Fluorescence photobleaching to evaluate flow velocity and hydrodynamic dispersion in nanoslits

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Velocity measurement is a key issue when studying flows below the micron scale, due to the lack of sensitivity of conventional detection techniques. We present an approach based on fluorescence photobleaching to evaluate flow velocity at the nanoscale by direct visualization. Solutions containing a fluorescent dye are injected into nanoslits. A photobleached line, created through laser beam illumination, moves through the channel due to the fluid flow. The velocity and effective diffusion coefficient are calculated from the temporal data of the line position and width respectively. The measurable velocity range is only limited by the diffusion rate of the fluorescent dye for low velocities and by the apparition of Taylor dispersion for high velocities. By controlling the pressure drop and performing measurements we determine the fluid viscosity. The photobleached line spreads in time due to molecular diffusion and Taylor hydrodynamic dispersion. By taking into account the finite spatial and temporal extensions of the bleaching under flow, we determine the effective diffusion coefficient, which we find to be in good agreement with the expression of the two dimensional Taylor–Aris dispersion coefficient. Finally we analyze and discuss the role of the finite width of the rectangular slit on hydrodynamic dispersion.

1 Introduction

Nanofluidics offers the opportunity to explore the interplay between bulk and interfacial phenomena.1 Interactions between the fluid and the channel walls are prominent in nanofluidic systems because of the large surface-to-volume ratio. The frequency at which a fluid molecule hits a wall by diffusion is greatly increased. Interfacial phenomena, such as adsorption and wall slippage, can influence both fluid diffusion and velocity profiles at the nanoscale.2 Therefore, flow detection through velocity measurements at the nanoscale is an active research field. Most techniques use fluorescent tracers to overcome the lack of sensitivity of conventional detection techniques.

Two main categories of velocimetry and anemometry techniques emerge in microfluidics, using either fluorescent particles or molecular tracers.3 Downscaling these techniques below the microscale is usually not straightforward, especially when we want to access the velocity profile.

Particle based methods (particle imaging velocimetry or particle tracking velocimetry) are not directly suitable to study nanoflows since the vertical resolution is limited by optical diffraction. However, by taking advantage of evanescent wave illumination, it is possible to overcome this difficulty, and thus acquire velocity profiles with a very good resolution close to the surface.4–7 This technique requires however an accurate calibration and particle–wall interactions may raise the measurements’ uncertainties.8

On the other hand, methods based on molecular dyes are non-intrusive and are thus well adapted to very small scales. Several approaches have been developed: measuring the displacement of tagged regions (see ref. 3 for a review) or using dual-focus fluorescence correlation spectroscopy.9 Nevertheless, to our knowledge, only one study has been able to acquire three dimensional velocity profiles below the microscale with molecular tracers, using a combination of stimulated emission depletion microscopy and laser-induced fluorescence photobleaching anemometry.10

When the acquisition of the full velocity profile is not required, simpler methods based on photobleaching have been proposed. Photobleaching offers various strategies to study nanoscale flows. Measuring the fluorescence recovery,11 or measuring the fluorescence decay12,13 leads, after calibration, to the determination of the mean velocity in the photobleached region. However, the most direct way to take advantage of photobleaching is to measure the displacement of the photobleached region, either by placing a detector at another position,14 or by imaging directly the line displacement in time.15 In this case no calibration is required. Furthermore, the line displacement can be measured with very good precision, in the case where the spreading due to diffusion and hydrodynamic dispersion is small. Mosier et al.15 verified that the stretching of the dye molecules, due to velocity gradients, results in the fast dispersion of the fluorophores and thus a fast reduction of the signal to noise ratio. The authors thus
conclude that electrokinetic flows in microchannels are good candidates for the technique.

Here we apply the fluorescence photobleaching technique to submicron flows. Apart from its simplicity, the technique offers a remarkable signal to noise ratio even in pressure driven flows. Indeed, the Peclet number is much lower in nanofluidics, since it is proportional to the characteristic size of the channel crosssection. Hence hydrodynamic dispersion rarely comes into play in nanofluidics, whereas it has been shown to raise measurement uncertainties in microfluidics. Fluorescence photobleaching is an appealing technique to investigate submicron pressure driven flows. However, it has been discussed that velocity gradients in both transversal directions will enhance the dispersion at long times. To minimize the effects of the photobleached line spreading due to both diffusion and hydrodynamic dispersion, one needs to be able to follow the line displacement at small time scales.

The main goal of this work is to apply the photobleaching technique to visualize flows below the micrometre scale. The effect of diffusion and dispersion in pressure driven flows is investigated in a commonly used geometry in nanofluidics: a rectangular channel, also called nanoslit. Measuring the mean velocity in pressure-driven flows gives access to the effective viscosity of complex fluids such as polymer solutions below the micron scale, for which we might expect some deviations from bulk properties.

The paper is organized as follow. First, the device used to achieve pressure driven flows in nanoslits is presented, as well as the photobleaching experimental procedure. Then, we recall theoretical considerations to account for concentration profiles under flow at short times. In the light of these predictions, we analyze the experimental intensity profiles in terms of mean flow velocity and effective diffusion coefficient. We discuss the influence of hydrodynamic dispersion in this geometry and experimental time range. Finally, the potential of the technique is demonstrated through its application to a polymer solution.

2 Materials and methods

2.1 Chip design and pressure control

The clogging of microfluidic devices is a major hurdle when reducing a channel dimension below one micrometre. In order to prevent flow interruption, one possible strategy is to avoid direct injection into the nanoslits and to use standard microfluidic channels onto which nanoslits are connected. Furthermore, it is more convenient to use not only one, but a large number of nanoslits in parallel, so that if one is clogged the others are still usable. Eventually, some of the nanoslits are clogged after a week of use, due to dust, particles or fluorescent dye crystallization. To ensure accurate control of the pressure in the entrance of the nanoslits, we have designed our chips so that the total flow rates in the nanoslits are negligible compared to those in the microchannels.

Our design is sketched in Fig. 1, where 8 to 115 nanoslits are connected perpendicularly to two microchannels. As shown below, such a device leads to an indirect pressure gradient control in the nanoslits. We fabricated four devices, varying the nanoslits’ heights, \( h \) from 0.27 to 4 \( \mu \text{m} \) (0.266, 0.551, 1.59, 3.87 \( \mu \text{m} \)). The height of the microchannels is of the order of 40 \( \mu \text{m} \). Due to isotropic glass etching, the channel width, \( w \) is much larger than the channel height (see section 2.2). The widths of the nanoslits and the microchannels are about 25 \( \mu \text{m} \) and 250 \( \mu \text{m} \) respectively.

A pressure-driven flow is established in the nanoslits through a pressure difference in microchannels. Both ends of the microchannels are connected to a pressure-control regulator (MFCS 4C, Fluigent). We apply pressures \( P_1 \) and \( P_2 \) at the inlet of microchannels 1 and 2 and \( P_0 \) at the outlets. The pressure difference \( P_1 - P_2 \) ranges from 5 mbar to 800 mbar. Since the hydrodynamic resistance of the nanoslits is very high compared to that of the microchannels, the contribution of the nanoslit flows to the microchannel ones is negligible. Thus both the flow rates and the pressure gradients are uniform in the microchannels. The latter is given by \( (P_k - P_0)/L \), where \( L \) is the microchannel length and \( k = 1 \) or 2, standing for the two microchannels. Let us consider the nanoslit \( i \), which end pressures are named \( P_{i1} \) and \( P_{i2} \). The pressure \( P_{ik} \) is simply given by \( P_{ik} = P_i - L(P_k - P_0)/L \), where \( L_i \) is the distance separating the inlet of a microchannel and the nanoslit \( i \). Therefore, the pressure drop \( \Delta P_i = P_{i2} - P_{i1} \) in the nanoslit \( i \) is expressed as

\[
\Delta P_i = (P_2 - P_1) \frac{L_i}{L}
\]

Thus, the pressure drop applied in the nanoslit \( i \) is simply proportional to the applied pressure difference \( P_2 - P_1 \).

2.2 Fabrication details

Standard photolithography and wet etching are used to make the micro-nano glass chips. A glass wafer (3 inches Borofloat 33, Sensor Prep) is protected by chromium and gold layers (Vacuum coater, BOC Edward Auto 500) of 30 nm and 70 nm respectively. A positive photoresist (Microdeposit S1818, Rohm and Haas) is then spin-coated on the wafer and insulated with a UV-light (Aligner, Suss Microtec MJB4) through a mask. Two successive etching steps are performed. The protection step is repeated in between. Microchannels are etched first with a solution at 20% of...
hydrofluoric acid in DI-water. The etching rate is about 1 μm per minute, depending on the glass properties. Nanoslits are then etched with BOE (Buffer Oxide Etchant) containing about 7.7% of hydrofluoric acid (60 g of NH₄F, 25 ml of HF 40%, 5 ml of HCl and 100 ml of DI-water). The BOE has a slower etching rate of about 100 nm per minute, which allows a better control of both channel height and wall flatness. The etched wafer is cleaned with a mixture of sulfuric acid and hydrogen peroxide (400 ml of H₂SO₄ at 95% and 400 ml of H₂O₂ at 30%), prior to thermal bonding. In order to control the channel geometry, particular care is given to wet etching and thermal bonding steps. Hydrofluoric acid solutions allow us to etch microchannels and nanoslits with flat walls. Nanoslits have rectangular cross-sections from 0.27 to 4 μm × 25 μm.

2.3 Imaging and bleaching

Image acquisition is performed with a confocal microscope (Observer.Z1 LSM 5 Live, Zeiss) and a 63× immersion oil objective at acquisition rates ranging from 10 to 60 images per second. In order to visualize the flow, we use a solution of fluorescein isothiocyanate. This dye is known to be easily photobleached. We create a ‘dark’ line in the first illumination step, a defined region of the channel is etched with BOE containing about 7.7% hydrofluoric acid (60 g of NH₄F, 25 ml of HF 40%, 5 ml of HCl and 100 ml of DI-water). Nanoslits are then cleaned with a mixture of sulfuric acid and hydrogen peroxide (400 ml of H₂SO₄ at 95% and 400 ml of H₂O₂ at 30%), prior to thermal bonding. In order to control the channel geometry, particular care is given to wet etching and thermal bonding steps. Hydrofluoric acid solutions allow us to etch microchannels and nanoslits with flat walls. Nanoslits have rectangular cross-sections from 0.27 to 4 μm × 25 μm.

2.4 Fluids

The solutions used for all experiments are water/glycerol mixtures containing fluorescein isothiocyanate (FITC), purchased from Sigma-Aldrich at 0.1 × 10⁻¹ mol L⁻¹. However, the fluorescence intensity of FITC is maximum at pH equal to 7,18 so we add a few drops of NaOH at 2.5 × 10⁻³ mol L⁻¹ to reach this pH. Solutions are then filtered through a 0.2 μm pore diameter EKV membrane (Acrodisc, Pall). Table 1 reports the viscosity of different solutions. Viscosity measurements are performed on a stress-controlled rheometer (Advanced Rheometer 1000, TA Instruments) with a cone-plate geometry at 22 °C.

3 Analysis

Let us consider the transport properties of a solute in a pressure driven flow inside a rectangular cross-sectional channel. The solute concentration c (in the dilute limit) follows the convection-diffusion equation

\[ \frac{\partial c}{\partial t} + v(x, t) \frac{\partial c}{\partial x} - D \Delta c = s, \]

where D is the molecular diffusion coefficient, v the velocity and s the source term. This equation is greatly simplified thanks to the experimental geometry. Given the small channel height, the characteristic time necessary for a solute molecule to diffuse along the z-direction is rather small compared to the acquisition time scale. The diffusion time is estimated as \( h^2/2D \). Thus, for small heights, the condition is always fulfilled, since \( h^2/2D \) is in the order of a few milliseconds. As a consequence, the concentration is homogeneous throughout the height and it is natural to consider z-averaged quantities. Furthermore, given the aspect ratio of channels—the width is from 6 to 100 times larger than the height—we consider that the problem is invariant in the y-direction. Under these assumptions, the problem reduces to the situation described by Taylor and then refined by Aris,19,20 who showed that the z-averaged concentration \( \bar{c} \) is given by:

\[ \frac{\partial \bar{c}}{\partial t} + v_0 \frac{\partial \bar{c}}{\partial x} - D_{eff} \frac{\partial^2 \bar{c}}{\partial x^2} = s(x, t), \]

where, in between two infinite plates, \( D_{eff} = D(1 + Pe^2/210) \) and \( Pe = \nu_0/D \) with \( \nu_0 \) the mean velocity. In the mean velocity frame of reference, this equation reduces to:

\[ \frac{\partial \bar{c}}{\partial t} - D_{eff} \frac{\partial^2 \bar{c}}{\partial x^2} = s(x' + \nu_0 t, t) \]

where \( x' = x - \nu_0 t \). The impulse response of this equation is expressed as:

\[ c(\cdot, t) = \frac{H(t)}{\sqrt{4\pi D_{eff}t}} \exp \left( -\frac{x^2}{4D_{eff}t} \right), \]

where H is the Heaviside function. It allows to calculate the solution for an arbitrary source using a convolution, which reads

\[ c(x', t) = \int c(\cdot, \tau) s(x' + \nu_0 (t - \tau) - \lambda, t - \tau) d\tau. \]

Both the bleaching duration \( \tau_b \) and spatial width \( w_b \) cannot be neglected since they are in the order of the acquisition time scale. We assume that the source \( s(x, t) \) is given by \( s(x, t) = s_0 \nabla^2 (x/w_b) \nabla^2 (t/\tau_b) \), where \( \nabla^2 \) is the rectangular function. Finally, we obtain for \( t > \tau_b/2 \):

\[ c(x', t) = \frac{s_0}{2} \int_{t-\tau_b/2}^{t+\tau_b/2} \psi(w_b, \tau) - \psi(-w_b, \tau) d\tau, \]

where

\[ \psi(w_b, \tau) = \text{erf} \left[ \frac{w_b/2 + X' + \nu_0 (t - \tau)}{\sqrt{4D_{eff} \tau}} \right]. \]
This expression is easily estimated numerically. An example of the resulting concentration profiles, using typical experimental parameters, is shown in Fig. 2, in the case where the flow velocity influences the concentration profile. We observe that the concentration profile is symmetric and looks very similar to a Gaussian function. Its standard deviation is higher than for an impulse response, $\sqrt{2D_{\text{eff}}t}$. For small velocities, $v_0 \ll w_0/D$, the second moment is in the order of $w_0/2 + \sqrt{2D_{\text{eff}}t}$. For high velocities, the standard deviation increases. Indeed, during the photobleaching step, molecules have time to travel across the bleaching region and even further. The second moment of the distribution is estimated just after the bleaching by $(w_b + v_0 t_b)/2$.

We notice that the concentration profiles deviate from a Gaussian shape only at very short times. When high velocity is coupled with fast diffusion, i.e. for intermediate values of $v_0 w_0/D$, the concentration profile could be asymmetric at short times (see the first profile after the bleaching in Fig. 2). These situations are avoided in the experiments by decreasing the bleaching time, which also raises the contrast of images in this regime.

As soon as the profile is symmetric, the maximum value of the concentration profile simply follows $v_0 t_d$ and could be used to measure the mean flow velocity. However, it is necessary to take into account the width and duration of the bleaching to measure $D_{\text{eff}}$. For the sake of simplicity, the experimental results are fitted with Gaussian functions. The velocity is directly given by the temporal evolution of the Gaussian peak position and the above solution is used to interpret effective diffusion coefficients from Gaussian standard deviations (see sections 4.1 and 4.2).

Let us now briefly discuss the approximations we have made. In particular, we neglect the dispersion due to velocity gradients in the $y$-direction. This should come into play at long times, when molecules have enough time to diffuse along the width during the experiment. In this regime, the effective diffusion coefficient should be larger than the one predicted by a 2D analysis. Since the experimental times considered in this article are shorter or in the order of the channel width diffusion time, we can neglect the effect of the transverse direction. Furthermore, we show in section 4.2 that the experimental standard deviations of the concentration profiles are in very good agreement with 2D predictions, which validates $a$ posteriori the approximation. However, for fluids of low viscosity and for high values of $Pe = Pe_{\text{w}} w_l/\eta$, we observe that the line starts to bend due to dispersion in the $y$-direction. This point will be discussed further in section 4.3. In all other sections, only the central region of a channel is analyzed.

### 4 Results and discussion

The mean velocity in a nanoslit is determined from the temporal evolution of a photobleached region, as shown in the image sequence in Fig. 3.

For each photobleaching experiment, the acquisition of two image sequences is required. In the first sequence, a region of the channel is photobleached and its movement is recorded. In the second sequence, no photobleaching is made; instead, the gradual fluorescence intensity decrease, due to laser exposition during routine image acquisition, is monitored. The latter sequence is used as reference images for the first. Images from the two sequences are subtracted and averaged in the $y$-direction, transverse to the flow, considering a region of interest as shown in Fig. 3. The resulting intensity $I_0 - I$ is then normalized by the reference intensity $I_0$. Fig. 4 shows a typical outcome of the experiment. The quantity $(I_0 - I)/I_0$ is plotted as a function of the $x$-position in the nanochannel for different times in the image sequence.

Each concentration profile is fitted with a Gaussian function, $c(x) = A \exp[-(x - x_0)^2/2\sigma^2]$. As shown in Fig. 4, the Gaussian functions are in excellent agreement with the data. This is consistent with the analysis presented in the previous section since the theoretical concentration profile (eqn (7)) is at long times well approximated by a Gaussian function. To verify the proportionality between the dye concentration and the fluorescence intensity, we integrate the concentration profiles over space to obtain the total intensity. It remains constant over time (see the inset in the Fig. 4), which indicates that the fluorescence intensity is proportional to the dye concentration.

In the following, we use the temporal evolution of the fitting parameters: the line mean position $x_0$ and its characteristic half-width $\sigma$.

**Fig. 2** Predicted time evolution of the concentration profile under flow (solid lines, eqn (7)), compared with the impulse response (dashed-dotted lines, eqn (5)). Concentration profiles during the photobleaching are also shown (dashed lines). The parameters used for this example are: $t_b = 60 \text{ ms}$, $w_b = 10 \mu\text{m}$, $v_0 = 10^{-4} \text{ m s}^{-1}$, $D = 10^{-10} \text{ m}^2 \text{ s}^{-1}$. During the bleaching, the successive profiles displayed are calculated for $t/t_b = -0.25, 0, 0.25$ and 0.5. After the bleaching, $t/t_b = 1, 2, 3, 4$ and 5.

**Fig. 3** Image sequence of a photobleached line in a 266 nm high nanoslit, acquired at 30 images per second. A 7.5 $\mu\text{m}$ wide region is photobleached for 100 ms. The fluorescence intensity is analyzed over a region of interest, which is displayed on the first image of the nanoslit. The mean velocity is $v_0 = 2.5 \times 10^{-5} \text{ m s}^{-1}$ and $Pe_{\text{w}} = 0.093$. 

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4.1 Velocity

The mean position of the concentration peak \( x_0 \) as a function of time is shown in Fig. 5. The mean line position is perfectly linear with respect to the time, such that

\[
x_0 = v_0 t,
\]

leading to a precise measurement of the mean \( z \)-averaged velocity, \( v_0 \). To increase the precision, we systematically carry out 5 photobleaching experiments and average the resulting velocities. A typical error of 2\% is obtained, indicating a good reproducibility of the velocity measurements. We verify the uniformity of the velocity along the nanoslit, i.e. the velocity is independent of the observation location.

The mean velocity in the central region of the channel is given by the Poiseuille law:

\[
v_0 = \frac{1}{12} \frac{h^2 \Delta P}{\eta L},
\]

Knowing that \( \Delta P \propto (P_1 - P_2) \) for a given nanoslit (see eqn (1) in section 2.1), the velocity is proportional to the inlet pressure difference. This is verified in Fig. 6a and b with various fluid viscosities. The accessible velocity range is quite large and goes from \( 10^{-6} \) to \( 10^{-3} \) m s\(^{-1}\). Note that the positive and negative displacements align perfectly, which means that there is no effect of the scan direction of the laser. Indeed, we limit our study to small velocities as compared to the scan speed of the confocal microscope, which is about 15 mm s\(^{-1}\).

All parameters in eqn (9) are known with a good precision \( a \ priori \), except for the nanoslit height. The channel depth is

\[ h_r \]

† Due to isotropic chemical etching, the cross-section of nanoslits is not strictly rectangular. Given the high aspect ratios of nanoslits, the shape of the channel sides will not influence the maximum velocity of the profile. If we focus on measuring velocities in the central region of the channel, the mean velocity follows the Poiseuille law between two infinite plates, which depends on the channel height.
measured with a stylus profilometer (Veeco Dektak) just after the etching step, but glass wafers may deform during thermal bonding. The channel height can slightly change. The latter can be precisely determined by fitting the experimental linear relation between the mean velocity and the pressure drop for a reference fluid (50/50 water/glycerol mixture) of known viscosity. Eqn (1) and (9) are then combined to calibrate the channel height. The values obtained are close to the profilometer measurements. A significant decrease is obtained for the smallest channel heights (up to 50% for a 266 nm high channel).

This hydrodynamic determination of the channel height is validated by an optical measurement. Using an objective of small numerical aperture, we integrate the fluorescence intensity in both the micro- and nanoslits. We assume that buckling of the glass during the thermal bonding is negligible in the microchannels and that the fluorescence intensity is proportional to the channel height. So, we calculate the channel height knowing the fluorescence intensity ratio between the microchannel and the nanoslit. This second method is less accurate than the hydrodynamic one, but leads to similar channel heights.

Finally, the height being calibrated with a reference fluid, we validate the viscosity dependence of the velocity–pressure drop relationship. Fig. 6c compares the viscosity determined in the nanoslits with the viscosity of water/glycerol mixtures measured with a rheometer. For every channel height from 0.27 to 4 μm, the correlation is rather satisfying. We conclude that our devices coupled with a fluorescence photobleaching technique are of great use to measure viscosities below the microscale, in pressure-driven flows.

### 4.2 Effective diffusion coefficient

The second parameter we extract from the intensity profiles is the standard deviation \( \sigma \), i.e., the half width of the photobleached line. As explained in the analysis section 3, the relation between standard deviation and effective diffusion coefficient is not simply given by \( \sigma = \sqrt{2D_{\text{eff}}t} \). Fig. 7 shows some examples of the variation of \( \sigma \) versus \( \sqrt{t} \), which is obviously not linear, due to the finite size of the bleaching width \( w_b \) and duration \( \tau_b \). These data are systematically fitted with the second moment of the predicted concentration profile defined in eqn (7), i.e., \( \sigma(t) = \int x^2e^{x^2/2}dx \). The only fitting parameter is the effective diffusion coefficient, since \( w_b \) and \( \tau_b \) are experimental parameters and \( w_b \) is estimated from the temporal evolution of the maximum position, as detailed in the previous section. As shown in Fig. 7, the fitting curves are in reasonable agreement with the data. It validates our approach for a wide range of Peclet numbers \( \text{Pe}_z \), from 10^{-3} to 100. If we now compare the curves at different Peclet numbers, i.e., velocities, we observe that the standard deviation increases with the velocity. We see two main reasons. First the initial width of the line is larger for high velocities. Indeed, a larger number of molecules have traveled through the photobleached region during the bleaching step. Second, while increasing the Peclet number, hydrodynamic dispersion comes into play and the effective diffusion coefficient increases. It results in a faster spreading.

For each fluid and channel height, we determine the effective diffusion coefficient as a function of the velocity, i.e., Peclet number, as shown in Fig. 8. In all cases, \( D_{\text{eff}} \) is constant for low Peclet numbers and then increases significantly. According to the analysis, we expect the effective diffusion coefficient to be given by

\[
D(1 + \text{Pe}_z^2/210)
\]

We take advantage of the low Peclet number regime to determine for each solution the molecular diffusion coefficient \( D \). \( D \) is the mean value of \( D_{\text{eff}} \) for \( \text{Pe}_z < 1 \). The results are displayed in the inset of Fig. 8 as a function of the inverse of the viscosity \( \eta \) of the solutions. The values obtained for different heights of nanoslits are similar. The error bars denote one standard deviation and represent a typical error of 20%. The molecular diffusion coefficient obtained in water is \( \sim 3 \times 10^{-10} \text{ m}^2\text{s}^{-1} \) at 22 °C, which is in quite reasonable agreement with values published in the literature (\( D = 4.17 \times 10^{-10} \text{ m}^2\text{s}^{-1} \) at 25 °C in ref. 22). Besides, in the inset of Fig. 8, \( D \propto 1/\eta \), which is in agreement with the Stokes–Einstein equation \( D = k_BT/6\pi\eta R \). A linear fit to the data leads to an estimation of the hydrodynamic radius \( R \) of the fluorescein molecule of 6.9 Å. We can thus conclude that fluorescence photobleaching method also leads to the determination of molecular diffusion coefficients in a flowing fluid.

In Fig. 8 we report the values of effective diffusion coefficients normalized by the molecular ones as a function of Peclet number. The data for water/glycerol mixtures in 0.27 to 4 μm high channels fall on the same curve and follow the law \( D_{\text{eff}} = D(1 + \text{Pe}_z^2/210) \). Our results thus agree well with the predicted evolution of the effective diffusion coefficient, coming from a 2D analysis of the hydrodynamic dispersion, for a wide range of Peclet numbers. It validates the assumption that the diffusion in the y-direction is negligible for the time range accessible in our experiments, which lies between \( h^2/D \) and \( w^2/D \). Besides, we limit the intensity measurement to the central region of the channel.

### 4.3 Lateral dispersion

Until now, we quantified the contribution of the Taylor hydrodynamic dispersion in the z-direction to the spreading of the
This measurement has been performed on six water/glycerol mixtures of decreasing viscosities (from dark to light color symbols) ranging from $8.8 \times 10^{-4}$ to $3.1 \times 10^{-2}$ Pa s. In 0.27 to 4 μm high nanoslits. Each point corresponds to a single time sequence, without filtering or averaging, which explains the dispersion of the data. For each set of parameters $h$, $v_0$, $D$, at least five time sequences are acquired. Thanks to automation, more than 10 000 data points are displayed in the Figure. The solid line represents Taylor–Aris dispersion coefficients as a function of Pe number in a rectangular cross-sectional channel. Inset: measured molecular diffusion coefficient $D$ that 10 000 data points are displayed in the Figure. The solid line represents Taylor–Aris dispersion coefficients as a function of Pe number in a rectangular cross-sectional channel. Inset: measured molecular diffusion coefficient $D$ that 10 000 data points are displayed in the Figure. The solid line represents Taylor–Aris dispersion coefficients as a function of Pe number in a rectangular cross-sectional channel. Inset: measured molecular diffusion coefficient $D$.

The diagram describes the evolution of the logarithm of the ratio $w/R_c$ as a function of $Pe_y = Pe_y w / h$ at long times, where $h$ is the channel width, $w$ is the channel width, and $R_c$ is the radius of curvature of the photobleached line. We define $R_c$ as the radius of curvature of the photobleached line, $w$ the channel width and $x / h$ with $x$ the position of the line in the channel. The solid lines define a region in this diagram for $Pe_y > 1 / x$ and $Pe_y > x$, where a curvature of the line is predicted due to lateral dispersion (see text).

Dutta et al.16 considered a pressure-driven flow of a solute through an isotropically etched channel. They demonstrated with numerical simulations the apparition of non-Gaussian tails at the side of the channel at short times ($t \ll w^2/D$), which should evolve toward a Gaussian distribution at long times.

The line curves only if convection dominates the diffusion process in the lateral direction. Thus, $Pe_y > 1$.
(ii) At long times, the diffusion should homogenize the concentration and the curvature of the line should disappear for \( t > w^2/D \). Since \( x_0 = v_0 t \), this condition reads \( \text{Pe}_l < \tilde{x} \).

(iii) At short times and for low \( \text{Pe}_l \), the increase of the profile width is of the order of \( \sqrt{D t} \). \( v_0 t \) is an estimation of the dispersion of velocities in the \( y \)-direction. Thus the curvature of the bleached line is only significant for \( v_0 t > \sqrt{D t} \), equivalent to \( \tilde{x} > 1/\text{Pe}_l \).

In fact the two last conditions contain the first one. They define a region in the diagram \( \tilde{x} - \text{Pe}_l \) that is drawn in Fig. 10. The region agrees remarkably well with the diagram domain, where \( w/r_e \geq 1 \), i.e. the curvature of the line is significant.

4.4 Application to a polymer solution

One of the interesting applications of the technique is the study of the flow properties of complex fluids below the micron scale. To give an example of the possibilities offered by this technique, we measure the viscosity of a solution of a \( 2 \times 10^5 \) g mol\(^{-1} \) molecular weight polyacrylamide (Acros Organics) at 5 wt\% in water in our nanofluidic devices and on a stress-controlled rheometer. In both cases, we scan a range of shear rates from 10 to 250 s\(^{-1} \), for which the viscosity remains constant. Thus, the polymer solution does not exhibit any shear-thinning in the bulk or in confinement.

As shown in Fig. 11, we notice however a significant decrease of the effective viscosity with confinement, which may involve slippage at the wall. Indeed, polymer solutions are well known to exhibit large slip length. Further interpretations will be the object of a future publication.

**Fig. 11** Viscosity of a \( 2 \times 10^5 \) g mol\(^{-1} \) molecular weight polyacrylamide solution at 5 wt\% in water as a function of the channel height \( h \) for a given shear rate of 100 s\(^{-1} \), estimated by \( \tilde{\gamma} = 2v_0/h \). The bulk viscosity, measured on a stress-controlled rheometer is given for a shear rate of 100 s\(^{-1} \).

5 Conclusions

This work lays the foundation for the study of nanoscale flows using fluorescence photobleaching. A wide range of mean velocity measurements are obtained, together with an evaluation of diffusion coefficients. Considering fluorescein in water/glycerol mixtures, the accessible velocity range goes from \( 10^{-6} \) to \( 10^{-3} \) m s\(^{-1} \). The temporal evolution of the signal to noise ratio determines the measurement accuracy. As a result, the measurement of low velocities is limited by molecular diffusion. The minimum velocity for a small dye molecule in water is about \( v = 10^{-6} \) m s\(^{-1} \), which corresponds to \( \text{Pe} \approx 10^{-3} \). To measure high velocities, the limiting factor is the apparition of Taylor dispersion for \( \text{Pe} > 10^2 \), which corresponds to \( v = 10^{-3} \) m s\(^{-1} \) in our experimental conditions. Interestingly, by reducing the channel height, we push back the highest limit of the velocity range as compared to the one in the microchannels. Furthermore, 3D effects of the hydrodynamic dispersion are small as long as we focus on short times. Thus, fluorescence photobleaching benefits from excellent sensitivity below the micron scale. As the signal to noise ratio remains very good at a few hundreds of nanometres, we can reasonably hope that the technique is still usable with smaller channel heights. We noticed that thermal bonding of glass wafers often induces a buckling of the channel walls. Decreasing the channel height will require other bonding methods, such as anodic bonding. Our setup brings accurate measurements of fluid viscosities in nanoslits. Future work will deal with applications of fluorescence photobleaching techniques to the study of complex fluid flows in nanoslits.

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References


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