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Hollow fiber Mid-IR spectrometer with UV laser ablation sampling for fine spatial resolution of isotope ratios in solids

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ABSTRACT

We describe a system that combines isotope ratio analysis via mid-infrared (Mid-IR) laser absorption spectroscopy with fine spatial resolution sampling using a UV pulsed laser. The UV laser ablates a pit in a solid on the order of 10 microns in diameter. The sample-derived particulates resulting from laser ablation pass through a micro-combustor, and the resulting gas is analyzed using Mid-IR laser absorption spectroscopy in a capillary absorption spectrometer (CAS). The CAS uses a hollow fiber optic waveguide with a reflective inner coating and a small internal volume on the order of 1 ml. The hollow fiber both guides the laser light from source to detector and contains the gas sample at reduced pressure. Near unity overlap between the laser beam and sample enables sensitive analysis with ultra-small sample size. A prototype system has been demonstrated to enable stable carbon isotopic analysis ($\delta^{13}C$) with 1 per mil precision using < 1 picomole of carbon and is currently being used to study nutrient exchange in soil/root/microbial rhizosphere studies. The smaller sample size of this system is enabling fine spatial resolution analysis (on the order of 10 microns), which is roughly an order of magnitude smaller than was possible with an isotope ratio mass spectrometer (IRMS). In addition to rhizosphere studies, the system can provide a useful tool for fine scale isotope analysis with applications in biological, forensic, and environmental science.

Keywords: Isotope analysis, mid-infrared, laser absorption spectroscopy, hollow fiber optic waveguide, laser ablation sampling, rhizosphere, forensic

1. INTRODUCTION

Stable isotope analysis of solid samples has wide ranging applications including pharmaceutical tracers, biological labeling, and forensic analysis. Isotope ratio mass spectrometry (IRMS) provides an effective solution, but for some sample-limited applications the requisite sample size (on the order of 10 nanomoles) can be significantly greater than feasible. NanoSIMS instruments can provide the desired sensitivity, but utilization of these full-lab-scale devices is typically not practical due to their high-cost (on the order of $1M), high degree of sample preparation, and challenges in obtaining high precision isotope measurements.

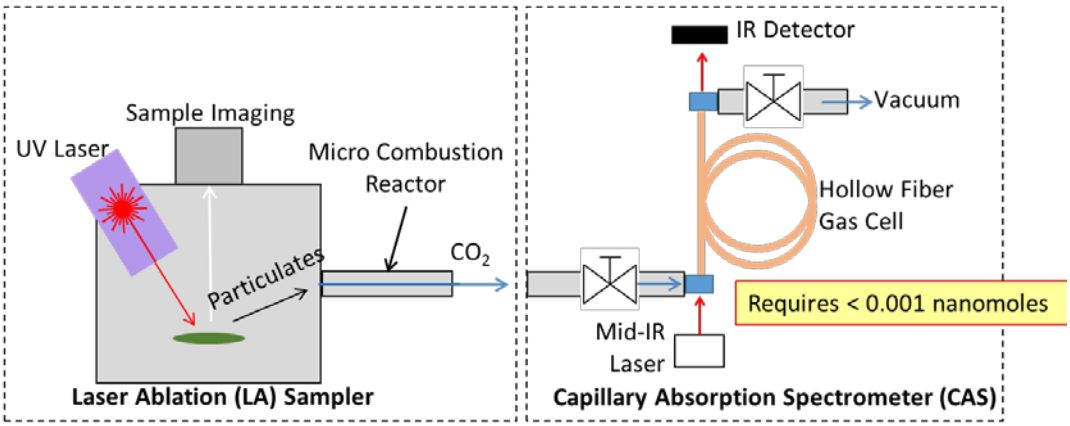

Figure 1. UV laser ablation (LA) sampling is combined with a CAS to analyze isotope ratios within a small-volume hollow fiber cell.

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To fill an unmet need, we developed a new bench-top tool that enables fine scale, spatial analysis of the isotopic composition of solids. A UV laser ablates a pit in a solid on the order of 10 microns in diameter. The sample-derived particulates resulting from laser ablation pass through a micro-combustor, and the resulting gas is analyzed using mid infrared (Mid-IR) laser absorption spectroscopy in a capillary absorption spectrometer (CAS)[1], see Figure 1. The CAS uses a hollow fiber optic waveguide[2] with a reflective inner coating and a small internal volume on the order of 1 ml. The hollow fiber both guides the laser light from source to detector and contains the gas sample at reduced pressure. Near unity overlap between the laser beam and sample enables sensitive analysis with ultra-small sample size.

1.1 Laser Ablation Sampling for Isotope Analysis

PNNL has previously developed and demonstrated laser ablation sampling techniques for accurate isotope analysis of solids, an example of sampling pits for analysis of horse hair is shown in Figure 2[3]. The laser ablation setup is based on a commercial UV device (wavelength, λ = 266 nm) that has been augmented with a micro-combustion reactor to convert the carbon in ablated particulates to CO_2. The stable carbon isotope ratio ($\delta^{13}C$) in the original solid sample is obtained by measuring the resulting $^{13}CO_2$ / $^{12}CO_2$ ratio. In general, the IRMS requires > 10 nanomoles of carbon to make an accurate measurement, which in turn requires about a 50 µm diameter pit in relatively high carbon material (e.g., horse hair) and a 100 µm ablation pit for relatively low carbon material (e.g., soil). In the present work, we have replaced the IRMS with a CAS, which has been previously shown to produce precise isotope ratios ($\delta^{13}C$ ~ 1‰) with as little as 0.02 nanomoles of carbon[1] and with recent advances the requisite sample can be reduced to < 0.001 nanomoles[4].

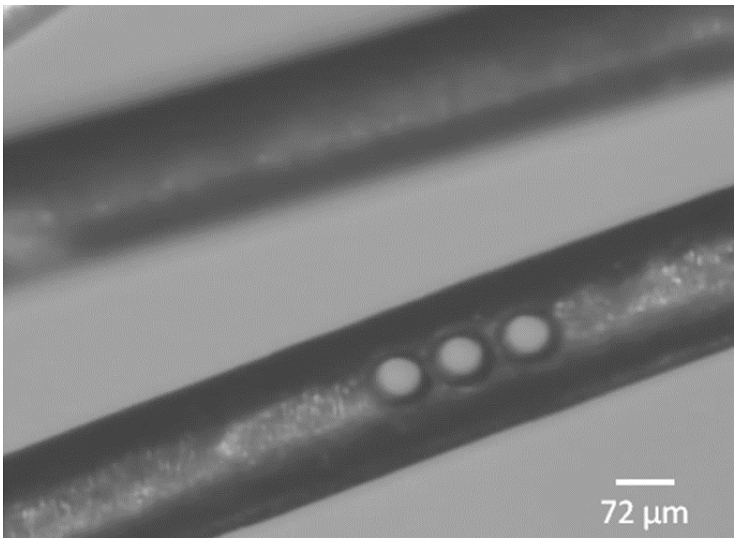

Figure 2. Picture of horse hair used in demonstration of isotope analysis of solids with laser ablation sampling (from Moran, et. al., 2011)[3]

1.2 Capillary Absorption Spectrometer (CAS)

The CAS concept utilizes hollow core fiber optic waveguides produced at OKSI, Figure 3. These fibers have excellent light guiding properties in the Mid-IR wavelength range[5] including being an excellent solution for single mode laser delivery[6]. The hollow fiber lengths in these systems typically range from L = 1 to 5 meters, which provides a moderate path length for absorption spectroscopy in a compact form factor. In addition to being demonstrated for isotope analysis, the systems have also been demonstrated for trace analysis of various gases including mixtures of methane, SO_2, and other volatile organic compounds (VOCs)[7]. Systems can be customized for specific applications utilizing a range of fiber internal diameters (ID = 0.2 to 1.5 mm) to optimize the trade-off between lower sample volume and higher gas throughput. In addition, we have the ability to optimize the dielectric layer thickness for a specific laser wavelength range. Furthermore, new systems are being developed at OKSI utilizing hollow fibers based on plastic tubing instead of glass tubing, where use of plastic tubing enables relatively large diameter fibers that are flexible and very robust, see Figure 3.

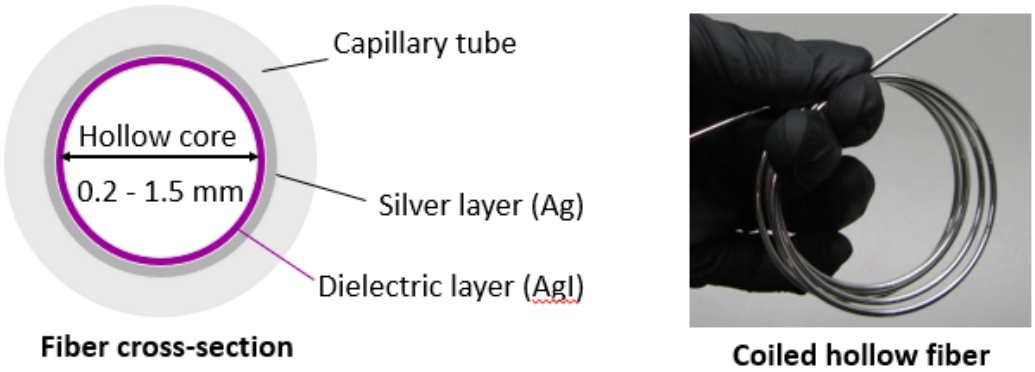

Figure 3. (Left) Diagram of hollow fiber cross-section showing internal coatings. (Right) Picture of a coiled plastic hollow fiber.

2. LASER ABLATION + CAS FOR RHIZOSPHERE ANALYSIS

2.1 Isotope Ratio Analysis in the Rhizosphere

Soil contains high degrees of spatial heterogeneity, plant roots navigate this complex environment in search of nutrient and water resources required to support their growth, and in the process, they alter the surrounding soil ecosystem (i.e., rhizosphere), see Figure 4. For instance, root exudates and other forms of rhizodeposits produce localized regions of increased organic carbon content, which can fuel active rhizosphere microbial communities. In turn, these communities can provide multiple services to the host plant by assisting in nutrient acquisition, protection from pathogen invasion, and improved resistance to water stress. Despite the high importance the rhizosphere plays in overall plant health and productivity, it occupies a very small spatial region along and adjacent to root surfaces.

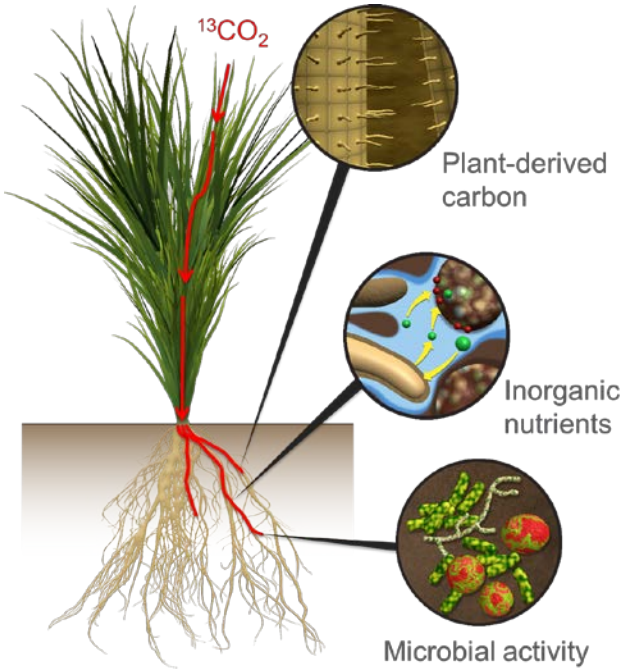

Figure 4. Analyzing the fine-scale spatial dependence of the $\delta^{13}C$ isotope ratio in the rhizosphere can help identify specific regions of active root exudation and/or root growth within the soil environment.

Stable isotope tracers, such as ^{13}C, provide a key tool for tracking carbon input from plant hosts into surrounding rhizosphere communities and have widespread use in studies of the rhizosphere[7]. PNNL has been developing

techniques to effectively study the rhizosphere using IRMS[9][10]; however, the small spatial scale can confound efforts to fully assess fundamental biological principles driving interactions between rhizosphere communities, and thus the higher spatial resolution of the LA-CAS is needed. The ultimate goal of this specific work is to study processes in the soil that impact plant growth. These studies will inform efforts to improve productivity of biofuel crops (including on marginal soil quality) to enable higher efficiency growth with less impact on the environment.

2.2 Laboratory Methods

To provide a preliminary evaluation of the LA-CAS approach, we produced a series of switchgrass samples in rhizobox systems, see Figure 5. After approximately 6-8 weeks of growth we injected $^{13}CO_2$ into the growth chamber and continued plant growth for 48 hours to isotopically label plant photosynthate produced during this time. We performed analysis of harvested leaf and root materials by elemental analysis isotope ratio mass spectrometry to confirm isotopically labeling of plant biomass.

To collect samples for analysis, we removed one side of the rhizobox to expose the subsurface plant biomass and used a coring device to preserve spatial orientation in the extracted samples that were one-half inch in diameter, Figure 6. We then dried the samples and placed them into a custom-built ablation chamber which we inserted into a Cetac LSX-500 laser ablation system. We measured samples with both laser ablation coupled to isotope ratio mass spectrometry (LA-IRMS) and laser ablation coupled to capillary absorption spectroscopy (LA-CAS). In both cases, we sampled spots on the root and the adjacent soil at locations with increasing perpendicular distance from the root. We performed LA-IRMS with multiple laser shots producing a 100 µm dimeter sample area, while for LA-CAS, we were able to use a smaller 25 µm sampling size. After ablation, a helium carrier flow is used to entrain the solid particles resulting from the ablation and transport them through a microcombustion reactor to convert the entrained carbon to CO_2. Example laser absorption spectroscopic data of CO_2 taken with the LA-CAS system is shown in Figure 7.

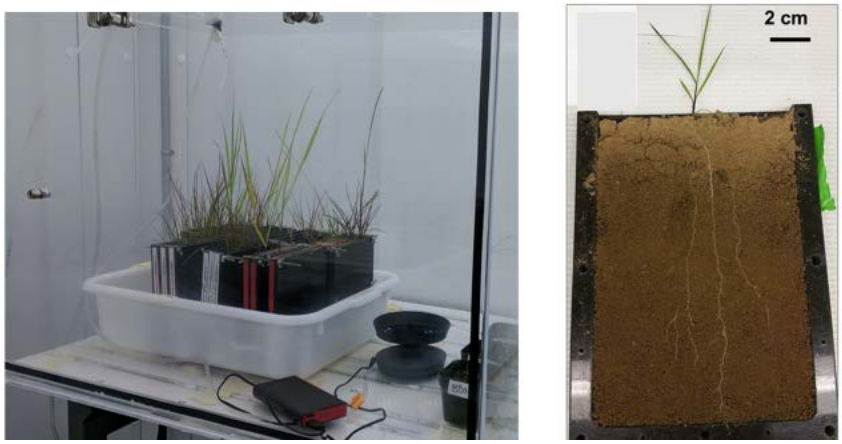

Figure 5. (Left) $^{13}CO_2$ was added to the headspace inside a plant growth chamber to isotopically label plant biomass and photosynthate. (Right) Plants were grown in rhizoboxes with removable sides to allow sampling of roots and the rhizosphere.

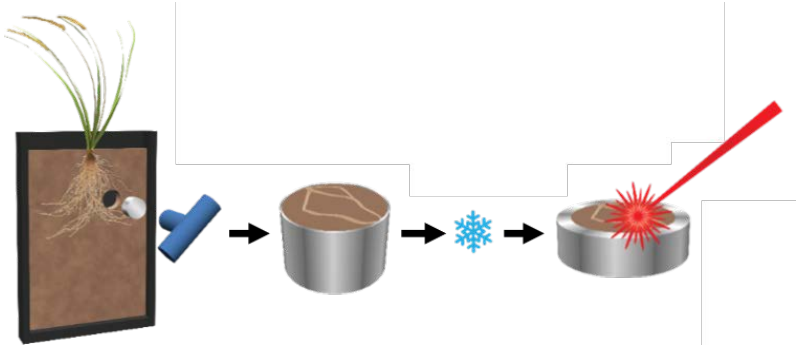

Figure 6. We used a sample coring device with one half inch inner diameter to preserve spatial orientation while excising the sample. We then dried the samples and placed them into the ablation chamber for analysis (adapted from Denis et. al., [9]).

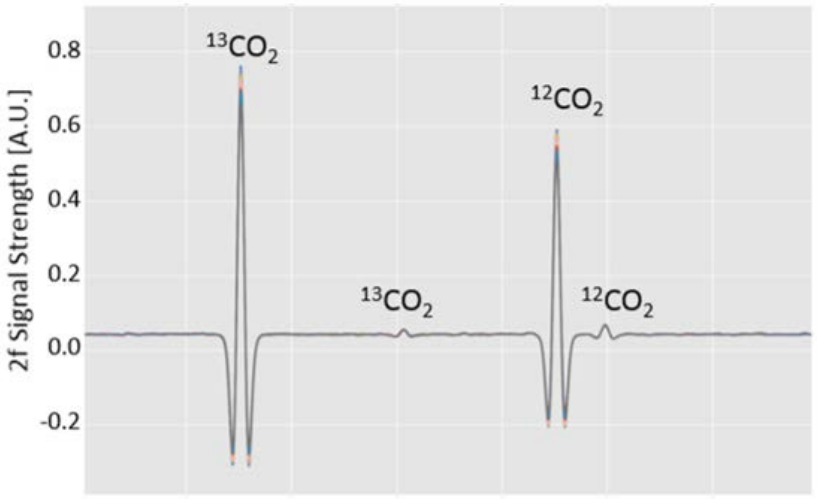

Figure 7. Typical laser absorption spectroscopy data showing the separation between different ^{12}C and ^{13}C absorption peaks. This figure shows 2f frequency modulated data.

2.3 Experimental Results

Results from both the LA-IRMS and LA-CAS approaches show agreement in the overall trend, namely decreasing root exudates (as measured by a drop in $\delta^{13}C$) as the distance from the root increases. Due to the inherent variability within the root-rhizosphere system together with the fact that different spots were used for the different devices, a one-to-one comparison is not appropriate. There are differences between the data sets, which may be the result of analytical offset, actual spatial differences between the respective isotopic transects, or a combination of the two. Still, the similarity between the two data types provided some degree of assurance that the approaches provided comparable results.

There was a major difference between LA-IRMS and LA-CAS in terms of the physical impact that the measurements had on the actual sample. LA-IRMS has much lower instrument sensitivity than LA-CAS and therefore requires both a larger laser ablation spot size for LA-IRMS as well as a higher number of ablation pulses to remove suitable material for isotope analysis (120 shots versus $\leq$ 30 shots per analysis respectively). As a result, sampling for LA-IRMS produces much larger pits (diameter ~ 100 μm) on the surface than sampling for LA-CAS (diameter ~ 25 μm). Larger pits can limit analysis for at least two reasons: 1) removal of material may make it more challenging to perform complementary sample analysis (e.g., representative DNA extraction, proteomic analysis, etc.) and 2) the larger sample pits effectively reduce the spatial resolution of the analysis. Based on the size of the needed laser ablation sampling, the LA-CAS offers at least a four-fold increase in spatial resolution versus LA-IRMS in this example. Continuing efforts are underway to enable reduction in the laser spot size diameter used for LA-CAS to further increase its resolution. Given the thinness of the rhizosphere and the steep gradients that transect the rhizosphere, the increased resolution with use of the LA-CAS technique will provide a valuable tool for elucidating carbon exchange processes in this dynamic system.

We performed additional LA-CAS transects crossing from soil into rhizosphere and then the root including some in which we continued analysis over two parallel roots in the same sample, Figure 8. In all cases, we observed the expected general pattern, namely decreased root exudation with distance from the root. Interestingly, however, is that in some cases the root itself does not display as high a measured $\delta^{13}C$ as the adjacent rhizosphere. We are continuing to process additional samples to confirm this pattern. A leading hypothesis to explain this observation, however, is that the root segment in this region was not actively growing so it wasn't incorporating large amounts of ^{13}C-labeled carbon into the root itself. Rather, the root may have served as a conduit for ^{13}C-labeled photosynthate which accumulated (over the short time duration of this experiment) in the rhizosphere. Additional studies are required to accept or refute this concept, but it should be noted that the phenomenon was only made visible by the higher spatial resolution afforded by the LA-CAS, as this trend has not been observed in LA-IRMS data.

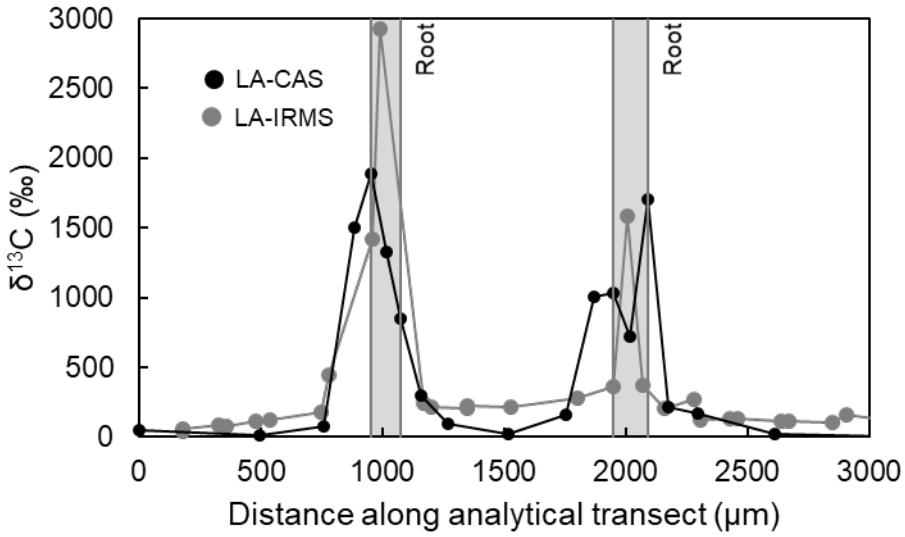

Figure 8. Analysis of two parallel roots. We performed LA-CAS analysis of two parallel roots and observed transport of ^{13}C-labeled root exudate into the rhizosphere. The data presented for both the LA-IRMS and LA-CAS was normalized to the same in-house isotope standard (a section of monofilament fishing line having $\delta^{13}C$= -27.67 ‰).

3. FUTURE AND RELATED WORK

The immediate near-term goal of the described effort is to produce a laboratory tool for analyzing the carbon isotope ratio of solids, see Figure 9. Such systems will not only be useful for rhizosphere studies, as shown here, but also myriad forensic and biological applications. In particular, the system will be useful in sample limited and/or applications requiring fine spatial resolution analysis. Related efforts are also being pursued for nitrogen isotope in solids, as well as direct N_2O analysis in soils. In addition, systems utilizing the CAS spectroscopic engine are being developed for methane isotope analysis from airborne drones, as well as, underwater submersible vehicles.

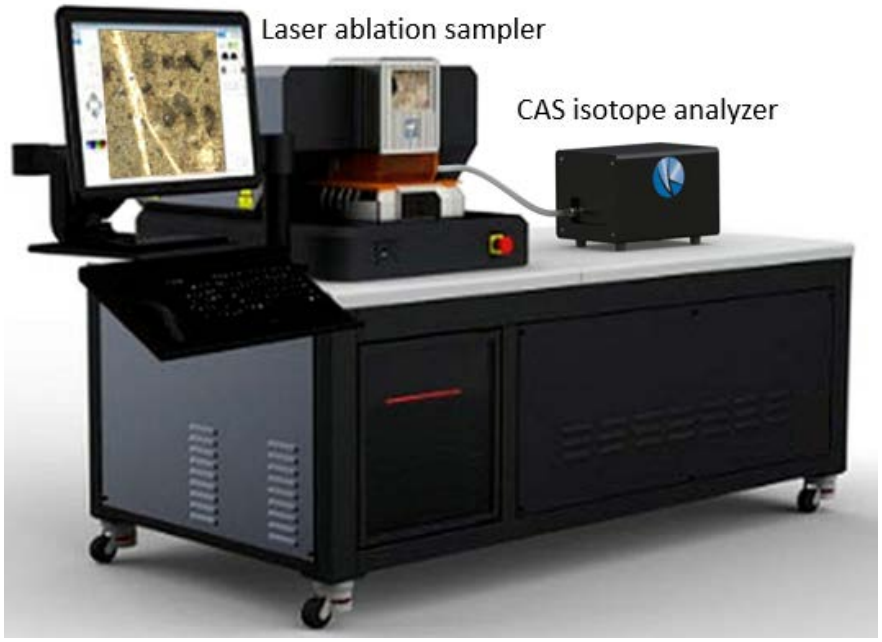

Figure 9. Conceptual image of Laser Ablation[11] + CAS system

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