

A Microreactor Workflow for Spatial Proteomics Sample Preparation

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Summary:

We present MR-SP² [1] (microreactor-based sample preparation workflow for spatial proteomics) that addresses key loss mechanisms in laser capture microdissection (LCM) coupled to liquid chromatography–tandem mass spectrometry (LC-MS/MS). MR-SP² integrates specimen capture, one-pot processing, and pipetting-free transfer to LC-MS/MS via Evotip precolumns. Evaluated using formalin-fixed paraffin-embedded (FFPE) murine kidney tissue, MR-SP² reduced the adsorptive surface area by 64% compared to tube-based workflows, yielding more than a 3-fold increase in protein identifications.

Keywords: spatial proteomics, sample preparation, LCM, LC-MS/MS, few-cell proteomics, FFPE

Background, Motivation and Objective

Spatial proteomics enables protein mapping within tissue at cellular resolution, with laser capture microdissection (LCM) coupled to liquid chromatography–tandem mass spectrometry (LC-MS/MS) being the predominant approach [2]. The legacy Zeiss PALM MicroBeam remains the second most widely used LCM platform despite its discontinuation [1], yet no dedicated low-loss sample preparation workflow exists for it. Conventional tube-based workflows suffer from three compounding loss mechanisms: incomplete specimen transfer during pressure-catalyzed LCM capture, adsorptive losses of proteins and peptides to reaction vessel surfaces during processing, and a final pipetting step into the LC-MS/MS interface. At the few-cell level, a single pipetting step alone can reduce proteome identifications by 50% [3]. These losses disproportionately affect low-abundance peptides, fundamentally limiting the achievable proteomic depth in spatially defined tissue microregions [4].

Description of the New Method or System

MR-SP² centers on a novel microreactor fabricated from cyclic olefin copolymer (COC) foil via thermoforming, with a 3D-printed COC cap and holder (Fig. 1 A+B). The microreactor geometry for MR-SP² (Fig. 1C) is compatible with the Zeiss PALM RoboMover, occupying the position of a conventional tube cap for direct specimen

capture in 20 μ L acetonitrile – half the volume of the conventional workflow (Fig. 1D). This eliminates the centrifugation step required to transfer specimens from tube cap to tube body, which is a primary source of specimen loss. All processing steps (protein extraction at 95°C, reductive alkylation, tryptic digestion, and acidification) are performed in the sealed microreactor in a total volume of 20 μ L, minimizing the inner surface area exposed to the sample from 92.8 mm² in the conventional workflow to 33.4 mm² (64% reduction). Sample transfer to LC-MS/MS is achieved by clipping the microreactor directly onto an Evotip disposable precolumn, entirely eliminating pipetting of the sample. The complete workflow is depicted in Fig. 1. Workflow performance was benchmarked against a conventional tube-based workflow using FFPE murine kidney tissue sections analyzed on a timsTOF flex coupled to an Evosep One system.

Results

MR-SP² improved specimen capture success rate from 75% (12/16) to 94% (15/16) compared to the conventional workflow. At 22-cell input (50,000 μ m³), both workflows performed comparably in protein identifications (3381 $\pm$ 80 vs. 3174 $\pm$ 59 proteins, Fig. 2B), with MR-SP² showing improved peptide depth (16,021 $\pm$ 1009 vs. 13,514 $\pm$ 740 peptides, p < 0.05 Mann-Whitney U (MWU), Fig. 2A). The advantage of MR-SP²

became pronounced at lower inputs where adsorptive losses are more severe. At 5–6 cells ($12,500 \mu\text{m}^3$), MR-SP² identified 1145 ± 188 proteins with match-between-runs (MBR) versus 302 ± 126 proteins for the conventional workflow (>3-fold increase, $p < 0.05$ MWU). At 1–2 cells ($3,125 \mu\text{m}^3$), MR-SP² reached 695 ± 112 proteins (MBR) compared to 206 ± 51 for the conventional workflow (>3-fold increase, $p < 0.05$ MWU). Quantitative reproducibility (Fig. 2C) remained high across all input levels, with mean coefficients of variation (CV) below 0.10 for MR-SP².

References

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Illustrations, Graphs, and Photographs

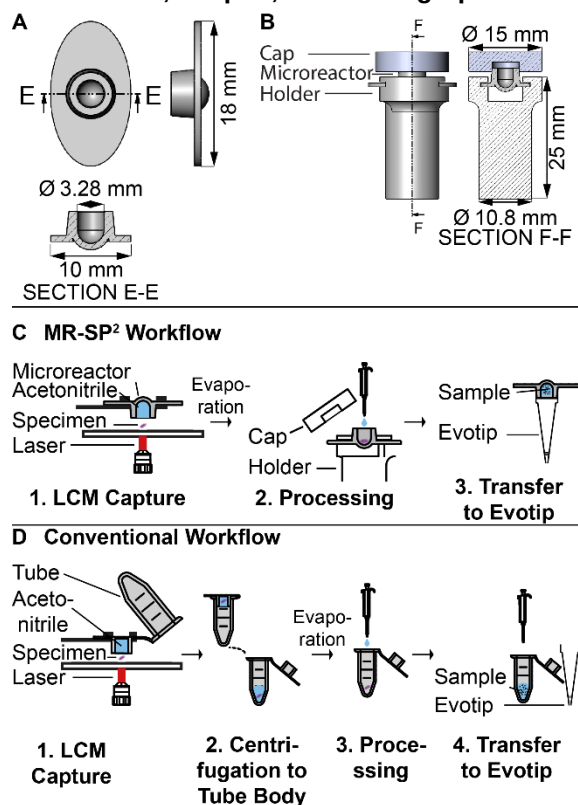


Fig. 1. (A) Microreactor and dimensions. (B) Components used for MR-SP² including microreactor, cap and holder. (C) The MR-SP² workflow including LCM capture, processing, and Evotip transfer. (D) Conventional workflow using a standard tube including LCM capture, centrifugation of specimen to tube body, processing, and Evotip transfer.

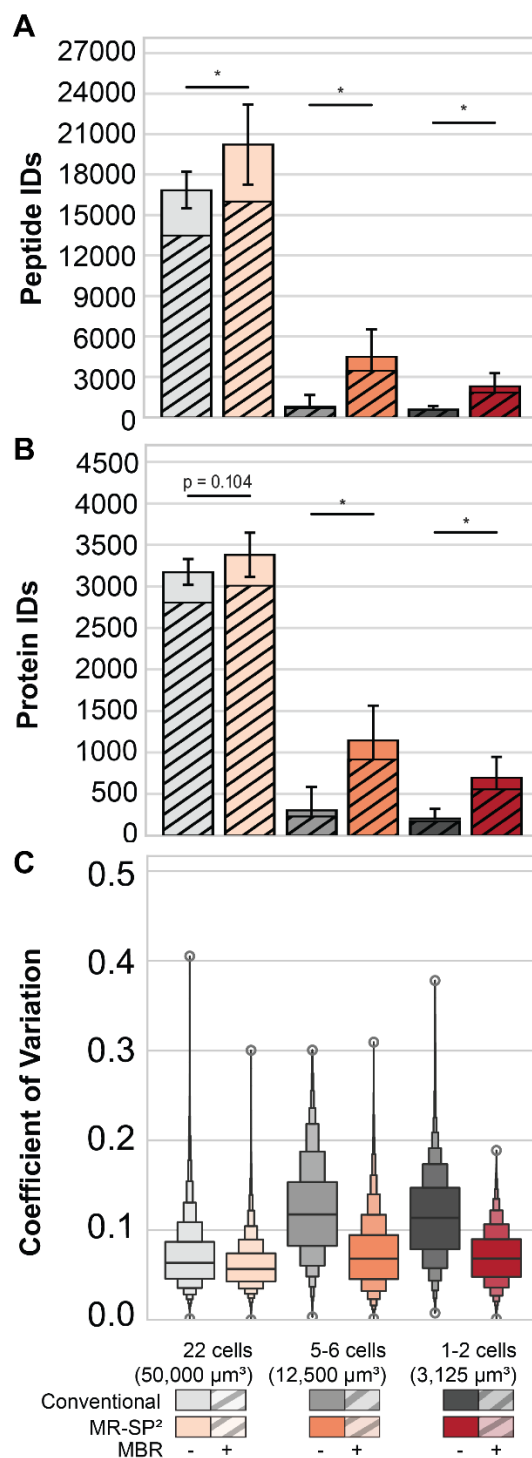


Fig. 2. Results of LC-MS/MS analysis for different input volumes including (A) peptide identifications, (B) protein identifications, and (C) coefficient of variation for $\log_2$ peptide intensities among peptides identified in at least 3 precursors.