

PHOTONIC PROFILING TOWARDS MONITORING ENDOTHELIAL CELL DYSFUNCTION AT SINGLE CELL LEVEL

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ABSTRACT

We integrate microfluidic and label-free optical technologies with highly efficient cell-to-light coupling to measure the real-time response of individual cells in a population to specific stimuli. The innate optical response of the cell comprises of scattering, absorbance and auto-fluorescence signals as well as cell morphology. We demonstrate that the so-obtained ‘‘photon fingerprint’’ acts as a key reporter for the dysfunction of cells and may thus inform on a patient’s medical condition.

KEYWORDS: centrifugal microfluidics, label-free detection, living photonics, photonic fingerprint, single cell analysis.

INTRODUCTION

Biophotonics has by now emerged as a powerful tool for real-time analysis in the life sciences and medicine due to its high sensitivity, non-destructiveness, minimum or even non-invasive analysis and low limits of detection. It is therefore deemed to launch the next generation of diagnosis and prognosis tools. Without doubt, microfluidic and photonic technologies will have to be synergistically combined to leverage real-time measurement of relevant analytes in very small sample volumes.

In a conventional photonic approach, isolated peaks are studied and correlated to the concentration of a given analyte [1]. We here measure the innate, label-free spectral response termed ‘‘Photon Fingerprint’’ (PIN) [2] which includes inherent or acquired bands (scattering, absorbance and / or auto-fluorescence) of a cell culture or tissue. We show well distinguishable PINs for healthy and non-healthy cell cultures or tissue (Table 1).

Table 1. PIN Parameters of single-cell analysis

| Optical Parameter | Single Cell Parameter |
|---|---|
| Light backscattered (polarized) | Size, shape and RI of nucleus Size, shape, RI and number of mitochondria |
| Light scattered at low angle ($< 5^\circ$) (non-polarized) | Size, morphology, refractive index, viability |
| Light scattering at large angle ($> 130^\circ$) (non-polarized) | Membrane roughness, refractive index |
| Absorbance bands | Specific indicators |
| Auto-fluorescence bands | Specific proteins |

OPERATIONAL PRINCIPLES

The biochip design is based on cell capture within an array of geometrical traps as described in detail by Burger *et al.* [3]. A very high capture efficiency is achieved through a merely centrifugally driven sedimentation, i.e. with the liquid bulk at rest. The V-cup shaped capture elements (diameter: 13 μm) are arranged in a staggered array of 47×24 cups, thus providing the capacity to individually align 1128 cells. The centrifugal test stand utilised in this work are derived from the system initially reported by Grumann *et al.* [4].

EXPERIMENTAL

The base part of the microfluidic chip exhibiting the inlets and V-cup array has been fabricated in PDMS (Sylgard 184, Dow Corning GmbH, Germany). Moulds for PDMS panel featuring the V-cup array and the reservoirs are surface micro-machined using SU8-3025 (Microchem, USA). For replication, PDMS was mixed at a ratio of 10:1 (base to curing agent by weight), poured on the mould and degassed under a vacuum for 1 hour. The PDMS was then cured in an oven at 70 $^\circ\text{C}$ for 3 hours and then removed from the mould using a standard scalpel. 1-mm diameter access holes were then punched at the inlet ports. The PDMS is then cleaned using nitrogen. The middle Poly(methyl methacrylate) (PMMA) layer (chip support holder) is structured by a laser cutter (Epilog Zing Laser, Epilog, USA). A pressure-sensitive adhesive (PSA) attaches the middle layer to the base plate of the chip; it consists of a standard borosilicate microscope slide. The so-obtained chip is then treated by air plasma (1000 mTorr for 5 minutes) and bonded together with the PDMS base to form the biochip. The selected materials and fab-

rication methods for the biochip all satisfy the key criteria of transparency, biocompatibility, absence of leaks and long term stability.

RESULTS AND DISCUSSION

To support the formal process of obtaining ethical approval for access to patient samples, we demonstrate functionality on commercial human umbilical vein endothelial cells (HUVECs) in various health states in the meantime. Differential inflammation has been induced by exposing the cells to various concentrations of protein tumour necrosis factor alpha (TNF- α) during culture. Bright field imaging (BFI) records cell morphology which notably changes with the level of cell inflammation which, in turn, leads to an increased number of mitochondria (Fig. 1). Broadband light interrogation (BBLI) measures the scattering and absorbance bands (Fig. 2). It can be found that a unique photonic cell profile is obtained for each condition investigated. Light transmission is highest for healthy HUVECs and decreases with the inflammation-induced protein levels in the cells. Auto-fluorescence (AF) signals from each cell type have been examined in relevant wavelength bands (Table 2), confirming that each condition of a given cell type results in a distinctive photonic response. AF intensities grow with the concentrations of TNF- α in each of the three wavelength bands of interest (Fig. 3).

Table 2. Single Cell Analysis PIN Measurements

| Broadband Light interrogation / nm | AF Band 1 / nm | AF Band 2 / nm | AF Band 3 / nm |
|------------------------------------|--|--|--|
| $\lambda_{ex} = 360 - 800$ | $\lambda_{ex} = 403 \pm 32$ $\lambda_{em} = 465 \pm 20$ | $\lambda_{ex} = 492 \pm 15$ $\lambda_{em} = 530 \pm 20$ | $\lambda_{ex} = 572 \pm 15$ $\lambda_{em} = 630 \pm 20$ |

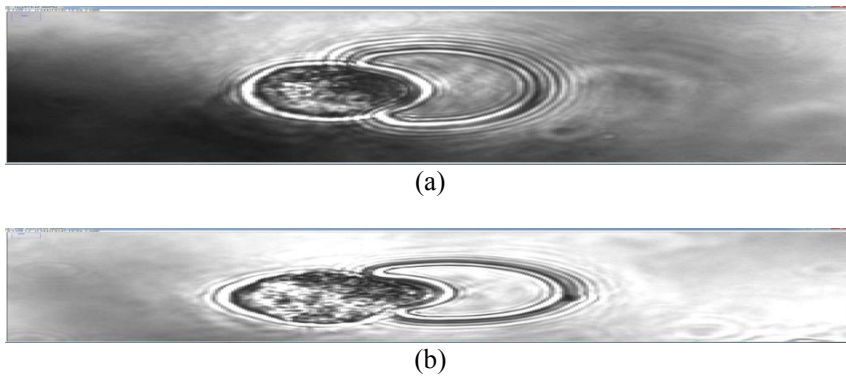


Figure 1: Cell morphology profiles for (a) healthy HUVEC (b) HUVEC treated with 100 ng ml^{-1} TNF- α for 24 hours. Treatments with 50 ng ml^{-1} TNF- α & 20 ng ml^{-1} TNF- α for 24 hours have also been performed. With increasing level of induced inflammation, increased changes are also observed in the size, shape and refractive index of the cell nucleus. An increased number of mitochondria are also observed, along with changes to the cell membrane roughness.

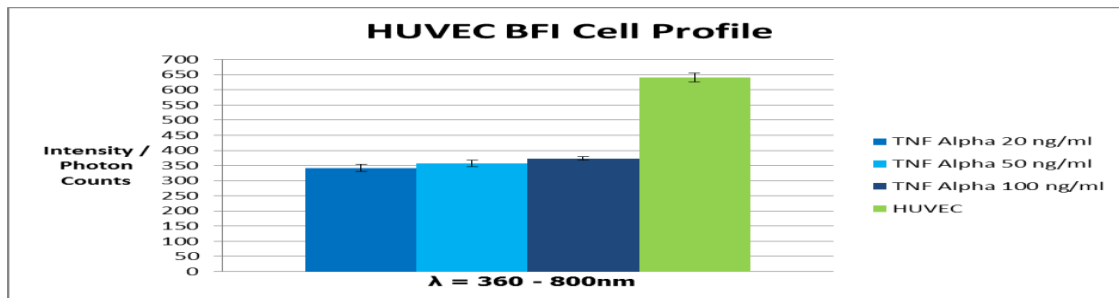
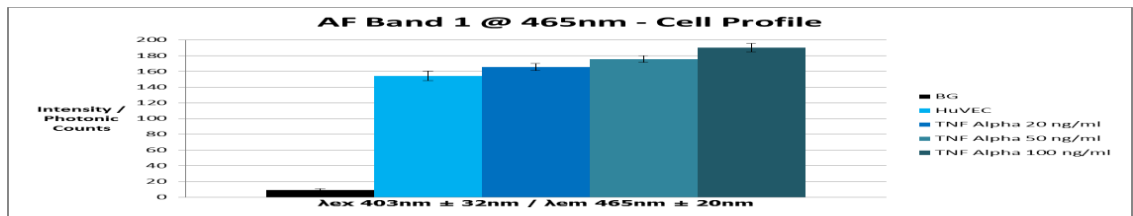
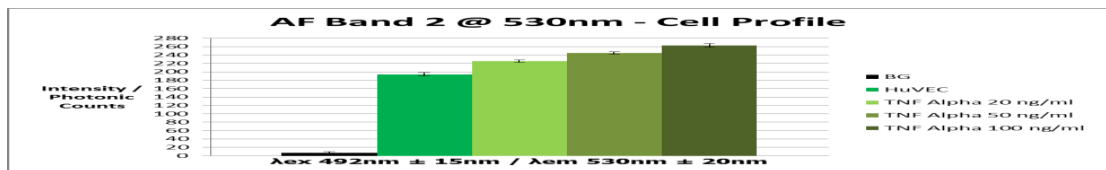


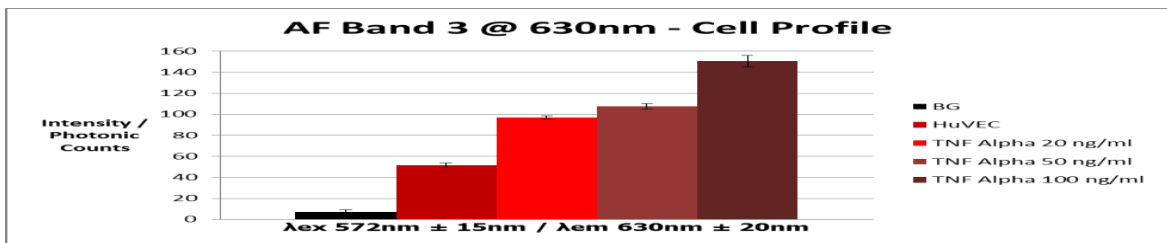
Figure 2: Broadband light interrogation (BBLI, between $\lambda_{ex} = 360 \text{ nm}$ and 800 nm) PIN for HUVECs which are healthy and have undergone exposure to TNF- α for 24 hours at various test concentrations.



(a)



(b)



(c)

Figure 3: Auto-fluorescence properties have been measured in the key wavelength bands (a-c). Each cell type condition provides a unique signal response. The dark segment on each plot is the contribution of the acquired background signal.

CONCLUSION

On our centrifugal microfluidic platform, we have established an experimental method for high-efficiency capture of cells over an array of V-cup shaped geometrical traps. Through the single-occupancy distribution imposed by the scale-matching with the capture elements, we were able to record “photonic fingerprints” (PINs) of individual cells comprising of scattering, absorbance and auto-fluorescence signals. These PINs were well correlated with increasing levels of cell dysfunction induced by exposure to inflammatory TNF- α . We now plan to advance this “lab-on-a-disc” platform towards a cell- and tissue-based device for patient diagnostics, e.g. in the field of cardiovascular disease.

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REFERENCES

- [1] Pantanelli S.M., Li Z., Fariss R., Mahesh S.P., Liu B. and Nussenblatt R.B., Differentiation of Malignant B-lymphoma Cells from Normal and Activated T-cell Populations by their Intrinsic Autofluorescence, *Cancer Research* **2009**, *69(11)*, 4911–4917.
- [2] <http://www.liphos.eu/> European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 317916.
- [3] Burger R., Reith P., Kijanka G., Akujobi V., Abgrall P. and Ducrée J., Array-based Capture, Distribution, Counting and Multiplexed Assaying of Beads on a Centrifugal Microfluidic Platform, *Lab on a Chip* **2012**, *12*, 1289–1295.
- [4] Grumann M., Brenner T., Beer C., Zengerle R. and Ducrée J., Visualization of Flow Patterning in High-speed Centrifugal Microfluidics, *Review of Scientific Instruments* **2005**, *76(2)*, 025101.

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