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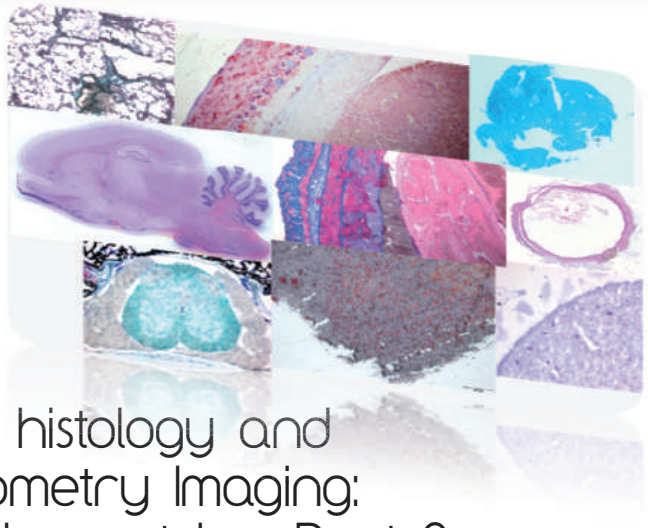
Application Note #MSI-08



Gather classical histology and Mass Spectrometry Imaging: Here is the match - Part 2

Introduction

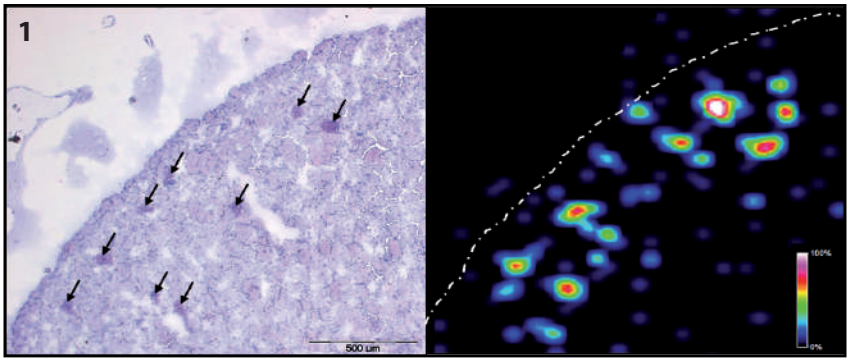
In this application note, we will show the benefits of merging histological stains and Mass Spectrometry Imaging (MSI). The correlation between histological structures or disease related alterations and molecular species localization provides more information about biology or disease progression/mechanism taking place within tissue. In fact, histological features can drive molecular imaging interpretation and vice versa [1]. Some pathological changes are not visible by staining or immunohistochemistry (IHC) and MSI provides, in this case, a real added value in order to go beyond the visual inspection. In drug discovery, histological stains enable the visualization of biological tissue types, whereas MSI provides molecule localization and quantification and can also monitor disease state biomarkers level.

Moreover, it is possible to evaluate toxicity induced by a treatment thanks to biomarkers quantification. These biomarkers can be spatially correlated with local tissue's damages. In this second part of this application note, we'll present four other histological stainings which are listed in the table below with their target tissue and related therapeutic area applications.

Staining	Targeted Tissue/Structure	Therapeutic Area or Disease	Molecular Species
PAS	Glomeruli	Diabetes	Lipids/ Metabolites
Azan Blue	Bone/ Muscle	Musculoskeletal disorders	Metabolites
Oil Red O	Lipids	Metabolic diseases	Lipids
Masson's trichrome	Collagen	Toxicity (Fibrosis)	Lysophospholipids

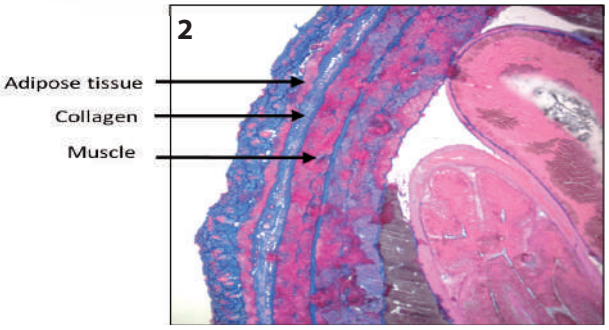
Periodic Acid-Schiff reaction (PAS)

The PAS stain is based on the action of Schiff reagent that reacts with molecular aldehyde groups. PAS stains carbohydrates and carbohydrate rich macromolecules in magenta for example glycogen, mucus in cells, basement membranes, brush borders of kidney tubules, or small and large intestine reticular fibers.

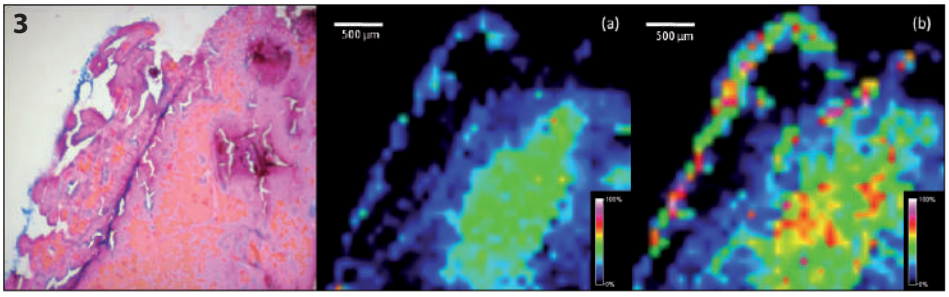


Picture 1 shows several glomeruli within a kidney tissue section stain using PAS. MS Images of sulfatide ion (*m/z* 1042.68) show co-localization with glomeruli. This illustrates that combining PAS stain and MSI can be used to study glomerular dysfunction, such as glomerulonephritis [5].

Azan blue



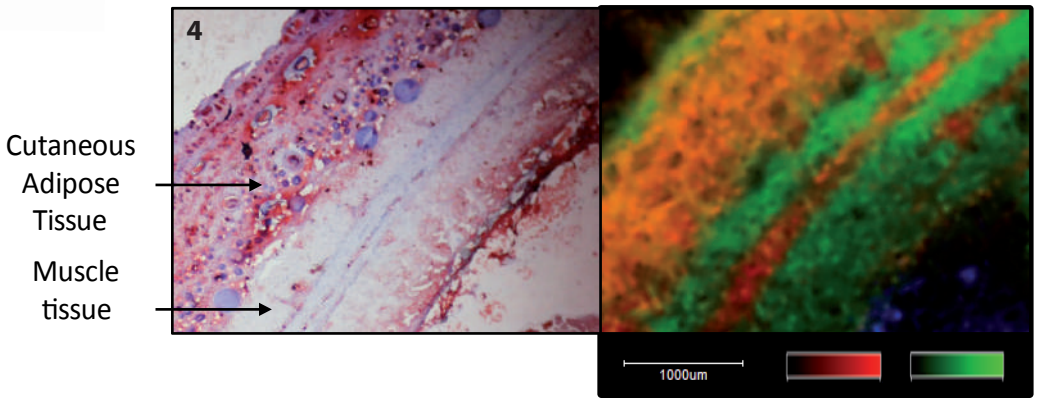
Azan blue staining uses azocarmine B or G species followed by aniline blue to differentiate histological structures. Nuclei and erythrocytes are stained in bright red whereas collagen basement membrane and mucin are stained in blue. Muscle and red blood cells highlight an orange to red color. The **picture 2** shows a part of a mouse whole body section stained with Azan blue. Muscle layer can be clearly visualized from other structures such as adipose tissue or collagen. This staining can be useful to assess musculoskeletal disorders.



Moreover, MSI provides spatially preserved molecular information on specific metabolites or lipids contained in muscle fibers as presented in **picture 3**. This picture shows a transversal tissue section of a mouse back leg. Muscle fibers are highlighted in orange thanks to Azan blue staining. The distribution of two ribonucleotides, the adenosine monophosphate (AMP, *m/z* 346.0584, 2a) and adenosine triphsosphate (ATP, *m/z* 505.9934, 2b) coincides with fiber-rich regions. Because these ribonucleotides are involved in muscle metabolism, combining Arzan blue staining with MSI enables to assess their distribution and modulation to study musculoskeletal or metabolic disorders.

Oil Red O

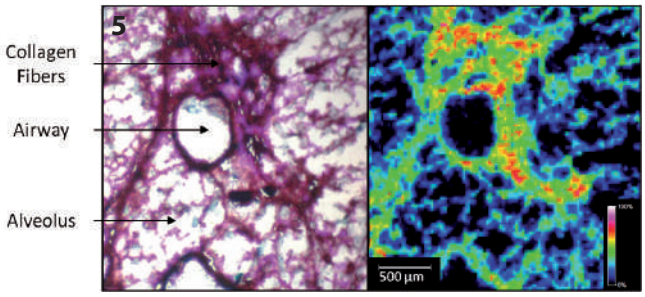
Oil Red O is a lysochrome diazo dye used to mark neutral triglycerides, lipids, fatty acids and lipoprotein. Lipids accumulation in tissue is stained in deep red. Nuclei stains blue/black.

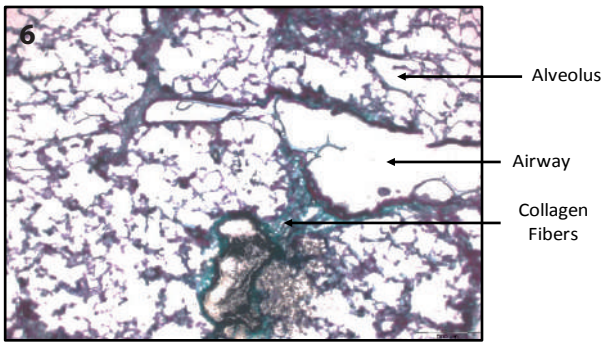


Skin lipids are well observed on the Oil Red O staining image of the adipose tissue as presented in **picture 4**. The overlay distribution of two lipids reveals their typical localization in skin substructures. Adipose tissue differences are correlated with two lipids distribution, *m/z* 734,58 in red and *m/z* 758,58 in green respectively PC (32:0) and PC (34:1). Metabolic diseases and inflammation can be related to lipid synthesis dysfunction within discrete histological tissue. Combining Oil Red O and MSI of constitute lipids is a powerful approach to study lipid synthesis dysfunction in the skin, as well as in other tissues such as atherosclerotic tissues.

Masson's trichrome

Masson's trichrome is often used to stain connective tissue and to distinguish cellular items from extracellular items. Thus, nuclei and other basic-liking structures are stained in blue, cytoplasm, muscle, erythrocytes and keratin are stained in bright-red. Then, nuclei is stained in black and collagen is stained green.





In **picture 5 & 6**, Masson's trichrome is used to study a toxicity induced by a chemical compound in lung tissue. In fact, it reveals fibrotic area within lung tissue structure (airways and alveolus) thanks to green staining of collagen fibers as displayed. On molecular image, lysophosphatidic acid (LPA 16:0) ion ($[M-H_2O-H]^-$ *m/z* 391.2253) is clearly distributed and concentrated in the fibrosis area. In this case, MSI aims to identify some molecular biomarkers of this phenomenon such as LPA. In the same time, MSI keeps regional specificity and so explains this toxicity associated to atypical figures or biological processes. The content of collagen fibers will emerge thanks to the use of Masson's trichrome combined to MSI.

Conclusion

Classical histology combined to MSI provides a valuable tool for the optimisation and validation of drug targeting process.
Thus, we highlight that matchinghistological staining and MSI offers a real added value.

Avantages

- Staining adapted to several biological investigations
- Differenciation of tissue types with staining
- Molecular composition of tissue types thanks to MSI

Benefits

- Accurancy
- Relevance
- Confidence

ImaBiotech provides a wide range of histological staining possibility combined with MSI unique attributes to improve histopathological understanding.

References

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Keywords

- Histology
- Staining
- Molecular imaging
- Biomarkers
- Pathology

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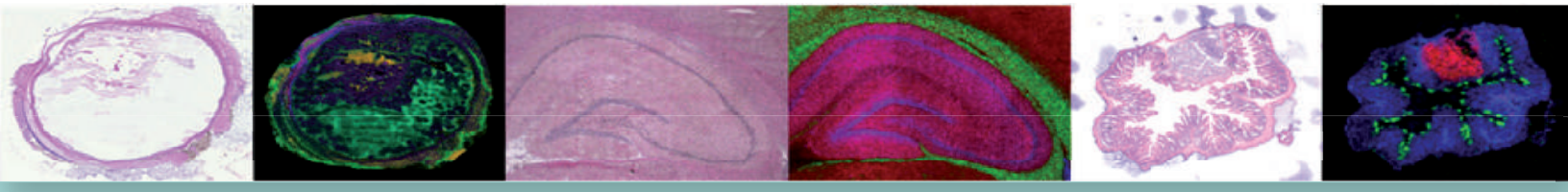

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