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BeamMap: Beam Enabled Accurate Mapping and Molecular Analyte Profiling for topography and biochemistry spatial correlation

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ABSTRACT

BeamMap is a multimodal bioimaging instrument that integrates scanning electron microscopy with vacuum electrospray-enabled mass spectrometry to enable in situ correlation of biosample structure and untargeted chemistry. It has potential to achieve subcellular topographical imaging alongside chemical mapping via vacuum nanoelectrospray soft ionization with tunable energy for broad range of analyte detection. Demonstrated on a model imaging system, the workflow links morphology and molecular analysis concurrently within the same analytical environment. The BeamMap approach aims to expand discovery-driven single-cell and multiomics investigations across diverse biological samples and tissues.

INTRODUCTION

Interplay of cellular organization and intercellular communications is central to understanding biological function and disease pathogenesis.¹ Because of the chemical complexity of biological tissues, imaging technologies play a crucial role in resolving heterogeneity at the single-cell level, where diverse cellular structures and microenvironments coexist in close proximity.^{2, 3} Most high-resolution imaging methods target specific biomarkers and require chemical labeling. While these approaches offer robust chemical mapping at cellular scales, they limit the number of biomolecules that can be analyzed simultaneously, constraining discovery potential.⁴ Mass spectrometry imaging (MSI) offers untargeted chemical mapping, allowing broader chemical access with high molecular specificity. However, current applications of MSI methods are challenged by the trade-offs between spatial resolution⁵, detection sensitivity, and ionization softness.⁶

At the same time, dependence on a single imaging modality restricts the breadth of information that can be captured from complex biological systems. Multimodal imaging strategies have

emerged to address these limitations by acquiring complementary datasets in parallel or orthogonal measurements⁷, but many require extensive sample preparation⁸ or acquisition steps⁹ that can perturb sample integrity, introduce variability, and ultimately compromise biological interpretation. An approach that enables simultaneous, *in situ* access to cell/sub-cellular topography while probing untargeted cell-scale biochemistry would substantially expand the scope of biological discovery.¹⁰

RESULTS

BeamMap Instrument and Operation Principles

BeamMap is a multimodal MSI technique that integrates scanning electron microscopy (SEM) with vacuum electrospray-enabled mass spectrometry within the same imaging environment (Fig. 1a). Biosamples are introduced into the SEM chamber, where topographical imaging captures subcellular structural elements while a finely focused vacuum electrospray ionization (vESI) source locally extracts and ionizes surface analytes (Fig. 1b). Ionized gas-phase analytes are transported from the SEM over a one-meter distance via, SPion®, an RF-guided ion transfer device, to a downstream mass spectrometer for chemical detection identification.

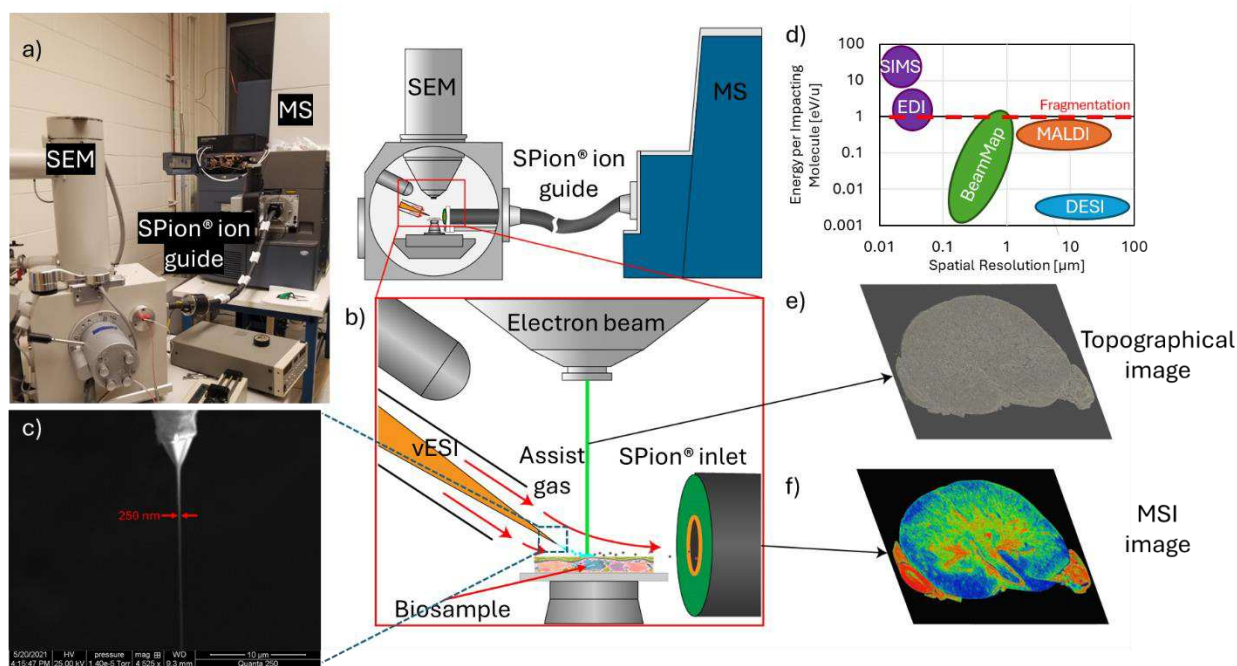


Figure 1. BeamMap (Beam Enabled Accurate Mapping & Molecular Analyte Profiling) instrument a) combines high-resolution SEM and vESI-MSI for *in situ* topographical and chemical mapping at a single-cell scale. b) Inside the SEM chamber, the electron beam acquires a topographical image e) while the vESI source and assisting gas extracts and ionizes surface analytes. f) An ion guide transfers the analytes from the SEM to the MS for chemical detection to generate a chemical map. c) SEM high vacuum image of vESI generation depicts the Taylor cone

emanating from a sub-5 μm electrospray tip producing a 250-nm diameter beam of droplets used to precisely extract sample analytes. d) Performance map comparing the spatial resolution of various MSI methods and “softness” of analyte ionization (function of energy supplied for extraction). BeamMap’s vESI source is tunable by the applied electric field for extracting/ionizing a broader range of biomolecules with minimal fragmentation.

A key feature of the BeamMap system is the use of a tightly-focused vESI beam used to extract surface analytes to improve MSI resolution. In the SEM high vacuum operation, an aqueous ammonia solution delivered at a nanoliter-per-minute flowrate produces charged droplet beams with diameters on the order of hundreds of nanometers (Fig 1c). The tightly focused beam is a result of the lack of ambient drag in vacuum, allowing droplets to accelerate to high speeds and reduces the time for repulsion between droplets that causes plume dispersion¹¹. An assisting sheath gas surrounds the vESI source to improve ion desolvation and provide vESI droplet transport assistance, enabling efficient molecular extraction and ionization. *In operando* adjustments to the vESI tip-to-sample distance and gas flow rates allow precise control over the droplet impact energy and transport trajectories¹².

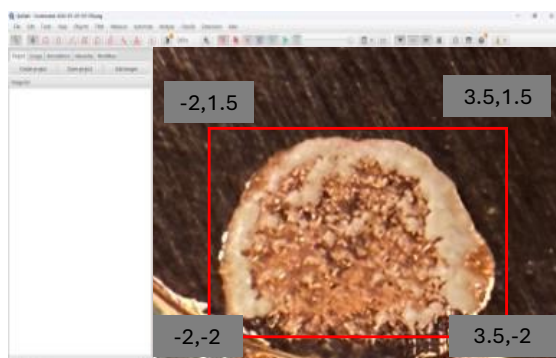
BeamMap’s tunable desorption/ionization energy enables broad biomolecular coverage at high spatial resolution. The performance map (Fig. 1d) compares the spatial resolution across MSI modalities as a function of the energy per impacting molecule, a parameter that determines the extent of ion fragmentation. Secondary ion mass spectrometry (SIMS) and electrospray droplet impact (EDI) yield the highest spatial resolutions but rely on high energy projectiles that readily fragment fragile species^{13, 14}. Desorption electrospray ionization (DESI) provides much softer ionization, but its spatial resolution is limited by large sampling spot sizes¹⁵. Matrix-assisted laser desorption ionization (MALDI) is widely used, but the matrix chemistry often constrains the range of targets that can be analyzed^{16, 17}. By contrast, BeamMap integrates the best features of these techniques by allowing the soft ionization capability of DESI but with the finely focused sampling of SIMS in vacuum. The adjustable vESI source geometry and supersonic gas jet assist open an opportunity for detection of lipids, metabolites, and proteins at the single-cell level, enabling detailed characterization that spans multiple biomolecular classes.

SEM imaging (Fig. 1e) and vESI-MSI (Fig. 1f) can achieve, in their limits, a topographical spatial resolution of ~ 10 nm (in high vacuum mode) or 100 nm (in environmental mode) and a chemical resolution of ~ 250 nm (diameter of the liquid vESI beam), respectively, allowing interrogation of intracellular regions and revealing biochemical relationships that cannot be resolved by any other MSI methods.

Correlated SEM and vESI-MSI imaging workflow

BeamMap integrates topographical and chemical data from the same region of interest (ROI) without the need for sample transfer and is demonstrated on a reserpine sample deposited onto a copper substrate. Using the SEM stage coordinates of the sample position before analysis, fiducial points and a sampling trajectory were generated in QuPath, an open-source image analysis platform, to define an ROI (Fig. 2a) for chemical interrogation. A vESI source (Fig. 2b) with a $\sim 5\text{-}\mu\text{m}$ emitter produced a sub-500-nm vacuum electrospray in ESEM mode (10^{-1} Torr) under an estimated electric field strength of $\sim 10^7$ V/m. Using an $\text{H}_2\text{O}/0.1\%$ acetic acid solution at 100 nL/min, the electrospray beam impinges on the surface and locally extracts analytes as it raster scans the ROI. MS detection (Fig. 2c) of vESI-desorbed reserpine acquired directly from the SEM chamber demonstrates efficient molecular transfer through the SPion[®] ion guide for chemical identification. A 40-psi (at the supply source) supersonic gas jet was used to accelerate droplets and enhance analyte desolvation. The dominant MS spectral signal corresponds to singly protonated reserpine (m/z 609.6) with an intensity of $\sim 2 \times 10^5$, while fragment ions at m/z 397.4 and 195.2 indicate partial fragmentation under the present vacuum vESI conditions. Compiled MS data were processed using METASPACE to generate an ion intensity heatmap of reserpine (Fig. 2d). After vESI scanning, an SEM image (Fig. 2e) of the sample was acquired in ESEM mode at 1 Torr with $\sim 500\text{-nm}$ resolution, revealing distinct morphological features that map to spatial film nonuniformities, including the characteristic “coffee-ring effect” arising from solvent evaporation during sample preparation drying. Co-registration of the SEM and MSI images (Fig. 2f) reveals strong spatial agreement between morphological and molecular features, demonstrating BeamMap’s ability to directly correlate surface topography with localized chemical distributions within the same field of view.

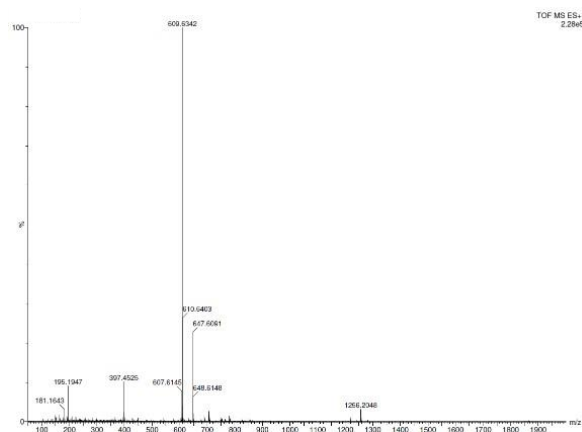
a) Selecting ROI for vESI scanning



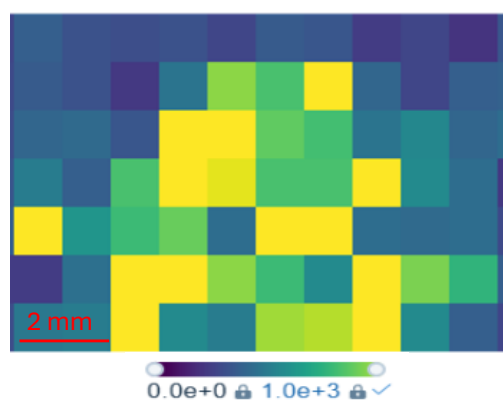
b) Snapshots of vESI sampling



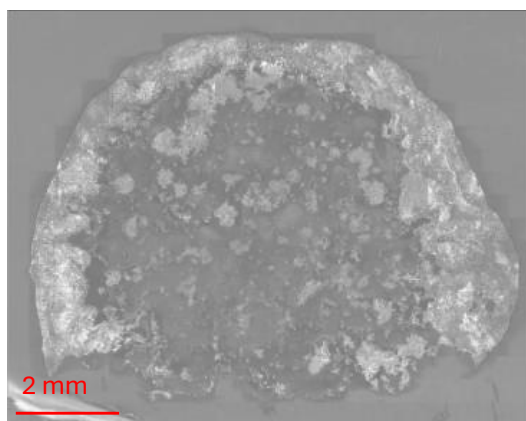
c) MS detection



d) MSI Image generation



e) Topographical Imaging



f) Topographical and chemical image co-registration

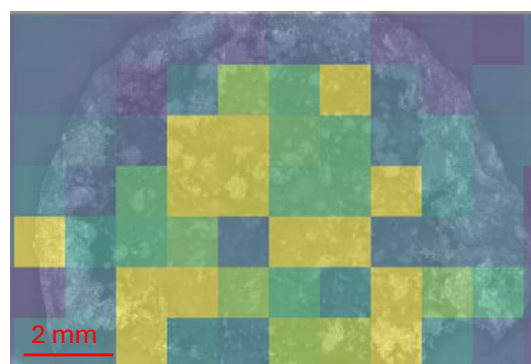


Figure 2. *In situ* imaging workflow illustrates simultaneous topographical and chemical mapping within the BeamMap platform. a) An ROI and fiducial points of a reserpine spot deposited onto a copper substrate are generated in Qupath to establish a path for vESI scanning b) a sub-500 nm vESI droplet beam impinges on the sample and extracts surface analytes as it raster scans (the spot size/resolution is controlled by impingement duration). c) Extracted reserpine analytes (m/z 609.6) are transported through SPion® and detected by the MS. d) MS datasets are compiled in METASPACE to generate an ion intensity heatmap of reserpine. e) SEM image of the reserpine spot captures topographical sample features. f) MSI and SEM co-registration reveals strong spatial agreement between surface topography and chemical distributions, validating BeamMap's *in situ* multimodal imaging capability.

DISCUSSION

BeamMap addresses a central challenge in biological imaging by permitting correlation between structural elements with untargeted chemical characterization of samples within a single instrument to enhance discovery-driven biological investigation. The *in situ* imaging workflow eliminates experimental variability while maximizing the information extracted from biological specimens. The ability to tune desorption/ionization conditions enables access to lipidomic, metabolomic, and proteomic data at spatial resolutions approaching the subcellular level.

While the present study focuses on establishing a proof of principle using a model bio-sample, BeamMap shows potential for broad applicability to biological systems, including cells and tissues that are compatible with the SEM environment. Continued instrument development focuses on expanding the molecular coverage for chemical mapping, optimizing spatial resolution for single-cell investigations, and applying the platform to relevant biological applications where correlation between structure and chemistry is essential.

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METHODS

Chemicals

HPLC-grade reserpine and acetone were purchased from Sigma-Aldrich (MO, USA). LC-grade Methanol, Water, and Formic Acid were purchased from Thermo Fisher Scientific (MA, USA).

Sample preparation

Reserpine was dissolved in acetone at a 1 mg/mL concentration. 15 mL of the solution was pipetted onto a copper substrate and allowed to dry in the ambient environment for 10 minutes. This step was repeated four times, leaving a total deposit of 60 mg on the substrate.

SEM imaging

An FEI Co. Quanta 250 Environmental SEM (OR, USA) acquired topographical images in environmental mode at a 0.5 Torr pressure. The SEM beam energy was set to 10 kV with a spot size of 6 (3.6 nA beam current) and a dwell time of 1 s to acquire topographical features at 500-nm resolution. FEI Maps 2.0 software was used to generate a 9.2 mm by 4.9 mm tileset that acquired images, each with a 256 μm x 256 μm field of view. Once all images were acquired, they were stitched together in the software to create a composite topographical map.

Image analysis and vESI path creation

In QuPath 0.5.1, a polygon area setting tool was used to define the region for MSI sampling area, and the rectangle setting tool was used to pin the corner points. These points define the XY limits of MSI sampling that covers the entire sample area. The stage coordinates for the corner points were obtained by visually checking the location of the vESI emitter with respect to the sample, and these values were saved into a GeoJSON output file via the annotation tool. In python, using the corner marks the affine transformation was used to obtain the SEM stage coordinate of the MSI sampling area. The SEM stage coordinates for the vESI target points were then generated in 500 μm raster pattern that spans the MSI sampling area.

vESI-MS detection

The vESI source consists of an electrospray emitter (fused silica, 3- μm I.D., 360- μm O.D.) that is housed within a 500- μm gas capillary. The emitter produced a charged droplet beam from a 98/2 v/v MeOH/H₂O with 5% formic acid solution flowing at 40 nL/min while a 30-psi nitrogen sheath gas flow assisted droplet transport and gas-phase ion extraction through SPion[®]. The emitter tip-to-sample distance was 1 mm, spray-to-sample angle was 70°, and tip-to-SPion[®] inlet distance was 3.5 mm. The sample moved on the computer-controlled SEM XYZ stage to allow raster scanning. Chemical detection was completed on a Waters Corp. Xevo G2 QToF (MA, USA) mass spectrometer.

Data Processing and MSI image generation

MSConvert (ProteoWizard) was used to convert raw files into mzML format. MS signal intensities for hydrogen and potassium adducts of reserpine (609 and 647, respectively) were obtained and summed using pymzML. Registration data matching vESI measurements to corresponding MS spectrum and their sample coordinate data were paired with the reserpine intensities data within the python environment. The final ion map for reserpine signal was generated using the matplotlib library. The ibd and the corresponding imzML file were generated in pyimzML where the data files were uploaded into METASPACE for visualization.

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