**Ungated Cardiac CEST Data Processing Guide**

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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’.
   1. Set the ‘directory’ variable to the top-level data directory (e.g., /Users/jonah/Documents/MRI\_Data/Berkeley/Manuscript\_Data/).
   2. Set ‘animal\_id’ to the scan ID corresponding to the Bruker data folder (e.g., 20240524\_125104\_214100\_1R\_1\_5)
   3. Set the ‘exp’ variable to the experiment number corresponding to cestsegCSUTE acquisition, keeping ‘Cest’ as the label.
   4. Set ‘save\_as’ to the name of the directory within your experiment folder you would like processed data to be saved under.
   5. 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.
   1. 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).
   2. You will be prompted to draw ROIs in the following order (see Fig. 2):
      1. RV insertion points (anterior to inferior)
      2. LV epicardium
      3. LV endocardium
   3. 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:
   1. Per-segment z-spectra and Lorentzian difference plots
   2. Example anatomical images (from reference images)
   3. ROIs and segmentation
   4. A pickled ‘fits’ file, which includes the following (per segment):
      1. Per pool CEST contrasts (under key ‘Contrasts’)
      2. Two-step Lorentzian fitting parameters (under key ‘Fit\_Params’)
      3. Raw offsets, B0 corrected offsets, raw Z-spectrum data, interpolated Lorentzian fits, and Lorentzian difference data (under key ‘Data\_Dict’)

**Fig 1.**

**A close-up of a person's eye

Description automatically generated**

**Fig 2.**

