Human Umbilical Vein Endothelial Cells (HUVEC) Label Free Single Cell Photonic Fingerprinting

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Method Article

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Abstract

This protocol provides the required information to obtain a single cell analysis profile for HUVEC which have been exposed to differential levels of induced inflammation via lipopolysaccharide \(LPS) exposure.

Reagents

Reagent preparation protocol Differential inflammation has been implemented by exposing the cells to various concentrations of LPS during the cell culture process \(below). LPS was obtained from Sigma Aldrich Ireland \(L2654-1MG) and was diluted to a final stock concentration of 1,000 µg/mL. A further dilution of stock concentration to diluent at a ratio of 1:5 was then applied to give a working concentration of 200 µg/mL. The diluent used was distilled water \(DH2O) with 0.1% BSA, twice filtered with a 0.22 µm filter. Each concentration of LPS added to the cells under culture was incubated for a 24-hour period under the conditions described below. The fluidic volumes of LPS, along with their corresponding concentrations for a 20-mL cell culture flask are summarised in Table 1. See figure in Figures section. **Cell culture protocol** HUVEC cells were obtained from PromoCell GmbH, Germany \(C-12203 HUVEC-c pooled). Cells were cultured in PromoCell Endothelial Cell Growth Medium \(C22110), supplemented with Fetal Calf Serum \(0.02mL/mL), Endothelial Cell Growth Supplement \(0.004 mL/mL), Epidermal Growth Factor \(recombinant human) \(0.1ng/mL), Basic Fibroblast Growth Factor \(recombinant human) \(1ng/mL), Heparin \(90µg/mL), and Hydrocortisone \(1µg/mL). Cells were cultured in 75cm^2 flasks at 37°C and 5% CO_2. When harvesting or passaging the HUVEC cells, the PromoCell Detach Kit \(C41200) was used. Cells were isolated by centrifugation at 220 x g for 3 minutes. The cell pellet was finally resuspended in culture media.

Procedure

Biochip preparation Full details of the V-SCA-EC BDT have been provided in D3.2. 1. To assure complete and bubble-free filling, place the device in a vacuum prior to introducing the liquids for a minimum of 30 minutes. 2. To prime the biochip, load PBS \(phosphate buffer saline) buffer with 1% bovine serum albumin \(BSA) via the loading chamber on the top right section of the biochip. Once the chip has been primed, introduce the cells via the loading chamber on the top left section of the biochip. **Biochip Microfluidic Testing** 3. Once the chip has been primed, introduce the cells via the loading chamber on the top left section of the biochip. All pumping is performed using the previously described centrifugal test stand \(Table 2) and a 3D printed chip holder which allows three biochips to be tested in parallel \(Fig. 1). Since the sedimentation takes place with the liquid bulk at rest, i.e. in the absence of flow, the cell capture efficiency of the V-cup array system is significantly higher than that of typical flow-driven systems. Initial cell capture tests were performed using 20-µm polystyrene beads to emulate cell behaviour and then these tests were repeated using HUVEC and EA.hy926 cells. In all cases, a high V-cup occupancy rate \(> 95%) was observed \(Fig. 2). See figure in Figures section. Table 2: Centrifugal Test Stand Testing Protocol See figure in Figures section. Figure 1: LiPhos single cell viewgraph. \(a) Biochip schematic. \(b) Three biochips under centrifugal microfluidic testing. \(c) Single cell capture, analysis

and PIN readout principles. See figure in Figures section. Figure 2: V-cup array occupancy distribution for 20-µm polystyrene beads, HUVEC and EA.hy926 cells. Each test case shows an occupancy rate \(one bead / cell per cup) of >95%. An empty V-cup or V-cup containing more than one bead / cell is classified as an error. N = 10, p < 0.05.

Anticipated Results

Optical Measurement Protocol _System measurements_ The optical detection system utilised has been described in D3.2 and is summarised in Figure 3. The operational wavelengths utilised are summarised in Table 3. The camera settings for each measurement are summarised in Table 4. See figure in Figures section. Figure 3: \(a) Setup of the centrifugal test stand with integrated fluorescence detection and optical tweezers module. \(b) Detailed view of the optical module for fluorescent imaging and particle manipulation. \(c) Schematic of the optical system. \(c) Royal Society of Chemistry 2015 \[1] Burger R., Kurzbuch D., Gorkin R., Kijanka G., Glynn M., Mc Donagh C. and Ducrée J., An Integrated Centrifugo-opto-microfluidic Platform for Arraying, Analysis, Identification and Manipulation of Individual Cells, Lab On A Chip 2015, 15, 378-381. See figure in Figures section. Table 3: Operational wavelengths utilised for single cell analysis See figure in Figures section. Table 4: Excitation and detection parameters utilised for single cell analysis _Image Analysis_ Images were analysed by the software ImageJ \(version 1.46r). Cell signal intensity was quantitated by 'region of interest \(ROI)' analysis. Background signal levels are removed and an average intensity was obtained for each cell through the time series. _Data Presentation_ Bright field imaging \(BFI) records cell morphology which changes with the level of cell inflammation which, in turn, leads to an increased number of mitochondria \(Fig. 4). Whilst changes are present and detected, they are not as pronounced as the corresponding changes observed under TNF-α exposure. Broadband light interrogation \(BBLI) measures the scattering and absorbance bands \(Fig. 5) and it is observed that light transmission is lowest for healthy HUVEC and increases with the inflammation-induced protein levels in the cells. AF intensities grow with the concentrations of LPS in each of the three wavelength bands of interest \(Fig. 6) but the AF signal magnitudes are smaller than those observed under TNF-α inflammation conditions. See figure in Figures section. Figure 4: HUVEC which are \(a) healthy and treated with LPS for 24 hours at \(b) 5 ng/mL and \(c) 10 ng/mL. Cell morphology changes are present and detected for increasing levels of LPS exposure; the observed changes are not as pronounced as the changes observed under TNF-α exposure. See figure in Figures section. Figure 5: Single cell profiles for broadband light \(λex = 360 – 800nm) interrogation measuring inherent absorbance and scattering bands for HUVECs treated for various concentrations of LPS. Light transmission is lowest for healthy HUVECs and increases with the inflammation induced protein levels in the cells. \(For each cell test condition: n = 210 cells, p<0.05) See figure in Figures section. Figure 6: Single cell auto-fluorescence profiles for HUVEC treated for various concentrations of LPS. The presence and levels of induced inflammation are confirmed by an increase in auto-fluorescence on each of the wavelength bands examined. Each cell test condition produces a unique photonic signal response. \(n = 210 cells, p<0.05)

Acknowledgements

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Figures

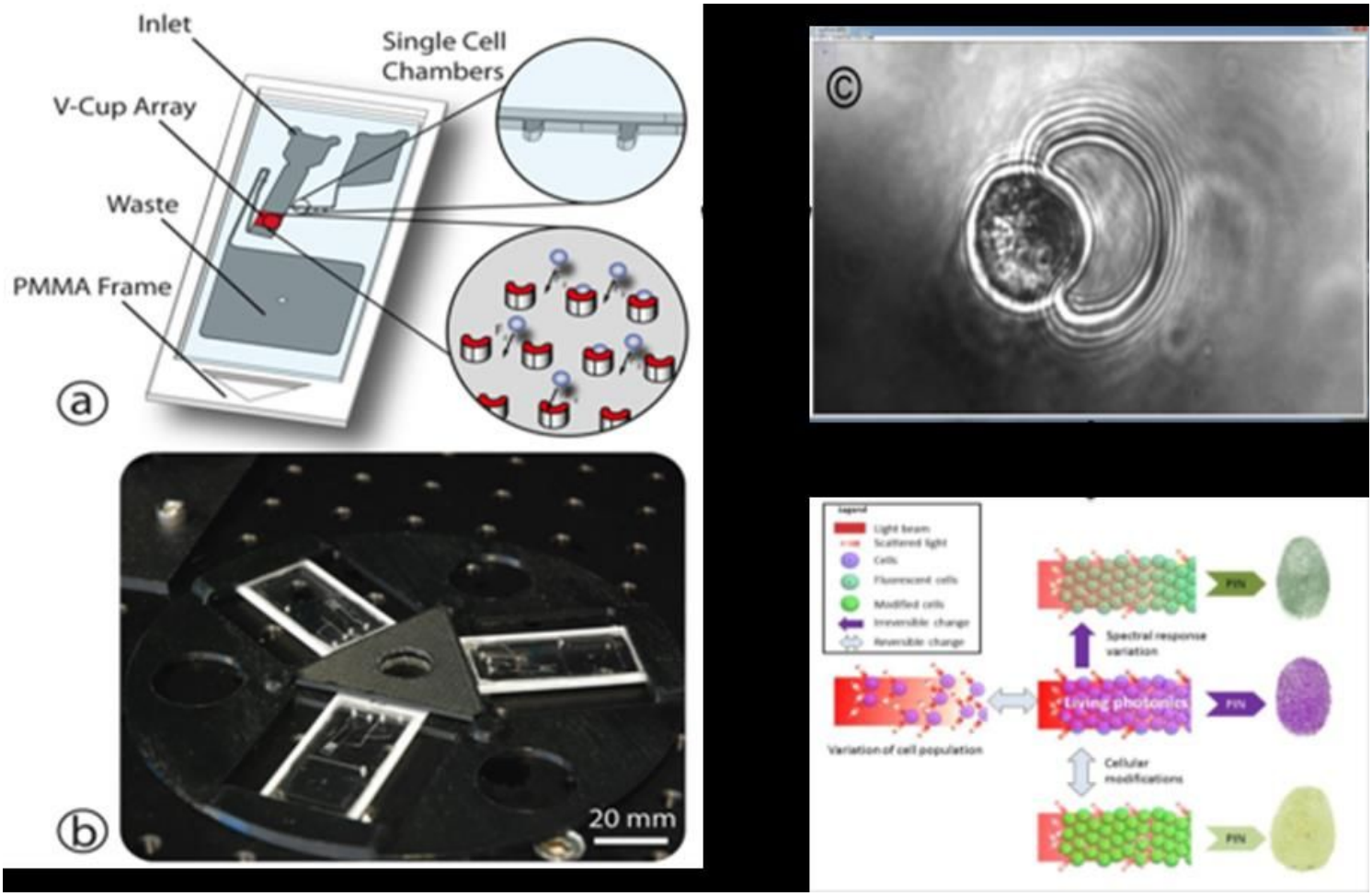

Figure 1

LiPhos single cell viewgraph

LPS	Stock Concentration (µg / mL)	Fluidic Volume (µL)
	1000 (diluted 1:5 to give a working concentration of 200)	250
	Test Concentration (ng / mL)	**Fluidic Volume (per standard 20 mL Cell Culture Flask) (µL)**
	100	10
	50	5
	20	2
	10	1

Figure 2

Table 1 Preparation volumes and concentrations utilised for LPS-induced inflammation

Microfluidic Step	Fluidic Volume / µL	Spin Rate / Hz	Duration / Minutes
Chip Priming	6	0	3
Cell Capture	8	10	3
		20	25
		10	3
		8	0.5
		6	0.5
		4	0.5
		1	0.5
		0	10

Figure 3

Table 2 Centrifugal Test Stand Testing Protocol

Wavelength Band	Excitation Wavelength / nm	Detection Wavelength / nm
Absorbance / Scattering	360 – 800	360 – 800
Auto fluorescence 1	403 ± 32	465 ± 20
Auto fluorescence 2	492 ± 15	530 ± 20
Auto fluorescence 3	572 ± 15	630 ± 20

Figure 4

Table 3 Operational wavelengths utilised for single cell analysis

Wavelength Band	Excitation Power / %	Camera Exposure Time / ms	Minimum Detection Intensity / Photon Counts	Maximum Detection Intensity / Photon Counts
Absorbance / Scattering	2.5	20	0	3000
Auto fluorescence 1-3	100	1000	0	1500

Figure 5

Table 4 Excitation and detection parameters utilised for single cell analysis

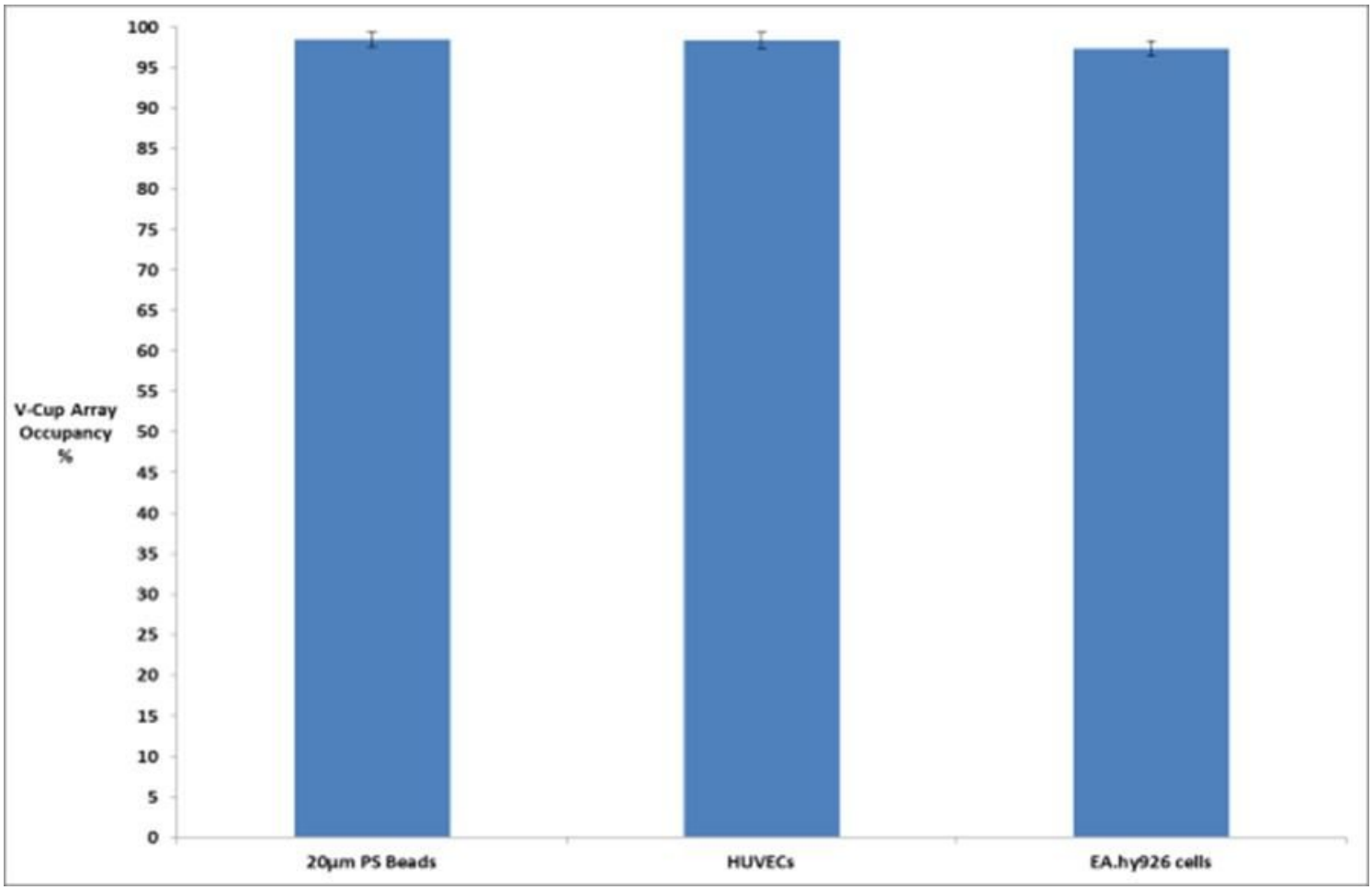

Figure 6

Figure 2 V-cup array occupancy distribution for 20-µm polystyrene beads, HUVEC and EA.hy926 cells

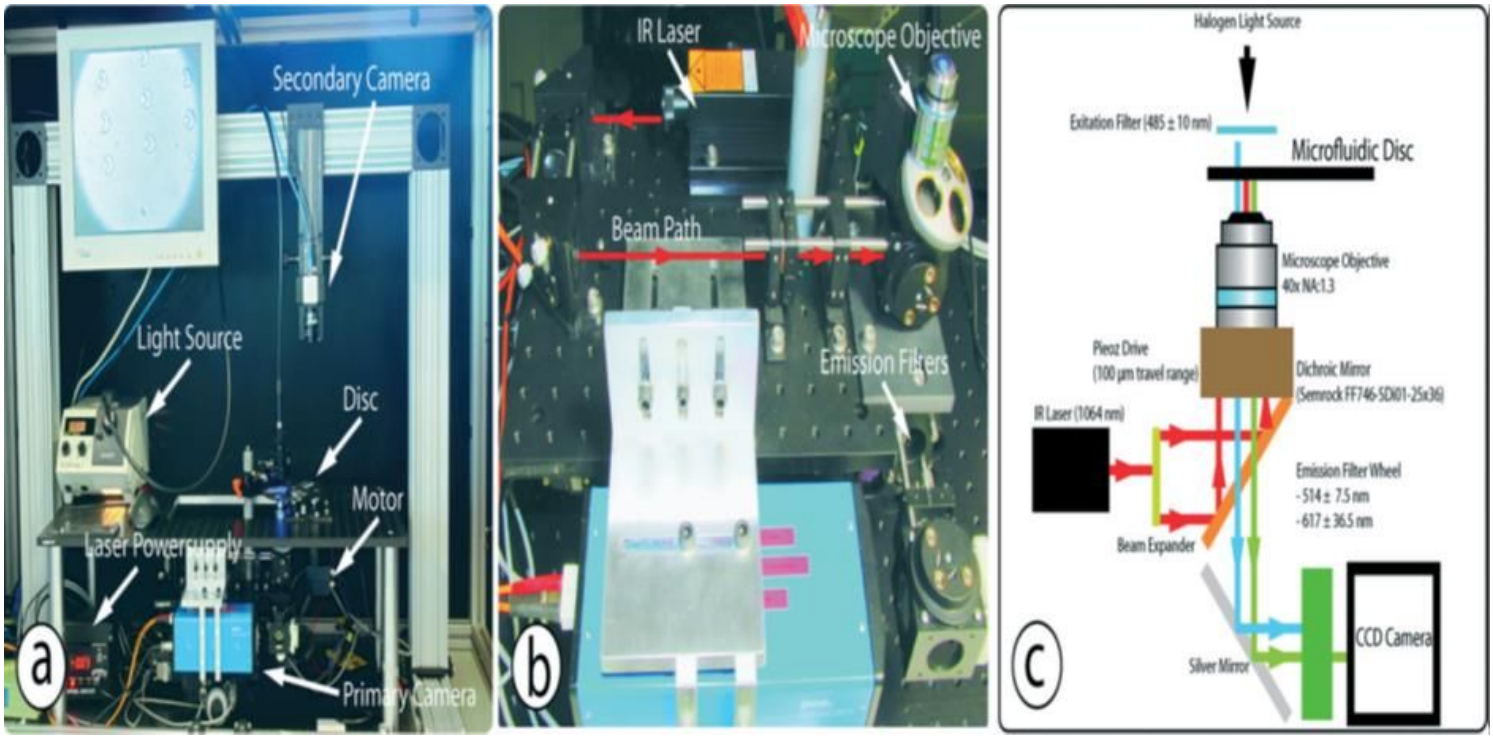

Figure 7

Figure 3 Setup of the centrifugal test stand with integrated fluorescence detection and optical tweezers module

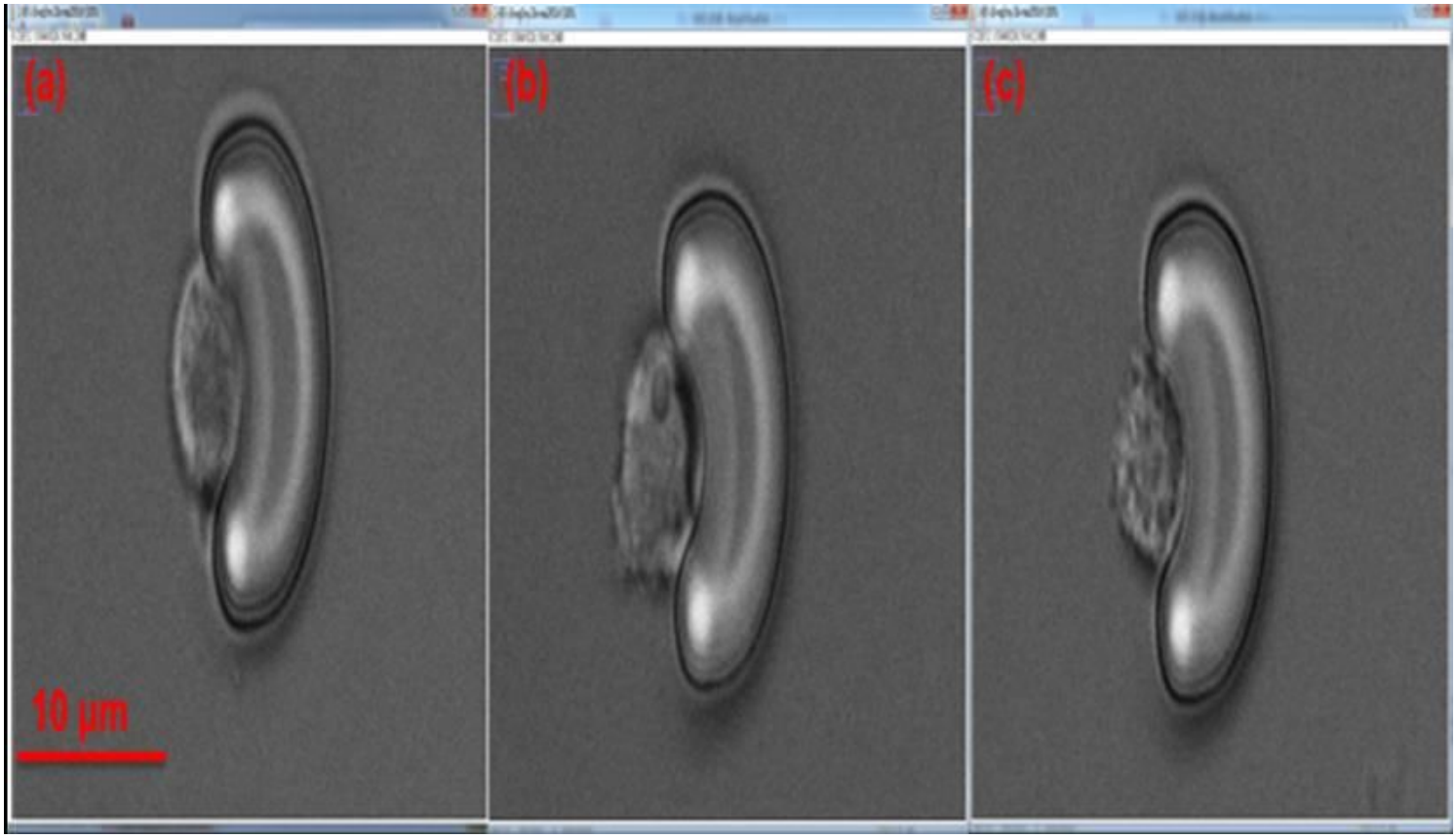

Figure 8

Figure 4 HUVEC which are (a) healthy and treated with LPS for 24 hours at (b) 5 ng/mL and (c) 10 ng/mL.

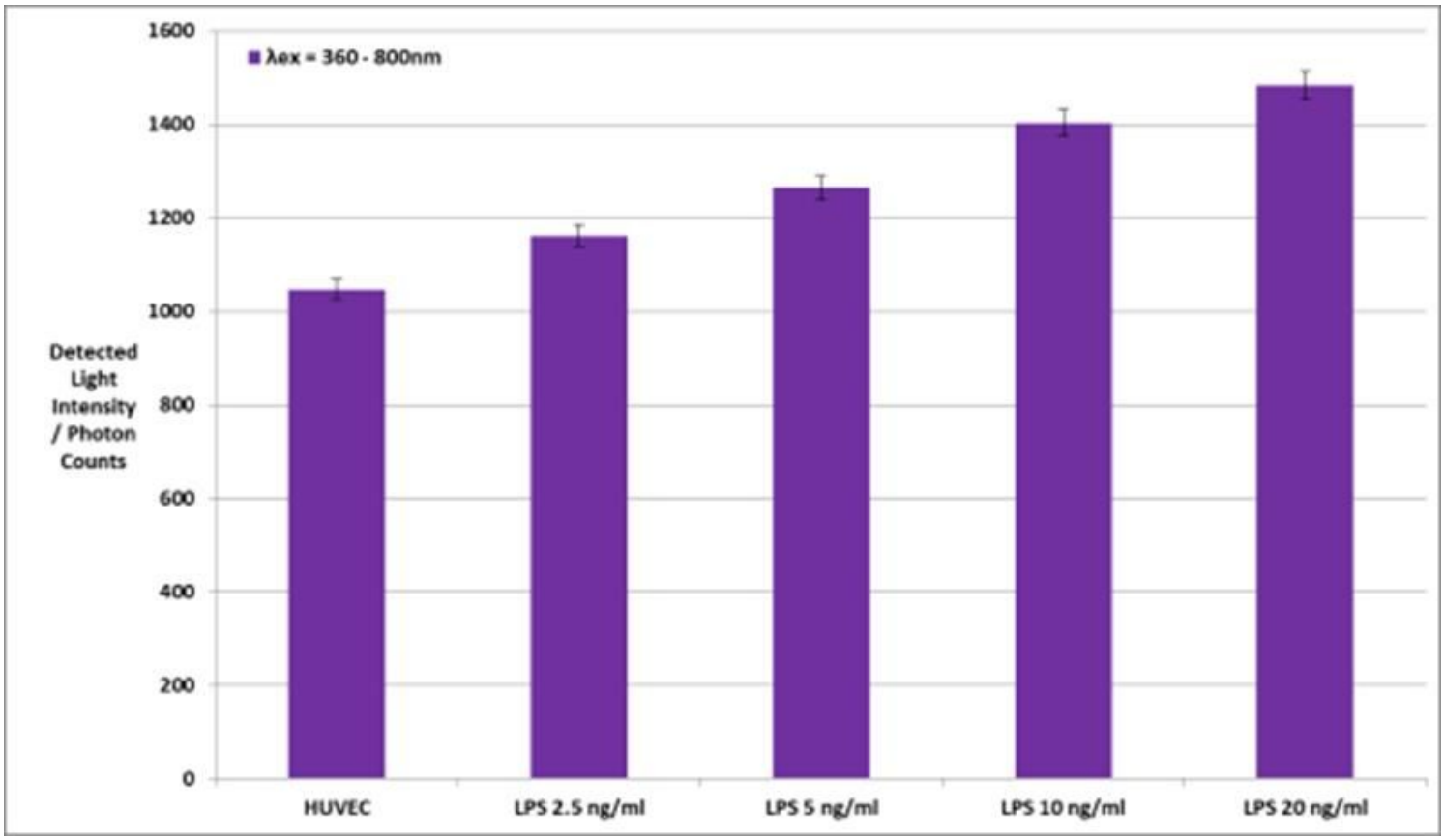

Figure 9

Figure 5 Single cell profiles for broadband light (λex = 360 – 800nm) interrogation measuring inherent absorbance and scattering bands for HUVECs treated for various concentrations of LPS

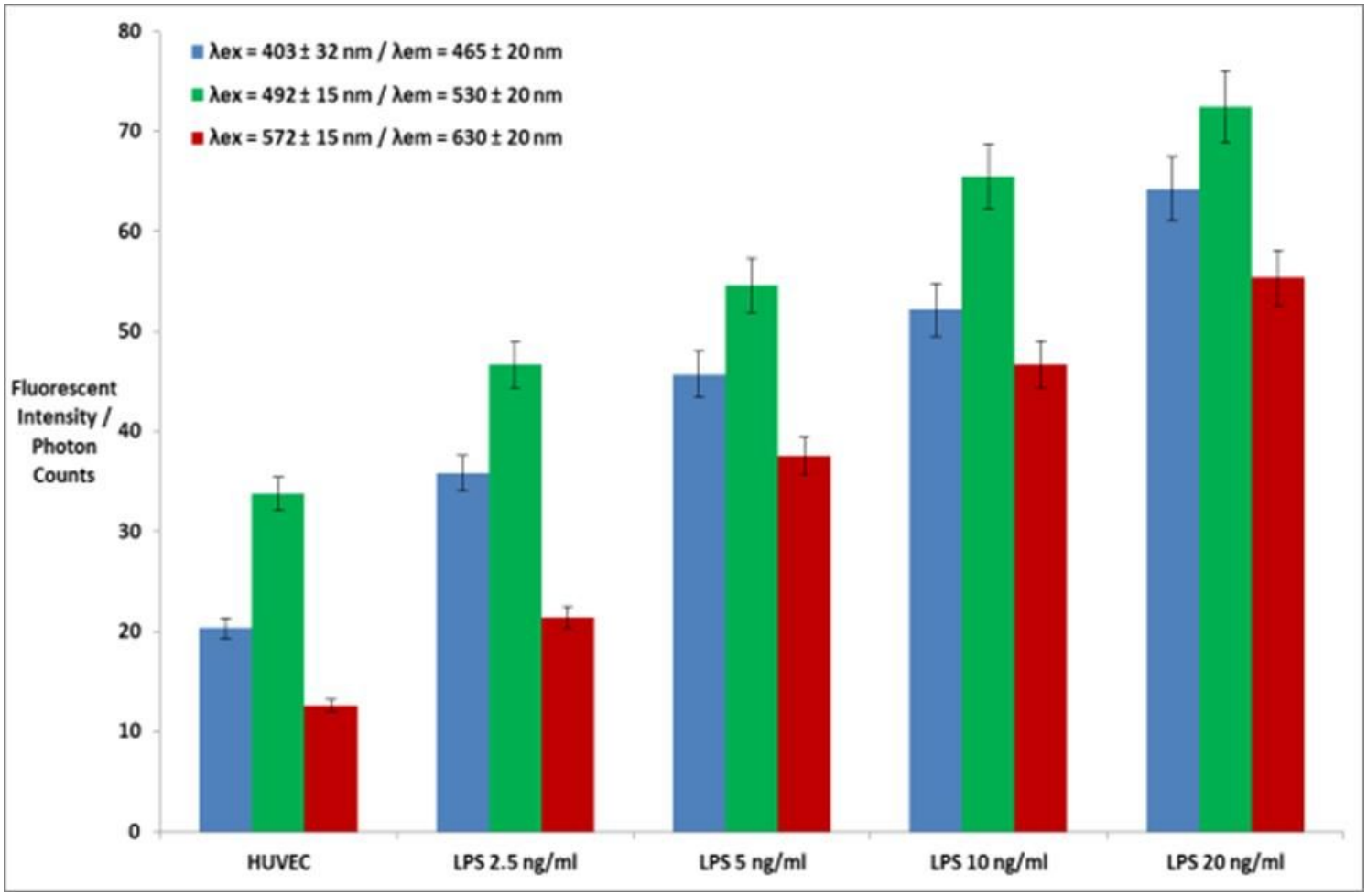

Figure 10

Figure 6 Single cell auto-fluorescence profiles for HUVEC treated for various concentrations of LPS