De novo spatial proteomic profiling of immune synapses using machine learning-guided Microscoop

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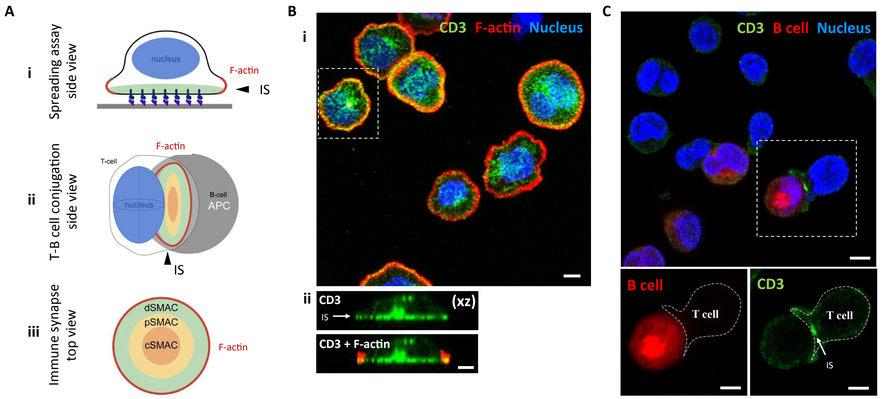
Abstract

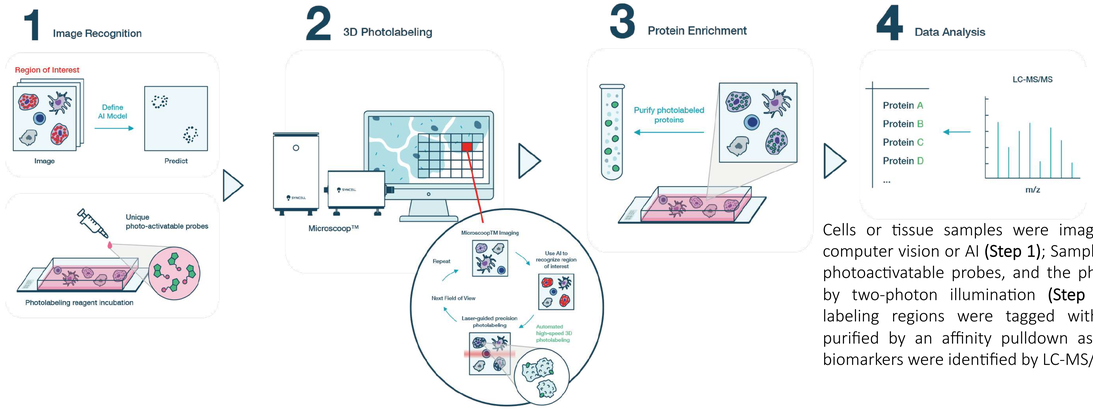
The spatial proteome of the immune synapse (IS) between a target cell and a lymphocyte is fundamentally important to understand the mechanism of cell-mediated immunity for both immuno-oncology and therapeutic applications. In this research, we used Microscoop™, a fully-automatic microscope system integrated with a machine learning-based algorithm, to best determine the proteome of the IS. We used Raji B cells as antigen-presenting cells (APCs) and induced the formation of IS by incubating with Jurkat T cells. Multiple IS images were applied to train our algorithm using convolution neural network-based deep learning. A sequential process including fluorescence imaging, deep learning-enabled pattern generation, and photochemical labeling was implemented to achieve spatial-specific biotinylation of the IS proteins. Moreover, Microscoop is capable of repeating the process automatically on thousands of fields of view, such that sufficient amount of immune-synaptic proteins are biotinylated for protein identification with mass spectrometry. We have successfully labeled and isolated proteins from spreading T-cell and spatially reorganized interfaces between T cells and APCs. Following MS-based proteomic analysis, several hundreds of proteins were identified, including the proteins known to be specifically associated with T cell receptor (TCR) activation such as LcK, one of the major factors involved in TCR signaling at IS. More interestingly, we identified several proteins novel for IS, including proteins involved in phosphatidylinositol signaling. Our data showcases the capability of subcellular *de novo* spatial proteomics of the Microscoop technology, revealing novel factors responsible for initiating the immune response of a lymphocyte and shedding light on immune checkpoint signaling and tumor immunotherapy.

Spreading T cells and T-B cell conjugation models are used to reveal novel biomarkers on IS



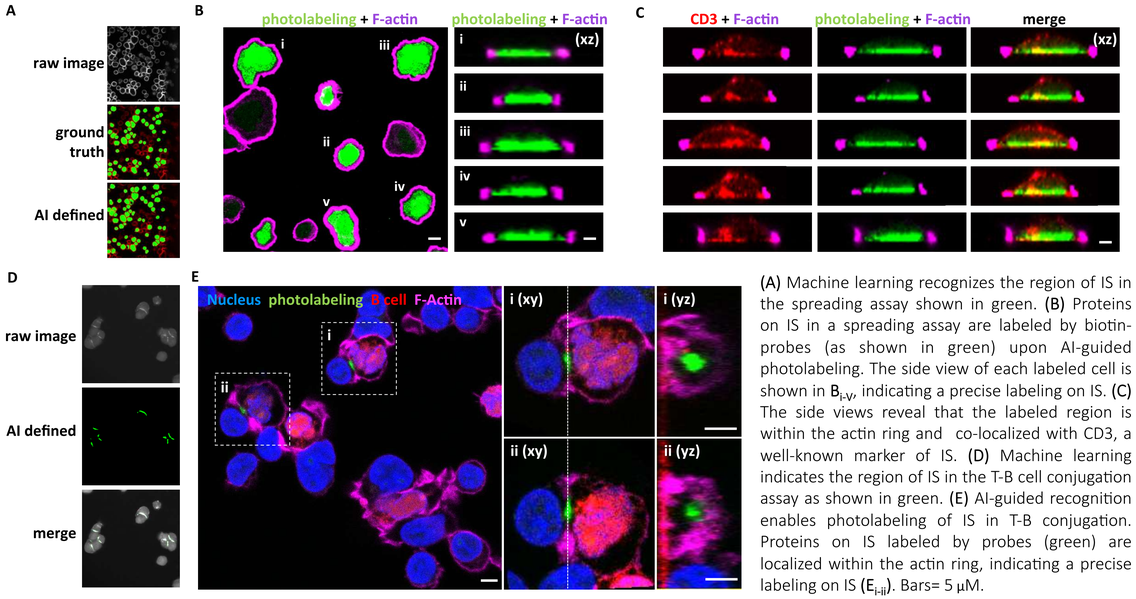
Schemes show the cellular localization of IS in a T cell spreading assay (A_i) and T-B cell conjugation (A_{ii}). IS is also known as supramolecular activation cluster, SMAC (A_{iii}), This structure is composed of segregated rings with cluster of proteins, including the central ring, cSMAC; the peripheral ring, pSMAC and the distal ring, dSMAC. **(B)** In the spreading assay, cells were seeded on the CD3 and CD28 antibodies-coated coverslips for 15 min, and fixed and stained with indicated antibodies and dyes. Side view of the cell in the inset in B_i. IS indicated by CD3 is present at the bottom of the cell. **(C)** In T-B conjugation, T cells were first seeded on poly-D lysine coated coverslips. After incubating for a few minutes, SEE-activated B cells were added and incubated for another 10 minutes. IS denoted by CD3 staining is indicated by arrows. Bars= 5 μM.

Spatial proteomic profiling via machine learning-guided microscopic photolabeling



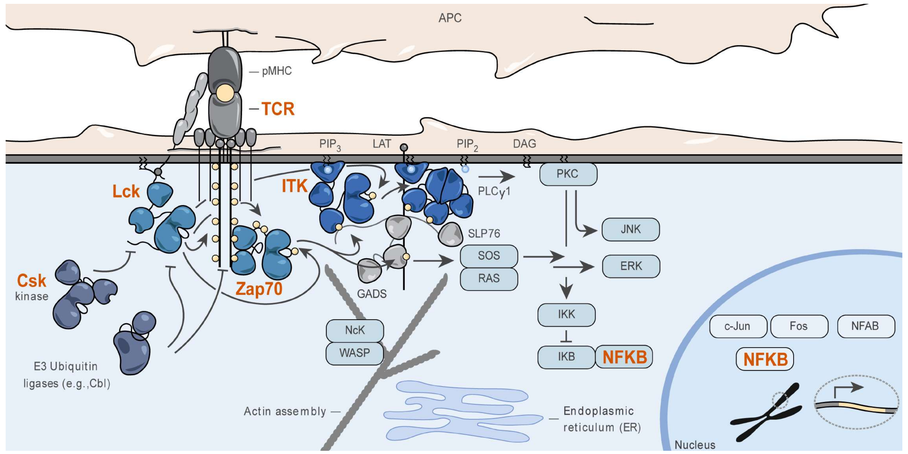
Cells or tissue samples were imaged and recognized by computer vision or AI **(Step 1)**; Samples were incubated with photoactivatable probes, and the photolabeling was driven by two-photon illumination **(Step 2)**; Proteins in these labeling regions were tagged with probes and further purified by an affinity pulldown assay **(Step 3)**; *De novo* biomarkers were identified by LC-MS/MS **(Step 4)**.

Immune synapses are recognized by AI and precisely labeled by photoreactive probes



(A) Machine learning recognizes the region of IS in the spreading assay shown in green. **(B)** Proteins on IS in a spreading assay are labeled by biotin-probes (as shown in green) upon AI-guided photolabeling. The side view of each labeled cell is shown in B_{i-v}, indicating a precise labeling on IS. **(C)** The side views reveal that the labeled region is within the actin ring and co-localized with CD3, a well-known marker of IS. **(D)** Machine learning indicates the region of IS in the T-B cell conjugation assay as shown in green. **(E)** AI-guided recognition enables photolabeling of IS in T-B conjugation. Proteins on IS labeled by probes (green) are localized within the actin ring, indicating a precise labeling on IS (E_{i-ii}). Bars= 5 μM.

Spatial components of immune synapse are identified using Microscoop



Modified from Courtney et al., 2018. doi.org/10.1016/j.tibs.2017.11.008

Known	*De novo*
CD3D	*LCP2*
CD3E	*INPP4A*
LcK	*GRAP2*
ITK	*PTPN6*
Zap70	*Galectin-9*
PI3K	*VAV*
Csk	*family*
NFKB	

Molecules involved in T cell receptor (TCR) signaling, and a list of potential *de novo* components are identified using Microscoop.

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