WHITE PAPER ON MICRO AND NANOFLOW RATE MEASUREMENT

Defining the regions (1 – 1000) µl/min as microflow and (1 – 1000) nl/min as nanoflow, this white paper describes various principles for a primary realization of the measurement. In this document we briefly describe some common principles for flow rate calibrations. Typically, the gravimetric approach is used for microflow rates. For nanoflow rates potential methods are based on volumetric expansion and optic measurement of the dispensed volume. These three principles are discussed in the following sections.

GRAVIMETRIC MEASUREMENT OF DISPENSED MASS OVER TIME

THE BASIC PRINCIPLE

A reference for flow is often the gravimetric set-up. A gravimetrical setup requires a scale, a measuring beaker, a flow-generator, a water reservoir and connections between them. Gravimetric calibration of water mass flow is determined by measuring the delivered or removed mass from a vessel during a measured time period:

$$Q = \frac{\Delta m}{\Delta t}$$

Where *Q* is mass flow, *m* is mass and *t* is time. For volume flow rates the density of the liquid has to be included. Typically the flow exits a submerged needle because otherwise droplets can occur that will give significant errors. To be able to measure in the micro range, it is first of all necessary to limit the evaporation. At a flow rate of, say 1 µl/min, the evaporation can, if left untreated, be more than a hundred times higher than the flow rate. Furthermore, the following measures are recommended:

- To get a stable foundation, the balance should be placed upon a vibration-free base.
- Demineralized/ distilled and degassed water should be used to avoid entrapped and dissolved air. Travelling air bubbles will greatly affect the measurement or can (partly) block the flow.
- The system should be sufficiently primed in order to remove entrapped or dissolved air. A good measure for a fully degassed system is a quick responds of a flow meter when the flow is started or halted.
- One should use tubing that has no porosity, e.g. stainless steel tubing.
- The dispensing needle should be submerged in the liquid in order to avoid droplets formation. This, however, requires that a buoyancy correction for the immersed needled needs to be applied.
- The measurement beaker should be placed inside a draft shielded environment. For example, place the measurement beaker inside a small chamber.

UNCERTAINTY SOURCES

The **environment** of the calibration setup should be controlled or at least the values recorded in order to correct for any considerable change in the environmental conditions. The following parameters can cause errors in the mass

determination: temperature, humidity, pressure, air density, spatial or temporal environmental gradients, drafts and vibrations (if any). Electrostatic and RF noise may be relevant in case the 'device under test' is sensitive to it.

The **medium (calibration liquid)** is typically (pure) water, but can also be other fluids. The medium density and purity is important. The fluid temperature should also be considered as it affects the density and viscosity of the liquid.
The fluid density is required to determine the buoyancy (correction) as well as to convert from mass flow to volume flow. If the medium is not ultrapure water, it is a good practice to measure the density of the liquid.
A temperature difference between the water and the surrounding air will also produce a convection draft from warmer towards colder. Furthermore, a temporal temperature gradient will generate a flow rate and can be significant if very low flow rates are studied.

The **mass measurement** using weighing cells or microbalances have beside a calibration uncertainty further uncertainties from the resolution, nonlinearity, repeatability, reproducibility, eccentricity, zero point stability, response time, density of the mass standards and lag and drift since last calibration. Changes in buoyancy during calibration of both the offset masses (e.g. the vessel) as well as of the medium will affect the mass measurement.

The **calibration time** with respect to the beginning and the end of the flow measurement for the reference and the DUT needs to be simultaneous and for fully developed flows. One could use a diverter; however this is hardly used in micro fluidic measurements. Alternatively the flow is measured continuously to ensure measurement on a stable flow.

The **connections** from the device under test to the reference setup needs to be leak tight and not water absorbing. It is also good especially for small tubing to minimize air-traps, dead volumes and clogging.

The **outflow pipe** connection to the balance should also be considered. Capillary forces, intermediate interaction and buoyancy correction due to tube contact or tube immersion into the measurement beaker needs to be properly taken into account. Also the increasing water level causes an increasing hydrostatic pressure, which affects the stability of the flow rate.

The **flow generator** might produce flow pulsations. Furthermore, the flow rate may drift over time. This should be taken into account when the effect on the device under test is not known.

The **device under test** has a resolution and a repeatability which has to be taken into account at each calibration.

The **evaporation rate** should be determined in order to make the appropriated corrections. The uncertainty associated with this evaporation correction should be taken into account.

After evaluating these uncertainty sources, it is of common metrological practice to verify the measurement uncertainty by means of an intercomparison with other flow standards. An approach to check the reproducibility and calibration model is to cross check by varying various physical parameters and to predict the effect of variations in the set-up. Examples of such variations are changing the calibration and stabilization time, changing the outflow pipe diameter going into the measurement beaker, changing the vessel dimensions etc. Such an approach, however, cannot, replace validation by Intercomparison.

EVAPORATION COVER

The main difference between the gravimetric principles developed and validated in MeDD (Metrology for Drug Delivery) project is the realization of an effective evaporation cover.

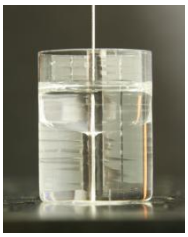
Saturated air

This method requires an evaporation trap. This device is part of the balance setup and consists of a glass chamber attached to the balance plate. In the middle of the chamber there is a reservoir full of water that saturates the air greatly reduces evaporation. Alternatively, the chamber is simply made as airtight as possible such that the air around and in the measurement beaker is saturated. CETIAT (France), IPQ (Portugal) and VSL (Netherlands) use one of these approaches for the gravimetric set up. Contact Benoit Savanier (benoit.savanier@cetiat.fr), Elsa Batista (ebatista@ipq.pt) or Harm Tido Petter (hpetter@vsl.nl), respectively, for more information.

Absorbing foam and saturated air

In this case the water enters the measurement beaker via a glass filter. The capillary forces in the glass filters suck through the filter before any droplets are formed at the surface. At the other side of the filter there is water-absorbing foam which prevents the water to stay at the glass filter and therefore further minimizes evaporation. METAS (Switzerland) uses this approach for the gravimetric set up. Contact Hugo Bissig for more information (Hugo.Bissig@metas.ch).

Oil based



This method requires an oil of lower density to cover the water. The outflow tube is then immersed into the water below the oil cover. This approach yields an unknown force on the immersed needle which can be characterized using a translation stage. DTI (Denmark) uses this approach for the gravimetric set up. Contact Anders Niemann for more information (akn@teknologisk.dk).

THERMAL BASED VOLUMETRIC EXPANSION OVER TIME

A reference for nanoflow rate can be based on the volumetric-expansion principle. Here, the flow rate follows from:

$$Q = \frac{dm}{dt}$$

Where *Q* is mass flow, *m* is mass contained in some reservoir and *t* is time. The reservoir is gradually heated which will cause expansion of the fluid inside the reservoir. By measuring the temperature gradient in time one has a measure for a flow rate. The above equation can be expanded into:

$$Q = -V_{H_0}\left[\left(1+\beta(T_R(t)-T_0)\right)\left\{A(T_M)\left(k_1(t)+\frac{\partial c(t)}{\partial t}\right)+B(T_M)k_1(t)c(t)\right\}+\rho(T_R)\beta k_2(t)\right]$$

where:

- A is the partial derivative of the density with respect to temperature measured in the reservoir
- B is the second order partial derivative of the density with respect to temperature measured in the reservoir
- $k_1(t)$ is the temperature gradient measured **in** the reservoir
- $k_2(t)$ is the temperature gradient measured **of** the reservoir
- c is a constant (steady state, constant temperature gradient)
- T_M is the temperature measured **in** the reservoir
- T_R is the temperature measured **of** the reservoir
- $V_H(t)$ is the temporal volume of the water contained in the reservoir and tubing that are inside the temperature controlled bath
- V_{H_0} is the volume of the water contained in the reservoir and tubing that are inside the temperature controlled bath, *at the reference temperature*

– β is the volumetric thermal expansion coefficient of the reservoir

The **reference volume** follows from the difference in mass between the empty and full reservoir. The **temperature** of the reservoir and the liquid in the reservoir follow from small NTCs placed inside and outside of the reservoir. The spatial **inhomogeneity** of the **temperature** and temperature gradient are determined with numerical modeling. Finally, the **fluid properties** (density and first and second order derivative) follow from the Tanaka equations.

VSL (Netherlands) uses this approach for flow rates down to 10 nL/min. Contact Peter Lucas (plucas@vsl.nl) for more information.

OPTIC MEASUREMENT OF DISPENSED VOLUME OVER TIME

An alternative method to measure flow rates less than 100 nL/min is the tracking of a liquid front in a capillary or in an etched channel. Figure 1 shows the principle set-up of a front tracking system: the experimental setup relies on a tele-centric CCD imaging system mounted on a high-precision, computer controlled linear stage to track a moving liquid meniscus in a glass capillary. The position of the linear stage can be automatically adjusted to track the motion of the liquid front.

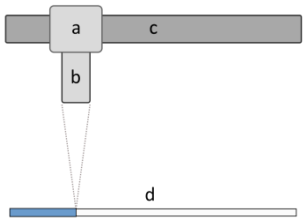


Figure 1: (a) camera, (b) tele-centric lens, (c) linear high-precision motion stage, (d) high-precision capillary

The measurements will need to be done in a temperature controlled environment and the evaporation should be minimized with an long thin tube attached at the open side of the capillary. The captured image is first filtered using a Canny-edge algorithm and the relative position of the meniscus`s edge (liquid-side) is then determined using a line segmentation function. The average volumetric flow rate $\dot{V}$ for the sampling period Δt is determined by the displacement Δx of the front during Δt and the radius R of the capillary:

$$\dot{V} = \frac{\Delta x}{\Delta t} \cdot \pi \cdot R^2$$

The uncertainty $u_{\Delta x}$ in the determination of the front`s displacement Δx can be expressed as:

$$u_{\Delta x} = u_{drift} + u_{stage} + u_{imaging} + u_{fluctuations}$$

where u_{drift} is the contribution due to effects like evaporation at the meniscus surface and/or leakages, u_{stage} is the uncertainty introduced by the positioning accuracy of the linear stage, $u_{imaging}$ the contribution from the accuracy of the imaging system (camera + lens) and $u_{fluctuations}$ represent the combined effect of random fluctuations of the experimental conditions (i.e. chamber temperature, room temperature, vibrations, ambient pressure and humidity).

FH Lübeck (Germany) uses this approach for flow rates down to 1 nL/min. Contact Martin Ahrens (ahrens@fh-luebeck.de) for more information.