Combined AFM-Confocal Microscopy of Oil Droplets: Absolute Separations and Forces in Nano-Films - Supporting Information

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Materials

Perfluorooctane (PFO, 98%) was obtained from Sigma and purified by column chromatography over silica (Flurosil, Sigma). The fluorescent dye, acid Red 88, was obtained from Sigma (95%) and used as received. Decanethiol was from Sigma (99%) and used as received. Water used was obtained from an Elga Maxima reverse-osmosis purification unit, and exhibited a minimum resistivity of 18.4 mΩ cm.

Atomic force microscopy

Measurements were made on a JPK NanoWizard 3 Bioscience AFM. This instrument has the Z-axis piezo assembly decoupled from the X and Y directions, and uses capacitative sensors that report the actual distance traveled in each axis. This feature is vital to measuring accurate force-displacement curves, as the piezo travel is slightly non-linear with applied voltage. Reported 'nominal' velocities are an average of the distance traveled over the entire time of the measurement, although the theoretical model takes into accound the precise spontaneous velocity at each point by parsing the reported distance/time function. Paddle-shaped silicon AFM cantilevers were custom made, with a beam of 450×50×3 μm, and a circular region at the end of the cantiever of diameter 60 μm.[1,2] The circular region at the end of the cantilever was coated with gold of thickness ≈20 nm, and the back of the cantilever was coated with chromium (8 nm) and gold (20 nm) to increase the reflectivity of the AFM laser. The gold region on the cantilever was rendered hydrophobic by reaction with decanethiol (1 mM) in ethanol for 2 hours. Cantilever spring constants, K, were determined by the method of Hutter and Bechhoeffer.[3]

Performing interaction measurements

Doplets of perfluorooctane were generated underwater in a specially-made fluid cell by gently discharging a syringe which had been backfilled with a few microliters of PFO in 0.3 mL water. The total volume of liquid in the fluid cell was 0.6 mL. The bottom of the fluid cell comprised a glass cover slip (Menzel-Gläser #0, 22×22 mm) which had been boiled in ethanol for 2 hours before assembly, to give a slightly hydrophobic surface (water contact angle 40 degrees). The fluid cell and cantilever were then mounted in the AFM, and the AFM cantilever was used to pick up a PFO drop of 70-100 μm diameter. A concentrated acid red 88 solution was then added to the fluid cell, to give a final dye concentration of 0.05 mg/mL. The drop on the cantilever was then brought over another drop, or an area of clear surface, in order to perform an interaction measurement. The confocal microscope was used in 'live' mode to axially align the drops in case of drop-drop interaction measurements.

Confocal microscopy

The AFM was mounted on a Nikon Eclipse Ti-E inverted microscope. This instrument was equipped with a Nikon A1 confocal imaging system. The objective used was a 40× Nikon water-immersion lens. The dye used in these experiments (Acid Red 88) was excited by a solid-state laser (488 nm, 20 mW), and the emission was recorded between 570-650 nm. Confocal images were captured at 512×512 pixels, with a vertical slice height between 0.2 and 0.5 μm.

Frame and line averaging were not used, in order to minimize acquisition times. Image intensity was adjusted by the camera pinhole and detector sensitivity in the Nikon Elements control software. No further manipulation of images was performed.

Images were analyzed to extract the droplet interface profiles using the software platform Igor Pro 6.04. TIFF files of each image slice were exported in grayscale from the Nikon Elements Viewer software and imported into Igor Pro. A built in line-profile tool was used to measure the intensity across the slice at the centre of the circle representing the droplet perimeter. The point of maximum intensity was assumed to represent the location of the interface. This was performed in 2 orthogonal directions (X and Y) to give 4 points in space on the droplet perimeter. These points were compiled using the known Z-height in space of each slice to provide profiles of the drop perimeter. A circle was then fitted through the points and the sum of the mean-square errors between the confocal profile point and circle fit were minimized. The error in the fitted value was taken as the distance that the separation could be increased or decreased that caused the summed mean-square error to double. In each case of droplet fitting, only slices where the profile was not distorted by the lensing effect of the interface were used. For the case of the droplet interacting with the flat surface, this represented all slices for the bottom hemisphere. For the droplet-droplet interaction case, the regions fitted are seen as experimental points in Fig. 3 of the main paper. 3D images were compiled directly from slices using the Nikon Elements Viewer software.

Surface force parameters

The zeta potential for perfluorooctane droplets in the Acid Red solution were determined using a Malvern Zeta-Sizer 2000. The conversion of electrophoretic mobilities to zeta potentials was based on the Smoluchowski formula.[4] Reproducibility was checked by comparing results at different emulsion concentrations of 0.1 %(w/w) and 0.06 (w/w), and the value was found to be -80 ± 5 mV. The perfluorooctane/water interfacial tension in acid red 88 solution was measured using a Dataphysics OCA tensiometer, and found to be 52 ± 2 mN/m.

Confocal/AFM measurements between rigid surfaces

In order to demonstrate the effectiveness of confocal microscopy for measuring absolute separations in AFM experiments, measurements were made of rigid systems. In this case, the rigid surfaces used were the AFM cantilever, and the glass surface of the fluid cell. The cantilever was positioned a few microns above the bottom of the fluid cell, and a confocal image taken

of the surface and cantilever. The AFM was used to bring the cantilever down to contact the surface directly after the confocal image. By comparing the point of cantilever-surface contact with the distance travelled by the cantilever (as measured by the displacement sensor) the initial separation could be obtained. In the case of hard contact, this is an absolute rather than modelled, value. The confocal image was also analyxed to obtain the absolute separation as for the drop case (Fig. S1). The agreement between the two methods was within 50 nm.

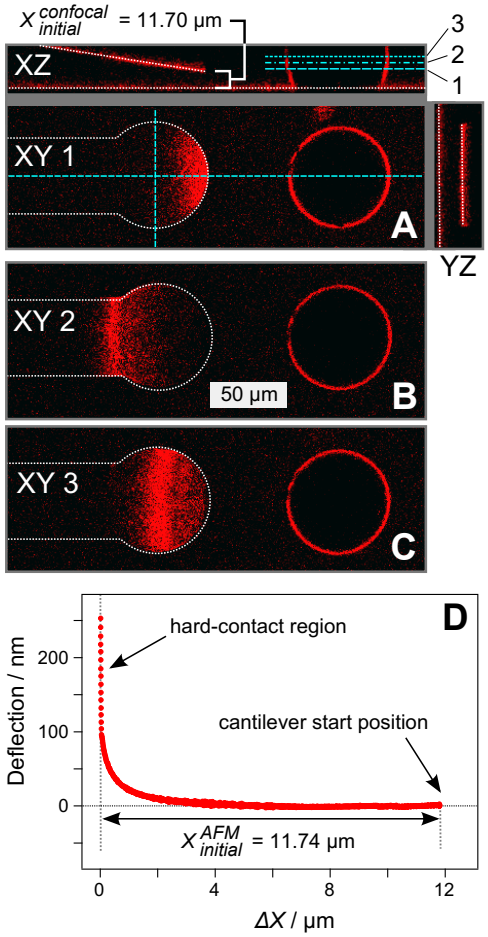

Figure S1: A - confocal 'slices' showing the AFM cantilever positioned near the solid surface. The dashed lines show the axial positions at which the slices are taken. The other feature on the surface is an immobilized drop; B and C - XY slices at 5 and 10 μm higher than the XY slice in A, showing the shape of the cantilever; D - force interaction between the cantilever and surface, measured with the AFM.

Agreement between separations from confocal data and theoretical modeling of AFM data

To ensure that measurements were reproducible and not subject to any systematic variations, a range of measurements were performed of drop-drop and drop-surface interactions. For each, the absolute separation from confocal imaging was compared with that obtained by theoretical modeling. These results are shown in Fig. S2.

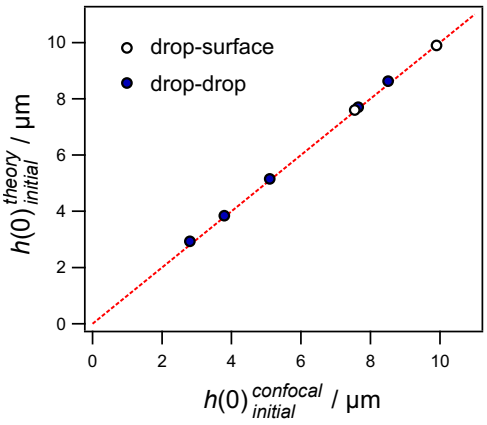

Figure S2: Correlation between absolute separations obtained from confocal imaging and from theoretical modeling. Circles are experimental data, and the dashed line represents $y = x$. The size of data point symbol is chosen such that it also serves as an error bar.

References

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