Published in final edited form as:

Analyst. 2018 February 07; 143(3): 654–661. doi:10.1039/c7an01828b.

Quantitative Mass Spectrometry Imaging of Glutathione in Healthy and Cancerous Hen Ovarian Tissue Sections by Infrared Matrix-Assisted Laser Desorption Electrospray Ionization (IR-MALDESI)

Milad Nazari¹, Mark T. Bokhart¹, Philip L. Loziuk¹, and David C. Muddiman^{1,*}

¹Department of Chemistry and Molecular Education, Technology, and Research Innovation Center (METRIC), North Carolina State University, Raleigh, NC 27695

Abstract

A quantitative mass spectrometry imaging (QMSI) method for absolute quantification of glutathione (GSH) in healthy and cancerous hen ovarian tissues using infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI) is presented. Using this technique, the ion abundance of GSH was normalized to that of a structural analogue, which was sprayed on the slide prior to mounting the tissue sections. This normalization strategy significantly improved the voxel-to-voxel variability; the variability is attributed to the overall ionization process. Subsequently, a series of calibration spots of stable isotope-labeled (SIL) GSH were pipetted on top of the tissue to construct a spatial calibration curve, and calculate the concentration of GSH in both tissue sections. The QMSI results were verified by LC-MS/MS quantification of GSH for the same tissues. GSH was extracted from tissue sections in a slightly acidic buffer and was then alkylated using *N*-ethylmaleimide to minimize autooxidation of GSH to glutathione disulfide. The alkylated GSH was separated from other contaminants using reversed phase liquid chromatography (RPLC) coupled to a triple quadrupole mass spectrometer, and the *z*-ion transition of NEM-GSH was used to quantify GSH in each tissue section. While the absolute values obtained using IR-MALDESI QMSI and LC-MS/MS were different, a ~2-fold increase in the concentration of GSH in cancer tissue compared to the healthy tissue was observed using both techniques. Possible reasons for the difference between absolute concentration values obtained using IR-MALDESI QMSI and LC-MS/MS are also discussed.

Graphical abstract

* Author for Correspondence: David C. Muddiman, Ph.D., Department of Chemistry, Molecular Education, Technology, and Research Innovation Center (METRIC), North Carolina State University, Raleigh, North Carolina 27695, Phone: 919-513-0084, david_muddiman@ncsu.edu.

Conflicts of Interest

The authors declare no competing financial interests.

IR-MALDESI quantitative mass spectrometry imaging of glutathione in healthy and cancerous hen ovarian tissues

Keywords

IR-MALDESI; Glutathione; Absolute Quantification; Mass Spectrometry Imaging; Q Exactive Plus

Introduction

Over the past two decades, mass spectrometry (MS) has become one of the most valuable tools in cancer research.^{1–3} Mass spectrometry imaging (MSI) is one of the recent and rapidly evolving applications of MS in cancer research.^{4,5} MSI can provide the spatial distribution and relative abundance of hundreds of analytes in a label-free manner. Typically, analytes are desorbed from discrete positions across the tissue sections, ionized, and then sampled by a mass spectrometer, while the exact location from which the sample was desorbed is also recorded. The mass spectra, along with the locations they were acquired from, are used to generate ion images of analytes of interest. Numerous combinations of ionization methods and mass spectrometers have been used for cancer research in different fields such as proteomics, metabolomics, and drug distributions.⁵

While MSI has been extensively used for relative quantification of analyte abundance in tissue sections, its applications for absolute quantification of analytes are nascent. Matrix-assisted laser desorption/ionization (MALDI) is the most commonly utilized method for quantitative MSI (QMSI) analyses⁶; however, it suffers from drawbacks such as the need for high vacuum and deposition of an organic matrix and partitioning of analytes into the matrix crystals, which varies between different analytes and tissue types.⁷ Recently, many groups have focused on developing ambient ionization methods for MSI, which allow for analyzing biological tissue sections at conditions that are much closer to their native state and reduce the sample preparation steps.⁸

Matrix-assisted laser desorption electrospray ionization (MALDESI) is an example of such an ambient ionization technique. In MALDESI analyses, a laser pulse is used to resonantly excite the endogenous or exogenous matrix to facilitate the desorption of material from the sample surface. The plume of mostly neutral desorbed material interacts with an orthogonal electrospray, where analytes partition into the charged droplets of electrospray and are ionized in an ESI-like manner.⁹ The current version of the source utilizes a mid-infrared (IR) pulse at 2940 nm to resonantly excite the O-H stretching of water present in the sample and an exogenous ice layer matrix. The ice matrix accounts for variations in water content of different tissues or different compartments in heterogeneous tissues, and it has been shown

to greatly enhance desorption and the consequent ion abundances.^{10,11} It is important to note that the ice matrix is used solely to enhance desorption and does not appear to participate in the ionization process. Therefore, unlike MALDI MSI experiments where the choice of matrix requires considerations based on tissue type or analyte size¹², ice can be used universally for different tissue types and analytes with different size and biochemical properties. Furthermore, using ice as matrix allows for analysis of small molecules without concerns about matrix-related peaks that are commonly observed in the lower m/z regions of MALDI mass spectra.

One of the applications of IR-MALDESI is in the field of metabolomics. Metabolomics refers to large-scale study of small molecules (metabolites) in an organism at given conditions. Aberrations in the metabolome have been linked to a variety of diseases such as diabetes, heart disease, inborn errors of metabolism, and cancers.^{13–16} In a recent untargeted metabolomics study, IR-MALDESI was utilized to discern the differences in relative abundance and spatial distribution of metabolites between healthy and cancerous hen ovarian tissues.¹⁷ The domestic egg-laying hen model was chosen for this analysis, since it is the only non-human animal model that exhibits spontaneous development of ovarian cancer (OVC) with a high prevalence^{18,19}, and similar to humans, its prevalence increases with age.^{20,21} One of the metabolites that was significantly more abundant in the cancerous hen ovarian tissue was glutathione (GSH). GSH is a tripeptide consisting of glutamic acid, cysteine, and glycine, and with concentrations in the 1–10 mM range, it is the most abundant non-protein molecule in cells.^{22,23} GSH is involved in a variety of cellular functions such as DNA repair, cell cycle regulation, and regulation of enzyme activity; however, its most important role is to act as a scavenger of reactive oxygen species (ROS) and protect the cell from the oxidative stress.^{22,24} Considering the importance of GSH in maintaining cellular homeostasis, it is not surprising that elevated levels of GSH have been reported in many cancers such as breast, colon, lung, and ovary.^{23–25}

In this work, we present an application of IR-MALDESI for QMSI of GSH in healthy and cancerous hen ovarian tissue sections. A structural analogue of GSH was used as normalization standard and was sprayed on the slide prior to thaw-mounting the tissue sections to reduce voxel-to-voxel variability in the ion images. In addition, the IR-MALDESI QMSI results were validated using LC-MS/MS. GSH was extracted from the adjacent tissue sections, and then alkylated using *N*-ethylmaleimide (NEM), prior to LC-MS/MS analyses.

Experimental

Materials

HPLC-grade methanol, water, and acetonitrile were purchased from Burdick and Jackson (Muskegon, MI, USA). Natural (NAT) glutathione standard, *N*-ethylmaleimide (NEM), 7.5 M ammonium acetate buffer (molecular biology grade), and acetic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stable isotope-labeled (SIL) glutathione ($^{13}\text{C}_{21}^{15}\text{N}_1$) was purchased from Cambridge Isotopes Laboratories (Tewksbury, MA, USA), and γ -L-glutamyl-L-cysteine ammonium salt (γ -EC) was purchased from Bachem (Torrance, CA, USA). Nitrogen gas (purity 99.999%) used for purging the IR-MALDESI imaging

enclosure was obtained from Arc3 Gases (Raleigh, NC, USA). The pH of ammonium acetate buffer was adjusted to 5.8 by adding glacial acetic acid. All other compounds were used without further modifications.

Hen Ovarian Tissues

Ovarian tissues from age-matched healthy and cancerous C-strain white leghorn commercial egg laying hens were obtained from an in-house biorepository. The birds were managed in accordance with the Institute for Laboratory Animal Research Guide, and all husbandry practices were approved by North Carolina State University's Institutional Animal Care and Use Committee (IACUC). The tissue sections were flash-frozen in cold isopentane in liquid nitrogen bath, and stored at -80°C until the time of analysis.

Sample Preparation for IR-MALDESI QMSI

The workflow of sample preparation for IR-MALDESI QMSI is shown in Figure 1. Microscope slides were coated evenly with γ -EC using a pneumatic sprayer (TM Sprayer, Leap Technologies, Carrboro, NC, USA) using the previously optimized parameters.²⁶ The tissues were cut into 10- μm thick sections using a Leica CM1950 cryostat (Buffalo Grove, IL, USA) and then thaw-mounted onto the slides that were sprayed with γ -EC. A dilution series of $^{13}\text{C}_{21}^{15}\text{N}_1$ -GSH at concentrations of 1.0, 0.5, 0.25, 0.13, 0.062, 0.031, and 0 mg/mL were prepared in 50:50 (v/v) methanol/water. One hundred nanoliters of each solution was pipetted on top of the tissue using a Hamilton 7000.5 syringe (Hamilton Company, Reno, NV, USA), and the tissues were immediately analyzed by IR-MALDESI.

IR-MALDESI QMSI

The details of the IR-MALDESI source and MSI analyses have been described elsewhere.^{10,27} In short, the slides were placed on a XY-controlled Peltier stage, which is housed inside an acrylic enclosure. The relative humidity (RH) inside the enclosure was reduced to $\sim 5\%$ by purging it with nitrogen gas, and then the Peltier stage was cooled to -9°C . The temperature was held constant for 10 minutes to allow for the tissue to come to thermal equilibrium, at which point the enclosure door was opened to expose the tissue to the ambient RH and facilitate the deposition of a thin layer of ice on top of the tissue. Once a sufficient amount of ice (roughly 2–3 times the thickness of the tissue) was formed over the tissue, the enclosure was closed and purged with nitrogen to reduce the RH to $\sim 10\%$ to maintain the layer of ice throughout the experiment. A mid-IR laser (IR Opolette 2371, Opotek, Carlsbad, CA, USA) operated at 2940 nm is used to resonantly excite the O-H stretching of water molecules present in tissue as well as the ice matrix, and facilitate the desorption of molecules from the tissue sections. The plume of desorbed material is then intercepted by an orthogonal electrospray where neutrals partition into the charged droplets of electrospray and are ionized in an ESI-like manner.⁹ MSI data were collected in negative ion mode since in a preliminary study glutathione was observed at an order of magnitude higher ion abundance in negative ion mode compared to positive ion mode. Per previous optimizations, a solution of 50/50 (v/v) methanol/water with 1 mM acetic was used in all QMSI experiments¹⁷

The IR-MALDESI source was coupled to a Q Exactive Plus (Thermo Scientific, Bremen, Germany) mass spectrometer. Two laser pulses ($f_{\text{rep}} = 20$ Hz) were used to achieve complete and reproducible desorption of material at each voxel. Due to the pulsed nature of ionization in IR-MALDESI MSI analyses, the automatic gain control function (AGC) of the instrument was disabled. In turn, the ions generated from both pulses were accumulated in the C-trap for a fixed injection time (IT) of 75 ms, and then injected into the Orbitrap for a single measurement. The m/z range of 200–350 Th was measured in negative ion mode with a resolving power of 140,000 (FWHM, m/z 200), and low parts per million (ppm) mass measurement accuracy (MMA) was achieved by using peaks of palmitic acid at 255.2329 $[M-H]^+$ and stearic acid at 283.2643 $[M-H]^+$ as lock masses.²⁸ The desorption diameter (spot size) of the laser was measured at 150 μm on tissue¹⁰, and oversampling^{29,30} was used to achieve a spatial resolution of 100 μm .

IR-MALDESI QMSI Data Processing

The .RAW files generated by the Q Exactive Plus instrument were converted to mzML using the MSConvert tool by Proteowizard³¹, and the mzML files were then converted to imzML format³² using the imzML Converter.³³ The imzML files were subsequently loaded into MSiReader³⁴, a vendor-neutral and open-source software designed for processing high resolving power MSI datasets. All images were generated using a ± 2.5 ppm tolerance.

The MSiQuantification function in MSiReader was used to calculate the concentration of endogenous GSH in healthy and cancerous tissue sections. Heatmaps and histogram of MMA of endogenous GSH in both tissues (Figure S1) were generated using the built-in MMA Heatmap function in MSiReader.³⁵

Sample Preparation for LC-MS/MS Quantification

Unless otherwise noted, all stock solutions and samples were prepared in a 95/5 (v/v) ammonium acetate/acetonitrile buffer (pH 5.8), hereinafter referred to as “Buffer”. The pH of Buffer was adjusted to be slightly acidic to prevent autooxidation of GSH during the extraction process, as previously reported in the literature.^{36–39} Hen ovary tissues were weighed accurately (184.0 mg for healthy tissue and 98.1 mg for cancerous tissue) and homogenized in 1.0 mL of cold methanol for 30 seconds using an Omni TH-01 homogenizer (Omni International, Kennesaw, GA, USA). SIL GSH at a final concentration of 295 μM was added to the homogenates and the mixture was centrifuged at $1,000 \times g$ at 4 °C for 5 minutes using a Sorvall ST16R centrifuge (Thermo Fisher Scientific, Waltham, MA, USA). One hundred microliters of the supernatant was transferred to a clean centrifuge tube, where the solvent was evaporated under dry N_2 stream and then reconstituted in 1 mL of Buffer, and then diluted by a factor of 100. Alkylation of free thiols was performed according to a previously optimized process⁴⁰, where 1 mM NEM in Buffer was added to the reconstituted sample. The reaction was allowed to proceed at room temperature for 30 minutes, and then quenched by addition of 1% (v/v) acetic acid to the mixture. The alkylated samples were analyzed right away by LC-MS/MS.

LC-MS/MS Analysis

A nanoLC-2D system equipped with an AS1 autosampler (Eksigent, Dublin, CA, USA) coupled to a TSQ Vantage triple quadrupole mass spectrometer (Thermo Scientific, San Jose, CA, USA) was used for LC-SRM quantification. An analytical column was constructed using a 75 $\mu\text{m} \times 15\text{ cm}$ PicoFrit column (New Objective, Ringoes, NJ) that was self-packed with Kinetex C18 2.6 μm (Phenomenex, Torrance, CA). Mobile phase A and mobile phase B comprised of 98:2:0.2 water/acetonitrile/formic acid (v/v/v) and 100:0.2 methanol/formic acid (v/v), respectively. Each sample was loaded using a 4 μL metered injection of a 2 μL sample using 100% mobile phase A at 500 nL/min. GSH-NEM was eluted using a 30-minute gradient at 500 nL/min, where the mobile phase was held constant at 100% A for 10 minutes, then ramped to 90% B over 2 minutes, and finally held at 90% B for the next 18 minutes. The eluents were ionized using ESI by applying a 1,600 V potential to the capillary held at 325 °C. A selected reaction monitoring (SRM) method was developed using the EZ Method setting in the accompanied XCalibur software (Thermo Fisher Scientific, San Jose, CA, USA) with a cycle time of 2.0 s, chrom filter peak width of 45 s, Q1/Q3 (FWHM) of 0.7, and a collision pressure of 1.5 mTorr. The collision energy (CE) for each transition was optimized by injecting a solution of NAT GSH standard alkylated with NEM and choosing the CE that resulted in the most abundant peak for each transition (Figure S3). The healthy and cancerous tissue extract samples were analyzed in triplicates and blanks were injected between each replicate.

LC-MS/MS Data Analysis

The .RAW files from LC-SRM analyses were imported into Skyline⁴¹ for peak alignment and integration. The z-ion transitions of NAT (433.1 $\rightarrow$ 287.1) and SIL (436.1 $\rightarrow$ 290.1) GSH-NEM were integrated and used for quantification. The peak areas were exported into Excel for subsequent analyses and calculation of GSH concentration in tissue sections.

Results and Discussions

Reducing Voxel-to-Voxel Variability in Quantitative IR-MALDESI MSI

Variability from one scan to another is one of the biggest challenges in QMSI analyses. This variability can be caused by many different factors such as differences in ionization efficiency or differences in morphology across the tissue section. Different approaches such as normalizing the ion abundance to total ion current (TIC) or to a structural analogue have been proposed. A detailed review of various normalization strategies in MALDI QMSI experiments was recently published by Rzagalinski & Volmer.⁴² While the variation due to tissue morphology cannot be circumvented, incorporating a normalization standard has been shown to greatly reduce the voxel-to-voxel variability due to differences in ionization efficiency in IR-MALDESI experiments.²⁶ One of the advantages of IR-MALDESI for QMSI analyses is that the tissue is completely desorbed at each voxel, and therefore, the amount of tissue sampled at each voxel remains constant throughout the experiment. In addition, complete desorption of sample allows for incorporation of the normalization standard below the tissue section.

To obtain the best results, it is crucial for the normalization compound to have similar ionization efficiency as the analyte of the interest. In this work, γ -EC was sprayed on a glass slide using a pneumatic sprayer as the normalization compound. The concentration used (1 mg/mL) was optimized empirically to obtain similar ion abundances of γ -EC and GSH in tissue. Ion images of NAT GSH (m/z 306.0766, $[M-H^+]^-$) in healthy and cancerous hen ovarian tissue sections before and after normalization to γ -EC are shown in Figure 2. Examples of locations across each tissue section that benefited greatly from normalization to a structural analogue are shown using dashed lines in Figure 2. It can be observed that by normalizing the ion abundance of NAT GSH to γ -EC (m/z 249.0545, $[M-H^+]^-$) the voxel-to-voxel variability is significantly reduced and morphological features such as follicles can be more readily distinguished in each tissue section to gain an appreciation for biological complexity and the heterogeneity of GSH content across each tissue section. Since the deprotonated adducts of both NAT GSH and γ -EC were used, this normalization strategy can reduce variations in ionization efficiency on a per-voxel basis and allows for absolute quantification of NAT GSH in the healthy and cancerous tissue sections.

Absolute Quantification using IR-MALDESI

In order to generate a calibration curve to calculate the absolute concentration of GSH in tissues, a spatial calibration curve of SIL GSH was pipetted directly on the tissue prior to IR-MALDESI MSI. As mentioned above, at each voxel the tissue is completely sampled; therefore, abundances of γ -EC, NAT GSH in tissue, and SIL GSH calibration on tissue can be monitored simultaneously on a per-voxel basis. The MSiQuantification function in MSiReader was used to construct calibration curves and calculate the absolute concentration of NAT GSH in healthy and cancerous tissue sections. This function automates the absolute quantification MSI data processing which is described in detail below.

For all analyses the ion abundances of NAT GSH in tissue and the SIL GSH spotted on the tissue were normalized to that of γ -EC (Figure 3A–B). The concentration of SIL GSH in each calibration spot was then calculated by dividing the total amount of SIL GSH by the corresponding spot volume in cubic millimeters (defined by the area of the spot multiplied by the tissue thickness) to account for variations in the size of the pipetted calibration spots. Calibration curves were constructed by plotting the normalized abundance of SIL GSH to γ -EC against the concentration of SIL GSH on tissue in units of μg GSH per gram tissue, assuming a tissue density of 1.0 mg/mm^3 . The average normalized abundance of endogenous GSH to γ -EC over the entire tissue was then used to determine the concentration of GSH (Figure 3C). Eventually, the concentration of GSH in each tissue was converted from $\mu\text{g/g}_{\text{tissue}}$ to mM for comparison with LC-MS/MS results and previous literature. As shown in Table 1, the IR-MALDESI QMSI results demonstrate a 2.4-fold increase in average absolute concentration of GSH across the cancerous tissue ($7.97 \pm 0.41 \text{ mM}$) compared to the healthy tissue ($3.33 \pm 0.32 \text{ mM}$). It is worth noting that GSH is not homogeneously distributed across the tissue sections, and its absolute concentration varies in different locations. The concentration heatmaps of GSH across each tissue section and the histogram of concentration frequency in each tissue (Figure S1) demonstrate the heterogeneity of GSH concentration. The overlap in the lower concentration region of the histograms (1–5 mM) between the cancerous and healthy tissues indicates that the developed

IR-MALDESI QMSI method has the potential to distinguish healthy regions within the cancerous tissue. As discussed above, observation of this remarkable heterogeneity is possible after normalization of GSH ion abundance to that of γ -EC.

Since in MSI experiments the sample is analyzed without any prior cleanup or chromatographic separations, it is imperative that high MMA is conserved throughout the analysis, which could consist of 3–4 hours. In order to ensure the mass measurement of NAT GSH in tissue sections was not compromised during the QMSI analyses, MMA heatmaps of GSH in both cancerous and healthy tissues, along with a histogram of MMA in all scans, were generated using the built-in function in MSiReader. As shown in Figure S2, for all the scans across the tissue the MMA of GSH falls within the ± 2.5 ppm tolerance used to generate the ion images and calculate the concentration.

Comparison of IR-MALDESI QMSI with LC-MS/MS

To compare the results obtained using IR-MALDESI QMSI to a more conventionally used method, GSH was extracted from adjacent tissue sliced and analyzed using LC-MS/MS. GSH is known to be a labile metabolite that can oxidize to glutathione disulfide (GSSG). In order to minimize this autooxidation, the extraction was performed in a cold Buffer with a slightly acidic pH. In addition, the thiol group of GSH was protected via alkylation by NEM, which reacts selectively with the –SH group. These conditions were previously shown to minimize the rate of conversion of NAT GSH to GSSG in tissue.^{36,40,43} Another added benefit of alkylation with NEM is that GSH-NEM is much less polar than GSH, and therefore reversed phase liquid chromatography (RPLC) can be used to separate GSH-NEM from other contaminants in the extract. Previous studies have shown that reacting NEM with GSH for 30 minutes is sufficient to achieve complete alkylation^{37,40}, and we found no evidence of under- or over-alkylation^{40,44} in our study (i.e. no peaks for GSH or NEM-GSH-NEM were observed).

A representative mass spectrum of NAT GSH-NEM, along with the chemical structure of the molecule, are shown in Figure 4A, where different fragment ions can be observed. Once the collision energies for different fragment ions of GSH-NEM were optimized (Figure S3), extracts from healthy and cancerous tissues were analyzed in triplicate and the z-ion transitions of NAT ($433.1 \rightarrow 287.1$) and SIL ($436.1 \rightarrow 290.1$) GSH-NEM were used to calculate the absolute quantification of GSH in these tissues. Representative extracted ion chromatograms (XIC) of the z-ion of both NAT and SIL GSH-NEM in healthy tissue are shown in Figure 4B. By integrating the area under each peak the absolute concentrations of GSH in healthy and cancerous tissues were calculated to be 1.68 ± 0.03 mM and 3.60 ± 0.09 mM, respectively. The values obtained for concentration of GSH in each tissue as measured by IR-MALDESI QMSI and LC-MS/MS are shown in Table 1. It can be seen that while the absolute values are different between the two modes of measurement, the relative increase in cancerous tissue compared to healthy tissue remains constant in both analysis modes. The reason for the difference in absolute values could be attributed to the fact that two completely different methodologies on different instruments were used for quantification.

In IR-MALDESI QMSI experiments, the samples were thaw-mounted onto slides and directly analyzed without any extraction or modification steps. The analysis was performed

in negative-ion mode since in a previous study of hen ovarian tissues GSH demonstrated much higher ion abundance in negative-ion mode, compared to positive ion mode.¹⁷ In contrast to IR-MALDESI QMSI, LC-MS/MS quantification was performed in positive-ion mode after extraction of GSH from tissues and subsequently alkylating GSH with NEM. The extraction and alkylation steps can introduce a systematic bias in the data when compared to IR-MALDESI QMSI results. Since in IR-MALDESI analyses the tissue is completely sampled, it is assumed that cells are lysed and all the GSH content is measured at each voxel. However, there is a possibility that some cells were not lysed during the homogenization and extractions steps prior to LC-MS/MS. Therefore, we hypothesize that the major difference between the two techniques that could lead to a systematic underestimation of GSH in tissues are the homogenization and extraction steps.

Conclusions

An application of IR-MALDESI QMSI for obtaining absolute concentration of the endogenous antioxidant glutathione in healthy and cancerous hen ovarian tissues was presented. The voxel-to-voxel variability in IR-MALDESI QMSI analyses was reduced by normalizing the ion abundance of GSH to that of γ -EC, and SIL GSH was pipetted directly on the tissue at different concentrations to construct a spatial calibration curve. In addition, GSH was extracted from the same tissues and alkylated using NEM prior to analysis by LC-MS/MS. The values obtained using IR-MALDESI QMSI were compared to the LC-MS/MS results and while the absolute values were different, a consistent ~2-fold increase in the concentration of GSH in the cancerous tissue was observed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors gratefully acknowledge financial assistance received from the National Institutes of Health (R01GM087964), the W. M. Keck foundation, and North Carolina State University.

References

1. Wulfschlegel JD, Liotta LA, Petricoin EF. Nat Rev Cancer. 2003; 3:267–275. [PubMed: 12671665]
2. Diamandis EP. Mol Cell Proteomics. 2004; 3:367–378. [PubMed: 14990683]
3. Liesenfeld DB, Habermann N, Owen RW, Scalbert A, Ulrich CM. Cancer Epidemiol Biomarkers Prev. 2013;1–21.
4. Chughtai K, Heeren RMA. Chem Rev. 2010; 110:3237–3277. [PubMed: 20423155]
5. Arentz G, Mittal P, Zhang C, Ho Y, Briggs M, Winderbaum L, Hoffman MK, Hoffman P. Adv Cancer Res. 2017; 134:27–66. [PubMed: 28110654]
6. Ellis SR, Bruinen AL, Heeren RMA. Anal Bioanal Chem. 2014; 406:1275–89. [PubMed: 24281323]
7. Chaurand P, Cornett DS, Angel PM, Caprioli RM. Mol Cell proteomics. 2011; 10:O110.004259.
8. Robichaud G, Barry JA, Muddiman DC. Encycl Anal Chem. 2014
9. Sampson JS, Hawkridge AM, Muddiman DC. J Am Soc Mass Spectrom. 2006; 17:1712–1716. [PubMed: 16952462]
10. Robichaud G, Barry JA, Garrard KP, Muddiman DC. J Am Soc Mass Spectrom. 2013; 24:92–100. [PubMed: 23208743]

11. Robichaud G, Barry JA, Muddiman DC. *J Am Soc Mass Spectrom.* 2014; 25:319–328. [PubMed: 24385399]
12. Schwartz SA, Reyzer ML, Caprioli RM. *J mass Spectrom.* 2003; 38:699–708. [PubMed: 12898649]
13. Gowda Nagana GA, Zhang S, Gu H, Asiago V, Shanaiah N, Raftery D. *Expert Rev Mol Diagnostics.* 2008; 8:617–633.
14. Xu J, Chen Y, Zhang R, Song Y, Cao J, Bi N, Wang J, He J, Bai J, Dong L, Wang L, Zhan Q, Abliz Z. *Mol Cell Proteomics.* 2013; 12:1306–18. [PubMed: 23397110]
15. Courant F, Antignac JP, Dervilly-Pinel G, Le Bizec B. *Proteomics.* 2014; 14:2369–88. [PubMed: 25168716]
16. Johnson CH, Ivanisevic J, Siuzdak G. *Nat Rev Mol Cell Biol.* 2016; 17:451–459. [PubMed: 26979502]
17. Nazari M, Muddiman DC. *Analyst.* 2016; 141:595–605. [PubMed: 26402586]
18. Johnson PA, Giles JR. *Nat Rev Cancer.* 2013; 13:432–436. [PubMed: 23676850]
19. Hawkrige AM. *Proteomics Clin Appl.* 2014; 8:689–699. [PubMed: 25130871]
20. Barua A, Bitterman P, Abramowicz JS, Dirks AL, Bahr JM, Hales DB, Bradaric MJ, Edassery SL, Rotmensch J, Luborsky JL. *Int J Gynecol cancer.* 2009; 19:531–539. [PubMed: 19509547]
21. Treviño LS, Buckles EL, Johnson PA. *Cancer Prev Res.* 2012; 5:343–349.
22. Van Haften RIM, Haenen GRMM, Evelo CTA, Bast A. *Drug Metab Rev.* 2003; 35:215–253. [PubMed: 12959415]
23. Balendiran GK, Dabur R, Fraser D. *Cell Biochem Funct.* 2004; 22:343–352. [PubMed: 15386533]
24. Gamcsik MP, Kasibhatla MS, Teeter SD, Colvin OM. *Biomarkers.* 2012; 17:671–691. [PubMed: 22900535]
25. Estrela JM, Ortega A, Obrador E. *Crit Rev Clin Lab Sci.* 2006; 43:143–181. [PubMed: 16517421]
26. Bokhart MT, Rosen E, Thompson C, Sykes C, Kashuba ADM, Muddiman DC. *Anal Bioanal Chem.* 2015; 407:2073–2084. [PubMed: 25318460]
27. Nazari M, Bokhart MT, Muddiman DC. *J Vis Exp.* 2016; e53942:1–7.
28. Olsen JV, de Godoy LMF, Li G, Macek B, Mortensen P, Pesch R, Makarov A, Lange O, Horning S, Mann M. *Mol Cell Proteomics.* 2005; 4:2010–21. [PubMed: 16249172]
29. Jurchen JC, Rubakhin SS, Sweedler JV. *J Am Soc Mass Spectrom.* 2005; 16:1654–9. [PubMed: 16095912]
30. Nazari M, Muddiman DC. *Anal Bioanal Chem.* 2015; 407:2265–2271. [PubMed: 25486925]
31. Kessner D, Chambers M, Burke R, Agus D, Mallick P. *Bioinformatics.* 2008; 24:2534–6. [PubMed: 18606607]
32. Schramm T, Hester A, Klinkert I, Both JP, Heeren RMA, Brunelle A, Laprévote O, Desbenoit N, Robbe MF, Stoeckli M, Spengler B, Römpp A. *J Proteomics.* 2012; 75:5106–5110. [PubMed: 22842151]
33. Race AM, Styles IB, Bunch J. *J Proteomics.* 2012; 75:5111–5112. [PubMed: 22641155]
34. Robichaud G, Garrard KP, Barry JA, Muddiman DC. *J Am Soc Mass Spectrom.* 2013; 24:718–721. [PubMed: 23536269]
35. Bokhart MT, Nazari M, Garrard KP, Muddiman DC. *J Am Soc Mass Spectrom.* 2017 In Press.
36. Norris RL, Eaglesham GK, Shaw GR, Smith MJ, Chiswell RK, Seawright AA, Moore MR. *J Chromatogr B.* 2001; 762:17–23.
37. Camera E, Rinaldi M, Briganti S, Picardo M, Fanali S. *J Chromatogr B.* 2001; 757:69–78.
38. Carroll D, Howard D, Zhu H, Paumi CM, Vore M, Bondada S, Liang Y, Wang C, St DK. *Free Radic Biol Med.* 2016; 97:85–94. [PubMed: 27212018]
39. Iwasaki Y, Saito Y, Nakano Y, Mochizuki K, Sakata O, Ito R, Saito K, Nakazawa H. *J Chromatogr B.* 2009; 877:3309–3317.
40. Shuford CM, Poteat MD, Buchwalter DB, Muddiman DC. *Anal Bioanal Chem.* 2012; 402:357–366. [PubMed: 21956263]
41. Maclean B, Tomazela DM, Shulman N, Chambers M, Finney GL, Frewen B, Kern R, Tabb DL, Liebler DC, Maccoss MJ. *Bioinformatics.* 2010; 26:966–968. [PubMed: 20147306]

42. Rzagalinski I, Volmer DA. *Biochim Biophys Acta*. 2017; 1865:726–739. [PubMed: 28012871]
43. Paulech J, Solis N, Cordwell SJ. *Biochim Biophys Acta*. 2013; 1834:372–379. [PubMed: 22910378]
44. Smyth DG, Blumenfeld OO, Konigsberg W. *Biochem J*. 1964; 91:589–595. [PubMed: 5840721]

Figure 1.

The workflow of quantitative IR-MALDESI MSI of hen ovarian tissue sections. In short, healthy and cancerous tissues were harvested and stored at -80°C until time of analysis. The microscope slides were sprayed with a normalization compound that is a structural analogue of GSH using a pneumatic sprayer. The tissues were sectioned using a cryostat, and then thaw-mounted onto the coated slides. Then a spatial calibration curve of the SIL-GSH was placed on each tissue section and tissues were analyzed by IR-MALDESI.

Figure 2. Ion images of glutathione in healthy and cancerous tissues before (top) and after (bottom) normalization to γ -EC, demonstrating the importance of normalization to a structural analogue for accurate observation of the heterogeneity of GSH abundance across each tissue section. The dashed lines show examples of locations across each tissue section that particularly benefited from normalization.

Figure 3.

A) Ion images of GSH normalized to γ -EC in healthy and cancerous tissues, **B)** ion images of SIL GSH normalized to γ -EC placed on top of each tissue, **C)** resulting calibration curves constructed from the SIL calibration spots along with the average abundance of GSH/ γ -EC over the tissue area for each tissue section. The error bars indicate the 95% confidence intervals in the mean.

Figure 4.

A) Representative tandem mass spectrum of NAT GSH-NEM showing all the transitions from healthy hen ovarian tissue. Only the z-ion transition (highlighted with an asterisk) was used to quantify GSH in tissue sections. **B)** XIC of z-ions of NAT and SIL GSH-NEM in healthy hen ovarian tissue demonstrating the co-elution of the two species.

Table 1

Absolute concentration of glutathione in healthy and cancerous hen ovarian tissue sections, along with the cancer/healthy fold change, obtained by IR-MALDESI QMSI and LC-MS/MS. The values are the mean concentration of glutathione in each tissue along with the 95% confidence interval of the mean (CIM_{95%}). The number of voxels analyzed for IR-MALDESI analyses and the number of technical replicates for LC-MS/MS analyses are presented in parentheses. Interestingly, the CIM_{95%} for IR-MALDESI are an order of magnitude larger than LC-MS due to the significant heterogeneity of GSH in the tissue.

	Healthy Tissue	Cancerous Tissue	$\frac{\text{Cancer}}{\text{Healthy}}$
IR-MALDESI	3.33 ± 0.32 mM (n = 12,786)	7.94 ± 0.41 mM (n = 4,855)	2.4
LC-MS/MS	1.68 ± 0.03 mM (n = 3)	3.60 ± 0.09 mM (n = 3)	2.1