Registration of CT data

AIMS

CT data should be warped to Allen Space and the inversion (Atlas to native space) should be possible.

PROBLEMS

[1] CT does not have enough image contrast for brain registration/segmentation.

SOLUTION: using t2w-image of the same animal & skull extraction from CT + creating inner mask of the skull with refers to the 'brain' volume. This volume is registered to the Allen-brain mask. The Allen-brain mask is created after registering the t2w-image to the Allen brain template and after back warping the Allen-brain mask. Thus, registration parameters from t2w registration can be applied to the CT image or other images.

[2] Mouse was differently positioned compared to berlin (t2w image is differently oriented) -> header of the t2w image has to be changed

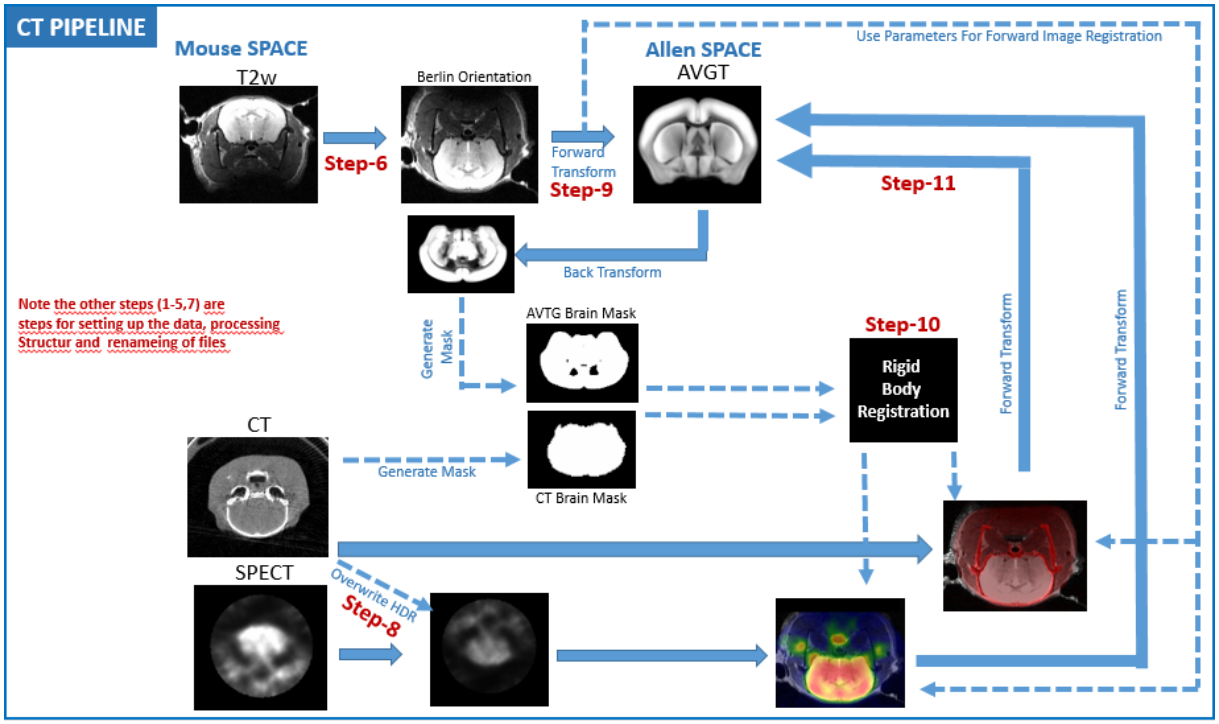
[3] t2w image is different regarding the position/origin of the CT image

[4] CT image is different regarding the position/origin of the t2w image

[5] SPECT image is different regarding the position/origin of the CT image

PIPELINE

The figure below depicts the steps to transform the present CT/SPECT data to Allen Mouse Space



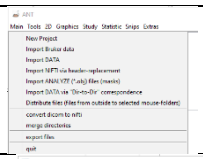
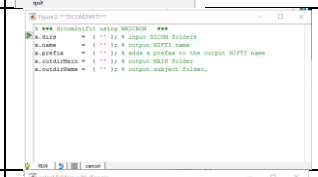
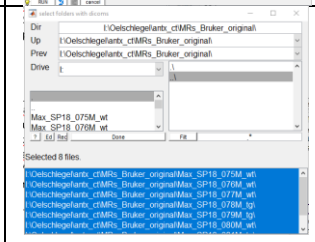
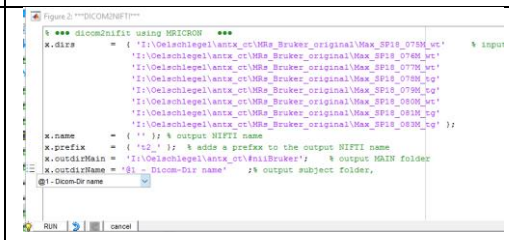
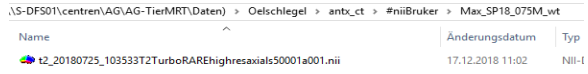
STEPS

1. Dicom to NIfti Conversion – Bruker data
2. Dicom to NIfti Conversion – CT data
3. Merge CT and t2w files/folders
4. Setup data pipeline
5. Rename files
6. Reorient t2w images to match with Berlin's mouse orientation
7. Rename 'ht20.nii to 't2.nii'
8. Reorient 'SPECT.nii' to match 'CT.nii'
9. Warp 't2.nii' to Allen Space
10. Registration of 'CT.nii' to 't2.nii'
11. Warp CT and SPECT image to Allen Atlas space

Add 10b: Manual POST-Registration of the CT-images (source) to 't2.nii' (target)

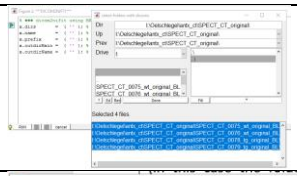
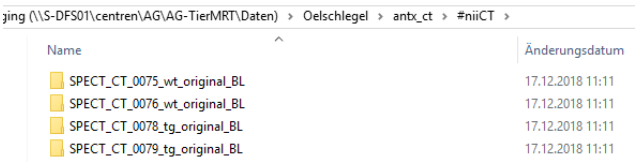
1. Dicom to NIfti Conversion – Bruker data

-**AIM**: convert Bruker Dicom data for each mouse folder
- set matlab working dir to the upper main folder that contains the BrukerDicom data
- start ant by typing ant in the command line

- from the ant menu bar select **MAIN**>' '**convert dicom to nifti'**	
- Select mice dicom directories. For this, set cursor to x.dirs, click on the green icon (left to x.dirs)	
- From the **left panel** select the main folder. This folder should contain the mouse/mice dicom folders. - All children folder of the main folder should now appear in the right panel. Select 1/more or all of these mouse folders from the **right panel** These folders contain the t2w-dicom image and potentially other images. If selected, these folders should disappear in the right window and reappear in the **lower panel** -click '**Done'**.	
-Assign a prefix for the output data by setting **x.prefix** to 't2_' -Select **x.outdirmain**>click green icon > select a directory to assign the output location, i.e. where Nifti's should be saved. If needed, create a new folder. →Here '#niiBruker' is the output folder for the Bruker-data -select **x.outdirName**> click on the left icon and select '@1 Dicom Dir name' (in this case the folder of the dicom contains the name of the upper directory) -hit '**Run'**	
OUTPUT: 	 Example: Contents of 075M folder. For each mouse dicom folder a corresponding nifti folder is created containing the respective Nifti-files after dicom conversion

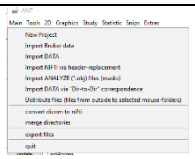
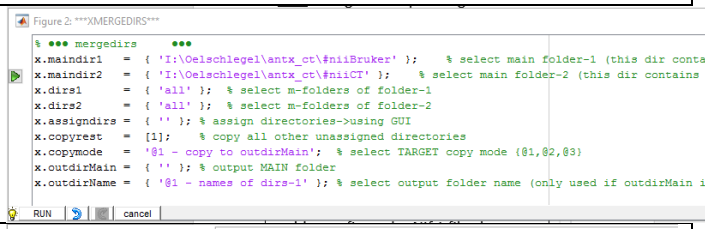
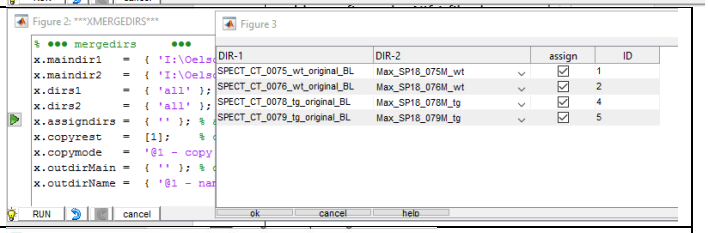
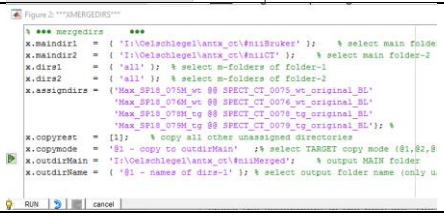
2. Dicom to NIfti Conversion – CT data

AIM: convert CT-Dicom data for each mouse folder

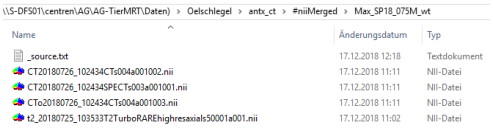
-from the ant menu bar select **MAIN>'convert dicom to nifti'**-select CT folder: click green icon left to **x.dirs**. From the Folder selection gui's right panel select the CT-folders (should disappear in the right panel and reappear in the bottom panel), then click done -add a prefix to the Nifti-files by setting **x.prefix** to 'CT'.	
-Select **x.outdirmain**>click green icon > select a directory to assign the output location, i.e. where Nifti's should be saved. If needed create a new folder. →Here '#niiCT' is the output folder for the CT-data -select x.outdirName> click on the left icon and select '@1 Dicom Dir name' -hit 'Run'.	
OUTPUT: 	 Example: Contents of 075M folder. For each mouse dicom folder a corresponding nifti folder is created containing the respective Nifti-files after dicom conversion.

3. Merge CT and t2w files/folders

AIM: merge corresponding CT data and Bruker data for each mouse

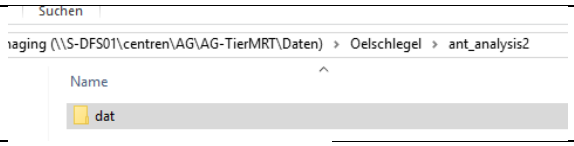
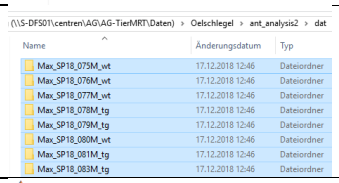
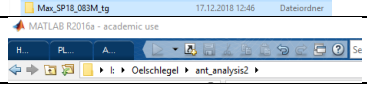
-from the ant menu bar select MAIN>'merge directories'	
-set cursor to x.maindir1, click green icon (left side) and select the '#niiBruker' folder from the dialogs' right panel, then click 'Done' -set cursor to x.maindir2, click green icon (left side) and select the '#niiCT' folder from the dialogs' right panel, then click 'Done'	
-set cursor to x.assigndirs, click green icon (left side) and check the assignment in the new gui. The assign-checkbox has to be check to merge the respective folders. If he matching of 'Dir1' and 'Dir2' fails use pulldown menu of 'Dir2' to select the corresponding folder -click 'OK'	
-set cursor to x.outdirMain, click green icon (left side) and select the output directory. If needed create a new folder. →Here '#niiMerged' is the output folder for the merged data.	

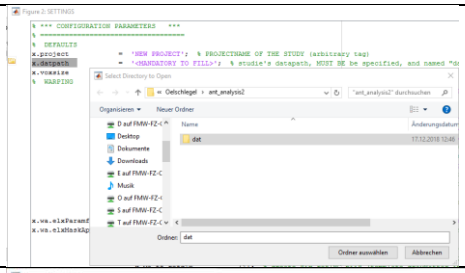
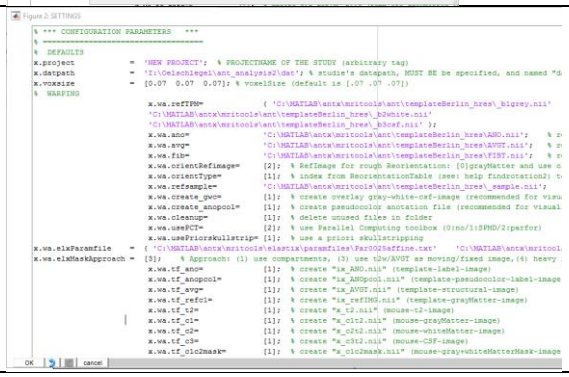
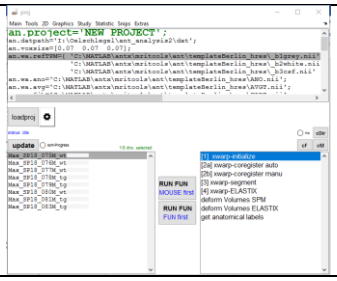
Information for other parameters (no need to change them here)
x.copyrest = [1]; % copy all other unassigned directories
-specifies whether all other directories should be copied as well. Here we want to copy all
	x.copymode = '@1 - copy to outdirMain' ;% select TARGET copy mode {@1,@2,@3}
	-here we want to copy/copy&merge the files to a new output directory instead of copying the data to either the #niiCT or #niiBruker folder MAIN folder
	x.outdirName = { '@1 - names of dirs-1' }; %
	-here we want to keep the folder names from dirs-1, i.e. the Bruker folder names for the output

OUTPUT: 	 Example: contents of 075M folder -the _source.txt file contains information regarding the origin of the merging procedure -Note: Mricron's dii2nii was used because SPM produced errors for the CT data. dii2nii produces two identical files *.002 and *.003, so only one is needed.

4. Setup data pipeline

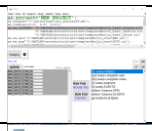
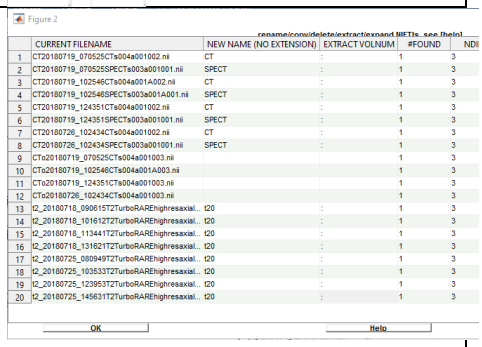
Aim: Create data structure. Copy data to the 'dat'-folder

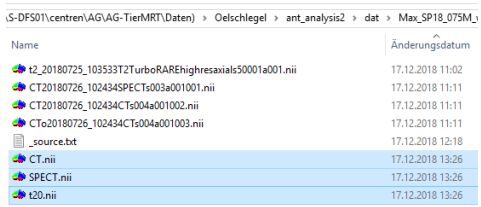
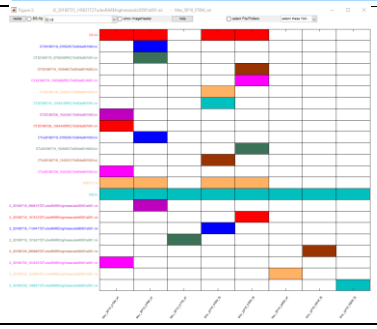
- Make an analysis folder. This folder will contain all data and templates, thus ensure enough storage space. Here, the analysis folder is names 'ant_analysis2' -Within the analysis folder create a folder and term is 'dat'	
-copy all merged mouse folder into the dat folder	
- Set matlab's current working directory to the the analysis folder (here 'ant_analysis2')	
-if not done before, start ant-tbx by typing 'ant' in Matlab' command line	

-create a new project file. For this go to ‘Main/New Project’ from the Ant Menu. In the opening gui, set cursor to x.datpath, then click on the icon appearing on the left side. From the opening gui select the ‘dat’-folder, than click ‘ok’/’elect folder’	
-set `x.wa.elxMaskApproach = [3] !!!` -click ‘OK’	
-the next dialog asks whether to save the config-file: keep the config. file name as ‘proj.m’ and select ‘speichern’/‘save’ -best practice is to save this m-file in the analysis folder -the next dialog asks whether to load the project: select yes. - the next time you can load the project file by selecting the ‘loadproj’-button from the ANT main panel	
RESULT: -project is loaded and mouse folders should be recognized as seen in the ANT’s left panel	

5. Rename files

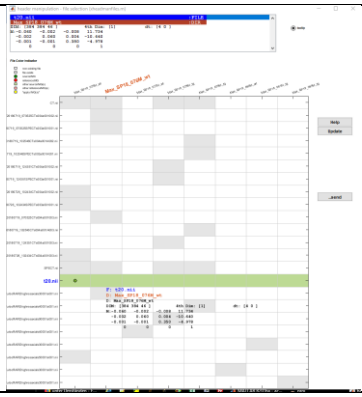
-AIM: rename NIFTI-files (here we use copies instead of overwriting the original data)

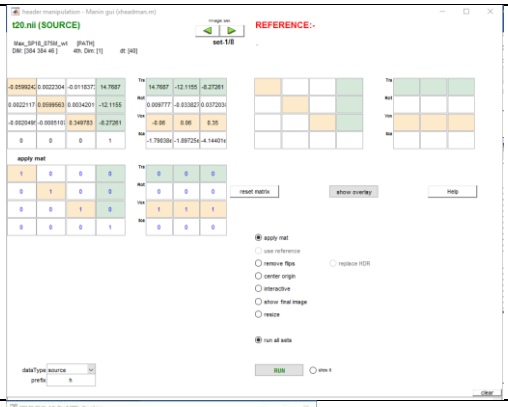
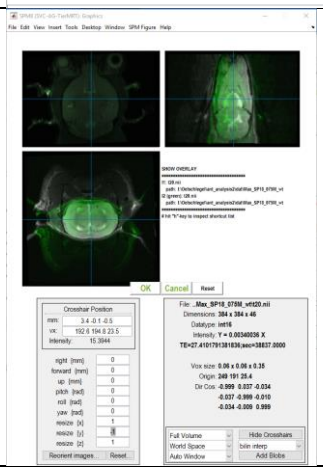
-select all mouse folders from ANT’s left panel	
-from ANT menu bar select TOOLS/manipulate files 1# - for the CT*002.nii data type ‘CT’ in the NEW NAME COLUME (you may copy and paste the name) - type a ’:’ in the EXTRACT VOLUME’ column to make copies of the orig. files instead of renaming the files -not the CT*002.nii data and CT*003.nii data are identical, so we don’t need them 2# - for the CT*SPECT*001.nii data type ‘SPECT’ in the NEW NAME COLUME - type a ’:’ in the EXTRACT VOLUME’ column to make copies of the orig. files instead of renaming the files 3# - for the t2*TurboRare+.nii data type ‘t20 in the NEW NAME COLUME - type a ’:’ in the EXTRACT VOLUME’ column to make copies of the orig. files instead of renaming the files - note that there are more t2w-images than CT images -select ‘OK’	

RESULT: -for all selected mouse folders copies were made for the t2w/CT and SPECT images with unified names -the right figure shows the content of folder 075M	
CHECK: -from ANT menu bar select graphics/show-case-file-matrix -we see that the same 4 mouse folders contain the CT.nii image (red cells, top row) and SPECT.nii image (orange cells, ~ middle row), while all mouse directories contain the t20.nii image (cyan, ~middle row)	

6. Reorient t2w image to match with Berlins mouse orientation

AIM. Mice in this data set is positioned differently than in Berlin

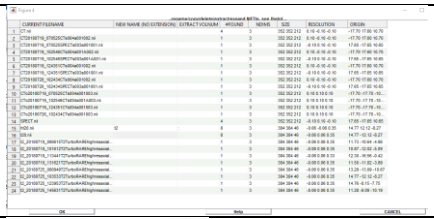
-select all mouse folders from ANT's left panel (if not done before)	
-from ANT menu bar select TOOLS/manipulate header 1. Single left-click onto the cell corresponding to the 't20.nii' image of the first mouse (indicated by a green dot) 2. Hit key 'e' to expand the selection, i.e. selecting 't20.nii' from all mouse folders (indicated by green background of the cell) 3. select 'send'	

- in the header manipulation window do the following: -select 'apply mat' -deselect 'remove flips' -select 'run all sets'	
-select 'show overlay' and wait until a new gui opens.. **<u>- important step:</u> in the SPM window type -1 in the field 'resize {y}' and don't forget to hit enter** -select 'OK'	
RESULT: The file 'ht20.nii' is created with an orientation similar to Berlin's mouse	

orientation NOTE: SPECT.nii and CT.nii are differently oriented compared to the t20.nii. Thus the have to be treated differently. In fact, after reorientation of the t20.nii to Berlin's orientation the CT.nii image matches the new t20.nii. However, the origin is sill different. The Spect.nii, however, is also oriented differently	

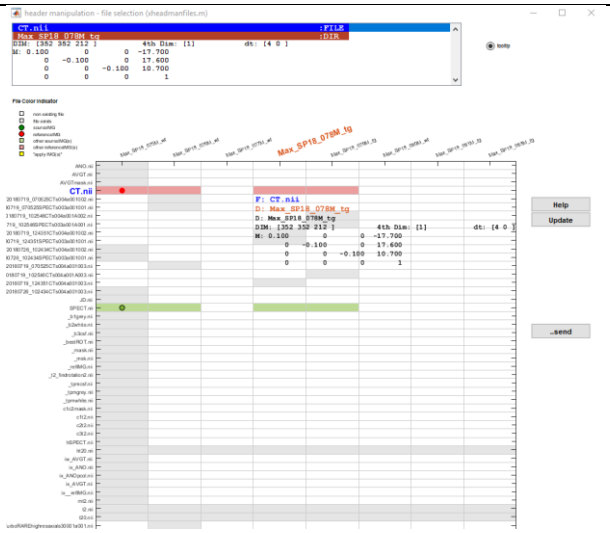
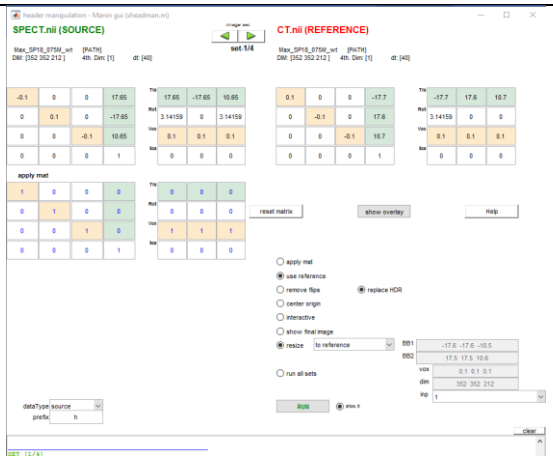
7. RENAMING ht20.nii to 't2.nii'

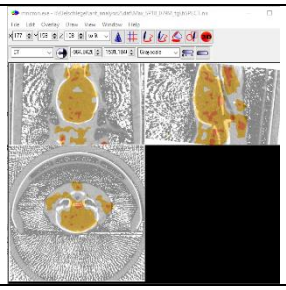
AIM. 't2.nii' is the standard input for image registration thus we rename the file ‚ht20.nii' to 't2.nii'. For documentation purpose we copy the file and rename the new file instead of overwriting the image

-select all mouse folders from ANT's left panel (if not done before)	
-from ANT menu bar select TOOLS/manipulate files 1# - for the file ‚ht20.nii'.nii type 't2 in the NEW NAME COLUME - type a ':' in the EXTRACT VOLUME' column to preserve the original file - select 'OK'	
Result: now the file 't2.nii' will be created for the selected mouse folders, which is the input for the registration. The existence of 't2.nii' is indicated by the yellow box in the left mouse-folder box in the ANT main gui.	

8. REORIENT SPECT.nii to match CT.nii

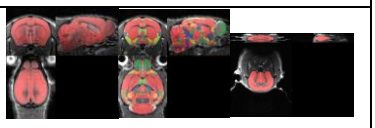
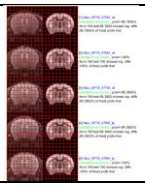
AIM. SPECT.nii image must be reoriented+resliced to match the CT.nii regarding positioning and origin

-select all mouse folders from ANT's left panel (if not done before)	
-from ANT menu bar select TOOLS/manipulate header 1. SOURCE image selection: **Single** left-click onto the cell corresponding to the 'SPECT.nii' image of the first mouse (indicated by a green dot) 2. TARGET image selection: **DOUBLE** left-click onto the cell corresponding to the 'CT.nii' image of the first mouse (indicated by a red dot) 3. EXPAND selection to all folders. Hit **key 'e'** to expand the selection, i.e. selecting all 'CT.nii' and corresponding 'SPECT.nii' images from all mouse folders (indicated by red and green background of the cells, respectively) 4. select 'send'	
- in the header manipulation window do the following: -deselect 'apply mat' -select 'use reference' -select 'replace HDR' -select 'resize': and there select 'to reference' from the pulldown menu and set the interpolation (inp) to 1 -select 'run all Sets' - you may select 'show it' right to the 'RUN' button to obtain an output overlay of the CTdata with the SPECTdata via MRIcron for each Source/Target Set (mouse dirs) -hit 'RUN' button -afterwards you may inspect the overlay of the CT and SPECT image by selecting the blue hyperlink in Matlab's command window. Don't forget to set the dynamic range of the CT data properly (such as 1 and 5000) otherwise the overlay is black	

RESULT: A new Image ‘hSPECT.nii’ is created that matches with the ‘CT.nii‘ regarding orientation and origin. -as an example, the right figure shows the overlay of the ‘CT.nii’ an ‘hSPECT.nii’ for mouse 079M.	

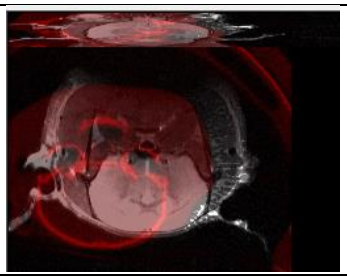
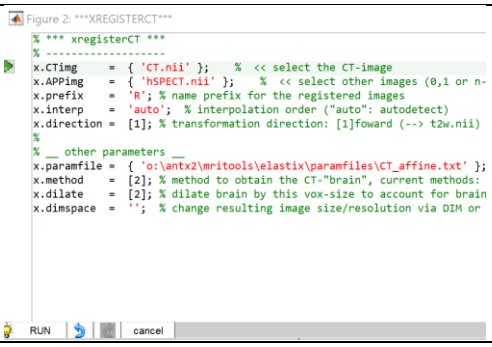
9. Warping t2.nii to Allen Space

AIM. **‘t2.nii’** is registered and warped to the Allen template. The transformation parameters for Native-to-Allen Space and its inverse is calculated.

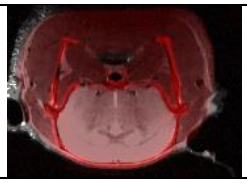
-select all mouse folders from ANT’s left panel (if not done before) * The left panel in the right image indicates that mouse 075M has been already skull stripped, roughly oriented, segmented and warped to Allen space as indicated by the colored boxes right to the mouse name (I had to run this mouse in a previous testing step .. but data will be overwritten now) -select the steps [1],[2a],[2b],[3] and [4] from the right box of the ANT main gui. -If Matlabs parallel-TBX is installed select the upper ‘RUN FUN’ button otherwise use the bottom ‘RUN FUN’ button to start the image registration * For each mouse (and this registration approach, i.e. 3) this will take ~30min	
RESULT: After processing the transformation parameters for registration of Images in native mouse space to Allen space and vice versa can be used to transform other images: The right fogire depict the overlay of the t2w image (‘x_t2.nii’) with the Allen template (AVGT.nii, red color, left), the pseudoAtlas (ANOpcol.nii, middle), both in Allenspace and the t2w image (t2.nii) and the backtransformed AllenTemplate (ix_AVGT.nii, red color, right panel)	
-also check registration using contextmenu from the left mouse folder box (e.g. cgeck coreg panel elastix)	

10. Registration of ‘CT.nii’ to ‘t2.nii’

AIM. Register CT.nii to t2.nii and apply registration to ‘hSPECT.nii’

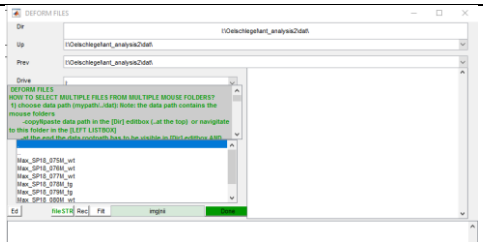
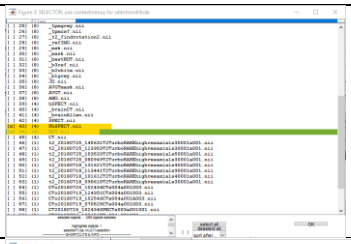
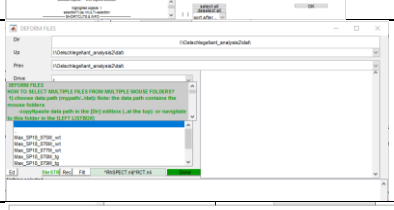
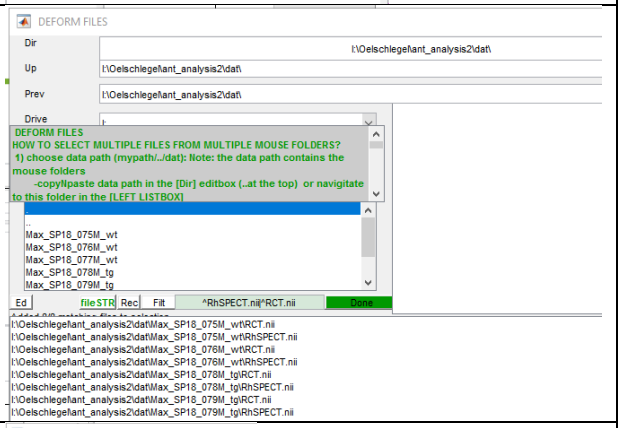
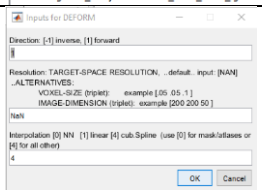
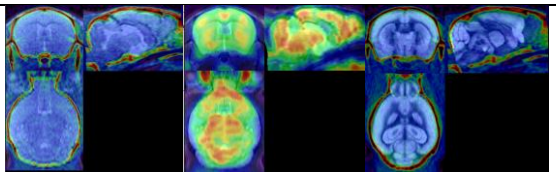
PROBLEM: -STATUS: orientation of CT.nii and t2.nii is fine after using performing step-6 (using [-1] for the ‘resize {y}’ parameter) - BUT: the two images are not co-located (see right figure) →New PROBLEM: CT.nii has no contrast regarding brain tissue compartments/structures → SOLUTION: create a brain mask from the CT’s skull-signal and register that with the back transformed Allen template brain mask (ix_AVGT.nii →mouse brain mask). Then apply this rigid transformation to CT.nii and ‘hSPECT.nii’., such that both images are co-located with ‘t2.nii’	
-select all mouse folders from ANT’s left panel (if not done before) -from ANT menu bar select TOOLS/registerCTimage	
Use left icons in the new gui to: - select x.CTimg: here ‘CT.nii’ -select x.APPimg: here ‘hSPECT.nii’ -the transformation is calculated using ‘CT.nii’ and applied to ‘hSPECT.nii’ - for affine transformation click icon of x.paramfile and select the respective parameterfile (“CT_affine.txt”) - set x.method to 2 -set x.dilate to 2 -hit ‘RUN’	

```
% *** xregisterCT ***
% ------------------
x.CTimg     =  { 'CT.nii' };    %  << select the CT-image
x.APPimg    =  { 'hSPECT.nii' };    %  << select other images (0,1 or n-
x.prefix    =  'R'; % name prefix for the registered images
x.interp    =  'auto';  % interpolation order ("auto": autodetect)
x.direction =  [1]; % transformation direction: [1]foward (--> t2w.nii)
%
% __ other parameters __
x.paramfile =  { 'o:\antx2\mritools\elastix\paramfiles\CT_affine.txt' };
x.method    =  [2]; % method to obtain the CT-"brain", current methods:
x.dilate    =  [2]; % dilate brain by this vox-size to account for brain
x.dimspace  =  '';  % change resulting image size/resolution via DIM or
```

RESULT: New files will be created ‘RCT.nii’ and ‘RhSPECT.nii’, both aligned with ‘t2.nii’ -example: mouse 075M overlay of ‘t2.nii’ and ‘RCT.nii’	

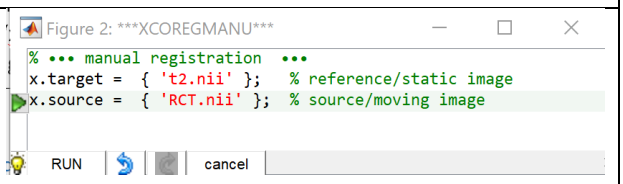
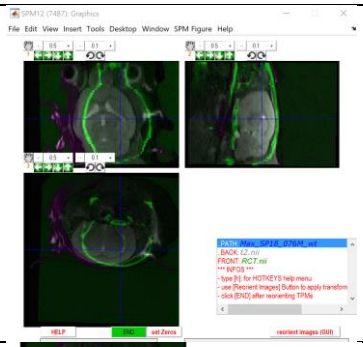
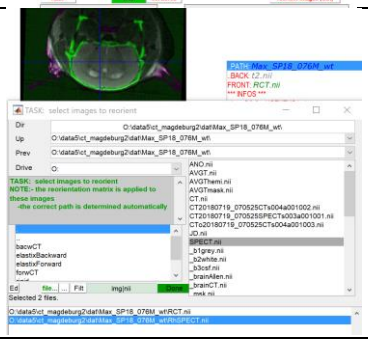
11. Warp CT and SPECT image to Allen Atlas space

AIM. The ‘t2.nii’ image was used to estimate the linear and nonlinear registration parameters to transform images aligned with ‘t2.nii’ into AllenAtlas space. Using these transformation parameters ‘RCT.nii’ and ‘RhSPECT.nii’ are brought into Atlas space.

-select all mouse folders from ANT’s left panel (if not done before)	
-select ‘deform Volumes Elastix’ from the right box of the ANT main gui -select the lower ‘RUN FUN’ button	
- in the new window select the ‘fileSTR’ button	
-in the new window select ‘RCT.nii’ and ‘RhSPECT.nii’ (note that the [x] tag marks a selected file while the [ ] tag marks unselected files) - hit ‘OK’	
- now, the the filter field in the file selection window should shows ‘^RhSPECT.nii|^RCT.nii’ -select the ‘Rec’ button to recursively find these files across mouse folders	
- now, the found files should appear in the lower listbox -hit ‘Done’	
-in the new Window, hit ‘OK’	
RESULT: now, ‘RCT.nii’ and ‘RhSPECT.nii’ are brought into Atlas space. The respective new files have the prefix ‘x_’ (, ‘x_RCT.nii’ and ‘x_RhSPECT.nii’) - The right figure shows the overlay of ‘x_t2.nii’ & ‘x_RCT.nii’ (left), ‘x_t2.nii’ & ‘x_RhSPECT.nii’ (middle) and ‘AVGT.nii’ (AllenTemplate) & ‘x_RCT.nii’ (right) for mouse 075M	

Add 10b: Manual POST-Registration of the CT-images (source) to ‘t2.nii’ (target)

AIM. In case of suboptimal registration of ‘CT.nii’ to ‘t2.nii’ use this function (‘xcoregmanu.m’).
USE THIS FUNCTION BEFORE warping the CT and SPECT image to ALLEN SPACE.

-Select the respective animals from the left listbox. -Note: Reorientation changes of the source image and other images are only set if the respective files are selected in the listbox after clicking the [reorient images (GUI)] button from the Graphics window. Thus, it is also possible to select all animals, inspect each case and to manually register only the cases you like.	
Select "**Tools/register images manually**" - In the parameter gui use the icon next to **x.target** and **x.source** and select the images: target: 't2.nii' → this is the image with the target orientation source: 'RCT.nii'→ this image should be reoriented -hit 'RUN'	
→ reorient image manually - In the Graphics window you have three options to manually register the source image (green image) to the target image (gray image): 1) shortcuts : hit 'h' to see the shortcut list 2) use panels above coronal/sagittal/axial images -use the 'hand icon' to drag the panel to a position of your choice - upper row controls to change the step size for translation and rotation - lower row controls to translate or rotate the source volume 3) SPM's default edit fields: enter value in the respective field	
→ apply reorientation to source image (and other images) If the manual registration is sufficient the next step is to apply the reorientation to the source image (and other images). For this, click **[reorient Images (GUI)]** button and select the images. - Here the reorientation should be applied to 'RTC.nii' & 'RhSPECT.nii'. - Accordingly, the header of 'RTC.nii' & 'RhSPECT.nii' will be changed. **NOTE: This step is mandatory to apply the reorientation to one/more images. Otherwise the orientation is not applied!** Click [**Done**].	
Finally, click [**END**] in the Graphics Window to close the GUI	

BATCHES

```
% % ====================================================
% % #g FUNCTION:      [xdicom2nifti.m]
% % #b info :         #wg function to convert dicoms to nifti
% % ====================================================
z=[];
z.dirs     = { 'O:\data5\ct_magdeburg2\raw\MRs_Bruker_original\Max_SP18_075M_wt'      % % input DICOM folders
             'O:\data5\ct_magdeburg2\raw\MRs_Bruker_original\Max_SP18_076M_wt'
             'O:\data5\ct_magdeburg2\raw\MRs_Bruker_original\Max_SP18_077M_wt'
             'O:\data5\ct_magdeburg2\raw\MRs_Bruker_original\Max_SP18_078M_tg'
             'O:\data5\ct_magdeburg2\raw\MRs_Bruker_original\Max_SP18_079M_tg'
             'O:\data5\ct_magdeburg2\raw\MRs_Bruker_original\Max_SP18_080M_wt'
             'O:\data5\ct_magdeburg2\raw\MRs_Bruker_original\Max_SP18_081M_tg'
             'O:\data5\ct_magdeburg2\raw\MRs_Bruker_original\Max_SP18_083M_tg' };
z.name     = { '' };                                   % % output NIFTI name
z.prefix   = { 't2_' };                                % % adds a prefxx to the output NIFTI name
z.outdirMain = 'O:\data5\ct_magdeburg2\raw\#bruker';                    % % output MAIN folder
z.outdirName = '@1 - Dicom-Dir name';                          % % output subject folder,
xdicom2nifti(1,z);

% % ====================================================
% % #g FUNCTION:      [xdicom2nifti.m]
% % #b info :         #wg function to convert dicoms to nifti
% % ====================================================
z=[];
```

```
z.dirs      = { 'O:\data5\ct_magdeburg2\raw\SPECT_CT_original\SPECT_CT_0075_wt_original_BL'       % % input DICOM folders
            'O:\data5\ct_magdeburg2\raw\SPECT_CT_original\SPECT_CT_0076_wt_original_BL'
            'O:\data5\ct_magdeburg2\raw\SPECT_CT_original\SPECT_CT_0078_tg_original_BL'
            'O:\data5\ct_magdeburg2\raw\SPECT_CT_original\SPECT_CT_0079_tg_original_BL' };
z.name      = { '' };                                                   % % output NIFTI name
z.prefix    = { 'CT' };                                                 % % adds a prefxx to the output NIFTI name
z.outdirMain = 'O:\data5\ct_magdeburg2\raw\#CT';                                    % % output MAIN folder
z.outdirName = '@1 - Dicom-Dir name';                                       % % output subject folder,
xdicom2nifti(1,z);


% % ====================================================
% % #g FUNCTION:       [xmergedirs.m]
% % #b info :          function to merge the  contents of pairwise assigned directories
% % ====================================================
z=[];
z.maindir1   = { 'O:\data5\ct_magdeburg2\raw\#bruker' };              % % select main folder-1 (this dir contains other dirs)
z.maindir2   = { 'O:\data5\ct_magdeburg2\raw\#CT' };                  % % select main folder-2 (this dir contains other dirs)
z.dirs1      = { 'all' };                                   % % select m-folders of folder-1
z.dirs2      = { 'all' };                                   % % select m-folders of folder-2
z.assigndirs = { 'Max_SP18_075M_wt @@ SPECT_CT_0075_wt_original_BL'       % % assign directories->using GUI
            'Max_SP18_076M_wt @@ SPECT_CT_0076_wt_original_BL'
            'Max_SP18_078M_tg @@ SPECT_CT_0078_tg_original_BL'
            'Max_SP18_079M_tg @@ SPECT_CT_0079_tg_original_BL' };
z.copyrest   = [1];                                         % % copy all other unassigned directories
z.copymode   = '@1 - copy to outdirMain';                          % % select TARGET copy mode {@1,@2,@3}
z.outdirMain = 'O:\data5\ct_magdeburg2\raw\#merged';                    % % output MAIN folder
z.outdirName = { '@1 - names of dirs-1' };                         % % select output folder name (only used if outdirMain is defined),
xmergedirs(1,z);

% ••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
% BATCH:       [xrename.m]
% descr:  #bc [xrename] RENAME/DELETE/EXTRACT/EXPAND/COPY file(s) from selected ant-mousefolders
% ••••••••••••••••••••••••••••••••••••••••••••••••••••••••••••
z=[];
z.files={ 'CT20180719_070525CTs004a001002.nii'     'CT'         ':'
        'CT20180719_070525SPECTs003a001001.nii'   'SPECT'    ':'
        'CT20180719_102546CTs004a001A002.nii'      'CT'         ':'
        'CT20180719_102546SPECTs003a001A001.nii'               'SPECT'    ':'
        'CT20180719_124351CTs004a001002.nii'         'CT'         ':'
        'CT20180719_124351SPECTs003a001001.nii'   'SPECT'    ':'
        'CT20180726_102434CTs004a001002.nii'         'CT'         ':'
        't2_20180718_090615T2TurboRAREhighresaxials30001a001.nii'    't20'        ':'
        't2_20180718_101612T2TurboRAREhighresaxials40001a001.nii'    't20'        ':'
        't2_20180718_113441T2TurboRAREhighresaxials90001a001.nii'    't20'        ':'
        't2_20180718_131621T2TurboRAREhighresaxials40001a001.nii'    't20'        ':'
        't2_20180725_080949T2TurboRAREhighresaxials30001a001.nii'    't20'        ':'
        't2_20180725_103533T2TurboRAREhighresaxials50001a001.nii'    't20'        ':'
        't2_20180725_123953T2TurboRAREhighresaxials30001a001.nii'    't20'        ':'
        't2_20180725_145631T2TurboRAREhighresaxials30001a001.nii'    't20'        ':' };
xrename(1,z.files(:,1),z.files(:,2),z.files(:,3) );
```