Standard Operating Procedure: Animal MRI Data Segmentation and Processing

1. Purpose

This procedure details the steps taken to load, segment, and prepare magnetic resonance imaging (MRI) data acquired in pre-clinical mouse models of breast cancer.

2. Scope

Successful application of this procedure results in quantitative MRI maps a from one visit with saved segmentation files of tumor margins.

3. Definitions

DICOM	Digital Imaging and Communications in Medicine
MRI	magnetic resonance imaging
ROI	region of interest
DCE-MRI	Dynamic contrast-enhanced MRI
ADC	Apparent diffusion coefficient
SER	Signal Enhancement Ratio

3. Responsibility

Researchers trained in MR image loading and analysis will carry out this procedure.

4. Procedures

1. *Organize data*. Copy pre-clinical imaging data into a directory organized first by subject ID and then by imaging visit.

2. *Load MRI data into MATLAB workspace*. Use our custom function *load_u24_preclinical.m* by providing as input the directory where an individual subject MRI data is stored (*start_folder*) and where you want the *MATLAB '.mat'* to be stored (*save_folder*). Within the '.mat' file there will be individual data structures for each imaging visit.

   e.g.: *load_u24_preclinical*(*start_folder,save_folder*);

   Note: *load_u24_preclinical.m* assumes that you have Bruker's provided *pvtools* within your MATLAB path to run. *pvtools* handles the the reading and loading of raw imaging data and scan headers, while *load_u24_preclinical.m* interfaces with *pvtools* to organize, label, and store the data.

3. *Draw tumor and muscle ROIs*. After the data is loaded use our custom function *roi_u24_preclinical.m* to draw ROIs around the tumor and muscle for each animal and imaging visit. The muscle ROI is

used for DCE-MRI analysis to either scale the arterial input function for individual scans or used for reference region analysis in DCE-MRI. *roi_u24_preclinical.m* is executed by providing the location of the ".mat" file generated for an individual animal from step 2. After executing *roi_u24_preclinical.m* it will append the generated ROIs to individual animals ".mat" file.

Note: *roi_u24_preclinical.m* requires the use of vuOnePaneROIDrawer (from the Vanderbilt University Tools MATLAB distribution). MATLAB's *roipoly* could be substituted instead of vuOnePaneROIDrawer

4. *Perform T_1 mapping, ADC mapping, signal enhancement ratio (SER) calculation, and pharmacokinetic analysis of DCE-MRI data*: Use our custom MATLAB script *imagefit_u24utbs_v1.m* to perform all quantitative imaging analysis. *imagefit_u24utbs_v1* is executed by supplying the data structure for an individual visit loaded from the animal-specific ".mat" file. For all mapping or curve fitting, we utilize *lsqcurvefit* to minimize the residual between the model and the measured values. *load_u24_preclinical* will organize the image arrays and label them so that each individual subfunction (T_1-mapping, *ADC* mapping, SER calculation, and DCE-MRI analysis) within *imagefit_u24utbs_v1.m* will utilize the correct set of data. At the end of the execution of *imagefit_u24utbs_v1.m* it will append the results of the quantitative analysis to the animal-specific ".mat" file.

   a. T_1-mapping via variable flip angle method: We fit the equation below voxel-wise to variable flip angle T_1-weighted MRI data with at least four flip angles to return estimates of T_1 and S_0.

$$S\left(T_1, S_0\right) = \frac{S_0\left(\sin\left(\alpha\right)\left(1-\exp\left(-TR/T_1\right)\right)\right)}{\left(1-\exp\left(-TR/T_1\right)\cos\left(\alpha\right)\right)} \quad (1)$$

   where S_0 is constant related to scanner gain and proton density, α is the prescribed set of flip angles, *TR* is the repetition time, and T_1 is the longitudinal relaxation time. T_1 is constrained between 0 and 5 s, while S_0 is constrained to non-negative values.

   b. *ADC mapping*: We fit $S(ADC, S_0) = S_0 \exp\left(-b \cdot ADC\right)$ voxel-wise to diffusion weighted MRI data collected with at least two *b*-values to return estimates of *ADC* and S_0.

   c. *SER calculation*: The SER is calculated voxelwise from the DCE-MRI with the following formula: $SER = \left(S_{peak} - S_{baseline}\right) \Big/ \left(S_{washout} - S_{baseline}\right)$. S_{peak} is the peak or maximum signal for intensity for an individual voxel, $S_{baseline}$ is the averaged signal intensity prior to the injection of the contrast agent, $S_{washout}$ is the averaged signal intensity from the last 10 dynamics.

   d. *Pharmacokinetic analysis of DCE-MRI data*: The temporal evolution of tissue concentration of the contrast agent ($C_t(t)$) is modeled using the standard model, Eq. (2):

$$\frac{dC_t\left(t\right)}{dt} = K^{trans} \cdot C_p\left(t\right) - \left(K^{trans} \Big/ v_e\right) \cdot C_t\left(t\right) , \quad (2)$$

   where K^{trans} describes the movement of the contrast agent from the plasma space to the tissue space (i.e., the volume transfer constant), $C_p(t)$ is the concentration of the contrast

agent in the plasma space at time *t*, and v_e is the extravascular extracellular volume fraction. To estimate K^{trans} and v_e, the signal intensity measured during the MRI experiment must be related to $C_t(t)$. We first solve Eq. (1) for the R_1 ($R_1 \equiv 1/T_1$) time course $R_1(t)$ shown in Eq. (3):

$$R_1(t) = \frac{1}{TR} \cdot \ln\left[\frac{S_0 \cdot \sin(\alpha) - S(t) \cdot \cos(\alpha)}{S_0 \cdot \sin(\alpha) - S(t)}\right], \quad (3)$$

where $S(t)$ is the measured signal intensity at time *t*, and S_0 is defined in Eq. (4):

$$S_0 = S_{pc} \cdot \left[\frac{1 - e^{-TR \cdot R_{10}} \cdot \cos(\alpha)}{\left(1 - e^{-TR \cdot R_{10}}\right) \cdot \sin(\alpha)}\right], \quad (4)$$

where S_{pc} is the measured signal intensity prior to the contrast agent injection, and R_{10} is the measured R_1 prior to the contrast agent injection. For tissue, $R_1(t)$ is related to $C_t(t)$ using Eq. (5):

$$C_t(t) = (R_1(t) - R_{10}) / r_1, \quad (5)$$

where r_1 is the relaxivity of the contrast agent. r_1 is set to 3.7 $mmol^{-1}$ sec^{-1} [1]. We use a population C_p(t) from [2] which is scaled (as described in [3]) to achieve a v_e 0.13 in muscle. This scaled C_p is then used for all analysis. K^{trans} and v_e were constrained from 0 to 10.

5. *Saving pre-clinical MR images as DICOM images*: After all quantitative MR analysis is performed, we then save out the analysis as DICOM images for sharing *via ePAD*. Using our custom script *preclinical_dicom_writer_v1.m,* DICOM images are written to a folder labeled *pDCM* located within your current working directory in MATLAB. *preclinical_dicom_writer_v1* takes a single input of the animal specific ".mat" file which stores all images and analysis. This script requires the uses of *dicomwriter_u24_v1.m* (for writing DICOM images for individual image series) and *write_DSO.m* (for writing digital segmentation objects).

5. Review and Revision

State how often the SOP is reviewed, or under what circumstances it is to be revised and indicate who is responsible for reviewing the SOP.
<Insert text here>

6. References

[1] Rohrer M, Bauer H, Mintorovitch J, Requardt M, Weinmann H-J. 2005; Comparison of magnetic properties of MRI contrast media solutions at different magnetic field strengths. *Invest Radiol* [Internet]. United States; **40** 11 715–24. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16230904

[2] Loveless ME, Halliday J, Liess C, Xu L, Dortch RD, Whisenant J, Waterton JC, Gore JC, Yankeelov TE. 2012; A quantitative comparison of the influence of individual versus population-derived vascular input functions on dynamic contrast enhanced-MRI in small animals. *Magn Reson Med*. **67** 1 226–36.

[3] Hormuth II DA, Skinner JT, Does MD, Yankeelov TE. 2014; A comparison of individual and population-derived vascular input functions for quantitative DCE-MRI in rats. *Magn Reson Imaging*. **32** 4 397–

401.

7. History

Version	Revision Date	Section(s) Revised	Comments
1	2021-AUG-30	N/A	Initial Draft