DYNAMIC MEASUREMENT OF NANOLITER PER MINUTE FLOW BY SCALED DOSAGE OF FLUORESCENT SOLUTIONS

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ABSTRACT

This work describes a new method for continuous flow measurements over the range of 1 nL/min to 1000 nL/min in a microfluidic channel using integrated waveguides to excite and collect fluorescence from a photobleachable dye. By relating intensity of the dye to the energy it receives as a function of flow rate, our device extrapolates the measurement of a calibrated flow meter to a regime where the latter has large or unknown uncertainties. Coupled to a new way to determine zero flow, we demonstrate a first-of-its kind ability to accurately measure < 30 nL/min with uncertainties on the order of 5 %.

KEYWORDS: Flow Metrology, Optofluidics, Fluorescence, Zero Flow

INTRODUCTION

Many instruments, including high performance liquid chromatography, drug delivery systems, and emerging microfluidic technologies require accurate measurement of microflows. Indeed, precise flow measurement is necessary for determining shear stresses, controlling droplet formation, establishing concentration gradients, and ensuring nutrient or drug delivery rates in medical and biotech applications [1]. However, as technologies shrink, they face challenges associated with reproducibility, regulatory approval, and maintaining competitive advantage. Thus, increasingly accurate methods are needed to both measure and control uncertainty at smaller flows.

Conceptually these challenges have been addressed through careful miniaturization of bulk-scale methods and control of environmental conditions. Such approaches have largely been successful at achieving high-precision flow measurements on the order of 1 μL/min [2], and further optimization has allowed for measurement on the order of 10's of nL/min to within a few percent uncertainty [3,4] to as low as 5 % uncertainty at 5 nL/min with 5 min measurement time [5]. However, these and related techniques [6] are increasingly constrained by practical limitations characterizing geometry, temperature, or interfacial properties at the microscale. Thus, further reduction in relative uncertainty may be hard to achieve with engineering approaches alone.

In this work, we report on a technique that leverages a surprisingly assumption-free scaling relationship that relates the output efficiency of fluorescent molecules in flow to the dosage of light received while passing through an optical interrogation region. The approach can extend a calibrated measurement to unexpectedly low flow rates and is largely agnostic to sources of uncertainty that affect other measurement techniques – namely, knowledge of the system geometry or the flow profile.

EXPERIMENTAL

(Identification of commercial products does not imply recommendation or endorsement by NIST. The materials and equipment used may not necessarily be best for purpose.) Master silicon wafers were coated with a 100 μm thick layer of SU-8 2075 (Microchem) and exposed using a maskless aligner (MLA 150, Heidelberg Instruments). The master wafers were then developed and coated with trichloro(1H,1H,2H,2H-perfluorooctyl)silane (Sigma-Aldrich). Optofluidic devices were fabricated from masters by micromolding of poly(dimethylsiloxane) (PDMS) (Sylgard 182, Dow Corning) using soft lithography methods [7]. Prior to oxygen plasma bonding the molded device to a flat PDMS substrate (Plasmatics Systems Inc), inlet ports were created using a 0.75 mm Harris Micro-Punch. Waveguide channels of the device were filled with optical adhesive (Norland 88, Norland Products) and degassed. Stripped and cleaved optical fibers (FG105UCA, Thorlabs) were inserted into the tapered ends of the waveguide channel followed by UV curing. Channels around the waveguides were filled with black PDMS (Sylgard 170, Dow Corning) to prevent scatter or stray light from interacting with dye outside the interrogation region.

A fiber-coupled diode laser (LuxX 488nm-60 mW, Omicron-Laserage) as excitation source and photodetectors (400-110nm Silicon detector with OD2 attenuator, Newport Corp.) coupled to a power meter (2936-R, Newport Corp.) were attached to fibers that delivered and received light, respectively, from the device. A fluorescein emission filter (ET520/40m, Chroma) was placed in the emission photodetector to exclude excitation light. Flows

were controlled via gravity with a motorized stage (LTS300, Thor Labs) and monitored in series with a flow meter (LG16-0150D, Sensirion AG). Fluorescein (30181, Sigma Aldrich) dissolved in phosphate buffered saline (pH 7.4, Thermo Fisher) was used create contrast within the optofluidic devices. Volumetric flow rate was determined by scaling reservoir height between zero flow (described below) and measured flow using a calibrated Sensirion flow meter at maximum reservoir height (300 mm). Dosage curves were generated by scanning laser power (in 5% increments) over a range of reservoir heights followed by plotting the resultant fluorescence efficiency (ratio of fluorescence intensity to incident laser power) against dosage (ratio of effective power to flow rate).

A nanoscale flow meter with optical waveguides is shown in Figure 1. Fluid in a single microchannel is passed through optical interrogation regions along the flow path. A fluorescein solution in the microchannel is excited by 488 nm excitation light that is transmitted to an interrogation region in the flow channel by an optical adhesive-filled microchannel waveguide. Transmitted light and emitted fluorescence are collected by waveguides in and out of the excitation path, respectively. Microchannels filled with black PDMS were created between waveguides to reduce exposure of the fluorescein to excitation light outside the interrogation region and to minimize leakage of excitation light into the emission waveguide.

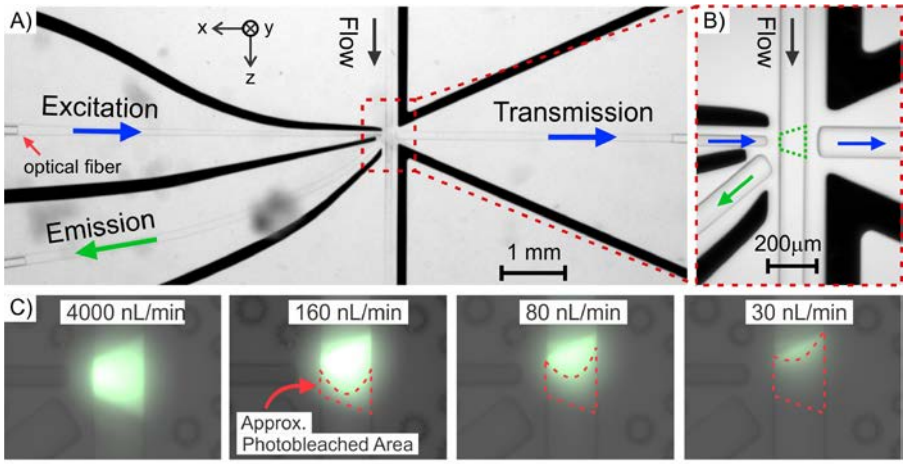

Figure 1: (A) Optical images show an overview of one optical interrogation zone along a microchannel. Excitation, emission, and transmission waveguides are marked to indicate direction and primary wavelength of light in each path (blue = 488nm, green = 520 nm). Close up view of the interrogation zone (approx. green trapezoid). (C) At high flow rates (low dosages), fluorescein is fully fluorescent and matches the laser illumination profile (left). Panels moving to the right show decreasing flows (increasing dosages) at fixed power. Fluorescein near the walls, and increasingly nearer to the entrance of the interrogation region, is photobleached sooner into the laser path. Fluorescence efficiency is calculated by dividing the emitted fluorescence by transmitted laser power. Channel width is 100 μm.

THEORY

A scaling relationship provides the foundation for the measurement technique based on the observation that the fluorescence efficiency of a dye is a function of the total radiation it receives. Mathematically, we assume that the concentration of unbleached fluorescein, c, in the interrogation region changes as a function of time due to convection, diffusion, and photobleaching (e.g. irreversible destruction of the molecule). We can express this as

$$\partial_t c = D\nabla^2 c - v_v u(x,y)\partial_z c - f(p)\phi(x,y,z)c \qquad (1)$$

where D is the diffusion coefficient of fluorescein, $v_v u(x,y)$ is the velocity profile, v_v is the volumetric flow rate, $f(p)\phi(x,y,z)c$ models the photobleaching rate, p is the laser power, and $f(p)$ is the effective power adjusted to correct fluorescein photobleaching nonlinearity. The latter was determined to be $f(p) = p^{1.174}$, consistent with [8]. We also emphasize that $u(x,y)$ and $\phi(x,y,z)$ are unknown in general. Fluorescence emission in the interrogation region produces a time-dependent intensity signal, which can be expressed as

$$I(t) = p \int dxdydz\, \psi(x,y,z)c(x,y,z,t) \qquad (2)$$

where $\psi(x,y,z)$ is an unknown scaling factor that accounts for excitation and emission losses in the channel.

Explicit solutions to Eq. (2) are not obtainable since most model inputs are unknown. However, diffusion effects can be eliminated by ensuring that the microchannel width is on the order of 10s to 100s of microns, so that the Peclet number is large. Moreover, it can be shown that the steady-state fluorescence efficiency $\mathcal{F} = I(t \to \infty)/p$ has a one-to-one relationship with the dosage $\xi = f(p)/v_v$. Physically, this amounts to observing that fluorophores moving through the interrogation region receive a total amount of light jointly dependent on the velocity of the molecules and laser power. Thus, a different flow rate will yield the same dosage if the power is changed by the same proportion. Critically, this fact is sufficient to develop a measurement, since the efficiency can be determined at a known flow rate by varying the power. A forthcoming manuscript fully describes the underlying theory [9].

RESULTS AND DISCUSSION

Flow meters are limited in their ability to measure flows close to zero, either because of expanding uncertainties due to contributions from evaporation or capillary forces, or from lack of precision in system geometry. The opto-fluidic flow meter has a unique ability to bound the convective contribution to flow around zero, where equal diffusion of fluorescent molecules from both sides of the interrogation region establishes a minimum fluorescence signal. Determination of zero flow is accomplished by finding the bounds of reservoir height at which the fluorescence signal is minimized (*e.g.* all dye is photobleached except for diffusion of unbleached dye into the interrogation region). This phenomenon can be validated by observing photobleaching as the flow shifts from positive to negative (Figure 2). A slight positive flow leads to replenishment of fluorescein on the upstream side; a slight negative flow leads to replenishment of fluorescein on the downstream side.

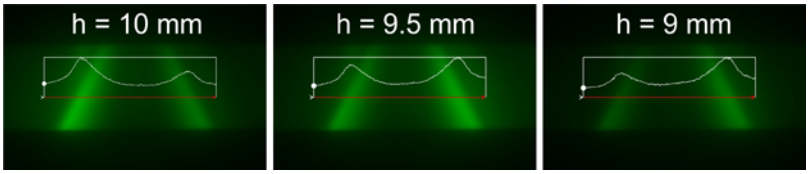

Figure 2. *Microscopy images show fluorescence at heights near zero flow; intensity profiles shown in white. Brighter intensity on the left indicates positive flow, brighter intensity on the right indicates negative flow. When flow is near zero, all fluorescein in the channel is bleached, except for balanced diffusion of new fluorescein into the laser path.*

We could repeatably determine the height of zero flow to within ± 0.5 mm (0.29 mm standard uncertainty for rectangular distribution) on a 100 μm wide flow region. Given that conductance is proportional to the ratio of flow and pressure (relative height, h), conductance can be expressed as

$$G = \frac{v_{v1} - v_{v2} + \varepsilon_{v1} + \varepsilon_{v2}}{\rho g (h1 - h2 + \varepsilon_{h1} + \varepsilon_{h2})} \qquad (3)$$

Where ρ is density, g is the gravitational constant, and ε_i corresponds to the absolute uncertainty of measurand *i*. Using a Sensirion flow meter calibrated to 5 % uncertainty (k=2; 95% confidence) with a gravimetric method [3] at two heights, where $h2 \to v_{v2} = 0$, we determined the conductance of the system to be ≈ 0.26 nL/min Pa^{-1} (2.5 nL/min $mm^{-1}(H_2O)$). Thus, we can achieve roughly ± 0.73 nL/min uncertainty in volumetric flow. Rearranging Eq. 3, *any* volumetric flow rate can be estimated from the reservoir height (or flow can be defined by reservoir height), and the 5 % uncertainty on the flow meter at 500 nL/min can be used to choose a height for flow as small as 15 nL/min with the same 5 % relative uncertainty. Further improvement in the zero flow estimate may be possible by decreasing flow channel width, which enhances sensitivity to height by decreasing conductance and increases the Peclet number to improve discernment of convection. Although the zero-flow correction does not provide dynamic measurements of flow *per se*, it does enable initialization of a gravimetric control system.

Dynamic flow measurements can be made with a technique based on the observation that the fluorescence efficiency is a one-to-one function of the dosage. First, a "master curve" of efficiency over a range of laser dosage is determined at a flow rate within the range of low uncertainty for a calibrated flow meter. Figure 3 shows a master curve generated with 50 μmol/L fluorescein solution flowing through a 100 μm wide interrogation region.

After creation of a master curve, unknown flow rates can be determined by mapping a measured fluorescence efficiency back to dosage for a given laser power. To demonstrate rescaling over a broad range of flows and laser powers, we show the alignment of other curves (corrected to zero flow, as above). We find that the fluorescence efficiency-dosage relationship approximates a continuous, convex function over approximately 3 orders of

magnitude in flow from 1 µL/min to nearly 1 nL/min. Relative percentage errors from a convex fit to the data are less than 4 % in both fluorescence efficiency and dosage to 27 nL/min (which is the lowest point overlapping the master curve), indicating that the scaling technique effectively preserves relative error over a range of flow measurements that extends well below the specified range of traditional flow meters. We are evaluating a bootstrapping approach to scale down the master curve to even lower flows.

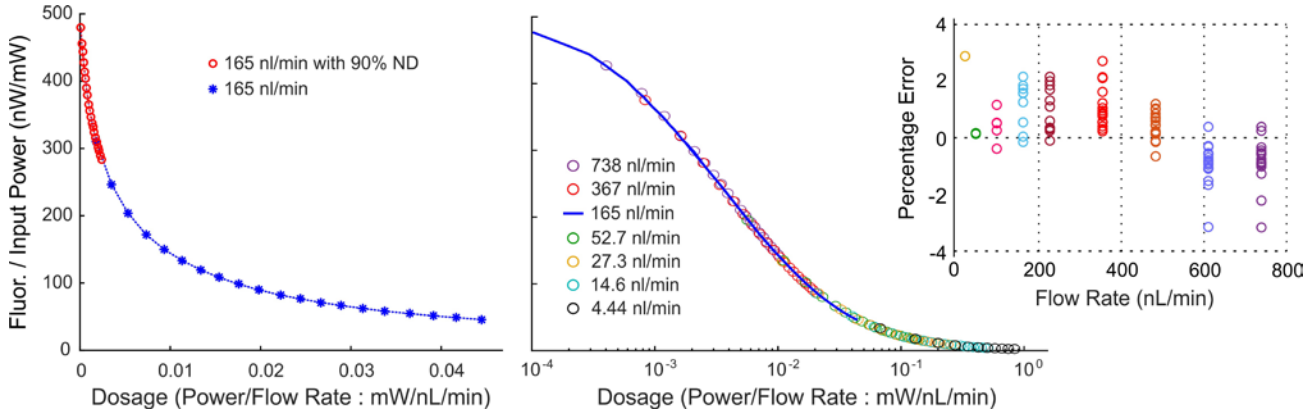

Figure 3. (Left) Fluorescence efficiency was measured at 165 nL/min in 5% increments of laser power with (∗) and without (o) a 90 % blocking neutral density filter. (Right) Fluorescence efficiencies for other flow rates overlaid on 165 nL/min data show agreement with rescaling over 3 orders of magnitude. Plot is log (x) to enable visualization over the range of dosages. (Inset) Percentage errors of residuals to master curve created using flow rates above 165 nL/min. Errors for lower flows are shown for dosages that overlap with the master curve.

CONCLUSION

The new optofluidic method to measure nanoscale fluid flows can re-scale an existing calibration without expanding the relative uncertainty to lower flow regimes where its uncertainty was large or unknown. With precise determination of zero flow, flow rates can be extrapolated to a few nL/min within best-case meter error, which is typically above 1000 nL/min. Importantly, the method requires neither precise measurement of channel cross section nor an imaging system; only a dosage-scaling relationship need be determined at a known flow rate.

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REFERENCES

[1] Y.-C. Tan, J.S. Fisher, A.I. Lee, V. Cristini, and A.P. Lee, *Lab Chip*, 4, 292, 2004. G. Guan, L. Wu, A.A. Bhagat, Z. Li, P.C.Y. Chen, S. Chao, C.J. Ong, J. Han, *Sci. Rep*,, 3, 1475, 2013. B.K. McKenna, J. G. Evans, M. C. Cheung, and D. J. Ehrlich, *Nat. Meth.*, 8, 401, 2011. V. Hessel, H. Lwe, and F. Schnfeld, *Chem. Eng. Sci.*, 60, 2479, 2005.

[2] C. Melvad, U. Kruhne, J. Frederiksen, *Meas. Sci. Technol.*, 211-6, 2010.

[3] Schmidt JW, Wright JD, *Proc. 9th Int. Symp. Fluid Flow Meas.*, 14-17, 2015.

[4] H. Bissig, et. al, *Biomed. Eng.-Biomed. Tech., 60, 301-16, 2015*.

[5] M. Ahrens, St. Klein, B. Nestler, C. Damiani, *Meas. Sci. Technol.*, 25, pp 1-9, 2014

[6] J.T.W. Kuo, L. Yu, E. Meng, *Micromachines*, 3, 550-73, 2012. Salipante P, Hudson SD, Schmidt JW, Wright JD, *Exp. Fluids*, 58 (7), 85, 2017.

[7] G. A. Cooksey, C. G. Sip, A. Folch, *Lab Chip*. 9(3), pp 417-426, 2009. J. C. McDonald, D. C. Duffy, J. R. Anderson, *et al. Electrophoresis*. 21(1), pp 27-40, 2000.

[8] G.H Patterson, D.W. Piston, *Biophys. J.*, 78, 2159-52, 2000.

[9] P.N. Patrone, G.A. Cooksey, A. Kearsley, *Phys. Rev. Appl.*, submitted.

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