**CellProfiler Workshop – Practice 1**

1. We will use CPW1\_Example\_Human.
2. There are 3 images in this file: d0 = Nuclei DNA Stain, d1 = phospho-Histone 3, d2 = cell body (actin).
3. Open CellProfiler so you have a new project file.
4. In Images: drag the folder ‘CPW1\_Example\_Human’ into the box.
5. In NamesandTypes: use ‘Images matching rules’ to assign the names **DNA**, **PH3** and **Cell\_Body** to the 3 images. Click update to check the images are recognised correctly.

**Building the pipeline:**

1. Add a new Module (+).
2. Add **Identify Primary Objects** to the pipeline on the **DNA** image. Do not use advanced settings. Name the objects as ‘Nuclei’.
3. Typical diameter of objects = 50-200, Discard objects outside range and touching the border.
4. Start Test Mode, Step:

How many nuclei are identified? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. In the window that opens, zoom and measure a few nuclei to get an approximate size.
2. Change the range to 10-50 and Step.

How many nuclei are identified? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. Change the Typical diameter of objects = 8-80 and Step.

How many nuclei are identified? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. Add a second **Identify Primary Objects** to the pipeline and apply it to the **PH3** image.
2. This time use Advanced Settings and name the objects as ‘PH3’.
3. Typical diameter of objects = 7-80.
4. Threshold = Global, Minimum Cross Entropy. Use Step to test the segmentation.

How many objects are identified? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

What problems can you identify? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. Threshold = Global, Otsu with two classes. Use Step to test the segmentation.

How many objects are identified? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

What problems can you identify? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. Change to Otsu with 3 classes and Foreground. Use Step to test the segmentation.

How many objects are identified? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. Change the Method to distinguish clumped objects to ‘Shape’.

How many objects are identified? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. We will now relate the Nuclei and PH3. Add the **RelateObjects** module to the pipeline. Assign Nuclei as Parent, and PH3 as child.
2. Add **Identify Secondary Objects** to the pipeline with the **Cell\_Body** as the input image.
3. Input Objects = Nuclei. Name the objects as ‘Cells’.
4. Method to identify secondary objects = Propagation.
5. Threshold = Global, Minimum Cross Entropy. Use Step to test the segmentation.

What problems can you identify? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

What is the Threshold value? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. Change the Threshold correction factor to 0.8 and use step to test the segmentation.

What is the new Threshold value? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. Add **Identify Tertiary Objects** to the pipeline: Larger objects = Cells, smaller objects = Nuclei.
2. Name the Tertiary object to be identified as ‘Cytoplasm’. Use step to test the segmentation.
3. Add **Measure object intensity** to the pipeline. Images to measure = **DNA** and **PH3**. Objects to measure = ‘Cells’, ‘Cytoplasm’ and ‘Nuclei’. Use step to test.
4. Add **Measure object size shape** to the pipeline. Object sets to measure = ‘Cells’, ‘Cytoplasm’ and ‘Nuclei’. Do not calculate Zernike or advanced features. Use step to test.
5. Add **Overlay outlines** to the pipeline. Do not display the outlines on a blank image. Select the DNA image on which to display the outlines.
6. Name the output image as ‘Overlay’. Colour, Thick outlines.
7. Add three outlines, one for Cells, once for Nuclei and one for PH3 (on the same Overlay outlines module). Use colour-blind friendly colours. Use step to test.
8. Add **Save Images** to the pipeline. Save the ‘Overlay’ as an Image. Construct the file name ‘From image filename’ and prefix with DNA. Append a suffix called ‘\_Overlay’.
9. Save as a .png file format. Make a folder on your Desktop called ‘CP-Workshop1’ and output the file to this location. All other settings as ‘No’ and save Every cycle.
10. Add **Export to Spreadsheet** to the pipeline. Use Comma delimiting and output to the ‘CP-Workshop1’ folder on your Desktop. Prefix the filename with the date and your initials.
11. ***Change Select the measurements to export and Calculate the per image mean values to Yes. Press the button and tick the following: Image-> Count and FileName only. Cells->AreaShape ->Area only. Cytoplasm->AreaShape ->Area only. Nuclei->AreaShape ->Area and Children->PH3. PH3->Parent->Nuclei. \*Ask if you need help with this, we can go through it together\*.***
12. Exit test mode and check everything is ticked green. Save your Project File as CP-Workshop1.
13. Analyse Images. When finished open the .png image. Open the .csv files in Excel.

In the Image file, how many Nuclei are counted? \_\_\_\_\_\_\_\_\_\_\_\_ How many are PH3 positive? \_\_\_\_\_\_\_\_\_\_\_

What is the mean cell area? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ What is the mean nucleus area? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_