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Fluorescence Microscopy Visualization of Contacts Between Objects**

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**Abstract:** The area of contact between two objects was detected by using the strong enhancement of the fluorescence of rigidochromic probe molecules attached to one of the surfaces. Confinement of the molecules suppresses nonradiative decay and turns on the fluorescence. The approach is demonstrated by imaging of the contact area of a plastic sphere in contact with a flat glass surface. Our results agree excellently with the prediction of Hertz’s classical theory based on elastic deformation.

The study of contact mechanics dates back to 1882 with the publication of “On the contact of elastic solids” by Hertz. For the behavior of virtually all mechanical systems, the mechanics of the contact between their constituents is crucial. Friction, for instance, is a direct consequence of contact mechanics and is responsible for about 30% of the world energy consumption. Surprisingly little is known about how the physical contacts between objects arise, although this is essential for understanding their mechanics. The main challenge is that since most (if not all) surfaces possess a certain roughness, the actual contacts may occur on microscopic length scales, even for large macroscopic bodies. Bowden and Tabor were the first to emphasize the importance of surface roughness for bodies in contact.

Herein we describe the first direct visualization of mechanical contacts at the microscale by means of fluorescence microscopy, using specifically developed probe molecules that fluoresce when confined in a contact. To achieve this goal we synthesized rigidochromic fluorescent molecules that fluoresce only very weakly in (low-viscosity) solutions owing to the presence of rapid non-radiative relaxation pathways for the excited state. This fast non-radiative decay is triggered by the rotation around a specific bond in the molecule. When the rotation of the bond is hindered, the non-radiative decay is suppressed, and the excited state decays by emitting a photon. When rigidochromic molecules are incorporated in a very viscous medium, such as a glassy polymer matrix, a strong fluorescence is observed. This effect can be used to measure local viscosities in polymer films and study their free volume and glass transition, and to investigate the viscosity of membranes and intracellular media. We show that the confinement between two surfaces also impedes the non-radiative relaxation of the probe molecule 1 that starts fluorescing strongly when confined. This effect then allows the detection of the physical contacts between surfaces on a molecular scale.

For our experiments, we synthesized a new member of the DCDHF class of compounds that has in recent years been developed by Moerner, Twieg, and co-workers for single-molecule imaging. The compounds investigated in this study.

**Scheme 1.** The compounds investigated in this study.

Supporting Information. This chromophore has advantages over previously used viscosity sensitive probes such as dicyanovinyljulolidines and BODIPY dyes: excitation and emission in the visible part of the spectrum, good photostability, and particularly low fluorescence in low-viscosity polar solvents. Compound 1 was chemically linked to the surfaces of glass coverslips to investigate the imaging of contact areas. Compound 2 was used for comparison.

As a first step to characterize their photophysical properties, we measured absorption and emission spectra of compounds 1 and 2 in a series of solvents. The data listed in the Supporting Information (Table S1) show that there is little difference in the properties of 1 and 2, as expected. Both show a weak solvatochromic effect in absorption and in emission. The fluorescence quantum yields $\Phi_f$ are low, and tend to...
decrease with increasing solvent polarity. Fluorescence decay times \( t_f \) follow the same trends as the quantum yields. In some solvents they were shorter than the time resolution of our instrument (< 10 ps). The quantum yields and decay times are larger in solvents of higher viscosity. For example, in cyclohexanol \( t_f = 0.46 \text{ ns} \) and \( \Phi_f = 0.11 \), while in 2-propanol \( t_f = 0.040 \text{ ns} \) and \( \Phi_f = 0.010 \). The reason for the difference is that in low-viscosity solvents rapid nonradiative deactivation of the excited states occurs by twisting of the exocyclic \( C=\text{C(CN)}_2 \) bond, as was reported previously for DCDHF chromophores.\(^{[8]}\) In some solvents we found a bi-exponential fluorescence decay, indicating that the photophysical behavior of this chromophore is more complicated than was suggested previously.\(^{[9]}\)

We systematically studied the effect of solvent viscosity with minimal effect of polarity by subjecting solutions of compound 1 in acetonitrile to different hydrostatic pressures. To convert the hydrostatic pressures to changes in viscosity, we used the relationship between viscosity of acetonitrile and pressure described by Martin et al.\(^{[18]}\) using data from Dymond et al.\(^{[19]}\) The results are shown in Figure 1.

We find that the fluorescence intensity can be described well by the Förster–Hoffmann equation:\(^{[12]}\)

\[
\log(I_f) = A \log(\eta) + C \tag{1}
\]

In Equation (1), \( A \) is a constant that depends on the dye and the solvent.\(^{[13]}\) From the slope of the line shown in Figure 1b we find \( A = 0.66 \pm 0.04 \). For other systems with the same type of rotor unit, values of \( A \) between 0.5 and 1.2 were recently reported, depending on the nature of the solvent.\(^{[4,15]}\)

To be able to look at the contact of an object with a flat surface, we covalently attached probe 1 to glass cover slips. The latter were functionalized with \( N-(2\text{-aminoethyl})-3\text{-amino-}

![Image](85x133 to 258x367)

**Figure 1.** a) Fluorescence spectra of 1 in acetonitrile at different pressures. b) Fit of the intensity data according to Equation (1).
When the bead is pressed onto the cover slip, the confinement leads to a clear fluorescence increase owing to the rigidochromic effect: a roughly circular fluorescent spot appears and increases in size as the force is increased (Figure 2). When the bead is retracted and placed again with the same load, the contact area is reproduced within ±5%.

To compare with the classical Hertz theory, which was exactly devised for this situation,[1,12] we estimated the macroscopic contact area by fitting a circle to the fluorescent area. In Hertz theory, the radius $a$ of the contact area between a sphere (radius $R$) and a flat surface pressed against each other with force $F$ is described by:

$$a^2 = \frac{3R(1-\nu^2)}{4E} F$$  \hspace{1cm} (2)

In Equation (2), $E$ is the Young’s (shear elastic) modulus and $\nu$ is the Poisson ratio of the sphere material ($\nu = 0.37$ for PMMA). The modulus of glass can be ignored because it is much higher than that of PMMA. By relating the area to the load according to Equation (2), we can derive the Young’s modulus of the PMMA sphere (Figure 3). The value found $E$(PMMA) = 2.0 GPa is a bit lower than the literature value for bulk PMMA, which is presumably due to a slight softening of the PMMA sphere by DMSO.[23] Most importantly, we observe that the theory agrees remarkably well with the experiments, which strongly supports the validity of using immobilized compound I as a probe for mechanical contact.

We note that the fluorescent spot is not perfectly circular, and shows a significant amount of structure within it, implying that there are many small contacts at the microscopic scale, rather than one large homogeneous contact, as is commonly assumed in contact mechanics. At the same time, contact mechanics has been tested many times, and seems to be valid even when the microscopic structure of the contact is not taken into account. This presumably holds as long as the typical scale of the roughness is much smaller than both the radius of the bead and the contact area,[6,24,25] which is the case for this experiment.

In summary, the present approach offers a unique method to directly observe the detailed structure of the contact area between two surfaces. We obtain diffraction-limited resolution in the imaging plane, but the resolution in the axial direction is determined by the thickness of the monolayer of dye molecules on the flat glass surface (roughness < 1 nm). We are currently investigating the application of this new method to different problems in contact mechanics.

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