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Autofluorescence Microscopy Data Acquisition

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1 Works for me dx.doi.org/10.17504/protocols.io.7e3hjgn

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ABSTRACT

Scope:

Obtain autofluorescence microscopy images of tissues.

Expected Outcome:

An RGB autofluorescence microscopy image of the tissue section that enables registration and correlation of different imaging modalities on a pixel by pixel basis.

GUIDELINES

Carefully handle slides with gloves. Finger prints and dust on slide can alter final image.

MATERIALS TEXT

Zeiss AxioScan Slide Scanner

Slide Adapters for Scanner

- 1 If sectioned tissue is frozen, return to room temperature (~20°C) within a vacuum desiccator (~30 min), otherwise proceed directly to step 2.
- 2 Place microscope slide within adapter and insert into the Zeiss AxioScan Slide Scanner
- 3 Perform coarse focusing of the tissue using:
DAPI filter set (ex. 335-383 nm; em. 420-470 nm, blue)
Lamp power (~90%) and moderate exposure times (~150 ms)
- 4 Perform fine focusing of the tissue using the following to build a focus map:
DAPI (blue)
GFP (ex. 450-490 nm; em. 500-550, green)
DsRed (ex. 538-562 nm; em. 570-640, red)
Lamp power (~90%) and moderate exposure times (~150 ms)
- 5 Define the imaging region that includes the tissue.
- 6 Acquire autofluorescence image.

7 Export autofluorescence image as an OME-TIF with the following options:

"BigTIFF"

"Use Tiles"

"Compress"

"Convert to 8 Bit"

Alternatively, other image file types, such as "BigTIFF" and "pngs" can also be useful, depending on the application.



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