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Academic and Industrial Trends in Dielectrophoresis

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Current trends in the dielectrophoresis (DEP) literature show that contributions from electronic engineers and materials scientists are increasingly being focused on the development of DEP-based protocols for chemical and biochemical analyses that involve nanoparticles. Two modes of DEP are predicted to dominate, namely metal-electrode based (eDEP) and insulator-based (iDEP). Where high values of the applied electric field and its gradient are required for the spatial manipulation of nanoparticles (*e.g*., exosomes, proteins, viruses, carbon-nanotubes), the preferred option is likely to be eDEP. This is also the case for applications such as the selective isolation of biological cells; the development of cell-based drug discovery protocols; electronic sensors and the assembly of nanoparticles (*e.g*., carbon nanotubes). For the separation or detection of bioparticles such as DNA, RNA, proteins or bacteria, where manipulation selectivity is based on differences in surface charge and counter-ion mobility, then iDEP will be appropriate.

Six commercial products incorporating DEP will be outlined, namely: the Panasonic bacteria counter; the Shimadzu IG-1000 nanoparticle analyzer; Silicon Biosystems DEPArrayTM; the DEPtech, 3DEP, system for characterizing the dielectric properties of cells; the ApoStreamTM technology for isolating tumor cells; and the microarray devices developed by Biological Dynamics for isolating nanoparticulate biomarkers. All of these technologies employ iDEP.

Dielectrophoretic cell rotation for label-free phase optical cell tomography

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Achieving quantitative information about suspended cells in a noninvasive and label-free manner is of utmost importance in the fields of biomedicine and live cell analysis. While conventional phase contrast imaging methods are not quantitative and present significant imaging artifacts, tomographic approaches that capture the complex wave fronts of the light transmitted through the cell provide information about the cell 3-D refractive index distribution.
For that, either the cell or the illumination source have to be rotated at highest possible coverage of the rotation angular range on any axis while at the same time knowledge about the viewing angle is preserved. This significantly limits many approaches. Rotating illumination is typically limited to angles of 140°, causing missing data points in the angular spectrum. Even though other approaches allow full angular range, by either rotating the entire sample or by rotating micropipette aspirated single cells, they are only applicable to adherent cells or do not allow for noninvasive 3-D imaging.
In this work, we present a novel approach for complex-wave front tomography with 360° rotation of suspended single cells on any axis, with an angular resolution of less than 2.5° and without physically touching the cells. For that, we employ a microfluidic protocol based on dielectrophoretic (DEP) forces.

In a microfluidic channel individual cells are trapped and rapidly rotated by means of a DEP field cage in a noninvasive and precise manner. Complex-wave front projections of the rotated cells are acquired and processed into the cellular 3-D refractive index map. By this, we obtained label-free imaging data of both large cancer cells and three-types of white blood cells. Importantly, the cells can be recovered after inspection, which is important to label-free live cell sorting, monitoring cellular pathological conditions in body fluids as well as for therapeutic purposes.

Rapid Prototyping and 3D Printing Technology for Microfluidic Lab-on-a-Chip Systems

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There are a number of different ways that modern rapid prototyping and additive manufacturing techniques, such as 3D printing, can be applied in the fabrication of microfluidic and lab-on-a-chip systems. This talk will begin by reviewing the previous work on the development of 3-dimensional microfluidic devices. It will go on to describe a process developed at the University of Edinburgh [1, 2], which allows the integration of functional fluidic components such as valves and mixers, along with sensing. This has been used to create a demonstrator system capable of automated extraction of Michaelis-Menten enzyme kinetics. Initial results from these experiments will be presented as a proof of principle of the manufacturing technique.
Presenter: Dr. Stewart Smith.

[1] A. Buchoux, P. Valluri, S. Smith, A.A. Stokes, P.R. Hoskins and V. Sboros, "Manufacturing of microcirculation phantoms using rapid prototyping technologies," 2015 37th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), Milan, 2015, pp. 5908-5911. doi: 10.1109/EMBC.2015.7319736
[2] A. Buchoux, S. Smith, D.J. Clarke and A.A. Stokes, “Development of a 3D microfluidic Lab on a Chip for
light spectrometry analysis of enzyme kinetics”, poster presentation at IEEE-NIH 2016 Special Topics Conference on Healthcare Innovations and Point-of-Care Technologies, Cancun, Mexico.

Real-time monitoring of the metabolic activity of hepatocytes in a microbioreactor

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The establishment of valuable cell models and their exploitation for addressing important issues in a number of disciplines such as tissue engineering, drug development or toxicology, is a complex task that requires control of a multitude of processes. Recently, together with colleagues from Jerusalem we have introduced a novel perfusion microbioreactor for the cultivation of hepatocytes. Through the integration of microbeads that contain oxygen-sensitive chromophores it is possible to measure the O2-concentration in real-time in the medium in the immediate vicinity of the cells. By adjusting the flow of the medium and the number of cells allows the monitoring of their metabolic activity over several weeks. Based on this approach we were able to assess the toxicity of a number of hepatotoxic compounds. Recent work includes the integration of a second class of microsensor beads that allows the monitoring of the pH in the cell clusters and the establishment of more relevant cells models. Results will be presented based on modified hepatocytes based on the cell line HepG2.

[1] S. Prill et al. (2014), Biomicrofluidics; 8: 034102-1-034102-9.
[2] S. Prill et al. (2015), Archives of Toxicology; 90: 1181-1191.
[3] D. Bavli et al. (2016), PNAS; 113; E2231-E2240.

Microfluidic systems for flow cytometric analyses of blood cells

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Differentiation and counting of cells in blood is routinely used in laboratory medicine to support medical diagnosis in various fields including haematology, immunology and oncology. Many measurements are carried out in central laboratories capable of analysing several thousand samples per day utilizing optically and impedance based high throughput instruments. On the other hand, there is an increasing request for analytic point of care tests. For example, a rapid and reliable test for the determination of platelet concentrations during surgery would facilitate decisions on transfusions. In addition, rapid tests would also be valuable tools for emergency services.

We developed and characterised various microfluidic chips for flow cytometric cell differentiation. Lithography, ultraprecision milling and etching was applied to fabricate 2 ½ - or 3 - dimensional devices. All structures feature hydrodynamic focussing stages to position cells that are moving with velocities between 1m s-1 and 5m s-1 in the centre of the flow channel in one or two dimensions. Cells are characterised with a throughput of 1kHz to 5kHz according to their light scattering properties, fluorescence intensity when specifically labelled by monoclonal antibodies or impedance at different frequencies. From such observations, measurands like size, shape, antibody expression, membrane capacity and resistance of the intracellular contents may be derived by applying adequate mathematical modelling and data analysis.

The standard protocol to determine concentrations of white blood cells include destruction of erythrocytes by lysis. However, in leukemia patients, abnormal white blood cells might be significantly modified or destroyed and in newborns red blood cells might be resistant against lysis. For both applications, a lysis free procedure would simplify the interpretation of concentration measurements. To this end we developed a microfluidic device for combined electrical and optical flow cytometric analyses of blood cells [1]. Using two different frequencies between 1MHz and 15Mhz for impedance measurements, simultaneous lysis-free and label-free differentiation of blood platelets, erythrocytes, granulocytes, monocytes and lymphocytes in human whole blood was demonstrated. Impedance based cell identification was validated using standard immunostaining protocols including CD45, CD235a and CD61 antibodies. Since only a dilution process is involved in sample preparation, our AC-impedance based cell differentiation chip is well suited as sensor component in a microfluidic system, which include sample handling.

[1] P. Simon et al. (2016), Lab Chip, 16:2326–2338

AC electrokinetic manipulation of nanoparticles and Molecules

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AC electrokinetic phenomena like dielectrophoresis (DEP) and AC electroosmosis have been applied to the spatial manipulation of biological cells over many years. They are increasingly exploited for the separation and immobilisation of nanoparticles and molecules in micro- and nanoelectrode systems. Especially the dielectrophoretic immobilisation of antibodies and enzymes on electrodes is quite attractive for a well controlled functionalisation of sensors. With proteins being about three orders of magnitude smaller than cells, both the size dependence of DEP and the increased influence of thermal motion call for much higher field gradients when targeting molecules. Such an increase can be achieved by increasing the voltages applied, which, however, leads to heating and additional fluid flow that interfere with DEP action. A better approach is to use electrodes with sizes and curvatures reaching those of the target molecules. Therefore, we have developed several electrode types: Interdigitated electrodes with electrode gaps below 1 µm, planar triangular electrodes at around 100 nm distance, and regular arrays comprising up to 1 million pin-like electrodes with curvatures reaching the size of proteins (< 10 nm). Successful immobilisation is demonstrated for polystyrene nanospheres, single molecules of the autofluorescent protein R-phycoerythrin [1], for horseradish peroxidase and for antibodies [2], as well as for DNA. Fluorescence microscopy shows that protein function is preserved in the course of DEP immobilisation. Localisation is achieved by scanning force, scanning electron and optical microscopy. Fluorescence polarisation microscopy reveals the immobilisation of the autofluorescent eGFP in a properly aligned manner [3] and allows to determine the orientation of the protein's fluorescing subunit in relation to the whole molecule. Ongoing work is aimed at the field controlled manipulation of virus particles, of even smaller molecules, e.g. peptides, a deterministic positioning of molecules as singles, and the combination with surface enhanced Raman spectroscopy.

[1] R. Hölzel, N. Calander, Z. Chiragwandi, M. Willander and F. F. Bier. *Phys. Rev. Lett.* 95, 128102 (2005).
[2] S. Otto, U. Kaletta, F. F. Bier, C. Wenger and R. Hölzel. *Lab Chip* 14, 998 (2014).
[3] E.-M. Laux, X. Knigge, F. F. Bier, C. Wenger and R. Hölzel. *Small* 12, 1514 (2016).