Tutorial: Running ANTx on machines without graphic support "working without graphical user interfaces, GUIs"

This tutorial shows how to perform some basic steps without GUIs, for instance when running on a HPC-machine.

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1) OPTIONAL: How to set the paths of ELASTIX in UNIX (LINUX)-systems:

```
MAKE ELASTIX RUNNING ON UNIX/LINUX-Systems
1) SET PATH OF ELASTIC IN .bashrc-FILE
FOR INFORMATION:
see: Elastix-manual (section 3.2)
see: https://usermanual.wiki/Document/elastix490manual.1389615963/html#pf17
Linux: Add the following lines to your .bashrc file:
export PATH=folder/bin: $PATHexport
LD_LIBRARY_PATH=folder/lib:$LD_LIBRARY_PATH
...where "folder" is the path to the linux-Elastix-folder (which is located in the antx2-
folder): YOUR-DRIVE\antx2\mritools\elastix\elastix linux64 v4.7
My linux-elastix-folder is "/sc-projects/sc-proj-agtiermrt/Daten-
2/ressources/antx2/mritools/elastix/elastix linux64 v4.7/". Thus, my bahrc-file it modified as
follows:
           -----[ELASTIX-PATH in bashrc]-----
export PATH=/sc-projects/sc-proj-agtiermrt/Daten-
2/ressources/antx2/mritools/elastix/elastix linux64 v4.7/bin:$PATH
export LD LIBRARY PATH=/sc-projects/sc-proj-agtiermrt/Daten-
2/ressources/antx2/mritools/elastix/elastix linux64 v4.7/lib:$LD LIBRARY PATH
2) RELOAD .bashrc-FILE & TEST ELASTIX
_____
- save .bashrc-file, exit editor, than type the following to reload the .bashrc-file again:
source .bashrc
- check installation, by typing:
elastix
- if successful, a message is displayed:
Use "elastix --help" for information about elastix-usage.
```

2) OPTIONAL: Open interactive session on HPC and start Matlab

OPEN INTERACTIVE JOB on HPC (optional)

srun --time 7-00 --mem=64G --ntasks=8 --pty bash -i

-Please check the parameters, here for 7 days, 64 Gb Ram, 8 cores

LOAD MATLAB-MODULE AND START MATLAB (optional)

module load scientific/matlab/R2021b matlab

-to access Matlab might be different on another machine!

3) BASICS

FROM NOW ON type in the MATLAB CMD-WINDOW...

ADD ANTx-PATHS

Go to the ANTx-patd and link all necessary paths using "antlink"-command:

cd /sc-projects/sc-proj-agtiermrt/Daten-2/ressources/antx2/antlink

GO TO STUDY-FOLDER

Create an empty study folder (here "groeschel"). The study-folder is the folder where the registration of several animals of a study is performed.

cd /sc-projects/sc-proj-agtiermrt/Daten-2/mri/projects/groeschel/

UPDATE ANT-TOOLBOX

This is not mandatory... just type **updateantx(2)** to update the toolbox, i.e. obtain the latest version from GitHub. For more info type help updateantx.

updateantx(2)

CREATE AN ANTx-PROJECT-FILE:

makeproject('projectname',fullfile(pwd,'proj.m'), 'voxsize',[.07 .07 .07],'wa_refpath','/sc-projects/sc-projagtiermrt/Daten-2/ressources/anttemplates/mouse Allen2017HikishimaLR','wa species','mouse');

-here the project-file "proj.m" is created using a target voxel size of 0.07 x 0.07 x 0.07 mm, the <u>animal</u> template is "mouse Allen2017HikishimaLR", with the species 'mouse'.

- a suitable template has to be downloaded from google-drive :

https://drive.google.com/drive/folders/1q5XOOVLvUYLqYsQJLqNRF7OK8fNwYhl9

The template should be unzipped and stored where it could be reached (do not save the downloaded template in the current project-folder!). Creating a project-file has to be done only once!

LOAD THE PROJECT-FILE "proj.m"

Here we load the created project-file "proj.m":

loadconfig(fullfile(pwd,'proj.m'));

CHECK WHETHER THE PROJECT-FILE IS LOADED

global an; an

The global variable "an" (struct) contains the basic information for this study. In the CMD-window you should the following:

project: 'NEW PROJECT'

datpath: '/sc-projects/sc-proj-agtiermrt/Daten-2/mri/projects/groeschel/dat'

voxsize: [0.0700 0.0700 0.0700]

wa: [1x1 struct]

4) IMPORT BRUKER-DATA

Before doing this step, I just created the 'raw'-folder within the study's folder and copied the datasets of two animals into the 'raw'-folder. First we just read the file-information of the Bruker raw-data from the "raw"-folder (fullfile(pwd,'raw')) which is located in the current study folder.

The file-information will be stored in the resulting w-struct.

w=xbruker2nifti(fullfile(pwd,'raw'),0,[],[],'gui',0,'show',1); % first read all data and show it

The w-struct contains the file-information table "d" with header "hd". This table is listed in the CMD-window when running this command. You could save & reload the struct and import some data later on (advantage: loading time is reduced). To show the table again just type:

w.showtable(w); %to show the table in CMD-window

If graphic is supported you could also visualize the table in an extra window via w.showtable 2(w) .

Here, the raw-data folder contains two data-sets (i.e. the data from two animals):

```
| 1 | 20200925MG | LAERWRT | MCR000025 | 1 | 9 | 1 | Dilipi | 02_5_DTT_EPI_seg_b1600_25dir | 76.67712_20-Oct-2020_15:04:13_/sc-projects/sc-proj-agtiermrt/Daten-2/mri/proje | 129.7613_20-Oct-2020_15:04:41_/sc-projects/sc-proj-agtiermrt/Daten-2/mri/proje | 129.7613_20-Oct-2020_15:04:41_/sc-projects/sc-proj-agtiermrt/Daten-2/mri/proje | 129.7613_20-Oct-2020_15:04:41_/sc-projects/sc-proj-agtiermrt/Daten-2/mri/proje | 129.7613_20-Oct-2020_16:5:04:41_/sc-projects/sc-proj-agtiermrt/Daten-2/mri/proje | 129.7613_20-Oct-2020_16:5:04:41_/sc-projects/sc-proj
```

Here we want to import the turboRARE-image ("03 T2 TurboRARE CRP.nii") and the image "DTI EPI seg b100 6dir".

To visualize the filtered table run the following command (note that the w-struct is used as 1st argument): w2=xbruker2nifti(w,0,[],[],'gui',0,'show',1,'flt',{'pro','03_T2_TurboRARE_CRP|EPI_seg_b100'}); the table now looks as follows:

Now let's import these data. For this just set the 'show'-parameter to 0:

w2=xbruker2nifti(w,0,[],[],'gui',0,'show',0,'flt',('pro','03_T2_TurboRARE_CRP|EPI_seg_b100' });

5) VISUALIZE FILES AND FOLDERS

-type **dispfiles** to see a file-by-folder matrix in the command-window (see dispfiles for further help): dispfiles

The following is displayed in the CMD-window:

Here we see that the study's "dat"-folder now contains two animal-folders ("20200925MG_LAERMRT_MGR000025" and "20200925MG_LAERMRT_MGR000027"). Each Folder contains the two imported files ("02 1 DTI EPI seg b100_6dir 1.nii" and "03_T2_TurboRARE_CRP_1.nii").

6) SELECTION OF ANIMALS

Before renaming the files let's first select the animals we want to process.

Here we will select all animals:

mdirs=antcb('getallsubjects')

Note that the variable "mdirs" contains the full paths names of the selected animals:

```
{'/sc-projects/sc-proj-agtiermrt/Daten-2/mri/projects/groeschel/dat/20200925MG_LAERMRT_MGR000025'}
{'/sc-projects/sc-proj-agtiermrt/Daten-2/mri/projects/groeschel/dat/20200925MG_LAERMRT_MGR000027'}
```

Alternative selection of all animals:

mdirs =antcb('selectdirs',[1:2])

mdirs=antcb('selectdirs','all')

7) RENAME FILES

Here we rename the file <u>'03_T2_TurboRARE_CRP_1.nii'</u> to 't2.nii'. Note that the name convention of <u>"t2.nii"</u> is mandatory, because this file is used for registration to standard space. I.e., the registration relies on the name 't2.nii'!

The renaming of the file <u>'03_T2_TurboRARE_CRP_1.nii'</u> is just because I hate long names.

Let's rename '03 T2 TurboRARE CRP 1.nii' to t2.nii':

xrename(0,'03_T2_TurboRARE_CRP_1.nii','t2.nii',':','dirs',mdirs);

```
and rename '02_1_DTI_EPI_seg_b100_6dir_1.nii' to 'dti_b100.nii':
```

```
xrename(0,'02_1_DTI_EPI_seg_b100_6dir_1.nii','dti_b100.nii',':','dirs',mdirs);
```

Here for safety reasons, we make a copy of the original file and rename the copied file. Note that copying and renaming of the copied version is defined via the colon-symbol (:); the 4th input arg). Alternatively, to rename the original files just keep the 4th arg empty.

Now, we check whether the new files exist via dispfiles:

8) REGISTER "t2.nii" TO TEMPLATE SPACE (STANDARD SPACE)

Registration of "t2.nii" to standard space is done in 4 steps: [1] initialization, [2] rough rigid registration, [3] segmentation and [4] warping. You can perform these steps ('task') isolated & sequentially or combined,. Note however, that task '2' can be only performed when task '1' has been already performed. Here the rough registration is done automatically (autoreg', 1). We also use parallel processing across animals ('parfor',1); Please check the memory and number of cores when using parallel processing:

```
xwarp3('batch','task',[1:4],'autoreg',1,'parfor',1, 'mdirs',mdirs(:));
```

Now let's check whether the <u>"t2.nii"</u> is transformed to standard-space (new name is: <u>"x t2.nii"</u>) and the template <u>("AVGT.nii")</u> and atlas <u>("ANO.nii")</u> is back-transformed to native-space (new names: <u>"ix ANO.nii"</u> & <u>"ix AVGT.nii"</u>) via:

Here we see that all three files were created for each data set

9) Extract the first 3d-volume from the 4D-vlume 'dti b100.nii'

Use the following command to extract the 1st volume (4th input arg: 1) of 'dti_b100.nii' and save it as 'dti_b100_1stlMG.nii' for all animals (mdirs):

```
xrename(0,'dti_b100.nii','dti_b100_1stIMG.nii','1','dirs', mdirs );
```

Again, check the existence of the new files via:

```
dispfiles('flt','^dt')

FILE x FOLDER

20200925MG_LAERMRT_MGR000027 counts

tit_b100.nii + + 2/2

tit_b100_istMG.nii + 2/2

counts

2/2 2/2
```

10) COREGISTER 'dti_b100_1stIMG.nii' to 't2.nii'

```
Now let's coregister the <a href="file" dti_b100_1stIMG.nii"">file 'dti_b100_1stIMG.nii</a> (moving image) to the 't2.nii'-image (fixed image): z=[]; z.TASK={ '[2]' }; ;% flag 2 (or '2') signals to perform coregistration via SPM z.targetImg1={ 't2.nii' }; ;% TARGET-IMAGE z.sourceImg1={ 'dti_b100_1stIMG.nii' }; ;% SOURCE-IMAGE z.sourceImgNum1=[1]; ;% IN CASE OF 4D-vol use 1st 3d-volume of SOURCE z.applyImg1= ";% HERE, THE TRAFO IS NOT APPLIED TO OTHER IMAGES z.cost_fun='nmi'; z.sep=[7 2 1 0.5 0.1 0.05]; z.tol=[0.01 0.01 0.01 0.001 0.001 0.001]; z.fwhm=[4 4];
```

```
z.centerering=[0];
z.reslicing=[1]; ;% OUTPUT-IMAGE IS RESLICED TO MATCH WITH TARGET
z.interpOrder='auto';
z.prefix='r3'; ;% OUTPUT FILE-PREFIX
z.warping=[0]; %WARPING IS "OFF"
z.isparallel=1; ;% PARALLEL PROCESSING ENABLED
xcoreg(0,z, mdirs);
```

Again, check existence of the registered file:

Here we see that that the file <u>'r3c_dti_b100_1stIMG.nii'</u> exists for each animal. The file <u>"r3c_t2.nii"</u> is just a copy of the unchanged target-file ('t2.nii', fixed image).

11) TRANSFORM ANOTHER IMAGE TO STANDARD-SPACE

Now, we want to transform the image <u>"r3c dti b100 1stlMG.nii"</u> to standard-space (1st arg: 1) for all selected animals (mdirs), using b-spline interpolation (4th arg: 4), using the local reorientation information estimated from the rough rigid registration step (5th arg: 'local') and indicate that the input-file is located in the animal folder (6th arg: struct('source', 'intern'). This will create the file <u>"x r3c dti b100 1stlMG.nii"</u> in standard-space:

fis=doelastix(1, mdirs,{'r3c_dti_b100_1stIMG.nii'},4,'local',struct('source','intern')); Let's check the existence of the file "x_r3c_dti_b100_1stIMG.nii":

12) TRANSFORM ANOTHER IMAGE TO NATIVE-SPACE

In the same way we could also transform an image from standard-space to the the template space (for instance the Atlas). Here we transform the template's hemispheric mask <u>('AVGThemi.nii'</u>) to native space (new name: <u>'ix_AVGThemi.nii'</u>). For transformation to native space the 1st arg is -1, we use NN-interpolation to preserve numbers/hemispheric-IDs (4th arg: 0):

fis2=doelastix(-1, mdirs,{'AVGThemi.nii'},0,'local',struct('source','intern'));

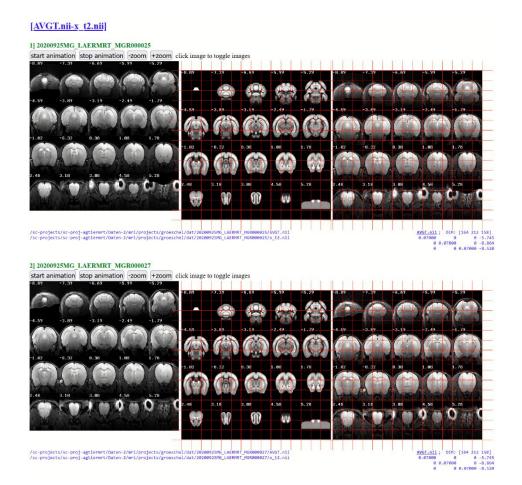
Let's check the existence of the file 'ix_AVGThemi.nii':

13) CHECK REGISTRATION in STANDARD-SPACE - CREATE HTML-FILE

Next, we will create an HTML-file to visualize the overlay of the template <u>"AVGT.nii"</u> and <u>"x_t2.nii"</u> (animal in standard-space). The 3rd arg defines the output-folder, the 4th input, defines the size of the images and the number of slices to visualize:

checkreghtml(mdirs,{'AVGT.nii', 'x_t2.nii' },fullfile(pwd, 'checks'),struct('size',300, 'slices', 'n20'))

Now you can visualize the registration with your Web-browser. The 1st image is an animated gif, showing the overlay of the 'AVGT.nii' and 'x t2.nii' images. Click onto the image to toggle between the two images, or hit 'start animation'-button to see the animated gif. The 2nd ('AVGT.nii') and 3rd image ('x t2.nii') shows the two images side-by-side.

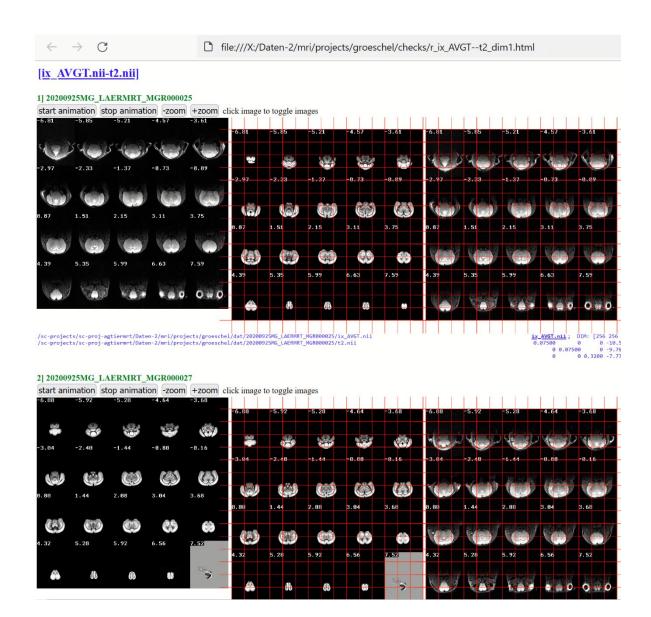


14) CHECK REGISTRATION in NATIVE-SPACE - CREATE HTML-FILE

In the same, we can create an overlay of images in native-space. Here we compare the images <u>"t2.nii"</u> and "ix_AVGT.nii" for all animals and save the output as HTML-file.

Note that the slicing is now done from the 1st-dimension ('dim': 1)

checkreghtml(mdirs,{'ix_AVGT.nii','t2.nii'},fullfile(pwd,'checks'),struct('size',300,'slices','n20','dim',1))



...End of this tutorial. More progress is hopefully coming soon.