Supporting material.

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A New Instrument for Time Resolved Static and Dynamic Light Scattering Experiments in Turbid Media

Christian Moitzi, Ronny Vavrin, Suresh Kumar Bhat, Anna Stradner, and Peter Schurtenberger

1. Alignment of the multiangle 3D cross-correlation instrument.

The multiangle 3D cross-correlation instrument is a high precision optical instrument. It requires precise alignment of the mechanical and optical components. In particular it has be assured that both laser beams are focused into the same scattering volume and that the pair of detectors which is placed at a particular angle detects light scattered in this volume at exactly the same *q*-vector. In this case it is not sufficient to detect at the same angle, i.e. the same norm of the *q*-vector. Also the orientations of the two detectors have to coincide. It is only under these strict conditions that a correlated signal will be detected in the cross correlation function. For the multiangle instrument this means that two illuminating laser beams and 8 detector "beams" must intersect in a volume smaller than 100µm in well defined orientations. Every small deviation leads to a reduction in the degree of correlation that results in a reduction of the intercept. Once the deviations are too large either in orientation or overlap volume, no correlation between the two signals is detected any more. Due to the large number of degrees of freedom of the alignment (tilt and translation of fibers, lasers, and lenses) it is practically impossible to find a correlated signal by unsystematically turning one screw after the other. Therefore a robust alignment procedure that results in at least some correlation has

to be applied. Once there is a non-zero intercept in the correlation function the alignment can then be optimized by optimizing one degree of freedom after the other. The alignment procedure can be divided into three main parts: the mechanical alignment, the optical alignment, and the optimization of the initial alignment.

Mechanical alignment.

The purpose of the mechanical alignment is to match the rotational axis of the sample cell and the index matching vat with the rotational axis of the goniometer, which will later carry the laser. For the alignment of the index matching vat its position is checked by a micrometer gauge which is mounted onto the goniometer arm and touches the surface of the vat. The tilt of the vat can be adjusted by two micrometer screws. The micrometer gauge is mounted such that it touches the upper surface of the vat. A sufficient alignment is reached if the value shown by the gauche is not varying more than 50μm during one full rotation of the goniometer arm. Afterwards the index matching vat is translated horizontally in two directions by micrometer screws. The micrometer gauge is now mounted such that it touches the lateral surface of the vat. Again the value shown by the gauche should not vary more than 50μm during one rotation of the goniometer. The sample cell is aligned in complete analogy to the index matching vat.

Optical alignment.

As a first step the index matching vat is filled with decalin. Then the optical fiber that guides the illuminating laser beam to the instrument is mounted onto the goniometer arm. One has to make sure that the incident laser beam is horizontal and intersects the rotational axis of the goniometer arm. To check that two pinholes are placed on the optical table in a way that the laser beam passes through both of them. Only if the beam passes both pinholes again after a rotation of 180° one can be sure that the beam is horizontal and intersects the rotational axis.

As a next step the beam splitter is mounted. It splits the primary laser beam into 3 parallel beams, two intense ones and a central beam with only about 5% of the intensity. All three beams are parallel. The positions of the intense beams are 15mm above and 15mm below the central beam. One has to make sure that all beams are horizontal and intersect the rotational axis of the goniometer.

For the alignment of the lenses a small pinhole is placed exactly on the rotational axis of the goniometer in the index matching vat in a way that the central laser beam passes through it. The lenses $L_0 - L_4$ have to be adjusted to focus all three laser beams through that pinhole at the same time. To make a proper alignment of the detection lenses $L_1 - L_4$ possible they are mounted upside down, so that the goniometer arm can be moved below the detection lenses. Of course this is not possible any more as soon as lens L_0 is mounted. Therefore the detection lenses $L_1 - L_4$ are aligned first. Afterwards L_0 is mounted onto the goniometer arm and aligned as well. Doing so, all five lenses have their focal points at the same point on the rotational axis of the goniometer.

The next step is the alignment of the detection fibers. All of them have to point towards the same scattering volume. For the alignment the laser is coupled into the fibers from the back side, where they are normally connected to the photomultipliers. The detection lens is covered by a mask having two holes, 15mm above and 15mm below the center. Then the fibers are tilted and moved until the laser passes one of the holes in the mask and the pinhole at the sample position. The laser coupled into the other fiber at the same detector has to pass through the other hole in the mask but through the same pinhole at the sample position. After repeating the alignment procedure at all four angles, both lasers and all 8 fibers are aligned in a symmetrical way pointing towards the same spot in the center of the sample cell.

Optimization of the initial alignment.

After the initial alignment is finished the detection fibers are connected to the photomultipliers, the central beam is blocked, and a singly scattering sample is placed in the instrument instead of the pinhole. After measuring a couple of seconds one should get a non-zero intercept of the cross-correlation function at all four angles. If this is not the case the initial alignment is not good enough to give a correlated signal, and the procedure has to be started from the beginning. To improve the alignment the tilt and the position of the lenses and of the optical fibers can be varied. In practice, each degree of freedom is optimized separately by finding the maximum of the cross-correlation function of a singly scattering sample. The theoretical maximum of the 3D cross-correlation technique is an intercept of 0.25. With our present instrument values between 0.16 and 0.21 are reached at intermediate angles. At very small (<25°) and very large (>130°) angles the intercept values decay significantly.

2. Choice of the angular regime for the Guinier extrapolation.

In general there are problems using the Guinier extrapolation if the sample is very polydisperse. In our case the particle size distribution is very broad, ranging from 30nm to 300nm in radius. The maximum of the size distribution is located at about 100nm, but the distribution exhibits a weak tail to large radii (e.g. C. G. de Kruif, Journal of Dairy Science 1998, 81, 3019-3028). Most of the micellar mass is distributed in the main peak of the size distribution around 100 nm and dominates accordingly the relevant viscoelastic properties of the system as it undergoes the sol-gel transition that is at the focus of our investigation. However, the trace amounts of large aggregates also present contribute measurably to the scattering at very low values of q. As we are interested in the main component we have opted for an instrument configuration where we limit the q-range to values that are optimized for the

main peak of the distribution, but are outside of the Guinier regime (given by $qR_G<1$) for the large particles.

In addition one has to consider that the casein micelles are not homogeneous but possess a core-shell structure with a relatively dense core and a shell of relatively low scattering contrast. This results in a smaller radius of gyration then for homogeneous spheres with the same size and consequently leads to a larger Guinier region. Therefore the Guinier plot of casein micelles of 200nm in radius appears to be almost perfectly straight in the used *q*-regime.

The static scattering curve, which is shown in Figure S1, shows the problem of the Guinier extrapolation. There is no linear Guinier regime in the Guinier plot (Fig. S2) even if very small angles are measured. At small angles the contribution of the large particles to the overall signal becomes large. If, for instance, the angular regime between 10° and 20° is used, an R_G of about 200nm is obtained, which corresponds to a physical size of almost 300nm in radius. Centrifugation of the sample (removal of the largest particles) reduces the upturn at small angles and has only little influence on the slope at higher angles. As a conclusion it can be stated that the results of the Guinier extrapolation of very polydisperse samples like casein micelles depend on the chosen *q*-range, which can thus be used to discriminate between the very large particles and the main peak and highlight structural changes in the region of interest in the size distribution. We were choosing a *q*-regime for our extrapolation which includes the majority of the particles within the Guinier regime and where we are not sensitive to a small number of large particles only. We emphasize that a different choice of this regime would cause a shift in the absolute values of R_G. The trends, however, are still preserved. It is important to note that our choice of the *q*-range for the Guinier extrapolation is only suited to look at intra-micellar rearrangements, where the size is rather decreasing than increasing during the acidification. Any investigation that aims at characterizing the evolution of the

cluster size distribution due to casein micelle aggregation would have to rely on much lower *q*-values.

3. Structure factor effect in undiluted milk.

As mentioned in the main article we are able to measure the static intensities even in undiluted milk. However, a quantitative determination of R_G and M_W by a Guinier extrapolation is not possible due to the significant influence of repulsive interactions at the highest concentrations. This is actually not surprising, as the swelling ratio of casein micelles is approx. 3. Therefore the volume fraction of the casein micelle in undiluted milk is about 0.1. For such a concentration a significant decrease of the forward scattering due to the contribution from the static structure factor is expected. For pure hard sphere interactions *S(0)* is about 0.46 at a volume fraction of 0.1. The Figures S3 and S4 show the experimentally determined concentration dependence of the forward scattering intensity *I(0)* and the apparent radius of gyration R_G. Obviously the quantitative determination of R_G and *I(0)* by a Guinier extrapolation is only possible for weakly diluted samples.