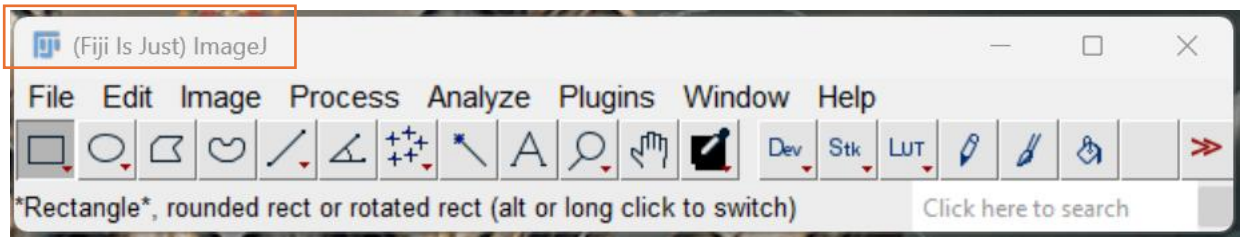
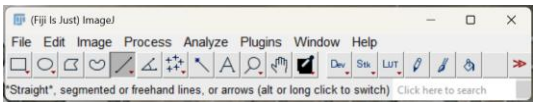
# General:

***Before/For the analysis***

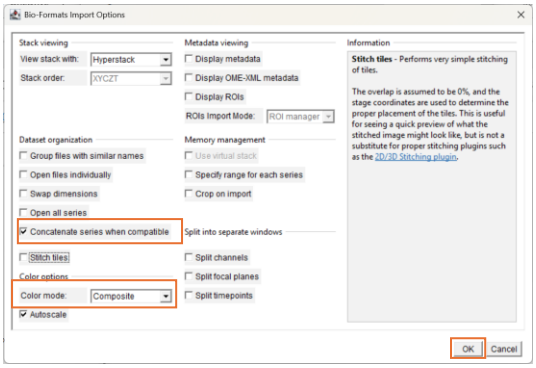
- bring computer mouse, if possible, to make our live easier
- download Fiji/ ImageJ
  version suitable for your operation system



# General 1: open images from .lif files

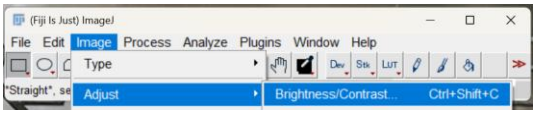



- drag and drop .lif file




Open via Bio-format
- Concatenate series when compatible
  ⇨ it will open images with the same "format" (channels)
- Color mode: Composite
- OK


Open via Bio-format
- Select All
- OK




Brightness/contrast
- to see all images for further analysis
- adjust minimum and maximum

Brightness/contrast
- adjust min and max

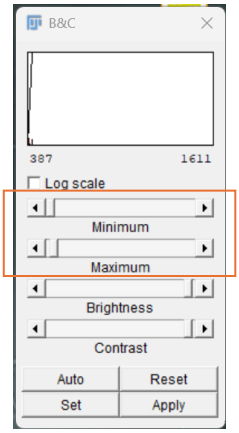
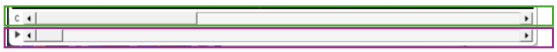


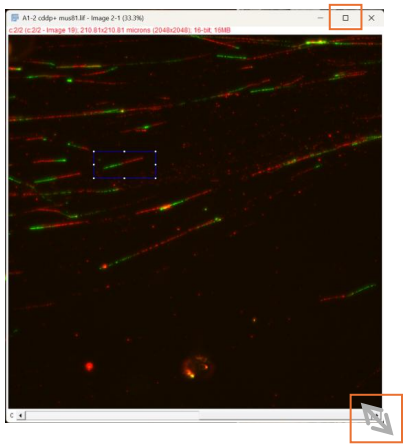

Image or Concatenate window
- lower bar



- to change channels
- to change images

***Note:***
zoom in/out:
- Shift + mouse wheel
- or + /- on keyboard

maximalize images window:
- first zoom in → max (upper bar) → move window around "space + right button on mouse
- or double side arrow drag from the corner

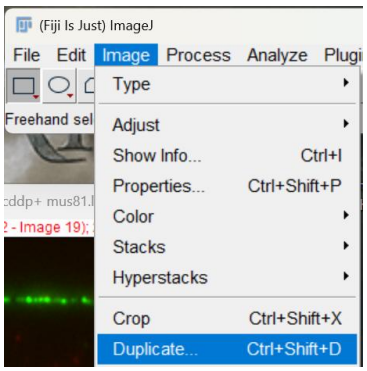


maximalize with "drag arrow", if max on upper bar doesn't work

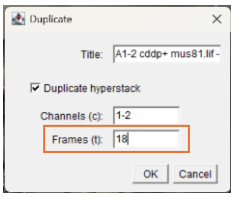
***Note:***
If there is problem with opening img or looks weird, e.g. open only one channel; check settings and properties in another software e.g. LASX again.

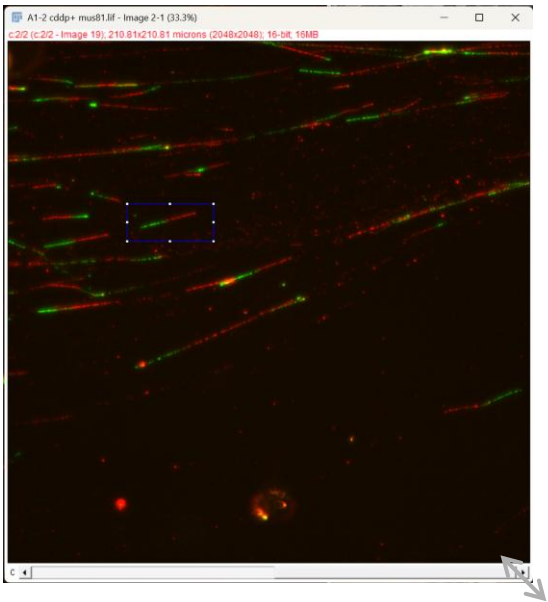
# General 2: Representative images/region of interest with scale bar; saving


- Select rectangle tool
- draw rectangle around object of interest
- if you want to rotate – select do more space around it

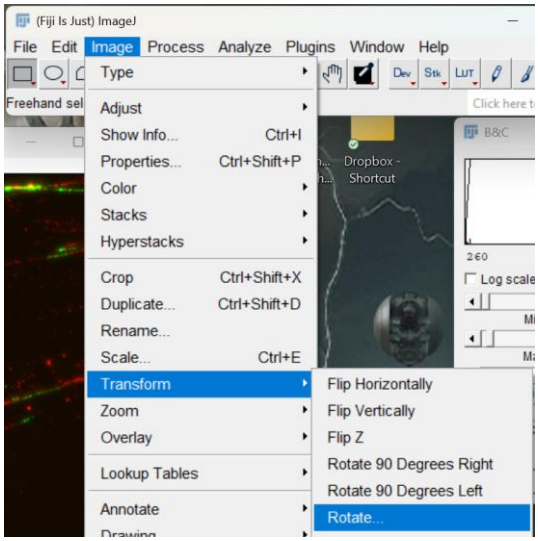


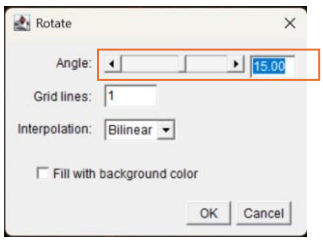

Duplicate
- just to have backup
- choose Frames (imgs) of interest

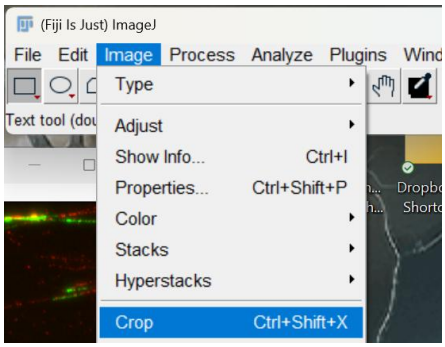


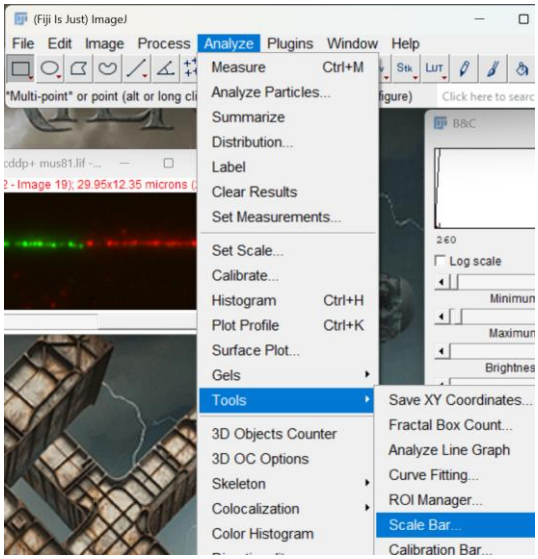



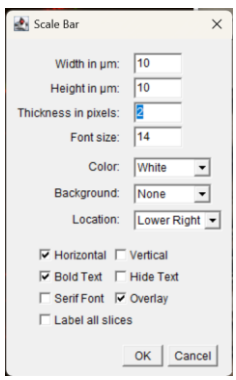

maximalize with “drag arrow”, if max on upper bar doesn’t work

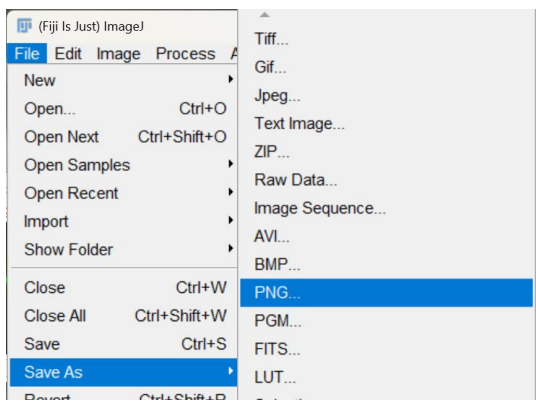


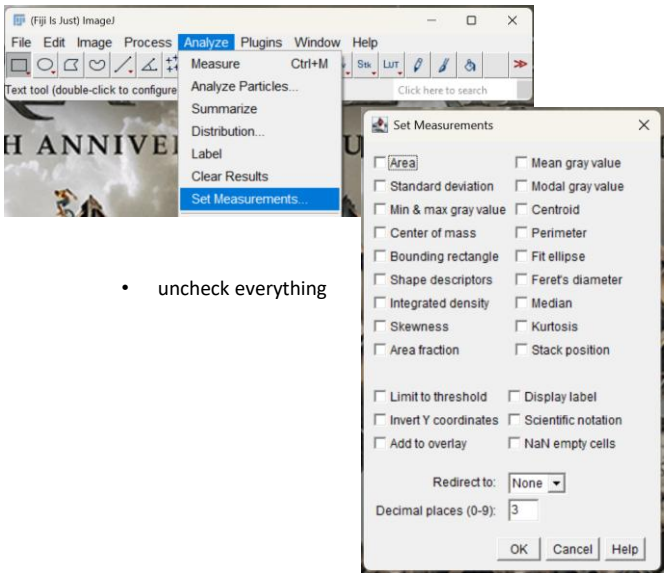



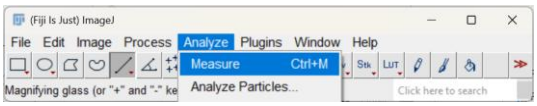



# 3. Analysis of the effect of R-loops on replication fork progression



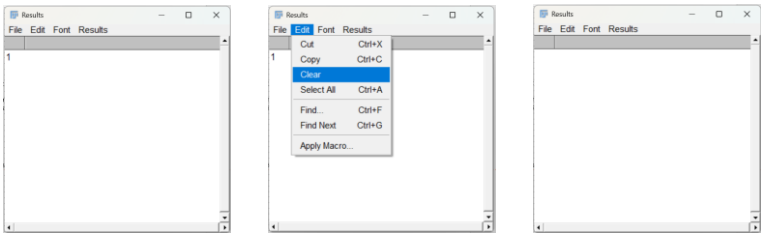

- uncheck everything

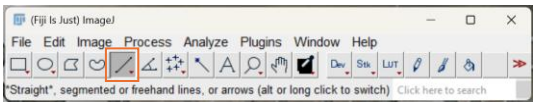


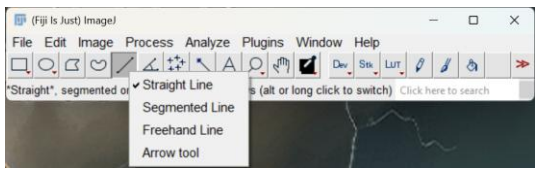

- delete the first – common mistake

Measurement
- Analyze → Measurement
  delete the first measurement

or
- press “M” on keyboard after first measurement
  no first measurement problem

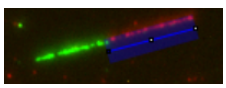


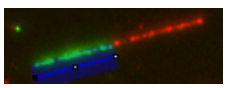




Measurement
- Select Straight line or other
- measure red part of fiber
- press “m”
- measure green part of the same fiber
- press “m”

Double click on tool to open window for changing Line Width – if you need, usually default setting are good for measurment





- measure 150 fibers in total (300 measurements)
- in Results table → Select All (ctrl + A)
- copy to Excel
- process in Excel
- option: finalize graph in GraphPad

measure red part of fiber
1. right button on mouse at the beginning of the fiber and hold & drag
2. press “m”

measure green part of the same fiber
1. right button on mouse at the beginning of the fiber and hold & drag
2. press “m”

## *Excel*

| | ↓↓ insert mearusment ↓↓ | | copy here |
|---|---|---|---|
| | Angle | Length | 11.459 |
| FALSE | 1 | 14.036 | 11.459 | 9.814 |
| TRUE | 2 | 15.202 | 9.814 | 13.324 |
| FALSE | 3 | 11.136 | 13.324 | 13.122 |
| TRUE | 4 | 11.31 | 13.122 | 8.867 |
| FALSE | 5 | 3.327 | 8.867 | 6.897 |
| TRUE | 6 | 0 | 6.897 | 3.846 |
| FALSE | 7 | -15.524 | 3.846 | 5.769 |
| TRUE | 8 | -15.524 | 5.769 | 15.464 |
| FALSE | 9 | 22.297 | 15.464 | 8.529 |
| TRUE | 10 | 17.56 | 8.529 | |

| | ↓↓ insert mearusment ↓↓ | | copy here |
|---|---|---|---|
| | Angle | Length | 11.459 |
| FALSE | 1 | 14.036 | 11.459 | 9.814 |
| FALSE | 3 | 11.136 | 13.324 | 13.122 |
| FALSE | 5 | 3.327 | 8.867 | 6.897 |
| FALSE | 7 | -15.524 | 3.846 | 5.769 |
| FALSE | 9 | 22.297 | 15.464 | 8.529 |

| CldU | IdU | both | ratio |
|---|---|---|---|
| =MEDIAN(B7:B102) | | #NUM! | #NUM! |

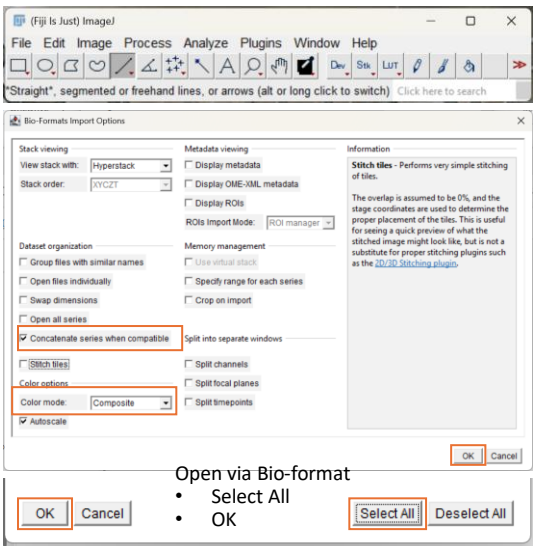
MEDIAN(number1, [number2], ...)

| CldU | IdU | CldU + IdU | IdU/CldU |
|---|---|---|---|
| #NUM! | #NUM! | #NUM! | #NUM! |

- first column FALSE/TRUE
  =ISEVEN(“next column”)
- 2nd – 4th …. insert measurement from FIJI
- copy 4th column to next, one row up
- set filter for first column

- create representative images

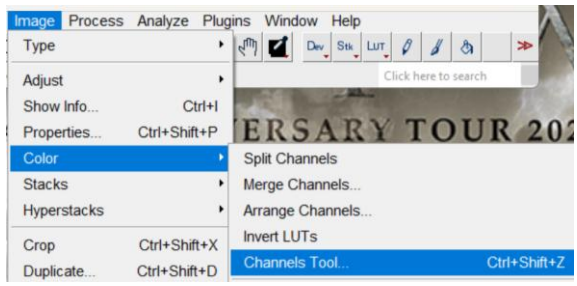
# 2. Monitoring the formation of R-loops in human cell

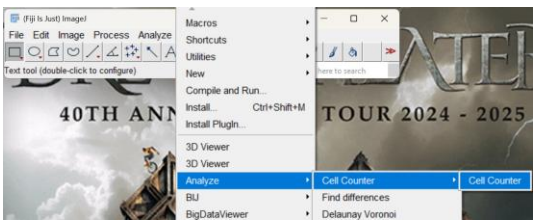



- drag and drop .lif file

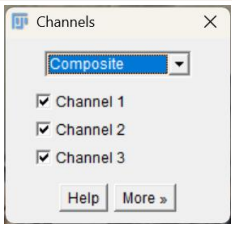
Open via Bio-format
- Concatenate series when compatible
  ⇨ it will open images with the same "format" (channels)
- Color mode: Composite
- OK

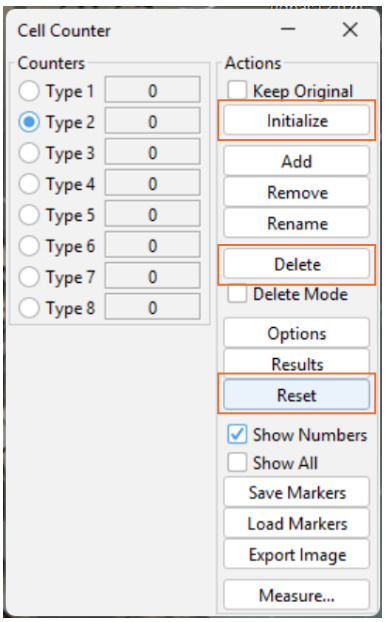
Open via Bio-format
- Select All
- OK




Channels Tool
- switch ON/OFF visibility of individual channels in concatenate
- can be useful for counting foci




Initialize
- to start
- for each concatenate
- it will reset previous image adjustment (e.g. B&C)

Counters (Type)
- select
- each has different color choose best contrast

Delete
- delete the last point

Reset
- delete all
- at the end of counting

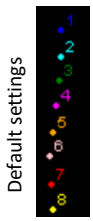
Rename
- change name of types

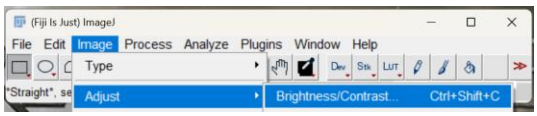
Option
- change color of types

***Note:***
Initialize change the image window to normal size (as it was open); for maximalize – it has to be adjusted again.

***Note:***
Numbers are visible only on the channel, where you count them. When you change channel, you will not see previous – but you can make a new one (add to previous in counting) ??? Therefore, count always on one channel. ???

***Example:***
Type … : PCNA-, RNH1-
Type …. : PCNA-, RNH1+
Type … : CNA+,RNH1-
Type … : PCNA+, RNH1+




Brightness/contrast
- to see all images for further analysis

Brightness/contrast
- adjust min and max

- to change channels
- to change images

Analysis
For each condition (NT, CPT, CDDP, MMC, APH), determine
- the percentage of PCNA-negative and
- PCNA-positive cells with >10 RNH1D210N-GFP foci.
- score at least 150 PCNA (-) and 150 PCNA (+) cells for each condition

Plot the data using GraphPad Prism and select representative (page 2, General 2) images of cells.