ALLEN INSTITUTE *for* BRAIN SCIENCE
Fueling Discovery

ALLEN **Mouse Connectivity Atlas**

ACCESSING ANNOTATION AND GRIDDED PROJECTION DATA MAPPED TO CCF V2 (OCTOBER 2014)

OVERVIEW

In the May 2015 data release, we introduced a next generation common coordinate framework (CCF v3) based on a population average to support the integration of new mouse brain datasets in the Allen Brain Atlas Data Portal. See the Allen Mouse Common Coordinate Framework whitepaper for detailed construction information.

All data from the Allen Mouse Brain Atlas were remapped to the new CCF v3. This document details how to access archival annotation, structure-level projection summary and gridded data from the October 2014 release as mapped to CCF v2.

3-D REFERENCE MODEL

Five volumetric data files are available for download:

File	Description
atlasVolume	UCHAR (8bit) grayscale Nissl volume of the reconstructed brain at 25 µm resolution http://download.alleninstitute.org/informatics-archive/october-2014/annotation/atlasVolume.zip
P56_Mouse_annotation	UINT (32bit) gray matter structural annotation volume at 25 µm resolution based on the **Allen Reference Atlas**. The value represents the ID of the finest level structure annotated for the voxel. http://download.alleninstitute.org/informatics-archive/october-2014/annotation/P56_Mouse_annotation.zip

P56_Mouse_annotationFiber	UINT (32bit) fiber tract structural annotation volume at 25 µm resolution based on the **Allen Reference Atlas**. The value represents the ID of the finest level structure annotated for the voxel. http://download.alleninstitute.org/informatics-archive/october-2014/annotation/P56_Mouse_annotationFiber.zip
averageTemplate	USHORT (16bit) average brain template used as registration target at 25 µm resolution http://download.alleninstitute.org/informatics-archive/october-2014/annotation/averageTemplate.zip
P56_Mouse_gridAnnotation_100micron	UINT (32bit) combined structural and fiber tract annotation volume at grid (100 µm) resolution for projection analysis. http://download.alleninstitute.org/informatics-archive/october-2014/annotation/P56_Mouse_gridAnnotation_100micron.zip

All volumetric data is stored in an uncompressed format with a simple text header file in [MetaImage]() format. The raw numerical data is stored as a 1-D array as shown in the figure below.

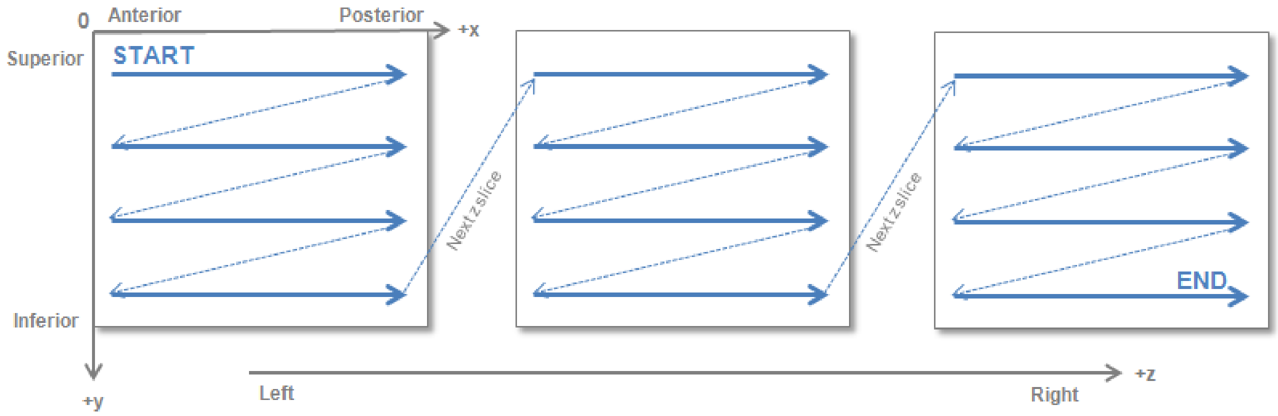

Figure: Packing of 3-D volumetric data into a 1-D numerical array.

Example Matlab code snippet to read in the 25μm atlas and annotation volumes:

```
% ------------
% Download and unzip the atlasVolume, annotation, annotationFiber and averageTemplate zip files
% ------------

% 25 micron volume size
size = [528 320 456];

% VOL = 3-D matrix of atlas Nissl volume
fid = fopen('atlasVolume/atlasVolume.raw', 'r', 'l' );
VOL = fread( fid, prod(size), 'uint8' );
fclose( fid );
VOL = reshape(VOL,size);

% ANO = 3-D matrix of structural annotation labels
fid = fopen('P56_Mouse_annotation/annotation.raw', 'r', 'l' );
ANO = fread( fid, prod(size), 'uint32' );
fclose( fid );
ANO = reshape(ANO,size);

% FIBT = 3-D matrix of fiber tract annotation labels
fid = fopen('P56_Mouse_annotationFiber/annotationFiber.raw', 'r', 'l' );
FIBT = fread( fid, prod(size), 'uint32' );
fclose( fid );
FIBT = reshape(FIBT,size);

% AVGT = 3-D matrix of average template volume
fid = fopen('averageTemplate/atlasVolume.raw', 'r', 'l' );
AVGT = fread( fid, prod(size), 'uint16' );
fclose( fid );
AVGT = reshape(AVGT,size);

% Display one coronal section
figure;imagesc(squeeze(VOL(264,:,:)));colormap(gray);
figure;imagesc(squeeze(ANO(264,:,:)));colormap(lines);
figure;imagesc(squeeze(FIBT(264,:,:)));colormap(lines);
figure;imagesc(squeeze(AVGT(264,:,:)));colormap(gray);

% Display one sagittal section
figure;imagesc(squeeze(VOL(:,:,220)));colormap(gray);
figure;imagesc(squeeze(ANO(:,:,220)));colormap(lines);
figure;imagesc(squeeze(FIBT(:,:,220)));colormap(lines);
figure;imagesc(squeeze(AVGT(:,:,220)));colormap(gray);
```

Example Matlab code snippet to read in the 100µm grid annotation volume:

```
% -----------
% Download and unzip the 100 micron gridAnnotation zip files
% -----------

%  grid volume size
sizeGrid = [133, 81, 115];

% ANOGD = 3-D matrix of grid-level annotation labels
fid = fopen( 'P56_Mouse_gridAnnotation_100micron/gridAnnotation.raw', 'r', 'l' );
ANOGD = fread( fid, prod(sizeGrid), 'uint32' );
fclose( fid );
ANOGD = reshape(ANOGD,sizeGrid);

% Display one coronal and one sagittal section
figure;imagesc(squeeze(ANOGD(73,:,:)));colormap(lines);caxis([0 3000]);
figure;imagesc(squeeze(ANOGD(:,:,78)));colormap(lines);caxis([0 3000]);
```

DOWNLOADING PROJECTION GRIDS

Expression summary for each SectionDataSet from October 2014 release (mapped to CCF v2) have been archived on our download server:

http://download.alleninstitute.org/informatics-archive/october-2014/mouse_projection/

The index file mouse_projection_data_sets.csv list all SectionDataSet from the Allen Mouse Connectivity Atlas and includes links to download grid data as a zip file and ProjectionStructureUnionize results as a csv file.

For each dataset, the gridding module creates a low resolution 3-D summary of the labeled axonal trajectories and resamples the data to the common coordinate space of the 3-D reference model. Casting all data into a canonical space allows for easy cross-comparison between datasets. The projection data grids can also be viewed directly as 3-D volumes or used for analysis (i.e. target, spatial and correlative searches).

Each image in a dataset is divided into a 100 x 100 µm grid. Pixel-based statistics are computed using information from the primary image and the segmentation mask:

- **projection density** = sum of detected pixels / sum of all pixels in division
- **projection intensity** = sum of detected pixel intensity / sum of detected pixels
- **projection energy** = projection intensity * projection density

The resulting 3-D grid is then transformed into the standard reference space.

Example Matlab code snippet to read in the 100 µm density grid volume:

```
%------------
% Download and unzip the density grid file for VISp SectionDataSet
% -----------

%  grid volume size
sizeGrid = [133, 81, 115];

% DENSITY = 3-D matrix of projection density grid volume
fid = fopen('11_wks_coronal_126862385/density.raw', 'r', 'l' );
DENSITY = fread( fid, prod(sizeGrid), 'float' );
fclose( fid );
DENSITY = reshape(DENSITY,sizeGrid);

% Display one coronal and one sagittal section
figure;imagesc(squeeze(DENSITY(73,:,:)));colormap(hot);caxis([0 1]);
figure;imagesc(squeeze(DENSITY(:,:,78)));colormap(hot);caxis([0 1]);
```

Comparing Projection Data Grids and Gene Expression Grids:

Due to section sampling density, projection data grids are at 100µm resolution while gene expression grids are at 200µm resolution. Upsampling with appropriate interpolation of the gene expression data is necessary in order to numerically compare between the two different types of data. When interpolating the data, "no data" (-1) voxels need to be handled specifically.

Example Matlab code snippet to upsample gene expression grid with "no data" handling:

```
% Download and unzip energy volume file for gene Rasd2 coronal SectionDataSet 73636089
mkdir('Rasd2_73636089');
urlwrite('http://api.brain-map.org/grid_data/download/74819249?include=density', 'temp.zip');
unzip('temp.zip','Rasd2_73636089');

% Download and unzip density volume file for BLAa injection SectionDataSet 113144533
mkdir('BLAa_113144533');
urlwrite('http://api.brain-map.org/grid_data/download/113144533?include=density', 'temp.zip');
unzip('temp.zip','BLAa_113144533');

% Gene expression grids are at 200 micron resolution.
geneGridSize = [67 41 58];
fid = fopen('Rasd2_73636089/density.raw', 'r', 'l'  );
Rasd2 = fread( fid, prod(geneGridSize), 'float' );
fclose(fid);
Rasd2 = reshape( Rasd2, geneGridSize );

% Projection grids are at 100 micron resolution
projectionGridSize = [133 81 115];
fid = fopen('BLAa_113144533/density.raw', 'r', 'l'  );
BLAa = fread( fid, prod(projectionGridSize), 'float' );
```

```
fclose(fid);
BLAa = reshape( BLAa, projectionGridSize );

% Upsample gene expression grid to same dimension as projection grid using linear interpolation
[xi,yi,zi] = meshgrid(1:0.5:41,1:0.5:67,1:0.5:58); %note: matlab transposes x-y
d = Rasd2; d(d<0) = 0; % fill in missing data as zeroes
Rasd2_100 = interp3(d ,xi,yi,zi,'linear');

% Handle "no data" (-1) voxels.
% Create a mask of "data" vs "no data" voxels and apply linear interpolation
m = zeros(size(Rasd2));
m(Rasd2  >= 0) = 1; mi = interp3(m,xi,yi,zi,'linear');

% Normalize data by dividing by interpolated mask. Assign value of "-1" to "no data" voxels.
Rasd2_100 = Rasd2_100 ./ mi;
Rasd2_100( mi <= 0 ) = -1;

% Create a merged image of one coronal plane;
gimg = squeeze(Rasd2_100(52,:,:)); gimg = max(0,gimg); gimg = gimg / 0.025; gimg = min(1,gimg);
pimg = squeeze(BLAa(52,:,:)); pimg = max(0,pimg); pimg = pimg / 0.8; pimg = min(1,pimg);
rgb = zeros([size(gimg),3]); rgb(:,:,1) = gimg; rgb(:,:,2) = pimg;
```

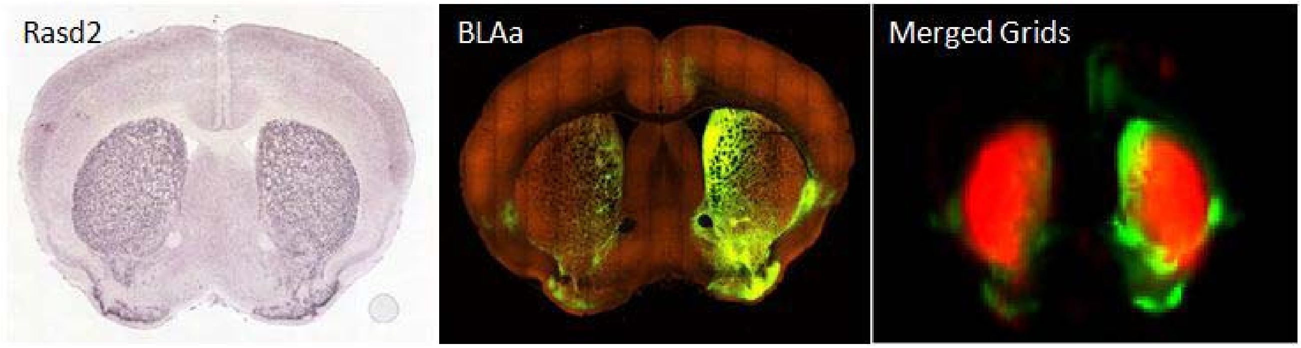

Figure: ISH SectionDataSet (id=73636089) for gene Rasd2 showing enriched expression in the striatum (left). Projection SectionDataSet (id=73636089) with injection in the anterior part of the basolateral amygdalar nucleus (BLAa) showing projection to the striatum and other brain areas (center). One coronal slice of the BLAa projection density grid (green) merged with an upsampled and interpolated Rasd2 expression density grid (red)

Projection Structure Unionization

Projection signal statistics can be computed for each structure delineated in the reference atlas by combining or unionizing grid voxels with the same 3-D structural label. While the reference atlas is typically annotated at the lowest level of the ontology tree, statistics at upper level structures can be obtained by combining measurements of the hierarchical children to obtain statistics for the parent structure. The unionization process also separates out the left versus right hemisphere contributions as well as the injection versus non-injection components.

Projection statistics are encapsulated as a ProjectionStructureUnionize object associated with one Structure, either left, right or both Hemispheres and one SectionDataSet. ProjectionStructureUnionize results from the October 2014 release can be downloaded through the link in the index file.