



**HAL**  
open science

## Improved on-chip impedimetric immuno-detection of subpopulations of cells toward single-cell resolution

Rémi Manczak, Marc Fouet, Rémi Courson, Paul-Louis Fabre, Armelle Montrose, Jan Sudor, Anne Marie Gué, Karine Reybier

### ► To cite this version:

Rémi Manczak, Marc Fouet, Rémi Courson, Paul-Louis Fabre, Armelle Montrose, et al.. Improved on-chip impedimetric immuno-detection of subpopulations of cells toward single-cell resolution. *Sensors and Actuators B: Chemical*, 2016, 230, pp.825-831. 10.1016/j.snb.2016.02.070 . hal-01305407

**HAL Id: hal-01305407**

**<https://hal.science/hal-01305407>**

Submitted on 22 Apr 2016

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15

**IMPROVED ON-CHIP IMPEDIMETRIC IMMUNO-DETECTION OF  
SUBPOPULATIONS OF CELLS TOWARD SINGLE-CELL RESOLUTION**

**Rémi MANCZAK<sup>a,b</sup>, Marc FOUET<sup>b</sup>, Rémi COURSON<sup>b</sup>, Paul-Louis FABRE<sup>a</sup>, Armelle  
MONTROSE<sup>a</sup>, Jan SUDOR<sup>a</sup>, Anne-Marie GUE<sup>b,§</sup>, Karine REYBIER<sup>a,§\*</sup>**

<sup>a</sup> Pharma-Dev, UMR 152, Université de Toulouse, IRD, UPS, France  
<sup>b</sup> LAAS-CNRS, 7 avenue du Colonel Roche, F-31077 Toulouse cedex 4, France

<sup>§</sup> These authors contributed equally to the work.

**\*Corresponding authors:** Faculté des Sciences Pharmaceutiques, Université Paul Sabatier  
Toulouse 3, 35 chemin des Maraîchers, 31069 Toulouse cedex 9, France  
tel: +33 562259804; fax: +33 562259802, karine.reybier-vuattoux@univ-tlse3.fr

1

2 **ABSTRACT**

3 Impedance spectroscopy has gained interest for the quantitative detection of specific cells mainly  
4 due to a label-free detection and their miniaturization capability required for integration on chip and  
5 development of point-of-care diagnostics. In this paper, we report the study of impedimetric  
6 microfluidic devices with improved sensitivity targeting the immuno-detection of cells. The sensitivity  
7 of our system was evaluated in terms of the capacity of the electrodes to trap monocytes by  
8 immune-reaction with CD14 antibody immobilized on micro-electrode surface. All measurements  
9 were performed in faradic mode using a redox probe. The sensitivity was evaluated as a function of  
10 the impedance increase  $\Delta Z$  recorded at 100 Hz caused by the insulating character of the cell trapped  
11 on electrodes. Analyses first confirmed that the sensing performances were significantly improved by  
12 using microfluidic. This increase could originate from an increase in the probability of cell trapping  
13 and a better organization of cells on the electrode due to the laminar flow. The great sensitivity was  
14 recorded with interdigitated electrodes for which the influence of the gap value was evaluated. The  
15 maximum sensitivity was reached with the smallest inter-electrodes gap tested (50  $\mu\text{m}$ ). This  
16 performance was in part attributed to the redox cycling taking place between neighboring fingers  
17 that was strongly affected when cells were trapped on the electrodes edges. Furthermore we also  
18 demonstrate that the slice of cell concentration for which the sensitivity is maximized is correlated to  
19 the area of electrodes. Moreover, the smallest area of interdigitated electrode (0.1 mm length)  
20 allowed the detection of as low as 5 cells per mL

21

22 **KEYWORDS:**

23 Impedance, interdigitated electrode, microfluidic, immune-detection, cell detection

24

## 1 1. INTRODUCTION

2 The quantitative detection of specific cells is usually carried out by flow cytometry due to its high  
3 sensitivity and reliability. Fluorescence-activated cell sorting (FACS) analysis which combines fluidic  
4 techniques and optics is the reference method used to count cells via fluorescently labeled antibody  
5 conjugates. However, this technique is time-consuming, expensive and not suited to routine  
6 screening and point-of-care diagnostics. The development of total analysis solutions in microfluidic or  
7 lab on-chip devices for integrated cell-based detection increased considerably over last ten years [1].  
8 Miniaturized cell detection devices have several advantages such as rapid detection, low cost, use of  
9 small volumes of biological materials, portability and improved sterile conditions. The most widely  
10 used techniques for cell counting are optical techniques [2,3] (fluorescence, surface plasmon  
11 resonance, interferometric detection) or electrical methods [4,5]. Electrochemical methods have  
12 gained interest mainly due to a label-free detection and their miniaturization capability required for  
13 integration on a chip, but also for their low cost and short analyses times. Among electrochemical  
14 methods, Impedance is a powerful electrochemical method that uses periodic small AC amplitudes  
15 and responds to modifications caused by the target cells (coulter counter for single cell analysis) [6-  
16 8]. Electrochemical impedance spectroscopy (EIS) is the method of choice in the case of cells trapped  
17 on functionalized electrodes surface. In this latter case, the change in the electrode impedance is  
18 measured and can be correlated to the amount of trapped cells [9]. Bio-impedance monitoring has  
19 already been employed for example to study cellular kinetics [10], cancer drug screening [11], or  
20 parasitized cells [12-14].

21  
22 Electrochemical measurements rely on the use of at least 2 electrodes. Currently, combination with  
23 microfabrication-based technologies allows fabrication of micron-sized electrodes. Microelectrodes  
24 have many advantages over conventional electrodes among which economy due to batch fabrication  
25 [15], large current densities (low currents) due to enhanced mass transport [16], very short time  
26 constants, low ohmic drop and possibility of integration and the development of portable systems  
27 [17]. Microelectrodes, due to smaller currents, have higher impedances [18] and allow the study of  
28 high resistivity samples [19]. Furthermore, the spatial resolution of microelectrodes offers the ability  
29 to explore several cells [20] or even a single cell [21]. The measured impedance response is  
30 consequently influenced by the geometry of the electrodes and optimization of electrode geometry  
31 is a key factor in cell analysis as demonstrated by the number of paper dealing with this subject  
32 [18,22,23]. Furthermore the sensing performance can be significantly improved by using  
33 microfluidics to provide effective liquid manipulation and greater flexibility.

34 In this paper, we report the study of impedimetric microfluidic devices with improved sensitivity  
35 targeting the immuno-detection of cells. The sensitivity of the system was evaluated as the capacity  
36 of the electrodes to trap monocytes by immune-reaction with CD14 antibody immobilized on the  
37 electrode surface [13] and to produce a significant impedance change. Several designs including  
38 simple or interdigitated electrodes were tested and also compared to microfluidic measurements.

39

## 40 2. MATERIALS AND METHODS

### 41 2.1. Materials

#### 42 2.1.1. Products

1 6-mercapto-1-hexanol (MH), 11-mercaptopundecanoic acid (MUA), N-ethyl-N-(dimethylaminopropyl)-  
2 carbodiimide (EDC), N-hydroxysuccinimide (NHS), phosphate buffered saline (PBS) solution,  
3 recombinant protein G (PG), bovine serum albumin (BSA), lipopolysaccharide (LPS), hydrogen  
4 peroxide and sulfuric acid were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). The  
5 CD 14 and CD 16 antibodies were purchased from Miltenyi Biotec (Paris, France). Absolute ethanol  
6 was purchased from Fisher Scientific (Illkirch, France) and deionized water was obtained using the  
7 Milli-Q water system Millipore (Molsheim, France). DF-1050 films (Engineered Materials Systems,  
8 Inc.), SU8 3050 resin (Microchem), 4" glass substrates (Schott AF32), Polydimethylsiloxane (PDMS)  
9 (Dow Corning), hexamethyldisiloxane (HMDS) (Sigma Aldrich), and AZ Nlof 2035 (AZ Electronic  
10 Materials) were used for the microfluidic device.

### 11 2.1.2. *Monocyte cell culture*

12 Monocytes (THP-1) were cultivated in Roswell Park Memorial Institute 1640 medium (RPMI 1640;  
13 Sigma-Aldrich) supplemented with sodium bicarbonate (1.5 g/L; Lonza (Amboise, France)), d-(+)-  
14 glucose (4.5 g/L; Sigma-Aldrich), L-glutamine (2 mM; Lonza), sodium pyruvate (1mM; Sigma-Aldrich),  
15 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (10 mM; Sigma-Aldrich), 2-mercaptoethanol  
16 (0.05 mM; Sigma-Aldrich) and 10% foetal bovine serum (Lonza). The cells were maintained at 37 °C in  
17 a 5% CO<sub>2</sub> atmosphere.

18 The cell suspensions were prepared par dilution successives et comptage sur??

## 19 2.2. Methods

### 20 2.2.1. *Devices manufacturing process*

21 Devices were manufactured on glass substrates combining standard microfabrication processes  
22 (electrodes) and thick photoresists technologies (microfluidics).

#### 23 ***Microelectrodes fabrication (Figure 1a)***

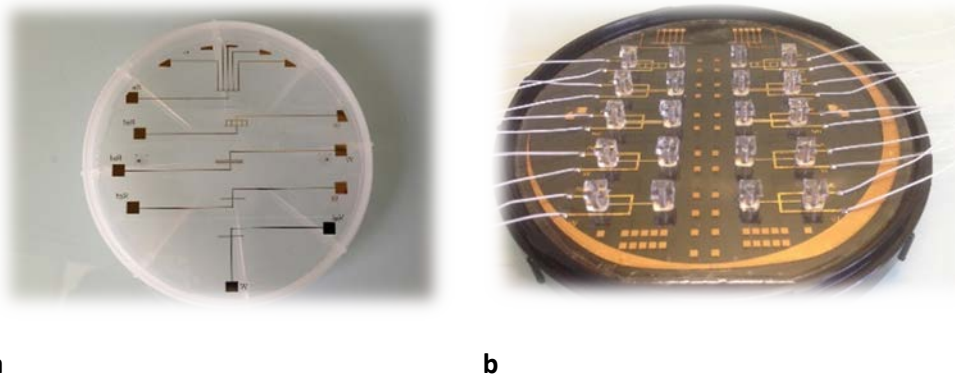
24 The glass substrates were first cleaned with a piranha solution to remove any organic residues. After  
25 rinsing with deionized water, drying under a nitrogen flow, and exposure to an O<sub>2</sub> plasma (Tepla  
26 600), the substrate was treated with hexamethyldisiloxane (HMDS) to improve adhesion of  
27 photolithographic resists. The structuration of gold electrodes was processed following a classical  
28 "lift off" technique. An AZ Nlof 2035 resist was spin-coated at "vitesse" and exposed to UV (635nm)  
29 during "durée" with a "masqueur utilise". Then a titanium-gold layer respectively 50nm and 200 nm  
30 thick was obtained by evaporation in a "marque machine" reactor.i. After the photoresist revelation  
31 in acetone, the patterned gold layer was then covered with a 5 µm thick SU-8 photoresist  
32 (Microchem) . SU-8 was spin coated at "vitesse" during "durée" and prebaked (PEB) at  
33 "temperature" during "durée". The SU-8 was patterned using standard photolithography in order to  
34 cover electric tracks and to protect electrodes periphery. This step allows to control precisely the  
35 electrodes geometry and dimensions and to avoid unexpected electrochemical effects at the Ti/Au  
36 interface. The last residues of unexposed resins were removed by O<sub>2</sub> plasma.

#### 37 ***Microfluidic integration (Figure 1b)***

38 Microfluidic channels were patterned by standard lithography of a 100 µm thick SU8 layer deposited  
39 on top of the electrodes wafer. As described extensively in Abgrall et al (ref) open channels were

1 then covered by lamination of a 25  $\mu\text{m}$  thick “home-made” dry film of SU-8 and a final  
2 photolithographic step allowed the realization of microfluidic inlets and outlets. To reduce  
3 phenomenon of cracks and delamination of the layer originating from residual stress in the SU-8  
4 photoresist, a 120°C hard bake was then applied to the system.

5 Fluidic connections were obtained either with the help of PDMS pads glued at each inlet/outlet of  
6 the channels or with a mechanical support especially designed to handle the fluid and power supplies  
7 of each chip. PDMS pads were obtained with Polydimethylsiloxane (PDMS) mixed with curing agent  
8 at proportion of 10/1, degassed with nitrogen for 1 h, then coated on a clean silicon master, and  
9 finally, annealed at 90 °C in an oven during 1 h. After 15 min exposure to an O<sub>2</sub> plasma, the pads  
10 were stuck onto the wafer using a UV-sensitive glue.



11 **Figure 1: pictures of the devices built for the study** a) without and b) with microfluidic channels and  
12 PDMS pads allowing circulation of fluids.

13 To reduce fabrication time and cost the SU-8 resist was also replaced by a novel epoxy dry film DF-  
14 1050 (EMS) following the process described by Courson *et al.* [24]. Note that in this latter case the  
15 mechanical support was used exclusively.

16 Finally, the wafer was diced using a diamond pen to individualize each microelectrode system.

### 17 2.2.2. Electrode modification

18 The cleaned electrodes were modified as described by Montrose *et al.* [13]. In absence of channels  
19 the surfaces were modified through successive deposition of drops of the modifying agents for  
20 various incubation times: a mixture of 11-mercaptoundecanoic acid (MUA) 1 mM and 6-  
21 mercaptohexanol (MH) 10 mM for 18h/ a mixture of aqueous solutions of N-hydroxysuccinimide  
22 (NHS) 15 mM and 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) 75 mM for 30 min / protein  
23 G 100 g.mL<sup>-1</sup> in PBS 10 mM for 18h / BSA 143 g/L for 30 min and CD14 antibody X mM for 18h. In the  
24 case of microfluidic devices the modification of electrodes were carried out under a flow of the  
25 modifying agents using a Fluigent® MFCS-8C with an inlet pressure of 10 mbar and no outlet pressure  
26 for different times : SAMs: 3 hours / NHS EDC: 30 min / PG: 3h / BSA: 30 min / Ab: 2h. This procedure  
27 considerably reduced the time of the surface bio-functionalization to about 10 hours.

28

### 29 2.2.3. Impedimetric measurements

30 The cell trapping were carried out, either by placing a drop of cells suspension during 2 hours on  
31 functionalized electrodes in the case of non-microfluidic devices or by applying a continuous flow of

1 cells suspensions for 30 min in channels (pressure drop of 10 mbar) before rinsing and impedance  
2 measurements.

3 EIS measurements were performed using an Autolab PGSTAT 128n (Metrohm) and the NOVA  
4 software. Experiments were performed in PBS 10 mM (pH = 7.4) containing 5 mM Fe<sup>III</sup>/Fe<sup>II</sup>  
5 (Fe(CN)<sub>6</sub><sup>3-/4-</sup> (1:1)). The impedance spectra were recorded from 100 mHz to 50 kHz at the free  
6 potential with an alternating voltage of 10 mV. All experiments were performed at room  
7 temperature in a Faraday cage.

8 In the case of open chips, the electrochemical measurements were performed in a drop of  
9 electrolyte containing the redox probe using a SCE as reference immersed in the drop (scheme 1b).  
10 In the case of microfluidic chips, the measurements were performed under a flow of electrolyte,  
11 using a two electrode set-up, the reference and counter electrode being short-cut.

### 12 **3. RESULTS AND DISCUSSION**

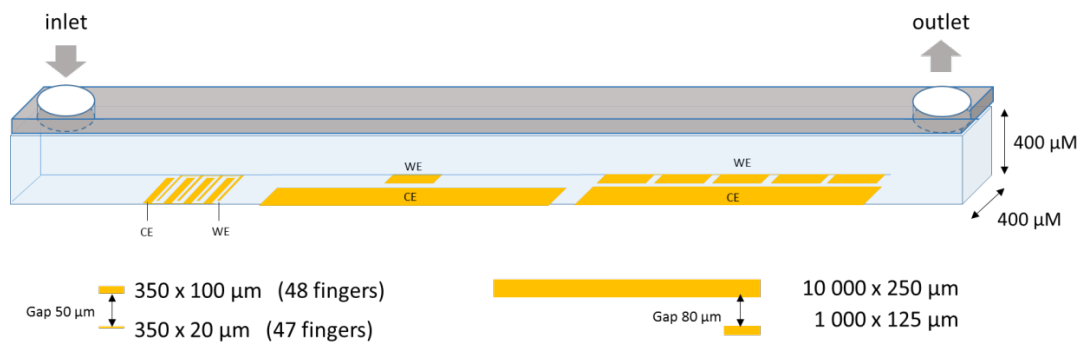
13 Previous experiments performed on macro-electrodes with different areas (1, 0.5, 0.04 cm<sup>2</sup>) have  
14 demonstrated a strong increase in sensitivity when decreasing electrodes areas (90% of  
15 improvement in  $\Delta Z$  for 0.04 cm<sup>2</sup> area compare to 1 cm<sup>2</sup>) without any increase in the noise [13]. This  
16 is in agreement with inverse proportionality between charge transfer resistance and the electrode  
17 area. For this reason we decided to miniaturize working and counter electrodes using classical  
18 technologies of microfabrication such as photolithography associated with lift off. Three geometries of  
19 micro-electrode arrays were tested: simple, multiple and interdigitated electrodes as presented in  
20 scheme 1a.

#### 21 **3.1. Static measurements**

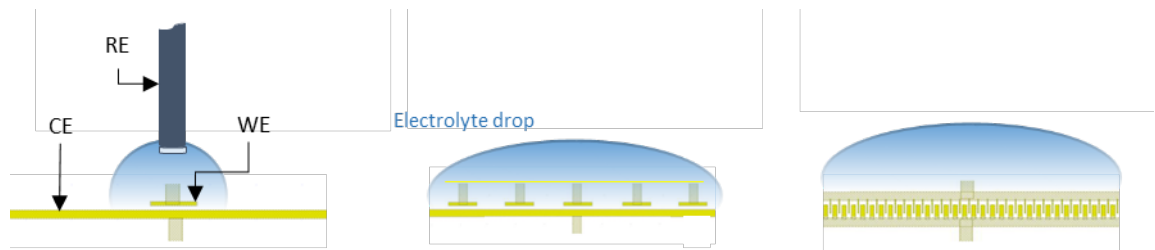
22 In order to qualify micro-electrodes behavior, static measurements were first performed. In this case,  
23 both biofunctionalization of electrodes and impedimetric measurements were carried out using  
24 drops as illustrated in Scheme 1b. The functionalization of electrodes with specific antibodies CD14  
25 was carried out using self-assembled monolayers associated with protein G as previously described in  
26 the literature [13]. Each modification step was characterized using electrochemical impedimetric  
27 spectroscopy (data not shown). It was verified by applying the modification procedure to SU8 surface  
28 that none of the reagents reacts with the resins surface. To study the sensitivity of the  
29 immunosensor, monocyte concentrations ranging from 10<sup>3</sup> to 10<sup>9</sup> cells/mL were first deposited on  
30 each microelectrode of various geometry previously functionalized with CD14 antibody. The  
31 corresponding Nyquist diagrams are presented in Figure 2a-c. The semi-circle diameter increases  
32 with the cell concentration due to the insulating character of cells which cover the electrodes and  
33 block efficiently the electron transfer towards the redox probe. The maximum variations of  
34 impedance were recorded at low frequency at 100 Hz (data not shown) demonstrating that the  
35 diffusion of the redox probe was also modified by the presence of cells [25]. The variations of  
36 impedance relative to the antibody layer ( $\Delta Z_{/AB} = Z - Z_{AB}$ ) measured at 100 Hz, as a function of the  
37 logarithm of cells concentrations are presented in Figure 2d. One can consider that the impedance  
38 measured is the sum of the signal corresponding to the free and graft parts of the surface, which  
39 means that the impedance is related to the surface coverage ratio. A free surface presents the lowest  
40 impedance change  $\Delta Z_{/AB}=0$  while a totally covered surface would present the highest one,  $\Delta Z_{/AB}$  max.  
41 The best sensitivity was recorded with simple electrodes in particular in the range of 10<sup>3</sup> and 10<sup>5</sup>

1 cells/mL with a sensitivity  $\Delta Z_{/AB} = 800 \Omega/\text{decade}$ . The sensitivity was decreasing when the electrode  
 2 surface increases to reach  $\Delta Z_{/AB} = 200 \Omega/\text{decade}$  for multiple and interdigitated electrodes for the  
 3 same range. The graph clearly demonstrates different profiles of sensitivity depending on the  
 4 geometry; the impedance for electrodes having the smallest surface areas seems to saturate as when  
 5 totally covered, whereas for electrodes with large areas, impedances increase exponentially with  
 6 increasing concentrations of cells. The difference between simple and multiple electrodes can be  
 7 easily explained by considering that the multiple electrodes corresponds to electrodes in parallel and  
 8 in this case their impedance is given by :  $1/Z = \Sigma (1/Z_i)$ . The impedance of multiple electrodes would  
 9 be equal to  $Z_{\text{simple}}/5$ . Furthermore one can note that impedances recorded with interdigitated  
 10 electrodes were weaker than impedances recorded with other electrode types. This result could be  
 11 explained by the hemi-spherical diffusion field at each interdigitated microelectrode overlapping  
 12 with neighboring diffusion layers [26], and the concomitant redox cycling between electrode fingers  
 13 enhancing the current and then decreasing the impedance.

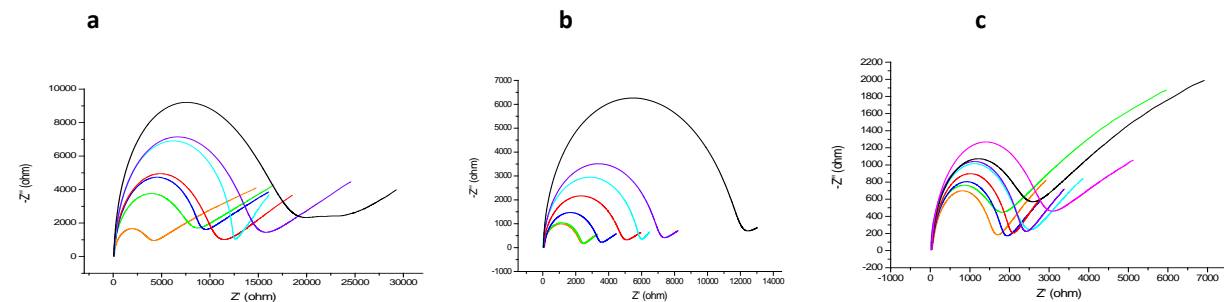
14 a)



15 b)  
 16

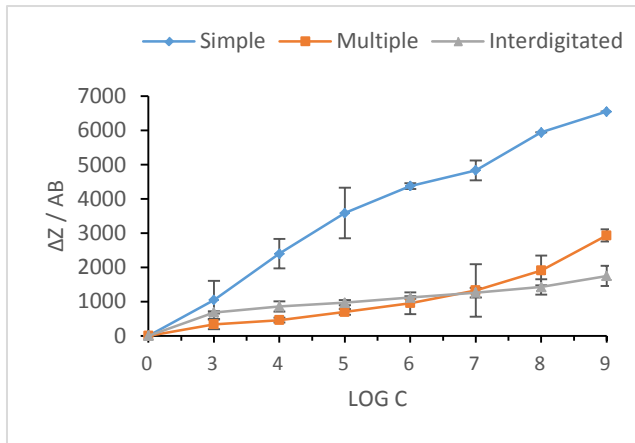


17 **Scheme 1:** a) three electrode geometries tested; b) impedimetric measurements were carried out  
 18 through deposition of drops and immersion of the SCE reference electrode.  
 19



20 d)  
 21





2 **Figure 2- Influence of the electrode geometry in static mode.** Nyquist plot of CD 14 antibody  
 3 modified electrodes, after incubation with monocytes suspensions (0 (–),  $10^3$  (–),  $10^4$  (–),  $10^5$  (–),  $10^6$   
 4 (–),  $10^7$  (–),  $10^8$  (–),  $10^9$  (–) cells/mL) measured in 5 mM  $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$  for (a) simple, (b)  
 5 multiple, and (c) interdigitated microelectrode. Frequency range: 0.1 Hz to 50 kHz. d) Corresponding  
 6 variation of impedance relative to the antibody layer measured at 100 Hz, as a function of the  
 7 logarithm of cells concentrations.

8 We are aware that our static system consisting of a drop of cell suspension sitting on the  
 9 microelectrodes is not ideal as the interaction of cells with a functionalized surface takes place  
 10 mainly through sedimentation. Taking into account the radius of the sample drop (around 3 mm for  
 11 200  $\mu$ L of electrolyte) and a sedimentation speed of cells of around  $1 \times 10^{-6}$  m/s, the fraction of cells  
 12 reaching the modified surface within 2 hours is weak. For example for  $10^6$  cells/mL i.e. 200 000 cells  
 13 in the drop, only 600 will be trapped on a single-electrode while the full coverage is reached for 1 000  
 14 cells (cell radius estimated to 12  $\mu$ m). This low trapping ratio explained why the saturation appear for  
 15 very high cell concentrations. Furthermore, trapping through sedimentation favors the non-specific  
 16 grafting leading to not well organized layers and stacking.

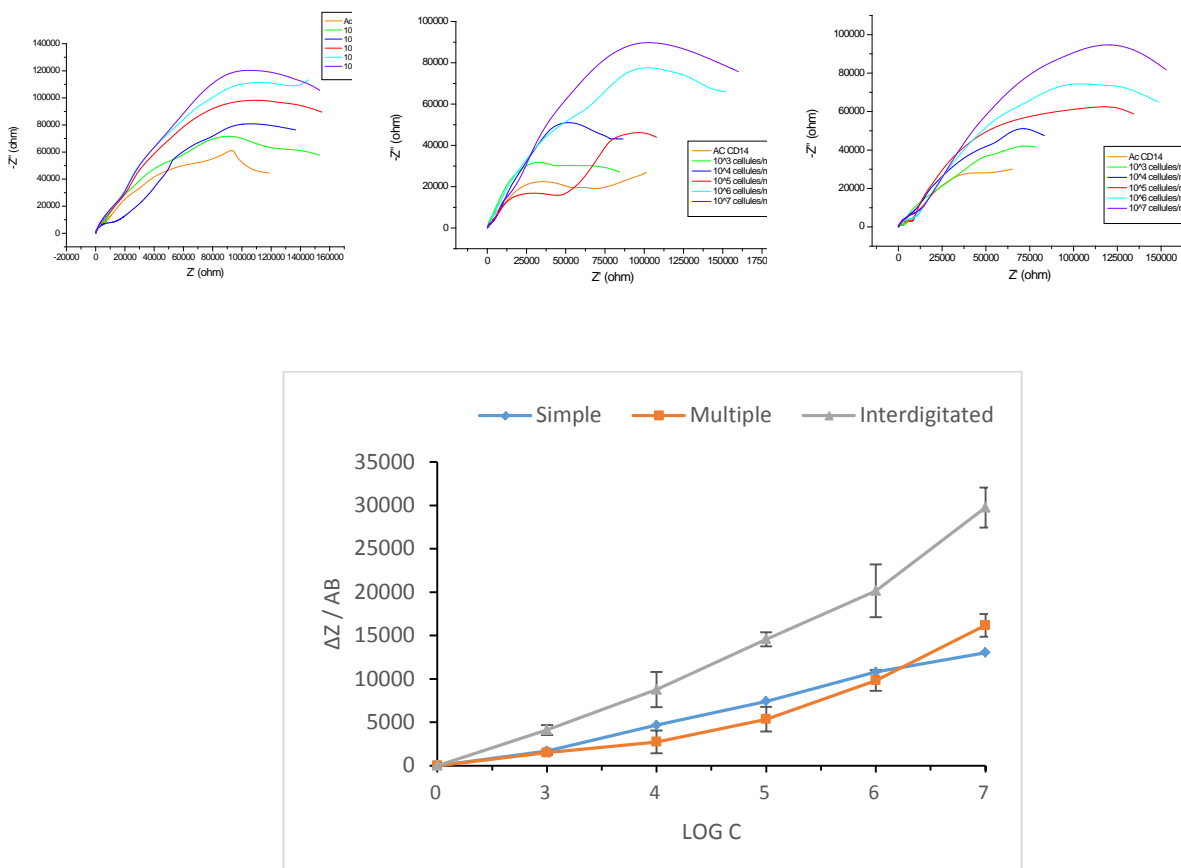
### 17 3.2. Microfluidic approach

18 Microfluidic integration offers numerous advantages compared to static approach. Beyond well-  
 19 established potentialities of fluid handling, such as sample pretreatment, assays parallelization and  
 20 others, in our case it also minimizes the sedimentation phenomenon and increases specific cell-  
 21 electrodes interaction.

22 We manufactured microfluidic devices with channels (length: 14800  $\mu$ m, width: 500  $\mu$ m, height: 100  
 23  $\mu$ m) and integrated electrodes of different designs. The surface of electrodes was functionalized  
 24 inside assembled chips, which strongly shortened the functionalization time to only 10 hours and  
 25 dramatically improved the homogeneity of the attached layers.

26 The impedimetric analyses were performed using a two electrodes set-up including a pseudo  
 27 [counter + reference] electrode. The cell trapping was performed by applying a continuous flow of  
 28 cells suspensions for 30 min on functionalized electrodes. After rinsing the electrodes, the impedance  
 29 measurements were then performed under a flow of the PBS electrolyte containing the redox probe.  
 30 The Nyquist diagrams and the corresponding variations of impedance recorded at 100 Hz for are  
 31 presented for each geometry in Figure 3a and 3b respectively. The microfluidic mode induced a  
 32 transition to a stationary mode (semi-infinite diffusion) characterized as demonstrated on diagrams  
 33 by the apparition of dissymmetric loops of Leminscate type for all geometries (Figure 3a) instead of

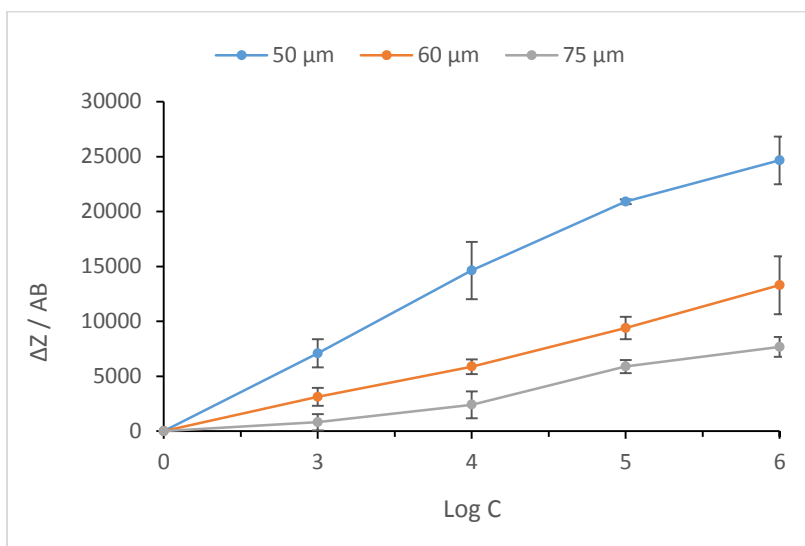
1 the classical Warburg line recorded in static mode. The values of the impedance changes  $\Delta Z_{/AB}$   
 2 recorded in this case are much greater than the ones recorded in the static mode. The variation of  
 3 impedance  $\Delta Z$  increases for  $10^6$  cells/mL by a factor 2, 12 and 18 for simple, multiple and  
 4 interdigitated electrodes respectively. This increase could originate from different factors, including  
 5 an increase in the probability of cell trapping and a better organization of cells on the electrode due  
 6 to presence of a laminar flow, and the change of the measuring mode from three to two electrodes.  
 7 For interdigitated electrodes the sensitivity increased from 200 to 4 000  $\Omega$  per  $\log[\text{cells}]$ , making this  
 8 geometry the most sensitive in the microfluidic mode. This could originate from the fact that  
 9 interdigitated electrode structures distribute the detection of localized changes to a larger sensing  
 10 surface [27] (length 20 times larger than for simple electrodes). Furthermore, the trapping of cells on  
 11 electrode edges modifies the inter-electrode space and consequently, the conductivity between  
 12 electrodes has even stronger impact on the impedance when the distance between the electrodes is  
 13 small. This can explain the great impedance changes recorded for interdigitated electrodes having a  
 14 gap of 50  $\mu\text{m}$  compared to the others with a gap of 180  $\mu\text{m}$ . These results are in accordance with the  
 15 works of Couniot and co-workers [28] which have demonstrated (using bacteria) a correlation  
 16 between the interdigitated inter-electrode space and the detection sensitivity. The mean sensitivity  
 17 is indeed larger for smaller electrode gaps but the electrical response is noisier. They established that  
 18 the optimal electrode gap is approximately four times the bacteria diameter [28].



19 **Figure 3- Influence of the electrode geometry in microfluidic mode** ; Nyquist plot of CD 14 antibody  
 20 modified electrodes, after incubation with monocytes suspensions ( $0$  (–),  $10^3$  (–),  $10^4$  (–),  $10^5$  (–),  $10^6$   
 21 (–),  $10^7$  (–),  $10^8$  (–),  $10^9$  (–) cells/mL) measured in 5 mM  $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$  for (a) simple, (b)  
 22 multiple, and (c) interdigitated microelectrode. Frequency range: 0.1 Hz to 50 kHz. Corresponding

1 variation of impedance relative to the antibody layer measured at 100 Hz, as a function of the  
2 logarithm of cells concentrations for measurements carried out in a microfluidic mode with different  
3 geometries.

4  
5 Therefore, 3 different gap values were tested: 50, 60 and 75  $\mu\text{m}$ . After functionalization of the  
6 electrodes, a range of cell concentration of  $10^3$  to  $10^6$  cells/mL was applied on each interdigitated  
7 design. The corresponding variations of the relative impedance module  $\Delta Z_{/AB}$  as a function of the  
8 logarithm of cell concentrations are presented in Figure 4. The results clearly demonstrate that the  
9 sensitivity increases when the gap decreases. The sensitivity recorded on the range tested varies  
10 from 1280  $\Omega$  /decade for the gap of 75  $\mu\text{m}$  to 2200  $\Omega$  /decade for 60  $\mu\text{m}$  and 4111  $\Omega$  /decade for the  
11 gap of 50  $\mu\text{m}$ . These results are in accordance with the factor 4 between the gap and the scale of the  
12 target cell mentioned in literature [28], assuming that the diameter of monocytes used (THP1) varies  
13 around 12-13  $\mu\text{m}$ .

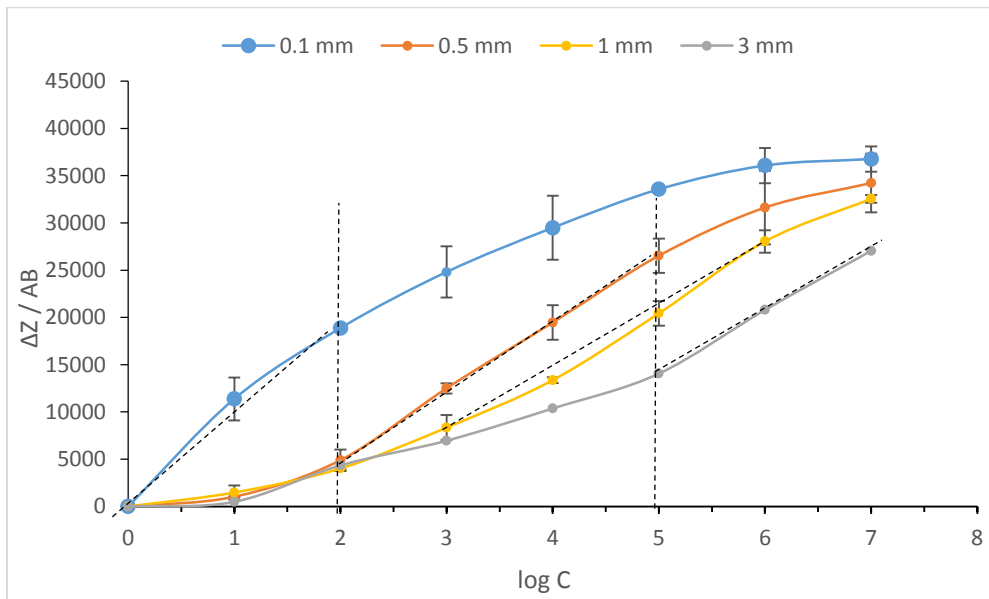


14

15 **Figure 4: Influence of the gap.** Variation of impedance relative to the antibody layer measured at 100  
16 Hz, as a function of the logarithm of cells concentrations for measurements carried out in a  
17 microfluidic mode with interdigitated electrodes for different values of gap: 50, 60 and 75  $\mu\text{m}$ .

18 Another parameter has to be taken into account and it concerns the area of the electrode and more  
19 specifically the length and number of strands. Four different lengths were tested: 0.1, 0.5, 1 and 3  
20 mm which comprise respectively 1, 2, 4 and 14 fingers of same dimension (20  $\mu\text{m}$  x 350  $\mu\text{m}$ ) with a  
21 given gap equal to 50  $\mu\text{m}$ . For this study, the fabrication process was slightly modified by replacing  
22 SU8 resin by laminated DF films to reduce processing time. Furthermore, to overcome **micro-welds**  
23 and PDMS bonding pads allowing microfluidic circulation of fluids, a new measurement system was  
24 set up at LAAS. Both electrical and fluidic parts were managed by the mechanical support, in which  
25 each chip was incorporated to be tested individually. The corresponding variations of the impedance  
26 module are presented in Figure 5. According to the surface, it is clearly observed that for the 0.1 mm  
27 a rapid saturation due to a total coverage appear while for the 3 mm (surface ratio 14), the  $\Delta Z_{/AB}$   
28 increases continuously with cell concentration due to a partial coverage. The two others systems  
29 present intermediate behaviors: an increase of the signal tending to saturate. All interdigitated size  
30 have comparable maximum sensitivities (> 6000  $\Omega$ /decades) but for specific cell concentration range.  
31 The 0.1 mm electrode has a maximum sensitivity until  $10^2$  cells, and after that the sensitivity

1 decreases due to the surface saturation. Furthermore with this area the device allowed a detection  
 2 limit below 5 cells/mL (LOD = 3xSD/slope). For the 0.5 mm microelectrode, the sensitivity is  
 3 maximum in a cell concentration ranging from  $10^2$  to  $10^5$  cells/mL and  $10^6$  for 1 mm electrodes  
 4 before it starts to decrease. In the case of 3 mm electrode, the sensitivity increases from  $10^5$   
 5 cells/mL. These results demonstrate that there is a threshold beyond which a surface is exploitable  
 6 for a given slice of concentration. A too large area is not suitable to detect rare events. For early  
 7 diagnostic i.e. detection of low number of cells, the 0.1 mm microelectrode seems to be the most  
 8 suitable.



9

10 **Figure 5: Influence of the electrode length.** Variation of impedance relative to the antibody layer  
 11 measured at 100 Hz, as a function of the logarithm of cells concentrations for measurements carried  
 12 out in a microfluidic mode with interdigitated electrodes for different lengths: 0.1, 0.5, 1 and 3 mm  
 13 (surface ratio 1, 2, 4 and 14).

14

## 15 CONCLUSION

16 In this paper we demonstrate by employing monocytes, how the immunodetection of  
 17 subpopulations of cells is strongly enhanced by using interdigitated electrodes with small inter-  
 18 electrodes gap compared to electrodes with simple geometries. These performances are linked to  
 19 strong minimization of the redox cycling between electrode fingers upon cell trapping. Furthermore  
 20 we also demonstrate that the slice of cell concentration for which the sensitivity is the maximum is  
 21 proportional to the electrode size. Moreover, the interdigitated electrodes with smallest area that  
 22 were fabricated (0.1 mm length) allowed to detect cells in solutions with concentrations smaller than  
 23 5 cells/mL, opening thus new perspectives for the detection of rare events such as the tracking of  
 24 circulating stem cells, for example.

25

## 26 ACKNOWLEDGEMENTS

1 The authors would like to thank the Région Midi-Pyrénées and the University of Toulouse for  
2 financial support.

### 3 REFERENCES

- 4 [1] H. Andersson, A. Van den Berg, Microfluidic devices for cellomics: a review, *Sensor. Actuat. B-*  
5 *Chem.* 92 (2003) 315-325.  
6
- 7 [2] S.Y. Yang, S.K. Hsiung, Y.C. Hung, C.M. Chang, T.L. Liao, G.B. Lee, A cell counting/sorting system  
8 incorporated with a microfabricated flow cytometer chip, *Meas. Sci. Technol.* 17 (2006) 2001-2009.  
9
- 10 [3] C.C. Lin, A. Chen, C.H. Lin, Microfluidic cell counter/sorter utilizing multiple particle tracing  
11 technique and optically switching approach, *Biomed. Microdevices* 10 (2008) 55-63.  
12
- 13 [4] K. Kiilerich-Pedersen, N. Rozlosnik, Cell-Based Biosensors: Electrical Sensing in Microfluidic  
14 Devices, *Diagnostics*, 2 (2012) 83-96.  
15
- 16 [5] U. Hassan, N.N. Watkins, C. Edwards, R. Bashir, Flow metering characterization within an electrical  
17 cell counting microfluidic device, *Lab Chip.* 14 (2014) 1469-1476.  
18
- 19 [6] X. Cheng, Y.S. Liu, D. Irimia, U. Demirci, L. Yang, L. Zamir, W.R. Rodríguez, M. Toner, R. Bashir, Cell  
20 detection and counting through cell lysate impedance spectroscopy in microfluidic devices, *Lab Chip.*  
21 7(6) (2007) 746-55.  
22
- 23 [7] S. Gawad, L. Schild, P. Renaud, Micromachined impedance spectroscopy flow cytometer for cell  
24 analysis and particle sizing, *Lab Chip.*, 1 (2001) 76-82.  
25
- 26 [8] A.C. Sabuncu, J. Zhuang, J.F. Kolb, A. Beskok, Microfluidic impedance spectroscopy as a tool for  
27 quantitative biology and biotechnology, *Biomicrofluidics*, 6(34103) (2012) 1-15.  
28 [9] B. Pejdic, R. De Marco, *Electrochim. Acta*, Impedance spectroscopy: Over 35 years of  
29 electrochemical sensor optimization, 51 (2006) 6217- 6229.  
30
- 31 [10] J. Wegener, C.R. Keese, I. Giaever, Electric cell-substrate impedance sensing (ECIS) as a  
32 noninvasive means to monitor the kinetics of cell spreading to artificial surfaces, *Exp. Cell Res.*, 259  
33 (2000) 158-166.  
34
- 35 [11] C. Caviglia, K. Zór, S. Canepa, M. Carminati, L.B. Larsen, R. Raiteri, T.L. Andresen, A. Heiskanen, J.  
36 Emnéus, Interdependence of initial cell density, drug concentration and exposure time revealed by  
37 real-time impedance spectroscopic cytotoxicity assay, *Analyst* 140(10) (2015) 3623-3629.  
38
- 39 [12] Q. Liu, C. Wu, H. Cai, N. Hu, J. Zhou, P. Wang, Cell-Based Biosensors and Their Application in  
40 Biomedicine, *Chem. Rev.*, 114 (12) (2014) 6423–6461.  
41
- 42 [13] A. Montrose, S. Cargou, F. Nepveu, R. Manczak, A.M. Gué, K. Reybier, Impedimetric  
43 immunosensor for the detection of circulating pro-inflammatory monocytes as infection markers,  
44 *Biosens. Bioelectron.* 49 (2013) 305-311.  
45
- 46 [14] Reybier K., Ribaut C., Coste A., Launay J.P., Fabre P.L., Nepveu F., 2010. *Biosens. Bioelectron.*  
47 25(12), 2566-72.  
48

- 1 [15] J.W. Judy, Microelectromechanical systems (MEMS): fabrication, design and applications, *Smart*  
2 *Mater. Struct.*, 10 (2001) 1115-1134.  
3
- 4 [16] G. Justin, S. Finley, A. Abdur Rahman, A. Guiseppi-Elie, Biomimetic hydrogels for biosensor  
5 implant biocompatibility: electrochemical characterization using micro-disc electrode arrays  
6 (MDEAs), *Biomed. Microdevices*, 11(1) (2009) 103-105.  
7
- 8 [17] T.H. Park, M.L. Shuler, Integration of cell culture and microfabrication technology, *Biotechnol.*  
9 *Prog.*, 19 (2003) 243-253.  
10
- 11 [18] W. Franks, I. Schenker, P. Schmutz, A. Hierlemann, Impedance characterization and modeling of  
12 electrodes for biomedical applications, *IEEE Trans. Biomed. Eng.* 52 (2005) 1295-1302.  
13
- 14 [19] Matysik F.M., Meister A., Werner G., Electrochemical detection with microelectrodes in capillary  
15 flow systems, *Anal. Chim. Acta*, 305 (1995) 114-120.  
16
- 17 [20] D.W. Greve, X. Huang, D. Nguyen, M.M. Domach, Modeling of impedance of cell-covered  
18 electrode, *Proceedings of the IEEE Sensors 2003 (IEEE Cat. No. 03CH37498)*, 2 (2003) 1358-1363.  
19
- 20 [21] Y. Huang, B. Rubinsky, *Sens. Actuat. A-Phys.*, Microfabricated electroporation chip for single cell  
21 membrane permeabilization, 89(3) (2001) 242-249.  
22
- 23 [22] S. Cho, H. Thielecke, *Physiol. Meas.*, Design of electrode array for impedance measurement of  
24 lesions in arteries, 26(2) (2005) S19-S26.  
25
- 26 [23] D.T. Price, A.R. Rahman, S. Bhansali, Design rule for optimization of microelectrodes used in  
27 electric cell-substrate impedance sensing (ECIS), *Biosens. Bioelectron.* 24(7) (2009) 2071-2076.  
28
- 29 [24] R. Courson, S. Cargou, V. Conedera, M. Fouet, M.C. Blatche, C.L. Serpentin, A.M. Gue, Low-cost  
30 multilevel microchannel lab on chip: DF-1000 series dry film photoresist as a promising enabler,. *RSC*  
31 *Adv.*, 4 (2014) 54847.  
32
- 33 [25] C.E. Nwankire, A. Venkatanarayanan, T. Glennon, T.E. Keyes, R.J. Forster, J. Ducreé, Label-free  
34 impedance detection of cancer cells from whole blood on an integrated centrifugal microfluidic  
35 platform, *Biosens. Bioelectron.*, 68 (2015) 382-389  
36
- 37 [26] O. Ordeig, J. Del Campo, F.X. Munoz, C.E. Banks, R.G. Compton, Electroanalysis Utilizing  
38 Amperometric Microdisk Electrode Arrays, *Electroanal.*, 19 (2007) 1973-1986.  
39
- 40 [27] S.O.P. Blume, R. Ben-Mrad, P.E. Sullivan, Characterization of coplanar electrode structures for  
41 microfluidic-based impedance spectroscopy, *Sens. Actuat. B-Chem.*, 218 (2015) 261-270.  
42
- 43 [28] N. Couniot, D. Flandre, L.A. Francis, A. Afzalian, Signal-to-noise ratio optimization for detecting  
44 bacteria with interdigitated microelectrodes, *Sens. Actuat. B-Chem.*, 189, (2013) 43-51.  
45  
46  
47

