Fundamental limits of optical microrheology

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Abstract

We estimate the fundamental limits of different microrheological techniques based on optical detection. It is suggested that particle tracking systems using nondifferential detection have a minimum detectable displacement given by $0.2\left(\frac{\lambda_0}{NA}\right)\left(1/\sqrt{SNR}\right)$, where $\lambda_0$ is the wavelength, NA is the numerical aperture of the focusing objective, and SNR is the signal-to-noise ratio of the system. This limit has important consequences in microrheology, since the noise contributes with an apparent diffusion constant of $D_l \approx 0.02\left(\frac{\lambda_0}{NA}\right)^2\left(\frac{B}{SNR}\right)$, where $B$ is the bandwidth of the detection unit. As the SNR of ordinary microscopes is limited, one should be extra careful when probing soft materials with low diffusion constants. On the other hand, in differential systems based on laser detection, the SNR is considerably increased due to reduced laser noise, and the minimum detectable displacement is given by $0.4\left(\frac{\lambda_0}{NA}\right)\left(1/SNR\right)$. One may therefore expect to measure the diffusion constant with higher accuracy if the SNR is large. Finally, we find that total internal reflection microscopy (TIRM) has a minimum detectable displacement given by $0.1\lambda_0/SNR$.

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1. Introduction

Optical microrheology has gained considerable popularity the last decade due to the development of new techniques, as well as the advances in computational techniques and image analysis [1–12]. It has been used to probe the local rheological properties (e.g., shear modulus) of a number of different soft materials with considerable success. In a typical experiment, a submicron fluorescent bead is immersed in the material of interest, and then the lateral Brownian motion is recorded, assuming that the particle stays within the microscope’s field of depth during the measurement interval. After sampling enough data points of the particle’s position, one may extract the mean-square displacement and also the diffusion constant. The latter can be related to the shear modulus through the Stokes–Einstein relationship. At the center of optical rheology is the ability to detect small displacements, and recent studies using video microscopes claim displacement resolutions down to 10 nm with limited signal-to-noise ratios [6,7]. However, nanometer displacements are not easy to calibrate, and one should be extremely careful when claiming such resolutions, also because errors may have an impact on the rheological data obtained. In particular, no calculations based on waveoptics and noise theory have been performed, and it is therefore quite difficult to assess whether the resolutions claimed are physically correct or not.

Another microrheological technique based on optical detection is total internal reflection microscopy (TIRM). In a typical TIRM experiment, the colloidal particle levitates above the interface by colloidal forces such as electrostatic double-layer repulsion. A small particle placed a distance above an interface (in an optically less dense medium) will scatter evanescent waves back into a detection system. By monitoring the optical signal, one may track the position and height of, e.g., colloidal particles at interfaces, thus enabling studies of, e.g., Brownian motion and colloidal interactions [13,14].

The aim of the current study was to estimate detection limits for these two classes of rheological instruments, based on classic waveoptics and noise theory. We discuss the influence of noise on the displacement and positional measurements, and show that one must be careful when measuring diffusion coefficients of high-viscosity liquids.

2. Probability and noise

At the heart of a detection system is our detector, which here is assumed to be ideal. That is, it can in principle de-
tect infinitely small position changes. Thus, we neglect the inherent clipping usually present in most systems detecting spatially varying signals. The signal current in the detector is related to the optical power $P_0$ incident on the detector by $i_0 = n_s P_0$, where $n_s$ is the sensitivity of the photodetector, typically between 0.1 and 0.9 A/W. In optical data detection systems, knowledge of the spatial and amplitude variations of the intensity at the detector plane is used to deduce, e.g., the particle position. However, due to noise, the signal current is distributed around the mean value $i_0$. If white noise sources are present in the system, and a large number of photons are incident on the detector, the probability density function for the signal current $i_s$ from the detector follows a Gaussian distribution,

$$P(i_s) = \frac{1}{\sqrt{2\pi} n_s^2} \exp \left[ \frac{(i_s - i_0)^2}{2 n_s^2} \right], \quad (1)$$

where $i_0$ is the mean signal current, $n_s$ is the standard deviation due to noise, and the signal-to-noise ratio is defined as $\text{SNR} = i_0/n_s$.

To this end, the probability density for the signal current can be related to the probability density for the position $p(x)$ by observing that the number of observations of intensity in the range $i_s$ to $i_s + di_s$ is equal to the number of observations of position in the corresponding range $x$ to $x + dx$; i.e., $P(i_s) dx = p(x) dx$, or [15]

$$p(x) = P(i_s) \left| \frac{di_s}{dx} \right|, \quad (2)$$

The absolute sign occurs because the probability density is defined to be nonnegative, and we consider only linear detection here.

Now it remains to find the SNR. In the systems studied here there are three important contributions to the noise: laser (or light source) noise, shot noise, and thermal noise in the detector. Laser noise is often caused by instabilities generated by, e.g., mode competition in the laser cavity. The magnitude of the noise generated by the laser can be estimated by assuming that the electric field fluctuates as $E = E_0[1 + b(t)]$, where $b(t) \ll 1$. On evaluating the intensity (using the root mean square value as the measure of noise), we find that $i_s = i_0(1 + 2\Delta t)$, where $\Delta t = \langle \text{Re}^2[b(t)] \rangle^{1/2}$. Thus, the laser noise is represented by $i_l = 2n_s P_0 \Delta t$, where a reasonable value is $\Delta t \sim 10^{-4}$ for a good laser system [16]. Although every stage of the amplification process contributes to the overall noise performance, it is usually the case that the first stage is most important, simply because the noise from this stage is amplified also in the other stages. We consider here only two main sources of noise in the detector: thermal noise and shot noise. Thermal noise depends on the detector’s bandwidth $B$ and shunt resistance $R$.

$$i_{th}^2 = \frac{4kT B}{R}, \quad (3)$$

where $i_{th}$ is the time-averaged current noise, $k = 1.38 \times 10^{-23}$ J/K is Boltzmann’s constant, and $T$ is temperature (here taken to be $T = 300$ K). On the other hand, shot noise is related to the signal power as

$$i_{sh}^2 = 2e\eta_s P_0 B, \quad (4)$$

where $e$ is the electron charge.

Since all the noise sources are assumed to be uncorrelated, their sum represents the total noise, and the SNR is given by

$$\text{SNR} = \frac{\eta_s P_0}{\sqrt{(4kT B/R) + 2e\eta_s P_0 B + (2\eta_s P_0 \Delta t)^2}}, \quad (5)$$

3. Particle tracking systems

Tracking particles in suspension is a key feature of many scientific and industrial systems. Usually, one or more small particles are imaged through the optical system. Here we assume a radiating scatterer much smaller than the wavelength. If we neglect all polarization effects (which tend to broaden the focal spot), and assume that the reflection and transmission coefficients are unity, then the electric current is related to the position at the detector plane by the formula [17]

$$i_s(r) = \eta_s P_0 \left| \frac{\gamma}{\gamma} B(\theta) \sin \theta J_0 \left( \frac{2\pi}{\lambda_0} r \sin \theta \right) \right| \exp \left( i \frac{4\pi}{\lambda_0} z \cos \theta \right) d\theta, \quad (6)$$

where $B(\theta)$ is an apodization factor of the imaging system, $J_0$ is the Bessel function of the first kind (order 0), $z$ is the distance between the geometrical focal plane and the plane where the point scatterer is located, and the integral is taken over the angular aperture of the objective lens, which has a numerical aperture NA of $\sin \gamma$. The magnification of the imaging system is here assumed to be unity, but this requirement is easily relaxed if needed (although not of interest here, since our detector can detect infinitely small displacements). It should be clear that changing $z$ also changes the electric current in the detector. However, here we are mostly interested in particles located at the geometrical focal plane $z = 0$. Since the focal depth of the system is larger than the wavelength, it is reasonable to assume that small variations in $z$ (due to, e.g., thermal agitation) do not change the signal, and we therefore neglect these and set $z = 0$. Moreover, we want to examine the system with the best possible resolution. We therefore collect only light with high spatial frequencies, assuming that no light is lost in the process. This is naturally an idealization, but we are here interested in the fundamental limit of the system. In Fig. 1 we show the spatial signal distribution (Eq. (6)) as a function of position $r$ on the detector. The solid line corresponds to $B = 1$ and $\gamma = 90^\circ$, whereas the dashed line corresponds to an annular aperture where $B(\theta) = 1$ when $90^\circ \geq \theta > 80^\circ$ ($B = 0$ when $\theta < 80^\circ$). Thus, a more localized intensity distribution occurs if we block the low-angle part of the aperture at the cost of higher sidelobes.
To obtain the best possible resolution of the system considered here (ignoring the sidelobes), we therefore require that $B(\theta) = \delta(\theta - \gamma)$, which corresponds to collecting only the high spatial frequencies of scattered light. For a small displacement $r$ of the scatterer, the signal current is

$$i_s \approx \eta_s P_0 \left| J_0 \left( \frac{2\pi}{\lambda_0} r \eta_s \right) \right|^2 \approx \eta_s P_0 (1 - \beta r^2),$$

(7)

where we have set $\beta = 2\pi^2 (\eta_s \lambda_0)^2$. One may wonder whether our small-distance approximation influences the result. As we will see, this is not the case, since the probability density Eq. (1) falls off very quickly, thus limiting the possible particle positions very close to $r = 0$ and fulfilling $r \ll \lambda_0/2\pi \eta_s \lambda_0$. Without lack of generality, we consider fluctuations on the $x$ axis only, such that the probability distribution Eq. (2) is

$$p(x) = \frac{2i_0 \beta}{in\sqrt{2\pi}} |x| \exp \left( -\frac{1}{2} \beta^2 SNR^2 x^2 \right).$$

(8)

It is interesting to see that the probability density is exactly zero at $x = 0$, which is due to the very small change in signal $dI_s/dx$ found in this area. We are here most interested in the fluctuations around $x = 0$, which can be found as

$$(\Delta x)^2 = \frac{1}{\sqrt{\beta SNR}} \frac{\lambda_0}{\eta_s \lambda_0} \frac{0.2}{\lambda_0} = \frac{1}{\sqrt{\beta SNR}} \frac{\lambda_0}{\eta_s \lambda_0} \frac{0.2}{\lambda_0}$$

(9)

and therefore we have

$$\Delta x = \left( \frac{2}{\pi} \right)^{1/4} \frac{1}{\sqrt{\beta SNR}} \approx \frac{\lambda_0}{\eta_s \lambda_0} \frac{0.2}{\lambda_0}.$$  

(10)

In a practical microscope we may have $\lambda_0 = 0.55 \mu m$, $\eta_s \lambda_0 \approx 1$, and $SNR \approx 50$, giving $\Delta x \approx 16 \text{ nm}$. This is within the requirements of our small $x$ approximation, $x \ll 90 \text{ nm}$. One should remember that Eq. (10) represents the apparent mean-square displacement due to noise, and we must require that the actual mean-square displacement (due to, e.g., diffusion) is larger than $\Delta x$. On digitizing the images one also encounters additional sources of error, such as clipping of images due to the digitizing grid [6]. Our analysis suggests that a displacement resolution of 10 nm is at the lower limit given a SNR less than 100.

3.1. Diffusion measurements

As an example of how noise-induced apparent motion may influence practical measurements, consider thermally fluctuating microparticles which are used to probe the rheology of complex fluids [4]. Here a small displacement may be due to thermally induced (random) motion or simply a result of noise. More clearly put, the detector is fooled to see motion due to the noise. Since the two contributions are comparable, one should be careful when extracting data. In particular, the noise-induced apparent motion influences the measured diffusion coefficient and therefore also the rheological (e.g., viscosity) data extracted from this. Let us now assume that we want to track a particle undergoing one-dimensional Brownian diffusion. The random walking particle takes a total of $n = j + k$ steps, where $j$ are taken to the right and $k$ to the left. Using standard probability theory one finds that the probability for a given $j$ is

$$P_n(j) = \frac{n!}{j!(n-j)!} 2^{-n},$$

(11)

where the total distance to the right is $\Delta x_r = j \Delta x_b = (n-j) \Delta x_b = (2j - n) \Delta x_b$. Here the particles use time $\Delta t$ to move distance $\Delta x_b$. The mean-square deviation is then

$$\langle x^2 \rangle = \sum_{j=0}^{n} (\Delta x_b^2) P_n(j) = n(\Delta x_b^2).$$

(12)

If we define $t$ as the total time interval needed for $n$ steps, then $t/\Delta t = n$, and we may write

$$\langle x^2 \rangle = \frac{(\Delta x_b)^2}{\Delta t} t = 2Dt.$$

(13)

where $D$ is the usual diffusion constant, which here is related to the elementary parameters through $D = (\Delta x_b)^2/2 \Delta t$. Here $D = D_t + D_i$ consists of two parts: one part generated by the thermal motion of the particle ($D_t$) and another induced by the noise in the system ($D_i$). As has been pointed out before, noise gives rise to apparent motion at the detector plane and, therefore, places a lower limit on the measurable diffusion constant. That is, if we want to measure the properties of media with low $D$, e.g., high-viscosity materials, then it is important to maximize the signal-to-noise ratios. To have a useful measure of $D$, we must require that $D_i \gg D_t$.

To obtain an explicit expression for the lowest possible diffusion constant, we assume that the scatterer is at rest ($D_t = 0$), and that the system has a finite SNR. Since $1/\Delta t$ represents the effective dynamic range of the system, $B \approx 1/\Delta t$. The typical fluctuations are estimated from Eq. (10) ($\Delta x_b \rightarrow \Delta x$), and we find that

$$D_t \approx \frac{1}{50} \left( \frac{\lambda_0}{\eta_s \lambda_0} \right)^2 \left( \frac{B}{\text{SNR}} \right).$$

(14)
As an example, consider the case \( \text{SNR} = 50, \ NA = 1, \ B = 50 \ \text{Hz}, \) and \( \lambda_0 = 500 \ \text{nm} \). This gives \( D_t \sim 10^{-14} \ \text{m}^2/\text{s} \).

In a recent article it was suggested that the ordinary diffraction pattern (from, e.g., a single fluorescent molecule) seen on the detector will broaden due to diffusional processes, and that this broadening can be used to extract the diffusion constant \( D_t \) [18]. In Ref. [18] small diffusion coefficients were measured in this way, and found to be in agreement with those using mean-square displacement analysis. Our analysis suggests that the smallest possible diffusion constant obtained this way should be about \( D_t \sim 10^{-14} \ \text{m}^2/\text{s} \).

It is well known that the thermal diffusion coefficient of a sphere is related to the viscosity \( \eta \) of the liquid through Einstein’s relationship, \( D_t = kT/6\pi \eta a \) [19]. Thus, we must require that the viscosity fulfills

\[
\eta \ll 50kT \left( \frac{NA}{\lambda_0} \right)^2 \left( \frac{\text{SNR}}{6\pi aB} \right).
\]  

(15)

Thus, assuming \( a = 0.1 \ \mu\text{m}, \ T = 300 \ \text{K}, \) and the parameters above, we find that \( \eta \ll 0.2 \ \text{N}\text{s}\text{m}^{-2} \). This means that the viscosity of water (\( \eta \approx 10^{-3} \ \text{N}\text{s}\text{m}^{-2} \)) can be measured with this method, whereas high-viscosity materials such as glycerol (\( \eta \approx 10 \ \text{N}\text{s}\text{m}^{-2} \)) must be treated with more care.

3.2. Differential detection

One may imagine that differential detection could improve the measurement accuracy. Here a small displacement \( x \) from the optical axis could be detected as an asymmetric signal by a quadrant photodiode. To obtain an estimate of the apparent mean-square displacement in the case of such a differential detection system we consider two photodiodes, one that detects an optical power \( P_r \), and another that detects \( P_t \). Each photodiode has an effective width \( D \), which is a measure of the extent of the light spot. The physical width is assumed to be larger, so that no loss of signal due to offset occurs. An infinitiesimally small part of the detector located at \( x' \) receives a power \( P_0 d(x') \), where \( P_0 \) now is defined to be the total power incident on the two detectors, and \( d(x') \) is a distribution function. To obtain the total power incident on the two photodiodes, we must integrate over the power distribution and divide by 2\( D \) (which is the extent of the distribution),

\[
P_r = \frac{P_0}{2D} \int_0^P d(x' - x) \, dx',
\]

\[
P_t = \frac{P_0}{2D} \int_0^P d(x' + x) \, dx',
\]  

(16)

which to first order gives an effective signal \( i_s = \eta_s (P_r - P_t) = \eta_s P_0 (x/D) \), i.e., is linear in the displacement. Note that \( i_0 = \eta_s P_0 \) is the total signal current generated in the differential system (i.e., after the differential amplifier). The probability density is still Gaussian,

\[
p(x) = \frac{1}{\sqrt{2\pi} (i_n D/\eta_0)} \exp \left[ -\frac{x^2}{2(i_n D/\eta_0)^2} \right],
\]  

(17)

and the apparent mean-square displacement is

\[
\Delta x = \frac{D}{\text{SNR}}.
\]  

(18)

We here use the extent of the power distribution as a measure of \( D \), and set \( 2\pi NAD/\lambda_0 \approx 2.4 \) (where \( \lambda_0 \approx 2\pi NAx/\lambda_0 \) has its first zero, see Eq. (7)), i.e., \( D \approx 0.4\lambda_0/NA \). Then we get

\[
\Delta x \approx 0.4 \frac{\lambda_0}{NA \ \text{SNR}} \left( \frac{2eB}{\eta_s P_0} \right)
\]  

(19)

which in the shot noise limit becomes

\[
\Delta x \approx 0.4 \frac{\lambda_0}{NA} \sqrt{\frac{2eB}{\eta_s P_0}}.
\]  

(20)

We see that only in the limit of high SNR one can expect to obtain subnanometer resolution. Indeed, if \( \lambda_0 = 0.5 \ \mu\text{m} \) and \( NA = 1 \), then we must require that \( \text{SNR} > 200 \) to reach this limit. It should also be noted that only one particle can be kept in the focused laser beam, which limits the possibility of probing rheological data at multiple points. On using a laser array, one could in principle avoid this, but at the cost of reduced quality of the laser beam due to off-axis aberrations.

In Refs. [4,5] a focused laser beam (\( \lambda_0 = 0.67 \ \mu\text{m} \)) with \( NA = 1.4 \) was scattered off a small sphere, and the differential signal was measured with a quadrant photodiode. A displacement resolution of 0.01 nm Hz\(^{-1/2} \) when \( 50 > B \geq 0.5 \ \text{kHz} \) was claimed. Assuming \( \eta_s P_0 = 0.01 \ \text{mW} \), we get a lower fundamental limit of \( 3 \times 10^{-5} \ \text{nm Hz}^{-1/2} \) from Eq. (20). However, this is only in the shot noise limit, and neither the optical power incident on the quadrant detector, nor the signal-to-noise ratio was given in Ref. [4], so that it is impossible to know whether our calculation applies the correct numbers. Moreover, one should bear in mind that photodiodes usually carry dark currents (typically 1 nA) in addition to thermal noise sources, which increase the apparent mean-square displacement considerably. However, we can conclude that the method has indeed subnanometer resolution prospects, but one expects that in a practical system the realizable limit is considerably higher than the shot noise limit given here.

4. Total internal reflection microscopy

It is well known that a plane wave in an optically less dense medium with refractive index \( n_1 \) impinging on an optically more dense medium with refractive index \( n_2 \) can excite evanescent waves above the critical angle. A small particle placed a distance \( z \) from the interface (in the optically less dense medium) will scatter the evanescent wave back into a detection system, and the power incident on the detector is
to a good approximation given by
\[ P_d = P_0 \exp(-\alpha z), \quad \alpha = \frac{4\pi}{\lambda_0} \sqrt{n_1^2 \sin^2 \theta_i - n_2^2}, \quad z \geq 0, \]

where \( P_0 \) is the incident power, \( \lambda_0 \) is the wavelength of vacuum, \( n_1 \) and \( n_2 \) are the refractive indices of the first and second media, respectively, and \( \theta_i \) is the incident angle. Here we are interested in small distances \( z \), which results in
\[ i_s \approx i_0(1 - \alpha z), \]
as long as \( \alpha z \ll 1 \).

In a typical TIRM experiment, the colloidal particle levitates above the interface by colloidal forces such as electrostatic double-layer repulsion. Due to Brownian motion, they are seen to fluctuate around an average height \( z_m \), and if the gravity is too strong one finds \( z_m = 0 \). However, this is only possible in the absence of noise. In a real system, the measured position appears to fluctuate due to noise sources. In general, if the noise fluctuations are superimposed on those generated by Brownian motion (both of which are independent processes), the total probability density is obtained by taking the convolution of the two corresponding probability distributions. However, here we neglect the Brownian processes to obtain a simple measure of the apparent position fluctuations as a function of the signal-to-noise ratio alone.

The probability density is still Gaussian, and is given by
\[ p(z) = \begin{cases} \frac{1}{\sqrt{2\pi \sin^2 \theta_i - n_1^2 \sin^2 \theta_i}} \exp\left[-\frac{z^2}{2\sin^2 \theta_i - n_1^2 \sin^2 \theta_i}\right] & \text{if } z \geq 0, \\ 0 & \text{if } z < 0. \end{cases} \]

As a measure of these fluctuations near \( z = 0 \), we use the mean-square value defined as
\[ (\Delta z)^2 = \int_{-\infty}^{\infty} z^2 p(z) dz, \]

and which can be evaluated as
\[ \Delta z = \frac{\lambda_0}{4\pi \sqrt{2\sin^2 \theta_i - n_1^2 \sin^2 \theta_i}} \]

Again we define the maximum signal-to-noise ratio as
\[ \text{SNR} = i_0/i_n. \]

We here assume that \( n_1 = 1.5 \) (glass) and \( n_2 = 1.3 \) (water), thus giving a critical angle of \( \theta_c \approx 60^\circ \). If we set \( \theta_i = 70^\circ \), then
\[ \Delta z \approx \frac{0.1 \lambda_0}{\text{SNR}}. \]

Using Eq. (5), we find that (neglecting the temperature-induced fluctuations of the interface)
\[ \Delta z \approx \frac{0.1 \lambda_0}{\eta_s P_0} \sqrt{(4kT B/R) + 2\eta_s P_0 B + (2\eta_s P_0 \Delta_1)^2}. \]
noise by both electronic and optical methods. These methods usually introduce noise on their own [13,16], and the shot noise limit discussed above is therefore a good measure of the lowest possible error in the axial position. An alternative method for improving the resolution is to attract the colloidal particle to the interface using an electric (or magnetic) field gradient, so that to a good approximation the Brownian fluctuations can be neglected [14]. Then only the laser and electronic noise is present, and one can try to subtract this from the real signal. One could also imagine employing differential detection to reduce the laser noise. However, a detailed study of these effects is outside the scope of the current work.

5. Conclusion

We have estimated the fundamental limits of different microrheological techniques based on optical detection. It is suggested that particle tracking systems using nondifferential detection have a minimum detectable displacement given by $0.2(\lambda_0/\text{NA})(1/\sqrt{\text{SNR}})$, where $\lambda_0$ is the wavelength, NA is the numerical aperture of the focusing objective, and SNR is the signal-to-noise ratio of the system. Since the SNR of ordinary microscopes is limited, one should be extra careful when probing soft materials with low diffusion constants. For example, when probing the viscoelastic properties of biological networks, the small Brownian displacements involved place constraints on what one can measure. In a differential system based on laser detection the SNR is considerably increased due to reduced laser noise, and one may therefore expect to measure the diffusion constant with higher accuracy. We have seen that the SNR is the main limitation of the techniques discussed here. In principle one could imagine improving the SNR by using stochastic resonance; see, e.g., [20]. However, this is outside the scope of the current study.

References