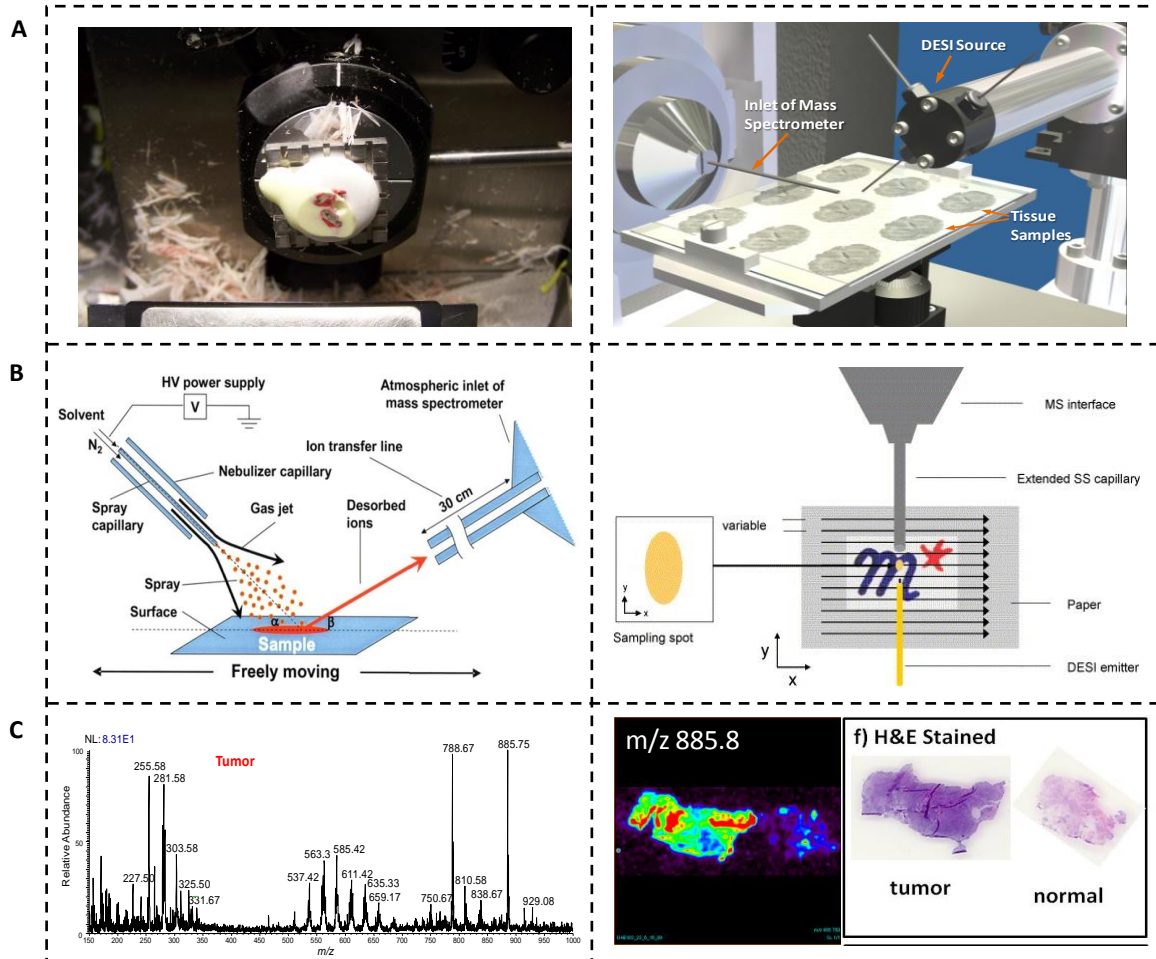


## Analysis of Human Brain Cancer Using Tissue Smears by Desorption Electrospray Ionization – Mass Spectrometry

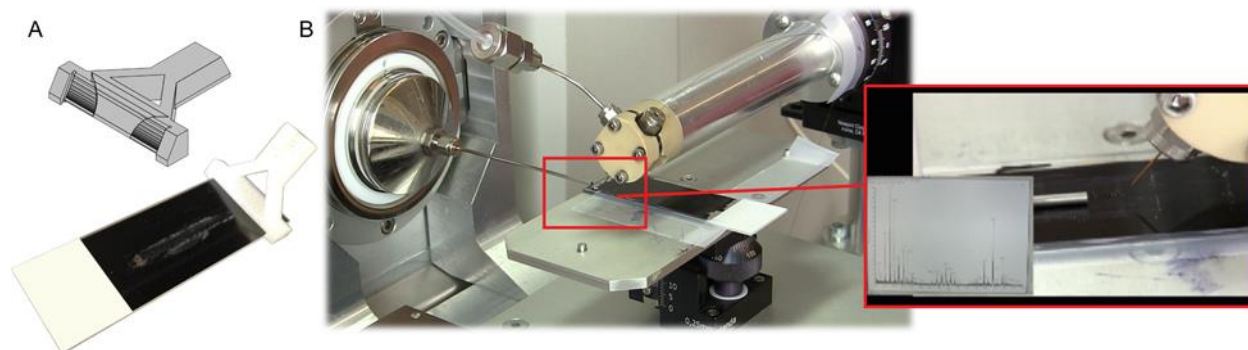
### Introduction

Mass spectrometry (MS) imaging is a well-established tool for mapping the spatial distribution of biomolecules. It offers rapid, sensitive, specific, and simple analytical protocols for tissue analysis. Ambient ionization refers to MS analysis in which ions are generated and sampled under native environmental conditions (e.g. pressure, temperature, humidity) and they contrast methodologies where ionization occurs under vacuum such as matrix-assisted laser desorption ionization (MALDI) and secondary ion mass spectrometry (SIMS). Ambient ionization techniques emerged early in this century and are defined by minimal-to-no sample preparation and no separation of molecules (e.g. chromatography) prior to MS analysis. The mass spectrometer alone is tasked with translating the ions detected from complex biological mixtures into insight on disease state, metabolic activity, physiological response, or developmental time point. Ambient MS has expanded into a variety of techniques that differ in their desorption and ionization mechanisms as well as the coupling of these two processes in time [1]. Desorption electrospray ionization mass spectrometry (DESI-MS) was the first ambient ionization technique to be developed [2]. In a DESI imaging experiment (Figure 1), a charged aerosol is sprayed on the sample (e.g. a tissue section) in a small spot (around 100-300 $\mu$ m), forming a thin liquid film that extracts material. Subsequent aerosol impacts splash charged droplets from the liquid film, and ions are generated from the droplets via electrospray like processes. The ions are then sampled by the mass spectrometer. Full scan mass spectra are collected pixel-by-pixel by rastering the spot across the sample continuously in the x-direction. The potential scope of DESI for tissue diagnostics increased significantly by the use of solvent systems that do not alter tissue morphology [3]. This approach allows for histopathological examination of the same tissue after DESI-MS analysis. DESI imaging has been used to study flash-frozen tissue sections of a number of different organs including prostate [4], germ-cell, bladder, kidney [5], breast, intestine [6], and brain [7,8]. In each case, the mass spectral profiles ( $m/z$  values and associated abundances) of detected oncometabolites and cell membrane lipids (such as phospholipids, sulfatides and sphingolipids) proved characteristic of different tissue types and diseases states.



**Figure 1.** Schematics of DESI-MS imaging of tissue sections with example mass spectra and ion images. **A.** Tissue is flash-frozen and cryo-sectioned to 10-15 $\mu$ m and then thaw-mount onto glass microscope slides. The slide is then fixed to the DESI moving stage. **B.** A charged aerosol is sprayed on the sample (e.g. a tissue section) in a small spot (around 100-300 $\mu$ m), forming a thin liquid film that extracts material. Subsequent aerosol impacts splash charged droplets from the liquid film, and ions are generated from the droplets via electrospray like processes. The ions are then sampled by the mass spectrometer. Full scan mass spectra are collected pixel-by-pixel by rastering the spot across the sample continuously in the x-direction. **C.** Example of DESI mass spectra obtained from a piece of tumor tissue. An ion image shows distribution of  $m/z$  885.8 as a heat map, and hematoxylin and eosin (H&E) stained tissue sections.

DESI imaging of tissue sections takes approximately 30-60 minutes for the typical 10x10 mm<sup>2</sup> tissue section. An alternative approach is rapid DESI-MS profiling of tissue smears which provides molecular information on multiple compounds in less than five minutes, making it amenable to intraoperative cancer diagnostics [8,9]. Tissue smearing eliminates the flash freezing and tissue sectioning steps (Figure 2), and it has been recently shown that the act of smearing does not compromise the chemical information detected by DESI, whereas the histological examination loses accuracy and precision as morphology and architecture of the cells are altered by the smearing process. The MS signal is dependent on the quantity and the physical distribution of the tissue that is smeared on a glass microscope slide [8,9].



**Figure 2.** (A) Smear device and illustrative brain tissue smear. (B) Rapid DESI-MS analysis of tissue smears, inset shows the spray and associated signal obtained.

Here we present the fundamental aspects of DESI-MS and describe its evolution from laboratory-based DESI-MS imaging of frozen tissue sections to intraoperative DESI-MS analysis of fresh tissue smears.

### Aims

- Perform rapid examination of brain tissue smears by DESI-MS.
- Evaluate the smear method's value for intraoperative diagnosis of human brain cancers.
- Explore the DESI mass spectra of tissue smears. Understand the spectral differences between normal grey matter and normal white matter. Attempt the deconvolution of mixed tissue specimens.
- Perform MS/MS experiments and tentatively identify the main lipid species in mouse brain tissue.

### Prelab Activity

1. Introduction to DESI-MS. Basics of DESI-MS profiling and imaging in full scan on a linear ion trap mass spectrometer.
2. Discussion of how ion images are created.
3. Discussion of histopathological examination and criteria the pathologist uses when making diagnosis.

### Experimental procedure

1. Set up the DESI moving stage and sprayer with necessary solvent and nebulizing gas connections. Obtain background mass spectra using blank glass slides, and use mouse brain to observe expected lipid profiles.
2. Smear a small quantity of fresh mouse brain tissue on a glass slide, with the aid of a custom 3D-printed device. Analyze the smear by a rapid DESI-MS methodology and record the MS data in negative and positive ion mode. Compare the spectra with a DESI-MS spectral library for recognition of patterns.

3. H&E stain the tissue smear that was analyzed with DESI-MS imaging in Procedure 2. Observe the morphology under a microscope. Discuss the main morphological features and how they relate to the chemical profiles observed by DESI-MS.

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