REGISTER_CT App README

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Before you begin

You will need your CT scan in a 3D .tif format. In other words, you will need to convert your CT from its original format (often DICOMS, but maybe something else) into a 3D .tif format. The orientation of the .tif doesn't matter – that will be accomplished subsequently.

There are many ways to accomplish this. You might have your own processing code to read in your CT and save out a 3D .tif, or you might already have image processing software you like (e.g., 3DSlicer).

Here, I'll provide brief instructions using FIJI/ImageJ:

1. Open your file: try either File > Import or Plugins > Bio-Formats > Bio-Formats Importer

2. Do a quick check that your CT looks right

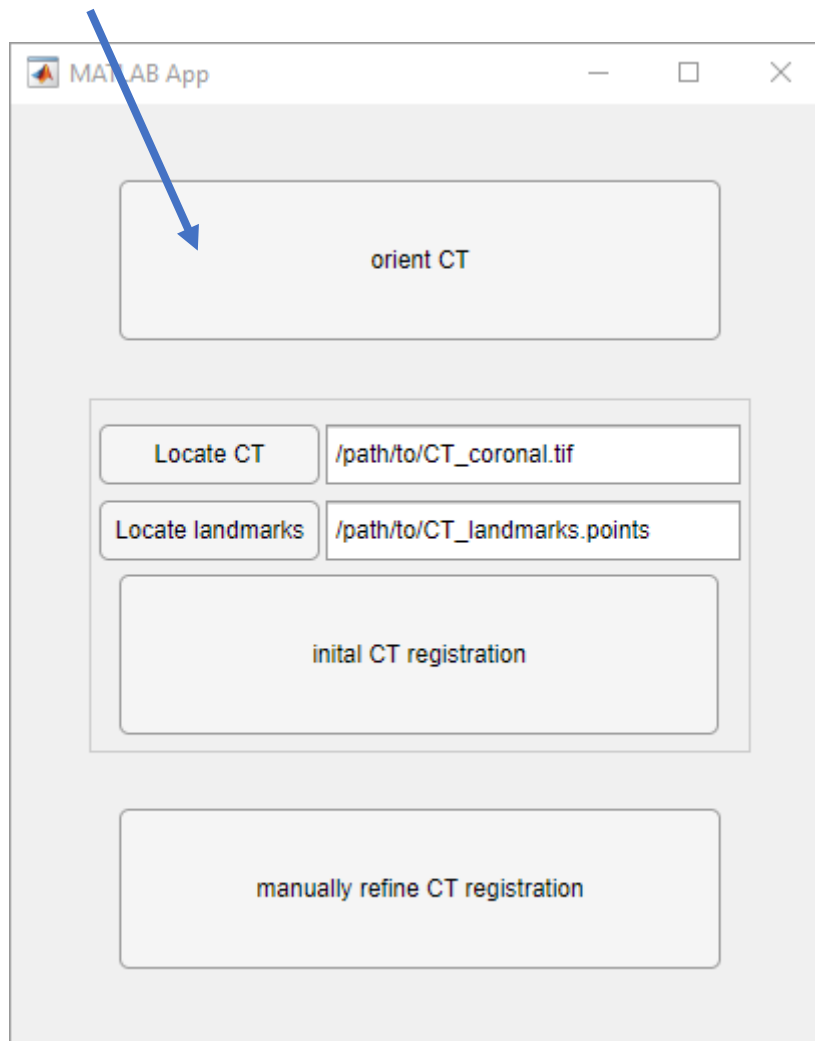3. File > Save As > Tiff…

Main steps
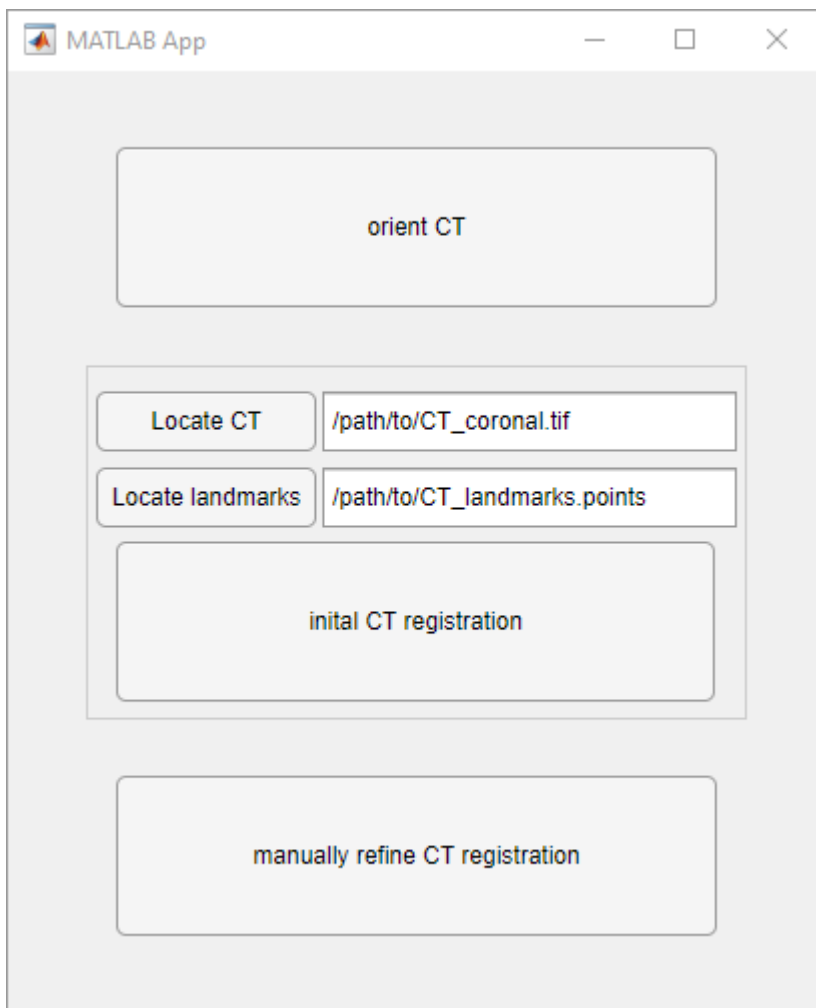
1. [Orient CT](#)

2. [Landmark-based registration](#)

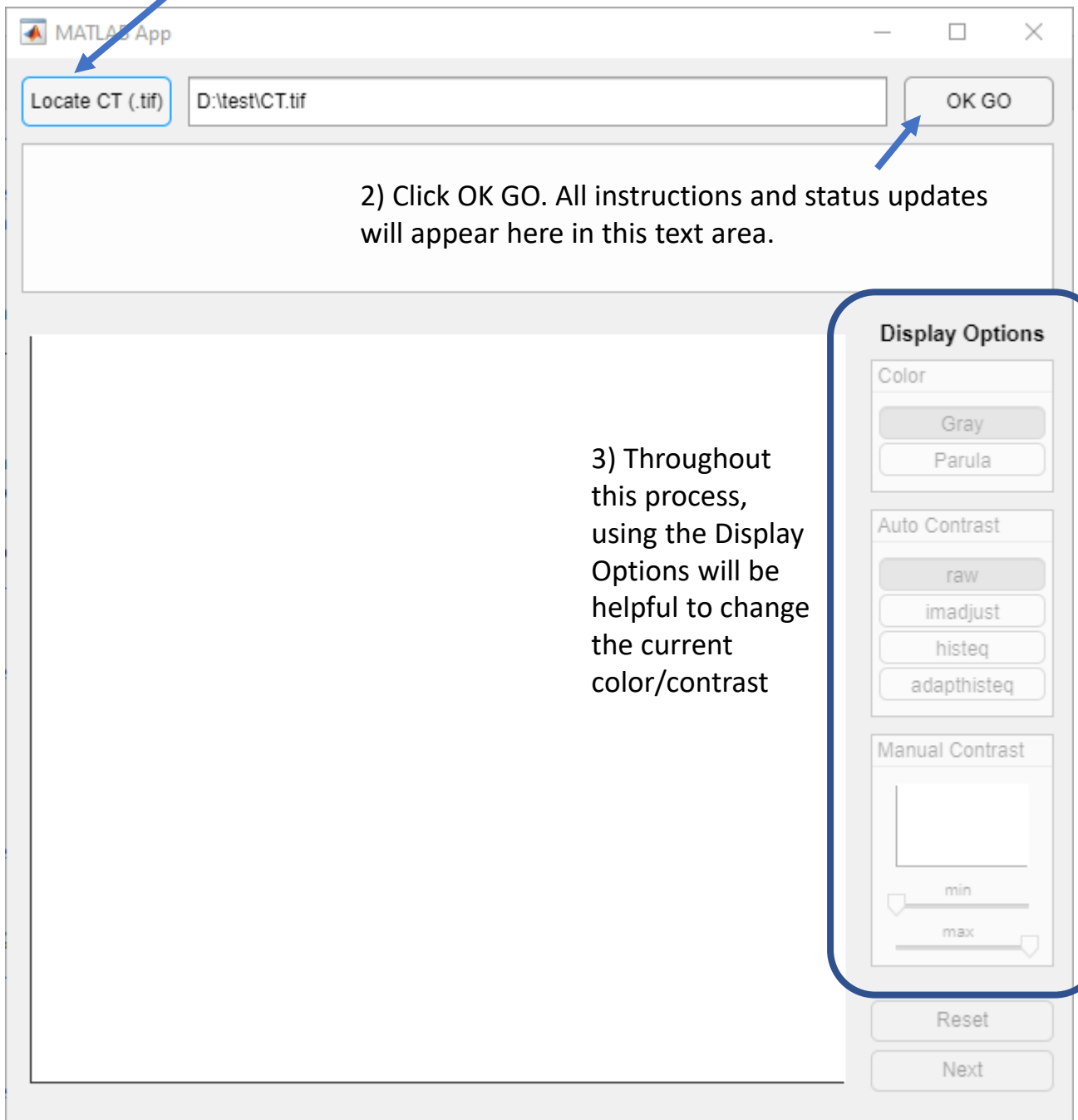3. [Manual registration refinement](#)

Step 1: Orient CT

Clicking this runs the MATLAB app
orient_CT.mlapp, which you can also run from
outside of this GUI.

orient_CT.mlapp

1) Click here to locate your CT 3D .tif file, or
enter the path in the text field.

MATLAB App — □ ×

| Locate CT (.tif) | D:\test\CT.tif | OK GO |

2) Click OK GO. All instructions and status updates
will appear here in this text area.

Display Options

Color

Gray

Parula

Auto Contrast

raw

imadjust

histeq

adapthisteq

Manual Contrast

min

max

3) Throughout
this process,
using the Display
Options will be
helpful to change
the current
color/contrast

Reset

Next

choosing the orientation to use

Instructions

MATLAB App

Locate CT (.tif)   D:\test\CT.tif   OK GO

You will eventually be choosing points along the midsagittal plane, so first let's figure out the best orientation for doing that. In each row you will see a few slices from one orientation. Click the orientation (row) in which you will best be able to visualize the midsagittal plane. The axial (horizontal) view tends to work well, though the coronal view will also work. Use the display options to adjust the display as needed. Click Reset if you need to change your mind. Click Next once you are satisfied with your choice.
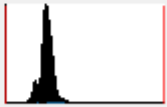
Some sample slices from your scan will be displayed. You'll want to click the <u>row</u> that contains the view/orientation on which you'll best be able to identify the midsagittal plane. Here I've chosen the 3rd row (axial view). It's okay if your scan isn't oriented cleanly along sagittal, coronal, axial axes. Just pick the view that will work best.

Display Options

Color

Gray

Parula

Auto Contrast

raw

imadjust

histeq

adapthisteq

Manual Contrast

min

max

Reset

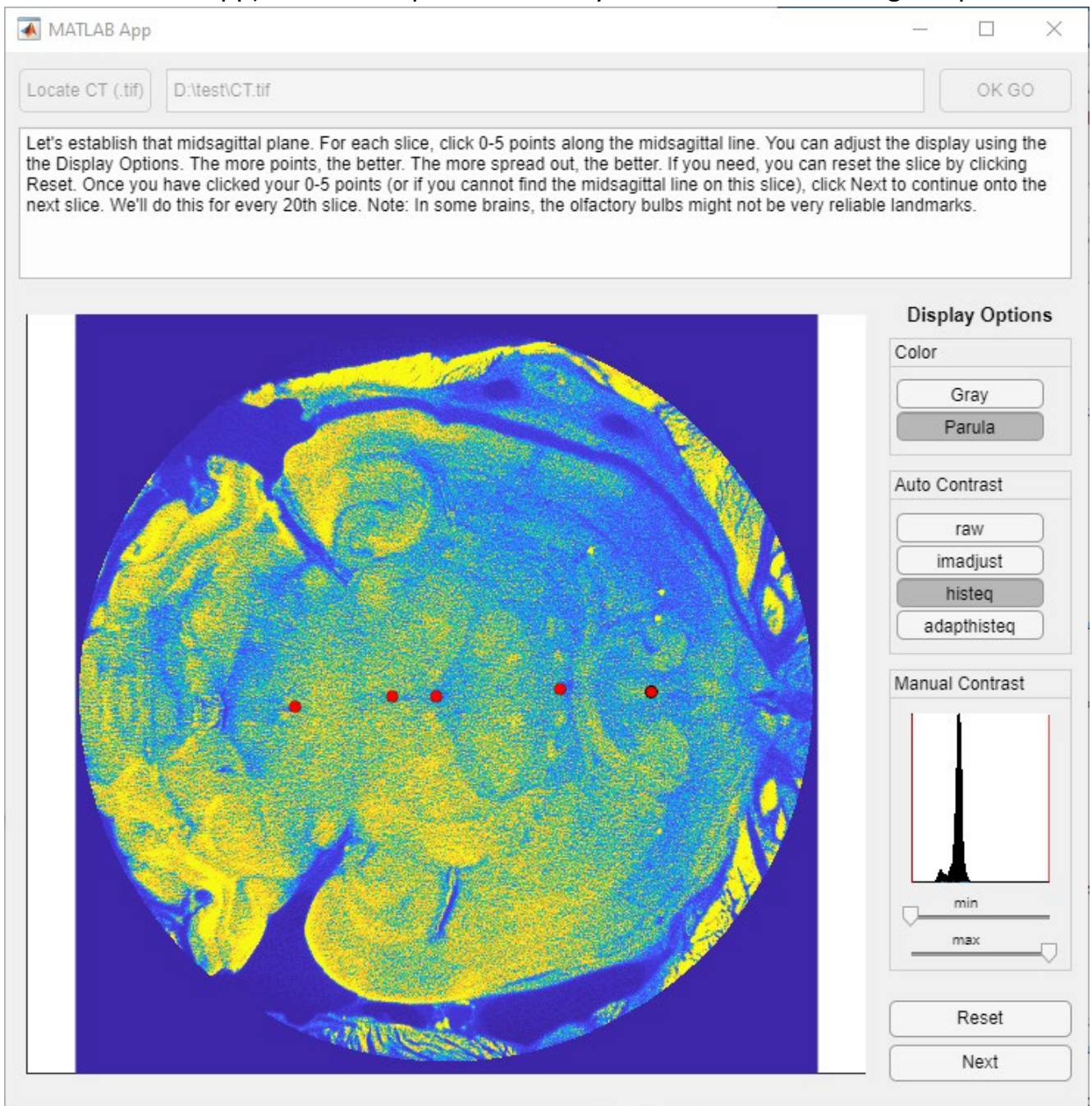Next

6

marking the midsagittal plane

Now you'll mark the midsagittal line on multiple slices using up to 5 points per slice. Look for where you can clearly decide where the midsagittal plane is. You'll do this every 20 slices (this can be changed by editing the app.output.midsag_slice_step variable in the app). You can skip slices where you can't see the midsagittal plane.
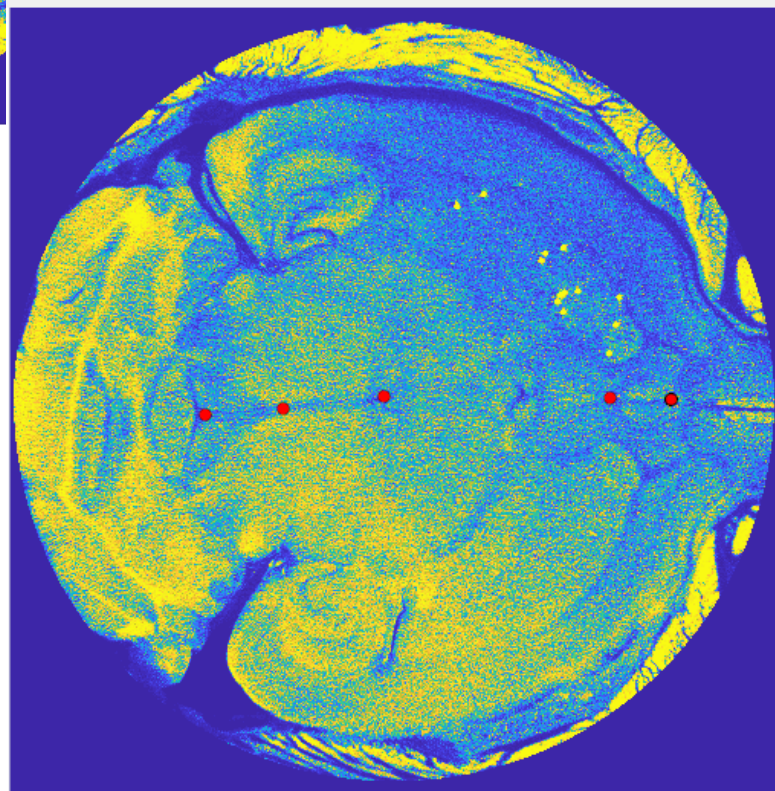
marking the midsagittal plane
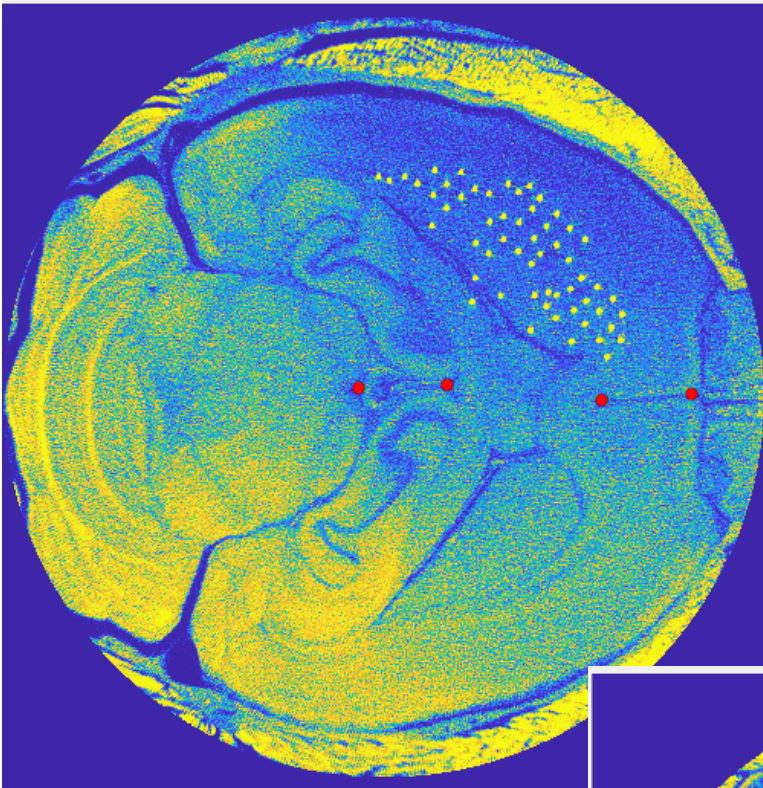
Some more examples

Pay attention to the text field updates. Once you reach the end, the text area will display "Calculating midsagittal plane… "
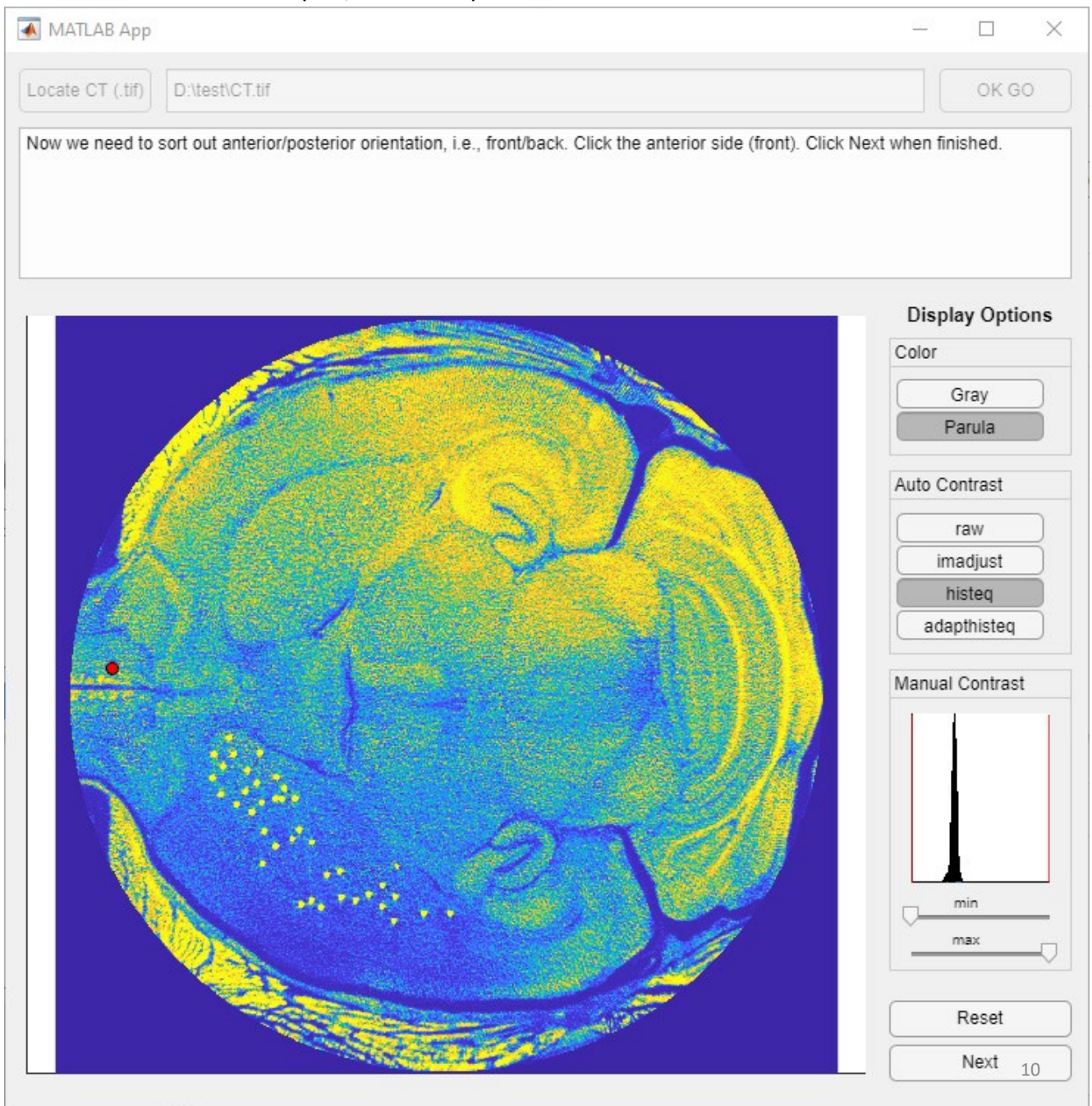
orienting: part 1

Now you'll see the midsagittal slice, and you'll draw an approximate line along the long axis (anterior-posterior) of the brain. The purpose is to get a brain in approximately the orientation you would use when you slice it coronally, so that it'll be easy to mark landmarks going forward.
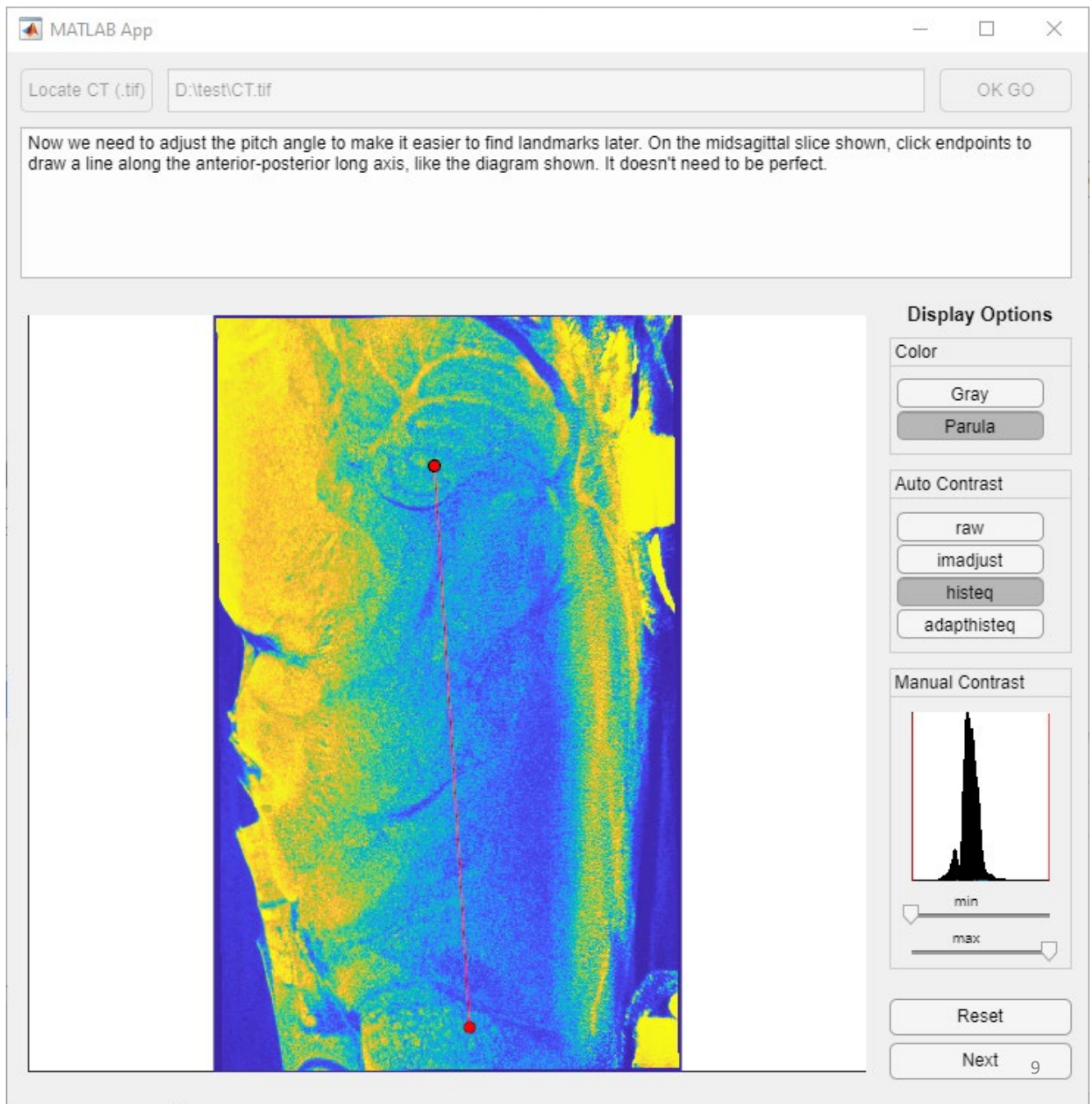
orienting: part 2

Now you'll see the middle axial/horizontal slice (note if this slice is too far dorsal or ventral for you to make sense of it, it means your CT scan is padded dorsally or ventrally with a bunch of blank space. You could crop it and then run this app again). Click the anterior side (i.e., the front) of the brain.

orienting: part 3

Now you'll see the middle coronal slice (note if this slice is too far dorsal or ventral for you to make sense of it, it means your CT scan is padded anteriorly or posteriorly with a bunch of blank space. You could crop it and then run this app again). Click the dorsal side (i.e., the top) of the brain.
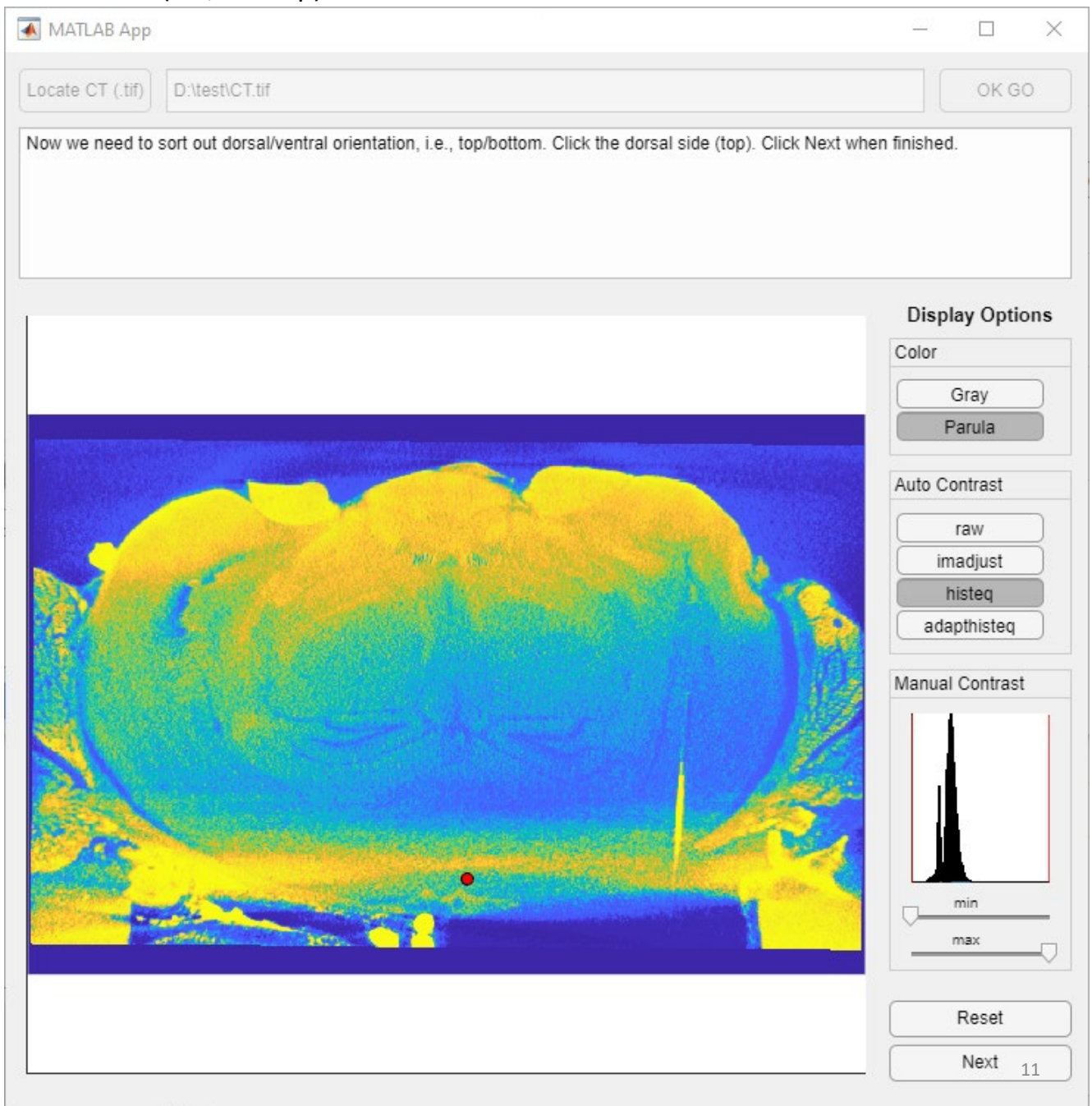
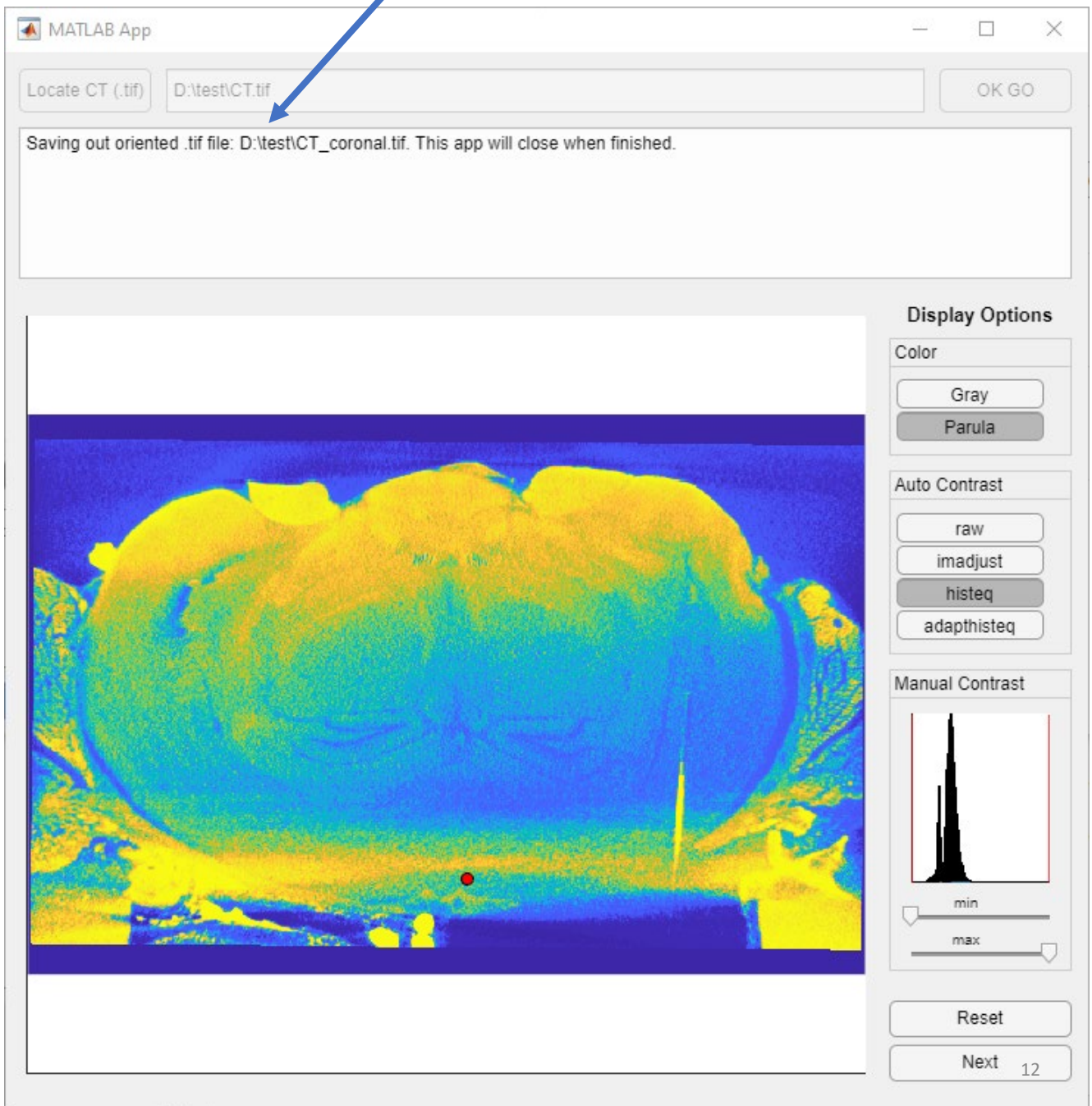# saving

Your newly-oriented CT will be saved and will have "_coronal.tif" at the end of the file name. It may take a few or several minutes.

Step 2: Landmark-based registration

The next step will be landmark-based registration. But before you do this, you'll need to mark and save your landmarks. You'll do this in FIJI. The next several slides will provide more instructions.
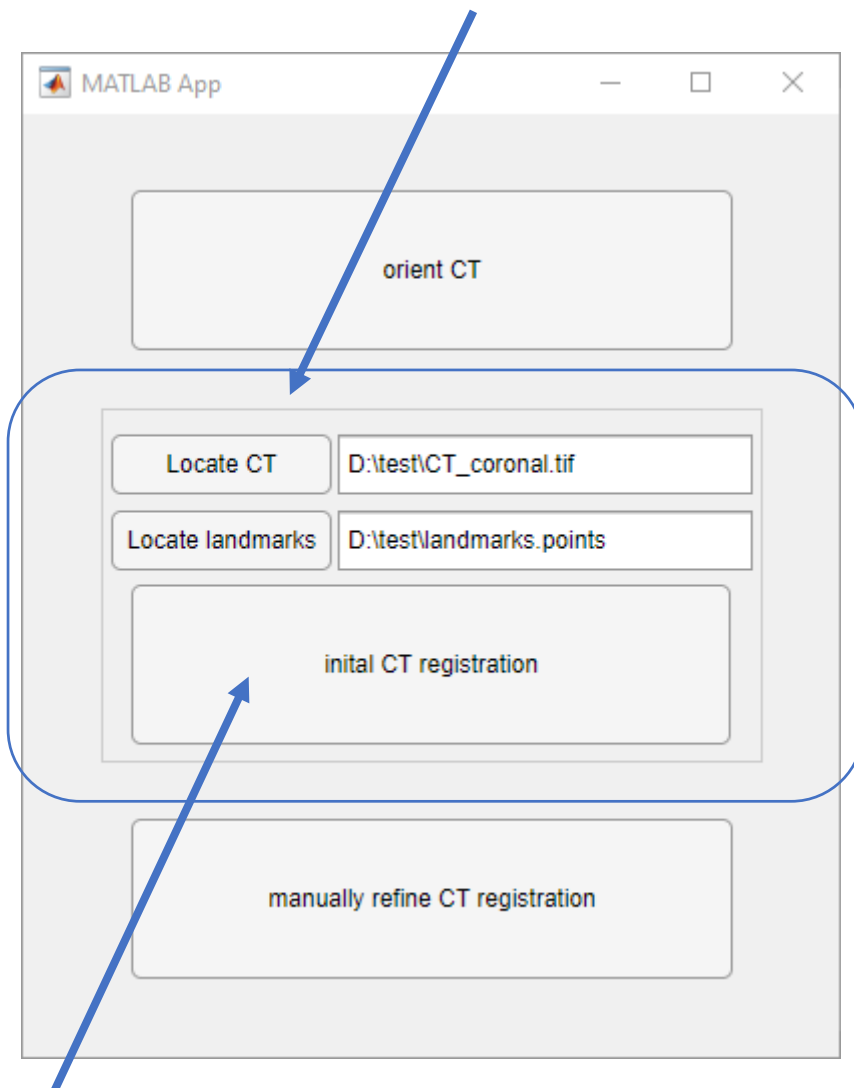
Note: this calls the function landmark_registration.m, which you can also run from the MATLAB command line. Open the function for more details.

Landmarks

Reference: these landmarks have been adapted from Sergejeva et al., 2015

Computational Neuroscience

Anatomical landmarks for registration of experimental image data to volumetric rodent brain atlasing templates

CrossMark

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[d] Institute of Neuroscience and Medicine (INM-6), Research Center Jülich, 52425 Jülich, Germany
[e] Brain Research Network (BreNt) and RIKEN Advanced Science Institute, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan
[f] Informed Minds, 2100 NE 16th Ave, Wilton Manors, FL 33305, USA

HIGHLIGHTS

- 16 anatomical landmarks reliably recognized in T_1, T_2, and T_2^* mouse brain MRI.
- All landmarks identified in Nissl histology and block-face images from the mouse brain.
- Most landmarks identified in MRI and histological images from the rat brain.
- Guidelines for locating each landmark presented in the Scalable Brain Atlas.
- Facilitates landmark-based registration to Waxholm Space and thus worldwide datasharing.

Instructions

- On the following slides you'll see descriptions and images of the reference landmarks (average of seven independent raters) shown on the Allen Mouse Brain CCF Atlas.
    - You can use FIJI/ImageJ to open the atlas and landmarks (which you generated via GENERATE_ATLAS_FILES, see README)

- Use the Name Landmarks and Register plugin in FIJI/ImageJ to establish your set of landmarks.

- Be sure to **name them exactly as shown.** This is important because the registration program will look for specifically-named landmarks.

- Skip any that you can't find.

- Once you've finished, click through them to double check their placement.
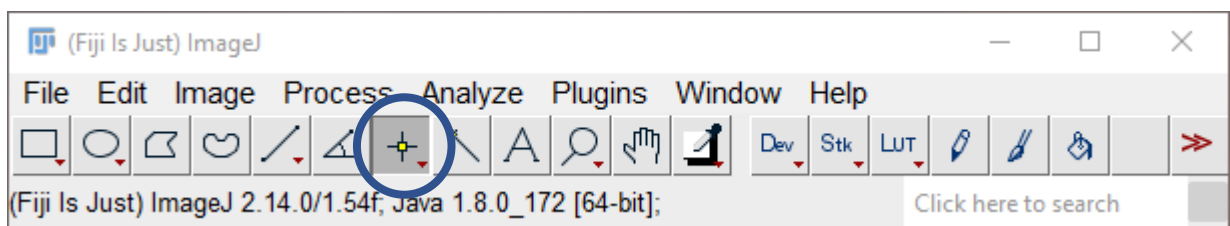
Name Landmarks & Register
Instructions

1. Open your coronally-oriented CT in FIJI
2. Plugins > Landmarks > Name Landmarks and Register
3. Load your landmarks file if you already started one.

For each landmark:

1. Go to the coronal slice that has your landmark
2. Make sure you are in point selection mode

3. Click Add New Point if necessary
4. Click Rename to name the current point
5. Click the name of the point to set it
6. Use Show/Reset/Rename/Delete as necessary
7. Save as you proceed, and once you're finished

Name Landmarks & Register
Example

Helpful hints and notes

1. You may find it helpful to look at your scan from multiple views. In FIJI/ImageJ you can do this using orthogonal views: Image > Stacks > Orthogonal Views or use the shortcut Ctrl+Shift+H
   - Note that you can't mark landmarks in this mode, so you'll have to return to the default view (close the other views or Ctrl+Shift+H again) before it'll let you mark landmarks

2. You may find it helpful to consult multiple atlases to get a sense of the anatomy as you go. Here are links to some:
   1. Allen Brain Atlas – there are a bunch of different tools here
   2. Kim Lab atlas

LANDMARKS

The following slides will show you reference landmarks and descriptions from both Sergejeva et al., 2015, and as defined by our group on the Allen Mouse Brain CCF Atlas.

The **name** of the landmark will be the slide title.

KM

Definition of landmark KM - Cortex middle   X

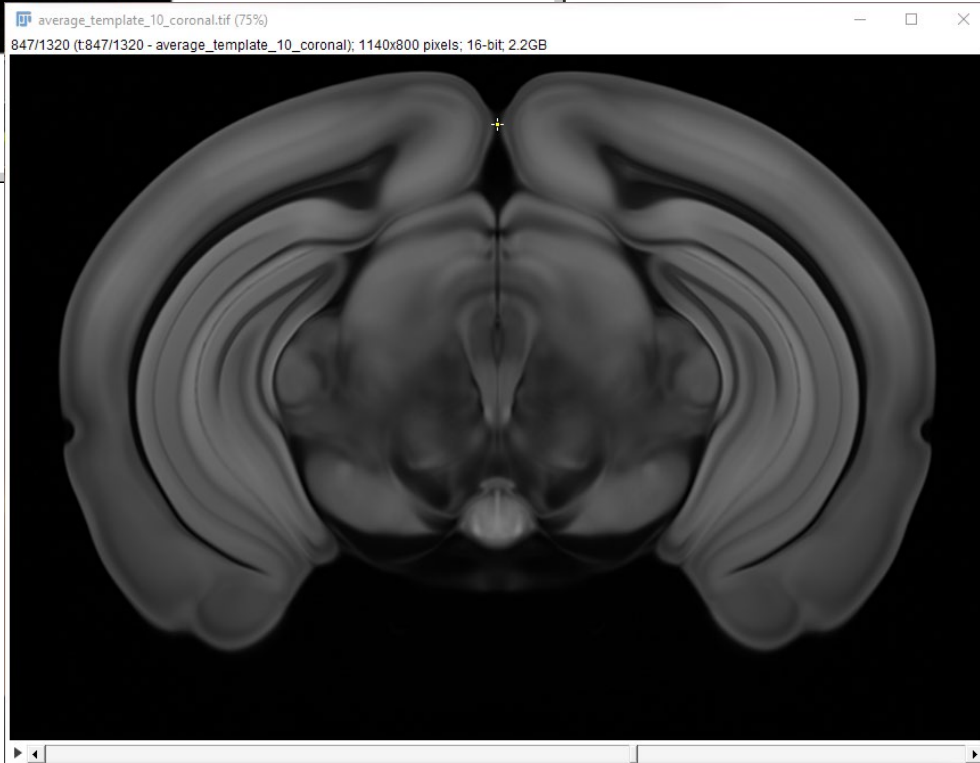T1(MDEFT): Move anteriorly until the last slice with clearly separated cortex halves. KM is between them
Note: the image below differs from the WHS standard in cutting angle, contrast and extent.

KM

The last slice with obviously separated cortex-halves

Click between them

average_template_10_coronal.tif (75%)   —   □   ×
847/1320 (t:847/1320 - average_template_10_coronal); 1140x800 pixels; 16-bit; 2.2GB

PM

Definition of landmark PM - Pontine nucleus middle

T1(MDEFT): Move anteriorly until the last slice where Pontine Nuclei are connected. PM is the center of this connection.
Note: the image below differs from the WHS standard in cutting angle, contrast and extent.

PM
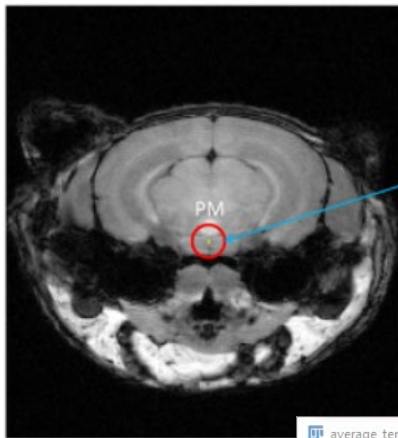
1. The last slice where pontine nuclei are connected with each other
Click in the center of this connection

average_template_10_coronal.tif (75%)
901/1320 (t:901/1320 - average_template_10_coronal); 1140x800 pixels; 16-bit; 2.2GB
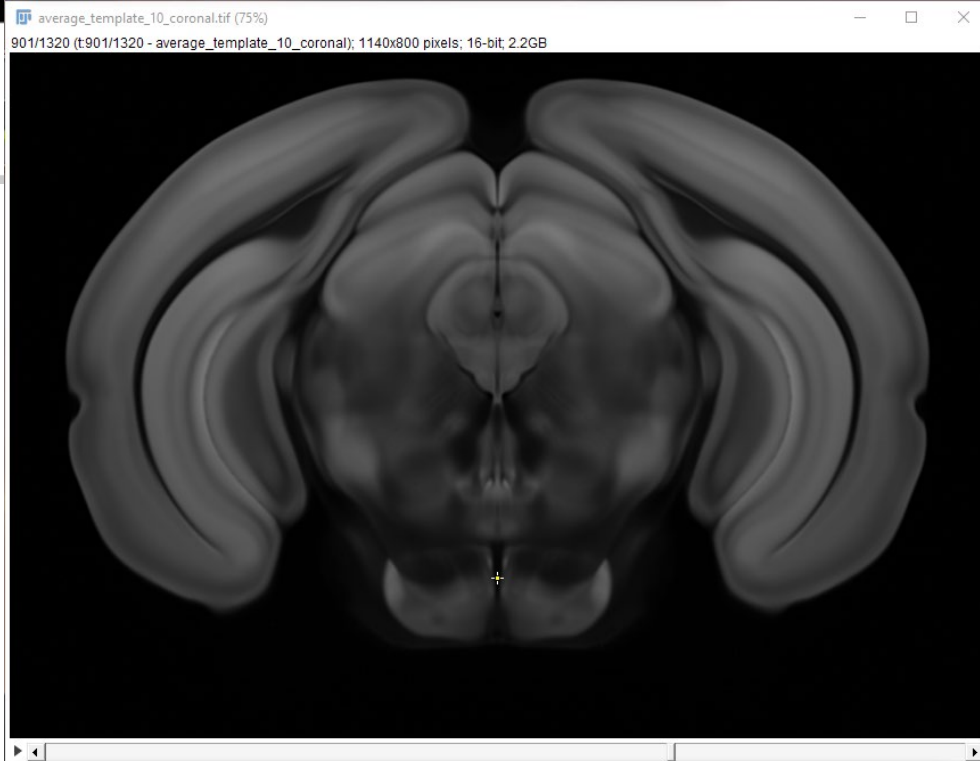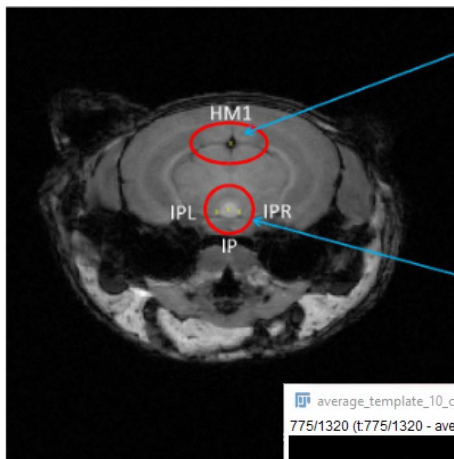
HM1

Definition of landmark HM1 - Hippocampus middle 1   X

T1(MDEFT): Move anteriorly until Corpus Callosum gets continuous in the middle. HM1 is in the opening at the crossing of midline and borderline between hippocampus and thalamus/superior colliculus.
Note: the image below differs from the WHS standard in cutting angle, contrast and extent.

1. Corpus callosum gets continuous in the middle (faint on this image)

Click in the opening underneath: at the crossing of the midline with the borderline between hippocampus and thalamus

2. Interpeduncular nucleus, its middle slice. It is the largest and the most sharply bounded here

HM1

IPL   IPR
IP

average_template_10_coronal.tif (75%)   —   □   ×
775/1320 (t:775/1320 - average_template_10_coronal); 1140x800 pixels; 16-bit; 2.2GB
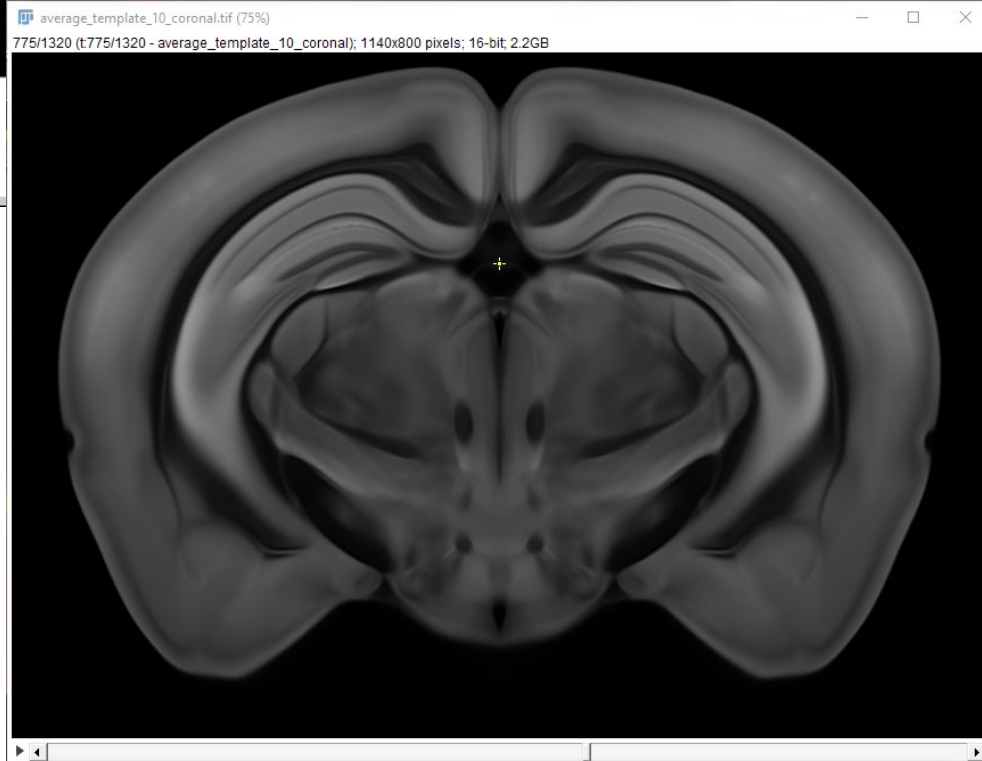
IP

Definition of landmark IP - Interpeduncular nucleus middle   X

Amended definition: Find the coronal slice where the IP extends the most ventrally. IP is at the ventral-most point in the middle.

HM1

IPL   IPR

IP

1. Corpus callosum gets continuous in the middle (faint on this image)

Click in the opening underneath: at the crossing of the midline with the borderline between hippocampus and thalamus
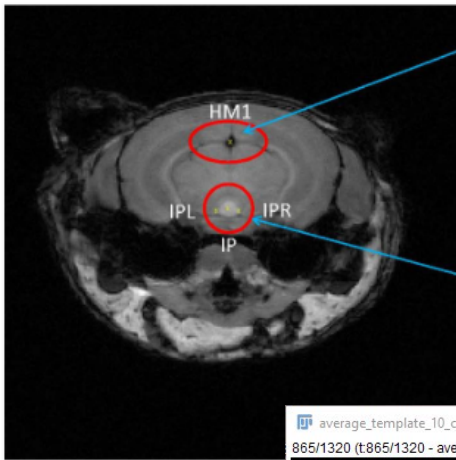
2. Interpeduncular nucleus, its middle slice. It is the largest and the most sharply bounded here

average_template_10_coronal.tif (75%)

865/1320 (t:865/1320 - average_template_10_coronal); 1140x800 pixels; 16-bit; 2.2GB

IPL

Definition of landmark IPL - Interpeduncular nucleus left   X

Amended definition: Find the coronal slice where the IP extends the most ventrally. IPL is at the left-most point of the IP.
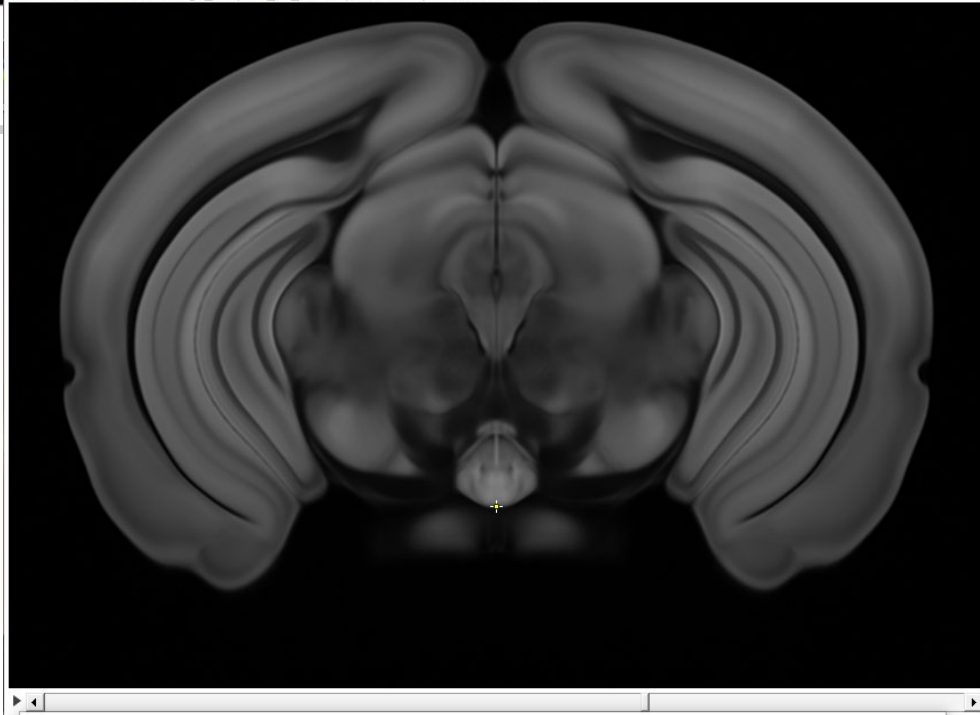
1. Corpus callosum gets continuous in the middle (faint on this image)

Click in the opening underneath: at the crossing of the midline with the borderline between hippocampus and thalamus

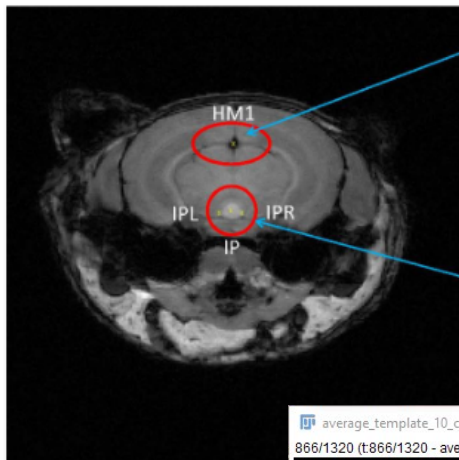2. Interpeduncular nucleus, its middle slice. It is the largest and the most sharply bounded here

HM1
IPL   IPR
IP

average_template_10_coronal.tif (75%)
866/1320 (t:866/1320 - average_template_10_coronal); 1140x800 pixels; 16-bit; 2.2GB

IPR

Definition of landmark IPR - Interpeduncular nucleus right   X
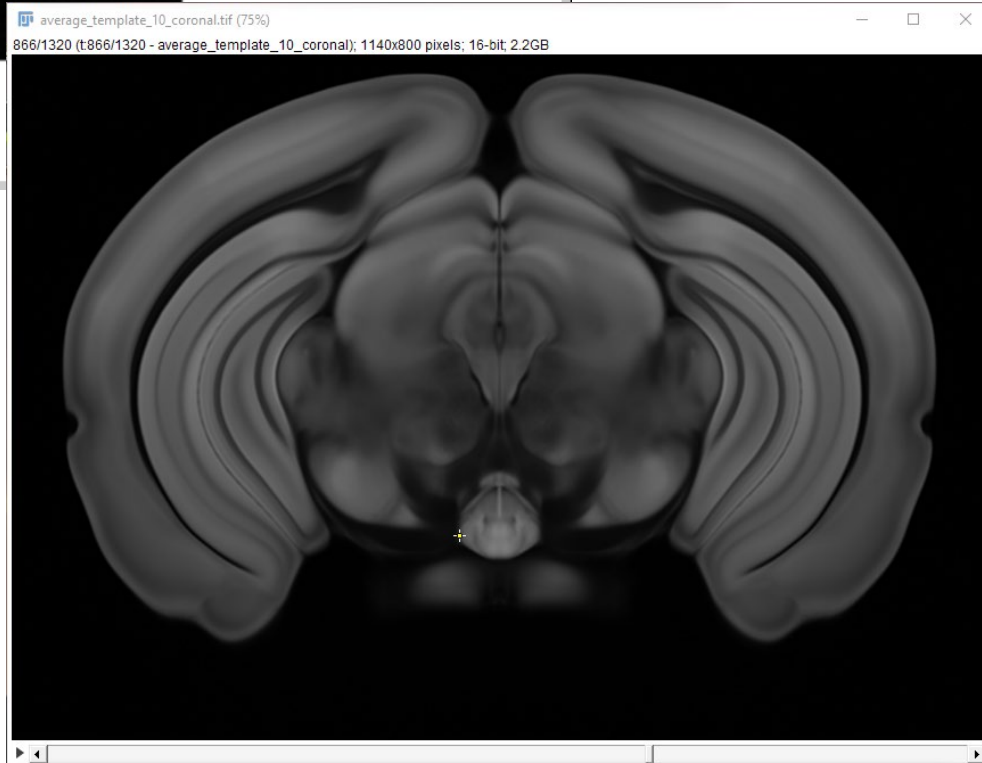
Amended definition: Find the coronal slice where the IP extends the most ventrally. IPR is at the right-most point of the IP.

1. Corpus callosum gets continuous in the middle (faint on this image)

Click in the opening underneath: at the crossing of the midline with the borderline between hippocampus and thalamus
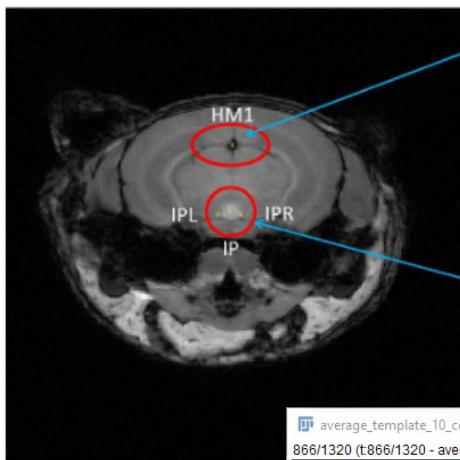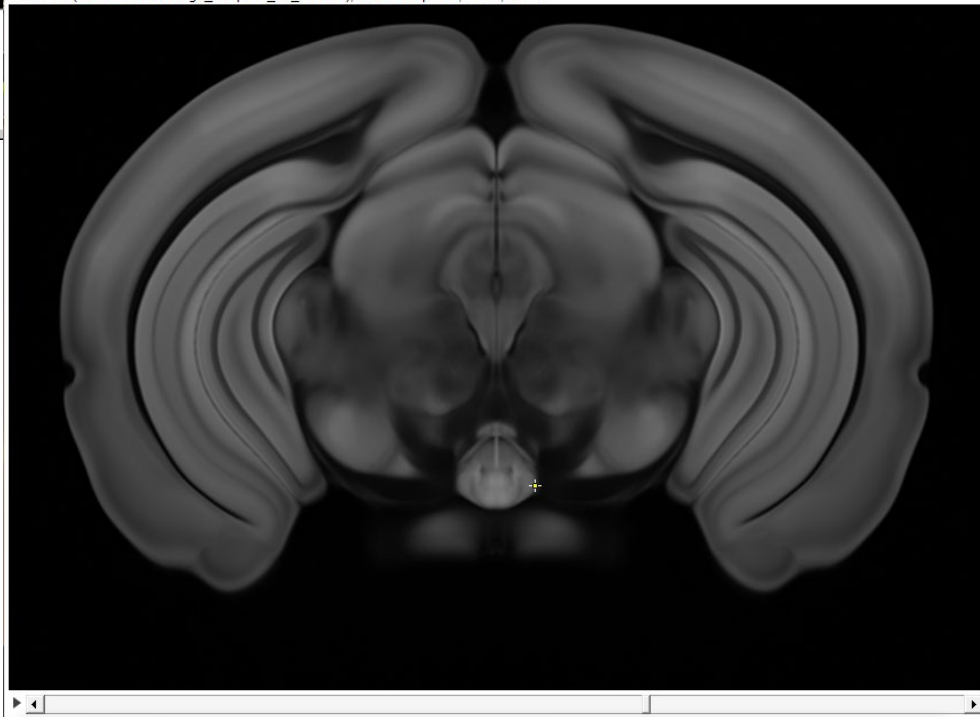
2. Interpeduncular nucleus, its middle slice. It is the largest and the most sharply bounded here
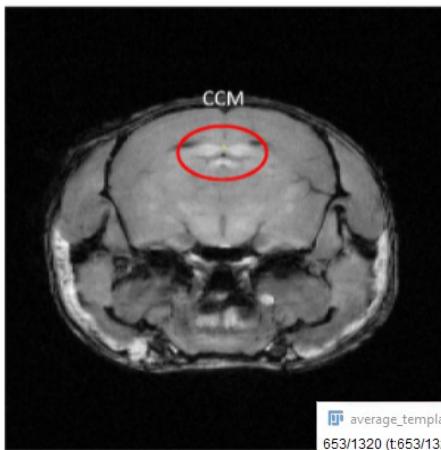
HM1

IPL   IPR

IP

average_template_10_coronal.tif (75%)

866/1320 (t:866/1320 - average_template_10_coronal); 1140x800 pixels; 16-bit; 2.2GB

CCM

Definition of landmark CCM - Corpus Callosum middle   X

Amended definition: Move posteriorly and use the last slice where the hippocampi are still connected at the midline. CCM is at the connection point.

CCM

The last slice with this obvious butterfly

Click on the middle of the borderline between corpus callosum and its fore wings

average_template_10_coronal.tif (75%)

653/1320 (t:653/1320 - average_template_10_coronal); 1140x800 pixels; 16-bit; 2.2GB
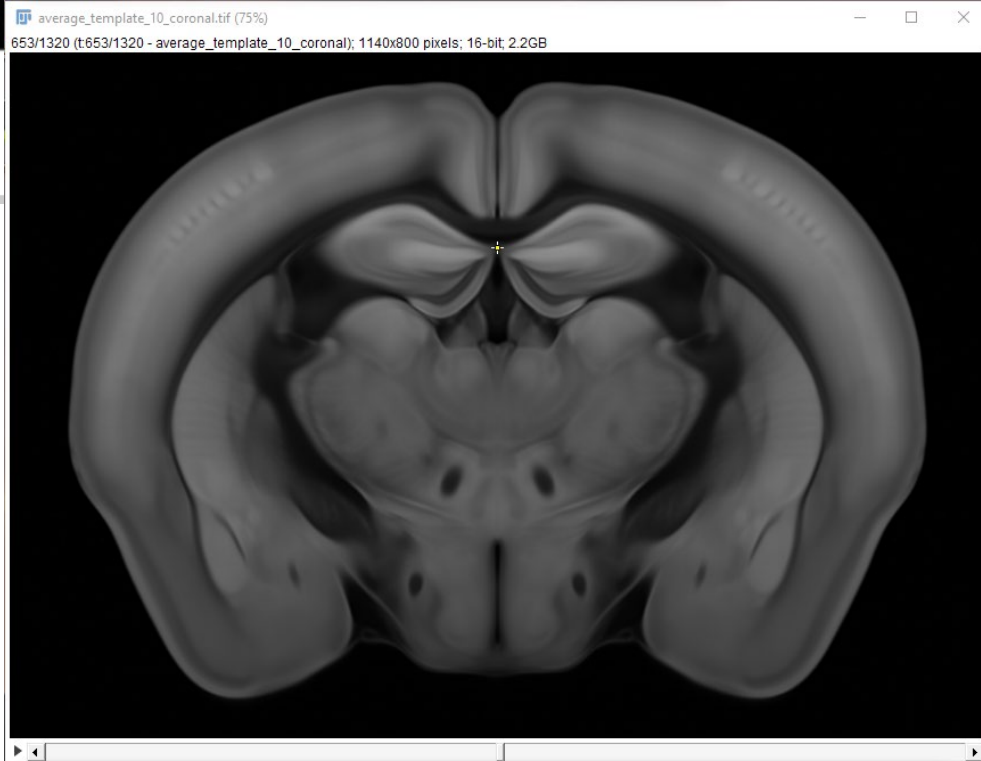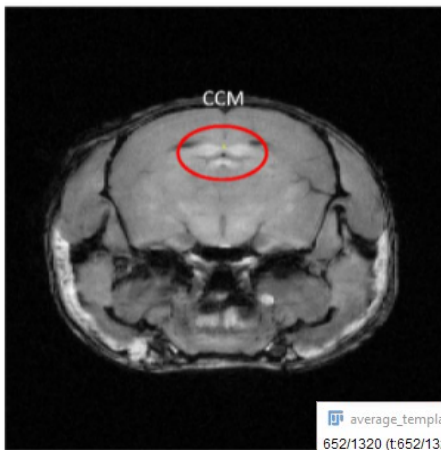
CCML

Definition of landmark CCM - Corpus Callosum middle   X

Amended definition: Move posteriorly and use the last slice where the hippocampi are still connected at the midline. CCML is the left-most point of the brain on this coronal slice (the DV coordinate doesn't matter here).

CCM

The last slice with this obvious butterfly

Click on the middle of the borderline between corpus callosum and its fore wings

average_template_10_coronal.tif (75%)

652/1320 (t:652/1320 - average_template_10_coronal); 1140x800 pixels; 16-bit; 2.2GB
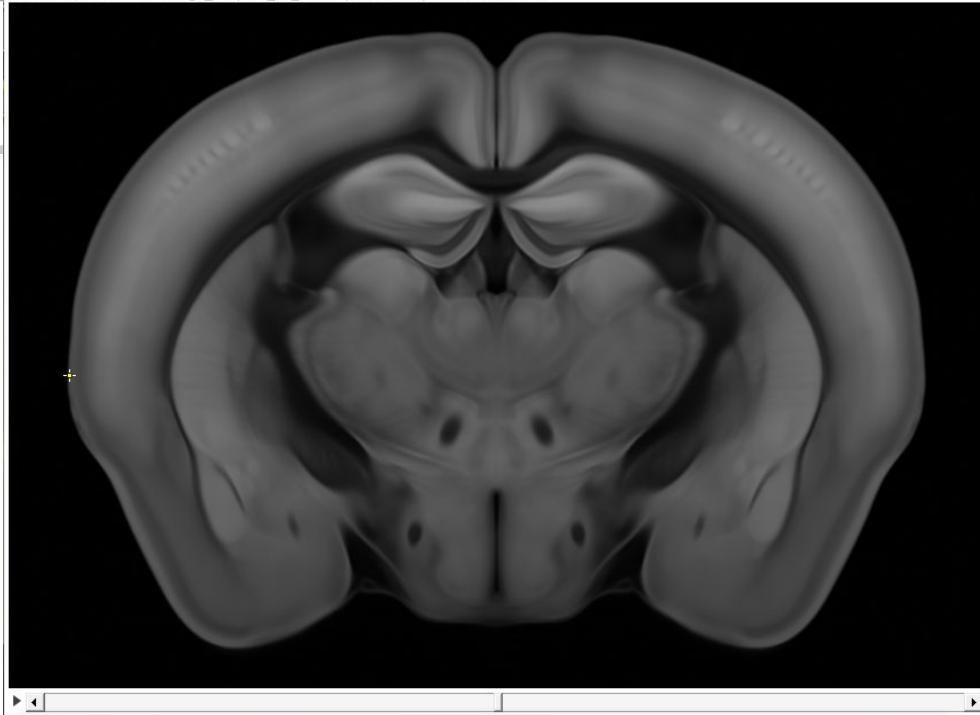
CCMR

Amended definition: Move posteriorly and use the last slice where the hippocampi are still connected at the midline. CCMR is the right-most point of the brain on this coronal slice (the DV coordinate doesn't matter here).

CCM

The last slice with
this obvious butterfly

Click on the middle of the
borderline
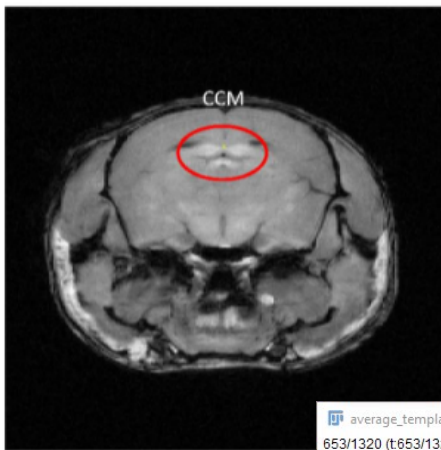between corpus callosum
and its fore wings

average_template_10_coronal.tif (75%)

653/1320 (t:653/1320 - average_template_10_coronal); 1140x800 pixels; 16-bit; 2.2GB
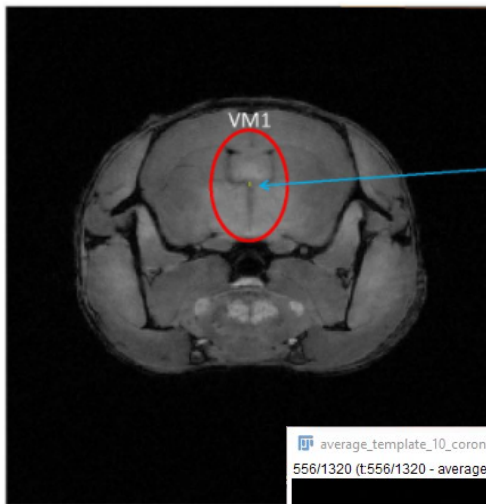
VM1

Definition of landmark VM1 - Ventricle middle 1   X

T1(MDEFT): Move anteriorly until you get to the most articulate "Wineglass"-slice, where the lateral ventricles are still connected to the "glass stem". VM1 is between stem and bowl. Note: the image below differs from the WHS standard in cutting angle, contrast and extent.

VM1

„Wine-glass"-slice;
as distinct from the next slice,
lateral ventricles are still
connected to the „glass stem",
and the wine glass
is the most articulate
here

Click on the junction
between the stem and the bowl

average_template_10_coronal.tif (75%)   —   □   ×
556/1320 (t:556/1320 - average_template_10_coronal); 1140x800 pixels; 16-bit; 2.2GB

VM1L

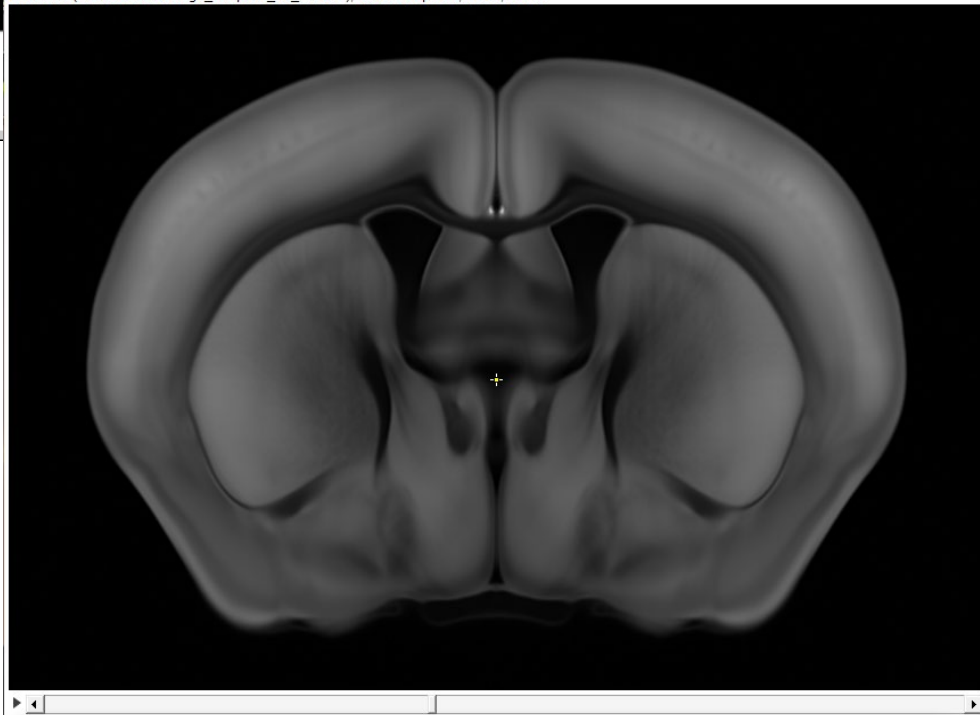**Definition of landmark VM1 - Ventricle middle 1**   X

T1(MDEFT): Move anteriorly until you get to the most articulate "Wineglass"-slice, where the lateral ventricles are still connected to the "glass stem". VM1 is between stem and bowl. Note: the image below differs from the WHS standard in cutting angle, contrast and extent.

VM1L is the left-most point of the brain on this coronal slice (the DV coordinate doesn't matter here).

VM1

"Wine-glass"-slice; as distinct from the next slice, lateral ventricles are still connected to the "glass stem", and the wine glass is the most articulate here

Click on the junction between the stem and the bowl

average_template_10_coronal.tif (75%)   □   ✕

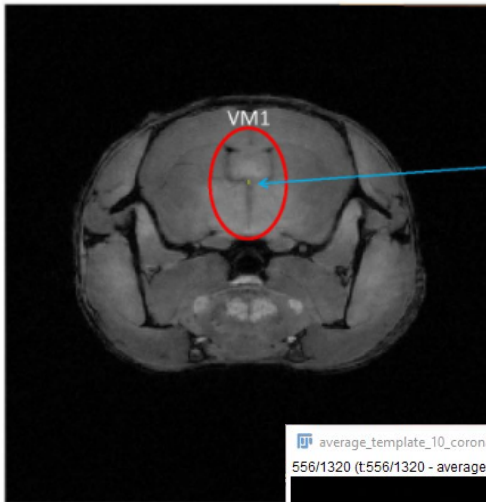556/1320 (t:556/1320 - average_template_10_coronal); 1140x800 pixels; 16-bit; 2.2GB

VM1R

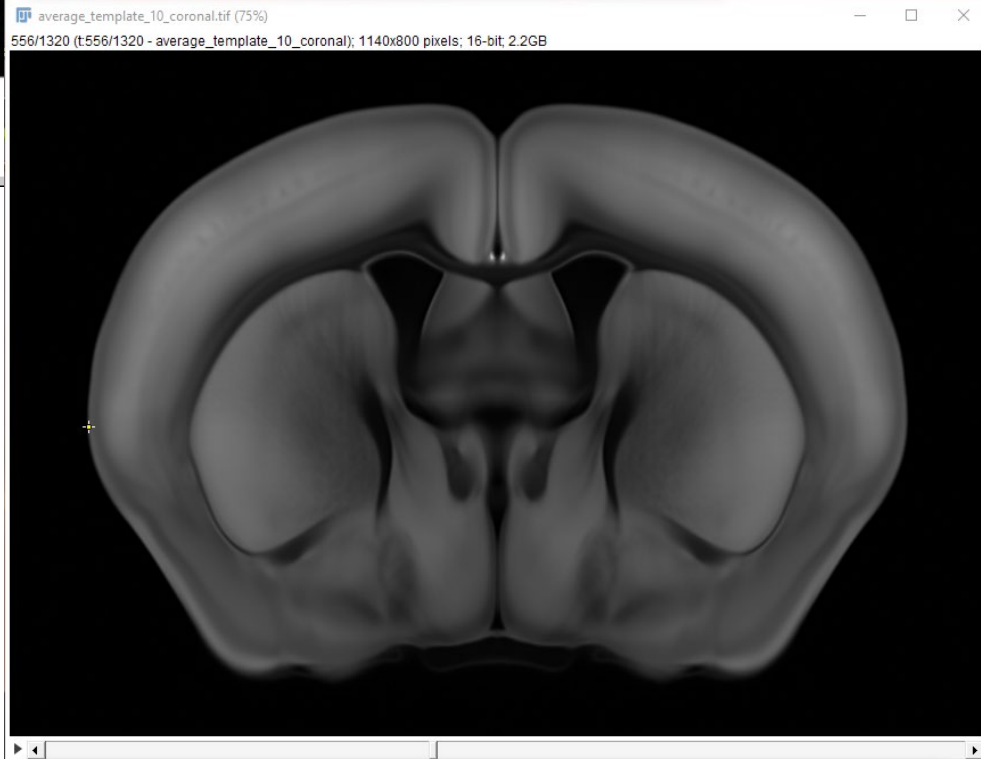**Definition of landmark VM1 - Ventricle middle 1**   X

T1(MDEFT): Move anteriorly until you get to the most articulate "Wineglass"-slice, where the lateral ventricles are still connected to the "glass stem". VM1 is between stem and bowl.
Note: the image below differs from the WHS standard in cutting angle, contrast and extent.

VM1R is the right-most point of the brain on this coronal slice (the DV coordinate doesn't matter here).

VM1

„Wine-glass"-slice;
as distinct from the next slice,
lateral ventricles are still
connected to the „glass stem",
and the wine glass
is the most articulate
here

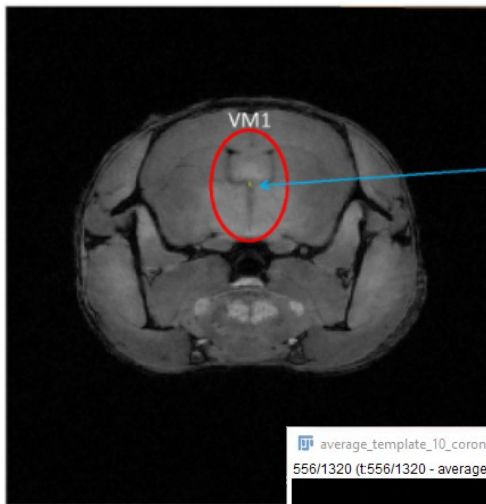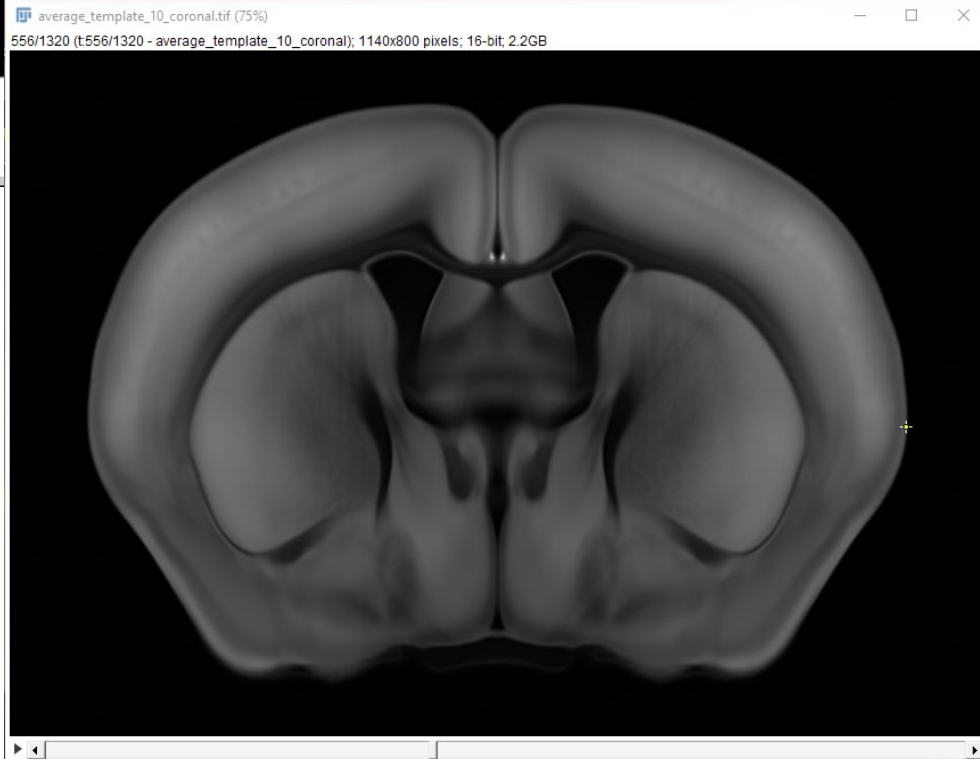Click on the junction
between the stem and the bowl

average_template_10_coronal.tif (75%)   — □ ✕
556/1320 (t:556/1320 - average_template_10_coronal); 1140x800 pixels; 16-bit; 2.2GB

ACL

Definition of landmark ACL - Anterior Commissure Left   X

T1(MDEFT): Move anteriorly until the first slice with no shade of the anterior commissure in the middle. ACL is on the remaining 'islet' on the left, on its left border
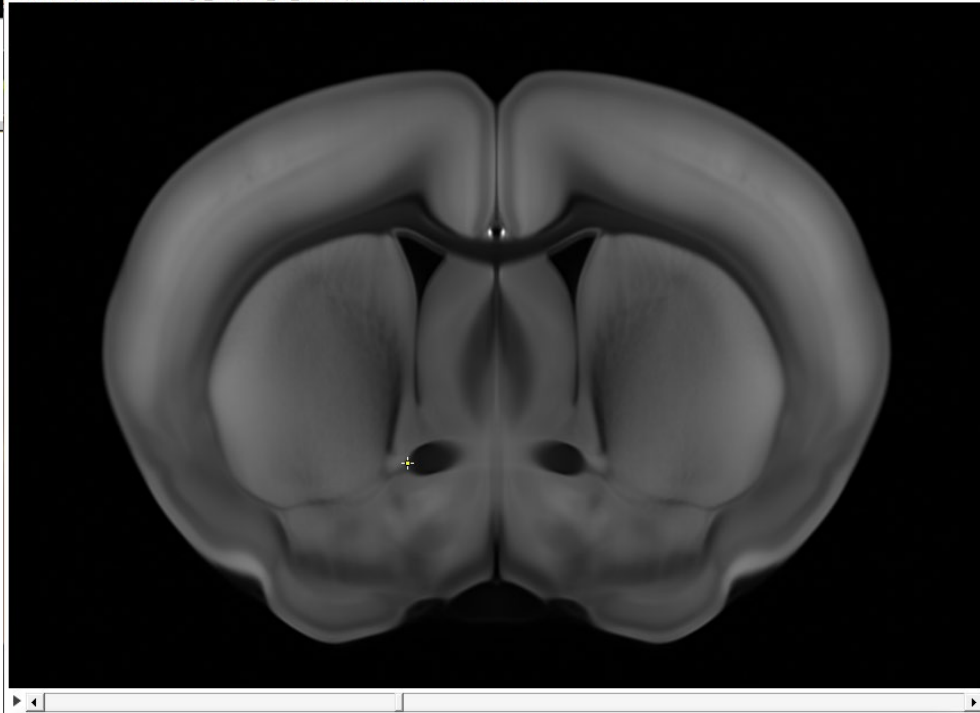Note: the image below differs from the WHS standard in cutting angle, contrast and extent.

ACL   ACR

The first slice with
no shade of the
anterior commissure
(which is vary vague here)
in the middle

Click on the outer points of
the remaining „islets" of it

average_template_10_coronal.tif (75%)   — □ ✕
507/1320 (t:507/1320 - average_template_10_coronal); 1140x800 pixels; 16-bit; 2.2GB

ACR

Definition of landmark ACR - Anterior Commissure Right   X

T1(MDEFT): Move anteriorly until the first slice with no shade of the anterior commissure in the middle. ACR is on the remaining 'islet' on the right, on its right border
Note: the image below differs from the WHS standard in cutting angle, contrast and extent.
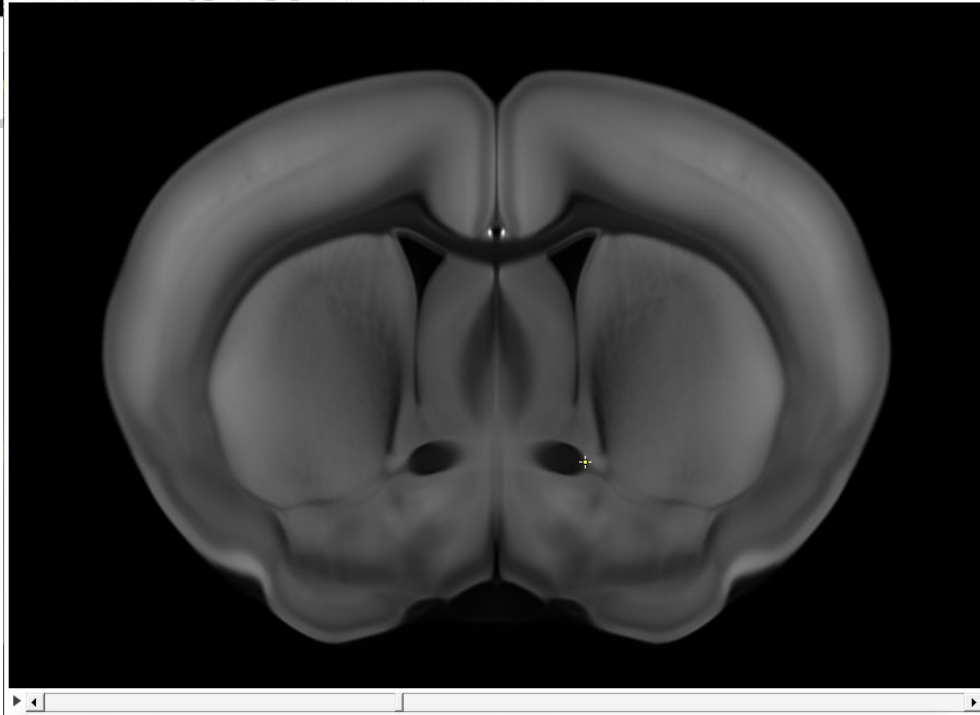
ACL   ACR

The first slice with no shade of the anterior commissure (which is vary vague here) in the middle

Click on the outer points of the remaining „islets" of it

average_template_10_coronal.tif (75%)   —   □   ×
507/1320 (t:507/1320 - average_template_10_coronal); 1140x800 pixels; 16-bit; 2.2GB

FM1

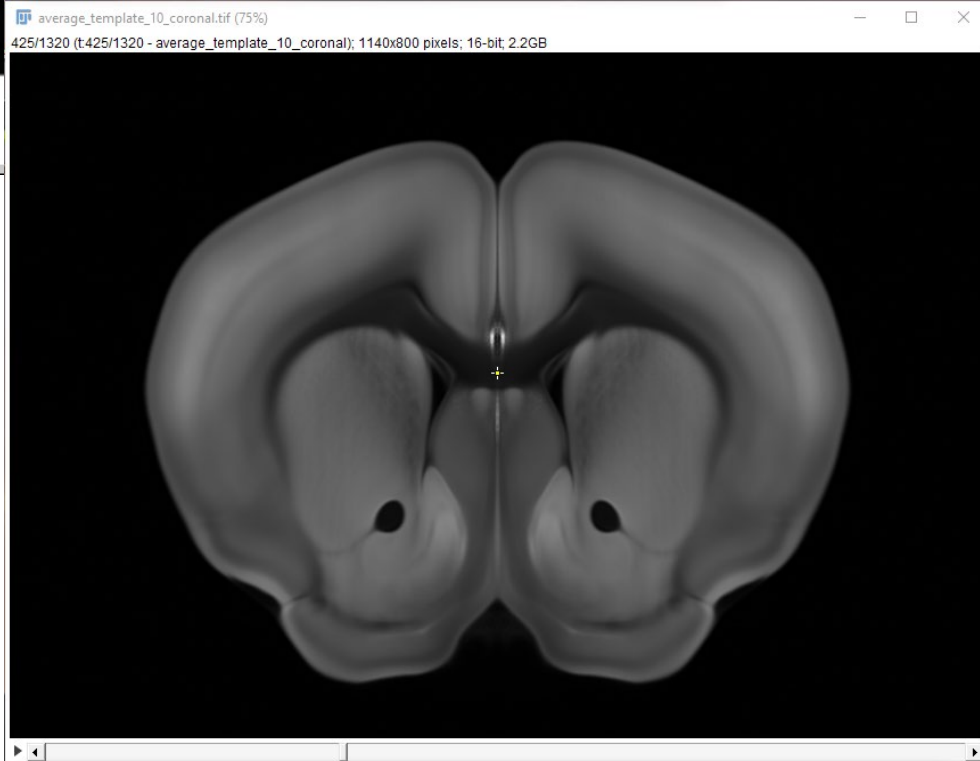Definition of landmark FM1 - Frontal middle 1   X

T1(MDEFT): Move anteriorly until the last slice where the Corpus Callosum is uninterupted.
FM1 is in its middle.
Note: the image below differs from the WHS standard in cutting angle, contrast and extent.

FM1
FL2 • • • FR2

Corpus callosum is uninterrupted
for the last time;
on the next slice the vertical slit
on it cuts it through

Click on its middle and
on the inner ventricle tips

average_template_10_coronal.tif (75%)   □   ×
425/1320 (t:425/1320 - average_template_10_coronal); 1140x800 pixels; 16-bit; 2.2GB

FL2

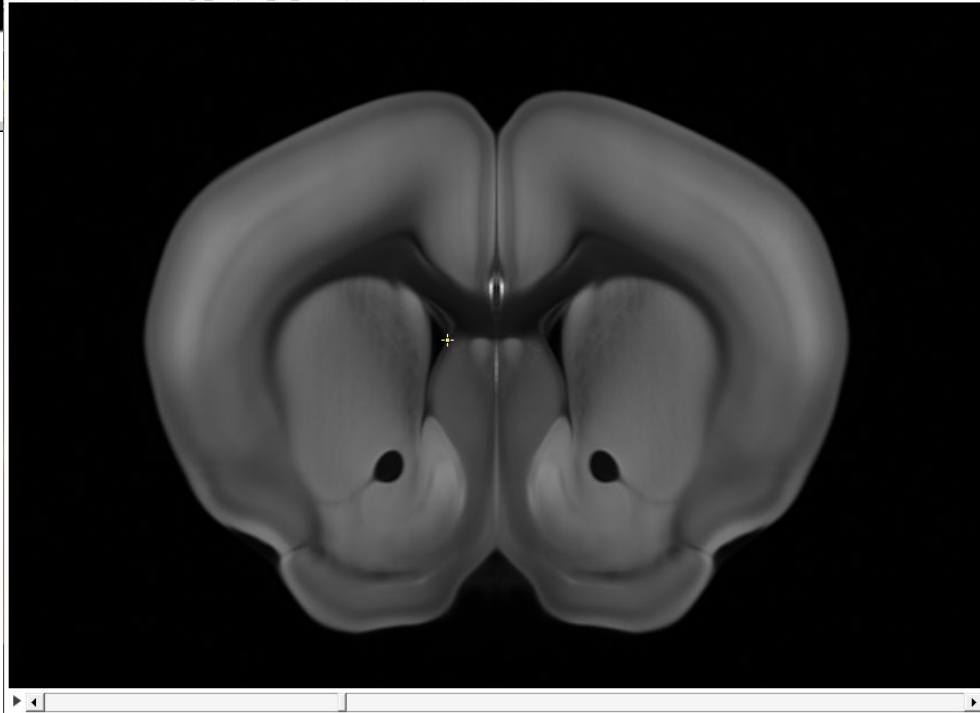**Definition of landmark FL2 - Frontal left 2**   X

T1(MDEFT): Move anteriorly until the last slice where the Corpus Callosum is uninterupted. FL2 is the left inner ventricle tip
Note: the image below differs from the WHS standard in cutting angle, contrast and extent.

FM1
FL2   FR2

Corpus callosum is uninterrupted
for the last time;
on the next slice the vertical slit
on it cuts it through

Click on its middle and
on the inner ventricle tips

average_template_10_coronal.tif (75%)   — □ ×
425/1320 (t:425/1320 - average_template_10_coronal); 1140x800 pixels; 16-bit; 2.2GB

# FR2

Definition of landmark FR2 - Frontal right 2 X

T1(MDEFT): Move anteriorly until the last slice where the Corpus Callosum is uninterupted. FR2 is the right inner ventricle tip
Note: the image below differs from the WHS standard in cutting angle, contrast and extent.

FM1
FL2   FR2

Corpus callosum is uninterrupted for the last time;
on the next slice the vertical slit on it cuts it through

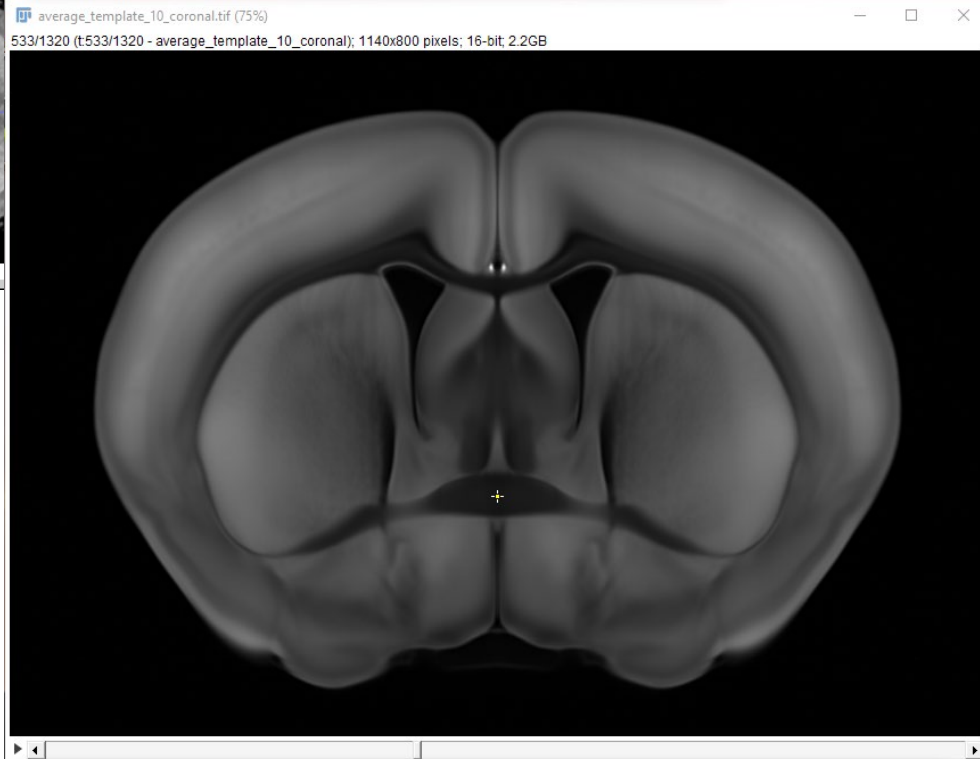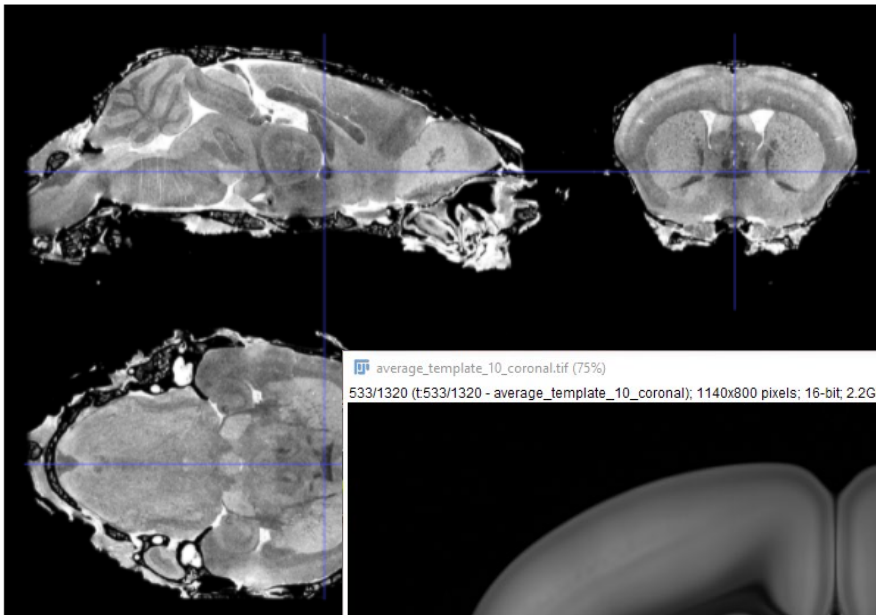Click on its middle and on the inner ventricle tips

average_template_10_coronal.tif (75%)   □ ×
425/1320 (t:425/1320 - average_template_10_coronal); 1140x800 pixels; 16-bit; 2.2GB

36
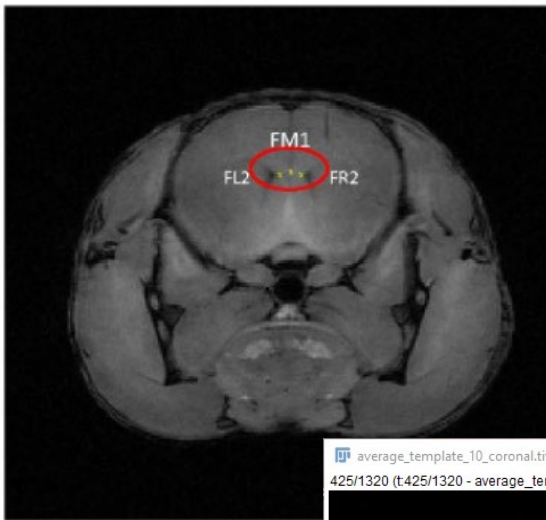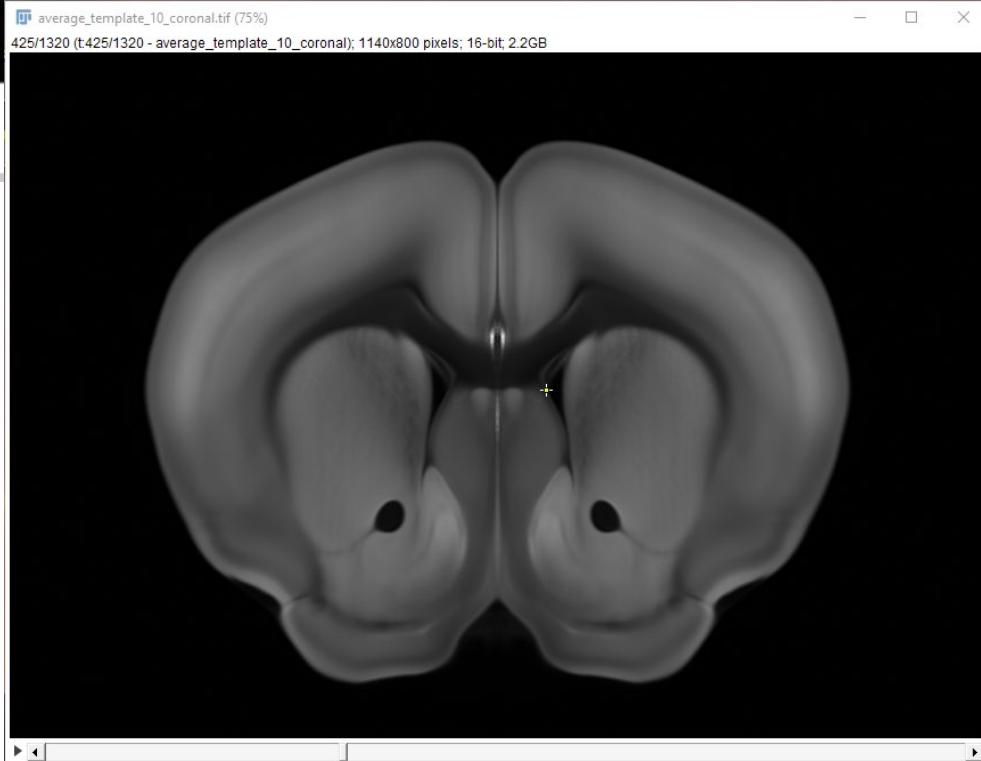
AC

Definition of landmark AC - Anterior Commissure: WHS origin   X

AC is used as the origin of the Mouse-WHS coordinate space. It is located in the anterior commissure (AC) at the intersection between:

- The mid-sagittal plane,
- A coronal plane passing midway (rostro-caudal) through the anterior and posterior branches of AC,
- A horizontal plane passing midway through the most dorsal and ventral aspect of the AC.

Note: the image below shows the actual orientation of the WHS mouse.

average_template_10_coronal.tif (75%)

533/1320 (t:533/1320 - average_template_10_coronal); 1140x800 pixels; 16-bit; 2.2GB

Step 2: Landmark-based registration

Click here to locate your coronally-oriented CT 3D .tif file, or enter the path in the text field. Similarly, locate your landmarks .points file.
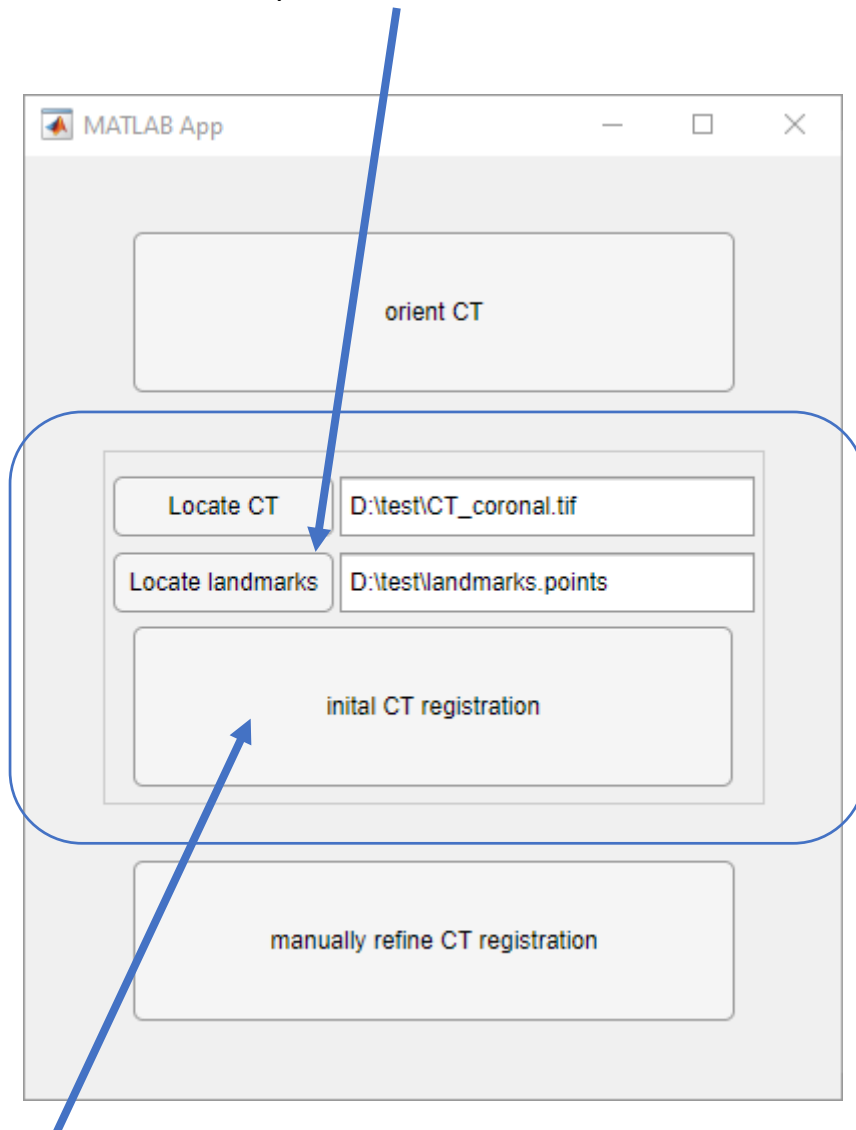
Note: this calls the function landmark_registration.m, which you can also run from the MATLAB command line. Open the function for more details.

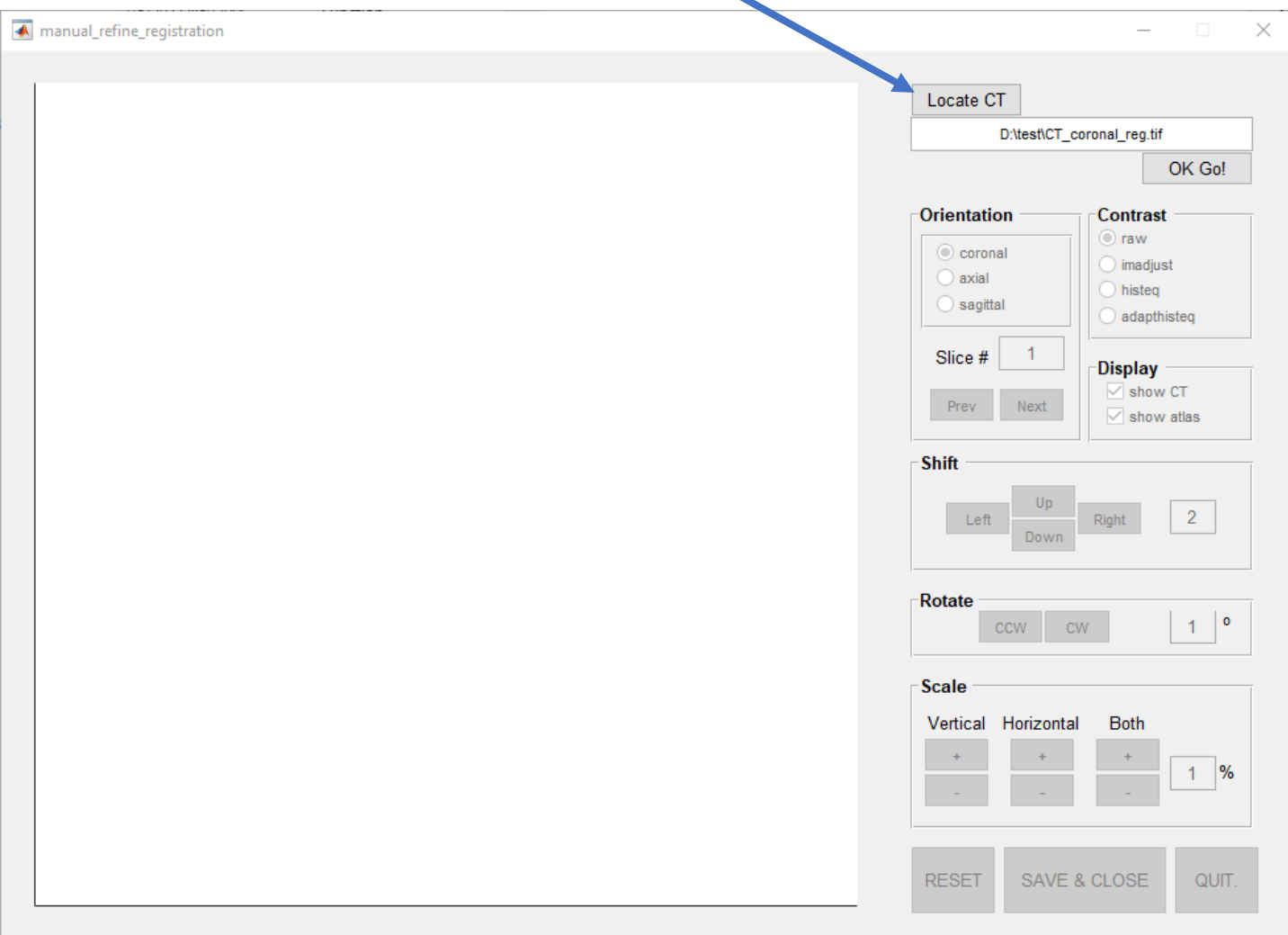Your resulting file will have _reg.tif at the end of the file name.
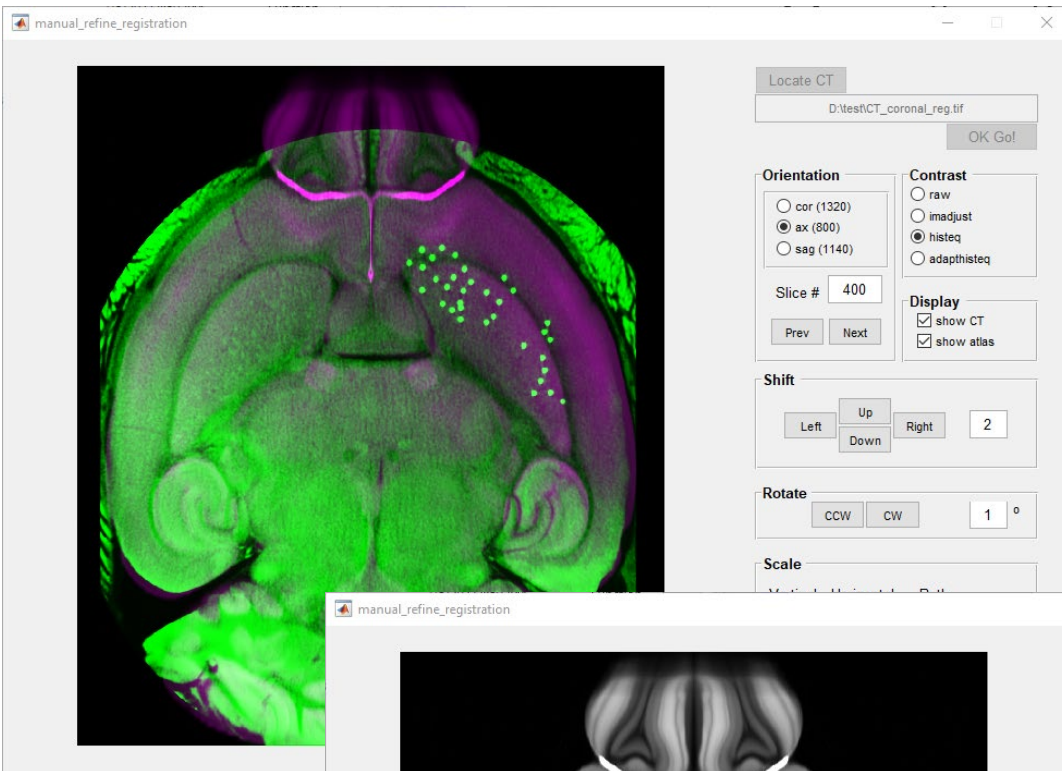
Step 3: Manually refine registration
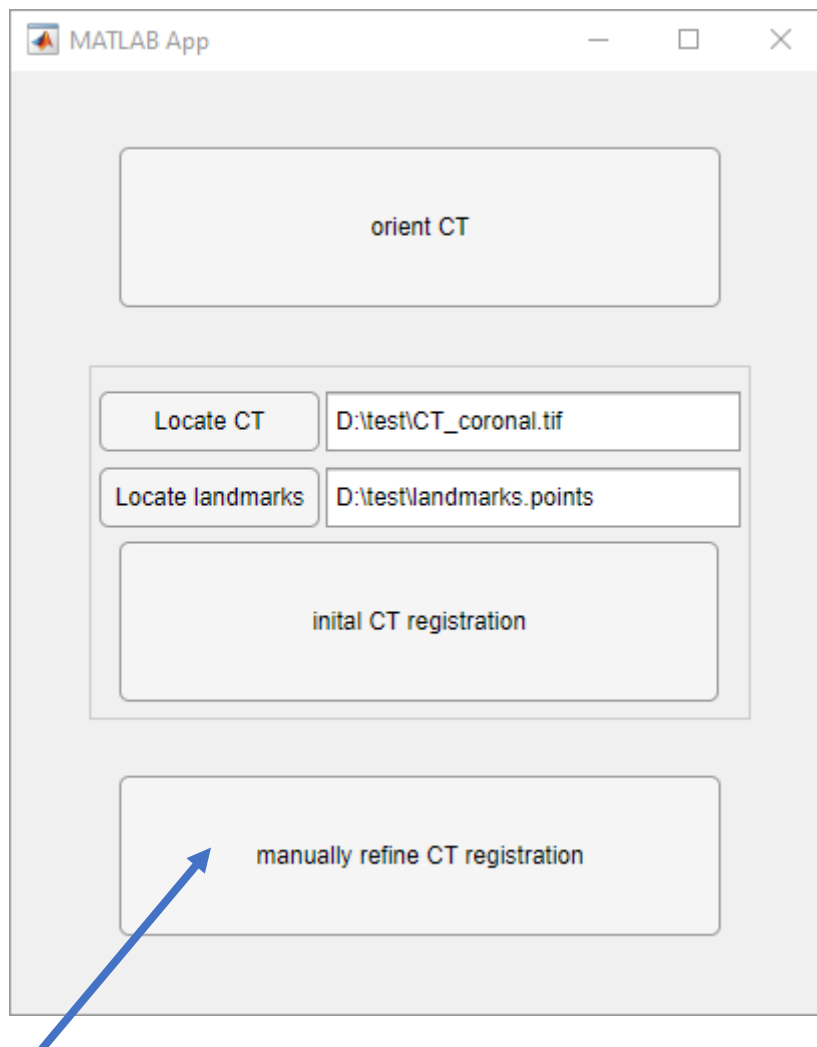
Note: this opens the GUI manual_refine_registration.m, which you can also run from the MATLAB command line.

Manually refining your registration

Click here to locate registered CT .tif file, or enter the path in the text field. Then click OK Go!

Manually refining your registration

Use different views, slices, and display options to refine the registration of your CT to the atlas.

Prioritize the alignment of the regions you most care about.

Save when you're done. It will save. Your resulting file will have _manually_adjusted.mat at the end of the filename.