

# WELCOME (







Hanna



Stéphane



Sujan



Marcela

#### A bit of housekeeping

- Everyone has a computer? Everyone has access to an outlet?
- Everyone has a mouse?
- Everyone has all the software installed?
- Everyone is fed and watered and ready to go?

#### What to expect

#### Bioimage Analysis for Quantitative Microscopy

Course program 30.9-4.10.2024

#### Trainers:

Hanna Grobe Elnaz Fazeli Joanna Pylvänäinen Sujan Ghimire Stéphane Rigaud

\*open to all interested

#### Locations

Auditorium Biokemi, Biocity 3<sup>rd</sup> floor Auditorium Biologi, Biocity 2<sup>rd</sup> floor Putous Auditorium, Joki Conference Center

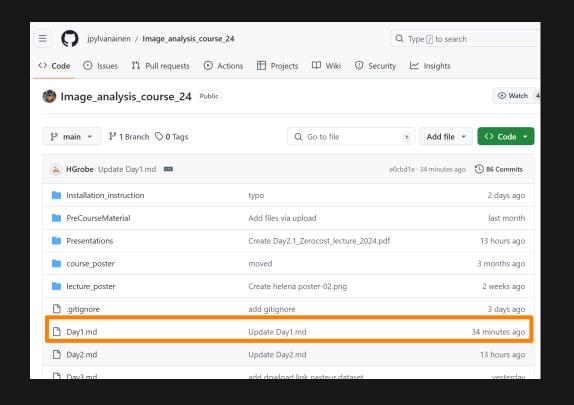
	Mon	Tue	Wed	Thu	Fri
9.00_10.00	<b>Intro + Fiji Basics</b> Hanna Grobe	<b>DL lecture</b> Joanna Pylvänäinen	<b>QuPath</b> Stéphane Rigaud	TrackMate + CellTracks Colab lecture Joanna Pylvänäinen	
10:00-10:15					Presentation: TBI image data team Pasi Kankaanpää
10:15-10:30	break	break	break	break	Project presentations
	<b>Fiji Basics</b> Hanna Grobe	<b>DL</b> demo annotation and training Sujan Ghimire	<b>QuPath</b> Stéphane Rigaud	Cell tracking with TrackMate Joanna Pylvänäinen	
12-13	Lunch (at own cost)	Lunch (at own cost)	Lunch (at own cost)	Lunch (at own cost)	Lunch (at own cost)
13:00-13:15	Presentation: Euro-Biolmaging Jiri Funda, Susanne Vainio Fiji Macros	Science talk *: Deep Learning in Microscopy Guillaume Jacquemet	Science talk *: Deep Learning in Histopathology Pekka Ruusuvuori	Keynote talk *: How to not lie with charts - better data visualisations for life sciences	Science talk *: Next-generation file formats, version control, and publishing your data
	Elnaz Fazeli	<b>DL demo quality control</b> Joanna Pylvänäinen	<b>QuPath</b> Stéphane Rigaud	Helena Jambor  Track Analysis using  CellTracksColab  Hanna Grobe	Junel Solis  Work with your own data
15:15-15:30	break	break	break	break	break
	<b>Fiji Macros</b> Elnaz Fazeli	<b>DL demo apply to own data</b> Joanna Pylvänäinen	<b>QuPath</b> Stéphane Rigaud	GPU accelerated Fiji image processing Stéphane Rigaud	Work with your own data Goodbye and farewell
17:00-21:00				Course dinner in Mauno	

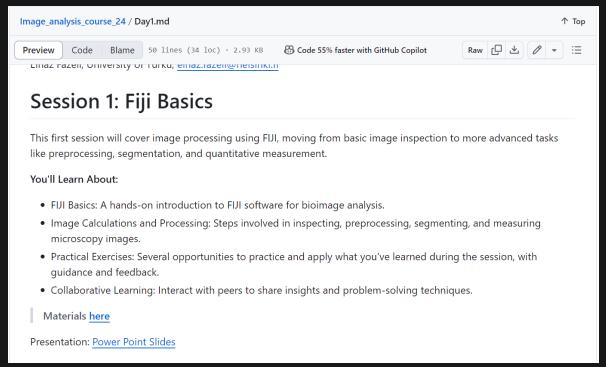
The info for each day can be found on the GitHub page for this course:

.../Image analysis course 24

(You should also have an email with the link in your inbox.)

#### **How the GitHub works**





#### Before we get started for real...



Set a goal for the week

Get to know your neighbor

#### Get to know your neighbor



Bealfwhitetpietstoitethietethilagreathoretethibitee?now?

#### ... now to the real stuff

#### **FIJI Basics**

**Bioimage** Mon Intro + Fiji Basics **Analysis for** 9-10.00 Hanna Grobe Quantitative 10.00-10:15 **Microscopy** 10-15-10.30 break Fiji Basics 30.9-4.10.2024 Hanna Grobe 10.30-11.45 Trainers: Hanna Grobe Elnaz Fazeli Joanna Pylvänäinen Sujan Ghimire Stéphane Rigaud \*open to all interested Locations: Auditorium Biokemi, Biocity 2nd floor Auditorium Biologi, Biocity 3rd floor Putous Auditorium, Joki conference center

Part 1

Take homes from the precourse material A walk through the image analysis pipeline

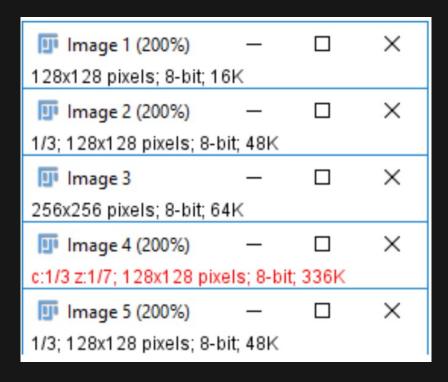
Part 2

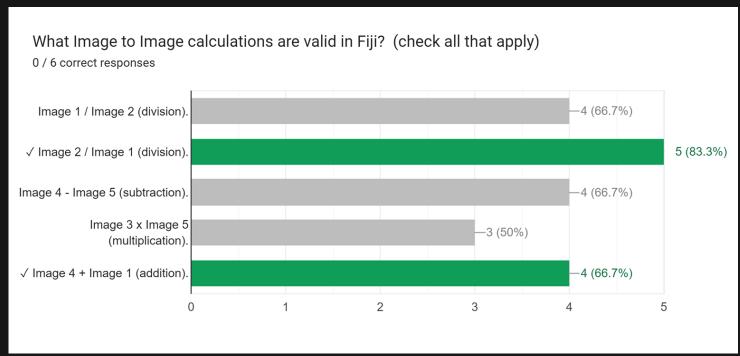
The struggle is real - get your FIJI warmed up

#### Take home from the pre course

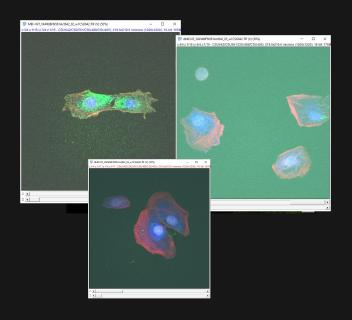
- ? Image conversion is affected by the image display YES (<a href="here's why">here's why</a>)
- ? What Image to Image calculations are valid in Fiji? (check all that apply) [1/3; 128x128pix; 8bit] / [128x128pix; 8-bit] [c:1/3; z:1/7; 128x128pix; 8bit] + [128x128pix; 8-bit]
- ? What is the normalization factor for a 3 x 3 filter kernel, where all elements are 1? 1/9 (here's why)
- ! Saving an image with an ROI in TIFF format from Fiji also saves the ROI.

#### Image calculations



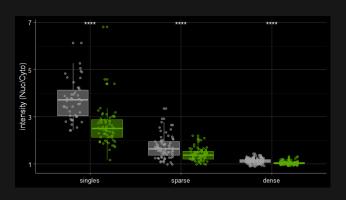


### Let's get going



AIM: Analyze YAP localization

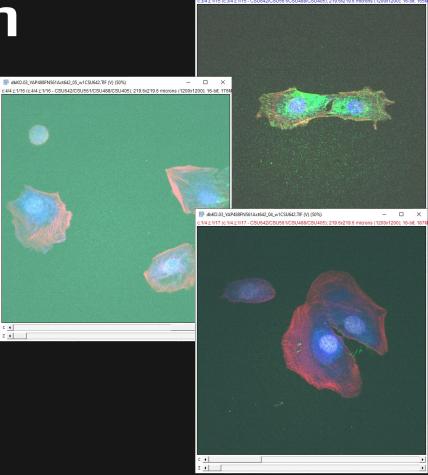
What are the steps?



#### Step 1 - Image inspection

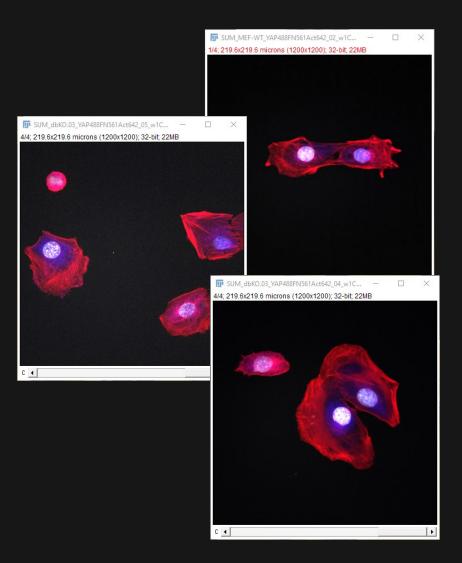
- Channels what was stained
- Stack do we have z stacks (and do we need them)
- Are there artefacts
- Are the images calibrated
- Did our traget stay in focus

• ...



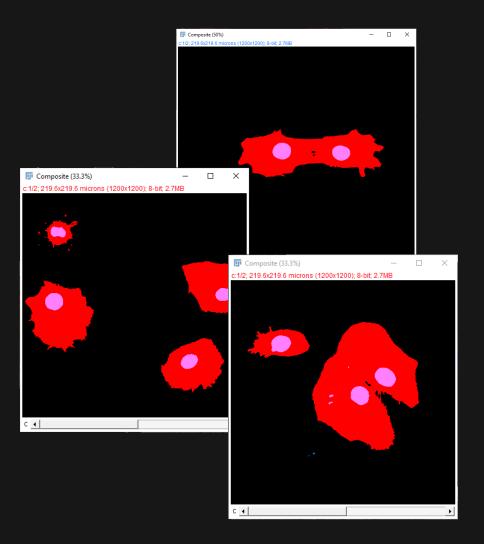
### Step 2 - Preprocessing

- Filter
- Remove background
- Make projections
- Change bit depth
- Crop
- •



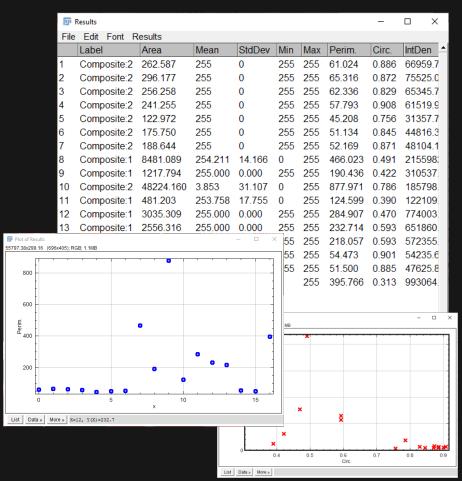
### Step 3 - Segmentation

- What needs to be segmented?
- What kind of thresholding
- Binary operations



#### Step 4 - Image measurement

- Set measurements
- Analyse particles
- Inspect results table



#### **Break time**











### Now you go!



Sample data - Raw



https://tinyurl.com/BAQM-D1raw



Data inspection

Preprocessing



15 min (10:45) 5 min

work time check in

### Now that you went:

TASK	MY SOLUTION	YOUR SOLUTION
Reduce z dimensions	Sum slices (Z-Project > SUM slices)	
Duplicate channels for manipulations	Duplicate nuclei and actin	
Filter	Median filter for both nucleus and actin (radius = 5px)	

See "Script\_1.ijm"

#### Now you go!



Sample data - Filtered / Your own processed data



https://tinyurl.com/BAQM-D1filtered



Segmentation

Binary operations



15 min (11:00) words 5 min che

work time check in

## Now that you went:

TASK	MY SOLUTION	YOUR SOLUTION
Duplicate channels for manipulations	Duplicate nuclei and actin	
Thresholding nuclei	Adaptive threshold	
Thresholding actin	MinError	

See "Script\_2.ijm"

#### Now you go!



Sample data - Segmented / Your own segmented data



https://tinyurl.com/BAQM-D1labelled



Image measurements



15 min (11:15) work time

## Now that you went:

TASK	MY SOLUTION	YOUR SOLUTION
Duplicate channels for manipulations	Duplicate nuclei and actin	
Measure nuclear YAP intensity	Analyse particles on thres. DAPI (size = 50 - Infinity) Measure on YAP signal	
Measure cytoplasmatic YAP intensity	Create selection on thres. actin Add to ROI manager ROI manager XOR Measure on YAP signal	

See "Script\_3.ijm"

#### **Lunch time**













