Fiji Exercises Collection

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This is a collection of exercises for the open-source Fiji software.

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!

1 - Working with Single-Channel Images

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 - 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 - 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 ovarlay scale bar in the saved image **only** if you open it in Fiji
   - continue with 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 - Composite images - channels display, splitting and merging

1. `File > Open Samples > Fluorescent Cells` (IJ:26.4)
2. `Image > Color > Channels Tool` (IJ:28.7.5)
3. toggle the `Channel` checkboxes to show/hide the respective channel
4. change the dysplay mode:
    - using the dropdown menu, switch between `Composite`, `Color` and `Grayscale` modes and observe the changes in the image display
5. select back the `Composite` dysplay mode
6. `Image > Color > Arrange Channels`
    - click on `New 1`, and select `Magenta` to change the LUT of the first channel
7. re-arrange the order of the channels:

    - in the `New channel order` settings, modify the order of the channels by changing the order of the `123` numbers (e.g. 213)
    - click `OK` to apply the changes
8. split channels
    - `Image > Color > Split Channels` (IJ:28.5.1)
9. merge channels to composite
    - `Image > Color > Merge Channels` (IJ:28.5.2)
    - check `create composite` checkbox (IJ:28.5.2)
10. LUT (look-up table) (IJ:19.17 & IJ: 28.15)
    - change `LUT` of the channels (`LUT` in the `startup tools` or `Image > Lookup Tables`)
11. color blindness
    - convert to RGB Color: `Image > Type > RGB Color` (IJ:7)
    - `Image > Color > Simulate Color Blindness`
    - `Image > Color > Dichromacy`

2.2 - RGB images - replace red with magenta

1. `File > Open Samples > Fluorescent Cells` (IJ:26.4)
2. convert to RGB Color: `Image > Type > RGB Color` (IJ:7)
3. `Image > Color > Replace Red with Magenta`

3 - Image Processing: Filters

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 - 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 - 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 - 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 - 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 - 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 - 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 - DAPI segmentation with Labkit

1. open the hela.tif image (drag & drop, or `File > Open`)
2. change `LUT` to Grays
3. `Plugins > Labkit > Open Current Image With Labkit`
4. sidebar, under `Segmentation`: click `Labkit Pixel Classification`
5. topbar: select the pencil tool
6. sidebar: select `foreground`. Draw a line inside a nucleus
7. sidebar: select `background`. Draw a line outside a nucleus
8. sidebar: click the play button next to `Labkit Pixelclassification`
9. repeat the last three steps until happy with result
10. click the drop down menu next to `Labkit Pixel Classifier`. Select `Show Probability Map in ImageJ`
11. inspect the probability maps, do you understand the meaning of the values of the pixels in the different channels?
12. export the segmentation: click the drop down menu next to `Labkit Pixel Classifier`: `Segmentation > Show Segmentation Results in ImageJ`
13. inspect results, do you understand the meaning of the pixel values?
    - you now have a binary image, but not the kind Fiji likes
    - to measure, proceed by thresholding (`Image > Adjust > Threshold...`): “set” both threshold values to 1, then Analyze Particles, etc
    - alternatively, multiply all values in the Labkit output image by 255, then apply Binarize, etc
14. save the resulting image with name “myLabkitHeLa1.tif”: `File > Save As > Tiff`

4.4 - DAPI double-segmentation with Labkit

1. open the hela.tif image (drag & drop, or `File > Open`)
2. change `LUT` to Grays
3. `Process > Enhance Contrast`. check `Equalize histogram`. Then, `OK`
4. `Plugins > Labkit > Open Current Image With Labkit`
5. sidebar, under `Segmentation`: click `Labkit Pixel Classification`
6. sidebar, under `Labeling`: click `add label`
7. rename `Label 1` by doublecklicking. For instance into `cytoplasm`. Optional: choose a different label color by clicking onto the color swatch.
8. topbar: select the pencil tool
9. sidebar: select `foreground`. Draw a line inside a nucleus
10. sidebar: select `cytoplasm`. Draw a line inside the cytoplasm
11. sidebar: select `background`. Draw a line where there is no cell
12. sidebar: click the play button next to `Labkit Pixelclassification`
13. repeat the last four steps until happy with result
14. click the drop down menu next to `Labkit Pixel Classifier`. Select `Show Probability Map in ImageJ`
15. inspect the probability maps, do you understand the meaning of the values of the pixels in the different channels?
16. export the segmentation: click the drop down menu next to `Labkit Pixel Classifier`: `Segmentation > Show Segmentation Results in ImageJ`
17. inspect results, do you understand the meaning of the pixel values?
    - you now have an image with three values
    - to measure, proceed by thresholding (`Image > Adjust > Threshold...`) at 0, 1, and 2, to extract each class (use Set and then set both thresholds to 0, 1, or 2)
    - then proceed with Analyze Particles, etc for each of the classes of interest (nuclei and cytoplasm)
18. Bonus round: play with Settings

5 - Spot Detection

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 - 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 - 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 - 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 - 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?