1. Preparation

- Clean the desktop
- Download all the resources files on the desktop (talk, example images, macros, plugins, etc…)
- Download sample files on the desktop
- Ensure internet connection is working
- Do a clean install of Fiji and keep installation files

2. Basic Fiji Philosophy

2.1. Tour of the menus

2.1.1. Explain what each menu is for

- **File** is for I/O
- **Edit** is for selections and copy/paste
- **Image** is for tools close to what you will find in photoshop (channels, layers, type, info, LUTs)
- **Process** is for filtering and image math
- **Analyze** is for working with filtered images and do measurements
- **Plugins** is for adding new features and macro automation
- **Help** is for anything related to Fiji and updating
- Explain **role of status bar** and **tools**

2.1.2. Plugins: show example of added feature

- Use **mean vs mean 3D** in *plugin>process>mean (3D)*

2.1.3. Cookbook

- Explain plugins can also appear as new menus, be containers of macros, etc.
- **Show** https://imagej.net/Cookbook page (menu on the upper right)
- Show example using **Fly Brain** sample: (Add scale bar with options)

2.2. Updating Fiji

2.2.1. Explain the updater concept and what it updates

- Use *Help>Update…*
- Show *advanced* mode to get more info on plugins

2.2.2. Show what libraries are important

- Bioformats
- BIG EPFL
- Coockbook
- CMCI-EMBL

2.2.3. Update menus

2.3. Show how to refresh menus:

- *Help>refresh menus*
- Explain why you have to restart also

2.4. Updating image J core

- Show you can change the IJ core version using *help>update ImageJ*

3. Working with an image

3.1. Using samples

- Open samples >Fluorescent cells 400k
- Describe the image window
- *Image>Show info* (or [i]) and quickly describe the text

3.2. Play with channels

3.2.1. Channel tools

- *Image>Color>Channel Tools*
- Enable or disable channels using checkboxes
- Switch to grayscale or color and show slider and option
- Use more to change color of one channel

3.2.2. Split channel

- Using the channel tools split channels

3.2.3. Select the info text on DAPI channel and remove it

- Select using different tools (use large selection), double click to close polygon
- *Edit>clear*

3.2.4. Convert to 8bit only and adjust brightness

- *Image>type>8bit* even if already checked, explain difference with 8bit color
- Use *Image>adjust>brightness/contrast* and show the damage of the previous edit and compression artifacts

3.2.5. Threshold the image

- *Image>adjust>threshold* and explain the options and what a binary image is
- Select nucleus using magic wand
- Restore selection on green channel image

3.3. Calibrate the image and set scale

- Select **green channel**
- Draw line across a cell, typical 10 um
- Add scale bar using *Analyze>tools>Scalebar…*
- Set scale using *Analyze > Set scale…* and assign it the 10um value
- Calibrate the image using *Analyze>Calibrate…* use 2nd degree polynomial, give 0-128-255 on the left (grey level), 50-100-125 (real value) on the right, say unit is ug/L
- Add calibration bar using *Analyze>Tools>Calibration Bar…*

3.4. Show search commands

3.4.1. Look for median filter

- Use the *FIJI quicksearch* bar to look for median
- Configure the search tool using … and deactivate the [L] shortcut

3.4.2. Show source

- Using the ImageJ Command Finder (shortcut [L]) look for median
- Display source code and show commands and comments

4. Open big CZI tile scan image and crop it

4.1. Open CZI slide image

- Explain hyperstack dialog options – insist on generic nature
- Ignore stitching and select 2nd image from the stack – explain google maps like nature of pyramid image

4.2. Select a region

- *Edit>Selection>Specify* and select a 1024x1024 ROI
- Move the ROI to a suitable area

4.3. Crop it

- *Image>Crop*

4.4. Duplicate image

- *Image>Duplicate* and give it a suitable name like "copy"

4.5. Convert to RGB

- *Image>type>RGB color*

4.6. (Skip these steps if it doesn't work)

- Show pre-made macro

5. Process the image

5.1. Open the first image from "Whole Dataset folder"

- *Process>Filter>Median* – with preview, 2 pixels
- *Image>Adjust>Color Treshold -*
- *Process>Binary>Make binary*
- *Process> Binary >Open*
- *Process> Binary >Fill Holes*

5.2. Measure typical cell

- Use wand tool to select typical cell
- Set measurements to measure area
- Measure area to get typical value

5.3. Use analyze particles

- Set size with 50%-200% of measurement
- Set circularity min at 0.2
- Show overlay masks
- Summarize
- Exclude on edges
- Add to ROI manager

5.4. Show Roi Manager

- Show regions on original image using "*show all*" option while on the original image

6. 1st Macro

- Redo the steps to binarize the image using the macro recorder
- Save macro as *binarize.ijm*
- Add interactivity using *getNumber("title", defaultvalue)*
- Install macro with shortcut using *macro "title [shortcut]" {}*
- *Load macro « FilterAndAnalyze.ijm »*

7. Batch

7.1. Show how to apply batch

- *Process>batch>macro*
- Explain limits and remove interactivity
- Create a test folder and test on a few files

8. Show macro for building tiles

- Load and edit macro, explain and demo.

9. Show Weka