



# QuPath

*Open source software for analysing (awkward) images*

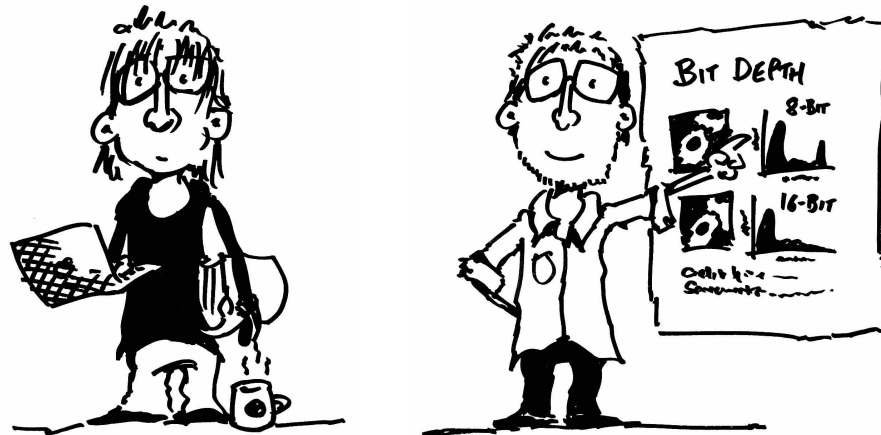
Peter Bankhead

Melvin Gelbard & Mahdi Lamb

University of Edinburgh

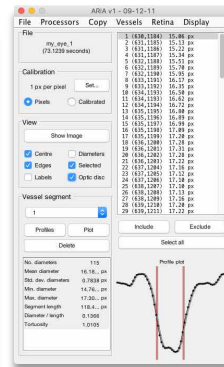
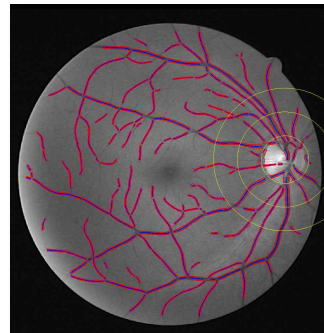
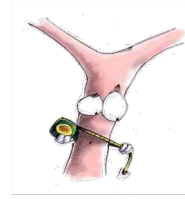
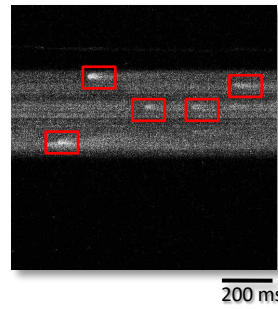
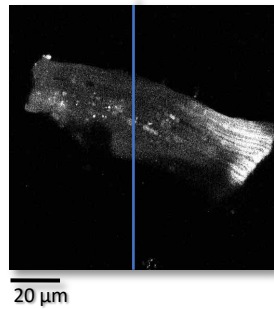
[p.bankhead@ed.ac.uk](mailto:p.bankhead@ed.ac.uk)

# Why *QuPath* exists

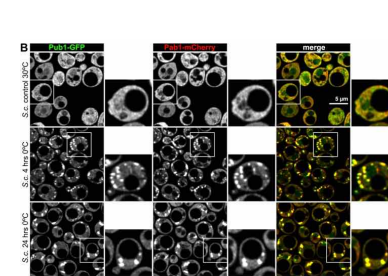


# My background is in bioimage analysis

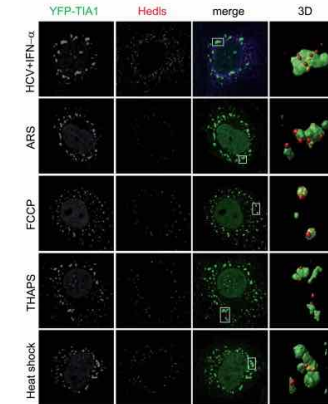
## Extracting information from microscopy & biomedical images



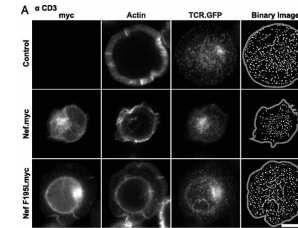
PhD @ Queen's University Belfast (2005 – 2009)  
Analyzing  $Ca^{2+}$  signals & retinal vessels



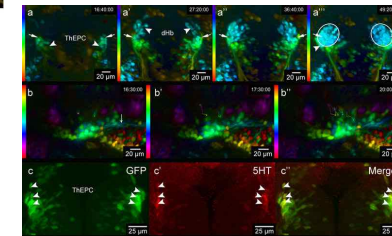
Hoffmann et al. *Mol Biol Cell.* (2012)



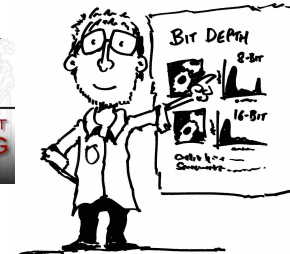
Ruggieri et al. *Cell Host Microbe.* (2012)



Abraham et al. *J Immunol.* (2012)



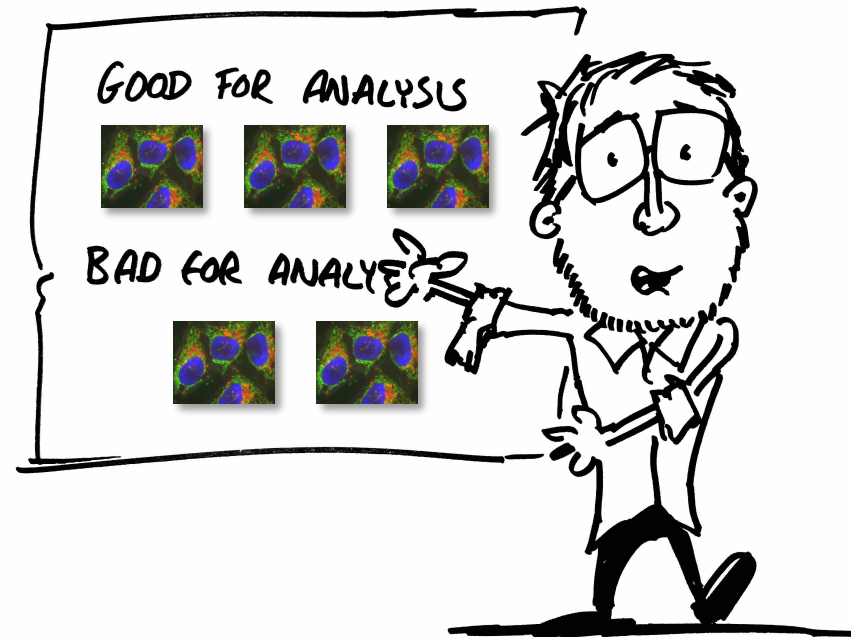
Beretta et al. *Neural Dev.* (2013)



Postdoc @ Heidelberg University (2010 – 2012)  
Image analysis specialist at Nikon Imaging Center

# Bioimage analysis is hard!

There is no right way to do it, but lots of wrong ways –  
*Understanding the key concepts is essential!*





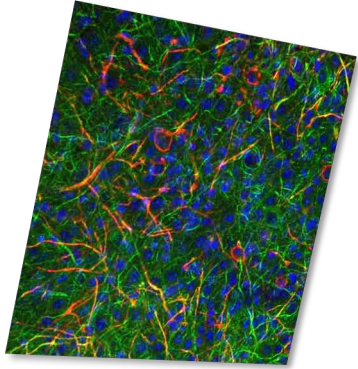


Even if you *do* know the concepts,  
*bioimage analysis is still hard!*

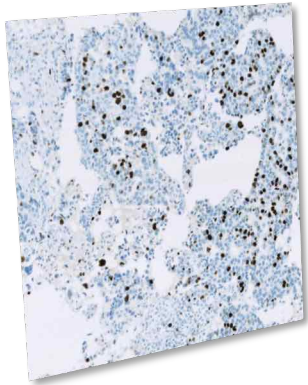


Almost nothing ‘just works’ –  
existing algorithms & software weren’t designed  
for the specifics of your case or mine

# Image analysis is often seen as a black box



Images go in

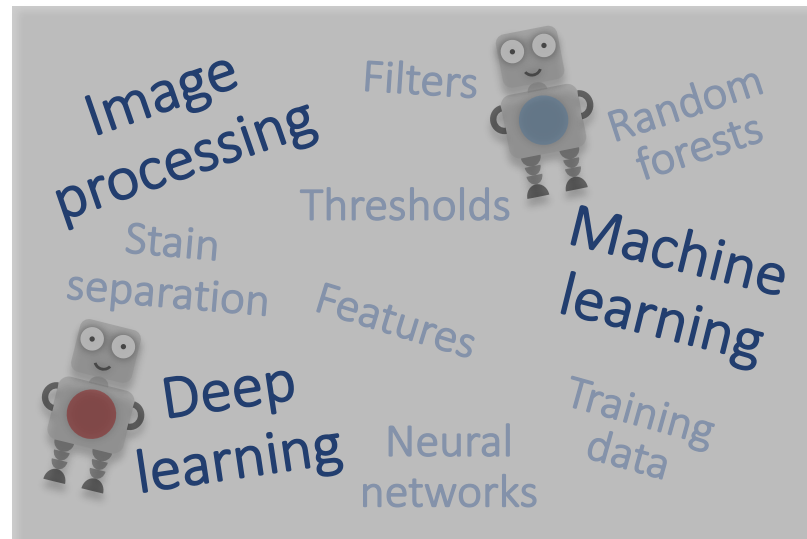


Stuff  
happens

Results come out

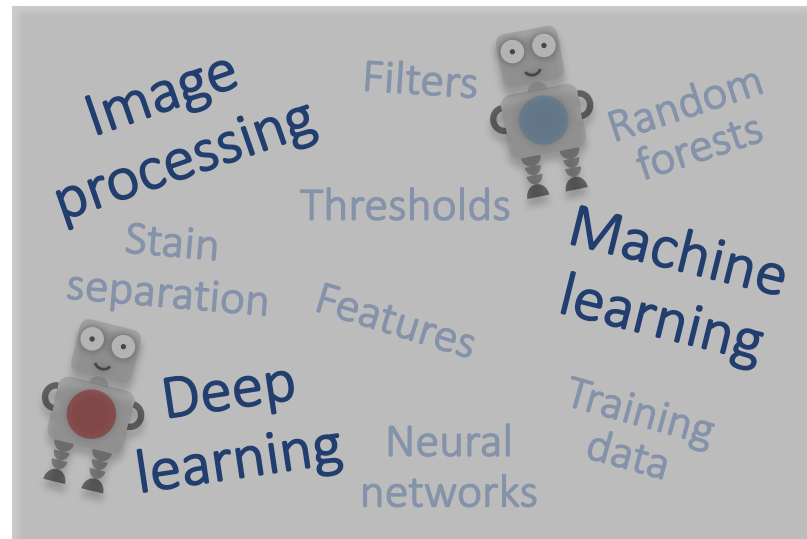
# Image analysis is often seen as a black box

The people who interpret the data need to understand what happens in the box...



# Image analysis is often seen as a black box

The people who interpret the data need to understand what happens in the box...



...which means we need transparent algorithms & software

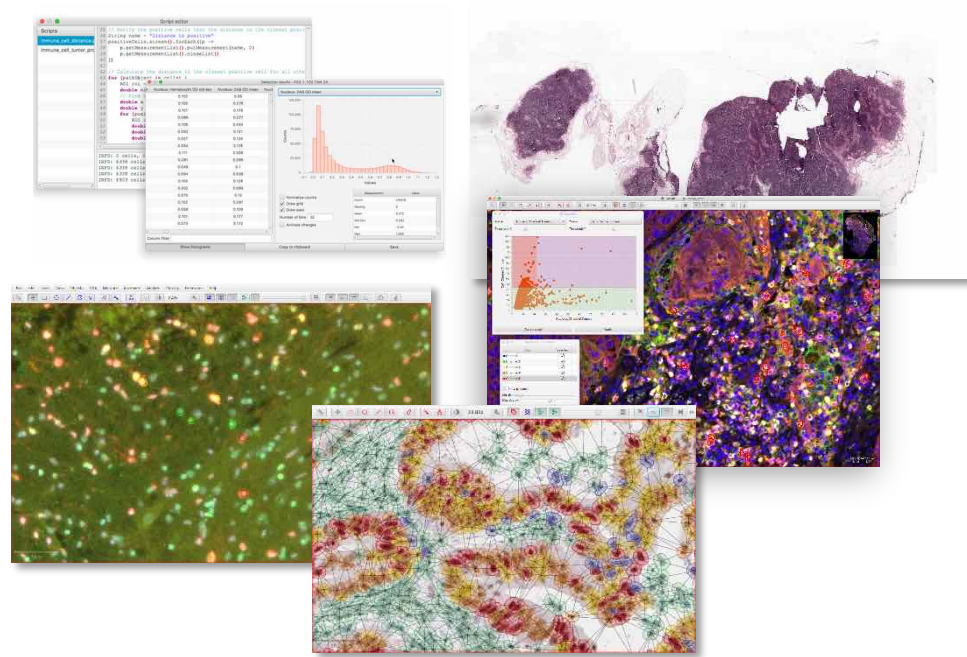
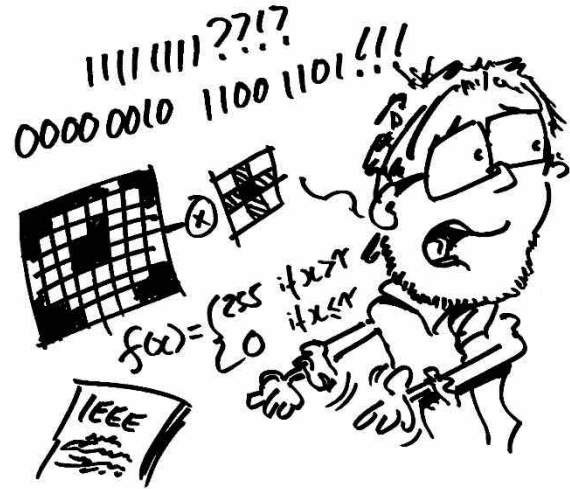


Doing bioimage analysis **effectively**  
requires communicating across disciplines





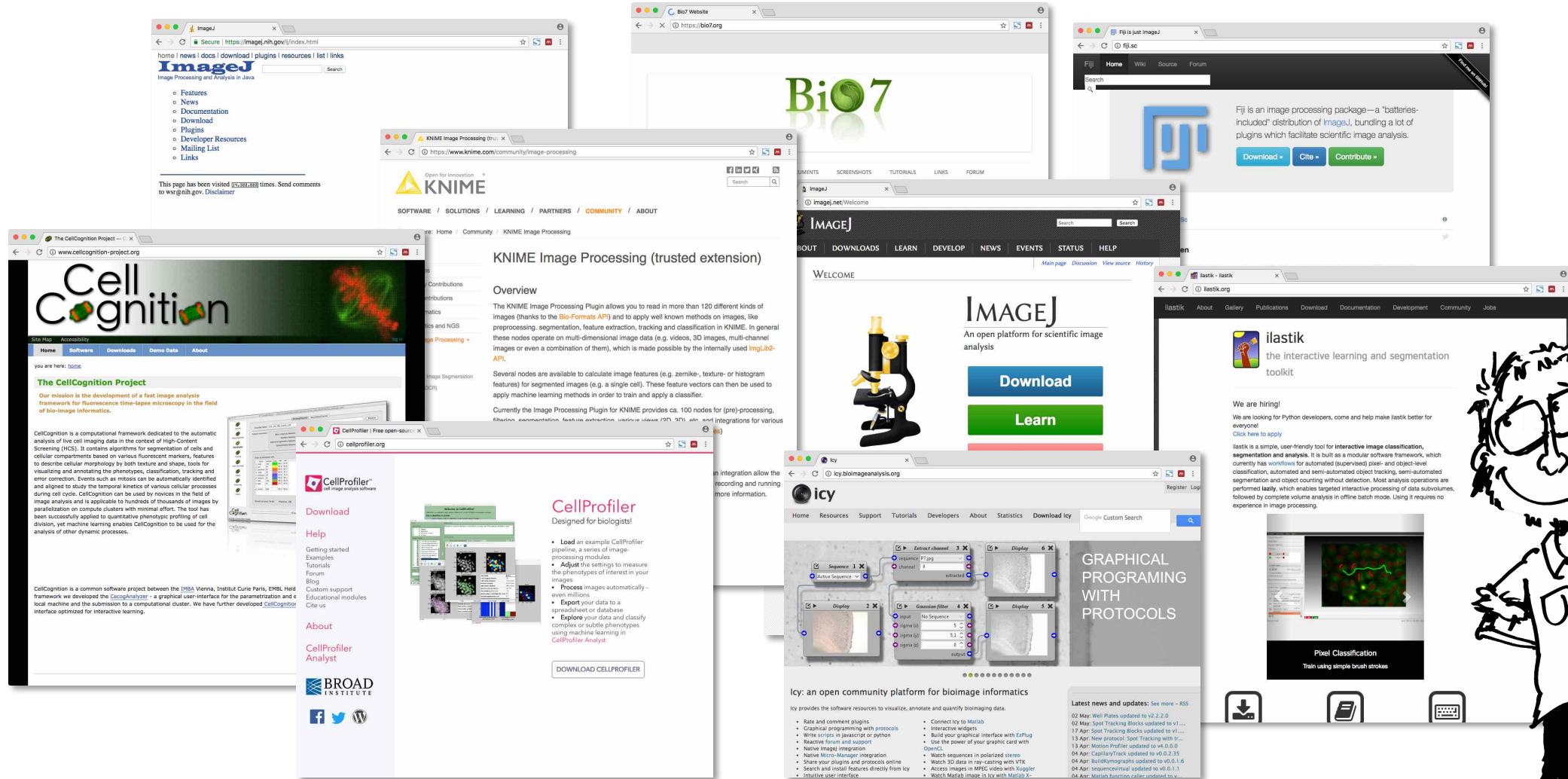
# Doing bioimage analysis **effectively** requires communicating across disciplines



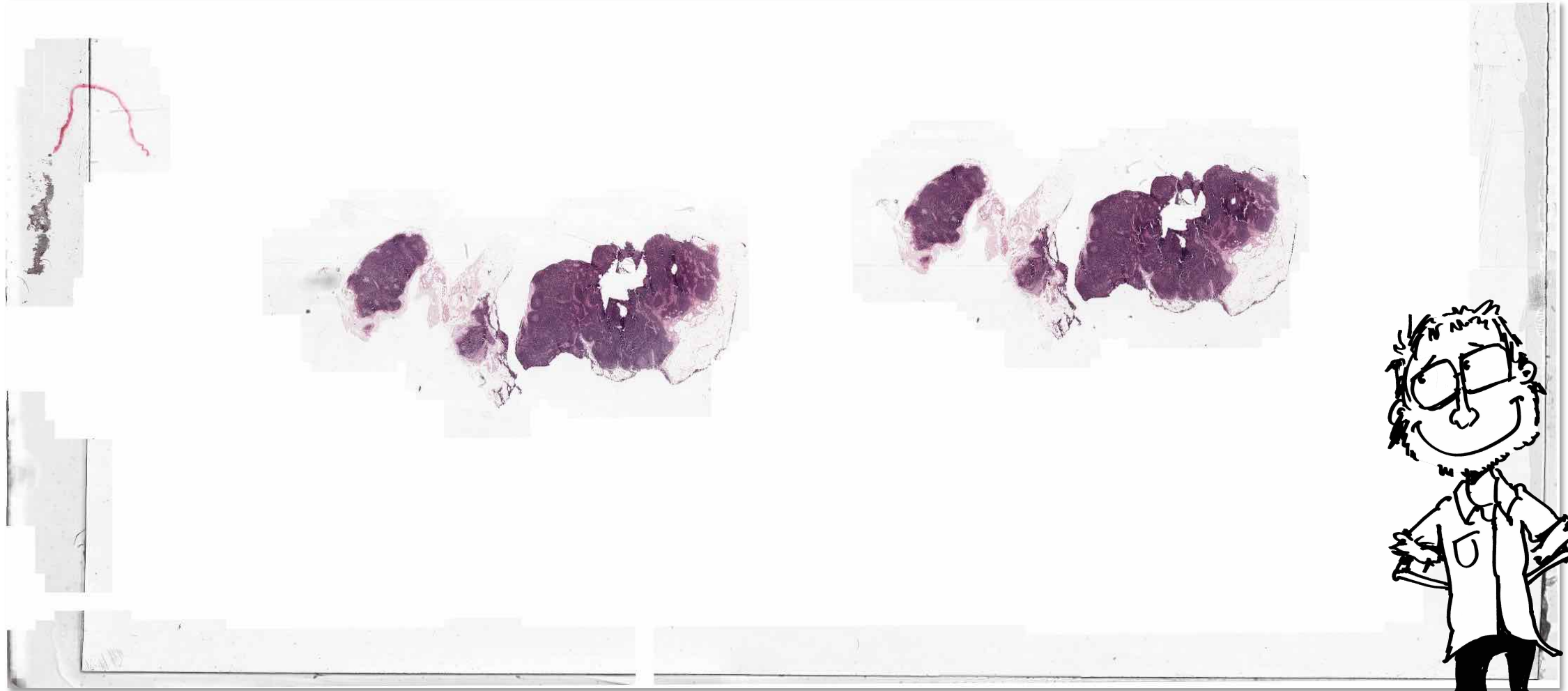
Clever algorithms are sometimes part of this – but these need software to make them accessible



# A fabulous ecosystem of open source bioimage analysis software makes this possible...

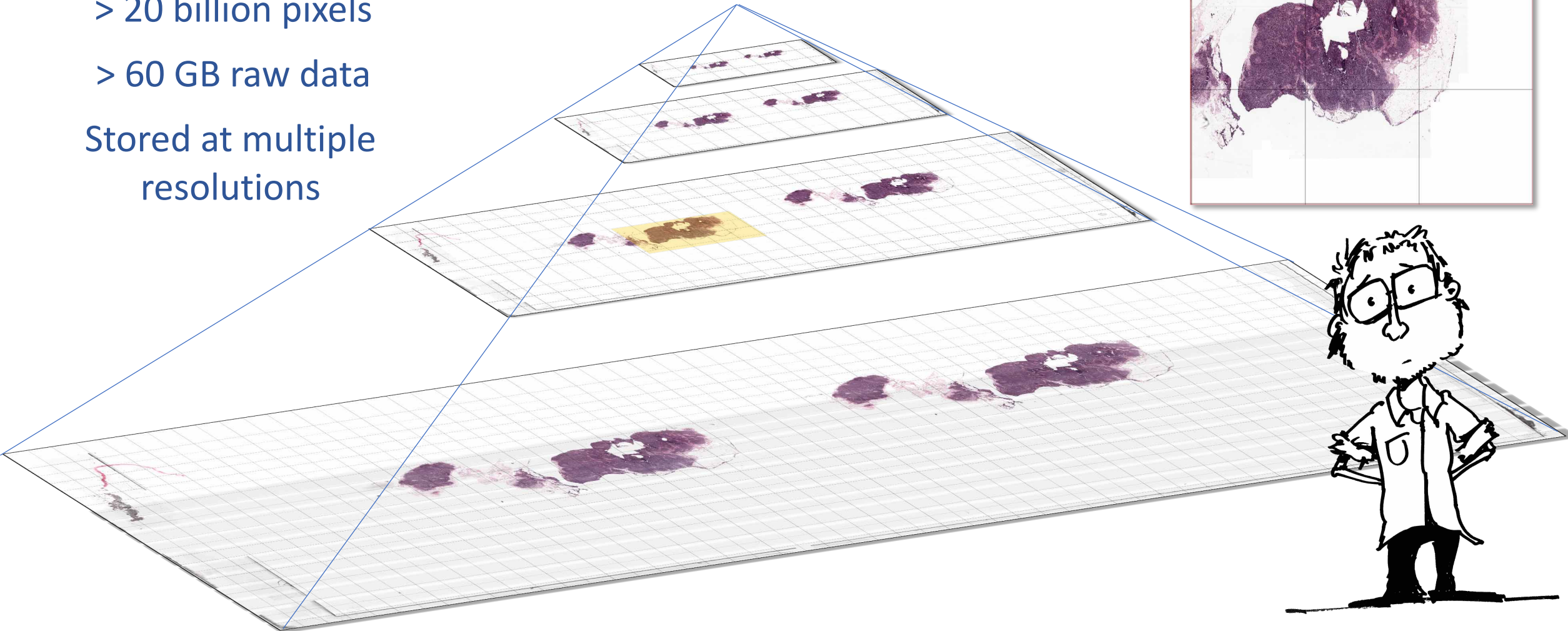
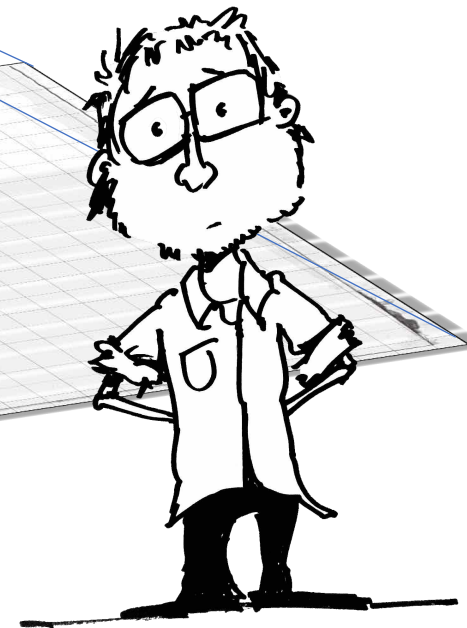
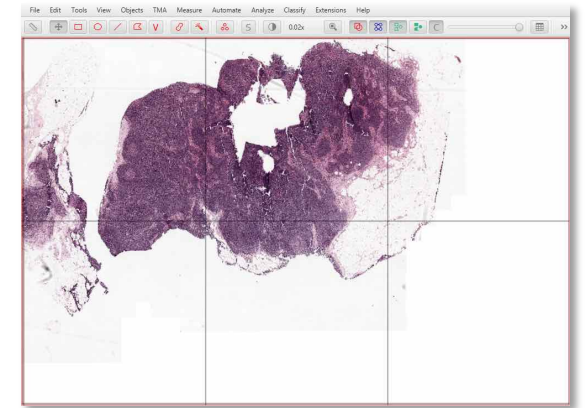


...but there can still be applications  
that require something new



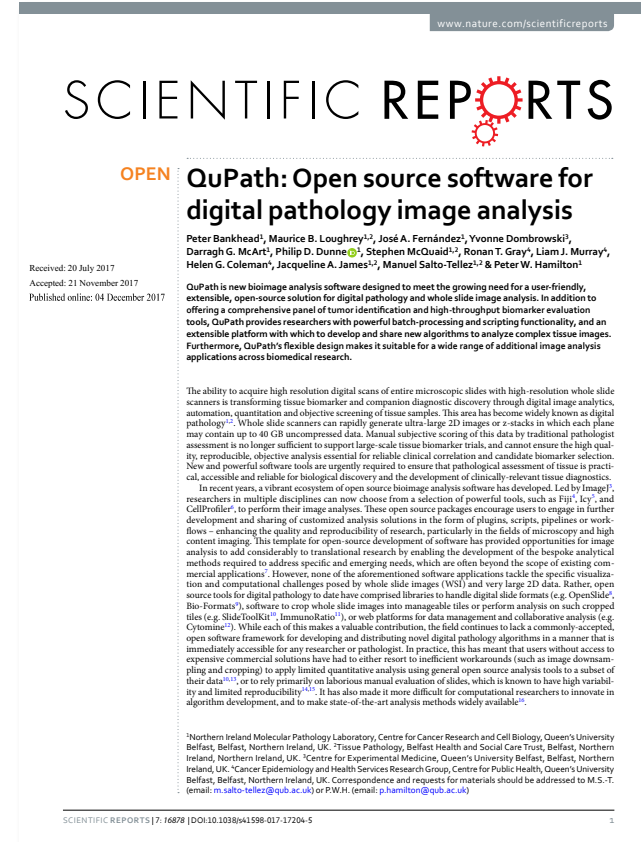
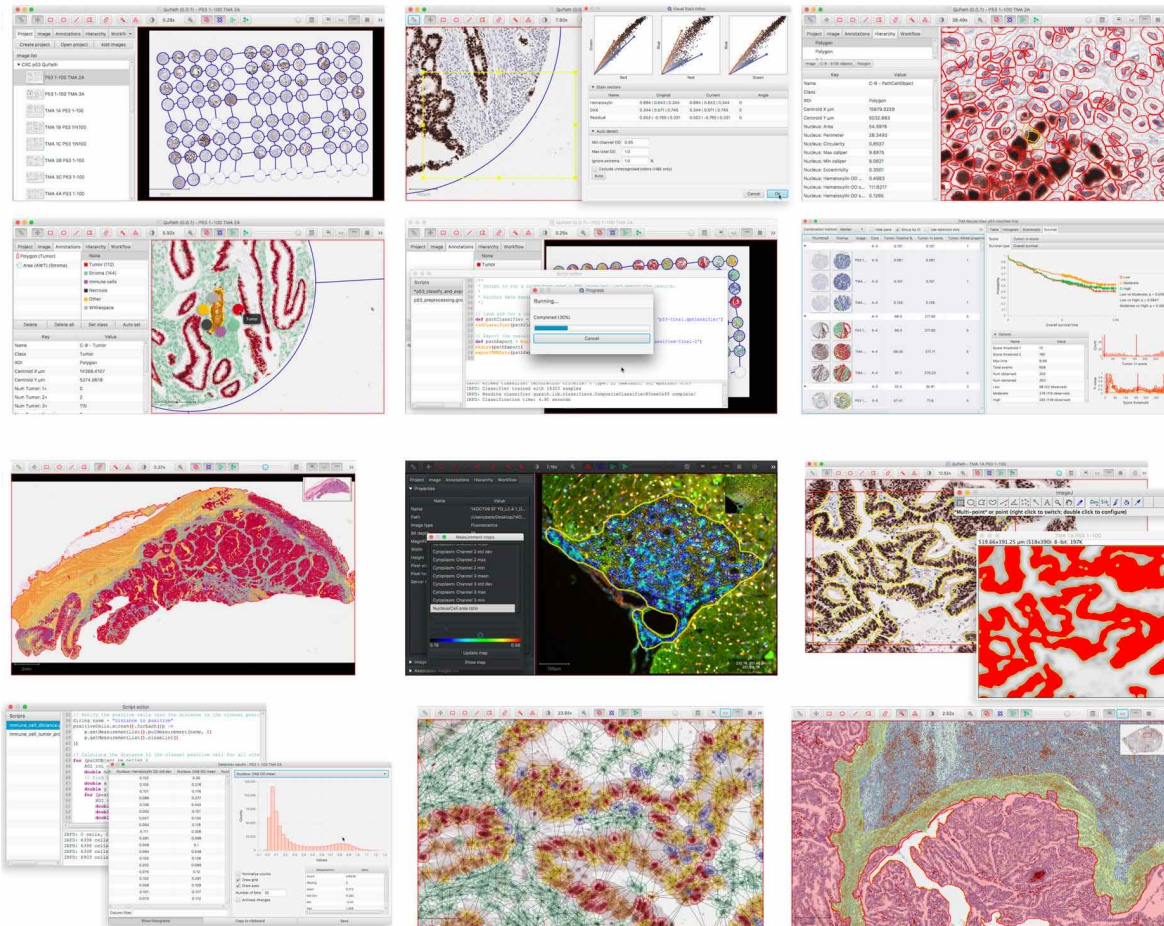
# Digital pathology requires specialised software designed to handle huge, complex images

- > 20 billion pixels
- > 60 GB raw data
- Stored at multiple resolutions





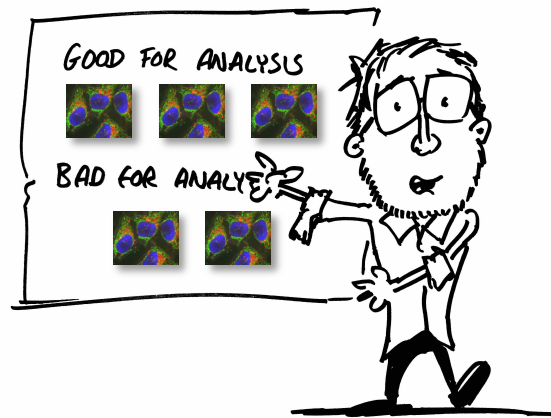
# QuPath exists to fill this gap: Open source software for whole slide analysis (and more)



Bankhead et al. *Sci Rep* (2017)

# *QuPath's* goal is to provide...

1. An open source platform for whole slide image analysis
2. New tools to address other bioimage analysis challenges

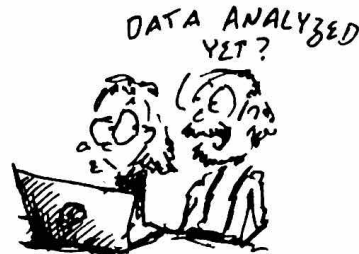


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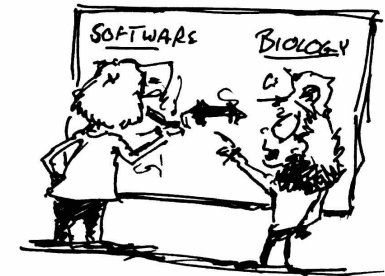
1. An open source platform for whole slide image analysis
2. New tools to address other bioimage analysis challenges



The hard part of analysis  
should be defining the question  
*(not wrestling with the software)*

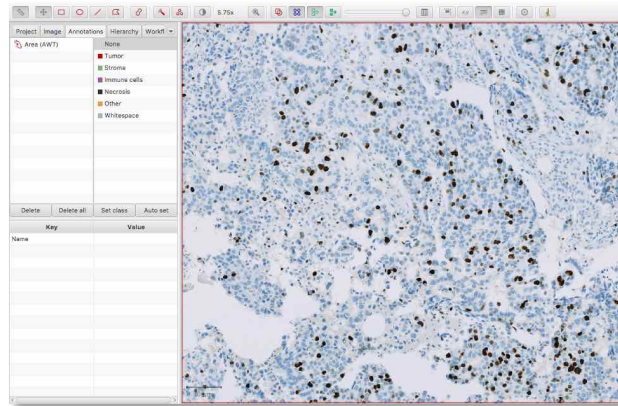


Analysis should be  
verifiable  
*(it's important to be correct)*

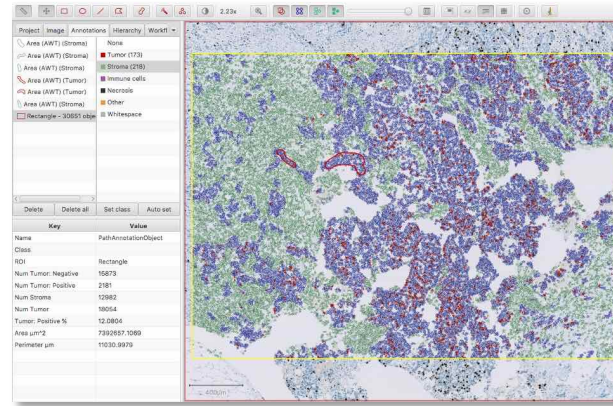


A single solution can  
solve many problems  
*(it's worth trying to do it well)*

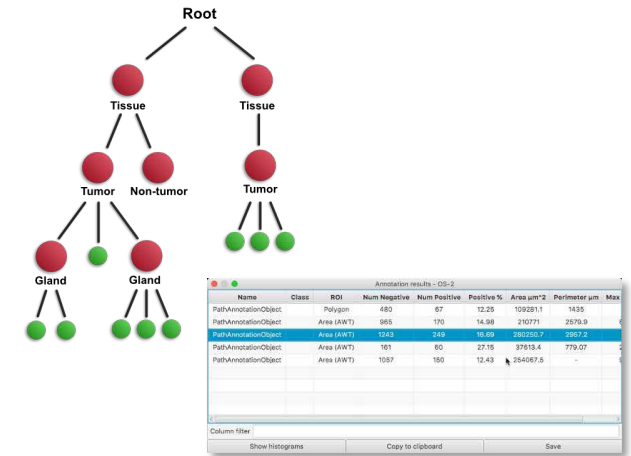
# QuPath's approach to image analysis



Start with pixels



Identify objects



Query the objects

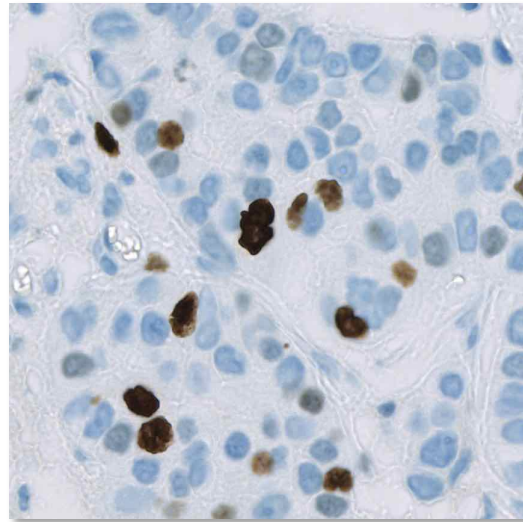
If your application fits with this model, you might find QuPath a good choice



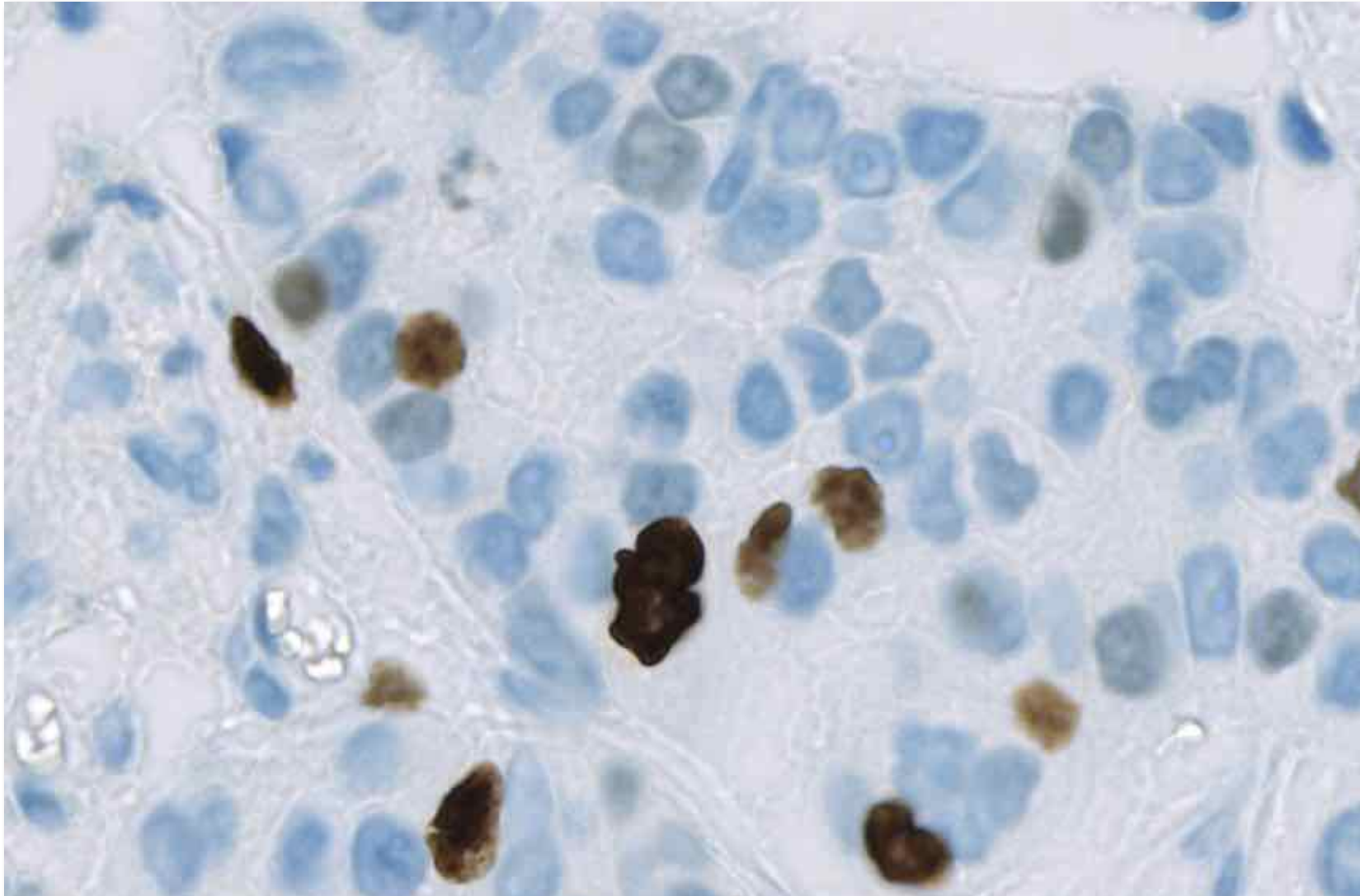
# How *QuPath* is used



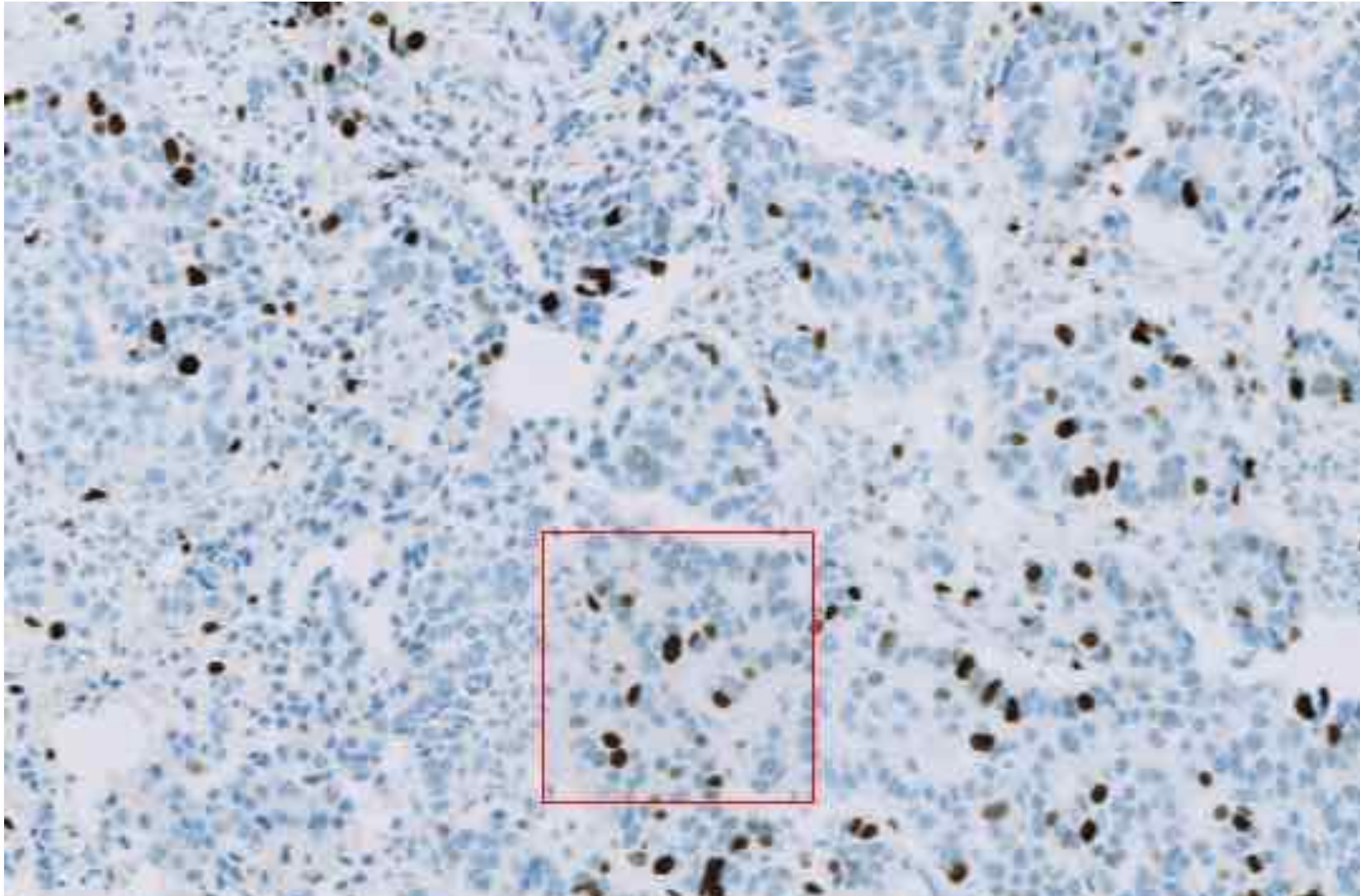
An easy problem in pathology –  
how many nuclei are brown?



An easy problem in pathology –  
how many nuclei are brown?

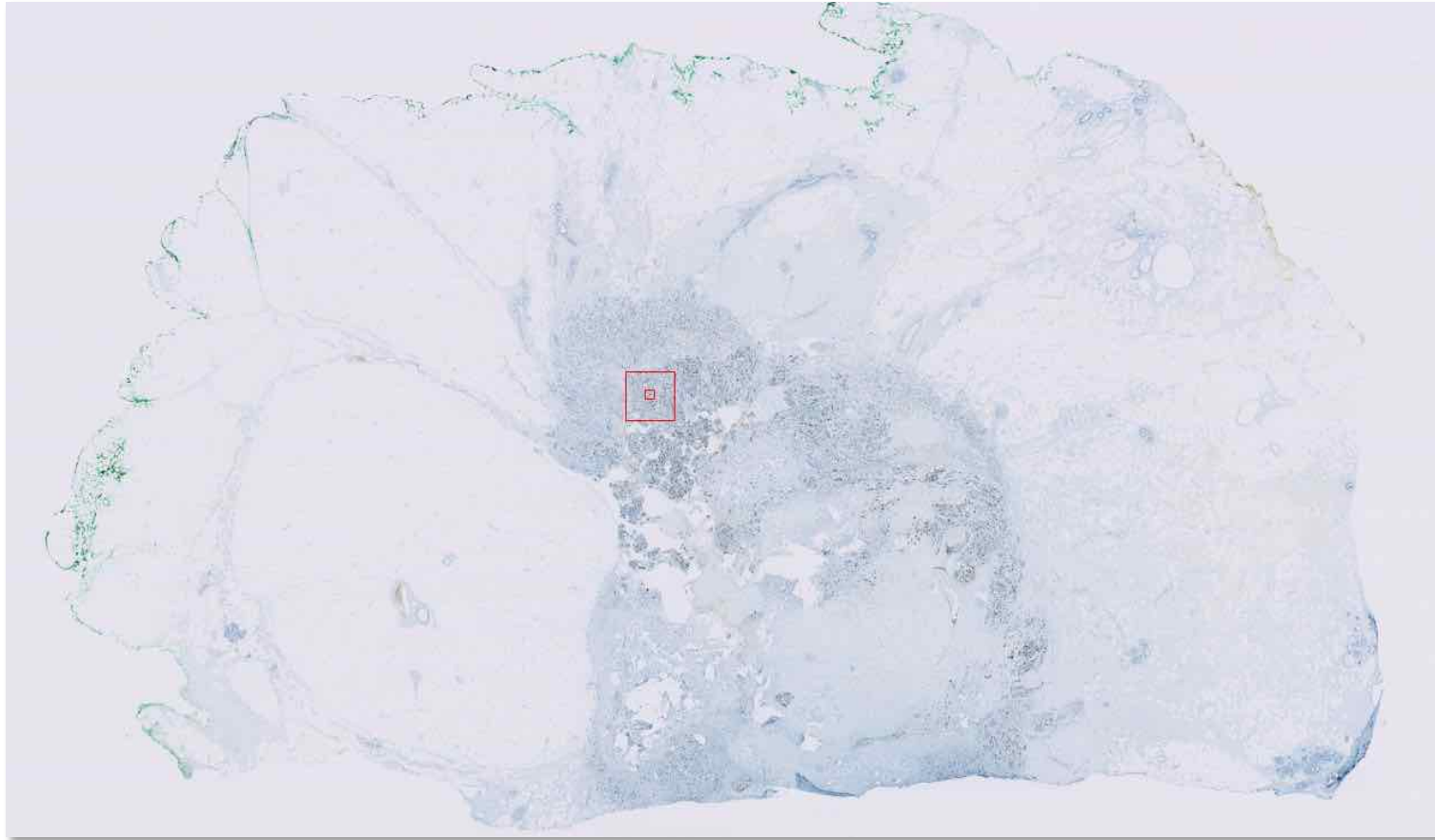


An easy problem in pathology –  
how many nuclei are brown?

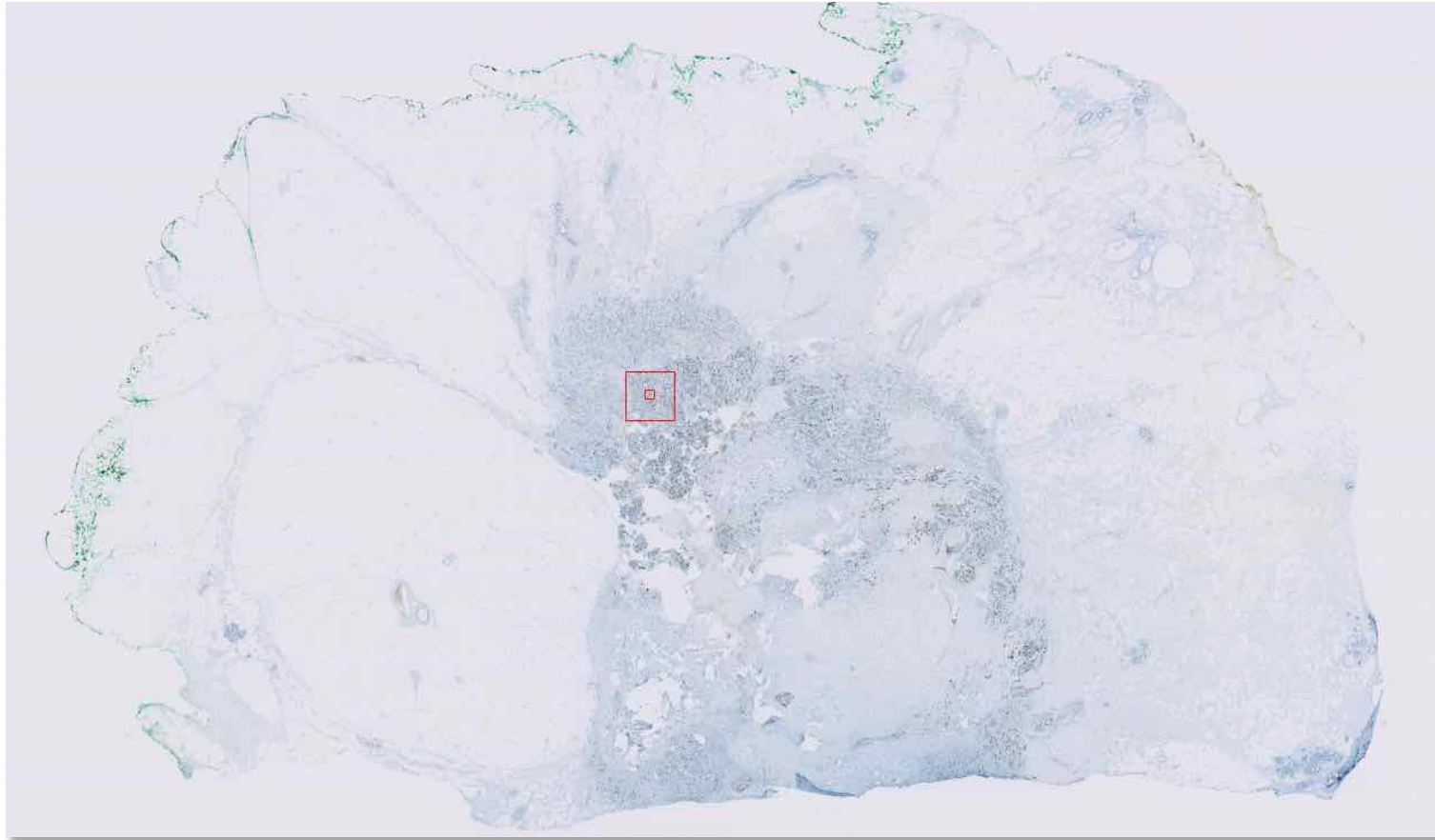




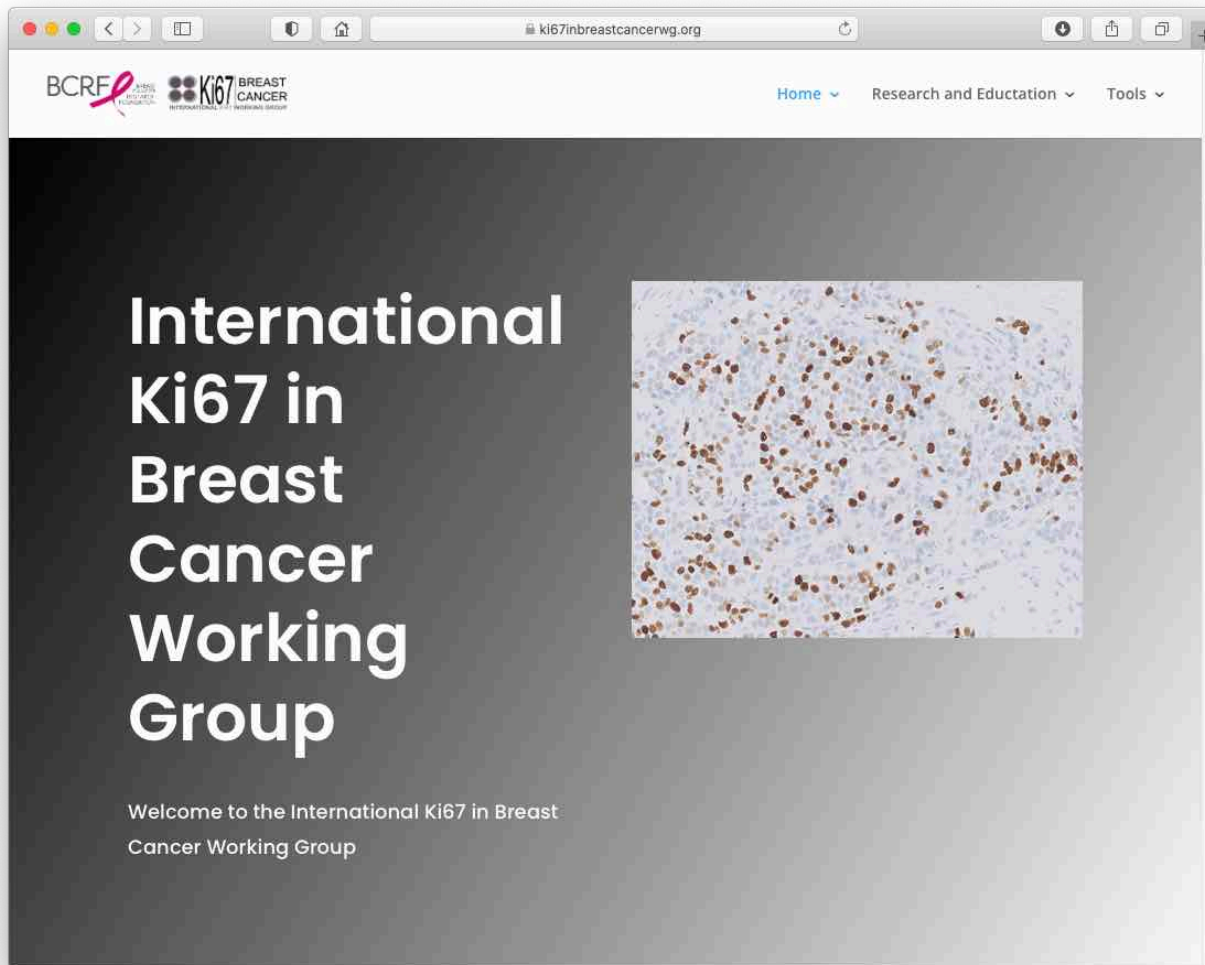
An easy problem in pathology –  
how many nuclei are brown?



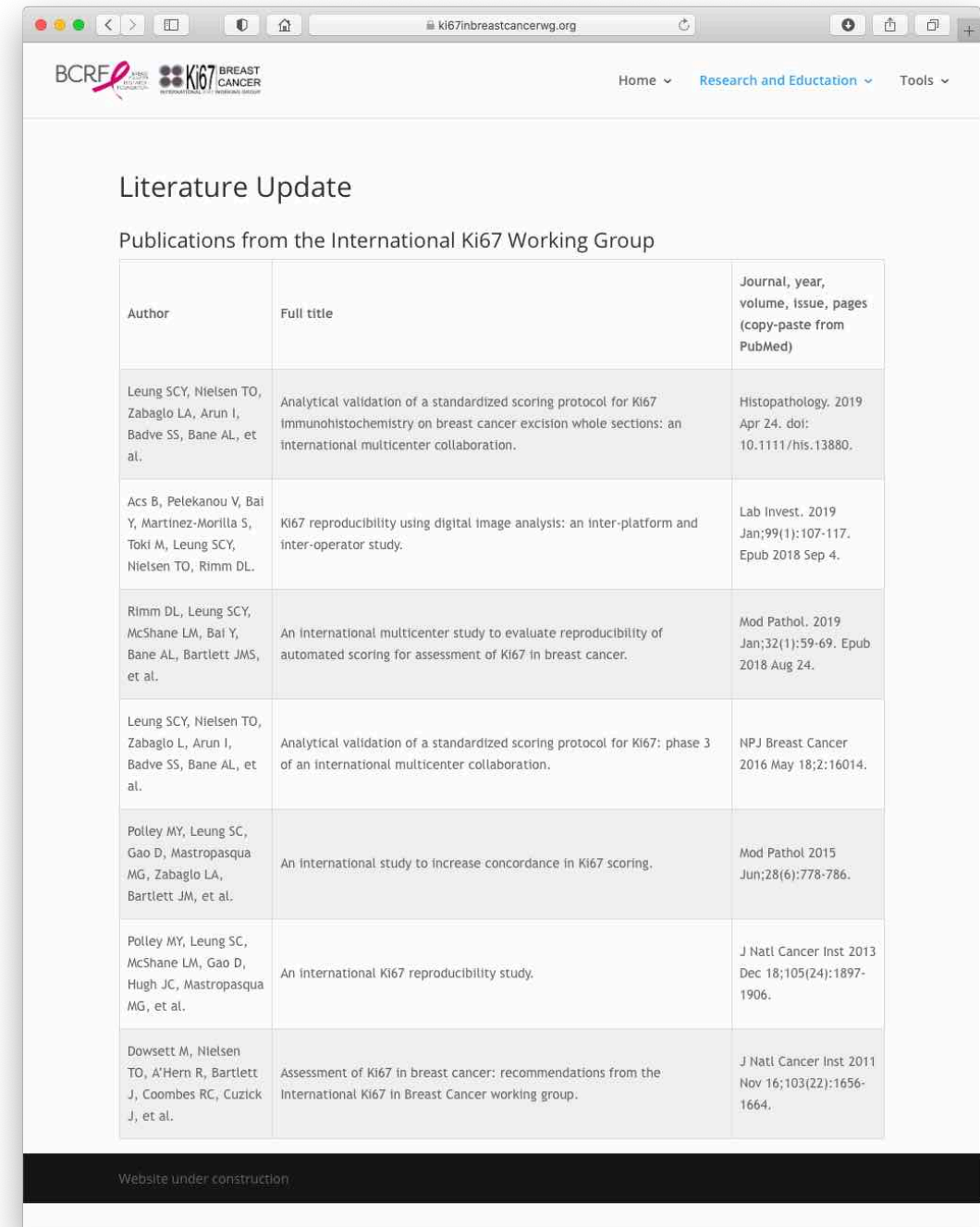
A *not so* easy problem in pathology –  
how many *tumour* nuclei are brown?



# Accurate & reproducible Ki67 scoring is hard!



The screenshot shows the homepage of the International Ki67 in Breast Cancer Working Group. The page features the BCRF and Ki67 Breast Cancer logos at the top. The main heading reads "International Ki67 in Breast Cancer Working Group". Below the heading is a large image of a breast cancer tissue section stained for Ki67, showing numerous brown-stained nuclei. At the bottom, a welcome message reads: "Welcome to the International Ki67 in Breast Cancer Working Group".



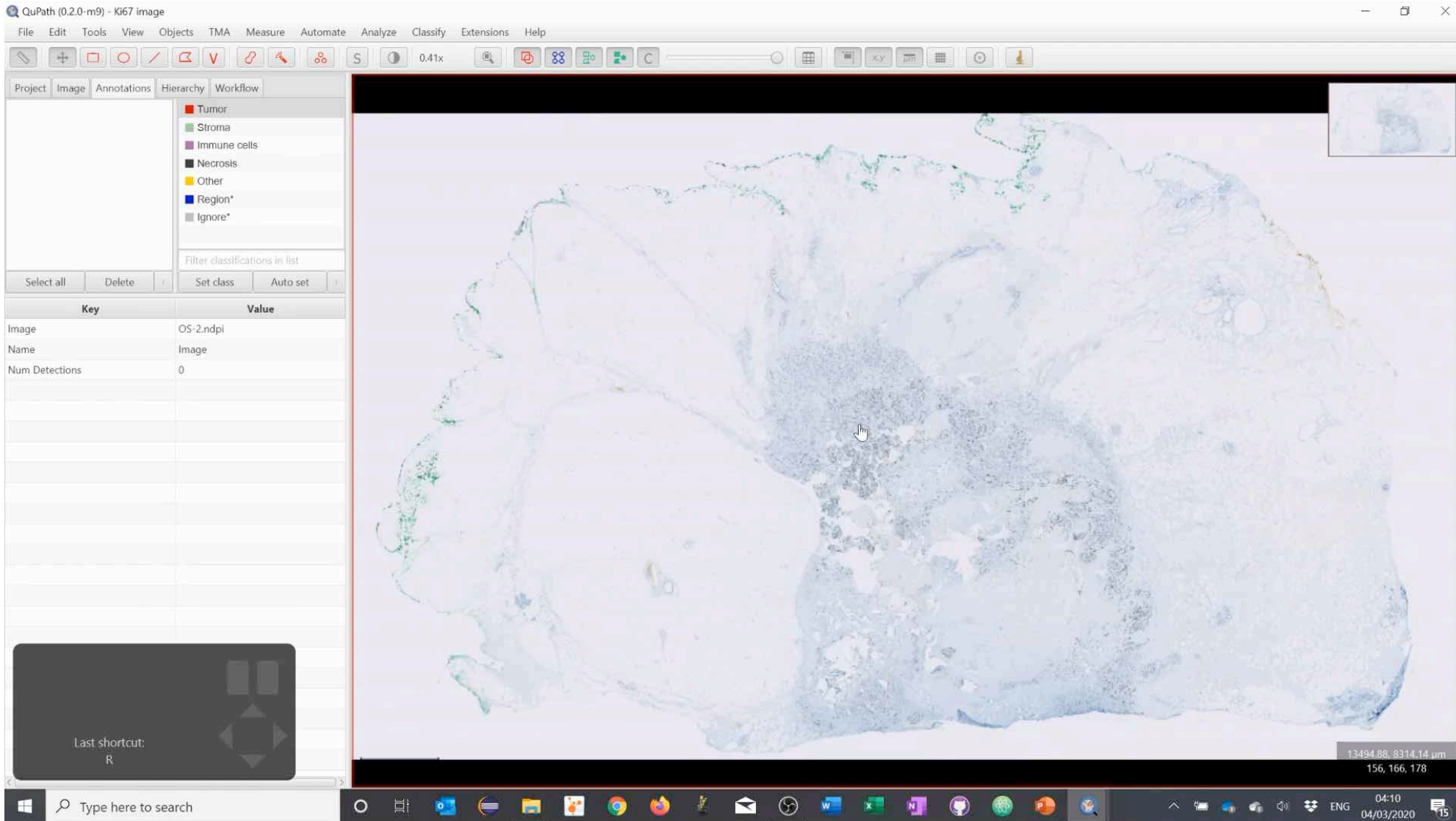
The screenshot shows a "Literature Update" page from the International Ki67 Working Group. The page lists several publications in a table format. The table has three columns: Author, Full title, and Journal, year, volume, issue, pages (copy-paste from PubMed).

Author	Full title	Journal, year, volume, issue, pages (copy-paste from PubMed)
Leung SCY, Nielsen TO, Zabaglo LA, Arun I, Badve SS, Bane AL, et al.	Analytical validation of a standardized scoring protocol for Ki67 immunohistochemistry on breast cancer excision whole sections: an international multicenter collaboration.	Histopathology. 2019 Apr 24. doi: 10.1111/his.13880.
Acs B, Pelekanou V, Bai Y, Martinez-Morilla S, Toki M, Leung SCY, Nielsen TO, Rimm DL.	Ki67 reproducibility using digital image analysis: an inter-platform and inter-operator study.	Lab Invest. 2019 Jan;99(1):107-117. Epub 2018 Sep 4.
Rimm DL, Leung SCY, McShane LM, Bai Y, Bane AL, Bartlett JMS, et al.	An international multicenter study to evaluate reproducibility of automated scoring for assessment of Ki67 in breast cancer.	Mod Pathol. 2019 Jan;32(1):59-69. Epub 2018 Aug 24.
Leung SCY, Nielsen TO, Zabaglo L, Arun I, Badve SS, Bane AL, et al.	Analytical validation of a standardized scoring protocol for Ki67: phase 3 of an international multicenter collaboration.	NPJ Breast Cancer 2016 May 18;2:16014.
Polley MY, Leung SC, Gao D, Mastropasqua MG, Zabaglo LA, Bartlett JM, et al.	An international study to increase concordance in Ki67 scoring.	Mod Pathol 2015 Jun;28(6):778-786.
Polley MY, Leung SC, McShane LM, Gao D, Hugh JC, Mastropasqua MG, et al.	An international Ki67 reproducibility study.	J Natl Cancer Inst 2013 Dec 18;105(24):1897-1906.
Dowsett M, Nielsen TO, A'Hern R, Bartlett J, Coombes RC, Cuzick J, et al.	Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer working group.	J Natl Cancer Inst 2011 Nov 16;103(22):1656-1664.

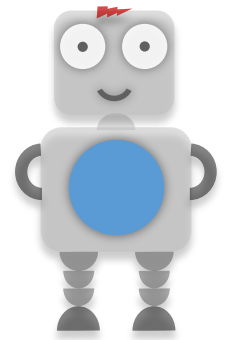
At the bottom of the page, there is a black bar with the text "Website under construction".



# Early *QuPath* applications focussed on brightfield IHC analysis



Calculate  
Ki67 positive %  
in tumour cells



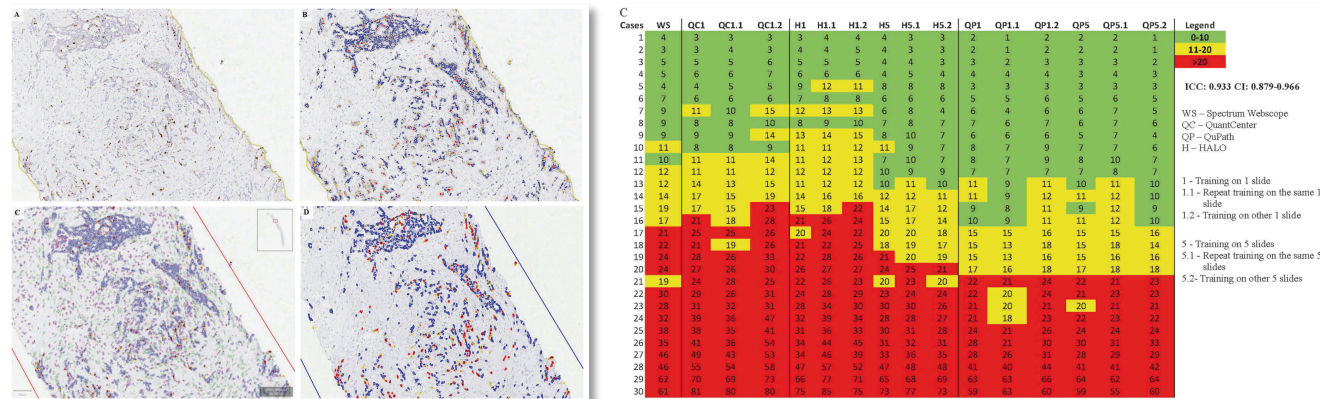
Combines image  
processing + AI

Sample image:  
*OpenSlide*

# Independent comparison of digital pathology software

## Ki67 scoring in breast cancer biopsies using HALO, QuPath, QuantPath

Acs et al. *Lab Invest* (2018)



Laboratory Investigation  
<https://doi.org/10.1038/s41374-018-0123-7>



### ARTICLE

### Ki67 reproducibility using digital image analysis: an inter-platform and inter-operator study

Balazs Acs<sup>1</sup> · Vasiliki Pelekanou<sup>1,2</sup> · Yalai Bai<sup>1</sup> · Sandra Martinez-Morilla<sup>1</sup> · Maria Toki<sup>1</sup> · Samuel C. Y. Leung<sup>3</sup> · Torsten O. Nielsen<sup>3</sup> · David L. Rimm<sup>1</sup>

Received: 21 June 2018 / Revised: 16 August 2018 / Accepted: 16 August 2018  
 © United States & Canadian Academy of Pathology 2018

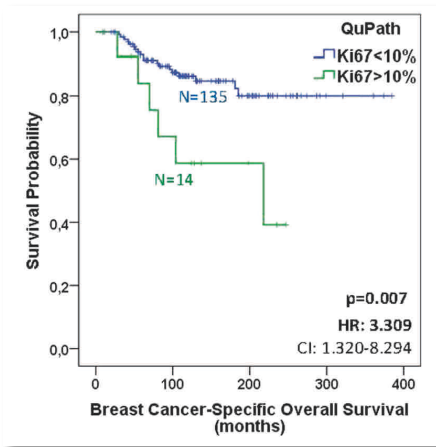
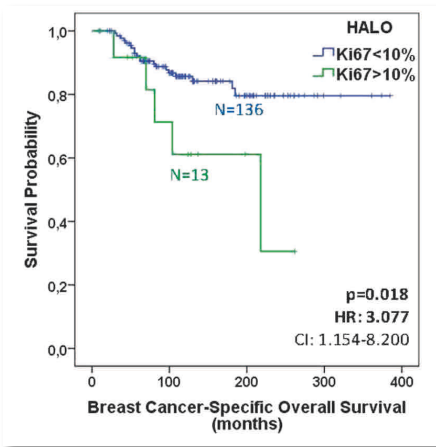
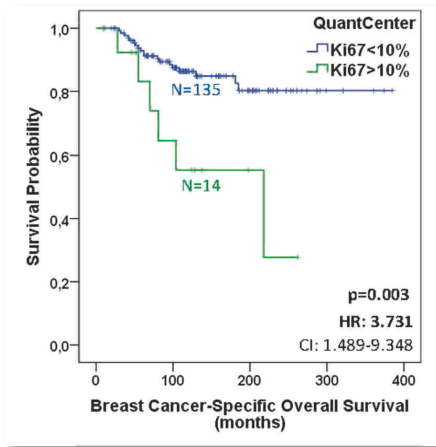
### Abstract

Ki67 expression has been a valuable prognostic variable in breast cancer, but has not seen broad adoption due to lack of standardization between institutions. Automation could represent a solution. Here we investigate the reproducibility of Ki67 measurement between three image analysis platforms with supervised classifiers performed by the same operator, by multiple operators, and finally we compare their accuracy in prognostic potential. Two breast cancer patient cohorts were used for this study. The standardization was done with the 30 cases of ER+ breast cancer that were used in phase 3 of International Ki67 in Breast Cancer Working Group initiatives where blocks were centrally cut and stained for Ki67. The outcome cohort was from 149 breast cancer cases from the Yale Pathology archives. A tissue microarray was built from representative tissue blocks with median follow-up of 120 months. The Mib-1 antibody (Dako) was used to detect Ki67 (dilution 1:100). HALO (IndicaLab), QuantCenter (3DHistech), and QuPath (open source software) digital image analysis (DIA) platforms were used to evaluate Ki67 expression. Intraclass correlation coefficient (ICC) was used to measure reproducibility. Between-DIA platform reproducibility was excellent (ICC: 0.933, CI: 0.879–0.966). Excellent reproducibility was found between all DIA platforms and the reference standard Ki67 values of Spectrum Webscope (QuPath-Spectrum Webscope ICC: 0.970, CI: 0.936–0.986; HALO-Spectrum Webscope ICC: 0.968, CI: 0.933–0.985; QuantCenter-Spectrum Webscope ICC: 0.964, CI: 0.919–0.983). All platforms showed excellent intra-DIA reproducibility (QuPath ICC: 0.992, CI: 0.986–0.996; HALO ICC: 0.972, CI: 0.924–0.988; QuantCenter ICC: 0.978, CI: 0.932–0.991). Comparing each DIA against outcome, the hazard ratios were similar. The inter-operator reproducibility was particularly high (ICC: 0.962–0.995). Our results showed outstanding reproducibility both within and between-DIA platforms, including one freely available DIA platform (QuPath). We also found the platforms essentially indistinguishable with respect to prediction of breast cancer patient outcome. Results justify multi-institutional DIA studies to assess clinical utility.

### Introduction

Ki67 labeling index (Ki67 LI) is currently one of the most promising yet controversial biomarkers in breast cancer [1]. The European Society for Medical Oncology (ESMO)

Clinical Practice Guidelines suggests that Ki67 LI may provide useful information, if the assay can be standardized [2]. The St. Gallen Consensus Conference in 2017 also agreed that Ki67 LI could be used to distinguish between HER2-negative luminal A-like and luminal B-like breast cancer subtypes [3]. However, the panel also emphasized the reproducibility issue of Ki67 LI, suggesting calibration of Ki67 scoring [3]. The American Society of Clinical Oncology recommended against the use of Ki67 LI for prognosis in newly diagnosed breast cancer patients because of lack of reproducibility across laboratories [4]. The International Ki67 in Breast Cancer Working Group (IKWG) has nevertheless published consensus recommendations for the application of Ki67 IHC in daily practice [5]. According to this group, parameters that predominantly influence the Ki67 IHC results include pre-analytical



✉ David L. Rimm  
 david.rimm@yale.edu

<sup>1</sup> Department of Pathology, Yale School of Medicine, New Haven, CT, USA

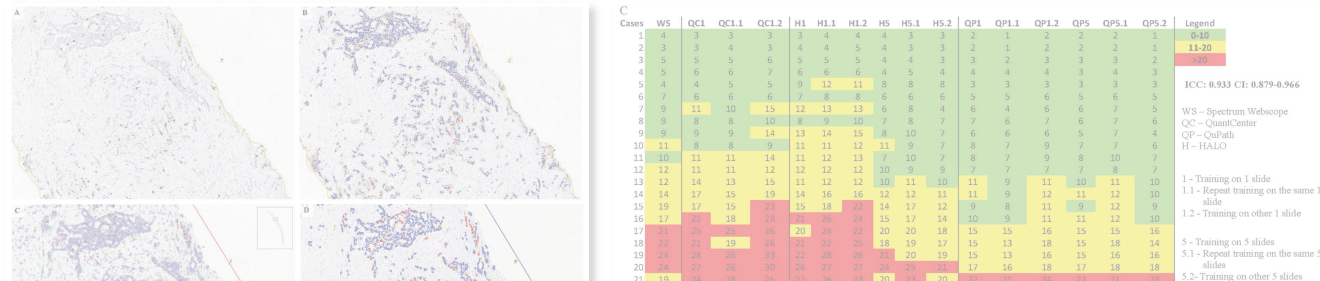
<sup>2</sup> Precision Oncology, Sanofi US Services Inc, Cambridge, MA, USA

<sup>3</sup> Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada

# Independent comparison of digital pathology software

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Acs et al. *Lab Invest* (2018)



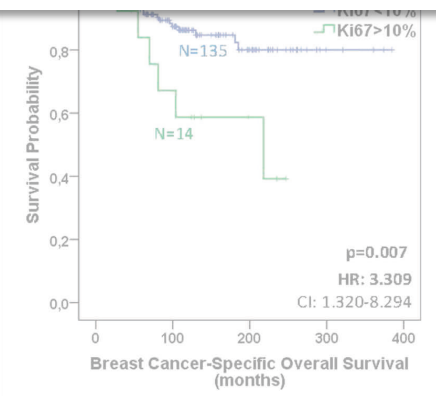
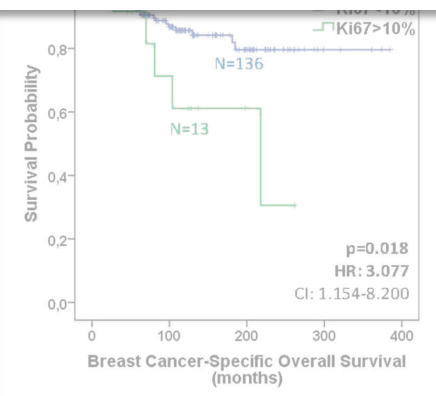
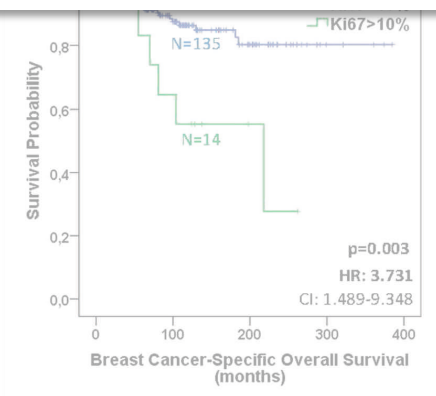
Laboratory Investigation  
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ARTICLE

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<sup>1</sup> Department of Pathology, Yale School of Medicine, New Haven, CT, USA

<sup>2</sup> Precision Oncology, Sanofi US Services Inc, Cambridge, MA, USA

<sup>3</sup> Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada





# Training, support & documentation are essential!

The image.sc forum website is shown with a list of topics under the 'qupath' category. A video player is embedded, showing a tutorial titled 'Quantitative Pathology & BioImage Analysis: QuPath - [NEUBIASAcademy@Home] Webinar'. Below the video, there are several other video thumbnails and a 'QuPath tutorial #1 - Getting started' section.

A Twitter thread by Pete Bankhead (@petebankhead) from August 19th. The thread discusses various tools in QuPath, such as polygon and polyline tools, and zoom-dependent brushes. It includes screenshots of the QuPath software interface showing these tools in use on histology images.

The QuPath documentation page for ImageJ integration. It explains that QuPath was created by someone who was a big fan of ImageJ and that it uses ImageJ as an extension. The page includes a table of contents and a section titled 'Sending image regions to ImageJ' which shows a screenshot of the QuPath toolbar with the 'Send region to ImageJ' button highlighted.

*QuPath* is still mostly used for pathology applications

But it *can* do more...



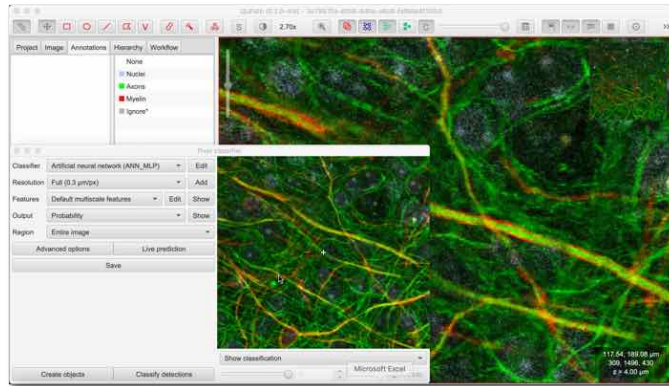
# What *QuPath* can do



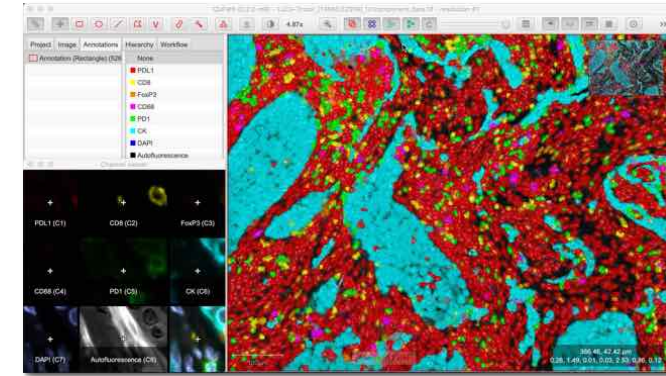
The first new stable release since 2016  
was made in **June 2020**



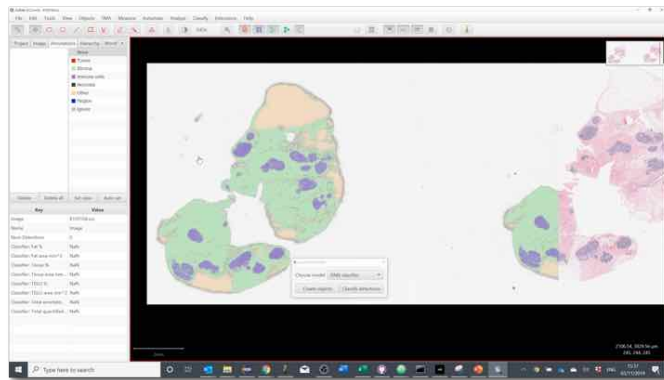
# What *QuPath* can do



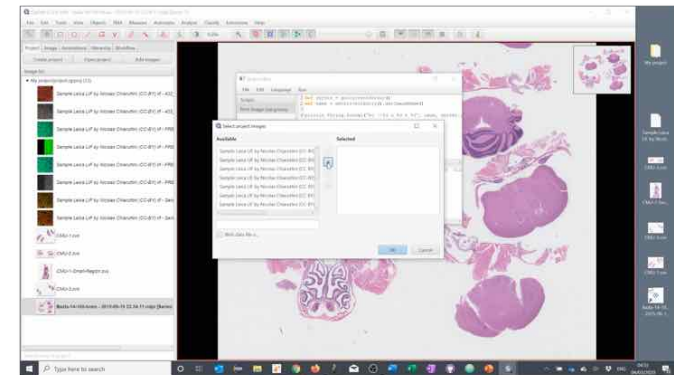
Pixel classification



Multiplexed analysis

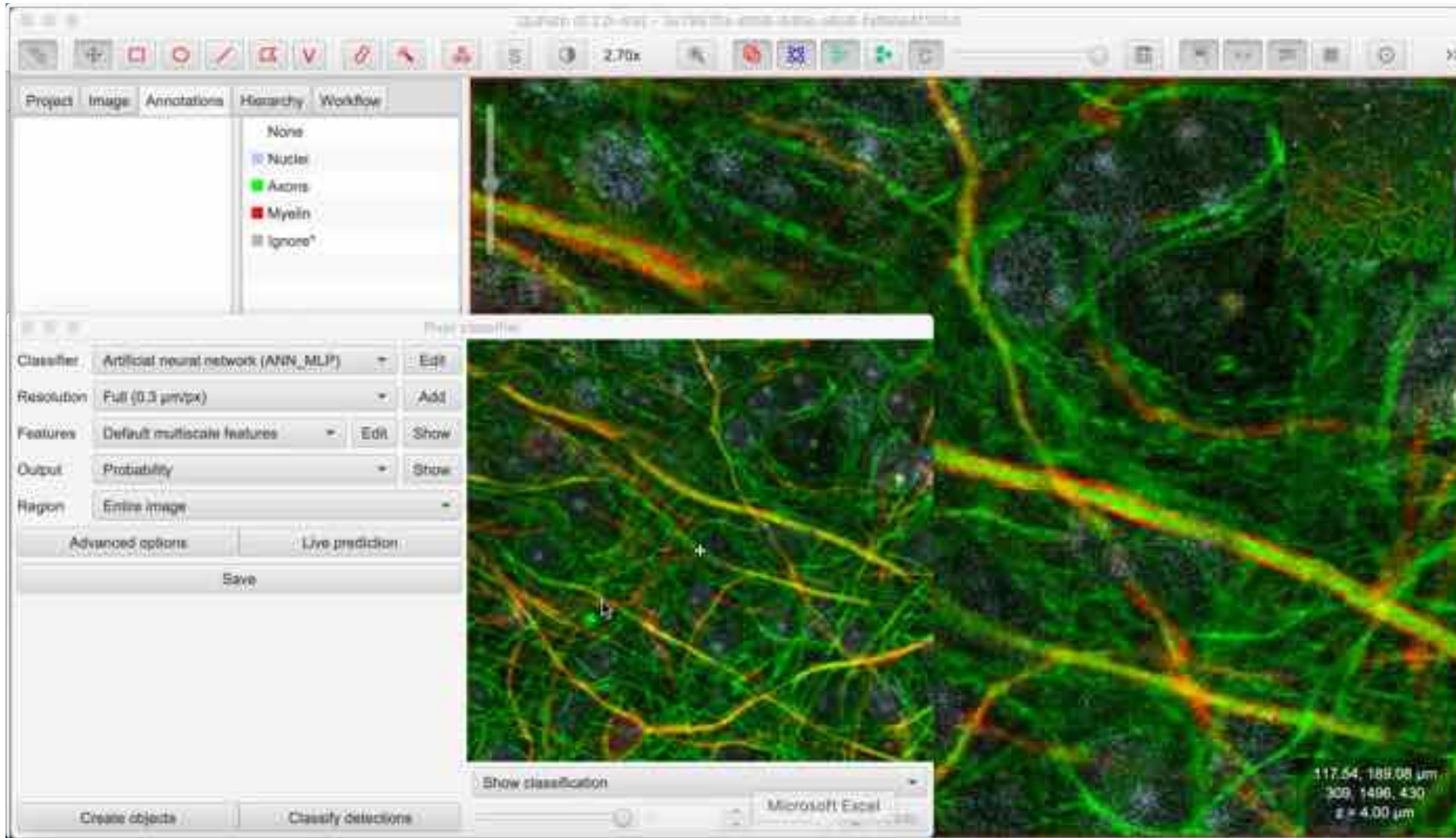


Deep learning



Workflow integration

# Pixel classification



Train interactively  
Support z-stacks

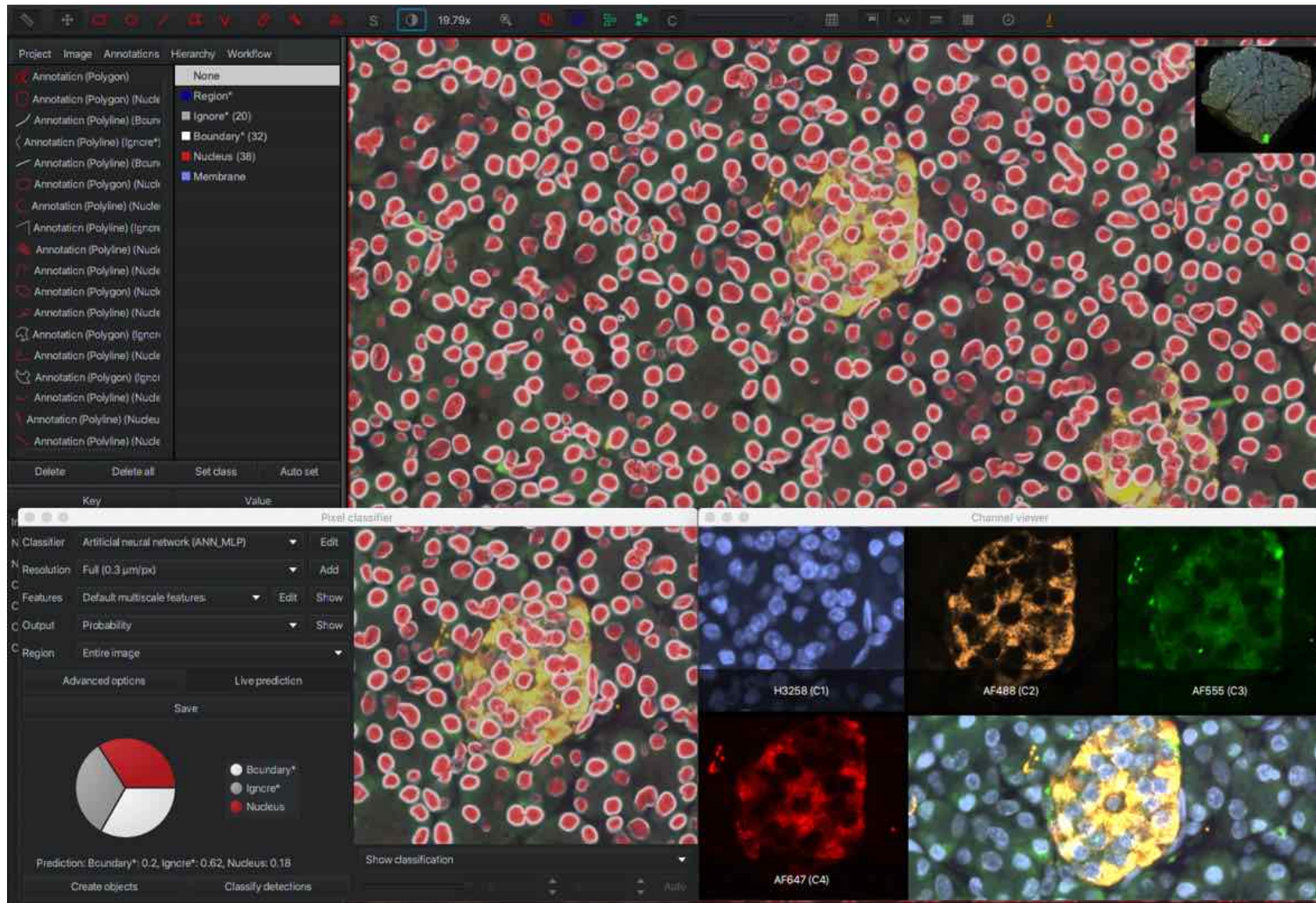
Identify  
myelinated axons

Collaboration with  
Dr Yvonne Dombrowski  
*Queen's University Belfast*





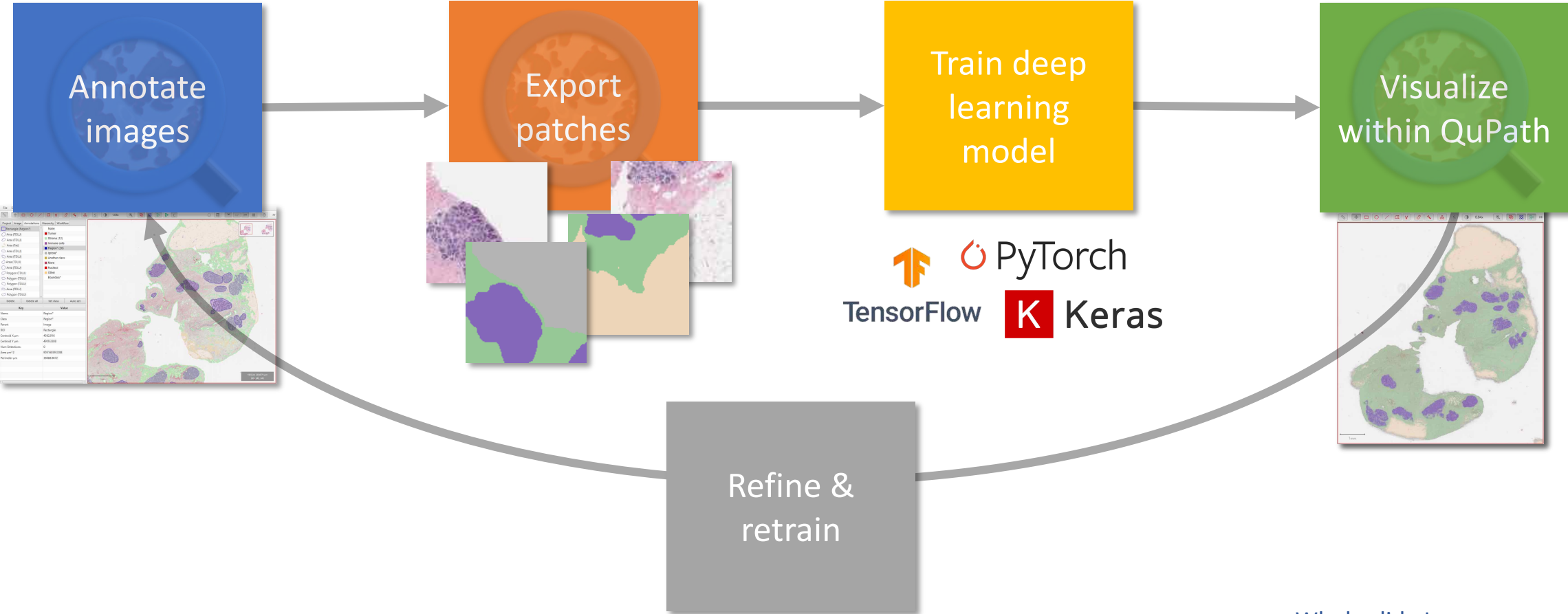
# Pixel classification



Train to aid  
object separation

Assign boundary class,  
Specify line thickness,

# A deep learning workflow in *QuPath* (in progress!)



Whole slide Image source:  
Virtual Tissue Bank of the  
Susan G. Komen for the Cure® Tissue Bank  
at the IU Simon Cancer Center



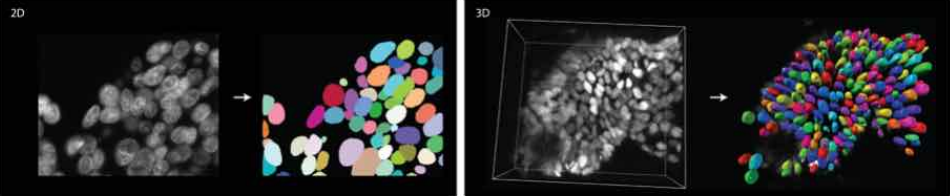
# QuPath + StarDist can help resolve tricky nucleus-identification problems

*StarDist by  
Uwe Schmidt & Martin Weigert*

README.md


pypi package 0.5.0 build passing build passing

## StarDist - Object Detection with Star-convex Shapes



This repository contains the implementation of star-convex object detection for 2D and 3D images, as described in the papers:

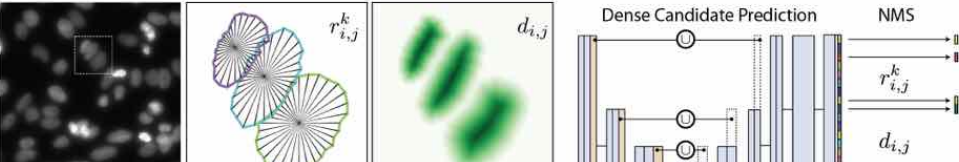
- Uwe Schmidt, Martin Weigert, Coleman Broaddus, and Gene Myers.  
[Cell Detection with Star-convex Polygons](#).  
International Conference on Medical Image Computing and Computer-Assisted Intervention (MICCAI), Granada, Spain, September 2018.
- Martin Weigert, Uwe Schmidt, Robert Haase, Ko Sugawara, and Gene Myers.  
[Star-convex Polyhedra for 3D Object Detection and Segmentation in Microscopy](#).  
The IEEE Winter Conference on Applications of Computer Vision (WACV), Snowmass Village, Colorado, March 2020



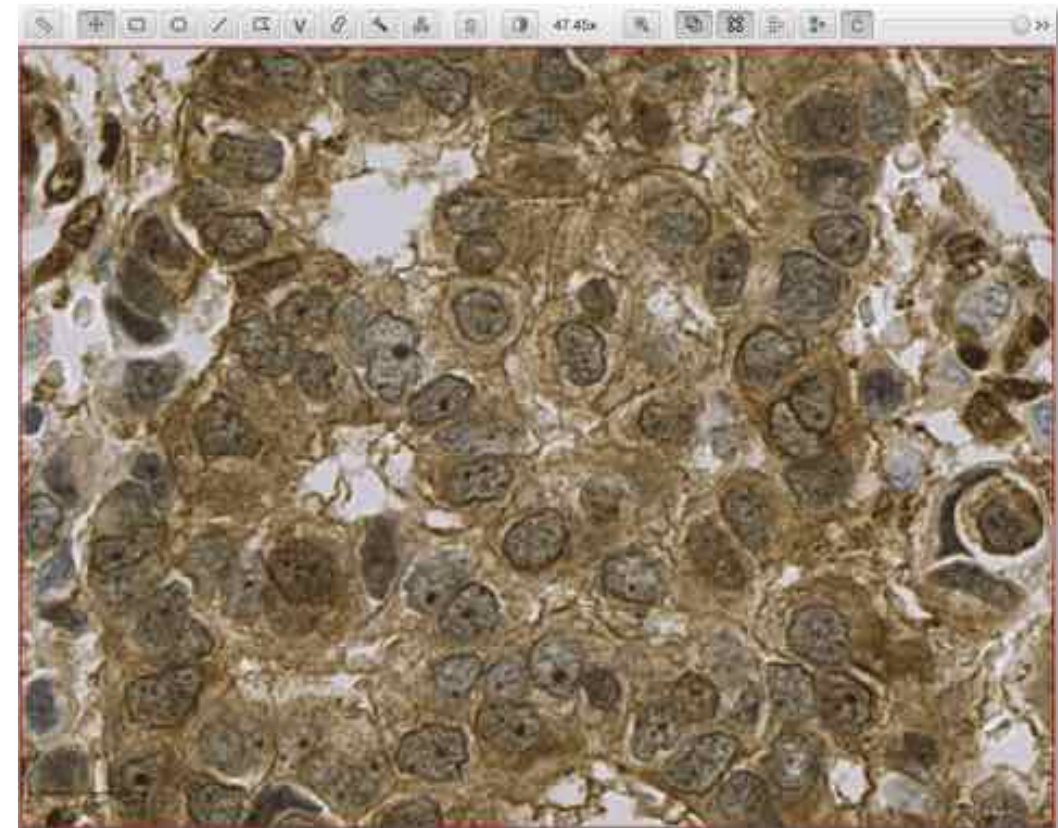
Please [cite the paper\(s\)](#) if you are using this code in your research.

### Overview

The following figure illustrates the general approach for 2D images. The training data consists of corresponding pairs of input (i.e. raw) images and fully annotated label images (i.e. every pixel is labeled with a unique object id or 0 for background). A model is trained to densely predict the distances ( $r$ ) to the object boundary along a fixed set of rays and object probabilities ( $d$ ), which together produce an overcomplete set of candidate polygons for a given input image. The final result is obtained via non-maximum suppression (NMS) of these candidates.



The diagram illustrates the StarDist pipeline for 2D images. It starts with an input image (raw) and a corresponding label image (fully annotated). The model predicts distances ( $r_{i,j}^k$ ) and object probabilities ( $d_{i,j}$ ). These are used for Dense Candidate Prediction, which produces a set of candidate polygons. Non-Maximum Suppression (NMS) is then applied to these candidates to produce the final result, showing the predicted object boundaries and distances.





# QuPath + StarDist can help resolve tricky nucleus-identification problems

*StarDist by  
Uwe Schmidt & Martin Weigert*



QuPath

latest

Search docs

CONTENTS:

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- Getting started
- Tutorials
- Concepts

Advanced

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- Exporting images
- Exporting annotations
- StarDist
  - Building QuPath with TensorFlow
  - Getting pretrained models
  - Detecting nuclei
  - Customizing detection
  - Differences from StarDist Fiji

Scripting

Reference



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Docs » Advanced » StarDist [Edit on GitHub](#)

## StarDist

StarDist is a fantastic, deep-learning-based method of 2D and 3D nucleus detection from Martin Weigert and Uwe Schmidt. It exists as a [Python library](#) and [Fiji plugin](#).

This page describes how to start using StarDist 2D directly within QuPath as an alternative method of cell detection.

### Cite the paper!

If you use StarDist in a publication, be sure to cite it:

- Uwe Schmidt, Martin Weigert, Coleman Broaddus, and Gene Myers. [Cell Detection with Star-convex Polygons](#). *International Conference on Medical Image Computing and Computer-Assisted Intervention (MICCAI)*, Granada, Spain, September 2018.

(And if you use it in combination with QuPath, be sure to [cite the QuPath paper too!](#))

### Warning

This is a provisional feature, hastily created for the NEUBIAS webinar on QuPath in April 2020, and released under mild duress from attendees. You can find both the StarDist and QuPath webinars on the [NEUBIAS YouTube channel](#).

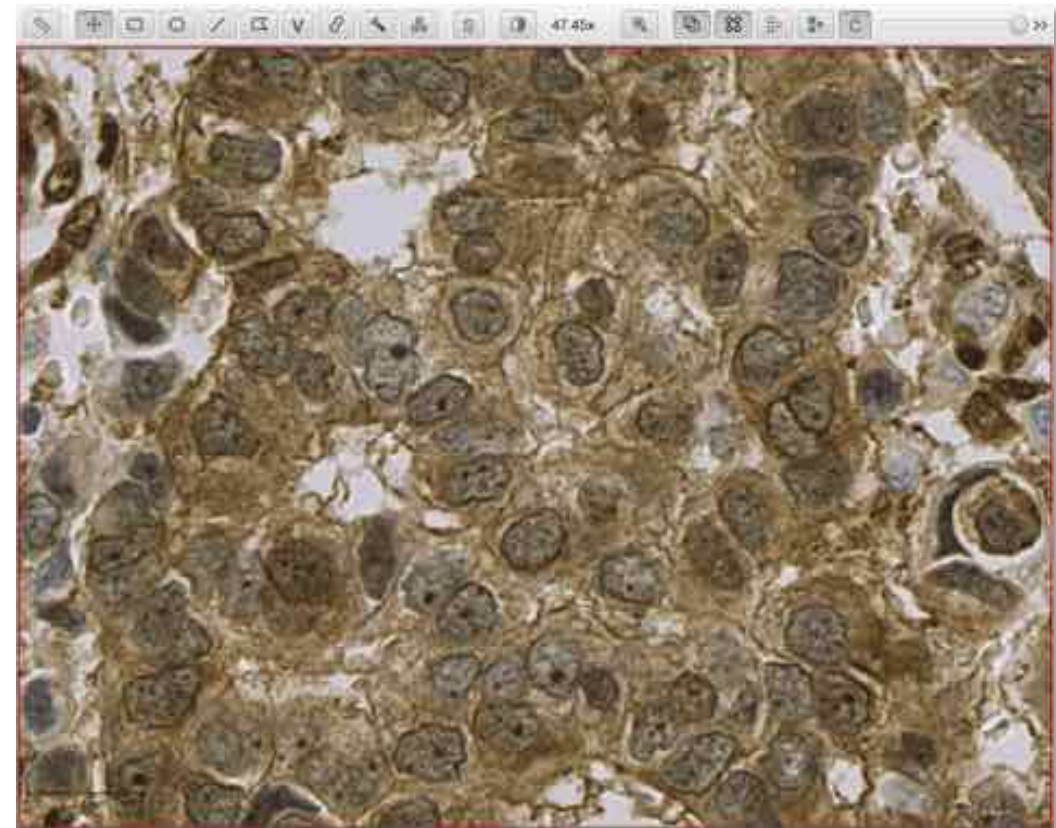
If it already works for you, great! If not, please be patient – and be aware that it may change substantially as QuPath is developed further.

## Building QuPath with TensorFlow

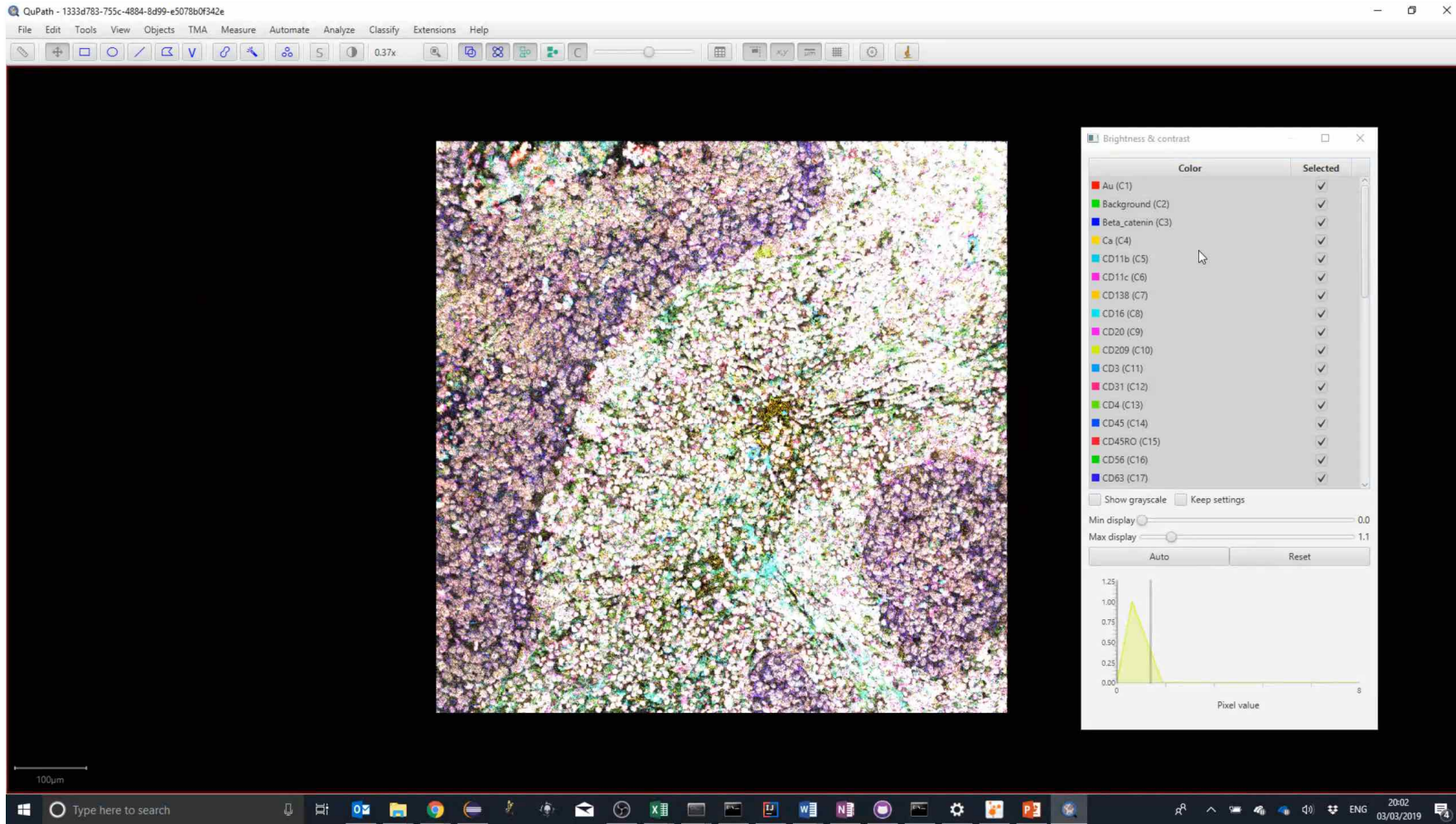
StarDist requires TensorFlow, which is not currently included in the main QuPath distributions.

To get this, you will need to build QuPath and enable the optional TensorFlow module. See [Building from source](#) for details.

You will need to add either `-Ptensorflow-cpu=true` or `-Ptensorflow-gpu=true` parameters, depending upon whether TensorFlow should use a graphics card or not. For example:



# Multiplexed analysis



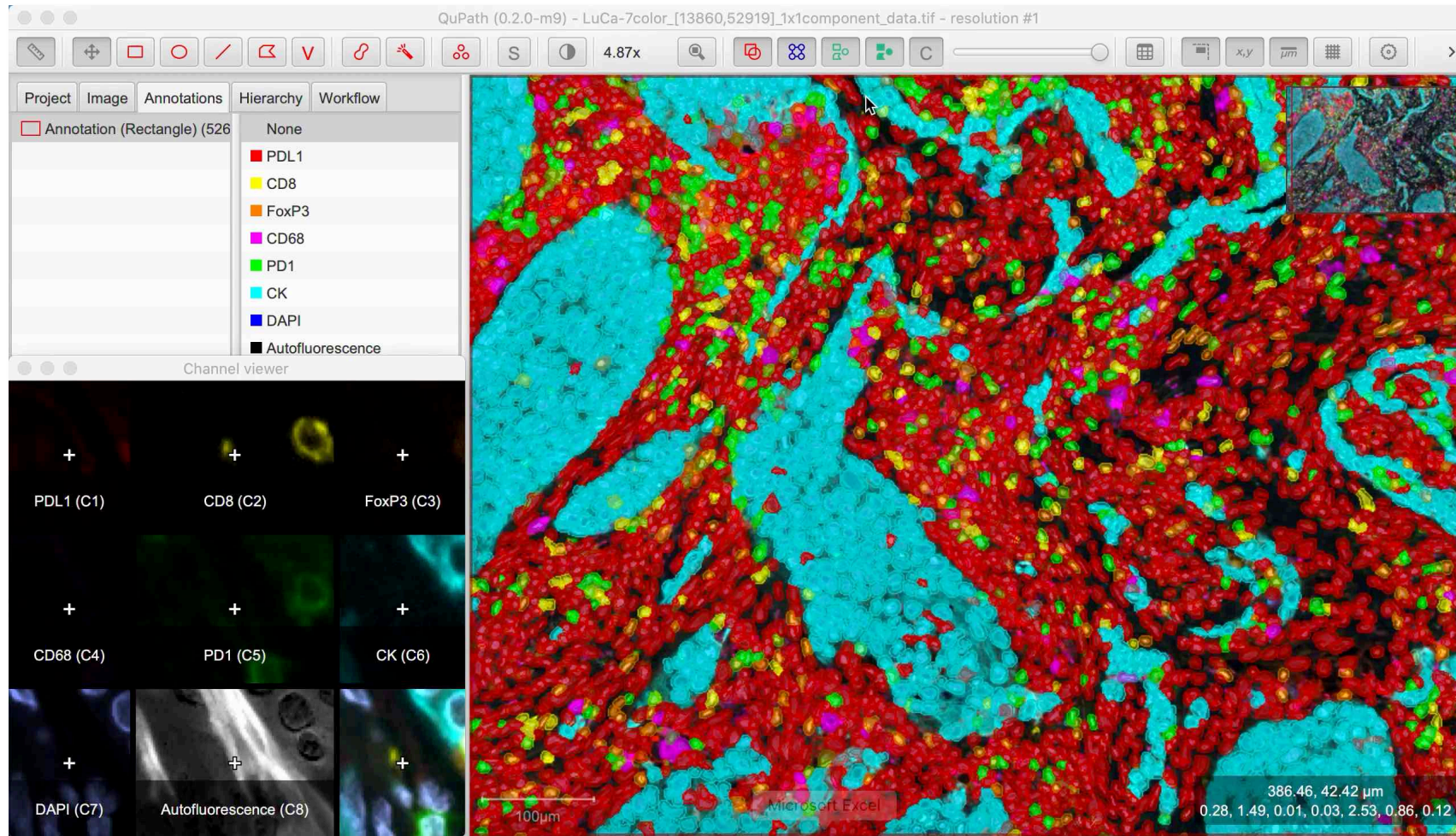
Support for  
multiplexed images

Channel viewer to  
visualize  
> 40 channels  
simultaneously

Multiplexed Ion Beam Image source:  
Keren et al. *Cell* (2018)  
<https://mibi-share.ionpath.com>



# Multiplexed analysis

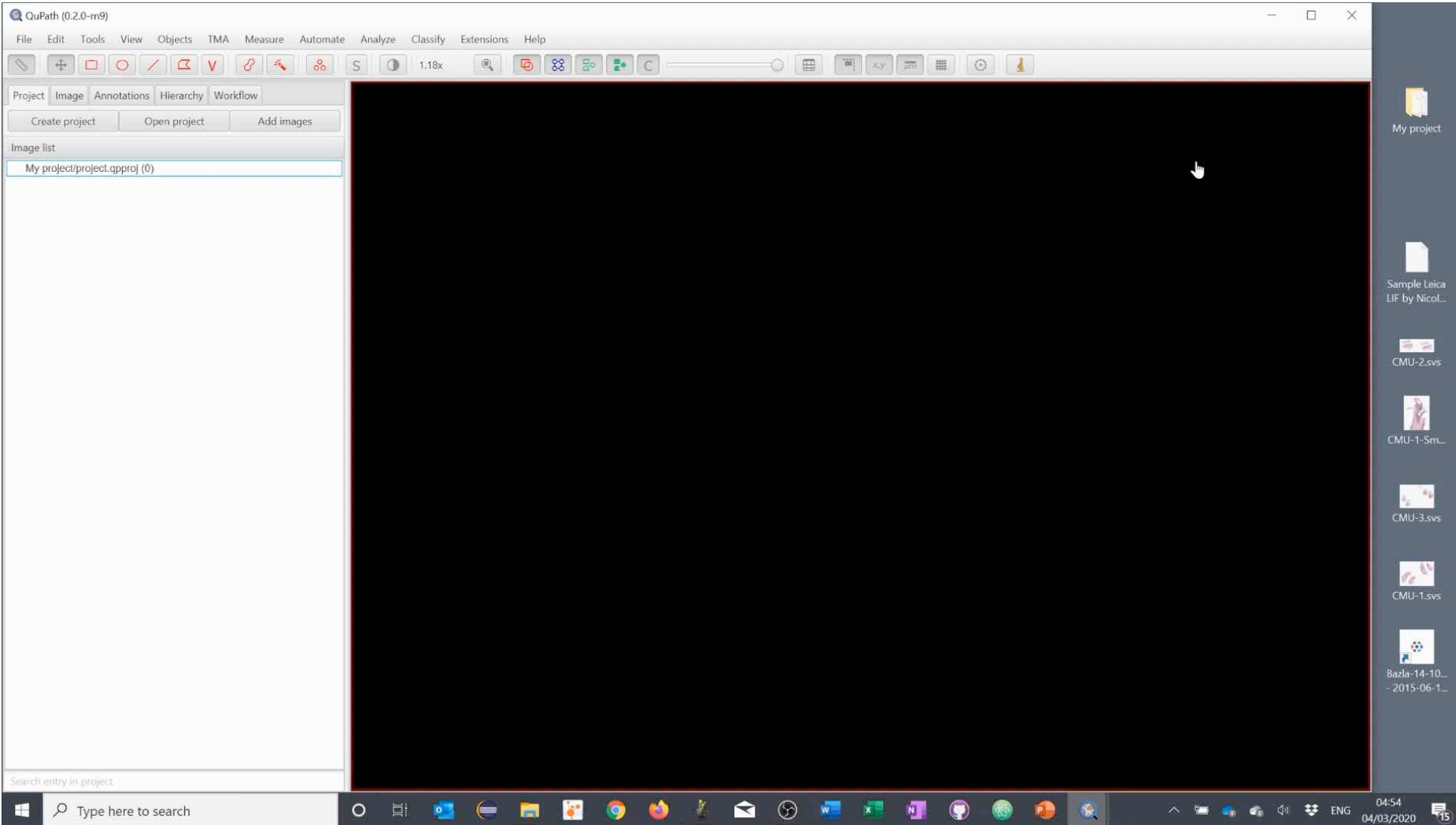


Single-class &  
composite classifiers

Cell phenotyping,  
Toggle visibility,  
Query locations &  
distances

Original image source:  
*LuCa-7color\_[13860,52919]\_*  
*1x1component\_data.tif*  
© Perkin Elmer, CC-BY 4.0

# Workflow integration



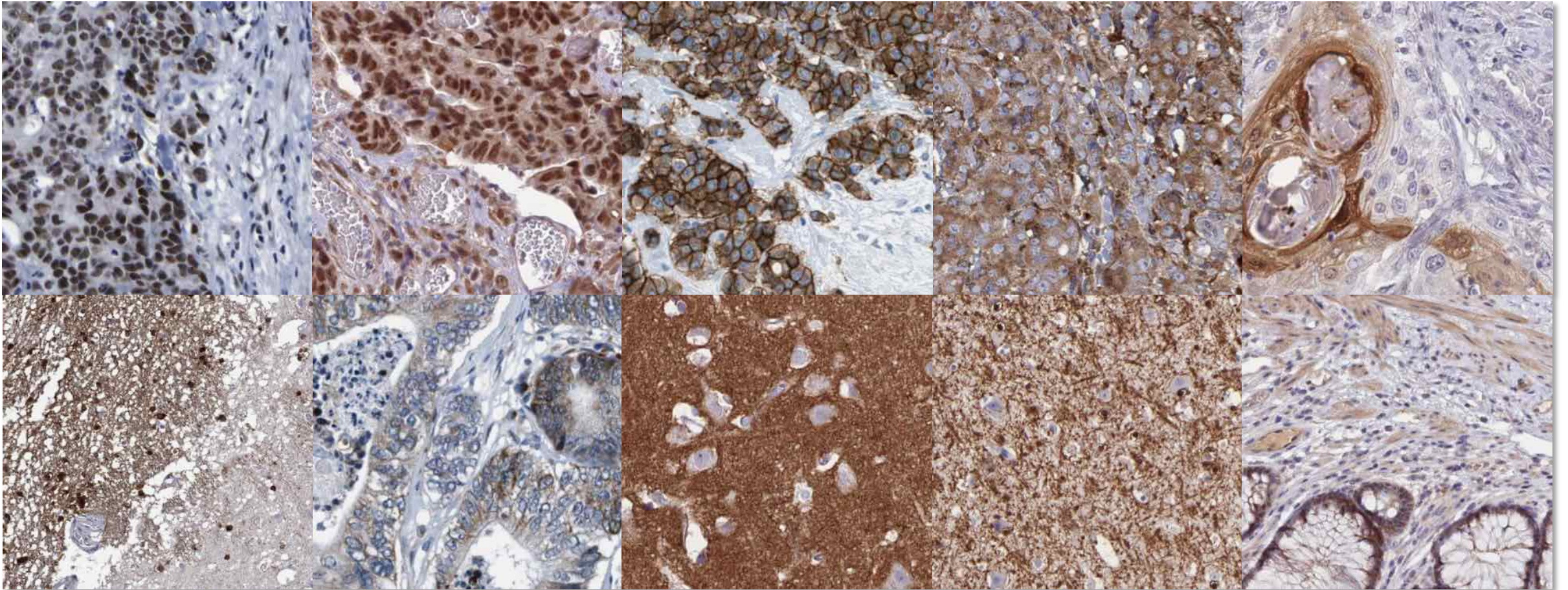
Projects to  
manage data

Add images  
(local & remote),  
Mask file names,  
Run batch scripts

*A plea to users of  
bioimage analysis software...*



Similar-sounding problems often pose *very* different computational challenges!



Haematoxylin & DAB images from the Human Protein Atlas

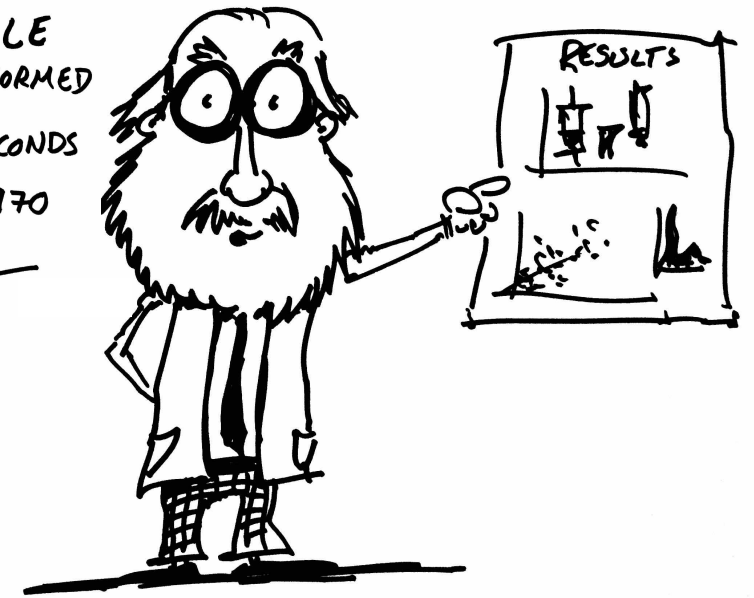


# Automated does not equal unbiased!

TO AVOID MANUAL BIAS,  
WE TRIED ALL 17 AUTOMATED  
THRESHOLDS IN IMAGES  
AND CHOSE THE ONE THAT  
WORKED



THIS ANALYSIS IS  
FULLY REPRODUCIBLE  
AS LONG AS IT'S PERFORMED  
15414530574.03 SECONDS  
AFTER 1 JANUARY 1970



Replicability is hard to achieve!

Even with all the software tools available  
*bioimage analysis is still hard!*



Creating & supporting user-friendly software  
for researchers is important!

# Thanks!

Chan  
Zuckerberg  
Initiative 

  
wellcome



CANCER  
RESEARCH  
UK



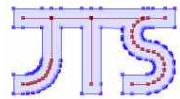
*The creators & maintainers of  
many other open source projects*

OpenJDK

 BIO-FORMATS



OpenSlide



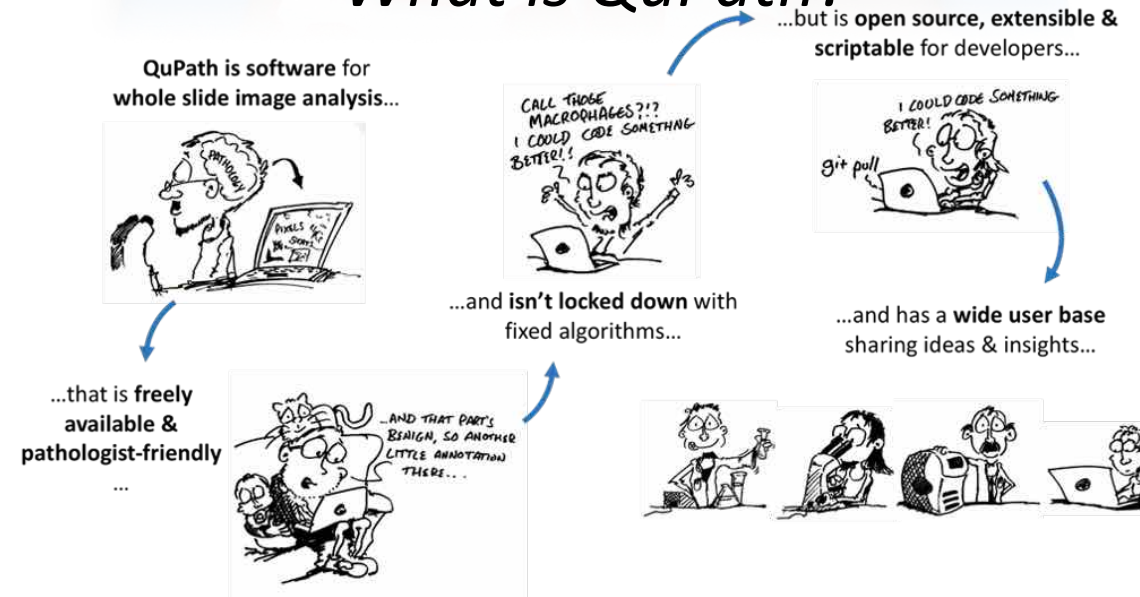
 AdoptOpenJDK

*Everyone who participates on the community forum*

*Everyone who makes datasets available*



## What is QuPath?



<https://qupath.github.io>