Image Analysis with QuPath

Ofra Golani, March 2023

MICC Cell Observatory,
Weizmann Institute of Science

Image by Pete Bankhead, QuPath

מכון ויצמן למדע
WEIZMANN INSTITUTE OF SCIENCE

The de Picciotto-Lesser Cancer Cell Observatory

Establish a leading platform in the field of advanced light microscopy and image analysis. Support and advance research on campus through cutting edge technologies and applications


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QuPath Overview

Developed for Digital Pathology, by Pete Bankhead

Large 2D, Multiplexed data

Dr. Pete Bankhead
MRC Institute of Genetics and Molecular Medicine
University of Edinburgh

Open Source

Written in Java, Scripting in Groovy

ImageJ bridge

Extensions bridge into the Python world eg: StarDist, Cellpose

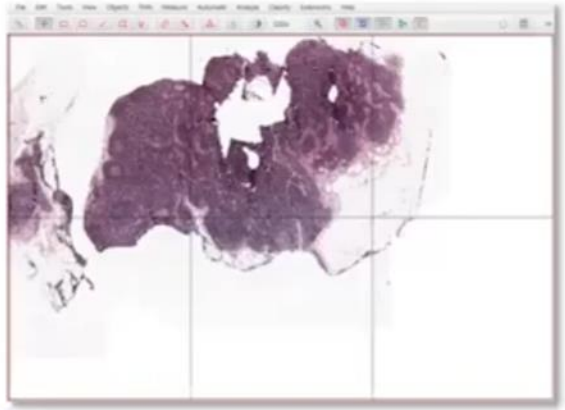
Works in Windows, Mac, Unix

Digital pathology requires specialised software designed to handle huge, complex images

> 20 billion pixels

> 60 GB raw data

Stored at multiple resolutions



Slide by Pete Bankhead, QuPath

2020-04-29 15:42:00

QuPath Overview

Handle big whole slide images:

100,000 * 100,000 pixels – 10GB uncompressed in single channel

Efficient Visualization is a challenge: Multi-scale pyramids

Efficient Analysis: Block-processing, GPU

Immuno-Histochemistry (H&E, H-DAB,…), Fluorescent, Multiplexed

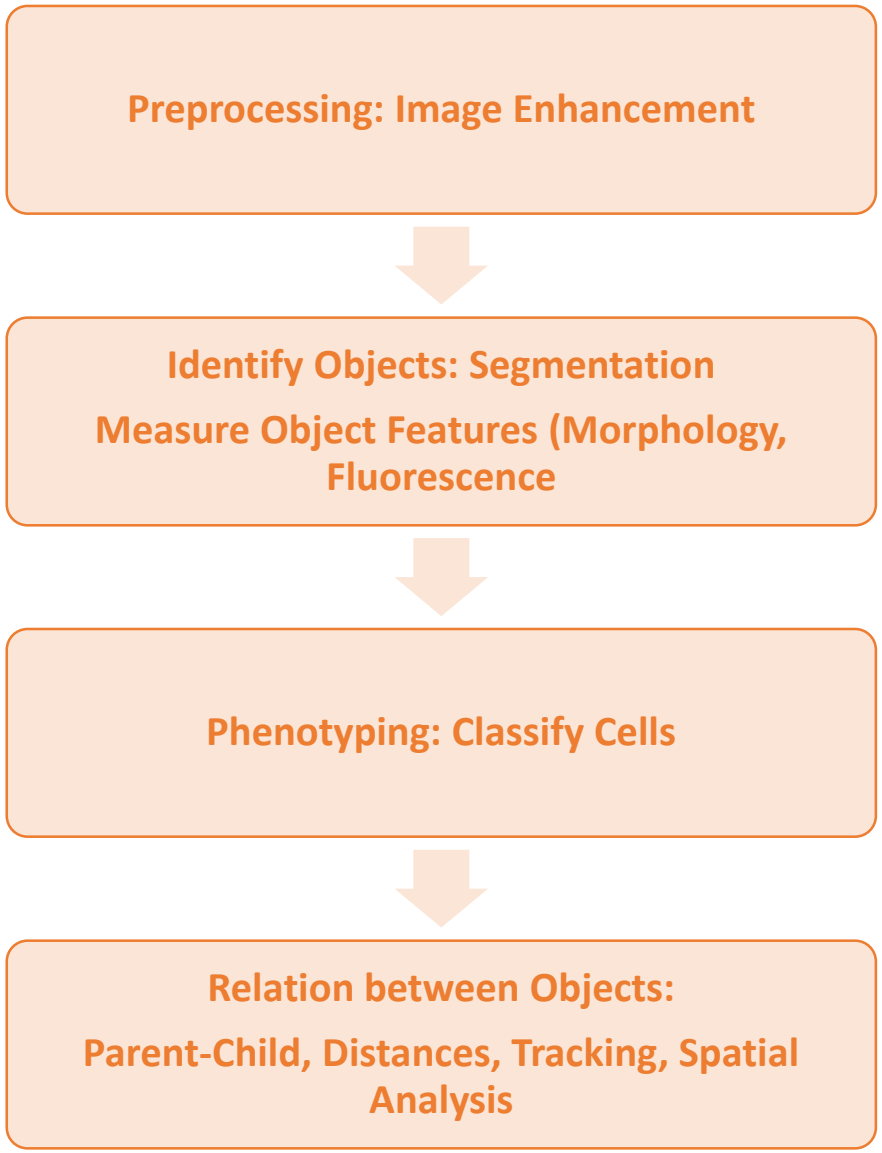
Image Analysis Workflows

- Break the analysis into sequential steps
- In each step: choose from modules / components of the corresponding category
- Interactive optimization of the analysis steps and tuning components and user-defined parameters
- Automation: Create pipe-line, looping on many images
- Statistics: Group results and Compare
- Visualization and Inspection (sometimes Manual correction)

Which objects ?

What to Measure ?

Which module suites for each step ?



QuPath Components

Pixel classifier: Threshold based, ML based

Region segmentation (connected component) based on pixel classifier

annotations or detection

Cell segmentation: threshold based, DL based – StarDist, Cellpose

- nuclei segmentation + optional expansion until it touch another cell
- Intensity measurements
- Shape measurements

Object Classifier: single value / ML

Annotation expansion

Object relations

(limited) Spatial analysis: Density, neighborhood

Dataset: Whole slide Tonsil tissue

Stained , Imaged with PhenoImager, unmixed

DAPI	Nuclei	white
Opal480	CD8	cyan
Opal520	PDL1	green
Opal570	Ki67	yellow
Opal620	CD68	orange
Opal690	PanCK	red
Opal780	CD20	magenta

Building Analysis Workflows in QuPath

Which Objects ?

Whole Tissue,

Regions: Follicle (yellow), Epithelial (red)

Cells: border, classes

What to Measure ?

Cell type composition in each region and surrounding

Distances between cells and regions

Which Component to use for each step ?

We'll see through the workshop…

Building Analysis Workflows in QuPath

Find Whole Tissue

Cell Segmentation

Phenotyping / Classify Cells

Positive Cells / Double Positive Cells

Find Follicle and Epithelial Regions

Expand regions

Measure and Export Results

Region area, Cell types distributions within Regions and Surroundings, …

Building Analysis Workflows in QuPath

Find Whole Tissue

↓

Cell Segmentation

↓

Phenotyping / Classify Cells

Positive Cells / Double Positive Cells

↓

Find Follicle and Epithelial Regions

↓

Expand regions

↓

Measure and Export Results

Region area, Cell types distributions within Regions and Surroundings, …

Basics & Concepts

QuPath GUI
Projects
Annotations / Detections
Classes

Pixel Classifiers

Threshold based & ML

Cell segmentation

Threshold based / DL

Object classifiers

Single measurement / ML

Additional goodies …

Workflow Examples

QuPath - Getting Help

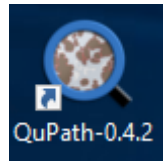
Resources

Image.sc Forum: QuPath
https://forum.image.sc/tag/qupath

ReadTheDocs Documentation
https://qupath.readthedocs.io/en/latest/

Youtube Tutorials
https://www.youtube.com/channel/UCqepVnS1QsB7B8nBA9T91EQ/playlists

QuPath Workshop – Get Prepared



1. **Login / remote login** (Remote to Maldives / Bora / Galapagos / Santorini)

Workshop Material (Data, PDFs, Scripts) on Local computer: D:\QuPathWorkshopMaterial / D:\ QuPathWorkshopMaterial2 (remote)

Laptop users: Copy from \\lscf-storage\shared\QuPathScriptsAndProtocols\QuPathWorkshopMaterial

2. **Open QuPath**

3. **Install Extensions** Drag & Drop extensions:

from C:\Programs\QuPath-0.4.2\extensions to QuPath window

qupath-extension-stardist-0.4.0.jar,

qupath-extension-biop-2.0.0.jar

qupath-extension-cellpose-0.6.1.jar

Choose default settings

4. **Setup Cellpose**

Extensions > Installed Extensions > Open Extension folder

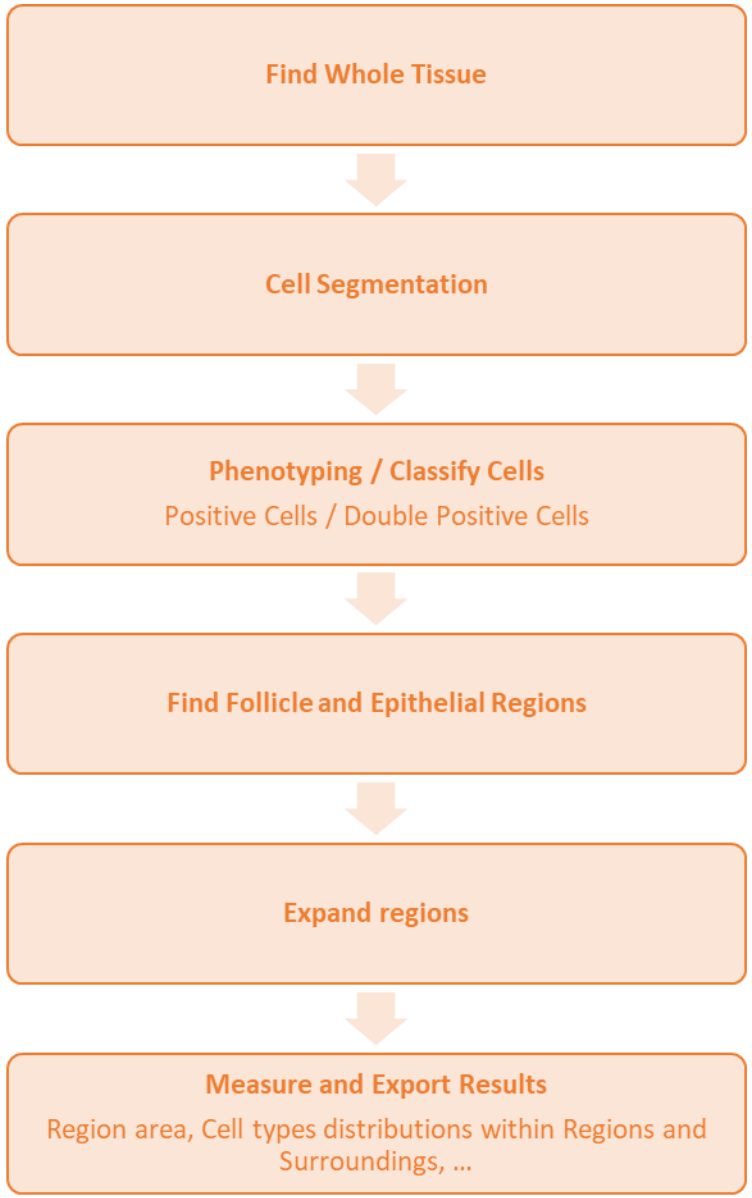
(D:\Users\USERID\QuPath\v0.4\extensions)

Copy run-cellpose-qc.py from C:\Programs\QuPath-0.4.2\extensions to Extensions folder

Edit > Preferences > Cellpose

QuPath Basics - Outline

- Create project, load images
- Inspect meta data
- Create whole tissue region, Annotations
- Segment cells – (simple way) StarDist
- Classify cells – Simple way / ML
- Region Segmentation
- Combine Regions & Cells information
- Export measurements
- Run for whole project

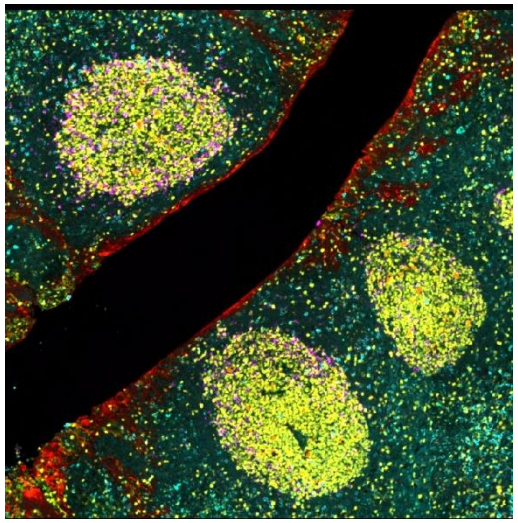


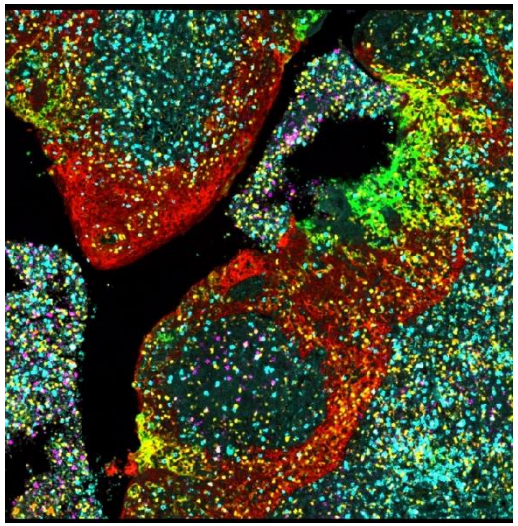
Dataset: Whole slide Tonsil tissue

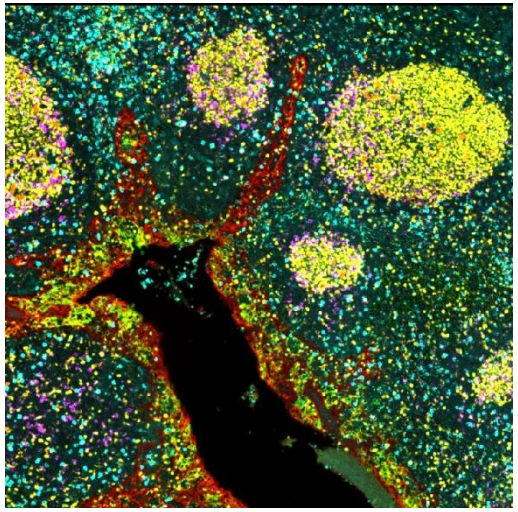
Stained , Imaged with PhenoImager, unmixed

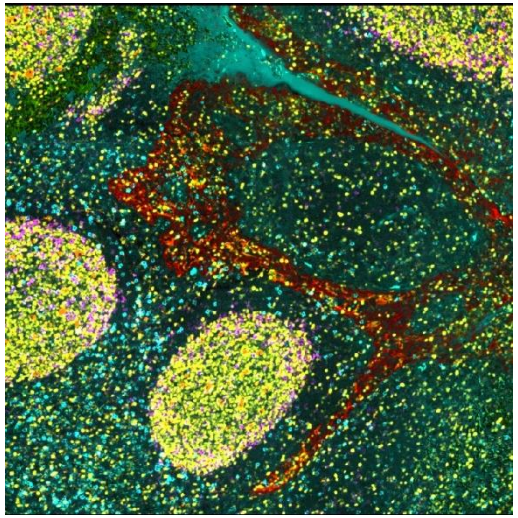
DAPI	Nuclei	white
Opal480	CD8	cyan
Opal520	PDL1	green
Opal570	Ki67	yellow
Opal620	CD68	orange
Opal690	PanCK	red
Opal780	CD20	magenta

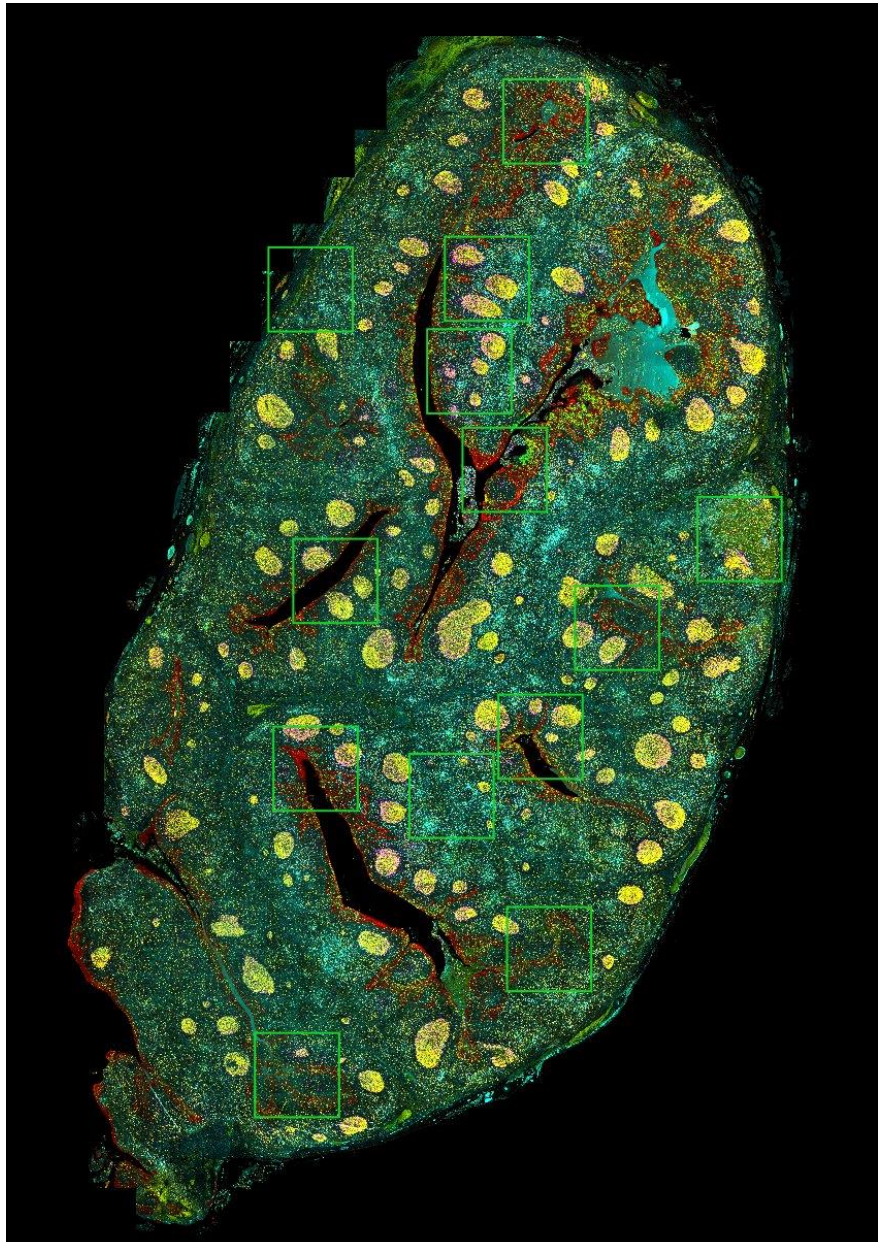
Dataset: Whole slide Tonsil tissue







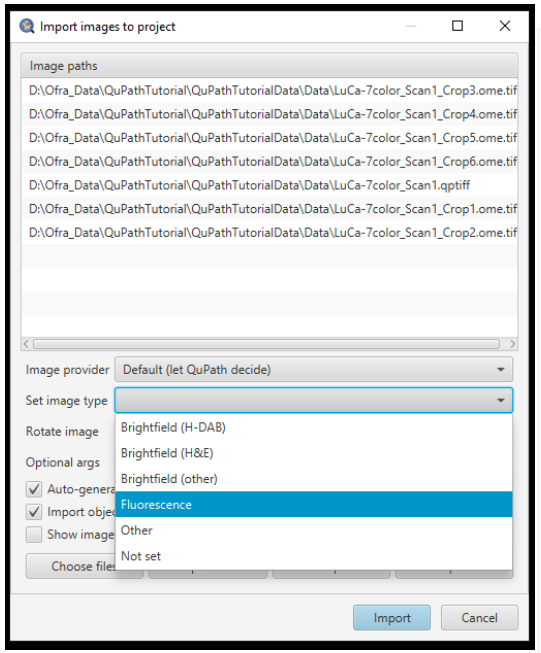


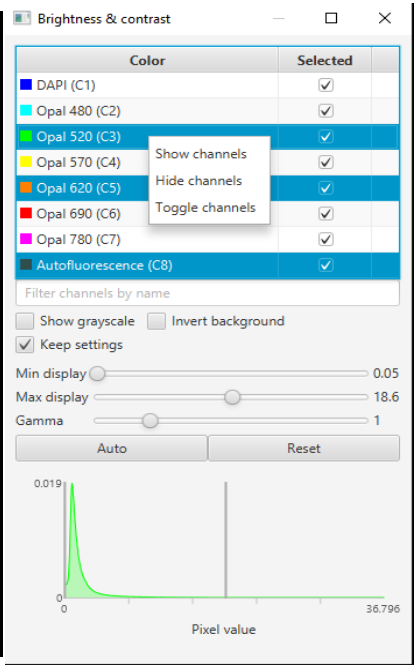


Ex 1: Create Project, Import and Inspect images Metadata, Control Display, Channel Names

- Create Project
- Open Images
- Double-click to Explore different images

What is image size, pixel size, bit depth?





- Show/Hide channels, change channel name and
- Ctrl+L to look for script editor

```
setChannelNames('Nuclei','CD8','PDL1','Ki67','CD68','PanCK','CD20','AF')
```

- *Scripts\ChangeChannelNamesColors-Tonsil.groovy* > **Run for Project**

Tips: 1) Drag & Drop 2) Ctrl+L to search for commands

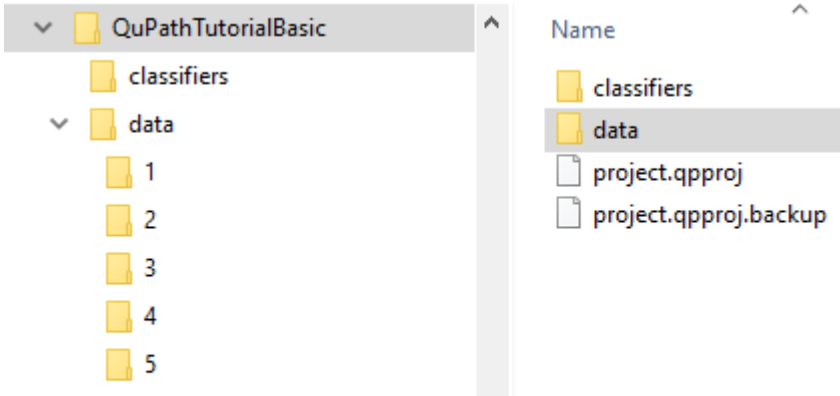
Projects in QuPath

Projects do not include images, but only extra information

Save Changes do not affect the image, It saves the appearance, metadata and objects

- Group related images
- Easily switch between images
- Organize data, scripts, classifiers and other useful things
- Thumbnail
- Run scripts for a whole project
- Open Project: select **project.qpproj** or drag&drop

Inspect the project folder structure along the workshop



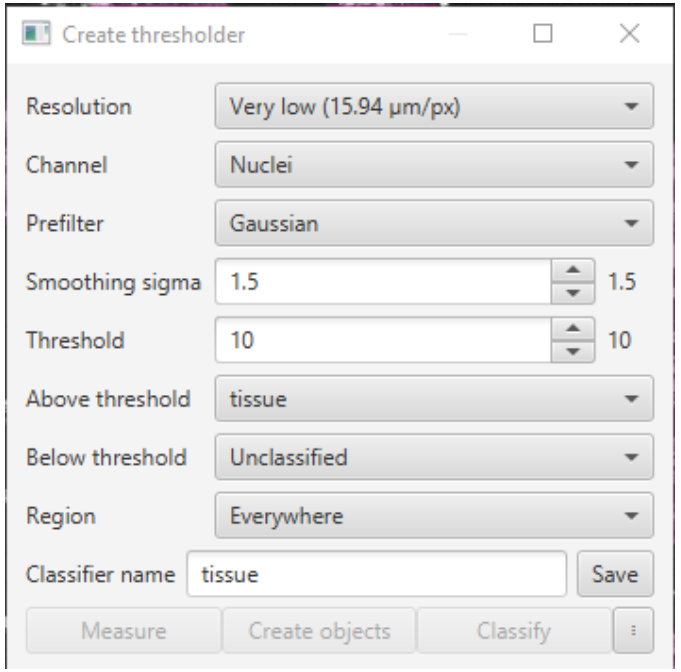
Ex 2: Define Tissue border, Manual and automatic Annotations

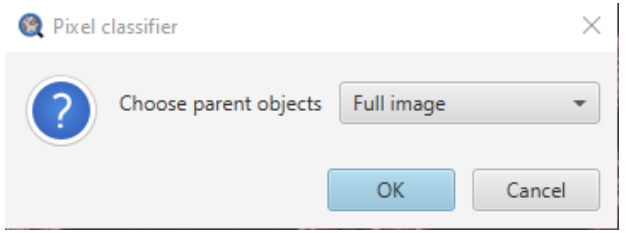
Manual Annotations

Image: Whole Slide

- Try: rectangle [R], polygon [P], brush [B], magic wand [W] to draw tissue border
- Dynamic brush and wand size, **Brush+Alt /Shift to delete/add**

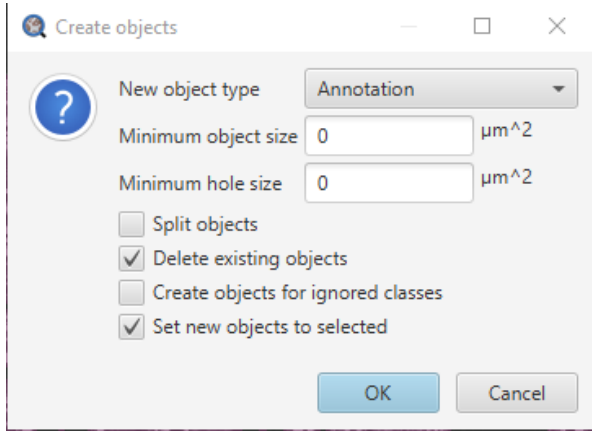
  To avoid overlap: deselect Annotations **Brush+Ctrl+Shift**. Shift+F to Show Filled
- Annotation Tab: Selected annotation, delete, Locking, Set properties
- Anything annotation Tweetorial





Automatic Thresholder

- Add class “WholeTissue”
- Ctrl+L > Create thresholder
- Zoom in to tissue border, explore different resolutions, change threshold and smoothing sigma
- Save the classifier
- Create objects
- Find good value for “Minimum Object size”, “Minimum Hole Size”
- “Split Objects”
- ‘a’, ‘c’, transparency



Ex 3a: Segment Cells – The Classical way

- Duplicate image
- Create annotation with diverse nuclei appearance
- Ctrl+L > Cell Detection
- Inspect Pixel value, Tune Intensity
- Change Cell Expansion

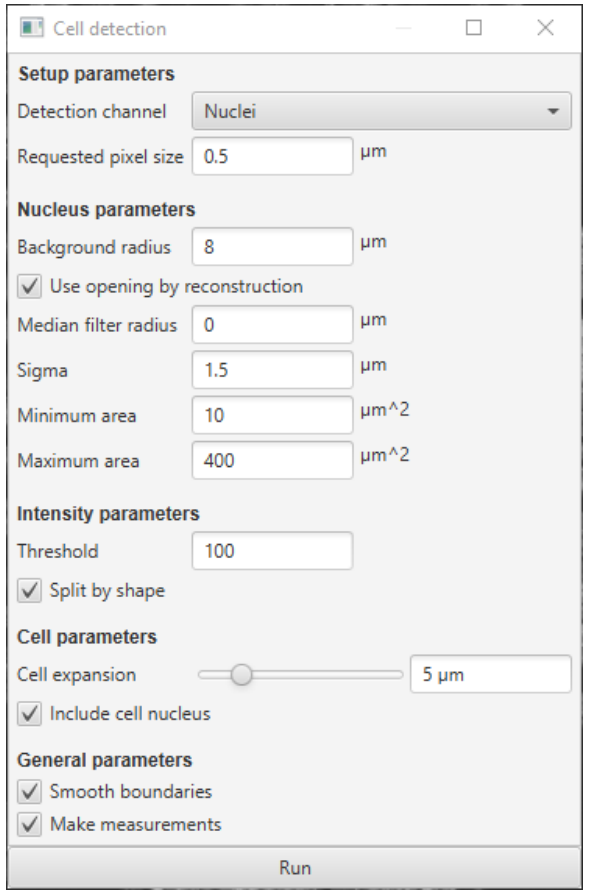
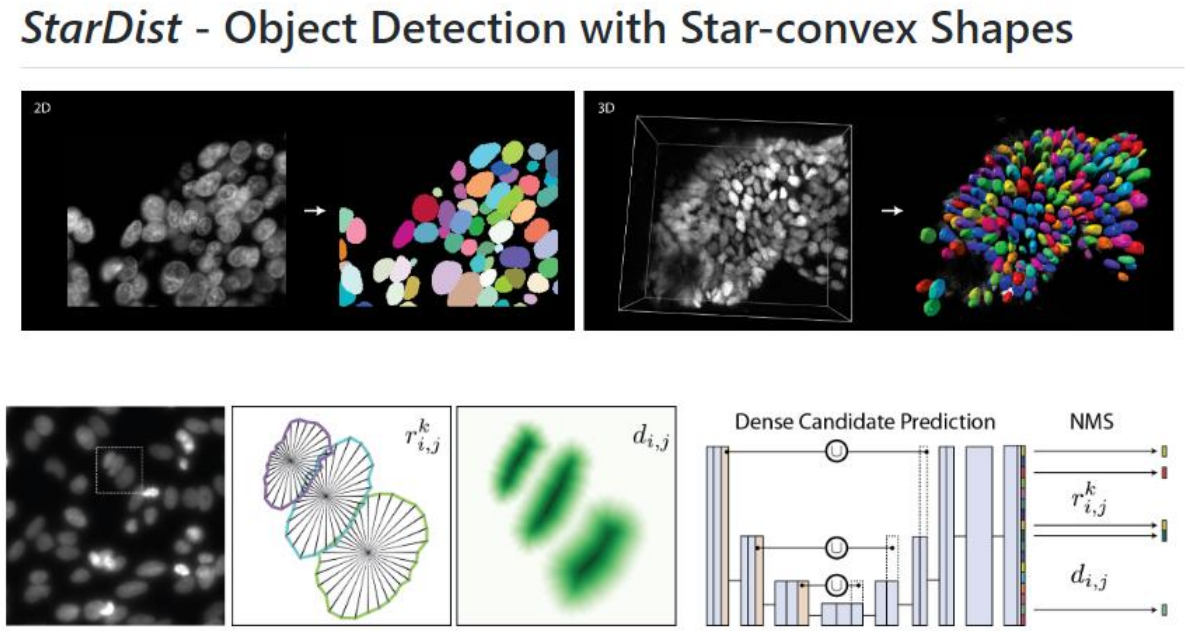


Image: C09

Nuclei Segmentation with StarDist

By: Uwe Schmidt, Martin Weigert



- Open source
- Fiji Plugin
- Out-of-the-box models for
  2D Fluorescent and H&E images
- Training Notebooks available via StarDist and ZeroCostDL4Mic
- QuPath implementation: Extension, Running through script, Cell Expansion

Uwe Schmidt, Martin Weigert, Coleman Broaddus, and Gene Myers.
Cell Detection with Star-convex Polygons. MICCAI, 2018.

https://github.com/stardist/

Ex 3b: Segment Cells with StarDist

- Duplicate image
- Create annotation with diverse nuclei appearance
- ***RunStarDistOpenCV.groovy*** > Run
- Inspect segmentation: miss-detection, false detections, over/under segmentation
- Remember: cell expansion is just geometrical

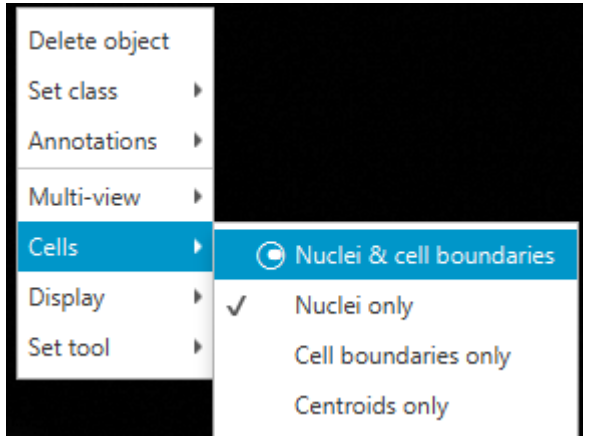
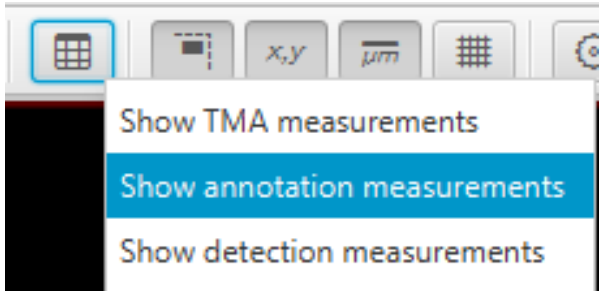


Image: C09

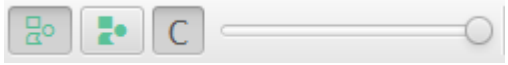
```
// Specify the model .pb file (you will need to change this!)
var pathModel = 'A:/shared/QuPathScriptsAndProtocols/QuPath_StarDistModels/dsb2018_heavy_augment.pb'

def NucChannel = 'Nuclei'
def PixelSize = 0.2485
def CellExpansion = 5
```

Ex 4: Inspect Mesasurments

- Double-click on a cell
- Annotation Table: **How many cells**?
- Detection Table: Click to find related cell, Sort, Histograms
- Measurement Maps



Annotations and Detections

Annotations	Detections
Created by user, or algorithm	Created by algorithm
Usually for large region of interest	Usually something small: cell or nucleus
Usually no more than few hundred	Often hundred of thousands (or millions)
Flexible	Efficient
Can be edited, Manually, Expand, Fill, …	Cannot be edited, only deleted
Can include annotations and detections	
Measurments	Measurements List (Shape, Intensity, Distance)
Show Names, Fill (Shift+'f') 	Fill ('f'), Density, Measurements Maps 

Relation between objects: Object Hierarchy (Resolve Hierarcy)

Other Objects: TMA

Possible to convert Annotations ↔ Detections

Ex 5: Classify Cells - Single measurement Classifier

- Create Single measurement classifier for CD8, Ki67
- Try different *Measurement* and *Threshold*
- Use: Live Preview, ‘c’, transparency
- Save the classifiers
- Apply to whole image: Create Full Image Annotation, Run StarDist, Load Classifier, Apply Sequentially
- Inspect classification:

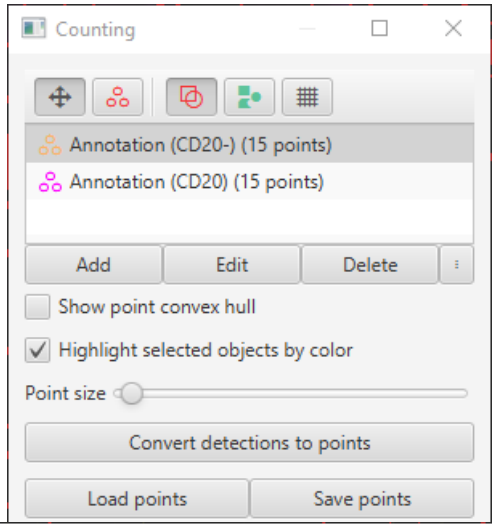
  Are all positive cells identified? Look for Double positive

  How many cells in each class ?

Image: C09

Ex 6: Classify Cells - Supervised Learning Classifier

- Annotate classes with the Point Tool
- Use Shortcuts when annotating
- Train Object Classifier
- Save intermediate points and classifiers
- Live Update, Iterative



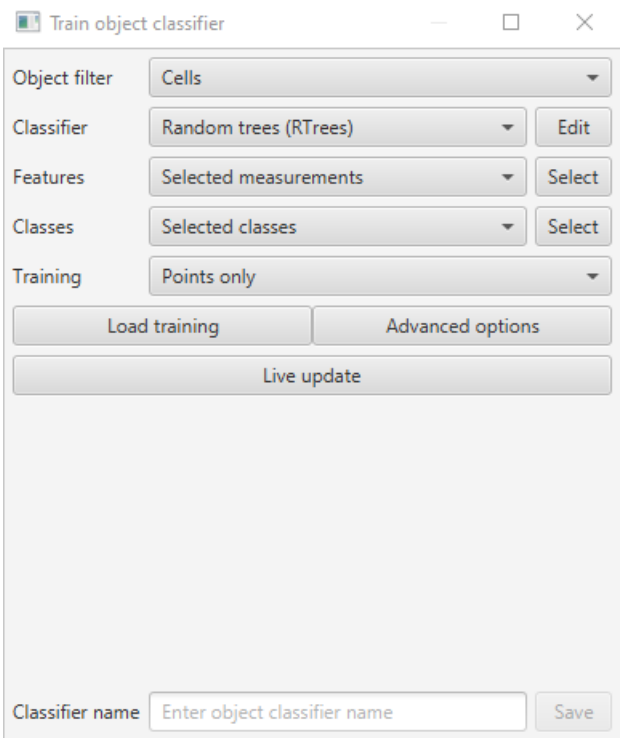


Image: C09

Classes

- Classes provide information about Object
- Used to distinguish regions of interest for data quantification and categorize segmentation results
- Used for training classifiers
- Objects can each have one class
- But the Class can be combined: CD8-Positive:Ki67-Positive
- ‘null’ indicates ‘unclassified’
- Ignored* classification, end with *, in some circumstances they are ignored (not used for creating objects, not counted)
- Provide a way to select objects and independently Show/hide them
- Can be populated from channels & objects

Ex 7: Segment Regions with Pixel Classifier

- Goal: Segment *Follicles* and *Epithelial* regions
- Duplicate the image: XX_ForTraining
- Display only the *Nuclei*, *Ki67* and *PanCK* channels
- Add Classes
- Draw annotations, use *AutoSet* while Annotating
- Train pixel Classifier
- Live Prediction
- Explore channels, scales, features
- Save Classifier
- Switch to original image, Load Classifier
- Create Objects, Choose values for Min object sSize, Min Hole Size

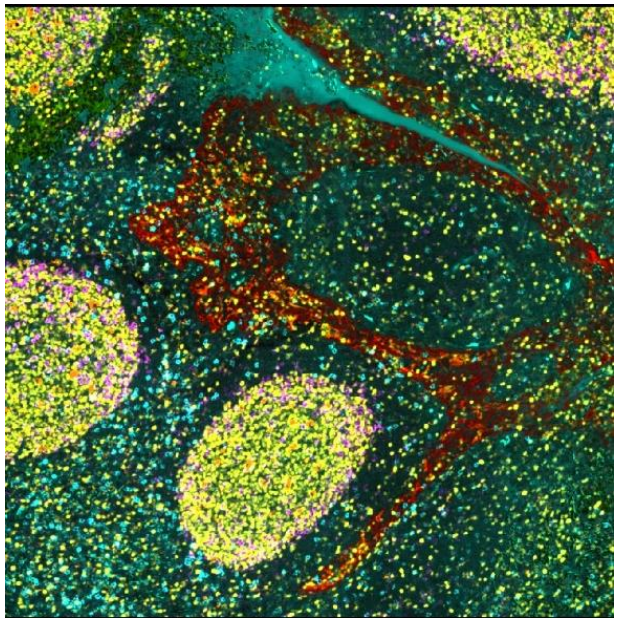


Image: C06

Ex 8: Combine Regions and Cells Analysis

- Create Full image Annotation
- Run StarDist
- Apply your cell classifier of choice

- How many Cells within Epithelial / Follicle regions?
- What is the percentage of cells of class X in each region?
- How many Cells are within 50 um around the Follicle?
- What is the distance between cells and specific regions?
- Create Cell Density Map - for all cells , for class X only

Image: C06

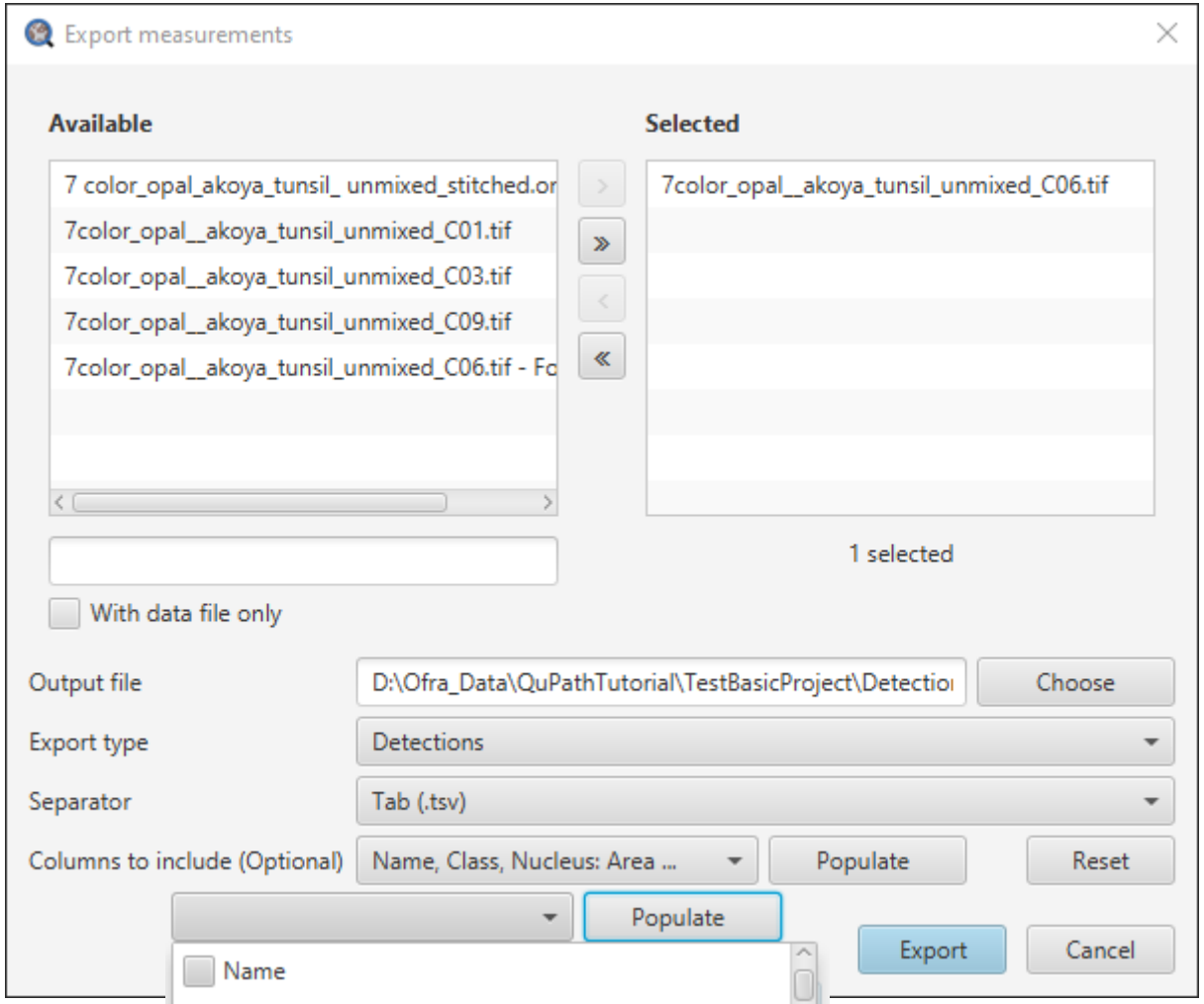
Ex 9: Export Results

- Export Measurements
- Select the measurement type to be exported
- Use Populate to select the columns to include

Use script to export annotations and detections as **labeled images**

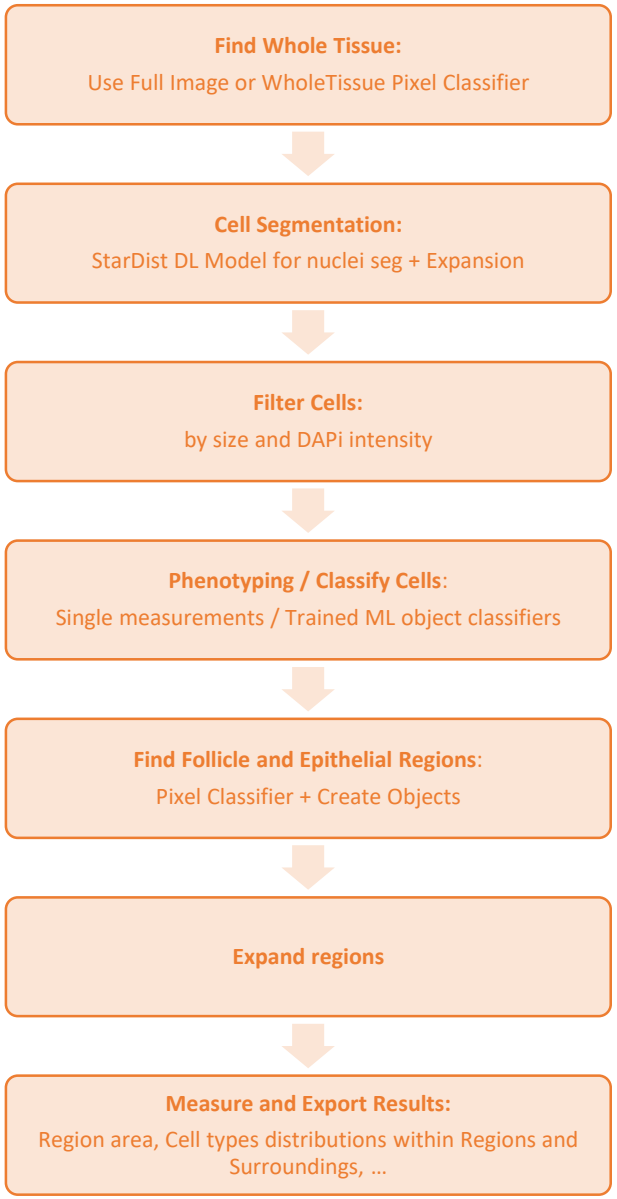
ExportObjectsAsLabelImage.groovy

Image: C06



Ex 10: Apply Workflow for the Whole Project

- Script: ***FullAnalysis_ForProjects.groovy***
- Tune parameters:
  - Which steps to perform
  - Class Names
  - Classifiers Names
  - Minimal Object Size …
- Run on single image — **Image: C03**
- Run > Run For Project — **Images: C01, C03, C09**



Ex 10: Apply Workflow for the Whole Project

```
// Script Operation Parameters
def UseFullImageAnnotationInsteadOfSelectedClass = 1

def RunWholeTissueClassifier = 1
def deleteExistingObjects = 1
def runStarDist = 1
def filterSomeCells = 1
def classifyCells = 1

def RunPixelClassifier       = 1
def runAnnotationExpansion    = 1
def calcDistanceToAnnotations = 1
def exportLabelImages        = 1

// Parameters for Whole Tissue Selection
def ClassToSelect           = "WholeTissue"
def WholeTissueClassifierName = "WholeTissueFinder"
def MinWholeTissueSize      = 200000 // um^2
def MinWholeTissueHolesize   = 10000 // um^2

// StarDist based Cell Segmentation parameters

// Specify the model .pb file (you will need to change this!)
def pathModel = 'A:/shared/QuPathScriptsAndProtocols/QuPath_StarDistModels/dsb2018_heavy_augment.pb'

def NucChannel = 'Nuclei'
def ProbabilityThreshold = 0.5
def PixelSize          = 0.2485  // Resolution for detection, make sure to set it to the Pixel Size of your data
def CellExpansion       = 5.0 // pixels

// Further cell filtering parameters
def MaxNucArea       = 300   // um^2
def MinNucArea       = 10    // um^2
def MinNucIntensity   = 20     // remove any detections with an intensity less than or equal to this value

// Cell Classification Parameters
def CellClassifierName = 'CD8_Ki67_CD20_ML_v1'

// Pixel Classifier Parameters
def PixelClassifier = "FollicleEpithelialFinder"
def Minimum_ObjectSize = 10000
def Minimum_LumenSize  = 2000

// Annotation expanstion parameters
def AnnotationClassToExpand = "Follicle"
def ExpandRadius_um = 50.0

// Results parameters
def ResultsSubFolder = 'export'
def downsample     = 1 // 10
def labelsSubFolder = "image_export"
def outputSuffixCellLabels       = "_CellLabels.tif"
def outputSuffixAnnotationsLabels = "_AnnotationLabels.tif"
```

Find Whole Tissue:
Use Full Image or WholeTissue Pixel Classifier

↓

Cell Segmentation:
StarDist DL Model for nuclei seg + Expansion

↓

Filter Cells:
by size and DAPi intensity

↓

Phenotyping / Classify Cells:
Single measurements / Trained ML object classifiers

↓

Find Follicle and Epithelial Regions:
Pixel Classifier + Create Objects

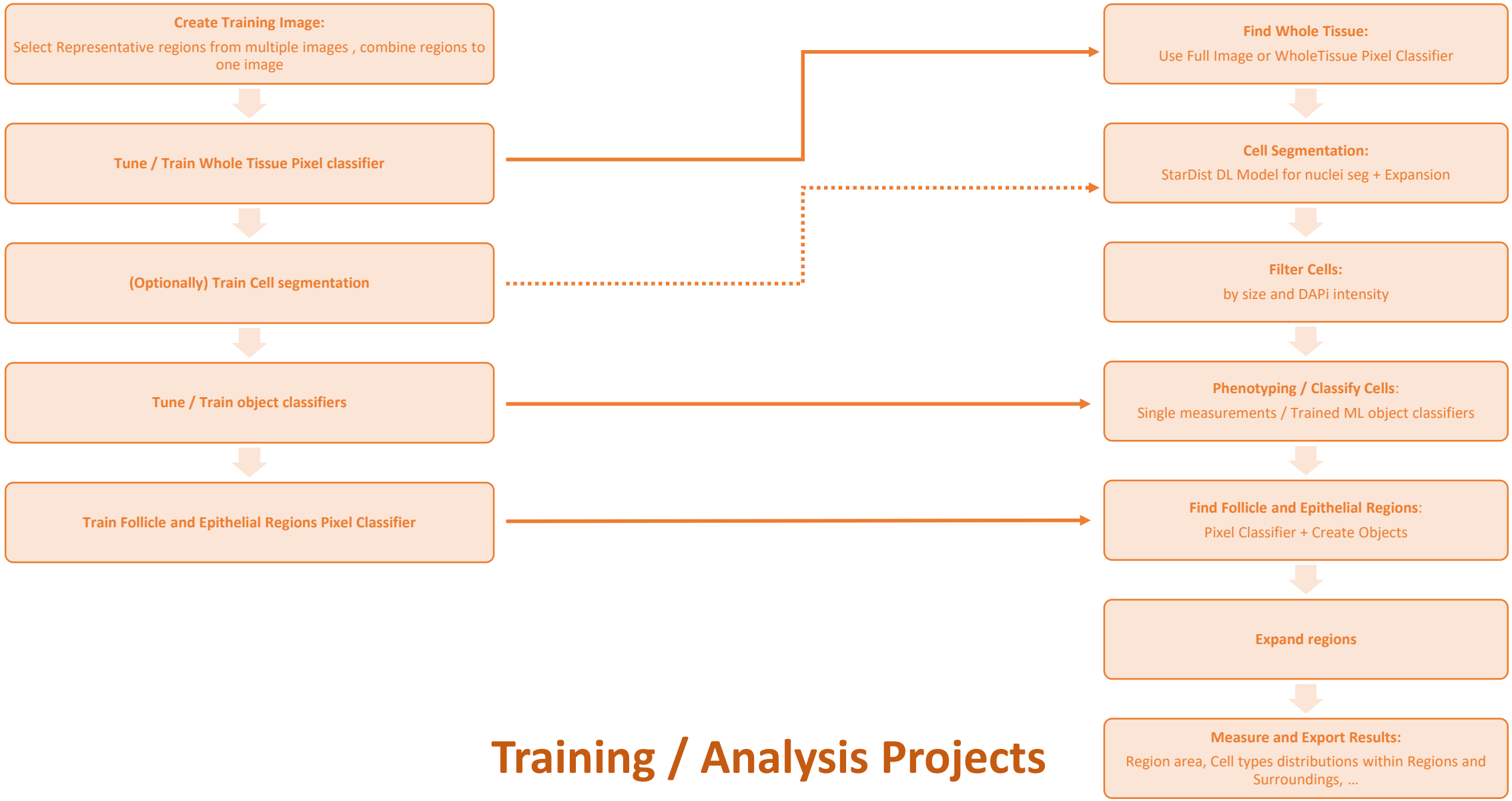
↓

Expand regions

↓

Measure and Export Results:
Region area, Cell types distributions within Regions and Surroundings, …

Building Analysis Workflows in QuPath



Training / Analysis Projects

Building Analysis Workflows in QuPath

Duplicate projects: 1) for classifier training 2) analysis

Training Project

- **Select representative regions from multiple images**
- **Combine the regions into Training Image**
- **Train different classifiers on duplicates of the Training image to avoid annotations overlap**

Analysis Project

- Typically use a script to apply all steps of the desired workflow using the classifiers trained on the Training Project
- Apply to multiple images / all project

Ex 11: Create Training Image

- Create Training Project, load images
- For each image
  - *Create Region annotation*, 'Unclassified'
  - Multiple representative regions
  - Preferred size – should enable to understand the context
- *Create Training Image*: Combine all regions into one image
- On the new "Mosaic" image – create full image annotation
- Segment regular cells
- Duplicate the image for each classifier you want to train
- Train object Classifier:
  - Single-class or combined
  - **Don't annotate on the borders between different regions**
  - Remember to save intermediate annotations + classifiers
- Copy classifier(s) to Analysis project
- Tune the analysis script and apply ***FullAnalysis_ForProjects.groovy***

BYOD

Short Presentations & Discussion

Which objects ?

What to Measure ?

What should be the workflow ?

Which module suites for each step ?

BYOD Session: Bring Your Data Data

Which objects ?

What to Measure ?

What should be the workflow ?

Which module suites for each step ?

- Separate into Training and Analysis projects
- Tune / Train Classifiers on representative regions from multiple images and conditions
- Train each classifier on different copy of the training
- Copy the classifiers to the analysis project
- Tune the Analysis script for your own workflow and classifiers

Thank You

Resources

Image.sc Forum: QuPath
https://forum.image.sc/tag/qupath

ReadTheDocs Documentation
https://qupath.readthedocs.io/en/latest/

Youtube Tutorials
https://www.youtube.com/channel/UCqepVnS1QsB7B8nBA9T91EQ/playlists

Dr. Pete Bankhead
MRC Institute of Genetics and Molecular Medicine
University of Edinburgh

Olivier Burry, Nicolas Chiaruttini, Romain Guiet, Arne Seitz
The EPFL BIOP group

The QuPath Community on image.sc forum, especially:
Zbigniew Mikulski,
Mike Nelson
Mark Zaidi
Anonymous **Research Associate**