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The Measure of Confidence

Agilent Technologies

Characterization of Single Cell Multiplex Proteins using ICP-QQQ

Amir Liba, Jonathan Wanagat

Agilent Technologies Inc., USA and UCLA, CA, USA

Introduction

To better understand aging and complex diseases such as heart disease, stroke, and cancer, a single cell approach is necessary. Current methods for assaying protein levels within tissues rely on various molecular biology techniques (e.g., Western blots) that require milligram to gram quantities of tissue homogenates or immunofluorescent (IF) techniques that are limited by spectral overlaps. Novel techniques are required to bring protein measurement sensitivity to the single cell level and offer spatiotemporal resolution and scalability. Here we describe a new approach to protein measurement by exploiting the inherently low concentration of rare earth elements (REE) in biological systems. By coupling REE-antibody immunolabeling of cells with laser capture microdissection (LCM) and ICP-QQQ, multiplexed protein measurement in histological sections of single cells is possible. This approach will complement evolving single cell techniques and our ability to understand cellular heterogeneity in complex biological systems and diseases.

Materials and methods

Tissue staining with REE

Immunolabeling of mouse quadriceps femoris muscle samples with ^{141}Pr, ^{171}Yb, ^{167}Er and ^{103}Rh (DNA intercalator) was performed as described previously [1]. Stained slides were dehydrated quickly through an ethanol and xylene series and dried completely before LCM.

Laser capture microdissection (LCM) of individual muscle fibers

Single skeletal muscle fibers were captured using a Leica LMD7000 laser dissecting microscope into individual wells of a 48-well microslide (Figure 1). The cells were digested with 1uL of 70% nitric acid and digested samples taken up in 9uL of water. Samples were stored at 4°C until analysis by triple quadrupole ICP-MS (ICP-QQQ).

ICP-QQQ analysis of REE-immunolabeled muscle fibers

Ten uL samples were either injected into the Agilent 8800 ICP-QQQ via a syringe pump or by an Agilent 1260 Cap-LC at a flow rate of 10 uL/min. The ICP-QQQ was equipped with a low-flow nebulizer and a total consumption spray-chamber. Sample introduction parameters are given in Table 1. Sensitivity and ion lens voltages were auto-tuned via MassHunter 4.1 software using 1 ppb tune solution.

Table 1. 8800 sample introduction parameters

RF power	1550 W
Carrier gas	1 L/min
Dilution gas	0.15 L/min
Sampling depth	8 mm
TRA mode	0.2 sec integration time

To ensure the entire sample was delivered to the ICP-QQQ, the injected samples were bracketed by air injection.

Statistical analysis

All data with normal distribution were presented as means ± SEM (Standard Error of the Mean) calculated with GraphPad Prism software.

Results and Discussion

REE and protein measurements by ICP-QQQ

Table 2 shows the ICP-QQQ data obtained from the analysis of five representative single muscle fibers.

The highest counts were detected for ^{141}Pr and ^{103}Rh that correspond to single cell myosin and DNA content, respectively, while lower counts were detected for ^{167}Er and ^{171}Yb that correspond to single cell IL-6 and GAPDH content (Figure 2). The variability in ^{141}Pr (myosin) signal is likely due to fiber size, while the variability in the ^{103}Rh (DNA) signal is likely due to differences in nuclei number between different cells. The presence of REE in biological samples is extremely low, resulting in negligible ICP-QQQ background from REE. The average signal-to-noise ratio across the five samples and the four REE tags in Table 2 was 85. Captured muscle fibers that were not immunolabeled with REE showed no detectable signals (data not shown).

We are developing protocols to normalize the REE counts to cell size or other housekeeping proteins as is routinely done with protein detection in Western blots.
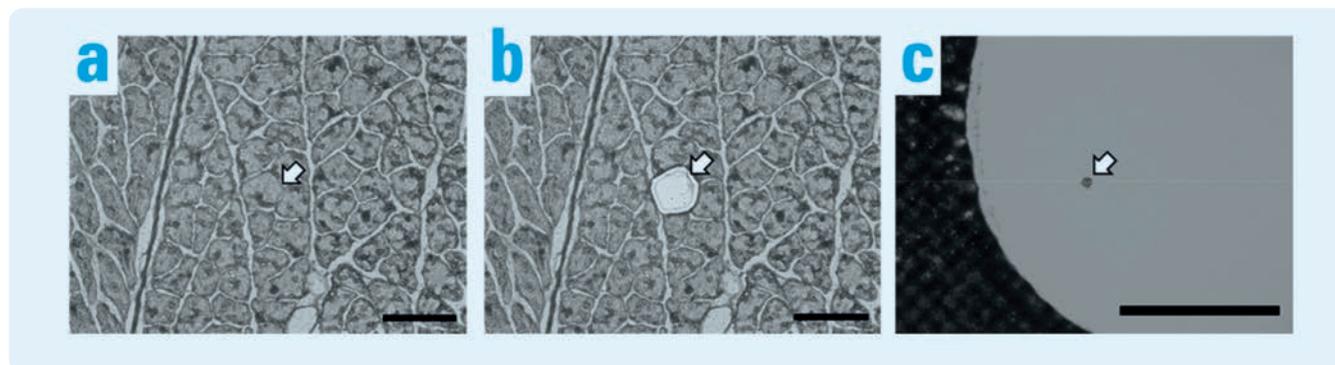

Figure 1. Laser capture microdissection of an individual skeletal muscle fiber before capture (a), the tissue section after capture (b) and the captured fiber (c). The arrows denote the targeted fiber (a), the selective laser cutting of only the targeted fiber (b) and the captured fiber (c). Black bar is 50 microns (a and b) and 500 microns (c)

Table 2. ICP-QQQ data from single, REE-labeled muscle fibers

REE label	Fiber 1	Fiber 2	Fiber 3	Fiber 4	Fiber 5	Average counts[b]
[141]Pr-Ab-Myosin	2562[a]	2726	2835	3198	1976	2659 ± 200
[167]Er-Ab-IL6	178	273	345	299	233	266 ± 28
[171]Yb-Ab-GAPDH	105	126	160	138	110	127 ± 10
[103]Rh-DNA	877	712	1482	776	1132	996 ± 141

[a]Data are represented as counts for each respective m/z analyzed (103, 141, 167, and 171).
[b]Average counts for each REE label ± SEM.
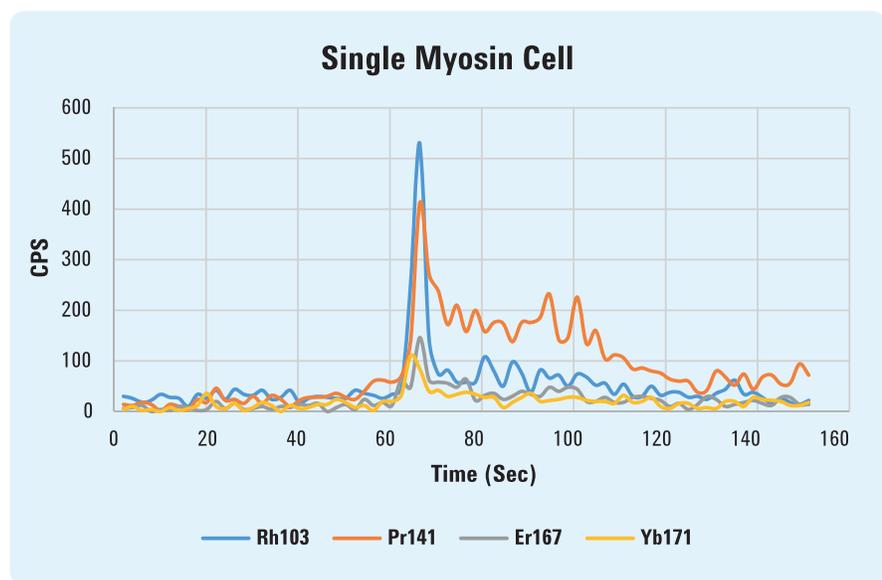
Figure 2. Time resolved spectrum showing the non-chromatographic signal from a single muscle fiber cell.

Conclusions

Our approach to measure single cell protein content using REE immunolabeling, LCM and ICP-QQQ is based on combining standard and emerging techniques in order to facilitate studies in a wide variety of biological samples at the single cell level. Together with other single cell analyses, this approach will help uncover heterogeneous cellular events in complex diseases and add to an integrated view of the underlying pathology.

References

1. Wanagat J, Cao Z, Pathare P, Aiken JM (2001), Faseb J 15 (2):322-332

7900 ICP-MS Automated Method Wizard used for Analysis of Food CRMs

Kazuhiro Sakai, Junichi Takahashi and Ed McCurdy

Agilent Technologies Japan and UK

Introduction

In food analysis, as the potential toxicity of trace elements becomes better understood, the trend is for a wider range of elements to be monitored, and for the regulated levels to decrease. As a result, many food laboratories are now considering or already using ICP-MS for these measurements.

The implementation of ICP-MS creates several challenges for laboratories that are currently using more traditional and well-established techniques for metals analysis. Trace level analysis requires tighter control of general laboratory practices to ensure that the low detection limits can be achieved routinely. ICP-MS is also generally considered more difficult to learn and use than the well-established ICP-OES and AAS techniques, especially in the more complex method development for new sample types. Selection of suitable analyte isotopes and internal standard elements requires some experience, while identifying and overcoming potential interferences requires an understanding of mass spectrometry. The recent introduction of collision reaction cell (CRC) technology for ICP-MS makes it possible to eliminate most common polyatomic interferences that occur in the samples typically encountered in a food laboratory, but choosing the optimum cell gas conditions for trace levels analysis of foods also requires some expertise.

The Method Automation function of ICP-MS MassHunter 4.1 simplifies method development, making it easier for users of all levels of experience to develop reliable methods for their particular sample types.
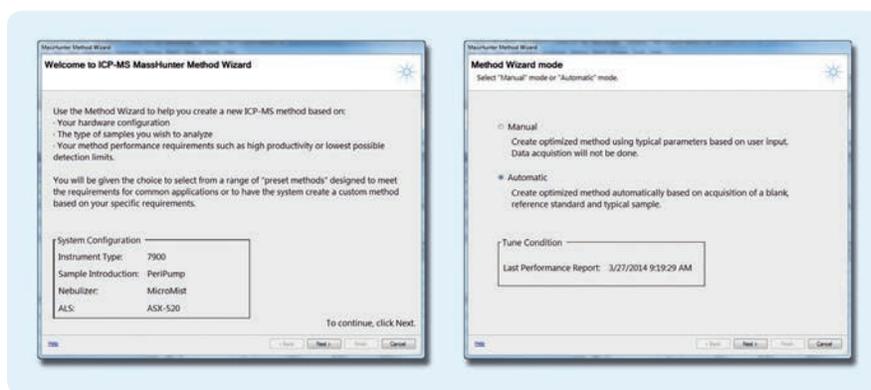

Figure 1. Method summary and hardware configuration are displayed, and operator selects Method Wizard mode: Manual or Automatic (7900 only)

Experimental

Instrumentation and Reagents

All analyses were carried out using an Agilent 7900 ICP-MS with Ultra High Matrix Introduction (UHMI) and H_2 cell gas options, and a standard sample introduction system consisting of a glass concentric nebulizer, quartz spray chamber and quartz torch with 2.5 mm internal diameter injector. The standard Ni-tipped interface cones were used. An Agilent ASX-520 autosampler delivered the samples, which were held in 50 mL vials. For digestion of food CRMs, a Milestone ETHOS 1 Advanced Microwave Digestion System was used. Details are given in Reference [1].

The CRMs used for this study were DORM-4 (fish protein) from NRC-CNRC, CRM 7402-a (cod fish tissue) and CRM 7403-a (swordfish tissue) from National Metrology Institute of Japan (NMIJ). High purity reagents 68% HNO_3 and 36% HCl (Ultrapur-100 grade) were purchased from Kanto Chemicals, Japan. The calibration solution was prepared from Agilent Mixed Stock Standard (4183-4682) with Hg and Sn added from single element standards (SPEX Certiprep).

Method Building

The "Method Wizard" function of ICP-MS MassHunter can operate in either manual or, for the 7900 ICP-MS, fully automatic mode (Figure 1). In manual mode, available for all supported Agilent ICP-MS mainframes, the user is prompted to enter some information about the total dissolved solids (TDS) concentration of their samples and to define the analyte elements. The Method Wizard then optimizes the acquisition for either fastest sample

throughput or lowest possible detection limits. The manual mode is suitable for familiar sample types when the operator has some expertise in ICP-MS and is mainly interested in simplifying the task of method development.

In automatic mode, the Method Wizard selects the most appropriate plasma mode and tuning conditions, analyte isotopes, integration times, cell gas modes and assigned internal standards. These selections are based on the current instrument configuration and the composition of the user's typical sample, which is measured as part of the automated process. The standard 7900 with general purpose plasma conditions and He cell mode provides analytical results that meet the requirements for routine food analysis. However, in this case, the 7900 included the UHMI and H_2 cell gas options, so operating conditions that use these options were automatically selected where they offered improved performance.

Once all required information (location of tuning, calibration and unknown sample solutions, composition of calibration and ISTD stocks, and so on) has been entered, the method building starts automatically.

From the measurement of the tuning solution, the sample uptake and rinse times are calculated. The TDS level and major element composition are derived from the results of the semi-quantitative analysis of the typical sample. The measured TDS level is used to determine the appropriate plasma mode (Low Matrix, General Purpose, UHMI aerosol dilution factor), and the major element composition is used

Table 1. Analytical results of food CRMs measured using method created by ICP-MS MassHunter's Method Wizard

Analyte	Unit	DORM-4 (fish protein) Concentration	Certified	7402-a (cod fish tissue) Concentration	Certified	7403-a (swordfish tissue) Concentration	Certified
9 Be [No gas]	mg/kg	0.01 ± 0.00		N.D. (<0.0008)		N.D. (<0.0008)	
23 Na [No gas]	g/kg	12.9 ± 0.3		3.4 ± 0.1	3.6 ± 0.2	3.57 ± 0.07	3.57 ± 0.12
24 Mg [No gas]	g/kg	0.81 ± 0.01		1.29 ± 0.03	1.34 ± 0.03	1.60 ± 0.03	1.58 ± 0.04
31 P [HEHe]	g/kg	7.6 ± 0.2		10.8 ± 0.1	12	14.5 ± 0.2	14.5 ± 0.4
34 S [HEHe]	g/kg	8.7 ± 0.2		10.4 ± 0.1		8.43 ± 0.06	
39 K [H_2]	g/kg	12.6 ± 0.6		21.3 ± 1.2	22.3 ± 1.0	25.5 ± 0.8	26.3 ± 1.1
40 Ca [H_2]	g/kg	2.18 ± 0.11		0.46 ± 0.03	0.52 ± 0.05	0.196 ± 0.014	0.189 ± 0.009
51 V [He]	mg/kg	1.50 ± 0.01		N.D. (<0.014)		N.D. (<0.014)	
52 Cr [He]	mg/kg	1.75 ± 0.09	1.87 ± 0.16	0.67 ± 0.00	0.72 ± 0.09	0.058 ± 0.001	
55 Mn [He]	mg/kg	3.02 ± 0.11		0.41 ± 0.03	0.41 ± 0.03	0.190 ± 0.004	0.201 ± 0.010
56 Fe [H_2]	mg/kg	339 ± 20	341 ± 27	11.2 ± 0.5	11.2 ± 0.9	13.6 ± 0.7	13.1 ± 0.5
59 Co [He]	mg/kg	10.7 ± 0.09		0.030 ± 0.003	0.04	0.015 ± 0.001	
60 Ni [He]	mg/kg	1.26 ± 0.11	1.36 ± 0.02	0.40 ± 0.10	0.38 ± 0.05	0.076 ± 0.037	
63 Cu [He]	mg/kg	15.8 ± 0.1	15.9 ± 0.9	1.13 ± 0.02	1.25 ± 0.07	1.26 ± 0.02	1.31 ± 0.04
66 Zn [He]	mg/kg	49.3 ± 0.5	52.2 ± 3.2	20.5 ± 0.2	21.3 ± 1.5	33.3 ± 0.2	33.6 ± 1.0
75 As [HEHe]	mg/kg	6.73 ± 0.08	6.80 ± 0.64	36.4 ± 1.1	36.7 ± 1.8	6.77 ± 0.13	6.62 ± 0.21
78 Se [H_2]	mg/kg	3.47 ± 0.12	3.56 ± 0.34	1.8 ± 0.1	1.8 ± 0.2	2.11 ± 0.06	2.14 ± 0.11
88 Sr [He]	mg/kg	9.72 ± 0.10		1.74 ± 0.03	2	1.08 ± 0.02	1.13 ± 0.03
95 Mo [He]	mg/kg	0.261 ± 0.005		0.010 ± 0.006	0.01	N.D. (<0.0008)	
107 Ag [He]	mg/kg	0.022 ± 0.001		N.D. (<0.0050)		N.D. (<0.0050)	
111 Cd [He]	mg/kg	0.304 ± 0.001	0.306 ± 0.015	0.009 ± 0.000	0.009	0.152 ± 0.003	0.159 ± 0.006
118 Sn [He]	mg/kg	0.077 ± 0.004	0.056 ± 0.010	0.016 ± 0.002		0.036 ± 0.001	
121 Sb [He]	mg/kg	0.009 ± 0.000		0.014 ± 0.001	0.02	0.002 ± 0.001	
137 Ba [He]	mg/kg	5.01 ± 0.03		0.027 ± 0.002		2.4 ± 0.02	
202 Hg [He]	mg/kg	0.358 ± 0.004	0.410 ± 0.055	0.53 ± 0.01	0.61 ± 0.02	5.02 ± 0.02	5.34 ± 0.14
205 Tl [He]	mg/kg	0.001 ± 0.002		N.D. (<0.010)		N.D. (<0.010)	
208 Pb [He]	mg/kg	0.405 ± 0.007	0.416 ± 0.053	0.03 ± 0.00	0.04	0.006 ± 0.003	
232 Th [He]	mg/kg	0.177 ± 0.002		N.D. (<0.0008)		N.D. (<0.0008)	
238 U [He]	mg/kg	0.056 ± 0.005		N.D. (<0.0010)		N.D. (<0.0010)	

to identify potential matrix-based interferences and to select the most appropriate cell mode (no gas, He, High Energy He, H_2), isotope, integration time and ISTD for each analyte. After automatic optimization of these settings, the Method Wizard creates batch acquisition parameters that are ready to run for the target sample type.

Results and Discussion
DORM-4 CRM was used as the "typical sample matrix" required by the Method Wizard to optimize the analysis parameters. The plasma mode is selected based on the TDS concentration of the typical sample measured during the method setup and, since in this case the digestion resulted in a TDS level of below 1000 ppm, "Low Matrix" mode was chosen.

A range of elements from major to trace levels was determined, focusing on the analytes which have certified reference values in one or more of the 3 CRMs. The results obtained with the 7900 ICP-MS are shown in Table 1, together with the available certified values. Good agreement with the reference values was obtained in all cases.

Conclusions
The method created by the Method Automation function of the ICP-MS MassHunter software was demonstrated to generate accurate data for all major and trace elements certified in a range of food reference materials. The same approach can be used for other sample types such as environmental, geological, clinical and pharmaceutical, which greatly simplifies method development for routine laboratories.

Reference and More Information
1. Agilent Application Note: **Application of the Agilent 7900 ICP-MS with Method Automation function for the routine determination of trace metallic components in food CRMs**, 5991-4556EN.

New Agilent ICP-MS Mobile App - Free to Download

Steve Wilbur

Software Product Manager, ICP-MS
Systems, Agilent Technologies Inc. USA

ICP-MS Mobile
Agilent Technologies, Inc

★★★★★   Free

Imagine...

You worked all day on Friday preparing a large batch of ICP-MS samples and the results need to be on the boss's desk on Monday morning. It's 8:00 Friday night, you're still in the lab making sure your calibrations and QC samples are in control. Everything looks great so far. You have an hour commute home. What do you do?

1. Go home, enjoy the weekend and hope for the best.
2. Stay and wait till the samples and final QC have completed sometime on Saturday afternoon.
3. Come back to the lab over the weekend, to allow time to rerun the batch if there was a problem?

Or...

4. Check the status of the batch remotely, without disrupting your weekend, from the shopping mall, golf course, pool side, sofa ... using your smart phone!

Or, what if...

- Your office is in a separate building from the lab, and you just want to be sure your ICP-MS analysis is under control without leaving your desk.
- It's early Sunday morning and you can't remember whether you set the Queue to switch off the plasma at the end of the run when you left on Friday night.
- You want the plasma ignited and the ICP-MS instrument warmed up and autotuned when you arrive at the lab on Monday morning, ready to run samples.
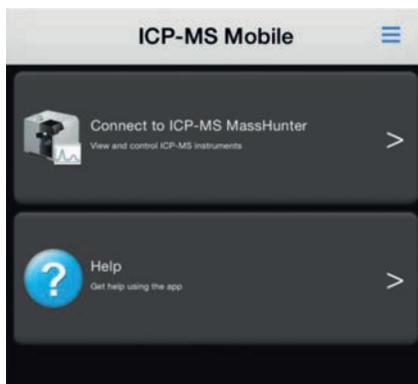
If your ICP-MS is an Agilent 7900, 7700 Series or 8800 ICP-QQQ running the latest MassHunter 4.1 software, you can use the **free mobile app** on your iOS or Android smartphone or tablet to eliminate the uncertainty and stress from the above situations.

The app is downloadable free of charge from the Apple App Store for iOS devices or the Google Play store for Android devices. Just search for Agilent, or ICPMS Mobile. It is easy to find.

The ICP-MS Mobile app enables:

- Direct connection, in real time, to any configured Agilent ICP-MS instrument running MassHunter 4.1 with web access. If your laboratory is protected by a firewall which permits remote access via a Virtual Private Network (VPN), you can still connect if your mobile device has access to the VPN.
- High level of security. Initial configuration of the connection requires the user to know the IP address, user ID, and password of the MassHunter PC. This can be typed into the mobile app if the user is away from the MassHunter PC, or all the necessary information can be imported by using the mobile device to scan the QR code which MassHunter displays on the PC screen.

Once connected, you can:

- View the instrument status (offline or online)
- Switch the plasma on or off. "Plasma On" can include a preconfigured startup routine including warm-up and autotune
- View the Queue status and pause or restart the Queue
- View the QC or internal standard recovery charts in real time as they are updated after each sample

The Agilent ICP-MS Mobile app is only the beginning. Come with us while we explore 21st century possibilities in laboratory automation.

Consumables Guide for the New 7900 ICP-MS

Eric Vanclay

Supplies Product Manager, Agilent Technologies Australia

As more and more Agilent 7900 ICP-MS systems are installed, thoughts in those labs begin to shift to day-to-day operation - including consumables. Many supplies for the new Agilent 7900 ICP-MS are the same as those used with the 7700 Series ICP-MS and 8800 ICP-QQQ (e.g. sample cone, peristaltic pump tubing), however there are a number of unique supplies for the 7900 ICP-MS.

Here we aim to clarify those parts that are unique to the new 7900 ICP-MS and those that are common to the equivalent model of 7700 and 8800 - see Table 1 and 2.

Locate the Right Supplies to Support Your ICP-MS Operation

ICP-MS Quick Reference Guide available to download

Table 1. ICP-MS supplies where the same part is used for the 7700 Series, 8800 and the new 7900 ICP-MS.

Description	Part Number
Peristaltic pump tubing - for sample uptake (white/white) - for internal standard (blue/orange) - for drain	G1833-65569 G3280-67047 G1833-65570
Sample uptake tubing 0.5 mm id, 1.6 mm od, 5 m	G1820-65105
Online ISTD addition kit	G3280-60590
Spray chamber (for non-UHMI configuration)	G3280-80008
Connector tube (for non-UHMI configuration)	G3270-80025
Shield plate	G1833-65419
Torch	G3280-80053
Torch bonnet	G1833-65421
Ni sampling cone Retaining ring	G3280-67040 G3280-20504
Interface wrench for sampling cone	G3280-01507
Foreline pump oil (1L)	6040-0834
PFA inert sample introduction kit (with 2.5 mm id sapphire injector)	G4912-68000

Table 2. ICP-MS supplies that are unique to the 7900 ICP-MS. The equivalent supplies for the 7700 Series and 8800 ICP-QQQ are not interchangeable as the designs are different.

Description	Part Number
MicroMist nebulizer (features U-series sample inlet) Sample tubing for nebulizer	G3266-80004 G3266-80012 (0.5 mm ID)
Spray chamber for UHMI	G8400-67150
Clamp for ball joint	G8400-60327
Ni skimmer cone for standard 7900 with x-lens	G8400-67200
Skimmer base for standard 7900 with x-lens	G8400-60624
Extraction lens 1 for standard 7900 with x- lens Extraction lens 2 for standard 7900 with x- lens	G8400-60215 G8400-60216
Omega lens for standard 7900 with x-lens	G8400-60217
Omega bias lens for standard 7900 with x-lens	G8400-00240
Oil filter element for the rotary pump	9499201

To help you locate the supplies you need for the 7900 ICP-MS, we've updated the two page **ICP-MS Quick Reference Guide**, 5990-8182EN. It now clearly lists those parts that are suitable for the 7900, 7700 and 8800, and the supplies that are unique to the new 7900 ICP-MS. Existing supplies for the 7700 and 8800 have been retained, so this one guide now covers the current 7900 and 7700 Series ICP-MS and 8800 ICP-QQQ.

To download the guide, go to **agilent.com** and search using the publication number 5990-8182EN. Alternatively, you can access it from Agilent's atomic spectroscopy supplies web page at: **agilent.com/chem/supplies**

Catch-up with the 7900 ICP-MS On-demand Webinar

Title: Raise Your Expectations: Learn How the New Agilent 7900 ICP-MS Redefines ICP-MS Performance

Presenter: Ed McCurdy, Agilent ICP-MS Product Marketing

More than 500 delegates tuned in to hear Agilent's Ed McCurdy present an overview of the Agilent 7900 ICP-MS at two webinars in February and April 2014. The high level of interest and attendance reflects the impressive capabilities of the new 7900, particularly in redefining the limits of matrix tolerance for ICP-MS.

If you missed the live broadcast, and would like to learn more about the 7900, you can catch up now. No phone line is necessary as audio for this event is broadcast direct to your PC along with the slide show via the Internet. The webinar is supported by a new Application Note on the subject of high salt matrix analysis – see panel below.

Simply log-in or register for this FREE Webcast at: spectroscopyonline.com/ performance

Agilent ICP-MS Publications

To view and download the latest ICP-MS literature, go to **www.agilent.com/chem/icpms** and look under **"Literature Library"**

- **Application note:** Performance of the Agilent 7900 ICP-MS with UHMI for high salt matrix analysis, 5991-4257EN
- **Application note:** Single particle analysis of nanomaterials using the Agilent 7900 ICP-MS, 5991-4401EN
- **Application note:** Application of the Agilent 7900 ICP-MS with Method Automation function for the routine determination of trace metallic components in food CRMs, 5991-4556EN
- **Application note:** Determination of P, Si and S in acid digested lubricating oil using the Agilent 8800 Triple Quadrupole ICP-MS, 5991-4513EN
- **Technical note:** Reaction data for 70 elements using O_2, NH_3 and H_2 gases with the Agilent 8800 Triple Quadrupole ICP-MS, 5991-4585EN

This information is subject to change without notice.

Agilent Celebrates Shipment of First 7900 ICP-MS to Mitsubishi Semicon QA Lab
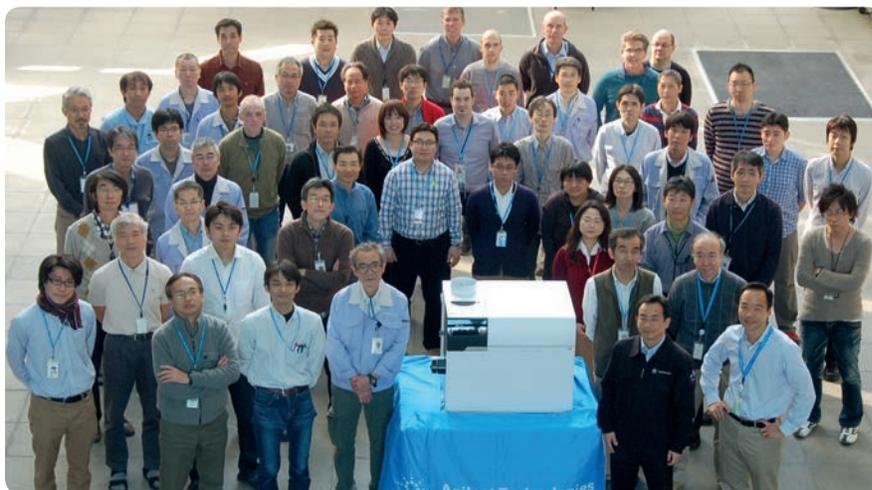
Agilent's ICP-MS team gathers to celebrate the shipment of the first 7900 ICP-MS from the Tokyo Analytical Division (TAD) manufacturing site in Japan to to the Kurosaki Plant of Mitsubishi Chemical Corporation, Japan, where it is now safely installed in the Quality Assurance Section of the Inorganic Materials Center.

The Mitsubishi QA team is already familiar with Agilent ICP-MS as the 7900 will replace a 7500cs, and colleagues in Taiwan are highly satisfied with the performance of their 7700s ICP-MS. The 7900 will be used to analyze semiconductor grade chemicals such as HCl and H_2SO_4 six days a week for 8 hours per day! Mitsubishi's Daisuke Iwakiri (pictured with the 7900) is confident that the 7900 has the robustness necessary to manage the workload as well as delivering improved performance compared to their existing ICP-MS.

Conferences. Meetings. Seminars.

- **European Workshop on Laser Ablation 2014**, July 8-11, 2014, Royal Holloway University of London, UK, **ewla.rhul.ac.uk**
- **NEMC**, August 4-8, 2014, Washington, DC, USA, **nemc.us**
- **ACS Fall**, August 10-13, 2014, San Francisco, CA, USA, **acs.org**
- **Thailand, Malaysia and Singapore:** Spectroscopy users are welcome to join our Annual Atomic Spectroscopy User Meetings and learn about new developments from Agilent and share experiences. The dates are:
  - Monday, August 18, Bangkok
  - Wednesday, August 20, Kuala Lumpur
  - Friday, August 22, Singapore

Register online at **agilent.com/chem/atomic_user_meeting**
- **Society of Minerals Analysis,** September 16-18, 2014, Elko, NV, USA, **sma-online.org**
- **CMA Saskatoon**, September 21-25, 2014, Saskatchewan, Canada, **cma2014saskatoon.ca**
- **SciX**, September 28-October 3, 2014, Reno, NV, USA, **scixconference.org**
- **SupplySide West**, October 6-10, 2014, Las Vegas, NV, USA, **west.supplysideshow.com**

Agilent ICP-MS Journal Editor   Karen Morton for Agilent Technologies
e-mail: icpms@agilent.com

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