Published in final edited form as:

Anal Bioanal Chem. 2015 March ; 407(8): 2311–2320. doi:10.1007/s00216-015-8489-5.

A Method to Prevent Protein Delocalization in Imaging Mass Spectrometry of Non-Adherent Tissues: Application to Small Vertebrate Lens Imaging

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Abstract

MALDI imaging requires careful sample preparation to obtain reliable, high quality images of small molecules, peptides, lipids, and proteins across tissue sections. Poor crystal formation, delocalization of analytes, and inadequate tissue adherence can affect the quality, reliability, and spatial resolution of MALDI images. We report a comparison of tissue mounting and washing methods that resulted in an optimized method using conductive carbon substrates that avoids thaw mounting or washing steps, minimizes protein delocalization, and prevents tissue detachment from the target surface. Application of this method to image ocular lens proteins of small vertebrate eyes demonstrates the improved methodology for imaging abundant crystallin protein products. This method was demonstrated for tissue sections from rat, mouse, and zebrafish lenses resulting in good quality MALDI images with little to no delocalization. The images indicate, for the first time in mouse and zebrafish, discrete localization of crystallin protein degradation products resulting in concentric rings of distinct protein contents that may be responsible for the refractive index gradient of vertebrate lenses.

Keywords

Imaging Mass Spectrometry; Matrix Assisted Laser Desorption Ionization (MALDI); Lens; Crystallins

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Introduction

Following the first publication of matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) in 1997 by Caprioli and collaborators [1] many sample preparation methods have been developed for imaging a variety of analytes, including metabolites [2, 3], drugs [2, 4, 5] agrochemicals [3], lipids [6–8], peptides [9, 10] and proteins [11, 12] in multiple tissue types. The diversity of analytes and tissue types from a wide variety of species in studies that utilize MALDI IMS has resulted in diverse sample preparation techniques; each of which needed to be optimized for the specific application in order to obtain good quality data. Essential steps for optimal sample preparation include: tissue sectioning, mounting, washing, and matrix application [13–18]. Each step requires optimization to produce homogeneous matrix coverage, high analyte signal-to-noise (S/N) ratios, and minimal analyte delocalization.

Analyte delocalization is a problem that negatively impacts observed spatial resolution and image quality. Analyte delocalization can occur at multiple stages during the sample preparation process including during solvent-assisted mounting (in the case of lens tissue), tissue washing, and matrix application. Thaw mounting of biological samples onto a conductive target is routinely used in MALDI IMS and involves adherence of a frozen tissue section to a surface by thawing prior to drying. It is assumed that no significant delocalization of analytes occurs at this point due to the small amount of cytosolic liquid present within a thin tissue section. However, thaw mounting is not always suitable for all tissues types, such as plant, vertebrate ocular lens, and human skin [19], since variations in physical properties for these tissue types can cause a loss of adherence to the target surface once desiccated.

In addition to tissue mounting, tissue washing can also contribute to analyte delocalization. Aqueous washing of biological samples improves spectral quality for protein and lipid analysis by removing salts present in cells and interstitial fluids; salts that interfere with analyte ionization [20] or produce spectra with multiple cationic species for each analyte [6]. Washing can also help to improve matrix-analyte crystal formation upon matrix deposition and has become commonplace for IMS analysis of proteins. Although sample washing is commonly used, it is possible that highly soluble proteins may be displaced or may diffuse completely out of the tissue, resulting in an inaccurate determination of their localization and distribution within the sample. Furthermore, washing methods are typically not suitable for small soluble molecule imaging since the soluble analytes are often removed or migrate away from and across the tissue. Additionally, washing protocols can also exacerbate loss of sample adherence to the target plate.

Matrix application can also result in dissociation of the tissue section from the target surface and can contribute to analyte delocalization; therefore, choosing the appropriate matrix application technique based on the type of biological sample is crucial to obtaining quality images. The techniques and instrumentation for matrix application have progressed rapidly in recent years since matrix application is a crucial step to obtaining high quality spectra. The main objectives of the matrix application step are to provide homogeneous matrix coatings and to prevent over-wetting of the tissue by solvent, since excessive solvent usage

can result in analyte delocalization. The use of solvent-free or low solvent volume methods during the matrix application step can prevent delocalization [21,-23]; however, efficient extraction of analytes may be compromised.

Analyte delocalization and poor tissue adherence are issues that affect successful imaging of multiple sample types, particularly as the sample sizes become small. One such sample type is the ocular lens that is composed of highly abundant crystallin proteins. Lens transparency is required for proper delivery of images to the retina and is established by close packing of fiber cells that contain high concentrations of crystallin proteins. The organized spatial arrangement of specific crystallin proteins is believed to play a crucial role in maintaining the refractive properties of the lens [24, 25]. In order to focus images onto the retina a gradient of refractive index (GRIN) is established from the anterior surface to the posterior surface; however, the exact molecular nature of the GRIN has not been completely characterized.

Lens crystallins are highly concentrated (300–900 mg/mL) water-soluble proteins and account for ~60% of the total lens protein [26]. As fiber cells differentiate, they lose their nuclei and other organelles to prevent light scatter on the optical axis. The result of this differentiation process is that little or no protein turnover occurs in mature fiber cells; therefore, lens proteins accumulate modifications such as truncation, deamidation, oxidation, and crosslinking over the lifetime of the organism [27]. The three classes of crystallins, the α -, β -, and γ -crystallins, are so named according to the order in which they elute from a gel filtration column [28]. There are two forms of the α -crystallins, the lens specific form α A-crystallin and the more ubiquitously expressed α B-crystallin, both of which are believed to have both structural and chaperone-like functions within the lens [29, 230]. The α -crystallins, therefore, play a major role in maintaining the solubility of damaged or denatured proteins within the lens to protect against protein aggregation and light scattering.

Previous IMS analyses have shown that the α -crystallins in the outer cortical region of the lens in human and bovine tissue are present in their full-length form. Post-translationally modified and truncated α -crystallin products are present in distinct locations within the nuclear and cortical regions of the lens [26, 27]. During sample preparation for MALDI IMS analyses, washing or matrix application can result in disruption of the unique and defined localizations of these proteins and their modified/truncated products. In addition, lens tissue sections have a propensity for lifting off of the MALDI target plate during these steps. Soft landing of lens tissue for ocular lens protein imaging was first described by Han *et al.* [26], where a thin film of ethanol applied to an ITO coated glass slide was used to assist in mounting the sample. Grey *et al.* described utilizing a methanol soft landing for the analysis of human lens tissue [27]. However, when similar techniques were applied to smaller vertebrate lens samples (i.e. rodent and zebrafish), high levels of analyte delocalization were observed. This relative increase in delocalization is due in part to the small size of the rodent and zebrafish lenses since the slightest migration of analytes off tissue is detectable at the spatial resolutions used to image these tissues. Previous images published on MALDI IMS of rat lenses by Stella *et al* [31] were acquired only from the tissue surface, not from the adjacent target surface, so the extent of delocalization was not detected in the resulting MALDI IMS data.

Here we describe a method to address many of the aforementioned issues observed in the sample preparation process for protein IMS analyses. Our method employs a conductive adhesive tape target to enable imaging of samples with poor adherence without the need for thaw mounting or solvent-washing that cause analyte delocalization. Conductive adhesive tape has previously been utilized to improve whole body animal section IMS [32, 33], for the analysis of the drug methamphetamine within hair samples [34], imaging of plant stem and leaf tissues [3] and imaging of wheat and rice grains [35, 36]. The method described here involves attaching tissue cryo-sections to electrically conductive carbon tape or tab and lyophilization of the sample without allowing it to thaw, thereby minimizing analyte delocalization. We examine the effects of solvent-assisted mounting and tissue washing on protein delocalization and demonstrate the utility of the carbon tape/pad method for imaging ocular lens proteins from small vertebrates including rat, mouse, and zebrafish having radial dimensions as small as 1mm.

Methods

Animal Strains

Rat lens tissue was obtained from the Wistar-strain derived hereditary cataractous ICR/f rat [37] at 21 days and ~100 days of age. Whole mouse eye tissue was obtained from 7 and 11.5 month old C57BL/6 mice. Zebrafish lens tissue was obtained from an adult (approximately 12 months old) *Danio rerio* wild type fish.

Slit-lamp Ophthalmoscopy

Eyes of non-anesthetized mice were examined in a Nikon FS-2 slit lamp ophthalmoscope, as previously described [38, 39]. Mouse eyes were dilated with a 1:1 mixture of 1% tropicamide (Alcon, Fort Worth, TX, USA) and 10% phenylephrine hydrochloride (Akorn, Abita Springs, LA, USA). The slit lamp angle was 40 degrees and the slit width was 0.2 mm. Images were recorded using a Canon Optura Pi digital camcorder and captured with Adobe *Premiere* Software.

Carbon tape/tab method

Frozen whole mouse and zebrafish eyes with lenses *in-situ* and lenses from dissected rat eyes were sectioned at 20 μ m thickness at a temperature of -20°C in a Leica CM3050S cryostat. Sections were then adhered to either pre-cooled (-20°C) electrically conductive tape (3M XYZ axis acrylic filled adhesive containing conductive microfibers, 76 μ m thickness; Ted Pella, Inc. Redding, CA, USA) cut to desired size or pre-cooled (-20°C) electron microscopy ultra-thin carbon conductive adhesive tabs (carbon filled conductive adhesive with a nonconductive cloth core material, total thickness 160 μ m, 12 mm diameter; Electron Microscopy Sciences, Hatfield, PA, USA) by applying gentle pressure from a gloved fingertip. Tissue sections were carefully mounted onto adhesive surfaces that were held with forceps to prevent heat transfer from fingers that could thaw the tissue. Mounted sections were then transferred to a pre-cooled -80°C lyophilizing vessel using forceps and freeze dried for at least three hours to ensure the samples did not thaw. The lyophilized sections on adhesive tape/tab surfaces were mounted onto gold-coated MALDI target plates before matrix deposition.

Methanol soft landed and washed tissue experiments

Two separate experiments were carried out to compare images acquired from methanol soft landed/unwashed and methanol soft landed/washed lenses. Dissected rat lenses were sectioned at 20 μm thickness at a temperature of $-20\text{ }^{\circ}\text{C}$ in a Leica (Buffalo Grove, IL, USA) CM3050S cryostat. Equatorial sections were obtained from approximately the center of the lens in order to obtain a representative view of the cortical through nuclear regions of the lens. A 21 day old lens section from the central region of the tissue was mounted onto an Applied Biosystems (Framingham, MA, USA) gold target plate using a thin layer of methanol to help the section adhere to the plate surface as described by Grey *et al* [27]. The tissue section was not washed and sample plate was then vacuum desiccated until dry. A second rat lens section (100 day old) was methanol soft landed onto a gold target plate and washed with sequential submersions in 70% and 95% ethanol for 60 seconds each. The section was dried under vacuum before matrix application. In order to demonstrate tissue lifting of methanol soft landed lens tissue from older eyes a mouse eye from an 11 month old C57 mouse lens was methanol soft landed onto a gold target plate and dried in a vacuum desiccator without washing. For tissue lifting measurements, optical profilometry images were taken of the mounted mouse lens section with a Zeta Instruments (San Jose, CA, USA) Zeta 20 profilometer over a z distance of 225 μm at 4x magnification. Zebrafish lens sections were mounted on carbon conductive tabs and one section was washed three times with 10 μL of 50:50 acetonitrile:water using a pipette to provide a washed/unwashed tissue comparison.

Matrix deposition

Matrix was deposited onto the samples using a Portrait 630 acoustic spotter (Labcyte Inc., Sunnyvale, CA, USA). The matrix composition was 20 mg/mL sinapinic acid (Sigma Aldrich, St. Louis, MO, USA) in 50:45:5 ACN:H₂O:formic acid for mouse and rat lenses, and in 50:49.9:0.1 acetonitrile:water:trifluoroacetic acid for the zebrafish lens. Matrix was applied in a 200 μm array with 40 passes of one droplet per pass for mouse and rat tissue, and a 150 μm array for zebrafish lens. The instrument minimum repeat time was set to 120 seconds for the first five passes to prevent spots merging on the tissue. Time was then reduced to 90 seconds for the next 10 passes and 70 seconds for the remaining passes for the rodent lens tissue experiments. The minimum repeat time was set to 10 seconds to allow merging of the spots on tissue in the zebrafish experiment. The merger of the spots on tissue allowed for a higher spatial resolution image for the small lens with data acquired at 80 μm pitch rather than the original 150 μm array. This approach was necessary to achieve good matrix/analyte co-crystallization resulting in high sensitivity while minimizing analyte delocalization as a result of the small droplet volume.

Data Acquisition

Data were acquired in linear mode using a Bruker AutoFlex Speed III instrument (Bruker Daltonics Billerica, MA, USA) equipped with a Smartbeam II 1 kHz, 355 nm laser with 500 shots per pixel and a mass range of 4,000–30,000 Da. Delayed extraction was set to 450 ns for rodent lens images and 390 ns for the zebrafish lens image. The laser attenuator was adjusted to a 61% offset with a range of 40% for rodent lens imaging and 60% offset with a

30% range for zebrafish lens imaging. The spatial resolution for data acquisition was 200 μm for the rodent lens tissues. Data from the zebrafish lens tissue were acquired at 80 μm spatial resolution. Global spectra were exported from FlexImaging 3.0 into mMass, an open source mass spectrometry tool (www.mMass.org) [40], to display global spectra acquired from washed and unwashed rat lens tissue. Images were generated using FlexImaging 3.0 and normalized to the total ion count; the relative intensity scales were adjusted for each m/z value as indicated in the figures to improve image quality. The instrument was calibrated prior to data acquisition using a mixture of insulin, cytochrome C, apomyoglobin and trypsinogen.

Results and Discussion

To determine the utility of conductive adhesives as surfaces for MALDI IMS of lens tissue, we compared MALDI spectra acquired from rat lens sections prepared in typical fashion for optimal signal, i.e. washed on a gold target and prepared as unwashed tissue on a carbon tab. Figure 1 displays average mass spectra obtained from two MALDI-IMS analyses of rat lens sections (~100 days of age). Figure 1a shows a mass spectrum from a methanol soft landed/ethanol washed lens where major α A-crystallin protein products were observed. Sequence assignments were made based on previously published MS/MS analyses for full-length and C-terminally truncated rat α A-crystallin products [31]. Figure 1b displays a mass spectrum from an unwashed carbon tab mounted and lyophilized lens section with the same signals labeled. Since salts are still present in the unwashed carbon tab-mounted sample, a potassium adduct at m/z 9,428 corresponding to the α A-crystallin sequence 1–79 and a sodium adduct at m/z 9,867 corresponding to the α A-crystallin sequence 1–83 were observed. Despite signal suppression in the spectrum acquired from the carbon tab-mounted sample, all crystallins observed in the spectrum from the ethanol washed sample can be observed in the spectrum from the carbon tab mounted sample with a signal-to-noise ratio sufficient for imaging.

Analyte delocalization of three α A-crystallin products in the rat lens images is compared in Figure 2 for ethanol washed (left panel), unwashed (middle panel) and carbon tape mounted/unwashed tissue (right panel). Note that the tissue sections were mounted on the gold target plates using the methanol soft landing method. The images from the first two panels provide data on the effect of tissue washing on protein delocalization. The images generated from the carbon tape mounted samples (right panels) display data from five sequential equatorial rat lens sections (~100 days of age) obtained from the center of the lens with ~80 μm distance between imaged sections. These data provide information on protein delocalization using the carbon tape method and show the reproducibility of the method. Tissue sections on carbon tape were spotted within the same array of matrix and data were acquired within the same image file.

The signal intensity and localization for the truncated α A-crystallin 1–53 product at m/z 6,413, (predicted m/z) in Figure 2a is similar for all three sample preparation methods. This highly truncated α A-crystallin product is present in the central nuclear region of the lens, as expected [31]. The less abundant signal, indicated by blue/green pixels on the very outside of the ethanol washed tissue (left panel), is attributed to background noise based on the

intensity observed and localization outside of the tissue margins (indicated by the dotted line). Figure 2b displays the distribution of the truncated α A-crystallin 1–83 product at m/z 9,844 that has previously been shown to have a circular distribution similar to the natural concentric layers of fiber cells that elongate to surround the lens nucleus [31]. In the analysis of methanol soft landed/ethanol-washed tissue, the distribution appears to be highly delocalized throughout the tissue section; however, a perinuclear circular pattern of signal is observed with the methanol soft landed/unwashed sample and is replicated among multiple lens sections using the carbon tape method. The delocalization observed using the ethanol wash method is extensive towards both the center and outside of the lens tissue.

The distribution of intact, full-length α A-crystallin 1–173 (Figure 2c) shows that delocalization of this protein occurred toward the outside of the ethanol washed tissue onto the surrounding target plate. The signal intensity present on the surrounding target plate appears to be much higher than on tissue; a feature that could be attributed to better ionization efficacy of protein located on the gold surface compared to protein located on the tissue surface since the delocalized protein may be subject to fewer suppression effects than on tissue. This explanation could also account for the increase in signal observed in the average mass spectrum of the ethanol washed sample in Figure 1a. The green signal observed within the tissue boundary is the expected distribution of the intact α A-crystallin 1–173 (confirmed by the carbon tape method) suggesting that minimal migration towards the central region occurs and that delocalization primarily occurs towards the outside of the tissue. This notion is confirmed by the replicate images observed using the carbon tape method shown in Figure 2c of α A-crystallin 1–173 in the rat lens. Only slight signal delocalization was observed for the α A-crystallin 1–173 signal in the images from methanol soft landed/unwashed tissue and from carbon tape mounted tissue. Note that the wider distribution of the intact α A-crystallin 1–173 signal in the methanol soft landed/unwashed tissue is similar to that reported by Stella *et al.* [31] and is most likely not due to delocalization, but due to the younger age of the animal. The few pixels observed outside of the tissue edge are most likely due to the deposition of matrix spots from the acoustic spotter landing partially on and partially off tissue. This delocalization is unavoidable with the discrete spotting method of matrix application; however, due to the very small volume of the matrix droplets (~170 pL) the spots do not spread further than 200 μ m or one image pixel. The effect of delocalization of the truncated and full-length α A-crystallin proteins is clearly apparent in the overlaid images (Figure 2d) where the nuclear and perinuclear ring regions cannot be clearly discerned from one another in the washed tissue samples, but show well-defined localizations with the carbon tape method. Comparisons of images from methanol soft landing/washed to methanol soft landing/unwashed samples indicate that the extensive delocalization of crystallins in rodent lens samples is primarily due to the ethanol wash method.

Although minimal crystallin delocalization was observed using the methanol soft landing/unwashed method, older and cataractous lenses frequently exhibit detachment of the nuclear region of the lens from the target surface. Figure 3 displays a 3D profilometer image of an 11 month C57 methanol soft landed/unwashed mouse lens where substantial cracks and lifting (up to 225 μ m) of the tissue can be seen in the nuclear region. Ethanol washing can

prevent tissue lifting since it fixes the tissue to the sample surface; however, signal delocalization occurs with this sample preparation method as shown in Figure 2. The carbon tape/pad method reduces signal delocalization and eliminates tissue lifting from the target surface.

The carbon tape method was used for imaging smaller mouse lenses and resulting protein images were compared to those from rat lens. Figure 4 displays images generated from mouse and rat lens (21 day old) tissue mounted on electrically conductive tape showing the distribution of multiple α -crystallin protein products. Protein identification for signals observed in the mouse lens tissue was based on identified signals from rat tissue due to high sequence homology [α A-crystallin rat (UniProtKB A0JN13) and mouse (UniProtKB Q569M7) share 100% sequence identity; α B-crystallin rat (UniProtKB P23928) and mouse (UniProtKB P23927) share 98% sequence identity]. Images were generated for four C-terminally truncated α A-crystallin products: 1–53, 1–93, 1–157, and 1–168 as well as for full length α A-crystallin 1–173 and α B-crystallin 1–175 (assignment based on the predicted mass obtained from the Uniprot website, www.uniprot.org). Images acquired using the carbon tape method produced similar distributions of the same truncated species within the mouse and rat lenses. The more truncated proteins were found near the lens nucleus and the more intact proteins were observed in the outer cortical region of both the rat and mouse lenses. The rat lens images confirm localizations previously observed by Stella et al. [31] using an ethanol mounted and ethanol washed tissue prior to MALDI IMS analyses. The overlaid images clearly display distinct distributions of crystallin products in the nuclear, inner cortex, and outer cortex regions. Figure 4a displays images for two truncated α A products present within the nuclear region of mouse and rat lenses having the sequences 1–53 (m/z 6,412) and 1–93 (m/z 11,049). Figure 4b displays truncated α A-crystallin products 1–168 (m/z 19,369) and 1–157 (m/z 18,214) that are found in the inner cortex region. Figure 4c displays the distribution of the full-length α A 1–173 (m/z 19,817) and full-length α B 1–175 crystallin (m/z 20,105) present in the cortical region of the lens for both mouse and rat. Figure 4d displays overlaid images of the protein products for both mouse and rat which form concentric ring distributions.

The concentric ring distributions are reminiscent of the patterns observed in optical lens images as a result of light scattering via slit lamp experiments. Figure 5 displays such a slit lamp image which provides an optical view of the spherical lens in the eye of a living mouse. Externally, the first surface is the cornea which is largely collagen matrix. The fluid-filled aqueous chamber is located behind the cornea and appears as a black band. Just interior to the aqueous is the lens capsule; the basement membrane for the lens epithelium. Fiber cell differentiation from the single epithelial cell layer is highly symmetric and results in concentric layers of transparent, refractive cells with varying protein composition [41]. The MALDI IMS images of vertebrate lenses presented in this report confirm concentric regions of differing protein composition.

The carbon tape method was applied to image even smaller lens sections from 1-year-old zebrafish, *Danio rerio*. Although it is known that fish lenses contain high concentrations and multiple forms of γ -crystallins compared to mammalian lenses, virtually nothing is known about the spatial distribution of fish lens crystallins. Figure 6a shows an optical image of a

washed (top) and an adjacent unwashed (bottom) section of a zebrafish lens mounted on a carbon tab after matrix deposition. The diameter of the unwashed section was measured to be 984 μm . Figure 6b shows regions where data were acquired indicating a significant distance between the edge of the tissue and the edge of data acquisition. This margin was included so delocalization could be observed as signal outside of the tissue boundary. The images in Figure 6c are generated from a signal at m/z 20,569, a putative γ -crystallin, indicating that the washing step improved the sensitivity of the measurement but caused significant signal delocalization compared to the signal observed from unwashed tissue. The signal at m/z 20,569 from the unwashed section on a carbon pad is present within the tissue boundary.

Figure 6d displays image data generated from the unwashed section mounted on a carbon tab from an unidentified protein at m/z 8,561 (potentially ubiquitin) present in the nuclear region of the zebrafish lens. Another unidentified protein at m/z 9,822 was found in the inner cortex region of the lens. Combining these signals with the previous signal observed in figure 6c to form an overlay image (bottom panel) clearly shows the concentric ring formation that is consistent with protein distributions observed in mammalian lenses. Very little lateral migration was observed in the unwashed section compared to the washed section of the zebrafish lens tissue. This is important because as the sample size of the tissue becomes smaller the relative extent of delocalization becomes larger and more apparent. For example if an analyte migrates 200 μm off tissue from a 1 mm diameter size sample such as the zebrafish lens the migration is 20% the diameter of the tissue. 200 μm migration off tissue from a 4 mm size sample such as the rodent lens the migration is only 5% of the original sample size.

In summary, the data presented herein demonstrate that adhesive conductive tape mounting of vertebrate lens tissue sections provides a solution to tissue lifting and signal delocalization in MALDI IMS experiments. By extension, our results suggest that this sample protocol can provide a useful alternative for tissue types where thaw mounting, solvent-assisted mounting, and/or washing results in analyte delocalization or sample detachment from the target surface. A study of tissue washing effects indicates that the carbon tape/pad method minimized lens protein delocalization primarily caused by washing procedures. Slight delocalization was encountered using the carbon tape/pad method for proteins at the tissue edges; however, the distance of delocalization was no further than the diameter of the matrix spots or a single image pixel. Attempts to wash tissue mounted on conductive material also resulted in delocalization as high signal intensity was observed off tissue (data not shown). An additional advantage of using carbon tape or adhesive carbon tabs for tissue mounting is that more control over sample positioning is obtained..

The carbon tape method was successfully applied to image ocular lens crystallins from lenses as small as 1 mm in diameter. Due to the very high abundance of crystallins in lens tissue, the signal intensities observed were adequate for imaging purposes and washing steps were not required during the sample preparation. The distinct concentric ring formations previously observed in the rat lens are also observed for the first time in mouse and zebrafish lenses and correspond to specific protein products observed in these defined regions. The variable distributions of lens proteins occur as a result of lens growth and

protein modification as the tissue ages. Moreover, the variable expression of these protein constituents is believed to produce a refractive index gradient across the lens that makes the lens a focusing optical element [42]. Further studies using MALDI IMS to observe protein distributions in lenses obtained from genetically modified animal models as well as animals subjected to specific diets and environments will lead to a better understanding of the biological processes involved in cataract formation.

Conclusions

Conductive, adhesive targets provide a useful surface for MALDI IMS of tissues with poor adherence properties. A study of protein delocalization indicated that tissue washing protocols can lead to substantial protein delocalization and obscure important localization patterns and that this delocalization becomes significant for small tissue samples. Based on these data, a sample preparation protocol is presented for imaging of proteins in lens tissues using conductive, adhesive surfaces that can easily be applied to other challenging tissue types. Use of this protocol revealed, for the first time, concentric ring distributions of modified crystallin proteins in mouse and zebrafish lenses; distributions that could not be easily deciphered by standard IMS methods.

Acknowledgments

This work was supported by the following grants: EY-019728 (KLS), EY04542 (JIC), EY012018 (HM) and EY020963 (SB) and GM103391 from the National Eye Institute.

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Figure 1. MALDI spectra from (a) methanol soft landed/washed rat lens tissue and (b) carbon tape mounted and lyophilized rat lens tissue showing major signals due to αA -crystallin products.

Figure 2.

MALDI IMS comparison of a methanol soft landed/washed 100 day old rat lens tissue sample (left panel), a methanol soft landed/unwashed 21 day old rat lens (center panel), and replicates of carbon tape mounted/unwashed 100 day old rat lens tissue (right panel) displaying the distribution of (a) m/z 6,413 α A crystallin 1–53, (b) m/z 9,844 α A crystallin 1–83, (c) m/z 19,835 α A 1–173 and (d) overlay of the previous m/z values. Scale bars represent 2 mm. Tissue margins are indicated by dashed white lines.

Figure 3. 3D optical profilometry image (4x magnification) of an unwashed 11.5 month old mouse lens methanol soft landed onto a gold coated MALDI plate displaying the cortex and nuclear regions where the tissue can be seen to be lifting from the surface.

Figure 4. MALDI IMS images displaying α -crystallin products from mouse and rat lens tissue located in (a) nuclear regions, (b) cortex region, (c) outer cortex region and (d) overlay of multiple signals. Scale bars represent 1 mm.

Figure 5.
Slit lamp image of a normal mouse eye *in vivo*.

Figure 6.

Optical and MALDI IMS images from a carbon tape mounted adult zebrafish lens (a) Optical image of washed (top) and unwashed (bottom) zebrafish lens sections and spotted matrix array. (b) Optical image of regions of interest where data were acquired. (c) MALDI images generated from putative γ -crystallin at m/z 20,569. (d) Multiple MALDI images displaying concentric rings observed in the zebrafish lens.