

Whole Slide Image (WSI) Analysis with QuPath

Tutorial



EMPAIA Academy Hands-On Workshop – ECDP2022

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Whole Slide Image (WSI) Analysis with QuPath



Objectives:

- 1. learn how to get started with the open-source software QuPath
- 2. train QuPath to distinguish tumor / non-tumor
- 3. use QuPath for Ki67 Analysis
- 4. apply the trained algorithm to other regions of interest



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(A) Set-Up Project in QuPath

(1) Download & Install QuPath
(2) Create Project & Add Whole Slide Images
(3) View & Explore WSI

(1) Download, Install and Open QuPath

- Download & install the QuPath Software suitable for your operating system from <u>https://qupath.github.io/</u>
- 2. After successful installation, you can find the QuPath Icon on the desktop:



(2) Create Project in QuPath and Add Images

1. Create Project:

"File" > "Project" > "Create project"



- 2. Add WSI to Project:
 - a. "File" > "Project" > "Add images"
 - b. drag&drop Ki67 image to pop-up window, and set image type "Brightfield (other)"

🍭 QuPath



Set image type	•
Rotate image	Brightfield (H-DAB)
Optional args	Brightfield (H&E)
Auto-genera	Brightfield (othry)
Import object	Fluorescence
Choose files	Other
Choose line.	Not set
	Import Abbrechen

(3) View & Explore WSI in QuPath

1. Open WSI

(double-click on image name in the image list)

🍳 QuPath	
File Edit Tools View Objects TMA Measure Automate	Analy
S + C / C V 2 * & S	
Project Image Annotations Hierarchy Workflow	
Create project Open project Add images	
Image list	
 myQuPath-ECDP-WS/project.qpproj (2) 	
DigitalSlide_1.svs	
DigitalSlide_2.svs	
	QuPath File Edit Tools View Objects TMA Measure Automate File Edit Tools View Objects TMA Measure Automate Froject Image Annotations Hierarchy Workflow Create project Open project Add images Image list myQuPath-ECDP-WS/project.qpproj (2) DigitalSlide_1.svs DigitalSlide_2.svs

 Explore the image: scroll to zoom in and out... press left mouse key to drag the image...

(B) Train Classifier for Tumor/Non-Tumor

- (1) Make ROI with Region*
- (2) Annotate Tumor Areas within that region
- (3) Annotate Non-Tumor Areas within that region
- (4) SELECT that annotation
- (5) Train Classifier

(1) Annotate Region of Interest

- 1. Step 1: Select Annotation-Class "Region*":
 - > 1. click on "Annotations"
 - > 2. click on "Region*"
 - > 3. click on "Auto set"
- 2. Step 2: Select Annotation Tool: (e.g. Rectangle)



QuPath - 3.svs File Edit Tools View Objects TMA Measure Automate Analyze Classify Project Image Annotations Hierarchy Workflow Annotation (Rectangle) (Region Tumor Stroma Immune cel Necrosis Othe Region* I lanore Positive Negative Non-Tumor

3. Step 3: Draw Annotation to mark Region of Interest

(2) Annotate Tumor Areas

 Step 1: Select Annotation-Class "Tumor": > 1. click on "Annotations"

- > 2. click on "Tumor"
- > 3. click on "Auto set"
- 2. Step 2: Select Annotation Tool: (e.g. Polygon or Bru



3. Step 3: Draw Annotations to Mark Tumor Areas



🍭 QuPath - DigitalSlide_1.svs



(3a) Create Annotation Class "Non-Tumor"



(3b) Annotate Non-Tumor Areas

- 1. Step 1: Select Annotation-Class "Non-Tumor"
 - > 1. click on "Annotations"> 2. click on "Non-Tumor"> 3. click on "Auto set"
- 2. Step 2: Select Annotation Tool: (e.g. Polygon or Brush)

3. Step 3: Draw Annotations to Mark non-tumor Areas







(3d) Train Classifier

- 1. Select the Region of Interest
- 2. Step 2: Select "Train Pixel Classifier"

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1	Object cla	assification	Þ	8 2	с —
_	Pixel class	ification	٠	Load pixel cla	assifier
	Training i	mages	۲	Train pixel cla	assifier Ctrl+Shift+P
				1	S

- 1. Step 3: Configure Pixel Classifier
 - a. set Resolution to "Moderate"
 - b. set Region to "Everywhere"
 - C. click on "Live prediction" (and wait for result)
 - d. use the slider to adjust the transparency of the result visualisation so that you can check the plausibility of the result

 \rightarrow if the result is not good yet, try another resolution (e.g. "Extremely low" ...)

2. Step 4: Name and Save your Classifier

Pixel classifier

Classifier	Artif	ficial neu	ral network (ANN_MLF	?)	_	-	Edit				
Resolution	Mod	derate (4.	00 µm/px)		а		-	Add			· ····	
Features	Defa	ault multi	scale feature	25	-	E	Edit	Show		×.,	10	5
Output	Clas	sification	l				-	Show	1	e f	100 m	12
Region	Ever	ywhere		b				*	1 Par	ST.	AL.	
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Classifier na	ame	Enter na	me					Save	Show c	assific	ation	
Me	asure		Create ob	jects		Class	ify	:	_)—	d	-

Objects Edit Tools View TMA Mea 0 S V D (4) Automatic Tumor Annotations Project Image Annotations Hierarchy Workflow Annotation (Polygon) (Stroma) None Tumor (6) Annotation (Polygon) (Stroma) Stroma (4) 🕅 Annotation (Geometry) (Stroma) Step 1: Select Region of Interest from Annotations' list Immune cells 🗞 Annotation (Polygon) (Tumor) Necrosis Annotation (Polygon) (Tumor) Other Step 2: In Pixel classifier window select "Create objects" 2. Annotation (Polygon) (Tumor) Region* (1) 🗱 Annotation (Geometry) (Tumo Ignore* 3. In pop-up windows choose parent object 🛤 Annotation (Geometry) (Strom: Positive Negative Annotation (Polygon) (Tumor) Pixel classifier X "Current selection" Annotation (Rectangle) (Regio Choose parent objects Current selection and object type "Annotation" Pixel classifier Abbrechen Artificial neural network (ANN MLP) Classifier Edit 4. \rightarrow find automatic annotation of Ŧ Resolution Very low (7.76 µm/px Add detected tumor areas in the Create objects \times Default multiscale featu Features Edit Show Annotations' list New object type Annotation Classification Show Output um^2 Minimum object size 0 Region Any annotations QuPath - DigitalSlide 2.svs µm^2 Minimum hole size 0 Load training Advanced options Edit Tools View Objects TMA Measure Automate Analyze Classify Extensic Split objects Live prediction U D / C V ~ 1 S Delete existing objects 0.89x Create objects for ignored classes Project Image Annotations Hierarchy Worflow -Set new objects to selected Annotation (Polygon) (Stroma) None Classification: Strop Tumor (5) Abbrechen Annotation (Polygon) (Stroma) OK Classifier name tumor-cla Save Stroma (3) 🔧 Annotation (Polygon) (Tumor) Immune ce Measure Create objects Classify Annotation (Polygon) (Tumor) Necrosis 🔷 Annotation (Polygon) (Tumor) Other 🗱 Annotation (Geometry) (Tumor) Region* (1 M Annotation (Geometry) (Stroma) Ignore* Annotation (Polygon) (Tumor)

Positive

QuPath - DigitalSlide 2.svs

(C) Ki67 Analysis

(1)Select/Define Area for Analysis(2)Detect Positive Cells

ad (C) Ki67 Analysis: Background Information

Ki-67 is a commonly used indicator of cellular proliferation in cancer



Ki67_index [%] = [number of positively stained tumor cells] [total number of tumor cells]

(1) Select/Define Area for Analysis

 in the Annotations' list select the annotation of a tumor area (e.g. the annotation of the automatically detected tumor areas)



(2) Ki67 Analysis: Detection of Positive Cells

2. from the QuPath menu select "Analyze">"Cell detection">"Positive cell detection"



3. in the "Positive cell detection" pop-up window click on "Run"

4. Wait for the result...

Positive cell detection			(2	\times
Setup parameters					
Detection image	Hematoxylin OD				*
Requested pixel size	0.5	μm			
Nucleus parameters					
Background radius	8	μm			
Median filter radius	0	μm			
Sigma	1.5	μm			
Minimum area	10	µm^2			
Maximum area	400	µm^2			
Intensity parameters					
Threshold	0.1				
Max background intensity	2				
✓ Split by shape					
Exclude DAB (membrar	ne staining)				
Cell parameters					
Cell expansion	-0		5 µm		
Include cell nucleus					
General parameters					
✓ Smooth boundaries					
 Make measurements 					
Intensity threshold paran	neters				
Score compartment	Nucleus: DAB OD	mean			-
Threshold 1+	-0		0.2		
Threshold 2+	_0		0.4		
Threshold 3+			0.6		
✓ Single threshold	\frown				
	Run	3	3		

(3) Ki67 Analysis Result

4. Find the result

QuPath - DigitalSlide_2.svs Edit Tools View Objects TMA Measure Automate Analyze File 0 8 2 00 4 V S 0 Project Image Annotations Hierarchy Work 🝷 🚧 Annotation (Geometry) (Tumor) (None Tumor 💊 Annotation (Polygon) (Tumor) (14) Strom: Annotation (Rectangle) (Region*) Immur Annotation (Polygon) (Stroma) Annotation (Polygon) (Tumor) Delete Select all Key Value Image DigitalSlide_2.svs Name Tumor Class Tumor Parent Image ROI Geometry Centroid X µm 7634.3482 5113.0687 Centroid Y µm Num Detections 62508 Num Negative 51986 Num Positive 10522 Positive % 16.833 1267.3066 Num Positive per mm^2 8302647.3819 Area µm^2 562593.7585 Perimeter µm

Classify

14.37x

(D) Apply Trained Algorithm to Other Region

- (1) Define another Region of Interest
- (2) Load Pixel Classifier
- (3) Cell Detection

(1) Define Region of Interest

- 1. In the Project's Image list double-click on the file name to open the respective WSI
- 2. Draw Region of Interest (e.g. rectangle annotation)



🎯 QuPath - DigitalSlide_1.svs
File Edit Tools View Objects TMA Mea
Project Image Annotations Hierarchy Work 🕤
Create project Open project Add images
Image list
 myQuPath-ECDP-WS/project.qpproj (2)
Digital Slide_1.svs
DigitalSlide_2.svs

(2) Load Pixel Classifier

- From the menu bar select "Classify">"Pixel classification">"Load pixel classifier"
- In the "Load pixel classifier" pop-up window choose the (previously trained) tumor classifier → result: classifier is applied and tumor areas are detected
- Create the annotation object for the detected tumor areas Step 3a: in the Annotations' list select the Region of Interest Step 3b: in the "Load pixel classifier" window click on "Create objects" Step 3c: in the pop-up window choose "Current selection" as parent object

Step 3d: select "Annotation" as new object type

→ result: find annotation for the detected tumor areas in the Annotations' list





Extensions

Object classification

vixel classification

Training images

Help

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(3) Cell Detection

- 1. In the Annotations' list select the previously generated annotation of the detected tumor areas
- 2. In the menu bar go to
 - "Analyse" > "Cell detection" > "Positive cell detection"



3. In the "Positive cell detection" window click on "Run" ...and wait for result...

Setup parameters			
Detection image	Hematoxylin (D	
Requested pixel size	0.5	μm	
Nucleus parameters			
Background radius	8	μm	
Median filter radius	0	μm	
Sigma	1.5	μm	
Minimum area	10	μm^2	
Maximum area	400	μm^2	
Intensity parameters			
Threshold	0.1		
Running			
Ce 735 nuclei detected	(61%)		
Ce 735 nuclei detected	(61%)	•	
Ce 735 nuclei detected	(61%) Cancel	1	
Ce 735 nuclei detected Ce Ge W Make measurements	(61%) Cancel	J	
Ce 735 nuclei detected Ge Make measurement: Intensity threshold par	(61%) Cancel	I	
Ce 735 nuclei detected Ge Make measurement: Intensity threshold par Score compartment	(61%) Cancel s rameters Nucleus: DAB	OD mean	
Ce 735 nuclei detected Ge Make measurements Intensity threshold par Score compartment Threshold 1+	(61%) Cancel s rameters Nucleus: DAB	OD mean	
Ce 735 nuclei detected Ge Make measurements Intensity threshold par Score compartment Threshold 1+ Threshold 2+	(61%) Cancel s rameters Nucleus: DAB	OD mean 0.2 0.4	
Ce 735 nuclei detected Ge Make measurement: Intensity threshold par Score compartment Threshold 1+ Threshold 2+ Threshold 3+	(61%) Cancel s rameters Nucleus: DAB	OD mean 0.2 0.4 0.6	

(4) Cell Detection Result = Ki67 Analysis Result

File

Image

Name

Class

Parent

ROI



Further Reading:

https://qupath.readthedocs.io/en/stable/docs/tutorials/cell_detection.html#

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