# TEM-Analysis using *analySIS* and *temanalysis.nb*

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***19-05-2003***

*Please note that TEM-measurements are not very suitable to determine the absolute value of the radius of a colloidal sphere. First of all, if the focuss is not quite correct, it will be very difficult to set the threshold. Secondly, the TEM is only calibrated once every three years, so it might be off the scale. You can use a TEM-grid to get around this problem. Finally, silica particles, for example, are known to shrink (±2%) during TEM-measurements. Static light scattering (SLS) is a better way to determine the radius of a colloidal sphere.*
*If you want to know the polydispersity of your particle system, SLS would be a bad choice, since it overestimates the polydispersity. TEM is the preferred technique for measuring polydispersities.*

- Start the *analySIS* program.
- Load a TEM-image (go to the *File* menu of the menu bar, left-click *Open* and select the required image using the browser). This picture will be loaded to the first image slot by default (see the rectangle on the left side of the screen containing the numbered 'television screens').
- Set the threshold (left-click *Set Thresholds* in the *Image* menu in the menu bar). This is the most crucial step of the analysis, since it detemines which grey values are assigned to the particle and which are assigned to the background when you binarize the image (necessary for image processing). First, zoom in on the particles until you have approximately 10 particles in view. In the *Set Thresholds* menu, select *Histogram* under *Diagram*. Set the red vertical line at the right end of the left black shoulder, such that the particles in your view are just completely colored (green by default). Press *OK*.
- Left-click *Binarize* in the *Oper* menu of the menu bar.
- Left-click *Erosion* in the *Morphological Filter* menu of the *Oper* menu of the menu bar.
- Repeat the *Erosion* operation until all particles are no longer connected. You can use *Ctrl-y* to repeat the last operation you performed. Just above the image slots, there is a rectangular box in which the sources and the destination of the next operation can be defined (I call it the *Flow control* box). Before every consecutive erosion step, make sure that source and destination of these erosion steps are the same (3 in most cases). Otherwise, the program will put the result of every step in a new image slot. Since their number is limited (the program only uses the first 8 slots), you may lose the first few slots. You should avoid this, since you need the binarized image later on in the analysis. You can change the destination by dragging the destination slot to the folder in the *Flow control* box marked *Dest*.
- After eroding the particles, left-click *Skeleton Dark* in the *Morphological Filter* menu of the *Oper* menu of the menu bar.
- Make sure that *Src* in the flow control box refers the binarized image and *Src2* to the skeleton picture. Go to the *Image Calculator* in the *Oper* menu of the menu bar. Define the operation: *Src&Src2*, press = and close the *Image Calculator*. This will perform a logical AND.

- Left-click *Separate Particles* in the *Morphological Filter* menu of the *Oper* menu of the menu bar to separate the particles.
- Left-click *Set Frame* in the *Image* menu of the menu bar; a red rectangle will appear in the image. Position the upper left corner of the red rectangle at a suitable position near the upper left corner of the image, hold down the left mouse button, drag the lower right corner to a suitable position near the lower right corner of the image and press the right mouse button. This frame can be used to get rid of border particles. Make sure the scale bar is outside of this frame.
- Left-click *Define Detection* in the *Analysis* menu of the menu bar. Select *Exclude* under *Border particles* and *Frame* under *Search area* and left-click *execute*. Close the menu by left-clicking *OK*. Now only spheres that are completely within the frame have been selected. Single spheres are dark green by default, aggregates have different colors.
- Left-click *Delete Particles* in the *Analysis* menu of the menu bar. Left-click on all non-dark-green particles. Check whether there are other particles within the frame which should not be taken into account during the analysis and delete these too. When you are done, press the right mouse button.
- Left-click *Define Measurement* in the *Analysis* menu of the menu bar. Select, at least, *Diameter Mean, Area* and *Shape Factor*. Leave the menu by left-clicking *OK*.
- Left-click *Particle Results* in the *Analysis* menu of the menu bar. This will generate a sheet containing the acquired data. Right-click on the title bar (blue by defaut) and select *Save As*. Save this data sheet to any file you like in txt-format.

You can treat several TEM-images like this and obtain *n* files containing data on your particle system. If all this data refers to the same particle system, it is easiest for later analysis that the data is combined in a single file. Take one of the *n* files and use it as a basis for the total file. Use *Copy* and *Paste* to transfer data from the other files into the basis file. Copy only the numerical data, not the notes at the top of the file. Append this numerical data below the data already in the basis file, such that it seems to have come from a single TEM-image. However, do not worry about the *Particle ID*.

Once all data is in a single txt-file, it is quite easy to obtain final results using the *Mathematica* notebook *temanalysis.nb*, available via the internal site of the SCM website (http://www.colloid.nl/). The notebook requires an installation of *Mathematica* to run and contains an explanation on how to run it within *Mathematica*.