Rotational diffusion coefficient measured by Polarized Fluorescence Recovery After Photo bleaching (pol-FRAP)

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Introduction

We have improved the pol-FRAP method to measure the rotational diffusion coefficient D^r of fluorescently-labeled colloidal spheres as well as anisotropic particles[1]. Our set-up has a broad time scale from tens of μs to tens of seconds or more.

Theory

The time scale for reorientation of Brownian particles is characterized by the rotational diffusion coefficient D^r, for non-interacting spheres given by the Stokes-Einstein-Debye relation:

$$D_0^r = \frac{k_B T}{8\pi\eta_0 a_T^3} \qquad (1)$$

k_B = Boltzmann's constant
T = absolute temperature
η_0 = solvent viscosity
a_T = (hydrodynamic) particle radius

Using the Debye[2] differential equation for the probability $P(\hat{D},t)d\hat{D}$ for finding a particle with orientation $\hat{D}$ at time t due to rotational diffusion, we can deduce the anisotropy in the fluorescence intensity r(t) :

$$r(t) = r_0 e^{-6D_0^r t} \qquad (2)$$

r_0 = initial anisotropy
t = time

with a characteristic decay time $\tau^r = 1/(6D_0^r)$ for the rotational diffusion of non-interacting spherical particles.

Experiment

A short intense, linear polarized laser pulse bleaches dye molecules inside the particles, with their absorption dipole moment preferentially parallel to the polarization direction of this exciting ('*pump*') beam.
When they are *probed* with a linear polarized, continuous-wave laser beam, the resulting fluorescent intensity will be high or low, depending on whether the polarization direction of the probe beam is parallel or perpendicular to the initial bleach polarization, resulting in an anisotropy of the signal. Due to the rotational Brownian motion of the particles the anisotropy disappears. The experimental set-up is schematically depicted in Fig 1.
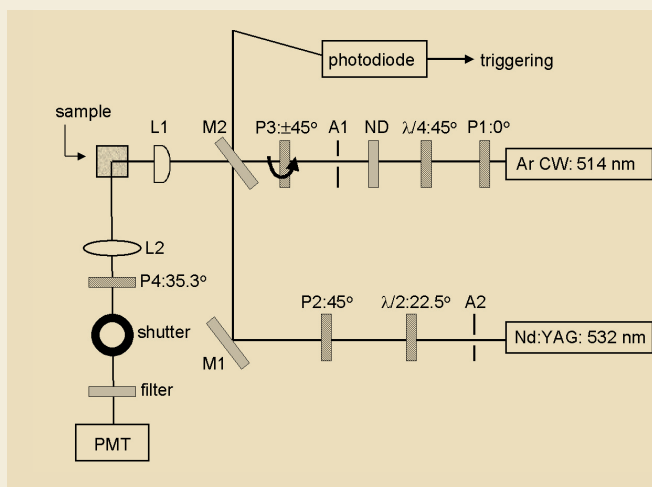

Figure 1. Instrumental set-up. Most important parts:
- *Pump beam: Bleaching pulses of 5 ns from an Nd-Yag laser at λ=532 nm. The vertical polarization direction is turned by a λ/2 platelet to a polarization direction of +45⁰*
- *Probe beam: Cw attenuated Ar-ion laser at λ=514 nm, linearly polarized*
- *λ/4 platelet converts linearly into circularly polarized light*
- *P3 polarizer takes one linear polarization direction out of the circularly polarized light. The polarization direction is made alternately parallel (+45⁰) or perpendicular (-45⁰) to the pump beam (+45⁰)*
- *L1 best form laser singlet lens is used to focus both laser beams into the sample*
- *P4 sheet polarizer is oriented at an (so-called 'magic') angle of 35.3⁰ from the vertical direction to fulfill the condition of measuring the absorption dipole moment only. This tabletop set-up allows for an easier interpretation of the data than for the microscope epi-fluorescence geometry used elsewhere*
- *Shutter consisting of a fast ferro liquid crystal shutter to screen the sensitive detector to the high intensity pump laser beam pulse of 5 ns (peak power 200 kW). The rise time of 55 μs to open the shutter limits the shortest accessible time scale*
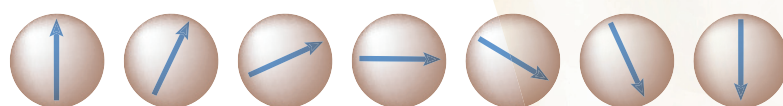
Results

Tracer silica spheres with a dye core are easier to synthesize than optically anisotropic particles needed for other techniques. Pol-FRAP proves to be very sensitive to low fluorescence intensities and therefore requires only small tracer concentrations while the host colloidal suspensions can be highly concentrated.
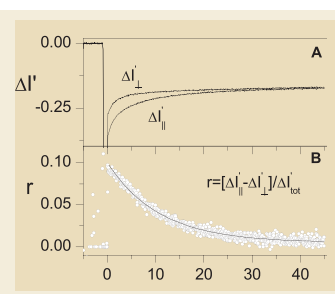

Figure 2. Photo bleaching measurement of the rotational diffusion of rhodamine labeled silica particles (a_T=230 nm, volume fraction 0.1 %) dispersed in DMF/DMSO,
A. The normalized fluorescent intensity for the polarization direction of the probe beam parallel or perpendicular to the polarization direction of the pump (bleach) beam,
B. Due to the orientational relaxation of the bleached particles the anisotropy in the fluorescent intensity shows a single exponential decay to zero with a characteristic decay time τ^n of 10.2 ms, giving a rotational diffusion coefficient D^r=16 s^{-1}.
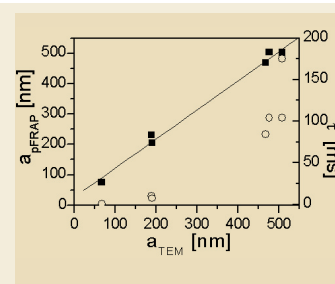

Figure 3. Comparison of pol-FRAP with TEM.
The hydrodynamic particle radius as determined by pol-FRAP (closed symbols) plotted versus the bare radius measured with TEM. On the right y axis the corresponding decay time τ^n (open symbols).

An example of an experiment to study the influence of a host matrix (concentrated fd virus rods) on the rotational diffusion of immersed tracer spheres is demonstrated in Fig. 4. The rotational diffusion time τ^r in the concentrated dispersion of fd virus rods is 60 times larger than at infinite dilution.
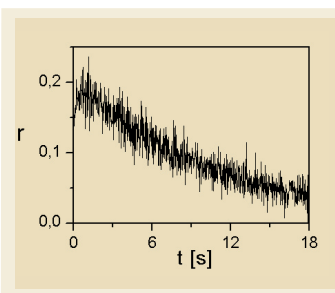

Figure 4. Long- time orientational relaxation of rhodamine labeled silica spheres (a_{TEM}=508nm) in a concentrated (4.5 mg/ml, about 100 times the overlap concentration) dispersion of fd virus rods.
The rotational diffusion time is τ^r=12 s in this dispersion and τ^r=0.21 s at infinite dilution.

Conclusion

- Broad rotational diffusion time window: tens of μs to tens of seconds.
- Fluorescently-labeled tracer particles are relatively easy to synthesize.
- Pol-FRAP can be applied to concentrated colloidal suspensions with slow particle dynamics.
- Very low concentration of labeled tracer particles is needed.
- The tabletop geometry used in our set-up allows for easier interpretation of data than for microscope geometries used elsewhere.

References
[1] M. P. Lettinga, G. H. Koenderink, B. W. M. Kuipers, E. Bessels, and A. P. Philipse; Rotational dynamics of colloidal spheres probed with fluorescence recovery after photo bleaching; to be published in J. Chem. Phys. probably in **120-9**, 515409 (march 2004)
[2] P.Debye; Polar molecules (Dover, New York, 1929)