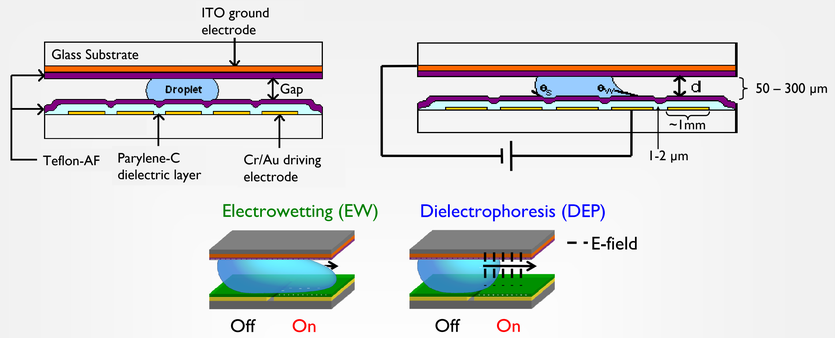
Fluorinated liquid-enabled protein handling and surfactant-aided crystallization for fully in situ digital microfluidic MALDI-MS analysis

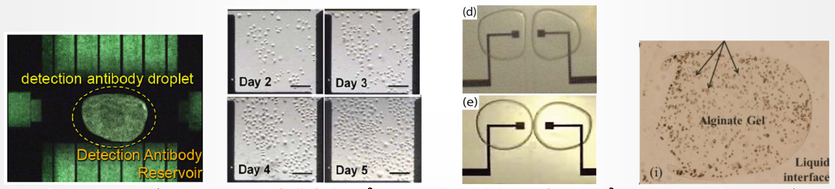
Andrew P. Aijian, Dr. Debalina Chatterjee, Professor Robin L. Garrell
Department of Chemistry and Biochemistry, University of California Los Angeles, Los Angeles, CA 90095-1569

Introduction

Digital (droplet) microfluidics is a method for handling discrete droplets of small amounts of liquid (pico-microliters) through the spatially controlled application of electric fields. A cross section schematic of a digital microfluidic device is shown below.

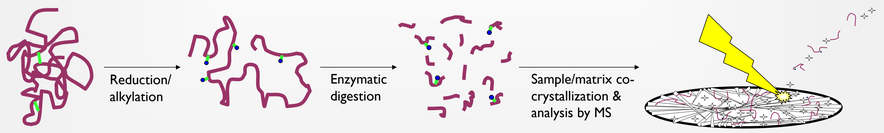


Because droplet movement is driven entirely by electric fields, digital microfluidics can be completely automated, allowing complex or tedious liquid handling protocols to be performed with minimal manual effort. For this reason, digital microfluidics has been shown to be effective at enhancing various chemical and biological assays.



Immunoassays[1] Cell Culture[2] Lipid Bilayer Creation[3] Drug Screening[4]

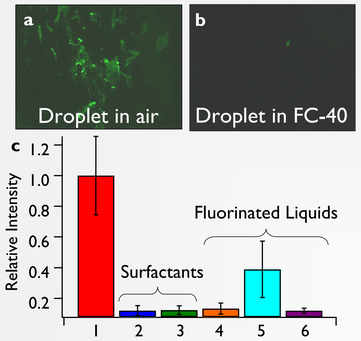
In this work we use digital microfluidics to streamline the sample preparation process for peptide mass fingerprinting (PMF), a common technique for studying proteins by Matrix Assisted Laser Desorption Ionization Mass Spectrometry MALDI-MS. The PMF process is shown below:



Limitations to performing PMF assays on the digital microfluidic platform include the non-specific adsorption of proteins to the device surface, which inhibits droplet movement, and poor sample/matrix co-crystallization on the hydrophobic device surface. While the addition of nonionic surfactants such as Pluronics® can facilitate protein droplet movement, these additives often interfere with the MALDI spectra. In this work, we investigated alternative methods for enabling the movement of protein solutions on digital microfluidic devices and techniques for enhancing sample/matrix co-crystallization to achieve high quality mass spectra directly from the device surface.

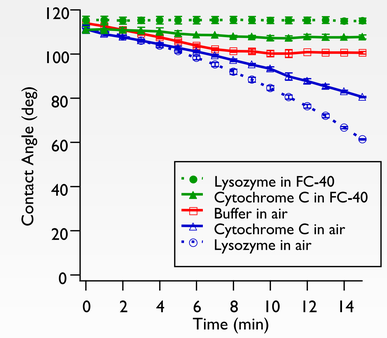
Results

We found that fluorinated liquids are effective at preventing non-specific protein adsorption to the device and facilitating the movement of protein solutions. The fluorinated liquids examined here were Fluorinert® FC-40 and FC-75 and Novec™ Engineering Fluid HFE 7500 and HFE 7100 (3M).

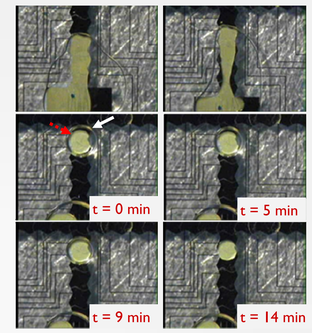


a,b) Fluorescent protein adsorption from a droplet sitting on the device surface is minimized by engulfing the droplet in fluorinated liquid.

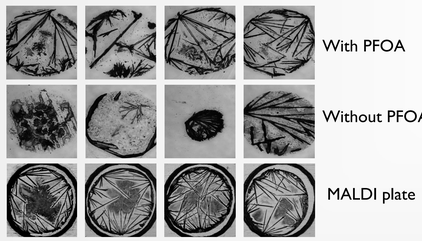
c) The use of fluorinated liquids (4,5,6) can be as effective as Pluronics® surfactants (2,3) at reducing protein adsorption.



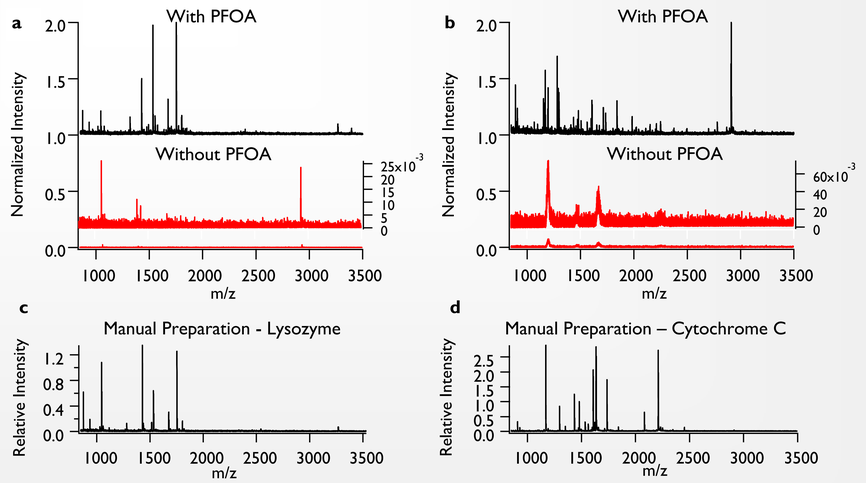
When proteins adsorb to a solid surface, the interfacial energy decreases which can be detected by a decrease in the contact angle. It is clear that the addition of fluorinated liquid prevents the adsorption of proteins to the device surface compared to a droplet sitting on the surface in air.



Fluorinated liquids (solid arrow) can be removed from the sample drop (dashed arrow) via evaporation at room temperature. This is advantageous compared to the use of Pluronics® or non-volatile oils which are difficult to remove from the sample and can cause spectral interference if not removed.



Crystal morphology is critical to obtaining quality MALDI-MS spectra. Protein samples crystallized with matrix solution containing PFOA on a device surface consistently exhibited the typical needle-like crystal morphology of DHB crystals and mimicked samples crystallized on a traditional stainless steel MALDI plate.

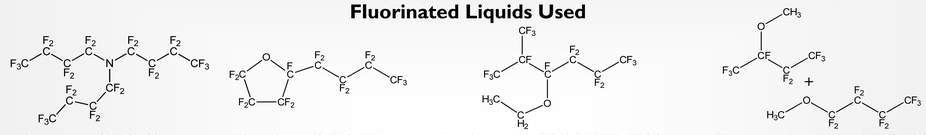


Using fluorinated liquids and surfactants to promote sample movement and crystallization, mass spectra can be obtained from microfluidic sample preparation (a & b) that are comparable to those obtained from traditional, manual protocols (c & d).

Approach and Methods

We hypothesized that engulfing a droplet in a fluorinated liquid shell could enable the movement of protein solutions on the microfluidic device by minimizing the aqueous/solid interface and thus non-specific protein adsorption. We used contact angle goniometry and fluorescence microscopy to monitor protein adsorption.

Fluorinated Liquids Used

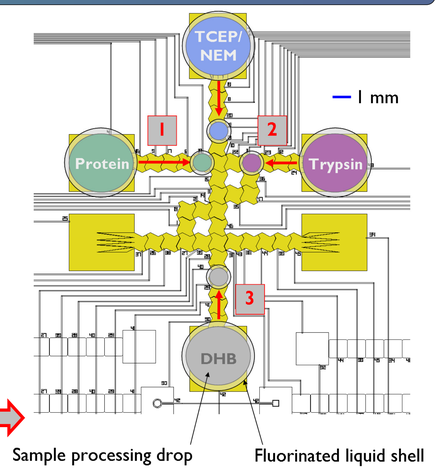


Fluorinert® FC-40 **Fluorinert® FC-75** **Engineering Fluid HFE 7500** **Engineering Fluid HFE 7100**

To address the problem of poor sample/matrix co-crystallization, small amounts of fluorinated surfactant (pentadecafluorooctanoic acid, PFOA) ware added to the MALDI matrix solution to provide hydrophilic domains to aid in the nucleation of matrix crystals on the hydrophobic surface of the device.

Protein samples were prepared for MALDI-MS analysis according to the following protocol:

1. Disulfide bond reduction/alkylation with tris(2-carboxyethyl)phosphine and N-Ethylmaleimide - 5 min.
2. Tryptic digestion - 15 min.
3. Sample/matrix co-crystallization - 15 min.



Conclusions

- Fluorinated liquids facilitate the movement of protein solutions on digital microfluidic devices.
  - Minimize protein adsorption
  - Evaporation of fluorinated liquid = easy removal, no spectral interference
- Small amounts of fluorinated surfactants promote crystallization of the matrix on the device surface
  - Good crystals = good spectra
- Digital microfluidics can be used for completely in-situ MALDI sample preparation.

References & Acknowledgements

References:
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