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Measurement of Metals in Tissue Samples Using Laser Ablation-ICP-MS and an Organic Matrix-Based Standard

Tetsuo Kubota[1*], Raimund Wahlen[1], Bert Woods[1]
Pierre Burckel[1], Yuki Sugiura[2],
Koichi Tsuneyama[3]

[1]Agilent Technologies, Inc.

[2]Multiomics Platform, Center for Cancer Immunotherapy and Immunobiology, Kyoto University Graduate School of Medicine, Japan,

[3]Department of Pathology and Laboratory Medicine Institute of Biomedical Sciences, Tokushima University Graduate School, Japan

Introduction

Laser Ablation-Inductively Coupled Plasma-Mass Spectroscopy (LA-ICP-MS) can provide localization information of elements on solid samples. Therefore, bioimaging techniques using LA-ICP-MS have been developed to investigate the distribution of metals on thin sections.

Primary biliary cholangitis (PBC) is an autoimmune disorder that primarily targets the interlobular bile ducts. The resulting bile duct damage causes chronic cholestasis in the liver, which can eventually progress to cirrhosis. It is well-established that individuals with PBC exhibit impaired copper (Cu) excretion, leading to Cu accumulation within the liver.

Pathologists have employed orcein or Victoria blue staining to visualize Cu deposition in pathological specimens.[1] Orcein staining is commonly used to assess the stage of PBC. However, both staining methods measure Cu levels indirectly by detecting Cu-binding proteins, which introduces significant variability in measurement accuracy across different facilities and yields relatively low detection sensitivity.

The aim of the study was to directly quantify Cu in PBC specimen using LA-ICP-MS, a highly sensitive technique, and compare the results with that obtained from normal-looking tissue in liver. The elemental concentrations were determined from external calibration curves generated using an organic matrix-based standard (Solid scale^{TM}, FUJIFILM Wako Chemicals).

Conclusions

This study demonstrates the high performance of the Agilent 8900 ICP-QQQ equipped with an ESL 213 laser ablation system for bioimaging human liver specimens.

- An organic matrix-based standard (Solid scale^{TM}) provided quantitative results of metals in human liver specimens.
- Patterns of metal distribution (^{24}Mg, ^{31}P, ^{44}Ca, ^{56}Fe, ^{63}Cu, ^{95}Mo) in human liver showed accumulation of Cu in a PBC specimen and its unique localization pattern along the regenerative nodule.

These results demonstrate that determining metal concentrations in thin sections using LA-ICP-MS provides valuable insights into the mechanisms of PBC and other metal-related diseases.

Experimental

Hardware Configuration

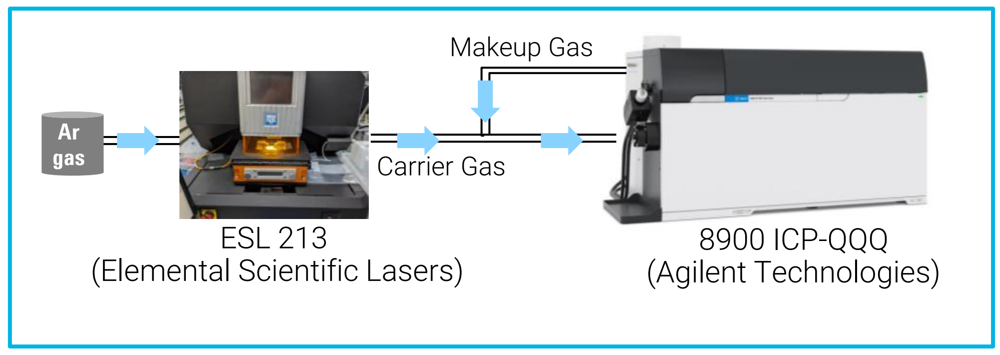

Figure 1. LA-ICP-MS system

Hardware Parameter	Setting
RF Power	1600
Sampling Depth	8.0
Makeup Gas Flow Rate	0.25
Cell Gas and Flow Rate	H_2 3 mL/min, O_2 10%

Table 1. 8900 ICP-QQQ system parameters

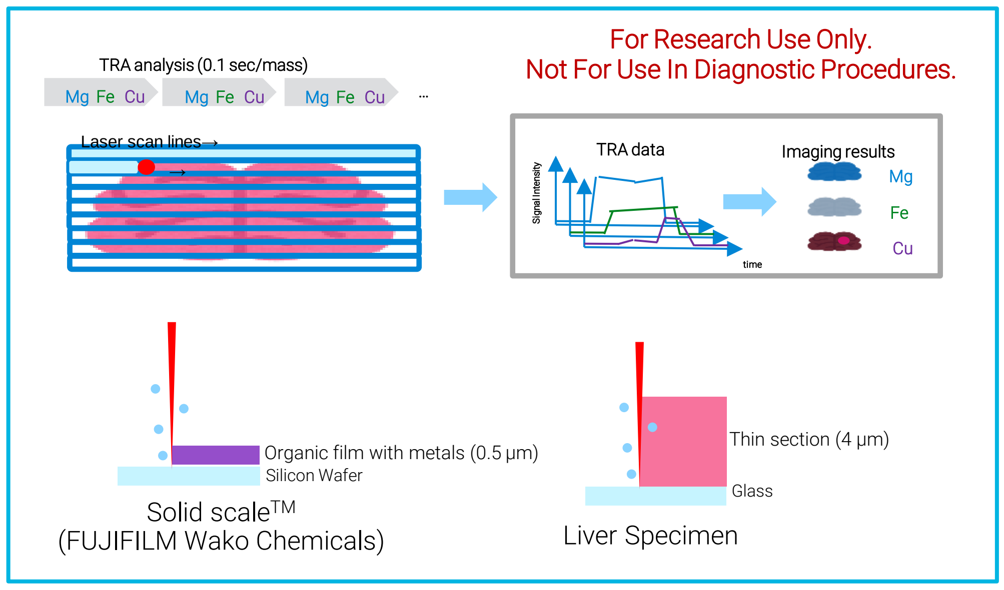

Figure 2. Overview of LA-ICP-MS and ablated samples

Parameter	Solid scale^{TM}	Liver specimen
Laser Output (%)	25 (≒1.0 J/cm^2)	
Repetition Rate	20	
Spot Size (µm)	30	
Thickness (µm)	0.5	4
Density (g/cm^3)	≒1.05	
Scan Speed (um/sec)	30	
Number of Analytes	6	

Table 2. Laser Ablation method, sample, and analysis method parameter

Since most of parameters are common between Solid scale^{TM} and liver specimen, assuming that

- all samples are ablated
- there is no sensitivity variation due to the differences in carbon content

,the conc. of elements in the liver specimen can be calculated by the following equation;

$$(Conc.) = \{ (Signal\ Intensity) - b \} / a * 0.5 / 4$$

where *a* and *b* correspond to the slope and y-intercept of the calibration curve, respectively.

Results and Discussion

Calibration curves

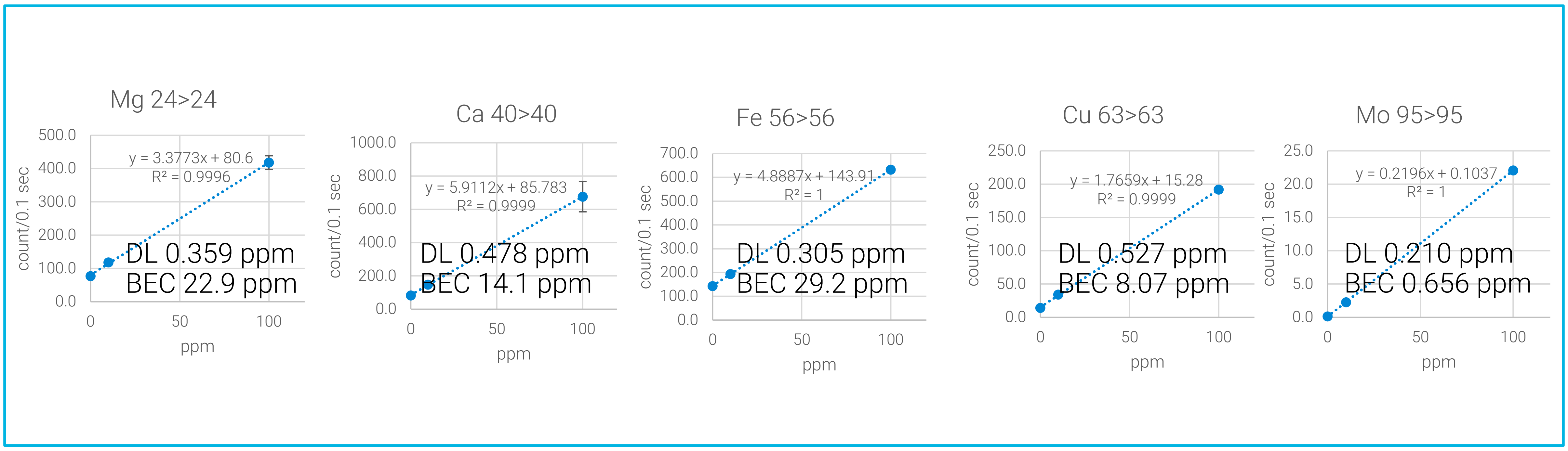

Figure 3. Calibration curves generated using an organic matrix-based standard. (Phosphorus is not contained in the Solid scale™ standard.)

Quantitative Measurements

Sample	Mg	Ca	Fe	Cu	Mo
Reference data[2]	662	214	849	31.8	2.1
Reference data[3]	102	104	138	3.84	4.30
Normal-looking liver	264.2	328.6	511.0	14.1	0.6
PBC specimen	766.9	567.3	756.9	79.6	6.1

Table 3. Mean concentration (µg/g) of metals in whole liver specimens

Any points on the specimens with a P signal count of 200 or more were considered as tissue, so the mean concentration of Mg, Ca, Fe, Cu, and Mo in whole liver specimens were calculated for these points. The Cu signal was highest in the PBC specimen. For the other analytes, there was both agreement and discrepancies with the reference values, likely due to differences with patients or sample preparation methods.

Calibration using the organic matrix-based standard provided quantitative results of mean conc. of metals in human liver specimens.

References

1. Henwood, "A. Current applications of orcein in histochemistry. A brief review with some new observations concerning influence of dye batch variation and aging of dye solutions on staining", Biotechnic & Histochemistry, 78(6), 2003, 303–308

2. Katoh, Y, *et al.*, "ヒト臓器中の元素濃度 肝臓および腎臓", keitaikinou2002, 1, 2–13

3. Baj J., *et al.*, Assessment of the Concentration of 51 Elements in the Liver and in Various Parts of the Human Brain—Profiling of the Mineral Status *Nutrients*, 2023, 15, 2799

Results and Discussion

Distribution of metals in human liver samples

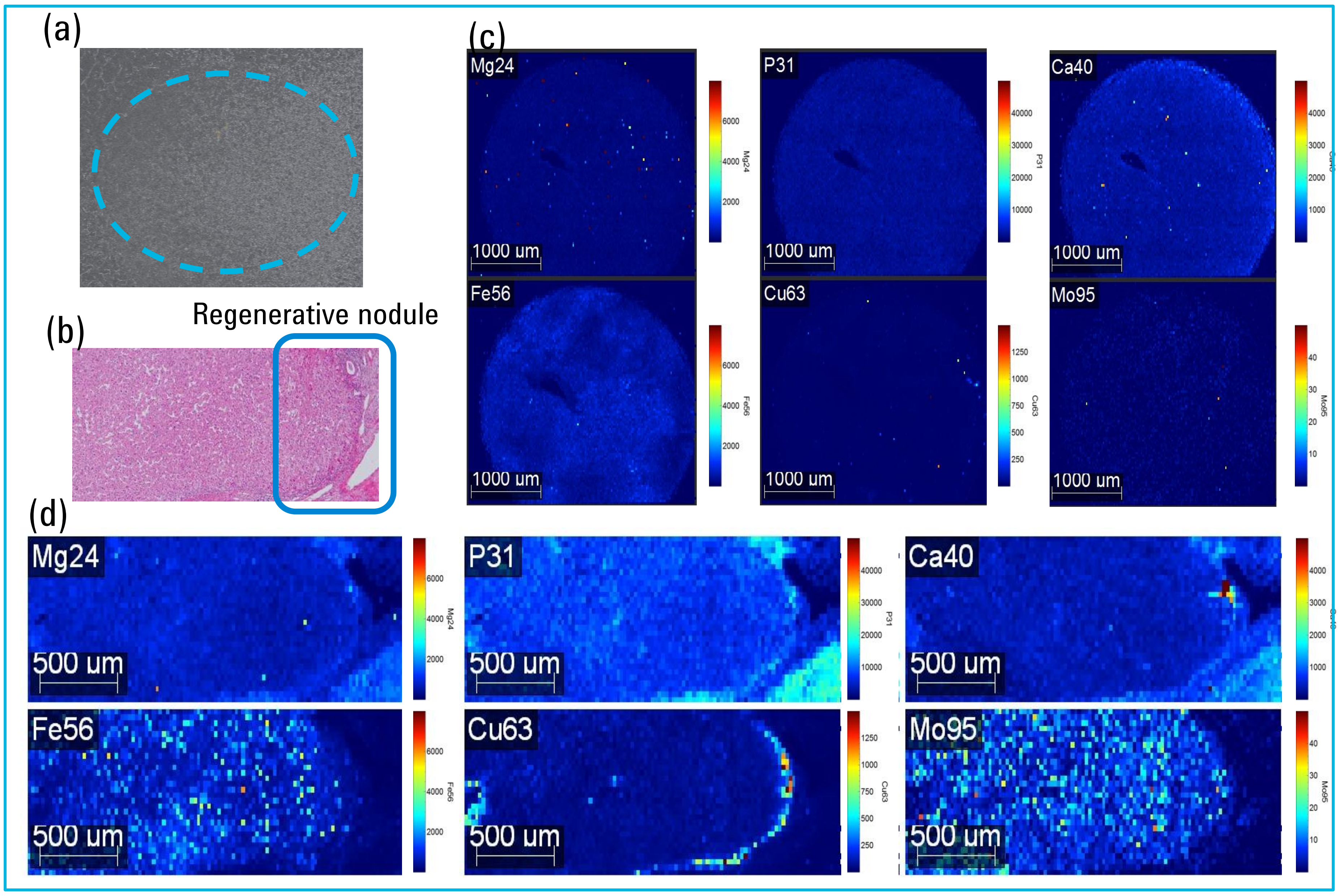

Figure 4. Microscopic picture of (a) normal-looking liver, (b) PBC specimen, metal distribution in (c) normal-looking liver and (d) PBC specimen. Units of color scale are ppm (Mg, Ca, Fe, Cu, and Mo), and counts (P), respectively.

Signal intensities of the TRA data plots were converted to concentrations of metals using the calibration curves (Figure 3). Figure 4 shows quantitative imaging results of a normal-looking liver and PBC specimen. In PBC, a high accumulation (1000 to 2000 ppm) of Cu was observed along the regenerative nodule (a non-cancerous growth in the liver that forms in response to liver damage). In the control liver specimen, low Cu signals were detected. These results confirm Cu accumulation in PBC liver specimen, as suggested by orcein staining.

Patterns of metal distribution (^{24}Mg, ^{31}P, ^{44}Ca, ^{56}Fe, ^{63}Cu, ^{95}Mo) in human liver specimens show a very high-level accumulation of Cu in PBC specimen and its unique localization along the regenerative nodule.