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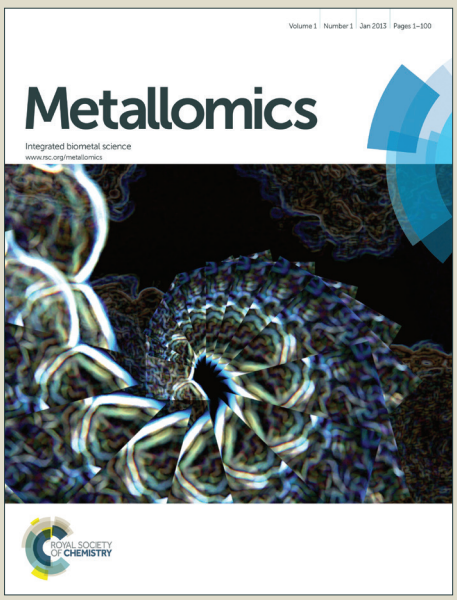
Accepted Manuscript



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Complementarity of MALDI and LA ICP mass spectrometry for platinum anticancer imaging in human tumor

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Abstract

The follow-up of the Heated Intraoperative Chemotherapy (HIPEC) of peritoneal carcinomatosis would benefit from the monitoring of the penetration, distribution and metabolism of the drug within the tumor. As tumor nodules can be resected during the therapy, mass spectrometry imaging is a suitable tool for the evaluation of treatment efficacy, and, as a function of the result obtained, the therapy can be re-optimized. In this work we demonstrate the complementarity of laser ablation (LA) ICP mass spectrometry and MALDI imaging to study the penetration and distribution of two Pt-based metallodrugs (cisplatin and oxaliplatin) in human tumor samples removed from patients diagnosed with colorectal or ovarian peritoneal carcinomatosis. LA ICP MS offered a sensitive (LOD for ^{195}Pt 4.8 pg·s^{-1}) imaging of platinum quasi-independently of the original species and sample matrix and thus an ultimate way of verifying the penetration of the Pt-containing drug or its moieties into the tumor. MALDI imaging was found to suffer in some cases from signal suppression by the matrix leading to false negatives. In the case of oxaliplatin metallodrug, studies results from ICP and MALDI MS imaging were coherent whereas in the case of cisplatin, species detected by ICP MS imaging could not be validated by MALDI MS. The study is the first application of the dual ICP and MALDI MS imaging to the follow-up of metallodrugs in human tumors.

Introduction

Heated Intraoperative Chemoterapy (HIPEC) is one of the most recent treatments of cancer; it consists of treating the residual post-operational cancerous tissues with a local application of high concentrations of warmed anti-cancer drugs for a short period of time.[1,2,3] Undoubtedly,

the understanding of the tumor response following HIPEC requires the data from the monitoring of the drug intake, distribution and metabolism within the tumor.

Platinum derivatives, such as cisplatin (cis-diamminedichroplatinum*(III)*), oxaliplatin ([(1R,2R)-cyclohexane-1,2-diamine](ethanedioato-O,O')platinum*(II)*) and carboplatin (cis-diammine(cyclobutane-1,1-dicarboxylate-O,O')platinum*(II)*), are a class of popular metallodrugs used in cancer therapy [4]. Their pharmacokinetics has usually been studied by the measurement of the total platinum concentrations by atomic absorption spectrometry (AAS) or ICP MS or by the measurement of the residual drug and its metabolites by the coupling of a separation technique (chromatography and electrophoresis) and ICP MS [5,6]. However, these techniques fail to supply information on the spatial distribution of the drug and, in the case of hyphenated techniques, fail for solid samples as the extraction recoveries prior to chromatography are never quantitative.

Imaging mass spectrometry is based on the measurement of the intensities of elemental or molecular ions generated locally by means of a laser and offers a typical resolution of 20-50 µm[2,7] Elemental imaging by laser ablation ICP MS, optimized to become a routine tool by Becker group [8,9,10] makes use of the low detection limits of ICP MS (fg level per pixel of 20-200 mm^2) and the large (10^8) linear range of quantitative response. The results acquired at a rate of 1 cm^2/h are visualized using suitable imaging software. These advantages are set off by the limitation to heteroatom-containing molecules only and the loss of speciation information.

A more popular alternative is the use of matrix-assisted laser desorption ionization (MALDI) MS imaging introduced by Caprioli group based on the monitoring of specific mass-to-charge (*m/z*) ratios of molecules of interest (usually peptides, lipids or metabolites).[1, 11] The measurement is usually carried out by TOF MS or - in order to assure more selectivity - tandem mass spectrometry (TOF/TOF). As histological features remain intact throughout the analysis of a tissue section, distribution maps of multiple analytes can be correlated with histological and clinical features. Unlike in ICP MS, the images are hardly quantitative and interferences due to the complexity of the biological matrix are a serious limitation leading in extreme cases to false positives or negatives.[11] Indeed, the ionization efficiency is critically dependent on the molecule structure and biological environment and the accurate measurement of the m/z value without interferences from concomitant species requires high resolution mass spectrometers.

Applications of mass spectrometry imaging to metallodrug studies have been rather scarce and limited to model animal studies [12]. They included multi-element imaging of mouse[13] and rat[14] kidney treated with cis-platin and imaging of Gd in mouse tumors subjected to magnetic fluid hyperthermia[10] by LA-ICP MS whereas MALDI MS imaging was used to for the determination of the distribution of oxaliplatin in rat kidney. [10]

The synergy between elemental and molecular mass spectrometry was convincingly demonstrated for hyphenated techniques[15, 16, 17] but, to our, knowledge our study is the first example correlating ICP and MALDI MS imaging data for the analysis of human tumors. The tumor nodules resected during the therapy represent valuable clinical material for mass spectrometry imaging techniques; the information acquired allows for the understanding of the processes taking place and – as a result - the optimization of the therapy.

The objective of this study was to investigate the synergy of MALDI and ICP MS imaging for the analysis of human tumors collected after heated intraoperative chemotherapy with two platinum-based metallodrugs. LA-ICP MS imaging was used in order to validate and complement the MALDI MS results.

Materials and methods

Tissue preparation for MALDI and ICP MS imaging

Tissues collection and sample preparation

Four tumor samples were surgically removed from human donors diagnosed with colorectal or ovarian peritoneal carcinomatosis. before and after HIPEC treatment with two different Pt-drugs and analyzed after the approval of the local ethic committee. HIPEC treatment was based on the administration in an open laparotomy of a heated solution of platinum based drug oxaliplatin and cisplatin with respectively 460 and 75 mg of the drug per square meter, at 42°C for 30 or 60 minutes. The tumors removed before the HIPEC treatment were analyzed as reference samples and the ones removed after the treatment were used to image the penetration of the drugs into the cancerous tissue.

The samples were immediately frozen in liquid nitrogen and stored at -80°C until analysis. Frozen tumoral tissue sections of 10 µm were sliced using a Microm HM 550 OPVD cryostat (Thermo Scientific), set at -20°C.

Imaging Mass Spectrometry

For MALDI MS imaging analysis, 10-µm slices of the tissue were thaw mounted on Indium-Tin Oxide ITO coated conductive glass slides (LaserBiolabs, Nice, France) and dehydrated in a desiccator. They were then coated manually with a 10 mg/ml α-cyano-4-hydroxycinnamic acid (LaserBiolabs, Nice, France) dissolved in 50% acetonitrile and 0.1% trifluoroacetic acid. For ICP-MS analysis, 10 µm tissue slices were thaw mounted on 2.5cm×2.5cm glass slides and desiccated. The chemical species monitored by MALDI MS included the intact compounds and their and monomethionine complexes forming signal clusters at m/z 456–460 and 359-363 for oxaliplatin and cisplatin, respectively.

For the parallel histological analysis consecutive 10-µm thick slices were collected and mounted onto specific supports; hematoxylin and eosin staining was used. The summary of the experimental protocol is shown in Fig. 1.

Instrumentation

MALDI MS imaging

MALDI imaging was performed using the 4800 Plus MALDI TOF/TOF™ Analyzer (AB Sciex). Image acquisition was achieved using the 4800 imaging tool software. Imaging of tumor tissue sections was performed in a reflector positive mode, in the mass range of m/z 300-700, with a 100×100 µm raster and laser intensity set around 50% of full laser intensity as selected within the 4000 Series Explorer™. At each position of the tissue section, an averaged mass spectrum is generated from 750 consecutive laser shots. The laser beam was determined to be circular with a diameter of 50 µm.

The two dimensional (2D) ion image of the tissue section was constructed using Tissue View™ software (Applied Biosystems, MDS analytical technologies). A color scale representing signal intensity is exported from TissueView™ software to provide semi quantitative information.

LA ICP MS Imaging

A Collision Cell Inductively Coupled Plasma Mass Spectrometry (ICP-MS) instrument (AGILENT 7500cs, Yokogawa Analytical Systems, Tokyo, Japan) was used. Operating conditions are given in Table 1. A New Wave Research (CA, USA) UP-213 Nd:YAG deep UV system was used for laser ablation.

The LA system was directly coupled to the ICP torch using a 60-cm Tygon tube (5.0 mm i.d.). The ablated material from the LA chamber was swept by a He carrier gas, mixed with an Ar makeup gas immediately prior to introduction to the plasma. Working conditions were

optimized in terms of detection limits and speed by the ablation of a raw tissue slices partially impregnated with a Pt drug solution. The LA ICP MS imaging was carried out with medium spatial resolution as its goal was the validation of the MALDI MS imaging data rather than fine structural characterization of the tissue. The used conditions are given in Table 1. Each LA ICP MS image is composed of ca. 25 laser scans (spot size 200 μm, 200 μm between each line). The signal intensity heatmaps were obtained using a lab-written application for Matlab R2009b (Mathworks, Natics, MA).

Results and discussion

Pt localization inside tumor tissues

The goal of the study was the localization of Pt-drugs and their possible metabolites in the tumor in order to check their penetration into the cancerous tissue; the presence of metabolites may indicate deactivation of the drug. A comparison of the behavior of two drugs (oxaliplatin and cisplatin) was carried out. The results of MALDI MS and LA ICP MS imaging for the use of oxaliplatin and cisplatin are shown in Fig. 2 and Fig. 3, respectively.

The platinum containing signature of the compounds detected by MALDI MS is asserted by the cluster due to the four major isotopes of the platinum ^{194}Pt, ^{195}Pt, ^{196}Pt and ^{198}Pt (relative abundances 32.9, 33.9, 25.9 and 7.2 %, respectively). The species monitored by MALDI MS was exclusively the monomethionine conjugates of the drug. The detection limit was estimated at 1-50 μg/g dry tissue. [12]

In Fig. 2 MALDI MS images (A1 and B1) show the peripheral location of the oxaliplatin complex. The LA ICP MS image (A2 and B2) of the same tumor, confirms that the Pt is mostly present on the outside of the sample thus confirming that no unknown Pt-containing metabolite is present in another region of the sample. For this Pt drug, the results of both molecular and elemental MS imaging methods are in agreement showing poor penetration of the drug into the cancerous tissue.

In the case of cisplatin (Fig. 3), it was not possible to detect any known drug-related molecule by MALDI MS. As a result, MALDI MS did not allow the drawing of any conclusions on the drug penetration into the tumor tissue. At the same time, LA ICP MS image (Fig. 3) confirms the presence of Pt inside the tumor thus indicating that the drug did enter the tissue but that MALDI ionization of the Pt-containing molecule present in the tumor was not successful. No characteristic Pt isotopic pattern was detected. Unfortunately, although LA ICP MS is able to confirm the presence of Pt in the tissue, it does not allow the identification of the structure of the Pt species.

Pt quantities in the tumor linked to the doses given to the patient

Both platinum compounds used were detected in the tumor tissue by LA ICP MS (LOD for ^{195}Pt 4.8 $pg \cdot s^{-1}$, corresponding to 20 fg per spot) demonstrating that the drugs reached their targets. However, the oxaliplatin response is ca. twice higher than that of cisplatin in the corresponding tumor taking into account the the drug concentrations in the HIPEC bath during the HIPEC protocol (molar ratio [oxali-Pt]/[cis-Pt] = 4.6). This preliminary conclusion is subject to further verification as, due to the differences in the geometry of the images, the fully quantitative data in the whole tissue would be necessary for definitive interpretation. The comparable intensities of the signals for Se, Cu and Zn (data not shown) detected in the tumors rule out the possibility of a tissue matrix influence on the LA ICP MS analysis.

Conclusion

LA ICP MS was successfully used in this work for the imaging of Pt drug in human tumoral tissue to complement the MALDI MS data. The synergy of both MALDI molecular MS and more sensitive, matrix independent and semi-quantitative LA ICP mass spectrometry imaging techniques was clearly shown. LA ICP MS imaging of metallodrugs in malignant tissues opens the way to a better understanding and dose-efficiency studies of clinical treatments. In the present study, the differences in the behavior of oxaliplatin and cisplatin were confirmed by the LA ICP MS images obtained for tumors treated with the two drugs; it was shown that oxaliplatin is mostly found at the periphery of the tumors whereas cisplatin penetrates deep into the tumor tissue.

Acknowledgments

The financial support of the Conseil Régional d'Aquitaine (20071303002PFM), INCa-DGOS-Inserm 6045 grant and the FEDER program (31486/08011464) is acknowledged. JB acknowledges the fellowship of the French Ministry of Education

Table 1: ICP MS and LA operational conditions

ICP Q MS Agilent 7500cs	
ICP ion source and ion transmission	
Forward power, W	1300
Plasma Ar gas flow rate, l min^{-1}	15.00
Auxiliary Ar gas flow rate, l min^{-}	0.80
Carrier Ar gas flow rate, l min^{-1}	0.76
Carrier He gas flow rate, l min^{-1}	0.50
Collision cell gas	He
Collision cell gas flow	3,6 $ml.min^{-1}$
Cones	Platinum
Data acquisition	
Measured isotope	^{13}C, ^{58}Ni, ^{60}Ni, ^{63}Cu, ^{64}Zn, ^{64}Cu, ^{66}Zn, ^{77}Se, ^{78}Se, ^{89}Y, ^{103}Rh, ^{194}Pt, ^{195}Pt, ^{196}Pt,
New Wave Research UP-213 LA	
Wavelength, nm	213
Pulse energy, mJ	0.45 (50%)
Fluence, J cm^{-2}	1
Spot size, µm	200
Distance between lines, mm	200
Scan speed, mm s^{-1}	50
Carrier He gas flow rate, l min^{-1}	0.50

Captions to figures:

Fig.1.
Scheme of the analytical protocol.

Fig 2:
Oxaliplatin imaging in the tumor tissues: A, B - pictures of tumor slices; A1, B1 - MALDI MS images; A2, B2 - ^{194}Pt LA-ICP MS images .

Fig 3:
Cisplatin imaging in the tumor tissues: C, D - picture of tumor slices; C2, D2 - ^{194}Pt LA-ICP MS images.

Fig. 1SI.
Calibration of the LA-ICP MS system.

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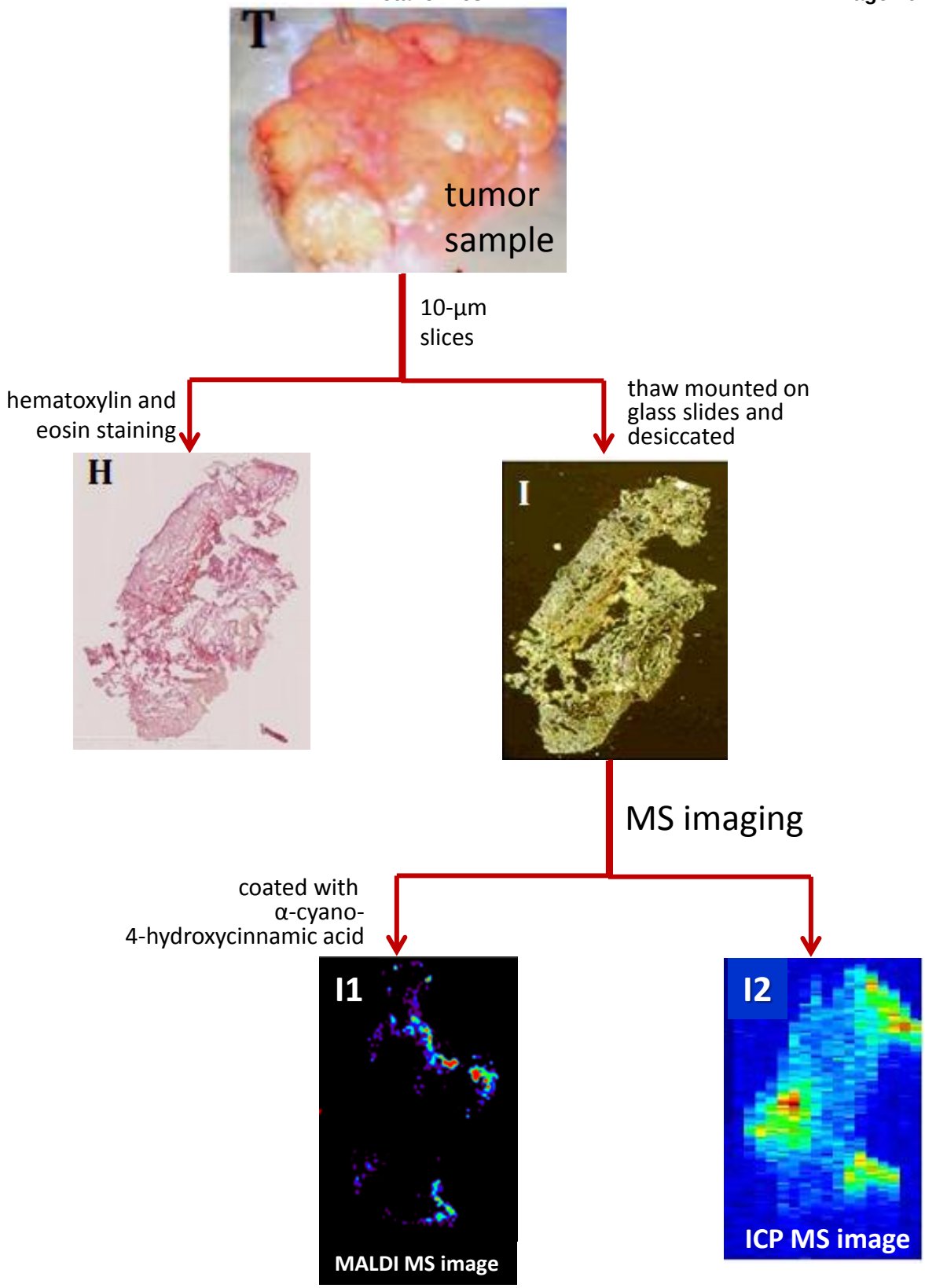

Fig.1

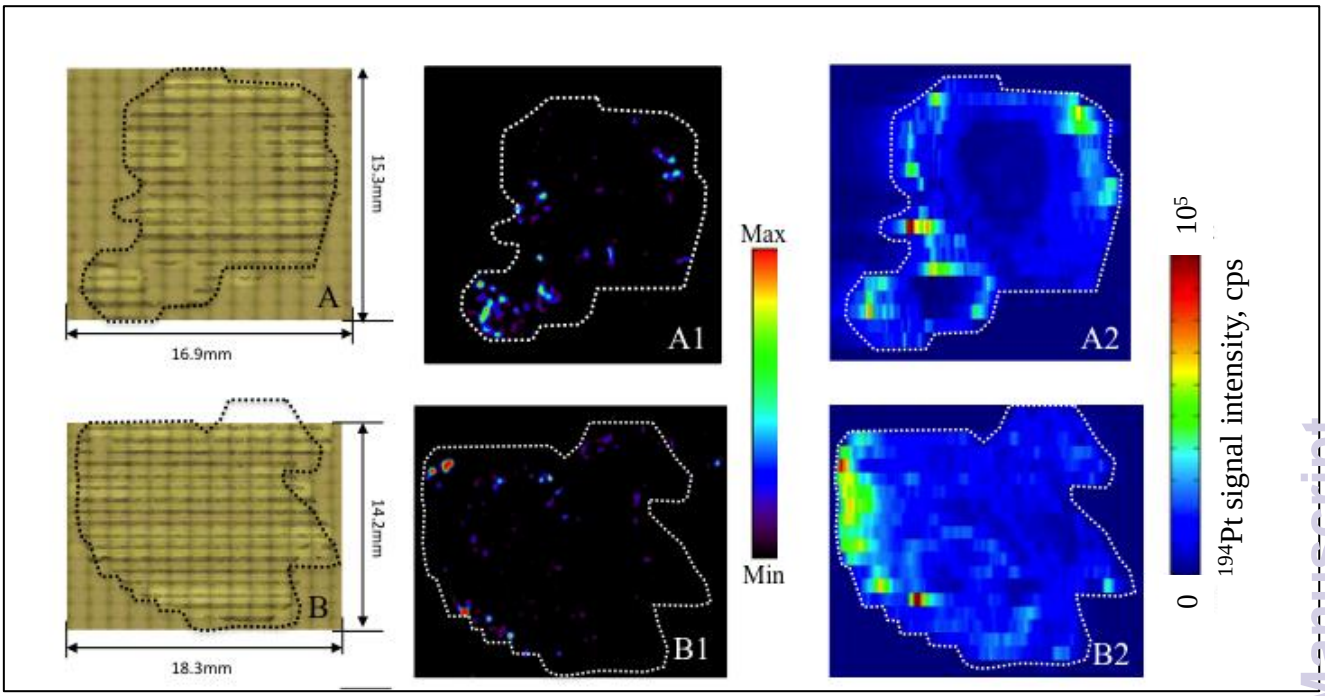

Fig. 2

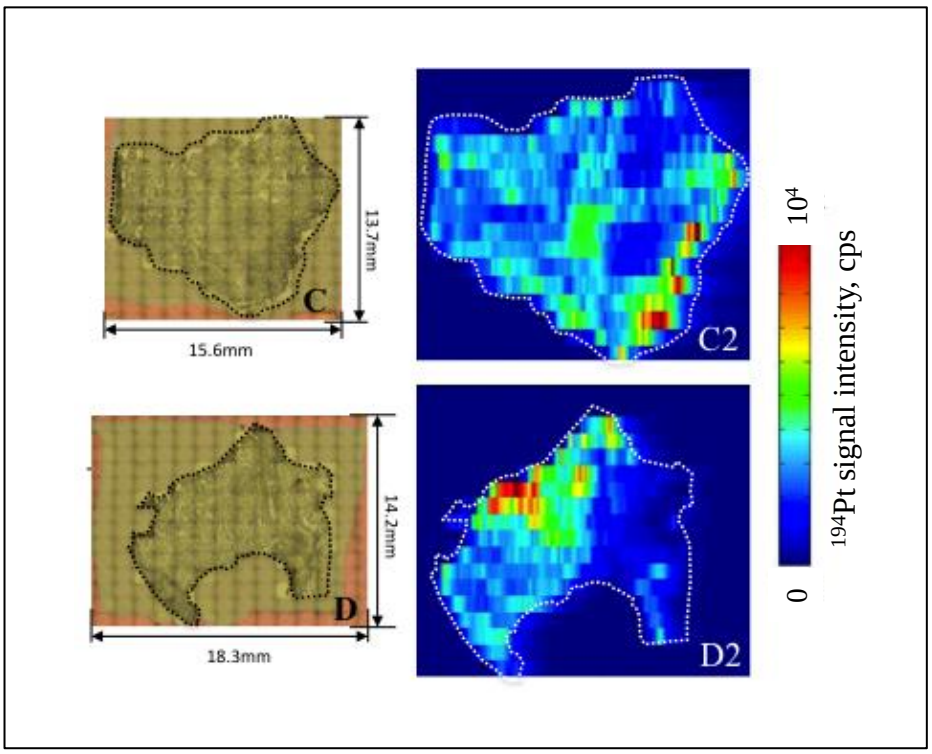

Fig. 3

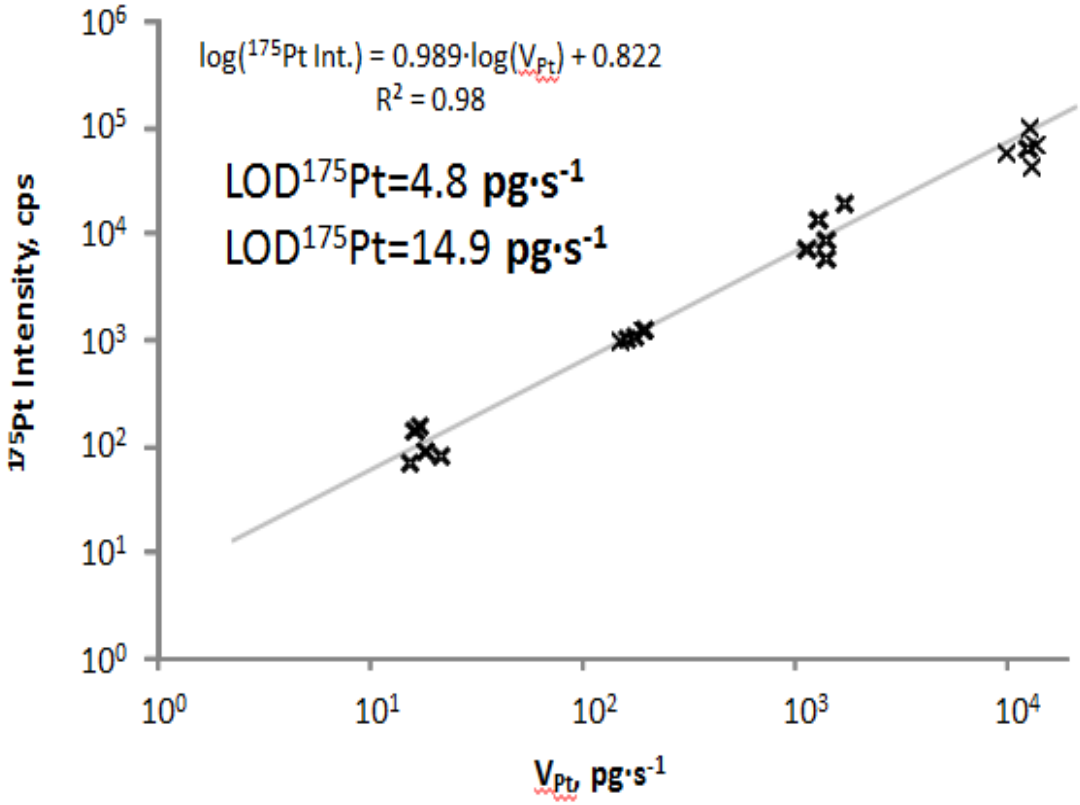

Fig. 1SI