APR 13, 2020

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.beumjeu6

Protocol Citation: Guanshi Zhang, Dusan Velickovic, Annapurna Pamreddy, Jessica Lukowski, Theodore Alexandrov, Christopher R Anderton, Kumar Sharma 2020. Optical Image Collection. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.beumjeu6>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
 We use this protocol and it's working

Created: Apr 08, 2020

Last Modified: Apr 23, 2020

PROTOCOL integer ID:
 35437

Optical Image Collection

Dusan Velickovic¹, Annapurna Pamreddy¹,
 Guanshi Zhang¹, Theodore Alexandrov³,
 Jessica Lukowski², Christopher R Anderton²,
 Kumar Sharma¹

¹University of Texas Health San Antonio;

²Pacific Northwest National Laboratory;

³European Molecular Biology Laboratory

KPMP

Metabolomics Protocols & Workflows

Guanshi Zhang
 UT Health San Antonio

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

MATERIALS

100% Ethanol Contributed by users
NovaUltra H&E Stain Kit IHC World Catalog #IW-3100
NovaUltra PAS Stain Kit IHC World Catalog #IW-3019
Eosin Solution Contributed by users
Mayers hematoxylin solution Contributed by users
Mastertech Bluing Solution American Master Tech Scientific Catalog #HXB00288E
Mastertech Differentiating Solution Contributed by users Catalog #HXD00188E
BBC Biochemical Optic Mount I Xylene Scientific Supply & Equipment Catalog #BBC 7722
95% Ethanol Thermo Scientific
10% Formalin Contributed by users
0.5% Periodic Acid Solution (0.5g periodic acid in 100mL DI water) Contributed by users
37% Formalin Contributed by users
Coverslips Contributed by users
Chemical Permount Mounting Medium Fisher Scientific Catalog #SP15-100
Aperio ScanScope Contributed by users

✕ Leica TCS SP8 Confocal Microscope Contributed by users

STEP MATERIALS

✕ Mayers hematoxylin solution Contributed by users

✕ Mastertech Differentiating Solution Contributed by users Catalog #HXD00188E

✕ Mastertech Bluing Solution American Master Tech Scientific Catalog #HXB00288E

✕ Eosin Solution Contributed by users

✕ 75% Ethanol Contributed by users

✕ 95% Ethanol Thermo Scientific

✕ 100% Ethanol Contributed by users

✕ Xylene Contributed by users

✕ Coverslips Contributed by users

✕ Chemical Permunt Mounting Medium Fisher Scientific Catalog #SP15-100

✕ 0.5% Periodic Acid Solution (0.5g periodic acid in 100mL DI water) Contributed by users

✕ Schiff's reagent Merck MilliporeSigma (Sigma-Aldrich) Catalog #3952016-500ML

✕ Mayers hematoxylin solution Contributed by users

✕ 95% Ethanol Thermo Scientific

✕ 100% Ethanol Contributed by users

✕ Xylene Contributed by users

✕ Coverslips Contributed by users

✕ Chemical Permunt Mounting Medium Fisher Scientific Catalog #SP15-100

✕ 75% Ethanol Contributed by users

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.

Note

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 $-80\text{ }^{\circ}\text{C}$) 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 $-20\text{ }^{\circ}\text{C}$
- 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 Contributed by users

- 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 staining under the microscope. If signal is too weak, 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 Contributed by users Catalog #HXD00188E
- 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 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 Contributed by users
- 14 Wash/dehydrate slides with 75% ethanol for 00:01:00 to 00:02:00
75% Ethanol Contributed by users

- 15 Dehydrate slides with 95% ethanol for 00:01:00 to 00:02:00
95% Ethanol Thermo Scientific
- 16 Dehydrate slides with 100% ethanol for 00:02:00 (twice)
100% Ethanol Contributed by users
- 17 Immerse slides in xylene for 00:02:00 (twice)
Xylene Contributed by users
- 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 Contributed by users
Chemical Permount Mounting Medium 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.

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) Contributed by users

26 Rinse in tap water four times.

27 Place in Schiff reagent for 00:15:00

Schiff's reagent Merck MilliporeSigma (Sigma-Aldrich) Catalog #3952016-500ML

Note

Sections become a light pink color

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

Note

Sections immediatly become a dark pink color

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

Mayers hematoxylin solution Contributed by users

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

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

75% Ethanol Contributed by users

95% Ethanol Thermo Scientific

100% Ethanol Contributed by users

Xylene Contributed by users

Coverslips Contributed by users

Chemical Permunt Mounting Medium 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.