Reduced Hemoglobin Signal and Improved Detection of Low Abundance Proteins in Blood-Rich Tissues for MALDI Mass Spectrometry Imaging

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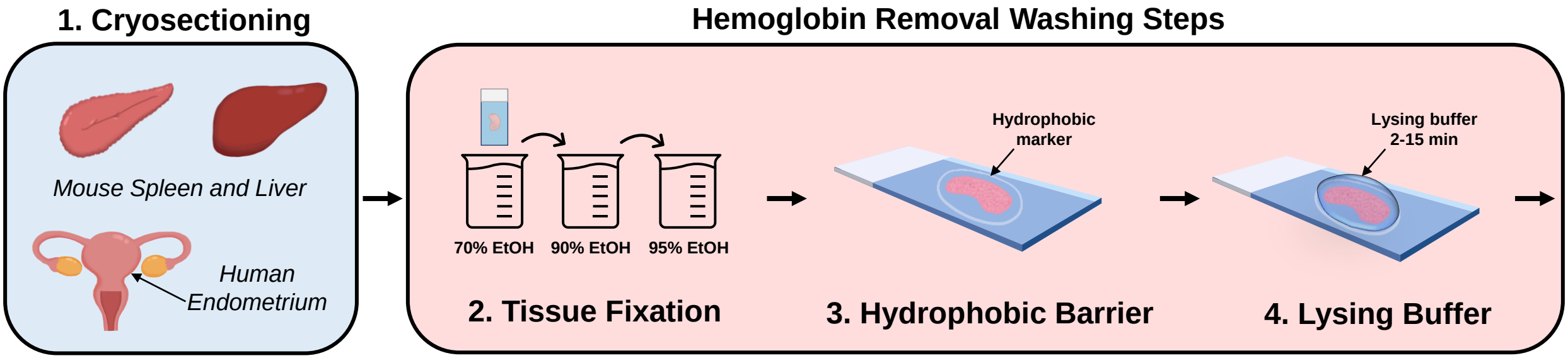
Introduction

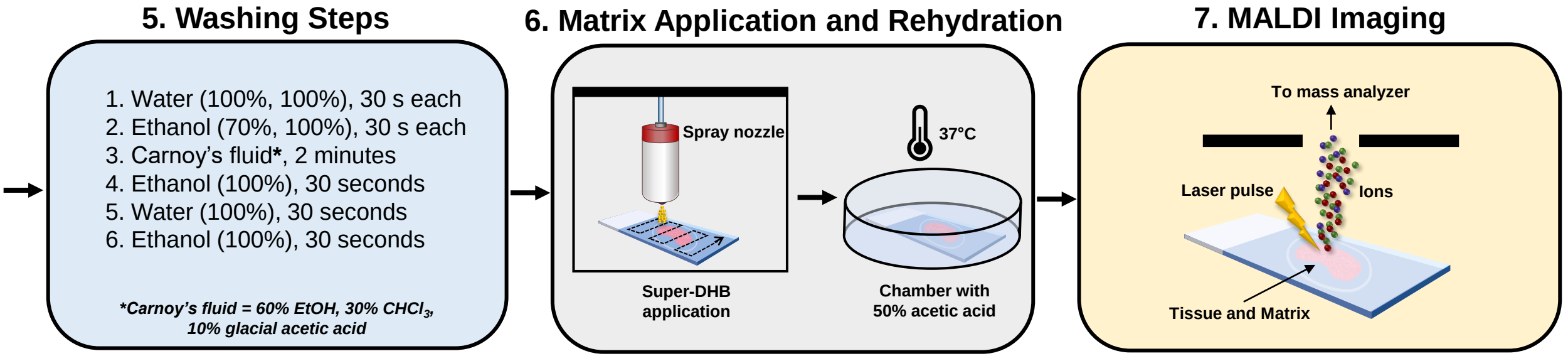
- Mass spectrometry imaging provides a powerful approach for the direct analysis and spatial visualization of molecules in tissue sections.[1]
- Matrix assisted laser desorption ionization (MALDI), intact protein imaging has been widely investigated for biomarker analysis and diagnosis in a wide variety of tissue types and diseases.[2]
- However, blood rich or highly vascular tissues present an increased challenge in molecular imaging due to the high ionization efficiency of hemoglobin, thus resulting in ion suppression of low abundance proteins.[3]
- Here, we describe a protocol to selectively reduce hemoglobin signal in blood-rich tissues that can easily be integrated into routine imaging workflows.

Methods



- Mouse liver, mouse spleen, and human endometrial curettage were sectioned at 12 µm and mounted onto indium tin oxide (ITO) slides **(Step 1)**.
- Tissue sections were submerged in ethanol (70, 90, 95%) for 30 seconds each for tissue fixation **(Step 2)**.
- A hydrophobic barrier was drawn around each tissue section, then an ammonium-chloride potassium (ACK) lysing buffer (Gibco) was aliquoted within the barrier for 2, 5, 10, 15 minutes **(Step 3 and 4)**.

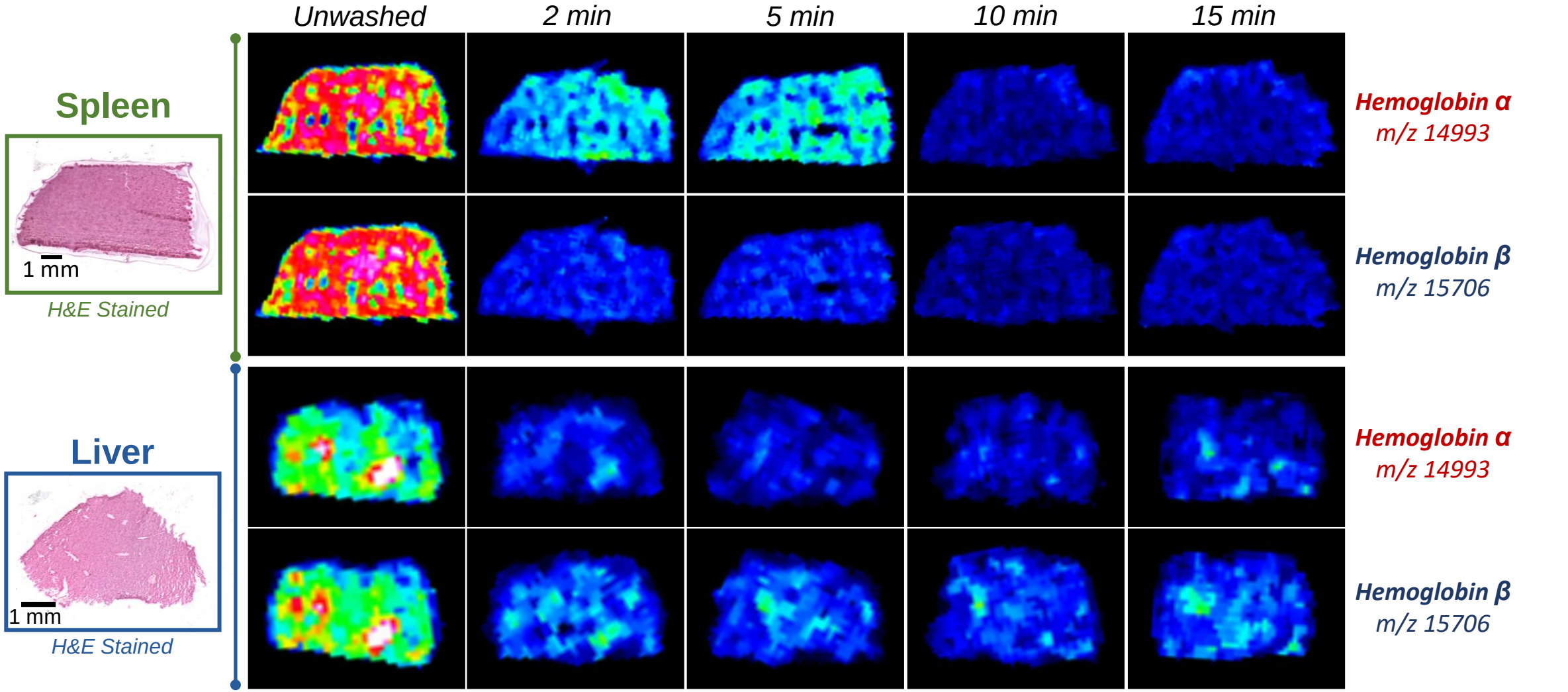
Methods



- After lysing, the tissue sections were washed in various solvents for salt and lipid removal **(Step 5)**.
- Matrix application of super-DHB was performed using an HTX M5 Sprayer and protein extraction was enhanced through rehydration in a chamber containing 50% acetic acid for 5 minutes at 37°C **(Step 6)**.
- MALDI imaging data was acquired on a Bruker autofleX Max MALDI TOF/TOF mass spectrometer operated in linear positive ion mode from *m/z* 2000-40000 using a spatial resolution of 150 µm **(Step 7)**.

Results and Discussion

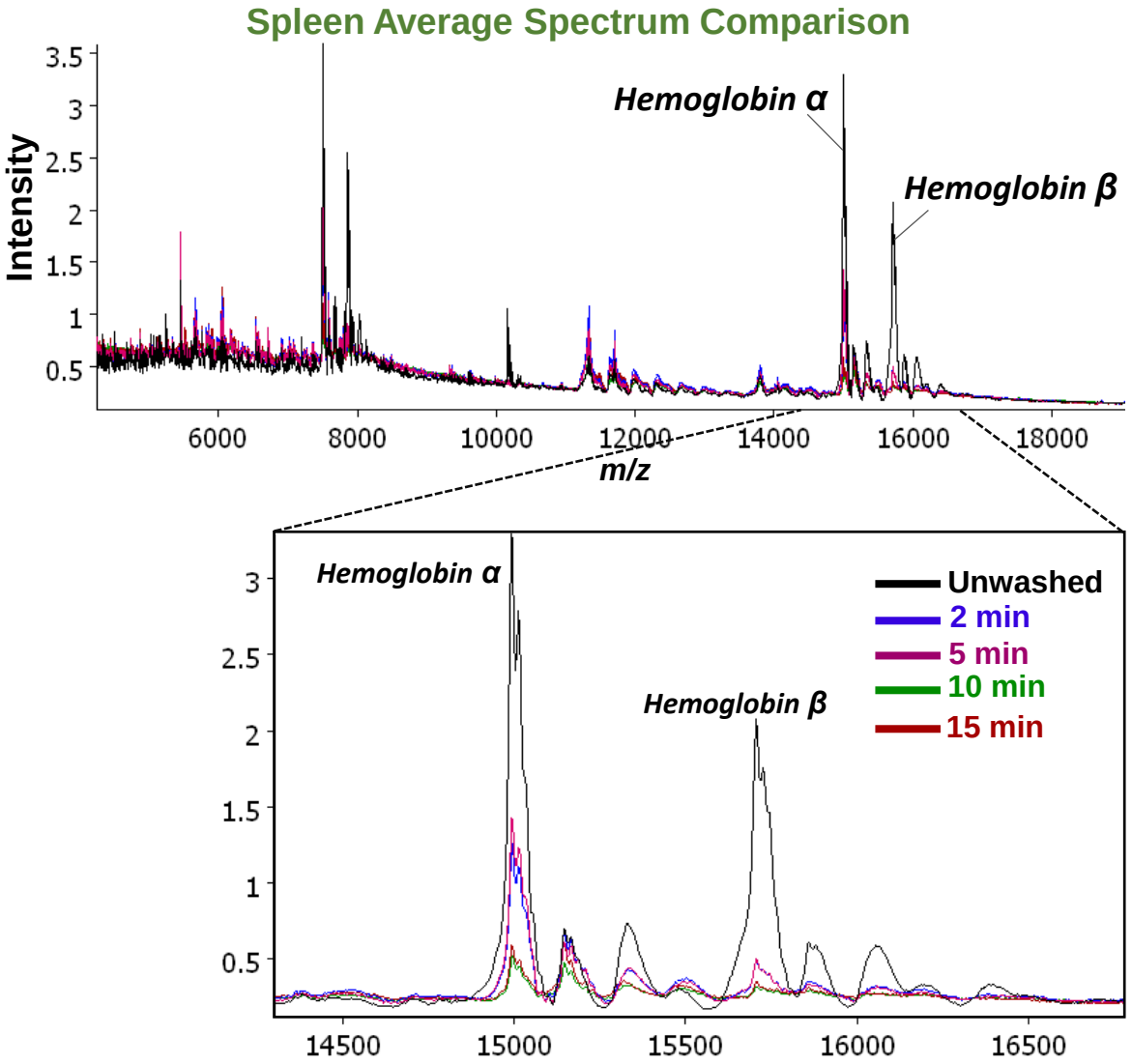
MALDI Images of Spleen and Liver with Varied Lysing Buffer Washing Times



- Molecular imaging of mouse spleen and liver revealed a significant reduction in signal from hemoglobin α and β subunits after application of the lysing buffer.

Results and Discussion

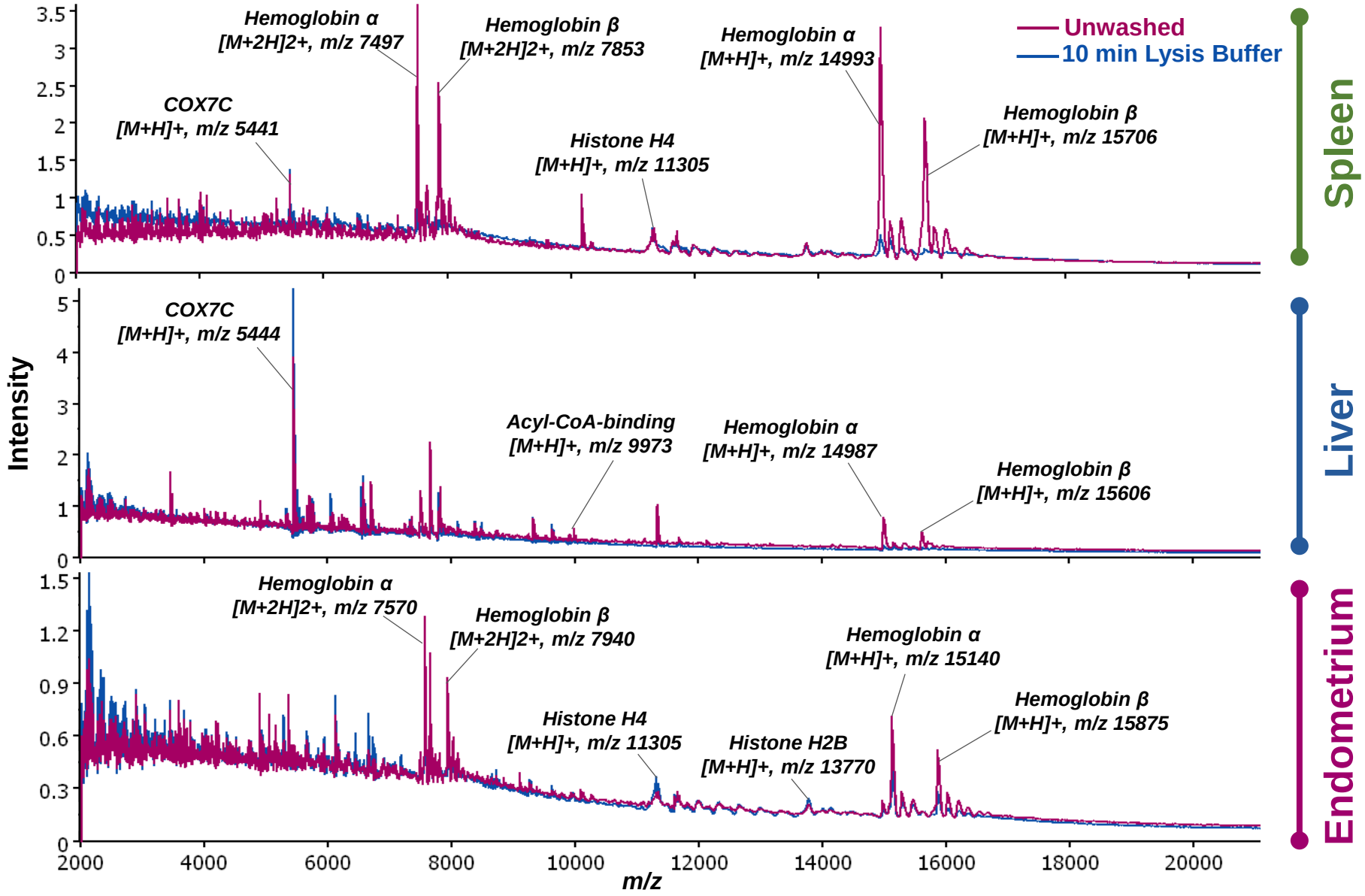
Effect of Lysing Buffer Washing Time on Hemoglobin Signal



- Time optimization of the lysing buffer protocol was performed by varying the time of application between 2-15 minutes on mouse liver and spleen (data for liver not shown)
- In spleen, hemoglobin α and β decreased in absolute signal intensity by 57-76% after 2 or 5 minutes of lysis buffer application while a decrease of 82-84% was observed after 10 or 15 minutes of lysis buffer application
- Washing time of 10 minutes in lysing buffer was determined to be optimal

Results and Discussion

Average Spectrum for Unwashed vs. 10 min Lysis Buffer Washed Tissue Sections



- Treatment with a lysing buffer for 10 minutes was found to be effective to decrease signal from hemoglobin
- Signal from other proteins are retained or enhanced

Results and Discussion

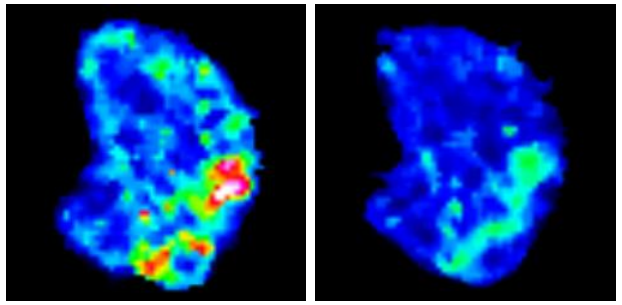
Application: Analysis of Human Endometrium

- Protein imaging analysis of endometrial curettage can be challenging as the rich blood content and high ionization efficiency of hemoglobin can result in ion suppression.

- After treatment with lysis buffer for 10 minutes, both hemoglobin α (*m/z* 15140) and β (*m/z* 15875) subunits were observed to decrease in absolute signal intensity by 40-45%, while the relative abundance from other proteins such as Histone H4 (*m/z* 11305) was observed to be enhanced by 24%.

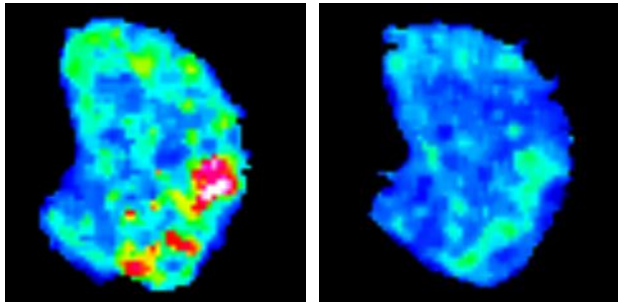
Hemoglobin α
m/z 15140

Unwashed 10 min Lysis Buffer



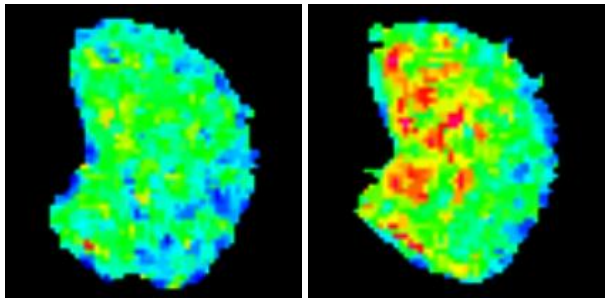
Hemoglobin β
m/z 15875

Unwashed 10 min Lysis Buffer



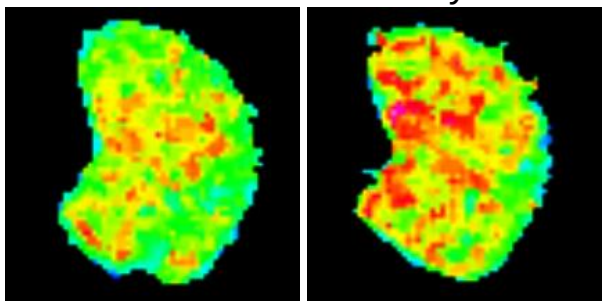
Histone H4
m/z 11305

Unwashed 10 min Lysis Buffer



Histone H2B
m/z 13770

Unwashed 10 min Lysis Buffer

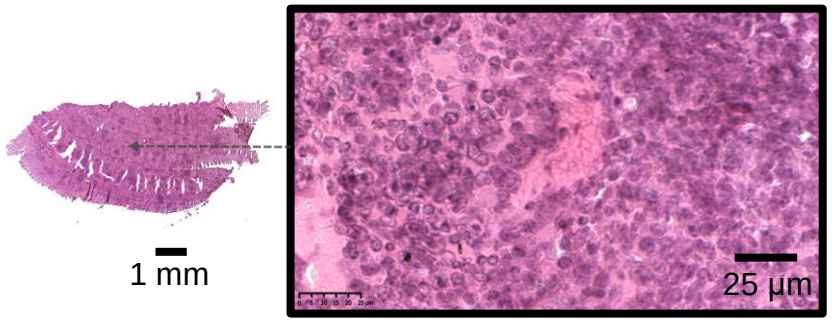


Results and Discussion

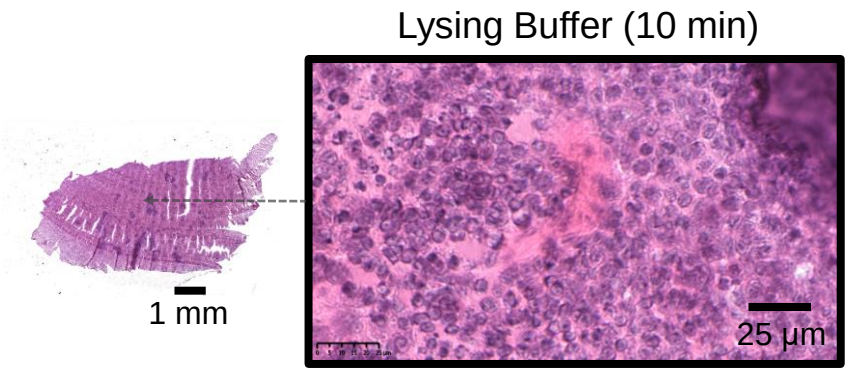
Hematoxylin and Eosin (H&E) Stained Images Before and After Lysing Buffer Application

Spleen

Unwashed

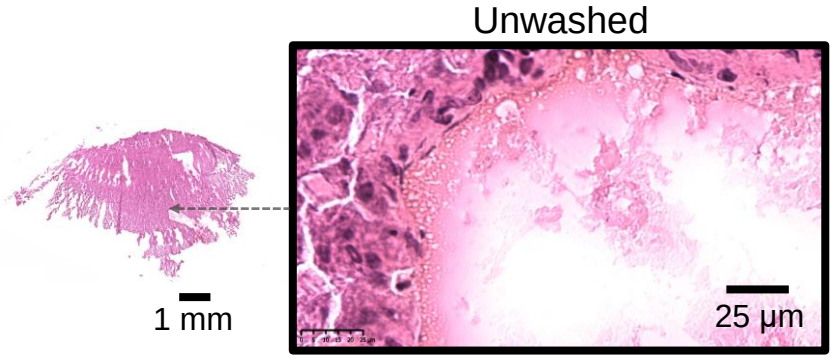


Lysing Buffer (10 min)

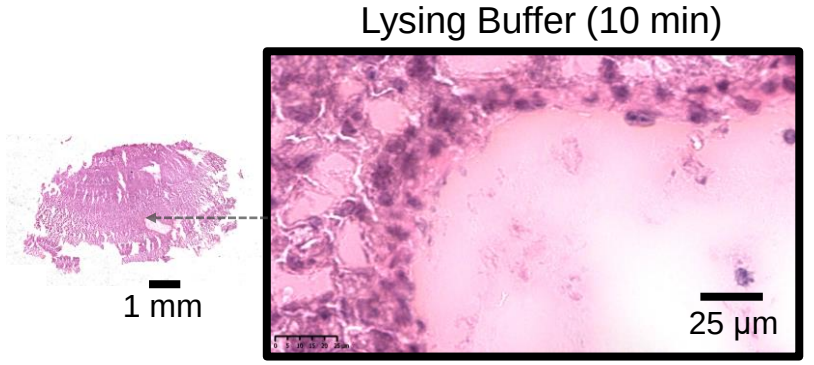


Liver

Unwashed

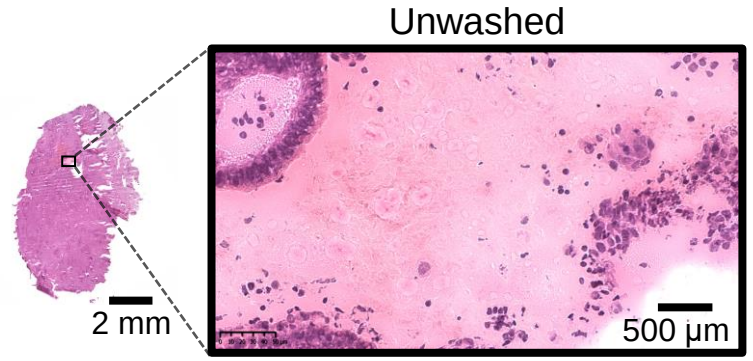


Lysing Buffer (10 min)

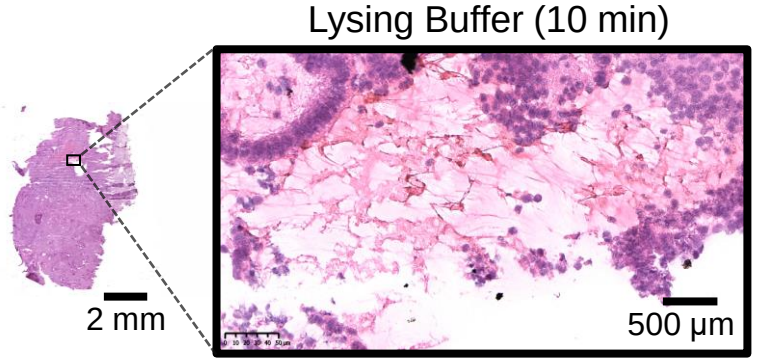


Endometrium

Unwashed



Lysing Buffer (10 min)



- H&E stained images depict a decrease of blood in tissue sections after treatment with lysing buffer

Conclusions

- This protocol using the lysing buffer to reduce hemoglobin signal offers an advantage for molecular imaging of tissues containing high blood content by reducing the susceptibility of low abundant proteins to ion suppression from hemoglobin.
- Treatment with a lysing buffer for 10 minutes was found to be effective to remove 82-84% of signal from hemoglobin α and β subunits from spleen while retaining or enhancing signal from other proteins.
- Lastly, this lysing buffer protocol can be introduced prior to other lipid/salt removal steps and easily integrated into current protein imaging workflows.

References and Acknowledgments

1) Cornett, D.S. *et al. Nature methods.* **2007**, *4*(10), 828-833
2) Seeley, E.H. *et al. PNAS.* **2008**, *105*(47), 18126-18131
3) Kuprowski, M.C. *et al. J. Am. Soc. Mass Spectrom.* **2007**, *18*(7), 1279-1285

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