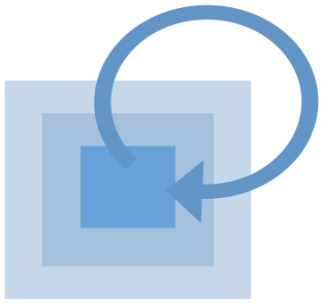

2012

Matrix for Chemical IT
(MATCHIT)

Deliverable
Report
D5.3

TABLE OF CONTENTS

D5.2 Iterative chemtainer processing (Demonstrator)

A demonstration of integrative chemistry using iterative chemtainer processing.

The demonstrator will be presented at the review. This document provides some background information and context.

1 Introduction and objective

Recursive and iterative processing lies at the core of both computer science and chemistry. In chemistry, traditional experimentation performs recursive processing at the level of test tubes, emphasizing work with pure chemicals in cycles of mixing, reaction, and product isolation by means of separation and concentration (e.g. crystallization). After a chemical processing cycle, product chemicals have been purified ready to act as inputs in a new round of processing. In MATCHIT, where we seek to use DNA addresses to tag chemtainers to allow chemistry to be performed with addressed chemtainers, establishing recursive processing of chemtainer contents is a key to algorithmic integration of production and computation. In this deliverable demonstration, we will demonstrate our ability to support iterative processing of chemtainers, without resorting to mechanical valves, which are simply a continuation of macroscopic chemical experimentation down to the microscale. Instead we will use electrodes for the fine-grained programmable control of the iterative processing, limiting the electronic control by conventional fluidic processing equipment to the starting and stopping of pumps, to stop and start in parallel entire chains of chemtainers in the form of microscopic plug droplets.

The chemistry we employ will be based on the core synthetic process of template directed DNA ligation, and is deliberately constructed to be an enzyme-free process, not dependent on extant biology for biological enzymes. The synthetic chemistry is a further development of the novel disulphide ligation chemistry of the von Kiedrowski lab, in a collaboration between the two groups at RUB, and makes heavy use of the electronically reversible triplex hybridization process developed by RUB-BioMIP. We planned to demonstrate iterative processing of DNA exhibiting exponential growth, and most of the work done by the electronic microfluidics team was to support this achievement. By the end of the project, however, it became clear that a strong template-free background reaction prevents successful implementation of the full exponential scheme using triplex DNA ligation, and the simplified scheme of linear amplification was targeted instead. Most of this description concerns the full exponential iterative scheme, as that was the subject of most work, but the demonstration will occur with the linear iterative scheme only. A gain in amplification is a precondition for chemical iteration, since otherwise material loss ultimately prevents continued processing. Amplification consumes resources and can in principle be replaced by simple fixation of external resources, if no chemical autonomy in the amplification products are required. In this demonstration we preferred to attempt the autonomous amplification process involving template synthesis, rather than use simpler externally directed syntheses.

The basic scheme of iterative processing being targeted in MATCHIT is shown in fig. 5.3.1. It was chosen to be operationally independent of the pending achievement of purine-purine ligation with disulphide chemistry, and is novel in using the DNA triplex scheme to support exponential amplification with only a single pyrimidine-pyrimidine ligation process. Normally, DNA replication requires both of the Watson Crick complementary sequences to be replicated, but we (Minero and McCaskill, 2012) have postulated that in the triplex structure, a non-ligated purine sequence bound to the pyrimidine template can also serve as a template for pyrimidine ligation. The scheme proposed can

also be tested in principle using full length (ligated) purine strands instead of the two purine building blocks. More details of the iterative processing protocols and high level description in the MATCHIT-calculus are presented in Appendix 1 and 2 respectively.

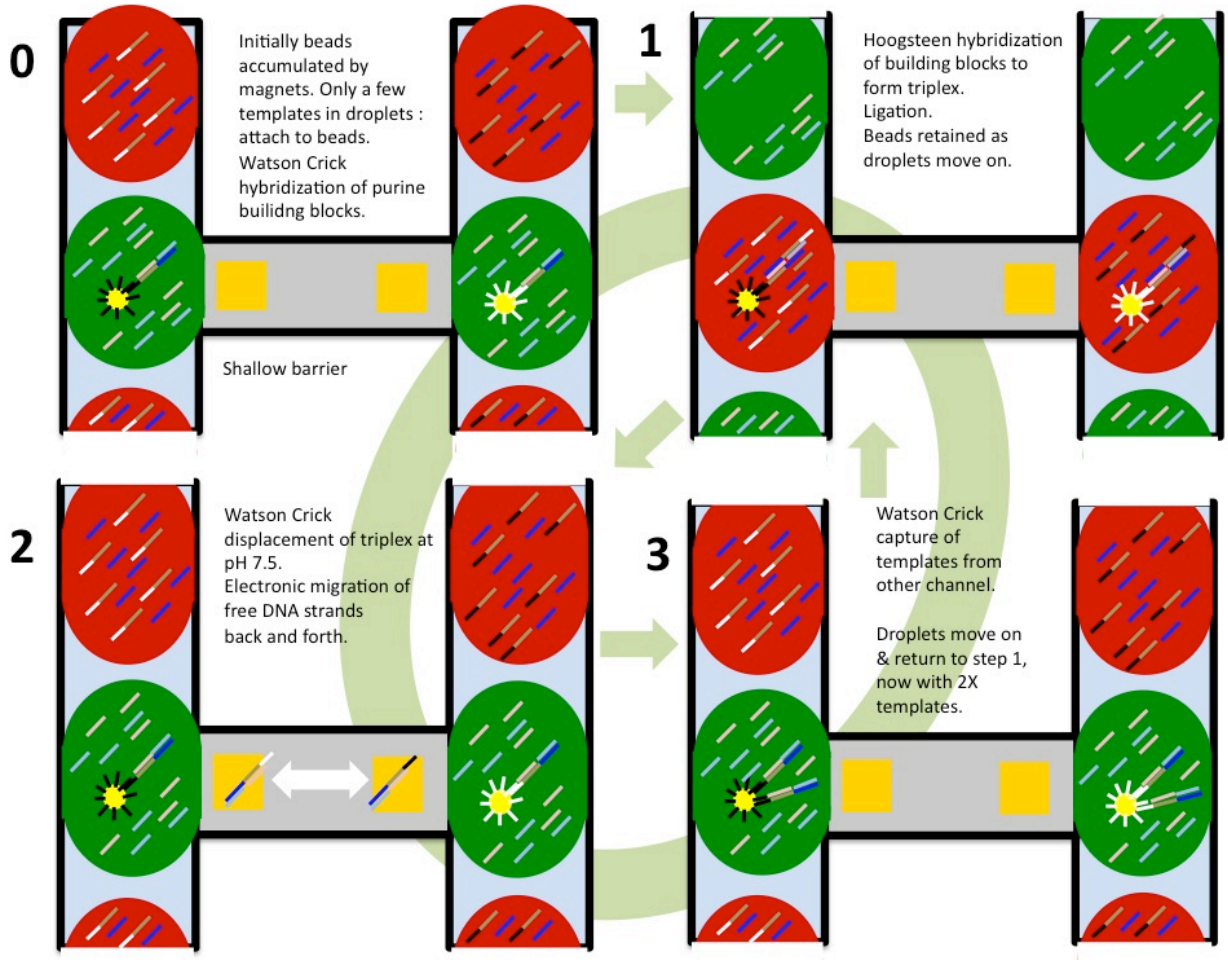

Figure 5.3.1: Iterative scheme for droplet-based DNA amplification. The scheme clocks the two droplet chains synchronously in two channels, which are connected by an electronically switchable hydrodynamic barrier (shallow channel) that prevents mixing unless electronically activated. **0:** Initially, beads are collected at the junction site (only one is depicted) and only a small number of template single stranded DNA are added (one copy depicted**) 1-3:** Thereafter the processing consists of cycles of (**1**) moving the droplets forward one step to allow triplex formation on beads at pH 5.5 and ligation (**2**) moving the droplets forward one step to convert triplex strands to two duplexes at pH 7.5 and electronic mixing DNA content (not beads) (**3**) picking up new templates from other channel and electronic barrier established again. When the droplets are clocked forward, the DNA solution is washed off to departing droplet but the beads stay put on the channel walls, held by the magnetic field. [Minero & McCaskill, 2012, RUB]

2 Choice of chemtainers: aqueous droplets

2.1 Why droplets as chemtainers?

Aqueous droplets function as chemtainers consisting of pico- or nanolitre volumes, thereby facilitating reduced reagent consumption and background noise.[1] Microfluidic droplets are self-assembling easily in oil based as well as ionic liquids and are able to self-repair because of their surface tension properties. They can be generated in a T or Y-junction or in a flow-focusing channel.[2][3][4] The control of

1 M. T. Guo, A. Rotem, J. A. Heyman and D. A. Weitz, Lab Chip, 2012, 12, 2146.
2 T. Thorsen, R. W. Roberts, F. H. Arnold and S. R. Quake, Phys. Rev. Lett., 2001, 86, 4163.

droplet formation is the requirement for cycles (using the slow reaction processing tracks = hydrogel filled electro osmotic flow channels) in the proposed programmable matrix in MATCHIT.

All water-soluble material can be encapsulated inside as well as hydrogel gel beads, DNA-tagged silica or polymer-beads, nanoparticles, micelles and vesicles. RUBa has noted that hydrophobic oil droplets as well as reverse micelles and vesicles lead to interaction with the hydrophobic carrier fluid, which may disrupt the droplets. Droplets are stable miniaturized reaction containers. High salt concentrations are possible with no leakage of the water-soluble material into the carrier fluid (e.g. ionic liquids). They are also pH stable. It is noted that certain detergents in ionic liquids can destabilize the aqueous droplets.

Droplets are easily monitored by optical and fluorescence microscopy and confocal microscopy. The main idea in MATCHIT was to physically localize droplets at surface sites containing immobilized DNA complementary to DNA contained in the droplet. The surface site clearly needs to be hydrophilic to achieve this, and one strategy proposed to enhance the specific interaction was to have the DNA in the droplet attached to hydrophilic beads. Once hybridization takes place, one expects that the beads will not want to leave the droplet, so the droplet will stick to that site (figure 5.3.2).

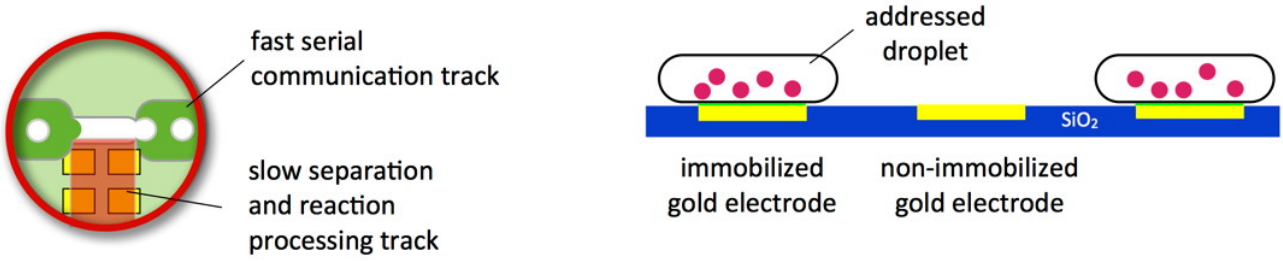

Figure 5.3.2: Left: Concept of microfluidic chemtainer processing. Right: DNA-hybridization dependent molecular droplet docking via immobilized DNA on gold electrodes and specific bead/nanoparticle binding.

An intermediate step shows the binding of DNA-labelled beads to surfaces. One way to achieve long processing times required with droplets for MATCHIT is by using the concept of trapped or parked droplets. This can be combined with the MATCHIT- meander concept to allow content release to gel based interconnecting channels. The main steps are: 1) pattern surfaces with DNA (e.g. via thiol-groups) 2) show specific bead binding 3) use these beads in aqueous droplets to show DNA addressed droplet processing.

2.2 Additional capabilities of droplets with magnetic beads

Droplets as chemtainers are versatile in being able to contain other chemtainer object hierarchically. In MATCHIT we have invested special attention to magnetic beads as embedded chemtainers for two reasons: (i) the magnetic beads allow DNA interactions to have an influence on whole droplet mobility, so that DNA addressing addressing within a droplet can determine whether a droplet stops at a specific site or not (ii) magnetic beads can serve as a high surface area replaceable immobilization site, with greater ease of repeatable processing than with electrodes embedded in the channel walls. With suitable electromagnets, or permanent magnets mounted on translation stages, the magnetic retention of magnetic beads can also be switched electronically, but we do not need to make use of this

3 B. Dollet, W. van Hoeve, J. P. Raven, P. Marmottant and M. Versluis, Phys. Rev. Lett., 2008, 100, 034504.
4 T. Nisisako, T. Torii and T. Higuchi, Lab Chip, 2002, 2, 24.

additional functionality here. Instead, for the iterative processing explored here, we simply use magnetic beads as convenient immobilization surfaces that do not move with the droplet chemtainers.

2.3 Gel channel segments as extra chemtainer

Electric fields stemming from arrays of microelectrodes can concentrate molecules in microfluidic channel environments using a combination of electrophoretic and electroosmotic driving forces. Such chemtainers without walls can be enhanced by gelation of the entire solution and by channel networks with narrow-necked openings to further limit diffusion. The challenge is to label such containers so that their processing is DNA dependent. This is of course possible via the chemical microprocessor technology, provided there is a (typically optical) sensing of the presence of certain DNA sequences (e.g. fluorescent hybridization assay). Then the electronics establishes the relationship between containment and the presence of certain DNA sequences.

In the iterative processing scheme, we have chosen to stay close to the MATCHIT calculus conception in which the intervening gel processing structures play a subordinate role. Rather than try to employ the full power of DNA length separation electrophoretically in the gel channel network, we have discovered an iterative scheme in which the electronic transport of all mobile DNA between two droplets suffices to support iterative processing, when complemented by magnetic bead or electrode surface based immobilization of DNA and the resulting specific transfer of hybridized DNA from one droplet to another through an ionic liquid spacer fluid. This is in line with the general decisions in the project consortium to target simpler scenarios for realizing integration goals rather than the full complexity of the electronic microfluidic network.

3 Droplet manipulations for iterative processing

The droplet manipulations necessary for implementing this scheme have been outlined in Deliverable 5.4, along with the major steps in experimental realization. We merely list these results here, summarizing the relevant aspects for our example, and refer to D5.4 for details.

1. Alternating and other “on demand” droplet chains
   In the scheme proposed here we discovered that two alternating droplet chains are sufficient to support a complex multistep amplification protocol without any switching of surface binding. Our attention was therefore devoted to establishing alternating content droplet chains rather than more general “on demand” droplet chains of arbitrary sequence. However the approach we adopted allow generalization to more complex droplet sequences, including for example additional washing droplets between the A,B alternating sequences as shown in Fig. 5.3.1.
2. Droplet braking
   Droplet braking at the bridging channel junction was established using special droplet braking structures, and it was not required for this to be DNA address dependent in the protocol investigated.
3. Droplet stop and start
   Robust control of droplet chain start and stop with single droplet stepping between parked structures is necessary to allow the time for reaction and for the exchange of DNA in the second phase of processing. This was achieved with back pressure regulation via electronic micropumps (Bartels M5 & M6).
4. Electrical chemtainer content extraction, separation and reinjection
   Only an elementary version of the full complexity of these operations achieved in MATCHIT, as documented in the extension of Deliverable 5.2, is required to implement the iterative scheme. All non-immobilized DNA must be extracted from droplets into an intervening shallow channel segment (that does not need to contain a reversible gel solution) and then

transferred to the second droplet at the opposite end of the connecting channel. Then the same operation is required in reverse. Since this operation does not require full length separation, no branching of channels for waste disposal is required in the separating channel network.

5. Magnetic bead transfer of DNA between droplet chemtainers
   The use of magnetic beads to transfer DNA between solutions is a standard part of the biotechnological repertoire and has also been integrated in diverse microfluidic systems. Here we have shown successful transfer of DNA between successive droplets in an integrated microfluidic systems, using ionic liquid spacers which in contrast with oil spacer systems are compatible with electrode mediated processes. High performance NdFeB permanent magnets with sizes down to 1 mm were used to implement locally defined magnetic fields which were applied at the rear of the chemical processor system to avoid interference with the optical imaging system.
6. Design of local magnetic bead manipulator
   We investigated the ability to produce a tightly confined magnetic field with 100μm resolution for specific site dependent manipulation quantitatively using FEM simulation. The results were discouraging, in the absence of active magnetic elements within several hundred μm of the fluidic plane, in contrast with magnetic storage media, and meant that either thin film magnetic components need to be integrated in the microsystem, or optical monitoring sacrificed. Because of our local electrode control however, we found that only weakly local (>1mm) magnetic fields sufficed for the iterative protocol. See Appendix 3.

4 Iterative chemtainer processing based on triplex ligation and magnetic beads in droplets

4.1 Microfluidic setup

The basic microfluidic setup for this demonstration was designed and constructed in year 3 of MATCHIT and is shown in Fig. 5.3.2, both in the twin droplet channel format as described in Fig. 5.3.1 and in the simplified single channel format as described in Section 4.2 below. The extensive work on droplet control described in Section 3 and Deliverable 5.4 on these and a variety of other test microfluidic structures has led to a robust system for implementing iterative processing in MATCHIT.

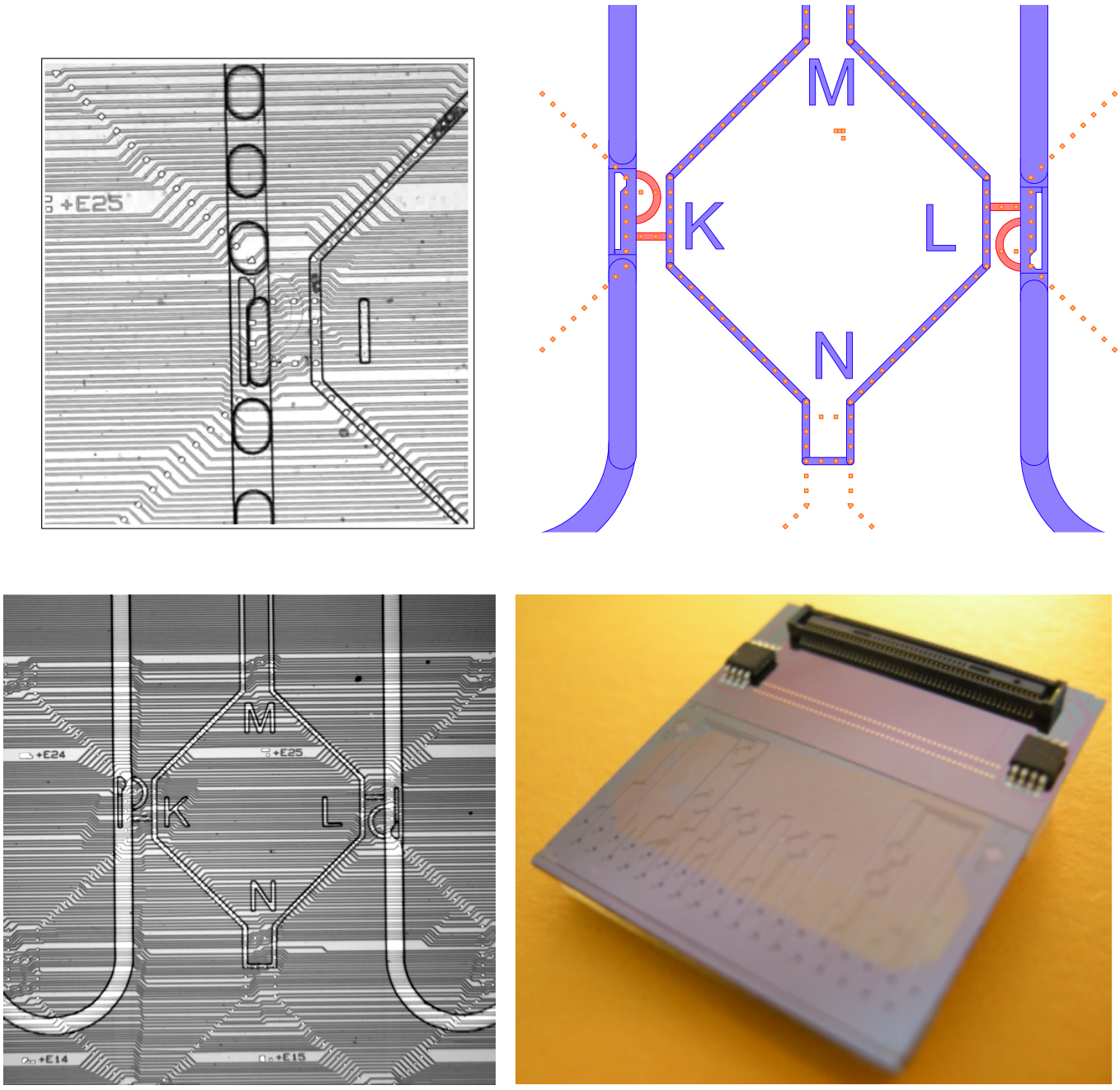

Fig. 5.3.2 Simplified microfluidic design for MATCHIT matrix to demonstrate iterative chemtainer processing. The original MATCHIT droplet meander with numerous gel processing connections is simplified to two droplet channels with an electronically switchable interconnecting channel, or even further to one droplet channel with in built alternating droplet generation on chip. **Top Left:** Close up of droplet braking structure with aqueous droplets in ionic liquid carrier fluid. **Top Right:** Overlaid mask design of simplified matrix processor with channels in purple and electrodes shown in pink. The narrow channel in the centre (KNL) is the separation channel connecting the two droplet streams. The orange channels are shallow (*ca.* 1µm) depth and serve as hydrodynamic barriers modularizing the flow in the system. **Bottom Left:** Photo of entire chemical microprocessor chip for these simplified designs, with electrical (top) and fluidic connectors (beneath). **Bottom Right:** Microscopic image of the fluidic structure: the thick channels are *ca.* 100µm wide.

4.2 Simplified design of iterative demonstration with linear amplification

The full scheme shown in Fig. 5.3.1 showed a strong background reaction, probably as a result of a half-length triplex complex between two pyrimidine and one purine building blocks. This makes it difficult to detect any template effect in the coupled process when the purine building blocks are added. On the other hand, the ligation reaction in the triplex starting with a hairpin duplex as template,

in the absence of additional purine template, shows a very low background reaction rate. In the absence of pH cycling, only as much product as template can be created, because the product strands do not dissociate readily from the triplex complex at pH 5.5. Thus this direct chemical reaction does not generate enough product to serve as an amplification reaction *per se*. We first investigated pH cycling in bulk by titration and found a linear increase in product with the number of pH cycles, as evidenced by gel product analysis. Linear amplification can then serve as a basis for iterative chemtainer processing of DNA in droplets.

In the simplified scheme, shown in Fig. 5.3.2, iterative isothermal processing is achieved by transferring droplets over stationary surfaces (for convenience magnetic beads). In each new acidic droplet, the templates on the magnetic beads can iteratively produce single stranded DNA products via ligation of pyridine oligomer building blocks in the triplex strand of the resulting complex. In the alternate droplets at neutral pH, the triple strand is released to the solution, freeing the template again for action in the following acidic droplet. Thus with stop start unidirectional droplet transfer, iteratively, every second droplet is filled with single stranded template, separated from the reactants. This process requires only pulsed electronic control of the back pressurizing pump, and can proceed in parallel for numerous droplets subsequences. The progress of the reaction is monitored by fluorescence, using the quencher leaving group strategy developed in the von Kiedrowski lab. The magnetic bead based transfer could also be enhanced with an electric field based separation step. With forward and backup stop and start movement between just two droplets, a linear increase in DNA product concentration with increasing iteration cycle can be achieved, up until the concentration where product inhibition by product binding proceeding faster than building block binding occurs.

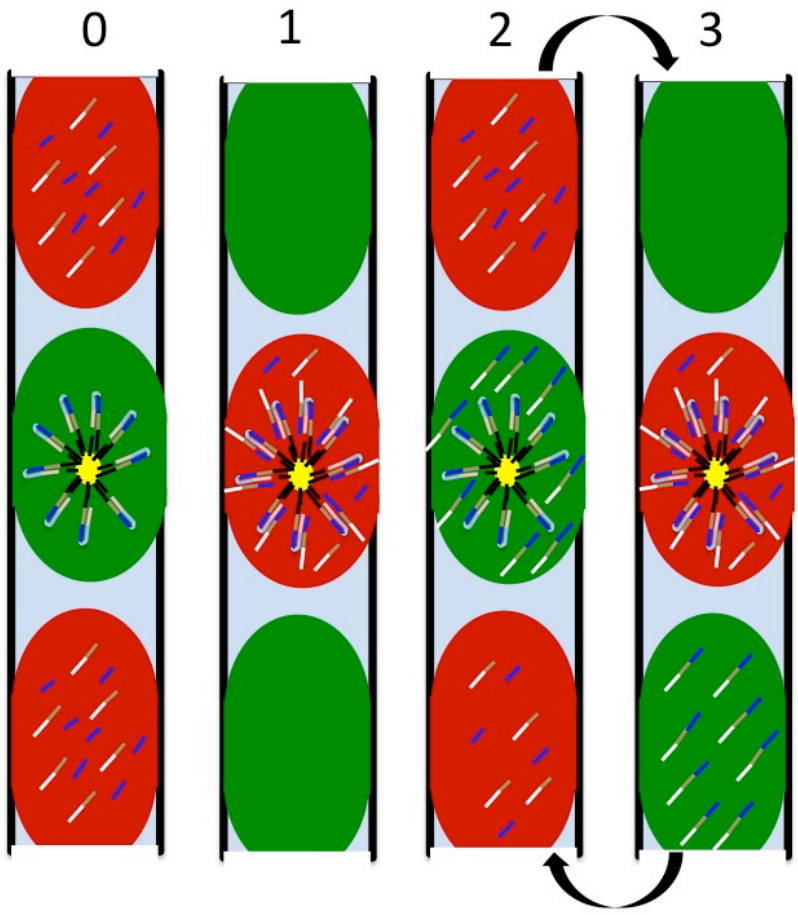

Figure 5.3.3: Simplified iterative scheme for droplet-based DNA linear amplification. The scheme as shown in minimal form uses only one alternating droplet channel, and magnetic beads. **0:** Initially, beads are collected at one site (only one is depicted) and contain hybridized template hairpin DNA **1:** The droplets are clocked one step and pyrimidine strand ligation takes place on beads at pH 5.5. Thereafter the processing consists of cycles of steps 2-3. (**2**) moving the droplets forward one step to dissociated single stranded product at neutral pH 7.5 and (**3**) moving the droplets forward one step to acidic pH 5.5 where renewed ligation of triple helical bound pyrimidine strands takes place When the droplets are clocked forward, the DNA solution is washed off to departing droplet but the beads stay put on the channel walls, held by the magnetic field. [RUB]

4.3 Experimental demonstration

The demonstration of iterative DNA processing with simplified product cleanup, as in the above protocol, will be presented in the form of video microscopy films at the review.

5 Summary and conclusions

The major deliverable of achieving iterative processing is tackled with an iterative amplification scheme involving novel pH cycling of triplex-duplex structures in chemtainer droplets. Computational variants with more complex sequences of droplets (e.g. three sequences) and no longer unidirectional processing of the droplet chains are possible, but the simple iterative scheme shown in this deliverable demonstrates that programmable control of complex chemical processing at the chemtainer level can be achieved in electronic microfluidic systems. The magnetic beads used in the implementation are merely for convenience, in order to allow replenishable surfaces to be employed and the combination with electronic separation protocols in the full exponential system will be an interesting subject of future research when the problems with background reactions in triplexes are resolved.

List of films for demonstration.

To be supplied at the review Apr 30, 2013.

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Appendix 1 MATCHIT Procedure for Iterative Processing : Triplex DNA Amplification

J.S.McCaskill & G.A. Minero RUBa.

The following describes in more detail the procedure for iterative amplification using only electronically controllable steps and not making any use of valves for traditional chemical buffer changes or wash steps. The only physical processes are (i) start and stop of the droplet chain movement, which is a parallel operation using a pulsed micropump (ii) local electronic mixing of DNA strands induced by electrodes. The underlying disulphide bond forming pyrimidine oligos were designed and prepared by V. Patzke (RUBb).

Inlets

(1L,2L) ionic liquid

(1A) pH5.5 1μM Y1• Y2 + mag. bead sat. with ◆ and few copies of bound Y◆

(2A) pH5.5 1μM Y1◆ Y2 + mag. bead sat. with •

(1B,2B) pH 7.5 1 μM R1 R2

Channels

1 and 2, each with 3 inlets, each supporting alternating droplet chains

3 interconnecting shallow channel (not allowing bead transport because of gel or mag.)

optional braking structures in channels 1 &2 located opposite channel 3

Magnets (static)

Located over the bridging channel 3 between channels 1 and 2

Electrodes

In bridging channel to support bidirectional content exchange (leaving magnetic beads fixed)

Procedure

1. Pump channels 1 and 2 with ionic liquid
2. Pulse pumps alternately A and B on inlets (1A,2A) and (1B,2B) to create alternating droplets in channels 1 and 2
3. Use magnets to collect and retain beads from droplets in channels 1 & 2 at junction to ch 3
4. Use epump to stop droplet chains in chs 1&2 with droplets type B at channel 3 junction. This action is assisted by the presence of droplet braking structures there.
5. Activate electrodes to exchange contents between droplets 1B and 2B across ch 3

See detailed electrode protocol : Uwe Tangen.

6. Move droplet chain forward one droplet so that droplets type 1A and 2A are at junctions. Allow to react for 1-10 min. Monitor fluorescence.

7. Go to step 4 and iterate.

Short description of mechanism

The beads (or surfaces) in the two channels differ with respect to one is immobilizing for ◆ and the other for •. This is implemented with a DNA tag sequence attached to the bead covalently or with a streptavidin-biotin link: the tag matches an elongated sequence on single stranded builidng block Y1.

With the two chains synchronized, the transfer step between channels can be simple mixing (e.g. back and forth with electrodes) but keeping the beads or surfaces separate.

The mechanism depicted below in more detail uses * and circle instead of the close diamond and circle.

In step B, the first time only, purine oligos R1 and R2 bind in Watson Crick pairing with first copy of Y on bead. Only the beads with assembled cargo are transferred to next step as droplets move on. Interchange of contents other than beads between channels 1 & 2 doesn't hurt.

In step A, the building blocks Y1 and Y2 bind to nicked WC duplex on bead via Hoogsteen pairing in triplex and ligate. The tag on the Y1 is opposite to that of the bead in the channel.

In subsequent steps B, the Y strand triplexes dissociate, and form duplexes with R1&R2. Interchange of contents leads to matching tags immobilizing the Y strands coming from the other channel on the beads. R1 and R2 may be carried across too. This step creates new template copies on the beads, and supports the exponential amplification of template Y in each channel.

Antonio Minero and John McCaskill invented this SPREAD-like procedure because

(i) the R1 R2 ligation of purine sequences with disulphide chemistry was not ready

(ii) we wanted exponential amplification

(iii) using full R would lead to a strong background reaction of duplex ligation in solution and the information is added in high copy number defeating much of purpose of amplification

Experiments of Volker Patzke established that there is a background triplex complex of Y1,Y2 and R1 which supports ligation without template, so that the scheme cannot work as shown. It could work with R instead of R1+R2 if the washing step is good, so that only R attached to the beads is transferred to solution A. Nonetheless, we constructed the microfluidic machinery to run this, and also experimented with macroscopic versions using capillaries and CE.

Appendix 2 MATCHIT Calculus Description of Triplex DNA Amplification.

DNA amplification in MATCHIT

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REACTION EQUATIONS

Template directed ligation of complementary oligomers translates into the following reaction equation:

$$\gamma + \gamma_1^\top + \gamma_2^\top \longrightarrow \gamma^{||} \tag{1}$$

We allow for $\gamma^{||}$ to perform ligation via Hoogsteen bonding:

$$\gamma^{||} + \gamma_1 + \gamma_2 \longrightarrow \gamma \cdot \gamma^{||}, \tag{2}$$

and we allow for the triple helix to dissociate

$$\gamma \cdot \gamma^{||} \longrightarrow \gamma + \gamma^{||} \tag{3}$$

CALCULUS EXTENSION

We employ left side indices to tags and chemtainers (beads) to denote binding sites implemented as DNA sticky ends. For example, ${}^{\alpha}\gamma$ can bind to the chemtainer ${}^{\alpha\top}\mathbb{C}\ \mathbb{D}$ and we write the hybridization result with the usual syntax ${}^{\alpha}\gamma\,{}^{\alpha\top}\mathbb{C}\ \mathbb{D}$. We allow for such tagging to occurr spontaneously, by adding the autonomous reaction

$${}^{s}s' + t\,{}^{s\top}\mathbb{C}\ P\ \mathbb{D} \longrightarrow {}^{s}s'|t\,{}^{s\top}\mathbb{C}\ P\ \mathbb{D} \tag{4}$$

for any tags $s, s' \in \mathcal{T}$ and tag set t.

SYSTEM SETUP

The amplification protocol operates over four different types of locations, denoted by a, a', b, and b'. These are arranged in two droplet chains $a_i, b_i, a_{i+1}, b_{i+1}, \ldots$ and $a'_i, b'_i, a'_{i+1}, b'_{i+1}, \ldots$ We will use b and b' to feed $\gamma_1^\top$ and $\gamma_2^\top$, and a and a' to feed γ_1 and γ_2. Those locations, however, differ in that we feed ${}^{\beta}\gamma_1$ at location a and ${}^{\alpha}\gamma_1$ at location a'.

AMPLIFICATION PROTOCOL

Amplification starts with seeds ${}^{\alpha}\gamma\,{}^{\alpha\top}\mathbb{C}\ \mathbb{D}$ and ${}^{\beta}\gamma\,{}^{\beta\top}\mathbb{C}\ \mathbb{D}$ at appropriately initialized b and b' locations:

	$b_i : {}^{\alpha}\gamma\,{}^{\alpha\top}\mathbb{C}\ \mathbb{D} + \gamma_1^\top + \gamma_2^\top$ (5)
reaction (1)	$b_i : {}^{\alpha}\gamma^{||}\,{}^{\alpha\top}\mathbb{C}\ \mathbb{D}$ (6)
$\mathbf{feed}(a_i, {}^{\beta}\gamma_1 + \gamma_2); \mathbf{move}({}^{\alpha}\gamma, b_i, a_i)$	$a_i : {}^{\alpha}\gamma^{||}\,{}^{\alpha\top}\mathbb{C}\ \mathbb{D} + {}^{\beta}\gamma_1 + \gamma_2$ (7)
reaction (2)	$a_i : {}^{\beta}\gamma \cdot {}^{\alpha}\gamma^{||}\,{}^{\alpha\top}\mathbb{C}\ \mathbb{D}$ (8)
$\mathbf{feed}(b_{i+1}, \gamma_1^\top + \gamma_2^\top); \mathbf{move}({}^{\alpha}\gamma, a_i, b_{i+1})$	$b_{i+1} : {}^{\beta}\gamma \cdot {}^{\alpha}\gamma^{||}\,{}^{\alpha\top}\mathbb{C}\ \mathbb{D} + \gamma_1^\top + \gamma_2^\top$ (9)
reaction (3)	$b_{i+1} : {}^{\alpha}\gamma^{||}\,{}^{\alpha\top}\mathbb{C}\ \mathbb{D} + {}^{\beta}\gamma + \gamma_1^\top + \gamma_2^\top$ (10)

An equivalent program is performed in parallel on locations a', b':

$$b'_i : {}^{\beta}\gamma\,{}^{\beta}\overline{\mathbb{C}}\ \mathbb{D} + \gamma_1^\top + \gamma_2^\top \tag{11}$$

reaction (1) $$b'_i : {}^{\beta}\gamma^{||}\,{}^{\beta}\overline{\mathbb{C}}\ \mathbb{D} \tag{12}$$

$\mathbf{feed}(a'_i, {}^{\alpha}\gamma_1 + \gamma_2); \mathbf{move}({}^{\beta}\gamma, b'_i, a'_i)$ $$a'_i : {}^{\beta}\gamma^{||}\,{}^{\beta}\overline{\mathbb{C}}\ \mathbb{D} + {}^{\alpha}\gamma_1 + \gamma_2 \tag{13}$$

reaction (2) $$a'_i : {}^{\alpha}\gamma\,.\,{}^{\beta}\gamma^{||}\,{}^{\beta}\overline{\mathbb{C}}\ \mathbb{D} \tag{14}$$

$\mathbf{feed}(b'_{i+1}, \gamma_1^\top + \gamma_2^\top); \mathbf{move}({}^{\beta}\gamma, a'_i, b'_{i+1})$ $$b'_{i+1} : {}^{\alpha}\gamma\,.\,{}^{\beta}\gamma^{||}\,{}^{\beta}\overline{\mathbb{C}}\ \mathbb{D} + \gamma_1^\top + \gamma_2^\top \tag{15}$$

reaction (3) $$b'_{i+1} : {}^{\beta}\gamma^{||}\,{}^{\beta}\overline{\mathbb{C}}\ \mathbb{D} + {}^{\alpha}\gamma + \gamma_1^\top + \gamma_2^\top \tag{16}$$

At this point, we exchange the newly created templates between locations a_{i+1} and a'_{i+1}:

$$b_{i+1} : {}^{\alpha}\gamma^{||}\,{}^{\alpha}\overline{\mathbb{C}}\ \mathbb{D} + {}^{\beta}\gamma + \gamma_1^\top + \gamma_2^\top \circ b'_{i+1} : {}^{\beta}\gamma^{||}\,{}^{\beta}\overline{\mathbb{C}}\ \mathbb{D} + {}^{\alpha}\gamma + \gamma_1^\top + \gamma_2^\top \tag{17}$$

$\mathbf{move}({}^{\alpha}\gamma, b'_{i+1}, b_{i+1}) | \mathbf{move}({}^{\beta}\gamma, b_{i+1}, b'_{i+1})$ $$b_{i+1} : {}^{\alpha}\gamma^{||}\,{}^{\alpha}\overline{\mathbb{C}}\ \mathbb{D} + {}^{\alpha}\gamma + \gamma_1^\top + \gamma_2^\top \circ b'_{i+1} : {}^{\beta}\gamma^{||}\,{}^{\beta}\overline{\mathbb{C}}\ \mathbb{D} + {}^{\beta}\gamma + \gamma_1^\top + \gamma_2^\top \tag{18}$$

reaction (4) $$b_{i+1} : {}^{\alpha}\gamma|{}^{\alpha}\gamma^{||}\,{}^{\alpha}\overline{\mathbb{C}}\ \mathbb{D} + \gamma_1^\top + \gamma_2^\top \circ b'_{i+1} : {}^{\beta}\gamma|{}^{\beta}\gamma^{||}\,{}^{\beta}\overline{\mathbb{C}}\ \mathbb{D} + \gamma_1^\top + \gamma_2^\top \tag{19}$$

This completes one cycle of the iterative protocol. Comparing the state (19) state (5) $\circ$ (11), we recognize that after amplification both beads have an additional double strand $\gamma^{||}$.

Using the macros **amp_left** for the program (5) through (10), **amp_right** for the program (11) through (16), and **exchange** for the program (17) through (19) and neglecting parametrization, we can compose the entire amplification protocol as

$$!((\mathbf{amp\_left} \mid \mathbf{amp\_right}); \mathbf{exchange}), \tag{20}$$

where the semicolon denotes sequential composition, the vertical bar denotes parallel composition, and the exclamation mark repetition.

Appendix 3 Local vs. global magnetic control of MATCHIT matrix

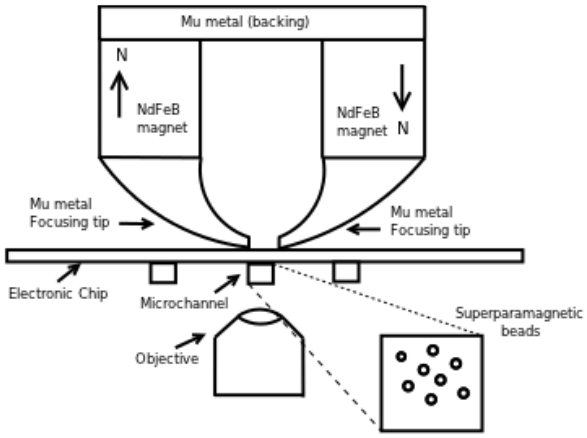
(A. Sharma, RUBa)

Design and optimization of magnetic beads focusing device in microchannels

Focusing of superparamagnetic beads in microchannels is an important step for iterative processing. These beads binds DNA molecules in droplets using biotin-streptavidin linkages.

We optimized the design for magnetic beads focusing device using NdFeB magnets. We used open source finite element packages FEMM (Finite Element Method Magnetics) [1] for two dimensional simulations and getDP (General Environment for the Treatment of Discrete Problems)[2] for three dimensional simulations. The key issue is to make the design compatible with inverted optical microscope. This allow us to simultaneously control the motion of beads together with their optical detection using fluorescence microscopy.

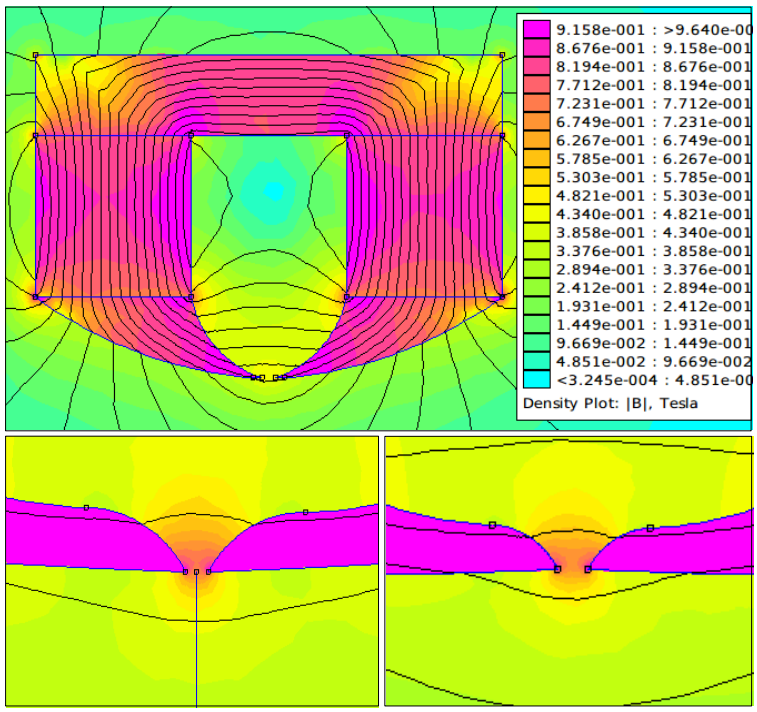
The basic idea was that the magnetic field should be strong enough in the channels with sharp gradients. This should concentrate the beads locally inside the channel. Our initial design consists of two strong NdFeB magnets (1 cm or 0.5 cm), connected to magnetic tweezers [3]. Materials with high magnetic permeability were used for building up magnetic tweezers. We check the performance with Mu metal and Perm alloy. Same material was used to create a backing on the top of the magnets to confine the magnetic fields. The critical part of the design is the magnetic tips of the tweezers. They should be designed such that maximum field should penetrate vertically into the material with sharp gradients. This would lead magnetic beads focused in a narrow region.



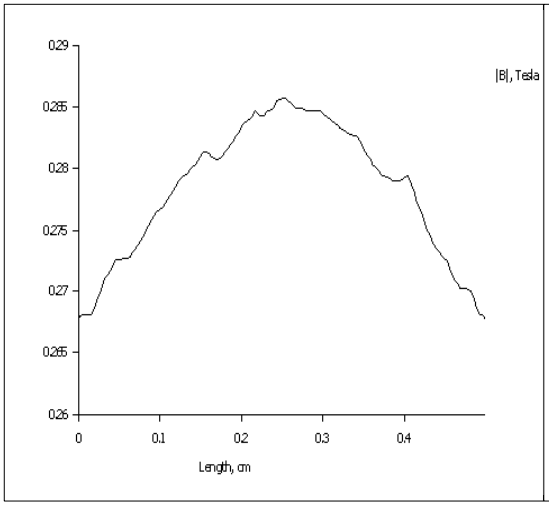
Schematic diagram of magnetic tweezers for focusing magnetic beads

FEM simulations were carried out on variety of magnetic tip designs and varying various geometric parameters which includes size of magnets, gap. The minimum distance of the magnetic beads from the magnetic tip was kept 600 μm, which is the thickness of the electronic chip (SiO_2).

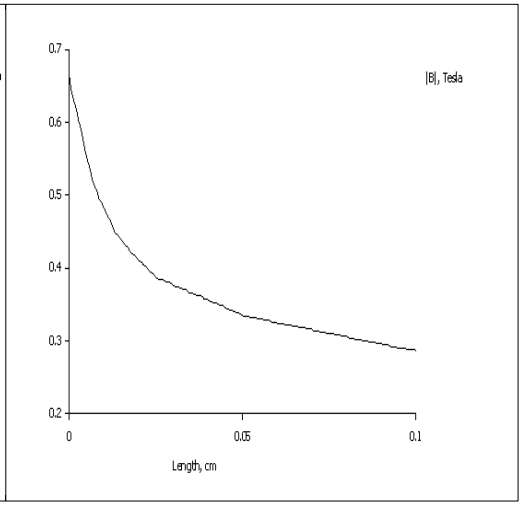
To check the performance of different setup's, we extracted the magnetic field profile at a distance 600 micrometers below the magnetic focusing tip.



Magnetic field distribution from FEMM calculations with magnified view at focusing tips.



Variation of magnitude of magnetic field along

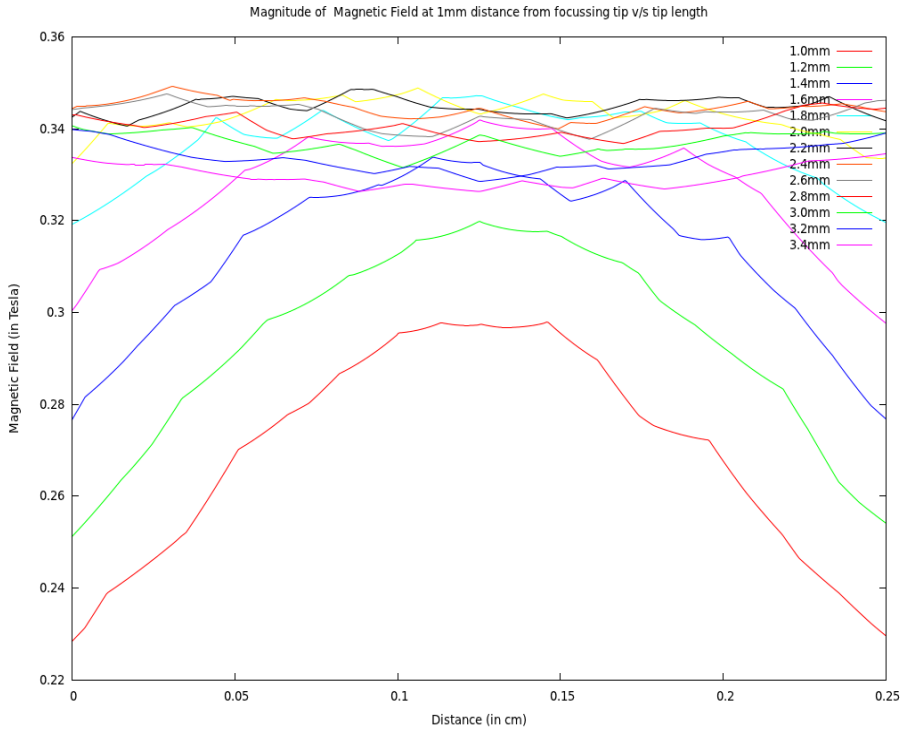


Variation of magnitude of magnetic field along

lateral direction (along X) vertical direction (along Z)

We observe that the performance of magnetic tips i.e. high penetration depth with sharp gradients works only in the close proximity of the tips. At distance around 600 µm to 1mm, the field spreads out and gradients falls down. Changing the gap between the magnets, improves the field penetration depth and makes gradient steeper but still not strong enough localize magnetic beads 10-50µm at 600µm depth.

So, we made further calculations on simpler trapezoidal geometry on NdFeB magnets. We observe better performance at 1mm depth as compared to previous design. The magnitude of magnetic field was comparable to previous setup but field gradients were sharper. Fig. describes the variation in magnitude of magnetic field with different tip lengths.



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2. C. Geuzaine, GetDP: a general finite-element solver for the de-Rham complex, PAMM Volume 7 Issue 1. Special Issue: Sixth International Congress on Industrial Applied Mathematics (ICIAM07) and GAMM Annual Meeting, Zurich 2007

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