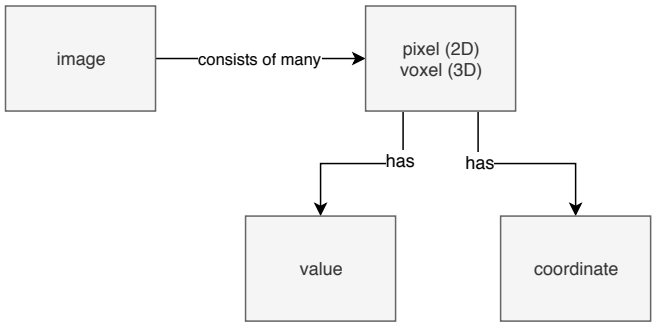
Basic image properties

- Scientific images are measurements. Thus, it is very important to know how to access the actual values of pixels.

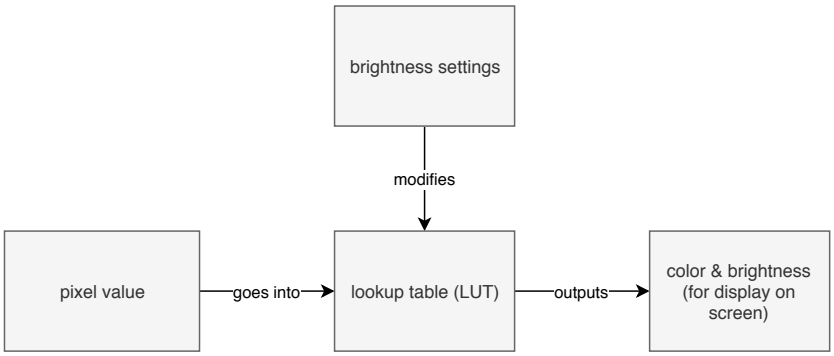


Activities

- Explore different ways of accessing pixel values and coordinates in an image

Lookup tables (LUTs)

- Scientific images are just a collection of many many numbers. However, human brains are typically not very good at taking the information in like this. Thus, lookup tables are used to convert the numbers into colored images that can be much better perceived by our brains.
- Changing the LUT severely changes the perception of an image! Thus, scientist have a great responsibility in carefully choosing the appropriate LUTs (=> "image ethics").

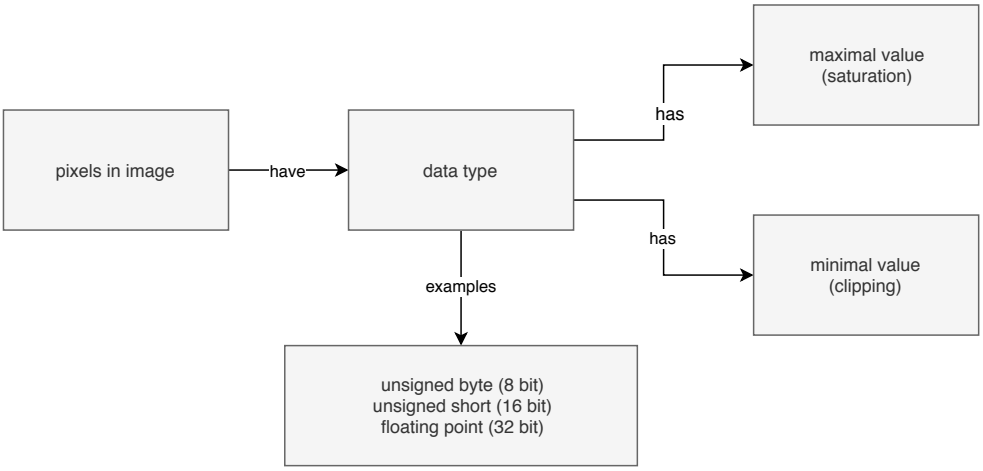


Activities

- Explore different LUT and Brightness settings and discuss their pros and cons
- Discuss how brightness settings can be (incorrectly) used to hide objects in a image
- Visualize current LUT setting by adding a calibration bar
- Demonstrate and discuss the importance of equal LUT settings for quantitative comparative image display

Image (pixel) data types

- Pixels in images are of a certain data type, which limits the values a pixel can take.
- Sometimes the data types of the pixels are changed. This can alter the values.
- In science, the value of a pixel often is an actual measurement. Thus, utmost care has to be taken when changing the data type.

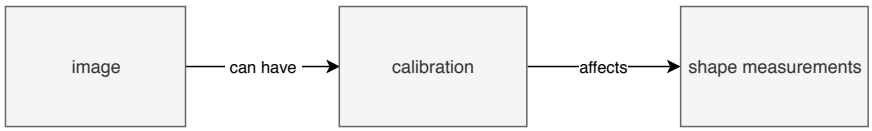


Activities

- Discuss the values range of different data types (looking at example images).

Calibration

- TODO

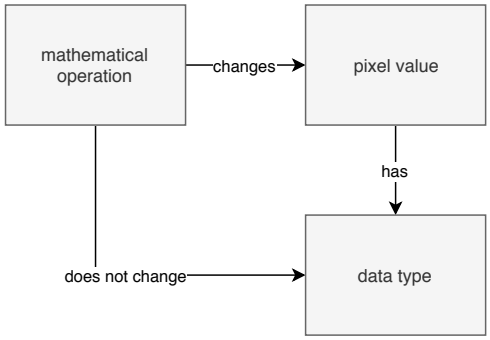


Activities

- Measure length of a line or area of a region while changing calibration of image

Image math

- Many image processing operations change the values of pixels in an image. However, due to the limitations of the pixel data types the results are not always correct. For proper scientific image analysis one needs to understand in which circumstances such incorrect results can occur and how to circumvent this.

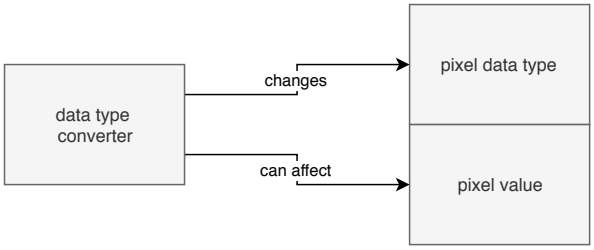


Activities

- Apply mathematical operations on images and observe how the pixel values change (the results can be unexpected, due to limitations of the respective data type).

Data type conversions

- Data type conversions are sometimes necessary, but one has to be very careful, because often they change the pixel values (often without explicit warning).
- For example, saving images in different file formats can change the pixel values.
- Uncontrolled data type conversions are a common source of errors in biological image analysis.

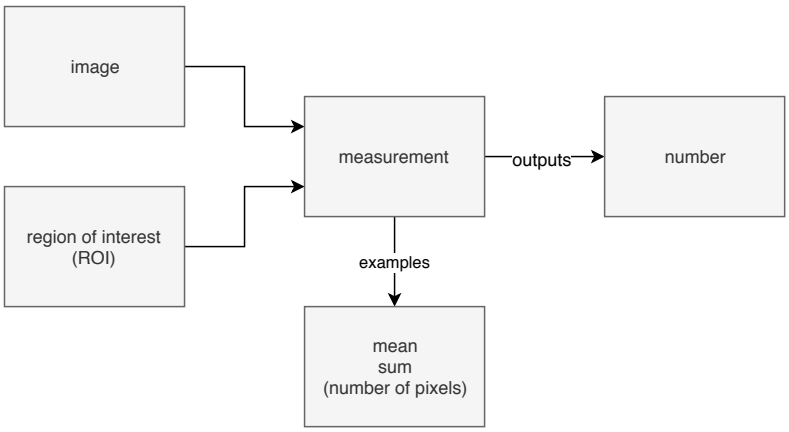


Activities

- Explore whether and how data type conversions change the pixel values.
- Explore how saving images in different file formats changes the pixel values.

Intensity measurements

- Intensity measurements in an image are achieved by mathematical operations on a collection of selected (region of interest) pixel values.
- Intensity measurements in images are very important in biology, e.g. to measure the concentration (or expression level) of proteins in certain locations.

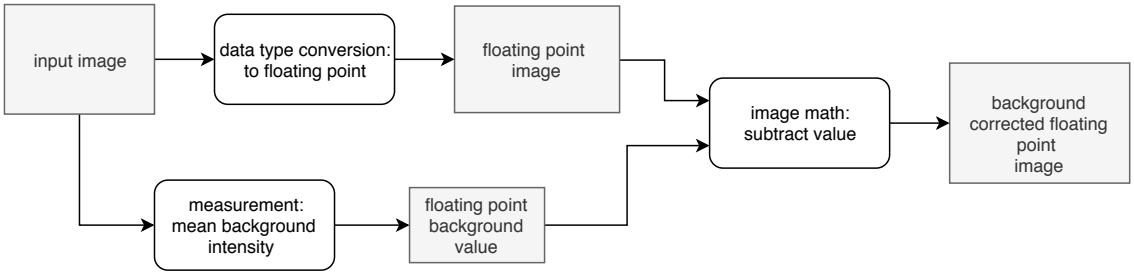


Activities

- Practice intensity measurements in an image
- Discuss the different names for the same measurement
- Discuss how some of the measurements relate to each other

Background subtraction workflow

- Most biological images have non-zero intensity values in regions outside of the objects of interest. For example, most cameras on microscopes have a read noise with can be many hundred gray values (for 12bit or 16bit detection). In order to properly quantify the intensities of objects such background must be taken into account. Camera read noise is typically constant across the whole image. Thus such read noise can be dealt with by subtracting a constant value from each pixel.
- Proper background subtraction is very important and needs to done with care.
- Automated background detection can be quite difficult and often is one of the most challenging aspects of biological image analysis.

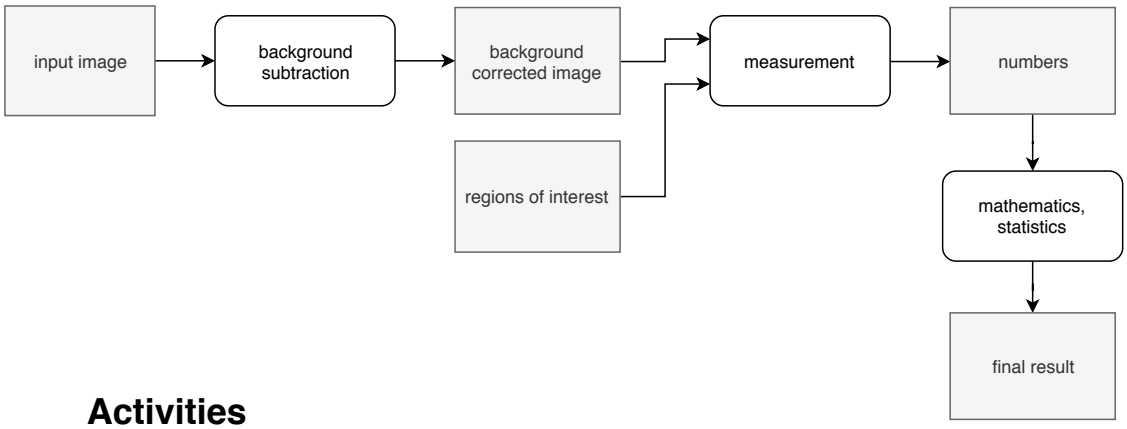


Activities

- Perform background subtraction on an image

Object intensity measurement workflow

- In biology, conducting meaningful and scientifically correct intensity measurements are often quite difficult, because they require advanced knowledge in the domains of biology, microscopy and image analysis. Thus, to achieve correct results, discussions between trained bioimage analysts and biological researchers are often required.
- The first step typically is background subtraction.

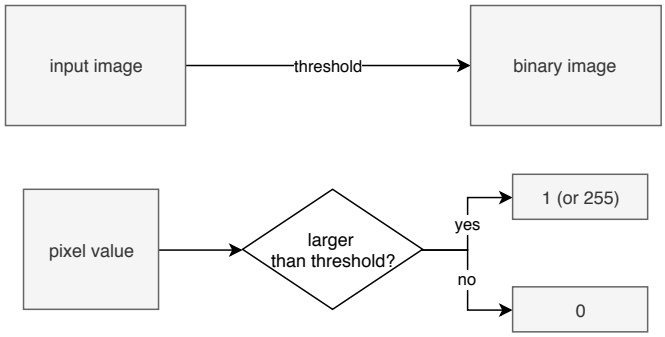


Activities

- Watch presentation about image intensity formation and interpretation in fluorescence microscopy
- Try to perform background subtraction on your own!
- Try measuring object intensities on your own!

Threshold

- In order to find objects in a image, the first step is to determine whether a pixel is part of an object or of the image background. In the vast majority of cases this is done by thresholding.

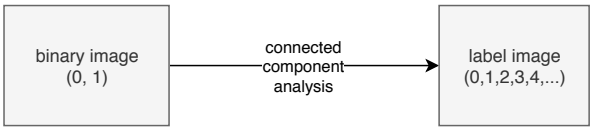


Activities

- Practice thresholding of an image (discuss the difference to gating).

Connected component analysis

- In order to introduce the concept of objects in an image connected component analysis is of utmost importance and is used in all image analysis packages.
- Label images are a great way of representing objects, because (i) it is a well accepted standard, enabling interoperability between different software packages, and (ii) it works in 2D and 3D, (iii) they can be compressed very efficiently.

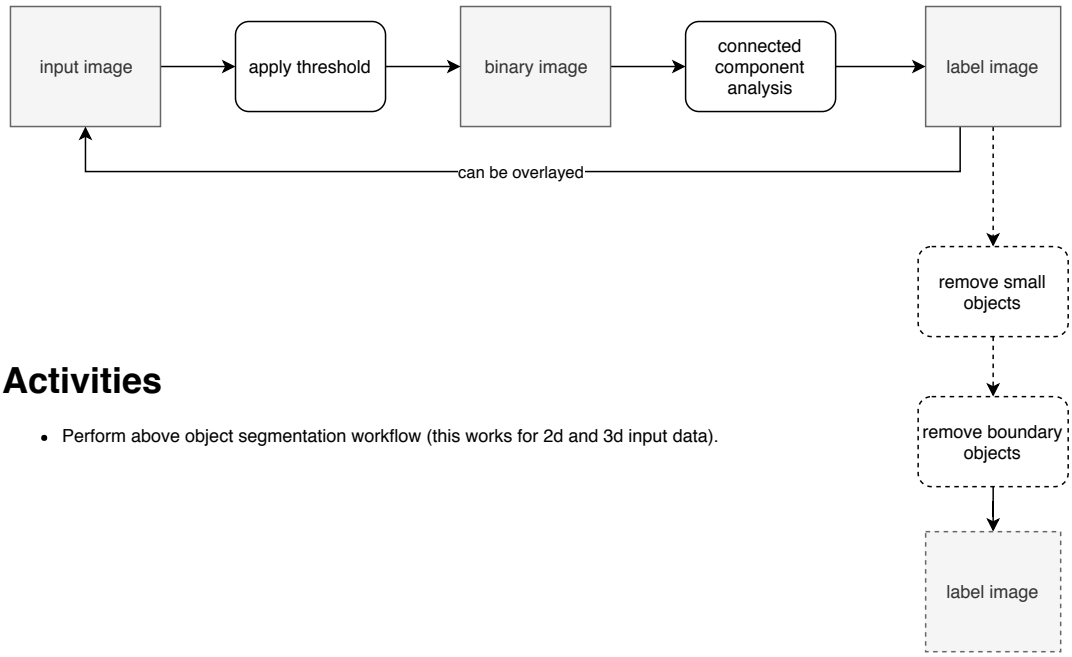


Activities

- Perform connected component analysis on a binary image
- Explore multi-color LUTs for visualization of the label images

Simple object segmentation workflow

- Object segmentation via an intensity threshold followed by connected component analysis is one of the most common workflows for analysis of fluorescence microscopy images.

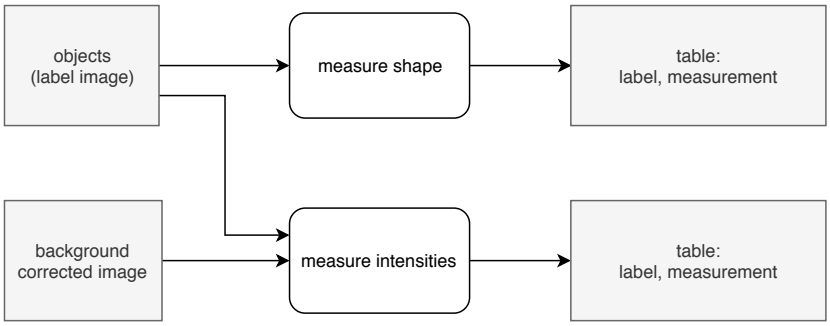


Activities

- Perform above object segmentation workflow (this works for 2d and 3d input data).

Object shape and intensity measurements

- ...

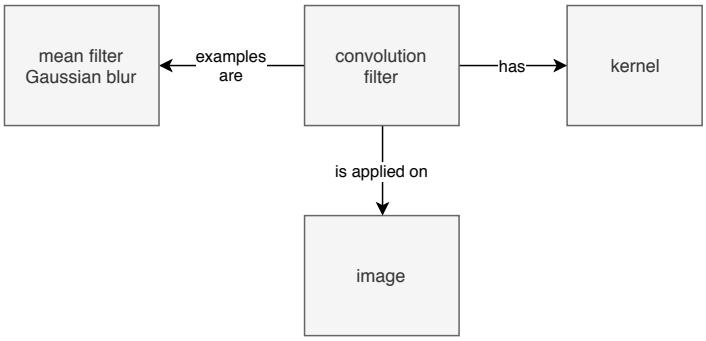


Activities

- Practice above workflows

Convolution filters

- Image filtering is very useful, e.g. to reduce noise and thereby make segmentation easier.
- Convolutional filters are a very important class of image filters.

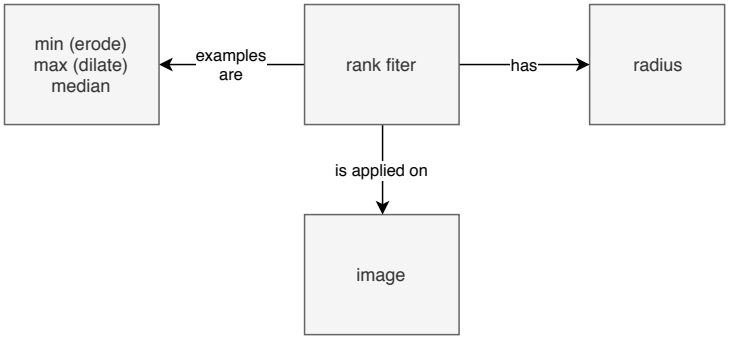


Activities

- Explore how different convolution filters change the values in an image
- Discuss how the kernels of a mean filter and a Gaussian filter look like
- Apply a mean filter to reduce noise and thereby make thresholding easier

Rank filters

- Rank filters are filters that cannot be described as a convolution.
- Rank filters work like this: (i) sort the pixel values in a certain neighborhood around a central pixel, (ii) pick one value based on its position (rank) in this sorted list of values, (iii) replace the central pixel by the value of the pixel that you picked.

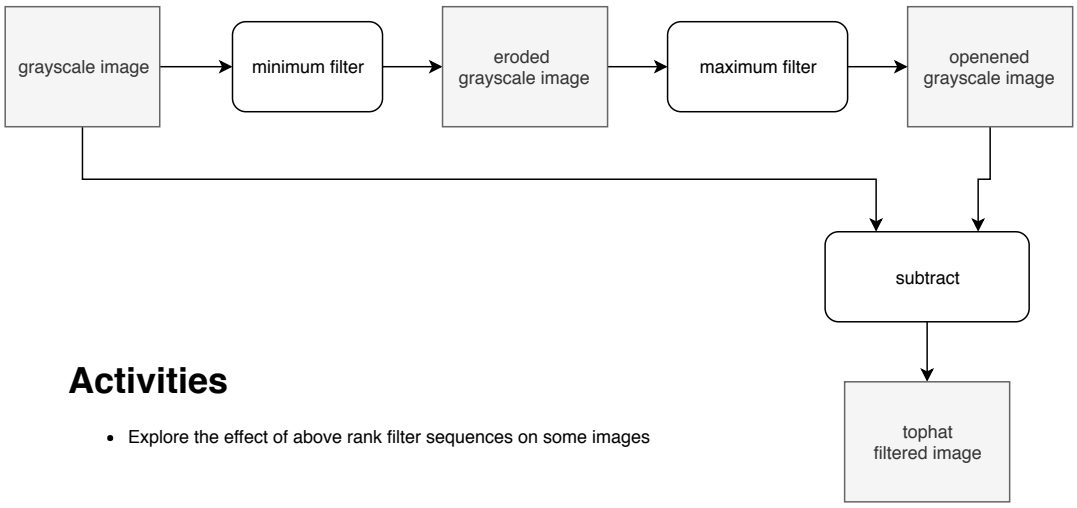


Activities

- Understand how rank filters work conceptually, looking at an example
- Explore how different rank filters change the values in an grayscale image
- See how a median filter is edge preserving (compare with mean filter)
- Explore applying rank filters to a binary images

Rank filter sequences (morphological filtering)

- Applying rank filters in all kind of sequences is very useful and one of the major toolkits of every image analyst.
- This important image analysis field is also called morphological filtering.
- Morphological filtering can be applied both to binary and grayscale images.

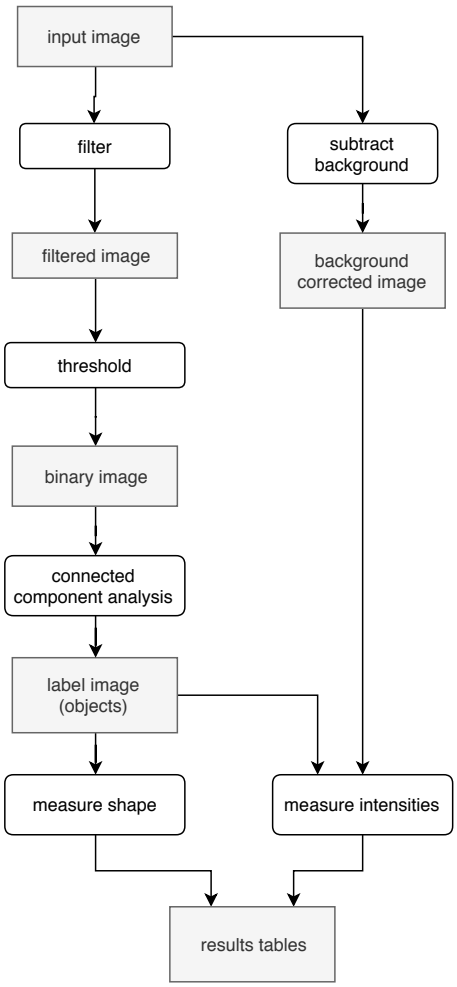


Activities

- Explore the effect of above rank filter sequences on some images

Advanced (typical) image analysis workflow

- ...

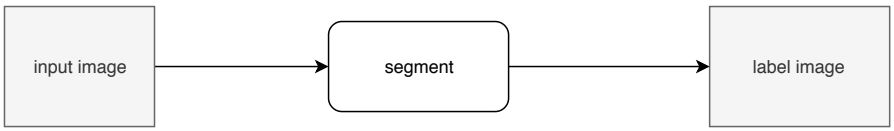


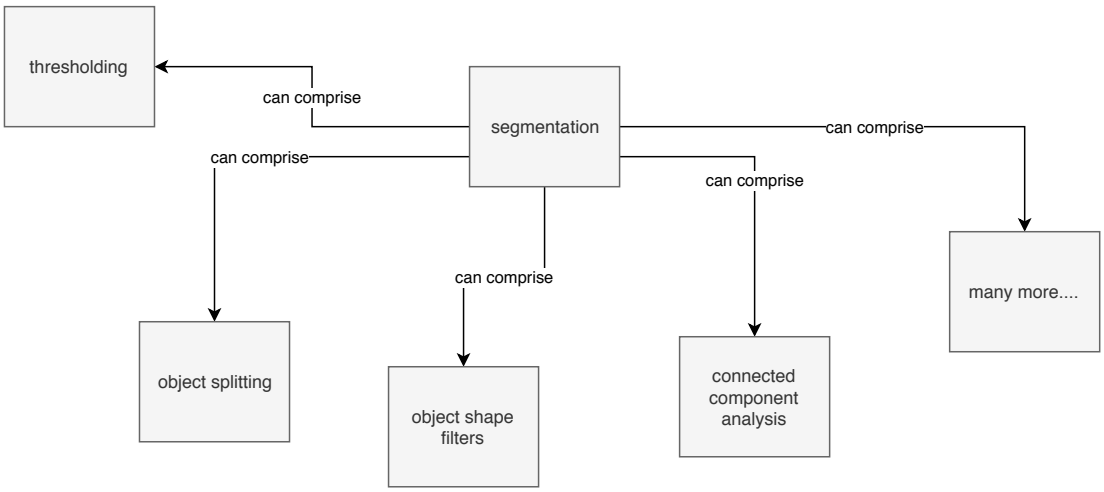
Activities

- Discuss each step in the pipeline and discuss alternative implementations.
- Discuss object filtering

Image segmentation

- https://en.wikipedia.org/wiki/Image_segmentation





Local background subtraction

- In biological images the background is often uneven, which means that it is not sufficient to just subtract one number from the whole image.
- The most common ways of local background subtraction are: tophat filter, median filter subtraction

Activities

- Discuss each step in the pipeline and discuss alternative implementations.
- Discuss object filtering

Object splitting using a shape based watershed

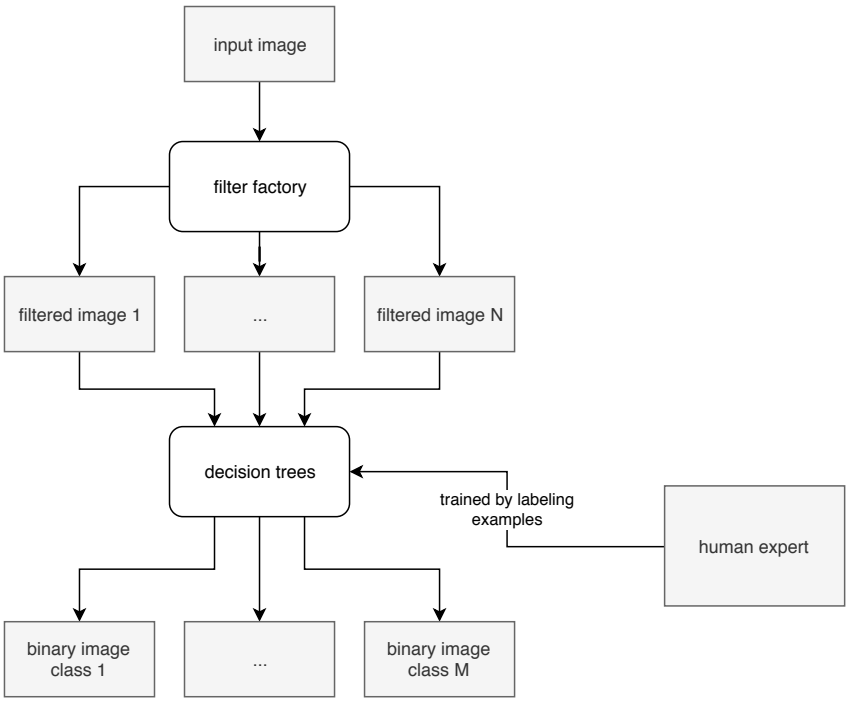
- Objects are often very close such that they might end up having the same label (=> connected compoments analysis).
- There are several ways how to split such "touching objects". The most common way is a "shape based watershed".

Activities

- Discuss each step in the pipeline and discuss alternative implementations.
- Discuss object filtering

Machine learning based pixel classification

- Finding the right combination of filters that lead to an image which can be easily thresholded can be tedious.
- It is possible to let a computer find the right combinations of filters to highlight certain structures in images.



Activities

- Discuss how thresholding can be viewed as a very simple form of "pixel classification"
- Try it out on an image

Automated threshold (global)

- ....

Activities

- Discuss each step in the pipeline and discuss alternative implementations.
- Discuss object filtering