

Some preliminary notes:

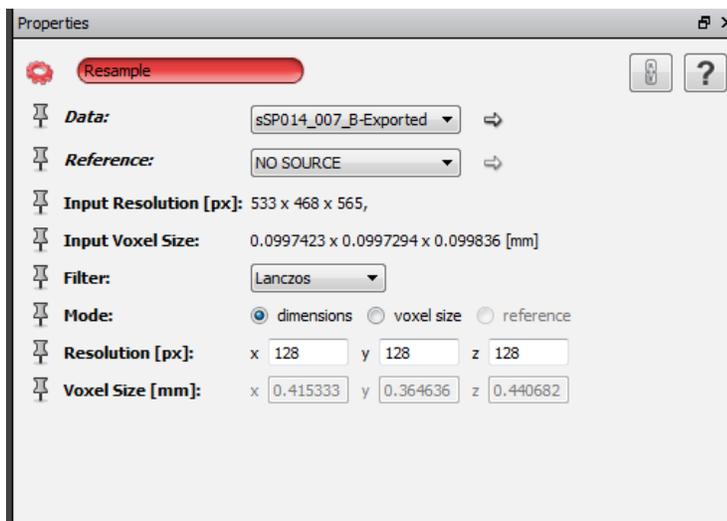
These instructions are for stitching two overlapping data sets together. If you have more than 2 data sets to stitch together, I'd recommend doing one set at a time: Stitch A to B, save as A+B, stitch A+B to C, save as A+B+C, etc.

### 1) Open all of the data sets you will be stitching together.

### 2) Downsample all of the data sets

(Note: This is not a necessary step, and the end result is that you will be decreasing the resolution of the data to be stitched together. But most data sets taken in the BRC CT facility are large enough that I'd recommend downsampling, so as to prevent very slow computing times and increased likelihood of the software crashing. Skip to step 3 if you are sure you don't want to downsample. If you are downsampling, you will want to do it for all of your data sets.)

Right click on the first green object in the project view that you want to downsample, search for "resample", click on it.



Set the resolution to your desired dimensions. Your starting dimensions are shown next to "Input Resolution" (NOT "Resolution": note that these values always start at 128 px. They do not describe the current file size.) Reducing all the numbers by half is a good starting point. Click Apply. A green object ending with .resample will show up in the project view tab. Click on it. File->Save Data As. Save as a dicom or tiff set in a new folder. When stitching the data together, you will be using this new, resampled data.

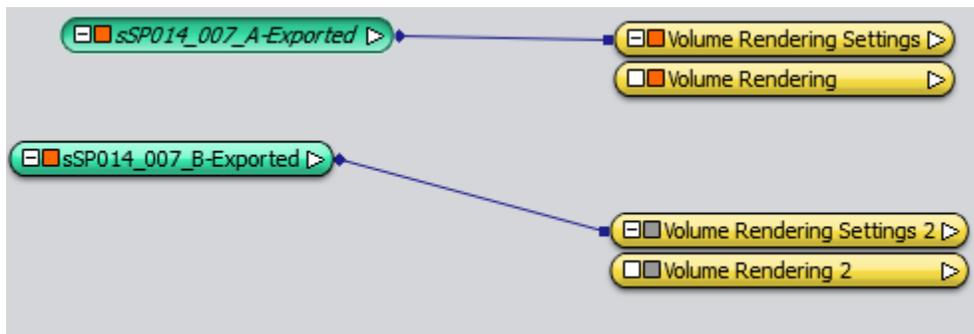
Do this for each data set to be stitched.

Once you have resampled, you don't need to keep the original data in the Project View. You may take all green objects associated with the original, un-downsampled data sets and drag them to the trash can.

### 3) Coarse Alignment of Data sets

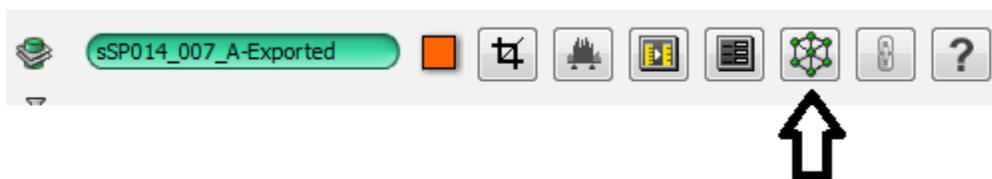
When a data set is visible in Avizo, it's located in what, for lack of a better term, is a real physical space in the window. The first thing we are going to do is to take the data we have and arrange them so that they are relatively close to their actual aligned positions. This will help in the next step.

Attach a Volume Rendering object to all of your data sets (note that for large data sets, setting an Ortho View may run more smoothly, though alignment may be a little more difficult. For now, remove the second volume rendering object from the display by clicking on the orange square (this turns it to gray and stops it from displaying on the main screen.)

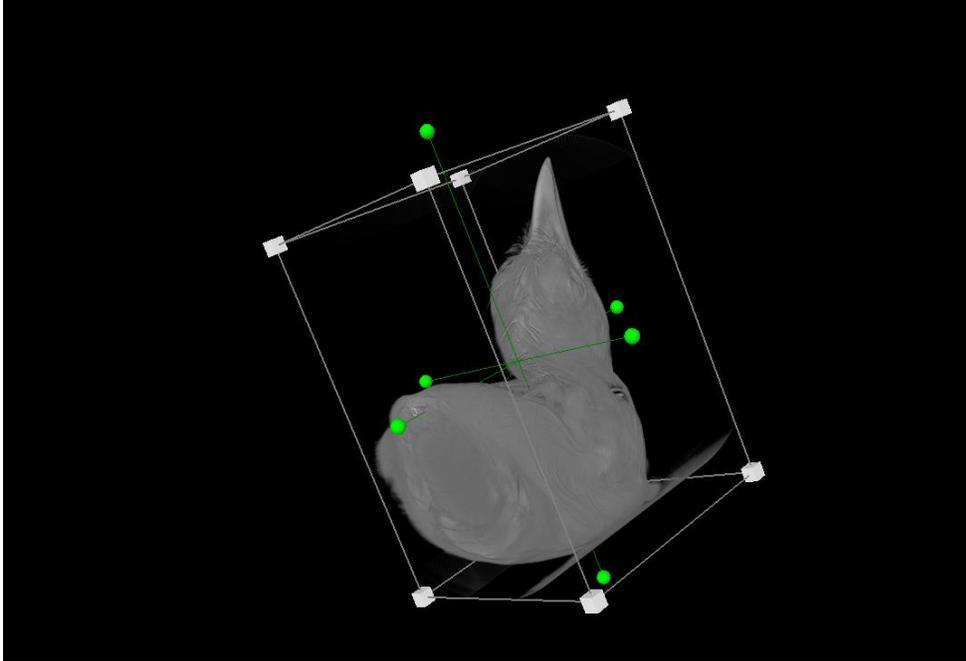


Make any adjustments to the volume rendering settings that you need to make the area of interest clearly visible.

Select the data file you will be working with (File A above has been selected.) In the properties window, select the Transform Editor. This is the icon with the wire frame cube; rolling over it will display "Transform Editor."



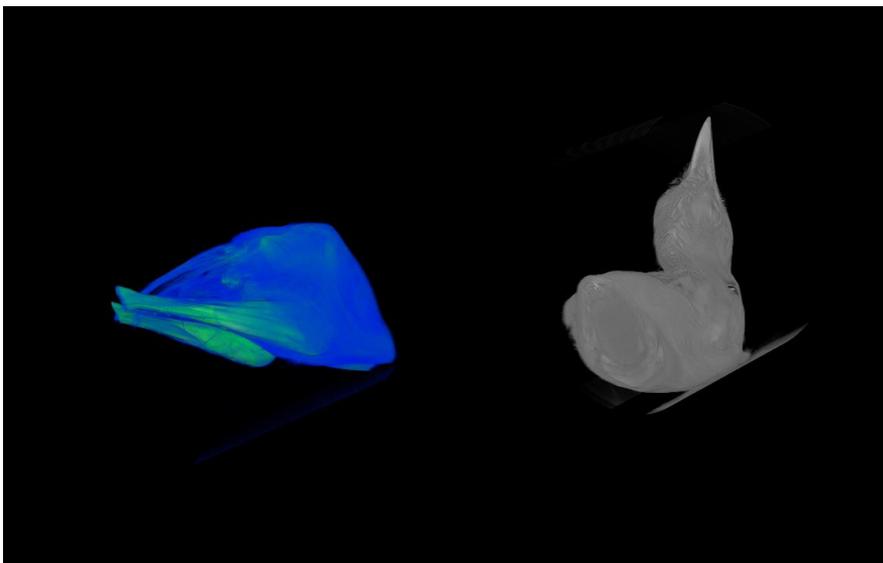
This will toggle a wireframe box seen below. The data set can be moved (translated), rotated, or rescaled relative to the other data sets if you have your mouse set to cursor mode (click on the mouse arrow button on the top menu).



*(Data Courtesy Eric Gulson and David Winkler lab)*

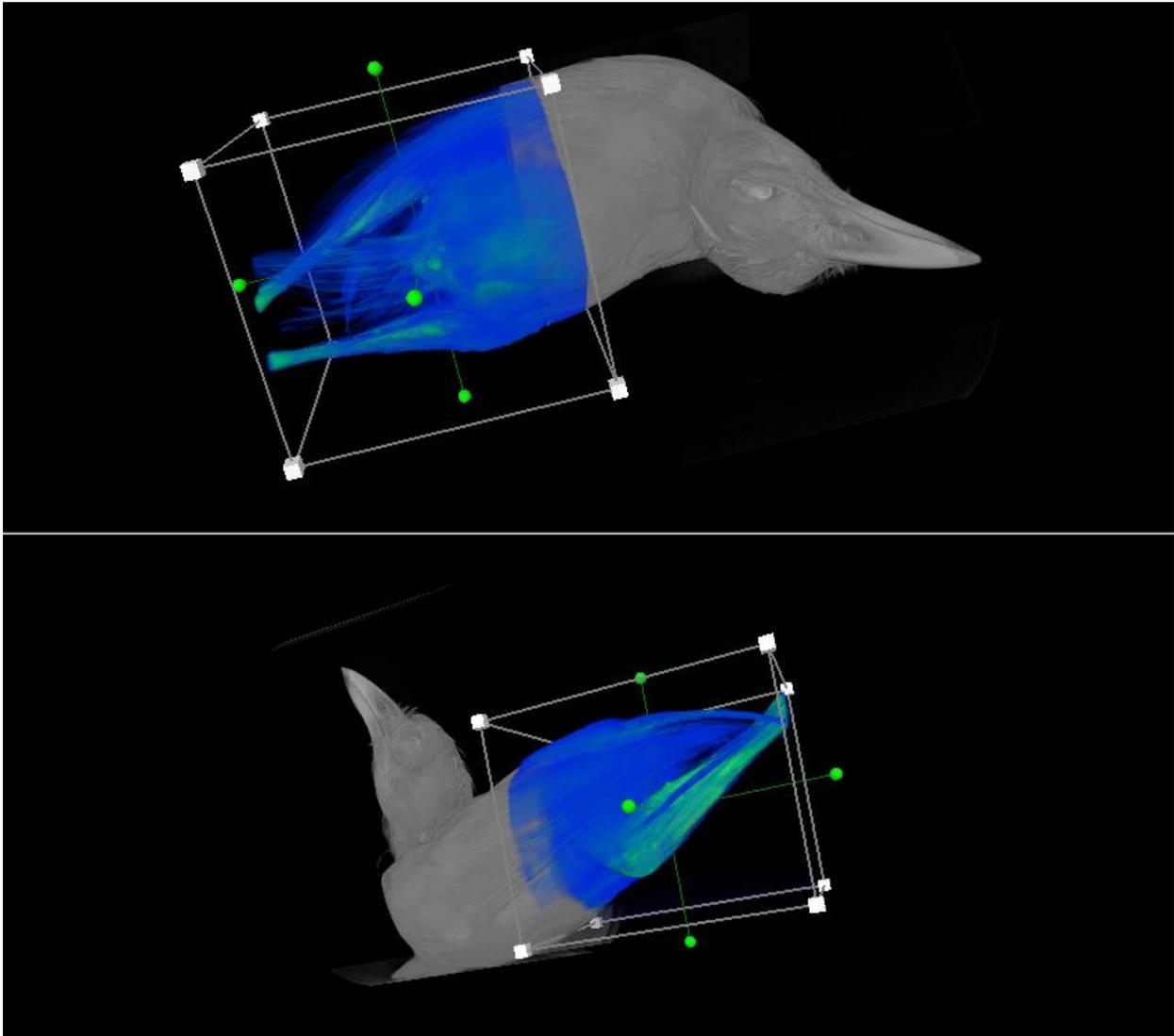
Using the cursor, click and drag the scan to one side, so that the next data set will not overlap when made visible. Any mistakes can be undone in the file properties menu under “Reset”.

Deselect the Transform Editor for the first data set, then display the volume rendering for the second data set, making any needed adjustments.



(Note: I have used a different colormap in this example for clarity.)

Toggle the Transform Editor on the second data set, and position it in its desired location.

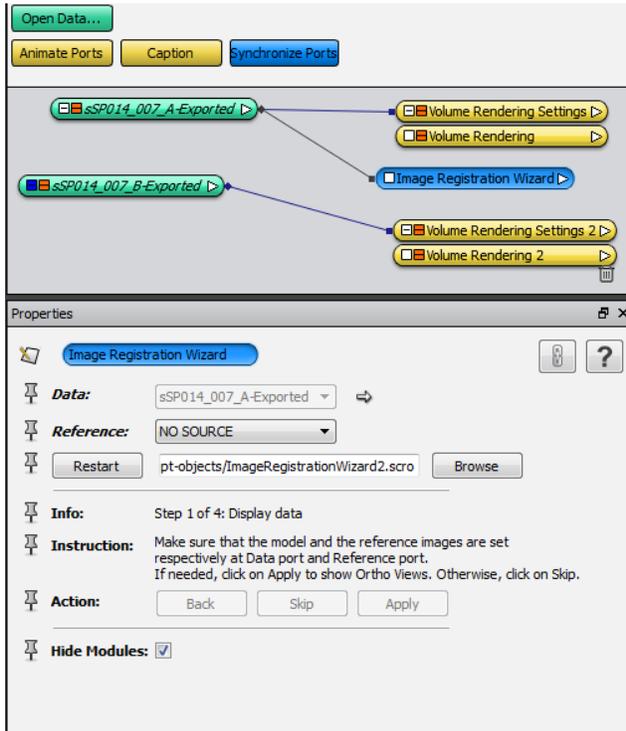


Toggle the Transform Editor off when you're done.

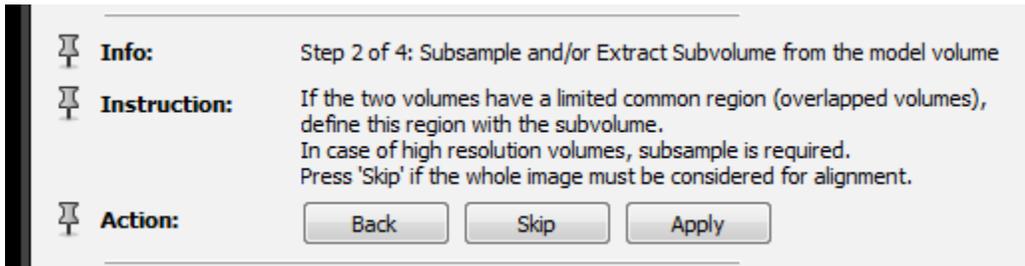
#### 4) Image Registration

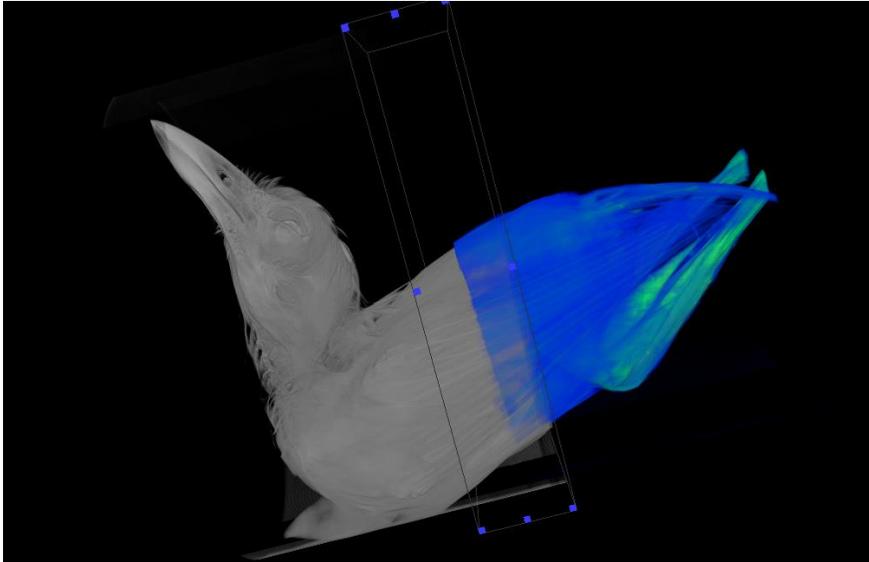
(Note: Using the Image Registration Wizard is an optional step. I sometimes find that it is faster and more effective to do this by hand using the techniques shown in step 3, particularly by comparing the three Ortho Views.)

Apply the “Image Registration Wizard” function to one data set. Click on that object to display its information in the Properties window.



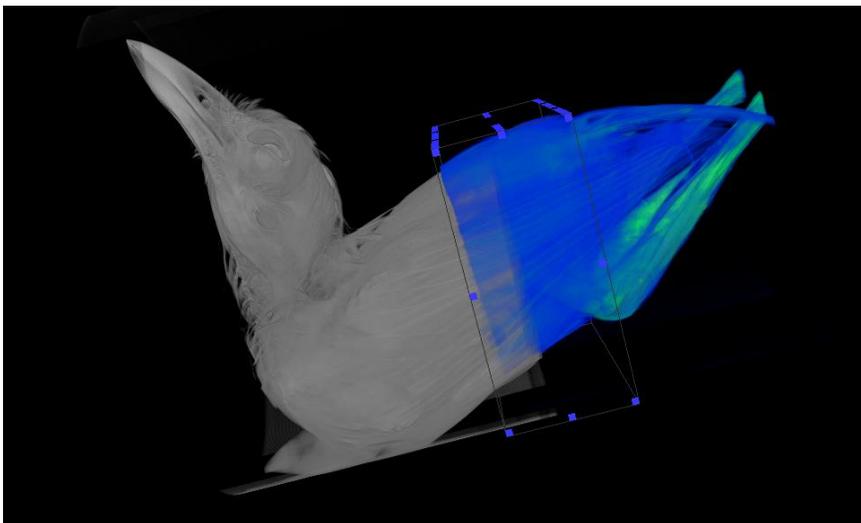
Using the drop-down menu, set the Reference to the second data set. If your data is not currently visible, either toggle it back on or click Apply to display an ortho view of both data sets, then click Skip.





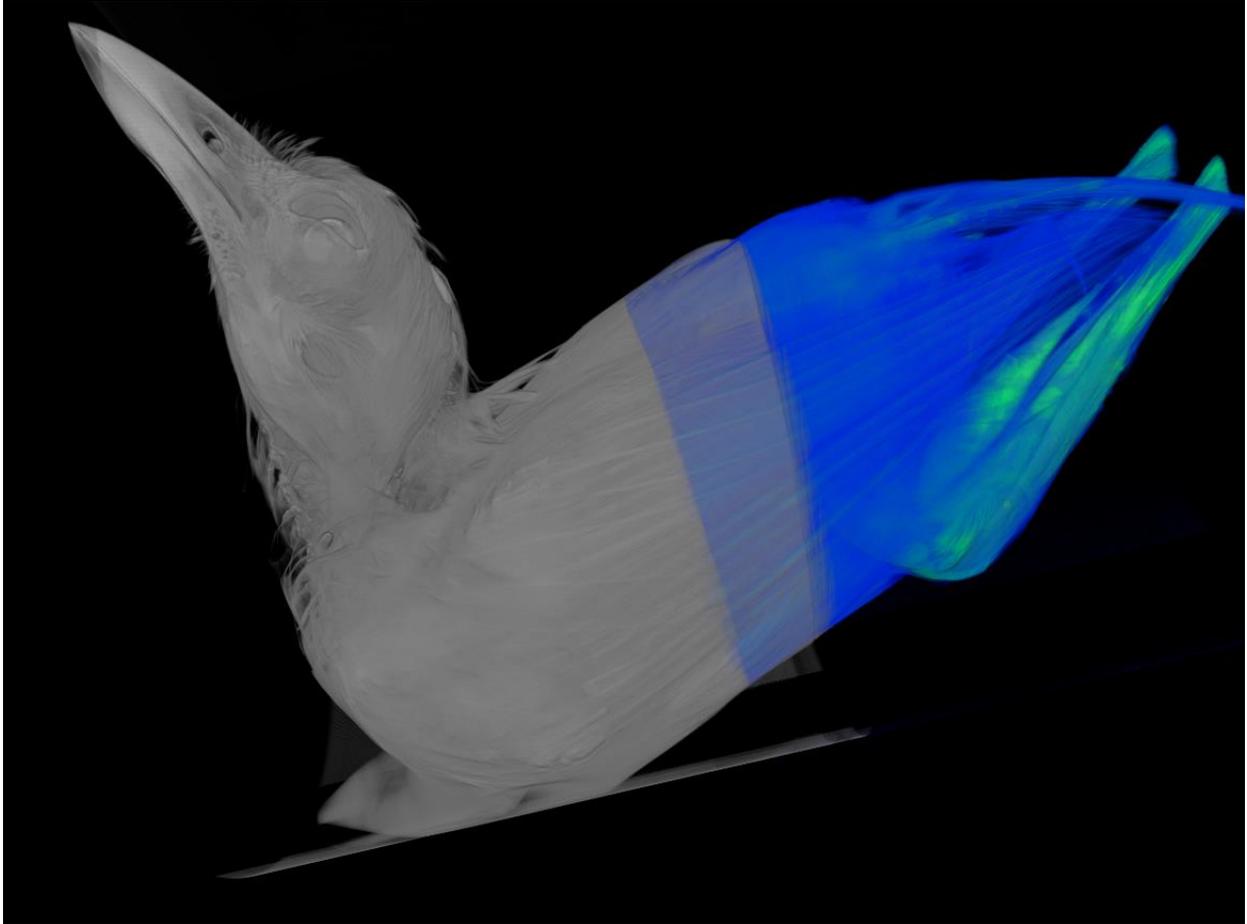
Using the cursor, drag the corners of the box until the box encloses the part of the first scan that overlaps the second. I prefer to be a little bit generous with my selection of this region; the larger the volume you select, the longer it will take the software to register the images, but there's a decreased likelihood of making a mistake that prevents the registration from being successful. Click Apply when the volume has been selected.

Next, you will do the same thing for the second scan.



Click Apply.

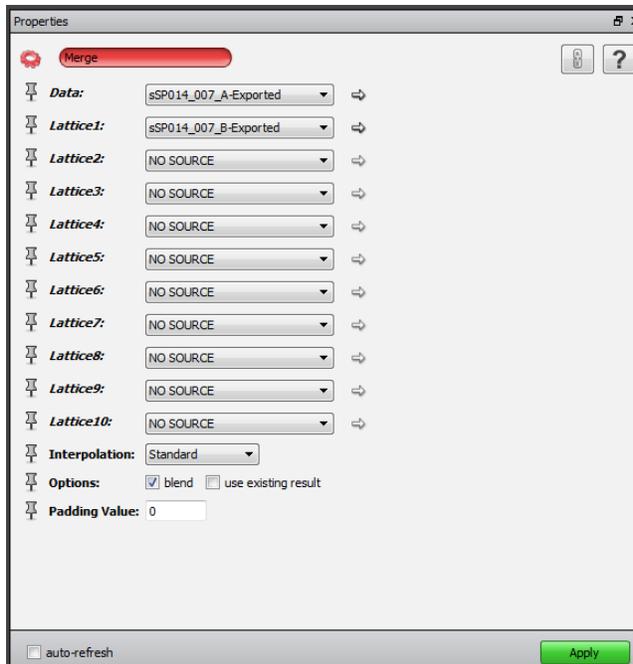
The Image Registration Wizard gives several choices in how it performs the registration. Experiment with these values if the defaults do not give you a satisfactory result. Click Apply. The registration will take a few moments.



The final computed registration—note that the overlap is somewhat greater than in my sloppily hand-aligned scans, and how the details of the feathers now register perfectly between the two scans.

### **5) Merge registered images**

Apply the Merge function to the first dataset. Select the Merge object to display its options in the Properties window.



Set the first dataset to “Data” and the second to “Lattice1” (note: these designations seem to be arbitrary; they don’t need to go in any particular direction.) The choices at the bottom allow you to favor one scan over another or to blend the two datasets. Click Apply.

To clarify, the “merge” command will combine two datasets no matter whether they are well-registered or not. If you load two datasets and skip to “merge” without aligning them, it will cheerfully average the data and give you two faded images on the same data slice.

After several minutes, a data object labeled “Merged-[filename]” will appear. Save this before doing anything to it; if Avizo crashes you will lose the merged data set if you don’t save it.

Look at the data to check for any registration errors. If you have another set of scans to add, repeat these steps with the new combined data and the third scan to be added.