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 an HPC-machine.

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1) OPTIONAL: How to set the paths of ELASTIX in UNIX/LINUX-system:

```
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 within the antx2-folder):
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 stmodified
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:
 - if successful, a message is displayed:
Use "elastix --help" for information about elastix-usage.
```

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

OPEN INTERACTIVE JOB on HPC-CLUSTER (optional)

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

-Please check the parameters

LOAD MATLAB-MODULE AND START MATLAB (optional)

module load scientific/matlab/R2021b

matlab

-To access Matlab is for shure different on another machine!

3) BASICS

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

ADD ANTx-PATHS

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

GO TO STUDY-FOLDER

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

The study-folder is the folder where the registration of several animals of one study will be performed

UPDATE ANT-TOOLBOX

Just type updateantx(2) to update the toolbox. For more info type help updateantx updateantx(2)

CREATE A 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 voxelsize of 0.07x0.07x0.07 mm, the animal template is "mouse Allen2017HikishimaLR", with species 'mouse'
- a suitable template has to be downloaded from google-drive :

https://drive.google.com/drive/folders/1q5XOOVLvUYLqYsQJLqNRF7OK8fNwYhI9

And should be unzipped and stored where it could be reached (do not store the downloaded template in the current project-folder!)

-This has to be done only once!

LOAD A PROJECT-FILE "proj.m"

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

CHECK WHETHER THE PROJECT-FILE IS LOADED

global an; an

now you should see some fileds stored in the global variable "an" (struct):

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

First we just read the file-information of the Bruker-raw from the raw-data folder stored in the current study folder (fullfile(pwd,'raw')) and store it in the w-struct.

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

The w.struct contains the a table "d" with header "hd" which is listed in the CMD-window when running this command. Now you can save & reload the struct and import some data later on (advantage: loading time is reduced). To show the table again, type:

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

If graphic is supported you could also see the table in an extra window via w.showtable2(w); The raw-data contain two data-sets (2 animals)

```
2 20200925MG_LAERMRT_MGR00027 1 3 1 RARE 03_T2_TurboRARE_CRP 6.5536 20-Oct-2020 14:57:06 /sc-projects/sc-proj-agtiermrt/Daten-2/mri/proje 2 20200925MG_LAERMRT_MGR00027 1 4 1 DtiEpi 02_1_DTI_EPT_seg_b100_6fir 129.7613 20-Oct-2020 14:58:07 /sc-projects/sc-proj-agtiermrt/Daten-2/mri/proje 129.7613 20-Oct-2020 14:58:07 /sc-projects/sc-proj-agtiermrt/Daten-2/mri/proje 0.004096 20-Oct-2020 14:59:08 /sc-projects/sc-proj-agtiermrt/Daten-2/mri/proje 0.004096 20-Oct-2020 14:59:09 /sc-projects/sc-proj-agtiermrt/Daten-2/mri/proje 0.004096 20-Oct-2020 14:59:09 /sc-projects/sc-proj-agtiermrt/Daten-2/mri/proje 0.004096 20-Oct-2020 14:59:09 /sc-projects/sc-proj-agtiermrt/Daten-2/mri/proje 0.004096 20-Oct-2020 14:54:33 /sc-projects/sc-proj-agtiermrt/Daten-2/mri/proje 0.004096 20-Oct-2020
```

Here we want to import the Turborare-image and the DTI EPI seg b100 6dir-image.

To visulaize 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:

```
BRUKER DATA

set SubjectId

1 20:000925MG_LAERWRT_MGR000025 1 3 1 RAFE 03.1Z_TurboRARE_CRP 6.5336_28-Oct-2020 15:05:38 /sc-projects/sc-
1 20:000925MG_LAERWRT_MGR000025 1 4 1 Dtifpi 02.1_DTI_EPI_seg_b100_6dir 20.6536_28-Oct-2020 15:05:38 /sc-projects/sc-
2 20:000925MG_LAERWRT_MGR000027 1 3 1 RAFE 03.7Z_TurboRARE_CRP 6.6536_28-Oct-2020 14:5756_05-Oct-2020 14:5756_65-
2 20:000925MG_LAERWRT_MGR000027 1 4 1 Dtifpi 02.1_DTI_EPI_seg_b100_6dir 20.64384_20-Oct-2020 14:5756_05-Oct-2020 14:5756_65-
2 20:200925MG_LAERWRT_MGR000027 1 4 1 Dtifpi 02.1_DTI_EPI_seg_b100_6dir 20.64384_20-Oct-2020 14:5756_05-Oct-2020 14:5756_65-
2 20:200925MG_LAERWRT_MGR000027 1 4 1 Dtifpi 02.1_DTI_EPI_seg_b100_6dir 20.64384_20-Oct-2020 14:58:07 /sc-projects/sc-
```

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 in the study's dat-folder now contains two animal-folders ("20200925MG_LAERMRT_MGR000025" and "20200925MG_LAERMRT_MGR000027") and each animal contain two files.

6) SELECTION OF ANIMALS

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

Here we select all animals:

mdirs=antcb('getallsubjects')

mdirs contains the fullpaths 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

```
Lets 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); and rename '03_T2_TurboRARE_CRP_1.nii' to t2.nii' xrename(0,'03_T2_TurboRARE_CRP_1.nii','t2.nii',':','dirs',mdirs);
```

Here for safety reasons, we make a copy of the original file and rename the copied fiel (copying and renaming is defined via the colon-sign (:) as 4th input arg). To really rename the file, keep the 4th arg empty.

Now, check the renaming via dispfiles:

```
dispfiles

FILE × FOLDER
```

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

Registration of "t2.nii" to standard space (SS) is done in 4 steps ([1] initialization, [2] rough rigid registration, [3] segmentation and [4] warping). You can perform these steps ('task') isolated but must applied in the order. Rough Registration 'is done automatically (autoreg', 1), here we use parallel processing across animals ('parfor',1); Please check the memory and number of cores for parfor-processing.

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

-let's check whether the "t2.nii" is now in standard-space ("x_t2.nii") and the template ("AVGT.nii") and atlas ("ANO.nii") is back-transformed to native-space ("ix_ANO.nii" & "ix_AVGT.nii") via:

```
dispfiles('flt','^x.*.nii|^ix.*.nii');

FILE x FOLDER

20209925MG_LAERMRT_MGR000025 20209925MG_LAERMRT_MGR000027 counts

ix_ANO.nii + 2/2
ix_ANGT.nii + 2/2
```

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) of 'dti_b100.nii' and save as 'dti b100 1stIMG.nii' for all animals (mdirs):

```
xrename(0,'dti_b100.nii','dti_b100_1stIMG.nii','1','dirs', mdirs); and check existence of file via:
```

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

```
FILE x FOLDER

20200925MG_LAERWRT_MGR000025 20200925MG_LAERWRT_MGR000027 counts

dti_bl00.nii + + 2/2

dti_bl00_stIMG.nii + 2/2

counts 2/2 2/2
```

10) COREGISTER 'dti b100 1stIMG.nii' to 't2.nii'

Now lets coregister the file 'dti_b100_1stIMG.nii' to the 't2.nii'-image:

```
z=[];
z.TASK={ '[2]' };
z.targetImg1={ 't2.nii' };
                                       ;% TARGET-IMAGE
z.sourceImg1={ 'dti_b100_1stIMG.nii' }; ;% SOURCE-IMAGE
z.sourceImgNum1=[1];
                                       ;% IN CASE OF 4D-vol use 1<sup>st</sup> 3d-volume of SOURCE
z.applyImg1= "
                                       ; % TRAFO IS NOT APPLIED TO OTHER Images here
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);
```

```
check existence of file:
```

```
dispfiles('flt','^r3')
```

FILE x FOLDER			
	20200925MG_LAERMRT_MGR000025	20200925MG_LAERMRT_MGR000027	counts
r3c_dti_b100_1stIMG.nii	+	+	2/2
r3c_t2.nii	+	+	2/2
counts	2/2	2/2	

11) TRANSFORM ANOTHER IMAGE TO STANDARD-SPACE

Now, we want to transform the image "r3c_dti_b100_1stIMG.nii" to standard-space (1st arg: 1) for all selected animals (mdirs), using b-spline interpolation (4th arg: 4), with local reorientation estimated from the rough rigid registration step (5th arg: 'local') and that the input-file is located in the animal folder (6th arg: struct('source','intern')). This will create the file "x_r3c_dti_b100_1stIMG.nii": fis=doelastix(1, mdirs,{'r3c_dti_b100_1stIMG.nii'},4,'local',struct('source','intern')); check existence of the file "x_r3c_dti_b100_1stIMG.nii":

12) TRANSFORM ANOTHER IMAGE TO NATIVE-SPACE

In the same way we could also bring the template's hemispheric mask ('AVGThemi.nii') to native space ('ix_AVGThemi.nii'). For transformation to native space the 1st arg is -1, we use NN-interpolation to preserve numbers (4th arg: 0):

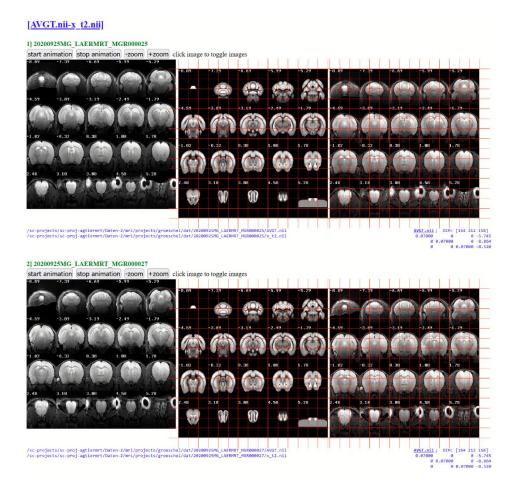
fis2=doelastix(-1, mdirs,{'AVGThemi.nii'},0,'local',struct('source','intern')); check existence of the file 'ix AVGThemi.nii':

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

Next we create an HTML-file to visualize the overlay of the template "AVGT.nii" and "x_t2.nii" (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 copy the checks-folder and visualize the registration with your Web-browser (1^{st} image: animated gif, 2^{nd} image: 'AVGT.nii', 3^{rd} image: 'x_t2.nii')



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

In the same, we can create the overlay of "t2.nii" and "ix_AVGT.nii" and save it as HTML-file. Note, here the slicing is done from the 1st-dimension ('dim': 1) checkreghtml(mdirs,{'ix_AVGT.nii','t2.nii'},fullfile(pwd,'checks'),struct('size',300,'slices','n20','dim',1))

