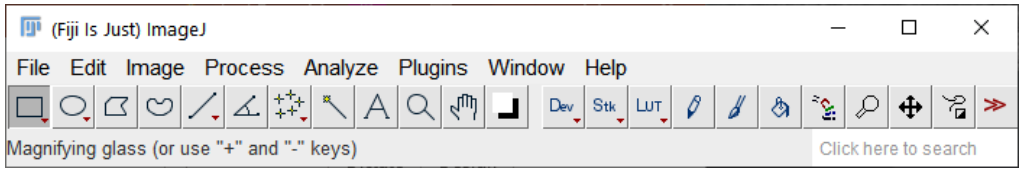


Fiji workshop 2025

- Day 1:
  - Image data visualization in Fiji
- Day 2:
  - Quantitative image analysis in Fiji
- Day 3:
  - One-on-one analysis support with your own image data

Joel Ryan, Philip Kesner, Barbara Da Rocha, Mar Garcia Ferrés

ABIF

March 4 to 6 2025

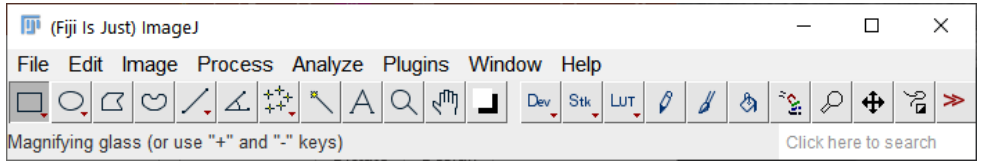
https://github.com/ABIF-McGill/ABIF-Fiji-Workshop-2025

Day 1: Data visualization in Fiji

- Today's goals
  - Get acquainted with Fiji!
  - LUTs, contrast
  - Composite images
  - Intro to scripting!
  - Look at data from 3D images
  - Look at data from timelapse images
  - Images for publications

https://github.com/ABIF-McGill/ABIF-Fiji-Workshop-2025

Fiji is awesome! But it has some drawbacks…



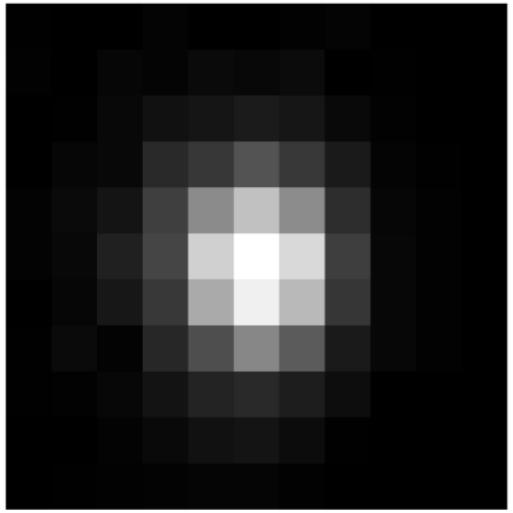
- Why should you use Fiji?
  - Free open-source software
  - Huge development community
  - Librairies to open nearly any microscope image (!)
  - Automation and scripting with multiple programming languages (!)
- When should you use something else than Fiji?
  - More powerful computation
  - Better 3D visualization
  - Many deep learning tools
  - Instrument-specific processing

https://github.com/ABIF-McGill/ABIF-Fiji-Workshop-2025

Digital images

- A digital image is simply a table of numbers
- Each number represents the intensity value of a pixel
- The intensity value is proportional to the amount of light collected at that location

- Software such as Fiji simply displays each number as a certain shade or colour



=

4106	4255	4198	4253	4395	4309	4277	4221	4188	4135	4140
4220	4280	4404	4487	4572	4586	4532	4446	4309	4198	4137
4262	4370	4549	4784	5076	5185	4879	4730	4404	4250	4188
4335	4480	4772	5404	6137	6635	6038	5066	4606	4274	4159
4347	4637	5067	6206	8438	10595	8548	5769	4599	4349	4099
4449	4612	5103	6472	10578	15650	11162	6041	4548	4171	4043
4376	4648	4985	6018	9530	13004	9980	5964	4581	4279	4124
4346	4456	4726	5370	6855	8213	6962	5261	4597	4339	4256
4230	4353	4627	4900	5444	5733	5288	4747	4438	4238	4198
4200	4329	4470	4651	4807	4746	4652	4461	4293	4231	4143
4193	4186	4372	4486	4518	4488	4407	4350	4249	4143	4122

Example images

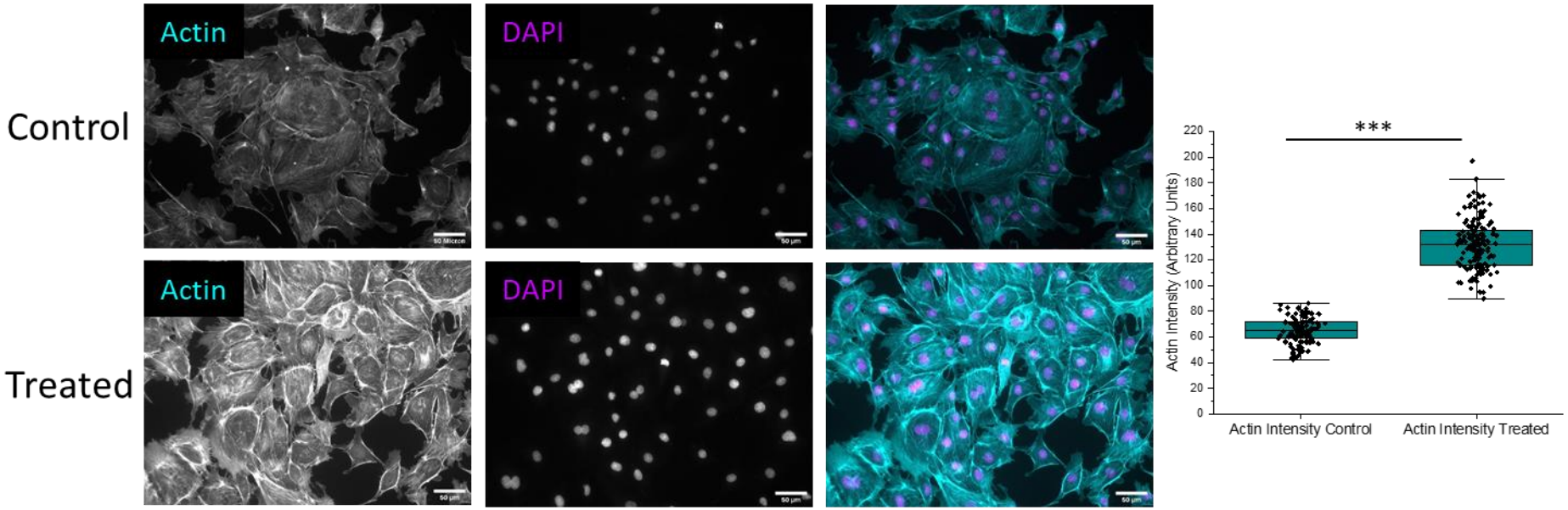
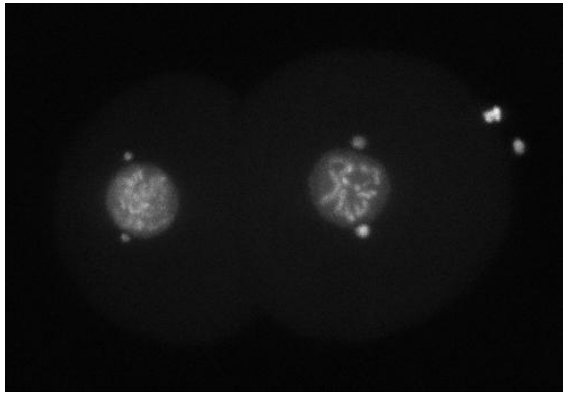
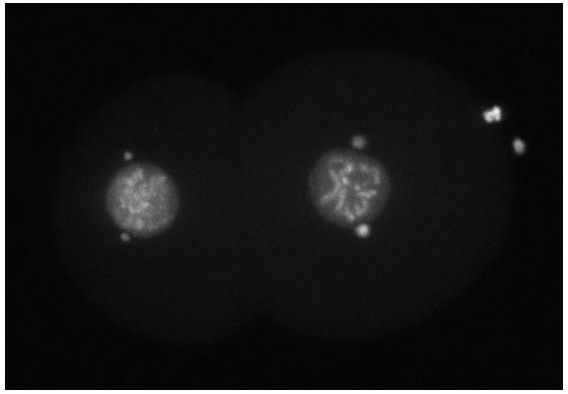


Figure 1: Images of CHO-K1 cells stained with Phalloidin Alexa Fluorophore 488 and stained with the nuclear probe DAPI. Images were flat field corrected using images of a fluorescent plastic slide imaged on the same microscope. Images were collected on an upright Zeiss Axioskop with a EC PlanNeoFluar 20x/0.5 NA objective lens with an AxioCam ICm1 camera. DAPI was imaged using a DAPI cube and an exposure time of 50 ms. Actin was imaged with a FITC cube and a 150 ms exposure. Brightness, gamma and contrast were adjusted to visualize the actin features well. The scale bar is 50 µm. Box plot of actin intensity for control (n=110) and treated (n=149) cells. T-test was done with a two sample t-test, unequal variance, two tailed. *** corresponds to $P<0.001$.

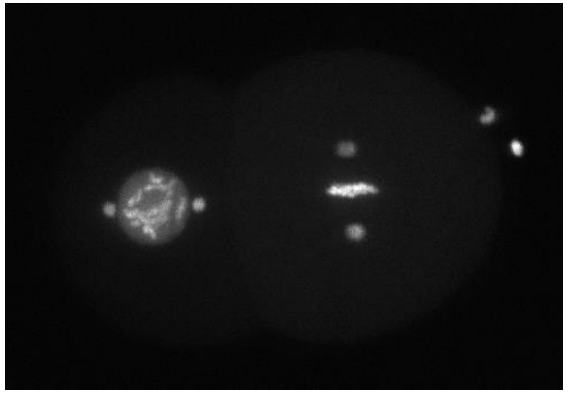
Example images



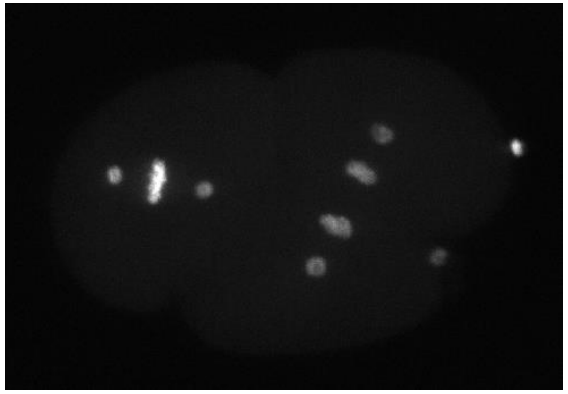
C. elegans **embryo**
eGFP-H2B
eGFP-gamma-tubulin
Max projection movie
One z-stack per minute acquisition
4 frames-per-second playback



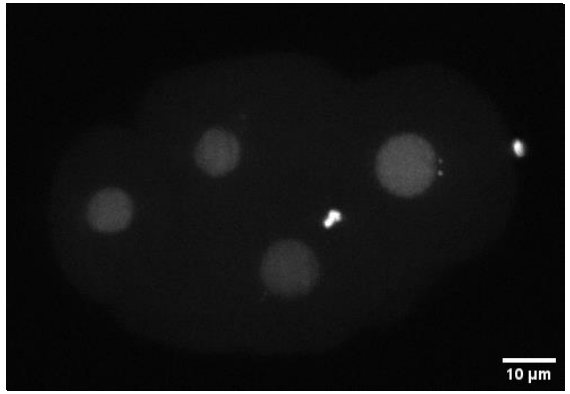
0 minutes



2 minutes

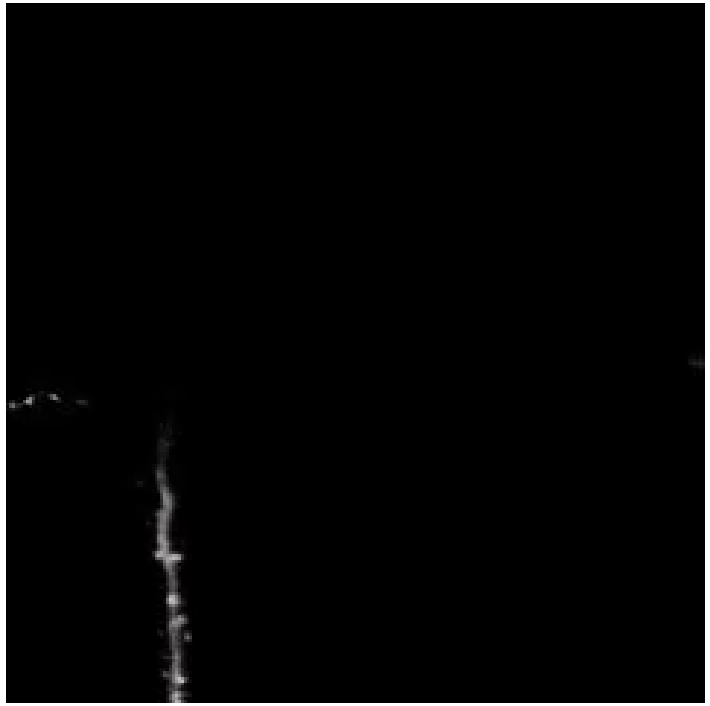


4 minutes



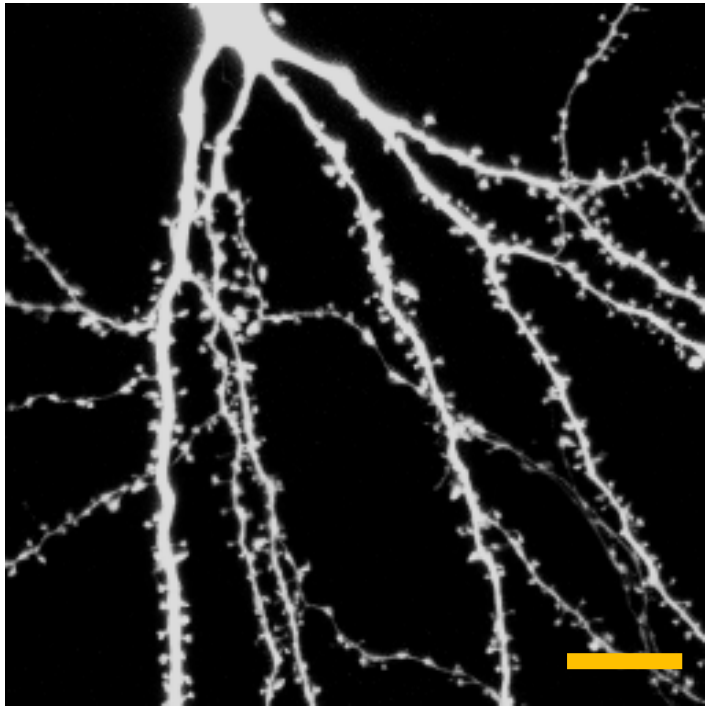
8 minutes

Example images



Imaris demo image

Pyramidal cell z-stack
69 z-slices
15 frames-per-second playback

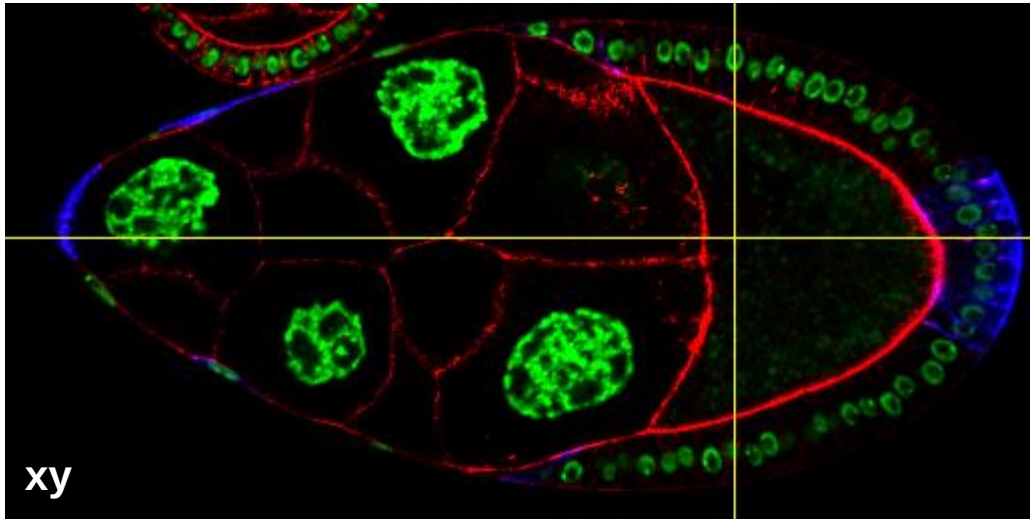


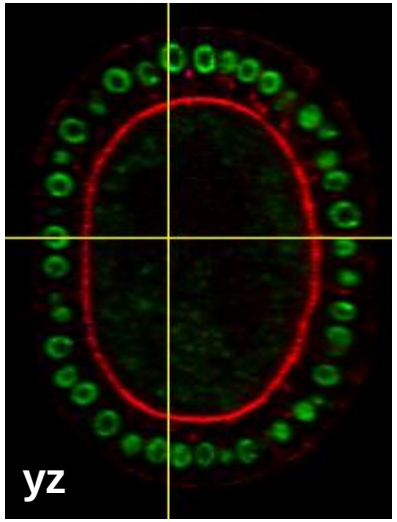
Maximum intensity projection (Fiji)
Scalebar: 10 um

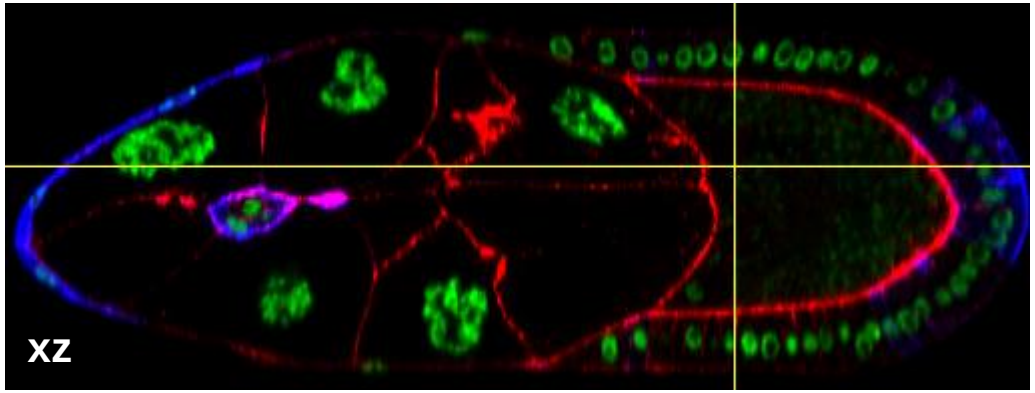
Example images

Imaris demo image

Drosophila egg Chamber
3 channels x 98 z-slices
16 frames-per-second playback

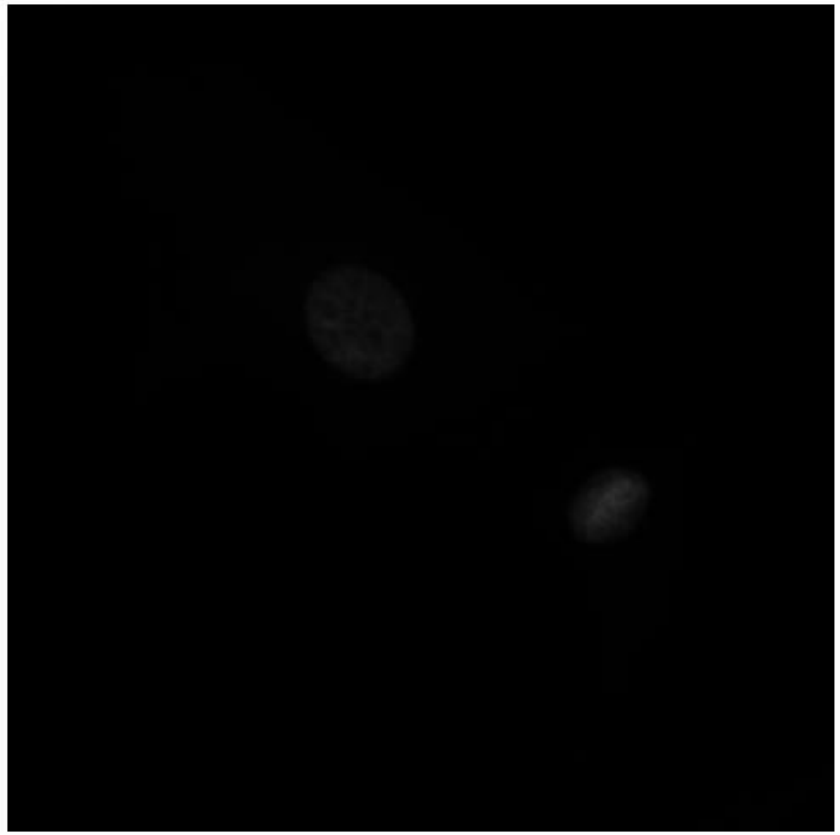






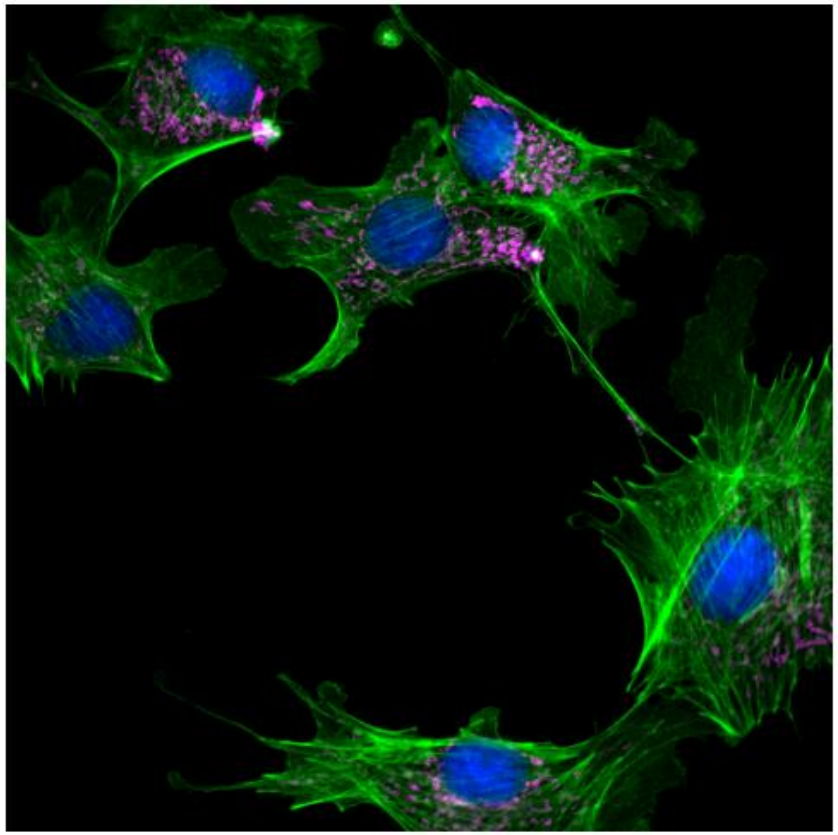
Orthogonal slice views

Exercise 1 – raw vs composite RGB images



demo_DAPI_Phalloidin_Mitotracker_001.tif

Raw image, drag-and-drop in powerpoint.



demo_DAPI_Phalloidin_Mitotracker_001.tif (RGB).tif

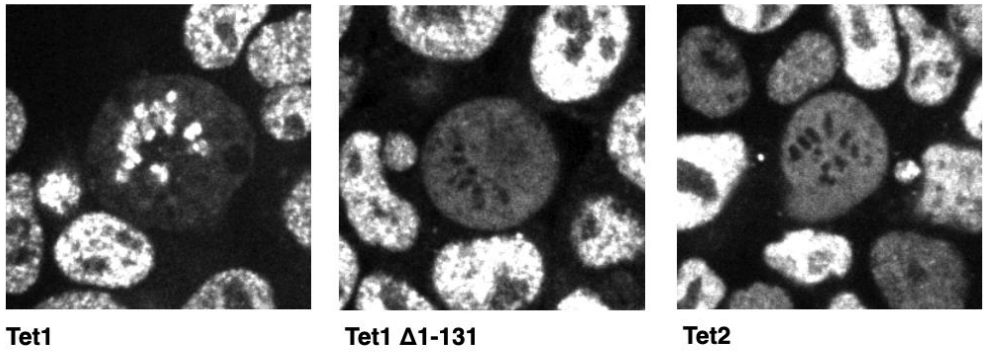
Composite RGB image generated with Exercise 1 macro, drag-and-drop in powerpoint.

Day 2: Quantitative imaging analysis in Fiji

- Goal:
  - Learn to use common image processing tools in Fiji in order to extract quantitative information

Introduction to quantitative imaging analysis

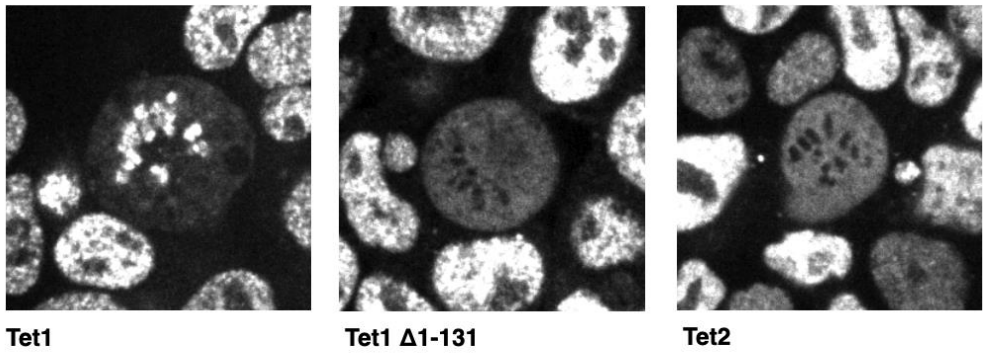
- Why quantitative analysis?

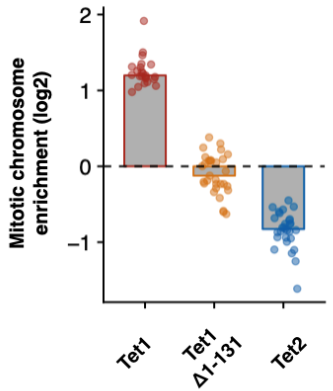


Ryan J., 2020

Introduction to quantitative imaging analysis

- Why quantitative analysis?

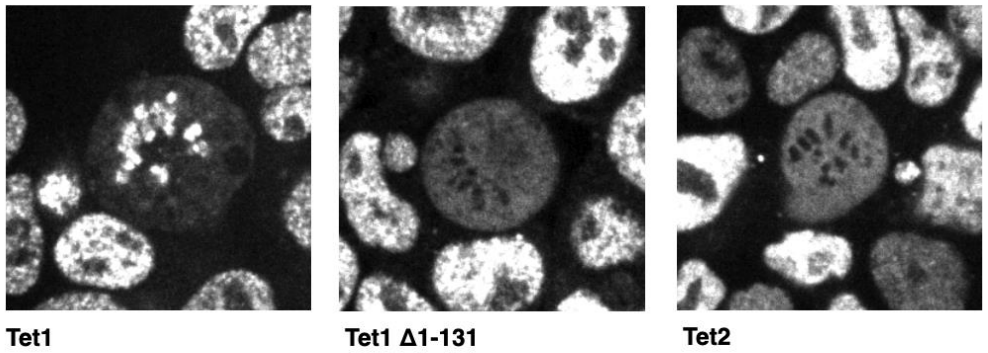




Ryan J., 2020

Introduction to quantitative imaging analysis

- Why quantitative analysis?



- Measured quantity (space, time, intensity etc)
- Increases robustness of observations
- Elucidate patterns that are not easily visible by eye

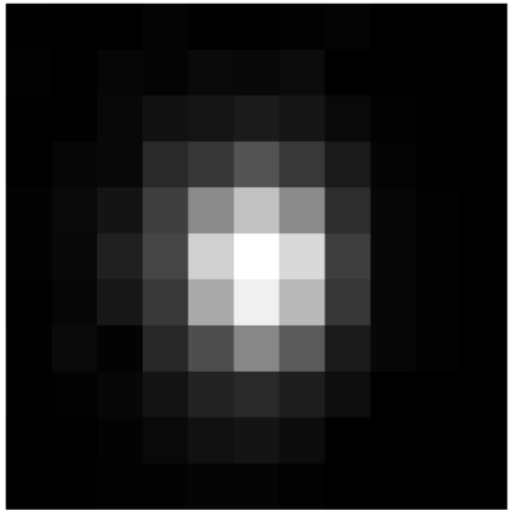
Better questions, better science!

Ryan J., 2020

Introduction to quantitative imaging analysis

Images can be quantitative:

- A digital image is simply a table of numbers
- Each number represents the intensity value of a pixel
- The intensity value is proportional to the amount of light collected at that location



=

4106	4255	4198	4253	4395	4309	4277	4221	4188	4135	4140
4220	4280	4404	4487	4572	4586	4532	4446	4309	4198	4137
4262	4370	4549	4784	5076	5185	4879	4730	4404	4250	4188
4335	4480	4772	5404	6137	6635	6038	5066	4606	4274	4159
4347	4637	5067	6206	8438	10595	8548	5769	4599	4349	4099
4449	4612	5103	6472	10578	15650	11162	6041	4548	4171	4043
4376	4648	4985	6018	9530	13004	9980	5964	4581	4279	4124
4346	4456	4726	5370	6855	8213	6962	5261	4597	4339	4256
4230	4353	4627	4900	5444	5733	5288	4747	4438	4238	4198
4200	4329	4470	4651	4807	4746	4652	4461	4293	4231	4143
4193	4186	4372	4486	4518	4488	4407	4350	4249	4143	4122

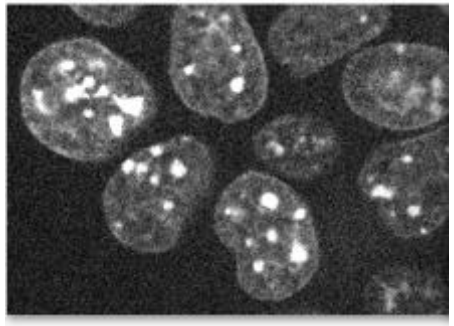
Value at each pixel = **Signal + Background + Noise**

Introduction to quantitative imaging analysis

- Goal:
  - Learn to use common image processing tools in Fiji in order to extract quantitative information

Introduction to quantitative imaging analysis

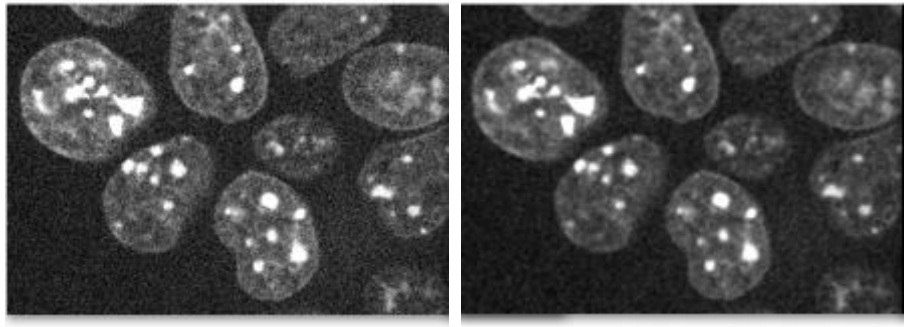
- Goal:
  - Learn to use common image processing tools in Fiji in order to extract quantitative information



Open an image

Introduction to quantitative imaging analysis

- Goal:
  - Learn to use common image processing tools in Fiji in order to extract quantitative information

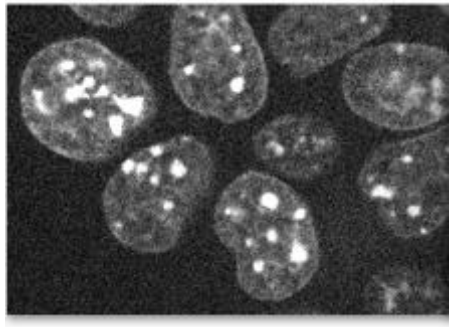


Open an image

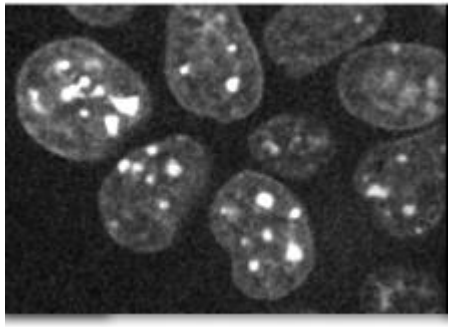
Processing such as filtering

Introduction to quantitative imaging analysis

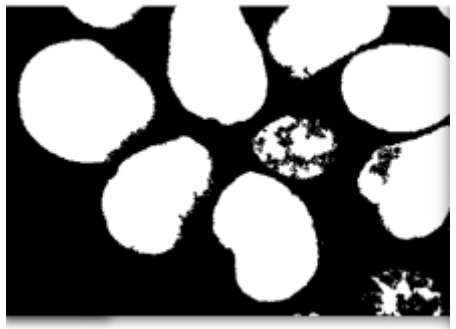
- Goal:
  - Learn to use common image processing tools in Fiji in order to extract quantitative information



Open an image



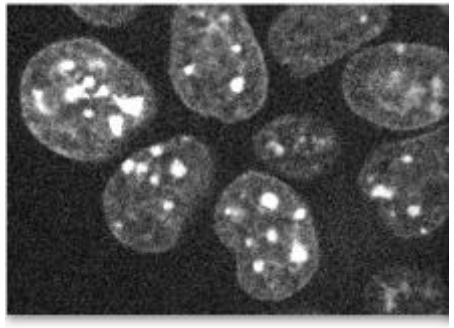
Processing such as filtering



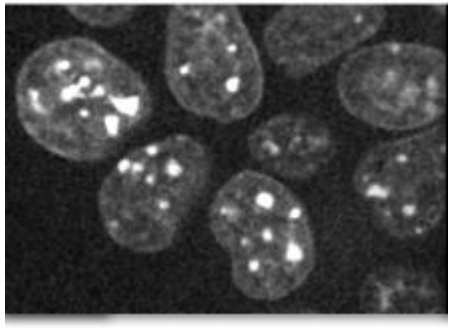
Segmentation or object detection

Introduction to quantitative imaging analysis

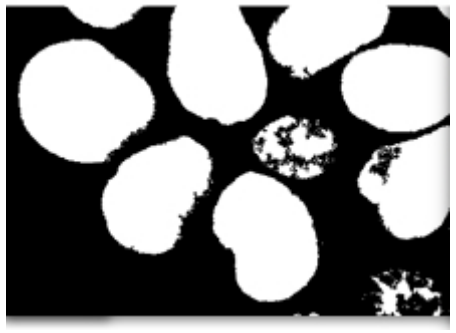
- Goal:
  - Learn to use common image processing tools in Fiji in order to extract quantitative information



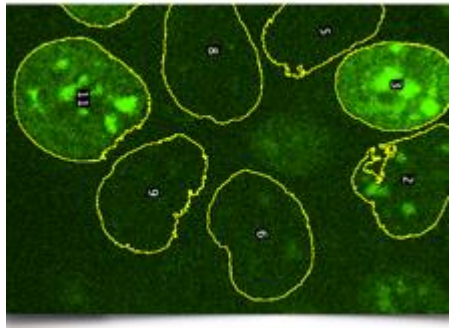
Open an image



Processing such as filtering



Segmentation or object detection

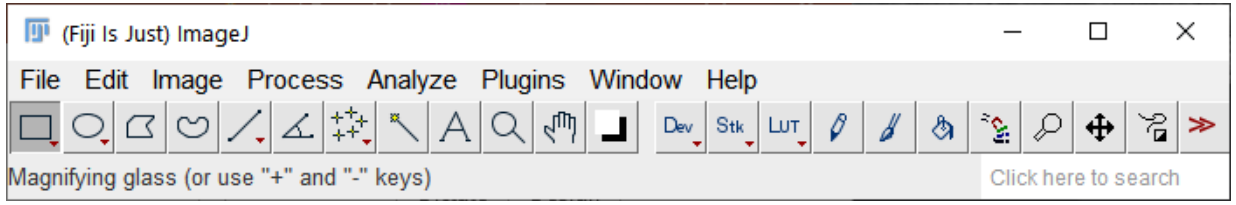


Data extraction from segmented objects

-Number of objects
-Shape / size
-Intensity within object…

Introduction to quantitative imaging analysis

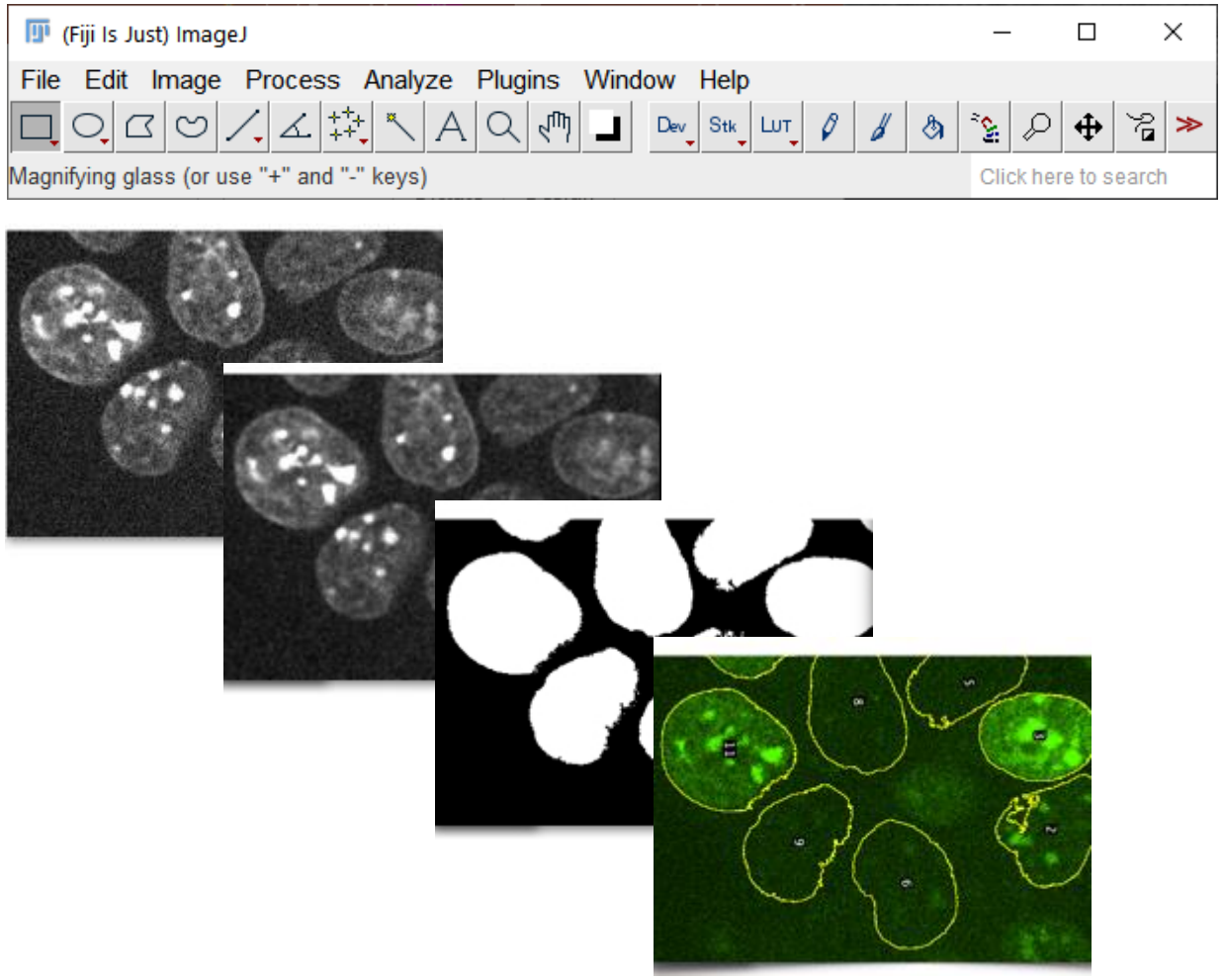
- Goal:
  - Learn to use common image processing tools in **Fiji** in order to extract quantitative information



- Why Fiji?
  - Free open-source software
  - Huge development community
  - Librairies to open nearly any microscope image (!)
  - Automation and scripting with multiple programming languages (!)

Introduction to quantitative imaging analysis

- **Today**:
  - Load images in Fiji, get familiarised with the program
  - Processing using convolutional filters
  - Segmentation with thresholding
  - Data extraction
    - …plotting?



for loop

```
for (i = 0; i < numROI; i++) {

    roiManager("Select", i);
    Stack.setChannel(2);
    run("Measure");

}
```

for loop

Start value

End value

Increment

```
for (i = 0; i < numROI; i++) {

    roiManager("Select", i);
    Stack.setChannel(2);
    run("Measure");

}
```

for loop

Start value

End value

Increment

```
for (i = 0; i < numROI; i++) {

    roiManager("Select", i);
    Stack.setChannel(2);
    run("Measure");

}
```

first, “build a sequence of integers going from the Start Value, to the End value, with an increment of 1”

for loop

Start value

End value

Increment

```
for (i = 0; i < numROI; i++) {

    roiManager("Select", i);
    Stack.setChannel(2);
    run("Measure");

}
```

first, “build a sequence of integers going from the Start Value, to the End value, with an increment of 1”

---in the case of numROI being 5:

0, 1, 2, 3, 4

for loop

Start value

End value

Increment

```
for (i = 0; i < numROI; i++) {

    roiManager("Select", i);
    Stack.setChannel(2);
    run("Measure");

}
```

first, “build a sequence of integers going from the Start Value, to the End value, with an increment of 1”

---in the case of numROI being 5:

0, 1, 2, 3, 4,

“Run the { content of the loop } replacing **i** with the first integer of the sequence.

Then run the { content of the loop } replacing i with the second integer in the sequence… “ and so on…

for loop

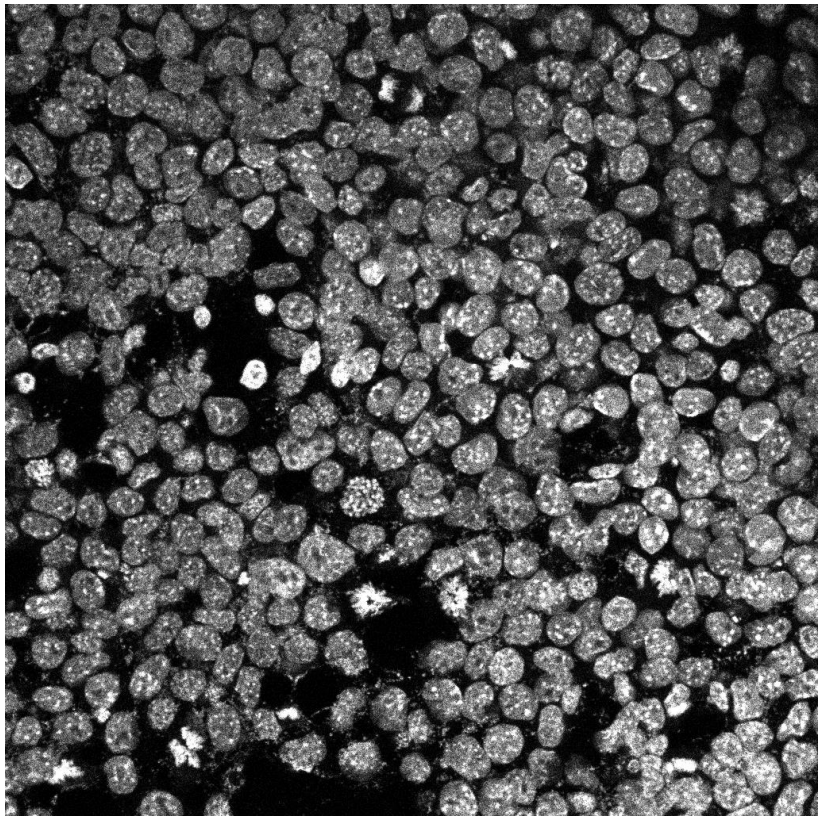
Start value End value Increment

```
for (i = 0; i < numROI; i++) {

    roiManager("Select", i);
    Stack.setChannel(2);
    run("Measure");

}
```

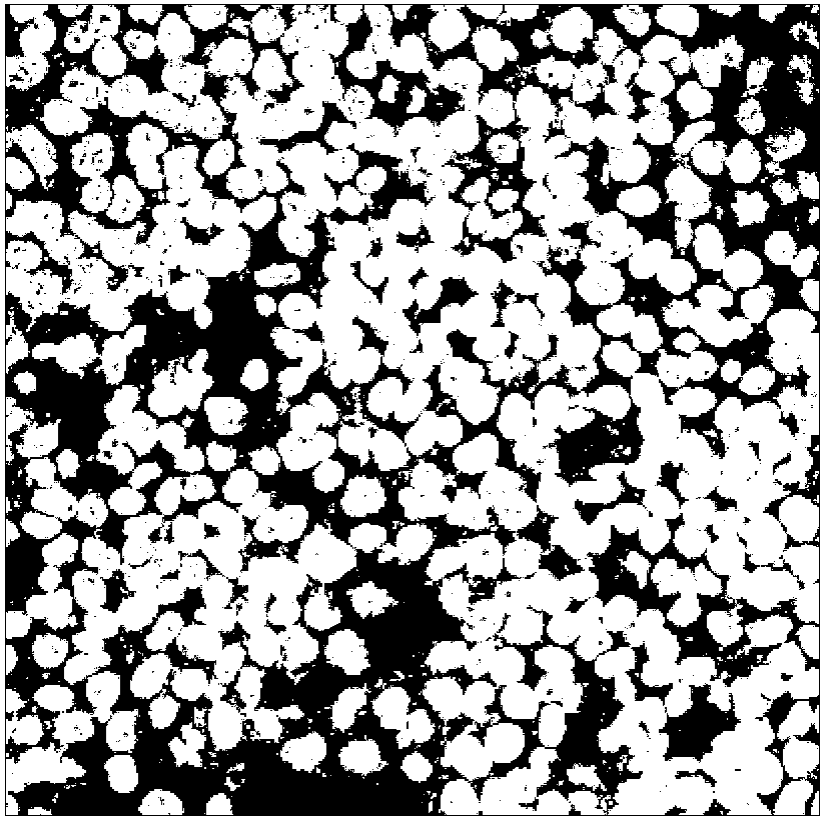
```
i = 0
roiManager("Select", i);
Stack.setChannel(2);
run("Measure");

i = 1
roiManager("Select", i);
Stack.setChannel(2);
run("Measure");

i = 2
roiManager("Select", i);
Stack.setChannel(2);
run("Measure");

i = 3
roiManager("Select", i);
Stack.setChannel(2);
run("Measure");

...
```

Deep Learning based segmentation

- Sometimes objects are too close for adequate thresholding



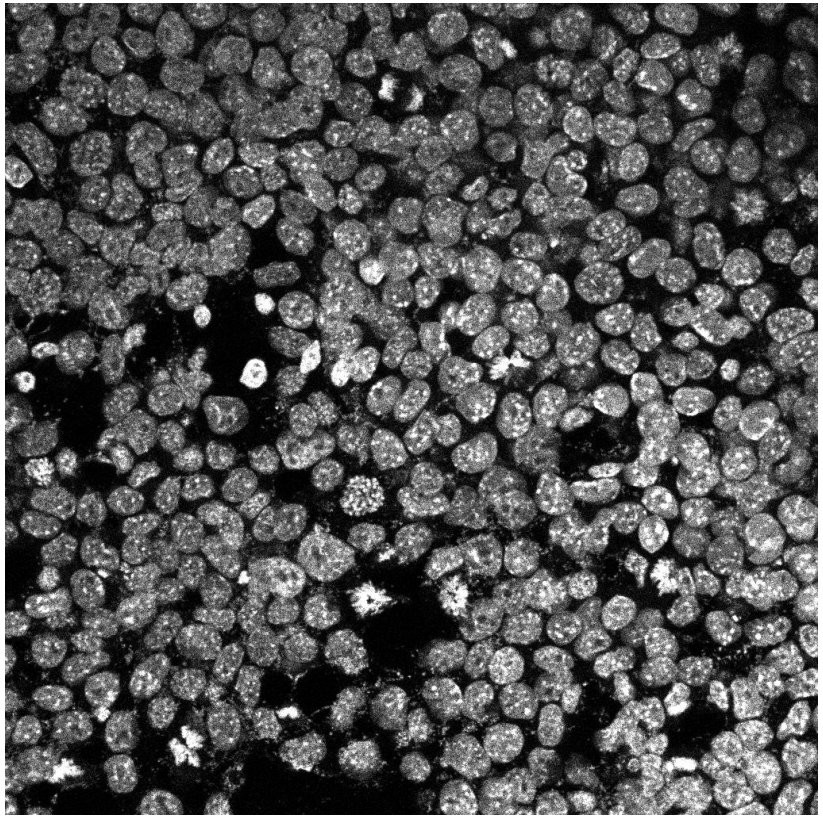
DAPI image



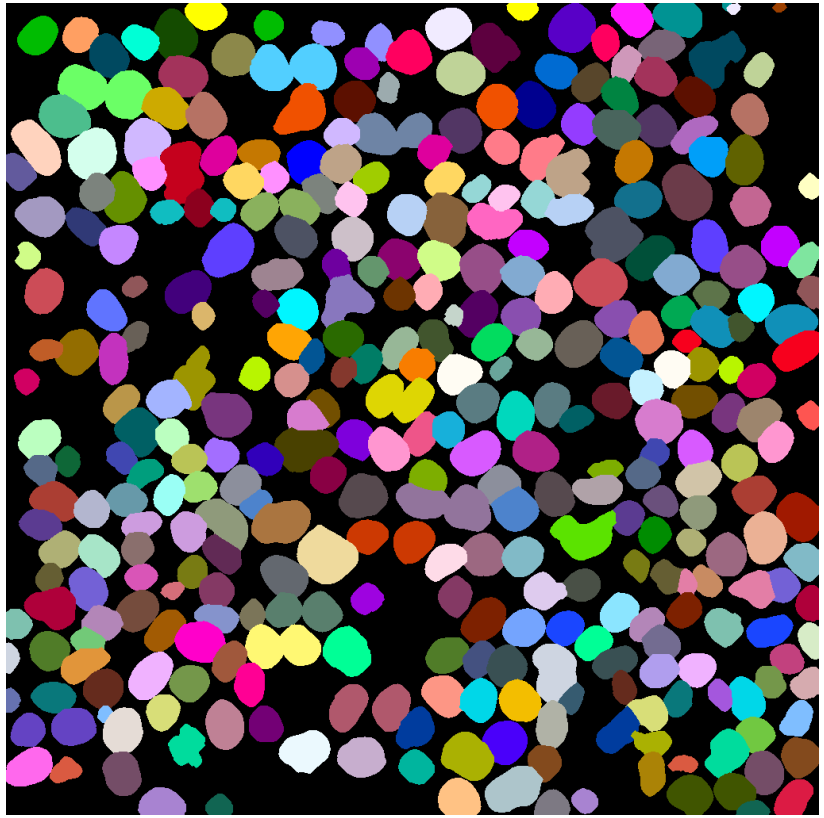
Otsu threshold image

Deep Learning based segmentation

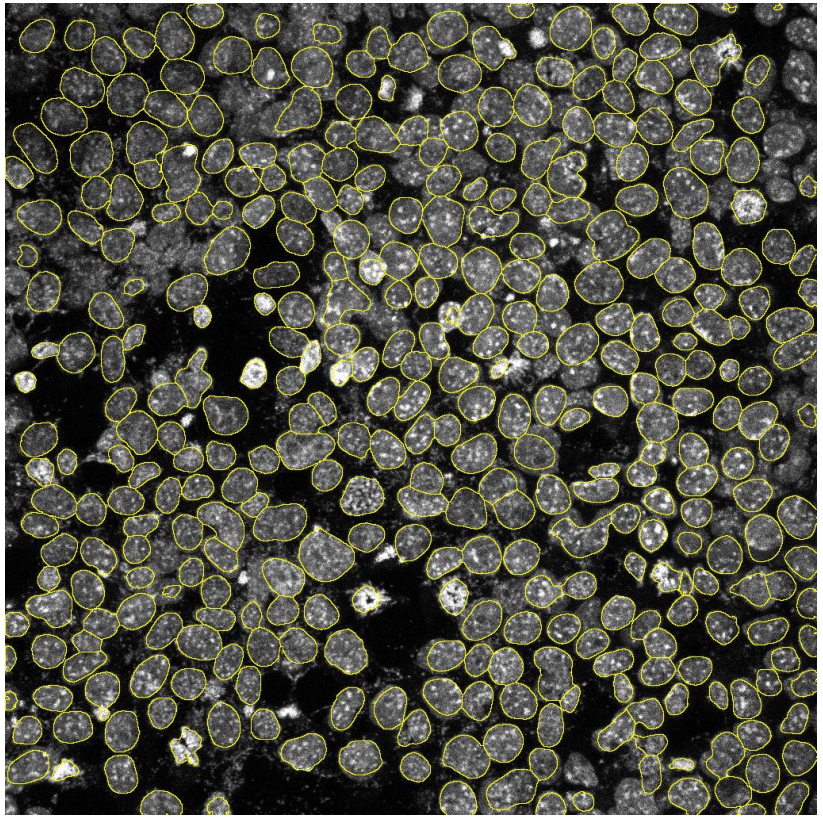
- Machine learning tools trained on cell images can help distinguish close objects



DAPI image



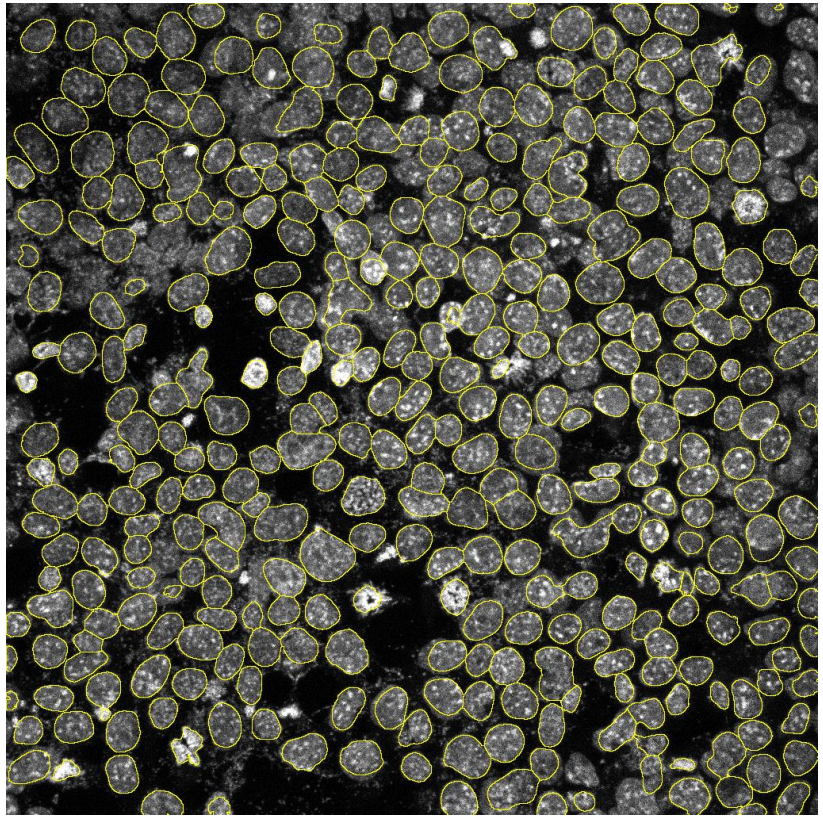
Nuclei masks identified with *Cellpose*



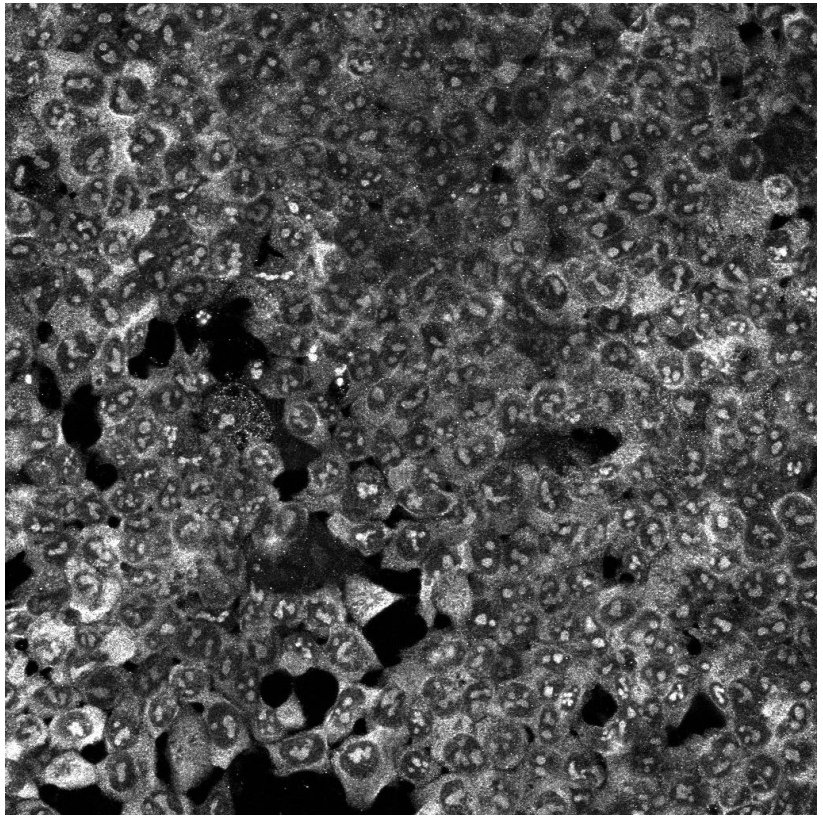
DAPI image + mask outlines

Deep Learning based segmentation

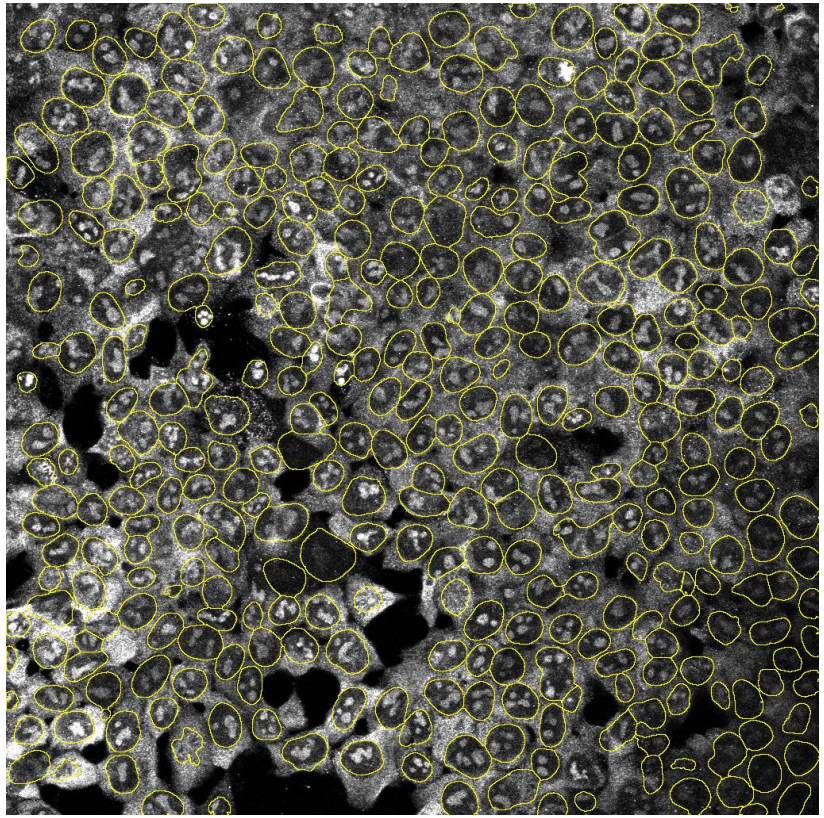
- Using the DAPI masks, we can then quantify intensities of other stainings in the nucleus



DAPI image + nucleus mask outlines



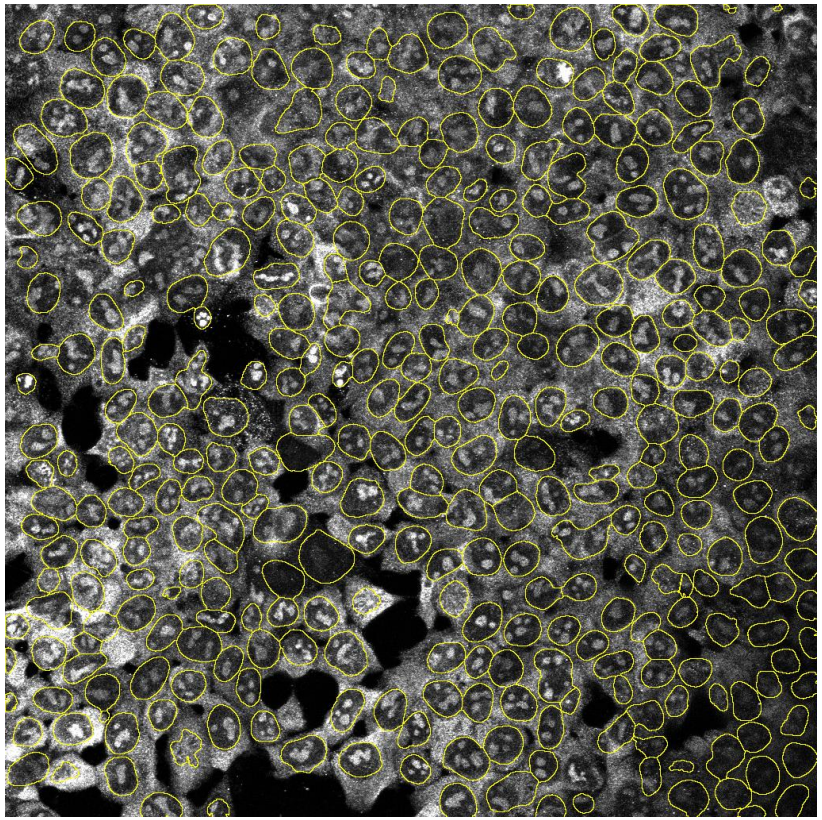
LIN28-GFP image



LIN28-GFP image + nucleus mask outlines

Deep Learning based segmentation

- Using the DAPI masks, we can then quantify intensities of other stainings in the nucleus

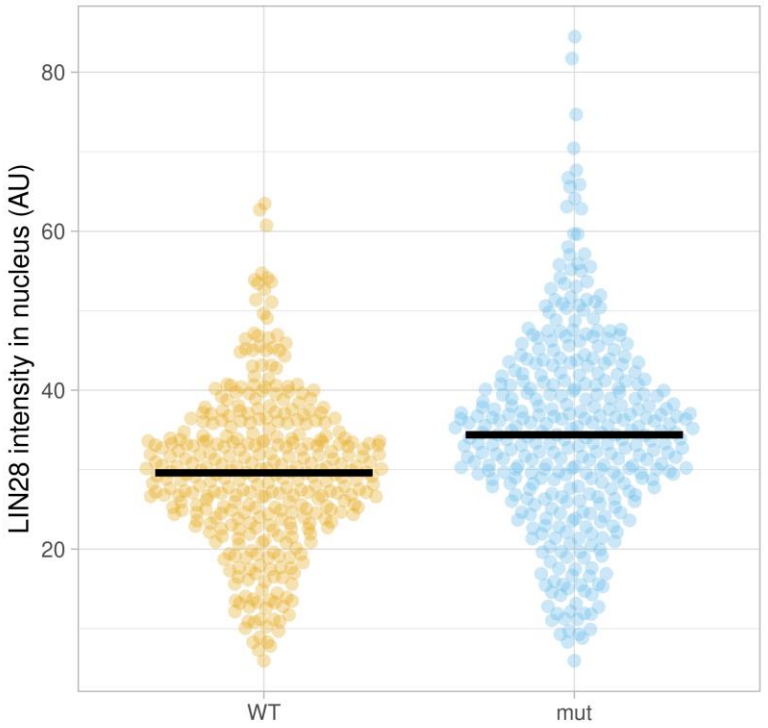


LIN28-GFP image + nucleus mask outlines

Results

File Edit Font Results

	Label	Area	Mean	StdDev	Mode
342	MAX_20240129_CGR8_iBidi_Timecourse_OTX2_SOX2_ESRRB_LIN28A_CGR8_OTX2_T0_1-2.tif:0949-0908	147.453	12.157	5.874	10
343	MAX_20240129_CGR8_iBidi_Timecourse_OTX2_SOX2_ESRRB_LIN28A_CGR8_OTX2_T0_1-2.tif:0944-0376	155.523	32.527	17.836	20
344	MAX_20240129_CGR8_iBidi_Timecourse_OTX2_SOX2_ESRRB_LIN28A_CGR8_OTX2_T0_1-2.tif:0935-0813	66.584	12.790	7.279	10
345	MAX_20240129_CGR8_iBidi_Timecourse_OTX2_SOX2_ESRRB_LIN28A_CGR8_OTX2_T0_1-2.tif:0950-0581	187.322	26.753	14.969	20
346	MAX_20240129_CGR8_iBidi_Timecourse_OTX2_SOX2_ESRRB_LIN28A_CGR8_OTX2_T0_1-2.tif:0951-0448	148.421	36.044	22.070	20
347	MAX_20240129_CGR8_iBidi_Timecourse_OTX2_SOX2_ESRRB_LIN28A_CGR8_OTX2_T0_1-2.tif:0952-1002	116.864	8.281	4.259	5
348	MAX_20240129_CGR8_iBidi_Timecourse_OTX2_SOX2_ESRRB_LIN28A_CGR8_OTX2_T0_1-2.tif:0960-0678	87.810	22.566	14.736	11
349	MAX_20240129_CGR8_iBidi_Timecourse_OTX2_SOX2_ESRRB_LIN28A_CGR8_OTX2_T0_1-2.tif:0963-0029	133.087	27.723	14.907	15
350	MAX_20240129_CGR8_iBidi_Timecourse_OTX2_SOX2_ESRRB_LIN28A_CGR8_OTX2_T0_1-2.tif:0963-0517	83.694	29.380	21.045	12
351	MAX_20240129_CGR8_iBidi_Timecourse_OTX2_SOX2_ESRRB_LIN28A_CGR8_OTX2_T0_1-2.tif:0971-0955	132.118	7.816	3.847	5
352	MAX_20240129_CGR8_iBidi_Timecourse_OTX2_SOX2_ESRRB_LIN28A_CGR8_OTX2_T0_1-2.tif:0972-0146	127.518	30.303	17.061	20
353	MAX_20240129_CGR8_iBidi_Timecourse_OTX2_SOX2_ESRRB_LIN28A_CGR8_OTX2_T0_1-2.tif:0972-0813	83.774	16.490	11.709	7
354	MAX_20240129_CGR8_iBidi_Timecourse_OTX2_SOX2_ESRRB_LIN28A_CGR8_OTX2_T0_1-2.tif:0959-0852	25.665	13.484	6.645	8
355	MAX_20240129_CGR8_iBidi_Timecourse_OTX2_SOX2_ESRRB_LIN28A_CGR8_OTX2_T0_1-2.tif:0967-0076	44.793	60.728	21.658	66
356	MAX_20240129_CGR8_iBidi_Timecourse_OTX2_SOX2_ESRRB_LIN28A_CGR8_OTX2_T0_1-2.tif:0991-0640	192.165	28.741	13.635	19
357	MAX_20240129_CGR8_iBidi_Timecourse_OTX2_SOX2_ESRRB_LIN28A_CGR8_OTX2_T0_1-2.tif:0994-0918	115.089	12.575	5.527	8

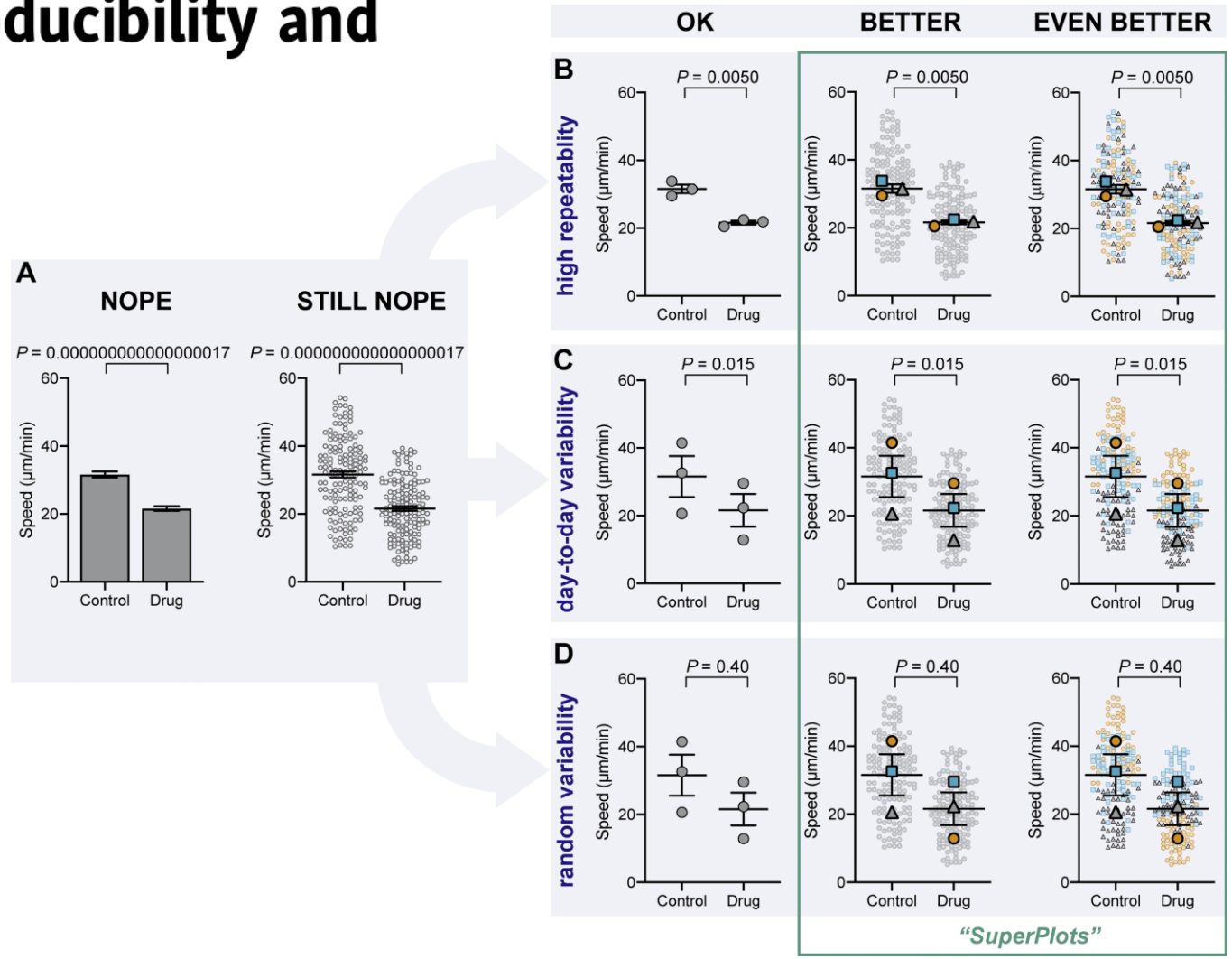


Plotting resources

SuperPlots: Communicating reproducibility and variability in cell biology

Samuel J. Lord[1], Katrina B. Velle[2], R. Dyche Mullins[1], and Lillian K. Fritz-Laylin[2]

- SuperPlots provides guidelines for presenting experimental data.
- Best to show all data points and colour code them per experimental replicate.
- For statistics the *n* value should be the number of experiments.



https://pubmed.ncbi.nlm.nih.gov/32346721/

Plotting resources

PlotsOfData - Plots all Of the Data

Data upload

- (•) Example 1 (wide format)
- ( ) Example 2 (tidy format)
- ( ) Upload file
- ( ) Paste data
- ( ) URL (csv files only)

[ ] These data are Tidy

Select and order:

Download in tidy format (csv)

[ ] Show information on data formats

Data offset

- (•) Quasirandom
- ( ) Random
- ( ) None; stripes
- ( ) None (for small n)

Visibility of the data

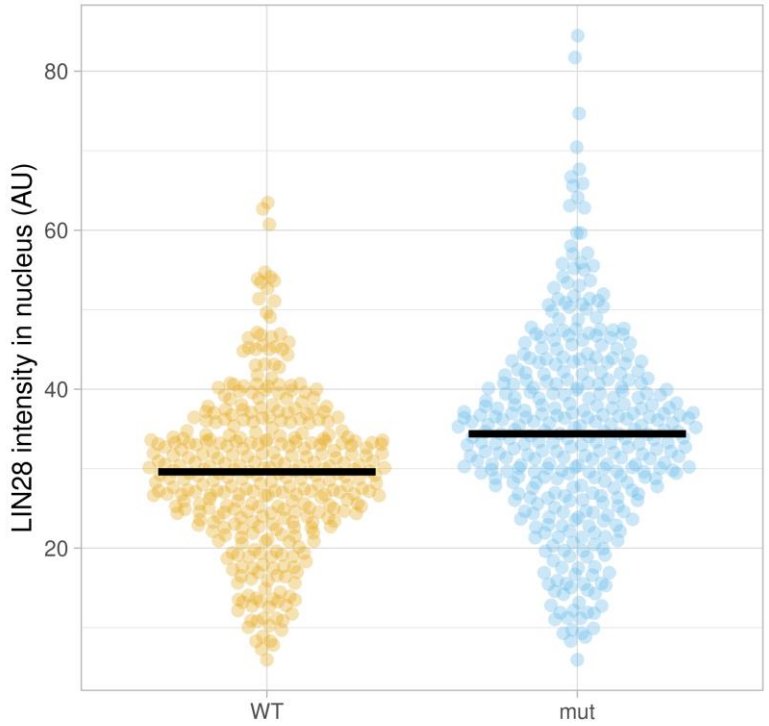
0 0.3 1

0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1

Statistics

- (•) Median
- ( ) Mean
- ( ) Boxplot (minimum n=10)
- ( ) Violin Plot (minimum n=10)

[ ] Add 95% CI (minimum n=10)



Generate plots with data points on PlotsOfData webapp

https://huygens.science.uva.nl/PlotsOfData/