NCL Method PCC-21

Measuring Size and Number Concentration of Metallic Nanoparticles using SP-ICP-MS

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This protocol assumes an intermediate level of scientific competency with regard to techniques, instrumentation, and safety procedures. Rudimentary assay details have been omitted for the sake of brevity.

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1. Introduction

This protocol describes how to measure the size and concentration of individual metallic nanoparticles using ICP-MS (inductively coupled plasma - mass spectrometry) in single particle (SP) mode. Accurately determining the size of individual nanoparticles on a per particle basis both quickly and accurately is an ever-increasing need within nanoparticle characterization. ICP-MS is capable of measuring a broad range of nanoparticle sizes with high resolution, thus allowing the measurement of multiple particle populations for the quality assessment of nanoformulations. Additionally, SP-ICP-MS can accurately determine particle concentrations without the need for concentration standards.

ICP-MS is an ultrasensitive technique which can determine the concentration of trace elements in solution down to the pg/g level. Delguard et al first demonstrated in 2006 that ICP-MS could accurately measure the mass of individual metallic nanoparticles in what has come to be known as SP-ICP-MS [1]. By introducing a nanoparticle solution into the ICP, the individual nanoparticles are ionized into an ion cloud which then is carried as an intact unit to the detector. After a brief rapid expansion of the ion cloud, the duration of this cloud, defined as the time it first hits the detector to the time the last ion hits, is approximately 100-650 μs for 30-150 nm particles, respectively. This allows for two methods for measuring nanoparticles in SP mode, both of which will be outlined in the protocols below.

In method 1, a large integration time (10 ms) with respect to the duration of the ion cloud is used in combination with a sufficiently dilute solution to measure individual nanoparticles. Since the integration time is much larger than the particle cloud duration, this method measures the entire particle cloud as a single event. Due to this, it is extremely important that the nanoparticle solution is sufficiently dilute to prevent multiple particles from hitting the detector during the integration window. This method has the advantage of being usable for many modern ICP-MS instruments that do not have a dedicated single particle counting mode since most of these instruments are capable of integrations times as low as 10 ms. Additionally, the long integration times allow for a longer time period for the detector to recover after a nanoparticle event, leading to a lower occurrence of detector saturation and small particle events.

Method 2 uses a shorter integration time than the duration of the ion cloud which allows for the measurement of nanoparticle events by rastering multiple points across the detection event.

The 50 µs integration time is generally available in instruments with a dedicated single particle mode and provides a few advantages over 10 ms integration measurements. The faster integration time allows for the collection of more information than the 10 ms integration which is limited to the total or summed intensity of the nanoparticle event. Three types of information are available due to the ability to raster across the peak. Like 10 ms integrations, the total or summed intensity is available, however, also available are the max height intensity of the peak and the duration of the ion cloud. Additionally, due to the faster integration time, samples about ten times higher in concentration than the 10 ms method can be accurately analyzed simplifying the sample preparation process.

2. Reagents, Materials and Equipment

Note: The NCL does not endorse any of the suppliers listed below; their inclusion is for informational purposes only. Equivalent supplies from alternate vendors can be substituted.

2.1 Reagents

- 2.1.1 Water, 18MΩ-cmresistivity
- 2.1.2 SRM 3121 Gold (Au) standard solution (NIST)
- 2.1.3 Citrate Stabilized gold nanoparticles (NP; 30, 40, 50, 60, 80, 100, 150 nm; Ted Pella/BBI solutions)
- 2.1.4 Citrate Stabilized Ag NPs (30, 50, 70, 100 nm; Ted Pella/ NanoComposix)
- 2.1.5 Concentrated Nitric Acid (HNO_3), trace metal grade (Fisher Scientific)
- 2.1.6 Concentrated Hydrochloric Acid (HCl), trace metal grade (Fisher Scientific)

2.2 Materials

- 2.2.1 2 mL microcentrifuge tube (Eppendorf)
- 2.2.2 Low density polyethylene (LDPE) sample bottles, 30 and 60 mL volume (Nalgene)
- 2.2.3 15 mL conical centrifuge tube, polystyrene (Falcon)

2.3 Equipment

- 2.3.1 NexION 200B ICP-MS with single particle mode (Perkin Elmer)

3. Methods

3.1 Measuring the flow rate of the peristaltic pump

3.1.1 The flow rate needs to be determined for the specific peristaltic pump, tubing, and the solvent system being used in the SP-ICP-MS. Due to the deterioration/ stretching of the tubing over time, the flow rate should be measured just prior to each SP sample run.

3.1.2 To measure the flow rate, move the sampling probe to a vial containing the running buffer, in this case 18 MΩ-cm resistivity water, and allow the sampling tube to fill with water until all air or residual solvent has been evacuated from the sample tubing.

3.1.3 Collect 4 microcentrifuge tubes. One will be used for a pre-run to ensure that there is no dead space left in the tubes, and the remaining three will be used to measure the flow rate.

3.1.4 Pre-weigh and record the weight for the three 2 mL microcentrifuge tubes which will be used to determine the flow rate.

3.1.5 Turn off the peristaltic pump and disconnect the sampling tube from the nebulizer and place the end of the tube into the un-weighed tube.

3.1.6 Set the speed of the peristaltic pump to the speed used during single particle measurements.

3.1.7 Start the peristaltic pump while simultaneously starting a timer.

3.1.8 Collect water for 1-2 minutes, stopping the pump when the timer completes.

3.1.9 Repeat 3 more times for the tared microcentrifuge tubes.

3.1.10 Cap and weigh the tared microcentrifuge tubes containing water.

3.1.11 Subtract the weight of the empty tube from the total weight of the tube plus solvent to get the total amount of liquid collected. Multiply this weight by the density of the liquid. For sufficiently dilute buffers in water if the density is not known, assuming a density of 1 mg/mL is adequate. Divide the calculated volume by the total time in minutes to get the flow rate for the subsequent set of measurements. Repeat two more times and average the results together to get the final flow rate.

3.1.12 Reconnect the tubing to the peristaltic pump.

3.2 Sample Preparation – Dissolved Elemental Standards (Au standards)

3.2.1 NIST SRM 3121 has an initial concentration of around 10 mg/mL (10 parts per thousand), so the dissolved standards need to be prepared by serial dilutions to achieve running concentrations of 10 – 100 parts per billion (ppb, ng/g) for 50 µs measurements or 0-10 ppb for 10 ms measurements.

3.2.2 The dissolved metal standards should be prepared in a buffer system that stabilizes the ion. For gold dissolved standards, a solution of 1.5% HNO3, 4% HCl is used to stabilize the gold ions.

3.2.3 To prepare the dissolved gold standards, first prepare a 50 parts per million (ppm, ug/g) solution of NIST SRM 3121 by first weighing a 30 mL LDPE sample bottle. Record the weight. Add 150 µL of stock NIST SRM 3121 to the pre-weighed bottle and record the weight. Dilute to a total volume of 30 mL using the HNO3: HCl solution.

3.2.4 Next prepare a 1 ppm (µg/g) solution of SRM 3121 by weighing a 60 mL of LDPE sample bottle. Add 1 mL of the 50 ppm Au solution prepared in 3.2.3 to the bottle and record the weight. Dilute to solution to a total volume of 50 mL using the HNO3: HCl solution and record the weight.

3.2.5 Next prepare the dissolved Au standards from the 1 ppm SRM solution prepared in 3.2.4. For example, to make a 10 ppb solution of SRM 3121, first weigh an empty 60 mL LDPE sample bottle. Next, add 500 µL of the 1 ppm solution of SRM 3121 prepared in 3.2.4 and weigh the sample bottle. Finally, dilute to a total volume of 50 mL using the HNO_3:HCl solution and record the final weight. Continue following this method until the desired dissolved Au solutions are prepared.

3.2.6 The exact concentration of each dissolved standards in ppb (ng/g) can be calculated using Equation 1 and solving for M2 and using the weights of the solutions added.

$$M_{STD1}W_{STD1} = M_{STD2}W_{Total} \quad (1)$$

3.2.7 To determine weight of the standards, the weight of the empty bottles should be subtracted from the weight recorded after adding the standard and the weight recorded after the final dilution.

3.3 Preparation of NP standard solutions

3.3.1 As stated, a single element nanoparticle standard solution is needed for accurate determination of the transport efficiency which will be used to accurately determine both size from a dissolved elemental standard and concentration of the NP solution. NIST RM 8012 30 nm Au and NIST RM 8013 60 nm Au have been used in literature as the NP standards due to the accuracy of their reported size. However, due to the limited availability of this RM, non-reference gold nanoparticle solutions can be used to create this calibration curve provided that they contain a monodisperse solution of nanoparticles. In order to use these solutions, the size should be independently verified via TEM to determine the exact geometric diameter of the nanoparticles and use this size while constructing the calibration curve. Additionally, ICP-MS or other elemental analysis method should be used to determine the total ionic concentration of the solution and estimate the concentration of the stock nanoparticle solution which will aid in determining the needed run dilution as well as verifying the concentration results.

3.3.2 To prepare the nanoparticle calibration standards, optimal nanoparticle flux, or rate that the nanoparticles hit the detector, needs to be determined. Two sources of instrumental uncertainty are dependent on the nanoparticle flux. Bias is introduced when sample flux is too high due to the increase frequency of multiple particles hitting the detector simultaneously. When this happens, it is difficult to distinguish between single and multiple particle events. Bias is related to the nanoparticle flux (f_{NP}) and the dwell time (t_{dwell}) by Equation 2 [2,3].

$$Bias = \frac{t_{dwell}}{2} \times f_{NP} \quad (2)$$

On the other hand, the relative standard deviation (RSD) decreases with increasing f_{NP} due to the larger total number of nanoparticles counted. Since the total NPs counted is proportional to the total time (t), their relationship with RSD is given in Equation 3 [2,3].

$$RSD_{NP} = \frac{1}{\sqrt{t \times f_{NP}}} \quad (3)$$

The target nanoparticle flux is determined from the point where the RSD is equal to the bias and is given by Equation 4 [3].

$$f_{NP} = \sqrt[3]{\frac{4}{t \times t_{dwell}^2}} \quad (4)$$

The number concentration and total dilution of the NP samples can be determined for each of the integration times according to Equation 5 [3].

$$N_{NP} = \frac{f_{NP} \times 60}{q_{liq} \times {}^{\eta_n}/_{100}} \quad (5)$$

Two additional factors must be determined before the number concentration in Equation 5 can be used to estimate the particle numbers: these are the flow rate (q_{liq}) and the nebulization efficiency (η_n). The flow rate is easy to manually measure as described in Section 3.1. The nebulization efficiency, however, must be determined experimentally after nanoparticle standards have been analyzed. Without prior knowledge or an estimate of the nebulization efficiency, it may be necessary to run single nanoparticle standard at a few dilutions to determine its response and use the response of a single dissolved standards of similar signal to obtain an estimate of the nebulization efficiency. The estimated particle flux and number concentration for particles in solution is given for 10 ms and 50 µs for an acquisition time of 100 s in Table 1. A nebulization efficiency of 0.1 and a flow rate of 0.285 mL/min were used to calculate the number concentrations in Table 1.

Table 2 gives a list of various sizes of Au NPs and the approximate total dilution necessary to achieve the number concentrations reported in Table

1 (based on the manufacturer supplied gold content of 0.01% gold chloride).

Table 1: Particle flux for SP-ICP-MS for dwell times of 10 ms and 50 µs for a 100 s acquisition.

t_{dwell}	f_{NP}	N_{NP} *(part./mL)*
10 ms	7.37	1.5×10^4
50 us	251	5.3×10^5

Table 2: Approximate dilution factors necessary for various sized Au NPs both calculated from Equation 5 (Calc), and actual run dilutions (Run).

Au NP Dia. (nm)	DF 50 µs	DF 10 ms
150	3,000	110,000
100	10,000	340,000
80	21,000	730,000
60	49,000	1,700,000
50	83,000	2,800,000
40	150,000	5,200,000
30	390,000	13,000,000

3.3.3 Serial dilutions should be utilized in order to make the proper run dilutions for each nanoparticle solution. The following dilutions will be made in 18 MΩ-cm resistant water. Total solution volumes will be 10 mL with the weights of all solutions substituted for volume. Conversion to volume units can be done by assuming a 1 mg/mL density of each solution. Typical dilutions will take place by first weighing an empty conical centrifuge tube. Then the sample solution is added to the tube and the weight of the tube and the stock is recorded. Finally, the diluent is added to the tube and the total weight is recorded. The dilution factor and concentration of each sample can be determined similar to the procedure described in sections 3.2.6. It is recommended that the minimum amount of sample to weigh out is 100 mg/ 100 µL of solution in order to ensure an accurate weight is obtained. Tables 3 and 4 give a summary of the serial dilutions targeting different dilution factors presented in Table 2. In the tables, the amounts for each solution are given in mL since it is easier to add amounts based on volume, but the weight of each solution should be recorded to ensure accuracy.

Table 3: Serial dilutions to achieve necessary dilution factors for SP-ICP-MS for 50 µs integration.

Solution ID (Sample Amount)	**Diluent Amount** (mL)	**Dilution Factor** (Au NP solution ID)
Stock (0.10 mL)	9.90 mL	1:100 (All)
1:100 solution (0.10 mL)	9.90 mL	1:10k (All except 150 nm; final dilution for 100 nm)
1:100 solution (0.33 mL)	9.67 mL	1:3k (150 nm)
1:10k solution (4.76 mL)	5.24 mL	1:21k (80 nm)
1:10k solution (2.04 mL)	7.96 mL	1:49k (60 nm)
1:10k solution (1.20 mL)	8.80 mL	1:83k (50 nm)
1:10k solution (0.67 mL)	9.33 mL	1:150k (40 nm)
1:10k solution (0.26 mL)	9.74 mL	1:390k (30 nm)

Table 4: Serial dilutions to achieve necessary dilution factors for SP-ICP-MS for 10 ms integration.

Solution ID (Sample Amount)	**Diluent Amount** (mL)	**Dilution Factor** (Au NP solution ID)
Stock (0.10 mL)	9.90 mL	1:100 (All)
1:100 solution (0.10 mL)	9.90 mL	1:10k (All)
1:10k solution (0.87 mL)	9.13 mL	1:110k (150 nm)
1:10k solution (0.28 mL)	9.72 mL	1:340k (100 nm)
1:10k solution (0.14 mL)	9.86 mL	1:730k (80 nm)
1:10k solution (0.10 mL)	9.90 mL	1:1M (30, 40, 50, 60 nm)
1:1M solution (5.78 mL)	4.22 mL	1:1.7M (60 nm)
1:1M solution (3.57 mL)	6.43 mL	1:2.8M (50 nm)
1:1M solution (1.92 mL)	8.08 mL	1:5.2M (40 nm)
1:1M solution (0.77 mL)	9.23 mL	1:13M (30 nm)

3.4 Preparation of Unknown Samples

Unknown samples should be diluted as outlined in section 3.3.3. To determine the amount of dilution for each sample, the particle concentration can be estimated using the size of the nanoparticle from a different method (DLS, TEM, etc.) and the total ionic concentration of the analyte in solution according to Equation 6.

$$N_{NP,estimate} = \frac{3 \times [Analyte\ Ion]}{\rho_{NP} \times \pi \times r^3 \times 4 \times 1x10^{-21}} \quad (6)$$

Where ρ_{NP} is the density and r is the radius of the NP. Once the particle concentration has been estimated, the necessary dilution can be calculated using Equation 5 and the solutions should be made as outlined in 3.3.3.

3.5 Measurement Procedure

3.5.1 Load the diluted samples into the auto sampler of the ICP-MS instrument. The samples should be loaded in increasing concentration (lowest to highest) for the dissolved standards, and smallest to largest diameter for the NP standards and unknowns.

3.5.2 While the instrument is warming up, move the sampling tube into a vial containing your dilution buffer for the nanoparticles. Measure the flow rate as outlined in Section 3.1.

3.5.3 (Optional) If extra sensitivity is needed for an analyte, move the sample tubing to a vial containing a dilute solution of the analyte (~50 ng/g) and tune the instrument to maximize the response of the analyte.

3.5.4 Measurements Using Dedicated SP-ICP-MS program

3.5.4.1 (If your instrument has a dedicated single particle mode): Open single particle application/ module.

3.5.4.2 And set up the method for either a 50 µs or 10 ms integration run.

3.5.4.3 Enter the total run time. The calculations for the data tables in Sections 3.3.2-3.3.3 were done using a run time of 100 ms. If you choose to use a different run time, the flux, nanoparticle run

concentration, and total dilutions will need to be recalculated using the formulas found in Section 3.3.2.

3.5.4.4 Choose the element corresponding to the NP and choose the specific isotope to be analyzed depending on abundance and possible interferences.

3.5.4.5 Create a sample sequence. Run at minimum of three buffer blanks before any samples have been run to get a stable background signal and make sure there is no residual analyte signal. Additionally, while setting up the sequence, it is advisable to run at least three buffer blanks after each of the calibration sets (dissolved and NP) in order to remove any analyte carryover. Run at least two buffer blanks between each sample.

3.5.4.6 Run the sequence.

3.5.5 (Alternate Method) If your ICP-MS does not have a dedicated SP-ICP-MS mode, you can make single particle measurements by collecting the data using the time resolved analysis mode (chromatography mode). To do this simply set up the timed resolved analysis for your total measurement time.

3.5.5.1 Create a time resolved analysis method to measure your analyte and set the integration time to 10 ms.

3.5.5.2 Set up and run the sequence as outlined above.

4. Data Analysis

4.1 Analysis of raw data.

4.1.1 You may perform the data analysis using either the built in SP-ICP-MS program, or using your own analysis template. The following data analysis description will describe manual data processing and analysis using a custom R script, but the basic procedure can be applied to manually analyzing the data in any other data processing or spreadsheet application as long as you have access to the raw data.

4.1.2 If your instrument does not have a dedicated single particle mode to analyze your data, your only option will be to manually analyze the raw data.

4.2 Determine the average and standard deviation (SD) of the buffer blanks to determine limits of detection (LOD) for peak intensity.

4.2.1 Determine the average signal for each buffer blank run during the experiment by taking the average value of all data points in each of the spectra and average them together. An example of the signal collected for a Buffer (Blank) sample is given in Figure 1.

4.2.2 Determine the standard deviation of each buffer blank and average them together.

4.2.3 Determine the limit of detection for the peak intensity. The limit of detection can be calculated by Equation 7.

$$LOQ = 3 \times SD \quad (7)$$

4.3 Determine the average and standard deviation of the signal duration for the buffer blanks to determine the LOD of the signal duration

4.3.1 Identify the peaks in the background signal by considering any non-zero intensity to be a peak, where consecutive non-zero intensities will be counted as a single peak.

4.3.2 The duration of the peak can be determined by counting the consecutive non-zero values (as illustrated by the drop lines in the inset in Figure 1). To convert to duration in seconds, multiply the total number of consecutive points by the integration time.

4.3.3 Determine the average signal duration for each buffer blank by averaging the duration of each signal spike and average them together.

4.3.4 Determine the standard deviation of the signal duration for each buffer blank and average them together.

4.3.5 Determine the limit of detection for the signal duration of the buffer blanks as outlined in Section 4.2.3.

4.4 Construct the dissolved standard calibration curve

4.4.1 Determine the average signal intensity of each of the dissolved nanoparticle standards. A typical spectrum of a dissolved standard is given in Figure 2.

4.4.2 Plot the average signal intensity as a function of the mass if analyte hitting detector during the integration period. The mass hitting the detector can be calculated by converting the concentration to mass per second and multiplying by the integration time. Equation 8 illustrates how this calculation is carried out.

$$\frac{Au\ ng}{sol\ g} \times \frac{1x10^{-9} g\ Au}{1\ ng\ Au} \times \frac{\rho_{sol} g}{mL} \times \frac{q_{liq} mL}{min} \times \frac{1\ min}{60\ sec} \times 5x10^{5}\ sec \quad (8)$$

4.4.3 Perform a linear regression on the plotted points to determine the dissolved standard slope. Figure 3 gives a typical calibration plot constructed from the dissolved standard data.

4.5 Construct the NP standard calibration curve

4.5.1 Isolate the individual nanoparticle signals from the raw data

4.5.1.1 Figure 4 demonstrates the raw signal from a 100 nm Au NP. Peaks associated with the NP need to be isolated with peaks caused to do random signals generated at the detector. To do this, a two-step thresholding of the data will be applied to filter out background noise.

4.5.1.2 First, filter out all the signal intensities that are not larger than the LOD for the signal intensity of the buffer blanks by setting those values below the LOD to zero.

4.5.1.3 (For 50µs measurements) Next, filter out any signals that have a duration smaller than the LOD for the signal duration of the buffer blanks by setting those values below the LOD to zero. This does not need to be done for the 10 ms measurements since the duration of each event is a single data point.

4.5.1.4 Filtered data should now be composed of a zero baseline with nanoparticle peaks appearing as the non-zero values. Figure 5 gives an example of what the final filtered data will look like for

the 50 µs measurements. In the table there are zeroes preceding and following the nanoparticle peak, and the peak itself consists of consecutive non-zero values which correspond to the ion cloud hitting the detector over a period of time. A graph of the resulting peak is given to the right of the table. For 10 ms measurements there will only be single non-zero peaks due to the integration time being much larger that the duration of the ion cloud.

4.5.2 (For 50 µs measurements) Sum the intensities of each of the non-zero intensities for each peak/ nanoparticle event. This will be used as the signal for each nanoparticle and used to construct a histogram for each nanoparticle standard.

4.5.3 (For 10 ms measurements) Use the single point intensity for each peak as the nanoparticle signal to using while constructing a histogram for each nanoparticle standard.

4.5.4 Construct a histogram of the signal intensity for each of the nanoparticle standards.

4.5.5 Fit the histogram using a gaussian fit and determine the center max height of the histogram for each nanoparticle standard. This will be used as the NP signal while constructing the nanoparticle calibration curve. A typical histogram with the corresponding gaussian fit is given in Figure 6.

4.5.6 Convert the known diameter of each nanoparticle standard to mass per particle. Plot the signal of each nanoparticle as a function of the mass of the particle. Fit using a linear regression and determine the slope of the line. By plotting the calibration curve as a function of the mass of the particle the calibration plot will be linear. Alternatively, the cubic root of the signal can be plotted vs diameter to also obtain a linear plot. An example of a calibration curve constructed from the nanoparticle standards is given in Figure 7.

4.6 Determine the Nebulization Efficiency

4.6.1 Divide the dissolved standard slope by the nanoparticle standard slope to get the nebulization efficiency. For example, using the calibration curves in Figures 3 and 7, the slopes for each linear regression are 3.88×10^{15} and 6.12×10^{16} for the dissolved standard curve and nanoparticle standard curve, respectively. This gives a transport efficiency of 6.3% (0.063)

4.7 Process and determine the signals for the unknown particles

4.7.1 Isolate the individual NP signals from the background according to sections 4.5.1.1-4.

4.7.2 (For 50 µs measurements) Sum the intensities of each of the non-zero intensities for each peak/ nanoparticle event. This will be used as the signal for each nanoparticle and used to construct a histogram for each nanoparticle standard.

4.7.3 (For 10 ms measurements) Use the single point intensity for each peak as the nanoparticle signal to using while constructing a histogram for each nanoparticle standard.

4.7.4 Construct a histogram of the signal intensity for each of the nanoparticle standards.

4.7.5 Convert the Nanoparticle intensity histogram to a histogram of size using the procedures outlined below in Section 4.8.

4.8 Determine the sizes of the unknown NPs

4.8.1 (Method 1; NP calibration; known density) Using nanoparticle calibration curve linear regression formula, convert the histogram to diameter in nm.

4.8.1.1 Convert the intensity signal to mass of analyte per particle using the nanoparticle calibration curve linear regression formula.

4.8.1.2 Using the density of the material, convert the mass per particle to volume per particle in nm^3.

4.8.1.3 Convert the volume of the nanoparticle to diameter using the formula for the volume of a sphere.

4.8.2 (Method 2; NP calibration; unknown density) Using nanoparticle calibration curve linear regression formula, convert the histogram to diameter in nm.

4.8.2.1 Construct a calibration curve of the cubic root of the intensity as a function of NP diameter.

4.8.2.2 Convert the cubic root of the measured intensity for the unknown NP to a size in nm using the linear formula from the linear regression

4.8.3 (Method 3; dissolved calibration; known density) Using dissolved standard calibration curve linear regression formula, convert the histogram to diameter in nm.

4.8.3.1 Convert the signal of the unknown sample to mass per nanoparticle using the dissolved standard calibration curve

4.8.3.2 Multiply this mass by the transport efficiency.

4.8.3.3 Using the density of the material, convert the mass per particle to volume per particle in nm^3.

4.8.3.4 Convert the volume of the nanoparticle to diameter using the formula for the volume of a sphere.

4.9 Determine the particle concentration of the unknown NP solution

4.9.1 Count the total number of nanoparticle events during the measurement.

4.9.2 Convert the counted number of particles to a particle concentration by using Equation 9. Where CNP is the undiluted particle concentration in particles per mL, n_{NP} is the number of particles counted, t is the total time in seconds, q_{liq} is the flow rate in mL/ min, and η_{neb} is the nebulization efficiency measured in Section 4.6.

$$C_{NP} = \left(\frac{n_{NP} \times 60}{t \times \eta_{neb} \times q_{liq}}\right) \times \text{Dilution Factor} \quad (9)$$

5. Precautions and Guidelines

5.1 All safety precautions per your lab SOPs should be followed for the nanomaterial that is being tested. At a minimum, one should wear a lab coat, gloves and protective goggles and work in a biological safety cabinet or a chemical fume hood to minimize exposure to the worker.

5.2 Dilutions used in SP-ICP-MS are often very large and can affect the stability of the nanoparticle. Figure 8 illustrates the sample stability of the diluted nanoparticle solutions over a six-day period. Samples should be used same day for concentration analysis and new samples should be prepared for each measurement. Size is not noticeably impacted over the 6-day period, and stale solutions will still give an accurate size analysis. Additionally, a stabilizing surfactant may be added to the NP solution provided there is ample evidence that it does not increase agglomeration of the sample. Due to the nature of the SP-ICP-MS measurement, the surfactant should not interfere with the measurements.

5.3 As stated in Section 3.3 and 3.4, it is necessary to ensure that the NP samples are sufficiently dilute to make accurate measurements for both size and concentration. Figure 9 demonstrates this importance by looking at the 150 nm Au NP at three different concentrations: 100k, 10k, and 1k. In Figure 9, the overlapping histograms show a consistent peak center regardless of concentration, demonstrating that accurate determination of nanoparticle sizes is still possible even at concentrations in which bias is relatively high. The main issues caused by concentrations too high are the inability to determine if the sample is monodisperse or if the multiple peaks are due to distinct nanoparticle populations, and how to deconvolute the overlap in the peaks to accurately determine the total number of particles measured which is necessary for determining the particle concentrations.

5.4 When making 50 µs measurements, the use of the summed intensity over the peak is used in place of the max height of the peak. However, both can be used to give an accurate determination of the diameter of the nanoparticle. While using the max height to determine the size of the nanoparticles the distribution of particles will be much wider than that of the summed intensity. This is due to the "flattening" of the peak signal caused by the spread of the ion cloud as it moves to the detector and is exacerbated by integration times shorter than 50 µs. This effect is demonstrated in the graphs below in Figure 10.

5.5 Detector saturation is a concern when running SP-ICP-MS measurements especially for 50 µs integration times and larger particles (~ > 150 nm) due to

saturation of the pulse detector. This effect can be demonstrated in Figure 11 where the signal from NPs larger than 150 nm begin to deviate from a linear response.

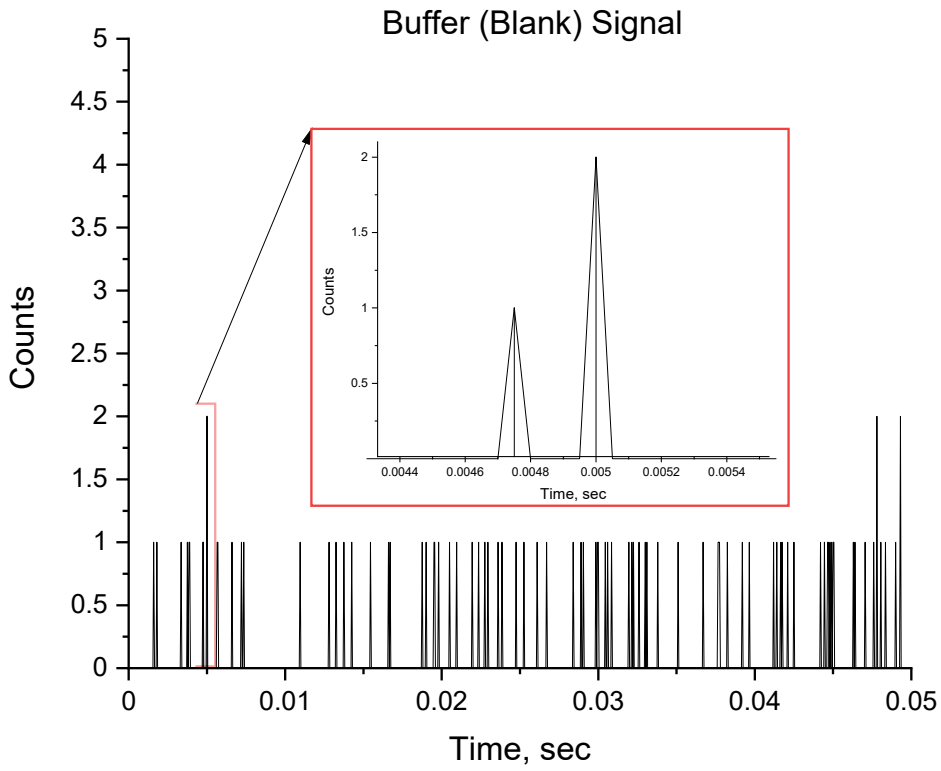

Figure 1: Buffer (Blank) signal obtained during a 50 µs integration SP-ICP-MS acquisition. The inset shows a blown up portion of two peaks to show the width of the duration of the spikes in background signal, with the number of dropdown lines showing the number of consecutive non-zero values for each peak.

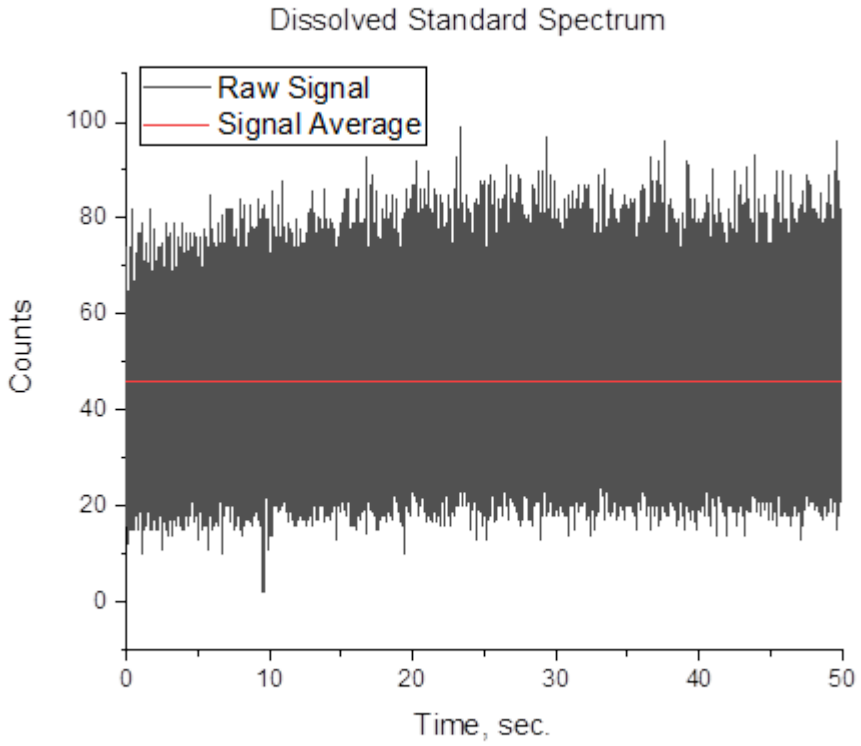

Figure 2: Dissolve standard spectrum for a 50 ppb dissolved gold standard to illustrate how the counts are determined for calibration. Because the signal of the dissolved standards is constant, there will be no peaks like in a discrete event. As the concentration of the dissolve standard increases, so does the height of the signal (both the max and min signal). By taking the average of this signal, indicated by the red line, the dissolved calibration curve can be constructed as average signal as a function of the mass of the calibrant (g Au) hitting the detector during the integration window.

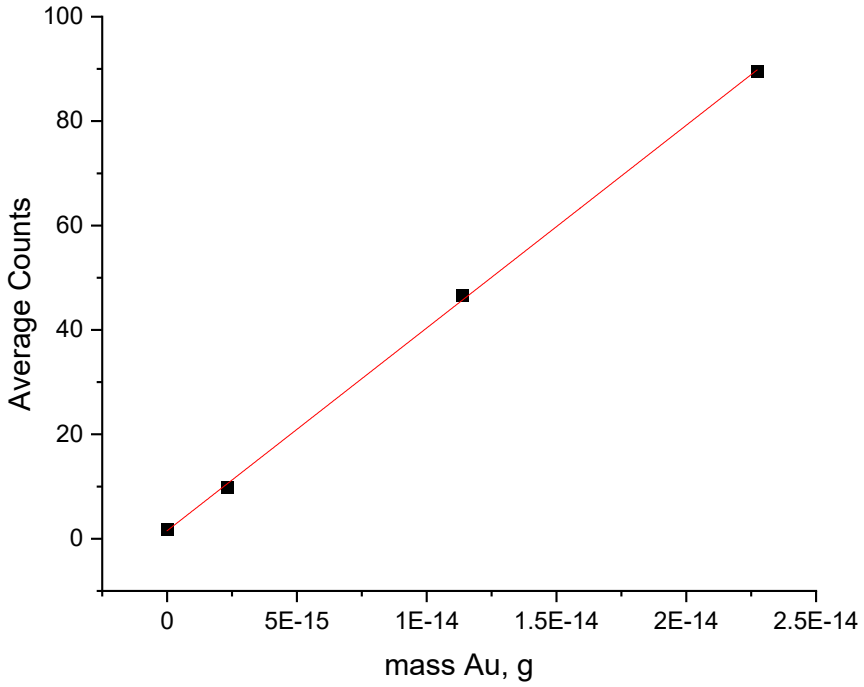

Figure 3: Calibration curve resulting from using the average signal as a function of the mass of the dissolved standard hitting the detector during a 50 µs integration time. The red line is the linear fit of the data showing that the points are linear.

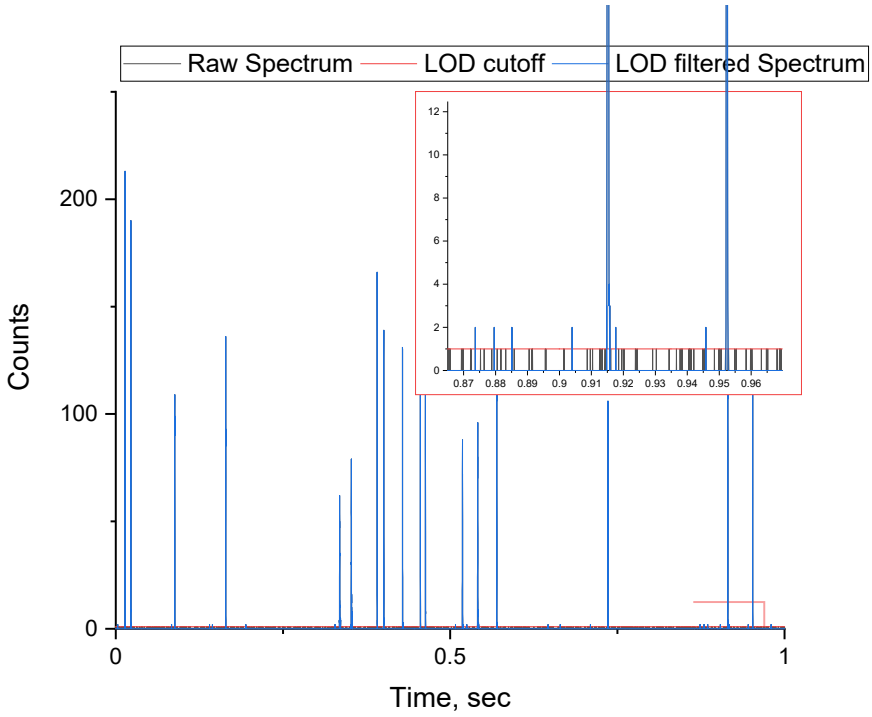

Figure 4: Typical spectrum of a 100 nm Au NP as measured by SPC-ICP-MS with a 50 µs integration time. The black trace represents the raw data, the red line represents the LOD cutoff used in the first filter pass, and the blue trace is the data filtered after removing the peaks that are below the limit of detection. The inset shows a zoomed view of the graph focusing on the low count region. As can be seen, there remains low count peaks even after filtering using the limit of detection. These peaks however last less than a few data points and can be removed by filtering against the LOD of the background peak duration.

5.00E-05	0
1.00E-04	0
1.50E-04	0
2.00E-04	0
2.50E-04	0
3.00E-04	0
3.50E-04	4
4.00E-04	5
4.50E-04	10
5.00E-04	40
5.50E-04	74
6.00E-04	119
6.50E-04	131
7.00E-04	127
7.50E-04	95
8.00E-04	57
8.50E-04	37
9.00E-04	18
9.50E-04	6
0.001	4
0.00105	0
0.0011	0
0.00115	0
0.0012	0
0.00125	0
0.0013	0
0.00135	0
0.0014	0
0.00145	0
0.0015	0

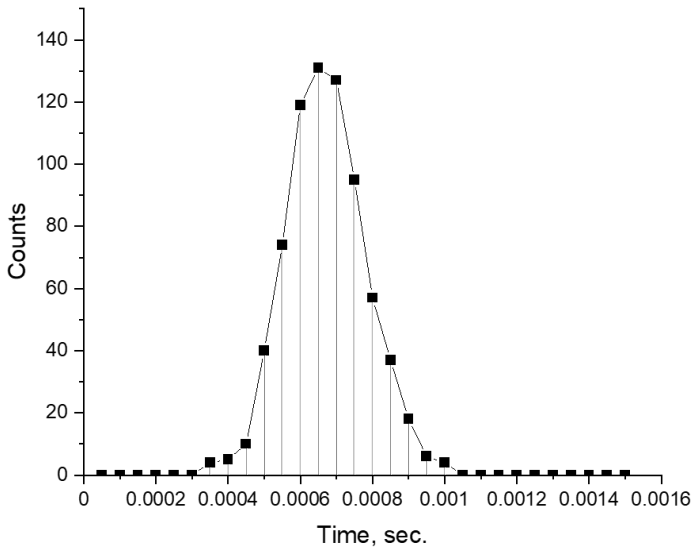

Figure 5: Final filtered data for a 100 nm Au NP obtained using 50 µs integration time using SP-ICP-MS. The signal of the peak appears as consecutive non-negative values bracketed by zeroes. The total signal of the peak is obtained by summing all of the non-zero values. The duration of each peak can be obtained by counting the consecutive non-zero values and multiplying that number by 50 us.

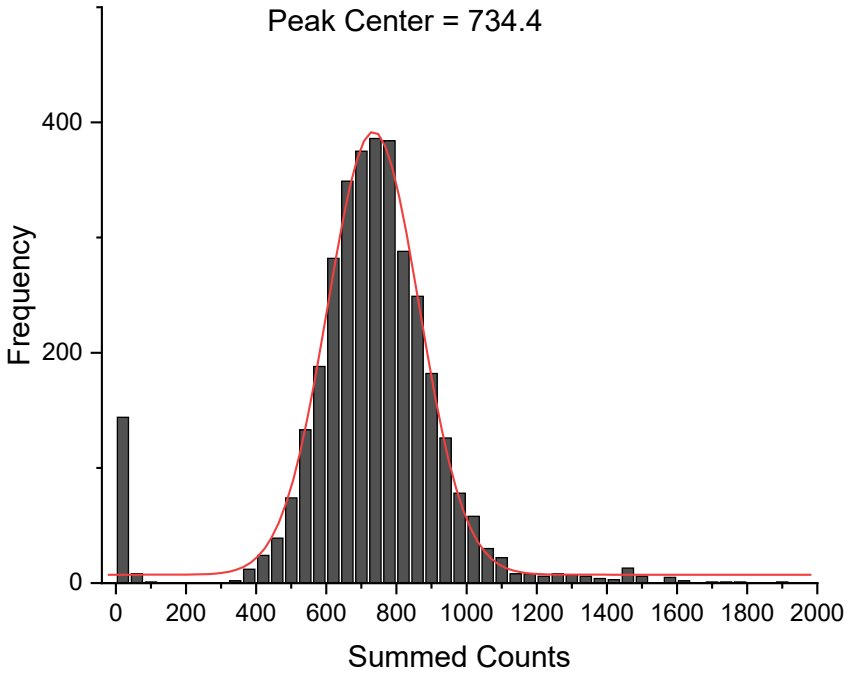

Figure 6: Histogram for the summed peak intensities for the 100 nm Au NP. The red trace is the gaussian peak fit for the peak. The fitted peak is centered at 734.4 counts. The counts at the center of the fitted curve are used to construct the NP calibration curve by plotting the peak center values as a function of the mass of the nanoparticle.

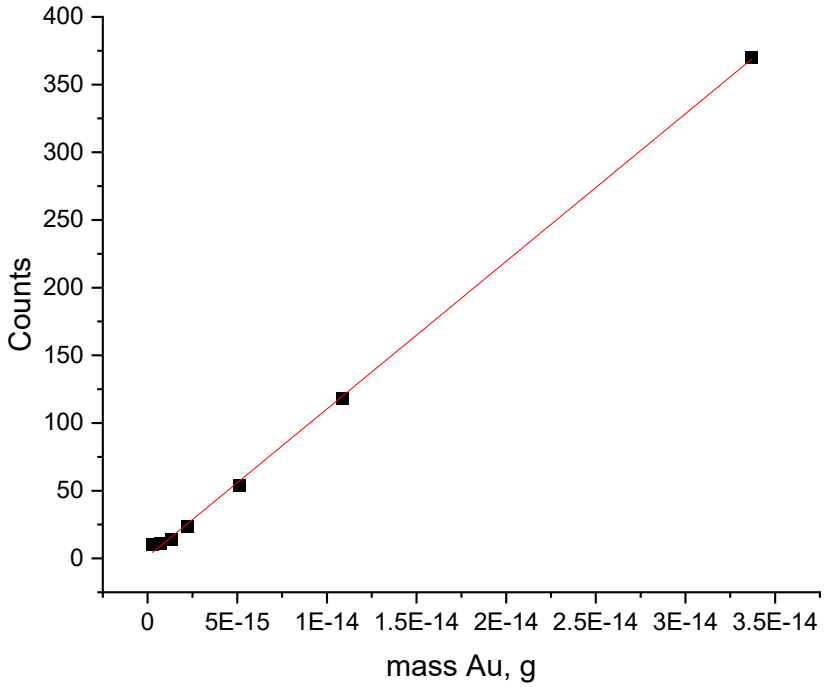

Figure 7: Calibration constructed from the nanoparticle calibration standards. The calibration is constructed from the summed intensity of the NP peaks as a function of the mass of the nanoparticle. The mass of the nanoparticle is determined from the reported diameter of the Au nanoparticles, and converted to a mass by converting the diameter to a volume per nanoparticle and using the density of Au to further convert that to a mass per nanoparticle. The red line is the linear fit of the data showing that it is linear up to 150 nm Au particles.

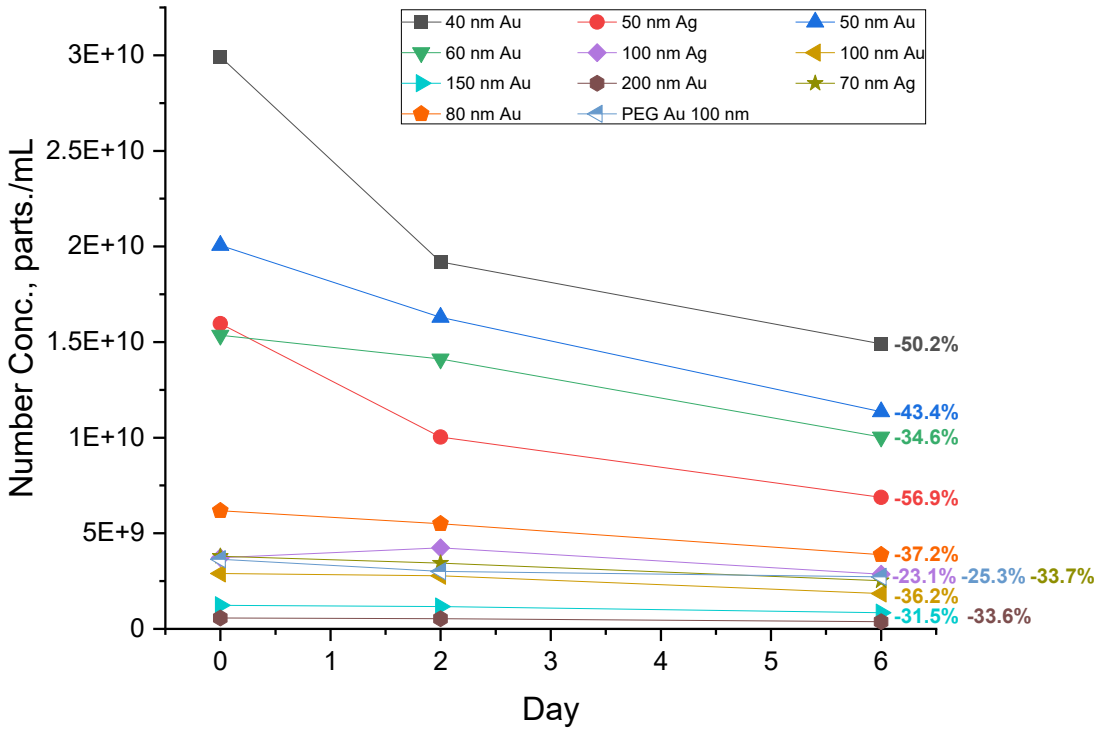

Figure 8: The six-day stability of the diluted nanoparticles is plotted in the graph and with each color indicating each different NP solution. The percent change is indicated by the color-coded number to the right of the day 6 data point. In all instances a decrease in the stability of the nanoparticles was observed showing a decrease in number concentration ranging from ~ 23-57% over the six-day period.

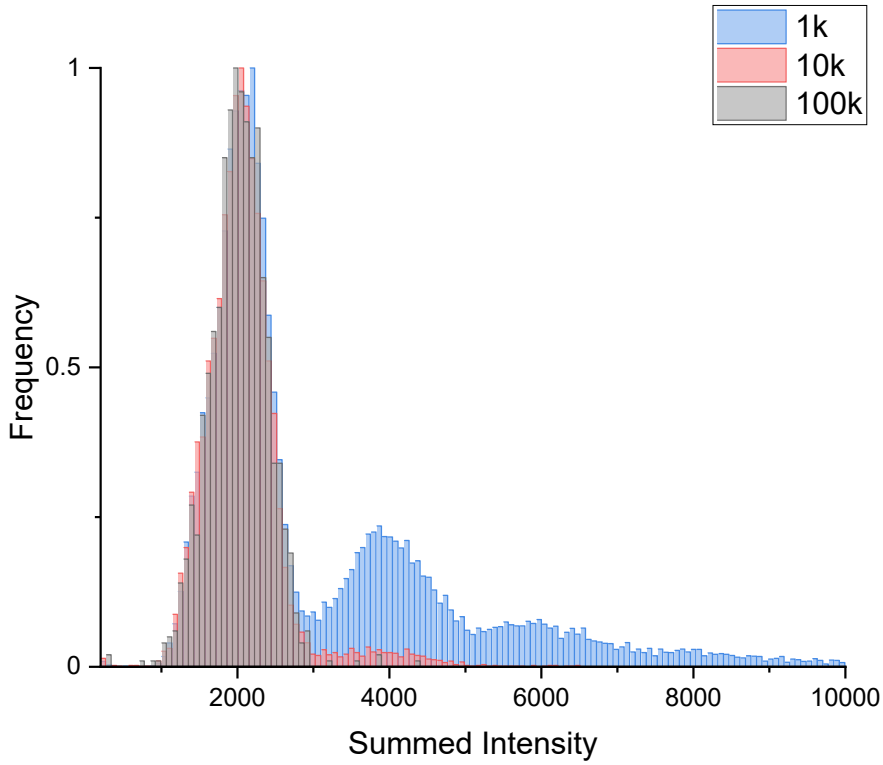

Figure 9: Demonstration of the concentration effect on the ability to detect nanoparticle using SP-ICP-MS. Displayed are three histograms of summed intensity of measured nanoparticle events for 150 nm Au NPs. At 100k dilution (black histogram) only a single distribution is observed for the nanoparticles and demonstrates what a properly diluted NP sample should look like. When the concentration is increased 10-fold to 10k dilution, (red histogram), a new peak appears at exactly 2x the summed intensity of the peak corresponding to the single particle events. This second peak corresponds to 2 particles hitting the detector simultaneously. As the concentration is increased 100-fold over the 100k dilution to 1k (blue distribution), this effect is exaggerated with additional peaks corresponding to 2, 3, and 5 NPs hitting the detector simultaneously.

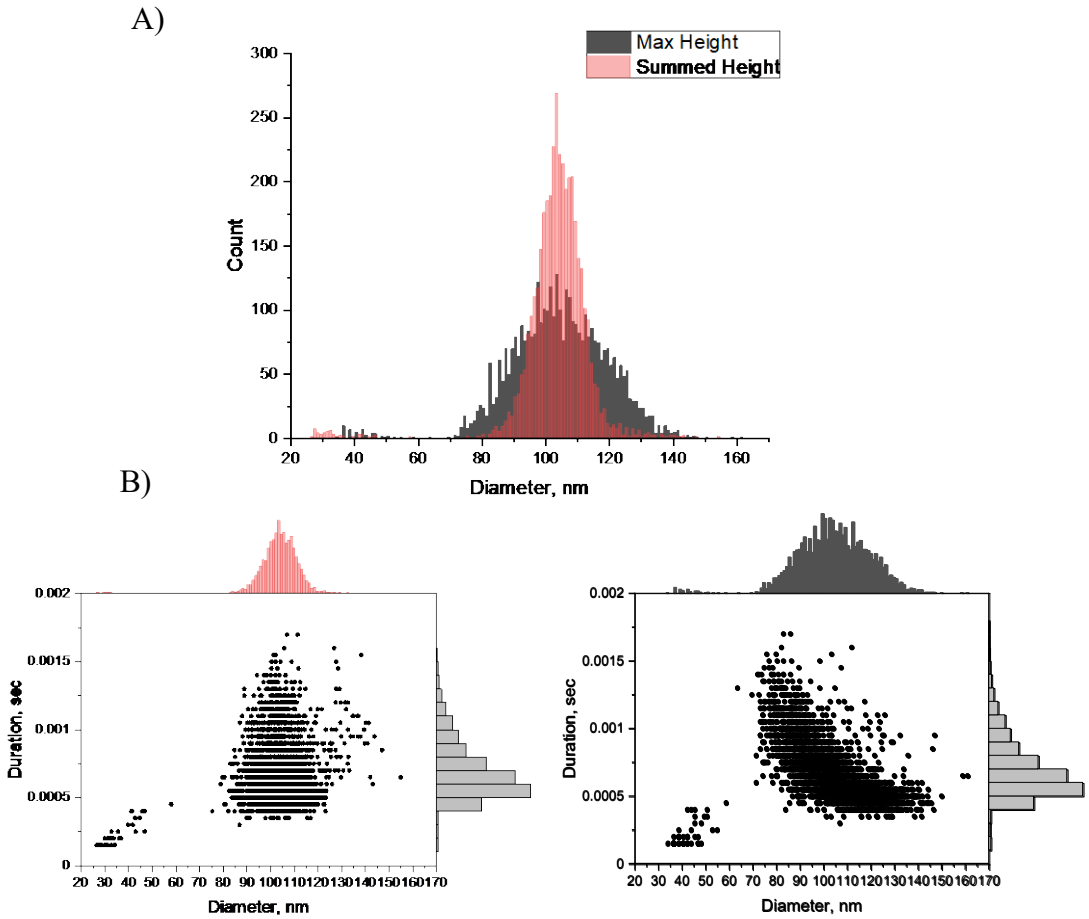

Figure 10: Plots showing the effects of ion cloud spread on the distributions of measured nanoparticles. In Figure A, histograms constructed from the max height of particle peak (grey) and plotted alongside a histogram constructed of the summed heights of the particle peak (red) for the same data with the same number of bins. It is evident that the distribution for the summed height is tighter than the max height histogram. In B) the same histograms (x-axis) are plotted in a 2-D scatter plot with the duration of the nanoparticle peak (y-axis). For the summed height histogram, the duration largely coincides with the size histogram showing an even distribution of durations over the different sizes. In contrast, the max height versus duration plot show a definite skew in both the lower and upper size limit, where most of the larger duration peaks correspond to smaller max intensities while smaller durations are more strongly correlated with larger nanoparticle sizes.

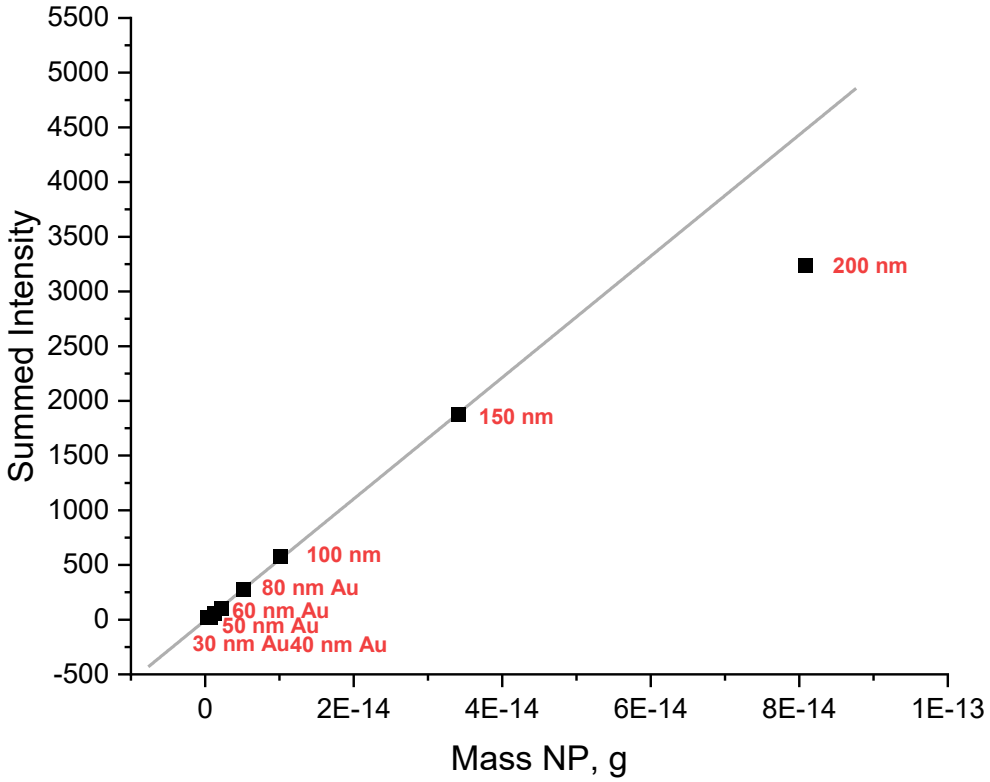

Figure 11: The summed intensity counts are plotted as a function of the mass of each nanoparticle. The nanoparticles from 30 nm to 150 nm are linear in response and the linear regression line for these particles is plotted on the graph in grey. Beyond 150 nm the response is no longer linear and can be seen by the 200 nm Au NP which lies well below the linear fit.

6. Waste Disposal

Follow your facilities recommended procedures for waste disposal of nanoparticle and acidic solutions.

7. References

1. Degueldre, C.; Favarger, P. Y.; Wold, S., Gold colloid analysis by inductively coupled plasma-mass spectrometry in a single particle mode. Analytica Chimica Acta 2006, 555 (2), 263-268.
2. Laborda, F.; Jiménez-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. Journal of Analytical Atomic Spectrometry 2013, 28 (8).
3. Murphy, K. E.; Liu, J.; Montoro Bustos, A. R.; Johnson, M. E.; Winchester, M. R. Characterization of nanoparticle suspensions using single particle inductively coupled plasma mass spectrometry; NIST SP 1200-21; National Institute of Standards and Technology: 2016.

8. Abbreviations

C_{NP}	concentration of nanoparticle solution
f_{NP}	nanoparticle flux
HCl	hydrochloric acid
HNO_3	nitric acid
ICP	inductively coupled plasma
LDPE	low density polyethylene
LOD	limit of detection
MS	mass spectrometry
M_{STD1}	concentration of standard before dilution
M_{STD2}	concentration of standard after dilution
NIST	National Institute of Standards and Technology
n_{NP}	number of nanoparticles

$N_{NP,est}$	estimated nanoparticle number concentration
NP	nanoparticle
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
q_{liq}	flow rate
r	radius
RSD	relative standard deviation
SD	standard deviation
SOP	standard operating procedure
SP	single particle
SRM	standard reference material
t	total time
t_{dwell}	dwell time
W_{STD1}	weight of standard added
W_{TOT}	total weight of solution
η_n	nebulization efficiency (%)
ρ_{NP}	density of the nanoparticle