Opening CT data (an image stack)

1. File > Import Image Files… > Open Folder
    a. Select folder where the stack of image files is located.
    b. When the console populates with image file names, confirm that only images that are part of the stack is included (sometimes there are extra files in the folder).
2. > Next
    a. Image spacing (in μm) needs to be specified for each scan. In File Explorer, go to the folder that includes the image stack folder and there should be an XTEKCT or PCA file. Open it in a plain text editor (e.g., Notepad). Copy and paste the value associated with voxel size (typically X, Y, Z resolutions are the same so no need to copy and paste each one). Go back to Dragonfly and paste the value into X, Y, and Z fields.
3. > Finish

Initial Setup

4. To change to quad-view, in the left menu: Layout > Views > click on 4-window icon.
    a. In this view option, you can always double click on a window to maximize that view. Then double click again to go back to 4-window view.
    b. View the data in 2D and 3D views until you get a hang of the controls and to help orient you with the specimen.
        i. Mouse control within 3D view:
            1. Rotate: left click drag
            2. Pan: Left + right drag
            3. Zoom: press on scroll wheel and drag
        ii. Mouse control within 2D view:
            1. Pan: Left + right drag
            2. Zoom: press on scroll wheel and drag
            3. Go through stacks: scroll using the scroll wheel. This is fairly slow, so it's faster to click on "Range Min: xxx Max xxx" text and drag up or down to go through the image stack more quickly.
    c. Adjust color range in 2-D view in left menu list: "Window Leveling" > adjust histogram (typically shift the left yellow bar to the right to make darker, low density areas all true black color)
5. Crop to remove empty spaces
    d. Right menu: "Properties" tab > right click on CT volume in project tree > Modify and Transform > Crop…
    e. Change the dimensions (can do this either in 2D or 3D views)
    f. Once area to keep is set, then uncheck "Create new dataset" and hit apply.

Save

- This is a good time to save the Dragonfly file. You can keep the file locally on the laptop until you're done segmenting and reconstructing. Once you're done, please upload the Dragonfly file to the appropriate Dropbox folder (specimen folder above the image stack folder).

Segmenting

6. Left menu: click on "Segment" tab.
7. As first step, it's good to segment based on greyscale value range, where you highlight all "white" parts of the image stack (i.e., mostly bone). Although not perfect, this will get you most of the way through reconstructing the skull.
    a. "Range" menu: Check "Define range"
    b. Adjust the dial below to specify the range of greyscale values to highlight (can see highlighted section in red on 2-D views). You want to make sure that all the bones are

included and any or most of the unwanted regions un-highlighted. If reconstructing avian skulls, then good to check that the keratinous beak (slightly less than bone) is not highlighted.

c. Click "Add to New." This will create a new ROI (Region of Interest), which will show up in the "properties" tab on the right of the screen. You can double click on the "new ROI" in the project tree and rename it to "Skull"
d. To the right of the ROI name, click on "3D" button (crossed out in default) to display the 3D model of what you segmented so far for that ROI. Turn off any other 3D buttons to only show the segmented ROI in the 3D viewer.
    i. I don't like the default 3D model rendering settings. To change, in the right menu underneath the project items: "3D opacity" > reduce "Highlight"; adjust "ROI".

8. Manually add or subtract by using the paint tool
    a. At this point, don't worry about small or isolated pieces that are separated from the skull. These can be easily removed later with another program (GeoMagic Wrap).
    b. The use of greyscale range above may sometimes miss some thin bony structures (e.g., typically the occipital region) or include unwanted parts (e.g., some parts of the keratinous beak).
    c. Make sure that the correct ROI ("skull") is selected on the right menu. Otherwise, you may not be able to modify its segmentation or if there are other ROIs, you will be changing the wrong ROI.
    d. When you have one of the 2D views clicked on, in the left menu, "ROI Painter" menu.
        a. Here is where you select a tool to add to or remove already-segmented regions of the ROI. The "paintbrush" (left-most button) will typically be the tool you will be using. Depending on the situation, you will select either "2D mode" or "3D mode". "2D mode" if you want to segment bit by bit with control at each image slice. "3D mode" allows you to segment across multiple image stacks depending on the size of your brush. One must be extra careful with "3D mode" because if may select or remove parts unintentionally.
        b. The paintbrush can either be circular or spherical; or square or cubic by clicking on one of the two buttons below the paintbrush button.
        c. To add to existing ROI, you hover the cursor over one of the 2D views. To readily change the size of the brush, you hold done "left shift" or "left control" and scroll using the scroll wheel. To add to the ROI, hold down "left control" key and drag the brush. To remove from ROI, hold down "left shift" key and drag the brush.
        d. You will notice that this will highlight or remove a circular or square region. Most of the time, you will want to highlight only bone or remove parts with certain greyscale values. In this case, you can go back to the "Range" section and check "Define range" then specify the greyscale range that you want to restrict segmenting to. This allows you to paintbrush only the red-highlighted parts of the image stack.
    e. Before we further edit the ROI, make a copy of the ROI so that you'll always have an unedited version in the file. To make a copy, select the ROI label on the right and click the blue copy button below. This will add a copy of the ROI in the list.
    f. There are a lot of thin trabeculae (struts) inside the skull, which will add tremendous amount of extra polygons in the model that we don't need for our research. To close this up, go to "Morphological operations" section > Select "3d" > Shape: "Cube" > "Kernel size: "15" (this value seems to work for some, but always check to see that space between individual bones are not being linked) > click "Close". This will take a while to complete. Repeat this "close" procedure several times until the skull becomes a solid unit.

Generate mesh (3D model)

- After you're confident that you've gotten the entire skull segmented, then you can generate a mesh file (3D polygon file).

- Still in “Segment” tab > Export
    - Change “To a Mesh” > “Normal”. After some time, this will create a mesh as one of the project items on the top-right. Make the mesh visible by clicking on the “eye” icon and the “3D” button next the mesh label.
    - Underneath the project tree, you can see how many vertices, edges, and triangles make the model. This will be fairly high, meaning very large file size, so we want to reduce these values. To do so, right click on mesh label again > “Mesh Decimator” > adjust the dial to reduce the number of triangles to just above 10,000,000.
    - Right click on new mesh > “Export” > “Mesh to File” > in the Save As window, “Save as type: “PLY (*.ply)” and name as specimen name with number and skull: e.g., PCC_GGP001_skull.ply. Check “Export as binary.” Please save it locally and then upload it to Dropbox in the appropriate specimen folder. Ideally, the file size of the exported PLY file should be in the order of 200–300MB.
    - To check out your new 3-D model, you can download the free software MeshLab and open in the program.