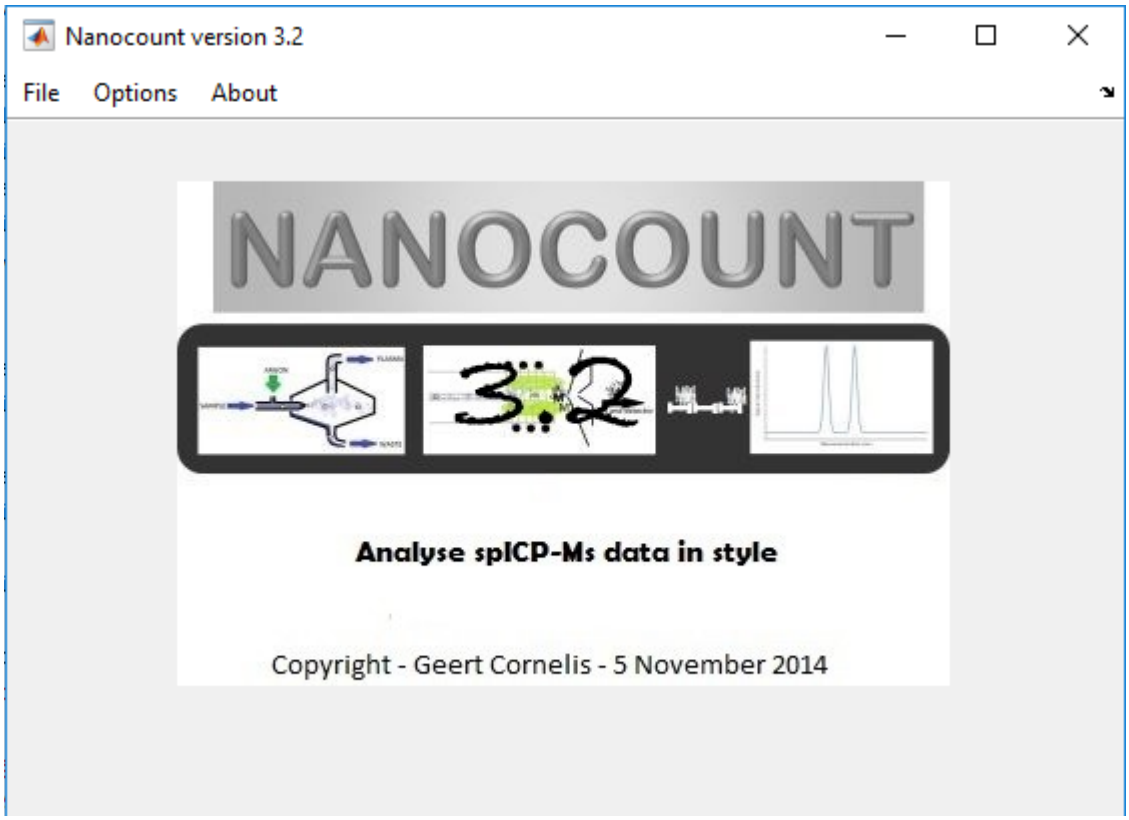
Manual to

Nanocount version 3.2



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Acknowledgments

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Improvements relative to the previous version

The following is a complete list of improvements done relative to the previous version

Major:

- Particle size distributions can be calculated with the X-axis being either corresponding spherical diameter or mass per particle
- It is now possible to calibrate diameter, mass per particle, and number concentrations using particle standards with the "grouping" tool that can be accessed via the nebulisation efficiency tool.
- Nebulisation efficiencies can be grouped so that their average is used as nebulisation efficiency value
- For each sample individually, diameter, mass per particle, and number concentration can be calculated using different nebulisation efficiencies if so desired
- All nebulisation efficiencies and averages thereof can be combined to extrapolate over time, with some restrictions

Minor

- 'Size' has been replaced with 'Diameter'
- Using the nebulisation efficiency calculation methods based on flow mass balance and flow mass balance with intensity correction are no longer available as it is increasingly becoming clear that these yield highly inaccurate results and few users are interested in using them. The method other is still available if the nebulisation efficiency is calculated separately using these methods.

What is Nanocount?

Nanocount is software to analyse single-particle ICP-MS (spICP-MS) data. Such data emerges as a series of datapoints that have to be worked with intensively to arrive at the desired result: a particle size distribution (PSD). The operations to be done are far too numerous to handle in e.g. spreadsheet format. Moreover, the size of the data files is usually far too large to handle easily in spreadsheets. Since the first papers on spICP-MS by Degueldre et al.[1], spICP-MS has gained much acclaim and has moved beyond the reconnaissance stage into a growing body of applications. Nanocount may help to further this development.

Software similar to Nanocount is available from Perkin Elmer as a separate module within their syngistix software environment controlling PE ICP-MS machines. This software is thus not available to users of other ICP-MS machines. Moreover Nanocount aims to offer more features to spICP-MS users. Key features are:

[1] Degueldre, C.; Favarger, P. Y., Colloid analysis by single particle inductively coupled plasma-mass spectroscopy: a feasibility study. *Colloids and Surfaces a-Physicochemical and Engineering Aspects* **2003,** *217* (1-3), 137-142.

- A customizable import tool for text files in any format holding spICP-MS data from any commercial ICP-MS machine.
- A data correction tool, allowing to correct data for long term drift or short term disturbances
- A deconvolution tool, allowing to accurately distinguish dissolved and particulate signals using various recently published methods.
- A nebulization tool, allowing to calculate and use nebulisation efficiency as accurately as possible using various methods

This manual does not comprise theoretical treatise of underlying concepts. The reader is referred to the proper references listed further in this manual. Only proper handling of tools and features within Nanocount is explained.

This first version is a beta version, so we do not guarantee full operation yet. We do, however, encourage interested users to report and bugs and inconveniences to us so we can possibly rectify those as much as possible.

References

The basic spICP-MS theory was developed by Degueldre et al.[2] and has been described by several authors.[3] Deconvolution tools were mainly based on work done at the Environmental Nanochemistry laboratory, Gothenburg University. [4] Methods for nebulisation efficiency determination are based on work done at the Colorado School of Mines[5] and Gothenburg University[6]. Drift correction procedures have been published.[7]

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[2] Degueldre, C.; Favarger, P. Y.; Wold, S., Gold colloid analysis by inductively coupled plasma-mass spectrometry in a single particle mode. *Analytica Chimica Acta* **2006,** *555* (2), 263-268.

[3] Pace, H. E.; Rogers, N. J.; Jarolimek, C.; Coleman, V. A.; Gray, E. P.; Higgins, C. P.; Ranville, J. F., Single Particle Inductively Coupled Plasma-Mass Spectrometry: A Performance Evaluation and Method Comparison in the Determination of Nanoparticle Size. *Environmental Science & Technology* **2012,** *46* (22), 12272-12280.

Laborda, F.; Jimenez-Lamana, J.; Bolea, E.; Castillo, J. R., Selective identification, characterization and determination of dissolved silver(I) and silver nanoparticles based on single particle detection by inductively coupled plasma mass spectrometry. *Journal of Analytical Atomic Spectrometry* **2011,** *26* (7), 1362-1371.

[4] Cornelis, G.; Hassellov, M., A signal deconvolution method to discriminate smaller nanoparticles in single particle ICP-MS. *Journal of Analytical Atomic Spectrometry* **2014,** *29* (1), 134-144.;

Tuoriniemi, J.; Cornelis, G.; Hassellöv, M., Size discrimination and detection capabilities of single-particle ICP-MS for environmental analysis of silver nanoparticles. *Analytical Chemistry* **2012,** *29*, 743-752.

[5] Pace, H. E.; Rogers, N. J.; Jarolimek, C.; Coleman, V. A.; Higgins, C. P.; Ranville, J. F., Determining Transport Efficiency for the Purpose of Counting and Sizing Nanoparticles via Single Particle Inductively Coupled Plasma Mass Spectrometry. *Analytical Chemistry* **2011,** *83* (24), 9361-9369.

[6] Tuoriniemi, J.; Cornelis, G.; Hassellöv, M., Improving Accuracy of Single particle ICPMS for Measurement of Size Distributions and Number Concentrations of Nanoparticles by Determining Analyte Partitioning during Nebulisation. *Journal of Analytical Atomic Spectrometry* **2014,** *29*, 743-752.

[7] Cornelis, G; Rauch, S., Drift correction of the dissolved signal in single particle ICPMS. Analytical and Bioanalytical Chemistry, **2016**, *408*, 5075-5087.

Data types in Nanocount

Nanocount means to be flexible with the sort of data input it can handle. There are two types of spICP-MS data:

- Conventional
- Fast acquisition speed technique (FAST)

The main difference between conventional and FAST spICP-MS is the number of data points have been acquired per nanoparticle event. In conventional spICPMS analysis this is always only one and more than one in the case of FAST-spICP-MS. Practically, FAST-ICP-MS is only possible on machines that can acquire data sufficiently fast, i.e. using dwell times of 0.1 ms or lower. Nanocount handles FAST data by searching for peaks in the time-resolved spectrum of a sample. Each peak is considered a nanoparticle event and the integrated area of the peak is the total signal intensity of the event. Overlapping peaks are taken into account in this process.

Note that the FAST data treatment algorithm is still under investigation. Its validity has not yet been fully tested and there are as of yet no scienitifc publications available on this algorithm.

Secondly, there are two types of projects:

- Batch
- Transient

The particle size dsitributions in one sample are considered static as a function of time in batch projects, whereas they can change with time in Transient projects. The latter is useful to treat data when spICP-MS was used to measure time-resolved data coming from chromatography-type techniques such as field-flow fractionation, size exclusion or hydrodynamic chromatography. However, it can also be used to measure the evolution of size and dissolved ion concentration in one sample during e.g. a dissolution/aggregation process.

Whether a project is conventional or FAST AND whether it is Batch or Transient has to be indicated at the very start of making a project and cannot be changed later on.

Installing Nanocount

Nanocount is currently only available for a windows environment. The installation file comes as “Setupnanocount64.exe” or “Setupnanocount32.exe”, depending on whether your operating system is Windows 10, 8, 7 or Vista (64 bit) or Windows XP (32bit). Run the executable.

If you are installing Nanocount for the first time or you do not have the correct compiler installed (version 8.4), it is required to have a working internet connection, because the installer will download a Matlab Runtime compiler, required to run the program. This is a large file and depending on the speed of your internet connection, the download process can take several minutes, up to an hour.

The window below appears (Figure 1). The internet connection settings can still be changed at this point to optimize the runtime compiler. Then a window appears, allowing to set the installation folder and to add a desktop icon. If a version of Nanocount is already present, the new version will be installed over it so a prior uninstall is not required. Clicking "Next" will automatically start the compiler download process. If the correct compiler is already installed, the installer immediately jumps to the next screens (Figure 2).

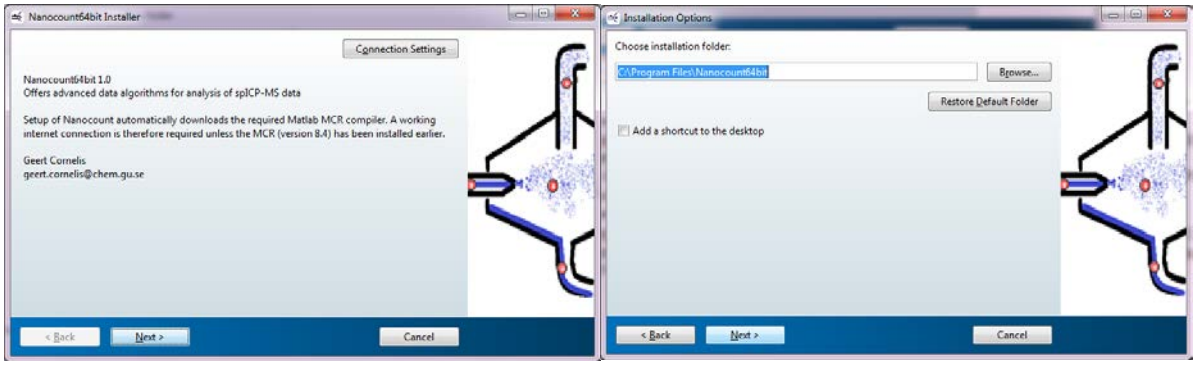


Figure 1 *Installation windows*

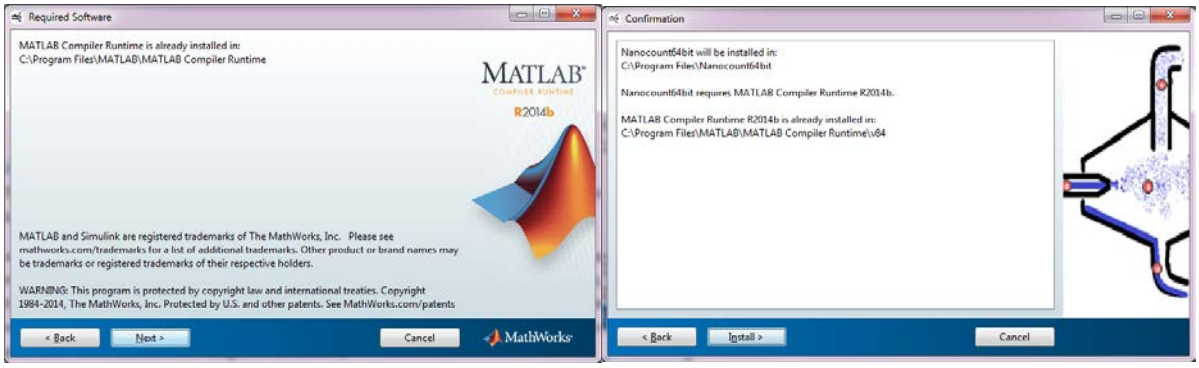


Figure 2 *Installation windows*

Clicking next brings the user to the final window before the actual installation of Nanocount occurs. Here one can check the installation folder for the last time. The installation process itself should not take much more than a couple of minutes.

If you used to have an older version of Nanocount on your computer, make sure to delete the file "settings.nan" in the folder" /Mydocuments/Nanocount" for your new licence to take effect.

Possible errors

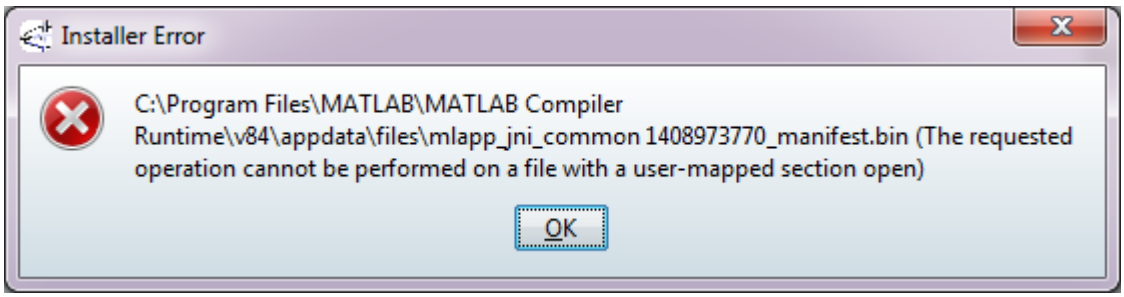
The most common error during installation is that there is no working internet connection, required to download the runtime compiler. Double check that you connection is working. Also check with your administrator that you have the correct rights to install software on your computer.

For people working with computers from countries that use non-ascii symbols in their language, the following error message may appear during installation:

> The message “INSTALLER ERROR. Error finding installer class. An exception occurred while looking for class…”.

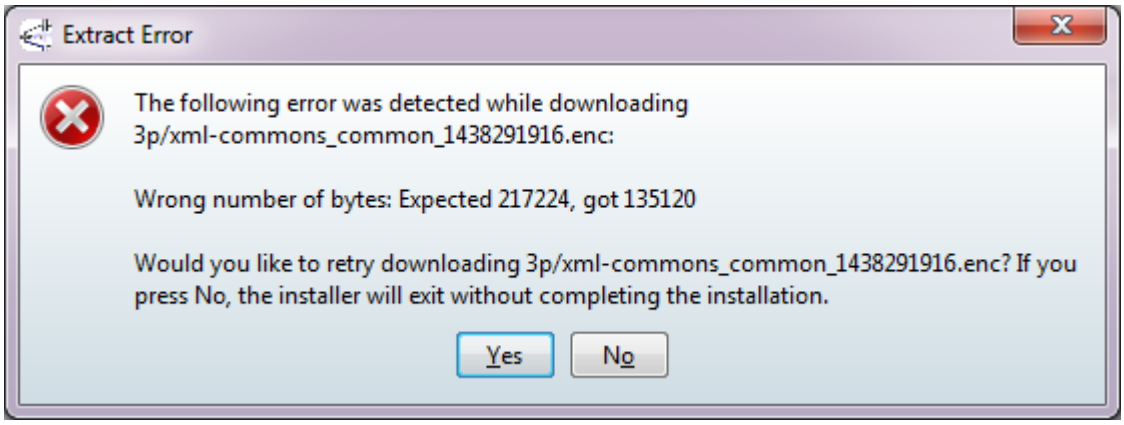
There is a bug in the installer on Windows in the way that it behaves with TEMP environment variables that point to a directory containing non-English or special characters. To work around this issue, make sure that your TEMP variable points to a directory name containing only standard ASCII characters. Sometimes a TEMP variable points to a directory inside of your profiles folders, so you may also want to check if your user name, in which the “my documents” folder is located” contains non-ascii characters (e.g. Greek, Russian or Turkish).

Another error can be the following



If the windows explorer is open this error may also appear. Close Windows explorer and try again.

Another error may cause an error message similar to the figure below to appear. In this case, there is a problem with your network and most likely, you need to go to a different network and download the software there.



General overview

The table below shows a typical workflow for a given project.

<table>
<tr><th>Batch</th><th>Transient</th><th>FAST</th><th>Comment</th></tr>
<tr><td colspan="3">Start project:
• Add name, isotopes, nanoparticles, input flow
Determine whether project is conventional or FAST and batch or transient</td><td>Selecting isotopes makes them available to import data to. More isotopes can be added later. Nanoparticles are selected for final PSD calculation, more nanoparticles can be added at a later stage.
Determining the project type can only occur at this stage</td></tr>
<tr><td colspan="3">Select files from which to input data</td><td>Only selecting files does not import them, this happens in the next step</td></tr>
<tr><td colspan="3">Import data</td><td>All data (histograms, PSDs, models,…) of existing components is overwritten when data is imported to them. Therefore, do NOT forget to put in the right dwell time, this can NOT be changed later</td></tr>
<tr><td colspan="3">Remove drift and outliers from dissolved standards, drift from samples</td><td>Especially important when using a deconvolution method</td></tr>
<tr><td colspan="3">Calibrate the project</td><td>If using deconvolution, a noise model must be chosen at this stage, in which case the calibration must be carefully checked.</td></tr>
<tr><td></td><td>Select ROIs and determine slicing</td><td></td><td></td></tr>
<tr><td colspan="2">Remove dissolved signals using different models</td><td>Remove signals AND determine integration method</td><td>Check out the decision scheme (Figure 24) to find the best method.</td></tr>
<tr><td colspan="3">Nebulisation efficiency determination</td><td>The diameter method is strongly advised, in which case removing dissolved signal must happen first from nebulisation efficiency checks.
Using particle standards is also possible at this point</td></tr>
<tr><td colspan="3">Calculate and visualise PSDs/show trends</td><td></td></tr>
</table>

Building blocks in Nanocount

Objects

Nanocount is organised into objects (as it is programmed object-oriented). An overview of the main objects will probably further the user's understanding of how to handle the software:

- **Projects**: Project objects contain all the information and objects (components, standards, samples, files, nebulisation efficiency checks, isotopes, nanoparticles) related to a sample run.
- **Files**: These are the text files that are imported into a Nanocount project.

- **Isotopes**: The isotopes that are measured in the ICP-MS method. These are the elements that are present in nanoparticles suspected to be present in the samples, but also in the internal/external standards as well as in the particle or solutions used to determine nebulisation efficiency. The user can define his/her own isotopes in Nanocount.
- **Nanoparticles**: Nanoparticles consist of elements. The user can define his/her own nanoparticles in Nanocount, provided that the stoichiometric composition and density is known.
- **Elements**: Isotopes are measured during spICP-MS, but the same isotope can be measured at different dwell times, which leads to different calculations. So for each isotope and each dwell time that that isotope was measured, a different element is created within the project.
- **Histograms:** An intensively used way of storing and recalculating data in Nanocount. This class is explained in more detail below. Particle size distributions are just one example of the histograms shown in Nanocount.
- **Tubes:** these simply represent the tubes that were in your autosampler racks. They just serve as vehicles to gather in data from the text files that were exported from the icp-ms. Nanopcount then uses this information to create components.
- **Import rules**: A set of conditions used to interpret data contained in text files and how to translate these into usable data for a particular element within components.
- **Components**: Components are the basic building blocks of a spICPMS project in Nanocount. For each element in a tube, a different component is created, whose type is one of four different ones:
    - **Standards**: These are the dissolved standards that are used to either calibrate the measured elements to allow PSD calculations or to calibrate the calculation of the nebulisation efficiency, e.g. when the size method is used for those (see further).
    - **Samples**: These are the actual samples in which the user wants to determine a PSD.
    - **Nebulisation efficiency checks**: These serve to calibrate the nebulisation efficiency, a crucial parameter in spICP-MS. Nanocount allows several checks during a run so that if drift is suspected, the measured nebulisation efficiency can be extrapolated. Nebulisation efficiency checks can be calculated using a variety of methods.
    - **Nebulisation efficiency groups**: These serve to use averages of dseveral nebulisation efficiencies or to use particle standards to calculate diameters, mass per particle, number concentrations using a calibration.
    - **Sensitivity checks or sensitivity checks:** Most commonly, only one element can be measured at a time in spICP-MS thus avoiding the use of an internal standard that otherwise can be used to compensate for drift and matrix suppression of the signal. To still allow correction, separate measurements of an element can occur, of which the data, often the average value, can be used to correct for drift. To do this, accurate time information on all components is required so that the spICPMS signal can be corrected as a function of time.
- **Calibrations**: These objects hold all the dissolved calibration information for a particular element. In the most basic version, i.e. “basic” calibrations, only the sensitivity and dissolved background noise are calibrated. In more advanced convolution methods, the calibration object

also holds information about relationships between different noise parameters that accurately describe how dissolved signals behave. This allows dissolved signals to be deconvoluted from signals holding nanoparticle signals as well.[4]

- **Models**: Models are the dissolved signal models that are used to calibrate a Nanocount project. The most basic one is the linear model, but there are also the normal, poissongaussian, negative binomial, polyagaussian, and holistic poissongaussian and holistic polyagaussian model that all can be used if a more advanced deconvolution method is used.
- **ROIs**: Region of interests are used for transient spICP-MS, where you want more imformation on a particular time range, in which case you have to define a ROI, which is in its turn divided into slices of time for which a PSD is calculated.

Histograms

Histograms are used intensively throughout Nanocount and deserve some discussion to improve the user's understanding of some of the used algorithms. Histograms essentially reflect the number of occurrences of a particular range of values in a data series. Figure 2 shows how this works. A histogram is calculated from a series of 10,000 values, e.g. a spICP-MS signal, by simply counting how many times each signal occurs. These sorts of histograms of the raw data are finally recalculated into PSDs using spICP-MS theory, where the ion counts are recalculated into diameters and the frequencies into number concentrations. PSDs are thus histograms in their own right as they just reflect the number of times a certain diameter occurs in a particular volume (Figure 3).

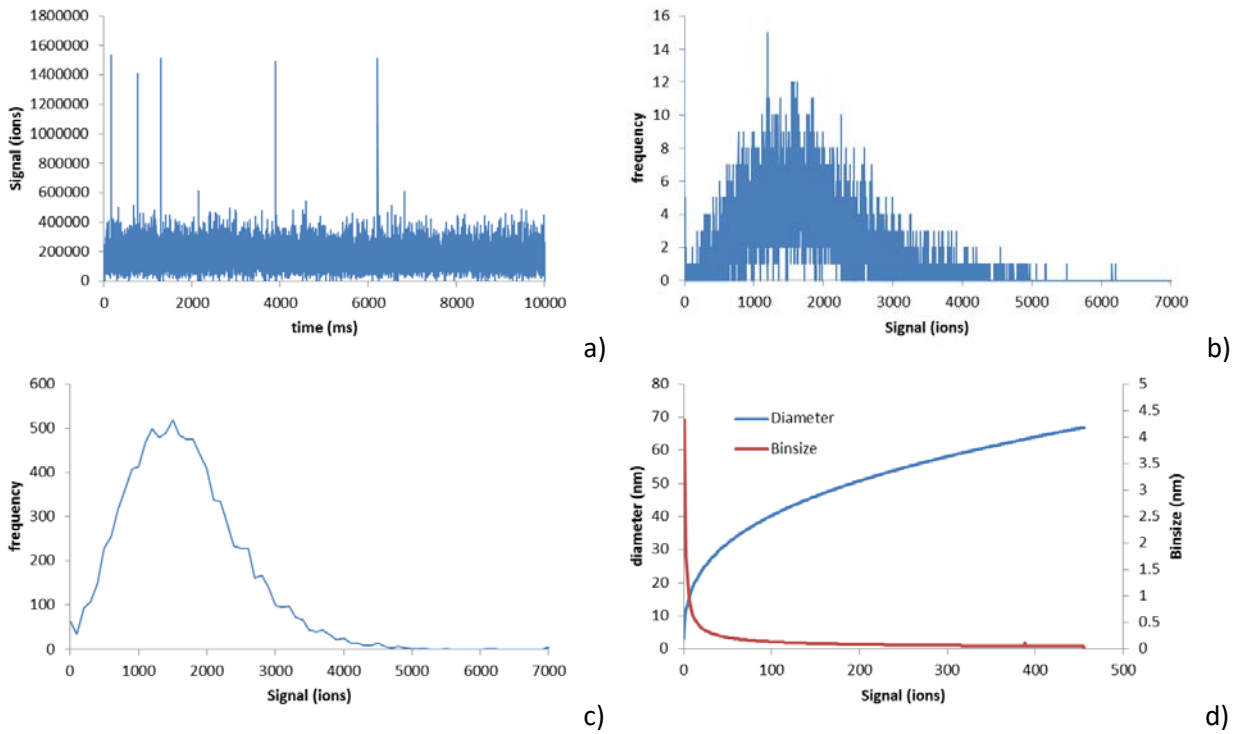


Figure 3. *a) Raw spICP-MS data: signal (in ions) as a function of time (in ms) b) The raw signal histogram made from the raw data in a: signal frequency as a function of signal (ion ions). c) The same histogram as in b), but now the values have been rebinned using a uniformly distributed bin vector having a broader bin size. d) A possible relationship between signal intensity (in ions) and diameter (in nm) and the corresponding binsize as a function of signal intensity that is generated if the raw histogram (b) is directly calculated into a PSD.*

In the first histogram shown in Figure 3, each discrete integer signal is considered, i.e. [0,1,2,3,…,max-1,max] where max is the highest measured signal. The vector [0,…,max] is called the binvector, whereas the number of occurrences is called the frequency vector. The difference between two subsequent binvector values is called the bin size. In the first case, this is 1, which is also the minimum binsize possible for spICP-Ms signals where at least one ion arrives at the detector (not e.g. ¾ of an ion). Using a binsize of 1 to describe this data, results in a rather low count for most values and a noisy histogram. However, we may choose to increase the binsize to say 100. Figure 3 shows that this results in a much smoother histogram that shows the same data, only using a higher binsize. In both cases, though, the binsize is said to be "uniform", i.e. all bins have the same binsize of either 1 in the first case or 100 in the second.

One can understand that increasing the binsize results in a clearer representation, but possibly also in the loss of information where fine features are lumped together. For this reason, the raw form (i.e. binsize = 1) is used the most throughout Nanocount, thus preserving the maximum of information. However, rebinning histograms may prove very useful when calculating the final result, i.e. the PSD. However, diameters are no longer discrete values and can thus be rebinned in an infinite number of ways. Figure 3 shows a typical relationship between signal magnitude (ions) and the diameter calculated using spICP-MS theory. This relation basically shows that there when calculating PSDs from a histogram with binsize 1, the calculated diameter binvector will have large binsize for small diameters, but the binsize quickly decreases to lower values. In many cases, we are however interested in a uniform diameter binsize, in which cases it becomes interesting to recalculate the PSD's diameter binvector. One may also wish to have finer features, and thus a smaller binsize, in a certain diameter range. Nanocount offers this possibility.

Default files

The files "nanoparticles.nan" and "isotopes.nan" may not be deleted. Doing such results in loss of the nanoparticles and/or isotopes list and a new list has to be created. Old projects can still be opened to read data in this case, but it will be impossible to add new data to projects, once the isotope and nanoparticle list have been deleted.

Files generated by Nanocount have different default extensions: *.nan, *.csc….. The files include

- "settings.nan": The file holding the licence information and all the default save locations.
- "*.prj": Project files holding saved data
- "nanoparticles.nan": The file holding the nanoparticles database.
- "isotopes.nan": The file holding the isotopes database.
- "*.csc": Lists of reusable component schemes
- "*.rul": Lists of reusable import rules

All files can be given different extensions, which may be desirable for the user to keep track of all the files that have been created. Alternatively, the user may choose to save them in different folders.

Graphs

All graphs in Nanocount have similar functionalities as shown in Figure 4. There are many fine, but important features in most graphs in Nanocount so the user must have clear visual access to all these features by zooming in. Figure 4 shows a graph after a zoom has been initiated, because there are scroll bars visible. The two buttons in the upper right corner:

Being in zoom mode in one graph, does not allow you to zoom into another graph in the same window. You will notice that the magnifying glass mouse pointer is not visible in the other graph. To zoom into this graph, one must first click on any "+" button and then click on the "+" button of the prefered graph.

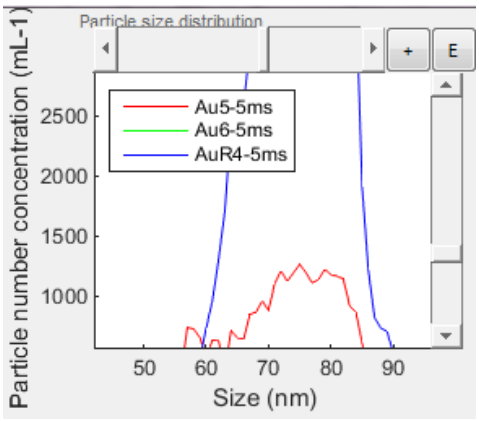


Figure 4 *Different functionalities associated with all graphs in Nanocount*

- "+": Clicking this button initiates the zoom utility for the selected graph only. After clicking this button, the mouse ion changes to a magnifying glass with a "+" when panning over the graph for which the zoom function is active. While the zoom utility is active, possible actions are:
    - Clicking anywhere: The picture will be zoomed in two times at the clicked position. X and/or Y Scroll bars will appear as shown in Figure 4 to navigate to other parts of the graph while keeping the same zoom level.
    - Shift-click: The same as just clicking, but this time to zoom out. If zero zoom is reached, the scroll bars disappear.
    - Click-hold and drag: A box will appear while dragging so that the user can indicate the zoom area to be magnified.
    - Mouse roller away: Same as clicking anywhere on the graph: two times zoom at the point indicated.
    - Mouse roller towards you: Same as shift-clicking anywhere on the graph.

Clicking any "+" button on any graph again closes the zoom utility. Note that you first have to close the utility for one graph to be able to subsequently activate it for another graph in the same tool.

All other interactive functionalities within the plot area (dragging reference lines, resizing boxes) are not available when in the zoom mode. You must first click "+" again to go out of zoom mode before you can handle any objects. This applies for all graphs in the active tool, i.e. if you are in zoom mode in one graph, you also cannot handle objects in another graph.

- "E": Exports all the data shown in the graph as tab separated data to the clipboard so that it can be pasted in e.g. excel or a text file.

The main menu

This menu (Figure 5) allows to start, save or open new projects as well as create new isotopes or nanoparticles.

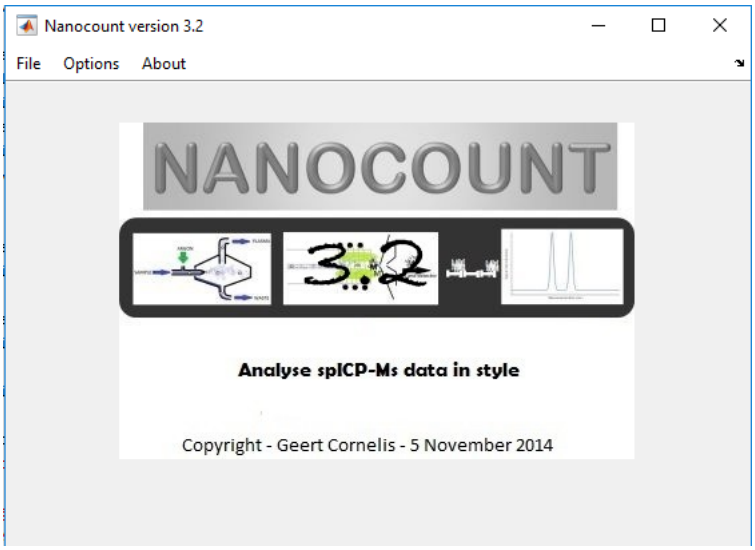


Figure 5 *The main menu.*

File

Here, the user can start new (New) projects or open previously saved ones under a new name (save as) or the current name. An arbitrary number of projects can be run at the same time so that the user can compare results. Selecting "Close" closes the currently selected project. The menu also shows up to 5 of the recently edited projects, serving as quick links to open them.

Options

Here, new isotopes or new nanoparticles can be created and default file locations can be set. Also, appearances can be changed.

Isotopes

There are some default isotopes in Nanocount as shown in Figure 6, but new ones can of course be created. The table on the left in Figure 6 is editable so the user can change the symbol string and molar mass of existing isotopes. "Symbol" is the symbols string of the isotope. In the current beta version,

isotopes are in fact the same as elements. “mass” is therefore the average element molar mass, or if preferred, the accurate isotope molar mass. This mass is used to calculate the total molar mass of nanoparticles as explained later. Hence, if an isotopically enriched nanoparticle is used, a new isotope needs creating having the accurate average mass of e.g. enriched ^{68}Zn. new isotope.

Handling isotopes occurs with the buttons on the right.

- “Add isotope” adds a new line at the bottom of the table where a new isotope can be given its symbol and default mass. Note that an empty box will be kept if the contents are never filled out
- “Delete isotope” deletes the currently selected isotope. Selecting an isotope occurs by clicking on a row anywhere in the body of the table (not on the numbered column).
- “Sort alphabetically” sorts the isotopes according to increasing alphabetical order of the symbol, for easy referencing that becomes relevant when the table contains many isotopes.
- “Sort according to mass” does the same as “Sort alphabetically”, but sorts according to the increasing default mass.
- “Save” saves the current isotope table in a file called “isotopes.nan” for reference in all Nanocount projects.
- “Exit” leaves the organise isotopes tool

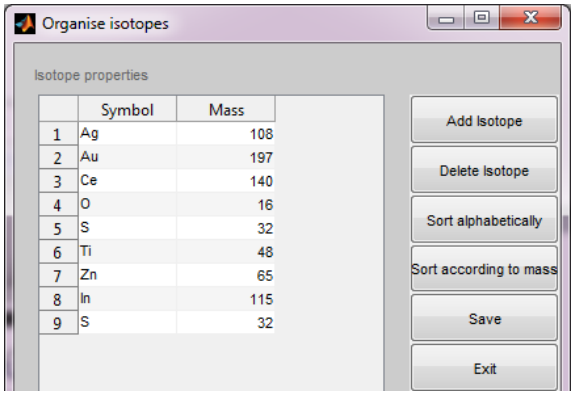


Figure 6 *The “organise isotopes” tool.*

Nanoparticles

Nanoparticles consist of isotopes. There are three some default nanoparticles in Nanount. TiO_2, for instance, consists of one isotope Ti and two isotopes O. Nanoparticles thus have associated with them:

- A symbol
- A composition (how many of which isotope)
- A molecular mass, calculated from the composition
- A density, required to calculate the PSD

There are two tables in the “organise nanoparticles” tool (Figure 7): one showing all the current isotopes in Nanocount and one showing all the nanoparticles. The isotope table is non-editable, at this point, except for the third column that is used to declare the stoichiometry of new nanoparticles. The Nanoparticle table is also non-editable, except for the density value in the column “Density”. The “Mass”

column holds the molar mass of the nanoparticle that is automatically calculated from the isotopic composition. Note that the user is responsible for finding the correct stoichiometry, i.e. Nanocount does not check whether charge balance has been reached. Lastly, the new nanoparticle's density has to be filled out in the column "Density". This column should not be left empty, as it will create problems when calculating the PSD later on.

Manipulating the nanoparticles occurs with the buttons on the right side.

- Both the isotope and nanoparticle tables can be manipulated similarly to the organise isotopes tool using "sort alphabetically" or "sort according to mass" buttons. Each table has its own set of those buttons
- "Save" saves the set of nanoparticles shown in a file called "nanoparticles.nan".
- "Exit" exits the organise nanoparticles tool.
- "Add": To create a new nanoparticle, the user must first fill out the stoichiometry of the new nanoparticle in the upper table in the column "NP stoech.". Otherwise, no new nanoparticle is created. For instance, if you want to create a Ag_2O nanoparticle, fill out "1" in the row for the O isotope and "2" for the Ag isotope and click "Add". A new line will appear holding the symbol "Ag2O" and the calculated molar mass of the nanoparticle.
- "Delete" deletes the selected nanoparticle.

Selection of rows or cells in tables in Nanocount is always within the body table, not column or row headings. This owes to an inherent limitation of the programming language (Matlab). Select multiple rows by holding ctrl on the keyboard and then clicking rows with the mouse or to select a whole range, click a cell, hold shift on the keyboard and then select the other end of the range.

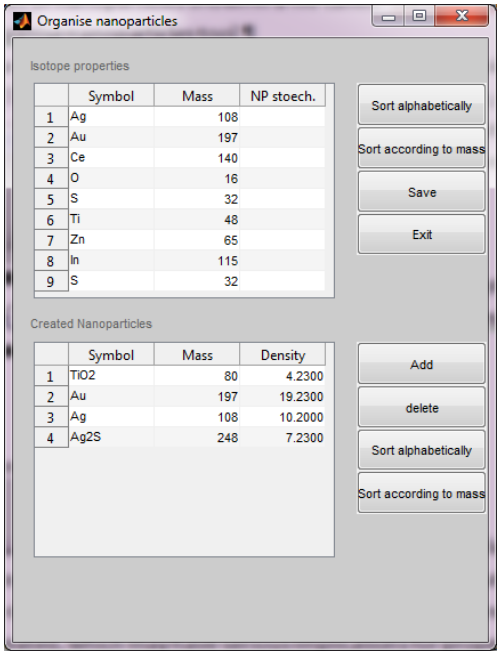


Figure 7 *The "Organise nanoparticles" tool.*

File locations

Use this option to set default file locations for the isotope and nanoparticles files (“isotopes.nan”, “nanoparticles.nan”). Note that setting another location here, would imply that Nanocount will be looking for these files in a different location. If the files are not found there, new files holding no isotopes or nanoparticles will be created, which may have serious implications for proper functioning of Nanocount. Use this option only if you want to reorganise and thus move the already existing “isotopes.nan” and “nanoparticles.nan” files.

The other three file locations have fewer implications. Nanocount will just look for needed files in these locations first when saving or loading projects, component or import schemes. The tools where this occurs usually work through a browser-type interface so the user can still look elsewhere than in the default locations.

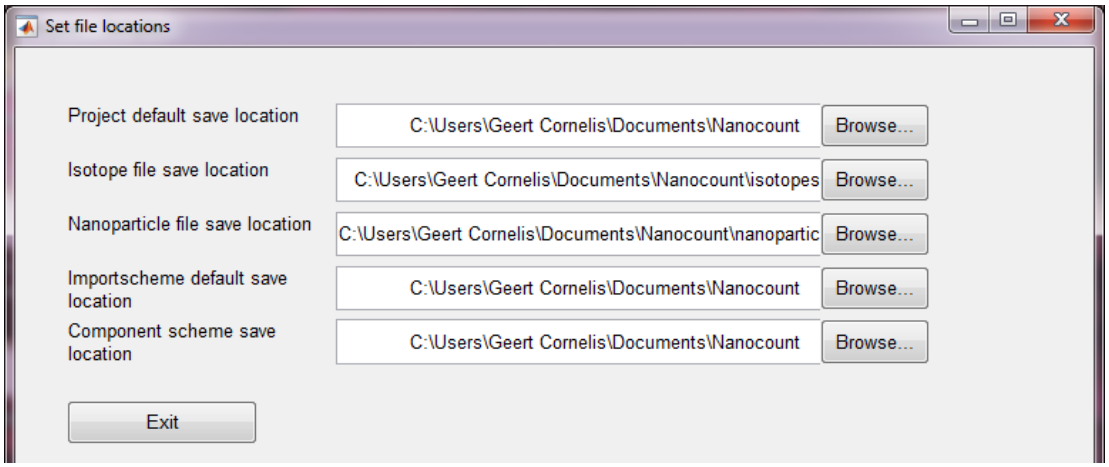


Figure 8 *The “Set file locations” tool.*

Appearances

A limited number of visual appearances can be changed in the appearances tool (Figure 9):

- Font size
- Font type

Upon closing the tool, the font size and font type will be changed in all currently active windwos, including tools, project window and main menu. However, some fonts will always be, by default Helvetica 10 pt, i.e. the top horizontal menus (main menu and project windows only) and table contents, because of limitations of the programming language (Matlab).

- Automaximization

Checking automaximization will make sure that every project window or tool that is opened, will henceforth always be maximized to the maximum screen size.

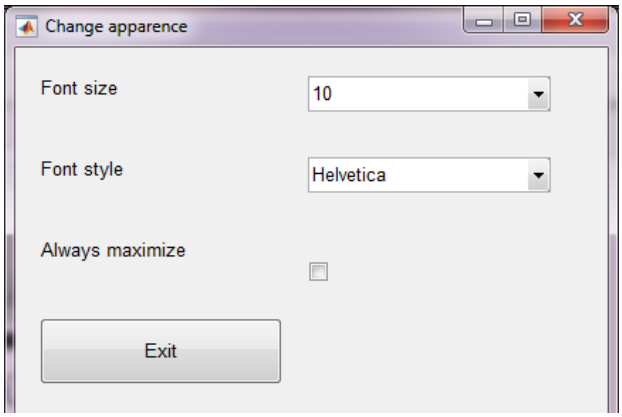


Figure 9 *The "Appearances" tool.*

Opening a new project

When selecting project > New in the main menu, Figure 10 appears, where basic data about the project can be given.

- "Project Name": Gives the name to the project. The name will also be used to create a default save file name.
- "Data Type": There are two options:
    - "Conventional": Each data point is regarded as containing a whole nanoparticle event or none at all. It is assumed that there is infinite time in between data pints, i.e. they do not influence each other.
    - "FAST": Particle events occur as peaks constituted of several consequent data points. It is assumed that zero time occurs in between data points.
- "Batch or Transient":
    - "Batch": The PSD of particles in the samples does not change over time. The data is regarded in a static way.
    - "Transient": The data streams describe dynamic systems, i.e. the PSD in the samples may change over time.
- "Change isotopes": Opens a simple tool to select the isotopes (NOT elements!) that will be used in the project. This includes e.g. isotopes necessary for calculating the nebulisation efficiency if the size method is used.
- "Change nanoparticles": A list of nanoparticles is shown. All nanoparticles in the database holding any of the selected isotopes can be selected. Selecting all nanoparticles at this stage is less crucial compared to selecting isotopes as there are less implications.

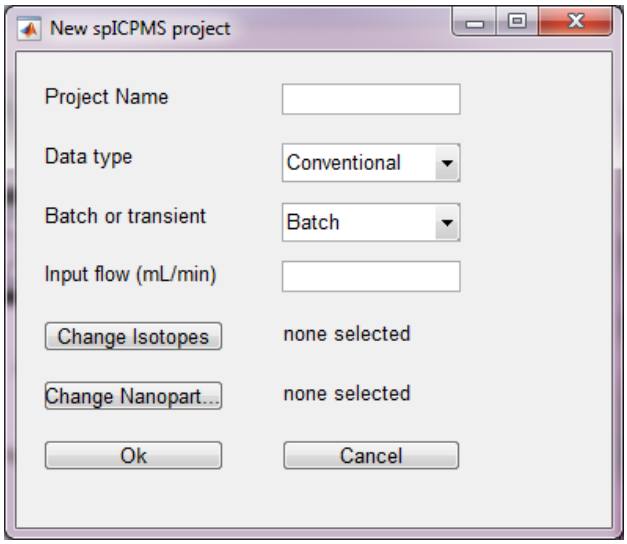


Figure 10 *The "Open new spICPMS project" tool.*

Change isotopes

When this button is clicked, a new window opens (Figure 11). A table with all available isotopes appears. Very similarly to the tool to create isotopes or nanoparticles explained earlier, the table can be manipulated by sorting alphabetically or according to molar mass. The table is non-editable, expect for the checkboxes by which desired isotopes can be selected. Deleting isotopes serves to limit the list of available isotopes in the import tool (see further) from which elements are created. Similarly to the "general" window, all changes are saved to the project at all times and closing the window or pressing exit, does not discard changes. If changes must be discarded, they have to occur manually.

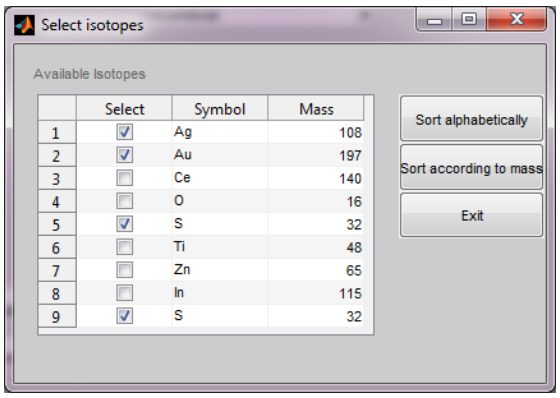


Figure 11 *Selecting isotopes for the spICP-MS project.*

Beware that only those isotopes that were actually measured need selecting. Isotopes that can also be in nanoparticles (e.g. O or S), but were not explicitly measured need not be selected here. It doesn't create problems, though, if they are still selected.

Change nanoparticles

The tool is again very similar to the select isotopes (Figure 12). The table lists all nanoparticles within "nanoparticles.nan" that have an isotope in their composition that has been selected by the user in the select isotopes tool. The selection here has implications for nebulisation efficiencies if the "size" method is chosen (see further) and for PSD calculations of sampels (see further) as it limits the list of different nanoparticles that can be chosen.

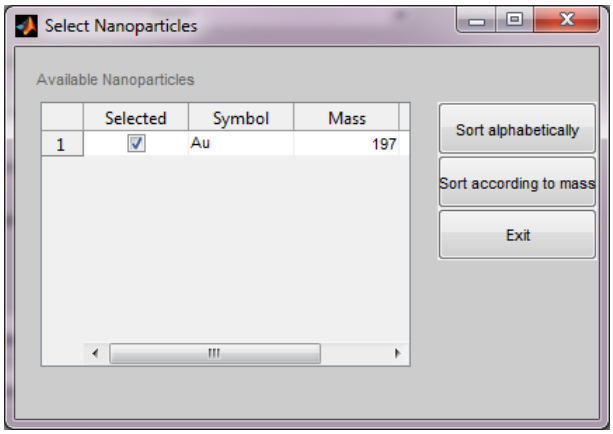


Figure 12 *Selecting nanoparticles for the spICP-MS project.*

Not all isotopes that may occur in nanoparticles have to be selected in the isotope list. For instance, oxygen does not necessarily have to be selected to be able to take the nanoparticle "TiO_2" in account. Only the isotope "Ti" had to be selected in this case. Selecting O anyway does not entail problems, though.

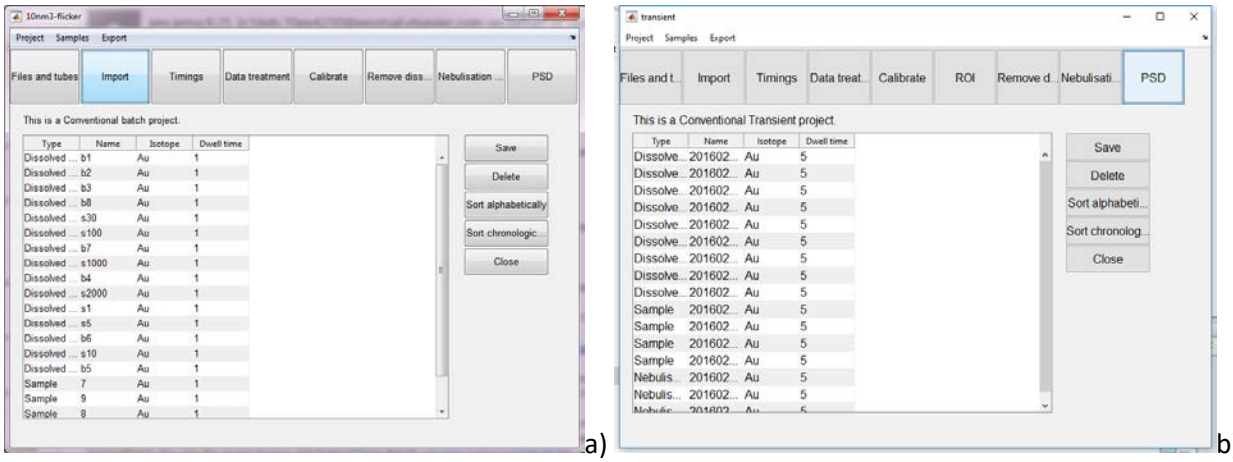
Similarly to the "general" window, all changes are saved to the project at all times and closing the window or pressing exit, does not discard changes. If changes must be discarded, they have to occur manually.

The project window

A project window opens when a new project is started or an existing one is opened from the main menu. Several project windows can be open at the same time and a project is selected by clicking within a project window, e.g. on the menu.

A project is not made active by clicking on the window border, selecting it from the bottom bar of the windows desktop, nor by using alt+tab or alt+start. It requires an actual mouse click anywhere within the window, including the menu. This is because of inherent limitations of the programming language (Matlab).

The project window summarizes some general info about the project such as the project name in the window head. The main body is a table listing all imported components. Before any data has been imported (using the import tool), no data is shown in this table.



a) b)

Figure 13 *The project window for a) a batch project and b) a transient project. Note that b) was obtained from a different operating system (Windows 10) than a) (Windows 7).*

The table lists

- The type of the component (Dissolved standard, Sample, Nebulisation efficiency check, or Sensitivity check).
- The name of the component
- The element of each component as defined y
    - The isotope symbol
    - The dwell time

The project window menu has three entries: “Project”, “Samples” and “Export”. Note that the export functionalities have not yet been developed, but will do so in future versions. The options in the menu are:

- Project
    - Save
    - Save as
    - General
    - Files and components’
    - Import rules
    - Timings
    - Data treatment
    - Nebulisation efficiency
    - Close
- Samples
    - Calibrate
    - Remove dissolved
    - Obtain PSD
    - Assign ROIs (Transient projects only)

"Samples" only becomes active as soon as there are components with imported data and dissolved standards. As samples are also created for nebulisation efficiency checks that hold data (e.g. because the nebulisation efficiency is calculated based upon a NIST gold standard), the number of samples can be nonzero, while there are actually no "true" samples, i.e. those where we want to calculate a PSD and for which there is a component defined as type "sample".

Then there are some "quick link" buttons in the main project window:

- "Files and tubes": Opens the import files tool
- "Import": Opens the import rules tool
- "Timings": Opens the timings tool
- "Data treatment": Opens the data treatment tool
- "Calibrate": Opens the calibration tool
- "ROI": Opens the Assign ROIs tool
- "Remove dissolved": Opens the deconvolution tool.
- "Nebulisation efficiency": Opens the nebulsiation efficiency tool
- "PSD": Opens the particle size distribution tool.
- "save": Saves the project, if there is no safe file declared, a browser opens.
- "Delete": Delete the currently selected component from the project.
- "Sort chronologically": Sort the components in the project according to their acquistion time
- "Sort alphabetically": Sort the components in the project according to their name.
- "Close": Closes the current project.

When sorting components, it applies to all tables in any tool you open from the project window. Sorting alphabetically may be useful when you have measured not in the same order as that you may want to group the data (e.g. according to element or dwell time). Deleting a component from the project automatically updates all data that is related to this component (e.g. calibration is changed when deleting a standard).

When selecting a tool from the project window, all options other than "save" and "save as" are disabled from the project window. This is to avoid the user taking interdependent actions in different tools. Of course, all options become available again, when a tool is closed.

Save, Save as and Close

These self-explanatory options apply to the project for which the project window is open. The close option always triggers a question if the project needs to be saved or not, even if no changes had been made. The same behaviour occurs if the window is closed by the "X" in the upper right corner of the window. Projects are also saved as "*.prj" files and the default project save location is opened first. The user can, however, change the extension if desired, but this is not recommend e.g. to keep an overview between the project files and other files (such as e.g. nanoparticles.nan). It is highly recommend not

using the project names “nanoparticles” or “isotopes”. Active projects can also be saved through the main window as outlined earlier.

General

This option serves to change some of the general information on the project. Most of this information was given when starting a new project, but can still be changed here. Note also that all info is automatically saved to the active project so closing the window or pressing “OK” does not discard the changes. (De)selecting isotopes will, again, only limit the isotopes that can be selected during data import. (De)selecting in the isotope or nanoparticle tables will therefore have no consequences whatsoever for already imported data. Note there is no quick link to general, so this tool has to be started via the project window’s menu.

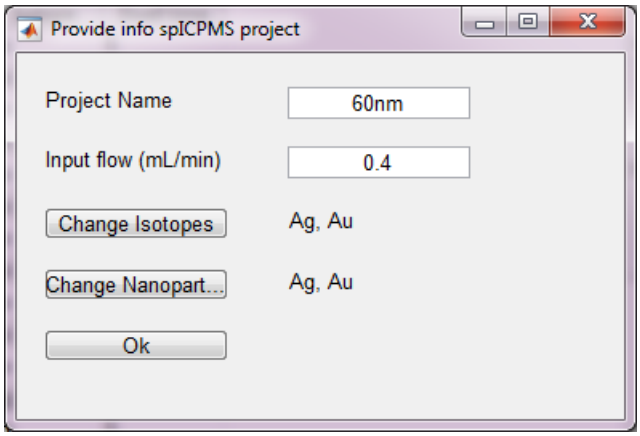


Figure 14 *Providing general info on the spICP-MS project.*

Importing data

Methods

Nanocount is set up to allow maximal flexibility in terms of data formats and how to combine multiple files and multiple elements in individual components. Some things to realize are:

- The same isotope but measured at different dwell times must be seen as two different elements
- Different isotopes, but measured at the same dwell time must be seen as two different elements
- Any file can hold data for one or more elements
- This data can be time series and signal series, but only the signal series is really required. If a time series is missing, data can still be imported, but extrapolation as a function of time becomes difficult.
- Files are combined into tubes using the “Import files” tool
- Tubes are transformed into components in the “Declare import rules” tool
- Each component holds data of only one element.

Figure 15 shows two examples of typical import sequences for a mono-element case and a multi-element case. Note that other examples can be thought of, e.g. a multi-element case where different

files holding data for different elements are combined first in one tube of a particular type, which will in the second stage be transformed in several components of the same type, but each holding data of different elements.

- If one tube has received data from several files, but all are for the same element, then only one component will be created of the same type as the tube, in which all the data is combined into one larger data set (Figure 15a)
- Tubes holding data for different elements are split up in several components of the same type as the tube, one for each element set (Figure 15b).
- All components that contain data have to follow this two-stager sequence
- Nebulisation efficiency checks that do not hold measured spICPMS data can also be declared in the nebulisation efficiency tool. If the user changes his mind later on and the latter type of component should still contain associated data, he/she should delete the component (in the project window) and create a tube and components in the normal way.

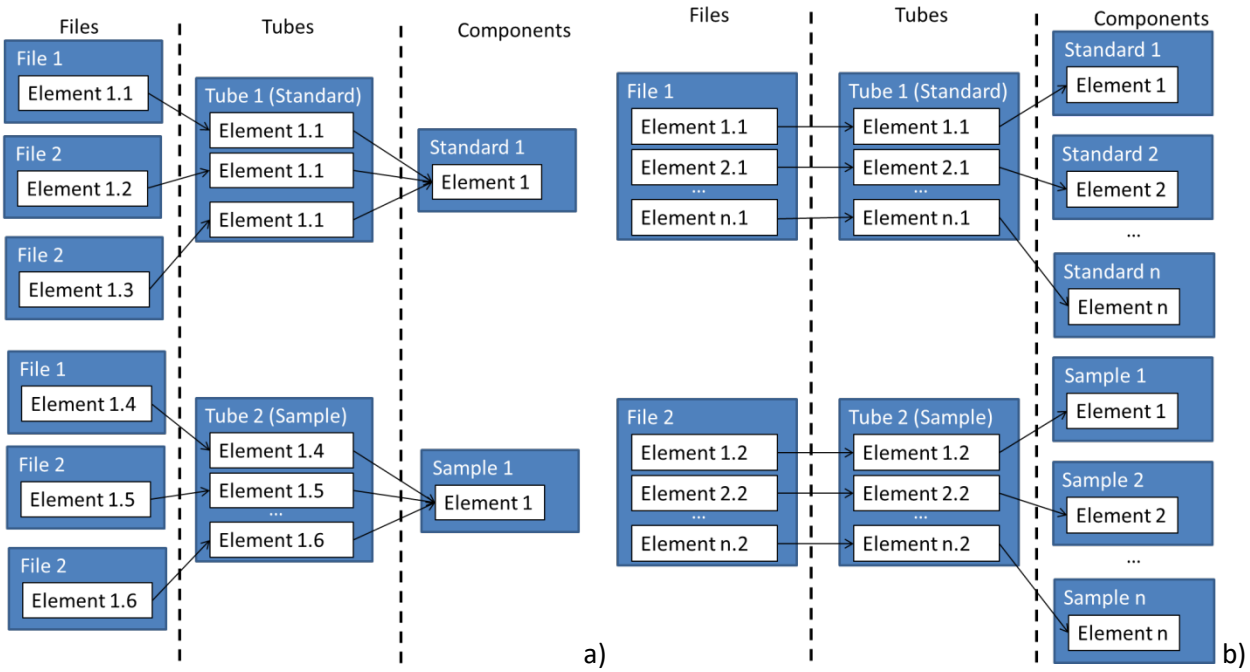


Figure 15 *Two typical import sequences for a) a single element case and b) a multi-element case. The first stage is performed in the "import files tool" where files and tubes are linked. The second stage, where tubes are transformed into components is done in the "declare import rules" tool.*

Import files tool

This tool (Figure 16) serves to define tubes in the project, i.e. which files they will take data from and which type of component the data has to feed into.

Note that attaching files does not mean that the tubes will immediately contain data. Such is only the case after data is imported using the import rules tool.

Three lists are shown in the import files tool:

- “Loaded files”: holding a shortlist of all the text files from which the user will potentially draw data.
- “Assigned files for tube #”: holding all the files that were assigned to the tube selected in the “tubes” table.
- “tubes”: Holding a table of all the existing tubes of the active project. The table can also be used to change the tube type

Files selected in the loaded files list will only be interpreted as text files. It is therefore useless to attempt to upload sample files form the ICP-MS software.

There are several buttons in the tool:

- “Add files”: allows selecting additional files to the list already present in “Loaded files”. An explorer-type window is opened where the user can navigate to the location for the desired text files.
- “Delete files”: Allows removing selected files from the “Loaded files” list. The files are not automatically removed from any existing tube. This has to be done using “Remove associated files”. Note also that delete does not actually delete the file as such, only from the project.
- “Sort files”: Sorts the selected files according to their acquisition time
- “>>>>” Assigns the selected file in the “Loaded files” list to the assigned file list of the tube. If the tube has no name yet, it will automatically get the same name (without extension) as the first assigned file.

Note that it is possible to select the same file more than once, i.e. one file can be associated with several different tubes (such as in Fig. 11b). This can be useful if the file contains data for several elements. One of these element’s data could e.g. be used to create a nanoparticle sample and should thus be assigned to a “sample” type tube, whereas another element’s data could be used to create a nebulisation efficiency check.

- “Remove assigned files”: Removes the selected files in the “Assigned files” list form the assigned files of the selected tube. The file is removed only from the selected tube, not from the Loaded file list.
- “Remove all assigned files”: Removes all assigned files from all tubes. Can be usefull e.g. after a tube scheme is uploaded and the uploaded files are not relevant anymore and therefore all need to be deleted.
- “Assign/create type”: Provides a quick way to create tubes and assigning files. When this button is clicked, a number of new tubes equal to the number of selected files will be created. Each new tube will have the same as the name (without extension) of the file that is also assigned to this new tube. The type of the tubes to be created can be set by the drop down below this button.

- A type dropdown which sets the type to add using the “Type” button.
- “Add tube”: Adds a new empty row below the currently selected component in the “tubes” table. A “dissolved standard” type is assigned per default, but can be altered in the table column “Type”.
- “Delete tube”: Deletes the select component from the components list and from the project. The files assigned previously to the tube are not deleted from the “Loaded files” list.
- “Move Up” and “Move Down”: Simply serve to change the tube order, which may be desirable to keep an overview. The selected tube is moved a position up or down.
- “Save scheme”: The tube scheme created in the “tubes” table can be saved to a file (see paragraph on files) so that it can be reused (and altered) in future projects. Note that both the scheme is saved as well as the assigned files.
- “Load scheme”: A previously saved tube scheme (“*.csc”) can be uploaded to save time. The user will automatically be redirected to the default save location for tube schemes, but can still alter the location where to look for these schemes. The name and type appear in the “tubes” table, and the assigned files of the current tube in the assigned files window. Note that these assigned files still refer to their original file location at the time that the scheme was saved. They will thus not be found if the file locations have changed in the meanwhile.
- “OK” leaves the tool. The changes are automatically saved so discarding changes can only occur manually.

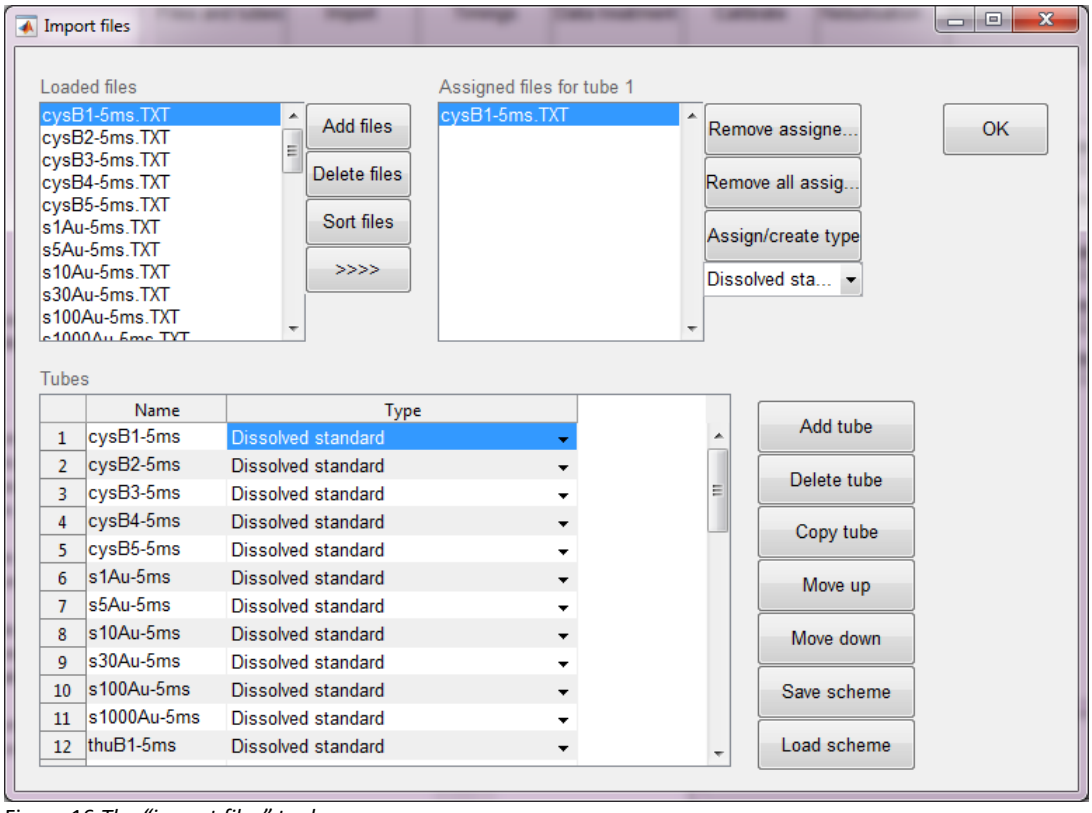


Figure 16 *The “import files” tool.*

The “tubes” table allows changing name and type of components. The table itself is editable except for the column “units” which changes automatically with component type. The name can simply be typed by clicking on the cell of the desired component in the column “Name”, whereas the type can be changed by a drop-down menu. This holds the following options:

- “Particle sample”: nanoparticle samples of which the user wants to calculate a PSD
- “Dissolved standard”: samples used to calibrate sensitivity and blank level for particle samples and nebulisation efficiency checks (if the size method is used) and possibly also dissolved noise parameters if a convolution model is used.
- “Nebulisation efficiency check”: Used to fix the nebulisation efficiency at a certain time point. If more than one is defined, the nebulisation efficiency can be extrapolated as a function of time.
- “Sensitivity check”: A one off measurement to correct the sensitivity of (the) element(s) that may drift as a function of time. The data of the sensitivity check can then be used as a sensitivity “sensitivity check” to extrapolate sensitivity as fixed by the calibration to other time points to account for drift.

The type of the tube selected here, defines the type of the components in wich the tuibe will eb transformed.

The Import tool

In this tool (Figure 17), the import rules can be defined to interpret the data extracted from text files into tubes. The tool has been made as flexibly as possible to accommodate the output generated by most icp-ms software packages, but in rare cases, some pre-arrangement of the data, e.g. using a spreadsheet or text processor, may be required.

Files have to be loaded and assigned to tubes in the import files tool first before the data they contain can be imported into components.

There are three windows in the tool.

- The “Available rules” window lists the created or loaded rules and allows changing them.
- The “Data” window shows the data of the file of a particular file-tube combination selected in the “Available combinations” window. This data is interpreted using the separation character defined by the cell both in the column “Separation” and in the row of the rule selected in the “Available rules” window. This window also allows selecting which cells, rows or columns should be assigned to a specific import rule. Note that this table will show nothing if no rule OR no combination is selected.
- The “Applicable combinations” window lists file tube combinations that were declared in the “import files” tool. This window is used to select one or more combinations. The data of the first file of those combinations that were selected is displayed in the “Data” window. Selections of multiple combinations can also be used to apply rules to several combinations at the same time.

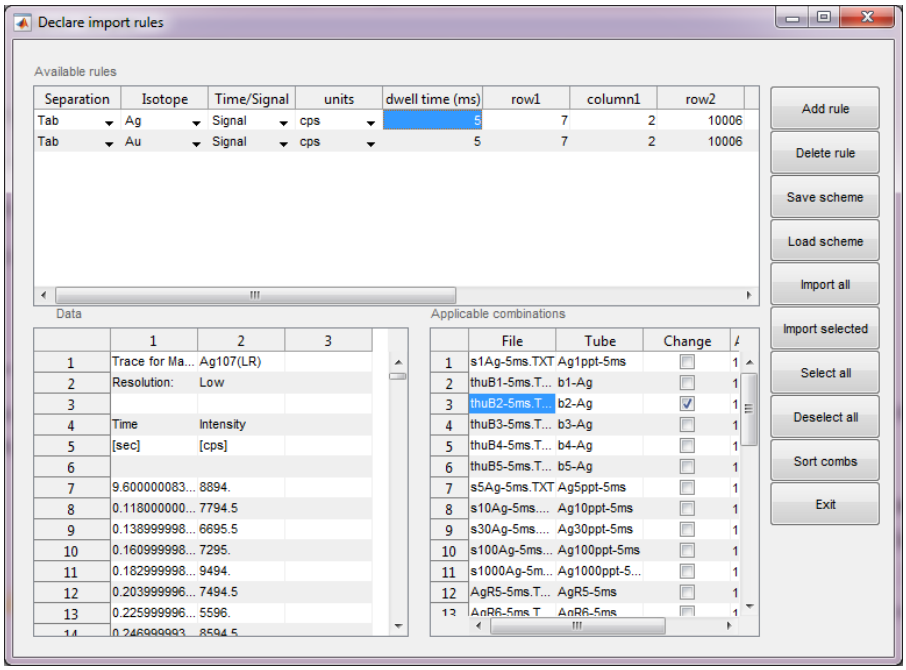


Figure 17 *The import rules declaration tool.*

Buttons

The buttons on the right side have following functions:

- “Add rule”: A new row is added in the “Available Rules” window where a new rule can be defined. Some default values will appear, but those can be changed. The rule will automatically be applied to all combinations.
- “Delete rule”: The rule currently selected in “Available rules” is deleted. Deletion is permanent and will automatically remove it from all assigned combinations.
- “Save scheme”: Saves the set of rules in “Available Rules” into a “*.rul” file (See paragraph on files) so that it can be opened for future projects. Note that the assigned combination numbers are not saved so these have to be assigned manually after opening a scheme.
- “Load scheme”: Loads a set of rules from a file into the “Available Rules” window. This upload will not include assignment to individual files. This has to occur manually. Note that the isotope of the uploaded csc file must already be contained in the project.
- “Import all”: The rules are checked for potentially generating conflicting data (see further) and are then saved to the project. All the data of all combinations is subsequently imported. This deletes all raw data previously contained within components. It also resets the altered data (via the alter data tool) of components to the raw data as well as resets all calibrations to basic ones and removes all operations to existing components.
- “Import selected”: Does the same as “Import all”, but only for the files selected in the “Change” column of the “Applicable combinations” window. Data of components that are not involved in the import of selected combinations are left intact.
- “Select All”: Selects all the combinations (i.e. all the boxes in the column “change” are ticked).

- “Deselect All”: Deselects all the combinations (i.e. all the boxes in the column “change” are unticked).
- “Sort combs”: Sorts the combinations according to the acquisition time of the files associated with the combinations
- “Exit”: exits the tool.

Defining rules

The rules in “Available rules” have many different properties to allow much flexibility in importing data.

- “Separation”: The separation character (Tab, comma, space, semicolon) defining different columns in the data within text files.
- “Isotope”: This drop down menu allows selecting for which one of the isotopes available in the project (as chosen within the “General” tool) the data range within the rule will apply.
- “Time/signal”: interprets the data range for the selected isotope either as data for time or signal. Note that selecting time is not strictly necessary for all applications. If there is no rule for importing time data but there is one for signal data, the data will be set to [1..#signal points] per default. However, certain applications require time, e.g. when drift correction is to be applied or in future Nanocount versions, transient data.
- “Units:” This drop down allows selecting different formats of the datacells. The data is then recalculated into the default units: seconds [s] for time and [ions] for signal (using the dwell time).
- “Dwell time”: The default dwell time of 1 ms is given here per default, but can be changed.
- “row 1; column 1; row 2; column 2”: These determine the data range in the text file for which the rule applies. It can be defined by typing in the row and column numbers of the upper left cell (row 1 and column 1) and the bottom right cell (row 2 and column 2), but most commonly the data is more conveniently selected using from the “Data” window as explained below. Note that only square data blocks can be selected this way, but the organisation of data within such a block may be specified further using segmentation.

It is important to provide an as accurate dwell time as possible as this parameter has a big influence on the final PSD result. The nominal dwell time (as selected for ICP-MS measurement) may be different from the actual dwell time. This can be observed when looking at the raw data expressed in cps. For instance, a true 1 ms dwell time should lead to exactly 1000 cps if only one ion arrived during a dwell (the lowest nonzero value in non-contaminated blanks). Often a lower number can be found, .e.g 998 cps, corresponding to a 1.002 ms dwell time.

- “Segments”: Defines whether there are segments in the chosen data range and how these are oriented. Segments can emerge when the data is not organised as one long row or one long column, but rather in subsequent shorter columns or rows within a bigger square data block. This drop-down therefore allows three choices: “None”, “Rows” or “Columns”. During import, the data of these segments is then arranged in one long chain of data, although the “timings”

tool allows to define intersegment times if such would be required, e.g. in view of drift correction.

- "Segment Length": When selecting a datablock and segmentation, it is assumed that the whole of the block needs to be imported and the total number of cells in rows or columns is therefore given by default. However, a lower number may be desirable in certain cases, which can be altered here manually.
- "Apply to": Is a drop down with different options to assign the current rule to certain File -Tube combinations. When a new rule is created, it is applied to all combinations by default. Using the options in this drop down in combinations with selected combinations, this can be changed using several options:
    - "All combinations": The selected rule is applied only to the files currently selected combinations in the "Change" column of the "Applicable combinations" window.
    - "Only these combinations": The selected rule is applied only to the combinations currently selected in the "Change" column of the "Applicable combinations" window.
    - "Also these combinations": The combinations currently selected for the current rule will be kept, but extend with the currently selected combinations in the "Change" column of the "Applicable combinations" window.
    - "Not these combinations": The combinations currently selected for the current rule will be removed if present in the currently selected combinations in the "Change" column of the "Applicable combinations" window.
    - "All combinations for these tubes": The currently selected rule will be applied to all combinations bearing the tubes present in the currently selected combinations.
    - "All combinations for these tubes": The currently selected rule will be applied to all combinations bearing the files present in the currently selected combinations.
- "Applicable combinations": Is a non-editable column listing the combination numbers to which the rule has been assigned. Similarly, the last column in the "Applicable combinations" shows all rules applicable to all combinations.

Assigning data ranges

Data ranges can be assigned manually in the "row 1"…"column 2" cells in the "Available rules" window, but are more convenient way is through the "Data" window. If a rule is selected in "Available rules" having an appropriate separation character for the data in the file selected in "Loaded files", the data in the "Data" window may be seen to be ordered neatly into cells, columns and rows as shown in Figure 18. Selecting cells in this table triggers different behaviours.

- Selecting a rectangular block of cells of one or more column and row makes a button "cells" visible (Figure 18). Clicking this button transfers ONLY the coordinates of the upper left and bottom right of the block of selected cells to the selected rule. The rule will thus be applied to the data highlighted in the selected data block. If necessary, the data range can still be modified manually as outlined earlier and segmentation can be applied.
- Selecting one column of more than one cell makes the "column" button visible. Clicking this button transfers the data range of the WHOLE column containing data to the selected rule, i.e.

from the most upward selected column to the last cell in the selected column holding data. The “cells” button is also visible to allow selecting only this limited highlighted range.

- Selecting one row of more than one cell makes the “row” button visible, allowing to select the whole row, i.e. from the leftmost highlighted cell until the last cell in the row holding data. The “cells” button is similarly also visible.

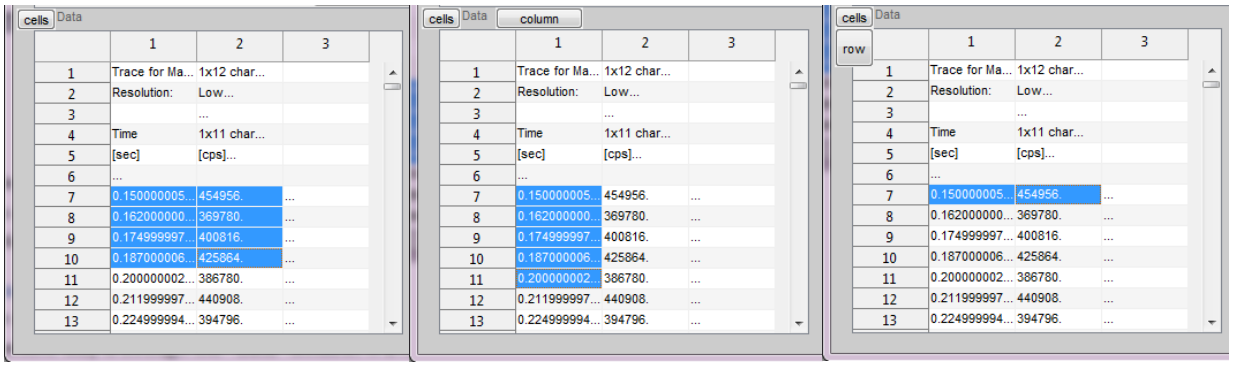


Figure 18 *Different ways of indicating data ranges for the declare import rules tool.*

Import check

When “Import all” or “Import selected” is clicked, a rule check is executed. The table below lists all conditions that rules need to fulfil for data import to proceed:

- There need to be rules
- The rules import data for an isotope that is not defined for the current project. This can occur if a component scheme was uploaded in which another isotope is defined.
- One of the rule property values has been left blank
- Wrong units have been assigned to the rule, .e.g. “ions” are assigned as units whereas the rule is for a “Time” data range.
- Not all the files assigned to components have a rule that provides them with a signal. Note that not having a data range does not trigger an error because if a time range is missing, a default time line is assigned to the data of this component. This is because time is not always necessary to calculate PSDs, unless it involves transient data or drift correction is desired.

The timings tool

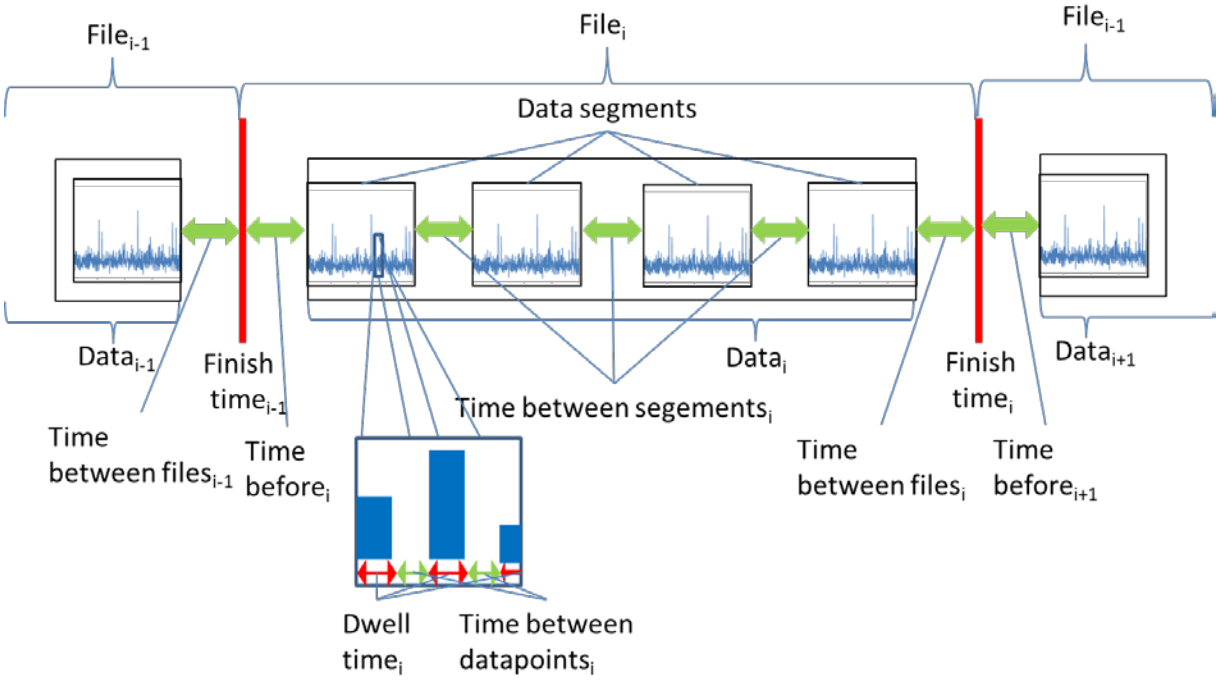


Figure 19 *Position of relevant timings of datafiles on the overall timeline.*

This tool allows precisely setting the timing of data. Knowing when the different data points were measured relative to each other is important when drift correction is used and/or when FAST or transient spICP-MS signals were acquired (not yet implemented in the beta version). There are different timings relevant for data as show schematically in Figure 19. Most of these times can be set for each data object in the table “File timings” (Figure 20), except the dwell times that had to be set during import of the files. The table also allows altering the time order of files, by typing in a new order position in the first column and clicking the button “Reorder” so that a new order position is also attributed to all other files. When opening the timings tool for the first time in a project, the data objects order is based on the “real” order, i.e. based on the information containing in the creation time of the files from which the data originates. It could be desirable to change the order of the files in some cases. The last column of the table displays the real finish time as calculated from the finish time and date contained in the text file attributes, expressed in the units defined by the drop-down “Time units”. This finish time is non-editable, because it is not used in any calculations. It merely serves to allow the user to apply real time order by clicking the “apply real times button”. It is actually all other times together with the file order that determine the timings of data as shown in Figure 19.

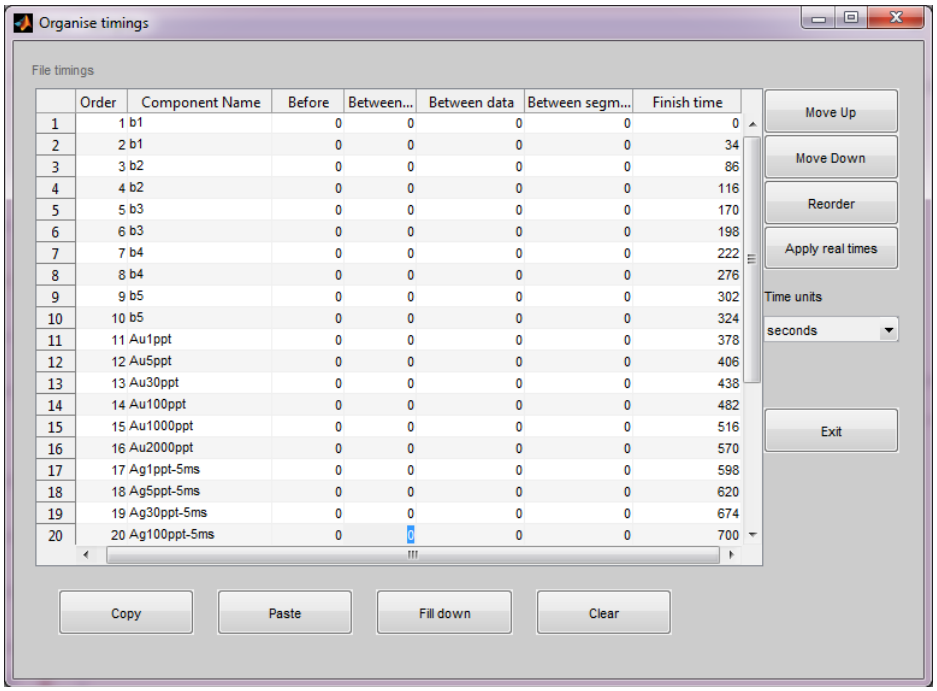


Figure 20 *The "Organise timings" tool.*

Several buttons allow manipulating the table (Figure 20).

- "Move up": The file selected in the table is moved up one order in the table
- "Move down": same but one order down
- "Reorder": The file order is reset based on the values in the first column ("Order").
- "Apply real times": The file order is set based on the finish time extracted from the text file attributes. In this process, the lowest finish time is set to zero and other times increment form there in the units defined in the drop down "Time units".
- "Time units": This drop down determines how all the numbers in the table should be interpreted. Changing the units also changes all existing datai n the table
- "Exit": Clicking this button, without first clicking save, discards all changes and leaves the tool.
- "Copy": A region of the table can be highlighted and by clicking "copy" the editable data can be used elsewhere within the same table when clicking "Paste".
- "Paste": Pastes previously copied data, as much as possible, in a highlighted region of the table or a region bordering a selected cell.
- "Fill down": copies and pastes all the information from a selected cell down to the last cell in a column of the table.
- "Clear": Deletes all values form a cell in the table.

The user does not have to use the real finish time of the samples. He/she can still rearrange the files differently if so wished. The real finish time only determines the order of the files by default so if a different order needs to be used, this can be done using the editable column "Order" and/or the "Move up" or "Move Down" buttons for individual files.

Data treatment

The data treatment tool (Figure 21) mainly serves to remediate as much as possible drift and experimental "accidents" incurred. spICP-MS relies on absolute stability, or at least predictable noise in the signal, but in practice, this stability may be disturbed at times. The data treatment tool can thus serve to compensate for deviations in the general signal level (drift), occurrences of unwanted nanoparticle peaks or the user can cut out unreliable parts of the data.

The data treatment procedure has several features:

Cuts

When correcting data, the data indicated in cuts is removed and not taken into account in any further calculations (red box in Figure 21). spICP-MS data arrays are typically long (> 10000) so cutting out smaller parts of the data often does not compromise accuracy of the final results. Highly compromised data regions can thus be cut out if none of the other data treatment tools suffices to obtain a satisfactory signal.

Sensitivity checks

The cut data is then corrected using sensitivity checks. Intensity sensitivity checks are components of the type "Sensitivity check". The raw data of the selected file will then be corrected linearly based on the time difference between these two sensitivity checks and the difference in measured signal level. This means that the sensitivity checks have to have data for the same element. Note that the data in intensity sensitivity checks can also be treated in this tool. In future Nanocount Versions, the option to select an internal standard will also become possible, which would be relevant only if more than one isotope can be measured at the same time.

Moving average

A simple moving average algorithm is used to discover the overall drift trend of the data (green line in Figure 21). The window length (a.k.a. "period") of this moving average can be changed and optimized in the data treatment tool.

Reference time

The intensity of the moving average at the **reference time** (vertical dotted line in Figure 21) defines the signal level to which the data will be corrected. This means the data will be "straightened" so that it is on average equal to the reference level over the whole time range. It is often, however, unclear where the true signal level should have occurred, e.g. when drift is pronounced as in Figure 21. Most often, it is the lowest average signal level. The local standard deviation at the reference time is also the standard deviation to which the data will be corrected if flicker correction is used as an option

Outlier level

The **outlier level** can also be set during data treatment in Nanocount. Occurrence of nanoparticle signals can have a major effect on the fitted splines that aim to find the average signal level. This effect is not desirable and therefore, nanoparticle events should not be taken into account when calculating the moving average. By setting the outlier level, n, a cut-off O is calculated as

$O = \mu + n \times \sigma$

where μ is the average and σ is the standard deviation. Data above this cut-off is removed before calculating the spline fit. O can be a function that changes with time. Either the overall average of the data or the local average, i.e. the one calculated using moving averages, is used depending on the correction method used. The same applies to the overall vs. local standard deviation. The table below shows which are used depending on which correction technique is used.

	Moving average	Flicker correction	Average	Standard deviation
1	No	No	overall	overall
2	Yes	No	local	overall
3	Yes	Yes	local	local

Flicker correction

Signals that occur at higher levels usually also have a higher standard deviation because the flicker noise increases with average intensity. This means that when signals are corrected only using the splines to the reference level that the standard deviation in parts of the data might differ from the rest of the data. For instance, the data occurring at time t < 3000 s in Figure 21 has a higher standard deviation than the data at t > 70 s, because drift has caused both a higher signal intensity as well as a higher standard deviation. When the Flicker correction option is chosen, not only the average level is corrected, but also the time dependent standard deviation of the signal.

The tool

In the right upper corner, the element of the currently selected component is shown. Beyond that, there are 3 panes and two graphs in the tool.

- "Sensitivity checks" allows using sensitivity checks to correct data.
- "Edit data" allows using moving averages.
- The "Components" pane allows selecting created components.
- The upper graph shows the raw, joined data from the selected component. In this case it may be recognised that the data consisted of only one segment (this is the case in Figure 21, but note that there was zoomed in on the raw data). This graph also shows several lines
    - The moving average (green line)
    - The local standard deviation, shown as moving average – standard devation (yellow line)
    - The outlier cut-off, the blue line.
    - The reference time line, i.e. the dotted vertical line
- The bottom graph shows the corrected data. It is this data that shall be used for all other calculations such as calibrations, nebulisation efficiency and PSD calculations. The pane to the right of this graph allows switching between the time-dependent corrected data or the histogram that will be calculated from this data. If it concerns a FAST project, the histogram is calculated from the integrated peak areas of nanoparticle events.

The options in “sensitivity checks” are

- Reference anchor: Select a predefined sensitivity check to serve as reference level for the linear correction
- Anchor 1: The first sensitivity check before the data
- Anchor 2: The second sensitivity check after the data

Using a low p value is recommended, both in the case of dissolved standards as NP samples. In the first case, you might be losing aspects of the noise that could be modelled and used in a deconvolution method, whereas in the case of NP samples, you might be losing spikes of small particles.

The Options in the “Edit data” pane are:

- “Drift correction”: If this box is ticked, all the options below appear. Unticking this box removes all corrections except cuts.
- “Window”: The period of the moving average. By default, the moving average is set equal to Infinity, meaning that no moving average is used at all. If the user sets the slider to the maximum again, no moving average is used again. Lowering the slider increases the detail of the moving average. Note that cut out data is not used to calculate the moving average.
- ”Outlier”: The outlier level, expressed as the number of times the standard deviation above the moving average. A good value is often 3. This value can either be typed in or the slider can be moved to the right or left. If the slider is moved to the far right, “Inf” appears in the text box and the outlier level disappears. It is assumed in this case that the user does not want to indicate an outlier level at all.
- “reference time” The reference time. Can also be altered by manipulating the vertical dotted line in the raw data graph. By default, this value is set to the average signal level, a level that is calculated ignoring all other corrections such as splines, outlier removal or cuts. This level is often not the one where one wishes to correct the data to. For instance, when there is massive drift, it is not necessarily the average level that is the correct level. This slider allows setting the level to any value between the minimum and maximum measured level. Alternaqtively, the vertical line on the graph can be selected and moved to indicate a good reference time visually.
- “Flicker correction”: Correct the local standard deviation or not, as explained earlier.
- “Remove outliers”: When this box is not ticked, outliers are only removed when calculating moving averages and/or local standard deviations. When the box is ticked, however, the outlier data is effectively removed from the cleaned data. This is shown in Figure 21 where this utility is especially useful for correcting data of dissolved standards. It may for instance happen that nanoparticle signals appear even in these (e.g. because of contamination). Cutting out extreme outliers is recommended in this case because doing such dramatically improves calibration, especially if a deconvolution option is used where noise models are to be fitted. Note that regardless of whether this box is ticked, the splines are always calculated without taking the outliers into account.

- "Optimize": An optimal value for window, the period for calculating moving averages, is sought by scanning many different values and calculating sum of squares (See publication[8]). This algorithm is still under construction, so the results should be critically reviewed.

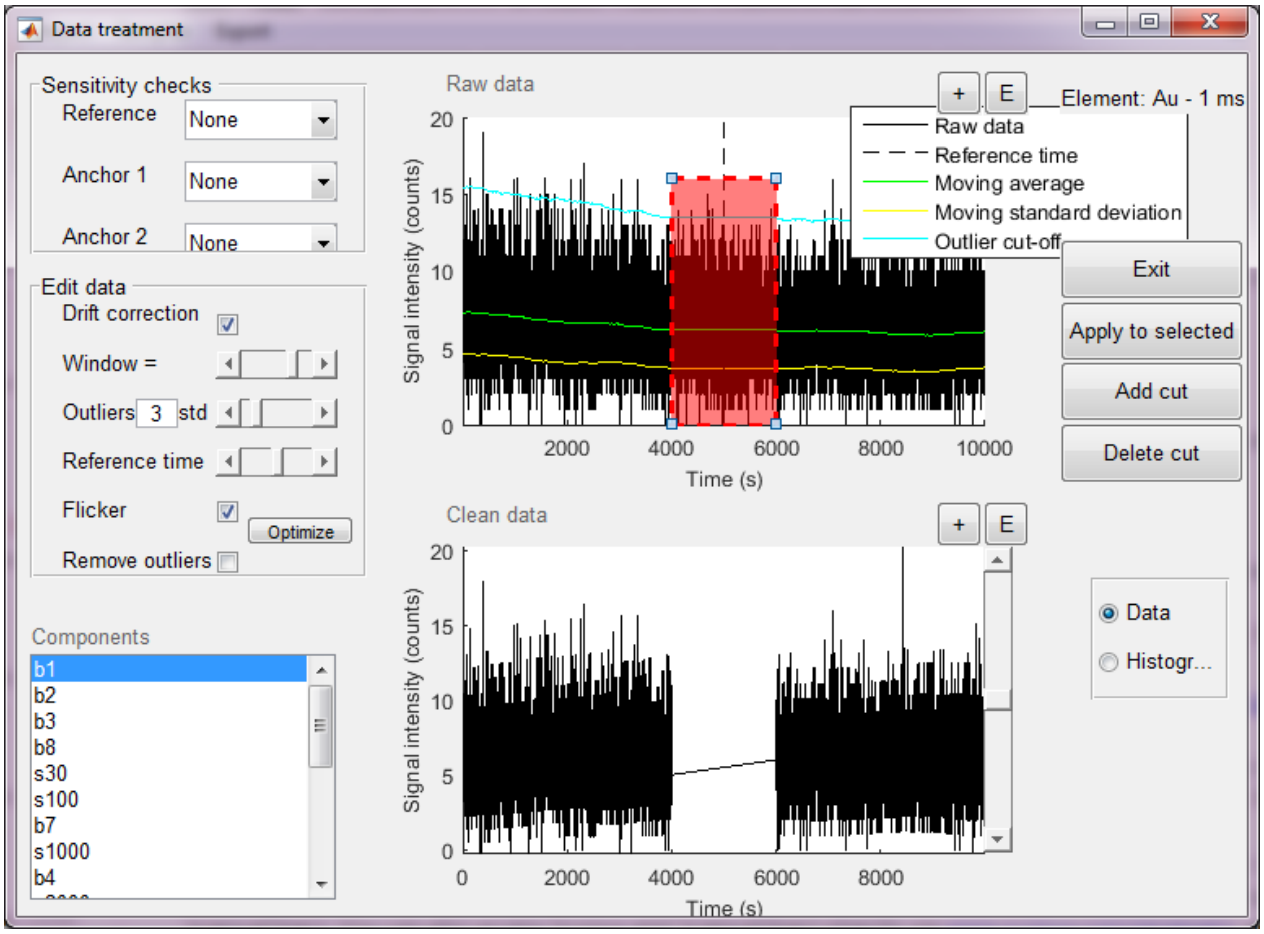


Figure 21 *The Data treatment tool.*

Other features of import are

- "Add cut": This introduces a box in the raw data graph as indicated in Figure 15. Data contained within the time range of this box is removed from the raw data and is also not taken into account when fitting splines. The time range of these cuts can be altered by first selecting the box by clicking on its edges. The selected box will be highlighted as shown in Figure 14. Then click anywhere in the raw data graph and hold the mouse button. The nearest cut edge will shift to the clicked time point. As soon as the mouse button is released, a new time range is defined, and the data is corrected. To deselect the cut, click another cut box or click anywhere outside the edges of the raw data graph.
- "Delete cut": If a cut is highlighted, it will be removed from the graph and the data will be restored.

[8] Cornelis, G. 2016. Drift correction for signle particle ICP-MS. Submitted to Anal. Bioanal. Chem.

- "Apply to selected": In many cases, data treatment can be highly similar for different data objects. By selecting several components in the "Components" table, and then clicking "Apply to selected", the currently shown data treatment settings (moving average, outlier level, flicker correction, remove outliers, sensitivity checks and cuts) are applied to all selected components.
- "Data/histogram": Allows switching the view of cleaned data between the raw data (i.e. intensity as a function of time) or histogram (i.e. frequency as a function of intensity).
- "Exit": Exits the tool. All changes are automatically updated throughout the project, which means that calibrations , model fittings, and/or PSDs should change.

Cutting data means losing data. Small losses are probably not a problem, but statistical significance drops if a too large proportion of the data is lost.

Calibration

Accurate calibration of the dissolved elements in the project is vital to correct calculations. A number of important parameters, specific to the spICP-MS project are determined at this stage:

- Sensitivity: The number of ions per dwell time per concentration unit of analyte that arrives at the detector.
- Blank level: The average background stream of ions arriving at the detector if blanks are measured
- Flicker factor: Only relevant if deconvolution methods are used, based on a normal, poissongaussian (hermite) or a polyagaussian model.
- Shot factor: Only relevant if deconvolution methods are used, based on a polyagaussian.

"Calibration" here only entails calibration using dissolved standards and not a calibration using particle standards, a process that is done in the "nebulisation efficiency" tool. When the latter type of calibration is done for either size or mass per particle AND for number concentration, a dissolved calibration is not necessary to calculate PSDs.

Calibration of noise models gives more confidence that, when deconvoluting dissolved signals from particle signals in the "remove dissolved" tool, that the number of false negatives is limited, i.e. as little true particle signals as possible are removed. Note that in the "remove dissolved" tool there is also an option to do noise fitting without any prior calibration of the noise models. Given the flexibility of some of the used models, there is a high chance that false negatives would occur, i.e. small particles being regarded as dissolved events.

When working with conventional spICPMS projects, calibration in Nanocount can go beyond the classical concentration vs. intensity calibration done in a normal ICP-MS measurement. Depending on the desired noise model, different relations need to be calibrated. There are six different noise models, all of which, except the basic model, are based on [9]:

- **"basic":** A basic linear model where only a classical calibration curve – average signal intensity vs. analyte concentration – needs to be optimized. This is the only model available when working with FAST data.
- **"normal"**: This model is based on a Gaussian distribution to be fitted to dissolved standards and is useful when the measured intensity in both blanks and dissolved standards is relatively high so that all fit a Gaussian distribution.
- **"holistic polyagaussian":** While [9] mainly describes a method based on model fits to separate dissolved standards, it also mentions a direct relation between average dissolved level, total standard deviation, shot factor and flicker factor (equation (15)). During this calibration that relation is fitted non-linearly to the experimental data.
- **"polyagaussian"**: This model is the method described in [9] and is also shown in Figure 18. A polyagaussian model is fitted to each blank and dissolved standard and the noise parameters are related to each other using equations (3),(5) and (6) in [9] to calculate the shot factor and the flicker factor.
- **"Negative binomial"**: Even though a polya and negative binomial distribution are essentialy the same, the different formula by which the negative binomial is calculated often leads to slightly different results compared to the polya distribution. The polya pmf works better in most cases, though.
- **"holistic poissongaussian"**: Similarly to the holistic polyagaussian model, this approach does not fit any model to data of separate dissolved standards. The relation, found in among others Laborda et al.[10], describes a relation between average dissolved level, total standard deviation, and flicker factor. During this calibration that relation is fitted non-linearly to the experimental data.
- **"poissongaussian"**: This approach involves, similarly to the polyagaussian model, a piecewise fitting to each dissolved standard, obtaining the average dissolved level and standard deviation describing a poissongaussian (i.e. hermite) noise model. The flicker factor is then found by linear regression of the standard deviation as a function of the average signal.

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[9] Cornelis, G.; Hassellov, M., A signal deconvolution method to discriminate smaller nanoparticles in single particle ICP-MS. *Journal of Analytical Atomic Spectrometry* **2014,** *29* (1), 134-144.;

[10] F. Laborda, J. Medrano and J. R. Castillo, Quality of quantitative and semiquantitative results in inductively coupled plasma mass spectrometryJ. Anal. At.
Spectrom., 2001, 16, 732–738.

It is highly recommend that one "plays" around with the different available models when using deconvolution. The fit of all standards should be carefully checked and compared with alternative models. Also thoroughly clean up data firs using the "data cleanup" tool. Most often, a good fit is required for the standards having low concentrations, because spICP-MS often have relatively low dissolved ion concentrations. Note also that when a shape factor of zero is fitted for one or more standards, that all standards are automatically excluded and the corrected standards have to be included manually.

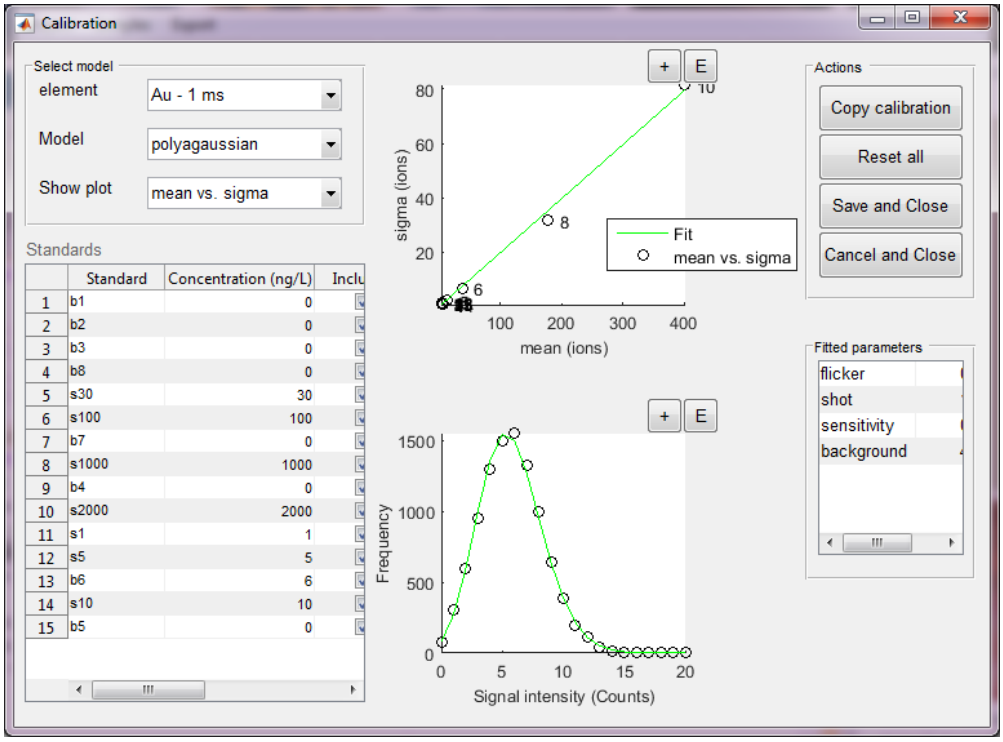

Figure 22 *The calibration tool.*

Features in the calibration tool (Figure 22) include:

- "**Select model" pane**
  - "Element": The element that is being calibrated. Each isotope can per spICP-MS project only hold one calibration. This drop-down box will list all the isotopes for which data has been imported.
  - "Model": The noise model used to describe noise of the data. If no deconvolution procedure is desired, the "basic" model should be selected here. If the model is changed here, the new choice is only saved to the project if "save this as optimal" or "save and close" is clicked. This means that the user can check out another model, even deselect standards and inspect relations without altering his previously saved calibration. If the noise model of the saved calibration is selected here, the originally saved calibration is again shown. This allows the user to "play" with different noise models.

  - "Show plot": To select which relation is to be optimized. There are different options, depending on which model is selected. Upon selection of a relation, the relation is shown in the upper graph, as well as the fit as it had been optimized previously. The table "Standards" also shows all the optimized settings for the specific selected plot.
- "**Standards" table**. This table shows the values, specific for the currently selected relation. The table allows optimizing the calculation of calibration parameters, e.g. by excluding certain standards from a relation, altering the concentration value or even manually adjusting fitted parameters. When a certain standard is clicked in this table, it also becomes the selected one and if the chosen model approach is not "linear" the model fit for that particular dissolved standard is shown in the lower graph. The columns are:
  - "Standard": A non-editable column showing the name of the standard. The name of dissolved standards can only be altered in the "Import files" tool.
  - "Concentration": An editable column showing the user-defined concentrations of the selected isotope in the dissolved standards (in ng L^{-1}). Changing concentrations here, will have an effect on the average intensity – concentration relation, regardless of whether it is the active plot or not.
  - "Included": Allows making particular dissolved standards being part or not from a particular relation. This can be useful, e.g. when a zero shape is fitted while a logarithmic relation of shape must be calculated or when model fit is poor. These dissolved standards can then be excluded from the currently active relationship so that calibration parameters can be calculated more precise. (De)selecting standards has, however, no effect on other relationships.
  - Parameter columns: Depending on the noise model shown, several parameters are shown here. The columns are editable so that the user can supply his/her own values directly. All relevant relationships are subsequently recalculated and shown and the model fit is adjusted as well. Note that if certain values are incompatible (e.g. zero or negative shape values), they are automatically not included in the current relation.
- **Graphs**: The upper graph shows the currently selected relationship with data in circles and the fit in a continuous (green) line. The Pearson correlation coefficient for the current fit is also shown. The bottom graph shows the model fit for the currently selected dissolved standard (via the standard table). The latter graph is not relevant in the case a linear noise model has been selected.
- **Copy calibration:** clicking this button allows copying a calibration of one element to another, provided that both elements have the same isotope. Clicking this button produces a list of all elements in the spICPMS project the calibration can be copied to. The user then selects the desired element. New standards are created from the standards having a different dwell time and the same concentrations and dissolved model are then applied, effectively creating a new calibration.
- **Other actions**
  - "Reset all": Refits the currently selected model and recalculates the relations, including all standards (if possible).
  - "Save and Close": Saves the current calibration and leaves the tool.

  - "Cancel and close": Leaves the tool without saving the current calibration.
- **Fitted parameters:** This pane shows all the fitted/calculated calibration parameters.

Determine regions of interest

Here, one can determine which time regions of a particular sample are of interest and should be analysed further. One also defines how the data will be split up. PSDs are always calculated from a large number of data points (> 1000) and acquiring these always takes some time. Hence, each PSD has to be calculated from some smaller time region called "slices". Each ROI can be divided up in slices in two ways:

- Non overlapping slices: There is no overlap between slices, so each PSD will be calculated from unique data
- Overlapping slices (see Figure 23): PSDs are calculated from moving windows over the whole ROI.

Whichever methods is more appropriate is as of yet unsure, but the overlapping slices have as an advantage that a much higher time resolution will be obtained, because much more data points will be generated if the slices are allowed to overlap. Slices can be added to a given ROI manually in the tool, or by a method that automatically generates are non-overlapping or overlapping slices of a given period.

Figure 23 shows the assign ROI tool's functionalities.

- **"Add ROI":** Adds an ROI to the currently selected sample. An extra ROI appears in the Define ROI graph at a default time that can then be adjusted.
- **"Delete ROI":** Deletes the currently selected ROI. This also removes all associated slices as well as data.
- **Slice method for this ROI:** If an ROI is selected, selecting a method here, automatically generates slices according to the following schemes and adds them to the graph "Define slices in selected ROI", but only if the required values in "slice period" or "slice distance" are given..
  - Equal time non-overlapping: The value in "slice period is interpreted as a time in seconds and the ROI is divided in non-overlapping slices having this period.
  - Equal data points non-overlapping: The value in "slice period is interpreted as a number of data points and the ROI is divided in non-overlapping slices all having this number of data points.
  - Equal time overlapping: The value in "slice period is interpreted as a time in seconds and the ROI is divided in overlapping slices having this period and the first data point of each slice is away from the previous one with the time value given in "Slice distance".
  - Equal data points overlapping: The value in "slice period is interpreted as a number of data points and the ROI is divided in overlapping slices all having this number of data points and the first data point of each slice is away from the previous one with the number of data points given in "Slice distance".

- **“Slice period”:** The length of the slices. Depending on the method chosen, the units are time (seconds) or a number of data points**.** A value here is required before slices can be generated.
- **“Slice distance”:** The distance between starting points of slices. A value here is only required if an overlapping method is chosen. Depending on the method chosen, the units are time (seconds) or a number of data points**.**
- **“Add slice”:** Slices can be added manually to the selected ROIs using this button. The appear at a default time and can then be adjusted in the “Define slices” graph.
- **“Delete slice”:** The currently selected slice is deleted as well as all of its data.
- **“Components:”:** All the samples defined for the current project.
- **“Define ROIs graph”:** This graph shows the whole data of the currently selected sample as well as all the ROIs that have been defined. Selecting ROIs works by clicking on the edges of one and the part of the data contained within this ROI will then be shown in the “define slices” graph. If there are any slices, they will then be shown too. The range of all the ROIs can be manipulated here.
- **“Define slices in selected ROI:”** This graph shows the data and all slices of the currently selected ROI. Slices can either be added using one of the aforementioned methods or manually using the “Add slice” button. The range of all the slices can be manipulated here and slices can be selected to be removed. Only one can be removed at a time.
- **“Exit”:** Exits the tool.

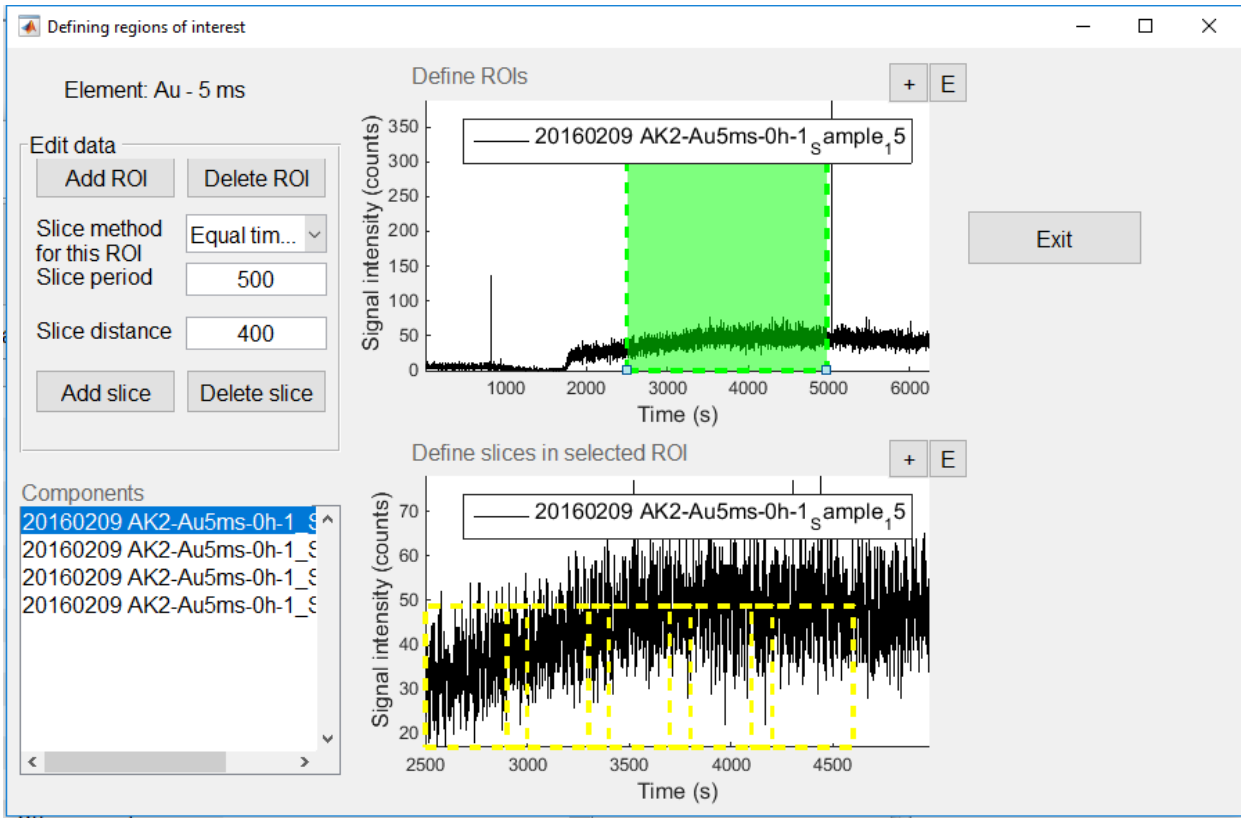

Figure 23. The Assign ROIs tool.

Remove dissolved signals

Methods

Method types

This tool allows removing the dissolved signal from combined NP – dissolved signals using several methods available in the literature.:

- **"None":** Choosing this option allows a manual setting of the dissolved level at an arbitrary value.
- **"n x sigma"**: This approach is based on outlier analysis[11] where nanoparticle signals are recognised as outliers if they exceed a user-defined number of times the standard deviation.
- **"Deconvolution"**: This approach is based on[9] where a calibrated noise model is fitted to the lowest-intensity data points of the signal histogram. The resulting estimate of the dissolved part of the combined signal can then be removed from the data. This option is not available for FAST data.
- **"Noise fitting"**: A statistical method is fitted to the low intensity signal intensities of the sample, similarly to the deconvolution method, but the model is not constrained by a calibration as in the deconvolution method. The method therefore also works when only a basic calibration has been done. The chosen statistical model is fitted by minimizing the least squares differences with a chosen subset (low intensity signals) of the sample histogram. A downside of this method is that large degrees of freedom exist when fitting models straight to sample data, possible resulting in inaccurate fitting compared to the more constrained deconvolution method, especially if a low number of fit points beyond the first peak maximum is chosen.
- **"Standard fitting"**: The histogram of a measured standard is fitted to the low intensity data of a sample, only by altering the number of dissolved data points. This method can be useful if none of the statistical models fit the background data, but it is very unflexible in that sense that if the dissolved level in your sample is not exactly the same as in one of the standards, the fit will probably be less than satisfactory. An advanced calibration using a statistical model is thus also not required.
- **"K-means"**: Based on a different algorithm[12] that identifies clusters the data in the raw histogram. The cluster with the lowest average data is the background signal.

Choosing a good method

Figure 24 gives an indication of how to find the best possible method to distinguish dissolved and particulate signals. More advanced methods are not always the best option, but may be necessary especially if there is a lot of overlap. Not that The deconvolution method is the best one ion the latter case, but it also requires that several standards have been measured and that one of the available

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[11] Tuoriniemi, J.; Cornelis, G.; Hassellöv, M., Size discrimination and detection capabilities of single-particle ICP-MS for environmental analysis of silver nanoparticles. *Analytical Chemistry* **2012,** *29*, 743-752.

[12] Bi, X.; Lee, S.; Ranville, J. F.; Sattigeri, P.; Spanias, A.; Herckes, P.; Westerhoff, P., Quantitative resolution of nanoparticle sizes using single particle inductively coupled plasma mass spectrometry with the K-means clustering algorithm. *Journal of Analytical Atomic Spectrometry* **2014,** *29* (9), 1630-1639.

statistical models fits the data and leads to linear relationships between the mean dissolved signal and other model parameters.

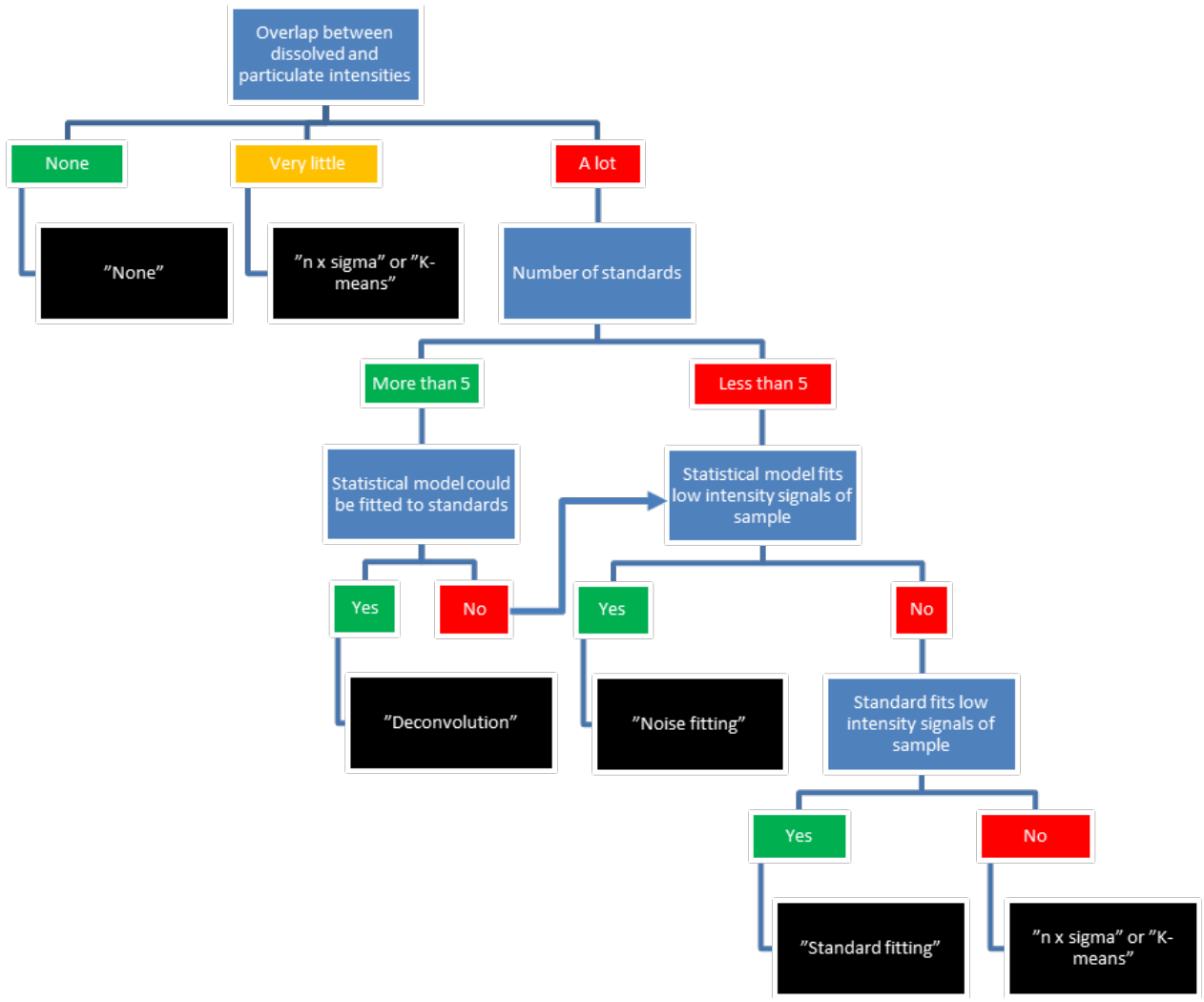


Figure 24 Decision scheme to come at the best possible method to distinguish dissolved and particulate signals.

Functionalities

Many of the functionalities in the tool vary with the chosen deconvolution method. Common elements in the tool are (Figure 25):

- **"Current element"**: This pane shows the element of the sample currently chosen in the "loaded samples" listbox.
- **"Deconvolution options"**
  - **"Select method"**: Select one of the above methods as the active method
- **"Loaded samples"**: Allows selecting samples. The data of the first selected sample is then shown in the graphs. Multiple selection allows extrapolating deconvolution settings to other samples.
- **"Raw data":** Shows the histogram of the raw data, the dissolved level as a vertical dotted line and, if applicable, a fitted dissolved noise model.

- **“Processed data”**: Shows the deconvoluted data.
- **“Apply to all”**: This button applies the settings for the current sample to all samples that are currently selected in the “Loaded samples” list.
- **“Exit”**: Exits the tool.
- **“Fit results”**: Depending on the deconvolution method chosen, this pane shows some useful results from the setting of the deconvolution procedure. Common for all methods are
    - **Sum of squares**: Is used only for the deconvolution method. The sum of squares of the currently displayed fit. Can be used to assess whether a change in parameters leads to a better or worse fit.
    - **Total number of data points:** this is determined by the number of data points in the raw data, remaining after data clean-up
    - **Particle events**: The number of data points that will be considered for calculation of the PSD
    - **Dissolved events:** the number of data points that will not be considered for calculation of the PSD

No specific method

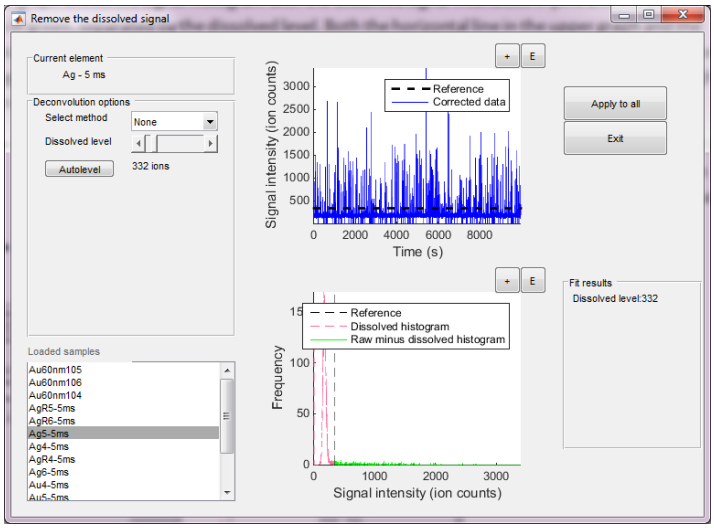


Figure 25 *The deconvolution tool usingthe method “None”.*

If no specific method is use (option “None”) the top graph reflects the raw data in time space and a horizontal black line shows the dissolved level, i.e. the signal intensity level below which data points are considered emanating from dissolved signals and above which they are considered particulate. The lower graph shows the signal histogram with the dissolved signal intensities in pink and the particulate ones in green, separated by the dissolved level. Both the horizontal line in the upper graph and the vertical one in the lower graph can be manipulated to alter the dissolved level. This is useful if the user recognizes visually features such as a peak maximum that suggests where NP signal intensities roughly begin and dissolved ones stop.

- **“Dissolved level”** (sliderbar): By de/increasing the dissolved level, the horizontal or vertical line in resp. upper and lower graph, the dissolved level can be changed. Note that the minimum

number is 0. The text below this slider shows the number of ions that is considered the dissolved level for the selected sample.

- **"Autolevel"**: This feature is explained to some detail below and serves to provide a more objective means to select the dissolved level.

n x sigma

An alternative method is the n x sigma method[11]. Figure 26 shows the window when this option is chosen. The default value for n is 3, but it can be increased below and above at will, even to negative values higher than -5. A maximum of n = 15 is allowed by Nanocount. In addition, n is a continuous variable (as opposed to the number of fit points in the deconvolution method) and can thus take on non-integer values. The top graph now shows the data in time space and the bottom graph the histogram, dissolved signals in pink and particulate ones in green. Again, several ways exit to find the optimum level of *n*.

- **"Outliers" (slider):** A slider is available to slide the *n* value up and down. Nanocount will then consider all signal levels higher than n x standard deviation as nanoparticles and all signals lower as dissolved signals. The resulting dissolved level is then shown, both in the raw data (upper graph) as in the processed data (lower graph). The processed data shows the dissolved signal in pink and the nanoparticulate signal in green.
- **Autolevel:** similarly to the deconvolution method, this button first allows the user to determine a search range (See further).
- **Graphs:** The dissolved signal level can also be set graphically in either the raw data graph or the processed data graph. Just click on the black dotted line to make it active. Then drag the line (up and down in the upper graph, left or right in the lower graph) to the desired dissolved level. Nanocount will update the slider level and the dissolved level in the other graph that was not manipulated, showing the new dissolved level.

After setting n, the dissolved signal histogram is shown in the bottom graph as a red dotted line, whereas the signal – the dissolved histogram, i.e. the nanoparticulate histogram, is shown in green.

If the dissolved level is set graphically (either via the raw data or processed data graph), Nanocount will calculate an n value so that n x standard deviation equals the desired dissolved level as close as possible. That is why you may see the lines in the graphs jumping around after you release the mouse.

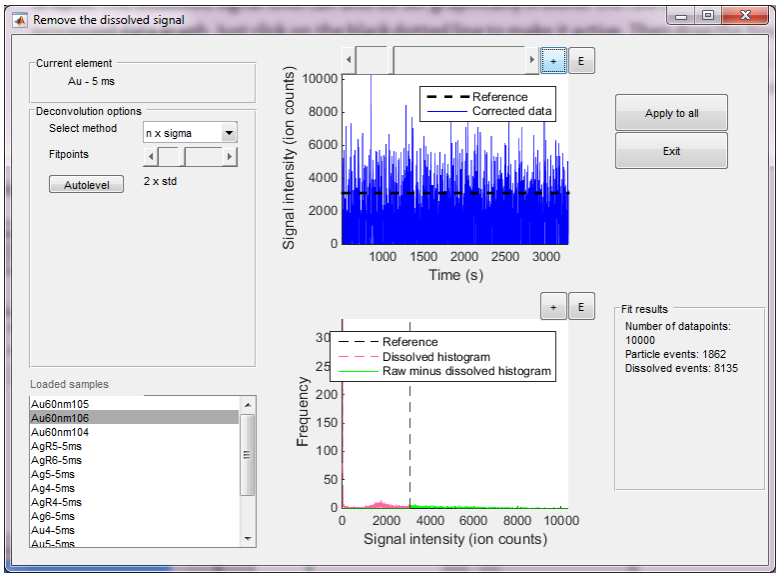


Figure 26 *The remove dissolved tool using the n x sigma method*

Deconvolution

This option is not available for FAST data. During a deconvolution method, a number of data points needs to be set to which a soluble noise model will be fitted. This implies that these data points must contain signal frequencies of non-NP signals. This implies that the number of fit points should be as low as possible, but enough to accurately fit a dissolved model. Note that the minimum number of fit points is two.

Dissolved signals have on average a lower signal intensity as NP signals, because NP signals always carry a dissolved component (see[9]). The first local maximum in a spICP-MS histogram is usually a dissolved peak so start by setting the number of fit points somewhat beyond the first maximum. Note that this maximum often occurs at signal = 0 in the case of a very low dissolved background concentration.

Nanocount offers several ways of setting the number of fit points:

- **"Fit points"** (sliderbar): by de/increasing the number of fit points, a higher number of data points will be used to fit the dissolved model set during the calibration stage. The number is also shown graphically in the upper graph by the dotted black vertical line. Note that the minimum number is 2. The vertical line in the data graph can also be selected and moved by clicking/dragging. This is useful if the user recognizes visually features such as a peak maximum that suggests where NP signal intensities roughly begin and dissolved ones stop.
- **"Autolevel"**: This feature is explained to some detail below and serves to provide a more objective means to select the number of fit points.
- **"Deconvolve"**: Regardless of whether this box is ticked, the fitted dissolved curve is always subtracted from the combined dissolved – nanoparticulate signal (The red dotted curve inFigure 27). However, this histogram still contains components from the dissolved noise (see [4]). The deconvolve option allows to mathematically deconvolve this noise out of the red dotted curve in

Figure 27 resulting in the green curve. It is the result of mathematical deconvolution of the dissolved signal fitted in the upper graph from the subtracted green curve in the bottom graph (total signal – dissolved signal). Deconvolution is a complex process that not always results in meaningful results. For instance, it is required that the information being deconvolved (i.e. the dissolved fit) contains less information (i.e. has a lower bin length) than the histogram from which deconvolution occurs (i.e. the raw histogram minus the dissolved fit). If this condition is not satisfied, deconvolution simply returns the same information as in the red dotted curve again and only the green curve is visible. There are, however, two slider bars to manipulate this process to some extent.

- **"Ignoring":** The signal intensity level below which the signal should be ignored: the ignore factor. The signals below this value are removed from the fitted dissolved curve before it is used in the deconvolution process. Very often, the ignore factor needs to be increased above the minimum level (where all the information in the dissolved fit is attempted to be used) for deconvolution to resort in some effect. The largest effect of deconvolution occurs with NPs with relatively low sizes[4]. Such histograms do not contain much information to start with so large, dissolved fits need to be shed of the relatively unimportant high signal values that often have a very low signal frequency. This process reduces the length of the bin of the dissolved signal so that it may become lower than the bin length of the NP data and thus be deconvolved from data such as the the red dotted line. In this case, as shown in Figure 27, both the red dotted line and the green curve are visible, because deconvolution was successful. Note, however, that the ignoring factor should be kept as low as possible (i.e. using the maximum of information possible) to arrive at accurate results. If the ignore factor needs to be set very high for deconvolution to work, it may be a better option to not use the deconvolution option at all.

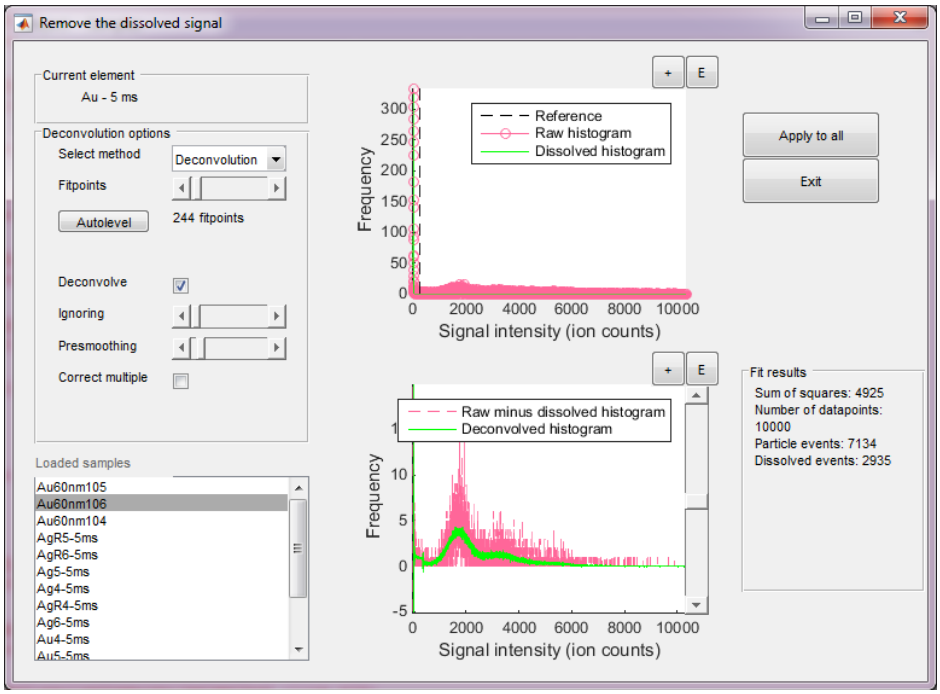


Figure 27 *The deconvolution tool using the deconvolution method.*

- **"Presmoothing":** The nanoparticle – dissolved histogram (i.e. the green curve) contains too much features so that a much distorted histogram is obtained after deconvolution, because the mathematical procedure of deconvolution often leads to meaningless results if "unsmooth" curves are used. The "pre-smoothing" tool can sometimes help to resolve such problems. By pre-smoothing the raw histogram minus the dissolved fit (i.e .the red dotted curve in Fig. 19), a successful deconvolution may be obtained. The resulting deconvolved histogram will of course be a lot smoother than the original raw histogram minus the dissolved fit, because smoothing always results in loss of data. The smoothing factor therefore needs to be at a minimum level that results in meaningful data. If over smoothing is applied, e.g. resulting in total loss of data or infinite values, an error screen may appear.

Pre-smoothing of the raw histogram – dissolved fit is not visualised, only the final deconvolution result is shown. This histogram will be already smoothed to some extent because it was calculated from smooth histograms. However, further smoothing of this deconvolved histograms itself is not possible in the dissolved signal removing tool. This is only possible in the PSD calculator tool.

- **"Correct multiple"**: Checking this box enables an experimental algorithm used to automatically compensate for incomplete and multiple particle events. However, the algorithm has not been validated yet and it is not recommended to use this option.

Noise fitting

This option is not available for FAST data. The noise fitting method works very similarly to the deconvolution except that a noise fitting calibration is not required. It will merely fit a user-defined noise model (using the "Select model" drop down in Figure 28) to the data using a user-defined number of fitpoints (Figure 28). The noise fitting method works with any calibration, including a basic one as it does not source calibration objects for information while fitting the user-defined noise level.

The noise fitting method is particularly useful if calibration of a noise model was not possible, e.g. because of drift, etc… However, the high flexibility of some of the models used, particularly the polyagaussian model, generate a high risk that false negatives will occur, ie. small particles being recognized as dissolved signals. The deconvolution method is therefore recommended if possible.

When using the noise fitting for the first time, the default model that is fitted is the polyagaussian model, but the user can select the Normal model or poissongaussian via "Select model". Other than that, all other featuruses are the same as in the deconvolution method.

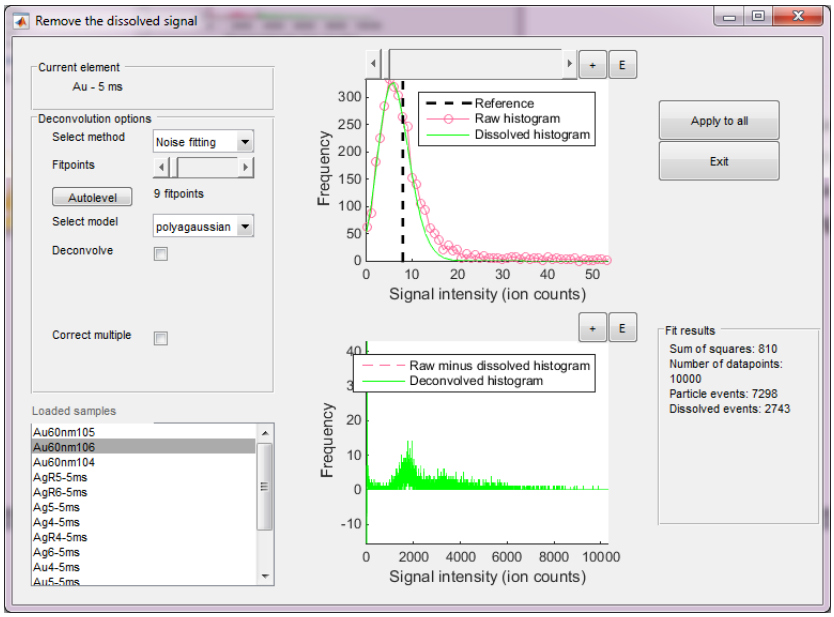


Figure 28 *The remove dissolved tool using the Noise fitting method*

Standard fitting

The histogram of a measured standard is fitted to the low intensity data of a sample, only by altering the number of dissolved data points. This method can be useful if none of the statistical models fit the background data, but it is very unflexible in that sense that if the dissolved level in your sample is not exactly the same as in one of the standards, the fit will probably be less than satisfactory. An advanced calibration using a statistical model is thus also not required.

Figure 29 shows the options when using the Standard fitting method, many of which are similar to the “Deconvolution” and “Noise fitting” methods and are explained there. Specific to this method is the drop down menu “Select standard” where one selects the measured standard to fit to the low intensity signals of the chosen sample. The number of fit points can similarly be altered than in other methods and is used to find the most appropriate number of dissolved events where the histogram of the chosen dissolved standard best matches that of the sample histogram.

Note that this method does not require an advanced calibration. This option is also available to use with FAST data.

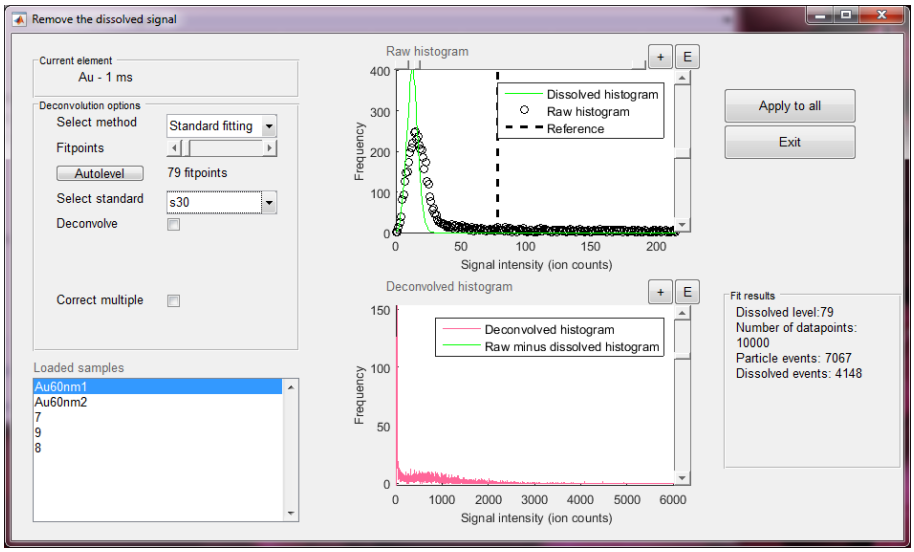


Figure 29 *The remove dissolved tool using the Standard fitting method*

K-means

The K-means algorithm identifies clusters of data in the raw histogram, based on their distance from the mean. Nanocount allows the user setting the number of possible clusters using the slide bar as shown in Figure 30 (where the number of clusters, k = 3). The minimum value for k = 2 as there is always one cluster for the dissolved signal and at least one for the particle signal. Nanocount has an arbitrary maximum of k = 12 clusters. The cluster having the lowest average signal intensity is the background signal and is shown in the Processed data graph in a red dotted line. The dissolved signal level is also indicated with a horizontal and vertical dotted line in the Raw data and Processed data graphs, respectively. Note, however, that in the case of the K-mean algorithm, that these lines cannot be manipulated as they could, e.g. in the n x sigma algorithm. The reason is that most dissolved levels are unlikely to converge to solution for a corresponding k value. Similarly, there is also no Autolevel function available for this algorithm.

Nanocount only shows the cluster with the lowest signal intensity as the background signal. However, k may have to be increased above 2, e.g. if the particle disitribution is multimodal. The user can visually recognise this in the Processed data graph. In addition, increasing k above 2 does not necessairily lead to more clusters being recognised if there are none. The user may thus find that increasing k has often little or no effect in a particular k range.

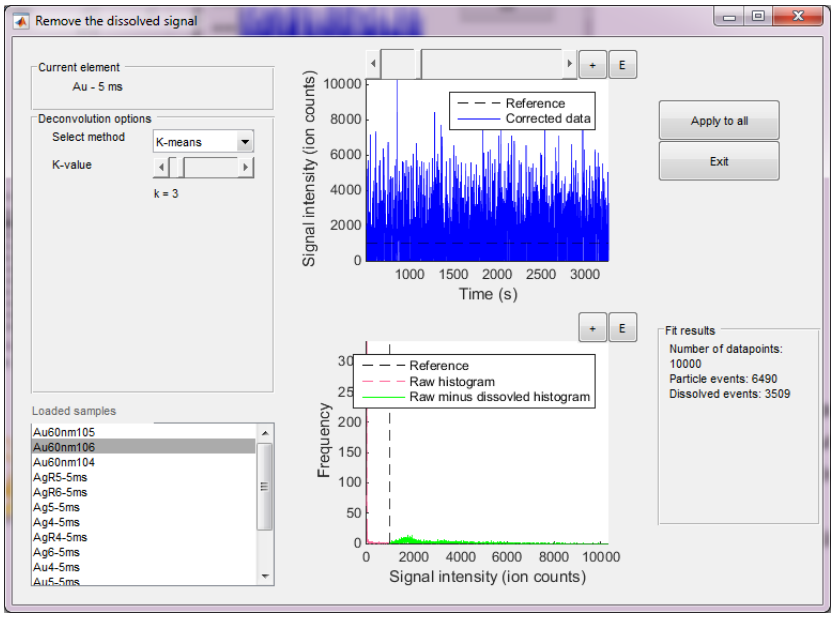


Figure 30 *The deconvolution tool using the K-means clustering method.*

The autolevel search engine

The autolevel search engine automatically applies the method defined by "set method" to the data, using a range of different numbers of "fit levels". The fit level depends on the method chosen. In the case of "none" it is the dissolved level, n for the n x sigma method and the number of fit points for the deconvolution and noise fitting options. Note that there is no autolevel option for the K-means method. A trend thus emerges, in the number of data points that is considered nanoparticulate or dissolved and different sums of square errors will be obtained. When a further increase in number fit level does not result in a change of nanoparticulate data points and/or the sum of squares no longer improves (or gets worse), this is an indication that an optimal number of fit points has been reached. When clicking the autolevel button, the dialog box in Figure 31 appears.

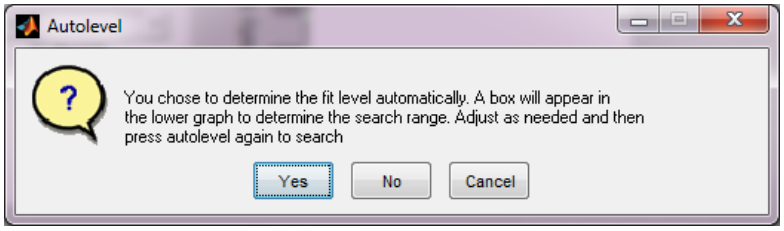


Figure 31 *The notification of the autolevel search engine.*

Clicking "No" or "Cancel" stops the autolevel functionality. Clicking "Yes" continues it. This feature was built, because the fitting procedure may require a lot of computing time on somewhat slower computers. Selecting a search range, e.g. before the first peak in the histogram, will thus skip unnecessary computing time. A red box will appear in the lower graph. The vertical lines of this box indicate the start and stop range over which the autolevel function will look for the optimal number of fit points. Default values are set (start range = 2 fit points, stop range: 12 fit points) and the box is adjustable by selecting it and then clicking and dragging.

The red box may not be clearly visible at first. The zooming functions of the graphs are always active so try zooming in the lower graph to make the red box more clearly visible so that you can adjust its boundaries.

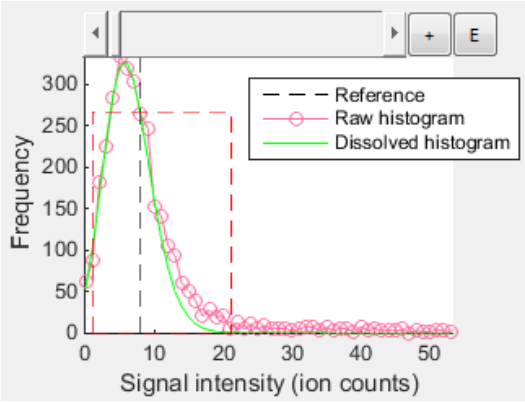
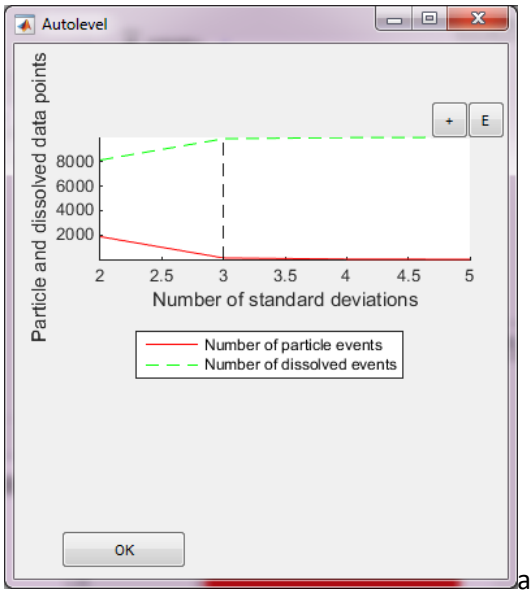
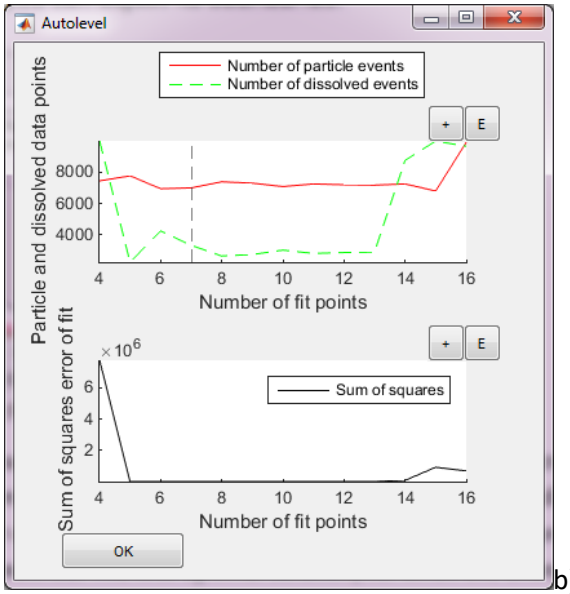


Figure 32 *Setting the search range for the autolevel function*

Once you have selected a search range, click autolevel again. Nanocount will increase the number of fit points and calculate the number of nanoparticulate data points, the number of dissolved datapoints and the sum of squares error for each selected number of fit points. It will halt this process when the end of the search range has been reached. The first optimal solution, i.e. when the change in calculated nanoparticulate data points is less than 5 % relative to the previous (lower) number of fit points, is remembered.



a)



b)

Figure 33 *The autolevel search engine results a) when using no specific method or the n x sigma method and b) when using the deconvolution option.*

A new window appears (Figure 33) showing the results of the search procedure. The number of nanoparticulate and dissolved data points is shown in the top graph, whereas the sum of squares error is shown in the lower graph in the case the deconvolution method is used. In the case the search was

successful; a dialog box appears (Figure 34). The graphs in the main window also show the result of the last fitting procedure graphically. The user has some options now:

- Accept the last solution by clicking “OK”.
- Using a different fit level by selecting the black dotted line and dragging it to the desired level and clicking “OK”.

Once the autolevel utility has been started, the red box remains in the lower graph and autolevel searching can be restarted. It is also possible to manually reset the number of fit points to any value.

Note that only integer fit levels are possible at all times, including for the n x sigma value. Morevoer, the n - search range for the n x sigma method is determined by first finding the n values that match the range set by the box in Figure 32, which is based on intensity values. If no solution is found for these values and thus no n – search range is calculated, no optimal solution for n is found.

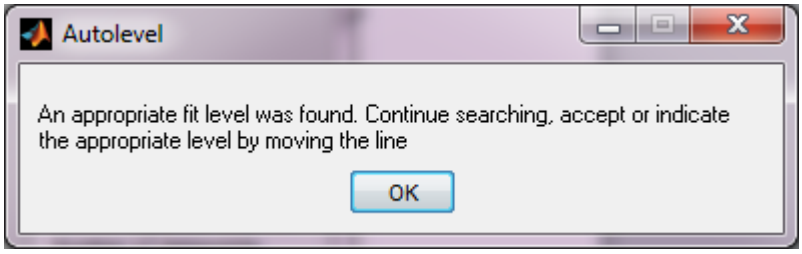


Figure 34 *The message appearing when an appropriate fit was found.*

Fast projects

Integration is a necessary step to process data from FAST projects into PSDs. The nanoparticle events drag out over time and the total intensity of the nanoparticle event has to be evaluated over some time range. The integration step is done in the “Remove dissolved signals” tool. Nanocount currently offers two methods: “none”, “fixed”. A third method is still to be programmed (“Variable”). Removing dissolved signals is then only possible using the visual, nxsigma, Standard fitting, and K-means methods. The latter occurs in much the same way as conventional projects.

“None” integration method

This method recognizes beginning of nanoparticle events as non-zero signals and endings of events when the signal goes back to zero. Additional beginnings and endings are added when so-called “valleys” are encountered, i.e. the signal decreases to a non-zero value and shortly thereafter increases again. Everything in between a beginning and an ending is considered the nanoparticle event and is integrated. The method as such has no additional integration parameters.

Fixed” integration method

This method was published by Tuoriniemi et al.[13] (Figure 35). Peaks are detected as second derivatives going through zero. All around this peak in a fixed integration window is considered belonging to a

[13] *Tuoriniemi, J.; Cornelis, G.; Hassellöv, M. 2015. New Peak Recognition Algorithm for Detection of Ultra Small Nano Particles with Single Particle ICP-MS Using Rapid Time Resolved Data Acquisition on a Sector-Field Mass Spectrometer. J. Anal. Atom. Spectr. 30, 1723 – 1729.*

nanoparticle events, regardless of whether there are any valleys within the window. The method thus has an integration window that must be set. The method also requires the lowest number of ions that can possibly be considered a particle event based on their shape. This is relevant when the dissolved background is extremely low. Nanocount will only consider Gaussian-like peaks of all events with an integrated ion count having this value or more. The maximum is four, because events of more ions than four can assume too many different shapes that may all be Gaussian.

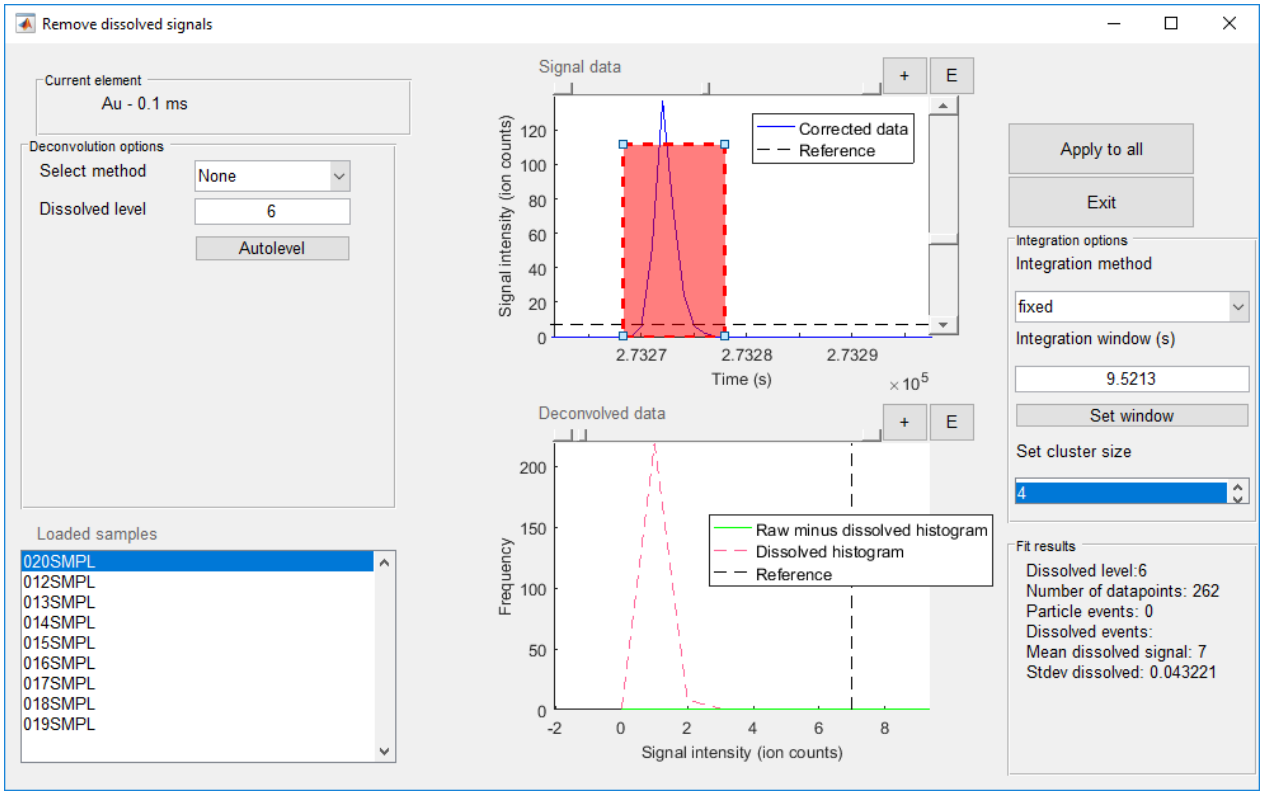


Figure 35. The "remove dissolved signals" tool in the case of a FAST project using the fixed integration method

Some extra features are in this case:

- **"Integration method"**: "None", "fixed" or "Variable", but the last method has not yet been programmed in
- **"Integration window"**: The units are seconds, the same as shown in the top graph in all methods except standard fitting. Typing a different value here alters the fixed integration window length and reintegrates the data.
- **"Set window":** clicking this button creates an adjustable box in the center of the screen. Adjusting the size of the box, e.g. so that a particle event is covered completely, changes the length of the fixed integration window and reintegrates the data.
- **"Set cluster size":** alters the minimum number of ions an event must integrate to in order to be recognized as a particle event based on their shape. The maximum is four.

When using the Standard fitting method, the top graph switches to a histogram view. The button set window will still work as before, but as the X-axis of the graph no longer shpows time, but signal intensity, non-sensical results will be reached. It is thus recommend to first set the integration window visually when using another deconvolution method (e.g. "None") and then switch to the Standard fitting deconvolution method to optimsie the number of fitpoints.

Transient projects

In the case of transient projects, signal discrimination has to occur for each slice. If a sample in a transient project contains no slices (e.g. nebulization efficiencies), the tool looks very much like it does for batch projects, but if ab ROI has been defined for a given sample, the tool looks like Figure 36. Two extra boxes appear at the right, where the different ROIs can be chosen, based on their time limits. A similar box appears for the slices. This means each individual slide can be analysed with different methods if need be, but in most cases, the same method is applied for all slices in a given ROI. The new button "apply all slices" applies the settings used for the currently displayed slice to all the slices in the ROI. There is no apply to all samples button anymore, because the number of samples in transient projects is usually much lower compared to batch projects.

All methods available for batch tests are also available for slices of transient signals. This means that if many slices are defined in an ROI, and a complicated method such as deconvolution is chosen, that signal discrimination can take a very long time.

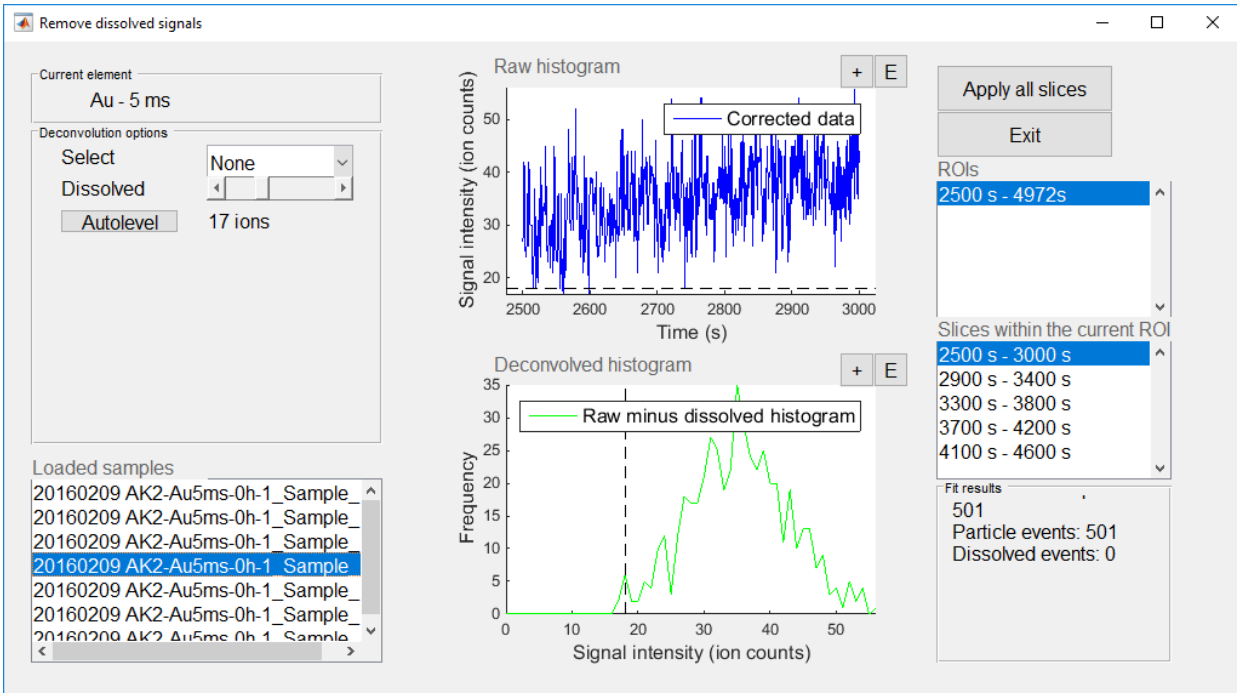


Figure 36 The "remove dissolved signals" tool in the case of a sample holding ROIs

Nebulisation efficiency

Methods

The nebulisation efficiency tool serves to allow Nanocount to calculate nebulisation efficiency (η) from the nebulisation efficiency checks. There can be several nebulisation efficiency checks so that η can vary throughout the sample series and can be extrapolated using the PSD calculating tool (see further) based on the time the nebulisation efficiency check occurred. The nebulisation efficiency tool allows using a couple of different methods to calculate η:

- **Diameter**: Based on[14], this method uses standards of known size (usually 10^6 x diluted NIST Au nanoparticles of 60 nm). A calibration curve of dissolved Au standards needs to be measured as well and analysed first using the calibration tool (see earlier) so that sizes can be calculated from signal intensities. A certain signal intensity is selected as the one corresponding to the certified size. Nanocount then alters η so that the certified size indeed corresponds to the indicated signal intensity.
- **mpp:** Is exactly the same as the previous method, only a certified mass per particle needs to be given. This method may be more appropriate as the size method if the used particles do not have a spherical shape.
- **Number**: Also based on[14], this method uses standards of known number concentrations. Even though certified number concentration standards do not exist, NIST standards are sometimes used for this purpose. A calibration curve of dissolved Au standards is not required for this method. Nanocount alters η so that the certified number concentration indeed corresponds to the total signal frequency.
- **Other**: Allows for manually setting η determined any method the user has to its disposition.

The data of nebulisation efficiency checks can be treated using the data treatment tool and subsequently filtered and deconvoluted just like any sample using the deconvolution tool. However, the deconvolution needs to be done using the deconvolution tool first, because the dissolved background is subtracted from the data during deconvolution, a procedure that may significantly affect the final result of the calculated nebulisation efficiency.

Figure 37 shows the functionalities of the nebulisation efficiency tool present when selecting the method "Other".
Several features shown are common for all methods:

- "Sample parameters": Shows, amongst others, the element of the currently selected nebulisation efficiency check. In the case of the size or number method it also shows the list of possible nanoparticles to indicate the particle the nebulisation efficiency contained. This is necessary to calculate sizes or number concentrations.

[14] Pace, H. E.; Rogers, N. J.; Jarolimek, C.; Coleman, V. A.; Higgins, C. P.; Ranville, J. F., Determining Transport Efficiency for the Purpose of Counting and Sizing Nanoparticles via Single Particle Inductively Coupled Plasma Mass Spectrometry. *Analytical Chemistry* **2011,** *83* (24), 9361-9369.

- The options pane
    - o "Select method": allows to select a nebulisation efficiency calculation method
    - o "Input flow": The flow rate of liquid going into the nebuliser, measured at the time of the nebulisation efficiency determination. This extremely important value needs to be determined accurately. The default value (filled in the "General info" tool) is initially filled out here.
- The table "Loaded Nebulisation efficiencies": Lists all nebulisation efficiency checks associated with the current spICP-Ms project. Only one check can be selected at a time. It also shows the method selected for each check and the relevant expected value. For the method "Other" this is simply the nebulisation efficiency itself, for the size method, it is the certified size, or certified number for the number method.
- "Results": Shows the calculated or user-supplied η value (not in %, but dimensionless, i.e. 0.02 means a η value of 2 %).
- "Exit": Exits the tool. The calculation of nebulisation efficiencies according to any method is automatically saved to the project. Hence there is no "save" button.
- "Create a neb. eff.": This box allows creating a new component of the type nebulisation efficiency check. Of course the same can be done in the "Import files" tool, but the user can also create checks here that do not contain data, and thus. Note, however, that files cannot be attached to checks created here. Hence, the methods "Particle standard – size", "Particle standard – number", "Measured flows and analyte" will only work if the user uses the "import files" tool to attach data files to the new nebulisation efficiency checks.
- "Delete check": Deletes the currently selected nebulisation efficiency check and all its data files.
- "Grouping": To group nebulisation efficiencies (see further)

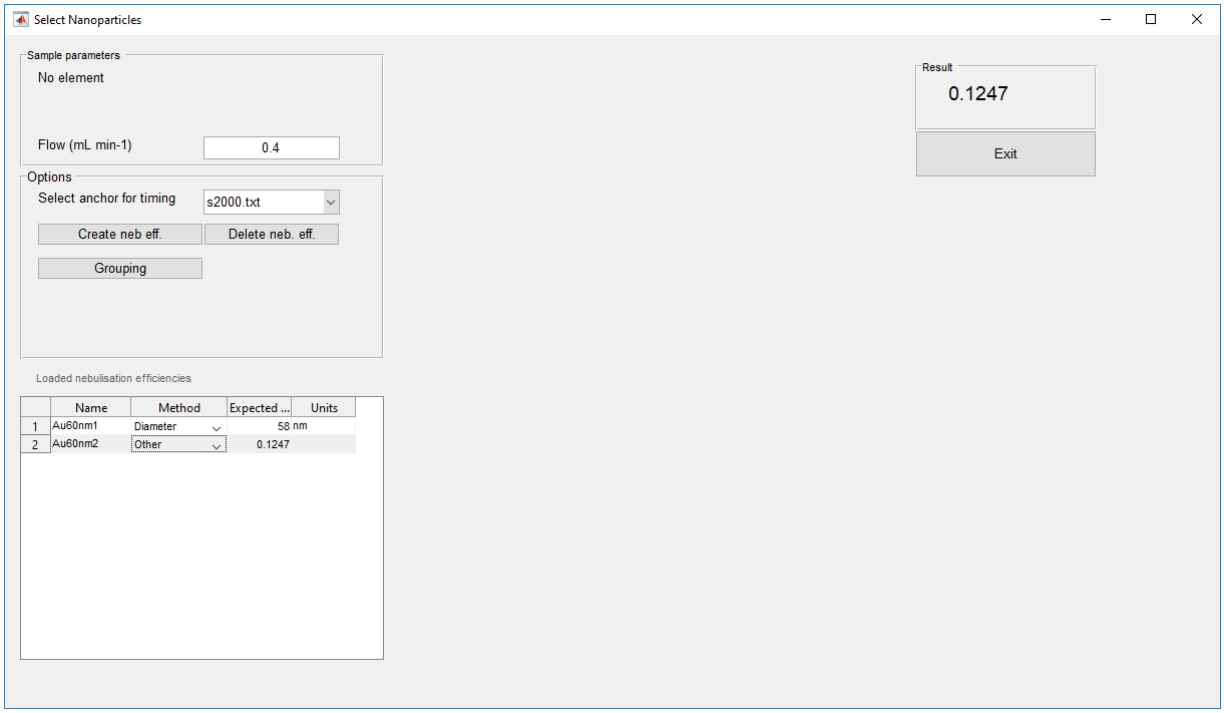


Figure 37 *The nebulisation efficiency tool when the "other" method has been chosen*

Particle standard methods

When selecting particle standard methods, the nebulisation efficiency is calculated based on the data of a certified standard. Most commonly a 60 nm Au nanoparticle suspension is used in this case. These methods can thus not be used if a nebulisation efficiency was not measured and/or when the nebulisation efficiency had no known diameter, mass per particle or number concentration. There are three methods here: the diameter, mass per particle (mpp) and the number method. All methods have some additional features present in the tool:

Calculating nebulisation efficiency using a certified diameter standard is experimentally the most cumbersome, as a separate calibration curve for Au needs to be set-up. However, it is currently considered the most accurate method.

Diameter

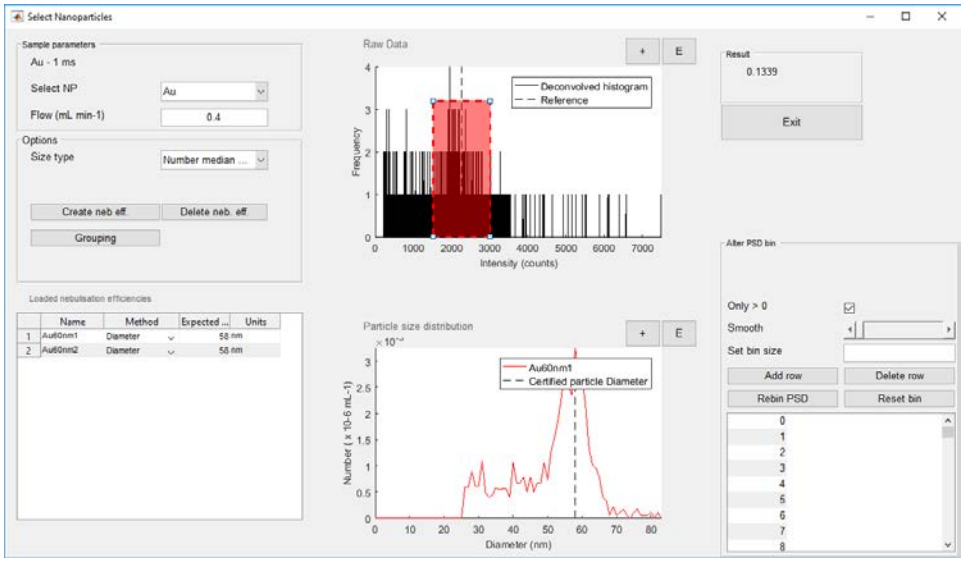


Figure 38 *The nebulisation efficiency tool when using the diameter method.*

In the “Sample parameters” and “Options” panes, several new options appears (Figure 38).

- “Select NP”: Select which nanoparticle is in the samples and is to be used to calculate the nebulisation efficiency.
- “Flow” can be seen in the sample parameters book. This is the sample flow into the nebuliser. By default, the project sample flow is given here, but it can be altered if for some reason the flow was different during measurement of the nebulisation efficiency.
- “Diameter type”: There are four options here, telling Nanocount which diameter the certified diameter has to be compared with.
    - Number average diameter
    - Number median diameter
    - Mass average diameter
    - Mass median diameter

The correct choice depends on which certified diameter one uses from the NIST documents. For instance, the number median diameter is best when the TEM diameter is taken from the NIST documents.

- "Certified diameter": in the table with loaded nebulisation efficiencies. The user can supply the certified diameter here (e.g. 58 for the certified 60 nm NIST Au particles). If no value is supplied, no value can be calculated for η and a default values of 0.02 is therefore set for η. This also occurs if Nanocount was unable to calculate a η based on the data supplied by the user.

The top graph shows the (possibly deconvoluted) histogram of the measured data and the PSD calculated using the fitted η in the lower graph. The upper graph ("Raw data") contains some functionality: A data region as a red box and a vertical line. The data range defines the region in which Nanocount will look for the signal to best use to calculate η. As a first guess, Nanocount searches for the signal with highest frequency in the data range and assuming that this is the signal intensity corresponding to the certified diameter, it then calculates η and uses it to calculate the PSD. It will then calculate the PSD parameter the user has indicated in "Diameter type". This value will often differ from the Certified diameter because the highest frequency signal in the deconvolved histogram is not necessarily the signal corresponding to the Certified diameter. Nanocount will then take another signal within the data range as guess and repeat the process until the calculated η results in a Number average diameter or whichever Diameter type that was selected with the exact value of the Certified diameter. The black dotted vertical line then indicates the best signal value. When activated (by clicking on it), the line can be dragged from left to right to manually change which signal value should correspond to the certified diameter to calculate η. The red box indicates the signal range over which Nanocount should search for the highest value.

The bottom graph shows the resulting PSD that is calculated using the fitted η to verify that the fitting procedure will lead to the desired result. As the "Diameter" method is used here, the X-axis shows the corresponding spherical diameter and the Y-axis shows the number concentration. Figure 38 also shows that a table appears and several buttons to manipulate the diameter bin of the PSD. Notes on how to operate these can be found in the section on the PSD calculator tool. Manipulating the PSD of nebulisation efficiencies can be useful for cases where a relatively low concentration of a 60 nm NIST Au NP standard was measured using e.g. a low dwell time resulting in statistically insignificant counts for individual signal bins. Calculating a PSD from such a histogram would result in an unclear maximum peak and thus calculating the nebulisation efficiency by selecting a particular peak is not possible.

Mpp

All inputs are the same as in the diameter method, except that a certified mass per particle needs to be given instead of a spherical diameter. The bottom graph will thus have mass per particle, expressed in fg, in the X-axis. The raw PSD is automatically rebinned in having a fixed 0.1 fg bin mass, but this can be changed using the tools in "Alter PSD bin".

Number

Many inputs for this method are similar to the diameter and mpp methods, except that, of course, a certified number concentration needs providing at "Number" rather than a diameter or a mass part

particle. Note that this number concentration should be expressed as a multitude of million counts. The red box in the “Raw data” histogram graph indicates which region of the whole intensity region needs to be considered when calculating the total signal frequency. This box is, again, adjustable by selecting it and then clicking and dragging. This is handy when the user does not want to include a region where he/she suspects background signals to occur. η is then calculated so that the total number concentration calculated from the total signal frequency matches the certified number concentration. The bottom graph shows the calculated PSD for comparison purposes. Note that this graph is only shown when a dissolved calibration calibration is available for the isotope that is being worked with, which may provide an extra check that the nebulisation efficiency was calculated correctly. A calibration is, however, not absolutely required to calculate the nebulisation efficiency for this particular method.

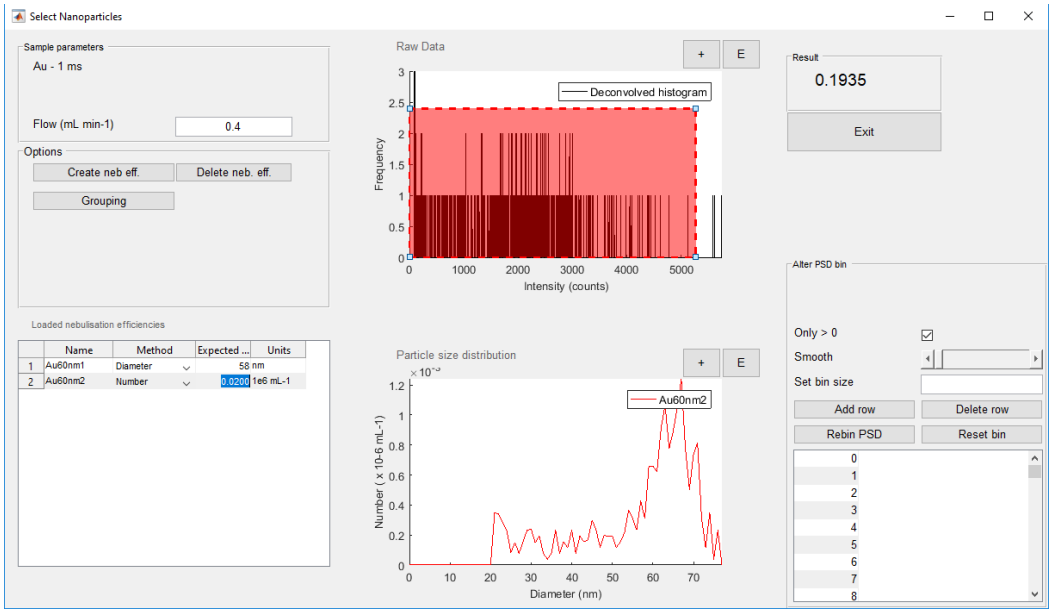


Figure 39. *The nebulisation efficiency tool when using the number concentration standard method.*

Grouping

There are two reasons to group nebulization efficiencies:

- Make averages of several values
- Construct particle calibrations

Figure 40 shows the basic components when using the average function.

- The result window
- A table with available nebulisation efficiency checks along with their expected values. This expected values is the certified diameter, mpp or number, depending on the checks’ respective method used to calculate the nebulization efficiency, or the nebulization efficiency itself, if the check used the method “other”. The table also shows wich checks are selected for the currently active nebulization efficiency group.
- A list of available nebulization efficiency groups.

- Buttons to create or delete groups. When a group is created, it initially has no checks associated and thus no results. Add checks by clicking in the “Included” column in the “loaded nebulization efficiencies” table.
- A drop down to indicate whether the group is an average or a particle calibration
- An exit button to go back to the nebulization efficiency wizard
- A box with relevant results for this group.

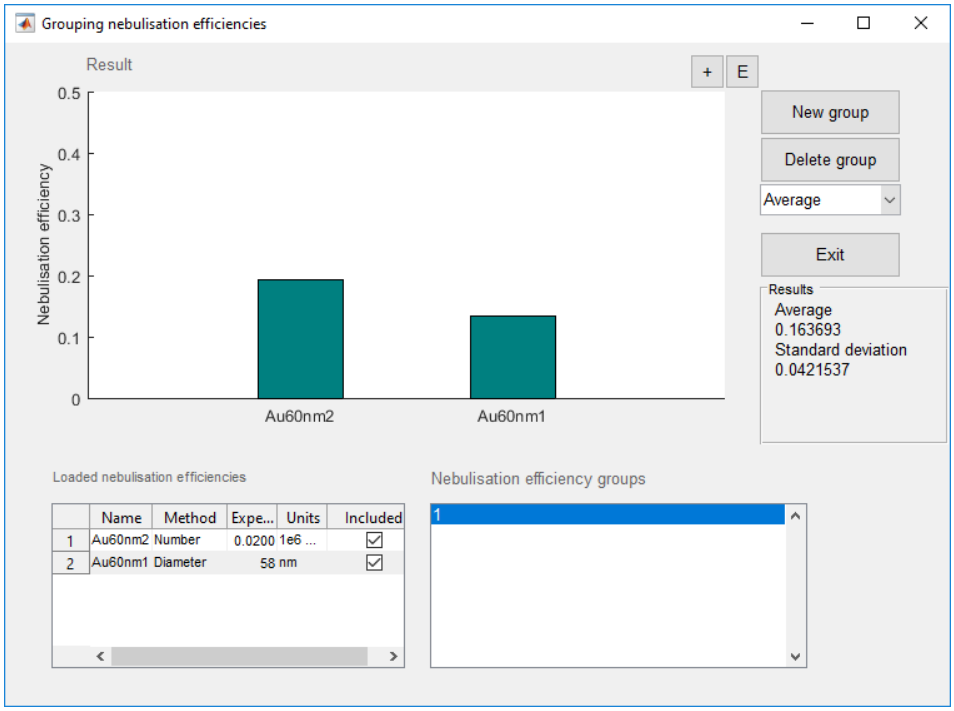


Figure 40. *The grouping tool within nebulisation efficiency determination when calculating averages.*

Averages

When the average method is used for a nebulization efficiency group, the average nebulization efficiencies and standard deviation is calculated all checks involved, provided that these checks have a calculated or given nebulization efficiency values associated with them. Groups for averages can hold checks using any method together. Figure 40 shows an average group for two checks, with one based on a diameter method and another on a number method. A result will only be shown in the graph and in the result box when at least two checks having a nebulization efficiency value are included in the group.

Particle calibration

Calculating number, diameter or mpp of unknown samples are usually based on dissolved standards. Diameter, number concentration and mpp are in this case calculated using formulae in which the nebulization efficiency is a required parameter. Another way of calculating is available if several particle standards of different known diameter, number concentration or mpp, but the same material exist. These can then be used to construct an empirical relation between the known value and the measured average particle event signal intensity or frequency. This calibration can then be used to calculate the diameter, number concentration or mpp of unknown samples.

Figure 41 shows how the grouping tool looks when calibrating for diameter. Note that only nebulization efficiency checks calculated for diameter, mpp or number can be used respectively for a calibration of diameter, mpp or number. This is not the case for diameter, mpp or number check groups using the average. These can be used for calculating any parameter. If a nebulization efficiency check of a wrong type is elected in the "Loaded nebulization efficiency checks" table, an error is produced. Otherwise, a calibration and some fitting results appear in respectively the results graph and box.

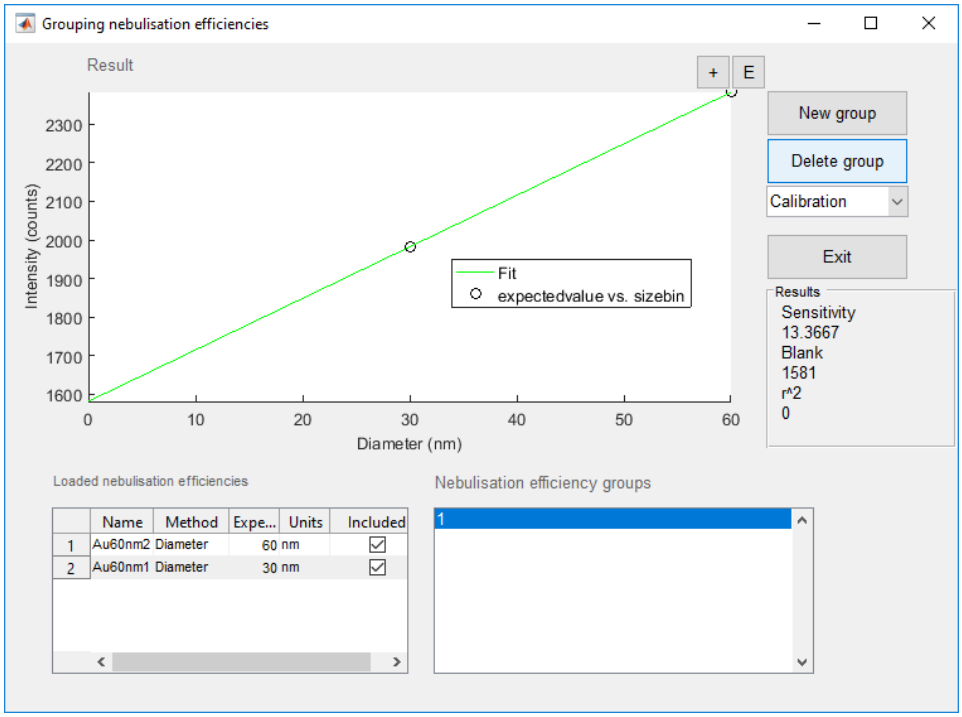


Figure 41. *The grouping tool within nebulisation efficiency determination when making a particle calibration.*

Particle size distributions

The final desired results can be obtained from the particle size distribution tool, activated by clicking "Obtain PSD" in the Sample submenu from the project window or the "PSD" quick link. Here, Particle size distributions of individual samples can be inspected and edited and trends of multiple samples can be obtained. Individual or multiple samples can be selected in the bottom left table. The functionalities in the tool are (Figure 42):

- **"Sample parameters" pane**
  - This pane shows the element of the currently selected sample, or - if more than one sample is selected- the element of the first of the selected samples. "Flow": The default flow (inserted in the "general" tool) is filled out automatically, but can be altered, e.g. if there was drift in the flow during the course of the experiment.
  - "Select Nanoparticle": Based on the current element, a list of possible nanoparticles (from the total project lsit as selected by the user) bearing the isotope that was measured is populated. The user can select which nanoparticle to assume was present.

Its molecular mass and density will then be used to calculate the PSDs. Note that the choice will be invalid if samples of different elements are chosen at the same time.

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Negative particle number concentrations or masses, while still shown in the PSD results, are not taken into account when calcualting properties of the PSD as shown in the trend graph. The motivation is that negative values are a consequence of a bad fitting of dissolved models and should not have happened (in an ideal world).

- **"Nebulisation efficiency" pane**

  The nebulization efficiency to be used for the currently selected sample(s) are to be chosen here. Names of individual checks are shown as well as numbers of created groups. Some basic data on their nature, such as whether it concern an average or particle calibration group is shown and in the latter case, for what the group was calibrated.

  Multiple checks can be selected for a given sample, but there are some restrictions:

  - Particle calibration groups are only used to calculate the parameter that they are calibrated for. This means that one has to select groups both for the desired X-axis property (diameter or mpp) AND for the desired Y-axis property (number).
  - As outlined lower, mass can also be shown in the PSDs Y-axis, but this does not require a nebulization efficiency value, but a dissolved calibration curve to be in place.
  - A PSD can be shown for a sample, even in the absence of a dissolved calibration curve if particle calibration groups are chosen for both X and Y axis.
  - Individual checks or groups used for averages, can be used to calculate diameters, numbers or mpps.
  - Up to two checks or groups of the proper type can be combined to extrapolate the relevant parameters as a function of time. For instance, if two groups with averages are sued, then the actual nebulisation efficiency used for a given sample is extrapolated between the average time of these two groups towards the time when the current sample was measured.
  - Particle calibration groups can only be combined with other groups of exactly the same type. In this case, both the slope and intercept of the calibration are extrapolated based on the average time of the group.
  - Groups of averages and individual checks can freely be combined with each other, but selecting more than two relevant checks or groups produces an error.

- **"Trends" pane**
  - "X-axis trend": After selecting one or multiple samples for which to show a trend, the user has several options to show on the X-axis of the trend graph. Note that for trends including expected concentrations, this data needs to be inserted, either in the bottom left table. Nothing will be shown for samples for which data is missing. The same

applies if the requested data cannot be calculated, e.g. in the case of logarithms of zero or negative values that would result in complex or infinite results.

- "Y-axis trend": Similarly to the X-axis, the user has several options to visualize trends of samples.
- "Add range": A box appears as shown in Figure 42. The trend shown in the "Sample ytrend" graph is now only calculated for the currently selected diameter range. More than one range can be added to the PSD graph. Selecting ranges only shows data for that particular range. Clicking outside the graph deslects all the ranges and the data in "Sample trend" is then calculated for all the data and not one partiuclar range.
- "Delete range": Removes the currently selected range.

Showing log(measured/expected) concentrations in the Y-axis is a particularly convenient way of checking accuracy of calculated (mass or number) concentrations. Even if the user has no knowledge of the expected concentrations he/she can still fill out the dilution factors as expected concentrations. If spICP-MS measurements occurred in the linear range, the calculated total (mass or number) concentrations should increase linearily with the dilution factor (or expected concentrations) and the log(measured/expected) concentrations should be on a flat line. If the expected concentrations are known and measurements are accurate, the flat curve should even occur at log(measured/expected) = 0.

- **"Loaded samples"**: This table allows to select one or more samples for which data should be shown. It also displays the expected total particle number concentration (in millions of particles per mL) and allows editing them. If more than one sample is selected, the PSD graph shows the PSD of the sample with the lowest sample number.
- **"Particle size distribution" graph:** Depending on whether mass or number is chosen here, a PSD of the selected nanoparticle will be shown here as rebinned and smoothed using the different PSD functionalities to the right of this graph. These functionalities are exactly the same as for the nebulization efficiency tool and are discussed below.
- **"Exit"**: exits the tool. The data is contiuously automatically saved to the project.

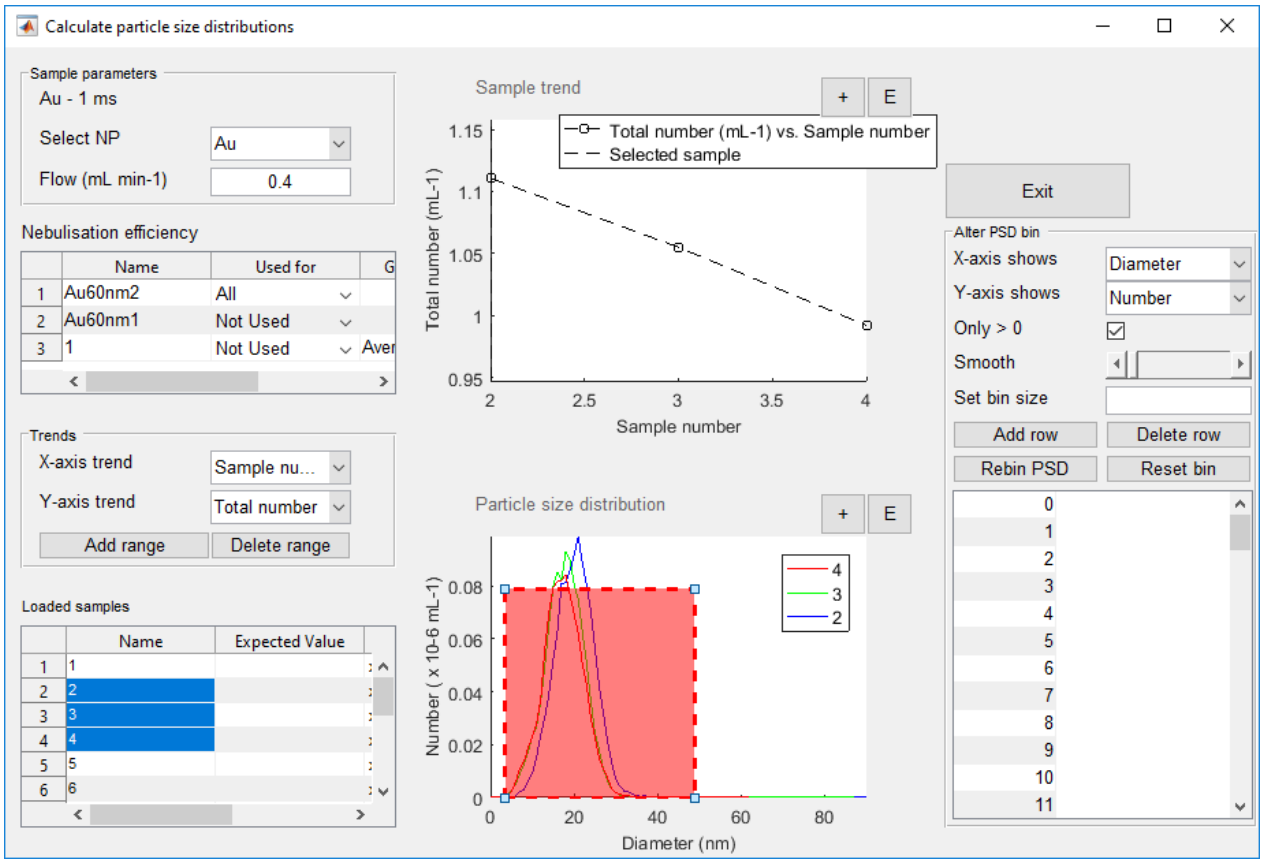

Figure 42 *The PSD calculation tool*

Alter PSD bin

These tools are available in the nebulization efficiency tool when using the size method or in the PSD tool and serve to change the appearance of the PSDs. In the case of the nebulization efficiency tool, changing the PSD may alter the calculated nebulization efficiency. In the case of the PSD tool, the PSDs of all currently selected samples is changed simultaneously, an action that may alter the values shown in the "Sample trend" graph. The different functionalities are:

- "X-axis shows": either diameter or mass per particle (mpp) can be selected here. Note that data is only shown if the correct nebulization efficiencies are associated with the sample.
- "Y-axis shows": either number concentration or mass can be slected here. Note that number concentrations are only shown if the correct nebulization efficiencies are associated with the sample. Mass requires a dissolved calibration.
- "Only > 0": Only positive calculated number concentrated are shown and not the negative ones that may arise e.g. when fitting of dissolved models during deconvolution was not perfect.
- "Smooth": A simple moving average algorithm is applied to smooth the PSD. The more the slider is moved upwards, the more smoothing is applied.
- "Set bin size": The value filled out here is used to populate the bintable with an equal bin all over, starting from the first value shown in the table further down among the PSD bin tools.
- "Add row" adds an empty row below the currently selected row in the bintable so that anew value can be inserted.

- "Delete row": Deletes the currently selected row from the bin table.

Transient projects

In the case of transient projects, the only trend one is interested in is how the different PSD properties (total number concentration, median size, broadness,…) evolve as a function of time. Figure 43 therefore shows that in the case of transient projects, the "X-axis trend" drop down menu has been replaced by selecting ROIs for the current sample (which in turn, is selected in "Loaded"). As soon as a PSD property is chosen in "Y-axis trend" (such as Number median size in Figure 43), the trend graph shows the trend as a function of time, using the average time for each slice in the X-axis.

The PSD of the first slice is shown in the PSD graph, but PSDs of other slices can be displayed as well. Click on the Y-axis of the sample trend where a dotted line will show up that can be dragged to a different slice in the time trend, for instance, the second slice in Figure 43. The PSD of that slice is then shown in the PSD graph.

Any alterations done to the PSD shown, i.e. changing nebulsiation efficiencies, flow rate, bins or smoothing degree, is automatically applied to all PSDs of the currently shown ROI. The effect on the trend is also automatically displayed.

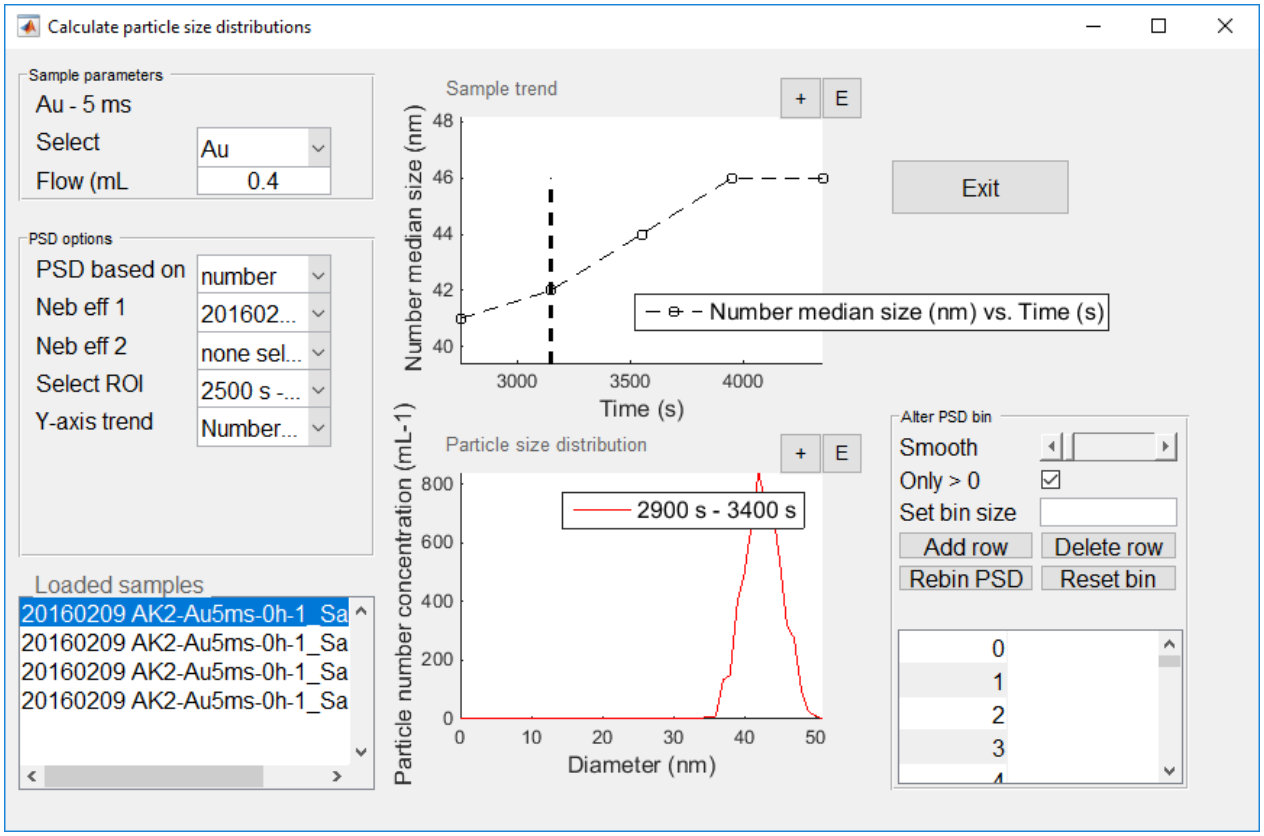

Figure 43 The Calculate particle size distribution tool for transient projects.

Support

For more information and much appreciated feedback please mail to

geert.cornelis@slu.se

Users are encouraged to feed back any issues or mssing functionalities that they feel should be added to Nanocount.