Bioimaging

Hyphenation of a high-speed laser ablation system to Quadrupole Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for imaging applications

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Abstract

Purpose: Demonstrate the capabilities and limitations of the combination of a new laser-ablation systems dedicated to high-speed mapping with quadrupole based ICP-MS systems.

Methods: An Elemental Scientific Lasers imageGEO193 Laser Ablation system fitted with a TwoVol3 Ablation chamber was directly coupled with a Thermo Scientific™ iCAP™ TQ ICP-MS using a compatible Dual Concentric Injector. Data was acquired using Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software and processed using Iolite™ Laser Ablation Data Reduction Software.

Figure 1. Thermo Scientific iCAP TQ ICP-MS Instrument, Elemental Scientific Lasers imageGEO193 Laser Ablation System with TwoVol3 Cell, and the ESL Dual Concentric Injector (DCI) for Thermo Scientific iCAP Qnova Series ICP-MS and Thermo Scientific Neoma Multicollector ICP-MS Systems

Results: A series of tooth samples containing a range of elements in localized phases was analyzed to demonstrate the ease of coupling to a commercially available high-speed mapping laser ablation system, ease of dwell-time optimization for best signal-to-background ratio and image contrast across all scanned elements, and finally fast analysis times from sampling to image.

Introduction

Laser Ablation (LA) coupled with ICP-MS is a well-established way to directly analyze solid samples. The main advantages of laser ablation include the ability to avoid lengthy and potentially contamination prone sample preparation protocols and the ability to obtain information about the spatial resolution of an analyte in a sample.

The interest in so-called mapping techniques has increased in recent years, calling for laser ablation systems to develop methods to improve sample transfer and therefore speed of mapping experiments. New laser-ablation systems dedicated to high-speed mapping are commercially available and can be easily coupled to quadrupole ICP-MS for such applications.

With the improvement in sample transfer and washout times from laser ablation systems, the time available to analyze discrete packets of sample from a laser pulse is drastically reduced. The limit for lateral resolution using a sequential ICP-MS, such as a quadrupole ICP-MS, is dependent on the dwell times chosen for each measured m/z channel. This has a direct impact on the signal-to-background ratio achievable for each m/z channel; therefore, a direct impact on the final image contrast for each mapped m/z channel.

Materials and methods

Sample Preparation

List samples, sample sources, and all relevant sample preparation steps and parameters.

Instrumentation

The choice of appropriate method settings (e.g. triple quad vs. single quad operation, reactive gas used and product ions to be analyzed in Q3) was accomplished using the Reaction Finder method development assistant in the Qtegra ISDS Software. Reaction Finder selects optimum analysis conditions despite the many available options inherent when using a triple quadrupole ICP-MS, but also leaves flexibility for testing different settings for a particular analyte.

Intelligent Mass Selection (iMS) was used on Q1 in all analyses to efficiently remove other ions potentially interfering on the product ion mass whilst maintaining highest detection sensitivity.

Method settings are summarized in Table 1.

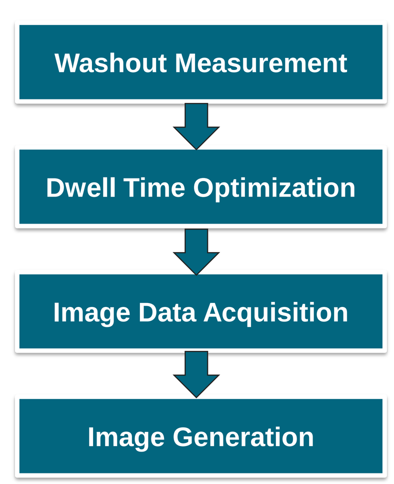
Table 1. Instrument Configuration and Operating Parameters

Thermo Scientific iCAP TQ ICP-MS	
Injector	2.0 mm i.d., quartz
Interface	Ni Cones using a High Sensitivity (2.8 mm) Skimmer insert
RF Power	1300 W
Make-Up Flow	0.66 L·min^{-1}
CRC Flow	0.320 mL min^{-1} O_2
Elemental Scientific Lasers imageGEO193 Laser Ablation System	
Total Cell Flow	0.800 L·min^{-1} (Analytical Cup)
Spot Size	25 µm Square
Scan Type	Image Lasso Scan
Laser Energy	~3.0 J·cm^{-2}
Repetition Rate	250 Hz
Overlap / Scan Rate	20 µm / 1,250 µm sec^{-1}

Data Analysis

Data acquisition was accomplished using Qtegra ISDS Software, dwell time optimization was performed using the Precognition™ plugin of Elemental Scientific Lasers ActiveView™ 2 Software and image generation was accomplished using Iolite Laser Ablation Data Reduction Software.

For high-speed imaging applications, after tuning and optimizing the ICP-MS Instrument and laser energy for the sample matrix, the imaging workflow is as follows:



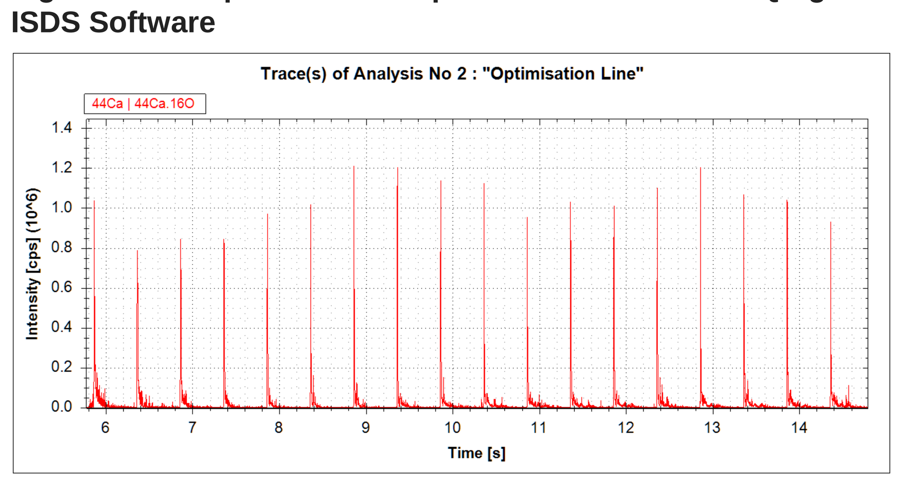
Results and Discussions

Washout Measurement and Optimization

For low-dispersion laser ablation set-ups, it is vitally important to assess the washout performance and peak shape of the laser pulses to determine the maximum scanning rate that can be used for a particular number of m/z channels without introducing blurring effects.

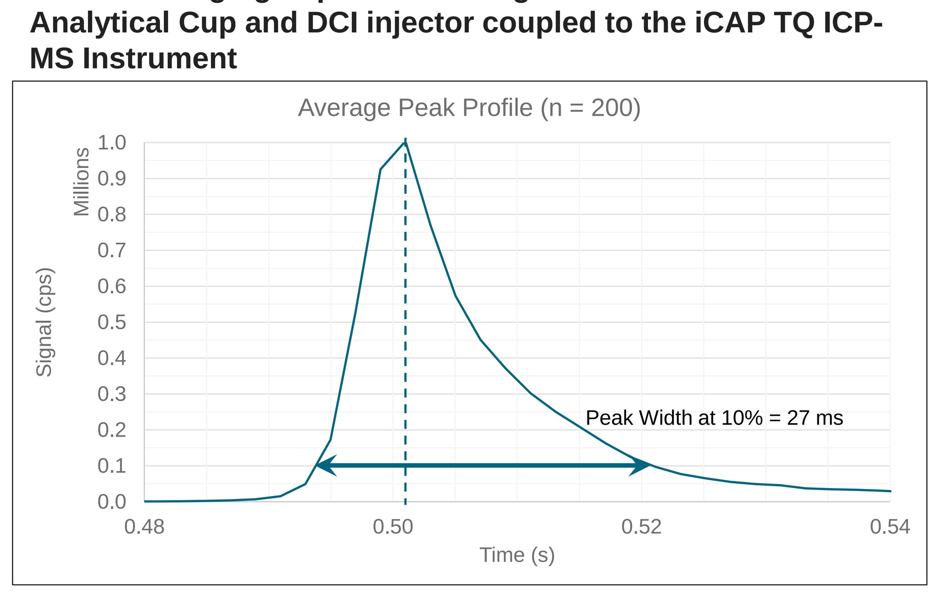
To do this, a low repetition rate line was ablated on the sample surface and a matrix element was measured at 1 ms dwell time (44Ca as 44Ca.16O in this case).

Figure 2. Example Washout Optimization Trace from Qtegra ISDS Software



The data were transferred to Microsoft Excel, using the Laser Data Reduction Export functionality in Qtegra ISDS Software, each peak was extracted, peak heights normalized to the first peak height and then all peaks were averaged. The peak width was then measured as the width of the peak at 10% peak height.

Figure 3. Average Normalized Peak Shape for the Laser Ablation Imaging Experiment using the ESL TwoVol3 Analytical Cup and DCI injector coupled to the iCAP TQ ICP-MS Instrument



Using this method, the measures peak width was 27 milliseconds, which was then used as a time constraint for subsequent dwell time optimization.

Dwell Time Optimization

Multi-elemental image generation typically suffers from artefacts inherent in the technique that can be minimized through proper optimization. The main artefacts that contribute to poor 2D image quality are aliasing, blurring (pixelation and smear) and contrast.[1-4]

Aliasing occurs, in essence, if the repetition rate and the cycle time of the laser ablation system and the ICP-MS system are not properly synchronized. This phenomenon causes periodicity in signal intensities that appear as bands in the final image. To combat this, the laser repetition rate and the sampling cycle frequency need to be matched so that an integer number of laser pulses fall within one quadrupole sweep.[1]

Pixelation blur is mainly a factor of the resolution at which the image is gathered, i.e., the laser spot size and the resulting pixel size.[2] To achieve high resolution (low blur), a small spot size must be used; however, this will directly decrease the signal-to-background ratio (i.e., contrast) and increase the total experiment time. The amount of acceptable blurring must therefore be balanced with the desired contrast and experiment time

Smearing, or motion blur, occurs when the transfer of material (washout) cannot keep up with the tracking of the laser across the sample surface (i.e., the scanning rate of the translation stages) and subsequent laser pulses are partially mixed. This is mitigated by adjusting the laser scanning time and repetition rates so that each laser pulse is fully resolved from each other when reaching the detector of the ICP-MS.

Contrast, as discussed briefly above, is a function of signal-to-background ratio and can be adjusted by the amount of material ablated per shot (spot size and/or fluence) or the dwell time of each scanned m/z channel during the quadrupole sweep. Given a set of lasing conditions, optimizing the distribution of dwell times between all m/z channels scanned will achieve the best possible contrast for all analytes in one run.

Optimization of the Dwell Times was performed using the Precognition Add-On from Iolite Software available for ActiveView2 Laser Ablation Software. First, signal intensity data for all desired isotopes were acquired using a simple line scan across a section of the sample surface with high perceived variability. The data were loaded into the Precognition module, which then optimized the dwell times to improve the Signal-to-Noise ratio (SNR) for each scanned isotope. There are two methods available:

1. The Hutchinson Method: Dwell times are distributed such that the SNR is increased to above 3 for all isotopes.
2. The Van Malderen Method: adjusts dwell times to improve SNR of isotopes whilst being constrained to the washout of the cell.

The Hutchinson method may result in cycle times much longer than the pulse width of the signal, so in this study the Van Malderen method was chosen. Resulting dwell times are summarized in Table 2.

Figure 4. Precognition Workflow for Dwell Time Optimization for Laser Ablation ICP-MS Imaging

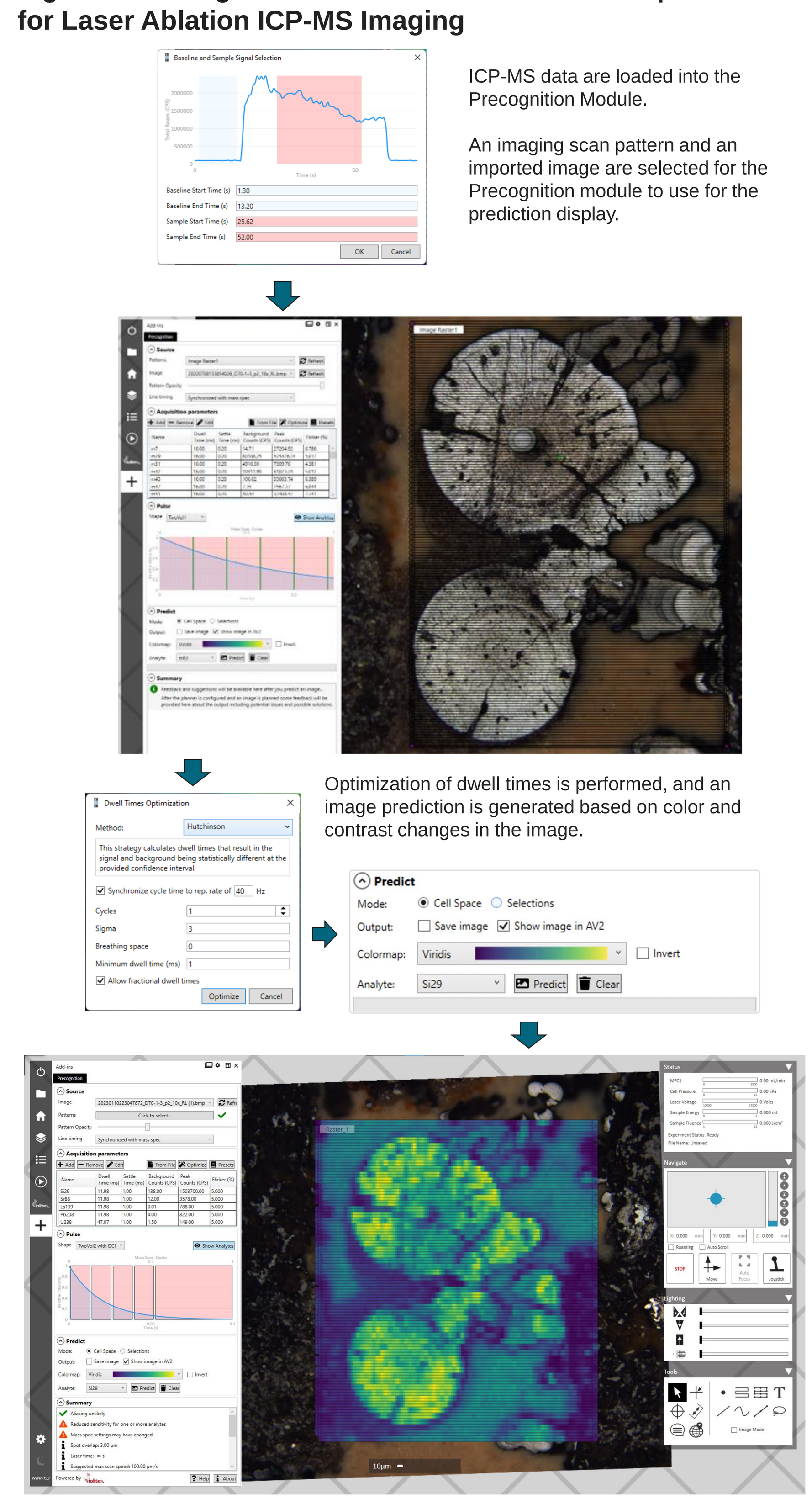


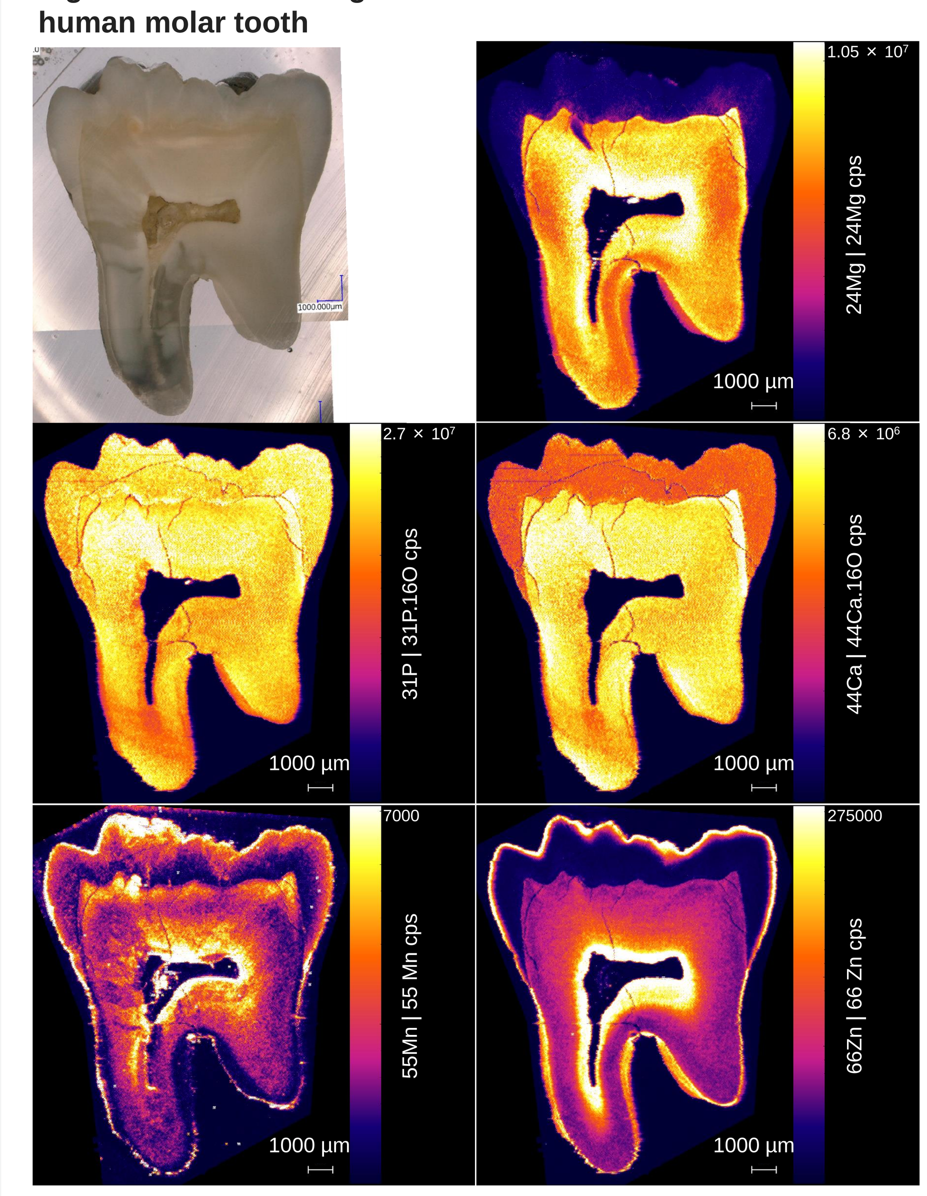
Table 2. Optimized Dwell Times for Tooth Imaging using the Iolite Precognition Add-On for ActiveView2 with the Van Malderen Method

Identifier	Dwell Time (s)	Q1 Resolution
44Ca | 44Ca.16O	0.0005	Normal
88Sr | 88Sr.16O	0.0006	Normal
138Ba | 138Ba.16O	0.001	Normal
31P | 31P.16O	0.0005	High
55Mn | 55Mn	0.0036	Normal
24Mg | 24Mg	0.0004	Normal
66Zn | 66Zn	0.0007	Normal
111Cd | 111Cd	0.0031	Normal
208Pb | 208Pb	0.002	Normal
238U | 238U.16O2	0.004	Normal
45Sc | 45Sc.16O	0.0004	Normal
89Y | 89Y.16O	0.0006	Normal

Human Tooth Images

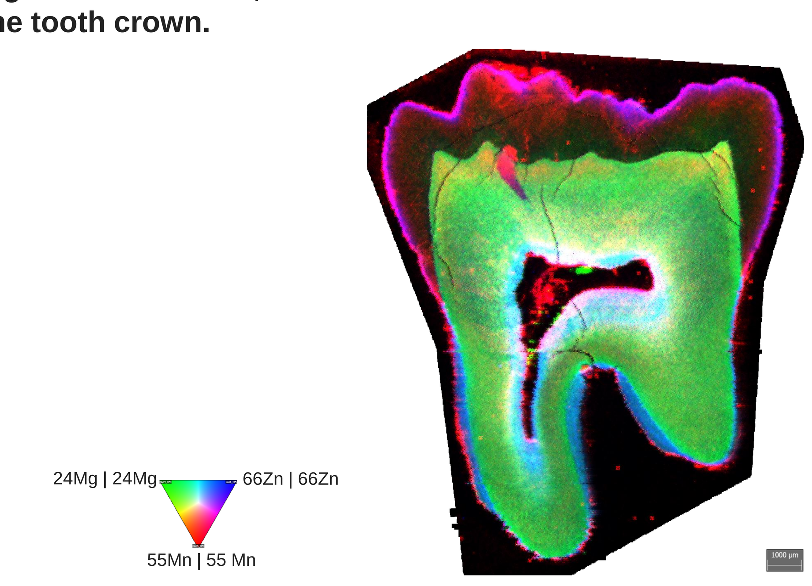
The following selected images were generated using an imaging lasso scan measuring 12,871.2 × 15,500 µm. At a scan rate of 1,250 µm/sec, the entire map was collected in just over 2 hours.

Figure 5. Selected Single Elements distributions within a human molar tooth



The images show certain elements are found preferentially in the crown of the tooth vs the dentine. Various elements can be stacked to show phase separation and/or co-localization as can be seen in the example image below.

Figure 6. Three-element RGB image showing separation of Mg from Mn and Zn, and some co-localization of Mn and Zn in the tooth crown.



Conclusions

Through careful optimization of laser ablation and ICP-MS parameters, it is possible to generate high quality multi-elemental images at a fast rate. The use of new low-dispersion laser ablation cells and aerosol transfer technology is not limited to simultaneous mass spectrometers and is also applicable to sequential scanning mass spectrometers such as Quadrupole ICP-MS.

The major limitation of coupling low-dispersion Laser Ablation systems to sequential mass spectrometers is the limited number of elements that can be scanned; however, this is offset by the sensitivity and the specificity of, especially, triple quadrupole ICP-MS systems for the reduction of interferences on select analytes.

Most imaging experiments can be reduced to three or four analytes of interest, and in these cases LA-ICP-MS coupled to Triple Quadrupole ICP-MS using a low-dispersion, fast imaging setup can provide the analytical data quality required to answer challenging research questions.

References

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