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Vitesse Note **NT04**

Optimizing instrumental, data collection and signal processing parameters for single particle analysis of nanoparticles in real samples using the Vitesse time of flight ICP-MS

- **Signal profile measured for each nanoparticle**
- **Skewed peaks minimised by reaction cell conditions**
- **Peak detection parameters per isotope**
- **Particle data quality tests available for**
  - o Element coincidence check
  - o False peaks due to baseline artifacts
  - o Particle overlaps
  - o Particle signal skew

Introduction

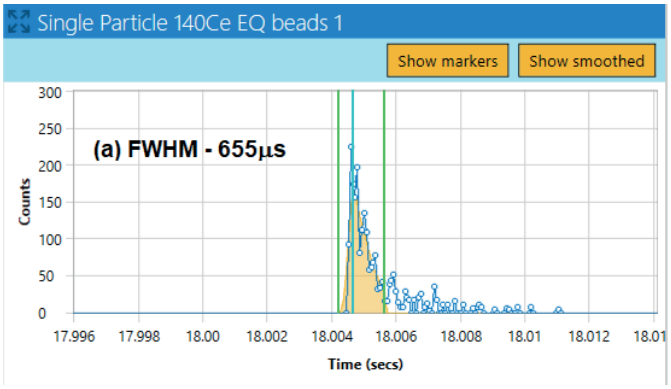
ICP-MS has proven to be a powerful analytical tool for the measurement of nanoparticles using the Single Particle mode [1]. Vitesse applications note NT01 describes the use of ICP-TOF-MS to measure multiple elements in individual nanoparticles and then generate useful representations of elemental distributions in different ranges of particles. To improve the quality of data reported, measurements need to be filtered to remove falsely identified signals, falsely identified element coincidences within particles and errors in particle size and particle number reporting that can arise from signal overlaps. This application note will describe the tools provided with the Nu Quant data processing software to improve the quality of single particle ICP-MS measurements.

Interference removal

The Vitesse uses an always on reaction cell, pressurised with 3-10 sccm of He and 3-7 sccm H_2 gas. This is a common gas mixture used with quadrupole ICP-MS to remove argon and nitrogen based interferences but further optimisation of the setup parameters is required for both time of flight ICP-MS and single particle mode analysis for nanoparticles compared to a conventional quadrupole ICP-MS performing routine analysis.

A pressurised reaction cell is most effective at removing interferences when the ion beam is slowed to near thermal energies[2]. However, under near thermal conditions a significant loss of ions can be seen due to collisional scattering which effects lower masses more than higher masses. The use of a small accelerating field gradient, by incrementally changing the voltage between the sections of the segmented hexapole assembly, reduces the losses for low mass elements and a field strength of between 0.1 and 0.3 V/cm was found to be optimal for the gas conditions used.

For nanoparticles, other researchers have observed that reaction cells can broaden the signals seen from individual particles as the ion packets are slowed in the reaction cell and ions diffuse in the beam[3]. Figure 1 shows the effect of reaction cell optimisation on the cerium signal when aspirating EQ calibration beads (Fluidigm, USA).



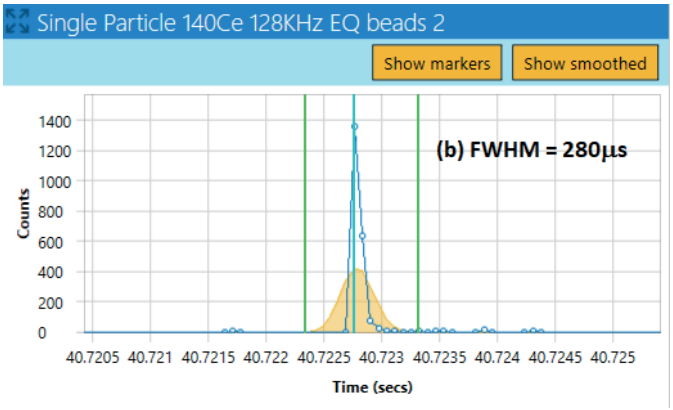


Figure 1a: showing the measurement of cerium contained in EQ calibration beads. Figure 1(a) shows the widening effect of using non-optimised reaction cell conditions and figure 1b shows the same sample once gas flows and axial field voltages are optimised for nanoparticle peak shapes.

Particle detection and element coincidence algorithms

The peak detection algorithm used for Vitesse data is the same as was previously reported in work on the Attom ES (1). However, to reduce the data processing resources required to conduct a peak search for every isotope measured by TOF-ICP-MS, Nu Quant allows target isotopes to be selected with each of these isotopes having individual parameters for signal smoothing and particle detection thresholds. This provides a higher level of flexibility so finding moderately sized particles above the ionic background noise for one isotope can be accommodated by extra smoothing without impairing the signal to background of a different isotope where there is minimal background and the particles are much smaller. The complete mass range of integrated isotopes per spectrum are maintained within the database of the experiment so further optimisation and reprocessing of particles is possible to look for additional target isotopes and alternate smoothing conditions as required.

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Once particle events have been found for each target isotope, the software generates a list of times for peak maxima with the start and end inflexion points found by the algorithm along with the measured full width half maximum values for the smoothed signals. The next processing step determines whether found peaks are individual particles or the same particle which contains different isotopes from the target set. By setting a value for a minimum expected peak width, the software can determine if peak maxima for different isotopes occur within this minimum peak width and if they do, the earliest start and latest end inflexion points found are then used to collate data for a single merged particle. Figure 2a shows the overlay of 56 Fe and 197 Au signals for a mixed nanoparticle sample where two different particles are almost coincident. Figure 2b and 2c shows the effect of changing the expected peak width from to 500 µs to 800 µs. If an appropriate width is not selected, coincidence is reported when they should be separate particles.

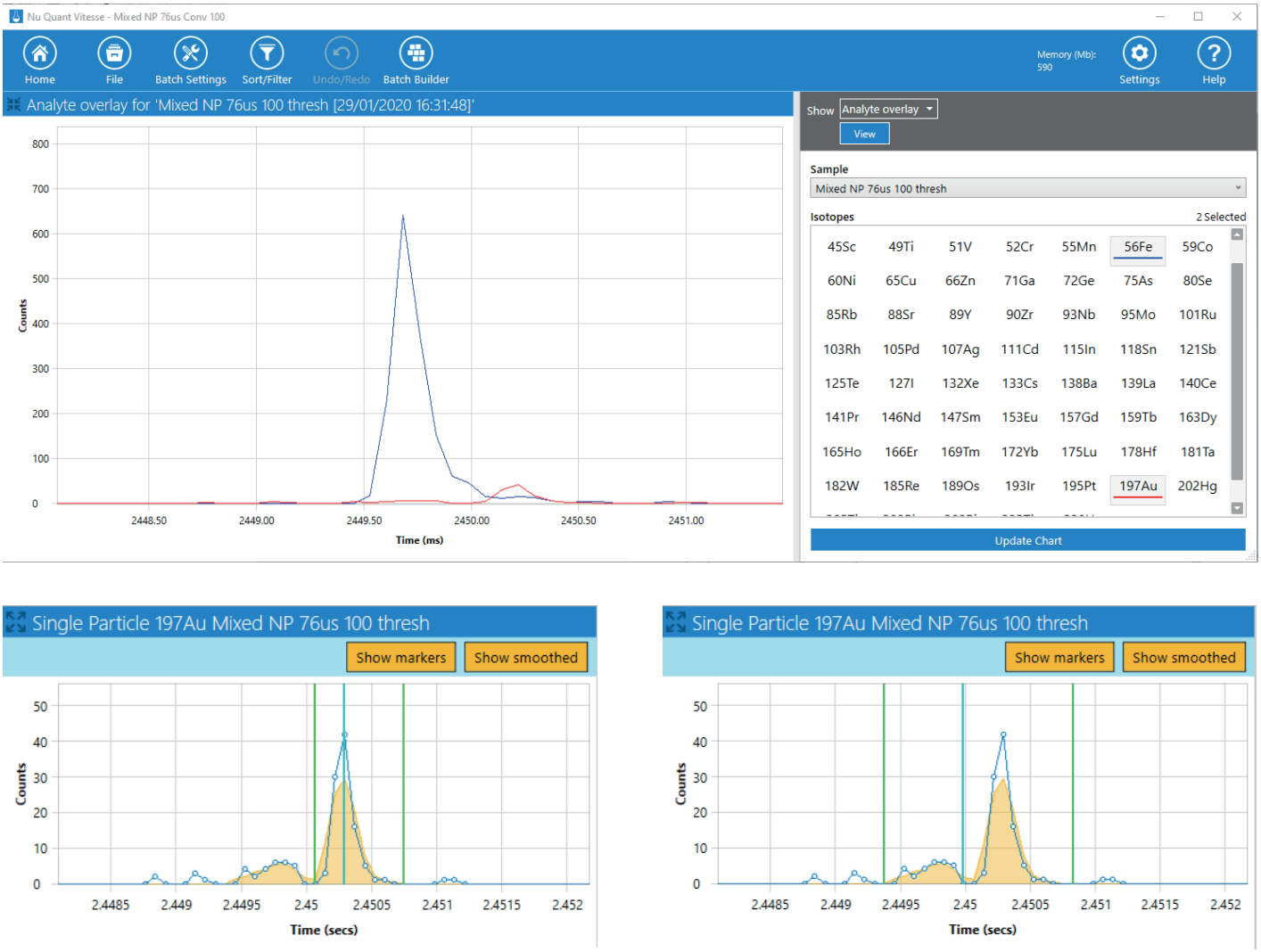

Figure 2a: showing overlays for Au and Fe for the same signal times, b: showing the correct idenitfication of the second Au only particle when 500 µs is used for a peak width and c: shows how the major peak for the Fe and the second smaller Au peak are merged in the reporting when the expected peak width is set too high.

Once the isotope coincidences have been determined, there is a single compiled list of peaks (maxima, start and end times and FWHM) for the sample. All collected data that is not included in one of the peaks is used to determine the background and standard deviation of the background for each isotope to be used in later calculations. All measured isotopes are then summed for the peaks between each start and end point and the background counts subtracted. This produces a list of isotope counts for each particle found along with the ionic backgrounds for each isotope and the peak meta data which is all available within the Nu Quant software python scripts and can be used for further data evaluation.

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Methods of validating data quality

A typical sample analysis requires the measurement of several hundred particles to provide a useful statistical distribution of particle masses or composition. To maintain accurate determinations of numbers of particles it has been established that samples need to be sufficiently diluted to prevent significant particle overlaps[4], classically analysts diluted samples to obtain <100 particles per second which would normally ensure that particle coincidences were only a few percent. With this experimental setup, it is possible to evaluate the degree of particle coincidence (as opposed to isotope coincidence within a particle) by comparing the start and end inflexions of peaks found by the search algorithm. A Python script is used to compare the start and end of each particle and report the number of adjacent peaks as a percentage of the total particle peaks found. Figure 3 shows an example where two particles are adjacent, they can be easily separated by the search algorithm and reported accurately but the proportion of adjacent particles is a good quality indicator of when a sample may need diluting.

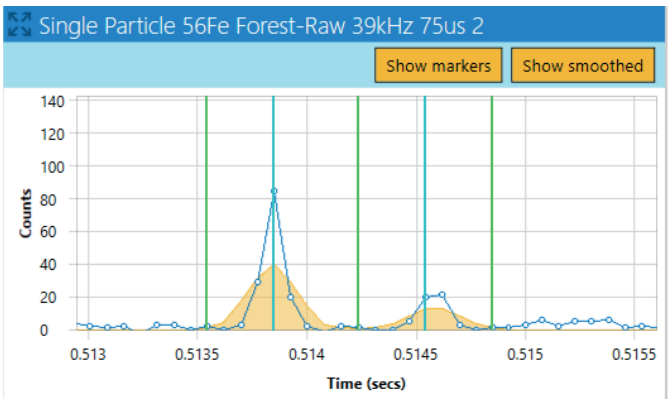

Figure 3: showing two adjacent particles which share start and end inflexion points

As the full width half maximum is calculated for each target isotope in each particle, it is also possible to use this combined with the start and end points to indicate when a higher level of particle coincidence has started to lead to particle signals overlapping. In many cases the particle signals can still be integrated but the number of particles where the full width cannot be measured at the half maximum is reported as the percentage of overlapping peaks and an option is provided for the user to choose whether to include these particles in further reporting or not. Figure 4 shows an instance where a larger particle has swamped a neighbouring one such that the FWHM can not be reported but the particle can be integrated with reasonable accuracy.

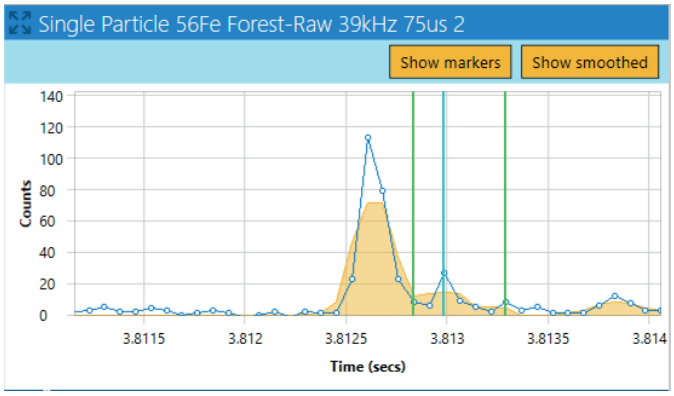

Figure 4: showing a small peak next to a larger peak which can still be identified and integrated but is indicative of a possible need to dilute the sample and rerun it.

When there is a significant ionic background signal for an isotope in nanoparticles of interest, the background is often irregular and can lead to artefacts that can be mis-identified as particles. If a particle is found with no adjacent particles but the full width half maximum can not be measured, the value reported is most likely not a real particle. By using the threshold for peak search and the standard deviation limit for inclusion in the reporting, baseline artefacts can be reduced whilst the standard Python script can be used to identify these anomalies and exclude them from further evaluation. Figure 5 shows a baseline artefact identified as a particle because the detection threshold and reporting standard deviation limits are not optimised. These parameters can be modified within Nu Quant and the acquired data reprocessed without the need to rerun the sample.

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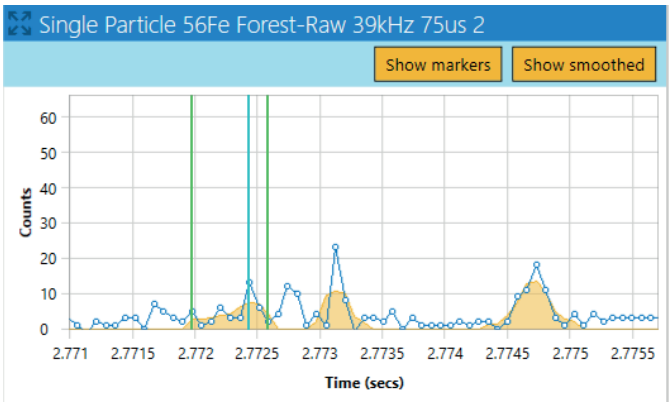

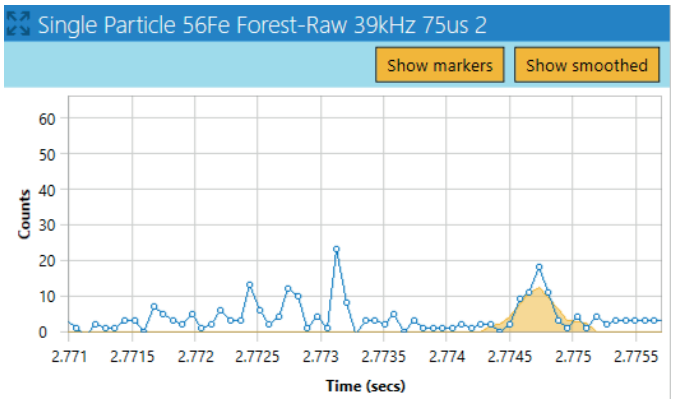

Figure 5a: showing a baseline artefact mis-identified as a particle which can be corrected post-acquisition by modifying the detection threshold and standard deviation for reporting, figure 5b.

The Python script provides a high level of flexibility for reporting not only the nanoparticle integrations but also the quality data for each particle and element coincidences. The script also uses the charting capabilities of Nu Quant to display histograms and scatter plots of the particle compositions. The scripts used can be the standard ones provided by Nu Instruments or customised ones edited by the user or shared between research groups. Figure 6 shows an example of the CSV output available with the scripts and figure 7 shows a typical histogram of particle composition for a forest water sample.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	BR	BS	BT	BU	BV	BW	BX	BY
1	Sample name	Forest-Raw 39kHz 75us 2																						
2	Sample time	31/10/2019 12:05																						
3	Total sample time (ms)	64256.175																						
4	Total particle number detect	5745	NOTE: not all particles detected will be reported as some will be below the limit of quantitation test!																					
5	Particles/second	89.40774953																						
6																								
7	Reporting as zero values below (3.0 * background count SD * half peak width)																							
8																								
9	Time	Average FWHM (us)	Peak Star	Peak End	23Na	24Mg	27Al	28Si	39K	40Ca	45Sc	48Ti	51V	52Cr	55Mn	(T) 56Fe	Peak sum		Peak Quality					
10	(ms)	Background counts			2062.305	362.4715	0.392525	430.9145	782.6921	4008.094	0.615791	19.67187	-0.00342	-0.18186	0.251225	1.660523			0.59 % Swamped by adjacent peak					
11		Background SD			81.83581	24.86126	1.278387	28.48574	48.45723	121.2215	1.27853	4.258007	1.187344	1.179101	1.792175	2.166961			11.37 % Measurable peaks adjacent to another peak					
12		Background cps			26958235	4738190	5131.05	5632869	10231270	52393390	8049.557	257148.6	-44.6897	-2377.31	3283.992	21706.18			0.21 % Poor quality peaks (possible background artefacts)					
13		Background SD cps			1069749	324983.8	16710.94	372362.6	633427.9	1584595	16712.81	55660.22	15520.84	15413.08	23427.13	28326.29			1.229 Average Skew (tail/lead)					
14																								
480	11801.3	230.291706	11800.97	11801.66	0	0	0	0	0	0	0	0	0	0	247.88	173.01	420.8929		0.8					
481	11807	237.4701925	11806.78	11807.7	0	0	244.79	0	0	0	0	0	0	0	0	1420.71	1665.5		3					
482	11822.2	236.6692707	11821.77	11822.46	0	0	232.73	0	0	0	0	0	0	0	0	348.98	581.7091		0.8					
483	11862.9	231.6858719	11862.7	11863.24	0	0	0	0	0	0	0	0	0	0	0	418.6	418.5964		Adjacent					
484	11895.8	237.418381	11895.52	11895.98	0	0	0	0	0	0	0	0	0	0	0	99.2	99.19679		0.5					
485	11959.6	237.3453323	11959.4	11959.93	0	0	64.14	0	0	0	0	0	0	0	0	110.94	175.0777		1.333333					
486	11979	229.9842548	11978.75	11979.36	0	0	0	0	0	0	0	0	0	0	354.46	0	354.4621		1.666667					
487	11984	233.2946418	11983.8	11984.41	0	0	0	0	0	0	0	0	0	0	91.3	992.3	1083.603		1.666667					
488	12008.3	237.065617	12007.98	12008.59	0	0	342.31	0	0	0	0	0	0	0	146.04	1695.04	2183.393		Adjacent					
489	12028.5	nan	12028.17	12028.55	0	0	0	0	0	0	0	0	0	0	0	130.6	130.5993		Swamped					
490	12028.7	nan	12028.55	12029.01	0	0	0	0	0	0	0	0	0	0	186.57	126.44	313.0135		Swamped					
491	12064.6	232.9793342	12064.36	12064.89	0	0	0	0	0	0	0	0	0	0	170.52	141.59	312.1036		1.333333					
492	12081.3	239.9086057	12080.96	12082.49	0	0	672.2	0	0	0	0	0	0	0	0	4370.98	5043.183		Adjacent					
493	12099.2	241.2666017	12098.86	12099.78	0	0	0	0	0	0	0	0	0	0	0	1048.33	1048.334		1.4					
494	12107	238.637983	12106.66	12107.43	0	0	0	0	0	0	0	0	0	0	0	204.05	204.0491		1.5					
495	12110.5	232.1433256	12110.18	12111.02	0	0	0	0	0	0	0	0	0	0	0	321.37	321.3685		1.75					
496	12131.8	235.730137	12131.37	12132.29	0	0	0	0	0	0	0	0	0	0	0	252.5	252.5017		1.4					
497	12135.3	233.6248997	12134.97	12135.81	0	0	0	0	0	0	0	0	0	0	0	180.59	180.5935		Adjacent					
498	12138.5	263.7855471	12138.03	12138.94	0	0	0	0	0	0	0	0	0	0	0	209.36	209.361		1					
499	12140.2	237.4510058	12139.94	12140.55	0	0	0	0	0	0	0	0	0	0	0	169.22	169.2198		1					
500	12161	244.3645033	12160.52	12161.28	0	0	0	0	0	0	0	0	0	0	0	255.14	255.1367		0.666667					
501	12182.1	229.6362112	12181.86	12182.32	0	0	0	0	0	0	0	0	0	0	0	140.07	140.0669		1					

Figure 6: showing an example of the CSV output from one of the default Python scripts available with Nu Quant for nanoparticle analysis.

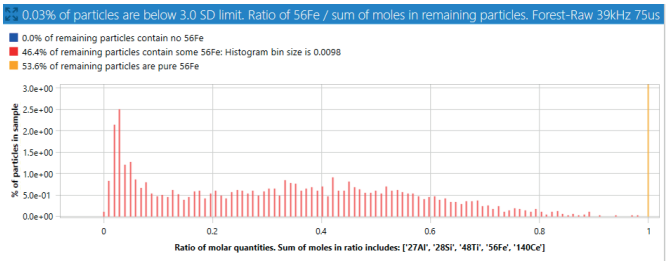

Figure 7: shows an example histogram of particle composition when using one of the default scripts available with Nu Quant.

Conclusion

Nu Quant provides a powerful tool for nanoparticle analysis with the ability to provide quality criteria for the found particles to assist with method development and validation. The flexible reporting and charting capabilities make reviewing data an easier task for new and experienced researchers.

References

1: Shaw and Donard: J. Anal. At. Spectrom., 2016,31, 1234-1242
2: Tanner and Baranov, J. Am. Soc. for Mass Spec., 1999, 10(11), 1083-1094
3: Bolea-Fernandez et. al., Anal. chimica acta, 2019, 1077, 95-106
4: Pace et. al., Environ. Sci. Technol. 2012, 46, 22, 12272–12280