Supporting Information

Fast 3D microscopy imaging of contacts between surfaces using a fluorescent liquid

Dina Petrova,[†] Bart Weber,[§] Clémence Allain,[‡] Pierre Audebert, [‡] Daniel Bonn,[⊥] and Albert M. Brouwer[*,†]

[†]van 't Hoff Institute for Molecular Sciences, University of Amsterdam, P.O. Box 94157, 1090 GD Amsterdam, The Netherlands

[§]Advanced Research Center for Nanolithography (ARCNL), P.O. Box 93019, 1090 BA Amsterdam, The Netherlands

[‡]PPSM, ENS Cachan, CNRS, Université Paris-Saclay, 94235 Cachan, France

[⊥]Institute of Physics, University of Amsterdam, P.O. Box 94485, 1090 GL Amsterdam, The Netherlands

*To whom correspondence should be addressed: A.M.Brouwer@uva.nl.

Experimental details

The refractive index of the fluorescent liquid was measured on an Abbe refractometer.

The fluorescence images (Figures 2-4, S2 and S4) were obtained with the microscopy setup described in ref. S1. In this setup, a rheometer (Anton Paar DSR 301) was mounted on top of an inverted confocal microscope (Zeiss Axiovert 200M). We glued a sphere to the rheometer plate and moved it into contact with a droplet of the fluorescent liquid on top of a coverslip. The rheometer measures normal force on the contact. The fluorescent liquid was excited by an argon-ion CW laser at 514 nm. The objective lens was a 63× 1.3 NA LD A-Plan, Zeiss. Filter sets used for filtering of excitation and emission light were dichroic mirror HFT 405/514 and emission filter BP 560|615. Imaging was performed using the Zeiss LSM 5 LIVE microscope control system.

The fluorescence lifetime traces were measured using time-correlated photon counting (TCSPC) using a TimeHarp 200 module incorporated in the MicroTime 200 confocal microscope (PicoQuant GmbH) with a 100× 1.4 NA objective (UplanSApo, Olympus), mounted on a piezo-scanning stage (Physik Instruments GmbH). Again, the rheometer (Anton Paar DSR 301) was mounted on top of the confocal microscope. The bead to be used was glued to the rheometer plate. An NKT SuperK Extreme pulsed (9 MHz) white-light continuum laser with the SuperK Select acousto-optical tunable filter (AOTF) was used for excitation at 514 nm. It excites the fluorescent liquid in between the glass coverslip and the bead, which was pressed with a controlled normal force. A detection pinhole with a diameter of 75 µm was used. For filtering excitation and emission light, the following set of filters was used: 510|20 Semrock (for filtering laser light after AOTF), 514|25 notch filter Semrock (for blocking excitation laser light in the detection pathway), 593 LP emission filter Semrock, 540 DCLP dichroic mirror Chroma. For measurements of spectra the emission filter was removed.

The average acquisition time for the TCSPC time traces was 60 s. On average, each histogram had 106 photons. Total decay curves were mono-exponentially fitted for each measurement using a Maximum Likelihood Estimation method, and deconvolution with the measured instrument response function

(IRF) was applied. The IRF was determined using scattered light at the excitation wavelength from a ludox sample, FWHM ~ 300 ps.

Spectra were measured using a Spectra Pro-150 spectrograph (Acton Research Corp.) with a PhotonMAX EMCCD camera (Princeton Instruments) connected to the MicroTime 200 microscope. An integration time of 100 ms was used for obtaining spectra at the points where the thickness of the liquid was 50 nm and larger; for smaller thicknesses an integration time of 1 s was used. 10 spectra were averaged in each point, a total 100 points were measured for each type of contact (dark, bright, none), and the spectra were smoothed with binomial smoothing filter (smoothing parameter set to 7) in Igor Pro software.

Atomic force microscopy (AFM) was used to characterize the surfaces prior to the contact measurements. A Dimension FastScan AFM from Bruker was used to perform the measurements. Images were obtained in tapping mode. Each image was flattened by a 2D spline fit in order to subtract the spherical tip shape from the surface profile, using the Nanoscope 1.4 software.

Determination of scaling factor

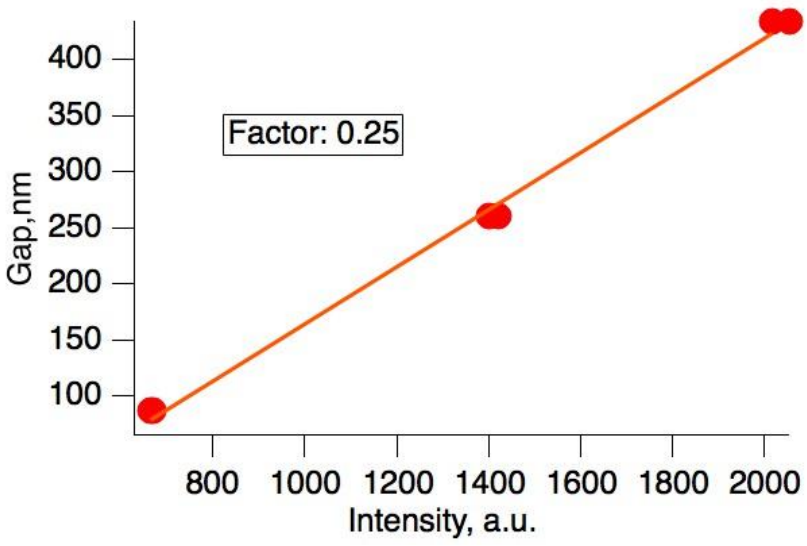

Figure S1. Determination of the scaling factor between the intensity of fluorescence and the thickness of the liquid layer, obtained from the Newton rings reflection pattern by using Eq. (1) in the main text.

To determine the scaling factor between fluorescence intensity and liquid height, two images were compared: fluorescence intensity and reflection intensity. A cross-section is taken through each of the images and the peak positions were identified from the interference pattern as indicated in panel (b) of Figure S2 and in Figure S3. For each of the peak positions, the heights calculated through Equation (1) in the main text were correlated to the fluorescence intensities at those positions. This analysis is shown in Fig. S1 and yields the calibration factor, in this case 0.25 arbitrary units (a.u.) per nm.

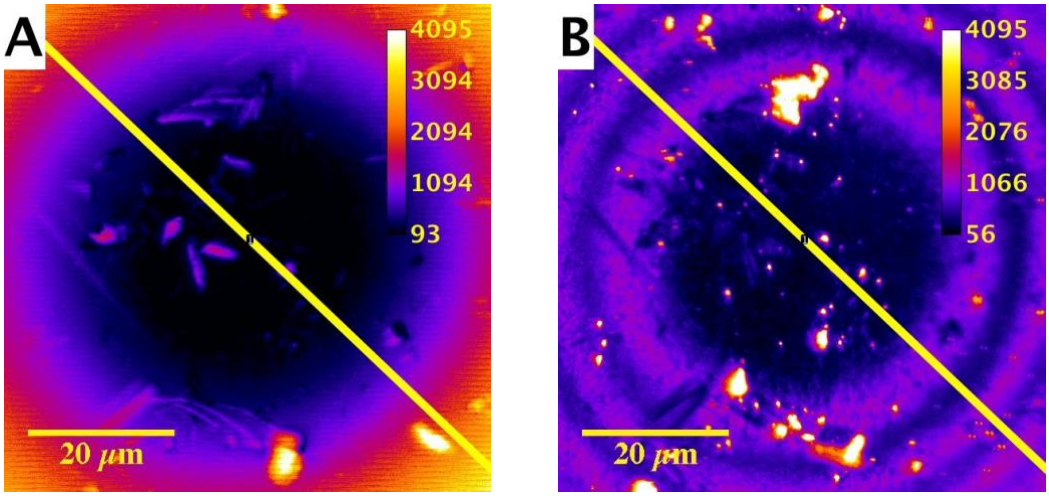

Figure S2. Contact area between the glass bead and a glass coverslip imaged with fluorescent liquid method. The cross-section for calibration of the scaling factor is indicated in each of the images. a) Fluorescence intensity image. b) Reflection of the laser light forming an interference pattern of Newton rings.

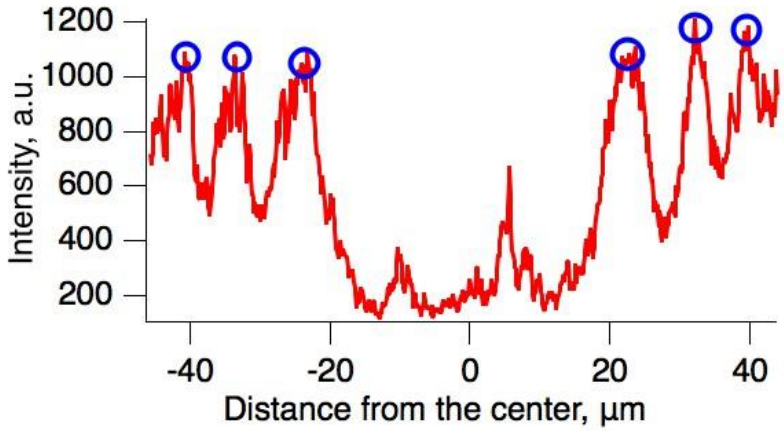

Figure S3. Cross-section of the Newton rings reflection pattern shown in Figure S2. Blue circles indicate the peaks of the interference pattern.

AFM vs fluorescence intensity images. Other materials

In Figure S4 the fluorescence intensity image of a polystyrene bead in contact with the fluorescent liquid on a glass coverslip (A) is compared with the AFM image of the same bead (B).

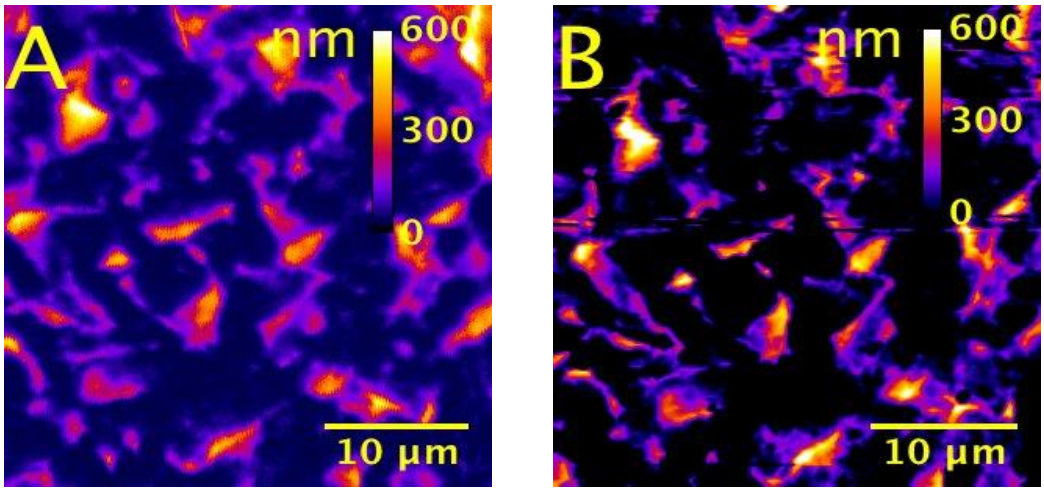

Figure S4. Rough PS sphere on smooth glass coverslip interface. For ease of visual comparison, zoomed areas of the overall surface are shown. A) Image obtained by the fluorescent liquid method. B) AFM image of the same PS bead. The height values in A) do not go down to zero because a thin boundary layer of the fluorescent liquid separates the two surfaces.

Supporting Information References

[S1] B. Weber, T. Suhina, T. Junge, L. Pastewka, A. M. Brouwer, D. Bonn, *Nat. Commun*. **2018**, *9*, 888.