=Getting Started=

==Setting Up AtlasFitter==

AtlasFitter was developed for MatLab 2007 and has been tested with MatLab 2008b. To run AtlasFitter first unzip all the files into a folder in your MatLab path. Second, in the file get\_atlas\_property.m on line 3, change the path string to the path containing the AtlasProperties.txt file. Third, in the AtlasProperties.txt file set the button\_path to the folder containing the AtlasFitter functions, set the raw\_atlas\_path to the folder containing the atlas panel images (see backwarping ROIs below), and set the default\_atlas\_path to the full path for the preferred default atlas file. Then run atlas\_fitter.m.

==Loading Files==

===Loading Images===

Use the Add Image button to add image files to a stack. A stack of images can be saved by clicking the Export Images button. This will save a .mat file with the paths to the image files in the stack. A stack can be loaded by clicking the Load Images button and finding the stack’s .mat file.

=====Viewing Images=====

The Slice slider (denoted by the S above it) can be used to move between images in a stack. The Image File window will show the file name of the current image being displayed. The number of the image in the stack will be shown in the window above the Slice slider. The Zoom button (indicated by a magnify glass icon) can be used to zoom in and out of the image. The Pan button (indicated by a hand icon) and the Horizontal and Vertical sliders (below and to the left of the image respectively) can be used to pan around an image.

=====Modifying Images=====

Before loading an atlas, or with the atlas disabled, one can rotate or flip an image by using the corresponding rotate (Clockwise or Counter-Clockwise arrow) or flip (horizontal or vertical) buttons. Regions within an image can be erased by clicking the Erase Area button. You will be prompted to outline the region to be erased. The color the region is set to is defined in the AtlasProperties.txt file under erased\_area\_color. Current choices are restricted to black or white. Once an image is modified it will be saved in the directory of the original image with \_mod added after the name. The current stack will also be amended to include the modified image file path.

===Loading an Atlas===

Use the Load Atlas button to select an atlas file to load. The default atlas, specified in the AtlasProperties.txt file under default\_atlas\_path, will be highlighted.

=====Viewing Atlas Panels=====

The Atlas slider (denoted by the A above it) can be used to scroll between panels in the atlas. The file name of the current atlas being displayed is shown in the Atlas File window. The panel of the atlas being displayed is shown in the window above the Atlas slider. To disable the atlas (makes it invisible and unresponsive to certain buttons) click the A button above the Atlas slider.

=Atlas / Image Registration=

==Surface Warp==

===Locating Tissue Surface===

The tissue is defined as a contiguous group of pixels with values above or below a given threshold defined in one of six color channels (red, green, blue, hue, saturation, or value). To set the threshold, move the Threshold slider to the desired value. The value is shown in the window to the left of the Threshold slider and ranges between 0 and 1. The threshold value is defined as the percent of the maximum allowed pixel value for the selected channel for that image, e.g. 0.5 in the red channel of an 8-bit image corresponds to a pixel value of 128 while 0.7 in the hue channel of any image corresponds to 0.7. Use the H, S, V, R, G, B check boxes to select the desired channel (hue, saturation, value, red, green, and blue respectively). If the tissue is darker than the background set Greater Than / Less Than button to <, while if the tissue is brighter than the background set it to >. Then simply click the Find Surface button followed by a click on the image inside the tissue section.

To help estimate the ideal threshold you can display a pseudo color intensity image of each of the color channels by either clicking the HSV button (will display the hue, saturation, and value channels in 3 separate figures) or the RGB button (will display the red, green, and blue channels in 3 separate figures), along with a color bar. The color channel images will have the lowpass filter applied as will be the case when the surface detection algorithm is applying your chosen threshold.

The surface detection algorithm pads the image with either white or black pixels based on the setting in the AtlasProperties.txt file under erased\_area\_color. If the tissue is darker than the background set this to white, and if the tissue is lighter than the background set this to black. Beyond simply setting the threshold the user is able to tune the surface detection algorithm with three parameters in the AtlasProperties.txt file. The lowpass\_filter\_size and lowpass\_standard\_deviation set the size and standard deviation of the Gaussian kernel respectively. The morphological\_filter\_size defines the diameter of the circular morphological element used to open and close the image. Set this value to the approximate size of the bumps or tears in the tissue section you want to smooth over.

The tissue outline will now be traced in red and saved for that tissue section.

===Applying the Surface Warp===

Use the Atlas slider to locate the most appropriate atlas panel. If your atlas has Left/Right symmetry you can select a single hemisphere to warp using the L and R buttons. If the traced boundary around the tissue section does not match the surface points on the atlas panel you can modify which points identify the atlas surface using the Modify Bound button. After clicking Modify Bound you will be prompted to click on points in the atlas panel identifying the new surface path. To remove points from the surface, simply slick the first and last point you want included in the surface. To add points to the surface, simply click on a surface point, then click the internal points in the order you want them included in the surface, and finally end on a surface point.

After the correct atlas panel is displayed and the surface of the tissue is outlined, click the Surface Warp button. You will then be required to identify a minimum of 3 points along the surface of the atlas and their corresponding location on the surface of the tissue (click atlas surface point, it will turn blue, then click its location on the tissue surface, a blue line will connect it to the atlas point). Once at least 3 point pairs are identified right click the mouse (right clicking after identifying an atlas surface point but before identifying its location on the tissue surface will cancel the warp).

Beyond simply warping the surface of the atlas onto the surface of the tissue section, you can choose to have the centroid of the atlas warped onto the centroid of the section by setting the surface\_warp property in the AtlasProperties.txt file to “add centroid”. Set it to none to ignore the centroid.

To ensure further applications of the interpolated warp algorithm or basic linear transformations do not move the surface, click the Lock Surface box to lock down the surface points. Immobile points are drawn in black.

==Internal Warp==

This can be applied by itself or after performing a surface warp.

===Define Mobile Points===

Click the Mobile Points button. First click an atlas point, this will highlight it in blue, then click the location you want it warped to, this will draw a blue line connecting the atlas point to that location. To remove a mobile point simply reclick that atlas point. To remove all points click the Reset Points button. To stop this function right click the mouse.

===Define Fixed Points===

Click the Fixed Points button. Clicking atlas points will highlight them in black. These points will remain immobile during the warp. To remove a point reclick it. To remove all points click the Reset Points button. To stop this function right click the mouse. If a surface warp has already been performed, the surface points can be defined as fixed points by checking the Lock Surface box.

===Apply the Internal Warp===

After all mobile and fixed points are defined, use the Warp button to apply the interpolated warp algorithm using the landmark points defined. After application of the warp, all fixed points will remain black (identifying them as fixed points) and all mobile points will now be fixed in their new location and highlighted in black. Numerous iterations of the interpolated warp algorithm can be applied.

==Manual Adjustments==

===Moving Points===

To move individual points click the Move Individual Points & Regions button, then select the point you’d like to move, and finally click on the new location you want to move the point to. You can continue to move points in this manner without clicking the button. When you are finished, click the Move Individual Points & Regions button to deactivate the function.

===Moving Regions===

To move a single or group of regions, click the Move Individual Points & Regions button, then click once within each region you’d like to move. After selecting the regions, right click the mouse to disable the selection function. The region or regions will remain active until the Move Individual Points & Regions button is clicked.

To move either the left or right hemisphere, do not use the Move Individual Points & Regions button. Simply click the L or R button (have both activated to move the entire atlas) then follow the instructions below.

The speed by which regions are moved is set by the Fast / Slow button. In slow mode regions will move by 1 pixel or 1 degree per click of the manual buttons described below. The number of pixels / degrees associated with the fast mode is set in the AtlasProperties.txt file under fast\_speed.

=====Rotating Regions=====

The Clockwise and Counter-Clockwise Arrow buttons can be used to rotate the active region(s).

=====Flipping Regions=====

The Horizontal and Vertical Flip button can be used to flip the active region(s).

=====Translating=====

The Up, Down, Left, and Right Arrow buttons can be used to move the active region(s).

=====Stretching / Shrinking=====

Use the Vertical DV / Horizontal ML button to select which axis you would like to stretch or shrink the atlas in. The Expanding Arrow buttons can then be used to stretch the atlas while the Compressing Arrow buttons can be used to shrink the atlas. The Absolute buttons will simply stretch or shrink the atlas in the horizontal or vertical direction relative to how they appear on the screen. Meaning if you have previously rotated the atlas 90 degrees, then stretching it horizontally relative to your computer screen will actually stretch the atlas vertically compared to how it was originally drawn. The Body buttons will stretch or shrink the atlas relative to their original axis. Here the program will take into account any rotation you may have already applied to the atlas. Note, if you apply a rotation by any means other than the Clockwise and Counter-Clockwise Arrow buttons, such as by moving individual points or via a warp, the Body buttons will not be able to take it into account.

=====Scaling=====

The atlas can be globally scaled by entering the scale factor in the window next to the Multiply button then clicking the button. You will then have the option to apply the scaling to just the current atlas or to all atlas panels.

=====Docked / Detached Regions=====

Use the Dock button found in the center of the Up, Down, Left, and Right arrows to either select docked or detached mode. In docked mode the active regions will always remain attached to the rest of the atlas. In detached mode any manual adjustment made to the active region(s) will have no affect on the remainder of the atlas.

=Fixing Mistakes=

===Resetting the Atlas===

There is no undo button to reverse changes made to the atlas (though all of the manual buttons come in pairs that are able to reverse each other’s actions). One’s only recourse is to reset the atlas to its original state by clicking the Reset Atlas button and begin again.

===Clearing Drawn Objects===

It is occasionally possible to click buttons fast enough that objects will be drawn but their previous position will not be deleted. This can include multiple atlases being drawn on the screen at once, or a tissue surface boundary being carried over to the next slice. If this ever happens simply click the Clear Objects button. To redraw objects tied to the current slice, simply click the Slice slider bar. To redraw the current atlas, click the Atlas slider bar.

=Saving Atlas Alignment=

===Locking an Atlas Panel===

Once you have an atlas panel in the position you like relative to the tissue section you can save that atlas for that section by clicking the Lock Atlas button. The A button will turn red indicating the current atlas panel is locked to the current tissue section. In this way you can work on aligning atlas panels to multiple images in a stack before performing any analysis. When you use the Slice slider to view an image with a locked atlas, that atlas will be displayed. To unlock an atlas panel from a tissue section simply click the Unlock Atlas button.

Note, the atlas is locked in its current state when the Lock Atlas button is clicked. While it is possible to make changes to the atlas after locking it, none of these changes will be saved unless you click Unlock Atlas and then Lock Atlas again. In this way you can use the lock feature as a temporary save of an atlas’ current state such as before applying the warp algorithm. To revert back to the locked atlas simply click the Slice slider bar.

===Saving / Loading User Data===

Use the Save User Data button to save all traced tissue boundaries, locked atlases, and ROIs. The saved file will also contain the path to the current atlas being used as well as the paths to the images in the current stack.

To load a data file click the Load User Data button. This will load all of the saved information described above. You can then choose to load the image files saved with this data file. After loading, the displayed tissue section and atlas section may not match. Simply click the Slice slider bar to display the atlas panel and tissue boundary associated with that tissue section.

=Using the Atlas Predictor=

The atlas predictor function can be used to help estimate which atlas panel best fits the current tissue section. This feature is only applicable when the tissue sections all originate from a single block sampled at uniform spacing. The atlas predictor does not actually look at the tissue section but rather uses linear interpolation to estimate which atlas panel should be used based on which atlas panels have been locked to other images in the stack. The linear interpolation uses the known spacing between the atlas panels in combination with which atlas panels have been locked to which tissue sections to generate the estimate. Therefore, the feature will not work until at least 2 atlas panels have been locked to tissue sections. As more atlas panels are locked the accuracy of the predictor will increase. When the atlas predictor is active and at least 2 atlas panels have been locked, as you scroll through the tissue sections using the Slice slider, the current atlas will automatically update to the best prediction (unless a tissue section already has a locked atlas, then the locked atlas will be displayed).

=Regions of Interest (ROI)=

===Drawing ROIs===

To draw a region of interest, click the Outline Region button. This will open an image of the current tissue section in a separate window (if a boundary has been traced around the tissue section, the image will be cropped to only display what is inside that boundary). Using a separate window is intentional as it prevents one from defining the region based on atlas boundaries and potentially biasing one’s data. Instead, ROIs are meant to be drawn based on features in the tissue section alone such as a lesion, cannula track, or the tip of a recording electrode. Use the left mouse button to trace the region, and when finished use the right mouse button to close the polygon. Then double click to return to AtlasFitter. The ROI will now be displayed in green on the tissue section.

Multiple ROIs can be drawn by simply clicking the Outline Region button again. You can choose to replace the current ROI or add an additional ROI. To delete all ROIs from the image click the Delete Region button.

===Back Warping ROIs===

If a ROI has been drawn over a tissue section on which an atlas panel has been aligned, the ROI can be back warped onto the original panel from the atlas. The path of the original atlas panels must be specified in the AtlasProperties.txt file under raw\_atlas\_path. Atlas panels must be saved with a file naming scheme that ends in the atlas panel number, e.g. fullpathname\atlaspanel\_001.tif, fullpathname\atlaspanel\_002.tif… Then simply click the Back Warp button. The ROI will now be displayed over the original atlas panel in a separate figure. At this point you are able to save the coordinates of the back warped ROI. These can be used to draw multiple ROIs over a single atlas panel.

=Flat Fielding Images=

Use the Flat Correct button to apply a flat field image correction to the current tissue section image (this correction requires that you have a flat field image saved on your computer). The correction divides pixel-wise the tissue image by the flat field image then rescales the pixel values in the tissue image.

=Analyzing Data=

Use the Analyze Regions button to execute a series of analysis functions specified in the AtlasPorperties.txt file. If the file you specify to save your data to already exists, that file will be loaded and any images that have already been analyzed for that file will be skipped.

The code as currently written passes through the following analysis options in the following order. Each function must be designed to receive a specified input and generate a specified output. The names of the functions called are specified in the AtlasProperties.txt file.

handles – the GUI data structure

output – user specified data structure, initialized as an empty matrix

Since handles and output serve as both input and output variables to all the functions described below, they can be used to pass data between them.

==Basic Analysis Steps==

===Whole Image===

This function is run on the entire image with format:

[handles, output] = whole\_image\_function(handles, Image, output);

Specify none in the AtlasProperties.txt file under analyze\_image to skip this step. If no atlas is locked to the current tissue section the analysis stops here and moves to the next tissue section.

===Whole Tissue Section===

This function runs on the image after it has been cropped using the Whole Section region in the atlas locked to that section as a mask. It uses the following format:

[handles, output] = whole\_section\_function(handles, Image, output);

Specify none in the AtlasProperties.txt file under analyze\_whole\_section to skip this step.

===Whole Left and Right Hemispheres===

This function runs on the image after it has been cropped using the Whole Left and Whole Right regions in the atlas locked to that section as a mask. It uses the following format:

[handles, output] = whole\_leftright\_function(handles, Image, output);

Specify none in the AtlasProperties.txt file under analyze\_whole\_leftright to skip this step.

===Individual Regions===

This function runs on the image after it has been cropped using a single region from the atlas locked to that section as a mask. The main code loops through each region calling the following function:

[handles, output] = individual\_region\_function(handles, Image, output);

Specify none in the AtlasProperties.txt file under analyze\_individual\_regions to skip this step.

At this point the output data structure is saved and the function moves to the next tissue section image to analyze.

==Specific Type of Analysis==

===ROI Atlas Overlap===

To quantify the overlap between user defined ROIs and the atlas regions specify overlap in the AtlasPropertiex.txt file under analyze\_user\_regions. This will run a predefined function before any of the other analysis and the data output will be saved to a separate file. It will loop through all tissue section images skipping only those that do not have an atlas panel locked to them.

===Automated Cell Identification===

The following functions were developed to automatically identify, analyze, and count cells in the tissue sections.

wholeimage\_findedge – operates on the whole image and detects sharp edges. The pixels near these edges are excluded from further analysis. Enter it under analyze\_whole\_section in AtlasProperties.txt.

wholeleftright\_findcells – operates on the region of the image outlined by the left and right hemispheres and identifies cells using the CellCounter algorithm. Enter it under analyze\_whole\_leftright in AtlasProperties.txt. Specifying ignore in the AtlasProperties.txt file under analyze\_user\_regions causes pixels within ROIs to be ignored.

individualregions\_countcells – operates on the region of the image outlined by individual atlas regions and outputs cell count, cell density (normalized count), area, mean cell intensity, and total cell signal for each atlas region. Enter it under analyze\_individual\_regions in AtlasProperties.txt. Specifying ignore in the AtlasProperties.txt file under analyze\_user\_regions causes pixels within ROIs to be ignored.

=Downloads=

AtlasFitter – Download

Atlas Panel Images – Download

Coronal Rat Brain Atlas Wireframe – Download

==Brain Region Abbreviation Index==

AA Anterior Amygdaloid Area

ACx1 Primary Auditory Cortex

ACx2 Secondary Auditory Cortex

AN Anterior Amygdaloid Nucleus

ANC Central Amygdaloid Nucleus

ANL Lateral Amygdaloid Nucleus

ANM Medial Amygdaloid Nucleus

B Basal Nucleus

BLA Basolateral Amygdala

BMA Basomedial Amygdala

cc corpus callosum

CPu Caudate Putamen

CgCx1 Primary Cingulate Cortex

CgCx2 Secondary Cingulate Cortex

Cl Claustrum

CxA1 Cortex Amygdala Transition

DPCx Dorsal Peduncular Cortex

DTT Dorsal Tenia Tecta

EA Sublenticular Extended Anygdala

EGP External Globus Pallidus

EctCx Ectorhinal Cortex

EnPirN Endopiriform Nucleus

FrACx Frontal Associational Cortex

FrCx Frontal Cortex

Hip CA1 CA1 Region of Hippocampus

Hip CA3 CA3 Region of Hippocampus

Hip DG Dentate Gyrus

IG Indusium Griseum

ILCx Infralimbic Cortex

IPAC Interstitial Nucleus Posterior Limb Anterior Commissure

InCx Insular Cortex

LEntCx Lateral Entorhinal Cortex

LOT Lateral Olfactory Tract Nucleus

LSN Lateral Septal Nucleus

MCx1 Primary Motor Cortex

MCx2 Secondary Motor Cortex

MEntCx Medial Entorhinal Cortex

MS Medial Septal Nucleus

Misc1 Miscellaneous

NAcbC Nucleus Accumbens Core

NAcbSh Nucleus Accumbens Shell

OlfTub Olfactory Tubercle

OrbCxDL Dorsal Lateral Orbital Frontal Cortex

OrbCxL Lateral Orbital Frontal Cortex

OrbCxM Medial Orbital Frontal Cortex

OrbCxV Ventral Orbital Frontal Cortex

PRhCx Perirhinal Cortex

PirCx Piriform Cortex

PrLCx Prelimbic Cortex

PtACx Parietal Association Cortex

RSCx Retrosplenial Cortex

SCx1 Primary Somatosensory Cortex

SCx2 Secondary Somatosensory Cortex

SHi Septohippocampal Nucleus

ST Stria Terminalis Bed Nucleus

SubD Dorsal Subiculum

SubV Ventral Subiculum

TACx Temporal Association Cortex

VCx1 Primary Visual Cortex

VCx2 Secondary Visual Cortex

VDB Vertical Limb Diagonal Band Nucleus

VP Ventral Pallidum

Whole Left Left Hemisphere

Whole Right Right Hemisphere

Whole Section Entire Atlas