

# Generating CBF Map from single PLD pcASL MRI

Sunnybrook Neuroimaging summer school Perfusion Module Tutorial 1

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If you want to try creating a CBF map on your laptop but have not installed the software libraries:

 VirtualBox

**Windows Users need to download a virtual machine:**

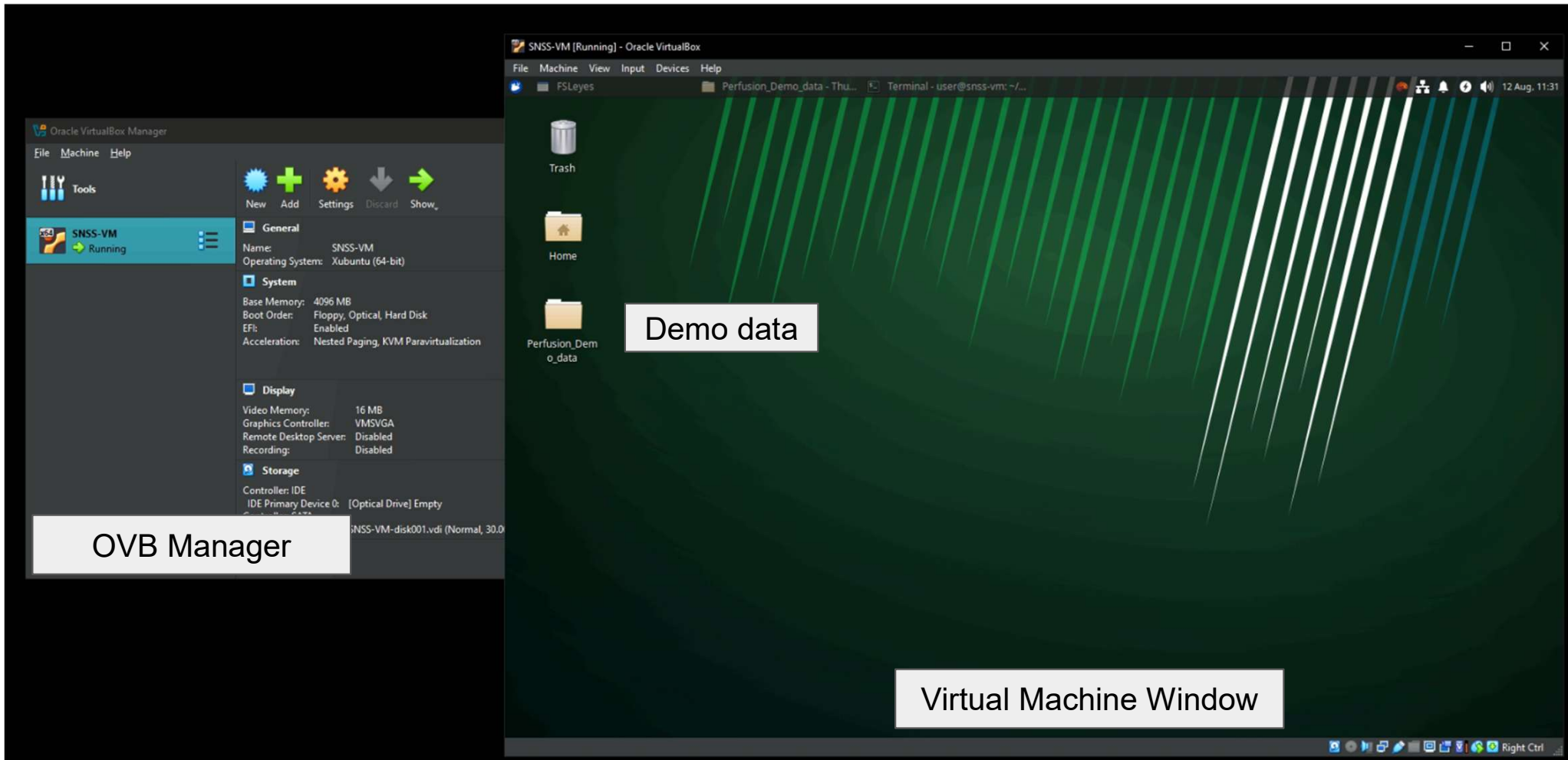
1. Install Virtualbox ([www.virtualbox.org/wiki/Downloads](http://www.virtualbox.org/wiki/Downloads))
2. Download the VM OVA file (<http://bit.ly/3HrkpwC>)
3. Open Virtualbox, and select “File—>Import Appliance”
4. Select the downloaded OVA file and press “Next—>Finish”



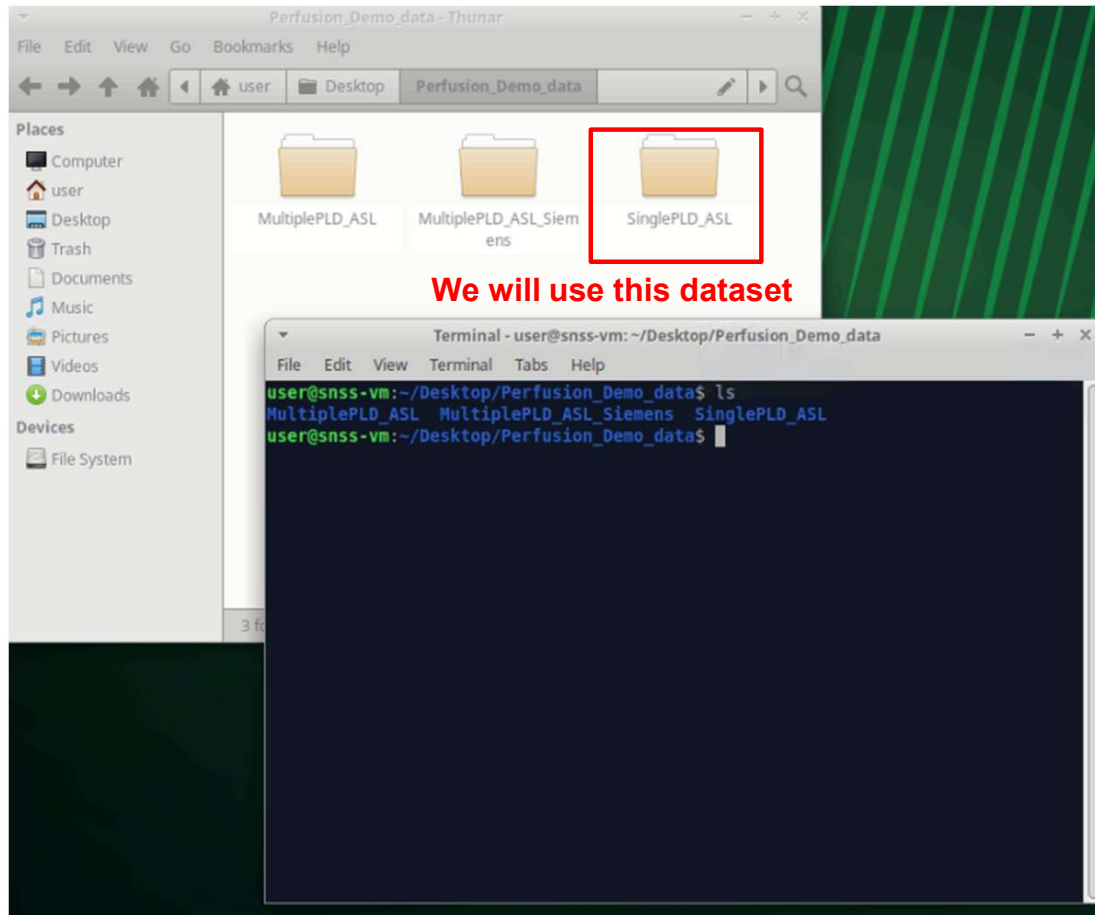
**Mac and Linux users can directly download the FSL, no virtual machine is needed:**

1. FSL (<https://fsl.fmrib.ox.ac.uk/fsl/docs/#!/install/index>)
2. Perfusion Dataset (<http://bit.ly/3J6H95K>)

If your VM boots correctly it will look like this:



## There is a demonstration dataset included in the virtual machine



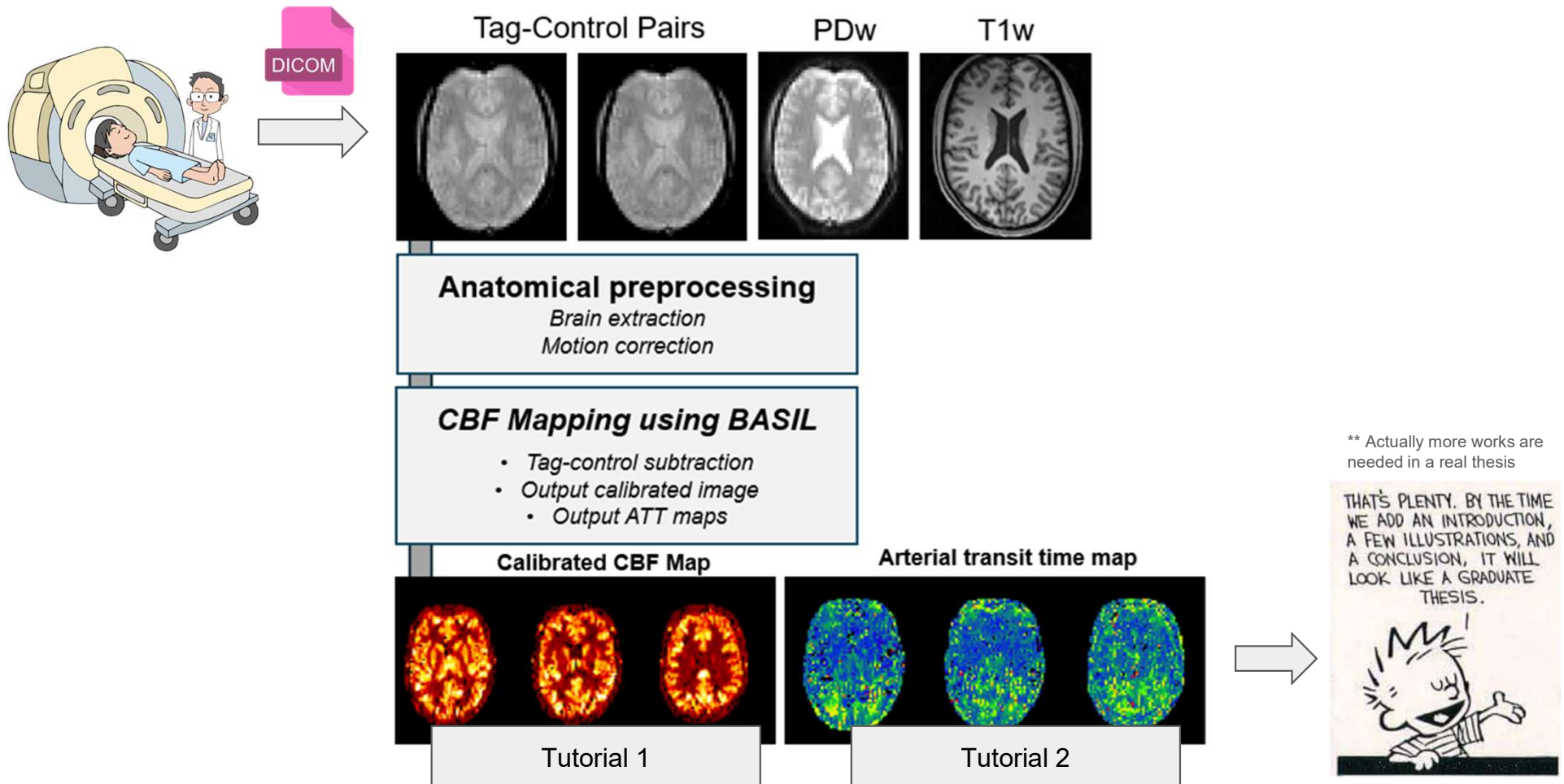
Data from Sunnybrook Siemens Prisma 3T scanner and GE 3T scanner.

Mac and Linux users can download the dataset: Perfusion Dataset (<http://bit.ly/3J6H95K>)

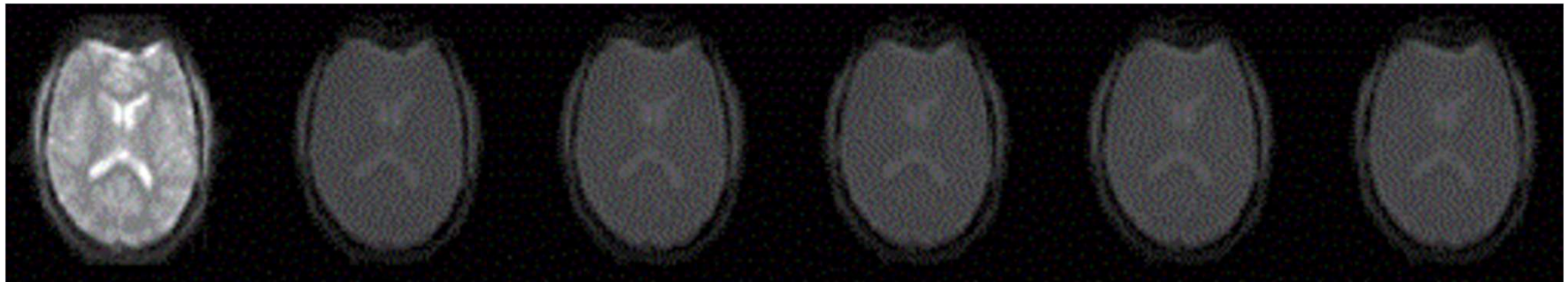
### Linux Users Tips:

1. Right click on the window and select "Open Terminal Here" allows you to call out the command line terminal
2. Use "**ls**" to list files in the current folder;  
Use "**cd**" to move between directories;  
Use "**pwd**" to show the current path

# Agenda: Creating an ASL-CBF map from single PLD pcASL data



## What does a typical single PLD pcASL dataset looks like?

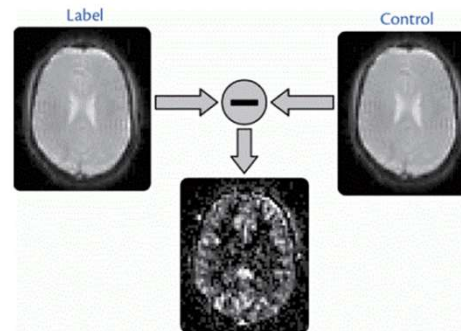


Calibration image

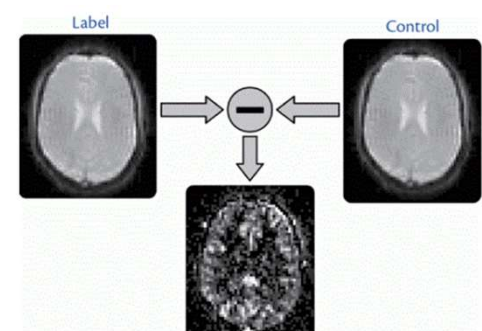
An extra image

Label-Control 1

Label-Control 2

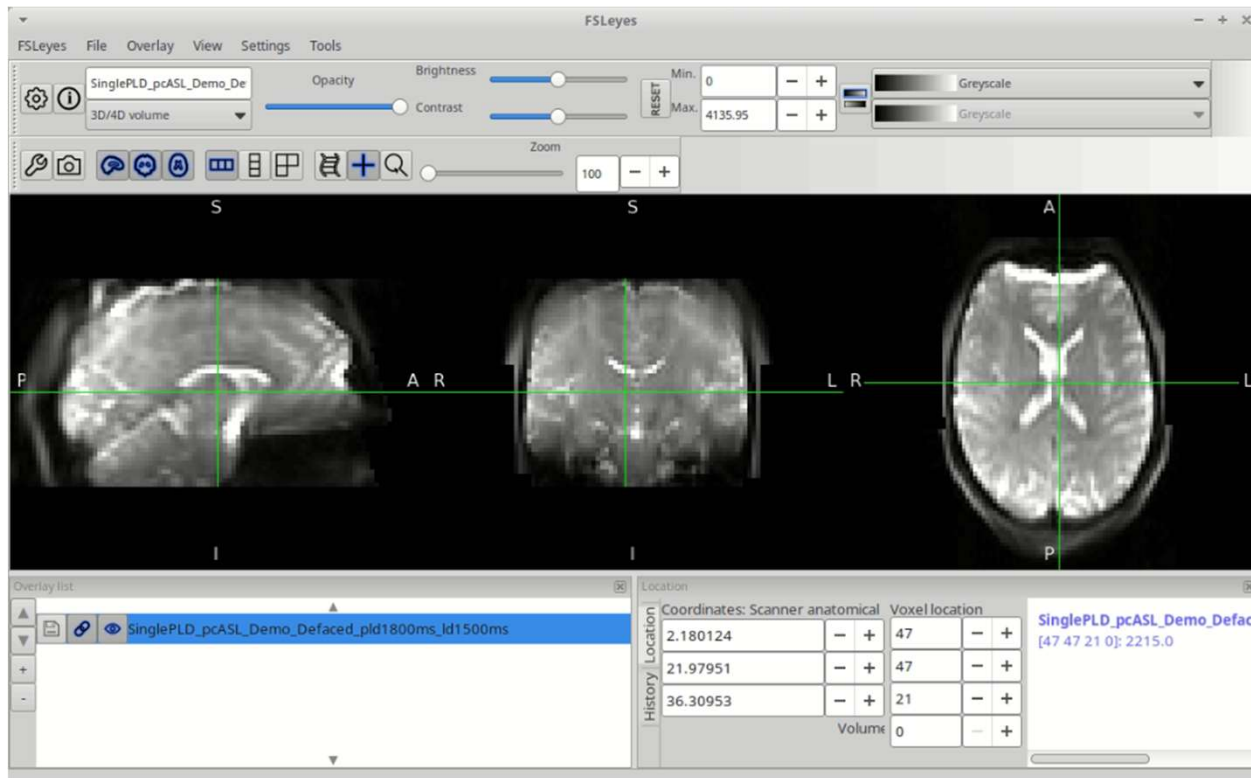


PWI 1



PWI 2

# Exercise 1: Visualization of the pcASL dataset



## Linux Users Tips:

1. To start the FSleyes program, start a terminal and type **"fsleyes"** and press enter.

## To-discuss:

1. If you increase the volume number, what you will see?
2. Why the first volume is much brighter than the next few volumes?
3. Will label image show higher or lower signal intensity than the control image?



## Exercise 2: Reading through the image data to identify ASL parameters



Modality: MR  
MagneticFieldStrength: 3  
Manufacturer: SIEMENS

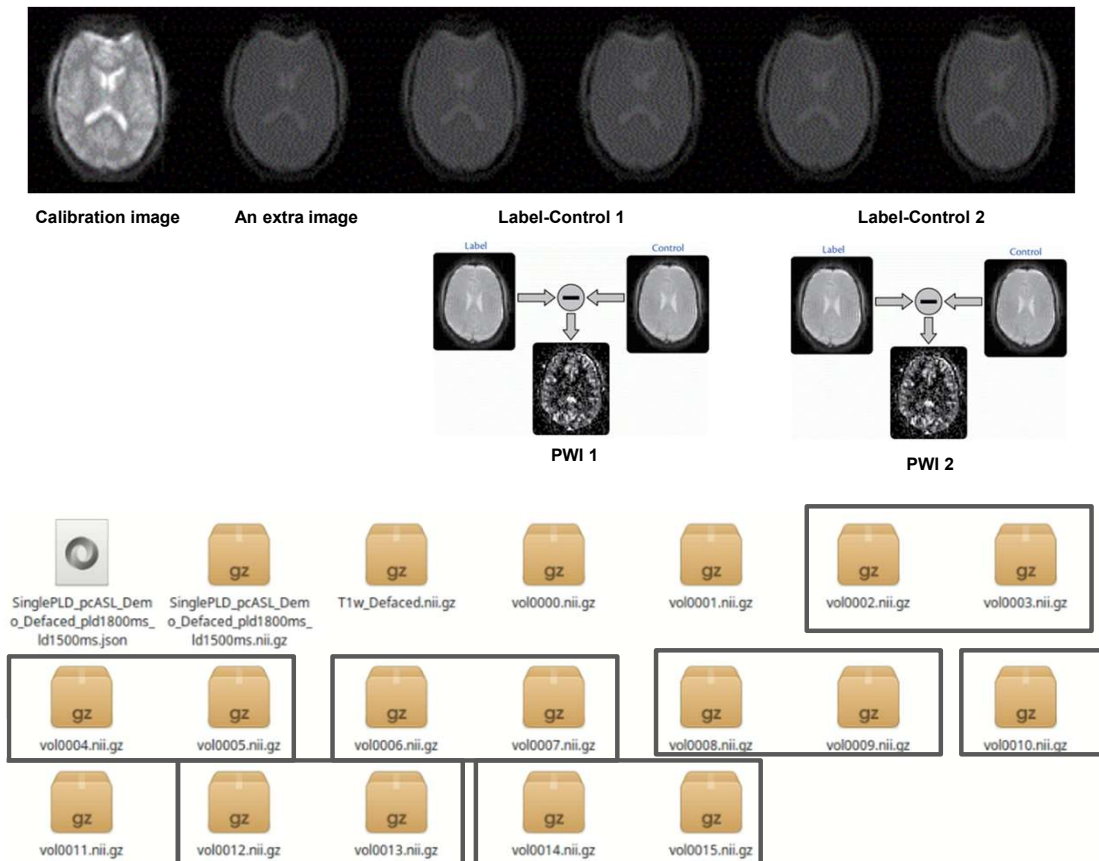
Repetition time (ms): 4190  
Echo time[1] (ms): 38.32

Slice thickness (mm): 2.5  
Voxel size x (mm): 2.5  
Voxel size y (mm): 2.5  
Number of slices: 48

Image type: "Original", "Primary", "ASL"  
LabelingDuration (s): 1.8  
PostLabelDelay(InversionTime) (s): 1.6

```
8 Series description: tgse_pcasl_PLD1800_2p5iso_48s1
9 Image type: ORIGINAL\PRIMARY\ASL\NONE\ND\MOSAIC
10 Manufacturer: SIEMENS
11 Model name: Prisma
12 Software version: syngo MR E11
13 Study id: 1
14 Series number: 8
15 Repetition time (ms): 4190
16 Echo time[1] (ms): 38.32
17 Flip angle: 120
```

## Exercise 3: Preparing the dataset for ASL analysis using **fslsplit** and **fslmerge** functions



### Bash Users Tips:

To show help message in any fsl comments, you can just type the command and press enter.

To select all files with part of file names in common, you can use \* sign (e.g. \*.nii.gz selects all NIFTI images in the current directory)

### To-dos:

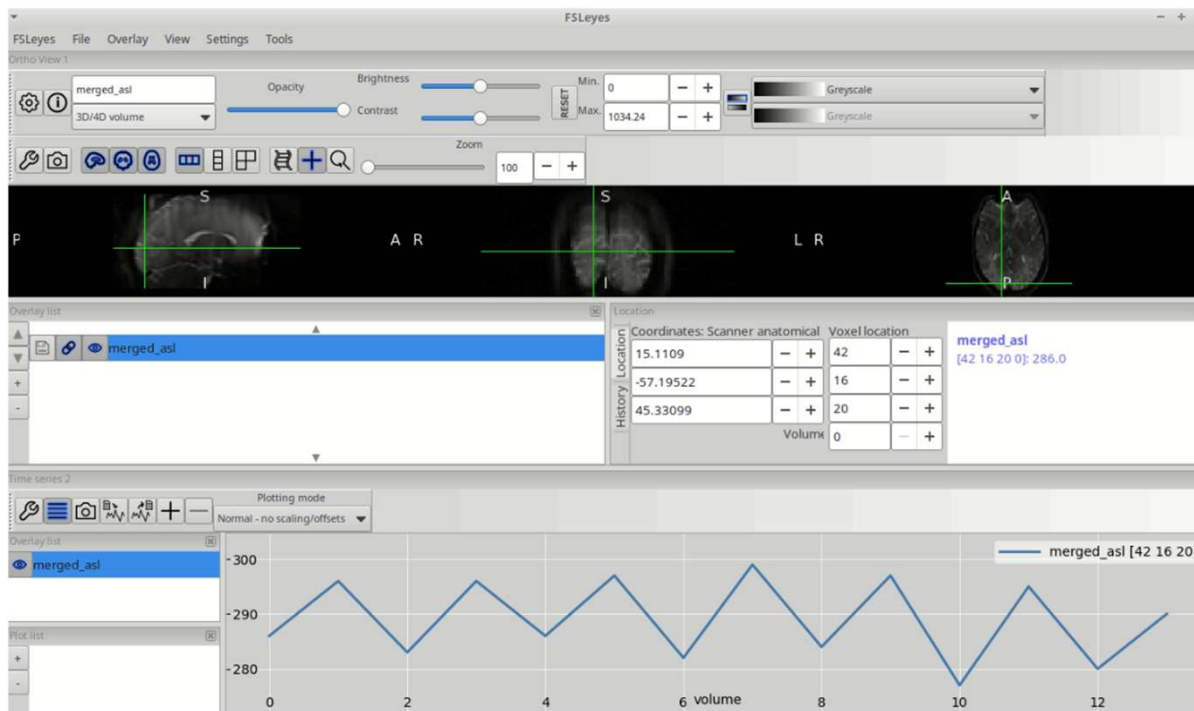
1. Use “**fslsplit**” command to split the 4D time series into 3D volumes.
2. Identify and rename the PDw image.
3. Use “**fslmerge**” command to merge the tag-control pairs back into the 4D time series.

### Hints:

1. **fslsplit <input.nii.gz> -t**
2. Identify and rename PDw image
3. Identify the 7 label-control pairs
4. Remove the one extra unused image
5. **fslmerge -t <output name> <all inputs vol\*.nii.gz>**



## Exercise 4: Visual inspection of label-control sequence using time series plot in FSLeyes



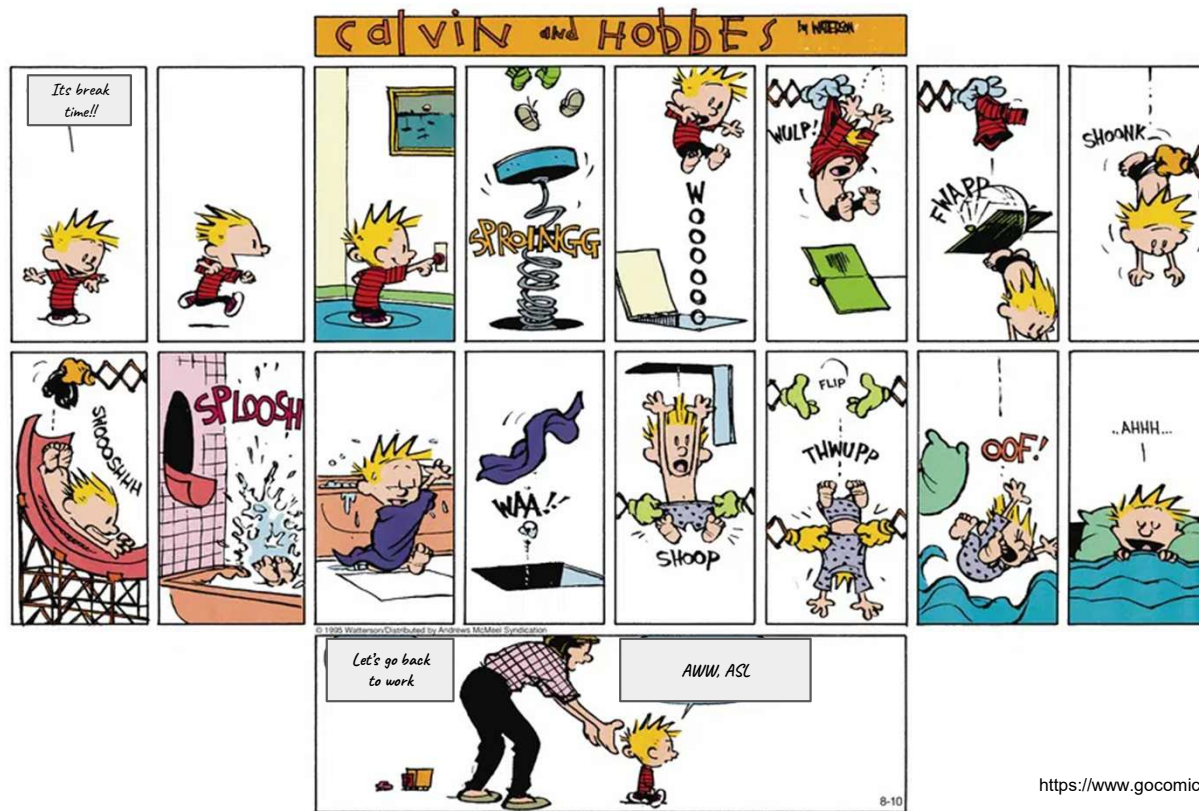
### To-dos:

1. Load the merged label-control images post “fslmerge” function to the fsleyes viewer.
2. Use green cursor to select a brain region of your interest.
3. Create a time series plot using “view” menu > “Time Series”

### Question:

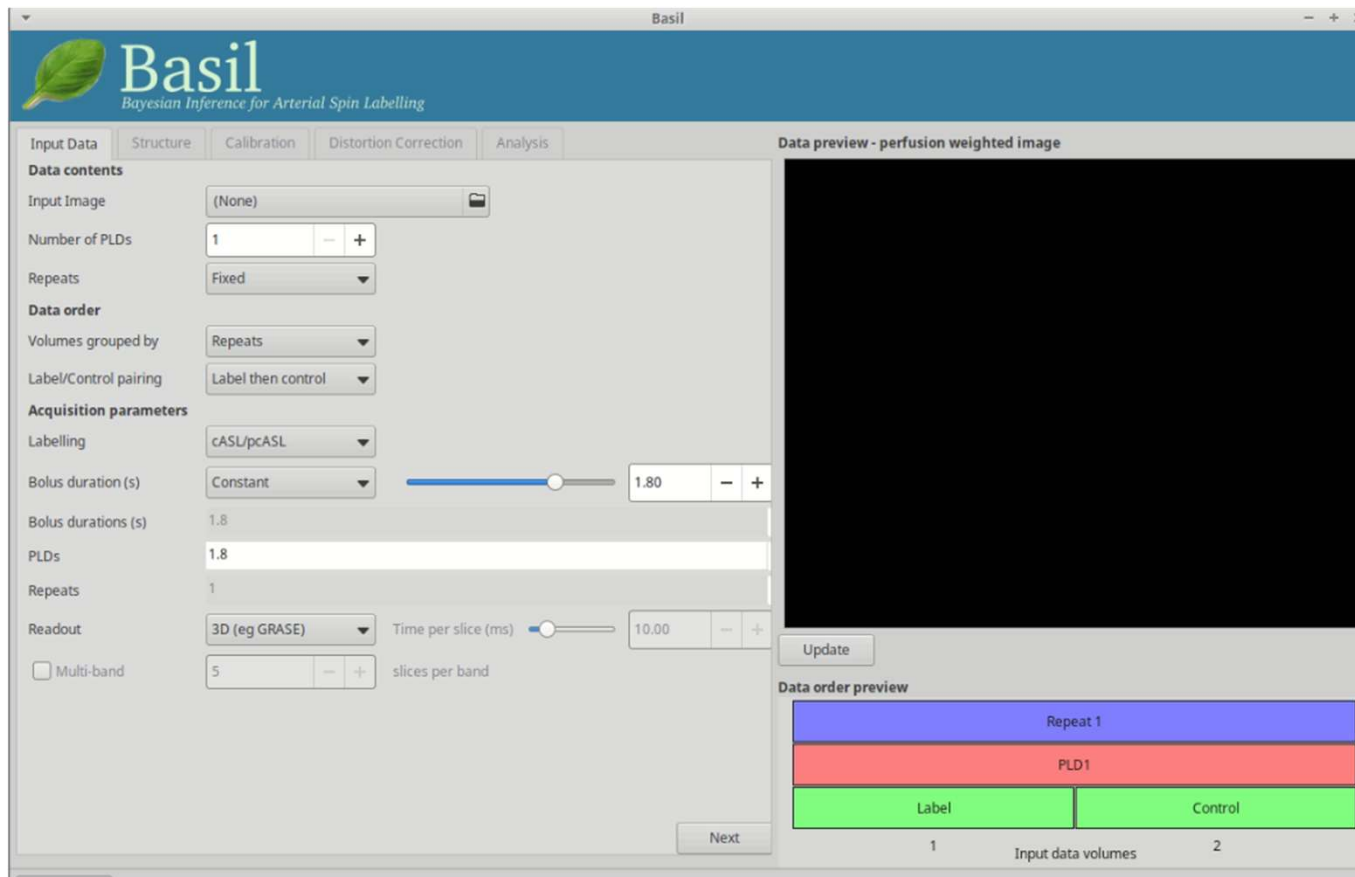
1. Is the first image a control image or a tag image?
2. Which brain region showed higher ASL signal fluctuation?

# Let's take a break ...



<https://www.gocomics.com/calvinandhobbes/2025/08/10>

## Exercise 5: Introduction to the Oxford ASL GUI



### Linux Users Tips:

For programs like OxfordASL and Fsleyes with GUI, each terminal usually can only run one of such program. If you close the terminal or force run another task on the same terminal, the program will stop. Thus you want to create a new terminal everytime when running a new GUI-based program on top of another.

### To-dos:

1. Call "asl\_gui" in a new terminal to open up the OxfordASL-BASIL program
2. Input the label-control pairs to the **Data contents > Input Image** tab
3. Conduct a data preview by clicking the "Update" button

## Exercise 5: The “Input Data” Tab

**Basil**  
Bayesian Inference for Arterial Spin Labelling

**Input Data** | Structure | Calibration | Distortion Correction | Analysis

**Data contents**

Input Image: merged\_asl.nii.gz

Number of PLDs: 1

Repeats: Fixed

**Data order**

Volumes grouped by: Repeats

**Label/Control pairing**

- Label then control
- Control then label
- Pre-subtracted

**Acquisition parameters**

Bolus duration (s): Constant, 1.80

Bolus durations (s): 1.8

PLDs: 1.8

Repeats: 7

Readout: 3D (eg GRASE)

Time per slice (ms): 10.00

Multi-band: 5

**Data preview - perfusion weighted image**

S S A

F A R L R L

I I

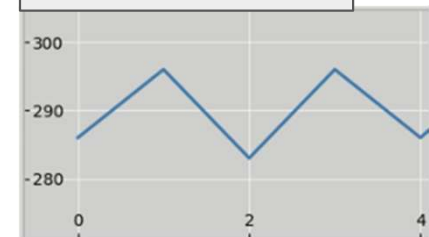
**Data order preview**

Repeat 1	...
PLD1	...
Label	Control

1 Input data volu

Next

Time series plot



### Question:

Is the first image a control image or a tag image?

How many repeats do we have?

What is the bolus duration, PLD, readouts?

From .json file

```
MRAcquisitionType: "3D"
SeriesDescription: "tgse_pcasl_PLD1800_2p5iso_48sl"
ProtocolName: "tgse_pcasl_PLD1800_2p5iso_48sl"
ScanningSequence: "EP"
SequenceVariant: "SK"
ScanOptions: "FS"
SequenceName: "tgse3d1_3528"
```

## Exercise 5: The completed “Input Data” tab:

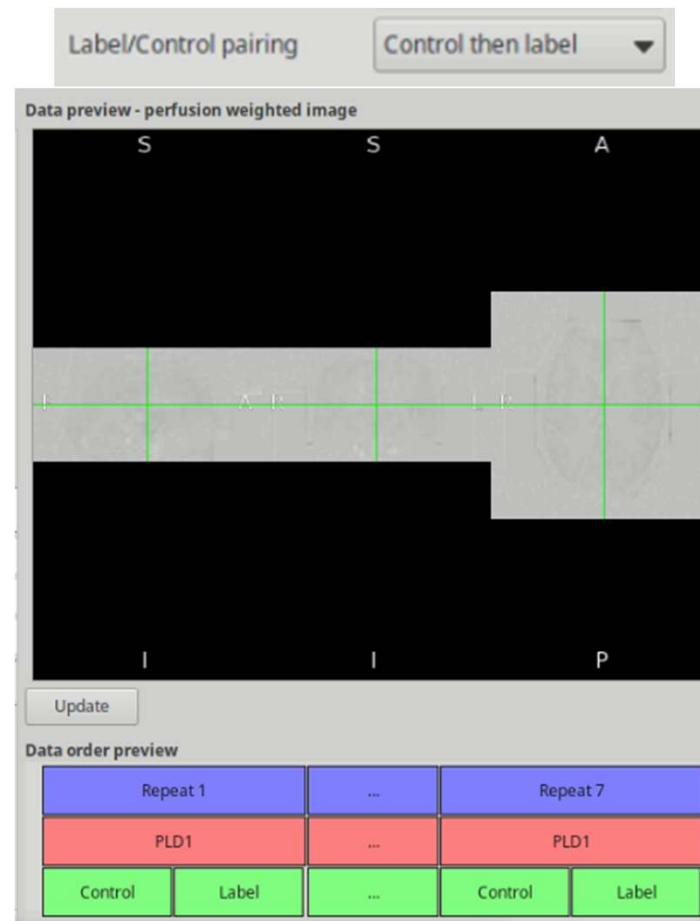
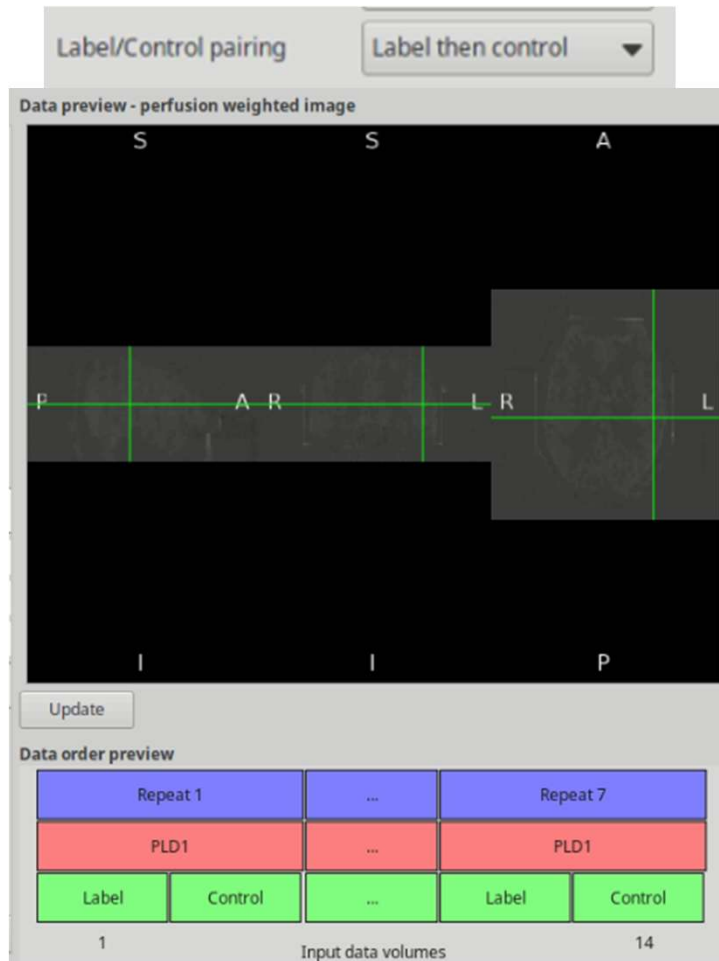
The screenshot shows the 'Input Data' tab of a software interface. The 'Data contents' section includes an 'Input Image' field with 'merged\_asl.nii.gz', 'Number of PLDs' set to 1, 'Repeats' set to Fixed, 'Data order' set to Repeats, 'Volumes grouped by' set to Repeats, 'Label/Control pairing' set to Label then control, 'Acquisition parameters' set to cASL/pcASL, 'Labelling' set to Constant, 'Bolus duration (s)' set to 1.50, 'Bolus durations (s)' set to 1.5, 'PLDs' set to 1.8, 'Repeats' set to 7, 'Readout' set to 3D (eg GRASE), 'Time per slice (ms)' set to 10.00, and 'Multi-band' set to 5 slices per band. The 'Data preview - perfusion weighted image' shows a brain scan with labels S, A, I, P, L, R, A, R, L, R, L. The 'Data order preview' table shows the sequence of volumes.

Data order preview				
Repeat 1	...	Repeat 7		
PLD1	...	PLD1		
Label	Control	...	Label	Control
1	Input data volumes			14

**This scan:**

1 PLD;  
Fixed repeats;  
pcASL imaging;  
LD=1.5s;  
PLD=1.8s;  
3D readouts

## Exercise 5: What happens if you select the wrong label-control pair

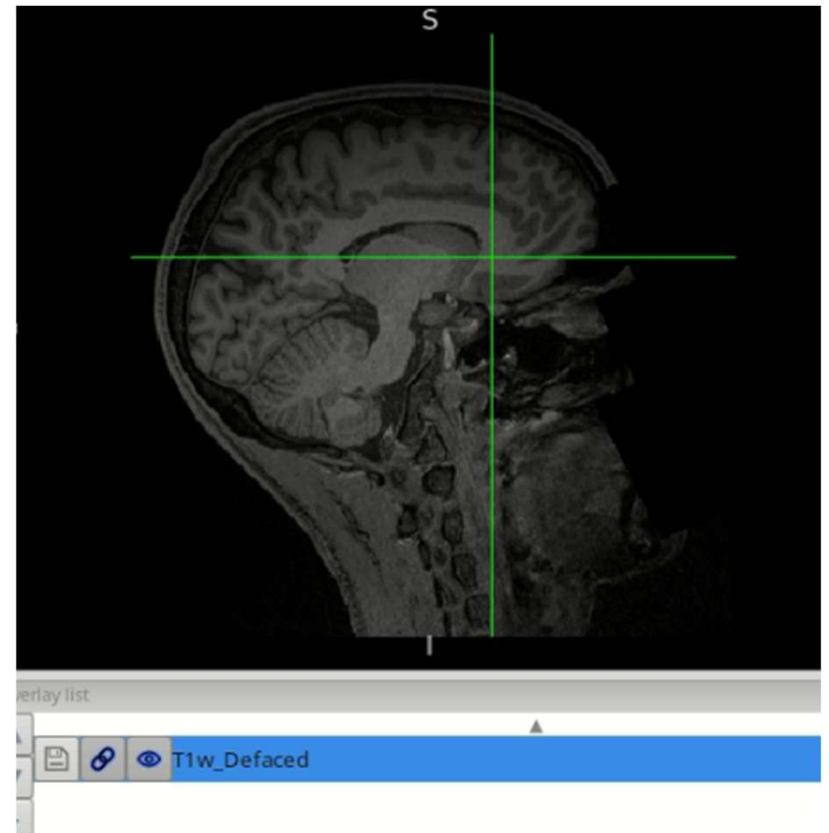
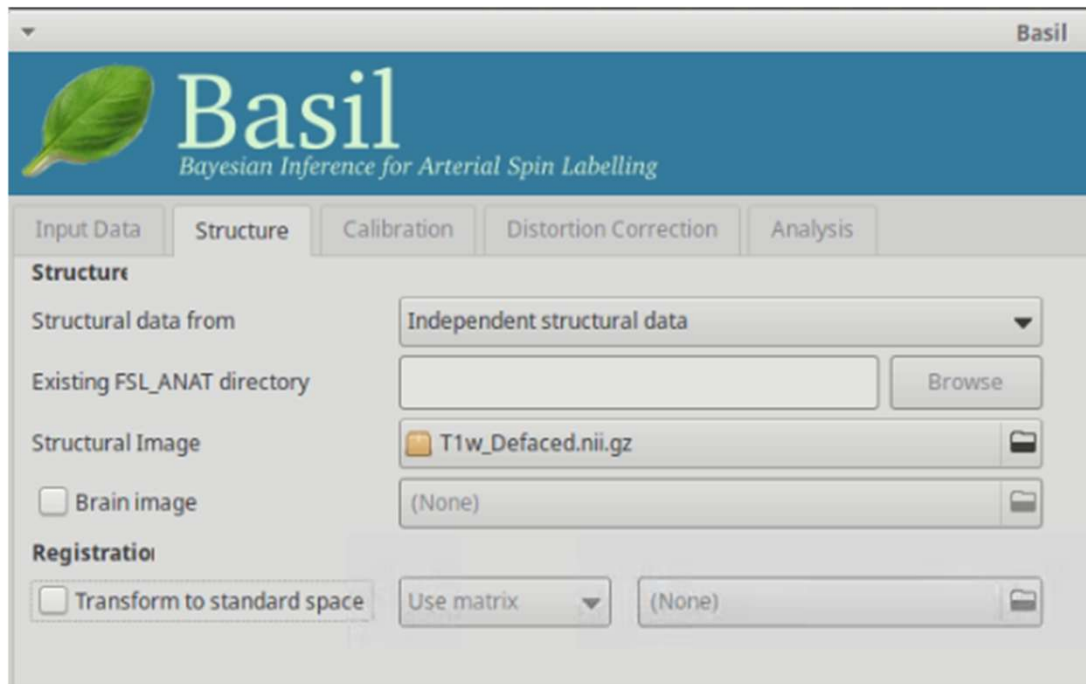


I was told my cerebral blood flow was negative 30 ml/100g/min ..?

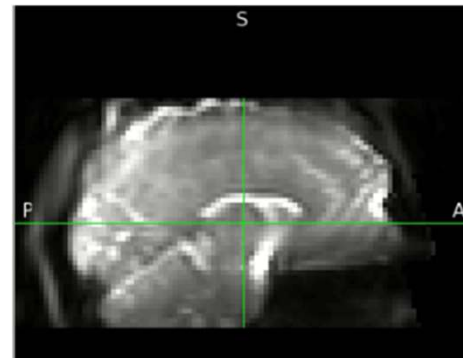
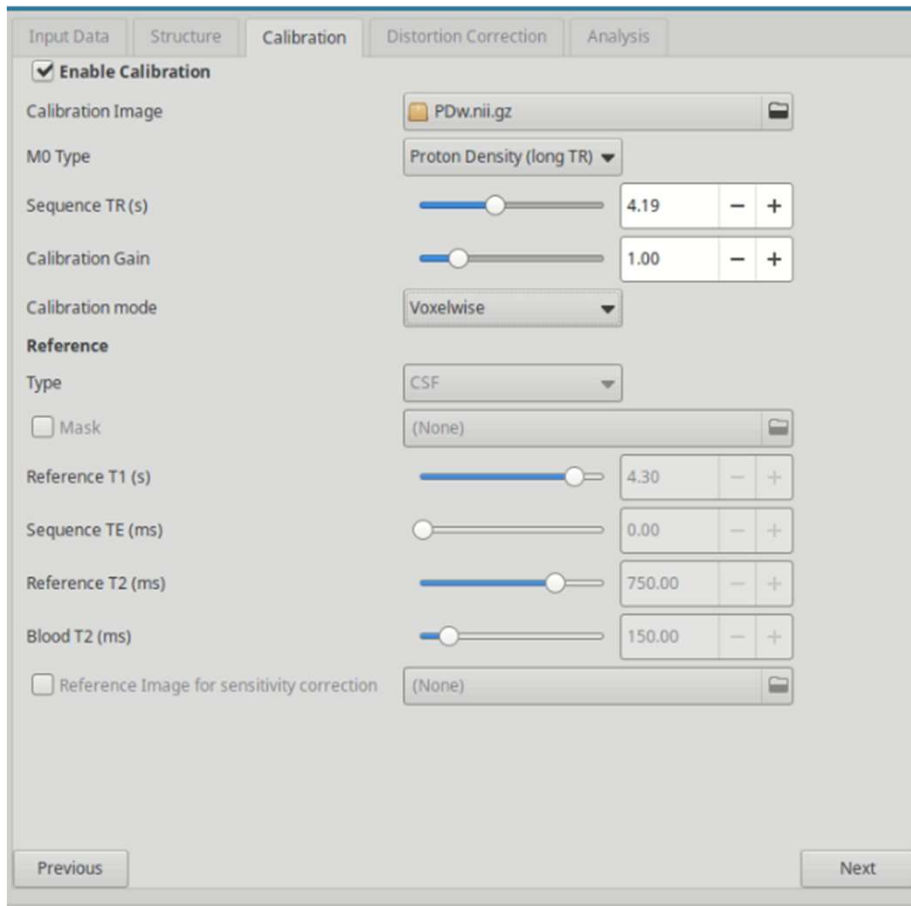




## Exercise 5: Loading the T1w image to the “Structural” tab



## Exercise 5: Loading the PDw image to the “Calibration” tab



### Question:

1. How will you find the sequence TR value?
2. Why we need a PDw image here?
3. If you got an ASL scan, and the sequence TR was 1.5s, how would you comment on the acquisition? (Hint: Short TR makes PDw image look closer as a T1w image. What will happen on signals in GM, WM, and CSF? We also know from kinetic model:  $CBF \propto (\Delta M/M_0)$ )


## Exercise 5: Introducing the “Distortion Correction” tab

Input Data   Structure   Calibration   **Distortion Correction**   Analysis


**Distortion**


☐ Apply distortion correction   Calibration image ▾


**Calibration Image**

Phase-encode-reversed calibration image (None) 


**Fieldmap**

Fieldmap image (in rad/s) (None) 

Fieldmap magnitude image (None) 

☐ Brain-extracted magnitude image (None) 

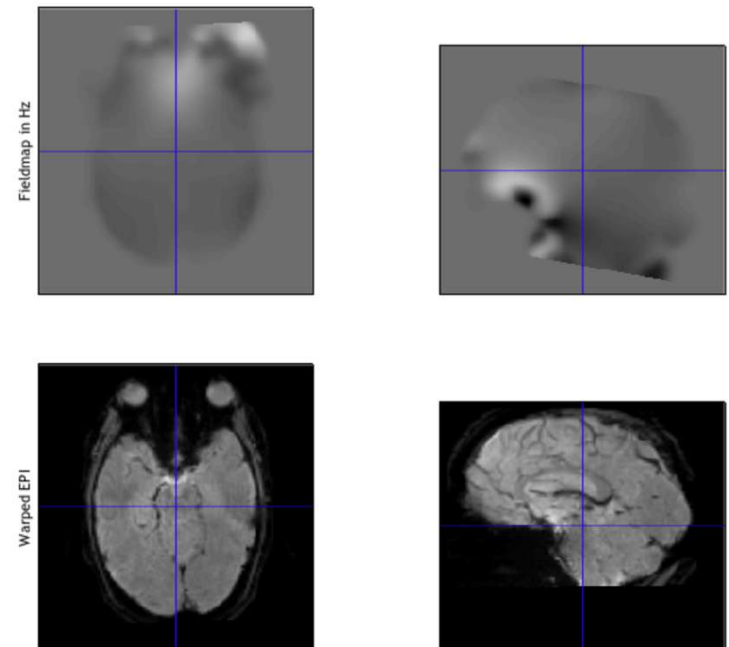
**General**

Effective EPI echo spacing (ms)  1.00000 - +

Phase encoding direction x ▾

If you acquire ASL with **anterior–posterior (AP)** phase encoding, reversing the PE direction to **posterior–anterior (PA)** flips the sign.

(Optional) The calibration image mode uses the two opposite-PE images to estimate a field map of displacements and applies this to correct distortions in ASL data.

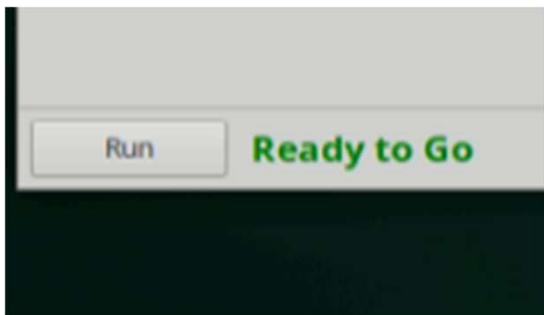


## Exercise 5: Introducing the “Analysis” Tab

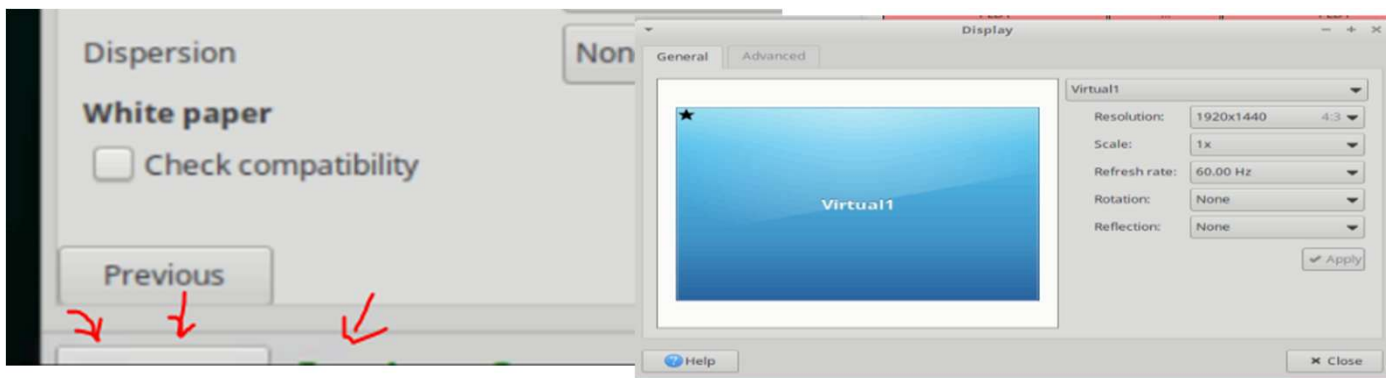
The screenshot shows the 'Analysis' tab of a software interface. The top navigation bar includes 'Input Data', 'Structure', 'Calibration', 'Distortion Correction', and 'Analysis'. The 'Analysis' tab is active and contains the following sections:

- Basic analysis**
  - Output Directory:
  - ☐ User-specified brain Mask:
- Initial parameter**
  - Arterial Transit Time (s):
  - T1 (s):
  - T1b (s):
  - Inversion Efficiency:
- Analysis**
  - ☒ Adaptive spatial regularization on perfusion
  - ☐ Incorporate T1 value uncertainty
  - ☐ Include macro vascular component
  - ☒ Fix label duration
  - ☒ Partial Volume Correction
  - ☒ Motion Correction
  - Exchange model:
  - Dispersion:
- White paper**
  - ☐ Check compatibility

## Exercise 5: Let's run the code - It will take some time.



```
Run
logfile: No such file or directory
oxford_asl -i=/home/user/Desktop/Perfusion_Demo_data/SinglePLD_ASF/
merged_asl.nii.gz --iaf=tc --ibf=rpt --casl --bolus=1.5 --xpts=7 --
tis=3.3 -s=/home/user/Desktop/Perfusion_Demo_data/SinglePLD_ASF/
T1w_Defaced.nii.gz -c=/home/user/Desktop/Perfusion_Demo_data/
SinglePLD_ASF/PDw.nii.gz --cmethod=voxel --tr=4.19 --cgain=1 -o=/home/
user/Desktop/Perfusion_Demo_data/ASL_outputs --bat=1.3 --t1=1.3 --
t1b=1.65 --alpha=0.85 --spatial=1 --fixbolus --mc --pvcorr --artoff
OXFORD_ASF - running
Version: v4.0.29-5-g09a5c3a-dirty Thu Jul 13 13:20:21 2023
Input file: /home/user/Desktop/Perfusion_Demo_data/SinglePLD_ASF/
merged_asl.nii.gz
Structural image: /home/user/Desktop/Perfusion_Demo_data/SinglePLD_ASF/
T1w_Defaced.nii.gz
Saving results in native (ASL acquisition) space to /home/user/Desktop/
Perfusion_Demo_data/ASL_outputs/native_space
Saving results in structural space to /home/user/Desktop/
Perfusion_Demo_data/ASL_outputs/struct_space
Pre-processing
```

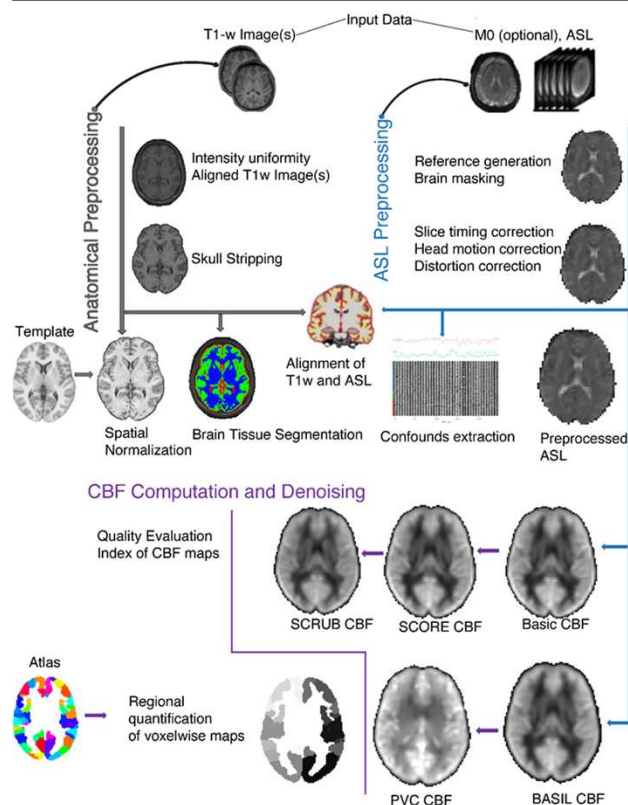


### Linux Users Tips:

If you found the GUI is too large and you can't rescale it because buttons are outside the window, try increase the resolution of your virtual machine via: Settings > Display

# Exercise 6: The CBF Mapper: Creating a pipeline to auto-run every cases for your future study

## An example ASL processing pipeline



## Raw ASL images> **ASLPipeline.sh** > CBF Map

```
#!/bin/bash
```

**# Define the constants - Which should be consistent over your study**

```
TC_Mark="tc"          # Input "tc" if 1st image is label.
LD=1.6                # second
TR_PDw=4.21          # second
T1_tissue=1.3         # second
T1_blood=1.65         # second
InvEff=0.85           # Inversion Efficiency (%)
rpts=7                # Number of repeats
```

**# Define the image inputs:**

```
Label_Ctrl=$1         # nii image of label-control pairs
T1w=$2                # nii image of structural MRI
PDw=$3                # nii image of calibration PDw MRI
```

**# Run the ASL pipeline (Example):**

```
oxford_asl -i ${Label_Ctrl} -o ASL_Analysis_xxxStudy \
-c ${PDw} --tr=TR_PDw --cmethod=voxel \
-s ${T1w} \
--t1=T1_tissue --t1b=${T1_blood} --alpha=${InvEff} \
--casl --bolus=${LD} --rpts=${rpts} --mc --pvcrr \
... # More costume arguments available
```



## Exercise 6: The CBF Mapper: Creating a pipeline to auto-run every cases for your future study

```
Run
/home/user/fsl/bin/oxford_asl -i=/home/user/Desktop/Perfusion_Demo_data/SinglePLD_ASL/merged_asl.nii.gz --iaf=tc --ibf=rpt --casl --bolus=1.5 --rpts=7 --tis=3.3 -s=/home/user/Desktop/Perfusion_Demo_data/SinglePLD_ASL/T1w_Defaced.nii.gz -c=/home/user/Desktop/Perfusion_Demo_data/SinglePLD_ASL/PDw.nii.gz --cmethod=voxel --tr=4.19 --cgain=1 -o=/home/user/Desktop/Perfusion_Demo_data/ASL_outputs --bat=1.3 --tl=1.3 --tlb=1.65 --alpha=0.85 --spatial=1 --fixbolus --mc --pvcorr --artoff
rm: cannot remove '/home/user/Desktop/Perfusion_Demo_data/ASL_outputs/logfile': No such file or directory
oxford_asl -i=/home/user/Desktop/Perfusion_Demo_data/SinglePLD_ASL/merged_asl.nii.gz --iaf=tc --ibf=rpt --casl --bolus=1.5 --rpts=7 --tis=3.3 -s=/home/user/Desktop/Perfusion_Demo_data/SinglePLD_ASL/T1w_Defaced.nii.gz -c=/home/user/Desktop/Perfusion_Demo_data/SinglePLD_ASL/PDw.nii.gz --cmethod=voxel --tr=4.19 --cgain=1 -o=/home/user/Desktop/Perfusion_Demo_data/ASL_outputs --bat=1.3 --tl=1.3 --tlb=1.65 --alpha=0.85 --spatial=1 --fixbolus --mc --pvcorr --artoff
OXFORD_ASL - running
Version: v4.0.29-5-g09a5cba-dirty Thu Jul 13 13:20:21 2023
Input file: /home/user/Desktop/Perfusion_Demo_data/SinglePLD_ASL/merged_asl.nii.gz
Structural image: /home/user/Desktop/Perfusion_Demo_data/SinglePLD_ASL/T1w_Defaced.nii.gz
Saving results in native (ASL aquisition) space to /home/user/Desktop/Perfusion_Demo_data/ASL_outputs/native_space
Saving results in structural space to /home/user/Desktop/Perfusion_Demo_data/ASL_outputs/
```

The “TIS” matrix in oxford ASL = PLD + LD

```
# Design matrix for the ASL timeseries:
```

```
repeats="2,2,2,2"
TISs="3.4,2.2,3.7,2.8,1.8"
```

```
# Tutorial: For example, we have 3 PLD, each PLD have two repeats:
#           For your ASL image sequence: PLD1(T,C,T,C); PLD2(T,C,T,C); PLD3(T,C,T,C)
```

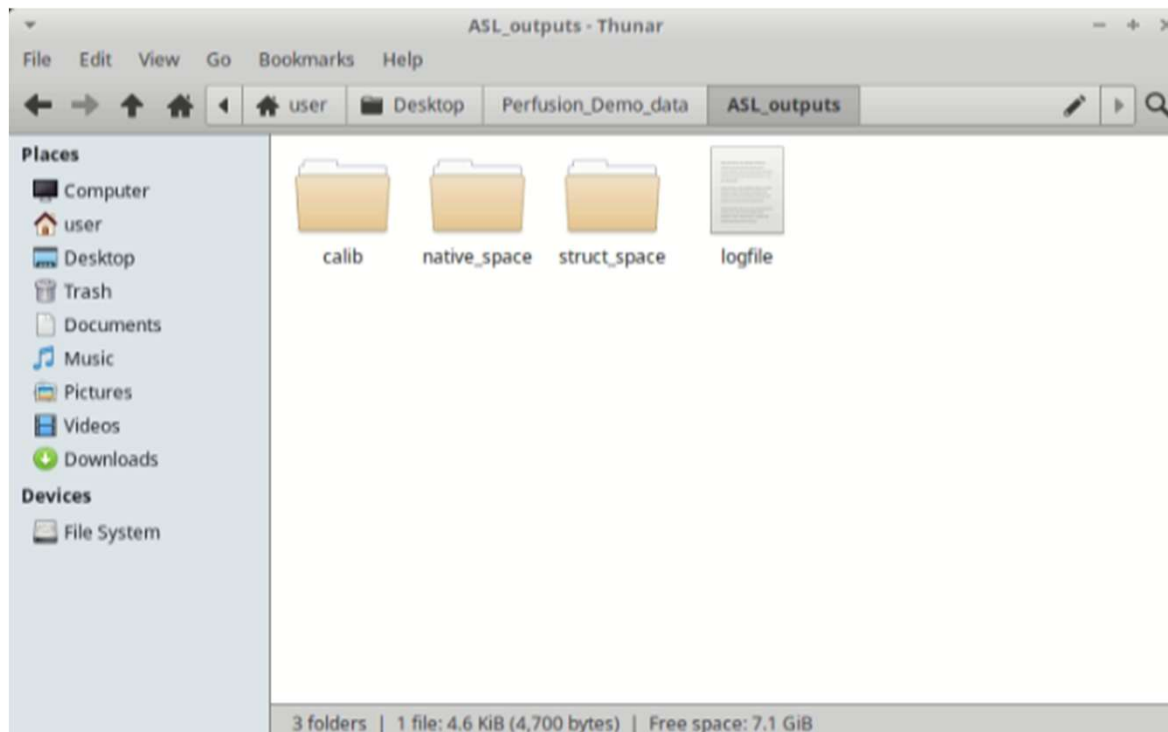
```
# First input the repeat number matrix:
#           repeats="2,2,2" (2 repeats for each PLD)
```

```
# Then for the TIS calculation:
#           TIS = PLD + Bolus time (Label duration)
#           e.g. TIS1 = 1.8 sec + 1.6 sec = 3.4 sec for first PLD
#           TIS2 = 1.0 sec + 1.6 sec = 2.6 sec for 2nd PLD
#           TIS3 = 0.2 sec + 1.6 sec = 1.8 sec for 3rd PLD
```

```
# Finally input your TISs matrix:
#           TISs="3.4,2.6,1.8"
```

- A good practice is to copy and save the code in the “Run” window for future use
- To learn more about Unix scripting: [https://andysbrainbook.readthedocs.io/en/latest/unix/Unix\\_Intro.html](https://andysbrainbook.readthedocs.io/en/latest/unix/Unix_Intro.html)

## Exercise 7: Let us take a look at the CBF map you generated!



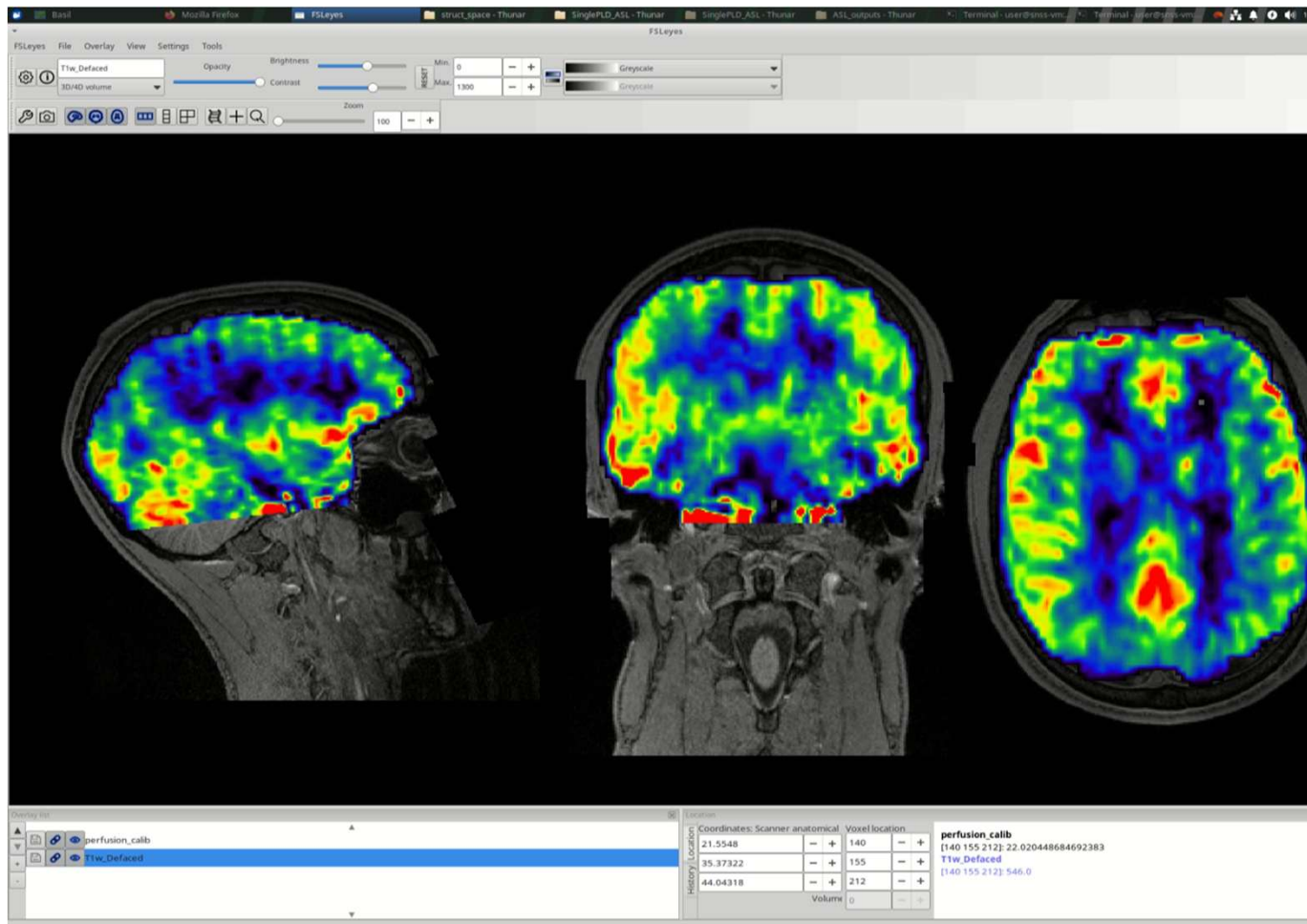
The **calib** tab contains the M0 image;

The **native space** contains the CBF image in its original ASL space (position).

The **struc\_space** contains the CBF image that is co-registered to the T1w images.

**\*\* We will examine the CBF images in the **struc\_space** folder.**

## Exercise 7: Let us take a look at the CBF map you generated!



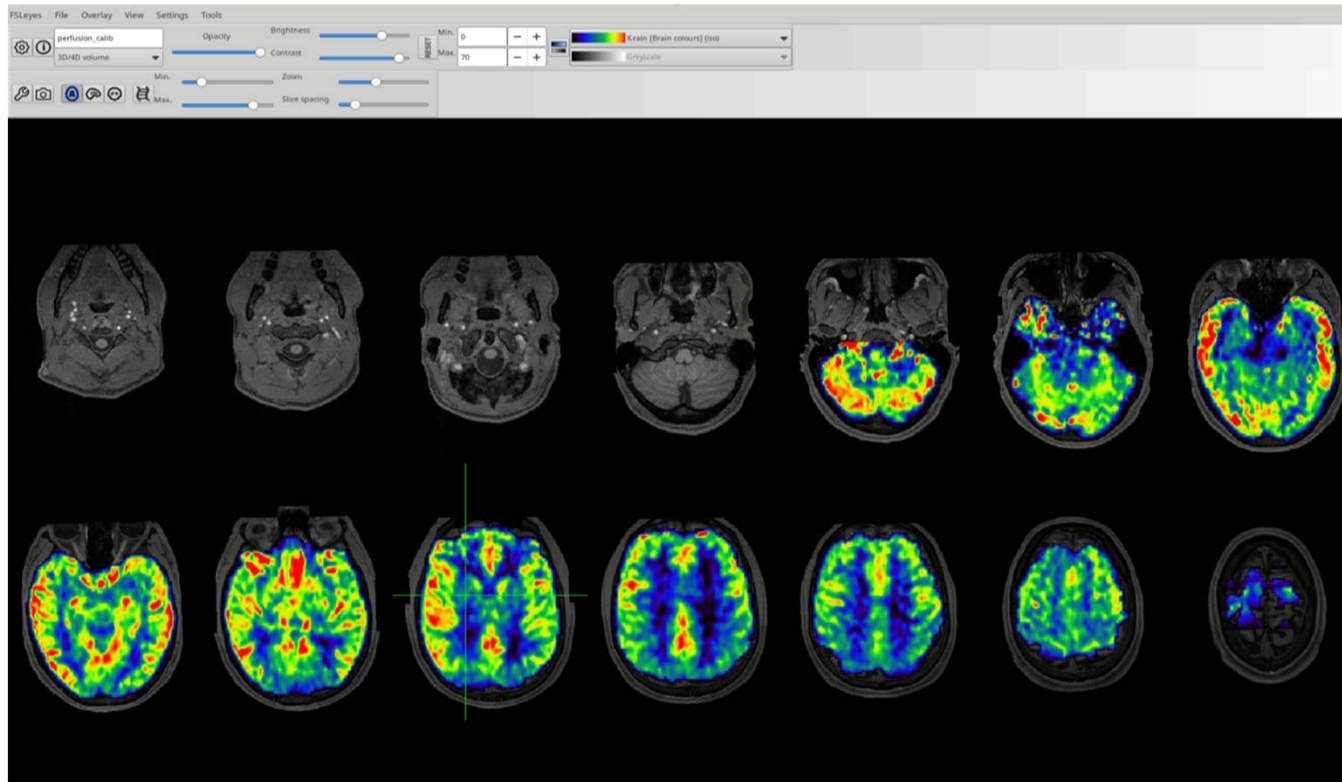
### To-dos:

1. Import **T1w** and **perfusion\_calib** images to the fsleaves.
2. Select the **perfusion\_calib** image and change the color bar to “X-rain”
3. Make sure **perfusion\_calib** image is on top of the **T1w\_Defaced**

### Questions:

1. What is the unit of each voxels in this CBF map?
2. Pick your favorite brain region and find an approximate CBF value.

## Exercise 7: Visually-inspect the CBF image using “Lightbox” view



# Summary of the workshop:

## Method:

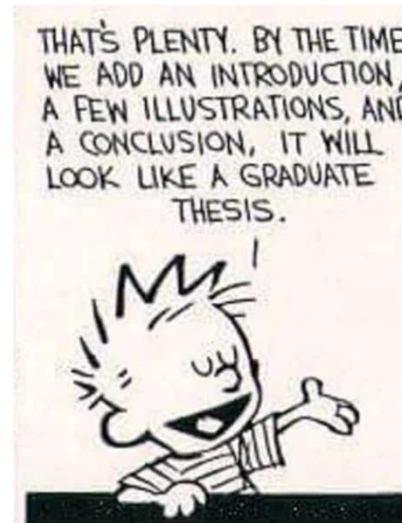
We recruited the healthy volunteer from University of Toronto (n=1). Neuroimaging was acquired using a \_\_\_\_\_(scanner name, field strength=\_\_\_\_). We collected T1-weighted images were acquired using \_\_\_\_ sequence for anatomical registration.

Cerebral blood flow (CBF) was measured using (pASL/cASL/pcASL) sequence (TR=\_\_\_\_, TE=\_\_\_\_, Spatial resolution=\_\_\_\_, Flip angle=\_\_\_\_). The labeling duration was \_\_\_\_ms, and we selected a single post-label delay of \_\_\_\_ ms. In total, \_\_\_\_ pairs of label-control images were collected. A proton-density-weighted image (TR=\_\_\_\_, TE=\_\_\_\_) were acquired for calibration.

Oxford ASL (version\_\_\_\_) were used for perfusion calibration and quantification...

## Results:

**Figure 1** shows the estimated CBF from pcASL experiment. The CBF in the \_\_\_\_\_ (Your ROI) was \_\_\_\_\_(unit).



\*\* Actually more works are needed in a real thesis