Atlas Registration Tutorial

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This tutorial shows how to:

(1) Setup ANT TBX,
(2) Organize the study folder
(3) Start ANT
(4) Create a new project and load a project file
(5) Import Bruker raw data
(6) Inspect the imported raw data
(7) Rename files, delete files, define the input image ("t2.nii") for atlas registration
(8) Perform the atlas registration
(9) Extract a 3d volume from a 4D volume and transform the 3d volume to Allen Space

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PREREQUISITES:

-Windows machine, 64 bit, tested Matlab Version: 2016a
-path of ANT TBX is properly set in Matlab (use antlink.m to set the paths, antlink.m is located in the antx-folder)
-you have raw data from a Bruker machine

DATA ORGANIZATION

→ the study folder and a dat-folder within the study's folder must be created

- find a storage device with enough space. Note that the data size of each mouse will exceed 200Mb after atlas registration

Do the following 2 steps:

(1) Create a folder with a proper name for the study: This Folder will later on contain all data.
(2) Within the study's folder create a folder and name it 'dat'. (After importing the data, this dat-folder will later on contain the data for each mouse. Note that the data of each mouse is separately stored in folders within the dat-directory).
(3) <optional> Within the study's folder you may create an import folder containing the Bruker raw data

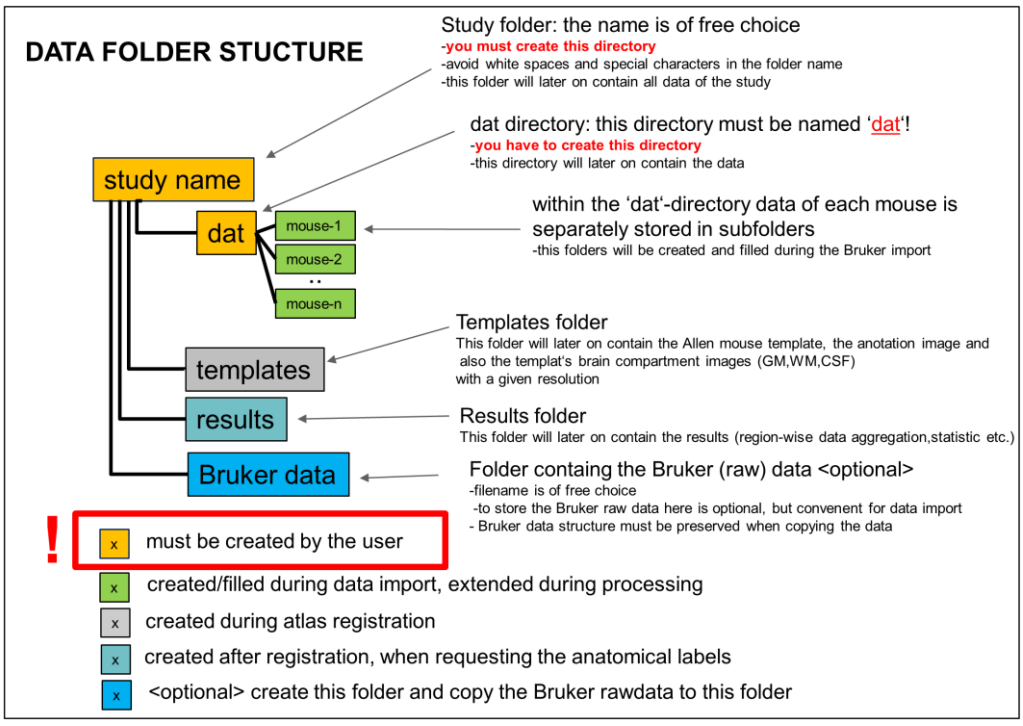


Figure: Data structure. The orange things you have to care about for now.

Example: For this example, I created a folder entitled 'sampleData_tutor' (left figure). Within the folder I created another folder 'dat' (which will later on contain the imported data). Finally, a folder named 'bruker_import' was created and Bruker

raw data from two mice were copied into the 'bruker_import' folder (right figure). Importantly, preserve the original data structure of the Bruker raw data!!!!

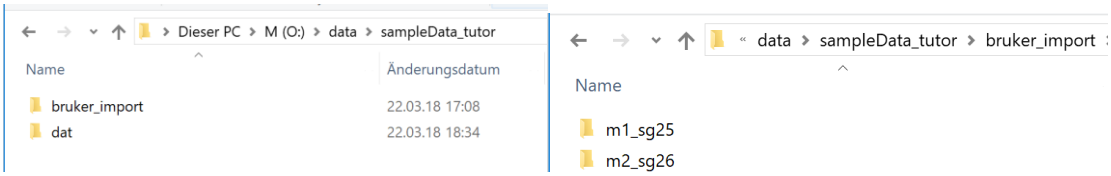


Figure: Example used int his tutorial

STARTING ANT

-set the current path of Matlab to your studies folder (# here: cd O:\data\sampleData_tutor)
-type "ant" in the command window to open the ANT gui.

CREATE a PROJECT

→ The project file (m-file) is usually stored in the studies folder and contains parameter settings. This project file must be created once. Note also, that the project file has to be loaded each time you want to work with the data. The project should be saved within the study's folder.

To create a project file do the following steps:

(1) from the ANT menu bar select [**main/new project**]
(2) in the settings menu you can give the project a name. Here, using the parameter "x.project", I termed the project 'tutor').
(3) Now the data path has to be linked. The parameter "x.datapath" has to point to the "../dat"-folder of the study. You may either type the full-path name /copy and paste it or select it. The latter can be done by clicking onto the line of 'x.datapath' to make an icon on the very left next to 'x.datapath' visible. Select the icon, to conveniently navigate and chose the "../dat" folder via gui. Hit [ok].

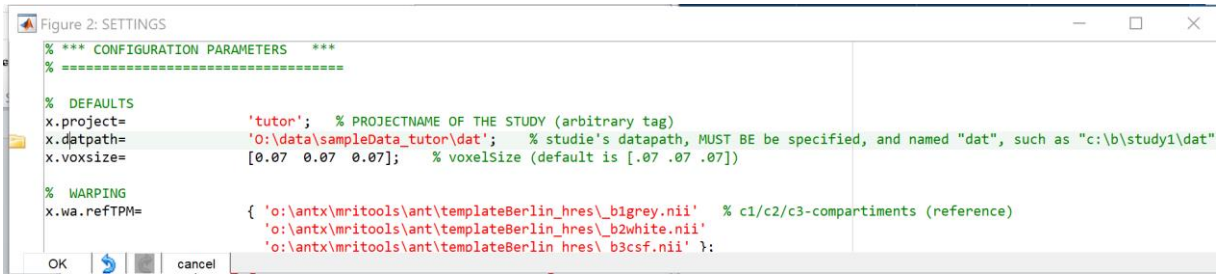


Figure: Setttings. Importantly you have to set the datapath ("…/dat"). For this you may navigate and select the dat-folder by hitting the left icon next to x.datapath

(4) A popup will ask you were to store the project file. In this example the project file entitled 'tutor_project.m' is saved as recommended within the study folder.
(5) Another popup will ask you whether the project should be loaded now. → Hit [yes].

LOAD A PROJECT

→he project file has to be loaded each time you want to work with the data.
-because the project is already loaded, this step can be skipped…
-hit [loadproj]-button from the ant main gui and select the project.file (here: 'tutor_project.m')

IMPORT BRUKER DATA

→This step shows how to import Bruker raw data

(1)<u>Select</u> [main/import Bruker data] from the ANT menu bar

<u>(2) DATA SET SELECTION</u>: in the [Bruker import] window select the directory containing the Bruker data from the right panel. Thereupon, the directory (here: "bruker_import") disappears from the right panel and reappears in the lower panel. Hit [Done]. Note that this would be the way to access all mouse data sets within the "bruker_import"-directory.
Alternatively (b), you can also select specific data sets: For this select the Bruker directory from the LEFT panel. Then, select the specific data sets from the RIGHT panel. Note that the selected mouse data sets disappears from the right panel and reappears in the lower panel. Hit [Done].

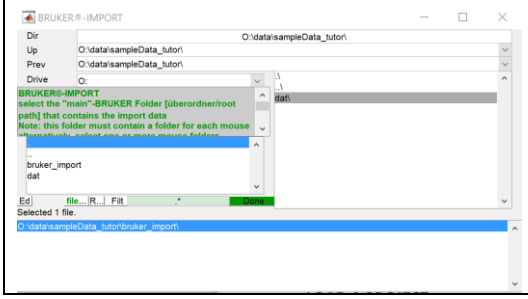
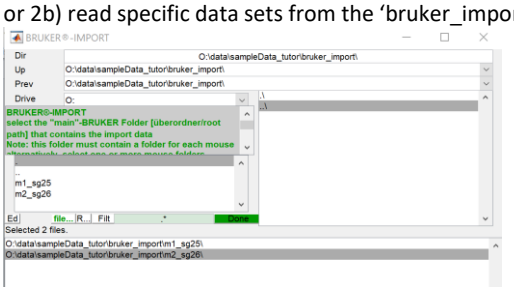
2a) read all data sets within the 'bruker_import' –folder	or 2b) read specific data sets from the 'bruker_import' –folder
	

Figure: Read all mouse folders from one directory (left), Read one/more selected mouse folders from one directory (right)

Here I selected two data sets.

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3) FILE SELECTION: The [Selector] window lists all volumes found in the selected data sets

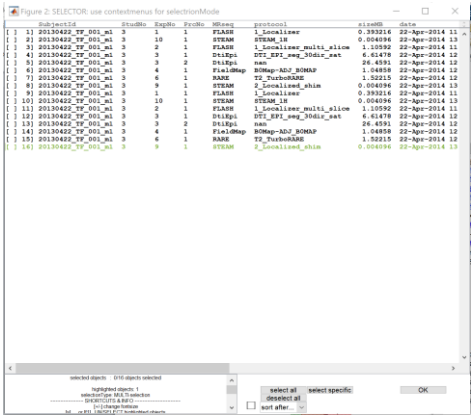


Figure: File selection, select the files you want to import

-You can now select specific files (double click, or select one/more volumes and hit [s] from the keyboard)
-to inspect a volume concerning acquisition parameters or display the volume you may use the context menu and select the respective item (see figure).

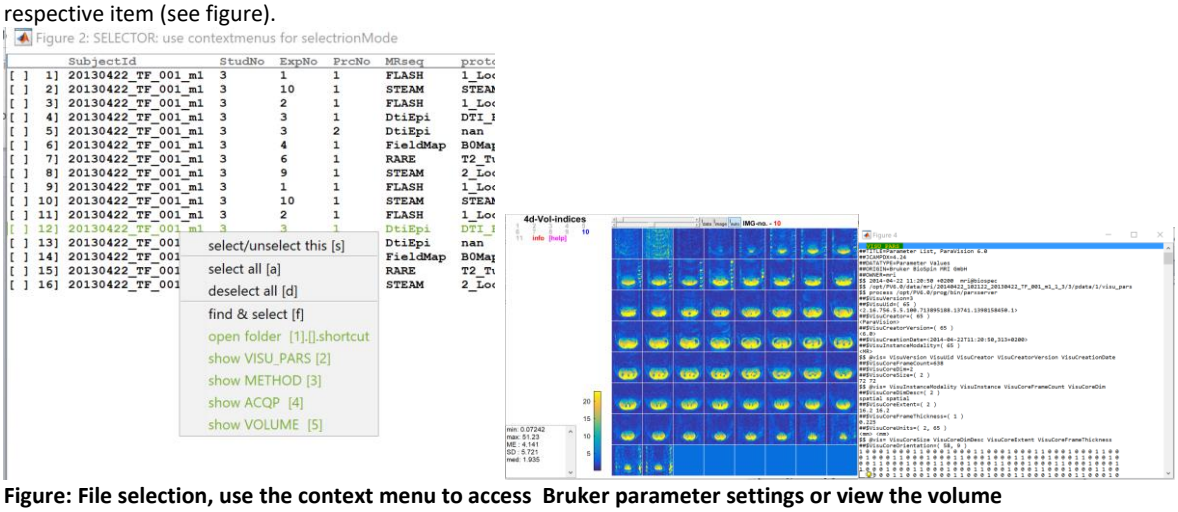


Figure: File selection, use the context menu to access Bruker parameter settings or view the volume

Here: all files will be imported: hit [select all], than hit [OK].

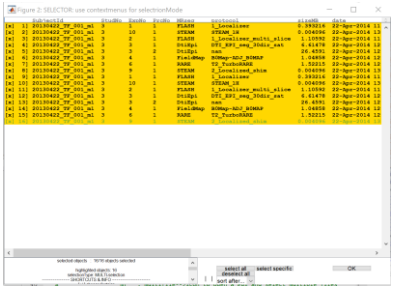


Figure: Example, all files are selected and will be imported

4) PARAMETER SETTING: The next window lists the import parameters (BrukerImport –parameterfile): Hit [RUN], don't change any parameters for now.

This process will create a unique directory for each of the selected data sets, i.e. for each mouse, in the "dat"-folder. Each of the selected volumes is converted to nifti-files and stored in the respective mouse-folder within the "dat"-directory.

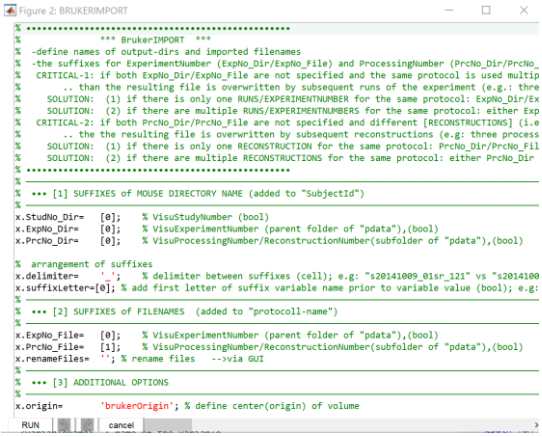


Figure: Parameter Settings for Bruker Import

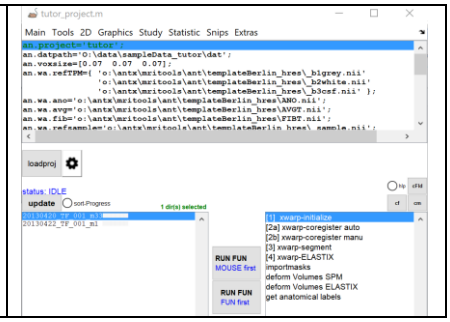
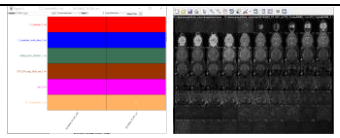
-Once the import step has been finished, the left panel of the ANT gui should show the imported mouse-folders, otherwise hit the [update] button. The mouse folders, located in the "dat"-folder, should now contain the imported files.	

Figure: ANT gui should now list the imported mouse folders in the left panel (here two mouse folders)

RAW DATA INSPECTION <optional>

Use one of the following options: a) Select one/more mouse directories from the left panel. In the context menu select "open folder in explorer". You can now visualize the data for instance using MRICRON	
b) Select one/more mouse folders from the left panel. Select [Graphics/show case file matrix] to visualize all mouse folders and existing files in matrix arrangement. You can than [left click] a cell (i.e. a specific image of a mouse) to show this volume (or later as overlay), [right click] to show this volume in MRICRON or [left double-click] to open the directory and highlight the selected file in windows explorer and use another visualization tool (e.g. MRICRON)	 case x file matrix (left), select a cell to visualize the data (right figure)

RENAME FILES/DELETING FILES, DEFINE "t2.nii" <mandatory>

→ This step has to be done to define the input image for the atlas registration. The input image must be named "t2.nii" in order to be found by the pipeline.

-This can be done manually (open each folder and rename or copy/rename the respective file to “t2.nii”).

-Alternatively do the following steps:

(1) Select all mouse directories or directories of interest from the left panel

(2) Select [Tools/”rename/copy/delete/extract/expand files (simple)”] from the ANT menu bar

(3) The file table allows to rename, copy&rename, delete, expand or extract images.

→ Here, the aim is to make a copy of the image “T2_TurboRARE_1.nii” and name it “t2.nii”.

For this, type “t2” or “t2.nii” in the [new name] column of the respective row and enter a colon (:) in the [extract volnum] column. The colon indicates to create a copy of the original file and rename the copied version. (Note, without the colon the original file is renamed). Hit [OK].

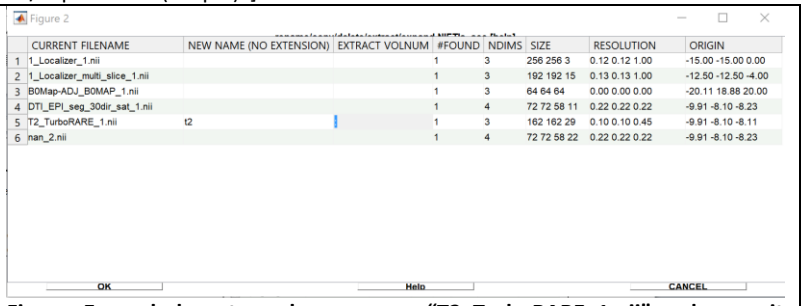


Figure: Example how to make a copy or “T2_TurboRARE_1.nii” and name it “t2.nii”. Use the colon (:) to rename the copy instead of renaming the original file. Note, copying/renaming is applied to all “T2_TurboRARE_1.nii” files found across selected mouse folders

REMOVING FILES

→Additionally you can **remove** files from the same gui

- In this example, we delete all localizer files (“1_Localizer_1.nii” and “1_Localizer_multi_slice_1.nii”): To do this, type a double hash (##) in the [New Name] column of the respective rows (images). Hit [OK]. Note that these files will be removed from all selected mouse folders.

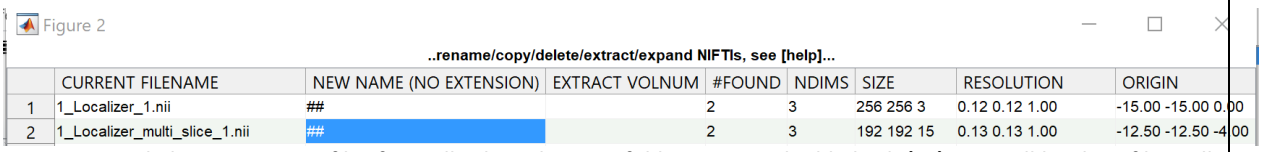


Figure: Example how to remove files from all selected mouse folders using a double hash (##). Here all localizer files will be deleted.

Back to the renaming of “T2_TurboRARE_1.nii” to “t2.nii”…

At this point, the left panel of the ANT gui should indicate the existence of the “t2.nii”-files (yellow box right to the mouse folder names). **NOTE: “t2.nii” is the mandatory name of the input image for atlas registration. In other words, a mouse brain can only registered to atlas space if the respective mouse folder contains an image entitled “t2.nii”.**

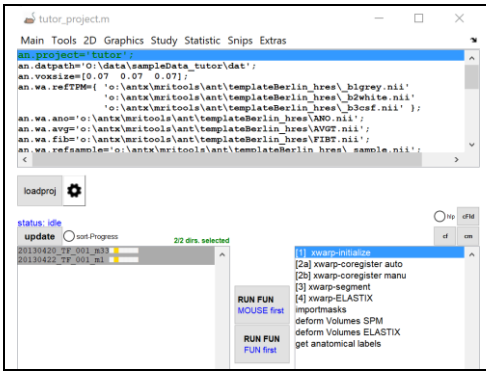


Figure: In this example we have data of two mice, structured in two mouse folders (left panel). Right to the mouse folder’s name the yellow box indicates that both folders contain a t2-weighted image with the name “t2.nii”. Note that “t2.nii” is per default the name of the input image for atlas registration (next topic)→ Hence, atlas registration can be started for the two mice.

ATLAS REGISTRATION

→ Here, the aim is to find the transformation parameters (rigid followed by affine followed by nonlinear b-spline transformation) to register the “t2.nii” image to the Allen template (Allen space/atlas space). The obtained parameters for forward (Allen space) and backward (mouse space/native space) transformation will later on allow to transform images to the respective space. Note, for atlas registration a mouse folder must contain a file named “t2.nii” (This is usually a renamed copy of the T2-weighted image), which is used as input image (moving image) for the registration pipeline.

Do the following steps:

(1) Select one/several/all mouse folders from the left panel of the ANT gui.

(2) In the right panel select [1, 2a ,3 and 4]. Do not select [2b], which is a rough manual (!) pre-registration. In most cases [2a] (automatic pre-registration) should work fine. Use [2b] only if the automatic mode fails or produce suboptimal results.

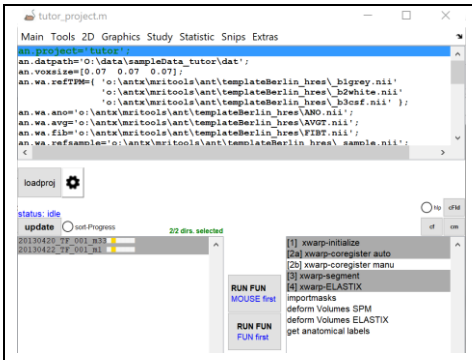


Figure: Atlas registration (AR). For AR select the desired mouse folders from the left panel. Then, select [1,2a,3 and 4] from the right panel and hit [Run Fun, Fun first] (lower button)

Meaning of [1,2a,3 and 4]:

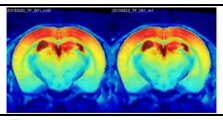
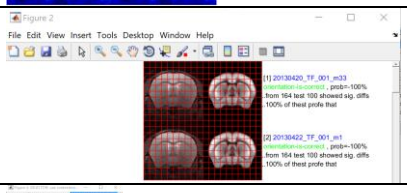
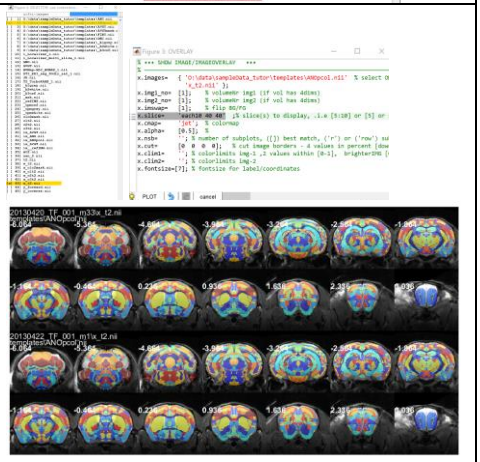
(1a) generate a templates folder below the studies folder with all necessary files (TPMS, ATLAS, template). Copy files from the templates folder to the specific mouse folder. Extract brain from "t2.nii" for rough preregistration. (2a) rigid registration. (3) SPMs unified approach (segementation, normalization, bias field correction). (4) registration of a template of the derived TPMS to Atlas space. Generation of forward & backward transformation parameters, transform some of the images (TPMs, TPM-mask, t2.nii to atlas space; AVGT.nii (template), ANO.nii (anotation file/atlas), ANOpcol (Atlas with pseudocolors) to mouse space)

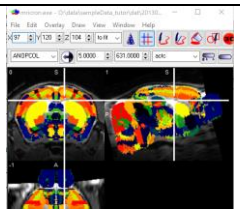
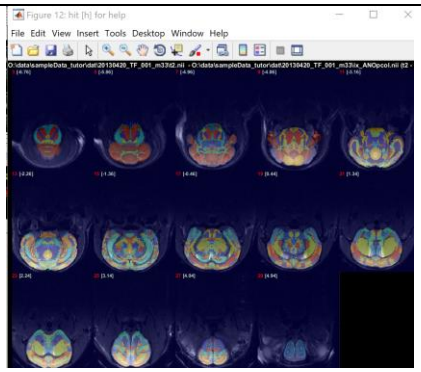
(3) Hit [run fun, fun first] from the ANT gui.

-atlas registration will take some time (15-20min per mouse)

VISUAL INSPECTION of ATLAS REGISTRATION <optional, but recommended>

→ visual inspection can be done to check the registration quality (eye-balling seems to be the best way)

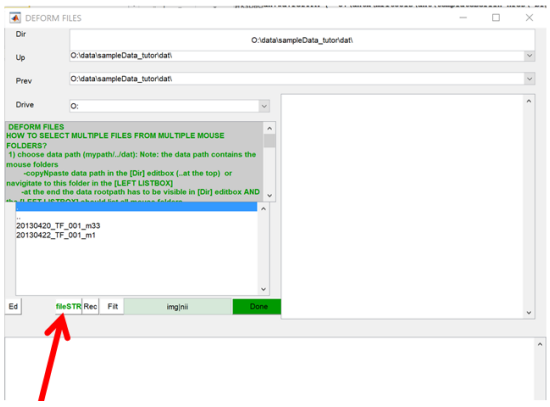
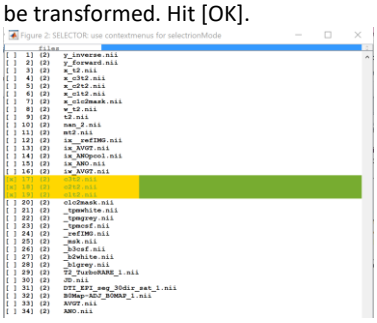
several optional ways:	
a) select the respective mouse folders from the left panel→ from the context menu select [check registration quality of x.t2]	
b) select the respective mouse folders from the left panel→ from the context menu select [check coreg - panel (elastix)]	
c) select the respective mouse folders from the left panel than go to [Graphics/Gui overlay image2] -from the selector window select "../templates/ANOpcol.nii" and "x_t2.nii" -in the Overlay-window click into the row of "x.images" to activate the green icon. Select the green icon to import "ANOpcol.nii" and "x_t2.nii" from the selector window. Set: x.imswap to 1, set x.slice= 'each10 40 40' and hit [plot] -use keyboard shortcuts to inspect/swap foreground/background images and fused image → This example shows the Allen Atlas (pseudocolorized→ "ANOpcol.nii") overlayed onto all selected "x_t2.nii" images (i.e. the "t2.nii" images transformed to atlas space). (x.imswap: swaps foreground and background images. Here every 10th. slice is displayed starting from the 40th slice up to the last-40 slices	

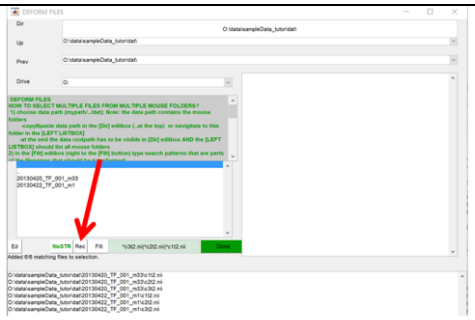
d) select the respective mouse folders from the left panel than go to [Graphics/case file matrix] from the ANT menu bar. In the case file matrix gui set [BG]-radio button to '1', chose "ANOpcol.nii" from the upper pull-down menu and select one of the cells of "x_t2.nii", click left of right mouse button to show the overlay between "x_t2.nii" and Allen atlas in Matlab or Mricron (e.g. you may use the left mouse button to obtain a fused image in Matlab→ use keyboard shortcuts to inspect/swap foreground/background images and fused image)	
→ Using the case-file matrix you can also inspect the overlay between atlas und "t2.nii" in mouse space. For this, set [BG]-radio button to '0', chose "t2.nii" from the upper pull-down menu and select one of the cells of "ix_ANOpcol.nii". Note that ix_ANOpcol.nii", refers to the pseudo-colorized Allen Atlas, transformed to native space (prefix: "ix_") →use keyboard shortcuts to inspect foreground/background and fused image → in native space you can check the overlay between "t2.nii" and all types of back-transformed images with the prefix "ix_" → you can also inspect the tissue compartments in native space (c1t2.nii, c2t2.nii, c3t2.nii) or in Allen space (x_c1t2.nii, x_c2t2.nii, x_c3t2.nii)	

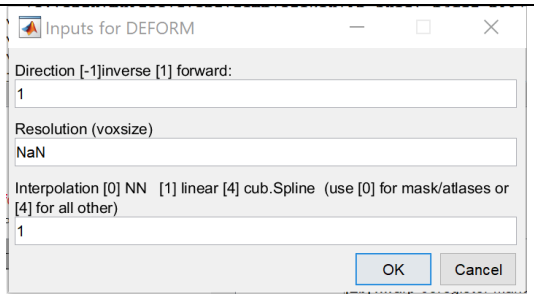
TRANSFORM OTHER IMAGES

→Once the "t2.nii" image has been transformed to Allen space ("x_t2.nii") and the transformation parameters have been estimated, other images (in register with 't2.nii') from native space can be transformed to atlas space and vice versa.

To do this do the following steps:

(1) Select one/more/all mouse folders from the left panel of the ANT gui	(2) In the right panel select [deform Volumes ELASTIX], than hit [RUN FUN, FUN first].
3) If [RUN FUN, FUN first] was selected, a window will pop up. Here, hit [fileStr] to recursively find all images of the search directory "Dir" (here: "O:\data\sampleData_tutor\dat\") 	4) All recursively found files will be listed in another window. - Select the files you want to transform, but select only those files at once with the same value range (binary or indexed masks vs. TPMs (range 0-1) vs. other images. This is because the interpolation parameter has to be set and should be different for each of those cases. → Here GM, WM and CSF should be transformed to Allen space. Note that this step is not nessessary because the initial "t2.nii" registration already transformed the TPMs to Allen space (x_c1t2.nii, x_c2t2.nii, x_c3t2.nii). This step is only exemplarily performed to show how other images can be transformed. Hit [OK]. 
5) Next, hit the [Rec] button to recursively find all selected files in the apriori selected mouse folders. The lower panel lists all found files across mouse folders. Hit [Done].	6) In the Deform Parameter window: set interpolation to 1. Hit [OK]





Notes on Deformation Parameters:

→ direction: (1) transform image to Allen space, (-1) transform image to native space

→ resolution: NaN, (keep this parameter as it is). It means use the resolution of the target image ("t2.nii" or "x_t2.nii") depending on the target space (direction)

→interpolation: (0) next neighbour interpolation → use NN for masks (brain masks or indexed atlas volumes), (1) linear, this should be fine for TPMs or images with a well defined value range (range of TPMS: 0-1), [3] spline interpolation→ for all other images (those that are not binary or those with unbounded value range).

Add on: Extract a 3d volume from a 4D volume and transform it to Allen Space

→ This example shows how to extract a specific 3d volume from a 4d volume for all selected mouse folders and transform this 3d image to Atlas space

***** NOTE: This is just an example. There is no rationale to extract exactly this 3d-volume from that 4d volume and transform it to Allen space!!!**

Do the following steps:

1) First, select all mouse directories or the directories of interest from the left panel

2) select [Tools/"rename/copy/delete/extract/expand files (simple)"] from the ANT menu bar

3) The aim is to extract the 4th volume of "DTI_EPI_seg_30dir_sat_1.nii" (4d-volume) and save this 3d-volume as "AAA.nii". To do so type a new name ("AAA") in the [New Name] column of the respective image and type "4" in the [extract volnum] column. The latter indicates that that that the 4th. Volume should be extracted.

Figure 2

..rename/copy/delete/extract/expand NIFTIs, see [help]...

	CURRENT FILENAME	NEW NAME (NO EXTENSION)	EXTRACT VOLNUM	#FOUND	NDIMS	SIZE	RESOLUTION	ORIGIN
1	ANO.nii			2	3	164 212 158	0.07 0.07 0.07	-5.75 -8.86 -8.52
2	AVGT.nii			2	3	164 212 158	0.07 0.07 0.07	-5.75 -8.86 -8.52
3	B0Map-ADJ_B0MAP_1.nii			2	3	64 64 64	0.00 0.00 0.00	-20.11 18.88 20.00
4	DTI_EPI_seg_30dir_sat_1.nii	AAA	4	2	4	72 72 58 11	0.22 0.22 0.22	-9.91 -8.10 -8.23
5	JD.nii			2	3	164 212 158	0.07 0.07 0.07	-5.75 -8.86 -8.52
6	T2_TurboRARE_1.nii			2	3	162 162 29	0.10 0.10 0.45	-9.91 -8.10 -8.11

4) Atlas registration: Now you can transform the "AAA.nii" image to Allen space as described above (for interpolation use spline interpolation(3))