Miniaturized Fluorescence Biosensing Reader for Multiplexed Allergen Screening at the Point-Of-Care

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INTRODUCTION

With the steady rise in cases of immune-mediated diseases, extensive studies on environmental, genetic, and epigenetic factors are essential [1]. To facilitate such studies, we developed a platform for advanced allergy profiling, consisting of a microfluidic device with micropillars and an automated processing system. This system is capable of detecting allergy-specific IgEs through the utilization of fluorescence-labelled antibodies. To ensure the platform's accessibility and utility in point-of-care settings and smaller laboratories, we have developed a compact, cost-effective fluorescence reader, enabling the automated readout of the microfluidic chip fluorescence. This novel integration offers promising advancements in the diagnosis and study of allergic conditions, providing a feasible tool in various healthcare and research settings.

METHODS

The microscope slide-shaped microfluidic system was fabricated by injection molding of polycarbonate, containing 384 micropillars (Figure 1) of 300 µm diameters. The slides are coated with a photo-linker polymer (OptoDex®), for immobilizing the allergens onto the micropillars, passivating the surface to suppress non-specific bindings, and to hydrophilize the microfluidic channel. The allergen extracts and recombinant proteins (Timothy grass, cat and dog epithelium, house dust mite, and common birch, from Bühlmann Laboratories AG and Indoor Biotechnologies Limited) were deposited (GeSiM GmbH, Nanoplotter 2.0) on defined pillars. After dispensing, a photo-immobilization is performed with a UV chamber (2 min at 20 mW/cm2, Beltron GmbH). After a film lamination (Simport T329-1, US) to close the channel, control serum samples (PathTROL) were used to validate the multiarray. Parallelized sample handling (up to 6 slides) is performed using the custom IncaTrace technology, injecting 80 µL of serum sample per slide. Finally, detection antibodies (Mouse anti-hIgE) and AlexaFluor 647 Goat-Anti-mIgG (80 µL each) are flown in the system and subsequent fluorescence images can be acquired. Measurements were performed both with a commercial microarray reader (InnoScan 710) and with the custom-made portable reader and the performance of the two devices was compared.

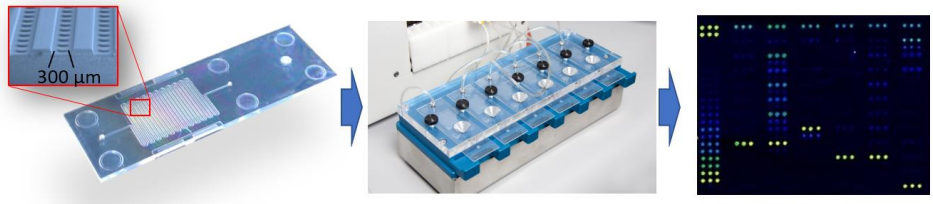

Fig. 1: Multiplex system for allergen screening. Left, Picture of the microfluidic system; middle, image of automated microfluidic system; right, the fluorescent signals on the micropillars

The portable reader employs fluorescence imaging instead of confocal scanning utilized by commercial slide scanners. This enables a reduction in size and cost and improves speed, however, sacrificing the sensitivity. The reader is comprised of several components including a camera, collection and beam-forming optics, a light source, emission and excitation filters, and a light trap (Figure 2).

From the spectral properties of the selected dye (Alexa-647), we selected a 642 nm laser (Roithner Laser Technik GMBH) as light source. Light-forming optics consists of a fiber collimator, an excitation filter to

remove unwanted emission from the laser, and an engineered top-hat diffusor (Thorlabs) to ensure uniform sample illumination. A combination of a color glass filter and an interferometric filter was used for optimal performance of the fluorescence image. Fluorescence emission was then collected with a camera objective (Kowa, 12 mm), modified with an additional 2 mm camera-to-objective distance to allow macro-imaging. A monochromatic CMOS camera (TheImagingSource DMM37UX265) based on a Sony IMX265 sensor chip was employed. Camera and chip feature manual control of integration time, gain, gamma, and 12-bit data output. Finally, a trigger from the camera is used to activate the laser during the measurement.

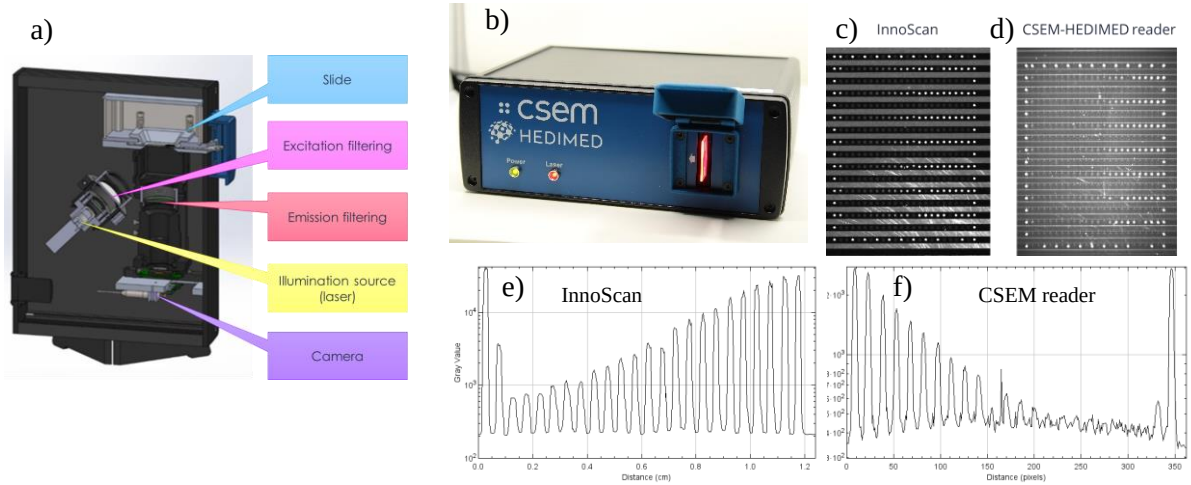

Fig. 2: a) Portable fluorescence reader structure; b) photo of the portable reader; c,d) comparison of the data acquired by InnoScan and the portable reader; (e,f) correspondent line profile in log-scale

RESULTS

To evaluate the performance of the portable reader it was compared to the commercial microarray reader Innoscan 710. We prepared slides with different dye concentrations and analyzed them using both readers. The brightest spot corresponded to the calculated dye concentration of 5000 molecules/µm^2, the faintest corresponds to 10 dye molecules/µm^2. Figures 2c and 2d shows the same slide as read by the commercial Innoscan system and our portable reader, respectively, with corresponding line profiles illustrated in Figures 2e and 2f. All 24 printed points can be resolved with an Innoscan reader, with a standard deviation of the signal of 7 dye molecules/µm^2. Our portable reader has a higher background level and noise, only 15 out of 24 points can be differentiated from the noise, reaching a sensitivity of ~100 dye molecules/µm^2 with a standard deviation of $\sigma = 140$ dye molecules/um^2. We determined the detection limit of the portable device to be 3σ, approximately equivalent to 500 dye molecules/µm².

DISCUSSION & CONCLUSIONS

Despite the lower sensitivity, the performance of the portable reader is suitable for the detection of IgEs-mediated allergic reactions, considering the intensity of measured control serum samples of low and high levels (167 and 274 IU/mL total IgE concentrations). Overall, while the distinct differences in noise levels and background between the two devices underscore the trade-off between portability and precision, our portable reader represents a viable option for point-of-care applications, due to its practical sensitivity in allergen detection. Such fluorescence imager allows to use fluorescent multi-array analysis for allergens screening. The novelty relies in its compact design, enabling to perform measurements at any place. Compared to commercial readers such as the previously used InnoScan, our portable reader is 20x smaller and 40x less expensive.

REFERENCES

Huang, F., Jia, H., Zou, Y., Yao, Y. & Deng, Z. (2020). Exosomes: an important messenger in the asthma inflammatory microenvironment. J. Int. Med. Res. 48, 1–11

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