REPORT:

A1.2.5 : Calibration methods for measuring the response or delay time of drug delivery devices using Newtonian liquids for flow rates from 5 nL/min to 100 nL/min

This report was written as part of activity A1.2.5 from the EMPIR Metrology for Drug Delivery (MeDD II) project. The three-year European project commenced on 1st June 2019 and focused on providing traceable measurements of volume, flow and pressure of existing drug delivery devices and mixing behaviour and occlusion phenomena in multi-infusion systems. For more details about this project, please visit www.drugmetrology.com

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Introduction to the EMPIR Metrology for Drug Delivery project

The overall aim of this project is to improve dosing accuracy and to enable the traceable measurement of volume, flow and pressure in existing drug delivery devices and in-line sensors operating at very low flow rates. This will be achieved through the development of new calibration methods and by expanding the existing metrological infrastructure. This project will also investigate fast changing flow rates, which are step changes between two flow rates within a second, the physical properties of mixtures of liquids and occlusion phenomena in multi-infusion systems in order to prevent inaccurate measurement results and thus to improve patient safety.

The specific objectives of the project are:

1. To develop new traceable techniques for generating and measuring the response or delay time of drug delivery devices regarding changes in flow rate, from 5 nL/min to 100 nL/min, using Newtonian liquids (WP1). For steady flow rates an uncertainty of 1 % (k=2) or better is expected, whereas for fast changing flow rates an uncertainty of 2 % (k=2) or better is expected. The techniques developed will be used to characterise and validate the different response times of at least 3 different types of drug delivery devices (including infusion analysers) (WP3 and WP4) and one type of flow sensor, to accurately measure the administered flow and volume with the required uncertainties.
2. To upgrade the existing flow facilities and knowledge of the partner NMIs in order to enable the traceable in-line measurement of the dynamic viscosity of Newtonian liquids, as a function of the flow rate and pressure difference, with a target uncertainty value of 2 % (k=2). The measurement uncertainty will be validated using Newtonian liquids with traceable dynamic viscosity calibration. Additionally, tests with non-Newtonian liquids will be performed in order to prove the concept. To calibrate transfer standards for the in-line measurement of dynamic viscosity and other physical properties of liquids, in order to use these transfer standards for flow measurement and to determine the mixing behaviour of different liquids.
3. To develop and validate novel calibration procedures for existing medical flow devices (e.g. infusion pumps, pain controllers and infusion pump analysers) with traceability to a primary standard and with a target uncertainty value of 2 % (k=2) for a range of 5 nL/min up to 600 ml/min and also to develop a proof-of-concept on-chip microfluidic pump used as a transfer standard in drug discovery and organ-on-a-chip applications for flow rates lower than 100 nL/min.
4. To design and develop a multi-infusion system containing check valves, with several options for testing how liquids, with different viscosities mix and flow and how this affects drug concentration. The flow rates and pressures will be traceably calibrated in all infusion lines, as well as at the outlet of the syringe pump, to be able to analyse the effects of pressure-equalising devices and to detect occlusion phenomena and bad mixing configurations.
5. To facilitate the take up of the technology and measurement infrastructure developed in the project by the measurement supply chain (i.e. accredited laboratories, instrumentation manufacturers, etc.), standards developing organisations (ISO/TC 30, ISO/TC 48, ISO/TC/SC 62D, ISO/TC 69, ISO/TC 76, ISO/TC 84, ISO/TC 150, ISO/TC 210) and end-users (i.e. hospitals and health centres).

Activity 1.2.5 – Calibration methods for measuring the response or delay time of drug delivery devices using Newtonian liquids for flow rates from 5 nL/min to 100 nL/min

Overall Objective: Using input from A1.2.1-A1.2.4, CETIAT, NEL, IPQ, CMI, RISE, METAS and THL will write a report on calibration methods for measuring the response or delay time of drug delivery devices using Newtonian liquids for flow rates from 5 nL/min to 100 nL/min.

Once agreed by the consortium, the coordinator will then submit the report to EURAMET as D1: 'Report on calibration methods for measuring the response or delay time of drug delivery devices using Newtonian liquids for flow rates from 5 nL/min to 100 nL/min'.

The following Institutes have contributed to this Report:

no	Participant Type	Short Name	Organisation legal full name	Country
1	Internal Funded Partner	NEL	TUV SUD Limited	United Kingdom
2	Internal Funded Partner	CMI	Cesky Metrologicky Institut	Czech Republic
3	Internal Funded Partner	IPQ	Instituto Português da Qualidade, I.P.	Portugal
4	Internal Funded Partner	METAS	Eidgenössisches Institut für Metrologie METAS	Switzerland
5	Internal Funded Partner	CETIAT	Centre Technique des Industries Aérauliques et Thermiques	France
6	Internal Funded Partner	RISE	RISE Research Institutes of Sweden AB	Sweden
7	Internal Funded Partner	DTI	Teknologisk Institut	Denmark
8	External Funded Partner	HSG-MIT	Hahn-Schickard-Gesellschaft für angewandte Forschung e.V.	Germany
9	External Funded Partner	THL	Technische Hochschule Lübeck	Germany
10	Unfunded Partner	BHT	Bronkhorst High-Tech BV	Netherlands

The progress within this report covers the Period 1st June 2019 to end of August 2020.

1 - Response or delay time measurement: definitions and examples

The response or delay time (also called "start-up" time) can have a different meaning depending on the application. Several definitions for the response or delay time can be encountered in the scope of drug delivery devices, hence the definition used should always to be agreed with the end-user and stated in the test report. The following examples explain the different definitions.

1.1 Response or delay time to reach 100% of target flow rate

Time between the physical starting of the drug delivery process (by pressing a "start" button on the device, for example) and reaching, by increasing values, the desired flow rate, as illustrated in Figure 1.1.1.

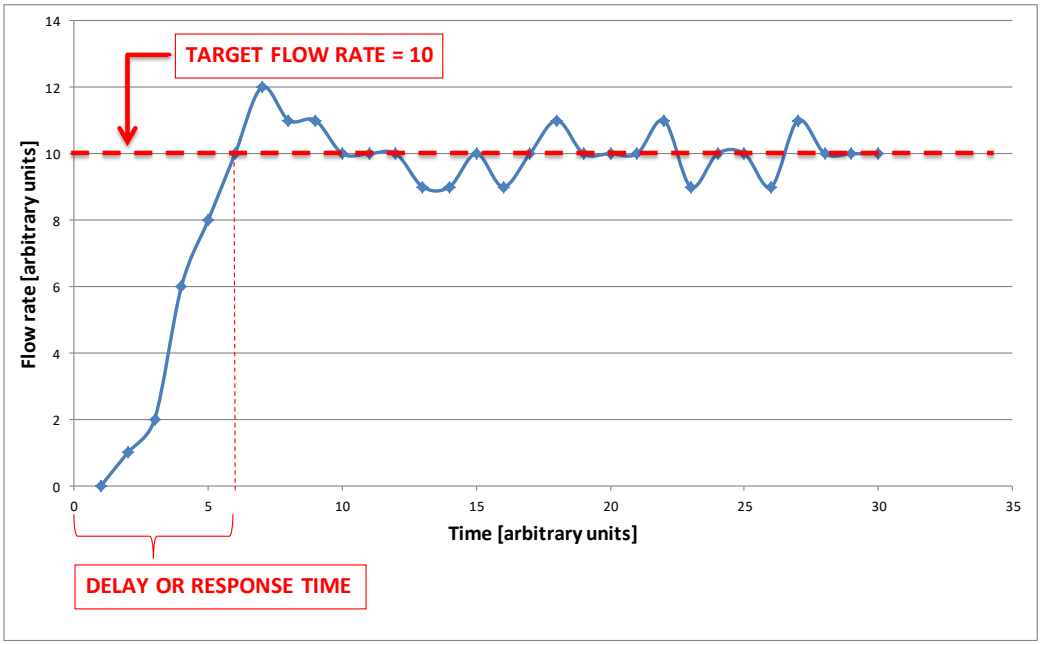


Figure 1.1.1: Illustration of delay/response time defined as reaching 100% of target flow rate

1.2 Response or delay time to reach 95% of target flow rate

Time between the physical starting of the drug delivery process (by pressing a "start" button on the device, for example) and reaching, by increasing values, a given percentage (95% in the example below) of the target value, as illustrated in Fig. 1.2.1.

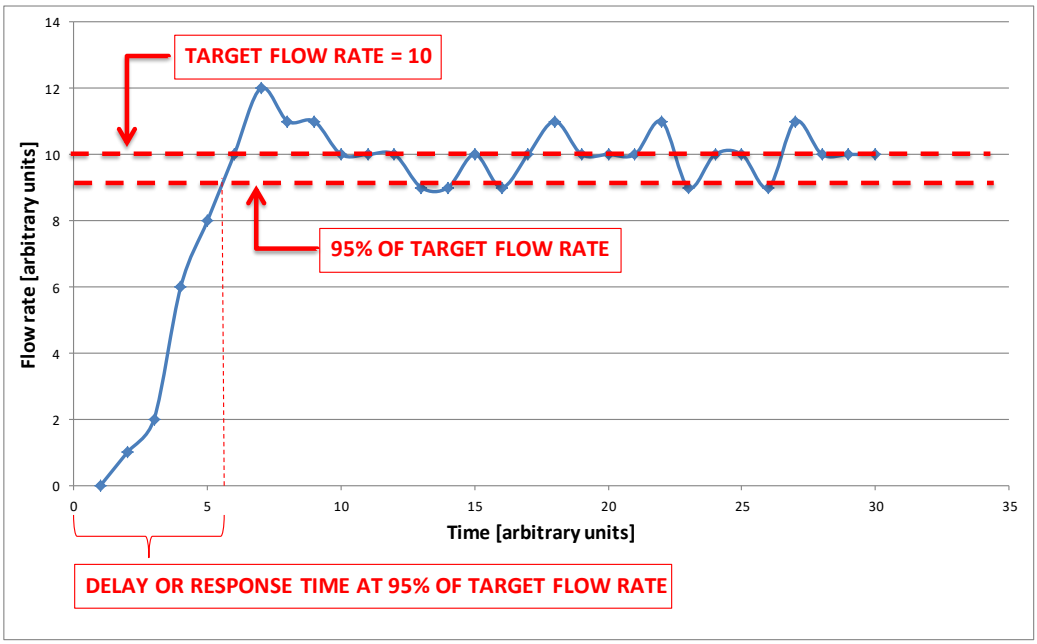


Figure 1.2.1: Illustration of delay/response time defined as reaching 95% of target flow rate

1.3 Response or delay time to be within ± 5% of target flow rate

Time between the physical starting of the drug delivery process (by pressing a "start" button on the device, for example) and reaching a flow rate stability within a given percentage (+/-5% in the example below) of the target value, without leaving this interval, as illustrated in Figure 3.

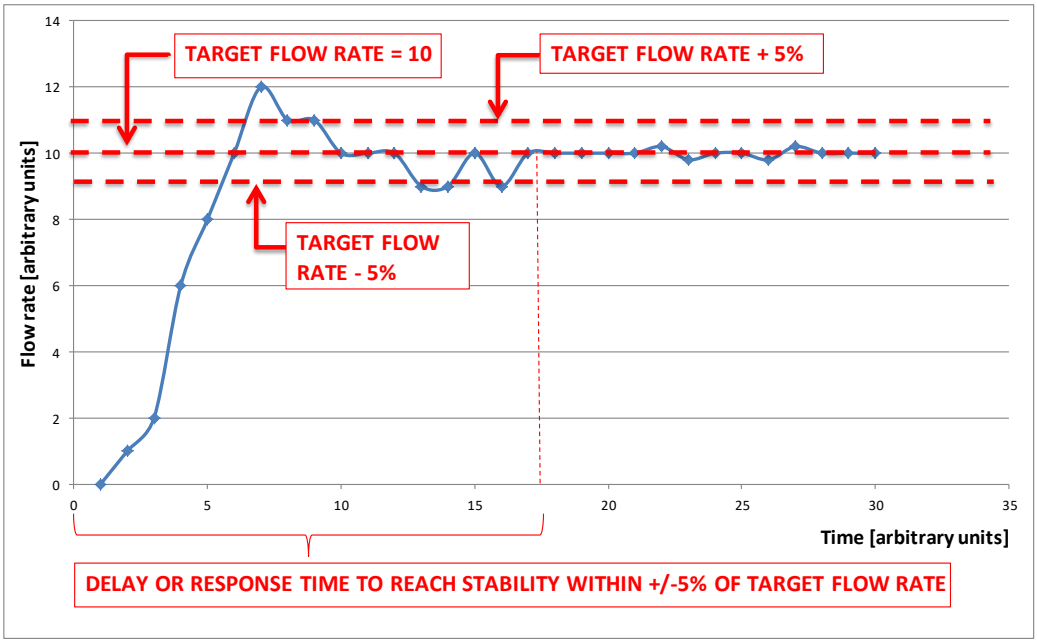


Figure 1.3.1: Illustration of delay/response time defined as being with ± 5% of target flow rate

1.4 Response or delay time defined by switch-on delay and rise time to reach 90% of target flow rate

Using conventions from electrical engineering the:

- turn-on delay is defined as the time between the physical starting of the drug delivery process (by pressing a "start" button on the device, for example) and reaching 10% of the target flow rate
- rise time (t_{rise}) is defined as the time between 10% and 90% of the target flow rate

This is illustrated in Figure 1.4.1.

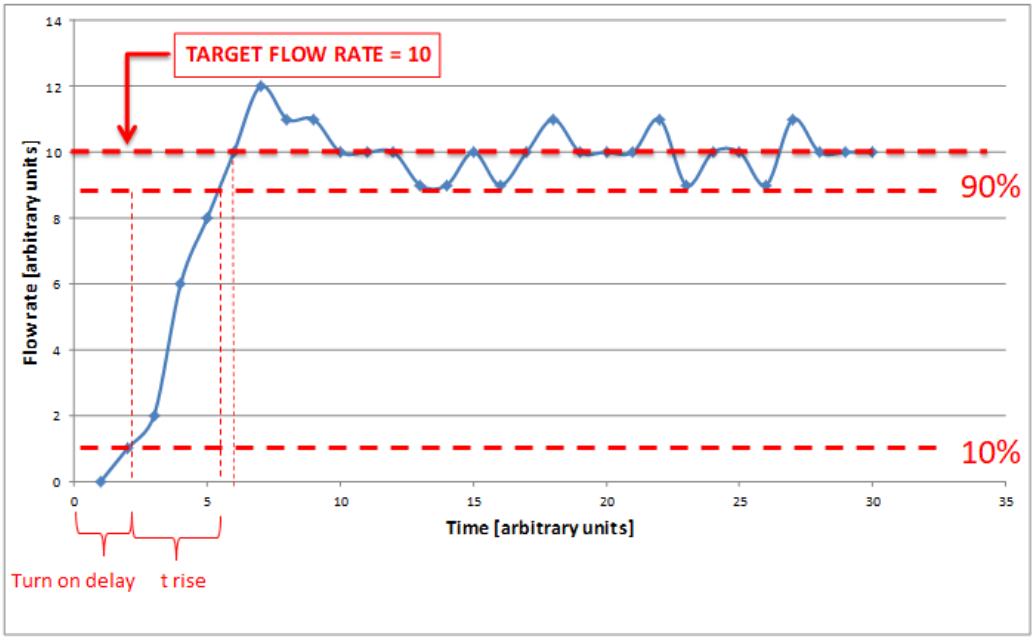


Figure 1.4.1: Illustration of delay/response time defined with turn-on delay time and rise time

2 - Response or delay time measurement: general method and recommendations

In order to quantify the delay or response time of a given flow generating device, the following recommendations should be considered:

- Use a calibrated chronometer or software/instrument allowing the timestamping of the measured reference flow rate;
- Evaluate the uncertainties on both the reference flow rate and the response or delay time measured. For example, a known (and calibrated) sampling frequency of mass, volume, or flow data can be used to calculate a time interval;
- Use a defined and stated method to synchronize the starting of the delivery process and the timestamping of the mass, volume or flow data. For example, an operator can start the mass data recording of a weighing scale when they simultaneously push the "start" button on the drug delivery device. In any case, the uncertainty on synchronization should clearly appear on the time uncertainties evaluation;
- Make sure the definition of the response or delay is clearly stated in the test report;
- Clearly describe all test conditions likely to influence the response or delay time of the device under test:
    - accessories (syringe type/volume /material, perfusion kit),
    - tubing length and material for connecting the device to the reference system
    - fittings types (in case of important dead volume)
    - liquid used (with or without degassing: bubbles can increase significantly the response time measured)
    - Ambient conditions (room temperature, pressure and humidity intervals during the test)

By satisfying the recommendations above and analyzing the flow rate data recorded since the start of the drug delivery process from the device under test, then each facility is able to determine, by one of the definitions presented above, the response or delay time.

In the scope of this project, the methods described in Report A1.1.8 and outlined below can all be used to measure and record dynamic flow rates within their stated rates.

The last chapters present uncertainty calculations for gravimetric, optical, microPIV and displacement methods.

3 - Calibration facilities

3.1 TUV SUD Limited – NEL

Description of Facility	The facility includes a system comprising five main parts (Fig 3.1.1): a microfluidic chip, a pressure system, a fluorescent microscope, a CCD camera and a software interface. Fluids with a known concentration of fluorescent beads (1 µm diameter) are introduced into the microfluidic chip from a fluid reservoir under adjustable positive pressure (ranging from 50mbar to 200 mbar). The geometry of the microfluidic channel in the chip determines the resulting flow rate according to the pressure value applied. A fluorescent microscope is used to illuminate the flow behavior inside the chip. A camera (1-500 Hz acquisition rates) is used to record images of beads moving in the flow using a LabView interface. An *in house* developed software routine (Matlab) is then used to calculate the velocity of the beads and extrapolate the flow rate according to the geometry of the microfluidic channel used. The microfluidic chips are made with polydimethylsiloxane (PDMS), enabling dimensions down to a few microns to be obtained. The chips are transparent.
Description of Measurement Principle	The measurement relies on tracking fluorescent beads suspended in water-based fluids at approx. 21 ° C flowing into a microfluidic serpentine of known dimensions. To measure different flowrates at approximately the same flow velocity and applied pressure values, the fluidic resistance of the microfluidic serpentine is varied by changing the length and width of the microfluidic channel while fixing its height. The cross section of the channel is rectangular. The fluidic resistance is derived from Hagen-Poiseuille's law: $$R_h = \frac{12\mu L}{wh^3\left(1-0.63h/w\right)}.$$ where R_h is the fluidic resistance, µ is the dynamic viscosity, *L* is the length of the channel, *w* is the width and *h* is the height of the channel. Different values of R_h will determine the flow rate achieved inside the serpentine (Fig.3.1.2). Other options for flow measurements are available which include the use of immiscible phases. The flow rate measurements will rely on tracking a moving water/oil interface.
Can the Facility be used for Static / Dynamic or Both	The facility can handle both steady-state and transient flowrate measurements. To achieve this, the applied pressures must be programmed to the appropriate transient values (through a software interface controlling the pressure system) or connecting a second devices which creates intermittent flow. Prior to each measurement, fluids are degassed to reduce air bubbles in the liquids to minimize the fluidic compliance. The walls of the microfluidic channels can be treated to become hydrophilic and low adhesion to prime water fluids, avoid air bubbles and beads adhesion to channel walls.

Facility Flow Ranges	The minimum flowrate the facility is expected to be able to measure is 5 nL/min. The maximum flowrate that has been designed to be measured, according to project specification, is at 100 nL/min.
Temperature / Pressure Ranges	The applicable full pressure range of the facility is from 30 to 1000 mbar. However, pressure values from 50 to 200 mbar will be used. Currently, the facility will be operated at room temperature (~21.7 °C). However, further measures can be implemented (such as a Peltier chamber and/or a humidity chamber) to maintain temperature and compensate liquid evaporation.
Other Fluids	The primary fluid used is distilled water (density 0.9982 g/mL at 20 °C). Any fluids (Newtonian or Non-Newtonian fluids) that are in aqueous phase can be used within the facility, e.g. blood.
Uncertainty Budget	The overall uncertainty of the flowrate measurement is calculated based on the variation in dimension of the microfluidic channel and the uncertainty of the position of the bead in each frame depending on the frame rate used. Because of the fabrication processes, there are variations on the dimensions of the channels. The channels will be characterized using a scanning electron microscope (SEM) and an Alpha-Step surface profiler.

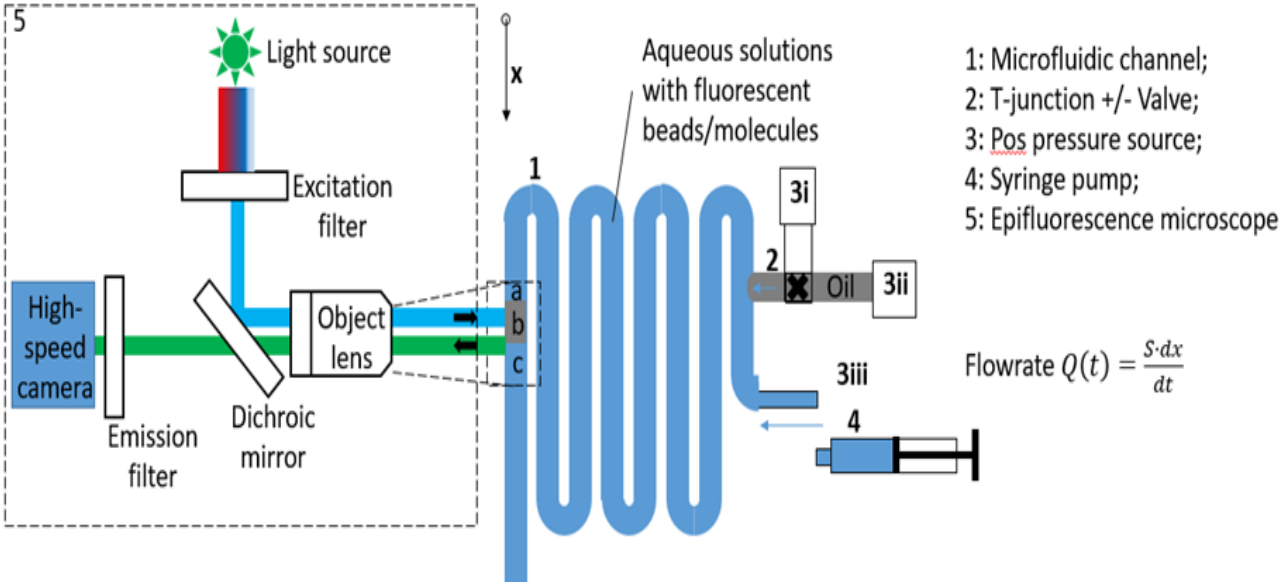


Figure 3.1.1 Flowrate measurement facility. It includes a microfluidic chip (1) with a T-junction (+/- PDMS Quake valve), a positive pressure source or syringe pump for fluid actuation, an epifluorescence microscope and a CCD camera. Images are recorded and analysed to extract fluid velocity.

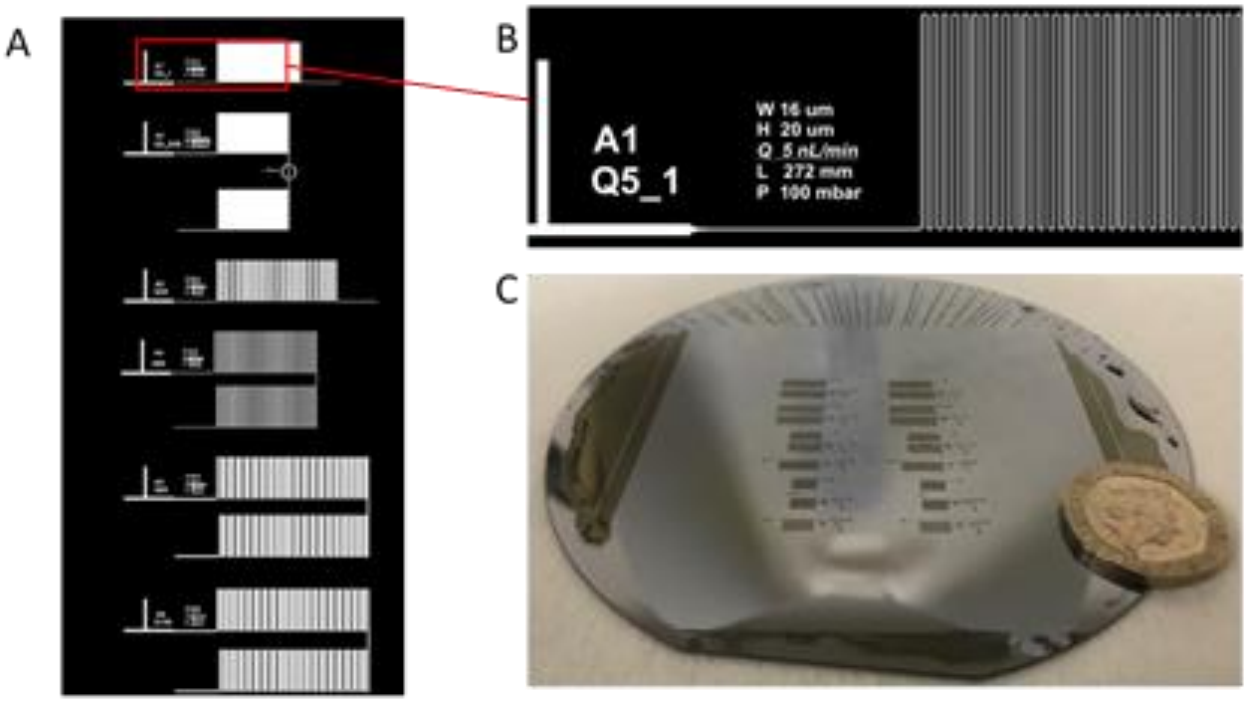


Fig.3.1.2. Designs and fabrication of the microfluidic chips. (A-B) Examples of microfluidic serpentine channels. (C) Microfluidic master obtained from photolithographic processes for casting microfluidic chip in PDMS.

3.2 Cesky Metrologicky Institut – CMI

Description of Facility	Within the activity A1.1.4 CMI is developing a special beaker eliminating systematic errors due to changing buoyancy and capillary forces when used in the gravimetric method of micro-flow calibrations. The beaker itself is not a complete calibration facility. It is just a part of equipment which can be used in any gravimetric facility to improve an uncertainty of the facility.
Description of Measurement Principle	The principle of the beaker is based on keeping constant water level in the area where a needle of the gravimetric system is inserted into the water in the beaker. Scheme of the beaker can be found in Fig. 3.2.1. Part A of the beaker is the part where a needle is inserted. The purpose of the construction is to keep the water level constant in this part. The construction contains a lid with a tube inserted into water in the part A such that the water surface inside the tube can be covered by an oil film to reduce evaporation without contact of the oil with the top of the cylindrical capillary drainage channel B. The capillary drainage channel B is realized by a space between a glass cylinder and the stainless steel internal beaker. The distance of the glass from the stainless steel is around 0.2 mm. Purpose of the capillary drainage channel B is to lead water from the part A to the collecting beaker C, such that volume of the water in the part A and consequently the water level in this part remains constant. In Fig. 3.2.2 photos of a prototype currently tested are shown. The collecting beaker in the photos is provisional. It can be replaced by any other standard beaker of suitable dimensions. In Fig. 3.2.3 (left) a detail of the inner stainless-steel part of the beaker is shown. In this design water flows over a sharp blade. This design turns out to be extremely sensitive to the blade surface properties. E.g. several days of oxidization on air after manufacturing significantly improves the beaker performance. Therefore, other designs not so sensitive to the surface properties are also tested, e.g. a design with horizontal slid as shown in Fig. 3 (right).
Can the Facility be used for Static / Dynamic or Both	The facility is intended mainly for a stationary flow but it can be tested also for dynamic flows to see its performance.
Facility Flow Ranges	Maximal flow rate is given by dimensions of the capillary drainage channel. Minimal flow rate is not limited by the beaker itself but by the gravimetric facility it is used with. At this moment (August 2020) a large size prototype of the beaker is tested at the CMI gravimetric facility for flow rates (1 – 100) g/h.

	A downscaled prototype is going to be manufactured for testing at the DTI gravimetric facility for flow rates down to 1 mg/h.
Temperature / Pressure Ranges	Given by the gravimetric facility the beaker is used with. The beaker itself is used at atmospheric pressure. The temperature of water should not exceed value where the material of the beaker can be damaged.
Other Fluids	Designed for water. Other designs for other fluid also possible.
Uncertainty Budget	Given by the gravimetric facility the beaker is used with. The purpose of the beaker is to reduce certain uncertainty components: - uncertainty of buoyancy correction by keeping the buoyancy force between a needle and a scale constant - noise in scale reading during the beaker filling by eliminating the slip-stick effect of a moving water-air interface along a needle

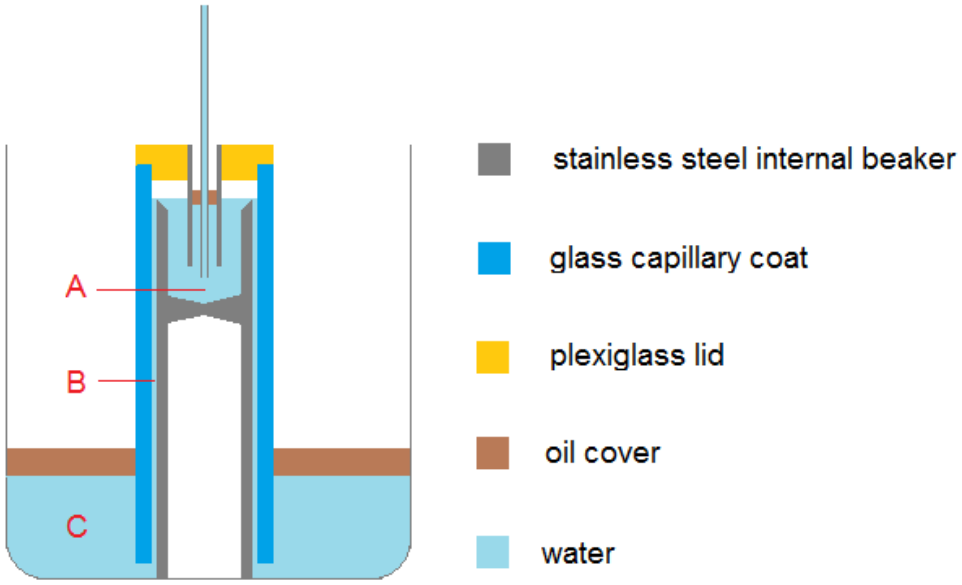


Figure 3.2.1 Scheme of the beaker; A – constant level internal beaker, B – cylindrical capillary, C – collecting beaker

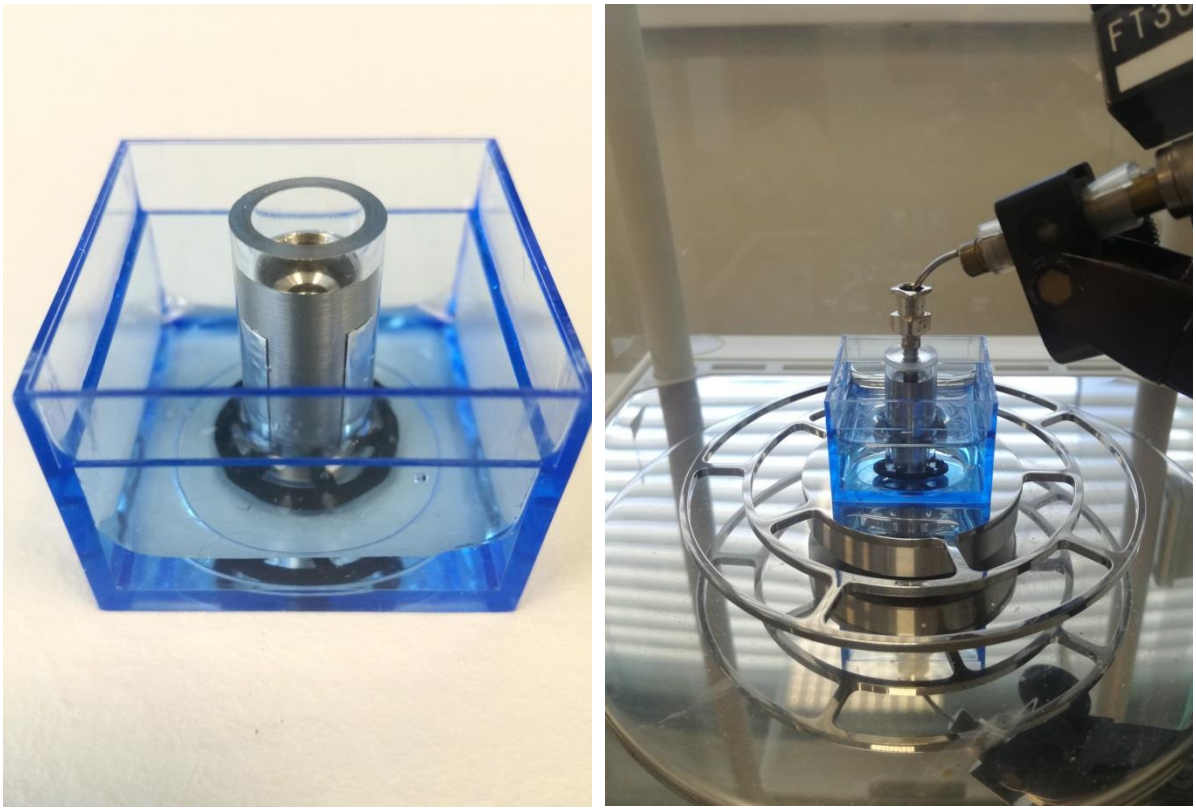


Fig. 3.2.2 - Prototype of the beaker; detail without the plexiglass lid and the tube (left), complete setup on a scale (right)

Figure 3.3.3 - Detail of the internal beaker with the blade design (left) and an alternative design with horizontal slid instead of the blade (right)

3.3 Instituto Português da Qualidade – IPQ

Description of Facility

Description of the overall facility including the calibration method used, how it functions, how it is controlled, Data Acquisition etc.

All the methods are conducted in a climate-controlled room insulated from vibration (reference temperature of 20 ºC, > 50 % relative humidity). The time is acquired by the computer.

Gravimetry

The flow is generated by a programmable syringe pump. First, the syringe is filled with high ultrapure water. A high flow rate is imposed to the system in order to purge the flow line from air bubbles which can significantly affect flow stability and introduce flow errors. This procedure is repeated until the flow circuit is completely purged (normally 10 minutes of continuous water flow is enough). Additionally, the tube ending is immersed in the weighing vessel to prevent flow oscillations due to droplet detachment. The data is collected from the balance using a LabVIEW application every 250 ms during 15 min. The flow is determined every 30 seconds. An AX26 Mettler balance and XP205 Mettler balance are used.

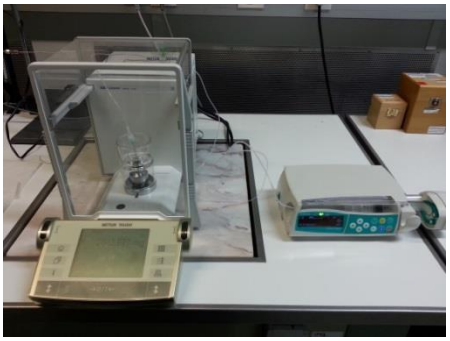


Figure 3.3.1 – Calibration of a syringe pump by gravimetry

Table 3.3.1 – Nexus 3000 gravimetric calibration using 1 ml syringe

Measured flow balance (ml/h)	Uncertainty (ml/h)	Error (%)	Uncertainty (%)
0,92	0,23	8,7	25,00
10,80	0,50	-7,4	4,63
99,30	2,40	0,7	2,42
496,80	5,80	0,6	1,17
994,50	23,70	0,6	2,38

Interferometry

The Interferometric method uses an interferometer (Hewlett-Packard, model 5528A; it operates at 633 nm, and the signal is processed using a LabVIEW application) to monitor the distance travelled by a pusher block of the syringe pump in order to determine the flow rate. The use of interferometry for flow measurement involved the use of the following components: a laser unit (A) with a detector incorporated (an optical arrangement composed by two retroreflector cubes (C) (one of which with a beam splitter attached (B)), a Control Unit, a pusher block, the syringe pump Nexus 3000 (D) with the removable glass syringe (E), (Figure 3.3.2).

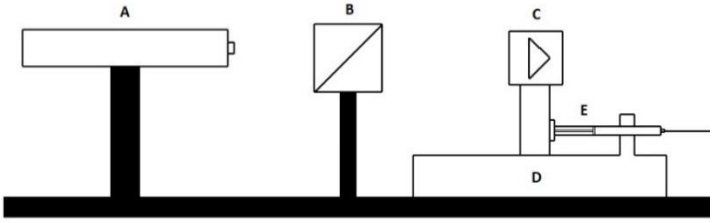


Figure 3.3.2 – Assembly of interferometric method setup

In practice, the generation of flow was accomplished by a stepper motor which drove a screw connected to a pusher block that itself pushed the syringe piston. Therefore, knowing the internal diameter of the syringe with very high precision, the travelled distance, and the time needed for that travelled distance, it was possible to calculate the flow rate of the fluid inside the syringe and its uncertainty.

Figure 3.3.3 – Picture of the interferometric method setup

Table 3.3.2 – Nexus 3000 interferometric calibration using 1 ml syringe

Nominal Flow (µl/h)	Measured flow (µl/h)	Uncertainty (µl/h)	Error (%)	Uncertainty (%)
0,1*	0,0970	0,0027	3,9	3,14
1	1,08	0,03	-7,6	2,87
10	10,13	0,19	-1,3	1,88
100	101,87	3,00	-1,8	2,94
500	503,57	13,00	-0,7	2,58
1000	1011,32	19,00	-1,1	1,88

*The syringe used was of 0,1 ml

Optical method – Drop

As an alternative to the gravimetric and the interferometric method, a new methodology based on the application of optical technology was also developed to measure flow rates.

The syringe pump is set to a specific flow rate, the delivered liquid flow was determined by observing the volume increase of a drop at the end of the flow line placed inside an evaporation trap (Figure 3.3.4). This observation was performed at a specific time by using photographs taken by a digital USB microscope with 400X magnification.

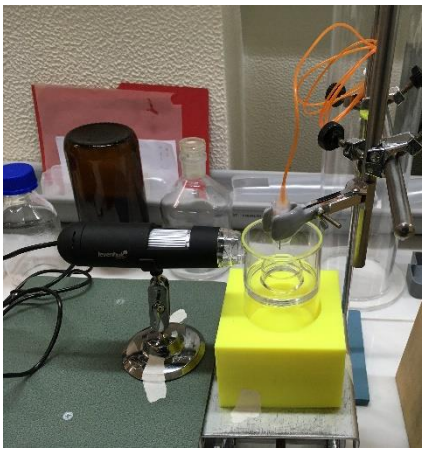


Figure 3.3.4 – Picture of the drop setup

The projected area of the drop was obtained geometrically with the assistance of a Pyton software (by dividing the image into slices), with pixel resolution, and considering the tube diameter where the drop is hanging, 1,49658 mm, measured by interferometry, as the reference value for length (Figure 3.3.5). This value was then converted to an equivalent sphere volume and later to a flow rate based on time acquired and on volume change.

Figure 3.3.5 – Image using Python software

Table 3.3.3 – Nexus 3000 optical calibration using 1 ml syringe

Nominal Flow (µl/h)	Measured flow (µl/h)	Uncertainty (µl/h)	Error (%)	Uncertainty (%)
1,00	0,74	1,22	35,1	164,86
10,00	10,27	3,24	-2,6	31,55
100,00	104,26	10,03	-4,1	9,62
500,00	496,87	35,64	0,6	7,17
1000,00	1083,50	49,50	-7,7	4,57

Optical method – Front track

The delivered liquid flow is determined by observing the movement of the liquid meniscus in a capillary/tube using the same microscope of the drop method. The flow is calculated based on the meniscus movement variation using pictures frames and the time variation between of each frame.

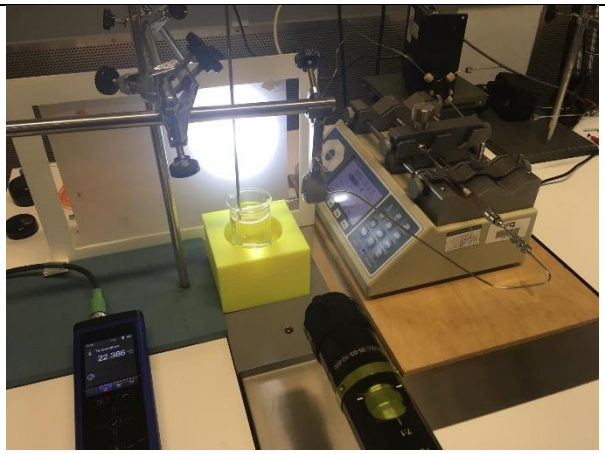


Figure 3.3.6 – Front track setup

In order to determine the coordinates of the blue point represented in figure 6 a Pyton algorithm was developed. The variation of these coordinates in each frame gives the travel distance of the meniscus in pixels that are later converted to length units using the external diameter of the capillary as reference.

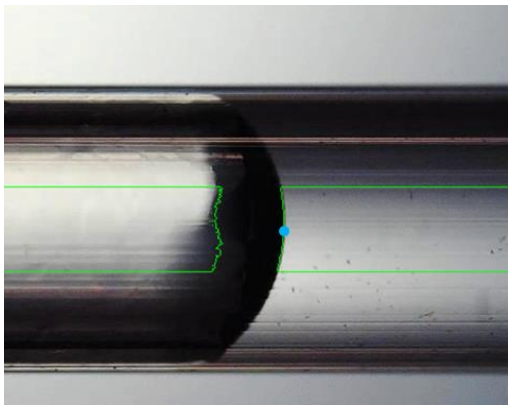


Figure 3.3.7 – Central point in meniscus base

Table 3.3.4 – Nexus 3000 front track calibration using 1 ml syringe

Nominal Flow (µl/h)	Measured flow (µl/h)	Uncertainty (µl/h)	Error (%)	Uncertainty (%)
1	0,99	0,06	-0,87	6,34
10	9,97	0,35	-0,29	3,49
100	101,83	2,02	1,83	1,98
500	499,76	6,77	-0,05	1,35
1000	997,06	14,46	-0,29	1,45

Description of Measurement Principle

Description of the measurement principle behind the facility/method

Gravimetry

The gravimetric method relies on weighing the mass of the working fluid delivered by the instrument under test for a set time. The mass is converted to volume at a reference temperature. The volumetric flow rate (Q) is then determined by the quotient of the volume of the reference liquid and the time interval, including corrections which consider evaporation, buoyancy and environment effects.

$$\text{Model: } Q = \frac{1}{t_f - t_i}\left[\left((I_f - I_i) - \left(\delta m_{buoy}\right)\right) \times \frac{1}{\rho_w - \rho_A} \times \left(1 - \frac{\rho_A}{\rho_B}\right) \times [1 - \gamma(T - 20)]\right] + \delta_{evap}$$

Interferometry

Laser interferometry is used to measure the intensity of a wave resulting from the overlapping of two or more waves that have travelled over different distances and are superimposed on a single point therefore this methodology can be applied to measure the distance of the pusher block of a flow generator connected to a glass syringe in order to determine the flow rate.

Model: $\boldsymbol{Q = v \times A = \frac{x_2 - x_1}{\Delta t} \times \pi r^2 = \frac{d\pi r^2}{t}}$

Optical method – Drop

The grow of a drop is monotonized by taken a photo at a specific time. The image of the drop is then treated with Pyton software allowing the volume determination of the drop volume per unit of time, this permits flow determination.

Model: $\boldsymbol{Q = \frac{\Delta V}{\Delta t}}$

Optical method – Front track

The movement of the meniscus in a capillary is monotonized by taken a photo at a specific time. The image of the meniscus is then treated with Pyton software and algorithms allowing the volume variation per unit of time, this permits flow determination.

Model: $Q = \frac{d}{\Delta t} \times \pi \times r^2$

Comparison of methods

The Nexus syringe pump was calibrated using the 4 methods and different glass syringes. The results are in figure 3.3.8 and 3.3.9.

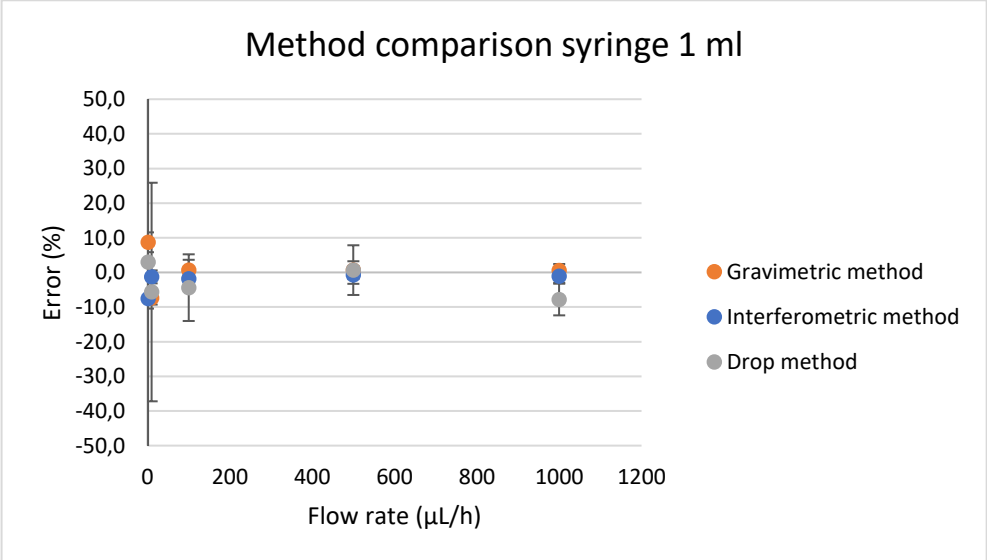


Figure 3.3.8 – Method comparisons syringe 1 ml

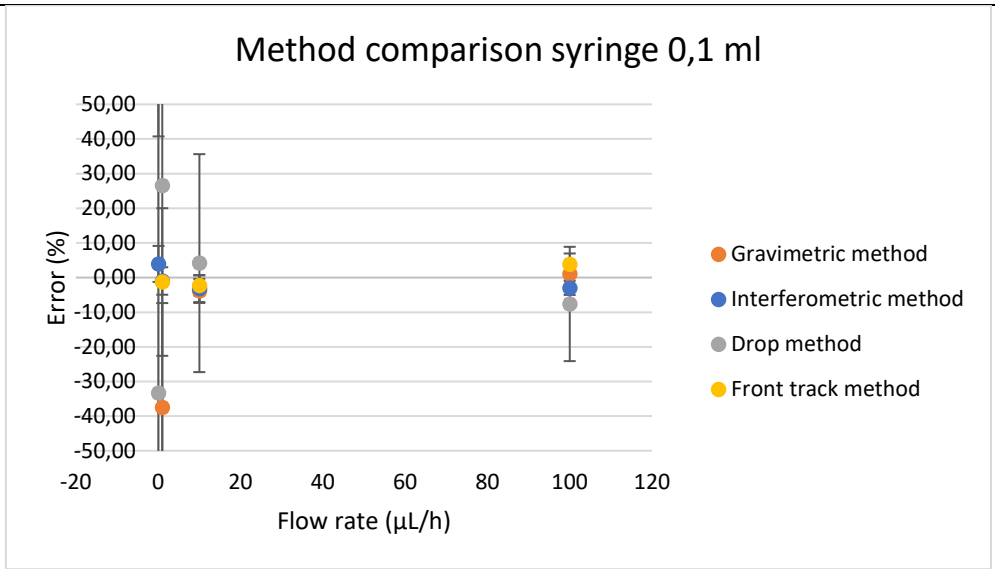


Figure 3.3.9 – Method comparisons syringe 0,1 ml

All results are consistent with each other.

Can the Facility be used for Static / Dynamic or Both

Comment on how or if the facility can handle dynamic or transient conditions (fast flow changes) & quantification of the change

The gravimetric method and the interferometric method can be used for dynamic measurements.

Figure 3.3.10 – Calibration of BL100 Bronkhorst meter using interferometry

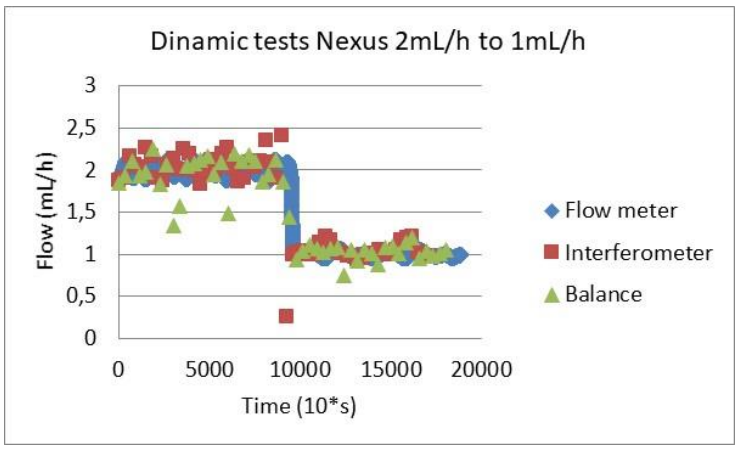


Figure 3.3.11 – Test for dynamic measures using BL 100 meter

Facility Flow Ranges	State the maximum and minimum flow ranges that the Facilities can cover **Gravimetry** 2000 mL/h to 0,001 mL/h, *U*= 0,3 % to 25 % **Interferometry** 1 mL/h to 0,0001 mL/h, *U*= 2% to 3 % **Optical method – Drop** 1 mL/h to 0,01 mL/h, *U*= 4% to 30 % **Optical method – Front track** 1 mL/h to 0,001 mL/h, *U*= 2% to 6 %
Temp / Pressure Ranges	State the temperature/pressure maximum and minimum ranges that the facilities can cover For all methods the temperature can go from 17 to 23 ºC at atmospheric pressure.
Other Fluids	Describe the Primary fluid used and any additional fluids that could be used within the Facilities For all methods the primary fluid is water but any other fluid can be used.
Response or delay time (also called "start-up" time)	At IPQ facilities the response or delay time is considered to be the time between the physical starting of the device under test and reaching, by increasing values, a 95 % of the target value, this is normally achieved after 15 minutes. Also, we use only start the data recording (in a balance or by other means) after we reach the stabilization conditions described above.
Uncertainty Budget	Brief description of the uncertainty components of the facility/method used and what your calculated uncertainty is for your Facility/method. **Gravimetry** The main standard uncertainties considered are: mass measurements (m), density of the mass pieces (ρ_B), density of the water (ρ_W), density of the air (ρ_A), evaporation rate (δQ_{evap}), water temperature (T), time (t), expansion coefficient (γ), standard deviation of the measurements (δQ_{rep}) and buoyancy on the immersed dispensing needle (δQ_{mbuoy}). **Interferometry** The main standard uncertainties considered are: distance (d), time (t), radius of the syringe (r), stability of the setup (δQ_{sta}), water temperature (T), time (t), expansion coefficient (γ), standard deviation of the measurements (δQ_{rep}). **Optical method – Drop** The main standard uncertainties considered are: Volume determination by drop method (V), that includes the inaccuracy of the contour of the drop and the pixels determination, evaporation (δQ_{evap}), time (t) and standard deviation of the measurements (δQ_{rep}). **Optical method – Front track** The main standard uncertainties considered are: Volume determination by front track method (V) , that includes the inaccuracy of the point coordinate determination, stability of the setup (δQ_{sta}), water temperature (T), time (t), expansion coefficient (γ), time (t), the radius of the tube (r), and standard deviation of the measurements (δQ_{rep}).

3.4 Eidgenössisches Institut für Metrologie – METAS

Description of Facility	The METAS Microflow and Milliflow facilities consist of homemade piston provers to generate the flow and the gravimetric method to determine the flow rate and calibrate the volumes and the volume flow rates of the piston prover. The flow generator, is filled with water and the water is pressed at the desired flow rate through the DUT and collected in the beaker on the balance, as shown in Figure 3.4.1. Weighing data are continuously collected by a real time system (RT), which communicates with the balance at 20 Hz via RS232. The weight value is directly paired with the time stamp of the RT. The other sensor values such as water pressure upstream and downstream of the DUT, the water temperature at various positions and the ambient conditions are recorded as well at a frequency of 1 Hz. The position the piston prover is determined by counting the pulses sent by the linear measuring system by means of an FPGA, which is a Field Programmable Gate Array with hard coded program code running on a defined constant cycle time of the order of 25 ns (40 MHz). For each additional pulse in any direction, a time stamp of the FPGA is recorded and a pair with the position and the timestamp is formed. These pairs of values are then read from the main software at a lower frequency. The real time position is used to calculate the real time speed by means of linear least square fit over a time window that is adjustable. Multiplying the speed with the cross section of the piston gives the volume flow rate. In the mode of fast changing flow rates, the data of the pressure sensors are collected with a FPGA and are paired with a timestamp. These pairs of data are also then read from the main software at a lower frequency. To synchronize the RT and the two FPGA-systems, a trigger signal is produced by one FPGA system and the other FPGA system is recording this trigger signal allowing a perfect match of the two timescales.
Description of Measurement Principle	Flow is generated by the piston prover. The real time position is used to calculate the real time speed by means of linear least square fit over a time window that is adjustable. Multiplying the speed with the cross section of the piston gives the volume flow rate. The gravimetric method consists of weighing the collected water in a beaker and applying several corrections such as evaporation, buoyancy correction, etc. The ambient conditions must be well controlled and recorded to avoid any virtual flow incident due to temperature instabilities in the absolute temperature and temperature gradients.
Can the Facility be used for Static / Dynamic or Both	The facilities operate in static and dynamic mode. The facilities can produce dynamic or transient conditions (fast flow changes). The changes occur within a time of less than 1 second and a ratio of 1:500 can be obtained. The range of flow rates is set by the choice of the piston used. The gravimetric methods can detect these fast flow changes in the range of 400 mL/min down to 50 nL/min. The piston provers can generate these fast flow changes in the range of 400 mL/min down to 20 nL/min.

	Steady flow rates are also generated in the range from 400 mL/min down to 20 nL/min and measured by the gravimetric method in the range from 400 mL/min down to 50 nL/min. Volumes of the delivered liquid are determined either by the piston prover or the gravimetric method.
Facility Flow Ranges	Dynamic mode: 20 nL/min - 400 mL/min (Piston Prover) 50 nL/min - 400 mL/min (Gravimetric Method) Steady mode: 20 nL/min - 400 mL/min (Piston Prover) Steady mode: 50 nL/min - 400 mL/min (Gravimetric Method)
Temperature / Pressure Ranges	Temperature: ambient Pressure: 0 – 10 bar
Other Fluids	Primary fluid: distilled water Additional fluids: any non-harmful fluid
Uncertainty Budget	Dynamic mode: 20 nL/min (50 nL/min) - 400 mL/min, 2.0 % - 0.2 % Steady mode: 20 nL/min (50 nL/min) - 400 mL/min, 1.0 % - 0.07 % Uncertainty components of the gravimetric method includes • drift, calibration and reading uncertainty of the balance • uncertainty of linear least square fit • evaporation buoyancy correction • correction from conventional weight to real weight • stability of ambient temperature conditions • stability of water temperature and temperature gradients at different positions along the piping (piston, DUT, Balance) • repeatability and stability of the flow detection • stability of the capillary forces acting at the water bridge to the measurement beaker Uncertainty components of the gravimetric method includes • Linearity of the linear stage • Repeatability and stability of the flow generation • Influence of torque and force on the accuracy of the position of the plunger

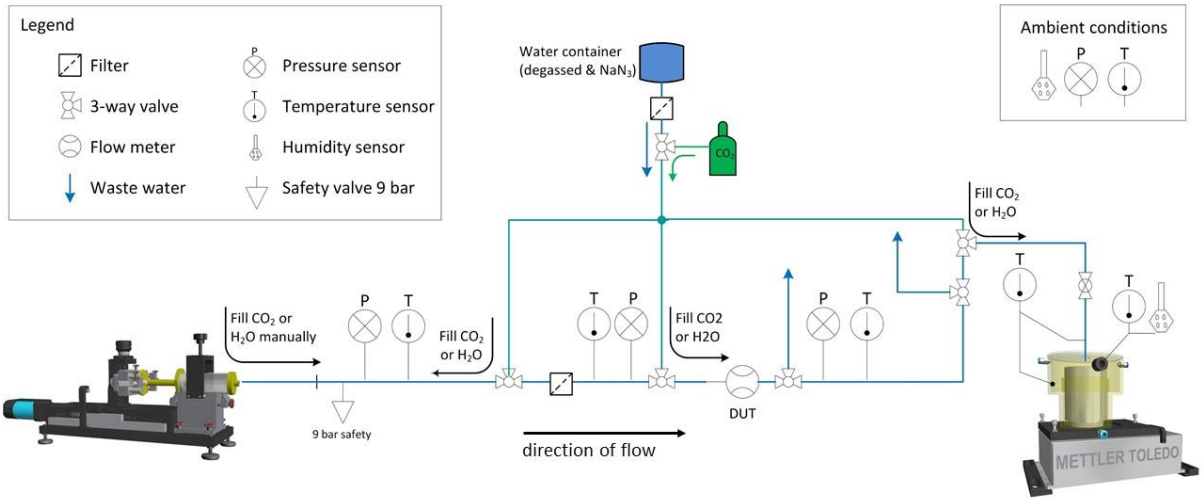


Figure 3.4.1 - Scheme of the updated Microflow and the developed Milliflow facility at METAS. The METAS piston prover presses the water through the DUT in the beaker on the balance, where it is continuously collected. Other components are the pressure sensors, temperature sensors, pressure relief valve and the water reservoir.

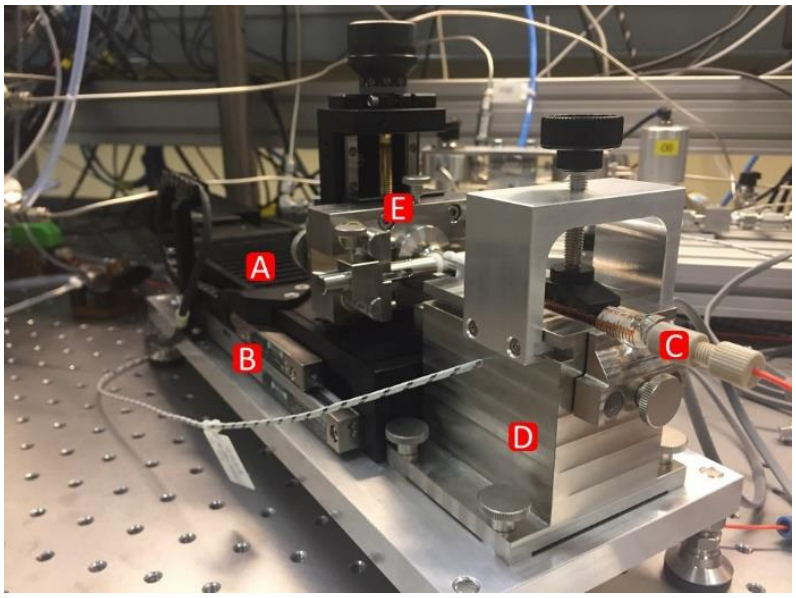


Figure 3.4.2 - METAS piston prover of the Microflow facility with a speed range from 0.1 mm/s to 0.1 µm/s. (A) high precision linear stage, (B) linear measuring system, (C) syringe, (D) mounting syringe body, (E) mounting and positioning for syringe plunger. The same design and components are used for the METAS piston prover of the Milliflow facility, but with a different speed range from 4 mm/s to 4 µm/s.

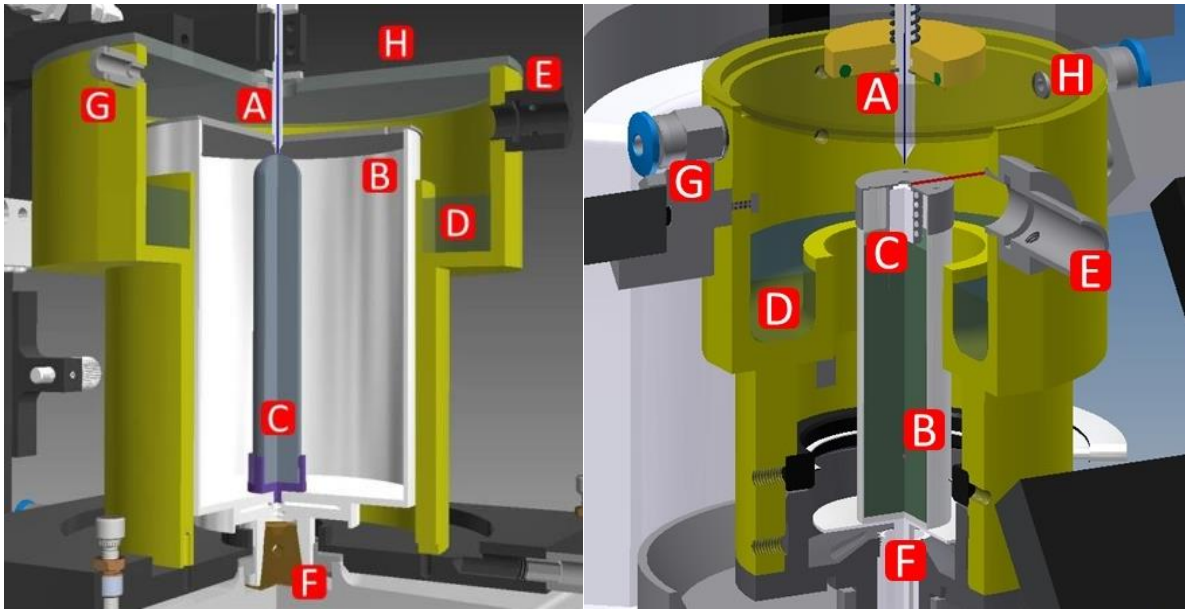


Figure 3.4.3 - Weighing zone on the balance of the Milliflow (left) and Microflow (right) facilities. (A) outlet needle, (B) beaker with cover, (C) glass filter, (D) water in evaporation trap, (E) mount for T and rH sensor, (F) balance, (G) tubing for humidity exchanger, (H) cover.

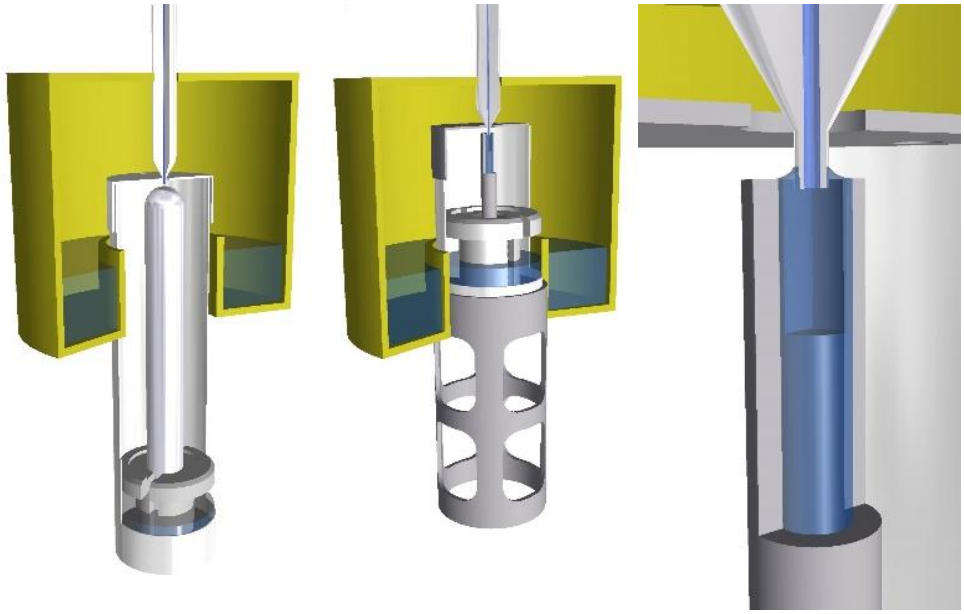


Figure 3.4.4 - Other measurement beakers for the Microflow facility. (left) beaker with glassfilter like the beaker for the Milliflow facility. (center and right) Capillary beaker, where the outlet needle is placed in the center of the capillary. Capillary beaker is suitable for the detection of fast changing flow rates.

3.5 Centre Technique des Industries Aérauliques et Thermiques – CETIAT

Description of Facility	CETIAT's optical measurement system consists of a JAI SP-12000M-CXP4-F camera (188 fps at 12MP), Qioptiq Optem FUSION Lens System 7:1 with a motorized zoom controller and a KL 2500 LED backlight for image acquisition, Quartz glass capillaries with inner diameters ranging from 100 µm to 1 mm to circulating water or water and oil, and 4 translation and 1 rotary stages (see Fig.3.5.1) Camera calibration is realized using a calibrated Olympus OB-M transmitted light objective micrometer. A signal generator that is calibrated against an atomic clock is used for triggering the camera to start image acquisition. Liquid flow is generated by CETIAT's micro-flow bench for flow rates going down to 16 µL/min and by a CETONI Nemesys syringe pump (using 1mL to 10µl syringes) below that limit. A valve will be used to introduce oil droplets in the flow in case of tracking Water/Oil interfaces. Measurement results will be compared to the ones obtained by Bronkhorst BL 100 flow meter down to 20 nL/min and possibly by a Quartz Crystal Microbalance.
Description of Measurement Principle	Meniscus tracking consists in measuring the displacement as a function of time of a liquid/air or liquid/liquid interface moving inside a glass capillary tube that is connected to a flow generating device. Images of the moving meniscus are acquired by a high-speed camera. A computer program written in Python language is used to measure the flow velocity from the frequency of the signal generator i.e. the frame rate and the interface displacements measured using Digital Image Correlation, and then to deduce the flow rate from the calculated velocity and the previously measured inner diameter of the capillary.
Can the Facility be used for Static / Dynamic or Both	The nano-flow facility is used in both Static and Dynamic conditions. The dynamic flow profiles are generated using either Bronkhorst (ML120, BL 100, etc.) flow controllers or the Nemesys Syringe pump with dedicated software. Generated flow changes are comprised up to a factor ten of the flow rate value in less than one second. The meniscus tracking by image correlation in 100 µm inner diameter capillaries enables the measurement of flow rates down to 1 nL/min within time intervals as small as 1s. For all flow conditions, a reference flow rate value over a time interval of one second is available. This allows the study of flow rate fluctuations and thus, a complete characterization of flow generating devices.
Facility Flow Ranges	The nano-flow facility can cover flow rates ranging from 1 nL/min to 16µL/min. The micro-flow (gravimetric, developed during MeDD1) can cover flow rates ranging from 1 µL/min to 167 mL/min.
Temperature / Pressure Ranges	Liquid flow temperature is controlled at +/-1°C from 10 to 50°C, as the device under test and/or the flow generator (depending on the calibration conditions needed) is placed inside a climatic chamber.

Other Fluids	Measurements are carried out using ultra-pure water flowing through the device under test. CETIAT degassed ultra-pure water properties are density of 998.2 kg/m3 at 20°C and conductivity of 0.06 µS/cm.
Uncertainty Budget	The different measured quantities in our system are: Inner diameter of the capillary tube, positions of the interface inside the capillary, timestamps corresponding to each interface position and velocity of the interface, to which are associated the following uncertainty components : 1-camera calibration and specifications, pixel intensity profile and digital image correlation methods for the diameter and position measurements, 2-frame rate calibration and exposure time for time measurements and 3- linear regression parameters for velocity. Additionally, the uncertainty budget includes the system's drift and temperature effect as well as the uncertainties corresponding to the correction of physical phenomena e.g. evaporation, thermal expansion and stick-slip effect. The current relative expanded (*k*=2) uncertainty is between 10% and 1% from 1 nL/min to 16 µL/min.

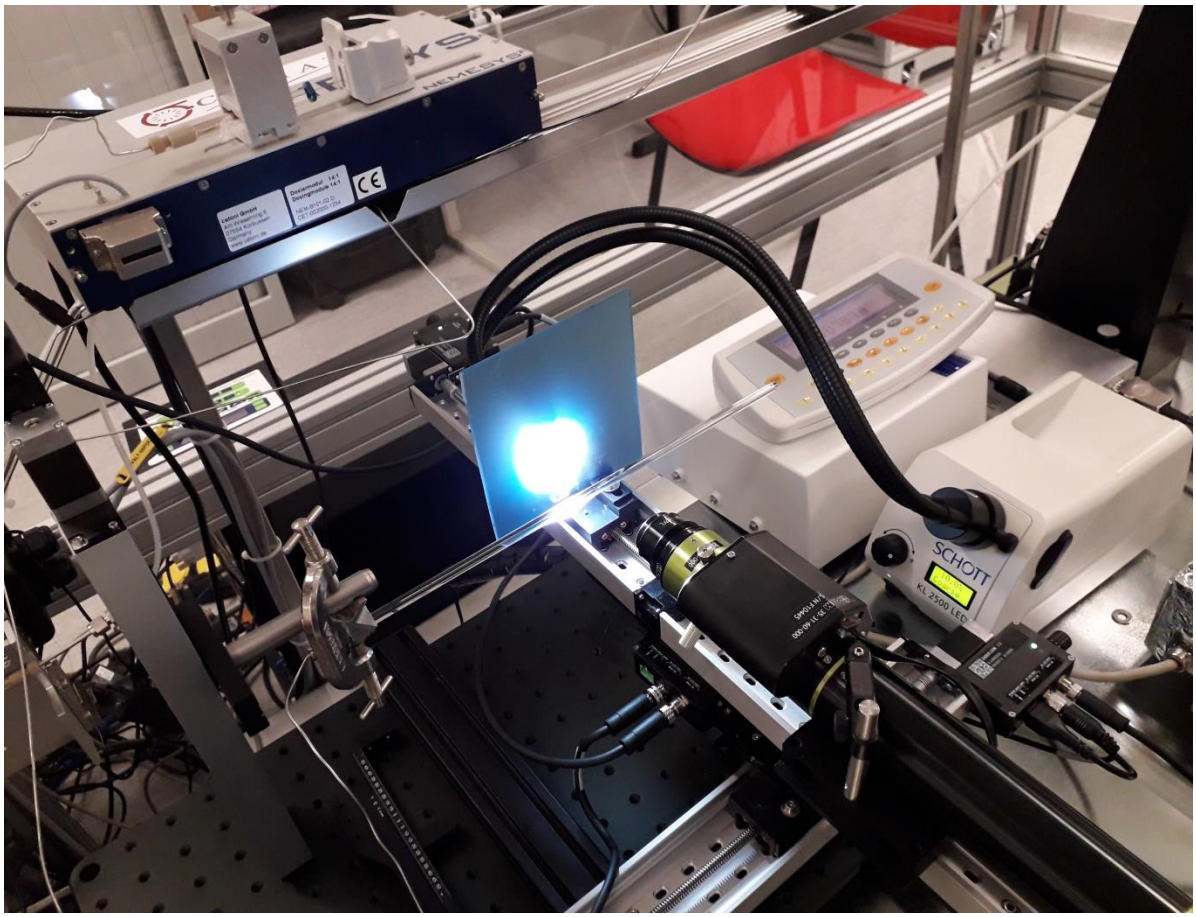


Figure 3.5.1 - photograph of CETIAT's Interface tracking measurement system

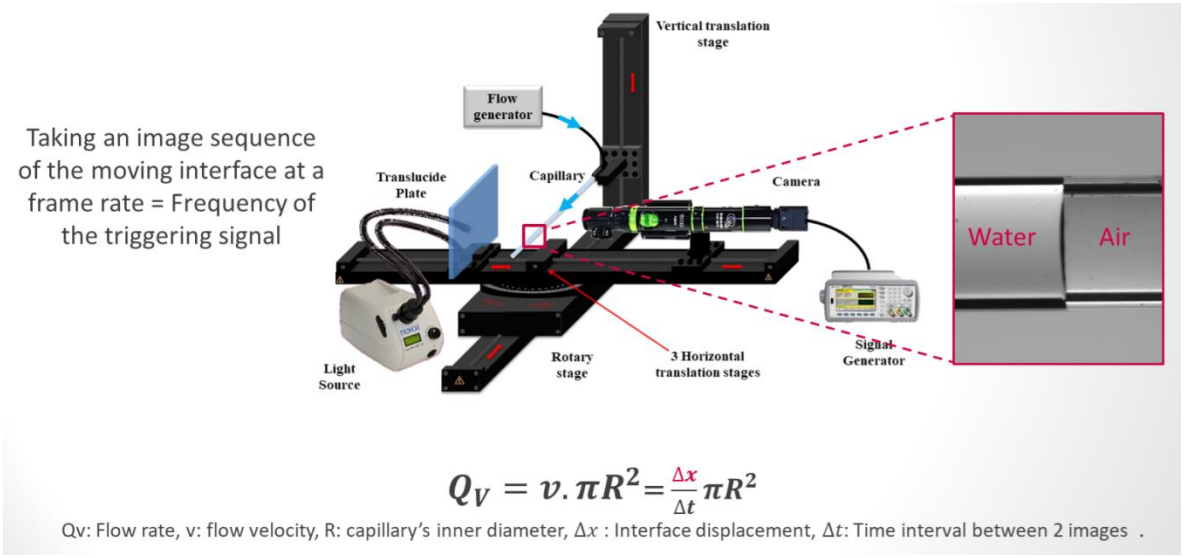


Figure 3.5.2 - Schematic of CETIAT's Interface tracking measurement system

3.6 RISE Research Institutes of Sweden AB – RISE

Description of Facility	The micro flow facility at RISE was upgraded with a new flow generator which is mainly a pressure vessel in a temperature-controlled bath. The flow rate is generated by pressurized air using a very-precise flow regulator. In order to generated very low flow rates (down to 100 nL/min) capillary tubes are used. The traceability is primarily realized by gravimetric weighing method. Therefore, a new weighing scale (10 mg) was purchased. A new evaporation trap and a new beaker was fabricated at RISE workshop. As a second possibility to generate low flow rates a syringe pump will be equipped with a glass scale (Integrated Measuring System, IMS) in order to obtain (total) volume information at any time during calibration. Data from the linear scale will (most likely) be read-out by using a 25 bits SSI interface. The data acquisition is mainly performed by a PC with a 4-port RS232 card to control and read-out the syringe pump, the flow meter and the weighing scales (MT AT201 205 g and MT UMT5 5g). The weighing scales are read-out by means of RS232 at a frequency of 2.5 Hz. The new weighing scale (MT XPR10 10 g) will be read-out by USB with a frequency of 10 Hz. The pressure signals (4…20 mA) are logged by means of an additional DAQ card. The raw data is collected by the same PC using LabVIEW (also as GUI) and saved with timestamps in an EXCEL file. The data post processing is also performed in Excel by using another tab in the same Excel template.
Description of Measurement Principle	Flow can be generated either by means of the syringe pump equipped with different syringes of various sizes or by using the pressure vessel. The desired flow rates and pressures (and pressure drops) can be further adjusted by means of capillary tubes of different sizes. The weighing method is performed by collecting the water (or another liquid) in a beaker. The weighing scale is continuously read-out. After the measurements several corrections for evaporation and buoyancy effects, etc. are applied. The ambient conditions (ambient temperature and atmospheric pressure, etc.) are measured but are not be recorded.
Can the Facility be used for Static / Dynamic or Both	The facility can be operated in static and dynamic mode (not tested yet). In the future the facility should handle dynamic transient conditions (fast flow changes). The quantification of the change is mainly depended on the read-out of weighing scale used.
Facility Flow Ranges	The flow rate range of the facility is 100 nL/min to 10 ml/min with the requirement of some modifications (tubing etc.).
Temperature / Pressure Ranges	Temperature: ambient temperature (around 20 °C, climatic room) Pressure: 0 to 4 barg (upgradeable to max. 10 barg)
Other Fluids	Primary fluid: ultra-pure and degassed water Additional fluids: liquids other than water (non-toxic)

Uncertainty Budget	Steady (static) flow: Target uncertainties: 0.5% (higher flow rates) to 2.0% (lowest flow rate) Unsteady (dynamic) flow: Target uncertainties: 0.5% (higher flow rates) to 5.0% (lowest flow rate)

Figure 3.6.1 – Flow generator 1: Syringe pump Chemyx Nexus 3000

Figure 3.6.2 – Flow generator 2: Syringe pump CETONI NEMESYS

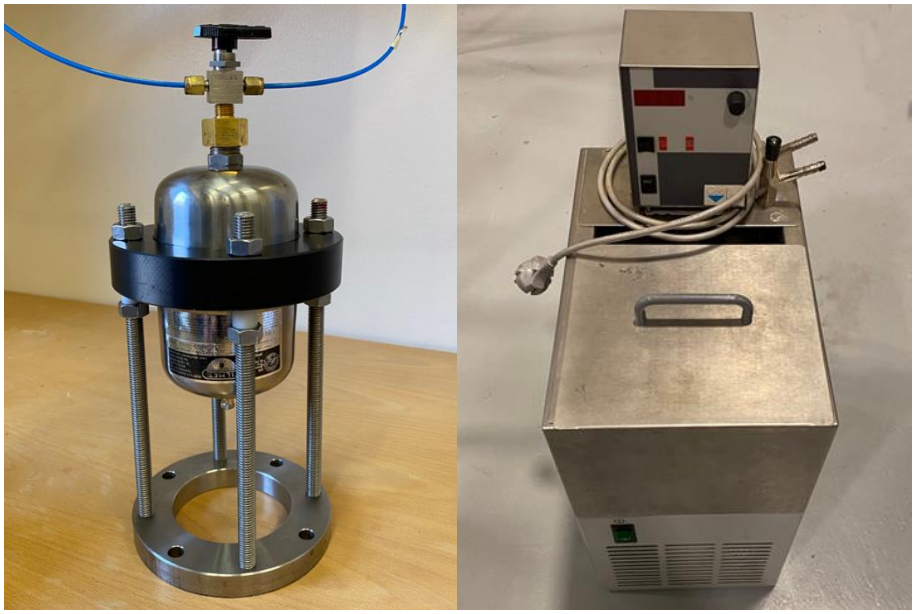


Figure 3.6.3 – Flow generator 3: Pressure vessel Zilmet (1 L, 10 barg max.) and temperature bath

Figure 3.6.4 – Weighing scale Mettler Toledo XMR10

Figure 3.6.5 – Pressure sensor (pressure range 0-5 bar) and I/O distribution box

Figure 3.6.6 – Flow meter Bronkhorst M12 and pressure sensor (4…20 mA)

3.7 Teknologisk Institut - DTI

Description of Facility	At DTI the primary standard covers a flow range from 100 ml/h (1.6 ml/min) to 1 µl/h (17 nl/min) with uncertainties from 0.05 % to 5 % with calibration time from 10 - 75 min. The micro flow laboratory at DTI is accredited to a flow rate from 600 ml/h (10 ml/min) to 1 ml/h (17 µl/min) with uncertainties from 0.05 % to 4 %. Design of the facility The setup is based on gravimetric measurements, i.e. the flow is determined from $$Q = \frac{\Delta V}{\Delta \mathrm{t}} = \frac{\Delta m/\rho_w}{\Delta \mathrm{t}}.$$ The time, Δt, is determined from an oscillator in order to make traceability feasible. The water density, ρ_w, is determined using a formula from literature and checked by measurements. The measuring beaker used is thoroughly cleaned and acclimatized to the balance. Demineralized degassed water is used as fluid. The most difficult parameter to determine accurately for small flow rates is the mass change, Δm. It is based on measurements made with a laboratory scale with microgram resolution. Several corrections are required, e.g. for buoyancy by air, displacement by the inlet ("needle"), evaporation, capillary forces and sticking. To allow for accurate weighing the entire setup is place on a granite table, which in particular provides an almost vibration-free base. Furthermore, the temperature of the setup is stabilized. Rapid temperature variations are prevented by placing the entire setup in a custom-built isolation chamber. This chamber also minimizes draft and convection effects. The chamber consists of metal box which is shielded from the surroundings by an isolating layer. Long-term stability is ensured by the laboratory's climate control. Evaporation is dealt with in different ways depending on the flow rate. In the case of the smallest flows the evaporation is almost completely eliminated by covering the water by an oil layer (see below). Alternatively, evaporation can be limited by increasing the relative humidity near the beaker and/or reducing the reducing the opening aperture of the beaker. For small flow rates it may be needed to measure the evaporation rate regularly in order to reduce the resulting uncertainty. The liquid oil cover used as evaporation trap is very efficient as only an evaporation rate of < 9 nl/h is remaining. However, the water needs to be delivered below the oil surface through the outlet pipe, which leads to several challenges e.g. capillary forces, buoyancy, inertia, stiction and friction, absorption, adsorption, stick/slip and vibration transferal. To limit these effects, the outlet pipe is made of 0.4 mm stainless steel tubing. The operational volume of the beaker is < 5 ml, yet the setup includes a system for emptying the beaker automatically when it becomes full. This system provides significant benefits for measurements at relative high flow

	rates, by reducing the amount of manual handling required and increasing the thermal stability of the setup. If the Device under test (DUT) is a flow meter then the demineralized and degassed water runs from pump and into the DUT. For that purpose, three syringe pumps are installed. Two Cavro XLP 6000 pumps are working in parallel and used for flow rates > 50 µl/min, whereas a Cavro Centris pump is used for smaller values; the minimum rate for continuous flow is 1 µl/hr ≈ 17 nl/min. In addition to steady flows the pumping system can also provide dynamic flow-rate profiles. If the DUT is a pump, the water exits the DUT and is lead to the scale via stainless steel tubing. This configuration has e.g. been used for some medical devices. <u>Dynamic gravimetric weighing</u> The balance is connected to a computer, enabling measurements with a frequency of 10 Hz and traceable timestamps with a dedicated timing hardware. By having continuous read out it becomes possible to detect the actual flow rate as a function of time in contrast to the case of static weighing where the delivered mass is measured and divided by elapsed time. The resolution of the balance is 1 µg, the output stability is below 10 µg.
Description of Measurement Principle	The facility is based on the gravimetric principle with an optional oil layer in the beaker as evaporation trap.
Can the Facility be used for Static / Dynamic or Both	The scale can deliver data with a frequency of 10 Hz – and it seem suitable for measuring dynamic and transient conditions. In MeDD I the dynamic behavior of a syringe pump was measured/check on this setup.
Facility Flow Ranges	The maximum flow rate is 60 mL/hr (1 mL/min), if the automatic system for emptying the beaker can be employed (e.g. if the DUT is a flow meter). Otherwise, the maximum flow rate may be limited to 10 mL/hour (0.167 mL/min). The facility is tested (with success) for 5 µL/hr (0.083 µL/min), however a realistic aim is 1 µL/hr (0.017 µL/min).
Temperature / Pressure Ranges	The facility can only operate at ambient conditions, meaning room temperature in the lab (18 – 25 degrees Celsius)
Other Fluids	Normally degassed demineralized water is used, however it will be possible to use other fluids as well. Prior to the project insulin mimicking fluid has been tested.
Uncertainty Budget	The dominant uncertainty contribution (>90 %) for the setup comes from the uncertainties of the corrections for the forces between the outlet pipe, the water and oil in the measurement beaker. The measurement uncertainty is approx. 0.5 % (k=2) at the flow rate of 10 ml/h. Below flow rates of 300 µl/h

	the balance uncertainty and uncertainty of the correction for buoyancy become dominant (90 % at 5 µl/h), while the level of water rise within the beaker becomes insignificant (about 20 µm). The measurement uncertainty rises to about 5 % (k=2) at the lowest flow rate of 1 µl/h.

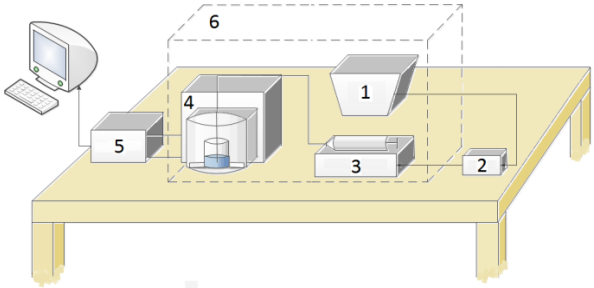


Figure 3.7.1: DTI facility. Setup where the DUT is a syringe pump. Water is lead from the reservoir (1) through the degasser (2) and into the syringe pump (3). From the syringe pump water is lead to the balance (4) using stainless steel tubing. The outlet tube may be traversed through the oil-based layer and into the water in the beaker. The balance is connected to balance electronics (5) and to a PC. The setup is enclosed in a chamber (6). Flow meters are installed between pump (3) and scale (4).

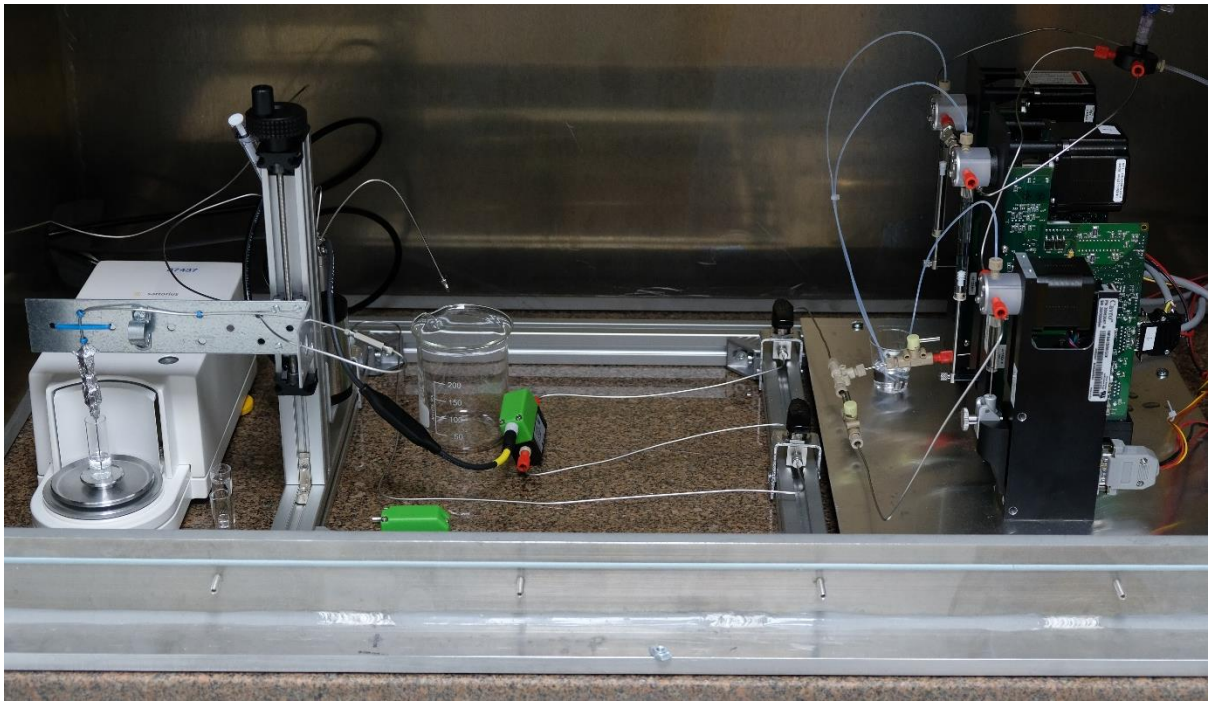


Figure 3.7.2: Photo of the DTI facility (central parts). The syringe pumps are visible to the right, the flow meter (green) in the center and the weighing system to the extreme left. The height-adjustment system for the inlet and the six-port valve for the emptying system are located towards the left side between scale and flow meter. Different beakers are available depending on the flow rate. For this photo, the draft screen of the scale has been removed and the insolating box around the setup is opened.

3.8 Hahn-Schickard-Gesellschaft für angewandte Forschung e.V. – HSG-MIT

Description of Facility	Hahn-Schickard (HS) have developed a flow rate measurement device based on micro particle image velocimetry (micro-PIV) method to address flow rates below 100 nL/min. Shear rate, maximum velocity, velocity profile shape, and flow rate can be derived from this nonintrusive method for the flow rate measurement at microscales.
Description of Measurement Principle	Micro-PIV is an optical measurement technique for flow velocity determination. It is based in on the displacement of tracer particles between two points in time. The experimental set-up of a PIV system consists of several subsystems: a transparent micro channel with seeded tracer particles, an illumination to expose the particles in a plane or volume of the flow at least twice within a short time interval, a camera to record a sequence of frames and a post-processing evaluation to determine the flow velocity. Within the first 9 months of the project, three different imaging methods are investigated at Hahn-Schickard: the confocal laser microscopy, digital holography, and simple stereo lens set-up (standard microscopy) with a CCD camera. Exemplary pictures taken with each setup are shown in figure 3.8.1. A channel with a height and width of 50 x 50 µm was used and beads with a diameter of 5 respectively 1 µm. To minimize the sedimentation of the beads, a mixture of deionized water and heavy water was used (50% v/v), resulting in a density of $\rho_{medium} = \rho_{beads}$=1,05 g/cm³. Confocal microscopy provides images with a very good quality, but has the disadvantage of a complex setup and expensive equipment. The digital holography set-up is shown in figure 3.8.2. An advantage of this setup is that multiple planes can be reconstructed out of one image minimizing impact of unwanted interference patterns; via thresholding unwanted artifacts can be removed. Therefore, the system doesn't have to scan through all the different planes in the channel. However, the channels appear dark in holographic image because the outer channel structures diffracts a lot of light. Interference patterns of channel wall hides interference patterns of beads in holographic setup. Therefore, the beads within the channel cannot be seen clearly. Only Artifacts/dirt particles outside the channel are very well visible. It can be concluded that for these small channel sizes below 50 µm our holographic set-up is not suitable. The standard microscopy seems to be the most suitable option to image beads in small channels. The setup is show in figure 3.8.3. With the transmission light setup different standard stereo lenses can be used (10x to 50x). For the first experiments, a 10x magnification is set with the transmitted light setup. A channel with a size of 50 x 5 x 0.85 mm is placed underneath the camera and the lens. Polystyrene beads with a size of 20 µm are seeded inside the channel and a mixture of deionized water and heavy water was used (50% v/v) as medium resulting in a density of $\rho_{medium} = \rho_{beads}$=1,05 g/cm³. Images are taken from one focal plane in the channel at a specified time interval (figure 3 bottom). Particles that do not move are sedimented and adhere to the channel bottom. To get more information about the flow profile, one could improve the set-up and scan different focal planes in the channel. An advantage of standard microscopy is that no fluorescent setup is necessary for imaging.

	Current work focuses on the optimization of the set-up: searching and testing beads, which do not cluster, test the holographic set-up with larger channels, and evaluate different algorithms for post processing to determine the flow rate. A first prototype will be available in June 2020.
Can the Facility be used for Static / Dynamic or Both	The described method could be used for static and dynamic measurements, because it only depends on the framerate of the chosen camera. In this case, a camera with a framerate of 10 fps is used. Therefore, dynamic changes in the range of 100 ms can be detected.
Facility Flow Ranges	Flow range 5 to 500 nL/min (planned) Flow range 60 to 6000 µl/min (current status)
Temperature / Pressure Ranges	Pressure 0 to 2 bar
Other Fluids	For the PIV system Deionized water together with D2O is mixed (50/50 v/v) to achieve a similar density then the latex beads used as tracer particles in the channel (density of 1,05 g/cm³).

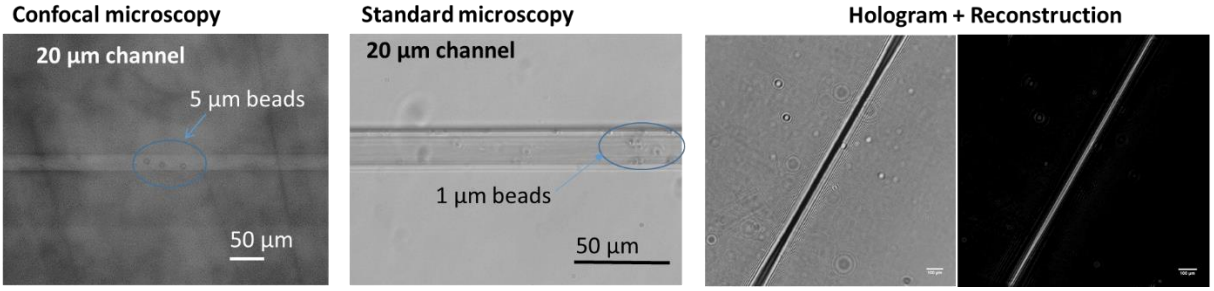
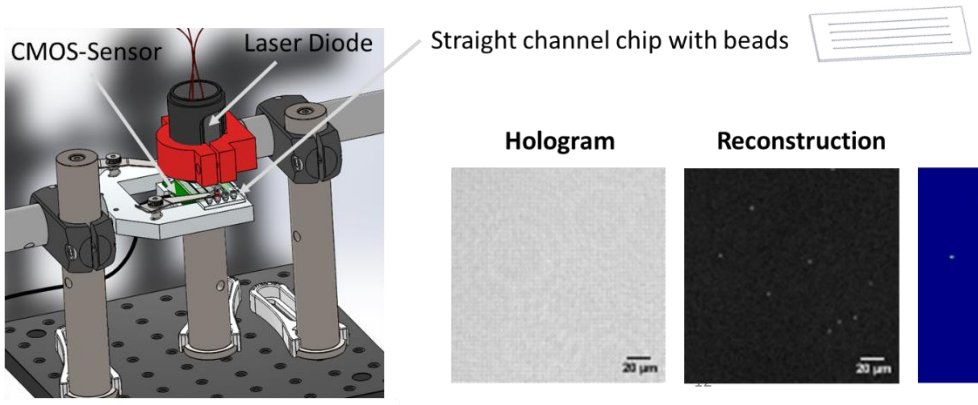


Figure 3.8.1: Comparison of three different imaging methods which are currently investigated at Hahn-Schickard: the confocal laser microscopy, digital holography, and simple stereo lens with a CCD camera. A channel with a height and width of 50 x 50 µm was used and beads with a diameter of 5 respectively 1 µm.



Test setup for holographic imaging.

Holographic analysis of silica beads (d_{bead}= 1 µm) on a microscope slide.

Figure 3.8.2 shows the test setup for the holographic imaging method on the left. The setup consists of a laser diode, a hole, the straight channel chip with seeded beads and a CMOS sensor to capture the images. On the right, you can see a typical holographic analysis consisting of the recorded hologram, the reconstructed image and the edited one. Here not the straight channel was used, but beads on a microscope slide.

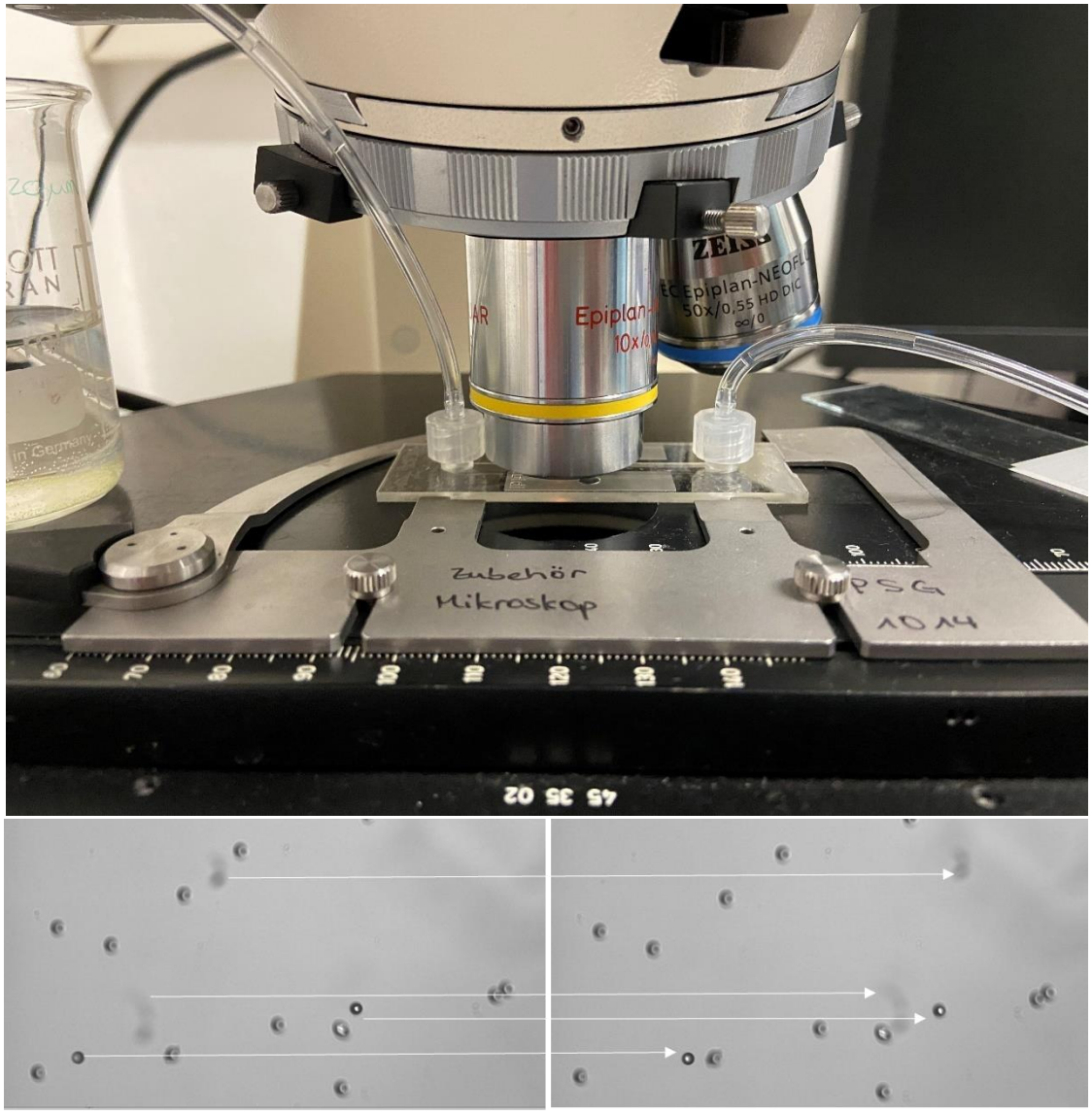


Figure 3.8.3 shows the test setup for the standard microscopy. A 10x magnification is set with the transmitted light setup. A channel with a size of 50 x 5 x 0.85 mm is placed underneath the camera and the lens. Beads with a size of 20 µm are seeded inside the channel. Images are taken from one focal plane in the channel at a specified time interval (bottom). Particles that do not move are sedimented and adhere to the channel bottom.

3.9 Technische Hochschule Lübeck – THL

Description of Facility	*Camera-Front-Tracking-System*: High precision capillaries (0.15 mm to 1 mm inner diameter) in combination with telecentric lenses and a high-speed camera were used to examine flows between 50 nl/min and 500 µl/min at sample rates up to 5 kHz (5000 fps), Figure 3.9.1. Acquisition times between 2 and 60 s are possible. The acquisition time and resolution can be adjusted via different capillary diameters (150 µm, 300 µm, 600µm, 1000µm), different magnifications(2x, 4x, 5x) of the measuring lenses, Table 3.9.1, and via the recording speed. The camera is mounted on a linear stage to adjust the distance after a lens change. The capillary is mounted with an adjustable holder on a linear stage, Figure 3.9.2. Calibration method: The inner diameters of the capillaries are determined with a Keyence digital microscope VHX600. The conversion factor (pixel to µm) is determined with a glass scale from Leica. The resolution also depends on the resolution of the camera used.(SpeedCam MiniVis-Eco2, 1280 x 1024 px) The facility is primarily designed to characterize the dynamic properties of various medical sensors or devices. Different flow sources, syringe pumps (Nemesys, Cetoni; Nexus 3000), pressure sources Fluigent MFCS-EZ (2 channels 0-1 bar), valves (Bürkert, Giga, Asco) or sensors, pressure sensors (Wika), flow sensors (Sensirion, Bronkhorst) can be used. Data acquisition is realized either via the software of the respective device manufacturers or in LabView with NiDAQ cards.
Description of Measurement Principle	The measurement principle is optical front tracking. The volumetric change inside a cylindrical geometry is measured with high precision by monitoring the displacement of the liquid front and the related time. The volumetric flow rate Q is determined by the displacement Δx of the liquid front during a time interval Δt and the radius R of the capillary or of the syringe. $Q = (\Delta x/ \Delta t)\, \pi R^2$
Can the Facility be used for Static / Dynamic or Both	The facility can handle dynamic conditions. The volume can be determined every ms. The camera is able to sample 1000 frames per second.
Facility Flow Ranges	The facility has been used between 50 nL/min and 500 µL/min.
Temperature / Pressure Ranges	The facility can cover only room temperature. Pressure range is limited between ambient pressure and 6 bar
Other Fluids	Primary fluid is water. Water based solutions could be used too.
Uncertainty Budget	Uncertainty is 4% for flow rates higher than 50 nL/ min.

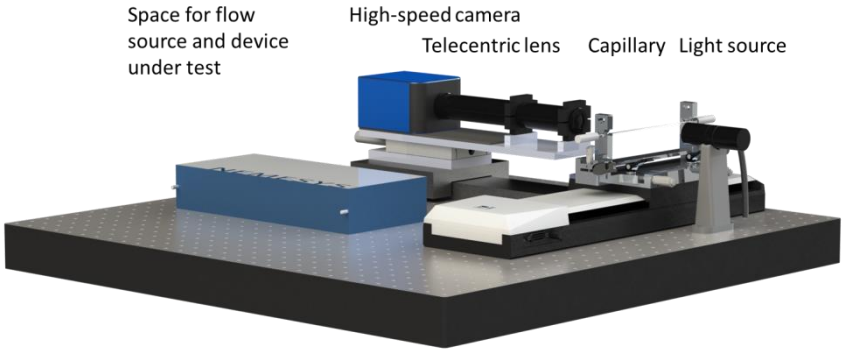


Figure 3.9.1. Camera-Front-Tracking-System. High precision capillary in combination with telecentric lenses, a collimated LED light source and a high-speed camera.

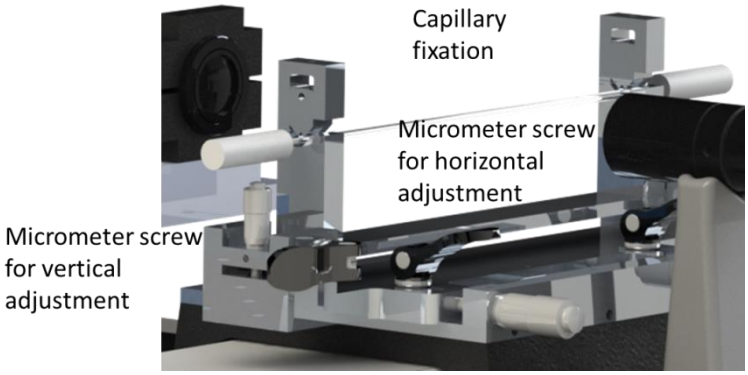


Figure 3.9.2. Adjustable holder for the capillary.

Table 3.9.1

Capillary ID	resolution [nl/px] 5.0L / 4.0L / 2.0L	Volume [µl]
150	0.04 / 0.05 / 0.10	0.05/ 0.07 / 0.13
300	0.17 / 0.21 / 0.42	0.21/ 0.27 / 0.54
600	0.66 / 0.84 / 1.68	0.85/ 1.07 / 2.15
1000	1.84 / 2.33 / 4.66	2.35/ 2.98/ 5.57

3.10 Bronkhorst High-Tech BV

Description of Facility	Description of the overall facility including the calibration method used, how it functions, how it is controlled, Data Acquisition etc. https://www.degruyter.com/view/j/bmte.2015.60.issue-4/bmt-2014-0145/bmt-2014-0145.xml http://www.mfhs2014.uni-freiburg.de/conference/proceedings Pages 165-168
Description of Measurement Principle	Description of the measurement principle behind the facility/method https://www.degruyter.com/view/j/bmte.2015.60.issue-4/bmt-2014-0145/bmt-2014-0145.xml http://www.mfhs2014.uni-freiburg.de/conference/proceedings Pages 165-168 Gravimetric.
Can the Facility be used for Static / Dynamic or Both	Comment on how or if the facility can handle dynamic or transient conditions (fast flow changes) & quantification of the change Under ISO17025 accreditation, only steady state flows can be calibrated. Dynamic or transient conditions & quantification of the change is under investigation, potentially up to 5 Hz.
Facility Flow Ranges	State the maximum and minimum flow ranges that the Facilities can cover https://www.rva.nl/system/scopes/file_ens/000/000/448/original/K127-sce.pdf?1556107221 https://www.bronkhorst.com/getmedia/bee86d4d-d3d5-43f6-8d7a-413cbc0587c2/Bronkhorst-Calibration-Center-960003.pdf?v=1 For liquids: 1 g/h – 30 kg/h under ISO17025 accreditation. < 1 g/h down to 1 mg/h or less under investigation. High flows up to 1000 kg/h.
Temperature / Pressure Ranges	State the temperature/pressure maximum and minimum ranges that the Facilities can cover https://www.bronkhorst.com/getmedia/bee86d4d-d3d5-43f6-8d7a-413cbc0587c2/Bronkhorst-Calibration-Center-960003.pdf?v=1 Pressure: 1 – 9 bar absolute; (for liquids) Temperature: 19 – 23 ºC
Other Fluids	Describe the Primary fluid used and any additional fluids that could be used within the Facilities https://www.bronkhorst.com/getmedia/bee86d4d-d3d5-43f6-8d7a-413cbc0587c2/Bronkhorst-Calibration-Center-960003.pdf?v=1

	Water (only water calibrations under ISO17025 accreditation; other liquids not ISO17025 upon request.
Uncertainty Budget	Brief description of the uncertainty components of the facility/method used and what your calculated uncertainty is for your Facility/method. https://www.degruyter.com/view/j/bmte.2015.60.issue-4/bmt-2014-0145/bmt-2014-0145.xml http://www.mfhs2014.uni-freiburg.de/conference/proceedings Pages 165-168 https://www.rva.nl/system/scopes/file_ens/000/000/448/original/K127-sce.pdf?1556107221 https://www.bronkhorst.com/getmedia/bee86d4d-d3d5-43f6-8d7a-413cbc0587c2/Bronkhorst-Calibration-Center-960003.pdf?v=1 Uncertainty ranges from 0.65% for 1 g/h to 0.1% for 200 g/h up to 30 kg/h.

4 - Uncertainty calculations

4.1 Gravimetric method uncertainty evaluation

Description of facility	Gravimetric facilities for micro-flow calibrations consist of a flow generator (e.g. a piston generator, syringe pump, etc.) and a flow rate reference realized by a scale and a clock enabling to measure a mass of liquid passed in time. Gravimetric micro-flow calibration facilities require special treatment of liquid transport from a tubing connected to a meter under test to a beaker standing on a scale. This can be realized by various methods like: a needle immersed into the liquid in the beaker; liquid bridge between the needle tip and an element causing a capillary rise of the liquid above its level in the beaker, etc. Another effect which needs a special treatment in case of very low flows is evaporation. The facilities use various methods to suppress the evaporation such as: water vapor saturation chambers; oil covers of water surfaces, etc.
Traceability	The method is traceable to: mass, time
Model function	The reference mass flow rate Q_m is given as $$Q_m = \left((Q_w - Q_{Drift}) \cdot f_{b,s} + Q_{Cap}\right) \cdot f_{b,m} \cdot f_{b,t} + Q_{Evap} + Q_{Leak} + Q_T$$ where Q_w ... mass flow rate determined from the raw indication of the scale Q_{Drift} ... apparent indicated mass flow rate due to the scale drift Q_{Cap} ... apparent indicated mass flow rate due to time dependent capillary force between a tube leading water to the beaker and the liquid in the beaker Q_{Evap} ... the evaporation rate of the water in the beaker collecting the water Q_{Leak} ... possible leakage through the tubing or the connectors Q_T ... flow rate correction due to temperature instability during MUT calibration and consequent thermal expansion of tubing $f_{b,s}$ ... air buoyancy correction for conditions during the scale calibration $f_{b,m}$ ... air buoyancy correction for actual conditions during MUT calibration $f_{b,t}$ ... immersed tube buoyancy correction Q_w can be determined as a linear fit of the measured dependency of the indicated mass on time $$Q_w = Linest(m_{ind}, t)$$ where m_{ind} ... scale mass indication t ... time or it can be determined from the initial and final scale readings as $$Q_w = \frac{m_{ind,f} - m_{ind,i}}{t_f - t_i}$$ where $m_{ind,f}$ ... final indication of the scale (corrected by the scale error obtained from the scale calibration) $m_{ind,i}$ ... initial indication of the scale (corrected by the scale error obtained from the scale calibration) t_f ... final time

t_i ... initial time

If the scale is calibrated using weights with density ρ_c and the density of air surrounding the weights during the scale calibration is $\rho_{a,s}$ and the error of the scale is determined by directly comparing the scale indication with the true mass of the weights, i.e. no other corrections to conventional conditions are used, then the following buoyancy correction for scale calibration conditions is used

$$f_{b,s} = 1 - \rho_{a,s}/\rho_c$$

where
ρ_c ... density of calibration weights
$\rho_{a,s}$ ... density of air during the scale calibration

The air buoyancy correction for actual conditions during MUT calibration is determined as

$$f_{b,m} = \frac{1}{1 - \rho_{a,m}/\rho_{w,b}}$$

where
$\rho_{w,b}$... density of water in the beaker during a MUT calibration
$\rho_{a,m}$ ... density of air surrounding the beaker during a MUT calibration

The immersed tube buoyancy correction is applicable for setup with a needle (tube) immersed into water in a beaker such that the length of the immersed part of the needle increases with water level increase in the beaker. It is given as

$$f_{b,t} = 1 - \left(\frac{d_t}{d_b}\right)^2$$

where
d_t ... outer diameter of a tube immersed into the water in the beaker (if used)
d_b ... inner diameter of the beaker (if cylindrical)

The reference volume flow rate Q_V is given as

$$Q_V = \frac{Q_m}{\rho_{w,M}} f_{syringe}$$

where
$\rho_{w,M}$... density of water in a MUT during a MUT calibration
$f_{syringe}$ ... correction factor for thermal expansion of a calibrated syringe

The $f_{syringe}$ factor is applicable for calibrations of syringe pumps only and it is given as

$$f_{syringe} = 1 - \gamma_s(T_s - T_{s0})$$

where
T_{s0} ... working temperature of the calibrated syringe
T_s ... temperature of the calibrated syringe during calibration
γ_s ... thermal expansion coefficient of the material of the calibrated syringe (volumetric or area coefficient depending on setup)

Uncertainty of the indicated mass (m_{ind}, $m_{ind,i}$, $m_{ind,f}$)	
Description of the physical background of the standard uncertainty component	This component is given by the uncertainty of the scale calibration, digit of the scale and repeatability of the scale reading.
Calculation is completed	
The method used for the calculation	$$u(m_{ind}; m_{ind,i};\ m_{ind,f}) = \left[u_{cal}^2 + u_A^2 + \left(\frac{\mathrm{dig}}{2\sqrt{3}}\right)^2\right]^{1/2}$$ where u_{cal} ... standard uncertainty of the scale calibration dig ... digit of the scale u_A ... standard uncertainty of average from repeated readings (if applicable) If the scale is not corrected for its error from the calibration, the error should be included in this uncertainty component.
Type of evaluation	Type A and B
Sensitivity coefficient	For linear fit: Propagation of uncertainties in linear fit to be used to get uncertainty of Q_m from the uncertainties of particular mass and time measurements. For measurement of initial and final mass: $$\frac{\partial Q_m}{\partial m_{ind,f}} = \frac{1}{t_f - t_i} f_{b,s} \cdot f_{b,m} \cdot f_{b,t}$$ $$\frac{\partial Q_m}{\partial m_{ind,i}} = -\frac{1}{t_f - t_i} f_{b,s} \cdot f_{b,m} \cdot f_{b,t}$$
The result obtained (if available)	METAS examples: $u(m_{ind}) = 10\ \mu g$ from calibration certificate IPQ example: see table 4.1.1
Uncertainty of time (t, t_i, t_f)	
Description of the physical background of the standard uncertainty component	The time necessary to deliver an amount of fluid
Calculation is completed	
The method used for the calculation	IPQ: Done, but not final because a time stamp will be soon implemented. For now we are using a chronometer to check the values of the computer.

	$$u(t) = \frac{U(\text{chronometer})}{2}$$
Type of evaluation	B
Sensitivity coefficient	For linear fit: Propagation of uncertainties in linear fit to be used to get uncertainty of Q_m from the uncertainties of particular mass and time measurements. For measurement of initial and final mass and time: $$\frac{\partial Q_m}{\partial t_f} = -\frac{m_{ind,f} - m_{ind,i}}{(t_f - t_i)^2} f_{b,s} \cdot f_{b,m} \cdot f_{b,t}$$ $$\frac{\partial Q_m}{\partial t_i} = \frac{m_{ind,f} - m_{ind,i}}{(t_f - t_i)^2} f_{b,s} \cdot f_{b,m} \cdot f_{b,t}$$
The result obtained (if available)	IPQ example: see table 4.1.1
Uncertainty of the scale drift (Q_{Drift})	
Description of the physical background of the standard uncertainty component	A short time drift of the scale due to the scale heating can be observed. Also a long time drift can appear. If the scale is drifting during the MUT calibration process a fictive flow rate appears in the measurement data which must be corrected or included in the uncertainty budget.
Calculation is completed	
The method used for the calculation	Set a weight on the balance and collect data to determine the mass flow rate. As the mass is constant and the balance reading is corrected for buoyancy correction, the mass flow rate expresses the drift of the balance. The maximum value can be taken for calculating a rectangular distribution of the uncertainty component. The drift per month is determined by repeated calibrations with traceable weights.
Type of evaluation	Type A
Sensitivity coefficient	$$\frac{\partial Q_m}{\partial Q_{Drift}} = f_{b,s} \cdot f_{b,m} \cdot f_{b,t}$$
The result obtained (if available)	METAS example: $u(\Delta m_{drift}) = 17\ \mu g$ per typical measurement time interval; assuming a rectangular distribution
Uncertainty due to instability in the capillary force (Q_{Cap})	
Description of the physical background of the	As the outlet needle is in contact with water in the beaker by means of the water bridge or the needle is immersed into the water in the beaker, there is a capillary force by which the water acts to the needle. The stability and changes of the capillary forces have to be determined since they influence the scale reading.

standard uncertainty component	Immediate value of the apparent flow rate due to capillary force changing during the MUT calibration Q_{Cap} is given as $$Q_{Cap} = \frac{1}{g}\frac{\mathrm{d}F_c^z}{\mathrm{d}t}$$ where g... local gravitational acceleration F_c^z... capillary force by which the liquid acts to the needle (positive when downwards) In case of initial and final reading only it is given as $$Q_{Cap} = \frac{1}{g}\frac{F_{c,f}^z - F_{c,i}^z}{t_f - t_i}$$ where $F_{c,f}^z$ ... the capillary force at end of the measurement $F_{c,i}^z$ ... the capillary force at start of the measurement t_f ... time at end of the measurement t_i ... time at start of the measurement In case of immersed needle setup there can be jumps in the shape of the water-air-needle interface due to sticking of the interface to impurities on the needle (slip-stick effect). If there are repeated jumps as the water surface climbs up along the outlet needle it appears as noise in the scale reading. If the passed volume is very small and the water-air-needle interface is trapped by an impurity during the filling process it can cause a systematic shift in the slope of the scale indication recorded in time. This type of effect should not occur for the setups with "water bridge" (e.g. METAS) where the water level does not climb up along the needle. However, also in the "water bridge" setups other systematic changes of the water-air-needle interface shape during the beaker filling should be taken into account, such as a possible change of the interface curvature due to pressure change in water near the interface during the filling.
Calculation in progress	
The method used for the calculation	One method to determine this contribution is to determine the flow rate stability. However, several contributions are included at the same time: stability of the capillary force, stability of the gravimetric method including the drift of the balance. Another method (probably challenging) would be a direct observation of the evolution of the interface shape during the beaker filling.
Type of evaluation	Type A or B, depending on the type of evaluation
Sensitivity coefficient	$$\frac{\partial Q_m}{\partial Q_{Cap}} = f_{b,m} \cdot f_{b,t}$$
The result obtained (if available)	-

Uncertainty of the densities in the buoyancy corrections $f_{b,m}$ and $f_{b,s}$	
Description of the physical background of the standard uncertainty component	The difference between changing air buoyancy acting on the beaker filled with water and air buoyancy acting on weights during the scale calibration must be taken into account through the correction factors $f_{b,m}$ and $f_{b,s}$ which are defined in the "model function" section. These factors depend on the density of air surrounding the beaker during the MUT calibration $\rho_{a,m}$, density of water in the beaker $\rho_{w,b}$, density of air surrounding the weights during the scale calibration $\rho_{a,s}$ and the density of calibration weights ρ_c.
Calculation is completed	
The method used for the calculation	The temperature, pressure and humidity measurements are used to calculate the density of the air and the water during the MUT calibration. The stabilities of the sensor measurements are also taken into account for the temperature, humidity and pressure stability. Additionally, the gradient of temperature and humidity can be determined along the height of the beaker to calculate the integrated air density. The humidity and the temperature can show a gradient in the weighing zone due to the evaporation trap, the heating of the balance and the holes for equilibration in the weighing housing. The uncertainty of density of water and air is then given by propagation of the uncertainties of temperature, pressure and humidity through the formula for the dependency of density on T, p, RH. For water the density at certain reference conditions should be determined by a measurement in a density calibration laboratory.
Type of evaluation	Type A and B
Sensitivity coefficient	$\frac{\partial Q_m}{\partial \rho_{w,b}} = \left((Q_w - Q_{Drift}) \cdot f_{b,s} + Q_{Cap}\right) \cdot f_{b,m} \cdot f_{b,t} \frac{-\rho_{a,m}/\rho_{w,b}}{\rho_{w,b} - \rho_{a,m}}$ $\frac{\partial Q_m}{\partial \rho_{a,m}} = \left((Q_w - Q_{Drift}) \cdot f_{b,s} + Q_{Cap}\right) \cdot f_{b,m} \cdot f_{b,t} \frac{1}{\rho_{w,b} - \rho_{a,m}}$ $\frac{\partial Q_m}{\partial \rho_c} = (Q_w - Q_{Drift}) \cdot f_{b,m} \cdot f_{b,t} \cdot (\rho_{a,s}/\rho_c^2)$ $\frac{\partial Q_m}{\partial \rho_{a,s}} = (Q_w - Q_{Drift}) \cdot f_{b,m} \cdot f_{b,t} \cdot (-1/\rho_c)$
The result obtained (if available)	METAS example: $u(f_{b,m})$ = 1.5*10^{-6} $u(\rho_{a,m}) = 8.5 \cdot 10^{-4}\ kg/m^3$ from measurements of temperature, humidity and pressure and calculating the integrated air density IPQ example: see table 4.1.1 (for volumetric flow rate)

Uncertainty of the beaker and needle diameters in $f_{b,t}$	
Description of the physical background of the standard uncertainty component	The tip of the outlet needle pushes to water with increasing force as the hydrostatic water pressure increases at the tip with increasing water level in the beaker. The corresponding correction $f_{b,t}$ is defined in the "model function" section and it depends on the needle outer diameter d_t and the beaker inner diameter d_b (in case of cylindrical beaker).

Calculation is completed	
The method used for the calculation	It is sufficient to measure the dimensions e.g. by a caliper and the standard uncertainty of the caliper measurement is then used as the standard uncertainty of d_t and d_b.
Type of evaluation	A and B
Sensitivity coefficient	$\frac{\partial Q_m}{\partial d_t} = \left((Q_w - Q_{Drift}) \cdot f_{b,s} + Q_{Cap}\right) \cdot f_{b,m} \cdot \frac{-2d_t}{d_b^2}$ $\frac{\partial Q_m}{\partial d_b} = \left((Q_w - Q_{Drift}) \cdot f_{b,s} + Q_{Cap}\right) \cdot f_{b,m} \cdot \frac{2d_t^2}{d_b^3}$
The result obtained (if available)	IPQ example: see table 4.1.1
Uncertainty due to evaporation of water from a beaker (Q_{Evap})	
Description of the physical background of the standard uncertainty component	Evaporation of water from the water collection beaker. Either the air has to be saturated with humidity to minimize the evaporation or the water has to be covered by oil to reduce considerably the evaporation. Covering with oil is not possible with the “water bridge” setup.
Calculation is in progress	
The method used for the calculation	METAS: The evaporation rate of the capillary beaker is determined by the gravimetric method. To validate the obtained evaporation rate, the piston prover can generate flow rates between 2000 nL/min and 5 nL/min. The deviation of the piston prover should be constant over the full range in case that the evaporation rate is correct. Another option is to calibrate the piston prover with oil and to collect the oil in the capillary beaker. The evaporation rate can also be determined, but it is expected to be negligibly small for the oil.
Type of evaluation	Type A, B
Sensitivity coefficient	1
The result obtained (if available)	METAS: In progress IPQ: see table 4.1.1
Uncertainty due to leak (Q_{Leak})	
Description of the physical background of the standard uncertainty component	Leaks in tubing can cause that a part of water is lost between the MUT and the scale.
Calculation is in progress	
The method used for the calculation	Close the end of the piping and set a pressure in the tubing. Check the decay time of the pressure curve. This gives an indication for the leak rate. The internal volume has to be estimated.
Type of evaluation	
Sensitivity coefficient	
The result obtained (if available)	

Uncertainty due to thermal expansion of tubing (Q_T)	
Description of the physical background of the standard uncertainty component	The volume in the tubing will expand or shrink due to temperature variations in any tubing. This is causing virtual flow. The lower the flow rate the more important get this contribution. This is as important as evaporation.
Calculation is in progress	
The method used for the calculation	The volume in the tubing is determined. The material of the tubing has to be taken into consideration. Measurements can be performed by isolating the tubing with Aluminum foil and to create several air isolation gaps. Compare stability measurements over the weekend (the stability should be the best) and measurements, where a heat source is disturbing the temperature stability and the temperature gradient stability.
Type of evaluation	Type A
Sensitivity coefficient	1
The result obtained (if available)	In progress

Table 4.1.1. Numerical example of the uncertainty components of the IPQ gravimetric facility

Standard uncertainty component $u(x_i)$	Source of uncertainty	Value of standard uncertainty $u(x_i)$	Distribution	Evaluation	$c_i \equiv \frac{\partial f}{\partial x_i}$	$u_i(V_0) \equiv \lvert c_i \rvert u(x_i)$ (L)	ν_{eff}
$u(m_{ind,f})$	Final mass (g)	2,86E-05	Normal	B	1,06E-03	3,02927E-08	50000
$u(\rho_{w,b})$	Density of the water (g/ml)	6,21E-04	Rectangular	B	-2,60E-04	1,61314E-07	50
$u(\rho_a)$	Density of the air (g/ml)	2,89E-06	Rectangular	B	2,28E-04	6,56797E-10	50000
$u(\rho_c)$	Density of the mass pieces (g/ml)	2,50E-03	Normal	B	4,79E-09	1,19675E-11	50
$u(T)$	Temperature (ºC)	7,02E-01	Normal	B	-2,59E-09	1,81839E-09	50
$u(\gamma_s)$	Expansion coefficient (/ºC)	2,89E-07	Rectangular	B	-5,94E-04	1,71591E-10	50000
$u(m_{ind,i})$	Initial mass (g)	2,86E-05	Normal	B	-1,06E-03	3,02926E-08	50000
$u(Q_{Evap})$	Evaporation (g/ml)	1,47E-08	Rectangular	B	1,00E+00	1,46693E-08	50000
$u(t_f)$	Final time (s)	7,00E-04	Normal	B	2,74E-07	1,91629E-10	50
$u(t_i)$	Initial time (s)	7,00E-04	Normal	B	-2,74E-07	1,91629E-10	50
$u(\delta Q_{mbuoy})$ $\delta Q_{mbuoy} = Q_w(d_t/d_b)^2$	Buoyancy (ml/s)	2,38193E-05	Normal	B	1,06E-03	2,52604E-08	50000
$u(\delta Q_{rep})$	Repeatability (ml/s)	1,22744E-06	Normal	A	1,00E+00	1,22744E-06	24

Flow (ml/s)	0,000259099
Veff	24,91998211
k	2,11
Expanded uncertainty (ml/s)	2,61409E-06
Expanded uncertainty (%)	1,01

References – gravimetric method

[1] H. Bissig *et al.*, *Primary standards for measuring flow rates from 100 nl/min to 1 ml/min – gravimetric principle*, Biomed. Eng.-Biomed. Tech., 2015; 60(4): 301–316

[2] E. Batista, N. Almeida, I. Godinho and E. Filipe, *Uncertainty Calculation in Gravimetric Microflow Measurements*, AMCTM X, 2015, vol 86, pp 98–104 (London: World Scientific)

[3] J.A. Sousa *et al., Method selection to evaluate measurement uncertainty in microflow applications*, 2019 J. Phys.: Conf. Ser. **1379** 012033

[4] EURAMET Calibration Guide No. 18, *Guidelines on the Calibration of Non-Automatic Weighing Instruments*

4.2 Optical method: meniscus tracking uncertainty evaluation

<table>
<tr><td>Description of facility</td><td>Meniscus tracking consists in measuring the displacement as a function of time of a liquid/air or liquid/liquid interface moving inside a glass capillary tube that is connected to a flow generating device. Images of the moving meniscus are acquired by a high-speed camera. A computer program written in Python language is used to measure the flow velocity from the frequency of the signal generator i.e. the frame rate and the interface displacements measured using Digital Image Correlation, and then to deduce the flow rate from the calculated velocity and the previously measured inner diameter of the capillary.</td></tr>
<tr><td>Traceability</td><td>The method is traceable to length through camera calibration and to time through the time stamping, ideally using a frequency transfer standard</td></tr>
<tr><td>Model function</td><td>The liquid flow rate $Q_{volumetric}$ is deduced from the following equation:
$$Q_{volumetric} = \pi \frac{d^2}{4} . v = \pi \frac{d^2}{4} . \frac{\Delta x}{\Delta t}$$
d: inner diameter of the glass capillary tube
v : the displacement velocity of the liquid/liquid or liquid/air interface
Δx : the horizontal displacement of the interface during the time interval Δt

The Model function shows 4 main uncertainty sources, listed in the table below:
<table><tr><th>Uncertainty Source</th></tr><tr><td>Inner Dimensions of Channel/Capillary</td></tr><tr><td>Interface Displacements</td></tr><tr><td>Time Stamp</td></tr><tr><td>Flow Velocity</td></tr></table>
Each uncertainty source is itself composed of several uncertainty components, as listed in the tables below:

For the inner dimensions of the capillary/channel:
<table><tr><th colspan="2">Inner Dimensions of Capillary Uncertainty Components</th></tr><tr><td colspan="2">Camera Resolution</td></tr><tr><td rowspan="3">Camera calibration</td><td>Micrometer Object/Standard</td></tr><tr><td>Position in the focal plane</td></tr><tr><td>Position in the depth of field</td></tr><tr><td colspan="2">Intensity profile</td></tr><tr><td colspan="2">Drift</td></tr><tr><td colspan="2">Temperature influence</td></tr></table>
For the Interface (meniscus, "front") displacements:</td></tr>
</table>

Interface Displacements Uncertainty Components	
Camera Resolution	
Camera Calibration	Micrometer Object
	Position in the focal plane
	Position in the depth of field
Motion plane angle	
Motion blur	
Correlation Function	
Drift	
Temperature influence	

For the Time stamp:

Time Stamp Uncertainty Components
Calibration
Resolution
Exposure time
Drift
Temperature influence

For the Velocity:

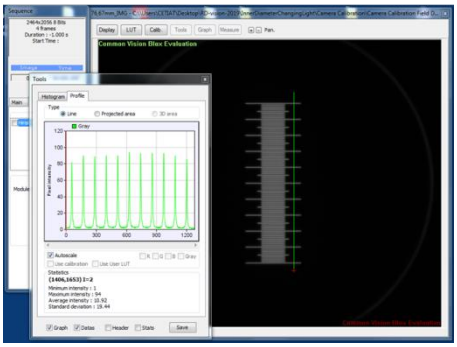
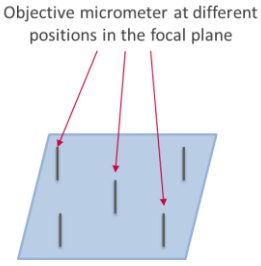
Flow Velocity Uncertainty Components
Method of velocity determination (e,g, linear fit residus)
Thermal Expansion
Evaporation
Interface phenomena: Stick-Slip Effect,..

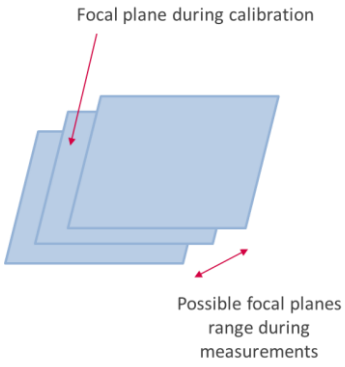
The sensitivity coefficients, as partial derivatives of the model function, are:

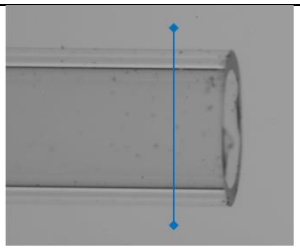
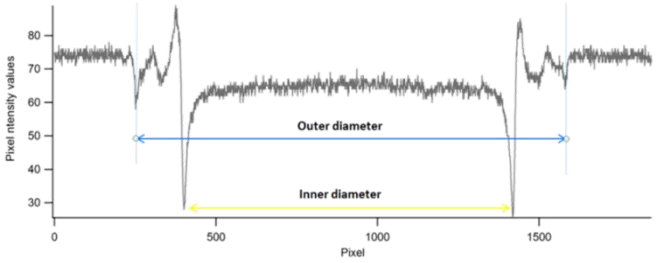
- For the capillary inner dimension (diameter): $\frac{\partial Q}{\partial d} = \frac{\pi d x}{2t}$
- For the interface displacement: $\frac{\partial Q}{\partial x} = \frac{\pi d^2}{4t}$
- For the time: $\frac{\partial Q}{\partial t} = \frac{\pi d^2 x}{4t^2}$

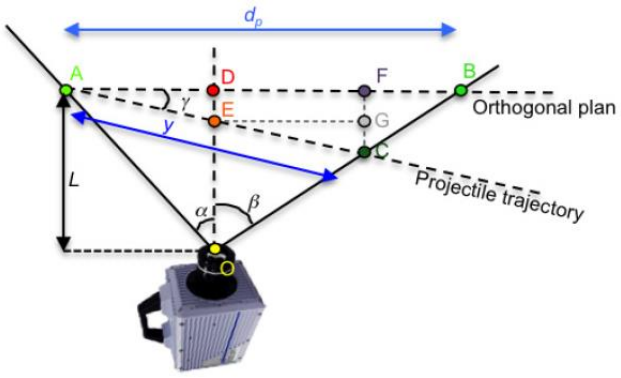
Uncertainty due to camera resolution

Description of the physical background of the standard uncertainty component	Physical limitation due to pixel size of the camera's sensor.
Calculation of the standard uncertainty component is completed or to be done?	Calculation completed
If calculation is completed or in progress	
The method used for the calculation	pixel size at the applied zoom

Type of evaluation	Type B
Sensitivity coefficient	1
The result obtained (if available)	See table 4.2.1 for CETIAT's example.
Uncertainty due to camera calibration	
Description of the physical background of the standard uncertainty component	Uncertainty due to camera calibration, using an objective micrometer (standard traceable to length), which depends on the following components: Component 1: the calibration of the objective micrometer itself (see images below).   Component 2 : the position of the micrometer in the focal plane, as shown below for a micrometer object (standard):  depending on which the pixel size may be more or less affected by optical distortions Component 3 : the position of the micrometer in the depth of field which affects the pixel size if the focal plane in which the micrometer was placed during calibration is different from the one in which the interface moves during measurement (given that both focal planes are included in the depth of field), as illustrated below:

	
Calculation of the standard uncertainty component is completed or to be done?	The calculation of the standard uncertainties corresponding to components 1 and 2 described above is completed. The uncertainty corresponding to the position in the depth of field (component 3) is, for now, assumed to be the same as the one corresponding to the position in the focal plane (component 2). Nevertheless, it will be experimentally evaluated in the future.
If calculation is completed or in progress	
The method used for the calculation	Component 1 : Value given in the calibration certificate (example for CETIAT: LNE, ISO17025 accredited lab) Component 2 : Experimental standard deviation of pixel sizes at 9 different positions of objective micrometer in image Component 3: equal to component 2 for now, to be experimentally evaluated and calculated as described below.
Type of evaluation	Type B for all components
Sensitivity coefficient	1 for all components
The result obtained (if available)	See table 4.2.1 for CETIAT's example.
If the component is not calculated yet	
Proposed way how to calculate the uncertainty component	The uncertainty due to the position in the depth of field (component 3) can be calculated from the standard deviation of pixel sizes measured using the objective micrometer at different distances from the camera but still within the depth of field.
Uncertainty due to intensity profile	
Description of the physical background of the standard uncertainty component	Uncertainty due to the extraction of information (mainly lengths and distances) from the pixel intensity profile and which can be affected by: - Camera resolution - Method used to extract information e.g. calculation of a pixel threshold value to detect the capillary tube's edges for the measurement of its inner diameter The following picture illustrate the intensity profile extraction from an image to determine outer and inner diameters of a capillary:

	 
Calculation of the standard uncertainty component is completed or to be done?	Standard uncertainty calculation completed
If calculation is completed or in progress	
The method used for the calculation	Method described in N. Pousset's paper about Objective Micrometer calibration. See the following article for a description of the method: Pousset, Nicolas & Salgado, José (2009). Calibration of line scale standards by optical microscopy.
Type of evaluation	Type B
Sensitivity coefficient	1
The result obtained (if available)	See table 4.2.1 for CETIAT's example.
Uncertainty due to motion plane angle	
Description of the physical background of the standard uncertainty component	Uncertainty due to object motion outside of the plane perpendicular to the camera
Calculation of the standard uncertainty component is completed or to be done?	Calculation completed
If calculation is completed or in progress	

The method used for the calculation	Deduced as y from equation (7) of article : https://www.metrology-journal.org/articles/ijmqe/abs/2014/02/ijmqe140007/ijmqe140007.html; $y = \frac{d_p}{\cos\gamma + \tan\beta \sin\gamma}$. as The figure below illustrates the motion plane angle calculation variables:  Note: The angle is taken as the step of the CETIAT's current system's rotary stage in CETIAT's example.
Type of evaluation	Type B
Sensitivity coefficient	1
The result obtained (if available)	See table 4.2.1 for CETIAT's example.
Uncertainty due to motion blur	
Description of the physical background of the standard uncertainty component	Interface motion during exposure time, see https://www.metrology-journal.org/articles/ijmqe/abs/2014/02/ijmqe140007/ijmqe140007.html
Calculation of the standard uncertainty component is completed or to be done?	Calculation completed
If calculation is completed or in progress	
The method used for the calculation	Distance D travelled by the interface during exposure time: $D = v.t_{exposure}$ *V*: Interface displacement velocity $t_{exposure}$: exposure time
Type of evaluation	Type B
Sensitivity coefficient	1
The result obtained (if available)	See table 4.2.1 for CETIAT's example.
Uncertainty corresponding to the correlation function	
Description of the physical background of the standard uncertainty component	Uncertainty corresponding to the resolution of the correlation function (used for distance measurements) and errors in image matching due to possible changes in measurement conditions (e.g. changes in light conditions).

Calculation of the standard uncertainty component is completed or to be done?	Calculation completed
If calculation is completed or in progress	
The method used for the calculation	Assumed, for now, to be equal to the resolution of the correlation function i.e. the pixel size.
Type of evaluation	Type B
Sensitivity coefficient	1
The result obtained (if available)	See table 4.2.1 for CETIAT's example.
Uncertainty due to timestamp	
Description of the physical background of the standard uncertainty component	Uncertainty associated with the recording of the images' timestamps which includes different components: Component 1: Accuracy of the reference frequency device used to timestamp images. Component 2: Timestamp resolution. Component 3: Exposure time.
Calculation of the standard uncertainty component is completed or to be done?	Calculation completed
If calculation is completed or in progress	
The method used for the calculation	Component 1: Accuracy specified by the characteristics of the frequency reference device (CETIAT's Rubidium atomic clock). Component 2: Resolution at 10^{-9} s, Component 3: Exposure time equal to 1/frame rate.
Type of evaluation	Type B
Sensitivity coefficient	1
The result obtained (if available)	See table 4.2.1 for CETIAT's example.
Uncertainty due to thermal expansion	
Description of the physical background of the standard uncertainty component	Possible displacement of the liquid/air or liquid/liquid interface due, in addition to the liquid flow, to the thermal expansion caused by temperature fluctuations.
Calculation of the standard uncertainty component is completed or to be done?	Theoretical calculation completed Experimental calculation to be carried out in the future.
If calculation is completed or in progress	
The method used for the calculation	Theoretical value determined from the ratio of water density values for 1°C temperature variation, multiplied by flow rate
Type of evaluation	Type B
Sensitivity coefficient	1
The result obtained (if available)	See table 4.2.1 for CETIAT's example.

If the component is not calculated yet	
Proposed way how to calculate the uncertainty component	The component will be experimentally evaluated by measurement of the displacement of an interface, inside the capillary tube, as a function of temperature changes generated along the capillary.
Uncertainty due to evaporation	
Description of the physical background of the standard uncertainty component	Possible displacement of the liquid/air interface due, in addition to the liquid flow, to the liquid evaporation near the meniscus.
Calculation of the standard uncertainty component is completed or to be done?	Theoretical calculation completed Experimental calculation to be evaluated in the future
If calculation is completed or in progress	
The method used for the calculation	Evaporation rate measured from equation given in : Enginneringtoolbox.com Water surface evaporation calculator for d=100µm , xs=0,014659 , x=0,0098 , hwe=2454 for v=0m/s
Type of evaluation	Type B
Sensitivity coefficient	1
The result obtained (if available)	See table 4.2.1 for CETIAT's example.
If the component is not calculated yet	
Proposed way how to calculate the uncertainty component	The component will be experimentally evaluated by measurement of the evaporation rate from the retreat of a water/air interface inside the capillary tube as a function of time

Table 4.2.1. CETIAT's Numerical example of the uncertainty components for **1 second** flow measurement at 1nl/min (lowest flow rate and highest uncertainty)

Standard uncertainty component	Source of uncertainty		Value of standard uncertainty $u(x_i)$	Distribution	Evaluation	$c_i \equiv \frac{\partial f}{\partial x_i}$	$u_i(V_0) \equiv \lvert c_i \rvert u(x_i)$ (L)	ν_{eff}
Inner dimensions of the glass capillary tube	Camera resolution		2,89E-08	Uniform	Type B	1	2,89E-08	2
	Camera calibration	Objective Micrometer	7,50E-08	Normal	Type B		7,50E-08	
		Position in the focal plane	3,30E-11	Uniform	Type B		3,30E-11	
		Position in the depth of field	3,30E-11	Uniform	Type B		3,30E-11	
	Intensity profile		2,89E-08	Uniform	Type B		2,89E-08	
	/		**8,54E-08**	/	/	**3,33E-10**	**2,85E-17**	
Interface displacement	Camera resolution		2,89E-08	Uniform	Type B	1	2,89E-08	300
	Camera calibration	Objective Micrometer	7,50E-08	Normal	Type B		7,50E-08	
		Position in the focal plane	3,30E-11	Uniform	Type B		3,30E-11	
		Position in the depth of field	3,30E-11	Uniform	Type B		3,30E-11	
	Motion plane angle		4,73E-11	Uniform	Type B		4,73E-11	
	Motion blur		4,08E-09	Uniform	Type B		4,08E-09	
	Correlation function		5,77E-08	Uniform	Type B		5,77E-08	
	/		**9,90E-08**	/	/	**7,85E-09**	**7,78E-16**	
Time Stamp	Calibration		1,67E-09	Normal	Type B	1	1,67E-09	300
	Resolution		9,62E-04	Uniform	Type B		9,62E-04	
	Exposure time		1,92E-03	Uniform	Type B		1,92E-03	
	/		**2,15E-03**	/	/	**-1,67E-14**	**-3,59E-17**	
Flow Velocity	Thermal expansion		1,92E-18	Uniform	Type B	1	1,92E-18	150
	Evaporation		2,10E-16	Uniform	Type B		2,10E-16	
	/		**2,10E-16**	/	/	**1**	**2,10E-16**	

Combined Uncertainty (nL/min)	4,84E-02	*Expanded uncertainty*	**9,68%**
Veff	/	*k*	2

4.3 Optical method: pendant drop uncertainty evaluation

Description of facility	The grow of a drop is monotonized by taken a photo at a specific time. The image of the drop is then treated with Python software allowing the volume determination of the drop volume per unit of time, this permits flow determination. The main standard uncertainties considered are: volume (*d*), time (*t*) and, standard deviation of the measurements (δQ_{rep})
Traceability	The method is traceable to: length
Model function	$$(Q) = \frac{V}{t}$$ $$V = \sum_{i=1}^{n} \pi \times r^2 \times h$$
Uncertainty of volume V	
Description of the physical background of the standard uncertainty component	The volume is calculated by the sum of cylindrical portions with a height of one pixel, considering that the droplet is symmetrical about its axis of revolution.
Calculation of the standard uncertainty component is completed or to be done?	Completed
If calculation is completed	
The method used for the calculation	$$u_v = \sqrt{\left(\frac{u_{scale}}{2}\right)^2 + \left(\frac{u_{focus} \times V}{\sqrt{3}}\right)^2 + \left(\frac{u_{contour} \times V}{\sqrt{3}}\right)^2}$$ - Uncertainty associated with the scale calibration (u_{scale}) using the tube diameter, determined by interferometry, with the value of 0.01 mm, converted into volume to 0.000001 mm^3. - Uncertainty associated with focus (u_{focus}), with the value of 0.01%. - Uncertainty associated with the contour determination method ($u_{contour}$), with the value of 0.005%.
Type of evaluation	Type B evaluation
Sensitivity coefficient	$$\left(\frac{\partial Q}{\partial V}\right) = \frac{1}{t}$$
The result obtained (if available)	See table 4.3.1 for IPQ's example

Uncertainty of time	
Description of the physical background of the standard uncertainty component	The time of the growth of the drop
Calculation of the standard uncertainty component is completed or to be done?	Done, but not final because a time stamp will be soon implemented. For now we are using a chronometer to check the values of the computer.
If calculation is completed or in progress	
The method used for the calculation	$u_t = \sqrt{\left(\frac{u_{crono}}{2}\right)^2 + \left(\frac{u_{delay}}{\sqrt{3}}\right)^2}$ (s) - Chronometer uncertainty (u_{crono}), with the value of 0.0014s. - Uncertainty associated with time delay (u_{delay}), with the value of 0.01s.
Type of evaluation	B
Sensitivity coefficient	$\left(\frac{\partial Q}{\partial t}\right) = -\frac{V}{t^2}$
The result obtained (if available)	See table 4.3.1 for IPQ's example
Uncertainty due to evaporation	
Description of the physical background of the standard uncertainty component	To calculate the uncertainty associated with evaporation the standard deviation of the average value of the measured evaporation flow Q_{evap} is used.
Calculation of the standard uncertainty component is completed or to be done?	Done
If calculation is completed or in progress	
The method used for the calculation	$u_{evap} = \frac{s\,(Qevap)}{\sqrt{n}}$ (µL/s)
Type of evaluation	A
Sensitivity coefficient	$\left(\frac{\partial Q}{\partial evap}\right) = 1$
The result obtained (if available)	See table 4.3.1 for IPQ's example

Uncertainty due to repeatability	
Description of the physical background of the standard uncertainty component	Standard deviation of the mean flow
Calculation of the standard uncertainty component is completed or to be done?	Done
If calculation is completed or in progress	
The method used for the calculation	$u(rep) = \frac{SDm}{\sqrt{n}}$ [µL/s]
Type of evaluation	A
Sensitivity coefficient	$\left(\frac{\partial Q}{\partial rep}\right) = 1$
The result obtained (if available)	See table 4.3.1 for IPQ's example

Table 4.3.1 - Example of the drop method for 1 mL/h

Standard uncertainty component $u(x_i)$	**Source of uncertainty**	**Value of standard uncertainty $u(x_i)$**	**Distribution**	**Evaluation**	$c_i \equiv \frac{\partial f}{\partial x_i}$	$u_i(V_0) \equiv \lvert c_i \rvert u(x_i)$ (L)	ν_{eff}
$u(V)$	Volume (mm^3)	9,53E-04	B	Normal	0,0204	0,004409	50000,00
$u(t)$	Time (s)	5,05E-03	B	Normal	-0,0061	3,1E-05	50,00
$u(evap)$	Evaporação (µL/s)	-2,21E-04	A	Normal	1,0000	0,000221	50000,00
$u(rep)$	Repeatability (µL/s)	0,000002	A	Normal	1	2,31773E-06	5,820E+02

Flow (ml/s)	0,300973
Veff	128
k	2,03
Expanded uncertainty (ml/s)	6,8126E-06
Expanded uncertainty (%)	4,6

References – optical methods

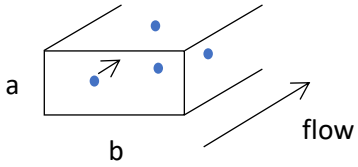
[1] N. Pousset, Nicolas & Salgado, José (2009). Calibration of line scale standards by optical microscopy, https://www.researchgate.net/publication/43107674_Etalonnage_de_micrometres_objets_par_microscopie_optique

[2] C. Robbe, N. Nsiampa, A. Oukara, A. Papy, Quantification of the uncertainties of high-speed camera measurements, Int. J. Metrol. Qual. Eng.Volume 5, Number 2, 2014, https://doi.org/10.1051/ijmqe/2014007

[3] F. Ogheard, P. Cassette, A.W. Boudaoud, Development of an optical measurement method for "sampled" micro-volumes and nano-flow rates, Flow Measurement and Instrumentation, Volume 73, June 2020, https://doi.org/10.1016/j.flowmeasinst.2020.101746

[4] EURAMET Calibration Guide No. 18, *Guidelines on the Calibration of Non-Automatic Weighing Instruments*

4.4 MicroPIV/MicroPTV method uncertainty evaluation

Description of facility	Flow rate in a micro-channel is determined based on observation of velocity and position of tracer particles dragged by the flow. For a large number of particles this is referred to as partial image velocimetry and for few particles as partical tracking velocimetry.
Traceability	The MicroPIV/MicroPTV method is traceable by the exact position of a tracer particle and the time stamp on the individual pictures recorded.
Model function	Channels featuring rectangular cross section are used.  Volumetric flow can be calculated according to $Q_{\mathrm{vol}} = v * a * b = \frac{x}{t} * a * b.$ Particles in the volumetric flow are recorded by a camera featuring a magnification lens with magnification *M*. The formula for Q_{vol} must thus be extended to $Q_{\mathrm{vol}} = v * a * b = \frac{1}{M} * \frac{x}{t} * a * b.$
Uncertainty due to a camera time stamp	
Description of the physical background of the standard uncertainty component	Variation of time stamp lead to an error in volumetric flow equation
Calculation of the standard uncertainty component is completed or to be done?	Completed
If calculation is completed or in progress	
The method used for the calculation	A high-speed camera is used which is capable of recording up to 200.000 frames/second. Although not mentioned in the data sheet it can be expected that the time stamp error for such a camera is in the femto second range.
Type of evaluation	Type B
Sensitivity coefficient	Q_{vol}/t
The result obtained (if available)	Can be neglected
Uncertainty due to an effective resolution of the optical setup	
Description of the physical background of the standard uncertainty component	Magnification of lens and pixel size of sensor result in an effective maximum resolution of setup.

Calculation of the standard uncertainty component is completed or to be done?	Completed
If calculation is completed or in progress	
The method used for the calculation	Effective resolution in µm/pxl. is calculated via the magnification of the lens which amounts to 10 x. The pixel size of the sensor amounts to 8 µm x 8 µm, Effective resolution amounts to 0.8 µm/pxl.
Type of evaluation	Type B
Sensitivity coefficient	Q_{vol}/x
The result obtained (if available)	Resulting uncertainty is ½ pxl and therefore 400 nm.
Uncertainty due to angular deviation of camera and substrate from 90°	
Description of the physical background of the standard uncertainty component	Beads appear to have travelled a shorter distance than they actually have if plane and camera are not perpendicular.
Calculation of the standard uncertainty component is completed or to be done?	Completed
If calculation is completed or in progress	
The method used for the calculation	Assumed error: 3° $x_{\mathrm{measured}} = x_{\mathrm{real}} * \cos(3°)$ $x_{\mathrm{measured}} = x_{\mathrm{real}} * 0.998$ Typical travel length between two pictures: 10 µm $x_{\mathrm{measured}} = 10\ \mu\mathrm{m} * 0.998 = 9.98\ \mu\mathrm{m}$ Maximum error for 1000 µm field of view therefore amounts to 2 µm.
Type of evaluation	Type B
Sensitivity coefficient	Q_{vol}/x
The result obtained (if available)	Error ranges from some nanometers to 2 micrometers
Uncertainty of peak locking	
Description of the physical background of the standard uncertainty component	Peak locking plays a role when the size of the particles is in the range of the pixel size of the used camera. For a pixel size of 8 µm and a magnification optic of 10 x this becomes relevant for particles of about 1 µm. During analysis, the particle is shifted toward the closest integer pixel [1].

	Peak locking can be avoided for particles which are at least two pixels in size. (see also [2])
Calculation of the standard uncertainty component is completed or to be done?	Completed
If calculation is completed or in progress	
The method used for the calculation	
Type of evaluation	B
Sensitivity coefficient	Q_{vol}/x
The result obtained (if available)	It can be approximated that peak locking will not play a role since particles cover about 10 pixels.
Uncertainty due to motion blur	
Description of the physical background of the standard uncertainty component	Long exposure times can lead to image blur and thus lead to an error in detection of the particle's position.
Calculation of the standard uncertainty component is completed or to be done?	Completed
If calculation is completed or in progress	
The method used for the calculation	Propagation length (d) of a bead during exposure time (t_{exposure}) can be calculated according to $$d = t_{\mathrm{exposure}} * v_{\mathrm{bead}}$$
Type of evaluation	B
Sensitivity coefficient	Q_{vol}/x
The result obtained (if available)	Assuming an exposure time of 30 µs and a traversing speed of 20 µm/s, the propagation length can be calculated to $$d = 30\ \mu\mathrm{s} * 60\ \frac{\mu\mathrm{m}}{\mathrm{s}} = 1800 * 10^{-12}\ \mathrm{m} = 1.8\ \mathrm{nm}$$ The resulting error is three orders of magnitude below the resolution limit.
Uncertainty due to image distortion	
Description of the physical background of the standard uncertainty component	Due to image distortion, particles can appear to move faster (barrel distortion) or slower (pincushion distortion), especially at the edges of the field of view.
Calculation of the standard uncertainty component is completed or to be done?	Completed

If calculation is completed or in progress	
The method used for the calculation	The maximum field curvature of the telecentric lens that will be used amounts to 0.07 % barrel distortion at the edge of the frame. Distortion in the mainly used middle of the frame is zero according to datasheet.
Type of evaluation	B
Sensitivity coefficient	-
The result obtained (if available)	-
Uncertainty due to random Brownian motion	
Description of the physical background of the standard uncertainty component	Random deviations in the velocity field can have their origin in Brownian motion. see e.g. [3]
Calculation of the standard uncertainty component is completed or to be done?	-
If calculation is completed or in progress	
The method used for the calculation	Can be neglected for particles featuring d > 1 µm
Type of evaluation	Type B
Sensitivity coefficient	
The result obtained (if available)	
Uncertainty due to channel fabrication errors	
Description of the physical background of the standard uncertainty component	Variation in channel height and width lead to a linear error in volumetric flow.
Calculation of the standard uncertainty component is completed or to be done?	Fabrication errors in both directions (a and b) are estimated to be ±5 µm.
If calculation is completed or in progress	
The method used for the calculation	Fabrication error
Type of evaluation	B
Sensitivity coefficient	Q_{vol}/a or Q_{vol}/b
The result obtained (if available)	±5 µm estimated

Uncertainty related to spatial resolution of flow velocity field due to tracer particle size	
Description of the physical background of the standard uncertainty component	Flow velocity inside a microPIV channel is not constant. There is the velocity decrease from a maximum in a center of the channel to zero at a wall. In case of larger tracer particles it might happen that the flow velocity field variation across the particle diameter is significant. In that case a question arises what fluid flow velocity should be ascribed to a particle if the velocity and position of the particle is known.
Calculation of the standard uncertainty component is completed or to be done?	to be done
If the component is not calculated yet	
Proposed way how to calculate the uncertainty component	Analytical approach could work here based on the paper of Maxey and Riley [4].

Since no substantial uncertainty for *t* was found and the uncertainty sources are independent from each other, the total uncertainty can be expressed by

$$\frac{\Delta Q}{Q} = \sqrt{\left(\frac{\sum \Delta x}{x}\right)^2 + \left(\frac{\Delta a}{a}\right)^2 + \left(\frac{\Delta b}{b}\right)^2} = \sqrt{\left(\frac{2.5\ \mu m}{x}\right)^2 + \left(\frac{5\ \mu m}{a}\right)^2 + \left(\frac{5\ \mu m}{b}\right)^2}$$

and consequently

$$\Delta Q = Q * \sqrt{\left(\frac{2.5\ \mu m}{x}\right)^2 + \left(\frac{5\ \mu m}{a}\right)^2 + \left(\frac{5\ \mu m}{b}\right)^2}.$$

References – microPIV and microPTV method

[1] A. Sciacchitano, Meas. Sci. Technol., 2019, 30 092001

[2] S. Scharnowski and C.J. Kähler, *Particle image velocimetry - Classical operating rules from today's perspective, Optics and Lasers in Engineering*, https://doi.org/10.1016/j.optlaseng.2020.106185

[3] 10.1146/annurev-fluid-121108-145427

[4] M. R. Maxey, J. J. Riley, *Equation of motion for a small rigid sphere in a nonuniform flow*, Phys. Fluids **26** (4), 1983

4.5 Displacement method uncertainty evaluation

Description of facility	The displacement method uses a device to monitor the distance travelled by a pusher block of a syringe pump in order to determine the flow rate. Knowing the internal diameter of the syringe with very high precision, the travelled distance, and the time needed for that travelled distance, it was possible to calculate the flow rate of the fluid inside the syringe and its uncertainty. A device that can be used to determine the travel distance of the pusher block is an interferometer. The movement of the piston can also be controlled by the motor motion and the high precision linear stage. Either a Linear measuring system or the motor encoder signal can be used to monitor the position of the piston plunger in time. The piston plunger is fixed in the axis of the piston. However, the linear measuring system is fixed on the side of the moving stage, which induces additional possible measurement uncertainty components. The motor encoder is in the axis of the motion, but under the horizontal line of the piston due to construction purposes. The Abbé offset must be taken into account. Pitch motion due to forces are possible, even if the mountings are stiff and very strongly fixed. Between the motor and the linear stage is often also a gear, which induces more fluctuations in the motion of the stage than the continuous and smooth motion of the motor. Some syringes have an incremental encoder output. For example, CETONI syringe pump (Nemesys) the control system has 2048 pulses/edges per rotation. Taking spindle pitch and gear ratio into account, the Nemesys pumps can resolve a 1 mm travel distance into approx. 59400 encoder increments. The max. travel of the module is approx. 64 mm. The volume of the syringe we use is in any case distributed over 60 mm, which then corresponds to approx. 3.5 million increments. The main standard uncertainties considered with the displacement method are: distance (d), time (t), radius of the piston (r), stability of the motion (δQ_{sta}), heating effects of the motion of the piston plunger (δQ_{heat}), standard deviation of the measurements (δQ_{rep})
Traceability	The method is traceable to: length and time
Model function	$Q = \frac{d\pi r^2}{t} \cdot f_{stab\ motion} \cdot f_{heating\ effects}$
Uncertainty of travel distance *d* from linear measuring system or from motor encoder signal	
Description of the physical background of the standard uncertainty component	Travel distance of the piston plunger, which is measured by the linear measuring system at the border of the moving stage or by interferometer. Influence factors like the Abbé offsets such as pitch yaw and roll must be taken into account, also linear error and angular error can be considered.

Calculation of the standard uncertainty component is completed or to be done?	Investigations of the motion and the position of the linear stage with respect to the position of the piston plunger. Add a prism at the position of the piston plunger to measure the offset, the repeatability, the torsion of the stage, the reproducibility of the position with respect to the signal from the linear measuring system. Check whether a hysteresis in the motion back and forth is observed and the positions are reproducible In case of the signal from the motor encoder, all these influences have also to be determined with the prism at the position of the plunger position. If the piston is in position and under use the forces on the mountings can induce bending (pitch). This stability must be investigated somehow as we talk about some micrometers. Of course, we can assume that under constant flow the pressure on the mounting is constant and the force is in equilibrium. One option is to test the delivered volume with the gravimetric method and compare the volumes obtained. If the encoder signal is used, the same tests must be performed and the uncertainty from the gear in motion has to be taken into account. For the interferometer the uncertainty is completed.
If calculation is completed	
The method used for the calculation	For the interferometer: $u(d) = \sqrt{u_A^2 + (u_L^2 d^2)}\ \ [mm];$ d has been assumed as the travel distance of the syringe, u_A is the uncertainty of the calibration of the interferometer and u_L is the uncertainty of the distance
Type of evaluation	Type B evaluation
Sensitivity coefficient	$\frac{\partial Q}{\partial d} = \frac{\pi r^2}{t}$
The result obtained (if available)	See table 4.5.1
Uncertainty of time	
Description of the physical background of the standard uncertainty component	The time of the traveled distance
Calculation of the standard uncertainty component is completed or to be done?	Time stamps are set by the internal clock of the FPGA, which collects the pulses of the motion. The internal clock can be calibrated with a 40 MHz signal. Therefore, this uncertainty contribution is negligible. It is also important to check if the data acquisition works without a delay for all parameters (real time system), in case some values are recorded delayed the measurement accuracy is reduced. Another possible but not so accurate solution is to use chronometer to check the values of the computer.

If calculation is completed or in progress	
The method used for the calculation	Calibrate the time clock of the FPGA with the 40 MHz signal In case a chronometer is used: $$u(t) = \frac{Uchron}{2}[s]$$
Type of evaluation	B
Sensitivity coefficient	$$\frac{\partial Q}{\partial t} = -\frac{\pi r^2 d}{t^2}$$
The result obtained (if available)	See table 4, negligible
Uncertainty of inner radius of the piston (syringe) and its variation over the length of the measuring distance	
Description of the physical background of the standard uncertainty component	The inner radius of the piston is used to convert speed into volume flow rate. An average inner radius is taken for this calculation. The deviation to a round cross section and the variation of the inner radius over the length of the measuring distance must be determined.
Calculation of the standard uncertainty component is completed or to be done?	Length department: tactile measurement or µCT measurements A caliper may be used, but this method is not recommended because it has a large uncertainty. The gravimetric method can also be used to determine the volume of the piston and therefore the internal radius can be obtained.
If calculation is completed or in progress	
The method used for the calculation	In case a calliper is used $$u(r) = \frac{U_{calliper}}{2}[mm]$$
Type of evaluation	B
Sensitivity coefficient	$$\frac{\partial Q}{\partial r} = \frac{2d}{t}\pi r$$
The result obtained (if available)	See table 4 Under investigation. Variation of the inner diameter along the travel distance is negligible with respect to the measurement uncertainty of the inner radius.
Uncertainty due to repeatability	
Description of the physical background of the standard uncertainty	Standard deviation of the mean flow

component	
Calculation of the standard uncertainty component is completed or to be done?	Done
If calculation is completed or in progress	
The method used for the calculation	$u(rep) = \frac{SDm}{\sqrt{n}}$ [µL/s]
Type of evaluation	A
Sensitivity coefficient	$\left(\frac{\partial Q}{\partial rep}\right) = 1$
The result obtained (if available)	See table 4
Uncertainty due to instability of the motion	
Description of the physical background of the standard uncertainty component	The travel distance *d* is often calibrated at rest. The stability of the motion must be determined. One option is to determine the stability of the flow rate with the gravimetric method. However, temperature stability must be guaranteed not to induce additional fluctuations.
Calculation of the standard uncertainty component is completed or to be done?	Gravimetric method to determine flow rate stability and determine the stability of the motion. Only investigate the differences between the speed and the flow rate determined gravimetrically. Relative stability is important because it will determine the fluctuations of the deviations. For the interferometer it's possible to measure this uncertainty component by taking the travel results under a stationary stage of the motor.
If calculation is completed	
The method used for the calculation	For the interferometer $u(stab) = \frac{SDs}{\sqrt{n}}$ [µL/s]
Type of evaluation	Type B evaluation
Sensitivity coefficient	$\left(\frac{\partial Q}{\partial stab}\right) = 1$
The result obtained (if available)	See table 4, Under investigation
Uncertainty due to heating effects of the motion of the piston plunger	

Description of the physical background of the standard uncertainty component	The motion of the piston plunger can induce heating effects due to friction. We have seen this with piston made of stainless steel and industrial sealing. We never had this problem with glass piston and PTFE sealings.
Calculation of the standard uncertainty component is completed or to be done?	Determine temperature variations that could induce virtual flows and volume expansion.
If calculation is completed	
The method used for the calculation	
Type of evaluation	Type B evaluation
Sensitivity coefficient	
The result obtained (if available)	Under investigation
Uncertainty due to leakage of the sealing	
Description of the physical background of the standard uncertainty component	Depending on the sealing on the piston plunger, leakage could occur at high pressures
Calculation of the standard uncertainty component is completed or to be done?	We don't know yet how to calibrate this. Measurement are calibrated with the gravimetric method. Maybe it can be perform a static high-pressure test over a longer time. The (possible) pressure loss could give you an idea about the (possible) leakage.

Table 4.5.1. Example for the interferometer method 0.001 mL/h (16nL/min)

Standard uncertainty component $u(x_i)$	**Source of uncertainty**	**Value of standard uncertainty $u(x_i)$**	**Distribution**	**Evaluation**	$c_i \equiv \frac{\partial f}{\partial x_i}$	$u_i(V_0) \equiv \lvert c_i \rvert u(x_i)$ (L)	ν_{eff}
$u(d)$	Distance (mm)	0.000185	B	Normal	0.000950202	1.75869E-07	5.000E+07
$u(t)$	Time (s)	0.000700	B	Normal	-1.7184E-08	1.20288E-11	5.000E+01
$u(r)$	Radius (mm)	0.015000	B	Normal	0.000261347	3.9202E-06	5.000E+01
$u(rep)$	Repeatability (µL/s)	0.000002	A	Normal	1	2.31773E-06	5.820E+02
$u(stab)$	Stability (µL/s)	2.104E-08	A	Normal	1	2.10384E-08	5.820E+02

Flow (ml/s)	0.000301
Veff	90
k	2.03
Expanded uncertainty (ml/s)	0.0000092
Expanded uncertainty (%)	3.1