Available online at www.sciencedirect.com

SciVerse ScienceDirect

Current Opinion in Chemical Biology

Single-cell imaging mass spectrometry

Melissa K Passarelli and Andrew G Ewing

Single-cell imaging mass spectrometry (IMS) is a powerful technique used to map the distributions of endogenous biomolecules with subcellular resolution. Currently, secondary ion mass spectrometry is the predominant technique for single-cell IMS, thanks to its submicron lateral resolution and surface sensitivity. However, recent methodological and technological developments aimed at improving the spatial resolution of matrix assisted laser desorption ionization (MALDI) have made this technique a potential platform of single-cell IMS. MALDI opens the field of single-cell IMS to new possibilities, including single cell proteomic imaging and atmospheric pressure analyses; however, sensitivity is a challenge. In this report, we estimate the availability of proteins and lipids in a single cell and discuss strategies employed to improve sensitivity at the single-cell level.

Addresses
Kemivagen 10, Gothenburg, Sweden

Corresponding author: Passarelli, Melissa K (andrewe@chalmers.se)

Current Opinion in Chemical Biology 2013, **17**:854–859

This review comes from a themed issue **Analytical techniques**

Edited by **Milos V Novotny** and **Robert T Kennedy**

For a complete overview see the Issue and the Editorial

Available online 12th August 2013

http://dx.doi.org/10.1016/j.cbpa.2013.07.017

Introduction

There is a fundamental flaw associated with analytical measurements of cell ensembles; the chemistry of an individual cell is often lost in the average chemistry of the cell population [1,2]. The same principle can be applied to the subcellular environment, as chemical heterogeneities within a single cell may be lost with single-cell analyses. Fortunately, single-cell imaging mass spectrometry (IMS) can elucidate intercellular chemical heterogeneity. The techniques ability to elucidate fundamental cellular processes by connecting chemical distributions with cell morphology makes it a valuable tool for molecular biology, cancerology, and pharmacology.

IMS is a popular and rapidly growing bio-analytical method that encompasses a number of ionization techniques, including desorption electrospray ionization (DESI) matrix-assisted laser desorption ionization (MALDI) and secondary ion mass spectrometry (SIMS) [3–7]. SIMS is a label-free, matrix-free technique that utilizes a focused primary ion beam to desorb and ionize analyte molecules. The high surface sensitivity and submicron lateral resolution of the technique make it ideal for single-cell imaging. [8,9] However, recent methodological and technological developments in the field of MALDI have pushed its spatial resolution from the 20–100 μm range to the 1–10 μm range (Figure 1). With the average mammalian cell having a diameter of approximately 20 μm, this advancement has allowed MALDI to cross the barrier from single-cell 'profiling' to single-cell 'imaging'. In this report, we examine the progress and the potential advantages of the SIMS and MALDI platforms in single-cell IMS.

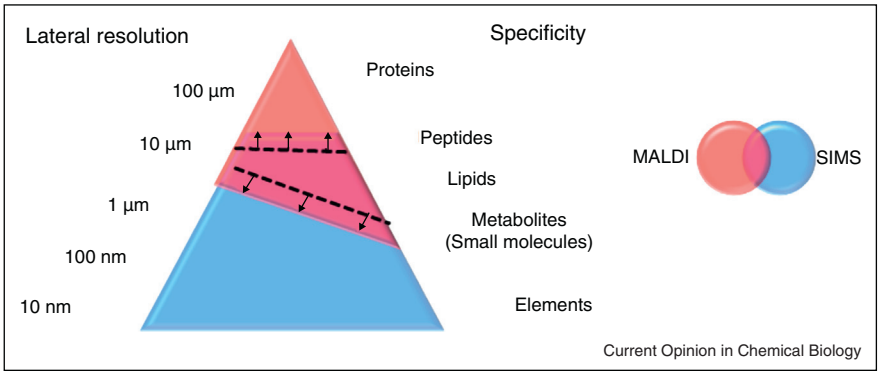
Sensitivity challenge

Sensitivity is a twofold challenge in single-cell IMS. First, there is a sensitivity limitation related to the nature of the sample itself. A single cell contains a diverse population of biomolecules with varying abundances. A typical mammalian cell is about 1–20 pL in volume and the abundance of a particular biomolecule can range from a few to a few million copies per cell.

In order to elucidate the demand single-cell analysis places on mass spectrometry detection, we have estimated the abundance of proteins and lipids in a single mammalian cell (Table 1). On the basis of a comprehensive proteomic map of the mammalian cell line, U2OS, reported by Beck *et al.*, the most abundant cellular proteins were present within a range of 4.5×10^3 to 2.5×10^6 copy numbers per cell [10]. Similarly, lipid abundances were extracted from data collected the murine cell line RAW 264.7 by LipidMAPS [11]. When directly compared, these data reveal that the most abundance lipid species is approximately 10 times more abundant than the most abundance protein species. Therefore, even with an attomole detection limit, a reasonable limit of detection (LOD) for most commercial mass spectrometers, only the most abundant proteins and lipids are detectable.

The second major challenge is the inherent limitation associated with the imaging modality itself. Sensitivity declines with increasing lateral resolution as reducing the analysis area or pixel diameter to improve the lateral resolution reduces the amount of material available to measure. McDonnell and coworkers calculated that the biomolecule concentration would have to be in the millimolar (mM) range in order to be detected, assuming an attomole limit of detection [12]. Again, this restricts detection to the most abundant biomolecules or localized regions of high concentration.

Figure 1



The overlap between MALDI and SIMS methodologies is growing, in part by the various strategies independently developed to improve the lateral resolution of MALDI and extend the dynamic range of SIMS.

Improving the sensitivity of ToF-SIMS and its impact on single cell-IMS

There are two regimes in SIMS, static and dynamic SIMS. These two regimes have independently developed methods to improve the sensitivity of SIMS.

Static SIMS

Static SIMS instruments typically employ a pulsed ion source and a ToF mass analyzer. The development of cluster ion sources has significantly improved the sensitivity of ToF-SIMS. The advantage of cluster sources is twofold; cluster ion beams improve the molecular ion yields of organic biomolecules and reduce the damage accumulation cross-section, allowing molecular information to be retained during depth profiling [13•,14••,15]. The energy from cluster projectiles is deposited closer to the surface with minimal damage to the subsurface compared to traditional atomic ion sources. The reduced altered layer improves the retention of molecular information during sputtering and allows molecular depth profiling and 3D mass spectrometry imaging to be carried out. This is a unique characteristic of SIMS-based IMS.

Table 1

The estimated number and concentration of various biomolecules in a single mammalian cell. The concentration was estimated based on a homogeneous distribution of the biomolecules in a 4.2-picoliter cell volume (20-µm sphere)

Omics	Species	Units per cell	Concentration
Lipidomic[a]	PC (36:1)	200 attomole	50 µM
	Cholesterol	4.3 femtomole	1.0 mM
Proteomic[b]	High abundance proteins	33 attomole	1.65 µM
	Low abundance proteins	830 yotomole	198 pM

[a] Information taken from LipidMAPS. The data have been pooled from the control cases at various time points from the KDO_2-lipid A experimental dataset, available at LipidMAPS.org [11]. To estimate the phosphocholine contribution, data have been pooled from 35 glycerophoscholine and 16 sphingomylin species. The lipidomic information is reported in pmol per microgram of DNA, we assume 6 pg of DNA per diploid cell.
[b] Information obtained from proteomiccommons.org. [10].

The sample preparation step provides a valuable opportunity to improve sensitivity by pre-treating the sample before analysis. A variety of matrices, including organic acids, ionic liquids and deposited metals, have been used to improve bimolecular ion yields. In addition, washing procedures designed to remove salts (i.e. ammonium formate) have improved biomolecule signals by mitigating salt-related ion suppression effects. Unfortunately, this step is also susceptible to artifacts, as perturbing the sample can introduce variances into the analysis [16•]. The need to introduce and maintain the cells in a vacuum environment is an unfortunate drawback of SIMS analyses. The development of flash freezing methods combined with analyses of cells in a frozen hydrated state, not only preserves the integrity of the cell, but also improves secondary ion yields compared to freeze dried cells [17].

Sensitivity and image contrast have been improved by employing data processing procedures to reduce the dimensionality of the dataset. One of the most common techniques is principal component analysis (PCA). In this technique, mass spectral peaks are compiled based on commonalities in the variance. This method has been successful in differentiating cell types in a heterogeneous culture [18]. Similarly, the chemical signature of vitamin E obtained from an *in situ* tandem MS spectrum has been used to extract and compile vitamin E-related fragment peaks from a ToF-SIMS image of an *Aplysia* neuron (Figure 2e) [19••].