An ultrasensitive digital nanoassay for immunotherapy effectiveness

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Abstract

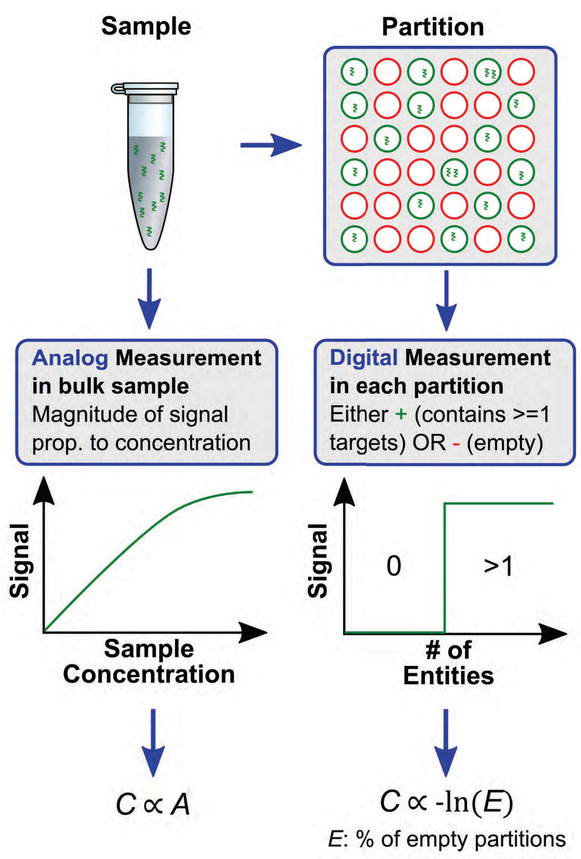
The detection of biomarkers is a key component in early disease detection, as well as to monitor treatment effectiveness, thus lowering the threshold at which some pathologies can be detected can have a significant impact on prognosis. The go-to method to measure protein levels in biological samples is the enzyme-linked immunosorbent assay (ELISA), however many biomarkers of interest are present at or below the sensitivity limit of conventional ELISA (~0.1 pM).

Digital ELISA allows for up-to a thousand-fold more sensitive detection than conventional ELISA. In this technique, the sample is partitioned so that each partition will contain a discrete number of molecules of the biomarker. The assay is then performed on each partition separately and the level of signal measured informs of the number of molecules in the sample.

Here we report a novel approach which relies on the capture of the target biomarker on magnetic beads for a chamber-less partitioning using digital microfluidics to perform assays on very small volumes of biological sample and fluorescence microscopy for the detection. The entire assay can be conducted at room temperature and most steps can be automated for an easy, highly sensitive biomarker detection.

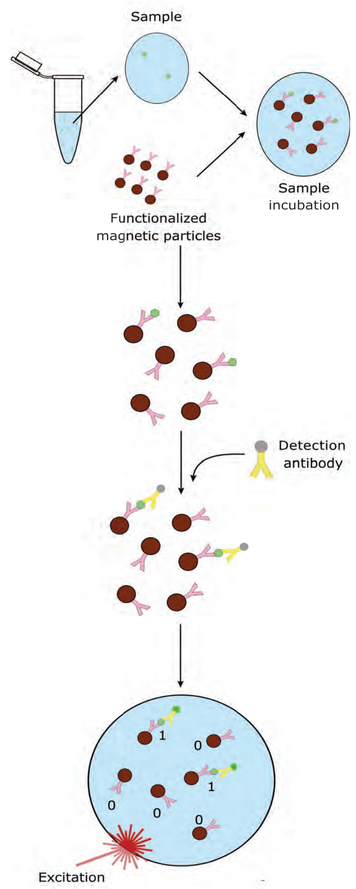
Approach & preliminary results

A digital immunoassay ...



Comparison of digital assays vs. conventional analog biological assays.
From: Basu 2017, SLAS Technology

… without microwells ...



Graphical representation of the different steps of the proposed digital immunoassay.

… on a digital microfluidics device!

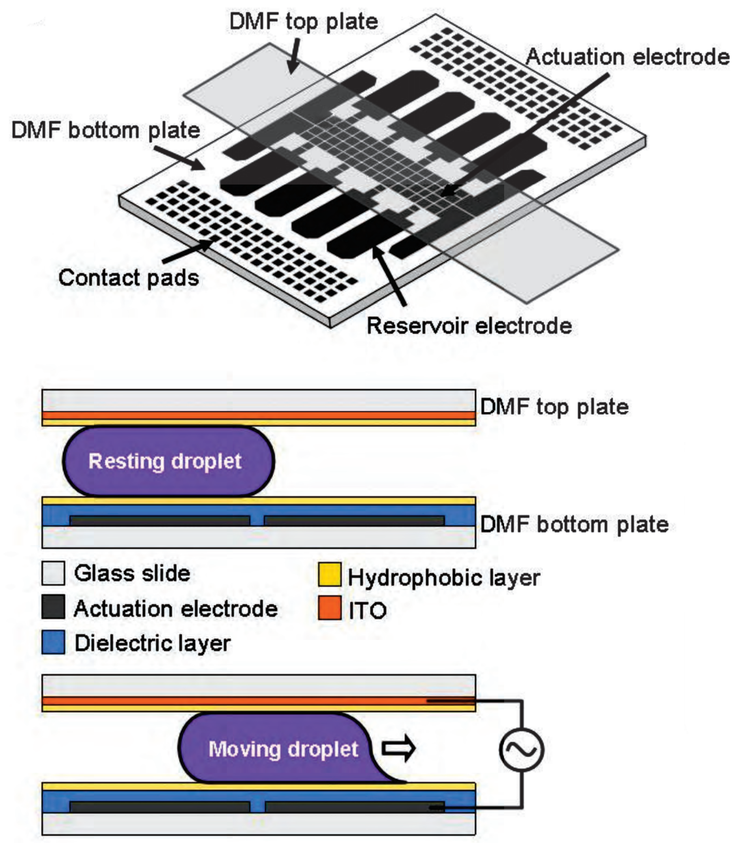
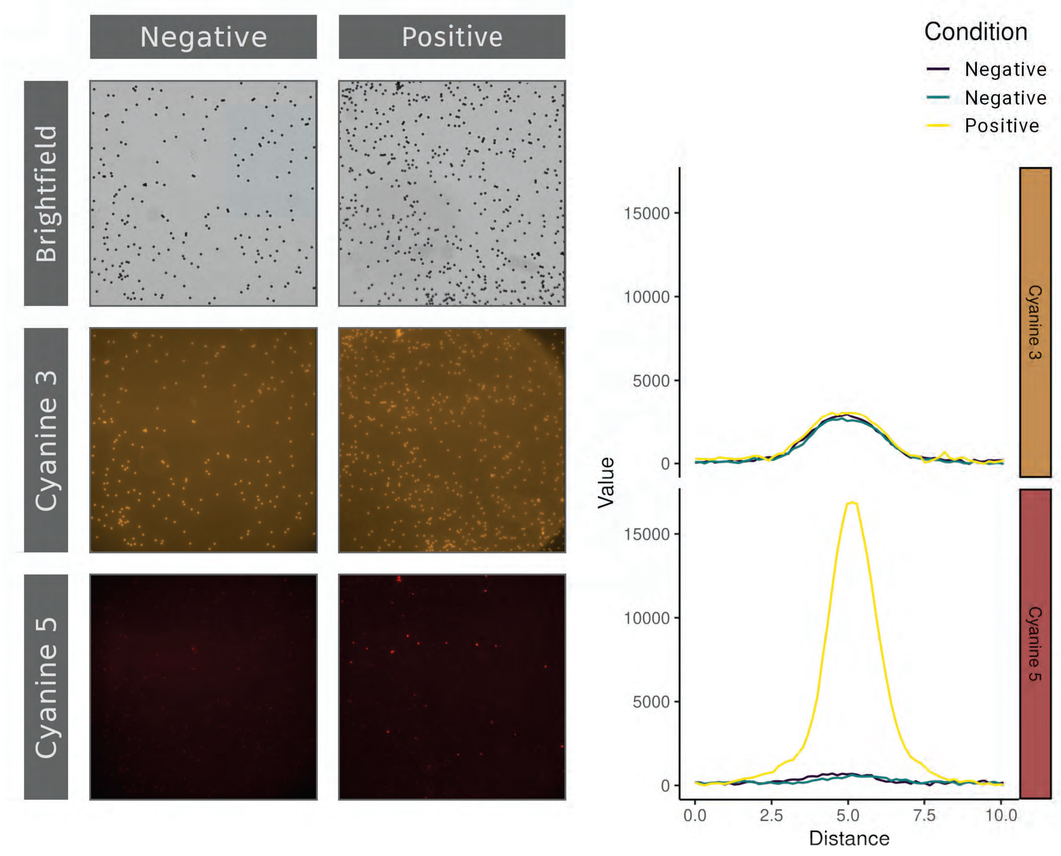
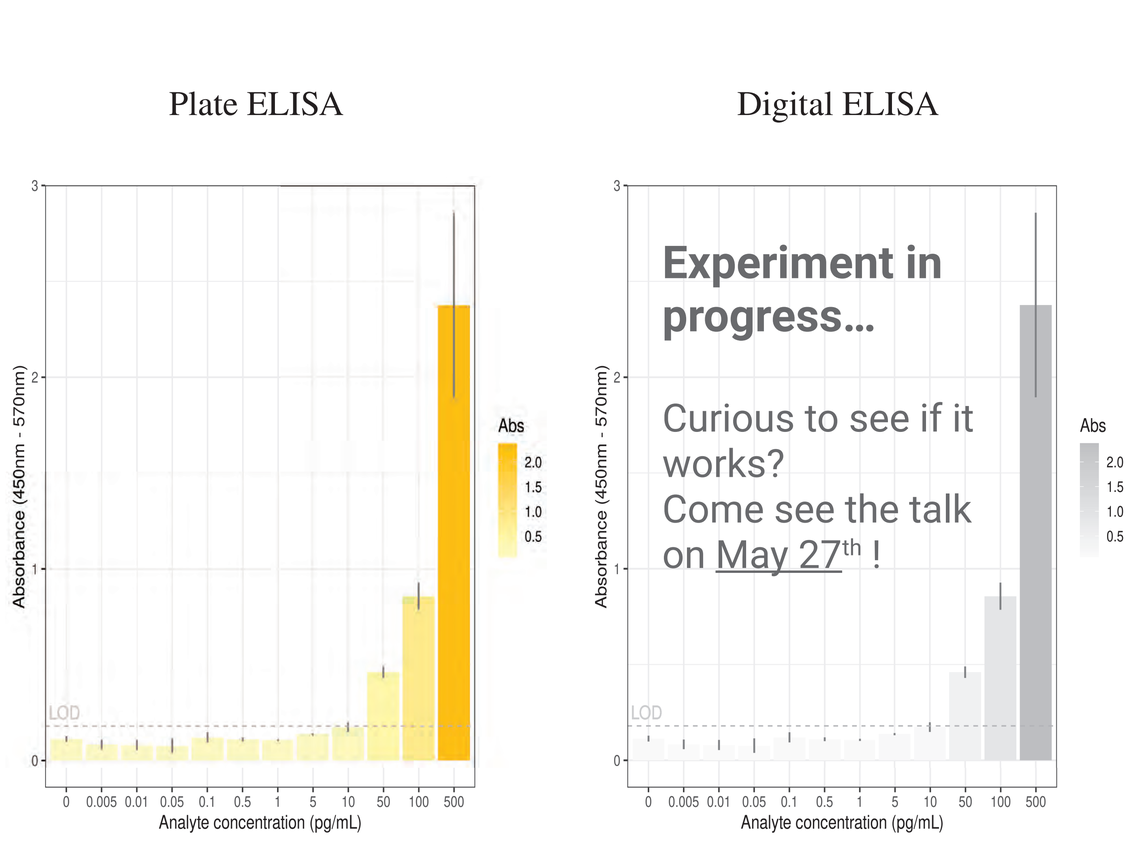


Illustration of an assembled digital microfluidics device (top).
Side view of a resting (top) or moving (bottom) between the top and bottom plates of a digital microfluidics device. The different layers comprising the top and bottom plates are listed in the legend.



Validation of the detection method on magnetic beads without analyte.
On the right are microscope images of magnetic beads at 400x magnification in brightfield (top row), cyanine 3 (middle row) or cyanine 5 (bottom row) channel. The beads are auto-fluorescent in cyanine 3 while the positive signal is detected in cyanine 5.
On the left is a fluorescence intensity histogram of a representative bead for each condition.



Barplot showing the absorbance values obtained for a plate ELISA against TNF-α using a standard at different concentrations.
The dotted line indicates the limit of detection (LOD).

Conclusion

Here, we propose a new approach to digital immunoassays that doesn't rely on microwells for sample partitioning but instead localizes the signal onto magnetic beads using a very efficient signal amplification method. This eliminates the need for oil to prevent sample evaporation, reducing the cost of the instruments.
The assay can be performed on a digital microfluidics device, allowing for small reaction volumes (~1µL) and automation of most of the experiment.