06 3D Shape, Pre-processing & Visualization

Assignment 6, 30 points in total; 20% of the total score for the practical part.

This assignment consists of 2 parts. **First** *chromo3d* is used. Both DIP, Huygens and VAA3D can be used. DIP-Image is used for some pre-processing of the images. For visualization and deconvolution VAA3D reps Huygens are used. The first part uses a CLSM image of plant chromosomes (Crepis sp.) and is given in the Assignment 6 archive. The second part uses the zebrafish images that you have made with the CLSM yourselves; these are all 3D images.

Part 6.1

1. (1) How many slices are in this image? And what is the aspect ratio?
2. (1) Make a function to display the image content of the original image as a panel of image planes so that the viewer can understand the content of the 3D image.

In order to get a good impression on the content of the file (plant chromosomes) several display functions are being explored and some new ones are added. The idea is to generate visual information on the content of the 3D image from 2D and 3D displays. In DIPLIB a 3D image can be simplified to a 2D image using a convert function. Some display functionality needs to be made by yourselves. In addition, VAA3D, or in some cases Huygens can be used. Use your own functions and these explore these other software packages to assess content of the 3D image and generate 3D displays.

Part 6.2

3. (1) Apply thresholding to the image. What is the best threshold value?
4. (3) Construct an algorithm to improve the visualisation of the thresholded image. We will explore depth cueing: each plane in the 3D image is attributed an intensity value. The lower the plane value to lower the grey value attributed. Make sure that the values for the planes are truly distinct.

Another way of displaying a three-dimensional image is to use the simulated fluorescence process which is typically used in CLSM. The ***sfp*** algorithm is part of Huygens Essential (*sfp* function). It is also part of the view display functionality of Huygens Software. Below results of the chromosome image using *sfp* are depicted.

5. (1) Explain the differences between figure 1 and figure 2

Figure 1

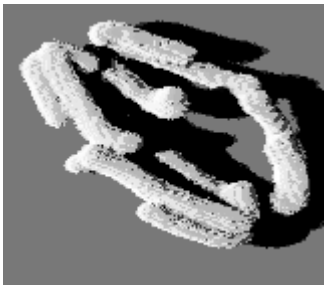

Figure 2

Part 6.3

The chromo3D image can be read in VAA3D. Here a number of display functions are available. This image has only one channel, and so this one channel is considered the "red" channel. Use the "see in 3D (entire image" function to generate a 3D view. This will be rendered as a maximum projection. We have looked at the aspect ratio in the plane. For good 3D display, the z-axis ratio must be set. We first start with the Volume visualization.

6. (3) Use an aspect ratio of 1:1:3 and apply the *maximum projection* function – this is the default display mode in VAA3D. Play with the contrast function as you think necessary. Generate a view with the maximum projection and with the alpha function. You can generate an animation to show your result.

Next, we continue with surface visualization. To that end you will use the "Load/Save Surf" function. You will choose channel One to create a surface for and you will use the option of a range surface.

7. (3) In order to produce a good surface visualization, you will have to explore the parameters, "lower range, upper range and mesh density. Make sure that the resulting visualization is displayed with the correct aspect ratio and at the same time that contents are visualised well, meaning that the chromosomes are well separated. Produce 2 to 3 views of your result. You can mix it with the volume visualization and use the threshold function to show less/more of the volume data. Explain the parameters that you have used.

The thresholded image and the depth cueing image can also be used for a visualization in VAA3D along the same lines as 6 and 7.

8. (2) From saved versions of the binary and the depth cueing image produce a volume and surface visualization form these images. Make for each of the versions, binary and depth cueing, at least 2 views. Explain the parameters that you have used for the visualizations (volume and surface).

Part 6.4

You have acquired 3D images of zebrafish with the CLSM. These images will be used in the next part. We will look into ways of improving the quality of the image for visualization and measurement. To that end, both VAA3D and Huygens are used; this is in addition to DIPLIB which has a lot of filters that can be useful.

9. (2) For your images produce a static 3D visualization. You can do this for separate channels or join them for the visualization. If necessary, first separate the channels and then join them; such can be accomplished with Huygens where you can use *sfp* and maximum projection.
10. (3) Improve the signal strength by processing the separate channels and produce a new 3D visualization of the images that you have used in (9). Explain the processing you have used with the parameters you have chosen.

The Huygens software is originally designed to do deconvolutions. This will be applied to your own images to see how much these can be improved through deconvolution.

11. (3) Use the automated (option for) deconvolution (Huygens) software to improve the image S/N. The initial result from the *sfp* visualization and maximum projection can be included in your report. Explain the differences from your efforts with preprocessing in (10).
12. (3) Use the manual deconvolution on your images and assess the parameters for deconvolution. Explain the results using the visualizations of your images for the parameters you have chosen in the manual deconvolution.
13. (4) Make the visualizations dynamic and publish the results as *mp4* movies. This should be done both 3D images. Per movie include the different results that are obtained so that, in a dynamic manner, the differences are demonstrated. Both Huygens and VAA3D have a good tool for making animations.
    Motivate the steps that you have used to come to the result; make this *mpeg* available to the course administration in the upload of the results to BrightSpace. These movies should be uploaded as **A6[team#]_[movie#].mp4**

In the report make good documentation of the movies so that they are referred to in the right manner and thus can be well understood. General remark; All images are provided in native format – if your images are not good enough for this assignment, please notify the course administration.