Why process images from a microscope?

Images are often pretty, but the really useful information is in the pixels.
Why process images from a microscope?
In the real world, images need processing all the time...

Terahertz time-gated spectroscopic imaging for content extraction through layered structures

Camera Culture
MIT Media Lab

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In the real world, images need processing all the time…
Why process images from a microscope?
In the real world, images need processing all the time…

Radius of Curvature: 769.441 m
Center Distance: 0.51 m
Starting from the pixel up
What makes up an image?
Starting from the pixel up

What makes up an image?
Starting from the pixel up
What makes up an image?
Starting from the pixel up
What makes up an image?
What’s a pixel?
What’s a pixel?

Photons...

Microscope body...
What’s a pixel?

Microscope body...

Pixels!
What’s a pixel?

Pixels!

Pixel value, gray value, gray level, colours, ...

Pixels!
Bit-depth and dynamic range

\[ \text{# gray values} = 2 \times \text{bits} \times \text{colours} \]

- 256 gray values = \( 2^8 \times 1 \)
- 65,536 gray values = \( 2^{16} \times 1 \)
From gray values to masks

# gray values = 2 \#bits \times \#colours

Finding relevant objects
From gray values to masks

$\# \text{ gray values} = 2 \times \#\text{bits} \times \#\text{colours}$

Finding relevant objects
Creating a mask
Thresholding - fluorescence intensity

- All grays
- Low grays
- High grays
Creating a mask

Edge detection

- All grays
- Variance filter (not yet binary)
- Canny edge detection
Adjusting a mask

Filters

Fluorescence intensity thresholded mask

Erode
(or minimum filter of 1)

Dilate
(or maximum filter of 1)

Filtering kernels
(here: circular radius of 1.5 pixels)
Generate quantitative information
Images are data in themselves, but contain more information than what is immediately available

Area
Perimeter
Circularity
Aspect ratio
Longest distance
...
Tutorial #1

Quantify nuclear size
Tutorial #2

Quantify nuclear fluorescence intensity
Processing exercises

Intensity distribution + dynamic range
Filtering
Thresholding
Analyzing particles
Measurements
Tutorial 1: shape descriptors - nuclei

- Open image: File > Open Samples > HeLa cells
- Separate channels: Image > Color > Split Channels
- Duplicate the nucleus image for #2
- Thresholding: Image > Adjust > Threshold
- Add to ROI manager: Analyze > Analyze Particles
- Adjust measurements: Analyze > Set Measurements > Area + Perimeter + Shape descriptors + Feret’s diameter
- Measure: From ROI manager, click measure

Tutorial 2: fluorescence intensity

- Open image: Select original nucleus image
- Overlay: Image > Overlay > From ROI manager
- Adjust measurements: Analyze > Set Measurements > Mean + STD deviation + Min & Max + Mean, etc.
- Measure: From ROI manager, click measure

Tutorial 3: macro recorder

- Do #1 with macro recorder on
- Run it on new image...