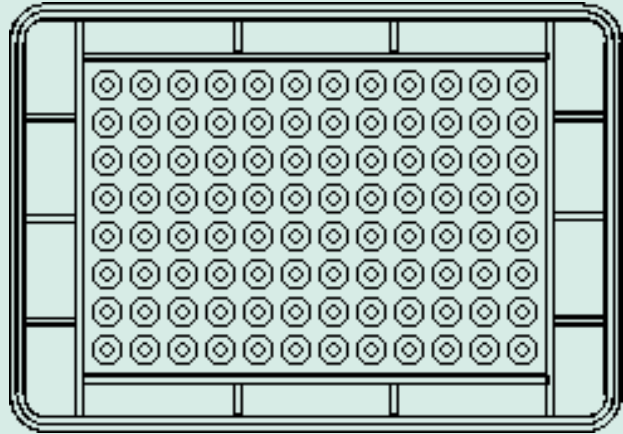


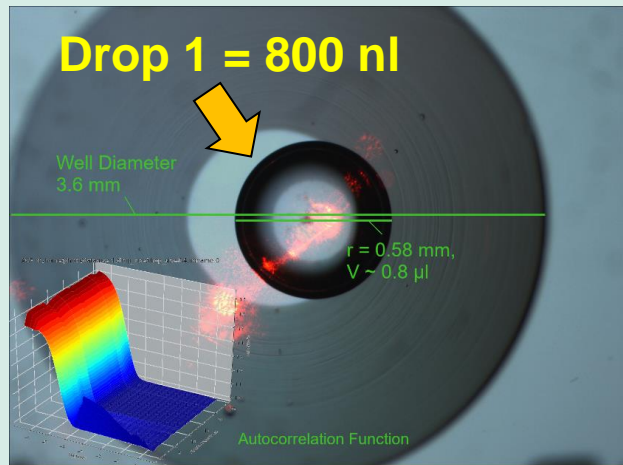
SpectroLight 600's technical realization of *in situ* DLS enables particle size determination in unmatched small sample volumes. Limitations in volume sizes are only given by state of the art drop setting technologies with their minimum dispensing volume constrains. Proven sample volumes and tests on the lower limits of SpectroLight 600 sample volumes for *in situ* DLS are provided in this document.

Used Plate Type:

The most abundant plate type for *in situ* DLS is the "Douglas Instruments Vapor Batch Plate". When applying *in situ* DLS on small volumes, they have to be always covered with paraffin oil, otherwise such small sample volumes will dry out which would bias the results, significantly.

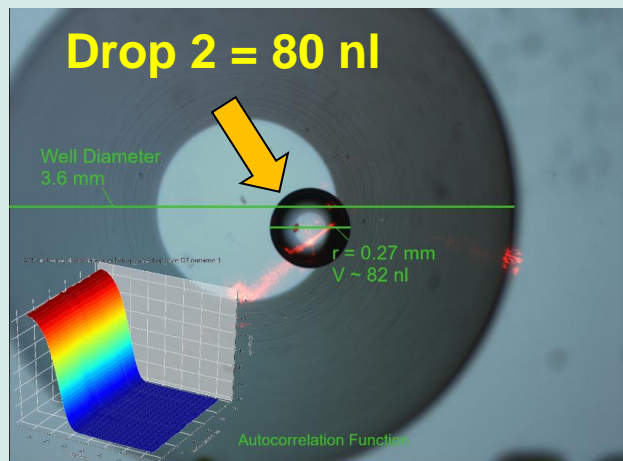


Manual dispensed droplets are usually dispensed in volumes ranging between 0.5 – 1.0 μl . The drop volume is already significantly smaller as the well itself. Hence, the drop resides at a random position. To cope with that, SpectroLight 600 is equipped with a unique droplet finding algorithm allowing to center the droplet automatically.



Drop sizes beyond manual dispensing:

State of the art dispensing systems are capable to dispense droplets as small as ~80 nl. Nevertheless, even such small sample volumes provide more than enough space for *in situ* DLS laser positioning. Thus, a plate loaded with sample droplets as small as this one can be still measured fully automated as well as in a scanning mode in a 96 well format plate. If they are protected from rapid evaporation via a layer of paraffin oil, even long term stability analysis up to several weeks are feasible.



Experimental Sample Volumes:

Some highly advanced dispensing systems might be able for for reliably dispensing of droplets as small as 15 nl. Even this small sample volume can still be automatically centered and subsequently analyzed via *in situ* DLS with a signal to noise ratio as in normally sized droplets.

Sub-nanoliter small Sample Volumes:

In order to determine the smallest sample volume that still can be used in a reasonable daily experimental approach, some artificially created droplets, due to dispensing errors, have been selected. A criteria for usefulness of a sample volume was required time for manual laser positioning. Automated laser positioning to find a good *in situ* DLS-laser position has already failed at this point. Although manual aiming of the laser was still achieved after a few trials. Thus, in an exceptional situation when the amount of sample is rather limited, this small volume would be an option to apply DLS provided a method for dispensing is available.

The Limit of Sample Volumes: Finally a sample volume has been tested that marks the limit of *in situ* DLS at least in the step motor controlled configuration realized in SpectroLight 600. Although, DLS could be still measured, the signal became noisy and positioning of the laser focus required some time. Nevertheless, a volume of this size can be considered to be more tech demo than of any practical use.

