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Post-IMS Autofluorescence Microscopy

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1 Works for me dx.doi.org/10.17504/protocols.io.879hzr6

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ABSTRACT

Scope:
Obtain autofluorescence microscopy images of tissues after IMS analysis

Expected Outcome:
A brightfield and green autofluorescence microscopy image of the tissue section that enables registration and correlation of different imaging modalities on a pixel by pixel basis.

1 Place microscope slide within adapter and insert into the Zeiss AxioScan Slide Scanner.

2 Perform Coarse focusing of the tissue using:
GFP filter set (ex. 450–490 nm; em. 500–550, green)
High lamp power (~90%) and moderate exposure times (~ 150 ms).

3 Perform Fine focusing of the tissue using:
GFP filter set (ex. 450–490 nm; em. 500–550, green)
Brightfield lamp at similar power and exposure time used in step 2 to build a focus map.

4 Define the imaging region that includes the tissue.

5 Acquire brightfield and autofluorescence image.

6 Export autofluorescence image as an OME-TIF File with the following options:
“BigTIFF”, “Use Tiles”, “Compress”, and “Convert to 8 Bit”.

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