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Review article

An accessible, scalable ecosystem for enabling and sharing diverse mass spectrometry imaging analyses

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ABSTRACT

Mass spectrometry imaging (MSI) is used in an increasing number of biological applications. Typical MSI datasets contain unique, high-resolution mass spectra from tens of thousands of spatial locations, resulting in raw data sizes of tens of gigabytes per sample. In this paper, we review technical progress that is enabling new biological applications and that is driving an increase in the complexity and size of MSI data. Handling such data often requires specialized computational infrastructure, software, and expertise. OpenMSI, our recently described platform, makes it easy to explore and share MSI datasets via the web – even when larger than 50 GB. Here we describe the integration of OpenMSI with IPython notebooks for transparent, sharable, and replicable MSI research. An advantage of this approach is that users do not have to share raw data along with analyses; instead, data is retrieved via OpenMSI's web API. The IPython notebook interface provides a low-barrier entry point for data manipulation that is accessible for scientists without extensive computational training. Via these notebooks, analyses can be easily shared without requiring any data movement. We provide example notebooks for several common MSI analysis types including data normalization, plotting, clustering, and classification, and image registration.

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Mass spectrometry is usually carried out on homogenized samples. Homogenization, whether achieved by mechanical pulverization or chemical extraction, destroys information on the spatial distribution of analytes in the sample. Mass spectrometry *imaging* (MSI) seeks to eliminate this information loss and to obtain both chemical and spatial information on analyzed samples. As such, it cannot rely on complete homogenization of samples.

Recent years have seen tremendous improvements in instrumental capabilities for MSI [22,47]. In this perspective, we discuss how the advances in instrument design and sample preparation have led to new challenges for MSI users, especially the size and complexity of the resulting datasets. We then show how OpenMSI—a web-accessible repository for MSI data and a platform for sharing and analyzing data—in combination with sharable programming notebooks based on IPython can address these challenges, and demonstrate practical application of this idea by providing example notebooks performing two common types of MSI analyses. The article focuses primarily on soft-ionization techniques due to the greater chemical information content they provide.

1. Better instrumentation leads to bigger, more complex data

Here we review instrumental advances that are leading to increasing spatial resolution of MSI data and also the orthogonal instrumental improvements that increase chemical "resolution". Higher spatial resolution leads to an increase in the number of pixels in an MSI image, and higher chemical resolution increases the complexity (or number) of the mass spectra recorded at each pixel. Simultaneously making use of improvements in both spatial and chemical resolution thus strongly increases the data size and complexity of MSI images, as we discuss below.

1.1. Instrumental improvements: spatial resolution

Spatial resolution in the single μm range, required for single-cell analysis of bacteria and also of many eukaryotic cells, has been generally inaccessible for MSI analyses that rely on soft ionization techniques such as matrix-assisted laser desorption (MALDI) or desorption via electrospray impact (DESI). Hard-ionization techniques, such as SIMS or nanoSIMS, easily reach these resolutions, and have been the subject of several recent reviews [26,41], but the harsh ionization conditions lead to analyte fragmentation into atoms or very small molecular fragments. Here our focus is on soft-ionization techniques, which can provide information on the abundance of intact metabolites, lipids, peptides, and proteins. Recent improvements discussed below are allowing even soft-ionization techniques to approach this single-cell limit. The x-axis of Fig. 1A shows obtainable spatial resolution for the most widely used ionization techniques.

1.1.1. Laser rastering techniques

The most popular and widespread means of collected spatially resolved mass spectra is by laser rastering over a sample surface. Matrix-assisted laser desorption ionization (MALDI) mass spectrometers usually use this imaging mode. Vendors usually fix the laser optics in a single position, and an XY-stage moves the sample across the incident laser beam. Spatial resolution can thus be limited by (a) the laser spot size and (b) the precision of the XY-stage [64]. Today's commercial MALDI-MS instruments have laser spot sizes that are in the range of 10–200 μm, and XY-stages with 1–10 μm translational precision.

Laser spot sizes thus usually constrain obtainable resolution. Oversampling techniques can improve spatial resolution to the limits of the XY-stage [23]. In Jurchen's implementation of this technique, a laser with a 100 × 200 μm spot size was repeatedly fired at a fixed position until the sample ions were no longer detected. A translation of the XY stage by 25 μm increments after analyte depletion brings fresh sample into the laser spot, allowing attribution of new signal to the 25 μm-region newly moved into the beam. Improved laser optics have also been reported that reduce laser spot sizes in MALDI-MS to about 2 μm [13,62]. A limitation of all rastering techniques is that increases in spatial resolution also increase data acquisition time.

"Ion microscopy". Heeren and co-workers have accelerated MSI acquisition times by developing a unique imaging mode for MSI that relies on spatially resolved *detection* of ions ejected from a sample, rather than spatially resolved ionization. They term this mode "ion microscopy" and have demonstrated it using MALDI [22] ionization techniques. Their technique takes spatially-resolved mass spectra "inside of" the laser spot of a MALDI-type ionization system, allowing for much larger laser spot sizes (~200 μm) and faster acquisition times. Large sample images are constructed as mosaics of these single-spot images as the laser rasters across an image surface. Spatial resolution is thus independent of the laser spot size and is instead dictated by both the magnification inherent to the ion optics of the mass spectrometer, as well as the pixel sizes of the ion detector. Imaging resolution with ion microscopy of 6 μm has been reported. The same detector style can also be used for secondary-ion based ionization [28]; spatial resolving power of 7 μm was demonstrated.

1.1.2. Desorption electrospray ionization (DESI)

Desorption electrospray ionization simplifies sample preparation, and unlike many laser ionization techniques, can work at atmospheric pressure. In DESI, an electrospray of solvent droplets impacts the sample surface, causing desorption and ionization of analyte molecules. The electrospray is generated from two nested capillaries. The inner capillary contains the electrosprayed solvent, and the outer one contains a heated gas stream. The first example of using a solvent electrospray to perform MSI was reported in 2004 [51]. DESI does not require a matrix or initiator to absorb laser energy and initiate ionization.

Early examples of DESI MSI reported spatial resolution of 100–250 μm [52,58], but other studies have identified variables that control spatial resolution in DESI and improved resolution to 35 μm [8,10]. Variables strongly affecting spatial resolution include solvent flow rate, XY-stage step size, and the geometric orientation of the electrospray emitter relative to the mass spectrometer [8]. Additionally, the penetration of solvent from electrospray droplets into the sample can partially dissolve and distort the sample, decreasing spatial resolution, but altering solvent composition can ameliorate this effect. *N*,*N*-dimethylformamide:ethanol mixtures were superior to methanol:water mixtures in this regard. Use of