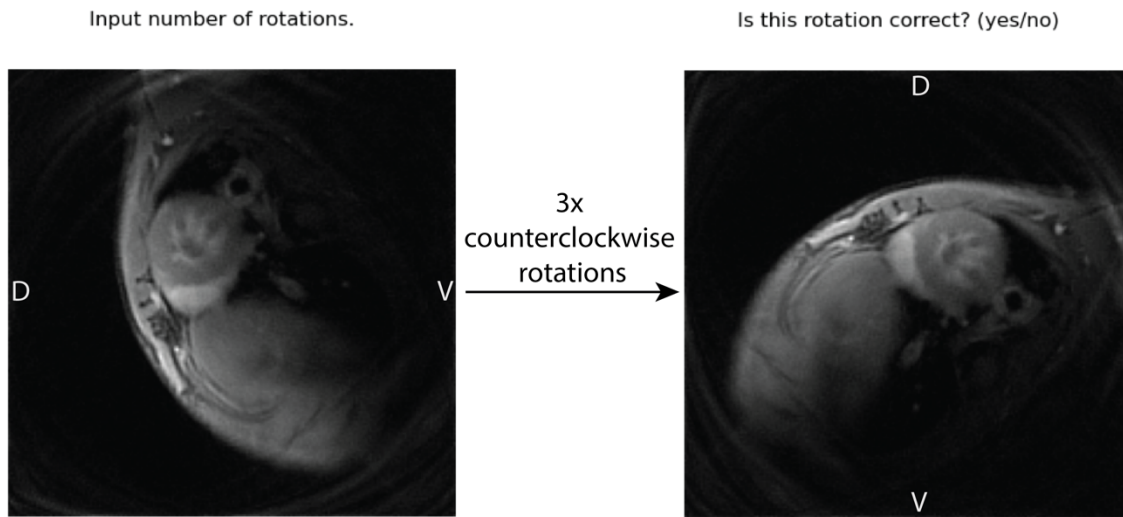**Ungated Cardiac CEST Data Processing Guide**
Jonah Weigand-Whittier
Department of Bioengineering, UC Berkeley
Last edited: May 29, 2024

1. Download the cest_scripts packages from GitHub and install dependencies from requirements.txt.
2. Install the latest release version of BART and add 'BART_TOOLBOX_PATH' or 'TOOLBOX_PATH' to the path variable per the BART readme.
3. Set variables in 'aha.py'.
   a. Set the 'directory' variable to the top-level data directory (e.g., /Users/jonah/Documents/MRI_Data/Berkeley/Manuscript_Data/).
   b. Set 'animal_id' to the scan ID corresponding to the Bruker data folder (e.g., 20240524_125104_214100_1R_1_5)
   c. Set the 'exp' variable to the experiment number corresponding to cestsegCSUTE acquisition, keeping 'Cest' as the label.
   d. Set 'save_as' to the name of the directory within your experiment folder you would like processed data to be saved under.
   e. Set undersampling. By default, we use 3x radial oversampling for acquisitions. Here, undersampling refers to the level of undersampling relative to 3x oversampling (i.e., by default we sample 1000 spokes, an oversampling value of 0.3 would reconstruct an image from the first 300 spokes). If you would like to experiment with "undersampling" (e.g., Nyquist sampling from 3x radial oversampled data), set this here; otherwise, use 'None'.
4. Run 'aha.py' in the terminal or through your IDE.
   a. You will be prompted to rotate your reconstructed images. Often, images are reconstructed Sometimes, if the angle of the cardiac short axis is particularly oblique, no rotations are required. Note that the input is the number of counterclockwise, 90-degree rotations (see Fig. 1).
   b. You will be prompted to draw ROIs in the following order (see Fig. 2):
      i. RV insertion points (anterior to inferior)
      ii. LV epicardium
      iii. LV endocardium
   c. The script will display selected ROIs with automatic AHA segmentation—if you are satisfied, type 'yes'.
5. CEST data is processed and saved under 'directory/Data/save_as', the following outputs are returned:
   a. Per-segment z-spectra and Lorentzian difference plots
   b. Example anatomical images (from reference images)
   c. ROIs and segmentation
   d. A pickled 'fits' file, which includes the following (per segment):
      i. Per pool CEST contrasts (under key 'Contrasts')
      ii. Two-step Lorentzian fitting parameters (under key 'Fit_Params')
      iii. Raw offsets, B_0 corrected offsets, raw Z-spectrum data, interpolated Lorentzian fits, and Lorentzian difference data (under key 'Data_Dict')

Fig 1.

Input number of rotations.

Is this rotation correct? (yes/no)

3x counterclockwise rotations

Fig 2.

Draw ROIs ("New ROI" to start)

'New ROI' to draw first ROI

Draw ROIs ("New ROI" to start)
Draw ROI 'Insertion Points (anterior --> inferior)'

Right click to close ROI, 'New ROI' to proceed

Draw ROIs ("New ROI" to start)
Draw ROI 'Endocardium'

After all three ROIs are closed, 'Finish' to confirm

Inferoseptal
Anteroseptal
Anterior
Anterolateral
Inferolateral
Inferior

Insertion Points (anterior --> inferior)
Epicardium
Endocardium

ROIs and segmentation will be displayed, input 'yes' if you are satisfied and 'no' to redraw