Electronic Supplementary Information for

"Assembling cellular networks of colloids via emulsions of partially miscible liquids: A compositional route"

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1 Experimental methods

Materials

Ethylene glycol (anhydrous, 98.5%), Tetraethyl orthosilicate (TEOS, reagent grade 98%), and hexamethyldisilazane (HMDS, reagent grade, ≥99%), Nile Red (technical grade), propanal (propionaldehyde ≥97%, FG, Kosher) were used as received from Sigma Aldrich. Nitromethane (99+%, stored under nitrogen) was used as received from Acros Organics. Ammonium hydroxide solution (35%) of reagent grade was obtained from Fisher and ethanol (absolute) from VWR. For the particles Fluorescein Isothiocyanate (FITC)-labeled silica spheres were used, which were synthesized following a modification of the Stöber method [1, 2].

Particle modification

FITC labeled particles were coated with an additional layer of silica, since the bare particles were too easily wettable by the ethylene glycol-rich phase. Dried FITC-labeled silica particles (4 g) were dispersed in ethanol (180 g) using sonication. Next, 14.8 g ammonium hydroxide solution (35%) was added, followed by 3 additions (dropwise and under stirring, 30 minute intervals) of 0.6 mL TEOS/ethanol mixture (1:1 v/v). After leaving this under stirring overnight, the particles were washed several times with ethanol, and stored in ethanol as a stock dispersion. From images obtained using transmission electron microscopy (Philips/FEI CM120) the average particle radius was determined to be 346 nm with a polydispersity of 12% (Figure S1a).

Hydrophilic particles: Particles from the stock dispersion were dried at ∼100 °C (Binder VD23). First for 30 minutes without vacuum, after which the solid layer of particles was broken up with a spatula, followed by another 60 minutes under vacuum.

Hydrophobic silica particles: The stock dispersion was diluted with ethanol/ammonia to obtain a final mixture containing 1.7 wt% particles, and 8.3 wt% ammonium hydroxide solution (35%). Finally, HMDS was added to a final concentration of 9 wt%. Particles were kept in this mixture under stirring overnight. Subsequently, after several washes with ethanol, they were dried at ∼100 °C (Binder VD23). First for 30 minutes without vacuum, after which the solid layer of particles was broken up with a spatula, followed by another 60 minutes under vacuum. The HMDS modification only happens on a molecular scale and does not affect the particle size (Figure S1b).

Contact angle measurements

To characterize the change in the particle wettability before and after HMDS modification, contact angles were measured on similarly modified glass cover slides (18×18 mm, Menzel Gläser). The glass slides were first left immersed in an aqueous 1.5 M NaOH solution overnight to generate a clean surface with exposed silanol groups. Afterwards, they were rinsed thoroughly with deionized water. Like the FITC-labeled silica particles, these slides were coated with silica by immersing them in an 7.6 wt% ammonium hydroxide solution (35%) in ethanol and adding a TEOS/ethanol mixture (1:1 v/v) to a total concentration of 1 wt%. Some of the slides were then modified with HMDS by leaving them immersed overnight in a stirred suspension of the hydrophilic particles in an ethanol/ammonia/HMDS mixture with the same composition as described above for the preparation of the hydrophobic particles. Afterwards, the slides were rinsed thoroughly with ethanol and dried, like the particles, at ∼100 °C (Binder VD23) for 30 minutes without vacuum and then another 60 minutes under vacuum.

Contact angles of the slides were measured with a Krüss EasyDrop instrument (model FM40Mk2) in combination with an environmental chamber (Thermostat TC3010) which was connected to

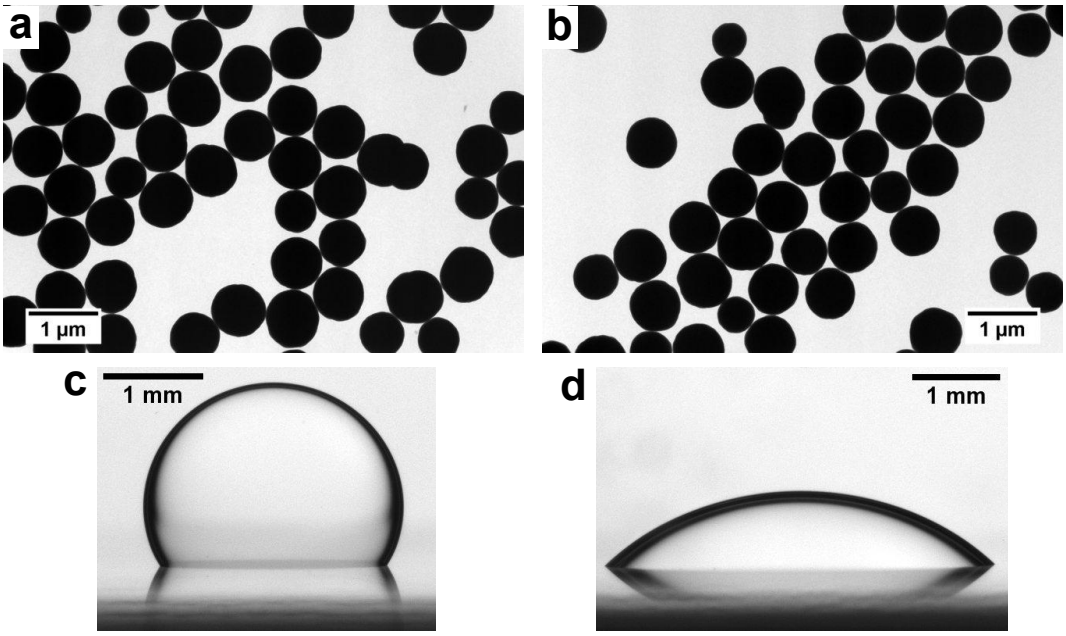

Figure S1: TEM images of FITC-labeled silica particles after coating them with an additional layer of unlabeled silica, (a) before and (b) after modification with HMDS. Below these are example images of a nitromethane-rich droplet deposited onto a (c) silica coated slide and (d) a HMDS-modified silica coated slide, which were used to determine the contact angle as described in the main text.

a thermostatted circulator (Julabo F12). The sample temperature was kept at 23.5±0.1°C. Nitromethane/ethylene glycol mixtures (65/35 w/w) were prepared, shaken and left to phase separate. The slides were immersed in the ethylene glycol-rich phase (the least dense phase). A pendant droplet of nitromethane-rich phase (∼8 μL) was then created from a glass syringe with a computer-controlled dosing unit (2 × 4 μL at a dosing rate of 30 μL/min). The substrate was then gently raised to press it against the droplet, allowing the droplet to attach to the substrate. By taking images like those in Figures S1 c & d and using the "Tangent method 2" in the software supplied by the manufacturer, the contact angle could be extracted. For both types of modified glass slide, 8 droplets were measured (4 droplets on 2 different slides). The contact angle of each droplets was followed over 10 minutes, taking a measurement when the droplet had just attached to the substrate and then approximately 2, 5 and 10 minutes after initial attachment. The measured contact angles were then averaged to give the values presented in Table S1.

Table S1: Results of contact angle measurements.

Slide	Contact angle (°)
Silica coating	118.9±1.8
HMDS modification	42.7±2.1

Sample preparation and imaging

Appropriate amounts of particles and nitromethane were measured out using a balance, followed by sonication to disperse the particles (∼15 minutes in a VWR sonic bath). In most cases Nile Red

was added to the sample by dissolving it in nitromethane first. Ethylene glycol was then added to obtain the desired sample composition (mostly giving a 35/65 ethylene glycol/nitromethane mass ratio), and subsequently the vial was vortex mixed (30 seconds) for emulsification. With a pipette a fraction of the emulsion was deposited directly onto a coverslide (Menzel-Gläser, 22×50 mm), after which evaporation could proceed and, depending on the sample, lead to network formation. Samples were observed with a Zeiss LSM confocal microscope using 40× and 63× oil-immersion objective, as well as a 20× objective. FITC dye in the silica particles was excited with a 488 nm laser line, and Nile Red in the liquids was excited with a 555 nm laser line. Emitted fluorescence from the 2 dyes present in a sample was sent to separate photomultipliers, split at 530 nm using a variable dichroic mirror. The light going to the PMT receiving $\lambda > 530$ nm (from Nile Red) was passed through a 560 nm long-pass filter.

For the larger scale network (Figure 3a) an emulsion of ∼0.75 mL (4 vol.% silica, 35/65 w/w ethylene glycol/nitromethane) was prepared as described above and transferred into a 1 cm path-length cuvette (Starna Scientific). This was left overnight in a fume cupboard for evaporation to proceed, leading to the formation of a network.

2 Additional experimental results

Movie

The supplementary movie shows the complete time series of images on which Figure 2 is based. It plays at ∼180× real time, with the entire video showing the structural and compositional evolution occurring over ∼25 minutes. The left hand image shows fluorescence from the particles, while the right hand image shows fluorescence from Nile Red.

Figures

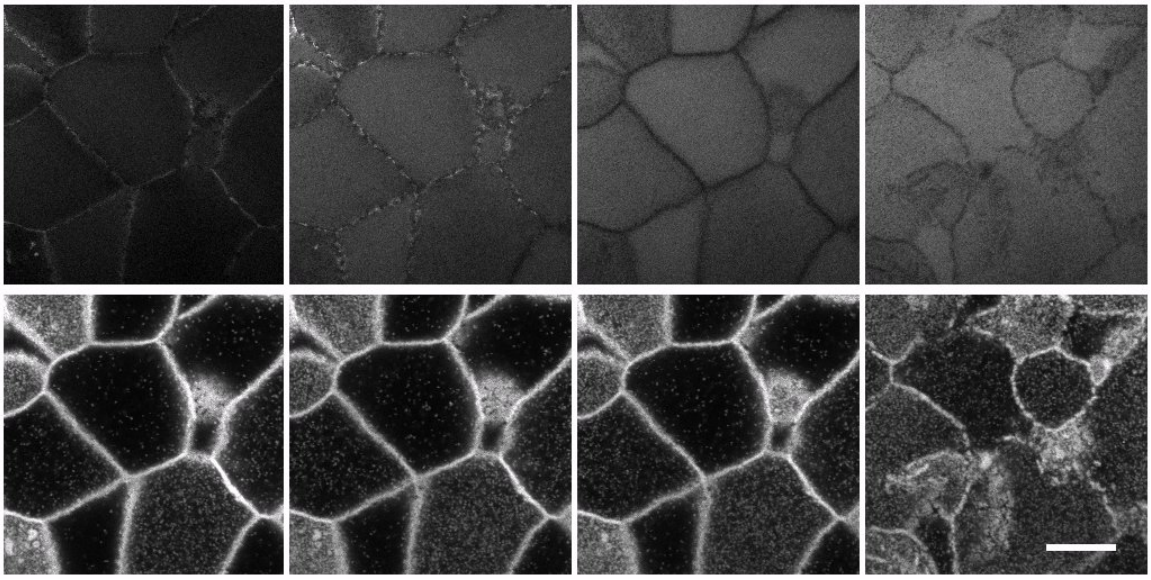

Figure S2: **More-detailed images from the time sequence showing structural collapse for the hydrophilic particles.** The images in Figure 1c were taken from the same image sequence. This Figure presents pairs of corresponding images of (top) Nile Red fluorescence from the liquids and (bottom) FITC fluorescence from the particles. From left to right the pairs of images were taken (far left images) just after structural arrest and, respectively, 4, 4.5 and 9.5 minutes after the far left pair of images. From these images, which are at single particle resolution, it appears that particle desorption from the interface is not significant with respect to the fate of the cellular structure. The scale bar is 25 μm.

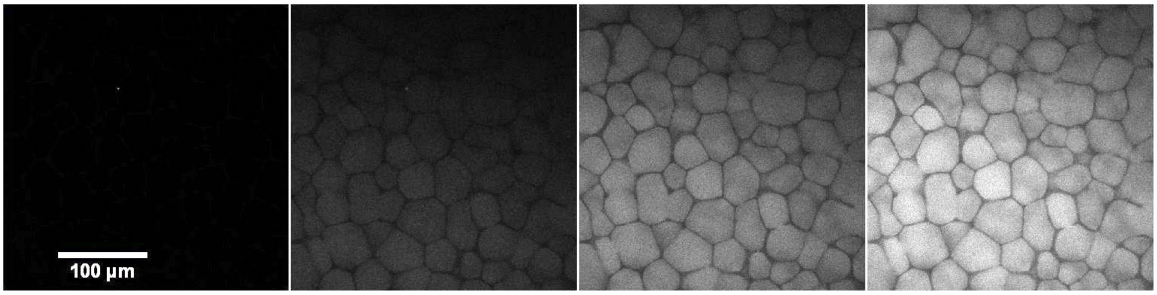

Figure S3: **Diffusion of ethanol/Nile Red into a cellular network of silica particles.** Time series of CLSM images (∼20 minutes pass, with time progressing from left to right). The network was formed in the absence of Nile Red (2 vol.% silica particles, 35/65 ethylene glycol/nitromethane mass ratio) and brought into direct contact with a Nile Red in ethanol solution.

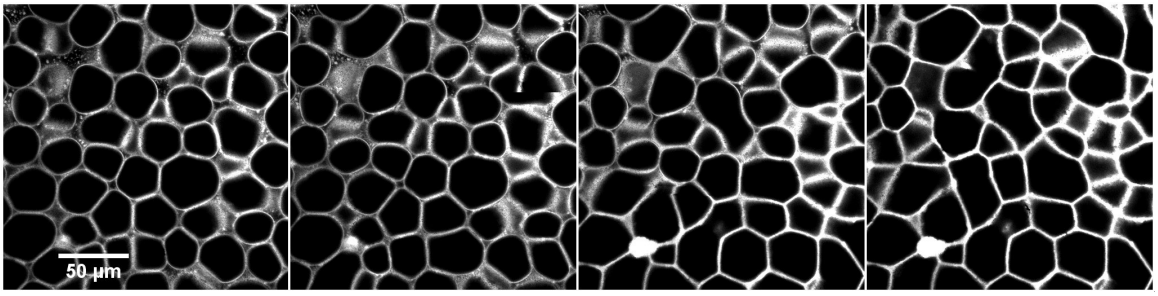

Figure S4: **Network formation on a slightly larger scale.** A time series (30 s intervals between images) showing the final moments leading up to the formation of the final structure shown in the far right image. Structure formation was imaged fairly close to the cover slide, and shows the effect of coalescence on final cell size. Sample composition: 4 vol.% silica, 35/65 w/w ethylene glycol/nitromethane.

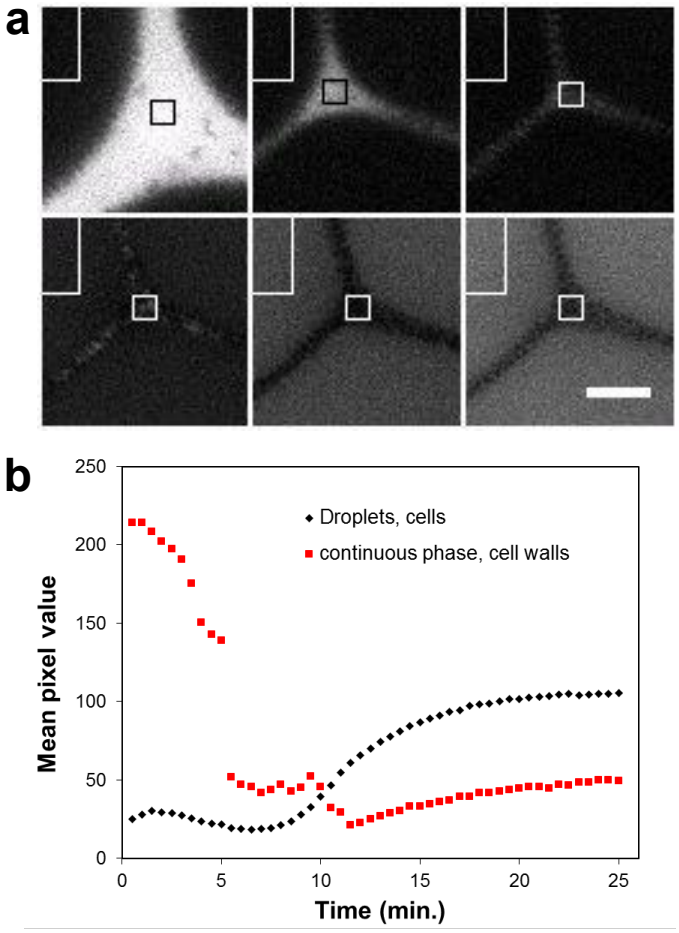

Figure S5: **Details on how the relative intensities presented in Figure 2c were determined.** (a) Example images (the scale bar is 5 μm) from the time sequence of images from which mean pixel values were determined using plugin "Measure Stack" in combination with ImageJ[3]. This was done using small areas for both a section of the continuous phase/cell wall and a section of a droplet/cell (as outlined in the different frames) at all different times. (b) The absolute intensities obtained in this way, which each were divided by their counterparts from the same image to obtain the relative intensities plotted in Figure 2c. The decrease in relative fluorescence intensity from the cells after point 4 has to do with changes in the (local) liquid composition in the respective parts of the sample. It is effectively caused by an increase of the fluorescence intensity inside cell walls. This could well be due to a change in refractive index caused by further mixing after the local mixing causes the interface to disappear. If the refractive index becomes more similar to the particles refractive index, the particle structure will scatter less fluorescence resulting in an increasing detected fluorescence signal from the cell walls. The fluorescence signal from the cells is also still increasing slightly at this stage, which is either due to Nile Red or nitromethane diffusing into the cells, thus indicating that the liquid composition is not yet completely homogeneous.

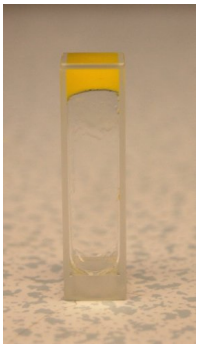

Figure S6: **A sample like that in Figure 3a, after network formation, positioned upside down.** The sample height is ∼4 mm with sides of 10 mm, then, from the initial sample composition, the weight of the sample can be estimated to be ∼0.5 g. This would give an order of magnitude of the yield stress $\frac{Mg}{A} \approx \frac{0.5 \cdot 10^{-3} \cdot 9.81}{(10^{-2})^2} \approx 50$ Pa.

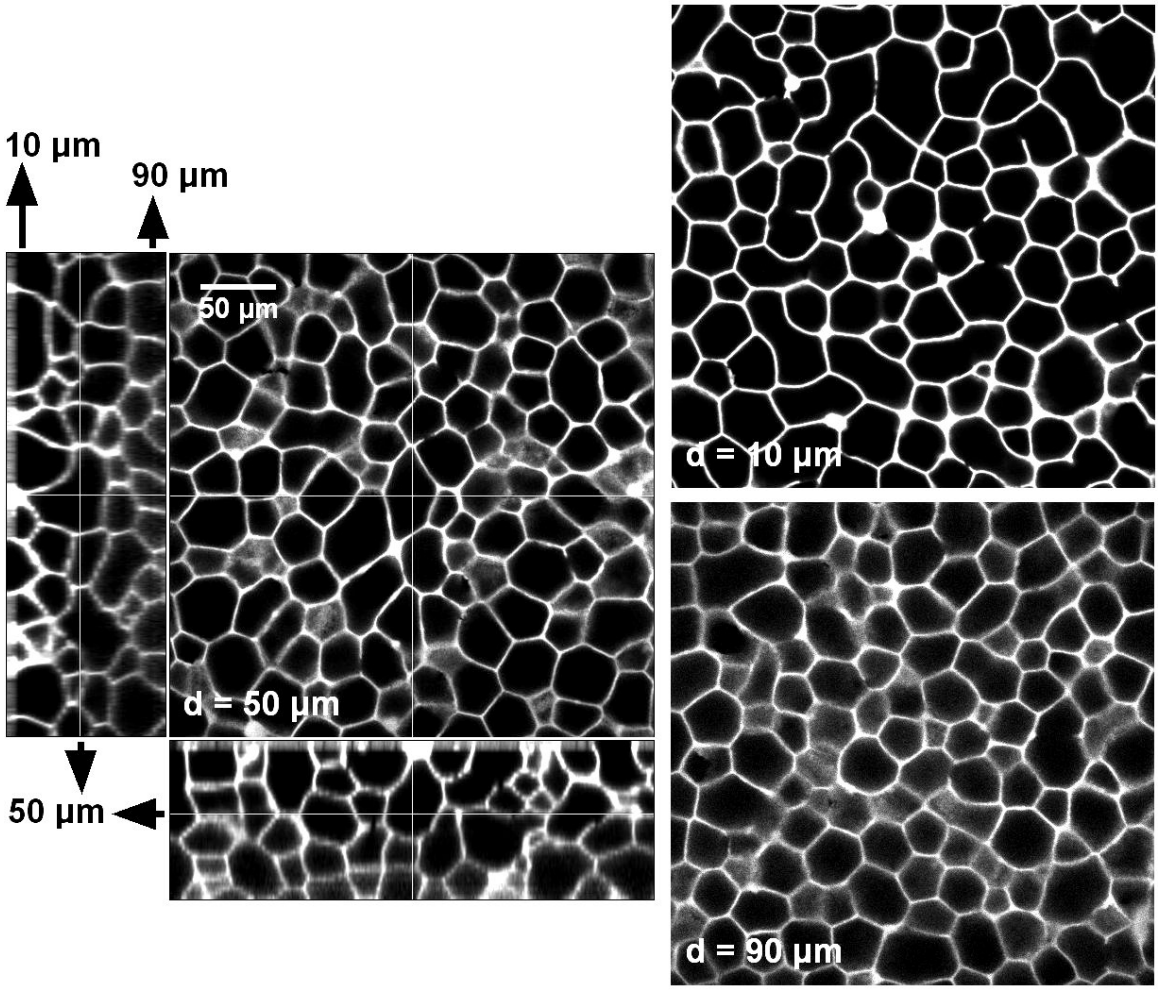

Figure S7: **Images from a CLSM z-stack**. For each image, the depth into the sample at which it was recorded is indicated. This structure formed from an emulsion with 4 vol.% silica particles. The side projections were generated using ImageJ.

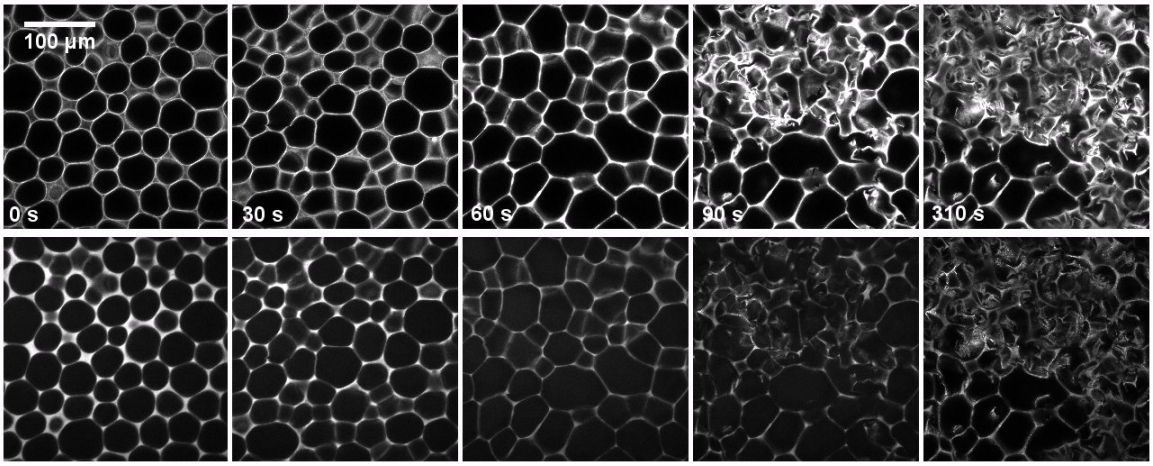

Figure S8: **Time sequence of images taken during evaporation of the propanal-rich continuous phase in a water/propanal emulsion.** The liquid composition is 30 wt% water and 70 wt% propanal and the water-rich droplets are stabilized by the hydrophilic particles (2 vol.%). CLSM images of colloids are displayed in the top row, and corresponding images of the propanal-rich phase visualized through Nile Red are shown in the bottom row. The network collapses quickly after structural arrest. Since Nile Red does not dissolve in water, its diffusion throughout the sample at a later stage, is not observed in this case. The mixing of the liquids is observed as a slowly decreasing Nile Red fluorescence intensity within the network, with bright pixels suddenly turning dark. This is still proceeding during and after collapse, and happens much more slowly than liquid mixing in the nitromethane/ethylene glycol mixture. This might suggest that residual propanal-rich phase is sticking particles together, explaining why many structural features stay intact. Hydrophilic particles used here are normally well-charged in water, so it would be expected that they disperse as the propanal-rich phase between the particles disappears.

References

[1] W. Stöber, A. Fink, and E. Bohn. Controlled growth of monodisperse silica spheres in the micron size range. *Journal of Colloid and Interface Science*, 26:62–69, 1968.

[2] A. van Blaaderen and A. Vrij. Synthesis and characterization of colloidal dispersions of fluorescent, monodisperse silica spheres. *Langmuir*, 81(2):2921–2931, 1992.

[3] W. Rasband. Imagej 1.46r. *http://imagej.nih.gov/ij/*.