06 3D Shape, Pre-processing & Visualization

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

This assignment has two main parts. For image pre-processing, you will use **DIP-Image (PyDIP)**. For visualization and deconvolution, you use **VAA3D** and **Huygens**.

1. In **part 1, 2 & 3**, you will work with a CLSM image of plant chromosomes (Crepis species) (file name: chromo3D), which is provided in the Assignment 6 archive on Brightspace.
2. In **part 4**, you will use the 3D CLSM images of zebrafish that you acquired yourself

Part 1

1. How many slices are in the **chromo3D** image? What is the aspect ratio? (1)
2. Write a function that extracts and displays the individual 2D slices from the 3D TIFF image. This is to make sure that the viewer can understand the contents of the 3D image. Show the resulting figures in the report. (1)

To better understand the content of the file (plant chromosomes), you will explore several display methods and create some new ones yourself. The goal is to generate informative visualizations of the 3D image using both 2D and 3D display techniques. In **DIP-Image/PyDip**, you can view a 3D image by displaying it as multiple 2D images. Using **VAA3D** and **Huygens** it is possible to further explore and visualize the 3D structure. Use a combination of your own functions and these software tools to assess and display the contents of the 3D image.

Part 2

1. Apply thresholding to the chromo3D image. What is the best threshold value? (1)
2. Develop and describe an algorithm to enhance the visualization of a thresholded 3D image by applying depth cueing. This entails assigning distinct grayscale intensity values to each z-plane in the 3D volume such that deeper (lower-indexed) planes appear darker, and shallower (higher-indexed) planes appear brighter. (3)

Another way to display a 3D image is by using the **simulated fluorescence process (SFP)**, which is commonly applied in **Confocal Laser Scanning Microscopy (CLSM)**. The SFP algorithm is available in Huygens Essential (through the **sfp function**) and is also included in the view display options of the Huygens software. Below, you will see the results of applying the SFP method to the chromosome image.

3. Explain the differences between Figure 1 and Figure 2. (1)

Figure 1

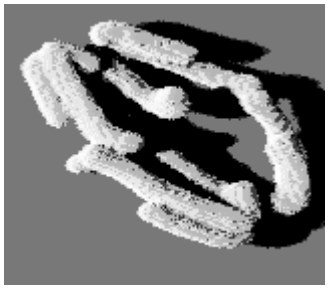

Figure 2

Part 3

The **chromo3D** image can be opened in **VAA3D**, where various display options are available. This image contains only one channel, which is treated as the "red" channel by default. To create a 3D view, use the **"See in 3D (entire image)"** function in VAA3D — this will display the image using a maximum intensity projection. For accurate 3D visualization, it's important to adjust the **z-axis scaling** to correct the aspect ratio. We will begin by using the **Volume Visualization** feature for this purpose.

1. 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. Generate animations for both views and ***submit the .mp4 files along with the report.*** (3)

Next, we continue with **surface visualization**. For that, you can 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.

2. To produce a good surface visualization, explore multiple parameters like lower range, upper range and mesh density. Make sure that the resulting visualization is displayed with the correct aspect ratio and that the contents are visualised well (chromosomes are well separated). Produce 2 to 3 views of your result. You can combine 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 in the report and justify your choice.** (3)
3. From the results of the binary and the depth cueing image (in Part 2, save as TIFF), produce a volume and surface visualization from these images. Make at least 2 views for each of the versions. **Explain the parameters that you have used for the visualizations (volume and surface)**. (2)

Part 4

You will have acquired 3D images of zebrafish with the CLSM (from the practical sessions). These images will be used in the next part. We will investigate ways of improving the quality of the image for visualization and measurement. For this, both **VAA3D** and **Huygens** can be used; this is in addition to **DIPLib** which has a lot of filters that can be useful.

1. From your images, produce a static 3D visualization. You can do this for separate channels or combine them for the visualization. If necessary, first separate the channels and then join them. This can be accomplished with **Huygens** (where you can use sfp and maximum projection). (2)
2. Improve the signal strength by processing the separate channels and produce a new 3D visualization of the same images (used in question 4.1). **Explain the processing you have used with the parameters you have chosen.** (3)

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.

3. Use the automated (option for) deconvolution software to improve the image S/N. The initial results from the sfp visualization and maximum projection can be included in your report. Explain the differences from your efforts in question 4.2. (3)
4. 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. (3)
5. Make the visualizations dynamic and publish the results as mp4 movies. This should be done for both 3D images. For each 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. ***Submit the .mpeg/.mp4 files along with the report in Brightspace***. These movies should be uploaded as **A6[team#]_[movie#].mp4**. (4)

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.