Three-dimensional visualization of contact networks in granular material
Carpentier, C.E.

Citation for published version (APA):
Carpentier, C. E. (2013). Three-dimensional visualization of contact networks in granular material
A solvatochromic fluorescent marker for the visualization of contacts in a granular system

As described in chapters 4 and 5, solvatochromic fluorescent probes have been attached to particles and surfaces. In this chapter, quantitative fluorescence microscopy measurements with those particles are described with the purpose of visualizing forces between them. Fluorescence decay times, intensities and spectra turn out not to differ significantly between contact and non-contact points, nor between no force and force. Contact networks between 10 μm PMMA particles, however, can be clearly visualized.
6.1. Introduction

9-Amino substituted perylene monoimides such as compound 17 (figure 6.1) exhibit good solvatochromic sensitivity (see section 2.3.3.1).\textsuperscript{1} This molecule exhibits the behavior common to push-pull conjugated systems, with a modest solvatochromic shift in absorption and a significant shift in fluorescence. “Push-pull” refers to the presence of an electron donating group at one end of the conjugated system (the amine moiety of 17) and an electron withdrawing group at the other end (the imide moiety). In the excited state, the amine side of the conjugated system will become more positively charged and the imide part more negatively charged, which leads to an increased dipole moment. Solvents of different polarities respond differently to this change in dipole upon excitation of 17, and as a result the absorption and emission spectra display positive solvatochromic shifts with increasing solvent polarity.

In chapter 4, the synthesis is described of compound 46, the carboxylic acid derivative of compound 17. This allows to connect the solvatochromic probe to surfaces via amide bonds. In this respect, reference compound 47 has been prepared, which resembles the covalently attached probe (figure 6.1). The absorption and emission spectra of 47 were recorded in a series of solvents, which showed that its absorption maximum ranges from 514 nm (in apolar cyclohexane) to 537 nm (in polar methanol), and its emission maximum from 628 nm (cyclohexane) to 732 nm (methanol). Thus, the amide analogue 47 exhibits solvatochromic behavior just like 17, and it is thus to be expected that the immobilized probe will as well. The fluorescence quantum yields of 47 decrease with increasing solvent polarity; $\Phi_F = 0.54$ in cyclohexane and $\Phi_F = 0.22$ in methanol.

As discussed in section 2.3.1. and 4.1.1, the phenomenon of solvatochromism can be employed in the investigation and visualization of force networks within granular materials.

Figure 6.1. Solvatochromic perylene probe 17 and reference compound 47.
Granules, functionalized with solvatochromic probes are envisioned to display different fluorescent properties at the “contact points”, where the particles touch each other, than at the “non-contact points”. Our hypothesis is that at the contact points between the particles the polarity is locally lower than at the non-contact points. This is because the solvent is not able to enter this volume and therefore less solvent molecules are present to stabilize the excited state of the solvatochromic probe. As a result, less solvent relaxation is envisioned to occur and it is expected that at the contact points between particles a shift of the emission maxima to shorter wavelength (hypsochromic or blue shift) will be observed. This shift may be larger when more force is applied to the granules, as more solvent will be pushed away, resulting in a less polar environment. Thus, the fluorescent properties are expected to depend on the amount of force applied to the granular system.

Secondly, the extent of solvatochromism is dependent on the viscosity of the medium. Complete solvent stabilization around the excited state can only be reached when the solvent molecules have enough mobility. Thus, in highly viscous media, relaxation of the solvent takes more time than the lifetime of the excited state and therefore cannot be fully completed. The solvent molecules cannot reorient themselves fully around the dipole. It is envisioned that when force is applied to a system of granular particles functionalized with a solvatochromic probe, the regions of the particle that experience forces, i.e. where they touch each other, will be more rigid. Due to this locally increased viscosity, a solvatochromic probe in that region can be expected to exhibit a hypsochromic shift in emission wavelength. Although the hypothesis is reasonable, it remains to be seen if the effects are large enough to be detectable, which will be investigated in this chapter.

In chapter 4, it is shown that applying liquid pressure (in acetonitrile) slightly shifts the emission wavelength of compound 47. This is caused by a change in dielectric properties of acetonitrile when pressure is applied. The density of the liquid is higher when compressed and thus more molecules are condensed in a particular volume. Thus, the electronic polarization per volume unit is increased by applying a force. Moreover, the effect of applying a force is more pronounced in polar solvents, because of the increased short-range interactions between neighboring molecules. When going from 1 to 3900 bar, the dielectric constant of acetonitrile increases from 35.95 to 43.68. This results in an emission maximum red shift for compound 47 in acetonitrile from 732 nm (1 bar) to 743 nm (3900 bar).

To make the particles transparent for the microscopy measurements, a medium is required between the particles that matches the refractive index of the PMMA particles ($n = 1.4914$). In most cases, we have used DMSO containing some sodium iodide for this purpose. It is known that the dielectric constant of DMSO is also affected by the application of force to the liquid, although the effect is smaller than
for acetonitrile. Thus, the measurements discussed in this and the next chapters are designed such that a force is applied to the particles only, not to the liquid. A special sample holder and plunger were designed to ensure that the DMSO can pass through the plunger, but the particles cannot. In this way, the density of the solvent stays the same throughout the measurement, and the probe on the particles will not experience a change in dielectric constant. Any shift in emission maximum should then be caused by the force applied to the particles, and not by force effects on the properties of the DMSO. Figure 6.2 depicts a schematic representation of the cylindrical sample holder and plunger made of a glass filter (see section 3.2.4. for a detailed description of the experiment). A suspension of PMMA particles in a solution NaI in DMSO that matches the refractive index of the particles was placed in the sample holder. As the plunger is permeable for the DMSO/NaI medium, a force can be applied on the particles by placing a weight on top of the plunger.

![Figure 6.2. Schematic representation of the specially designed sample holder with plunger (green), used for the confocal microscopy force measurements. Additional weight can be placed in top of plunger, for additional application of force. Sample cell dimensions: $\varnothing_{\text{inner}} = 0.8 \text{ cm}; h = 1.7 \text{ cm};$ plunger dimensions: $\varnothing = 0.8 \text{ cm}$.](image)

![Figure 6.3. Fast FLIM images: contacts are red (A) with a lifetime of 3.07 ns; Non-contacts are green (B) with a lifetime of 2.65 ns; image size = 80 x 80 $\mu$m; $z = 5 \mu$m.](image)
6.2. Results and discussion

6.2.1. Fluorescence lifetime vs. applied force
The first attempts to visualize the contact and force networks in a granular system were done with the functionalized PMMA particles of 10 \( \mu \text{m} \) diameter. The obtained fast FLIM (fluorescence lifetime imaging microscopy)\cite{6-8} images are shown in figure 6.3, in which the color scale indicates the corresponding fluorescence lifetime. Here, the green color represents short lifetimes of about 2.6 ns and the red color longer lifetimes of about 3.1 ns.

From these images, it seems obvious that the lifetimes at the “contact points” between the particles are significantly longer (figure 6.3 (A)), while the “non-contact points” exhibit shorter lifetimes (figure 6.3 (B)). However, one should take into account that these pictures are made in the fast FLIM mode with a high scan rate, resulting in too low photon count per pixel to achieve reliable results. In general, large errors are contained in fast FLIM images. When the fluorescence intensity is low, which is the case at the non-contact points, as will be discussed in section 6.2.2., photon counts are even lower, which results in underestimated lifetimes.\cite{9} This results in a distorted picture in the lifetime measurements.

In view of the above, the difference in fluorescence lifetime at the contact points and at the non-contact points was established by accurate measurements at many points throughout the sample. The lifetime was measured at a specific point by focusing the laser at that point for several seconds.

Figures 6.4 and 6.5 depict four FLIM images at two different locations in the sample (the z-position was kept constant at 5 \( \mu \text{m} \), halfway the bottom layer of particles, while the x and y position are scanned). In the left images, taken without applied force, the lifetime was measured at various contact points and non-contact points. These measurements were repeated with a force of 2.9 N applied to the system (right images) by putting weight on top of the sample holder.

All obtained lifetimes are represented in the histograms in figures 6.6 and 6.7. For the systems without force, the average lifetime \( \tau \) at the contact points (figure 6.6 (A)) is 2.93 ± 0.07 ns, and at the non-contact points (figure 6.6 (B)) it is 2.86 ± 0.08 ns. Indeed, the lifetime is measured to be somewhat shorter at the non-contact points with respect to the contact points in the system without force. However, the difference between both values is so small that they fall within each other’s error range. For the system with force applied, the average lifetime at the contact points (figure 6.7 (A)) is 2.83 ± 0.03 ns, and at the non-contact points (figure 6.7 (B)) 2.73 ± 0.06 ns. Again, the non-contact points exhibit a slightly shorter lifetime. All lifetimes observed here are comparable to the lifetime of the carboxylic acid functionalized probe 46 in a DMSO
solution ($\tau = 2.9$ ns, see section 4.2.2). In general, the fluorescence lifetimes appear to decrease when a force is applied to the system, but the changes are not significant.

**Figure 6.4.** Fast FLIM image of the functionalized PMMA particles; no force on the sample (A) and with force (B); image size = 80 × 80 μm; $z = 5$ μm.

**Figure 6.5.** Fast FLIM images of the functionalized PMMA particles; no force on the sample (A) and with force on the sample (B); image size = 45 × 45 μm (A); 40 × 40 μm (B); $z = 5$ μm.

**Figure 6.6.** Histograms of fluorescence lifetimes measured at contact points (A) and non-contact points (B), with Gaussian fits; no force applied to the system; $z = 5$ μm.
A solvatochromic fluorescent marker for the visualization of contacts

When a force is applied to a granular system from above, the contact points which are located between two particles more or less above one another will experience the majority of this force. Horizontal contact points (between two adjacent particles in a horizontal plane) may experience some force, as the two particles are pushed sideward against their neighbors. Because of their spherical nature, these particles sediment into a more or less closely packed arrangement, in which a perfect vertical arrangement, which would experience most of the force, does not occur. To study such vertical contact points, a single layer of particles was sedimented onto the bottom of the sample holder. Vertical contact points are thus created between the particles and the coverslip at \( z = 0 \mu m \). These contact points experience the vertically applied force, which enables the direct analysis of the force sensitivity of the solvatochromic dye.

In this respect, the lifetimes of sedimented particles without force applied on them are investigated by fast FLIM (figure 6.8 (A)). Different forces were applied to this system; \( 0.02 \text{N} \) (weight of the plunger = 2 g,) and \( 2.7 \text{N} \) (plunger + additional weight of 269 g). The force exerted by the plunger may vary a little, as frictional forces exist between the plunger and the glass wall of the sample holder. The corresponding fast FLIM images are depicted in figure 6.8 (B) and (C) respectively. Thus, these fast FLIM images are recorded in the \( x,y \)-plane, at \( z = 0 \mu m \). In addition, figure 6.9 shows fast FLIM images of the \( x,z \)-plane (A) and \( y,z \)-plane (B) for force = 0.02 N force. These images clearly demonstrate that the functionalized granules are arranged in a single layer.

For each of the three situations (i.e. the sedimented particles without force, 0.02 N of force and 2.7 N of force), the fluorescence lifetime was measured in fifty different contact points between the particles and the coverslide. In figure 6.10, these lifetimes are represented as histograms, together with the corresponding Gaussian fit.

---

**Figure 6.7.** Histograms of fluorescence lifetimes measured at contact points (A) and non-contact points (B), with Gaussian fits; force applied to the system; \( z = 5 \mu m \).
Figure 6.8. Fast FLIM images of the PMMA particles sedimented; force = 0 N (A), 0.02 N (B) and 2.7 N (C); image size = 80 × 80 μm; z = 0; images taken at different xy-positions.

Figure 6.9. Fast FLIM images of the PMMA particles sedimented; x,z-plane (A) and y,z-plane (B); F = 0.02 N.

Figure 6.10. Histograms of the fluorescence lifetimes of the sedimented particles; force = 0 N (A); 0.02 N (B); 2.7 N (C); z = 0 μm.
For the system without force, the probe molecules on the surface of the sedimented particles exhibited an average lifetime of $\tau = 3.24 \pm 0.13$ ns (figure 6.10 (A)). This value decreased slightly upon application of a force of 0.02 N, to $\tau = 3.08 \pm 0.10$ ns (figure 6.10 (B)), and increased again when the force rose to 2.7 N, giving an average lifetime of $\tau = 3.21 \pm 0.07$ ns (figure 6.10 (C)).

The difference between these values is hardly significant, and no trend in the lifetimes is observed upon application of more force. It can be concluded that measuring the variations in fluorescence lifetimes is not suitable to visualize forces between granules.

6.2.2. Fluorescence intensity vs. applied force

Analogous to the fast FLIM measurements, fluorescence intensity images were recorded for the granular systems of PMMA particles with a 10 μm diameter (at $z = 5$ μm). The intensity image in figure 6.11 clearly demonstrates that the intensity at the contact points is higher than at the non-contact points. This phenomenon may be caused by a mere doubling of the concentration of fluorescent probes, as two surfaces are present at the contact points, or there could be a genuine intensity rise induced by
the environment. This hypothesis was tested by measuring various cross sections through the sample, from which the integrals of fluorescence intensity were calculated. An example of such a cross section analysis is depicted in figure 6.12.

These areas of the cross section intensities obtained are plotted in two histograms, one for the contact points (figure 6.13 (A)) and one for the non-contact points (figure 6.13 (B)). Again, the results in these histograms are fitted with a Gaussian curve to obtain an average value with corresponding error. For the contact points, this average value is \(5.1 \pm 0.4 \times 10^3\) counts, compared to \(3.0 \pm 0.3 \times 10^3\) counts for the non-contact points, which is only a little bit more than half the value for the contact points. This implies that the increased intensity at the contact points is most probably indeed caused by a mere addition of probe concentrations. The slight reduction in intensity at the non-contact points may be due to some self-quenching.

Figure 6.13. Histograms of the intensity cross section at the contacts points (A) and the non-contact points (B); \(z = 5 \, \mu m\).

Analogous intensity images have been recorded further below in the same sample at the contact plane between the particles and the coverslip (\(z = 0 \, \mu m\)), which are depicted in figure 6.14. At this point, the particles press down on the cover slide, where they emit fluorescence, in analogy to the previously described measurements of the fluorescence lifetimes. At these contact points, the force applied to the particles can be measured directly. In figure 6.14, intensity images are depicted of sedimented particles without force applied (A), force of 0.02 N (B) and 2.7 N (C).

Again, the fluorescence intensity (number of counts at the emission maximum) was analyzed in fifty contact points, but now by recording emission spectra in each of those points, for the situation without force and with application of force (0.02 N and 2.7 N). The results of change in emission intensity are represented as histograms with Gaussian fit, depicted in figure 6.15. Table 6.1 summarizes the results. Fluorescence intensities vary from \(28 \pm 6 \times 10^3\) counts for the particles without force to \(25 \pm 8 \times 10^3\) counts for 0.02 N of force and \(28 \pm 7 \times 10^3\) counts for 2.7 N of force.
A solvatochromic fluorescent marker for the visualization of contacts

Figure 6.14. Fluorescence intensity images made with confocal microscopy; force = 0 N (A), 0.02 N (B) and 2.7 N (C); image size = 80 × 80 μm; z = 0 μm.

Figure 6.15. Histograms of the fluorescence intensity of particles experiencing 0 N (A), 0.02 N (B) and 2.7 N (C) of force; z = 0 μm.

The fluorescence spots of figure 6.14 were also analyzed for their cross section. Average cross sections for each of the three images are given in figure 6.16. The width of the spots, measured as their FWHM values, is greater than the theoretically expected value of 5.6 μm (see section 3.2.2). This deviation may be caused by slight out-of-focus measurements. Also the dent in fluorescence intensity at the center of the particles at 0 N and 0.02 N, suggest that the focal plane was slightly above z = 0 μm, as outlined in section 3.2.2. However, care must be taken with this conclusion, as the images are quite noisy with some extremely intense pixels, which have a large influence on the average intensity.
Chapter 6

Figure 6.16. Cross section analysis of the fluorescence spots of figure 6.14; FWHM values are 7.10 μm (0 N); 7.44 μm (0.2 N); 6.65 μm (2.7 N).

Table 6.1. Fluorescence intensity and emission maxima.

<table>
<thead>
<tr>
<th>Force applied (N)</th>
<th>Intensity max (counts)</th>
<th>Emission maximum (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>27.8 ± 5.6 × 10^3</td>
<td>689 ± 4</td>
</tr>
<tr>
<td>0.02</td>
<td>24.9 ± 7.8 × 10^3</td>
<td>694 ± 4</td>
</tr>
<tr>
<td>2.7</td>
<td>28.7 ± 7 × 10^3</td>
<td>693 ± 5</td>
</tr>
</tbody>
</table>

Figure 6.17. Histograms of the emission wavelength of particles experiencing 0 N (A), 0.02 N (B) and 2.7 N (C) of force; z = 0 μm.
In conclusion, the changes in intensity with varying force are insignificant, when the solvatochromic particles are used as granules. This is also apparent in figure 6.16, in which the total intensities for the three situations are almost the same.

6.2.3. Fluorescence wavelength vs. applied force
In the same investigation as depicted in figures 6.14, thus at $z = 0 \, \mu m$, the change in emission wavelength upon force application to the particles was determined. Thus, emission spectra were recorded at fifty contact points between the particles and the cover slip of the sample cell. The corresponding histograms with Gaussian fit are depicted in figure 6.17 and summarized in table 6.1. The average wavelength of emission shifts from $\lambda_{em} = 689 \pm 4 \, \text{nm} \, (F = 0 \, \text{N})$, to $\lambda_{em} = 694 \pm 4 \, \text{nm} \, (F = 0.02 \, \text{N})$ and $\lambda_{em} = 693 \pm 5 \, \text{nm} \, (F = 2.7 \, \text{N})$.

Clearly, these results demonstrate that the differences in photophysical properties, in fluorescence lifetime, intensity and wavelength of emission, are not significant when the situations without and with force are compared.

6.3. Conclusion
Fluorescence from PMMA particles functionalized with solvatochromic probe 46 could easily be detected using confocal microscopy, giving bright luminescence with low background signals. In this chapter, the visualization of the contact points is investigated, as well as the dependence of the fluorescence on the application of force.

The contact points exhibited brighter luminescence, although their fluorescence lifetimes hardly differed from those at the non-contact points. Thus, the location of the contact points could clearly be visualized, exhibiting fluorescence with almost double intensity as the intensity in the non-contact points. This phenomenon enables reconstruction of the entire contact network between the particles, as is described in chapter 8. This visualization of contacts can be used in further investigations of the statistical mechanics of granular material.

The dependency of the fluorescence lifetimes, emission maxima and fluorescence intensities on the application of force was investigated for both the contact and the non-contact points. The major goal of these measurements was to determine the extent of force sensitivity of the immobilized probe. The measurements in which the force was most directly transferred to the contact points, i.e. the single layer of particles on top of a cover slide, showed that no variations in the photophysical properties were observed. These investigations showed that the solvatochromic probe is not directly suitable for the visualization of forces. However, care should be taken, because the force on the particles is difficult to arrange. Images in the x,z-plane
Chapter 6 showed that one layer was obtained while doing the force measurements. Particles can move very easily through the sample and the particles are not completely monodisperse. Particles having a diameter up to 20 μm were observed, and it may be possible that those particles experience the majority of the force.

6.4. Experimental section

The samples of solvatochromic PMMA particles are prepared as described in section 3.2.4. A description of the fluorescence confocal microscopy measurements is given in section 3.2. The integration time for recording spectra was 10 s. Fluorescence lifetimes were recorded using the confocal microscopy set-up as described in section 3.2.3.1. Time correlated single photon counting histograms were prepared using the same number of photon counts (about 2.3 × 10⁶ photons for the measurements at z = 5 μm, about 1.0 × 10⁶ photons for the measurements at z = 0 μm). The same part of the decay curve was fit for each measurement (60% of the decay curve for the measurements at z = 5 μm, 84% of the decay curve for the measurements at z = 0 μm). No deconvolution with IRF was applied. Data was fit using a Maximum Likelihood Estimation method.

6.5. Acknowledgement

Joanna Siekierzycka and Michiel Hilbers are kindly acknowledged for their assistance with the confocal microscopy experiments. Gertjan Bon is kindly acknowledged for the development of the sample cell with plunger.

6.6. References