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Paving the Way for Future Emerging DNA-based Technologies: Computer-Aided Design and Manufacturing of DNA libraries

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1 As specified in Annex I

2 i.e. name of the person(s) responsible for the preparation of the document

3 Short name of partner(s) responsible for the deliverable

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Deliverable 3.9 Annex

1 List of microfluidic designs and fabricated chips for CADMAD

The following list contains all fabricated microfluidic chips that are used for experimental tests to investigate on-chip DNA processing steps for CADMAD accurately described in Deliverable 3.9. Of the 10 chip designs employed in CADMAD, 4 (Figs 1,2,3,7 as detailed in footnotes) were either designed or built and tested in synergy (shared costs) with one of two other EU projects (MATCHIT and ECCell) which also addressed in part the general issues of processing DNA in droplets. The remaining 6 designs were specific and unique to CADMAD.

1.1 DNA microprocessor chip

1.1.1 DNA microprocessor - full design (1st year activity)

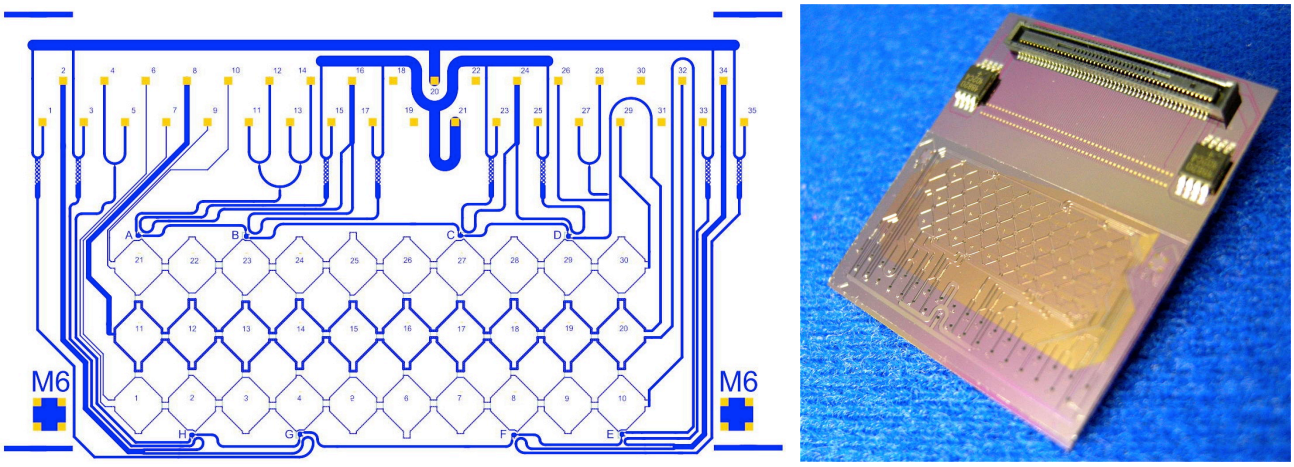


Fig. 1 Left: Overall fluidic CAD design of full DNA processor; Right: Image of full assembled DNA microprocessor with silicon micro-machined electrode layer and micro-molded PDMS fluidics.[7]

[7] Produced in first year of CADMAD project and reported in activity report year one by RUB. Design and fabrication as generic DNA processor for MATCHIT, ECCell and CADMAD projects.This enabled us to proceed rapidly and efficiently in CADMAD year to begin to address the CADMAD specific issues of steps 2-6. CADMAD specific features include the long DNA separation paths (to support separation of longer DNA) and the two horizontal layers of droplet processing to support the integration of two or more Y operations. In the MATCHIT and ECCell projects, the synergy with CADMAD was explicitly cross-referenced.

1.1.2 DNA microprocessor - sub designs (1st and 2nd year activity)

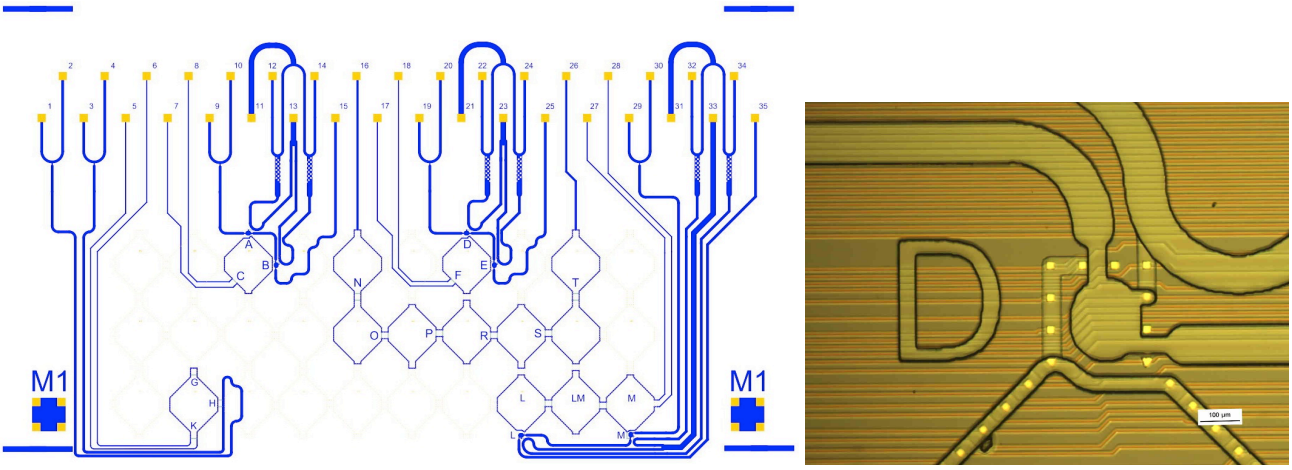


Fig. 2 Left: CAD design of DNA processor sub-functionalities for experimental investigation of droplet formation, injection, separation and reinjection; Right: Light microscopic image of a chip section.[8]

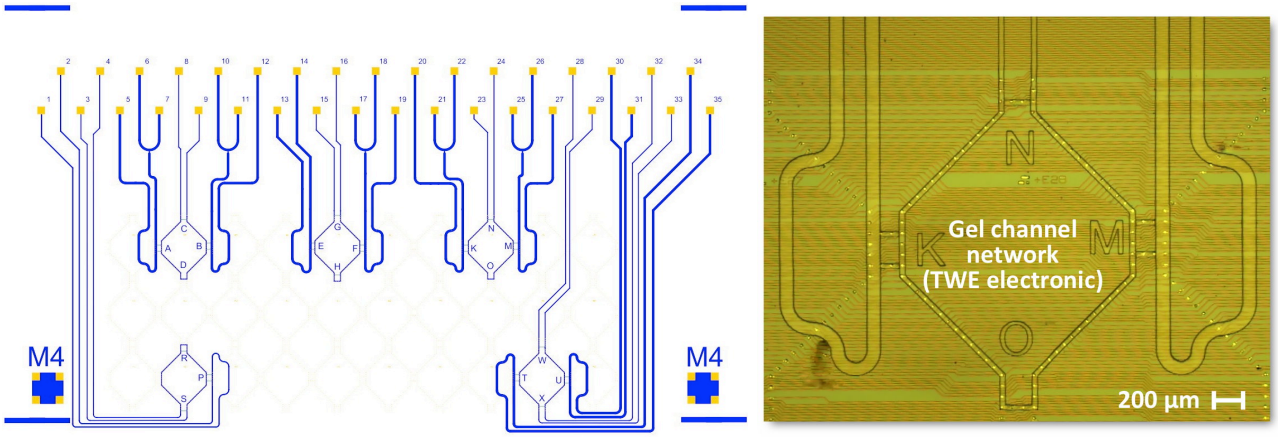


Fig. 3 Left: CAD design of DNA processor sub-functionalities for experimental investigation of droplet formation, injection, separation and reinjection; Right: Light microscopic image of a chip section.[9]

[8] Designed in ECCell project to exemplify generic DNA droplet processing. First fabricated and tested both for chemtainer processing in MATCHIT and for first steps of microfluidic DNA library synthesis in CADMAD: 50% time and effort, for synergy reasons in 2 projects.

[9] Design, fabrication and tests shared effort (50:50) in MATCHIT and CADMAD projects. DNA separation testing only continued in year two of CADMAD.

1.2 Droplet processor (2nd year activity)

1.2.1 Droplet processors – 1st design iteration

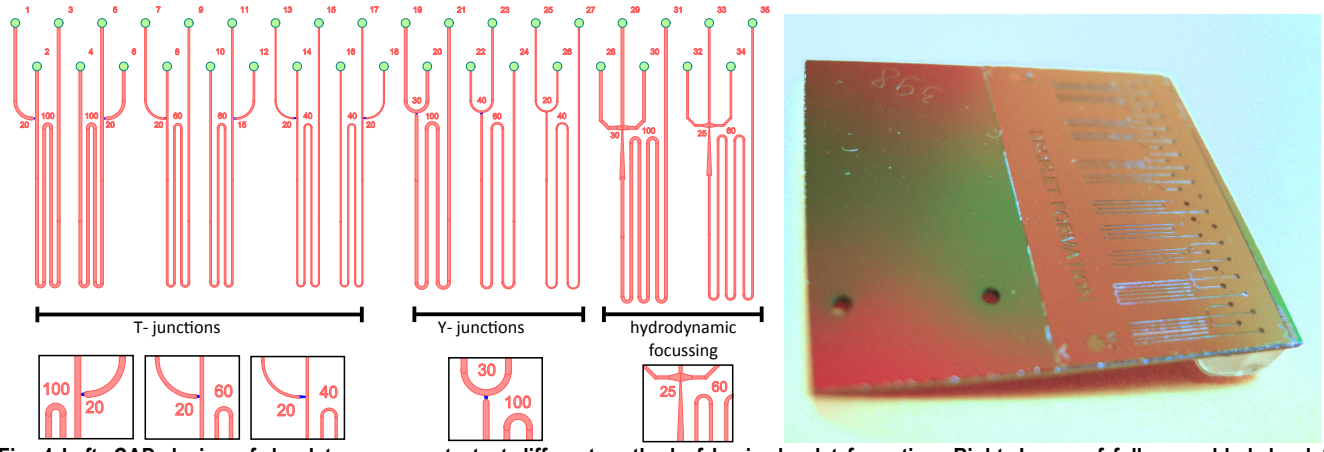


Fig. 4 Left: CAD design of droplet processor to test different method of basic droplet formation; Right: Image of full assembled droplet processor on silicon with micro-molded PDMS fluidics and backside interconnection.

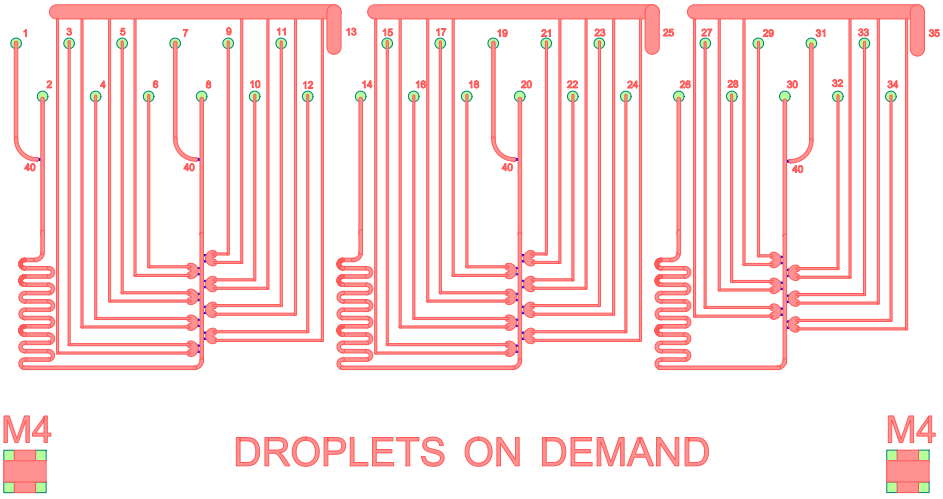


Fig. 5 Left: CAD design of droplet processor to test droplets on-demand formation.

1.2.2 Droplet processors – 2nd design iteration

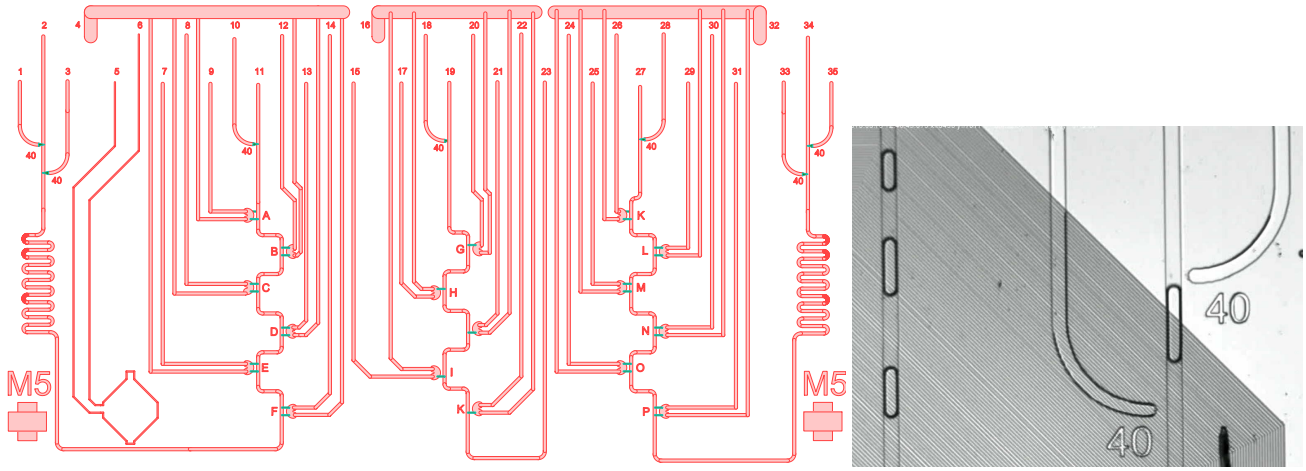


Fig. 6 Left: CAD design of droplet processor for experimental investigation of alternating droplet formation and droplet mixing; Right: Light microscopic image of a chip section for alternating droplet generation.

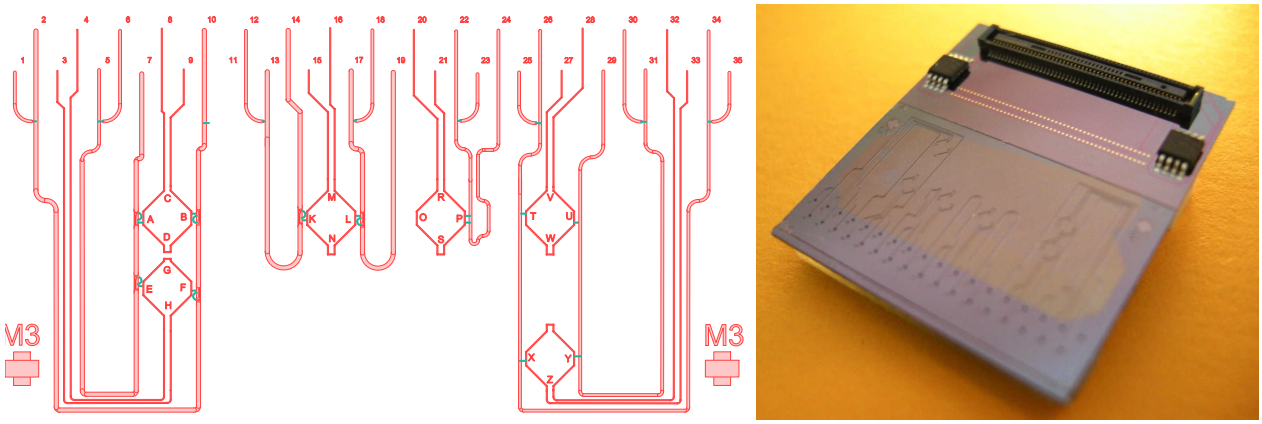


Fig. 7 Left: CAD design of droplet processor. The functionalities are: droplet formation, droplet braking, injection, reinjection and separation of DNA; Right: Image of full assembled DNA microprocessor with silicon micro-machined electrode layer and micro-molded PDMS fluidics.[10]

1.2.3 Droplet processors – 3rd design iteration

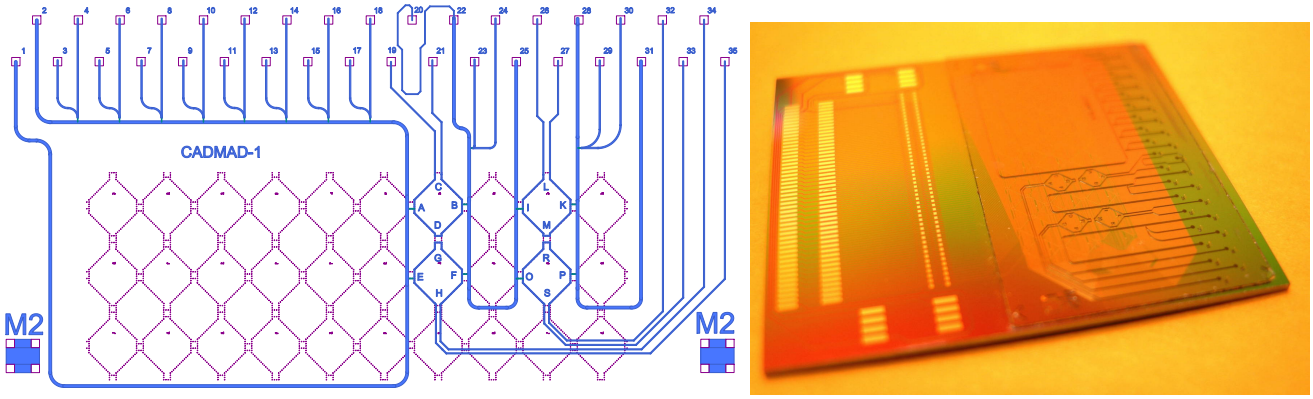
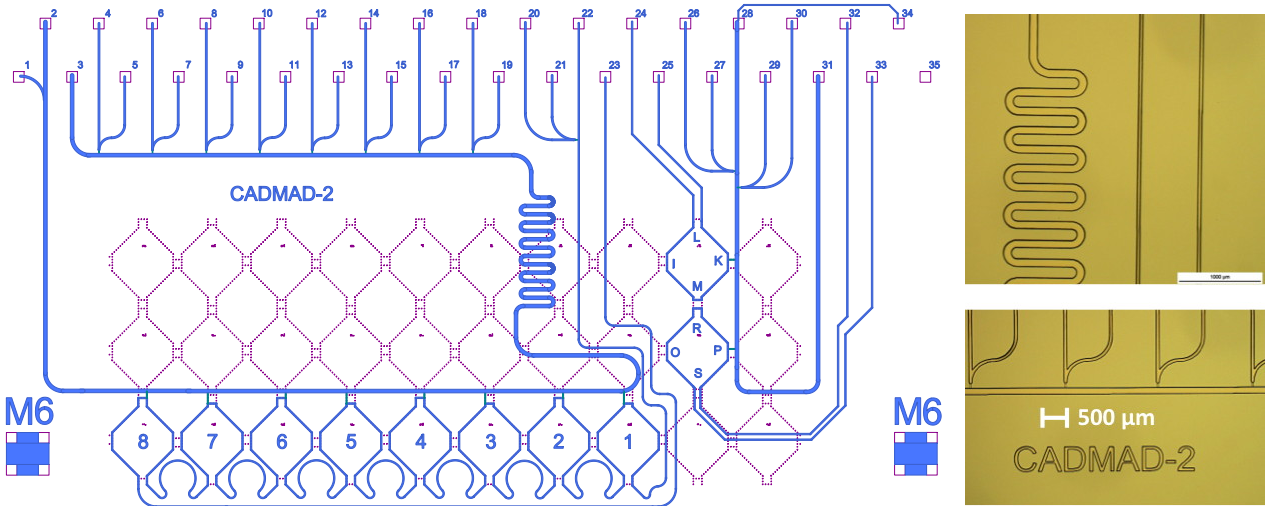


Fig. 8 Left: CAD design of further developed droplet processor. Functionalities: combinatorial droplet formation & mixing, injection, separation, reinjection; Right: Image of full assembled chip with silicon micro-machined electrode layer and micro-molded PDMS fluidics.



[10] Design, fabrication and tests jointly in MATCHIT (e.g. D5.2) and CADMAD (D3.9) with shared costs for synergy reasons. MATCHIT: "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."

Fig. 9 Left: CAD design of further developed droplet processor. Functionalities: combinatorial droplet formation & mixing, on-chip DNA synthesis, injection, separation and reinjection; Right: Examples of light microscopic images of selected chip sections.

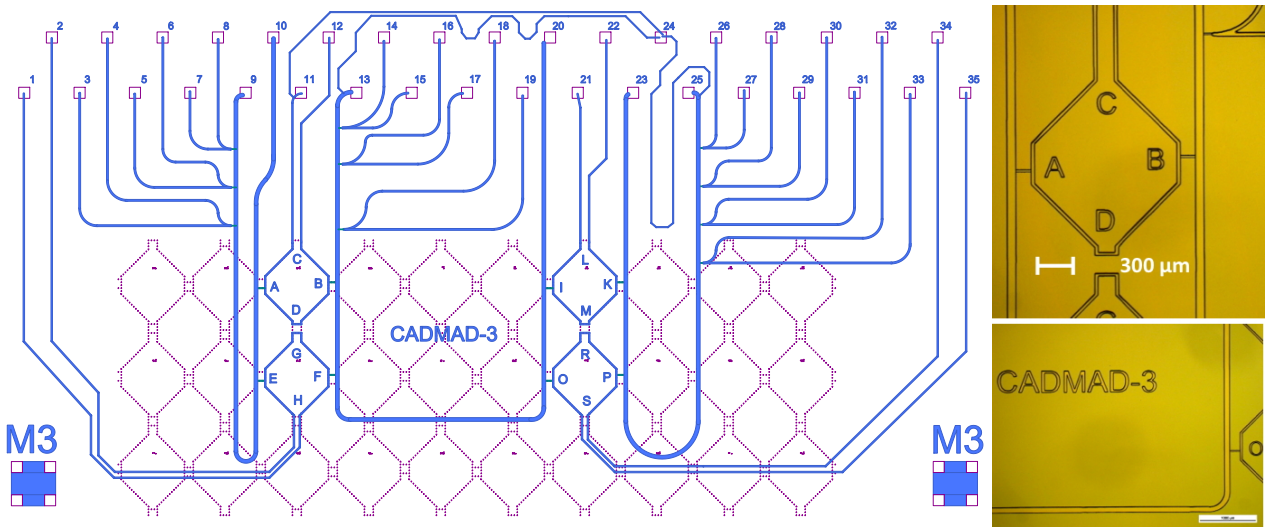


Fig. 10 Left: CAD design of further developed droplet processor. Functionalities: droplet formation & mixing, injection, separation, reinjection of DNA as well as cyclic iterative droplet processing; Right: Examples of light microscopic images of selected chip sections.

2 Demonstrator functionalities (movie list)

Demonstrator movies can be downloaded at:
https://sibelius.biomip.rub.de/bmcmyp/Data/CADMAD/Year%2B2/Review
Please use the QuickTime player to view the movies!
Note that 2 of videos in the 6 steps documented here, in this voluntary demonstrator supporting our deliverable in CADMAD, were the result of a common development with the MATCHIT project, as documented in footnotes below. The other 4 steps and 8 videos are the result of work in CADMAD only.

1. Product injection into droplet from hydrogel matrix: D3_9_droplet_reinjection.mp4
A droplet flow was created using an aqueous solution (50mM His buffer) and a carrier fluid based on an ionic fluid system (1-Ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide) in the right channel (see also D3.9, figure 16). The left channel was filled with a mixture of Pluronic gel and 8nt DNA-Alexa647. DNA oligos have been extracted from the separation gel into an aqueous droplet (50mM His buffer). (see also D3.9 Section 4.3.6)

2. Product extraction from droplet into separation gel: D3_9_droplet_extraction.m4v[11]
A droplet flow was created using an oligomer solution (8nt, DNA-Alexa647) and carrier ionic fluid system (butylmethylpyrrolidinium bis(trifluoromethylsulfonyl)imide [MeBu][NTF]) in the left channel (see also D3.9, figure 10). The right blue channel was filled with Pluronic gel and 30nt DNA-Alexa488. Small samples of the red labeled DNA have been extracted from droplets in the separation channel. (see also D3.9 Section 4.3.4)

3. Droplet generation and combinatorial droplet mixing:
D3.9_droplet_mixing_20130425_114626_0_elec.mp4; D3.9_droplet_mixing_20130425_114225_0_elec.mp4

[11] The extraction of DNA products to droplets following an on chip separation step was a process investigated in both the MATCHIT (for iterative chemtainer processing via product cleanup) and in the CADMAD project (50:50), and this video was also shown in the MATCHIT project final report.

A droplet flow was created using an aqueous solution and ionic liquid (1-Ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide) as a carrier (separating) fluid. The light microscopic movies show the programmable generation of alternating droplets and subsequent mixing of the droplets.
(Contribution for D3.9 Section 4.3.2 and 4.3.3)

4. Droplet braking using backpressure from µ-pumps: D3_9_droplet_braking_pressure_20130327.mp4
A droplet flow was created using an aqueous solution and ionic liquid (1-Ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide) as a carrier fluid. The light microscopic long-term movie shows the programmed droplet generation as well as stopping the droplet chain with relative amplitude 30 and frequency 100 Hz (Bartels micropumps).
(Contribution for D3.9 Section 4.3.4 and 4.3.6)

5. Droplet braking at defined locations for content processing: D3_9_droplet _braking.mp4[12]
A droplet flow was created using an aqueous solution and ionic liquid (1-Ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide) as a carrier fluid. The light microscopic movie shows the braked droplet displacement and release in Pluronics stabilized droplets.
(Contribution for D3.9 Section 4.3.4 and 4.3.6)

6. Gel-separation of two oligos in Pluronic-gel: D3.9_separation_20121024_150305_30sec.mp4, D3.9_separation_20121024_152100_15sec.mp4, D3.9_separation_20121029_120846_15sec.mp4, D3.9_separation_20121029_142851_15sec.mp4
These fluorescence microscopic movies show the separation in a 30% F87 Pluronic + 1mM NaCl in 50mM His pH7 channel.

1. DNA: OligoN2b(24nt, Alexa488) 1*10e-7M (blue color in movie)
2. DNA: OligoAlphaY1(45nt, Alexa647) 1*10e-7M (red color in movie)

(Contribution for D3.9 Section 4.3.5)

[12] Droplet braking was developed for general chemtainer processing in the MATCHIT project and for DNA synthesis processing in CADMAD, and this video was also shown in the MATCHIT project final report.