CUSTOMER APPLICATION NOTE 44449

ICP-MS as a tool in elemental brain imaging

Authors: Olga Minaeva[1,2,3], Ning Hua[1,2], Nicola Lupoli[1], Douglas S. Parsons[1,2], Lee E. Goldstein[1,2,3,4], [1]The Center for Biometals & Metallomics, Boston University School of Medicine, Boston, MA; [2]Department of Radiology, Boston University School of Medicine, Boston, MA; [3]Boston University Photonics Center, Boston, MA; [4]Boston University College of Engineering, Boston, MA

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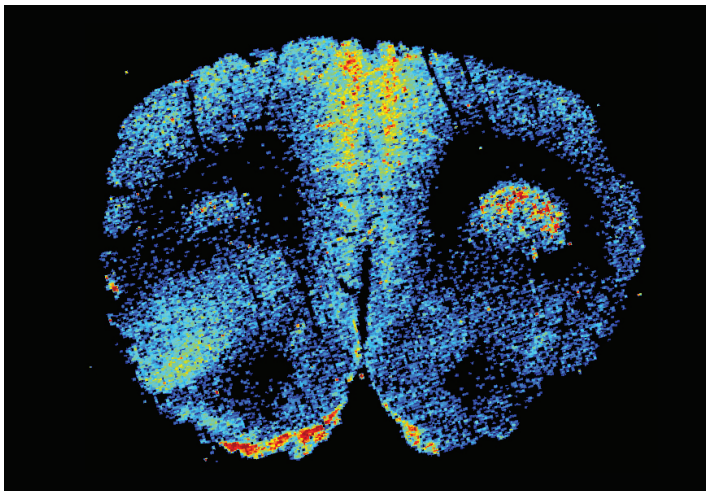

Goal

To demonstrate elemental brain imaging by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) using single quadrupole ICP-MS.

Introduction

LA-ICP-MS imaging is gaining recognition as a powerful new tool for neuroscience research. This technique enables precise anatomical localization and ultra-trace elemental quantitation in brains from humans and laboratory animals. LA-ICP-MS enables definitive elemental identification, ultra-trace analytical sensitivity (ppb-ppt), large dynamic range, and high spatial resolution (single micron) without labels or tags. In addition, multiple elemental maps can be generated simultaneously from the same brain section. Biologically ubiquitous elements such as phosphorus can be used to localize different brain regions and differentiate gray matter (neurons, glia, and blood vessels) from white matter (nerve fiber bundles). Phosphorus mapping greatly facilitates accurate localization and quantitation of other target elements that may be of interest (Cu, Zn, Fe, Gd, etc.). LA-ICP-MS imaging of brain specimens is a novel and powerful tool for exploring fundamental brain mechanisms in health as well as accelerating development of new diagnostics, treatments, drug delivery systems for neurodegenerative diseases, traumatic brain injury, brain cancer, demyelinating diseases, epilepsy, and other disorders of the central nervous system. This note demonstrates how elemental brain imaging can be generated using a laser ablation platform hyphenated to single quadrupole ICP-MS.

Experimental

Instrumentation

A laser ablation system (LSX-213 G2+, Teledyne CETAC Technologies) was coupled to a Thermo Scientific™ iCAP™ Q ICP-MS system for elemental brain imaging. Solution analysis was used to optimize the mass spectrometer to ensure maximum sensitivity and stability of the instrument. Optimization was conducted following careful inspection, testing, and if required, cleaning and/or replacement of critical instrument components, including skimmer and sampler cones. After this initial step, the mass spectrometer was connected to the laser ablation platform and tuned for this sample introduction method using the autotune routines provided in the Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software. An example of the parameters used for LA-ICP-MS can be found in Table 1.

Table 1. Instrument parameters for LA-ICP-MS measurements

iCAP Q ICP-MS	
Injector	2.5 mm i.d. quartz
Interface	Pt cones with High Sensitivity (2.8 mm) insert
RF power	1550 W
Nebulizer gas flow	0.93 L·min^{-1}
Autotune	Source Tune Laser (using NIST® SRM® 612 glass reference material)
LSX-213 G2+ LA System	
Ablation cell	HelEx II cell
Spot size	50 µm
Scan speed	100 µm·s^{-1}
Line separation	0 µm
Repetition rate	20 Hz
Fluence	5.10 J·cm^{-2}
Ablation cell gas flow	MFC1 = 0.75 L·min^{-1} He MFC2 = 0.20 L·min^{-1} He

Sample preparation

Rat brains: Rats were sacrificed by CO_2 asphyxiation followed by transcardial perfusion with 200 mL phosphate-buffered saline (to remove blood in the cerebrovasculature by fluid-exchange exsanguination) and in situ perfusion fixation with 200 mL 4% paraformaldehyde (PFA). Harvested brains were submerged in 4% PFA for 24 h at 4 ˚C, embedded in paraffin, and sectioned at 10 µm thickness. Luxol fast blue, hematoxylin, eosin (LHE) staining was performed on adjacent brain sections to confirm histological neuroanatomy. These experiments were approved by the Institutional Animal Care and Use Committee, Boston University School of Medicine, and conducted in accordance with guidelines from the Association for Assessment and Accreditation of Laboratory Animal Care and National Research Council Guide for the Care and Use of Laboratory Animals.

Human brains: Postmortem human brain was received as fixed tissue in formalin. Sections from the cortical region were embedded in paraffin and sectioned on a microtome at 10 µm thickness. These experiments were approved by the Institutional Review Board at Boston University School of Medicine.

Calibration standards: Analytical calibrations were performed with NIST reference standard SRM-612 (NIST, Gaithersburg, MD) and gelatin (10% w/v) spiked with Gd (NIST-3118a, NIST, Gaithersburg, MD). The gelatin standards were prepared using Type-A 300 Bloom strength gelatin powder (Electron Microscopy Sciences, Hartfield, PA). The gelatin and Gd solution was vortexed and heated in a 60 ˚C water bath. A small amount of the gelatin was then pipetted onto a chilled cryostat chuck and placed in a cryotome maintained at -20 ˚C. Once hardened, the gelatin standard was sectioned at 10 µm thickness and transferred to a microscope slide. Care must be taken during the transfer to avoid introducing tissue wrinkles and folds. Gelatin standard slides were stored at -20 ˚C until used for analysis. This method is described in detail by Gholap *et al.*[1] and Fingerhut *et al.*[2]

Data acquisition and processing

The iCAP Q ICP-MS system can be triggered by an electrical pulse from the laser ablation platform for synchronization or set to send a trigger pulse to the laser ablation platform. The second option is preferable for long-duration scans as this arrangement prevents sample ablation in the absence of ICP-MS data acquisition. When conducting line-by-line area scans, the iCAP Q ICP-MS system sends a trigger pulse to the laser ablation platform at the start of each line. If for some reason the mass spectrometer is not ready (for example, the plasma is unexpectedly extinguished), no trigger will be sent to the laser and sample will not be ablated. This system setup is especially important for high-value samples such as human brain.

LA-ICP-MS data sets were exported to a customized MATLAB® program (Mathworks, Natick, MA) and ImageJ (NIH open-source software) for anatomical mapping and analytical quantitation. ^{158}Gd-LA-ICP-MS imaging analyses were calibrated for total Gd concentration (c(Gd), $\mu g \cdot g^{-1}$) using calibration curves with eight points covering a tissue concentration range above and below expected values (Gd: 0.0–12.8 $\mu g \cdot g^{-1}$).

Results and discussion

Relative distributions of elements in rat and human brains

The distribution and concentration of elements in the brain provide important information about brain structure and function. Carbon is useful for identifying processing artifacts such as tissue wrinkles, folds, tears, and other technical issues during processing, sectioning, and transfer. Carbon maps provide critical information about tissue quality that may not be detected by macroscopic imaging. For instance, it is often difficult to distinguish a tissue section without processing artifacts (Figure 1, A) from one with wrinkles (Figure 2, A) or folds (Figure 3, A) by photomicrography. In this work, the ^{12}C signal was used to detect such artifacts (Figures1–4, B).

Phosphorus is highly abundant in the brain, with the greatest relative enrichment in gray matter with high cellular density of small neurons (e.g., dentate gyrus of the hippocampus, granule cell layer in the cerebellum). Therefore, phosphorus is an ideal element for differentiating gray and white matter. The ^{31}P isotope can be easily mapped with the iCAP Q ICP-MS system. ^{31}P has several polyatomic interferences ($^{14}N^{16}O^{1}H^{+}$, $^{15}N^{15}N^{1}H^{+}$, $^{15}N^{16}O^{+}$, $^{14}N^{17}O^{+}$, $^{13}C^{18}O^{+}$, $^{12}C^{18}O^{1}H^{+}$), but due to the high phosphorus concentration in human and rodent brain tissue, ^{31}P provides detailed structural information without post-processing optimization. Kinetic energy discrimination (KED) was therefore not used for interference removal, whereas higher detection sensitivity was obtained in standard mode for the main analyte, Gd. In order to demonstrate that the above-mentioned polyatomic interferences do not affect the observed signal for ^{31}P (and hence the maps), the threshold was lowered to zero counts per second (Figures 1-4, C). As can be seen from the resulting images, gray-white matter differentiation was still unambiguously possible. Brain images using the ^{31}P channel were also used to generate structural masks that can be applied to other elemental images to isolate brain from surroundings.

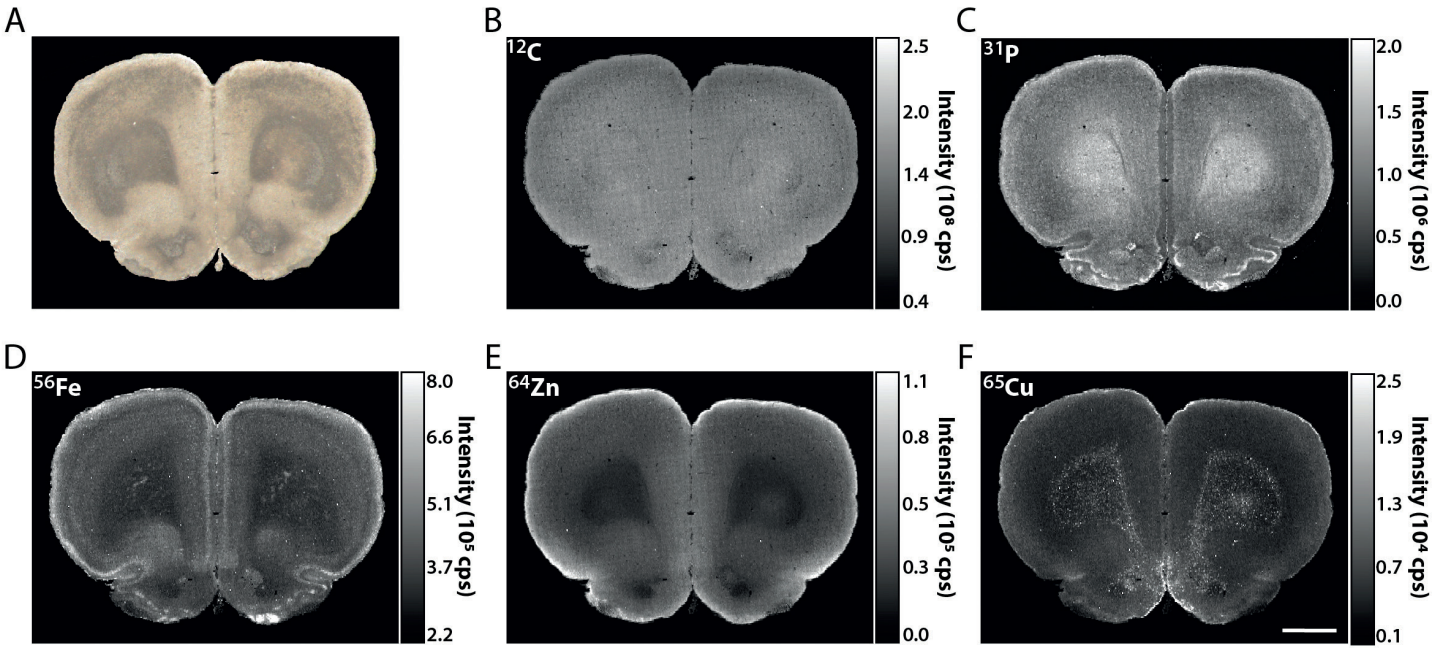

Figure 1. Elemental distribution maps for ^{12}C, ^{31}P, ^{56}Fe, ^{64}Zn, ^{65}Cu in rat anterior cingulate cortex and low power photomicrograph of brain section before ablation. All images are scaled the same. Scale bar, 2 mm

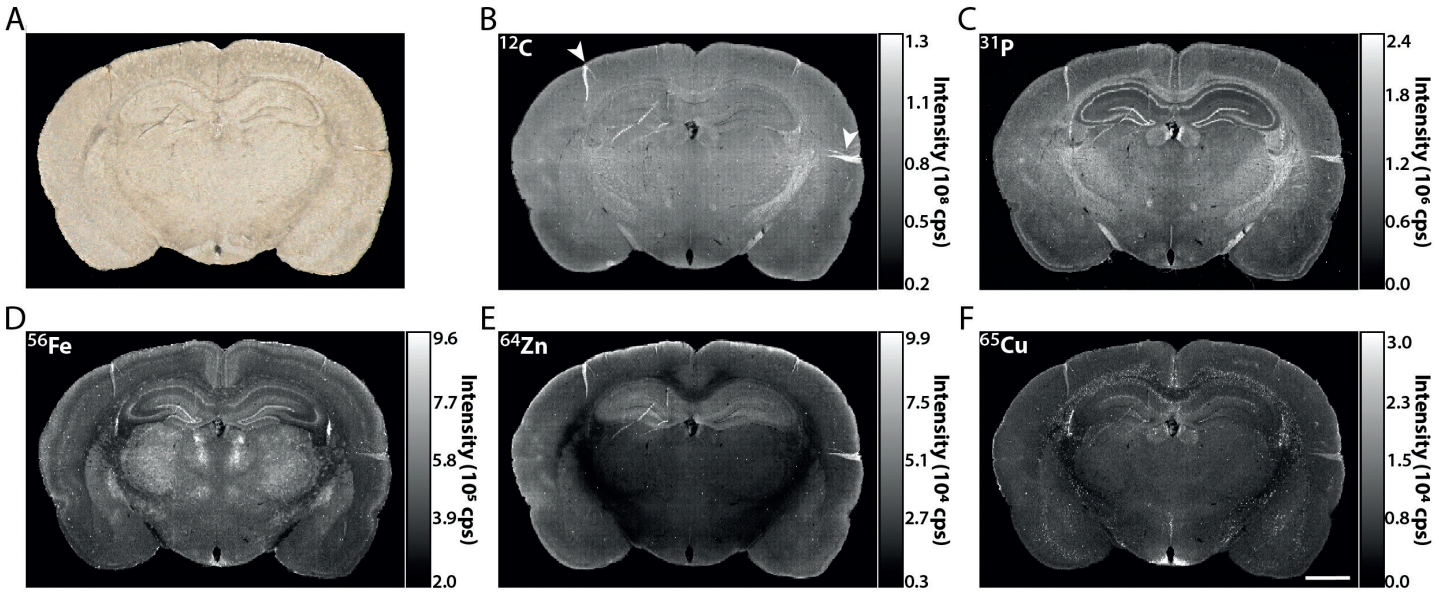

Figure 2. Elemental distribution maps for ^{12}C, ^{31}P, ^{56}Fe, ^{64}Zn, ^{65}Cu in rat rostral hippocampus and low power photomicrograph of brain section before ablation. All images are scaled the same. Scale bar, 2 mm Arrows indicate wrinkles in the tissue.

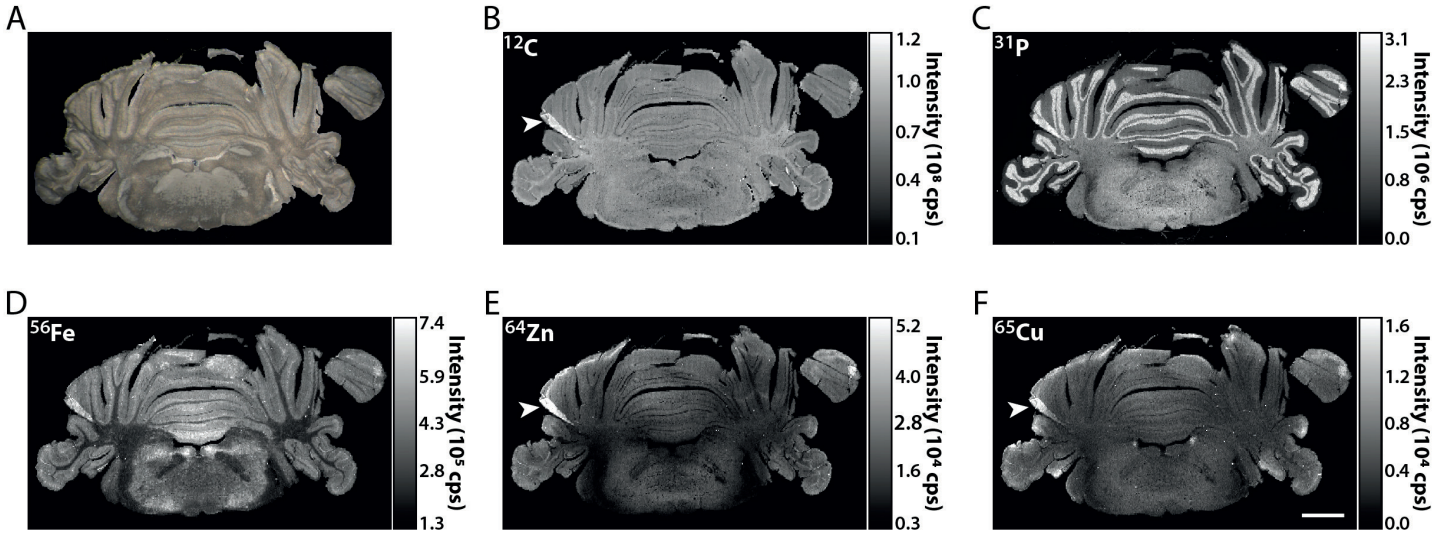

Figure 3. Elemental distribution maps for ^{12}C, ^{31}P, ^{56}Fe, ^{64}Zn, ^{65}Cu in rat cerebellum and low power photomicrograph of brain section before ablation. All images are scaled the same. Scale bar, 2 mm Arrows indicate a tissue fold.

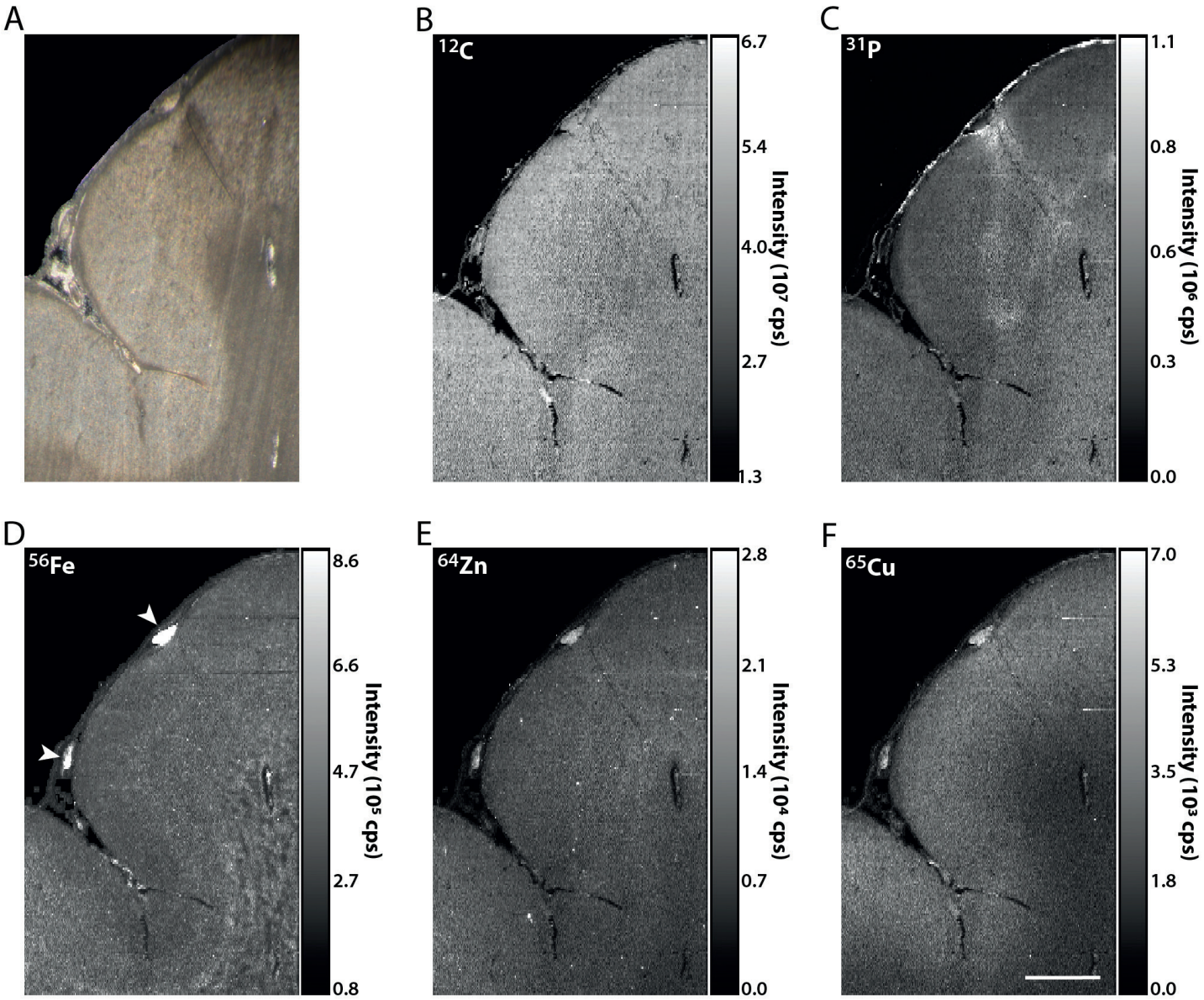

Figure 4. Elemental distribution maps for ^{12}C, ^{31}P, ^{56}Fe, ^{64}Zn, ^{65}Cu in rat anterior cingulate cortex and low power photomicrograph of brain section before ablation. All images are scaled the same. Scale bar, 2 mm Arrows indicate hematogenous (intravascular) blood.

^{56}Fe and ^{57}Fe channels yield similar signal-to-noise ratios in LA-ICP-MS brain imaging, so either Fe isotope (or both) can be used for routine elemental brain imaging. In this work, ^{56}Fe images are shown for four brain regions from rats and humans: rat anterior cingulate cortex (Figure 1, D), rat hippocampus (Figure 2, D), rat cerebellum (Figure 3, D), and human cerebral cortex (Figure 4, D). ^{56}Fe and ^{57}Fe have multiple polyatomic interferences that can influence LA-ICP-MS imaging results. However, the relative spatial distribution of brain iron can be achieved with single quadrupole ICP-MS. High quality relative images of brain iron can be obtained by applying thresholds to raw images. The ^{56}Fe maps presented here have been masked and thresholded to increase image contrast. High-resolution mass spectrometers such as the Thermo Scientific™ Element 2™ or Thermo Scientific™ Element XR™ High Resolution ICP-MS systems are preferred for analytical mapping of absolute concentrations of brain iron.

Special note should be made about conclusions drawn from brain iron maps. The cerebral vasculature in rat brains can be cleared of blood (by transcardial perfusion with phosphate-buffered saline) to avoid confounding contributions of iron-rich hemoglobin in red blood cells. The ^{56}Fe maps presented here show non-hematogenous (parenchymal) iron in the rat brain. By contrast, it is not possible to obtain perfused human brains, thus the ^{56}Fe maps presented here include contributions from both hematogenous (intravascular) and non-hematogenous (parenchymal) iron.

Information obtained from carbon (e.g., tissue uniformity, artifacts) and phosphorus (e.g., grey-white matter differentiation) channels greatly facilitate analysis and mapping of other elements of interest in the brain (e.g., zinc, copper, gadolinium, and other metals). Zn and Cu brain maps are shown in Figures 1-4, E, F. Note that the high levels of Zn and Cu in the rat cerebellum (arrows, Figure 3, E, F) are artifacts (i.e., tissue folds) ascertained by ^{12}C mapping of the same brain section.

Absolute distribution of Gd in rat and human brains

To convert brain maps from relative to absolute concentration, calibration curves were created based on gelatin standards spiked with elements of interest.[1,2] Other calibration strategies can also be used.[3,4] In this work, the LA-ICP-MS system was used for quantitative imaging of gadolinium in the brain. The ^{158}Gd channel was used to map the total gadolinium concentration in the rat brain after systemic administration (intravenous injection) of gadopentetate dimeglumine, a gadolinium-based contrast agent used during magnetic resonance imaging (MRI) examinations.[5] The ^{158}Gd channel was selected for these studies since its natural abundance (24.84%) is the highest of the six stable isotopes of this lanthanide series element.

Other factors that were considered include the absence of polyatomic interferences and high signal-to-noise ratio. Figure 5 shows that the calibration curve for Gd was linear and without drift over time. The coefficient of variation for the calibration curve slopes conducted on three different days was 1%. Accurate LA-ICP-MS calibration and imaging require system optimization with NIST reference standards before each scan. Examples of calibrated Gd brain maps are shown in Figures 6 and 7. Staining adjacent brain sections with Luxol fast blue, hematoxylin, and eosin is useful and informative as it allows to visualize different functional compartments in the overall system. Pink staining (eosin) reveals cell bodies, blue staining (hematoxylin) shows nuclei, and Luxol fast blue stains myelin (nerve fiber tracts). However, staining the same brain section used for LA-ICP-MS imaging is not recommended to avoid contamination from the stains used.

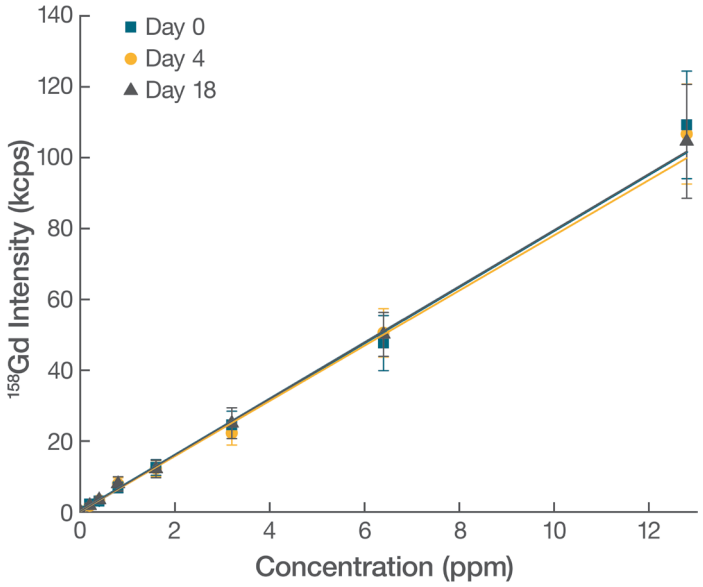

Figure 5. Calibration curve for ^{158}Gd based on gelatin standards

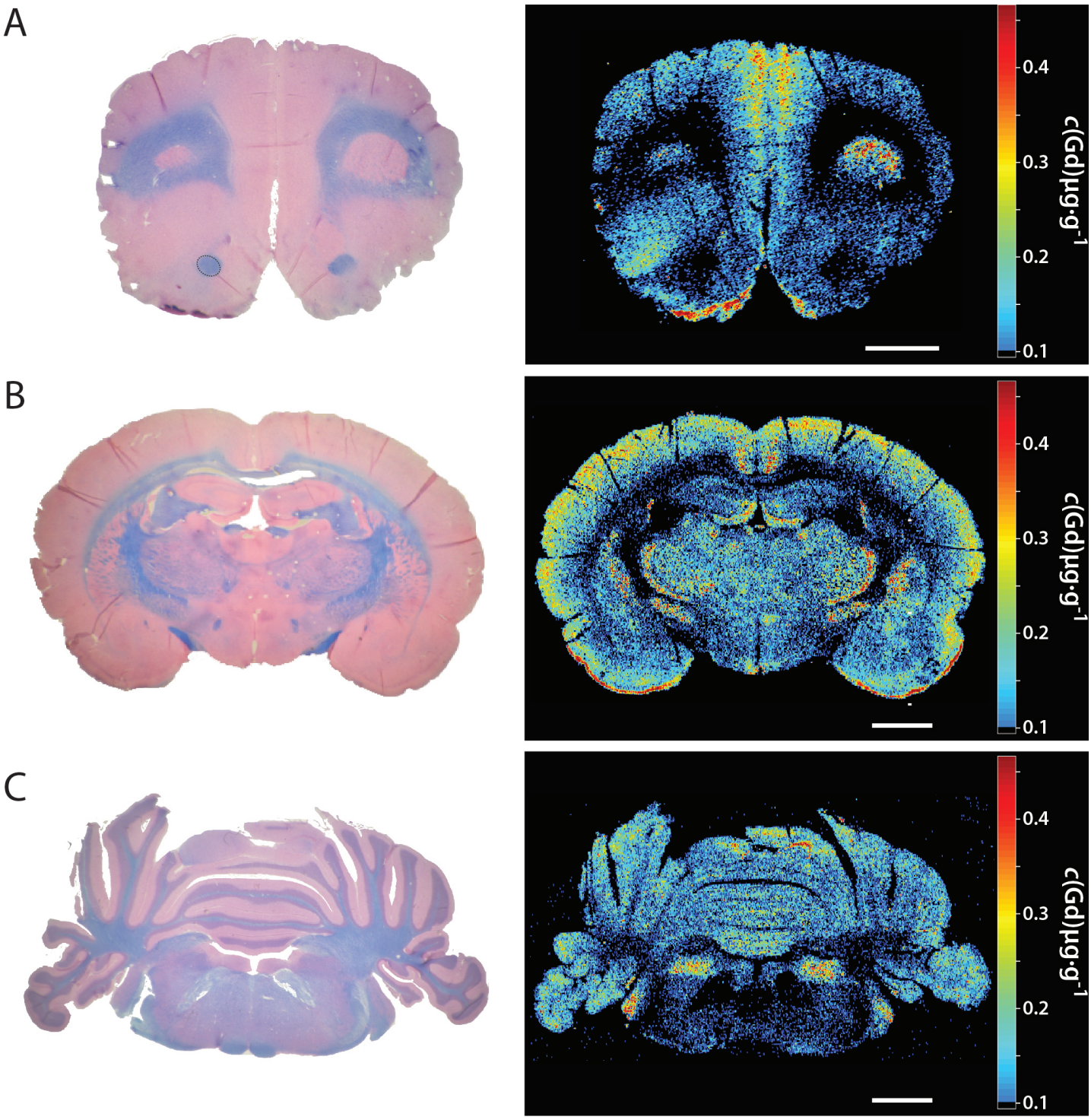

Figure 6. Calibrated LA-ICP-MS images of Gd in rat cerebral cortex after repeated exposure to intravenous gadopentetate dimeglumine. **A**, anterior cingulate cortex (ACC); **B**, rostral hippocampus (HIP); **C**, cerebellum (CER). *Left panels*, low power photomicrographs of representative coronal brain sections. Luxol fast blue, hematoxylin, eosin (LHE) staining. *Right panels*, LA-ICP-MS images (^{158}Gd) calibrated for total Gd concentration (*c*(Gd), µg·g^{-1}). Scale bars, 2 mm

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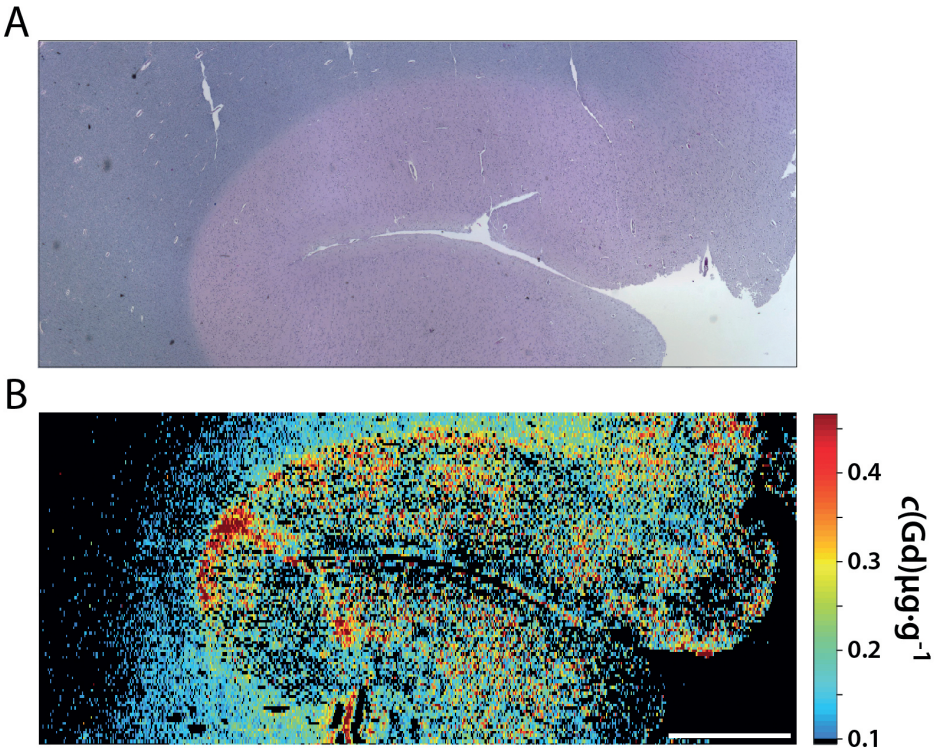

Figure 7. Calibrated LA-ICP-MS image of Gd in human cerebral cortex. A, Low power photomicrograph of the region of interest stained with Luxol fast blue, hematoxylin, and eosin. **B**, LA-ICP-MS brain map for gadolinium (^{158}Gd) calibrated for total Gd concentration (*c*(Gd), µg·g^{-1}). Scale bar, 2 mm

Conclusion

Use of the iCAP Q ICP-MS system in tandem with the LSX-213-G2+ laser ablation instrument can be exploited to produce high-quality, analytically calibrated maps of elements in brain specimens from laboratory animals and humans. This elemental mass spectrometry imaging modality provides new information about brain structure and function in health and disease. LA-ICP-MS imaging complements other neuroimaging techniques and brings new opportunities to advance brain research for preclinical and clinical applications.

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