Metrology research on bioimaging and protein analysis by using ID-LA-ICP-MS in NIM

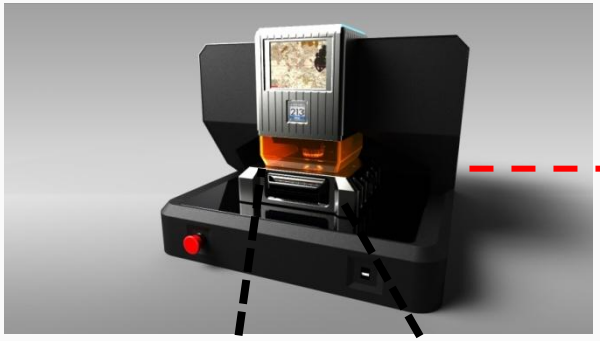
Feng Liuxing

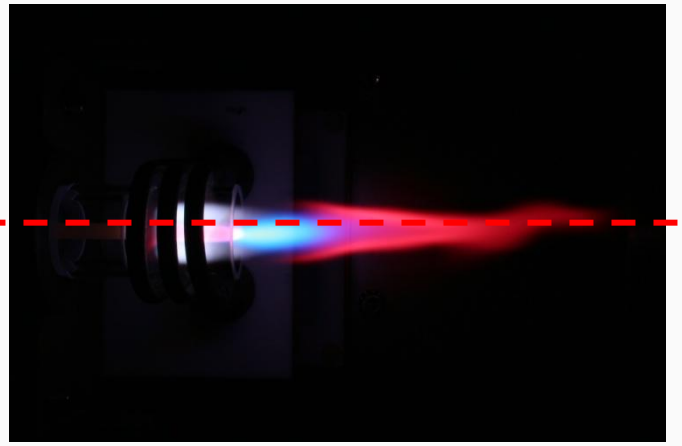
National Institute of Metrology, P.R. China

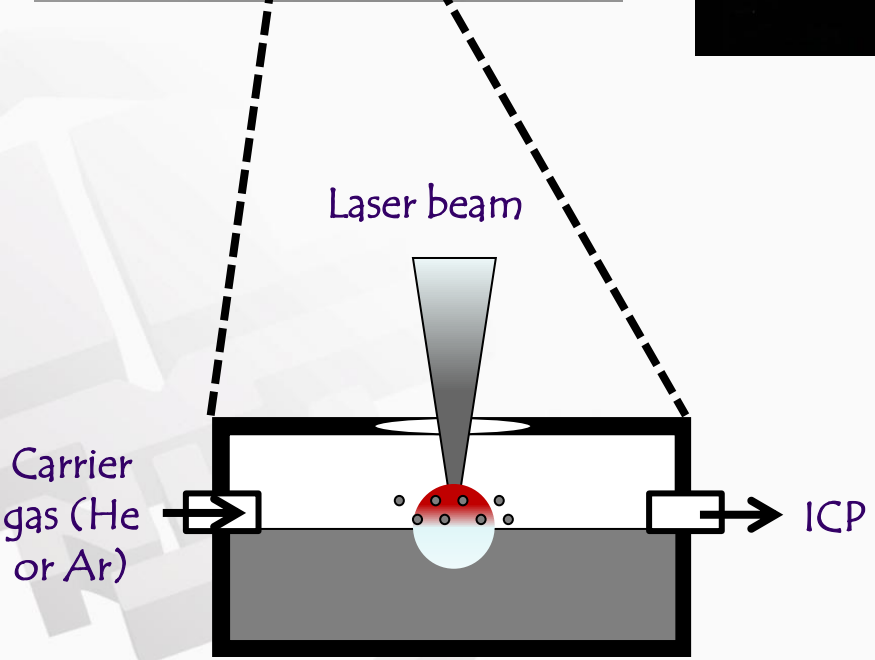
Content

- Introduction of Laser Ablation-ICP-MS
- Quantitative imaging of Fe, Cu and Zn in biological tissue by ID-LA-ICP-MS
- Quantification of proteins in serum by ID-LA-ICP-MS
- Conclusion

Introduction of LA-ICP-MS







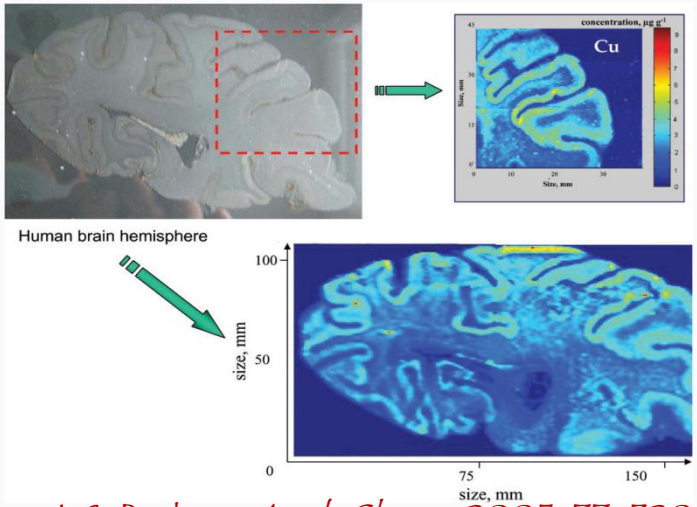
- ~2 μm resolution
- Element analysis ^{6}Li-^{241}Am
- 6 orders of magnitude detection
- Low limit of detection
- Isotope ratio information

➢ *Advantage*

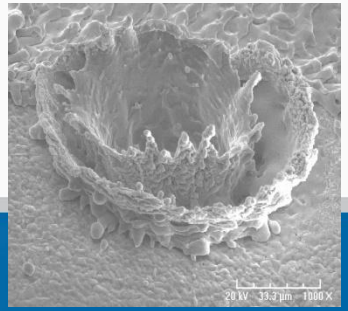
- Measurand: most inorganic elements and isotopic analysis
- DL: ppt ~ ppb
- Stability: RSD <5%
- Sample preparation: Non labor-intensive
- Analysis speed: Fast, direct, *in-situ,* elemental image

➢ *Disadvantage*

- Sample destroying
- Serious matrix effect
- Difficult for quantitative analysis
- Memory effects of the aerosol



J. S. Becker., *Anal. Chem.*, 2005, 77, 3208.



Sample after ablation

➢ *Challenge for LA-ICP-MS?*

Quantification!

Up to now, some calibration strategies have been used, including:

✓ External calibration using a matrix-matched CRM

fully matrix-matched CRMs is hard to acquired!

✓ Internal calibration by doping with a specific or naturally existing element in the sample

naturally internal standard may be not suitable for the target analyte.

doping is labor-intensive and hard to assure the homogeneity.

✓ Calibration with solution by a dual flow system

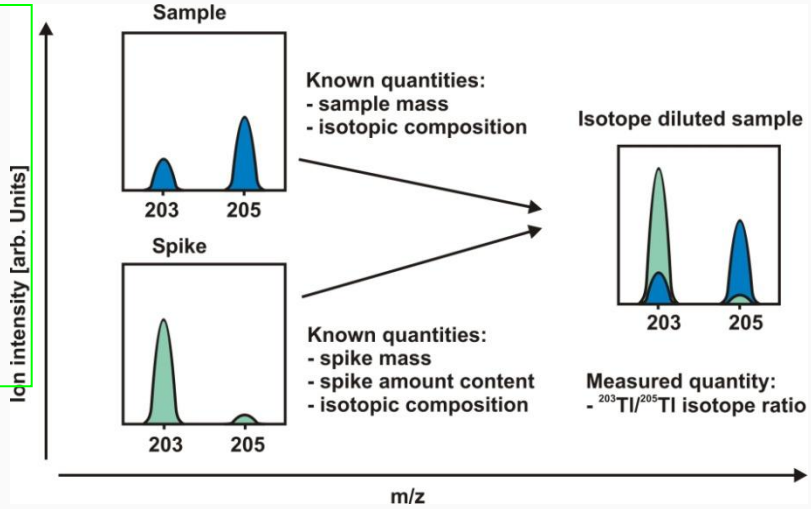
can not control the ablation process, and aerosol is different with solution!

Element isotope dilution MS (IDMS)

Equation:

✓ $$C_X = \frac{R_y - R_b}{R_b - R_x} \cdot \frac{\sum_{i=1}^{n} R_{ix} M_i}{\sum_{i=1}^{n} R_{iy} M_i} \cdot \frac{m_y}{m_x} \cdot G_y$$ directly traceable to SI unit

✓ High accuracy and small uncertainty



Aplication area:

✓ Isotope analysis with high accuracy, atomic weight measurement

✓ CRM certification, international comparisons

Development of element IDMS

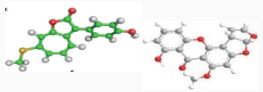
Food safty, enviroment

Life science area

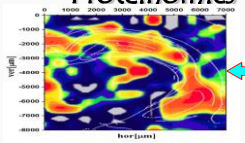
Area

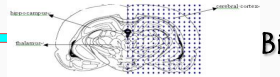
Processing

Proteinomics



Metallomics





Bioimaging

Analyte

Elements

Protein, biomarkers

metrology

Traditional IDMS

IDMS for *in-situ* analysis

Challenges on element based IDMS:

- ✓ Elements ⟶ macro moleculars?
- ✓ Bulk analysis ⟶ *in-situ*, micro-analysis?

LA-ICP-MS can analysis solid sample directly, and IDMS can get accurate, precise measurement. How to combine the two technique together efficiently?

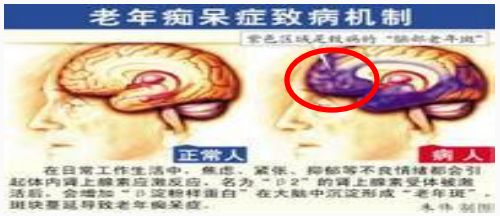
That is the task for metrologist!

Quantitative imaging by ID-LA-ICP-MS

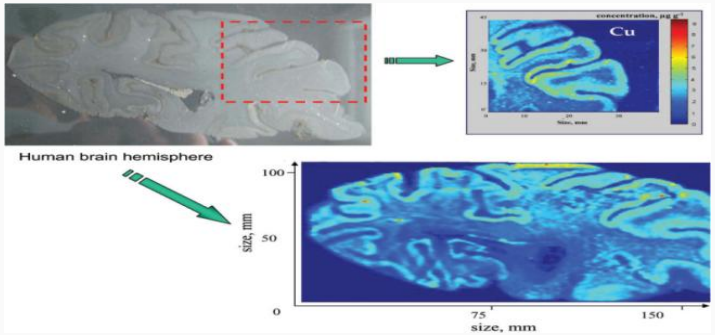
Why in-situ analysi?

- High space resolution
- Intuitively bioimaging
- Application in life science， but no quantification

AD disease

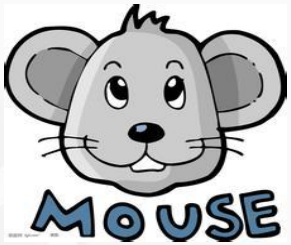


Different distribution of elements

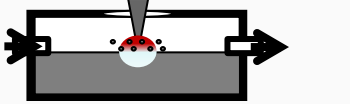


However, only imaging, no quantification!

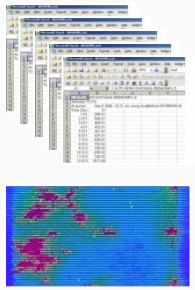
Scheme

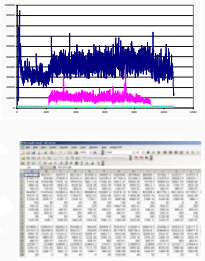


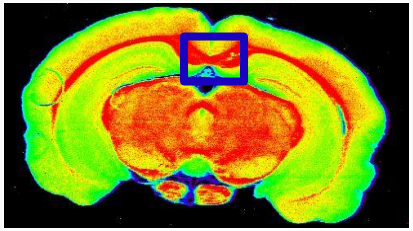
LA-ICP-MS



Isotope dilution



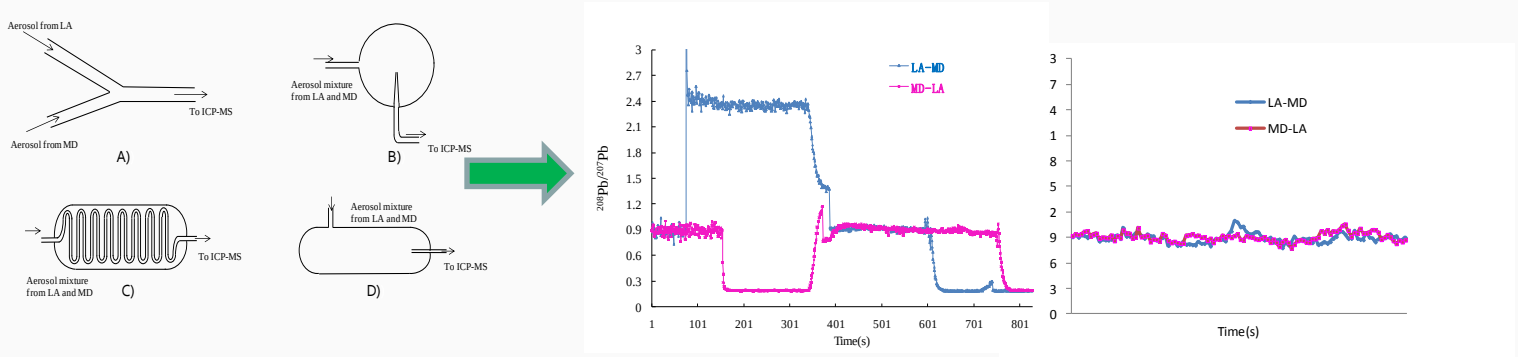




Combine IDMS and LA-ICP-MS

Absolute in-situ bioimaging

➢ *Optimization of LA aerosols mixing system*



Make LA-ID-ICP-MS more accurate and precise!

To realize satisfactory isotopic equilibrium and improve the accuracy and stability of the blended isotope ratio, four mixing devices with different shapes and sizes were designed and compared to improve the aerosol mixing efficiency.

J. Anal. At. Spectrom., 2014, 29: 2183-2189.

➢ *Preparation of homogeneous mouse brain sections*

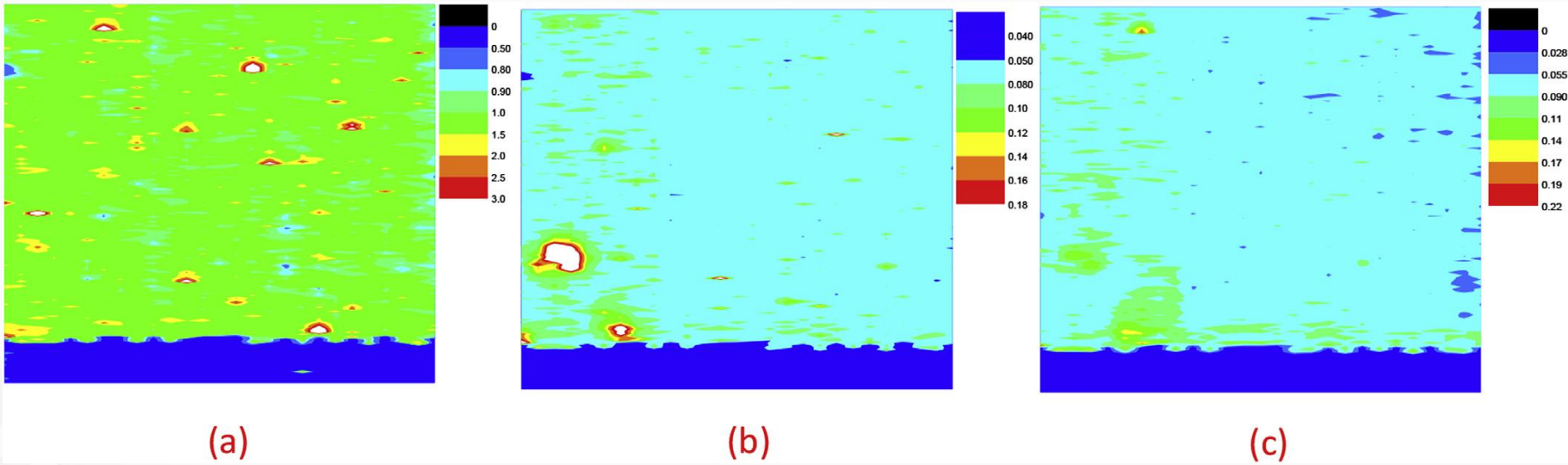


Fig. 1. LA-ICP-MS spatial distribution plots for (a) $^{56}Fe/^{13}C$, (b) $^{63}Cu/^{13}C$ and (c) $^{64}Zn/^{13}C$ of matrix-matched *in-house* standard.

The RSD of elements of the unspiked *in-house* standard were calculated. RSDs (from 40 lines) of 6.1%, 7.4% and 8.2% were obtained for $^{56}Fe/^{13}C$, $^{63}Cu/^{13}C$ and $^{64}Zn/^{13}C$, respectively. All the three elements are distributed homogeneously in most area.

➢ *Optimization of LA conditions*

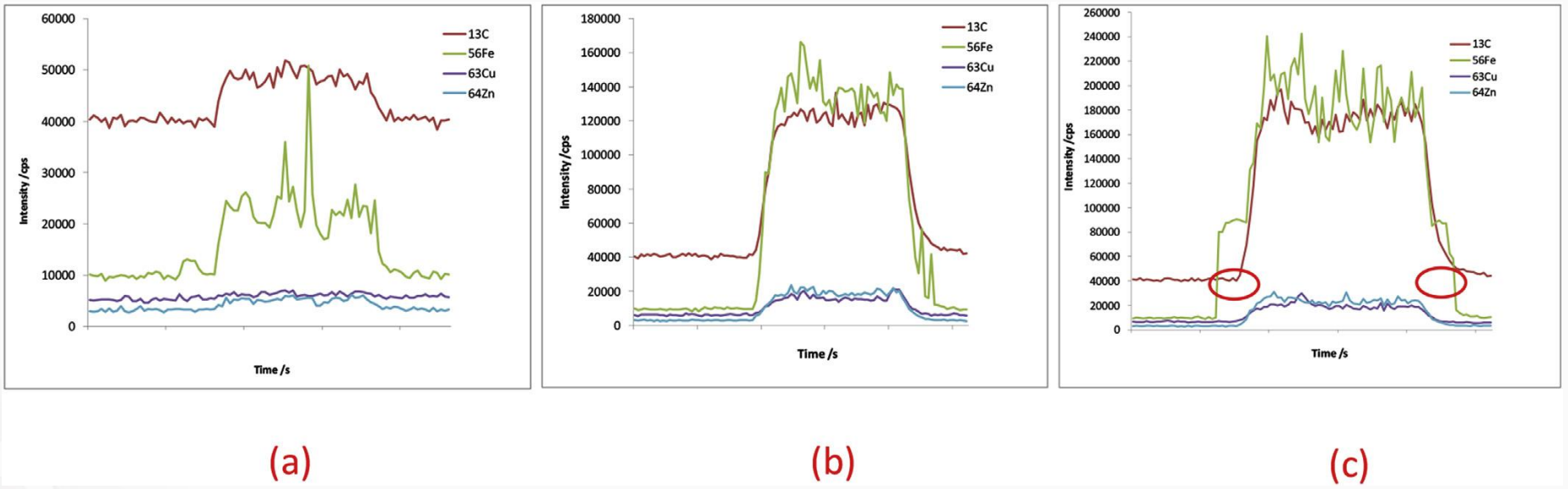


Fig. 2. Signals of 56Fe, 63Cu and 64Zn of one line LA scan on homogeneous standard with (a)LA energy 3.9 mJ/pulse, (b) LA energy 4.2 mJ/pulse and (c) LA energy 4.5 mJ/pulse.

The LA energy of 3.9, 4.2 and 4.5 mJ/pulse were investigated, and the optimal LA energy was fixed on 4.2 mJ/pulse in the following experiments.

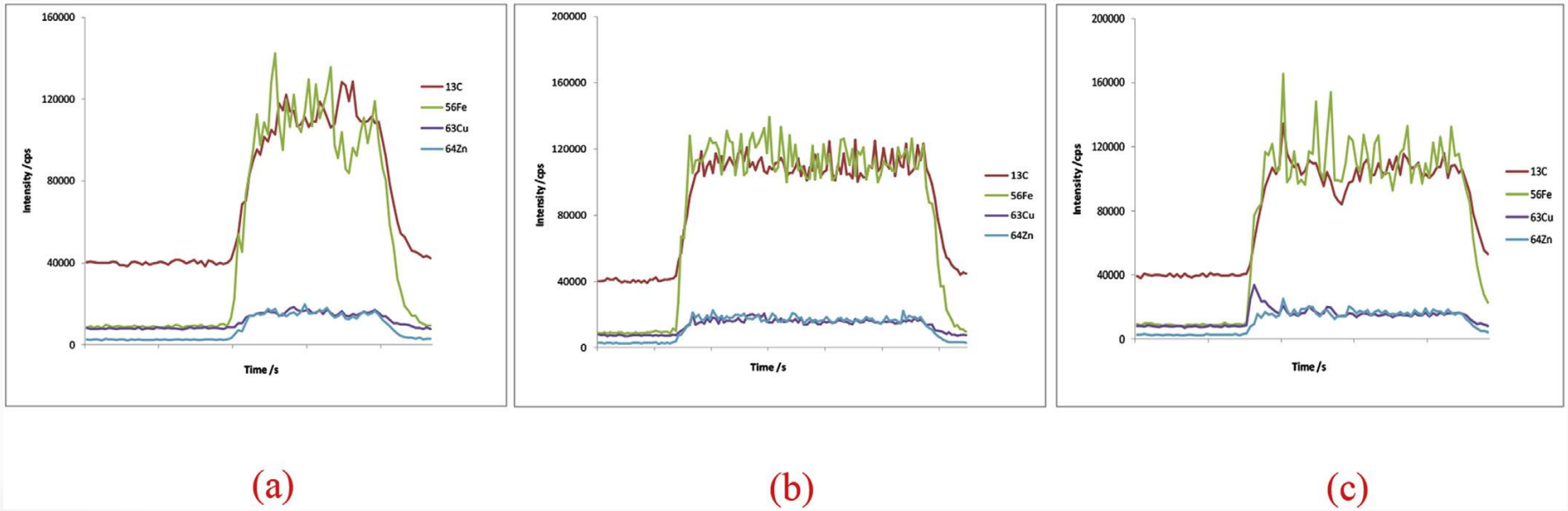
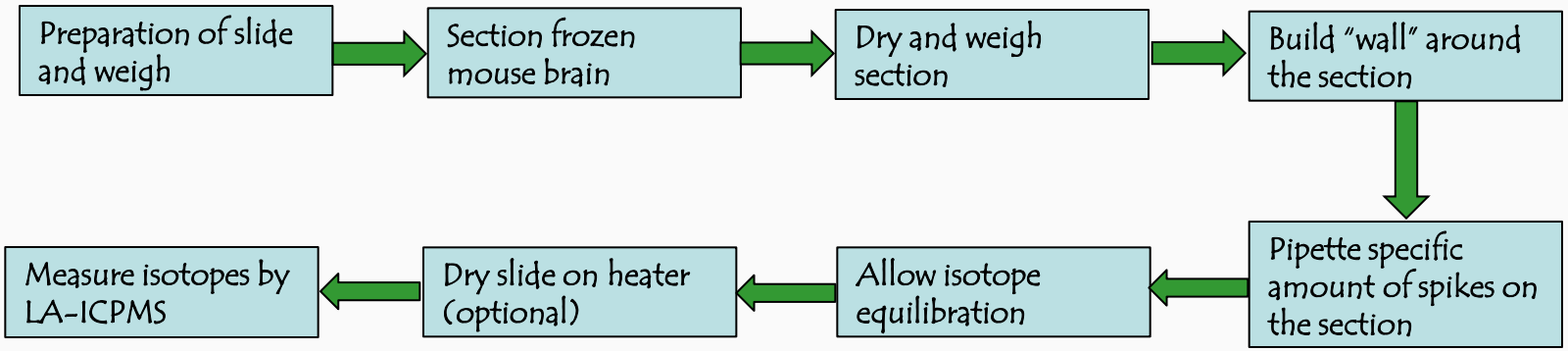


Fig. 3. Signals of 56Fe, 63Cu and 64Zn of one line LA scan on homogeneous standard with (a) 30μm s-1 , (b) 40 μm s-1 and (c) 50 μm s-1.

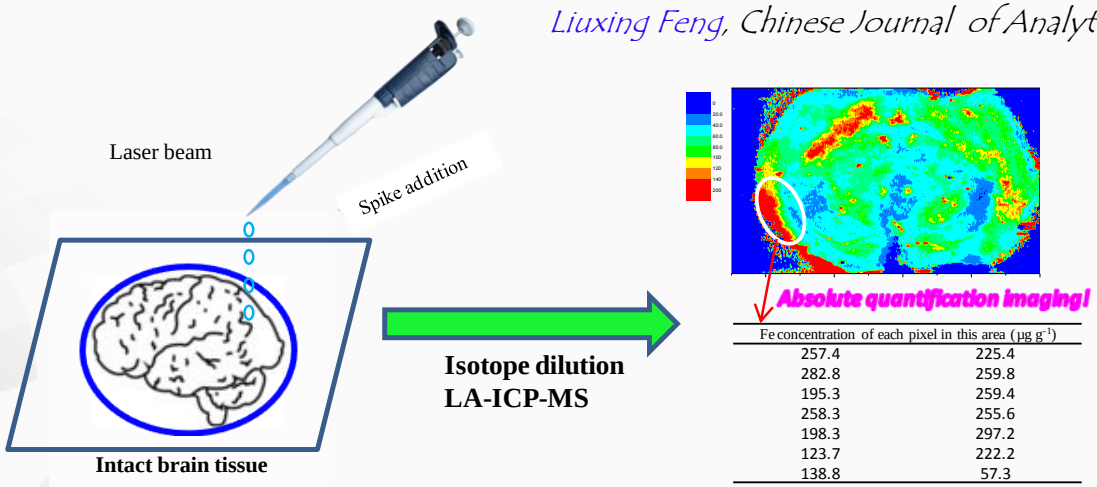
The RSD of ^{56}Fe, ^{63}Cu and ^{64}Zn of both 30 and 50 μm s^{-1} scan speed are approximately 14%, 8% and 12%, respectively. However, the RSD of the three elements with 40 μm s^{-1} are approximately 8%, 9% and 8%, which are much better than that of 30 and 50 μm s^{-1}. 40 μm s^{-1} seems to be the most appropriate scan speed.

➢ *Preparation of intact mouse brain sections for ID-LA-ICP-MS analysis*



- *6 replicate tissue sections prepared*
  - 5 lines scanned per section (total n = 30)

Liuxing Feng, Chinese Journal of Analytical Chemistry, 2014, 43, 536.



➢ *Evaluation of the "border" for isotope exchange*

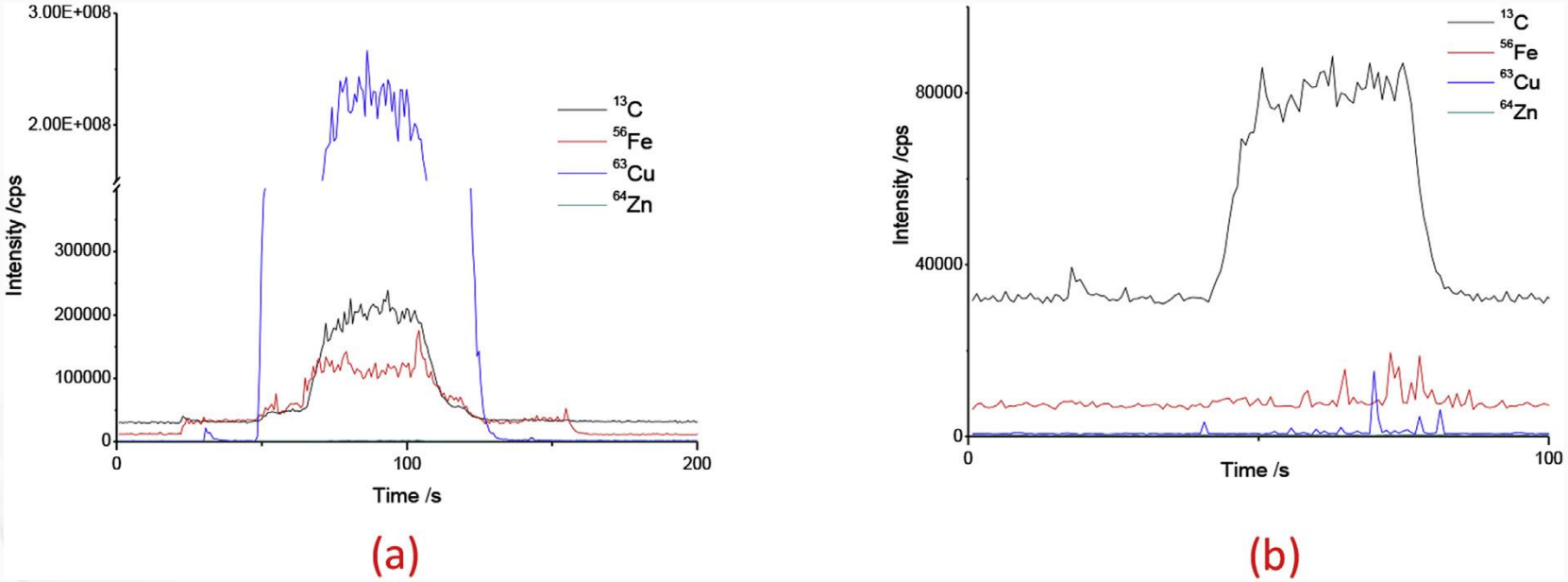


Fig. 4. Elements signal intensities obtained by LA-ICP-MS scanning across (a) liquid blocker pen and (b) silicone grease.

Liquid blocker pen and silicone grease were investigated, and silicone was chosen as the border in this work.

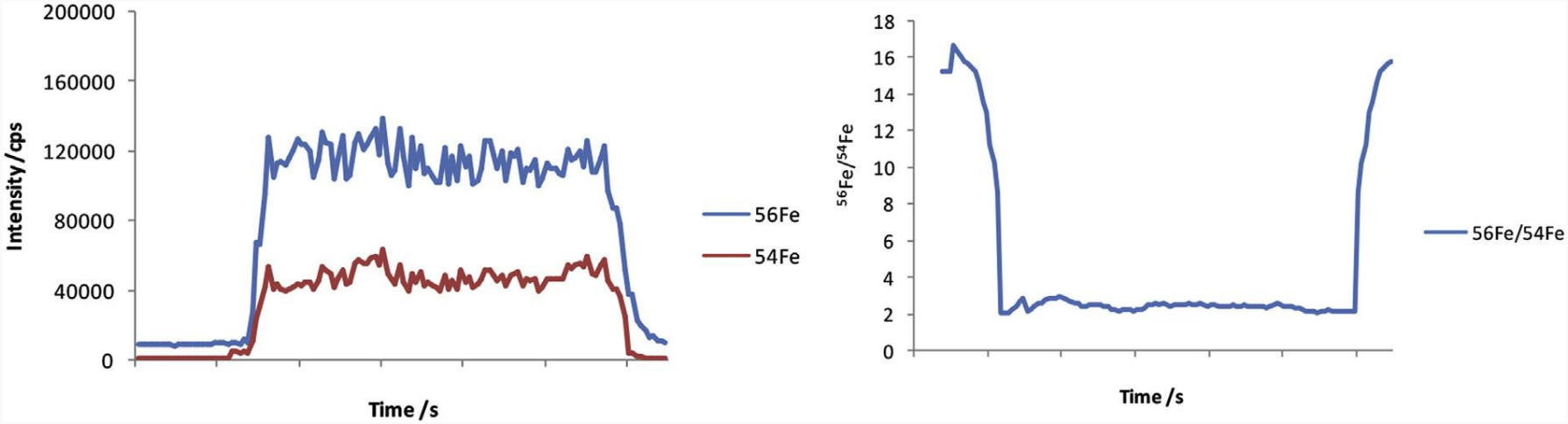


Fig. 5. ^{56}Fe, ^{54}Fe intensities (left panel) and $^{56}Fe/^{54}Fe$ (right panel) by line scan of standard section.

It can be seen clearly that almost no spike signals are observed (equal to blank) when the laser crossed the border and the adjacent space. No any reaction between the spike and the border.

➢ *Evaluation of isotope exchange time between spike and tissue*

- Isotopic equilibration for homogenised mouse brain
  - *Different immersion times investigated*
  - *Optimal ratio close to 1*

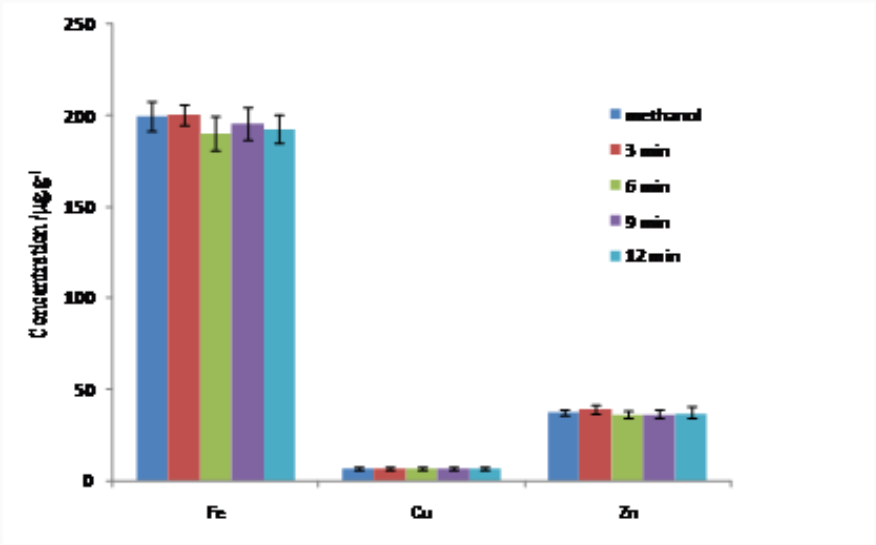


Fig. 6. Calculated results of Fe, Cu and Zn with different isotope exchange time

- *6 line scans ablated per tissue section*
- *RSDs ranged from 3 – 8%*
- *Equilibration observed after 3 minutes*

➢ *Method validation of ID-LA-ICP-MS by using in-house homogeneous standard*

– *Bulk analysis carried out for total Fe, Cu and Zn comparison*

	Fe	**Cu**	**Zn**
ID-LA–ICP-MS (±SD) ($\mu g\ g^{-1}$); n=6	121 ±10	7.0 ±0.6	39.5 ±3.8
Bulk Analysis (±SD) ($\mu g\ g^{-1}$); n=6	128 ±4	7.2 ±0.2	37.6 ±1.2

– *Shows good agreement between IDMS and bulk analysis!*

➢ ID-LA-ICP-MS quantitative imaging of intact brain sample

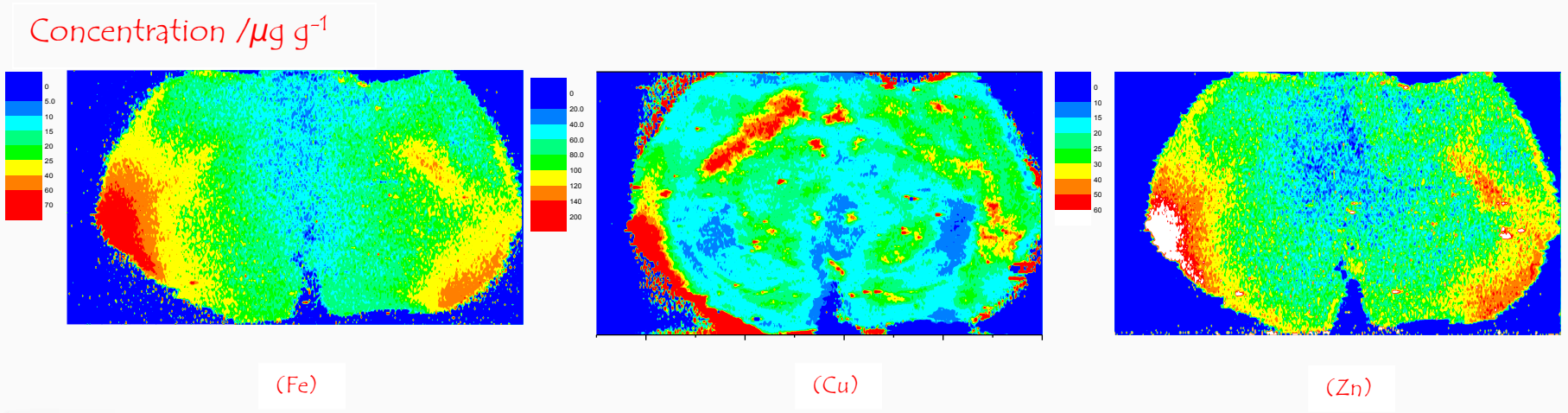


Fig. 7. Quantitative image of Fe, Cu and Zn in AD mice brain by ID-LA-ICP-MS

- ✓ The in-situ concentration was acquired by isotope dilution LA-ICPMS.
- ✓ The colour in the image not only represent the distribution but also the absolute concentration of Fe, Cu and Zn .
- ✓ Because of the isotope dilution approach, the RSDs (8~10%) are also better than those of external LA-ICP-MS reported by previous work.

➢ Assessment by μ-XRF and immunohistochemistry

μ-XRF conditions:

AD mouse brain section (50 μm thick) was used to underwent μ-XRF and immunohistochemical imaging analysis.

HV generator	Max. voltage: 50 kV; max. power: 50 W
X-ray optic	Polycapillary
Spot size	10 μm
Shutter-filter	25 μm Ti
Amp time	12.8 μs
Dwell time	300 ms

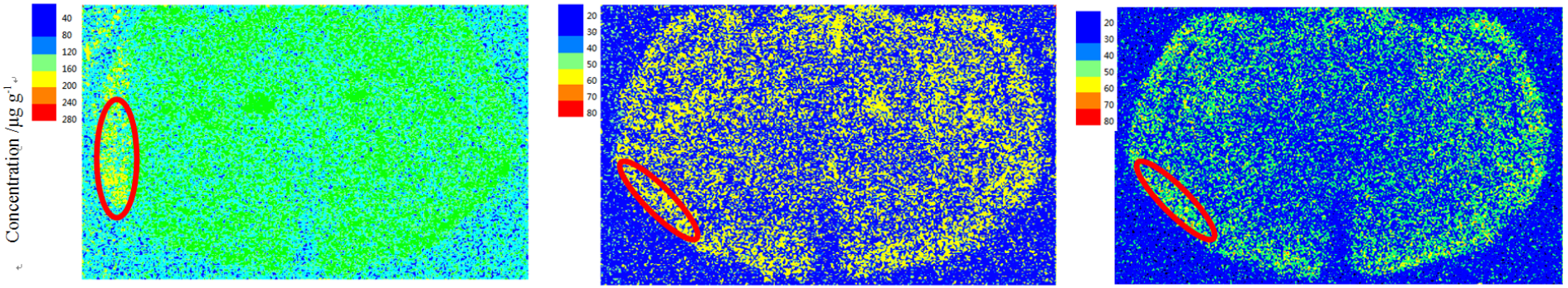


Fig. 8. Quantitative image of Fe, Cu and Zn in AD mice brain by μ-XRF

- ✓ *Because of the relative high detection limit and less pixels, the resolution is not as good as that of ID-LA-ICP-MS.*
- ✓ *However, all the three elements have similar distribution patterns with them of ID-LA-ICP-MS.*
- ✓ *In the "hot region" areas (about 20 spots), the elements concentration measured by using both μ-XRF and ID-LA-ICP-MS are much higher.*

Comparative Fe, Cu and Zn concentrations (in μg g-1) obtained by ID-LA-ICP-MS and μ-XRF for hot region of AD mouse brain section

	Fe	Cu	Zn
ID-LA–ICP-MS (±SD) (20 spots, n=20)	201 ±16	62 ±6	75 ±7
μ-XRF (±SD) (20 spots, n=20)	188 ±25	55 ±10	72 ±14

- ✓ *The average Fe, Cu and Zn concentrations in selected tissue regions obtained by μ-XRF fell well within the scope of ID-LA-ICP-MS values.*
- ✓ *The RSDs of the three elements by ID-LA-ICP-MS are much better than those by μ-XRF.*

Immunohistochemical staining was also carried out to investigate the distribution of Aβ protein, which attempt to correlate the interactions between metals and Aβ protein.

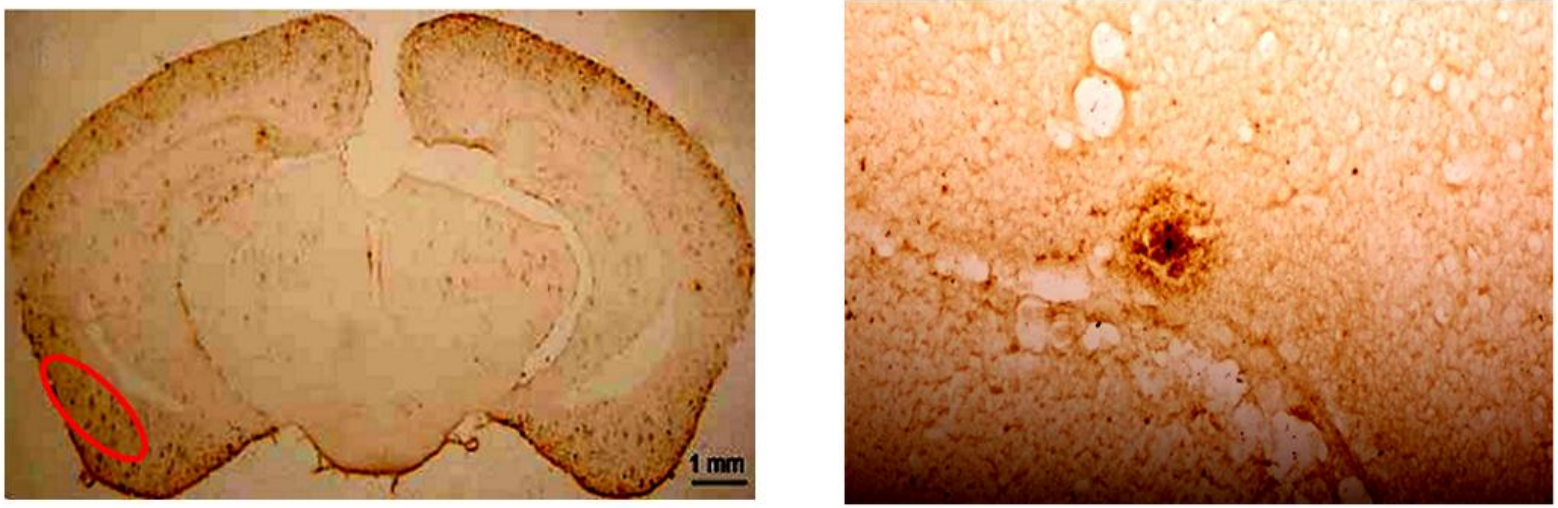


Fig. 9. Aβ42 plaques in brain sections of AD mouse

- ✓ *APP and Aβ has been reported to have metal binding and high affinity for Fe, Cu and Zn, suggesting a definitive role for APP/Aβ in metal ion homeostasis in brain.*
- ✓ *The images of ID-LA-ICP-MS, μ-XRF and immunohistochemistry do demonstrate some correlations between metals and Aβ protein.*

Anal Chim Acta, 2017, 984，66-75.

Anal Chim Acta, 2015, 884, 19-25.

Conclusion

✓ A novel quantitative imaging strategy for biological thin sections based on isotope dilution LA-ICP-MS was proposed.

✓ To evaluate if the isotope exchange reached, different isotope exchange conditions were investigated.

✓ The homogeneous mouse brain prepared as *in-house* matrix-matched standard was used for method validation and good consistency was acquired.

✓ The novel ID-LA-ICP-MS strategy was applied to intact AD mouse brain for absolute quantitative imaging.

Thank you!