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Static micromixers for modular chip reactor arrangements in two-step reactions and photochemical activated processes

T. Kirner [a,*], J. Albert [b], M. Günther [a], G. Mayer [b], K. Reinhäckel [a], J.M. Köhler [a]

[a] *Department of Physical Chemistry and Microreaction Technology, Institute of Physics, Technical University of Llmenau, Weimarer Str. 32, D-98693 Ilmenau, Germany*

[b] *Institute for Physical High Technology Jena, Winzerlaerstr. 10, D-07745 Jena, Germany*

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Abstract

Static mixing in chip devices can easily be monitored if a construction with transparent channel segments is used. Therefore, a mixer consisting of a three-layer arrangement glass/Si/glass was developed. All channel segments etched in the central Si chip can be visually observed or used for optical measurements in transmittance due to the completely transparent glass layers. The other channel segments are etched in both glass layers and are observable in reflection mode. The chip mixer contains eight split and recombine units. It was tested by the formation of a coloured product from two non-coloured aqueous solutions as well as by the interdiffusion of a fluorescent dye in glycerol/water mixtures. The microscopic measurements suggest that good mixing efficiency is achieved after all eight split and recombine units up to flow rates of 200 μl/min even in case of more viscous liquids. In case of lower flow rates (20 μl/min and less), sufficient mixing by interdiffusion already takes place in the first three conversion transducers. Additionally, the use of the reactor is demonstrated in a micro modular photochemical experimental arrangement.

1. Introduction

Mixing of fluids is a process step which is important for many applications of chemical analysis [1] and synthesis. This also applies to microsystems. It is essential to have micromixers as modules, in order to construct microstructured chemical plants (“lab-on-a-chip”). Several micromixers using different ways of mixing were developed [2–4]. Microsystems are characterised by low Reynolds numbers (<50), which means strictly laminar flow. Dynamic mixers [5], as used in macroscopic chemical plants and experimental set-ups are not easily suitable. Static micromixers are very well suited for easy mixing of reactants in micro fluidic systems [6–9]. In static micromixers, mixing is based on diffusion. Diffusion is a time-dependent process. This time can be provided to the microsystem by low flow rates or by long diffusion channels. The small dimensions in microsystems allow mixing by diffusion on relatively short timescales. In the microchip, introduced in the following, the mixing process is accelerated by splitting and recombining the liquid flow. The split-and-recombine principle is particularly suitable for flow-through mixing. Anodic bonding of microfabricated half shell chips leads to mechanical and chemical stable reactor modules. In mixer types with a Si/glass/Si construction, observation is only possible in the infrared region. We constructed a glass/Si/glass three-layer mixer in order to ensure optical transparency (visibility) in each step, providing the possibility of fast control of the mixing efficiency. For another transparent micromixer compare, for example [10].

Time-dependent reactions can be monitored by photometric or spectrophotometric transmittance measurements in 24 observation windows. The efficiency of mixing has been tested by using two different coloured liquids. A fast chemical reaction was performed in the reactor. A multistep reaction including a photochemical reaction step is presented as an example for using the module in a more complex micro chemical arrangement.

2. Experimental

2.1. Construction of the static micromixer

In contrast to static mixers developed earlier in Si/glass technology [6,8], the chip sandwich consists on a core chip of Si and two cover chips of glass (Fig. 1). All three chips

* Corresponding author.
E-mail address: thomas.kirner@tu-ilmenau.de (T. Kirner).

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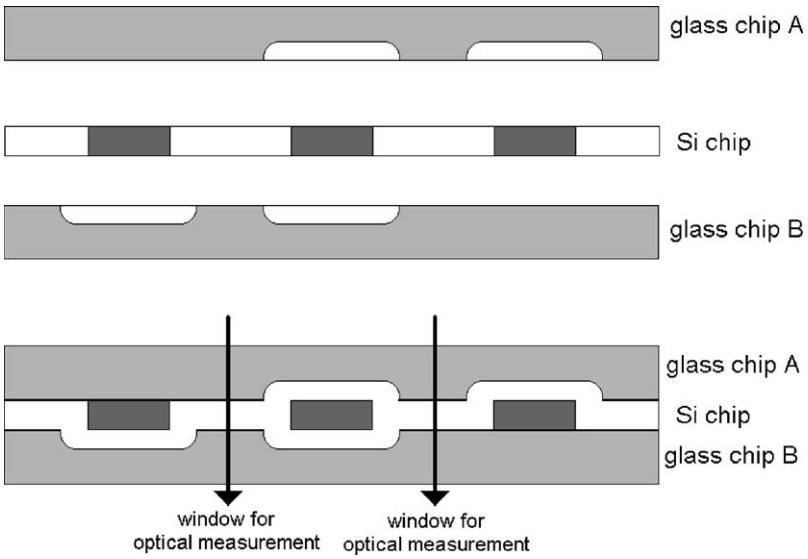


Fig. 1. Construction principle of static chip mixer (cross section).

are structured microlithographically in order to realise the channel structures and the microfluidic branches and reunification elements as well as the areas for the attachment of the fluid ports. Channels etched into one side of each glass chip define the two levels of fluid transport. The other sides of the glass chip possess no structures (plane) in order to support best conditions for optical imaging. The only exceptions are the drillings for the fluid ports. The micropatterned channel levels in both glass chips are separated by the interlaying Si chip. The Si chip was etched through completely in order to get fluidic connections between the both channel planes of the glass chips. Inside these Si channels, there are the best conditions for optical measurements namely in transmissive arrangement as a result of the smooth optical planes at all four interfaces defined by the quality of the original glass surface.

The chip size is 22 mm × 14 mm. It includes three fluid ports (two inlets and one outlet) and eight split and recombine units (Fig. 2). The flow channels possess a channel width between 0.2 and 0.7 mm. The arrangement of the channel elements was designed for an optimal reshaping of the cross section of stacked fluid columns parts. The change of flow directions from horizontally to vertically and back at branching points and reunification points supports the efficient reshaping and interdiffusion. The internal volume of the chip device including the fluid interconnectors amounts to about 8.5 μl. This results in mean residence times between 510 and 2.55 s at a pump rate between 1 and 200 μl/min. Therefore, the static mixer is well suited for applications in modular arrangements for chemical and biochemical experiments with small volumes. There are different channel diameters in the reactor resulting in different flow velocities. With a pump rate of 80 μl/min for example the velocity varies from about 12×10^{-3} to 30×10^{-3} m/s. The corresponding Reynolds numbers are 3.5 and 6 for water and 2.4 and 4 for glycerol, respectively. The numbers were calculated while neglecting the special etching profiles in silicon.

2.2. Materials and methods

All used chemicals were p.A. grade. Solutions were prepared by dispensing using Eppendorf pipettes and mixing in test tubes or Eppendorf tubes.

A two-channel UV/Vis spectral photometer Specord (Analytik Jena) was used for spectrophotometric measurements

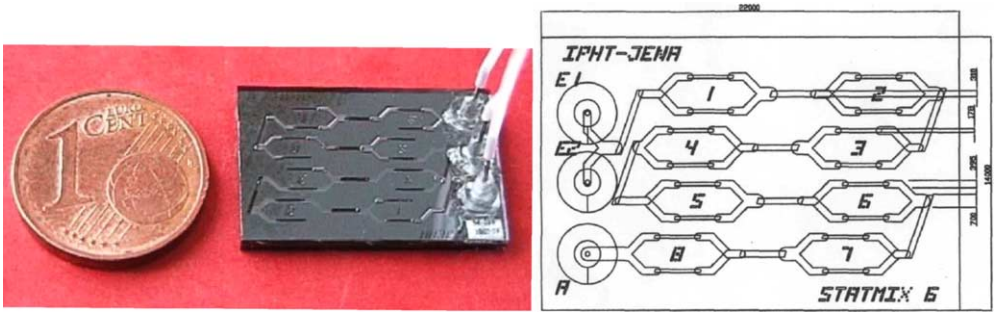


Fig. 2. Static chip micromixer (“Statmix 6”): optical image (left) and sketch of arrangement of the fluid ports and the eight split-and-recombine units.

of dye solutions as well as for the determination of product concentration in photochemical experiments.

The optical images were taken by use of a video camera (Cohu 2252, BFI-Optilas, Germany) in connection with a microscope (Axioplan 2 Imaging, Zeiss, Germany) and the Epiplan-Neofluar 5x/0.15 HD DIC or the LD Achroplan 20×/0.40 Korr. objectives. The images were taken in transmission or in reflection using a 100 W halogen lamp or a 100 W mercury vapor short arc lamp (HBO 100 W). In order to characterise the mixing process, two different coloured liquids were pumped through the reactor using syringe pumps (540-200, TSE, Germany), which provides the same flowrate at both inlets. For experiments, 1 ml polypropylene syringes were used (Roth, Germany).

2.3. Experimental arrangements

In all cases, modular constructed experimental set-ups were used. Syringes and chip modules were connected by PTFE tubing with an inner diameter of 0.5 mm. The principle of modular reaction systems enables the chemist to rearrange the reactors quickly and to optimise the experimental arrangement efficiently. In addition, it allows the comparatively simple construction of numerous different experimental set-ups by the use of a few types of different chip reactors. Each module is suited for multiuse and can be applied in different experiments.

In case of the photochemical experiment, an arrangement of four chip reactor modules was mounted on a microscopic slide. All modules were fluidically connected by short teflon tubes. The fluid inlets were directly connected with the syringes. In addition, two optical interfaces had to be integrated. A flexible light conductor was applied for the irradiation. The end of the fibre bundle was mounted in close contact to the first optical micro flow-through cell (Fig. 3). A second optical micro flow-through cell was placed behind the second mixer (addition of reagent for products) in order to realise the possibility of a on-line monitoring of products.

3. Experimental results

3.1. In situ characterisation of mixing by interdiffusion in viscous liquids

At first, the flow behaviour inside the channels of the micromixer was characterised by means of the interdiffusion of two aqueous solutions containing 20 and 15% of glycerol. The addition of glycerol leads to an increase of viscosity in comparison with aqueous solution without glycerol. So, the laminarity of flow should be stabilised by the additive. One of the solutions, which were guided through the mixer contained 1 mmol/l fluorescein sodium salt. So, the interdiffusion of both solutions could be visualised by the distribution of fluorescent liquid and the dispersion of fluorescence in dependence on the progress in the split and recombine steps.

The microscopic imaging of streaming solutions in Si windows in the different mixing steps shows a characteristic change in the spatial distribution of fluorescence (Fig. 4). The experiments were carried out at a flow rate of 20 μl/min in each input channel, that means an overall flow rate of 40 μl/min. At the beginning (first and second mixing step), the solution with low fluorescein content was streaming in the centre of the channel marked by a grey strip in the centre window. On the sides, the fluorescence-rich solution is streaming (Fig. 4, left hand). The grey strip in the centre becomes reduced during the next split-and-recombine steps. The ongoing mixing by diffusion is very well reflected by the fluorescence microscopic images of the 4th and 5th step (Fig. 4, centre). A complete mixing after the 7th step is proved by a homogenously fluorescent streaming liquid in the 8th split-and-recombine zone (Fig. 4, right).

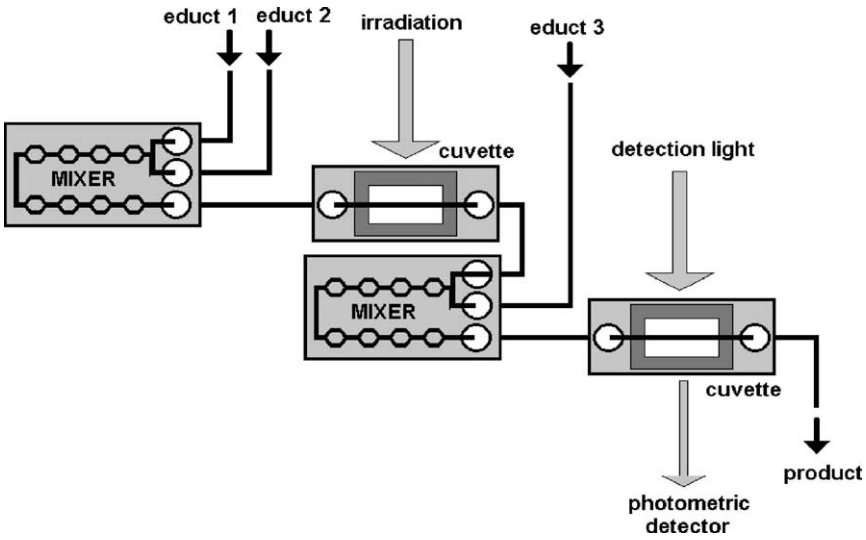


Fig. 3. Modular microreactor arrangement for photochemical experiments.

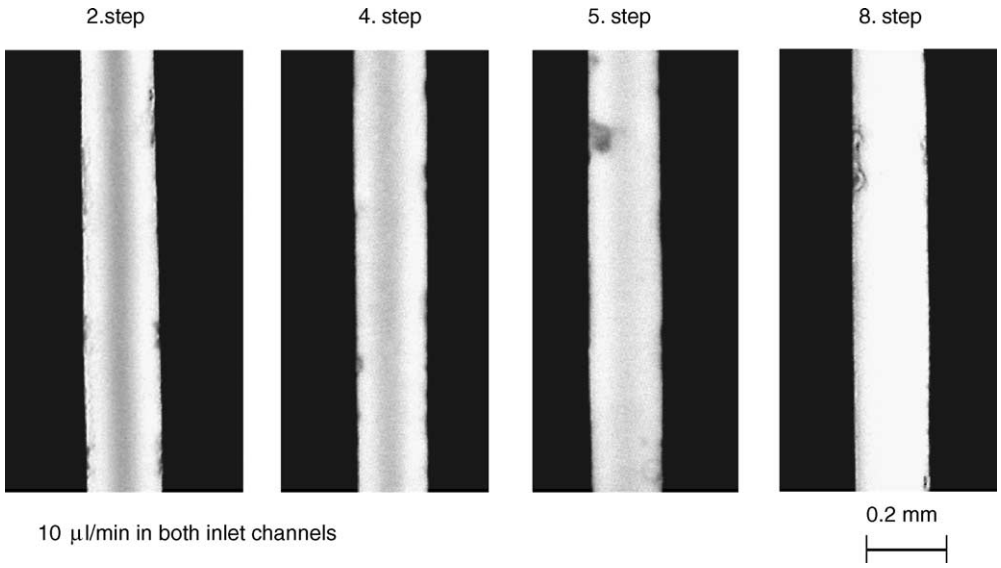


Fig. 4. Fluorescence microscopic image of optical windows (Si channels) in different mix steps inside chip mixer in case of interdiffusion during passage of fluorescent viscous solutions (20% glycerol in water). The disappearance of grey strip (fluorescein-poor solution) indicates increasing mixing efficiency from steps 2 to 8 (flow rate: 10 µl/min in both input channels).

The time need of diffusion limits the mixing effect at high flow rates. This can be seen clearly from the difference in the fluorescence distribution in the two windows in the central part of the mixer chip (Fig. 5). In both cases, the glycerol content amounts to 15%. Whereas the liquid lamellas are nearly completely disappearing in case of the lower flow rate (40 and 20 µl/min in each input channel), the lamellas of fluorescein-rich and fluorescein-depleted solutions show still significant contrast in case of higher flow rate (100 µl in each input channel, resulting in 200 µl/min as total flow rate). The laminarity of flow can be seen from the distribution of darker and brighter fluid lamellae in the transition zone between glass channel segments (larger channels) and Si channels (smaller channels).

The effect of flow rates becomes particular obvious in the starting phase of the mixing process between the split-and-recombine steps 1 and 3. A homogeneous fluorescence could be detected, if the flow was stopped after a few seconds (Fig. 6B). The falling gradient of fluorescence intensity from the centre to the sides in case of the glass channel (larger channel) is due to the curvature in the cross section of glass channels caused by the isotropic wet etching process. This leads to a decrease in the thickness of whole liquid column and, so, to a reduced density of fluorescent molecules

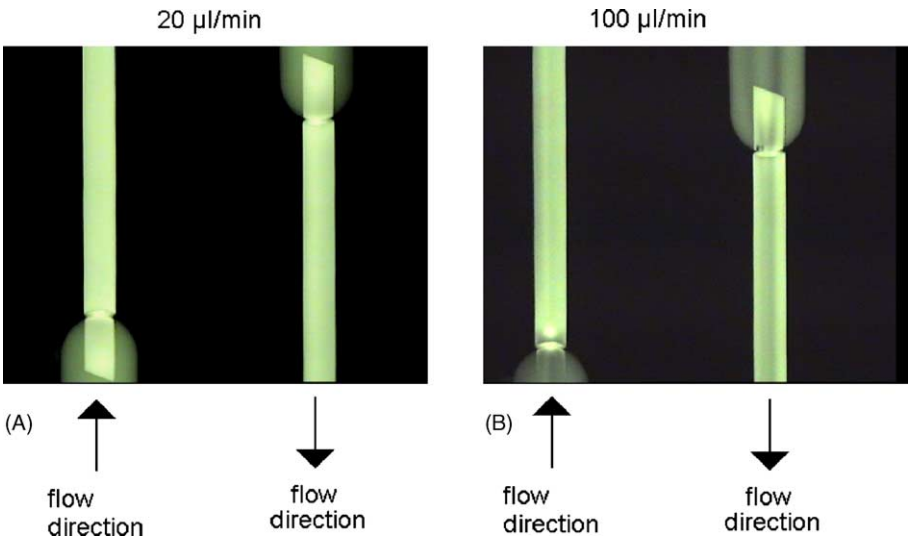


Fig. 5. Differences in mixing efficiency in dependence on flow rate: (A) 20 µl/min: in the centre of the chip device a nearly complete mixing was achieved (15% glycerol in water, fluorescein-labelled); (B) 100 µl/min: in the centre of the chip device complete mixing was achieved (15% glycerol in water, fluorescein-labelled).

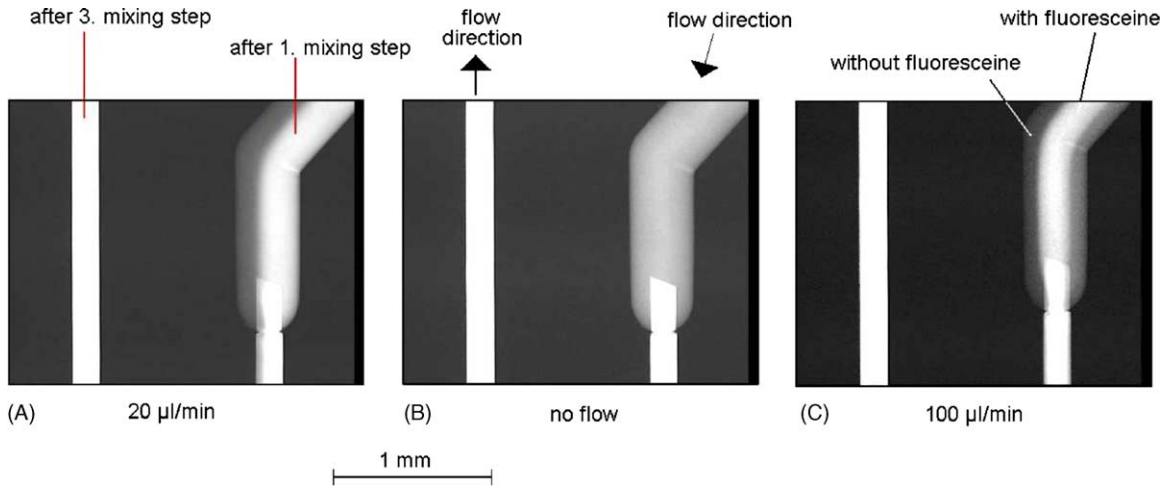


Fig. 6. Mixing efficiency in dependence on mixing step and flow rate: in all three images an input channel (right) and a channel after the third split and recombination step is shown: (A) flow rate: 20 μl/min; (B) no flow; (C) flow rate: 100 μl/min (15% glycerol in water, fluorescein-labelled).

per area even in case of a homogeneous distribution of the fluorescence molecules in the whole liquid. In the Si window, the optical path length and, therefore the area density of fluorophores, is always constant due to the planar parallel arranged optical interfaces defined by the locally plane surfaces of the both glass chips covering the Si channel. This corresponds well to the spatially homogeneous fluorescence in the Si windows without flow (Fig. 6B, left). The outlet region of the first split and recombine step supplies two lamellas of liquid at a flow rate of 20 μl/min (Fig. 6A, right). The laminar flow is conserved during the transition from the glass into the Si channel segment (Fig. 7a, right bottom). But, after the third split-and-recombine step, the mixing by interdiffusion is nearly complete at a flow rate of 2×20 μl/min,

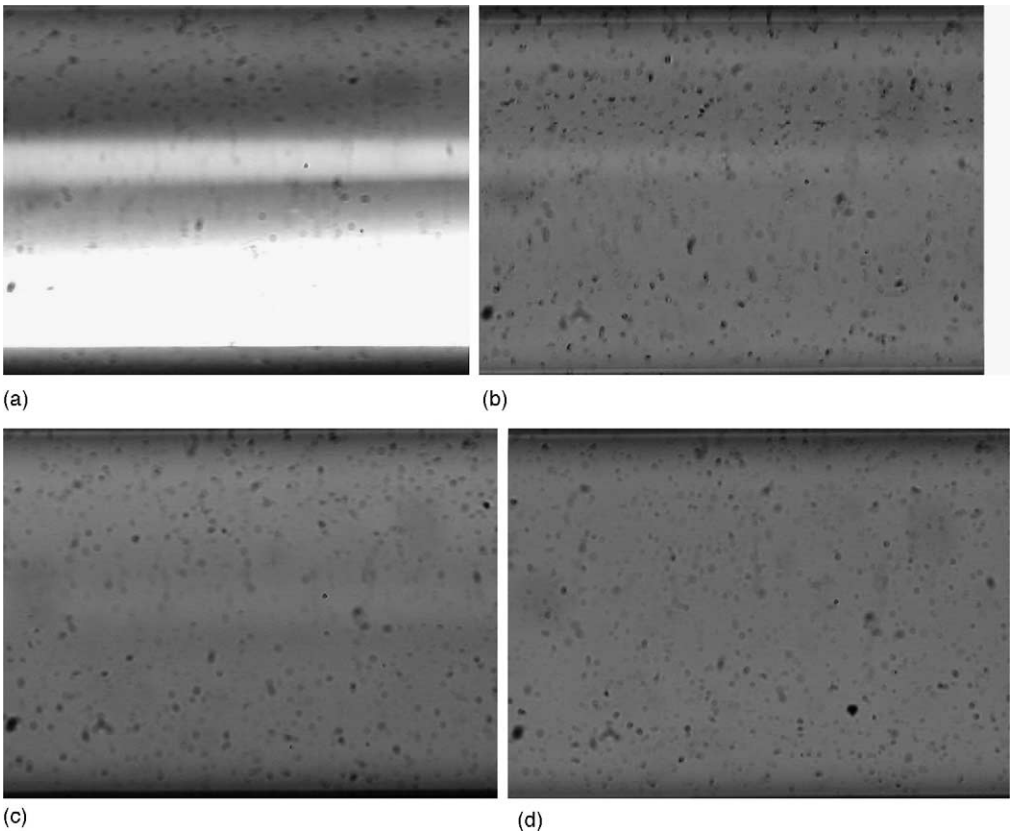


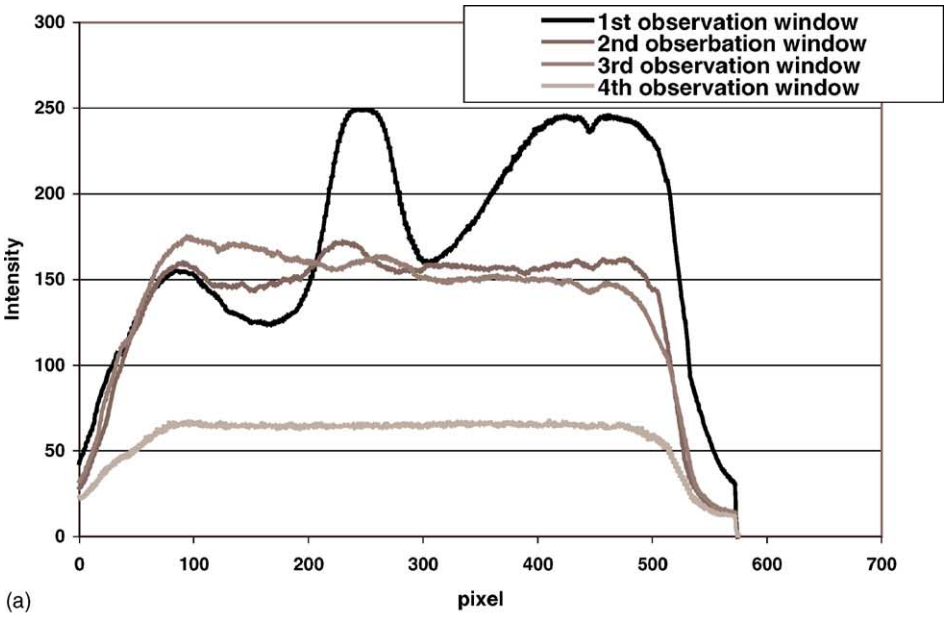
Fig. 7. Reaction of ammonium rhodanide and iron(III) chloride. Images of the four observation windows in the middle of the micromixer. The pumping rate was 40 μl/min.

which can easily be detect in a Si window (Fig. 6A, left). In case of a flow rate of 2 × 100 μl/min, three lamellas of liquid were observed after the first split-and-recombine step (Fig. 6C, right). Three steps are not sufficient for complete mixing at such high flow rates. This is evident from the fluorescence image of the Si window (Fig. 6C, left).

3.2. Formation of a dyed product by interdiffusional mixing

A simple chemical experiment was realised by the reaction of a iron(III) salt with rhodanide ions (thiocyanate) in aqueous solution. Thereby, the deeply red coloured complex $[Fe(SCN)_3(H_2O)_3]$ is formed instantaneously from a slightly yellow solution ($FeCl_3$ in water) and a colourless solution (NH_4SCN in water). The formation of the red coloured ferric complex compound indicates the mixing of the streaming educt solutions by interdiffusion of both components.

Both educt solutions were pumped through the chip mixer at a flow rate of 40 μl/min. The experiment showed, that no back diffusion takes place. As a result, the input channels before the first split-and-recombine step remain colourless or less coloured. But the interdiffusion starts during the first combination of both input streams already indicated by the



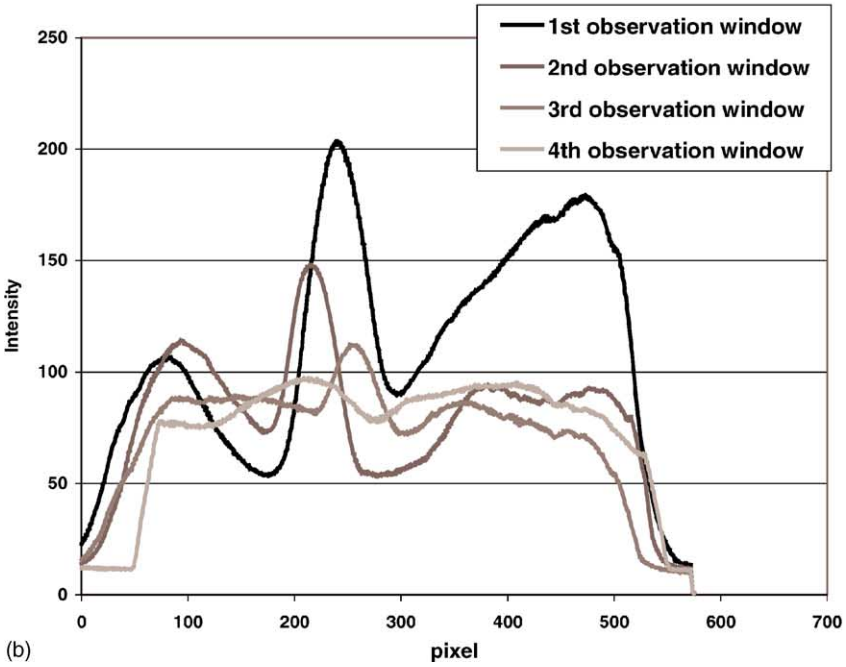


Fig. 8. (a) Cross sections through the images in Fig. 7. (b) Cross sections for an experiment with a pumping rate of 80 μl/min.

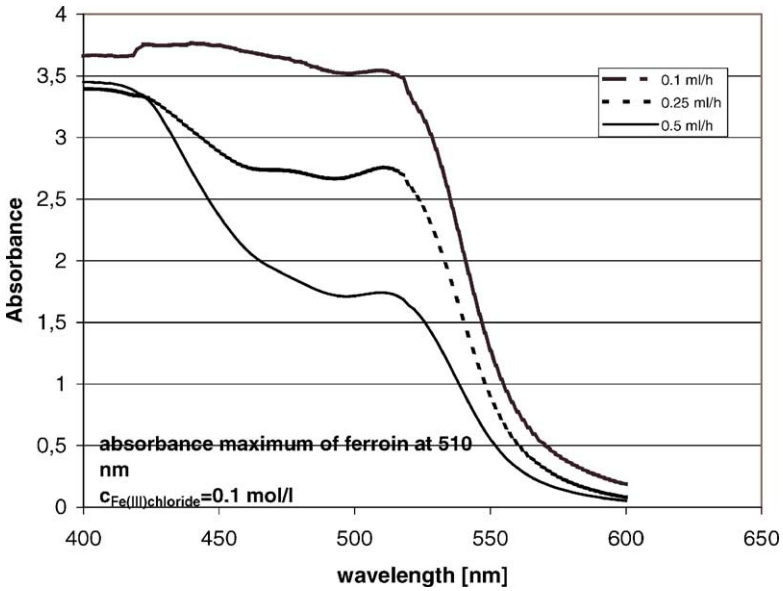


Fig. 9. Photolysis of oxalate in presence of Fe(III). *o*-Phenanthroline hydrochloride reacts with Fe^{2+}-ions to the intensively coloured Fe(II)–phenanthroline complex. Absorbance curves (UV/Vis, Jasco, V570, Germany) of the product ferroin at three different pump rates. The absorbance maximum of ferroin is at 510 nm. One can see that in this case higher pump rate leads to less product.

appearance of the red colour of the rhodano iron complex in the first mixing step (Fig. 7). The red colour is represented in the images gray scale images by dark colour. Fig. 7a shows the reaction after the first split and recombine unit. Three different stripes can be seen in the observation window. Fig. 7b shows the reaction after the third mixing unit. Mixing is nearly completed after the third mixing unit. This result is in good agreement with observations for diffusing

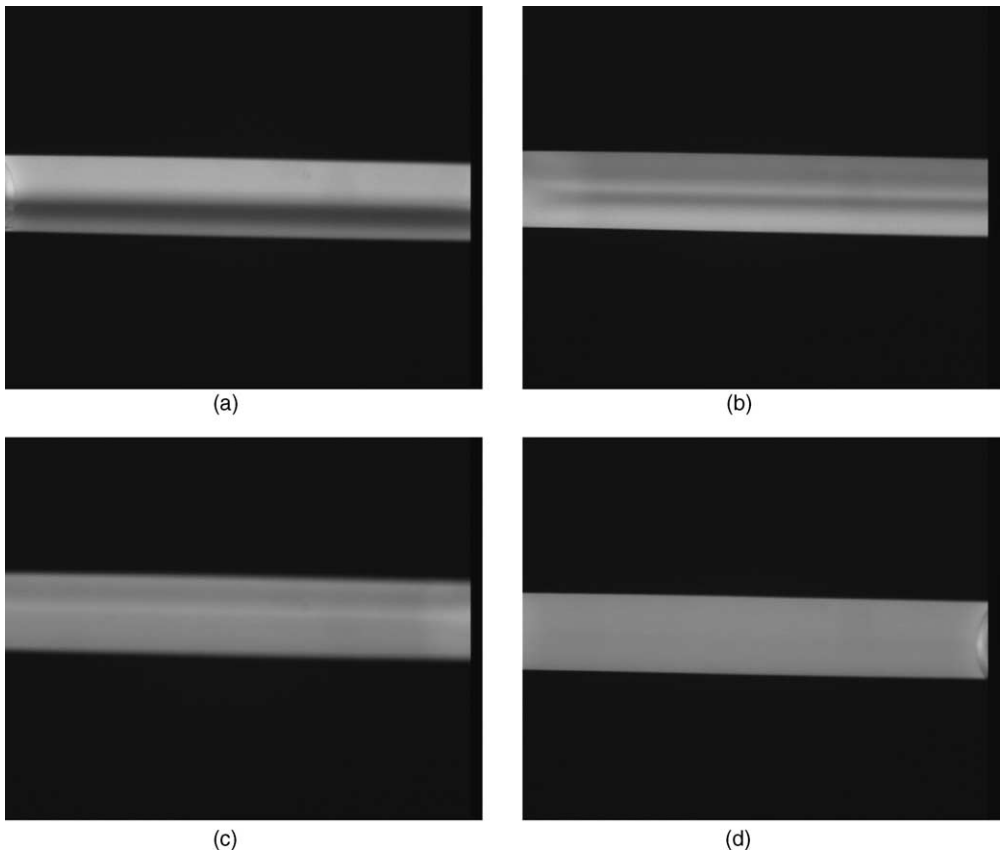


Fig. 10. Mixing of a fluorescein solution in 20% glycerol in water and 20% glycerol solution. The images were taken in transmission at the four observation windows after the first, third, fifth and seventh mixing unit.

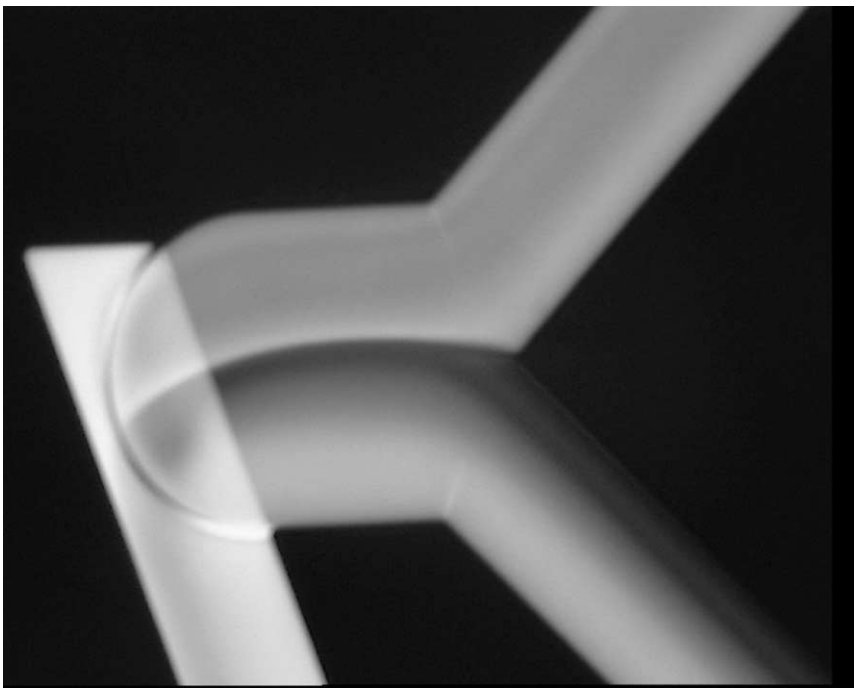


Fig. 11. Mixing process at the exit of the second split and recombine unit.

fluorescein at flow rates of 20 μl/min. In Fig. 8, the process is shown by plotting the cross sections of the observation windows of Fig. 7. Fig. 8a represents the reaction with pumping rate of 40 μl/min and Fig. 9b at 80 μl/min. The cross section were calculated with the red channel of the colour images. At a pumping rate of 80 μl/min, the reaction mixture is not completely homogeneous after the seventh mixing unit.

3.3. Photochemical formation of Fe(II) from Fe(III) in a modular micro reactor arrangement

The photochemically initiated decomposition reaction of iron(III) oxalate was chosen for testing the new static micromixers in a more complex modular micro reaction arrangement. The photosensitive complex is formed in a first mixing step of the two stable educts $FeCl_3$ and oxalic acid in the first chip mixer. This mixer is fluidically connected to a chip cuvette. Here, the photochemically reaction takes place. The irradiation can easily be executed by application of a flexible light guide arranged over the transparent window of the micro flow-through cuvette. Fe(II) ions and CO_2 (both dissolved) are formed by the light-induced redox reaction.

The chemical reaction rate can easily be determined by measuring the concentration of formed Fe(II) ions. Therefore, an additional reagent is mixed with the reaction solution. This mixing process is realised by a static chip mixer, too. The fluid outlet of the irradiation cuvette is, therefore, directly fluidically connected with the fluid inlet of the second mixer. *o*-Phenanthroline hydrochloride solution is added as reagent in order to form the deeply coloured Fe(II) phenantrholino complex (ferroin) [11–13]. The concentration of this complex can be measured either in a micro photometric arrangement with a second flow-through micro cuvette for on-line detection (Fig. 3) or by spectrophotometric measurements.

The photochemical formation of Fe(II) in the micro modular reaction arrangement was investigated in dependence of flow rate. In case of constant light power of the irradiation source, a increasing Fe(II) concentration was found with decreasing flow rate, as expected (Fig. 9). The reaction depends on the flow rate because the time of illumination by the UV-light differs dependent on the flow rate. The degree of mixing has no direct influence to the reaction. This experiment is an example for the application of the mixing module, but it is not possible to measure the degree of mixing in this experiment.

4. Conclusions and discussion

The developed static micromixer can advantageously used for the mixing of liquids of lower viscosity like aqueous solutions as well as for the mixing of solutions of mediate viscosity (up to 15–20% glycerol in water). At lower and mediate flow rates (up to 2×20 to 2×30 μl/min) about three split-and-recombine steps are sufficient for a efficient mixing of educts in cause of components with lower molecular weight. At higher flow rates (2×100 μl/min), six to eight split-and-recombine steps are necessary for a complete mixing in case of glycerol-containing liquids.

Fluorescein has a diffusion constant in water of about $D \approx 5.9 \times 10^{-6}$ cm^2/s. The volume of the micromixer was estimated to 8.5 μl. Considering a straight stream in a simple channel, mixing by diffusion would be possible in 17 s with $t \sim d_l^2/D$ [14]. The channel width differs between 178 and 700 μm, hence we assume a width of the lamellae d_l to 200 μm. With a pumping rate of 40 μl/min, the liquids stay about 12.75 s in the mixing module. Considering a viscosity of 1.76 mPa s for 20% glycerol solution in water and for fluorescein a hydrodynamic radius of 0.4 nm [15], the diffusion constant is about $D \approx 3 \times 10^{-6}$ cm^2/s according to the Stokes–Einstein equation [16]. Mixing in a straight channel would be then possible in 33 s. With the applied pumping rates of 40–200 μl/min mixing in a straight channel especially in glycerol would be completely impossible. The experimental results show that the micromixer provides for significant faster mixing. Following the two different coloured liquids in the micromixer, they pass the to inlets E1 and E2 (Fig. 2) before they were brought together in the first channel. Due to the laminar flow, the liquids are separated in two lamellae. In the first splitting step, the lamellae are split horizontally. That means a split flux is in the upper layer and one split flux is in the lower layer of the chip. Recombining the two split fluxes in the next step should lead to more than two lamellae. In an exemplarily experiment with a pumping rate of 80 μl/min after the first split and recombine unit more than two lamellae can be seen (Fig. 10a). The dark non-fluorescent liquid is enclosed by a broader fluorescent lamellae at the upper side and a smaller one at the lower side. After the third mixing unit, the several lamellae are still to see. They become blurred after the following mixing units till the mixing process is finished (Fig. 10b and c). The mixing process additionally is supported by the different channel widths (Fig. 11). The image in Fig. 11 was taken in transmission and represents the transition between the second and the third mixing unit. The outlet of the second unit is broader (700 μm) as the connection to the third unit (310 μm). Thus, the two fluxes were folded into another. Therefore, the mixing process is not only due to the split and recombine units but also to the different channel sizes. Also, in Fig. 11 one can see that there is some death volume at the transitions between different channel sizes. Characterisation of the micromixer with respect to death volumes and residence time was not part of this work and is currently under investigation.

The glass/Si/glass sandwich construction is very well suited for the monitoring of streaming, mixing and local interdiffusion processes. This could be shown by photometric detection in transmittance arrangements as well as by higher spatially resolved detection of the distribution of fluorescence markers inside single channel segments. These measurements can be executed either in transmittance (Si channel segments) or in reflection mode (glass channel segments or Si channel segments). The plan parallel arranged smooth glass surfaces of both covering chips support a very good microscopic imaging of substance distributions in the Si windows and allow quantitative measurements. These measurements or a visual control or monitoring can be done in parallel for all split-and-recombine steps due to the multiplicity of analogue Si windows. Pairwise arranged windows in the branched channel arrangements between the single split steps and recombine steps allow in addition a comparison of product distribution during the single mixing steps.

The static mixer was tested in a simple thermally activated solution reaction (at room temperature) and by a photochemical reaction in a more complex modular micro reactor arrangement. Both types of experiments shows the good performance of the micromixer and the easy use in freely configurable micro modular reaction arrangements.

Acknowledgements

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