

Motivation

The main focus of this PhD project lies in getting to know and further developing the microfluidic lab-on-chip flow cytometer developed at NanoSyd, to a point where an entire flow cytometer is embedded into a chip. This includes investigating microfluidic flow control, pressure sensing, including optical components, light sources and detectors, as well as parallelizing measurement channels and data collection and processing hard- and software in order to achieve high throughput and reliability.

Flow cytometry

The principles of flow cytometry involve combining the three fields of Optics, Fluidics and Electronics in order to map different properties of a large number of particles suspended in a fluid.

In figure 1, the principles of a classic flow cytometer setup are presented. On the left an excitation source (a blue laser) is seen. From above the sample (prepared with different fluorophors, attached to the particles to be investigated) passes through a flow cell, resulting in a stream of particles dropping through the laser beam. A detector picks up forward scatter signals, and a row of other detectors pick up different side scatter signals. All signals are monitored, processed and presented in the attached computer, resulting in scatter plots (figure 2) that provide information about the composition of the sample.

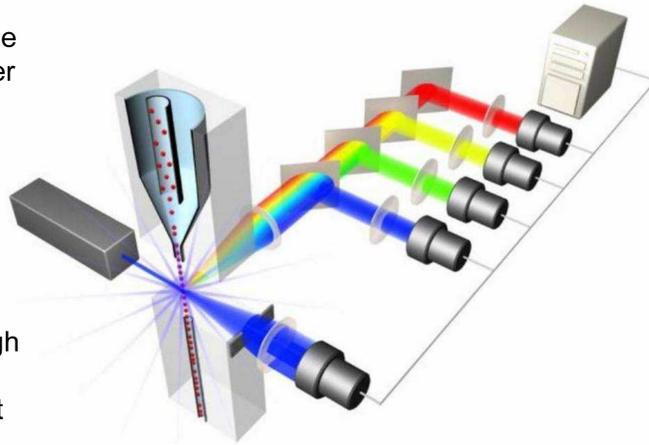


Figure 1: Principle diagram of classic flow cytometer setup.

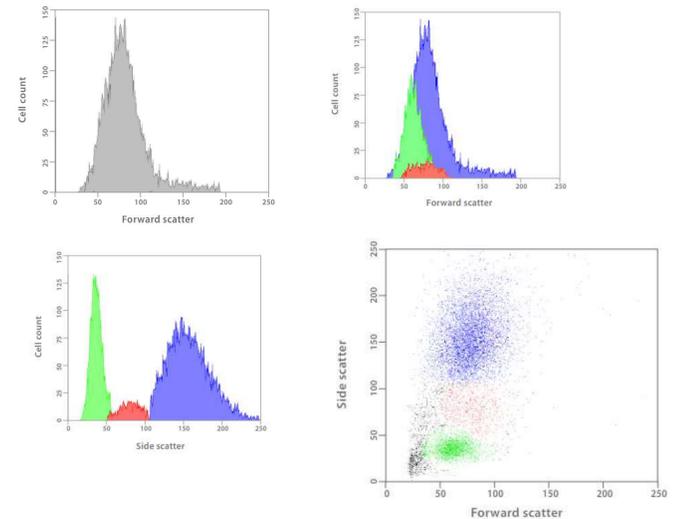


Figure 2: Illustration of how different detectors capturing signals from different fluorophors combine into 2-dimensional scatter plots of a sample composition.

PDMS Lab-on-Chip Flow cytometry

So far, a PDMS Lab-on-Chip Flow cytometer has been developed as part of the "Fluorescence detection of harmful microorganisms in liquid food using on-chip flow-cytometry" project. An experimental setup has been developed, containing fluid pumps, a 488 nm Argon-ion laser light source, detectors for scattered light as well as fluorescence, optics for coupling light into and out of the chips and software for collecting, processing and displaying the measurement data. A novel method for producing the chips has been developed, in which optical lithography is used to create a master mold off of which the individual chips are made, with great versatility in the connections to and from the flow channels.

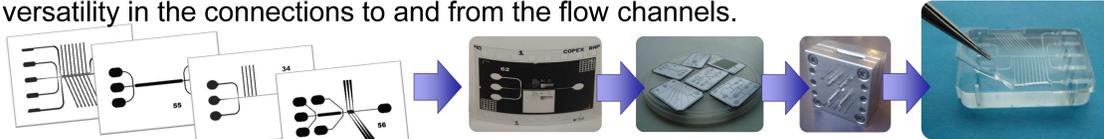


Figure 3: Illustration of the process developed for producing PDMS microfluidic chips – from mask designs on the left, through a photolithographic process to create mold masters towards molding and sealing of the finished chip.

Scatter plots from measurements using PDMS Lab-on-Chip Flow cytometer

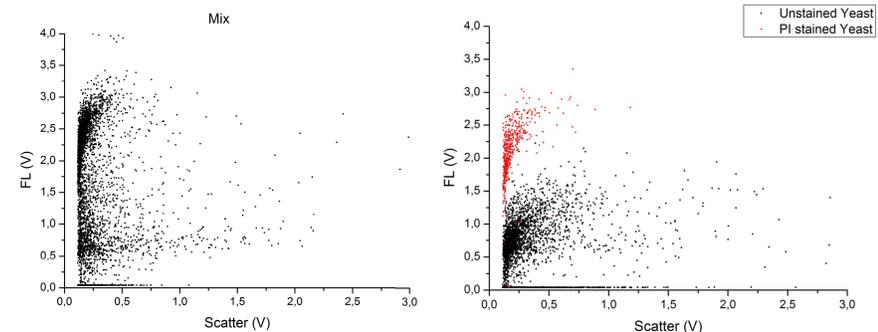


Figure 4: Scatter plots from measurements on samples of yeast cells – left, a plot of a sample with different cells mixed in a sample, right an overlap of measurements performed on 2 different yeast cell populations, with and without fluorophor staining.

PhD project milestones and end goal

This PhD project is based on the previous results outlined above. The timeline for the experimental work runs from July 2013 until the end of the year 2015, after which a thesis should be prepared for hand-in and defense. A timeline of work expected to be done is outlined below.

2013

Familiarization with current project and state-of-the-art technology. Initial upscaling attempts, with a goal of incorporating 8 parallel LoC flow cytometers on one setup in order to maximize throughput and minimize sample processing time – using 3-dimensional chip designs. Investigations on pressure distribution, manipulation and measurements on individual channels and methods of incorporating these.

2014

Further work on the parallelization of multiple LoC flow cytometers – focus on electronics and software for data collection and processing, as well as feedback for the pressure driving and distribution network. Investigations of integration into existing lab infrastructure, such as 96 well plate pipetting robots. Initial investigations on moving towards glass- or siliconbased microfluidics, developing methods for producing channels using e.g. Reactive Ion Etching.

2015

Working on glass/silicon-based microfluidic chips in order to integrate sensors, light sources, enhanced optics components etc. directly into the chip. Investigations of how plasmonic structures, diffractive optics etc. can improve the sensitivity of the fluorescence detection. Final goal will be to produce a microfluidic flow cytometer with integrated optics, fluid handling and control as well as electronics.

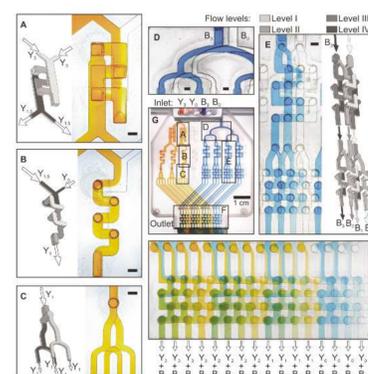


Figure 6: Example of 3-dimensional microfluidic structure, used to achieve four different titrations of two dyes and deliver all 16 possible combinations of these at separate outlets.

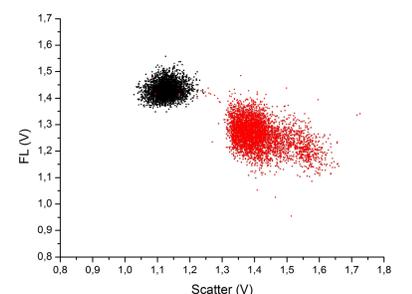


Figure 5: Scatter plot of 2 measurement series performed on different sized oil droplets



Figure 7: Example of 96-well plate with sample pipettes, used for automating preparation, transport and processing of multiple samples in an efficient way.

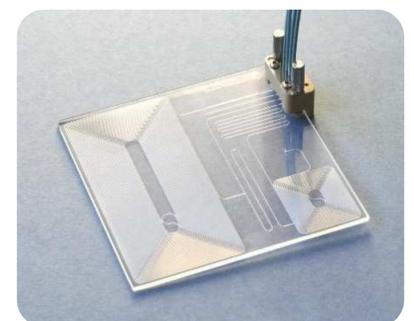


Figure 8: Example of integrated glass microfluidics device incorporating electronics and optics. A miniaturised gas chromatography column with on-chip resistive heating and absorption column for activated carbon particles.

References

- Figures 1+2: Invitrogen Flow Cytometry introduction (http://probes.invitrogen.com/resources/education/tutorials/4Intro_Flow/player.html)
 Figure 3: Images illustrating the production process developed at NanoSyd by Casper Kunstmann-Olsen and James Hoyland (NanoSyd, MCI, University of Southern Denmark)
 Figures 4+5: Scatter plots from experiments performed using the existing Lab-on-Chip PDMS Flow Cytometer setup by Casper Kunstmann-Olsen (NanoSyd, MCI, University of Southern Denmark)
 Figure 6: Neils, C. M., Tyree, Z., Finlayson, B., and Folch, A. "Combinatorial Mixing of Microfluidic Streams", *Lab on a Chip* 4 (4), 342 (2004).
 Figure 7: 96-well plate from ibidi Labware (<http://ibidi.com/xproducts/en/ibidi-Labware/m-Plates/m-Plate-96-well>)
 Figure 8: AZoNano - Fabricating a Microfluidic Chip for Environmental Testing with Miniaturised Gas Chromatography (<http://www.azonano.com/article.aspx?ArticleID=3476>)