Summary of evaluation of 21st Century Medicine Rabbit Brain Submission #6

Prepared by Kenneth Hayworth (January 2016)

This directory contains a subset of the electron microscope images taken for evaluation of the rabbit brain (Submission #6) submitted by 21st Century Medicine (21CM) in fulfillment of the requirements for the Brain Preservation Prize.

Kenneth Hayworth (KH) (President of the Brain Preservation Foundation (BPF)) and Michael Shermer (member of BPF advisory board) witnessed (on Sept. 25, 2015) the full Aldehyde Stabilized Cryopreservation surgical procedure performed on this rabbit at the laboratories of 21CM under the direction of 21CM lead researcher Robert McIntyre. This included the live rabbit's carotid arteries being perfused with glutaraldehyde and subsequent perfusion with cryoprotectant agent (CPA).

Full details of the procedure have been published by Robert McIntyre and Greg Fahy (21CM Chief Scientist) in the paper "Aldehyde Stabilized Cryopreservation" in the Journal of Cryobiology. Here is a link to that paper:

http://dx.doi.org/10.1016/j.cryobiol.2015.09.003

The directory contains the following files:

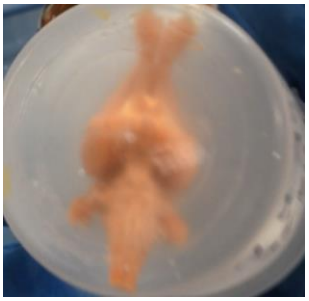

Cryobiology

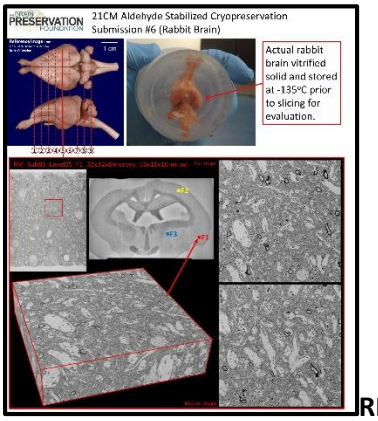
Aldehyde-stabilized cryopreservation

Robert L. McIntyre*, Gregory M. Fahy

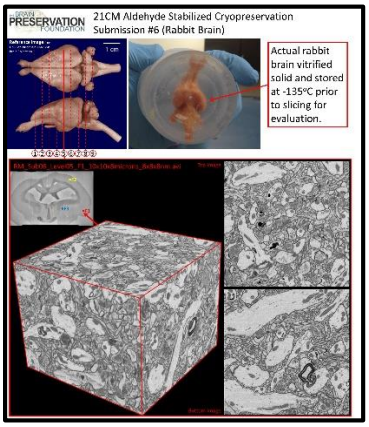
McIntyreAndFahy _Cryobiology2015.pdf –Copy of published paper.

MovieOfRabbitBrainBeingPutIntoStorage.avi -A movie (taken by KH) of the rabbit brain floating in fixative+CPA after being removed from skull, being put into a -135°C freezer storage unit.

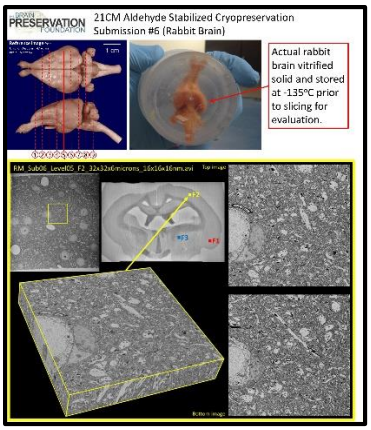

MovieOfVitrifiedRabbitBrainBeingTakenOutOfStorage.avi - A movie (taken by KH) of that same rabbit brain being taken out of the -135°C freezer unit after overnight storage. It is clearly visible that the brain has vitrified solid at this temperature.



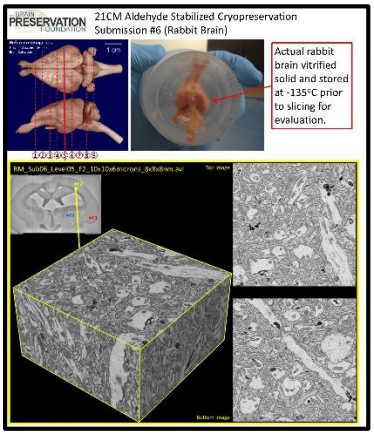
RM_Sub06_Level05_F1_32x32x8microns_16x16x16nm.avi -3D FIB-SEM volume "movie" (acquired by KH) of a 32x32x8 micron volume at location "F1" in cortex region of vibratome slice level #5 shown in Figure 9 below. Voxel size is 16x16x16nm.



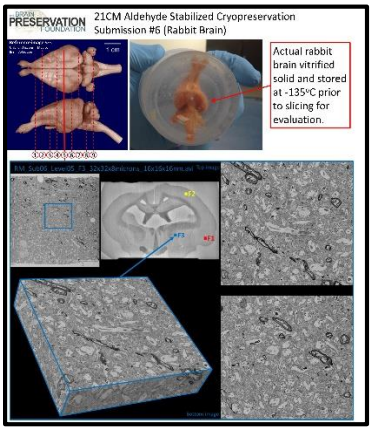
RM_Sub06_Level05_F1_10x10x8microns_8x8x8nm.avi -3D FIB-SEM volume "movie" (acquired by KH) of a 10x10x8 micron volume at location "F1" in cortex region of vibratome slice level #5 shown in Figure 9 below. Voxel size is 8x8x8nm.



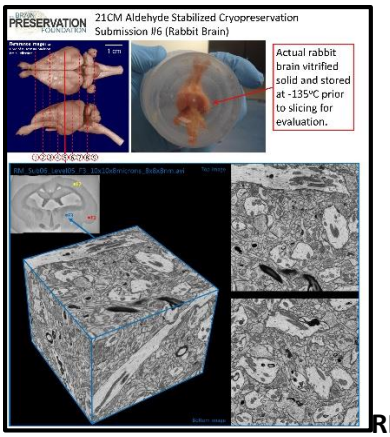
RM_Sub06_Level05_F2_32x32x6microns_16x16x16nm.avi -3D FIB-SEM volume "movie" (acquired by KH) of a 32x32x6 micron volume at location "F2" in cortex region of vibratome slice level #5 shown in Figure 9 below. Voxel size is 16x16x16nm.



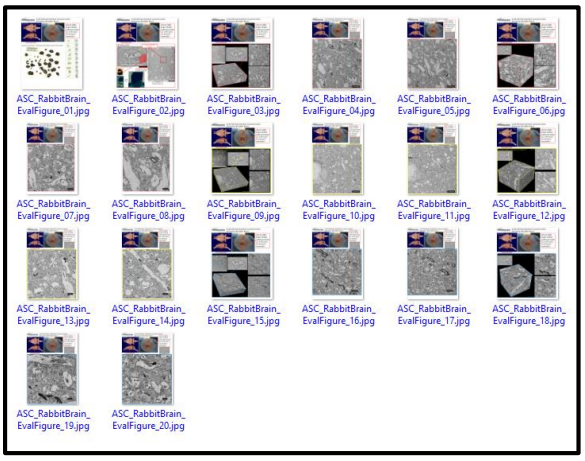
RM_Sub06_Level05_F2_10x10x6microns_8x8x8nm.avi -3D FIB-SEM volume "movie" (acquired by KH) of a 10x10x6 micron volume at location "F2" in cortex region of vibratome slice level #5 shown in Figure 9 below. Voxel size is 8x8x8nm.



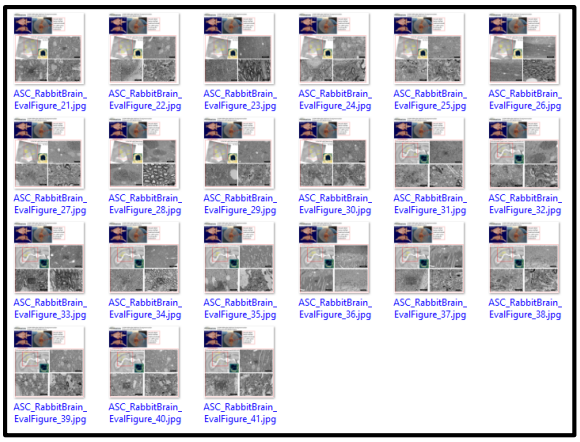
RM_Sub06_Level05_F3_32x32x8microns16x16x16nm.avi -3D FIB-SEM volume "movie" (acquired by KH) of a 32x32x8 micron volume at location "F3" in striatum region of vibratome slice level #5 shown in Figure 9 below. Voxel size is 16x16x16nm.



RM_Sub06_Level05_F3_10x10x8microns_8x8x8nm.avi -3D FIB-SEM volume "movie" (acquired by KH) of a 10x10x8 micron volume at location "F3" in striatum region of vibratome slice level #5 shown in Figure 9 below. Voxel size is 8x8x8nm.



\3D_FIBSEM_Pages1-20 -Subdirectory containing high resolution images showing locations of FIB-SEM datasets and example images.



\2D_SEM_Pages21-41 -Subdirectory containing 2D SEM survey images of vibratome levels 4 and 6. These include images of cortex, basal ganglia, hippocampus, and thalamus.

\2D_SEM_LowerResolution_Pages42-72 -Subdirectory containing 2D SEM survey images (made with slightly lower resolution SEM settings) of vibratome levels 4, 6, and 8. These include images of cortex, basal ganglia, hippocampus, thalamus, and cerebellum. Note: additional 2D survey images were taken from vibratome levels 2, 3, and 5, but these are not included in this directory.

Additional pictures showing steps of the Aldehyde-Stabilized Cryopreservation procedure and evaluation procedure:

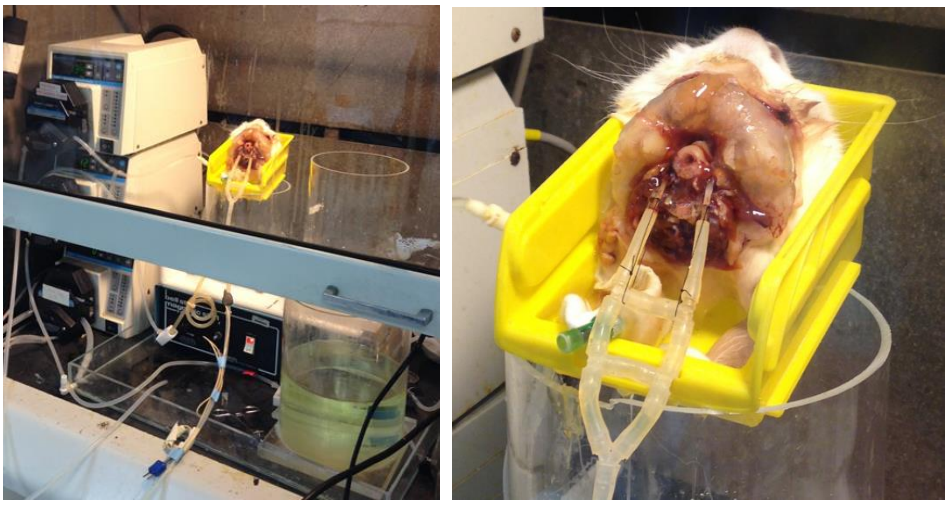


Figure 1. Rabbit head being perfused with CPA (photographed and witnessed by KH).

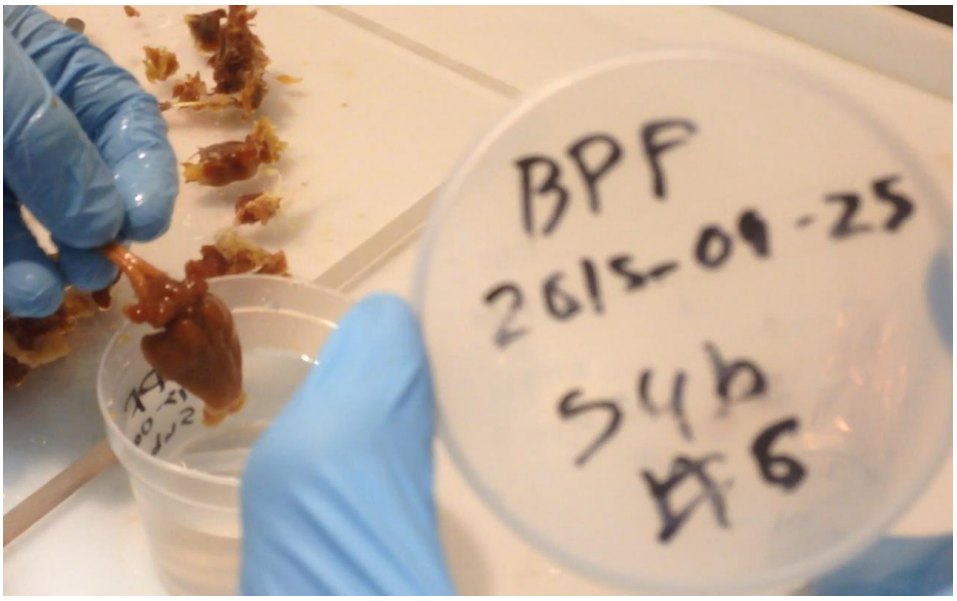


Figure 2. Robert McIntyre surgically removing rabbit brain and dropping in plastic container filled with fixative+CPA prior to storage. (Photographed and witnessed by KH.)

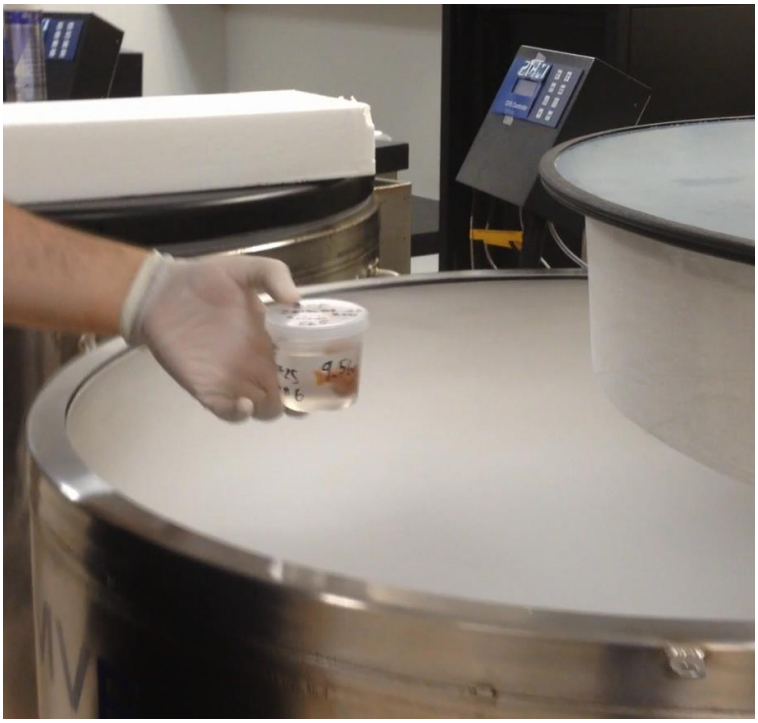


Figure 3. Robert McIntyre placing rabbit brain in -135^{o}C freezer unit. (Photographed and witnessed by KH.)

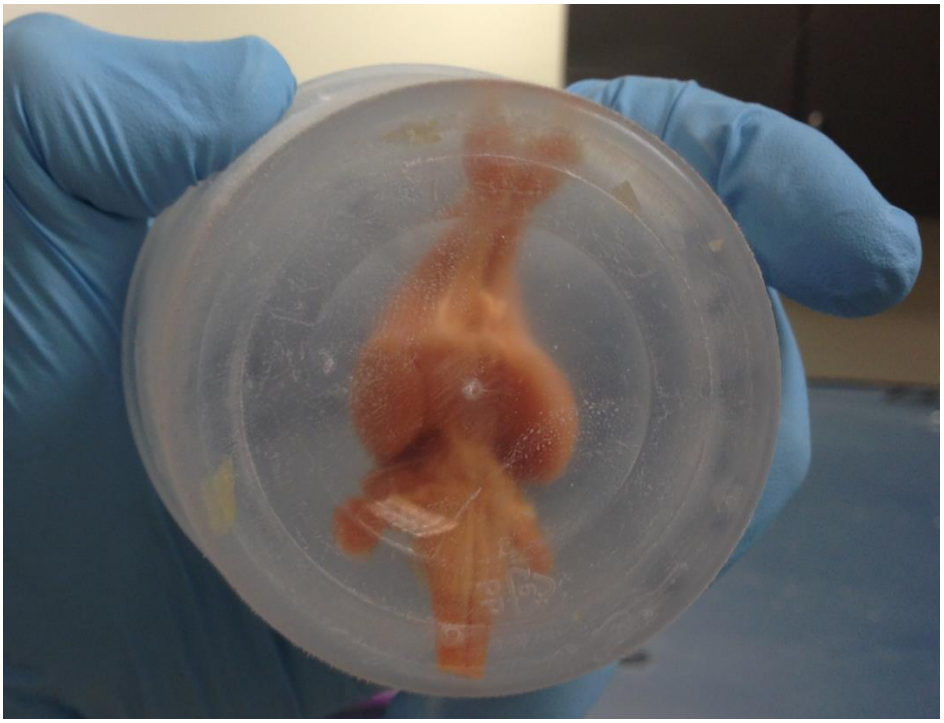


Figure 4. Robert McIntyre taking rabbit brain out of -135^{o}C freezer unit after overnight storage. Brain and block of CPA is completely solid. (Photographed and witnessed by KH.)

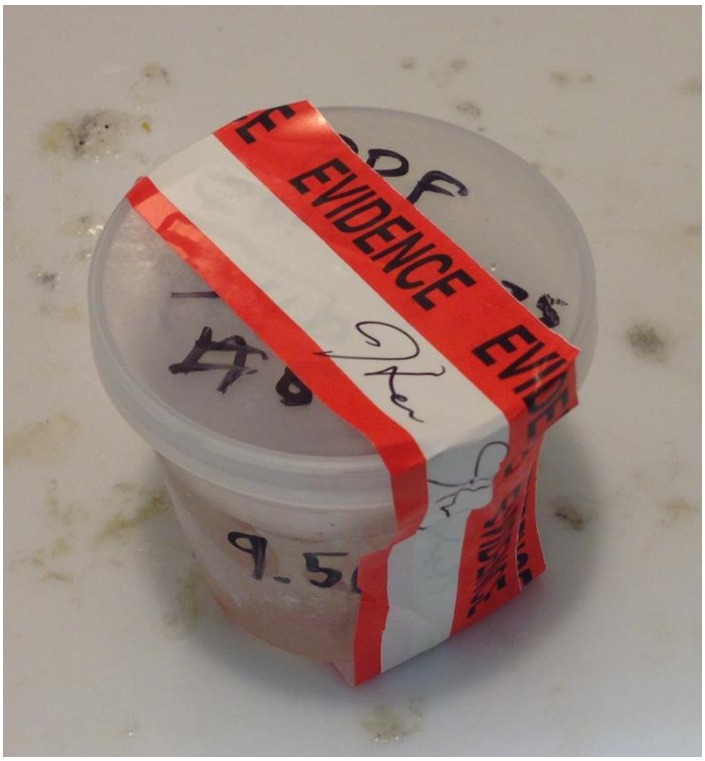


Figure5. Evidence tape was used to prevent tampering after removal from -135^{o}C storage. (Photographed and witnessed by KH.)

Figure 6. Rabbit brain embedded in agar in preparation for vibratome sectioning. (Photographed and witnessed by KH.)

Figure 7. Vibratome sectioning of rabbit brain. (Photographed and witnessed by KH.)

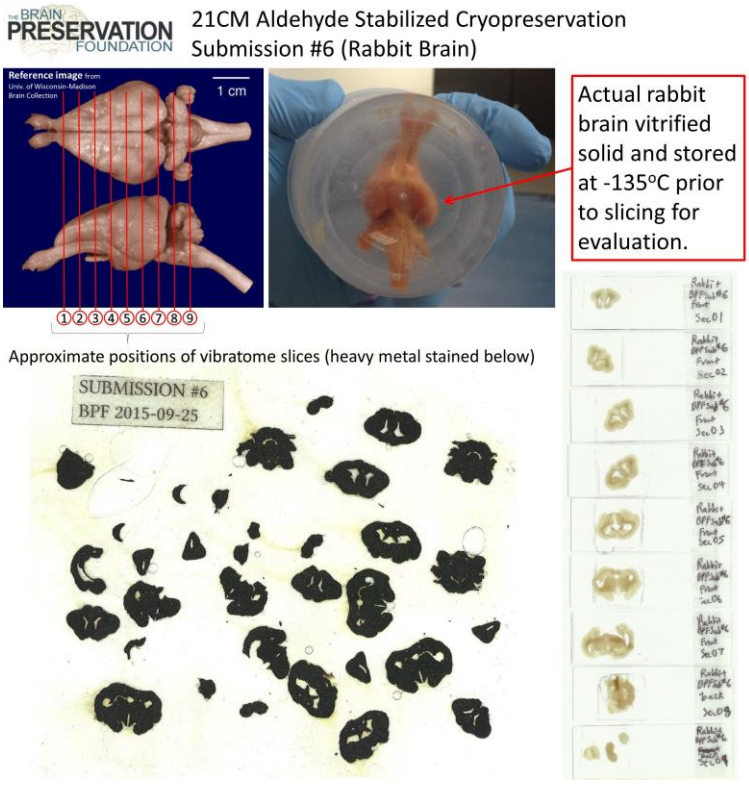


Figure 8. Figure showing approximate locations of vibratome sections overlaid on reference rabbit brain image. Nearby sections were put through heavy metal staining and plastic embedding procedure necessary for electron microscopy.

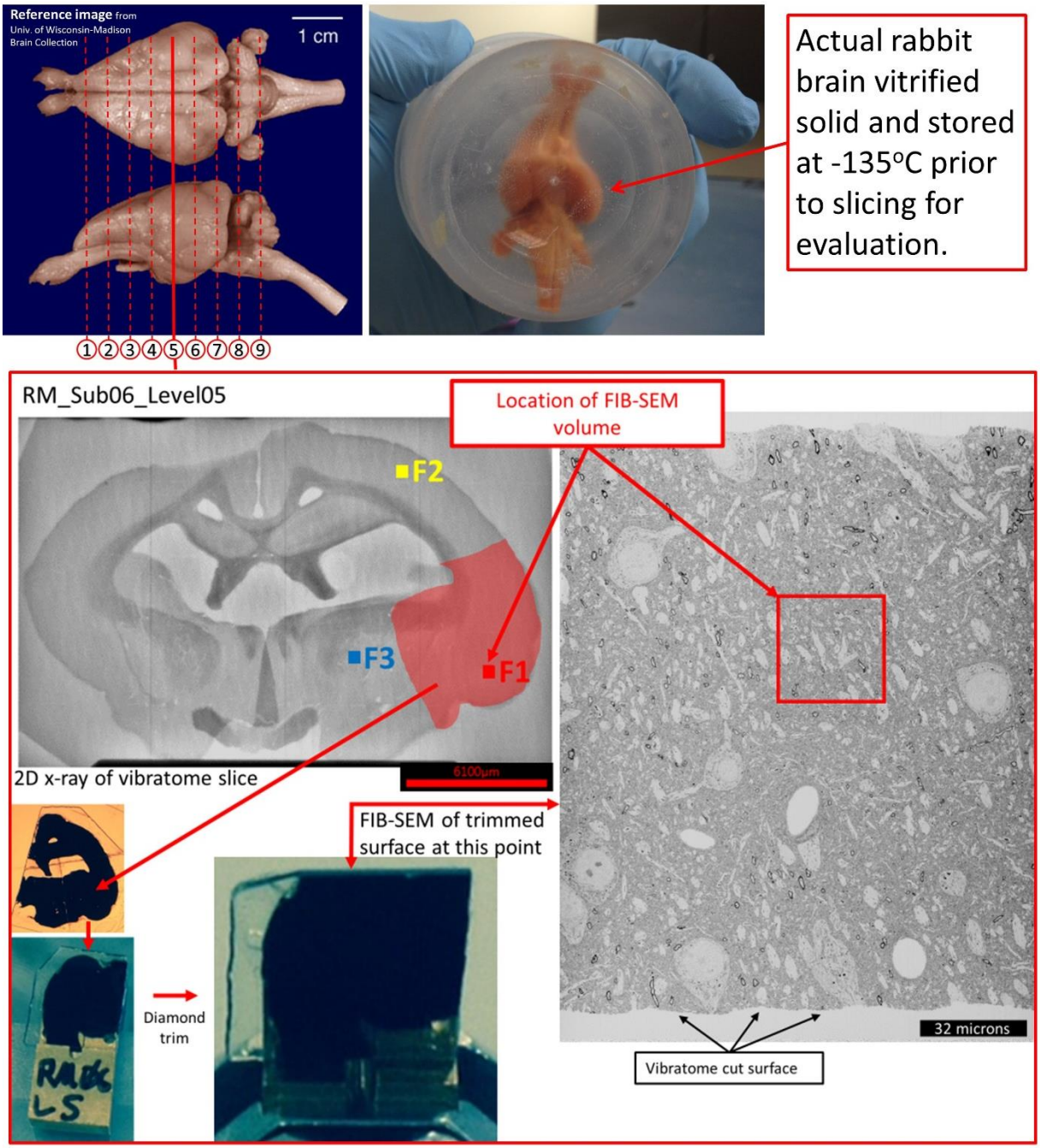


Figure 9. Figure showing location of the three FIB-SEM volume datasets each taken in different regions of the middle vibratome slice level #5.