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Optical Image Collection

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1 Works for me dx.doi.org/10.17504/protocols.io.beumjeu6

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ABSTRACT

Mass spectrometry imaging is an exciting technology, which enables simultaneous analysis of multiple molecular components directly from single cells, tissues, and organs. In combination with histological methods, this technique provides information about the spatial distribution of molecules in various biological tissues. Particularly, MALDI-MS imaging increases the coverage of metabolites by using different matrices and ionization modes. In coordination with in situ analysis of proteins, transcripts and epigenetic marks, the complementary spatial information on metabolites will establish metabolic pathways that are dominant and characteristic of disease states. We have recently developed and optimized a spatial metabolomics approach to image small molecules in human kidneys and biopsy sized material. With our combined expertise at UTHSA, PNNL and EMBL and recent advances, we have established methods for identifying metabolites in human kidneys, employed ultra-high mass resolution MS imaging for tissue analysis, and developed a bioinformatics resource (METASPACE) to annotate metabolites for anatomical localization and 3-D reconstruction. Our integrated technology can easily connect with other TIS sites to provide biochemical readouts of genes/proteins in specific tissue and cellular compartments.

GUIDELINES

Overview

1. AF and bright field images are captured on a confocal microscope before the matrix application.
2. H&E and PAS staining
   a) H&E and PAS staining is performed on both the serial section glass slide mounted samples and MALDI analyzed samples.
   b) MALDI analyzed samples are washed with 70% EtOH, 2 min, 3x to remove matrix
   c) Washed slides are dried in vacuum dessicator for 20 minutes prior to staining
3. Defrosted sections on glass slides are used as is.

MATERIALS

NAME	CATALOG #	VENDOR
100% Ethanol		
NovaUltra H&E Stain Kit	IW-3100	IHC World
NovaUltra PAS Stain Kit	IW-3019	IHC World
Eosin Solution		
Mayers hematoxylin solution		
Mastertech Bluing Solution	HXB00288E	American Master Tech Scientific
Mastertech Differentiating Solution	HXD00188E	
BBC Biochemical Optic Mount I Xylene	BBC 7722	Scientific Supply & Equipment

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NAME	CATALOG #	VENDOR
95% Ethanol		Thermo Scientific
10% Formalin		
0.5% Periodic Acid Solution (0.5g periodic acid in 100mL DI water)		
37% Formalin		
Coverslips		
Chemical Permount Mounting Medium	SP15-100	Fisher Scientific
Aperio ScanScope		
Leica TCS SP8 Confocal Microscope		

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Mayers hematoxylin solution		
Mastertech Differentiating Solution	HXD00188E	
Mastertech Bluing Solution	HXB00288E	American Master Tech Scientific
Eosin Solution		
75% Ethanol		
95% Ethanol		Thermo Scientific
100% Ethanol		
Xylene		
Coverslips		
Chemical Permount Mounting Medium	SP15-100	Fisher Scientific
0.5% Periodic Acid Solution (0.5g periodic acid in 100mL DI water)		
Schiff's reagent	3952016-500ML	Sigma Aldrich
Mayers hematoxylin solution		
75% Ethanol		
95% Ethanol		Thermo Scientific
100% Ethanol		
Xylene		
Coverslips		
Chemical Permount Mounting Medium	SP15-100	Fisher Scientific

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Autofluorescence (AF) and Bright Field Imaging

1 Before the matrix application, right after defrosting in desiccator, the AF image (pre-AF) is captured on a confocal microscope.

UTHSA: 10× objective on a Leica TSC SP8
PNNL: 20x objective on a Zeiss 710 LSM; 10x objective PALM MicroBeam (Zeiss inverse microscope Axiovert)

495 nm-720 nm, 404-488 nm and bright field channels are used for AF imaging with pinhole size 239 µm.

2 Matrix application and MALDI-MSI (duplicates for each sample at each spatial resolution).

3 AF image are uploaded to METASPACE and registered to corresponding MSI data.

H&E Staining

4 Air dry slides (after storage at **-80 °C**) for **00:03:00** to **00:05:00** under the hood.

5 Fix slides in ice cold acetone:methanol solution (1:1) for **00:10:00** to **00:15:00** at **-20 °C**

6 Wash/rehydrate slides in distilled H2O for **00:10:00** to **00:15:00**

7 Stain slides with hematoxylin for **00:00:20** to **00:00:30**

Mayers hematoxylin solution

8 Wash slides with distilled H2O for **00:02:00**

9 Rinse slides with differentiating solution by moving slides up and down 3-4 times within **00:00:05** . Then check the intensity of nuclear stianing under the microscope. If signal is too week, then go back to step 8 and do staining again for **00:00:05** to **00:00:10** , then rinse slides with distilled water again for **00:00:01** to **00:00:02** .

Mastertech Differentiating Solution
Catalog #: HXD00188E

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10 Wash slides with distilled H2O for **00:02:00**

11 Rinse slides with Bluing solution by moving slides up and down for **00:00:05** to **00:00:10**

Mastertech Bluing Solution
by American Master Tech Scientific
Catalog #: HXB00288E

12 Wash slides with distilled H2O for **00:02:00**

13 Stain slides with Eosin for **00:00:15** to **00:00:20**

Eosin Solution

14 Wash/dehydrate slides with 75% ethanol for **00:01:00** to **00:02:00**

75% Ethanol

15 Dehydrate slides with 95% ethanol for **00:01:00** to **00:02:00**

95% Ethanol
by Thermo Scientific

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16 Dehydrate slides with 100% ethanol for **00:02:00** (twice)

100% Ethanol

17 Immerse slides in xylene for **00:02:00** (twice)

Xylene

18 Air dry slides under the hood for **00:03:00** to **00:05:00**

19 Mount sections with Permount and cover with coverslips

Coverslips

Chemical Permount Mounting Medium
by Fisher Scientific
Catalog #: SP15-100

20 Stained amples are imaged using an Aperio ScanScope XT

21 H&E images are uploaded to METASPACE and registered to the corresponding MSI data

22 Slides are then re-stained with PAS

PAS Staining

23 Place the slides in 10% formalin in 95% alcohol for **00:10:00** .

24 Rinse in tap water four times.

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25 Oxidize in 0.5% periodic acid solution for **00:05:00**

0.5% Periodic Acid Solution (0.5g periodic acid in 100mL DI water)

26 Rinse in tap water four times.

27 Place in Schiff reagent for **00:15:00**

Schiff's reagent
by Sigma Aldrich
Catalog #: 3952016-500ML

Sections become a light pink color

28 Wash in lukewarm tap water for **00:05:00**

Sections immediatly become a dark pink color

29 Counterstain in Mayer's hematoxylin for **00:03:00**

Mayers hematoxylin solution

30 Rinse in TBS solution and then rinse in deionized water four times.

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31 Dehydrate by the successive ethanol and xylene steps 14 to 18 and coverslip using a synthetic mounting medium.

75% Ethanol

95% Ethanol
by Thermo Scientific

100% Ethanol

Xylene

Coverslips

Chemical Permount Mounting Medium
by Fisher Scientific
Catalog #: SP15-100

32 PAS slides from same tissue section as the H&E slide is imaged using Aperio ScanScope XT and uploaded to METASPACE and registered to the corresponding data.

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