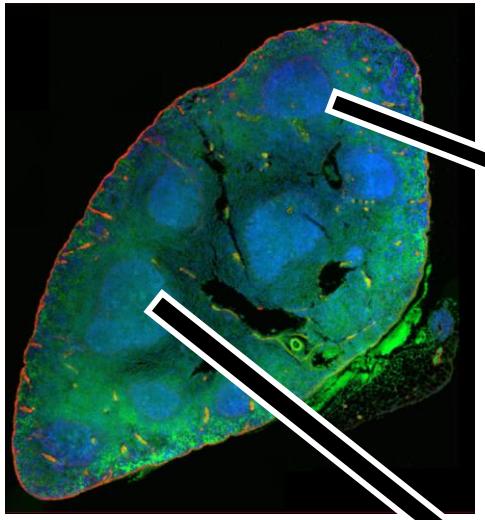
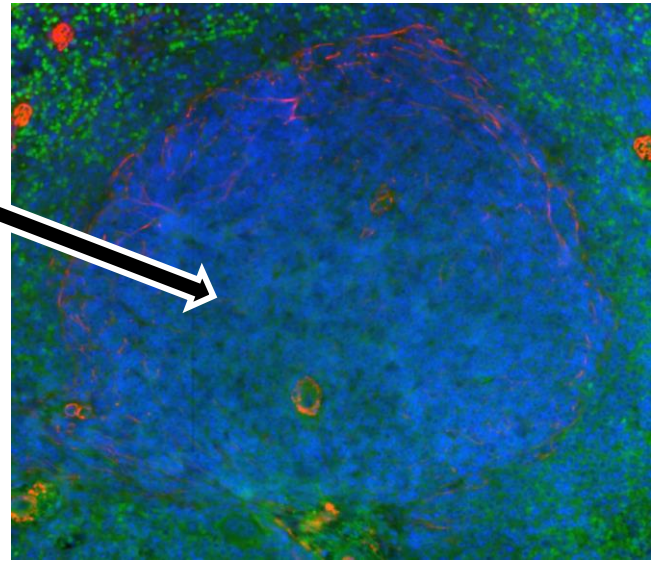
Exercises for the position of
Junior and Senior Engineer Biological Image analysis

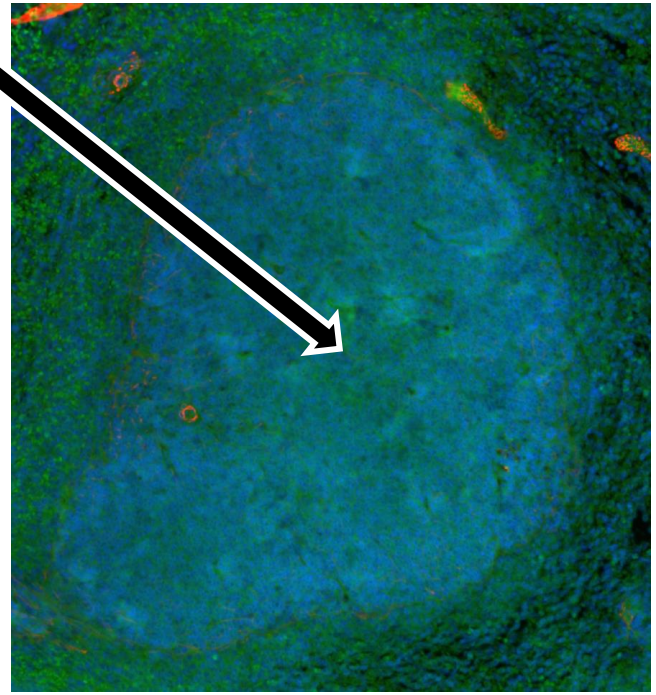
For each exercise, please:

- Perform the analyses, or explain how you would approach them
- You are free to solve the exercises in any way you think is useful
- Report the analysis (or approach) like a methods/results section in a scientific paper: succinctly but allowing reproducibility
- As your time might be limited, consider quality as much as quantity
- Please report your answers in a single PDF in English (*), and share larger parts of code or scripts on an online code repository
- We will only consider responses returned to us **before April 3rd** send to **stephen.whitmarsh@icm-institute.org.** Please use with subject title: [**Exercises image analysis**] to make sure your response is not lost

* The institute is bilingual, so don't worry too much about grammar







Markers

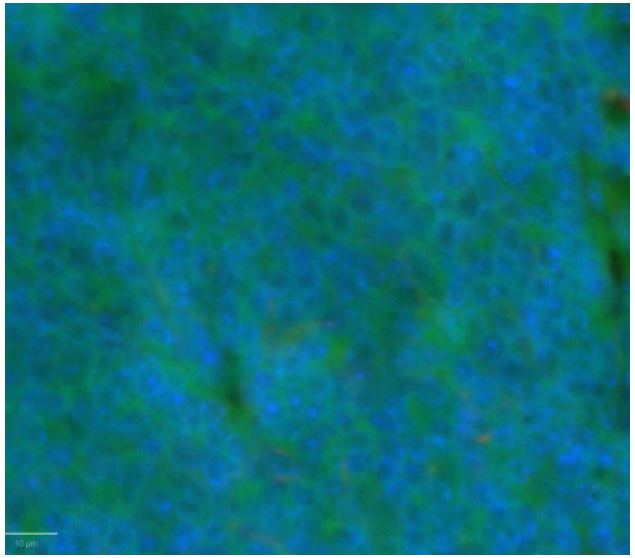
Color
H3258 (C1)
AF488 (C2)
Cy7 (C3)

Exercise 1

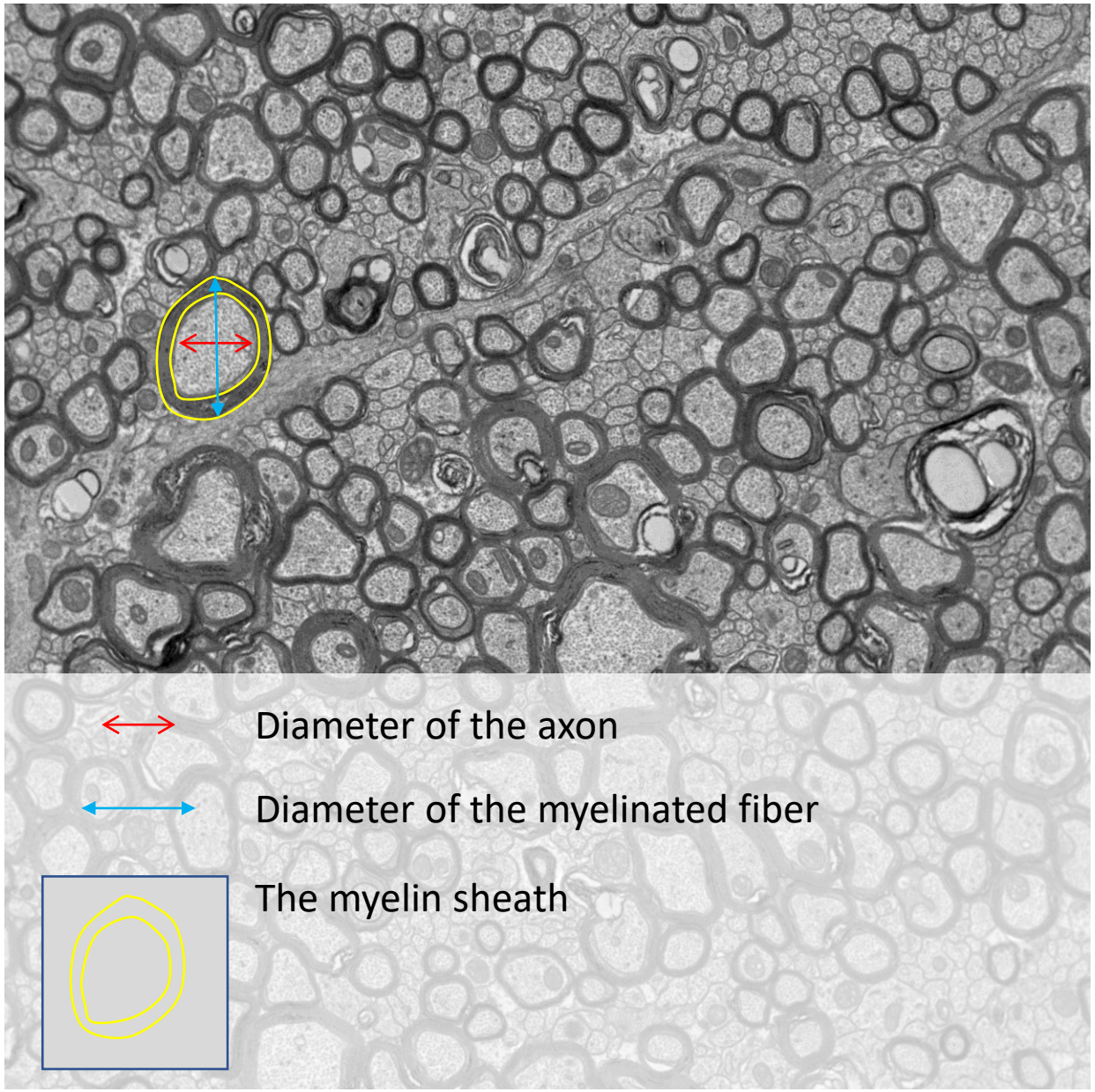
See file: *Spleen_Hoechst_AutoFL_SMA.ome.tif*
This is an image from a human spleen, and contains different layers that correspond to different markers

For this exercise:

- Count the <u>blue cell nuclei</u> in the white pulp of the spleen



Do not share these images



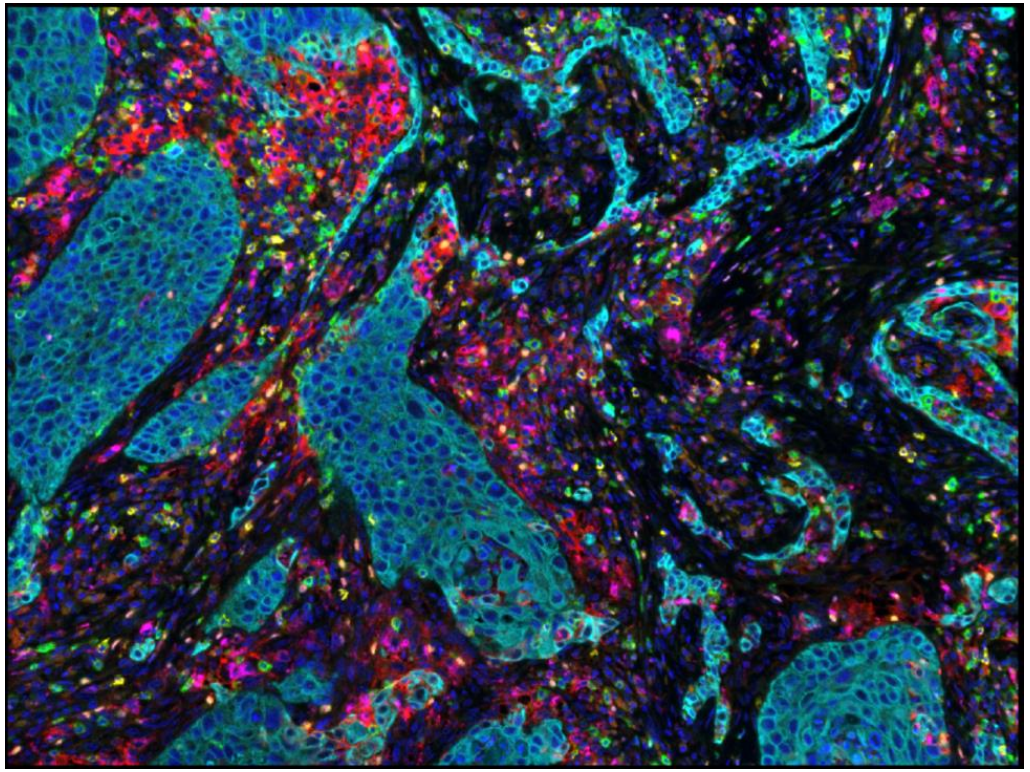
Exercise 2

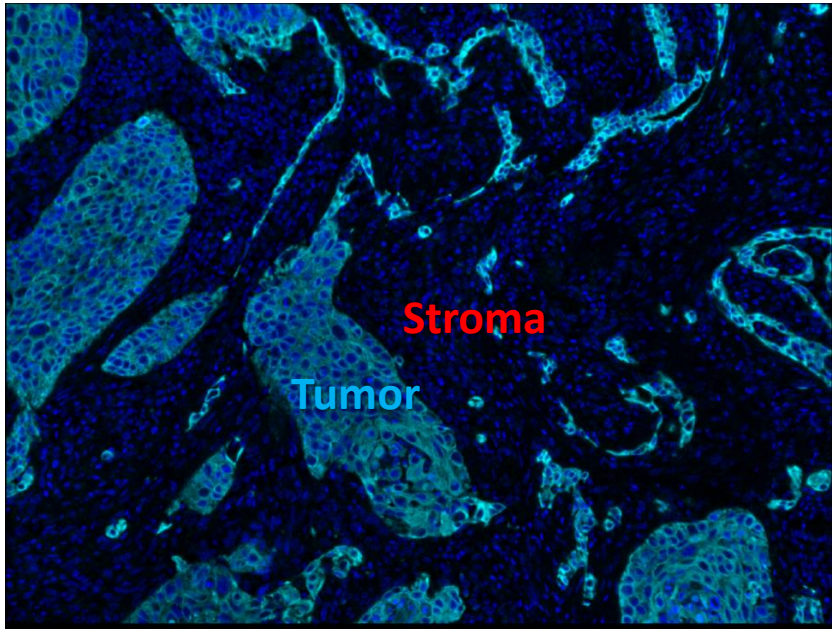
See file *axons.tif*

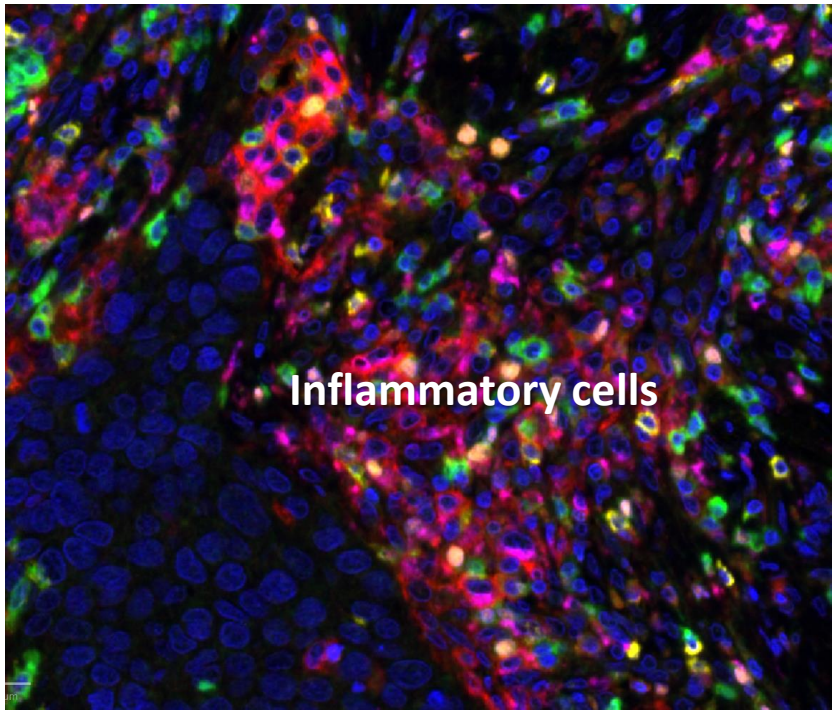
This is an image of myelinated fibers (serial section)

For this exercise:

- Segment the image to isolate the myelin sheaths (dark rings)
- Determine the diameters of the axons and the myelinated fibers







Markers

Color
PDL1 (Opal 520) (C1)
CD8 (Opal 540) (C2)
FoxP3 (Opal 570) (C3)
CD68 (Opal 620) (C4)
PD1 (Opal 650) (C5)
CK (Opal 690) (C6)
DAPI (C7)
Autofluorescence (C8)

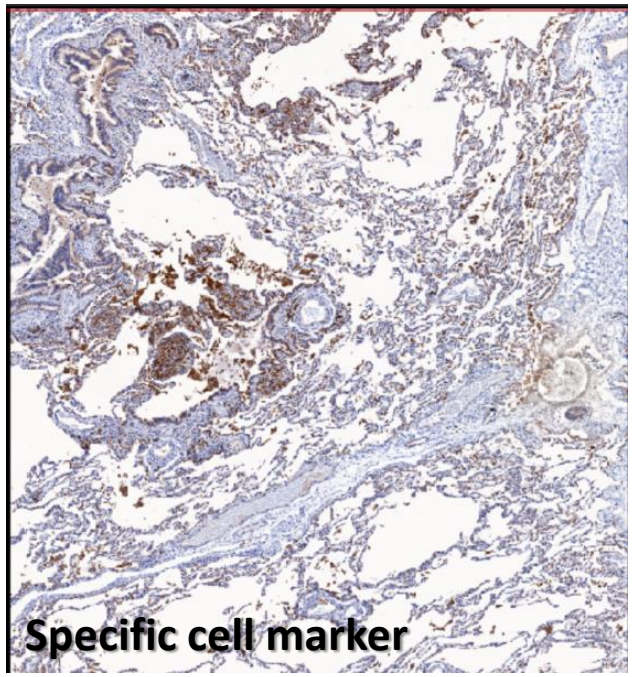
Do not share these images

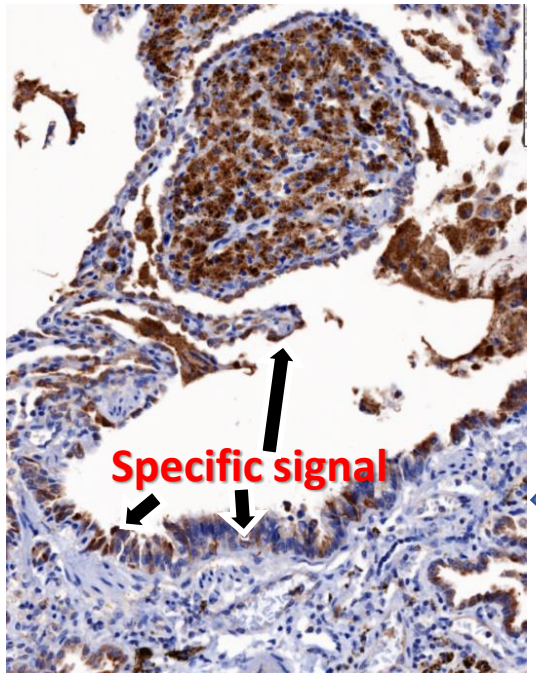
Exercise 3

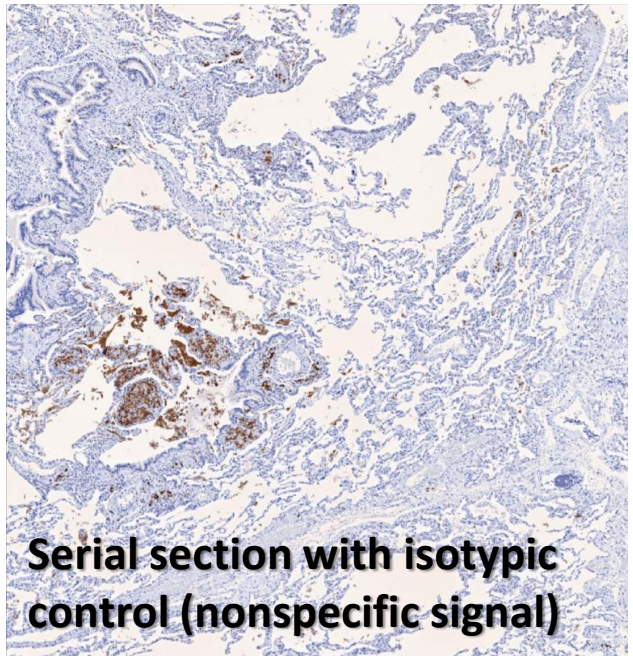
See file: *Multiplexing image_cancer-inflammation.tif*
This image is of an epithelial tumor, and contains different layers that correspond to different markers

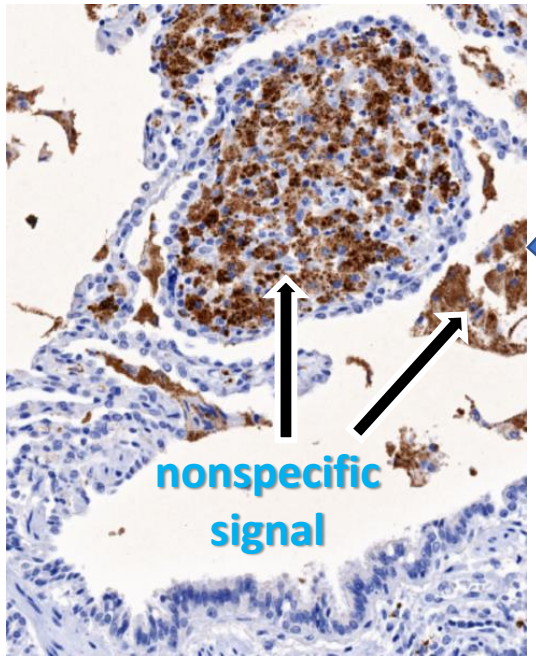
For this exercise:

- Segment tumor from stroma based on the CK positivity of the cells
- Segment all inflammatory cells from the stroma
- Attribute a phenotype of each inflammatory cell according to the available markers
- Give a relative number of each inflammatory cell type (phenotype) in this image
- Give a mean minimal distance of these cell types to the tumor tissue





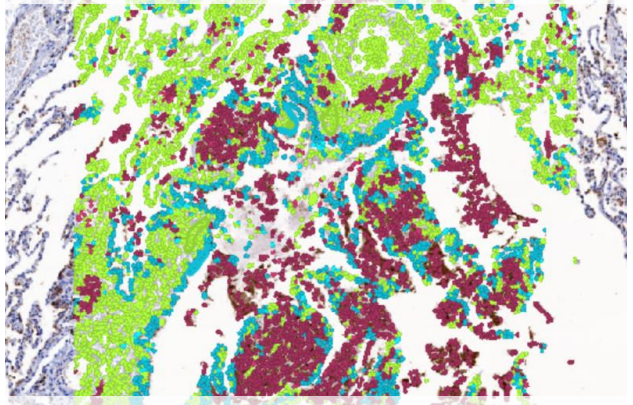




Exercise 4

See file: *Lung_isotypic control.svs* and *Lung_marker of interest.svs.* These contain serial sections taken from a lung stained with a specific cell marker and isotypic control using nonspecific staining

Figure A



Categories:

- Specific signal (blue)
- Nonspecific signal (red)
- Tissue (green)

For this exercise:

- Use both images to segment a specific staining (light blue in Figure A)
- Give the relative area of this stain compared to the area of lung the tissue.

Do not share these images