EURO-TELEPATH
"Telepathology Network in Europe"
COST Action IC0604

Basic Functioning of ImageJ *with practical exercises* 4

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Agenda

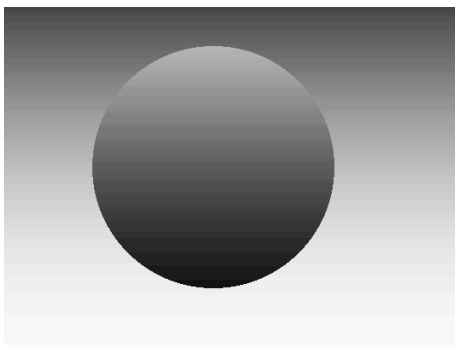
- ImageJ: what is, what it does
- Basic functioning of ImageJ
- Simple image processing operations
- **More complex, yet basic, exercises**

Systematic approach

- Rarely an image processing procedure is just one operation
- More often, we need to put together a sequence of operations,
  - Each of which with their own limits, damages on the image, and so on
- The next examples will try give an idea of things to consider when analysing images

1. Thresholding: limits?

- Let's create a difficult case
  - Open "smooth.jpg"
  - Draw circle selection in the middle
  - Edit: invert, then deselect
  - We clearly have an object in the image… try to threshold.



2. Measurement parameters

- Values for region characterization (e.g., for diagnostic interpretation)
  - **morphometry**: shape evaluation
  - **colorimetry**: color (including grey-level) evaluation
- morphometry:
  - area, perimeter, minimum and maximum diameter…
  - Form and regularity factors (how much roundish is an object?)
  - ...
- colorimetry:
  - Meand and standard deviation of grey levels
  - Texture measures
- Open “Blobs”, select an area, Analyze:measure, repeat (do not thrash!)

2. Quantitative measurements

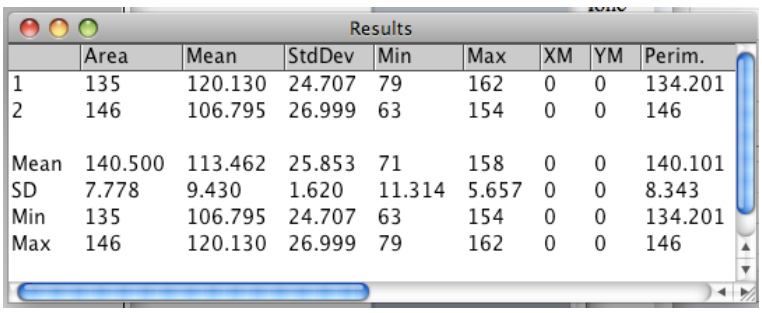
- If we need quantitative measures from the images, some correspondence between digital image and real scene should be given
  - **Size**: how large is a pixel (on X and Y)?
    - Simple problem: **image scale**
  - **Color**: to which grey intensity does the pixel value correspond?
    - More crucial problem: not often the pixel values are linearly dependent on real grey level (more often: gamma function). **Color calibration**
    - Anyway, the problem makes sense only if coloration/ staining has some quantitative meaning (e.g., stechiometric coloration)

2. Image scale

- Physical size corresponding to one pixel (not necessarily square!)
  - depends on acquisition device geometry and features
  - Can be set into ImageJ
- When geometry is fixed (e.g. microscope for one specific objective and camera):
  - can be set once and used many times
  - Open “Blobs” Analyze: Set scale (to 2.5µ) , try measures
- Sometimes geometry is variable (e.g. zoom, variable subject distance, etc)
  - Should be set at every acquisition
  - Open “Kidney”, follow me…

3. Reduction…

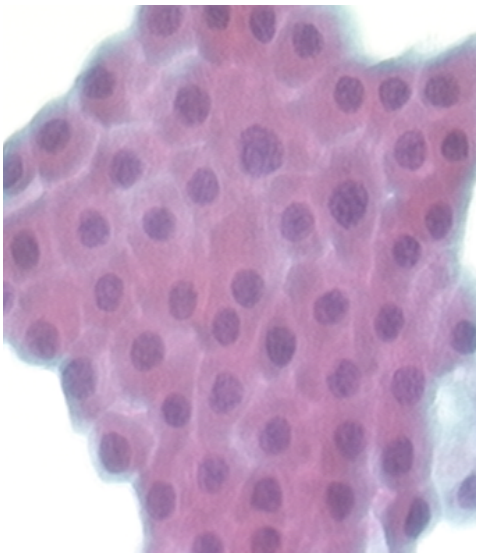
- Parameters can be further reduced when useful only as an aggregate
  - E.g.: just some statistical summary
  - Mean, std.dev, ….
- Select Results table, Edit:Summarize



	Area	Mean	StdDev	Min	Max	XM	YM	Perim.
1	135	120.130	24.707	79	162	0	0	134.201
2	146	106.795	26.999	63	154	0	0	146
Mean	140.500	113.462	25.853	71	158	0	0	140.101
SD	7.778	9.430	1.620	11.314	5.657	0	0	8.343
Min	135	106.795	24.707	63	154	0	0	134.201
Max	146	120.130	26.999	79	162	0	0	146

4. Automated measuring

- When possible, we try to reduce at minimum the human input into a measurement system,
  - to reduce work
  - To enhance objectivity
- We'll do now a full exercise
  - Input: an histologic image
  - Output: number of nuclei, average size, perimeter, roundness, etc
  - In the middle: restoration, enhancement, reduction , etc



First steps

- Open Histo.tiff
  - Color image: we need only morphologic features, so color not needed
  - let's choose the best channel…
  - And follow me, please!