MASS SPECTROMETRY IN ANALYTICAL APPLICATION TO RELEVANT CYTOCHEMICAL SPECIES

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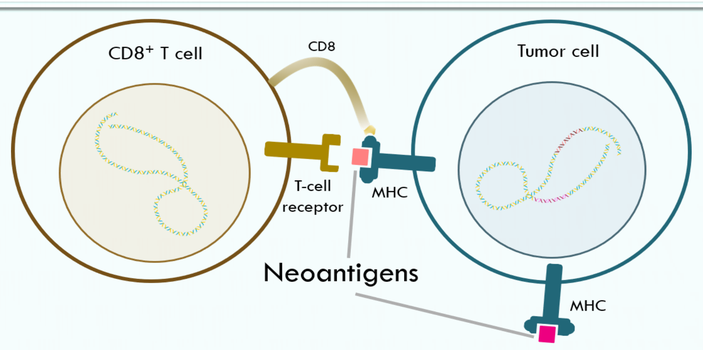
Target and objective

High-density chips are becoming available to allow measurement by mass spectrometry (MS) [1]. These micro-chip devices empower multi-omics ways to tease out the interrelationships between distinct classes of cellular component [2]. Combining analytical instruments to elucidate nonredundant cytological protein function is becoming widespread. Whether this be with microfluidics devices adapted with specialized design feature such as channels to the dimensionalities of individual cells or programmable electrode actuation [3], this novel technological field is opening up improved workflow methodologies in terms of miniaturization, parallelising, and integration [2].

Prompted by these advancements in microfluidic chip implementations, we are anticipating to yield MS cancer vaccine discovery outcomes in resolving the analytical specifications of neoantigens selected from cancerous immunological adjacencies (see Figure 1). The later will enable needed information on the molecular identity, fragmentation knowledge and oncogenic detail sought after by modern pharmaceutical strategies as well as the biomedical developments thereof, *i.e.* molecular immunotherapies (see Figure 3).

What is a neoantigen?

Figure 1. Neoantigen and neoantigen MHC complex at the exosome of a tumor cell and interaction with CD8+ T cell receptor.



Why is this work needed?

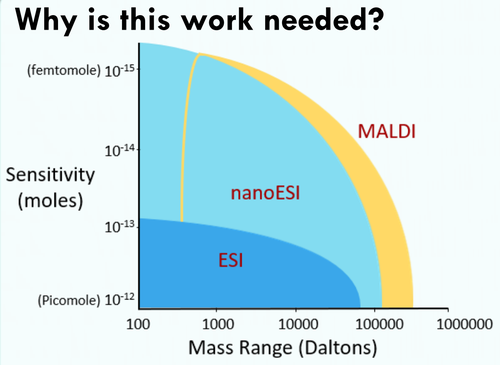


Figure 2. A glance at sensitivity of separate ion sources. Adapted from Springer Nature Customer Service Centre GmbH: Springer. JASMS. Schalley, C.A. J Am Soc Mass Spectrom (2004) 15: 625.

The small order of magnitude of single cells and scarce number of target molecules therein remains a challenge for analysers [4]. One strategy for overcoming the issues associated with selectively characterising the properties of single cells is to adapt microfluidic devices which can help to achieve in one part the required experimental scale and the other, the analytical flexibility to parallelise and integrate methodological steps [2]. Microfluidic designs are positioned into expanding the technological prefractionation toolbox to: (i) help derive more intact valuable biological information [4], (ii) better aptly exploit the sensitivity advantage from both desorption and spray ionization principles (see Figure 2) for analysis.

What is the focus of neoantigen therapies?

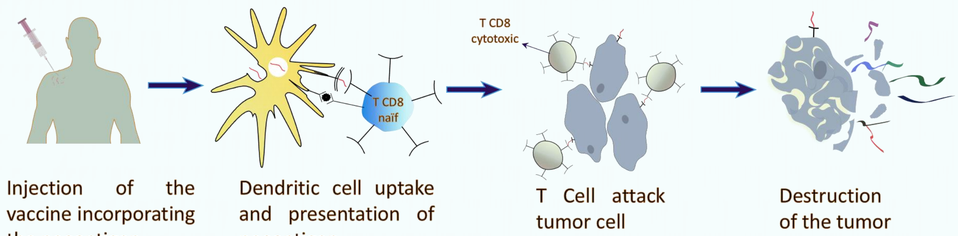


Figure 3. The biomedical program through which to utilize neoantigens to train the immune system to better eliminate cancerous cells.

Background

One of the possibilities to adapt for neoantigen discovery is the use of digital microfluidics. Opendrop V3.2. is an open project and the hardware and software is open source. The device is a platform for development. It consists of a digital microfluidic lab on a chip (LOC) type of liquid handling platform. Unlike with the microchannel designs, it relies on electrowetting on dielectric (EWoD, see Figure 4). This electrical polarization enables the individual control of droplets on an array of electrodes [5]. Planar efficiency of this dielectric is then moderated with the application of a 'thin film' hydrophobic coating, based on the user instruction of the developers (see Figure 6), to serve as an interacting layer for the system [3].

The special appeal of Opendrop, as with all digital microfluidics, is the seamless capability to pin down on microliter sized droplets which can be made to move, merge, split from one electro pad to another and dispense from reservoirs (see Figure 5) [5]. In its basic manipulation the cardinal or diagonal movement directions across the array is coordinated with a simple joystick (see Figure 7). A key aspect of the device is that OpenDrop is a modular machine with a simple fabrication design geometry conditioned for coupling together with other technologies such as mass spectrometry or operating software such as Arduino [3].

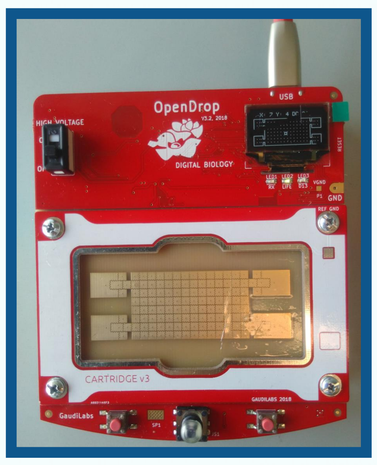


Figure 7. The Opendrop V3.2.

How does Electro-wetting on dielectric (EWoD) work?

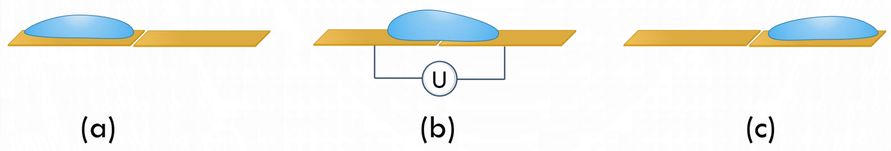


Figure 4. A schematic on how EWoD operates. (a) A occupied electrode at steady state. (b) An applied voltage actuates the droplet to move. (c) Droplet arrives at the neighboring charged electrode [3].

What operations can be run with Opendrop

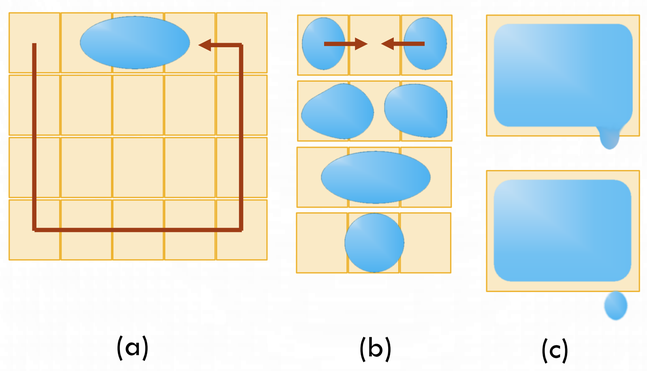
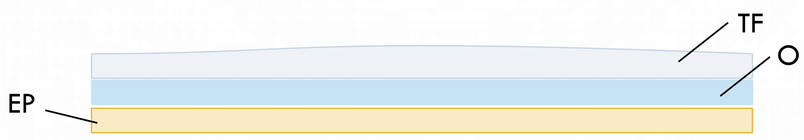


Figure 5. The microfluidic operations trialed and observed: mixing (a), merging (b) and dispensing (c) of small liquid droplets (4-24 µl) [3].

Coating technique and hydrophobic layer



EP: Electrode pad

O: Oil (hydrophobic substance) 3-10 µm.

TF: Thin film (Saran wrap)

Figure 6. The Opendrop specialised 'thin film' hydrophobic layer coating, with; electrode pad (EP), oil (3-10 µm thick hydrophobic substance) (O), thin film (saran warp) (TF).

Further approaches

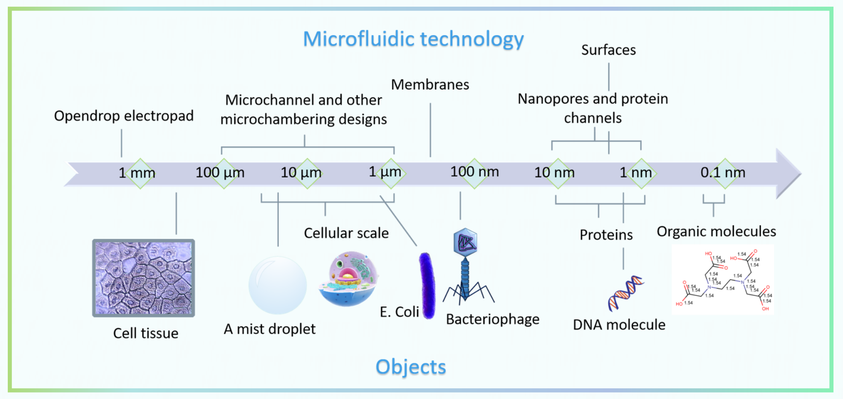


Figure 8. Microfluidic tools along the scale of magnitudes from the tissue to the molecule.

Other microfluidic based platforms specific to MS analysis

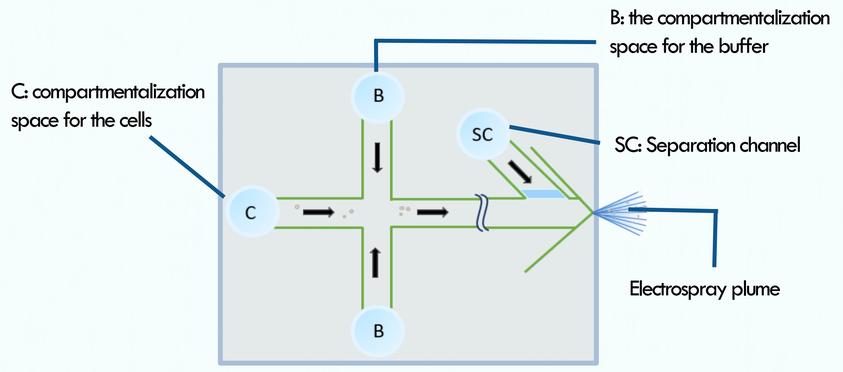


Figure 9. Illustrative diagram of a CE-ESI-MS microchip. Reprinted (adapted) with permission from (Anal. Chem.2010823967-973 Publication Date:January 8, 2010 https://doi.org/10.1021/ac902218y). Copyright (2019) American Chemical Society.

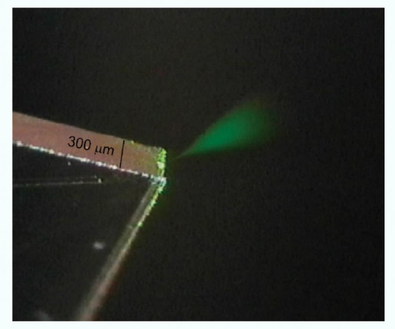


Figure 10. Corner electrospray exit of a channel microfluidic. Reprinted (adapted) with permission from (Anal. Chem.200880186881-6887. Publication Date:August 13, 2008 https://doi.org/10.1021/ac800428w). Copyright (2019) American Chemical Society.

Raw material

- Coper electrodes with a gold coating on top applied by the electroless nickel immersion gold (ENIG) plating technique.
- A frame made of a material abbreviated FR4, further information available at https://electronics.stackexchange.com/questions/127762/schematics-vs-pcb-designs.

Advantages of microfluidics

- It is possible to assay twenty or more functional protein results simultaneously from single cells [6]
- Cell behaviors (e.g. motility) may be correlated with protein assays [6]
- Third extensions to quantized cell populations enable measurement of cell to cell relationships [6]
- Rare cells can be functionally identified for further analysis or culturing [6]
- Reduced reagent and analysis time
- Potential of high-throughput

Follow up

In a last statement, Opendrop is a low budget release that offers the capability to elaborate original studies across and between different labs [3]. However:

(i) Reaching from one location to another suffers from an improbable reproducibility and remains a nature of undesirable inconsistency with Opendrop. (ii) Actuation is flawed as the droplet traction differs between the electro-pads of the board and the sawed surface trench that separates these. In a later phase, better polished surfaces are needed for the EWoD technique, that offer superior droplet adherence. Later stage development would also include what better interconnect to use with Opendrop or other digital microchips to spray or desorption MS interfaces.

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