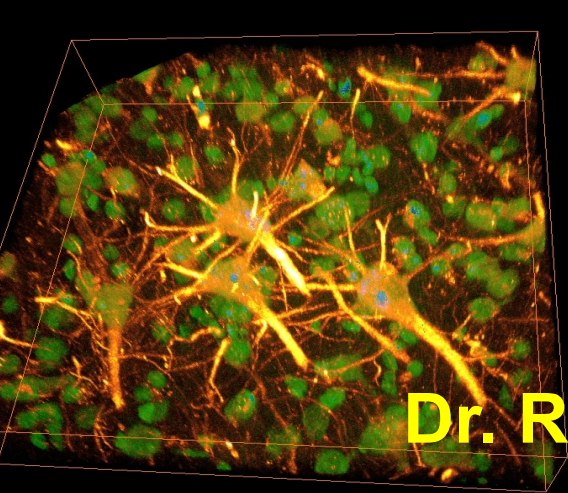
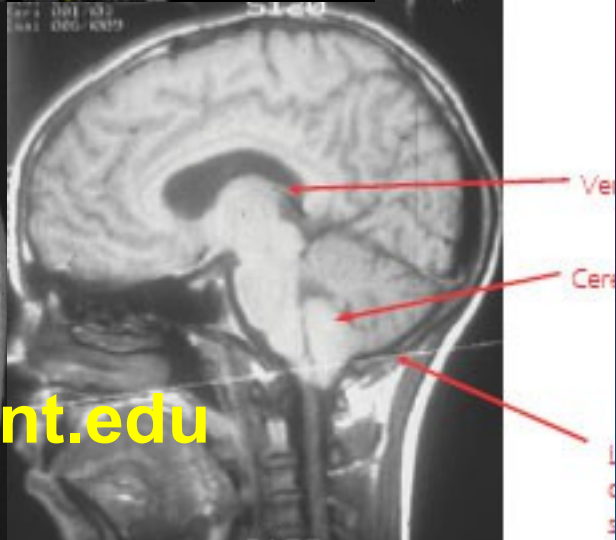
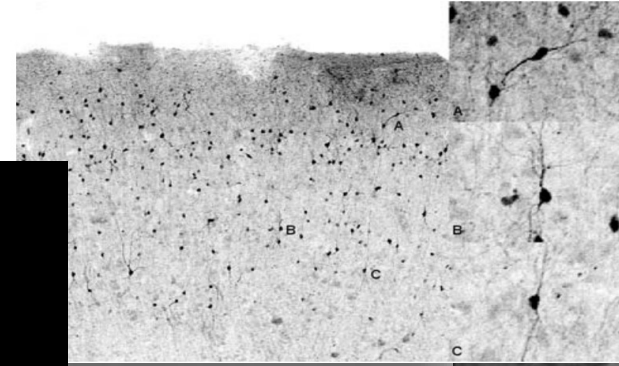
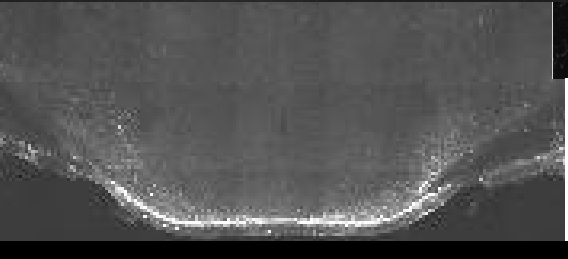
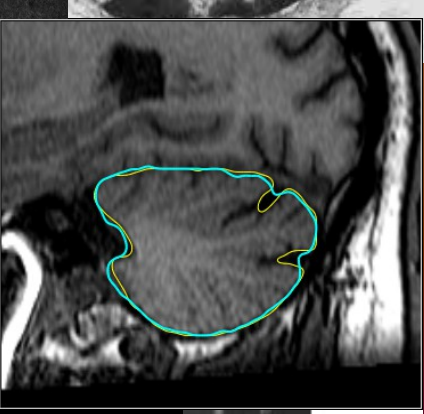
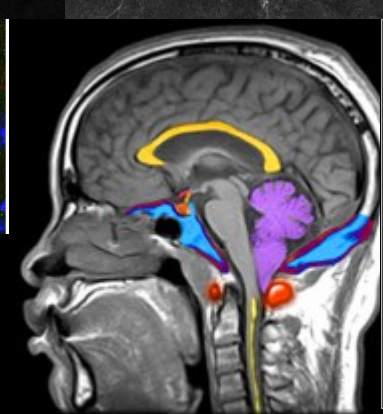
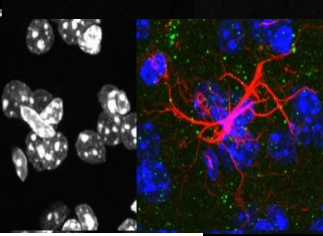
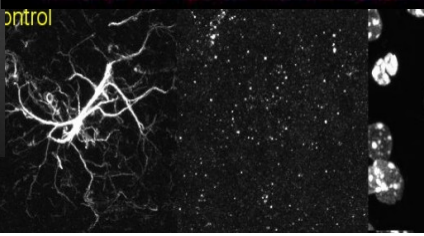
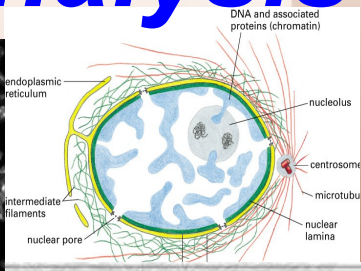
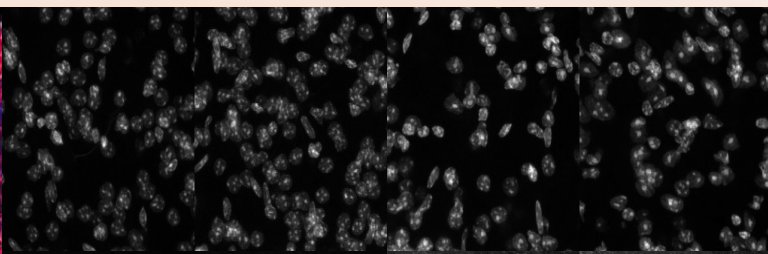
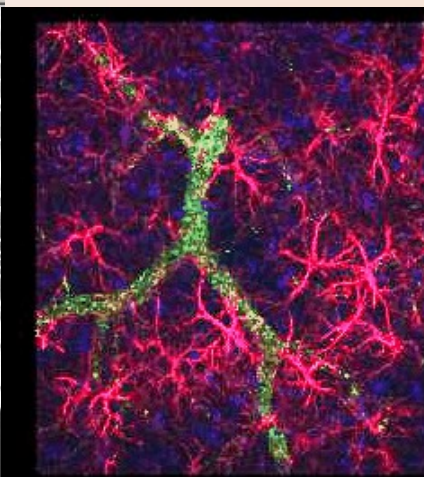
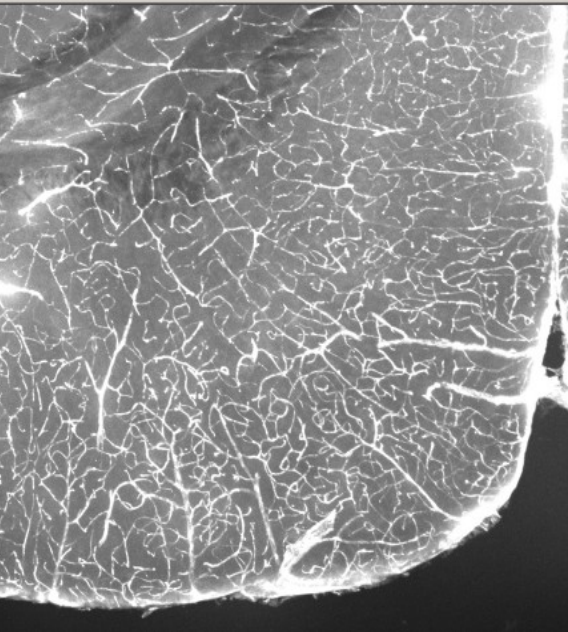


# Automated Image Processing & Analysis



Dr. Rob Clements - [rclement@kent.edu](mailto:rclement@kent.edu)

# Imaging Projects – *Plugins Creation*

## MICROSCOPY

1) Background estimation

2) Tiled data reconstruction and correction

3) Astrocyte classification

4) Microvessel analysis

CONFOCAL MICROSCOPY

5) Neuron classification

6) Nucleus classification

3D ELECTRON MICROSCOPY

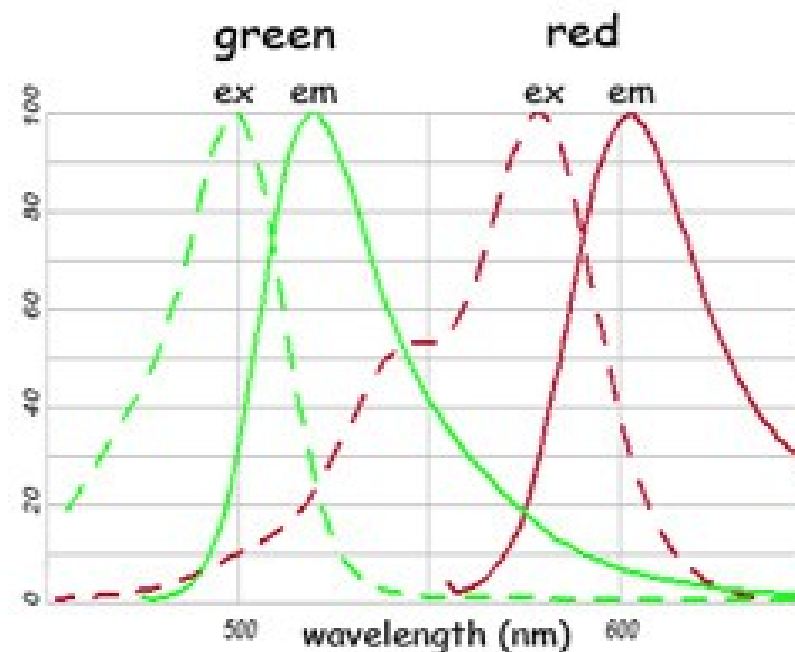
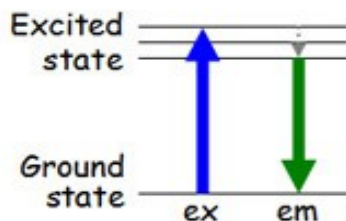
7) Cerebellum classification

MRI



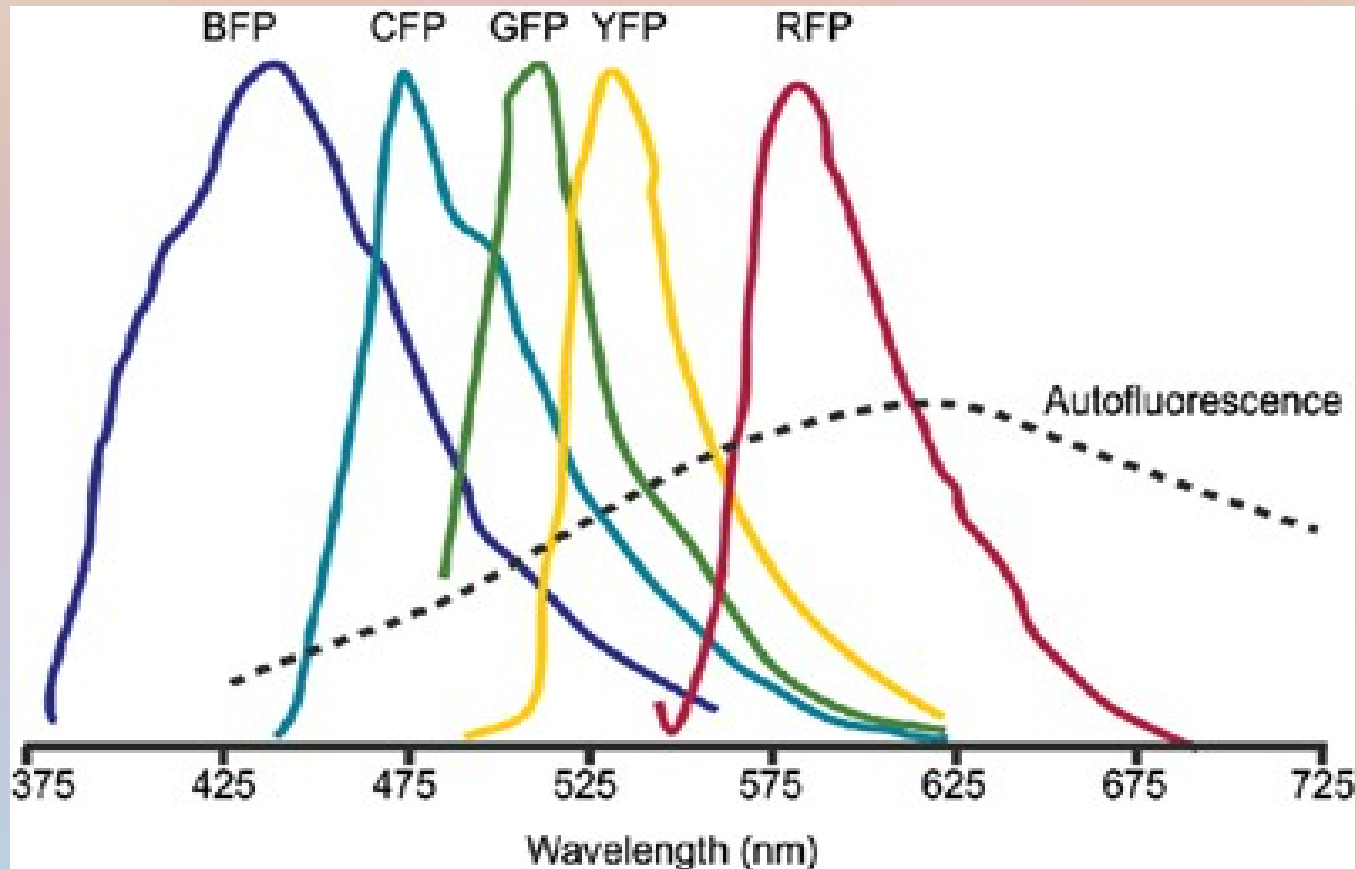
# Fluorescence Microscopy

- specimen can be labeled with a fluorophore (green fluorescent protein-GFP) directed at an object of interest
- specimen is illuminated with light of a specific wavelength which is absorbed by the fluorophores
- fluorophore emits longer wavelength (different color)
- green fluorophore absorbs a blue excitation photon and an electron changes from the ground state to an excited state.
- electron then decays back from the excited state to the ground state and releases a green photon



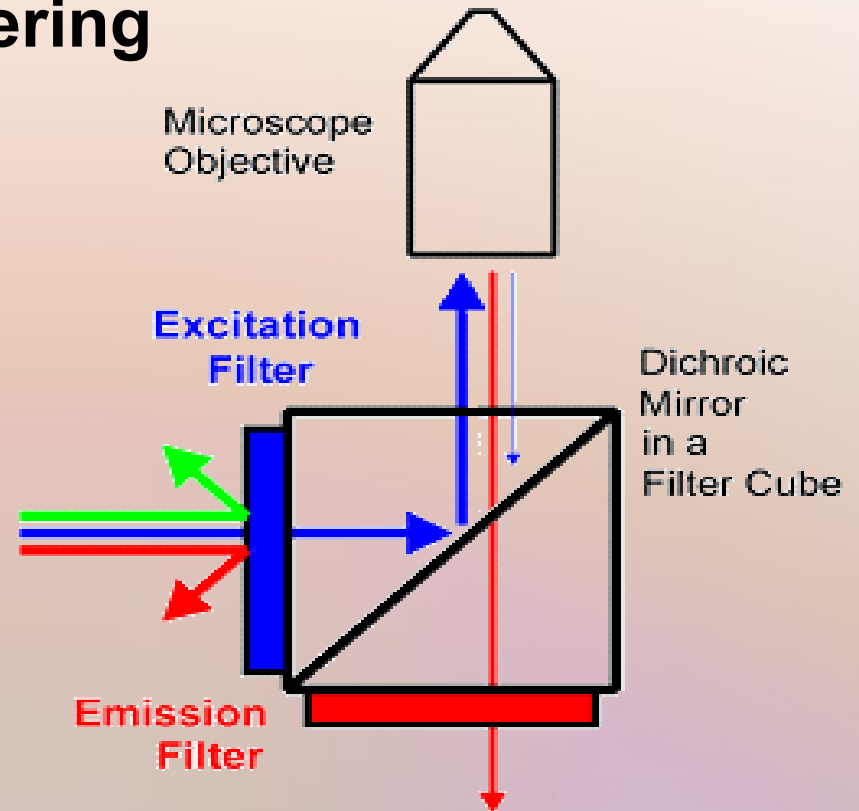
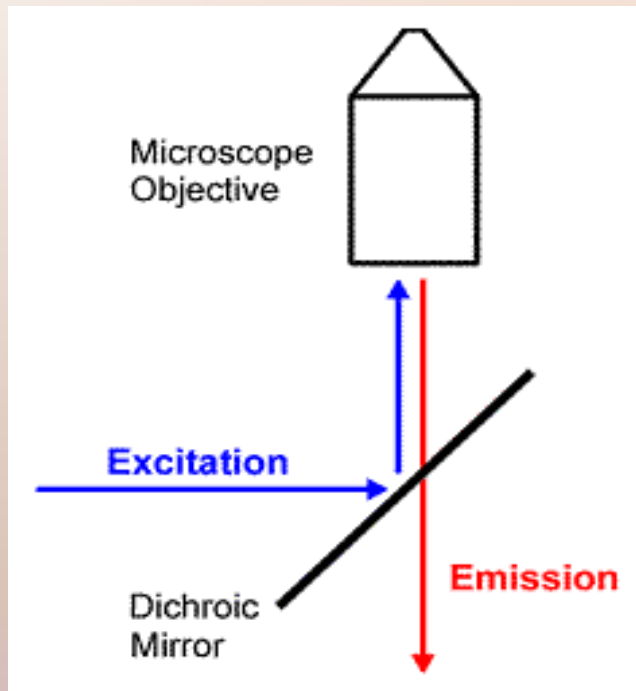
# Fluorescence Microscopy

- Possible to use concurrent fluorophores as long as excitation/emission wavelength different
- commercially available bound to organelle probes and antibodies
- permits probing multiple structures concurrently





# Fluorescence Microscopy Wavelength Filtering



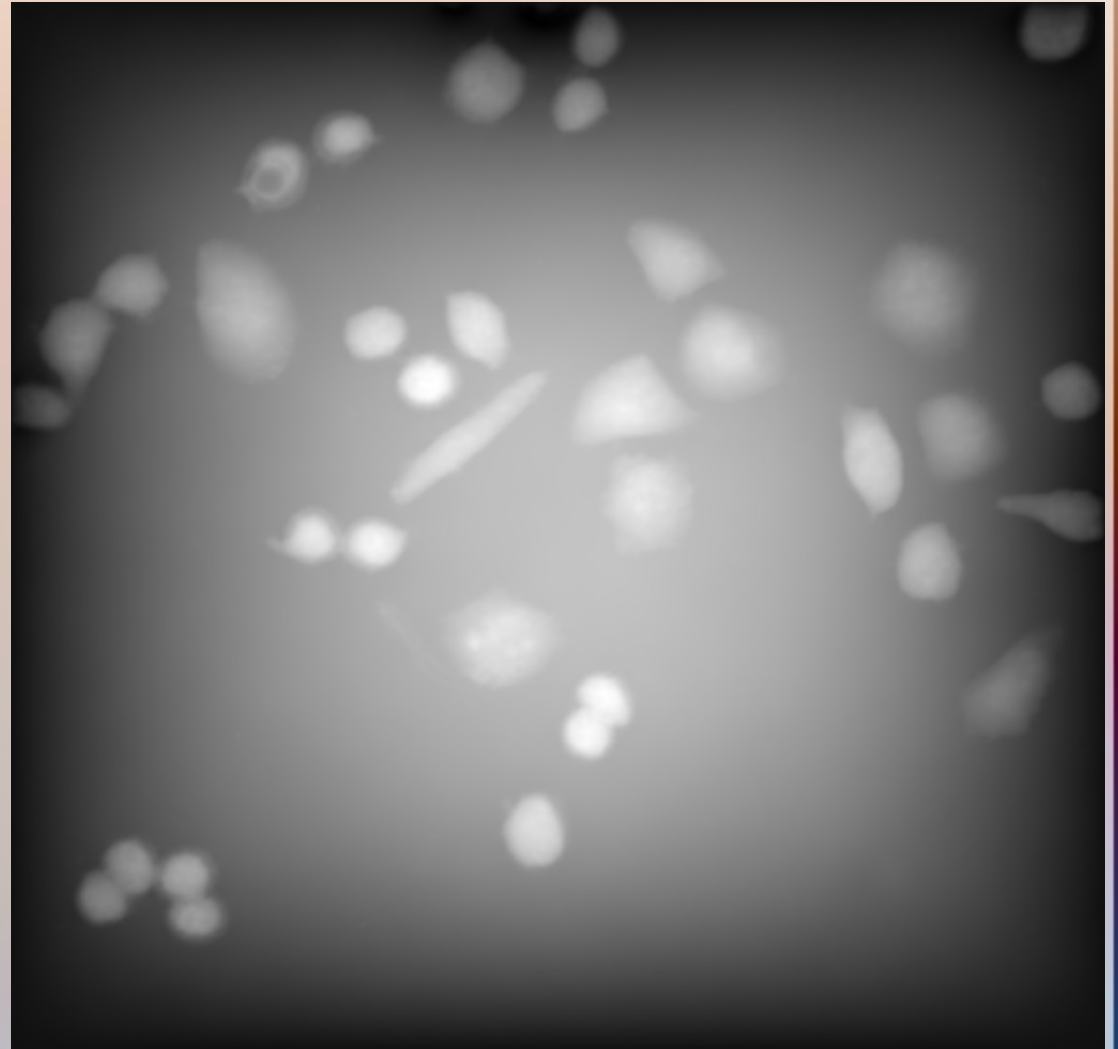
\* **Excitation filter** - the excitation path just prior to the dichroic mirror to select the excitation wavelength

\* **Emission filter** - to select the emission wavelength from the sample and remove excitation light, it is placed beneath the dichroic mirror.

# Imaging Projects

## 1) Background estimation

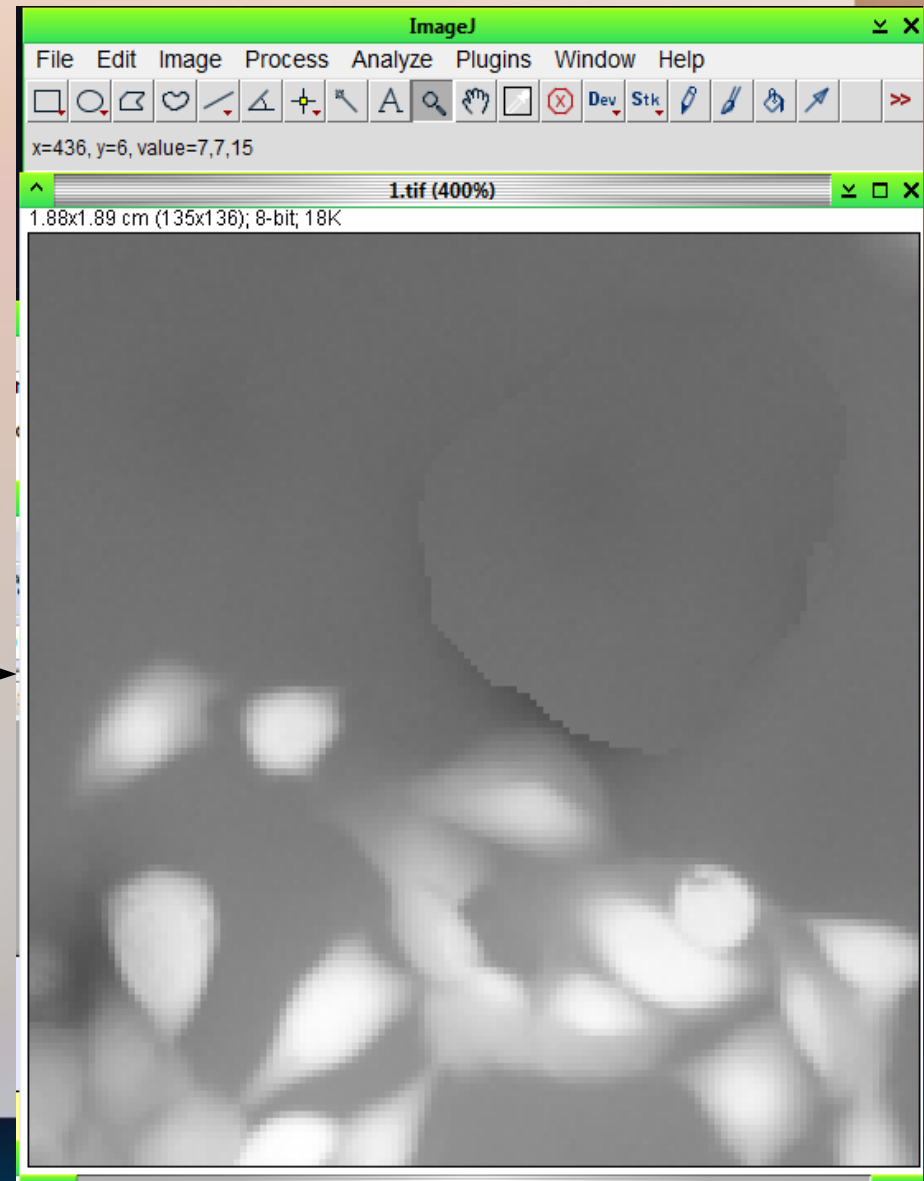
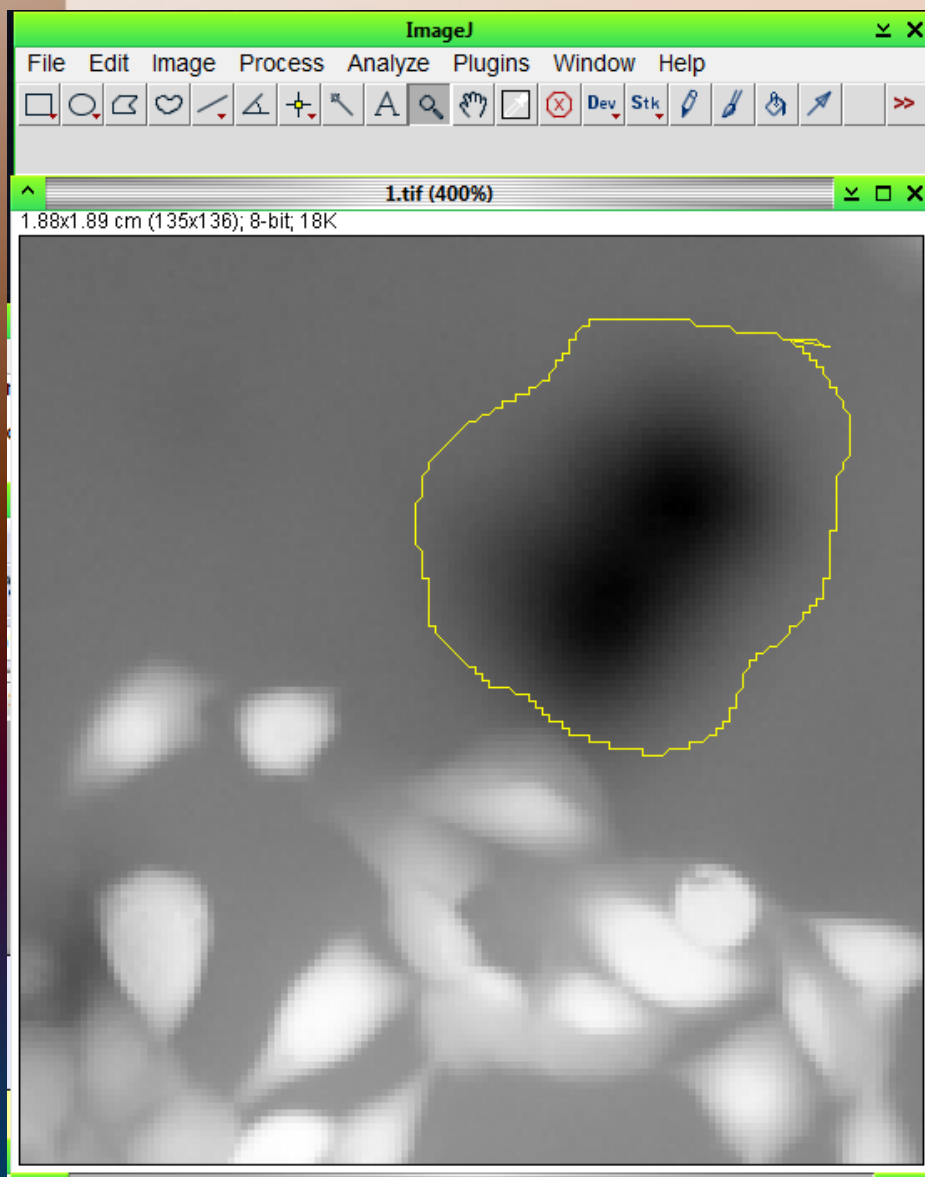
- fill in area of an image based on existing background
- user selects an area which is then filled based on surrounding pixels



# Imaging Projects

## 1) Background estimation

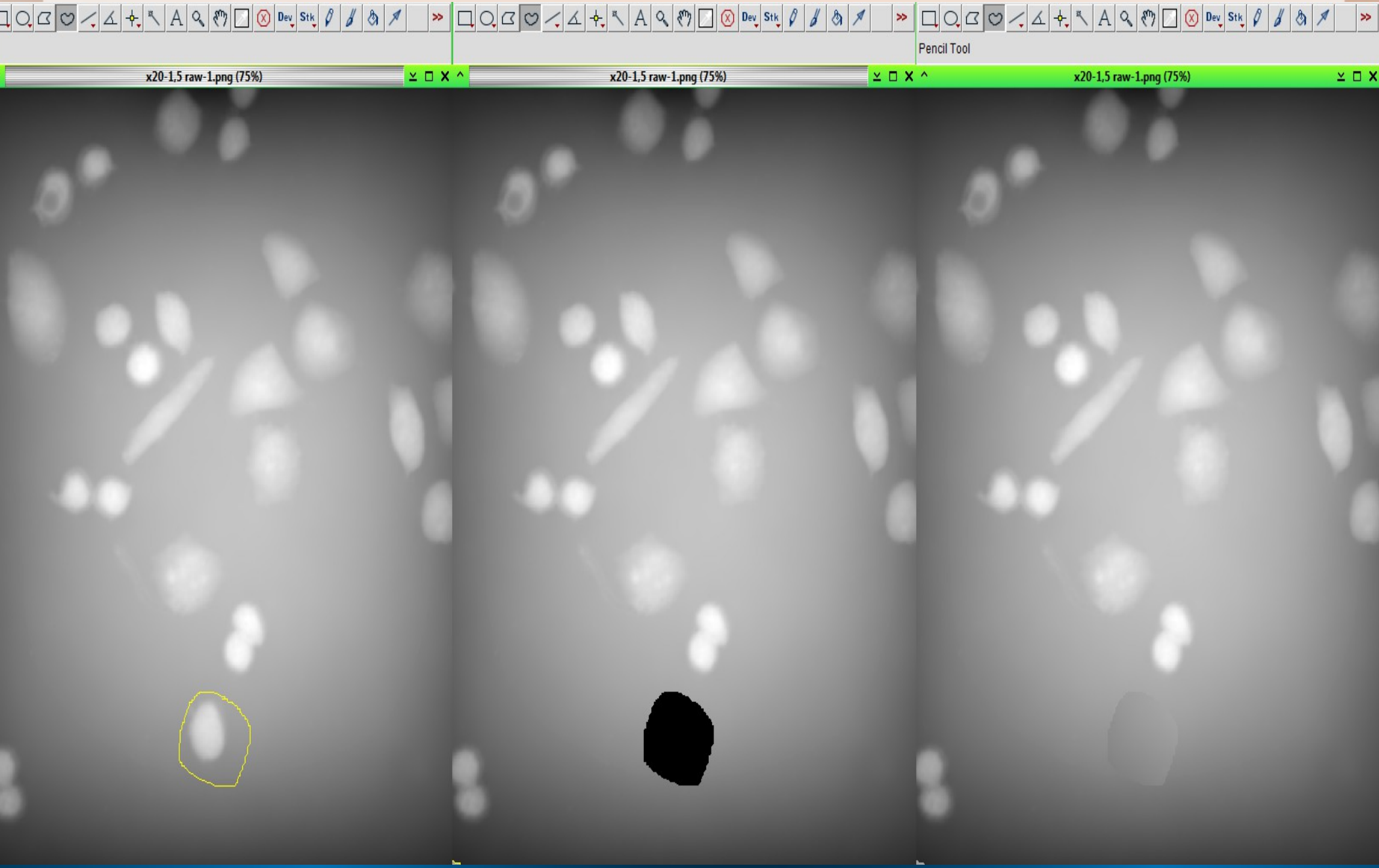
- fill in area of an image based on existing background
- user selects an area which is then filled





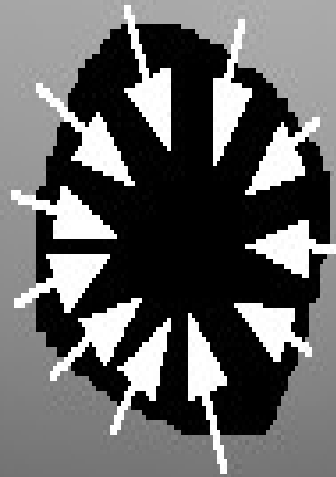
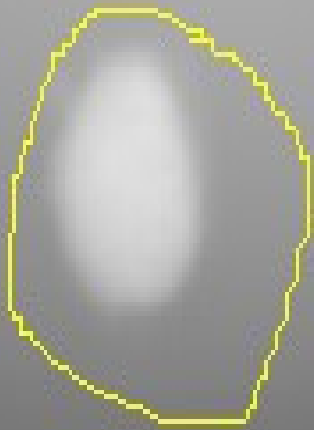
# Imaging Projects

## 1) Background estimation



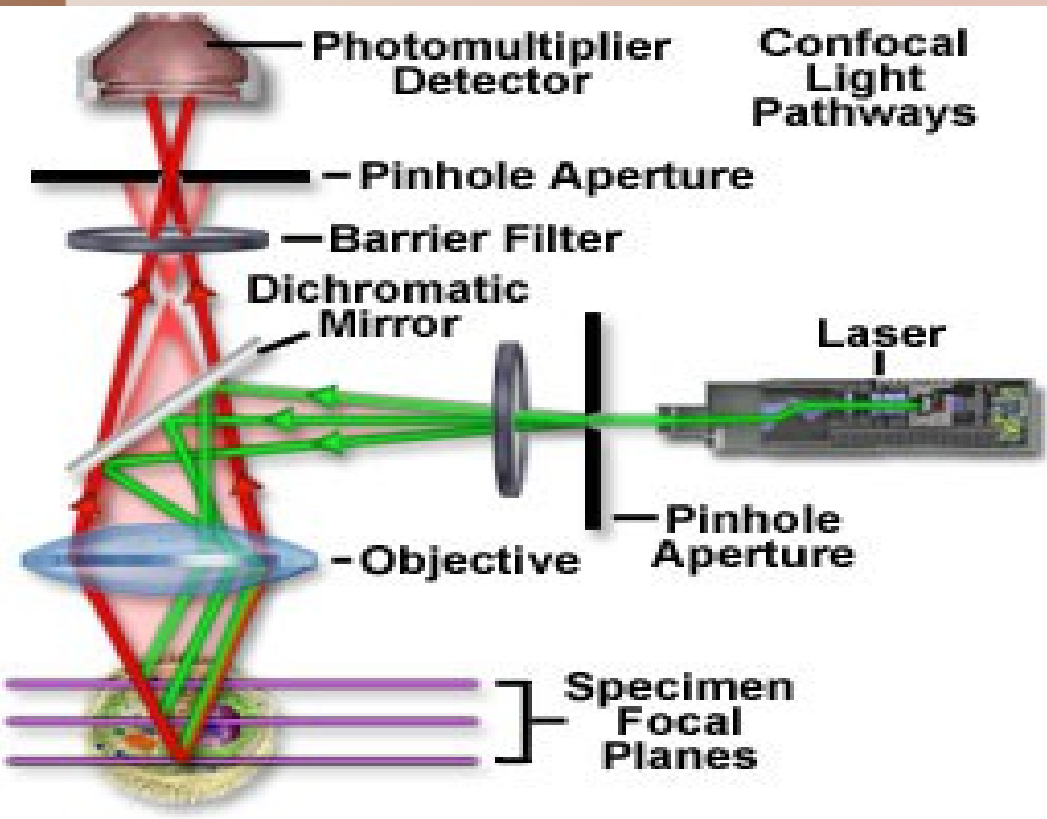
# Imaging Projects

## 1) Background estimation -smooth transitions



# Confocal Microscopy

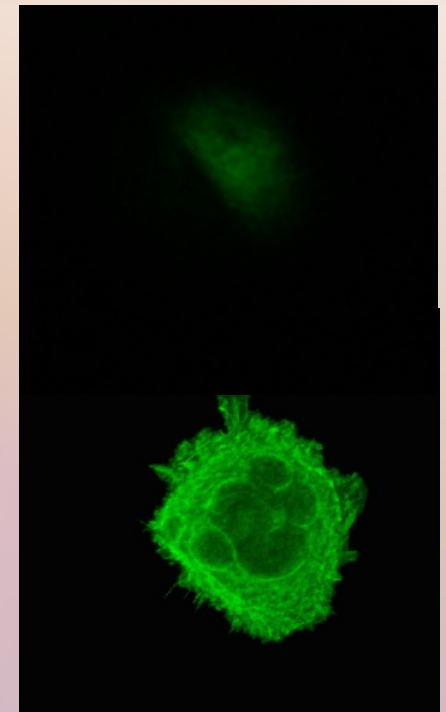
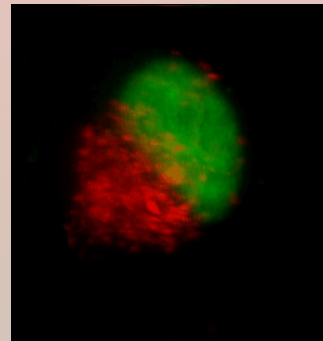
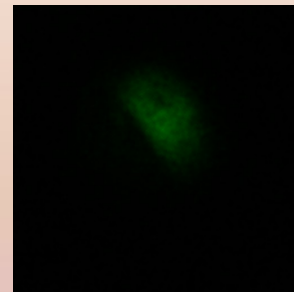
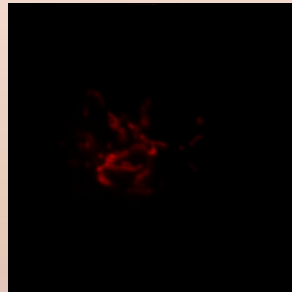
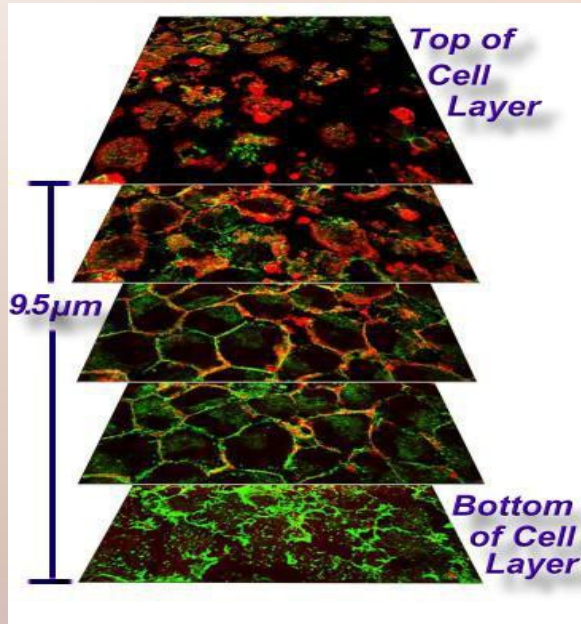
- laser line to illuminate and scan samples, with series of mirrors and filters
- uses point illumination and pinhole to eliminate out-of-focus light





# Confocal Microscopy

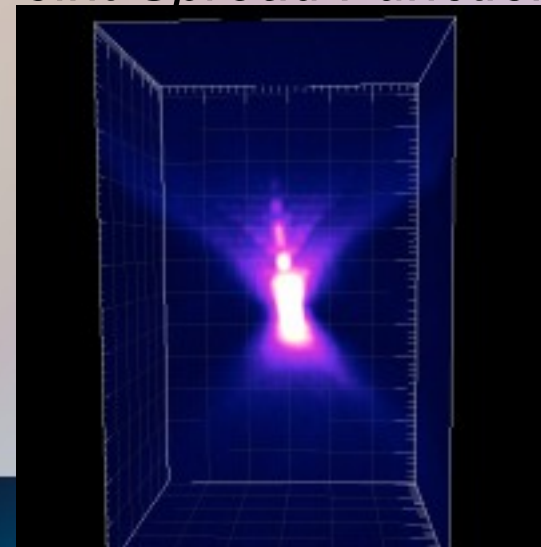
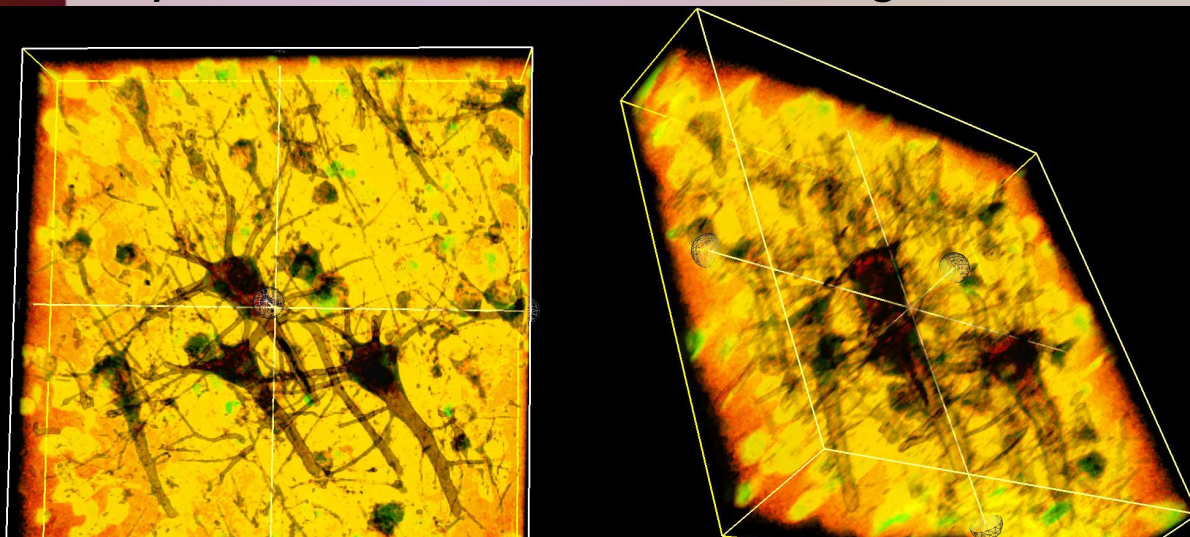
Permits 3D data collection by creating image stacks and 3D reconstruction  
-far better resolution by imaging single planes and recombining



## Problems

*Acquisition time , Axial Blurring----*

*Point Spread Function*



# Imaging Projects

## MICROSCOPY

**1) Background estimation**

**2) Tiled data reconstruction and correction**

**3) Astrocyte classification**

**4) Microvessel analysis**

CONFOCAL MICROSCOPY

**5) Neuron classification**

**6) Nucleus classification**

3D ELECTRON MICROSCOPY

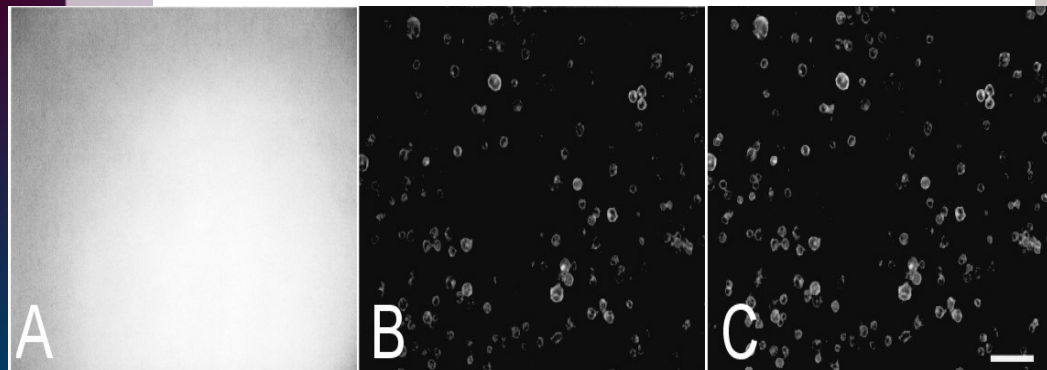
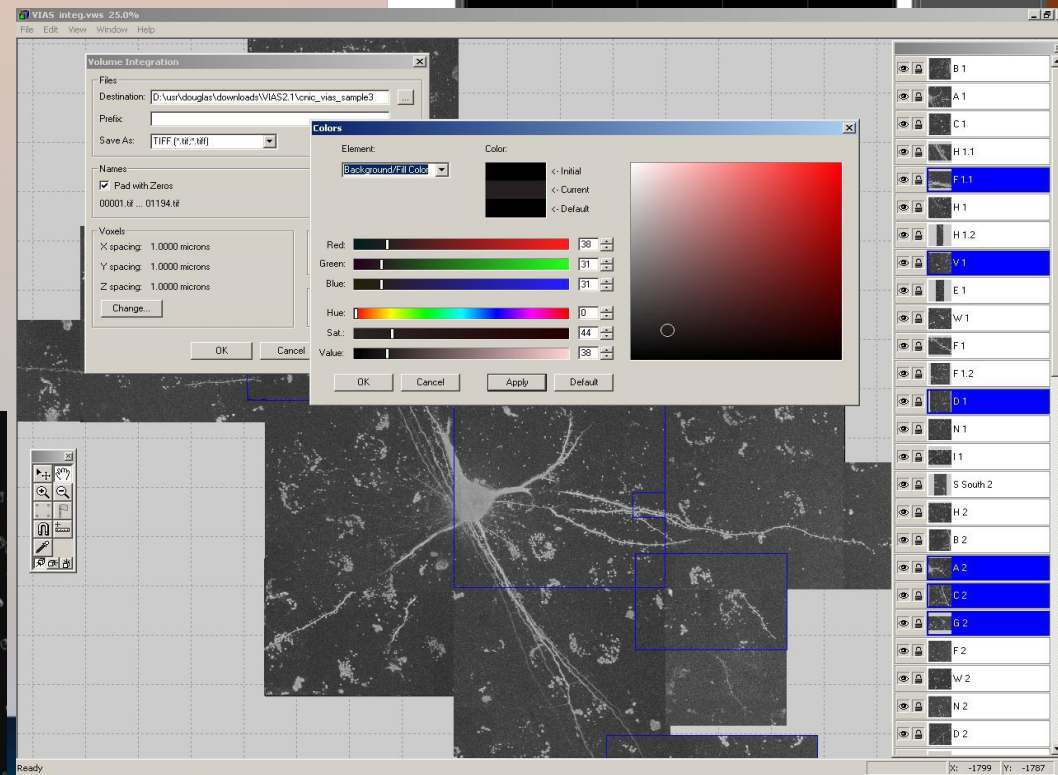
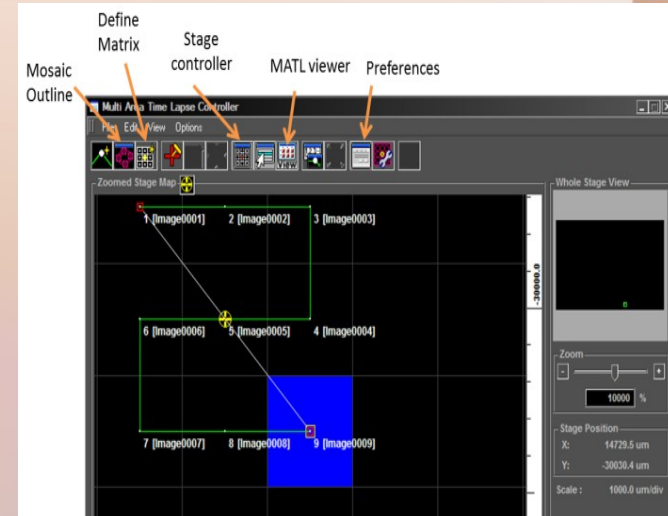
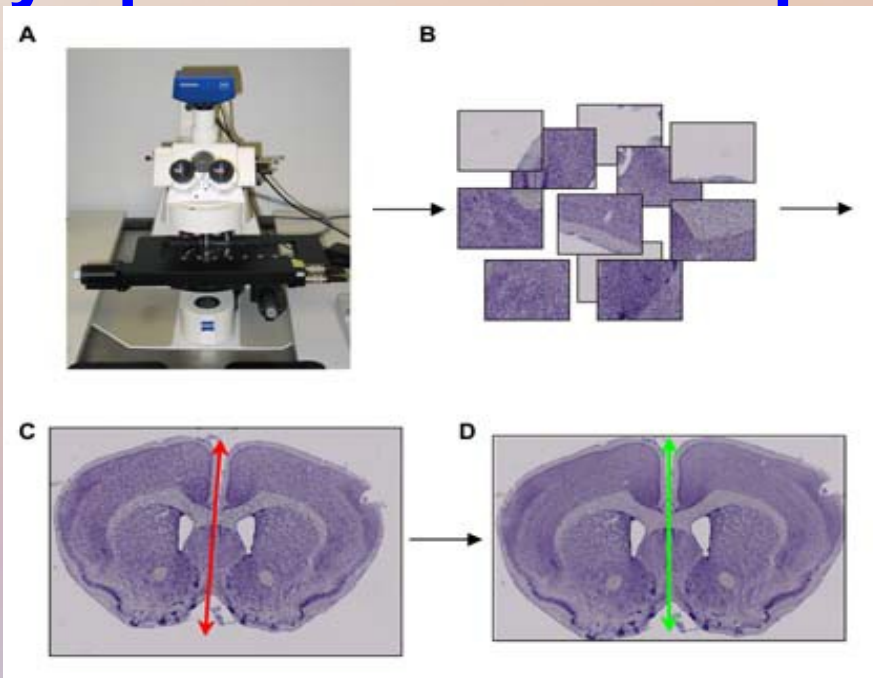
**7) Cerebellum classification**

MRI

# Imaging Projects

## 2) *Tiled data reconstruction and correction*

- accurately stitch multiple images together
- correct brightness across images
- existing plugin usage?
- olympus data format input



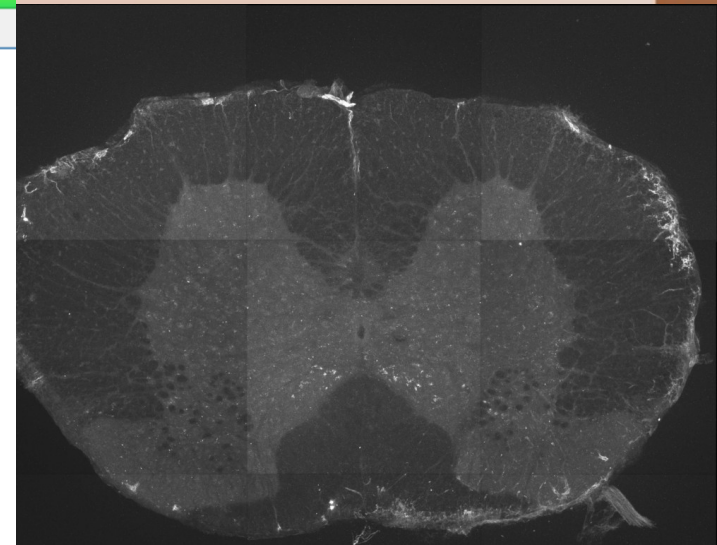


# Imaging Projects

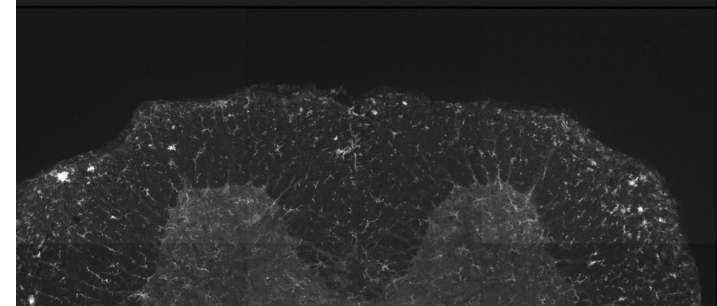
## 2) Tiled data reconstruction and correction

- accurately stitch multiple images together
- multichannel OIB and MATL input – rgbTiff/Select output
- stage drift-->predictable?

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MAI1_Mosaic - Notepad
File Edit Format View Help
/Stage>
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<YAxisDirection>TopToBottom</YAxisDirection>
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<Repeats>1</Repeats>
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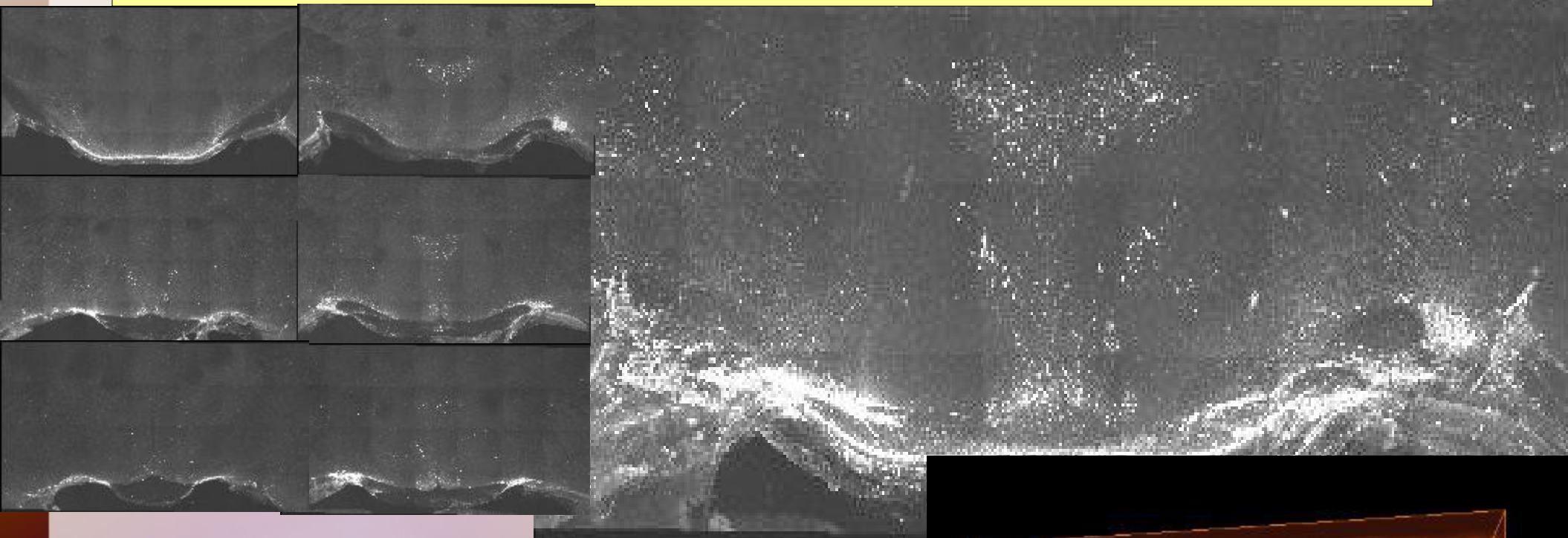
Collagen IV (vessels)



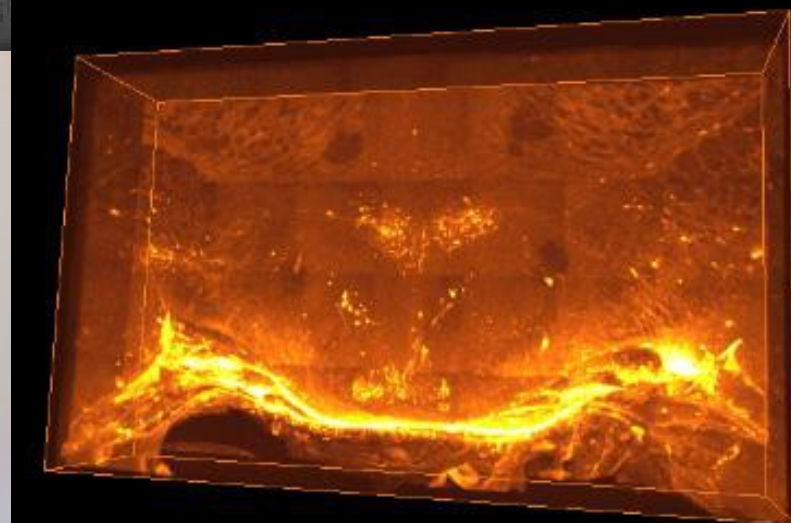
# Imaging Projects

## 2) Tiled data reconstruction and correction

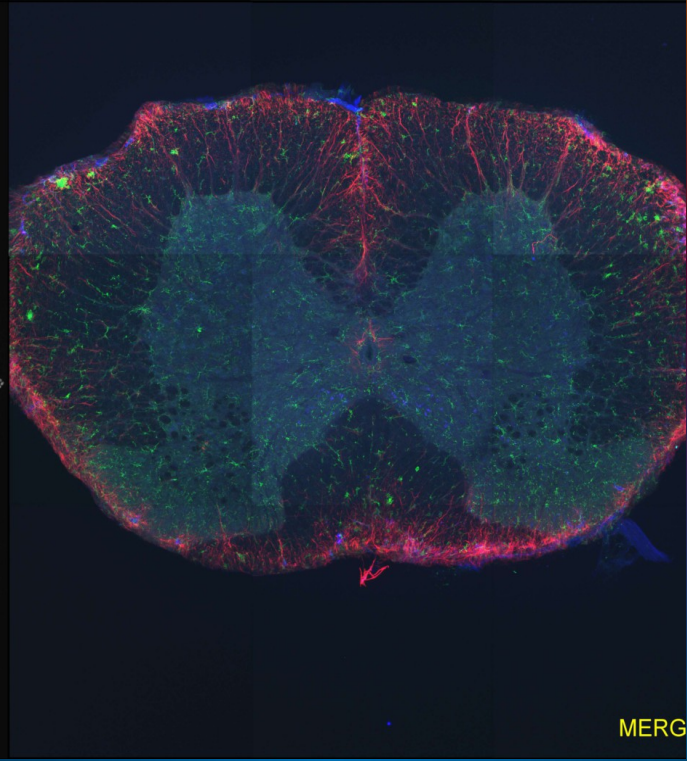
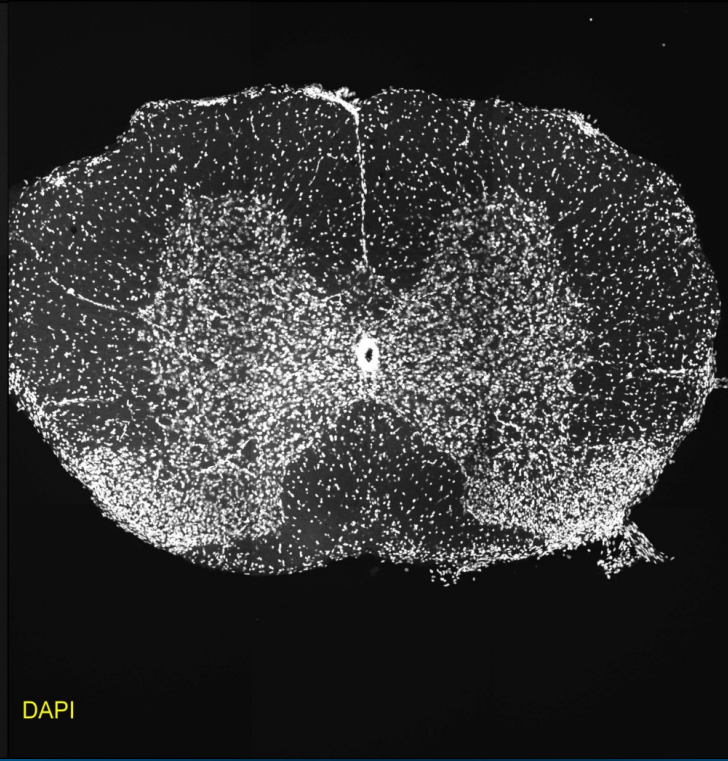
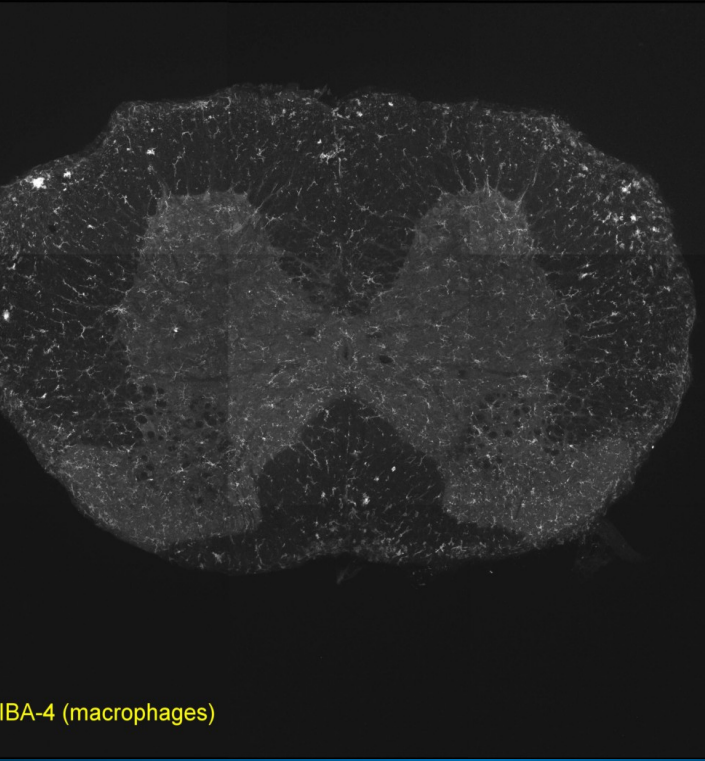
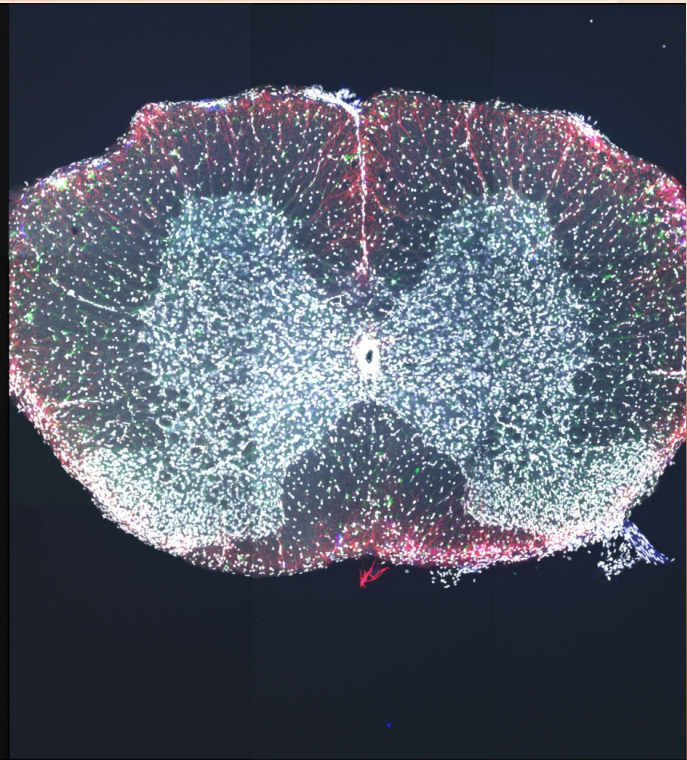
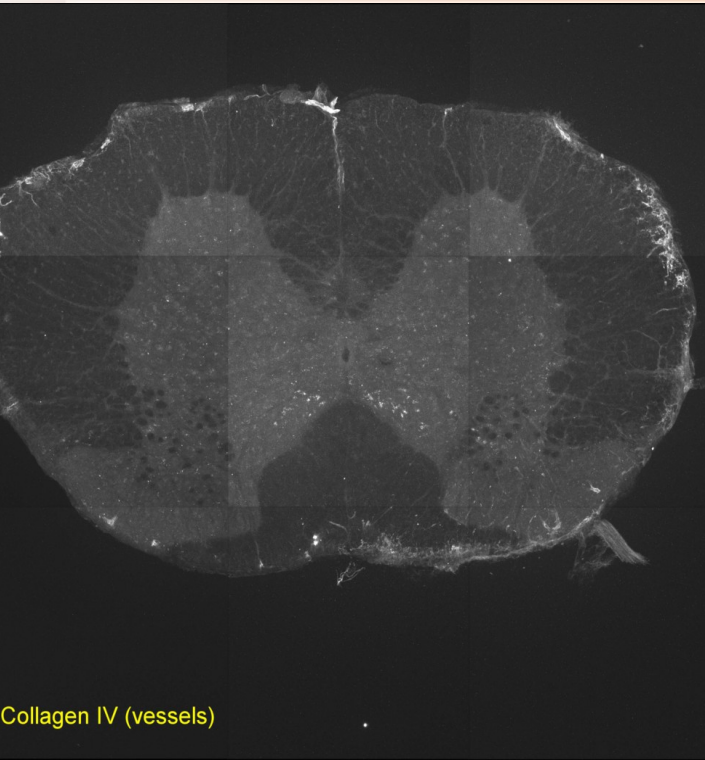
### TISSUE RECONSTRUCTION



- 4.35 mm x 2.6mm x 1.4 mm
- voxel resolution; 1.2 microns
- 3508x2072x700 pixels
- 19600 optical sections





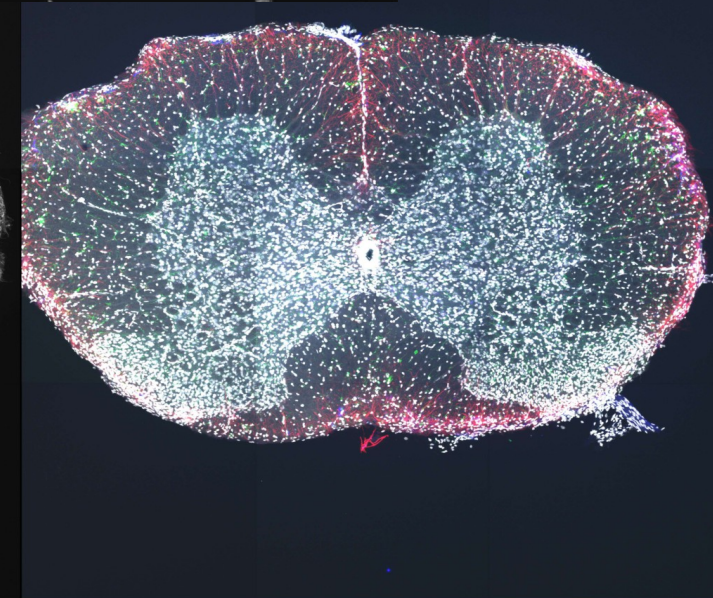
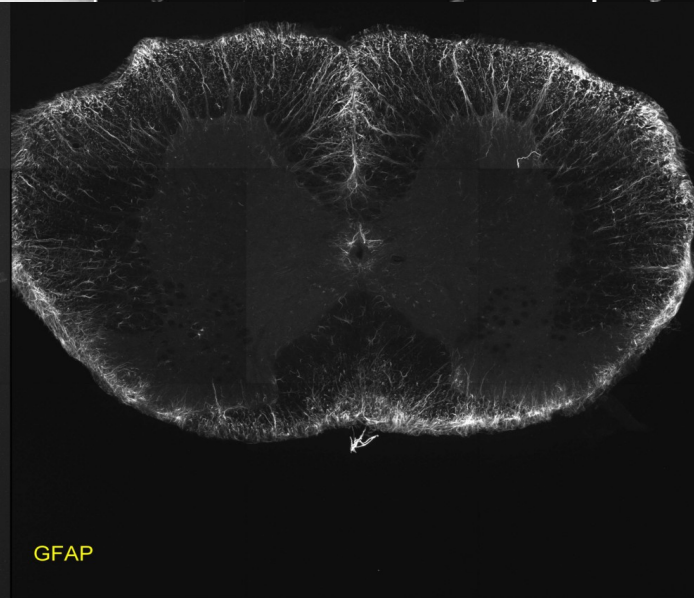
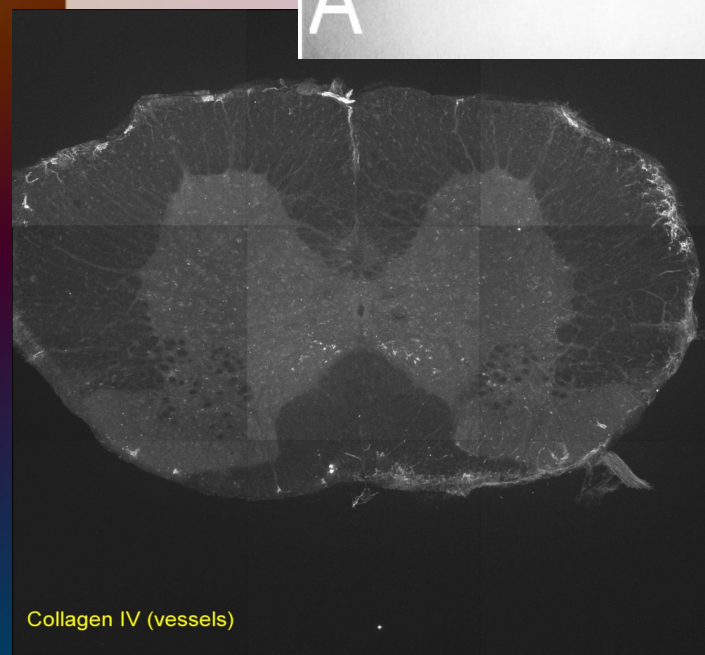
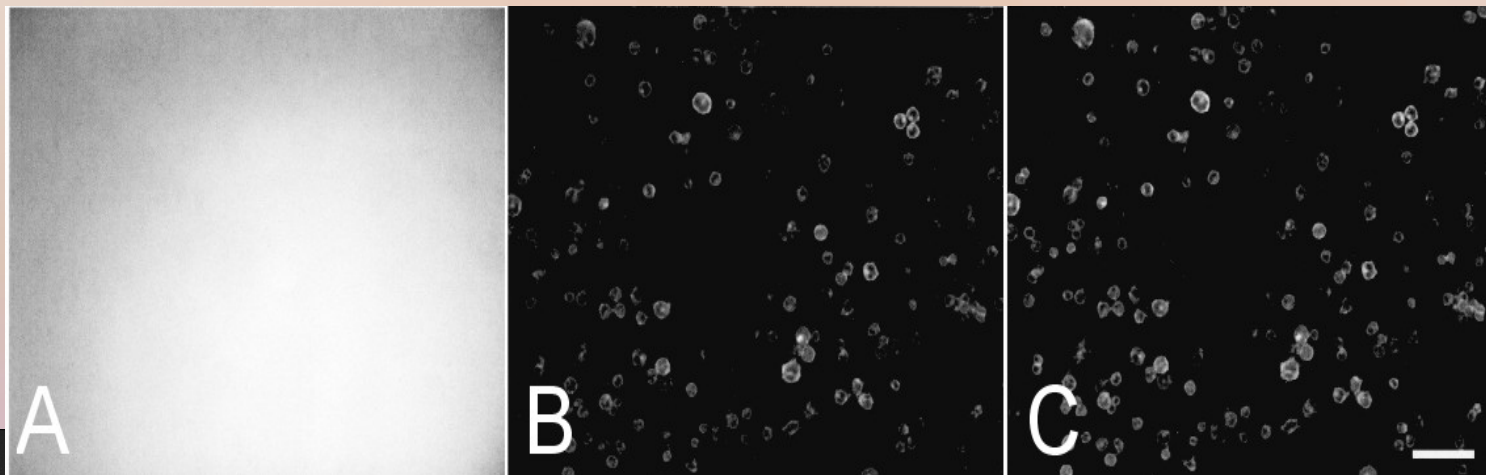




# Imaging Projects

## 2) *Tiled data reconstruction and correction*

- correct brightness across images/seems
- use adjacent tiles, (calibration image?)
- different channel issues?



# Imaging Projects

## MICROSCOPY

1) Background estimation

2) Tiled data reconstruction and correction

3) Astrocyte classification

4) Microvessel analysis

CONFOCAL MICROSCOPY

5) Neuron classification

6) Nucleus classification

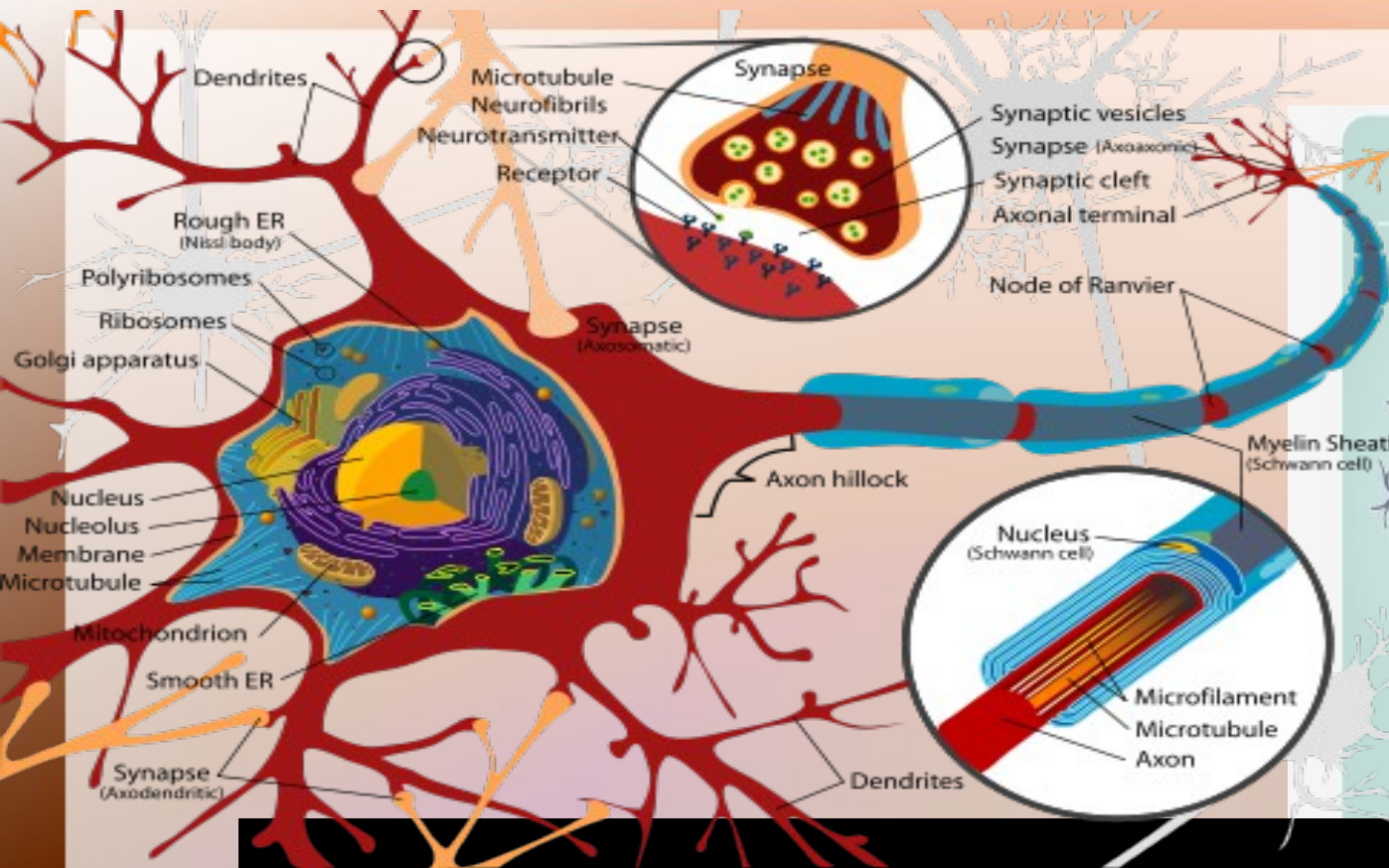
3D ELECTRON MICROSCOPY

7) Cerebellum classification

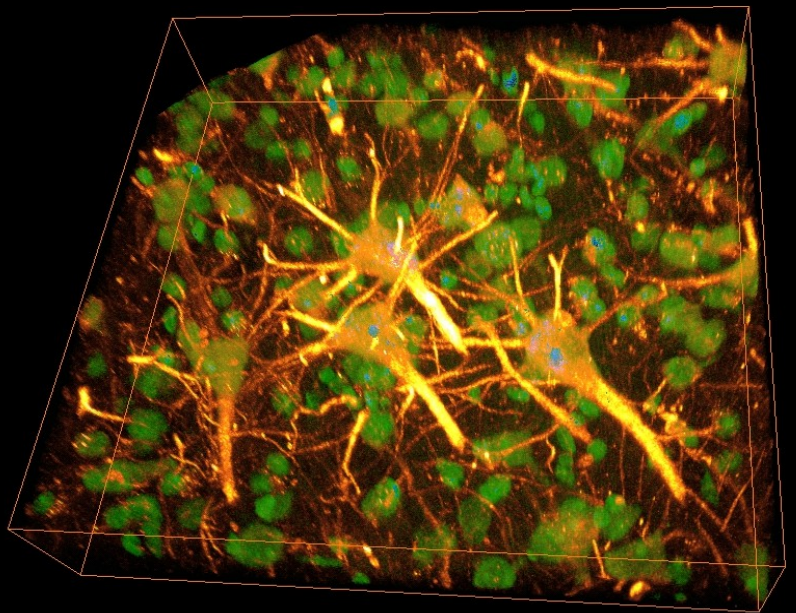
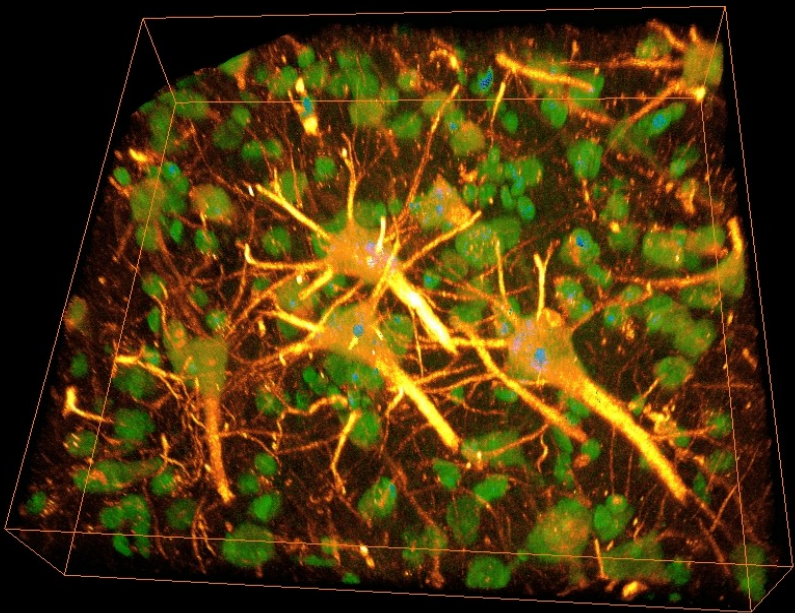
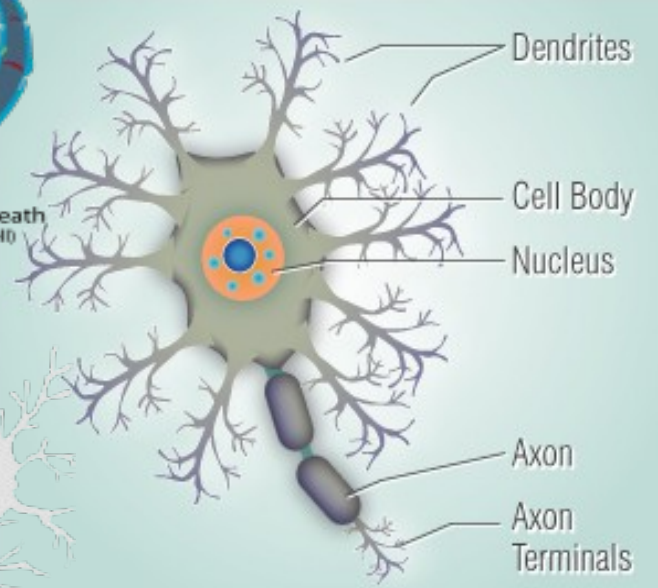
MRI



# Neurons



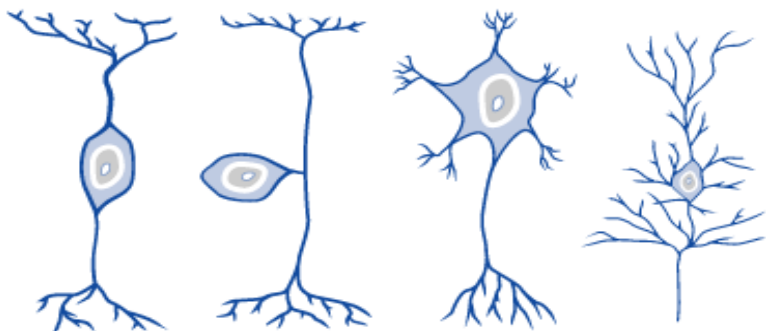
## The Neuron





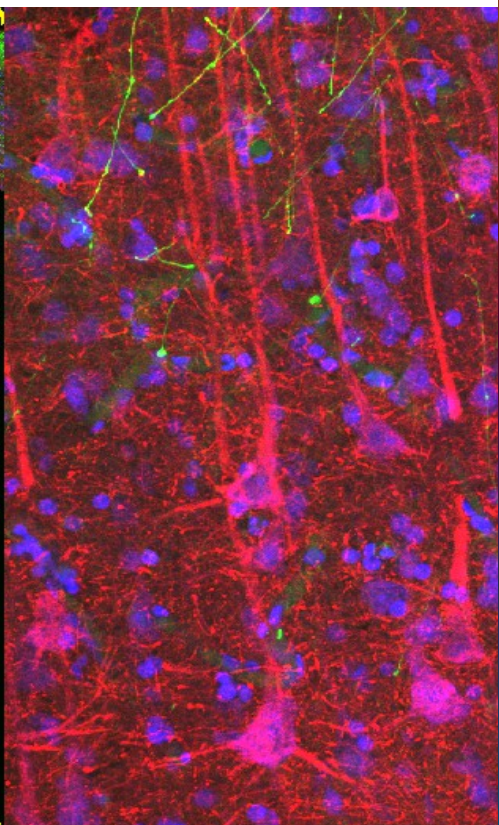
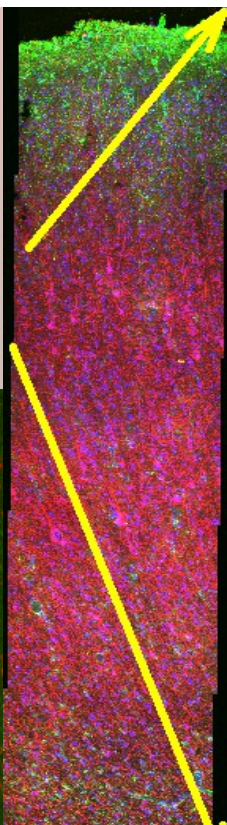
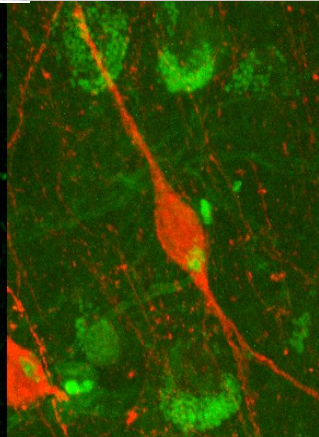
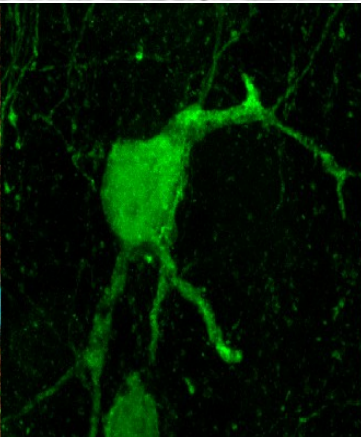
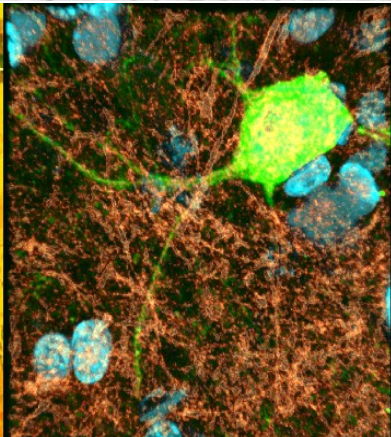
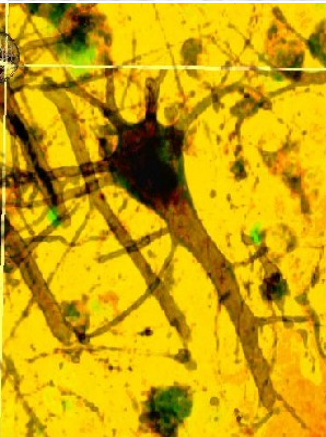
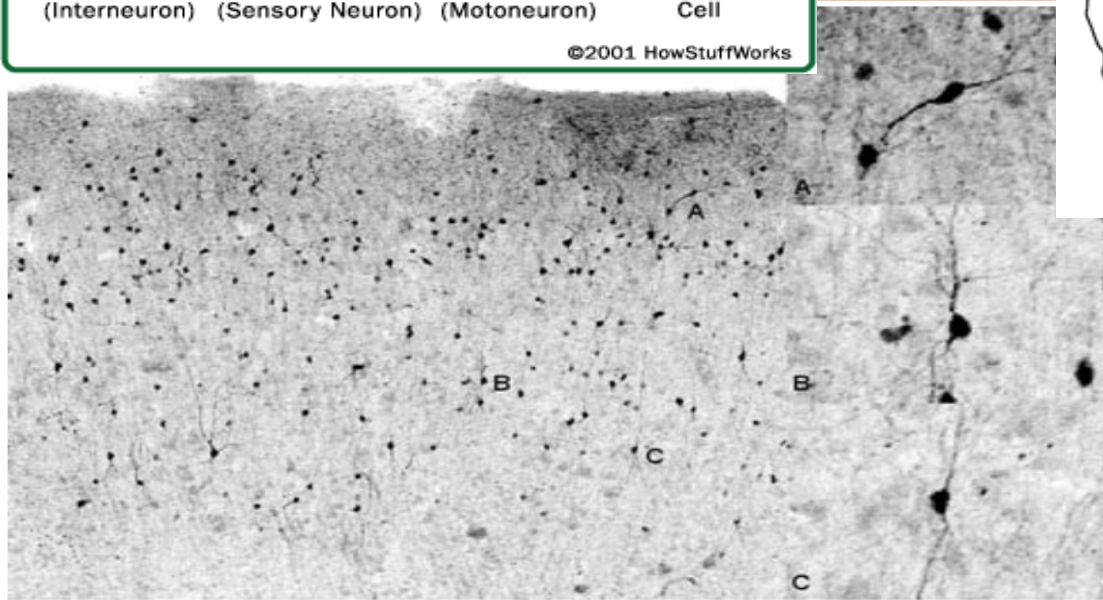
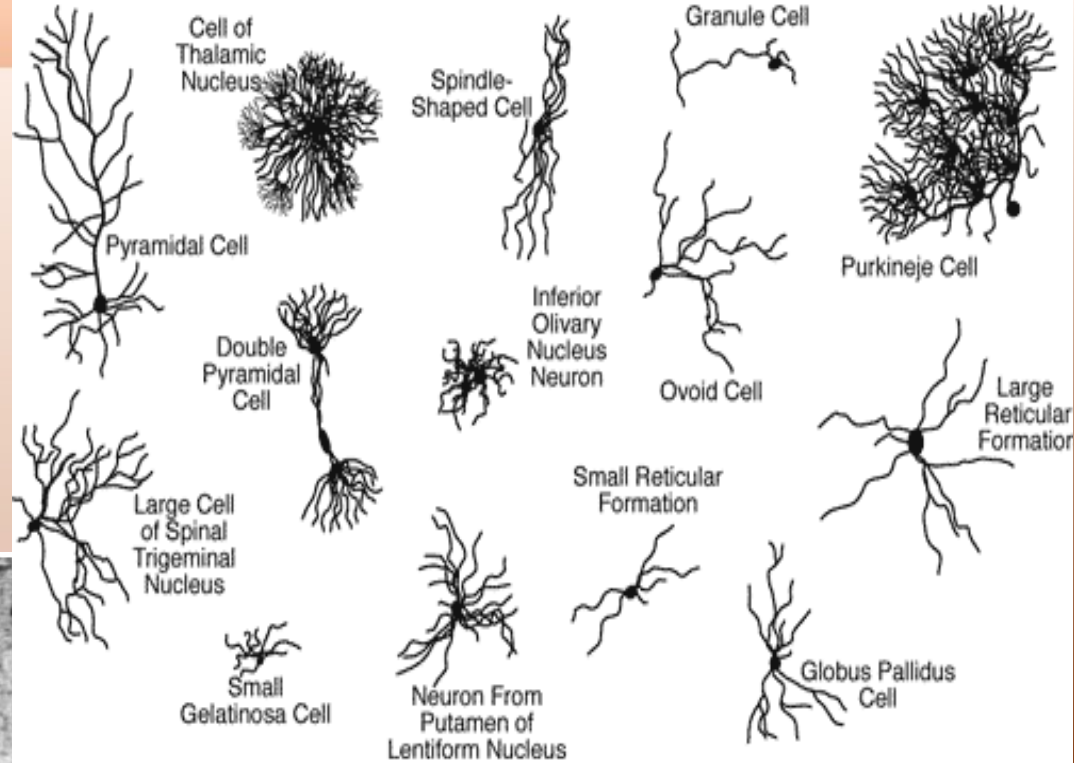
# Types of Neurons

## Basic Neuron Types



Bipolar (Interneuron)    Unipolar (Sensory Neuron)    Multipolar (Motoneuron)    Pyramidal Cell

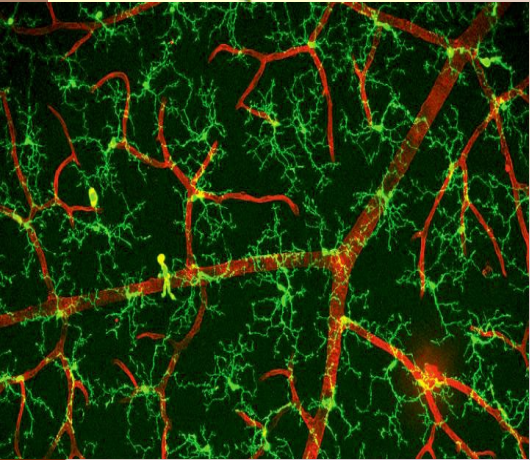
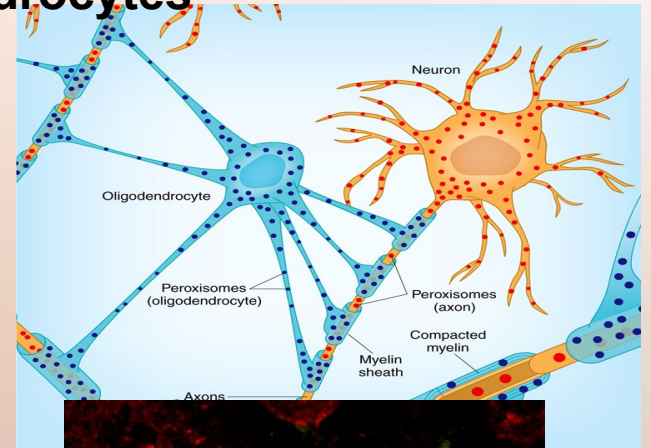
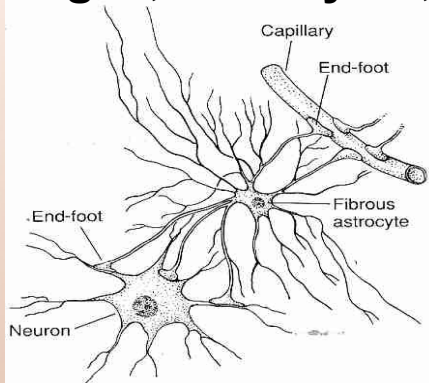
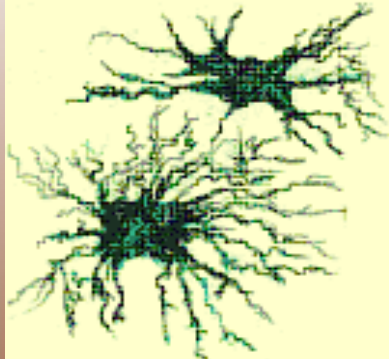
©2001 HowStuffWorks



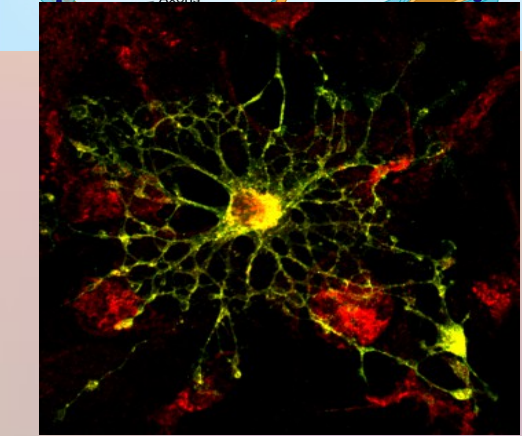
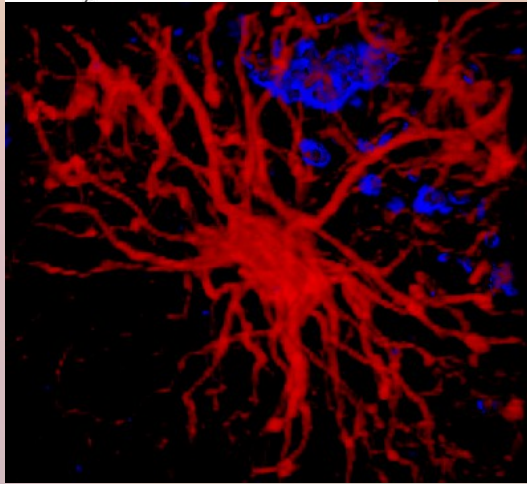


# Other Cell Types in the Brain

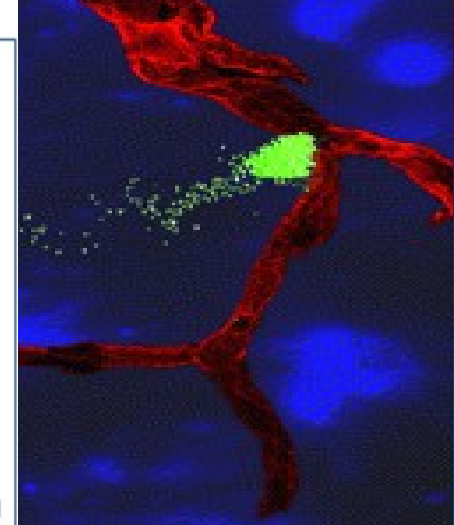
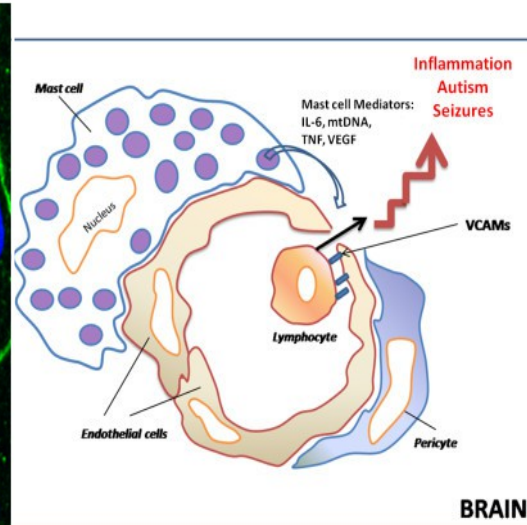
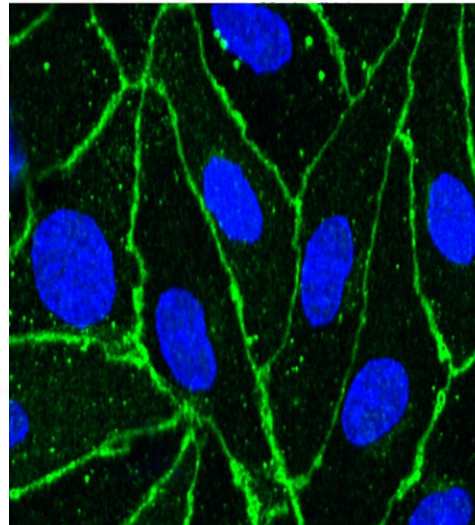
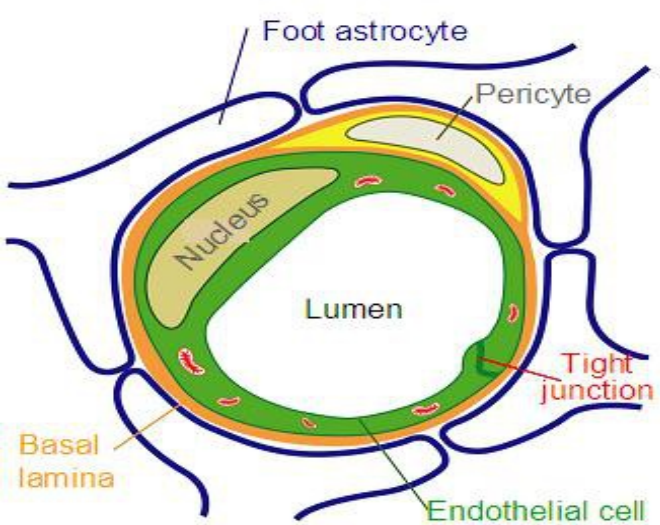
## Glial Cells -microglia, astrocytes, oligodendrocytes



**Endothelial**



**Mast Cells**

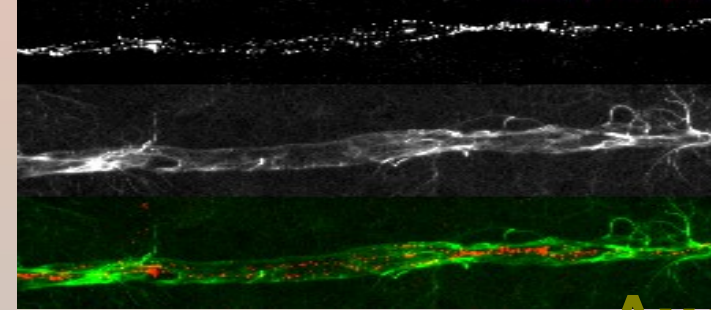
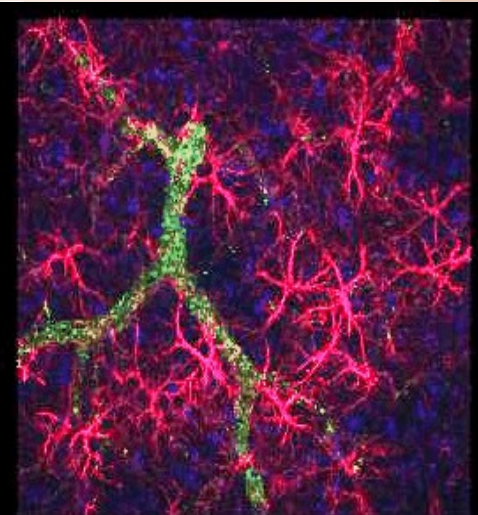
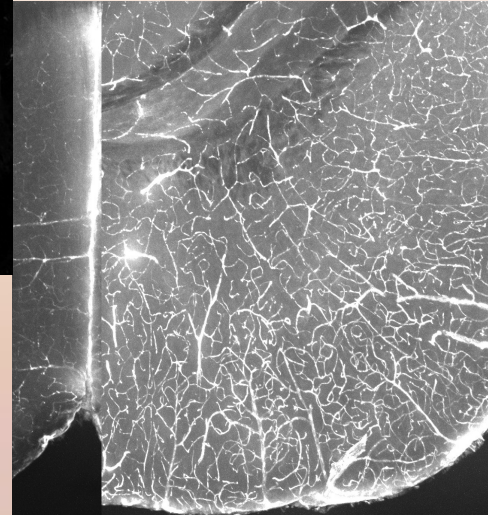
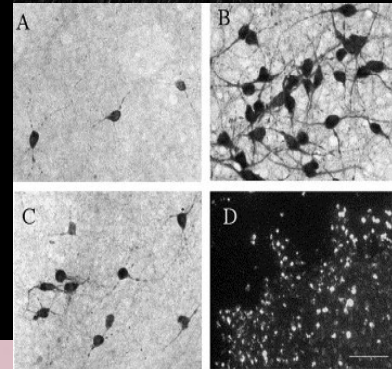
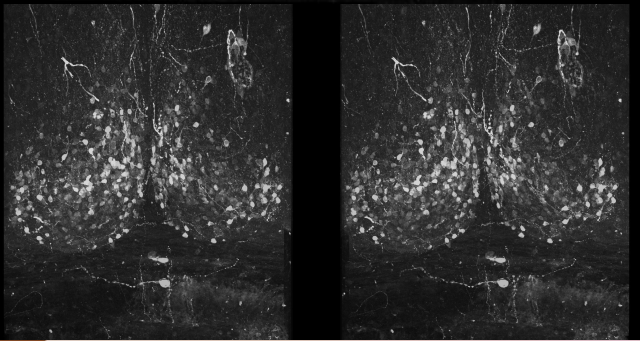
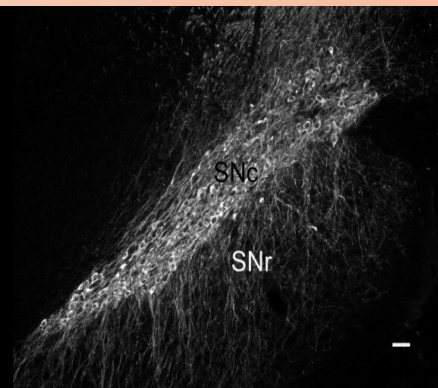




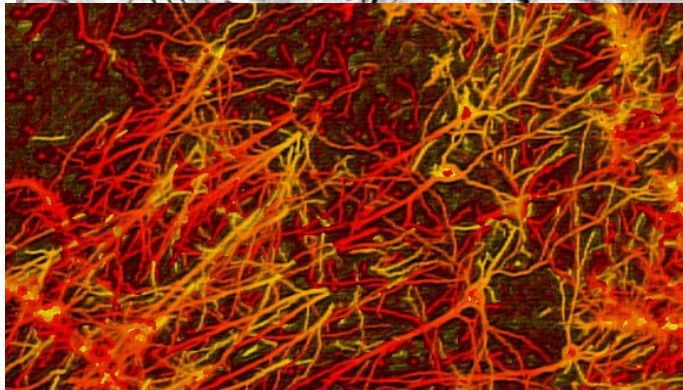
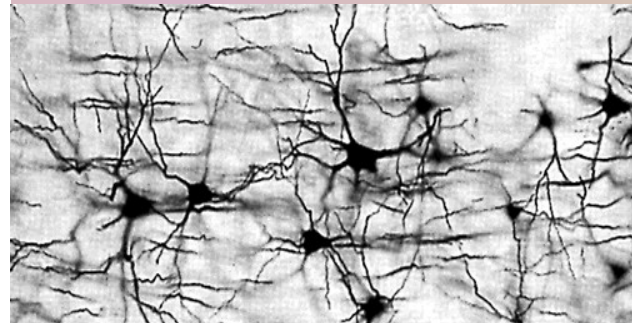
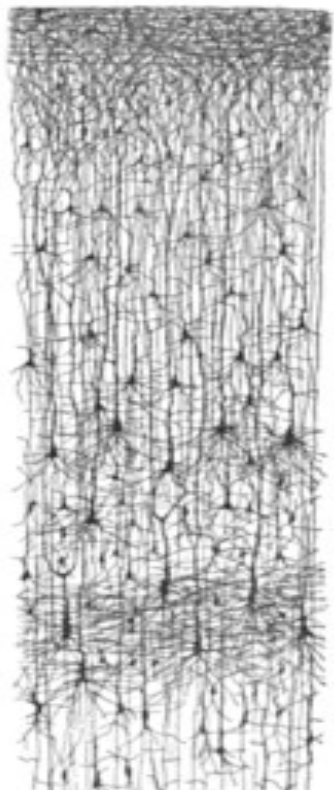
# Functional Units in the Brain

## Blood Brain Barrier

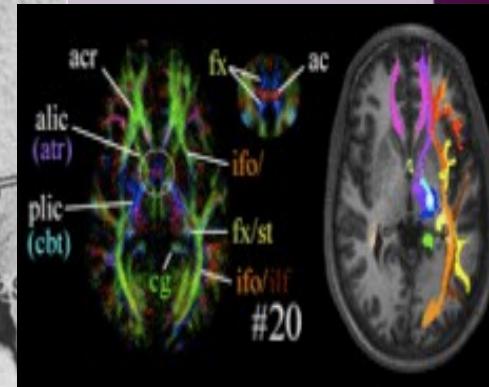
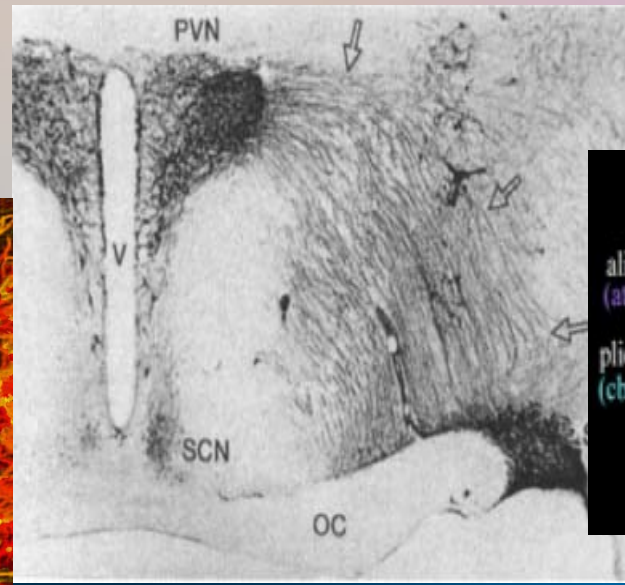
## Nuclei /Regional



## Neural Networks



## Axonal Tracts

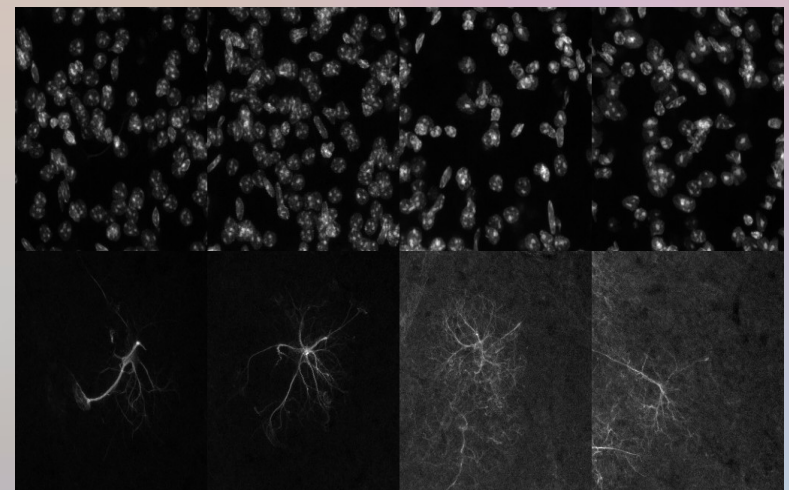
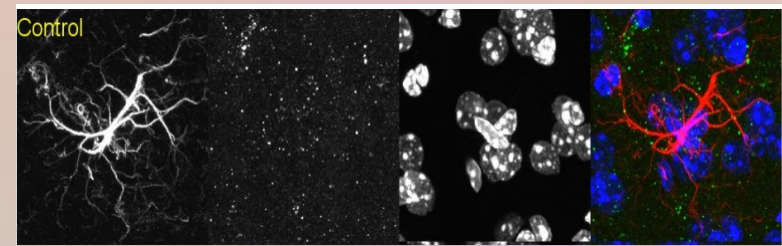
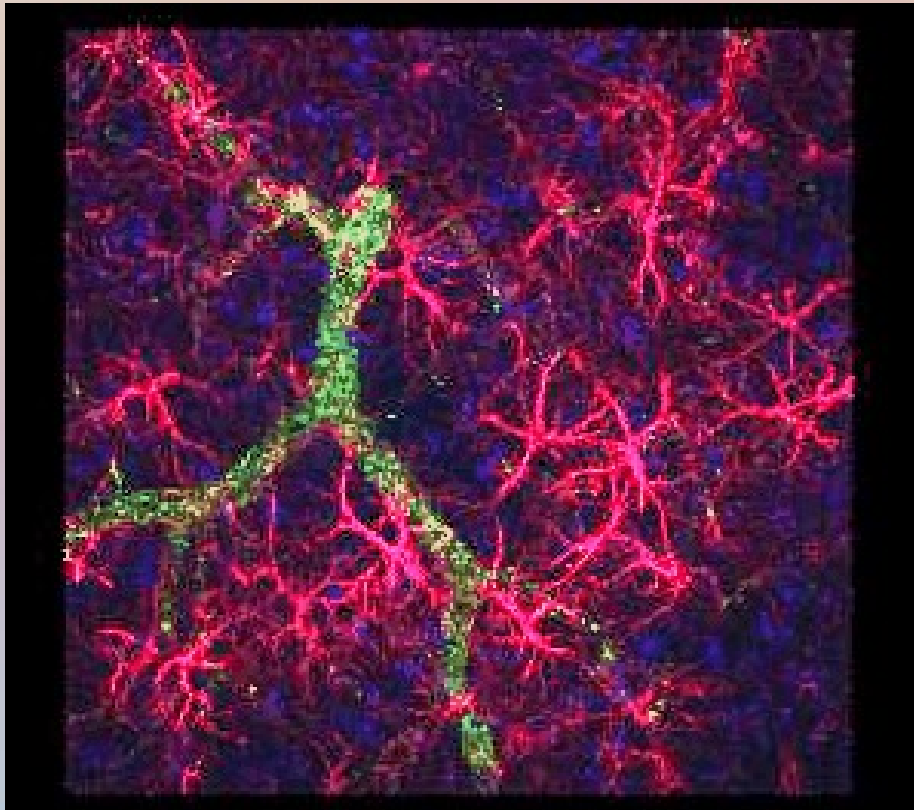




# Imaging Projects

## 3) *Astrocyte analysis*

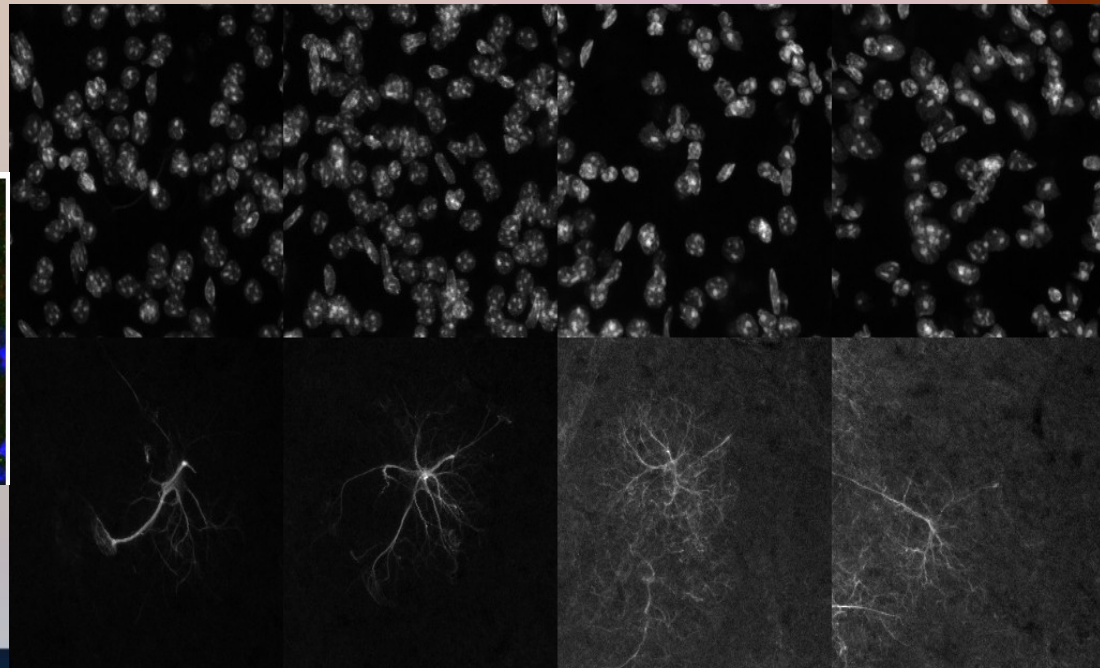
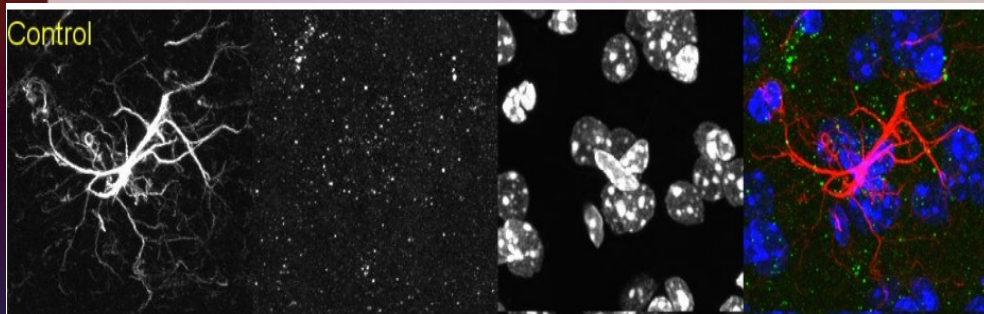
- automatically segment *Individual* Glial Cells in 3D
- classify astrocytes and provide volume and surface area
- extract and count individual glial cells
- measure degree of overlap with vessels
- use and extend existing plugins



# Imaging Projects

## 3a) *Astrocyte analysis*

- automatically segment *Individual* Glial Cells in 3D
- intelligent thresholding → brightness differences?
- segmentation → use of seed points
- extendable to larger data sets with multiple astrocytes
  - using seed points
  - overlapping/touching cell structures?

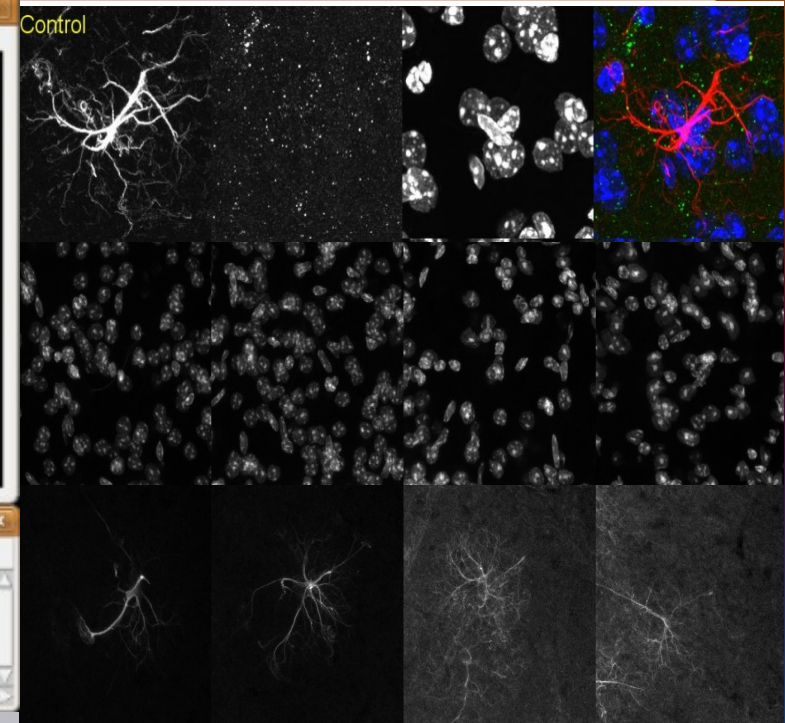




# Imaging Projects

## 3a) *Astrocyte analysis*

- classify astrocytes and provide volume and surface area
- existing tools for quantification
- describe number/length of cell extensions
- define cell body?
- describe number of bifurcations
- skeletonize3d/analyze skeleton plugins?
- group similar cells for classification



# Branches	# Junctions	Length	# Triple points	Maximum Branch Length
15	7		7	72.669
5	2		2	48.799
7	3		3	67.113

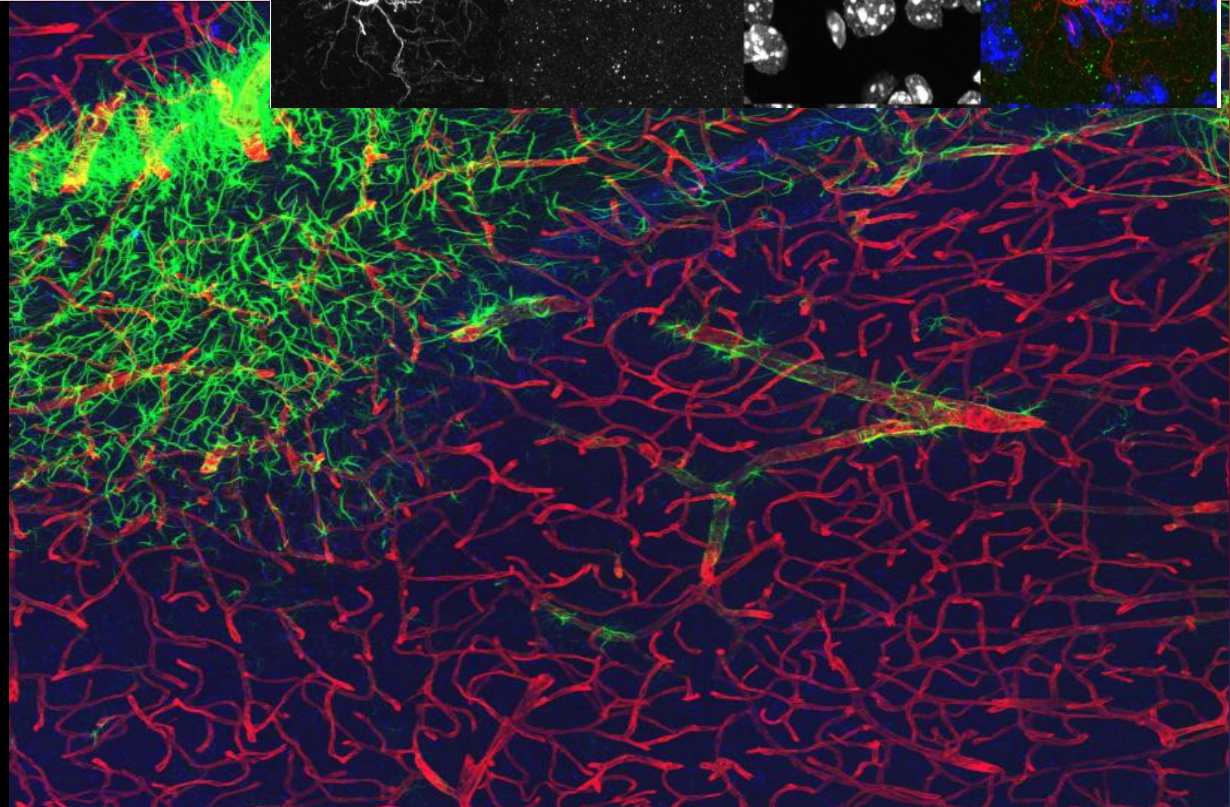
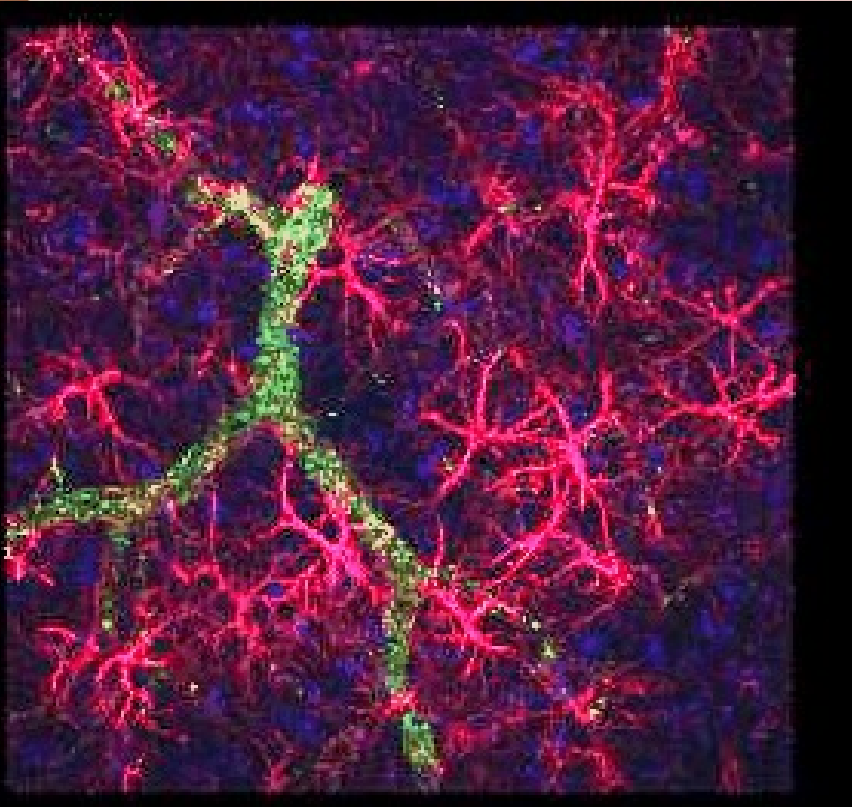
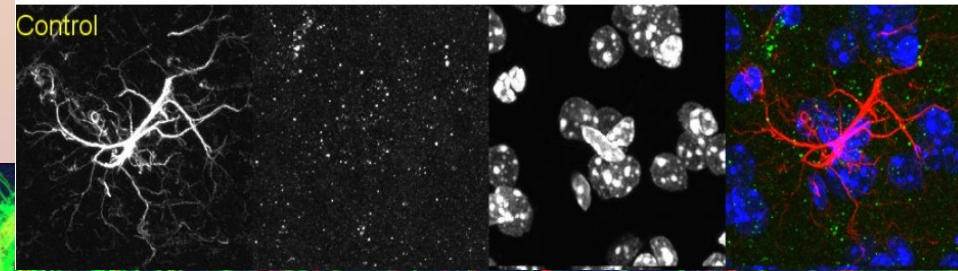




# Imaging Projects

## 3b) *Astrocyte analysis*

- extract, classify and count individual glial cells in larger image
- find seed points to start segmentation
  - use other channel information (nuclear stain)
- overlapping glial cells?
  - where to terminate segmentation?

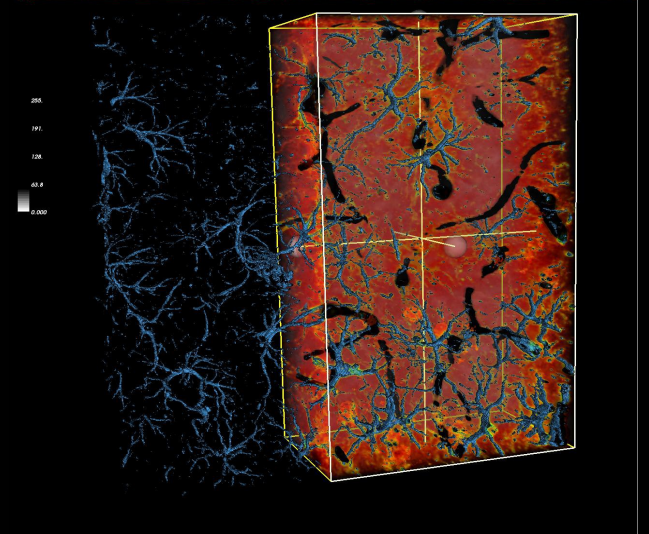
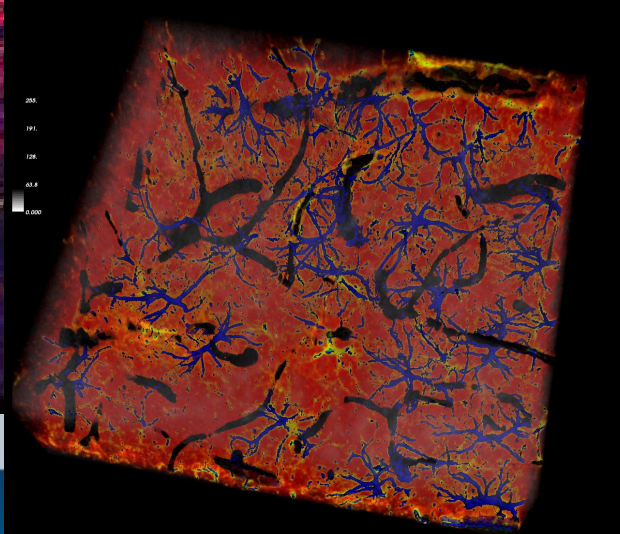
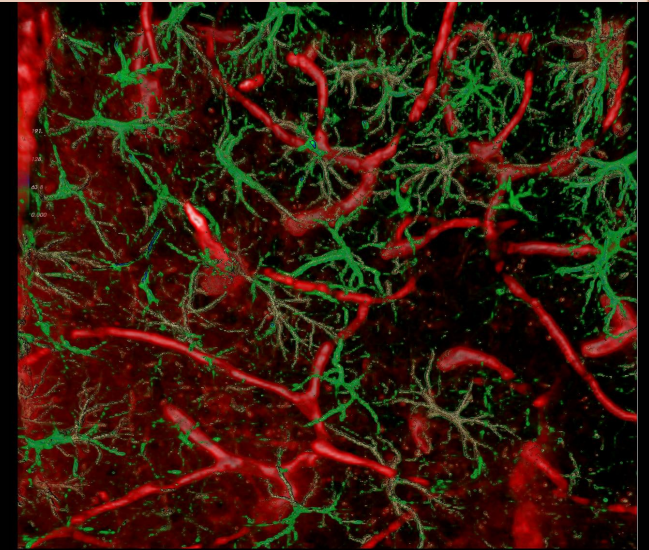
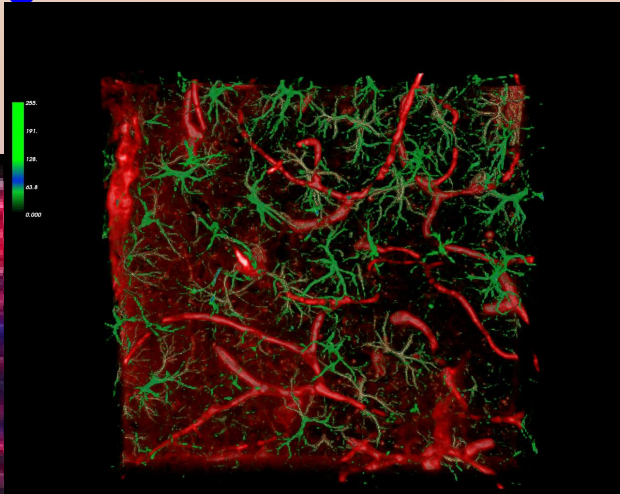
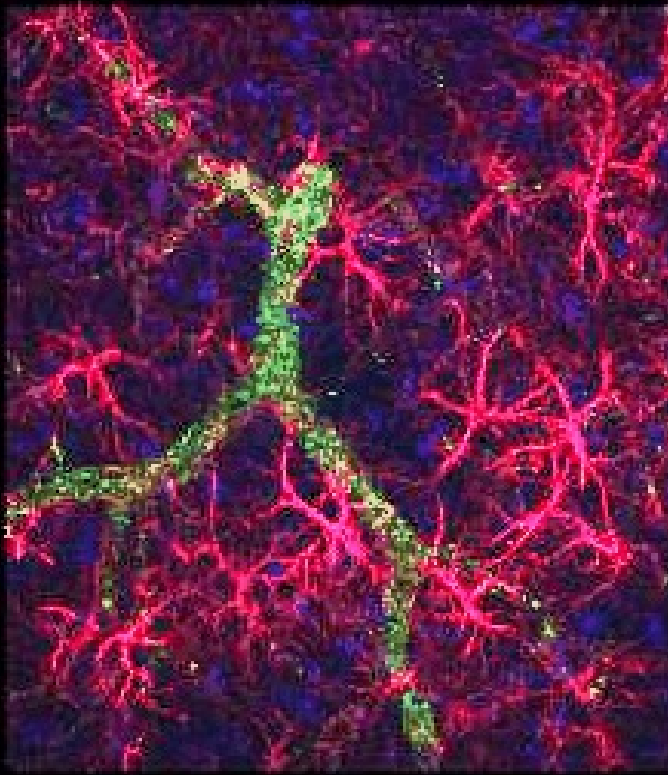




# Imaging Projects

## 3b) *Astrocyte analysis*

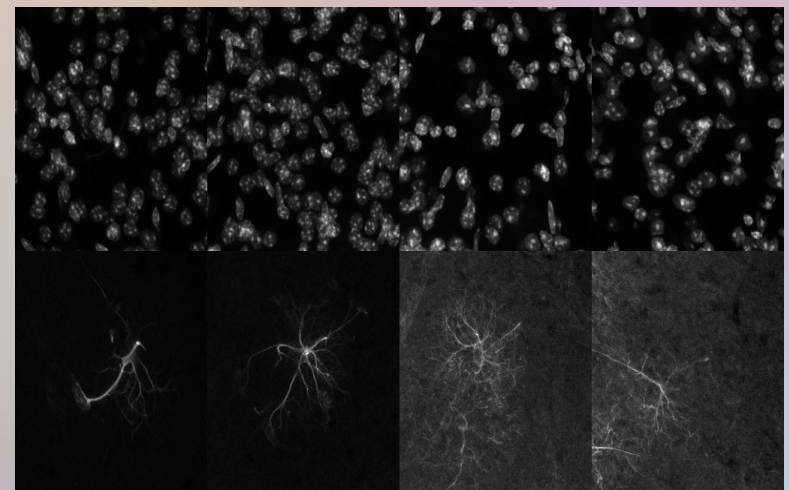
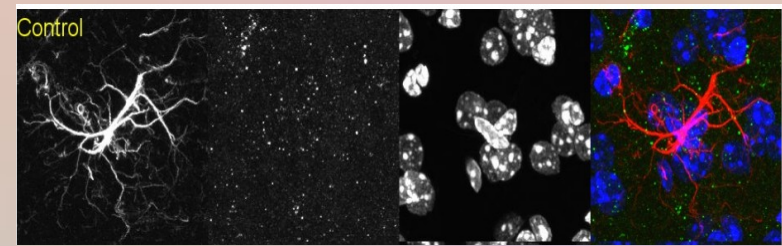
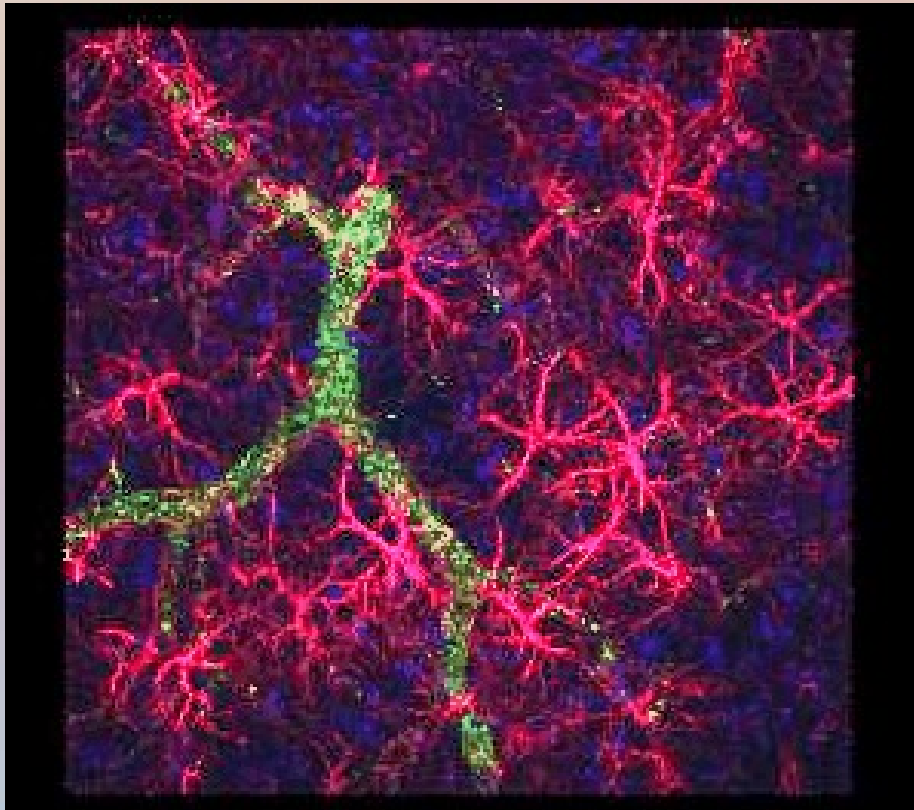
- measure degree of astrocyte overlap with vessels in secondary channel
- percentage of total cell overlap
- what part of the cell overlaps?
- relate this to groupings of cells?



# Imaging Projects

## 3) *Astrocyte analysis*

- automatically segment *Individual* Glial Cells in 3D
- classify astrocytes and provide volume and surface area
- extract and count individual glial cells
- measure degree of overlap with vessels
- use and extend existing plugins





# Imaging Projects

## MICROSCOPY

1) Background estimation

2) Tiled data reconstruction and correction

3) Astrocyte classification

4) Microvessel analysis

CONFOCAL MICROSCOPY

5) Neuron classification

6) Nucleus classification

3D ELECTRON MICROSCOPY

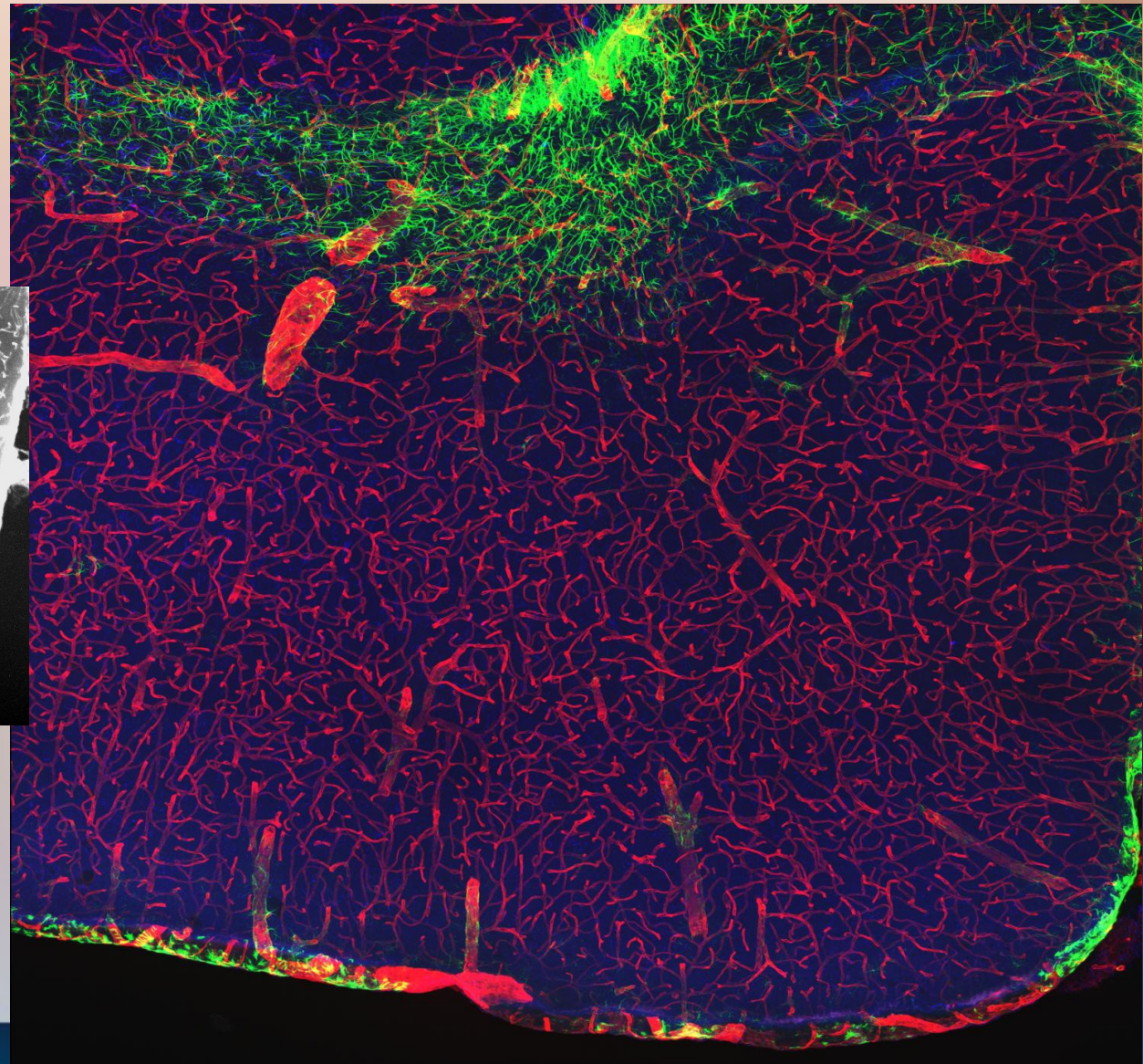
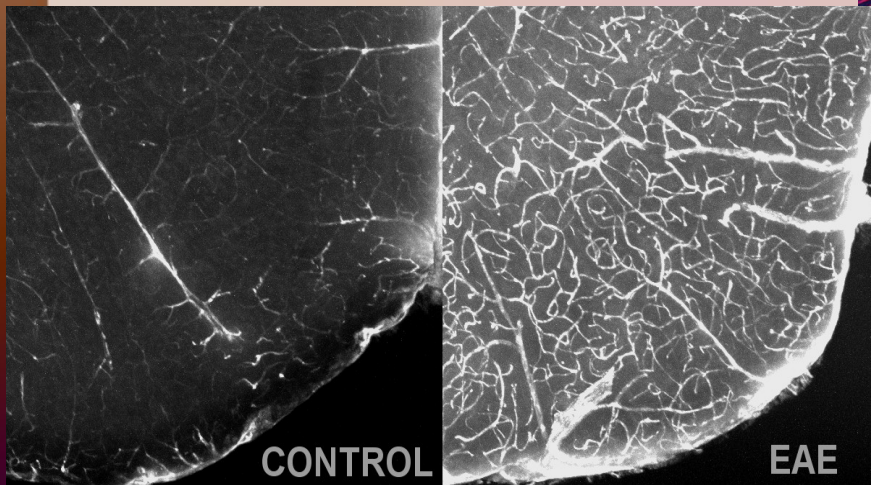
7) Cerebellum classification

MRI

# Imaging Projects

## 4) Microvessel analysis

- segment microvessels in 3D
- classify vessel segments
- surface area/volume
- segment width, density
- identify branching patterns



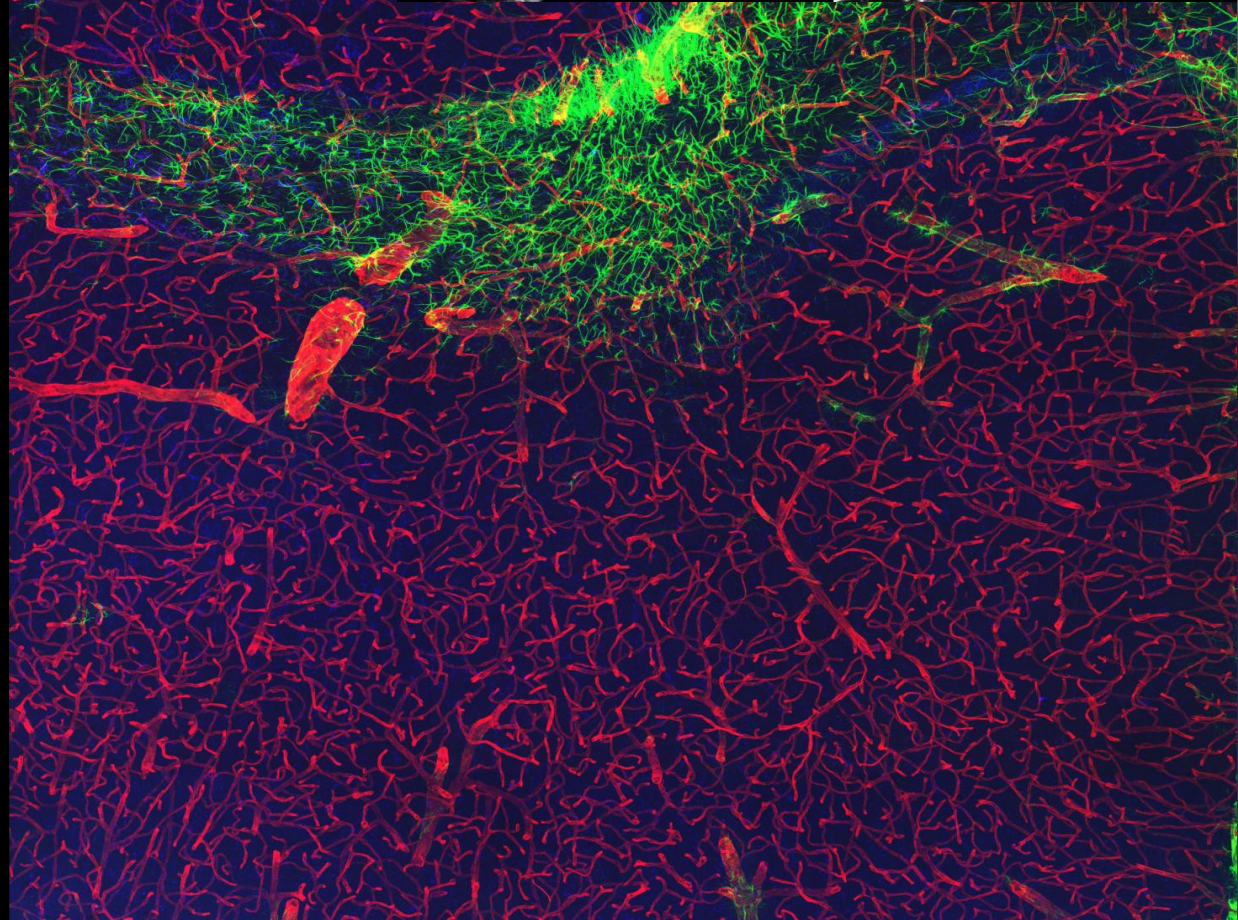
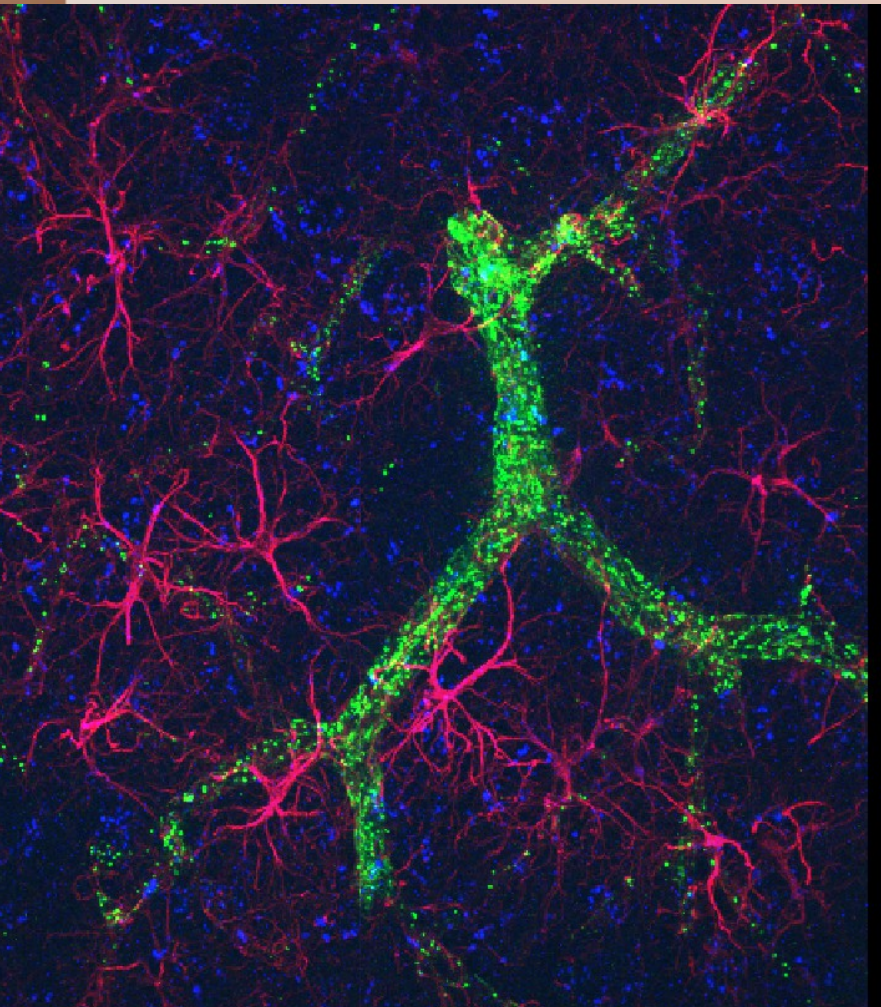
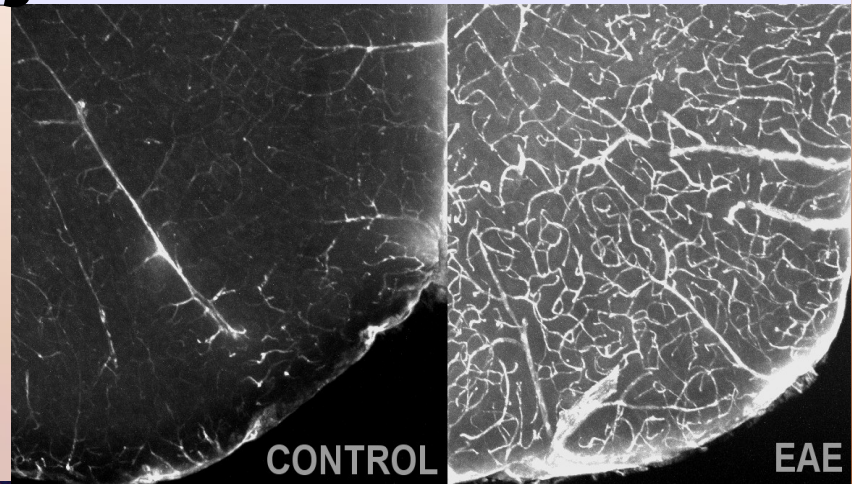


# Imaging Projects

## 4) Microvessel analysis

Segment microvessels in 3D

- intelligent thresholding
- resistant to partial staining techniques
- transected segments?



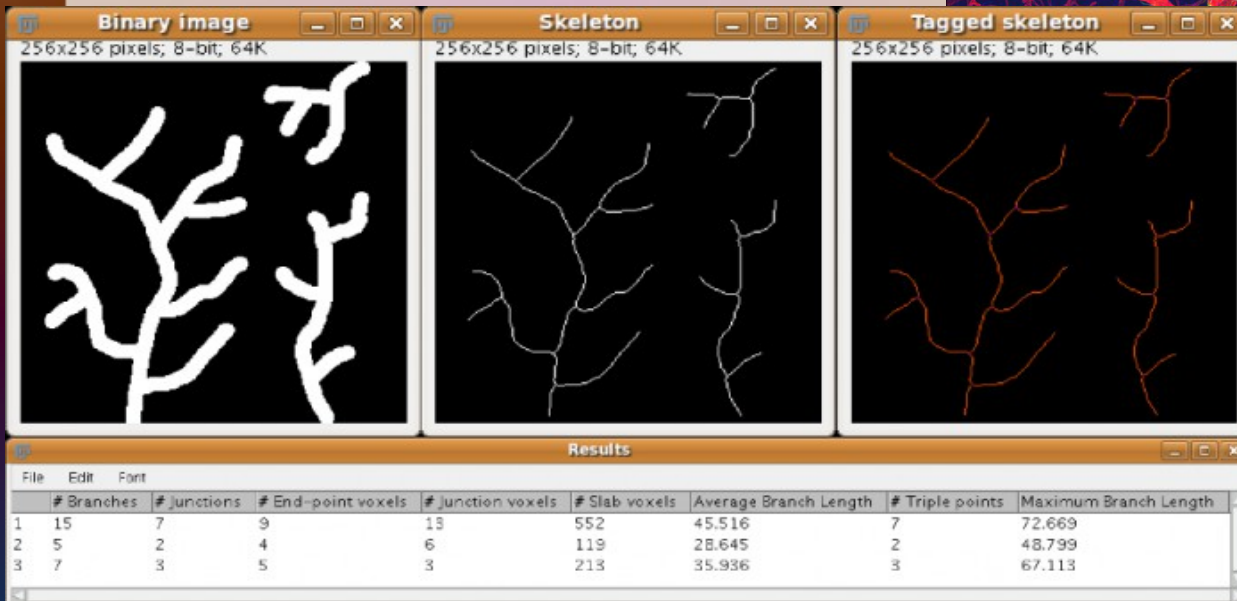
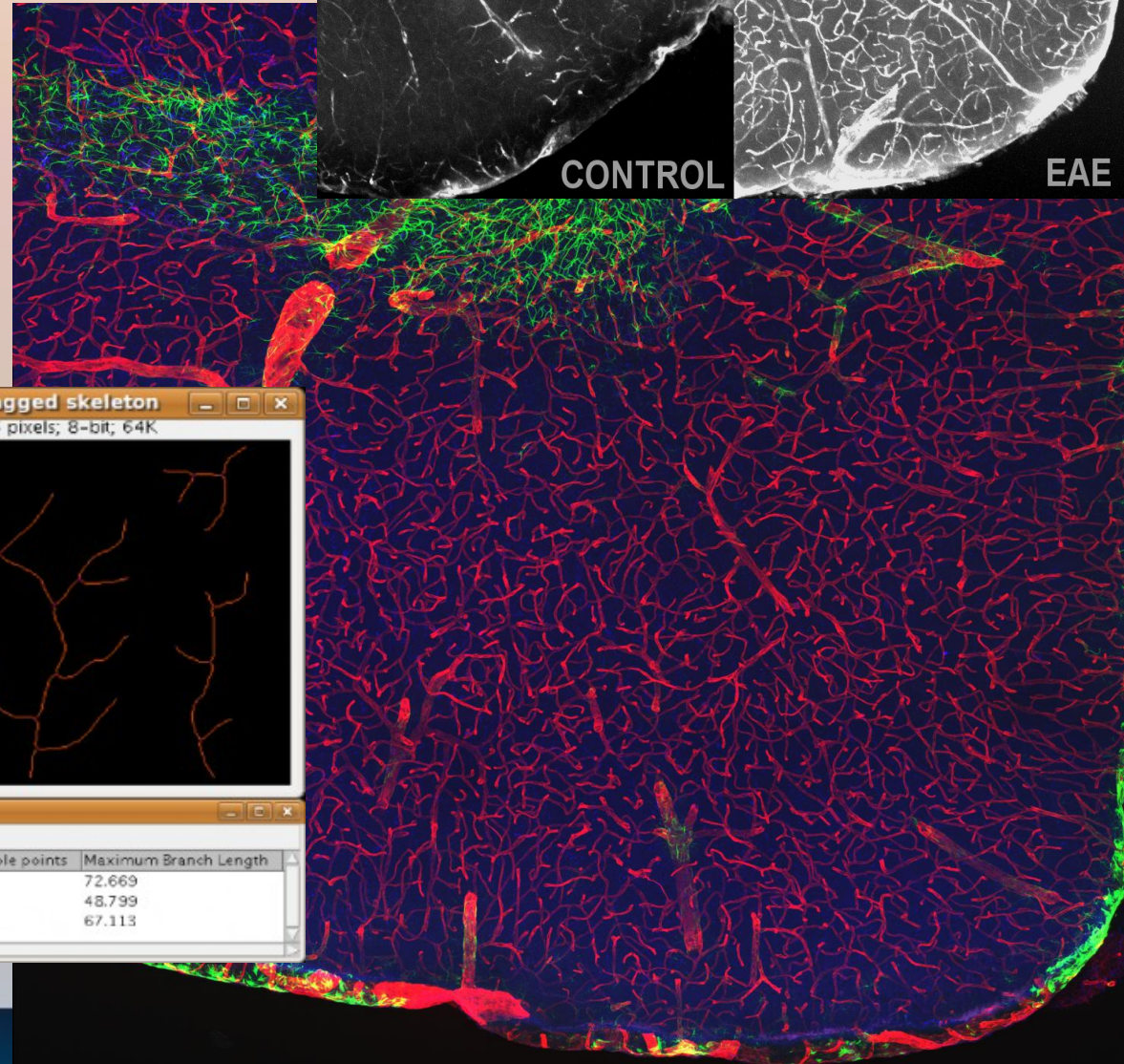
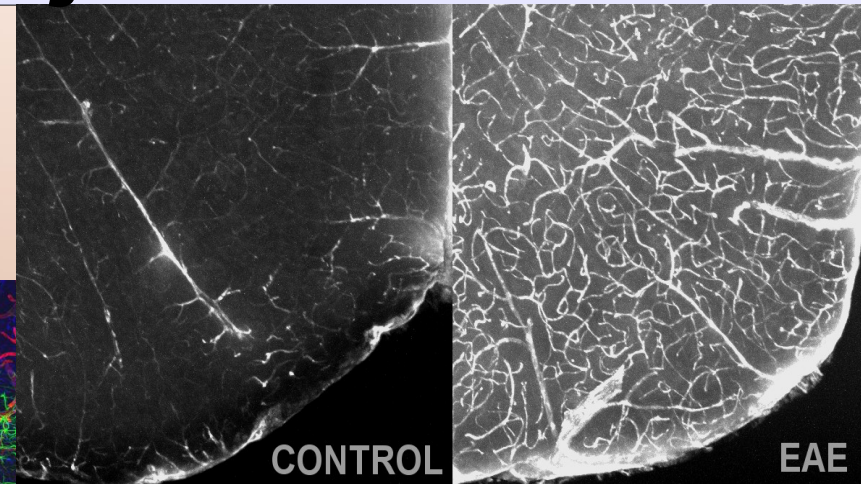


# Imaging Projects

## 4) Microvessel analysis

### Classify vessel segments in 3D

- number of segments
- surface area/volume
  - existing tools
- segment width
- vessel density
- identify branching patterns
  - existing plugins





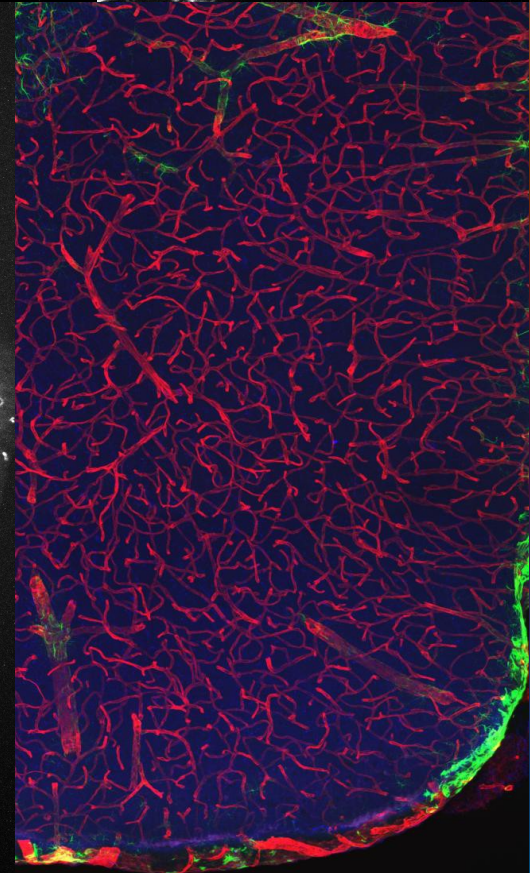
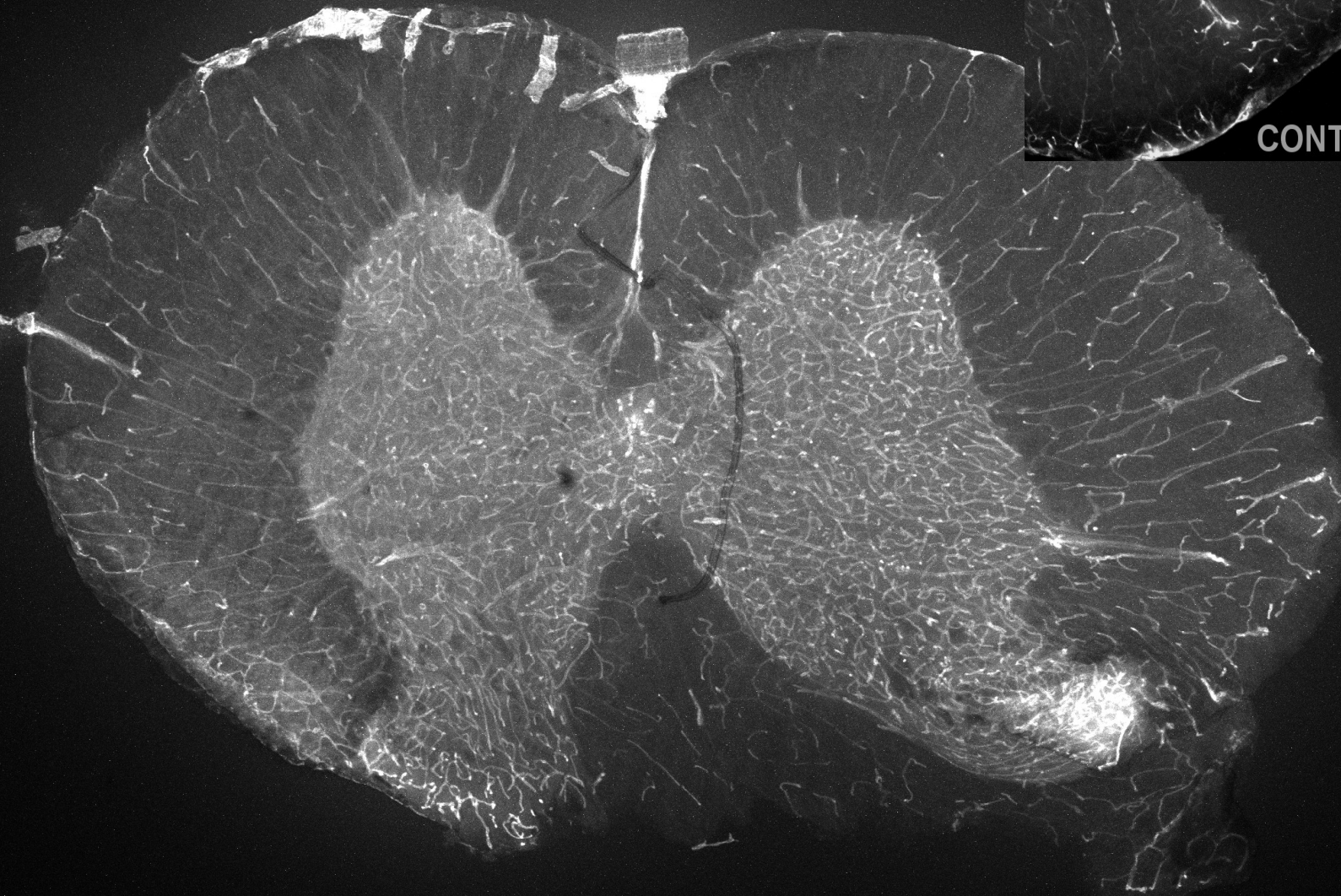
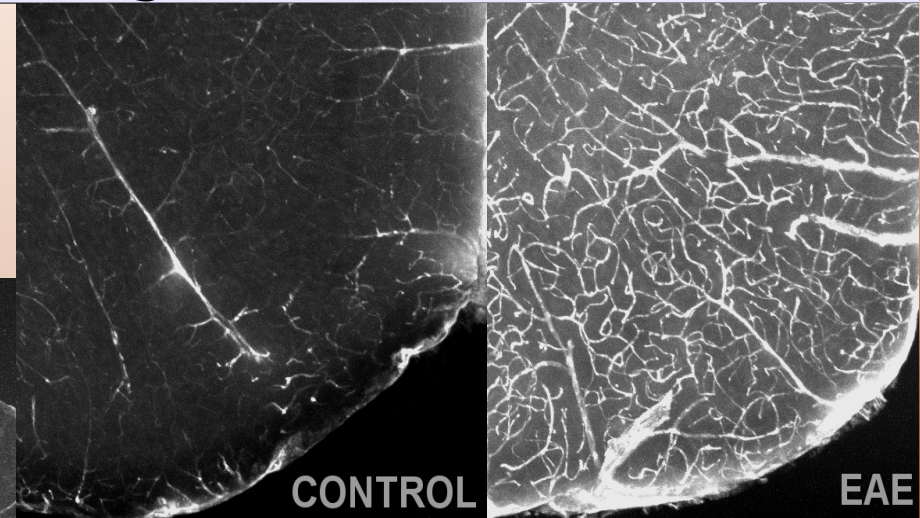
# Imaging Projects

## 4) Microvessel analysis

Test on Multiple datasets

Provide relevant metric output

User interface

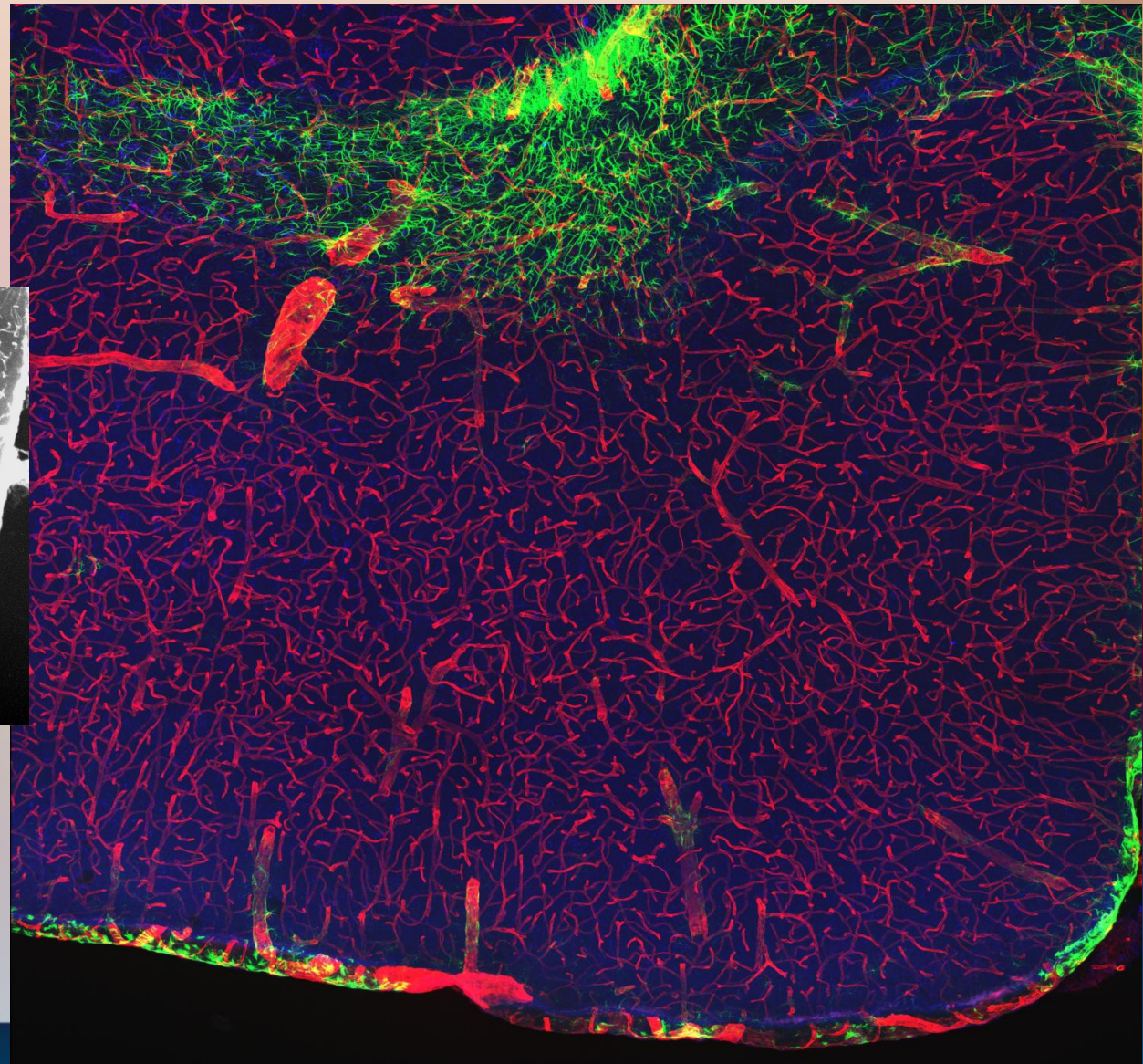
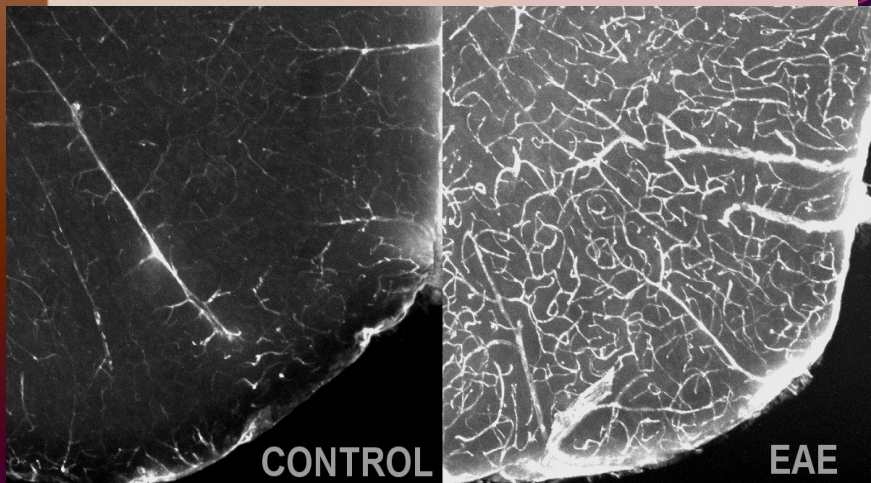




# Imaging Projects

## 4) Microvessel analysis

- segment microvessels in 3D
- classify vessel segments
- surface area/volume
- segment width, density
- identify branching patterns



# Imaging Projects

## MICROSCOPY

1) Background estimation

2) Tiled data reconstruction and correction

3) Astrocyte classification

4) Microvessel analysis

CONFOCAL MICROSCOPY

5) Neuron classification

6) Nucleus classification

3D ELECTRON MICROSCOPY

7) Cerebellum classification

MRI



# Imaging Projects

## 5) Neuron classification

Automatically Segment

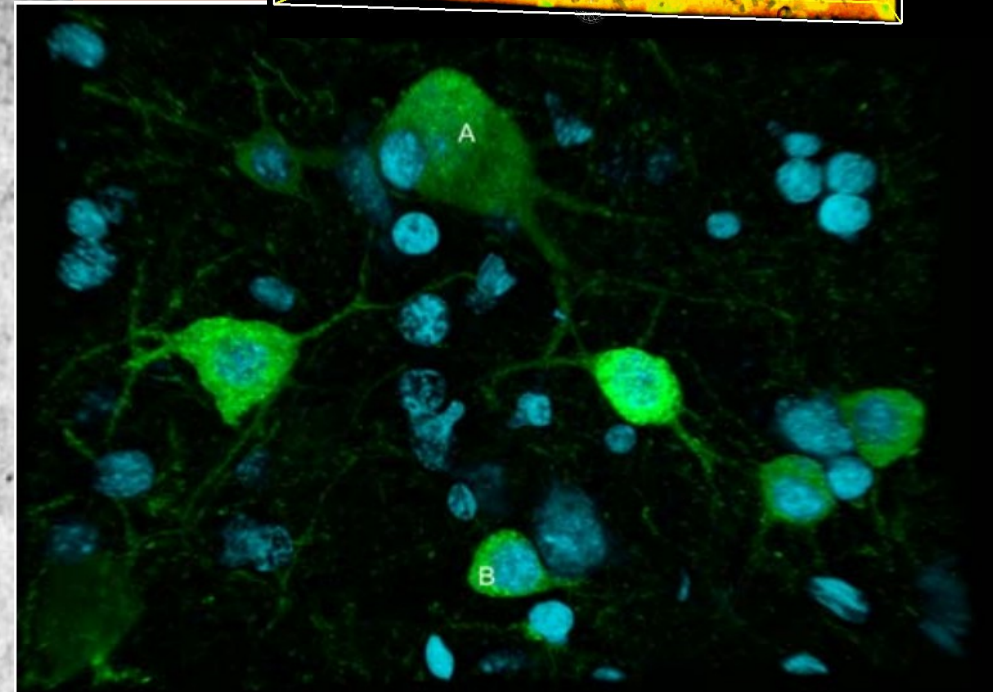
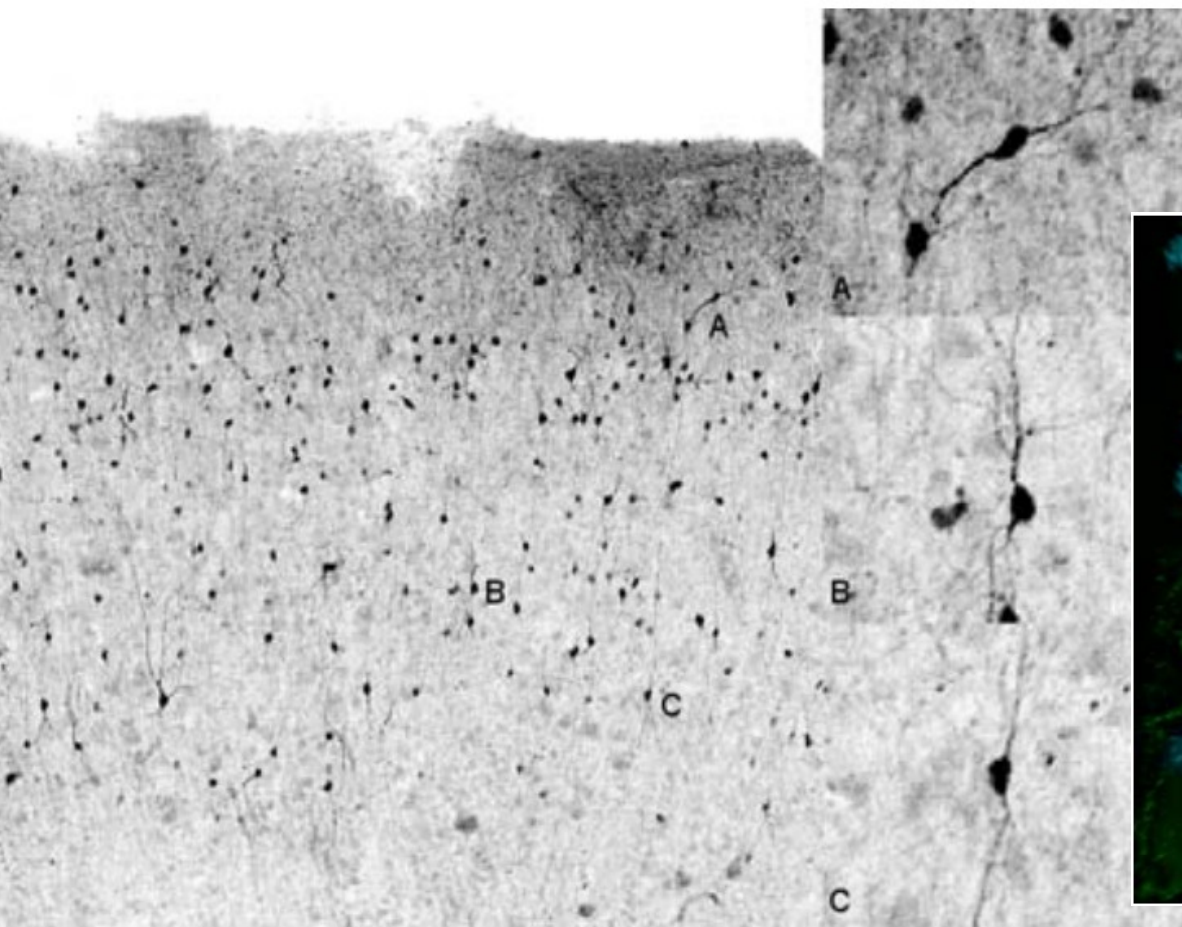
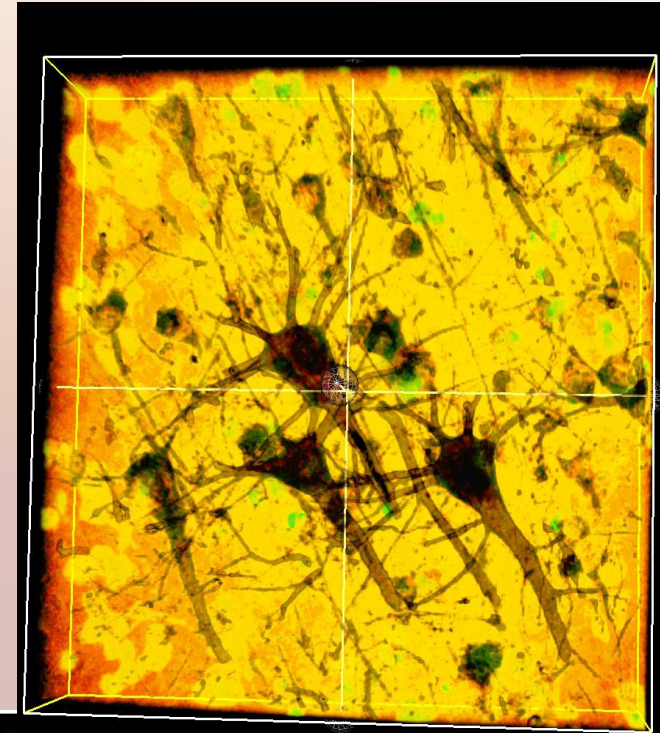
Quantify *Individual* neurons

Analyze neuronal branching patterns

Connectivity information

Analyze cellular location, clustering and proximity

Error, Other measures?...



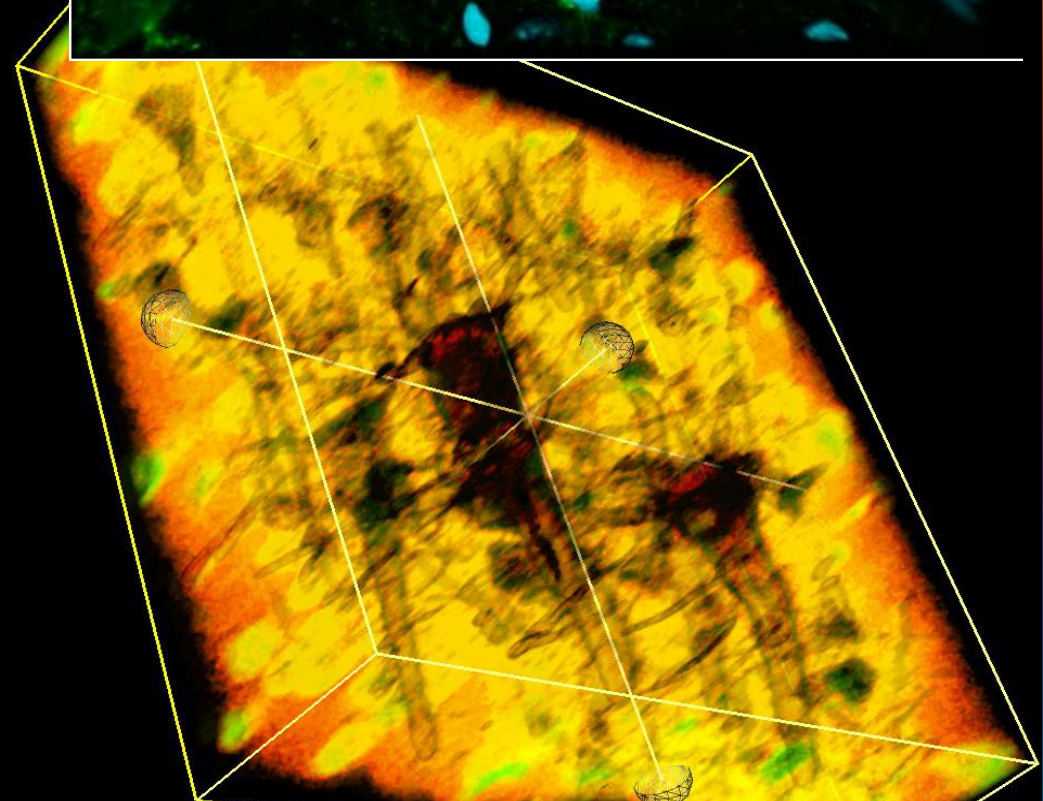
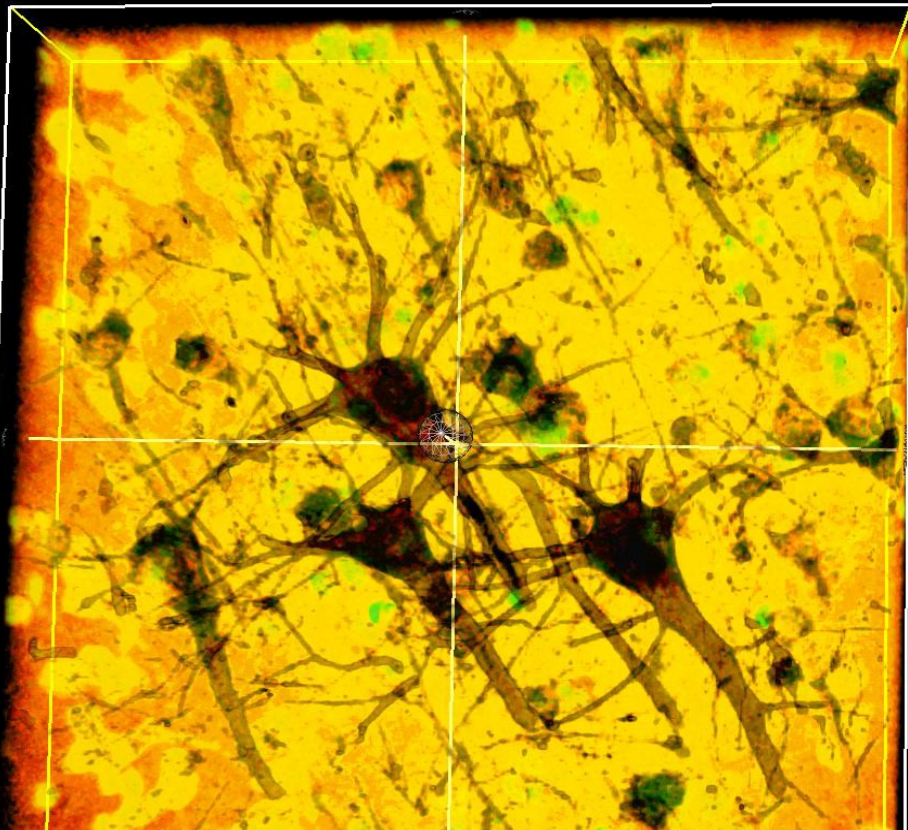
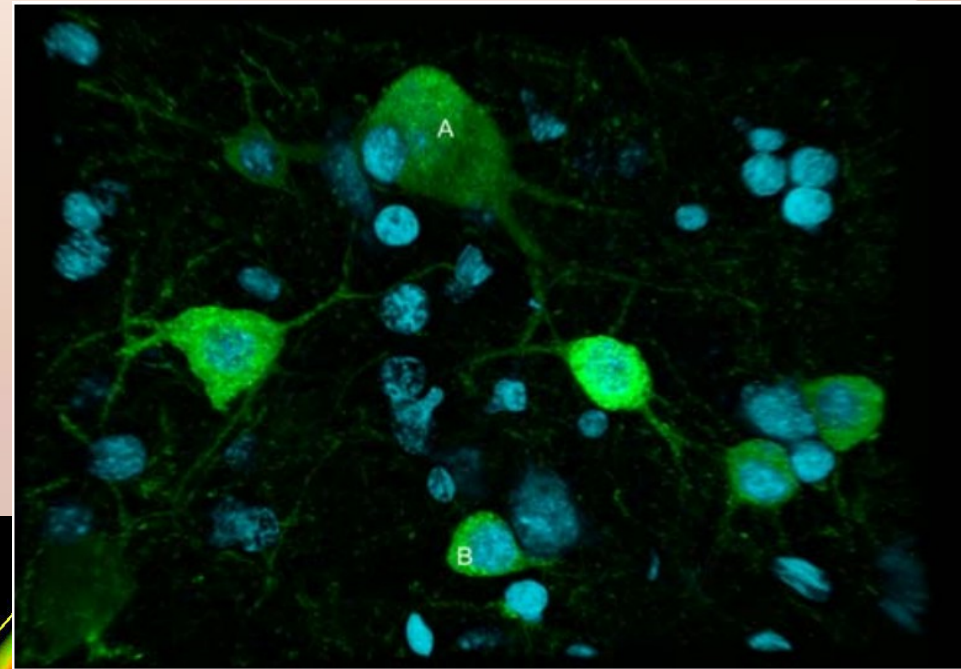


# Imaging Projects

## 5a) Neuron classification

### Automatically Segment neurons

- intelligent thresholding*
- resistant to partial staining*
- transected segments, adjacent cells?*
- seed points?*
- extendable to larger images*
- staining inconsistencies?*





# Imaging Projects

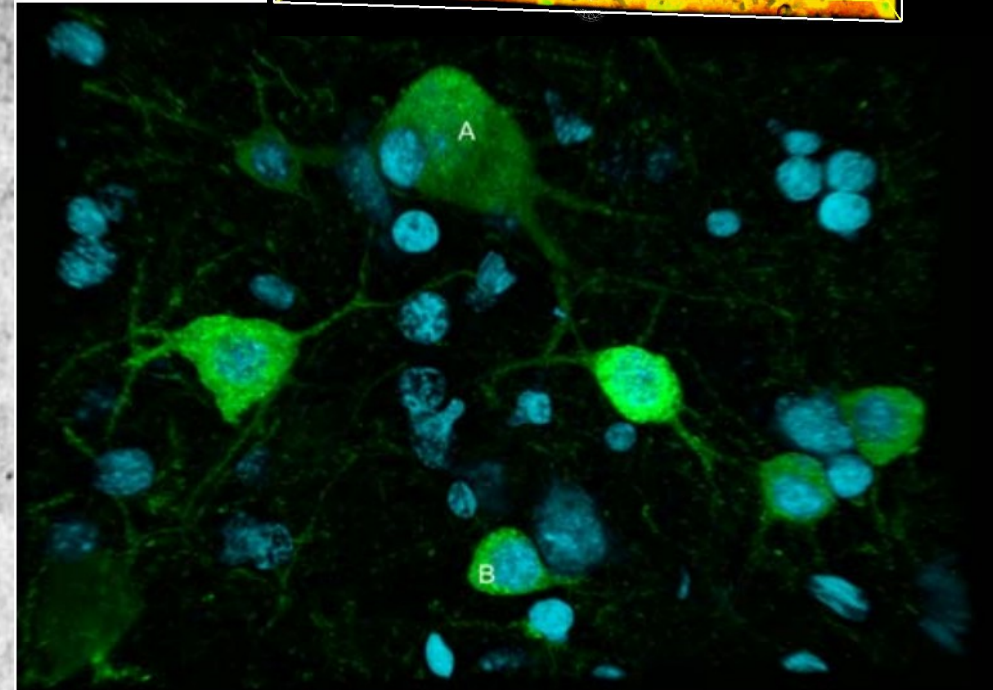
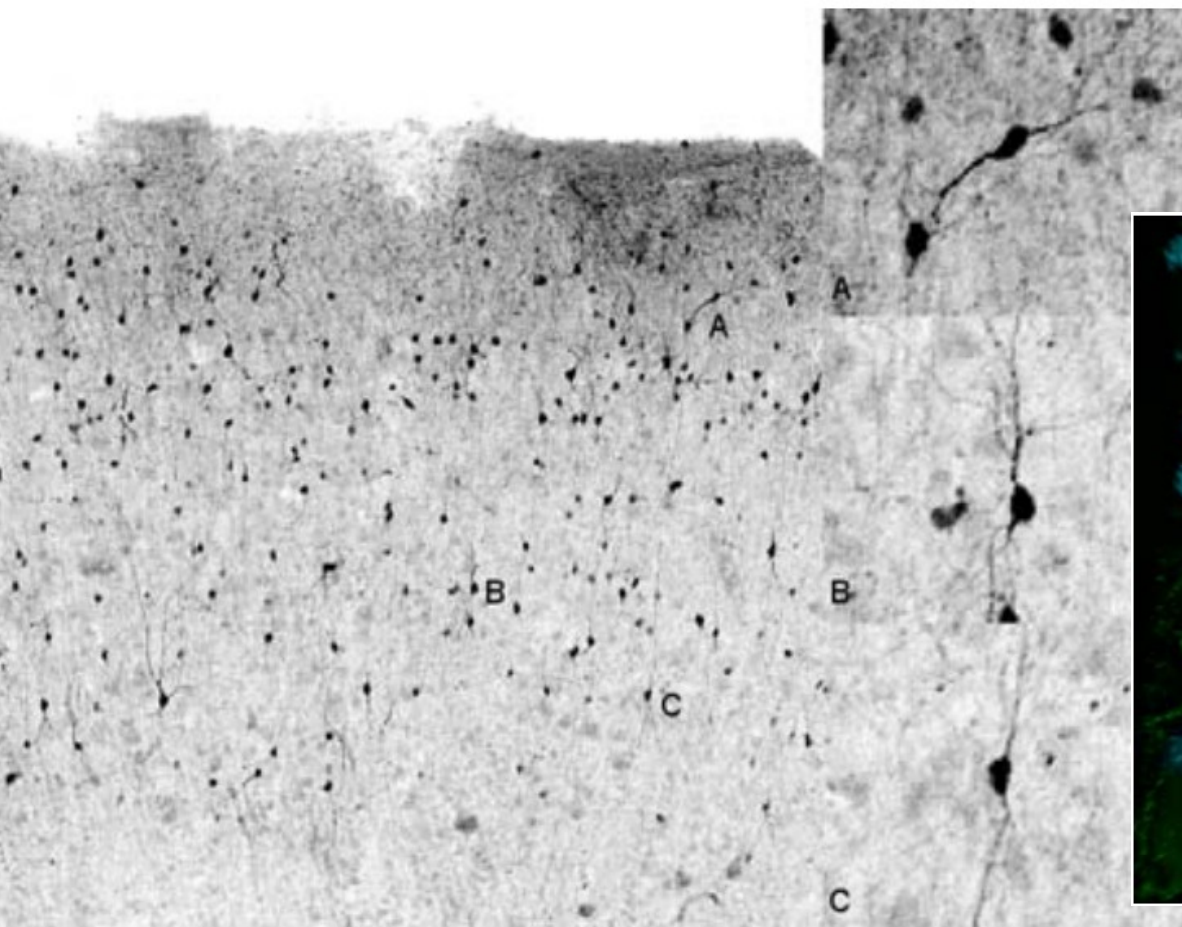
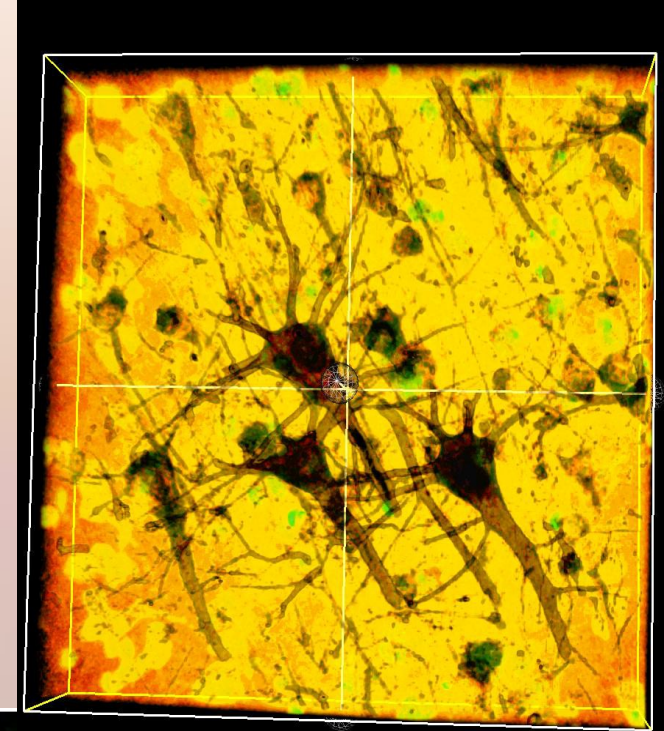
## 5a) Neuron classification

Quantify *Individual* neurons

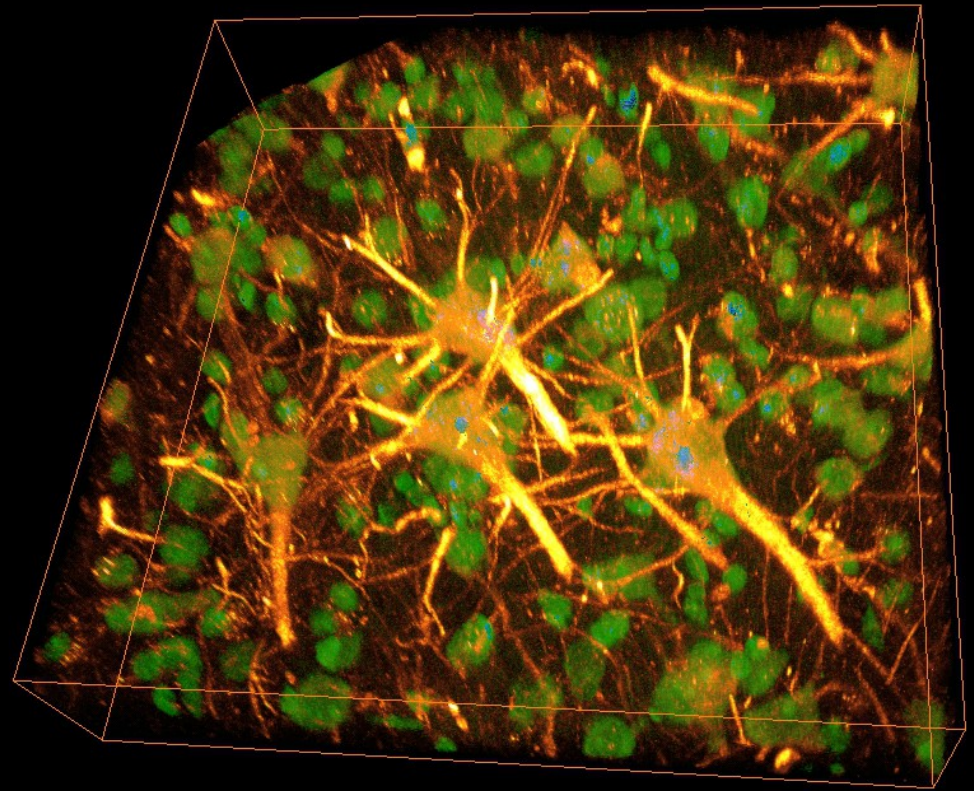
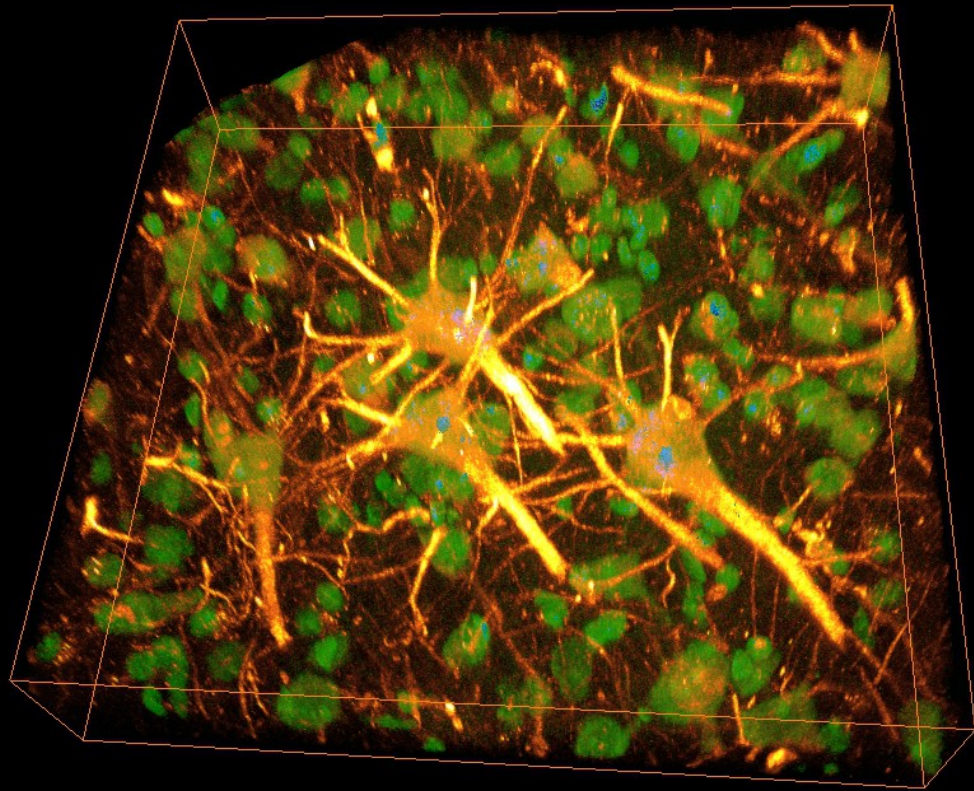
-volume, surface area

Segment multiple cells from larger images

2D vs. 3D?



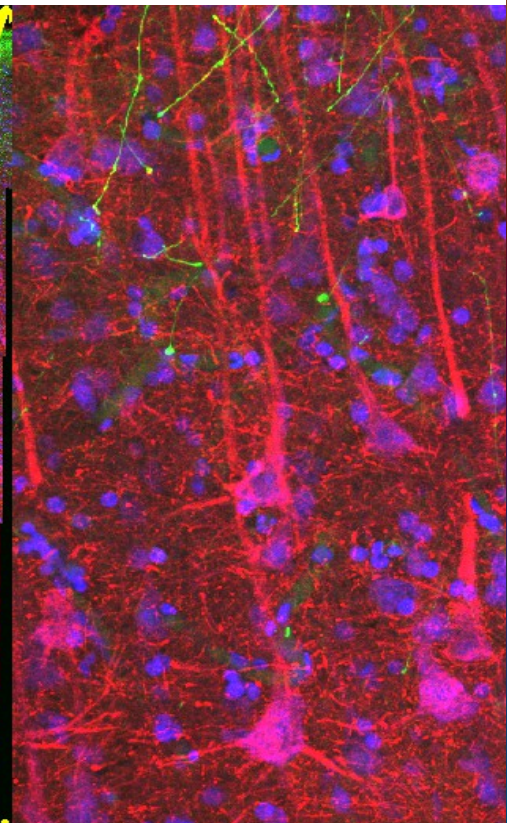
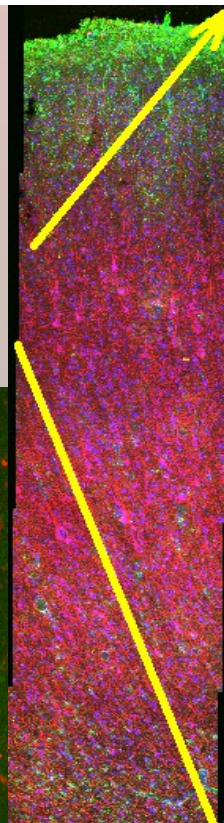
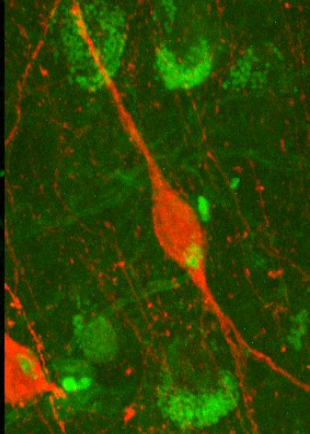
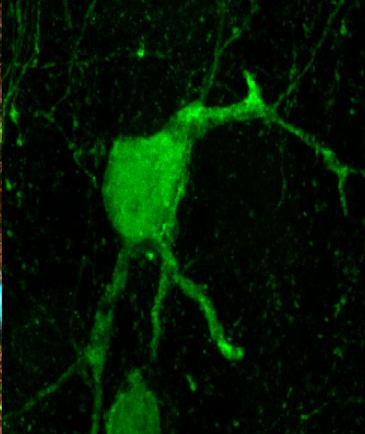
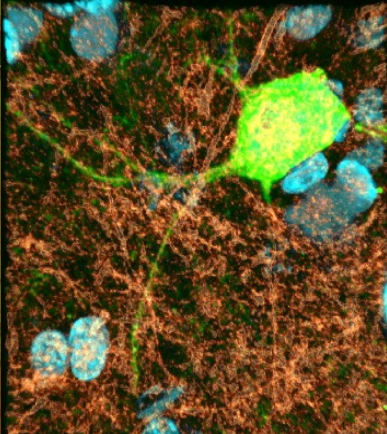
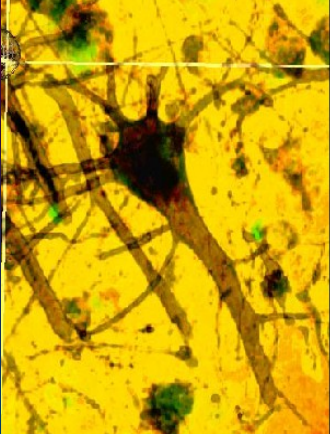
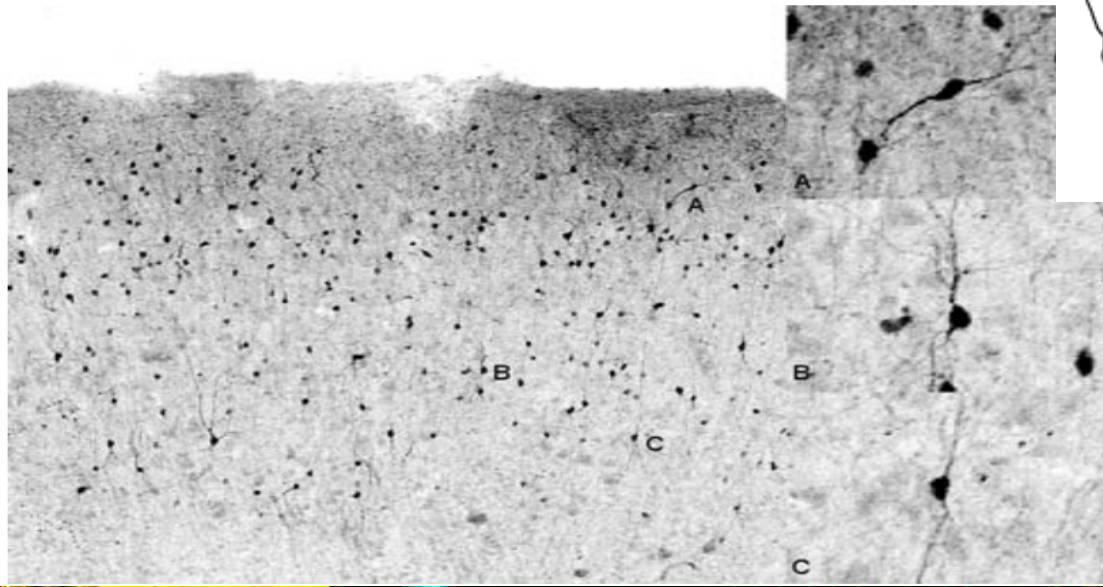
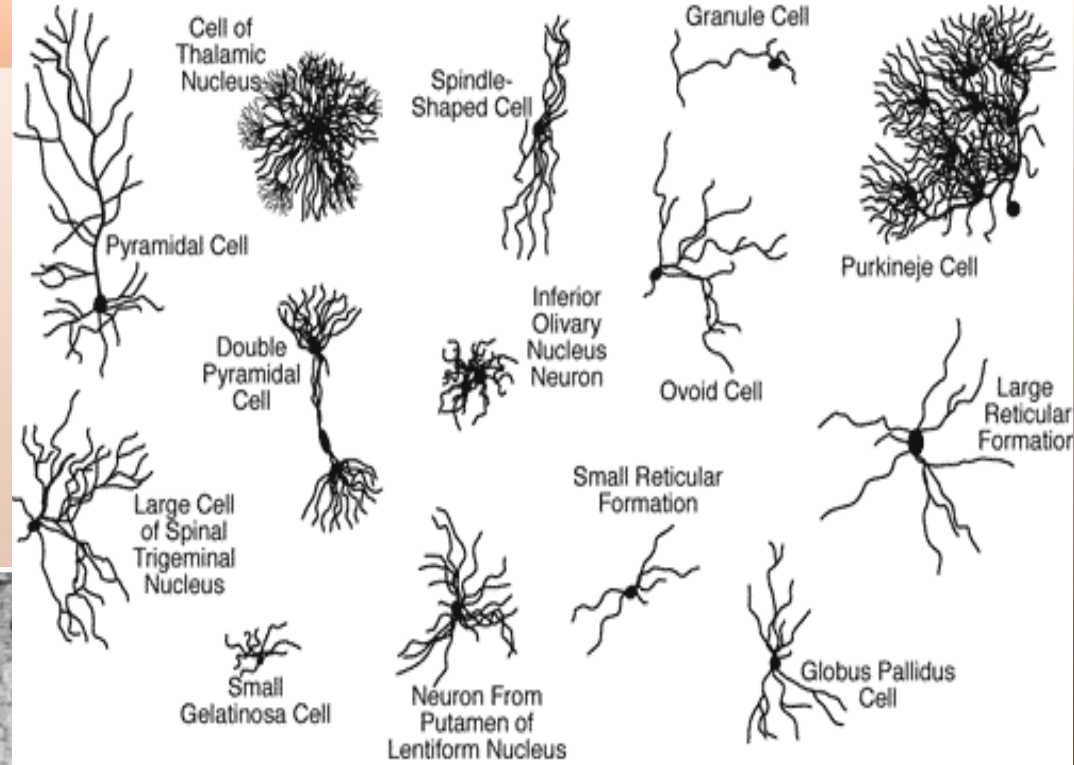
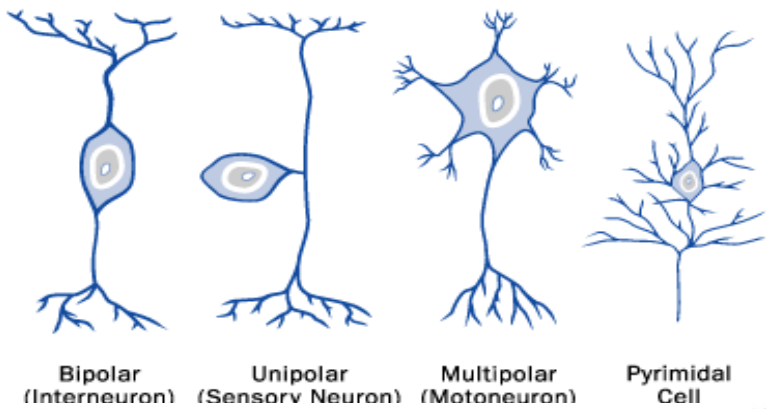






# Types of Neurons

## Basic Neuron Types



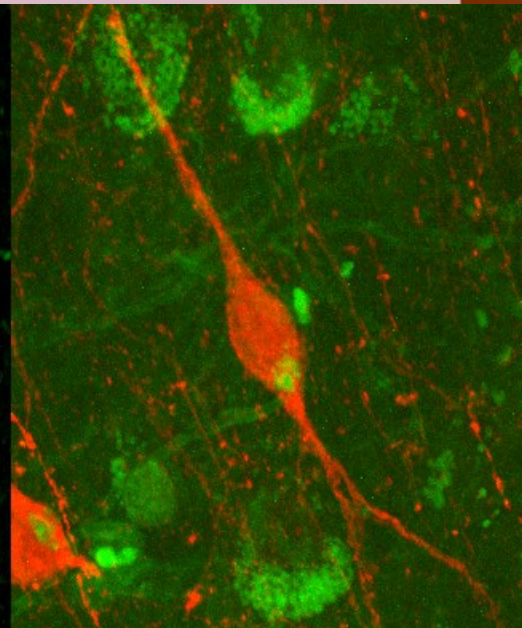
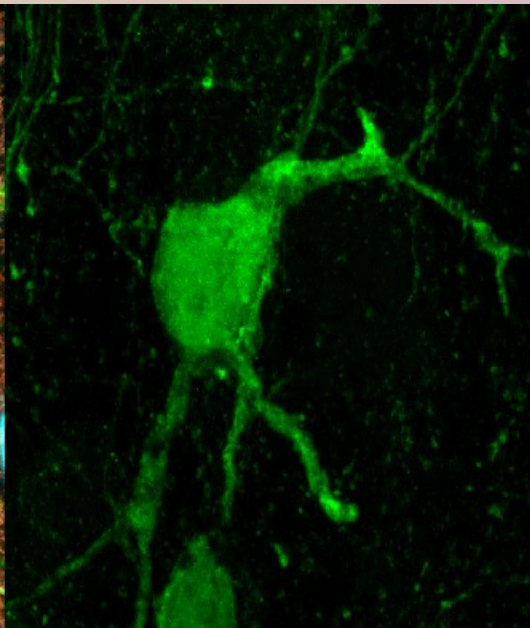
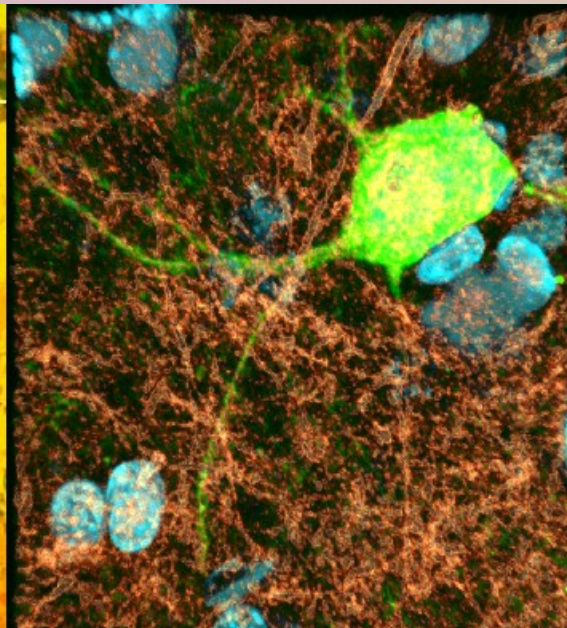
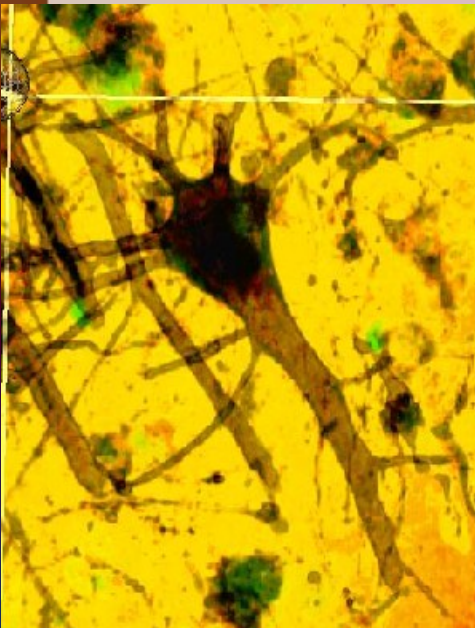
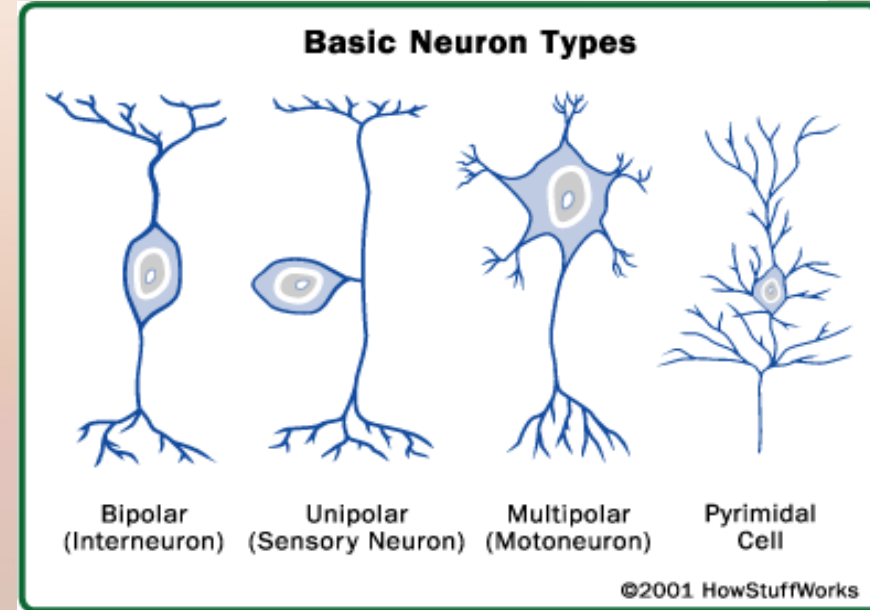


# Imaging Projects

## 5b) Neuron classification

Analyze neuronal branching patterns

- analyze segmented cells
- identify cell body
- number of projections (length/diameter/size)
- polarity (uni vs. multi?)
- number of bifurcations



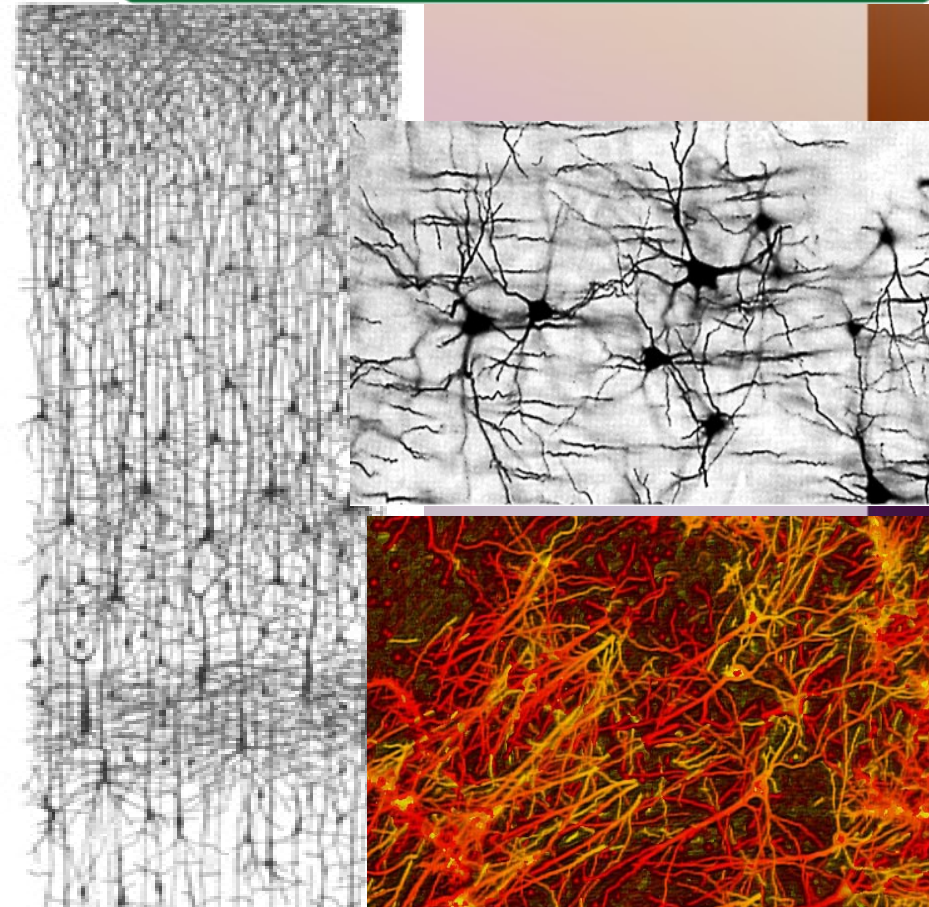
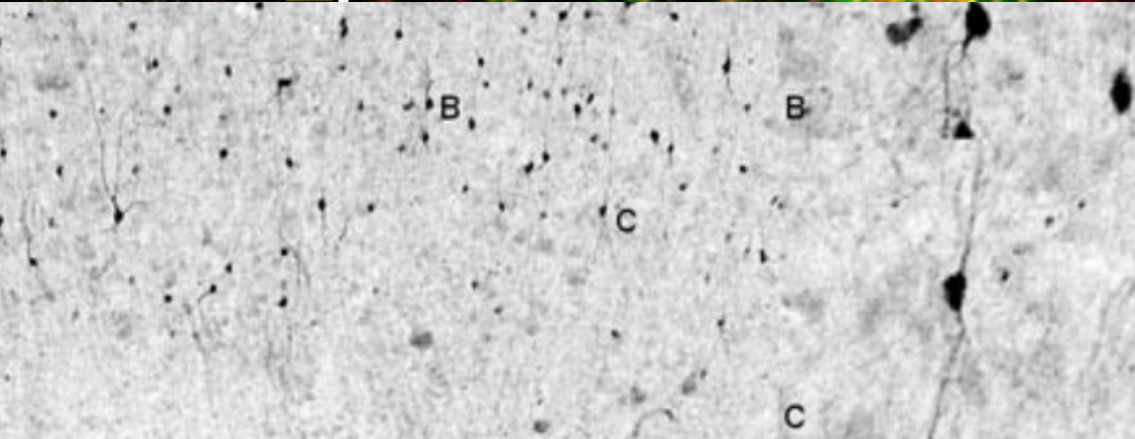
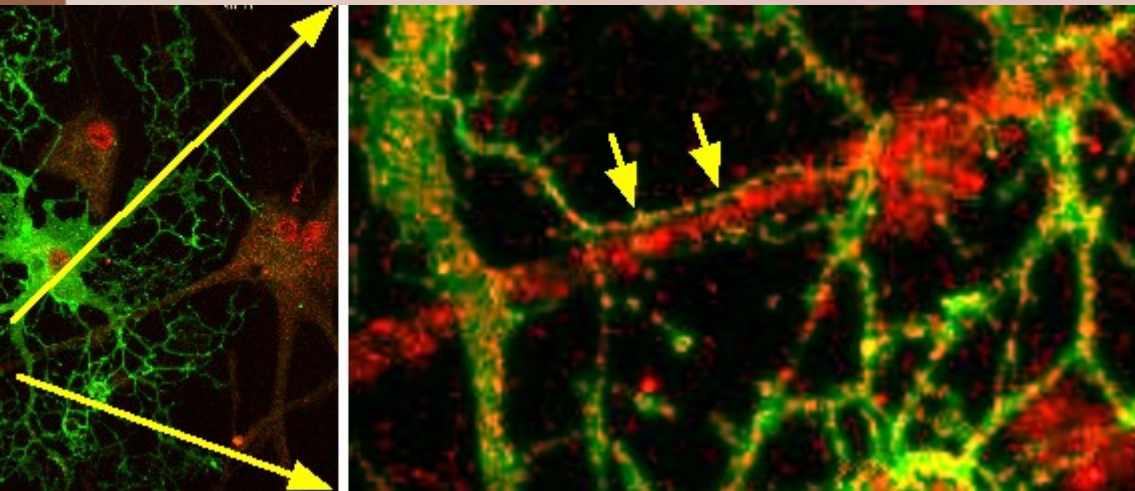
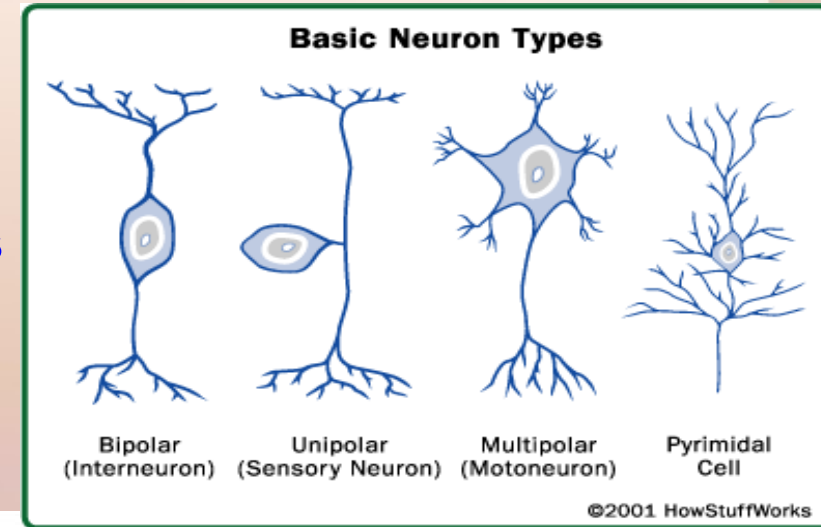


# Imaging Projects

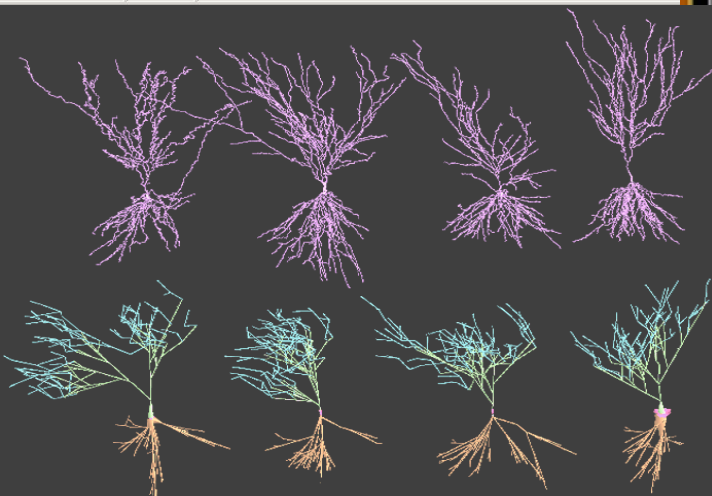
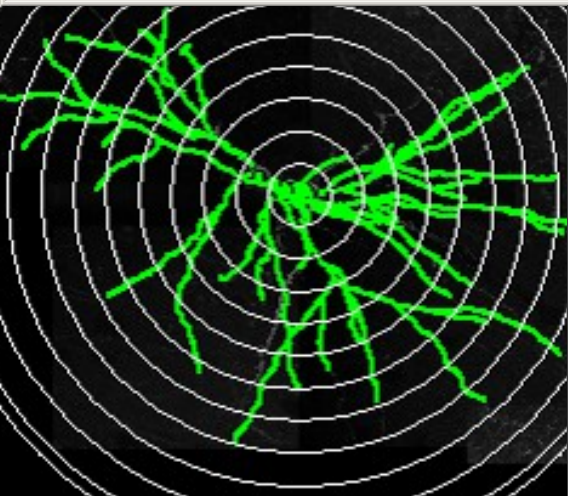
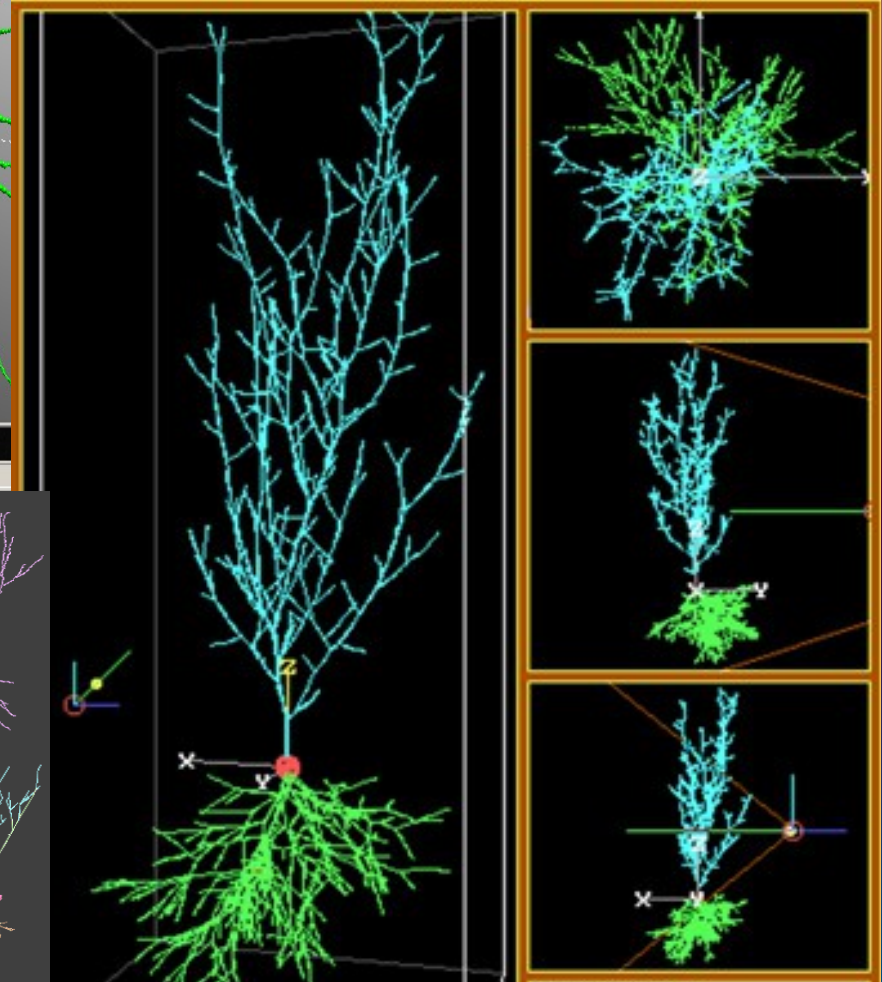
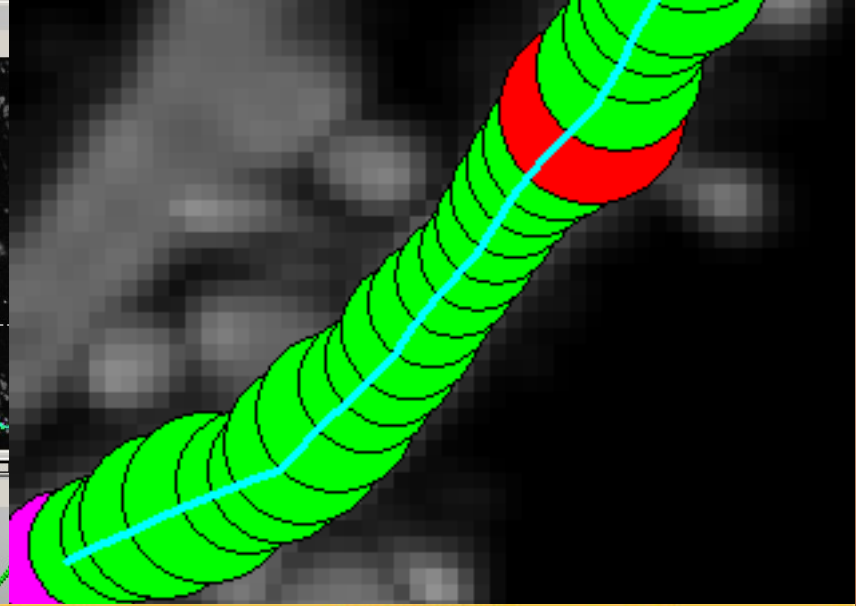
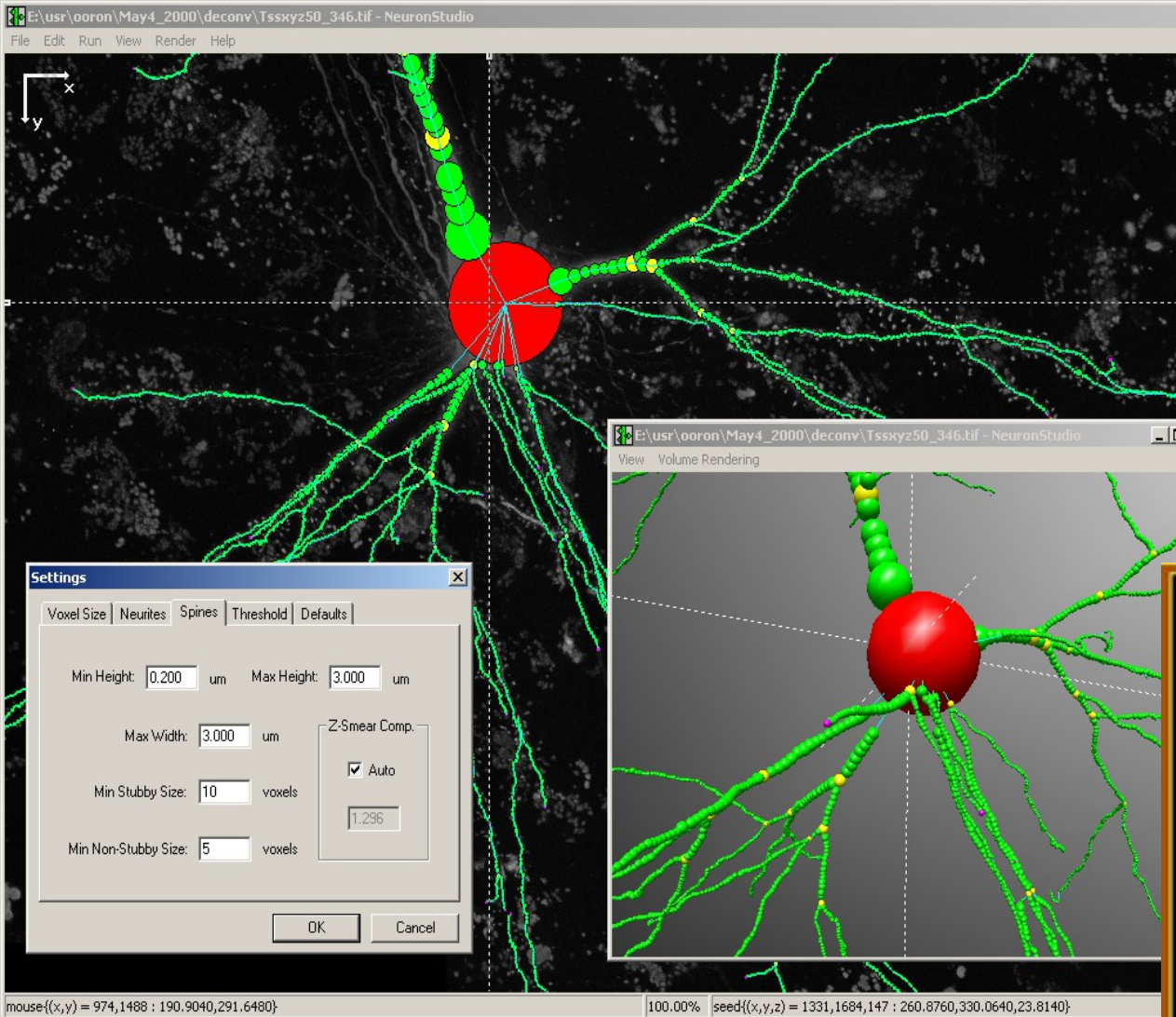
## 5b) Neuron classification

Analyze neuronal connectivity

- cell location
- clustering and proximity to other cell types
- cell contacts / synapses
- combine information to define networks
- use existing tools?









# Existing Tools

## DATABASES

[Cell Centered Database \(CCDB\),](#)

[SenseLab Project](#)

[L-Neuron](#)

[Neuromorph](#)

[brainmaps.org](#)

[Neuroscience Information Framework \(NIF\)](#)

[Connectome Project .](#)

## Free and Commercial Software

[cellprofiler](#)

[Xvoxtrace](#)

[Neurolucida](#)

[Imaris](#)

[Amira](#)

[SSECRETT and NeuroTrace](#)

## ImageJ/FIJI

[NeuronJ , Simple Neurite Tracer](#)

[Find Connected Regions](#)

[Neuron Morphology , TrakEM2](#)

[Simple Interactive Object Extraction \(SIOX\)](#)

[Advanced Weka Segmentation](#)

[Trainable Segmentation plugin .](#)

# Imaging Projects

## MICROSCOPY

1) Background estimation

2) Tiled data reconstruction and correction

3) Astrocyte classification

4) Microvessel analysis

CONFOCAL MICROSCOPY

5) Neuron classification

6) Nucleus classification

3D ELECTRON MICROSCOPY

7) Cerebellum classification

MRI

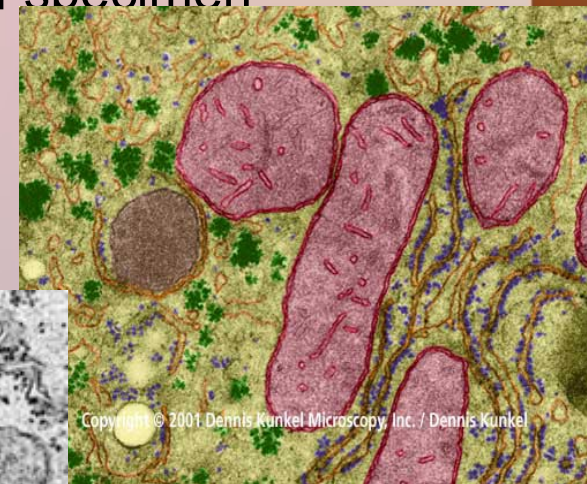
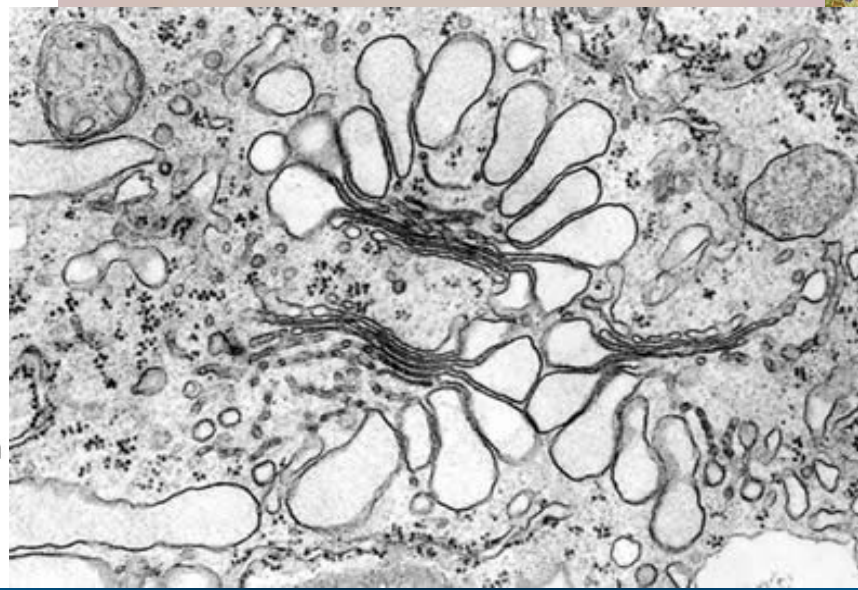
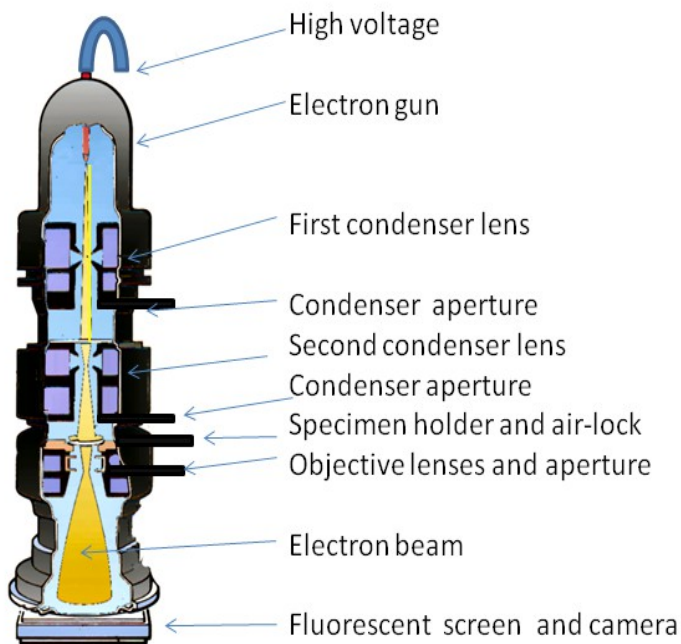


# Electron Microscopy

- uses a particle beam of electrons to illuminate the specimen and create a magnified image of it
- high magnification compared to light microscopy (1,000,000x vs 2000x)
- electromagnetic lenses control electron beam

## Transmission electron microscope (TEM) (.2 nm resolution)

- electron gun transmits electron beam through ultra thin specimen
- specimen is transparent as well scatters electrons
- transmitted beam carries information about structure of specimen
- beam focused on fluorescent screen, and captured



Transmission Electron Microscope

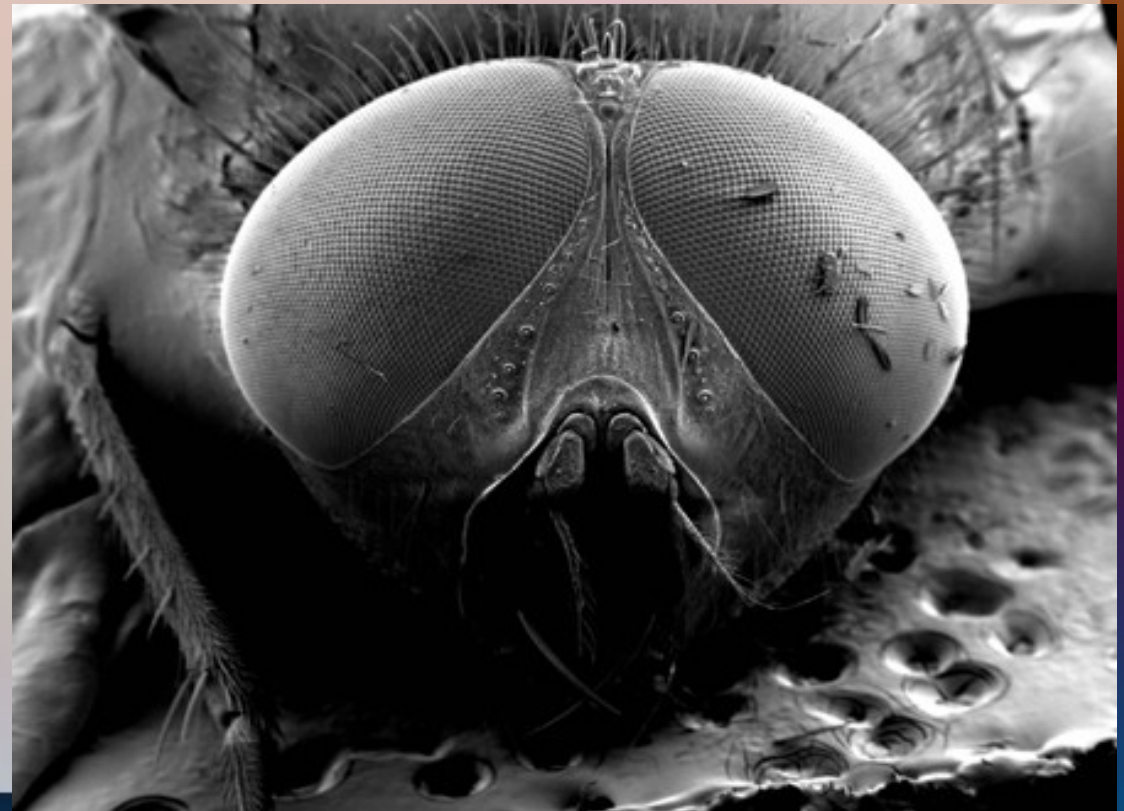
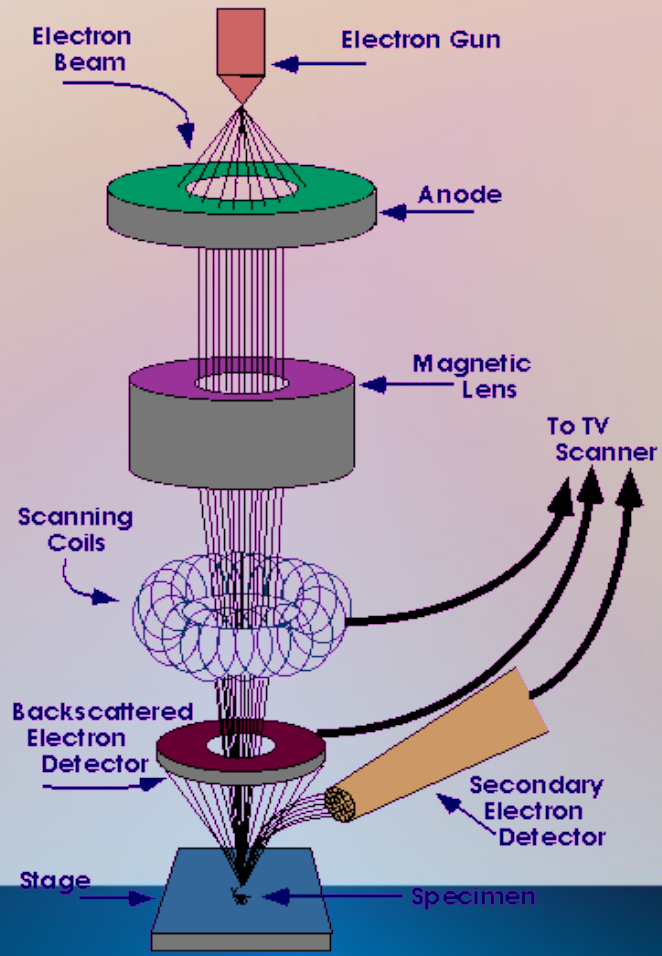




# Electron Microscopy

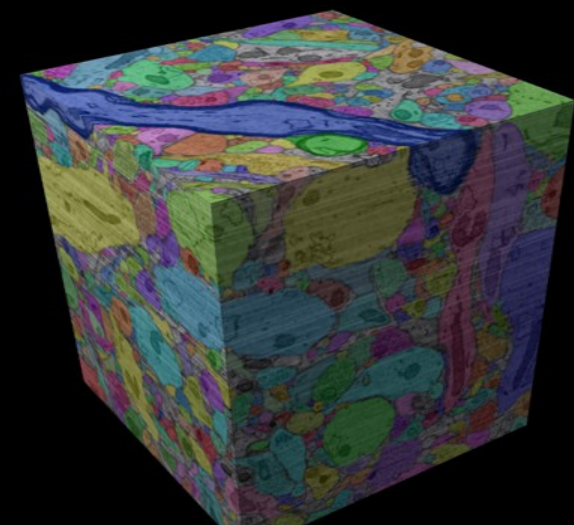
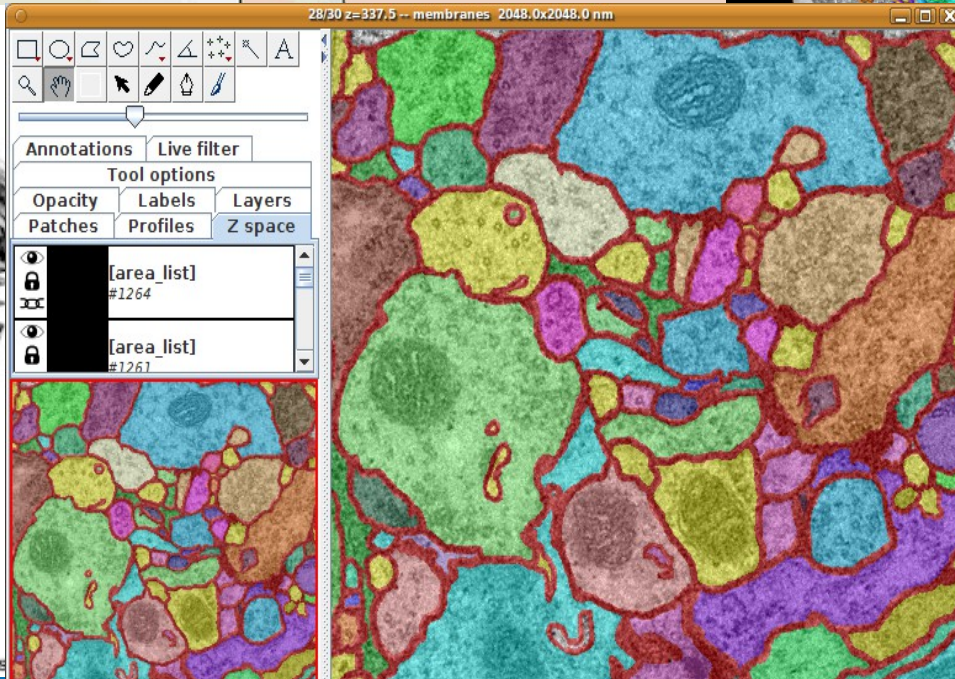
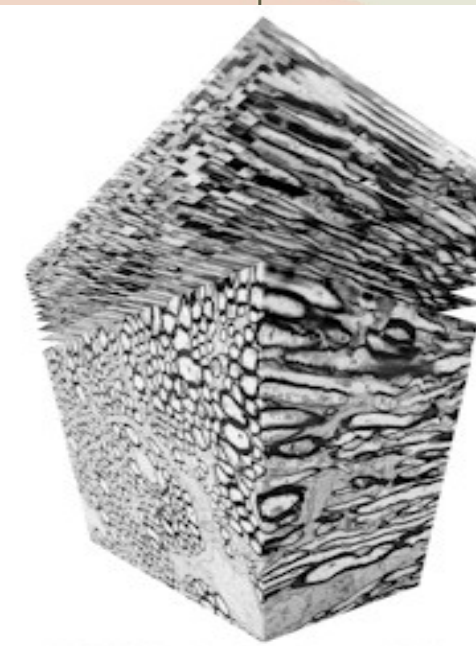
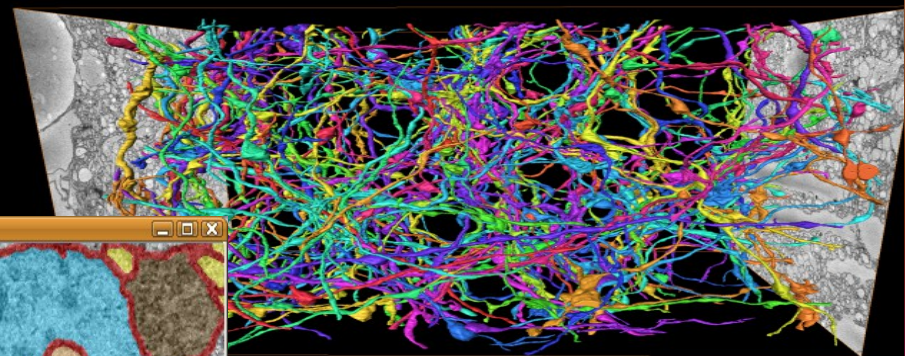
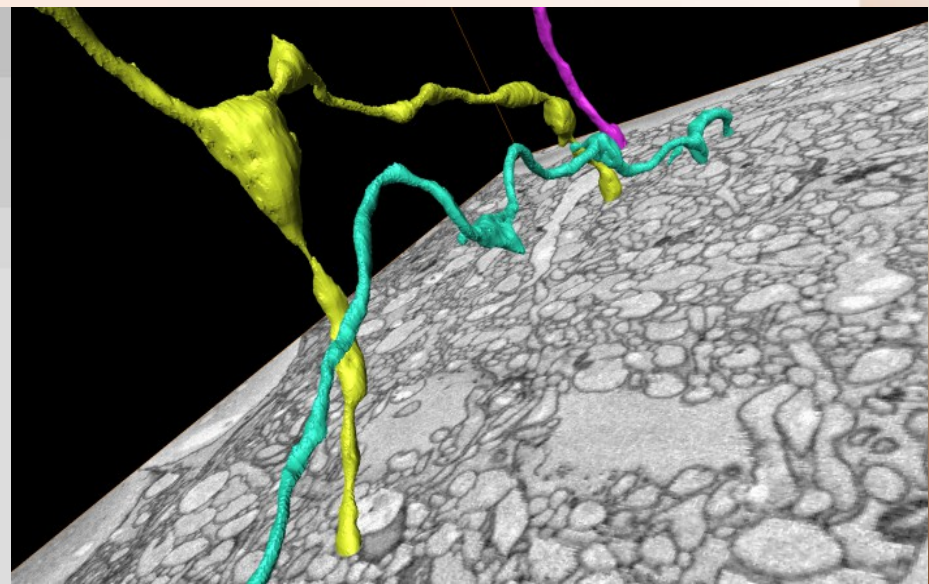
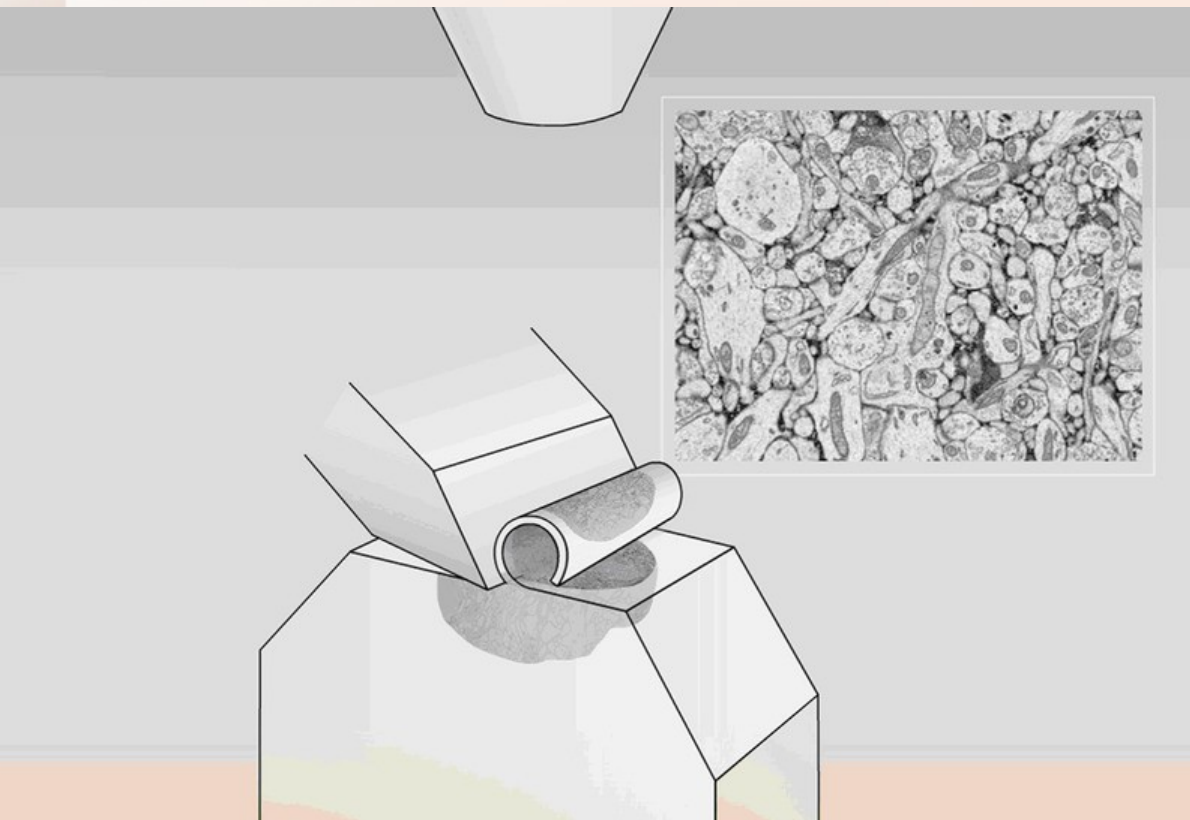
## Scanning electron microscope (SEM) (10 nm)

- focused beam scans an area of specimen
- beam loses energy and is converted to heat, secondary electrons, light or x-ray
- detector maps the intensity of the signals at the position of the beam
- depth of field provides a good 3D representation of the specimen





# Serial Blockface Scanning Electron Microscope

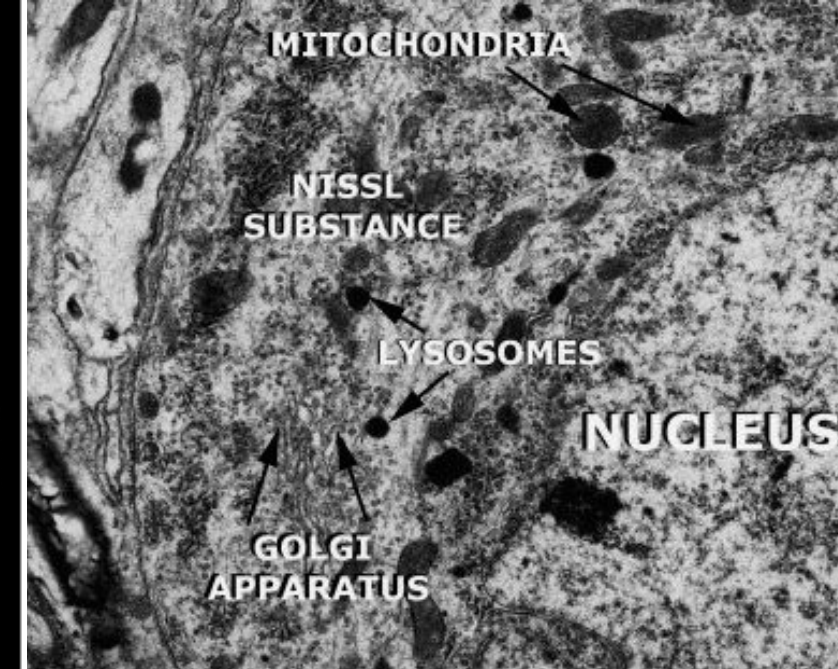
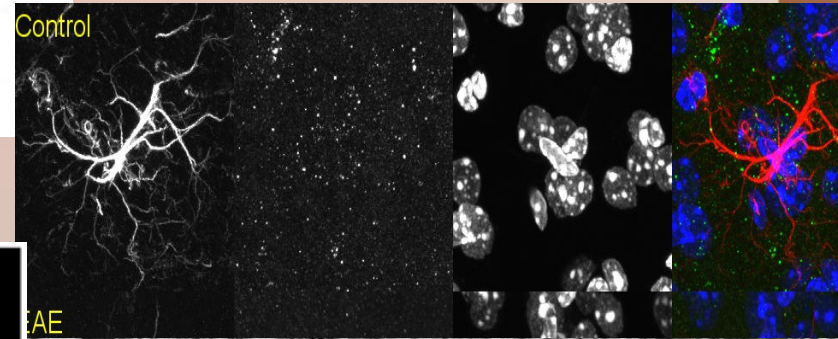
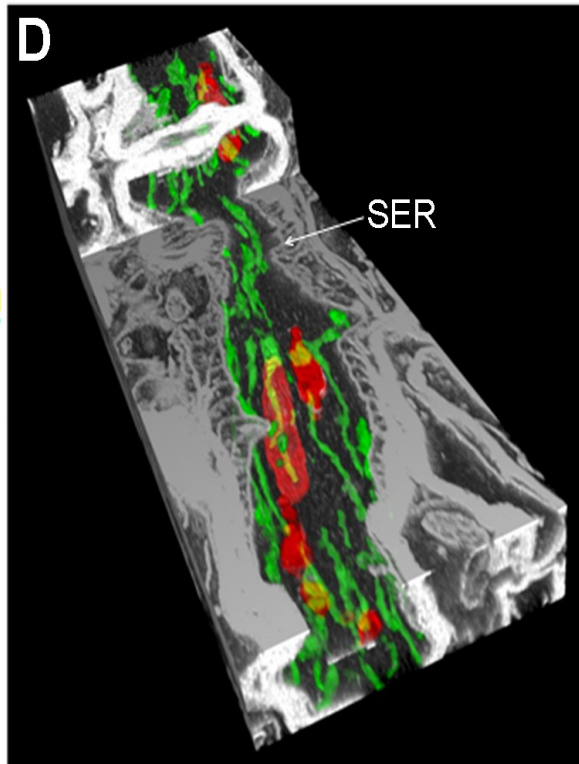
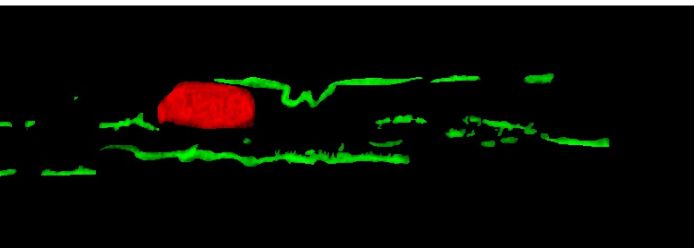
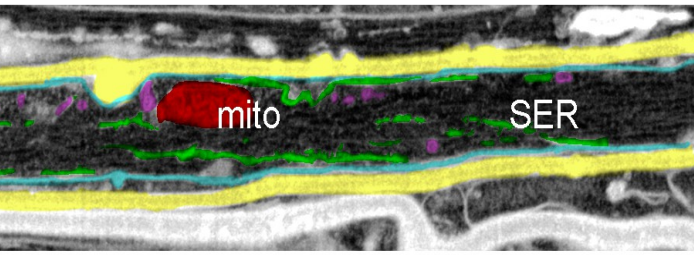
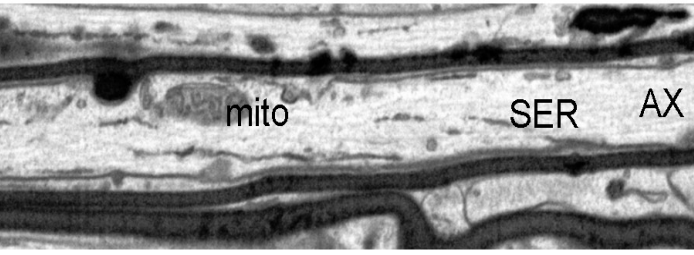
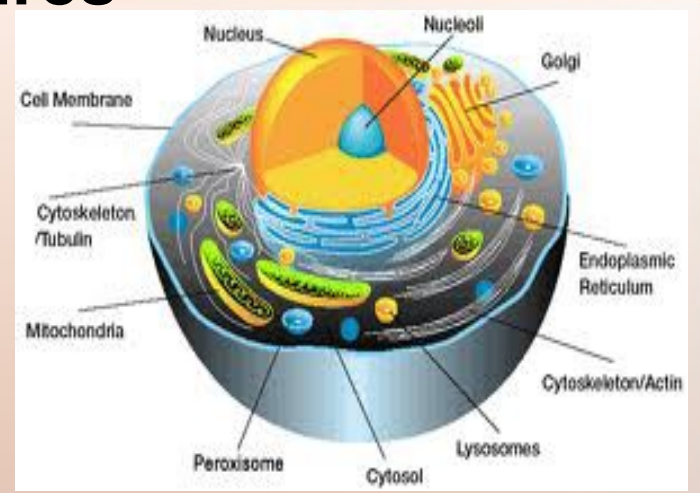
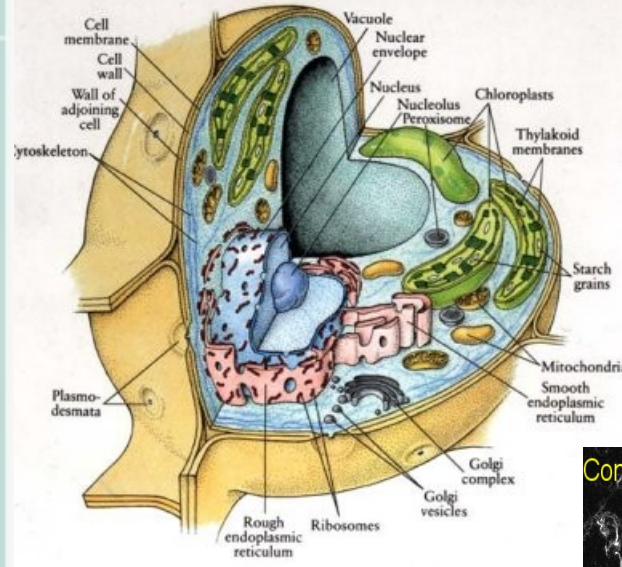
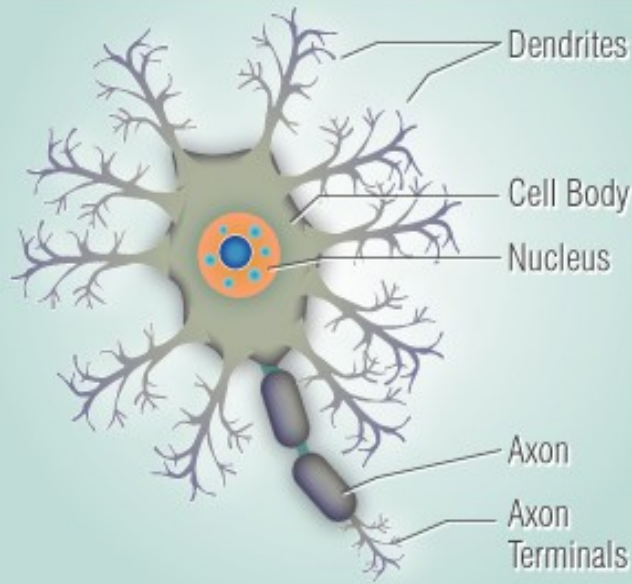


3D-EM Stack of Myelinated Fibres

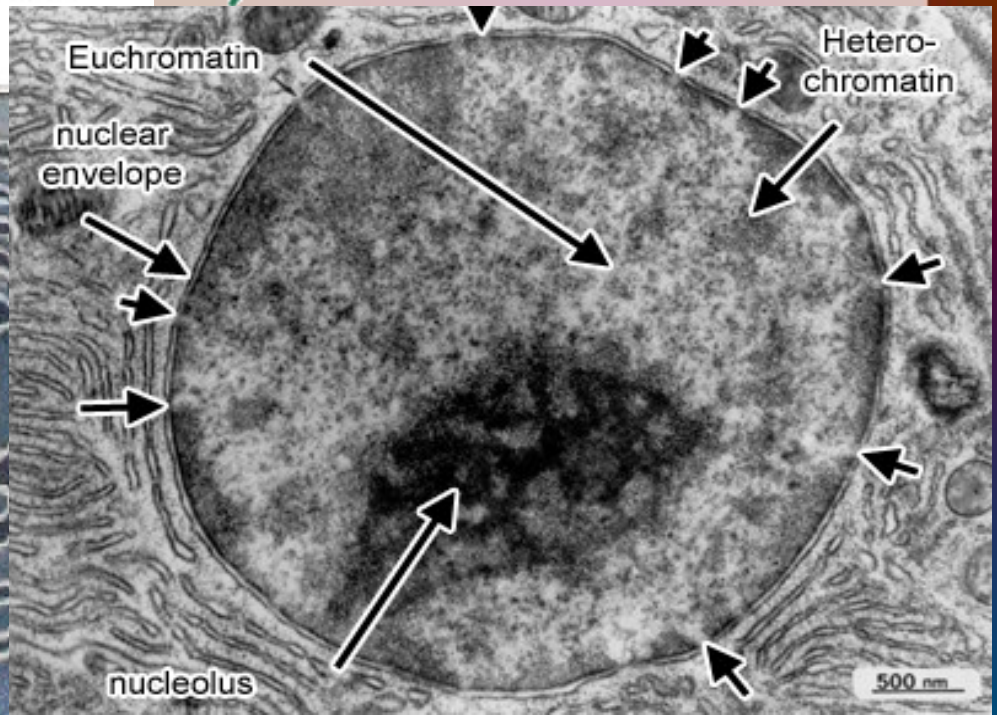
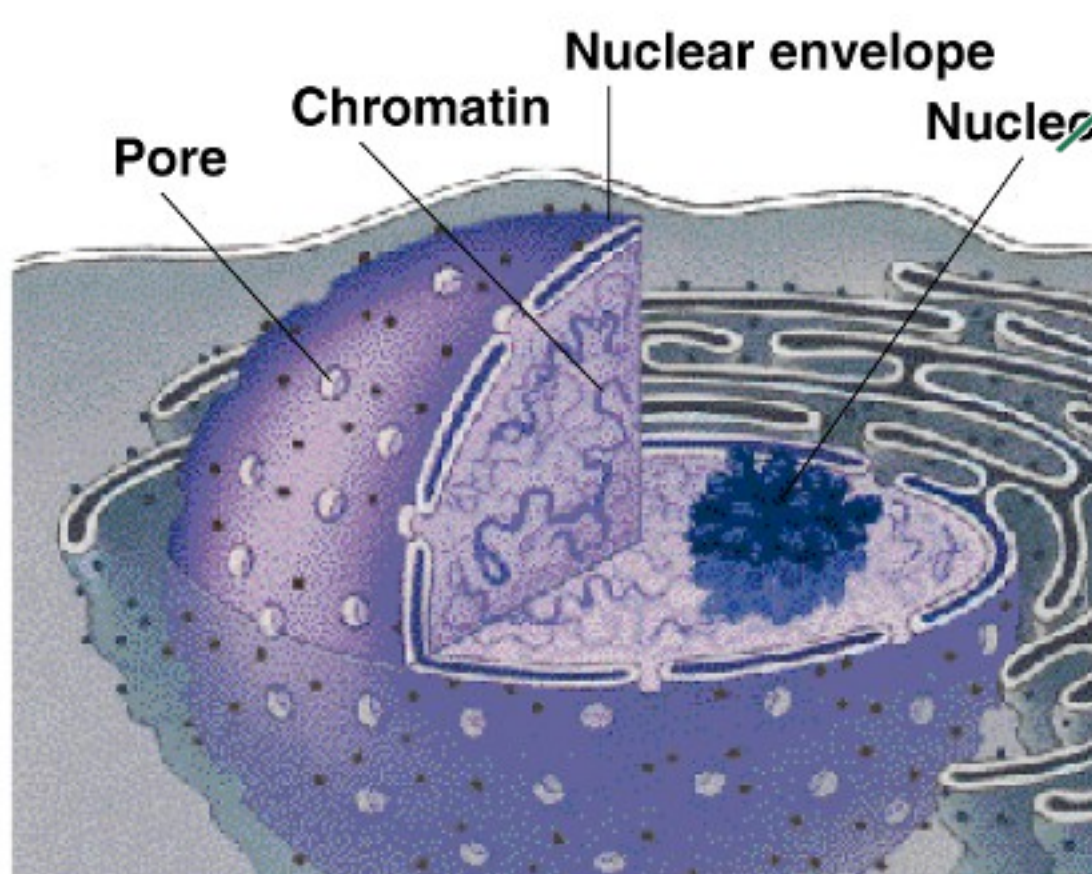
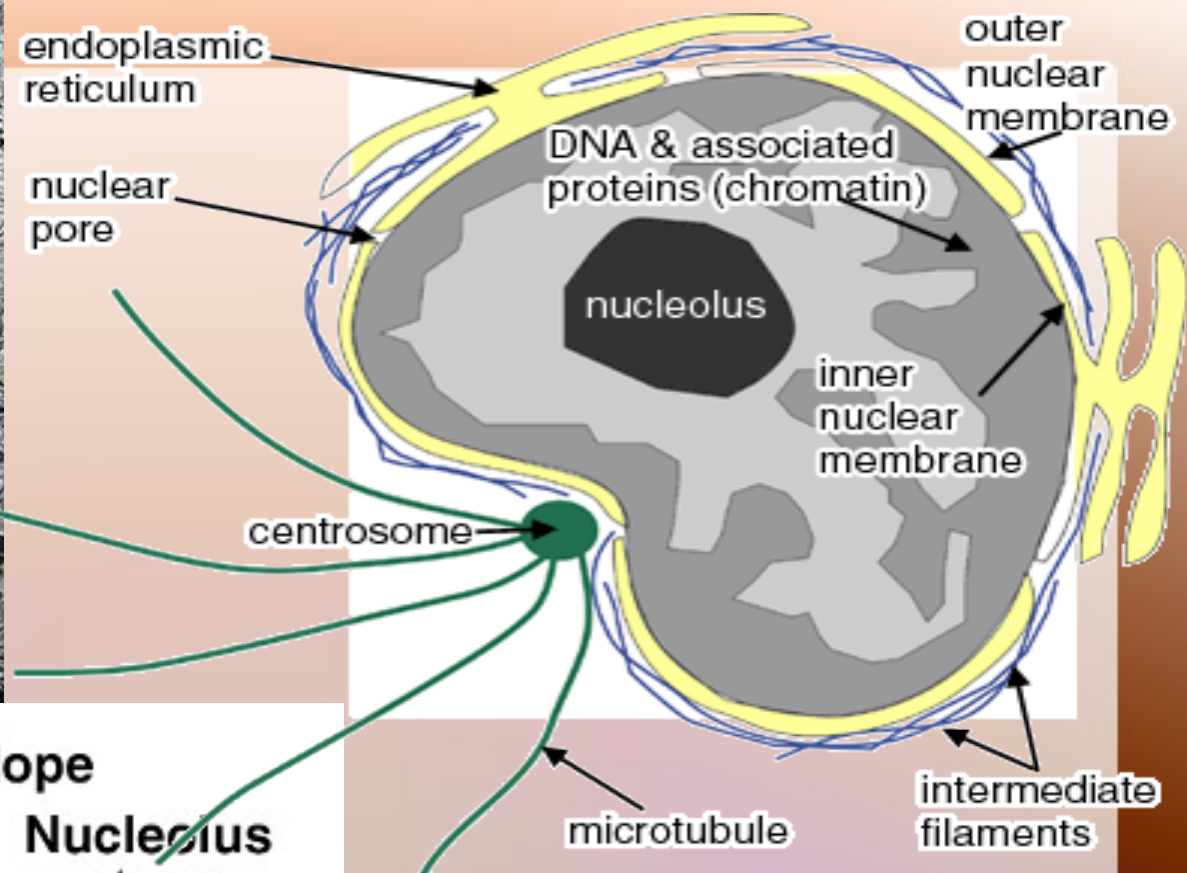
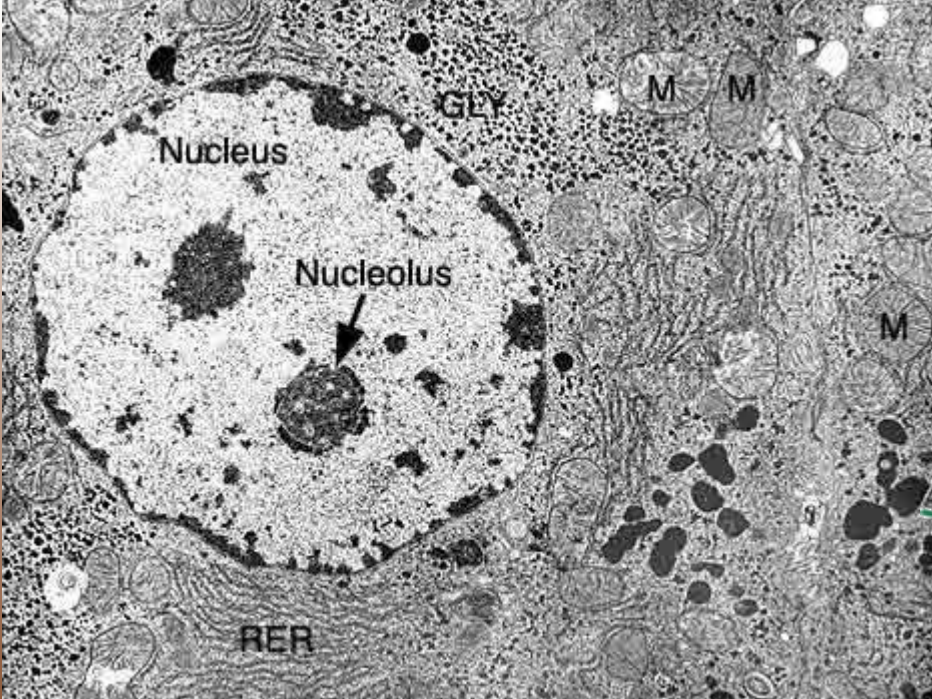


# Intra Cellular Structures

## The Neuron









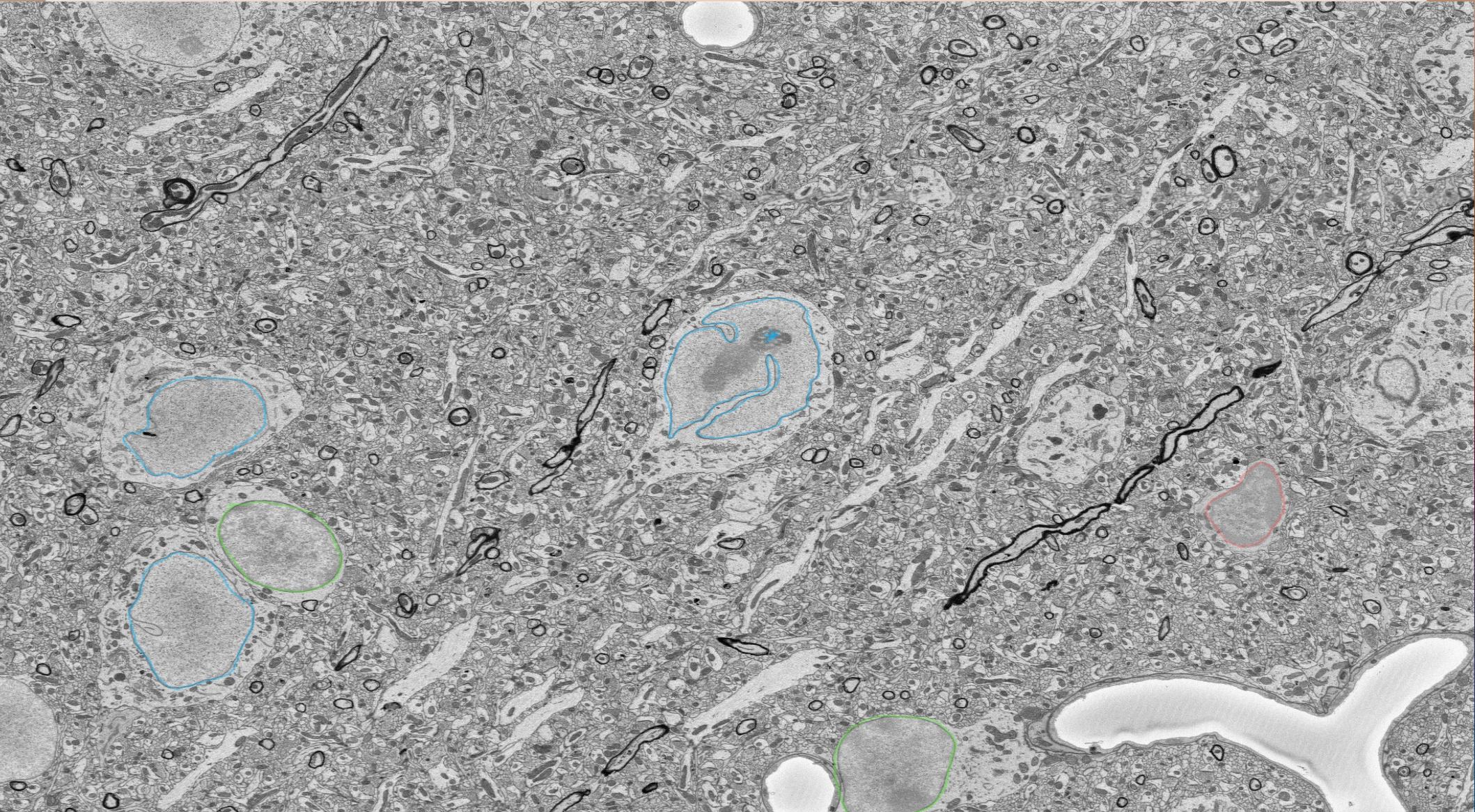
# Imaging Projects

## 6) Nucleus classification

Segment nuclei from 3DEM data

Analyze structure of extracted nuclei

Group nuclei into types based on classification





# Imaging Projects

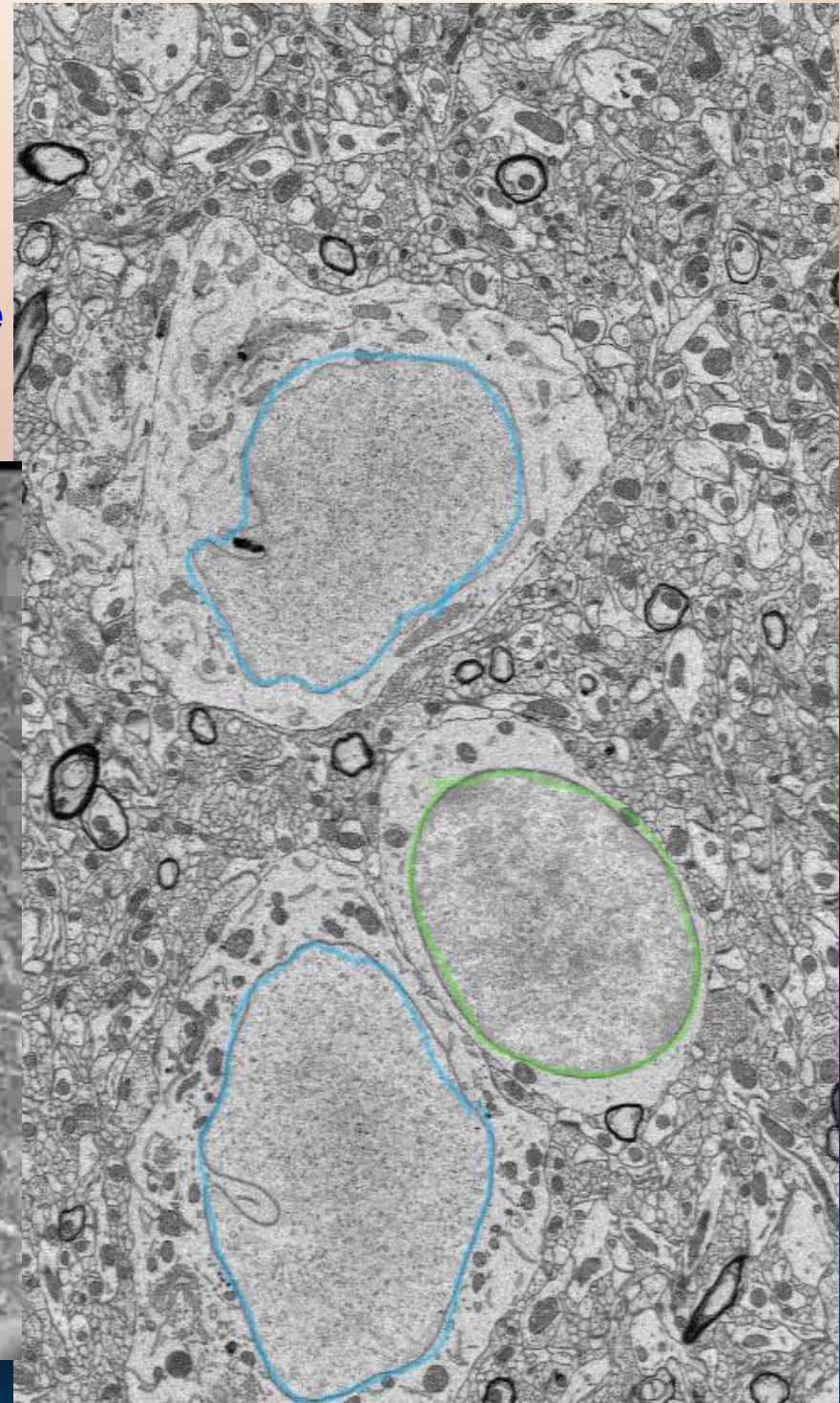
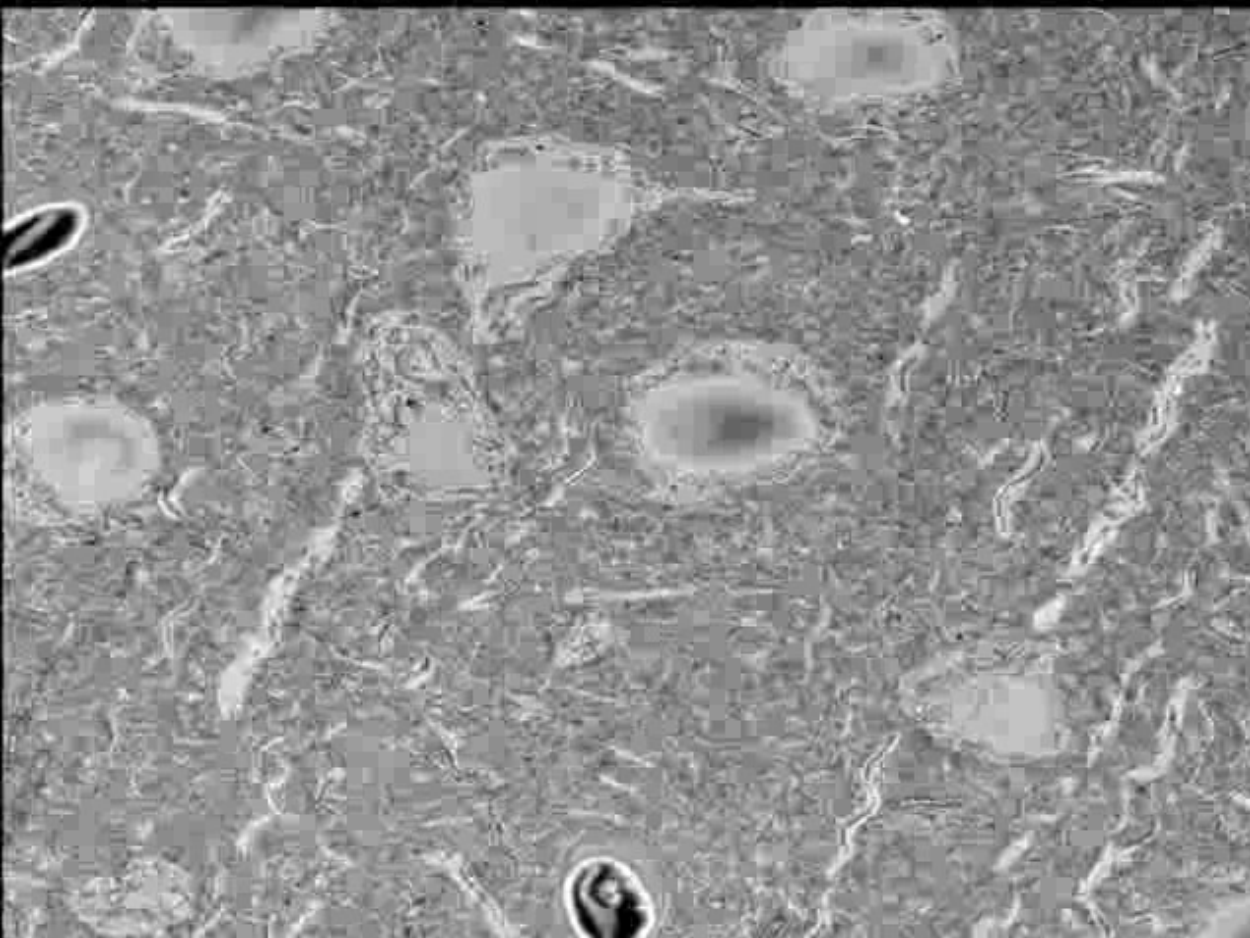
## 6) Nucleus classification

-segment all nuclei in 3D image

-seed points?

-3D vs. 2D

-analyze circularity, volume, area, density/texture





# Imaging Projects

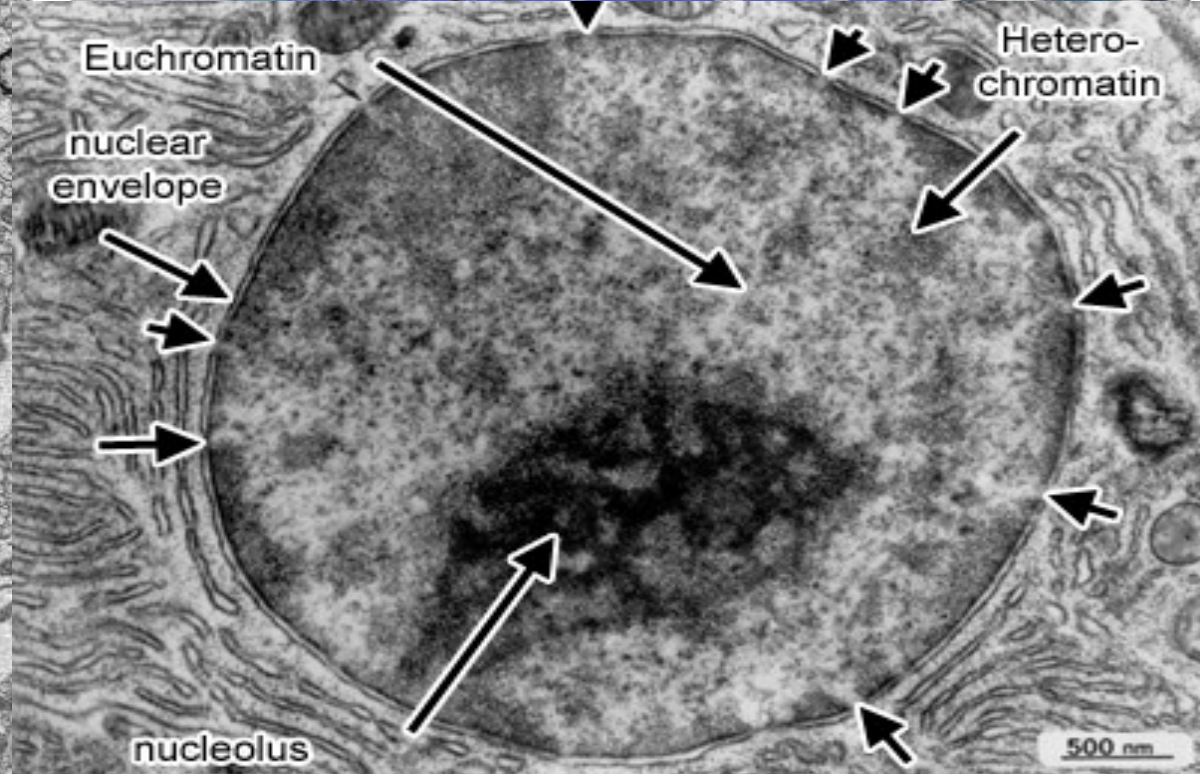
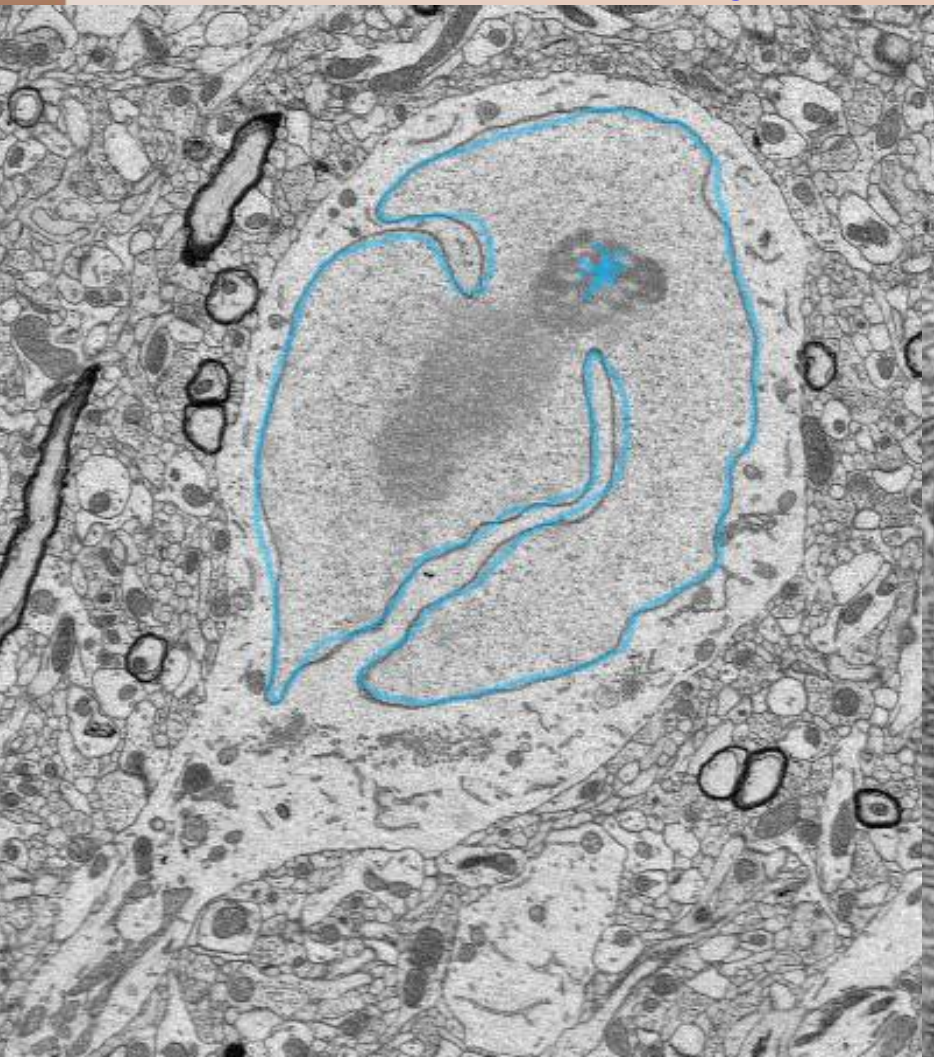
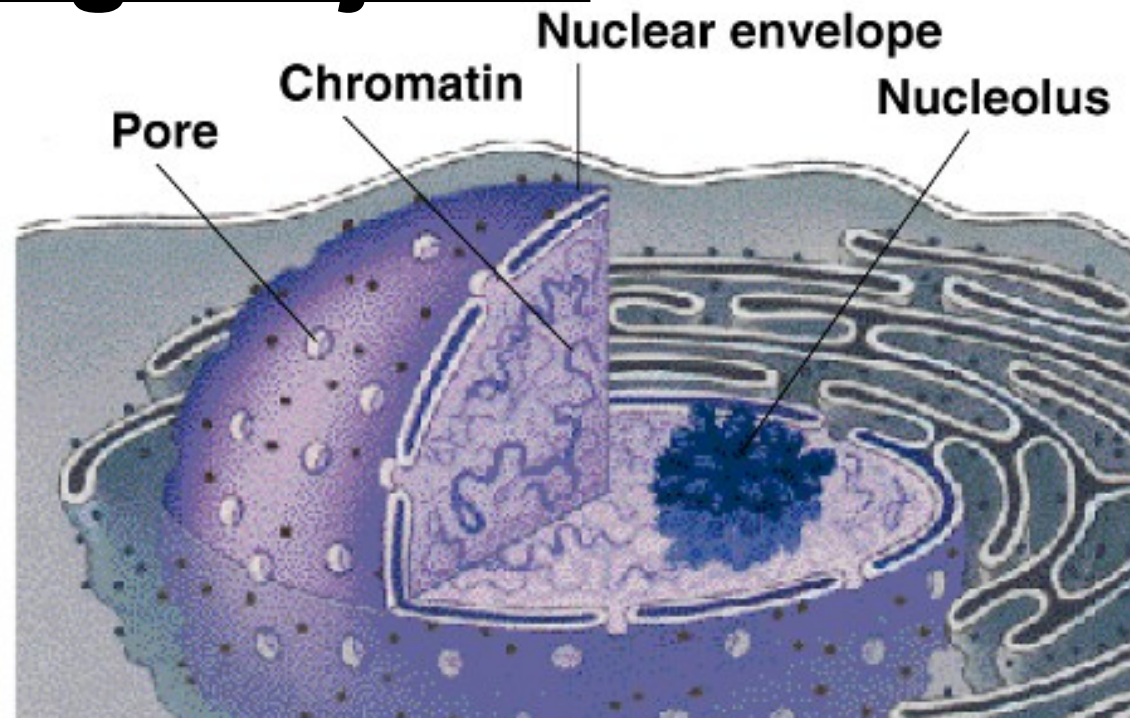
## 6) Nucleus classification -segment *within* nuclei

-nucleolus

-volume, area, density

-heterochromatin

-volume, area, density



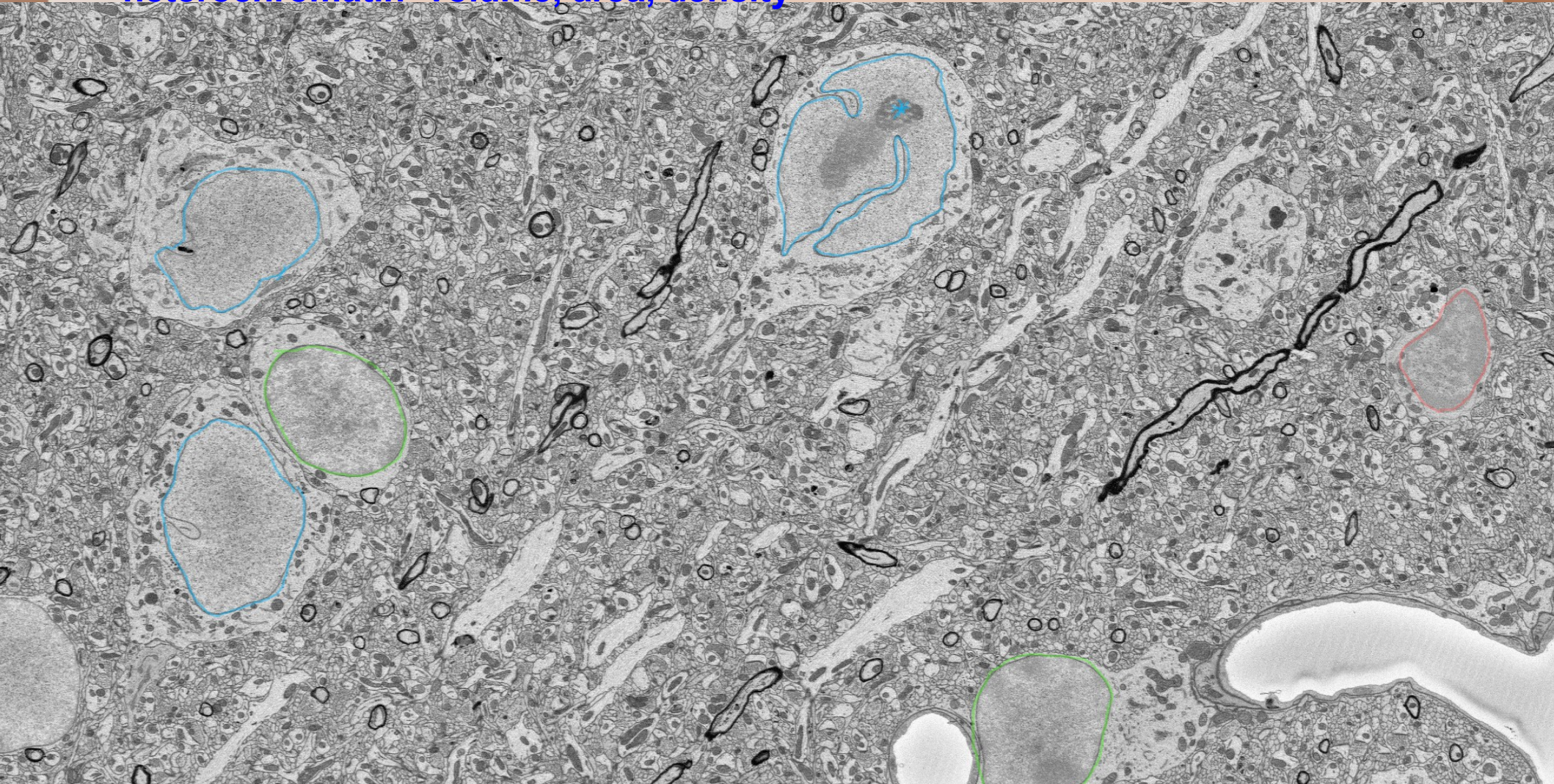


# Imaging Projects

## 6) Nucleus classification

**Classify Nuclei into Groups based on;**

- how many groups?
- nucleus -circularity, volume, area, density/texture
- nucleolus -volume, area, density
- heterochromatin -volume, area, density





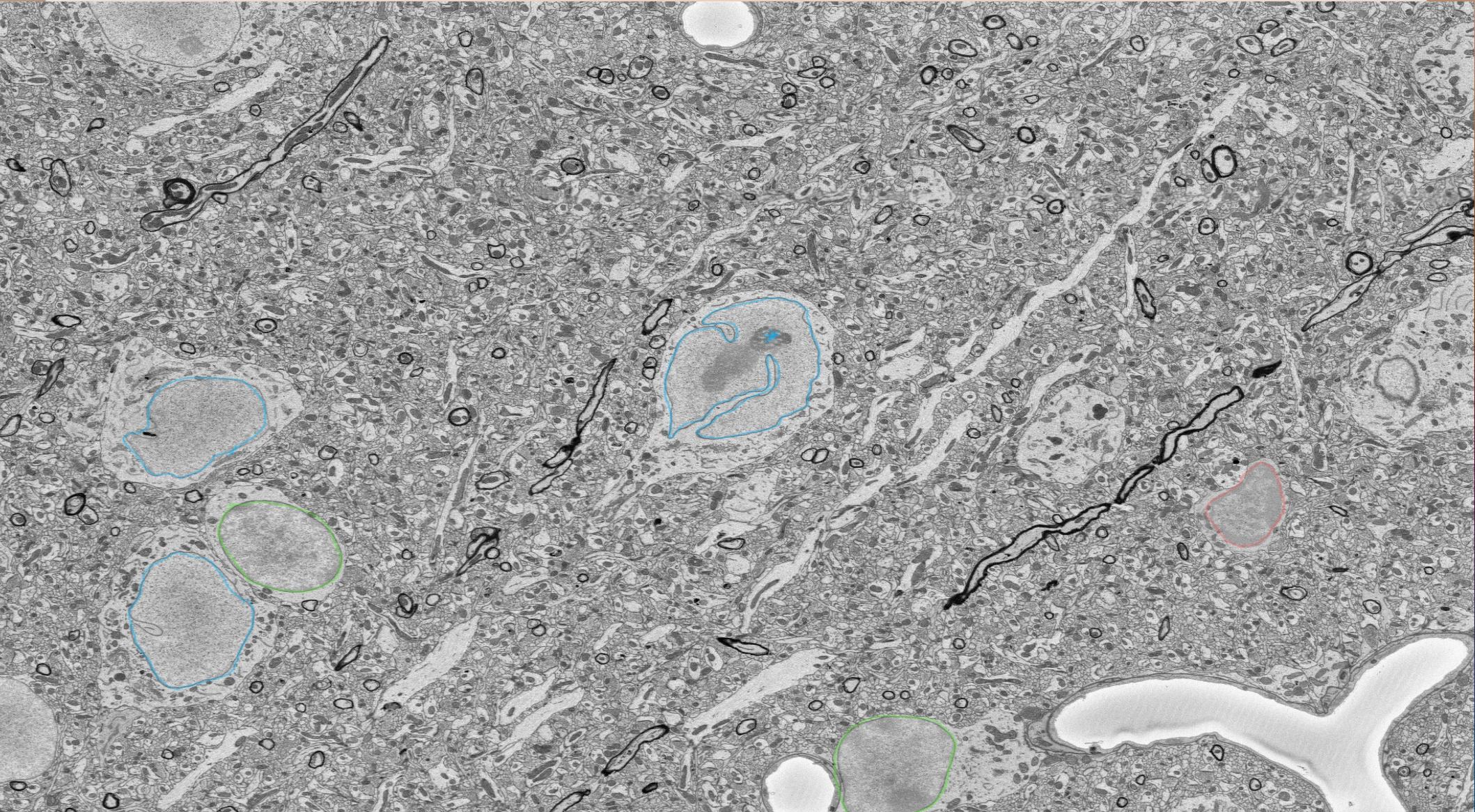
# Imaging Projects

## 6) Nucleus classification

Segment nuclei from 3DEM data

Analyze structure of extracted nuclei

Group nuclei into types based on classification





# Imaging Projects

## MICROSCOPY

1) Background estimation

2) Tiled data reconstruction and correction

3) Astrocyte classification

4) Microvessel analysis

CONFOCAL MICROSCOPY

5) Neuron classification

6) Nucleus classification

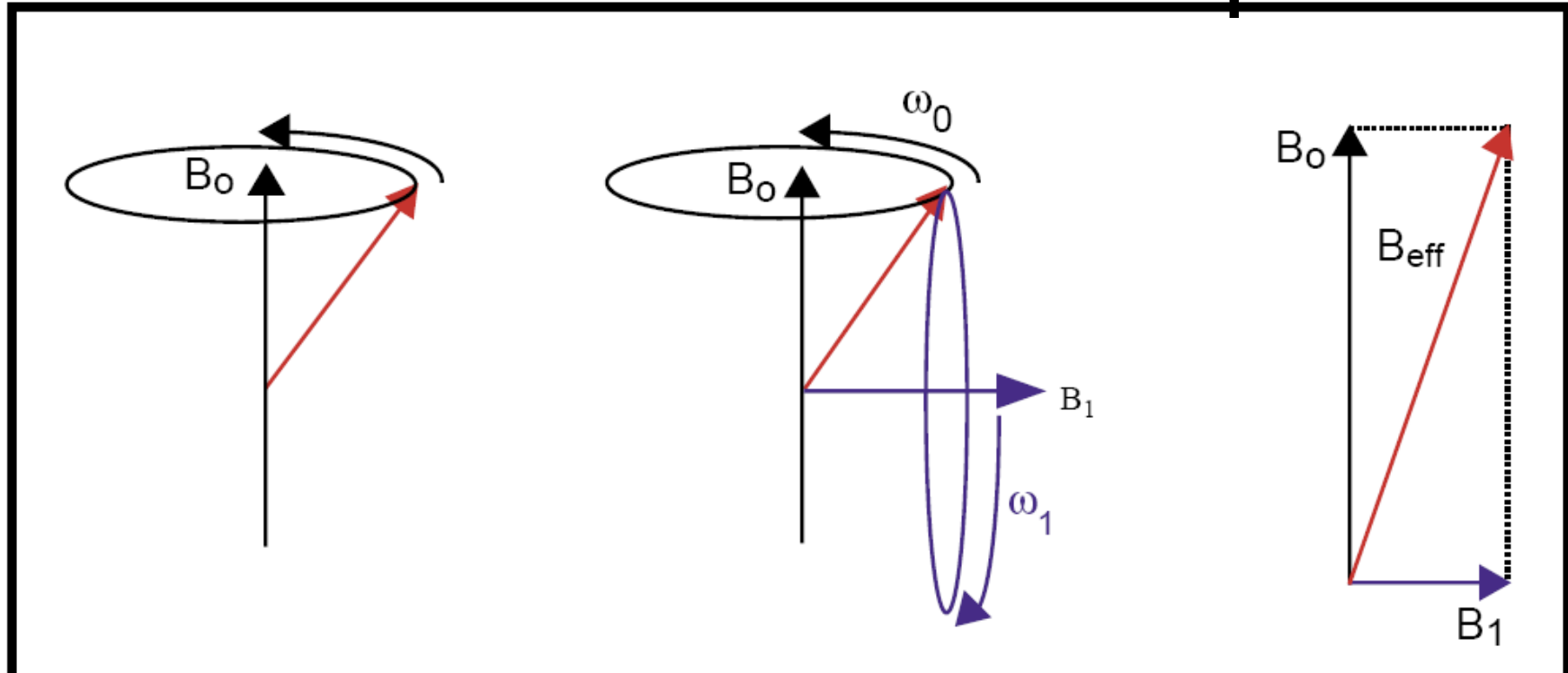
3D ELECTRON MICROSCOPY

7) Cerebellum classification

MRI

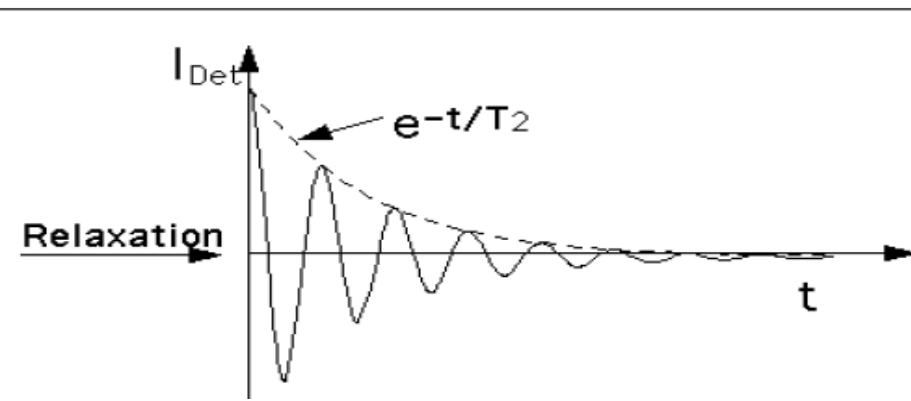


# MRI General Principle



- a static magnetic field ( $B_0$ ) is placed on the sample, each Hydrogen atom in the nuclei precesses (spins) around the field creating nuclear magnetic resonance signal (NMR)
- each H nucleus spins at a given frequency (Larmor frequency) dependent on the magnet strength (tesla) and its gyromagnetic constant (for hydrogen its 42.577 MHz per tesla)
- send an RF (radiofrequency) pulse  $B_1$  at the Larmor frequency across the magnetic field

# MRI General Principle



- adjust RF pulse characteristics and the magnetic field;  
→ changes position, thickness, and region being imaged
- NMR signals recorded in "k-space" (a data matrix containing spectral and spatial information about structures in the sample)
- FFT

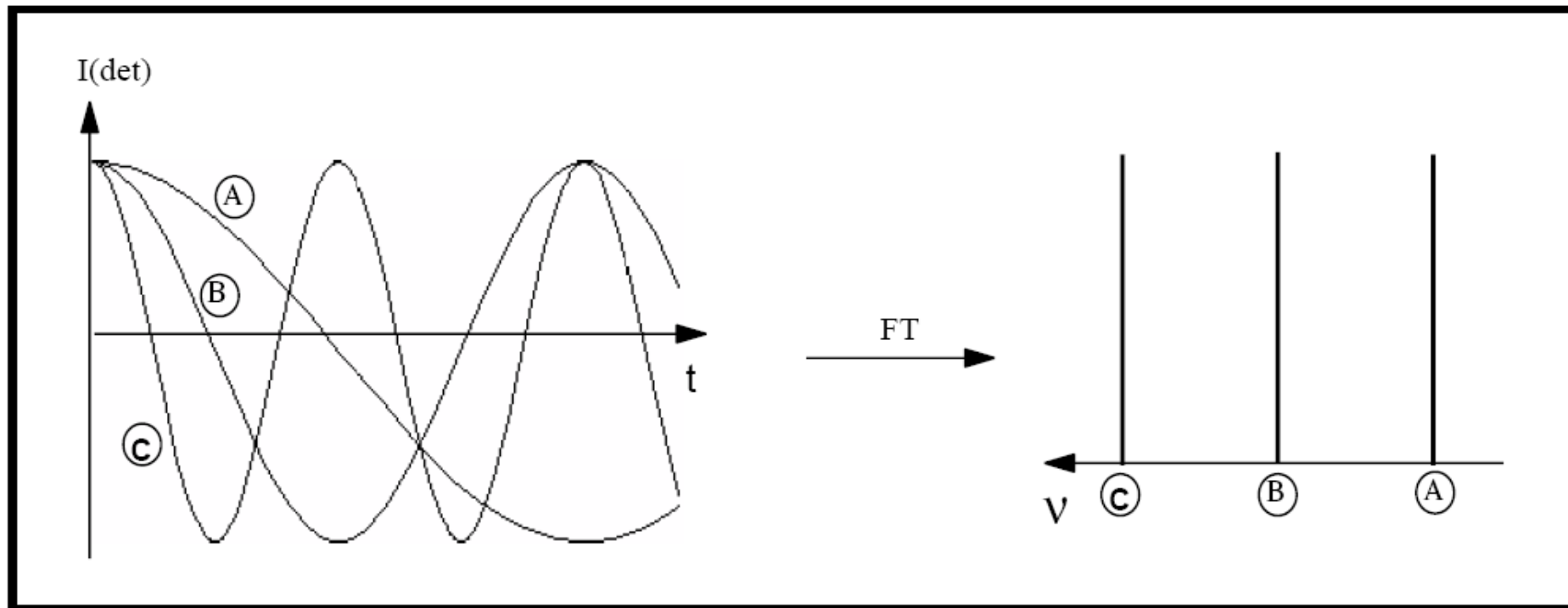
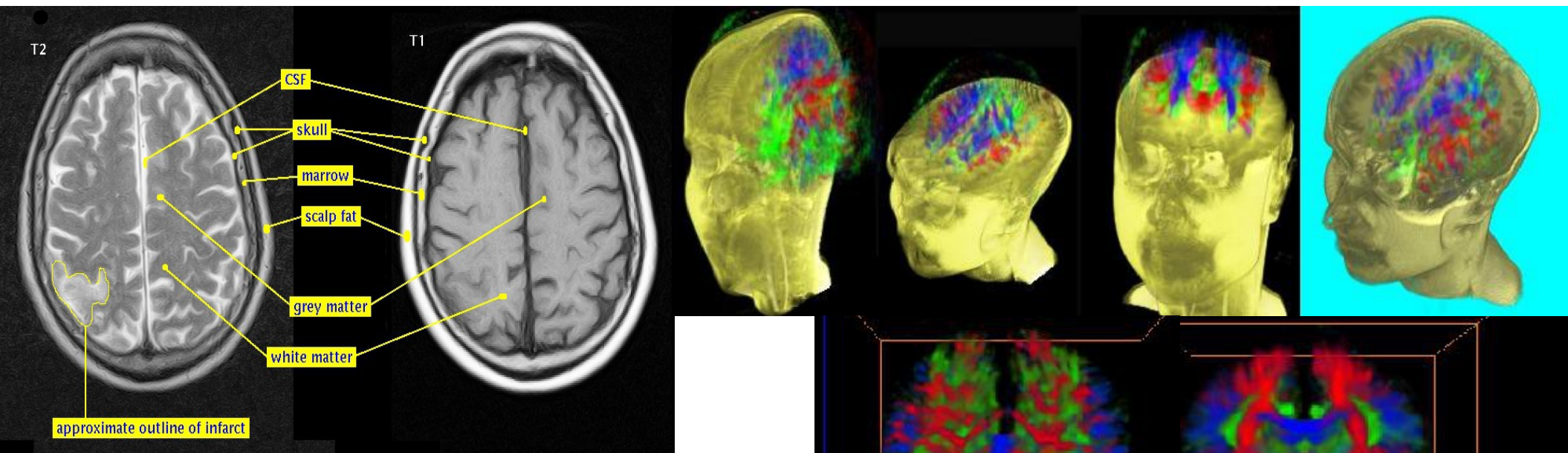


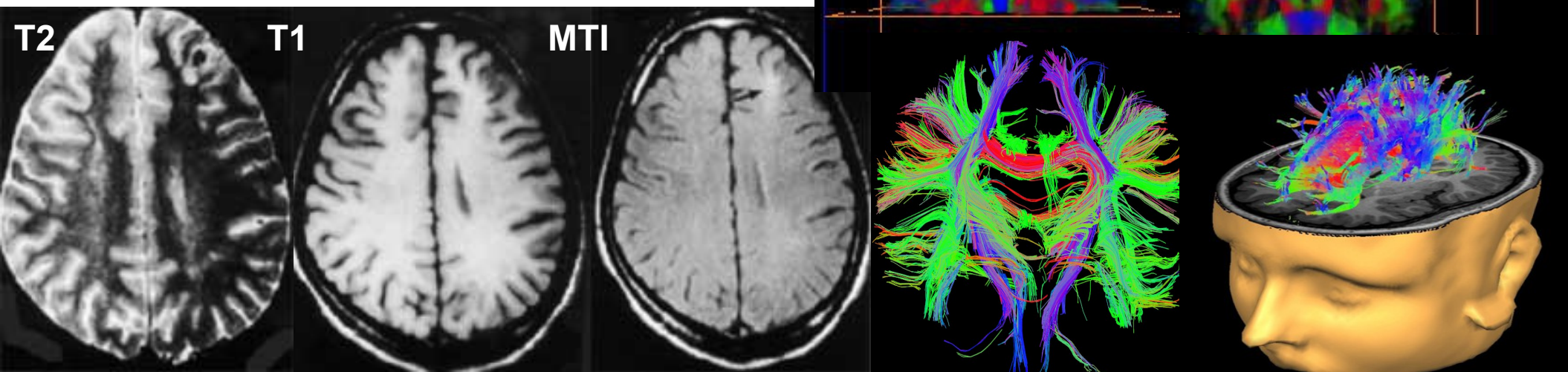
FIGURE 13. Signals from 3 spins with different precession frequencies (left) and their corresponding Fourier transforms (right)



- T1 (longitudinal relaxation) = time for magnetization to recover to equilibrium after RF pulse (grey/white matter)
- T2 (transverse relaxation) - transient decay of the rotating magnetization of all nuclei
- Diffusion Tensor Imaging (DTI)



• **Magnetization Transfer Imaging**



# Imaging Projects

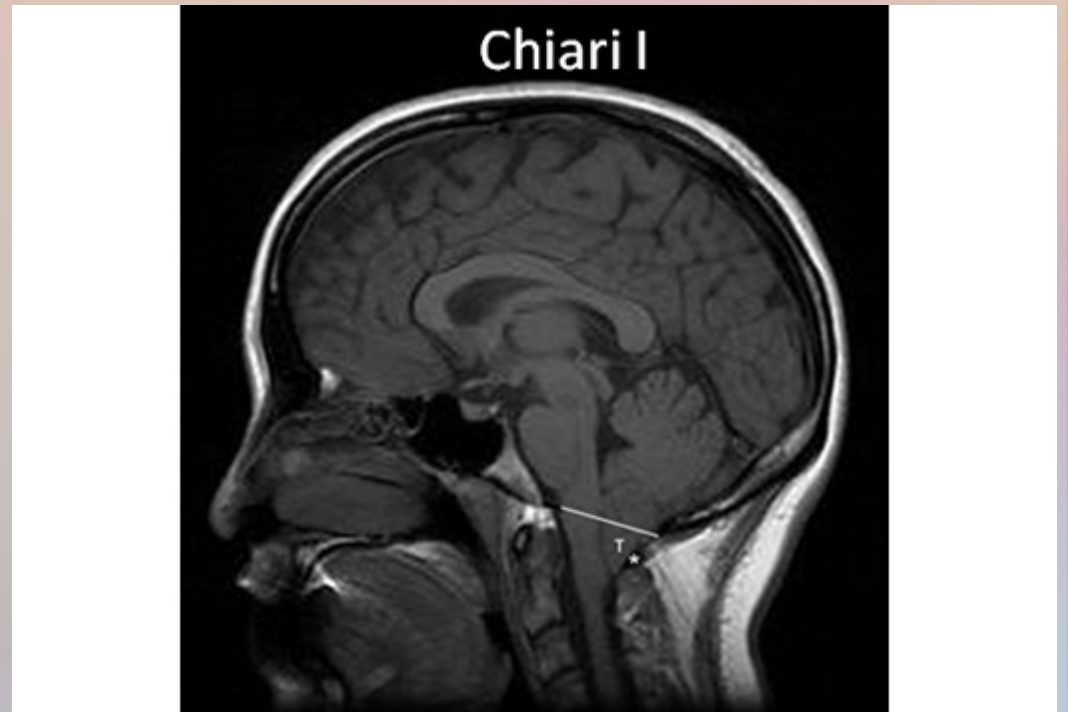
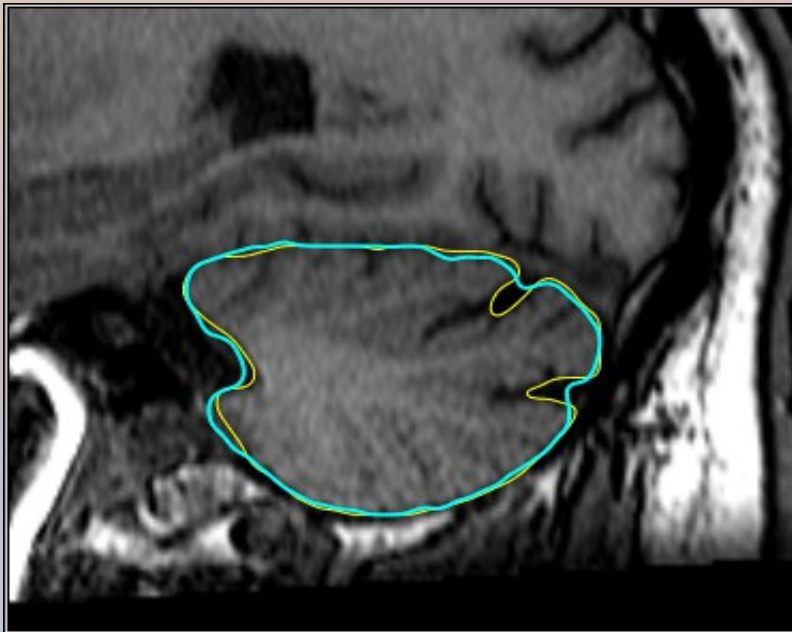
## 7) *Cerebellum Segmentation and Classification*

-segment cerebellum

-characterize structure of cerebellum

-draw line indicating where cerebellum should stop

-indicate possibility of Chiari malformation



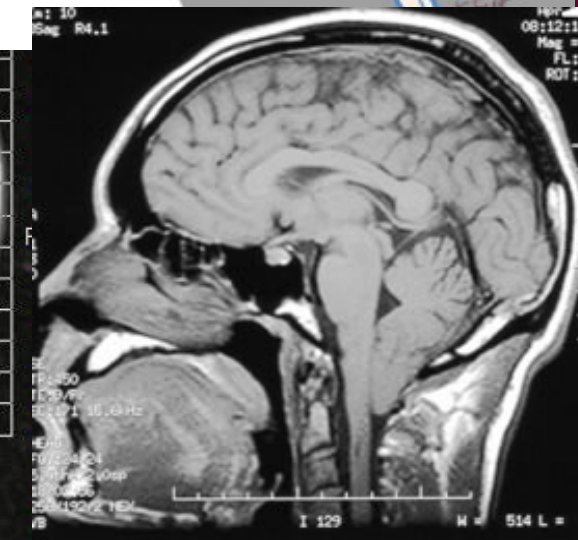
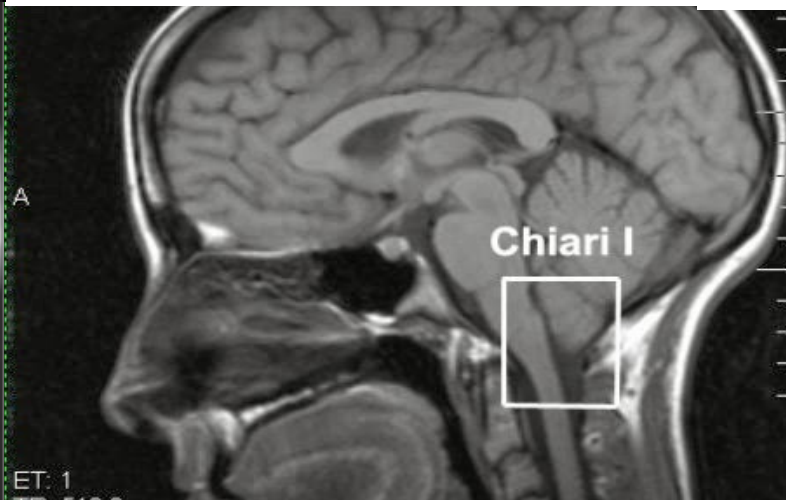
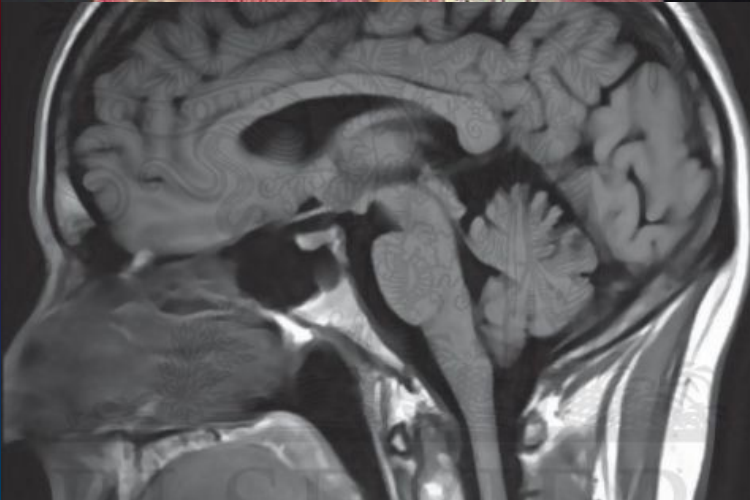
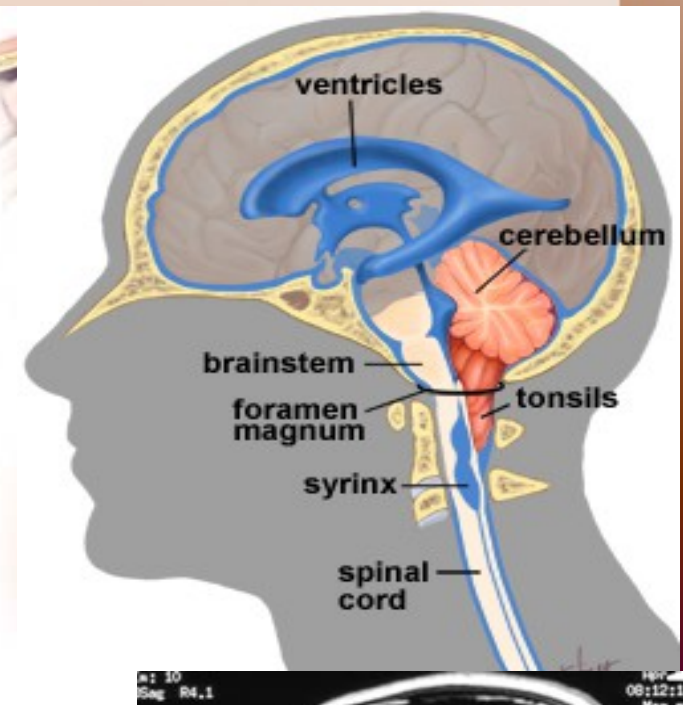
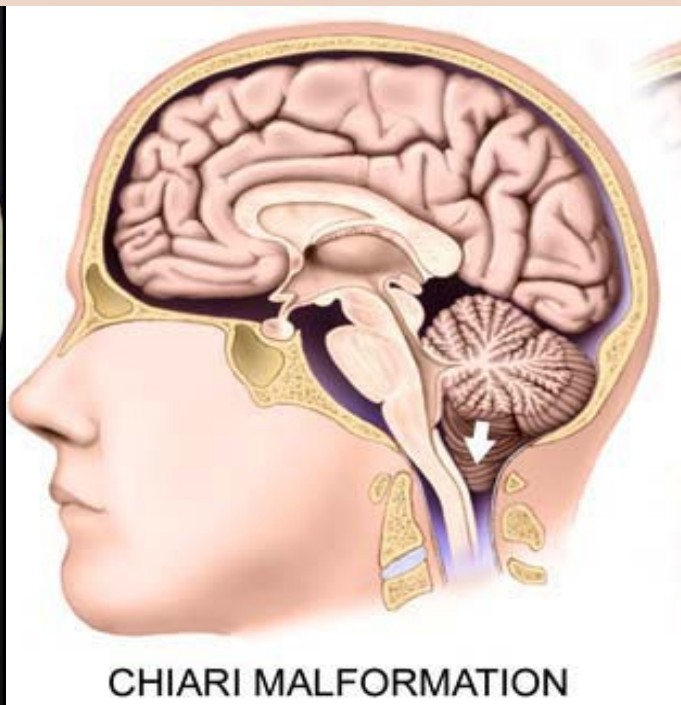
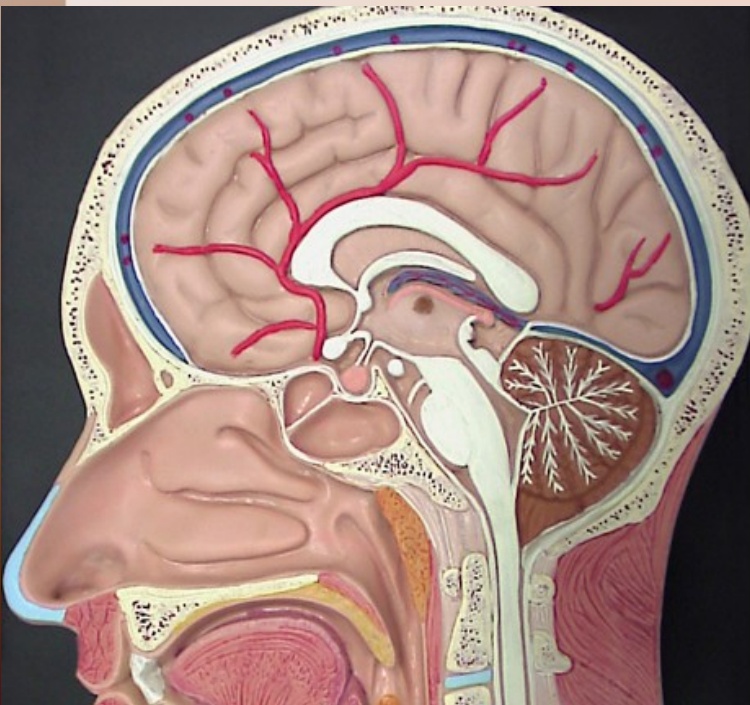
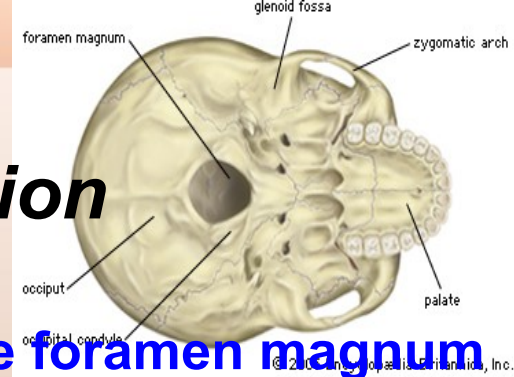


# Imaging Projects

## 7) *Cerebellum Segmentation and Classification*

### Chiari malformation (1-4)

-downward displacement of the cerebellar tonsils through the foramen magnum



# Imaging Projects

- 7) *Cerebellum Segmentation and Classification*
- automatically segment cerebellum
  - use landmarks as clues, brain stem, skull

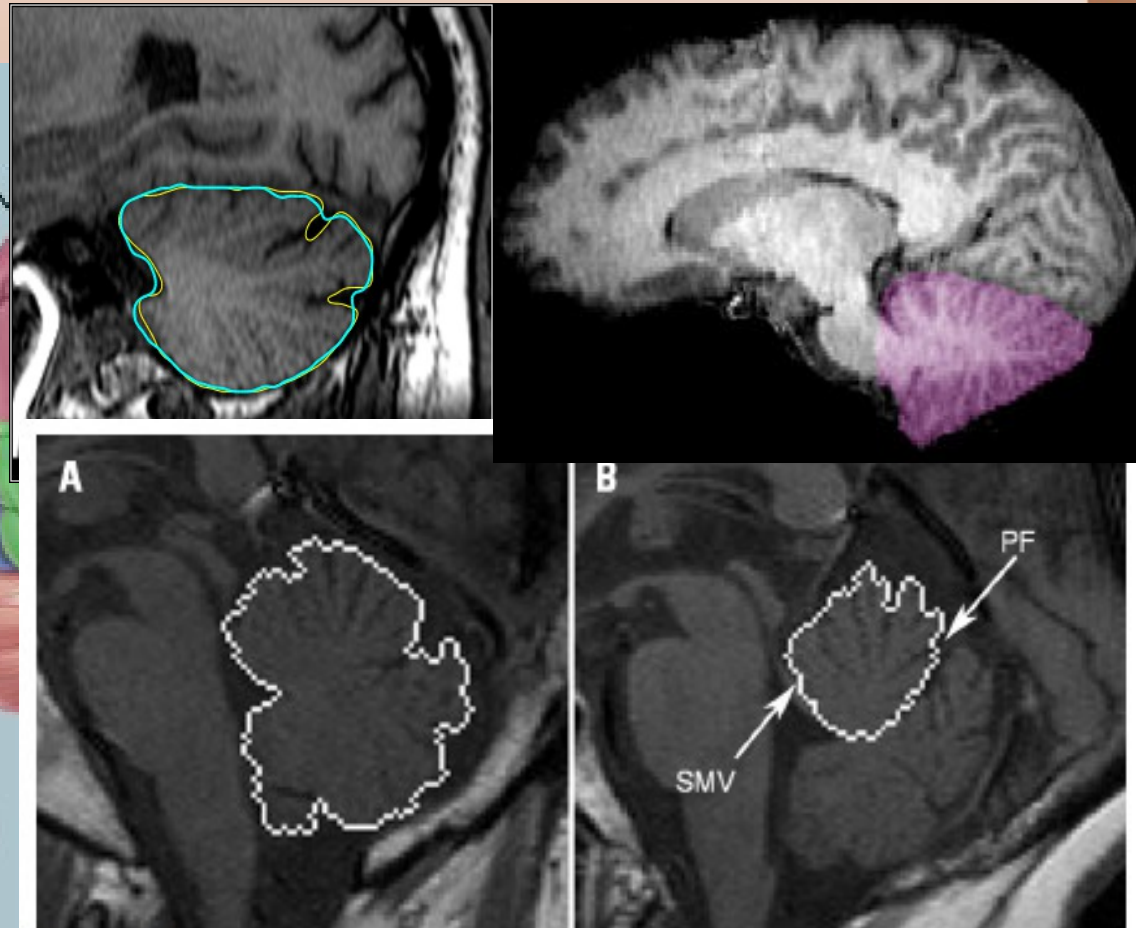
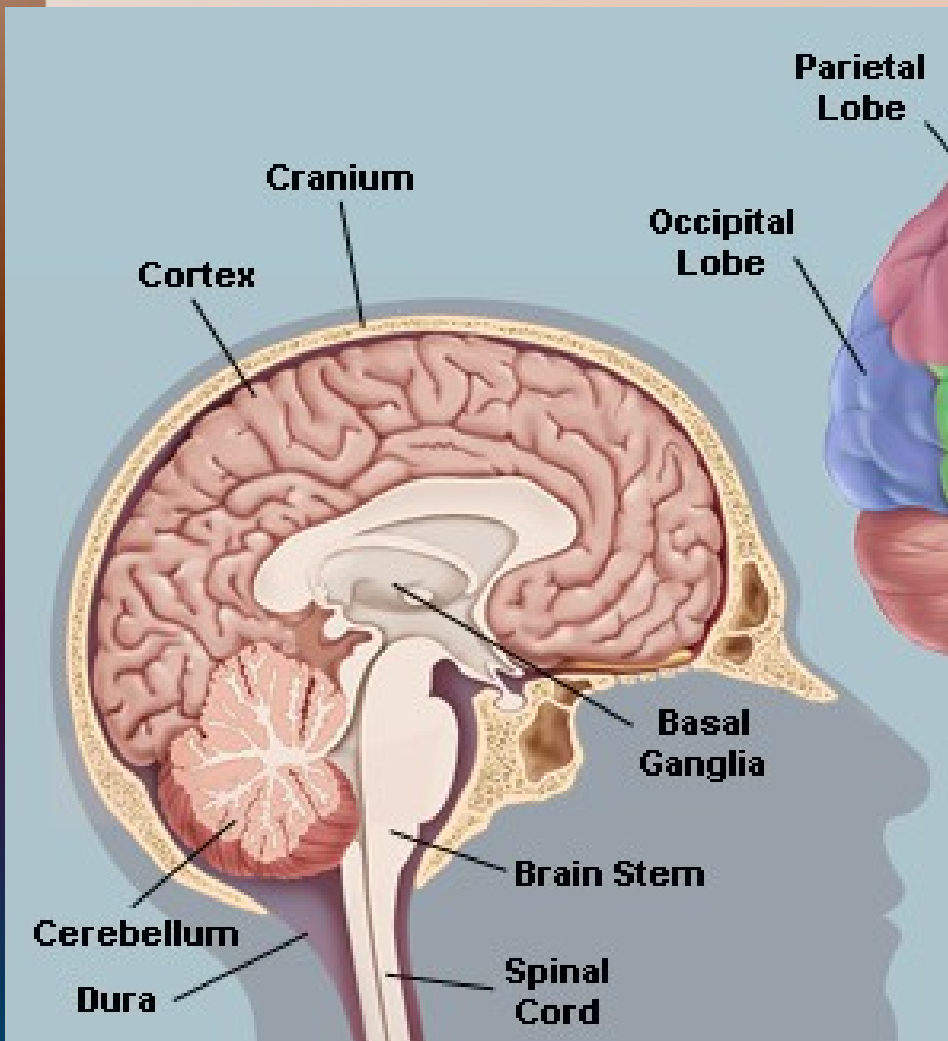


Fig 1. Cerebellum and anterior lobe segmentation. The cerebellum was manually outlined on sagittal slices [A]. The anterior lobe of the cerebellum was also outlined in sagittal slices [B]. Its



# Imaging Projects

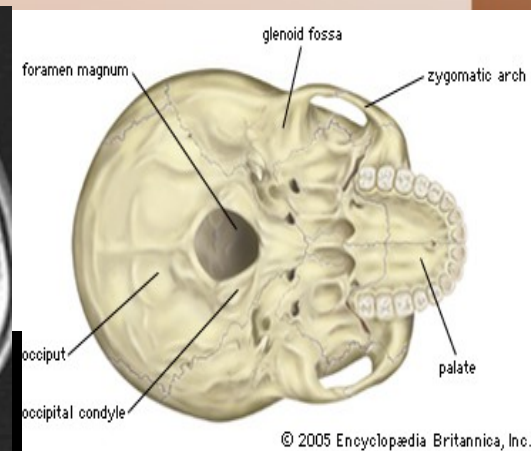
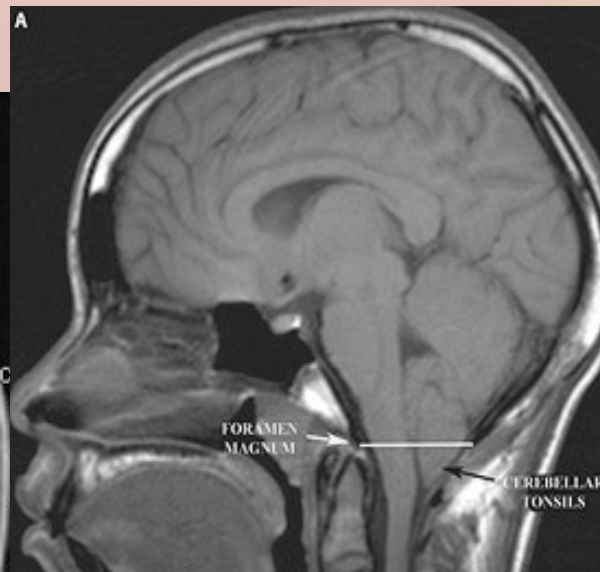
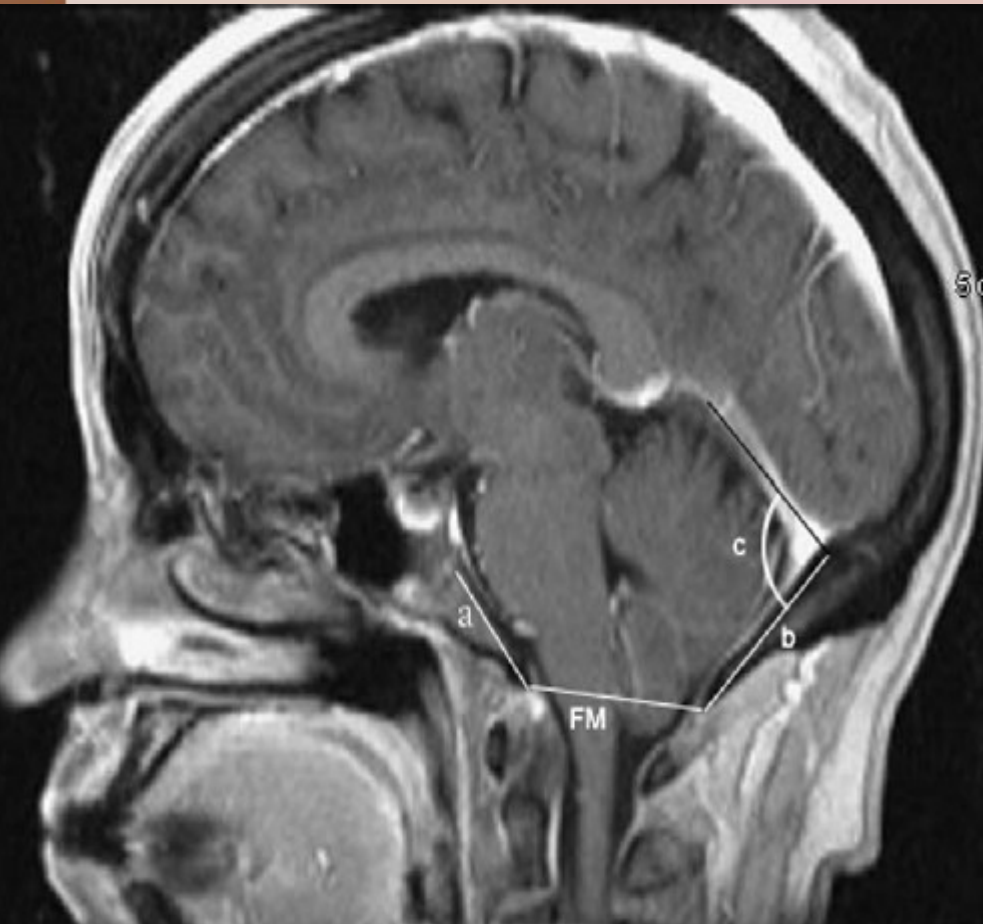
## 7) *Cerebellum Segmentation and Classification*

*-draw line indicating where cerebellum should stop*

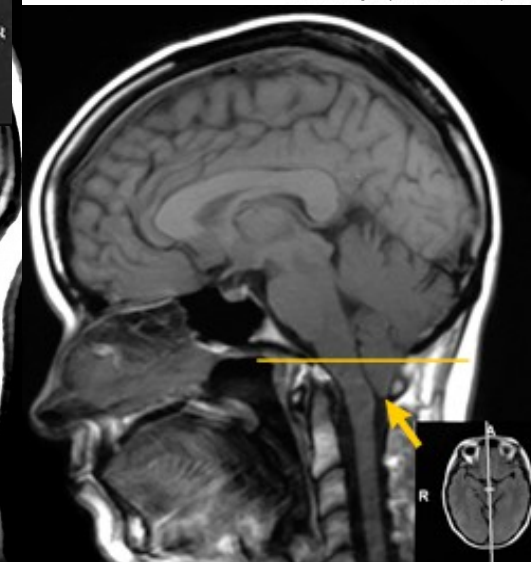
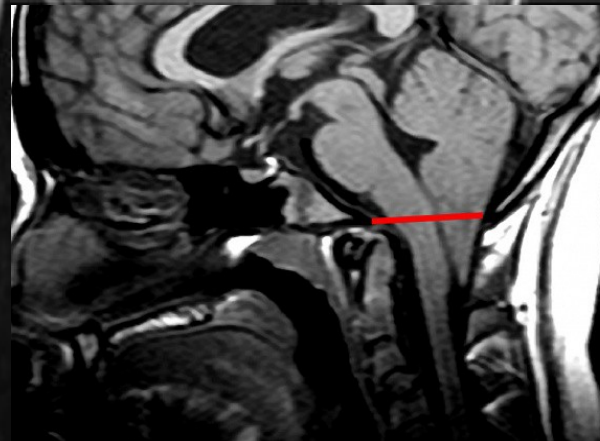
*-downward displacement of the cerebellar tonsils through the foramen magnum*

*-indicate possibility of Chiari malformation*

*-amount of cerebellar herniation*



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# Confounding Factors in Data

- **Brightness inhomogeneities (laterally and axially (PSF) –confocal)**
- **Sample auto fluorescence**
- **Poorly/incompletely stained**
- **Transected structures due to physical sectioning**
- **Alignment and recombination (tiled data or badly aligned image stacks)**
- **Noise associated with 3DEM**
- **Dense overlapping structures**
- **Sample variability**
- **Size**
- **Processing time**

**Error? – *confidence estimates***

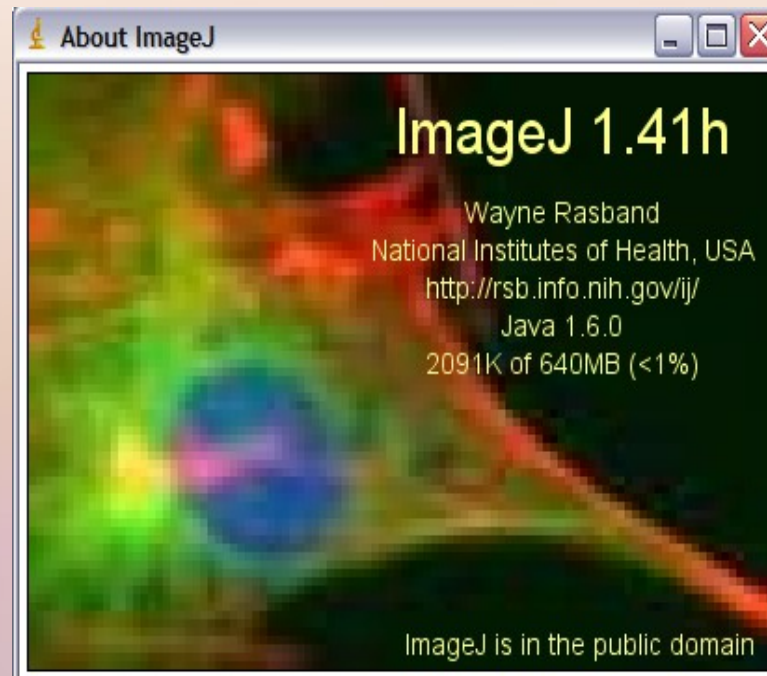


# ALL PROJECTS

- **Implemented/useable as ImageJ plugin**
  - *Call from command line?*
- **Extendable to other data**
  - *Test/works on all provided data*
- **Fully automated**
  - *minimal user input to trigger*
- **Simple Graphical Interface**
- **Multithreaded for cluster applications**
- **Sensible feedback of results**
  - *Non-destructive image overlay*
  - *Table of results/graphs*
- **Error warnings**

# IMAGEJ

<http://rsbweb.nih.gov/ij/>



## Plugins and Macros

<http://rsbweb.nih.gov/ij/plugins/index.html>

<http://rsbweb.nih.gov/ij/developer/macro/macros.html>

## Examples

<http://rsbweb.nih.gov/ij/docs/examples/index.html>

## Forum

<http://imagej.1557.n6.nabble.com/>



# Existing Tools

## DATABASES

[Cell Centered Database \(CCDB\)](#),

[SenseLab Project](#)

[L-Neuron](#)

[Neuromorph](#)

[brainmaps.org](#)

[Neuroscience Information Framework \(NIF\)](#)

[Connectome Project](#) .

## ImageJ/FIJI

[NeuronJ](#) , [Simple Neurite Tracer](#)

[Find Connected Regions](#)

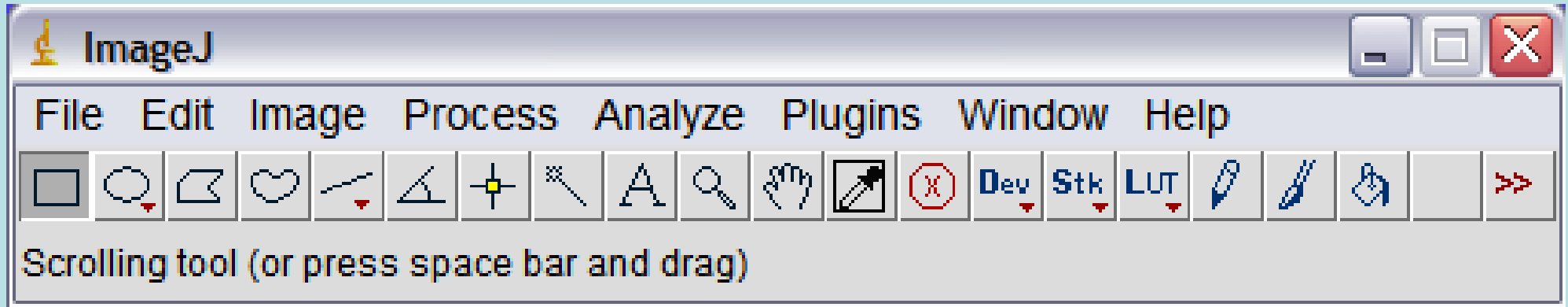
[Neuron Morphology](#) , [TrakEM2](#)

[Simple Interactive Object Extraction \(SIOX\)](#)

[Advanced Weka Segmentation](#)

[Trainable Segmentation plugin](#) .

# Introduction to the Main Menu

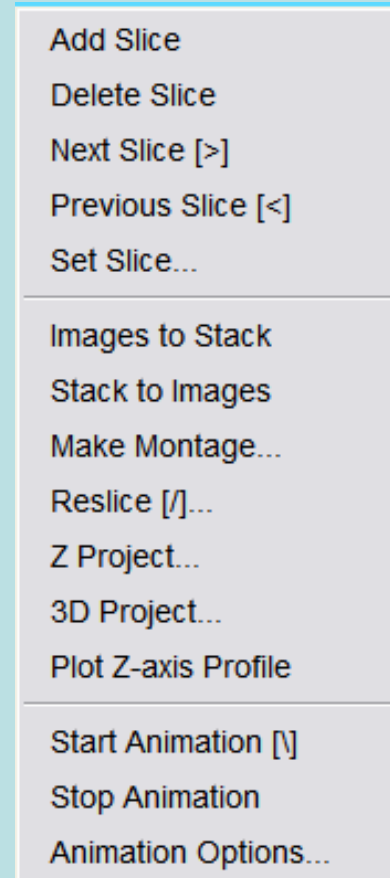
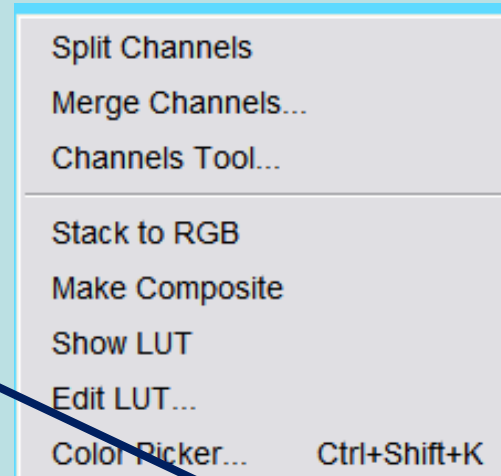
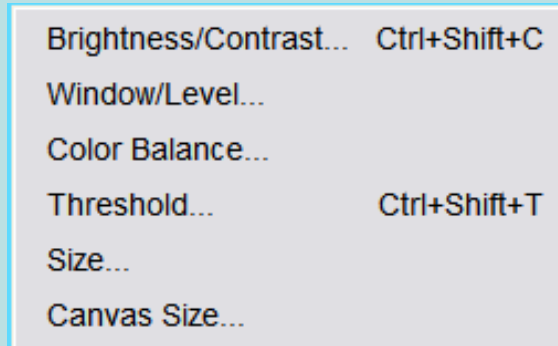
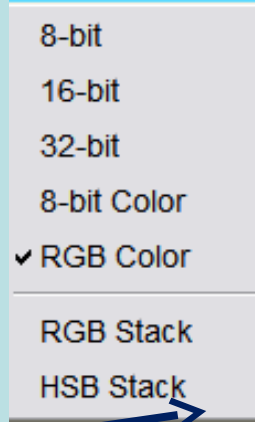
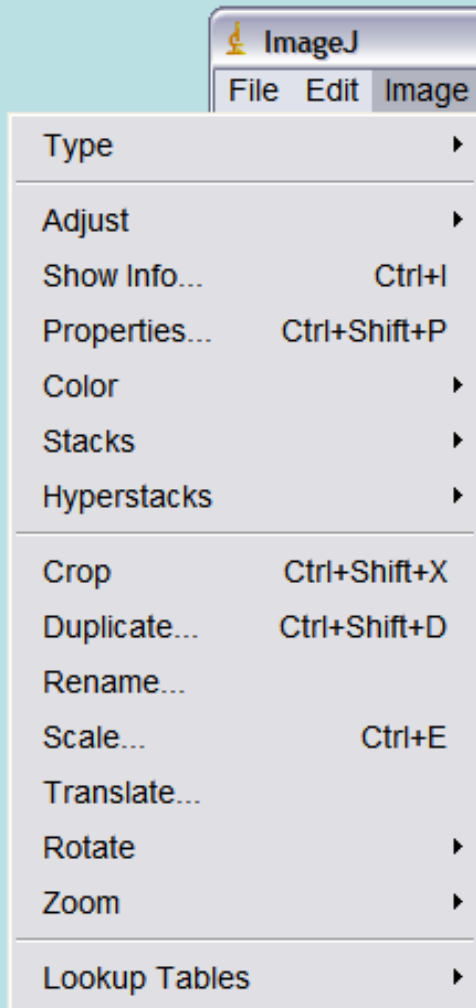


Of these, we'll concentrate on:

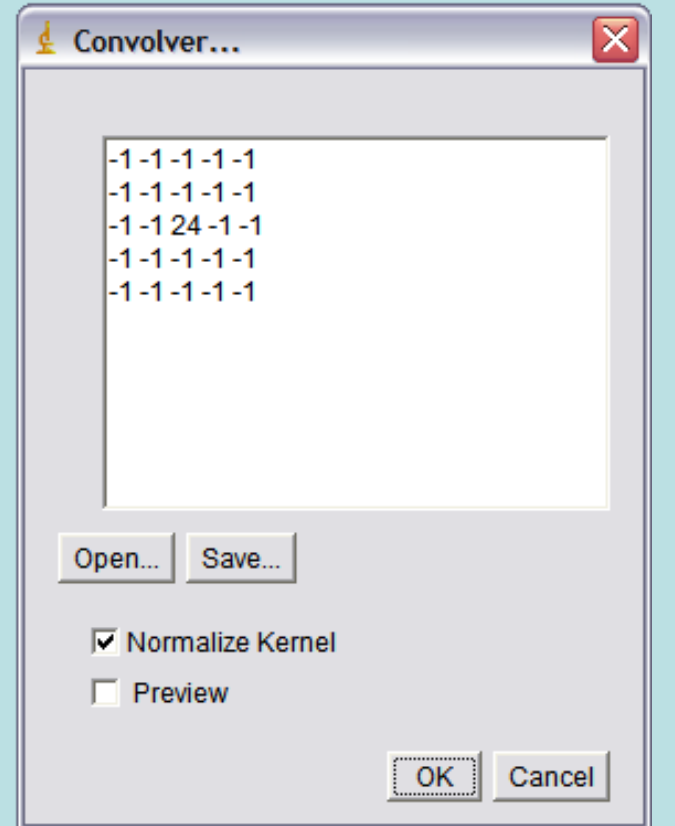
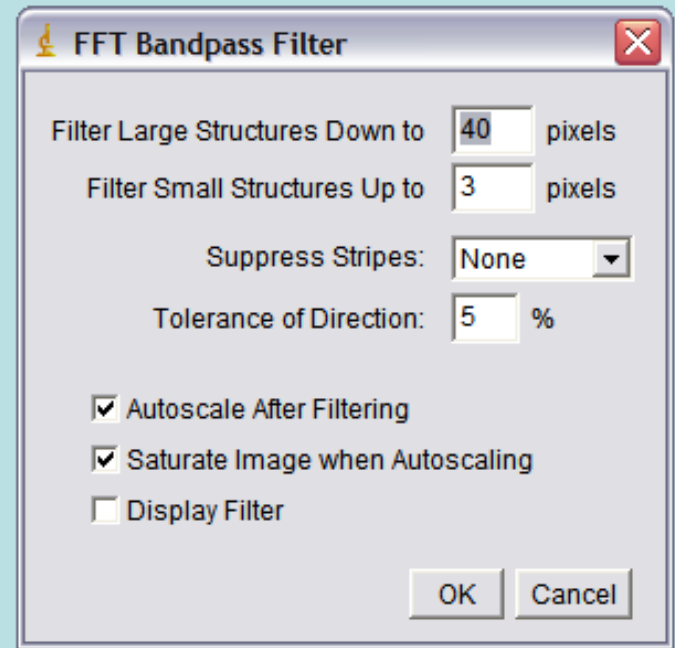
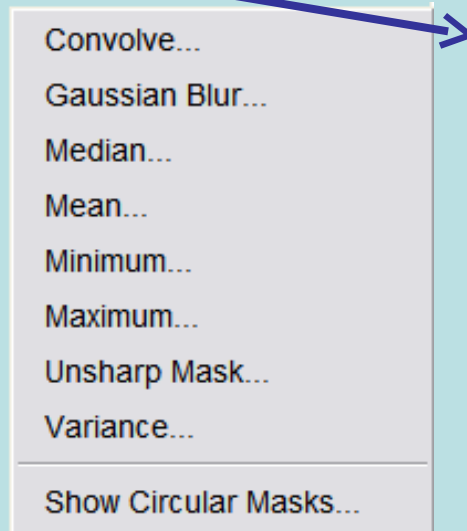
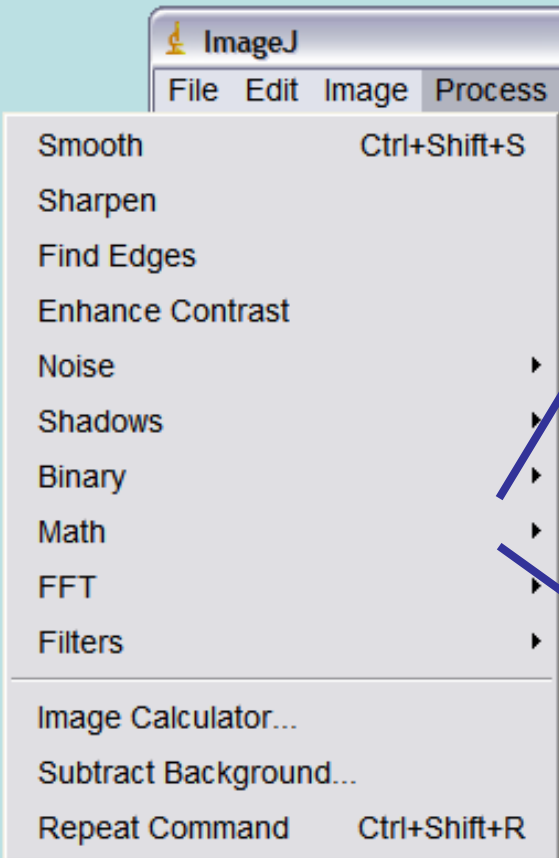
- Image
- Process
- Analyze
- Plugins
- Help



# Image Menu

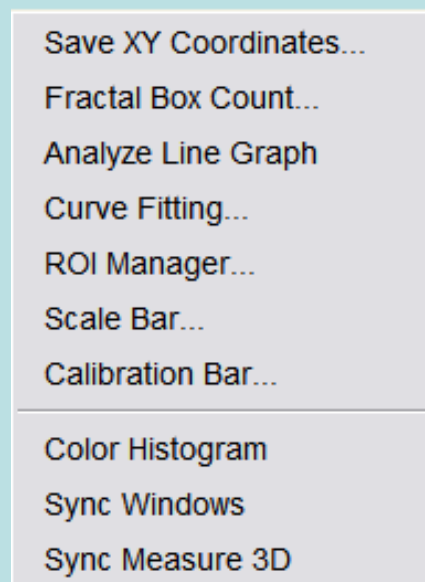
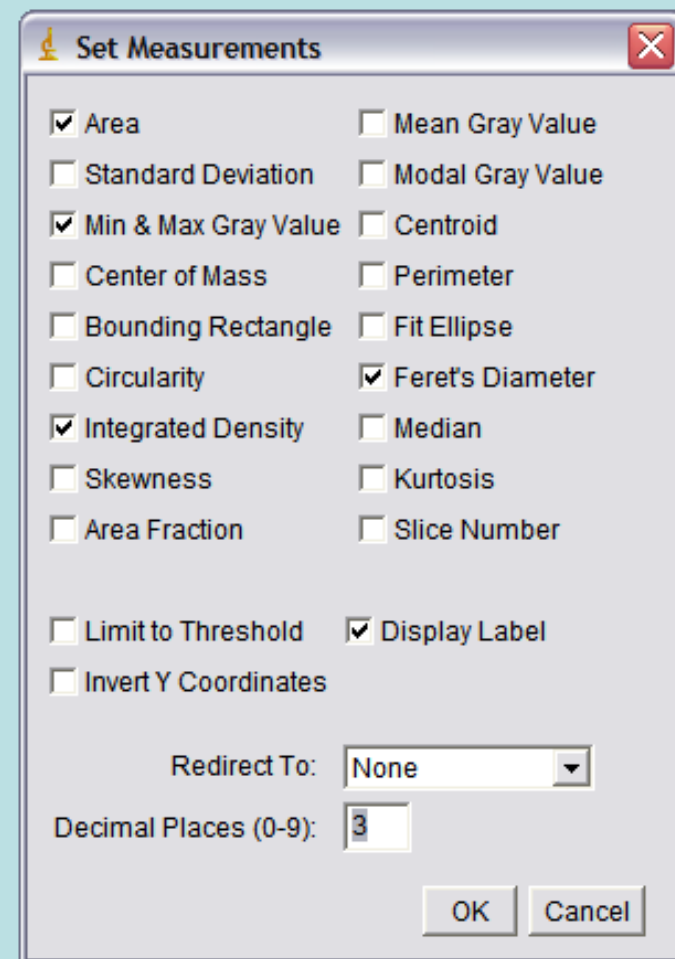
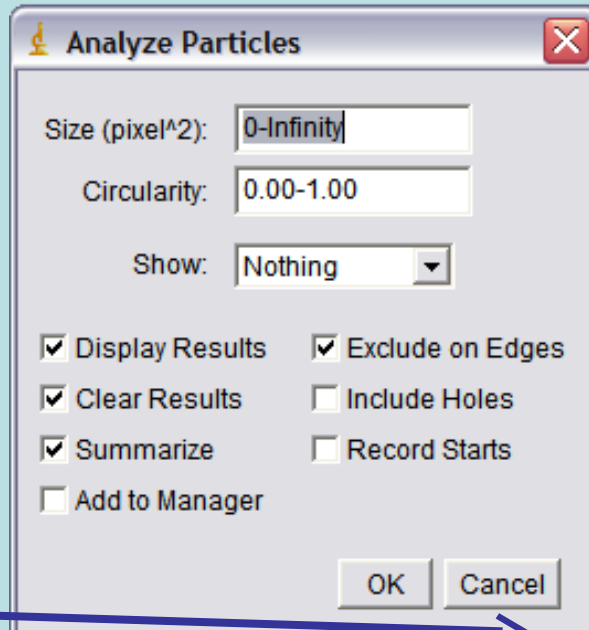
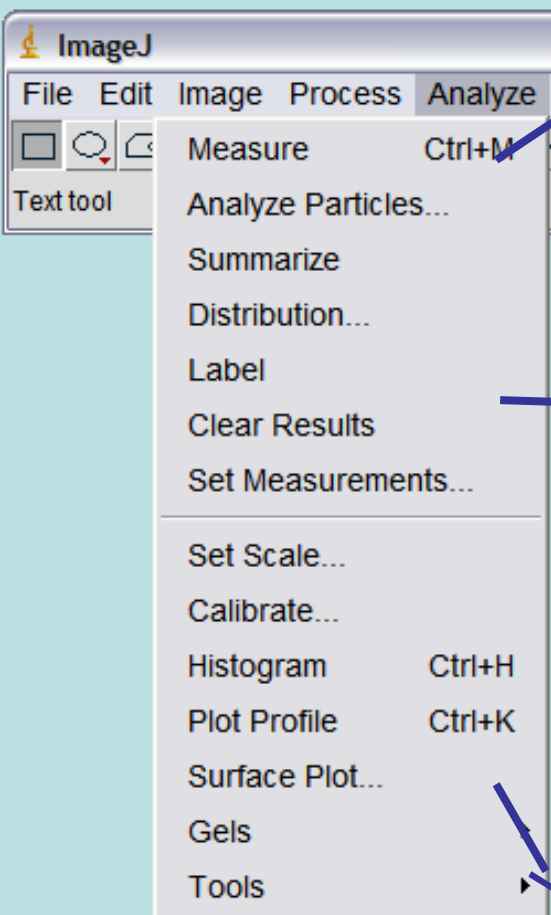


# Process Menu

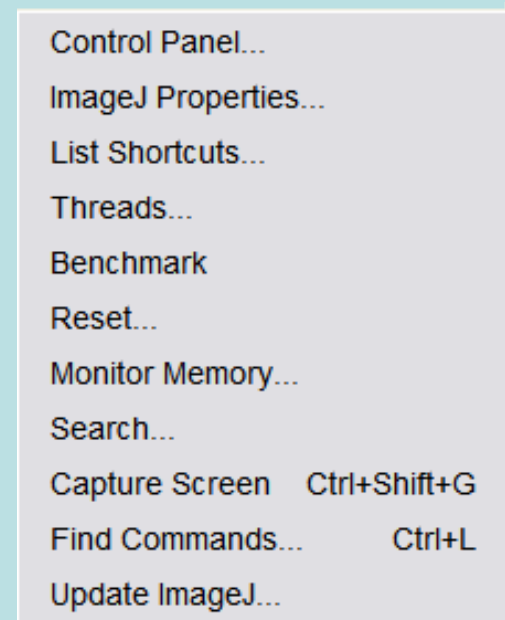
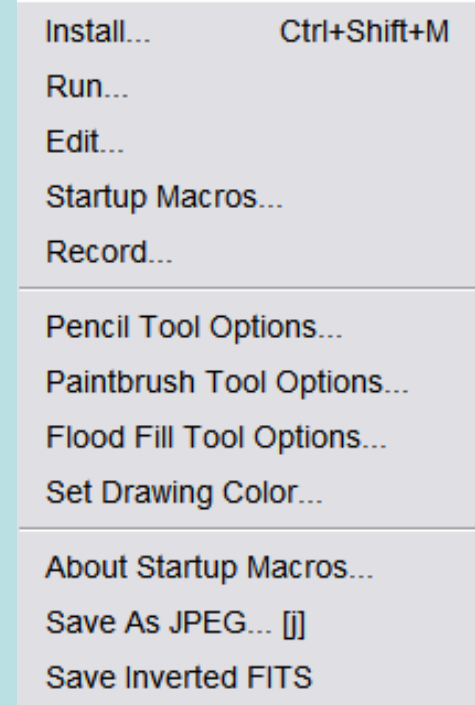
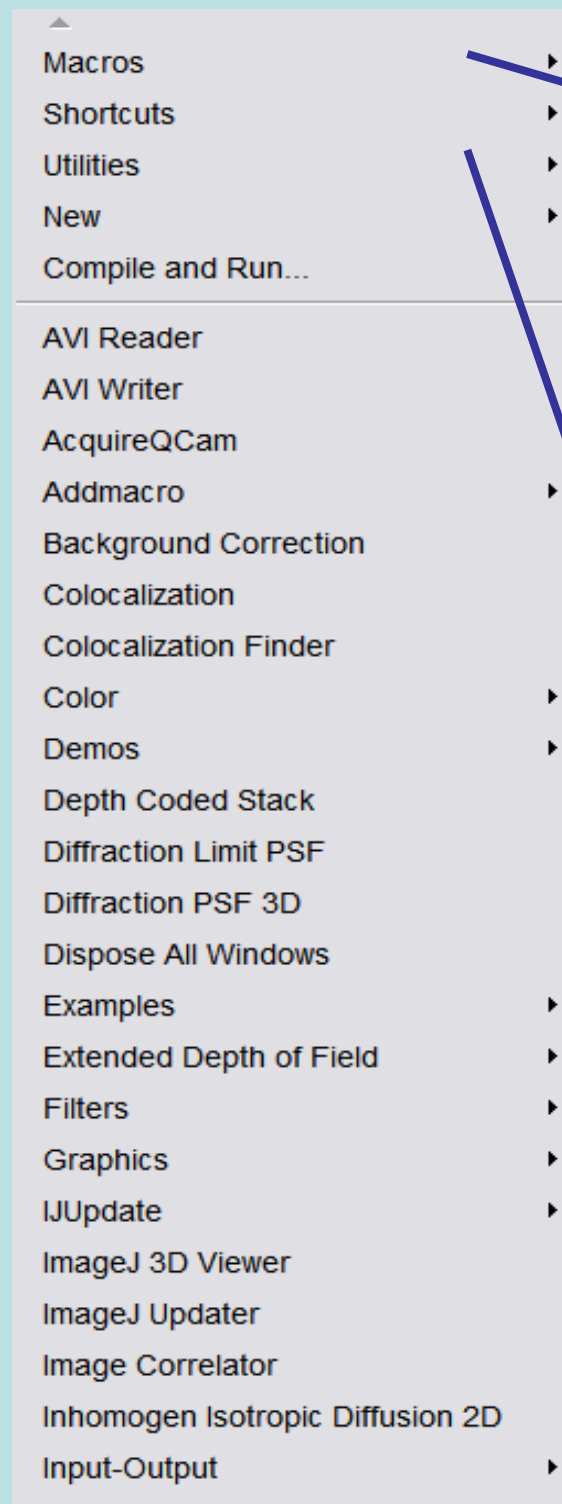




# Analyze Menu



# Plugins Menu





# FIJI

Fiji is an image processing package. It can be described as a distribution of ImageJ (and soon ImageJ2) together with Java, Java 3D and a lot of plugins organized into a coherent menu structure. Fiji compares to ImageJ as Ubuntu compares to Linux.

<http://fiji.sc/wiki/index.php/Fiji>



```
dir1 = getDirectory("Choose a Directory ");
```

```
list = getFileList(dir1);
```

```
ddir1 =dir1+"\\tiffs\\";
```

```
File.makeDirectory(ddir1);
```

```
for (i=0; i<list.length; i++) {
```

```
file = list[i];
```

```
file2 = dir1 + list[i];
```

```
open(file2);
```

```
//name1 = list[i] + " - C=0";
```

```
//name2 = list[i] + " - C=1";
```

```
//name3 = list[i] + " - C=2";
```

```
//selectWindow(name1);
```

```
run("Brightness/Contrast...");
```

```
setMinAndMax(0, 4095);
```

```
//selectWindow(name2);
```

```
run("Brightness/Contrast...");
```

```
setMinAndMax(0, 4095);
```

```
//selectWindow(name3);
```

```
setMinAndMax(0, 4095);
```

```
run("Brightness/Contrast...");
```

```
setMinAndMax(0, 4095);
```

```
//run("Concatenate...", "stack1=name1 stack2=name2 title=stack");
```

```
//run("Concatenate...", "stack1=name3 stack2=stack title=stacks");
```

```
name1 = ddir1 + i;
```

```
saveAs("Tiff", name1);
```

```
close();
```

## **IMAGEJ → Writing Macros**

*(use the macro-recorder under  
plugins—macro--record to get started)*

```
}
```



## IMAGEJ → Writing Macros

```
Dialog.create("TILER Z-Project")
numcols = 3;
numrows = 3;
Dialog.addNumber("ROWS", numcols);
Dialog.addNumber("COLUMNS", numrows);
Dialog.show();

numslices = nSlices;
numrows2 = numrows*numcols;
last=nSlices*numrows2;

run("Brightness/Contrast...");
setMinAndMax(0, 4095);
call("ij.ImagePlus.setDefault16bitRange", 0);
run("Make Montage...", "columns=numslices rows=numrows2 scale=1 first=1 last=last
increment=1 border=0 font=12");
run("Montage to Stack...", "images_per_row=numslices images_per_column=1
border=0");
run("Z Project...", "start=1 stop=numslices projection=[Max Intensity]");
run("Montage to Stack...", "images_per_row=1 images_per_column=numrows2
border=0");
run("Make Montage...", "columns=numcols rows=numrows scale=1 first=1
last=numrows2 increment=1 border=0 font=12");
```

```
//TRIPLE STAIN RGB ANALYZER
dir1 = getDirectory("Choose a Directory ");
list = getFileList(dir1);
ddir1 =dir1+"\\sum\\";
File.makeDirectory(ddir1);
ddir2 =dir1+"\\max\\";
File.makeDirectory(ddir2);
```

```
for (i=0; i<list.length; i++) {
file = list[i];
file2 = dir1 + list[i];
open(file2);
run("Brightness/Contrast...");
setMinAndMax(0, 4095);
call("ij.ImagePlus.setDefault16bitRange", 0);
run("Stack Splitter", "number=2");
r = "(stk_0002_)" + file;
g = "(stk_0001_)" + file;
b = "(none)" + file;
selectWindow(file);
run("Close");
run("Merge Channels...", "red=r green=g blue=*None* gray=*None*");
run("Z Project...", "projection=[Sum Slices]");
name1 = ddir1 + "SUM_RGB" + file;
saveAs("Tiff", name1);
selectWindow("RGB");
run("Z Project...", " projection=[Max Intensity]");
name1 = ddir2 + "MAX_RGB" + file ;
saveAs("Tiff", name1);
close();
close();
close();
}
dir2 = dir1+"\\max\\";
list = getFileList(dir2);
for (i=0; i<list.length; i++) {
file = dir2 + list[i];
match = indexOf(file, "RGB");
file = list[i];
if (match > 0) {
open(dir2 + list[i]);
run("Split Channels");
selectWindow(file+ " (green)");
setThreshold(94, 255);
run("Convert to Mask");
selectWindow(file+ " (green)");
wait(2000);
run("Invert");
wait(1000);
selectWindow(file+ " (green)");
wait(1000);
imageCalculator("Subtract create", file + " (red)",file + " (green)");
run("Measure");
run("Close All");
}
```

# IMAGEJ → Writing Macros

```
selectWindow("Results");
name = dir2 + "Results.xls";
run("Text...", "save=name");
run("Clear Results");
//print(f, list[2] + "\t");
//selectImage(-3);
//saveAs("Raw Data", "./2.raw");
//selectImage(-3);
//saveAs("Raw Data", "./3.raw");
//selectImage(-4);
//saveAs("Raw Data", "./3.raw");
dir2 = dir1+"\\sum\\";
list = getFileList(dir2);
for (i=0; i<list.length; i++) {
file = dir2 + list[i];
match = indexOf(file, "RGB");
file = list[i];

if (match > 0) {
open(dir2 + list[i]);
run("Split Channels");
selectWindow(file+ " (green)");
setThreshold(94, 255);
run("Convert to Mask");
selectWindow(file+ " (green)");
Wait(2000);
run("Invert");
wait(1000);
selectWindow(file+ " (green)");
wait(1000);
imageCalculator("Subtract create", file + " (red)",file + " (green)");
run("Measure");
run("Close All");
}
}
selectWindow("Results");
name = dir2 + "Results.xls";
run("Text...", "save=name");
run("Clear Results");
}
```



# IMAGEJ → Writing Plugins

```
import ij.*;
import ij.process.*;
import ij.gui.*;
import ij.plugin.*;
public class Demontager_ implements PlugIn {
    /** This plugin converts a montaged image to an image stack
        based on the number of rows and columns input by the user.
        This is the opposite of what the Image/Stacks/Make Montage
        command does.
    */
    public void run(String arg) {
        ImagePlus imp = IJ.getImage();
        GenericDialog gd = new GenericDialog("Demontager");
        gd.addNumericField("Columns:" , 1,0);
        gd.addNumericField("Rows:" , 1,0);
        gd.showDialog();
        if (gd.wasCanceled())
            return;
        int columns = (int)gd.getNextNumber();
        int rows = (int)gd.getNextNumber();
        ImageProcessor ip = imp.getProcessor();
        ImageStack stack = demontage(ip, columns, rows);
        new ImagePlus("Demontage", stack).show();
    }

    public ImageStack demontage(ImageProcessor ip, int columns, int rows) {
        int width = ip.getWidth();
        int height = ip.getHeight();
        int cropwidth = width/columns;
        int cropheight = height/rows;
        ImageStack stack = new ImageStack(cropwidth, cropheight);
        for (int j=1; j<=rows; j++) {
            for (int i = 1; i<=columns; i++) {
                int x = i * cropwidth - cropwidth;
                int y = j * cropheight - cropheight;
                ip.setRoi(x, y, cropwidth, cropheight);
                stack.addSlice(null, ip.crop());
            }
        }
        return stack;
    }
}
```

# Automating/ Calling ImageJ from other Programs

## *Call it from the command line*

### Plugins located in same directory:

```
java -cp ij.jar:. analyze blobs.tif > results.txt (Unix)
java -cp ij.jar;. analyze blobs.tif > results.txt (Windows)
```

### Or Define the Directory:

```
java -Dplugins.dir=/usr/local/ImageJ -cp /usr/local/ij.jar:. analyze blobs.tif > results.txt (Unix)
java -Dplugins.dir=C:\ImageJ -cp C:\ImageJ\ij.jar;. analyze blobs.tif > results.txt (Windows)
```

## *Run it in Batch mode → no GUI*

### *-batch path [arg]*

Runs a macro or script in batch (no GUI) mode, passing it an optional argument. ImageJ exits when the macro finishes.



# Imaging Projects

## MICROSCOPY

1) Background estimation

2) Tiled data reconstruction and correction

3a/b) Astrocyte classification

4) Microvessel analysis

CONFOCAL MICROSCOPY

5a/b) Neuron classification

6) Nucleus classification

3D ELECTRON MICROSCOPY

7) Cerebellum classification

MRI

## **DATA ACCESS**

**<ftp://neuro:n3uro@science.kent.edu/>**