Quantification of digital microfluidic fluorescence assays with the Varioskan LUX Multimode Microplate Reader

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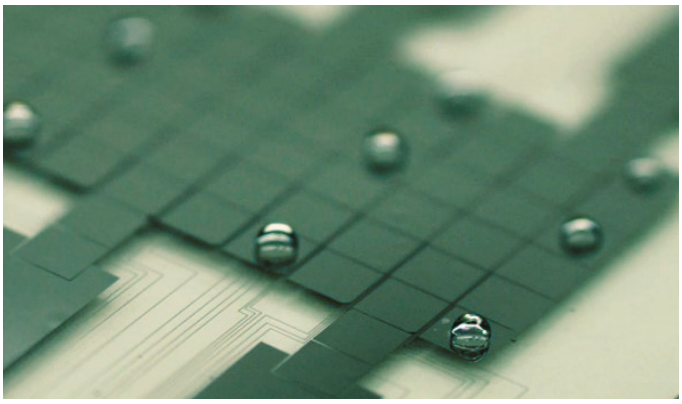
Goal

This application note demonstrates the feasibility of using the Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader for applications other than microplate-based assays—for instance, on-chip fluorescence-based quantification of digital microfluidic assays.

Introduction

Digital microfluidics (DMF) is a fluid manipulation technique based on electrowetting-on-dielectric principles [1]. In DMF, tiny droplets of sample in the range of hundreds of nanoliters to a few microliters are actuated on an array of electrodes coated with a dielectric polymer. By applying voltage to the electrodes in a controlled manner, the sample droplets can be split, merged, and mixed on the DMF device [2]. In contrast to in-channel microfluidic devices, DMF technology does not require auxiliary components (such as syringe pumps, tubing, or fluidic connectors) for sample introduction and manipulation, thus allowing better portability and straightforward implementation of parallel unit operations. DMF also allows lower reagent consumption and produces less waste compared with conventional well-plate assays, thus making possible more versatile sample manipulation prior to detection. An open-source DMF automation system, DropBot [3], was recently launched by Wheeler Microfluidics Laboratory, University of Toronto, which facilitates access to the technology by any user with minimal experience.



In recent years, DMF has been used as the platform for a range of biochemical and cell-based assays with integrated on-chip analysis via, for instance, electrochemical, nuclear magnetic resonance, and mass spectrometric detection [4]. The challenge with these detection methods is that they often demand a more customized analytical setup, including modifications to the DMF microchip design, such as manual addition of an external electrospray needle [5]. Optical microscopy (through the transparent top plate of the DMF chip) provides the most straightforward option for quantifying the products of DMF assays, but it suffers from low throughput by allowing monitoring of only a single spot at a time. The possibility of rapidly scanning the fluorescence of the full DMF plate using a standard microplate reader would increase the feasibility of DMF technology for high-throughput analyses without the need for customized chip designs.

Here we demonstrate the integration of a DMF chip with fluorescence intensity analysis using the Varioskan LUX Multimode Microplate Reader.

Materials

Instruments

- Varioskan LUX Multimode Microplate Reader (Thermo Fisher Scientific, Cat. No. VLBLATD2)
- DropBot automation system for sample actuation on the DMF device

Reagents and materials

- Resorufin, NADPH, and NaOH (Sigma-Aldrich)
- 7-ethoxyresorufin (Toronto Research Chemicals)
- Phosphate buffer—0.1 M potassium phosphate, 5 mM $MgCl_2$, pH 7.4 (Amresco, Solon, OH)
- $MgCl_2$ (Sigma-Aldrich, Steiheim, Germany)
- Custom-made DMF chip—with a transparent top plate and a bottom plate containing an array of 15 x 6 electrodes, a layer of SU-8 as a dielectric material, and a hydrophobic coating of 1% Cytonix FluoroPel™ PFC 1604V fluoropolymer
- Lid of a Thermo Scientific™ Nunc™ 96-microtiter well plate (Thermo Fisher Scientific, Cat. No. 163320)

Methods

An ethoxyresorufin-O-deethylase (EROD) assay [6] was chosen as the model for an on-chip enzymatic reaction [7] in this study. In this assay, the enzymatic deethylation of 7-ethoxyresorufin can be quantified by measuring the fluorescence emitted by resorufin (λ_{ex} = 570 nm, λ_{em} = 590 nm), the product of the deethylation reaction. The DMF chip with 50 x 75 mm (W x L) bottom plate and 25 x 75 mm (W x L) top plate was assembled on top of the well-plate lid as shown in Figure 1A. The sample droplets in the DMF chip were aligned along the wells using the embossed markings on the well-plate lid as a guide, and fixed with standard office tape. The stacked lid and chip were then flipped (upside down, clockwise) and rotated (180º, clockwise) to align the sample droplets with the holes of the well-plate tray (Figure 1B and 1C). Fluorescence signals originating from the sample droplets (Figure 1D) were measured with multipoint fluorescence analysis via the bottom optics and through the transparent DMF top plate. The fluorescence multipoint measurements were then averaged, using the "Multipoint Reduction" function of Thermo Scientific™ SkanIt™ software (Figure 1E).

To establish the calibration curve, resorufin samples with concentrations ranging from 0.01 to 5.0 µM were prepared in phosphate buffer containing the nonfluorescent substrate (2 µM 7-ethoxyresorufin) and 0.5 mM NADPH as the typical cofactor in enzyme assays [7]. The pH of the sample solution was adjusted with 2 M NaOH. The fluorescence intensity signal originating from the buffer samples (containing all components but resorufin) was recorded in the same manner and subtracted as the background.

Results and discussion

Compared with direct scanning of the DMF chip via top optics, the multipoint fluorescence analysis function using the bottom optics of the Varioskan LUX Multimode Multiplate Reader allowed more precise quantification of the fluorescence signal. As seen in Figure 1D, good linearity of signal (R^2 = 0.999) was reached even from relatively small droplets (area 4 x 4 mm^2, volume 3 µL). Fluorescence quantification using the top optics, with both 96- and 384-plate templates, resulted in much higher variability because the signal was averaged over a larger area without point-picking, and thus nonfluorescent areas were also included in the arithmetic average.

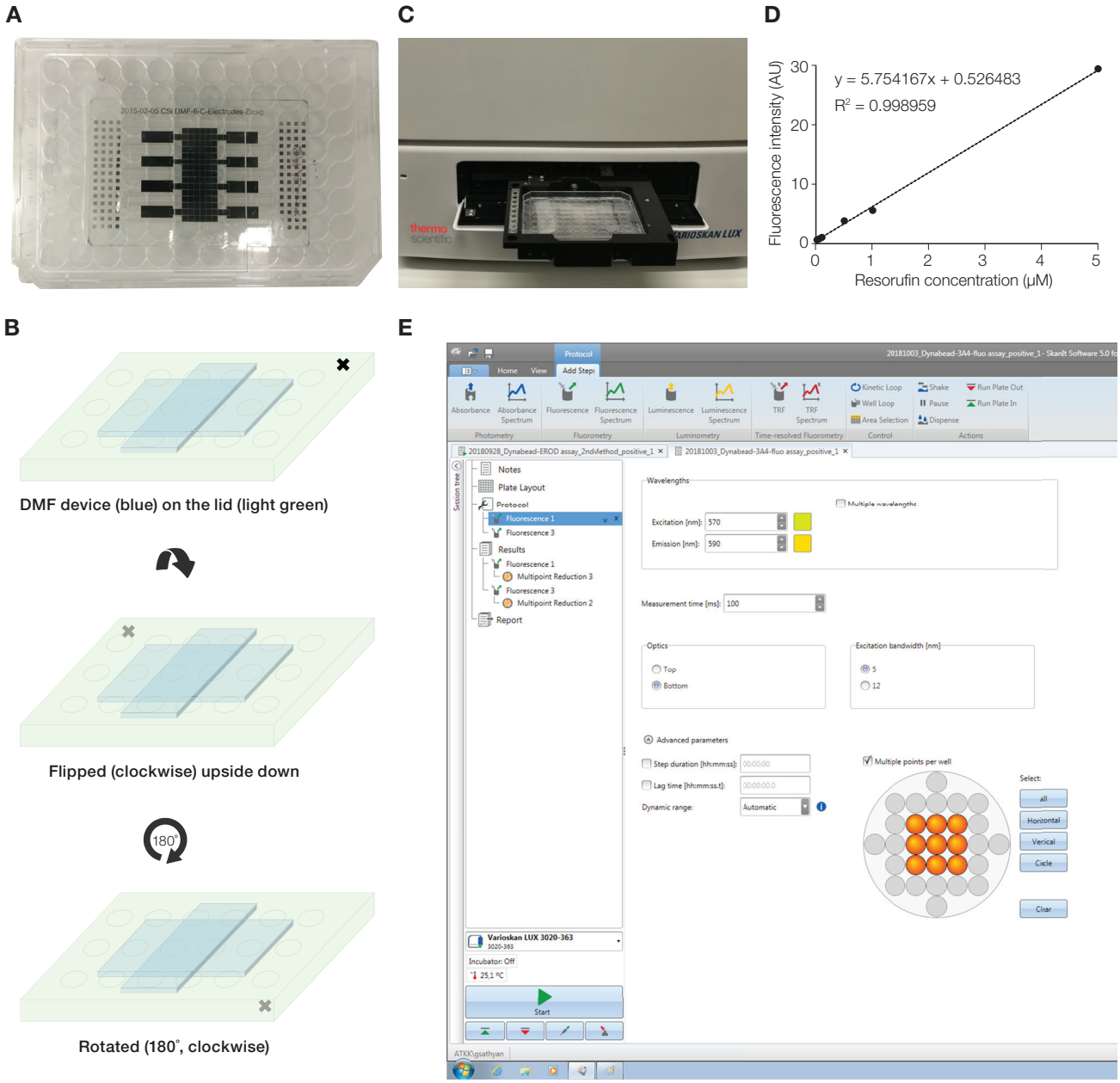


Figure 1. DMF chip assembly setup for EROD assay. (A) Assembly of DMF device on a well-plate lid. **(B)** Illustration of the flip and rotation steps followed by alignment of the DMF chip (areas of interest) with the wells of the reader tray. **(C)** DMF device on the reader tray. **(D)** Calibration curve of resorufin fluorescence measured with the Varioskan LUX microplate reader. **(E)** Settings used for 9-point bottom optics measurement with SkanIt Software.

Conclusions

Compared with optical microscopy, the Varioskan LUX microplate reader enables fluorescence measurements with a wide range of wavelengths at high speed, making the overall setup a powerful option for rapid bioanalysis. The possibility of defining a custom measurement area (here, 3 x 3 matrix) and averaging the results with a multipoint reduction step makes the fluorescence analysis of the microfluidic chips easier and faster compared to the single-point measurements performed with an optical microscope. The versatility of the Varioskan LUX microplate reader provides high flexibility in setting up assays for digital microfluidic technologies.

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References

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