1-42 aggregates. This proteome not only contained known Aβ-associated proteins, but also repeatedly revealed proteins that are not considered as Aβ-related proteins so far. Further validation is in progress to check whether novel Aβ-associated biomarkers can be identified by SYNCELL Microscoop.