

TOF SPI



Single Particle Investigator

USER MANUAL

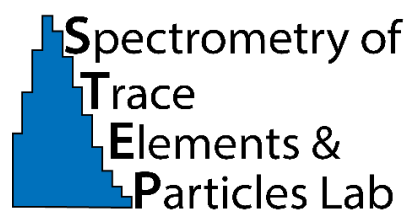
Developed by the Gundlach-Graham Research Group at Iowa State University

<https://gundlach-graham.chem.iastate.edu/>

<https://github.com/TOFMS-GG-Group>

Manual Version for TOF-SPI ver 2.7.3+

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Contents

1	TOF-SPI Overview	4
2	TOF-SPI Step 1: Settings, Critical Values, and Background	7
2.1	Detailed Instructions for TOF-SPI_Bkgd_LCs.exe	7
3	TOF-SPI Step 2: Determining Calibration Parameters	13
3.1	TOF-SPI Step 2b: Determining Calibration Parameters via Microdroplet Calibration	13
3.1.1	“02a_tofSPI_DropletCalib.exe” Instructions	13
3.2	TOF-SPI Step 2b: Determining Calibration Parameters via External Calibration	20
3.2.1	Instructions on 02b_TOF-SPI_LiquidCalib.exe	20
4	TOF-SPI Step 3: Finding Particles, Quantifying Particles, Generating Reports	29
5	Appendix I: Background on spICP-TOFMS and Online Microdroplet Calibration	46
5.1	spICP-TOFMS	46
5.2	Critical Values and Critical Masses in spICP-TOFMS	46
5.3	Online Microdroplet Calibration	48
6	Appendix II: Data Structure	50
6.1	icpTOF Data Structure	50
6.2	TOF-SPI Architecture	52

1 TOF-SPI Overview

The Time-of-Flight Single-Particle Investigator “TOF-SPI” programs are a set of data processing tools for the batch analysis of multi-elemental spICP-TOFMS data from icpTOF instruments (TOFWERK AG). The TOF-SPI programs are designed for spICP-TOFMS data collected with calibration either achieved by the “conventional” particle-size method of determining transport efficiency and absolute sensitivities¹ or the online microdroplet calibration method.^{2,3} TOF-SPI is written in LabVIEW and can be downloaded and installed as an executable function on 64-bit Windows PCs (<https://github.com/TOFMS-GG-Group>, repository: “TOF-SPI”). Installation guidelines can be found in the “READ ME” document on github. Example data files—“Test Data_Microdroplet Calibration” and “Test Data_External Calibration”—are also provided. TOF-SPI data processing steps are divided between three individual data processing steps, each with their own sub-program. In the first step, the analyte isotopes are selected, the single-particle region data boundaries are selected for data from online microdroplet calibration, the mass-dependent critical value expressions are calculated for each analyte,^{4,5} and the background signal levels are determined. In step 2, the absolute sensitivities and plasma uptake rate (q_{plasma}) are determined. In step 3, particle signals are found, split events are corrected,⁶ mass amounts of elements are quantified in each particle, particle number concentrations are determined, and data can be exported in a variety of formats. Work-flow diagrams for the use of the TOF-SPI with online-microdroplet calibration (Fig. 1.1.1) and with particle-size method for mass amount and number concentration calibration (Fig. 1.1.2) are provided on the following pages. In this manual, detailed instructions for the use of the TOF-SPI program are provided.

¹ 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. *Anal. Chem.* 2011, 83 (24), 9361-9369.

² Mehrabi, K.; Günther, D.; Gundlach-Graham, A., Single-particle ICP-TOFMS with online microdroplet calibration for the simultaneous quantification of diverse nanoparticles in complex matrices. *Environ. Sci.: Nano* 2019, 6 (11), 3349-3358.

³ Harycki, S.; Gundlach-Graham, A., Online microdroplet calibration for accurate nanoparticle quantification in organic matrices. *Anal Bioanal Chem* 2022, 414 (25), 7543-7551.

⁴ Gundlach-Graham, A.; Hendriks, L.; Mehrabi, K.; Günther, D., Monte Carlo Simulation of Low-Count Signals in Time-of-Flight Mass Spectrometry and its Application to Single-Particle Detection. *Anal. Chem.* 2018, 90 (20), 11847-11855.

⁵ Gundlach-Graham, A.; Lancaster, R., Mass-Dependent Critical Value Expressions for Particle Finding in Single-Particle ICP-TOFMS. *Anal. Chem.* 2023, 95 (13), 5618-5626.

⁶ Gundlach-Graham, A.; Mehrabi, K., Monodisperse microdroplets: a tool that advances single-particle ICP-MS measurements. *J. Anal. At. Spectrom.* 2020, 35 (9), 1727-1739.

Online Microdroplet Calibration Flow Chart

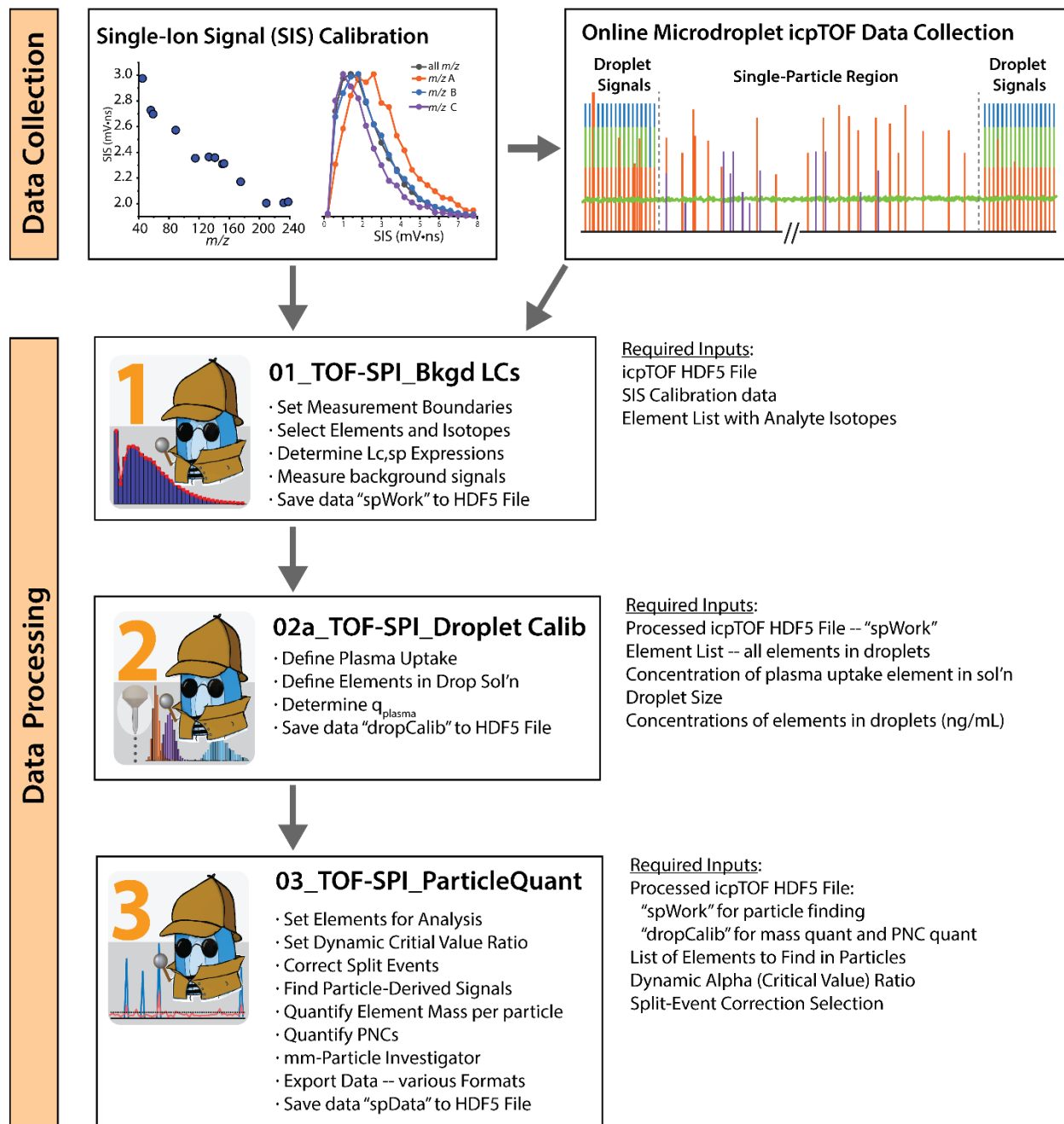


Fig. 1.1. Work-flow diagram for TOF-SPI with online microdroplet calibration.

External Calibration / Particle Size Method Flow Chart

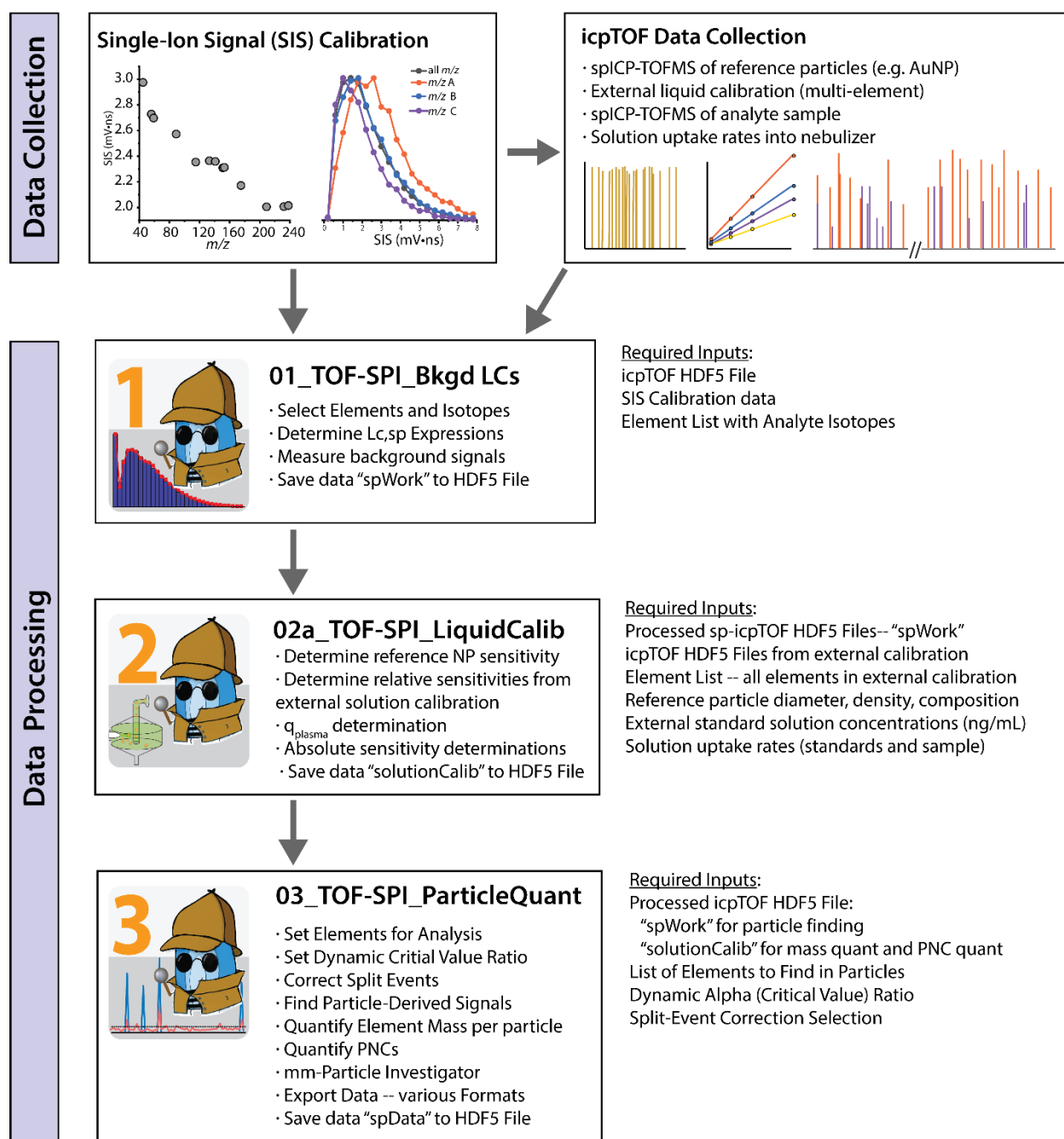
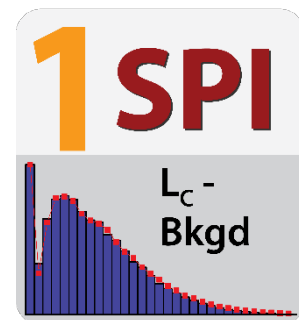


Fig. 1.2 Work-flow diagram for data analysis with TOF-SPI using the particle-size external calibration method.

2 TOF-SPI Step 1: Settings, Critical Values, and Background

In step 1 of TOF-SPI (“01_TOF-SPI_Bkgd_LCs.exe”), the user selects analyte elements and isotopes for particle analysis, defines the droplet regions and sp-regions for online microdroplet calibration, defines how mass-dependent compound-Poisson critical value expressions are generated, and selects a background calculation method. With these user-set parameters in place, the program can be used to calculate critical value expressions and background counts for all defined analyte elements in all



samples loaded, i.e. as a batch analysis procedure. Data generated from step 1 of TOF-SPI are saved in the “spWork” group in the HDF5 file. The 1st step of TOF-SPI is the most computationally intensive because critical value expressions are calculated via Monte Carlo simulation. It is recommended to run all files collected on a single day with the same basic structure (e.g. same sp-regions and data file length) in batch mode because this saves computer resources. (Note: The only feedback that the 01_TOF-SPI_Bkgd_LCs.exe program is running is the “running arrow” on the top right of the program window. This arrow is black when the program is running and turns back to white upon completion. Be patient: it could take a few minutes for the program to process a large batch of files; however, the results will be worth it!)

Check List / Overview of User Steps:

- ✓ Load “ICP-TOFMS Data File(s)”
- ✓ Toggle “Run All Files in Folder?” to process all HDF5 files in folder.
- ✓ Start and End of sp-Region
- ✓ Load or define isotopes for analysis
- ✓ Set parameters for SIS Histograms and Critical-Value Calculations
- ✓ Define how background counts will be determined (drift averaged or fit)
- ✓ Toggle “All Parameters Set, Ready to Run Bkgd Fitting?”
- ✓ Run Program with “Write Info to HDF5 File” Enabled

2.1 Detailed Instructions for TOF-SPI_Bkgd_LCs.exe

The workflow of 01_TOF-SPI_Bkgd_LC is split between two tabs. In the first tab (Load File_Set Parameters), all the user-input information is provided. In the second tab (Bkgd TofCt Values), the user selects the background signal determination method and verifies that all required user inputs are in place and that the complete program is now ready to be run. To switch between these tabs, just click on them. Detailed instructions of user data needed and how to run the program are provided below.

Tab 1: Load File_Set Parameters

This tab contains five different sections, and each section is shown by a different colored box in Figure 2.1.1, which is a screen capture of the front panel of the program prior to loading any icpTOF data or other user input.

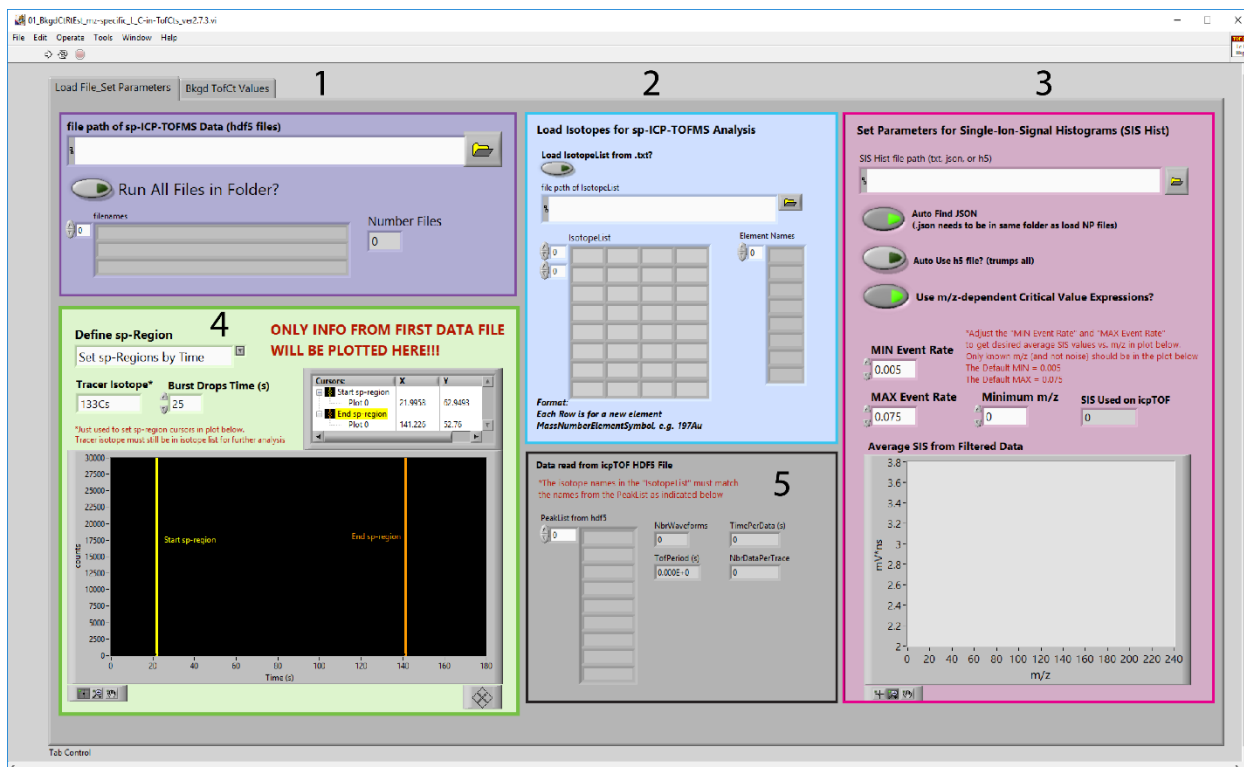


Figure 2.1.1. Graphical user interface (GUI) of the step 1 of TOF-SPI (01_TOF-SPI_Bkgd_LCs.exe) with no data loaded.

Steps in Workflow:

1. Upload or drag the icpTOF HDF5 data file into “file path of spICP-TOFMS Data (hdf5 files)” (box 1 in Figure 2.1.1)

Drag and drop a file into the box or browse for a file using the file browsing button. A single file or multiple files can be processed at a time. To do batch processing of multiple files, click the button “Run All Files in Folder?”

After the program is run, the names of all files being processed will be shown below in the “filenames” box

Note: For the batch processing to work, all files must have the droplet bursts in the same location. If this is not the case, files must be processed individually in step 1. Subsequent steps can then make use of the batch processing mode.

2. Input elements and isotope or load elements and isotope list (box 2 in Figure 2.1.1)

All analyte elements and isotopes that the user would like to use for quantification, or find as particles, must be input into the program in the “Isotope List”; this list can be populated

manually or through uploading a txt file. In both manual entry or in an “IsotopeList” txt file, the first column contains the name of each element to be analyzed, and subsequent columns contain the isotopes that correspond to that element (see Table 1 below). If multiple isotopes are listed in a single row, their signals will be summed together. The element name in column 1 is the only name TOF-SPI will recognize in further analysis steps. It is not necessary for the “element” to be the actual name of a real element. It is simply the name TOF-SPI will give to the summed isotopes listed.

To load the isotope list from a .txt file: click the button labeled “Load IsotopeList from .txt?” and browse for the desired file.

Table 1: Example of properly formatted Isotope List

Cs	133Cs			
Mg	24Mg	25Mg		
Al	27Al			
Ti	48Ti			
Ag	107Ag	109Ag		

3. Set parameters for SIS histogram (box 3 in Figure 2.1.1)

In this section SIS data obtained during the detector tuning step of the ICP-TOFMS instrument is loaded.

- If HDF5 files were collected using a workflow in TOFpilot (ver.2.11.5.0 or later), the SIS data should be saved to the HDF5 file directly. In this case, select the button labeled “Auto use H5 file?”.
- If the data was not collected using a workflow, a separate .json file is necessary. If the .json file is present in the same folder as HDF5 data files (recommended), then click on the button “Auto find JSON”; the button will turn green as shown in figure 2.
- Otherwise, deselect the “Auto find JSON” and load the .json SIS calibration file by selecting the path.

Highly suggested: Select the button “Use m/z dependent critical value expressions.” This reduces false-positive particle identifications by one to two orders of magnitude compared to thresholding criteria based on Normal or Poisson statistics. The MIN Event Rate and MAX Event Rate can be adjusted to select only SIS signals from known m/z in the SIS tuning solution.

Once section 1, section 2 and section 3 are completed, press the run button present on top left of the program (indicated by white arrow) or use the “CTRL+R” shortcut. After starting the program, the white run arrow will turn black indicating the program is running (when complete, it turns white again.) The GUI looks like figure 2.1.2 now which shows file names (section 1), isotope and element names (section 2), single-ion-signal histogram (section 3), droplet region and single particle region (section 4) and additional data information (section 5).

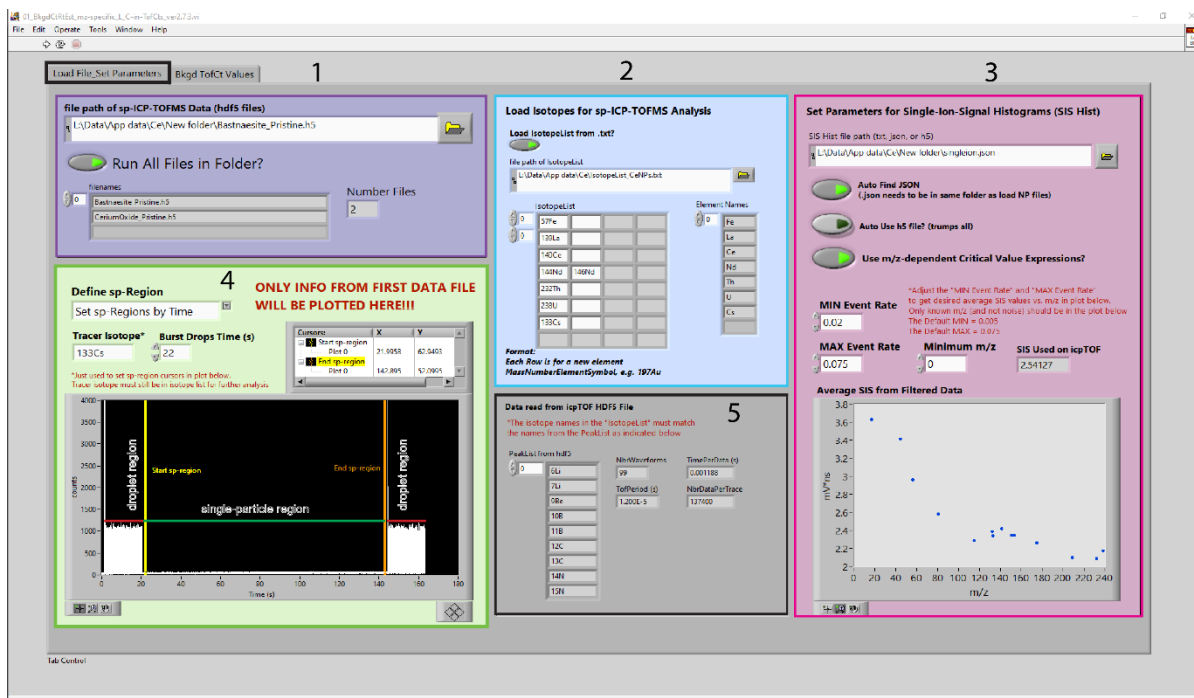


Figure 2.1.2. Graphical user interface (GUI) of step 1 (01_tofSPI-Bkgd-CritValues.exe) of TOF-SPI. The program looks like Figure 3 when data files, isotope list (.txt), droplet elements list with concentration (.txt), and single-ion-solution histogram (.json) are uploaded and run. And sp-region is selected for further data processing.

4. Selection of the single-particle region (box 4):

The default tracer element for droplets is Cs. If using an element other than Cs, enter it in the “Tracer Isotope” box and run the program again to make the droplet signals appear. There are two lines that define the bounds of the single-particle region. The yellow bar sets the start of the region, and the orange bar is the end of the region. There are several ways to adjust the location of these bars, which can be changed using the “Define sp-Region” dropdown box:

- Set “sp-Regions by Cursor”: Use the crosshair tool in the lower-left corner of the time trace to manually drag the cursors to the proper locations.
- Set “sp-Regions by Time”: enter the length of the droplet burst in the “Burst Drops Time (s)” box. (e.g., if the droplet burst is always < 25s long, enter 25 in the box. The program will set the cursors 25 s from either end of the acquisition.
- Set “No Drops: sp-Region Full Time Trace No Drops”: This will remove the droplet regions so that the full time trace is the sp-Region. This is useful for analysis without online microdroplet calibration, like when external calibration via the particle-size method is used. No time trace will appear on the plot when this option is selected.

If everything is good on this tab, then move on to the second tab “Bkgd TofCt Values” for additional data processing steps.

Tab 2: Bkgd TofCt Values

This tab contains three different sections, as shown in Figure 2.1.3. There are two options for how to do the background fitting: drift correction (default) or sum squared error (SSE) fitting. Directions for each background determination method are provided below. The background fitting step can take a long time to process because the Monte Carlo simulations of compound Poisson critical values are computationally intensive. Double-check that all data in Tab 1 is loaded in properly before running the program with the “All Parameters SET? Ready to Run Bkgd Fitting?”

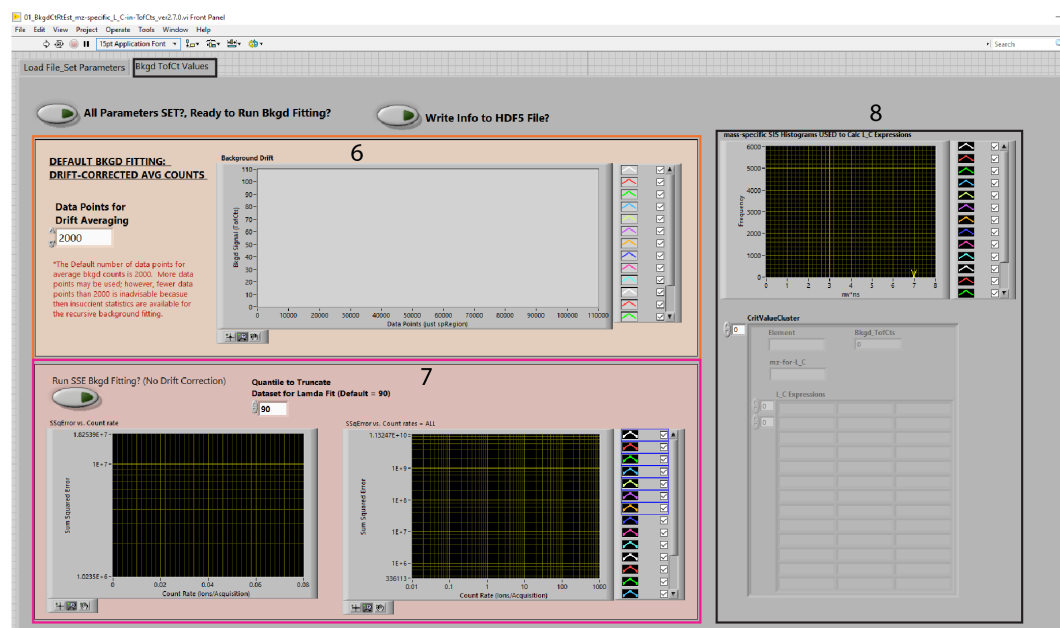


Figure 2.1.3. GUI showing the second tab selected and no data yet processed.

For Drift-Correct Background Determination (box 6 in Figure 2.1.3):

This fitting method is the default method and the one that should be used most of the time. It is especially useful (and necessary) for long acquisitions and for TOF background signals that drift across time. To modify the number of consecutive data points for which average background counts are determined, change the value in the “Data Points for Drift Averaging” box. The default value is 2000; and should work well in most cases. If the default drift-corrected bkgd fitting is desired, then simply click the click (i.e. toggle) the “All Parameters SET?, Ready to Run Bkgd Fitting?” and the “Write Info to HDF5 File?” buttons to save the information from this program to the HDF5 file. Run the program using “CTRL+R” or the white run arrow in the top left of the program window. An example screenshot after files have been processed with drift-correction background determination is provided in Fig. 2.1.4.

For Sum Squared Error Fitting Background Determination (box 7 in Figure 2.1.3)

If the data does not have any drift, then it can be processed without drift correction. Click the “Run SSE Bkgd Fitting ? (No Drift Correction)” button. Also press the “All Parameters SET?, Ready

to Run Bkgd Fitting” and “Write Info to HDF5 File?” buttons and run the program. Run the program using “CTRL+R” or the white run arrow in the top left of the program window. An example screenshot after files have been processed with SSE background fitting is provided in Fig. 2.1.5.

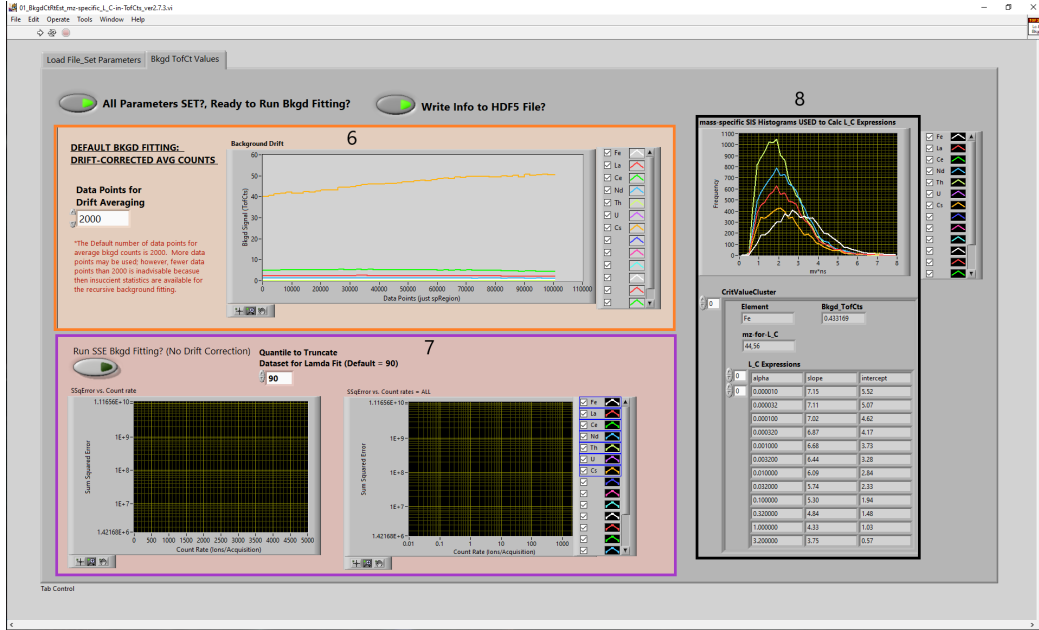


Figure 2.1.4. GUI of second tabl. At this point files are processed, and information is written on HDF5 file.

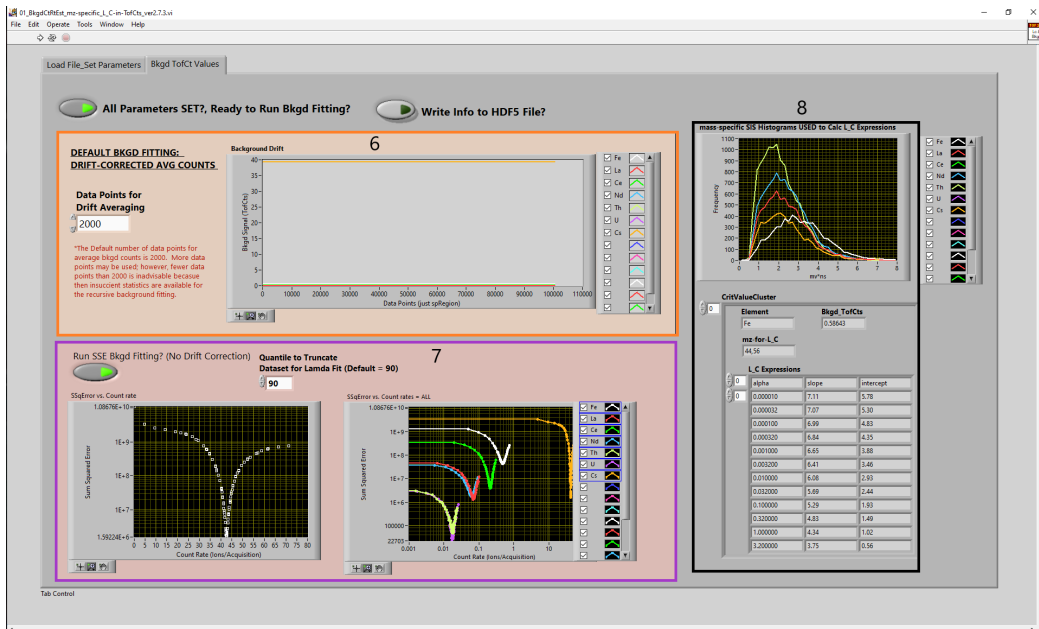
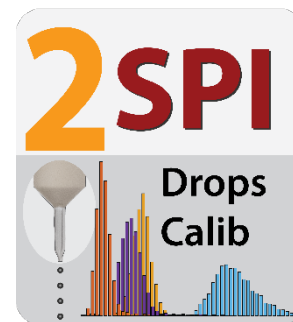


Figure 2.1.5. GUI of tab 2. At this point files are processed to check Sum Squared Error vs. Count Rate (TofCts/Acquisition).

3 TOF-SPI Step 2: Determining Calibration Parameters

There are two different programs for determining the calibration parameters (step 2a and step 2b); however, data should only be processed with either 2a or 2b. Step 2a should be used for samples calibrated with online microdroplet calibration. Step 2b should be used for samples calibrated via external calibration.



3.1 TOF-SPI Step 2b: Determining Calibration Parameters via Microdroplet Calibration

Check List / Overview of User Steps:

- ✓ Load spICP-TOFMS Data Files
- ✓ Select Plasma Uptake Standard, load Plasma Uptake concentration in nebulized sample
- ✓ Set droplet diameter, load in droplet concentrations, set plasma uptake concentration in drops
- ✓ Run program to check the droplet thresholding. Adjust if necessary.
- ✓ Run program with “Write Calibration info to H5 File” enabled so transport efficiencies and sensitivities are recorded to each data file.

3.1.1 “02a_tofSPI_DropletCalib.exe” Instructions

In Step 2a, the user will input the droplet size and concentration of the droplet solution to find the plasma uptake and absolute sensitivities of the elements in the droplet solution. All particle data used in this step must have already been processed by Step 1. Once samples are run in Step 2a, calibration data will be saved to the HDF5 files, in the “dropCalib” folder, to be used for quantitative analysis of selected isotopes within particles in Step 3.

Tab 1: File Settings

On the first tab (“File Settings”), upload your spICP-TOFMS H5 data files using the folder button or by dragging the desired file into the file bar. These files must be first processed by step 01 and contain microdroplet signals for calibration. To run all files in a folder, select one of files in the folder using the file input dialog. Ensure the button labeled “Run All Files in Folder?” is checked (green), see Fig. 3.1.2 . To run a single file, select one file using the file input dialog. Ensure that the button labeled “Run All Files in Folder?” is unchecked (grey).

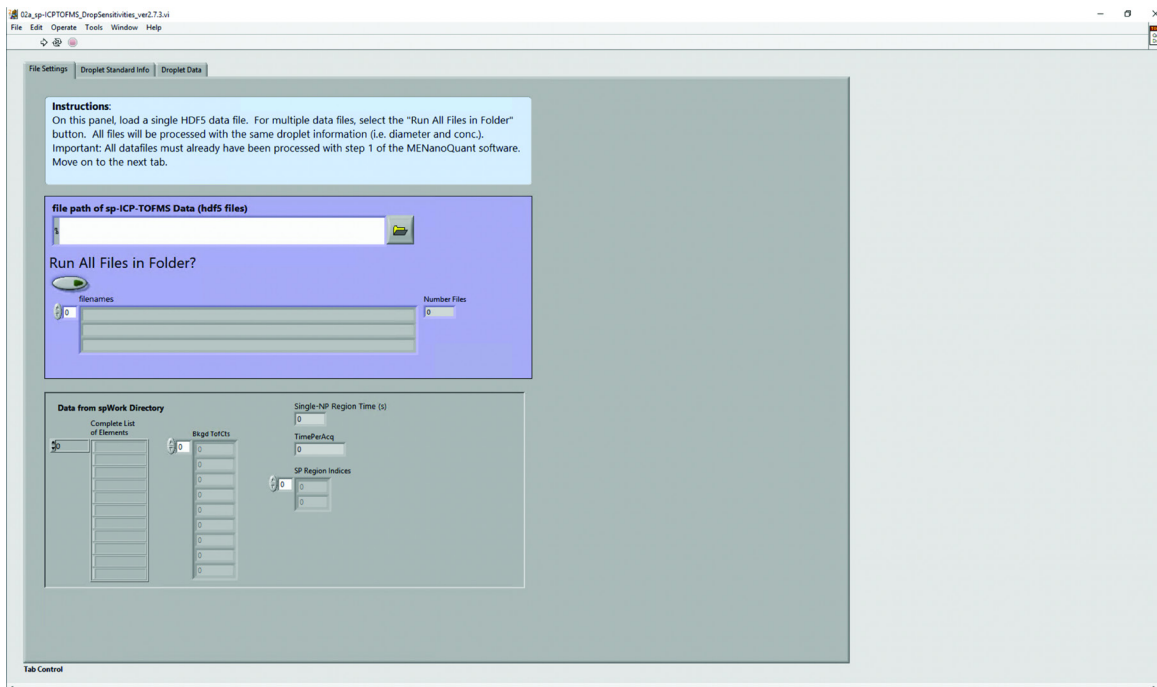


Figure 3.1.1. GUI of step 2a using microdroplet calibration, shown above without inputting data. Tab 1 is used only to select the spICP-TOFMS files.

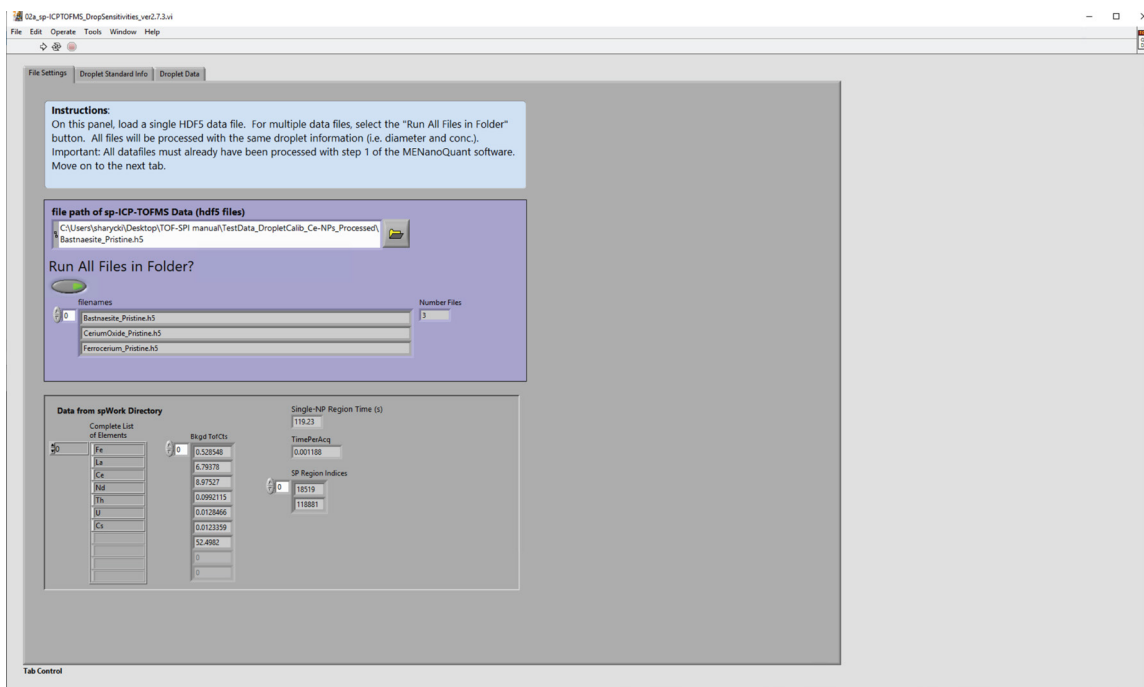


Figure 3.1.2. GUI of step 2a tab 1 with all sample data loaded. The information in the “Data from spWork Directory” box indicates that this data was properly processed with step 1.

Tab 2: Droplet Standard Info

On the second tab, “Droplet Standard Info,” information on the droplets used for online microdroplet calibration is provided by the user. In Fig. 3.1.3, a screenshot of this tab is provided with sections of the program numbered. The operations for each of these numbered sections of the program are provided below.

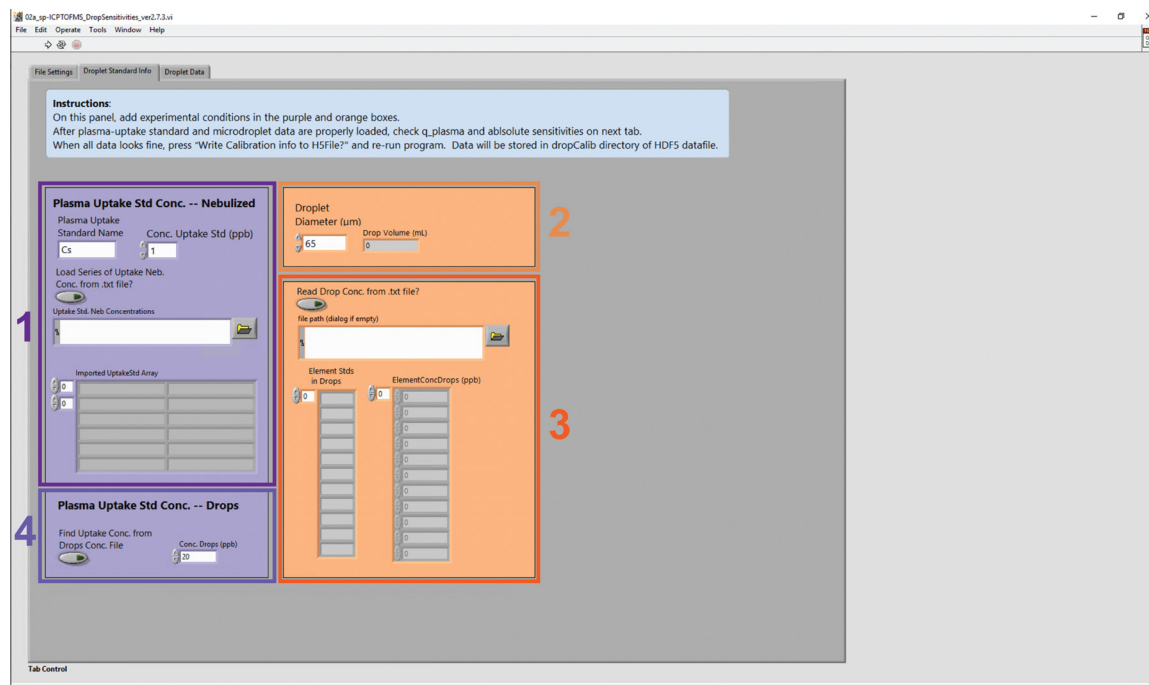


Figure 3.1.3. GUI of tab 2 with no data entered.

1. Input the nebulized plasma uptake standard concentration

In box 1, the information relating to the nebulized plasma uptake should be entered. The element used for the plasma uptake standard can be changed in “Plasma Uptake Standard Name” box. The name of this element must be an element in the isotope list entered in Step 01 of TOF-SPI. The nebulized uptake standard concentration can be entered in one of two ways:

Manual entry (single value)

If the concentration of the nebulized uptake standard is the same in all files being analyzed, it should be entered in the “Conc. Uptake Std (ng/mL)” box. (Note: previous versions of TOF-SPI (prior to ver2.7.4.2) had this box labeled “Conc. Uptake Std (ppb)”, as seen in Fig. 3.1.3).

Upload .txt file

If the concentration of the uptake standard varies between files, a text file can be uploaded that contains the concentration information for each file. The file should be formatted with the first column containing the name of the .H5 files and the second column containing the

concentration of the uptake standard in ng mL^{-1} . The first row of this file is reserved for column headers and will not be imported as data. An example is shown below:

Filename	Conc. (ng mL^{-1})
File1.h5	2.2
File2.h5	3
File3.h5	3.5

2. Input the droplet diameter

In box 2, enter the average diameter of the microdroplets in μm . TOF-SPI will automatically calculate the volume assuming that the droplet was spherical.

3. Enter the droplet concentration

In box 3, there are two ways to enter the elemental concentrations of the microdroplet standard: with a text file or manual entry.

Upload .txt file

To upload a text file, ensure that the button labeled “Read Drop Conc. from .txt?” is checked (green). The text file must be formatted with the first column containing the element name and the second column containing the concentration in ng mL^{-1} . The element names must be included in the isotope list from step 01 of TOF-SPI. An example is shown below:

Au	10.34
Ag	9.85
Cs	15.52

Manual entry

To manually enter concentrations, ensure that the button labeled “Read Drop Conc. from .txt?” is unchecked (grey). Enter the elements in the droplet solution in the column labeled “Element Stds in Drops” and their corresponding concentrations in ng mL^{-1} in the column labeled “ElementConcDrops in ng/mL (ppb).” (Previous versions (prior to ver2.7.4.2) of TOF-SPI had this box labeled “ElementConcDrops (ppb)” as seen in Fig. 3.1.4.)

4. Input the plasma uptake concentration from the droplets

In box 4, enter the concentration of the uptake standard in the droplets. If a text file was used to load in the droplet concentrations in step 3 above, *and* the uptake standard is listed in the text file, simply check the button labeled “Find Uptake Conc. from Drops Conc. File.” If the uptake standard was not listed in the droplet concentration .txt, or concentrations were manually entered, enter the concentration of the uptake standard in the droplets in the box labeled “Conc. Drops (ng/mL)”

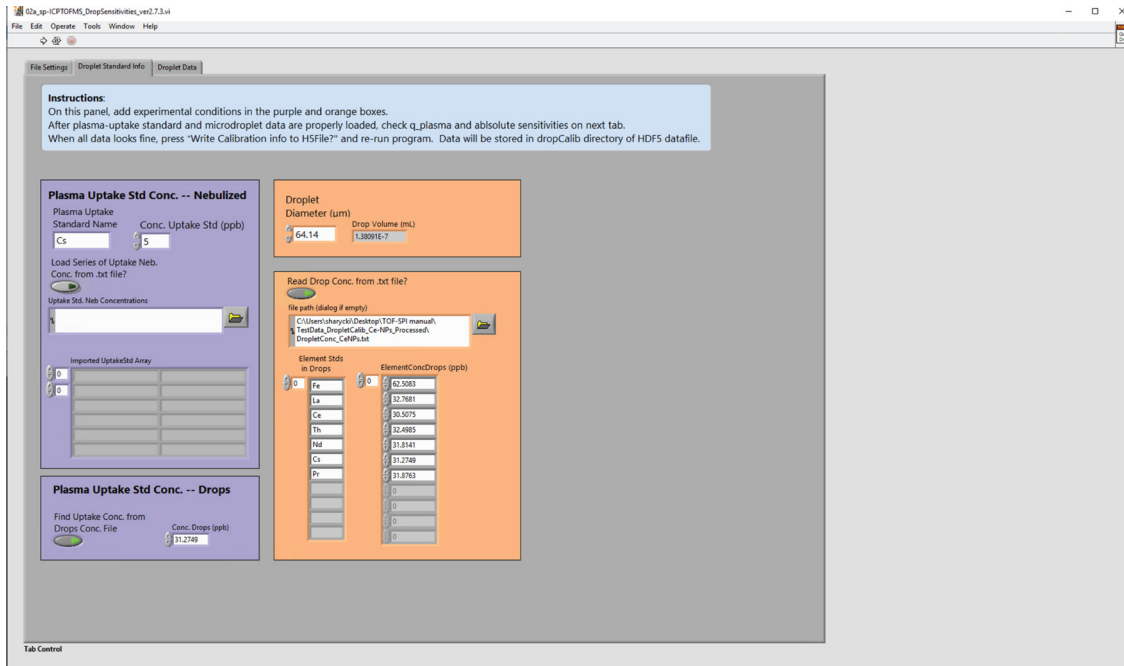


Figure 3.1.4. GUI of TOF-SPI step 2a, tab 2 with all sample data loaded.

Tab 3: Droplet Data

On the third tab, “Droplet Data,” the user checks to make sure that all droplet data loaded correctly and determined absolute sensitivities and q_{plasma} values make sense. In Fig. 3.1.5, a screenshot of this tab is provided with sections of the program numbered. The operations for each of these numbered sections of the program are provided below.

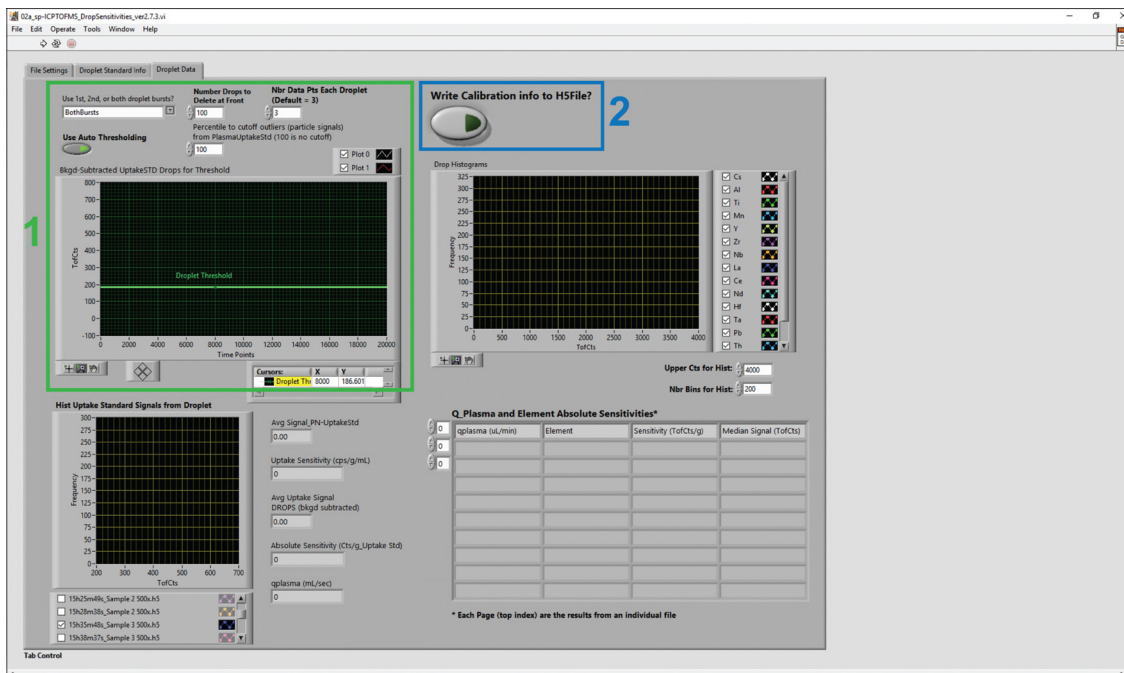


Figure 3.1.5. GUI of tab 3 with no data entered.

Steps in Workflow on Tab 3:

1. Adjust droplet calibration settings

At the top of this page (box 1), the settings for the droplet calibration can be modified. There is a drop-down box to change which droplet burst to use for calibration: the first burst, second burst, or an average of both bursts. The box labeled “Number Drop to Delete at Front” changes how many droplets from the beginning of the droplet burst will be excluded from the calibration. By default, the signals from the first 100 droplets at the beginning of each burst are omitted for the median signal calculation to account for solvent evaporation in the tip of the microdroplet generator. This solvent evaporation happens before and between bursts of droplets and causes the first droplets to be more concentrated with trace elements, and so produce elevated signals. This number can be adjusted to exclude more or fewer droplets. The “Nbr Data Pts Each Droplet” box adjusts the number of adjacent data points summed together for each droplet signal. This may need to be adjusted depending on the mass-spectral acquisition rate, but the default of 3 is good in most cases.

2. Set the droplet threshold

The threshold level for the microdroplets needs to be set prior to calibration. There are two ways to set the threshold for the microdroplets, auto thresholding and manual:

Auto Thresholding

If the “Use Auto Thresholding” button is checked, TOF-SPI will automatically determine the threshold for finding microdroplet signals for each file individually from the signal of the uptake standard in the droplets. This is particularly useful when sensitivities vary across files and is the generally recommended approach.

Manual Thresholding

To manually set the threshold for the droplet signals, uncheck the “Use Auto Thresholding” button. Run the program by clicking the white arrow in the upper left corner or type ‘CTRL + R.’ This should load in the droplet time trace in the “Bkgd-Subtracted UptakeSTD Drops for Threshold” window on the upper left side of the page. Click on the crosshair selection toolbox in the lower left corner of the droplet time trace window. Drag the green bar labeled “droplet threshold” to the desired threshold location on the time trace.

3. Write calibration information to HDF5 file

When all parameters are set as desired, check the button labeled “Write Calibration info to H5File” and run the program.

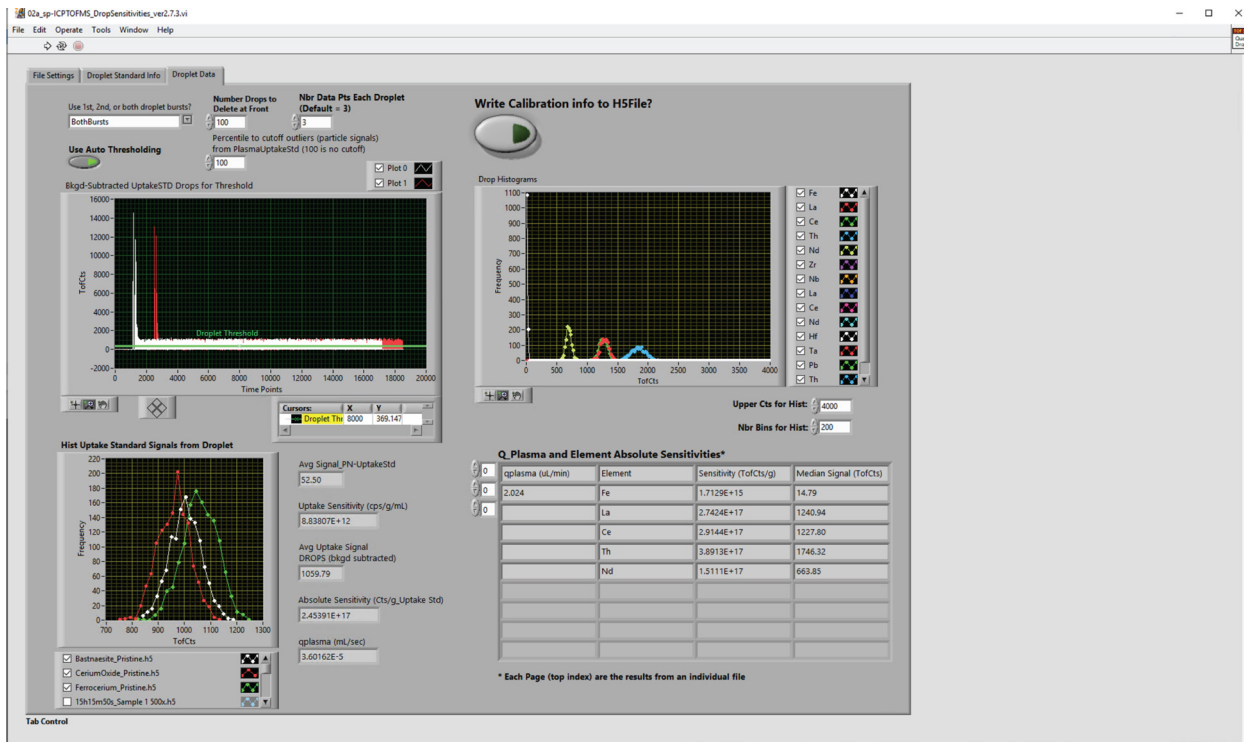


Figure 3.1.6. GUI of tab 3 with all sample data loaded. The histogram in the lower left corner labeled “Hist Uptake Standard Signals from Droplet” shows the overlaid histograms of the uptake standard for all files. The histograms on the upper right (“Drop Histograms” are the overlaid droplet signal histograms for the last file that was analyzed only. The histogram display settings (upper range and number of bins) can be adjusted using the “Upper Cts for Hist” and “Nbr Bins of Hist” boxes. It is necessary to rerun the program for the display to update after changing these numbers. The array in the lower right corner displays plasma uptake (q_{plasma}) and droplet sensitivity information for every element in the droplet concentration list. Information from different files can be viewed by adjusting the first index counter. The rows and columns displayed can be shifted by adjusting the second and third index counters, respectively.

3.2 TOF-SPI Step 2b: Determining Calibration Parameters via External Calibration

In step 2b of TOF-SPI (“02b_TOF-SPI_LiquidCalib.exe”), the user will input both particle and dissolved calibration data to find transport efficiency and sensitivities of selected isotopes. All particle data used in this step must have already been processed with Step 01 of TOF-SPI. Once samples are run in Step 2b, calibration data will be saved to the HDF5 files, in the “solutionCalib” folder, to be used for quantitative analysis of selected isotopes within particles in Step 3.



Check List / Overview of User Steps:

- ✓ Load reference particle spICP-TOFMS data file, ICP-TOFMS files for calibration of reference particle element, calibration curve concentrations, and uptake rates
- ✓ Run program to check reference particle time trace, reference element calibration curve, and the transport efficiency reported
- ✓ Load Multiple Element (ME) calibration curve ICP-TOFMS files, calibration curve concentrations, isotopes for quantitative analysis, and uptake rates
- ✓ Run program to check the ME calibration curves, and look over the absolute sensitivities now calculated for the selected isotopes
- ✓ Load sample spICP-TOFMS data files and uptake rates
- ✓ Run program with “Write Info to HDF5 File” enabled so transport efficiencies and sensitivities are recorded to each data file

3.2.1 Instructions on 02b_TOF-SPI_LiquidCalib.exe

The work-flow of 02b_TOF-SPI_LiquidCalib is split into three tabs. In the first tab (Liquid Quant TE Workup), icpTOF HDF5 data files from analysis of standard reference particles and dissolved solutions of the same element to create a calibration curve are used to calculate the plasma uptake rate (q_{plasma}) and the transport efficiency (TE). q_{plasma} is calculated via particle size method as the ratio absolute sensitivity of the particle reference signal (TofCts g^{-1}) over the relative sensitivity (TofCts $\text{s}^{-1} / \text{ng mL}^{-1}$). The TE is the q_{plasma} divided by the measured uptake rate of solution into the nebulizer. This ratio of sensitivities works to find TE because the signal of the solution depends on transport into the plasma whereas the signal measured from a particle is independent of TE, and masses (or concentrations) of both the solution and particle are known.⁷ In the second tab (Liquid Quant DEs), the multiple element calibration curves are input, and the detection efficiency (DE), also called absolute sensitivity (TofCts g^{-1}), and relative sensitivity (TofCts $\text{s}^{-1} / \text{ng mL}^{-1}$) of each element/isotope selected is calculated. Detailed instructions of user input within each tab are provided below.

⁷ M. D. Montaña , J. W. Olesik , A. G. Barber , K. Challis and J. F. Ranville , Anal. Bioanal. Chem., 2016, 408 , 5053-5074

Tab 1: Liquid Quant TE Workup

The first tab in step 02b contains two main sections, separated into blue and red boxes, as shown in Figure 3.2.1. In the blue box, data from the spICP-TOFMS analysis of the reference particle sample is input and processed. In the red box, ICP-TOFMS signals from the dissolved solution calibration standards are input, calibration curves and generated, and q_{plasma} and TE are determined.

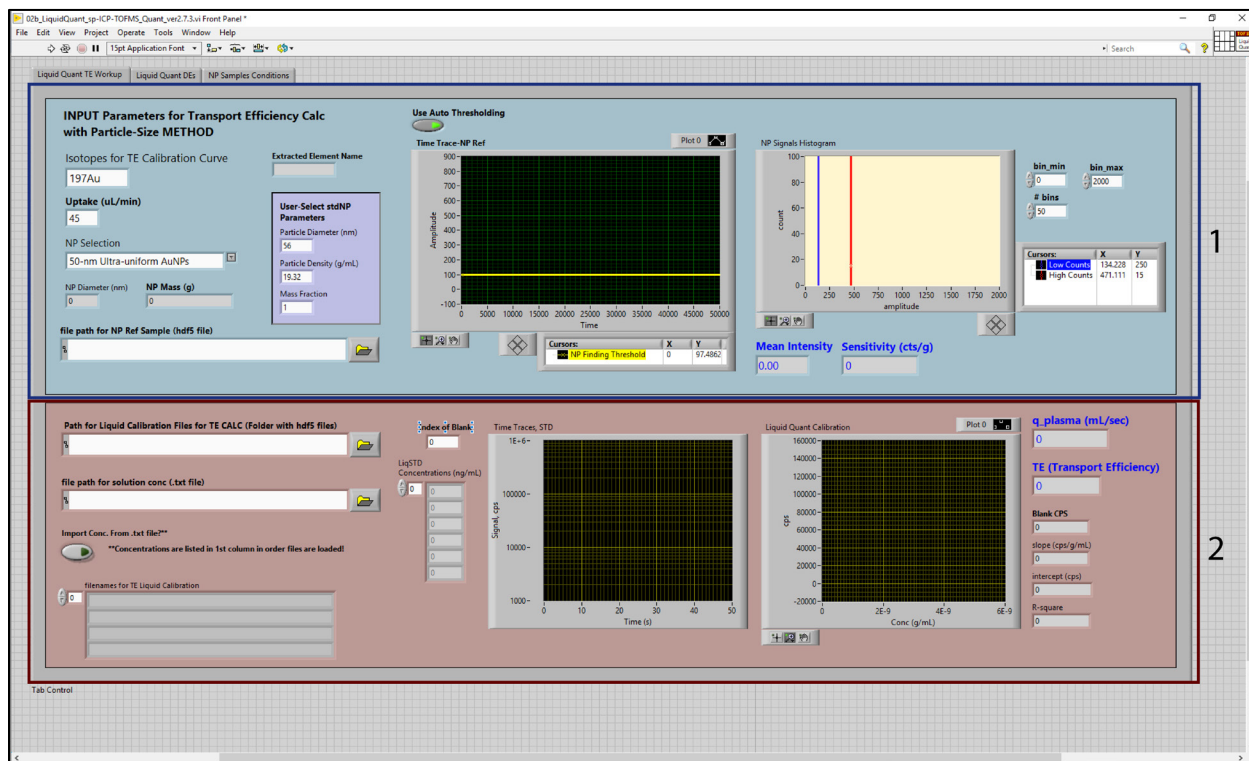


Figure 3.2.1 GUI of step 2b using solution calibration and reference standard particles for calculation of transport efficiency, shown above without inputting data.

Steps in Workflow on Tab 1:

1. Upload and define the standard reference particle used (box 1)

The first step of 2b is to upload and define the reference particle used to calculate TE. First, drag and drop (or browse for) the data file into the box indicated under “file path for NP Ref Sample (hdf5 files).” This should be a single file where only the reference particle was run. Note: This file must be processed with step 1 of TOF-SPI prior to analysis in step 2b.

Enter the isotope to be used for the standard reference particle calibration, the liquid sample uptake rate (i.e. flow rate), and the type of particle used from the drop-down menu. The uptake rate is given in μL per min, and is obtained via flow-meter, or from the setting used on a syringe pump or autosampler. If the particle type used is not in the drop-down menu provided, select “User selected” and use the inset purple box “User-Select stdNP Parameters” and fill in particle diameter, density, and mass fraction of the selected element.

Run the program to ensure particle signals are plotted in the time trace and the auto-thresholding button is selected (shown by the yellow line) used to create the histogram on the right. The histogram is the distribution of counts of the selected isotope within each particle detected, given the particle has counts above the yellow line in the time trace. The yellow line threshold can be manually moved by deselecting the auto-threshold button and dragging the line up or down and should be moved if the corresponding histogram is not Gaussian in shape. The blue (low count) and red (high count) lines on the “NP Signals Histogram” can also be manually moved so only particles within the two lines are used to calculate TE. For example, the red line may be moved in order to remove double events. Mean intensity (in TofCts), as well as the sensitivity of the standard reference isotope, are given below the histogram.

2. Upload or drag the calibration curve files for the standard reference particle (box 2)

The first file input in the red (lower) box on the first panel is for the calibration curve using the selected isotope from the standard reference particle. The file input is for an entire folder, so all calibration data files should be uploaded together. Be sure that the files are labelled numerically or alphabetically, as they will appear and be plotted in that order. The position of the ‘blank’ data file can be indicated, if not at the beginning, by the “Index of the Blank” input. Note that numbering starts at ‘0’. Run the program to check the file names and order, which will populate in the lower left corner in the “filenames for TE Liquid Calibration” list.

3. Input standard reference calibration concentrations and re-run

The next input is for the calibration solution concentrations. Users can enter this in two ways: either through uploading a .txt file with listed concentrations in the same order as the calibration data files, or by manually entering concentrations. If using a pre-made .txt file, simply drag or search for the file name in the “file path for solution conc (.txt file)” box and select the button for “Import Conc. From .txt file?”. If manually entering, enter concentrations in the order of the files under the “LiqSTD Concentrations (ng/mL)” table. The unit used for concentration is ng mL^{-1} in either case. Run the program, and make sure the concentrations are correct, the time traces of each calibration data file are plotted. Also make sure the “LiqSTD Concentrations” table populates correctly if being read from a .txt file.

Check the q_{plasma} (the mL of liquid sample introduced to the plasma per second) and the TE. Finally, check the calibration curve shown in the lower right of the first panel; blank counts per second, slope, intercept, and R^2 values generated. Now the user can move to the second tab.

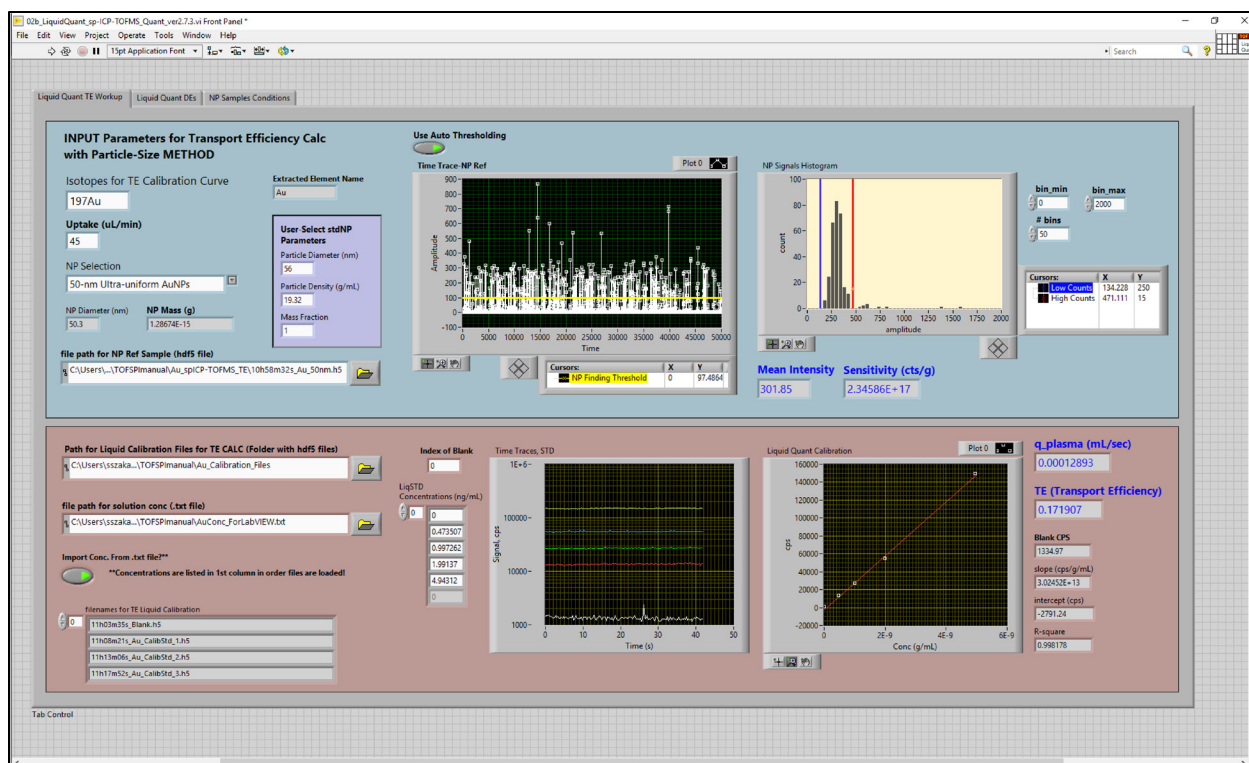


Figure 3.2.2. GUI of step 2b tab 1 with all sample data input for 50 nm Ultra-uniform standard reference Au particles in the top blue box, and the corresponding Au calibration curve (with concentrations) in the lower red box. The q_{plasma} and TE are displayed in the lower right side of the tab.

Tab 2: Liquid Quant DEs

The second tab in step 02b requires input of a ME calibration curve to calculate the absolute sensitivities for elements the user is interested in quantifying within particles. The whole panel, without data entered, is shown in Figure 3.2.3.

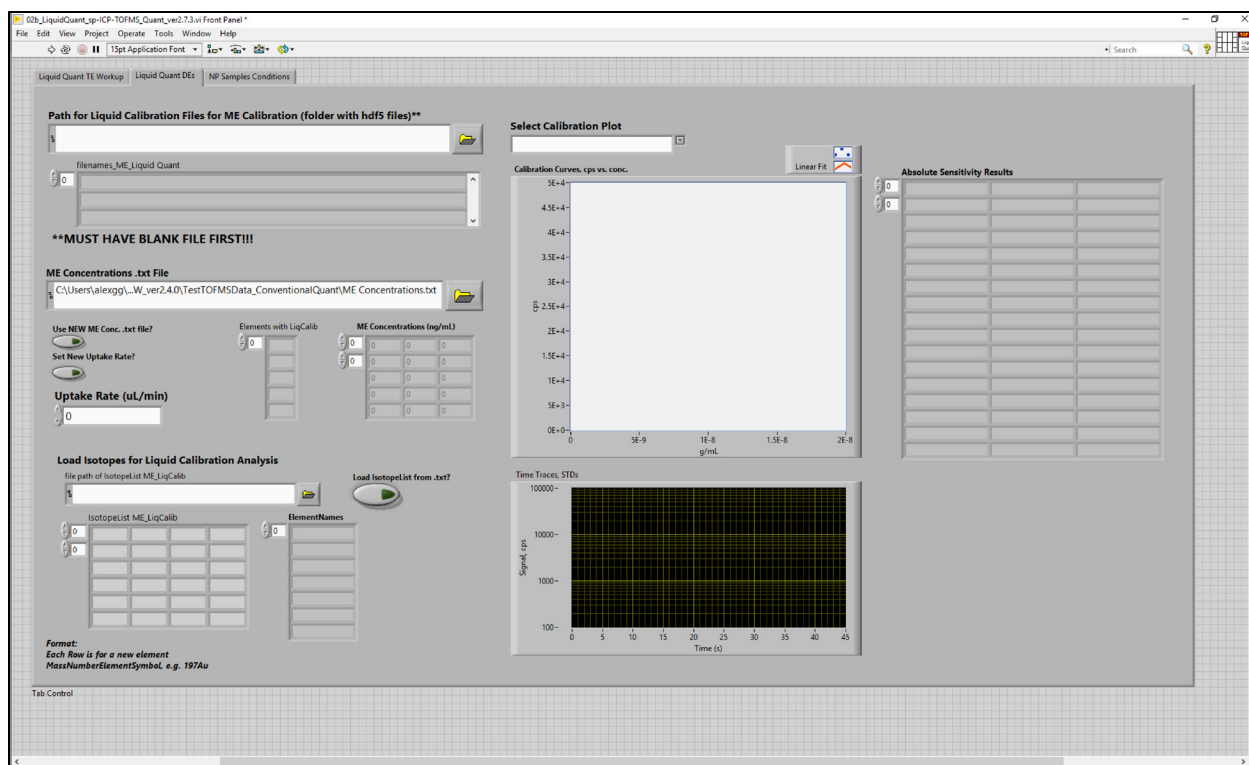


Figure 3.2.3. GUI of tab 2 with no data entered.

Steps in Workflow on Tab 2:

1. Upload or drag the folder containing Multi Element Calibration data files

Drag and drop a folder, or browse for a folder, under the “Path for Liquid Calibration Files for ME Calibration (folder with hdf5 files)” that contain all files used in the ME calibration curve. Similar to the first tab, the files should be in order of concentration (least to most), but on this tab, the blank run must be first. Be sure to label files accordingly, and hit run to double check they populate in order beneath the folder selection box.

2. Input ME calibration concentrations

Like tab 1, the next input is for the calibration curve concentrations. Again, users can enter this by inputting a .txt file under the “ME Concentrations .txt File”. If a .txt file is being used, and contains any differences from the concentration file used for the reference standard in tab 1, select the button “Use NEW ME Conc. .txt file?”. Be certain the unit used for concentrations in the calibration solutions is ng mL^{-1} . Run the program, and make sure the concentrations are correct and the time traces of each calibration data file are sufficient. An example of how to format the ME Concentration .txt file is shown in Table 3.2.1. If uptake rate changed from what was used for the data files on the first tab, the new rate for the ME calibration curve can be input under the “Uptake Rate ($\mu\text{L}/\text{min}$)”. If a new rate is input, make sure the “Set New Uptake Rate?” button is selected as well. Otherwise, it is assumed all files have the same uptake rate set in the first tab.

Table 3.2.1. Sample of how to format the text file for the ME concentrations of the calibration solutions. Each element in the solution is indicated in the top row. The element names need to match the element names used in step 1 of TOF-SPI. Each row beneath corresponds to a single calibration solution. The concentrations of a single element within the calibration solutions are found by going down each column. For all elements and calibration solutions, the concentration goes from least (blank) to most concentrated.

La	Ce	Nd	Pb	Th
0	0	0	0	0
0.5194	0.5007	0.508	0.5173	0.482
1.0542	1.0163	1.031	1.05	0.9783
1.9945	1.9227	1.9506	1.9865	1.8509
4.0536	3.9077	3.9644	4.0374	3.7617

3. Load Isotopes for Liquid Calibration Analysis

The final step for the second tab is to load the isotope list being calibrated. The isotope list should follow the same format as in Step 1 (01_TOF-SPI_Bkgd_LCs). Drag and drop the .txt file or browse for the .txt file under the “file path of IsotopeList ME_LiqCalib” and select the “Load IsotopeList from .txt” button. Analyte elements in this should only include those found in the ME solutions. Alternatively, element names and isotopes can be entered manually. If, in Step 01, some analyte elements included summed isotope signal, then the same isotopes must be summed here for such an analyte element.

Run the program. The “Select Calibration Plot” drop-down menu allows the user to select a specific isotope defined and once a selection is made, re-run the program to see it plotted.

All calibration element’s sensitivities are given on the right side of the panel.

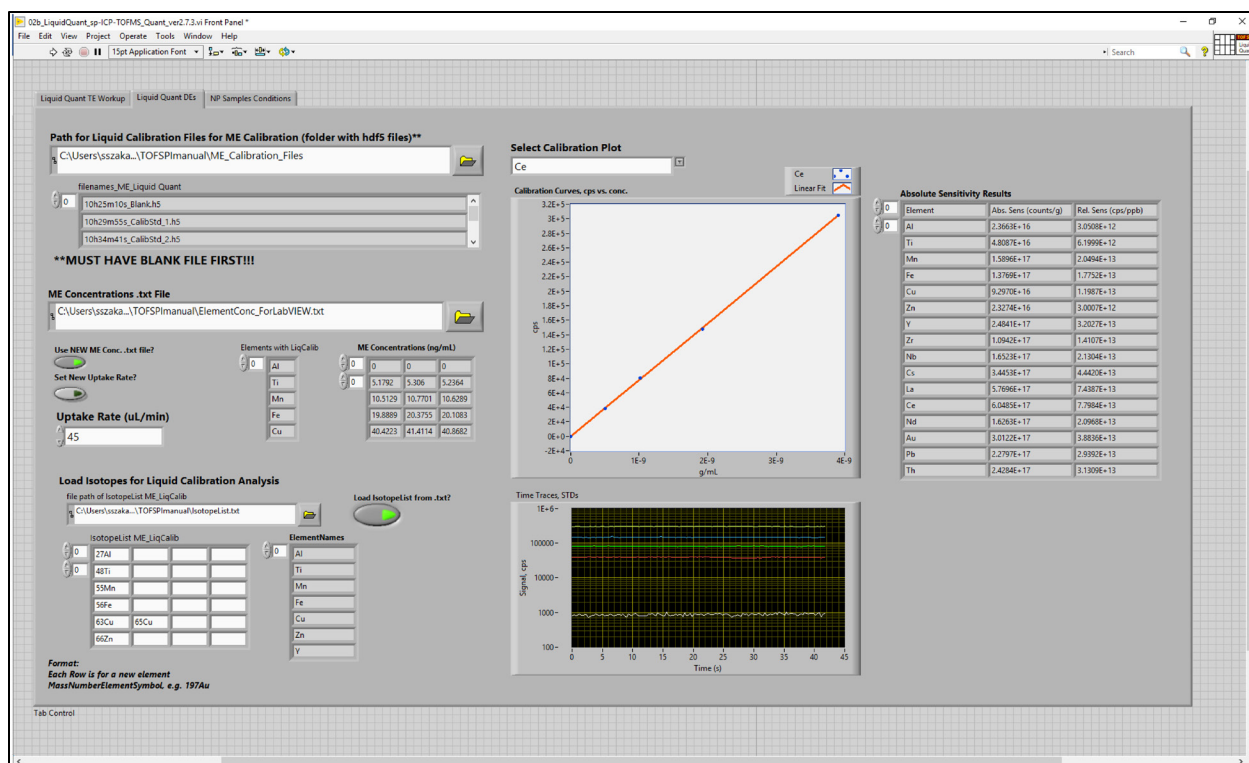


Figure 3.2.4. The ME calibration curve here included five different concentrations (including a blank), each made of 18 elements. After loading the hdf5 files, concentration text file, and isotope analysis text file, the calibration plot for Ce was selected. The five data points from each calibration solution are plotted as counts per second (cps) vs g mL^{-1} (middle with red line) and each solution’s time trace is also shown (bottom middle). On the right side of the screen are the absolute sensitivities in counts per gram, as well as the relative sensitivities in cps per ng mL^{-1} .

Tab 3: NP Sample Conditions

The third and final tab in step 02b (see Figure 3.2.5) is where the TE and DEs calculated in the first two tabs are saved to the sample spICP-TOFMs data files.

Steps in Workflow on Tab 3:

1. Input particle sample sp-ICP-TOFMs hdf5 files

Drag and drop a folder, or browse for a folder, under the “Path of sp-ICP-TOFMS Data (folder with hdf5 files)” that contain all sample files. If uptake rate for the samples is not the same as that indicated input on the first tab, continue to the green inset box. If the sample rate is the same, click on the “Write Solution Calibration Data to HDF5?” button and run the program. All calibration and TE data will be written to the sample’s HDF5 data file and can be found in the group “solutionCalib” of each HDF5 file.

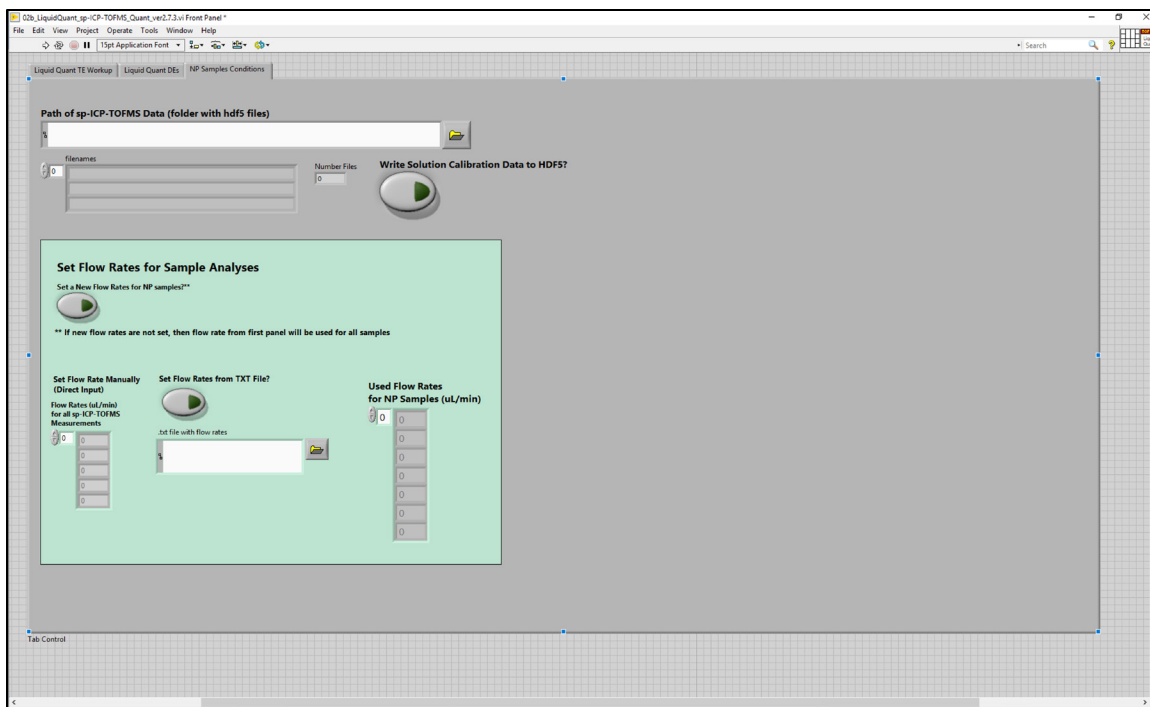


Figure 3.2.5. GUI of tab 3 with no data loaded.

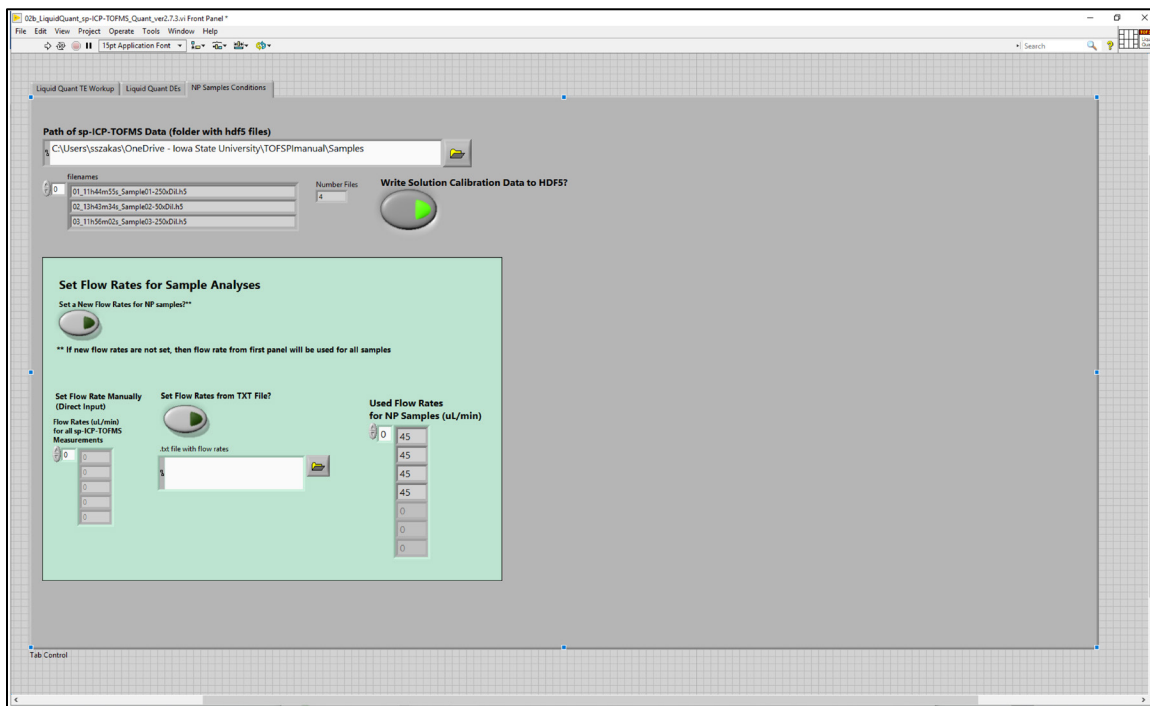


Figure 3.2.6. GUI of tab 3 with particle sample files loaded. The names of the files are visible beneath the file upload box, as well as the total number of files.

2. If sample uptake rates are different (green inset box) than first tab

The green inset box, labeled “Set Flow Rates for Sample Analysis” allows the user to set individual uptake rates for each sample. If this is used, first select the button for “Set a New Flow Rates for NP samples?”

The user can choose to set each file manually by typing the flow rate into the left-most panel, under “Set Flow Rate Manually (Direct Input)”. Otherwise, the user can upload a .txt file with flow rates for each data file by selecting the “Set Flow Rates from TXT File?” button and uploading the .txt file. When the program is run, the rates in the .txt file should populate the box to the right, under “Used Flow Rates for NP Samples”. An example of how to format the flow rate .txt file is shown below (all in $\mu\text{L}/\text{min}$). (Note: the input flow rates need to be in the same order as the HDF5 files loaded.)

50
47
100
45

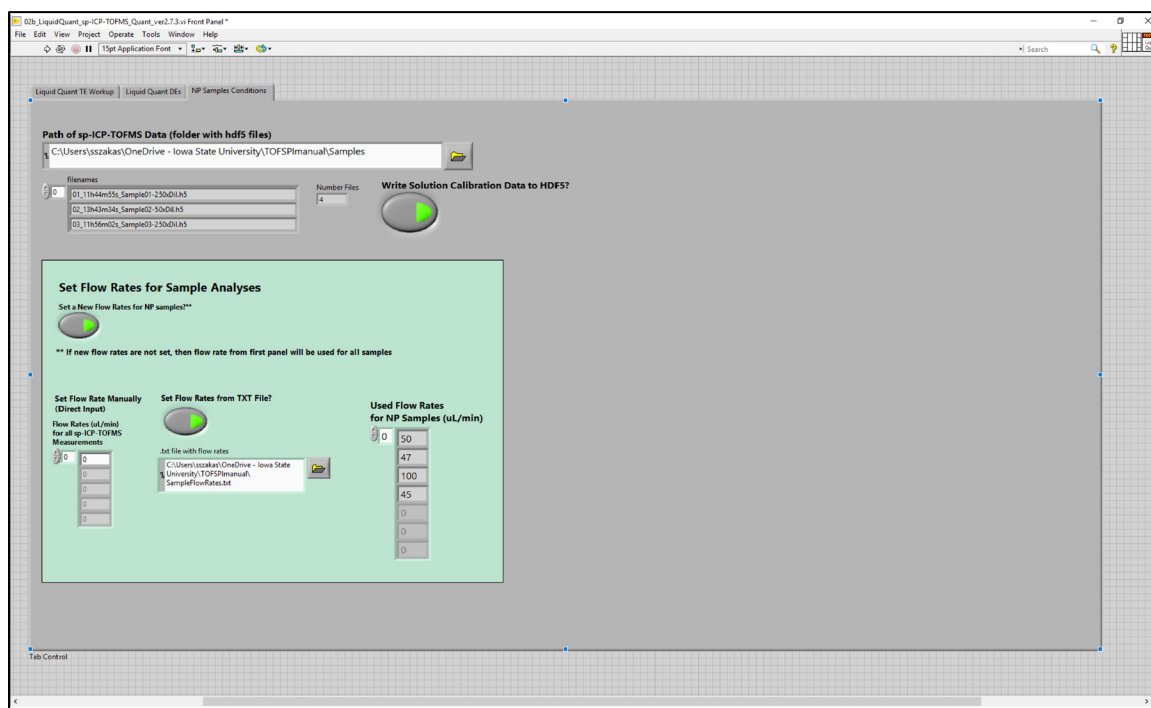
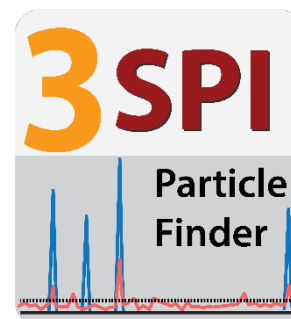


Figure 3.2.7. If new uptake rates for the sample files need to be set, the user will need to upload, or manually enter, the new uptake rates into the green box on this tab before writing this calibration data to the HDF5 sample files. Here, a text file was uploaded indicating each sample’s uptake, which is displayed on the right side of the green box. Always check these are correct by running the program, and then click the button to “Write Solution Calibration Data to HDF5?” and re-run before moving to step 3.

4 TOF-SPI Step 3: Finding Particles, Quantifying Particles, Generating Reports

Step 03 of tofSPI (“03_tofSPI_ParticleFinder.exe”) is used for the detection and quantification of single-particles events. This step features several options for split-event correction and multiple formats for single-particle data export. This program also allows for the exploration of particle data with single-particle time traces and histograms displayed in the GUI.



Check List / Overview of User Steps:

- ✓ Load ICP-TOFMS Data file(s)
- ✓ Toggle “Run All Files in Folder?” to process all HDF5 files in folder if desired
- ✓ Set the “Dynamic Critical Value: NPs:Bkgd Ratio”, “Max Alpha Rate”, and “Maximum NP Signal” parameters
- ✓ Specify the form of Split-Event Correction to use
- ✓ Toggle the desired report format(s) for export
- ✓ Run Program

4.1 Detailed Instructions on 03_tofSPI_ParticleFinder.exe

The spICP-TOFMS HDF5 files must first be processed with steps 01 and 02 of TOF-SPI before using step 03; Step 03 is broken down into five tabs. In the first tab (Load File_Set sp-Region), all the user-input information is provided. In the second tab (SE Corr / Element Mass Calibration), the user selects the type of split-event (SE) correction. Also, in this tab element mass and signal distributions can be viewed. In the third tab (Particle Signal Viewer / PNCs), all particle signals, only multi-metal signals, and the noise background can be viewed; a summary of single-particle event results is also displayed in a table. Additionally, a bar graph showing the number of particle events sorted by the number of elements detected can be viewed. In the fourth tab (Report Settings), the user may determine the types of reports to export. In the fifth tab (mmNP Data Interrogator), the user can view, sort, and save specified multi-metal particle signals.

Tab 1: Load File_Set sp-Region

The first tab of step 03 is used to upload single-particle data files. Additionally, this tab can be used to edit the desired elements for analysis, view the sp-region of the time trace, and view a summary of the results from the previous TOF-SPI processing steps.

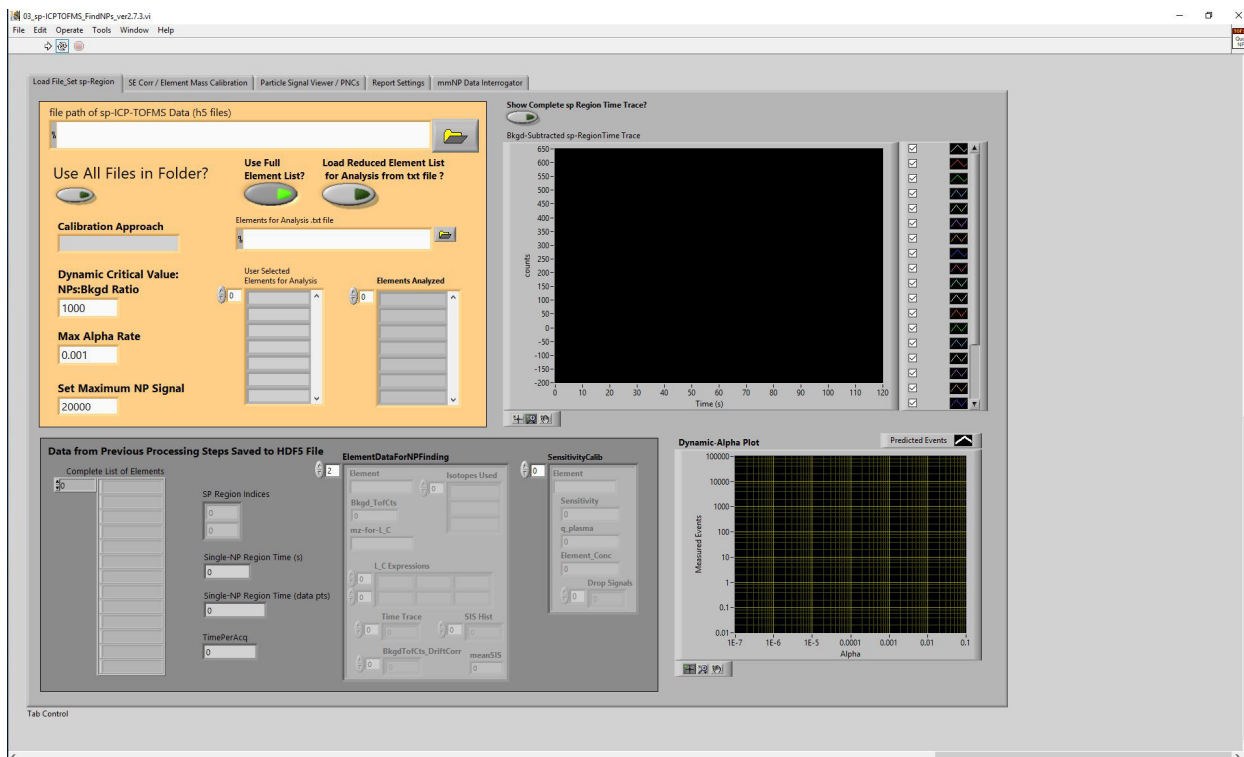


Figure 4.1.1. GUI of step 03 (tab 1) for the detection of single-particle events, shown above without inputting data.

Steps in Workflow for Tab 1:

Upload HDF5 file(s) First, in the orange box, load the spICP-TOFMS HDF5 file using either the folder button or by dragging the desired file into the file bar. These files must be processed by step 01. For quantitative results, the files must have also been processed with TOF-SPI Step 02a or 02b. To process multiple HDF5 files at once, ensure all files are in the same folder and toggle the “Run All Files in Folder?” button to ON (green indicator).

1. Select elements for analysis

By default, the “Use Full Element List?” button is toggled; this results in the elements analyzed originating from the initial isotope list populated in Step 01. An abbreviated list can be uploaded as a .txt file or entered manually by typing the element symbols (or specific names of elements as specified in Step 01) into the table provided. If the shortened list is a .txt file, click the “Load Reduced Element List for Analysis from .txt file?” button and load the file by clicking the folder button or drag the file to the file path box. To analyze elements not specified in the initial isotope list, return to Step 01 and reprocess all files with a new isotope list.

2. Set critical value parameters

The calibration approach is automatically populated from the HDF5 file based on which Step 02 processing was performed. Below the calibration approach indicator, the critical value parameters “Dynamic Critical Value” and “Max Alpha Rate,” can be adjusted. In spICP-TOFMS, critical

values are the threshold (in background-subtracted TofCts) used to distinguish between background-derived and particle-derived signals. In TOF-SPI the type-I false-positive error rate is set dynamically based on the ratio of total number of particle events measured to the number of false-positive events we would expect for a given alpha value. For example, if 100,000 spICP-TOFMS spectra are measured and critical values are determined with an alpha value of 0.001, then it is expected that ~100 false positive events (from background signals) will be registered above the critical value. Setting a dynamic critical value particle-to-background number ratio (PBNR) relates the number of recorded particle signals to the number of predicted false positive events. By default, the dynamic PBNR is 1000, which means that there should be 1000x more particles than false positive events registered. For example, if 100,000 spICP-TOFMS spectra are recorded and 1000 particle events are recorded when the alpha value is 1×10^{-5} , then the dynamic critical value would be achieved because we measure 1000 particles and expect 1 false positive: the ratio is 1000:1. On the other hand, if we measure only 10 particle events, then an alpha value of 1×10^{-7} would be required to reach the desired PBNR. The inverse of the PBNR is the fraction of false positive events to true particle events predicted; so a PBNR of 1000 produces 0.1% error in particle number and a PBNR of 100 produces 1% error. In TOF-SPI, critical values are adjusted automatically to reach the desired PBNR for all elements measured; PBNR between 100 and 1000 are suggested. If you would like to force very conservative (i.e. low) false positive results, then the “Max Alpha Rate” can be adjusted to a low number such as 1×10^{-5} (the lowest alpha possible is 1×10^{-7}).

Below the input for critical values, the “Set Maximum Nanoparticle Signal” field can be set; a default value of 20,000 (TofCts) is used for this parameter. The maximum NP signal is the ceiling for the total particle intensity of a single-particle event; if a NP signal is above this threshold, it will not be included in the exported data. This parameter is related to the dynamic range of the instrument and is used to avoid inaccurately reporting signals that result in detector saturation.

3. Data Display on Tab 1

In the top right corner, the sp-region time trace can be populated for the file that is being processed; however, it should be noted that displaying the time trace(s) will slow the processing speed. This figure will not display data until the program is initialized and will only display the time trace of the last HDF5 file processed.

At the bottom of this tab, a summary table of data acquired from the previous steps is displayed. If these fields are empty after running step 03, ensure that the HDF5 file was modified from the previous steps.

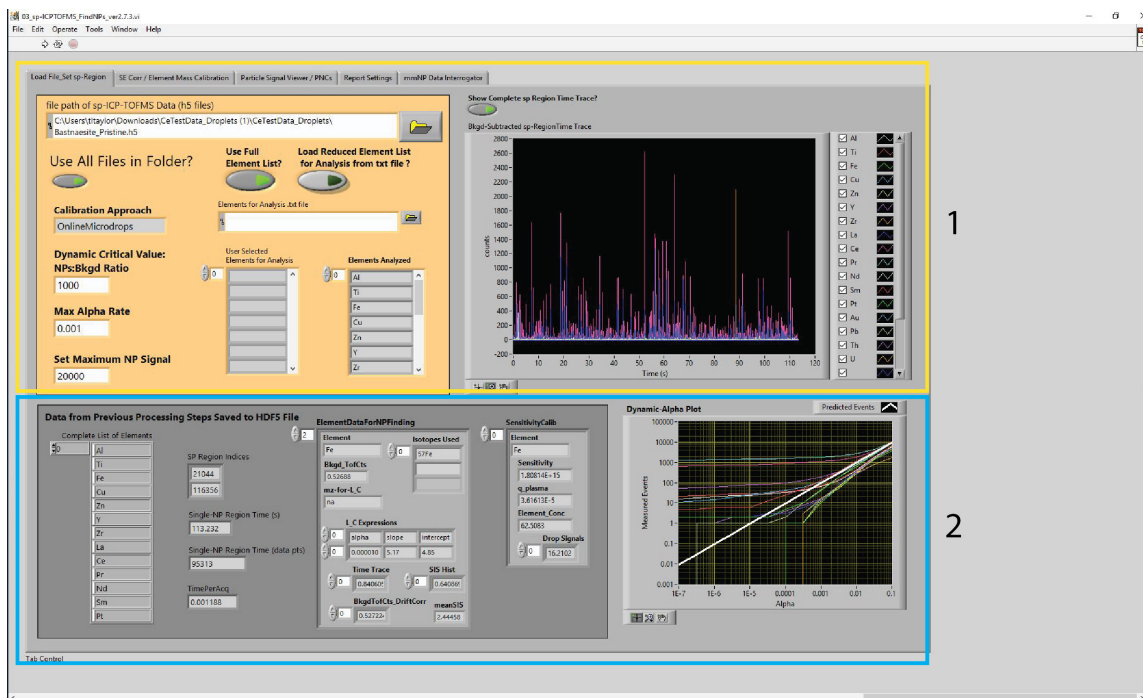


Figure 4.1.2. Tab 1 with microdroplet test data entered. In box 1, on the left, HDF5 files are uploaded, and full isotope list is used from step 1. On the right, the time trace shows data for “Bastnaesite_Pristine.h5” data file. Box 2 shows results from previous steps on the left and the dynamic alpha plot for the data on the right.

Tab 2: SE Corr/Elemental Mass Calibration

In the second tab, the type of split-event (SE) correction method may be selected. The options include: “no event correction,” “two-event correction,” “multi-event correction,” “adjacent event correction,” or “sum-spectrum correction.” A bar graph will appear depicting the number of split-events detected by element index. Additionally, the median nanoparticle mass, elemental sensitivities, and critical mass values will be shown below the split-event correction box. To the right, histograms of the element masses and intensities can also be observed in the second tab.

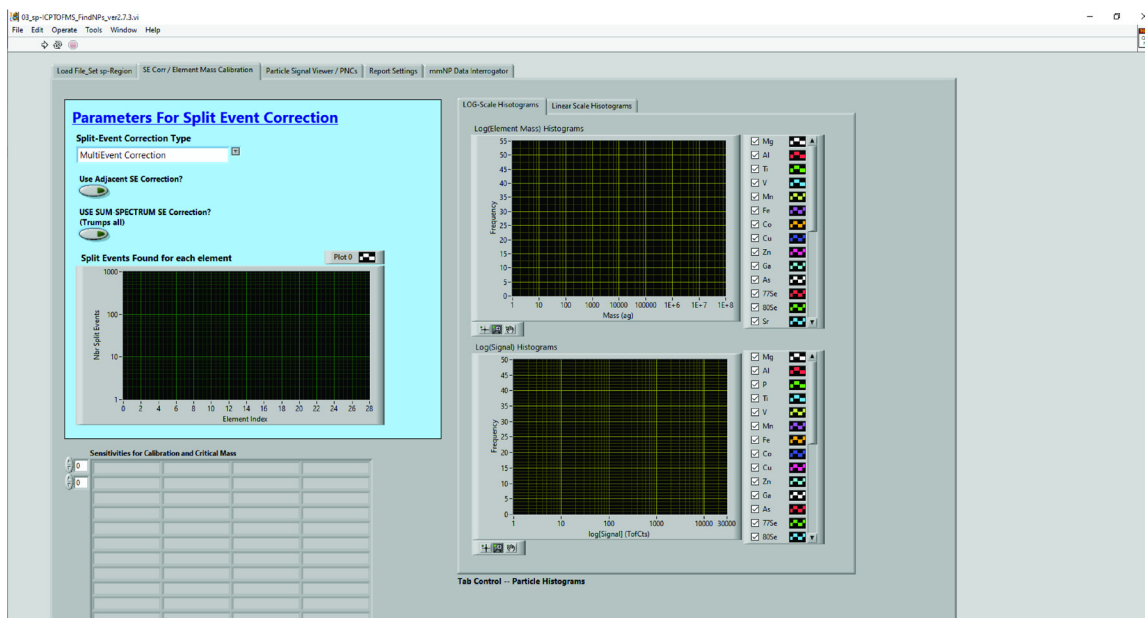


Figure 4.1.3. GUI of step 03 (tab 2) for split-event correction, shown above without inputting data.

Steps in Workflow for Tab 2:

1. Set Split-Event Correction parameter

Split-event (SE) correction parameters are set in the blue box. There are four methods of split-event correction in this program as well as the option to apply no event correction. Graphical representation of each type of split-event correction is shown in Figure 4.1.4.

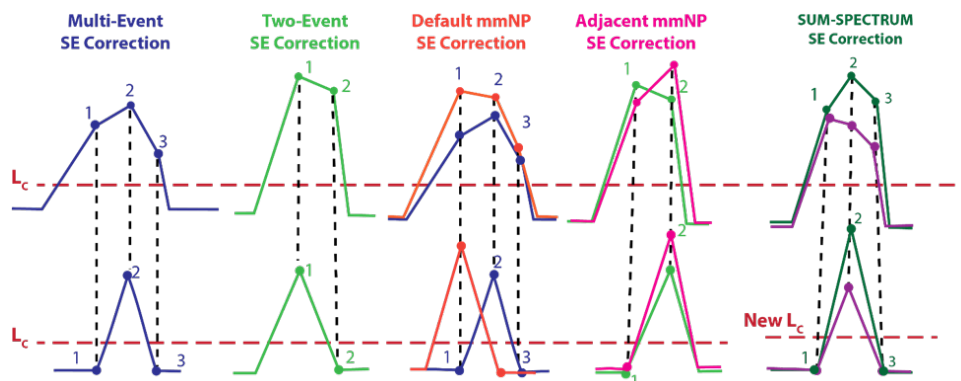


Figure 4.1.4. Graphical representation of how each slit event correction type processes nanoparticle data.

The drop-down menu is limited to no event correction, two-event correction, or multi-event correction. **The default case is multi-event correction and should be used in most cases.** In two-event correction, only adjacent events above a defined split-event critical value ($L_{c,SE}$) will be combined and the sum of these events will be combined into the bin of the highest signal with a zero inserted in the other bin. Sometimes particle signals can span more than two data points. In this case, multi-event correction should be used. In multi-event correction, all consecutive signals

above $L_{C,SE}$ are summed together into a single event with the summed signal combined into the bin with the highest original signal, the other bins are replaced with zeros. Multi-event correction is most accurate when the spICP-TOFMS signals are sparse. Users can look at the histograms of masses and signals for each split-event correction strategy to help in selecting the “best” approach.

When using the aforementioned correction methods, each individual mass channel is treated separately. This can result in some true mmNPs appearing as separate particle events; especially for mmNPs composed of high and low mass elements that have different transit times through the ion optics upstream of the TOF mass analyzer. To correct for split mmNP signals, adjacent or SUM-SPECTRUM split-event correction methods may also be used. “Adjacent SE correction” is ideally implemented when particle signals derive from particles containing both high and low mass elements such as rare-earth-element (REE) doped microplastics.⁸ This method is initialized under the same conditions as two-event and multi-event correction; however, after single-element split-events are corrected, element-signals that are adjacent to one another are indexed together. Combing the signals into the same indexing bin preserves the multi-elemental nature of the particle. Adjacent SE correction should only be used for sufficiently dilute suspensions. “SUM-SPECTRUM SE correction” uses a summed time trace of all elemental signals and a recalculated $L_{C,SE}$ for find and correct split events. This SE correction trumps all other options.

2. Data Display on Tab 2

On the right of the tab, elemental mass and signal histograms are shown. Using the tabs for this specific section, the user can choose to view the data on linear or log scales. These histograms will appear after this step has been completed.

Below the split-event correction parameters, the number of detected split-events is displayed numerically and graphically by element index. The element index correlates to the order in which the elements are listed in the isotope list.

Below the blue box, median NP mass (g), absolute sensitivity (TofCts g^{-1}), and critical mass (g) can be found for each element.

⁸ Harycki, S.; Gundlach-Graham, A., Characterization of a high-sensitivity ICP-TOFMS instrument for microdroplet, nanoparticle, and microplastic analyses. *J. Anal. At. Spectrom.* 2022, 38 (1), 111-120.

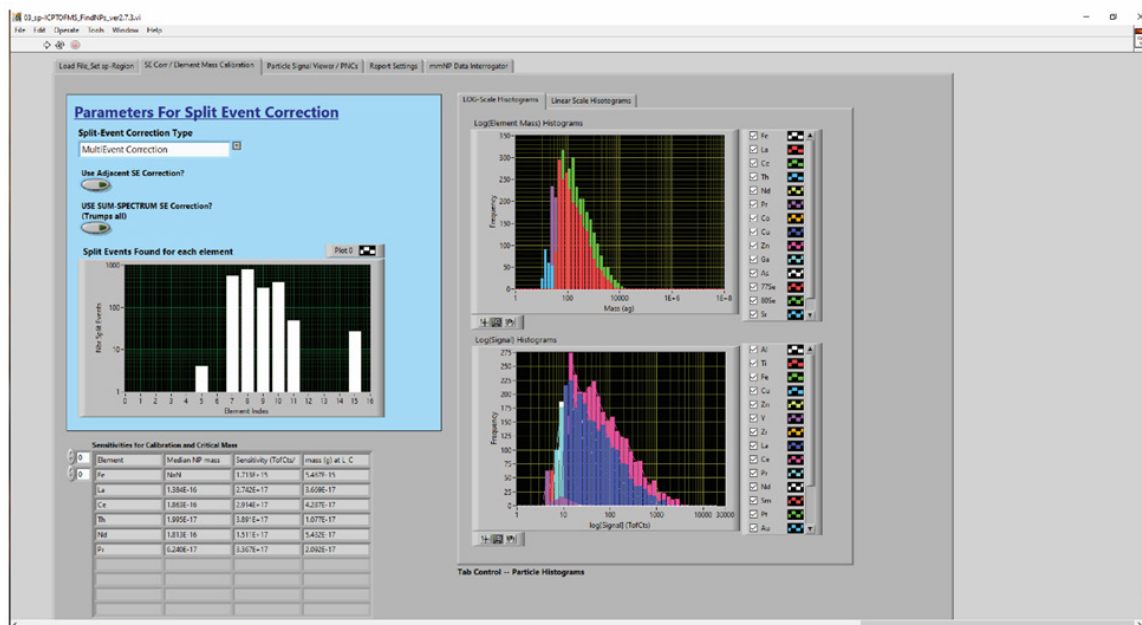


Figure 4.1.5. Tab 2 showing results of multievent correction with microdroplet test data. In the blue box, multievent correction is selected. The bar chart shows the number of split-events per element where the element index corresponds to the position of the element on the isotope list. Histograms on the right show the element mass distribution on a logarithmic scale.

Tab 3: Particle Signal Viewer/PNCs

In this tab, the single-particle region is displayed after step 03 is initialized. There are three display tabs that feature all particle signals, only multi-metal nanoparticle signals, and no nanoparticle signals (noise only display). To the right of the data display, several indicators will populate with data collected after particle detection, including a bar chart displaying the number of elements detected in a particle event. Toward the bottom of this tab, a summary of the single-particle data is displayed as a table. Tab 3 is present for the user to check results and do some initial data investigation in TOF-SPI prior to exporting data.

Steps in Workflow for Tab 3:

1. Data Display Tab 3

In the top left portion, the single-particle region time trace is featured. Using the tabs at the top of this box, select between viewing all nanoparticle signals, multi-metal nanoparticles (mmNPs) only, or noise only signals. Select which elements to feature on these plots by checking, or unchecking, the boxes to the right of the figure.

To the right of the time trace, key nanoparticle event information is displayed. This includes, the total number of particle events detected, particle number concentration (particles mL⁻¹), as well as the number of single-metal and multi-metal events. Also featured in this section are the number of unique mmNP signatures, the number of single-particle events, and the fraction of NP signals from

mmNPs. The number of elements in each particle event is displayed as a histogram to the right as well as the number of events above the maximum threshold set in the first tab of step 03.

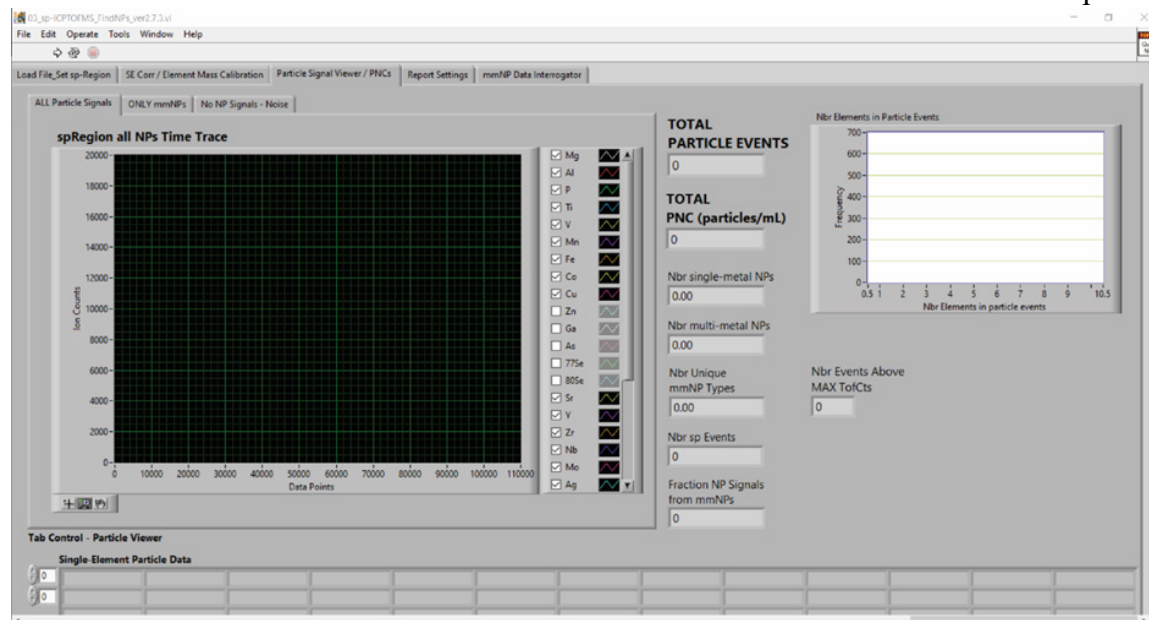


Figure 4.1.6. GUI of step 03 (tab 3) for the detection of single-particle events, shown above without inputting data.

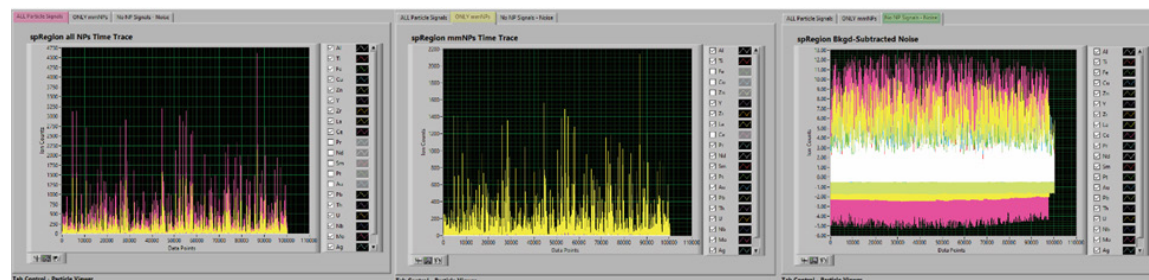


Figure 4.1.7. Three different viewing options for the single-particle time trace for the “Bastnaesite_Pristine.h5” data file. Left, shows all particles events detected. Middle, only multi-metal particles. Right, shows just noise without nanoparticles.

Tab 4: Report Settings

In this tab, data from step 03 can be saved in four formats: Excel Report, TofPilot Format Reports (.csv), Report ML-Format (.csv), and Save NP Data to HDF5 Group – “spData”. The report style is selected by toggling the buttons next to each output format.

Steps in Workflow for Tab 4:

1. View single-particle time trace and particle event information

To select format of report for export click the button next to the report type. Excel report, TofPilot Format, and machine learning (ML)-Format reports files will appear in the file directory with the data files after the program has completed. “Save NP Data to HDF5 Group” option will add the

data to the HDF5 data file in a new group titled “spData”. Report file paths are shown to the right of each button. **See section 4.2 for details on exported data.**

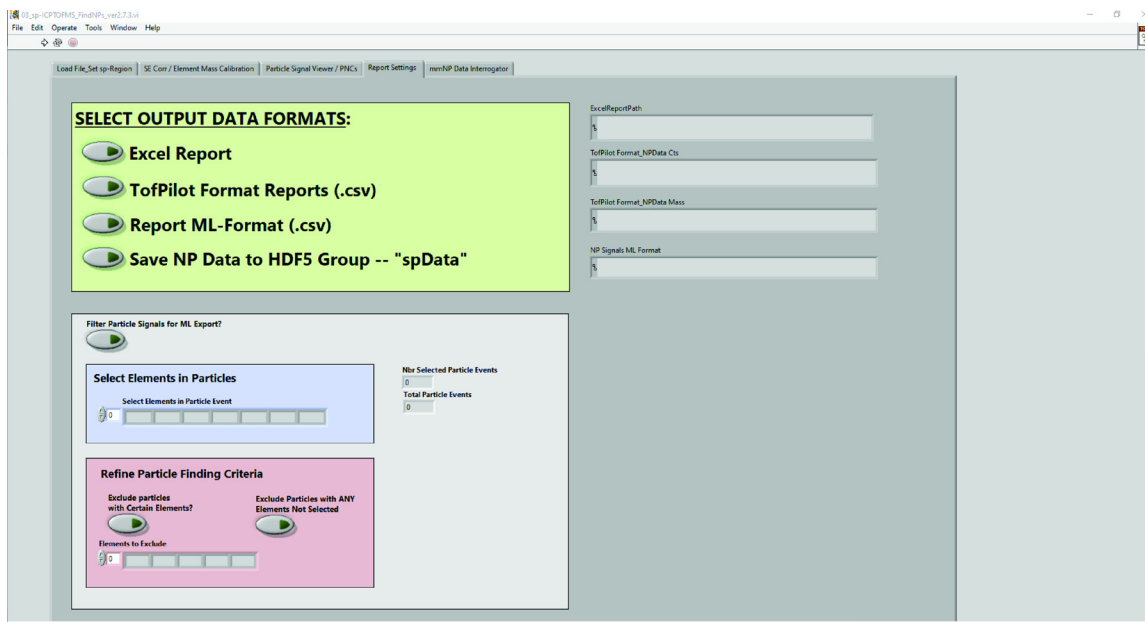


Figure 4.1.8. GUI of step 03 (tab 4).

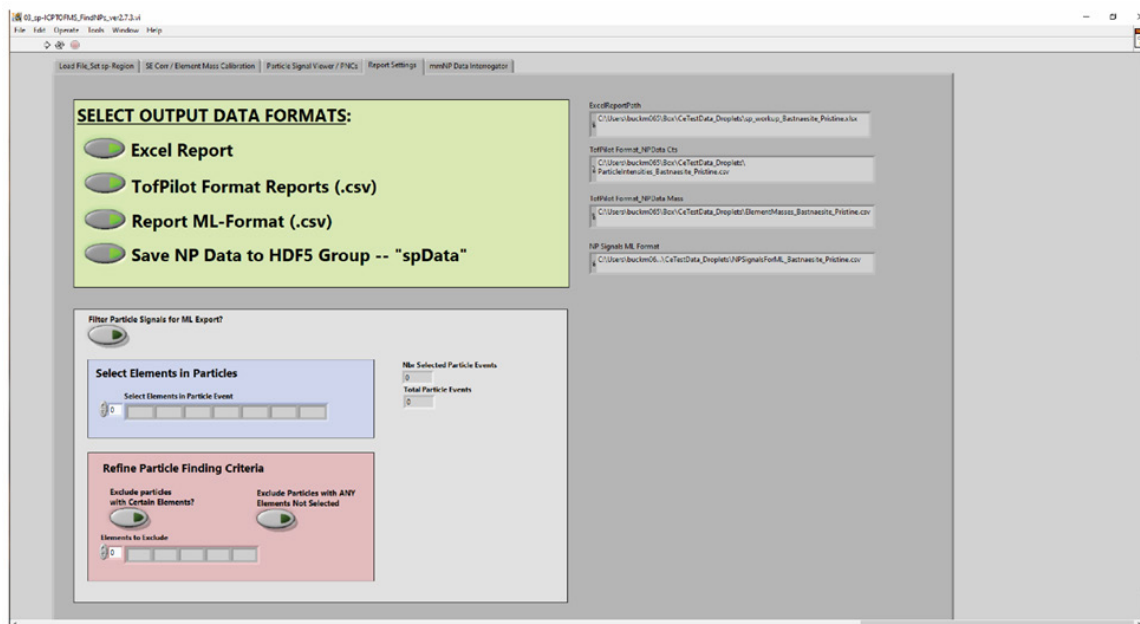


Figure 4.1.9. Data export options used for “Bastnaesite_Pristine.h5”; all report types were enabled. To the right, the file path for each data file is displayed. Below, a filter can be applied to the elements in the ML report.

Tab 5: mmNP Data Interrogator

This tab can be used to begin filtering the data for specific particles and elements of interest. This tab displays filtered data in the GUI and also gives the option to write data for the selected NP signals to a .csv file.

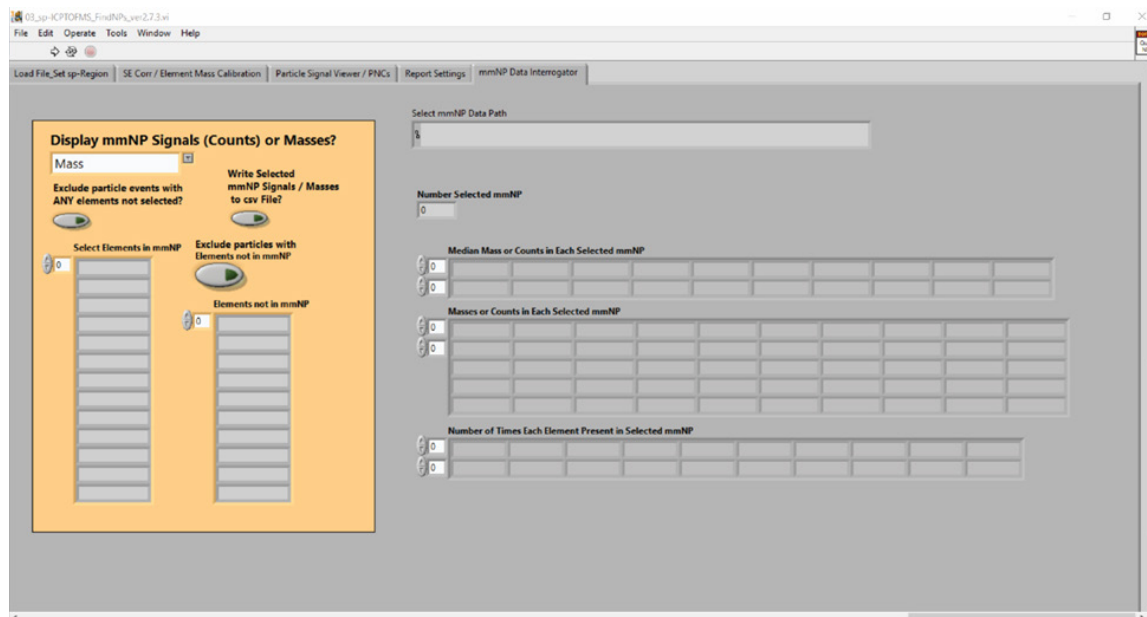


Figure 4.1.10. GUI of step 03 (tab 5) for the interrogation of particle events, shown above without data.

Steps in Workflow for Tab 5:

1. Data Filtering

The “mmNP Data Interrogator” tab can be used to filter and view mmNP data. In the orange box, select the corresponding drop-down menu option to view this data in mass or in counts. With the default settings, e.g. an empty array and the ‘exclude non-selected elements’ button off, all mmNPs will be displayed. When elements are entered in the array entitled “Select Elements in mmNP,” all mm particle events that include these elements will be displayed. When elements are entered in the “Select Elements in mmNP” array and the “Exclude particles with ANY elements not selected” button is toggled ON, then data will be filtered to show particles that exclusively contain the elements in the “Select Elements in mmNP” list. To select some elements that should be NOT in particle events found, then toggle the “Exclude particles with Elements not in mmNP” button and add the elements not to be included in the filtered mmNP list to the “Elements not in mmNP” array. Run step 03 again to display newly specified elements.

To export this data, select the ‘write selected mmNP signals/masses to .csv files’ button and rerun the program.

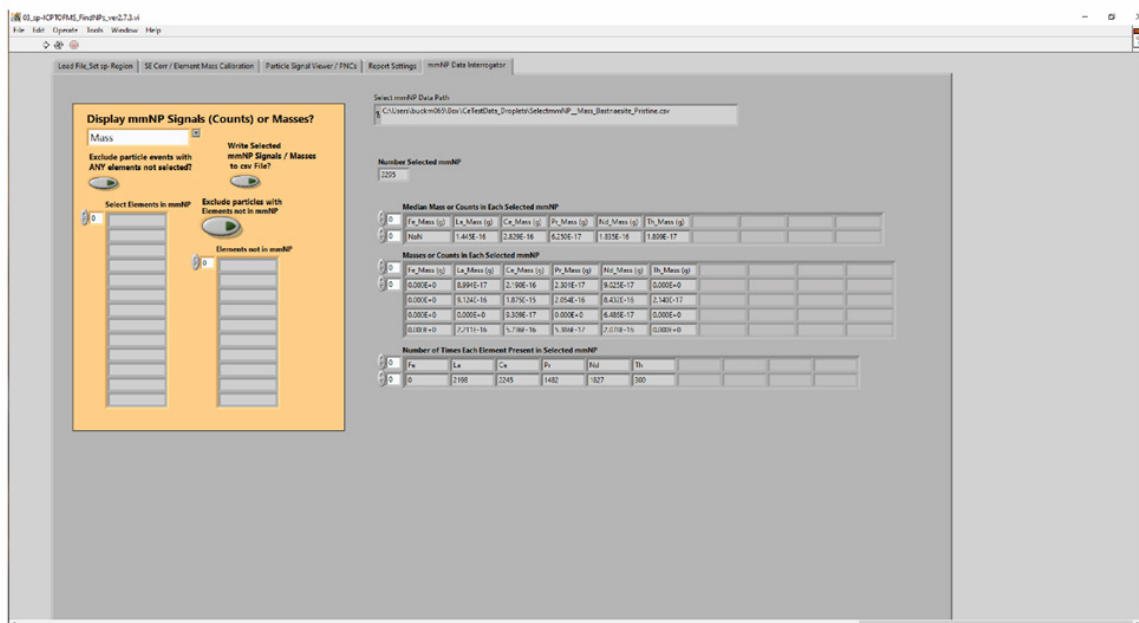


Figure 4.1.11. “mmNP Data Interrogator” tab with the “Bastnaesite_Pristine.h5” data file entered. In the orange box, nanoparticle signals are set to export as mass. Also, in this box the option to enter specific elements for viewing allows for the exclusion of all other elements. For each of the selected elements, median mass, mass of each particle, and number of particle events are displayed on the right.

4.2 Data Export Details

In Step 03 of TOF-SPI, there are multiple methods of exporting and saving the single-particle data for analysis. As described briefly in Section 4.1 Tab 4, the single-particle data can be saved as an excel report, a TofPilot mimicking format, a condensed report format that can be utilized for machine learning (ML), and/or directly to the HDF5 file. In addition to setting the report parameters, the file paths to each of the reports generated in step 03 will be displayed to the right of the report selection box. After the program has completes running, all reports are saved to the same folder as the HDF5. Figure 4.2.1 illustrates the folder contents when all report types are selected.

Bastnaesite_Pristine	H5 File
singleion	JSON Source File
ElementMasses_Bastnaesite_Pristine	Microsoft Excel Comma Separated Values File
NPSignalsForML_Bastnaesite_Pristine	Microsoft Excel Comma Separated Values File
ParticleIntensities_Bastnaesite_Pristine	Microsoft Excel Comma Separated Values File
sp_workup_Bastnaesite_Pristine	Microsoft Excel Worksheet
DropletConc_CeNPs	Text Document
DropletDiam_CeNPs	Text Document
IsotopeList_CeNPs	Text Document
TOF-SPI_Parameters_DropletCalibration_Updated	Text Document

Figure 4.2.1. Example of all files and file types used for processing the single-particle data file “Bastnaesite_Pristine.h5” with TOF-SPI steps 01-03 with online microdroplet calibration (step 02a). Also included in this figure are the four Microsoft Excel documents generated with step 03.

4.2.1 Report Settings – Excel Report

This report type exports files with a name format of “sp_workup_filename.xlsx”. The sp-workups are detailed reports that contain eight sheets of information from the TOF-SPI analysis.

Settings

The first sheet of this report (Figure 4.2.2) features a summary of some important settings during the processing of the single-particle data file. These include the calibration approach used, the time per acquisition, total acquisition time, duration of the single-particle region, total measurement time, plasma uptake standard, q_{plasma} , and a final copy of the isotope list used for the analysis in step 03.

Data Acquisition and Analysis Settings	
Calib Approach	OnlineMicrodrops
TimePerAcq(s)	0.001188
Time_sp-Region(s)	119.231
Time_TotalMeas(s)	163.231
PlasmaUptakeStd	Cs
q_{Plasma} (mL/s)	0.000034
Element Name	Isotopes Used
Al	27Al
Ti	48Ti
Fe	57Fe
Cu	65Cu
Zn	64Zn+66Zn
Y	89Y
Zr	90Zr
La	139La
Ce	140Ce
Pr	141Pr
Nd	144Nd+146Nd
Sm	147Sm+149Sm
Pt	194Pt+195Pt
Au	197Au
Pb	206Pb+208Pb
Th	232Th
U	238U

Figure 4.2.2 sp_workup data settings tab.

smNPs

In the ‘smNPs’ sheet (Figure 4.2.3), single-metal particle events are displayed as particle intensity in ToFCts (left group) or element mass in g (right group). Each column has the single-particle signal or mass for that element in a particular particle. **IMPORTANT:** on this sheet, all the particle events are single-metal, i.e. only one element was detected in each particle. There is no association between element signals or masses that are in the same row.

ME Comp	Al	Ti	Fe	Cu	Zn	Y	Zr	La	Ce	Pr	Nd	Sm	Pt	Au	Pb	Th	U	Fe_Mass (g)	La_Mass (g)	Ce_Mass (g)	Pr_Mass (g)	Nd_Mass (g)	Th_Mass (g)
1	0	0	0	0	0	0	0	123.6731536	15.2071013	25.41881955	22.82906163	11.49989489	0	91.47672862	0	257.4371081	0	0	4.51E-16	5.22E-17	7.55E-17	1.51E-16	6.62E-16
2	0	0	0	0	0	0	0	12.96437962	15.17107507	40.23785438	26.27554401	7.981984118	0	77.85015168	0	4.798169957	0	0	4.73E-17	5.21E-17	1.19E-16	1.74E-16	1.23E-17
3	0	0	0	0	0	0	0	29.16509024	13.0575523	15.17928033	9.306084334	6.078266457	0	187.9276668	0	10.19585778	0	0	1.06E-16	4.48E-17	4.51E-17	6.16E-17	2.62E-17
4	0	0	0	0	0	0	0	11.45111251	14.34848193	23.62022739	10.51122233	11.32559627	0	0	0	9.824756331	0	0	4.18E-17	4.92E-17	7.01E-17	6.96E-17	2.52E-17
5	0	0	0	0	0	0	0	17.78289757	16.75621567	8.414901673	17.51183899	16.63843718	0	0	0	9.630424851	0	0	6.48E-17	5.75E-17	2.50E-17	1.16E-16	2.47E-17
6	0	0	0	0	0	0	0	31.71088797	14.48658123	7.343557354	11.22779814	7.246772436	0	0	0	4.2167394	0	0	1.16E-16	4.97E-17	2.18E-17	7.43E-17	1.08E-17
7	0	0	0	0	0	0	0	10.0988945	13.98221851	9.759728862	9.450952444	6.03863237	0	0	0	9.274382314	0	0	3.68E-17	4.80E-17	2.90E-17	6.25E-17	2.38E-17
8	0	0	0	0	0	0	0	108.880008	29.26922793	14.46381714	35.38377425	6.338130293	0	0	0	145.0703896	0	0	3.97E-16	1.00E-16	4.30E-17	2.34E-16	3.73E-16
9	0	0	0	0	0	0	0	13.96614244	25.25833974	7.016441074	102.860369	0	0	0	0	31.6640029	0	0	5.09E-17	8.67E-17	2.08E-17	6.81E-16	8.14E-17
10	0	0	0	0	0	0	0	10.25382318	30.68211906	8.08679812	24.75124201	0	0	0	0	8.25314255	0	0	3.74E-17	1.05E-16	2.40E-17	1.64E-16	2.12E-17

Figure 4.2.3. sp_workup smNPs tab.

mmNPs

In the ‘mmNPs’ sheet (Figure 4.2.4), multi-metal nanoparticle events are displayed by multi-elemental composition as particle intensities inToFCTs (left group) or element mass in g (right group). In this sheet, the signal from all elements in every recorded mmNP event are provided. Each particle event is a row on the spreadsheet. If an element was not detected as a particle event in a given particle, then 0 ToFCTs and 0 g are reported for that element. Only elements for which absolute sensitivities were recorded are quantified in terms of mass. The particle signals are listed in the order in which they were detected.

ME Comp	Al	Ti	Fe	Cu	Zn	Y	Zr	La	Ce	Pr	Nd	Sm	Pt	Au	Pb	Th	U	Fe_Mass (g)	La_Mass (g)	Ce_Mass (g)	Pr_Mass (g)	Nd_Mass (g)	Th_Mass (g)
1	LaCePnd	0	0	0	0	0	0	24.665	63.836	7.747	13.638	0	0	0	0	0	0	0.00E+00	8.99E-17	2.19E-16	2.30E-17	9.03E-17	0.00E+00
2	LaCePndTh	0	0	0	0	0	0	250.211	546.404	69.165	127.414	0	0	0	0	8.326	0	0.00E+00	9.12E-16	1.88E-15	2.05E-16	8.43E-16	2.14E-17
3	CeNd	0	0	0	0	0	0	27.132	0	9.801	0	0	0	0	0	0	0	0.00E+00	0.00E+00	9.31E-17	0.00E+00	6.49E-17	0.00E+00
4	LaCePnd	0	0	0	0	0	0	60.629	167.169	18.134	31.395	0	0	0	0	0	0	0.00E+00	2.21E-16	5.74E-16	5.39E-17	2.08E-16	0.00E+00
5	LaCe	0	0	0	0	0	0	16.127	51.779	0	0	0	0	0	0	0	0	0.00E+00	5.88E-17	1.78E-16	0.00E+00	0.00E+00	0.00E+00
6	LaCePndSm	0	0	0	0	0	0	180.704	355.242	54.101	115.623	7.958	0	0	0	0	0	0.00E+00	6.99E-16	1.22E-15	1.61E-16	7.65E-16	0.00E+00
7	LaCePndSmTh	0	0	0	0	0	0	254.102	502.068	84.962	134.481	13.596	0	0	0	9.461	0	0.00E+00	9.27E-16	1.72E-15	2.52E-16	8.90E-16	2.43E-17
8	LaCePnd	0	0	0	0	0	0	73.288	203.693	35.566	55.2	0	0	0	0	0	0	0.00E+00	2.67E-16	6.99E-16	1.06E-16	3.65E-16	0.00E+00
9	LaCePnd	0	0	0	0	0	0	115.071	205.668	28.439	54.577	0	0	0	0	0	0	0.00E+00	4.20E-16	7.06E-16	8.45E-17	3.61E-16	0.00E+00
10	LaCePnd	0	0	0	0	0	0	31.954	60.906	13.991	22.17	0	0	0	0	0	0	0.00E+00	1.17E-16	2.09E-16	4.16E-17	1.47E-16	0.00E+00

Figure 4.2.4 mmNPs tab of sp_workup.

All NPs_PNC

In the ‘All NPs_PNC’ sheet (4.2.5), summarized information about element-specific sp data are provided. These include particle number concentration (PNC, NPs mL⁻¹), number of NPs measured, particle frequency (NPs per data point), median signal (ToFCTs), average critical value (LC_{sp}, ToFCTs), average background (ToFCTs), critical value slope and intercept, dynamic alpha value, minimum NP-background number ratio, measured NP-background number ratio, split-event critical value, and the number of split events found.

Element	PNC (NPs/mL)	Nbr NPs Measured	Particle Freq (NPs/Data Pt)	Median Signal	Avg L_C Value (TofCts)	Avg Bkgd TofCts	L_C Slope	L_C Intercept	Dynamic Alpha	Min NP-Bkgd NbrRatio	Meas NP-Bkgd NbrRatio	Split-Event L_C	Nbr Split Events
Al	2.49E+02	1	9.96E-06	2.44E+01	11.39	0.489	7.72	5.99	1.00E-07	1000	100	10.82	0
Ti	0.00E+00	0	0.00E+00	NaN	7.89	0.153	5.51	5.74	1.00E-07	1000	0	7.41	0
Fe	0.00E+00	0	0.00E+00	NaN	9.37	0.433	5.51	5.74	1.00E-07	1000	0	8.88	0
Cu	0.00E+00	0	0.00E+00	NaN	9.03	0.466	5.59	5.21	1.00E-07	1000	0	8.54	0
Zn	0.00E+00	0	0.00E+00	NaN	12.46	1.682	5.59	5.21	1.00E-07	1000	0	11.93	0
Y	1.54E+04	62	6.18E-04	9.06E+00	5.51	0.081	5.32	4	3.20E-07	1000	1993	5.13	4
Zr	0.00E+00	0	0.00E+00	NaN	5.58	0.051	5.44	4.36	1.00E-07	1000	0	5.2	0
La	5.64E+05	2269	2.26E-02	3.80E+01	10.06	2.295	4.61	3.08	1.00E-05	1000	2819	9.31	556
Ce	7.19E+05	2892	2.88E-02	5.43E+01	12.49	5.022	4.39	2.66	3.20E-05	1000	1158	11.44	805
Pr	3.72E+05	1495	1.49E-02	2.10E+01	7.04	0.74	4.61	3.08	1.00E-05	1000	1766	6.43	291
Nd	4.61E+05	1855	1.85E-02	2.74E+01	8.21	1.271	4.48	3.16	1.00E-05	1000	2226	7.66	389
Sm	1.12E+05	449	4.47E-03	1.04E+01	5.42	0.163	4.63	3.55	3.20E-06	1000	1529	4.97	49
Pt	0.00E+00	0	0.00E+00	NaN	5.45	0.049	5.06	4.34	1.00E-07	1000	0	5.07	0
Au	7.46E+02	3	2.99E-05	9.15E+01	5.97	0.104	5.06	4.34	1.00E-07	1000	299	5.58	0
Pb	0.00E+00	0	0.00E+00	NaN	5.5	0.083	4.98	4.07	1.00E-07	1000	0	5.15	0
Th	7.76E+04	312	3.11E-03	7.76E+00	4.19	0.035	4.56	3.34	3.20E-06	1000	1040	3.79	27
U	0.00E+00	0	0.00E+00	NaN	4.99	0.013	4.88	4.44	1.00E-07	1000	0	4.62	0

Figure 4.2.5 All NPs_PNC tab of sp_data workup.

NP Mass Quant Dist

In the ‘NP Mass Quant Dist’ sheet (Figure 4.2.6), the masses (g) of each element in all smNPs and mmNPs are reported. IMPORTANT: on this spreadsheet, there is no relationship between element masses in the same rows. Also, there is no distinction between elements measured in smNPs and mmNPs. This sheet can be used to create element-specific mass histograms. Only elements with recorded absolute sensitivities are quantified in terms of mass per particle.

Element	Fe	La	Ce	Th	Nd	Pr
0	8.99E-17	5.22E-17	2.14E-17	9.03E-17	2.30E-17	
0	9.12E-16	2.19E-16	2.43E-17	8.43E-16	2.05E-16	
0	2.21E-16	1.87E-15	1.35E-17	6.49E-17	5.39E-17	
0	5.88E-17	5.21E-17	1.21E-17	2.08E-16	1.61E-16	
0	6.59E-16	9.31E-17	1.09E-17	7.65E-16	2.52E-16	

Figure 4.2.6 NP Mass Quant Dist tab of sp_data workup.

NP Mass Quant

In the ‘NP Mass Quant’ sheet (Figure 4.2.7), the median NP mass (g), the absolute sensitivity in (TofCts g⁻¹) and the critical mass for each element quantified in terms of mass are provided.

Element	Median NP mass (g)	Sensitivity (TofCts/g)	mass (g) at L_C
Fe	NaN	1.71E+15	5.47E-15
La	1.38E-16	2.74E+17	3.67E-17
Ce	1.86E-16	2.91E+17	4.29E-17
Th	2.00E-17	3.89E+17	1.08E-17
Nd	1.81E-16	1.51E+17	5.43E-17
Pr	6.24E-17	3.37E+17	2.09E-17

Figure 4.2.7 NP Mass Quant tab of sp_workup.

mmNP_PNC

In the ‘mmNP_PNC’ sheet (Figure 4.2.8), the number of nanoparticles with each recorded unique multi-elemental signature and their particle number concentration are provided.

	Nbr NPs Measured	PNC (NPs/mL)
LaCePrNd	871	216599.8714
LaCe	378	94000.86266
LaCeNd	342	85048.39955
LaCePrNdSm	214	53217.4196
LaCePrNdSmTh	155	38545.32728
LaCePrNdTh	69	17158.88763
LaCePr	60	14920.77185
CeNd	54	13428.69467
YLaCePrNdSmTh	52	12931.3356

Figure 4.2.8 mmNP_PNC tab of sp_workup.

ByElementNP-Nbr

In the ‘ByElementNP-Nbr’ sheet (Figure 4.2.9), the total number of particles per element as well as the number of times the element is measured as a smNP and in a mmNP are provided. Additionally, the unique elemental signatures of the mmNPs in which each element is detected are provided. These mmNP element signatures are ordered according to frequency. (Note: on this page there will be overlap (double counting) in the number of mmNPs. For example, if an AlFe NP is measured, it will be counted as a mmNP for both Al and Fe. Thus, the sum of the number off mmNPs on this page is NOT equal to the total number of mmNPs.)

Element	Nbr Total Particles	Nbr smNP	Nbr mmNP	Element Signatures
Al	1	0	1	AlYLaCePrNdSmTh
Ti	0	0	0	
Fe	0	0	0	
Cu	0	0	0	
Zn	0	0	0	
Y	62	0	62	YLaCePrNdSmTh YLaCePrNdTh YLaCePrNdSm YLaCePrNd AlYLaCePrNdSmTh YNdSmTh
Zr	0	0	0	
La	2269	71	2198	LaCePrNd LaCe LaCeNd LaCePrNdSm LaCePrNdSmTh LaCePrNdTh LaCePr Nd
Ce	2892	647	2245	LaCePrNd LaCe LaCeNd LaCePrNdSm LaCePrNdSmTh LaCePrNdTh LaCePr Nd
Pr	1495	13	1482	LaCePrNd LaCePrNdSm LaCePrNdSmTh LaCePrNdTh LaCePr Nd
Nd	1855	28	1827	LaCePrNd LaCeNd LaCePrNdSm LaCePrNdSmTh LaCePrNdTh LaCePrNdTh CeNd
Sm	449	8	441	LaCePrNdSm LaCePrNdSmTh YLaCePrNdSmTh LaSm CeSm YLaCePrNdSm
Pt	0	0	0	
Au	3	3	0	
Pb	0	0	0	
Th	312	12	300	LaCePrNdSmTh LaCePrNdTh YLaCePrNdSmTh LaCeNdTh LaPrNdTh YLaCePrNdTh
U	0	0	0	

Figure 4.2.9 ByElementNP-Nbr tab of sp_workup

4.2.2 Report Settings – TofPilot Format Reports (.csv)

This report setting will create two .csv files for each data file analyzed (Figures 4.2.10 and 4.2.11). The first will include particle intensities in counts with time stamps and index. The second csv file lists the mass of each element determined in each particle in grams with time stamps and index.

Index	TimeStamp (s)	La	Ce	Nd	Th	U
18148	21.5598	15.887	51.79	8.425	0	0
18177	21.5943	88.798	196.864	47.044	0	0
18209	21.6323	63.381	189.344	40.818	0	0
18228	21.6549	117.396	263.989	70.137	18.737	0
18257	21.6893	16.163	0	8.665	0	0
18292	21.7309	0	21.204	0	0	0

Figure 4.2.10 Exported excel sheet for ParticleIntensities created from the TofPilot Format Report.

Index	TimeStamp (s)	La (g)	Ce (g)	Th (g)	Nd (g)
18148	21.5598	5.79E-17	1.78E-16	0.00E+00	5.58E-17
18177	21.5943	3.24E-16	6.76E-16	0.00E+00	3.11E-16
18209	21.6323	2.31E-16	6.50E-16	0.00E+00	2.70E-16
18228	21.6549	4.28E-16	9.06E-16	4.82E-17	4.64E-16
18257	21.6893	5.89E-17	0.00E+00	0.00E+00	5.74E-17
18292	21.7309	0	2.12E-17	0.00E+00	0.00E+00

Figure 4.2.11 Exported excel sheet for ElementMasses created from the TofPilot Format Report

4.2.3 Report Settings – Report ML-Format (.csv)

This export style will create .csv files that include the element intensities in ToFCts for each particle and the recorded elemental composition (Figure 4.2.12). In addition, this export includes a table at the top of the file that includes the isotope(s) used for quantification, the absolute sensitivity, the median signal, the $L_{C,sp}$, the average background, and the dynamic alpha for each element.

	La	Ce	Nd	Th	U
1 Filename	Bastnaesite_pristine.h5				
2 spTime (s)	121.277				
3 qPlasma (mL/s)	0.000034				
4 Element	La	Ce	Nd	Th	U
5 Isotopes	139La	140Ce	144Nd+146Nd	232Th	238U
6 Sensitivity (ToFCts/g)	2.74E+17	2.91E+17	1.51E+17	3.89E+17	0.00E+00
7 MedianSignal (ToFCts)	4.38E+01	5.43E+01	2.75E+01	7.36E+00	NaN
8 Avg Lc (ToFCts)	12.45	12.59	8.25	4.09	4.86
9 Avg Bkgd (ToFCts)	2.335	5.014	1.269	0.034	0.013
10 DynamicAlpha	1.00E-05	3.20E-05	1.00E-05	3.20E-06	1.00E-07
11					
12 Particle Signals (ToFCts)					
13 Comp	La	Ce	Nd	Th	U
14 La+Ce+Nd	15.887	51.79	8.425	0	0
15 La+Ce+Nd	88.798	196.864	47.044	0	0
16 La+Ce+Nd	63.381	189.344	40.818	0	0
17 La+Ce+Nd+Th	117.396	263.989	70.137	18.737	0
18 La+Nd	16.163	0	8.665	0	0

Figure 4.2.12 Example ML-Format export excel sheet.

4.2.4 Report Settings – Save NP Data to HDF5 Group – “spData”

This output format does not create a new file, but rather saves the single-particle data to a new group in the HDF5 file labeled ‘spData’. The modified HDF5 file for Bastnaesite_Pristine.h5 after processing with TOF-SPI in Figure 4.2.13.

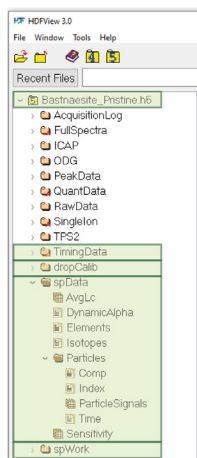


Figure 4.2.13 The modified HDF5 file for Bastnaesite_Pristine.h5 after processing with TOF-SPI has been completed. Single-particle data is saved under the ‘spData’ group; specifically, processed particle events are stored in the *ParticleSignals* dataset.

5 Appendix I: Background on spICP-TOFMS and Online Microdroplet Calibration

5.1 spICP-TOFMS

Single-particle Inductively Coupled Plasma Time-of-Flight Mass Spectrometry (spICP-TOFMS) is a high-throughput approach for the analysis of metal and metalloid-containing nanoparticles (NPs) and microparticles (μ Ps) at low number concentrations ($\sim 10^2$ – 10^6 particles mL^{-1}).⁹ In spICP-TOFMS, a dilute suspension of particles is introduced into the ICP via conventional liquid-to-aerosol sample introduction systems. In the ICP, discrete particles are vaporized, atomized, and ionized, to produce clouds of ions that—after being extracted into the mass analyzer—have temporal durations of ~ 200 to $500 \mu\text{s}$. If a sufficient number of ions from a NP or μ P are passed through the MS instrument, then the particle-produced transient pulse of ions is registered as a signal spike in the time-trace of the mass spectrometer. In the TOF mass analyzer, the discrete ion clouds are separated according to ions' mass-to-charge (m/z) ratios such that element- and isotope-specific transient signals are recorded for each particle. Based on coincidence of particle-derived element signals, multi-metal (mm) composition of individual particles can be determined. Through counting the number of these signal spikes, researchers can determine particle-number concentrations (PNCs) of analyte particles. Moreover, through calibrating the ICP-TOFMS response in terms of absolute sensitivity (counts g^{-1}) for analyte elements and/or isotopes, the amount of ion signal in each particle-produced signal spike can be related to the mass of an element in the particle. Some elements that are commonly present in NPs and μ Ps, such as carbon, nitrogen, oxygen, sulfur, and fluorine, are not readily detectable at the single-particle level by spICP-TOFMS due to their low masses, high ionization potentials, and/or high natural backgrounds.

5.2 Critical Values and Critical Masses in spICP-TOFMS

In spICP-MS, one must define the value above which particle signals are distinct from the steady-state background signals. This threshold value is a single-sided detection decision that only depends on the shape and mean of the background signal distribution and is called the critical value, L_c . Critical values can be defined for any alpha (α) value, which is the fraction of the background signal distribution that will (statistically) be above L_c . The α value is often called the “error of the first kind” or the “false-positive error” because α describes the relative amount of noise (i.e. background) that will be registered as detected, i.e. as “signal.”^{10,11} Critical values are expressed in signal units; on the other hand, critical masses (X_c^{mass}) are the critical value divided by a calibration factor to convert signal to mass. In conventional spICP-MS with quadrupole or sector field mass analyzers, critical values for separating particle signal from background signal

⁹ Gundlach-Graham, A., Multiplexed and multi-metal single-particle characterization with ICP-TOFMS. In *Analysis and Characterisation of Metal-Based Nanomaterials*, Elsevier: 2021; pp 69-101.

¹⁰ Currie, L. A., Limits for qualitative detection and quantitative determination. Application to radiochemistry. *Anal. Chem.* 1968, 40 (3), 586-593.

¹¹ Currie, L. A., Nomenclature in evaluation of analytical methods including detection and quantification capabilities. *Pure Appl. Chem.* 1995, 67 (10), 1699-1723.

are obtained using Normal statistics or Poisson-Normal statistics¹² and are usually set to $\mu+3\sigma$ or $\mu+5\sigma$, in which μ is the average background signal (μ_{bkgd}) that can arise from both dissolved analyte and plasma background species, σ is the standard deviation of the background signals, and 3 and 5 are z-scores representing alpha values of 0.00135 and 2.87×10^{-7} , respectively. In general, Poisson-Normal statistics are more applicable for low signal intensity backgrounds because these signals are Poisson distributed. For Poisson-distributed signal, the standard deviation of the signal can be approximated as $(\mu_{\text{bkgd}})^{1/2}$ and n-sigma critical values may be applied according to a Poisson-Normal approximation. However, these “n σ ” critical values are not appropriate for spICP-TOFMS because low-background signals from the icpTOF are not well described by either a Normal distribution or a Poisson distribution.

With icpTOF instruments (TOFWERK AG, Switzerland), ion signals are not counted; rather, electron currents from the microchannel plates (MCPs) are amplified and then recorded as voltages per unit time, i.e. $\text{mV} \cdot \text{ns}^{-1}$. To convert from voltage units to ion counts, the recorded voltage-time integral ($\text{mV} \cdot \text{ns}^{-1}$) signal is divided by a calibration factor to estimate true ion counts. With the icpTOF, this calibration factor is called the single-ion signal (SIS). A schematic diagram of the detection and ion-count calibration process is given in Fig. 5.2.1.

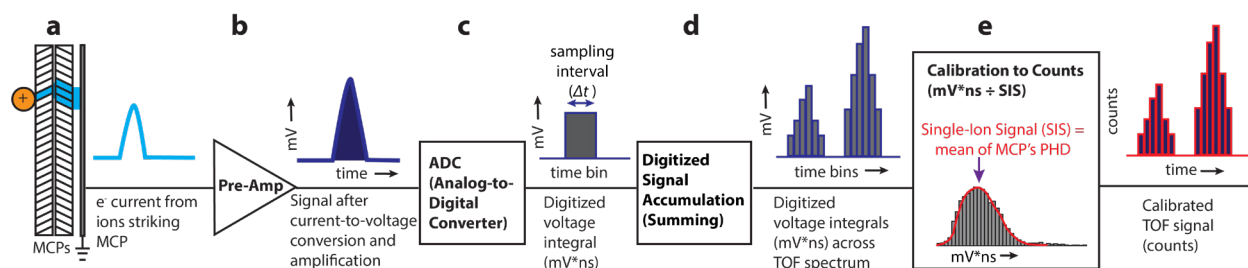


Fig. 5.2.1. Schematic of the detection process in TOF.

In a typical TOF analysis, the user calibrates the TOF detector via the TOFPilot “Detector Tuning” module in order to establish the Single-Ion Signal (SIS) for measurements for the day. The SIS is a single number and is actually the mean of the SIS histogram. The SIS histogram is a collection of $\text{mV} \cdot \text{ns}^{-1}$ detector responses from single-ion strikes on the MCP detector. The “SIS Hist” is analogous to the pulse-height distribution (PHD) of the MCPs, which is well-established for electron multiplier detectors.^{13,14,15}

Instead of following the Normal distribution or a Poisson distribution, signals from icpTOF instruments follow a compound Poisson distribution, in which ion arrival events are Poisson distributed and these events are “compounded” with the SIS response function of the icpTOF

¹² Laborda, F.; Jimenez-Lamana, J.; Bolea, E.; Castillo, J. R., Critical considerations for the determination of nanoparticle number concentrations, size and number size distributions by single particle ICP-MS. *J. Anal. At. Spectrom.* 2013, 28 (8), 1220-1232.

¹³ Dietz, L. A., Basic Properties of Electron Multiplier Ion Detection and Pulse Counting Methods in Mass Spectrometry. *Rev. Sci. Instrum.* **1965**, 36 (12), 1763-1770.

¹⁴ Wiza, J. L., Microchannel Plate Detectors. *Nuclear Instruments and Methods* **1979**, 162, 587-601.

¹⁵ Gedcke, D. A., Suppressing Noise in TOF-MS with Fastflight-2. *ORTEC Application Note AN62* **2001**.

detection system. In a series of publications, we have developed and verified the compound Poisson distribution that best characterizes low-flux icpTOF signals.¹⁶ In the icpTOF instruments, the counts reported (e.g. counts/extraction) are just an estimate of the true counts by dividing the recorded analog voltage (mV) by the average SIS value; for this reason, in this manual, we refer to all signals as TofCounts (TofCts for short).

Because icpTOF signals are fundamentally compound-Poisson distributed, the critical values used to distinguish particle-derived signals background signals (aka noise) must be derived in terms of a compound Poisson distribution. In TOF-SPI, compound Poisson critical value expressions are determined using Monte Carlo simulations and empirical SIS histograms recorded from the icpTOF instrument on the same day as the spICP-TOFMS analysis. The compound Poisson expressions can be mass-dependent or mass-independent, though mass-dependent critical values are generally more accurate and their use will produce fewer false-positive particle events. The general form of the critical value expressions is given in eqn 5.2.1, in which $L_{c,sp,i}$ is the critical value for some element, i , and $\lambda_{TofCts,i}$ is the average background signal in TofCts for element i . Critical value expressions can be determined for a range of alpha values; TOF-SPI creates expression for alpha values from 10% to 100 ppt (i.e. 1×10^{-7}). The critical mass for element i is the critical value divided by the absolute sensitivity ($S_{drop,i}$, TofCts g^{-1}) recorded for element i , as shown in eqn 1.2.2. Compound-Poisson critical values are used in Step 03 of TOF-SPI to separate particle-derived signals from background signals.

$$L_{c,sp,i} = a(\lambda_{TofCts,i})^{1/2} + b \quad (5.2.1)$$

$$X_{c,sp,i}^{mass} = \frac{L_{c,sp,i}}{S_{drop,i}} \quad (5.2.2)$$

5.3 Online Microdroplet Calibration

Online microdroplet calibration is an alternative to the size- or frequency-based calibration approaches typically used for spICP-MS. With this method, monodisperse multi-element microdroplets with known concentrations are introduced into the ICP at the same time as the particle sample. The droplets produce a transient signal similar to that produced by a particle and, as such, are an effective proxy for particle standards. Two bursts of droplets are used for calibration: one at the beginning of the measurement and one at the end.

Figure 5.3.1 depicts a typical online microdroplet calibration system. A microdroplet generator is used to produce microdroplets at the top of a metal tube. The droplets are transported through this “falling tube” and desolvated prior to mixing with a particle sample from the outlet of a spray

¹⁶ A. Gundlach-Graham, L. Hendriks, K. Mehrabi and D. Günther, Monte Carlo Simulation of Low-Count Signals in Time-of-Flight Mass Spectrometry and its Application to Single-Particle Detection, *Anal. Chem.*, **2018**, 90, 11847-11855.; L. Hendriks, A. Gundlach-Graham and D. Günther, Performance of sp-ICP-TOFMS with signal distributions fitted to a compound Poisson model, *J. Anal. At. Spectrom.*, **2019**, 34, 1900-1909.; 1. A. Gundlach-Graham and R. Lancaster, Mass-Dependent Critical Value Expressions for Particle Finding in Single-Particle ICP-TOFMS, *Anal. Chem.*, **2023**, 95, 5618-5626.

chamber upstream of the plasma torch. Since the droplets enter the plasma simultaneously with the particle-containing sample, any matrix effect from the sample that affects particle signals will also affect the microdroplet signals.¹⁷ A camera is used to size the droplets, and the volume of the droplet (V_{drop}) is calculated by assuming the droplets are spherical. The absolute sensitivity for each analyte in the droplet ($S_{drop,i}$) is then calculated by dividing the background-subtracted signal for an analyte by the mass of the analyte in the droplet (eqn. 5.3.1). Additionally, an uptake standard (typically Cs) is spiked into all samples. The same element is also in the microdroplets. By taking the ratio of the sensitivity of the uptake standard from the nebulized particle sample ($S_{Neb,Cs}$) to the sensitivity from the microdroplets ($S_{drop,Cs}$), the plasma uptake (q_{plasma}) can be calculated. The plasma uptake is then used to calculate the particle number concentration (PNC) (eqn. 5.3.3), where N_p is the number of particles measured and $t_{spRegion}$ is the time span of the single-particle region in the spICP-TOFMS time trace.

$$S_{drop,i} = \frac{I_{drop,i} - \lambda_{bkgd,i}}{C_{drop,i} \times V_{drop}} \quad (5.3.1)$$

$$q_{plasma} = \frac{S_{Neb,Cs}}{S_{drop,Cs}} \quad (5.3.2)$$

$$PNC = \frac{N_p}{(q_{plasma})(t_{spRegion})} \quad (5.3.3)$$

¹⁷ Hendriks, L.; Ramkorun-Schmidt, B.; Gundlach-Graham, A.; Koch, J.; Grass, R. N.; Jakubowski, N.; Günther, D., Single-particle ICP-MS with online microdroplet calibration: toward matrix independent nanoparticle sizing. *J. Anal. At. Spectrom.* **2019**, *34* (4), 716-728.; S. Harycki and A. Gundlach-Graham, Online microdroplet calibration for accurate nanoparticle quantification in organic matrices, *Anal. Bioanal. Chem.*, **2022**, 414, 7543-7551.

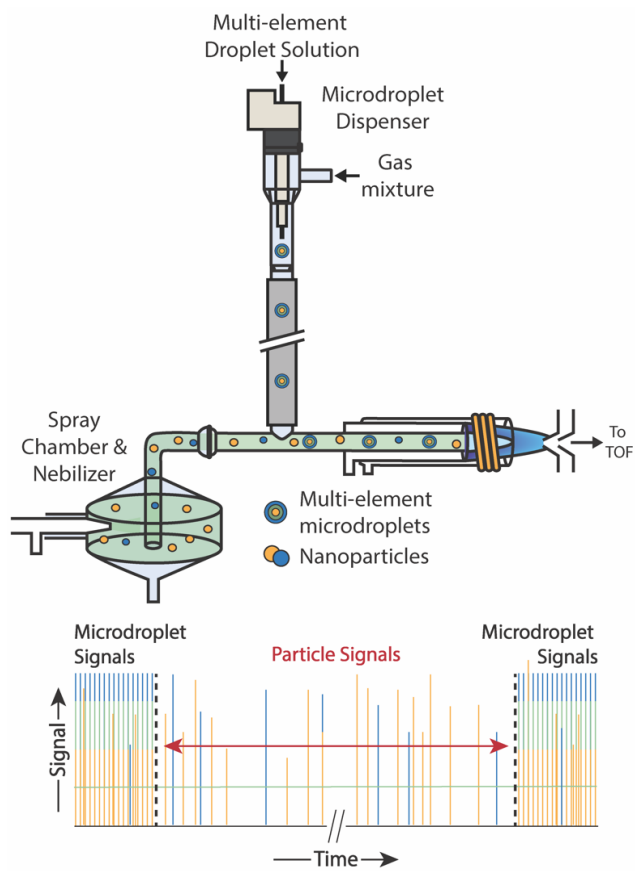


Figure 5.3.1. Schematic diagram of online microdroplet calibration system and data structure.

6 Appendix II: Data Structure

6.1 icpTOF Data Structure

A detailed description of the icpTOF data structure is beyond the scope of this manual; these details are provided in TOFWERK documentation. Here, a brief description is provided. HDF5 files saved by ToFDAQRec from the ICP-TOFMS software are used directly by TOF-SPI to gather data for processing, and also to store TOF-SPI generated data. The HDF5 (Hierarchical Data Format 5) file format is an open source file type well-suited for large files with heterogeneous data types. This file type allows for data to be efficiently read out and written into. In TOFMS HDF5 files, data are stored primarily in “groups,” “datasets,” and “attributes” of these groups and datasets. In Figure 6.1.1, we provide a directory view of all the groups stored in icpTOF HDF5 files. TOF-SPI does not use (or read) all of these groups and datasets. TOF-SPI only uses integrated data (stored in the “PeakData” group in the TOFMS HDF5 file) as well as a few other important datasets and attributes. The datasets and groups that are used by TOF-SPI are highlighted and a description of each is provided in Table 6.1.1.

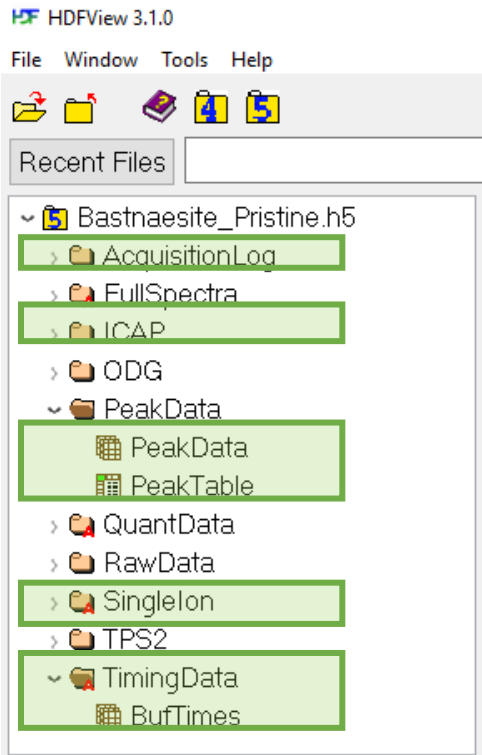


Fig. 6.1.1. Objects in icpTOF HDF5 files. Highlighted objects are used in the first step of TOF-SPI to read in data for single-particle analysis.

Table 6.1.1. HDF5 Objects in standard icpTOF HDF5 used in TOF-SPI.

HDF5 Name	HDF5 Object Type	Data Type	Description
/NbrWaveforms	Attribute	32-bit integer	Number of waveforms in averaged TOF spectrum
/NbrSegments	Attribute	32-bit integer	Number segments (usually just 1)
/NbrBufs	Attribute	32-bit integer	Number buffers
/NbrWrites	Attribute	32-bit integer	Number writes
/FullSpectra			
/Single Ion Signal		64-bit floating point,	SIS (mV*ns) used when data is collected to convert mV digitized to TofCts
/TimingData	Group		
/TofPeriod	Attribute	32-bit integer	Period of TOF measurement in nanoseconds

/BufTimes	Dataset	64-bit floating point, 2D-array	Time (sec) each buffer is started. Each row are the bufs for one write. Overall dimensions are NbrBufs × NbrWrites
/PeakData	Group		Group in which integrated data are stored
/PeakData	Dataset	32-bit floating-point, 4-D Array	Integrated signals in TofCts. Peak data can be integrated either in TofDAQ Viewer or in TOFWARE v3.2.0 or later using the “Integrate all peaks” in the “Fit&integr.” tab of the “Misc” tab.
/PeakTable	Dataset	Compound	Table of mass labels in [133Cs]+ format, centroid mass, and low and high integration boundaries
/SingleIon	Group	Group	Group with SIS histogram data. This object is only present if TofPilot Detector Tuning was done with histogram function activated and data were collected with TofPilot workflow with TofPilot v2.11.5 or later
/Data	Dataset	64-bit floating point, 2D-array	Average SIS Histogram, Column 1: SIS Value (mv*ns), Column 2: frequency
/NominalXX	Group		SIS Histograms of all nominal mass value, each mass has own group with attributes, data, and info

6.2 TOF-SPI Architecture

In TOF-SPI, three independent programs (steps 1-3) are used to process the data. **These programs should be completed in order.** Datasets created in each step are stored in the HDF5 files of the original icpTOF data. This way all processed data is stored along with the original data. The datasets created in one step of TOF-SPI are used in subsequent steps; these datasets are accessed through the HDF5 files. A schematic of the dataflow into and between TOF-SPI programs is provided in Fig. 6.2.1. The basic idea behind TOF-SPI is that that **the first two processing steps should be completed immediately following spICP-TOFMS data collection when the measurements are fresh in the minds of the experimentalist.** All the TOF-SPI programs can be run in batch mode, so that redundant data processing tasks are automated. In step 1 (“01_TOF-SPI_Bkgd LCs.exe”), the user selects analyte elements and isotopes, mass-dependent compound-Poisson critical value expressions are generated, and background TofCts signals for each selected element are calculated. Data from step 1 are saved in the “spWork” group in the HDF5 file. Step 2 is the calibration step in which absolute elemental sensitivities and plasma uptake rates are determined. This step is different for online microdroplet calibration (“02a_tofSPI_DropCalib.exe”) and external calibration via the particle size method (“02b_TOF-SPI_LiquidCalib.exe”). These data are saved in “dropCalib” or “solutionCalib” groups of the

HDF5 file, respectively. The first two steps of TOF-SPI create the list of elements to be analyzed and all the calibration factors needed to quantify particle-derived signals. This data is stored in each icpTOF HDF5 file; once this data is saved, the experimenter can come back and complete step 3 (“03_TOF-SPI_ParticleQuant.exe”) at any point—no need to look in the lab notebook for concentrations, filenames, etc.! In step 3 of TOF-SPI, particle signals are found, split events are corrected, mass amounts of elements are quantified in each particle, particle number concentrations are determined, and data can be exported in a variety of formats. The data generated in step 3 can also be saved to the icpTOF HDF5 file in the group “spData.” All the datasets saved to the HDF5 files are given below, along with a brief description.

HDF5 Data Workflow for TOF-SPI with Online Microdroplet Calibration

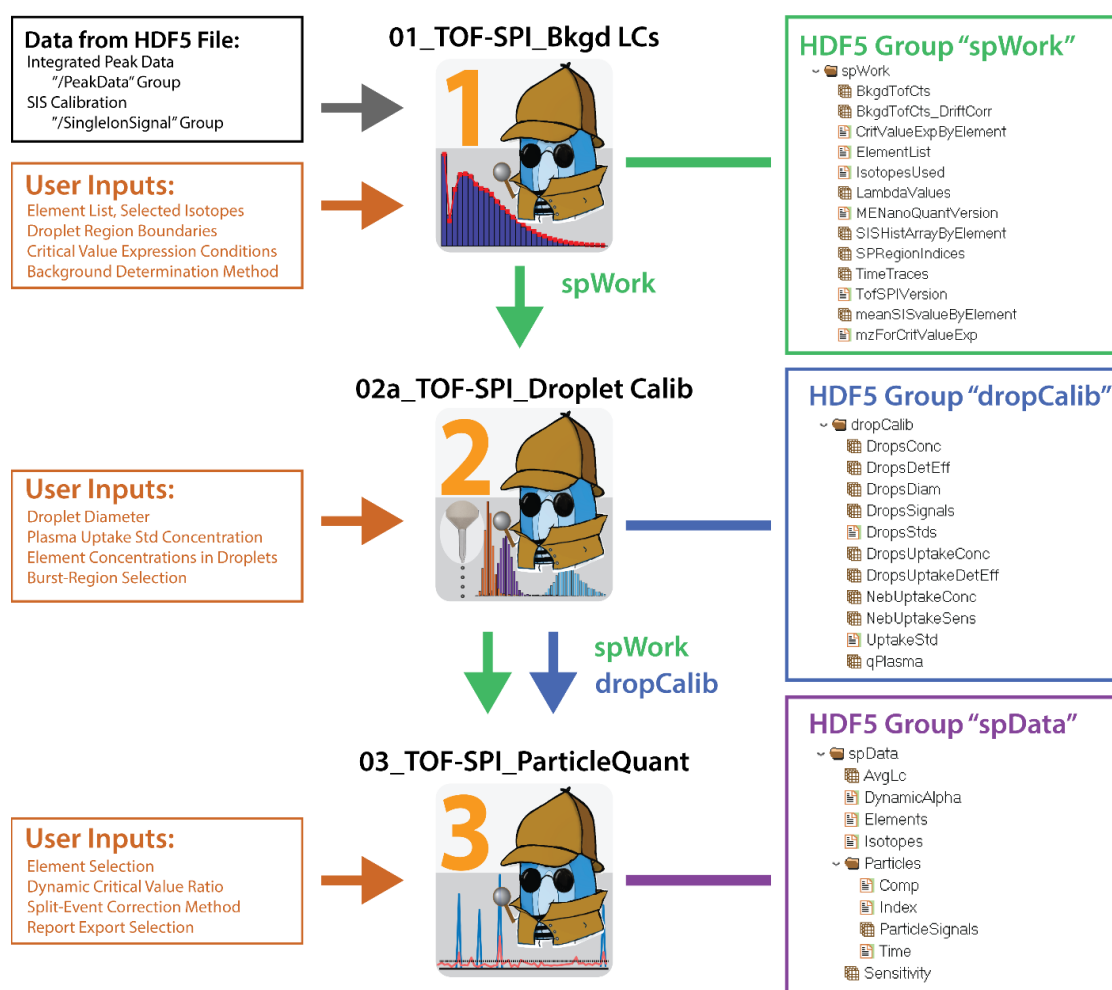


Fig. 6.2.1. Work-flow diagram of data input and generated with TOF-SPI when data is processed from online-microdroplet calibration.

HDF5 Data Workflow for TOF-SPI with Particle-Size Method External Calibration

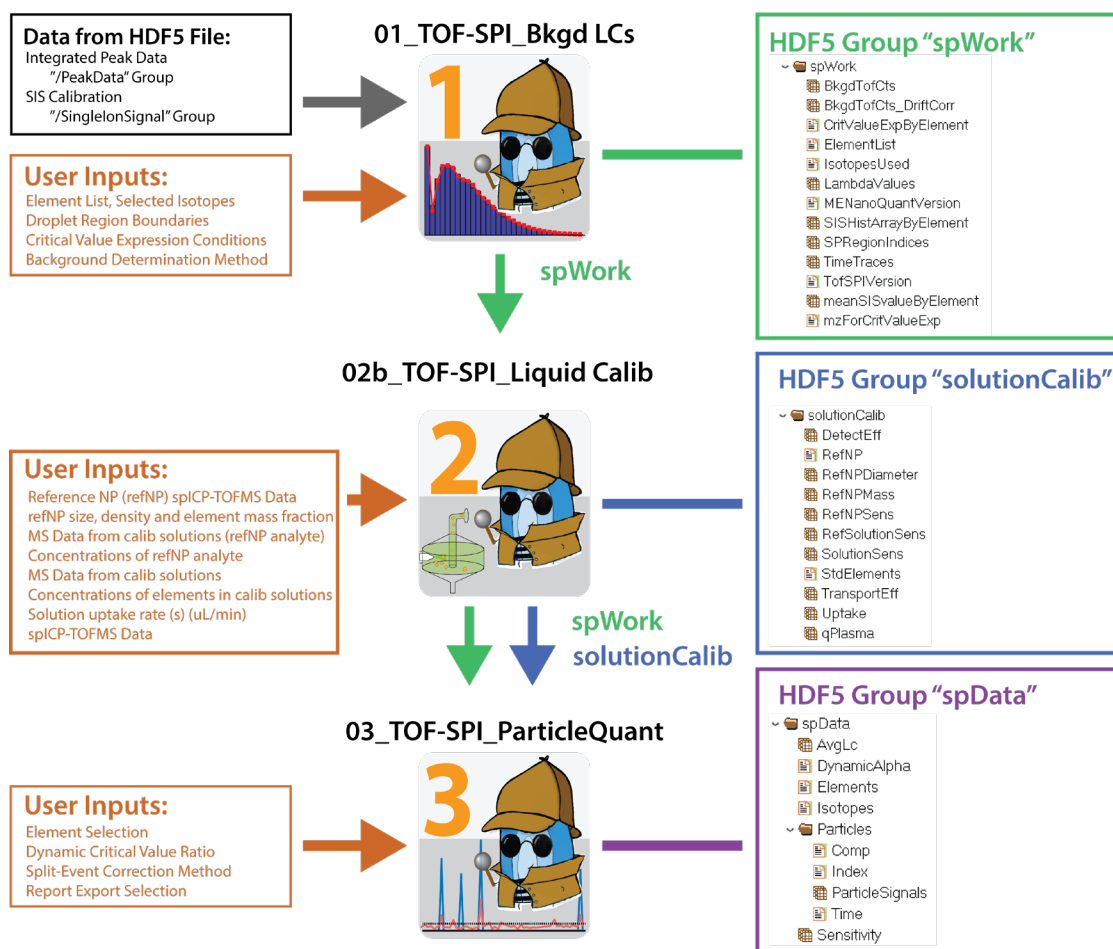


Fig. 6.2.2. Work-flow diagram of data input and generated with TOF-SPI when data is processed with calibration achieved via the particle-size method.

01_TOF-SPI_Bkgd LCs.exe, Saved datasets to HDF5:

spWork	HDF5 Group	Datasets saved from TOF-SPI_Bkgd LCs program
ElementList	String, 1D array	Symbols of all elements to be processed. These symbols may be defined by the user, but element symbols are recommended. The elements chosen here will be used in all subsequent TOF-SPI steps. A
IsotopesUsed	String, 2D array	This is a list of all the isotopes used for generating time trace of TofCt signals for each item in the the ElementList . Each row lists the isotopes used together to create summed time traces for each item on the ElementList
TimeTraces	64-bit floating-point, 2D array	Time traces of all elements used for the single-particle analysis. These time traces of each item in the ElementList have summed isotope signals as defined in IsotopesUsed . Each row is a unique element, each column is a time point. TimeTraces data are used for further data processing.
SPRegionIndicies	32-bit integer, 1D array	Boundaries of the single-particle region: first number is the starting index of the sp-region and the second number is the end of the sp-region
BkgdTofCts	64-bit floating-point, 1D array	Average TofCts of “dissolved” background signal. TofCts are per data point (not cps). Items are indexed as in ElementList .
LambdaValues	64-bit floating-point, 1D array	Same as BkgdTofCts . (Included for version compatibility.)
BkgdTofCts_DriftCorr	64-bit floating-point, 2D array	Array of background signal in TofCts at each data point along time trace of each element as defined in the ElementList . This background can account for signal drift. Each row is an element, each column is a time point (i.e. data index).
mzForCritValueExp	String	m/z values used to generate mass-dependent critical value expressions
SISHistArrayByElement	64-bit floating-point, 2D array	Array of signal-ion values that can be histogrammed to generate the characteristic mass-dependent SIS Histograms for each element in the ElementList .
meanSISvalueByElement	64-bit floating-point, 1D array	Mean SIS value in TofCts of each element-specific SIS histogram. Values are indexed according to ElementList .
CriticalValueExpBy Element	String, 3D array	This is an array with critical-value expressions for several false-positive levels, namely for alpha values (in %) of 0.00001, 0.000032, 0.0001, 0.00032, 0.001, 0.0032, 0.01, 0.032, 0.1, 0.32, 1.0, 3.2, 10. Each page (dim 0) is for the element as defined in the ElementList . dim 1 is the alpha value and dim 2 are the slopes and intercepts of the critical value expressions.
TofSPIVersion	String	Version info of TOF-SPI used for processing

02a_TOF-SPI_DropCalib.exe, Saved datasets to HDF5:

dropCalib	HDF5 Group	Data saved from TOF-SPI_dropCalib program
DropsStds	String, 1D array	List of the analyte elements in the droplets (i.e. not including the plasma-uptake std). This list gives the element references for the values in each of the other droplet arrays, i.e. in DropsConc , DropsDetEff , and DropsSignals .
DropsDiam	64-bit floating-point	Diameter of the droplets in each experiment.
DropsConc	64-bit floating-point, 1D array	An array of the concentrations (in ng mL ⁻¹) of all elements in the droplets, not including the concentration of the plasma uptake std
DropsDetEff	64-bit floating-point, 1D array	An array of the detection efficiencies (absolute sensitivities) of all analyte elements (i.e. not including the plasma-uptake std). Units of detection efficiencies are TofCts/g, where g is the total element mass of each element in the microdroplets not considering isotope abundancies.
DropsSignals	64-bit floating-point, 2D array	Signals from the droplets only. Droplets are found with uptake standard as the tracer element. A user-defined number of droplet signals (e.g. 100) are remove from the beginning of each droplet burst region. This array has combined signals from each set of droplet bursts. Each row is a different element and each column is signal (TofCts)from an individual droplet.
UptakeStd	String	Elemental symbol of the plasma-uptake std, e.g. Cs.
DropsUptakeConc	64-bit floating-point	Concentration of the plasma uptake standard in ng/mL in the microdroplets present in each of the samples being analyzed
DropsUptakeDetEff	64-bit floating-point	Detection efficiency (TofCts/g) of the uptake standard in the microdroplets
NebUptakeConc	64-bit floating-point	Concentration of the plasma uptake standard in ng/mL present in each of the samples being analyzed
NebUptakeSens	64-bit floating-point	Detection efficiency (absolute sensitivity) of the plasma-uptake analyte element in the microdroplets; units are TofCts/g. The detection efficiency of the plasma-uptake standard is corrected for background level of the plasma-uptake std in NP-containing solutions.
qPlasma	64-bit floating-point	Flow rate of solution into the plasma in units of mL/s.

02b_TOF-SPI_SolutionCalib.exe, Saved datasets to HDF5:

solutionCalib	HDF5 Group	Datasets saved from TOF-SPI_SolutionCalib program
StdElements	String. 1D array	Symbols of all elements with external calibration. These elements will be quantified in terms of mass per particle in step 3.
DetectEff	64-bit floating-point, 1D array	An array of the detection efficiencies (absolute sensitivities) of all analyte elements (i.e. the StdElements). Units of detection efficiencies are count/g, where g is the total element mass of each element in the microdroplets not considering isotope abundances.
Uptake	64-bit floating-point	Flow rate of solution into the nebulizer in units of $\mu\text{L}/\text{min}$.
TransportEff	64-bit floating-point	Fraction of solution uptake that reaches the plasma
qPlasma	64-bit floating-point	Flow rate of solution into the plasma in units of mL/s. Product of and Uptake and TransportEff .
RelativeSens	64-bit floating-point, 1D array	Relative sensitivities of solution calibrations of all elements (same order as StdElements list). Units: cps/(g/mL)
RefNP	64-bit floating-point	Element Name for Ref NP
RefNPDiameter	64-bit floating-point	Diameter of Ref NPs in nm
RefNPMass	64-bit floating-point	Mass of reference particle in g
RefNPSens	64-bit floating-point	Absolute sensitivity from reference element solution in TofCts/g
RefSolutionSens	64-bit floating-point	Sensitivity of reference solution in cps/(g/mL)

03_TOF-SPI_ParticleQuant.exe, Saved datasets to HDF5:

spData	HDF5 Group	Datasets saved from TOF-SPI_ParticleQuant program
Elements	String, 1D array	Symbols of all elements selected for particle finding and quantification. These elements can only include those specified in step 1; however, it must not include all elements.
Isotopes	String, 1D array	Isotopes of all elements selected for particle finding and quantification. These are defined in step 1.
DynamicAlpha	String, 1D array	Alpha value for which dynamic critical value ratio is satisfied. Dynamic critical value ratio is the ratio of found positive particle events to predicted false positive events for a given alpha and compound Poisson distribution. Each element may have a unique dynamic alpha value.
AvgLc	64-bit floating-point, 1D Array	Average critical value, in TofCts, for each element.
Sensitivity	64-bit floating-point, 1D Array	TofCts/g for each element. If no sensitivity is available for a given element, then the sensitivity is
Particles	HDF5 Group	Data of found particles
Particles\Comp	String, 1D array	Composition (Element symbol) of every found particle
Particles/Index	String, 1D array	Index of every found particle signal
Particles\Time	String, 1D array	Time (s) of every found particle signal. This is the same time as would be found on the timetrace using TofViewer
Particles\ParticleSignals	64-bit floating-point, 2D Array	2D array of the TofCts recorded for each element from each found particle. Each column in the 2D array is an element. This order is specified in the spData\Elements dataset. Each row is a unique particle. The time, index, and composition of the found particles are specified in the datasets: Time , Index , and Comp .