Photonic Lab on a Chip. Prospectives for Multiparametric Real-Time Cell Screening

Xavier Muñoz-Berbel
Centre Nacional de Microelectrònica (IMB-CNM, CSIC)
Campus UAB 08193 Bellaterra, Barcelona, SPAIN

Rosalía Rodriguez-Rodríguez
School of Pharmacy, Department of Pharmacology,
Universidad de Sevilla, Sevilla, SPAIN

Bergoi Ibarlucea
Centre Nacional de Microelectrònica (IMB-CNM, CSIC)
Campus UAB 08193 Bellaterra, Barcelona, SPAIN

Jordi Vila-Planas
Centre Nacional de Microelectrònica (IMB-CNM, CSIC)
Campus UAB 08193 Bellaterra, Barcelona, SPAIN

Stefanie Demming
Institut für Mikrotechnik, Technische Universität
Braunschweig, 38124 Braunschweig, GERMANY.

Stephanus Büttgenbach
Institut für Mikrotechnik, Technische Universität
Braunschweig, 38124 Braunschweig, GERMANY.

Andreu Llobera
Centre Nacional de Microelectrònica (IMB-CNM, CSIC)
Campus UAB 08193 Bellaterra, Barcelona, SPAIN
Andreu.llobera@imb-cnm.csic.es

***Abstract*—A Photonic Lab on a Chip (PhLoC) based on Poly(dimethylsiloxane) (PDMS) suitable to be used for multiparametric screening is presented. By recursive positioning of air mirrors at both sides of the system, light propagates via multiple internal reflection. In addition to its high level of integration, the system here presented only requires a single photolithographic step and the fabrication is based on low cost materials. Experimental results have shown that the proposed PhLoC is able to perform real time scattering or scattering+absorption measurements without any change in its configuration. Stained and non-stained monocytic cells were analyzing using a broadband light source. Limit of detection (LOD) of 54.9±0.7 cells (in scattering regime using unlabelled cells), 53±1 cells (in scattering+ absorption regime using labeled cells) were obtained. Finally, the system has also been used for measuring the dead/live cell ratio, obtaining a LOD of 7.6±0.4%.**

Keywords-component; Photonic lab on a chip, biophotonics, cell screening

I. INTRODUCTION

Photonics has emerged as a unique, extremely powerful technology for contactless real-time analysis in the life sciences and medicine. Using light as an interrogation mechanism in life sciences has major advantages, such as high-sensitive non-invasive analysis. Biophotonics is powerful and mature enough to provide the next generation of diagnosis and prognosis tools. In this context, technology mergers, such as the combination of microfluidics and photonics (obtaining the so-called photonic lab-on-a-chip), will be required to enable the real time measurement of relevant analytes in very small sample volumes. A first step towards spectroscopy on a chip was taken with the lens-less, ultra wide field cell monitoring array platform based on shadow imaging (LUCAS) [1]. Nevertheless, such platform has severe drawbacks for diagnosis, since it cannot perform multi-wavelength measurements (as could be the case of fluorescent labeling) and is not suitable for studies of cell growth or culture. Hence, the use of the LUCAS configuration is restricted to cell counting.

Nowadays, the first steps towards developing photonic lab-on-a-chip have been given. Such systems take advantage of the optical properties of the materials used to define micro-optical components inside or in the vicinity of the interrogation region [2,3]. When related to cell screening, however, it must be taken into account that a full understanding of the inherent process undoubtedly requires both an advanced architecture and protocol, since cells may undergo simultaneous events when affected by a disease. This issue is the current trend where most of the effort is currently being applied. In this context, the optimal configuration would a biophotonic system either able to work in different regimes depending on the cell culture under study or suitable to perform white light spectroscopy on a chip.

In this work, a photonic lab-on-a-chip able to work in two working regimes: LS (scattering), LS+ABS (scattering+ absorption), is presented. The acquisition protocol only requires a single measurement of 30 ms so as to record the spectral response of the cell culture placed at the interrogation region. If the cells are unlabelled or do not have absorption bands in the wavelength range under study, a scattering band (flat absorption band) is obtained. Conversely, when they are labeled, a superimposed absorbance band is observed. In both working regimes, the population can be determined. The applicability of the proposed system, it has been used for simultaneously determining the population of labeled and unlabeled cells, as well as the dead/live ratio.

II. Materials and Methods

Cell culture and labeling human monocytic cell line THP-1 (ECACC No.88081201) was maintained in RPMI 1640 medium (Gibco, 21875-034) with 20% fetal bovine serum (Gibco,10106-169) at 37 ºC in 5% CO2. Before each experiment, cells were rinsed twice in PBS tempered at 37ºC and kept alive in an incubator under controlled temperature and atmosphere. To obtain the dead cell population monocytes were fixed with 4% paraformaldehyde in PBS for 10 minutes. Afterwards, cells were stained with trypan blue (Gibco, 15250-061) in PBS (1:1) for 5 minutes and rinsed twice in PBS. For the dead/live ratio measurement, both populations were diluted to obtain the desired cell proportions.

The optofluidic system is presented in figure 1. It forms a complex system with a high degree of monolithic integration, since they comprise self-alignment systems for adequate positioning of fiber optics, lenses and focusing mirrors. Light propagates in the system following a zig-zag path with the help of integrated air mirrors [4]. In such conditions, the air mirrors meaningfully lengthen the optical path without increasing dramatically the overall size of the reactor. A broadband light source (Ocean Optics HL-2000, Dunedin, FL, USA) is coupled into the input multimode fiber optics with a diameter of 230 µm, which in turn is located in the self-alignment microchannels. At the end of this microchannel, a microlens corrects the numerical aperture of the fiber optics, having then parallel beams. Light enters into the interrogation region and interacts with the media that fills this region. The readout comprises an identical fiber optics, which carries the signal to a spectrometer (Ocean optics HR4000, Dunedin, Fl, USA) with a spectral resolution of 2 nm. The integration time has been fixed to 30 ms.

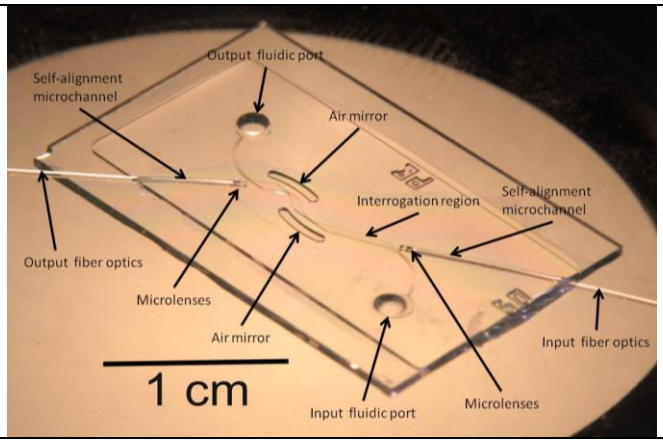

Figure 1: Picture of the PhLoC.

III. Characterization

When measuring unlabelled cells (LS regime), A plateau of maximal sensitivity is obtained at wavelengths between 580 and 700 nm, which simultaneously corresponds to an LOD minima, with values between 86 ± 1 and 54.9 ± 0.7 cells, respectively. Conversely, when using stained monocytes, the maximum sensitivity was obtained at the trypan blue absorption band (580-600 nm). At such range, a minimum LOD between 53±1 and 61±1 cells was obtained. Here, overall spectra is formed both by contributions of biomarkers and scattering.

Once the spectral response for both stained and non-stained monocytes has been achieved, the next step is the determination of the dead/live ratio.

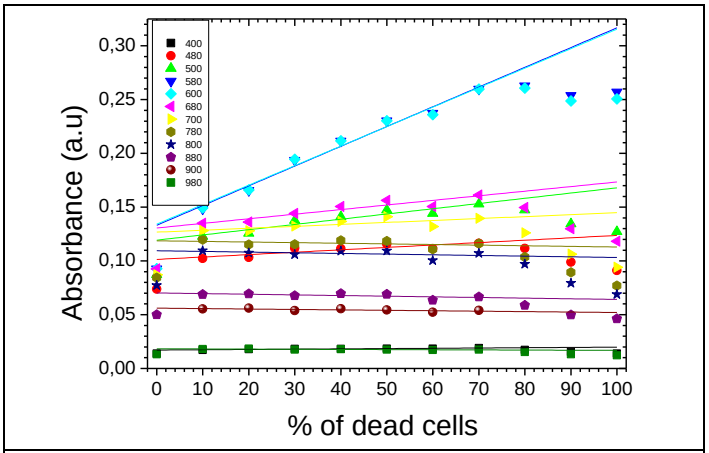

Figure 2: Absorbance vs % of dead cells using the PhLoC

To this effect, prepared samples ranging between 0% and 100% of labeled cells were measured. The spectra showed a transition from a flat scattering band (for 0% dead cells), to the conditions where the trypan absorption peak is very clearly visible (100% dead cells). The absorbance as function of % of dead cells is shown in Figure 2. The highest sensitivity corresponds to the trypan blue region (580-600 nm), with sharp decrease for both longer and shorter wavelengths. From these data, an experimental LOD of 7.6±0.4% is obtained. In other words, the PhLoC here presented has a performance comparable to the Neubauer cell counters, but only requiring 30 ms seconds for its measurements and with the possibility of multiparametric (by detecting different wavelengths) and continuous cell monitoring.

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