* Download and install ITK-SNAP (freeware)
  + <http://www.itksnap.org/pmwiki/pmwiki.php?n=Downloads.SNAP3>
  + ITK-SNAP version 3.6.0 was used for these analyses
  + Tutorials for using ITK-SNAP: <http://www.itksnap.org/pmwiki/pmwiki.php?n=Main.HomePage>
* Loading the Average Template
  + Under *File*, select *Open Main Image…*
  + Click *Browse* in popup window and select the 25um Average Template file (“average\_template\_25.nrrd”) and click *Open*
  + In the “Open Image – ITK-SNAP” popup window,
    - select File Format: NRRD
    - *Next >, Finish*
  + At this point, you should see the gray-scale Average Template in sagittal, coronal and horizontal views
* Loading the Reference Atlas onto the Average Template
  + Under *Segmentation*, select *Open Segmentation…*
  + *Browse,* select and *Open* the Reference Atlas (“CCFv3\_annotation\_25.nrrd”)
    - select File Format: NRRD
    - *Next >, Finish*
  + At this point, you should see the Reference Atlas overlaid upon the Average Template
    - If you do not, adjust the *Overall label opacity:* slide bar (located in the lower left of program window)
* Loading Structure Labels
  + Under *Segmentation*, select *Import Label Descriptions…*
  + *Browse,* select and *Open* label description file (“CCFv3\_annotation\_ITKSNAP\_labels.txt”)
    - Select File Format: Text Files
    - *Next >, Finish*
  + Alternately, for a label description file with structures colored according to our public-facing ontology tools, select and *Open* (“CCFv3\_annotation\_OntologyColor\_labels.txt”).
* Loading Transgenic data (i.e. Supporting Data)
  + Under *File*, select *Add Another Image…*
  + *Browse*, select and *Open* Supporting Data files
  + Choose between opening as a separate image or a semi-transparent overlay
    - As a separate image
      * Choose *As a separate image (shown beside other images)* option
      * *Next>, Finish*
      * There will now be two small thumbnail views in each orientation window; the top will be the Reference Atlas overlaid on the Average Template, the bottom is Reference Atlas overlaid on Supporting Data
    - As an overlay
      * Choose *As a semi-transparent overlay (shown on top of other images)* option
        + Use the *Overlay color map:* dropdown menu to choose a unique map color to distinguish between multiple Supporting Data overlays and the Average Template (which is default grayscale)
        + *Next>, Finish*
  + Adjusting *Overall label opacity:* slide bar will allow you to observe Supporting Data signal
  + Repeat to load additional Supporting Data files
  + Note that authors provided transgenic data files at this link, but other 25 um .nrrd files are accessible for connectivity atlas data through the AllenSDK (<https://allensdk.readthedocs.io/en/latest/connectivity.html>).
    - Loading Connectivity data (nrrd files not provided at this link location)
      * With the AllenSDK installed do the following in a python script, console, or notebook:
      * *Import* MouseConnectivityCache from the mouse\_connectivity\_cache module
      * Initialize MouseConnectivityCache
        + Set resolution to 25.
      * Use the *“get\_experiments”* method to get a table of connectivity experiments, listing ids, injection structures, etc.
        + Set dataframe to True to get this table a Pandas DataFrame.
      * For specific experiments, pass the experiment ids to the “*get\_projection\_density*” or “*get\_injection\_density*” methods to get the respective .nrrd files.
      * Load data as described above.
* Managing Supporting Data
  + In order manage the viewing of one or multiple data sets, open the Image Layer Inspector (*Tools 🡪 Layer Inspector…)*
  + Under the *Contrast* tab, all uploaded data sets are listed on the left under the “Additional Images” panel
    - Select image and adjust image opacity by manipulating the slider next to the eye
    - Adjust image contrast by selecting the *Auto* button in the “Linear Contrast Adjustment:” section or by manual adjustment of the contrast curve
  + Under the *Color Map* tab, change the color map of each image by selecting a hue gradient from the “Select a colormap:” dropdown or the “Color Map Editor:”
* Saving and Resuming ITK Sessions
  + You may save your uploads, gradients and contrast for expedited retrieval
    - Under *Workspace*, click *Save Workspace…*
    - Name the Workspace file, ensuring the suffix remains “.itksnap”; click *OK*
  + To restore your last session, open ITK-SNAP, then click on *Open Workspace…* button at bottom of page (alternatively you can select *Open Workspace…* from the *Workspace* toolbar)
    - *Browse* for saved file and *Open*