



The exam text is in English. You should answer the questions either in English or in Dutch. In either language and for all answers you should be **concise!** The exam consists of 3 questions on 2 pages. Mark each answer with the question number and use a **new page** for each of the answers. Clarity in the arrangement of the answers is much appreciated as it greatly helps correction of the exam. All answer sheets as well as this question list should be handed in! Incomplete sets are not considered for correction and marking. The exam starts at 11.15 hrs and ends at 13.00 hrs. Participation in the exam requires being present for at least 1 hrs. Good Luck!

**Question 1** (20 points)

Answer the following ten statements with correct/incorrect followed by a ONE-sentence motivation of the answer (motivation is required!!)

- Aliasing refers to the appearance of frequencies that are not in the original signal.
- In Confocal Microscopy a Look-up table is needed to produce colors on the screen that match the emission wavelength.
- The DC component of the FFT refers to the strength of the signal.
- On a microscope lens, the color code refers to the Numerical Aperture.
- The Gamut is a chromaticity diagram.
- For Phase Contrast Microscopy the specimen does not need to be fixed.
- For CCD camera the spectral response reflects how well it can be used for color imaging.
- The smaller the wavelength the higher the resolution that can be achieved.
- In Fourier Analysis Ringing is the effect of a poor cutoff filter.
- Safe colors are colors that every person directly understands.

**Question 2** (12 points)

For measurement, knowledge of the resolution of the optical system is very important. Therefore we like to characterize the system and we do so with the Nyquist Frequency. In this manner we can establish what can be achieved with a certain system. The Nyquist frequency is expressed as:

$$SD_{nyquist} = \frac{4 \cdot NA}{\lambda}$$

- Explain the formula for SD; how does it relate to the Nyquist criterion.

An optical system is referred to as band limited.

- How can we increase the sampling density in an opto-electrical system?

We check the power-spectrum of the FFT to characterize how the sampling is accomplished.

- Given the SD, how would that be seen in the spectrum?

We use the Raleigh criterion to get an idea of the resolution that can be achieved with an optical system, this is given by:

$$R = \frac{0.61 \cdot \lambda}{NA}$$

- With lens 10x, NA 0.25 what would be the minimal pixel size if we use a wavelength of 500 nm, taking the diffraction limit into account.



**Question 3** (13 points)

A biologist is preparing the acquisition of images on a bright field microscope. Due to uneven illumination it is difficult to use the whole of the dynamic range. From the histogram of the captured image it can be concluded that the microscope scene that was captured is underexposed; the histogram does show a typical bi-modal distribution.

- a) Sketch a histogram in the  $[0, 255]$  range from this underexposed image. Including, minimum and maximum values. You should choose the values of the minimum and the maximum here such that it can truthfully represent a histogram as indicated in the introduction of this question.

To suppress the effect of the under-exposure the histogram is rescaled by contrast stretching.

- b) Use the histogram of 3a to formulate a transform that rescales the image to 95% of the dynamic range. Write out the transform and sketch the resulting histogram.

In order to prevent the regular under-exposition the biologist is advised to adjust the illumination according to the Köhler principle.

- c) What should be done to do this?
- d) What other possibilities can the biologist try in order to obtain an even illumination in the image?