

## Prepare resting state Data for FSL

**NOTE: this tutorial is unfinished but will be finished asap  
(date: 24.11.20).**

## APPROACH-1

AIM: register the structural image to the template, use inverse registration pipeline to obtain the brain-mask in native space. Register structural image to EPI (RS-data), and bring brain mask into resting-state space.

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This tutorial for Approach-1 shows how to prepare resting state data for FSL with following steps:

1. Set ANT path, make study folder +start ANT GUI
  2. Download template
  3. Define a project
  4. Import Bruker data
  5. Import templates for this study
  6. Create a 't2.nii' image
  7. Examine Orientation
  8. Register 't2.nii' to the template
  9. Back-transform template brain mask to native space
  10. Extract 1st image of the 4D BOLD series
  11. Coregister 't2.nii' onto BOLD (RS-) Data
  12. Mask first EPI-image with brain mask
  13. Scale up 4D data for FSL
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## Alternative approach: APPROACH-2

AIM: register the structural image to 1<sup>st</sup> image of the RS-data. The structural image in register with the RS-data (t2.nii) is registered to the template. The inverse registration pipeline is used to obtain the brain-mask in native space (RS-DATA-SPACE).

Steps 1-5 as in Approach-1

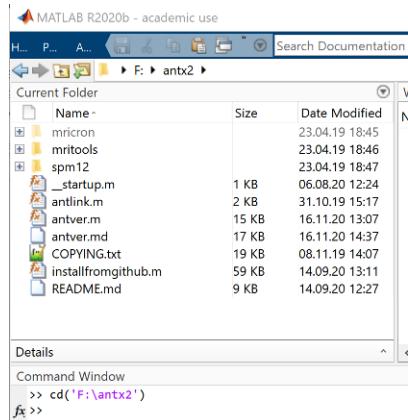
6. Extract 1st image of the 4D BOLD series (Approach2) → nearly identical to Approach1
7. Coregister 't2.nii' onto BOLD (RS-) Data (Approach2) → identical to Approach1
8. Create a 't2.nii' image (Approach2) → nearly identical to Approach1
10. Back-transform template brain mask to native space (Approach2) → same as Approach1
11. Mask first EPI-image with brain mask (Approach2) → nearly identical to Approach1
12. Scale up 4D data for FSL (Approach2) → identical to Approach1

# APPROACH-1

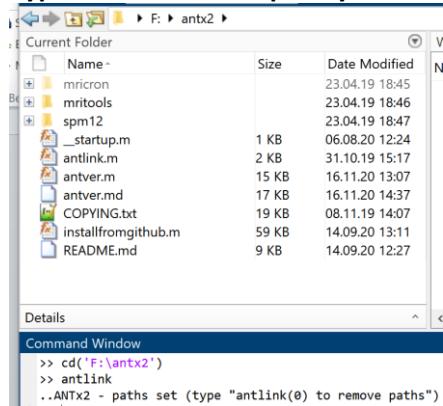
## 1. Set ANT path, make study folder +start ANT GUI

Set Matlab's current to antx2-TBX

Example: type: `cd('F:\antx2')`

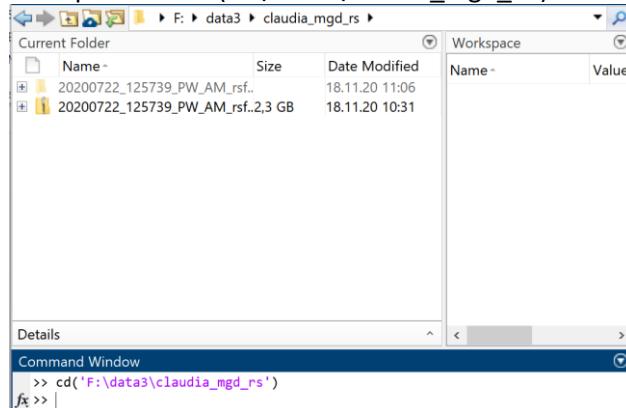


Type 'antlink' to temporary set the antx2'-paths



Go to project-folder

Example >>> `cd('F:\data3\claudia_mgd_rs')`



**NOTE:** I just created a folder 'claudia\_mgd\_rs'. This folder contains a folder with the unzipped raw-bruker data ('20200722\_125739\_PW\_AM\_rsfMRI\_Rat4\_200722\_PW\_AM\_rsfMRI\_Rat4\_1\_1'). Note that the raw-data can be stored somewhere else (... convenient).

### Start the GUI:

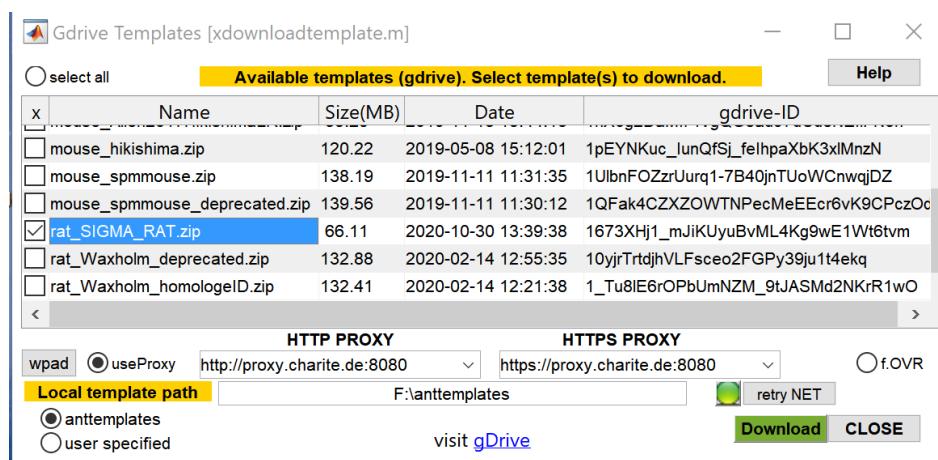
Type 'ant'



## 2. Download template

Here we use the Sigma Rat brain atlas.

Go to: ANT-MENU: EXTRAS/ download templates (Alternatively you can also download the template manually via EXTRAS/ get templates from googledrive).



Check 'rat SIGMA\_RAT.zip'. Here you have to examine whether you sit behind a proxy, if yes, you have to set the checkbox and the proxies properly (AGAIN: the alternative is download a template manually via ANT-MENU: EXTRAS/ get templates from googledrive). Use the [wpad]-button to examine whether you are behind a proxy and if so what the name of the proxies is (there is no guarantee that this works!. If you are working from home, you most likely have no proxies).

If successful, The template is downloaded and unzipped and stored in the 'anttemplates'-folder. Note that the anttemplates-folder is at the same hierarchical level as the 'ant'-TBX:

**For example after downloading the rat SIGMA\_RAT template located here:  
'F:\ANTTEMPLATES\RAT\_SIGMA\_RAT' and contains the following files:**

ANO.nii	Atlas labels
ANO.xlsx	Atlas (NIFTI)
AVGT.nii	template
AVGThead.nii	template (entire head)
AVGThemi.nii	Hemispheric brain mask

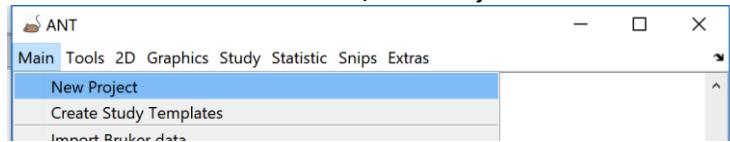
```

AVGTmask.nii Brain mask
changes.xlsx some info
parameter.m animal species parameter file
readme.txt readme with sources/links
_b1grey.nii GM
_b2white.nii WM
_b3csf.nii CSF

```

### 3. Define a project

From ANT-menu: select Main/New Project



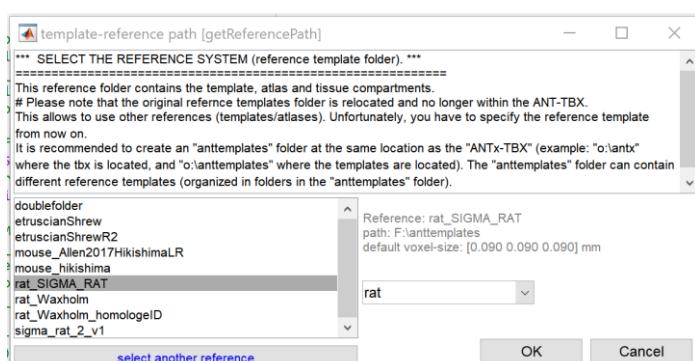
In the parameter window define the reference system (templates). This is important. The reference system defines the animal-related space and Atlas

```

% *** CONFIGURATION PARAMETERS ***
%
x.project      = 'NEW_PROJECT'; % PROJECT NAME (arbitrary tag)
x.datpath       = 'F:\data3\claudia_mgd_r5\dat'; % studie's datapath, MUST
x.voxsize       = [0.07 0.07 0.07]; % voxel size (default for Allen Mouse)
%
B x.wa.BiasFieldCor = [0]; % perform initial bias field correction (only needed)
B x.wa.usePriorSkullstrip = [1]; % use a priori skullstripping (used for automatic reg
B x.wa.fastSegment = [1]; % faster segmentation by cutting boundaries of t2.nii
B x.wa.orientationType = [1]; % index from ReorientationTable (see: help findrotate)
B x.wa.orientationParamfile = 'F:\antx2\mrifiles\elastix\paramfiles\trafoeuler5.txt'; %
B x.wa.elxMaskApproach = [1]; % used registration approach..click icon for further :
B x.wa.elxParamfile = { 'F:\antx2\mrifiles\elastix\paramfiles\Par0025affine.txt' }
B x.wa.cleanup = [1]; % delete unused files in folder
B x.wa.usePCT = [2]; % use Parallel Computing toolbox (@:no/1:SPMD/2:parfor)
%
TEMPLATE
B x.wa.refpath = 'MUST_BE_DEFINED'; % PATH of the used reference system (
B x.wa.species = 'mouse'; % animal species to investigate {mouse or rat}
%
VOLUMES TO TRANSFORM
B x.wa.tf_t2 = [1]; % create "x_t2.nii" (mouse-t2-image)
B x.wa.tf_avg = [1]; % create "ix_AVGT.nii" (template-structural-image)
B x.wa.tf_ano = [1]; % create "ix_ANO.nii" (template-label-image)
B x.wa.tf_c1 = [0]; % create "x_c1t2.nii" (mouse-grayMatter-image)
B x.wa.tf_c2 = [0]; % create "x_c2t2.nii" (mouse-whiteMatter-image)
B x.wa.tf_c3 = [0]; % create "x_c3t2.nii" (mouse-CSF-image)
B x.wa.tf_c1c2mask = [0]; % create "x_c1c2mask.nii" (mouse-gray+whiteMatterMask)

```

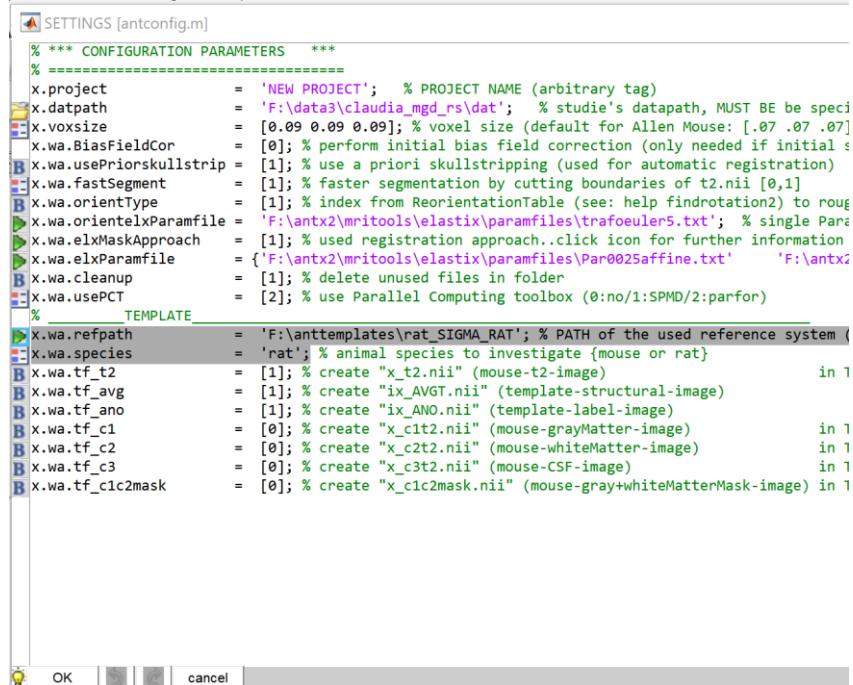
For this, select the green icon left to the 'refpath'-parameter.



Select the reference system from left listbox. Here, I selected the SIGMA RAT atlas (highlighted). Hit **[OK]**. Note that the left listbox contains only templates that have been already downloaded (To

download the templates use either ANT-Menu: EXTRAS/ get templates from googledrive or EXTRAS/ download templates ...see above).

The parameter window should now look as follows:

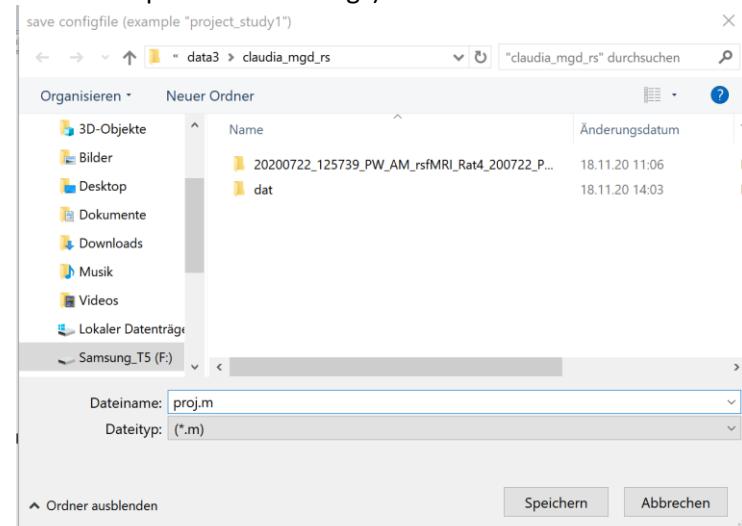


```
% *** CONFIGURATION PARAMETERS ***
%
x.project      = 'NEW_PROJECT'; % PROJECT NAME (arbitrary tag)
x.datpath       = 'F:\data3\claudia_mgd_rs\dat'; % studie's datapath, MUST BE specified
x.voxsize       = [0.09 0.09 0.09]; % voxel size (default for Allen Mouse: [.07 .07 .07])
x.wa.BiasFieldCor = [0]; % perform initial bias field correction (only needed if initial registration fails)
x.wa.usePriorSkullstrip = [1]; % use a priori skullstripping (used for automatic registration)
x.wa.fastSegment = [1]; % faster segmentation by cutting boundaries of t2.nii [0,1]
x.wa.orientType = [1]; % index from ReorientationTable (see: help findrotation2) to roughly align the image
x.wa.orientelxParamfile = 'F:\antx2\mrtools\elastix\paramfiles\trafoeuler5.txt'; % single Paramfile
x.wa.elxMaskApproach = [1]; % used registration approach..click icon for further information
x.wa.elxParamfile = {'F:\antx2\mrtools\elastix\paramfiles\Par0025affine.txt'} % single Paramfile
x.wa.cleanup    = [1]; % delete unused files in folder
x.wa.usePCT     = [2]; % use Parallel Computing toolbox (0:no/1:SPMD/2:parfor)

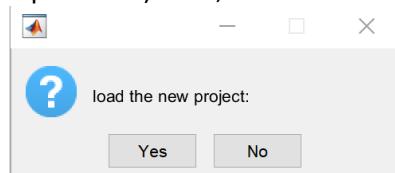
%
% TEMPLATE
%
x.wa.refpath   = 'F:\anttemplates\rat_SIGMA_RAT'; % PATH of the used reference system (rat)
x.wa.species    = 'rat'; % animal species to investigate (mouse or rat)
x.wa.tf_t2      = [1]; % create "x_t2.nii" (mouse-t2-image)                                in 1
x.wa.tf_avg     = [1]; % create "ix_AVGT.nii" (template-structural-image)
x.wa.tf_ano     = [1]; % create "ix_ANO.nii" (template-label-image)
x.wa.tf_c1      = [0]; % create "x_c1t2.nii" (mouse-grayMatter-image)                         in 1
x.wa.tf_c2      = [0]; % create "x_c2t2.nii" (mouse-whiteMatter-image)                        in 1
x.wa.tf_c3      = [0]; % create "x_c3t2.nii" (mouse-CSF-image)                             in 1
x.wa.tf_c1c2mask = [0]; % create "x_c1c2mask.nii" (mouse-gray+whiteMatterMask-image) in 1
```

I.E: the reference system and the animal species are defined. Hit [OK].

When hitting [OK], the user is prompted to save the project-file name (the project file is just a Matlab m-file with parameter settings).



Please save this file in the study folder. (Example: F:\data3\claudia\_mgd\_rs). Hit [SAVE] (german: 'Speichern'). If so, another window pops up and ask whether to load the project.



Hit [YES].

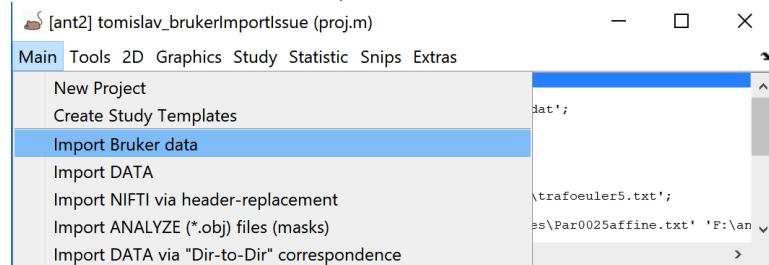
NOTE: In the next session just open the ANT GUI and select [loadproj]-button from the main GUI to load the studie's project file (aka parameter file).

The Study-folder "claudia\_mgd\_rs" now contains:

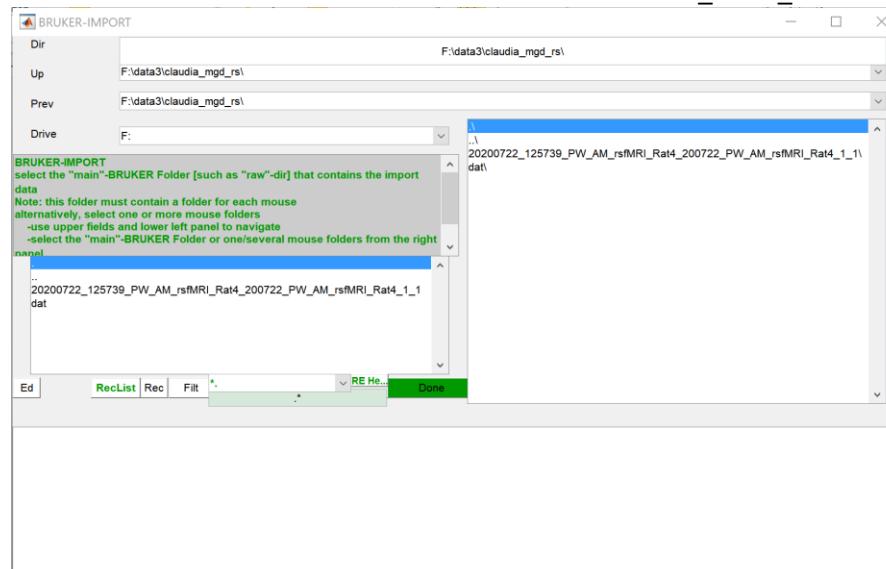
**20200722\_125739\_PW\_AM\_rsfMRI\_Rat4\_200722\_PW\_AM\_rsfMRI\_Rat4\_1\_1:** Folder with Bruker-raw data (btw. The data can be stored somewhere else ... it's more convenient for now)  
**Dat:** Folder, that is created when setup a new project. Later this folder will contain the data for all animals  
**proj.m** : parameter file (project file).

#### 4. Import Bruker data

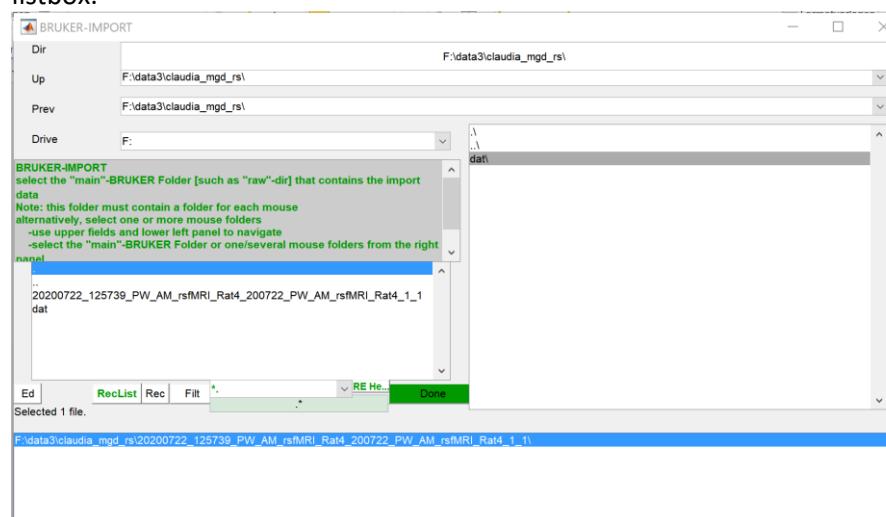
Go to ANT-MENU: Main/import Bruker data



In the Selection window select the Bruker raw data "RAW\_SCAN\_DATA" from the right listbox

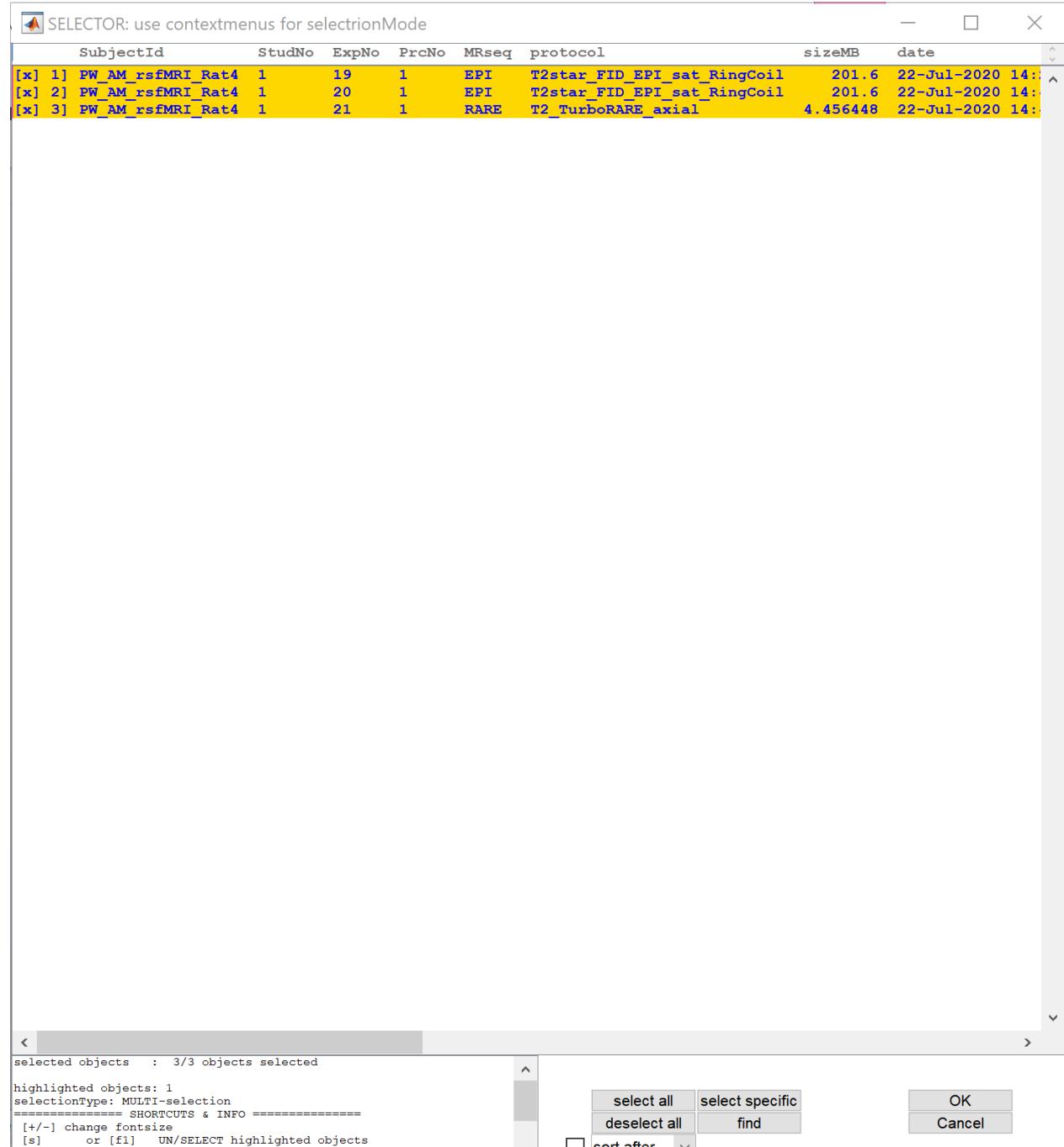


NOTE: The item(s) disappear(s) and reappear(s) in the lower listbox when selecting from the right listbox.



Hit [Done].

The Bruker-File selection window lists all available Bruker data.



Select the files you want to convert. Here I selected all files. Hit [OK].

Now the Bruker-parameter window pops up. This window shows all available and changeable parameters:

```

BrukerImport
=====
*** BrukerIMPORT ***
% -define names of output-dirs and imported filenames
% -the suffixes for ExperimentNumber (ExpNo_Dir/ExpNo_File) and ProcessingNumber (PrcNo_Dir/PrcNo_File) will critically determine the exi
% CRITICAL-1: if both ExpNo_Dir/ExpNo_File are not specified and the same protocol is used multiple times [ RUNS ] in the same mouse (= ...
% ... than the resulting file is overwritten by subsequent runs of the experiment (e.g.: three subsequent MPRAGE-runs --> [...]\\pdata\\1
% SOLUTION: (1) if there is only one RUNS/EXPERIMENTNUMBER for the same protocol: ExpNo_Dir/ExpNo_File is not necessary
% SOLUTION: (2) if there are multiple RUNS/EXPERIMENTNUMBERS for the same protocol: either ExpNo_Dir/ExpNo_File is necessary
% CRITICAL-2: if both PrcNo_Dir/PrcNo_File are not specified and different [RECONSTRUCTIONS] (i.e. processings) of the same protocol exist ...
% ... the the resulting file is overwritten by subsequent reconstructions (e.g: three processings in experimentNumber-4 [...]\\pdata\\4
% SOLUTION: (1) if there is only one RECONSTRUCTION for the same protocol: PrcNo_Dir/PrcNo_File is not necessary
% SOLUTION: (2) if there are multiple RECONSTRUCTIONS for the same protocol: either PrcNo_Dir or PrcNo_File is necessary
% =====
% [1] SUFFIXES of MOUSE DIRECTORY NAME (added to "SubjectId")
% =====
B x.StudNo_Dir = [0]; % VisuStudyNumber (bool)
B x.ExpNo_Dir = [0]; % VisuExperimentNumber (parent folder of "pdata"),(bool)
B x.PrcNo_Dir = [0]; % VisuProcessingNumber/ReconstructionNumber(subfolder of "pdata"),(bool)
% arrangement of suffixes
x.delimiter = '_'; % delimiter between suffixes (cell); e.g: "s20141009_01sr_121" vs "s20141009_01sr_1_2_1"
B x.suffixLetter = [0]; % add first letter of suffix variable name prior to variable value (bool); e.g: "s20141009_01sr_s1e2p1" vs "s20141009_01sr_1_2_1"
% =====
% [2] SUFFIXES of FILENAMES (added to "protocoll-name")
% =====
B x.ExpNo_File = [1]; % VisuExperimentNumber (parent folder of "pdata"),(bool)
B x.PrcNo_File = [1]; % VisuProcessingNumber/ReconstructionNumber(subfolder of "pdata"),(bool)
x.renameFiles = ''; % rename files -->via GUI
% =====
% [3] ADDITIONAL OPTIONS
% =====
x.origin = 'bukerOrigin'; % define center(origin) of volume

```

RUN | cancel

Set the **"EXPNO File"** to 1 (highlighted in fig). This adds the experiment number as suffix-string to the converted NIFTI file names and prevents that sequences with the same Protocolname and Protocolnumber will be overwritten.

Hit **[RUN]** and wait ...

At this step, all selected Bruker files should be converted to NIFTI-files and stored in the study's 'dat'-folder. After successful file conversion you will see the converted files in the Matlab command window

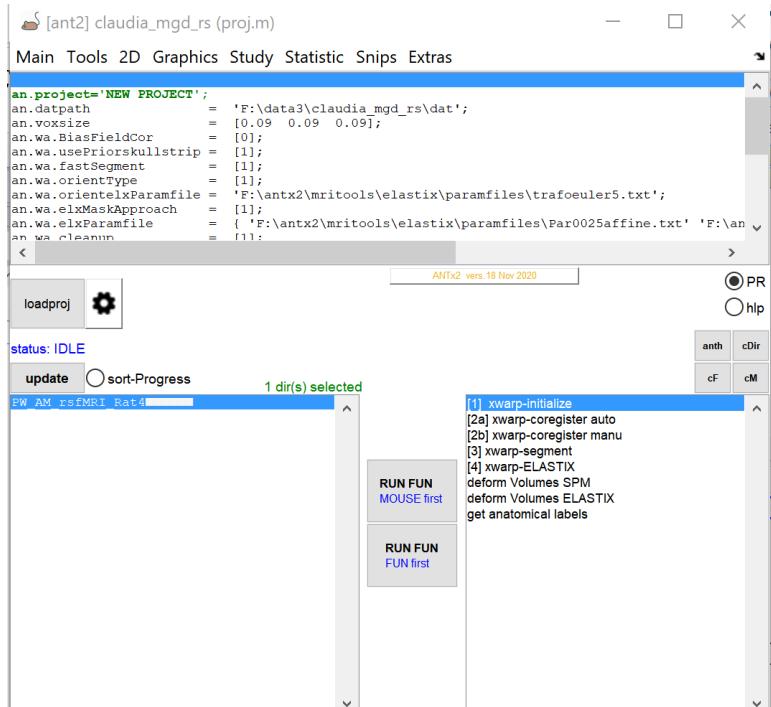
```

>>
0001] create F:\data3\claudia_mgd_rs\dat\PW_AM_rsfMRI_Rat4\T2star_FID_EPI_sat_RingCoil_19_1.nii; SOURCE: F:\data
0002] create F:\data3\claudia_mgd_rs\dat\PW_AM_rsfMRI_Rat4\T2star_FID_EPI_sat_RingCoil_20_1.nii; SOURCE: F:\data
0003] create F:\data3\claudia_mgd_rs\dat\PW_AM_rsfMRI_Rat4\T2_TurboRARE_axial_21_1.nii; SOURCE: F:\data3\claudia
[done]
fx >
```

Hit the hyperlinks to inspect (Location) the NIFTIs.

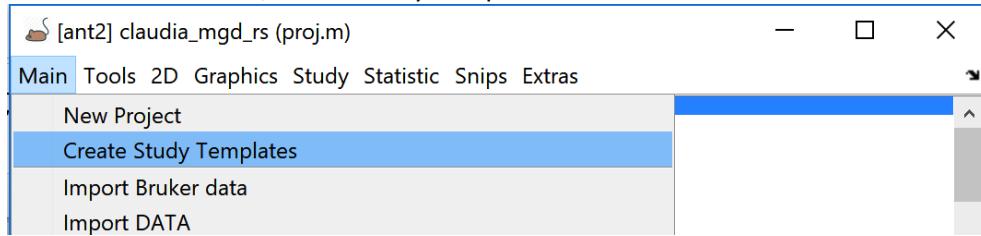
	Name	Änderungsdatum
ugriff	<a href="#">T2_TurboRARE_axial_21_1.nii</a>	18.11.20 14:12
e	<a href="#">T2star_FID_EPI_sat_RingCoil_19_1.nii</a>	18.11.20 14:12
	<a href="#">T2star_FID_EPI_sat_RingCoil_20_1.nii</a>	18.11.20 14:12

The main gui shows a new folder (i.e. one animal) in the left list-box. Here data of only one animal were converted.



## 5. Import templates for this study

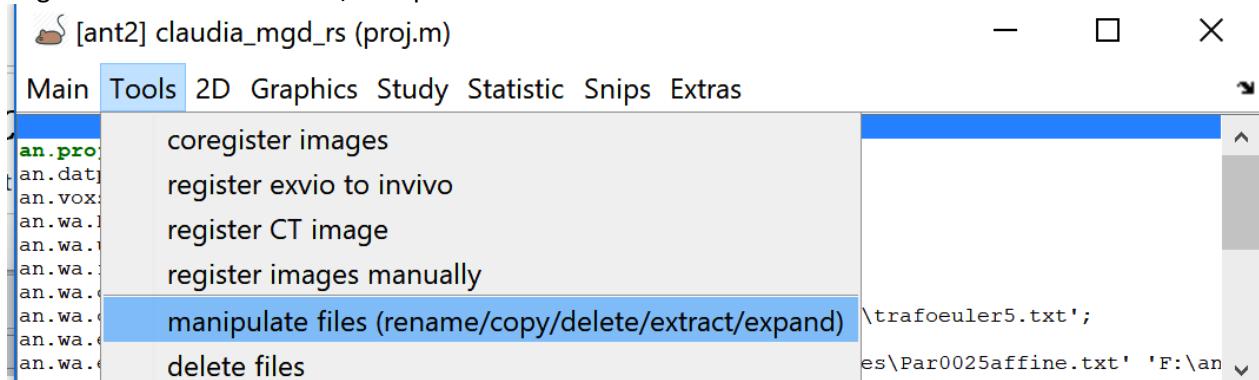
→ ANT-MENU: Main/Create Study Templates



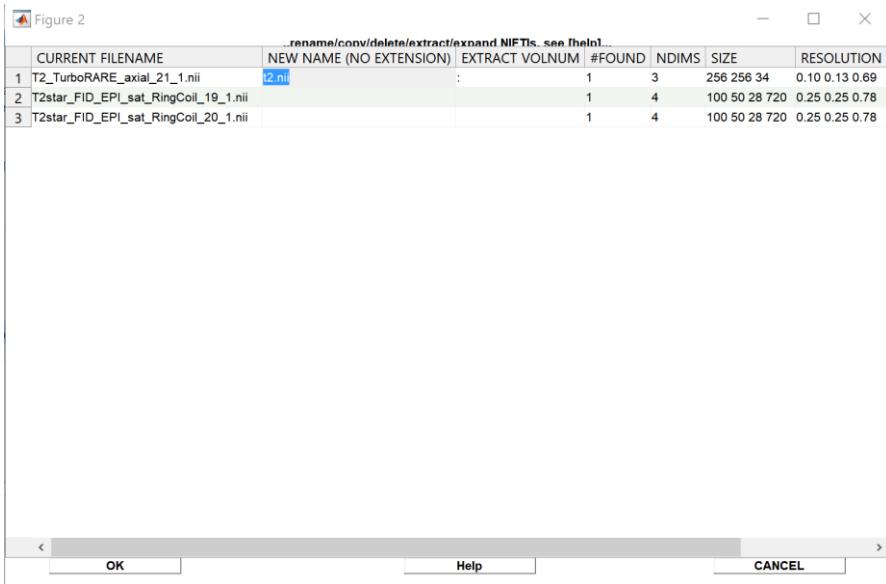
This step creates copies/reslices the template data from ‘anttemplates’-folder (specified in the proj.m-parameter file) to the studies folder. In the study-folder a new folder ‘templates’ is created.

## 6. Create a ‘t2.nii’ image

→ got to ANT-MENU: Tools/manipulate files..

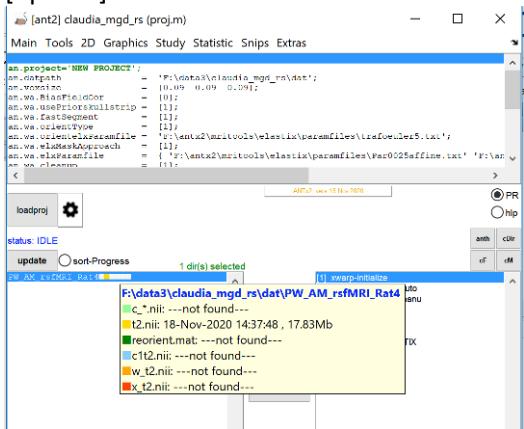


In the new GUI find the structural image that should be used as input for the registration to template ('T2\_TurboRARE\_axial\_21\_1.nii'). The “**new Name**”-column type ‘t2.nii’ and in the extract volnum column set a ‘:’ (colon –symbol). The colon symbol indicates that the file should be copied and renamed (instead of renaming the original file). Alternatively, you could also write ‘copy’ in this column.



Hit [OK].

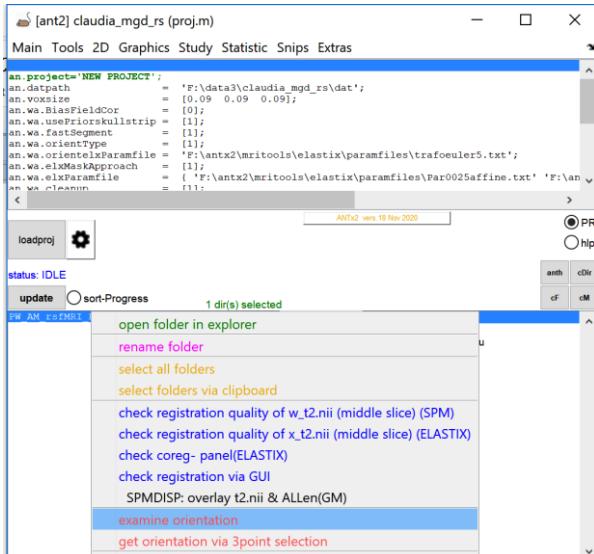
**IMPORTANT:** The 't2.nii' file is the starting file (input) for the registration-to-template pipeline. Therefore it is recommended to a proper structural image. The left listbox in the ANT-main gui indicates that the 't2.nii' for that animal now exists (yellow icon left to the animal folder name). If not, hit the [update] button.



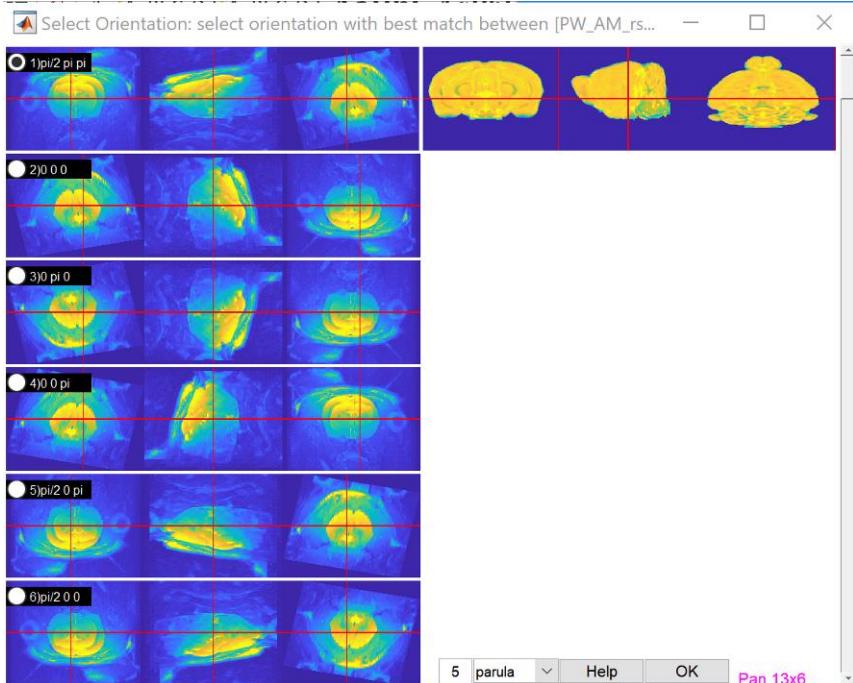
## 7. Examine Orientation

For animals of the same study the orientation is assumed to be roughly similar. However, we have to examine the orientation of one animal relative to the template.

To do this select an animal from the left listbox then open the context menu (right click) and select: **examine orientation**.



In the new window select the panel (via checkbox) from the left orientations that mostly fit with the right template



Here the first orientation ( panel-1) seems to be ok. Hit [OK].

If the rotation-ID is not [1] but another number you have to change the "orientationType"-Parameter in the project-file (parameter file..here proj.m). To do so use click the gearwheel icon from the ANT GUI and set the respective orientationType-value (figure: highlighted parameter). Hit [OK] and if asked, update the parameter file.

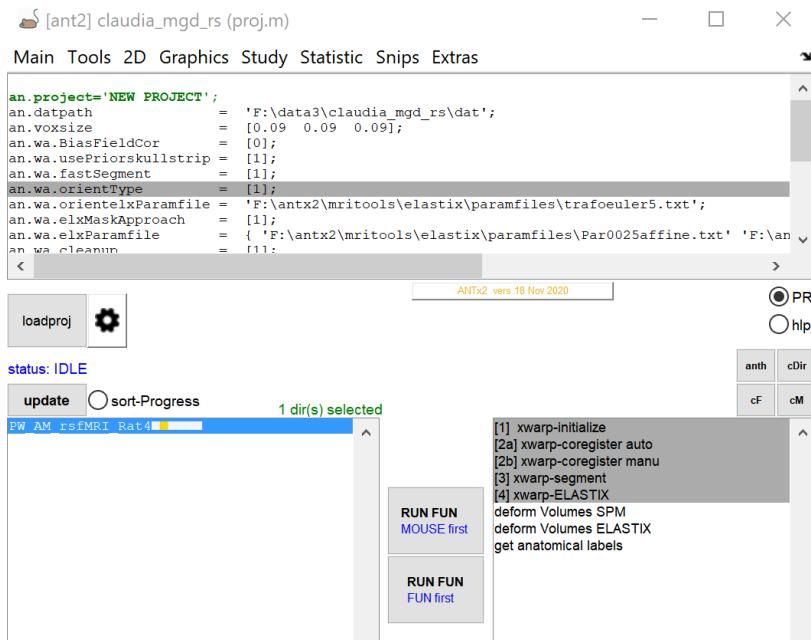
```

% *** CONFIGURATION PARAMETERS ***
%
X.project      = 'NEW PROJECT'; % PROJECT NAME (arbitrary tag)
X.datpath      = 'F:\data3\claudia_mgd_rs\dat'; % studie's data
X.voxsize      = [0.09 0.09 0.09]; % voxel size (default for A
%
B X.wa.BiasFieldCor    = [0]; % perform initial bias field correction (on
B X.wa.usePriorSkullstrip = [1]; % use a priori skullstripping (used for aut
B X.wa.fastSegment     = [1]; % faster segmentation by cutting boundaries
B X.wa.orientType      = [1]; % index from ReorientationTable (see: help
B X.wa.orientelxParamfile = 'F:\antx2\mrifiles\elastix\paramfiles\trafoeuler
B X.wa.elxMaskApproach = [1]; % used registration approach..click icon fo
B X.wa.elxParamfile    = { 'F:\antx2\mrifiles\elastix\paramfiles\Par0025aff
B X.wa.cleanup         = [1]; % delete unused files in folder
B X.wa.usePCT          = [2]; % use Parallel Computing toolbox (0:no/1:SP
%
% _____ TEMPLATE _____
B X.wa.refpath        = 'F:\antntemplates\rat_SIGMA_RAT'; % PATH of the u
B X.wa.species         = 'rat'; % animal species to investigate {mouse
%
% _____ VOLUMES TO TRANSFORM _____
B X.wa.tf_t2           = [1]; % create "x_t2.nii" (mouse-t2-image)
B X.wa.tf_avg           = [1]; % create "ix_AVGT.nii" (template-structural
B X.wa.tf_ano           = [1]; % create "ix_ANO.nii" (template-label-image
B X.wa.tf_c1           = [0]; % create "x_c1t2.nii" (mouse-grayMatter-ima
B X.wa.tf_c2           = [0]; % create "x_c2t2.nii" (mouse-whiteMatter-im
B X.wa.tf_c3           = [0]; % create "x_c3t2.nii" (mouse-CSF-image)
B X.wa.tf_c1c2mask     = [0]; % create "x_c1c2mask.nii" (mouse-gray+white

```

## 8. Register ‘t2.nii’ to the template

From the right listbox select the first 5 entries (note that “[2b] xwarp-coregister manu” manual registration is only used if “[2a] xwarp-coregister auto” is not selected). Here we want to register the t2.nii automatically.



Hit the **lower [RUN FUN]** button.

This steps will prepare the data (I.A: dirty fast skullstrip ‘t2’), rigid register ‘t2.nii’, segment and nonlinear register the data to the template. The steps are a little time consuming. In the meantime you could inspect the interim steps via the browser. If the browser did not, double click the “summary.html”- file from the studies folder.

← → C Datei | F:/data3/claudia\_mgd\_rs/summary.html

## Processing Report

Project: F:/data3/claudia\_mgd\_rs

75% processed  waiting ## start time: 18-Nov-2020 14:58:23; ## end time: ; ## last performed case: "PW\_AM\_rsfMRI\_Rat4" <>> Segmentation

processing..  PW\_AM\_rsfMRI\_Rat4 [inspect](#)

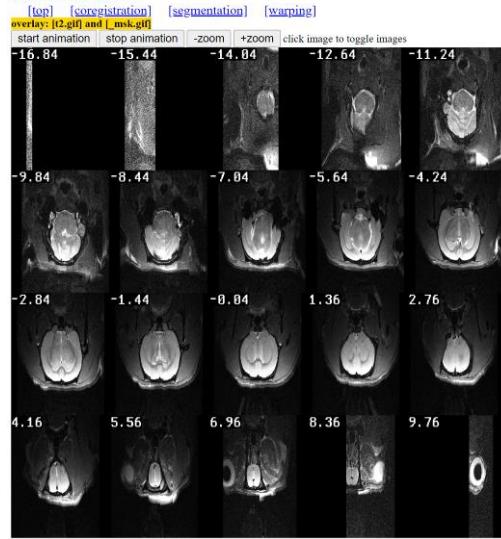
---

**LEGEND:**

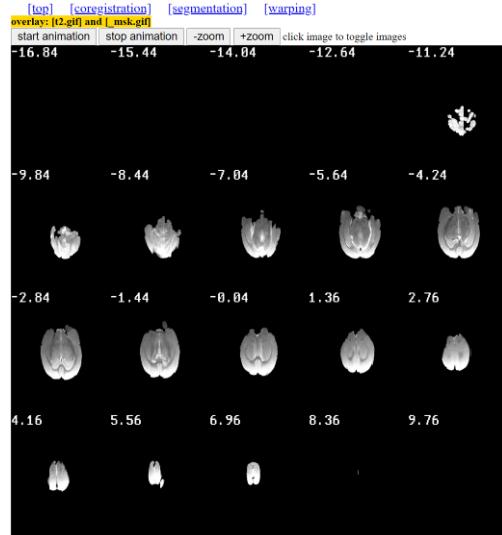
- [dataset-x] : data processed in this session
- [dataset-y] : data not processed in this session
- Yellow circle: ..selected tasks to process (marked yellow) {initialization, coregistration, segmentation, warping}
- Light blue circle: ..task never performed for this data set
- Orange circle: ..task has been performed before (and might be overwritten)
- Asterisk: \*..the currently running task
- Green circle: ● finished task

Click the hyperlink ‘inspect’ of the respective animal.  
Click on the images to see the task-specific output: Below: skullstripping

### INITIALIZATION

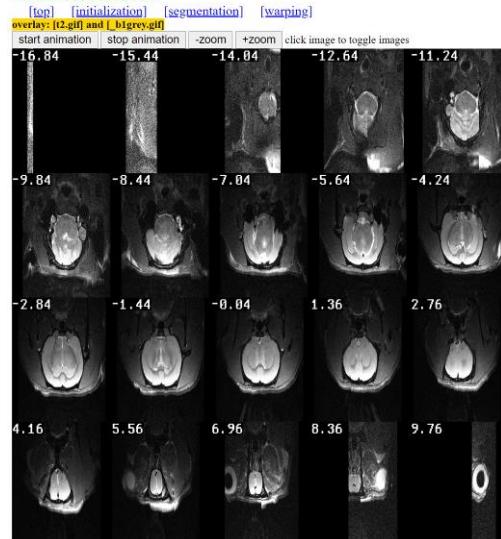


### INITIALIZATION

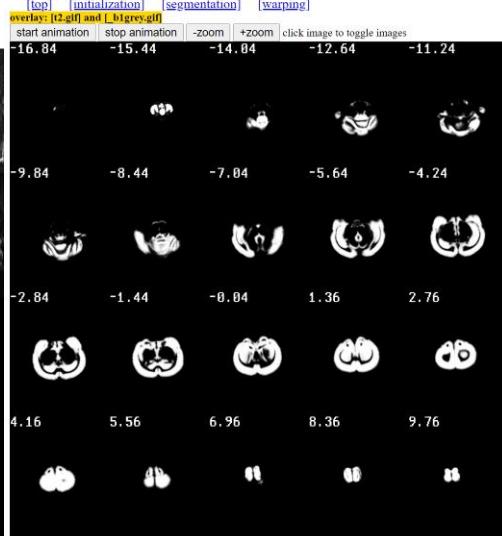


Below: rigid registration

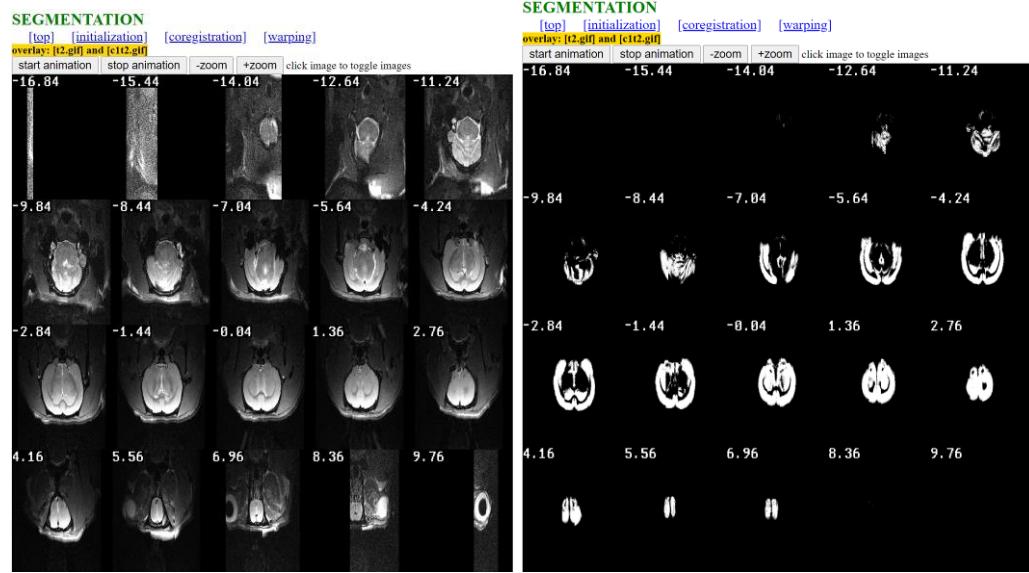
### COREGISTRATION



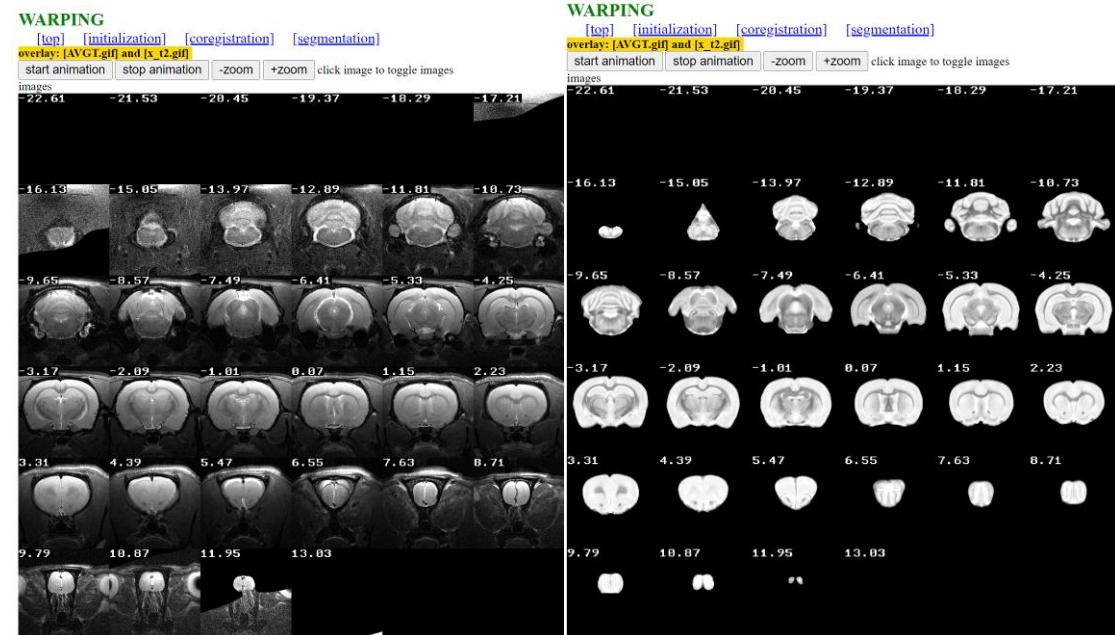
### COREGISTRATION



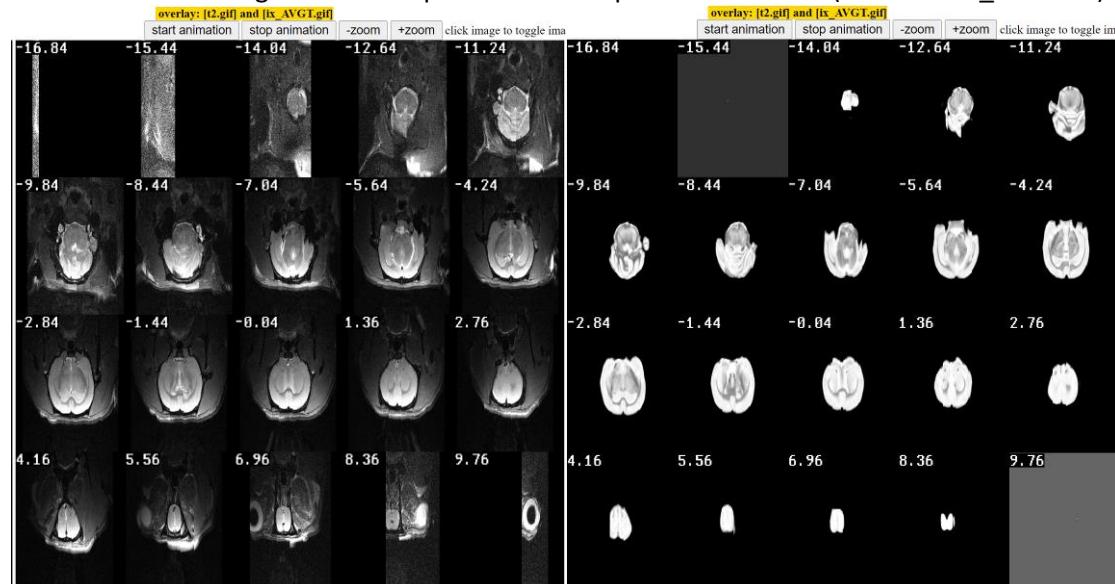
Below: Segmentation (GM is shown)



Below: Nonlinear Registration to standard Space (x\_t2.nii and AVGT.nii)



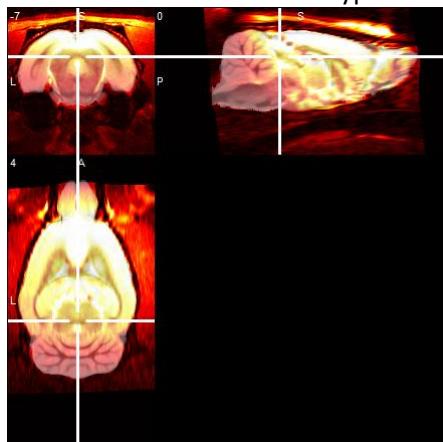
Below: Nonlinear Registration template to native space.. the inverse (t2.nii and ix\_AVGT.nii)



When finished you can select an image from the hyperlinks in the Matlab command window to inspect the overlay with the template

```
..calc forward..calc backward..done.
[0001][PW_AM_rsfMRI_Rat4]--> new image [x_t2.nii - AVGT.nii]: Explorer or MRicron
[f: doelastix.m]: batch
[0001][PW_AM_rsfMRI_Rat4]--> new image [t2.nii - ix_ANO.nii]:Explorer or MRicron
[f: doelastix.m]: batch
-[PW_AM_rsfMRI_Rat4] has new volume [t2.nii - ix_ANO.nii]:Explorer or MRicron
[0001][PW_AM_rsfMRI_Rat4]--> new image [t2.nii - ix_AVGT.nii]:Explorer or MRicron
[f: doelastix.m]: batch
-[PW_AM_rsfMRI_Rat4] has new volume [t2.nii - ix_AVGT.nii]:Explorer or MRicron
[0001][PW_AM_rsfMRI_Rat4]--> new image [x_t2.nii - AVGT.nii]: Explorer or MRicron
[f: doelastix.m]: batch
-[PW_AM_rsfMRI_Rat4] has new volume [x_t2.nii - AVGT.nii]: Explorer or MRicron
-[PW_AM_rsfMRI_Rat4] has new volume [t2.nii - iw_AVGT.nii]:Explorer or MRicron
-[PW_AM_rsfMRI_Rat4] has new volume [w_t2.nii - AVGT.nii]: Explorer or MRicron
..generate JACOBIAN
-[PW_AM_rsfMRI_Rat4] has new volume [JD.nii - AVGT.nii]: Explorer or MRicron
```

Hier I selected 'MRICRON' hyperlink of [x\_t2.nii - AVGT.nii]:

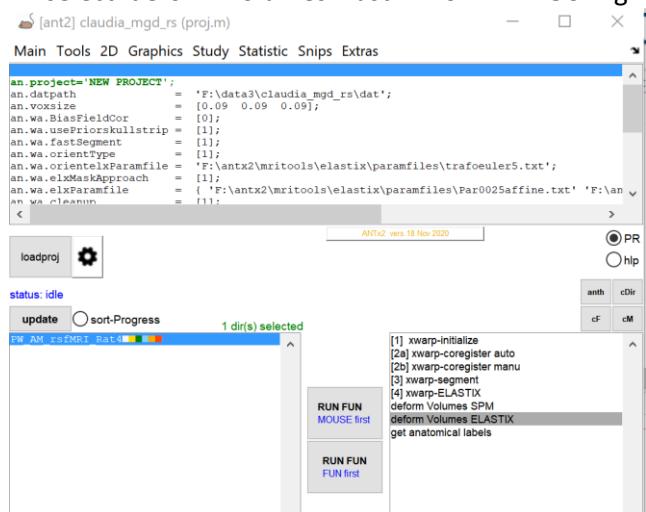


The registration looks fine (for me :-)

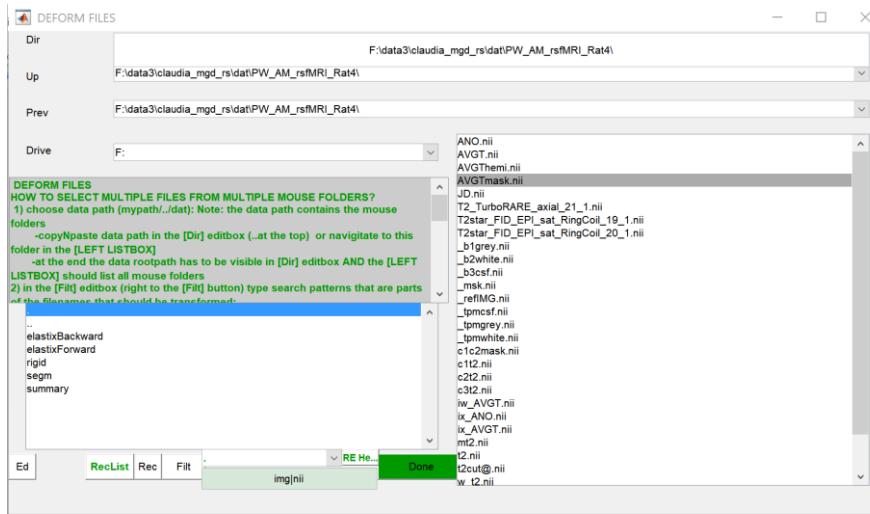
## 9. Back-transform template brain mask to native space

Here we want to back-transform the template brain mask ('AVGTmask.nii') to the animal's native space. If successful the back-transformed template brain mask ('ix\_AVGTmask.nii') is in register with the original 't2.nii'.

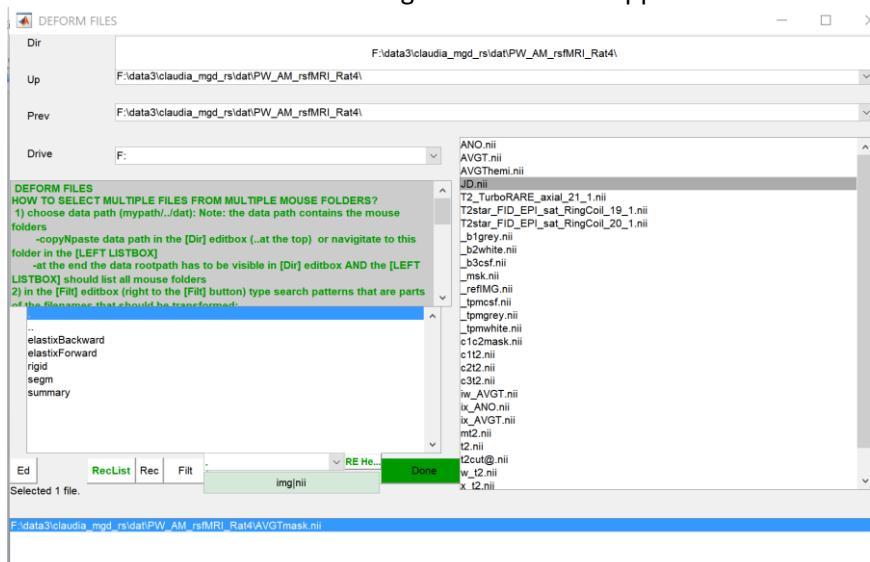
→ select 'deform Volumes Elastix' from ANT GUI right listbox.



Hit the **lower [RUN FUN]** button.

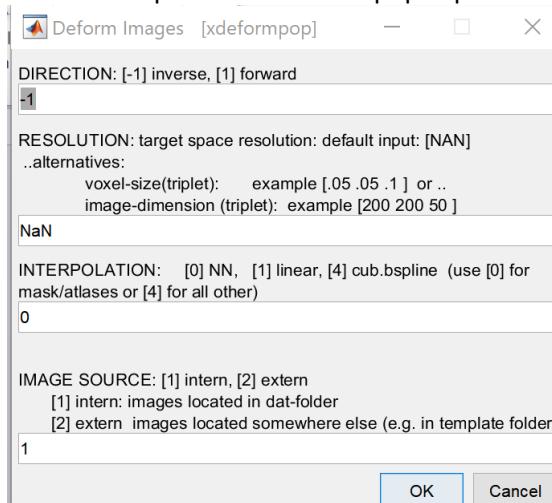


Select the 'AVGTmask.nii' from right listbox. It will appear in the lower listbox...



Hit [Done] button.

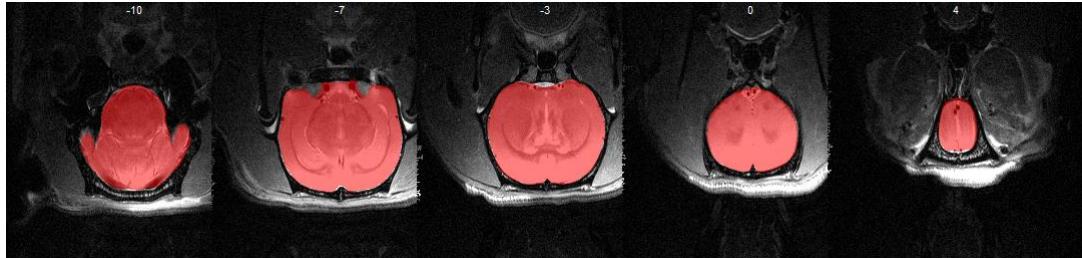
The deform parameter window pops up.



Here type **[-1]** for the direction (..i.e. back to native space) and **'0'** for the interpolation (next neighbour interpolation to preserve the binary values in the mask). Hit **[OK]** button. This step takes only a few secs ( $t < 5$ s), because the previous calculated inverse transformation parameters are only

applied to the image (thus the prefix 'ix' in 'ix\_AVGTmask.nii'). When done you can click on the MRicron hyperlink in Matlab's command window to inspect the overlay of 't2.nii' and 'ix\_AVGTmask.nii'

```
>> [0001][PW_AM_rsfMRI_Rat4]--> new image [t2.nii - ix_AVGTmask.nii]:Explorer or MRicron
[f: doelastix.m]: batch
done
```

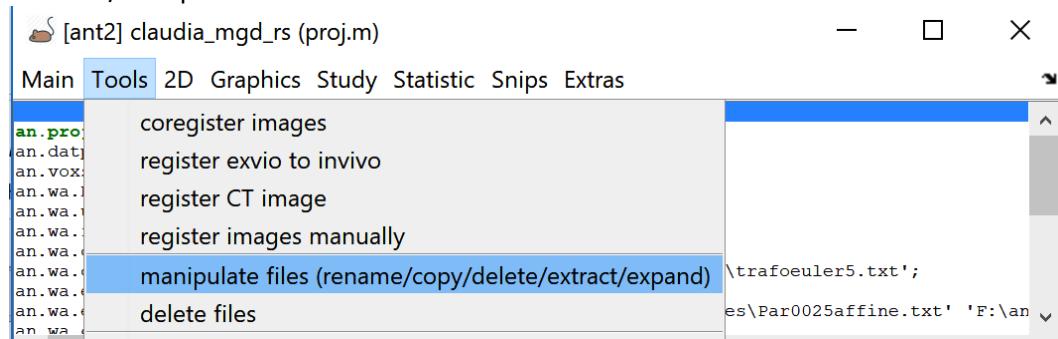


The overlay of 't2.nii' and 'ix\_AVGTmask.nii' looks ok, so the brain mask can be used in native space.

## 10. Extract 1st image of the 4D BOLD series

Here we extract the 1<sup>st</sup> image of the 4D resting-state data to use it as target-image for coregistering the 't2.nii' onto the 4D BOLD data.

→ Tools/manipulate files ...

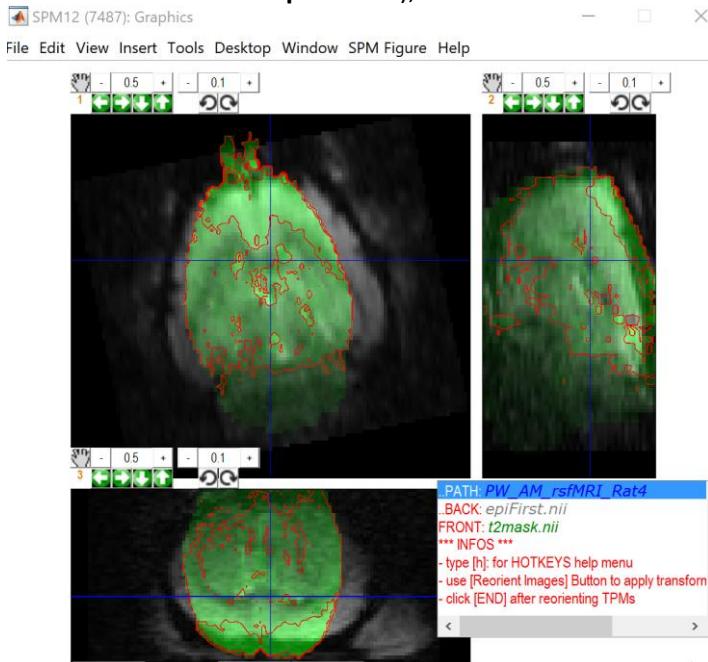


Search for 'T2star\_FID\_EPI\_sat\_RingCoil\_20\_1.nii' in the File listbox. Type 'epiFirst.nii' in the 'New Name'- column and '1' in the 'Extract'-column. With this, the 1<sup>st</sup> image from 'T2star\_FID\_EPI\_sat\_RingCoil\_20\_1.nii' is extracted and saved as 'epiFirst.nii'. Hit [OK] button.

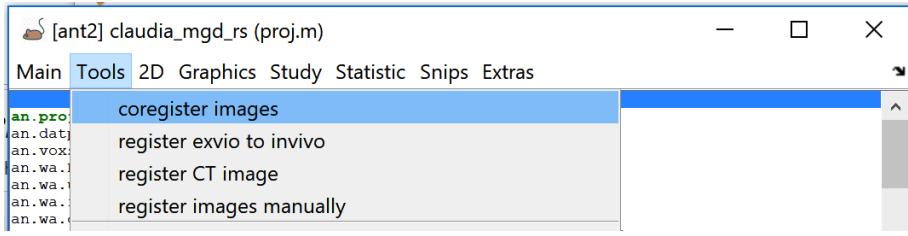
..rename/copy/delete/extract/expand NIFTIs, see [help]...								
	CURRENT FILENAME	NEW NAME (NO EXTENSION)	EXTRACT VOLNUM	#FOUND	NDIMS	SIZE	RESOLUTION	ORIGIN
1	ANO.nii			1	3	224 446 224	0.09 0.09 0.09	-9.60 -24.41 -9
2	AVGT.nii			1	3	224 446 224	0.09 0.09 0.09	-9.60 -24.41 -9
3	AVGThemi.nii			1	3	224 446 224	0.09 0.09 0.09	-9.60 -24.41 -9
4	AVGTmask.nii			1	3	224 446 224	0.09 0.09 0.09	-9.60 -24.41 -9
5	JD.nii			1	3	224 446 224	0.09 0.09 0.09	-9.60 -24.41 -9
6	T2_TurboRARE_axial_21_1.nii			1	3	256 256 34	0.10 0.13 0.69	-10.74 -18.29
7	T2star_FID_EPI_sat_RingCoil_19_1.nii			1	4	100 50 28 720	0.25 0.25 0.78	-5.06 -13.88 -1
8	T2star_FID_EPI_sat_RingCoil_20_1.nii	epiFirst.nii	1	1	4	100 50 28 720	0.25 0.25 0.78	-5.06 -13.88 -1
9	_b1grey.nii			1	3	224 446 224	0.09 0.02 0.02	-8.54 2.01 -27
10	_b2white.nii			1	3	224 446 224	0.09 0.02 0.02	-8.54 2.01 -27
11	_b3csf.nii			1	3	224 446 224	0.09 0.02 0.02	-8.54 2.01 -27
12	_msk.nii			1	3	256 256 34	0.10 0.13 0.69	-10.74 -18.29
13	_refIM0.nii			1	3	224 446 224	0.09 0.09 0.09	-9.60 -24.41 -9
14	_tpmcst.nii			1	3	224 446 224	0.09 0.09 0.09	-9.60 -24.41 -9
15	_tpmgrey.nii			1	3	224 446 224	0.09 0.09 0.09	-9.60 -24.41 -9
16	_tpmwhite.nii			1	3	224 446 224	0.09 0.09 0.09	-9.60 -24.41 -9
17	c1c2mask.nii			1	3	256 256 34	0.10 0.13 0.69	-10.74 -18.29
18	c1t2.nii			1	3	256 256 34	0.10 0.13 0.69	-10.74 -18.29
19	c2t2.nii			1	3	256 256 34	0.10 0.13 0.69	-10.74 -18.29
20	c3t2.nii			1	3	256 256 34	0.10 0.13 0.69	-10.74 -18.29
21	epiFirst.nii			1	3	100 50 28	0.25 0.25 0.78	-5.06 -13.88 -1
22	iw_AVGT.nii			1	3	256 256 34	0.10 0.13 0.69	-10.74 -18.29
23	ix_ANO.nii			1	3	256 256 34	0.10 0.13 0.69	-10.74 -18.29
24	ix_AVGT.nii			1	3	256 256 34	0.10 0.13 0.69	-10.74 -18.29
25	ix_AVGTMask.nii			1	3	256 256 34	0.10 0.13 0.69	-10.74 -18.29
26	mt2.nii			1	3	256 256 34	0.10 0.13 0.69	-10.74 -18.29
27	t2.nii			1	3	256 256 34	0.10 0.13 0.69	-10.74 -18.29
28	t2cut@.nii			1	3	198 132 41	0.10 0.13 0.70	-10.60 -12.30
29	w_t2.nii			1	3	224 446 224	0.09 0.09 0.09	-9.60 -24.41 -9
30	x_t2.nii			1	3	224 446 224	0.09 0.09 0.09	-9.60 -24.41 -9
31	y_forward.nii			1	3	224 446 224	0.09 0.09 0.09	-9.60 -24.41 -9
32	y_inverse.nii			1	3	256 256 34	0.10 0.13 0.69	-10.74 -18.29

## 11. Coregister 't2.nii' onto BOLD (RS-) Data

Here we register the 't2.nii' to the first volume ('epiFirst.nii') of the 4D time-series and apply the transformation to the brain mask ('ix\_AVGTmask.nii'). Because the BOLD data and the 't2.nii' images are not only translated and rotated but also slightly scaled differently (see fig below: 'green' masked version of t2.nii onto 'epiFirst.nii'), we will use an affine transformation.



→ANT-MENU: Coregister images



```

***COREGISTRATION***

% --- COREGISTRATION ---
% Block-1,Block-2...Block-3...: are independent coregistrations with independent targetImage/sourceImage and a
% coregister sourceImage[t1] onto targetImage[t1] and apply transformation to other images [applyImages]
%
x.TASK      = '[100] noSPMregistration, only elastix' ;% Task to perform (display before/after and or re
x.targetImg1 = {'epiFirst.nii'}; % target image [t1], (static/reference image)
x.sourceImg1 = {'t2.nii'}; % source image [t2], (moved image)
x.sourceImgNum1 = [1]; % if sourceImg has 4 dims use this imageNumber --> sourceImg(:,:,:,:sourceImgNum)
x.applyImg1  = {'c1t2.nii'
               'c2t2.nii'
               'c3t2.nii'
               'ix_ANO.nii'
               'ix_AVGT.nii'
               'ix_AVGTmask.nii'};
%
% PARAMETERS
x.cost_fun   = 'nmi'; % objective function: [nmi] norm. mutual Info, [mi] mutual Info, [ecc] entropy co
x.sep        = [4 2 1 0.5 0.1 0.05]; % optimisation sampling steps (mm)
x.tol         = [0.01 0.01 0.01 0.001 0.001 0.001]; % tolerances for accuracy of each param
x.fwhm       = [7 7]; % smoothing to apply to 256x256 joint histogram
x.centerering = [0]; % make copy of targetIMG & set origin to "center" ,than apply centeringTRafo to all co
%
% RESLICE VOLUME (OPTIONAL)
x.reslicing   = [0]; % reslice images, [0] no, do not reslice, [1] reslice to target Image (targetImg1)
x.interpOrder = 'auto'; % interpolation order [0]nearest neighbour, [1] trilinear interpolation, ["auto"]
x.prefix      = 'r'; % file prefix for the new resliced volume (if empty, overwrite the sourceIMG )
%
% NONLINEAR WARPING (OPTIONAL)
x.warping     = [1]; % do subsequent nonlinear warping [0|1],
x.warpParamfile = {'F:\antx2\mrifiles\elastix\paramfiles\par_affine038CD1.txt'}; % parameterfile used for w
x.warpPrefix   = 'c_'; % prefix out the output file after warping (if empty, it will overwrite the output
x.cleanup      = [1]; % remove interim steps

```

RUN | cancel |

#### In the parameter GUI do the following:

1) **TASK**: set to '[100]...' via left icon/pulldown menu : This circumvents SPM coregistration and uses Elastix registration

2) **targetImg1** : select 'epiFirst.nii' via left icon→ selection GUI. This is the target image

3) **sourceImg1**: select 't2.nii' via left icon→ selection GUI. This is the source image

4) **applyImg1**: aside the source image the following images will be also registered and brought in register with the target image ('epiFirst.nii'):

- \* c1t2.nii', 'c2t2.nii' and 'c3t2.nii' → the segmented GM,WM and CSF images
- \* 'ix\_ANO.nii' → the atlas file
- 'ix\_AVGT.nii' → the structural template of the reference system
- 'ix\_AVGTmask.nii' → brain mask

BTW: You can also register the 'ix\_AVGThemi.nii' (i.e. the hemispheric brain mask in native space) to the BOLD data. To do this, first you have to bring the 'AVGThemi.nii' to native space (analogous to 'AVGmask.nii' ...and then add 'ix\_AVGThemi.nii' in the '**applyImg1 -list**).

5) **warping** : set to [1]; → This allows the Elastix registration

6) **warpParamfile**: from left icon→selection GUI select the ELASTIX parameter file

['par\\_affine038CD1.txt'](#). This file will perform an affine registration. For reason to use affine...see above (scaling issue).

**Hit [OK]**. This step takes <2min.

When successful you will obtain the following files in register with '**epiFirst.nii**' : (using file-prefix 'c')

c\_ix\_ANO.nii    c\_ix\_AVGT.nii    c\_ix\_AVGTmask.nii  
 c\_c1t2.nii      c\_c2t2.nii      c\_c3t2.nii  
 and   c\_epiFirst.nii (Note that 'c\_epiFirst.nii' is just a copy of the target image)

**BEFORE REGISTRATION:** Below is the overlay of the 'epiFirst.nii' and 'ix\_AVGTmask.nii' (red)



**AFTER REGISTRATION:** Below is the overlay of the 'c\_epiFirst.nii' and 'c\_ix\_AVGTmask.nii'(red)



## 12. Mask first EPI-image with brain mask

Open the image calculator: → ANT-MENU: Tools/image calculator

```
% *** xcalc ***
%
x.niftis = {'c_epiFirst.nii' % << select IMAGE(S) to manipulate
             'c_ix_AVGTmask.nii' };
x.evalstring = '@i1.*@i2'; % string to evaluate->see help
x.outName = 'epiFirstMask.nii'; % outputname: <string><cell> or use
x.outDir = 'local'; % <<select the output directory: ["local"]
```

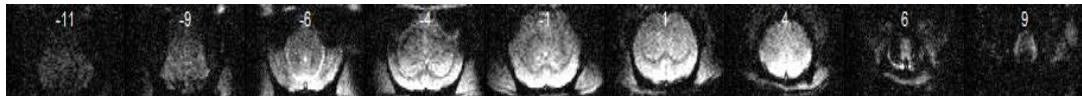
**Do the following steps:**

- 1) **niftis:** click green icon and select the two images 'c\_epiFirst.nii' and 'c\_ix\_AVGTmask.nii'
- 2) **evalstring:** type '@i1.\*@i2' → i.e multiply 1<sup>st</sup> image with 2<sup>nd</sup> image (voxelwise)... beware of the '\*' operation and note that 'c\_ix\_AVGTmask.nii' is binary thus this operation will zeroing all voxel values in 'c\_epiFirst.nii' if the same voxel in 'c\_ix\_AVGTmask.nii' contain a zero.
- 3) **outName:** type 'epiFirstMasked.nii' → this is the filename of the resulting Nifti file

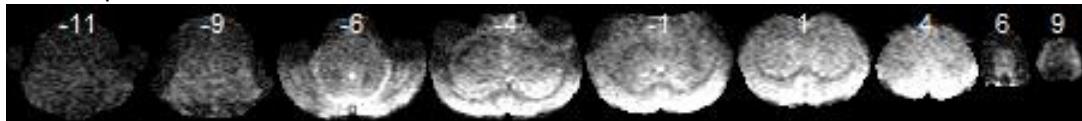
Hit [RUN].

Below: masked 1<sup>st</sup> volume of the RS-data before and after masking with the brainmask

Before: 'c\_epiFirst.nii'



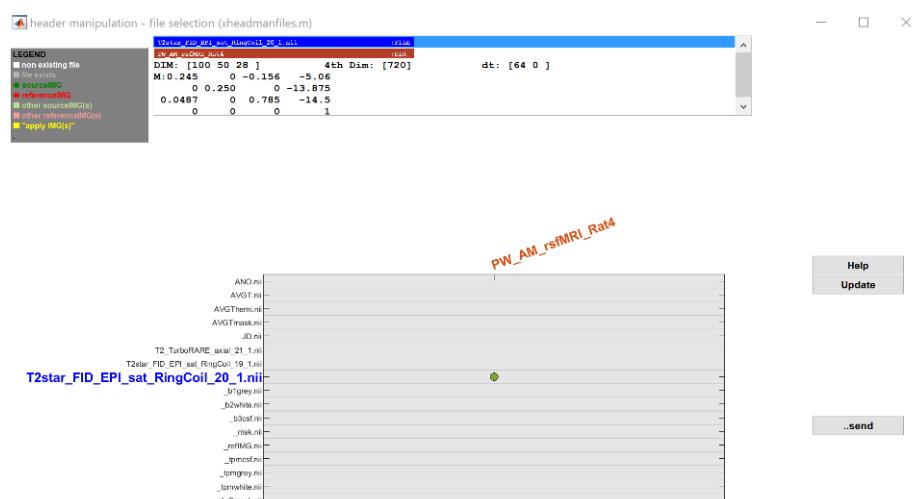
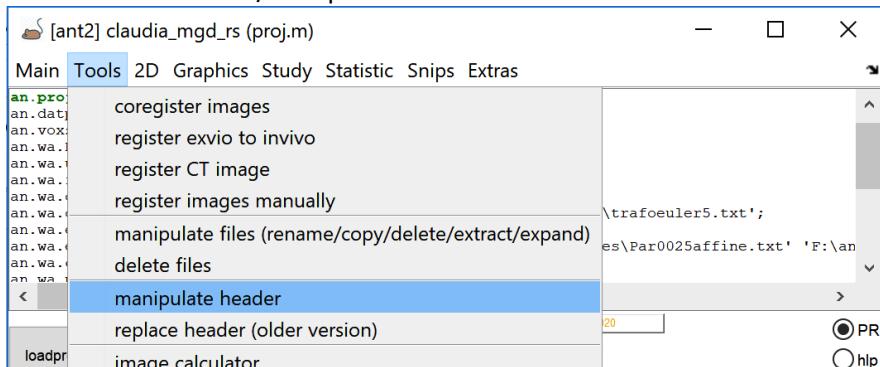
After: 'epiFirstMasked.nii'



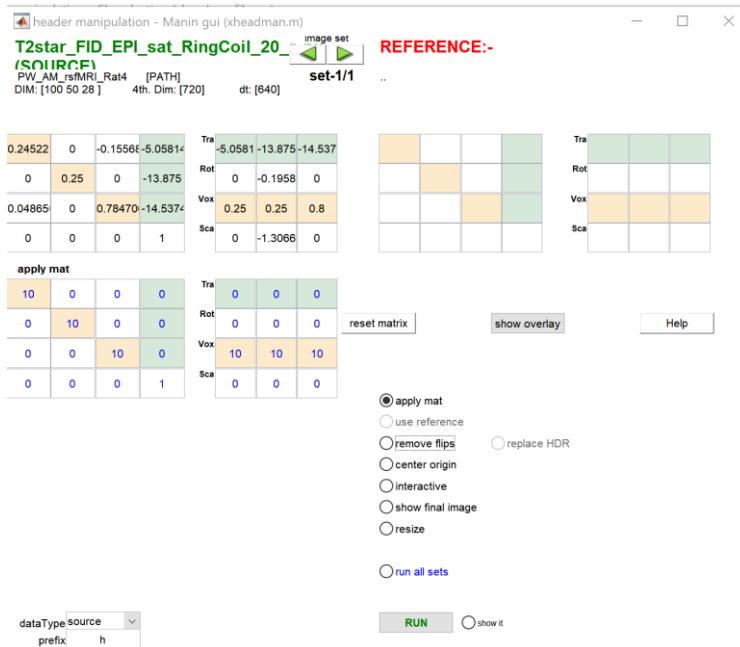
### 13. Scale up 4D data for FSL

Here 4d data are scaled up by factor 10

→ ANT-MENU: Tools/manipulate header



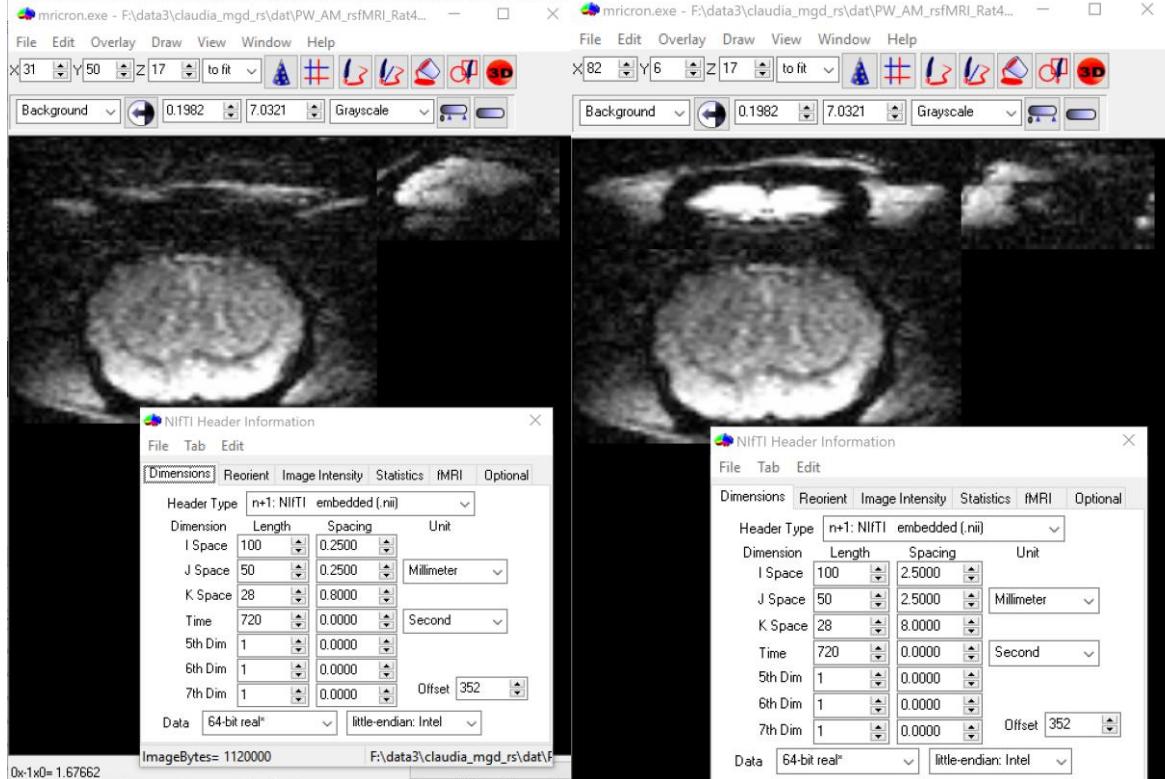
From header-manipulation file list select the 4D rs-Data “T2star\_FID\_EPI\_sat\_RingCoil\_20\_1.nii” (left click into cell). Make shure that right of the selected file name the icon is a ‘green dot’ (not a red dot!). Hit [**send**] button. This will open the header manipulation GUI.



### Do the following steps:

- 1) set [apply mat] radio to true, and set all other radios false
  - 2) in the right apply mat matrix (with rows: TRA,ROT,VOX,SCA) type 10 in each of the Vox-row's cell. This will effectively increase the voxel resolution in x,y,z dimension by a factor 10.
- Hit [RUN] button.

This step produces a new 4D time series with prefix 'h' 'hT2star\_FID\_EPI\_sat\_RingCoil\_20\_1.nii' scaled up by factor 10 (right image, compared to original timeseries, left image).



# Alternative approach: APPPROACH-2

→ Here the first step is to register the structur image 'T2\_TurboRARE\_axial\_21\_1.nii' to the first Image of the 4d RS-Data. The registered structur image is than renamed to 't2.nii'. Finally 't2.nii' is registered to the template (atlas). The advantage of this approach is that the RS-data directly function as native space such that images from and to native space can be much easier transformed. Remember: In the previous approach the 'T2\_TurboRARE\_axial\_21\_1.nii' was first renamed t 't2.nii' than registered to template space to obtain the brain mask in native-'t2.nii'-space. Finally the 't2.nii' and all necessary images were registered to a 3<sup>rd</sup> space, the RS-data-space.

## For other information

→ ANT-MENU: EXTRAS/documentation (docs)

What could be read next?: "[tutorial\\_orientation\\_and\\_manucoreg.doc](#)"

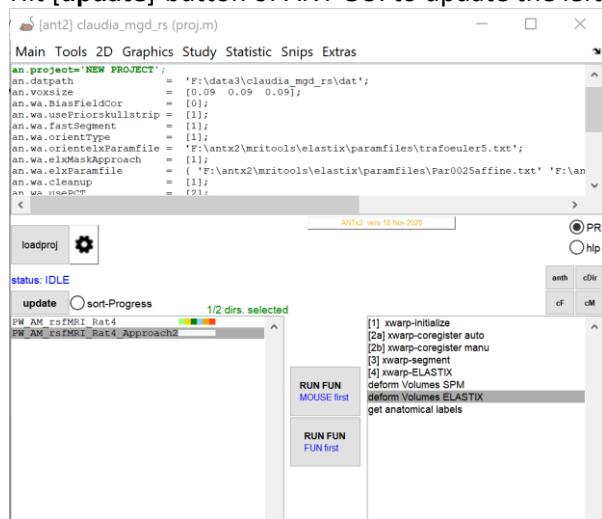
For this approach the steps 1-5 are the same (see above).

- 1. Set ANT path, make study folder +start ANT GUI**
- 2. Download template**
- 3. Define a project**
- 4. Import Bruker data**
- 5. Import templates for this study**

## PREPARATION

For approach-2. I created a new folder in the 'dat'-folder, named it '**'PW\_AM\_rsfMRI\_Rat4\_Approach2'**' and copied the following original NIFTI's : '**'T2star\_FID\_EPI\_sat\_RingCoil\_20\_1.nii'**' and '**'T2\_TurboRARE\_axial\_21\_1.nii'**' into this folder

Hit [update]-button of ANT GUI to update the left animal listbox:

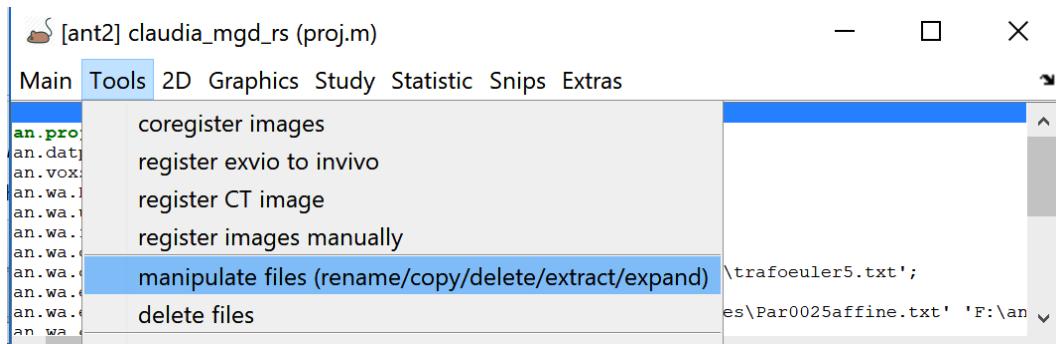


**Don't forget to select this animal from the left listbox to work with this data set**

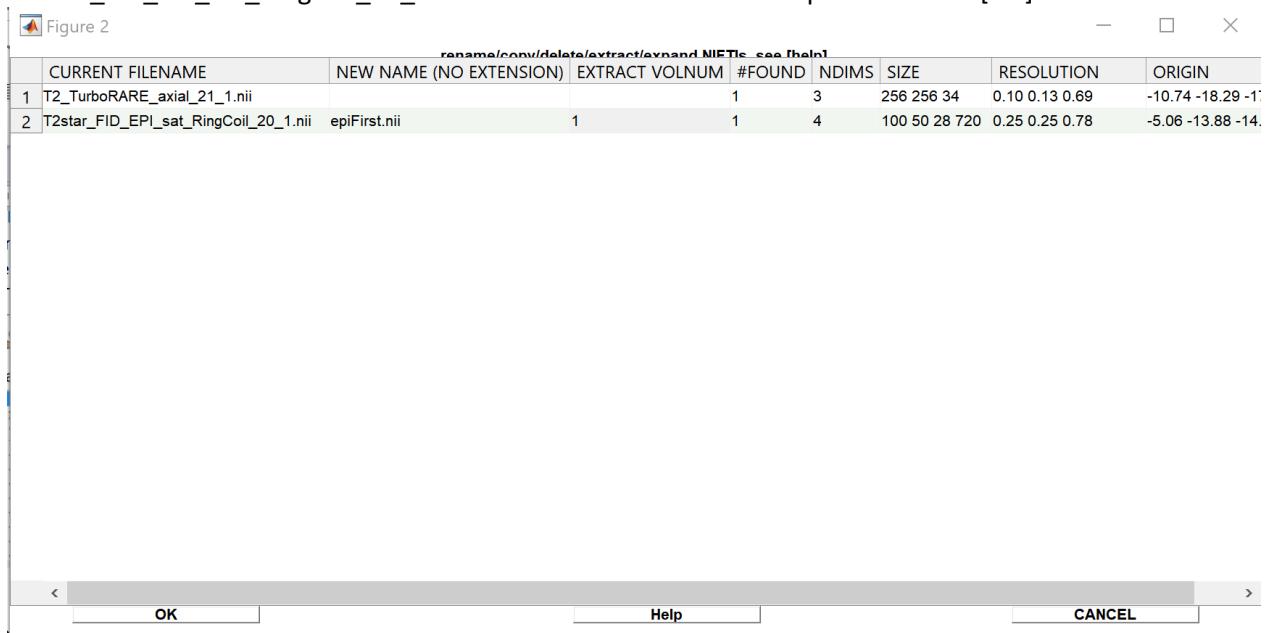
## 6. Extract 1st image of the 4D BOLD series (Approach2)

Here we extract the 1<sup>st</sup> image of the 4D resting-state data to use it as target-image for coregistering the 't2.nii' onto the 4D BOLD data.

→ Tools/manipulate files ...



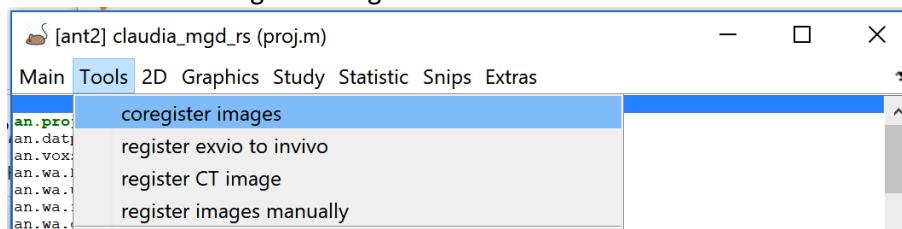
Search for '**T2star\_FID\_EPI\_sat\_RingCoil\_20\_1.nii**' in the File listbox. Type '**epiFirst.nii**' in the '**New Name**'- column and '1' in the '**Extract**'-column. With this, the 1<sup>st</sup> image from '**T2star\_FID\_EPI\_sat\_RingCoil\_20\_1.nii**' is extracted and saved as '**epiFirst.nii**'. Hit [**OK**] button.



## 7. Coregister 't2.nii' onto BOLD (RS-) Data (Approach2)

Here we register the structural image '**T2\_TurboRARE\_axial\_21\_1.nii**' to the first volume ('**epiFirst.nii**') of the 4D time-series. This allows to later use the resting state data space as 'native space'. PLEASE COMPARE THIS APPRACH WITH THE APPROACH ABOVE.

→ANT-MENU: Coregister images



```

***COREGISTRATION***
% --- COREGISTRATION ---
% Block-1,Block-2...Block-3...: are independent coregistrations with independent targetImage/sourceImgae and applyIMag
% coregister sourceImage[t1] onto targetImage[t1] and apply transformation to other images [applyImages]
%
x.TASK      = '[100] noSPMregistration, only elastix' ;% Task to perform (display before/after and or register)
x.targetImg1 = {'epiFirst.nii'}; % target image [t1], (static/reference image)
x.sourceImg1 = {'T2_TurboRARE_axial_21_1.nii'}; % source image [t2], (moved image)
x.sourceImgNum1 = [1]; % if sourceImg has 4 dims use this imageNumber --> sourceImg(:, :, :, sourceImgNum)
x.applyImg1 = {''}; % images on which the transformation is applied (do not select the sourceIMG again!)
%
% PARAMETERS
x.cost_fun   = 'nmi'; % objective function: [nmi] norm. mutual Info, [mi] mutual Info, [ecc] entropy corrcoef,[mm]
x.sep        = [4 2 1 0.5 0.1 0.05]; % optimisation sampling steps (mm)
x.tol         = [0.01 0.01 0.01 0.001 0.001 0.001]; % tolerances for accuracy of each param
x.fwhm       = [7 7]; % smoothing to apply to 256x256 joint histogram
x.centerering = [0]; % make copy of targetIMG & set origin to "center" ,than apply centeringTRafo to all coregiste
%
% RESLICE VOLUME (OPTIONAL)
x.reslicing  = [0]; % reslice images, [0] no, do not reslice, [1] reslice to target Image (targetImg1)
x.interpOrder = 'auto'; % interpolation order [0]nearest neighbour, [1] trilinear interpolation, ["auto"] to auto
x.prefix     = 'r'; % file prefix for the new resliced volume (if empty, overwrite the soureIMG )
%
% NONLINEAR WARPING (OPTIONAL)
x.warping    = [1]; % do subsequent nonlinear warping [0|1],
x.warpParamfile = {'F:\anTx2\mrifiles\elastix\parameterfiles\par_affine038CD1.txt'}; % parameterfile used for warping
x.warpPrefix  = 'c'; % prefix out the output file after warping (if empty, it will overwrite the output of the
x.cleanup    = [1]; % remove interim steps
%
% BLOCK-2 (optional)
% targetImg1
RUN | cancel |

```

#### In the parameter GUI do the following:

- 1) **TASK**: set to '[100]...' via left icon/pull-down menu: This circumvents SPM coregistration and uses Elastix registration
  - 2) **targetImg1**: select 'epiFirst.nii' via left icon → selection GUI. This is the target image
  - 3) **sourceImg1**: select the structural image '**T2\_TurboRARE\_axial\_21\_1.nii**' via left icon → selection
  - 4) **warping**: set to [1]; → This allows the Elastix registration
  - 5) **warpParamfile**: from left icon → selection GUI select the ELASTIX parameter file '[par\\_affine038CD1.txt](#)'. This file will perform an affine registration. For reason to use affine...see Approach-1/scaling issue.
- Hit **[OK]**. This step takes <2min.

When successful you will obtain '**T2\_TurboRARE\_axial\_21\_1.nii**' in register with '**epiFirst.nii**': (using file-prefix 'c') → '**c\_T2\_TurboRARE\_axial\_21\_1.nii**'. Also the file '**c\_epifirst.nii**' is created (This is just a copy of the target image).

**BEFORE REGISTRATION:** Overlay of 'epiFirst.nii' (blue) and 'T2\_TurboRARE\_axial\_21\_1.nii'(red)

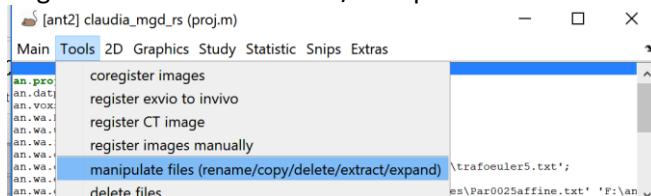


**AFTER REGISTRATION:** Overlay of 'c\_epifirst.nii' (blue) and 'c\_T2\_TurboRARE\_axial\_21\_1.nii'(red)



## 8. Create a 't2.nii' image (Approach2)

→ go to ANT-MENU: Tools/manipulate files..

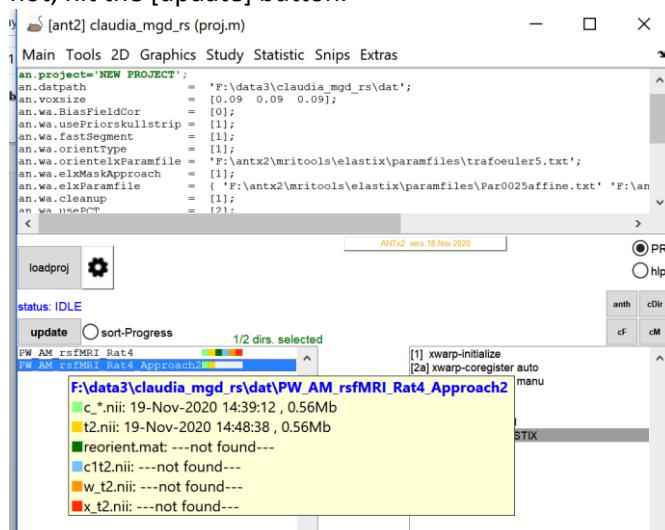


In the new GUI find the structural image that should be used as input for the registration to template ('c\_T2\_TurboRARE\_axial\_21\_1.nii'). The “new Name”-column type ‘t2.nii’ and in the extract volnum column set a ‘:’ (colon –symbol). The colon symbol indicates that the file should be copied and renamed (instead of renaming the original file). Alternatively, you could also write ‘copy’ in this column. **IMPORTANT:** use the RS-registered structural image ('c\_T2\_TurboRARE\_axial\_21\_1.nii') not the original ('T2\_TurboRARE\_axial\_21\_1.nii')!

CURRENT FILENAME	NEW NAME (NO EXTENSION)	EXTRACT VOLNUM	#FOUND	NDIMS	SIZE	RESOLUTION	ORIGIN
1 T2_TurboRARE_axial_21_1.nii			1	3	256 256 34	0.10 0.13 0.69	-10.74 -18.29 -1.
2 T2star_FID_EPI_sat_RingCoil_20_1.nii			1	4	100 50 28 720	0.25 0.25 0.78	-5.06 -13.88 -14.
3 c_T2_TurboRARE_axial_21_1.nii	t2.nii	:	1	3	100 50 28	0.25 0.25 0.78	-5.06 -13.88 -14.
4 c_epiFirst.nii			1	3	100 50 28	0.25 0.25 0.78	-5.06 -13.88 -14.
5 epiFirst.nii			1	3	100 50 28	0.25 0.25 0.78	-5.06 -13.88 -14.

Hit [OK].

**IMPORTANT:** The ‘t2.nii’ file is the starting file (input) for the registration-to-template pipeline. Therefore it is recommended to a proper structural image. The left listbox in the ANT-main GUI indicates that the ‘t2.nii’ for that animal now exists (yellow icon left to the animal folder name). If not, hit the [update] button.



## 9. Register ‘t2.nii’ to the template (Approach2)

→ Basically the same as in Approach1 ... → see: [8. Register ‘t2.nii’ to the template \(approach-1\)](#)  
From the right listbox select the first 5 entries (note that “[2b] xwarp-coregister manu” manual registration is only used if “[2a] xwarp-coregister auto” is not selected). Here we want to register the t2.nii automatically.



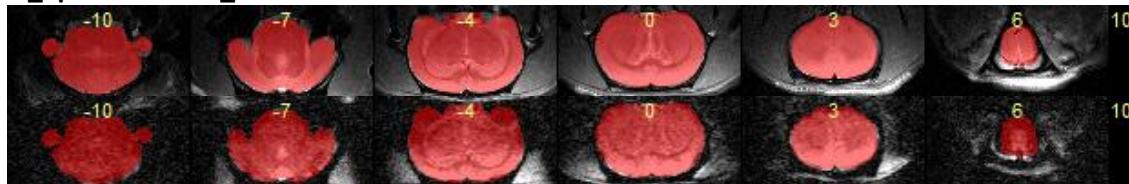
Hit the **lower [RUN FUN]** button.

This steps will prepare the data (I.A: dirty fast skullstrip 't2'), rigid register 't2.nii', segment and nonlinear register the data to the templat. The steps are a little time consuming. In the meantime you could inspect the interim steps via the browser. If the browser did not, double click the "summary.html"- file from the studies folder. → see: **8. Register 't2.nii' to the template (approach-1)**

## 10. Back-transform template brain mask to native space (Approach2)

→ see: **9. Back-transform template brain mask to native space (approach-1)**

→ result: All 3 images are now in the same space (i.e. in register): top: 't2.nii'+'ix\_AVGT.nii', bottom 'c\_epiFirst.nii +'ix\_AVGT.nii'.



## 11. Mask first EPI-image with brain mask (Approach2)

→ see: **12. Mask first EPI-image with brain mask (approach-1)**

Difference: There is a tiny difference compared to Approach-1: Here t2.nii is already in register with RS-data, thus we can use 'ix\_AVGTmask.nii'. In contrast to APPROACH-1, where t2.nii and 'ix\_AVGTmask.nii' was registered to RS\_data such that 'c\_ix\_AVGTmask.nii' was finally in register  
→ HERE WE USE 'ix\_AVGTmask.nii'

```

xcalc
% *** xcalc ****
%
=====
x.niftis = {'c_epiFirst.nii' % << select IMAGE(S) to manipulate
            'ix_AVGTmask.nii'};

x.evalstring = '@i1.*@i2'; % string to evaluate->see help
x.outName = 'epiFirstMask.nii'; % outputname: <string><cell> or use
x.outDir = 'local'; % <<select the output directory: ["local"]

```

**Do the following steps:**

- 1) **niftis**: click green icon and select the two images 'c\_epiFirst.nii' and '***ix\_AVGTmask.nii'*'  
**AGAIN (a)** 'c\_epiFirst.nii' ist just a copy of 'epiFirst.nii' → so you can use one of the two  
(b) use '***ix\_AVGTmask.nii'* here !!!****
- 2) **evalstring**: type '@i1.\*@i2' → i.e multiply 1<sup>st</sup> image with 2<sup>nd</sup> image (voxelwise)... preserve the '.\*' operation and note that 'ix\_AVGTmask.nii' is binary, thus this operation will zeroing all voxel values in 'c\_epiFirst.nii' if the same voxel in 'ix\_AVGTmask.nii' contain a zero.
- 3) **outName**: type 'epiFirstMasked.nii' → this is the filename of the resulting Nifti file  
Hit [RUN].  
→ the results are virtually the same as in Approach-1

**12. Scale up 4D data for FSL (Approach2)**

→ see: **13. Scale up 4D data for FSL (approach-1)**