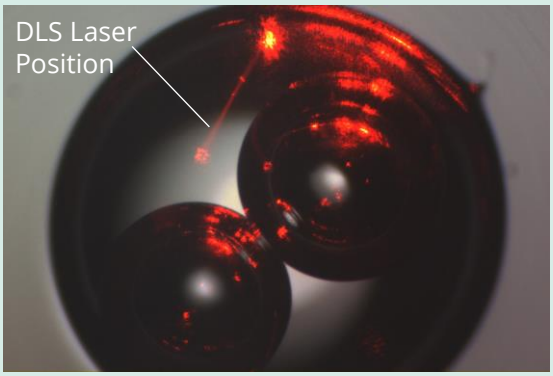
Robustness of *in situ* DLS

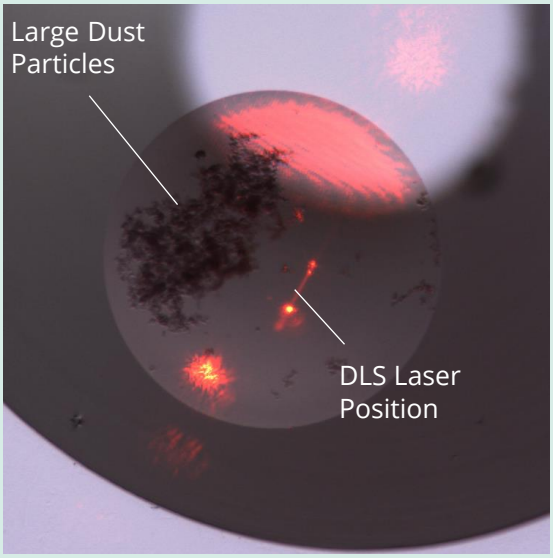
DLS is commonly known to be hindered by various factors e.g. sample heterogeneity, colloidal impurities, presence of solid phases (e.g. precipitated protein) and air bubbles. This chapter illustrates how robust *in situ* DLS is in terms of usually highly problematic solid impurities like fibres or air bubbles and inhomogeneous samples often occurring even under laboratory conditions.

A key feature for the robustness is a combination of automated X, Y and Z-micro meter precise positioning in combination with a drop recognition algorithm. This allows placing the detection point at a position that avoids such solid disturbing factors.

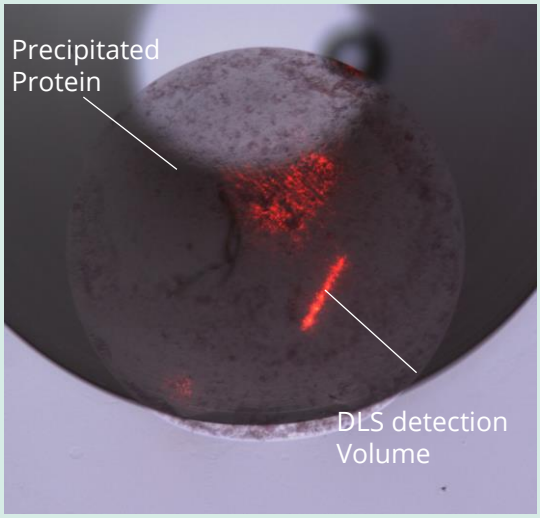
Air bubbles are usually caused by drop dispensing or pipetting. Often they stay in a sample drop for a long time without moving, even when their volume is as dominant as in this case. However, the drop volume still provides enough space for laser positioning and DLS detection.



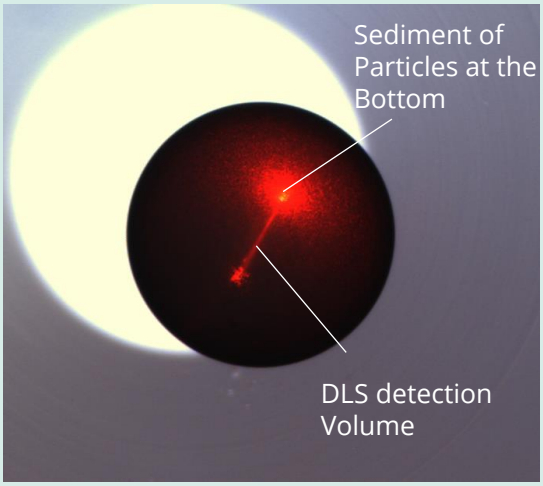
Dust and Dirt are frequently occurring disturbance factors, particularly in older samples. The presence of dust particles or fibres from clothes would be problematic if they are hit by the laser beam. However, this can be avoided by automated or manual laser positioning. The offset enables even long-term stability tests which can be extended over weeks.



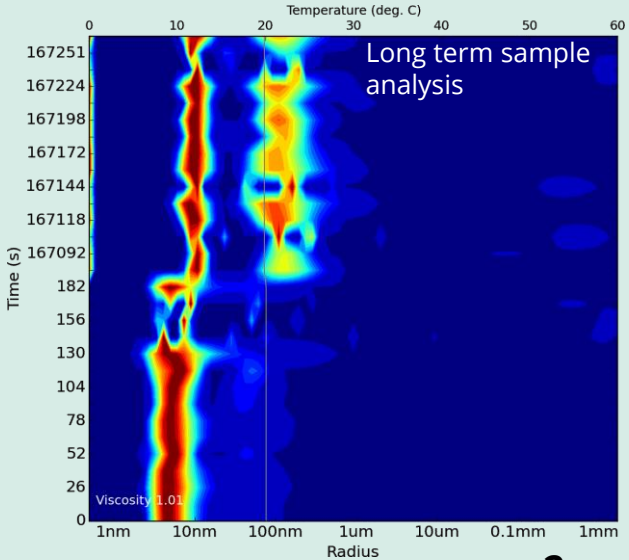
Highly aggregated samples can be analyzed as well, as long as particles show Brownian motion. SpectroLight 600 or *in situ* DLS can determine their sizes even in the µm range. The visible laser beam indicates that particles traverse the laser beam. Thus, the entire sample volume is still filled with moving particles instead of being completely precipitated.



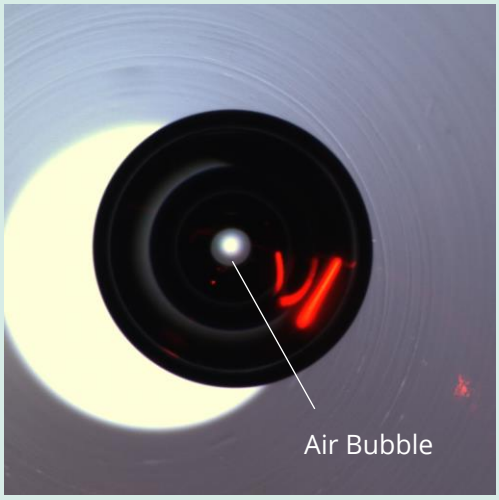
Inhomogeneous samples: Sometimes samples are remarkable different in terms of particle sizes. In this particular example three were two populations detected via DLS. But, these particle populations were detected in the supernatant. Another fraction was located at the bottom of the well as precipitate. They can be recognized by the immense amount of scattered light in this area.



Inhomogeneity was emphasized by size distribution analysis as well. The still soluble amount of the protein formed a bimodal distribution of 10 nm and soluble aggregates of approx. 42 nm respectively.



Laser positioning in order to avoid hitting an air bubble means in some cases to measure DLS in a very confined area nearby the air bubble. Despite the fact that such non-optimal optical conditions require a laser offset, the quality of the measurements were not affected. The circular volume surrounding the air bubble provides sufficient space for the laser to be positioned. In this case, even a long term stability analyis was feasible.



Long term analysis is possible even under such impaired conditions. In this case, the sample shows a significant change in the size. DLS measurements has been measured with an ~48 h break in the middle of the measurement series in order to monitor changes that the proteins undergo.