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# Water slippage over micro and nano structured surfaces

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# **Abstract (in English)**

The subject of this research is the systematic study of the dependence of water slippage inside microfluidic channels on the morphology and chemical nature of the solid wall.

A low-cost Micro Particle Imaging Velocimetry ( $\mu$ -PIV) apparatus has been designed and developed to perform the experimental characterization of the microflow dynamics.

The set-up is sufficiently versatile to image a pretty wide range of flow conditions present in microfluidic devices, allowing the determination of fluid velocity fields both in the bulk of the fluid by epifluorescence microscopy and at the interface with a transparent solid surface by the use of total internal reflection fluorescence microscopy (TIRFM).

We initially tested our apparatus against the recent results obtained by leading groups in the application of the  $\mu$ -PIV technique to the study of liquid-solid boundary conditions. In particular, we measured water slip length on flat surfaces as a function of the liquid-solid contact angle.

Then, we investigated the water slip dynamics in microchannels presenting a patterned floor.

Measurements on isotropically patterned surfaces, presenting a regular grid of pillars or dots as to model superficial roughness on the micron scale, generally showed a decrease in the value of the slip length with respect to the flat substrate.

Measurements performed as a function of the orientation of micrometric canals impressed on the floor of a microchannel with respect to the main flux direction, lead to new interesting experimental evidences. A confirmation of a reduction in the value of the slip length due to wall micropattering was obtained and, moreover, the effect that the anisotropy of a pattern on the floor has on the flow direction was investigated. By changing the orientation and the modulation of linear microstructures on a solid wall, it was possible to modify the flow direction even inside planar lithographic-made microchannels with a constant pressure gradient applied at both ends.

# **Abstract (in Italiano)**

L'argomento di ricerca riguarda lo studio sistematico della dipendenza dello scivolamento di acqua dalla morfologia e dalla natura chimica della superficie della parete su cui scorre il liquido all'interno di dispositivi microfluidici.

E' stato progettato e realizzato un sistema ottico a basso costo per misure di Micro Particle Imaging Velocimetry ( $\mu$ -PIV), per la caratterizzazione sperimentale della fluidodinamica su microscala. La versatilità dell'apparato è tale da permettere lo studio di flussi nelle diverse condizioni riscontrabili all'interno di dispositivi microfluidici: infatti, consente di determinare i campi di velocità del fluido nel bulk, utilizzando microscopia ad epifluorescenza, e all'interfaccia con una superficie solida trasparente, con microscopia a fluorescenza in condizioni di riflessione totale interna.

Le prime misure hanno riguardato la verifica di alcuni risultati recentemente ottenuti dai gruppi leader di  $\mu$ -PIV. In particolare, sono state effettuate misure della lunghezza di scivolamento di acqua su superfici piane al variare dell'angolo di contatto fra il liquido e il solido.

In seguito, è stata studiata la dinamica dello scorrimento dell'acqua all'interno di microcanali, in presenza di microstrutture impresse sulla superficie di base.

Misure su superfici che presentano strutture disposte isotropicamente (pilastri e buche), a simulare rugosità superficiali su scala micrometrica, hanno dimostrato in generale una riduzione della lunghezza di scivolamento rispetto alla superficie piana.

Misure effettuate al variare dell'orientazione di canali micrometrici impressi sulla base di un microcanale rispetto alla direzione del flusso, hanno portato a nuove interessanti evidenze sperimentali. E' stata ottenuta una conferma della riduzione nella lunghezza di scivolamento dovuta a microstrutturazione delle pareti ed, inoltre, è stato analizzato l'effetto che una strutturazione disposta anisotropicamente nel canale determina sulla direzione del flusso. Cambiando l'orientazione e la modulazione di una strutturazione lineare superficiale, è stato possibile modificare la direzione del flusso anche all'interno di canali microfluidici planari, fabbricati con tecniche litografiche, alle cui estremità venga applicato un gradiente di pressione costante.

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# Introduction

Microfluidics is an emerging, highly interdisciplinary field of research, lying at the interface between physics, chemistry, engineering and molecular biology, that presently attracts great industrial and academic interest due to its numerous potential applications and its scientific fascination.

Microfluidics is the science and technology of systems that process or manipulate small  $(10^{-9} \div 10^{-18} \text{ litres})$  amounts of fluids, using channels with dimensions of tens to hundreds of micrometres [1]. The possibility to extend liquid manipulations to small volumes, with precise dynamic control over concentrations, discovering and exploiting new phenomena occurring in fluids at the microscale level, represents microfluidics ultimate strength.

Miniaturization technology gave birth to this science in the 1990s: it is originally based on silicon micromachining, highly developed by the microelectronics industry. Since mid-1990, microfluidic fabrication technology moves towards plastic and polymer-based micromachining, together with the introduction of soft-lithographic techniques, that allow low costs and rapid prototyping for the devices.

A common feature to all microfluidic chips is their large surface-to-volume ratio. Accordingly, flow properties at the solid boundaries become a key factor in understanding the motion of liquids in microchannels.

A recently explored topic is the validity of the no-slip boundary condition, motivated both by the important role that drag reduction could play in microfluidic channels and the recent availability of accurate methods to measure the boundary slip lengths. The slip length is the distance behind the interface at which the liquid velocity extrapolates to zero. On nominally flat hydrophobic surfaces, it is generally rather small and lies in the range from tens to hundreds nm, which explains why the no-slip boundary condition is considered a good approximation in macroscopic flows, but the effect of a finite slip length on the fluid dynamics becomes important in channels of micrometer or nanometer size.

Moreover, very large slip lengths (up to a few  $\mu$ m) are observed on highly corrugated superhydrophobic surfaces. The origin of large slip lengths on these surfaces is related to the

trapping of extended zones of vapour between the wall and the liquid, inside the cavities of the surface roughness, that restricts the direct contact of the liquid with the wall to a very small fraction of the interfacial area, leading to the superhydrophobic properties of these surfaces.

Micro Particle Imaging Velocimetry ( $\mu$ -PIV) is an optical technique, that is widely employed in microfluidics to characterize flow patterns inside microchannels, and several authors have recently shown it to be very useful also for the determination of the slip lengths of water on different surfaces. Unfortunately the price tag of most  $\mu$ -PIV systems is still rather high, due to the use of expensive components such as high-power pulsed, single or dual, laser sources, high-end CCD cameras and synchronizing electronics.

The objectives of this PhD project were:

- the design and development of a low-cost μ-PIV system, that could be sufficiently versatile to image a range of flow conditions in microfluidic devices and that could allow the determination of fluid flow velocities both in the bulk of the fluid and at the interface with a solid surface;
- the study of water slip dynamics over flat surfaces and in particular the determination of water slip lengths on different substrates, to test the validity of our μ-PIV measurements when compared to the results of other research groups, leader in μ-PIV, present in literature;
- the application of the μ-PIV system to the study of water slippage over micro and nanostructured surfaces, measuring water slip lengths on variably patterned substrates and characterizing the effect that interface pattering might have on the flow geometry inside a microchannel.

The thesis is structured as follows.

**Chapter 1** introduces the physics of the fluids at the micron scale, including the hydrodynamics, the problem of the liquid-solid boundary conditions and a brief presentation of interface phenomena. **Chapter 2** deals with the experimental characterization of slip and provides a picture of the present results of boundary condition investigations. **Chapter 3** presents the fundamentals of  $\mu$ -PIV, an high spatial resolution optical technique to characterize microscopic flows. **Chapter 4** provides a detailed description of the  $\mu$ -PIV system that we have designed and developed and that we use to study water slippage in microchannels. **Chapter 5** shows the results of our  $\mu$ -PIV investigations on water slip dynamics over flat substrates and, in particular, presents slip length estimates on different substrates as a function of the contact angle. **Chapter** 

**6** collects the results of our  $\mu$ -PIV investigations on water slippage over patterned substrates, dwelling on the calculations of slip length on the differently microstructured substrates and on the effect that interface pattering has on flow geometry in a microchannel. The final chapter **Conclusions** summarizes the main conclusions of this project and points out the future work.

# **Chapter I**

# PHYSICS OF FLUIDS AT THE MICROMETRIC SCALE

#### I.1 Introduction

For the physics of the fluidic systems we are taking into consideration, we might think that the micrometer is a scale at which nothing out of the ordinary should happen. In fact, in simple liquids, for example, molecular sizes, intermolecular distances and correlation lengths are on the order of a nanometer, much smaller than the dimensions of an ordinary microsystem. Anyway, there are significant situations where the macroscopic description must be modified or even abandoned.

Arguing in terms of the scaling laws that govern physical quantities in a given system, it is evident that new force equilibria dominate the physics of microsystems. The characteristic geometries of microfluidic devices present large surface area-to-volume ratios and interfacial properties are accordingly given to play a great role. Forces of gravity determine effects that are generally negligible with respect to action of capillary forces. Mass transport in microfluidic devices is generally dominated by viscous dissipation and inertial effects may be ignored [2, 3]. Considerations concerning these unusual force balances lead to the evidence that it is often advantageous to work with miniaturized devices rather then with devices operating at the macrometric scales.

#### I.2 Hydrodynamics of microfluidic systems

#### Newtonian fluids

Figure (I.1) illustrates a shearing experiment, where a material of thickness *h* is confined between two parallel plates. The lower plate is held stationary and the upper plate is pulled with a force *F*, acting in direction *x* over the area *A* for a certain time *t*. The plate exerts a shear stress  $\sigma$  on the material immediately below it and causes a deformation from the equilibrium shape in it,  $\gamma = \tan \theta$ , where  $\theta$  is defined as in Figure (I.1). When the force is removed, the material will either behave elastically, recovering its original shape, or viscously, remaining in its new position (a flow has occurred), or plastically, if a partial recovery has taken place. These three types of response are characteristic of solids, liquids and plastic materials, respectively, but many materials exhibit a range of intermediate behaviours, depending on the time scale of the application of the stress and on the time of observation.



Figure (I.1). Liquid flowing between two parallel plates under an applied shear stress.

When the shear stress (shearing force/area fluid contact) is directly proportional to the rate of strain within the fluid, the fluid is said to be Newtonian and the flow is described with the following expression:

$$\sigma = \eta \cdot \frac{d\gamma}{dt}$$

where  $\eta$  is the dynamic viscosity (expressed in Pa·s) and  $\frac{d\gamma}{dt}$  is the velocity gradient within the distance *h*, the so-called shear rate (or strain rate). If the velocity of the upper plate is  $u_x$ , then

$$\frac{d\gamma}{dt} = \frac{d(\tan\theta)}{dt} = h^{-1} \cdot \frac{dx_s}{dt} = \frac{u_x}{h}$$

where *h* and *x<sub>s</sub>* are defined as in Figure (I.1). The viscosity of Newtonian liquids is simply a function of temperature and does not depend on shear rate, up to very high values of the shear rates. Newtonian liquids are normally made of low molecular weight molecules. Examples of liquids that in normal conditions behave in a Newtonian manner are water (at 20 °C,  $\eta = 1.002$  mPa s), olive oil ( $\eta = 99$ mPa s) and glycerol ( $\eta = 2330$  mPa s). Non-Newtonian liquids include a wide category of liquids (polymers, dispersions, etc.) whose behaviour is more complex, and in particular whose viscosity depends critically on the shear rate.

#### The Navier–Stokes equations

Hydrodynamics are governed by two differential equations, referred to as the Navier–Stokes equations, which can be derived in the hypothesis that the molecular nature of the fluid can be neglected and the liquid can be treated as a continuum. Considering the plot in Figure (I.2), the

continuum assumption holds whether the fluidic system presents a "fluid particle" (a volume containing a sufficiently large number of particles, much smaller than the size of the system) which can be located on the density plateau. The local values of the fluid's kinematic and transport properties for a fluid particle can be considered as the point values for the continuously varying fluid properties.



Figure (I.2). Continuum assumption in fluids illustrated by a thought experiment that consists in measuring the density of a fluid sample of volume L<sup>3</sup>.

Furthermore, we assume that the liquid is incompressible, which for water and aqueous solutions is a good approximation (the density of water changes by only 0.01% if the pressure increases from 1 to 2 atm). The incompressibility criterion, together with the principle of mass conservation, leads to the first of the two equations, the so-called continuity equation, which in vectorial form is:

$$\nabla \cdot \vec{u} = 0$$

where u is the velocity vector describing the liquid flow.

The second of the Navier–Stokes equations describing the velocity field in a Newtonian liquid is obtained by applying Newton's second law of motion to a small block of fluid:

$$\rho\left(\frac{\partial \vec{u}}{\partial t} + \vec{u}\nabla \vec{u}\right) = \vec{F} - \nabla p + \eta \nabla^2 \vec{u}$$

where  $\rho$  and  $\eta$  are the density and dynamic viscosity of the liquid, respectively, and p is the pressure.

The terms on the right of the equal sign represent the total force per unit volume acting on the block of fluid. *F* is the total long-range force per unit volume: for example, it may be the gravitational force  $F = \rho g$ . The terms  $(-\nabla p + \eta \nabla^2 \vec{u})$  express the stress force per unit volume and  $(\eta \nabla^2 \vec{u})$  represents the viscous force.

From Newton's second law of motion, the terms on the right of the equal sign can be expressed by the mass times the acceleration per unit volume. Writing the acceleration in terms of the velocity field, we obtain the term on the left.

When solving these equations it is necessary to specify boundary conditions. At a solid-fluid interface it is usually assumed that the normal component of the fluid velocity vanishes and the tangential component equals the local velocity of the surface (no-slip boundary condition).

#### **Reynolds numbers in microsystems**

Stationary incompressible flows characterized by the spatial scale l (a scale controlling all the microchannel dimensions and whose variations maintain constant aspect ratios) are described by one dimensionless number, the Reynolds number Re, which is expressed by:

$$\operatorname{Re} = \frac{UL}{v}$$

where v is the fluid kinematic viscosity

$$v = \eta / \rho$$

and U is the characteristic velocity of the fluid in a channel of length L and transverse dimensions b, along which a constant pressure difference  $\Delta P$  is applied

$$U \sim \frac{b^2 \Delta P}{\eta L} \sim \frac{\Delta P}{\eta} l.$$

The physical significance of the Reynolds number is that it is a measurement of the ratio between inertial forces and viscous forces. Flow patterns are functions of the Reynolds number: a value  $\text{Re} \leq 1500$  typically indicates a laminar flow and flows presenting  $\text{Re} \geq 1500$  are increasingly likely to be turbulent.

In terms of scaling laws, the Reynolds number satisfies the following expression:  $\text{Re} \sim l^2$ , which allows us to observe that miniaturization tends to favour low Reynolds numbers.

Typical fluid velocities in microfluidic systems do not exceed 1 cm/s and, for channel widths on the order of tens of  $\mu$ m, we are limited to Reynolds numbers lower than 10<sup>-1</sup>,: these values correspond to flows whose features clearly contradict the day-to-day human experience with fluids.

When Re is very small, the non-linear terms in the Navier-Stokes equation for momentum conservation disappear, resulting in the Stokes flow equation:

$$\vec{F} - \nabla p + \eta \nabla^2 \vec{u} = 0.$$

In the absence of free surfaces or interfaces, the flows governed by the Stokes equation posses remarkable properties: linearity in velocity, reversibility, uniqueness of solution, reciprocity, and the existence of a minimum of kinetic energy dissipation.

#### I.3 Boundary conditions and slip length

#### Historical foundations

Ample discussions on the nature of the hydrodynamic boundary conditions have been conducted by several scientists between the 18th and the early 20th centuries (see the historical reviews [4, 5, 6] and references therein).

Bernoulli, Coulomb, Navier, Poisson, Poiseuille, Stokes, Helmholtz, Couette, Maxwell, Taylor, just to mention a few distinguished names, faced the subject and proposed different solutions for the boundary conditions, not supported by clear experimental evidence, that can be summarized in the following three alternative cases (see Figure (I.3)).



Figure (I.3). Schematic representation of the no-slip (1), stagnant layer (2) and partial slip (3) boundary conditions. The length of the arrows is proportional to the velocity of the liquid.

1) The no-slip boundary condition

For a fluid flowing across a solid surface, no slip of liquids is observed. The fluid velocity at the solid surface is the same as that of the surface and gradually varies when moving away from the surface into the fluid.

#### 2) The stagnant layer boundary condition

A thin layer of liquid is firmly attached to the solid surface, and the rest of the bulk liquid slips over this layer. If the solid is of the same material everywhere, the thin liquid layer has a constant thickness that depends on the curvature of the wall, on the type of liquid and solid, and on the temperature. The attached liquid layer thickness becomes zero for liquids which do not wet the solid and liquid slips over the surface.

3) The partial slip boundary condition

A liquid may slip on a solid surface and the slipping would be opposed by a frictional force proportional to the velocity of the fluid relative to the solid.

Navier first formulated the partial slip boundary condition in 1823, in the form of a linear equation, introducing the idea of slip length as the distance beyond the liquid-solid interface at which the liquid velocity extrapolates to zero. The slip length b is related to the velocity of the liquid at the wall  $u_r$  by the expression:

$$u_r = b \frac{\partial u_b}{\partial z}$$

where  $u_b$  is the velocity of the fluid in the bulk and z is the direction perpendicular to the wall. Nowadays the Navies' characterization of slip is still the most commonly used.

Till the mid-20th century, there had been no unambiguous determination of the boundary condition at the liquid-solid interface. It was generally accepted that slip, if it did exist, could not be observed: a stagnant layer of liquid was too thin or a finite liquid-solid relative velocity at the surface was too small to make any observable difference in the experimental results on the macroscopic scale. The presence of slip would have been detected only using experimental techniques with resolution far beyond that available at the time.

Thus, the no-slip boundary condition has been widely employed for all practical applications.

#### Physics of slip for simple liquids

Given the liquid-solid boundary condition expressed in terms of the slip length (or Navier length)

$$u=b\frac{\partial u}{\partial z},$$

the length b can be related to the solid friction coefficient k, equating the expressions for the effects of the viscous shear stress at the wall and in the fluid, getting:

$$\sigma = ku_s = \eta \left| \frac{du(z)}{dz} \right| \Longrightarrow b = \frac{\eta}{k}$$

where  $u_s$  is the velocity of the fluid at the wall.

For a pure shear flow, b can be interpreted as the fictitious distance below the surface where the no-slip boundary condition would be satisfied (see Figure (I.4)) [7].



Figure (I.4). Interpretation of the Navier slip length.

Let us consider a molecule of liquid close to a solid surface. The molecule is subjected to the attractive Van der Waals forces exerted by the surface itself, which tend to trap the molecule at a molecular site, and to the action of the hydrodynamic stress, which tends to take the molecule far from the site. The condition for the detachment of the molecule is reached when the two effects are equal: the fluid is said to be subjected to a critical shear. When a fluid is subjected to a shear which is higher then the critical shear, slip is expected to occur.

Other situations where slip is expected regard flows of non-Newtonian fluids, such as polymer solutions [8, 9, 10, 11], and gas flows in devices with dimensions that are on the order of the mean free path of the gas molecules [12, 13]. Wall slippage was sometime suspected in flow on non-wetting surfaces [14].

Quite recently, substantial slip phenomena ( $b \sim 1 \ \mu m$ ) have been observed between ordinary fluids and atomically smooth surfaces, that have been made non-wetting with a deposition of a molecular layer, for values of shear lower then the critical shear [15, 16], which has been definitely surprising.

A possible explanation to these results was given by De Gennes [17], suggesting the formation of a gaseous film at the solid-liquid interface.

The origin of the film is not clear. If the liquid entering the channel is, for some reason, supersaturated with a certain gas, a pressure drop in the channel could indeed promote the release of gas bubbles. And, if the solid surface is not very wettable, the bubbles should preferentially nucleate at the liquid-solid interface, as they do in a glass of Champagne. Then, the shear flow should stretch them into a flat film, provided the shear stress is strong enough.

De Gennes calculates the corresponding slip length in the hypothesis of moderate a Knudsen number [18] for the gas:

$$b \cong \frac{\eta}{\rho u_{therm}}$$

which gives, choosing typical values for the kinematic viscosity ( $v = 0.15 \text{ cm}^2/\text{s}$ ) and for the thermal velocity in the gas ( $u_{therm} = 300 \text{ m/s}$ ), slip lengths on the order of a few micrometers.

#### Hydrodynamics of liquid flows with or without slip

Let us assume that a flat Poiseuille flow is established in a microchannel (height *h*, width *w* and length *L*), so that the velocity *u* only depends on the distance from the surface, which is described by the z coordinate (with the origin of the axes placed on the axis of symmetry of the channel, as in Figure (I.5)):  $\vec{u} = u(z)\vec{e_x}$ .



Figure (I.5). Representation of the microchannel, with corresponding notations.

We work in the hypothesis of an incompressible Newtonian fluid at small Reynolds numbers. A constant pressure gradient -G is applied along the channel:

$$-G = \frac{p_{out} - p_{in}}{L} = \frac{\partial p}{\partial x}$$
 and  $-G = \eta \frac{\partial^2 u}{\partial z^2}$ .

The transport efficiency is expressed by the volumetric flux:

$$Q = \int_{-h/2}^{h/2} u(z) w dz \, .$$

For negligible external volumetric forces, the equations for the stationary flow in the microchannel reduce to:

$$\nabla p = \eta \nabla^2 \vec{u}$$
 and  $\nabla \cdot \vec{u} = 0$ 

The velocity profile is can be determined assuming the appropriate boundary conditions. We examine three possible cases.

• Absence of slip:  $u(\pm h/2) = 0$ .

$$u(z) = \frac{G}{2\eta} \left( \frac{h^2}{4} - z^2 \right).$$

The volumetric flux is:

$$Q = \frac{Gwh^3}{12\eta}.$$

• Equal slip length at both walls:  $u(\pm h/2) = \mp b \frac{\partial u}{\partial z}$ .

$$u(z) = b\frac{Gh}{2\eta} + \frac{G}{2\eta}\left(\frac{h^2}{4} - z^2\right).$$

The liquid velocity is increased of a constant value given by the slip velocity at the walls:

$$u_{wall} = u(\pm h/2) = b \frac{\partial \gamma}{\partial t} = b \frac{Gh}{2\eta}.$$

The maximum velocity, which is reached in the middle of channel, is given by:

$$u_{\max} = u(0) = \frac{Gh}{2\eta} \left( b + \frac{h}{4} \right) = \frac{h}{4} \frac{d\gamma}{dt} \left( 1 + \frac{4b}{h} \right).$$

Therefore, the ratio of the two velocity can be expressed by:

$$\frac{u_{wall}}{u_{\max}} = \frac{4b}{h} \left( 1 + \frac{4b}{h} \right)^{-1} \approx 4\frac{b}{h} \,.$$

The volumetric flux is:

$$Q = \frac{Gwh^3}{12\eta} + b\frac{Gwh^2}{2\eta},$$

that is its value is increased of a factor  $\left(1+6\frac{b}{h}\right)$  with respect to the case of no-slip.

• Slip length *b* at one wall and no-slip at the other:  $u(-h/2) = b \frac{\partial u}{\partial z}$  and u(+h/2) = 0.

$$u(z) = \frac{G}{2\eta} \left( \frac{h^2}{4} - z^2 \right) + b \frac{Gh}{4\eta} \frac{1}{1 + b/h} \left( 1 - \frac{2z}{h} \right);$$
$$Q = \frac{Gwh^3}{12\eta} \left( 1 + 3\frac{b}{h} \frac{1}{1 + b/h} \right).$$

The expressions for the slip velocity and the volumetric flux, approximated to the first order in  $\frac{b}{h}$ , reduce to:

$$u_{wall}(-h/2) = b\frac{Gh}{2\eta} = 4u_{max}\frac{b}{h};$$
$$Q = \frac{Gwh^3}{12\eta} \left(1 + 3\frac{b}{h}\right).$$

In Figure (I.6), velocity profiles in a microchannel are shown for the three alternative boundary conditions, for the ratio  $b = \frac{h}{10}$ .



Figure (I.6). Velocity profiles in case of absence or presence of slip at the walls of a channel, for a slip length b=h/10. Three cases: no-slip boundary condition for both walls (straight line); no slip at the top wall and slip at the bottom wall (dashed line); slip at both walls, with the same slip length (dotted line).

#### I.4 Interfacial phenomena: wetting and capillarity

#### Surface energy

The large surface-to-volume ratios of microfluidic devices render surface effects increasingly important.

All interfaces are associated with a surface density energy Y, called "interfacial tension" or "surface energy", whose physical origin is related to the intermolecular interactions existing between surfaces.

For example, the energy *E* required to separate two planes bound by Van der Waals adhesion is given by:

$$E = \frac{A}{12\pi D_0^2} = 2\mathbf{Y} \,,$$

where A is the Hamaker constant (Lifshitz), strongly depending on the nature of the solids or liquids forming the interface, and  $D_0$  is a microscopic length scale.

#### Wetting and capillarity

The phenomenon of wetting takes place at three interfaces (solid-gas, liquid-solid and liquidvapour) connected by a contact line, as in Figure (I.7).

Working in terms of surface energies, the tendency for a fluid to adhere or "wet" the solid surfaces is described by the "spreading coefficient" *S*:



Figure (I.7). Diagram showing a solid-liquid-gas contact line.

$$S = Y_{SG} - Y_{SL} - Y_{LV}$$

where  $\mathbf{Y}_{SG}$ ,  $\mathbf{Y}_{SL}$  and  $\mathbf{Y}_{LV}$  denote the surface tension of solid-gas, solid-liquid and liquid-vapour interfaces, respectively. For example, the surface tension of water is well predicted by:

$$Y_{LV} = (0.076 - 0.00017T \text{ K}^{-1}) \text{ N/m}$$

where T is the temperature of the system.

The system is in equilibrium when the resultant of the forces is zero at the contact line. This condition is satisfied when:

$$Y_{SG} - Y_{SL} = Y_{LV} \cos\theta$$
 (Young's relation) or  $\cos\theta = \frac{S}{Y_{LV}} + 1$ .

There are three alternative situations according to the value of the spreading coefficient:

- 1.  $S \ge 0$ . Total wetting. Mechanic equilibrium cannot be reached. A small drop of the liquid on the solid surface spreads out to form a uniform layer. The contact angle  $\theta$  is zero.
- 2.  $S \le -2 Y_{LV}$ . No wetting. Mechanic equilibrium cannot be reached. The liquid adopts a shape that minimizes the contact area with the solid.
- 3.  $-2 Y_{LV} < S < 0$ . Partial wetting. Mechanic equilibrium is reached for a contact angle defined by the Young's relation.

A surface that a liquid can partially wet is said to be "hydrophilic" when the contact angle is smaller then 90°, and a surface is called "hydrophobic" when the contact angle is higher then 90°.

A consequence of the tensions present along the interfaces and of the force balance expressed by the Young's equation, is the so-called phenomenon of capillarity.

We see in Figure (I.8) how a glass capillary immersed into a reservoir of water is filled by water up to a certain height h so that the hydrostatic pressure given by the water column equals the pressure variation upon crossing the curved interface:

$$\rho_{water}gh = \Delta p_{air-water} = \frac{2Y_{LV}\cos\theta}{r_0} \,.$$



Figure (I.8). Capillarity of liquids filling small channels.

Capillarity is the tendency of liquids to draw themselves into small channels made of wettable materials. It makes difficult to fill hydrophobic channels and empty hydrophilic channels.

#### Wetting on rough surfaces

The wetting properties of a solid surface are affected by two factors: the surface energy and the surface geometry. The surface energy is an intrinsic property of a material that can be controlled by chemical modification, such as fluorination. The surface roughness of a hydrophobic solid can be tuned in order to increment its hydrophobicity and obtain a superhydrophobic solid surface [19, 20].

Two distinct theories are classically proposed to model the apparent contact angle on a rough surface. On one hand, roughness increases the surface area of the solid, which geometrically enhances hydrophobicity (Wenzel model) [21]. On the other hand, air can remain trapped below the drop, which also leads to a superhydrophobic behaviour, because the drop sits partially on air (Cassie model) [22].

For both the Cassie and Wenzel models of superhydrophobic states, minimizing the surface energy of a drop yields its apparent contact angle  $\theta^*$  on a rough hydrophobic substrate, as a function of the Young's contact angle  $\theta$ , determined on a flat surface of the same nature.

In the Wenzel model, the surface roughness r is defined as the ratio of the actual over the apparent surface area of the substrate (r > 1), and we have:

#### $\cos\theta^* = r\cos\theta.$

For a Cassie drop, the contact angle is an average between the value on air (that is 180°) and on the solid (that is  $\theta$ ). Denoting  $\phi_s$  as the fraction of solid in contact with the liquid ( $\phi_s$  is dimensionless and  $\phi_s < 1$ ), we find:

$$\cos\theta^* = -1 + \phi_s (1 + \cos\theta)$$



Figure (I.9). The two models of superhydrophobicity. For a moderate hydrophobicity (90°<  $\theta$  <  $\theta_c$ , where  $\theta$  is the contact angle on a flat surface and  $\theta_c$  is fixed by the texture design), the apparent contact angle  $\theta^*$  should be given by the Wenzel model. If  $\theta > \theta_c$ , air remains trapped below the drop, which sits on a composite surface made of solid and air;  $\phi$ s is the fraction of solid in contact with the liquid (Cassie regime). The Cassie regime can also be observed for  $\theta < \theta_c$ , in spite of a higher energy: this metastable situation is represented by the dotted line.

The Cassie equation holds for substrates either very hydrophobic (large  $\theta$ ) or very rough (large r). For these two limit situations, the Wenzel formula predicts a total drying of the surface ( $\theta^*=180^\circ$ ), which is not physical because of the contact that must exist between a drop and its substrate. Similarly, the Wenzel equation holds for solids that are slightly hydrophobic ( $\theta$  just above 90°): the presence of air pockets, which would imply that many liquid-vapour interfaces of high surface energy exist, is not favoured.

The two equations are successively obeyed as the contact angle increases, and the threshold value  $\theta_c$  between the two regimes is given by equating them. This yields to:

$$\cos\theta_c = \frac{\phi_s - 1}{r - \phi_s}$$

a quantity whose value lays between -1 and 0.

Figure (I.9) summarizes these results: the apparent contact angle  $\theta^*$  is plotted as a function of the Young angle  $\theta$ , and the expected behaviours are shown by a solid line. The Cassie regime is also represented at a moderate hydrophobicity (90° <  $\theta$  <  $\theta_c$ ; dotted line) to stress its metastability in this region.



Figure (I.10). Compression of a millimetric water drop between two identical microtextured hydrophobic surfaces. The apparent contact angle  $\theta^*$  is measured as a function of the imposed pressure  $\Delta P$  (open points). The filled points are obtained by varying the size of drops deposited on a single microtextured surface, which modifies the pressure  $\Delta P$  applied by the drop on the surface. The upper and lower dotted lines are, respectively, the values measured for a large drop initially formed either by deposition or condensation. [20].

A comparison between the interfacial energies associated with the Wenzel and the Cassie situations confirms that air pockets should be favoured only if  $\theta$  is larger than  $\theta_c$  [23].

The two states are very different from their adhesive properties: Wenzel drops are highly pinned and present high contact angle hysteresis, while Cassie drops are characterized by supehydrophobicity and by so-called self-cleaning effect.

Irreversible transitions between Cassie and Wenzel states can be induced, after deposition of a drop on a microtextured surface, by increasing the pressure exerted by this drop on its substrate. Pressing on a drop may change the contact angle and varying the drop size can also influence the value of  $\theta^*$  (see Figure (I.10)).

By applying a pressure on the drop, it is possible to induce an irreversible transition towards a new regime, that can be interpreted as a Wenzel state, for which the solid-liquid interface follows the texture of the solid surface. The contact angles in both states are comparable (the Wenzel

angle is slightly smaller than the Cassie one, but both are significantly larger than the Young angle  $\theta$ ), but the hysteresis is dramatically affected by the change of state (10 ÷ 20 times larger in the Wenzel regime): a drop in this regime adheres much better to its substrate, contrasting with what is expected in a superhydrophobic situation, and the self-cleaning effect is totally suppressed.

# **Chapter II**

# **EXPERIMENTAL CHARACTERIZATION OF SLIP**

#### **II.1 Introduction: slip or no slip for Newtonian liquids?**

The dynamics of flows in micron-sized fluidic devices can be accurately described only if the physics of the flows at the interface with solid surfaces is entirely understood. One important step to reach this goal consists in determining the correct boundary conditions.

Finding the exact boundary conditions that apply to flows in confined geometries is not only of great interest for the scientific community, but would also improve the understanding of several industrial and technological processes, such as flows in porous media, electro-osmotic flows, particle aggregation, sedimentation and lubrication. It would also contribute to a fundamental advance in the understanding of the physics of flows in biological processes.

The no-slip boundary condition cannot be derived from first principles or hydrodynamic considerations, but it has been demonstrated in numerous macroscopic experiments. The physical origins of this condition are believed to be mainly the trapping of liquid in cavities and crannies present on the solid surface and the attractive forces between the molecules of the solid and those of the liquid.

In recent years, the topic of the flow of Newtonian liquids at the micro scale has gained new interest and numerous research works have risen doubts regarding the assumption of universality of the no-slip boundary condition, showing that, under certain circumstances, fluid slip takes place at the solid boundary.

Several new modern methods of investigation of the boundary conditions for liquids flowing over solid surfaces are currently being employed in experimental, computational and theoretical studies.

The physical picture that emerges from the recent researches is that of a complex behaviour at the liquid-solid interface, involving an interaction of many physical and chemical parameters including wetting, shear rate, pressure, surface charge, surface roughness, impurities and presence of dissolved gas.

#### **II.2** Experimental techniques for the investigation of boundary conditions

The hydrodynamic boundary condition will always appear to be one of no-slip, unless the flow is examined on a length scale comparable to the slip length.

Given the small values of slip length for Newtonian liquids measured so far (from a few nanometres to a few micrometres), no consequences of slip on the flow of liquids on a macroscopic scale are expected.

Only extremely accurate techniques, with high spatial resolution and suitable for interfacial flow measurements, can detect the effects of slip.

These investigations require the confinement of a fluid in two dimensions between surfaces, as in capillary flows and surface force measurements, or the use of surface sensitive techniques, as the quartz crystal microbalance and the near surface imaging of flows using tracers.

As a consequence of the MEMS technology revolutionary application to chemistry, biology and biomedicine in the 1990s and of its subsequent development, a variety of techniques are now available that are capable of probing fluid flow on small scales and are therefore suitable for the study of boundary conditions.

Evidence for slip has recently been collected by different research groups using a range of techniques: the variety of employed methods assures that boundary slip is not an artefact of a single experiment.

Nowadays, a challenging goal for scientists would be to frame the obtained results in an accurate and consistent physical picture of the phenomena occuring at the interface.

There follows a brief description of the main experimental methods applied to measure slip lengths.

#### Techniques based on force or displacement measurement

The atomic force microscope and the surface forces apparatus are instruments used to measure either the hydrodynamic drainage force between two approaching surfaces or the displacement of one surface in response to the oscillation of the other surface [24].

The steady or oscillatory motion of a curved body is imposed perpendicularly to a solid surface, and the instantaneous resistive force, which may be compared with that from a model of the fluid motion in the gap, is measured assuming no-slip or slip boundary conditions.

The two most common narrow-gap geometries are either a sphere of radius a close to a planar surface or two crossed cylinders of radius a. For both cases, the viscous force F opposing the motion has the form [25]:

$$F = -f * \frac{6\pi\eta a^2 U}{d}$$

where U is the instantaneous velocity of the moving body, d the minimum distance between the two surfaces, and  $f^*$  the slip factor.

If the no-slip boundary condition is valid ,we have  $f^* = 1$ , while when a slip condition is realized,  $f^* < 1$  and depends on the slip lengths on both surfaces.

The expression for  $f^*$  in the case of equal slip lengths *b* is given by [26]:

$$f^* = \frac{d}{3b} \left[ \left( 1 + \frac{d}{6b} \right) \ln \left( 1 + \frac{6b}{d} \right) - 1 \right].$$

When  $d \ll b$ ,  $f^*$  goes to zero as  $f^* \sim d \ln(6b/d)/3b$ , so that the viscous force F only depends logarithmically on d.

#### • Surface force apparatus

The surface force apparatus (SFA) was originally designed to measure surface forces between two half cylindrical mica surfaces (see Figure (II.1)) [27, 28].

The separation between the two atomically smooth mica surfaces can be measured by interferometry with sub-nanometer resolution. One surface is mounted rigidly on a piezoelectric transducer and the other on a calibrated spring. In early measurements of drainage force, the surface mounted on the transducer is driven a known distance for a set period and then the drive is stopped. The surface separation is monitored as the fluid of interest is drained out from between the two surfaces: the difference between imposed and observed motions allows a calculation of the instantaneous force acting on the surfaces. [29]. In later models, oscillatory measurements are performed [16, 30, 31]: the surface mounted on the gap between the surfaces is monitored at a range of surface separations.

This is a well-established high-resolution technique, whose main disadvantages consist on the limited range of materials that can be utilized, on the complexity of the resulting flow field, and on the possible alteration of the measurements due to the presence of contaminating platinum particles on the mica surfaces (these surfaces are cut with hot platinum wires).

#### • Atomic force microscopy

Atomic force microscopy (AFM) was invented by Binnig, Quate and Gerber [32] and is used in a similar manner to SFA to measure drainage forces between two approaching surfaces [33, 34, 35, 36, 37, 38].



Figure (II.1). SFA. Atomically smooth mica surfaces are attached to cylindrical lenses which are arranged perpendicularly and immersed in a liquid. The distance between the surfaces is measured using interferometry. The drainage force arising from the normal approach or oscillatory motion is measured using the deflection of a spring, on which one of the surfaces is mounted [27].



Figure (II.2). Atomic force microscope block diagram.

Surface forces can be measured by placing a small colloidal sphere on the tip of a flexible cantilever beam. The substrate is driven towards the sphere, either at its resonance frequency or at fixed velocity, and the deflection of the beam is accurately monitored using laser light. Since the mechanical properties of the beam are known, deflection measurements can be used to infer the instantaneous drainage force on the colloidal particle.

The AFM technique is very accurate. A wide rage of substrates, even moderately rough, can be used and a large range of shear rates are accessible. Anyway, the exact determination of the approach rate and of the position of zero surface separation (in particular for rough surfaces) are not trivial. Moreover, the difficult interpretation of the instrumental response for oscillatory measurements, can be even more complicated by the presence of increasingly significant inertial effects for time scales on the order of the inverse of the resonance frequency of the cantilever.

#### Techniques tracing the fluid flow near a boundary

#### • Fluorescence recovery after photobleaching

The group of Leger uses fluorescent recovery after photobleaching (FRAP) to directly observe the flow of a fluid near a solid surface (see Figure (II.3)) [39, 15, 40, 41].

Fluorescent molecules are dissolved in the liquid being studied in a very low concentration and a region of the liquid is illuminated with an evanescent wave. Flow is observed by photobleaching the fluorescent molecules with a high intensity laser beam and monitoring the recovery in fluorescence as new molecules enter the photobleached region during a shear flow. Slip results in a faster fluorescence recovery, as the fluid motion at a given distance from the wall is faster when slip is present. Fluorescence recovery is sensitive to the average of the flow velocities within the evanescent field, as diffusion normal to the surface is rapid with respect to the experiment time scale.

There are two main advantages in the choice of this method: first, it is a direct investigation, for the actual flow of the liquid can be followed; second, the geometry of the flow is simple.

The main drawback of the technique is that it does not provide an absolute measurement of the fluid flow: only differences in slip behaviour can be observed by comparing fluorescence recovery under different surface conditions. Therefore, it is possible to quantify the slip in one system relative to another, but the slip in each system cannot be determined absolutely.

#### • Fluorescence cross-correlations

Fluorescent probes excited by two similar laser focuses are monitored in two small sample volumes separated by a distance of a few micrometers. Cross-correlation of the fluorescence intensity fluctuations due to probes entering and leaving the observation windows allows

determining both direction and intensity of the flow. The measured velocities are averaged over the focal size of microscope and the characteristics of the excitation laser.



Figure (II.3). Fluorescent molecules present in the liquid and in proximity to the bottom surface are excited using an evanescent wave. At time t = 0 the fluorescent molecules in a small region close to the surface are photobleached using a high intensity laser beam. As the liquid flows ( $t = t_1$ ), unbleached molecules enter the region of interest, replacing the bleached molecules. The recovery of the fluorescence intensity is measured as a function of time, and information is obtained on the average velocity of the particles within the region of interest [6].

This experimental method was first proposed in [42]. Also Joly, Ybert and Bocquet [43] used fluorescence correlation spectroscopy, but they measured the spontaneous diffusion dynamics (under no shear) of colloidal tracers in a confined geometry. Since the tracers' dynamics is affected by the boundary conditions at the wall, measured differences in diffusion coefficients of the tracers were converted into different slip conditions at the walls.

#### • Particle Imaging Velocimetry

For a detailed description of this experimental technique, the reader is referred to the next chapter.

#### Capillary techniques

Numerous experiments on the slip boundary conditions are conducted using thin capillaries [44, 45, 46], performing accurate measurements of liquid transport through the capillary.

A slip boundary condition increases the fluid flow, compared to the absence of slip condition, of a quantity that is proportional to the slip length (see Paragraph I.3 and Figure (II.4)).

The effect of different factors (pressure, surface roughness, geometry, etc.) on the flow of simple liquids in microchannels is investigated, but a model can explain all experimental results is not available yet.



Figure (II.4). Capillary technique. A liquid is driven through a capillary of radius r by a pressure difference applied along its length l. The flow rate of the liquid is measured and compared with the predicted values.

Rapid improvements in flow rate detection methods [47], along with perfected methods for the fabrication and characterization of capillaries, may result in this technique to be extremely useful. In fact, the greatest source of error in measurements of flow through microchannels comes from the measurement of the inner diameter of the channels. The fabrication of channels that are atomically smooth, of constant known width and very clean is still problematic. Moreover, when the surfaces are modified by adsorption of surfactants or self-assembled monolayers, the coverage and wettability inside the channels cannot be tested independently.

#### Quartz crystal microbilances

A quartz crystal driven at or near its resonance frequency, the so-called QCM, can be used to probe fluid properties and the adsorption of material to the surface of the crystal. A QCM provides a very sensitive measurement of the amount and viscoelastic properties of the material adsorbed at the interface (see Figure (II.5)). The presence of a liquid in contact with the surface of the crystal dampens its oscillation and changes the resonance frequency, by changing the acoustic impedance of the device. Since this effect depends strongly on the degree of coupling at the surface–liquid interface, QCM can be used as a sensitive technique to probe boundary slip [48, 49].

Crystals typically oscillate at 5 MHz or higher, with nanometer amplitude, and therefore high shear rates are accessible. A range of substrates can be used and the influence of a wide range of

adsorbates can be studied, anyway it is unclear how the finite surface roughness of the layer of gold that coats the crystals may affect the response of the crystal and the interpretation of slip. Relating the crystal response to the slip behaviour and to a slip length b is not trivial and is necessarily model-dependent, though theoretical developments are improving fast [50, 51].



Figure (II.5). Solid and bulk liquid velocity profiles showing no-slip and slip length boundary conditions. The dashed line shows the no-slip velocity profile [50].

#### Other techniques

Other methods to study liquid-solid boundary conditions include the study of flow of thin films on spinning disks [52] and on rotating cylinders [53], droplets moving down an inclined surface [54], particle sedimentation rates [55, 56] and excitation of surface waves [57].

#### **II.3** Molecular dynamics simulations

Molecular dynamics simulations are a theoretical tool which allows investigation of liquids, and it has been extensively used to probe boundary conditions [58].

Molecular dynamics simulations integrate numerically Newton's law of motion for single atoms (or molecules), subjected to an interatomic (or intermolecular forces) determined by the interaction potential. Potentials used in simulations range from the Lennard-Jones two-body potential to more realistic potentials, including many-body or orientation-dependent interactions. The equations of motion are integrated forward in time, with appropriate numerical cut-offs and

with specified initial and boundary conditions. Usually initial positions are random and initial velocities are taken from a Boltzmann distribution, corresponding to the temperature of the distribution.

It is possible to slightly modify the equations of motion, to model evolution at constant temperature, either by coupling the system of atoms to a heat bath or by a proper rescaling of the velocities at each time step.
Interactions with a solid can take place by adding different wall atoms, either fixed on a lattice or coupled to a lattice with a large spring constant, to allow momentum transfer from the liquid but to prevent melting.

The intermolecular constants appearing in the expression of the Lennard-Jones potential allow variation of the relative intermolecular attraction between liquids and solids, which therefore mimics wetting behaviour.

Two types of flow can be driven: atoms that constitute the wall(s) are driven at a constant velocity and bulk liquid has a Couette-flow profile, otherwise, each liquid atom is acted upon by a body force and liquid has a Poiseuille-flow profile.

The method described above is used to study slip in different types of liquids [59, 60, 61, 62, 63, 64, 65].

Early simulations show no slip except near contact lines [61, 64], while more recent studies report that molecular slip increases with decreasing liquid-solid interactions [66, 59, 62], liquid density [65], density of the wall [67], and decreases with pressure [66].

The model for the solid wall, the wall-fluid commensurability and the molecular roughness are also found to strongly influence slip [67].

A great volume of calculations is required to simulate a reasonably sized volume of space for a fair duration, for continuum behaviour to be observed.

For computational reasons, simulations to date are limited to tens of thousands of atoms, which restricts the size and time scale of the simulated physical system. The three control parameters in the simulations are the molecular/atomic mass, the interaction energy, and the molecular/atomic size. Lengths are measured in units of the molecular/atomic size (~ few Å) and times in units of the molecular interaction time (~  $10^{-12}$  s). Simulated systems are therefore limited to volumes measuring tens of nanometers and time scales are limited to nanoseconds.

A consequence of this observation is, for example, that molecular dynamics simulations always probe systems with much higher shear rates than any experimental setup [7].

A significant problem in the interpretation of results of molecular dynamics simulations in the continuum limit is pointed out by Brenner and Ganesan in the case of particle diffusion near a solid surface [68], and regards the scale separation between molecular and continuum phenomena.

In short, the correct boundary condition in the continuum should arise asymptotically as a matching procedure between the outer limit of the inner (molecular) system and the inner limit of the outer (continuum) system. In this way, the change in the physical behaviour within a few intermolecular length scales of the surface is explicitly taken into account. Thus, slip lengths

should not be measured literally at the molecular scale, but arise as the extrapolation, at the boundaries, of the far field hydrodynamic results.

# **II.4 Dependence of slip on physical parameters**

# Wetting properties

Friction at the liquid-solid boundary is a function of the physical and chemical nature of both the solid and the liquid and, in particular, wetting properties play a crucial role in the physics of interfaces.

Since surface tensions originated from intermolecular forces and can be related to the thermodynamic work of cohesion and adhesion, the slip length can, in principle, be related to molecular kinetic phenomena at the solid-liquid interface. The first contribution to connect the slip length with the contact angle was by Tolstoi. Blake [69] later reviewed Tolstoi's theory and discussed the results regarding liquid adsorption at the solid surface. By means of molecular and thermodynamic theories, in case of partial wetting, Tolstoi derived:

$$\frac{b}{\delta} \sim \exp\left(\frac{\alpha A \mathbf{Y}_{LV}(1 - \cos\theta)}{k_B T}\right) - 1$$

where *b* is the slip length,  $\delta$ , *A* and,  $\alpha$  are the average distances between the centers of adjacent liquid molecules, the effective surface, and the fraction of *A* composed of solid surface (the liquid molecule is considered as a sphere and  $A = \pi (\delta/2)^2$ ). Thus, the slip length can increase with respect to  $\delta$  of orders of magnitude.

Another theory uses the fluctuation-dissipation theorem and Green-Kubo expression to derive the slip length, at the molecular scale, from equilibrium thermodynamics [70]. Using the Onsager's hypothesis of linear regression of fluctuations (small fluctuations around equilibrium can be described by the same equations that describe the relaxation from non-equilibrium) leads to a formula for the time dependent momentum correlation function in the liquid, function of both the slip length and the wall position. The boundary condition is found to be applied about one molecular layer inside the liquid and the slip length is given by:

$$\frac{b}{\delta} \sim \frac{D^*}{S_t c_{LS}^2 \rho_c \delta^3}$$

where  $D^* = D_{\parallel} / D_0$ ,  $D_{\parallel}$  is the collective molecular diffusion coefficient and  $D_0 = \frac{k_B T}{3\pi\eta\delta}$  is the

Stokes-Einstein estimate for the bulk diffusivity,  $S_t$  the structure factor for first molecular layer (both  $D^*$  and  $S_t$  are dimensionless numbers of order unity),  $\rho_c$  the fluid density at the first

molecular layer (number per unit volume), and  $c_{LS}$  the dimensionless liquid-solid coefficient of the Lennard-Jones potential.

In the case of complete wetting, the slip length is essentially zero as soon as the roughness is a few percent of the molecular size.

In a non-wetting situation the slip length can be up to two orders of magnitude above the molecular size and increases with the contact angle; as  $\theta$  goes to 180°, the slip length diverges as

$$\frac{b}{\delta} \sim \frac{1}{\left(\pi - \theta\right)^4}.$$

Slip is measured for systems in complete wetting and partial wetting and the amount of slip is *usually* found to increase with the contact angle, either systematically or only for non-polar liquids. However, because of the presence of contrasting results, the influence of wetting properties on slip certainly needs to be further investigated.

The main experimental results are summarized in the left plot of Figure (II.8), which shows however an overall poor correlation between slip and contact angle. Data would suggest that the level of hydrophobicity is not a primary factor in determining slip, even if many research groups see an increase in slip after modification of the surface wettability.

# Surface roughness

The results from experiments investigating the influence of surface roughness on boundary slip present deep discrepancies: there are both indications that molecular-scale roughness suppresses slip or its shear-dependence [15, 38, 30], and hints that slip increases with roughness [35].

Indeed, experiments of this kind are challenging for several reasons: for instance, 1) it is difficult to produce suitable surfaces of controlled roughness, 2) altering the surface roughness may result in additional undesired changes in the interface properties, 3) the interpretation of the results is complicated by the uncertainty in the wall position, and 4) an appropriate description of realistic surface roughness is not available yet.

Extensive theoretical work indicates that the magnitude and the length scale of roughness are important, but there is no general agreement as regards the influence roughness has on boundary slip. Again, the calculations provide conflicting results: let us cite, for example, three basically different conclusions. According to Hocking [71], irregularities on the surface give rise to apparent slip at the moving contact line of a liquid, because the displaced fluid remains in the hollows on the surface, thus facilitating slip of the moving liquid. According to Richardson [72], the energy lost through viscous dissipation as a fluid passes over and around surface irregularities is sufficient to ensure that it is effectively brought to rest. Finally, according to

Galea and Attard [73], roughness influences slip in a complex manner: there is a no-slip boundary condition for surfaces commensurate with the fluid (when liquid and solid present molecules of comparable size) and the boundary condition tends to become of slip for rougher and smoother, but non-commensurate, surfaces.

The interaction of roughness with surface energies can lead to the spontaneous dewetting of a surface and the appearance of a superhydrophobic state; in this situation, roughness increases slip by producing regions of gas-liquid interface at the solid boundary.

Let us consider a surface *S* (see Figure (II.6)) covered with a fraction *c* of roughness elements of height *a* in a liquid at pressure *p* and denote by *r* the ratio of real to projected surface area (r>1).



Figure (II.6). SEM images of microstructured hydrophobic surfaces, presenting spikes, designed to study the influence of the surface pattern on the contact angle [74].

The change in free energy  $\Delta G$  to dewet the apparent area (1-c)S between the roughness elements arises from surface energies and work done against the liquid:

$$\Delta G = r(1-c)S(\mathbf{Y}_{SG} - \mathbf{Y}_{LS}) + (1-c)S(pa + \mathbf{Y}_{LV})$$

Using Young's law

$$\mathbf{Y}_{SG} - \mathbf{Y}_{SL} = \mathbf{Y}_{LV} \cos \theta \,,$$

we see that dewetting is energetically favourable when:

$$p < -\mathbf{Y}_{LV} \, \frac{1 + r \cos \theta}{a}$$

which, for a given value of the pressure, will occur if the surface is hydrophobic ( $\cos \theta < 0$ ) and *a* is small enough. The superhydrophobic state is therefore due to a combination of geometry and wetting characteristics.

#### Shear rate

Slip is *usually* found in experiments when the liquid partially wets the solid surface.

The measured slip lengths span four orders of magnitude (from molecular sizes to micrometers) and, in some investigations, are observed to depend on the shear rate at which the experiment or simulation is performed (see the left plot of Figure (II.8)), leading to a non linear boundary

condition, as  $b = b(\gamma)$ .

In particular, Zhu and Granick [16] report the largest shear-dependent slip lengths (up to  $\sim 2 \mu m$ ) in a squeeze flow experiment, in which two crossed cylinders oscillate about a fixed average distance. By measuring the viscous resistance, Zhu and Granick extract the slip length over a wide range of oscillation amplitudes, revealing the strong shear dependence.

The slip length is explicitly found to grow with the shear rate also in experiments using hydrophobic microchannels [46]. High-precision microchannels are treated chemically to enhance their hydrophobic properties and the flow rates of pure water at various applied pressure differences are measured. The slip length varies approximately linearly with the shear rate, with values of approximately 30 nm for water flow over hydrophobic surfaces at a shear rate of  $10^5 \text{ s}^{-1}$ .

Molecular dynamics simulations by Thompson and Troian [67] indicate that the Navier slip boundary condition is the low-shear rate limit of a more generalized relationship which is significantly nonlinear and divergent at a critical shear rate.

The critical shear rate is system dependent, as it decreases with decreasing corrugation of the surface energy: thus, the same liquid molecules on different substrates will experience varying amounts of slip and vice versa.

Lauga and Brenner [75] present a mechanical model, motivated by the observations of nanobubbles on hydrophobic surfaces, that claim to explain the observed shear dependence of slip in squeeze flow experiments.

The model considers the dynamic response of the gas bubbles, present on the solid surface, to variations in hydrodynamic pressure, due to the oscillation of a solid surface. Both the compression and diffusion of gas in the bubbles modifies the amount of liquid which is necessary to drain out at each cycle of the oscillation, modifying the viscous force on the oscillating surface. This sort of flow over a "leaking mattress" would determine the apparent shear-dependent slip.

An empirical model for shear dependence, inspired again by the measurements of Zhu and Granick, is proposed in [76]. Slip is envisaged to occur locally only when a critical surface shear stress is reached and, once slip begins, it takes place at a constant slip length; the no-slip boundary condition is assumed to remain valid otherwise.

# Dissolved gas, nanobubbles or gaseous films

The observed increase in flow rates in Newtonian liquids is explained by several authors with the presence of small gas bubbles trapped on rough and/or hydrophobic surfaces, that act as regions of perfect slip (for a liquid flowing over gas,  $b = \infty$ ) embedded in an otherwise no-slip boundary. The "effective" slip length is a means to describe this flow over a heterogeneous surface [77].

The flow of liquids in capillaries with inhomogeneously hydrophobized walls is theoretically investigated: Ruckenstein and Rajora [78] conclude that slip occurs over a gap, not directly at the solid–liquid interface; Vinogradova and Alexeyev [79] attribute the origin of slip to the decrease in viscosity in a very thin layer adjacent to a hydrophobic wall; later, Vinogradova and Andrienko [80] attributed slip to a prewetting transition, that is the formation of a film of vapour or phase-separated lubricant with lower viscosity between the fluid and the solid wall.

The stability observed for the nanobubbles against dissolution, even on hydrophobic surfaces, is not easily explained. Spherical gas microbubbles in liquid are unstable because the pressure inside the bubble is much higher than in the surrounding solution and the gas solubility is increased, leading to a rapid dissolution.

Moreover, there is growing evidence that nanobubbles are also present at completely wetting interfaces, such as water on mica [81] (see Figure (II.7)), which suggests that all surfaces submerged in liquid under some conditions may present nanobubbles.



Figure (II.7). AFM image (dimension 1  $\mu$ m × 1  $\mu$ m) of bubbles on mica surface in water, taken from [81].

In most cases where nanobubbles are observed, the surface coverage is low. This finding does not support the model proposed by Lauga and Brenner [75], as they found that a nearly complete coverage of the surface by nanobubbles is required to explain the observed slip effects.

It is well known that an air-water interface rapidly accumulates contaminants, that can increase the stability of the interface by forming a sort of skin at the gas-liquid interface.

Such a skin may act as a barrier to dissolution, influence the surface forces and alter the boundary conditions from one of infinite slip to partial slip or even, possibly, of no-slip.

Moreover, because of the presence of this skin of contaminant materials, the slip condition at the surface of a nanobubble is likely to vary from experiment to experiment and depends, for instance, on the total surface area of the nanobubbles, on the solvent and on the cleaning procedure.

A new approach to control nanobubble formation is proposed in [82] and involves the manipulation of the solubility of dissolved gases. A great number of nanobubbles can form in water on graphite surfaces (and a smaller number on mica surfaces) if the substrate is previously exposed to ethanol. Using variations in temperature and solvent the number density of nanobubbles can be influenced and, importantly, the use of outgassed solvents results in the absence of nanobubbles.

For the present, it seems that many published studies may indeed be affected by nanobubbles or entrained air at the interface, and particularly studies reporting slip lengths exceeding about 100 nm.

As regards the possible role that dissolved gases may play in inducing boundary slip, in [83] it is argued that slip occurs by two mechanisms: localized defect propagation and concurrent slip of large domains. Dissolved gases, omnipresent in most liquids, must provide interfacial defects and, therefore, could increase slip.

# **Electrical properties**

When studying slippage of electrolyte solutions and polar liquids, the amount of slip is found to vary with electrical properties, such as ionic strength and polarity.

In [84], the slip behaviour of a large number of liquids at alkylsilane coated solid surfaces is examined using the AFM technique. For non-polar liquids possessing small contact angles, the slip length is found to decrease with increasing hydrophobicity. For polar liquids possessing large contact angles, no obvious correlation is found between the slip length and the contact angle, but slip appears to be controlled by the dipole moment. One given possible interpretation is that dipole-dipole interactions between the two approaching surfaces generate a "surface lattice structure" in the polar liquid, and the cohesive energy of this lattice structure, which opposes the disruption of the lattice, generates the observed slip behaviour.

Moreover, when using small tracers to probe the fluid velocity, other electrical effects need to be carefully taken into account. If the surface and the particles are similarly charged, particles will

be repelled electrostatically and will not come within a distance equal to the screening length from the surface; if charges are opposite, tracers will stick to the surface.

Another potential problem is given by the influence of the streaming potential on the motion of small particles. If tracers are charged, their velocity will also include an electrophoretic component in response to the flow-induced potential difference; if they are sufficiently charged, this velocity will be able to overcome the electro-osmotic back-flow in the bulk and the particles will move faster than the local liquid, leading to an apparent slip length.

#### Pressure

In velocimetry experiments [85], the measured slip length is found to decrease with the value of the absolute pressure; for water, the no-slip boundary condition is recovered when the absolute pressure reaches 6 atm. These results hint at the probable role of surface-attached bubbles, presumably decreasing in size with an increase in pressure.

The possibility of surface slip due to gradients in liquid pressure should also be considered: in fact, the chemical potential of a liquid molecule depends on pressure, so a pressure gradient leads to a gradient in chemical potential, hence a net force on the liquid, determining a surface slip velocity and slip length.

#### **II.5** The state of the art

A rich review that collects the main published experimental results from liquid-solid boundary condition investigations, up to the beginning of 2005, is [7].

The plots in Figure (II.8), taken from the review, summarize the experimental results for boundary slip dependence on contact angle, thus level of hydrophobicity, and on shear rate.

From the left plot, it is evident that, although it is *usually* assumed that slip only occurs on hydrophobic surfaces, a large variety of hydrophilic surfaces with different wetting properties are capable of slip. However, data suggest that the level of surface wettability might not be a primary factor in determining slip.

In the right plot we see that, in many investigations, slip is observed to depend on the shear rate at which the experiment or simulation is performed.

Recollecting all the results shown so far, we might conclude that the slip lengths reported experimentally span many orders of magnitude, from molecular lengths up to hundreds of nanometers.



Figure (II.8). Experimental variation of the slip length *b* with the liquid-solid contact angle  $\theta$  (left plot) and with the typical experimental shear rate  $\gamma$  (right plot). The experimental results are given by the following techniques: pressure driven flow ( $\circ$ ), sedimentation ( $\bullet$ ), fluorescence recovery ( $\Box$ ), PIV ( $\triangleright$ ), streaming potential ( $\blacktriangledown$ ), fluorescence cross-correlations ( $\triangleleft$ ), SFA ( $\blacksquare$ ), and AFM ( $\triangleright$ ). When a solid line is drawn, the experimental results are given for a range of contact angles and/or shear rates.

Anyway, molecular theories are able to predict intrinsic slip lengths of up to tens of nanometers for hydrophobic systems, suggesting that any measurement of larger slip on flat surfaces is affected by factors that are not purely fluid dynamical.

The parameters that contribute to apparent slip include roughness-induced dewetting, presence of dissolved gas and contamination by impurities, while the slip magnitude is influenced by the contact angle, shear rate, electrical properties and pressure.

# **Chapter III**

# THEORY OF MICRO - PARTICLE IMAGING VELOCIMETRY

# **III.1 Introduction**

Particle Image Velocimetry (PIV) is a well-established measurement technique for macroscopic flows, and it is extensively described in literature (see [86, 87]).

Micro-Particle Imaging Velocimetry ( $\mu$ -PIV) is a modification of PIV in order to access the small scales of microfluidic devices, allowing to reach sufficiently small spatial resolutions.

The first successful  $\mu$ -PIV experiment is conducted in 1998 by Santiago et al. [88], to record the velocity field in a Hele-Shaw flow around a 30  $\mu$ m elliptical cylinder with a bulk velocity of approximately 50  $\mu$ m/s. The system utilizes an intensified CCD camera, an epi-fluorescent microscope, tracer particles tagged with a fluorescent dye, and an Hg-arc lamp for continuous illumination.

One year later, Meinhart et al. [89] demonstrate a  $\mu$ -PIV system consisting of the same general components with the exception that the continuous light source is replaced by two pulsed Nd:YAG lasers, as shown in Figure (III.1). The technique is applied to measure the flow field in a 30- $\mu$ m-high × 30- $\mu$ m-wide channel, with a maximum velocity three orders of magnitude higher then in the initial experiment performed just the year before, and results with high spatial resolution and accuracy are reported.

In 2000, Meinhart and Zhang [90] perform the highest speed measurements (up to 8 m/s) with the  $\mu$ -PIV technique, studying the flow inside a microfabricated inkjet printer head.

The aim of this chapter is to provide a theoretical and technical framework to understand a  $\mu$ -PIV system, its possibilities and limitations.

## III.2 Basic idea of µ-PIV

Micro-PIV is a full-field non-intrusive measurement technique where the flow velocity is obtained by recording the displacement of small tracing particles seeded in the working fluid, that faithfully follow the local fluid motion.



Figure (III.1). Schematic of the μ-PIV system in [89]. A pulsed Nd:YAG laser is used to illuminate fluorescent 200-nm diameter flow-tracing particles through an epi-fluorescent inverted microscope. A cooled interline-transfer CCD camera is used to record the particle images.



Figure (III.2). Displacement of the particle image pattern for the same interrogation region recorded at different times.

Two images of the particles are taken at different times and the time delay between the two photographs,  $\Delta t$ , is known. The two particle image fields are sectioned into smaller uniformly spaced regions, called *interrogation regions* (see Figure (III.2)).

The displacement of the group of particles within each interrogation region is determined using a statistical technique called *cross-correlation*. The cross-correlation function  $\Phi$  is expressed by:

$$\Phi(m,n) = \sum_{j=1}^{q} \sum_{i=1}^{p} f(i,j) \cdot g(i+m,j+n)$$

where f(i, j) and g(i, j) represent the array of gray values comprising the first image and the second image, respectively.

For a high quality set of PIV measurements, the cross-correlation function presents a welldefined peak, as shown in Figure (III.3): the location of the peak indicates how far the particles have moved in the time interval  $\Delta t$ .



Figure (III.3). PIV cross-correlation peak.

A first-order estimate of the local velocity of the fluid,  $\vec{u}$ , is obtained dividing the measured displacement vector,  $\Delta \vec{s}$ , by the time delay:

$$\vec{u} \approx \frac{\vec{x}_{t+\Delta t} - \vec{x}_t}{\Delta t} = \frac{\Delta \vec{s}}{\Delta t}$$

#### III.3 Typical µ-PIV system components

Figure (III.4) is a schematic of a typical  $\mu$ -PIV system. It is common to choose a pulsed monochromatic light source, such as a pulsed Nd:YAG laser system. It consists of two Nd:YAG laser cavities, beam combining optics and a frequency doubling crystal. The laser emits two pulses of light at  $\lambda = 532$  nm; the duration of each pulse is on the order of 5 ns and the time delay between light pulses can vary from hundreds of nanoseconds to a few seconds.

The illumination light is delivered to the microscope through beam-forming optics, which can consist of a variety of optical elements that deflect the light so that it fills the back of the objective lens, and thereby allows a broad illumination of the microfluidic device.

The illumination light is reflected upward towards the objective lens by an antireflective coated mirror, which is designed to reflect wavelengths of 532 nm and transmit 560 nm.

Another possible solution is to use continuous chromatic light sources, such as an Hg-arc lamp or halogen lamp, to provide illumination light; in this situation, an excitation filter must be used to allow only a narrow wavelength band of light to illuminate the test section. If a continuous light source is used, a mechanical or electro-optical shutter must be added to regulate the exposure to light of the image recording device, so that discrete particle images can form.



Figure (III.4). Schematic of a μ-PIV system using either a continuous or pulsed illumination light source.



Figure (III.5). Light collecting cone half-angle  $\theta$ , aperture diameter and working distance of a microscope objective.

The choice of the microscope lens properties is of crucial importance for high resolution measurements, and oil immersion (refraction index  $n_{oil}$ =1.515), high numerical aperture (NA, defined as the product  $n \cdot \sin \theta$ , where *n* is the refractive index of the objective's working medium and  $\theta$  is the half-angle of the light collecting cone, as in Figure (III.5)), high magnification M, low distortion, Plan Apochromatic lens are generally adopted.

Typically, the working fluid is directed through the microfluidic device by pressure or induced electro-kinetically. The working fluid can be any visibly transparent fluid (such as water) seeded with fluorescent particles.

The particles can be made of a variety of materials and are coated with a fluorescent dye with an excitation wavelength closely matched to the light source, and an emission wavelength closely matched to the barrier filter. Suitable particles can be purchased from a number of manufactures. The microfluidic chamber has at least one optically transparent wall, so that it can be viewed through the microscope lens. A barrier filter is positioned between the mirror and the relay lens.

The barrier filter filters out the illumination light that is reflected by the surface of the flow chamber or scattered by the particles.

A sensitive large-format interline-transfer CCD camera is commonly adopted to record the particle image fields. The large-format for the CCD array is desirable because it allows for more particle images to be recorded and increases the spatial dynamic range of the measurements; the interline-transfer feature allows high acquisition frequency, for two particle image frames can recorded with up to a few hundreds ns time delay.

#### III.4 Fundamental physics considerations of µ-PIV

Three main problems differentiate µ-PIV from conventional macroscopic PIV:

 $\Rightarrow$  the particles are small; their diameters are close to the wavelength of the illuminating light;

 $\Rightarrow$  the particles can be small enough that the effects of Brownian motion must be addressed;

 $\Rightarrow$  the illumination source is typically not a light sheet but an illuminated volume of the flow.

Let us come into the details of these three important aspects of the  $\mu$ -PIV technique.

# Small particles, comparable to the wavelength $\lambda$ of the illuminating light

The tracers' diameter *d* must be small enough that the particles do not alter the flow being measured and large enough to scatter sufficient light for their images to be detected. Consider that, if the Rayleigh scattering regime is reached ( $d << \lambda$ ), the amount of light scattered by the particle varies as  $d^{-6}$ . A solution to this imaging problem is given by the choice of fluorescently-labelled tracing particles, of a few hundred nm diameter, whose images are recorded by epi-fluorescence microscopy.

#### Effects of Brownian motion

When the size of the tracers is sufficiently small, the collective effect of collisions between these microtracers and the fluid molecules prevents the microparticle to follow the flow to some degree, generating the so-called Brownian motion. This phenomenon determines an error in the measurement of the flow velocity and an uncertainty in the location of the tracers.

First of all, let us consider how a particle suspended in flows behaves.

In an accelerating flow, a particle will tend to "hang" behind due to inertial effects: the velocity lag that generates depends on the particle diameter  $d_p$  and density  $\rho_p$ . Applying the Stokes' drag law to a spherical particle in a viscous low Re flow, the particle velocity can be estimated by:

$$u_p(t) = u_f \left( 1 - e^{-\frac{t}{\tau_s}} \right)$$

where  $u_p(t)$  is the particle velocity,  $u_f$  is the fluid velocity, and  $\tau_p$  is the response time given by:

$$\tau_p = \frac{d_p^2 \rho_p}{18\eta}$$

where  $\eta$  is the liquid dynamic viscosity.

The plot of Figure (III.6) shows how the particle response time decreases considerably with particle diameter. In  $\mu$ -PIV, d<sub>p</sub> typically lies in the range 200 nm ÷ 1  $\mu$ m. Considering a 200 nm polystyrene particle in water, the response time is ~10 ns, that is much smaller than the time scales of a realistic flow field. Therefore, errors due to velocity lag are negligible in  $\mu$ -PIV applications.

#### • Velocity errors

The problem of the effects of Brownian motion on the accuracy of  $\mu$ -PIV measurement is faced in [88].

Given time intervals  $\Delta t$  much larger than the particle inertial response time  $\tau_p$ , the mean square distance of diffusion is:

$$\left\langle s^{2}\right\rangle = 2D\Delta t$$

where the diffusion coefficient *D* is given by:  $D = \frac{k_B T}{3\pi \eta d_p}$ .

The effect of motion resulting from collisions between fluid molecules and suspended microparticles can be modelled as a white-noise process, which increases the uncertainty in the determination of the microparticle displacement.

An ideal particle that faithfully follows a steady flow, with a velocity  $\vec{u} = u_x \vec{i} + u_y \vec{j}$  over a time interval  $\Delta t$ , has a x- and y-displacement of  $\Delta x = u_x \Delta t$  and  $\Delta y = u_y \Delta t$ , respectively.

Thus, the relative error in the measurement of the *x*- and *y*-components of the particle velocity can be expressed by:

$$\varepsilon_x = \frac{\left\langle s^2 \right\rangle_x^{1/2}}{\Delta x} = \frac{1}{u_x} \sqrt{\frac{2D}{\Delta t}} \text{ and } \varepsilon_y = \frac{\left\langle s^2 \right\rangle_y^{1/2}}{\Delta y} = \frac{1}{u_y} \sqrt{\frac{2D}{\Delta t}}.$$

These errors determine a lower limit on the measurement time interval  $\Delta t$ : beyond the limit, measurements are dominated by uncorrelated Brownian motion.

Displacements grow as  $\Delta t$  while Brownian particle displacements are proportional to  $\Delta t^{1/2}$ , therefore, the relative error decreases with increasing time of measurement. But a longer  $\Delta t$  decreases the accuracy of  $\mu$ -PIV data, which are based on first order accurate approximation to the velocity. Moreover, Brownian motion becomes relatively less important for fast flows.

In practice, Brownian motion can be neglected for larger then 500-nm tracing particles and for 50-500-nm tracing particles with flow velocities higher then ~1 mm/s.



Figure (III.6). Particle response time to fluid acceleration for three different values of particle diameter:  $d_p = 10 \mu m$ ,  $5 \mu m$  and  $1 \mu m$ .

# • Particle position error

The random displacement due to Brownian motion, that might take place during the exposure time, determines an increase in the uncertainty associated with measuring the particle location. This uncertainty is practically negligible for exposure times where the typical Brownian displacement is small compared to the particle diameter, in the image plane.

# Volume illumination of the flow

In PIV, a thin sheet of laser light is generated in order to illuminate a single plane within the fluid flow and only particles within the laser sheet scatter light and are recorded by the camera. In  $\mu$ -PIV, illumination of a single plane within the fluid flow is not feasible for several reasons [91]:

(1) the optical access for silicon-based microfluidic devices is limited to one direction;

(2) special waveguides micromachined directly into the fluidic cell are needed to provide optical access for the light sheet, leading to increasing complexity and costs of fabrication;

(3) the difficulty to form a light sheet only a few  $\mu$ m thick and the practical impossibility to align it with the imaging optics' focal plane. Thus, in  $\mu$ -PIV, the flow field is entirely illuminated and the depth of the measurement plane is defined by the focusing characteristics of the recording optics. This mode of illumination is called *volume illumination*.

#### **III.5** Fluorescence

Due to the mode of illumination and related noise issues, elastic scattering light is not suitable for  $\mu$ -PIV and fluorescence imaging is adopted.

Fluorescence is the emission of photons by an atom or a molecule, following to a temporary excited electronic state caused by absorption of photons of a certain wavelength from an external radiation source. When a fluorochrome absorbs a photon with energy  $E_1$ , an electron is excited from its ground state  $E_0$  to a higher energy state (Excited state 1) corresponding to the energy of the absorbed photon, see Figure (III.7). Due to the fluorochrome's interactions with its molecular environment during the excited state, there is a loss of energy resulting in a relaxed excited state with energy  $E_2$  (Excited state 2) from which fluorescence emission originates. After a short time interval (typically  $10^{-9} \div 10^{-12}$  s) the excited electron collapses back to the ground state, emitting a photon of energy  $E = E_2 - E_0$  corresponding to the difference between the electron's excited and ground states. The energy of a photon is given by:

$$E = E_2 - E_0 = \frac{hc}{\lambda}$$

where *h* is Planck's constant, *c* is the speed of light, and  $\lambda$  is the wavelength. As  $E_2 < E_1$ , the wavelength for the fluorescent emission  $\lambda_{emit}$  is longer than for the absorbed radiation  $\lambda_{abs}$ . A fluorochrome can absorb photons over a spectrum of wavelengths, the *excitation spectrum*.



Figure (III.7). The principle of fluorescence. 1) A fluorochrome is excited by a photon to an energy level corresponding to *excited state 1*; 2) The excited electron loses energy due to interactions with the environment, falling to a relaxed excited state, *excited state 2*; 3) The electron collapses back to its *ground state* emitting a photon of energy given by the difference between excited state 2 and ground state.



Figure (III.8). Illustration of excitation and emission spectra of a fluorochrome with corresponding Stokes' shift. Several excitation and emission peaks may exist in general.

Due to variations in electrons' persistence in the excited state and, therefore, changes in energy loss prior to fluorescent emission, fluorochromes emit photons over a continuous spectrum of wavelengths, the *emission spectrum*, even when excitation is performed by a monochromatic light source. The excitation and emission spectra are distinct, although they often overlap and sometimes are almost indistinguishable. The difference between the peak excitation and emission wavelengths, for a fluorochrome, is the so-called *Stokes' shift* (see Figure (III.8)).

In  $\mu$ -PIV, the fluorescence properties of tracing particles are crucial for successful particle imaging. The excitation wavelength  $\lambda_{abs}$  of the fluorescent dye loaded on the tracers should closely match the wavelength of the illumination source, to ensure high fluorescence intensity. Moreover, the Stokes' shift of the dye should be sufficiently large for the optical filters and dichroic mirror to effectively filtrate the illumination wavelength, without loss of the emitted fluorescence signal.

# **III.6 In-plane spatial resolution limits**

The resolution of any optical system is limited by diffraction taking place when waves of light, upon passing a lens, converge and interfere with each other.

The diffraction pattern of a point source of light (such as a small fluorescent particle) imaged through a circular aperture, when observed in the focal plane, is termed the *Airy pattern*. This pattern consists of a central ring, the *Airy ring*, surrounded by concentric disks. The diameter of the Airy disk in the image plane, the so-called point-spread function, is given by:

$$d_s = 2.44M \frac{\lambda}{2NA}$$

where *M* is the magnification, *NA* the numerical aperture of the lens, and  $\lambda$  is the wavelength of light.

The actual recorded image can be estimated as the convolution of the point-spread function  $d_s$  with the geometric particle image  $Md_p$ . Approximating  $d_s$  and  $Md_p$  as Gaussians, the effective image diameter,  $d_e$ , can be written as:

$$d_{e} = \left[d_{s}^{2} + M^{2}d_{p}^{2}\right]^{1/2}$$

The value of the effective particle diameter projected back into the flow field is given by  $d_e/M$ .

The effective particle image diameter places a bound on the spatial resolution that can be obtained by  $\mu$ -PIV. Assuming that the particle images are sufficiently resolved by the CCD array, the location of the correlation peak can be resolved to within 1/10<sup>th</sup> the particle image diameter; thus, the uncertainty of the correlation peak location is:

$$\delta x \sim \frac{d_e}{10M}$$

The measurement error due to detectability,  $\varepsilon_d$ , can be written as the ratio of the correlation peak location uncertainty,  $\delta x$ , to the particle displacement,  $\Delta x$ :

$$\mathcal{E}_d = \frac{\delta x}{\Delta x}.$$

#### **III.7** Out-of-plane spatial resolution

#### Three-dimensional diffraction pattern

The intensity distribution of the three-dimensional diffraction pattern of a point source imaged through a circular aperture of radius a can be written in terms of the dimensionless diffraction variables (u, v) [91, ref. therein]:

$$I(u,v) = \left(\frac{2}{u}\right)^2 \left[U_1^2(u,v) + U_2^2(u,v)\right] I_0$$

and

$$I(u,v) = \left(\frac{2}{u}\right)^2 \left\{ 1 + V_0^2(u,v) + V_1^2(u,v) - 2V_0(u,v)\cos\left[\frac{1}{2}\left(u + \frac{v^2}{u}\right)\right] - 2V_1(u,v)\sin\left[\frac{1}{2}\left(u + \frac{v^2}{u}\right)\right] \right\} I_0$$

where  $U_n(u, v)$  and  $V_n(u, v)$  are the so-called Lommel functions, which may be expressed as an infinite series of Bessel functions of the first kind:

$$U_{n}(u,v) = \sum_{s=0}^{\infty} (-1)^{s} \left(\frac{u}{v}\right)^{n+2s} J_{n+2s}(v)$$

$$V_n(u,v) = \sum_{s=0}^{\infty} (-1)^s \left(\frac{v}{u}\right)^{n+2s} J_{n+2s}(v) \, .$$

The dimensionless diffraction variables are defined as:

$$u = 2\pi \frac{z}{\lambda} \left(\frac{a}{f}\right)^2$$
$$v = 2\pi \frac{r}{\lambda} \left(\frac{a}{f}\right)^2,$$

where *f* is the radius of the spherical wave approaching the aperture (which can be approximated as the focal length of the lens),  $\lambda$  is the wavelength of light, *r* is the in-plane radius and *z* is the out-of-plane coordinate, with the origin located at the point source (see Figure (III.9)). In the focal plane, the intensity distribution reduces to the expected result:

$$I(0,v) = \left[\frac{2J_1(v)}{v}\right]^2 I_0$$

which is the Airy function for Fraunhofer diffraction through a circular aperture. Along the optical axis, the intensity distribution reduces to:

$$I(u,0) = \left[\frac{\sin(u/4)}{u/4}\right]^2 I_0.$$

The three-dimensional intensity distribution pattern *I* expressed in diffraction units (u, v) is shown in Figure (III.10). The focal point is located at the origin, the optical axis is located at v = 0 and the focal plane is located at u = 0. The maximum intensity,  $I_0$ , occurs at the focal point. Along the optical axis, the intensity distribution reduces to zero at  $u = \pm 4\pi, \pm 8\pi$ , while a local maximum occurs at  $u = \pm 6\pi$ .



Figure (III.9). Geometry of a particle of diameter  $d_p$  imaged through a circular aperture of radius a by a lens of focal length f.

# Depth of field

The depth of field  $\delta z$  of a microscope objective is given by Inoue and Spring (1997) as:

$$\delta z = \frac{n\lambda_0}{NA^2} + \frac{ne}{NA \cdot M}$$

where *n* is the refractive index of the fluid between the microfluidic device and the objective lens,  $\lambda_0$  is the wavelength of the imaged light in vacuum and *e* is the smallest distance that can be resolved by a detector placed in the image plane of the microscope (for a CCD sensor, *e* is the pixel-to-pixel spacing on the CCD chip).

The equation for  $\delta z$  is the sum of the depth of field resulting from diffraction (the first term on the right-hand side) and from geometrical effects (the second term on the right-hand side).

The cut-off for the depth of field due to diffraction (the first term on the right-hand side) is chosen by convention to be one quarter the out-of-plane distance between the first two minima in the three-dimensional point-spread function, that is  $u = \pm \pi$  in Figure (III.10).

If a CCD sensor is used to record particle images, the geometrical term can be derived by projecting the CCD array into the flow field and then considering the out-of-plane distance the CCD sensor can be moved before the geometrical shadow of the point source occupies more than a single pixel. This derivation is valid for small light collection angles.

# Depth of correlation

The depth of correlation is defined as twice the distance from the object plane at which a particle can be located so that the intensity along the optical axis is an arbitrarily specified fraction of its in-focus intensity. Beyond this distance, the particle's intensity is sufficiently low, so that it does not contribute significantly to the velocity measurement.



Figure (III.10). Three-dimensional intensity distribution pattern I(u, v). The focal point is the origin, the optical axis is located along v=0 and the focal plane is located along u=0.

Let us denote with  $\phi$  this fractional intensity.

The PIV correlation depth is related to the depth of field of the optical system, but it is important to distinguish between these two concepts. It is worth recalling that the depth of field is defined as twice the distance from the object plane in which the object is considered unfocused in terms of image quality.

The theoretical contribution of an out-of-focus particle to the correlation function is estimated by considering ① the effect due to diffraction, ② the effect due to geometrical optics and ③ the finite size of the particle.

Let us fix the cut-off for the on-axis image intensity  $\phi$  to be one tenth of the in-focus intensity.

① The effect of diffraction can be evaluated considering the intensity of the point-spread function along the optical axis. We have:

$$\left[\frac{\sin(u/4)}{u/4}\right]^2 = \frac{I(u,0)}{I_0} = \phi = 0.1$$

that, once solved, tells that the intensity cut-off occurs at  $u \approx \pm 3\pi$ . Substituting this value for *u* into its definition and solving for 2*z* gives:

$$2z = 3\lambda \left(\frac{f}{a}\right)^2.$$

Using the definition of numerical aperture  $NA = n \sin \theta \approx na / f$  and substituting  $\lambda_0 = n\lambda$ , the measurement depth due to diffraction  $\delta z_d (= 2z)$  becomes:

$$\delta z_d = \frac{3n\lambda_0}{NA^2}$$

<sup>(2)</sup> Let us consider a particle of diameter  $d_p$  located on the optical axis of an imaging lens at a distance *z* from the focal plane, as in Figure (III.11).

The radius of the geometrical shadow  $r_{gs}$  cast by the particle onto the focal plane is given by:

$$r_{gs} = \frac{d_p}{2} + z \tan \theta \, .$$

Assuming that the out-of-focus distance z is small compared to the focal length of the imaging lens, the angle  $\theta$  may be approximated by the half-angle of the collecting cone of light appearing in the definition of NA.

The effect of geometrical optics on the correlation depth is estimated by considering the distance z from the object plane at which the particle intensity, along the optical axis, decreases by an amount  $\phi = 0.1$ , due to the spread of the geometrical shadow (that is the collection cone of the lens).



Figure (III.11). Geometrical shadow cast by an out-of-focus particle onto the focal plane. z is the distance between the particle and the focal plane,  $\theta$  is the collection angle of the imaging lens, and  $r_{gs}$  is the radius of the geometrical shadow.

Given uniform intensity within the geometrical shadow, the requirement is satisfied when the area of the geometrical shadow cast onto the focal plane by the out-of-focus particle is 10 times larger than that of an in-focus particle, that means:

$$\pi r_{gs}^2 = 10\pi \left(\frac{d_p}{2}\right)^2.$$

Substituting the previous expression for  $r_{gs}$  yields:

$$0.1\left(\frac{d_p}{2} + z\tan\theta\right)^2 = \left(\frac{d_p}{2}\right)^2.$$

Solving for  $\delta z_g (= 2z)$  gives:

$$\delta z_g = \frac{2.16d_p}{\tan\theta}$$

The above analysis assumes that the particle image is sufficiently resolved by the CCD array, which is true for most  $\mu$ -PIV experiments.

In situations where the pixel size is much larger than the particle diameter,  $\frac{e}{M} >> d_p$ , a different expression for  $\delta z_g$  is achieved, as shown in [91].

 $\ensuremath{\mathfrak{I}}$  For an estimate for the total measurement depth of a  $\mu$ -PIV system, also the finite size of the particles must be taken into consideration.

The total depth of correlation is obtained adding the three contributions:

$$\delta z_m = \delta z_d + \delta z_g + d_p = \frac{3n\lambda_0}{NA^2} + \frac{2.16d_p}{\tan\theta} + d_p$$

where  $\delta_{z_m}$  represents the actual measurement plane thickness for the  $\mu$ -PIV system.

# **Background noise**

In volume-illuminated  $\mu$ -PIV, all tracing particles in the cone of illumination emit light that contributes to the recorded image field, irrespectively of their being located inside or outside the focal plane, as shown in Figure (III.12). Out-of-focus particles increase the background noise level and reduce the signal-to-noise ratio of the image field.

The signal-to-noise ratio of the image field is defined as the peak image intensity of a typical infocus particle divided by the average background intensity. It depends on many parameters, such as the particle size and concentration, the test-section geometry, the illumination, the recording optics and the CCD detector.

For a fixed test-section depth, the level of background noise can be lowered by decreasing the concentration of tracing particles. A lower particle concentration requires the use of larger interrogation regions to obtain an adequate correlation signal, which reduces the spatial resolution of the measurements.

Higher spatial resolutions can be obtained in thinner test sections, because higher particle concentrations and, consequently, smaller interrogation spots can be used, maintaining an adequate signal-to-noise ratio.

In most cases, the microchannel of interest is designed to serve a certain purpose, its geometrical dimensions cannot be altered, and tracers' concentration is the only adjustable parameter. A lower concentration of tracers requires the use of larger interrogation regions, leading to a poorer spatial resolution. In order to avoid the loss of spatial resolution of the measurements, special interrogation or image processing techniques are commonly applied in  $\mu$ -PIV.



Figure (III.12). Volume-illuminated μ-PIV. All the particles in the cone of illumination emit light, but only those particles within the measurement plane are sufficiently focused to significantly contribute to the correlation function.

#### **III.8** Data interrogation in μ-PIV

When evaluating digital  $\mu$ -PIV recordings with conventional correlation-based algorithms, a sufficient number of particle images are required in the interrogation window to ensure reliable and accurate measurement results.

In many cases, the particle image density in the  $\mu$ -PIV recordings is not high enough (Figure (III.13, a)), leading to the so-called *low image density* (LID) recordings, that are usually evaluated with particle-tracking algorithms.

When using particle-tracking algorithms, the velocity vector is determined with only one particle and the reliability and accuracy of the technique are limited. Moreover, interpolation procedures are usually necessary to obtain velocity vectors on the desired regular grid points from the random distributed particle-tracking results (Figure (III.14, a)), adding further uncertainty to the final results.

There are two special processing methods to improve the accuracy of  $\mu$ -PIV measurements, avoiding the errors from the LID: the use of a digital image processing technique and the use of an improved evaluation algorithm. Both techniques work on the assumption that the examined flow field is steady and laminar.

#### Image overlapping

To obtain high-image-density (HID)  $\mu$ -PIV recording pairs, a series of LID recording pairs sampled at different times can be computationally overlapped.

An overlapped recording  $g_0(x, y)$  is given by:

$$g_0(x, y) = \max\{g_k(x, y), k = 1, ..., N\}$$

where  $g_k(x, y)$  is the grey value distribution of the k<sup>th</sup> LID  $\mu$ -PIV recording, for a total number of N recordings.

The maximum function is taken assuming that particle images are positive, that means bright particles and dark background. An example of image overlapping is shown in Figure (III.13, b). The maximum number of recordings that may be overlapped is limited by the number of particles in the individual images: in fact, adding too many recordings would lead to interference between particle images, not giving any further improvement in resolution.



Figure (III.13). Example of image overlapping from [92]: (a) one LID μ-PIV recordings; (b) result of overlapping 9 LID μ-PIV recordings. Image size of 256×256 pixels.



Figure (III.14). Effect of image overlapping, from [92]: (a) results for a single LID μ-PIV recording pair with a particle-tracking algorithm; (b) results for the overlapped μ-PIV recording pair with a correlation-based algorithm.

# Ensemble correlation method

The correlation function at a certain interrogation window of size of  $p \times q$  pixels is given by:

$$\Phi_{k}(m,n) = \sum_{j=1}^{q} \sum_{i=1}^{p} f_{k}(i,j) \cdot g_{k}(i+m,j+n)$$

where  $f_k(i, j)$  and  $g_k(i, j)$  are the grey value distributions of the first and second exposures, respectively, in the  $k^{th}$  µ-PIV recording pair.

For a single well-seeded image pair, without exceeding noise levels, the correlation function has a distinct maximum peak corresponding to the particle image displacement in the interrogation window. A number of substantially lower subpeaks, resulting from noise and mismatch of the particle images, are present in random positions of the correlation plane.

When the number of particle images within the interrogation window is not sufficient, or the noise levels is too high, the peak representing the true particle displacement may become smaller

than some of the subpeaks, as in Figure (III.15, a). An erroneous velocity vector may be generated.

For laminar and stationary flows, the velocity field is independent of the measurement time, so that the position of the main  $\Phi_k$  does not change for PIV recordings taken at different times. The subpeaks appear instead at random positions and with various intensities in the different image pairs.

Therefore, acquiring a large number N of PIV recording pairs, calculating their individual correlation functions  $\Phi_k$  (for k = 1, ..., N) and averaging the correlation functions for corresponding interrogation windows, the true displacement peak remains in the same position, while the randomly scattered subpeaks average to zero (see Figure (III.15)).

The ensemble correlation function is given by:

$$\Phi_{ens}(m,n) = \frac{1}{N} \sum_{k=1}^{N} \Phi_{k}(m,n).$$

In contrast with the image overlapping procedure, the ensemble correlation technique is not limited to LID  $\mu$ -PIV recordings or to a small number of recordings.



Figure (III.15). Effect of ensemble correlation, from [92]: (a) results with conventional correlation for one of the  $\mu$ -PIV recording pairs; (b) results with ensemble correlation for 101  $\mu$ -PIV recording pairs.

#### Background noise removal

Collecting a large number of  $\mu$ -PIV recording pairs allows removing the background noise. One solution to obtain an image of the background, from numerous  $\mu$ -PIV recordings, is given by averaging the recordings: in fact, particles are randomly distributed and quickly move through the camera field of view; hence their images disappear in the averaged recording. The image of the background, which shows contaminants on the glass covers, particles adhered to the wall, etc., maintains the same brightness distribution in the averaged recording as it does not move or change.

Another method is to generate the background image, associating to each pixel location a minimum of the ensemble of  $\mu$ -PIV recordings because the minimal grey value at each pixel may reflect the background brightness for the set of images.

The background noise can be successfully removed by subtracting the background image from the  $\mu$ -PIV recordings.

An ensemble correlation function for a large number of image sample pairs without background removal is shown in Figure (III.16, a): there is a dominant peak near zero displacement because the speckles do not move. When the background image is subtracted, the influence of the asperities is reduced and the particle displacement peak appears neat in the evaluation function in Figure (III.16, b).



Figure (III.16). Ensemble correlation function, taken from [92], for 100 image sample pairs without (a) and with (b) background removal.

# **Bias error reduction**

One effective technique to reduce the bias error of  $\mu$ -PIV measurements in complex flows is the Central Difference Interrogation (CDI) method.

The CDI method is developed by Wereley and Meinhart (see [93] for a detailed presentation) to substitute the adapting shifting Forward Difference Interrogation (FDI) technique. In FDI, the second interrogation window is shifted in the forward direction of the flow, an amount equal to the mean displacement of the particle images initially in the first window.

The comparison between the CDI and FDI methods is analogous to the comparison between central difference and forward difference discretizations of derivatives where in the central difference method is accurate to order  $\Delta t^2$ , while the forward difference method is only accurate

to order  $\Delta t$ . When using CDI, the first and second interrogation windows are shifted backward and forward, respectively, each by half of the expected particle image displacement (see Figure 3 in [93]).

As for most adaptive window shifting techniques, this technique requires iteration to achieve optimum results.

### **III.9** Total internal reflection fluorescence microscopy

Let us conclude the wide theoretical presentation of the  $\mu$ -PIV technique with a paragraph dedicated to the presentation of an elegant optical technique utilized to observe single molecule or tracers' fluorescence at surfaces and interfaces, the so-called Total Internal Reflection Fluorescence Microscopy (TIRFM).

The basic concept of TIRFM is simple, requiring only an excitation light beam travelling at a high incident angle through the solid glass coverslip. For our investigations, the glass coverslip represents the microfluidic cell floor, where the fluorophores adhere or slip, as in Figure (III.17). Refractive index differences between the glass and water phases regulate how light is refracted or reflected at the interface as a function of incident angle  $\theta$ .

For incident angles greater then a specific critical angle  $\theta_c$ , the beam of light is totally reflected from the glass-water interface, rather than passing through and refracting in accordance with Snell's Law. The critical angle  $\theta_c$  for TIR is given by:

$$\theta_c = \sin^{-1}(n_{liquid} / n_{solid}) = \sin^{-1} n$$

where  $n_{liquid}$  and  $n_{solid}$  are the refractive indices of the liquid and the solid respectively, and  $n = n_{liquid} / n_{solid}$ . The ratio *n* must be less then unity for TIR to occur.

The reflection generates a very thin electromagnetic field (usually of a few hundred nanometers) in the aqueous medium, the *evanescent field*, which has an identical frequency to that of the incident light and undergoes exponential intensity decay with increasing perpendicular distance z from the surface.

$$I(z) = I_0 e^{-z/d}$$

The characteristic decay distance *d* of the evanescent wave intensity is a function of the incident angle  $\theta$ , of the incident light wavelength in vacuum  $\lambda_0$  and of the liquid and solid refractive indices:

$$d = \frac{\lambda_0}{4\pi} \left( n_{solid}^2 \sin^2 \theta - n_{liquid}^2 \right)^{-1/2}.$$

Fluorophores residing in close proximity to the glass-water surface can be selectively excited by the evanescent field, provided they have electronic transitions occurring in the wavelength bandwidth of the illuminating light beam. Because of the exponential fall off of the intensity, fluorophores further away from the surface avoid being excited, leading to a dramatic reduction of unwanted secondary fluorescence emission from tracers that are not in the primary focal plane.

For the real situation, a finite-width beam incidence on a finite surface, the distance of propagation along the surface is called *Goos-Hanchen shift*. Intuitively, the evanescent wave can be pictured as the beam's partial emergence from the higher refractive-index into the lower refractive-index medium, travel for the Goos-Hanchen distance along the surface, and then reentrance into the solid. The Goos-Hanchen shift ranges from a fraction of a wavelength at

 $\theta = \frac{\pi}{2}$  to infinity at  $\theta = \theta_c$ .

For a Gaussian laser beam in TIR condition, the experimentally observed evanescent illumination is approximately an elliptical Gaussian profile, and the polarization and penetration depth are approximately equal to those of a single infinite plane wave.



Figure (III.17). Illustration of TIRFM working principle: an excitation light beam, travelling at a high incident angle through a glass slide, is totally reflected from the interface and generates an evanescent wave

A wide range of optical arrangements for TIRF can be employed.

Most configurations use an added prism to direct the light toward the TIR interface, but it is also possible to use an high numerical aperture (the highest commercially available, NA = 1.4) microscope objective for this purpose.

In the second case, the sample is epi-illuminated through the objective: if the incident beam is constrained to pass through the periphery of the objective's pupil, it can be refracted by the lens at an angle higher than the critical angle.

The main disadvantages of the prismless TIR configuration are that it requires very expensive objectives and, moreover, the image quality in terms of contrast is generally lower than for prism-based TIR arrangements.

For the detailed description of a prism-based TIRF configuration, we remand to the next chapter, which is devoted to the presentation of the  $\mu$ -PIV system we have realized.

The microscope configurations we reproduce are: epi-illumination for deep illumination of the bulk and a prism-based TIR for surface illumination of the sample.

# **Chapter IV**

# **MICRO - PIV SYSTEM DETAILS**

#### **IV.1** The imaging apparatus

#### Microscope and perfusion system

Figure (IV.1) shows a scheme of the imaging system that we have designed and assembled, and that we are currently employing as  $\mu$ -PIV recording system.



Figure (IV.1). Schematic of the optical measurement system set-up.

A microfluidic flow cell is realized either through a commercial close bath chamber (RC-30, Warner Instruments), housing a silicone gasket sandwiched between top and bottom glass coverslips (respectively 0.15 mm and 1 mm thick), held in place by mechanical pressure, or through flow chambers made of glass and thiolene-based optical adhesives (NOA, Norland Products) fabricated by using fast prototyping soft-lithographic techniques [94]. Both models of fluidic cell are provided with channels a few hundred µm deep and a few mm wide for the perfusate to traverse.

A peristaltic pump (Minipuls 3, Standard pump head, Gilson) equipped with a home-made pulsation dampener is connected to the chamber to generate a uniform flow of seeded water.

Red helium-neon laser illumination is chosen because of the wide availability and low cost of such lasers. A He-Ne laser of 5 mW maximum power at 632.8 nm (05-LHR-211, Melles Griot) is used in our experiments.

To further lower the cost of the system and to allow future economic solutions for changing excitation wavelength, we also test high brightness LEDs instead of a laser for epifluorescence illumination.

For  $\mu$ -PIV tests, we choose as tracer particles 1  $\mu$ m diameter polystyrene particles loaded with fluorescent dyes which absorb at  $\lambda_{abs} = 625$  nm and emit at  $\lambda_{em} = 645$  nm (Molecular Probes, Invitrogen) commercialised as 2% by weight solutions in water. Figure (IV.2) shows their excitation and emission spectra.



Figure (IV.2). Fluorescence excitation and emission spectra of FluoSpheres crimson fluorescent microspheres in H<sub>2</sub>O as a function of the wavelength (nm).

The microfluidic flow cell is mounted on a microscope that allows two different configurations for sample illumination: 1) epiluminescence and 2) TIR.

In the first mode, light is introduced through a horizontal aperture of a vertical tube used to hold a dichroic mirror, a filter, a lens, a camera and a microscope objective that can be manually and automatically focussed.

The dichroic mirror (model z633rdc, Chroma, USA) reflects the light of the source used to illuminate the sample from above, in the epiluminescence configuration, and it transmits the emission spectrum to the camera (nominal transmitted light shown in Figure (IV.3): 5% at  $\lambda = 633$  nm, 9% at  $\lambda = 635$  nm, and 63% at  $\lambda = 645$  nm). A filter (model HQ 675/50 m, Chroma, USA) is placed in front of the camera, in order to cut off the excitation scattered light. Both are chosen to be suitable for laser and red light LED illumination.

For the current experiments, fluorescent particles are excited through light coming from above, emitted by a series of seven super bright LEDs (Precision Optical Performance AlInGaP LEDs, Agilent Technologies, characterized by 635 nm peak wavelength, 17 nm spectral half-width,

9300 ÷ 16000 mcd luminous intensity at 20 mA and 6 deg viewing angle), shown in Figure (IV.4), reflected by the dichroic mirror and collected by the objective lens.



Figure (IV.3). Percentage of light transmitted by the dichroic mirror as a function of the wavelength.

We use a  $63 \times$  magnification, apochromatic corrected long working distance (2.2 mm) water immersion objective with a numerical aperture NA = 0.9 (HCX APO L U-V-I, Leica), whose vertical position can be fine adjusted with a piezoelectric nanopositioning system (MIPOS 250, Piezosystem Jena GmbH) with 5 nm resolution and 60 nm repeatability.

The TIR illumination configuration is obtained by placing a trapezoidal prism made of flint glass (the dimensions are: height of 26 mm and lower base surface of  $50 \times 20 \text{ mm}^2$ ; the refraction index  $n_{flint} = 1.599$  at  $\lambda = 633$  nm) below the sample. The system is aligned so that the TIR interface is given by the surface of the bottom glass coverslip of the flow cell, which is put in optical contact with the prism upper base surface by a layer of immersion oil. The flow cell top coverslip is put in optical contact to the objective by ophthalmic gel.



Figure (IV.4). The light source in epi-illumination: a series of seven super bright LEDs.

The evanescent field extends inside the microchannel from the glass base surface in the liquid for about 130 nm, with exponentially decreasing intensity. For more details, refer to the next section of this paragraph.

Figure (IV.5) shows the laser light path inside the prism. The light beam, directed upwards, traverses normally the prism lower base, is reflected at the  $60^{\circ}$  inclined lateral surface and reaches the prism upper base under an angle of  $60^{\circ}$ , which is sufficiently high to satisfy the TIR condition on the surface of the sample.

Flint glass is used not only for its high refraction index value (higher than n = 1.520 of ordinary optics glass), making the realization of the geometric conditions to obtain TIR easier, but also because it is characterized by low native luminescence at the working wavelengths. We find that the light intensity from a 5 mW maximum power He-Ne laser is sufficient to get useful TIR images.



Figure (IV.5). Laser light path inside the prism for TIR illumination configuration.

We also try using LED illumination in TIR configuration but with no success: in fact, the relatively large LED light beam divergence makes the alignment procedure necessary to realize the TIR condition definitely difficult. As a consequence, the evanescent field is masked by the unavoidable presence of spurious transmitted light.

Since our aim is to build a versatile  $\mu$ -PIV set-up able to image flow velocities in a wide range of flow velocities and channel diameters, it is necessary to choose a camera that is as sensitive as possible. Indeed, at high velocities, a relatively short time interval between consecutive exposures and correspondingly brief exposure times are necessary to obtain meaningful PIV images. High sensitivity is also required to record flows in very narrow channels as, in this case, very small tracing particles (typical diameter ranging between hundreds nanometers and a few micrometers) with correspondingly low intensity florescence emission are needed.

Recently commercialised Electron Multiplying Charge Coupled Device (EMCCD) detectors are probably the best and most economic solution to satisfy our requirements. In fact, their sensitivity can approach that of Multichannel Plate (MCP) Intensified CCD cameras (ICCDs) at
a much lower cost. The spatial resolution for EMCCDs is higher than for ICCD cameras and image distortion, affecting many ICCD cameras, does not take place in EMCCDs. Moreover, there is no risk of damaging the detector for accidental overexposures: in fact, we are given the possibility to switch in real time from intensified imaging to normal CCD imaging by regulating the electron multiplier gain via software. This last feature is also crucial for rapid alignment and optimization of exposure conditions in  $\mu$ -PIV.

For our set-up, we choose a recently commercialized EMCCD detector with a  $658\times496$  pixel array (corresponding to a  $6.58\times4.96$  mm<sup>2</sup> real image), with 14 bit resolution, and real time electron multiplier gain control (Luca, Andor Technology). The camera is cooled down to -20 °C to lower the readout noise from the CCD detector. Moreover, binning allows charge from two or more pixels to be combined on the EMCCD chip prior to readout, giving even better noise performance, at the expenses of spatial resolution. Using the 63× microscope objective described above, the field of view is of  $104\times79 \,\mu\text{m}^2$ .

Figure (IV.6) shows a picture of the assembled experimental apparatus.



Figure (IV.6). Picture of the optical home-build experimental apparatus.

# TIR configuration: the evanescent field depth

In order to satisfy the TIR condition at the interface between the solid floor of the microchannel and the working fluid, an intermediate layer of refractive index  $n_2$  (see Figure (IV.7)) is

interposed between the flint glass prism ( $n_1 = 1.599$ ) and the microchannel floor (for glass,  $n_3 = 1.520$ ). In this way, laser light escape due to reflections at the interface is significantly reduced. Immersion oil of refractive index  $n_2 = 1.516$  is chosen.

The presence of an intermediate layer does not influence the laser beam incident angle on the surface of the sample, as it can be easily shown by applying Snell's law.



Figure (IV.7). TIR system reproduced in the microscope

Using the notations of Figure (IV.7), for a water solution of refractive index  $n_4 = 1.33$ , we have:

$$\begin{bmatrix} n_1 \sin \vartheta_1 = n_2 \sin \vartheta_2 \\ n_2 \sin \vartheta_2 = n_3 \sin \vartheta_3 \end{bmatrix}$$

that implies:  $n_1 \sin \vartheta_1 = n_3 \sin \vartheta_3$ .

TIR is expected to occur at the  $n_3 - n_4$  interface, for a certain value of the angle, higher than the critical value,  $\vartheta_3 > \vartheta_c^{n_3-n_4}$ . For  $\vartheta_1 = 60^\circ$ , explicit calculations give:

$$\vartheta_{c}^{n_{1}-n_{2}} = \sin^{-1}\left(\frac{n_{2}}{n_{1}}\right) = 71.36^{\circ} \qquad (\text{no TIR at the } n_{1} - n_{2} \text{ interface: } \vartheta_{1} < \vartheta_{c}^{\eta_{1}-n_{2}} )$$
$$\vartheta_{2} = \sin^{-1}\left(\frac{n_{1}\sin\vartheta_{1}}{n_{2}}\right) = 66.06^{\circ}$$
$$\vartheta_{3} = \sin^{-1}\left(\frac{n_{2}\sin\vartheta_{2}}{n_{3}}\right) = 65.72^{\circ}$$
$$\vartheta_{c}^{n_{3}-n_{4}} = \sin^{-1}\left(\frac{n_{4}}{n_{3}}\right) = 61.04^{\circ} \qquad (\text{TIR takes place: } \vartheta_{3} > \vartheta_{c}^{n_{3}-n_{4}} ).$$

The laser beam penetration depth is:

$$d = \frac{\lambda_0}{4\pi} \left( n_3^2 \sin^2 \vartheta_3 - n_4^2 \right)^{-1/2} = 130 \text{ nm.}$$

Notice that *d* heavily depends on the incident angle: in fact, for  $\vartheta_1 = 57^\circ$ , which is the limit incident angle for TIR to take place at the  $n_3 - n_4$  interface, we have d = 283 nm, while for example for  $\vartheta_1 = 68^\circ$ , the evanescent field depth reduces to d = 77 nm.

Let us consider the situation in which we introduce another intermediate layer, consisting for example in a cured thiolene resin (see Paragraph V.1), that adheres to the glass substrate and is in contact with the working fluid, having a refractive index  $n_{NOA} = 1.56$ . In this case, the laser beam penetration depth for  $\vartheta_1 = 60^\circ$  is still d = 130 nm.

We give a 100 nm conservative estimate of the possible variation in penetration depth due to the set-up alignment.

#### IV.2 Data acquisition and analysis system

In addition to the components shown in Figure (IV.6), the complete µ-PIV system comprises of a Personal Computer for image visualization, acquisition and analysis.

#### Experimental procedure for data acquisition

The channel is filled by a solution of fluorescent beads that is prepared by diluting the commercial product in deionised water so that a suitable quantity of liquid and density of beads in the  $\mu$ -PIV images is obtained (~ 80  $\mu$ l commercial bead solution in a few ml deionised water), taking care that no air bubbles are trapped inside the perfusion cell during its initial filling.

First, the position of the bottom channel wall is determined using white light from a mercury arc lamp by bringing into focus the beads that are in contact with the bottom glass slide. For a more precise determination, white light is turned off and the He-Ne laser is switched on.

The laser beam is aligned to yield the TIR condition on the base surface of the channel, within the field of view of the microscope objective (see Figure (IV.8)). The objective position is fine regulated through the piezoelectric actuator in order to image those tracers whose fluorescence is excited by the evanescent field, that extends from the microchannel floor up in the liquid to a  $\sim$ 130 nm height. The presence of some fluorescent probes stuck to the flow chamber bottom coverslip help us determining the actual position of the wall.

 $\mu$ -PIV measurements in TIRF configuration are of crucial importance for the analysis of particle dynamics close to the channel bottom surface.

After switching on the peristaltic pump,  $\mu$ -PIV double exposure images and/or sequences of single exposure images are recorded using the Andor Solis software.



Figure (IV.8). Picture showing laser beam reflections in its path towards the flint glass prism, at whose faces the TIR condition in realized.

We set exposure times and time intervals between exposures so as to acquire "meaningful"  $\mu$ -PIV images from the middle of the channel, where the highest fluid velocity is expected. For example, for a nominal flow rate of 30÷130 nl/s the CCD array is typically exposed for  $\Delta t_{exp} = 2$  ms and the time delay between successive images in a series of single exposures is  $\Delta t_{del} \approx 70$  ms (~14 Hz). The frame rate of the camera in single exposure mode is limited to about 15 Hz, which restricts possible flow measurements to relatively small tracer velocities (< 300 µm/s, for a 63× objective).

By varying the voltage applied to the piezoelectric nanoposition device,  $\mu$ -PIV images can be recorded for different position of the focus within the channel, spanning from the bottom wall up to a 200  $\mu$ m distance, that is the maximum elongation of the piezoelectric stage.

## Data analysis procedure

The series of acquired images are analyzed using EDPIV demo software [95].

First, single or double exposure images are processed by applying EDPIV image processing procedures, consisting of normalizations of grey values,  $\mu$ -PIV filters and thresholds on grey values, in order to reduce diffuse background fluorescence and optimize tracing particle identification.

Then, ensemble correlation algorithms are applied to analyze the sequences of single exposure  $\mu$ -PIV images and obtain the velocity field relative to each vertical position of the tracers inside the microchannel. The single processed double-exposure images are analyzed again by applying the correlation method.

A different analysis procedure can be applied to the same series of images as a useful comparison, to provide in first approximation a proof of validity of the results of a  $\mu$ -PIV analysis. It simply consists on adding couples of successively acquired single exposure images, removing the residual background fluorescence, using MATLAB 6.1 MathWorks software (see Figure (IV.9)), and eventually measuring directly particle displacements with a ruler for different vertical positions inside the microchannel.

An estimate of the slip length can be extrapolated from each velocity profile obtained through the  $\mu$ -PIV technique in the different experiments. The analysis procedures followed to measure the slip length will be described in detail in the next chapter.



Figure (IV.9). Nine images, each one created by overlapping a series of 6 consecutive  $\mu$ -PIV recordings, after removing the residual background fluorescence, using MATLAB 6.1 MathWorks software. The red segments visualize particle displacements in (6· $\Delta t_{del}$ ) for nine different vertical positions inside the microchannel.

#### **IV.3 Effects of Brownian motion**

In the performed experiments, a characteristic velocity might be  $u \approx 50 \text{ }\mu\text{m/s}$  and  $\Delta t_{del} \approx 70 \text{ ms}$ ; this yields a relative error due to Brownian motion of:

$$\varepsilon = \frac{1}{u} \sqrt{\frac{2D}{\Delta t_{del}}} = 0.07 \quad (7\%)$$

for 
$$D = \frac{k_B T}{3\pi \eta d_p} = 0.43 \ \mu \text{m}^2/\text{s}$$
 at room temperature.

Since this error is a result of diffusion, it is unbiased and can be substantially reduced by averaging over several particle images in a single interrogation spot and by ensemble averaging over several realizations. Assuming each particle contributes equally to the average velocity vector, and that each measurement is statistically independent, the diffusive uncertainty is then proportional to:

$$\frac{\varepsilon}{\sqrt{N}} = \frac{1}{u} \sqrt{\frac{2D}{N\Delta t_{del}}}$$

where N is the total number of particles in the average.

In the  $\mu$ -PIV measurements, there are approximately five particle images per interrogation spot, and a set of eight consecutive recordings are used to average the vector field. This yields  $N \approx 40$ independent samples or approximately a 1% uncertainty in the averaged velocity.

#### **IV.4 Spatial resolution**

Let us first concentrate on the in-plane spatial resolution for our  $\mu$ -PIV set-up. The point-spread function is given by:

$$d_s = 2.44M \frac{\lambda}{2NA} \cong 54 \ \mu m$$

and the actual recorded image diameter is:

$$d_e = \left[ d_s^2 + M^2 d_p^2 \right]^{1/2} = 83 \ \mu \text{m}.$$

Therefore, the value of the effective particle diameter projected back into the flow field is:

$$d_e/M = 1.32$$
 µm.

Assuming that the location of the correlation peak can be resolved to within 1/10<sup>th</sup> of the particle image diameter, the uncertainty of the correlation peak location is:

$$\delta x \sim \frac{d_e}{10M} = 132$$
 nm.

The measurement error due to detect ability  $\varepsilon_d$ , for a particle displacement  $\Delta x$  of 40 pixel, for example, measured on the CCD array (that is equal to a real motion of  $40^*(10 \ \mu\text{m})/63 \sim 6 \ \mu\text{m}$  in the flow field), is:

$$\mathcal{E}_d = \frac{\delta x}{\Delta x} \cong 0.02 \quad (2\%).$$

and reaches a value of 20% for a 4 pixel displacement.

Let us now consider the out-of-plane spatial resolution for the  $\mu$ -PIV set-up. The depth of field  $\delta z$  of the microscope objective is:

$$\delta z = \frac{n\lambda_0}{NA^2} + \frac{ne}{NA \cdot M} = 1.29 \ \mu m$$

for n = 1.33 (refractive index of water),  $\lambda_0 = 645$  nm (wavelength of the imaged light in vacuum) and  $e = 10 \ \mu\text{m}$  (pixel-to-pixel spacing on the CCD chip).

The depth of correlation, which represents the actual measurement plane thickness  $\delta z_m$  for the  $\mu$ -PIV system, is:

$$\delta z_m = \delta z_d + \delta z_g + d_p = \frac{3n\lambda_0}{NA^2} + \frac{2.16d_p}{\tan\theta} + d_p = 6.53 \ \mu m$$

where the contribution to the total measurement depth due to diffraction is:

$$\delta z_d = \frac{3n\lambda_0}{NA^2} = 3.18 \ \mu \text{m}$$

and the contribution of the geometrical optics is:

$$\delta z_g = \frac{2.16d_p}{\tan \theta} = 2.35 \text{ } \mu\text{m}.$$

#### **IV.5** Sample images

Figure (IV.10) shows a sample of six single exposure unprocessed and processed images recorded inside a ~180 µm deep channel in epi-illumination, using LEDs as illumination source, with a  $\Delta t_{exp} = 2$  ms and a  $\Delta t_{del} = 70.16$  ms, for Q  $\approx 170$  nl/s. On the right, the image created by software overlapping the six recordings: the flow is clearly directed horizontally.

In Figure (IV.11), there is a sequence of nine single exposure images recorded in TIRF configuration with  $\Delta t_{exp} = 1$  ms,  $\Delta t_{del} = 80.16$  ms, 2×2 binning and EMCCD gain = 255, for a flow rate of about 1950 nl/s, together with the image created by software overlapping the nine recordings. The presence of fluorescent probes stuck to the channel bottom wall, that work as reference particles for the determination of the actual wall position, is evident in the overlapping.



Figure (IV.10). Sequence of six single exposure unprocessed and processed images (658×496 pixel) recorded inside a microchannel in epi-illumination, using LEDs as illumination source, with a  $\Delta t_{exp} = 2$  ms and a  $\Delta t_{del} = 70.16$  ms. On the right, the image created by software overlapping the six recordings: the flow is horizontally directed.



Figure (IV.11). (a) Sequence of nine successively recorded single exposure images in TIRF configuration ( $\Delta t_{exp} = 1 \text{ ms}$ ,  $\Delta t_{del} = 80.16 \text{ ms}$ , 2×2 binning and EMCCD gain = 255, for a flow rate of about 1950 nl/s) and (b) their overlapped image. The presence of fluorescent probes stuck to the channel bottom wall, reference particles for wall position determination, is evident in (b).

# **Chapter V**

# SLIP OVER FLAT HYDROPHILIC AND HYDROPHOBIC SURFACES

#### V.1 Introduction

This introductive paragraph consists in a brief overview of the common types of materials exploited for microfluidic chip manufacturing. In particular, we dwell into a quite detailed description of the materials and techniques we apply in the realization of the microfluidic cells, inside whose microchannels, dynamics of water slippage is studied.

Early stage fabrication of microfluidic devices takes advantage of the high-resolution wellknown techniques developed for microelectronics and leads to the realization of micromachined silicon and glass devices. However, the application of these materials to microfluidics presents a number of disadvantages:

• Glass and silicon are very rigid: devices can be fragile and difficult to assemble for the different expansion coefficients;

• Silicon is very expensive;

• Fluidic devices are considerably bigger than electronic devices: fewer units can be placed on a single wafer, increasing costs;

• Silicon is opaque and therefore incompatible with optical detection techniques;

• Glass is not crystalline: it does not allow the creation of sharp edges;

• Processing costs are very high in terms of chemicals (photoresist, developer, etc.) and set-ups (UV or X-ray source, alignment equipment, etc.);

• High temperature is often needed;

• Lithographic processes to create complex structures are definitely time-consuming.

Since mid-1990, microfluidic fabrication technology moves towards polymer-based micromachining.

The principal polymeric material used for microfabrication is polydimethylsiloxane (PDMS), a polymer with an inorganic siloxane backbone and organic methyl groups attached to silicon. A representation of the typical PDMS chain chemical structure is shown in Figure (V.1).



Figure (V.1). Chemical structure of PDMS, with the inorganic chain (Si-O-Si) and the organic side groups (-CH<sub>3</sub>).

PDMS is commercially available as a liquid pre-polymer made by siloxane oligomers, that reticulates after mixing with a reticulant agent (containing a platinum catalyst) and thermal curing. After curing, it becomes an optically transparent and flexible elastomer.

PDMS devices are commonly produced using *replica molding*, which is a technique that consists in the replication of a master, obtained pouring a liquid prepolymer on it and then promoting reticulation.

Masters are produced with common photolithographic techniques, using silicon, glass or photocurable polymers. To promote the detachment of the polymeric replica, an anti-stick self-assembled monolayer is placed on the master before its use, avoiding the formation of chemical bonds between prepolymer and master material. Afterwards, the prepolymer is poured on the master, cured and peeled off.

PDMS devices present many advantages, which include:

• Low cost of materials;

• Low cost of techniques, much cheaper than photolithographic techniques for silicon: this provides the possibility to obtain low-cost and mass-production devices;

• Reproducibility and rapid prototyping, allowing the production of devices in short time (less than 24 hours);

• Transparency, that enables optical detection;

• Resolution depends mainly on the master and not on the fabrication techniques. Provided a good master, even expensive, a large (virtually infinite) number of devices can be created, reducing costs;

• Devices are generally flexible, so fragility is not a problem. Flexibility enables PDMS to conform to surfaces and to reversibly bond;

• PDMS is oxigen-permeable and biocompatible: ideal for biological applications and especially for in-vivo cellular studies;

• It presents an external hydrophobic surface, that makes difficult for polar solvents (such as water) to wet the PDMS surface but promotes the adsorption of hydrophobic contaminants.

One important drawback advices against the use of PDMS devices in chemistry: the incompatibility with non-polar (in general organic) solvents. When a non-polar molecule is brought in contact with a PDMS surface, it has a great affinity with the methyl groups that are commonly exposed by the polymer. This promotes adsorption and, often, molecule penetration inside the material. PDMS undergoes swelling, that changes the aspect ratio of the designed structure and that can effect integration with other components (such as membranes, detectors, mixers or electrodes). Moreover, swelling changes surface properties and may cause microfluidic device desealing, when PDMS is bonded to a glass substrate.

Thus, to work with organic solvents, materials different from PDMS have to be used. There are two main alternatives:

1. To use glass as a material for producing microfluidic devices.

2. To employ solvent-compatible polymers, possibly more expensive than PDMS, abandoning soft lithographic techniques.

The solution we adopt consists in the use of thiolene resins. Harrison et al. first showed in 2003 the possibility to use thiolene resins as materials for microfluidic device fabrication [96].

Thiolene resins are commercially available as prepolymers that, under UV radiation exposure, polymerizes becoming hard and transparent, with a reaction involving a *thiol* group (R-SH) and an *ene* group (the double bond in  $H_2C=C-R$ '):

$$RS-H + \underset{R'}{\longrightarrow} \overset{hv}{\longrightarrow} \overset{RS}{\underset{R'}{\longrightarrow}} \overset{H}{\underset{R'}{\longrightarrow}}$$

This property makes thiolene resins widely used as optical adhesives and commercialized by Norland Products as Norland Optical Adhesive (NOA), followed by a number indicating different adhesive characteristics (such as viscosity).

All types of NOA have a transmission spectrum that shows no adsorption in visible light, while presents an adsorption peak around 365 nm.

For microfluidic chip fabrication, thiolene resins are put between two glass slides and exposed to UV radiation using an appropriate photomask to design microfluidic structures. After UV exposure, the non-cured prepolymer can be washed away, returning the desired microchannel network.

NOA photopolymerization is a frontal process [94, 97], because UV light is absorbed principally by the first layer of uncured prepolymer and only a little part of radiation reaches the deep uncured prepolymer.

The time intervals needed to have complete polymerization depend on several parameters, such as adhesive thickness, lamp power, lamp distance and external temperature.

For microfluidic applications the uncured prepolymer has to be removed after UV exposure. Two solvents are commonly used: ethanol and acetone. The former has only a mechanical effect, while the latter has also a chemical effect, because the prepolymer is soluble in acetone. For what concerns organic solvents, thiolene shows good compatibility with non chlorinated solvents, while for chlorinated solvents (dichloromethane) the swelling ratio is similar to that for PDMS.

#### V.2 Flow over flat hydrophilic substrates

As a first application of the developed  $\mu$ -PIV set-up, we study water slippage in microfluidic channels presenting flat surfaces and, in particular, whose floors are made of the different materials used in the fabrication of our microfluidic devices. Therefore, we consider water slippage on flat glass, flat NOA and flat PDMS. Varying the surface chemistry of glass, we study water slippage over a glass slide coated with a self-assembled monolayer of two different silanes. In this paragraph, let us start considering flat hydrophilic or slightly hydrophobic substrates: glass and NOA.

#### Preparation and characterization of the glass substrate

The microchannel floor is made of a microscope glass coverslip (Menzel glass), whose roughness and wetting properties are controlled. The microscope slide is washed with water, rinsed with ethanol and acetone, to remove dust particles and contaminants, and dried with  $N_2$ . The roughness is measured with an AFM (Digital Instruments, model CPII, equipped with a CSC21/50-A Veeco tip), in contact mode, before to assemble the RC-30 close bath chamber. The AFM image is shown in Figure (V.2) and the estimated roughness is less than 1 nm root-mean square. The water-glass slide contact angle is determined using the home-made apparatus in Figure (V.3) and it measures (22±3)° (see Figure(V.4)).

After characterization, the glass slide is mounted in the RC-30 chamber, to serve as microchannel floor. The silicone gasket sandwiched between top and bottom glass coverslips (respectively 0.15 mm and 1 mm thick) creates, in the fluidic chamber, a channel nominally 100  $\mu$ m deep, 3 mm wide and 4 cm long for the perfusate to traverse.

The microchannel actual depth can increase of an appreciable quantity (up to around 80  $\mu$ m) from its nominal value, due to both the presence of layers of vacuum grease among the different components of the flow chamber (necessary to guarantee water tightness) and the variable mechanical pressure that can be exerted to hold in place the assembly.



Figure (V.2). 30  $\mu$ m × 30  $\mu$ m contact mode AFM image of the glass surface; the rms roughness is less then 1 nm.



Figure (V.3). Picture of the liquid-solid contact angle measurement set-up realized in our laboratory.



Figure (V.4). Picture showing the spreading of a 0.5 ml water drop on the glass surface.

## Preparation and characterization of the flat NOA surface

To prepare a flat NOA surface, the method we follow is quite simple. A microscope slide is washed with water, rinsed with ethanol and acetone, and dried.

A thin layer of uncured NOA (previously diluted with acetone) is created on the glass slide by spin coating, then the sample is exposed to UV radiation for a long period ( $20 \div 30$  minutes), for a straightaway hard curing.

The roughness of the sample is measured, with the same set-up as for the glass surface, before to assemble the RC-30 close bath chamber. The AFM image is shown in Figure (V.5) and the estimated roughness is 4 nm root-mean square in the scanned 30  $\mu$ m × 30  $\mu$ m region. In detail, the surface of the sample presents an extended 1 nm root-mean square roughness, reflecting the roughness of the glass substrate, with sparsely distributed asperities that can reach an height of around 30 nm.



Figure (V.5). 30  $\mu$ m × 30  $\mu$ m contact mode AFM image of the flat NOA surface. The rms roughness is ~ 4 nm. Asperities are sparsely present, presenting an height of up to 30 nm.



Figure (V.6). Picture showing a 0.5 ml water drop put on the flat NOA surface.

The measurement of water contact angle on the flat NOA surface results in  $(77\pm3)^\circ$ , as shown in Figure (V.6).

The NOA-coated slide is mounted in the RC-30 chamber (see Figure (V.7,a)), to serve as microchannel floor. A nominally 100  $\mu$ m deep, 3 mm wide and 4 cm long channel is realized inside the fluidic chamber, by laying a silicone gasket on the flat NOA surface, and putting a glass coverslip (0.15 mm thick) on top, that serves as channel ceiling. Again, the microchannel actual depth can result higher than its nominal value, for both the presence of layers of vacuum grease among the components and the variable mechanical pressure that is exerted on the assembly.

We also fabricate a *2-layer* thiolene microfluidic device (see Figure (V.7,b)) by softphotolithographic techniques, that presents a 250  $\mu$ m deep and 6.5 mm wide channel of lateral and bottom walls in NOA and a top wall in glass, and investigate the water flow profile inside it. For a detailed description of the device fabrication process refer to [98].

#### Velocity profiles and slip length measurements

Figure (V.8) shows the velocity profile measured in the 182 µm deep channel presenting a glass floor and ceiling, for a volumetric flux Q  $\approx$  169 nl/s. As regards the µ-PIV acquisition parameters, the CCD array is exposed for  $\Delta t_{exp} = 2$  ms and a series of 8 successive single exposure images are acquired with a time delay  $\Delta t_{del} = 70.16$  ms (14.253 Hz).

Each experimental point in the plot represents the modulus of velocity average value of the velocity field, returned by the EDPIV ensemble correlation analysis, relative to each vertical position inside the channel. The direction of the velocity vectors in each velocity field can be considered constant within the (low,  $< 1^{\circ}$ ) errors, as expected in a microfluidic laminar flow.

The statistical error affecting the velocity measurements is lower than the point dimensions in the plot.

A first observation shows how the velocity reached by the tracers close to the middle of the channel results too high (>  $300 \mu m/s$ ), for an acquisition frequency close to 15 Hz, to be measured using the EDPIV ensemble correlation software.

However, the slip length is deduced from the experimental points by applying least squares fits to a parabola of the data. The fitting function is  $v = Az^2 + Bz + C$ , where the three parameters *A*, *B* and *C* are left free to vary.



Figure (V.7). (a) Warner Instruments RC-30 closed bath chamber. (b) Home-made 2-layer thiolene flow cell. A-B: masks for microchip production via soft photolithography; C: picture of the complete device. The chip is fabricated in two levels: the bottom level (600 μm thick NOA layer, 13 minutes UV pre-curing time) allows fluid inlet and outlet through the needles, while the top level (300 μm thick NOA layer, 10 minutes UV exposure time) forms a 250 μm deep and 6.5 mm wide channel with flat NOA floor and lateral walls and glass ceiling [98].



Figure (V.8). Velocity profile measured in a 182  $\mu$ m deep channel presenting a glass floor and ceiling for Q  $\approx$  169 nl/s.

Explicitly, the parameters must be determined in order to find the minimum for the Chi-square distribution:

$$\chi^{2} = \sum_{i=1}^{N} \left[ \frac{v_{i} - (Az_{i}^{2} + Bz_{i} + C)}{\sigma_{i}} \right]^{2},$$

where  $\sigma_i$  is the error relative to  $v_i$  and *N* is the number of experimental points to fit. The slip length is given by:

$$b = z_{wall} - \frac{-B - \sqrt{B^2 - 4AC}}{2A},$$

where  $z_{wall}$  is the actual vertical position of the wall. We determine  $z_{wall}$  using TIRF imaging, therefore, the value must be corrected taking into account the evanescent field depth  $(d = 0.13 \pm 0.10 \text{ }\mu\text{m})$ .

In the practice, we extrapolate at least five different estimates for the slip length, using five or more windows including different groups of experimental points, and average the result, to extract a more reliable value for *b*. The underlying idea is to keep into account the fact that some points sufficiently close to the interface can be pathologic: in fact, tracers flowing in proximity of the interface can move with a velocity that is appreciably different from the fluid velocity, due to hydrodynamic, electrostatic or electrokinetic interactions with the solid surface. A detailed discussion of the problem will be given in the Section V.4. Therefore, varying the data windows for the fits, a certain variable number of experimental points close to the interface are excluded. The slip length is the average of the values derived by the different fits and measures:

#### $b_{glass} = 0.10 \pm 0.09 \text{ (stat)} \pm 0.10 \text{ (syst)} \,\mu\text{m}$

where the statistical error is the standard deviation of the values, while the systematic uncertainty is given by the error in the determination of the actual position of the wall from TIR images, which is the uncertainty in the evanescent field depth.

The plot of Figure (V.9) shows the velocity profile measured in a 162 µm deep channel presenting flat NOA floor and glass ceiling, for a volumetric flux Q  $\approx$  79 nl/s. The CCD array is exposed for  $\Delta t_{exp} = 2$  ms and a series of 8 successive single exposure images are acquired with a time delay  $\Delta t_{del} = 70.16$  ms (14.253 Hz).

In this case, the velocity profile of the tracers is reconstructed by the experimental points throughout the channel.

Estimates of the slip length are again deduced by applying least squares parabolic fits to the data (considering variable data windows) and taking the opposite of the x-coordinate of the fitting-function crossing-point with the horizontal axis.

The slip length is the average of the values derived by the different fits and measures:

$$b_{NOA} = 1.35 \pm 0.03 \text{ (stat)} \pm 0.10 \text{ (syst)} \ \mu\text{m}$$

where the statistical error is the standard deviation of the values, while the systematic uncertainty is given by the determination of the actual position of the wall from TIR imaging.

Water flow inside the same typology of microchannel (with NOA floor and glass ceiling) is also investigated using the home-made flow cell previously described, for an higher value of the volumetric flux:  $Q \approx 117$  nl/s. The resulting velocity profile is shown in Figure (V.10).



Figure (V.9). Velocity profile measured in a 162  $\mu$ m deep channel presenting a flat NOA floor and glass ceiling for Q  $\approx$  79 nl/s.



Figure (V.10). Velocity profile measured in a 250  $\mu$ m deep and 6.5 mm wide channel, realized in the home-made microfluidic cell of Figure (V.7, b), presenting lateral and bottom walls in NOA and a top wall in glass, for Q  $\approx$  117 nl/s.

This time, the velocity profile cannot be completely reconstructed, for the maximum excursion of the objective piezoelectric actuator is limited to  $200 \ \mu\text{m}$ . Moreover, close to the middle of the channel, an experimental point dispersion with respect to the expected parabolic trend is particularly evident, probably due to the formation of small bubbles along the perfusion line or to some defective operation of the peristaltic pump pulsation dampener.

Therefore, a different fitting procedure is applied to obtain reliable results. The slip length measurement is made from the experimental points ( $z_i$ ,  $v_i$ ), for  $z_i < 60 \mu$ m, by making a linear regression, and taking the opposite of the intercept of the least square line. The fitting function is

z = B'v + A', where the two parameters A' and B' are left free to vary. Explicitly, the parameters must be determined in order to find the minimum for the function f(A', B'):

$$f(A',B') = \sum_{i=1}^{N} \left[ (B'v_i + A') - z_i \right]^2,$$

where *N* is the number of experimental points taken to be fitted. Thus, given  $z_{wall} = -0.13 \pm 0.10$ µm (from the evanescent wave penetration depth), we have:  $b = z_{wall} - A'$ .

Again, we calculate a few estimates for the slip length, using different groups of experimental points for each linear fit (excluding a variable number of possible pathologic points close to the surface) to extract a more reliable value for *b*.

The resulting slip length is:

$$b'_{NOA} = 1.40 \pm 0.31 \text{ (stat)} \pm 0.10 \text{ (syst)} \,\mu\text{m}$$

that shows a good compatibility with the previous determination of  $b_{NOA}$  within the errors.

#### V.3 Flow over flat hydrophobic substrates

Let us now deal with water slippage on flat hydrophobic surfaces. Varying the surface chemistry of glass, we study water slippage over a glass slide coated with a self-assembled monolayer of two kinds of silane: octadecyltrichlorosilane and perfluorooctyltrichlorosilane. We eventually consider the study of the water flow on a flat PDMS surface.

#### Preparation and characterization of the silanized surfaces

The first step is to clean a glass slide. A microscope glass coverslip is washed with water, and rinsed with ethanol and acetone. Afterwards, the glass is dried with N<sub>2</sub> and it is cleaned in a UV-ozone cleaner apparatus (Jelight Inc., model 42-220), in which a mercury lamp produces UV radiation (with  $\lambda_1$ =184.9 nm and  $\lambda_2$ = 253.7 nm).

Molecular oxygen strongly absorbs radiation at 184.9 nm and dissociates to produce atomic oxygen, which interacts with  $O_2$  to produce ozone. The 253.7 nm radiation is absorbed by the largest part of contaminants on the glass surface (hydrocarbons), that interact with ozone to produce volatile molecules (CO<sub>2</sub> and H<sub>2</sub>O). Schematically:

$$\frac{3}{2}O_2 + hv(184.9nm) \rightarrow O_3$$
  
hydrocarbons +  $O_3 + hv(253.7nm) \rightarrow CO_2 + H_2O_3$ 

These steps produce a clean surface, with exposed superficial -OH groups, due to the strongly oxidant environment.

The silanization process consists in the formation of a self-assembled monolayer (SAM) of a silane on the glass surface.

Two molecules are used to obtain two different coatings for the glass slides: octadecyltrichlorosilane ( $H_3C$ -( $CH_2$ )<sub>17</sub>—SiCl<sub>3</sub>, commonly known as OTS) and 1H,1H,2H,2H-perfluorooctyltrichlorosilane ( $F_3C$ -( $CF_2$ )<sub>6</sub>—( $CH_2$ )<sub>2</sub>—SiCl<sub>3</sub>, TPOS).

Both molecules present a long chain (alkylic, with or without fluorine atoms) and a reactive head (with three chlorine atoms), as schematically shown in Figure (V.11).

The glass slide is first treated for 30 minutes in UV-ozone cleaner, wetted with ultrapure water, lightly dried with nitrogen and then exposed to the silane vapours. The surface is covered by - OH groups and by a thin water layer, therefore, when the silane approaches the surface, its chlorine atoms are hydrolyzed by water:

$$R - SiCl_3 + H_2O \rightarrow R - Si(OH)_3 + 3HCl_3$$

Then, the molecules are adsorbed on the surface by hydrogen interaction with the superficial -OH groups. The same process takes place between adjacent molecules. This unstable configuration evolves, with water elimination, in the stable network shown in Figure (V.12).

The glass surface is now completely covered by a SAM of silane molecules and the wetting properties change from hydrophilic (typical of bare glass) to hydrophobic, for the presence of the alkyl or fluorinated chains.

In practice, a small quantity (~160  $\mu$ l) of silane is poured inside a vessel, in a low-vacuum chamber, and the glass slide is put on top of the vessel. A rotative pump provides the low vacuum (~10 mbar) and a liquid nitrogen trap prevents HCl vapours to interact with the pump. After a one hour treatment, external pressure is introduced inside the chamber by air reflux.

The estimated roughness of the resulting surface is 1 nm root-mean square, close to bare glass roughness, for both samples.

The contact angle of water on the glass surface coated with a SAM of OTS results in  $(108\pm2)^{\circ}$ , while it measures  $(103\pm2)^{\circ}$  on the glass surface coated with a SAM of TPOS (see Figure (V.13) and (V.14)), with approximately 20° hysteresis between advancing and receding angles.

Each silane-coated glass slide is eventually mounted in the RC-30 chamber, to serve as microchannel floor for  $\mu$ -PIV investigation.

Inside the fluidic cell, a silicone gasket creates a channel that is nominally  $250 \mu m$  deep, 3 mm wide and 4 cm long, presenting a ceiling of bare glass.



Figure (V.11): Chemical formula of OTS and TPOS, the two silanes used to functionalize the glass surfaces. The reactive head is shown in both molecules.



Figure (V.12). Schematic representation of the reactions involved in the silane SAM formation.



Figure (V.13). Picture showing a water drop put on the OTS-functionalized glass surface.



Figure (V.14). Picture showing a water drop put on the TPOS-coated glass surface.

# Preparation and characterization of the PDMS surfaces

To produce a flat PDMS surface, we apply the following procedure. A glass slide is cleaned, washing it with water and rinsing it with ethanol and acetone, to remove dust and contaminants. After drying the glass slide, it is ready to be used as surface for PDMS spin coating.

The polymer (Sylgard 184, Dow Corning) is prepared mixing the two components (prepolymer and reticulant agent) with a ratio 1:10 w/w. For the high viscosity of the prepolymer, many air bubbles remain trapped inside the material, while mixing the two components, and a degassing treatment is needed. The beaker containing the uncured PDMS is put inside a low vacuum chamber and a rotative pump is activated until bubbles disappear. A small quantity of PDMS is very gently poured on top of the glass slide to avoid new bubble formation, then the sample is loaded on the spin coater. When a flat PDMS layer coating the slide is formed, thermal curing (at

90 °C for 30 minutes or at room temperature for 24 hours) is performed: reticulation reactions take place producing the final elastomer.

The measurement of water contact angle on the PDMS surface results in  $(120\pm1)^{\circ}$  (see Figure (V.15)), while we cannot characterize its surface roughness for the AFM apparatus is temporary out of order.

The sample is mounted in the RC-30 chamber, to serve as microchannel floor. A silicone gasket forms, in the fluidic chamber, a nominally 250  $\mu$ m deep, 3 mm wide, 4 cm long channel sealed on top with a glass slide, for the perfusate to traverse.



Figure (V.15). Picture of a 0.5 ml water drop put on the flat PDMS surface.

#### Velocity profiles and slip length measurements

The plot of Figure (V.16) shows the velocity profile measured in a ~350 µm deep channel presenting a floor made of glass covered with a SAM of OTS and a ceiling of bare glass, for a volumetric flux Q  $\approx$  100 nl/s. The CCD array is exposed for  $\Delta t_{exp} = 2$  ms and a series of 8 successive single exposure images are acquired with a time delay  $\Delta t_{del} = 69.08$  ms (14.476 Hz).

The velocity profile of the tracers is only partially reconstructed by the experimental points, up to a distance of  $\sim$ 130 µm from the bottom wall.

One of our first prototypes of peristaltic pump pulsation dampener is used for this  $\mu$ -PIV acquisition, therefore, the wide dispersion of the measurements with respect to the expected parabolic trend might be determined by its defective operation. Another possible reason could be the formation of small bubbles along the feeding system.

Estimates of the slip length are deduced by applying least squares linear fits to the data points ( $z_i$ ,  $v_i$ ) for  $z_i < 40 \ \mu\text{m}$ , considering variable data windows, and taking the opposite of the intercept of the fitting line (and applying the correction for  $z_{wall} = -0.13 \pm 0.10 \ \mu\text{m}$ ).



Figure (V.16). Velocity profile measured in a ~350  $\mu$ m deep channel presenting a OTSfunctionalized glass floor and a bare glass ceiling for Q  $\approx$  100 nl/s.



Figure (V.17). Velocity profile measured in a 282  $\mu$ m deep channel presenting a TPOSfunctionalized glass floor and a bare glass ceiling for Q  $\approx$  70 nl/s.

The slip length is given the average of the values derived by the different fits and measures:

 $b_{OTS} = 0.56 \pm 0.15 \text{ (stat)} \pm 0.10 \text{ (syst)} \mu \text{m}.$ 

Figure (V.17) shows the velocity profile measured in the 282 µm deep channel presenting a TPOS-functionalized glass floor and a bare glass ceiling, for a volumetric flux  $Q \approx 70$  nl/s. As regards the µ-PIV acquisition parameters, the CCD array is exposed for  $\Delta t_{exp} = 1$  ms and a series of 8 successive single exposure images are acquired with a time delay  $\Delta t_{del} = 69.16$  ms (14.459 Hz). The statistical error affecting the velocity measurements, from the ensemble correlation analysis, is lower than the point dimensions in the plot.



Figure (V.18). Velocity profile measured in a 357  $\mu$ m deep channel with a flat PDMS floor and a bare glass ceiling for Q  $\approx$  90 nl/s.



Figure (V.19). Velocity profile measured in a 357  $\mu$ m deep channel with a flat PDMS floor and a bare glass ceiling for Q  $\approx$  83 nl/s.

The velocity profile can only be partially reproduced, exploiting the 200 µm maximum excursion of the objective piezoelectric actuator. Close to the middle of the channel, a quite wide point dispersion with respect to the expected parabolic trend is appreciable, probably due to a defective operation of the peristaltic pump pulsation dampener.

A linear fitting method is applied to obtain slip length measurements. Taking the experimental points  $(z_i, v_i)$ , for  $z_i < 60 \mu$ m, and making a linear regression, the slip length is given by the opposite of the intercept of the least square line (plus correction for  $z_{wall} = -0.13 \pm 0.10 \mu$ m).

Again, we calculate a few estimates for the slip length, using different groups of experimental points for each linear fit (excluding a variable number of possible pathologic points close to the surface) to extract a more reliable value for b.

The resulting slip length is:

$$b_{TPOS} = 0.81 \pm 0.30 \text{ (stat)} \pm 0.10 \text{ (syst)} \mu\text{m}.$$

The plots in Figures (V.18) and (V.19) show two velocity profiles measured in a 357 µm deep channel presenting a flat PDMS floor and a bare glass ceiling, for a volumetric flux Q  $\approx$  90 nl/s and Q  $\approx$  83 nl/s, respectively. In both µ-PIV acquisitions, the CCD array is exposed for  $\Delta t_{exp} = 2$  ms and a series of 8 successive single exposure images are acquired with a time delay  $\Delta t_{del} =$  70.16 ms (14.253 Hz) for each vertical position inside the microchannel.

In the plots, the velocity statistical error bars are comparable to the experimental point dimensions.

Because of the possible presence of small bubbles along the perfusion line or the defective operation of the peristaltic pump pulsation dampener (one of the first dampener prototypes is used here), the velocity measurements show a wide dispersion with respect to the expected parabolic trend. This is the reason why, multiple  $\mu$ -PIV acquisitions are made for this channel typology to obtain a reliable slip length measurement.

For each velocity profile, the slip length estimate is the mean of the slip length values obtained using different groups of experimental points ( $z_i$ ,  $v_i$ ), for  $z_i < 60 \mu$ m, for the linear regression analysis (excluding a variable number of possible pathologic points close to the surface).

The resulting slip length is the weighed average value of the b determinations from the two velocity profiles:

 $b_{PDMS} = 0.97 \pm 0.79 \text{ (stat)} \pm 0.10 \text{ (syst)} \mu\text{m}.$ 

#### V.4 Results on flat substrates

The plot in Figure (V.20) summarizes our experimental results for the boundary slip dependence on the contact angle. In case of multiple determinations for the same surface, the weighed average of the *b* values is reported. The vertical bars represent the statistical error on *b*, while the horizontal red dashed lines are meant to show the systematic uncertainty affecting *b*, that comes from the uncertainty in the actual wall position due to the variable TIR penetration depth.

Excluding the flat NOA surface case, we observe a mild increase of b with increasing surface hydrophobicity, even though, for the quite big errors affecting our determinations, we cannot precisely quantify, in each situation, the deviation from the no-slip boundary condition.

The slip lengths we find are possibly too large to reflect just the liquid slippage over the wall, especially for NOA, taking into account that we have almost a complete wetting situation.

The necessary comparison with the results available in literature is visually realized in the plot of in Figure (V.21), where our measurements are represented together with the determinations of b obtained with a number of different techniques. Our experimental points ( $\circ$ ) are located quite

close to PIV determinations of b ( $\blacktriangleright$ ) reported by other research groups. However, the overall plot still does not show any evident dependence of the slip length on  $\theta$ .



Figure (V.20). Experimental variation of the slip length *b* with the liquid-solid contact angle  $\theta$ . The vertical bars represent the statistical uncertainty on *b*. The horizontal red dashed lines show the systematic error affecting *b*, that comes from the uncertainty on the actual wall position.



Figure (V.21). Experimental variation of the slip length *b* with the liquid-solid contact angle *θ*, taken from [7], with our μ-PIV *b* measurements superimposed (○). The experimental results are given by the following techniques: pressure driven flow (○), sedimentation (●), fluorescence recovery (□), PIV (►), streaming potential (▼), fluorescence cross-correlations (◄), SFA (■), and AFM (▷). When a solid line is drawn, the experimental results are given for a range of contact angles.

It is worth underlying that, in our investigations, we observe that slip does not appreciably depend on the shear rate at which the experiment is performed.

Let us conclude the paragraph with a discussion of the possible interactions with the solid surface that tracers, flowing in proximity of the interface, can suffer from.

These phenomena can lead tracing particles to move with a velocity that noticeably differs from the underlying fluid velocity and, therefore, provide a justification of the analysis procedures we adopt to obtain reliable slip length values.

The interactions that determine the difference in tracer behaviour with respect to the working fluid have a different nature: hydrodynamic, electrostatic and electrokinetic. Let us consider each effect separately.

• The influence of pure hydrodynamic interactions of spheres seeding the fluid with walls [42, and ref. therein].

When a sphere of radius *R* is located in the central part of the channel (of half width *L*) in a Poiseuille flow, wall interactions are of the order of  $(R/L)^2$ , that is 10<sup>-5</sup> for us. The same is true for slip walls, since the fluid velocity in a channel with slip boundary conditions on the walls is equivalent to a Poiseuille flow displaced by a distance equal to the slip length *b*.

Interactions with walls only become important when a sphere moves close to either wall, at distances of few times R and less. At such distances, the flow profile practically is a shear flow.

Goldman et al. [99] calculate the velocity of a sphere, with the centre located at a distance z from

the wall, for a pure Couette flow (in the absence of the sphere, the shear  $\gamma$  does not depend on z) as:

$$\frac{v}{z \gamma} \approx 1 - \frac{5}{16} \left(\frac{R}{z}\right)^3 \quad \text{for } z/R >> 1,$$
$$\frac{v}{z \gamma} \approx \frac{0.7431}{0.6376 - 0.2000 \ln((z-R)\delta/a)} \quad \text{for } (z-R)/R << 1.$$

where  $\delta$  is the shear characteristic scale of variation.

The results are shown in Figure (V.22) where the points represents the exact numerical values and the dashed curves show the two limit cases given by the former equations.

The velocity of the particle is always lower than the velocity of the liquid in which it is suspended, but, as the distance of the particle from the wall gets more than three times its radius, the difference becomes less than 1%.



Figure (V.22). Speed of a sphere close to a wall in a simple shear flow, according to the ratio of the distance of the bead centre from the wall, and normalized by the fluid velocity in its absence. The insert shows Couette flow undisturbed (black line) and the speed of the ball. z / R = 1 is the contact point, where the marker velocity tends to zero logarithmically. The difference between the speed of the sphere and of the undisturbed flow is less than 1% when the distance from the

edge of the sphere to the plan is greater than its diameter (z / R = 3).

• The electrostatic interaction between surface-charges of the tracing particles and the ions on the solid surface.

Tracers typically present a negative surface charge that comes from sulphate groups  $SO_4^{2-}$  or carboxylic groups in the basic form -COO<sup>-</sup>. The solid wall is also negatively charged, mainly due to the fact that the silane terminals -Si-O-H at the surface (of glass, silicon or PDMS) get protonated in the presence of an aqueous solution.

The electric potential associated with these charges at a pH of 7 is on the order of -100 mV. This electrostatic repulsion affects the working solution, creating a sort of depletion layer.

Water is an electrolyte and, even if purified or deionised, it contains ions that couple to the electrostatic field and redistribute in order to screen its effect, forming a double layer near the immersed surface.

The first layer (the Stern layer) is a molecular film of counter-ions, bonded to the solid by an electrostatic interaction. The second layer (the Gouy-Chapman layer) is diffused and its structure is determined by the statistical equilibrium between thermal agitation, which tends to homogenize a charge distribution, and electrical forces, which tend to break the homogeneity by displacing charges of the same sign towards the surface.

Coupling the Poisson's equation for the electrostatic potential  $\varphi$  with a Boltzmann distribution for each of ion, for the simple case of an infinite slab normal to *z* coordinate, we have:

$$\frac{d^2\varphi}{dz^2} - \frac{\sigma}{\varepsilon D}\varphi = 0$$

where  $\varepsilon$  is the dielectric permittivity of the liquid medium,  $\sigma$  is the electrical conductivity and D is the diffusion coefficient of the ions of the double layer.

Considering that the potential of the surface at infinity is zero, the solution is given by:

$$\varphi(z) = \zeta \exp(-z/\lambda_D)$$

where  $\zeta$  is the potential for z = 0 and  $\lambda_D$  is the Debye-Hückel screening length, which expresses the order of magnitude of the thickness of the double layer, and is defined by the expression:

$$\lambda_D = \kappa^{-1} = \sqrt{\frac{D\varepsilon}{\sigma}}$$

In water, the semi-numerical formula  $\lambda_D = 0.3/\sqrt{c}$  can be used, where *c* is the concentration expressed as mol/l and  $\lambda_D$  in nm.

Because of water dissociation, the concentration *c* is necessarily higher than  $10^{-7}$  mol/l and the Debye length of pure water is about 1 µm (that is difficult to achieve practically anyway). In our investigations, a reasonable value for  $\lambda_D$  is a hundred nm, because of the presence of stabilizing ions in the solution of the tracing particles.

 $\zeta$  is the so-called Zeta potential of the solid, that is the potential at the boundary of the Stern layer, and it can be related to the surface charge density by the following expression:

$$\zeta = \frac{\sigma_e D}{\sigma \lambda_D}$$

where  $\sigma_e$  is the surface charge given by the sum of the charges frozen in the Stern layer and the charges appearing at the solid surface when it is submerged in the electrolyte.

Electrostatic interactions must be taken into consideration at sub-micrometer scales, especially for saline solutions, as they are responsible for a substantial depletion of the makers up to a distance of more than twice the Debye length from the solid wall. Moreover, a transition zone between exclusion and concentration identical to the solution is present and must be taken into consideration.

In the practice, for the reasonable values of  $\lambda_D \approx 100$  nm and  $\zeta \approx 50$  mV in our investigations, very few tracers are expected to have their center located within 300 nm from the wall and tracer distribution should be virtually uniform for a distance greater than 500 nm.

• An electrokinetic effect of the second order.

If an external or induced electric field is also applied to the microchannel, the presence of a net charge density near the wall, moving in response to the field, leads to a new velocity component, additional to the simple Poiseuille velocity, known as *electroosmotic flow*.

As the electrolyte solution flows down a pressure gradient, the cloud of counterions is advected by the flow and a streaming current is established.



Figure (V.23). Schematic representation of the flow between two parallel plates with charged surfaces (zeta potential  $\zeta_w$ ) and a charged suspended particle (zeta potential  $\zeta_p$ ). In the illustrated situation,  $\zeta_w < 0$  and  $\zeta_p < 0$ . The channel height is 2*h*, the particle radius is *R*, the smallest wall-particle distance is *d*, and the screening length  $\lambda_D = \kappa^{-1}$  [100].

If no short-circuit is present between the two ends of the capillary, accumulation of charge sets up a potential difference along the channel, termed the *streaming potential*.

Such a potential, opposes the mechanical transfer of charge by creating a reverse conduction current through the bulk solution, so that the total net electric current is zero. This induced axial electric field scales with the applied pressure gradient and leads to the creation of an induced *electroosmotic back-flow* which effectively slows down the fluid motion in the microchannel: a smaller flow rate for a given pressure drop is obtained compared to the case of the regular Poiseuille flow rate.

Let us now consider the tracing particles seeding the working fluid. The tracers, presenting a surface charge too, are advected by the fluid motion and they will also feel the influence of the induced streaming electric field: consequently, their velocity will not only reproduce the fluid velocity but will also include an induced *electrophoretic component* proportional to their zeta potential  $\zeta_p$  and the streaming electric field.

If the particle potential  $\zeta_p$  has the same sign as the channel surface potential  $\zeta_w$ , its electrophoretic component will be in the streamwise direction; furthermore, if  $\zeta_p$  is large enough, the electrophoretic velocity of the particle will be able to overcome the induced electroosmotic back-flow.

These particles would go faster than the expected Poiseuille pressure-driven profile, leading to the incorrect conclusion that the velocity profile has a non-zero slip velocity at the wall. Thus, even if the flow satisfies the no-slip condition, measurements of particle velocities would lead to non-zero apparent slip lengths.

This effect is formally described by Eric Lauga in [100] for the geometry of the Figure (V.23). In that article, Lauga proposes an estimate of a maximum value of apparent slip length that can be obtained in a typical  $\mu$ -PIV experiment, solely due to electrokinetic effects, that is around a few tens of nm. Although beyond molecular size, these value is much too small to explain the experimental data where  $b \approx 1 \mu m$  or more. Alternative mechanisms need to be invoked to explain the data, such as the presence of surface attached bubbles.

Drawing conclusions, the possible hydrodynamic, electrostatic or electrokinetic interactions between tracing particles and solid wall have relevant effects on the dynamics only when the particles move close to the wall, at distances of 2 or 3 times R and less. They might lead tracers to move with a velocity that differs from the underlying fluid velocity, either overestimating or underestimating its value.

Therefore, through the elimination of experimental determinations of the velocity fields up to a distance of  $\sim 2 \ \mu m$  from the interface, we make sure to get rid of possible pathologic measurements from the analysis procedure.

# **Chapter VI**

# SLIP OVER MICRO AND NANO STRUCTURED SURFACES

#### **VI.1 Introduction**

Nowadays, great interest is given to the research of methods to control the behaviour of fluids in microsystems for potential use in small integrated devices, to perform analytical and synthetic chemical tasks. Fluid handling for chemical applications typically requires control elements, such as pumps and valves, and also the ability to manipulate the position of streams within flows, e.g., for mixing or for the spatially resolved delivery of reagents.

In microfluidic systems, it is a challenge to create three-dimensional flows, because Reynolds numbers are small and channel geometries are typically long and narrow, with pressure and electric fields applied from the ends.

Another important practical constraint in designing microfluidic systems is that the common lithographic methods used in fabrication lead to planar, layered structures, while complex threedimensional structures are difficult to fabricate. Typically, the flow structure follows locally simple patterns, such as Poiseuille profiles for pressure driven flows.

A way to enlarge the forms of flow accessible with simple steady pressure gradients is to take advantage of the surface control allowed by microfabrication technology. For example, the creation of linear grooves, on (at least) one of the surfaces of the channel, makes the surface locally anisotropic.

This local anisotropy affects the geometry of the flow in a way that we try to characterize exploiting the  $\mu$ -PIV technique.

First of all, we concentrate on the effect that a pattern of holes or a pattern of pillars, present on the floor of a microchannel, has on water slip dynamics inside the microchannel.

Then, we consider the case of a microchannel with a floor bearing a set of parallel grooves, at a certain angle with respect to the channel axis, and characterize water flow inside it for a variable value of that angle.

Finally, we take into consideration the textured surfaces naturally occurring in Ginkgo Biloba leaves, which present hierarchical micrometer and nanometer sized structures. Water droplets on these surfaces readily sit on the apex of the nanostructures because air bubbles fill in the valleys of the structure under the droplet, resulting in extremely high contact angles and considerable superhydrophobic behaviour.

We fabricate a microchannel that presents on the floor an artificial surface that replicates the geometrical structures of a Ginkgo Biloba leaf and try again to characterize water flow inside it.

## VI.2 Master production

Micro patterned silicon masters produced with photolithography are supplied by the TASC National Laboratory (Trieste). Let us dwell into a brief description of their production process. The main lithographic steps are:

- sample cleaning;
- chrome deposition;
- photoresist deposition;
- UV exposure;
- photoresist development;
- chrome wet etching;
- silicon dry etching.

Si wafers (1 0 0), purchased by Siltronix (France), are cleaned in a clean-room environment with a Piranha solution (70:30  $H_2SO_4:H_2O_2$ ). They are immersed in the solution and heated to 90 °C for 30 ÷ 40 minutes. This process removes organic contaminants from the surface and forms a thin silicon oxide film, that is eventually removed dipping the wafer in a 10:1  $H_2O:HF$  solution for 5 minutes.

A mask reproduces the geometrical structures to be transferred. It is made of a thin Cr layer deposited on glass or quartz, for Cr absorbs UV light while glass or quartz transmit UV light to the underlying photoresist layer.

Photoresist is a photosensitive polymer: the luminous flux initiates physico-chemical reactions in the polymer which modify the solubility in certain solvents. In a so-called *positive resist*, light breaks or weakens the internal bonds and induces rearrangement of the molecules to a more soluble form. In a so-called *negative resist*, the action of light is to induce the formation of covalent bonds between principal or secondary chains, making light-exposed zones insoluble in a particular solvent while light-shielded zones can be dissolved in the same solvent.
Polymethylmethacrylate (PMMA) is the (positive) resist chosen. Before photoresist deposition, a ~ 100 nm thick Cr layer is evaporated on the clean Si surface.

A photoresist layer is created via spin coating, followed by slight heating (100 °C for a few minutes) to completely eliminate the polymer solvent.

The film is exposed to collimated UV light, across the photolithographic mask, for a certain time interval, that depends on several experimental parameters, such as resist layer thickness and light intensity.

The development step consists on immersing the system in a solvent to remove the irradiated zones of the resist layer, creating a pattern identical to the glass/quartz mask. Then, the resist mask is heated to 140 °C for 1 minute to improve resist adhesion.

Using a solution of  $Ce(NH_4)_2(NO_3)_6$ ,  $CH_3COOH$  and deionized  $H_2O$ , the so-called *Chrome etch*, portions of the Cr layer that are not protected by the resist are removed: thus, the pattern is reproduced in the Cr layer. The rest of the resist is eliminated using acetone.

The silicon surface, covered by a pattern of Cr, is now ready for the dry etching process, that is realized using an Inductively Coupled Plasma (ICP) system.

Plasma is produced by dissipation of electrical power to low pressure gas. An RF-driven coil surrounding the ICP chamber allows to couple inductively energy to the gas, allowing dense plasma formation. Electrons and ions in the plasma are accelerated by an external electric field. Accelerated light electrons leave the plasma cloud faster then ions and, therefore, the cloud acquires a positive electrical potential with respect to the surroundings. Ions are accelerated towards the chamber walls and the sample surface, determining the etching mechanism.

Figure (VI.1) shows a SEM image of a silicon master presenting a pattern of rectangular canals 8  $\mu$ m wide, 3  $\mu$ m deep and of 16  $\mu$ m period.



Figure (VI.1). SEM image of a silicon master patterned with canals 8 µm wide, 3 µm deep and of 16 µm period.

## VI.3 Preparation of the grooved surfaces: pillars, dots and canals

The aim is to prepare a transparent surface, patterned with the desired geometrical structures (pillars, dots or parallel linear canals), that could be used as floor for a microchannel in a fluidic cell.

The procedure we apply consists of the following two phases:

- replica molding with PDMS of the silicon master;
- UV-assisted replica molding with NOA of the PDMS master.

After silanization, the silicon master is ready to be used for replica molding with PDMS. A simple scheme of this technique is shown in Figure (VI.2).

The polymer (Sylgard 184, Dow Corning) is prepared mixing prepolymer and reticulating agent with a ratio 1:10 w/w. After the usual degassing treatment in a low vacuum chamber, PDMS is slowly poured on top of the master, inside a box to avoid spilling out.



Figure (VI.2). Schematic representation of the replica molding technique.

Thermal curing of the sample is then performed at 90 °C for 30 minutes, or at room temperature for 24 hours, to promote the reticulation reactions that produce the final elastomer. After these treatments, the PDMS block is detached from the master: it presents structures that are a negative replica of the master's patterns.

At this point, a microscope slide is cleaned, washing it with water and rinsing with ethanol and acetone, and dried with  $N_2$ . A thin layer of uncured NOA is deposited on the glass slide and the PDMS replica is gently put on top, trying to avoid air bubble trapping. A little pressure is exerted with a finger on the sample to make sure that uncured NOA properly fills indentations of the PDMS replica.



Figure (VI.3). UV-assisted replica molding technique with NOA.

The prepared sample is exposed to UV radiation for a long period (~ 30 minutes) for the NOA layer complete curing (see Figure (VI.3)). Afterwards, the PDMS replica is peeled off: the glass slide is covered with a layer of NOA that presents structures that are a negative replica of the PDMS master and a positive replica of the silicon master. To promote NOA complete curing and better adhesion of the polymer to glass, the sample is exposed again to UV radiation for further 15 minutes, after PDMS master removal. Finally, NOA spillover at the edges is carved with bistouries.

## VI.4 Flow over isotropic grooves: pillars and dots

Using the techniques described in Sections VI.2 and VI.3, glass slides coated with a patterned NOA layer are prepared.

The first pattern consists of a series of pillars, 8  $\mu$ m wide and 3  $\mu$ m high, located on a square grid of size 16  $\mu$ m, as shown in Figure (VI.4). The second pattern consists of a series of dots, 4  $\mu$ m wide and 3  $\mu$ m deep, located on a square grid of size 8  $\mu$ m.

One after the other, each patterned slide is mounted in the RC-30 chamber, to serve as microchannel floor. A nominally 250  $\mu$ m deep, 3 mm wide and 4 cm long channel is realized inside the fluidic chamber, by laying a silicone gasket on the patterned NOA surface, and putting a glass coverslip (0.15 mm thick) on top, that serves as channel ceiling.

The microchannel actual depth can result higher than its nominal value, for both the presence of layers of vacuum grease among the components and the variable mechanical pressure that is exerted on the assembly.



Figure (VI.4). White light recording of the microchannel floor consisting of a NOA flat surface patterned with pillars, 8 μm wide and 3 μm high, located on a square grid of size 16 μm.

## Velocity profiles and slip length measurements

The plot of Figure (VI.5) shows the velocity profile measured in a ~ 320 µm deep channel with a NOA floor presenting the regular pattern of pillars and a bare glass ceiling, for a volumetric flux  $Q \approx 60$  nl/s. For µ-PIV acquisitions, the CCD array is exposed for  $\Delta t_{exp} = 2$  ms and a series of 6 successive single exposure images are acquired with a time delay  $\Delta t_{del} = 68.00$  ms (14.706 Hz). Figure (VI.6) shows the velocity profile measured in a ~ 342 µm deep channel presenting a NOA floor patterned with dots and a bare glass ceiling, for a volumetric flux  $Q \approx 90$  nl/s. The CCD array is exposed for  $\Delta t_{exp} = 2$  ms and a series of 8 successive single exposure images are acquired

with a time delay  $\Delta t_{del} = 70.16 \text{ ms} (14.253 \text{ Hz}).$ 

The wall position is experimentally determined using TIRFM and white light imaging. The presence of the geometrical structuring of the interface at the micron scale determines, together with the evanescent wave formation, laser light scattering deeper in the liquid. Thus, white light imaging helps establishing the interface location.

We assume the origin of the *z* axis to be the plane supporting the pillars (pillars extend from  $z_{wall} = 0$  to  $z = +3 \ \mu\text{m}$ ) and housing the holes (dots extend from  $z_{wall} = 0$  to  $z = -3 \ \mu\text{m}$ ).

Both velocity profiles are only partially reconstructed by the experimental points. The wide dispersion of the measurements with respect to the expected parabolic trend might be determined by the defective operation of the peristaltic pump pulsation dampener used in these  $\mu$ -PIV acquisitions, together with the possible formation of small bubbles along the perfusion system.

Estimates of the slip length are deduced by applying least squares linear fits to the data points ( $z_i$ ,  $v_i$ ), for  $z_i < 40 \,\mu$ m, considering variable data windows, and taking the opposite of the intercept of the fitting line. The values are eventually corrected for  $z_{wall}$ , taking into account the evanescent field depth.



Figure (VI.5). Velocity profile measured in a ~ 320  $\mu$ m deep channel with a NOA floor presenting a regular pattern of pillars and a bare glass ceiling, for Q  $\approx$  60 nl/s. The zoomed section shows a window of data selected for a linear fit to determine an estimate of *b*.



Figure (VI.6). Velocity profile measured in a ~ 342  $\mu$ m deep channel with a NOA floor presenting a regular pattern of dots and a bare glass ceiling, for Q ≈ 90 nl/s. The zoomed section shows a window of data selected for a linear fit to determine an estimate of *b*.

The slip length is calculated as the average of the values given by the different fits and measures:

$$b_{pill} = -2.10 \pm 0.50 \text{ (stat)} \pm 0.10 \text{ (syst)} \ \mu\text{m}$$
  
 $b_{dots} = 0.10 \pm 0.66 \text{ (stat)} \pm 0.10 \text{ (syst)} \ \mu\text{m}$ 

in the case of a pattern of pillars or dots, respectively.

In both cases, we assist to an important reduction in the slip length value for a grooved NOA floor with respect to a flat NOA floor.

The presence of the pillars forces tracing particles not to follow the pressure gradient direction, as shown in Figure (VI.7), and to travel curved trajectories close to the interface. Everything goes as if the pattern of pillars on the floor of the microchannel determines an increase in the global resistance to flow, that traduces even in a negative value of the slip length.

The presence of dots does not significantly affect the flow direction, still it seems to determine an increase in the resistance to flow, as the value of  $b_{dots} < b_{NOA}$  within the errors.



Figure (VI.7). A: μ-PIV vector field measured at a 3 μm height from the channel floor, that is on top of the pillars. Pressure gradient direction information is lost. B: A few μm higher in the microchannel, the directional information is completely recovered.

## VI.5 Flow over an anisotropic pattern: a set of parallel linear grooves

Using the techniques described in Sections VI.2 and VI.3, we fabricate glass slides coated with a NOA layer, that presents a pattern of linear canals. The linear grooves are variably oriented with respect to the channel axis, corresponding to the applied pressure gradient direction too.

The first set of rectangular canals are 8  $\mu$ m wide, 3  $\mu$ m deep and of 16  $\mu$ m period (8-16 type), while the second set of canals, shown in Figure (VI.8), are 1  $\mu$ m wide and 1.5  $\mu$ m deep and of 6  $\mu$ m period (1-6 type). It is worth observing that tracers cannot penetrate inside the 1-6 type canals for their diameter is comparable to the canal width.

One after the other, the grooved surfaces are mounted in the RC-30 chamber, to serve as microchannel floor. A nominally 250  $\mu$ m deep, 3 mm wide and 4 cm long channel is realized in the flow chamber presenting a NOA floor with *8-16 type* canals, using a silicone gasket and sealing the channel with a bare glass coverslip. A microchannel with the same features but a nominal depth of 375  $\mu$ m is present when a NOA floor with *1-6 type* canals is tested.

As usual, the microchannel actual depth can be higher than the nominal values, for both the presence of vacuum grease among the flow cell components and the variable mechanical pressure that is exerted on the assembly.

The wall position is experimentally determined using TIRFM and white light imaging: in fact, the presence of microcanals at the interface determines, together with the evanescent wave formation, laser light scattering in the liquid. Thus, white light imaging helps establishing the interface location. Notice that we obtain neat laser light TIR only when the canals are directed parallel to the plane defined by the laser path inside the prism.



Figure (VI.8). White light recording of the microchannel floor consisting of a NOA flat surface patterned with linear canals 1  $\mu$ m wide, 1.5  $\mu$ m deep and of 6  $\mu$ m period. In this case, the pattern is obliquely oriented (angle of 44°) with respect to the pressure gradient direction, which is defined by the  $\mu$ -PIV vector field measured far from the microchannel walls.



Figure (VI.9). White light recording of the microchannel floor consisting of a NOA flat surface patterned with linear canals 8 μm wide, 3 μm deep and of 16 μm period. In this case, the pattern is perpendicularly oriented with respect to the pressure gradient direction, which is defined by the μ-PIV vector field measured far from the microchannel walls.

For 8-16 type linear grooves, the width of the canals is half their period: thus, the origin of the z axis is located at half pattern amplitude (as shown in Figure (VI.11)). For the 1-6 type linear grooves, the width of the canals is the sixth part of their period: thus, the wall position is located on top of the pattern and the canals can be considered as linear sinkings.

*Velocity profile and slip length measurement for (almost) parallely oriented 8-16 type canals* The plot in Figure (VI.10) shows the velocity profile measured in a 291  $\mu$ m deep channel with a NOA floor presenting a regular pattern of microcanals and a bare glass ceiling, for Q  $\approx$  118 nl/s.



Figure (VI.10). Velocity profile measured in a 291  $\mu$ m deep channel with a NOA floor presenting a regular pattern of 8-16 type microcanals and a bare glass ceiling, for Q  $\approx$  118 nl/s. The pattern of microcanals on the floor forms an angle of 17° with respect to the pressure gradient direction.



Figure (VI.11). Notations used in this paragraph.

In detail, the pattern of 8-16 type microcanals on the floor forms an angle of 17° with respect to the pressure gradient direction. During  $\mu$ -PIV acquisitions, the CCD array is exposed for  $\Delta t_{exp} = 2$  ms and a series of 8 successive single exposure images are acquired with a time delay  $\Delta t_{del} = 70.16$  ms (14.253 Hz).

Each experimental point in the plot represents the modulus of velocity average value of the velocity field, returned by the EDPIV ensemble correlation analysis, relative to each vertical position inside the channel. The statistical error affecting the velocity measurements is lower than the point dimensions in the plot.

The slip length is deduced from the experimental points by applying linear least squares fits to the experimental points  $(z_i, v_i)$ , for  $z_i < 60 \,\mu\text{m}$ , and taking the opposite of the intercept of the least square line. The fitting function is z = B'v + A', where the two parameters A' and B' are left free to vary. Given  $z_{wall} = -0.13 \pm 0.10 \,\mu\text{m}$ , from the estimate of the evanescent wave penetration depth, we have:  $b = z_{wall} - A'$ .

As usual, we calculate a few estimates for the slip length, using different groups of experimental points for each linear fit (excluding a variable number of possible pathologic points close to the surface) to extract a more reliable value for *b*.

The slip length is the average of the values derived by the different fits and measures:

$$b^{1/2}_{can8-16} = 0.70 \pm 0.20 \text{ (stat)} \pm 0.10 \text{ (syst)} \ \mu\text{m}$$

where the statistical error is the standard deviation of the values, while the systematic uncertainty is given by the error in the determination of the actual evanescent field depth.

An interesting further investigation regards to verify whether the slip lengths generated along the two main directions x and y (refer to the notations of Figure (VI.11)) are in general different. It is reasonable to expect that the anisotropy of the surface affects velocity components in the direction of the grooves or perpendicular to them in a different way.



Figure (VI.12). Profiles of the velocity components  $v_y$  (×) and  $v_x$  (+) that are parallel and orthogonal to the pressure gradient direction, respectively. The pattern of microcanals on the NOA floor forms an angle of 17° with respect to the pressure gradient direction

The same analysis procedure, applied to the velocity profiles for the components  $v_y$  and  $v_x$  in Figure (VI.12), leads to the following results:

$$(b^{17^{\circ}}_{can8-16})_{y} = 0.65 \pm 0.22 \text{ (stat)} \pm 0.10 \text{ (syst)} \ \mu\text{m}$$
  
 $(b^{17^{\circ}}_{can8-16})_{x} = 0.31 \pm 0.24 \text{ (stat)} \pm 0.10 \text{ (syst)} \ \mu\text{m}.$ 

These slip length values might be coherent with the idea that the channel gap appears wider for a flow parallel to the grooves: in fact,  $(b^{17^{\circ}}_{can8-16})_{y} \approx b^{17^{\circ}}_{can8-16}$  and  $(b^{17^{\circ}}_{can8-16})_{x} < b^{17^{\circ}}_{can8-16}$  within the errors.

If we plot the value of the angle between the direction defined by the  $\mu$ -PIV vector field at a certain distance from the interface and the direction of the microcanals on the channels floor, as in Figure (VI.15, B), we observe that up to a distance of a few  $\mu$ m from the interface, the flow slightly deviates from the direction of the applied pressure gradient towards the direction of the grooves. This is another clear effect of the anisotropy of the pattern: if a pressure gradient is applied at some angle with respect to the x y main directions, a sort of sideways shear develops, at a finite angle with respect to the applied gradient.

#### Velocity profile and slip length measurement for perpendicularly oriented 8-16 type canals

Let us now consider a 274  $\mu$ m deep channel with a NOA floor presenting a pattern of 8-16 type microcanals and a bare glass ceiling, for Q  $\approx$  46 nl/s, when the microcanals on the floor form an angle of 89° with respect to the pressure gradient direction.

During  $\mu$ -PIV acquisitions, the CCD array is exposed for  $\Delta t_{exp} = 2$  ms and a series of 8 successive single exposure images are acquired with a time delay  $\Delta t_{del} = 70.16$  ms (14.253 Hz).



Figure (VI.13). Velocity profile measured in a 274  $\mu$ m deep channel with a NOA floor presenting a pattern of 8-16 type microcanals and a bare glass ceiling, for Q  $\approx$  46 nl/s. The microcanals on the floor form an angle of 89° with respect to the pressure gradient direction.

The measured velocity profile is shown in Figure (VI.13).

The high dispersion of the experimental data with respect to the expected parabolic trend is almost certainly due to the formation of small bubbles along the perfusion system.

The slip length estimates are deduced from the experimental points by applying linear least squares fits to the experimental points ( $z_i$ ,  $v_i$ ), for  $z_i < 40 \mu$ m, using different groups of experimental points for each linear fit and applying the correction for  $z_{wall} = -0.13 \pm 0.10 \mu$ m.

The average of the *b* values derived by the different fits is:

 $b^{89^{\circ}}_{can8-16} = 0.45 \pm 0.35 \text{ (stat)} \pm 0.10 \text{ (syst)} \mu\text{m}.$ 

If we plot the value of the angle between the direction defined by the  $\mu$ -PIV vector field at a certain distance from the interface and the direction of the microcanals on the channels floor, as in Figure (VI.15, A), we observe that the flow follows the direction of the applied pressure gradient up to a distance of a few  $\mu$ m from the interface. At that distance, the average direction of the  $\mu$ -PIV field starts deviating from the x axis and, possibly due to the effect of the symmetry of the pattern on the floor, it randomly tilts to right or left, randomly increasing or decreasing the angle between the linear grooves and the channel axis.

### Velocity profile and slip length measurement for obliquely oriented 8-16 type canals

Let us now come to the case a 291  $\mu$ m deep channel with a NOA floor presenting a pattern of 8-16 type microcanals and a bare glass ceiling, for Q  $\approx$  40 nl/s, when the microcanals on the floor form an angle of 51° with respect to the pressure gradient direction.



Figure (VI.14). Velocity profile measured in a 291  $\mu$ m deep channel with a NOA floor presenting a pattern of 8-16 type microcanals and a bare glass ceiling, for Q  $\approx$  40 nl/s. The microcanals on the floor form an angle of 51° with respect to the pressure gradient direction.

For  $\mu$ -PIV imaging, the CCD array is exposed for  $\Delta t_{exp} = 2$  ms and a series of 8 successive single exposure images are acquired with a time delay  $\Delta t_{del} = 70.16$  ms (14.253 Hz).

The measured velocity profile is shown in Figure (VI.14).

Again we observe an high dispersion of the experimental points with respect to the expected parabolic trend. The slip length estimates are deduced from the experimental points by applying linear least squares fits to the experimental points ( $z_i$ ,  $v_i$ ), for  $z_i < 60 \mu$ m, using different groups of experimental points for each linear fit and correcting for  $z_{wall} = -0.13 \pm 0.10 \mu$ m.

The average of the *b* values derived by the different fits is:

 $b^{51^{\circ}}_{can8-16} = 0.31 \pm 0.27 \text{ (stat)} \pm 0.10 \text{ (syst)} \ \mu\text{m}.$ 

The plot in Figure (VI.15, C) shows the value of the angle between the direction defined by the  $\mu$ -PIV vector field at a certain distance from the interface and the direction of the microcanals on the channels floor. As a confirmation of the result already found in Figure (VI.15, B), we see how the flow slightly deviates from the direction of the applied pressure gradient towards the direction of the grooves, starting from a distance of a few  $\mu$ m from the interface downwards. This behaviour of the flux can be explained as an effect of the anisotropy of the pattern that leads to the development of a sideways shear flow.



Figure (VI.15). Plots showing the angle between the direction defined by the μ-PIV vector field and the direction of the grooves on the floor of the microchannel, as a function of the distance from the interface for (A) perpendicularly oriented grooves, (B) almost parallel-oriented grooves and (C) obliquely oriented grooves, with respect to the pressure gradient direction.

### Velocity profile and slip length measurement for parallely oriented 1-6 type canals

Let us now come to the tests involving channels with a floor presenting a pattern of 1-6 type canals.

The plot in Figure (VI.16) shows the velocity profile measured in a ~ 380  $\mu$ m deep channel with a NOA floor presenting 1-6 type microcanals, that are parallelly oriented with respect to the pressure gradient direction, and a bare glass ceiling, for Q ≈ 76 nl/s.

During  $\mu$ -PIV acquisitions, the CCD array is exposed for  $\Delta t_{exp} = 2$  ms and a series of 8 successive single exposure images are acquired with a time delay  $\Delta t_{del} = 69.08$  ms (14.476 Hz).



Figure (VI.16). Velocity profile measured in a ~ 380  $\mu$ m deep channel with a NOA floor presenting 1-6 type microcanals, parallelly oriented with respect to the pressure gradient direction, and a bare glass ceiling, for Q  $\approx$  76 nl/s.

The slip length is deduced from the velocity profile by applying linear least squares fits to the experimental points ( $z_i$ ,  $v_i$ ), for  $z_i < 60 \mu$ m, and applying the correction for  $z_{wall} = -0.13 \pm 0.10 \mu$ m. As usual, we calculate a few estimates for the slip length, using different groups of experimental points for each linear fit (excluding a variable number of points close to the wall). The slip length is the average of the values obtained by the different fits and measures:

 $b^{-0^{\circ}}_{can1-6} = 0.64 \pm 0.09 \text{ (stat)} \pm 0.10 \text{ (syst)} \mu\text{m}.$ 

#### Velocity profile and slip length measurement for perpendicularly oriented 1-6 type canals

Let us now come to the case a ~ 330  $\mu$ m deep channel with a NOA floor presenting a pattern of 1-6 type microcanals and a bare glass ceiling, for Q  $\approx$  34 nl/s, when the microcanals on the floor are pendicularly oriented with respect to the pressure gradient direction.

For  $\mu$ -PIV imaging, the CCD array is exposed for  $\Delta t_{exp} = 2$  ms and a series of 8 successive single exposure images are acquired with a time delay  $\Delta t_{del} = 68.00$  ms (14.706 Hz).

The measured velocity profile is shown in Figure (VI.14).

The slip length estimates are deduced from the experimental points by applying linear least squares fits to the experimental points ( $z_i$ ,  $v_i$ ), for  $z_i < 80 \ \mu$ m, using different groups of experimental points for each linear fit and correcting for  $z_{wall} = -0.13 \pm 0.10 \ \mu$ m.

The average of the *b* values derived by the different fits is:

$$b^{-90^{\circ}}_{can1-6} = -1.02 \pm 0.26 \text{ (stat)} \pm 0.10 \text{ (syst)} \ \mu\text{m}$$



Figure (VI.15). Velocity profile measured in a  $\sim$ 330 µm deep channel with a NOA floor presenting 1-6 type microcanals, perpendicularly oriented with respect to the pressure gradient direction, and a bare glass ceiling, for Q  $\approx$  34 nl/s.

## Velocity profile and slip length measurement for diagonally oriented 1-6 type canals

Finally, let us consider the case of a ~ 376  $\mu$ m deep channel with a NOA floor presenting a pattern of 1-6 type microcanals and a bare glass ceiling, for Q  $\approx$  120 nl/s, when the microcanals on the floor form an angle of 44° with respect to the pressure gradient direction.

For  $\mu$ -PIV imaging, the CCD array is exposed for  $\Delta t_{exp} = 2$  ms and a series of 8 successive single exposure images are acquired with a time delay  $\Delta t_{del} = 68.00$  ms (14.706 Hz).

The velocity profile of the tracers is only partially reconstructed by the experimental points, up to a distance of  $\sim$ 70 µm from the bottom wall.

The slip length estimates are deduced from the experimental points by applying linear least squares fits to the experimental points ( $z_i$ ,  $v_i$ ), using different groups of experimental points for each linear fit and correcting for  $z_{wall} = -0.13 \pm 0.10 \ \mu\text{m}$ .



Figure (VI.18). Velocity profile measured in a ~ 376  $\mu$ m deep channel with a NOA floor presenting 1-6 type microcanals, obliquely oriented (angle of 44°) with respect to the pressure gradient direction, and a bare glass ceiling, for Q ≈ 120 nl/s.



Figure (VI.19). Plot showing the angle between the direction defined by the  $\mu$ -PIV vector field and the direction of the 1-6 type grooves on the floor of the microchannel, as a function of the distance from the interface. The grooves are obliquely oriented (angle of 44°) with respect to the pressure gradient direction.

The average of the *b* values derived by the different fits is:

 $b^{44^{\circ}}_{can1-6} = 1.01 \pm 0.34 \text{ (stat)} \pm 0.10 \text{ (syst)} \ \mu\text{m}.$ 

The plot in Figure (VI.19) shows the value of the angle between the direction defined by the  $\mu$ -PIV vector field at a certain distance from the interface and the direction of the microcanals on the channels floor. There is a further confirmation of the results found in Figure (VI.15): in fact, starting from a distance of a few  $\mu$ m from the interface downwards, the flow clearly deviates from the direction of the applied pressure gradient towards the direction of the grooves. The anisotropy of the pattern is responsible for the development of a sideways shear flow in the liquid.

## VI.6 Flow over a micro and nano structured surface: an artificial Ginkgo Biloba leaf

In this paragraph, we present an attempt to experimentally characterize liquid flow slippage over a superhydrophobic surface incorporated in a microchannel.

The interest in working with superhydrophobic surfaces is associated to the Fakir effect (the socalled Cassie state): in highly rough hydrophobic surfaces, the liquid, instead of filling the pores present on the solid surface, remains at the top of the roughness, trapping some air in the interstices, and leading to a very small liquid-solid contact area. This behaviour considerably amplifies slippage.

Several natural surfaces show a superhydrophobic behaviour. For example, some naturally occurring plant leaves (such as Lotus or Ginkgo Biloba leaves) present well-demonstrated superhydrophobic surfaces and water drops can easily roll off over them, and carry away dust particles or dirt, a mechanism referred to self-cleaning.

In the case of leaves, water repellence is based on surface roughness caused by different microstructures and nanostructures, together with the hydrophobic properties of the wax coating. We fabricate a polymer surface presenting a patterned surface that directly replicates the geometrical structures of a fresh Gingko Biloba leaf (see Figure (VI.20)) and that exhibits analogous superhydrophobic properties.

## Preparation and characterization of the artificial Ginkgo Biloba leaf

Figure (VI.21) gives a schematic representation of the procedure we follow to create a PDMS superhydrophobic surface from a natural Ginkgo Biloba leaf.

It is possible to transform hydrophobic PDMS into a superhydrophobic surface by topographically modelling its surface at the micro and nano scale.



Figure (VI.20). Picture of a Ginkgo Biloba leaf.

In the practice, PDMS is prepared mixing prepolymer and reticulant agent (Sylgard 184, Dow Corning) with a ratio 1:10 w/w. Once degassed, the mixture is poured on a fresh Gingko Biloba leaf (the master). After solidification at room temperature for 24 h, the PDMS layer is peeled off: it presents the complementary surface structure of the leaf (negative template). After silanization with TPOS of the negative template, a second replication of PDMS on the negative template is made. In this way, the complex surface patterns of the leaf are transferred onto the surface of solid PDMS with high fidelity: we obtain an artificial Ginkgo Biloba leaf.



Figure (VI.21). Schematic representation of the replica molding technique to fabricate a PDMS replica of a Ginkgo Biloba leaf [101].

In order to characterize the positive and negative replica surfaces and to verify their superhydrophobic behaviour, water contact angles and contact angle hysteresis are measured using the apparatus in Figure (V.3).

Figure (VI.22) shows the CCD pictures of water drops on the PDMS replica: the contact angle on the positive replica surface is  $(145\pm3)^\circ$ , while the silanized surface of the negative replica has a contact angle of  $(142\pm3)^\circ$ .

To determine the hysteresis of a surface, we form and maintain a droplet on the test surface with the syringe and eventually increasing the drop volume (from 0.5 ml up to  $2\div3 \text{ ml}$ ). The hysteresis is given by the difference between the angle at which the contact line moves after droplet volume increase and initial contact angle.



Figure (VI.22). Pictures showing a water drop on the silanized PDMS negative template (A) and on the PDMS positive replica (B).



Figure (VI.23). Slowly moving the syringe from right to left allows a visualization of the advancing and receding contact angle for the silanized negative template, as shown in the two CCD pictures. The droplet in contact with the surface of the positive replica can be moved freely with the syringe without any adhesion to the surface.

Figure (VI.23) shows an effect of the contact angle hysteresis on the negative template: we see that, slowly moving the syringe to left or to right, the water drop cannot be separated from the surface of the negative template, when it is in contact with it. In contrast, the droplet in contact with the surface of the positive replica can be moved almost freely with the syringe, without any adhesion to the surface. We measure an hysteresis of about 6° and 14° for the positive and silanized negative replica respectively: these low values provide of proof of the surface superhydrophobicity.

After a 90 seconds treatment in UVO cleaner, the artificial Ginkgo Biloba leaf is layed on a clean microscope glass slide as support.

Finally, the sample is mounted in the RC-30 chamber, to serve as microchannel floor. A nominally  $250 \ \mu m$  deep, 3 mm wide and 4 cm long channel is realized inside the fluidic

chamber, by putting a silicone gasket on the patterned PDMS surface and sealing with glass coverslip (0.15 mm thick) on top.

## Problems with velocity profile measurement

This preliminary attempt to experimentally characterize water slippage over an artificial Ginkgo Biloba leaf does not lead to appreciable results.

The first and crucial problem to face consists in the determination of the actual wall position. The realization of TIRF imaging is practically impossible for the presence of micro and nano structures at the interface: the eventual evanescent field is masked by the unavoidable presence of spurious transmitted light.



Figure (VI.24). White light recording close to the surface of the artificial Ginkgo Biloba leaf (real dimensions:  $104 \times 79 \ \mu\text{m}^2$ ), on which water seeded with tracers is flowing.



Figure (VI.25). SEM image of the surface structures of a fresh Ginkgo Biloba leaf: a. stomata (scale bar =  $100 \ \mu m$ ), b. arrangement of the wax crystalloids (scale bar =  $1 \ \mu m$ ) [102].

White light imaging returns a picture of the surface (see Figure (VI.24)) that cannot be easily interpreted, even keeping in mind the expected geometry of structures present on the leaf. In fact, when viewed in an SEM (see Figure (VI.25)), a Gingko Biloba leaf shows micrometer-scaled

hills, that are textured by intricate nanostructures made by wax crystalloids. Where is it reasonable to fix the wall position on a surface presenting modulations even at the micron scale? Moreover, white light images clearly show the presence of several tracers that stuck to the surface (on top of hills, inside pits or on both structures?), which is in contrast with the realization of the Fakir effect.

One possible explanation is that the pressure exerted during flow chamber initial filling was too high, and has determined the direct passage from a Cassie to a Wenzel state.

However, the indubitable interest and fascination in investigating water slippage over an artificial Ginkgo Biloba leaf through the  $\mu$ -PIV technique requires further tests. A key factor will be to carefully control the pressure gradient value and to keep it as low as possible, during the initial filling phase.

## VI.7 Results on micro and nano structured substrates

Let us try to draw some conclusions from the slip length determinations collected in this chapter. First of all, it is worth to remind the value of the slip length of water on a flat NOA surface (see Section V.2), for all the microstructures fabricated on the channel floor are made of NOA. We have:

 $b_{NOA} = 1.35 \pm 0.31 \text{ (stat)} \pm 0.10 \text{ (syst)} \mu\text{m}.$ 

We start considering an isotropic pattern of pillars or dots whose characteristic dimensions are two orders of magnitude smaller than the channel height. We measure:

$$b_{pill} = -2.10 \pm 0.50 \text{ (stat)} \pm 0.10 \text{ (syst)} \ \mu\text{m}$$
  
 $b_{dots} = 0.10 \pm 0.66 \text{ (stat)} \pm 0.10 \text{ (syst)} \ \mu\text{m}.$ 

In both cases, we assist to an important reduction in the slip length value in the case of the grooved NOA floor with respect to a flat NOA floor.

The presence of the pillars forces tracing particles not to follow the pressure gradient direction, and to remain trapped among the structures or to travel curved patterns close to the interface. In a more physical picture, everything goes as if the pattern of pillars on the floor of the microchannel determines an increase in the global resistance to flow, that traduces even in a negative value of the slip length.

The presence of dots does not significantly affect the flow direction, still it seems to determine an increase in the resistance to flow, as the value of  $b_{dots} < b_{NOA}$  within the errors. If we consider the dots as a simple representation of some wall roughness for the channel (two orders of magnitude smaller than the channel height), then we might infer that its role on the definition of the boundary conditions needs to be taken into account. Then, we consider an anisotropic pattern of canals fabricated on the channel floor, whose characteristic dimensions are again two orders of magnitude smaller than the channel height. In the first case, for the 8-16 type linear grooves, the width of the canals is half their period, and the wall position is located at half pattern amplitude. We have:

$$b^{17^{\circ}}_{can8-16} = 0.70 \pm 0.20 \text{ (stat)} \pm 0.10 \text{ (syst)} \ \mu\text{m}$$
$$(b^{17^{\circ}}_{can8-16})_{y} = 0.65 \pm 0.22 \text{ (stat)} \pm 0.10 \text{ (syst)} \ \mu\text{m}$$
$$(b^{17^{\circ}}_{can8-16})_{x} = 0.31 \pm 0.24 \text{ (stat)} \pm 0.10 \text{ (syst)} \ \mu\text{m}$$
$$b^{89^{\circ}}_{can8-16} = 0.45 \pm 0.35 \text{ (stat)} \pm 0.10 \text{ (syst)} \ \mu\text{m}$$
$$b^{51^{\circ}}_{can8-16} = 0.31 \pm 0.27 \text{ (stat)} \pm 0.10 \text{ (syst)} \ \mu\text{m}.$$

This slip length value in the case of (almost) parallely oriented canals with respect to the channel axis,  $b^{17^{\circ}}_{can8-16}$ , results compatible with  $b_{NOA}$  within less than two sigma, as it could be reasonably expected. Moreover, as  $(b^{17^{\circ}}_{can8-16})_{y} \approx b^{17^{\circ}}_{can8-16}$  and  $(b^{17^{\circ}}_{can8-16})_{x} < b^{17^{\circ}}_{can8-16}$  within the errors, our results are coherent with the idea that the channel gap appears wider for a flow moving parallel to the grooves.

The remaining slip length measurements are affected by high statistical error and do not allow us to draw significant conclusions: in general, we can observe that the presence of 8-16 type linear grooves on the floor, forming a certain angle with the pressure gradient direction, lead to a reduction in the slip length value with respect to a flat NOA floor.

However, other important informations can be inferred from the velocity fields measured in channels with the 8-16 type linear grooved floors. In fact, another aspect of our investigations regards the determination of the effect that the pattern anisotropy has on the flow geometry.

We show clear evidence that, if a pressure gradient is applied at some angle with respect to the microcanal direction, a sideways shear flow develops, at a finite angle with respect to the applied pressure gradient. The measured deviation is quite high and might reach about a 10° deflection towards the direction of the grooves. This effect is visible up to a distance of a few  $\mu$ m from the interface, that is the length scale of the grooved structure; beyond that distance, the flow faithfully follows the direction of the applied pressure gradient with very low (< 1°) deviations, as expected in a microfluidic planar device.

In the second case, for the 1-6 type linear grooves, the width of the canals is the sixth part of their period, and the wall position is located on top of the pattern. The microcanals are linear sinkings where tracers cannot penetrate because of their dimensions. We measure:

$$b^{-9^{\circ}}{}_{can1-6} = 0.64 \pm 0.09 \text{ (stat)} \pm 0.10 \text{ (syst)} \ \mu\text{m}$$
  
 $b^{-90^{\circ}}{}_{can1-6} = -1.02 \pm 0.26 \text{ (stat)} \pm 0.10 \text{ (syst)} \ \mu\text{m}$   
 $b^{44^{\circ}}{}_{can1-6} = 1.01 \pm 0.34 \text{ (stat)} \pm 0.10 \text{ (syst)} \ \mu\text{m}.$ 

Here slip length values tell of a general decrease in the slip length due to the presence of the microcanals on the microchannel floor.

In case of an orthogonally oriented pattern, the negative value of the slip length might be justified by the possible formation of periodic three-dimensional recirculation structures localized in the vicinity of the bottom plate due to the modulation of the floor. The combination of this local flow, opposite to the principle flow direction, with the main Poiseuille flow, might result in a negative slip velocity at the interface and in a negative value for b.

However, the slip length value in the case of parallely oriented canals with respect to the channel axis,  $b^{-0^{\circ}}_{can1-6}$ , is much lower than  $b_{NOA}$  and compatible with it only within three sigma. On the contrary, we would reasonably expect  $b^{-0^{\circ}}_{can1-6}$  to be higher than  $b_{NOA}$  within the errors, to be coherent with the idea that the channel gap appears wider for the flow moving parallel to the grooves (and exactly for 1/6 of the tracers in average).

For further discussions on the validity or meaning of our slip length results, the demand is an analytical description that could theoretically model our experiments and provide a tool for comparison.

As regards the study of the effect that the anisotropy of this type of canals has on the flow geometry, we again obtain clear evidence that, if a pressure gradient is applied at some angle with respect to the microcanal direction, a sideways shear flow develops, at a finite angle with respect to the applied pressure gradient.

# Conclusions

Let us draw the conclusions of this research discussing separately the three main objectives to achieve and the results obtained.

## The µ-PIV system

A  $\mu$ -PIV system has been designed, home-assembled and tested.

The peculiarity of the microscope consists in the possibility to exploit two different configurations for sample illumination: in fact, it combines the typical and consolidated  $\mu$ -PIV epifluorescence imaging, to visualize tracers in the bulk of the liquid, with the elegant and powerful TIRF technique, to investigate the physics at the solid-liquid interface.

The final cost of the system is definitely lower than the price of recent, commercially available,  $\mu$ -PIV optical apparata, that are typically equipped with high-power pulsed single/dual laser sources, high-end CCD cameras and synchronizing electronics.

The innovative elements are:

- the use of high brightness LEDs as a source for epiluminescence light microscopy, which represents the most economic solution among illumination sources;
- the use of a commercial EMCCD detector, that is a highly cost-effective solution for an high sensitivity CCD camera; moreover, its flexibility allows to work in a pretty wide range of flow conditions.

The main problem with our experimental set-up has been to create a uniform and slow flux of water in the microfluidic chamber. Different perfusion systems have been tested in early  $\mu$ -PIV investigations (microsyringe, gravity or peristaltic pump generated flows), each system showing its pros and cons. The final choice has fallen on the use of a peristaltic pump equipped with home-made pressure pulsation dampeners. This solution allows to gradually feed tubing and flow cell with the liquid solution, applying the smallest pressure gradients and avoiding (or at least limiting) air bubble trapping. Once the right dimensioning of the pressure pulsation dampener has been reached, definitely uniform and slow fluxes have been obtained, with an economic perfusion system.

EDPIV software demo version is used to perform image processing and to compute crosscorrelation analysis. It has been chosen because it is freeware software, ready accessible and realiable.

### Water slip over flat surfaces

The investigations on water slippage inside microchannels presenting flat surfaces are mainly meant to provide a test our  $\mu$ -PIV set-up, by comparing our results to the recently obtained measurements by research groups, leaders in the application of the  $\mu$ -PIV technique to the study of liquid-solid boundary conditions.

Measurements of the water slip length on different flat surfaces, as a function of the liquid-solid contact angle, have been performed. The values we have obtained are well compatible within the errors to the slip length estimates in literature.

It is necessary to underline that our error bars are sometimes quite wide with respect to the uncertainties reported in  $\mu$ -PIV literature, because of the relatively low resolution of the imaging apparatus. In fact, we are limited by the illumination source power to use 1  $\mu$ m diameter tracers, and the relatively high tracer size together with the relatively low NA of the objective lens determines a significant increase in the depth of correlation of the  $\mu$ -PIV system.

Let us now take into consideration, in particular, the result on a smooth hydrophobic surface consisting in glass covered with a SAM of OTS. We have calculated:

$$b_{OTS} = 0.56 \pm 0.15 \text{ (stat)} \pm 0.10 \text{ (syst)} \mu\text{m}.$$

Our value is compatible within less than two sigma with  $b_{OTS} = -35 \pm 100$  nm (Joseph and Tabeling [103]) and within one sigma to  $b_{OTS} = 1 \ \mu m \pm 0.45$  nm (Tretheway and Meinhart [104]), while the two estimates reported by these two collaborations show a clear discrepancy,

given the same range of shear rates investigated (up to  $\gamma = 450 \text{ s}^{-1}$ ). Our measurement cannot clearly validate one of the two results. Moreover, it is worth pointing out that the window of shear rates at the wall that we have investigated is completely different, as we have explored up

## to $\gamma = 6 \text{ s}^{-1}$ .

### Water slip over micro and nano structured surfaces

Interesting experimental results have been obtained investigating water slip dynamics in microchannels presenting a variably patterned floor.

Measurements on isotropically patterned surfaces, presenting a regular grid of pillars or dots as to model a superficial roughness on the micron scale, have generally shown a decrease in the value of the slip length with respect to the flat substrate.

This result is coherent with the increase in liquid friction observed by a number of research groups for the case of surface roughness at the micron scale or lower.

Measurements performed as a function of the orientation of micrometric canals impressed on the floor of a microchannel, with respect to the main flux direction, have led to additional interesting conclusions.

A confirmation of a reduction in the value of the slip length due to wall micropattering has been obtained and, moreover, the effect that the anisotropy of a pattern on the floor has on the flow geometry has been shown. In fact, we have obtained clear evidence that, by changing the orientation of linear microstructures on a solid wall, it is possible to the modify the flow direction, even inside planar lithographic-made microchannels with a constant pressure gradient applied at both ends.

For a more quantitive approach and for an exhaustive discussion on the validity or meaning of our slip length measurements on patterned floors, the demand is an analytical description that could theoretically model our experiments and provide a tool for comparison.

The wish is that this preliminary study on the boundary-controlled flow directioning might stimulate the interest on new experimental and theoretical characterizations of the phenomenon.

So, what are the plans for the future?

This thesis has provided the opportunity to start investigating hydrodynamic boundary conditions with a powerful technique, that was definitely unfamiliar to our research group.

The acquired experience and the encouraging results obtained up to now lead to the clear necessity of a  $\mu$ -PIV system upgrade, that could allow to exploit more powerful illumination sources, smaller tracing particles and higher NA objectives, for higher resolution measurements.

The necessity to work under well controlled experimental conditions is also evident, in order to have the possibility to clearly discriminate the origin of the measured effects, trying to clear the physical picture at the solid-liquid interface.

With this improved set-up, it is then planned to study the effect that a both geometrically and chemically patterned surface has on the liquid flow. Furthermore, the flow of complex fluids will be investigated.

# **List of References**

[1] Whitesides G. M., The origins and the future of microfluidics, Nature, 442:368-373, 2006.

[2] Tabeling P., Introduction to microfluidics, Oxford University Press, 2006.

[3] Nguyen N. T., Wereley S. T., Fundamentals and applications of microfluidics, Artech House Microelectromechanical Systems Library, 2002.

[4] Goldstein S., Modern developments in fluid dynamics, Oxford University Press, 1938.

[5] Goldstein S., Fluid mechanics in the first half of this century, Ann. Rev. Fluid Mech., 1:1-29, 1969.

[6] Neto C., Evans D. R., Bonaccurso E., Butt H. J. and Craig V. S. J., Boundary slip in Newtonian liquids: a review of experimental studies, Rep. Prog. Phys., 68:2859-2897, 2005.

[7] Lauga E., Brenner M. P. and Stone H. A., Microfluidics: The no-slip boundary condition, Handbook of Experimental Fluid Dynamics, Tropea C., Yarin A., Foss J. F. (Eds.), Springer, 2007.

[8] Brochard F. and de Gennes P. G., Shear-dependent slippage at a polymer/solid interface., Langmuir, 8:3033-3037, 1992.

[9] Denn M. M., Extrusion instabilities and wall slip, Ann. Rev. Fluid Mech., 33:265-287, 2001.

[10] Leger L., Raphael E. and Hervet H., Surface-anchored polymer chains: their role in adhesion and friction, Adv. Polymer Sci., 138:185-225, 1999.

[11] Inn Y. and Wang S. Q., Hydrodynamic slip: polymer adsorption and desorption at melt/solid interfaces, Phys. Rev. Lett., 76:467-470, 1996.

[12] Kogan M. N., Rarefied gas dynamics, Plenum Press, New York, 1969.

[13] Muntz E. P., Rarefied-gas dynamics, Ann. Rev. Fluid Mech., 21:387-417, 1989.

[14] Schnell E., Slippage of water over nonwettable surfaces, J. Appl. Phys., 27:1149-1152, 1956.

[15] Pit R., Hervet H. and Leger L., Direct experimental evidence of slip in hexadecane: solid interfaces, Phys. Rev. Lett. 85:980-983 ,2000.

[16] Zhu Y. and Granick S., Rate-dependent slip of Newtonian liquid at smooth surfaces, Phys. Rev. Lett., 87:096105, 2001.

[17] de Gennes P. G., On fluid/wall slippage, Langmuir, 18:3413-3414, 2002.

[18] Knudsen M., Ann. d. Physik, 28:75, 1909.

[19] Patankar N. A., On the modelling of hydrophobic contact angles on rough surfaces, Langmuir, 19:1249-1253, 2003.

[20] Lafuma A. and Quere D., Superhydrophobic states, Nature Materials, 2:457-460, 2003.

[21] Wenzel R. N., Resistance of solid surfaces to wetting by water, Ind. Eng. Chem., 28: 988-994, 1936.

[22] Cassie A. B. D. and Baxter S., Wettability of porous surfaces, Trans. Faraday Soc., 40: 546-551, 1944.

[23] Bico J., Thiele U. and Quere D., Wetting of textured surfaces, Colloids Surf A, 206:41-46, 2002.

[24] Craig V. S. J., An historical review of surface force measurement techniques, Colloids Surf A, 129-130:75-94, 1997.

[25] Vinogradova O. I., Drainage of a thin liquid film confined between hydrophobic surfaces, Langmuir, 11:2213-2220, 1995.

[26] Hocking L. M., Effect of slip on motion of a sphere close to a wall and of two adjacent spheres, J. Eng. Math., 7:207-221, 1973.

[27] Tabor D. and Winterton R. H. S., The direct measurement of normal and retarded van der Waals forces, Proc. Roy. Soc. London A, 312:435-450, 1969.

[28] Israelachvili J. N. and Tabor D., The measurement of van der Waals dispersion forces in the range 1.5 to 130 nm, Proc. Roy. Soc. London A, 331:19-38, 1972.

[29] Campbell S. E., Luengo G., Srdanov V. I., Wudi F. and Israelachvili J. N., Very low viscosity at the solid-liquid interface induced by adsorbed C60 monolayers, Nature, 382:520-2, 1996.

[30] Zhu Y. and Granick S., Limits of the hydrodynamic no-slip boundary condition, Phys. Rev. Lett., 88:106102, 2002.

[31] Baudry J., Charlaix E., Tonck A. and Mazuyer D., Experimental evidence for a large slip effect at a nonwetting fluid-solid interface, Langmuir, 17:5232-5236, 2001.

[32] Binnig G., Quate C. F. and Gerber Ch., Atomic force microscope, Phys. Rev. Lett., 56:930-933, 1986.

[33] Craig V. S. J., Neto C. and Williams D. R. M., Shear-dependent boundary slip in an aqueous newtonian liquid, Phys. Rev. Lett., 87:054504, 2001.

[34] Bonaccurso E., Kappl M. and Butt H.-J., Hydrodynamic force measurements: boundary slip of water on hydrophilic surfaces and electrokinetic effects, Phys. Rev. Lett. 88:076103, 2002.

[35] Bonaccurso E., Butt H.-J. and Craig V. S. J., Surface roughness and hydrodynamic boundary slip of a Newtonian fluid in a completely wetting system, Phys. Rev. Lett., 90:144501, 2003.

[36] Vinogradova O. I. and Yakubov G. E., Dynamic effects on force measurements. 2. Lubrication and the atomic force microscope, Langmuir, 19:1227-1234, 2003.

[37] Vinogradova O. I. and Yakubov G. E., Surface roughness and hydrodynamic boundary conditions, Phys. Rev. E, 73:045302, 2006.

[38] Henry C. L., Neto C., Evans D. R., Biggs S. and Craig V. S. J., The effect of surfactant adsorption on liquid boundary slippage, Physica A, 339:60-5, 2004.

[39] Pit R., Hervet H. and Leger L., Friction and slip of a simple liquid at a solid surface, Tribol. Lett., 7:147-152, 1999.

[40] Hervet H. and Leger L., Flow with slip at the wall: from simple to complex fluids, C.R. Phys., 4:241-249, 2003.

[41] Leger L., Friction mechanisms and interfacial slip at fluid-solid interfaces, J. Phys.: Condens. Matter ,15:S19-S29, 2003.

[42] Lumma D., Best A., Gansen A., Feuillebois F., Radler J. O. and Vinogradova O. I., Flow profile near a wall measured by double-focus fluorescence cross-correlation, Phys. Rev. E, 67:056313, 2003.

[43] Joly L., Ybert C. and Bocquet L., Probing the nano-hydrodynamics at liquid-solid interfaces using thermal motion, Phys. Rev. Lett., 96:046101, 2006.

[44] Cheng J. T. and Giordano N., Fluid flow through nanometer-scale channels, Phys. Rev. E, 65:031206, 2002.

[45] Ou J., Perot B. and Rothstein J. P., Laminar drag reduction in microchannels using ultrahydrophobic surfaces, Phys. Fluids, 16:4635-4643, 2004.

[46] Choi C.-H., Westin J. A. and Breuer K. S., Apparent slip flows in hydrophilic and hydrophobic microchannels, Phys. Fluids, 15:2897-2902, 2003.

[47] Westin J. A., Choi C.-H. and Breuer K. S., A novel system for measuring liquid flow rates with nanoliter per minute resolution, Exp. Fluids, 34:635-642, 2003.

[48] Du B., Goubaidoulline I. and Johannsmann D., Effects of laterally heterogeneous slip on the resonance properties of quartz crystals immersed in liquids, Langmuir, 20:10617-24, 2004.

[49] McHale G. and Newton M. I., Surface roughness and interfacial slip boundary condition for quartz crystal microbalances, J. Appl. Phys., 95:373-380, 2004.

[50] Ellis J. S. and Hayward G. L., Interfacial slip on a transverse-shear mode acoustic wave device, J. Appl. Phys., 94:7856-7867, 2003.

[51] Ellis J. S. and Thompson M., Slip and coupling phenomena at the liquid-solid interface, Phys. Chem. Chem. Phys., 6:4928-4938, 2004.

[52] Yanagisawa M., Slip effect for thin liquid film on a rotating disk, J. Appl. Phys., 61:1034-1037, 1987.

[53] Watanabe K., Takayama T., Ogata S. and Isozaki S., Flow between two coaxial rotating cylinders with a highly water-repellent wall, AIChE J., 49:1956-1963, 2003.

[54] Gogte S., Vorobieff P., Truesdell R., Mammoli A., van Swol F., Shah P. and Brinker C. J., Effective slip on textured superhydrophobic surfaces, Phys. Fluids, 17:051701, 2005.

[55] Boehnke U. C., Remmler T., Motschmann H., Wurlitzer S., Hauwede J. and Fischer Th. M., Partial air wetting on solvophobic surfaces in polar liquids, J. Colloid Int. Sci., 211:243-251, 1999.

[56] Keh H. J. and Kuo J., Effect of adsorbed polymers on the slow motion of an assemblage of spherical particles relative to a fluid, Colloid Polym. Sci., 275:661-671, 1997.

[57] Letocart P., Radoev B., Schulze H. J. and Tsekov R., Experiments on surface waves in thin wetting films, Colloids Surf. A, 149:151-159, 1999.

[58] Koplik J. and Banavar J. R., Continuum deductions from molecular hydrodynamics, Ann. Rev. Fluid Mech., 27:257-292, 1995.

[59] Cieplak M., Koplik J. and Banavar J. R., Boundary conditions at a fluid-solid interface, Phys. Rev. Lett., 86:803-806, 2001.

[60] Cottin-Bizonne C., Barentin C., Charlaix E., Boequet L. and Barrat J. L., Dynamics of simple liquids at heterogeneous surfaces: molecular dynamics simulations and hydrodynamic description, Eur. Phys. J. E, 15:427-438, 2004.

[61] Koplik J., Banavar J. R. and Willemsen J. F., Molecular dynamics of Poiseuille flow and moving contact lines, Phys. Rev. Lett., 60:1282-1285, 1988.

[62] Nagayama G. and Cheng P., Effects of interface wettability on microscale flow by molecular dynamics simulation, Int. J. Heat Mass Transfer, 47:501-513, 2004.

[63] Sun M. and Ebner C., Molecular dynamics study of flow at a fluid-wall interface, Phys. Rev. Lett., 69:3491-3494, 1992.

[64] Thompson P. A. and Robbins M. O., Simulations of contact line motion - Slip and the dynamic contact angle, Phys. Rev. Lett., 63:766-769, 1989.

[65] Thompson P. A. and Robbins M. O., Shear flow near solids - Epitaxial order and flow boundary conditions, Phys. Rev. A, 41:6830-6837, 1990.

[66] Barrat J. L. and Bocquet L., Large slip effect at a nonwetting fluid-solid interface, Phys. Rev. Lett., 82:4671-4674, 1999.

[67] Thompson P. A. and Troian S. M., A general boundary condition for liquid flow at solid surfaces, Nature, 389:360-362, 1997.

[68] Brenner H. and Ganesan V., Molecular wall effects: are conditions at a boundary "boundary conditions"?, Phys. Rev. E, 61:6879-6897, 2000.

[69] Blake T. D., Slip between a liquid and a solid: D.M. Tolstoi (1952) theory reconsidered, Colloids Surf., 47:135-145, 1990.

[70] Barrat J. L. and Bocquet L., Influence of wetting properties on hydrodynamic boundary conditions at a fluid/solid interface, Faraday Disc., 112:119-127, 1999.

[71] Hocking L. M., A moving fluid interface on a rough surface, J. Fluid Mech., 76:801-817, 1976.

[72] Richardson S., On the no-slip boundary condition, J. Fluid Mech., 59:707-719, 1973.

[73] Galea T. M. and Attard P., Molecular dynamics study of the effect of solid roughness on the slip length at the fluid-solid boundary during shear flow, Langmuir 20:3477-3482, 2004.

[74] Bico J., Marzolin C. and Quere D., Pearl drops, Europhys. Lett., 47:220-226, 1999.

[75] Lauga E. and Brenner M. P., Dynamic mechanisms for apparent slip on hydrophobic surfaces, Phys. Rev. E, 70:026311, 2004.

[76] Spikes H. and Granick S., Equation for slip of simple liquids at smooth solid surfaces, Langmuir, 19:5065-5071, 2003.

[77] Lauga E. and Stone H. A., Effective slip in pressure-driven Stokes flow, J. Fluid Mech., 489:55-77, 2003.

[78] Ruckenstein E. and Rajora P., On the no-slip boundary condition of hydrodynamics, J. Colloid Int. Sci., 96:488-491, 1983.

[79] Alexeyev A. A. and Vinogradova O. I., Flow of a liquid in a nonuniformly hydrophobized capillary, Colloids Surf. A, 108:173-179, 1996.

[80] Andrienko D., Dunweg B. and Vinogradova O. I., Boundary slip as a result of prewetting transition, J. Chem. Phys., 119:13106-13112, 2003.

[81] Lou S. T., Ouyang Z. Q., Zhang Y., Li X. J., Hu J., Li M. Q. and Yang F. J., Nanobubbles on solid surface imaged by atomic force microscopy, J. Vac. Sci. Technol. B, 18:2573-2575, 2000.

[82] Zhang X. H., Zhang X. D., Lou S. T., Zhang Z. X., Sun J. L. and Hu J., Degassing and temperature effects on the formation of nanobubbles at the mica/water interface, Langmuir, 20:3813-3815, 2004.

[83] Lichter S., Roxin A. and Mandre S., Mechanisms for liquid slip at solid surfaces, Phys. Rev. Lett., 93:086001, 2004. [84] Cho J. H., Law B. M. and Rieutord F., Dipole-dependent slip on Newtonian liquids at smooth solid hydrophobic surfaces, Phys. Rev. Lett., 92:166102, 2004.

[85] Tretheway D., Stone S. and Meinhart C. D., Effects of absolute pressure and dissolved gases on apparent fluid slip in hydrophobic microchannels, Bull. Am. Phys. Soc., 49:215, 2004.

[86] Adrian R. J., Bibliography of particle velocimetry using imaging methods: 1917-1995, TAM Report, University of Illinois at Urbana-Champaign, 1996.

[87] Raffel M., Willert C. and Kompenhans J., Particle Image Velocimetry: A Practical Guide, Springer, 2002.

[88] Santiago J. G., Wereley S. T., Meinhart C. D., Beebe D. J. and Adrian R. J., A particle image velocimetry system for microfluidics, Exp. Fluids, 25:316-319, 1998.

[89] Meinhart C. D., Wereley S. T. and Santiago J. G., PIV measurements of a microchannel flow, Exp. Fluids, 27:414-419, 1999.

[90] Meinhart C. D. and Zhang H., The flow structure inside a microfabricated inkjet printer head, J. Microelectromech. Syst., 9:67-75, 2000.

[91] Meinhart C. D., Wereley S. T. and Gray M. H. B., Volume illumination for twodimensional particle image velocimetry, Meas. Sci. Technol., 11:809-814, 2000.

[92] Wereley S. T., Gui L. and Meinhart C. D., Advanced algorithms for microscale particle image velocimetry, J. AIAA, 40:1047-1055, 2002.

[93] Wereley S. T. and Meinhart C. D., Adaptive second-order accurate particle image velocimetry, Exp. Fluids, 31:258-268, 2001.

[94] Cabral J. T., Hudson S. D., Harrison C. and Douglas J. F., Frontal photopolymerization for microfluidic applications, Langmuir, 20:10020-10029, 2004.

[95] EDPIV is available at http://eo.yifan.net/users/l/lcgui/Edpiv\_intro.htm

[96] Harrison C., Cabral J. T., Stafford C. M., Karim A. and Amis E. J., A rapid prototyping technique for the fabrication of solvent-resistant structures, J. Micromech. Microeng., 14:153-158, 2004.

[97] Warren J. A., Cabral J. T. and Douglas J. F., Solution of a field theory model of frontal photopolymerization, Phys Rev E, 72:021801, 2005.

[98] Begolo S., Fabrication of microfluidic device resistant to organic solvents, Degree Thesis, University of Padova, 2007.

[99] Goldman A. J., Cox R. G. and Brenner H., Slow viscous motion of a sphere parallel to a plane wall - II Couette flow, Chem. Eng. Sci., 22:653-660, 1967.

[100] Lauga E., Apparent slip due to the motion of suspended particles in flows of electrolyte Solutions, Langmuir, 20:8924-8930, 2004.

[101] Sun M., Luo C., Xu L., Ji H., Ouyang Q., Yu D. and Chen Y., Artificial Lotus leaf by nanocasting, Langmuir, 21:8978-8981, 2005.

[102] Nguyen Tu T. T., Derenne S., Largeau C., Mariotti A. and Bocherens H., Evolution of the chemical composition of Ginkgo biloba external and internal leaf lipids through senescence and litter formation, Organic Geochemistry, 32:45-55, 2001.

[103] Joseph P. and Tabeling P., Direct measurement of the apparent slip length, Phys. Rev. E, 71:035303, 2005.

[104] Tretheway D. C. and Meinhart C. D., Apparent fluid slip at hydrophobic microchannel walls, Phys. Fluids, 14:L9-L12, 2002.
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