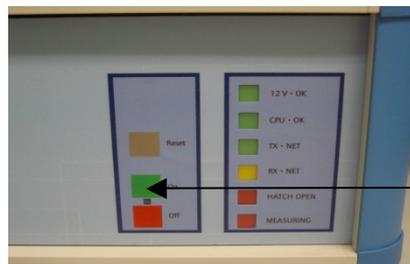


Tutorial for the dynamic light scattering system & Measurements



How to switch on the device:

- The SpectroSize 300 is simple to switch on, just be sure that the device is connected to a computer with the operating system Linux and the software is installed accordingly
- to switch the system on just press the start button

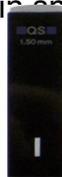


Start button

- after both, the computer - PC or Laptop - and the DLS instrument are switched on, it takes a few minutes until the laser and the cuvette holder has reached its working temperature

How to prepare a sample:

- the sample will be measured in small cuvettes of different types. For example:



- usually the cuvettes are stored in a box or in a 70% ethanol solution to prevent contamination caused by microorganisms. To avoid any denaturing or contamination of the protein, the cuvette should be cleaned just before a DLS measurement

Material required:

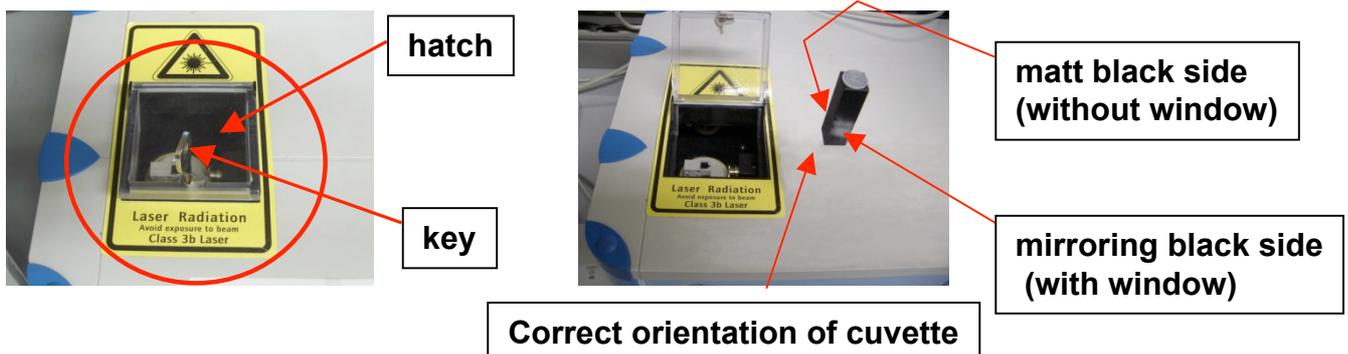
- cuvettes, 3 small beakers (< 80 ml), pipettes (10 μ l, 200 μ l) and its tips paper towels, gloves, DLS-Device, compressed nitrogen or air, Ethanol, dest. Water

Set up:

- fill a beaker with 20 ml of dest. water and a second one with 5 - 10 ml Ethanol. The third one is for the liquid waste

Preparation of measurements:

- take the cuvette carefully out of its storage vessel with e.g. soft tweezers. Avoid contacts with solid surfaces, you may accidentally scratch the surface of the cuvette
- invert the open end to a paper towel until the cuvette is empty
- fill in \sim 100 μ l of Ethanol and press up and down for several times to clean the cuvette inside. Than take out the Ethanol and put it to the waste (beaker). Invert the cuvette on the towel till all ethanol is gone
- fill in \sim 100 μ l of dest. water to rinse the cuvette. Take out the water with the 200 μ l pipette and also waste it. You should repeat this step three times to be sure that Ethanol is entirely removed and will not influence your measurement
- to avoid dilution of your sample it is better to dry the cuvette. Thus blow out the residue of water by pressured nitrogen or air. It is recommended to do it before you begin your measurement
- now the cuvette is ready to be used. For a standard measurement a volume of 20 μ l is sufficient and recommended
- pipette slowly, avoiding any bubbles inside the cuvette. Bubbles will disturb the measurement significantly
- turn the key to open the hatch, for safety it will also switch off the laser diode
- carefully place the cuvette in the basement. Important: the matt black side of the cuvette must point to the left. Close the hatch and lock it. The laser diode will be now switched on. The DLS system and sample are now ready for the measurement



Starting a DLS measurement:

- on the desktop of the PC an icon marked as Spectro is visible. Click it once with the left mouse button to start the DLS measurement program. Usually the first step is a test, whether the sample scatters light at all or not. For the standard autosave procedure and single measurement you can just enter a name at "Sample", "1" at "Number of measurements" and press start. The measurement will now begin. Before, check the default input-values, described on the following pages

The screenshot shows the Spectroscatter software interface. On the left, there are three plots: 'Autocorrelation Function of Protein_1 (pkg. 21)', 'Radius Distribution', and 'Signal Strength: 520.6kHz'. On the right, there are input fields for 'Measurement Duration (s): 30', 'Number of Measurements: 1', and 'Sample: Protein_1'. Below these are 'Threshold' settings (Time: 0.01, Value: 2.0) and a 'Log' window showing a list of events. At the bottom right, there are 'Start' and 'Stop' buttons. At the bottom left, there is a menu bar with options: Measure, Parameter, Distr., Histogram, Radius Plot, File List, Autopilot, Summary, SLS. Red circles and arrows highlight the 'Duration [s]', 'Number of measurements', 'Sample name', 'Start button', and 'Menubar'.

Duration [s]

Number of measurements

Sample name

Start button

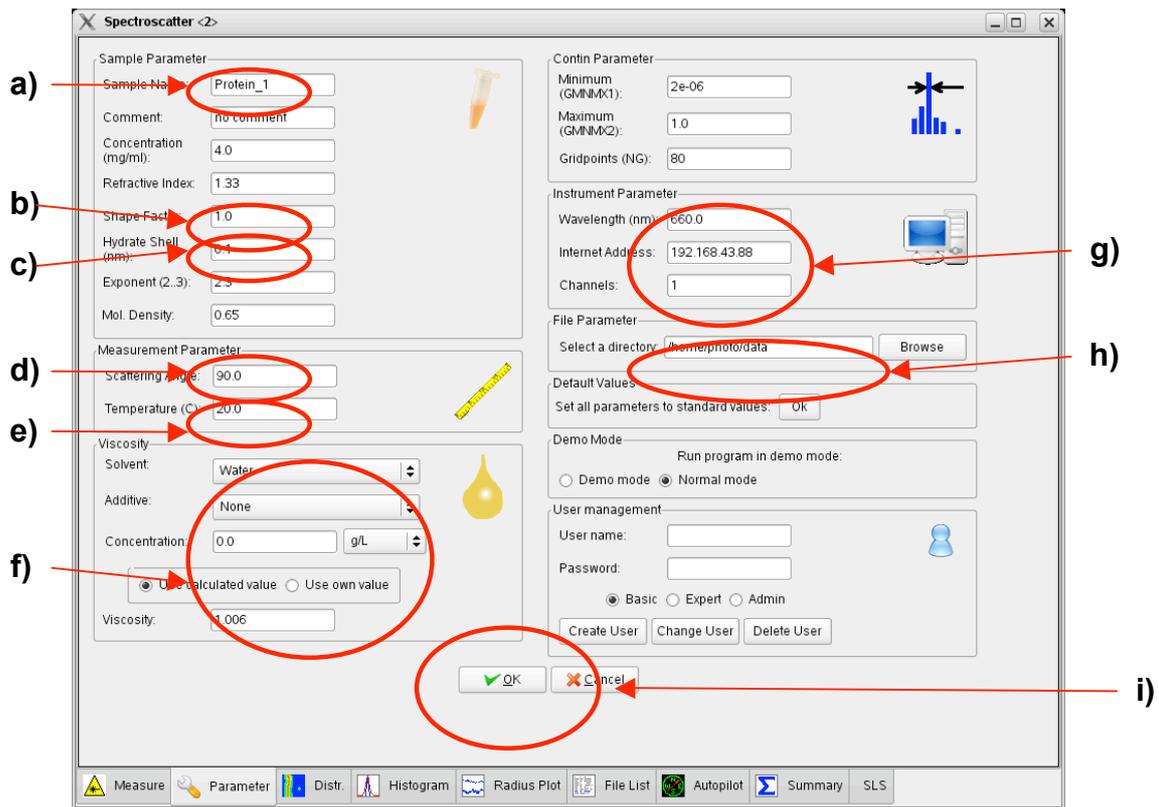
Menubar

Startwindow of the DLS software

- you can also measure more automatically by using the autopilot function. The first step is to prepare a working directory where the program can save the output of the DLS measurements
- a smart strategy to find your data later on is to prepare and create your own folders.
 - Create a subdirectory with the name of your sample inside of a directory with your name. In this subdirectory you can further place a directory with the day if required; this set up should be your working directory. Now press the "ok" button

Parameters

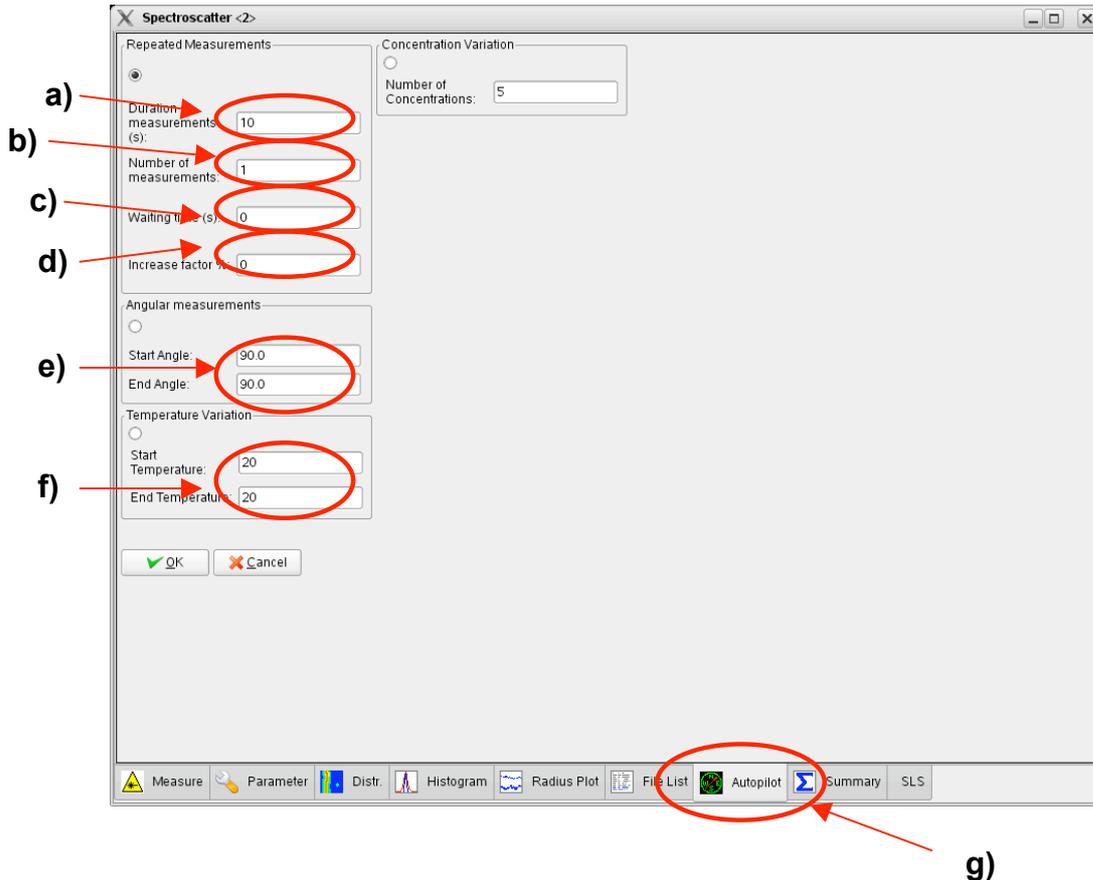
- After preparing your working directory you should check if all parameters for DLS measurements with your sample are correct. Also you have to tell the program in which directory (your working directory) the data acquired during the measurements should be saved. For this press the “Parameter” button in the menubar. The “Parameter” sub-menu appears:



- a) Samplename: enter your sample name
- b) Shapefactor: 1.000
- c) Hydrate: 0.1 nm
- d) Scatteringangle: default = 90°
- e) Temperature: default = 20°C, to measure at lower or higher temperature (10°C – 30°C) just enter the desired temperature
- f) Viscosity: default = 1.006, if your solvent is not water or contains glycerol or alcohols the viscosity is different from 1.006 and you have to enter the real value
- g) Wavelength: 660 nm; IP: 192.168.43.88
- h) Working directory: default = home/photo/pms, select your working directory
- i) Press “OK” to save changes

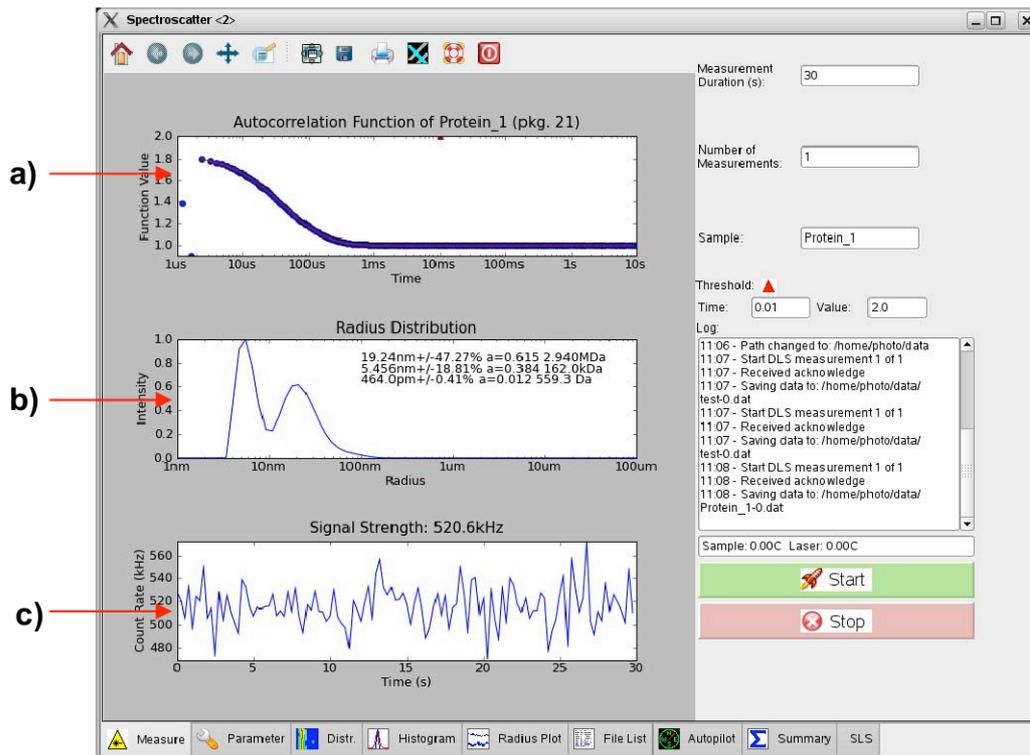
Autopilot

- for measurements with the autopilot function press the AUTOPILOT button in the MENUBAR. The Autopilot sub-menu opens:



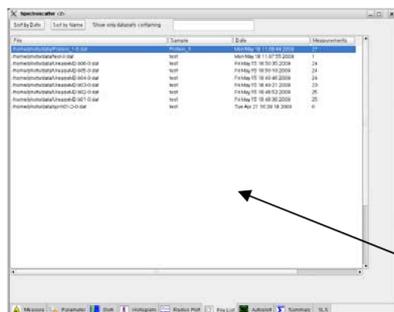
- a) Duration of measurements: usually a value between 10 and 60 seconds
- b) Number of measurements
- c) Waiting time: During long term measurements it is not needed to measure DLS all the time. By setting a waiting time between two measurements you can reduce data during long period measurements.
- d) Increase-factor: If you want to increase or decrease the waiting time within a set of measurements
- e) Angle: If you want to measure at different angles you can change the default settings (90/90)
- f) Temperature: For a temperature gradient use a start temperature different from the end temperature. Default = 20°C/20°C
- g) Autopilot button in menu bar

Measurement



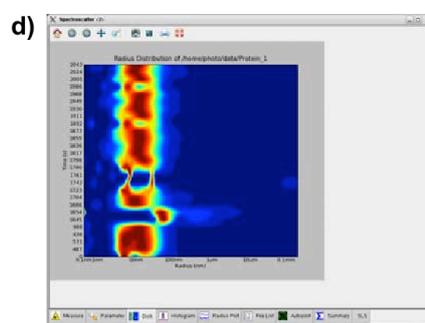
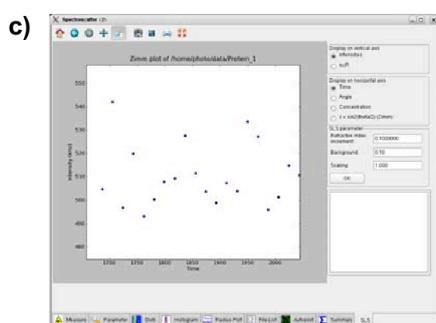
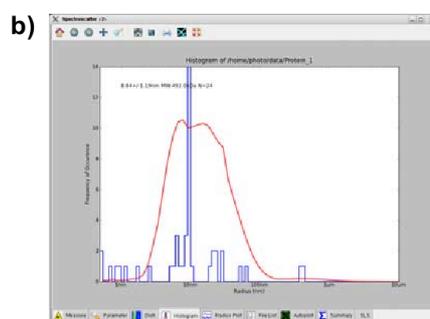
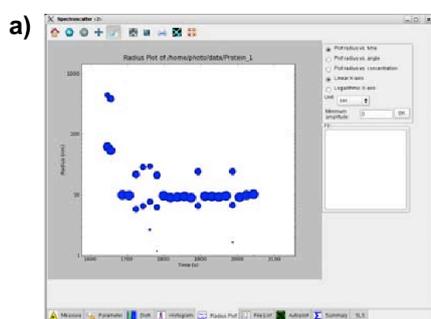
- a) Autocorrelation function (ACF) shows the time dependence of the intensity fluctuations
- b) CONTIN analysis of the ACF gives the radius distribution of the sample. Narrow peaks indicate that only one particle size is present. Broad peaks show that there is a mixture of particles of similar sizes in the sample. To appear as separate peaks, particle size must be at least a factor of about 3 apart. Also shown: Calculated values for molecular weight of the particles.
- c) Shows the count rate (number of photons registered by the detector) in kHz. Usually count rates between 10 and 1000 KHz are desired. The variation of the count rate within one measurement should not be too large to get meaningful results.

Evaluation



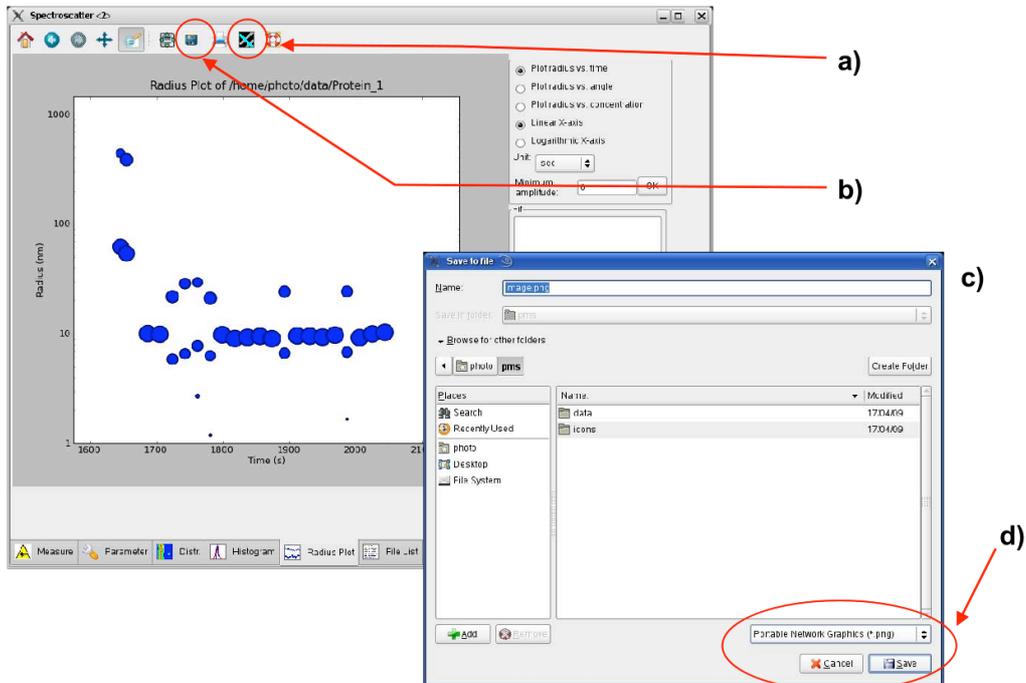
For evaluation of a set of measurements first press „File list“ in the menu bar. Select the dataset you want to evaluate and then press the buttons for the evaluation tools in the menu bar (Radiusplot, Histogram, Distr. or SLS)

File list sub-menu



- a) **Radiusplot:** shows the radius distribution of a set of measurements
- b) **Histogram:** shows a histogram of the radius values found for a set of measurements. The program tries to identify significant peaks and calculates the average value of each peak as well as the standard deviation.
- c) **SLS:** the development of the count rate during a set of measurements is displayed here. In an optional multi-angle set-up this can be used for evaluation of static light-scattering (SLS) data
- d) **Dist:** Shows the radius distribution with time plotted against radius. The color indicates the intensity of each particle as calculated by CONTIN.

Export of evaluated data



- In each graphical evaluation tool sub-menu you can easily export your data as raw data **(a)** for further evaluation with e.g. Excel or Origin and image **(b)** by just pressing the respective button in the upper menu bar. A new window appears **(c)** in which you can select the directory where the data should be saved and insert the name of the new file.
- If you want to export as an image you can select at **(d)** the filetype. Spectro so far supports png, eps, bmp, svg and pdf-format. If high-resolution (600 dpi) is required the best option would be eps. For PowerPoint-presentations the png-format is sufficient.