Supplementary Information: Results of an interlaboratory comparison for characterization of Pt nanoparticles using single-particle ICP-TOFMS

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	Lab1	**Lab2**	**Lab3**	**Lab4**	**Lab5**	**Lab6**	**Lab7**	**Lab8**	**Lab9**
icpTOF model	2R	R	S2	R	2R	2R	2R	R	S2
Plasma power [W]	1550	1550	1550	1550	1550	1550	1550	1550	1550
Nebulizer type and spray chamber	Standard setup (MicroMist nebulizer & Cyclonic Quartz Spray Chamber)	Thermo MicroFlow PFA-ST nebulizer Cyclonic Quartz Spray Chamber	Standard setup (MicroMist nebulizer & Cyclonic Quartz Spray Chamber)	Standard setup (MicroMist nebulizer & Cyclonic Quartz Spray Chamber)	Standard setup (MicroMist nebulizer & Cyclonic Quartz Spray Chamber)	Standard setup (MicroMist nebulizer & Cyclonic Quartz Spray Chamber)	Standard setup (MicroMist nebulizer & Cyclonic Quartz Spray Chamber)	Standard setup (MicroMist nebulizer & Cyclonic Quartz Spray Chamber)	Quartz Cyclonic Spray Chamber w/ ESI prepFAST ST nebulizer
Nebulizer flow [L/min]	1.08	1.09	1.01	1.13	0.97	1.07	1.04	1.05	0.99
Cool gas flow Nebulizer flow [L/min]	14	14	14	14	14	14	14	14	14
Auxiliary gas flow Nebulizer flow [L/min]	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Transport efficiency [%]	6.5	5.7	4.6	5.9	10.4	6.9	7.1	2.1	16.5
Sample uptake flow [mL/min]	0.40	0.37	0.35	0.44	0.12	0.39	0.3	0.4	0.05
Dilution factors	$1x10^6$ $1x10^5$	$1x10^5$	$1x10^6$ $5x10^5$	$1x10^6$ $5x10^5$	$2x10^6$ $5x10^5$	$2x10^5$ $7x10^5$	$1x10^5$	$1x10^5$	$2x10^5$ $7x10^4$

Standard Operating Procedure

Determination of elemental composition, particle number concentration and mass of inorganic nanoparticles in aqueous media by single particle inductively coupled plasma time-of-flight mass spectrometry (sp-ICP-TOFMS)

Contents

1. Scope and application

Following the rapid development of nanotechnology and their widespread use, engineered nanoparticles (ENPs) have become part of our daily lives. Through the use and disposal of ENP-containing products, the ENPs are released into the environment. Hence, in order to understand implications for human health and the environment, specific analytical methods, able to detect, quantify, and characterize these nanomaterials, are required. For nanotoxicology purposes, the detection of nanomaterials (NMs) at low concentrations is another key point. In this context, inductively coupled plasma-mass spectrometry (ICP-MS) used in single-particle mode (sp-ICP-MS) has become an established method for the detection and characterization of inorganic nanoparticles. Currently, sp-ICP-MS can be applied in routine analysis for the measurement of one or at most two isotopes at a time, hence limiting the available information. The monitoring of only one isotope per NP is particularly disadvantageous as it is the combined information of several elements or isotopes which makes it possible to understand the real composition of a particle and thereby its origin. Indeed, the determination of the "fingerprint" elements or isotopes in NPs is one of the most promising ways to distinguish natural (NNPs) and engineered nanoparticles (ENPs) and is therefore of great importance to the study of NPs in the environment[1]. In order to conduct multi-element single particle analysis, a clear solution is to employ simultaneous full-spectrum mass analyzers such as time-of-flight mass spectrometers (TOFMS), which enable the monitoring of multiple isotopes simultaneously further allowing the distinction of ENPs and NNPs.

The aim of this SOP is to provide a quantitative method for the determination of the elemental composition, the particle number concentration (PNC) and the mass (respectively size[1]) of inorganic NPs in aqueous solutions using an ICP-TOFMS (icpTOF, TOFWERK AG, Thun) with a conventional liquid sample introduction system. Although the data presented here were obtained using silver shelled gold core nanoparticles, the scope of this quantitative analysis of NPs can be extended to any inorganic NPs that produce a detectable signal by the icpTOF. The analysis guidelines are valid for multi-element NPs, consisting of metal and metal oxides with sizes ranging roughly from 20 to 250 nanometer[2] in aqueous suspensions, depending on the isotope and its corresponding sensitivity.

[1] Assuming shape is known and equivalent density to bulk material

[2] For larger particles, it is advisable to check for nonlinear detection effects. Non-linear response can be caused by incomplete atomization and ionization of large particles in the ICP or too intense signal at the detector of the MS.

2. Abbreviated terms

NM	Nanomaterial
NP	Nanoparticle
ENP	Engineered Nanoparticle
NNP	Natural Nanoparticle
sp-ICP-TOFMS	Single particle Inductively Coupled Plasma Time-of-Flight Mass Spectrometry
PNC	Particle Number Concentration
SOP	Standard Operation Procedure
TE	Transport Efficiency
TOF	Time-of-Flight

Table 1 Abbreviations used in this document.

3. Method principle

This SOP describes the quantitative analysis of silver shelled-gold core nanoparticles by sp-ICP-TOFMS as an example. The quantification is based on the method developed by Pace et al.[2] and requires the use of a nanoparticle standard, here 50 nm monodisperse Au NPs for the determination of the transport efficiency.
It is to be highlighted that sp-ICP-TOFMS is subject to the same matrix effects as standard ICP-MS and so unmatched solvent between ionic standards and particles samples may lead to erroneous results[3]. The procedure described in this SOP applies to the characterization of inorganic NPs in diluted aqueous suspensions. If the sample matrix is different, the accuracy of the results is not guaranteed. In such cases, a sample pre-treatment is recommended to produce an aqueous suspension.

4. Safety procedures and precautions

Standard personal protective clothing including lab coat, safety glasses and gloves, is required. When handling suspensions containing nanoparticles as well as preparing the solution standards with diluted acid, appropriate caution should be used.

5. Procedure

In this SOP, the analysis delivers information regarding the mass of each isotope in each single particle and the PNC per vial. The quantification is based on the method reported by Pace *et al.* where the transport efficiency is determined based on the known size of the reference particles [2]. The following substances and information are required:

- Particle samples with recommended PNC < 10^5 particles/mL.
  (If this concentration is unknown, measure your sample with the minimum available integration time – *1 ms for icpTOF S2, 1.8 ms for icpTOF and 3 ms for icpTOF 2R* – and dilute the sample until the number of particle signals for each analyte is < 15/second.)
- Calibration standards containing known concentrations of all analytes present in the particles. The signal of the particles in the sample should fall in the linear range of the calibration curve.
- Single element reference nanoparticles of a known size and density to determine the transport efficiency of the sample introduction system. Au NPs are recommended with a PNC < 10^5 particles/mL.
- Calibration standards containing the same element as the reference nanoparticles at known concentrations. The signal of the reference particles should fall within the linear range of the calibration curve.
- Liquid flow (i.e nebulizer uptake) must be determined externally prior to the particle analysis. The nebulizer uptake can be measured by weighing a blank solution before and after a defined aspiration time, e.g. 10 min. At least 1 g of solution should have been aspirated to reduce weighing errors and using the mean nebulizer uptake from 3 runs is recommended.

Additional information to the particle number concentration:

The ideal PNC is highly dependent on the sample uptake rate and transport efficiency. If a too high PNC is introduced into the plasma, the probability of double events will also increase proportionally, which will lead to an overestimation of the particle size and an underestimation of PNC. For example, with a sample flow rate of 300 µl/min, a transport efficiency of 3%, a PNC of 10^5 particles/mL, 15 particles are introduced per second into the plasma. Under such conditions, and with an integration time of 1 ms, the likelihood of an event being caused by multiple, concurrent NPs will be only 0.75% (ratio of statistical occurrence of double events to single events).

Additional information to the preparation of calibration standards:

Different matrices induce different matrix-effects in both the ICP and the sample uptake. Differences in the matrix composition (i.e. acid content, buffers, dissolved salts, organics) affect the nebulization, the size of the droplets transported into the plasma, which in turn affect the plasma load, temperature and ionization efficiency. A compromise was sought out here by preparing all calibration standards and sample dilutions with MilliQ.

- Because Au(III) stock solutions are usually stabilized in hydrochloric acid, while other standards are stabilized in nitric acid, it is advised to prepare the Au calibration standards separately from the multi-element calibration standards.
- Calibration standards must be prepared freshly since not all elements are stable in H_2O.

5.1. Apparatus and equipment

- Inductively Coupled Plasma Time-of-Flight Mass Spectrometer (TOFWERK AG, icpTOF S2, icpTOF R or icpTOF 2R) with standard liquid sample introduction system (i.e. pneumatic nebulizer and cyclonic spray chamber)
- Micropipettes & tips
- Analytical balance (e.g. Mettler Toledo)
- Ultrasonic bath
- Autosampler (optional)

It is important to clean the glassware and cones, as well as to replace old used tubings prior to measurements to guarantee minimum background signals.

5.2. Chemicals, reference materials and reagents

5.2.1. Ionic calibration standards solutions

Calibration standards for the element of interest, i.e. Au and Ag in this case, as well as for the determination of the transport efficiency are required.

In the presented example, Ag and Au calibration series were prepared from single-element standard solutions (Fluka and Inorganic Ventures). All dilutions were made with ultra-high purity water (MilliQ) and were carried out gravimetrically. Final concentrations ranged from 30 pg/g to 3 ng/g.

5.2.2. Wash solutions and blanks

- Rinse/wash solution (1% HCl and 1% HNO_3)
- Blank – ultra-high purity water (e.g. MilliQ from Milipore Corp, resistivity 18.2 MΩ x cm)

5.2.3. Nanoparticle standard

A well-characterized monodisperse nanoparticle standard, in terms of elemental composition, density, shape and size is required for the determination of the transport efficiency using the size method. A typical choice is NIST reference material SRM 8013, which consists of nominal 60 nm Au spherical NPs. However, due to scarcity of the material, any monodisperse well-characterized NPs of similar density to bulk material may be used. Additionally, the particle signal has to be clearly distinguishable from the dissolved and instrumental background to yield an unbiased mean or median sensitivity. Here monodisperse 50 nm spherical Au NPs from NanoComposix were used as a particle standard.

50 nm Gold Nanospheres, PEG Carboxyl, Ultra Uniform	
Diameter ± Std.Dev (TEM)	50.1 ± 1.8 nm
Particle mass (calculated)	1.26 fg
Mass Concentration Au	0.05 mg/mL
Particle Concentration	3.9E+10 particles/mL

Table 2 Example of standard reference particles to evaluate transport efficiency.

5.2.4. Sample

Bimetallic spherical Ag-Au NPs were used as sample in this example.

60 nm Silver Shelled Gold Nanospheres, Citrate, NanoXact	
Total Diameter ± Std.Dev (TEM)	59 ± 6 nm
Core Diameter (TEM)	30 ± 3 nm
Core Mass (calculated)	0.28 ± 0.08 fg
Shell Thickness (calculated)	14.5 nm ± 5.5 nm
Shell Mass (calculated)	0.98 ± 0.3 fg
Mass Concentration Au	0.80 mg/mL
Particle Concentration	$8x10^{11}$ particles/mL

Table 3 Example of a sample of known characteristics which was analyzed to evaluate the SOP described in this document.

5.3. Sample preparation

If the samples have been stored in the fridge, it is recommended that they reach room temperature before dilutions are performed. Vigorous shaking or sonication of the NPs suspensions is required prior to dilution. Here the 50 nm monodisperse Au NPs were diluted by a factor of 10^6 and the 60 nm Ag/Au coreshell NPs were diluted by a factor of 10^7 in ultra-high purity water.

Caution during sample preparation and storage of Ag NPs:

Ag NPs are susceptible to oxidation, hence exposure to light should be minimized.

- ➔ Store Ag NPs in amber bottles or bottles covered with aluminium containers in the fridge.
- ➔ Protect samples in aluminium foil.

5.4. Performing measurements

5.4.1. Operation of the equipment

Instrument Start:

1. Start TOFpilot. The TofDaqViewer application will start automatically.

Figure 1 'Splash Screen' showing up during start of TOFpilot application.

2. Make sure the tubing on the peristaltic pump is undamaged, hooked in with the plastic collars, and secured with the clamps. Check for the flow direction of the liquids.
3. In TOFpilot, on the top toolbar, choose the appropriate **Tune Setting** for your experiment, and click **Get Ready** to start the plasma. Wait for the startup sequence to finish. The indicator to the right of **Get Ready** will turn green and the **Get Ready** button will change to **Shut down**.

Assumption: The instrument has been previously tuned for optimal sensitivity, including optimization of ion optics, detector calibration and mass calibration. If not, it is recommended to run the autotuning sequence to optimize the instrument. (Refer to *icpTOF Reference Guide*)

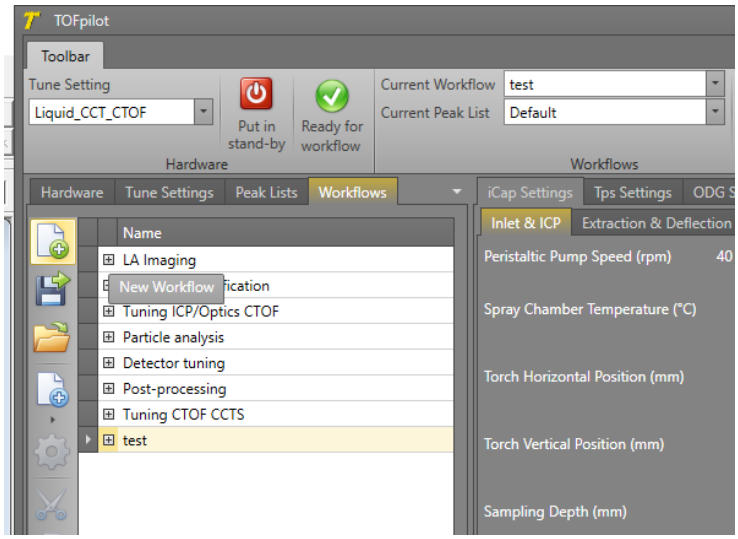
	icpTOF S2	icpTOF R	icpTOF 2R
Sensitivity ^{59}Co (kcps/ppb)	25	10	5
Sensitivity ^{115}In (kcps/ppb)	80	20	15
Sensitivity ^{238}U (kcps/ppb)	300	50	30
Mass resolving power ^{238}U	1000	3000	6000
CeO/Ce %	< 8	< 3	< 3

Table 4: Recommended specification to be reached in standard mode with 1 ppb Tune solution containing (Co, In, Ce and U) in STDS mode.

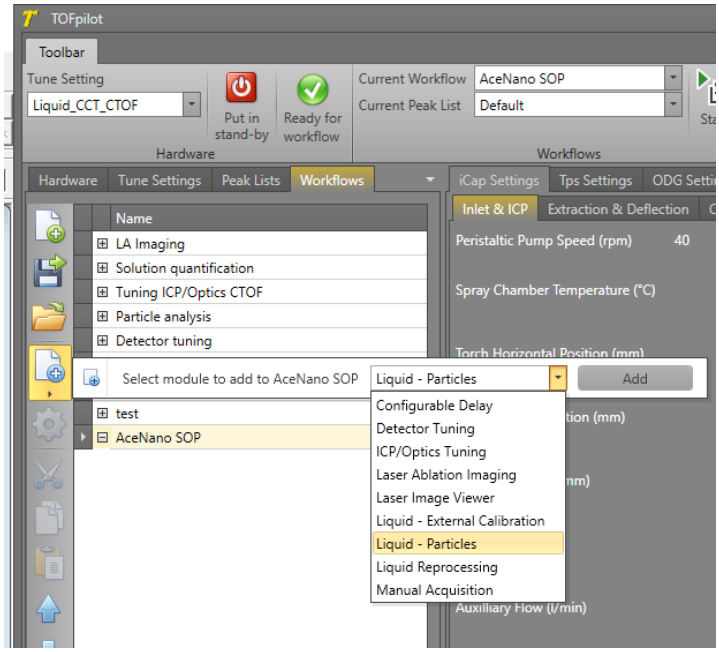
5.5. Measurement description

This section describes the set-up of a particle workflow in TOFpilot with a detailed step-by-step procedure:

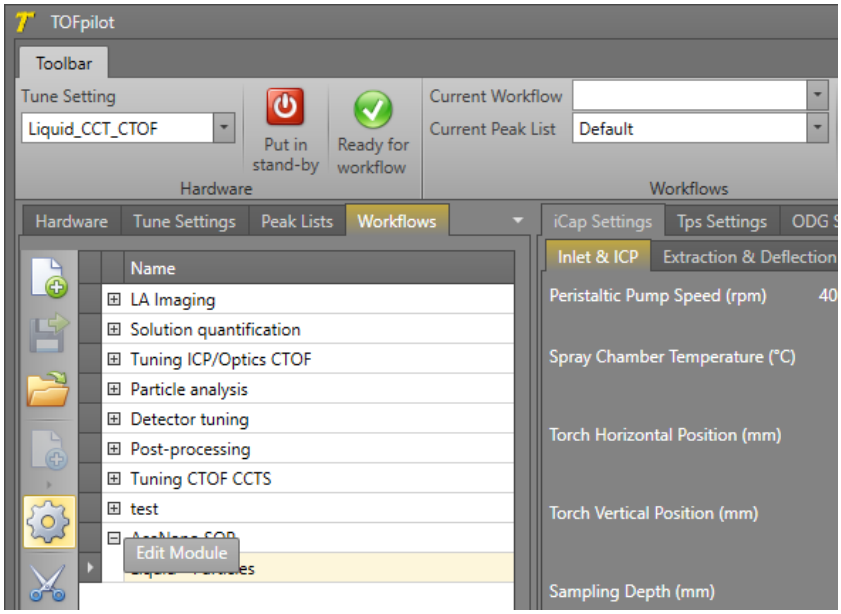
1) Setting up a new workflow by pressing the **New Workflow** button



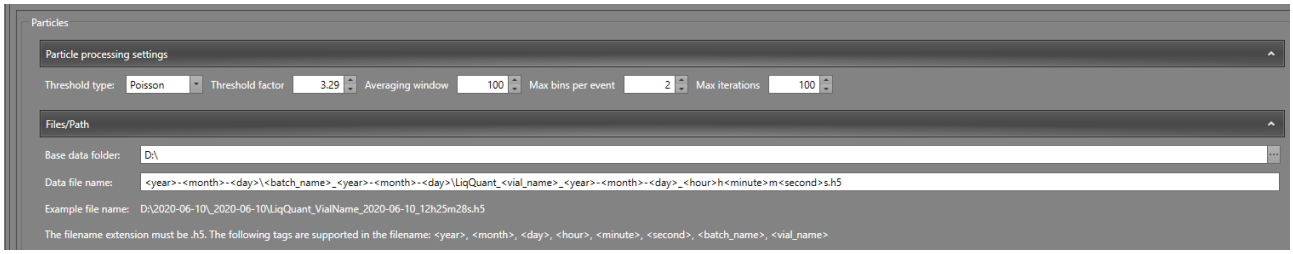
2) Selecting the **Liquid – Particles** module to add to the workflow and rename the workflow as desired



3) Click the **Edit** icon on the left bar to edit the measurement sequence



4) The particle processing settings uses per default a Poisson thresholding, an averaging window of 1000 datapoints, a maximum of 2 bins per event for split event correction, and a maximum of 100 iterations. These can all be modified in the particle processing settings.

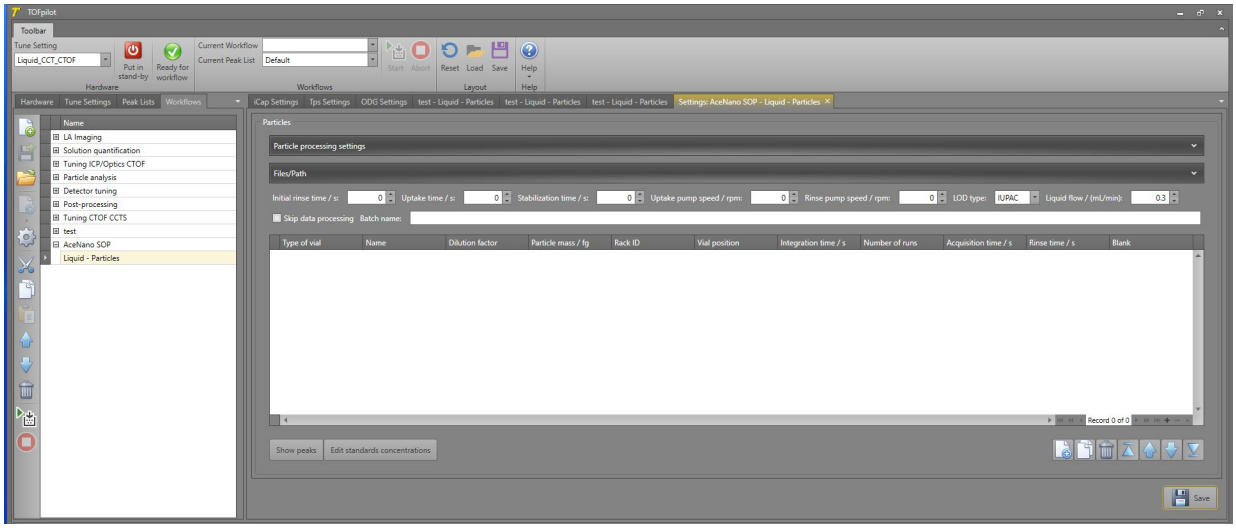


5) Check the Files/Path, for the path for the data in the data folder and the path for the results in the Results folder.

Additional information to particle processing settings:

- *Thresholding*: ICP-TOFMS noise is not gaussian-distributed, hence it is recommended to use the Poisson based thresholding rather than the sigma approach.
- *Averaging window*: A running average is used in the peak extraction step. The larger the window the more computational power is required, while with the smaller the window more artefacts due to edge effects will appear. Hence, 1000 is a good compromise.
- *Max bins per event*: A minimum of 2 bins is required for split event correction. Depending on the time resolutions used for the acquisition and the duration of the individual NPs transient signals, the number of bins per events will need to be increased, i.e elongated signals with use of CCT

6) Setting up a sequence



- Select the **Tune settings** which will be used for this analysis
- Insert **Initial rinse time/s** - rinse time required before the start of the entire sequence
- Insert **Uptake time/s** - time required for the solution to reach the plasma. This time depends on the configuration of the autosampler, e.g. length and internal diameter of tubing
- Insert **Stabilization time/s** - time required for the plasma to stabilize with the sample in order to reach a steady signal
- Insert **Uptake pump speed/rpm** - pump speed during sample uptake
- Insert **Rinse pump speed/rpm** - pump speed during rinse
- Select **LOD type** - IUPAC (LOD = 3.29*sigma + 2.71) or 3*Sigma (LOD = 3*sigma)
- Insert **Liquid flow/(ml/min)** - sample uptake rate for the introduction system measured externally prior to the particle analysis
- Tick **Skip Quantification** if you do not want the data to be automatically reprocessed after the sequence is complete
- Insert **Batch name** for the sequence
- Insert the first vial in the sequence by pressing **Add New Vial**

Types of vials	**Blank**	solution matrix with zero concentration
	Standard	Single-element or multi-element calibration standards of known concentration (e.g. Au calibration series for TE determination and multi-element calibration standards for NNPs analysis)
	Particles sample	sample containing particles
	Particles standard	dispersion with the reference particles for the transport efficiency determination (e.g. 50 nm Au NPs)

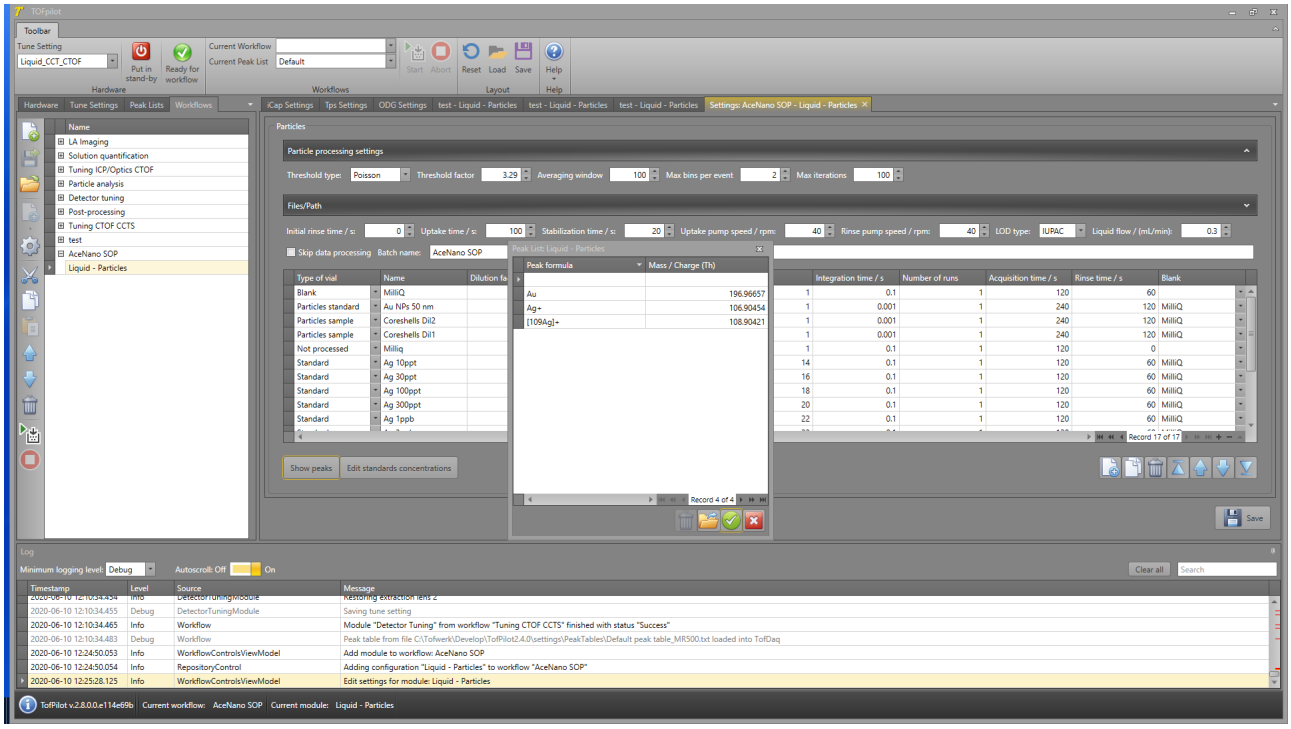
Table 5: Vial types available in TOFpilot liquid and particles workflow.

- If using **an autosampler** indicate rack and vial position

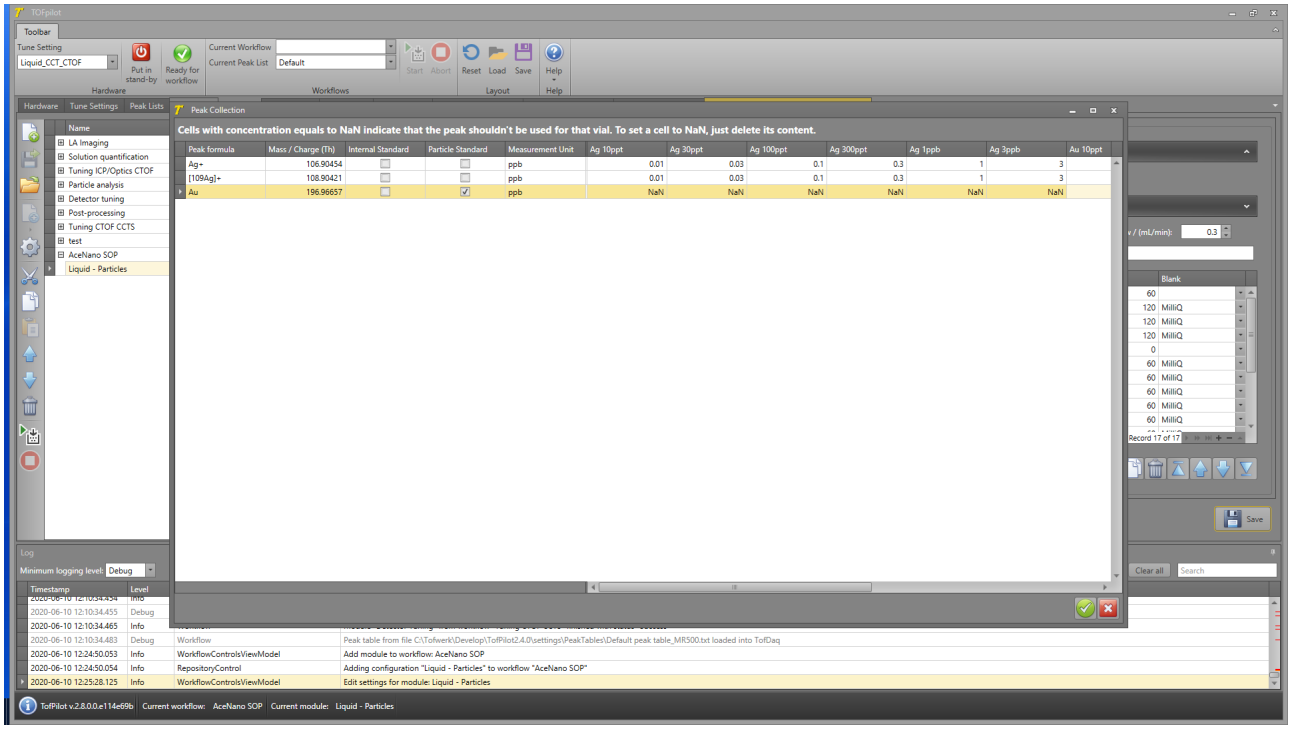
Additional information regarding integration time:
The integration time/s defines how many single TOF extractions are integrated into one datapoint, given in seconds. Robust timing settings allowing 100% data transfer are key for accurate results. It is recommended to use:

- > 0.1 s for standards (longer integration time result in better signal stability)
- Minimum available integration time for particle samples and standads (see specifications of instrument, 1 ms for icpTOF S2, 1.8 ms for icpTOF and 3 ms for icpTOF 2R)

7) Select all isotopes required for the analysis and TE determination

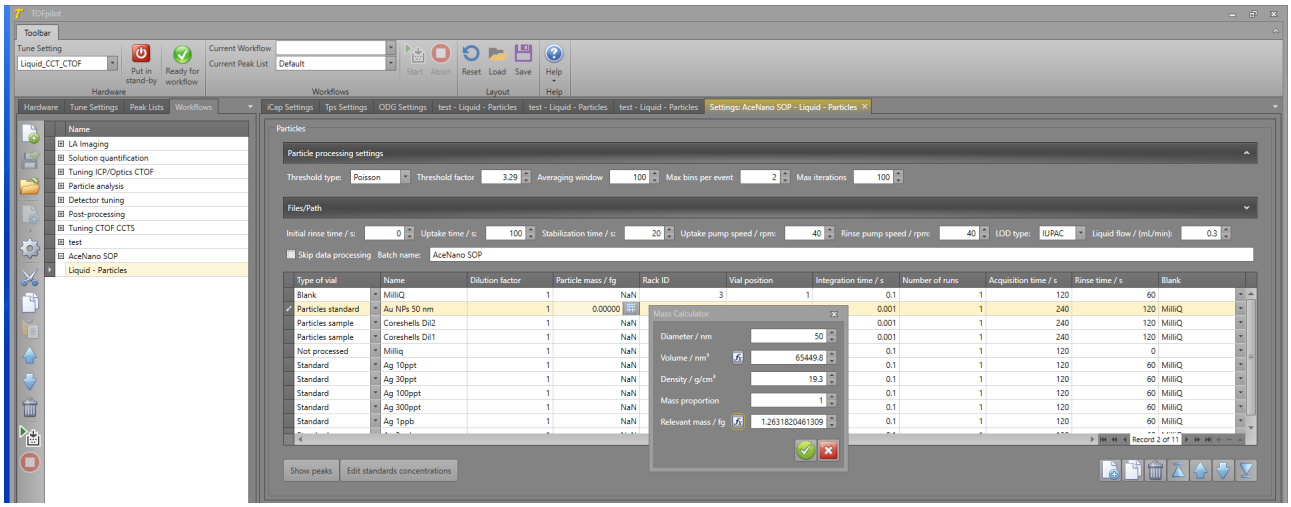


8) Edit the concentrations for the ionic standards and select the analyte for the particle standard vial.

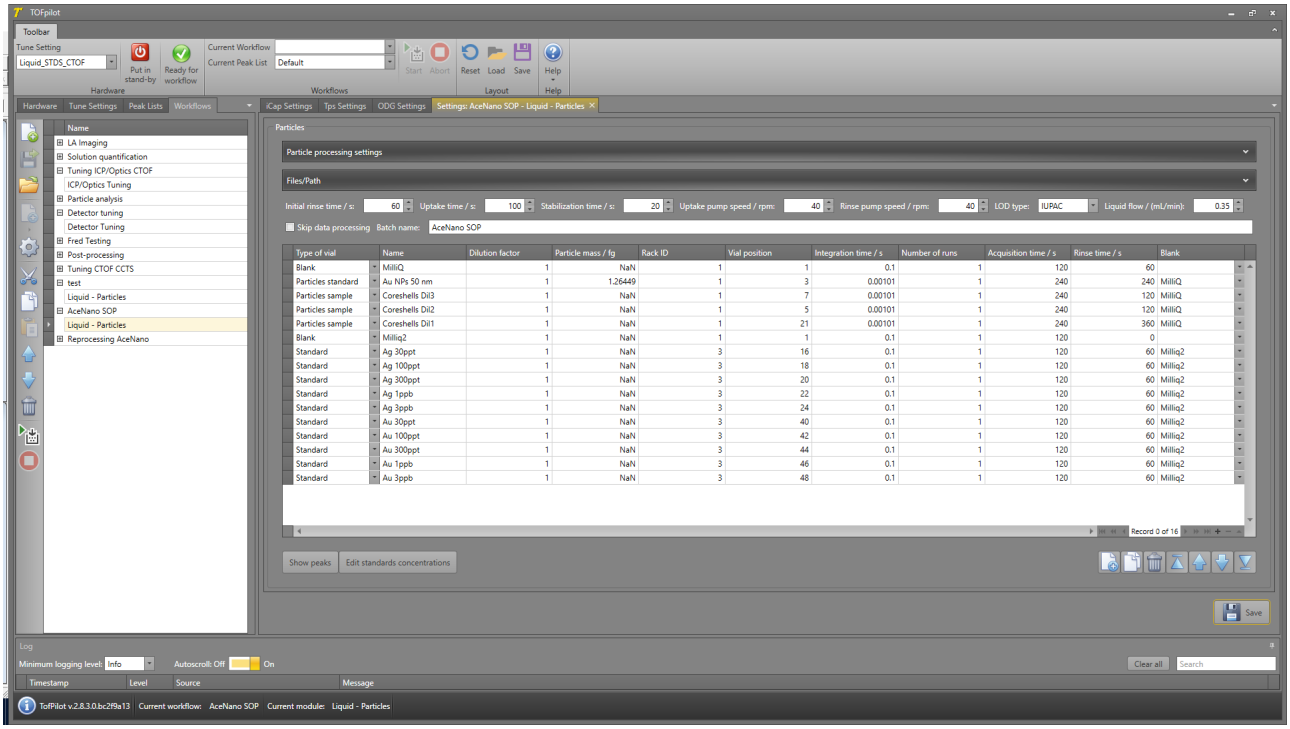


Note: it is recommended to work with gravimetric concentrations for improved accuracy, but these can be added in later with the reprocessing module.

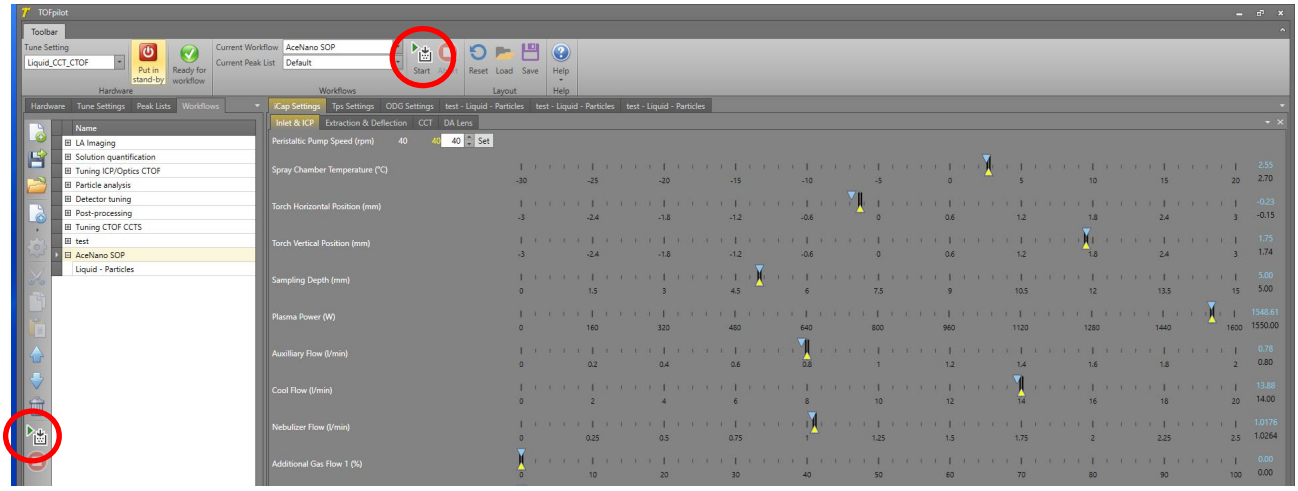
9) For the **Particle Standard**, the particle mass in fg needs to be specified. The **Mass Calculator** can be used to calculate this value from the specified diameter of the NP standard.



10) Example of a full sequence – Save and close

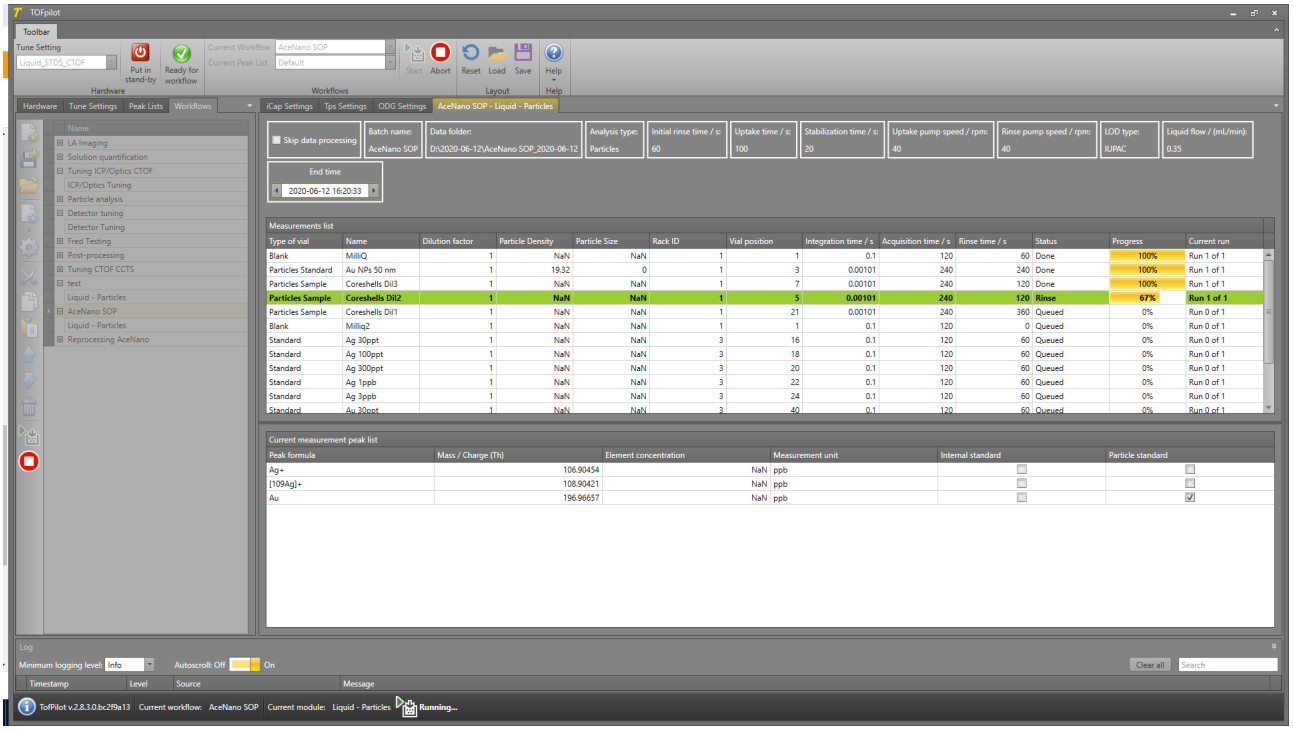


11) Start the workflow (there is no difference between the two start buttons)



12) Example of a running sequence

The example sequence started with the particle vials (standards and samples) to avoid long wash out times and elevated baseline from the ionic standards. Ultra-high purity water was used to rinse between vials. Ionic standards were measured from low to high concentration.

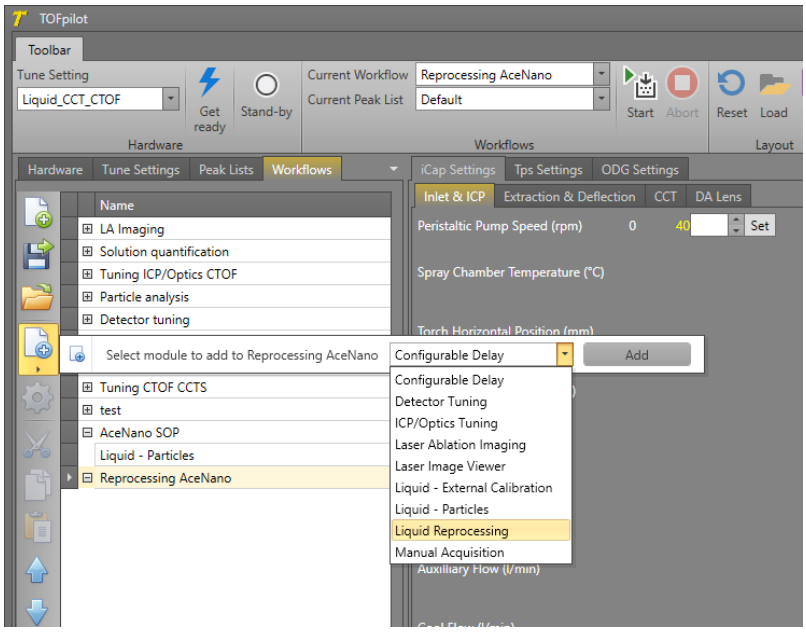


After the successful completion of the sequence, rinse the system with 1% HCl and 1% HNO_3. When all analytes have successfully been washed out and the baseline has returned to normal, rinse with MilliQ then run dry for a few minutes before switching off.

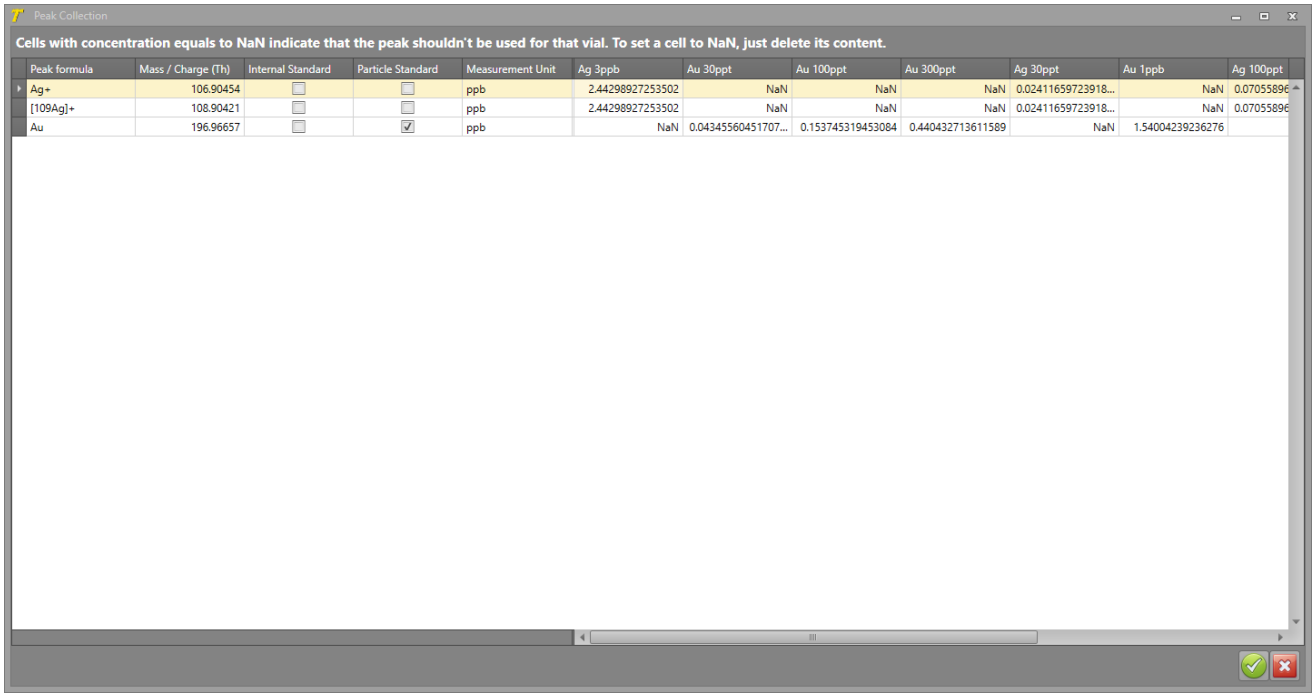
6. Data Analysis & calculation of results

6.1. Reprocessing module

The processing of data is done in TOFpilot either directly following the measurement or later using the dedicated reprocessing module as highlighted here below.



And can be used to insert exact concentrations:



6.2. Calibration curves

To build calibration curves, first the averages and standard deviations of all the vials marked as standards by the user need to be determined. The "raw" intensities (ions/extraction) are extracted from the peak data and converted into counts and cps. The average signal (A) and standard deviations (SD) are calculated for all analytes defined in the peak list as following:

$$A = \frac{\sum_{i=1}^{n} a_i}{n}$$

where, a_i is the signal intensity of analyte a per time bin i, given in counts per second, and n is the number of single data measurements in the run.

$$SD_{run} = \sqrt{\frac{\sum_{i=1}^{n}(a_i - A)^2}{n+1}}$$

where, a_i is the signal intensity of analyte a per time bin i, given in counts per second, A is the mean value of all a_i and n is the number of single data measurements in the run.

A weighted least squares fit is used to determine the calibration curve using $1/SD^2$ as weights[4].

The least squares fit gives a function on the form:

$$y_i = \alpha_i x + \beta_i$$

with associated uncertainties for slopes α_i (counts per second per ppb) and intercepts β_I (counts per second)

6.3. Limits of detection

Limits of detection (LOD) are calculated with the following methods (both are reported):

Formula 1: “IUPAC”[5,6]

$$LOD = \frac{3.29 * SD_{blank} + 2.72}{\alpha * \tau}$$

Formula 2: “3*Sigma”[7]

$$LOD = \frac{3 * SD_{blank}}{\alpha * \tau}$$

where *SD* is the standard deviation of the signal in blank in counts, α the slope of the calibration curve and τ the dwell time in s. $\alpha*\tau$ converts the slope of the calibration curve into counts/ppb, yielding LODs in ppb.

6.4. Particle events extraction

The following steps described the procedure performed automatically by the software. The procedure for identifying particle events is the same for the particle standards vials and particle sample vials. The required parameters can be set in the “particle processing settings” (chapter 5.5, step 4).

1. The raw data profiles are read out in counts/datapoints for all analytes.

2. The profiles are split into data chunks corresponding to the averaging window defined earlier (per default 1000 datapoints)
3. An iterative signal/background separation is performed on each data chunk:
   3.1. The average and SD of the data chunk is calculated
   3.2. Values which are higher than the threshold are marked as particles and are separated into a *particle* dataset. The threshold is calculated as either:
      3.2.1. Default formula, based on Poisson statistics

$$Thr = Avg + (3.29 * SD) + 2.72$$

      3.2.2. Gaussian threshold, where X is defined by the user

$$Thr = Avg + (X * SD)$$

   3.3. Repeat for the remaining dataset until no more values above the threshold are found or the specified maximum number of iterations has been reached.
4. The *particle* dataset is corrected for split-events: Peaks neighbouring other peaks are binned together, as it is assumed that these stem from the same particles. The maximum number of data points binned is configurable (default is 2). The timestamp of the highest of the binned points is taken as the timestamp for the binned data.
5. Analytes with identical bin times stem from the same particle and are stored on the same rows in the output csv files (see Annexe 1).

6.5. Particle quantification

The particle mass quantification for mono-metallic NPs is based on the work of Pace *et al*.[2] with some adaptations to match the TOF. Instead of working with peak heights, peak areas are used. In an effort to facilitate comparison, the same nomenclature was used. Multi-metal NPs or NPs containing elements not detectable by ICP-TOFMS are quantified by the same principle but including correction for the respective mass fraction of the element detected.

1. From the particle standard vial, the median signal response of the particles is determined.
2. The transport efficiency is calculated according to
   a. mNp = MedianCountsStandard /massOfStandardParticle (counts/g)
   b. dissolvedStandardEnteringNebulizerPerSecondPerPpb = massFlow * 1e-9 (g/s/ppb)
   c. mDiss = slopeOfDissolvedStandard / dissolvedStandardEnteringNebulizerPerSecondPerPpb (counts/ g)
   d. T_{eff} = mDiss / mNp
3. The calibration curve is transformed to mass units rather than concentration untis
   Slope_trans = slopeOfDissolvedStandard / (transportEfficiency*massFlow*1e-9) (counts/g)
4. The intensity of each particle event is converted to mass using the inverse transformed calibration.
   a. particleMass=I_event / Slope_trans (g)
5. The event frequency is determined from the number of recorded events and measurement duration
   a. NbrEvents/measurementTime
6. Subsequently, the particle number concentration (particles/ml) can be determined:

$$c_p[ml^{-1}] = \frac{eventFrequency * dilutionFactor}{T_{eff} * MassFlow}$$

6.6. Results of the quantification

In the automatically generated report, signal intensity and mass histograms are presented for each analyte of interest for each particle sample vial as well as for the particle standard vial. Additional statistics such as number of registered events, average, median and standard deviation are also provided.
Consequently, the following values can be extracted:

	Expected	Measured (average)
Mass Au core (fg)	0.28 ± 0.08	0.27 ± 0.19
Mass Ag shell (fg)	0.98 ± 0.3	0.90 ± 0.5
PNC (particles/mL)	$8x10^{11}$	$7.2x10^{11}$ based on Ag-events $6.34x10^{11}$ based on Au-events

Table 6: Quantified results of the 60nm Ag/Au coreshell sample

7. Further data processing

7.1. Size determination

TOFpilot stops at the determination of the particle masses as this workflow is not limited to spherical nanoparticles but may be used for any type of single entities (nanorods, nanocubes, cells…). However, if both the shape and density are known, then the determined masses in the .csv files can be further converted to volume and the corresponding particle diameters can be calculated using appropriate formulas. Table 7 presents the corresponding sizes for the Au core and Ag shell for the measured test sample. A detailed discussion regarding further data processing and quality assessment can be found in the Annexe 2.

	Expected	Measured (Guaussian fit)
Au Core Diameter (nm)	30 ± 3	29 ± 7
Ag Shell Thickness (nm)	14.5 ± 1.5	13 ± 5
Total diameter (nm)	59 ± 6	55.4 ± 9.4

Table 7: Sizing results of the 60nm Ag/Au coreshell sample

7.2. Multi-elemental fingerprinting

Further data processing steps regarding the multi-element composition of the particles can be performed from the provided .csv files (see Annexe 1). For example, composition filtering can be used.

8. Quality control

In order to assure accurate results, verify the following points:

- The shape of the **signal intensity distributions** for the gold standard NPs should be Gaussian or lognormal. A bimodal distribution would indicate insufficient dilution or particle agglomeration. In the case of a bimodal signal intensity distribution, the sample should be further diluted. If the shape of the signal intensity distribution does not change after dilution, then sample preparation needs to

be optimized or the quality of the samples needs to be assessed by an alternative method because the sample could have aged and degraded The same observation applies for known-homogeneous samples.

- Using the standard liquid sample introduction system of the icpTOF (pneumatic nebulizer and cyclonic spray chamber with peltier cooling), a transport efficiency ranging from 2 to 10% is expected. If this is not the case, apply the following measures:
    - Check clamps on peristaltic pump
    - Replace tubings
    - Check the performance of the nebulizer and spray chamber:
        - If the nebulizer is spraying correctly, a fine mist should be observed.
        - If coarse droplets are accumulating on the wall of the spray chamber, then it probably needs cleaning (for example, rinse 15 minutes with the rinsing solution).

9. Reporting of results

Results are automatically saved in a folder entitled *QuantificationResults_<date>_<time>*, which includes the following files:

- ***AnalysisReport <datetime>.pdf*** : Compilation of results
- ***h5_metadata.csv*** : experimental details relevant to the measurement of every vial (vial position, duration of measurement,…)
- ***liquid_average_signals_raw.csv*** : raw average signal of every before any processing
- ***liquid_average_signals_corrected.csv*** : average signal of every liquid vial after processing (blank subtraction and internal standard correction if applicable)
- ***calibration_curves.csv*** : slope, intersect and error estimations for each calibration curve
- ***lod.csv*** : Limits of detection estimates for each blank – analyte combination
- ***liquid_results.csv*** : summary of results for the liquid samples. Contains intensity values (cps) and resulting concentrations (ppb), plus error estimates.
- ***particle_intensities_<name>_<uid>.csv*** : conversion of h5 data into a more user friendly format. The .csv files display the timestamp and intensity (in counts) for each particle event. Data has been corrected for split events and blank subtracted. Simultaneous events will be shown on shared rows. One file for each particle and particle standard vial.
- ***particle_masses_<name>_<uid>.csv*** : Equivalent structure for particle intensities, but with calculated masses with one individual file for each particle vial.

10. **Validation status**

Results from the interlaboratory comparison study showed that all laboratories could determine the particle mass, size and particle number concentration of the test samples using the developed SOP and workflow within TOFpilot. After in-house and external validation, it was concluded that this SOP is well adapted and validated for particle mass, size and particle number concentration.

11. **Literature references**

[1] A. Praetorius, A. Gundlach-Graham, E. Goldberg, W. Fabienke, J. Navratilova, A. Gondikas, R. Kaegi, D. Günther, T. Hofmann, F. von der Kammer, *Environ. Sci.: Nano*, **2017**,4, 307-314

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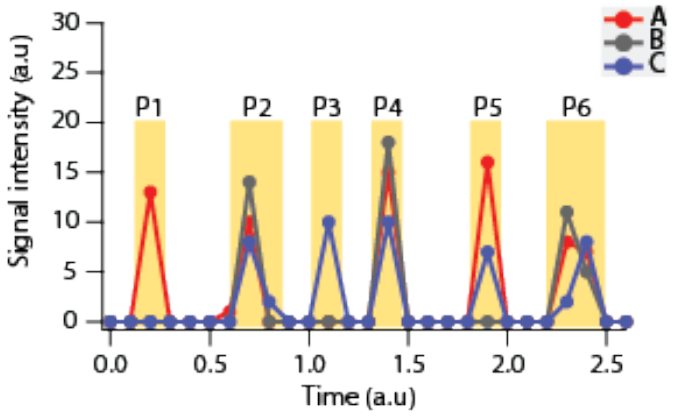
ANNEXE 1: Multi-elemental fingerprinting

A major feature of sp-ICP-TOFMS relies on its capability to distinguish single-element from multi-element NPs. For each particle which is transported into the plasma and ionized, full elemental spectra are recorded that allow the determination of the composition of the particle. Hence, it is important to understand how to recognize multi-element particles.

As mentioned, a full mass spectrum is acquired for each TOF extraction. The data is subsequently saved as a .h5 file but can be represented in a 2-dimensional array, where each row corresponds to a new time bin and each column to a different analyte/isotope. Consequently, signals which occur at the same time, are concurrent and are assumed to originate from the same particle.

0	0	0	0	
0.1	0	0	0	
0.2	13	0	0	P1
0.3	0	0	0	
0.4	0	0	0	
0.5	0	0	0	
0.6	1	0	0	
0.7	10	14	8	P2
0.8	0	0	2	
0.9	0	0	0	
1	0	0	0	
1.1	0	0	10	P3
1.2	0	0	0	
1.3	0	0	0	
1.4	15	18	10	P4
1.5	0	0	0	
1.6	0	0	0	
1.7	0	0	0	
1.8	0	0	0	
1.9	16	0	7	P5
2	0	0	0	
2.1	0	0	0	
2.2	0	0	0	
2.3	8	11	2	P6
2.4	7	5	8	
2.5	0	0	0	
2.6	0	0	0	



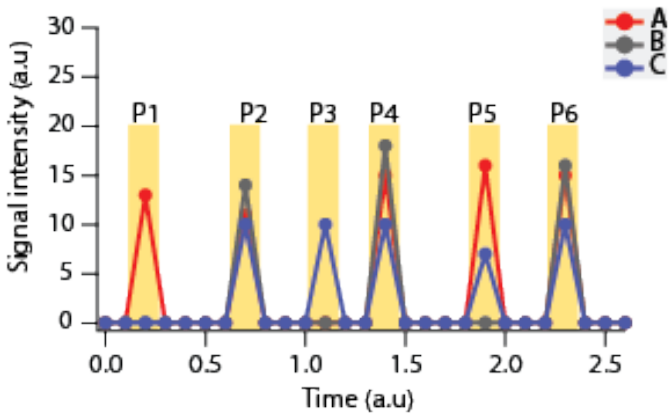
A synthetic dataset is presented here to illustrate single and multi-element particle. In the above figure, the transient time trace is shown for the different particles, which are composed of analytes A, B and C. The table on the left correspond to the output in the .csv files, with an analyte intensity versus time array. The first particle detected (P1) only contains analyte A, while the third particle (P3) only contains analyte C. For P2, P4 and P6, analytes A, B and C are all detected concurrently, hence these are considered as multi-element particles. P5 is also a multi-element particle, but of a different kind than P2, P4 and P6, as no signal is detected for analyte B.

Although concurrent signals are assumed to stem from a true multi-element single particle, it is always possible that they originate from multiple concurrent particles. Indeed, when two independent particles

reach the plasma at the same time, concurrent signals will be detected although they are not correlated. The probability of such cases is influenced by the PNC in the sample as well as the integration time used for the measurement and can be determined by event concurrency analysis. It is generally not possible to a posteriori distinguish whether a specific event results from multi-element NPs or from concurrent detection of multiple NPs. It is only possible to assign a probability for concurrent events from two particle types to occur by multiplying the probability of the occurrence of each particle type during the analysis. E.g. if 10^6 data sets (time bins) were collected and particle type P1 occurred 100 times and particle type P2 200 times, the probability of P1 and P2 occurring together would be $100/10^6 \times 200/10^6 \times 10^6 = 2\%$.

0	0	0	0	
0.1	0	0	0	
0.2	13	0	0	P1
0.3	0	0	0	
0.4	0	0	0	
0.5	0	0	0	
0.6	0	0	0	
0.7	11	14	8	P2
0.8	0	0	2	
0.9	0	0	0	
1	0	0	0	
1.1	0	0	10	P3
1.2	0	0	0	
1.3	0	0	0	
1.4	15	18	10	P4
1.5	0	0	0	
1.6	0	0	0	
1.7	0	0	0	
1.8	0	0	0	
1.9	16	0	7	P5
2	0	0	0	
2.1	0	0	0	
2.2	0	0	0	
2.3	15	16	10	P6
2.4	7	5	8	
2.5	0	0	0	
2.6	0	0	0	

In the data processing of the particle workflow, a split event correction procedure is applied it and sums successive signals together into one bin as illustrated below.



The same artificial data set as presented above has been corrected here for split events. P2 and P6 both had signals which were split over two time bins. Consequently, for P2 and P6, all signals are found in the same time bin, indicating a multi-element particle composed of analytes A, B and C.

ANNEXE 2 : Further Data Processing & Discussion of the results

In this document, results from the further analysis of the processed data are discussed.

Software:

- Excel was used for composition filtering
- Igor Pro 7 was used for data representation and fits.
- TOFDAQViewer was used for data visualization of the raw .h5 files.

Files :

- LiqQuant_Coreshells Dil3_2020-06-30_12h23m09s.h5 (raw file)
- *particle_intensities_Coreshells Dil3_d22b2e4c-9c1a-4522-9217-c733bf662de5.csv*
- *particle_masses_Coreshells Dil3_d22b2e4c-9c1a-4522-9217-c733bf662de5.csv*

Signal Intensity histograms

The signal intensity histograms can be directly plotted from the "*particle intensities"* file, as shown in Figure A1, for ^{107}Ag and ^{197}Au, respectively.

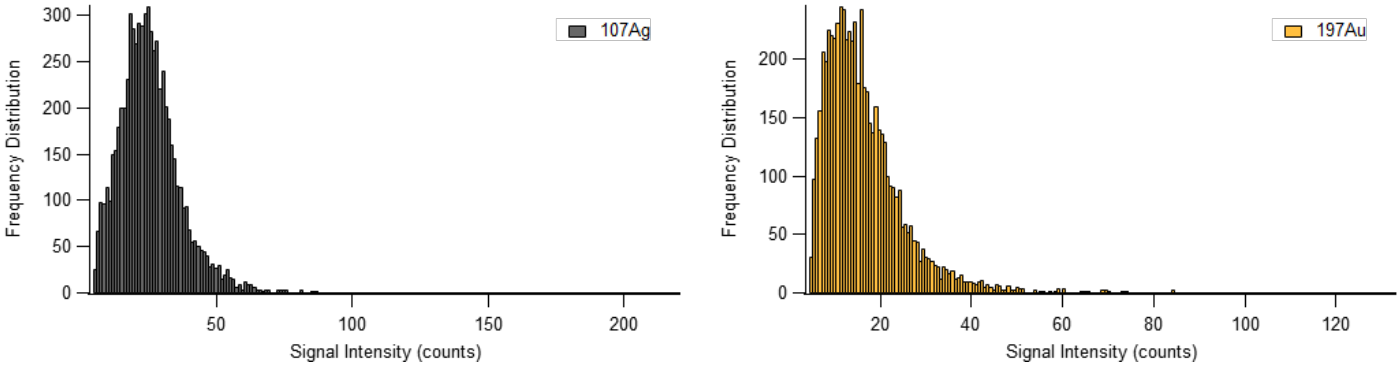


Figure A 1: Signal intensity histograms for silver (left) and gold (right).

Mass histograms

The mass histograms can be directly plotted from the particle masses file, as presented in Figure A2. It should be however noted that the masses are given in (g) and the conversion to (fg) leads to the artificial introduction of zeros into the dataset when multiplying blank cells and numbers using Excel[3] (blank cell*10^{-15} = 0). These artificial zeros need to be filtered out, otherwise an artificial bin at position zero will appear. Alternatively, blank cells need to be ignored when performing calculations.

[3] Using the excel formula: =if(isnumber(*A1*),*A1**1E-15,"") with "A1" being the source-cell, can avoid the introduction of zeros. Depending on the settings, it may be necessary to replace the comma by a semicolon.

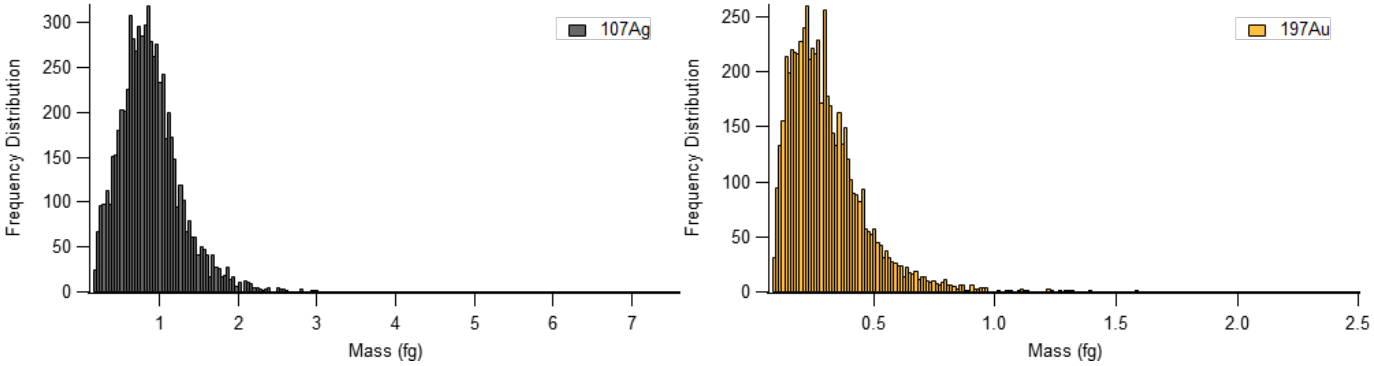


Figure A 2: Mass histograms for silver (left) and gold (right) after conversion from g to fg, and filtering of the artificial zeros.

Size histograms – Core diameter and shell thickness

From the particle mass (g), the diameter of the gold core D_{Au} is calculated using the following formula, where D_{Au} is the diameter in cm, R_{Au} is radius in cm, $m_{Au}$is the calculated gold mass in g, ρ_{Au} is the density of gold in g/cm^3. Results are displayed in Figure A3.

$$D_{Au} = \sqrt[3]{\frac{6 \cdot m_{Au}}{\pi \cdot \rho_{Au}}} \text{ and } R_{Au} = \frac{D_{Au}}{2}$$

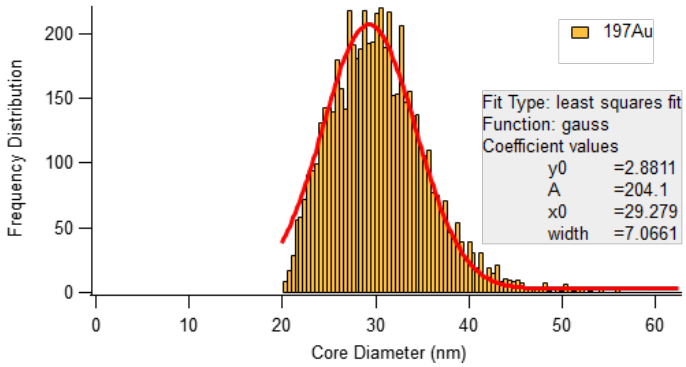


Figure A 3: Size histogram for the gold core after conversion from mass to diameter.

The hollow sphere formula is used to calculate the thickness of the silver shell, where R_{Tot} is the sum of the gold core radius R_{Au} and the shell thickness R_{Ag} in cm, m_{Ag} is the mass of silver in g, and ρ_{Ag} the density of silver in g/cm3.

$$R_{Tot} = \sqrt[3]{\frac{3 \cdot m_{Ag}}{4 \cdot \rho_{Ag}\, \pi} + R_{Au}{}^3} \text{ and } R_{Ag} = (R_{Tot} - R_{Au})$$

The shell thickness R_{Ag} and total diameter R_{Tot} are displayed in Figure A4 and A5, respectively.

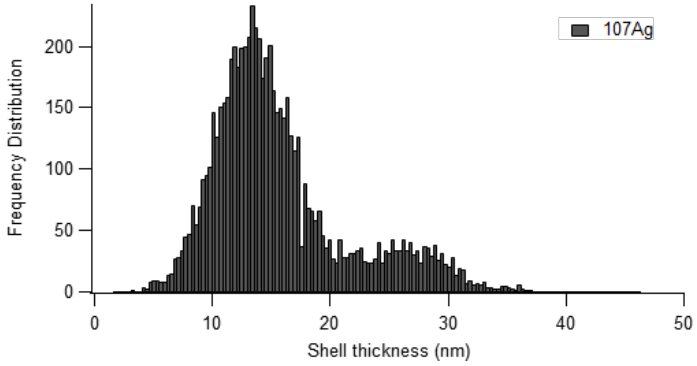


Figure A 4: Size histogram of the shell thickness after conversion from mass.

An unexpected second hump of smaller magnitude can be observed in Figure A4 after conversion from mass to shell thickness. It should be highlighted that this hump was not previously observed in either the signal intensity histogram (Figure A1) or the mass histogram (Figure A2) and only appeared after calculations involving both the Ag signals and its "concurrent" Au response (Au signal on the same row).

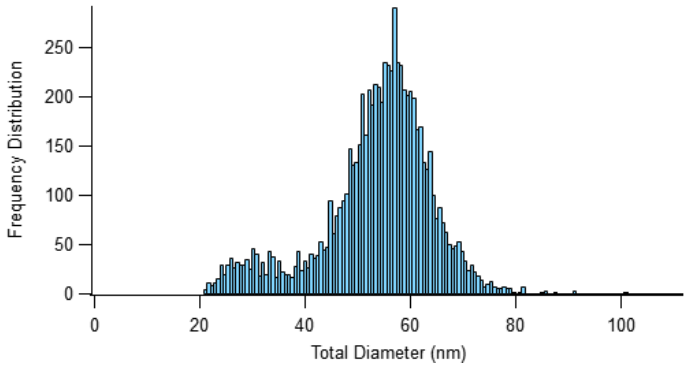


Figure A 5: Histogram of the total diameter

In Figure A5 displaying the total diameter of the coreshell NPs, a similar hump to that observed in Figure A4 can again be observed. In both cases, the hump is centred around 25-30 nm. A closer look into the .csv files revealed that Ag occurrences were not always associated with a gold signal but sometimes with a blank cell. Because the composition of the test NPS is known, namely that they are composed of a gold core and a silver shell, the data can be filtered with respect to particles composed of both elements. Figure A6 shows the shell thickness distributions after filtering with respect to the simultaneous occurrence of Ag and Au signals. From Figure A6, it becomes clear that the smaller magnitude hump observed in Figure A4 and Figure A5, corresponds to Ag signals without any concurrent Au signals. Consequently, if the particle signals are filtered based on their dual composition of silver and gold, a monomodal histogram is obtained for the total diameter (see Figure A7).

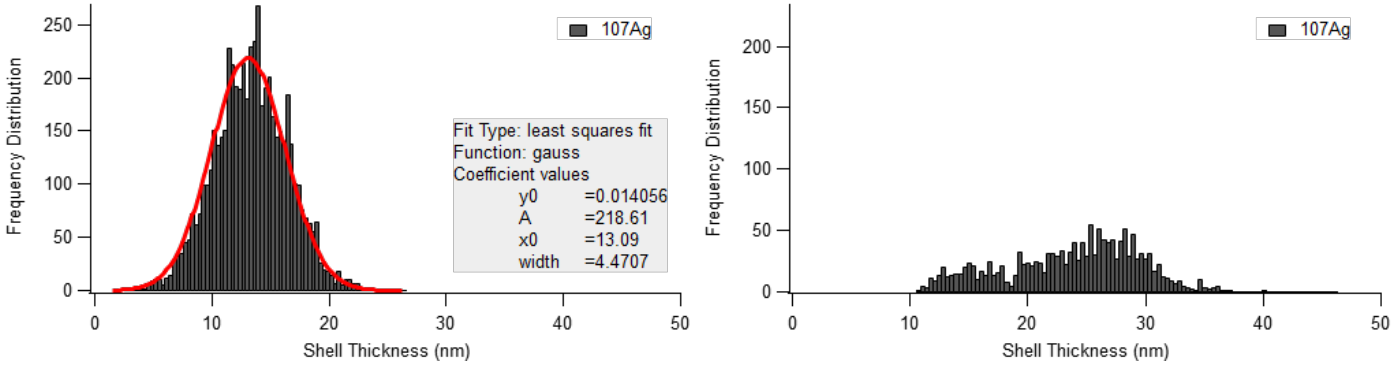


Figure A 6: (Left) Shell thickness distribution from Ag signals which are concurrent with Au signals. (Right) Shell thickness distribution from Ag signals with no concurrent Au signals.

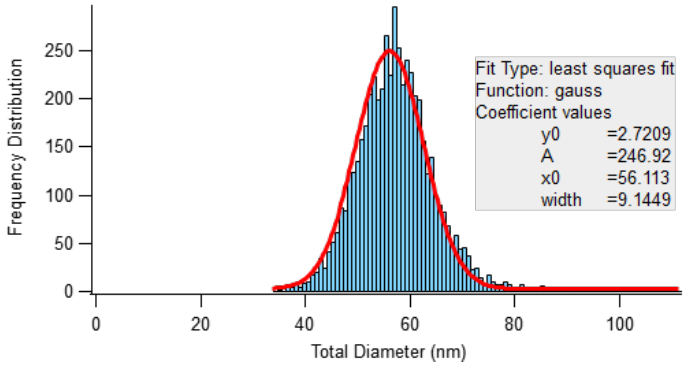


Figure A 7: Size histogram for the calculated total diameter of particles consisting of both Ag and Au.

After composition filtering, results are in good agreement with the expected values and are summarized in table A1. Although care needs to be taken in the interpretation of the data, it should be noted that only TOF data allows for such in depth investigation of multi-element particles.

	Expected	**Measured (Guaussian fit)**
Au Core Diameter (nm)	30 ± 3	29 ± 7
Ag Shell Thickness (nm)	14.5 ± 1.5	13 ± 5
Total diameter (nm)	59 ± 6	56 ± 9

15% of the 6850 observed Ag signals were not associated with gold signals. Hence in order to better understand why some Ag signals are not associated with Au signals, the raw .h5 file was analyzed using TOFDAQViewer. The time traces for ^{107}Ag (red), ^{109}Ag (pink) and ^{197}Au (blue) were monitored. The instances of Ag signals without concurrent Au signals (blank cells in the Au column of the processed .csv files) can be explained by three cases:

1) The particles are exclusively composed of silver (see Figure A8)
2) The particles have a silver shell and a small gold core, whose signal is below the thresholding limit. (see Figure A9)
3) The particles are composed of both a silver shell and a gold core, but their signals are split over multiple bins. The split event correction integrates the analyte signals into the maximum bin, but as

the Au signal and Ag signal have different maximum bins, they are separated and appear as “two different” particles in the processed .csv file. (see Figure A10 and Annex 1)

Based on the assumption that the test sample contained exclusively silver shelled gold core NPs and taking in to account that the sensitivity was sufficient to ensure that Ag and Au events were above LOD for all NPs, the number of Au and Ag events would have to be identical resulting in a ratio of PNC_{Au}/PNC_{Ag} =1. Even in the case of a single particle split in two events (case 3), the number of Au or Ag containing particles would not change. Hence, the higher PNC_{Ag} compared to the PNC_{Au} supports the occurrences of cases 1) and, eventually, 2). The presence of apparently pure Ag NPs was here unexpected and shows that the chosen test sample was either not as monodisperse or compositionally pure as initially assumed and reported.

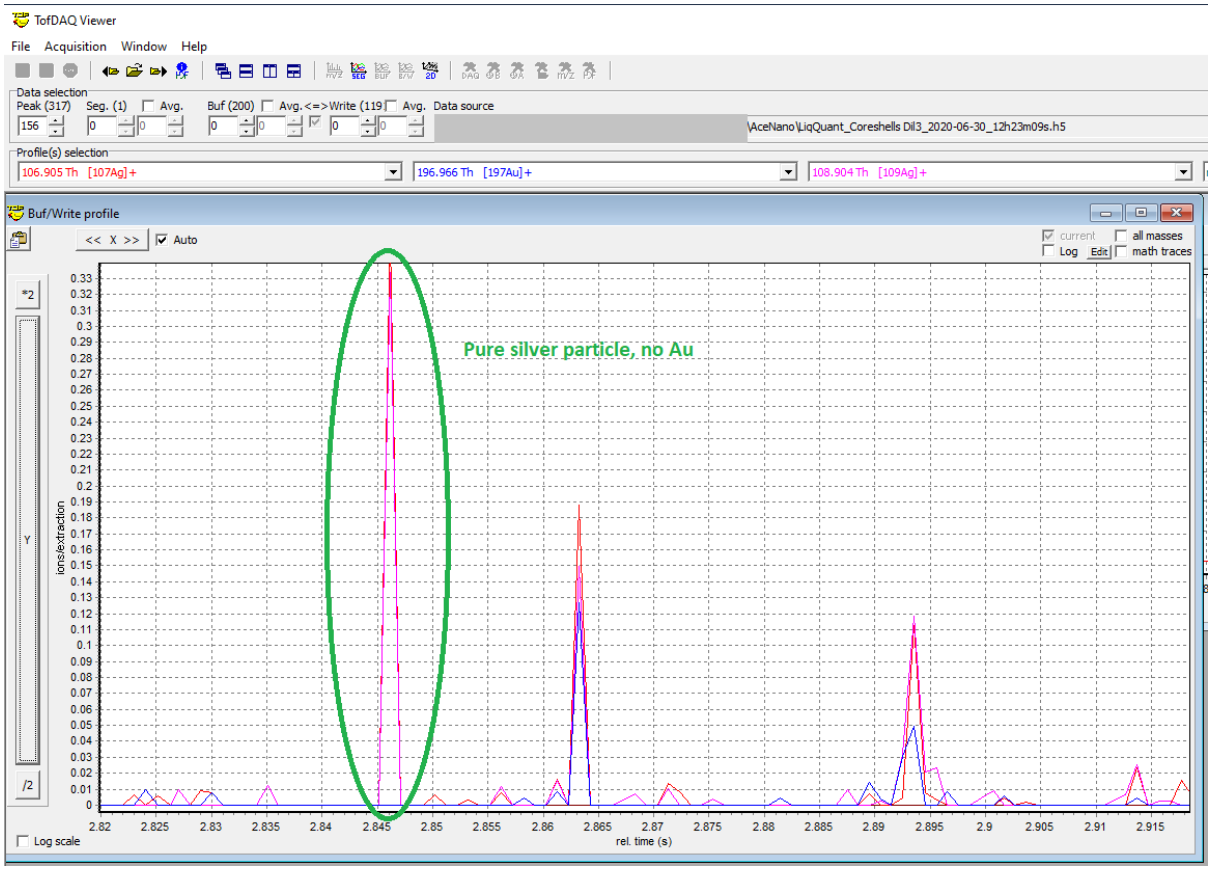


Figure A 8: The raw data was opened in TOFDAQViewer, where the isotopes ^{107}Ag (red), ^{109}Ag (pink) and ^{197}Au (blue) were monitored. Clearly recognizable is a pure silver nanoparticle without any gold.

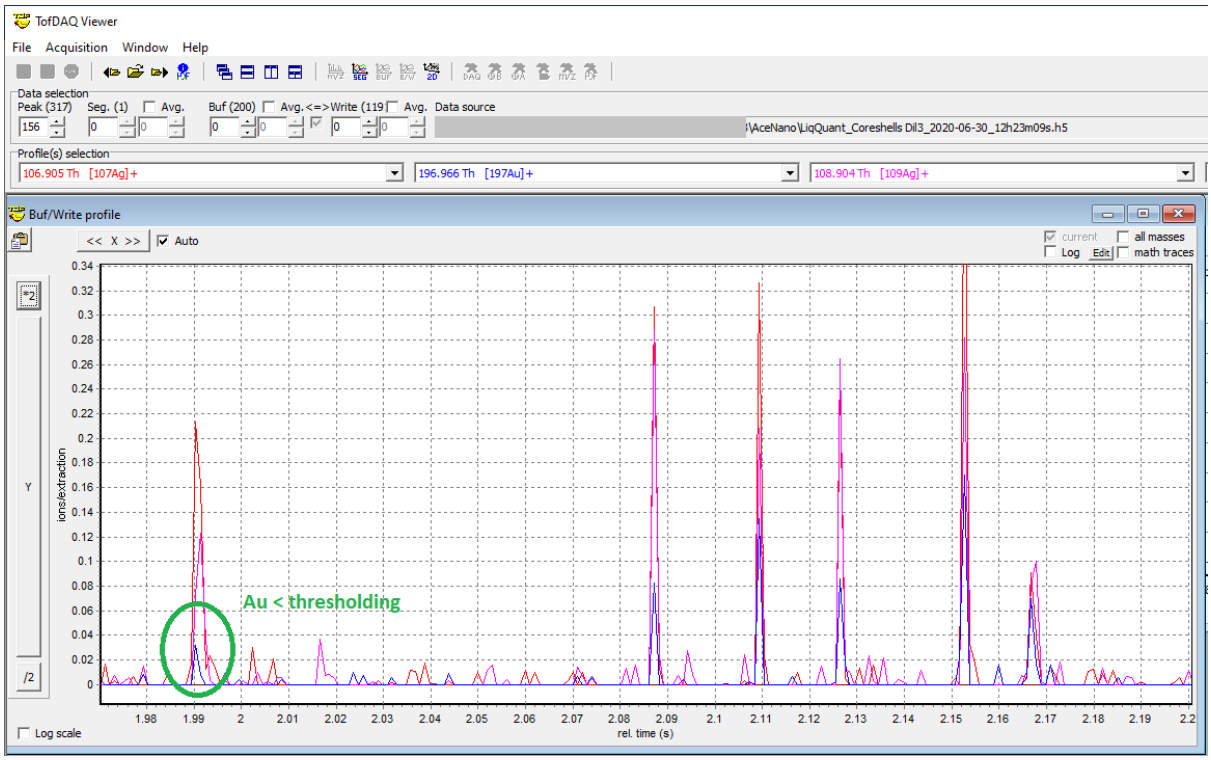


Figure A 9: The gold signal highlighted here has a values of approximatively 2 counts, which makes it difficult to distinguish from the background noise.

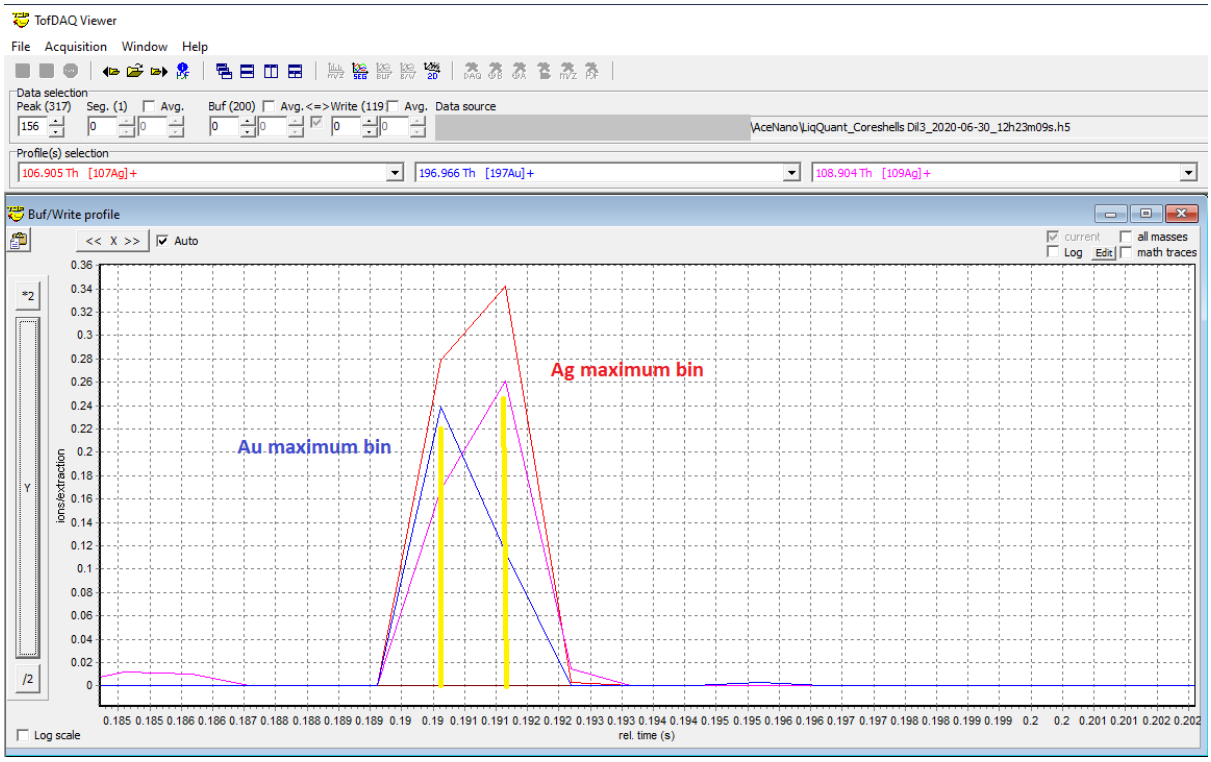


Figure A 10: Split particle which, after split event correction, will appear as two separate particles, consisting of Au and Ag exclusively.