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Digital reaction technology by micro segmented flow—components, concepts and applications

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Abstract

Segmented fluids can easily be processed in micro reactors and micro capillaries, if several boundary conditions are fulfilled. The ability of formation of highly regular segments and segment distances in combination with the ability of controlled manipulation, splitting and fusion of carrier liquid columns and single droplets opens the way to a new class of miniaturized chemical and biological operations. The subdivision of a certain reaction or a cultivation volume in a lot of aliquotes and the individual handling of the subvolumes leads to the possibility of realizing serial processes and statistical investigations by numerous highly comparable, but well separated reactor volumes. Combination of different substances, e.g. for testing of synergetic effects in drug development or catalysts screening, can be performed to large extent. The concept of "digital" reaction technology by micro fluid segments means the introduction of a digitalization principle by use of a large number of small reaction volumes handled serially in flow channels and flow-through micro devices. For the realization a set of modules including special interconnectors, T-junctions and other injector elements, transfer units and fluid resistance elements are necessary. Flow fusion modules and segment fusion modules are needed for the reorganization and recombination of serially flowing sequences of fluid segments and for the initiation of reactions by mixing of pairs and triplets of single segments. The principle can be used for quantitative chemical analyses like titration realized by use of micro fluid segments (digital micro segment titration). Micro segments could also be applied in modular synthetic chemistry, particularly in combinatorial chemistry so far as the educts and products of the single synthesis steps are compatible with liquid/liquid two phase system. The segmented flow principle is of particular interest in microbiological experiments and screening procedures. First result show the practical advantages in the highly parallelized production of monoclonal cell cultures in culture sets of very high diversity and for the cultivation of slowly growing microbial species. In addition, the possibility of producing of separated cell cultures in high density opens a series of applications in the screening and testing of drugs.

Keywords: Micro reactor; Segmented flow; Fusion; Splitting; Cloning; Serial processing; Cell cultivation

1. Introduction

Compartmentation is one of the key strategies in the organization of molecular processes in the living nature. It supports the separation of reaction volumes and reaction sites and enables the system to perform different chemical operations simultaneously. Thus, it is a precondition for parallelization of molecular processes. In living nature, compartmentation is realized in tissues, cells and cell organelles. Membranes separate the different compartments.

Micro system devices are under development and investigation for different problems in analytical and synthetical chemistry, in molecular biology and for screening purposes [1–4]. The model of compartmentation is applied in several approaches for micro chemical processes. A high degree of parallelization of well-defined reaction sites is realized in micro titerplates, nano titerplates [5,6] and biochips [7–9]. Automated manipulators, dispenser and pipettes are used in order to address the different compartments [10–13]. All these parallelization tools allow to operate chemicals with stepwise variation of qualitative and quantitative composition. So, libraries of substances can be created by stepwise variation of molecular building units during the combinatorial synthesis on chips or in nano titerplates. Assays can be optimized by stepwise variation of concentrations

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of effectors in the arrays. By use of parallelized carriers, synthetic as well as analytical problems are experimentally treated in form of stepwise varied parameters, that means ordered in a digital way.

Micro and nano titerplates as well as biochips represent two dimensional arrays. The principle of digitalization of chemical operations is still more obviously if the digitalization principle is transferred into linear arrays, that means streams of samples or strings of varied parameters. The stepwise variation and/or combination of parameters in a fluid stream corresponds to such an approach. Unfortunately, the fluid transport in micro channels and capillaries is usually laminary, in homogeneous liquid phase. This results in a fluidic dispersion, that means in a broadening of concentration pulses.

A real serial processing of liquid samples becomes possible in slug-like transport using heterogeneous systems. Liquid/liquid two phase systems offer the possibility of complex sample handling in chemical, biomolecular and microbiological operations using the special flow behavior of embedded liquid segments. Segmented fluids can easily be processed in micro reactors and micro capillaries, if some boundary conditions are fulfilled. Segments having volumes between about 100 and a few nanoliters can be produced with high accuracy and high reproducibility of segment volume. The use of such segmented fluids was earlier proposed for micro serial PCR [14] and other purposes [15–21].

The ability of formation of highly regular segments and segment distances in combination with the ability of controlled manipulation, splitting and fusion of carrier liquid columns and single droplets opens the way to a new class of miniaturized chemical and biological operations. The subdivision of a certain reaction or a cultivation volume in a number of aliquotes and the individual handling of the subvolumes lead to the possibility of realizing serial processes and statistical investigations by numerous highly comparable “reactors.” The application of stepwise varied concentrations for analytical, synthetical screening and test processes can be performed with small steps. Combination of different substances, e.g. for testing of synergetic effects in drug development or catalysts screening, can be performed to large extent. “Digital reactorics” by micro fluid segments means the introduction of the digitalization principle by use of many small reaction volumes handled serially in flow channels and flow-through micro devices. In the following, some components, concepts and applications of serial processing by use of segmented flow are discussed with the scope of digital processing in micro reaction technology.

2. Experimental

For the realization, a set of operation modules and methods is necessary. This includes special interconnectors, T-junctions and other injector elements, transfer units and fluid resistance elements. Special nozzles and double T-junctions are necessary for the separation and splitting of single segments. Flow fusion modules and segment fusion

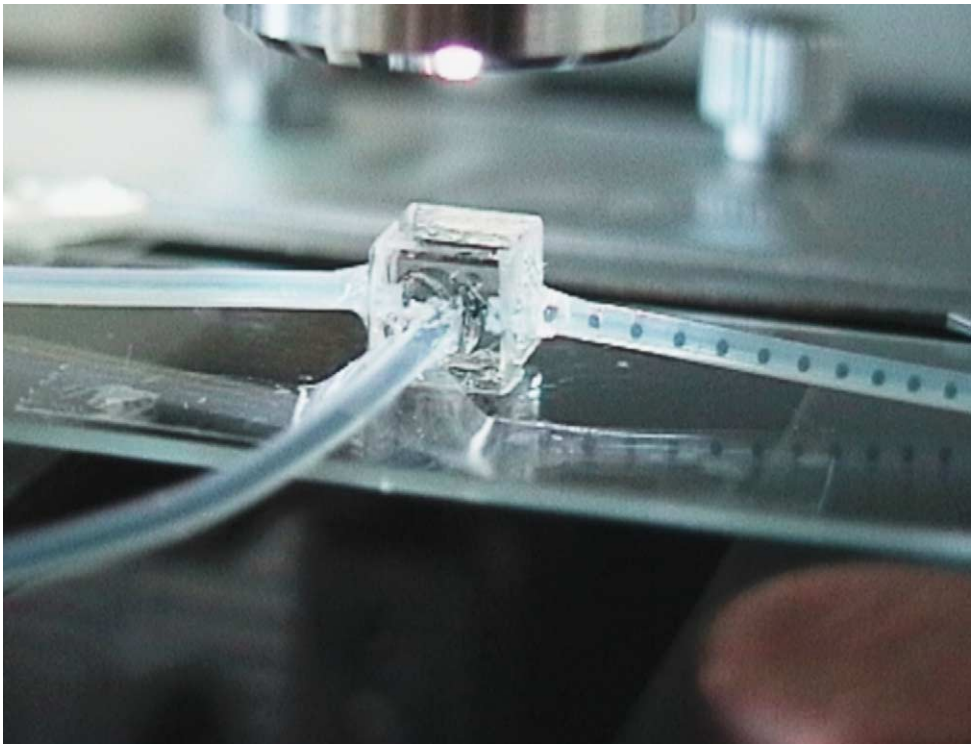

Fig. 1. PMMA-injector for production of a serial flow mounted on a microscope stage (inner diameter of tubes 0.5 mm).

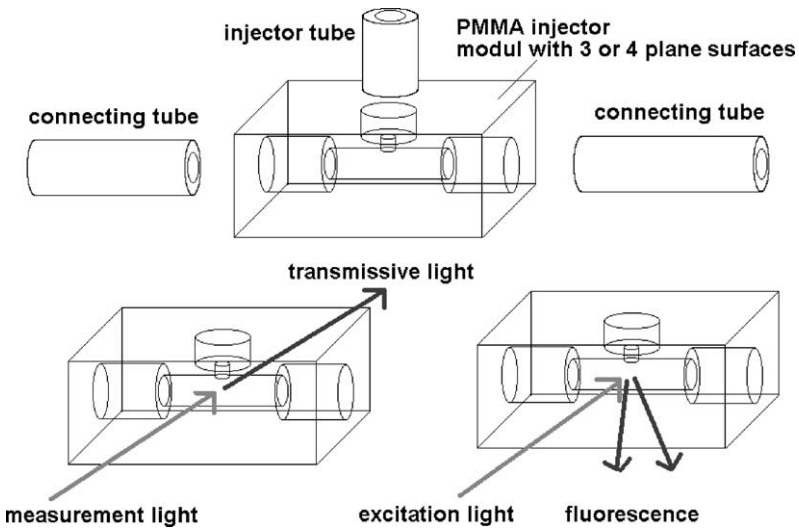

Fig. 2. Construction and fluid interconnection schemes for PMMA reactor modules for segmented flows and configuration for photometric and fluorimetric measurements using PMMA cuvette modules.

modules are needed for the reorganization and recombination of serially flowing sequences of fluid segments and for the initiation of reactions by mixing of pairs and triplets of single segments.

A series of modules was prepared by classical mechanical techniques. In this way, modules of glass as well as modules of polymethylmethacrylate (PMMA) were prepared. Fig. 1 shows an injector prepared by drilling in PMMA. The smallest inner diameter is 0.3 mm. The use of two different drilling diameters enabled us to prepare elements for segmented flow passage without change in inner diameter at the interfaces between tubes and injectors or cuvette-like modules

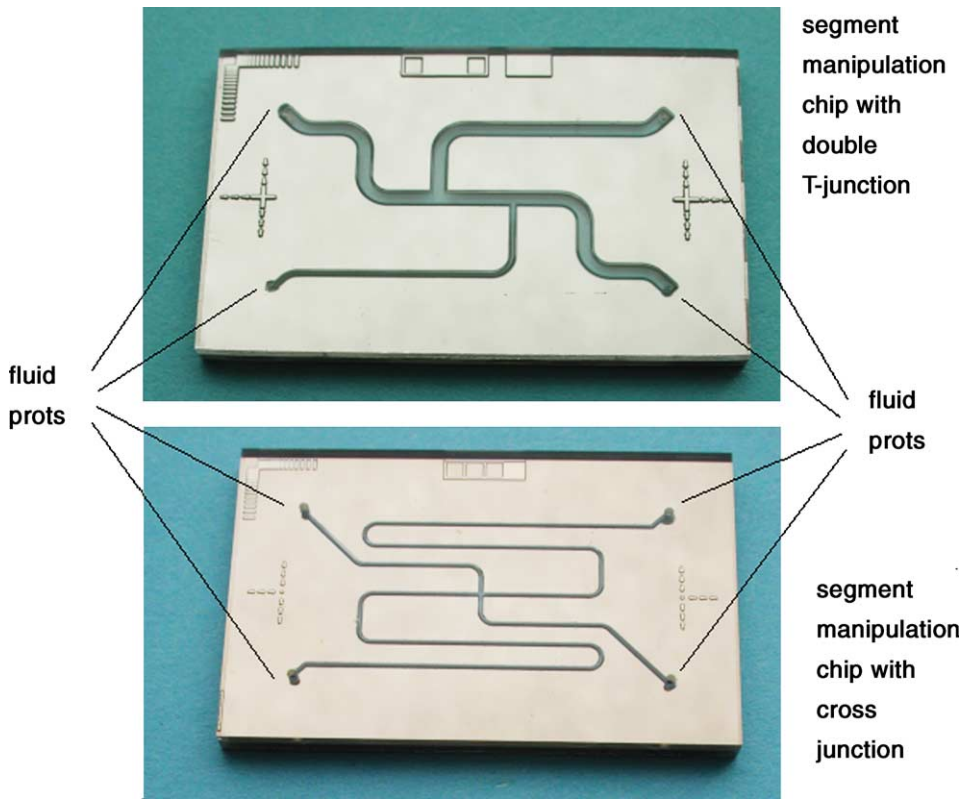

Fig. 3. Chip modules (14 mm × 16 mm) for generation and manipulation of fluid segments.

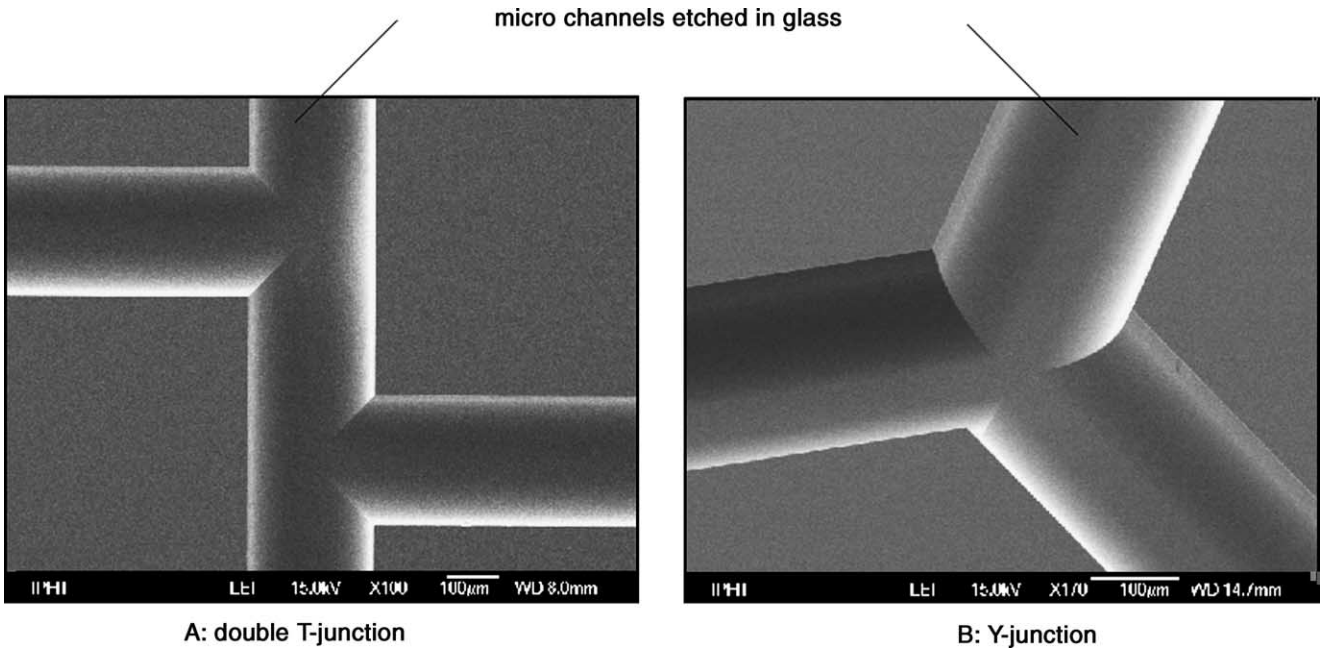

Fig. 4. Smooth surfaces of fluid channels in junction-configurations, made by photolithography and isotropic wet etching of glass (bars: 100 µm, SEM image; A: double T-junction; B: Y-junction).

for transmissive as well as for fluorescence measurement (Fig. 2). Optical measurements can easily be performed even with round channels in PMMA and high roughness of inner wall surfaces since the refractive indices of modul material (PMMA) and embedding liquid (aliphatic hydrocarbons) can be adapted very well to each other. Smaller channels and junctions are realized by photolithography and isotropic wet etching in glass substrates at a much higher precision (Figs. 3–5).

3. Concepts and results

3.1. Basic principle

The basic principle is the formation of larger series of fluid segments and the subsequent operation of this segments in chemical, biomolecular or microbiological processes. Therefore, the chemically isolated segments can be stored in micro capillaries or operated together (in parallel). Alternatively, it

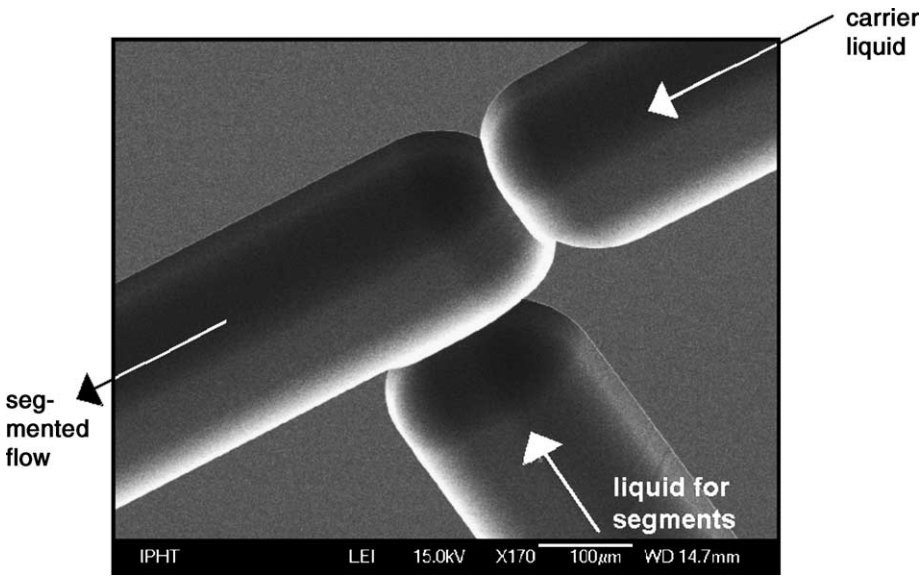

Fig. 5. Nozzle arrangement for the formation and/or manipulation of fluid segments made in glass substrate (bar: 100 µm, SEM image).

is possible to realize a stepwise variation of concentrations of one or more component in a series of segments. This can be achieved either by the application of a continuous shifting composition during the formation of the segments or by serially fusion of the single segments of a sequence with single segments of other segment sequences.

A chain of segments (sequence) can be understood as a string. These strings are distinguished by the arrangement of segments of different size or composition. Sequences of identical segments are monotonous strings. A multitude of different strings can be produced by the introduction of only two different segment types. They can differ either in concentration of only component or simply in the presence or absence of a chemical component. As result, binary sequences (denoted as Markov chains) are obtained. The variability of such sequences amounts to 2^z, where z is the number of segments in a sequence. The presence or absence of two or three substances leads to a variability of 4^z or 8^z, respectively. Still higher variabilities can be achieved by differentiated concentrations. If the concentration of one component in each segment can be defined precisely enough to distinguish 100 different concentrations, the number of segment types is 100 and therefore the variability of a segment sequence of the length z amounts to 100^z. That means, for example, that 10^{30} different sequences of only 30 segments can exist. Very high variabilities in segment sequences result from the variation of concentrations of more than one component in the segments. With four different chemical species 256 different segments can be produced, if only four concentrations are distinguishable (e.g. 0, 25, 50 and 100% of maximum value). This gives the opportunity to generate 256^z different sequences. This fact refers to the enormous variability even in the case of short sequences of segments.

The experimental trick consists of the very easy way of formation of regular segments with of stepwise varied composition by a highly reproducible fragmentation a liquid column with monotonously changed composition. So, continuously changed flow rates can be transferred into segment sequences with discrete variation of content (Fig. 6). The fragmentation of laminary flowing liquid columns into plug-like flowing chains of single segments is the most elementary operation of digital chemistry by segmented flow. A constant size and distance of segments can be realized, if continuously changing flow rates of the fluid actuators are adapted to a constant total flow rate. This is not only important for the generation of fragmented liquids, but also in the case of injection of additional components into segments of a previously generated segmented flow (example in Fig. 7).

3.2. Formation of binary segment sequences and sequences with concentration variations

Binary segment sequences can be produced by the controlled generation of two different types of segments through two different injector elements. Therefore two different micro fluidic arrangements are used. Either the injectors are applied in parallel and connected by fluid channels guiding both segment streams to a T- or Y-junction (Fig. 8a) or the injectors are arranged serially in a main channel (Fig. 8b). The first module type has the advantage of equivalent geometry and pressure conditions in both channels. But the flow rate in both carrier streams has to be adapted precisely in

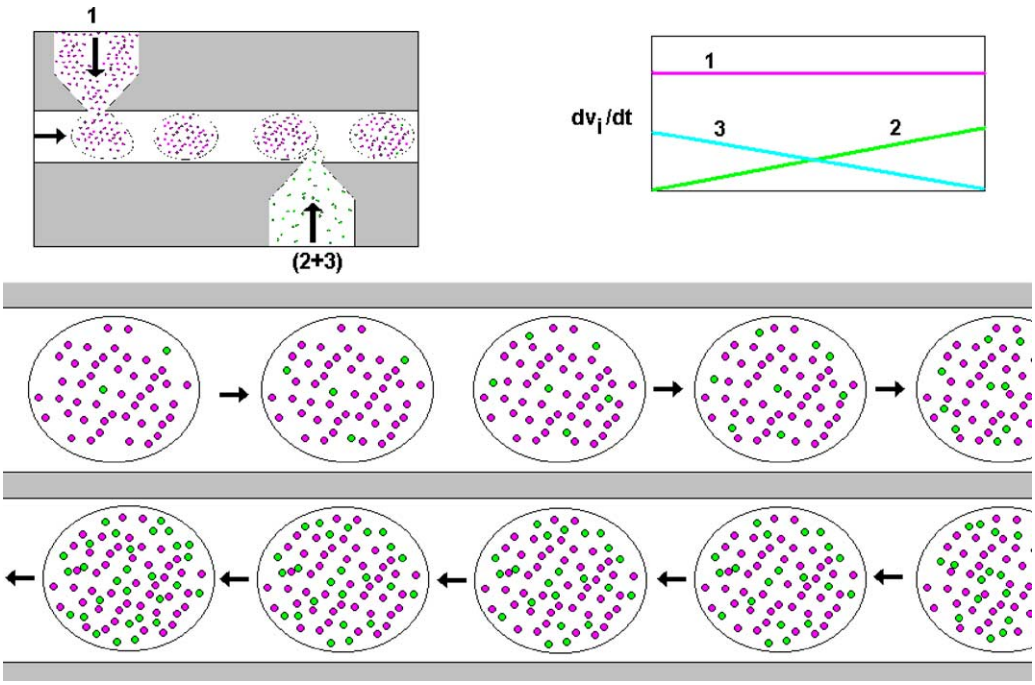

Fig. 6. Principle of transfer of continuously shifted flow rates into stepwise varied concentrations of closed reaction volumes inside segments as the key component for digital chemistry in segmented flow arrangements.

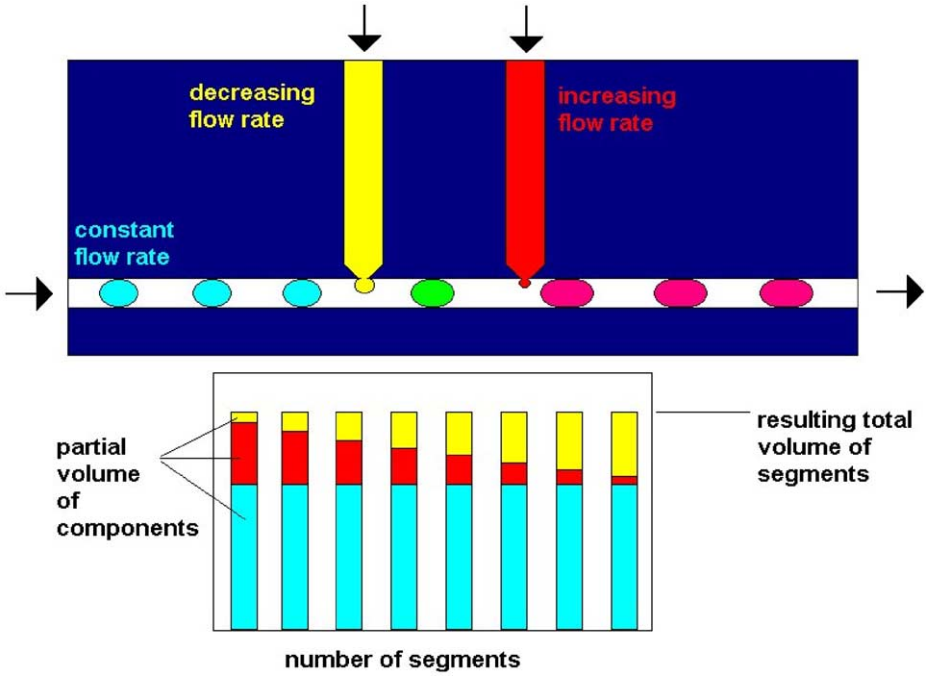

Fig. 7. Production of segment sequences with stepwise varied composition in a double injector arrangement.

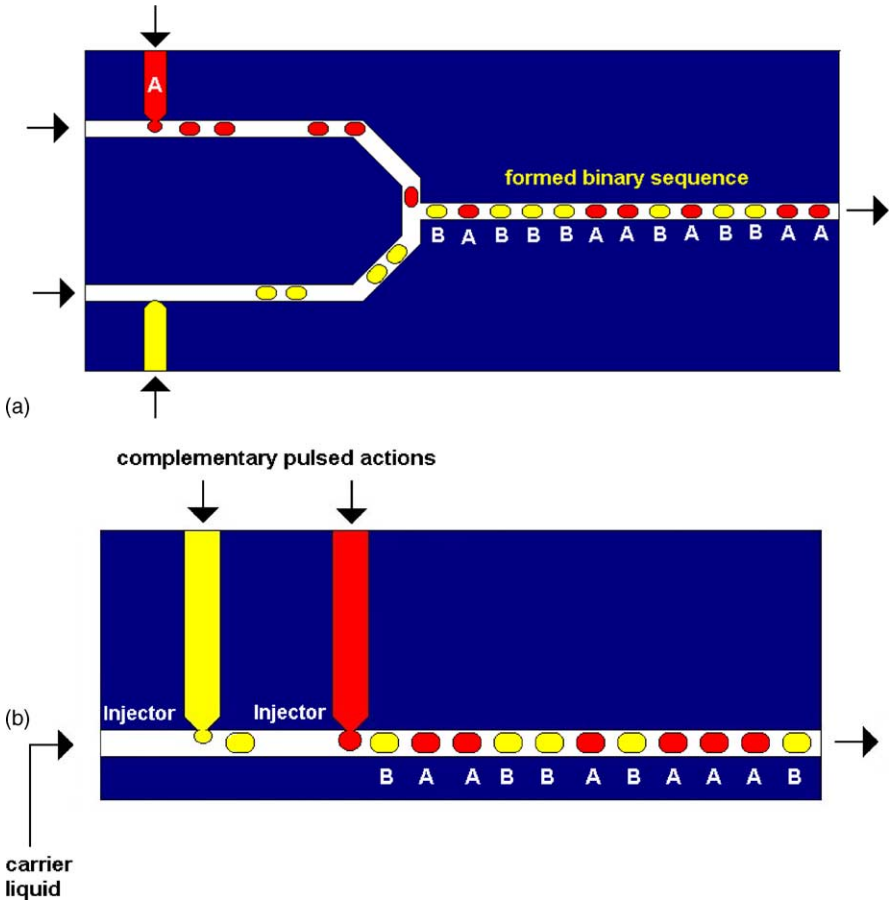

Fig. 8. Formation of a binary sequence of segments: (a) a parallel two-injectors arrangement integrated in a channel configuration with Y-junction; (b) a double injector arrangement in a single channel (serial configuration).

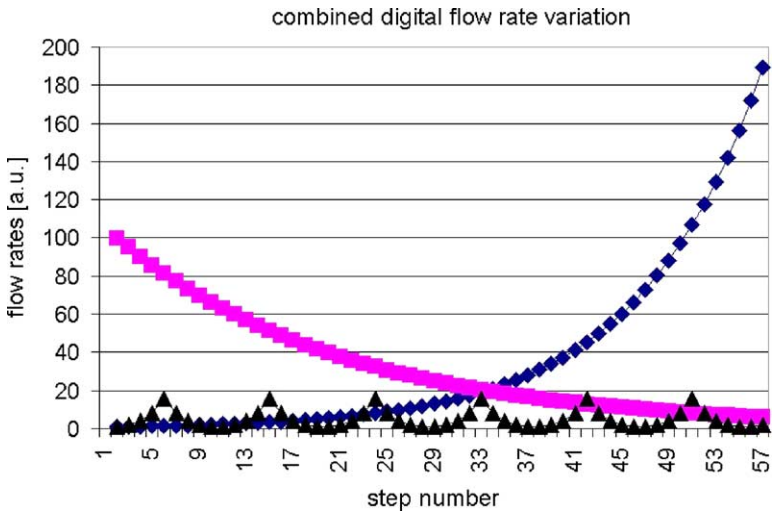

Fig. 9. Preparation of two- and more dimensional arrays of segment composition in segment sequencies: (a) example of continuous variation of flow rates in a micro fluid system with three programmable fluid actors; (b) resulting composition in segments in a three component system at fluid rate variation of (a) shown by ratios of parameters P2/P3 vs. P1/P3.

order to achieve the correct stacking of segments at the junction. The second one needs only one channel (one carrier stream), but it includes both injectors in an asymmetric situation. In this arrangement, the flow and pressure conditions at the second injector are dependent on the action of the first injector. It can be necessary to compensate the resulting effects by a time and pressure protocol for the second injector.

The controlled generation or release of segments of certain compositions in two, three or even more channels gives the possibility to generate complex sequences. Their pattern can become still more diverse by a continuous change of concentrations in the channels of single components. An example for the generation of a complex composed segment sequence from three micro fluidic segment generation channels is given schematically in Fig. 9. It is possible to create such sequences by controlled stacking of previously generated sequences of the original types or by an in situ generation process under control of the injectors.

The construction of segment sequences covering two or more dimensional spaces of concentrations of different components can be achieved by the application of continuously changed flow rates or combinations of them with flow rate cycles for a set of components. An example for three

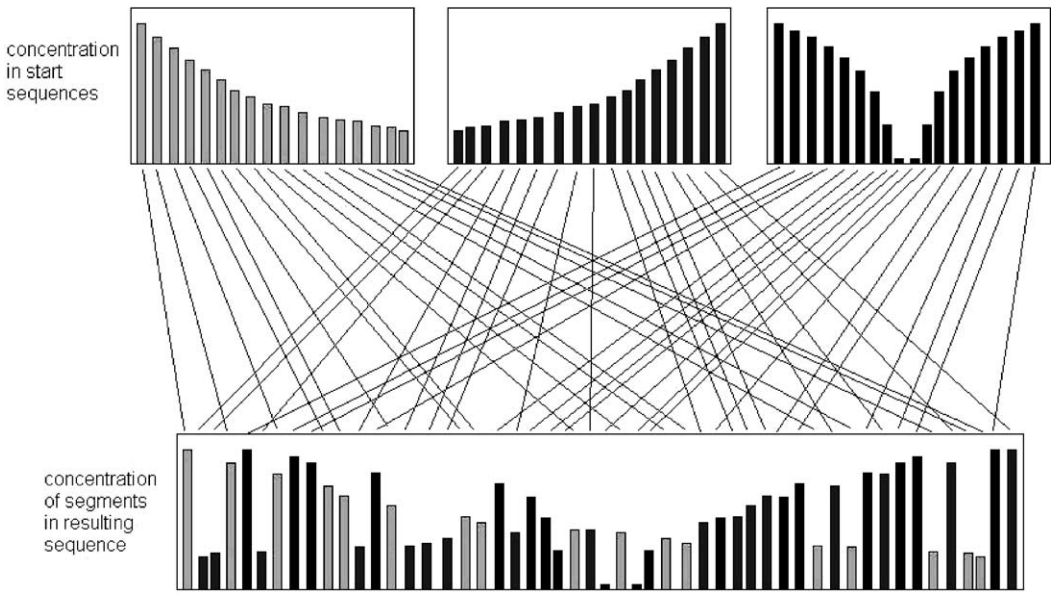

Fig. 10. Preparation of complex composed sequences (“nano fluidic strings”) from sequences with stepwise varied concentrations of single components.

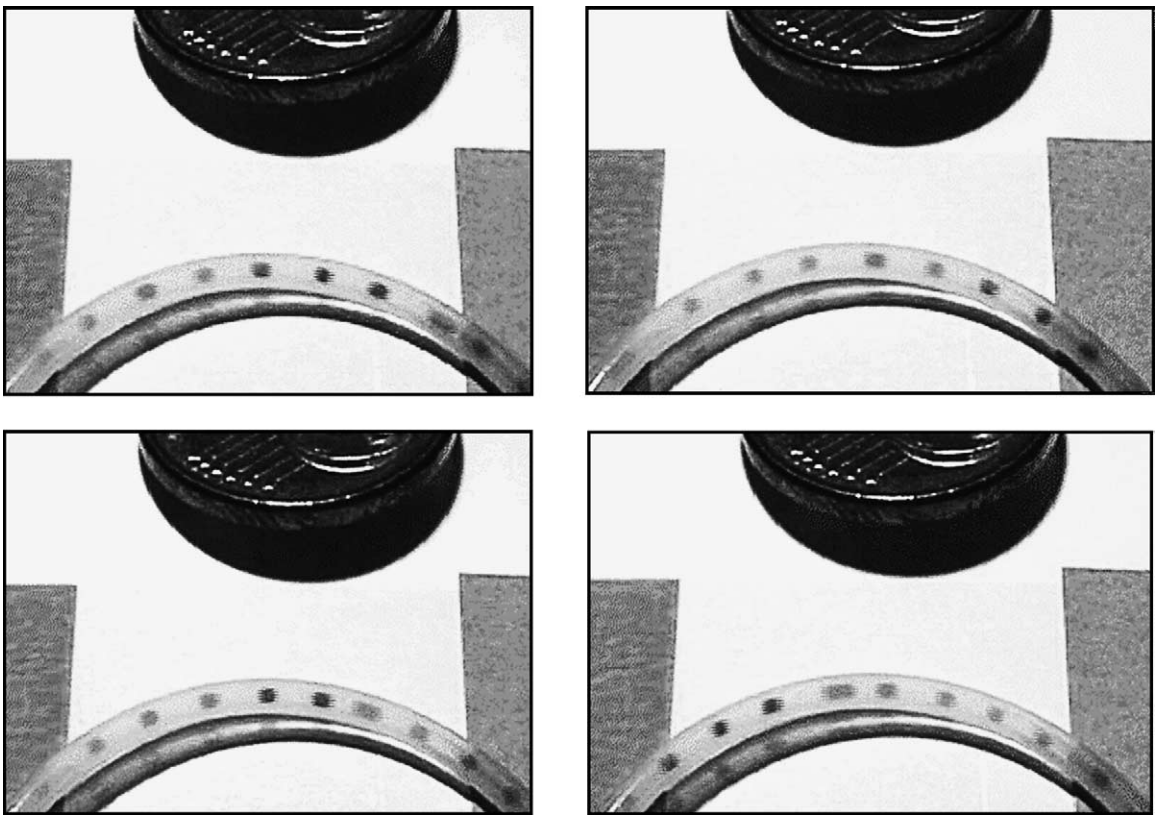

Fig. 11. Four examples of groups of aqueous segments with different content of two dyes embedded in tetradecane (“nano fluidic strings”) inside a plastic tube with an inner diameter of 0.5 mm. Different dye content is shown by different grey level of the segments.

different components is given in Fig. 9a. The log/log-plot of concentration ratios of the three components illustrates the possibility of covering larger areas of concentrations (Fig. 9b). Complex sequences of segments can also be produced by a controlled injection from three supplying channels with stepwise varied concentrations of components into one new segment sequence (Fig. 10). A simple example for chains of segments with different composition is given in Fig. 11. Therefore dyes were injected in different concentrations into aqueous segments. Dark spots inside the tube represent segments with higher concentration of blue dye.

3.3. Fusion and splitting of segments

The pure generation of different sequences of segments can be interesting for some purposes, for example aliquotation for screening of cells. For most chemical applications further operations with segments and segments sequences are important. The fusion of two segments to one larger segment on the one hand and the splitting of a segment in two parts on the other hand are the two most important elementary operations.

The most simple way of unification of two segments is the pairwise fusion of adjacent segments within a segment stream. This process can be initiated by stopping of one segment as a result of a fluid resistance element or a suited other change in the cross-section of the fluid channel. Stopping, fusion and release can be reproducibly controlled, if the total flow rate of the streaming sequence is adapted to the properties of the trap (Fig. 12). The necessity of a

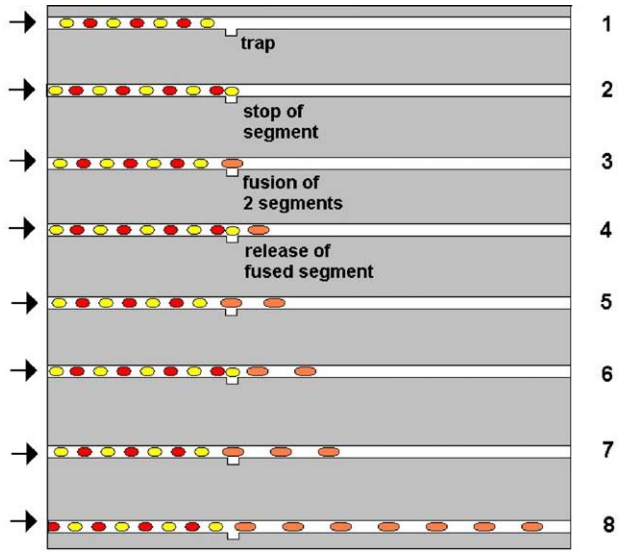

Fig. 12. Principle of pairwise fusion of fluid segments induced at fluid traps.

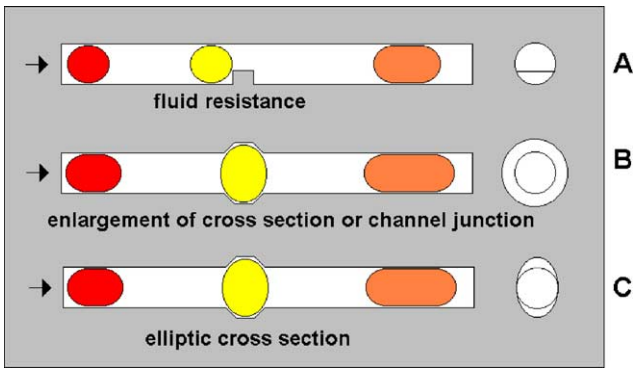

Fig. 13. Examples of fluid traps for pairwise fusion. Cross-sections are shown right: (A) fluid resistance, wall step; (B) enlargement of channel diameter; (C) deformation of cross-section.

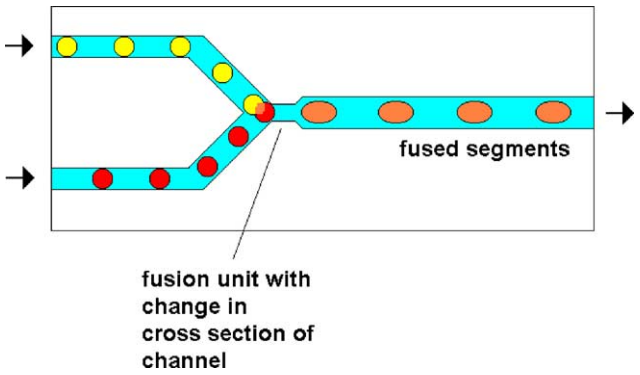

Fig. 14. Principle of fusion of segments in a Y-configuration of fluid channel in connection with a fluid resistance.

change in the shape of the segment, that means the need of interface energy or an increase of the bypass-stream of carrier liquid are responsible for the stopping and subsequent fusion process. Therefore, three different types of traps can be used: a hindrance inside the channel (Fig. 13A), a locally enlarged diameter of fluid channel (Fig. 13B) or a local asymmetry in the cross-section shape (Fig. 13C).

Alternatively, segments can be fused at channel junctions (Fig. 14). The fusion occurs on spontaneously if the phases of both origin streams are synchronized. Otherwise the position of segments in both supplying channels can be controlled by pressure. The situation can be improved by

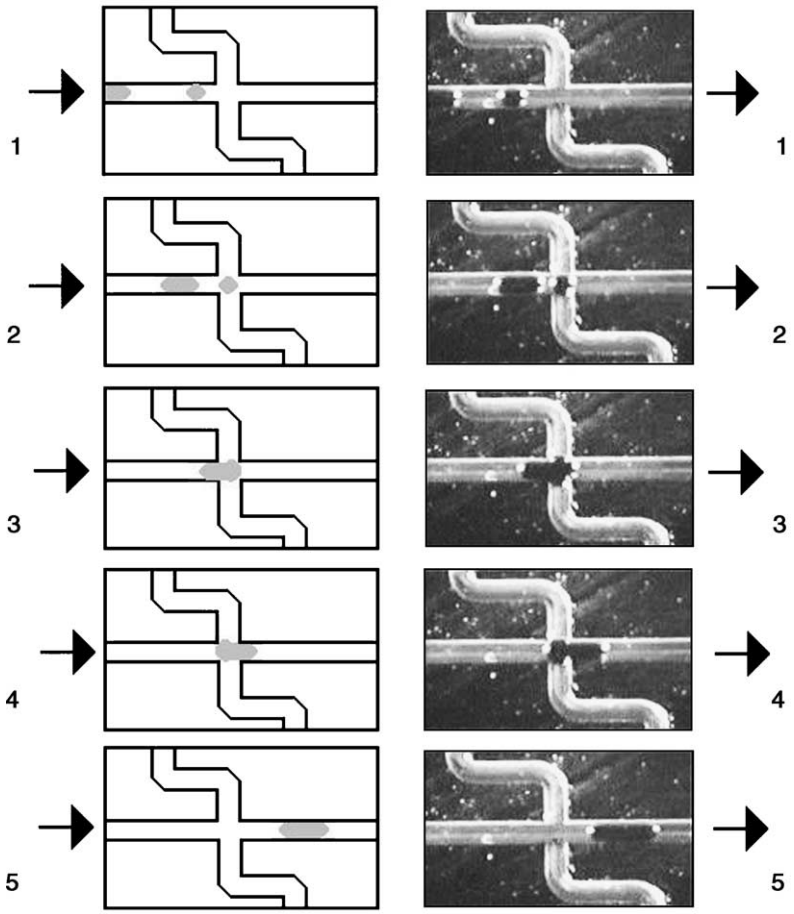

Fig. 15. Fusion of two segments at a cross junction inside a chip device. Both segments come from the left. The first one stops at the channel junction, is unifying with the second segment and the resulting large segment is moved-on to the right.

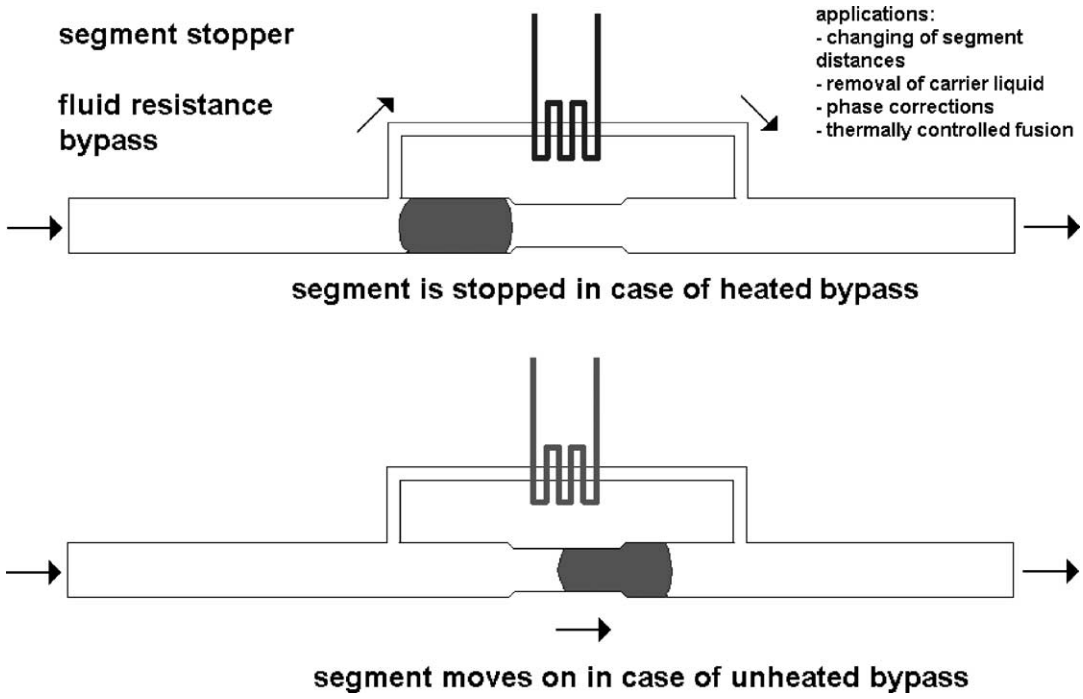

Fig. 16. Thermally controlled stopping of fluid segments in a channel configuration of reduced cross-section with an integrated by pass of high fluid resistance by means of a viscosity-controlled fluid motion ("microelectrocaloric stopping").

the combination of the junction with a fluid resistor or an other type of fluidic hindrance. If this element, viscosities and flow rates are well adapted, then a segment of one sort is stopped at the junction or at the fluid resistor and is waiting for a second segment in order to fuse with it. A similar fusion behavior was found on channel junctions. So, at suited flow rates, small segments stop at a cross junction and become fused with following segments (Fig. 15). After fusion, the formed larger segment is transported away automatically, that means without a change of the flow rate.

It is possible to realize a stopping and a fusion of segments by thermal pulses using the effect of decreasing viscosity of carrier liquid with increasing temperature. Therefore, thin film heaters are arranged over fluid resistors, e.g. at a fluid bypass (Figs. 16 and 17).

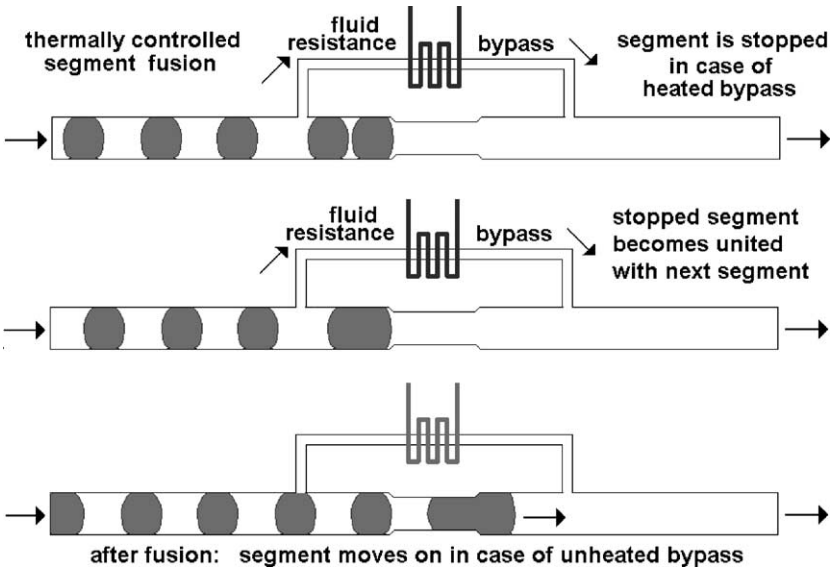

Fig. 17. Thermally controlled stopping and pairwise fusion of fluid segments in a channel configuration of reduced cross-section with an integrated by pass of high fluid resistance by means of a viscosity-controlled fluid motion ("microelectrocaloric fusion").

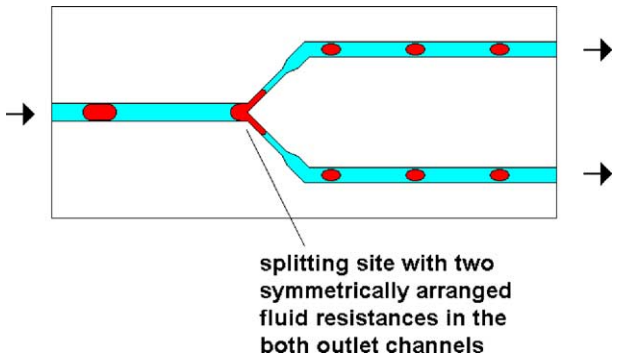

Fig. 18. Passive splitting of a segment into two parts by a branched channel configuration with a reduction of channel diameters.

A splitting of a larger segment into two smaller can be achieved at a bifurcation of a channel. A symmetric splitting should be based on a symmetric construction of the bifurcation unit. In addition a transformation of segment shape from spheric into cylindric is necessary to assist the splitting process. Interface energy has to be introduced into the system for shape transformation of segments as well as for splitting. A configuration with two constricted outlet channels (fluid resistors) behind the bifurcation site is advantageous (Fig. 18). Slightly asymmetric splitting units can be used for the production of pairs of unequal daughter segments. But, stronger asymmetries and disturbances can cause a passage of the whole segment volume into the left or right outlet channel. Splitting fails in this case.

More robust splitting units consists of a splitting chamber with a volume adapted to the volume of the mother segment (Fig. 19a). The mother segment is applied by a channel leading into the central region of this chamber (A). The channel for splitting pulses (B) is closed by an outside valve during the filling of the chamber. The positioning of the mother segment within the splitting chamber is assisted by two fluid resistances between the chamber and the outlet channels (C and D). After filling, the channel A is closed (outside valve). The splitting is induced by a pressure pulse in channel B. The configuration can also be applied for the production of asymmetrically splitted segments (Fig. 19b). A splitting in more than two daughter segments demand for further pulse channels. Fig. 20 shows an arrangement for the division of a segment into three parts. Electrical switching for sorting of segments is possible by branched channel structures, which are connected with thermally addressable fluid resistors (Fig. 21).

3.4. Cloning of segment sequences

Segment splitting is the elementary precondition for the automated production of copies of whole sequences. This

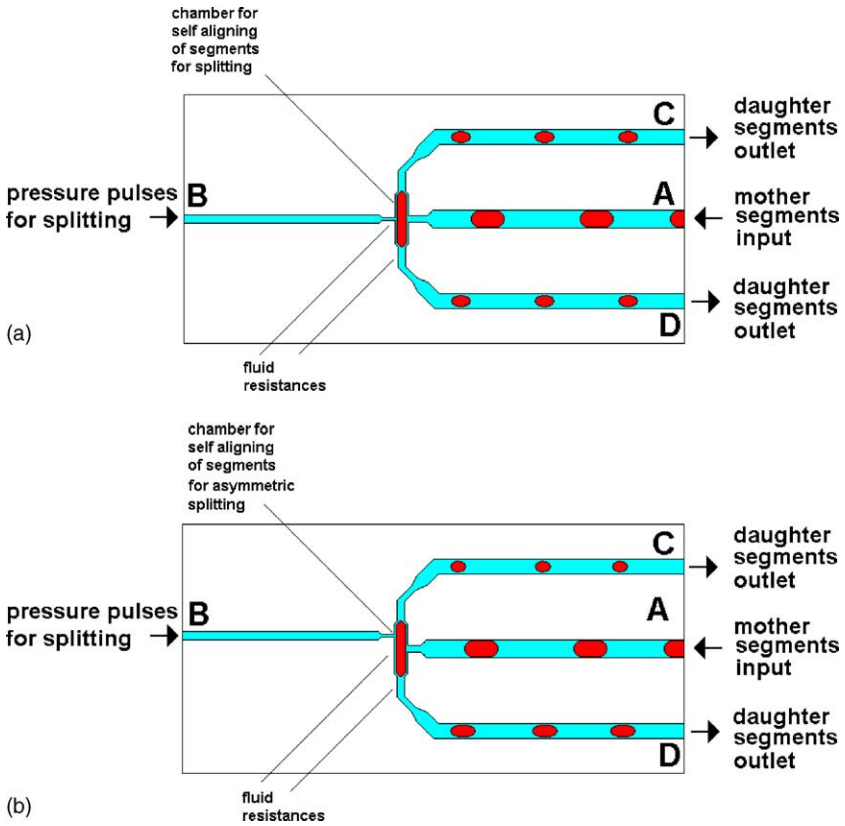

Fig. 19. Active splitting of a segment into two parts by a branched channel configuration with a volume-adapted segment chamber for self-positioning and fluid resistances: (a) configuration for symmetric splitting; (b) configuration for asymmetric splitting.

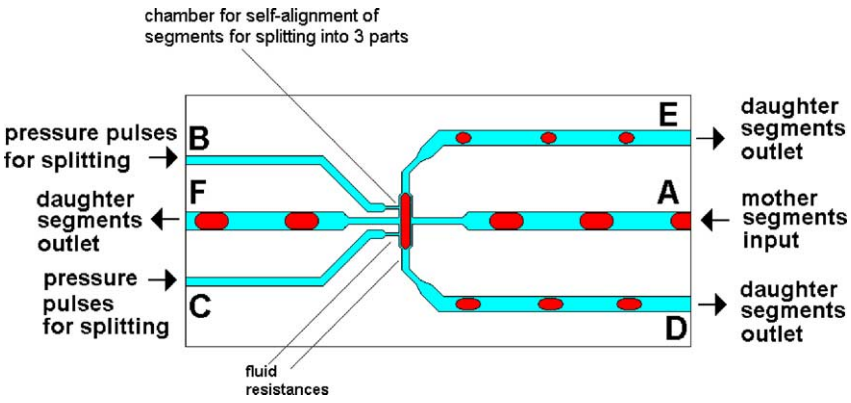

Fig. 20. Active splitting of a segment into three parts by a complex branched channel configuration with a volume-adapted segment chamber for self-positioning and fluid resistances.

operation is called "cloning" here because it is analogous to the production of syngenic genetically homogeneous offspring of living beings. The procedure is of importance for chemical systems, particularly, if different screening procedures have to be carried out with numerous samples and different test substances.

In chemical systems the amount of each substance inside a segment remains constant. For cloning either the daughter segments are much smaller than the mother segment (simple subdivision of volumes, Fig. 22A) or the solution in the segments has do be diluted in order to remain the size and volume of segments (Fig. 22B).

Sequence cloning is of particular interest in case of biological samples like miniaturized cell cultures. In this case, the number of cells inside mother and daughter segments can stay the same if an increase of cell number by cell division (cultivation) within daughter segments is initiated after splitting and dilution of segments by culture medium. The term "cloning" is matching this operations in a two-fold meaning, because the cells of a mother segment are transferred into real biological clones of this cells by the cultivation within the daughter segments.

3.5. Integrated systems for the generation and manipulation of segment sequences

Modular micro reactor arrangements are most often preferred during research and development as well as for educational purposes due to the easy possibility of changing components and single improvements of them. But always the fluidic connection of modules demands for fluid interfaces. Fluid interfaces are not only marked by an increase of parasitic volumes and dead volumes but they are in general connected with changes in the cross-sections of liquid

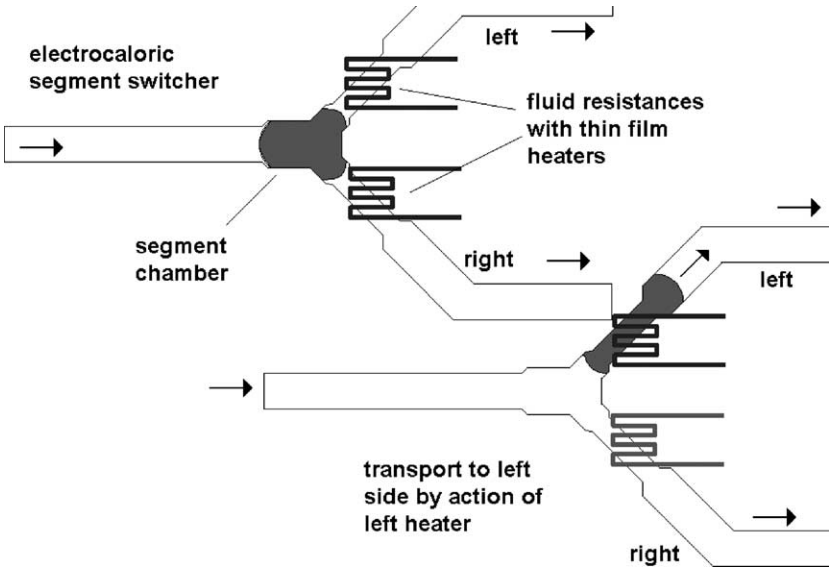

Fig. 21. Thermally controlled direction of motion of fluid segments in a branched channel configuration with reduced cross-sections ("microelectrocaloric switching").

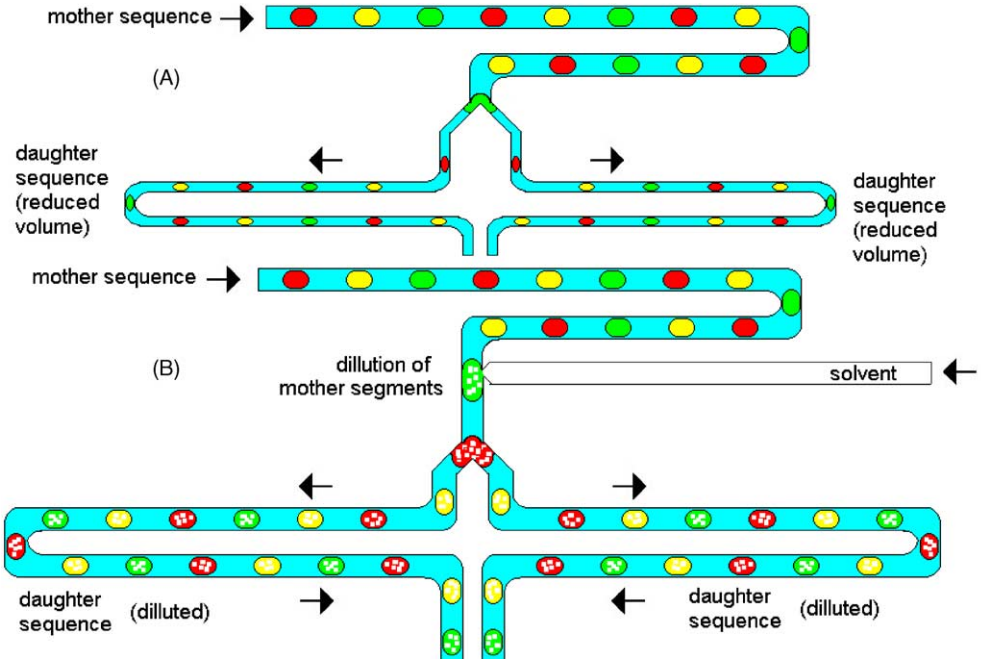

Fig. 22. Splitting operations with fluid sequences: (A) splitting without volume compensation, concentrations remain constant; (B) splitting with volume compensation, concentration is lowered.

columns passing through them. This is a serious problem for modular arranged micro fluidic systems for segmented flows. Changes in the channel diameters, undesired fluid resistances and other interferences must be strictly avoided. Consequently, standard fluid interconnectors cannot be applied in most cases and the development of special fluidic interfaces is necessary for modular composed segmented flow arrangements.

These difficulties can be overcome by integration of functions for segment operations in fluidic systems. The integration of several injectors is of interest for the generation of complex sequences, for example. The injectors can be arranged serially (Fig. 23A) or in parallel (Fig. 23B).

For further operations the transformation of segments plays a key role. The formation of cylinder-like from spherical segments is connected with the introduction of interface energy into segments. The best way to do it is the use of a conical reduction of diameters of fluidic channels. The reverse process, the formation of spherical segments from cylinder-like segments, is much easier due to the spontaneous relaxation of interfaces if cylinder-like segments are

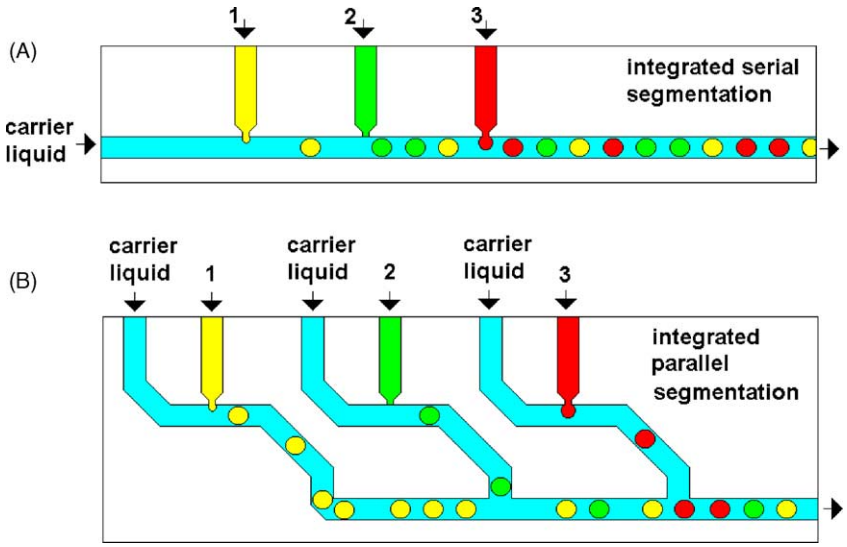

Fig. 23. Series of injectors for the preparation of complex segment sequences: (A) serial arrangement; (B) parallel arrangement.

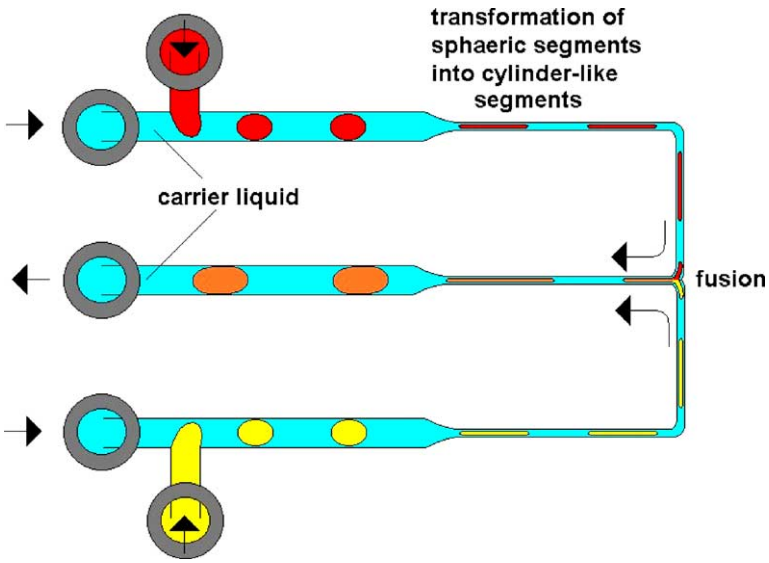

Fig. 24. Integrated arrangement for injection, segment shape transformation and fusion.

flowing from a channel segment with smaller diameter into a larger one.

There is a multitude of possibilities of combining fluidic elements for different operations or process chains. An example of a parallel segment generation followed by transformation and fusion is shown in Fig. 24. The transformation of spherical segments into cylinder-like makes the system more tolerant against minor changes in pressure, flow rate and, therefore phases of both supplying segment channels. In addition, it is in general more convenient to apply a position control by optical sensing in case of cylinder-like segments.

3.6. Application in miniaturized biotechnology

The principle can be used for a large variety of applications. Several techniques of quantitative chemical analysis can be realized by use of micro fluid segments, e.g. the principle of flow injection analysis can be transferred to segments as well as titration (digital micro segment titration), for example. Micro segments could also be applied in modular synthetic chemistry, particularly in combinatorial chemistry as far as the educts and products of the single synthesis steps are compatible with liquid/liquid two phase system.

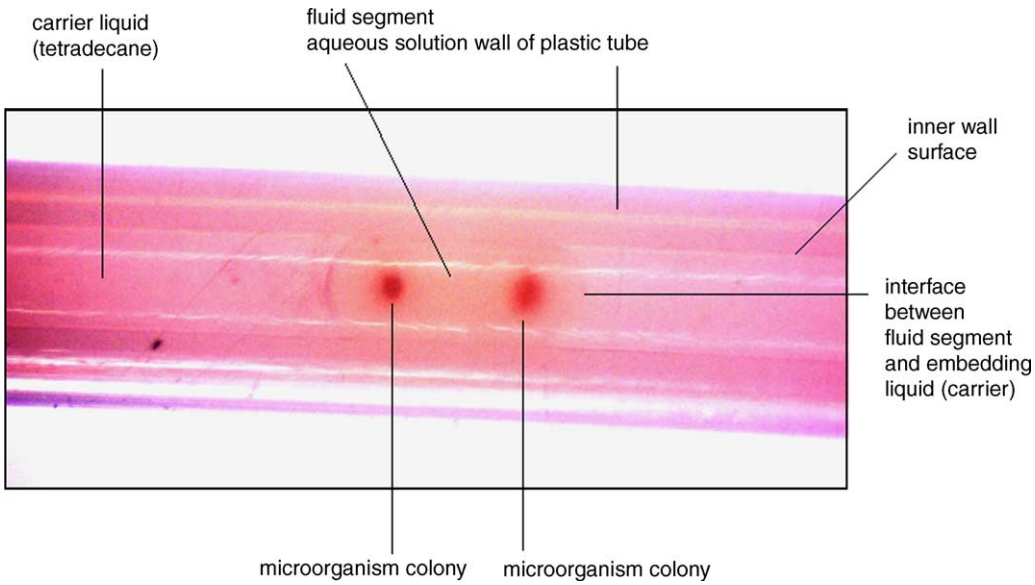

Fig. 25. Aqueous fluid segment containing two colonies of microorganisms embedded by an aliphatic carrier liquid inside a plastic tube (inner diameter 0.5 mm).

The segmented flow principle is of particular interest in microbiological experiments and screening procedures. First results show the practical advantages in the highly parallelized production microbial cultures of very high diversity and for the cultivation of species with low growth rates [21]. It was shown, that different microorganisms can be cultivated within the small segments (Fig. 25). In addition, it is possible to produce sequences of segments with different cell populations. In case of suited aliquotation of complex composed cell suspensions, it is possible to get a high portion of segments with monoclonal cell cultures, as shown in Fig. 26.

Besides cultivation, the possibility of production of separated cell cultures in high density opens the way to further applications in screening and testing of drugs. The combination of chemical and cell biological operations in sequences of segments gives very promising possibilities for the study of influences of substances on cells and also for cell–cell interactions. Nanoliter segments embedded in an non miscible inert liquid represent an ideal environment for operations with single cells. So it is assumed that this technique will become important for molecular cell technologies and single cell characterization procedures.

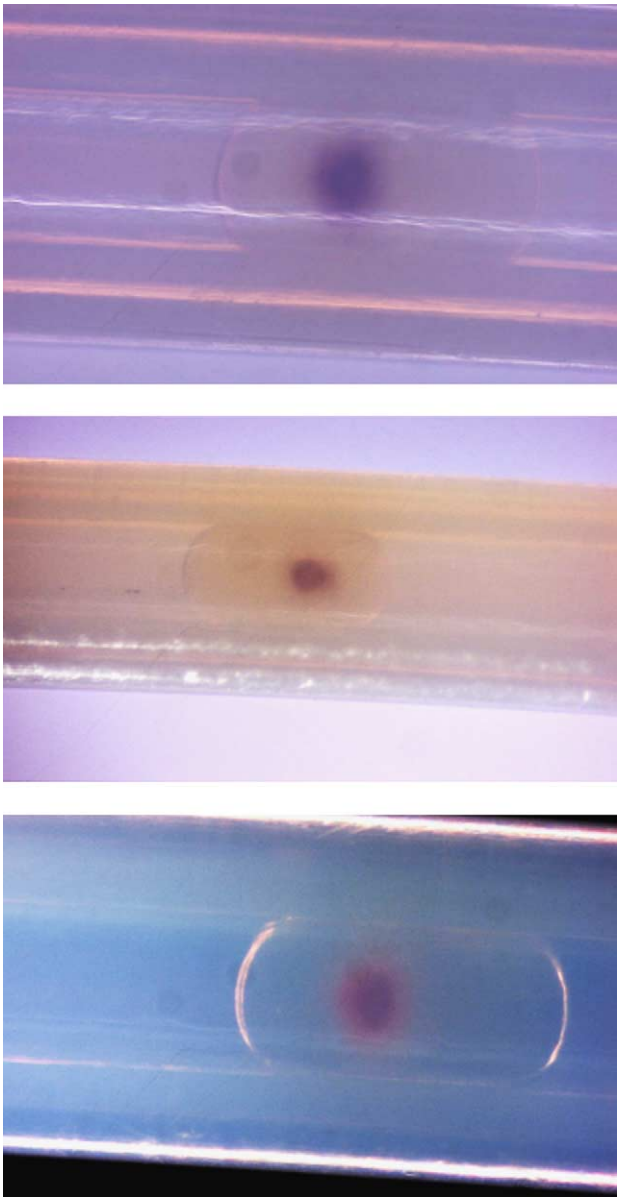

Fig. 26. Three examples of monoclonal cultures obtained in micro fluid segments (inner diameter of tubes 0.5 mm).

4. Conclusions

The application of fluid segments with volumes in the nanoliter range for serial flow processing is a very promising new technique in micro reaction technology. It overcomes the dispersion problem due to the laminary flow in micro fluidic systems. Segments can be produced and processed of high rates. Consequently serial processes with very high throughput can be realized.

Serial flow systems consist of a group of functional elements including injectors, segment splitters, fluid resistors and elements for transformation and fusion. These elements can be combined by modular arrangements as well as by integration in complex fluidic micro reactors.

Reaction volumes can easily be divided in a number of small isolated reaction volumes. Very high variabilities can be achieved in sequences of segments with different composition which can be described as fluidic strings. The simple connection of continuously changed fluid actuation (volume flow rate) with segmentation leads to a stepwise variation of concentration in the segments. Both strategies supply series of reaction compartments with digitally varied properties resulting in a so-called “digital chemistry” in serial flow.

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