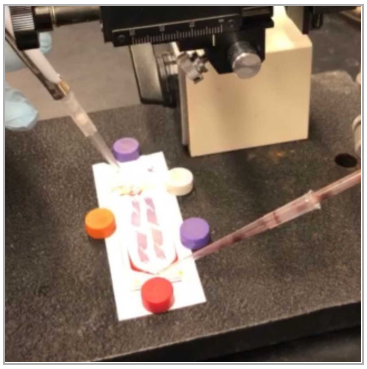

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# Indiana University H&E Staining on AKOYA PhenoCycler Flow Cell post Imaging —The Ferkowicz Method

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

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## ABSTRACT

This protocol was developed by Indiana University researcher (Michael Ferkowicz and colleagues). It described histological staining of tissue sections after imaging with the Akoya PhenoCycler (also known as CODEX) without removing it from Flow cell, which will help with near complete registration of the histology with the multi-fluorescence image obtained from CODEX. The innovative elements of this protocol include the strategic incorporation of an aspiration setup to allow flow of the staining solutions and washings across the Flow cell slide.
A video is attached to this protocol to show critical parts of the process.
This work was done part of the HubMAP and KPMP efforts

## ATTACHMENTS

IU protocol.mp4

## IMAGE ATTRIBUTION

Michael Ferkowicz and Angela Sabo

## GUIDELINES

**This protocol may require initially 2 persons until familiarity with the setup and steps.
**Positioning of the aspiration system attached to vacuum is key.
** Duration of Hematoxylin and Bluing agent may vary and should be adjusted to tissue type
**DI water can probably be used instead of 1X PBS but not tested.

## Setup

1 Following multiplex imaging with the AKOYA PhenoCycler (CODEX) system, the PhenoCycler slide with flow cell attached is stored in 1X CODEX Akoya Buffer at 4C until ready to stain

2 Attach an aspirator tipped with 200µL pipette tip (e.g., a standard laboratory vacuum) to a lab stand via clamp or a micro-manipulator with X/Y/Z adjustments.

3 Carefully secure PhenoCycler slide to base of lab stand (we use small round magnets on four sides to hold it in place)- see attached video

4 Position the aspirator tip over the distal PhenoCycler flow cell exit port (away from the slide label) just above opening (we orient the tip at 45→60° angle to the port). Be careful not to get too close and start drawing fluid, as this may result in a bubble in the flow cell- see attached video

5 Precise positioning of the aspirator is crucial to success. The perfect position is such that fluid is aspirated only when a pool of liquid exists over the entry port. If aspiration is more aggressive, then vacuum must be managed with a screw clamp on the vacuum line or less precisely, via the vacuum port valve. In this case, liquid must always be pooled over the inlet port while the vacuum is on. (see attached video).

## Staining

7m

6 All solutions should be added slowly with a P200 micropippetor such that a small pool above the entry port is maintained while vacuum is on. Adjust aspirator/vacuum for reasonable flow across slide without pulling air into entry port to avoid bubbles. Using volumes of 200 µL at a time is optimal to keep flow homogeneous when adding solutions to the entry port.

7 Rinse tissue with 600 µL of 1X PBS.

8 Immediately stain with 400 µL of 1X hematoxylin solution (Dako S330).

9 Allow hematoxylin exposure for 2 minutes. Start timing after first 200 µL addition or when hematoxylin starts to come out of exit port. Remove any excess hematoxylin around entry port. May have to stop vacuum to allow appropriate time for incubation. 2m

10 Wash with 600 ul 1X PBS

11 Add 400 µL of Bluing Buffer (Dako CS702) incubate for at least 1 minute (Start with 200 µL, and adjust aspiration and vacuum accordingly).

12 Wash with 600 µL 1X PBS. 1m

13 Immediately stain with 400 µL of eosin (Sigma Aldrich HT110216) and incubate for 4-10 min (range may depend on tissue type, thickness and scale). Adjust aspiration/ vacuum midway to ensure appropriate incubation time. 4m

14 Wash with 600 µL of 1X PBS.

## Adding mounting medium for imaging

15 Add 400 µL of 40% glycerol in PBS

16 Add 400 µL of 80% glycerol in PBS (you will need to increase aspiration via tip position and/or

vacuum strength to compensate for increased viscosity of the glycerol).

17 Allow tissue and media to equilibrate 8-24 hours at room temperature before imaging.

18 High resolution images can be obtained with a 20X air objective on a slide scanning-type microscope setup (e.g. Keyence BZ-X810).

19 Store at 4°C with ports covered with parafilm.