Lesson 5: Using Nonlinear Anisotropic Diffusion (NAD)

Filtering with NAD is relatively slow and involves adjusting two parameters to give the desired amount of filtering. The strategy is to do the operation on a small test volume and compare the results from different parameter settings in order to pick the right setting. After that, the full volume is filtered by breaking it into chunks so that multiple processors can be used.

1) `cd $WORKSHOP_HOME/IMOD_Labs/cryo-subvolume`

2) `etomo`

3) Press **Nonlinear Anisotropic Diffusion.**

4) First, we extract a test volume. Press the **file chooser** icon on the **Pick a volume** line and select *cryoSubvol.rec*. Press **View Full Volume**.

   Zoom the ZaP window up to 1 if necessary, and draw a rubber band in the middle that is about 300x300 pixels (see the size in the ZaP toolbar). Scroll through the slices and set the **Lo** and **Hi** limits to extract 25-30 slices. When choosing a test area, try and pick a region that exemplifies your region of interest. For this example, you might be interested in microtubules, actin, or ribosomes.

Press **Get Test Volume Range from 3dmod** to fetch the range into Etomo. Press **Extract Test Volume**. Press **View Test Volume** to make sure you got an appropriate selected area.

5) Now, we need to find the first parameter, the **K Value for the Test Volume**. For **List of K values**, enter **0.1,1,5,10,15,25,30,50,75.** And keep the **Iterations** at **10**. Press **Run with Different K Values**. The program computes a volume for each K value, using multiple processors. The K value controls how the diffusion of density occurs on each iteration. Where the image gradient is higher than K, this is considered to represent an edge, and density flows along the edge and not across it. Where the image gradient is lower than K, density diffuses uniformly.

6) When done, press **View Different K Values Test Results** to load the multiple volumes into 3dmod. The ZaP window has a second toolbar with **4th D** left and right arrows for stepping between the volumes (the keys **1** and **2** can also be used). The toolbar also shows the name of the file being displayed.

   One way to compare the volumes is to step between them in one window; this is the best way to see how they change from one to the next. To see the volumes side-by-side, select **Image / Linked Slicers**. A slicer will open for each volume, each one locked to that volume (note the red lock icon in the toolbar). The slicers will be kept at the same orientation and position, controlled by a single floating toolbar.

   Images look strange for intermediate K values (10 - 25), with regions of uniform intensity. Above this range, K is too high to stop the diffusion across places of high gradient, and diffusion is essentially isotropic. Within this range, diffusion is blocked across medium to high gradients, so the density probably gets trapped in medium-sized regions bounded by these gradients and becomes

evenly distributed. Below this K range, there are many, more closely-spaced, gradients to block diffusion, and this probably prevents the noticeable uniform regions. Close all 3dmod windows once you know the value you will choose.

7) Next, we need to choose the second parameter for NAD by **Finding Iteration Number for the Test Volume.**

   Enter your preferred K value in the **K value** field. For **List of iterations**, enter **2,5,8,11,15,21**. Press **Run with Different Iterations**. This time the nad_eed_3d program runs once, saving the results at each selected iteration.

   Press **View Different Iteration Test Results**. This time it is probably easier to assess the preferred filtering by stepping through the volumes in the ZaP window.

8) Now we can apply the 2 parameters by **Filtering the Full Volume**. Insert your selected K value in the **K value** field and number of iterations in the **Iterations** field. Press **Filter Full Volume**.

   When it is done, you can open it with **View Filtered Volume**, and delete the test volumes with **Clean Up Subdirectory**. Close Etomo and 3dmod windows when you are finished. The final volume is named by adding the extension *.nad* to the name of the original volume. Note that the extensions *.rec* and *.nad* are simply conventions and do not specify a file format; this volume is an MRC file.