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Integrated laser ablation and droplet collection system for efficient single cell isolation

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We use this protocol and it's working

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Abstract

State-of-the-art omics and imaging technologies for transcriptome, epigenome, and proteome measurements at the single-cell level have been successfully demonstrated and integrated into large tissue mapping consortia (e.g., HuBMAP, SenNet). However, current approaches for probing spatial distribution of the proteome typically rely on the use of antibodies, which limits multiplexing and requires a priori knowledge of protein targets. In this protocol, we describe a novel MS based spatial proteomic approach and deploy it for unbiased and deep mapping of proteins in tissues and specifically within senescent cells and their surrounding tissue microenvironment. The spatial single-cell proteomics (SSCP) technology enables efficient sample collection at high spatial resolution (5-10 μm) and opens the door to global cellular, subcellular and organelle proteomics. This approach is demonstrated here applied to a mouse tissue sample.

Laser capture microdissection (LCM)

1 Install in-house built robotic arm, which is used in the generation and delivery of a captured droplet.

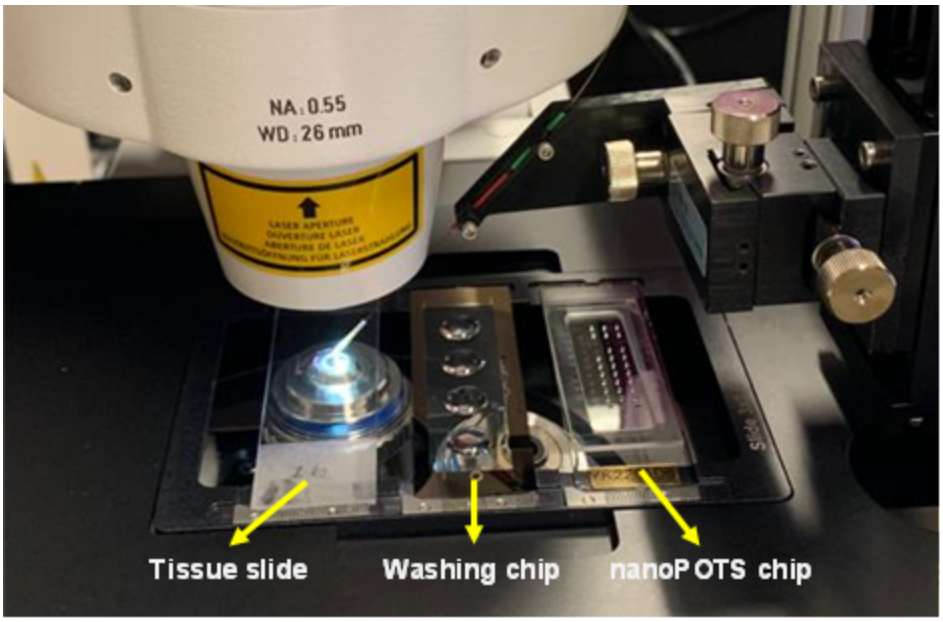
1.1 Wash the Syringe and glass capillary with MilliQ water

1.2 Turn on the PALM MicroBeam system

1.3 Position the robotic arm of the PALM MicroBeam system to ensure the capillary probe is sitting in the center.

2 Sample collection

2.1 Place the tissue slide (left), washing chip (middle), and nanoPOTS chip (right) in the slide holder, as shown below.



2.2 Scan and align the nanoPOTS chip.

2.3 Load buffers in the washing chip.

2.4 Scan the tissue slide.

2.5 Determine the safe operating height for the PALM MicroBeam system to enable unobstructed movement across the slide and chips..

2.6 Find the target region on the scanned image.

2.7 Navigate to the region of interest (ROI).

2.8 Select single cells of interest.

2.9 Load the laser settings (Speed 1, Cut energy 44, LPC Energy delta 20, Cut focus 21, LPC focus -2).

2.10 Locate the position information including selected tissue sections, nanoPOTS chip, move button, laser start button using the in-house developed robot software.

2.11 Use the robot software to start sample collection with the laser ablation function.

3 After sample collection: Properly dry and store samples

3.1 Set the incubator temperature to 70 °C.

3.2 Place the nanoPOTS chip in the incubator for ~20 min.

3.3 When the collection droplets are evaporated, cover the nanoPOTs chip with a glass slide and wrap it with foil.

3.4 Put the chip in -20 °C until further use.

NanoPOTS Proteomic Sample Processing for LCM tissue

4 Protein extraction and disulfide reduction

4.1 Turn on the nanoPOTS loading robot. Set temperature to 10 °C and humidity to 50%.

4.2 Prepare 100 µL of extraction buffer [1 mM DTT (dithiothreitol), 0.1% DDM (n-Dodecy-ß-D-maltoside), 50 mM ABC (Ammonium Bicarbonate) buffer (pH 8.5)] and place into the sample plate within the robot.

4.3 Place the sample chip into the robot and align the chip.

4.4 Dispense 200 nL of the extraction buffer into each well of the nanoPOTS chip.

4.5 Cover the nanoPOTs chip with a glass slide and wrap it with foil. Place the chip in the humidity box in a zipper bag and incubate at 70 °C for 60 min.

5 Alkylation

5.1 Prepare 100 µL of alkylation buffer [10 mM CAA (2-chloroacetamide) in 50 mM ABC buffer]and place into the sample plate within the robot.

5.2 Cool the chip to room temperature.

5.3 Place the sample chip into the robot and align the chip.

5.4 Dispense 50 nL of alkylation buffer into each well.

5.5 Cover the nanoPOTs chip with a glass slide and wrap it with foil.

5.6 Place the chip in the humidity box in a zipper bag and incubate in the dark for 30 min at room temperature.

6 Digestion

6.1 Prepare 100 µL of digestion buffer (0.01 ng/nL Lys-C and 0.04 ng/nL trysin) in 50 mM ABC buffer and place into the sample plate within the robot.

6.2 Place the sample chip into the robot and align the chip.

6.3 Dispense 50 nL of digestion buffer into each well.

6.4 Cover the nanoPOTs chip with a glass slide and wrap it with foil.

6.5 Place the chip in the humidity box in a zipper bag and incubate at 37 °C for 10 h.

7 Acid quenching

7.1 Prepare 100 µL of 5% formic acid in water and place into a sample plate within the robot.

7.2 Place the sample chip into the robot and align the chip.

7.3 Dispense 50 nL of 5% formic acid into each well.

7.4 Take out the chip from the nanoPOTS robot and place it into the desiccator until the droplets have evaporated.

7.5 Cover with the chip with glass slide, wrap with foil and store at -20 °C until analysis.

NanoPOTS-LC-MS/MS analysis

8 Inject each sample on the in-house assembled nanoPOTS autosampler utilizing an in-house packed SPE column (100 µm i.d., 4 cm, 5 µm, 300 Å C18 material,Phenomenex) for an online sample clean up, and an LC column (50 µm i.d., 25 cm,1.7 µm, 190 Å C18 material, Waters) which is heated to 50 °C using an AgileSleeve column heater (Analytical Sales and services, Inc., Flanders, NJ) for the peptide chromatographic separations. Dissolve samples with Buffer A [0.1% formic acid in water] on the chip, then load peptides on the SPE column and wash the trapped peptides with Buffer A for 5 min. After sample cleaning, separate and elute the peptides at 100 nL/min using a 60-min gradient, linearly increasing Buffer B [0.1% formic acid in acetonitrile] from 8% to 35%

9 Perform MS detection on an Orbitrap Lumos Tribrid mass spectrometer (Thermo scientific) with a FAIMS
interface operated in data-dependent acquisition (DDA) mode and a spray voltage of 2.4 kV

10 Perform a gas-phase fractionation of the ionized peptides using the FAIMS interface with a 3-stage stepped collision voltage (3-CV) method consisting of -45, -60, and -75 V. Fractionated ions with a
mass range 350-1600 m/z were scanned at 120,000 resolution with an injection time (IT) of 118 ms and an automatic gain control (AGC) target of 1E6

11 Generate a spectral library for a pooled tissue sample by selecting a single collision energy (CV) for each LC-MS run. During these runs, select precursor ions with intensities > 1E4 for MS/MS fragmentation at 30% highHCD and For the pooled tissue samples for generating a spectral library, a single CV was used for each LC-MS run. Precursor ions with intensities > 1E4 were selected for fragmentation by 30% higher-energy collisional dissociation (HCD) and scanned in an Ion trap with an AGC of 2E5 and an IT of 86 ms.

Data analysis

12 FragPipe (Ver. 21.1) powered by MSFragger (Ver. 4.0) search engine, Philosopher (Ver. 5.1.0) and IonQuant (Ver. 1.10.12) can be used used for the raw data processing in conjunction with the appropriate protein fasta.

13 For the mouse example dataset showcased here, the latest version of the Uniprot Mus musculus (Mouse) database was used for a data search and the below FragPipe parameter

settings selected.

14 *Fixed modification:* carbamidomethylation of cysteine / *Variable modification:* Protein N-terminal acetylation, oxidation of methionine.

15 C*leavage enzyme:* strict trypsin, peptide length: 7-50, max missed cleavage: 2, FDR 0.01

16 Match between runs (MBR) and MaxLFQ embedded in the FragPipe (minimum ions:1; minimum scans: 1; m/z tolerance 10 ppm; RT tolerance 0.7 min) was selected for peptide quantifications for the dataset featured here.