LAPASO – Label Free Particle Sorting

Final Report Summary

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An overarching scientific objective of the LAPASO project was to address important biomedical problems and advance diagnostics with microfluidics and nanobiotechnology integration.

Microfluidic label-free particle fractionation based on the inherent properties of particles (e.g. cells, microorganisms, organelles) offers significant advantages over conventional techniques in terms of ease of handling and usage, speed, and reduction in cost.

During this project, we trained 15 fellows within three interdisciplinary and inter-related themes based on understanding and exploiting label-free particle analysis: in parasitology improve diagnosis by enriching parasites that occur at low concentrations; in bacteriology to sort bacteria in different subpopulations based on morphology that is connected to pathogenicity; and for

To tackle these challenges we covered a wide range of technologies within materials, processing and simulation of micro- and nanosystem components, microfluidics and integrated optics and electronics.

Towards diagnosis of parasitic diseases (leishmaniosis, sleeping sickness and malaria) we have used the three technologies (1) deterministic lateral displacement (DLD), (2) real time-deformability cytometry (RT-DC) and (3) microfluidic impedance cytometry (MIC). DLD was successfully applied to the separation of *Leishmania mexicana* promastigotes from red blood cells. RT-DC measurements demonstrated that the changes in mechanical properties of infected macrophages are correlated to the infection progress culture. Also, the effect of *L. mexicana* infection on the dielectric properties of macrophages was studied by microfluidic impedance cytometry (MIC). Especially the design of deterministic lateral displacement (DLD) devices for separation of parasites (leishmania/trypanosomes) or malaria infected red blood cells from healthy erythrocytes was supported by modelling. Development of 2D and 3D simulation methods were developed for predicting particle trajectories through devices.

Our work on the enrichment of parasites and parasite-infected cells, as well as on biomarker discovery, has the potential to lead to the development of novel diagnostic techniques which are affordable, easy to use in the field, rapid, sensitive and specific. This could therefore have an important impact on the control of leishmaniosis as well as malaria.

DLD devices were used to sort different chain lengths of *Streptococcus pneumoniae*. It was also found that the devices were capable of sorting capsulated and non-capsulated strains of the same bacteria. A high throughput device was designed and tested with the aim to significantly increase the number of bacteria sorted for subsequent biomolecular analyses.

Our research has led to advancements in the understanding of the pathogenesis of pneumococcal meningitis. We have shown that pili, expressed in a subset of strains, increase the ability of pneumococci to invade the brain to cause meningitis. The expression of these pili could be correlated with the ability of pneumococci to form small single cocci in the brain. Strains which express pili are significantly better at entering the brain and causing meningitis than their non-piliated counterparts.

The cell size and dielectric properties, and the mechanical properties of skeletal stem cells (SSC) were investigated using a custom-designed microfluidic impedance cytometer and real time-deformability cytometry respectively.

Acoustophoresis as an initial step for further enrichment of rare cells was investigated. We tested the separation of mononuclear cells (MNC), a subpopulation of white blood cells accounting for 0.6% of all blood cells, from whole blood.

RT-DC is able to detect cytoskeletal alterations, distinguish cell-cycle phases, track stem cell differentiation into distinct lineages and identify cell populations in whole blood based on their mechanical fingerprints. In this work, we have demonstrated that by combining RT-DC with surface acoustic waves, high speed label-free cell sorting can be realized. Through this work we have gained knowledge on the biophysical properties of primary human skeletal stem cells. This information is important to understand the SSC phenotype in the bone marrow and under culture conditions, and pivotal to design efficient devices for SSC isolation using microfluidics. The final results constitute a label-free cell sorting device which is capable of significant SSC enrichment from human bone marrow providing homogeneous and pure sub-populations that can be used in developmental and pharmacological studies as well as, potentially, direct translation to the clinic using tissue engineering or stem cell therapy approaches.

A concept for a microfluidic drug delivery system and a microfluidic thermalisation apparatus for PCR-based diagnostics were developed

An integrated system with DEP and DLD was developed and tested with different cells and particles. Finally, DLD and MIC technologies were integrated into a single system, allowing us to combine label-free sorting with detection of particles based on their size and dielectric properties.

We achieved a high level of collaboration between the academic and industrial sectors. The network members from industry provided design guidelines for later commercialization and mass production.

In total, the work of the fellows resulted in 20 scientific publications, and 46 conference contributions (39 posters, 7 oral presentations). The fellows communicated their research to a broad audience during events such as science slams, research competitions and events targeted at young people.

This project has been a success mainly due to the highly multi-disciplinary expertise offered by the different project partners. PhD students and post-doctoral fellows were able to benefit from

collaborative sub-projects between partners and accelerate their learning process by interacting directly with experts from different scientific areas. The consortium also offered generous salaries that are in line with the typically high qualifications of PhD candidates and help attract experienced researchers from different parts of the world encouraging a multi-cultural research environment. The funding available to support the participation in career-developing activities such as training courses and international scientific conferences also promoted a fast learning process and enhanced the visibility of the research work produced within the consortium. All fellows share the belief that having participated in this project represents a boost to their career. Both, the supervisors and fellows agree that LAPASO has generated productive and long-lasting scientific collaborations.

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