# PHOT 533: Biomedical image analysis and image processing

### Final exam questions & solutions

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## Grading of the course

The project (and possible extension) will count for 50% of your grade. Points are given on the combined effort of the project and the oral explanation of it during the final exam.

The final exam comprises 50% of your grade and consists of 8 open questions. You will first answer the exam questions on paper and afterwards you explain your written answers individually.

## Exam questions

### Question 1: Image acquisition

Light captured by a camera is often taken as RGB color. When printing (with ink) however, often a different color scheme is used: Cyan, Magenta, Yellow, Black (CMYK). What is the relation between them or reason behind this?

**Answer**: Printing works with ink which absorbs part of the light and you observe the rest of the light. Adding a color makes the resulting absorption stronger, thereby reflected (observed) light less. For example the color cyan is the sum of blue and green light when working with light. However, when working with absorbing ink, you would construct green with cyan and yellow.

- Light: cyan = green + blue
- Ink (absorption): green = 1 - (red+blue) = cyan + yellow

## Question 2: Image acquisition

Staining tissue results often in nonlinear color intensities. This means that a cell of the tissue being twice as "brown" might not have twice the stained protein. Another challenge is that the color can change e.g. the "brown" color becomes black for very strong stained parts. How is this a problem for extracting the amount of protein using image processing? [optional] Can you think of a way to still get unbaised results up to a certain level?

**Answer**: As the intensity saved to the camera is "linear", we have less contrast for darker stained parts. As the color changes between dark and light stained pixels, the color change needs to be incorporated when trying to find the protein amount. When having multiple stains that both change their color depending on the protein amounts, the stainings become hard to untangle.

[optional] As a possible solution one can calibrate the staining up front using similar tissue samples as the ones of interest using only a single staining to find the intensity as function of the color adaptation. [optional] For the protein amount one should calibrate the intensity/color for similar tissue samples using a different technique, e.g. fluorescence to quantify the protein amount.

## Question 3: Image resolution

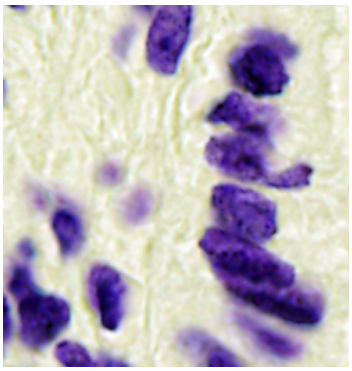
What properties of a microscope setup determine the point spread function (PSF) in the lateral direction?

**Answer**: The Numerical Aperture, the wavelength of the light, and the refraction index of the sample compared to the objective surrounding.

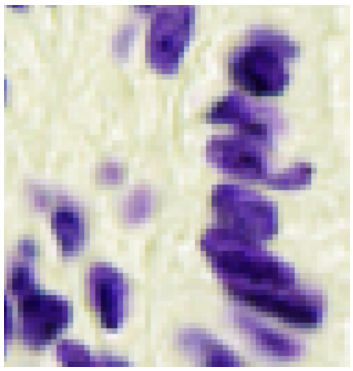
## Question 4: Image properties

The following images have different pixel sizes, what issues you can predict during image processing when the provided images have a too large pixel size?

Original image



4 times binned



8 times binned

**Answer**: To be able to discern objects or features the pixel size should be taken sufficiently smaller than those objects/features. In the second image background features are difficult to discern in the third image it is almost impossible to discern the location of the nuclei.

## Question 5: Image arithmetic and logic

If you multiply an 8-bit grey-valued image by a scalar larger than one, or if you add two such images together, you can obtain intensities higher than the maximum value 255 for 8-bit images. What is often done to prevent these kind of problems?

**Answer**: The integer intensity values of the image are converted to floating point values (32-bit or 64-bit). The intensities are often also normalized to fall between 0 and 1. The last promotes intensity values that are well within the lowest and highest floating point values. In certain cases it is not desired to convert integer values, and the issue can be prevented (for a limited number of operations) by using larger integer formats: e.g. signed 64-bit integers.

## Question 6: Neighborhood filters

What filter would be most appropriate to get rid of the noise in the following image with so-called pepper-and-salt noise? Chose out of following options, and explain why:

(a) A Gaussian filter with a large value of $\sigma = 10$
(b) A median filter (with a 3 by 3 kernel)
(c) A high-pass filter

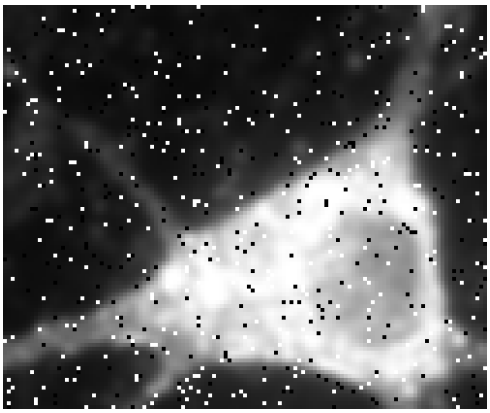
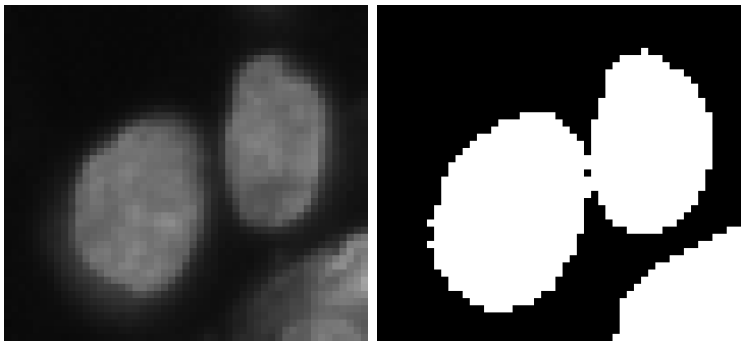


Figure 1: Image of neuron (Hippocampal region rat brain)

**Answer**: Answer (b), the median filter would be the best option because of the following: The noise exists out of mostly well separated pixels (either black or white). Therefore, a median filter would bring the pixel to the surrounding value (approximating the expected intensity). The Gaussian smoothing filter would remove a lot of detail, while still be suffering from taking the noisy data into account (since the errors of the noisy pixels are large). The high-pass filter would reduce the actual image content while keeping the noise.

## Question 7: Image Processing Pipeline

After segmentation often a post-processing step is performed. Splitting of foreground objects is often a challenge here. Can you describe a manner (a filter or combination of filters) in which you could possibly split the following two segmented objects?



**Answer**: An erosion or opening filter would remove the connection between the two nuclei. The last opening filter (erosion and subsequent dilation) would retain the overall size of the objects.

## Question 8: Object features and classification

There are many possible morphological/shape features that we can derive from detected objects in an image:

- Area,
- Perimeter (length of the "border"),
- Circularity,
- Average diameter

If you want to distinguish the two classes of objects in below image, which of the above features would be the most appropriate?

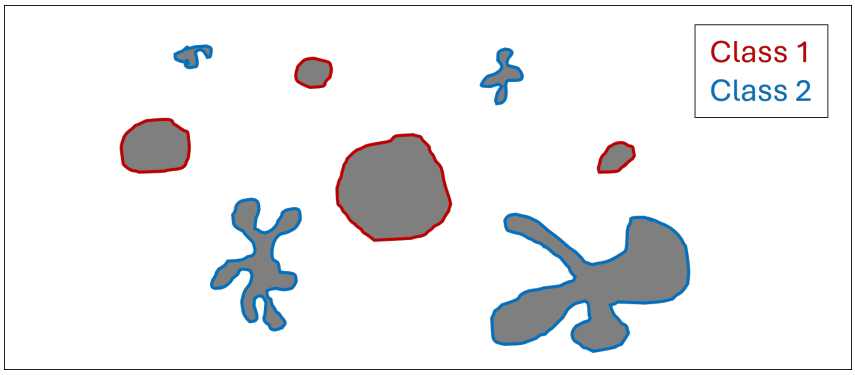



Figure 2: Shape classification

**Answer**: The image contains objects of different sizes and shapes, where objects within one class have a similar shape but can be scaled differently. Circularity would be best feature to separate the two classes, since it is scale-independent.