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High-throughput platforms for metabolomics

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Mass spectrometry has become a choice method for broad-spectrum metabolite analysis in both fundamental and applied research. This can range from comprehensive analysis achieved through time-consuming chromatography to the rapid analysis of a few target metabolites without chromatography. In this review article, we highlight current high-throughput MS-based platforms and their potential application in metabolomics. Although current MS platforms can reach throughputs up to 0.5 seconds per sample, the metabolite coverage of these platforms are low compared to low-throughput, separation-based MS methods. High-throughput comes at a cost, as it's a trade-off between sample throughput and metabolite coverage. As we will discuss, promising emerging technologies, including microfluidics and miniaturization of separation techniques, have the potential to achieve both rapid and more comprehensive metabolite analysis.

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Introduction

Metabolomics is a relatively new and fast-growing research field. Several different definitions of metabolomics exist, such as 'the analysis of set of small molecular mass compounds in a given biological condition' or 'methods to determine metabolite levels' [1]. Metabolomics, whatever its definition, is being broadly applied in fields such as biotechnology, in pharmaceutical and medical research, in synthetic biology, and environmental science. The broad scope of the metabolomics field is comprised of several distinct analytical approaches, including targeted metabolomics, metabolic fingerprinting, metabolic profiling and exometabolomics. Although major improvements of NMR based metabolomics have been achieved, mass spectrometry (MS) remains the most commonly used metabolomic approach [2,3].

Increasing throughput is highly desirable in that it both decreases costs and enables metabolomics to be applied to large-scale studies. Typically in the field of high-throughput screening (HTS), high-throughput is considered 10 000–100 000 samples per day [4]. In general, high-throughput in mass spectrometry based methods in metabolomics does not achieve this rate and hence the term 'high-throughput' in metabolomics is more a relative term to describe systems with an improved throughput compared to a standard of traditional liquid chromatography–mass spectrometry (LC–MS) methods. For example, where ~750 samples per day is considered high-throughput for LC–MS, desorption/ionization based MS methods can achieve ~10 000 samples/day [5•,6]. However, in MS methodology higher throughput comes at the cost of greatly reduced metabolite coverage, typically <10 metabolites.

The aim of this review is to give an overview of all developments to either improve the coverage or throughput of high-throughput mass spectrometry-based methods with a focus on metabolomic analysis. We describe a wide-range of MS techniques including those that we feel have the potential to enable higher coverage and throughput but do not attempt to comprehensively describe mass spectrometry desorption/ionization approaches. The reader is referred to several excellent recent reviews describing recent developments in high resolution MS and mass spectrometry imaging (MSI) approaches [7••,8,9].

Separation-based platforms

Several factors, including experimental setup or complex sample composition, could require separation of metabolites and/or sample matrix prior MS detection. For example, interference from the sample matrix can result in the decrease or absence of signals from metabolites present in the sample. Liquid-chromatography and gas chromatography are the most common separation techniques used with mass spectrometry (LC–MS and GC–MS, respectively). Capillary electrophoresis (CE) is another powerful approach, but is not as widely used. Both LC–MS and CE–MS most commonly use electrospray ionization (ESI) to produce ions for mass spectrometry analysis.

Although chromatographic-separation and electrophoretic separations are powerful tools for separating molecules in complex biological samples, they are time

consuming. Liquid handling is performed on a timescale of seconds, typically many seconds, and chromatography is an order of magnitude slower, typically requiring many minutes. Developments in both column and instruments technologies, including UHPLC (ultra-high performance liquid chromatography), monolithic columns and core-shell columns, have decreased analysis time and improved throughput [10,11••,12]. Reduction of analysis times to 1–5 min and ~1 min have been reported for LC–MS and GC–MS, respectively [13–17]. It should be noted that an alternative for increasing throughput is to use multiple columns in parallel or injecting multiple samples in series [18,19]. However, these methods will not decrease the actual analysis time.

Fast separation (millisecond timescale) of ionized analytes can be achieved using ion mobility separation (IMS) [20]. IMS separates ions based on the difference in mobility in an electric field in the gas phase, caused by their mass, shape/size and charge. Integration of IMS with MS (IMS-MS) can result in rapid analyte separation for MS-based measurements. An increasing number of commercial IMS-MS types from several different vendors are available [21•]. IMS-MS has been applied to analysis a wide variety of molecular classes, including secondary metabolites, lipids, drug metabolites and carbohydrates, and for the metabolic profiling of bacteria and blood [22–27]. Also, IMS can also be integrated into LC–MS system to allow faster chromatography and thus increase throughput of LC-based systems [28]. In theory, separation can be obtained on a timescale of ~100 ms, which makes IMS 2–3 orders of magnitude faster than LC separations [29]. Although the millisecond timescale of separation, corresponding sample throughputs of <1 second per sample have not yet been reported for IMS-MS. Nevertheless, IMS seems a promising separation technique for high-throughput metabolomics.

Separation-free platforms

Since separation techniques are time consuming, a much higher throughput can be achieved by simply omitting the separation and directly introduce the sample into the ionization source. This is known as direct infusion/injection (DI) or flow injection/infusion (FIE). In DI-MS a static sample is continuously introduced into the mass spectrometer using a syringe pump, or similar device. DI-MS has been applied for e.g. the metabolic profiling of fruit and human plasma, and detection of fatty acids in serum [30–32]. With FIE-MS, the sample is injected into a continuous stream of organic phase flowing to the ionization interface. FIE-MS has been applied in, for example, the detection of B vitamins in nutritional formulations, pesticides in food, and the global metabolic response to osmotic stress in *Escherichia coli* [33•,34,35]. Sample throughput of up to 2 samples/minute has been reported for both DI-MS and FIE-MS [5•,30,36].

Desorption-based platforms

Another group of direct analysis techniques are desorption based. In the last decade, a large number of different ionization techniques have emerged [9,37]. And although these platforms have primarily been used for MSI, they enable the comparison of spatially defined samples, where the throughput is dependent on the sample size and the scan rates.

ESI can be used for imaging of spatially defined samples by, for example, scanning the sample with electrosprayed solvent as in Desorption Electrospray Ionization (DESI) or scanning a droplet of solvent prior to ESI as in nanospray DESI (nanoDESI). Samples can also be desorbed using laser ablation with subsequent ionization using ESI (Laser Ablation ElectroSpray Ionization, LAESI) or simply extracted in situ and then electrosprayed (extractive electrospray ionization, EESI). DESI and nanoDESI have been used for lipid and metabolic profiling with a throughput of 2 seconds per sample [38•,39,40]. EESI and nanoEESI have been used to analyze small molecules in urine, milk and polluted water and has a throughput of up to 1.2 seconds per sample [38•,41]. LAESI has been applied to analyze metabolites and lipids in body fluids and tissue extracts with a sample throughput of 10 seconds per sample [42,43]. Also, most platforms, except for EESI, have already been commercialized for high-throughput applications [38•,44,45]. Another important technique, which is not based on ESI, is Direct Analysis in Real Time (DART) and uses metastable species for ionization. DART has been applied for metabolic fingerprinting of serum and can reach a throughput of 30 seconds per sample [45–47].

Laser DI is another common direct analysis technique. Since laser DI and ion extraction is very rapid (nanosecond timescale) these techniques can be extremely high-throughput. Matrix-Assisted Laser Desorption/Ionization (MALDI) is the most widely used laser DI technique. Here, analytes are co-crystallized with a matrix that absorbs laser light and transfers the lasers energy to the analyte [48••]. MALDI has primarily been used to analyze peptides, proteins and nucleic acids as abundant matrix ions <1000 Da can obscure or interfere with small molecule analysis. Due to advances in, for example, laser beams and the application of novel matrices, matrix interference can be minimized. MALDI has been applied for e.g. the metabolic profiling of cancer cells, identification of secondary metabolites in plant extracts, and dereplication of bacterial isolates [49–51]. Throughputs of about 3 seconds per sample have been reported for MALDI [52].

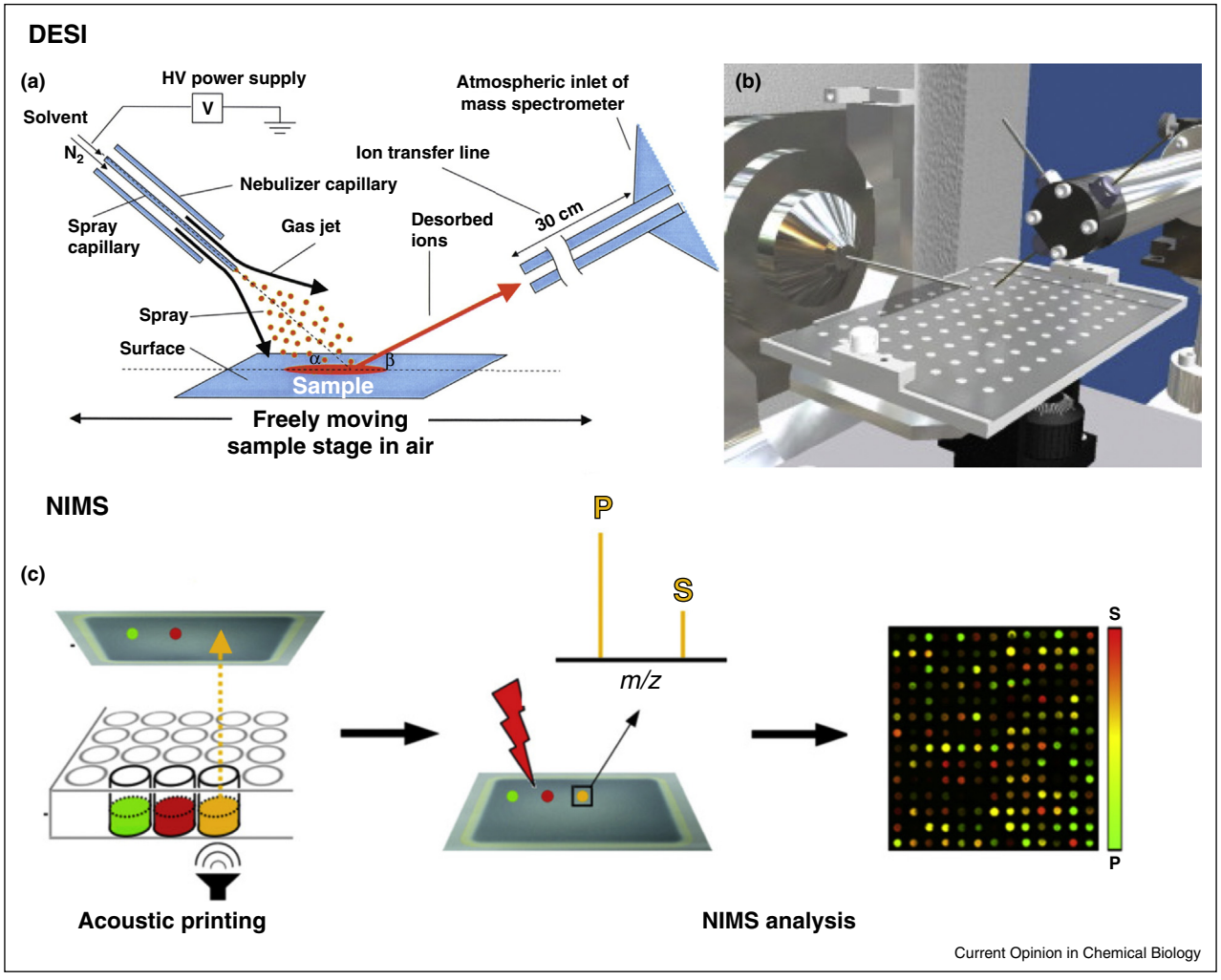
A wide-range of matrix-free, Surface Assisted Laser Desorption/Ionization (SALDI) approaches have been developed and have been reviewed in detail [53]. Many of these can be performed using commercial MALDI

instruments. Major SALDI approaches include Desorption/Ionization on Silicon (DIOS), Nanostructure-Initiator Mass Spectrometry (NIMS), and NanoPost Arrays (NAPA). The majority of these approaches are based on the direct laser desorption/ionization of analytes directly from nanostructured substrates. DIOS has been used for the analysis of metabolites in body fluids and enzyme activity screenings [48••,54••]. NIMS is a unique approach based on using liquid initiators, which are coated onto a nanostructured silicon surface [55]. An ionizing laser heats the surface, which results in the explosive vaporization of the trapped liquid coat as well as the adsorbed analyte molecules from surface applied samples [48••]. NIMS has been used to analyze lipids and xenobiotics in complex biological fluids, including saliva, urine and blood, and for high-throughput enzyme activity screenings in cellular extracts [54••,55,56].

Another matrix-free, desorption method is levitated droplet-MS [57]. This technique uses a laser for the ionization and desorption of acoustically levitated droplets. Although levitated droplet-MS has only been used to analyze small molecules in water, it seems a promising technique for high-throughput metabolomics.

Since desorption/ionization is surface based and loading/ejecting of plates takes several minutes, sample throughput can be significantly increased by increasing the number of samples spotted onto the planar surface and on the resolution and speed of the MALDI platform. By using acoustic deposition, which is a contact-free liquid transfer approach, we are able to spot 10 000 samples onto a NIMS surface. Analysis using a standard MALDI platform at a rate of ~10 000 samples/day takes ~13 h, and yields a throughput of ~5 seconds per sample [58••] (Figure 1).

Figure 1



Two examples of desorption based high-throughput MS approaches: DESI (**a** and **b**) and NIMS **(c)**. (a) Schematic of the DESI source and (b) DESI source equipped with a 96-sample plate (reprinted from [38•]). (c) Schematic of high-throughput NIMS screening (reprinted from [59]). Samples are acoustically printed and analyzed by NIMS.

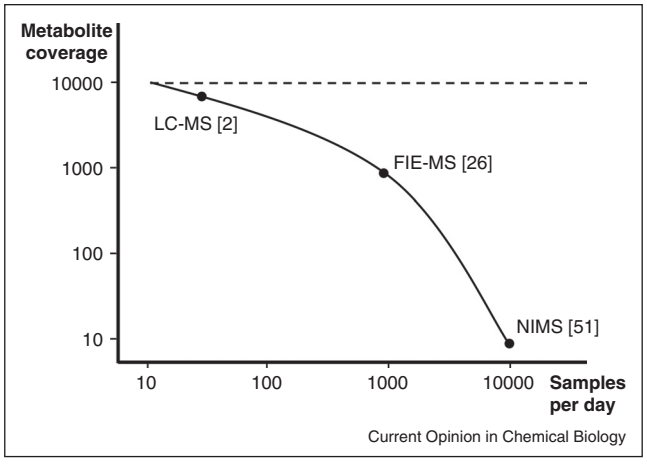
Sample throughput vs metabolite coverage

High-throughput approaches usually improve sample throughput at the expense of metabolite coverage. A common drawback of high-throughput MS approaches, for example, fast chromatography runs, direct infusion/flow injection-based and SALDI-based methods is a decrease in metabolite coverage (Figure 2). The coverage of detected compounds in complex samples is decreased due to sample matrix effects and incomplete resolution of analytes with similar mass-to-charge ratios. As the metabolites are co-ionized with the sample matrix, they suffer from ion suppression due to competitive ionization with the matrix components.

Ion suppression can be reduced by dilution of the samples, but this technique obviously lowers analyte concentrations and thus sensitivities [1]. Despite lower analyte concentrations, Fuhrer *et al.* were able to detect hundreds of metabolites in *E. coli* extracts using a high-throughput FIE-MS method [36].

Removing sample matrix, using techniques such as solid-phase extraction (SPE), liquid–liquid extraction (LLE) and protein precipitation, can also reduce ion suppression. Commercial platforms exist which couple DI/FIE-MS to an online automated extraction. The Agilent RapidFire platform couples SPE sample purification method with FIE-MS in a single automated system, and is capable of throughputs up to 5 seconds per sample [52]. The RapidFire platform has mostly been used to analyze drug and drug-like molecules in body fluids and in high-throughput enzyme screenings [60,61]. Raterink *et al.* coupled an automated LLE method with DI-MS and applied it to detect drugs and lipids in human plasma [62]. However, selectivity of the extraction method depends strongly on the analyte. The wide range of polarities and chemical diversity of metabolites poses a challenge for extracting all metabolites or even all metabolites in a subset and failure will provide a biased outcome and an incomplete view of the present metabolites. As a consequence, extraction methods are mainly applied when there is priori knowledge of metabolite composition or when a specific set of metabolites are targeted.

Figure 2



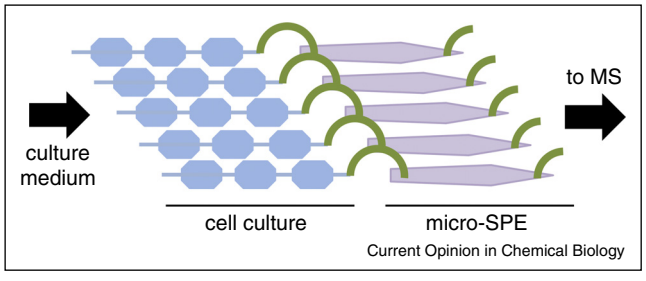
Schematic representation of sample throughput plotted against the total number of metabolites detectable by each platform. Solid line represents current throughputs of MS based platforms. Dashed line represents future direction of ideal throughput vs. coverage. Bracketed numbers indicate the respective references.

Microfluidics and MS

Microfluidics platforms provide a number of unique advantages relative to conventional bench-top systems, including reduced sample volumes, improved analysis speed and better multiplexing [63,64••]. The coupling of microfluidic devices to mass spectrometers is becoming more common and will likely play an important role in the development of high-throughput analysis systems. ESI has widely been exploited as an ionization method for on-line microfluidic-MS analysis, since it is compatible with low flow rates. MALDI has also frequently been coupled to microfluidics, as it allows for automated and high-throughput sample preparation and deposition [63,64••]. Furthermore, separation techniques can be miniaturized, enabling parallelization to increase analysis speed. Microchip-LC, microchip-CE, microchip-IMS and micro-SPE have all been coupled to MS and a commercial chip chromatography-MS instrument is available [21•,65•,66•]. ESI based approaches have been used for example, metabolomic studies of *E. coli* and the analysis drug metabolites in urine and MALDI based approaches have been used for example, profiling hormone release from Islets of Langerhans with sampling rates of 30 seconds (including deposition onto MALDI plate) [67–70].

Besides the previously mentioned examples, most microfluidic separation-technique and MS techniques have not yet been applied to high-throughput metabolomics. With the advantages of microfluidics, we anticipate that microfluidic-based MS platforms will be able to analysis thousands of metabolites at (ultra) high sample throughputs.

Figure 3



Schematic drawing of a possible microfluidic devices for (exo)metabolomics. Based on reference [72•].

One could envision devices capable of massive parallel sample separation using on-chip electrophoresis and/or chromatography or devices with microscale cell culture chambers and on-chip SPE for ultra high-throughput (exo)metabolomics (Figure 3) [71,72•].

Conclusion

The growth of metabolomics as a field means that the number of large-scale, multiplex and systems-wide studies will increase. This makes improving the throughput of MS-based analytical methods highly desirable. Currently, multiple MS-based platforms exist which are truly high-throughput, capable of analyzing 1000–10 000 samples a day, and which have been applied in a diverse range of metabolomic studies. Due to the trade-off between metabolite coverage and sample throughput, high-throughput MS-based platforms are complementary to existing high resolution, low-throughput separation-based MS methods. Both methods will be needed to cover both the metabolomic sample space and depth. With rapid developments in microfluidic-MS, these platforms will be able to bridge the gap between throughput and metabolite coverage.

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