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ADVANCING SINGLE-CELL AND SPATIAL PROTEOMICS WITH NOVEL LC-MS AND TISSUE DISSECTION WORKFLOWS

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This presentation highlights several complementary advances that push the limits of low-input and spatial proteomics. First, we introduce a high-throughput single-cell proteomics workflow that combines nanodroplet sample preparation, dual-column LC, TMTpro 32-plex labeling, and real-time spectral library searching to quantify thousands of proteins from hundreds of single immune cells in a single day. Using this approach, we generate the largest single-cell proteomic dataset on primary immune cells to date (1,275 cells), resolve eight immune cell types spanning monocytes, lymphocytes, and granulocytes, validate scRNA-seq–derived markers, and uncover novel protein-level signatures.

Second, to address the core throughput-sensitivity bottleneck in low-input LC-MS, we develop a narrow-bore open tubular LC platform that identifies on average ~2,500 proteins from ~20 pg of material at 720 samples per day, using ultralow-flow separations to maximize electrospray efficiency.

Finally, to improve spatial proteomics at cellular and near-cellular resolution, we introduce multi-tiered µDicers- two-photon-fabricated microblades that mechanically dissect tissue into uniform microtissues down to 10 µm, overcoming photothermal damage and material loss associated with conventional laser capture microdissection. Benchmarking on human squamous cell carcinoma shows that µDicers provide higher proteome coverage than LCM, particularly at cellular (10 µm) resolution.

Together, these advances establish a path toward routine, ultra-sensitive proteomic measurements at unprecedented throughput, enabling ever-smaller samples to be analyzed in ever-larger numbers for both basic research and clinical applications.

Keywords
Single-cell proteomics, nanoPOTS, open tubular LC, tissue microdissection, spatial proteomics