Basics of Processing and Analysis

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Outline

- Measurements and ROIs
- Manipulating pixels
- Thresholding
- Filters
- Masks
- Higher dimensions
- Macros

Measurements and ROIs

- Measurements are scaled by pixel size - another instance of metadata being essential!
- Measurements in image != measures in real life (blur, PSF, etc)
- ROIs can be any shape available on Fiji (which includes freehand)
- ROIs are always 2D
- ROI Manager is a good tool - ROIs in there are image-independent, though!
- If you need ROIs/overlays visible from other software, you need to flatten overlays

Point operations

- Operations that act on individual pixels
- Arithmetic operations are an example
- Inversion - does different things for different formats, inverting lookup tables looks the same but does different things
- Nonlinear contrast (always disclose when using these!)
- Multiple images/image calculator

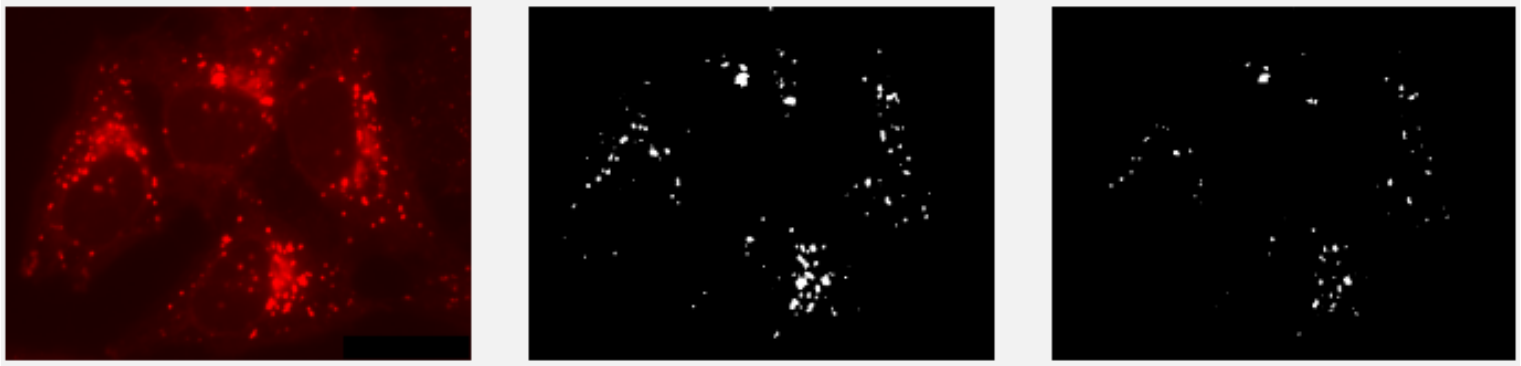
Exercises

- Try to figure out what "Invert" does for 8, 16 and 32-bit images.
- Explore nonlinear transforms via Math command
- Figure out the identical images!

Thresholding

- Why automatic segmentation? Faster, more reproducible, less bias
- Binary images and labelled images as results
- Global thresholding - tricky and gives you whatever result you "want"
- Fiji has a bunch of auto threshold algorithms
- Histograms can be useful here (keep binning in mind!)
- "Analyze particles" to count and label foreground regions

Exercise



Is global thresholding reliable?

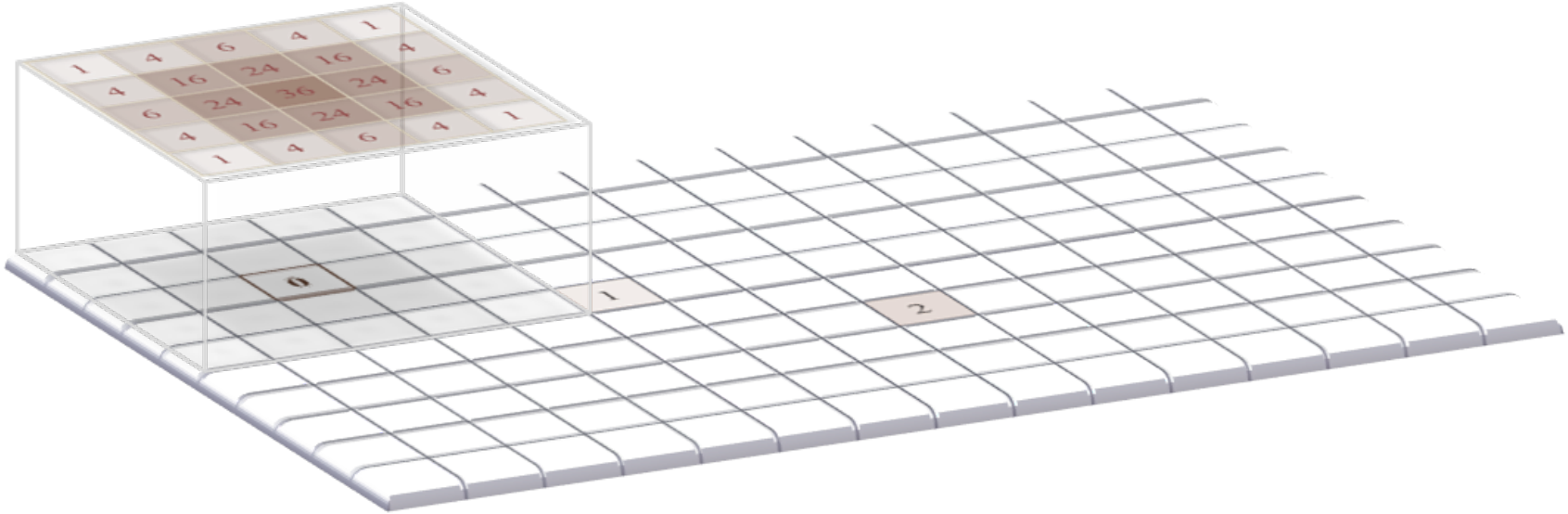
Challenges on thresholding

- Noise (Gaussian filters tend to be your friend!)
- Variable intensity backgrounds - rolling ball, subtract mean/median, auto local threshold
- Exercise: Explore several automated methods of thresholding the different channels of HeLa Cells sample

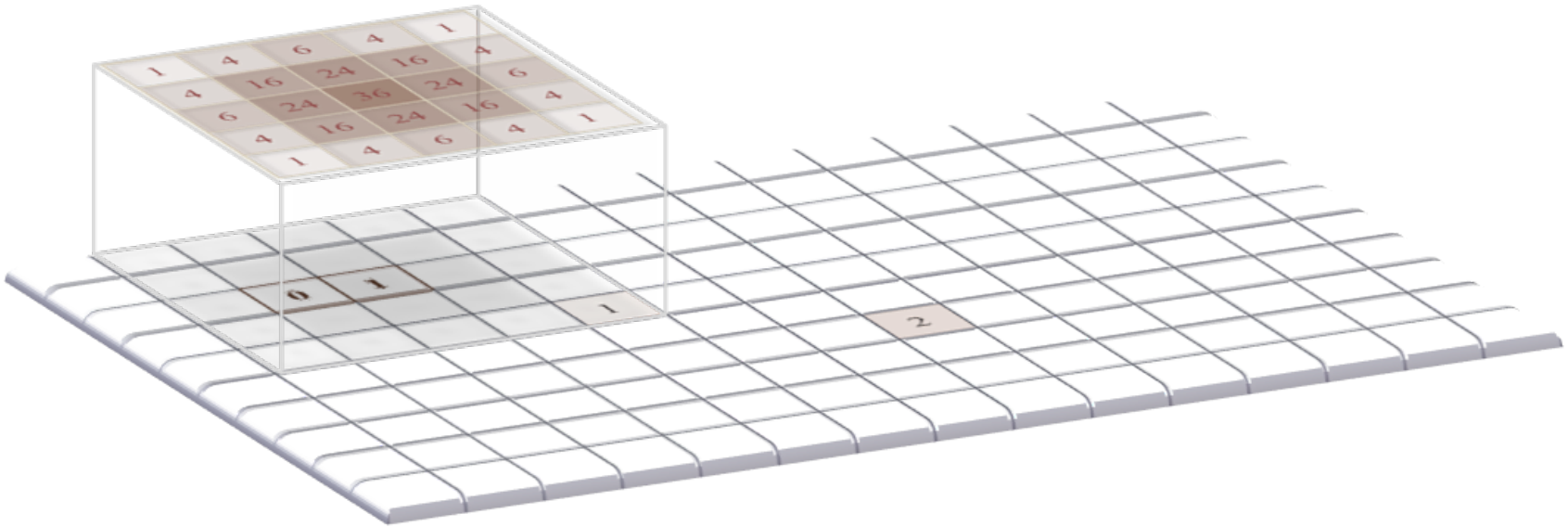
Filters

- Filters are our best friends
- Basic idea: each pixel is assigned a value that is a combination of nearby pixels
- Linear x nonlinear
- Mean filters, "smooth" command - reduces noise at cost of detail (blurriness)
- Linear filters, kernels, convolution
- Gradient filters (horizontal, vertical, magnitude)

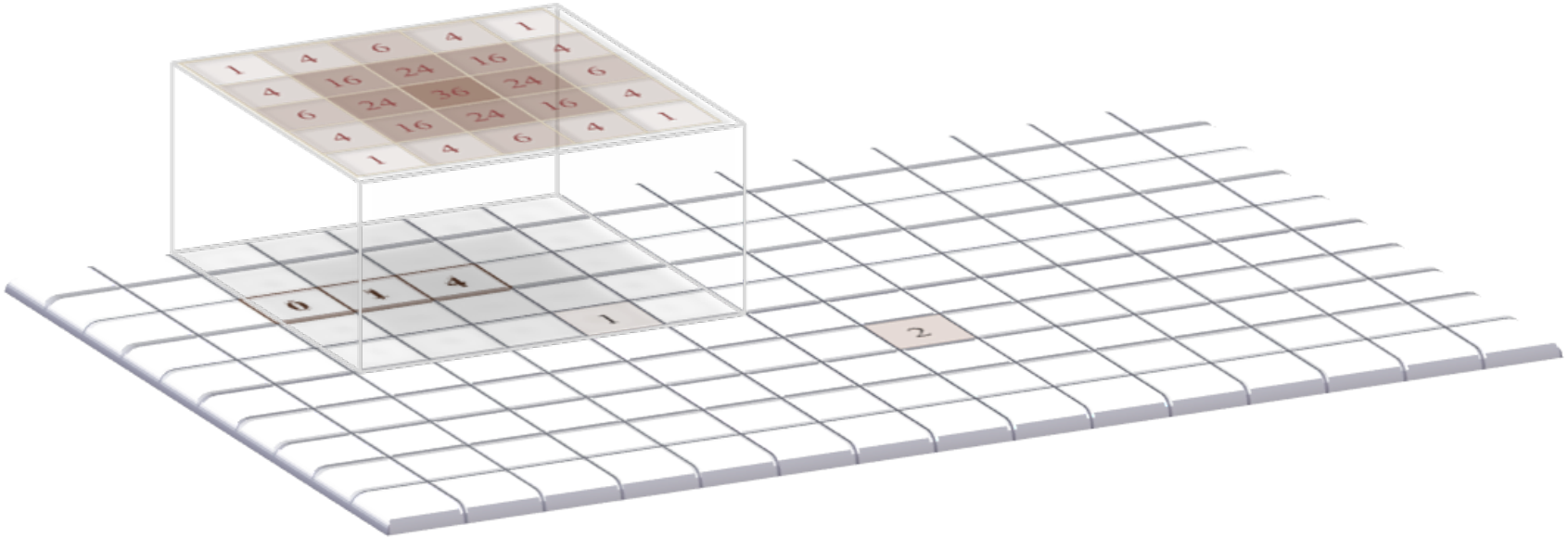
Applying a filter



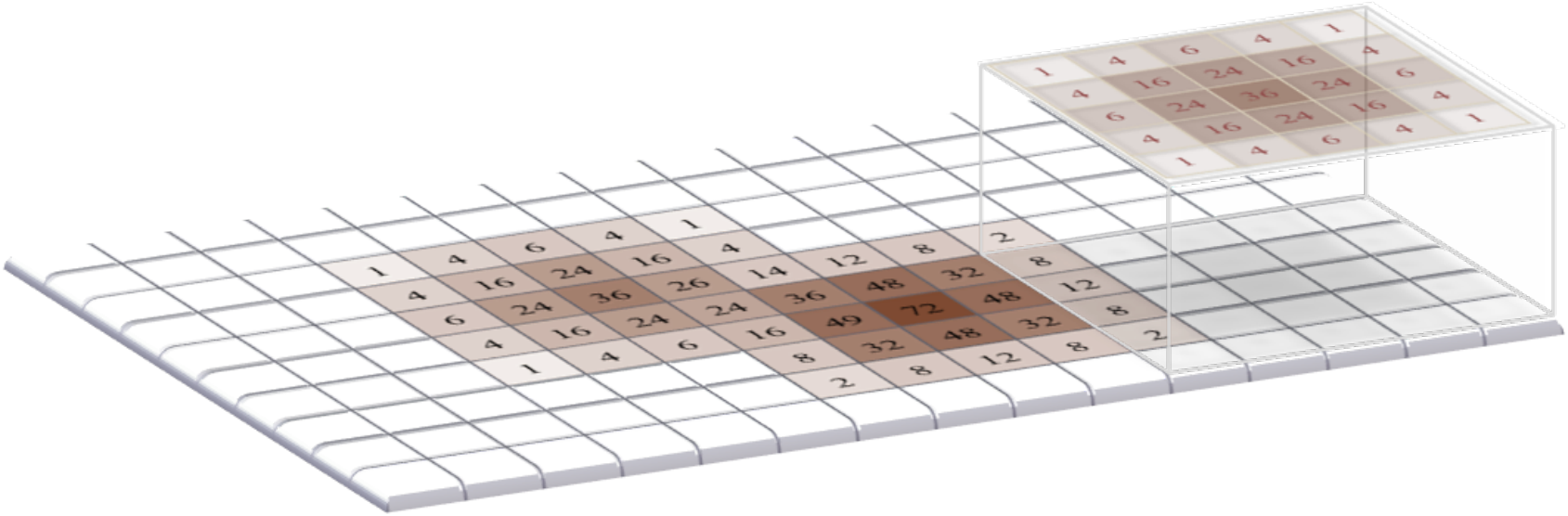
Applying a filter



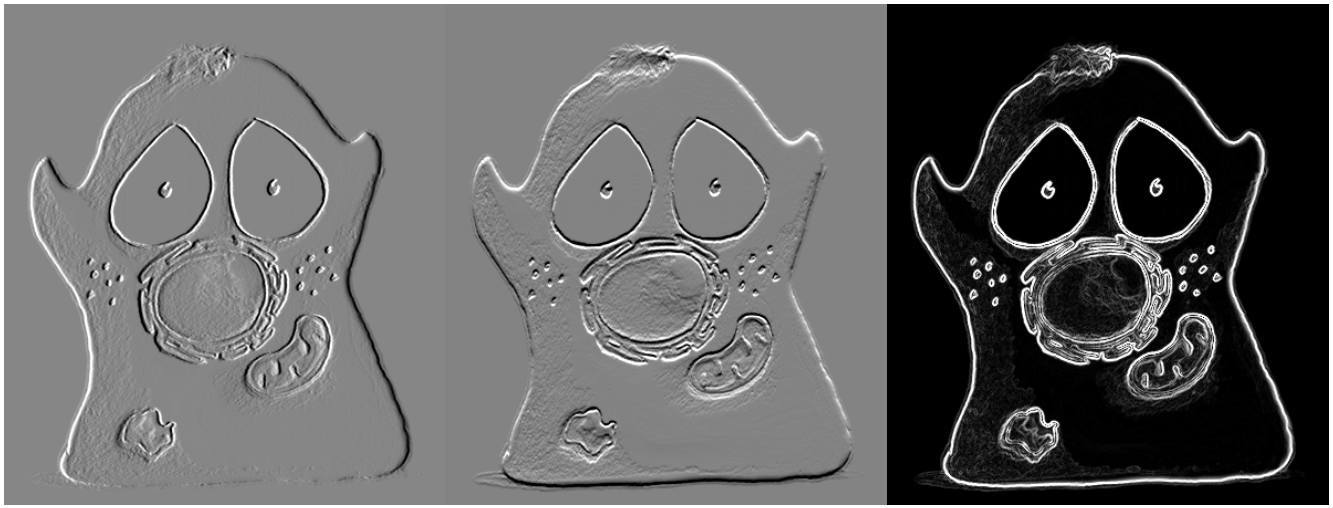
Applying a filter



Applying a filter



Exercise - applying gradients



Edge cases

- Filters relying on neighbourhoods need pixels "outside the image"
- There are many ways of assigning these pixels and these might affect the results
- Exercise! Can you figure out what Fiji assigns to out-of-picture pixels?

Nonlinear Filtering

- Rank filters: rank the neighbourhood and assign value based on the ordered list
- Most common example: median
- Median is like mean, but ignores outlier values
- Other examples: minimum/maximum

Gaussian Filtering

- Influence of neighbouring pixels decreases with distance
- In general, better smoothing result than mean
- Coefficients derived from a Gaussian function
- We'll talk more about these, but understanding their importance requires going into the Fourier world (which we probably won't)
- Unsharp: subtract a Gaussian, add it back to original (this is one of those "need to disclose" ones!)

Choosing a filter size

- Small filter will mostly suppress noise
- The bigger the filter, the bigger the structures being suppressed
- Defining filter size allows us to define a typical length scale for analysis
- Difference of Gaussians filtering: "bandpass"
- Laplacian of Gaussian: "inverse" of DoG

Binary Images

- Morphological Operations using rank filters
- Erode/Dilate and their relationships to max/min filter
- Opening/Closing as alternative that more or less preserve area
- In Fiji, these always use 3x3 neighbourhoods, but you can do it in larger neighbourhoods by just using max/min filters
- Outlines, fill holes, skeletonize

Image transforms

- Distance transform: each pixel gets the distance to the closest background pixel
- Is it useful? Ultimate points, possible merged structures on segmentation
- Distance transform in inverted image, Voronoi tesselation
- Watershed

Higher dimension data

- Point operations naturally extend, image arithmetic can be applied at each 2D slice
- Filtering and thresholding are possible, though computationally expensive
- Careful with brightness/contrast adjustment in Fiji - it's normally slice-specific and nothing guarantees that the rest of the stack will "like" it

3D filtering

- Some filters are separable, meaning they can be implemented as 3 1-dimensional filters instead
- These are much faster and should be preferred in 3D: Mean and Gaussian are two examples (though not median)
- Be wary of Z-direction "pixel size"

Macros

- Automation is VERY VERY IMPORTANT: it saves you time and it's better, more reproducible science
- Recording macros is your best friend (just remember to clean it up afterwards)
- Remember to make macros file-agnostic, add comments and make it as legible as possible

This is the last slide

- This material was inspired by Pete Bankhead's "Analyzing fluorescence microscopy images with ImageJ" e-book
- Please take 2 minutes to complete our feedback survey!
- tinyurl.com/imageanalysisfeedback