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Portable Fluorescence Microscope applied to Organ-on-a-Chip Models

Sergio Moreno[1], *Anna Vilà*[1], *Javier Ramón-Azcón*[2], *J. Daniel-Prades*[1], *Angel Diéguez*[1]
[1] *Department of Electronic and Biomedical Engineering, University of Barcelona, Martí I Franquès, 1 08028, Barcelona, Spain*
[2] *Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Baldiri Reixac 10-12, 08028 Barcelona, Spain*

sergiomoreno@ub.edu

Summary:
Compact microscopy represents a revolutionary concept that allows for qualitative and quantitative improvements in the analysis of samples. Recently, a compact bright-field microscope based on raster scanning has been developed. This work presents an update to this compact microscope with fluorescence capabilities. This novel capability has been validated using a sample of an Organ-on-a-Chip (OOC) of a muscle stained with Alexa Fluor 488. The objective of this process is to detect and evaluate immunofluorescence, which is used to assess the maturity status of the biological model.

Keywords: Ultra-compact microscope, fluorescence, microdisplay, single photon avalanche diode, organ-on-a-chip

Introduction

Biotechnology has profoundly impacted our ability to observe and analyze biological samples. This transformation is attributed to the advancement of conventional microscopy techniques such as brightfield [1] or fluorescence [2]. However, the devices used with these techniques have a very bulky setup and a fixed location that limit their usefulness in certain applications. The integration of compact microscopy into smaller, more portable systems would facilitate its use in in-situ settings, such as laboratories or even at the point of care (POC). The first highly compact microscope based on nano-illumination was presented by Franch *et al.* in [3]. The microscope sensor was based on a 16 x 16 pixel SPADs camera fabricated in a 0.35 µm HV-CMOS process. The emitting part consisted of an 8 x 8 Gallium Nitride (GaN) LED array with a size of 5 µm and a pitch of 10 µm. Vilà *et al.* describes in [4] a prototype of a electronically activated scanning transmission microscope based on a microdisplay and a CMOS imaging camera. This device has the significant advantage that, as it is based on only two chips, it has a high level of compactness and portability. In addition, it has a field of view of several millimeters and a resolution of up to 2 µm.

This work presents a new version of the compact microscope with fluorescence capabilities. This improvement over the bright field prototype enables the microscope to be used for a wide variety of biological applications. In particular, the microscope employs a microdisplay for lens-free fluorescence imaging in organ-on-a-chip (OOC) applications.

Materials and Methods

The compact fluorescence microscope integrates a single-pixel detector and microdisplay. The image sensor is a single-photon avalanche detector (SPAD), which replaces the conventional CMOS camera. The SPAD has a diameter of 10 µm, a dark count rate (DCR) of less than 1 kcps, and a photodetection probability (PDP) of approximately 10 %. The remaining electronic components are responsible for classifying the arrival times of the photons in the form of a histogram. This configuration allows for measurements to be made over a range of approximately 70 ns to 470 ns with a resolution of up to 150 ps. On the other hand, the display chip is a 0.13-inch AmµLED™ microLED display with a resolution of 640 x 480. The diameter of the light-emitting diodes (LEDs) is 2 µm, and they are arranged with a pitch of 4 µm, resulting in a range of gray intensity levels that can be represented by four bits. The display is equipped with a digital interface that employs serial peripheral interface (SPI) connectivity. The monochrome panel offers a luminance of 0.5 million nits for blue (455 nm ± 15 nm).

Figure 1 illustrates a representation of the setup used for the fluorescence microscope. The setup comprises no optical elements beyond a band-pass filter between the sample and the sensor. The purpose of the filter is to eliminate the light from the display from being detected. However, the lack of a lens to concentrate the light implies that we require the activation of more than a single LED to cause the excitation of the fluorophores. Therefore, N x N LED patterns are employed to enhance the excitation signal level.

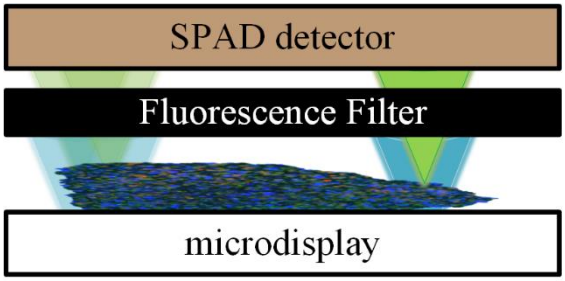

Figure 1. Representation of compact fluorescence microscope.

Results

A proof-of-concept experiment was conducted to validate the potential of the microscope for fluorescence visualization in OOC. The experiment was based on a 3D skeletal muscle on a chip. The OOC used by Fernandez-Costa et al. in [5] is employed in this study. This method involves the use of an alpha-actinin sarcomeric immunofluorescence stain to visualize sarcomeric structures within skeletal muscle tissues. This approach allows for the real-time study of the interaction between the muscle and the pancreatic islets. Figure 2 illustrates the image reconstructed with the microscope. The resulting fluorescence is represented in green and results from staining the sample with Alexa Fluor 488. The sample has been scanned in 2D using a 2 x 2 µLED illumination pattern. Additionally, a fluorescein isothiocyanate (FITC) emission filter with a central wavelength of 530 nm and a full width at half maximum (FWHM) of 43 nm is employed, thus eliminating the excitation light from the microdisplay.

The results demonstrate that the microscope can detect sarcomeric immunofluorescence emission in muscle tissue. Visualizing the presence of these sarcomeric structures allows for the evaluation of the functionality of muscle tissues on a chip and the confirmation of muscle tissue maturation.

Our results validate the microscope to be used for observing fluorescence in biological samples such as OOCs, which will benefit from improvements in microdisplay technology, including enhanced light power, reduced pitch, and switching capabilities. These advancements will enable higher image resolution and the possibility of implementing lifetime measurements.

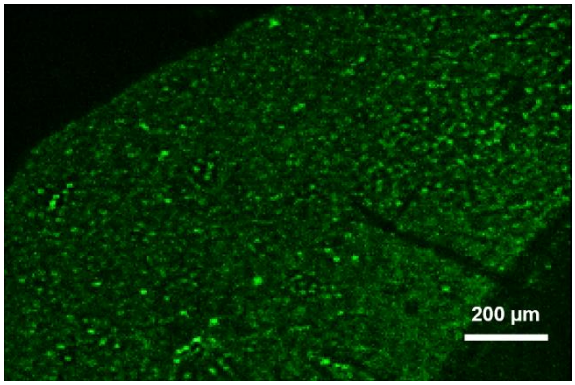

Figure 2. Captured fluorescence image of muscle tissue.

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