Introduction to data analysis in R

Quantifying protein co-localization in fluorescence images using the colocr package

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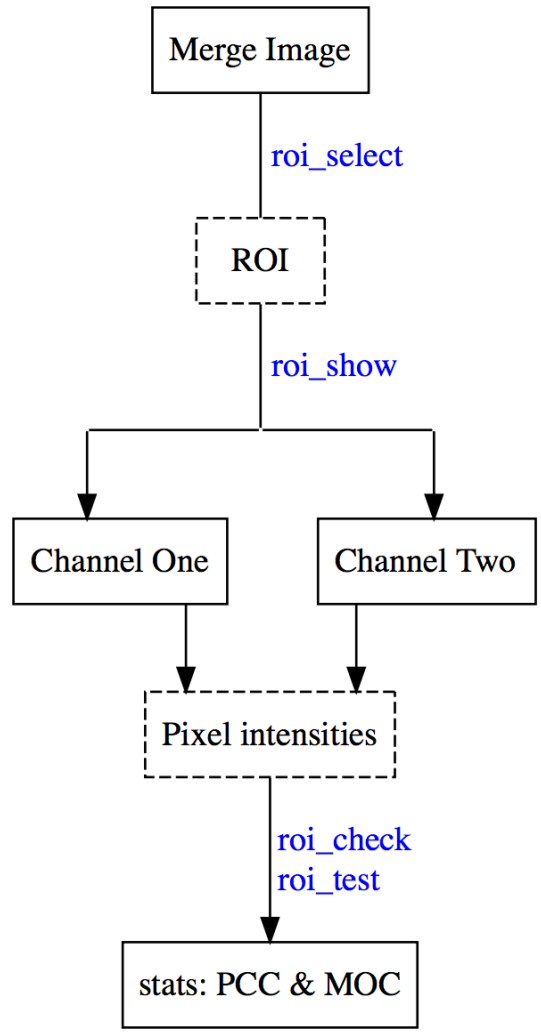
Fluorescence image analysis

A few R packages are available for conducting image analysis, which is a very wide topic.
The colocr package provides a simple straight forward workflow for

1. Loading images
2. Choosing regions of interest
3. Calculating co-localization statistics.

Included in the package, is a shiny app that can be invoked locally to interactively select the regions of interest in a semi-automatic way.

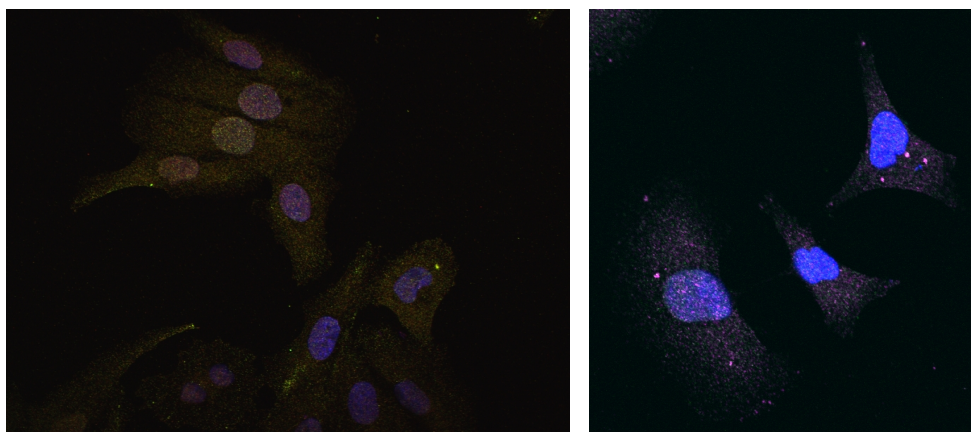


The dataset

The images we will be using come from the DU145 prostate cancer cell line. In this experiment, the cell line was treated with probes for two proteins RKIP and LC3.

The aim of this experiment is to determine, how much of the two proteins are co-localized or co-distributed in the particular cell line.

The files that contain these images are from left to 'image_1.png' and 'image_2.png'.



Loading images in R

We will first learn how to load these images in R and look inside the object to see how R represent images in numbers and coordinates.

```
# load colocr library
library(colocr)

# load image
img <- image_load('image_1.png')

# print img object
img
```

```
Image. Width: 800 pix Height: 600 pix Depth: 1 Colour channels: 3
```

This is a four dimensions object of class cimg that contains the image data.

Image representation as numbers

To get a deeper understanding of this object, we can transform it into a data.frame and have a look at it.

```
# transform the object into a data.frame and call str
str(as.data.frame(img))
```

```
'data.frame':   1440000 obs. of  4 variables:
 $ x    : int  1 2 3 4 5 6 7 8 9 10 ...
 $ y    : int  1 1 1 1 1 1 1 1 1 1 ...
 $ cc   : int  1 1 1 1 1 1 1 1 1 1 ...
 $ value: num  0.0392 0.0392 0.0392 0.0353 0.0353 ...
```

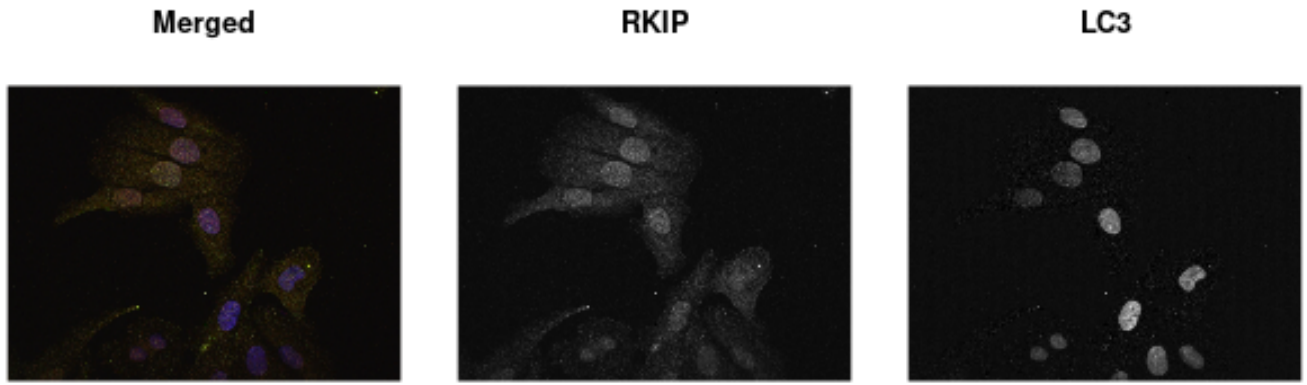
Here is a breakdown of the dimensions:

- x and y are the spatial dimensions
- cc is the color (channel)
- value is the intensity

Visualizing images

Channels 1, 2 and 3 refer to the red (RKIP), green (LC3) and blue colors (DAPI).

```
# show channels
library(imager) # to all channel
plot(img, axes = FALSE, main = 'Merged')
plot(channel(img, 1), axes = FALSE, main = 'RKIP')
plot(channel(img, 2), axes = FALSE, main = 'LC3')
```



Selecting regions of interest

To select a region of interest (ROI), we use roi_select. By default, the largest contiguous region of the image is selected.

Thresholding means all values below that number are set to 0, and above it to 1.

```
# select the region of interest
img_rois <- roi_select(img, threshold = 90)
str(img_rois)
```

```
 'cimg' num [1:800, 1:600, 1, 1:3] 0.0392 0.0392 0.0392 0.0353 0.0353 ...
 - attr(*, "label")= num [1:480000] 0 0 0 0 0 0 0 0 0 0 ...
```

The returned cimg object contains the original input image and an added attribute called label which indicates the 0 (not-selected) or 1 (selected) for each pixel.

Visualizing selected regions

To make sure the selection is appropriately encompassing the ROI, call roi_show, which shows

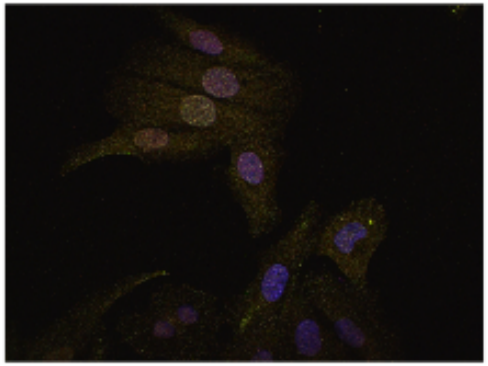
- The original merge image
- A low resolution picture of the ROI
- Highlighted ROIs in different channels

```
# select ROI and show the results
roi_show(img_rois, ind = c(1, 2))
```

Output in the next slide >>

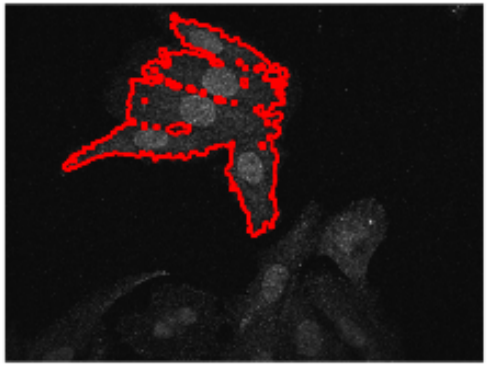
Visualizing selected regions

Merge

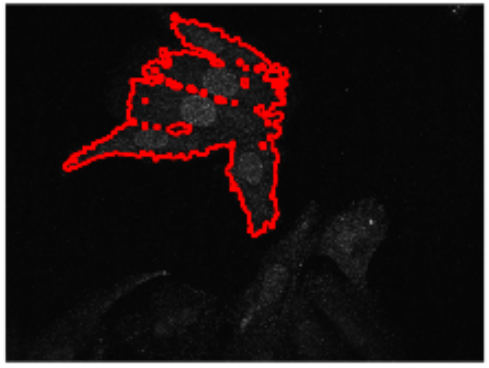


Pixel Set

Channel One



Channel Two



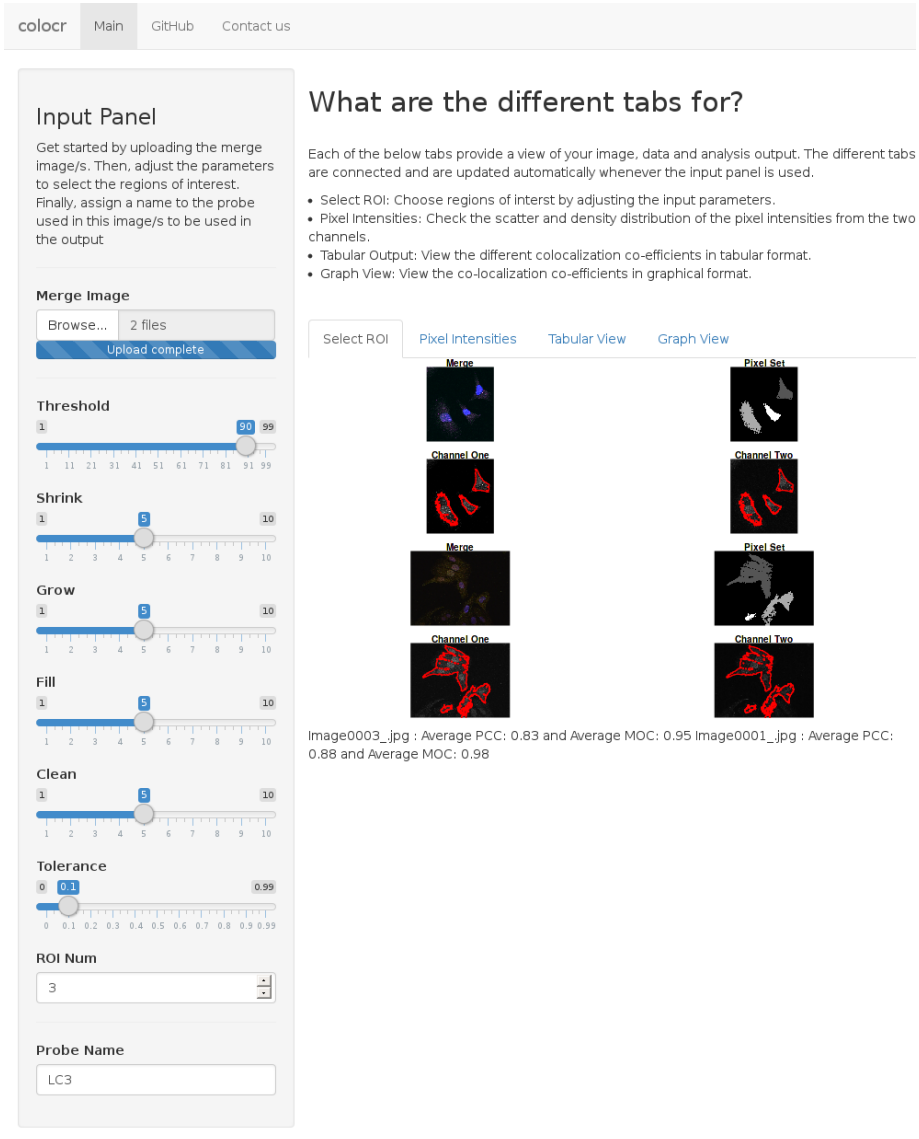
<< Code in the previous slide

The interactive web app

The package provides a simple shiny app to interactively determine these parameters and use it in the rest of the workflow.
To launch the app run the following

```
# run the shiny app
colocr_app()
```

Or visit this page.



Selecting multiple regions

If we want to select the all cells, we modify the different parameters in roi_select. We saw the threshold parameter before. The new parameters are

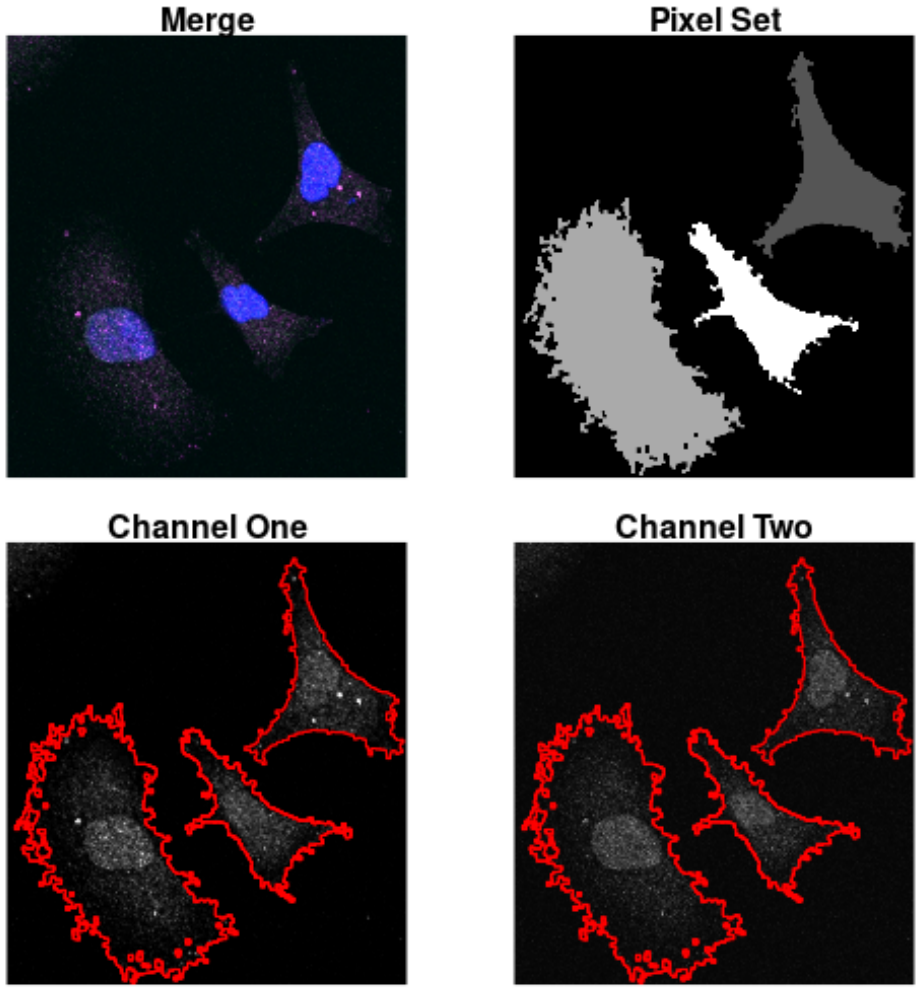
- shrink fitting pixels in a structure
- clear removing small isolated elements
- n the number of regions to select

```
# load 'image_2.png' as img2
img2 <- image_load('image_2.png')

# select and show three regions of interest
img2_roi <- roi_select(img2, threshold = 90,
    shrink = 10, clean = 10, n = 3)
roi_show(img2_roi)
```

Output in the next slide >>

Selecting multiple regions



<< Code in the previous slide

Pearson's correlation coefficient

Pearson's correlation coefficient (PCC) is the co-variance of the pixel intensity from two channels. The mean of the intensities is subtracted from each pixel which makes the coefficient independent of the background level.

The PCC is calculated as follows

$$\text{PCC} = \frac{\sum_i (R_i - \bar{R}) \times (G_i - \bar{G})}{\sqrt{\sum_i (R_i - \bar{R})^2 \times \sum_i (G_i - \bar{G})^2}}$$

where R_i and the G_i is the intensities of the red and green channels and the $\bar{R}$ and $\bar{G}$ are the average intensities.

PCC value of 1 means positive and -1 negative perfect correlation. 0 means there is no correlation between the pixel intensities.

Manders Overlap Coefficient

Manders Overlap Coefficient (MOC) does not require subtraction of the mean. Therefore, the values are always between 0 and 1. Also, the MOC is independent from signal proportionality.

$$\mathrm{MOC} = \frac{\sum_i (R_i \times G_i)}{\sqrt{\sum_i R_i^2 \times \sum_i G_i^2}}$$

where R_i and the G_i is the intensities of the red and green channels.

Calculating correlation statistics

The colocr package implements both PCC and SCC in roi_test.

Invoking the test is a one function call on the selected regions of interest.

```
# Calculate the PCC
tst <- roi_test(img2_rois, type = 'both')
tst
```

```
        pcc       moc
1 0.8798341 0.9303114
2 0.8963802 0.8990838
3 0.8882729 0.9386888
```

roi_test returns a data.frame with a column for each of the desired statistics: pcc and moc, and a row for each region of interest, in this case one row.

References

1. Ahmed M, Lai TH, Kim DR. colocr: an R package for conducting co-localization analysis on fluorescence microscopy images. PeerJ. 2019;7:e7255. doi:10.7717/peerj.7255.

1. Dunn KW, Kamocka MM, McDonald JH. A practical guide to evaluating colocalization in biological microscopy. Am J Physiol Cell Physiol. 2011;300(4):C723-C742. doi:10.1152/ajpcell.00462.2010.

Summary

What you learned

- Fluorescence image analysis
- Understanding images
- Loading images in R
- Selecting regions of interes
- Calculating correlation statistics

What's next

- Practice (Link)
- Homework (Link)
- What to learn next
  - Intermediate R Course (Data Camp Free Interactive Course)
  - R for Data Science (Book)