ESI-Mine™: Picodroplet Mass Spectrometry for High Throughput Single Cell Analysis

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Introduction

Mass spectrometry (MS) measures native molecules (*i.e.* label-free) and yields comprehensive metabolic profiles of the presence, absence and abundance of multiple, specific molecules. However, it is relatively low throughput (analyze ~10,000 samples/day). Recent developments in genetic design and assembly have greatly expanded biologists' capability to efficiently construct multiple gene pathway libraries in a combinatorial fashion. The vast number of strains generated in these libraries leads to a growing demand for the increased throughput of analytical tools such as MS.

Sphere Fluidics Limited (SFL) has developed an unique, high-throughput MS system based on its proprietary picodroplet (*i.e.* droplets of pL volume) technology, *i.e.* ESI-Mine™, which can test up to 200,000 biomolecular samples (*e.g.* peptides, enzymes and antibodies) per day using miniaturized input volumes of 500 - 700 pL, which is 1,400-fold lower than conventional MS. As an evolution from the previous work, we further improved ESI-Mine™ not only for high-throughput MS analysis of metabolites from single cell phenotypes, but also retaining its genetic information/cells. To achieve this, we developed a microfluidic MS emitter device which interfaces with a Perkin Elmer AxION 2 MS. Analyte picodroplets were re-injected at 1 – 3Hz onto the microfluidic MS emitter device and evaporated at the end of the nebulizing probe. Analyte molecules from picodroplets were ionized and analyzed giving saw-tooth shaped extracted ion current traces of selected ions. Each saw-tooth peak represented one picodroplet, comprising 5 – 8 scans. In order to extract the genetic information from the desired cell line or recover the cell itself of interesting MS 'hits', asymmetric microfluidic picodroplet splitting was established. One daughter picodroplet is analyzed by MS and the other enters a picodroplet sorting module for in-line retrieval of interesting 'hits' based on a novel picodroplet coding and decoding algorithm.

Materials and Methods

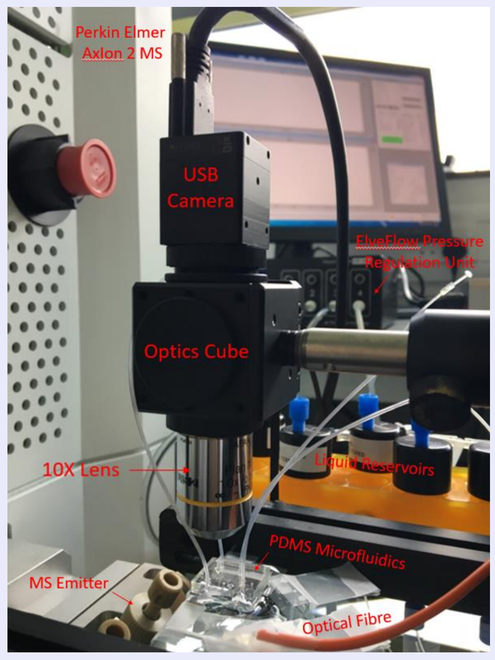

Figure 1 ESI-Mine™ Platform

- In-house developed ESI-Mine™ platform (Figure 1) and control software;
- In-house designed and fabricated microfluidics (Figure 2-b), and assembled microfluidic MS Emitter (Figure 2-a);
- Perkin Elmer Axlon 2 mass spectrometer;
- ElveFlow OB1 pressure controller comprising three 0-2 bars and one -1-0.9 bar regulators.

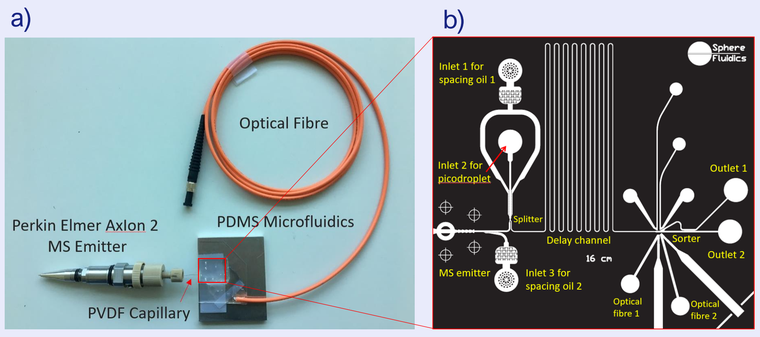

Figure 2 a) Microfluidic MS Emitter Assembly; b) Microfluidic Design

Results: Protein Picodroplet Library MS

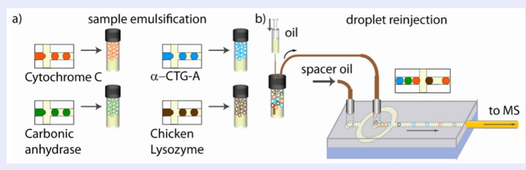

Figure 3 a) Four populations of picodroplets containing either cytochrome C, α-chymotrypsinogen A, carbonic anhydrase, or chicken lysozyme (each 25 μM); b) The picodroplet cocktail of the four populations was reinjected into microfluidic MS emitter interfacing ESI-Mine™.

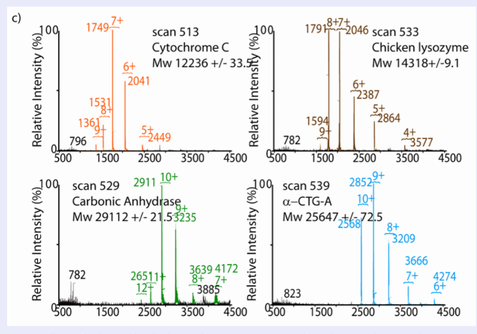

Figure 3 c) Individual scans of picodroplets containing cytochrome C (scan 513), carbonic anhydrase (scan 529), chicken lysozyme (scan 533), and α-chymotrypsinogen A (scan 539) respectively.

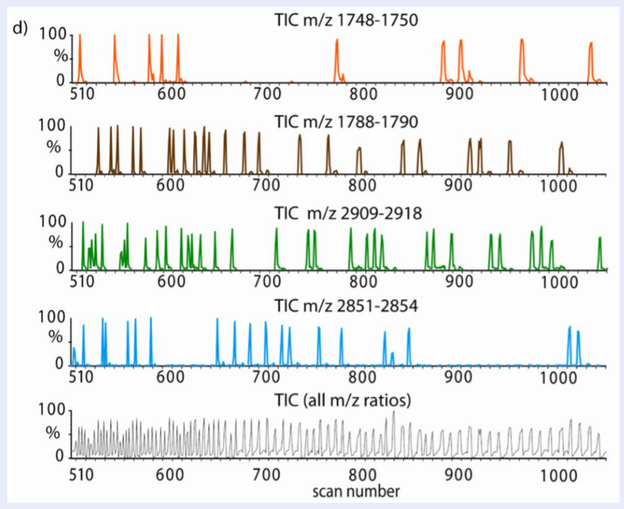

Figure 3 d) The TIC (551 scans, 33 s of data) and ion currents for 86 droplets recorded at m/z ranges 2851−2854, 2909−2918, 1788−1790, and 1748−1750 corresponding to the major charge states of α-chymotrypsinogen A, carbonic anhydrase, chicken lysozyme, and cytochrome C, respectively.

Ref.: C. A. Smith *et al.*, *Anal. Chem.*, 2013, 85(8), 3812-3816

Results : Small Molecule Picodroplet Library MS

Figure 4 Microfluidic MS emitter for picodroplet analysis

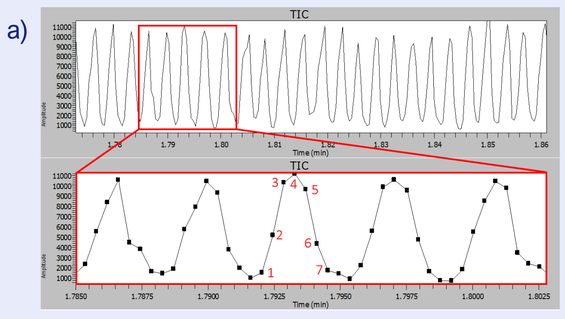

Figure 5 a) TIC of picodroplets containing 250 μM N-methyl-4-piperidinol at a reinjection frequency 4.6 Hz with 8-9 MS spectra per picodroplet; b) Individual MS spectra across a single picodroplet.

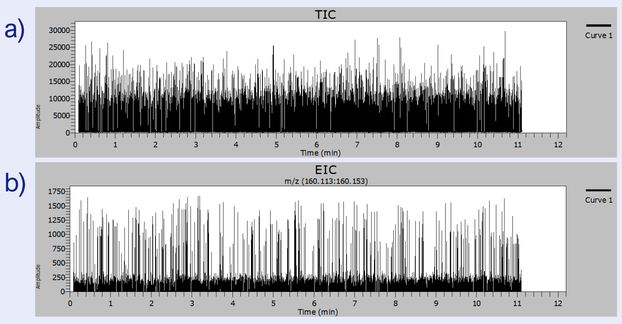

Figure 6 a) TIC of picodroplet mixture (9:1) of picodroplet A (150 μM HPAA, 160) and picodroplet B (1000 μM HPAA,160) at reinjection frequency 1-2Hz with MS scan rate of 30 scans/second; b) EIC of picodroplet mixture clearly shows the two populations.

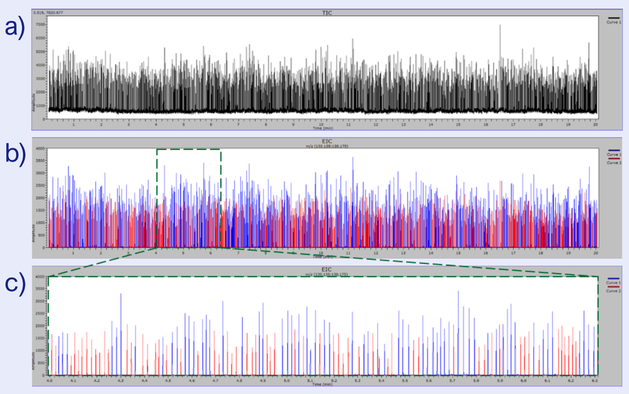

Figure 7 a) TIC of picodroplet mixture (1:1) of picodroplet A (containing 200 μM 4,4-dimethylmorpholin-4-ium iodide, DMM, 116) and picodroplet B (containing 200 μM 4-hydroxy -1,1-dimethylpiperidin-1-ium iodide, HDMP, 130) at frequency 1-2Hz with MS scan rate 30 MS scans/sec. b&c) EIC of picodroplet mixture clearly shows the two populations (DMM is red and HDMP is blue).

Results: Small Molecule Picodroplet Library MS Signal Based Picodroplet Sorting

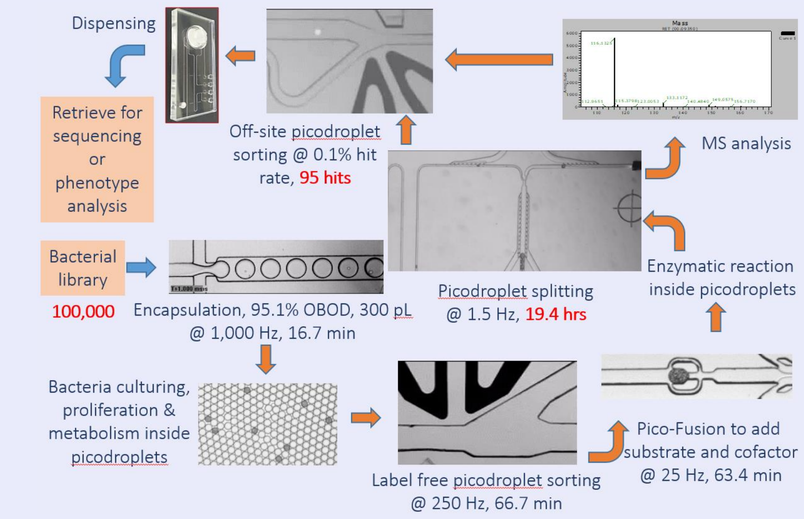

Figure 8 Complete work flow of single cell analysis and sorting on ESI-Mine™ platform

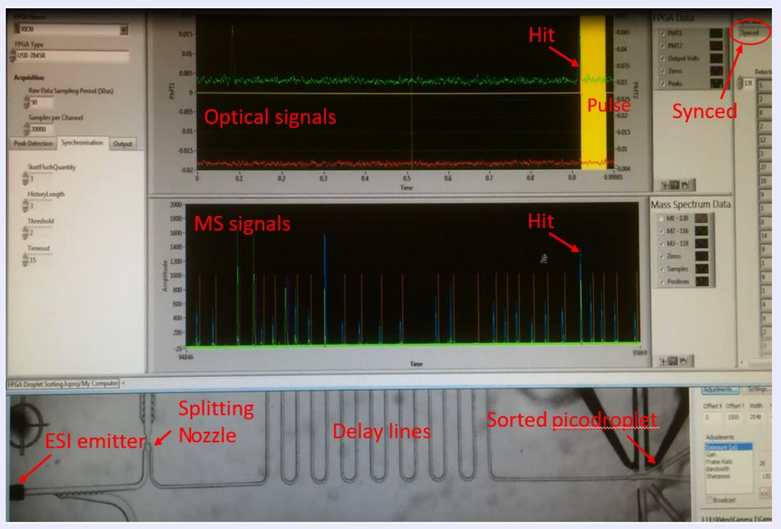

Figure 9 ESI-Mine™ software interface from an actual MS signal based small molecule picodroplet library analysis and sorting experiment (video is available)

Conclusion

- Both protein and small molecule picodroplet libraries can be analyzed on ESI-Mine™ platform in a high-throughput mode, *e.g.* ~10^5 per day, comparing with existing technologies (see the table on the right).
- Real time MS signal based small molecule picodroplet library sorting has been demonstrated.
- Next, we are exploiting the feasibility of MS signal-based protein picodroplet library sorting. Collaboration discussions are welcome.

Parameter	Microplate	Picodroplet
Throughput (samples/day)	10^2 - 10^3 (LC-MS) 10^4 (RF-MS)	10^5 - 10^6
Reagent volume (required to screen 1M mutants)	100 L (*e.g.* £500,000 for 10 mM NADPH)	1 mL (*e.g.* £5 for 10 mM NADPH)
Equipment	• Qpix colony picker • Multi-plate Incubators • Centrifuges • FX/Tecan liquid handling robots • LC-MS or RF-MS • MTPs (£20,000-£50,000)	• Syringe or air pressure pumps • Fabricated chips • Mass Spec • Biochip (£500).