

APR 16, 2024

OPEN  ACCESS

## DOI:

[dx.doi.org/10.17504/protocols.io.36wgqn55xgk5/v1](https://dx.doi.org/10.17504/protocols.io.36wgqn55xgk5/v1)

**Protocol Citation:** Emily Soja, Santhosh Sivajothi, William F Flynn, Elise T Courtois 2024. Hematoxylin and Eosin (H&E) Staining of Tissues following Multiplexed Imaging on Phenocycler-Fusion.

**protocols.io**

<https://dx.doi.org/10.17504/protocols.io.36wgqn55xgk5/v1>

**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

## Hematoxylin and Eosin (H&E) Staining of Tissues following Multiplexed Imaging on Phenocycler-Fusion

Emily Soja<sup>1</sup>, Santhosh Sivajothi<sup>1</sup>, William F Flynn<sup>2</sup>, Elise T Courtois<sup>1</sup>

<sup>1</sup>Single Cell Biology Lab, The Jackson Laboratory, USA; <sup>2</sup>The Jackson Laboratory



Santhosh Sivajothi  
The Jackson Laboratory

### ABSTRACT

Phenocycler-Fusion assay enables multiplexed antibody based imaging of FFPE or fresh frozen tissues. Hematoxylin and Eosin (H&E) staining is a gold standard method for observing tissue morphology. Obtaining multiplexed protein expression information and H&E images from the same tissue provides an additional layer of valuable information which can be used to interpret imaging data and used as input novel machine learning algorithms. This protocol details the steps to generate high quality H&E images from both FFPE and fresh frozen (FF) tissues following multiplexed imaging on the Phenocycler-Fusion.

*This protocol can also be used for H&E staining following multiplexed imaging on other platforms. In that case, skip the 'Flow cell removal' section, perform two washes of*

 00:05:00 mins each in 1X PBS and proceed directly to **Step 5**.

**Protocol status:** Working

We use this protocol and it's working

**Created:** Apr 09, 2024

**Last Modified:** Apr 16, 2024

**PROTOCOL integer ID:** 97990

**Keywords:** H&E, hematoxylin, eosin, codex, phenocycler, phephenocycler fusion, multiplex imaging

## MATERIALS

### Reagents:

- PBS (10X), pH 7.4 - Thermo cat# 70011044
- Xylene (Sigma cat# 534056) or Histo-Clear (National Diagnostics cat# HS-200)
- 100% (200 proof) ethanol - Sigma cat# E7023
- Hematoxylin Solution, Mayer's - Sigma cat# MHS32-1L
- Epredia Shandon Bluing Reagent - Thermo cat# 6769001
- Eosin Y Solution; Alcoholic - Sigma cat# HT110116-500ML
- DPX Mountant - Sigma cat# 06522-100ML
- DI water

### Materials and Equipment:

- Coplin jars
- Disposable transfer pipette- VWR cat# 10754-268
- Glass coverslip (1mm, 24 x 50) - Thermo cat# NC0106068
- Chemical fume hood

## Flow cell removal

**1** After a successful Phenocycler run, slides with attached flow cells can be stored in the storage buffer at  4 °C or used immediately for H&E staining.

**2** The flow cell needs to be removed from the slide in order to continue with H&E staining. Retrieve tissue slides with attached flow cells either from the storage buffer or directly after Phenocycler run.

**2.1** Fill a coplin jar with xylene or Histo-Clear. There must be sufficient volume to cover the entire flow cell.

**2.2** Place slides in the coplin jar containing xylene or Histo-Clear and incubate for  24:00:00 hours at  Room temperature 

1d

**2.3** Following incubation, gently remove the flow cell from the slide.**H & E staining**

- 3** Place the slides in 100% ethanol for  00:02:00 mins . Raise and dip the slides 10 to 15 times to fully cover the tissue. 2m
- 4** Place the slides in 95% ethanol for  00:02:00 mins . Raise and dip the slides 10 to 15 times to fully cover the tissue. 2m
- 5** Place the slides in DI water for  00:02:00 mins . Raise and dip the slides 10 to 15 times to fully cover the tissue. 2m
- 6** Proceed immediately from step 5 and place the slides in the following reagents:

- 6.1** Mayer's Hematoxylin -  00:04:00 mins 4m

**Note**

Lymphoid tissues or tissues with dense cellularity should be stained for only  00:03:00 mins . Rinse the slide in DI water to observe the stain. Return the slide to Mayer's hematoxylin if staining is not adequate.

- 6.2 DI water -  00:01:00 min 1m
- 6.3 Bluing Reagent -  00:01:00 min 1m
- 6.4 DI water -  00:01:00 min 1m
- 6.5 Alcoholic Eosin -  00:02:00 mins 2m
- 6.6 95% ethanol -  00:01:00 min without agitation or 20-30 dips to remove all excess reagent from previous step 1m
- 6.7 100% ethanol -  00:01:00 min without agitation or 20-30 dips to remove all excess reagent from previous step 1m
- 6.8 100% ethanol -  00:01:00 min without agitation or 20-30 dips to remove all excess reagent from previous step 1m
- 6.9 Xylene/Histo-Clear -  00:01:00 min without agitation or 20-30 dips to remove all excess reagent from previous step 1m

**6.10 Xylene/Histo-Clear - Hold in this solution as slides are mounted one by one****Slide mounting**

20m

- 7 To mount the slides, remove slides one at a time on to a paper towel inside the fume hood. Leave the other slides in the last xylene/Histo-Clear coplin jar to prevent over-drying the tissue.

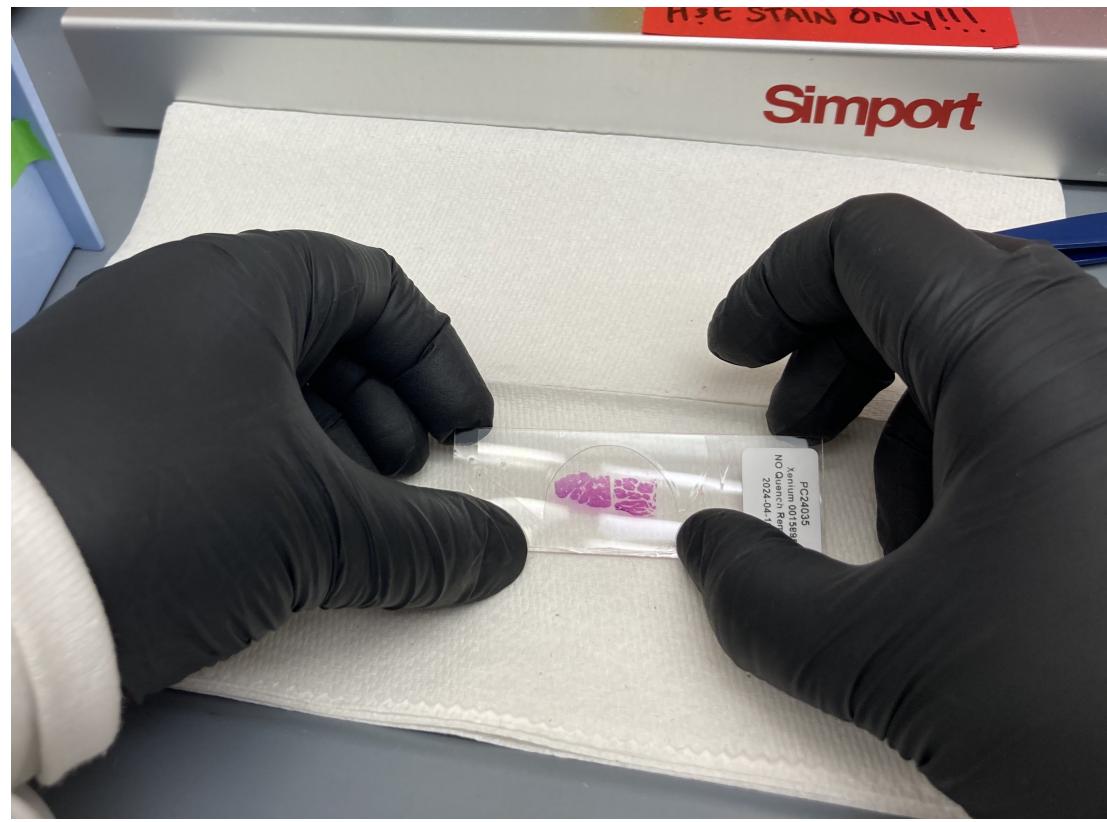
Dry the back of the slide and tilt to remove excess xylene, but do not let the tissues dry.

- 7.1 Using a disposable transfer pipette add enough DPX mountant (or other xylene-based mountants) to cover the tissue area (1 to 2 drops)



- 7.2 Clean a glass coverslip quickly and remove any particles on its surface. Place the long edge of the coverslip on the edge of the slide closest to you, tip the **slide** towards yourself so the mountant begins to reach the coverslip, and gently push down until the mounting media has spread evenly across the slide area containing tissue.





- 7.3** Remove excess mountant by placing the edges along the paper towel. Avoid mountant covering the top of the coverslip.

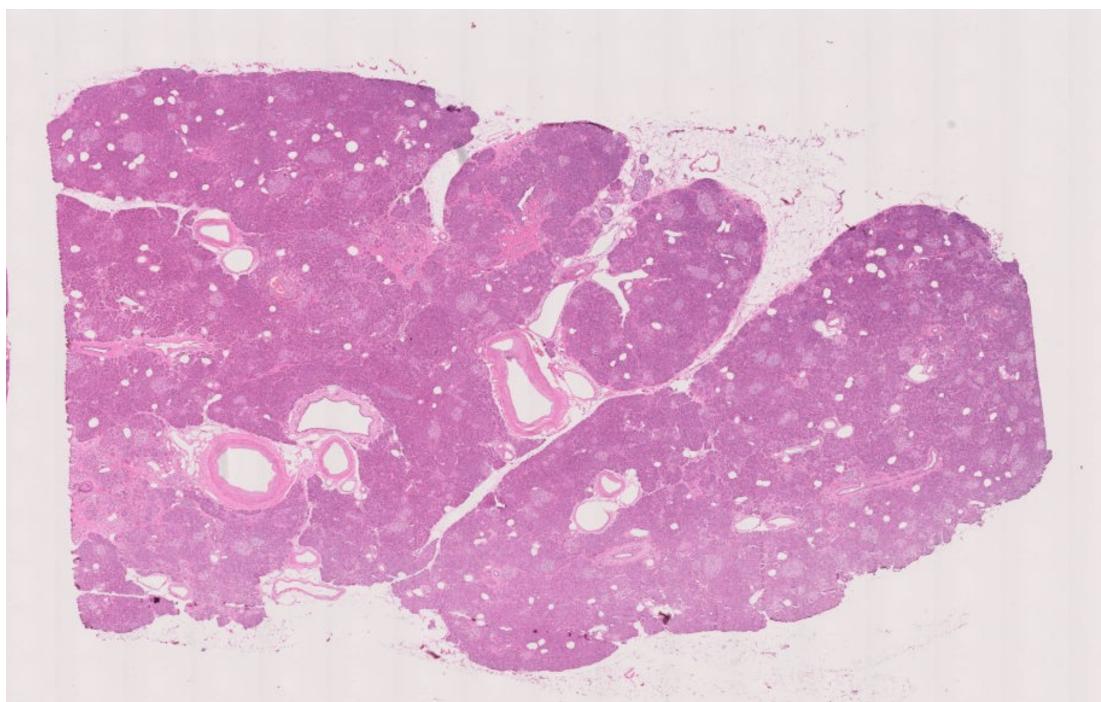


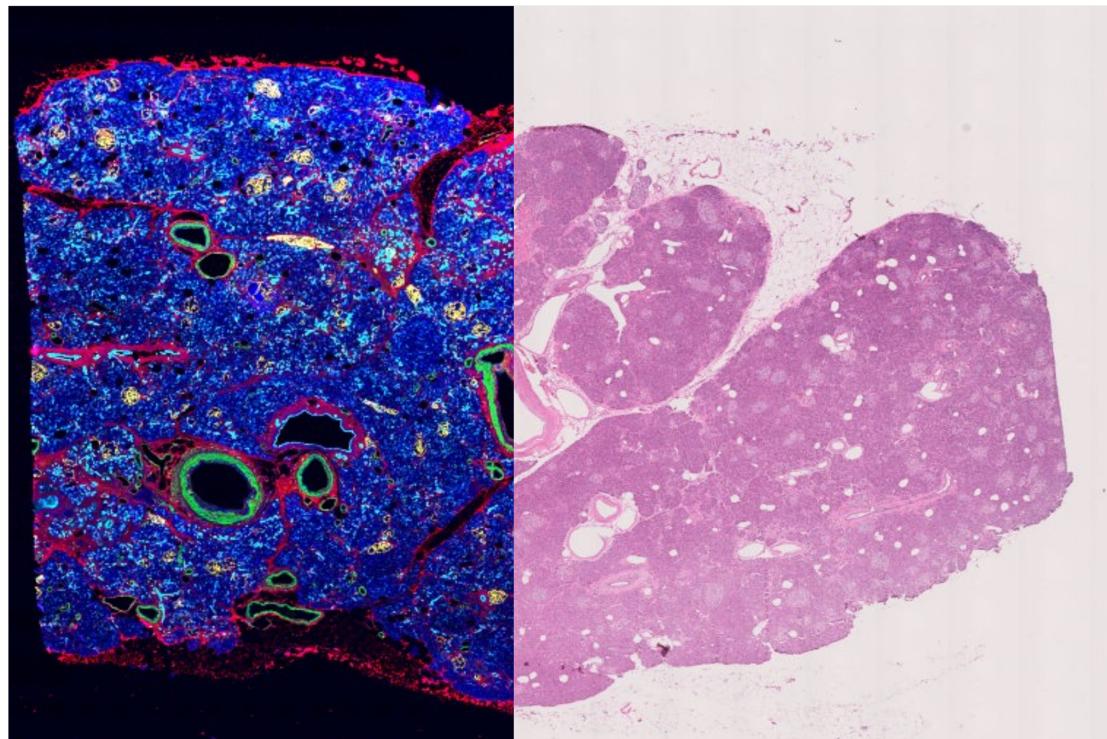
- 7.4** Gently press down on the coverslip to remove any bubbles that remain above the stained tissue.
- 8** Allow mountant to cure inside the hood for at least  00:20:00 mins before moving and imaging. Xylem-based mountants take up to 24 hours to cure completely. 
- 9**  go to step #7 and repeat mounting procedure for remaining slides.

- 10 Mounted slides can be imaged and stored in a slide storage box at  Room temperature

11 **Expected Result:**

H&E stained image of human pancreas FFPE tissue following multiplexed imaging on Phenocycler-Fusion  
*(Data courtesy of Prof. Paul Robson, The Jackson Laboratory)*





Overlay demonstrating multiplexed imaging and H&E staining on same tissue section (*Data courtesy of Prof. Paul Robson, The Jackson Laboratory*)