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# **Fiji Exercises Collection**

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**Jan 17, 2025**



# CONTENTS

<b>1</b>	<b>1 - Working with Single-Channel Images</b>	<b>3</b>
1.1	1.1 - Image inspection . . . . .	3
1.2	1.2 - Adjust brightness/contrast of all open images . . . . .	3
1.3	1.3 - File handling and non-invasive editing . . . . .	4
1.4	1.4 - File handling and invasive editing . . . . .	4
<b>2</b>	<b>2 - Working with Multi-Channel Images</b>	<b>5</b>
2.1	2.1 - composite images - splitting and merging . . . . .	5
<b>3</b>	<b>3 - Image Processing: Filters</b>	<b>7</b>
3.1	3.1 - Edge filters - vertical stripes . . . . .	7
3.2	3.2 - Edge filters - horizontal stripes . . . . .	8
3.3	3.3 - Edge filters - checkerboard . . . . .	8
3.4	3.4 - Morphological filters - Binary . . . . .	9
3.5	3.5 - Morphological filters - Gray . . . . .	9
<b>4</b>	<b>4 - Segmentation</b>	<b>11</b>
4.1	4.1 - DAPI segmentation with thresholding . . . . .	11
4.2	4.2 - DAPI segmentation with filters and thresholding . . . . .	12
4.3	4.3 - DAPI segmentation with Weka . . . . .	13
<b>5</b>	<b>5 - Spot Detection</b>	<b>15</b>
5.1	5.1 - Manual spot detection with the Multi-point Tool . . . . .	15
5.2	5.2 - Algorithmic spot detection with Find Maxima . . . . .	15
5.3	5.3 - Automatic spot segmentation with thresholding . . . . .	16
5.4	5.4 - Spot detection with noise . . . . .	16
5.5	5.5 - Spot detection with variable background . . . . .	17



By the Image Analysis Collaboratory @ Harvard Medical School

The exercises are designed to be used in a classroom setting, but can also be used for self-study. They are divided into sections, each of which covers a different topic and/or a different aspect of Fiji.

Here is an example illustrating the structure of the exercises:

### 1.1 - Image inspection

1. open the `single_channel.tif` image (drag & drop, or `File > Open`)
2. `Image > Duplicate (IJ:28.9)` (**shift + d**)
3. `Analyze > Histogram (IJ:30.10)` (**h**)
4. click `Live`
5. `Image > Adjust > Brightness/Contrast` (**shift + c**)
  - play with `Maximum`, `Minimum`, `Brightness` and `Contrast` sliders and with `Auto`, `Set` and `Reset`
  - observe the histogram window, what is changing, what is not?
  - click `Apply`
  - what changed?

You can perform each exercise using the **provided image(s)** (click the image name to download it - note that downloads may not work properly in Safari) or with **your own images**.

For certain Fiji commands, references to the ImageJ User Guide are included (e.g. **IJ:28.9**) as a helpful resource for learning more about Fiji's features and capabilities.

If a command has a keyboard shortcut, it is indicated in parentheses in **bold** (e.g. **shift + d**).

Have fun!

- Fiji Exercises
  - *1 - Working with Single-Channel Images*
  - *2 - Working with Multi-Channel Images*
  - *3 - Image Processing: Filters*
  - *4 - Segmentation*
  - *5 - Spot Detection*



## 1 - WORKING WITH SINGLE-CHANNEL IMAGES

### 1.1 1.1 - Image inspection

1. open the `single_channel.tif` image (drag & drop, or `File > Open`)
2. `Image > Duplicate (IJ:28.9) (shift + d)`
3. `Analyze > Histogram (IJ:30.10) (h)`
4. click `Live`
5. `Image > Adjust > Brightness/Contrast (shift + c)`
  - play with `Maximum`, `Minimum`, `Brightness` and `Contrast` sliders and with `Auto`, `Set` and `Reset`
  - observe the histogram window, what is changing, what is not?
  - click `Apply`
  - what changed?

### 1.2 1.2 - Adjust brightness/contrast of all open images

1. open the three `single_channel_...tif` images in folder `for_exercise_1.2` (drag & drop, or `File > Open`)
2. for all images: `Analyze > Histogram (IJ:30.10) (h)`
  - click `Live`
3. for one image: `Image > Adjust > Brightness/Contrast (shift + c)`
4. adjust contrast with `Maximum`, `Minimum`, `Brightness` or `Contrast` sliders or `Auto`
5. click `Set`
6. check `Propagate to all other open images` checkbox
7. click `OK`
8. what happened to the histograms?
9. what happened to the images?
10. did pixel values change?
11. pick another image and repeat steps 4–7
12. what happened, what is different, is it better/worse?

## 1.3 1.3 - File handling and non-invasive editing

1. File > Open Samples > Blobs (IJ:26.4) (**shift + b**)
2. Analyze > Tools > Scale Bar (IJ:30.14.6)
  - set Color, Background, Location
  - check: Overlay, Bold Text
3. File > Save As > Tiff -> blobs1.tif (IJ:26.10.1)
  - saving the image as tiff keeps the scale bar as an overlay, so pixel values below it are kept
  - NOTE: you can see the overlay scale bar in the saved image **only** if you open it in Fiji
  - continue with [1.4](#)

## 1.4 1.4 - File handling and invasive editing

1. open the blobs1.tif saved in [1.3](#) (drag & drop, or File > Open)
2. Image > Info (IJ:28.3) (**i**)
3. Image > Overlay > Remove Overlay (IJ:28.14.7)
  - the scale bar should disappear since it was saved as an overlay
4. Analyze > Set Scale (IJ:30.8)
  - set scale such as image is 100 um long in each dimension
5. Analyze > Tools > Scale Bar (IJ:30.14.6)
  - set Color, Background, Location
  - uncheck Overlay
6. File > Save As > Tiff -> blobs2.tif (IJ:26.10.1)
7. load again to check the difference (e.g. check info, hover over the scale bar and look at the pixel values)



## 2 - WORKING WITH MULTI-CHANNEL IMAGES

### 2.1 2.1 - composite images - splitting and merging

1. File > Open Samples > Fluorescent Cells (IJ:26.4)
2. Image > Color > Arrange Channels
  - click on New 1, and select Magenta
3. split channels
  - Image > Color > Split Channels (IJ:28.5.1)
4. merge channels to composite
  - Image > Color > Merge Channels (IJ:28.5.2)
  - check create composite checkbox (IJ:28.5.2)
5. LUT (look-up table) (IJ:19.17 & IJ: 28.15)
  - change LUT of the channels (LUT in the startup tools or Image > Lookup Tables)
6. color blindness
  - convert to RGB Color: Image > Type > RGB Color (IJ:7)
  - Image > Color > Simulate Color Blindness
  - Image > Color > Dichromacy



## 3 - IMAGE PROCESSING: FILTERS

### 3.1 3.1 - Edge filters - vertical stripes

1. open the vertical\_stripes.tif image (drag & drop, or File > Open)
2. Image > Duplicate (IJ:28.9) (**shift + d**)
3. apply horizontal Prewitt filter:
  - Process > Filters > Convolve
  - as kernel input (Prewitt):

```
-1 -1 -1
0  0  0
1  1  1
```

- click on OK.
  - do you understand the output of this process?
4. select the original vertical\_stripes.tif image and duplicate it again.
    - Image > Duplicate (IJ:28.9) (**shift + d**)
  5. apply vertical Prewitt filter:
    - Process > Filters > Convolve
    - as kernel input (Prewitt):

```
-1  0  1
-1  0  1
-1  0  1
```

- click on OK.
- do you understand the output of this process?

## 3.2 3.2 - Edge filters - horizontal stripes

The steps in this exercise are identical to 3.1, only the input image differs.

1. open the horizontal\_stripes.tif image (drag & drop, or File > Open)
2. Image > Duplicate (IJ:28.9) (**shift + d**)
3. apply horizontal Prewitt filter:
  - Process > Filters > Convolve
  - as kernel input (Prewitt):

```
-1 -1 -1
0 0 0
1 1 1
```

- click on OK.
  - do you understand the output of this process?
4. select the original vertical\_stripes.tif image and duplicate it again.
    - Image > Duplicate (IJ:28.9) (**shift + d**)
  5. apply vertical Prewitt filter:
    - Process > Filters > Convolve
    - as kernel input (Prewitt):

```
-1 0 1
-1 0 1
-1 0 1
```

- click on OK.
- do you understand the output of this process?

## 3.3 3.3 - Edge filters - checkerboard

The steps in this exercise are identical to 3.1 and 3.2 only the input image differs.

1. open the checkerboard.tif image (drag & drop, or File > Open)
2. Image > Duplicate (IJ:28.9) (**shift + d**)
3. apply horizontal Prewitt filter:
  - Process > Filters > Convolve
  - as kernel input (Prewitt):

```
-1 -1 -1
0 0 0
1 1 1
```

- click on OK.
- do you understand the output of this process?

4. select the original `checkerboard.tif` image and duplicate it again.

- Image > Duplicate (IJ:28.9) (**shift + d**)

5. apply vertical Prewitt filter:

- Process > Filters > Convolve
- as kernel input (Prewitt):

```
-1  0  1
-1  0  1
-1  0  1
```

- click on OK.
- do you understand the output of this process?

### 3.4 3.4 - Morphological filters - Binary

1. open the `exercise_morphology.tif` image (drag & drop, or File > Open)

2. duplicate the image 4 times and name each copy as follow:

- Eroded
- Dilated
- Closed
- Opened

3. apply binary morphological filters:

- Process > Binary (Erode, Dilate, Close, Open)
- Note: Make sure you apply the operation that corresponds to the name of each image.

4. report findings

### 3.5 3.5 - Morphological filters - Gray

1. open the `exercise_morphology.tif` image (drag & drop, or File > Open)

2. apply Gray Morphological filters:

- Process > Morphology > Gray Morphology

3. apply image **opening** with **circular** structuring element with **radius 3** - removes noise

4. Report: did it get rid of the noise?

5. apply image **opening** with **circular** structuring element with **radius 5**

6. report: did it get rid of the tentacles from the top-left object?

7. with the line tool (IJ:19.2.1), measure the diameter of the holes in the bottom-right object.

8. apply image **closing** with **circular** structuring element with **radius slightly larger than the measured radius** (diameter/2).

9. report: did it get rid of the tentacles from the top-left circle?



## 4 - SEGMENTATION

### 4.1 4.1 - DAPI segmentation with thresholding

1. open the DAPI.tif image (drag & drop, or File > Open)
2. change LUT to Grays
3. Image > Duplicate (IJ:28.9) (**shift + d**)
4. Image > Adjust > Threshold (IJ:28.2.4)
5. understand the function of the Dark Background checkbox (inspect pixel values)
6. try setting sliders manually. Can you find a good threshold range?
7. try different algorithms by selecting them in the left dropdown menu. Can you find one that gives a good result?
  - NOTE: Image > Adjust > Auto Threshold, if you want to see all at the same time
8. try different display options (Red, B&W, Over/Under) by selecting them in the right dropdown menu, do you understand what they show?
9. when happy with result, click Apply
10. save the resulting binary image: File > Save As > Tiff
11. apply watershed to divide touching objects
  - select the binary image
  - Process > Binary > Watershed
12. proceed with Analyze > Analyze Particles (IJ:30.2)
  - select Exclude on Edges and Add to Manager
  - click on OK
13. bonus: repeat step 12 but use the Size and Circularity options to try to exclude some particles and the Show dropdown menu to visualize different outputs.
14. set the parameters you want to measure:
  - Analyze > Set Measurement (IJ:30.2)
  - select Area, Mean gray value, Min & max gray value, Display label
  - click on OK
15. select the original image (open it again as in step 1 if you do not have it)
16. in the ROI Manager, click on Deselect and then on Measure

17. save the Results table as .csv: select the table and click on File > Save As

## 4.2 4.2 - DAPI segmentation with filters and thresholding

1. open the DAPI\_noise.tif image (drag & drop, or File > Open)
2. change LUT to Grays
3. Image > Duplicate (IJ:28.9) (**shift + d**)
4. Image > Adjust > Threshold (IJ:28.2.4)
5. understand the function of the Dark Background checkbox (inspect pixel values)
6. try setting sliders manually. Can you find a good threshold range?
7. try different algorithms by selecting them in the left dropdown menu. Can you find one that gives a good result?
  - NOTE: Image > Adjust > Auto Threshold, if you want to see all at the same time
8. apply a filter of your choice (Mean, Gaussian Blur, Median, ...)
  - Process > Filters
  - check the Preview checkbox
  - change the Radius / Sigma. What happens to the image?
  - when you are happy, click on OK
9. repeat steps 3, 7 and 8 until happy with result, then click Apply
10. save the resulting binary image: File > Save As > Tiff
11. apply watershed to divide touching objects
  - select the binary image
  - Process > Binary > Watershed
12. proceed with Analyze > Analyze Particles (IJ:30.2)
  - select Exclude on Edges and Add to Manager
  - click on OK
13. bonus: repeat step 12 but use the Size and Circularity options to try to exclude some particles and the Show dropdown menu to visualize different outputs.
14. set the parameters you want to measure:
  - Analyze > Set Measurement (IJ:30.2)
  - select Area, Mean gray value, Min & max gray value, Display label
  - click on OK
15. select the original image (open it again as in step 1 if you do not have it)
16. in the ROI Manager, click on Deselect and then on Measure
17. save the Results table as .csv: select the table and click on File > Save As



## 4.3 4.3 - DAPI segmentation with Weka

1. open the hela.tif image (drag & drop, or File > Open)
2. change LUT to Grays
3. Plugins > Segmentation > Trainable Weka Segmentation
4. draw a line outside a nucleus
5. click Add to class 1
6. draw a line inside a nucleus
7. click Add to class 2
8. click Train classifier
9. repeat the last four steps until happy with result
10. click Get probability
11. inspect the probability maps, do you understand the meaning of the values of the pixels in the different channels?
12. click Create result
13. inspect results, do you understand the meaning of the values of the pixels in the different channels?
  - you now have a binary image, but not the kind Fiji likes
  - to measure, proceed by thresholding (“set” both threshold values to 1), then Analyze Particles, etc
  - alternatively, multiply all values in the Weka output image by 255, then apply Binarize, etc
14. save the resulting image with name “myWekaHeLa1.tif”: File > Save As > Tiff
15. bonus round: play with Settings



## 5 - SPOT DETECTION

### 5.1 5.1 - Manual spot detection with the Multi-point Tool

1. read all the next steps before beginning
2. open the `beads_001.tif` image in folder `spot_detection` (drag & drop, or `File > Open`)
3. right-click (ctrl-click) on the `Point Tool` in the tool bar and select `MultiTool` (IJ:19.5)
4. double-click on the `Point Tool` in the toolbar and customize to your liking (test on image, clear points by `shift + a`)
5. count all beads by clicking on them one by one
6. how long did it take you to click on all the beads (measure with stopwatch the time it took to complete the previous step)?
7. add the points to the `ROI Manager` (e.g. press `t` on your keyboard)
  - NOTE: only one ROI will be added, it contains all the points
8. save the ROI to file (in the `ROI Manager` window, `More > Save`)
9. click `measure` in the `ROI Manager` to get number of spots

### 5.2 5.2 - Algorithmic spot detection with Find Maxima

useful [Find Maxima info](#) on the [image.sc](#) forum.

1. open the `beads_001.tif` image in folder `spot_detection` (drag & drop, or `File > Open`)
2. `Process > Find Maxima`
3. check `Preview point selection`
4. try different values for `Prominence` and the three checkboxes
5. try each of the possibilities in the pull-down (remember to click `OK` to apply your selection)
  - what is the difference and what could the different outputs be used for?
  - tip: **shift + u** allows you to pin sub-menus of the control panel for quick selection (e.g. the `Process` menu)
6. add you detected maxima to the `ROI Manager` and save to file
  - using your preferred settings, set the `Output type` to `Point Selection` and press `OK`
  - add the points to the `ROI Manager` (e.g. press `t` on your keyboard)

- NOTE: only one ROI will be added, it contains all the points.
  - save the ROI to file (in the ROI Manager window, More > Save).
7. load saved ROIs from the manual detection exercise (5.1) (e.g. drag & drop on Fiji the ROIs .zip file)
  8. compare results: how many beads do you get and how does the number compare to your manual count?

## 5.3 5.3 - Automatic spot segmentation with thresholding

1. open the beads\_001.tif image in folder spot\_detection (drag & drop, or File > Open)
2. Image > Adjust > Threshold (IJ:28.2.4)
  - select Otsu, then Apply
3. Analyze > Set Measurements (IJ:30.7)
  - select Area, Mean gray value, Display label
  - click on OK
4. Analyze > Analyze Particles (IJ:30.2)
  - select the Overlay Masks option in the Show dropdown menu
  - select Display results, Clear Results, Summarize, Add to Manager
5. save the ROIs to file (in the ROI Manager window, More > Save)
6. Compare results to the previous two approaches (5.1 and 5.2)
  - Do they differ significantly from each other?
  - Which performed better?

## 5.4 5.4 - Spot detection with noise

1. Repeat 5.2 with the with\_noise\_8000.tif image in folder spot\_detection
  - consider smoothing the image first. You can try any of the filter in the Process > Filters menu:
    - e.g. Process > Filters > Gaussian Blur, Process > Filters > Mean,...
    - NOTE: the Smooth operation under the Process > Smooth is a mean filter with radius 1 (3x3 kernel)
2. Repeat 5.3 with the with\_noise\_8000.tif image in folder spot\_detection
  - consider smoothing the image first. You can try any of the filter in the Process > Filters menu:
    - e.g. Process > Filters > Gaussian Blur, Process > Filters > Mean,...
    - NOTE: the Smooth operation under the Process > Smooth is a mean filter with radius 1 (3x3 kernel)
3. Compare and comment on the performance of the two methods
  - Which one is better?
  - Why/how is it better?

## 5.5 5.5 - Spot detection with variable background

1. Repeat 5.2 with the `beads_001_ramp.tif` image in folder `spot_detection`
2. Repeat 5.3 with the `beads_001_ramp.tif` image in folder `spot_detection`
3. Compare and comment on the performance of the two methods
  - Which one is better?
  - Why/how is it better?
4. What could be done to the image to make thresholding work better?