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Imaging mass spectrometry: From tissue sections to cell cultures ☆

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ABSTRACT

Imaging mass spectrometry (IMS) has been a useful tool for investigating protein, peptide, drug and metabolite distributions in human and animal tissue samples for almost 15 years. The major advantages of this method include a broad mass range, the ability to detect multiple analytes in a single experiment without the use of labels and the preservation of biologically relevant spatial information. Currently the majority of IMS experiments are based on imaging animal tissue sections or small tumor biopsies. An alternative method currently being developed is the application of IMS to three-dimensional cell and tissue culture systems. With new advances in tissue culture and engineering, these model systems are able to provide increasingly accurate, high-throughput and cost-effective models that recapitulate important characteristics of cell and tissue growth in vivo. This review will describe the most recent advances in IMS technology and the bright future of applying IMS to the field of three-dimensional cell and tissue culture.

Contents

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1. Introduction

Imaging mass spectrometry (IMS) is a rapidly developing technology that offers a unique perspective on biological systems. IMS can be applied to virtually any animal or human tissue and, more recently, to model systems grown in vitro [1]. The basic workflow of IMS is depicted in Fig. 1 and requires that the tissue be thinly sliced (10–20 μm), washed to remove debris and, depending on the ionization method, coated in a thin layer of matrix that assists in the ionization of proteins, peptides, lipids, drugs, xenobiotics, neuropeptides and drug metabolites [2–6]. While there are several ionization sources that can be used, the principle is the same for each: the ionization source rasters across the sample in an X,Y coordinate fashion while a mass spectrometer records a mass spectrum for each position. Each "pixel" in the resulting image is a mass spectrum from that specific coordinate position on the tissue. Data analysis programs combine this information with an optical image of the tissue and generate heat maps showing the spatial localization of any *m/z* value that was measured by the mass spectrometer. This technique allows for visualization of theoretically any ionizable species in a selected mass range while retaining spatial information. A single pixel in an IMS image contains all of the ionized molecules detected for a selected mass range. Depending on several variables, this could be tens or hundreds of ions/pixel with hundreds or thousands of pixels/image. A wealth of information is obtained from IMS experiments in the form of thousands of analyte signals being detected for each image. Image processing techniques that help highlight biologically relevant signals will be discussed in later sections of this review. IMS images can also be combined with other imaging techniques to provide additional, complimentary information about the samples. IMS can be thought of as "molecular histology" because acquired images can be correlated with H&E stains or other target-based immunostaining methods, and by themselves provide information within a spatially defined context [7].

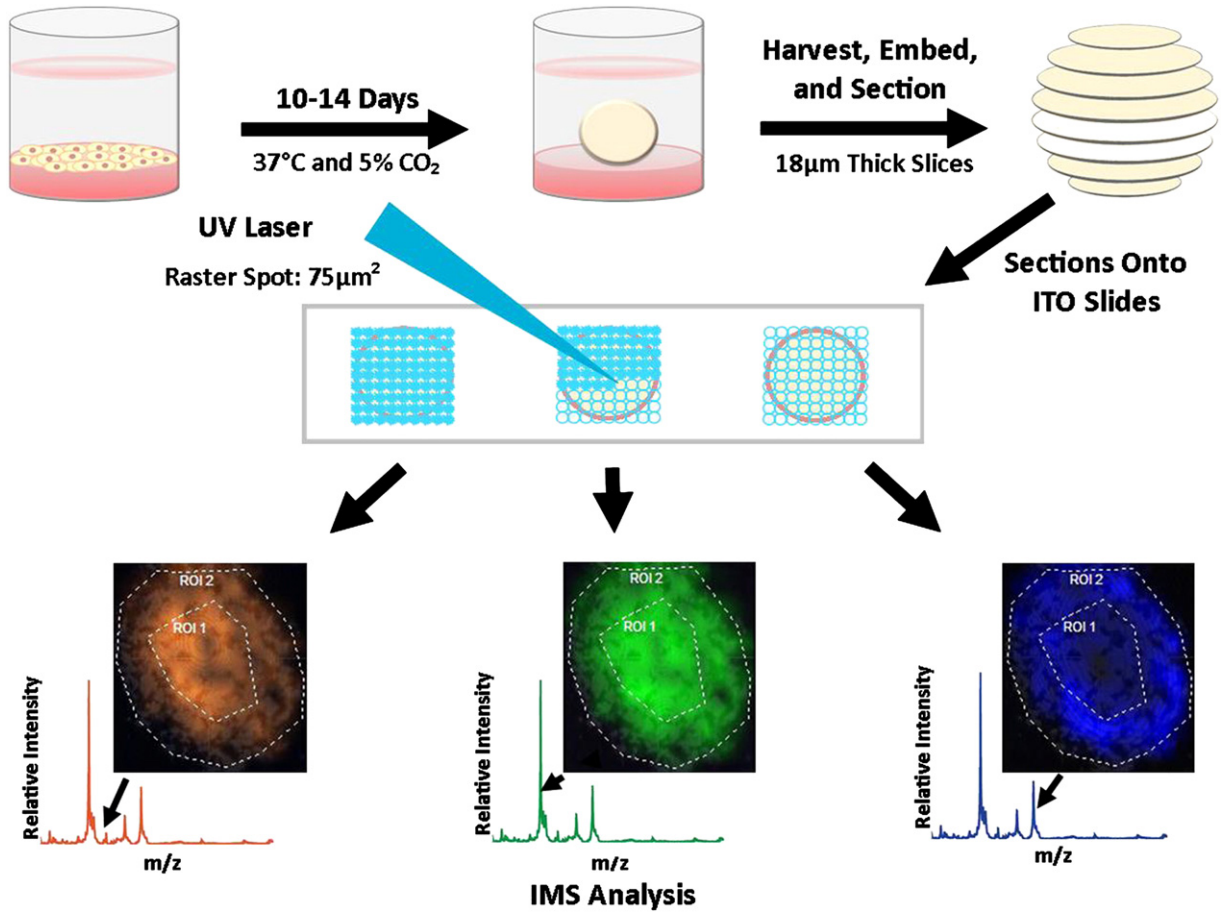

Fig. 1. An overview the general IMS workflow for 3D cell cultures. Cells are plated in 96-wells with a layer of agarose dissolved in cell culture media at the bottom of the inner 60 wells to facilitate 3D spheroid formation. After 10–14 days the spheroids grow to roughly 1 mm in diameter, which is large enough to establish nutrient and oxygen gradients mimicking tumor formation in vivo. The spheroids are then collected and embedded in gelatin for sectioning and subsequent IMS analysis. The workflow preserves spatially relevant proteomic information and enables examination of the distribution of proteins without the use of labels or pre-selection of analytes. ROI (Region of Interest) 1 corresponds to the spatial distribution of the proteins species in the necrotic core while ROI 2 delineates the outer perimeter of the spheroid sample.