Application Note

Microfluidics Innovation Center

MICROFLUIDIC CELL PERFUSION WITH A SYRINGE PUMP

https://www.microfluidics-innovation-center.com/application-notes/microfluidic-cell-perfusion-with-a-syringe-pump

Contents

Introduction **2**

Applications **2**

Setup **3**

Materials **3**

HARDWARE 3

REAGENTS 3

Design of the chip **4**

Quick Start Guide **4**

INSTRUMENT CONNECTION AND FILLING 4

CHIP PREPARATION AND SEEDING 6

CHIP CONNECTION TO FLUIDIC CIRCUIT 7

EXPERIMENT 8

Results **9**

Acknowledgements **11**

Introduction

Cell culture is a fundamental technique widely used in various areas of biological research and pharmaceutical development. In order to support the in vitro growth and viability of cultured cells, multiple factors must be carefully considered, including temperature, pH, and nutrients. Microfluidics is a technique that enables cells to be cultured on the microscale with very high control over environmental parameters. Among these factors, the ability to precisely regulate the flow of medium and other solutions over cells in a microfluidic chip is important for increasing the physiological relevance of their microenvironment compared to static culture models.

In this context, the pump plays an important role in the setup. Microfluidic pump selection should be considered with respect to flow stability and flow rate profile, in addition to experimental needs specific to the application (e.g. recirculation, perfusion volume and time etc), while keeping usability in mind (see: Microfluidic pump flow profiles: a comparative review). Syringe pumps are a commonly used option for cell culture applications. This application note demonstrates the setting up of a syringe pump for cell perfusion and measures the flow profile generated.

Applications

Cell perfusion on the microscale has wide applications in fundamental biological research, disease modelling and the medical and pharmaceutical industry, including:

- Live cell imaging and analysis (2D, 3D cultures)
- Microphysiological systems; Organ-on-chip; Barrier models
- Cell rolling and adhesion assays
- Shear stress studies
- Molecular transport
- Drug screening
- Behavioural and developmental studies (e.g. worm-on-chip)

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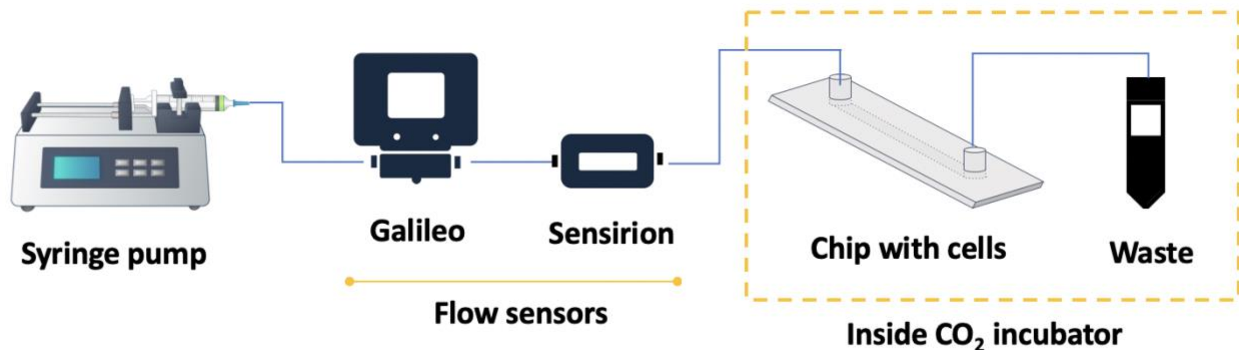
Setup



In this setup, a Standard Infuse/Withdraw PHD ULTRA™ Syringe Pump (Harvard Apparatus) was used to push the medium in one direction through a microfluidic chip seeded with cells.

Syringe pumps are commonly used to control the flow without the strict need for inline flow sensors. However, this can lead to over-reliance on factory instrument calibration and loss of high precision data such as flow profile or potential flow perturbations. In this application note we connected two flow sensors inline to verify the performance of the syringe pump and to measure the pulsatility of the flow profile. We used a commercially available Sensirion flow sensor (LG16-0431D, connected to a sensor reader; see image page 8: *Full fluidic setup*) and our new Galileo flow sensor that features a wide sensing range, clogging detection and measurement drift alert.

Materials

HARDWARE

- Syringe pump (Standard PHD ULTRA™ CP Syringe Pump, Harvard Apparatus)
- Syringe (e.g. 10 mL glass, Hamilton)
- Microfluidic chip (e.g. µ-Slide I Luer, ibidi ®)
- Tubings (PTFE, 1/16” outer diameter; OD), fittings
- 1 × 50 mL Falcon tube and reservoir cap
- [Optional] Galileo flow rate sensor (e.g. 1-200 µl/min cartridge; waterproof, if to be used in a CO_2 incubator)
- [Optional] Sensirion flow sensor (e.g. LG16-0431D, 2-80 µl/min) and sensor reader (e.g. MSR, Elveflow)

REAGENTS

- Cells (e.g. U-251 MG GFP; 0.6-0.8 × 10^6 cells/ mL)
- Medium: DMEM ([+] 4.5g/L D-Glucose; with 10% FBS, Penicillin/ Streptomycin (100 U/mL; 100 µg/mL)
- Phosphate buffered saline (PBS)
- Propidium iodide (PI) cell stain (75 µM)

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Design of the chip

This experiment used a straight channel chip (µ-Slide I Luer 0.4; ibidi ®). The simple design and large internal geometry of the chip make it straightforward to fill, seed and connect to the circuit. Its polymer coverslip base and microscope slide outer dimensions are ideally adapted for imaging, either as part of an endpoint staining protocol or for live cell imaging to monitor cell proliferation and morphology during perfusion.

µ-**Slide I Luer 0.4 (ibidi ®)**	**Features**
Interface type	Female Luer
Chamber volume	100 µl
Channel l × w × h	50 × 5 × 0.4 mm
Surface treatment	ibiTreat tissue culture treated
Slide base	#1.5 polymer coverslip

Quick Start Guide

INSTRUMENT CONNECTION AND FILLING

1. Connect your syringe pump to a power supply following the manufacturer's instructions.

2. Click the Galileo cartridge into its base.

3. Connect the Galileo base to a computer (USB type C-to-type C or USB type C-to-type 2). Open the Galileo software and click "Connect Galileo" on the interface.

4. Select your working liquid from the dropdown menu and click "Apply".

5. Connect the Sensirion flow sensor to the sensor reader and software as directed by the manufacturer.

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6. Select an appropriate syringe for your experiment and sterilize or disinfect it as required as per manufacture's recommendations. This experiment used a 10 mL glass syringe (Hamilton) disinfected with ethanol (70%).

Tips from the expert. Choose the right syringe volume: Determine your syringe volume based on your desired flow rate and length of perfusion time, then add your system fill volume. Note: consider if you will only push liquid through your setup in one direction or if you will alternately push and withdraw for bi-directional flow.

Tips from the expert. Sterilizing and disinfecting: The method used to sterilize or disinfect your syringe will depend on the syringe type used. For disposable syringes, use a new sterile syringe for each experiment. For re-usable syringes, refer to the manufacturer's information about autoclave, chemical and solvent compatibility, or clean using a disinfectant such as ethanol or aniosyme (1%).

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7. Fill the syringe with medium in a Biosafety hood to maintain sterility.

Tips from the expert. Pre-filtering medium through a 0.22 µm filter is suggested to reduce the chance of clogging small channels as a troubleshooting measure, if this issue is encountered.

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8. Attach tubing (1/16" ID PTFE) to the end of the syringe with a female threaded Luer adapter and a ¼"-28 threaded connector and ferule (see image below: *Full fluidic setup*).

Tips from the expert. Tubing lengths can be pre-cut and autoclaved, along with connectors, and assembled in a Biosafety hood to maintain sterility.

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9. Secure the syringe in the syringe pump as per the manufacturer's instructions.

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10. Connect the tubing from the syringe to the inlet of the Galileo flow sensor cartridge.

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11. Connect the outlet of the Galileo flow sensor cartridge to the inlet of the Sensirion flow sensor.

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12. Connect the Sensirion flow sensor to a sensor reader (e.g. MSR, Elveflow) and set up the sensor as per the manufacturer's instructions.

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13. Connect the outlet of the Sensirion flow sensor to a female Luer union (in place of the seeded chip for initial system filling) and connect the collection/ waste container.

Tips from the expert. Using a female Luer union in place of the chip for initial filling of the system with medium guards against air or fast liquid flow from disturbing the cells that are attached to the chip surface (see image page 7: *Chip connection*).

14. Start the syringe pump and completely fill the system with medium.

CHIP PREPARATION AND SEEDING

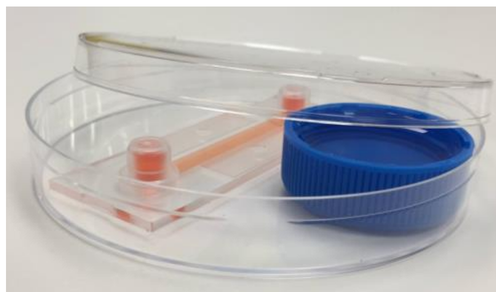
1. Add surface coating, if desired. Note that a hydrophilized surface is suitable for cell attachment without additional treatment.

2. Prepare cell suspension as per standard protocols. Ensure all cell clumps are gently but well dissociated and count carefully. Use the suspension immediately.

3. Seed a µ-Slide I Luer 0.4 chip with a cell density of 0.6-0.8 $\times$ 10^6 cells/mL using a pipette. Position the pipette tip at the base of the inlet right at the entrance to the channel and apply slow and gentle pressure to avoid generating air bubbles.

Tips from the expert. When seeding, add just enough cell suspension to fill the channel volume (in this case ~120 µl). Wait 10 min and then very gently fill the inlet and outlet reservoirs with fresh medium. Alternate between adding small volumes of medium to the inlet, then outlet, then inlet etc to avoid a hydrodynamic pressure difference between the inlet and outlet that can disrupt homogeneity of cell distribution in the chip.

4. Cover inlets/ outlets and leave 6-18 h in the CO_2 incubator for cells to attach, or as needed for the application.



***Cell attachment**: Straight channel chip freshly seeded with cells. Add a cap of water next to the chip during cell attachment incubation to limit evaporation of the small volume inside the chip.*

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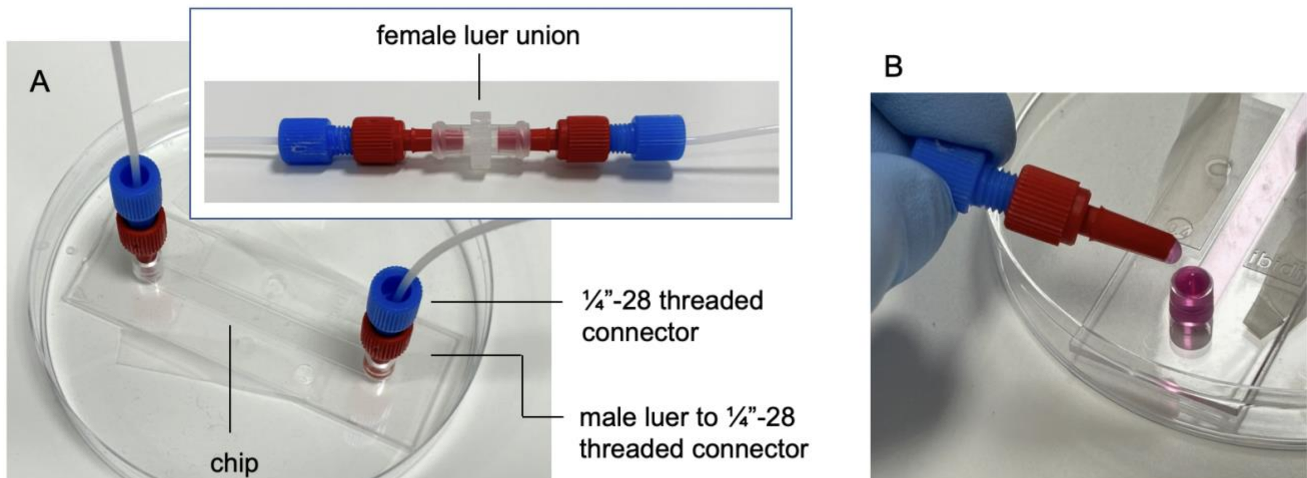
CHIP CONNECTION TO FLUIDIC CIRCUIT

1. Tape the chip to a sterile petri dish for maximum stability and mark on it the direction of flow.

2. Remove the female Luer union. Connect the chip by touching the small droplet of medium at the tip of the connector to the meniscus of the medium at the chip inlet, to avoid trapping air.

Tips from the expert. If no droplet of liquid is visible at the tip of the connector, utilise the principle of hydrostatic pressure and lower the tip of the tubing slowly to below the level of the reservoir, or until a small droplet is visible. Alternatively, start a slow flow, e.g. 10 µl/min, and hold the inlet connector vertically until a droplet is visible. Then proceed as above, touching meniscus to meniscus.

3. Connect tubing to the outlet of the chip. Carefully wipe away any medium from the outside of the chip. Perform a visual inspection about 1 h after starting the experiment to ensure there are no leaks.



***Chip connection**: A. Use a female Luer union in place of the chip for initial filling of the lines with medium. B. Once the system is filled with liquid, connect the pre-seeded microfluidic chip: wait until a small droplet of medium is visible at the tip of the connector, then touch this to the meniscus of medium at the chip inlet reservoir to avoid trapping air.*

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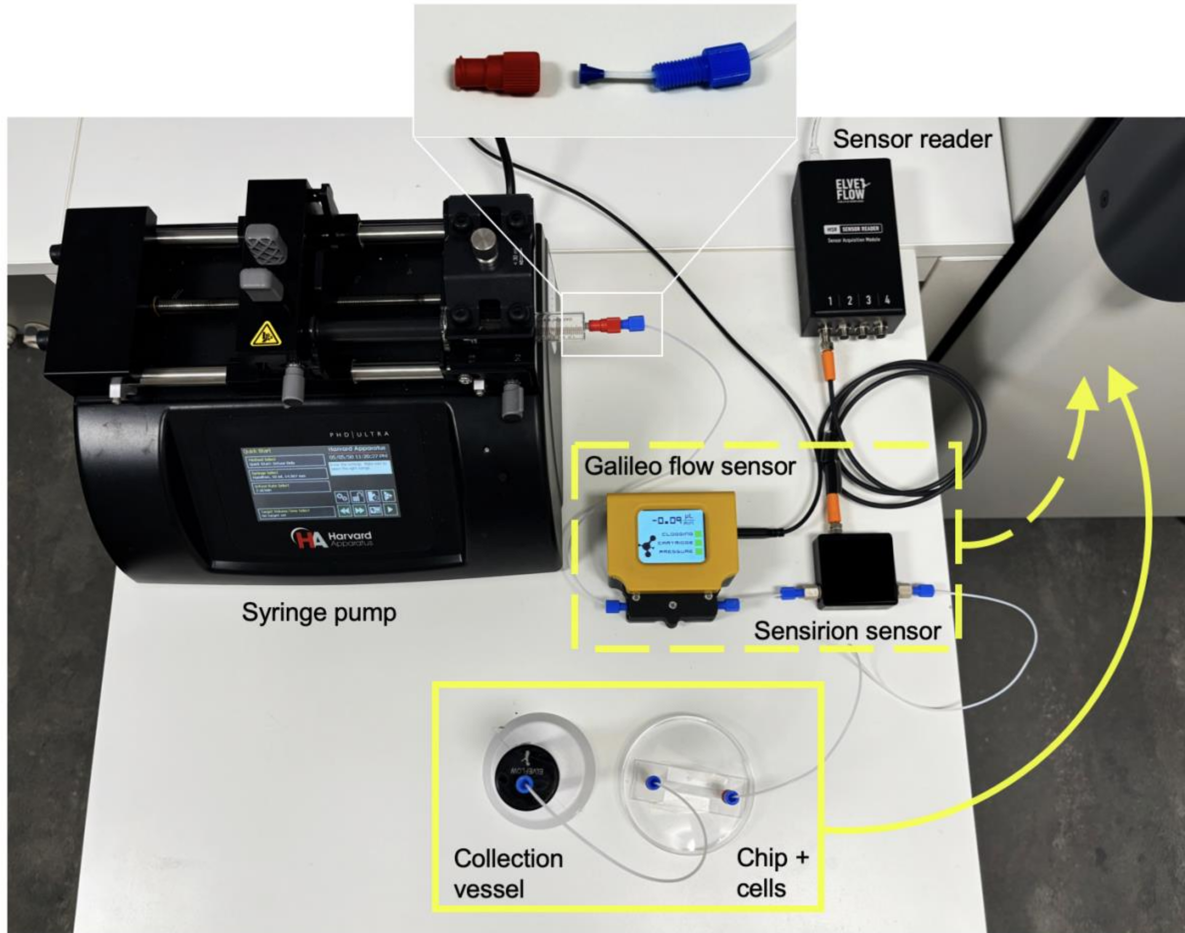

***Full fluidic setup**: Fluidic setup for cell perfusion in a microfluidic chip with a syringe pump. The chip and a collection container should be placed inside the CO_2 incubator (solid yellow box). Note, this setup shows two inline flow sensors to measure the flow profile of the syringe pump and to demonstrate our Galileo flow sensor performance. The MIC Galileo sensor displays the flow rate on the instrument screen. The Sensirion sensor is connected to a sensor reader. Both can record data when connected to a computer. Option: the flow sensors can also be placed inside the CO_2 incubator if waterproof versions are used (dotted yellow box).*

EXPERIMENT

1. Set desired flow rate on the syringe pump interface, e.g. 2 µl/min.

2. Start flow and leave for desired time, e.g. 24 h.

3. Analyze results of the cell perfusion experiment, e.g. stain and image cells in the chip or perform downstream analysis of collected aliquots of medium as desired.

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Results

Cells were cultured in a microfluidic chip with constant perfusion (2 µl/min) using a syringe pump. After 24 h, cells were stained in the chip with propidium iodide (Fig. 1). The accuracy and flow profile of the syringe pump was measured using 2 flow sensors (Figs 2, 3).

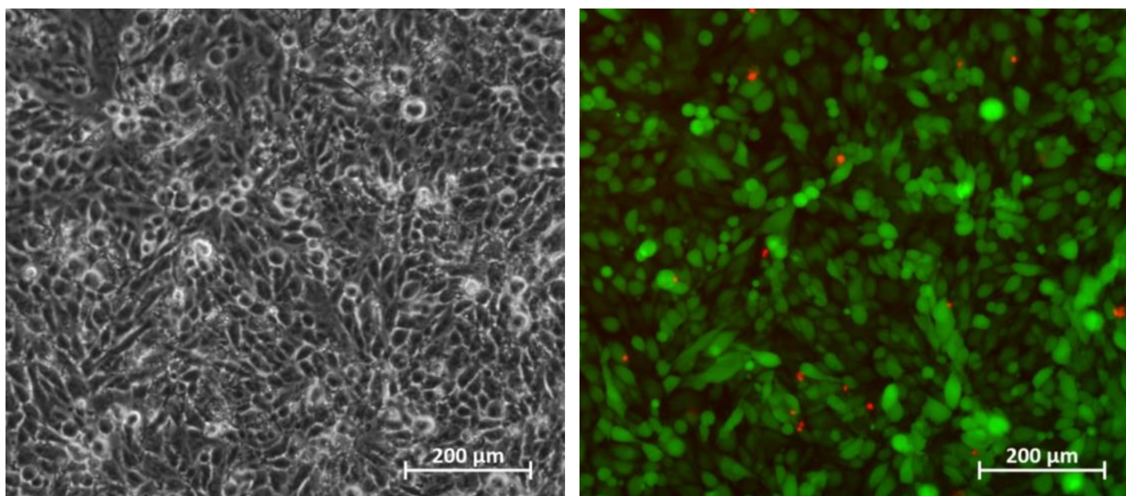

Figure 1. U-251 MG GFP cells after 24 h perfusion (2 µl/min, syringe pump). Phase contrast (left) and fluorescence images (right; GFP, green; propidium iodide, red).

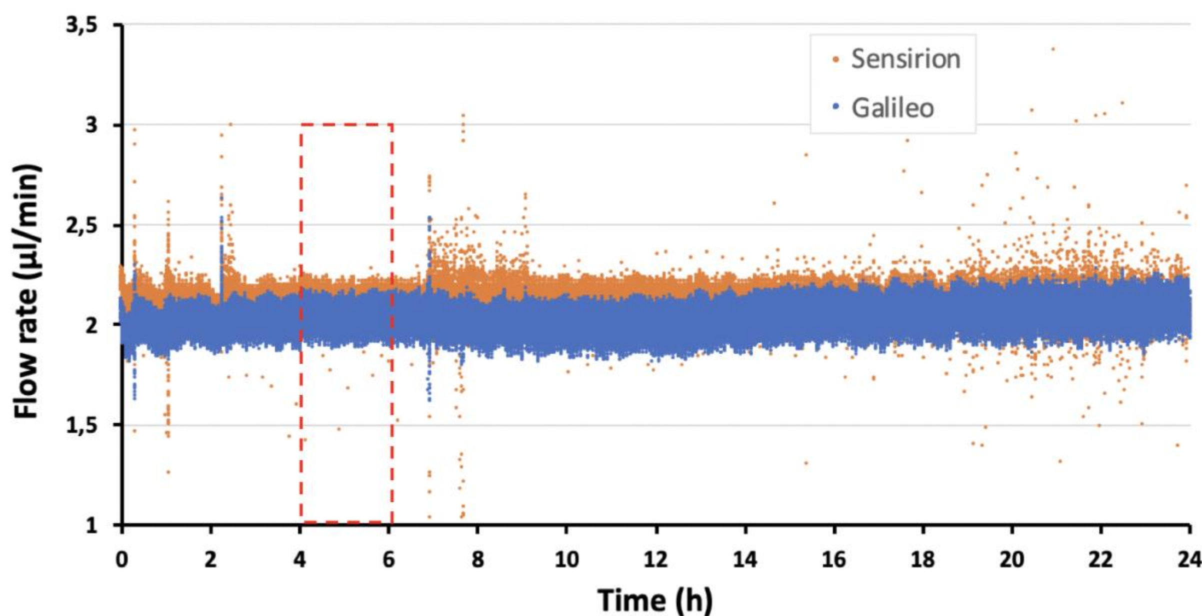

Figure 2. Syringe pump flow profile measured by a Galileo flow sensor (MIC) and a Sensirion flow sensor. The syringe pump was set at 2 µl/min and perfusion was run for 24 h. Zoom-in of flow profile (dotted red box) is shown below.

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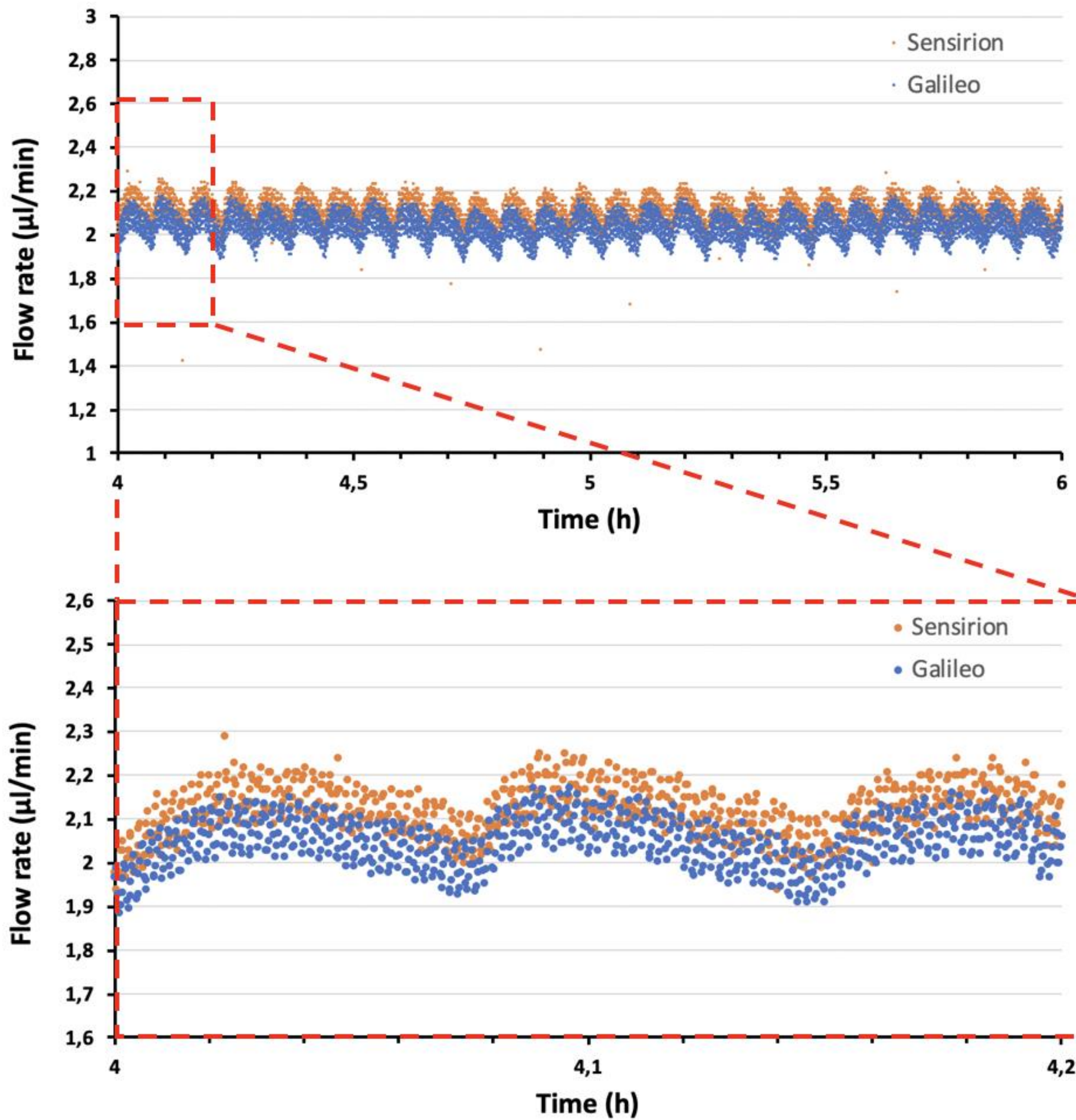
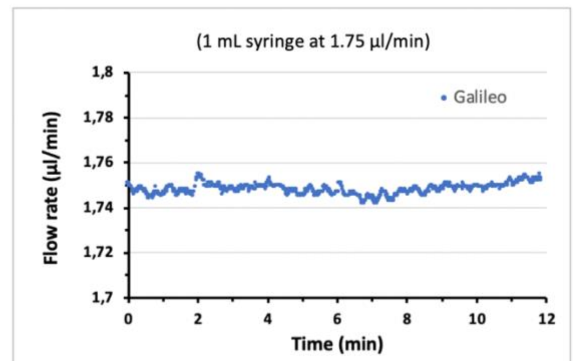


Figure 3. Zoom-in from Figure 1 of syringe pump flow profile measured by a Galileo flow sensor (MIC) and a Sensirion flow sensor. The syringe pump was set at 2 µl/min.

The syringe pump produced an oscillating flow profile (with a 10 mL syringe) due to the action of the step motor.

Note that using a smaller volume syringe (smaller barrel diameter) can result in smaller flow rate oscillations (*figure, right*). However, the syringe volume should be selected based on the flow rate and experiment length, e.g. a syringe of 1 mL volume can dispense liquid at a rate of 2 µl/min for a maximum of 8.33 h before becoming empty, while a 10 mL syringe can dispense at this flow rate for over 3 days.



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Acknowledgements

This application note has received funding from the European Union’s Horizon research and innovation program under HORIZON-EIC-2022-TRANSITION-01, grant agreement no. 101113098 (Galileo), and H2020-MSCA-RISE-2020, grant agreement no. 101007804 (Micro4Nano).

European Innovation Council

Funded by the European Union

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