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# Generating megapixel elemental images using Vitesse

- **Multi-element analysis of individual laser shots**
- **Sub microsecond timed integrations to individual laser pulses**
- **Stable acquisitions over many hours without recalibration**
- **Mega pixel image capable data acquisition, data processing and image generation software**

## Introduction

ICP-MS has proven to be a powerful analytical tool for laser ablation analysis of a variety of sample types. Historically, laser ablation systems have created transient sample signals of a few hundred milliseconds from a single shot which has been useful to obtain multi-element analysis of sample surfaces. To create multi-element images of sample areas, it has usually required spot sizes to be relatively high due to the dilution of the signal with time. Also, analysis times have been long as the repetition rate of the laser needed to be reduced to avoid blurring of data from one pixel into another. As laser ablation cells have developed, the washout times have reduced to a few tens of milliseconds, this has improved the sensitivity as the same amount of sample is compressed into shorter time and improved productivity as laser repetition rates have been able to be increased. Laser ablation is developing still with sample cells now being demonstrated with single figure millisecond washouts down to baseline between pulses. This leads to the interesting capability of being able to collect image data at up to 500 pixels per second. The only realistic technique to provide multi-element analysis on millisecond timescales is Time of Flight ICP-MS. However, the amount of data collected for larger images becomes a challenge for data storage and then processing of the mass spectra into elemental images. This technical note will describe how the Vitesse from Nu Instruments can collect the raw data required for mega pixel images and how this data is normalised and then displayed as element image maps. The ability to overlay different element maps will be shown as a tool to help understand the distribution of metals in centimetre sized biological samples with micron sized pixel resolutions.

## Data acquisition considerations

An Elemental Scientific NWR193 laser ablation platform was fitted with a prototype TV3 ablation cell. The output from the ablation site passed through to the ICP-MS torch via a single piece 0.8mm ID 1/16" OD PEEK tube. The capillary in the torch was inserted to where the injector began to taper and held concentric with the argon make-up flow passing concentrically to the capillary. A total of 1.2 Lpm of He was used as the ablation gas with 0.2-0.3 Lpm of argon added as a make up in the torch. The gas flows were optimised during tuning to obtain maximum sensitivity and best peak shapes for the washout of the ablated sample. Two He gas flows are used, one for the ablation cup and one as a general cell gas preventing loss of sample into the main chamber. The balancing of these two flows and the sample to cup distance were the primary parameters optimised for peak shape. The sample to cup separation can be maintained over a large image by using a refocusing function in the laser software where three points across an image can be focussed on and the remaining points all moved to fit a plane calibration from those three points. For each laser shot with the shutter open, a TTL electrical pulse is generated which is passed to the Vitesse electronics to coordinate acquired data against known laser shots.

Individual spectra were acquired every 25.5 µs with the ion signals being measured with an ETP MagneTOF detector, sampled every 0.5ns with an SP Devices 14 bit analogue to digital converter (ADC). The data from the ADC was passed directly to the graphics processing unit (GPU) fitted into the same PC at a rate of up to 6Gbyte/second without impacting the processor or memory of the PC itself. Within the GPU, spectra were summed and the electronic and mass spectral baseline removed every 2 spectra (51 µs) providing a significant improvement in abundance sensitivity as structured baselines from reinforced stray ion signals were not able to develop. After baseline subtraction, spectra are further summed within the GPU before peaks are integrated and the integrated isotope data is saved to solid state disks (SSD) continuously without interruption at a time set by the user in the analytical method. 2 ms spectra were used in this work with the raw data files for 1.3 mega pixel images using up 20 GB of SSD for each image. No recalibration of mass position was made during several days of analysing samples and each day, the sensitivity and peak shapes were optimised after the instrument plasma had been stabilised for 30 minutes prior to starting data collection for images.

## Interference removal

The Vitesse operates with the segmented reaction cell "always on". The Vitesse was setup with 3 sccm of He and 5 sccm of $H_2$ gases flowing into the reaction cell so all argon and nitrogen interferences were removed. This removed >99.9% of the total ion beam from the plasma and eliminated any significant space charge effects within the TOF. It also meant that the major isotopes $^{28}Si$, $^{39}K$, $^{40}Ca$, $^{52}Cr$, $^{56}Fe$ and $^{80}Se$ could be monitored for improved signal to backgrounds in the images.

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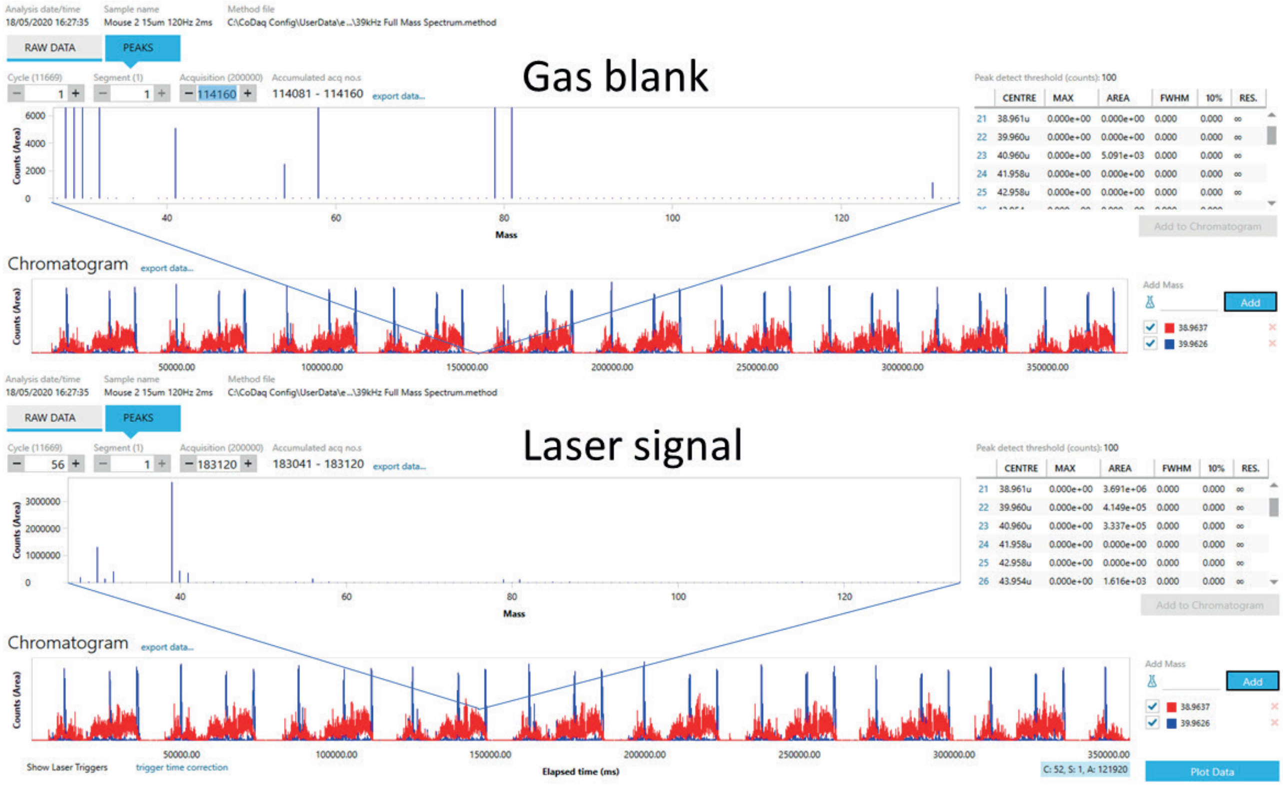


Figure 1: showing the gas blank with no counts (ADC counts before correction to ion cps) for $^{39}K$ (ArH interference) and $^{40}Ca$ (Ar interference) and sample signals from laser shots showing the correct isotope patterns for $^{39/41}K$ and $^{40/44}Ca$.

## Abundance sensitivity

Time of flight mass spectrometry has traditionally not been associated with good abundance sensitivity as the signals from scattered ions and large tails from major ions are reinforced over many acquired spectra to make the measurement of smaller signals 1 amu separated from a major ion more difficult. The Vitesse data acquisition technique subtracts the baseline early in the data accumulation process. Therefore signals which would contribute to peak tailing issues are removed before they can reinforce and high abundance sensitivity is observed.

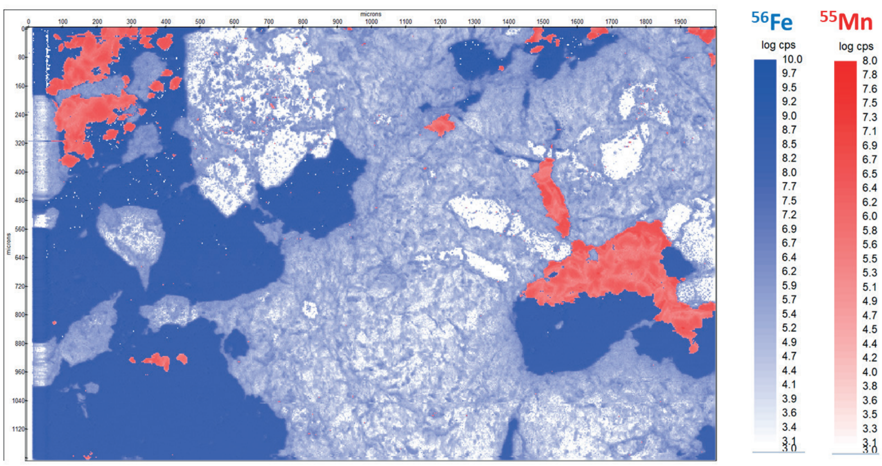


Figure 2: shows overlaid images for $^{55}Mn$ and $^{56}Fe$, the Mn is a minor component in parts of these minerals whereas the Fe is a constant across the sections where the Mn is found. The sharp boundaries into the Mn sections show the level of pixel to pixel separation using this imaging methodology and the wide signal range between Mn and Fe highlights the high abundance sensitivity possible with the Vitesse (data is displayed as ADC counts per second).

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### Data reduction

Large amounts of data are acquired by the ICP-MS when imaging samples. Acquired data was opened in the Vitesse quantitation software Nu Quant where Nu Instruments' Calculations Editor Python (NICE) scripts were used to normalise the data for each pixel. The data was first reduced using the measured laser triggers to demark times between which the data is averaged. The gas background is determined from data collected before the first laser trigger and subtracted from the averages between the laser markers. This integrated data is essentially the measured signal for each element in each pixel. The signal was then normalised for the abundance of each isotope and each element then corrected for the relative sensitivity factors calibrated from previous standards. Each isotope was then reported as the parts per million of that isotope relative to the sum of all normalised signals. These ppm values were associated with the X and Y laser stage positions to produce elemental map files with high levels of precision and no blurring between pixels.

### Samples for mega-pixel images

Mice were intravenously injected with silver nanoparticles and following previously used protocols(1), euthanised after different incubation times. The mice were frozen and whole-body sections taken with a Leica CM 3600 cryomacrotome. The sections were glued to glass slides and dried under vacuum. The samples were stored in a freezer and allowed to come to room temperature prior to laser ablation imaging.

### Image generation

MassImage from Münster University(2) was used to show the images from data reformatted by Nu Quant using a customisable Python script. MassImage can display images as huescales to better visualise wide dynamic range elements and as individual monochrome colours for different elements. The software allows the colour scales to be edited to suit different concentration ranges with the ability to set the minimum of the scale to be transparent. Images can be saved out as PNG format, allowing three elements as red, green and blue to be superimposed on a picture of the mouse section taken before the sample was ablated.

### Mega pixel images

Figures 3 and 4 show images generated with 30 µm square spots at 100 Hz ablation. An 18.5x54 mm area was ablated in 4.5 hours . The distribution of silver nanoparticles within different parts of the body can be observed.

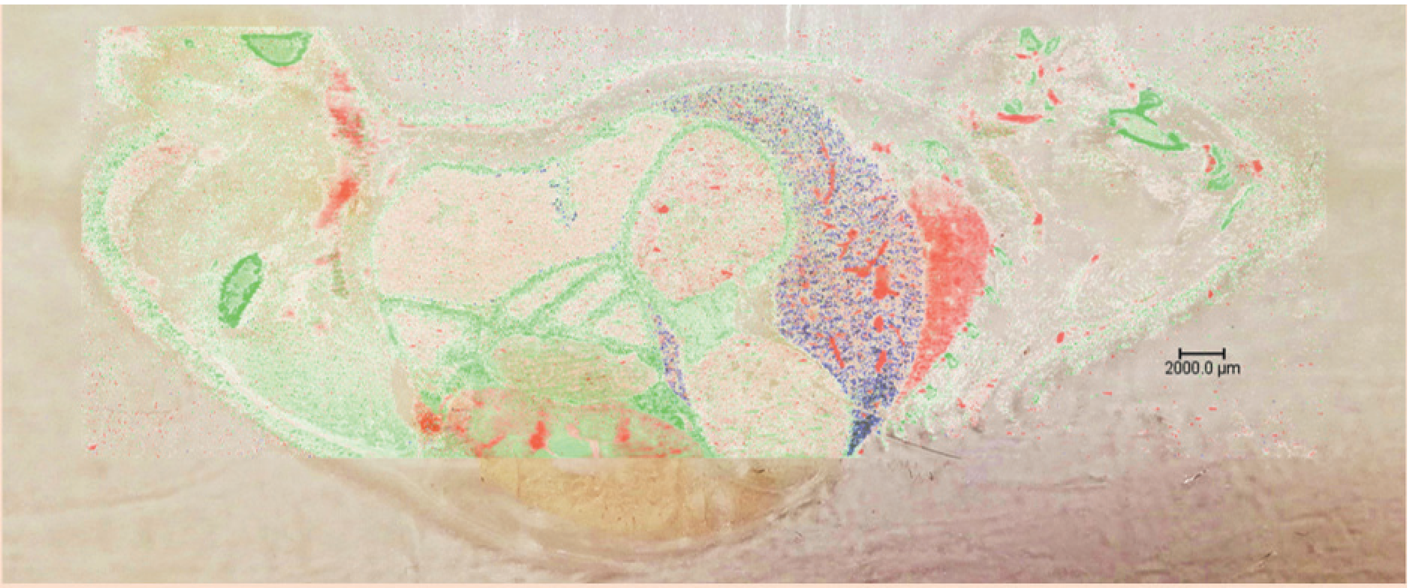


*figure 3* Fe (15% max) Ag (1% max) P (1.5% max)

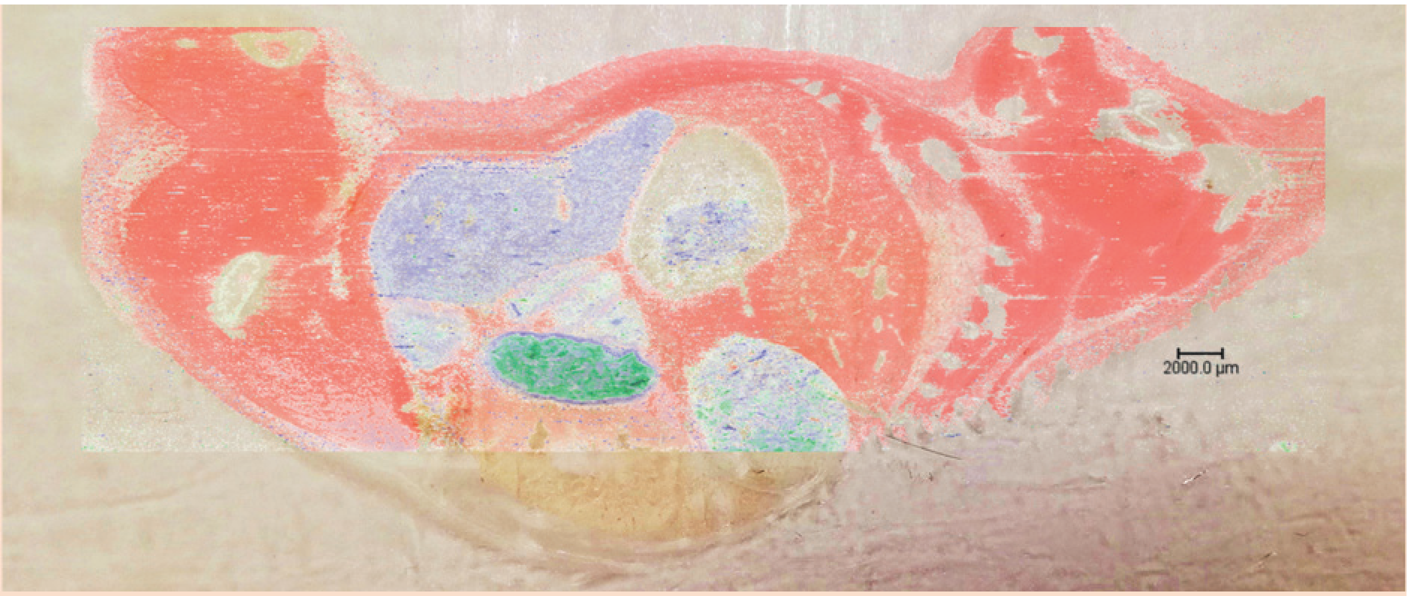


*figure 4*

Figures 5, 6 and 7: show images for a different mouse section generated with 15 µm square spots at 120 Hz ablation. A 22x54 mm area was ablated in 18 hours. Figure 7 shows a zoomed section where a feature of only a few tens of microns size can be clearly seen to contain uranium.

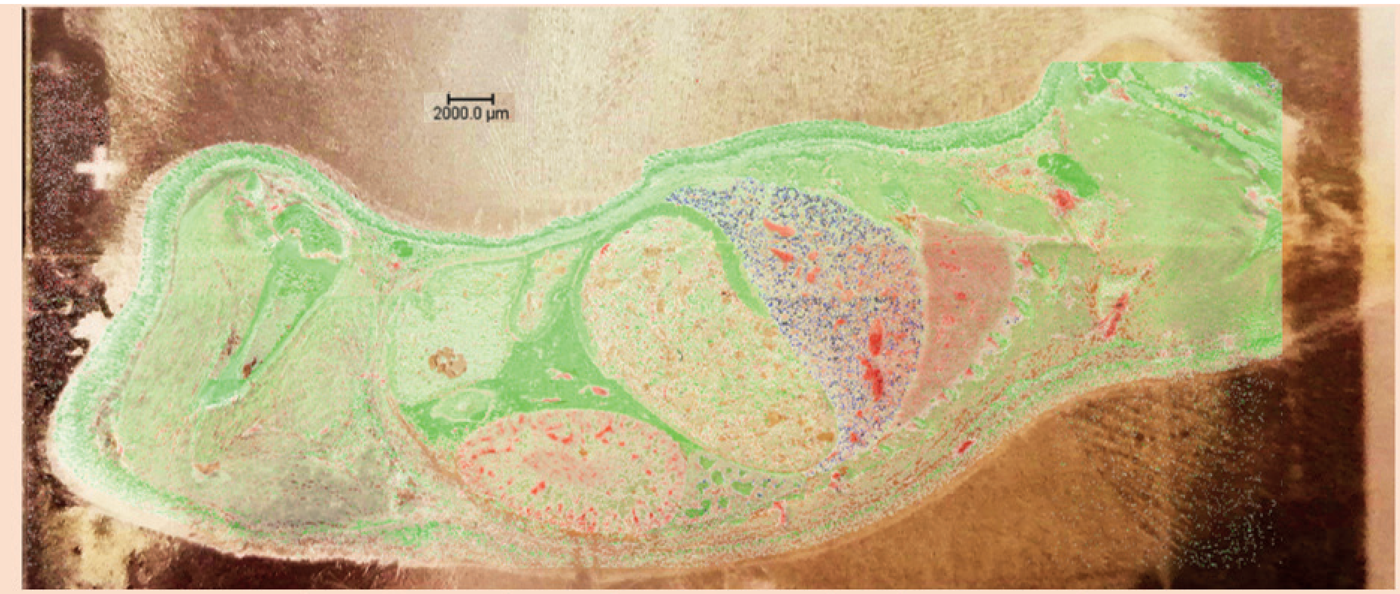


*figure 5*

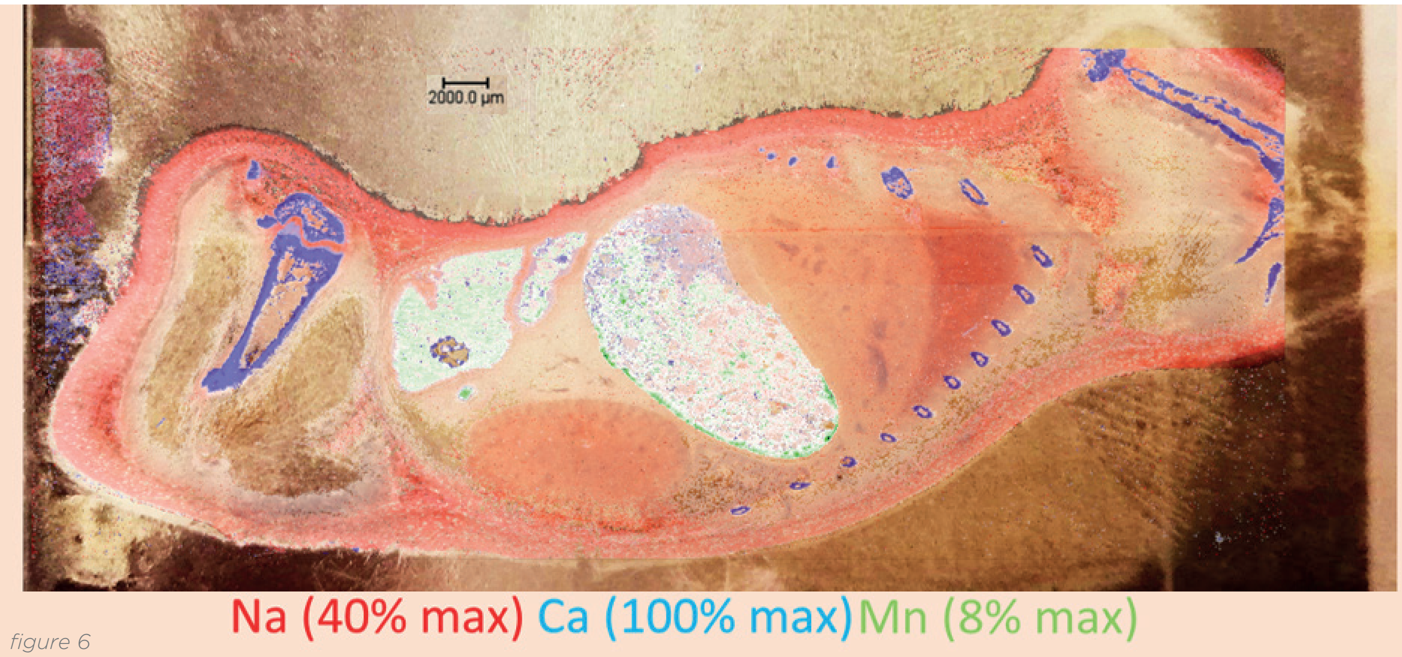


*figure 6*

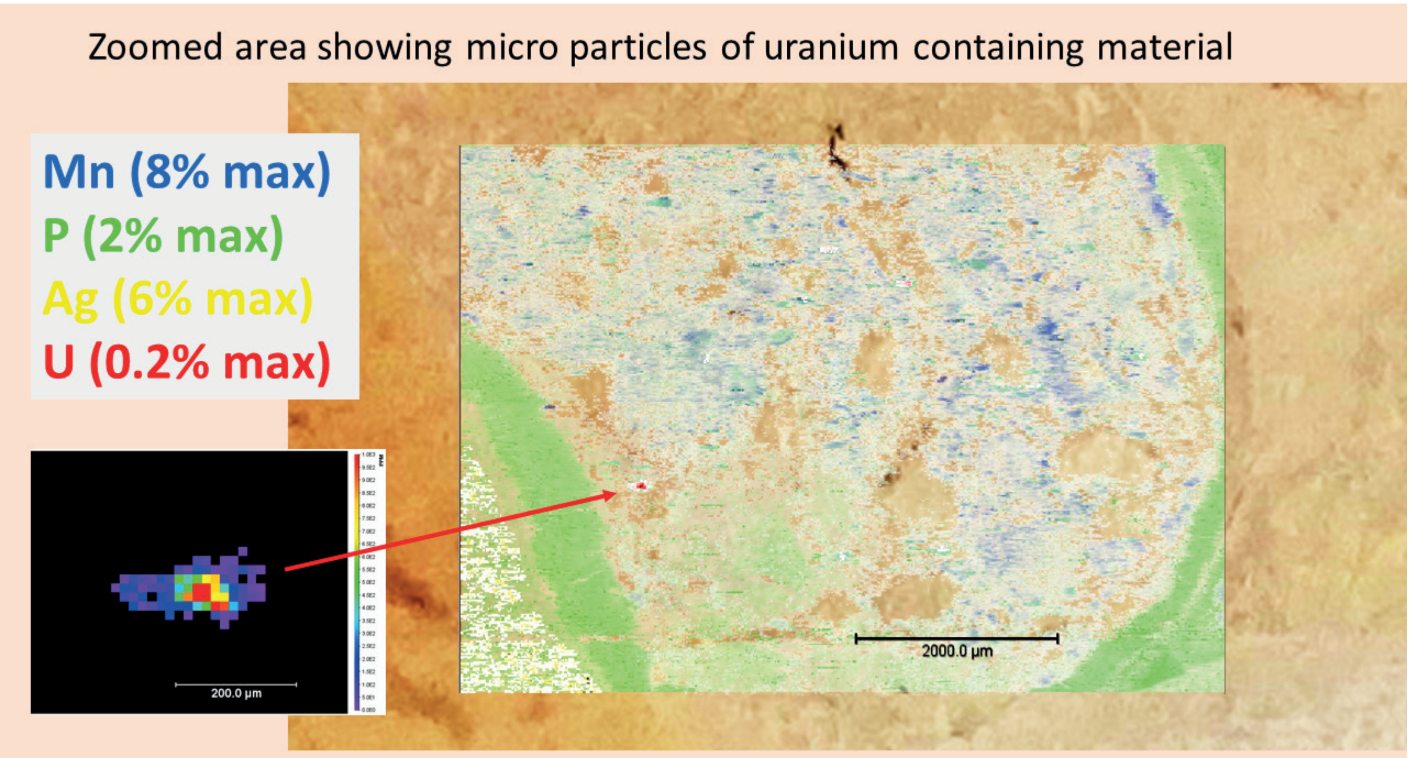


*figure 7*

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Summary

Nu Vitesse is proven to be a powerful and flexible TOF-ICP-MS with data acquisition and processing capabilities matched to the latest laser ablation cell designs. Fast, high resolution, multi-element mega pixel sized images can be created in a few hours. The use of Nu Quant gives the flexibility to match the data processing methods to the workflows of different users and output image data in a variety of formats compatible with common image rendering software packages.

*Acknowledgement:*

Thank you to Dr Meng Wang of the Institute of High Energy Physics in Beijing for providing the samples and giving us permission to use the data for this note and presentations.

*References:*

(1) Luo et. al, Anal. Chem. 2013, 85, 2977−2982
(2) https://uni-muenster.sciebo.de/s/EVOX9MGhGOqniDY