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### Cross References

- ▶ [Evanescent Wave Microscopy](#)

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## Evanescent Wave Illumination

### Synonyms

Total Internal Reflection Fluorescence (Microscopy); TIRM; TIRFM

### Definition

Evanescent wave microscopy uses the evanescent wave produced by the total internal reflection of light at a dielectric interface to illuminate a layer of material within the penetration depth of the evanescent field. The material of interest is imaged using a microscope objective typically within 100 to 200 nm of the interface.

### Cross References

- ▶ [Evanescent Wave Microscopy](#)
- ▶ [Fluorescence Measurements](#)
- ▶ [Fluorescence Labeling](#)
- ▶ [Quantum Dots](#)
- ▶ [TIRF](#)

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## Evanescent Wave Microscopy

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### Synonyms

Total Internal Reflection Fluorescence (Microscopy); TIRM; TIRFM

### Definition

When an electromagnetic wave (light) in a dielectric medium of index  $n_1$  is incident upon an interface of a different dielectric material of a lower optical density,  $n_2$  at an

angle  $\theta_1$  greater than the critical angle,  $\theta_c = \sin^{-1}(n_2/n_1)$ , total internal reflection (TIR) occurs. Although all of the incident energy is reflected, an electromagnetic field with exponentially decreasing intensity propagates in the less dense medium. This field is known as an [▶ evanescent wave](#) and has a decay length or penetration depth,  $d$ , on the order of the wavelength of the incident light,  $\lambda_0$ . The confined nature of the evanescent wave close to the interface is ideal for studying surface and near-surface phenomena pertaining to fluid mechanics and mass transport typically by fluorescence microscopy, which is known as total internal reflection fluorescence microscopy (TIRFM) or simply [▶ TIRF](#).

### Overview

As fluid mechanics reaches into the micro- and nano-scale, interfacial phenomena become increasingly important, and several diagnostic techniques have been adapted from other fields to quantify fluid phenomena at these scales. In addition to general and fluorescence microscopy, an important technique for fluid-surface studies is the use of [▶ evanescent wave microscopy](#).

### Origins

Evanescent wave microscopy or total internal reflection microscopy (TIRM) has been employed in the fields of biology and chemistry since the 1970s. The TIRM technique has long been used in cell biology studies and more recently cell-substrate contacts, vesicle fusion, and single-molecule observation. Here, cells on a microscope cover glass are illuminated by an evanescent field, which is produced through a total internal reflection (TIR) created at the cell/cover glass interface and imaged by a microscope. The technique has been used to measure chemical kinetics and surface diffusion, molecular conformation of adsorbates, cell development during culturing, visualization of cell structures and dynamics, and single molecule visualization and spectroscopy [1].

### Applications to Fluid Mechanics

In micro- and nano-scale fluid mechanics, measurements of diffusion and velocity are used to probe fundamental physical phenomena and evaluate the performance of devices. [▶ Evanescent wave illumination](#) has been combined with several other diagnostic techniques to make such measurements within the order of the penetration depth (about 100–200 nm) of fluid-solid interfaces with resolution sometimes as small as several nanometers. Laser-Doppler velocimetry has been applied to measure single point tracer particle velocities in the boundary layer

of a fluid within 1  $\mu\text{m}$  of a wall. By seeding fluid with fluorescent dye, total internal reflection fluorescence recovery after photobleaching (FRAP) has been used to measure near-wall diffusion coefficients and velocity (for a summary of initial applications see Zettner and Yoda [2]).

A critical contribution of **▶ evanescent wave microscopy** to micro- and nano-fluidics is the application of micro/nano-particle velocimetry techniques to measure velocity fields. Particle image velocimetry (PIV) and particle tracking velocimetry (PTV) have been viable macro-scale diagnostic techniques for measuring two-dimensional velocity fields since the 1980s. These techniques were later adapted to the micro-scale [3] (known as micro-PIV and micro-PTV), which made use of fluorescence microscopy. More recently, Zettner and Yoda [2] used the micro-PIV technique combined with evanescent wave illumination to measure the near-wall velocity field in a rotating Couette flow within about the penetration depth of the evanescent field from the wall. Micro-PTV has also demonstrated great success in measuring the near-wall velocity fields in an evanescent wave as well as the diffusion of tracer particles and even three-dimensional tracer displacements/velocities [4–6].

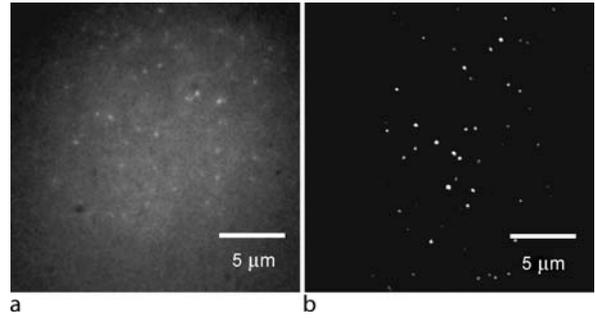
### Advantages

The small length scales involved in micro- and nano-fluidics do not allow for the convenient production of a light sheet to visualize distinct planes of fluid. Instead, imaging was typically limited to volumetric flood illumination as the only visualization technique, which used the depth of focus (DOF) of the microscope objective to designate distinct fluid planes [3]. The DOF is generally described by

$$\text{DOF} = \frac{\lambda_0 n_1}{\text{NA}^2} + \frac{n_1}{M \cdot \text{NA}} e. \quad (1)$$

where  $\lambda_0$  is the illumination wavelength,  $n_1$  is the index of the immersion medium,  $M$  is the magnification and  $e$  is the smallest distance that can be resolved by the detector. The DOF can be quite large for low magnification or low numerical aperture (NA) objectives, and even for high magnification, large NA objectives the DOF is on the order of several hundreds of nanometers. More importantly, flood illumination excites out-of-plane fluorophores outside of the DOF, which are imaged as background noise and significantly lower the signal-to-noise-ratio SNR).

Evanescent wave illumination provides an illuminated fluid layer of smaller dimension than by using the DOF alone, allowing for measurements closer to liquid–solid interfaces. The exponential decay of the light intensity away from the interface is well known, and studies have



**Evanescent Wave Microscopy, Figure 1** A comparison of (a) direct flood illumination versus (b) TIRF illumination of 200 nm diameter fluorescence polystyrene particles in water with a single 5 ns pulse from a Nd : YAG laser through a 100X objective with a NA = 1.45

shown that the intensities of fluorescent tracer particles excited by the evanescent field closely follow the local field intensity [5]. Thus, through careful calibration, tracer particle intensity can be mapped to relative distance from the interface allowing for three-dimensional tracking. Finally, since only a thin layer of fluid is illuminated, there is virtually no background noise from out-of-plane excitation resulting in a very high SNR (Fig. 1).

### Basic Methodology

#### Evanescent Field Theory

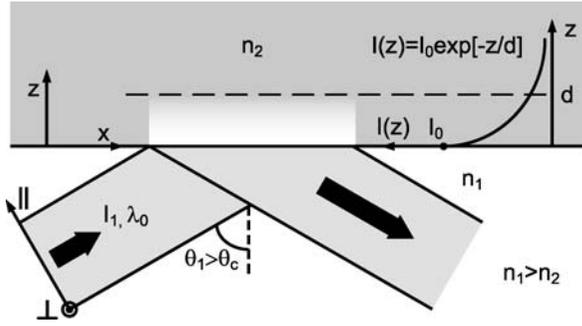
When an electromagnetic wave (light) in a dielectric medium of index  $n_1$  is incident upon an interface of a different dielectric material with a lower optical density,  $n_2$ , at an angle  $\theta_1$  Snell's law predicts the refracted angle,  $\theta_2$ , by:

$$n_1 \sin(\theta_1) = n_2 \sin(\theta_2). \quad (2)$$

For incident angles greater than the critical angle,

$$\theta_c = \sin^{-1}\left(\frac{n_2}{n_1}\right), \quad (3)$$

total internal reflection occurs as illustrated in Fig. 2. Although all of the incident energy is reflected, the solution of Maxwell's equations predict the existence of an electromagnetic field in the less dense medium with intensity decaying exponentially away from the interface. This evanescent wave propagates parallel to the interface and has a decay length,  $d$ , on the order of the wavelength of the illuminating light,  $\lambda_0$ . However, photons are not actually reflected at the interface, but rather tunnel into the low index material by optical tunneling. As a result, the reflected beam of light is shifted along the interface by a small amount ( $\Delta x = 2d \tan \theta_1$ ), which is known as the



**Evanescent Wave Microscopy, Figure 2** Illustration of geometry and parameters for an evanescent wave produced by the total internal reflection of plane waves incident on a dielectric interface

Goos–Haenchen shift. This shift is typically small for fluid diagnostic applications ( $\Delta x < 1 \mu\text{m}$ ) as compared to the field of view, which is on the order of  $100 \mu\text{m}$ .

The full details to the solution of Maxwell's equations are outlined in several other resources [1]. Here, only the results relevant to **evanescent wave microscopy** are presented, specifically the functionality of the intensity field in the low density material, which is used for imaging. It should be noted that these solutions assume infinite plane waves incident on the interface. In practice, however, one typically uses a finite, Gaussian laser beam, which is well approximated using these assumptions. The intensity field has the exponential form

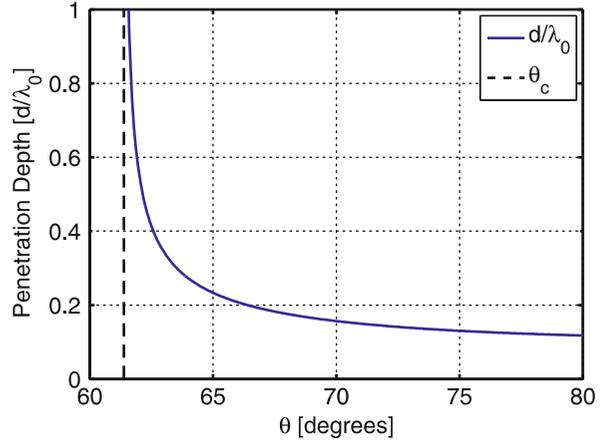
$$I(z) = I_0 e^{-z/d}, \quad (4)$$

where the decay length,  $d$ , is given by

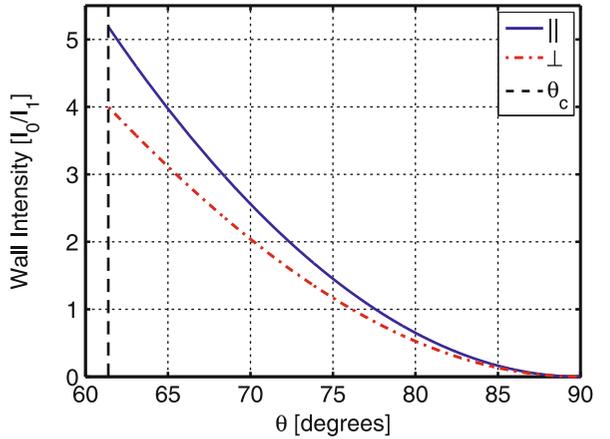
$$d = \frac{\lambda_0}{4\pi n_1} [\sin^2 \theta_1 - n^2]^{-1/2}, \quad (5)$$

and  $n = n_2/n_1 < 1$ . In a typical system, the substrate is a glass slide ( $n_1 = 1.515$ ) and the interrogation medium is water ( $n_2 = 1.33$ ), which gives  $n = 0.878$ . The behavior of  $d$  with respect to incident angle is shown in Fig. 3 for these conditions. For  $\lambda_0 = 514 \text{ nm}$  (Argon Ion), the penetration depth is about  $d = 140 \text{ nm}$  assuming an incident angle of  $\theta_1 = 64.0^\circ$ . The polarization of the incident beam does not affect the penetration depth, but it does affect the amplitude of the evanescent field. For plane waves incident on the interface with intensity  $I_1$  in the dense medium, the amplitude of the field in the less dense medium  $I_0$  is given by

$$I_0^\parallel = I_1^\parallel \frac{4 \cos^2 \theta_1 (2 \sin^2 \theta_1 - n^2)}{n^4 \cos^2 \theta_1 + \sin^2 \theta_1 - n^2}, \quad (6)$$



**Evanescent Wave Microscopy, Figure 3** Behavior of the penetration depth  $d$  normalized by the incident wavelength  $\lambda_0$  as a function of the incident angle  $\theta_1$  for a typical glass–water interface with  $n = 0.878$



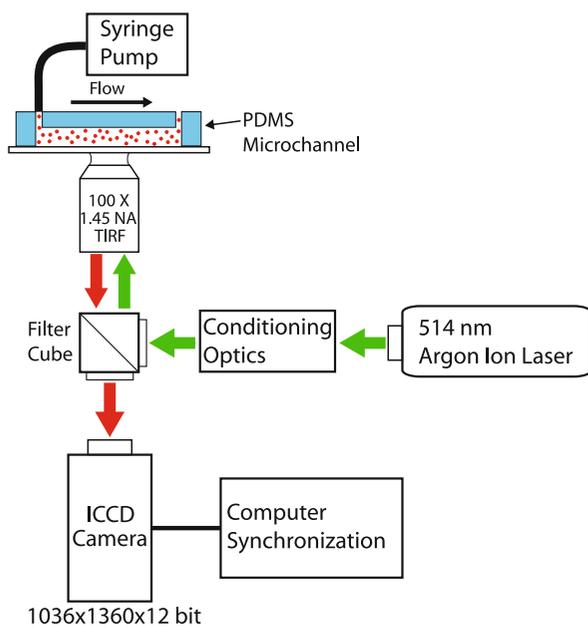
**Evanescent Wave Microscopy, Figure 4** Amplitude of the evanescent field intensity at the interface in the low index material  $n_2$  for both parallel and perpendicular polarization of the incident electromagnetic field

$$I_0^\perp = I_1^\perp \frac{4 \cos^2 \theta_1}{1 - n^2}, \quad (7)$$

for incident waves parallel and perpendicular to the plane of incidence, respectively, as shown in Fig. 4 [1].

### Experimental Implementation

Due to the length scales involved, most interesting phenomena probed by the evanescent wave require microscope optics for imaging. In the past, several TIRM studies have been conducted using only scattering from objects in the evanescent field for imaging. More recently, fluorescence microscopy systems have become the standard imaging technique for evanescent wave microscopy



**Evanescent Wave Microscopy, Figure 5** Schematic diagram of a possible TIRF system for micro- and nano-scale velocimetry using objective-based TIR illumination

(TIRF) due to superior SNR and the availability and variety of fluorescent probes. The basic components of a TIRF imaging system include: light source, illumination optics, specimen or flow cell, emission imaging optics and a camera (usually accompanied by a PC). An example of such a system is shown in Fig. 5.

Light sources are typically continuous-wave (CW) lasers (argon-ion, helium-neon, etc.) or pulsed lasers (Nd : YAG, etc.) for their collimation and single-wavelength characteristics. Non-coherent sources (arc-lamps) are not as common in typical homemade systems, but commercial versions are available. Conditioning optics are used to create the angle of incidence necessary for TIR and are of two types, which will be discussed below. The illumination optics must be held in contact with sample substrate, which can be glass, quartz or plastics as long as the surface is reasonably smooth (standard glass slides and cover slips are adequate). Finally, flow channels are available in a wide variety of forms (both commercial and homemade), which include PDMS channels bonded to glass slide substrates, etched glass channels and commercial glass or quartz flow chambers.

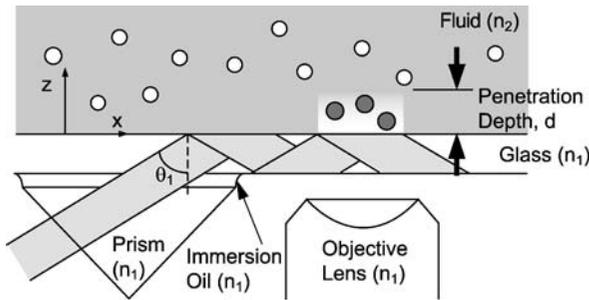
Fluorescence microscopy uses fluorescent molecules (fluorophores), which absorb light energy from the evanescent field created by the light source at wavelength  $\lambda_0$  and re-radiate photons at a longer wavelength  $\lambda_1$ . Optical filters isolate the excitation light from the emitted light, which

is then collected and imaged by a camera. High magnification microscope objectives (30X to 100X) serve as the collection optics and CCD cameras are used for capturing images. Depending on the level of light emitted from the probes, cooled or intensified CCD cameras may be necessary for better sensitivity. Fluorescent probes are commercially available in several forms with varying sizes: single fluorophores (fluorescein, rhodamine laser dye), polymer micro-spheres (50 nm to several  $\mu\text{m}$  diameter), **▶ quantum dots** (5–25 nm diameter), etc. Most dyes and particles are soluble in water for ease of use and application to biological systems. However, investigations of some interesting flow physics are possible with other materials (polymers, organics, etc.). In the case of imaging single particles, most tracers are sub-wavelength, and thus, typically limited to imaging the diffraction limited spot. However, sub-wavelength and sub-pixel resolution is possible through post-processing procedures.

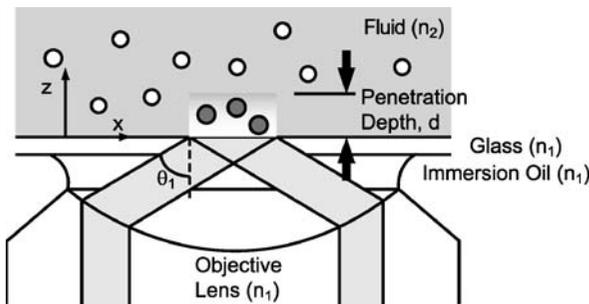
The two common types of illumination systems for creating evanescent wave illumination are prism-based systems and objective-based systems. There are several configurations for each, both of which have their own specific shortcomings, which are outlined elsewhere [1]. Two popular configurations for TIRM systems are presented below.

**Prism-Based Systems** Prism-based TIR illumination systems are typically home-built and low cost. A prism (triangular, square, hemispherical, etc.) is placed in contact with the sample substrate or flow channel (usually coupled to the substrate using an immersion medium). The laser beam is focused into the prism to illuminate the area of interest, then the angle is adjusted to create TIR at the interface of the sample. If the evanescent field is created on the surface of the flow channel opposite of the objective, then air, water or oil immersion objectives may be used provided they have the required working distance. Otherwise, if TIR is created on the same side as the objective, then oil immersion may not be used, since the beam will decouple from the system and fail to create an evanescent field. An example of a prism-based TIRM system is shown in Fig. 6.

**Objective-Based Systems** Objectives suitable for TIR are common (although expensive). They are typically limited to 60X to 100X magnification immersion objectives, and require very high numerical apertures ( $\text{NA} > 1.4$ ). In this method, the collimated light source is focused down onto the back focal plane of the objective to illuminate the sample. Light rays off of the optical axis are projected onto the sample interface at increasing incident angle. If the rays are localized and far enough off of the optical axis, then the full NA of the objective will be utilized and TIR



**Evanescent Wave Microscopy, Figure 6** Schematic diagram illustrating the general experimental set-up for prism-based TIRM, where the beam of light is coupled into a glass slide using a prism



**Evanescent Wave Microscopy, Figure 7** Schematic diagram illustrating the general experimental set-up for objective-based TIRM, where the beam of light is coupled directly into an objective. A large NA is necessary to achieve incident angles greater than the critical angle  $\theta_1 > \theta_c$

will occur at the sample interface creating an evanescent wave in the sample medium as shown in Fig. 7.

### Velocimetry and Tracking Techniques

With a TIRFM system in place, quantitative measurements can be made within 200 nm of the substrate surface. Typically, in fluid mechanics, a wealth of information about any given flow system can be found from the velocity field. As mentioned above, micro-PIV and micro-PTV are both well-established tools for extracting quantitative information from micro-scale fluid systems [3]. However, special care must be taken in applying these techniques to very near-wall flows with evanescent wave illumination. Both techniques require imaging the instantaneous positions of tracer particles seeded in the flow at two different instances in time to infer fluid velocities. Once a series of tracer particle images is obtained, PIV uses cross-correlations of segments from each image to determine the highest correlation for the movement of a group of particles from one image to the next and thus, the velocity of that group. PTV on the other hand, attempts to match individual tracer particles between the two images. This is performed

through identification and center detection of all particles then, matching particles usually through nearest-neighbor algorithms to find the displacement and subsequently the velocity. Since there is little to no slip at the wall, two-dimensional velocimetry within the evanescent field measures the average velocity in the evanescent field weighted by the concentration profile and detectability of the tracer particles. Three-dimensional measurements are typically made by tracking algorithms and rely on the characterization of the evanescent field intensity to map tracer particle intensity to distance from the wall [5, 6].

Particle tracking algorithms have also been used successfully in two- and three-dimensional diffusion measurements and simultaneous diffusion/velocity measurements. The random, thermal motion exemplified by small particles in a surrounding fluid, (Brownian motion), is typically characterized by a diffusion coefficient. By utilizing particle tracking, diffusion coefficients can be measured by observing many thousands of individual particle motions to build an ensemble of displacement statistics. Measurements of diffusion are even more interesting near liquid–solid interfaces, accessible by TIRF, where diffusion coefficients can be anisotropic [6, 7]. In addition to the implications for interface science, these effects should be used in velocimetry algorithms by correcting observed particle displacements to account for anisotropic particle diffusion characteristics.

### Near-Wall Effects

With the high resolution of the TIRM technique, one should be aware of several physical phenomena that are active on length scales comparable to the penetration depth. When an isolated particle is in the vicinity of a solid boundary, its Brownian motion is hindered due to an increase in hydrodynamic drag. The presence of the solid wall decreases the diffusion coefficient, resulting in hindered, anisotropic Brownian motion [7].

In many TIRM applications, it is important to know the concentration profile of tracer particles in the near-wall region. This is important for calculating average velocities in the evanescent field and mapping intensities to distances from the wall. Three physical phenomena may play a role of varying importance: (1) gravitational settling, (2) shear-induced lift (the Saffman effect) and (3) electrostatic attraction/repulsion. In all three cases, these effects compete with Brownian diffusion, which tends to mix the tracers into a uniform distribution throughout the fluid. The effect of gravitational settling is greatest for large particles ( $2a \sim 1 \mu\text{m}$ ) with density mismatch. Shear-induced migration or the Saffman effect is the migration of particles away from solid boundaries in the presence of

high shear. This becomes important for  $Re = 2a\rho U/\mu > 1$ , where  $U$  is the mean velocity [3]. Electrostatic attraction or repulsion between tracers and the wall can also have a substantial effect on the concentration profile, depending on the relative charges of the particle and substrate. This can be suppressed by the addition of electrolytes to the fluid medium to provide charge screening.

## Key Research Findings

### Velocimetry with Evanescent Wave Illumination

Until recently, there were no direct experimental measurements of near-wall nano-scale flows. The development of evanescent wave velocimetry has enabled contributions in several areas. If one applies an electric field along the length of a microchannel, ions near the charged surface move, dragging the bulk fluid along and thus creating a plug-type Electro-Osmotic Flow. TIRV has been applied to characterize the two wall-parallel velocity components in EOF within 100 nm of the wall, which includes the electric double layer (EDL). Analytical and numerical studies suggesting uniform flow near the wall were verified using a nano-PIV technique with an evanescent wave, demonstrating that the EDL is much smaller than 100 nm as predicted [8].

Another research focus has been the investigation of the no-slip boundary condition between a liquid and a solid - an assumption that has been challenged by recent experimental results and molecular dynamic simulations and the subject of many recent investigations. Experimental studies have reported a wide range of slip lengths, ranging from micrometers to tens of nanometers or smaller (including no-slip). Molecular dynamics simulations, on the other hand, suggest small slip lengths, mostly less than 100 nm. The experiments have been conducted under various direct and indirect measurement techniques with varying accuracy and uncertainty Huang et al. (2006) [5] used 3D [Total Internal Reflection Velocimetry](#) (3D-TIRV) to make direct measurements of slip length within 150 nm of the surface. Particle tracking in the wall-normal direction was approximately 10% of the evanescent field penetration depth (about 20 nm), and they were able to show that there appears to be minimal slip over hydrophilic surfaces, while hydrophobic surfaces do appear to introduce a discernable, but small boundary slip, with a slip length of the order of 10–50 nm.

Given the small size of the observation region generated using evanescent wave illumination, it is desirable to minimize the size of the tracer particles for better resolution, and the use of semiconductor nanocrystals or quantum dots (QDs) has been demonstrated. QDs are single fluorophores with fluorescence lifetimes similar to tradi-

tional fluorophores. They exhibit several qualities beneficial to nano-scale velocimetry including small diameter (5–25 nm), narrow emission spectra and controllable surface chemistry. Although they are much brighter than traditional fluorophores, QDs have significantly lower intensity than larger, more common tracer particles measuring several hundred nanometers. Velocimetry with QDs in aqueous solutions is made possible by TIRV because of the extremely high SNR. QD tracking and velocimetry has been successfully demonstrated by several groups using nano-PTV techniques [9, 10].

### Diffusion and Hindered Diffusion Measurements

When the distance  $h$  between a spherical particle of radius  $a$  and a solid boundary becomes sufficiently small ( $h/a \sim 1$ ), hydrodynamic interactions between the particle and wall hinder the Brownian motion (diffusion) of the particle. Such effects are critical to fundamental near-wall measurements and the accuracy of micro-velocimetry techniques, which rely on the accurate measurement of micro/nano particle displacements to infer fluid velocity. By applying the 3D TIRV technique to freely suspended fluorescent particles, simultaneous observation of three-dimensional anisotropic hindered diffusion has been measured for particle gap sizes  $h/a \sim 1$  with 200 nm diameter particles [6] and  $h/a \ll 1$  with 3  $\mu\text{m}$  diameter particles [7]. The latter results confirm the increase of hydrodynamic drag when a particle approaches a solid boundary, and such correction shall be applied to not only diffusion but also other translational motion of particles where the drag force is of concern.

### Future Directions for Research

Evanescent wave microscopy has already yielded a number of contributions to the fields of micro- and nano-scale fluid and mass transport, including investigation of the no-slip boundary condition, applications to electrokinetic flows and verification of hindered diffusion. With more experimental data and improvements to TIRF techniques the accuracy and resolution these techniques are certain to improve. Improvements that will assist include the continued development of uniform, bright tracer particles (such as quantum dots), the improvement of high NA imaging optics and high sensitivity camera systems, and further development of variable index materials for better control of the penetration depth characteristics.

### Cross References

- ▶ [Brownian Motion and Diffusion](#)
- ▶ [Brownian Motion](#)

- ▶ Evanescent Wave Illumination
- ▶ Fluorescence Measurements
- ▶ Fluorescence Labeling
- ▶ Micro-PIV-Based Diffusometry
- ▶ Quantum Dots
- ▶ TIRF

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## Evaporation Deposition

### Definition

A vacuum deposition technique where the material to be deposited is heated until it vaporises. This condenses on the substrate and forms a thin film

### Cross References

- ▶ Sputtering for Film Deposition

## Explosive Boiling in Microchannels

- ▶ Bubble Dynamics in Microchannels

## Extensional

- ▶ Viscoelasticity

## External Actuators

### Definition

A micropump or active microvalve is said to use external actuators when the component responsible for opening or closing the valve, or moving the fluid, is added to the device after fabrication. Examples would be a separate miniature solenoid valve used to pressurize or depressurize air chambers in a pneumatic actuator, or piezoelectric patches glued to a micromachined silicon or glass membrane.

### Cross References

- ▶ Electromagnetic Valves
- ▶ Electrostatic Valves
- ▶ Integrated Actuators
- ▶ Magnetic Pumps
- ▶ Membrane Actuation for Micropumps
- ▶ Microactuators
- ▶ Peristaltic Pumps
- ▶ Piezoelectric Valves
- ▶ Piezo/PZT in Microfluidics
- ▶ Pneumatic Valves
- ▶ Thermomechanical Valves
- ▶ Thermopneumatic Valves

## Externalization of Phosphatidylserine

- ▶ Microfluidics for Studies of Apoptosis