

Oct 09, 2024

QuPath to SCiLS instructions

DOI

<https://dx.doi.org/10.17504/protocols.io.kqdg32nq1v25/v1>

Naina Beishembieva¹, Brittney Gorman¹, Christopher Anderton¹

¹Pacific Northwest National Lab

KPMP

Tech. support email: info@kpmp.org



Naina Beishembieva

Pacific Northwest National Laboratory

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.kqdg32nq1v25/v1>

Protocol Citation: Naina Beishembieva, Brittney Gorman, Christopher Anderton 2024. QuPath to SCiLS instructions.

protocols.io <https://dx.doi.org/10.17504/protocols.io.kqdg32nq1v25/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

Protocol status: Working

We use this protocol and it's working

Created: August 30, 2024

Last Modified: October 09, 2024

Protocol Integer ID: 106768

Keywords: analyzing mass spectrometry imaging data, mass spectrometry imaging data, molecules from mass spectrometry image, mass spectrometry image, ionization mass spectrometry imaging, molecular data, molecular data from various region, qupath data, mass spectrometry, qupath segmentations with maldi, source software for digital pathology, data from qupath, microscopy in scil, molecular composition of specific tissue, tissue annotation, immunofluorescence, msi histology, msi data in scil, combining qupath segmentation, biological context for these molecular map, process of the tissue annotation, data from scil, annotation of tissue, immunofluorescence stain, annotated tissue, immunofluorescence signal, annotated molecule, molecular map, digital pathology, microscopy, instructions on qupath, manual selection of tissue, segmentation, msi data, specific tissue, maldi image, laser desorption, molecular abundances in segmented region, immunofluorescence channel, functional units in qupath, tissue functional unit, qupath, a

Funders Acknowledgements:

Kidney Precision Medicine Project

Grant ID: UH3 DK114920



Abstract

Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) is an analytical technique which provides the molecular composition directly from cells, tissues and organs. To provide biological context for these molecular maps acquired with MALDI-MSI histology or immunofluorescence is performed on the same or an adjacent tissue section. Methods for alignment, segmentation, and data analysis are required to extract molecular data from various regions within the MALDI images. We have developed a workflow to combine QuPath data and MALDI-MSI data in SCiLS. This data can be then exported to R for statistical analysis to study upregulation/downregulation of certain cell types at the areas of interest.

QuPath is an open-source software for digital pathology and whole slide image analysis. It allows for the annotation of tissue functional units and immunofluorescence stains. SCiLS (Bruker) is software for analyzing mass spectrometry imaging data. By combining QuPath segmentations with MALDI-MSI results, we are able to obtain the molecular composition of specific tissue functional units or immunofluorescence signals. It is also possible to combine metabolite annotations obtained from the open-source annotation platform METASPACE.

Part 1 QuPath to SCiLS (instructions on QuPath):

The process of the tissue annotation according to the immunofluorescence channel or by manual selection of tissue functional units in QuPath, as well as the export of this data into a file that can be recognized by SCiLS.

Part 2 QuPath to SCiLS (instructions on SCiLS):

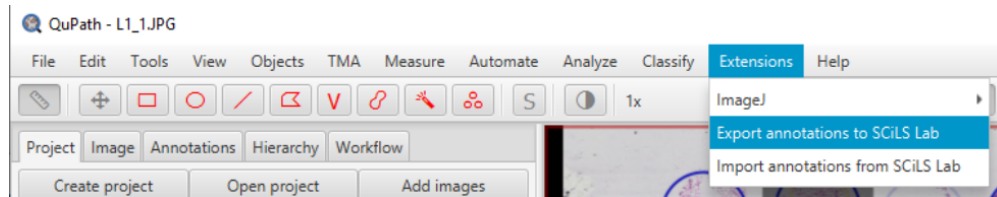
The process of importing the data from QuPath (annotated tissue functional units or areas of interest) and METASPACE annotations (annotated molecules from mass spectrometry images) to SCiLS, as well as the alignment of images from mass spectrometry and microscopy in SCiLS. This will allow for the further export of all the data from SCiLS to the R (SCiLS API) where statistical analysis can be performed to compare the molecular abundances in segmented regions.

Before start

Install QuPath

Part 1. QuPath to SCiLS (instructions on QuPath)

- 1 Install QuPathtoSCiLS extension
 - 1.1 Open QuPath
Find file "QuPathToSCiLS-1.4.jar".
My pathway is: C:\Program Files\SCiLS\SCiLS Lab\QuPathPlugin
 - 1.2 Drag the QuPathToSCiLS-1.4.jar file onto the running QuPath window
 - 1.3 If QuPath displays a message that the extensions directory is not specified accept the default or specify a different location.
 - 1.4 There should now be two entries "Export annotations to SCiLS Lab" and "Import annotations from SCiLS Lab" under QuPath Extensions:



If the entry does not show up restart QuPath.

- 2 Annotate files in QuPath either using tools to annotate such as magic wand (for example, when annotating H&E tissue)



Magic wand

or using thresholding for channels (for example, when have cyclic IF on the tissues)

- 2.1 Note (if using stained tissue):



This icon will allow to see all channels as shown below

Channel	Show
DAPI (C1)	☒
Ki67 (C2)	☒
CD31 (C3)	☒
CD45 (C4)	☒
CD163 (C5)	☒
SMA (C6)	☒
Collagen IV (C7)	☒
Podoplanin (C8)	☒
AGER (C9)	☒

Filter channels by name

Settings

☐ Show grayscale ☐ Invert background

DAPI (C1) ☐ Log histogram

Channel min: 28.8

Channel max: 2491.7

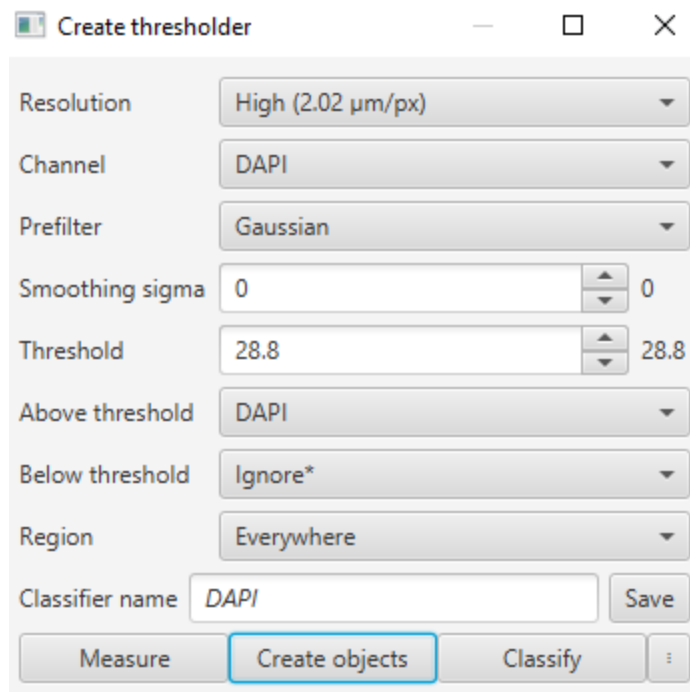
Viewer gamma: 1

Auto Reset

☒ Apply to similar images

To get rid of background, channel min value can be varied (usually ~ 10% of the max is a good setting)

- 2.2 Thresholding:
Classify > Pixel classification > Create thresholder
The following window will show up:



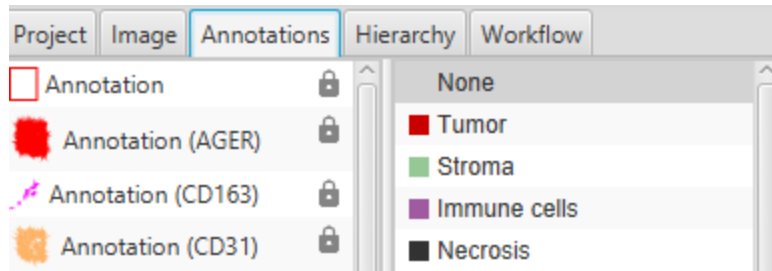
The screenshot shows a 'Create thresholder' dialog box with the following settings:

- Resolution: High (2.02 $\mu\text{m}/\text{px}$)
- Channel: DAPI
- Prefilter: Gaussian
- Smoothing sigma: 0
- Threshold: 28.8
- Above threshold: DAPI
- Below threshold: Ignore*
- Region: Everywhere
- Classifier name: DAPI

Buttons at the bottom include 'Measure', 'Create objects' (highlighted with a blue border), 'Classify', and 'Save'.

Test different resolutions and select the suitable one (it must show the selected areas in yellow), choose the right channel, prefilter-Gaussian and smoothing sigma 0 settings are kept at all times (they are the best), threshold-select the value based on what you got for channel min at 2.1, Above threshold - your channel, below threshold - Ignore*, classifier name - name of your channel

Then after this window was filled out, press: Create Objects > in the new window at the drop-down menu select either full image or all annotations (if selected only specific area of the tissue for the annotations) > at the next window press "OK" (Note: minimum object size can be changed from 0 to a pixel size IF those are not necessary for thresholding, then it will only mark cells larger than a size you have entered)
The annotation can be now checked here:



Important tip:

- When choosing resolution of your annotation, to make sure your file can be exported, try to use the lowest resolution that yet produces good results of annotation

- 3 After completing all annotations, go to Extensions > Export annotations to SCiLS lab
Save file

Part 2. QuPath to SCiLS (instructions on SCiLS)

- 4 Open SCiLS lab (Premium)
Press New File
- 4.1 Import/choose ImzML file
Press Next
- 4.2 Page "Mass axis settings" must appear
Check: bin size (it must match the bin size indicated in Metaspacer → dataset overview → m/z tolerance (ppm) value will indicate the bin size. If the value is not the same as in Metaspacer, unselect automatic and adjust this value
Change: constant type must be MRMS (Fourier - transform)
Press Next, then Import, name new SCiLS file, press save, press open imported file
- 5 Press File → open → choose the new created file from a previous step → press open
- 6 Press File → Import → choose Region SCiLS Exchange Format → choose here your annotated in QuPath file
- 6.1 The window with two pictures imported must appear – one from MSI and from QuPath (note: it will not appear annotated here)
Press Next

- It should now redirect to Image Co-registration page (provides instruction on the right side of the window)
After they are well aligned, press Next (good alignment is very important for precise results)
- 7 It will ask to select all needed regions from the list of annotation we have made in QuPath (usually need to select all of them)
Important: check the box "select new region" → name it
Press Finish (now the annotations of channels or anything is imported and appear in regions section of the main window)
 - 8 Press File → Attribute Editor → Press "Settings" icon
Select "Use as a region" for your annotations
Close
 - 9 Press File → Import → Feature list from file (flexImaging, MetaboSpace, SCiLS, CSV, TLST)
Choose file (for example, file imported from Metaspaces as CSV) → Open
Select m/z column and Name column → press Import
 - 10 Go to Feature list at the right side of the screen (At the bottom of the Visualization tabs)
Choose your file that you imported (for example, csv from Metaspaces) → press the globe icon
 - 11 Finally, the data now can be found at Ion Images
 - 12 Now that all data is gathered at SCiLS lab, the statistical analysis can be done in R (code provided, follow the instructions provided in the code)

Protocol references

Bruker software support. *QuPathToSCiLS*, 2024.

Dela Cruz, J. M. QuPath: Thresholding. <https://www.youtube.com/watch?v=BiiApbYzKak> (accessed 2024-08-31).

Bruker Daltonics. *Tutorial - Using SCiLS Lab and QuPath to integrate mass spectrometry imaging with histology*. YouTube. <https://www.youtube.com/watch?v=4KIM5MulUjg&t=587s> (accessed 2024-06-07).