

Outlining of the Research Progress in Hirata Laboratory

The Laboratory for Advanced Analytical Sciences (LAAS) is a new research unit established in 2016. Main objectivity of the research is to derive the elemental and isotopic data from various samples including geological rock, mineral, electric devices, and biological tissue samples using advanced mass spectrometry techniques. To do this, we are continuously developing analytical methods or instruments with the aim of extracting the new information that is not available to anyone else. Among the applications, we are currently developing the analytical systems for (a) precise and high-speed U-Th-Pb age determinations, (b) stable isotope geochemistry using heavy elements, (c) sensitive imaging analysis of metal and bio-molecules, (d) elemental analysis of nanoparticles (NPs) from meteorites and biochemical materials (Fig. 1). Six mass spectrometers with four in-house laser ablation systems are currently working in the research unit. We also have a chemical preparation facility (a shared facility of the Geochemistry Laboratory) for chemical analysis, where sample preparation, instrument cleaning and sample storage can be made.

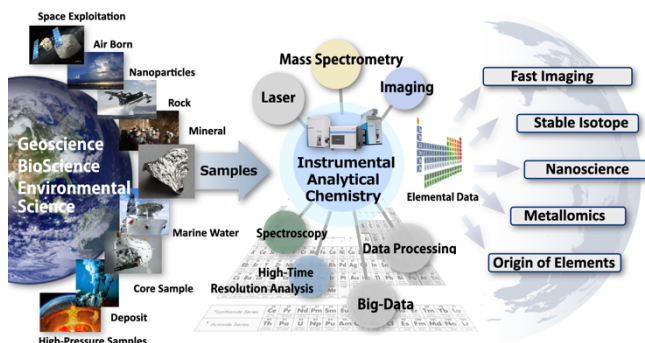


Fig. 1. Study Work Flow of Our Research Unit

Analytical technology advances rapidly, and even the most advanced analytical equipment can lose its performance advantages after about five years, and the number of collaboration researchers using our facility decreases within short time period. To maintain the function of our laboratory as world leader in analytical chemistry, continuous developments of both the analytical method and instruments are still key issue. In this section, we first outline our current instrumental setup, then present the results of our research over recent five years, and finally introduce future research direction of our laboratory.

a) High-mass resolution-ICP-mass spectrometer (Nu AttoM HR-ICPMS)

The ICP-mass spectrometer utilizing a high-transmission magnetic sector (ICP-SF-MS) can provide both the high elemental sensitivity and low background noise, and thus, the system is suitable for elemental and isotopic analyses of low concentration analytes. This instrument is equipped with a unique ion suppression device (attenuator). With the attenuator device, ion currents (ion flux) is reduced down to 1/100 to 1/500 level by passing through the metal-grids, which convert the ion signals of 100 Mcps to 100 kcps, obviating the risk of erroneous measurements originating the counting loss due to detector dead time. The switching the attenuator device requires only <math><1\text{ ms}</math>, so that the signal of a specific element or isotope can be suppressed (attenuated) during the high-speed mass scanning covering the whole mass ranges from Li to U. The wide dynamic range of the detector system is very important to detect and to measure the size of the nanoparticles. Important point is that the time-resolution of the signal integration did not change even with the attenuator device, and thus, ion signals being 1 – 100 Mcps can be quantitatively measured even from the transient signal events emanating from the single



Fig. 2 HR-ICPMS System

nanoparticles. The instrument's high elemental detection sensitivity enables rapid detection of small nanoparticles (<5 nm) and, when used in conjunction with the attenuator function, larger nanoparticles (>400 nm) can also be measured simultaneously. The system is currently being used to analyse the distribution of nanoparticles in biological tissue samples [imaging of nanoparticles] aiming at evaluating the toxicity of the nanoparticles.

b) ICP mass spectrometer for high precision isotope analysis (Nu Plasma II MC-ICPMS)

Multiple collector system setup applied to the magnetic sector-based ICPMS system (MC-ICPMS) has been widely used for the precise and accurate isotopic ratio measurements of various elements. The precisions being better than 0.01% in the isotope ratio measurements can be dramatically improved by the simultaneous detection of the analytes. Faraday detectors have been used as the bench mark ion detectors for the isotopic studies. Despite the obvious success in obtaining the precise isotopic ratio data, the Faraday ion detector is not capable for detection of single ions, mainly due to high background noise (Nyquist-Johnson noise). Faced with this, we have developed a multiple collector array based on six high-gain ion detectors (electron multipliers). The major drawback of the high-gain ion detector is poor gain stability of the system. Time-dependent changes of the gain of the ion detector equipped on MC setup causes systematical error in the measured isotope ratios. To overcome this, we have developed a new ion detection system based on Daly ion detectors. The system is currently used for age determination based on the U-Th-Pb isotope systems, hence, ^{202}Hg , ^{204}Pb , ^{206}Pb , ^{207}Pb , ^{208}Pb and ^{238}U (or ^{235}U) ions were simultaneously monitored by the six high-gain ion detectors. The MC-ICPMS system equipped with six high-gain ion detectors allows routine analysis of younger zircons (<1 Ma) and, more recently, of the zircon epithermis (<1 μm thick), which is used for paleoenvironmental deciphering, magmatic material evolution and tephra-chronology. The MC-ICPMS system combined with high-speed scanning laser ablation system is currently using for dating analysis of various rocks or minerals. The system is now used to support many geologists or geochemists outside the University.

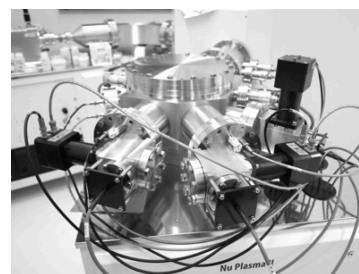


Fig. 3 Nu Plasma II equipped with six high-gain ion detectors

In 2020, high-time resolution amplifiers were applied to five of six high-gain ion detectors, enabling the isotopic ratio analysis of elements in nanoparticles. With the high-time resolution MC-ICPMS system (HTR-MC-ICPMS), isotopic ratios of individual nanoparticles can be made with very high analysis throughput (>100 grains per second). This is very important to search and to detect the solar nanoparticles (pre-solar grains) present in meteorites.

c) Triple quadrupole-ICP-mass spectrometer (ICP-QQQ)

Quadrupole-based mass spectrometer utilizing an ICP as an ion source has been widely used for elemental analyses. The collision/reaction cell technology equipped on our ICP-MS system can reduce various mass spectrometric interferences, resulting in better signal-to-noise ratio of the analytes. The ICP-QQQ system is applied for the in-situ analysis of trace-elements in solid materials using a laser ablation (LA) sampling technique. The sampling based on fast-scanning and high-repetition laser ablation enables us to monitor ultra-trace elements (ca. <math><0.1\text{ ng/g}</math> level) directly from solid materials. The combination of LA and ICP-QQQ system is currently used for quantitative elemental imaging analysis for both the geological and biological tissue samples. To enhance the elemental sensitivity of the system, newly developed vacuum interface was applied, and now the sensitivity is increased by a factor of 3 to 5 compared to conventional instruments.



Fig. 4 ICP-QQQ (Triple Quad)

d) Time-of-flight ICP mass spectrometer (ICP-TOF)

The ICP mass spectrometer utilizing a time-of-flight mass spectrometer (ICP-TOF) was introduced in 2019 through joint research with a Nuclear Regulation Authority (personnel training program). Unlike conventional ICPMS instruments (mass scanning-type mass spectrometer), it allows simultaneous analysis of all elements even from transient signals. It is used for short laser sampling or for nanoparticles, where the resulting signal is a transient signal of less than 1 ms in duration. It is currently being applied to the multi-spot analysis of zircon and the simultaneous analysis of U-Pb ages and REE (rare earth elements) abundance patterns from epithermal samples. By modifying the part of the instrument that draws ions from the plasma ion source (ICP) (the vacuum interface), the analytical sensitivity has been increased by a factor of 3 to 5 compared to conventional instruments. We are now using this instrument to develop a method for the analysis of individual elements in ultra-fine particles (particles less than 1 μm in size). As this analysis requires fast processing of huge amounts of data, we are developing our own data processing program (NP Shooter). We believe that such a high-speed analysis technology will become the major technology for promoting the Big Data Science in geochemistry.



Fig. 5 ICP-TOF (Time of Flight Mass Spectrometer)

e) Multiple-detector ICP mass spectrometer (Neptune XR MC-ICPMS)

This is an ICP mass spectrometer capable of multiple detection using a highly sensitive Faraday ion detectors, and was introduced in 2019 through joint research for human resources development program with a Nuclear Regulation Authority. The instrument has five Faraday detectors utilizing 10^{13} ohm amplifiers, which are nearly 100 times more sensitive than conventional Faraday detectors (using 10^{11} Ohm amplifiers). Due to the low response of this detector, a stable isotope signal is essential to take a full advantage of the 10^{13} ohm amplifiers. Recent applications strongly require isotope ratio measurements from small sample sizes or from

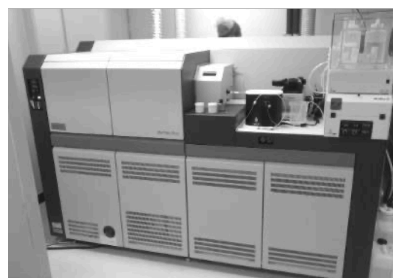


Fig. 6 Multiple Collector ICP-MS

ion signals with short time durations (i.e., transient signals) produced through the laser ablation sampling technique, and thus, no longer stable signals over the analysis period were obtained. Faced with this, a new data acquisition protocol called continuous ion monitoring (CIM) method has been developed (Yamamoto+, 2021), which enables the best performance of the new Faraday ion detector to be obtained even from transient signals. The method is currently being applied to the precise isotope analysis of Ca, Fe, Sr, Nd, Hf, W, and Pb. The main research target of our MC-ICPMS is focused on 1) analysis from small sample sizes, 2) elemental and isotopic analysis of nanoparticles, and 3) stable isotope studies using carbon and boron, being aware of the applications which are not done in other ICP-MS facility of the world.

(f) Ion trap type organic mass spectrometer (QTrap5500)

This is only the organic mass spectrometer capable of detecting ions with masses up to 1,000 amu (Da). The instruments were transferred through collaborative research with a drug company dealing with biomedical analysis of medicines. We have developed a new ion source that can be coupled with the laser ablation sampling technique, aiming at an imaging analysis of amino acids, peptides, vitamins, and sugars. To do this, we have developed a new atmospheric pressure ion source using AC barrier discharge for soft ionisation (ionisation with reduced fragmentation) of organic compounds. Combination of the new ion source and the laser ablation sampling technique, we have succeeded in the detection and imaging analysis of amino acids, vitamins, sugars and phthalate esters (endocrine disruptors). During this year, we are going to obtain (a) simultaneous imaging analysis of elemental imaging and biomolecules, and (b) distribution analysis of volatile organic compounds (VOCs) in meteorites. In the near future, we are going to apply this technique to the analysis of fluorine compounds to support international regulations on the use of fluorinated resins (PFAS, PFOS, etc.).



Fig. 7 Organic Mass Spectrometer equipped with in-house Ion Source

g) High-speed scanning nanosecond laser device (Nd:YAG 4HG Laser Ablation device)

This is an in-house developed new laser ablation system aiming at the in-situ sampling with spatial resolution of better than 8 μm . With the galvanometric optics, the laser ablation spots can be moved within 0.1 ms time period (high-speed multi-point ablation). The use of high-speed multipoint ablation enables simultaneous laser ablation of several solid samples, and the aerosol produced can be mixed in the cell, allowing mixing, addition of elements, dilution, etc., in the same way as for solution samples, thus enabling more flexible and user-friendly quantitative analysis. The instrument is now used for sampling using a laser ablation in liquid (LAL) technique, which enable the nanoparticulation of solid samples and the extraction of matrix particles from meteorites.

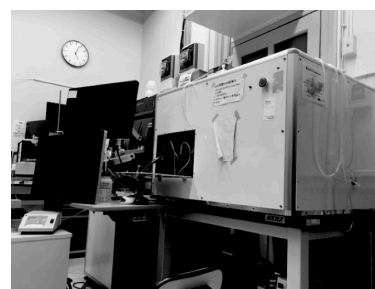


Fig.8 Fast Scan YAG Laser

h) Fast scanning femtosecond laser system (Jupiter Solid Nebulizer system)

This instrument is an in-house designed high-speed scanning and high-repetition rate laser ablation system using a state-of-the-art femtosecond laser with high energy density (introduced in 2019 in collaboration with the Nuclear Regulation Authority). We are currently using this instrument for various analyses such as U-Th-Pb dating, depth-profiling analysis, imaging analysis, and also applied for software developments. To take a full advantage of the multiple spot ablation protocol, geometry of the sample cell and gas flow conditions are highly important. For the elemental analysis for large-sized samples, we are developing “Large Cell” (Fig. 9). With this cell, elemental analysis from wider areas covering 30 cm circular shape can be made. For the imaging analysis for biological tissue samples, shorter sample washout time is important to improve the spatial resolution and also to reduce the analysis time. To achieve this, we have developed a “Small Cell”, and thus three different sample cells (Standard, large, and small cells) can be applied to cover various applications from geochemistry to biochemistry. The laser is also applied for the QTrap organic mass spectrometers to obtain imaging data of organic compounds (amino acids, peptides, sugars, phthalates, etc.) and biomolecules. The in-house software for the quantitative imaging analysis is also developed here (iQuant2 and NanoQuant).



Fig. 9 Fast Scan Femtosecond Laser

i) Fast scanning femtosecond laser system for laser ablation in liquids (LAL Laser system)

The laser system utilizes a state-of-the-art femtosecond laser with high energy density and high repetition rate, and was introduced in 2021 in collaboration with the Nuclear Regulation Authority. Currently developing a continuous sampling system (Continuous LAL device) for remote sampling for decommissioning measures and nanoparticulation of solid samples (Hirata, 2021). Continuous LAL technique can be applied for remote sampling, and the resulting sample aerosols can be delivered and stored in liquid media. The LAL technique can also be applied for the sampling/extracting the high-molecular weight organic compounds from tissue samples. We do believe that the LAL technique can be a principal technique for the imaging analysis of heavy molecules related with several disease.



Fig. 10 YAG Laser for Laser Ablation in Liquid (LAL) Sampling

j) High power nanosecond laser system for LAMIS (LAMIS Laser system)

A high-energy laser irradiation system dedicated to laser-induced optical emission spectroscopy, to be introduced in 2021 in collaboration with the Nuclear Regulation Authority. The system is specially designed for both the LIBS emission and laser ablation technique for the ICP-MS instruments. The LIBS technique utilizes the atomic emission spectrometry, whereas the LAMIS (Laser Ablation Molecular Isotopic Spectrometry) is based on emission from vibration/rotation spectrum of molecular ions. With the LAMIS technique, not only

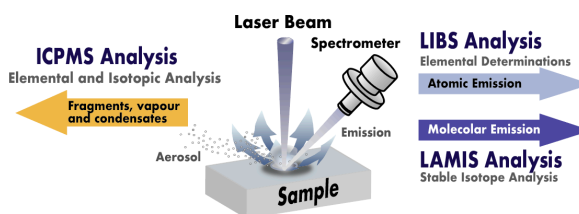


Fig. 11 Schematic diagram for hybrid analysis using LA-ICPMS and Optical Emission Spectroscopy (LIBS and LAMIS)

With the LAMIS technique, not only

abundance data but also isotopic ratio data can be derived directly from solid materials. The laser induced sample particles will be transported to the ICP ion source for further sensitive detection of elements/isotopes using the mass spectrometer. The high-power nanosecond laser was finely optimized for the LAMIS emission, and we are going to measure B, C, and O isotopes from geochemical rock/mineral samples.

2. Recent Research Achievements (2013-2021)

2.1 Stable isotope geochemistry

The application of stable isotopes to geochemistry already began in the late 1940s (e.g. Thode et al., 1949; Baertschi, 1950; Urey et al., 1951) and has accelerated in step with advances in mass spectrometry techniques. The study of stable isotopes started mainly with light elements such as hydrogen, carbon, oxygen, sulphur and noble gases (e.g. Hoefs, 2018). This can be summarised by the widespread presence of light elements in various samples, as well as the large relative mass differences ($\Delta m/m$) between isotopes. In terms of the former, light elements are abundant in samples as facies elements (elements that determine the combination of mineral phases) in rocks and as essential elements in living organisms. The latter, on the other hand, is related to the magnitude of the isotope fractionation effect (isotope fractionation coefficient: K_{ex}). Since the mass-dependent isotope fractionation (MDF) effect is proportional to the relative mass difference (Bigeleisen and Mayer, 1947), it is known that lighter elements produce a larger isotope effect. In contrast, the isotope fractionation effect is small for heavy elements, and isotopic composition changes in nature are small (Tanimizu et al., 2013; Ohno, 2014; Hirata, 2015; Munebayashi, 2016). Therefore, stable isotope studies using heavy elements have been limited in their applications. However, based on the Bigeleisen and Mayer isotope fractionation model, the isotope fractionation factor can be expected to have a large isotope effect in reactions at low temperatures, or in reactions where there is a large change in the binding energy of elements before and after the reaction (e.g. redox reactions). The Bigeleisen and Mayer model assumes isotope fractionation in reversible reactions (equilibrium reactions), but it is also expected to have a large isotope effect in kinetic reactions (e.g. solid-gas reactions), which may lead to new developments in stable isotope research.

Fifteen years ago, we focused on iron (Fe) in human red blood cells and investigated the isotopic changes. We focused on iron in living organisms because (1) biological reactions are low-temperature processes ($T = \text{about } 310 \text{ K}$) (T^2 rule of isotope effect), (2) iron changes its oxidation number in living organisms, and (3) it is abundant in blood and easy to sample. As a result, we reported that iron isotope ratios in human erythrocytes did not change throughout the year, and that iron isotope ratios differed between males and females (sex differences) (Ohno et al., 2004; Ohno and Hirata, 2004). This study is significant in that it shows that isotope ratios can be metabolic markers of metal elements in vivo. At the same time, Thomas Walczyk and his group in Germany reported similar results from human red blood cells. In their paper, they reported increased objectivity through data from a larger number of samples, and also reported that iron isotope ratios were altered in patients with specific iron metabolism abnormalities (Walczyk and Blanckenburg, 2002; Walczyk and Blanckenburg, 2005), and therefore did not take research priority. However, our research has not led to a priority. However, we understand that our work suggests that it is in line with new research and development trends in the world.

The study of stable isotopes of metallic elements in living organisms is gradually developing in both basic and applied research (Tanimizu et al., 2013; Tanaka et al., 2017a; Tanaka and Hirata, 2018). Recent trends in stable isotope research show that the research direction is accelerating towards the understanding of phenomena through physicochemical elementary processes in academic research, and towards clinical research in the life sciences in applied research. In particular, the development

of applied research into biochemistry has focused on copper as a new marker for metabolic changes. Changes in the isotopic composition of copper are expected to be useful in the future for the detection of Alzheimer's and Wilson's diseases and for elucidating the mechanisms of their expression (e.g. Albarède et al., 2016; Balter et al., 2015). Calcium has also been linked to trophic level and can be applied to the analysis of predation relationships (Walczyk and Blanckenburg, 2005), and increasingly to the detection of age- and disease-related metabolic changes (Skulan and DePaolo, 1999; Chu et al., 2006; Hirata et al., 2008; Heuser and Eisenhauer, 2010). In the future, we expect that the integration of calcium isotopes with elemental imaging techniques will accelerate the application of isotope stability chemistry to life chemistry research (Tanaka et al., 2017b; Tanaka et al., 2017c).

2.2 Development of the laser ablation method

When a laser beam of high energy density is irradiated on the surface of a solid sample, the laser beam penetrates into the sample ($<1\ \mu\text{m}$) and deliver its energy onto the sample. This can induce the increase of temperature up to 10,000 K, resulting in both the vaporisation of the constituent elements of the solid sample (also ionisation of some elements) and the explosive release of sample fragments (laser ablation phenomenon). This phenomenon is called as a laser ablation which can be used as a sample introduction technique for the ICP-Mass Spectrometry (Inductively Coupled Plasma-Mass Spectrometry: ICP-MS). Combination of the laser

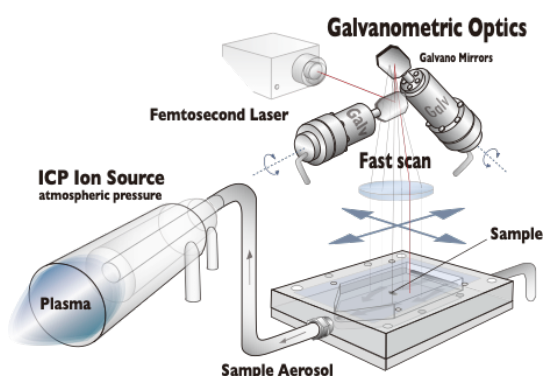


Fig. 12 Schematic diagram for fast-scan system using Galvanometric scanner equipped on our in-house laser ablation system

ablation sampling and ICP-MS technique (known as the LA-ICP-MS technique). The LA-ICP-MS technique has been widely used in geochemical and biochemical researches because the sensitivity of elemental detection and the spatial resolution of analysis have been dramatically improved by the high sensitivity of ICP-MS and the progress of laser oscillation and focusing technology (Fig. 12).

The spatial resolution achieved by LA-ICP-MS is little inferior to that of conventional probe techniques, including an electron beam microanalysis (EPMA), secondary ion mass spectrometry (SIMS), and microparticle X-ray fluorescence spectrometry (μXRF). However, the LA-ICP-MS method does not require the sample to be stored in a vacuum vessel, which allows elemental imaging analysis of wet samples (e.g. biological samples) and large area samples (several centimeters square), which is an important analytical feature which is not available with other probe techniques. For this reason, the improvement of the spatial resolution of LA-ICP-MS by narrowing down the laser beam has become a worldwide development trend. In our group, however, the improvement of the spatial resolution of LA-ICP-MS is not so positive. If spatial resolution of a few μm is required, it can be achieved by applying EPMA, SIMS, NanoSIMS, or μXRF using a high intensity X-ray source. Our idea is not to follow other advanced analytical methods, but to develop new analytical applications. We decided to focus on fast imaging analysis of large area samples (several mm or cm square) and improvement of analytical sensitivity, rather than improvement of analytical spatial resolution (Hirata et al., 2015).

Laser ablation from wide area ($> 20 \times 20\ \text{mm}$) can be achieved by the combination of high-frequency femtosecond laser and in-house fast-scan optics utilising a Galvanometric scanner (Fig. 12). The Galvanometric scanner is utilizing a pair of two moving mirrors, and the position of the laser ablation can be moved by changing the angle of the mirrors. With the original Galvanometric

scanner, ablation points can be jumped with a time interval of 1 ms, and laser ablation can be made from >1x1 mm within 1 sec duration, and therefore, the simultaneous laser ablation for two or more solid materials can be obtained. The sample aerosols released from two or three samples are well mixed in the laser ablation cell, and the mixed aerosols are introduced into the ICP. With the multiple spot laser ablation (msLA) protocol, spiking of internal standard elements, dilution of analytes, and calibration using the standard addition method can be applied, just like solution-based ICP-MS technique (Yokoyama et al., 2011; Makino et al., 2019). To take a full advantage of the solid sample mixing protocol, the new Yb:KGW femtosecond laser and faster response Galvanometric scanner was introduced in our ICP-MS facility. With the advanced msLA system, now the laser ablation can be made from large-areas covering >20x20 mm within 1 sec duration. With the better signal-to-noise ratio of the analytes, now we can monitor the ultra-trace elements (< 1 ppt in solid materials) directly from solid materials without any decomposition and dissolution procedures, obviating the risk of contamination or loss of analytes through the time-consuming chemical separation/purification procedures.

With the Galvanometric optics, a standard addition method can also be applied by mixing of aerosols released from sample and standard references. The mixing ratio [sample : standard] can be controlled by changing the number of laser shots for each component. Abundances of rare earth elements (REE) in natural zircon samples can be determined by the standard addition technique by mixing of REEs from glass standard material (Yokoyama et al., 2011). The Galvanometric optics can provide flexible calibration protocols for elemental analysis, where no proper (matrix-matched) calibration standard is available. In order to advance the application of this new laser ablation technique, we have started the product development of the device in 2018 and its commercialization in 2019.

One of the applications of the msLA technique is the elemental analysis of solid powder samples. Many solid samples have a heterogeneous composition and in order to extract information on their average chemical composition, they are homogenised by grinding, atomising and mixing. Powder X-ray fluorescence spectroscopy has been used as a benchmark method for the elemental analysis of powdered samples. However, X-ray fluorescence spectroscopy is limited to the analysis of the major components, making it difficult to analyse trace-elements. At present, the analysis is limited to standard rock samples, but we have succeeded in improving both the precision (repeatability) and accuracy (accuracy) of the analysis by obtaining a stable signal using fast multi-point ablation with a femtosecond laser (Fig. 13).

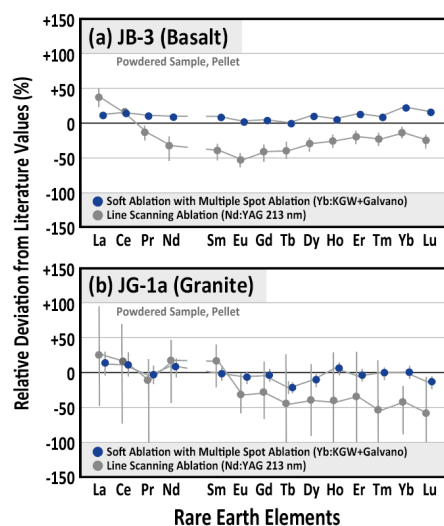


Fig. 13 Direct Analysis of REEs from powdered rocks

The reason for the improved signal stability can be attributed to the smaller size distribution of the aerosol particles introduced into the ICP. Femtosecond lasers apply energy to solids in a shorter time (200 fs) compared to conventional nanosecond lasers (oscillation time of 5-20 ns). This high energy density fractures the solid particles to a smaller size and allows for stable ionisation in ICP. Thus, in the elemental analysis of powder samples, the fast multi-point ablation method can contribute to the improvement of analytical sensitivity, accuracy and precision. At present, this method is applied to the analysis of geochemical samples, but in the future, it can be applied to the trace element analysis of electronic device materials such as SiC, SiN, GaO, GaN, etc. (these materials are insoluble even in aqua regia), and we would like to expand its application to the field of materials science, where more sensitive and rapid analysis is highly required.

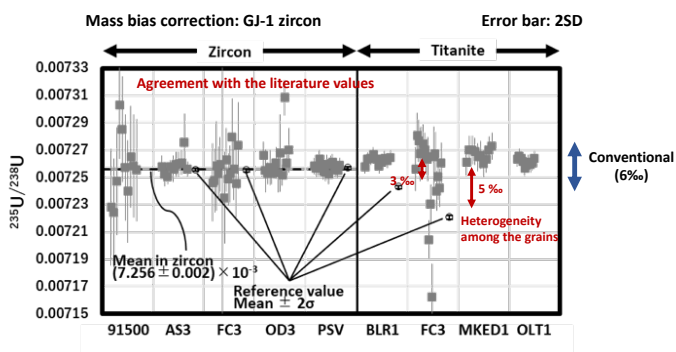


Fig. 14 Precise $^{235}\text{U}/^{238}\text{U}$ ratio analysis for zircon and titanites using the continuous ion monitoring (CIM) protocol.

2.3 Improvements in current measurement methods for ion detectors

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The Faraday detector is the most widely used ion detector in precision isotope analysis because of its long time stability and high signal input output linearity. In the Faraday detector, the current generated by the incident ions is measured as a voltage using a high-magnification amplifiers utilising high impedance registers (e.g., 10^{10} - $10^{11}\Omega$). Recently, measurement systems using further high-magnification amplifiers utilising 10^{12} - $10^{13}\Omega$ feedback resistors were described. With the amplifiers utilising the $10^{13}\Omega$ feedback resistors, the higher output voltage being about 100 times can be obtained than the conventional Faraday detectors utilising $10^{10}\Omega$ feedback resistors, resulting in better precision or repeatability of the isotope ratio measurements.

Despite the obvious success in obtaining the high precision with smaller sample sizes, major drawbacks of the technique is the slow response to time changes of the ion flux. To take a full advantage of the high-gain amplifiers, stable signal intensity profile is key issue. Small changes in the signal intensities can cause systematical errors in the measured isotope ratios. To minimise the effect of slow response of the Faraday amplifiers, the tau correction has been widely adopted. The tau correction adds or subtracts a constant signal proportional to the amplification or decay rate of the signal. With the tau correction, the response of the Faraday detector can be improved by a factor of several tens. However, scrutiny of the effect of signal intensity change onto the measured isotope ratio data, a simple tau correction does not fully compensate for the signal changes, and for larger signals, an overcorrection (overshoot) occurs, causing systematic errors in isotope ratio analysis (Hirata et al., 2003).

We have shown experimentally that the delay in the signal output is the same for increasing and decreasing signals, and that the number of ions lost when the signal increases and the number of ions overcorrected when the signal decays are the same. With the empirical corrections, the quantitative nature of the ion monitoring can be greatly improved. However, systematical errors in the measured isotope ratios cannot be corrected on the transient signals found in laser ablation of short time period (e.g., single shot analysis) or in signal events emanating from nanoparticles.

To overcome this, we have developed new signal integration protocol. The systematical errors due

to the slow response of the Faraday amplifiers can be dramatically reduced by integrating the ion signal over the entire time range from appearance to abstraction (continuous ion monitoring technique : CIM technique). Important point is that the technique does not require the tau correction, obviating the contribution of systematical errors originating the erroneous tau values. Moreover, the CIM technique allows accurate isotopic information to be extracted from unstable or transient signals. Using this method, it is possible to accurately measure the $^{234}\text{U}/^{238}\text{U}$ and $^{235}\text{U}/^{238}\text{U}$ ratios even from transients obtained from laser ablation, and to understand the small variations in nature (Fig. 4).

3 Prospects

3.1 Trends in the development of dating methods

We have been conducting U-Pb dating of zircon using the laser ablation-ICPMS technique. The research community is still growing up and more than 150 papers have been published in collaboration with domestic and international researchers. In order to respond to further diverse research requirements, we are also working on hardware developments aiming at dating of younger zircons (e.g. below 0.1 Ma) and dating on other various minerals.

(a) Dating of younger zircons

In young samples, the number of ^{206}Pb atom produced through the radioactive decay of ^{238}U becomes lower, so the detection of ultra-trace amounts of Pb isotopes is still key issue to derive accurate age information. To do this, new generation MC-ICP-MS instrument (Nu Plasma II from Nu Instruments) was installed in 2015, which utilising five high-gain ion detectors (Hattori et al., 2017). However, the sensitive ion detector used in this instrument, the electron multiplier (EMP), has a limited analysis range (dynamic range), and thus the system is not capable of the dating of young zircons. For example, young zircons (e.g., 100,000 years old or younger) have $^{206}\text{Pb}/^{238}\text{U}$ ratios of smaller than 0.00001, and further two significant digits of isotope ratio data is required to obtain reliable age data.

The problem of the EMP is that when strong ion signals ($>10^6$ cps) is measured for long time duration (e.g., > 1 hour), the amplification gain can change noticeably, and the reliability of the age values obtained is greatly reduced. We then turned our attention to the Daly detector, which developed in the 1970s. Daly detector has several unique features: (i) better long-term stability for over 10 hours, (ii) capable of detection of high-ion currents over 10^7 cps, and (iii) a simple non-expansive correction method can be applied to correct for counting off due to dead time (Hirata et al., 2016a; Kobayashi et al., 2017).

In collaboration with Nu Instruments (Wrexham, UK), we first introduce three Daly detectors onto our MC-ICPMS instruments installed at the University of Tokyo. Currently, ICPMS instruments equipped with multiple Daly detectors are becoming popular as one of the benchmark instruments for U-Th-Pb dating analysis.

In the U-Th-Pb dating method, Pb is not directly produced by the decay of U and Th. Various radionuclides are present during the decay process, each with a specific half-life, and lead (^{206}Pb , ^{207}Pb , ^{208}Pb) is produced by successive radioactive decay (radioactive decay series). Since the intermediate nuclides have different chemical properties from U and

Pb, the isotopic equilibrium is not maintained during crystallisation of minerals. In particular, in the ^{238}U radioactive decay series, ^{230}Th , which has a long half-life, undergoes fractionation and becomes present at a level different from the radiogenic equilibrium. Correction of this isotopic disequilibrium is important to derive reliable age data from young zircons.

The level of isotopic disequilibrium (magnitude of deviation from isotopic equilibrium) can be quantified by the differences in the partitioning for U and Th between magma and zircon (i.e., the difference in the partition coefficient). We developed new correction method for isotopic disequilibrium that assumes that (i) the radioactive equilibrium of the uranium decay series is achieved in the source magma from which zircon crystallizes, (ii) the $^{234}\text{U}/^{238}\text{U}$ ratio does not change between magma and zircon (^{238}U and ^{234}U remain in radioactive equilibrium), and (iii) the Pb incorporated during zircon crystallization is zero ($\text{Pb}^*=0$). Based on the correction method and a mass spectrometry technique with a new ion detector, it is now possible to date zircons to tens of thousands of years old. This "multi-chronology", which combines zircon U-Pb ages with FT and Ar-Ar ages, has led to a detailed age analysis of the Sangiran area and a breakthrough in the evolution of Homo erectus erectus (Java Protohuman) (Matsu'ura et al., Science, 2020).

(b) Dating of a wide range of mineral species

We are still modifying our analytical instruments to derive further reliable age data from zircons with various age ranges. In recent years, however, there has been a rapid increase in the scientific demand to extract age information from a wider variety of minerals. This is because the combination of ages from different closure temperatures can provide insight into the thermal history of the geology. At present, our group is preparing to carry out new U-Th-Pb age analyses from carbonates, apatite, monazite and garnet using laser ablation ICPMS. Recently, the ETH in Switzerland and the University of California in the USA have been vigorously developing dating methods. We are similarly working on mineral diversification, but not only that, we are also working in parallel to develop new dating methods that can be applied to decipher thermal history.

The U-Th-Pb dating method for zircon has the highest closure temperature of any dating system (Fig. 15). The high closure temperature makes it less susceptible to secondary denaturation and allows us to extract information on the age at which the sample was formed. On the other hand, age information at different closure temperature is important to elucidate the evolution of magma chambers, the age of sediment formation, and the thermal history of various geological events. For this purpose, it is important to combine dating methods with different closure temperatures. For the past five years, Iwano and Danhara et al. at Kyoto Fission Track and we have been trying to decipher the thermal history of zircons by combining the Fission Track Age (FT age) of zircons with the U-

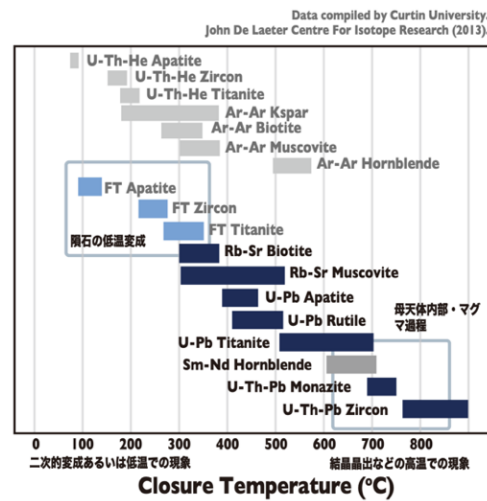


Fig. 15 Summary of Closure temperature of the isotopic chronometers (after John De Laeter)

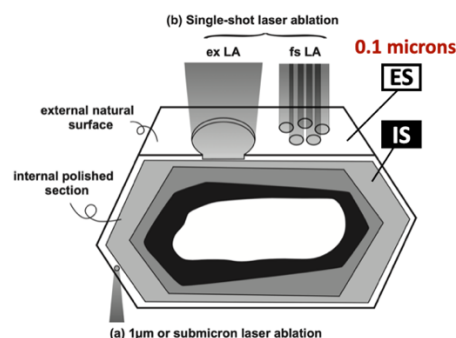


Fig. 16 High-resolution (<0.1 μm) depth profiling analysis of U-Pb ages for zircon multi-chronology studies (after Iwano et al., 2021)

Th-Pb age of zircons at lower closure temperatures (multichronology). Noting that the resulting U-Pb age of the zircon epidermis (<1 μm thick) is young (Fig. 16) and harmonic with the FT age, Iwano et al. have been actively analyzing the age of the zircon epidermis. The combination of this method with U-Th-Pb dating of zircon cores, rims, and epidermis, and comparison with FT ages, has the potential to decipher the growth process (thermal history) of zircon. In the future, we would like to apply this method to decipher the thermal history of magmas through systematic age analysis of zircons.

(c) Metallomics

Metallomics on the level of single cells is an ultimate goal in the development of the field. Since every single cell is treated as an individual entity, or particle, the methodology of NP analysis using ICP-MS can be applied for the analysis of elemental analysis of cell samples. Combination of the high-time resolution data acquisition and mass scanning achieved by time-of-flight mass

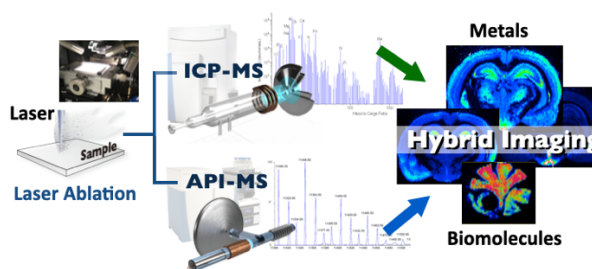


Fig. 17 Hyphenated Technique for Metallomics Study

spectrometers has blossomed ICP into becoming the key technique for a wide range of studies in Metallomics. However, we should not be too hasty to open the ceremonial champagne, as there remain many problems to be solved in order to make LA-ICP-MS a truly routine and mature analytical tool. Basic experimentation still remains crucial, and the struggle continues as we still face issues in obtaining reliable data from samples of small sizes or small areas. Moreover, the argon plasma used in the ICP is so powerful that that no species information on the introduced molecule remains, which is a shame because LA-ICP-MS has played such an important role in the frontiers of Metallomics. Therefore, we are trying to develop completely new ion source to couple with LA for the organic mass spectrometer, aiming for simultaneous imaging analysis of metal and biomolecules from a single sample (i.e., hybrid imaging analysis). The most significant advantage of the hybrid imaging analysis is that information concerning possible functional linkage between metal ions and biomolecules can be derived. The technique is also applied to derive clues for designing molecules in DDS (drug delivery system) studies.

One of the advantages of the laser ablation-ICPMS method is its ability to analyse the distribution state of a wide range of elements, from major to minor, in a solid sample (Imaging Mass Spectrometry). We have performed imaging analysis on rocks, minerals, fossils and biological tissue sections, and have developed and distributed free visualisation software (iQuant2) to make imaging analysis accessible to all. For these achievements, we are awarded the Best Paper Award of the Mass Spectrometry Society of Japan in 2017, 2021, and the Japan Society for Analytical Chemistry Award in 2020.

While the laser ablation ICPMS method can be used for the elemental mapping analysis (elemental imaging), the technique is not capable to derive the information concerning the status or chemical form of the elements. This is a major problem in environmental and biochemical research. For example, the toxicity of an element varies greatly depending on its chemical form. Inorganic mercury is relatively low in toxicity, whereas methylmercury is highly toxic. Inorganic arsenic is highly toxic, whereas methylated arsenic is less toxic. Iron, copper and zinc are essential elements in living organisms and are mostly present in the form of proteins. However, in their free form (elemental ions) they are known to be highly toxic to the body. For the evaluation of the behaviour of elements in living organisms (e.g. metabolic mechanism, function or toxicity), information on chemical forms is essential. For this reason, in addition to information on the distribution and concentration of

elements, information on the chemical form of the element or the coexisting proteins is important in modern metallomics research.

Extracting information from both elements and biomolecules (hybrid imaging) requires the ionisation of both elements and biomolecules. For elemental analysis, "hard ionisation" using ICP ion sources is required to improve quantification, and for biomolecular imaging analysis, "soft ionisation" is required to reduce fragmentation of the biomolecules. However, a soft ion source specifically designed for the laser ablation method has not been commercialised so far, which is why our research group decided to develop our own. The newly developed atmospheric pressure ion source is shown in **Fig. 20**.

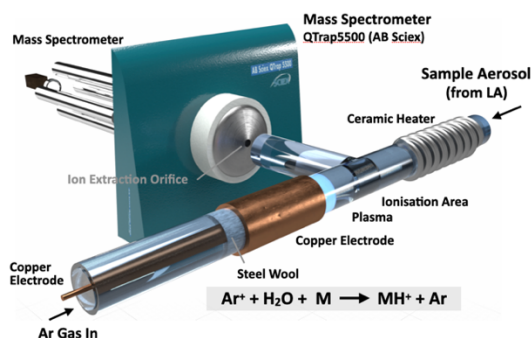


Fig. 20 New Soft Ion Source for Imaging Analysis of Biomolecules

This new ion source utilising a barrier discharge to ionise Ar, which collides with biomolecules such as amino acids, sugars and peptides emitted from the sample aerosol produced by the laser ablation method. Ion source based on the barrier discharge has several unique features such as (i) atmospheric pressure ion source, and thus the ion source is acceptable of dry aerosols produced through the laser ablation, (ii) very high excitation temperature, and (iii) low kinetic temperature, obviating the risk of decomposition of organic compounds. Through our preliminary results, we found that the $[M-H]^+$ base peak (the strongest ion signal) was obtained from most of the amino acids, sugar and vitamins, indicating that the molecules could be ionised without destruction (fragmentation).

The ion source developed here is also powerful enough to ionise the biomolecules in sample aerosols generated by the laser ablation technique, and thus, imaging analysis of organic compounds can be made by the identical data processing procedure established in the elemental imaging analysis using the laser ablation-ICPMS technique.

The resulting imaging data for some amino acids, sugars and vitamins obtained from a section of a commercial pellet (sugar-coated tablet) are shown in **Fig. 21**. It is clear that the sugar (sucrose) is present on the surface of the tablet due to the fact that it is a sugar-coated tablet. More importantly, the imaging results suggest that various amino acids and vitamins are not homogeneously distributed, but rather forms the clusters. It should be noted that the ionization efficiency for B6 is very low (Hui Hsin et al., submitted to Metallomics Research). Combination of newly developed ion source and the ICPMS technique, we expect to be able to perform simultaneous imaging of elements and biomolecules (hybrid imaging).

For the study of brain (e.g. brain tissue), fully integrated database for connecting the gene, protein, and metabolomes has already been established. A typical example is the Allen Brain Atlas. This database allows us to visually understand what genomes are expressed and what proteins are distributed in specific parts of the brain, and it also allows us to perform reverse lookups (i.e., to

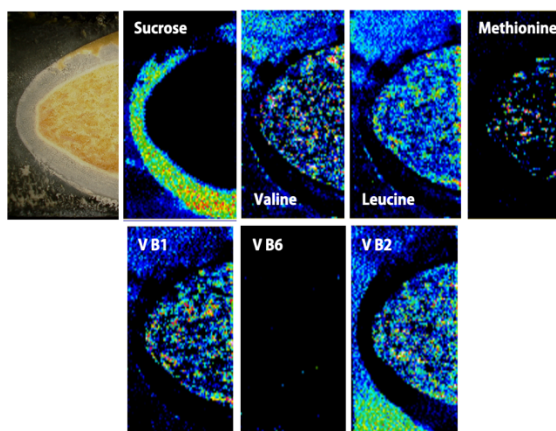


Fig. 21 Resulting Imaging of Amino Acids, Vitamines, and Sugar obtained by Laser Ablation with in-house Organic Mass Spectrometer

identify a location in the brain based on a specific genome sequence or protein), making it a powerful tool for linking genomics, proteomics and metabolomics. However, it should be noted that distribution of metal elements are very important to understand the full details of genomics, proteomics, and metabolomics. This is the major drawback of the Allen Brain Atlas. Hybridisation of elemental data with other omics data on the Allen Brain Atlas can accelerate the integration of various omics studies. We expect that the hybrid imaging system we are developing will play an important role in this purpose.

(d) Nanoparticle analysis

The newly developed ion detectors can be applied for the detection of ultra-fine particles (nanoparticles) using the ICP-MS technique. Ultra-fine particles are defined as particules with sizes ranging from 1 and 1,000 nm. It is widely recognised that physico-chemical nature of the solid materials can change dramatically with decreasing the size. This is mainly due to changes in the proportion of atoms present on the particle surface increases. As the size of the particle decreases, the proportion of atoms on the surface of the particle increases, so the physical properties of the particle are controlled mainly by the surface properties rather than the crystal structure of the constituent atoms. For example, the melting point of metallic Au can be lowered when size of Au particles becomes <50 nm, suggesting that melting point is no longer extensive variable. Furthermore, due to the quantum size effect, ultrafine particles exhibit specific chemical properties, reactivity, and colors, and this specificity has led to a wide range of applications in various research fields. On the other hand, the rapid expansion of the use of ultrafine particles has accelerated their release into the environment and their exposure to living organisms, raising concerns about their impact on the environment systems. This suggests that investigation of environmental dynamics and toxicity of the NPs is highly desired. To do this, we are trying to develop new analytical technique for fast and sensitive detection of NPs using the ICP-MS technique.

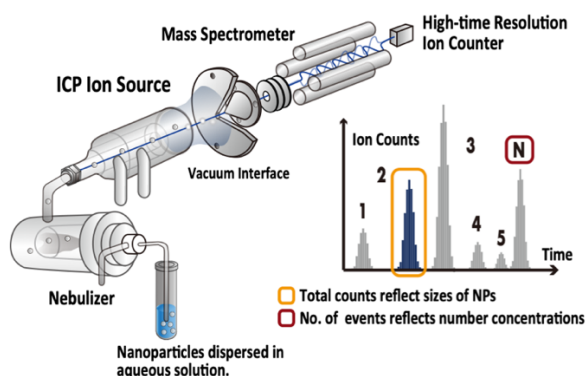


Fig. 22 Detection and size analysis of nanoparticles using high-time resolution ICPMS

Analysis of NPs using the ICP-MS is based on the powerful ion source achieved by both the high kinetic temperature and excitation temperature. When ultrafine particles are introduced into the ICP, the ultrafine particles are effectively vaporised, atomised and ionised within the ICP, forming a ion cluster (ion cloud) of constituting elements. The ion cloud is extracted into the mass spectrometer, and the ions are detected as a signal pulse (signal events) (Fig. 22). Typical time duration of the signal spike (corresponding to a single ion cloud) is about 0.2–0.6 ms, and thus, the high-time resolution ion detector is desired to obtain quantitative data. In the conventional ICPMS, data acquisition has been made with the time resolution of about 10 to 100 ms, whereas higher time resolution (i.e., shorter dwell time such as <0.03 ms) is required for the quantitative analysis of size of NPs. The signal intensity (total ion counts) of the signal events is basically proportional to the size of the NPs (volume of the NPs is proportional to the cube of the diameter), while the number of signal events per unit time is proportional to the concentration of the number of ultrafine particles in the sample solution, so the ICPMS method can be used to measure the composition, size and number concentration of the NPs in the analytical solution.

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In order to extract accurate size information of the NPs using the ICPMS method, the signal output linearity of the ion detector is still key issue. For example, there is a million-fold difference in the

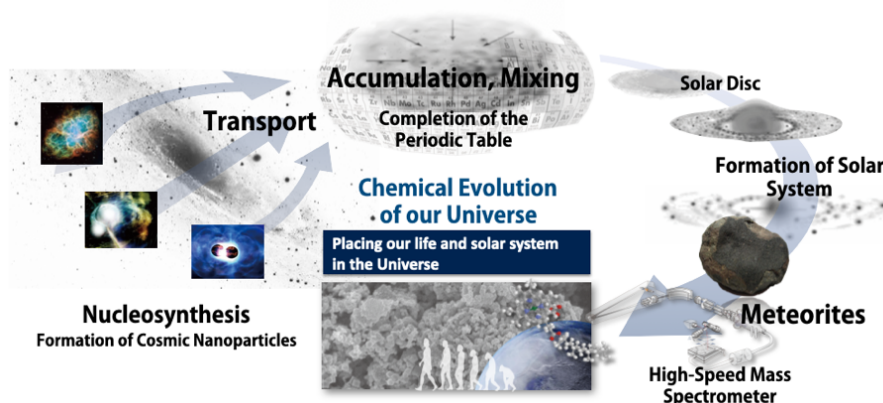


Fig. 23 Chemical Evolution of our Universe decoded by the Space Nanoparticles

measured ion counts between an 5 nm and 500 nm NPs. In other words, if one may obtain the 10 counts signal from 5 nm NPs, the measured counting exceeds 10^7 counts for a 500 nm NPs. With the conventional ion counting system, measurement of high ion currents being $>5 \times 10^6$ cps can be erroneous mainly due to (a) changes of detector gain, and (b) contribution of counting loss due to detector dead time, and thus, great care must be taken to achieve reliable size analysis of NPs. To address this problem, our group uses a platinum filter (grid) in front of the ion detector, and when measuring signals with high count rates, the signal is reduced by passing it through the platinum grid (attenuator method). The magnitude of attenuation can be controlled by changing the porosity of the platinum grid, and the attenuation gain of 1/100 or 1/500 was adopted in our analysis (Hirata et al., 2013; Sakata et al., 2014). When the attenuation gain of 1/100 was employed, signals of count rate being 10^7 cps can be suppressed down to 10^5 cps, and thus, the contribution of counting loss due to the detector dead time becomes negligibly small. Important feature of the attenuator is that switching on and off of the attenuator can be made within time period of <1 ms, allowing the user to decide whether turn on/off the attenuator during the fast mass scanning. This means that only certain elements and isotopes can be suppressed by the attenuator during the analysis sequence. The new ICP-MS system equipped with the high-time resolution ion counter is flexible enough to measure size of the NPs ranging from 5 to 400 nm. This range covers nearly whole range of the metal NPs present in nature, and thus, we expect that the system can be applied for source identification of various naturally occurring NPs, including PM_{0.1}.

The analysis of NPs using the ICP-MS is becoming increasingly popular due to its improved analytical sensitivity, analytical repeatability, and user friendly system setup. However, chemical composition (analysis of the abundance of constituent elements) and isotopic composition analysis of the NPs is difficult with conventional ICPMS equipment. As mentioned above, the signal events originating from single NPs are transient (pulsed duration of about 0.2-0.6 ms). Since this signal duration is shorter than the typical mass scanning rate (around 1 ms), only single element (isotope) information can be extracted from a single signal event (a single NPs). This means, in other words, that ICPMS cannot provide information on the elemental composition of NPs or the isotopes of their constituent elements. The inability to analyze the elemental or isotopic composition of NPs is fatal to the development of NPs analysis in the fields of geochemistry, environmental chemistry and biochemistry. Therefore, our research group has modified a multi-detector ICPMS system (MC-ICPMS system).

With the multiple detectors system configuration, it is possible to extract isotopic information from transient signals emanating from single NPs. However, conventional ion detectors have a signal integration time of 0.1 s (about 100 ms) and thus the system cannot follow the fast changes in the signal intensities (transient signals). In collaboration with Nu Instruments, we have developed a new

ion counter that improves the time resolution of four (now five) high-gain ion detectors, enabling ion measurements with up to 100,000 data readouts per second (integration time of 10 μ s). Here, we have also developed our own data processing software (NanoQuant), which can handle data volumes of up to 20 MB per second and enables isotopic analysis of elements (Suzuki et al., 2019).

This system is currently the only ICP-MS instrument in the world capable of precision isotope analysis of nanoparticle constituents. We performed $^{195}\text{Pt}/^{194}\text{Pt}$ ratio analysis on various sizes of platinum ultrafine particles (about 1,000 Pt nanoparticles of 30, 50 and 70 nm each) and found that the isotope ratio analysis accuracy was $\pm 30\%$ (2σ) for 30 nm, $\pm 20\%$ (2σ) for 50 nm and $\pm 10\%$ (2σ) for 70 nm. The resulting precision of the isotope ratio measurements is consistent with the uncertainty estimated based on the counting statistics of the ^{194}Pt and ^{195}Pt signals, and it is clear that the uncertainty in the isotope analysis obtained is mainly controlled by the counting statistical associated with the signal intensity (Hirata et al., 2019b; Yamashita et al., 2020; Hirata et al. Hirata et al., 2021). With the instrument developed here (high-time resolution multiple collector-ICP-MS: HTR-MC-ICPMS), we expect to be able to analyze the chemical composition and Pb isotopes of nanoparticles in environmental samples, such as PM_{0.1} and other fine aerosols, and to draw new insights into the formation and transport processes of aeolian dust.

In the present HTR-MC-ICPMS system, the precision and accuracy of the isotope ratio measurement is about ± 10 -20%. Little poor precision of the isotope ratio measurements may limit its application to the practical environmental samples. However, this analytical accuracy is sufficient for the research we are currently undertaking.

We are currently working with Dr. Tetsuya Yokoyama (Tokyo Institute of Technology) and Dr. Takuji Tsujimoto (National Astronomical Observatory of Japan) to detect fine particles originating outside the Solar system that would be produced during the formation of the elementals (pre-solar grain). The number fraction of the pre-solar particles is about 1/100,000 or even less than that in the ultrafine particles (matrix particles) formed in our solar system. The detection of pre-solar particles, which store the formation processes of heavy elements, requires elemental and isotopic analysis of a significant number of particles, making it a very risky "treasure hunt". The ICP-MS method, however, allows the analysis of about 100 particles per second (about 10^7 particles per day). By making the most of the high analytical throughput of the ICP-MS method, it is hoped that it will be possible to extract low abundance pre-solar particles (**Fig. 23**). We are currently to measure $^{196}\text{Pt}/^{195}\text{Pt}$ and $^{193}\text{Ir}/^{191}\text{Ir}$ isotope ratios from over 10^7 ultrafine particles in meteorites, and four grains of total 10^7 grains had significantly different $^{196}\text{Pt}/^{195}\text{Pt}$ and $^{193}\text{Ir}/^{191}\text{Ir}$ isotope ratios from both the terrestrial and chondrites materials. More importantly, the measured isotope ratios for both the Pt and Ir were also departed from those for the elements/isotopes produced through an s-process (slow neutron capture reaction). This suggests that the Pt and Ir presented in the four fine particles can be originating from the material produced by the r-process (rapid neutron capture reaction). We are keep trying to detect the isotopic anomaly from the meteoritic NPs.