



Aligning IVIS and Bruker Datasets

You should have a completed Bruker reconstruction, and a completed IVIS reconstruction to start. Here are examples:

IVIS reconstructions come as a series of single DICOMs (such as Z:\2019_July-Dec\Aaron Chiou\Siyong BonePlug Implantation Experiment\Week 2\IVIS_3D_reconstructions\M1_np_IVIS\dcm-2019070512402).

The **Bruker 3D reconstructions** come as DICOMs, tiffs or bmps. In order to do the alignment, the Bruker coreg software requires bmps (an example: Z:\2019_July-Dec\Aaron Chiou\Siyong BonePlug Implantation Experiment\Week 2\2019-_07_03_microCT\LP_bmps).

To align the datasets:

1. Go to the B46 workstation and use the *Coreg App* in the Bruker folder on the desktop.
2. Open the IVIS reconstruction by clicking on one of the images in the folder (bioluminescence data). They all will automatically load.
3. Do the same for the Bruker reconstruction (tomographic data).
4. Immediately click the scale and go to center buttons.
5. Note that you have different views and a contrast bar that you can choose.
6. Click and drag inside the boxes for translate and use the scroll bar for rotate.
7. Do not worry about the bottom of the mouse. Only fit to the top of the mouse as exactly as possible.
8. When you are done, save combined images.

Unfortunately, Bruker saves the images as 8-bit bmps in which the bioluminescence is red and everything else is grayscale. If you want your images in this format, you are ready for visualization.

If you want to visualize in any other color scheme, you need to change your data into two independent channels, rather than RGB bmps in which the R channel contains both the CT and the bioluminescence data. You can do this with the following ImageJ/FIJI macro:

```
//rw36@cornell.edu 190705
//script for changing RGB bmps from Bruker Coreg into two channel tiff stacks
//start out by loading bmp sequence
// File -> Import -> Image Sequence
```

```
rename("Work");
run("Split Channels");
outdir=getDirectory("Choose a Directory");
fname=File.nameWithoutExtension;
selectWindow("Work (blue)");
close();
imageCalculator("Subtract stack", "Work (red)", "Work (green)");
selectWindow("Work (green)");
saveAs("Tiff", outdir+"\\ "+fname+"_CT.tiff");
close();
selectWindow("Work (red)");
saveAs("Tiff", outdir+"\\ "+fname+"_BL.tiff");
close();
```

Get a scripting window via File > New > Script. Copy and paste the blue text (macro) above into the scripting window and follow the instructions in the first four lines of the script. Then run the macro. It will ask you to select a directory where you want the new data.

You are now ready for visualization.