NanoFinder 5.5 : Nanoparticle Analysis Software for *sp*-ICP-TOFMS

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# Introduction

NanoFinder program designed for the *sp*-ICP-TOFMS user who wants to analysis transient signal (normally Nanoparticle, droplet or cell) for multi element and multi transient events simultaneously. For simplicity we are going to stick to nanoparticle signal from here, however the same principle are also true for other transient signal. For the better understanding of the terms and abbreviations please refer to our publications listed at the end.1-3

In order to achieve the full picture, the raw data have to goes through 5

Detection:

Select elements and isotopes of interest, generate time traces of selected elements

Determine critical value (*LC*) expressions based on compound-Poisson modelling

Determine background (dissolved signal) count rates (*λbkgd*) for all elements

Background subtract all time traces

Correct all time traces for split events2

Find NP signals above the single-particle critical value (*Lc,sp*)

Concurrency:

Correct data set for particle-coincidence to remove spurious mmNP signals caused by concurrent measurement of two or more discrete particles with unique element fingerprints

Calibration:

Find microdroplet/Reference nanoparticle and or dissolve standard signals

Determine mass sensitivities for each element *i* (*Sdrop*,*i*) and sample flow rate to instrument (*qplasma*)

Clustering (and quantification):

Quantify elemental masses from detectable individual NP signals

Perform hierarchical clustering analysis of mmNP signals; discovery of conserved and non-conserved mmNP types

Quantify detectable PNCs of both smNPs and mmNPs

Report smNP and mmNP data

Except calibration step which needed for user to enter values from their calculation or using the complimentary software in our package DropCalib (release in 2021) the rest of the calculation done centrally in this program. Unlike the calibration steps which is user dependent and sometime need more care. The rest of the process could be done on an autonomous fashion as you will see here.

# Installation

You need to run the **Setup.exe** file form the package to installed “**NanoFInder.exe**” and its host software “Matlab\_Runtime”. The setup file automatically downloads the free version of “Matlab\_Runtime” for its installation. You do not need to have Matlab license for using this program. The only requirement is Microsoft EXCEL.

# Workflow

We represent a simple example of two data files for ease of explanation. One sample has only gold particle and the other one has Gold and silver core-shell particles. The data files are as Comma separated value (CSV). Generally, Tofwerk icpTOF HDF5 (h5) is also supported as input. A summary of required and generated files are summarized in Table 1 and 2.

Table 1: Files required for each analysis are marked as green in this graph. The orange color indicates cases where either of file would work.



Table 2: Files generated or modified in each analysis.



To run this program:

1. Place a meta\_data\_excel\_file (MDEF) to the same directory as your raw data files.
2. Fill the MDEF as explain in the following section.
3. Open the NanoFinder.exe program. In the user interface of the program select the required analysis.
4. When you ready press Run. Then there will be a pop-up file browser which need to be directed to the MDEF file.
5. Depending on the type of the process and number of sample the program starts to run through your data and show you the progress in the user interface and in the pop-up figures.
6. When the processed lamp next to Run bottom in the user interface turns green, it means that the program run is completed.
7. Result are save in the same directory with different file extension such xlsx, csv, pdf, fig and etc. depending on your analysis.

## Meta data Excel file (MDEF)

MDEF is an Excel spreadsheet and it contains multiple sheets of necessary information regarding your samples such as data files names, elements of interest, calibration values and so on. The Nanofinder program used it to complete its tasks and eventually write the summary of the analysis in there. In Table 3 you could find all the required sheet which need to be filed for each analysis.

Table 3: Meta data Excel file required sheets for every analysis indicated in green.



### Filename:

After placing the MDEF in the same directory as your data files, shown in the example below. You have two possibilities to filling the **Filename** sheet of MDEF.

**Possibility 1** is to fill it manually and put your desire sample name in first column, called “Sample name” and exact name(s) of your sample file name run(s) in the next column(s), called “File name Run 1, 2, …”.

First column (Sample name) is saved for user preferred names of each data file, since data file names are usually too crowded to be consider as the given name for that sample. Follow the necessary requirement for a file name. It should not contains special character ({, \, |, /, ?, :, [,…). Gives every row a unique name. feel free to keep the whole sample name column empty then your file name run 1 will be taken as your sample name.

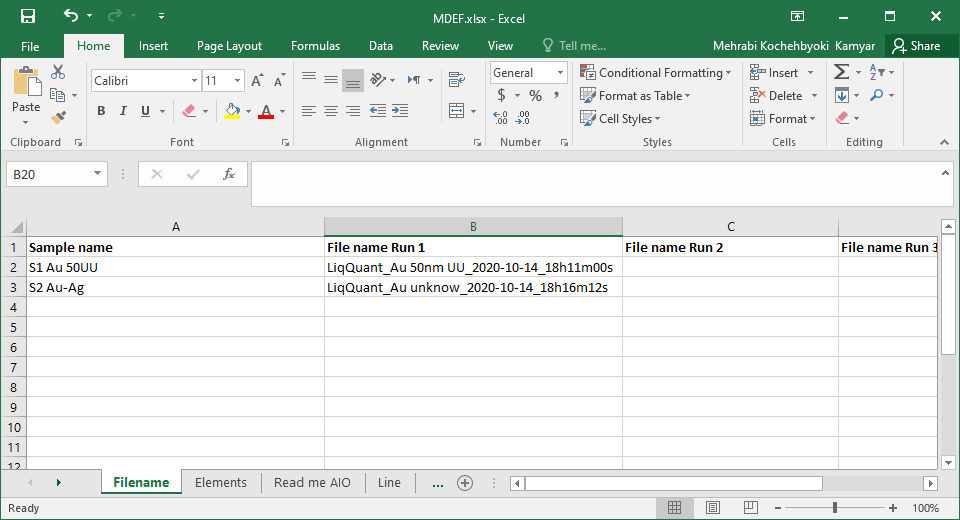
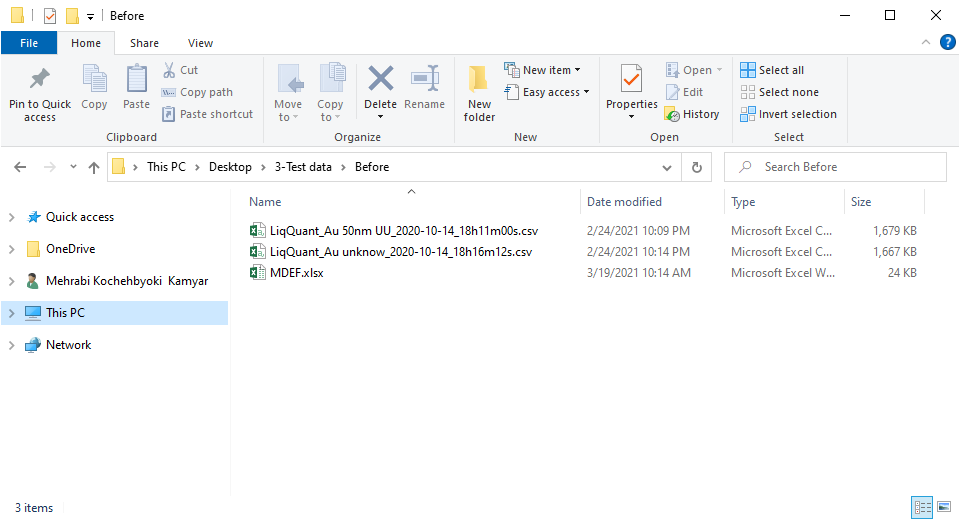
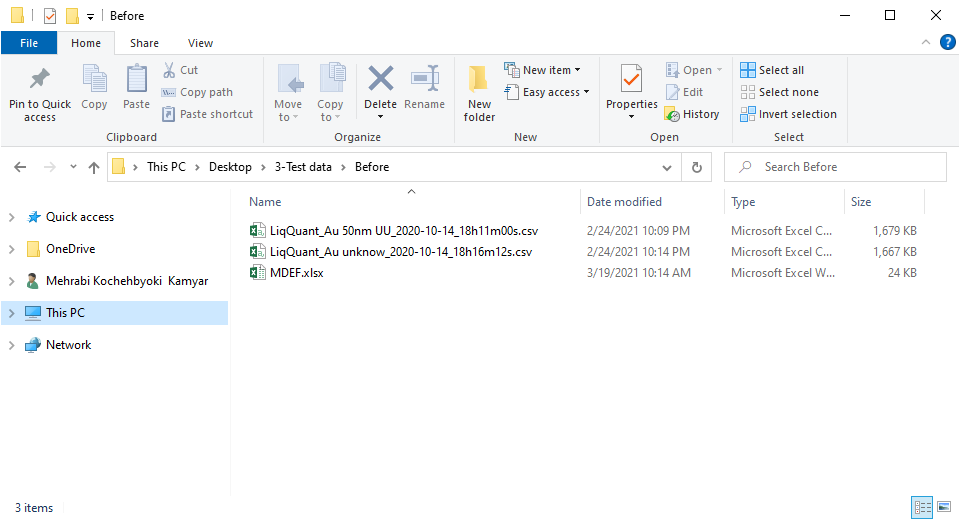


Figure 1: data directory and example of Filename sheet contents in case of **possibility 1**.

**Possibility 2** is to leave this sheet empty except keeping the headlines. After you executed the NanoFinder program, it will automatically select all of the files in the same directory as selected MDEF and desired format (.csv or .h5) as your data file and fill the Filename sheet. Notice in this case your sample name will be same as your first data file name Run 1. By specify number of replicates in the sheet of “Read me AIO” you may reshape your file name according to your preference here. the important task is all of your same need to have similar number of run then otherwise the program will rise an error.



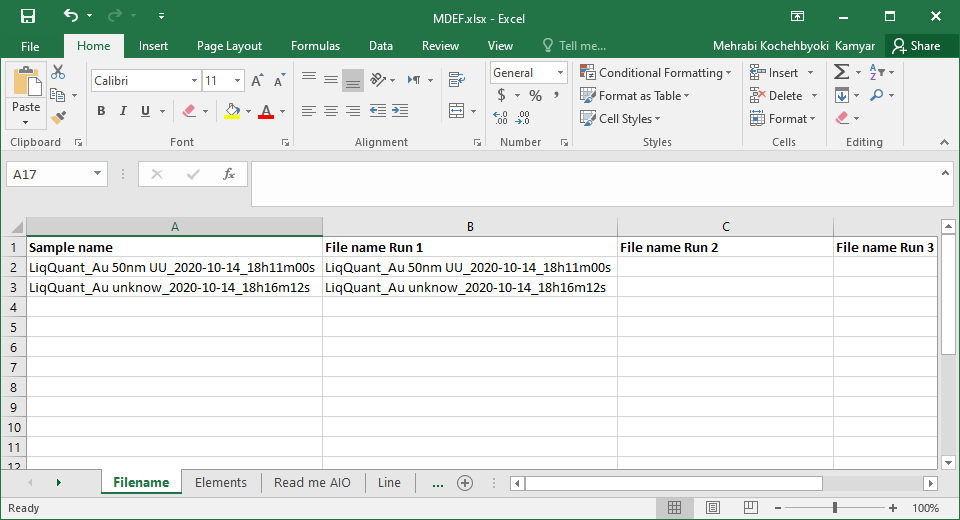
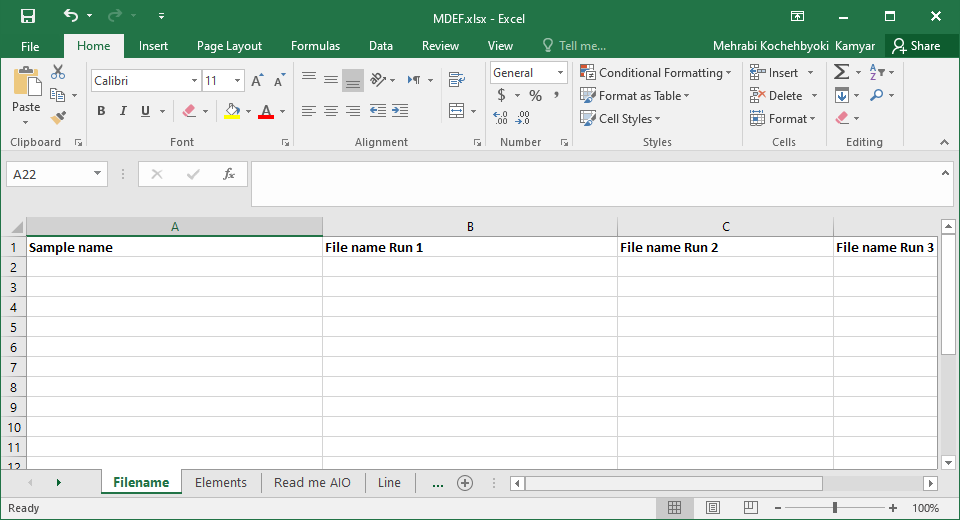


Figure 2: data directory and example of Filename sheet contents in case of **possibility 2**, before and After execution of program.

### Elements

Here you write the name of the interested element. Element name could be things like [56Fe]+, CeO+ or Au, its only need to be similar to your data file. If you wish to combine signal of isotopes or elements of interest, place them in the same row in cells next to each other. There is no limitation of the number of element that you want to analyzed or combined. as shown in Figure 3.

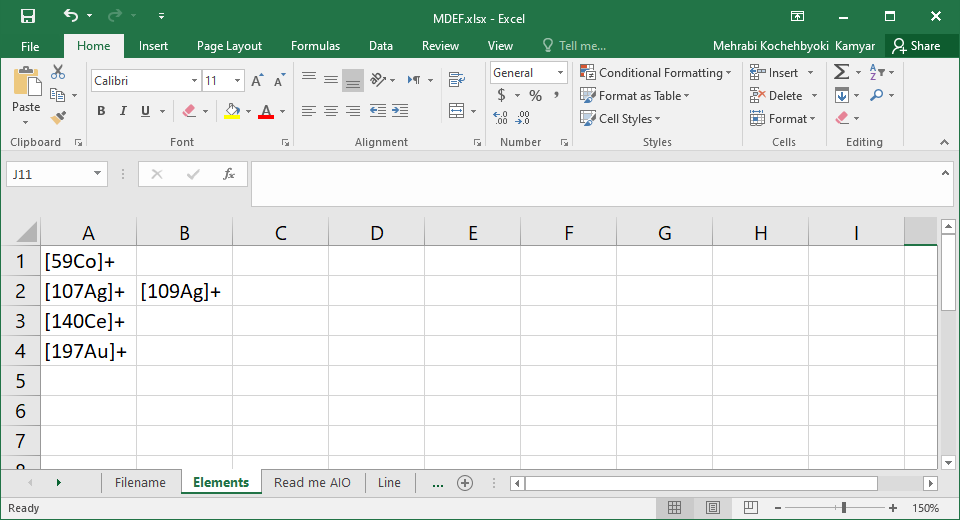


Figure 3: Example of filled Elements sheet of MDEF

### Read me AIO:

This sheet is the center of all analysis. It is also containing some of the necessary setting for **Detection** analysis. You only have to fill this sheet if you want to do DETET analysis. For filling the contents of this sheet, you could either directly use the graphical user interface (GUI) of NanoFinder or enter settings directly in MDEF. As it is shown in the fig, you must indicate your chose in the GUI.

**Conversion factor to counts:** Mass spectrometer raw data could be as count/extraction, count/s or etc. to conversion raw data to count Here one need to enter the conversion factor. If your data file is in HDF5(.h5), put 0 here and the program automatically copy “extraction into segment” from the HDF5 data files.

**Threshold lower boundary:** It is the lower threshold of particle detection. Place 0 if you want to eliminate it.

**Nanoparticle to Noise:** NanoFinder use dynamic thresholding. It allows the particle thresholding for each element be define with its own level of false detection rate. Basically this value is defining how many times your detected particle events of specific element is need to be higher than false positive events. For example, 100 here means that if we consider compound Poisson noise, for each 100 detected particles of specific element only 1 false positive predict to exist or 1% of error out of particle detected.

**True to false positive ratio:** This is related to compensate for signal drift. Place 1 in case you want to not have that. Generally, it is better to have it at 50.

**Start data point:** This indicate where in the data set software need to start the analysis. If you want it to start from beginning of the acquired time trace place 1 or 0 in this field.

**End data point:** This indicate where in the data set software need to terminate the analysis. If you want to processed complete file you could type 0 here.

**Number of Runs per sample**: If you measured your samples in more than one data file, specify the number of run per sample here. All of your samples must have same number of runs.

**Data file format (h5 or csv):** Raw data format for Detection analysis need to be specify here. there are two possibilities, HDF5(h5) file (default of the icpTOF) or comma separated value (CSV). For further clarification on how should the CSV file be filled in, please look at the test file.



Figure 4: Example of filled Read me AIO sheet of MDEF file. A, Read setting form GUI. B, Read setting form Excel and C, representation of the data in the Read me AIO.

### Line

In this page the line expression for the critical value calculation is placed. We encourage users to read more about fundamental of this expression from Gundlach-Graham, et al paper.1 If you have single-ion-signal histogram of your instrument (better to be measured at the same day of your obtained data) it is possible to calculate these value with current program. If not, you may use expression from other days or use values in the test data here. You may choose a more conservative **True to false positive ratio** (for example 100 or more) if your critical value expression is outdated.

If you have a single-ion-signal histogram and wish to calculate the critical value expression. In **Line** sheet delete all columns except the firs column (Alpha(rate)) and copy the single-ion-signal histogram to **SIS Hist** sheet as explained in following section. If you wish to calculate critical value expression for different set of Alpha rates from the one presented in Figure 6, just simply delete the presence values and add new ones.

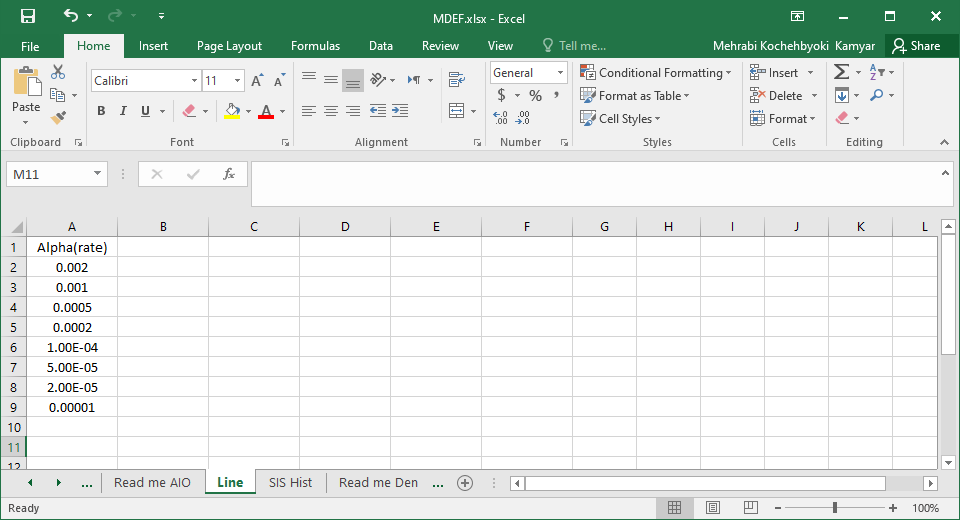


Figure 5:Example of emptied Line sheet for calculation of critical value expression.

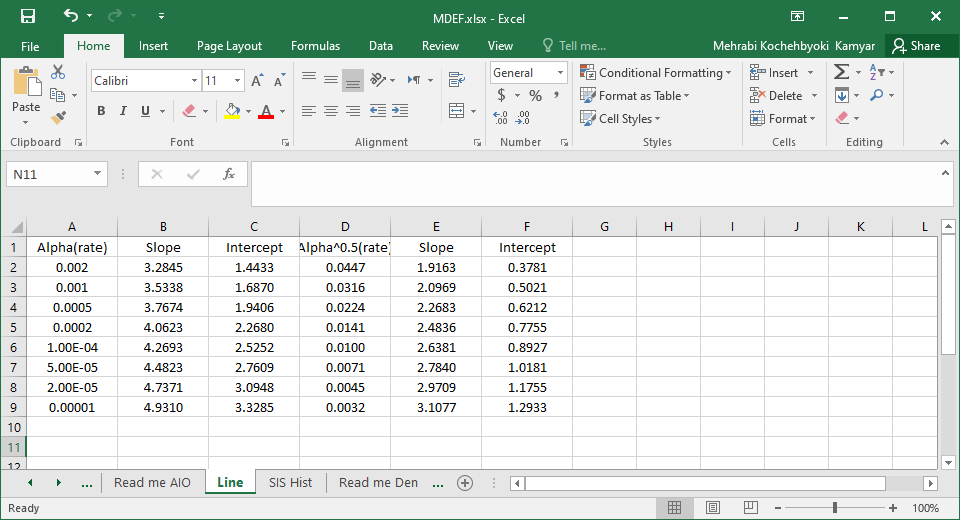


Figure 6: Example of filled Line sheet with critical value expression.

### SIS Hist

This sheet only required if you wish to calculate critical value expression of your instrument for **Detection with SIS** analysis. If you already have the critical line expression this sheet will not be used. Single-Ion-Signal in first column and its repetition in the second column as shown in Figure 7.

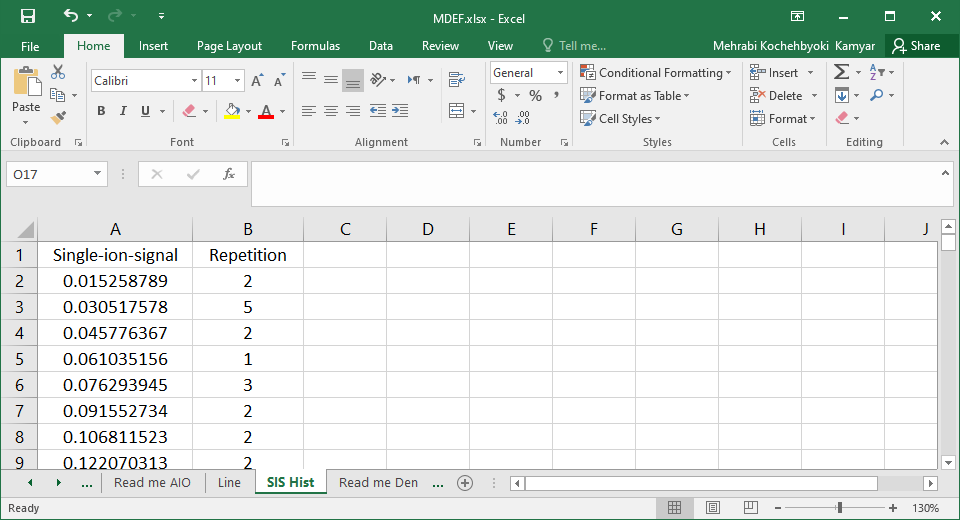


Figure 7: Single-ion-signal histogram placed in the SIS Hist sheet of MDEF file

### Read me Dem:

This sheet only required for the **Clustering** analysis. In this sheet you need to enter information as are summarized below.

**Cutoff1**: A number between 0 to 2, for the first Clustering analysis. This cutoff is use for intra-sample clustering analysis.

\*The distance used for clustering is correlation distance which define as, one minus the sample correlation between points, and 0 means there is no distance high correlation and 2 mean highest distance and reverse correlation

**Occurrence in cluster rate**: The minimum ratio of the elements that need to happen in each cluster to be taken to account for cluster proxy of second hierarchical clustering.

**Max number of clusters**: Max number of cluster in each data file if this number excided error will rise and Cutoff 1 need to be increase. This error normally happens if cutoff1 is low.

**Cutoff2**: A number between 0 to 2, for the second clustering analysis. This cutoff is use for intra-sample clustering analysis and normally it is smaller than Cutoff1.

**File name addition text:** what your csv file has in addition to first column of filename add it here. for example for **concurrency** analyzed data use (**.hpCC.csv**), for not concurrency corrected file from **Detection** use (**.NP time trace.csv**) and for other files just make sure it is in comma separated format and add (**.csv**) here.

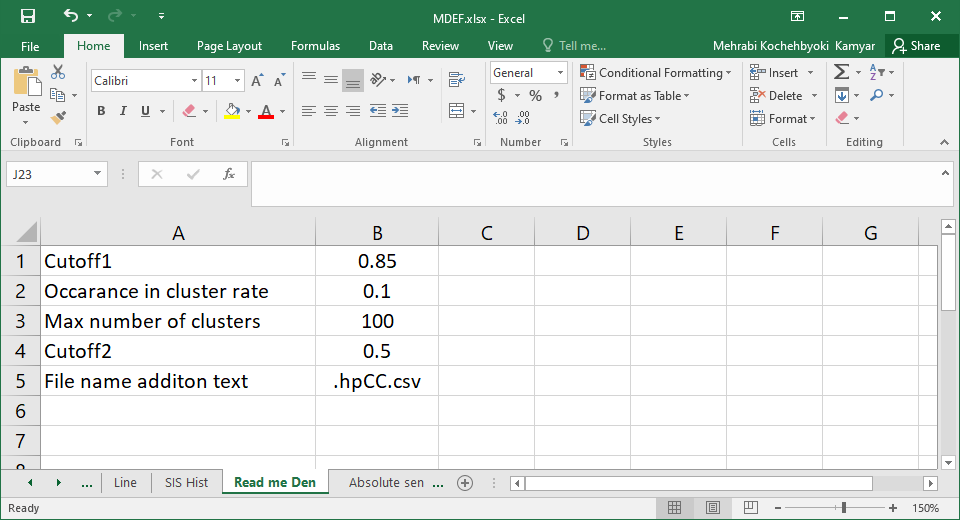


Figure 8: Example filled Read me Dem sheet of MDEF file.

### Absolute sensitivity(CountsPng)

This sheet only required if you wish to quantify and cluster your data (**Clustering** step). As the name suggest you need the absolute sensitivity of each element and sample that you have in the analysis in the unit of counts per Nano gram (Count/ng). The absolute sensitivity is the corrected for transport efficiency and it used for conversion of nanoparticle count to mass.

\*If you don’t have these calibration values just make a table full of value one (1) instead. This will still work but remember you will have an arbitrary unit and the reported data masses are not correct. But you can still see how your nanoparticle are clustered. Cluster may be slightly different if you put the actual mass rather than a table of all “1”.

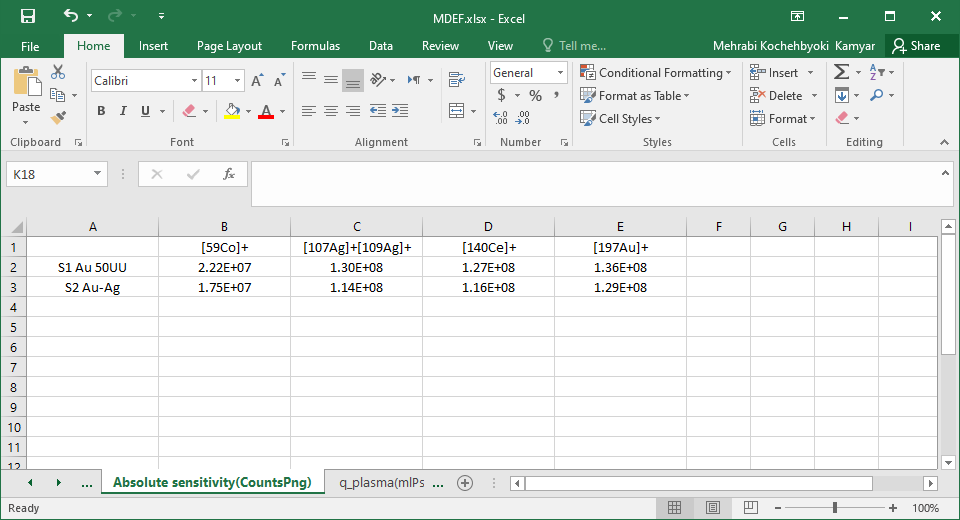


Figure 9: Example filled Absolute sensitivity(CountsPng) sheet

### q\_plasma(mlPs)

This sheet only required if you wish to quantify and cluster your data (**Clustering** step). In this MDEF sheet you have to fill two columns and one row per each sample in the similar order as in **Filename**. Firs column contains the q-plasma (uptake of sample to plasma) this mean you flow rate to nebulizer which is transport efficiency corrected. For example, if you have 100 **µL/min** nebulizer flow and 10% efficiency then this value is 0.000167 **mL/s**

The second column contains the measurement time divided by dilution factor of the sample for each sample in filename. For example, if your measurement time was 120s and dilution was 100 time then this number is 120s/100=1.2. Make sure to also add the time of all of your runs per sample of you are merge files.

\*If you don’t have these values just make a table full of value one (1). This will still work but remember you will have an arbitrary particle number concentration (PNC) and the reported data PNC are not correct

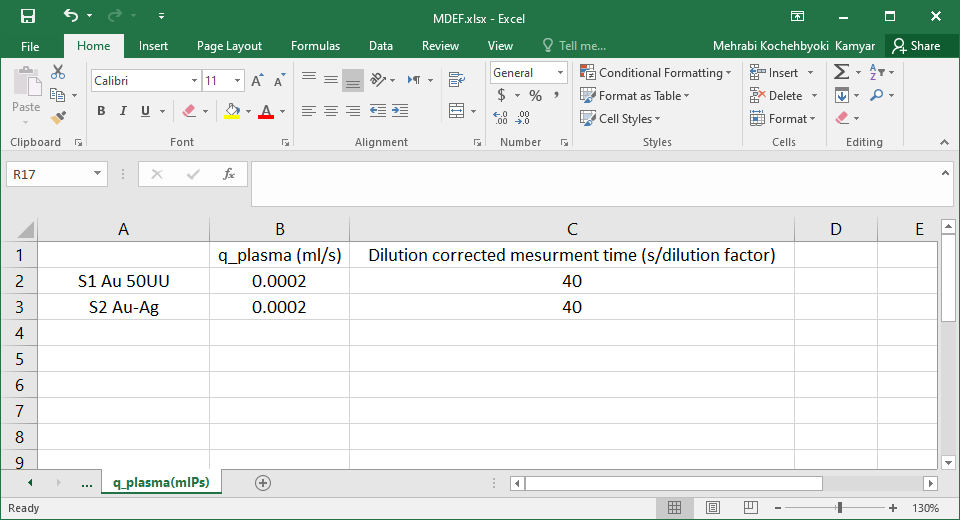


Figure 10: Example filled q\_plasma(mlPs) sheet

# 4.References:

1. A. Gundlach-Graham, L. Hendriks, K. Mehrabi and D. Gunther, 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.

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

3. K. Mehrabi, D. Gunther and A. Gundlach-Graham, Single-particle ICP-TOFMS with online microdroplet calibration for the simultaneous quantification of diverse nanoparticles in complex matrices, *Environmental Science-Nano*, 2019, **6**, 3349-3358.