

OTKA PD 73653

Digital microfluidics research grant

Project summary

Project duration: 01/09/2008 - 30/11/2011

Short summary of the results

The project proposal was based on an active research collaboration between our research group and Advanced Liquid Logic, LLC company based in Research Triangle Park, NC, USA. Due to the failure in the cooperation with our industrial partner for reasons related to sharing intellectual property, we had to focus most of our research efforts to reproduce and recreate digital microfluidic devices (originally expected to be provided by LLC): both the electrode matrices and the driving circuitry and software.

Despite of this hindrance I would conclude this research project as a successful one based on the following results. Our research group has been able to design, implement and test new digital microfluidic electrode arrays based on our extensive numerical simulations. Also, driving circuitry and software have been iteratively designed and tested to comply with the different electrode array structures. Furthermore, chemical and biological experiments have been conducted in newly designed microfluidic channel systems to be fitted to the application in digital microfluidic droplet systems. These channel systems have been designed and optimized using numerical simulation methods. One of these test systems have been developed into a prototype device, utilizing the camera (later a high-fps camera computer), the stereo microscope and the syringe pump, for blood cell counting used in flow cytometric devices. This prototype have been presented at professional meetings where it was favorably received. The related descriptions were published in two conference proceedings.

Detailed results of the project

1. Numerical model simulations of EWOD systems

The finite element numerical simulation software (COMSOL Multiphysics v3.5 at the time) purchased during the project have been extensively used to model the EWOD phenomenon. Modeling electrowetting on dielectric requires coupling between two physical model systems: two phase flow of compressible and incompressible species (e.g. water and air) and AC electrostatics. Also, 3 dimensional modeling of an electrode pair is highly challenging due to the high number of variables of the finite element method in 3D. (see e.g. Fig. 1.) The equation that links these physical models is credited to Lippmann and Young as early as 1875.

$$\cos\theta = \cos\theta_0 + \frac{C}{2\gamma_{LG}}V^2$$

Here, θ_0 is the contact angle without electric field, C is the capacitance of the dielectric layer that separates the liquid from the electrode under the droplet, γ_{LG} is the surface tension force between the solid and the gas, and V is the voltage. This coupling was implemented and used in the model.

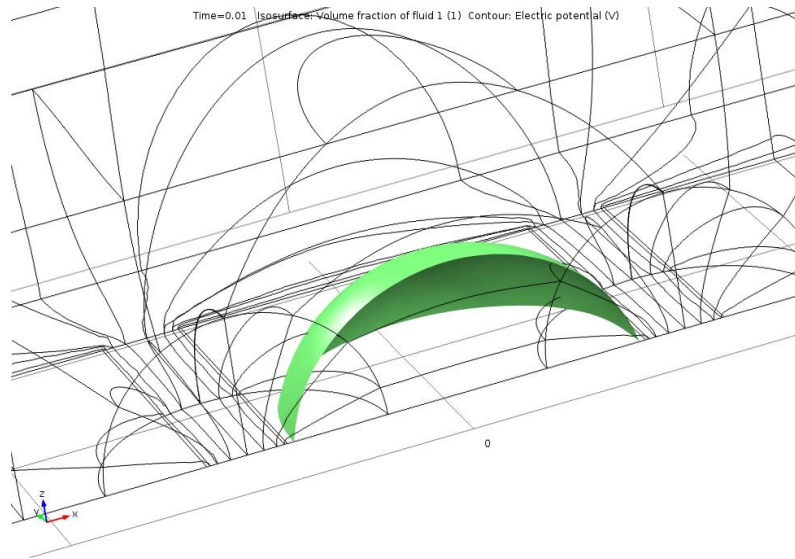


Figure 1. Numerical (finite element) simulation result of a droplet movement in electrostatic field using the Lippmann-Young equation as a coupling between wetting contact angle at the surface and electric potential. The green surface is depicting the droplet after 10 ms in time dependent simulation, the contour lines show 7 levels of the electric potential between 0 and 64 V. Symmetrical boundaries have to be used in 3D simulations to control memory usage.

2. Hardware developments of digital microfluidic systems

Based on previously developed digital microfluidic electrode arrays we have been experimenting with different designs of the electrode boundary facing other electrodes. Both electrode boundaries, electrode array arrangements and different gap sizes have been designed, fabricated and tested. All of these designs have been implemented on standard commercial PCB (printed circuit board) using circuit board design software and fabrication rules. Ultimately, the important part of these designs was the electrode distance (commercial PCB fabrication limits this at 150-200 μm) and edge curvature to minimize field enhancement and thus sparks that destroy the dielectric in the electrode surface. (some electrode arrangements can be seen on Fig. 2). The surface of electrodes had to be precisely coated with dielectric in order not to electrolyze the droplet (usually water). Using thin dielectric surfaces electrowetting is easily achieved using 80 V or above. Thin dielectric films were spin coated onto the surface of solderless PCBs. Spin coating is necessary to achieve 20-30 μm thin PDMS elastomer (polydimethylsiloxane, commonly used dielectric and structural material in microfluidic research) which is polymerized after spin coating. Another layer of Teflon AF 1600 fluoropolymer was spin coated onto the polymerized PDMS film to ensure highest possible hydrophobicity of the surface to water (Teflon has the highest hydrophobicity available). Spin coated Teflon films are 5-8 μm thin. These films ensure that no spark occurs even under the maximum operating voltage of 300 V.

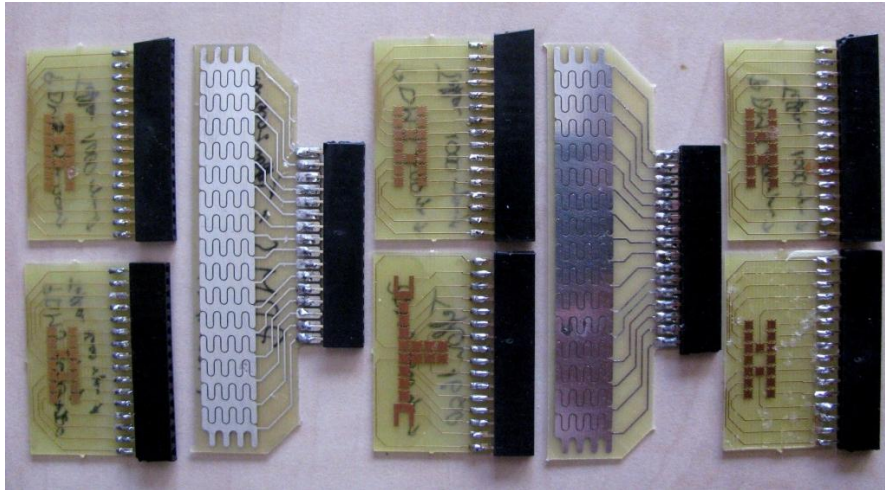


Figure 2. Some of the electrodes with different curvature, size and arrangement we tested (note the semi-transparency and the missing green color of standard PCBs, this is due to the fact that electrodes are native and not coated with the standard green soldermask finish)

(PDMS has a dielectric strength of 20 V/um). The simplest electrode arrangements are open platforms, meaning that the electrode pairs are implemented on the same PCB. Closed platforms require usually the ground electrode to be on top of the system on a separate surface (optimally glass or another transparent layer). Being more complex this way these closed platforms offer less measurement errors due to evaporation of the analyte. Closed platforms have been implemented also, using ITO (Indium Tin Oxide) coated glass clover slips.

Although the original project proposal included further developments and optical tests on the digital microfluidic platforms, the delays did not allow us to perform those analyses on these platforms. The time spent with developing these devices and applying surface modifications was fruitful, nevertheless.

3. **Hardware developments of EWOD driving circuitry and software**

Switchable AC power modules have been designed and implemented with an initial goal of running the EWOD driving circuitry from USB port (which is supplying 5V with a maximum of 1A). Due to the transformer and high voltage components the power dissipation of the circuitry exceeded 5W. Nevertheless, software control is achieved through USB connection. High voltage power switching is achieved using two components. Switching control is implemented on a Microchip PIC 18F87J50 USB microcontroller on a Demo Board. A custom designed and implemented high voltage (up to 600V) switching circuit is used for switching high voltage, ground and floating outputs to the electrodes (Figs 3 and 4). High voltage (up to 1500V) is supplied from the microsize power supply 1.5M24-P1 by Ultravolt (see Fig. 4 right hand side). This power supply requires 24V to operate. The driving circuitry is controlled by the microcontroller which is set to 10 kHz AC square wave output signals. This microcontroller is controlled by software (presently from MATLAB) to address different electrodes with certain patterns to achieve electrowetting and droplet motion.

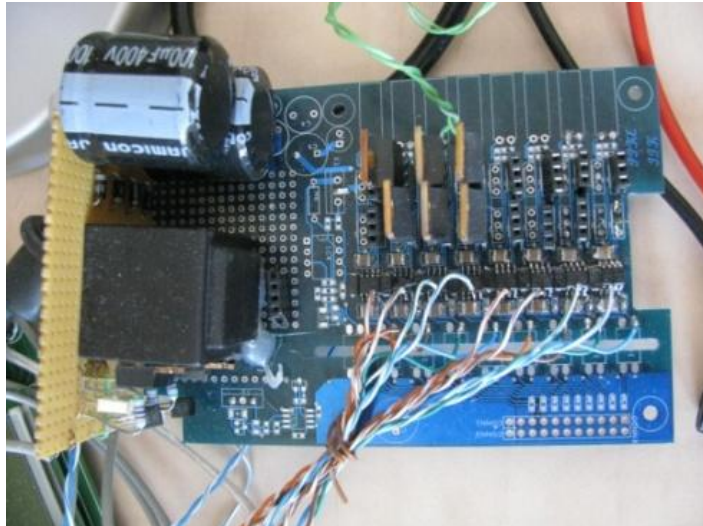


Figure 3. First generation of the high voltage switching circuit. The high voltage power supply with the huge condensers and the transformer can be found on the vertical PCB. Two green lines are available for high power switching on the electrodes at this stage of completion of the circuit.

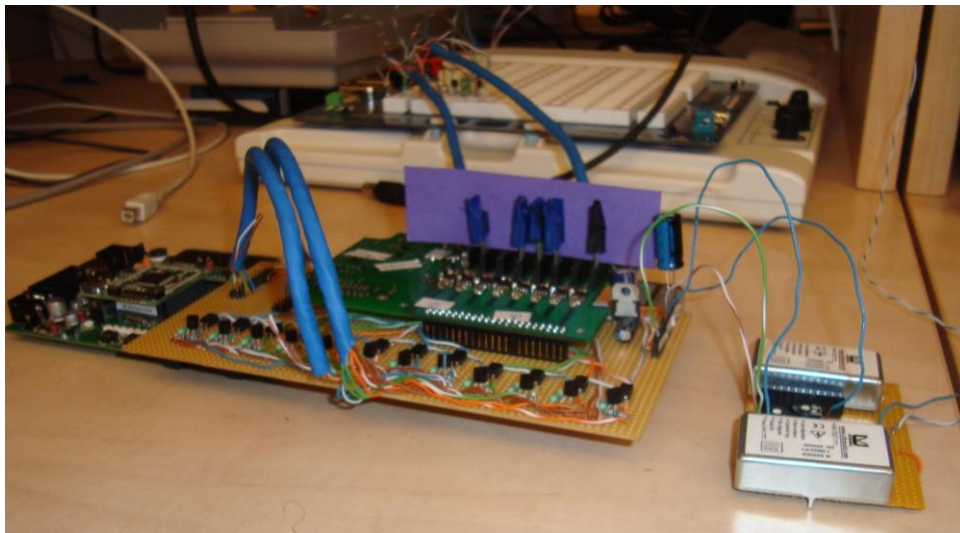


Figure 4. Second generation of the integrated high voltage switching control (left hand side, green PCB with USB microcontroller), switching circuit in the middle with 5 active output channels and Ultravolt's high-power power supplies on the right hand side.

At this stage we have good results with electrode designs, the control circuit is fully operational with a maximum voltage output of 600V and software control is implemented.

4. Numerical model simulations and geometry optimization of microfluidic channel systems

Preliminary experiments have been conducted to implement different chemical and biological measurements that can be performed in a digital microfluidic device, as well. The design considerations are different in microfluidic channels than in digital microfluidic electrode matrices so a different approach had to be adopted. Most of the microfluidic channels that we have designed and tested have been implemented (with the kind help of the MEMS Laboratory of the Research Institute for Technical Physics and Material Science of the Hungarian Academy of Sciences). PDMS was used as a structural element; all channels have been implemented in PDMS and

sealed with a glass slide to provide closed rectangular channel cross sections. A cell culturing device has been designed with different cell capture geometries. The implemented devices showed good cell capturing ability, though clogging have been observed in many areas due to cell adhesion to the PDMS surfaces (see Fig. 5). Furthermore, a different immune reaction based device have been modeled, fabricated and tested, based on the highly specific reaction between the allergen and IgE molecules present in the blood (see. Fig. 6).

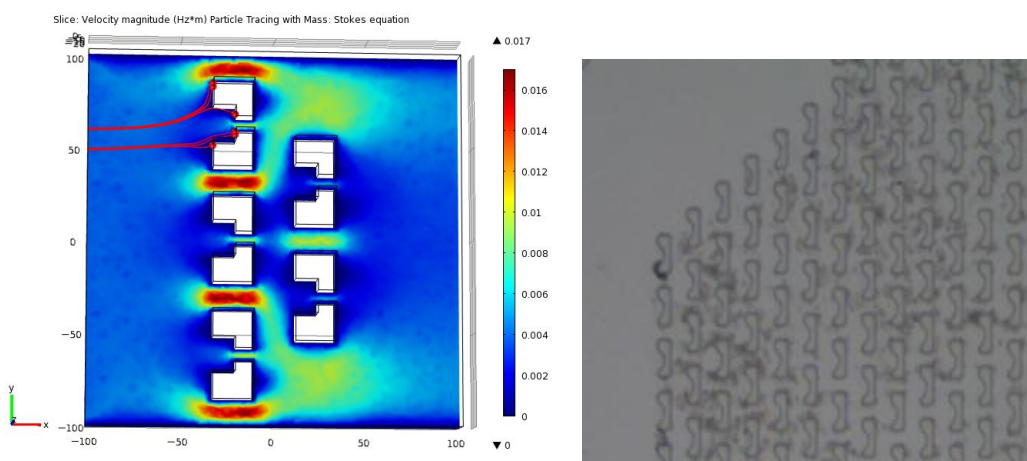


Figure 5. Numerically modeled (left) and implemented (right) microfluidic cell culturing device for different cellular response analysis. Numerical model shows that cells are likely to be captured at the defined geometry. The implemented device shows cell capture and clogging at narrow interconnects.

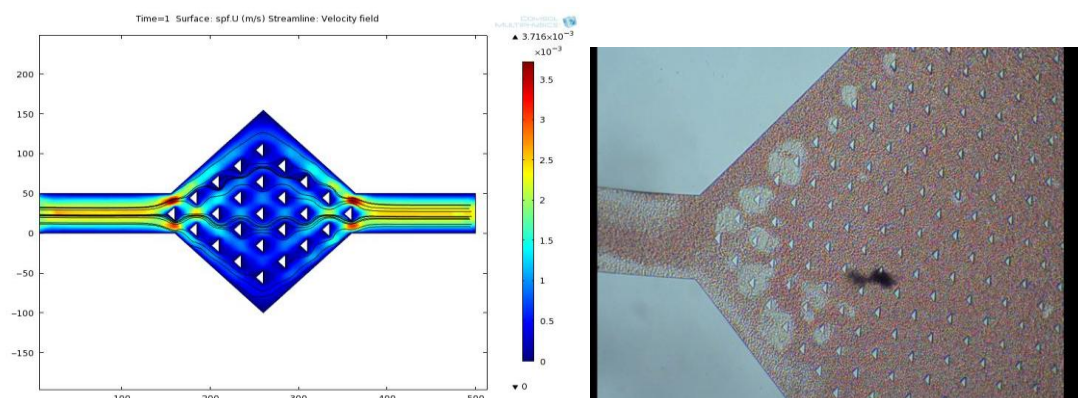


Figure 6. Numerically modeled (left) and implemented (right) microfluidic immunoassay-based system. Surface is coated with BSA-avidin and biotinated allergen (e.g. pollen), and the blood concentration of allergen-specific immunoglobulin-E molecules is measured with fluorescent method (planned).

These models, measurements and results are good preliminary test tools to implement on digital microfluidic platforms.

5. Development of real-time chip monitoring system for microfluidic devices

Both the stereo microscope and also biological microscopes (see Fig. 7) have been used to image digital microfluidic chips and also microfluidic test experiments with different channel systems. The stereo microscope provides maximum 40x resolution with upper light source that allows us to image non-transparent devices, e.g. digital microfluidic platforms (open or closed devices) since PCB material in itself is semi-transparent. Available biological microscopes provide up to 400x resolution with

lower light sources for transmitted light imaging, thus only transparent devices could be imaged with this setup.

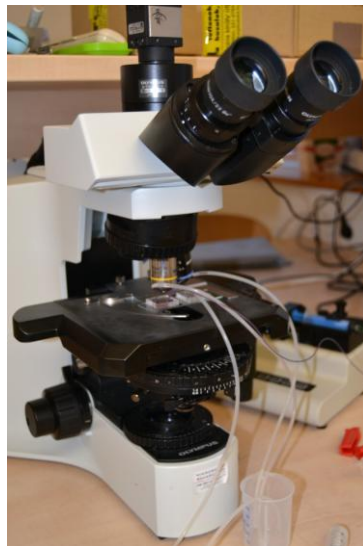


Figure 7. Biological microscope fitted with camera and microfluidic chip with syringe pump for imaging cell capture. (see Fig. 5 or Fig. 6 right hand side images obtained with this setup)

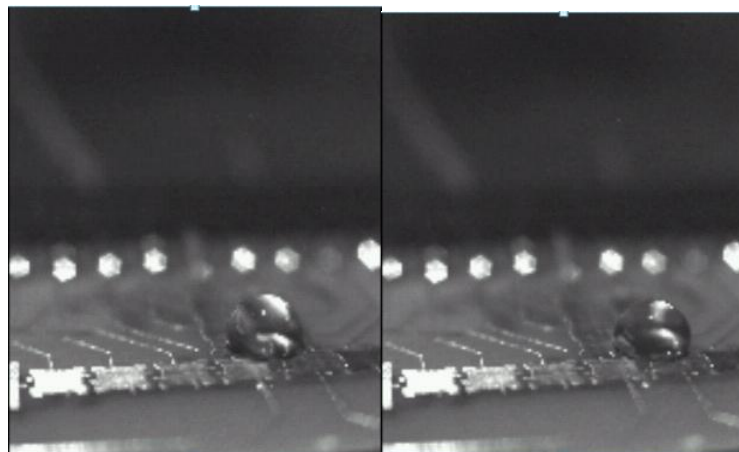


Figure 8. Droplet motion imaged with the stereo microscope and real-time imaging system (frames of a video).

6. **Prototype system for flow cytometric applications**

Microfluidic flow cytometry and cell detection is based on flow focusing microfluidic channels and high-fps camera computer (EyeRIS v1.3, using CNN-UM algorithms) systems. Flow focusing is achieved with a microfluidic channel design based on hydrodynamic focusing. Two side channels with fluid flows are introduced into a stream of liquid forcing it into a narrower geometry. The flow rates are set so that the output channel will have comparable width to blood cells (e.g. 10 microns). The flow is recorded and real time processed using a camera computer. Real-time CNN-UM based image acquisition and processing was part of another project.



Figure 9. Hydrodynamic flow focusing in a microchannel using intravenous blood (left side image). Original high-fps image using 60us exposition time (center), processed image with only cell like objects (right side image).



Figure 10. Prototype system for microfluidic flow cytometric applications. Main parts: syringe pump, upright biological microscope, hydrodynamic focusing microfluidic channel system, high-fps camera computer for real-time imaging.

The results of this part of the research have been accepted at 2 conferences and have been presented.

Differences between planned and actual results

Differences in the financial plan

1. Basic microfluidic devices have been purchased during the first year (e.g. standard connectors, plugs, converters) along with a syringe pump for the preliminary chemical and biological experiments.
2. A numerical modeling software have been planned, but this software have not been specified in detail in the proposal. During the first year we have researched and tried numerous finite element method softwares (e.g. Ansys, OpenFOAM, Fluent) and

considered COMSOL Multiphysics the best price/value bundle. This software have been purchased during the second year of the project and its subscription has been extended in the next year.

3. Slight adjustments have been made to the financial plan along the project to accomodate for over expenses in consumables.
4. Due to the delays in the readily available digital microfluidic platform the droplet routing optimization, biochemical sensor and detector integration and the implementation of an optical detection setup has been canceled, thus the funds separated for these parts have not been expended (e.g. equipment).

Differences in the work plan

The proposed work included the Advanced Liquid Logic company and their products. Without this head-start we had to design, develop and test new digital microfluidic platforms. This development took part of the planned advanced research time, therefore, we alternatively started involving students to develop experiments in other microfluidic devices. This allowed us to have preliminary results on the implementation of these important analyses on digital microfluidic platforms.