2012

Matrix for Chemical IT
(MATCHIT)

Deliverable
Report
D5.2 with
MS 15

Grant Agreement No. 249032

D5.2 Demonstration of chemtainer content extraction, selection and reinjection using integrated gel electrophoresis. (Demonstrator, RUB)

Prelude

This deliverable, first submitted in year 2, has been completed in year 3 with Milestone 15 (M30) using a new microfluidic design and improved control of electrodes. The integration of selected product reinjection into new droplets, which was missing in the year 2 submission, is shown in fig. 1A below. Decisive steps allowing the completion of this milestone (Product selection by electrophoresis in channel, with reinjection into droplet), and hence the deliverable 5.2, included the development of droplet braking structures, with ionic liquid bypass, and the transition to a new ionic liquids with better viscosity/hydrophobicity properties. The figure summarizes the key achievement of product reinjection from an integrated gel separation channel, which enables iterative processing of chemtainers. The integrated demonstrator will be displayed in video format at the final review. Electrophoretic separation of DNA was also perfected in year 3 of the project allowing separations in reversible pluronics gels for single strands in less than a minute over sub mm lengths.

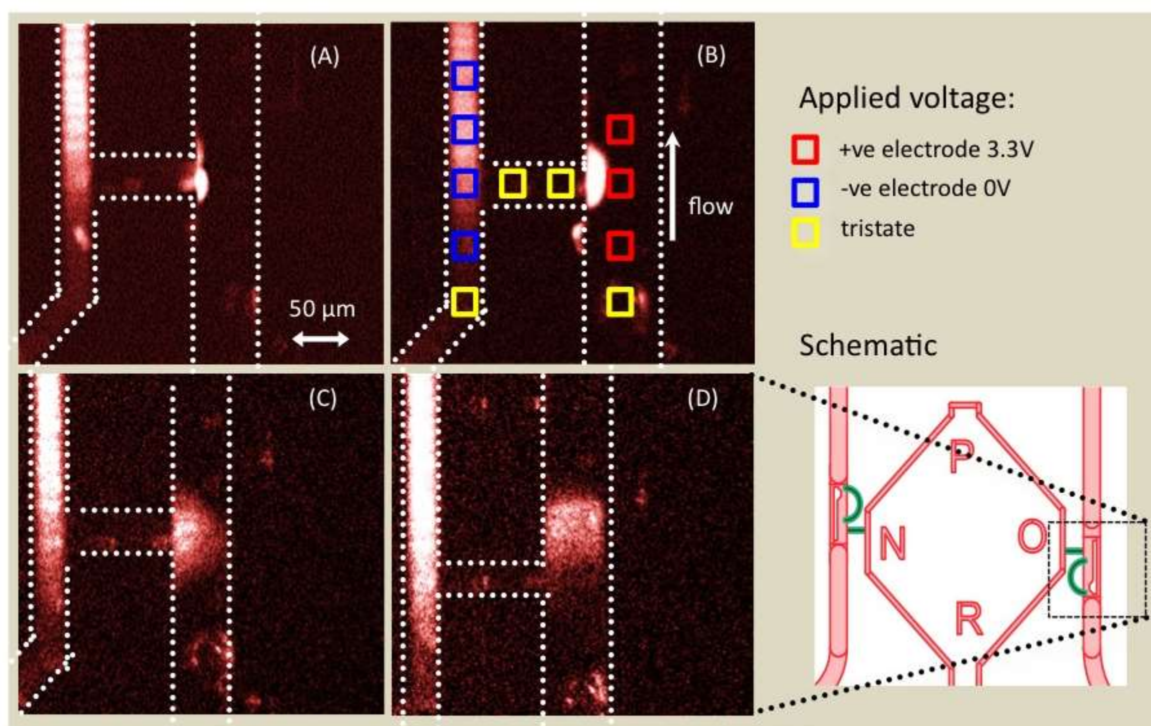

Fig 5.2.A Reinjection of electrophoretically separated product DNA into droplets.
Right: The schematic of the redesigned microfluidic structure is shown on the right. It consists of two droplet flow channels with the special braking structures developed in year 3 of the project. The latter serve to temporarily arrest the flow of droplets, easing the task of reinjection. The integrated electrophoretic gel separation channel (NPOR) is shown in the centre of the schematic.
Left: Images of fluorescent DNA in the closeup of the schematic near O. (A) separated DNA in the gel separation channel on left (B) Electrode configuration used to perform electronic transfer of selected DNA to form a nascent stationary minidroplet in the ionic liquid carrier channel on right. (C) Injected DNA concentrate fuses with incoming aqueous droplet in right channel (D) Droplet moves off down flow channel.
This figure also benefited from work in the CADMAD project applying chemtainer processing to DNA editing tasks, droplet reinjection was a necessary sub-process for both MATCHIT and CADMAD and required a significant investment of resources beyond what was available separately in either project.

1 Introduction

One of the main aims of the MATCHIT project is to establish a DNA-addressable container level chemical information processing that will allow sophisticated programmable and local on going activity in a chemical matrix. DNA-addressing of containers allows autonomous, sequence-directed processing (MIMD rather than SIMD) to take advantage of concentrated and refined chemicals in "clean" chemistry, rather than having to sustain non-equilibrium in the presence of varying mixtures of products and reactants for multiple reactions. Whereas biology employs complex enzymatic sequestration processes on membranes to orchestrate discrete micro-compartments in eukaryotic cells, operating in very complex mixtures through highly evolved information structures, in MATCHIT we are pursuing a purely chemical solution making use of physical feedback information via electrode arrays to separate and clean-up product mixtures. Thus the basic processing cycle that needs to be established is:

1. Set i=1
2. Dock DNA-labelled chemtainer with "purified" chemical set A_i
3. Inject chemical set B_i or fusion with chemtainer with chemical set B_i
4. React mixture A_i+B_i to new product set B_{i+1} with side product set C_i
5. Extract sample of all chemicals for cleanup and redirection
6. Separate product set B_{i+1} from side products C_i and reactant sets A_i and B_i
7. Undock docked chemtainer or move to new site
8. Set i= i+1 and go to step 2

In principle, the sample extraction step could be made selective, so that step 6 could be dispensed with, as in traditional formulations of membrane computing. However, the advantage of step 6, with feedback control is that it is fully electronically programmable, both in the choice of products and in the degree of separation. Of course there is always a trade-off between purity and yield in any extraction or separation process.

In MATCHIT we have succeeded in building an integrated microreactor for completing this entire chemtainer processing cycle, as described in more detail in section 1 of the WP5 activity report. This involved both the development of a novel two-layer electrode array for the separation matrix and a novel chemtainer processor, interfacing chemtainers in a daisy chain architecture to both resource/waste channels and the separation matrix as shown in fig. 1. The design also allows varying degree of separation through branching points in the separation matrix, and through the systematic use of hydrodynamic barriers and reversibly gelled separation media (custom pluronics mixtures) both upstream and downstream product transfer in the chemtainer (droplet) daisy chain.
The figure shows successive chemtainer droplets moving along the meandering daisy chain channel under white light. The chemtainers also allow the chemical processing "packages" to be transported on and off chip.

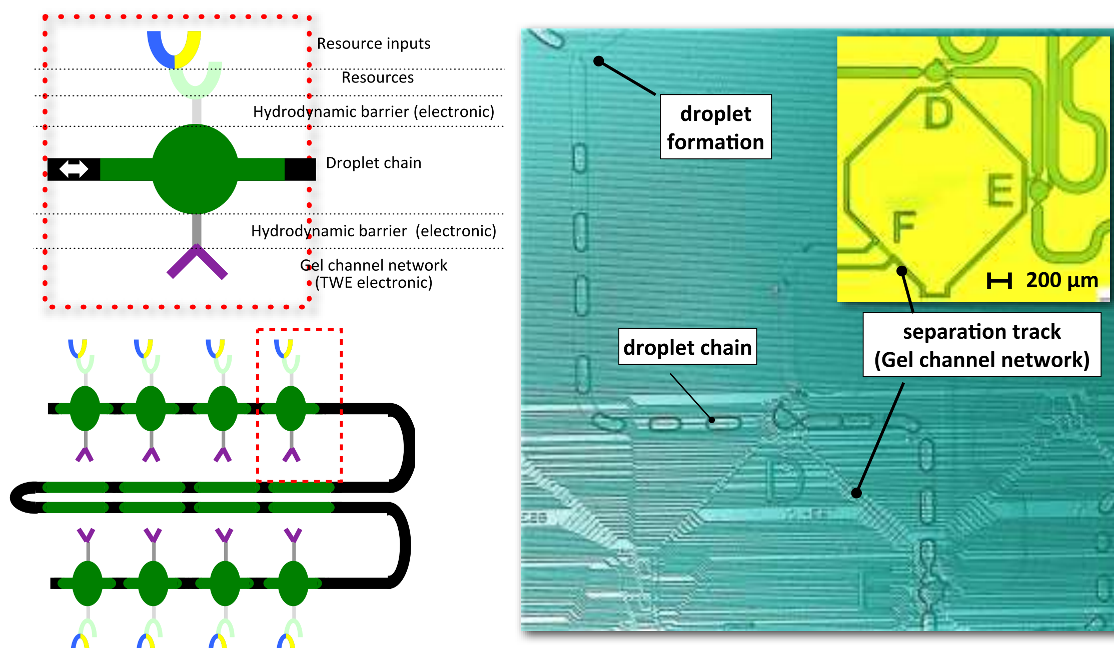

Figure 5.2.1: The left scheme shows a schematic view of the basic network module of the electronically programmable chemical matrix involving a fast serial droplet track (green/black) and slow (grey/violet) gel based reaction processing tracks. The right large microscope image illustrates the complete assembled PDMS microfluidics with electronic layer with droplet flow inside. The small yellow image shows the SU8 master structure for PDMS rapid prototyping. The resource channels are shown in the upper right corner of the image and the inset. The overall microfluidic design is shown in fig. 5.2.2.

The overall design concept is shown on the right of fig. 5.2.2. The droplet meander crosses the chip four times horizontally, compared with the three shown in the proposal schematic on the left, and material can be processed to both upstream or down stream droplets. In this design, extraction and reinjection is only at dedicated sites (8 of them are included in this stage of the processor, A-H).

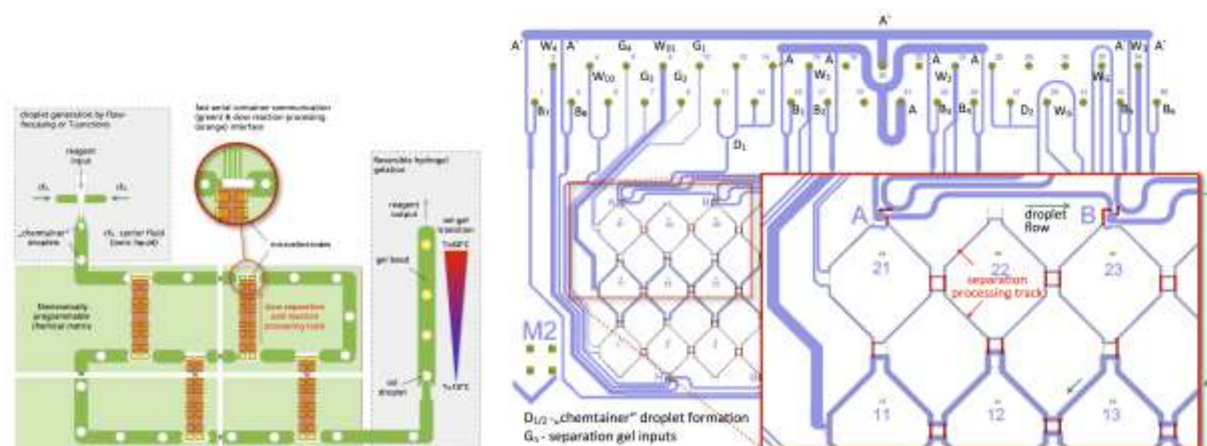

Figure 5.2.2: Electronically programmable chemical matrix. Left: original proposed integration scheme for a Chem-IT matrix involving a fast serial droplet track (green) and slow (orange) gel based reaction processing tracks. Iterative processing of material is possible via delivery of container contents to vertical gel segments (orange) that allow transfer to upstream containers. Right: full droplet processor photomask design.

Along the route to this overall solution for MATCHIT, a variety of self-contained test structures were developed to better explore sub-processes, such as extraction and separation. The electrode arrays with one dimensional on chip wiring and two dimensional on chip wiring were employed by all these test structures and the completed overall chemtainer processor. One such structure, used extensively to test the extraction and separation processes is shown in Fig. 5.2.3. We now turn to the demonstrations of all the separate processing steps. Their integration into the complete microreactor shown in fig. 5.2.2 is

only partially complete, as this reactor has only become available recently because of electronic layer construction problems (now solved).

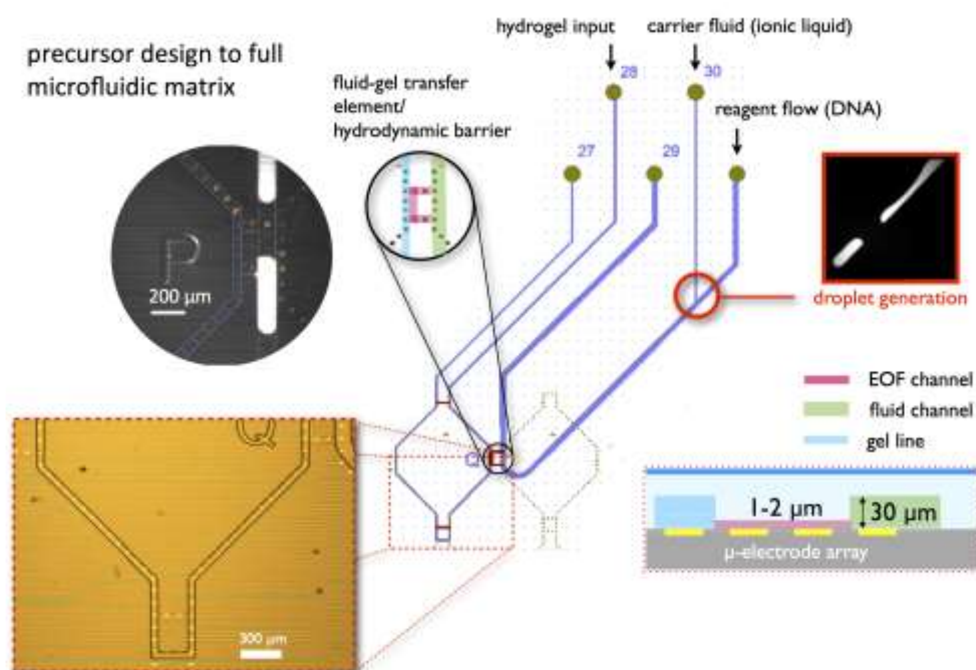

Figure 5.2.3: Integrated microfluidics for combined droplet generation and content processing. This important core functionality have been realised in a precursor design to full microfluidic matrix as shown as a mask design in the background. The experimental design shows two input channels - one used for the carrier fluid (30) and other for the reagent flow as well to generate droplets. Furthermore there is an input (28) for the hydrogel separation matrix. It contains also a fluid-gel transfer element used for Chemtainer extraction and injection, which also can act as a hydrodynamic barrier.

2 Chemtainer content extraction

2.1 Demonstrator functionalities (movie list)

Extraction of DNA from fluid into gel filled with other DNA: ng_biopro_vid_20111130_1455xxx_0_elec.mpg

This experiment (30nt, 200nM and 16nt, 200nM) shows the loading of the gel-phase (right blue channel filled with Pluronic gel and 30nt oligo) with the other DNA (left channel, red color, 16nt oligo).

Extraction of DNA from droplets into separation gel: D5_2_droplet_extraction.m4v

A droplet flow was created using an oligomer solution (8nt, DNA-Alexa647) and carrier ionic fluid (the ionic liquid system ([MeBu][BTA]) in the left channel (Figure 5.2.4). The right blue channel was filled with Pluronic gel and 30nt DNA-Alexa488. Small samples of the red labelled DNA have been extracted from droplets in the separation channel.

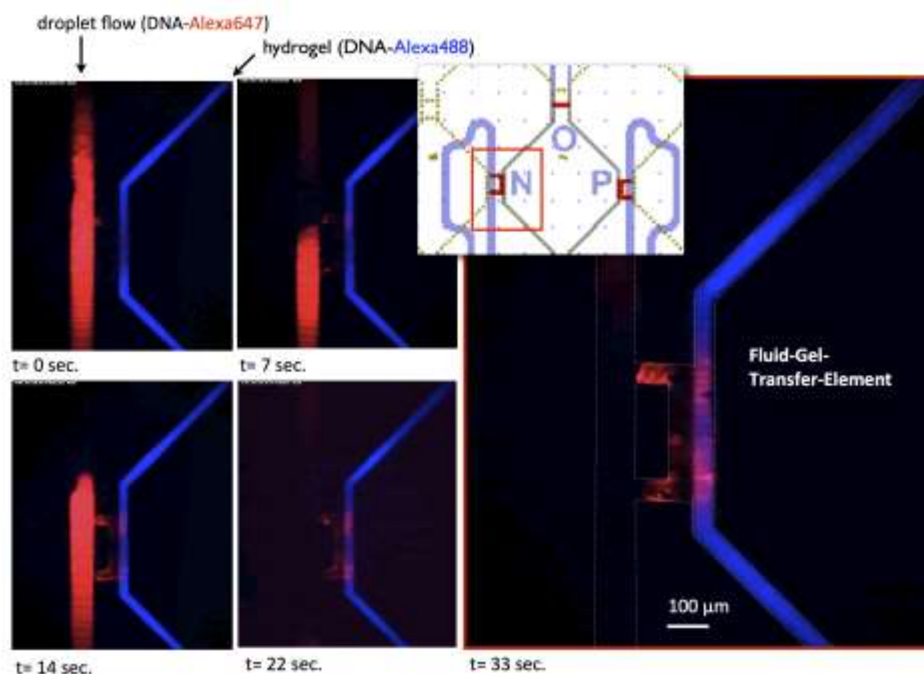

Figure 5.2.4: The movie sequences illustrate the droplet flow in the left channel, and the content extraction into the separation channel using the fluid-gel-transfer element.

Extraction into gel channel and separation: ng_biopro_vid_20120220_1541xxx_0_elec.mp4

This movie shows one of the issues with extracting DNA-material (24nt, 200nM and 12nt, 200nM) from the fluid resource channel into the gel-phase. In this case, after extraction, the DNA-cluster did not move properly in the separation channel. Our current explanation for this problematic behaviour is an interaction between the channel walls and DNA. However, hydrophilisation of the channel walls (data not shown) did not yet reveal a solution to this problem, so that we cannot attribute it uniquely to the hydrophobic fluorescent label. Saturation of the channel walls with DNA before the experiment appears to help.

Extraction with separation 24mer & 30mer Oligos: ng_biopro_vid_20111212_1643xxx_0_elec.mpg

The DNA mixture (24nt, 200nM and 30nt, 200nM) was extracted from the resource channel and moved to the separation lane. Separation though was not yet complete. Only a small difference in mobility between the two oligonucleotides was available for separation. After trying to separate the first cluster, a second cluster was collected and sent along the separation channel, it then collides with the first cluster.

3 Chemtainer content separation

The summary of all experiments comprises several phases of experimentation and software-development. First experiments were done with a mixture of two short DNA-oligomers which were loaded into the separation channels. In that phase, the first task was to create clusters of the formerly evenly distributed DNA-mixture in the gel and then try to separate the mixture. Several different buffers and gels were tested.

Date	Title	Module	Buffer	Salt	Gel	DNAs
28. Jun. 2011	TWE in TBE buffer	W367291210	TBE		Pluronic	8mer, 20mer
5. Jul. 2011	TWE two colors	W367291210	His 50mM pH7,2		25% Pluronic(87:127;2:1)	Cy5-Oligo 1*10e-6M, Oligo26(Alexa488) 0,5*10e-6M
11. Jul. 2011	TWE two colors II	W367291210	His 50mM pH7,2		25% Pluronic(87:127;2:1)	Oligo N (24mer)2e-7 M Alexa 647, Oligo 4 (30mer) 2e-6M Dy481XL
14. Jul. 2011	TWE two colors III	W367291210	His 50mM pH7,2		25% Pluronic(87:127)1:1	OligoN(24-mer)Alexa647 1*10e-7M, Oligo4(30-mer)Dy481XL 1*10e-6M
7. Sep. 2011	Feedback-TWE-II	W367291210	His 50mM pH7,2		25% Pluronic(127:87;2:1)	Oligo N (24mer)1e-7 M Alexa 647 + Oligo 4 (30mer) 5e-7M
15. Sep. 2011	Feedback-TWE-III	W367291210	His 50mM pH7,2		25% Pluronic(127:87;2:1	Oligo N (24mer)1e-7 M Alexa 647 + Oligo 4 (30mer) 5e-7M Dy481XL
28. Sep. 2011	TWE-IV	W367291210	His 50mM pH7,2		30% Pluronic(87:127;2:1)	Oligo1 (30nt, Oregon-Green) 1*10e-6M + Oligo20(8nt, Alexa647) 1.0*10e-6M
11. Okt. 2011	TWE-V	W367291210	TrisAcetat 50mM pH 7,5		30% Pluronic(87:127;2:1)	Oligo1(30nt, Oregon-Green) 2*10e-7M + Oligo20(8nt, Alexa 647) 2*10e-7M
12. Okt. 2011	TWE-VI	W367291210	His 50mM pH 7,2	1mM Na2HPO4	30% Pluronic(87:127;2:1)	Oligo1(30nt, Oregon-Green) 2*10e-7M + Oligo20(8nt, Alexa 647) 2*10e-7M
19. Okt. 2011	TWE-VII	W367291210	His 50mM pH 7,2	5mM Na2HPO4	30% Pluronic(87:127;2:1)	Oligo1(30nt, Oregon-Green) 1*10e-7M + Oligo20(8nt, Alexa 647) 1*10e-7M

Date	Title	Module	Buffer	Salt	Gel	DNAs	Movies
9. Nov. 2011	TWE-X	W367291210	His 50mM pH 7,2	1mM Na2HPO4	30% Pluronic(87:127;2:1)	Oligo1(30nt, Oregon-Green) 1*10e-7M + Oligo20(8nt, Alexa 647) 1*10e-7M	Loaded separation with moving camera and distributed feedback-control. ng_biopro_vid_20111109_1708xxx_0_elec.mpg
21. Nov. 2011	TWE-XI	W372231210	His 50mM pH 7,2	1mM Na2HPO4	30% Pluronic(87:127;2:1)	Oligo1(30nt, Oregon-Green) 1*10e-7M + Oligo22(16nt, Alexa 647) 1*10e-7M	
23. Nov. 2011	TWE-XII	W372291210	His 50mM pH 7,2		30% Pluronic(87:127;2:1)	Oligo1(30nt, Oregon-Green) 2*10e-7M + Oligo22(16nt, Alexa 647) 2*10e-7M	
29. Nov. 2011	TWE-XIII	W372231210	50mM TrisAcetate		25% Pluronic(87:127;2:1)	Oligo1(30nt, Oregon-Green) 2*10e-7M + Oligo22(16nt, Alexa 647) 2*10e-7M	
30. Nov. 2011	TWE-XIV	W366291210	His 50mM pH 7,2		30% Pluronic(87:127;2:1)	Oligo1(30nt, Oregon-Green) 2*10e-7M + Oligo22(16nt, Alexa 647) 2*10e-7M	Extraction of DNA from fluid into gel filled with other DNA. ng_biopro_vid_20111130_1455xxx_0_elec.mpg
5. Dez. 2011	TWE-XV	W373291210	50mM His		30% Pluronic(87:127;2:1)	Oligo1(30nt, OregonGreen) 2*10e-7M + Oligo22(16nt, Alexa 647) 2*10e-7M	
7. Dez. 2011	TWE-XVI	W373291210	50mM His	0.1mM Na2HPO4	30% Pluronic(87:127;2:1)	OligoN2(24nt, Alexa647) 2*10e-7M + OligoFN2(12nt, Alexa 488) 2*10e-7M	
12. Dez. 2011	TWE-XVII	W373291210	50mM His	10uM Na2HPO4	30% Pluronic(87:127new;2:1)	OligoN2(24nt, Alexa647) 2*10e-7M + Oligo2(30nt, Alexa 488) 2*10e-7M	Extraction of DNA with following separation 24mer and 30mer Oligos. ng_biopro_vid_20111212_1643xxx_0_elec.mpg
9. Feb. 2012	TWE-XIX	W381201211	50mM His pH7,2	10uM Na2HPO4	30% Pluronic(87:127;2:1)	Oligo22(16nt, Alexa647) 2*10e-7M + Oligo1(30nt, OregonGreen) 2*10e-7M	
14. Feb. 2012	TWE-XX	W381201211	50mM His pH7,2	10uM Na2HPO4	18% Pluronic127	OligoN2(24nt, Alexa647) 2*10e-7M + OligoFN2(12nt, Alexa488) 2*10e-7M	
20. Feb. 2012	TWE-XXI	W381201211	50mM His pH7,2		25% Pluronic(87:127;1:2)	OligoN2(24nt, Alexa647) 2*10e-7M + OligoFN2(12nt, Alexa488)	Extraction of DNA into gel channel and separation. ng_biopro_vid_20120220_1541xxx_0_elec.mp4

The second phase was a software-development-phase which introduced camera tracking of the active local region and which made the distributed feedback-control transferable into a moving feedback-controller.

The third phase is characterized by trying to extract a DNA-mixture from a fluid resource channel into a gel. This fluid resource channel can be seen as a very long droplet or plug. In the following four selected movies will be explained in more detail.

3.1 Demonstrator functionalities (movie list)

Separation with moving camera and distributed feedback-control: ng_biopro_vid_20111109_1708xxx_0_elec.mpg

Two oligomers (30nt, 100nM and 8nt, 100nM) where filled into a channel. The first part of the feedback-controlled algorithm collected the mixture in the channel and the created plug which was then subjected to separation in the gel. This experiment session tested the new moving camera with distributed feedback-control. Every electrode in the channel had its own controller that was activated by the preceding controller. The algorithm was such that after two separate clusters emerged a negative electrode in the middle of these two clusters switched to a negative potential and hence the controller moved the slower DNA back along the separation channel.

Extraction of DNA from fluid into gel filled with other DNA: ng_biopro_vid_20111130_1455xxx_0_elec.mpg

This experiment (30nt, 200nM and 16nt, 200nM) shows the loading of the gel-phase (right blue channel filled with Pluronic gel and 30nt oligo) with the other DNA (left channel, red color, 16nt oligo).

Extraction with separation 24mer & 30mer Oligos: ng_biopro_vid_20111212_1643xxx_0_elec.mpg

The DNA mixture (24nt, 200nM and 30nt, 200nM) was extracted from the resource channel and moved to the separation lane. Separation though was not yet complete. Only a small difference in mobility between the two oligonucleotides was available for separation. After trying to separate the first cluster, a second cluster was collected and sent along the separation channel, it then collides with the first cluster.

Extraction into gel channel and separationng_biopro_vid_20120220_1541xxx_0_elec.mp4

This movie shows one of the issues with extracting DNA-material (24nt, 200nM and 12nt, 200nM) from the fluid resource channel into the gel-phase. In this case, after extraction, the DNA-cluster did not move properly in the separation channel. Our current explanation for this problematic behaviour is an interaction between the channel walls and DNA. However, hydrophilisation of the channel walls (data not shown) did not yet reveal a solution to this problem, so that we cannot attribute it uniquely to the hydrophobic fluorescent label. Saturation of the channel walls with DNA before the experiment appears to help.

3.2 PDMS surface modification

"... there are several issues with poly(dimethylsiloxane) (PDMS) when it is applied in biomedical research, among which diffusion of small molecules into the PDMS matrix is the most serious one and still unsolved." (Lei et al., 2011 [1])

PDMS is the material of choice for microfluidic devices due to its prototyping capability using softlithographie, gas permeability, biocompatibility and optical transparency. The elastomer is able to bonding itself, glass as well as silicon using oxygen plasma treatment. However, the strong hydrophobic properties of PDMS and fast hydrophobic recovery after surface hydrophilization, negatively impacts on the performance of PDMS-based microfluidic device components.
A further disadvantage is also, that the electroosmotic/electrophoretic mobility, is often unstable, and hydrophobic small molecules (especially intercalating fluorophores) can adsorb onto the PDMS surface and interfering with the analysis. Penetration of molecules into PDMS can significantly change

[1] Lei Y, Liu Y, Wang W, Wu W and Li Z 2011 Studies on Parylene C-caulked PDMS (pcPDMS) for low permeability required microfluidics applications Lab Chip 11 1385

solution concentrations and could modify experimental results. The PDMS absorption of Nile red dye and its potential impact on microfluidic experiments was discussed by Beebe etal..[2]
The recent literature contains a vast number of PDMS surface modification methods. There are two important reviews, giving a good overview about the methods, which were used to change the surface properties of PDMS.[3][4]
Three main categories of PDMS surface modification methods are possible: gas-phase processing including plasma oxidation, UV irradiation and chemical vapor deposition (CVD). Furthermore, wet chemical methods like layer-by-layer deposition, sol-gel coatings, silanization, dynamic modification with surfactants as well as protein adsorption are available. Examples for covalent and noncovalent immobilisation of PDMS are e.g. multilayer poly(vinyl alcohol) coating,[5] polymer grafting[6], polyelectrolyte and surfactant[7] coating as well as phospholipid bilayer formation.[8]
Finally, there were reported combinations of gas-phase and wet chemical methods include silanization and LBL methods on PDMS surfaces. In most cases the latter methods based on a pretreatment by plasma oxidation.[9][10]
After careful examination, the covalent immobilisation of PDMS channel walls using wet chemical methods is the best choice to do an effective hydrophilisation in closed microchannels.

The following method based on poly(ethylene glycol) (PEG) grafted PDMS channel walls[11] was successfully tested in our microfluidic devices:
The first step is a channel solution oxidation using $H_2O/H_2O_2/HCl$ (5:1:1) to produce active Silanol groups, but there are only stable for a couple of hours, because of the recovery of hydrophobicity. After special washing and drying steps we have treated the microfluidic PDMS channels with 2-[methoxy(polyethylenxy)propyl]tri-methoxysilane using a slow continuous flow rate for 30 min. This hydrophilisation procedure should repeated after a two times use to refresh the wettability properties..

4 Chemtainer content injection

4.1 Demonstrator functionalities (movie list)

Injection using micro electrodes: D5_2_DNA_Processor_twin_chamber_Filling_elec.m4v

The left scheme in figure 5.2.5 illustrates a part of the CAD mask design of the microfluidic channel arrangement. The left and middle channel has been filled with gelated matrix of Pluronic (30% Pluronic F87 : F127 = 2:1 in His-buffer 50 mM pH 7.2) at 30°C. The right channel is flushed with a continuous flow of DNA-Alexa488 silution. The right image shows injected DNA solution using microelectrodes inside the hydrodynamic barrier.

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[2] Toepke, M. W., & Beebe, D. J. (2006). PDMS absorption of small molecules and consequences in microfluidic applications. Lab On A Chip, 6(12), 1484. doi:10.1039/b612140c
[3] Zhou, J., Ellis, A. V., & Voelcker, N. H. (2010). *Recent developments in PDMS surface modification for microfluidic devices.* (Z. El Rassi, Ed.)Electrophoresis, 31(1), 2–16. doi:10.1002/elps.200900475.
[4] Makamba, H., Kim, J. H., Lim, K., Park, N., & Hahn, J. H. (2003). Surface modification of poly(dimethylsiloxane) microchannels. Electrophoresis, 24(21), 3607–3619. doi:10.1002/elps.200305627
[5] D. Wu, Y. Luo, X. Zhou, Z. Dai, B. Lin Electrophoresis 2005, 26, 211–218.
[6] Hu, S. W.; Ren, X. Q.; Bachman, M.; Sims, C. E.; Li, G. P.; Allbritton, N. Anal. Chem. 2002, 74, 4117-4123.
[7] Liu, Y.; Fanguy, J. C.; Bledsoe, J. M.; Henry, C. S. Anal. Chem. 2000, 72, 5939-5944
[8] Rolland, J. P.; Van Dam, R. M.; Schorzman, D. A.; Quake, S. R.; DeSimone, J. M. J. Am. Chem. Soc. 2004, 126, 2322-2323
[9] Abate A R, Lee D, Do T, Holtze C and Weitz D A 2008 Glass coating for PDMS microfluidic channels by sol–gel methods Lab Chip 8 516–518.
[10] Kim B-Y, Hong L-Y, Chung Y-M, Kim D-P and Lee C-S 2009 Solvent-Resistant PDMS Microfluidic Devices with Hybrid Inorganic/Organic Polymer Coatings Adv. Funct. Mater. 19 3796–3803.
[11] Sui G, Wang J, Lee C-C, Lu W, Lee S P, Leyton J V, Wu A M and Tseng H-R 2006 Solution-Phase Surface Modification in Intact Poly(dimethylsiloxane) Microfluidic Channels Anal Chem 78 5543–5551.

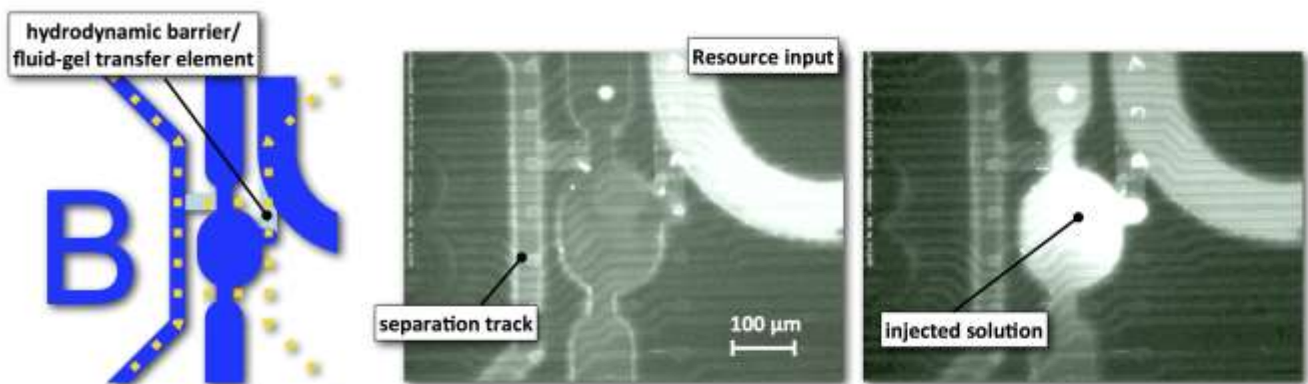

Figure 5.2.5: Injection of DNA content via resource channel using microelectrodes inside the hydrodynamic barrier.

Droplet parking via gelation and pressure injection:
D5_2_DNA_Processor_twin_chamber_Filling_droplets.m4v

The left scheme in figure 5.2.6 illustrates a part of the CAD mask design of the microfluidic channel arrangement including hydrodynamic barriers as well as the fluid-gel transfer element. The middle white light microscope image shows the continuous slow droplet flow (5 µl/h) using a solution of Pluronic (30% Pluronic F87 : F127 = 2:1 in His-buffer 50 mM pH 7.2) and air as a separating media at 10°C. Pluronic melting temperature: Tm = 22°C. The increase of chip temperature to 30 °C leads to the gelation of the Pluronic hydrogel and located microdroplets in microchannels. The right image shows the injected fluorescence labelled (Alexa488) DNA solution.

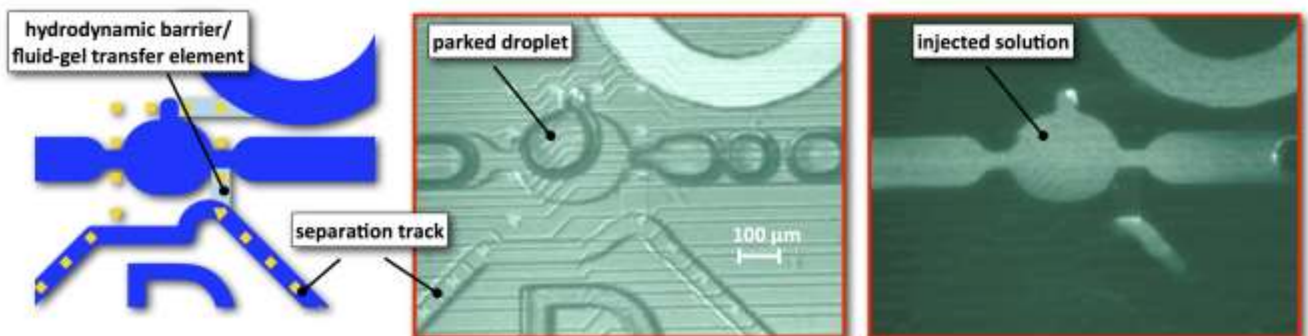

Figure 5.2.6: Parking of droplets via gelation using thermoresponsive hydrogels and injection of DNA content via resource channel.

Demonstrator movies can be downloaded at:
https://sibelius.biomip.rub.de/bmcmyp/Data/MATCHIT/Year+2+Report/wp5/movie
https://sibelius.biomip.rub.de/bmcmyp/Data/MATCHIT/Year+3+Report/wp5/movie
Please use the QuickTime player to view the movies!