03 Assignment Scalebar(s) & Restauration

Assignment 3, 25 points in total; 16.6% of the total score for the practical part.

This assignment consists of four (4) parts. You follow the steps as presented in the assignment and compile the results in a report. In the report for each part answers to the questions should be presented; a total of 10 questions. The answers are algorithms, (processed) images, tables, graphs and explanations. As indicated, if required images that result from processing can be added. Make sure you present the images in a concise manner in your report so that they do demonstrate the effect that you observed after processing. Strictly adhere to the order of this assignment.

This assignment is completed in Python/DipImage. For this assignment there are in total *5* scale-bar images which are obtained from a research microscope at different magnifications.

Part 3.1

For this part of this assignment there are *3* scale-bar images which are obtained from a research microscope at different magnifications. These are CamIm01.tif, CamIm02.tif and CamIm03.tif. The microscope/camera setup that these images are taken with needs to be calibrated. To that end, the size of the pixels needs to be expressed in SI units. The images of the ruler are taken at different magnifications and these are used to establish the pixel sizes. However, as can be seen through inspection of the images, there is some an uneven illumination in the background, you have to consider this in the processing of the result from the segmentation.

1. (3) Design a strategy to do the calibration for this microscope/camera using these three images: CamIm01.tif, CamIm02.tif and CamIm03.tif. In the strategy, indicate the *a priori* knowledge that will be included in the algorithmic approach.
2. (3) Design and implement an algorithm to come to an objective, precise and accurate measurement. Use binary mathematical morphology operations to be able to do a good measurement.
3. (2) Apply the algorithm you have implemented to the three scalebar images and provide the information on pixel sizes of this microscope/camera configuration in table form.
4. (2) The image CamIm03.tif is acquired with a 100x lens. From the calibration of this image derive the lens (magnification) with which the other two images were acquired in the form of a table. Explain the results that you obtained in terms of probable magnification and computed magnification.

Part 3.2

For part two of this assignment, two images are used: i.e., scale-im.tif and scale_im.ics. If you check the file size of the two images, you will notice that scale-im.tif and the scale-im.ics considerably differ. Moreover, the “ics” file consists in fact of two files; the header information (ics) and the data information (ids) are stored in separate files. This format is often used in

dip-image. You can save images in the ics-format. If you wish to write your files in another format you should indicate this as a parameter with command used for saving the images. Take the opportunity to study the structure of the ics-format.

5. (2) Explain the content of all items that are listed in the header (ics). What does the abbreviation stand for?
6. (2) Read both images scale-im.tif and scale-im.ics and compare the content of the two images. What are the differences? and show this in a numerical form. Explain the differences in file-size of both images.

Part 3.3

The image (part 3.2) contains a scale bar from which the pixel size in the image can be derived. For measurement on the scale bar segmentation is required. This is seriously hampered by the quality of the image. The image needs to be enhanced by first removing the uneven illumination in the background. This **should** be accomplished by **Mathematical Morphology,** applying grey-value mathematical morphology operations. Explore the possible parameters of the structuring elements in order to get a best possible result. For this particular image, at least two parameter approaches are compared.

7. (3) Design a strategy for pre-processing the images such that the image is sufficiently enhanced. Key to your approach is to remove the uneven illumination in the background. For the strategy clearly state the parameters that you have chosen and explain the result in the context of these parameters; write out the algorithm with different parameters.
8. (2) Apply segmentation on the result and motivate the procedure used. From the segmented image measure pixel size in units-per pixel.

Part 3.4

For this part image CamIm04 is used. This is an image of a different reticule compared to Part 3.1 but acquired with the same microscope/camera. The image quality is (even more) seriously hampered by an uneven illumination of the background. In part 3.3 you have developed an approach to get rid of the uneven illumination so that you can provide a good segmentation of the reticule. Please note the *a priori* information you get from the image which you can use for the answers.

9. (3) Apply background correction and segmentation on CamIm04. Show the result and motivate the procedure used. The approach can be similar to the approach taken in Part 3.3 (question 8), however, as the image is different, the parameters might be different.
10. (3) From the segmented image measure establish pixel size in SI-units and compare the result with that obtained from Part 3.1. Specifically, derive the magnification of the lens using the result that you obtained from Part 3.1 (question 4). Comment on the difference in the reticule used in CamIn04.

For your report on this assignment, include the processed images; the code should be added as an additional file. For each part of this assignment, you demonstrate image processing results by making a panel of image results with a clear legend. Make sure that the size of the image and the grey-value balance support what your results in the best possible manner.