The D4Scope: A Novel Low-Cost Fluorescence Detector for Point of Care Diagnosis

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Introduction: We have previously developed a new, highly sensitive point-of-care test —the D4 assay— that can detect analytes from a single drop of blood with femtomolar sensitivity. The D4 assay is based on printing Ab microarrays onto a non-fouling —protein-and cell-resistant— polymer brush on glass, that eliminates virtually all non-specific adsorption. All reagents are ink-jet printed on the D4 chip, so that no additional reagents are required, and the assay completes itself upon addition of a drop of blood by passive diffusion of reagents across the chip surface. However, the commercial microarray imaging detector —the Axon Genepix— that we have used to date for fluorescent readout is bulky, expensive, slow, and not suitable for use at the POC. To address this limitation, we have developed a handheld, battery-powered, low-cost fluorescence detector that can image fluorescent microarrays with high sensitivity. This detector combines the latest advances in microscopy, mobile computing, and telemedicine for protein microarray imaging and analysis in one platform.

Materials and Methods: The detectors' fluorescence elements are set in an oblique angle laser excitation format. The core fluorescence elements were first evaluated on an optical breadboard. This allowed each element—the laser, band-pass optical filter, and camera—to be tested, replaced, and iterated on rapidly without significant changes in alignment. The red laser, with high optical coherence in the fluorophore excitation bands, excites the fluorescent microarrays on the glass slide at a 45º angle. The fluorophores emit at a longer wavelength that is filtered by a bandpass optical filter, and the filtered wavelengths are then captured by a camera. These components were tested for sensitivity on D4 chips with a low fluorescence signal. Once a good combination was identified, the breadboarded elements were then modeled by a computer-assisted design software (Solidworks). A 3-D printable housing was then designed around these elements in Solidworks and 3-D printed on a Lulzbot Taz 6. The device was then assembled with the housing, optical elements, and a system-on-a-board computer and screen. The housing was iterated on for proper fit of all parts, portability, and durability. We also wrote custom software in Python for automated microarray analysis on each device. The software uses computer vision algorithms to quantify the microarray data from the fluorescence images and saves the images/data in a cloud MongoDB database within seconds, with a press of a button. All code is open-source, and available on Github.

Next, the performance and sensitivity of this detector was tested by generating dose-response curves with spiked analyte samples in serum. These samples were scanned by both the custom detector and a commercial Axon Genepix benchtop scanner to evaluate and benchmark its performance. We also beta-tested this detector to gather user feedback to guide future development. One such prototype has been already deployed with a D4 POCT for detection of Ebola virus at the Galveston National Laboratory's BSL4 lab in Texas on Ebola infected monkeys.

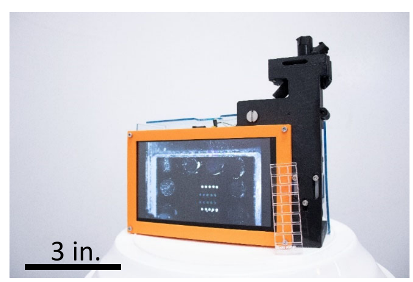

Figure 1: A D4 chip with 24 discrete microarrays and the prototype detector displaying a scanned microarray image from a D4 chip.

Results and Discussion: The device has shown a lower limit of detection than the conventional benchtop scanner for several biomarkers through internal testing. The total cost of parts is ~1500 USD, not yet adjusted for at-scale production. Further work will be done to reduce costs, improve sensitivity, and improve user-friendliness on both hardware and software fronts. In future work, we plan to incorporate brightfield imaging for traditional microscopy, such that this device can carry out brightfield and fluorescence imaging of cells and tissues, as well as fluorescence imaging of protein microarrays to provide a single, comprehensive platform for clinical immunoassays and pathology.

Conclusions: Together with the D4 POCT, this customized hand-low-cost hand-held detector promises to democratize sandwich immunoassays in a microarray format for clinical and research use.