Application Note

ACTIVE MICROMIXING ON CHIP

https://www.elveflow.com/microfluidic-applications/setup-microfluidic-flow-control/control-liquid-pressure-in-a-microchip-with-a-gas-pressure-source/

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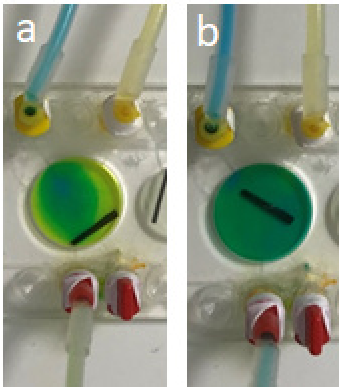
Introduction

Microfluidics has led to the development of miniaturized chemical and biological analysis platforms for several applications like chemical synthesis, clinical diagnostics, sequencing and synthesis of nucleic acids and enzyme reactions. For many of these applications mixing is a crucial step because efficient mixing significantly improves the detection sensitivity and reduces the analysis time.

At microscale, mixing can be achieved in two ways: passive and active.

- Mixing in passive micromixers is inherently slow, because it mainly occurs by diffusion and chaotic advection due to the kinetic energy of flow and the geometry of the micromixer; serpentine, herringbone, T and Y mixers are a few examples.
- Active micromixers employ disturbance generated by an external field for the mixing and the mixing process is rapid. Examples are magnetic, acoustic, pressure driven and magneto-hydrodynamic micromixers.



This application note focuses on the evaluation of active mixing using a magnetic stir-bar micromixer by performing dilution of a fluorescent dye using an Elveflow microfluidic setup.

Figure 1: Two ways to mix a blue and a yellow solution (a) passive method (stir-bar inactive), (b) active method (stir-bar active).

Applications

- Sample preparation [1,6,7]: Micromixers can be used for sample preparations, a preparatory procedure before analysis.
- Chemical reactions [2–4]: Micromixers are used as reaction platforms to perform chemical reactions at a microscale level.
- Dilution [5]: Micromixers are employed for dilution, which is an important step in many biochemical and pharmacological assays.

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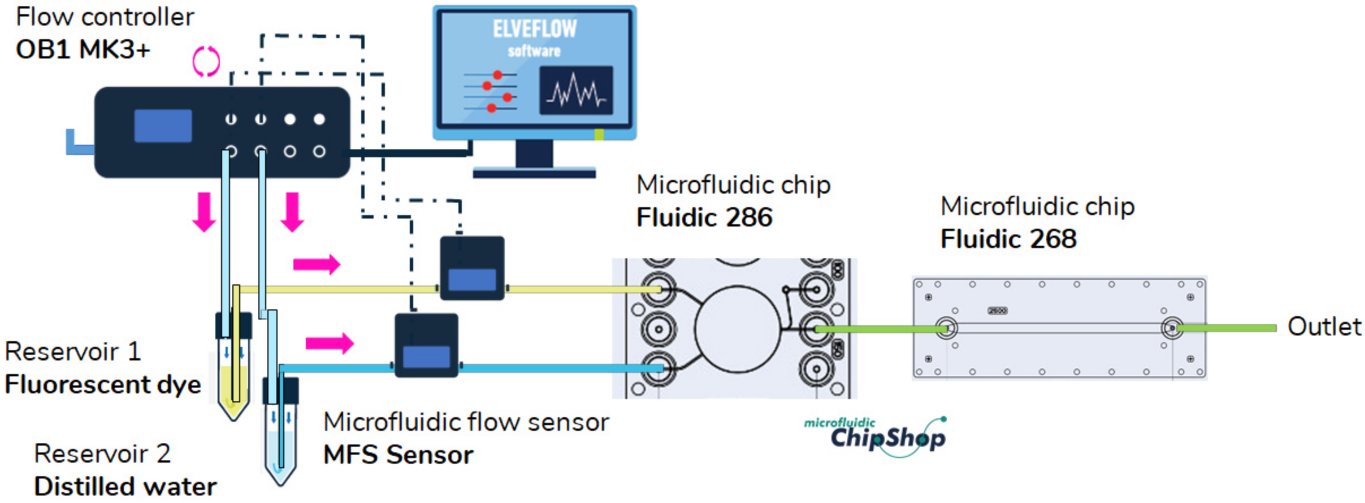
Principle

Utilizing the instantaneous mixing of the magnetic stir bar, predefined concentrations of solutions can be produced and collected at the outlet of the micromixer by calculating and applying appropriate flow rates at the inlet.

The microfluidic setup consists of a pressure controller, flow sensors, and a micromixer. The solutions in the micromixer chamber are stirred continuously using a magnetic stir-bar causing instantaneous mixing of the fluids. The pressure driven flow coupled with flow sensors enables precise control over the flow rate of the fluids entering the micromixer.

Setup



Materials

Hardware

- OB1 flow controller with at least two 0–2000 mbar channels, one channel -900/1000 mbar (option)
- 2 x flow sensors MFS3 2.4-80 µL/min
- Kit starter pack Luer Lock
- 3 x 50 mL Falcon reservoirs
- Microfluidic chip for mixing (Fluidic 286 with 1 pack of mini luer connectors from microfluidic ChipShop)
- Microfluidic chip for observation (Fluidic 268 with 1 pack of luer connectors from microfluidic ChipShop)
- 1 x pack of mini luer plugs from microfluidic ChipShop
- Digital magnetic stirrer plate
- Fluorescence microscope for observation

Chemicals

- Distilled water
- Fluorescent dye (e.g., fluorescein sodium salt)

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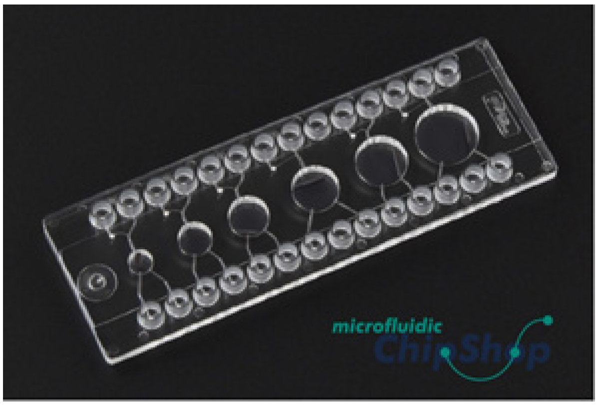
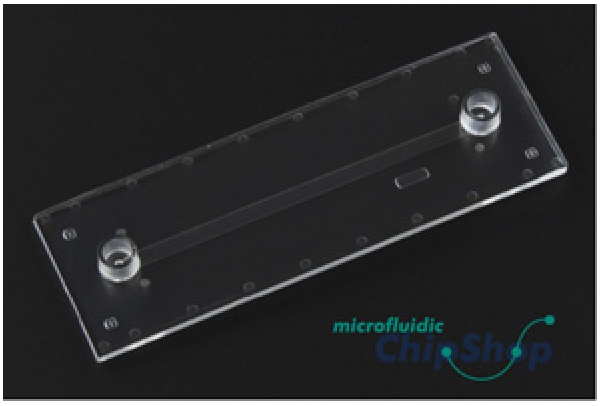
Design of the chip

Fluidic 286: Micro Mixer - Stir Bar Actuated Mixer



Interface type	Mini Luer
Volume chamber	20 - 40 - 60 - 80 - 100 - 150 µL
Lid thickness	175 µm
Material	PMMA

Fluidic 268: Straight channel chip with one channel



Interface type	Luer
Channel width	2.5 mm
Channel depth	150 µm
Channel length	58.5 mm
Lid thickness	175 µm
Material	PMMA
Surface treatment	Hydrophilized

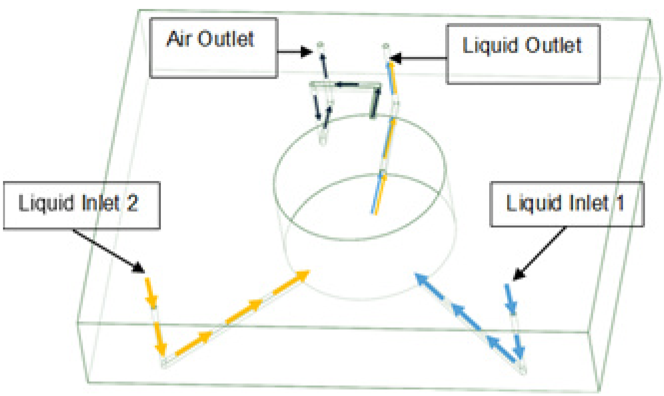


Figure 2: Schematic of the Fluidic 286.

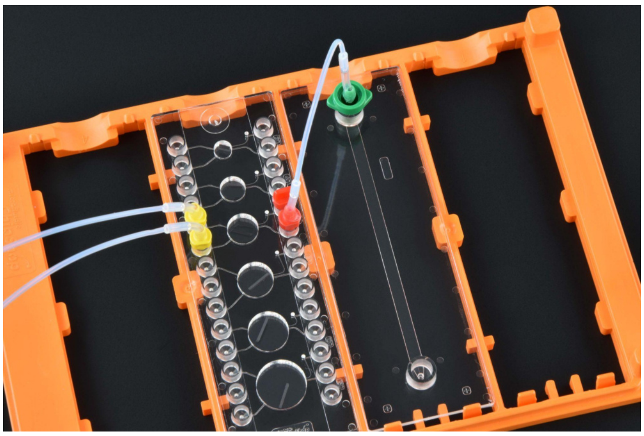


Figure 3: Picture of the setup combining Fluidic 286 and Fluidic 268.

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Quick Start Guide

INSTRUMENT CONNECTION

1. Connect your OB1 pressure controller to an external pressure supply using pneumatic tubing, and to a computer using a USB cable. For detailed instructions on OB1 pressure controller setup, please read the "OB1 User Guide".

2. Connect the flow sensors to the OB1. For details refer to "MFS user guide".

3. Turn on the OB1 by pressing the power switch.

4. Launch the Elveflow software. The Elveflow Smart Interface's main features and options are covered in the "ESI User Guide". Please refer to the guide for a detailed description.

5. Add the OB1 to the software: Press Add instrument \ choose OB1 \ set as MK3+, set pressure channels if needed, give a name to the instrument and press OK to save changes. Your OB1 should now be on the list of recognized devices.

6. OB1 calibration is required for the first use. Please refer to the "OB1 User Guide".

7. Add the flow sensors to the software: press Add sensor \ select flow sensor \ analog or digital (choose the working range of flow rate for the sensor if you have an analog one), give a name to the sensor, select to which device and channel the sensor is connected and press OK to save the changes. Your flow sensor should be on the list of recognized devices. For details refer to "MFS user guide".

8. Open the OB1 Window.

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SOLUTION PREPARATION

1. Fill the first reservoir with distilled water and connect the supplied 1/16" OD tubing and the 4mm OD coil tubing to the tank. For more details, refer to the video "Connector for the OB1".

2. Repeat step 1 with the stock solution of fluorescein sodium salt (10µM).

MICROFLUIDIC CHIP PREPARATION

1. Plug microfluidic tanks to the corresponding OB1 pressure controller outlet. For more details, refer to "Elveflow Microfluidic Reservoirs Assembly Instructions".

2. For flow measurement, connect the flow sensors between the microfluidic reservoirs and the chip. For more details, refer to the "MFS User Guide".

3. To fine-tune the system and to obtain the best performance in terms of flow rate control, add a resistance tubing (15 cm length of a 100 µm internal diameter) for the lines of distilled water and solution. For more details, please refer to the "Flow control tuning" document.

> Tips from the expert. The resistance should always be placed downstream of the MFS (between the MFS and the chip) to ensure a stable measurement.

4. Connect the distilled water reservoir to "liquid inlet 1" and add a plug to "liquid inlet 2", as well as to the "liquid outlet" (see the diagram in the "Design of the chip" section).

5. Set a low pressure (50 mbar) to fill the microfluidic chip with water and to remove any air bubbles.

> Tips from the expert. To remove the final air bubble from the microfluidic chip:
> - Option 1: tilt the microfluidic chip vertically until the air bubble is towards the outlet channel and apply a higher pressure (100 to 200 mbar) to force the air bubble out of the system.
> - Option 2 (To be applied when the air bubble is difficult/tricky to remove): Once the microfluidic chip is filled, connect the 'air outlet' to a falcon tube, which is connected to the vacuum channel of the OB1. Simultaneously, set a pressure of 100 to 200 mbar in "liquid inlet 1" and a vacuum of -50 to -100 mbar in "air outlet".

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CONTINUOUS DILUTION EXPERIMENTS

1. Connect the stock solution reservoir to "liquid inlet 2".

> Tips from the expert. To avoid adding air bubbles into the chamber, set a low pressure (50 mbar) until the solution starts dripping out of the tubing and then connect the tubing to the corresponding inlet.

2. Connect the "liquid outlet" to the inlet of the fluidic 268 microfluidic chip to analyse the final concentration obtained (in this application note, by the use of fluorescence) or you can directly collect your solution with the targeted concentration in a dedicated reservoir.

3. Place the digital magnetic stirrer plate under the fluidic 286 microfluidic chip (micromixer chip) and set a stirring rate.

> Tips from the expert. The stirring rate will depend on the volume of the chamber chosen and on the flow rates used for the experiments.

4. Switch your system to "Flow sensor mode" to activate the feedback loop. For more details, refer to "[Set a flow control feedback loop]".

> Tips from the expert. Thanks to internal experiments, we advise you to set the following values of P and I parameters:
> - for distiller water: P = 0.045 and I = 0.08
> - for stock solution: P = 0.08 and I = 0.1

5. Set a flow rate for the distilled water and the stock solution according to the final concentration wanted, and adjust the stirring rate to ensure a reliable mixing of the two solutions.

> Tips from the expert. Calculate the different flow rates for both solutions in advance to ensure using the best range of your flow sensor, taking into account the volume of the chamber used.

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AUTOMATED EXPERIMENTS

The dilution process can be automated following the next steps.

1. To create a sequence, click on the top middle button “Create Sequence” on the main window of the ESI software: a new window will appear.

2. Back to the OB1 window: set up the desired flow rates on both corresponding channels in order to get the targeted concentration and save the configuration by clicking on “Config”. For more details, refer to the “ESI User Guide”.

3. Step 2 can be repeated for all needed configurations.

4. Back to the OB1 window: save a “0” pressure or flow rate configuration for both of your channels and save the configuration by clicking on “Config”.

5. Back on the sequence window: on the left side of the window, click on the green “OB1” box (a line “OB1: Select instrument” appears in the middle part), on the right side, select the instrument (your OB1 pressure controller should appear when clicking on “Instrument”) and “load the configuration” saved previously.

6. On the left side of the sequence window, click on the “Wait” (clock symbol) box (a new line with a time frame appears): set a desired duration.

7. Repeat steps 4 and 5 for all the wanted concentrations.

8. On the left side of the window, click on the green “OB1” box (a line “OB1: Select instrument” appears in the middle part), on the right side, select the instrument (your OB1 pressure controller should appear when clicking on “Instrument”) and “load the configuration” zero pressure or flow rate.

9. On the left side of the sequence window, click on the blue “END” box (a new line “END” appears). This means that your sequence is over.

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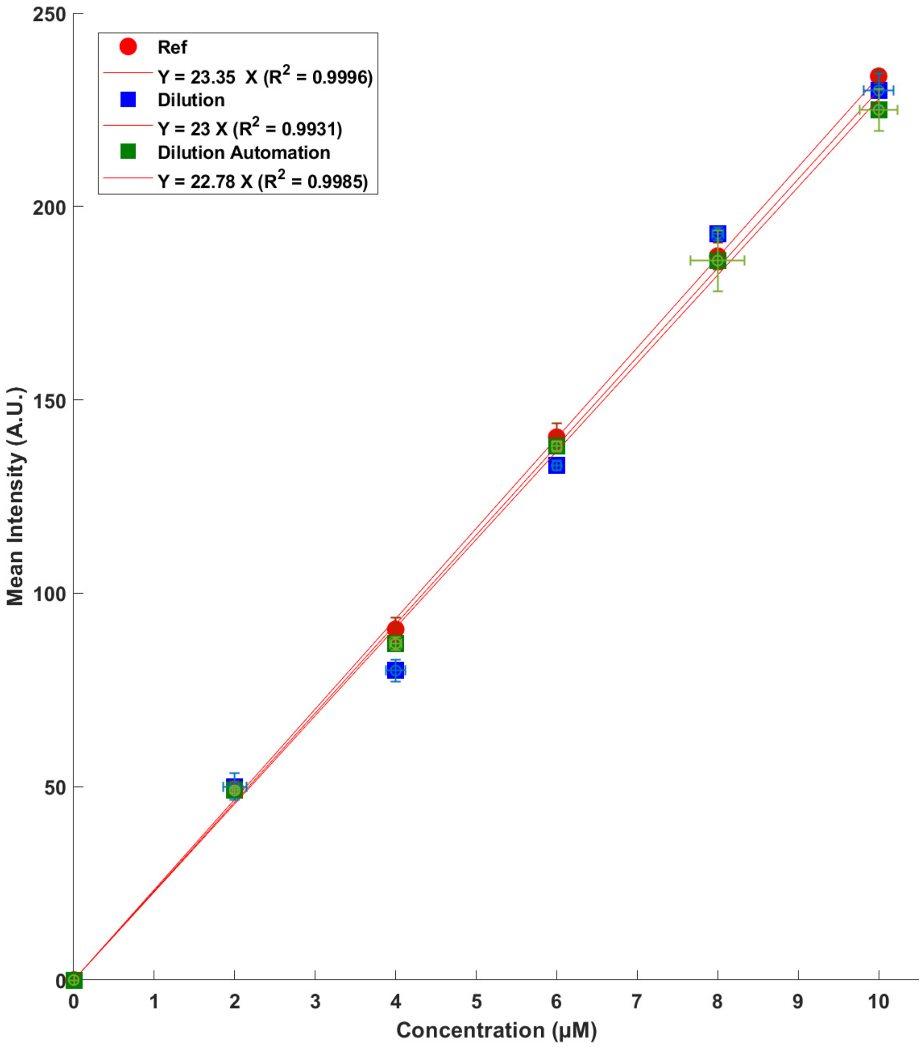
Results

In this application note, a known concentration of fluorescein dye (10μM) has been diluted with water using a microfluidic chip to achieve a targeted concentration of fluorescein through a controlled flow rate.
The following graph represents the fluorescence intensity as a function of the fluorescein concentration for:

- Ref: values obtained by manual dilution;
- Dilution: values obtained by dilution inside the microfluidic chip;
- Dilution Automation: values obtained by automated dilution inside the microfluidic chip.



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Acknowledgements

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Reference

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