

# ANT - WARPING MANUAL

## AIM: warp mcao/pt/sham data into Allen space

→ This tutorial treats the ‘32stack’-folder (mostly 32slices)

### Prerequisites

64bit windows, tested with Matlab v2015b & v2016

### Installation

- download and unzip the ANTX-toolbox
- save the toolbox to your preferred drive/path
- open Matlab, in Matlab set current path to the location of ANTX-Toolbox
- type and evaluate „antlink.m“ in command window to temporally add all necessary paths to the matlabpath (these paths are temporally added and will lost after restarting Matlab).
- *Alternatively you can create a hyperlink that occurs each time Matlab is started (select this hyperlink to temporally add all necessary paths of the toolbox). To do this, you can copy the ‘startup.m’ file (located in the ANTX-directory) to the matlab-root-path (in Matlab type: ‘matlabroot’ to obtain your matlab-root-path, see also: <https://de.mathworks.com/help/matlab/ref/startup.html?requestedDomain=de.mathworks.com>). Finally, in this ‘startup.m’-file you have to set your ANTX-path.*

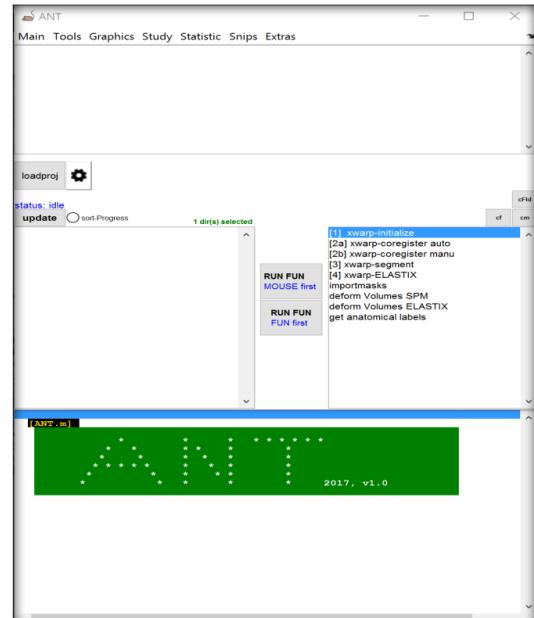
**set matlab’s current path to your mouse data** –use „cd studypath“, where studypath is the current study-folder or navigate to this path via Matlab’s current path (edit field in Matlab’s main window).

-example: suppose data are in the study-folder „O:\TOMsampleData\harms\\_\retest\_stack32“. This folder contains the folder ‘dat’ with subfolders (each subfolder of the ‘dat’-folder represents the data of one mouse) → thus set path to: O:\TOMsampleData\harms\\_\retest\_stack32

**Start ANT** : -type **ant** in Matlab editor to start ANT-GUI

The right figure shows the main panel, containing:

- an upper listbox to display information & parameters
- a left listbox to display all mouse-folders, i.e. subfolders in the ‘dat’-directory, data of each mouse is stored in its own folder
- a right listbox (main warping functions)
- some buttons (with tooltips)
- a menu-bar with functions and instant help if mouse pointer is hovered over a menu-bar-item (function’s help is displayed in the lower help figure)



## Define a project:

First, define a project-file (this has to be done once). This file stores some parameters.

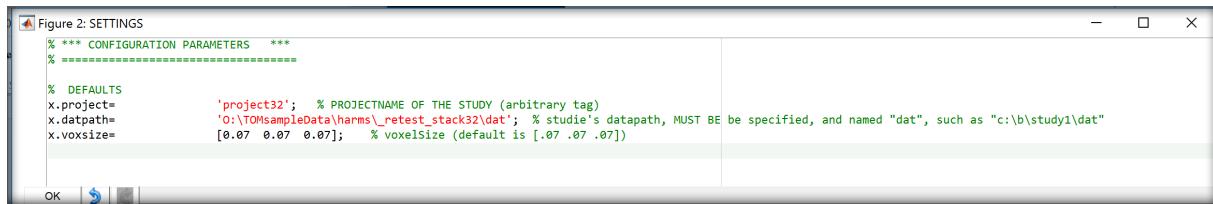
-select **main/new project** to define a new project.

- In the opening gui define the following paramters and delete (!) all other parameters:

```
x.project=      NEW PROJECT'; % PROJECTNAME OF THE STUDY (arbitrary tag)
x.datpath=      '<MANDATORY TO FILL>'; % studie's datapath, MUST BE specified,
x.voxsize=      [0.07 0.07 0.07]; % voxelSize (default is [.07 .07 .07])
```

where

- **x.project** is just an arbitrary name for the study
- **x.datpath** is the fullpath-link to the ‘dat’-folder: Either copy and paste the full path here (...\\dat) or use the icon on the left side: For this, move the cursor to line of **x.datpath**. If so, an icon will pop up on the left side. Select the icon and in the subsequent opening GUI select the data-path.  
→ in this example the following path was chosen: O:\\TOMsampleData\\harms\\\_retest\_stack32\\dat
- **x.voxsize**: define the aimed voxel-size for normalization and image warping (→ in this example the voxel-size is [0.07 x 0.07 x 0.07])
- Don’t forget to delete all other parameters. This ensures a dynamical path-setting of templates and other resources.

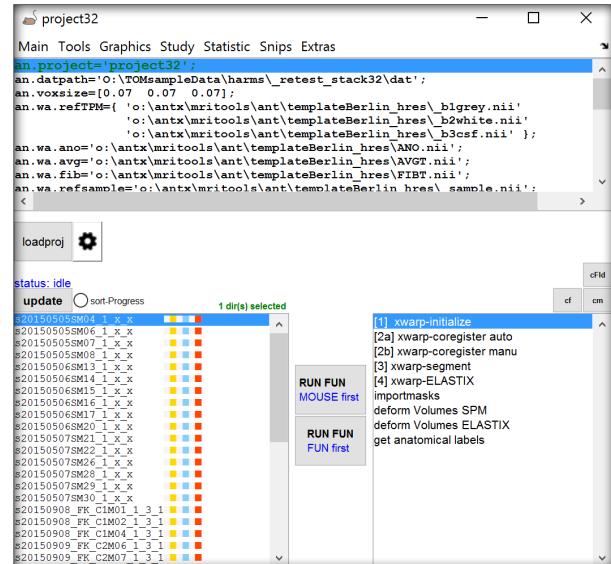


-click **[OK]**, and save the project in the study-folder (→ here folder

„O:\\TOMsampleData\\harms\\\_retest\_stack32, and give the project-file a name, (→ here: “project32”), >> then select “**YES**” in the next dialog to instantly load this new project-file (if “**NO**” was chosen, you have to load the project manually → see **load a project**).

**Load a project:** Once a project-file was created, you can load the project by selecting the [**loadproj**]-button located on the left side of the ANT-window.

Now the main ANT-window might look similar to the right figure: All mouse folder are displayed in the left panel (data of each mouse is stored in a separate folder in the “..\\dat\\”-folder). Here each item in the left listbox is simply the name of the mouse-folder with some additional suffixes (.from Bruker-import). Additionally, each folder has a color-coded bar. The color-coded bar, gives information over the progress of each mice (e.g. whether the t2.nii image exists at all (yellow) or whether data reorientation, segmentation or normalization has been performed).



→ Here the entire analysis was performed for all mice. However, we kept only the following files: The initial structural image (‘t2.nii’), the skull-stripped brain (‘\_msk.nii’) if exists, the lesion mask (‘masklesion.nii’), if exists, the hemispheric mask (‘hemi.nii’), the tissue compartments (GM, WM, CSF) after segmentation (‘c1t2.nii’, ‘c2t2.nii’, ‘c3t2.nii’) and the images after normalization into the Allen space (‘x\_t2.nii’, ‘x\_msk.nii’,

and if exists, ‘x\_masklesion.nii’ and ‘x\_hemi.nii’) and the jacobian determinant (‘JD.nii’). The color-coding shows that the following data exist: t2.nii (yellow), tissue compartments (blue) and the warped x\_t2.nii image (red).

You can hover over mouse folders to get more detailed information for each mouse. Alternatively use the right context-menu to open and inspect the selected folder(s).



..hovering mouse over mouse folder listbox

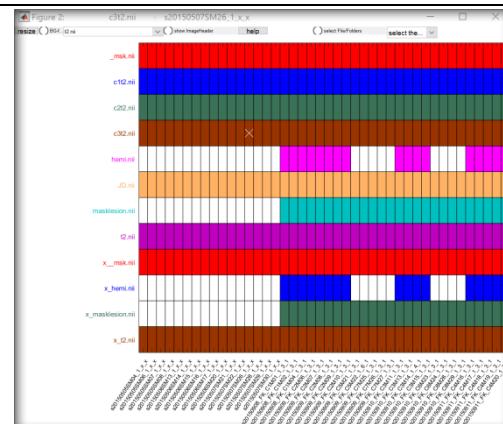


Context menu

### Sanity check:

To inspect the data of the mouse-folders select one/more/all mouse folders in the left mouse-folder listbox of the ANT window. You can also use the context menu of the mouse folder listbox and chose „**select all folders**“. In the main menu go to ‘[graphics/show case-file-matrix](#)’.

This matrix shows all selected mouse folders and the containing nifti-images. You can hover over each cell to see the name of the nifty-image and the respective mouse-folder (displayed in the figure title). You can also display and overlay two images here, or preselect mouse-folders, for instance to select mouse-folders with pending normalization (because the respective cells are ‘white’, i.e. do not exist in the folder) → see [[help](#)]-button



→ Here you can see that not all mouse-folders contain the ‘masklesion.nii’-file (sham group). Moreover, only a few folders (mice with MCAO protocol) contain the images ‘hemi.nii’ and ‘masklesion.nii’ and their warped complements (‘x\_hemi.nii’ and ‘x\_masklesion.nii’). However, all folders contain the structural image (‘t2.nii’), the tissue maps (‘c1t2.nii’, ‘c2t2.nii’ and ‘c3t2.nii’... equivalent to GM, WM and CSF, respectively), the Jacobian determinant (‘JD.nii’) and the warped structural image (‘x\_t2.nii’).

### **Data Processing-Step1: NORMALIZE DATA into ALLEN SPACE**

Select one/more or all mouse folders from the [[left mouse-folder-listbox](#)] of the ANT-window. Note that each mouse needs approx. 10-15 min to ‘run through the pipeline’. Thus, for now, it would be better to select one or two mice only.

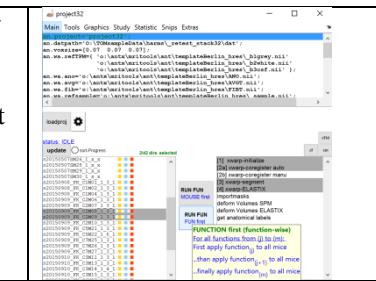
From the [[right functions listbox](#)] of the ANT-window select the following items (use [ctrl]-key for multiple selections): **[1] xwarp-initialize, [2a] xwarp-coregister auto, [3] xwarp-segment, [4] xwarp-Elastix**.

Select all this functions	description
[1] xwarp-initialize	-this step creates a “templates” folder with all necessary template files in the voxel-resolution defined in the project-file (this step is done only once!) -the template-folder is located in the study folder -then all necessary templates are copied in the currently processed mouse-folder
[2a] xwarp-coregister auto	-this step make an automatic affine coregistration of the structural image (t2.nii) of the currently processed mouse and the template image using ELASTIX → if this step fails, the [2b] xwarp-coregister manu should be used (see [ <a href="#">help</a> ]) For ELASTIX see: <a href="http://elastix.isi.uu.nl">http://elastix.isi.uu.nl</a>
[3] xwarp-segment	-this step uses the SPM’s unified approach together with the SPMMOUSE

	<p>toolbox and some outperforming functions from the Universitätsklinikum Freiburg to estimate the tissue compartment maps and to obtain the nonlinear transformation field</p> <p>see also:</p> <p>SPM: <a href="http://www.fil.ion.ucl.ac.uk/spm">http://www.fil.ion.ucl.ac.uk/spm</a>      SPMMOUSE: <a href="http://www.spmmouse.org">www.spmmouse.org</a>      FREIBURG: Universitätsklinikum Freiburg, Medizin Physik  <a href="https://www.uniklinik-freiburg.de/mr-en/research-groups/difffperf/fibertools.html">https://www.uniklinik-freiburg.de/mr-en/research-groups/difffperf/fibertools.html</a></p>
[4] xwarp-Elastix	<p>-this step performs the nonlinear transformation into and from Allen Space using ELASTIX and creates a transformation recipe for other images</p> <p>see: <a href="http://elastix.isi.uu.nl">http://elastix.isi.uu.nl</a></p>

→ in this example two mouse-folders were selected in the left mouse-folder listbox. The functions [1]..,[2a]..,[3].. and [4] were selected

-Next, hit **[RUN FUN, Fun first]-button** from the ANT main window to start the pipeline **and have a large cup of coffee.**



**-NOTE:** For this steps you may alternatively hit the **[RUN FUN, Mouse first]-button** to use parallel computing (prerequisite: you have to purchase Matlab's Parallel Computing Toolbox).

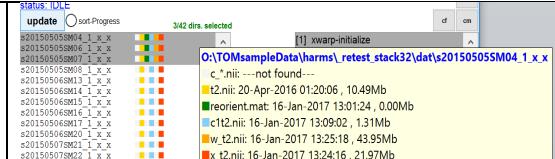
...

- if the [**IDLE**]-message in the ANT window appears again, the normalization should be done.

-click the **[update]**-button.. to update the ‘progress’-display of the mouse-folder listbox  
 → here for example, data from 3 mice were transformed into Allen space.

The color-coded bars indicate that additional files were created:

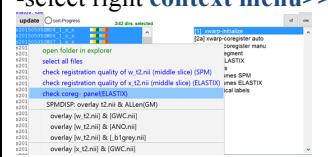
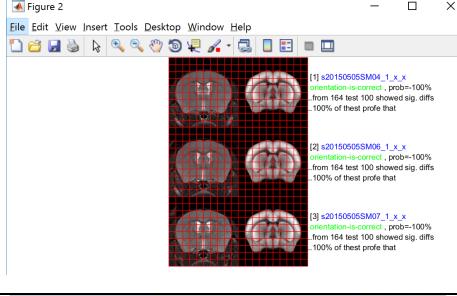
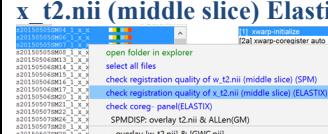
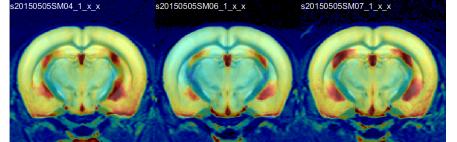
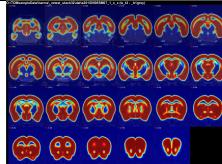
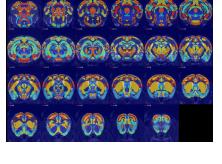
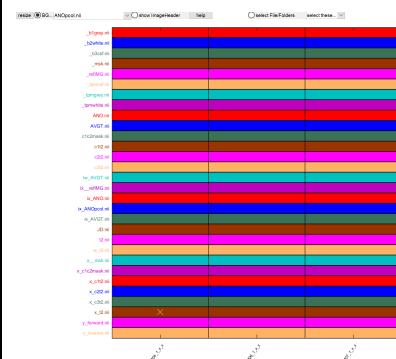
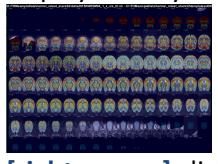
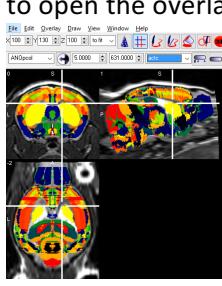
- (1): ‘reorient.mat’ (green color), that stores the affine transformation from original ‘t2.nii’ orientation to the template’s-orientation,
- (2) ‘w\_t2.nii’ (orange color), the normalized ‘t2.nii’ image using SPM
- (3) ‘x\_t2.nii’ (red color), the normalized ‘t2.nii’ image using ELASTIX



## Check Normalization

There exist several options to check the normalization. Here are some options and the resulting output:

Visual check of normalization	output
<b>CONTOUR: [x_t2.nii] &amp; ‘template GM’</b> -select the mouse-folders in ANT-mouse folder listbox Then hit <b>[w]-keyboard key</b> in the ant-mouse folder listbox →this shows several slices of the warped structural image (x_t2.nii) of each mouse-folder with the <b>contour</b> overlay of the templates gray-matter image (red)	

<p><b>SIDE-BY-SIDE [x_t2.nii] &amp; ‘template GM’</b></p> <ul style="list-style-type: none"> <li>-select the mouse-folders in ANT-mouse folder listbox</li> <li>-select right <b>context menu</b>&gt;&gt;<b>check coreg-panel Elastix</b></li> </ul>  <p>→ shows ‘x_t2.nii’ and the template’s GM map (middle-slice) with overlaid grid</p>	<p><b>Figure 2</b></p> 
<p><b>OVERLAY: [x_t2.nii] &amp; [AVGT.nii]</b></p> <ul style="list-style-type: none"> <li>-select the mouse-folders in ANT-mouse folder listbox</li> <li>-select right <b>context menu</b>&gt;&gt;<b>check registration quality of x_t2.nii (middle slice) Elastix</b></li> </ul>  <p>→ shows the overlay of ‘x_t2.nii’ and the templates “structural image” (avgt.nii) → hit <b>[h]-key</b> to get a list of keyboard shortcuts to: toggle between background and foreground image, change the color map, alpha transparency)</p>	
<p><b>OVERLAY: [x_t2.nii] &amp; ‘template GM’</b></p> <ul style="list-style-type: none"> <li>-select <b>one/more</b> mouse-folder in ANT-mouse folder listbox</li> <li>-select right <b>context menu</b>&gt;&gt;<b>overlay [x_t2.nii]&amp; [_b1gray.nii]</b></li> </ul> <p>→ to display the overlay of x_t2.nii and the template’s gray matter image → hit <b>[h]-key</b> to get a list of keyboard shortcuts to: toggle between background and foreground image, change the color map, alpha transparency)</p>	
<p><b>OVERLAY: [x_t2.nii] &amp; [‘ANO.nii’]</b></p> <ul style="list-style-type: none"> <li>-select <b>one/more</b> mouse-folder in ANT-mouse folder listbox</li> <li>-select right <b>context menu</b>&gt;&gt;<b>overlay [x_t2.nii]&amp; [ANO.nii]</b></li> </ul> <p>→ to display the overlay of x_t2.nii and the annotation/anatomical labelling image → hit <b>[h]-key</b> to get a list of keyboard shortcuts to: toggle between background and foreground image, change the color map, alpha transparency)</p>	
<p><b>CASE-FILE-MATRIX</b></p> <ul style="list-style-type: none"> <li>-select <b>one/more</b> mouse-folder in ANT-mouse folder listbox</li> <li>-go to <b>graphics/show case-file-matrix</b> of the ANT menu bar</li> <li>-select [ANOpcol.nii] from the left pull-down menu, activate [BG]-radiobutton to swap fore- and background, than [<b>left click</b>] onto one of the ‘x_t2.nii’-cells (white cross in snapshot below)</li> </ul>  <p>→ you can inspect other images in the same way.</p>	<p><b>[left mouse]</b> click onto ‘x_t2.nii’ to display overlay</p> 
	<p><b>[right mouse]</b> click onto ‘x_t2.nii’: to open the overlay with <b>MRICRON</b></p>  <p><b>[left mouse]</b> double-click to open the mouse-folder in windows explorer, and additionally selects the ‘x_t2.nii’-file, ready to be</p>

	<p><b>displayed with other viewing tools</b></p>
<p><b>gui-overlay - overlays with some adjustable parameters</b>  - select one/more mouse-folder in ANT-mouse folder listbox  - go to <b>graphics/gui-overlay-image</b> of the ANT menu bar  → the opening GUI allows <b>more parameters</b> to be set  - for now click <b>[plot]</b> to show the templates labelling image (pseudocolor: thus “ANOpcol.nii”) overlaid onto ‘x_t2.nii’  → Note, this GUI remains open: This allow to select other mouse-folder(s) and to display overlays with the same or modified parameter setting</p>	
<p><b>gui-overlay2- overlays with more adjustable parameters</b>  - select one/more mouse-folder in ANT-mouse folder listbox  - go to <b>graphics/gui-overlay-image2</b> of the ANT menu bar  → This function allows setting more displaying parameters. The function opens two windows (file-selection and parameter settings). In the left panel you can pick one or two images from a list (with images from the template folder and images found in the selected mouse folders).  → here we select ‘x_t2.nii’ and ‘ANOpcol.nii’  Than select <b>[x.images]</b> in the <b>[OVERLAY]</b> window, then click on the left icon (<b>green triangle</b>) to use the selected files from the file-selection window. Next, go to <b>[x.imshow]</b> and set this parmeter to 1. This option swaps background and foreground images (‘x_t2.nii’ should be the ‘bg’-image).  <i>Alternatively you can also select this parameter and click on the left icon [B] x.imshow= [1]; or press [F1] to change this parameter (toggle between 0 &amp; 1; this variable is of type Boolean)</i>  → hit <b>[plot]</b> to display the overlay (or press <b>[F3]</b>)</p>	<p>→ note that the two gui-windows remain open: This allow to select/unselect other mouse-folder(s) and to plot with the same parameter settings or to change parameters</p>

## DATA PROCESSING-STEP 2: WARPING OTHER IMAGES TO ALLEN SPACE

**AIM:** Warping of other images to Allen space based on an existing transformation ‘rule’.

**NOTE:** The transformation ‘rule’ for forward and backward direction has to be calculated in advance and only once using the ‘x\_t2.nii’-image. This step was performed **in the data processing step-1** (see above section). However, data-processing step-2 can be also used to warp images from Allen space (e.g. template images or the anatomical atlas) to the native mouse space as well.

-select the mouse folders in the ANT mouse folder-listbox. Note that only images from selected mouse-folders will be transformed. Than select **[deform Volumes Elastix]** from the right warping-listbox, than hit **[RUN FUN, Fun first]**-button from the ANT main window.

- This will open another GUI (**deform files**) -hit [**fileStr**]-button to recursively find all ‘unique’ nifti-files within the selected mouse-folders

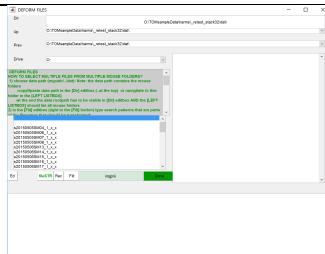


Figure 2: SELECTOR: use contextmenus for selectionMode

This will open the a window with all recursively found ‘unique’ nifti-files of the selected mouse-folders (The GUI also displays the number of found files across selected mouse-folders. *Example: In our case 3 mouse-folders were selected, thus the ‘t2.nii’ file should be found 3-times*

- here you can select one or more images that should be transformed

→ In this example, we want to transform only one image (across selected mouse folders), the ‘\_msk.nii’-file

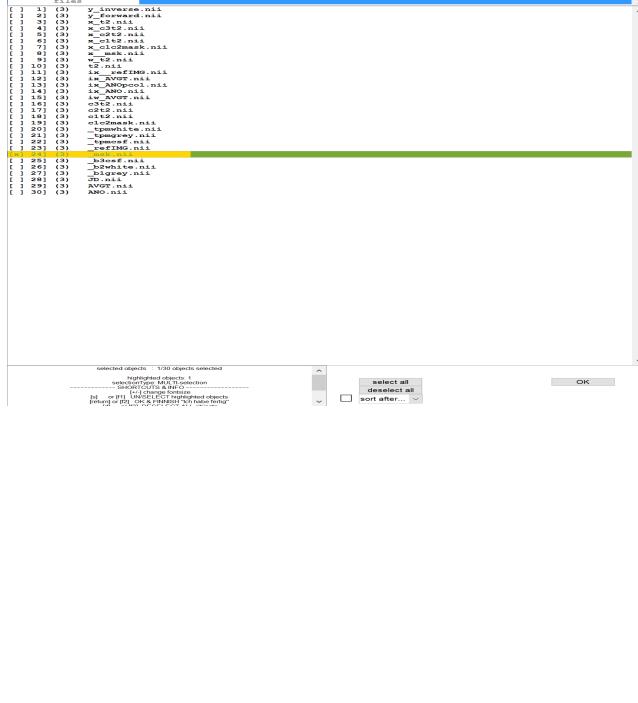
→ then click [**OK**]

<<note: several different images can be selected here: For example select all ‘c1t2.nii’, ‘c2t2.nii’, ‘c3t2.nii’, ‘masklesion.nii’ and ‘hemi.nii’ to warp all these images into Allen space in one step.

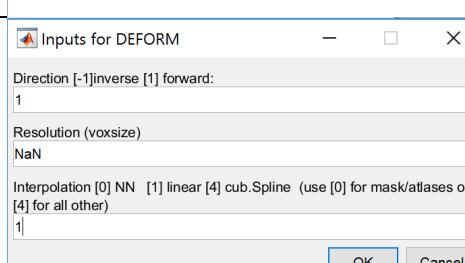
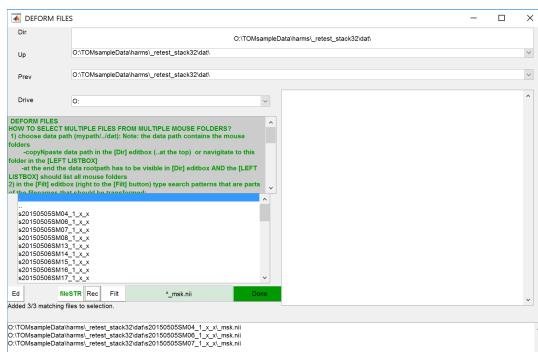
→ this function delivers only a search filter. In the [**deform files**]-window hit [**rec**] to recursively find files with a matching search filter in the selected folders. The found files will be depicted in the lower panel.

→ Optionally, single files can be deselected. For this, click on the respective files in the lower panel.

→ finally click [**DONE**]



selected objects: 103 objects selected  
highlighted objects: 3  
unselected objects: 100  
selected by ID: 103 highlighted objects  
selected by name: 103 highlighted objects  
selected by path: 103 highlighted objects  
selected by type: 103 highlighted objects



- In the final GUI you have to indicate:
    - the **direction** of transformation (to Allen space: 1),
    - the resulting **voxel-size** (here determined by the templates: nan is always fine) and
    - the **interpolation method** (for now set to next-neighbour interpolation: 0)
- finally click [**OK**]

-In this way you can transform multiple images to and from the Allen-Space. Importantly, several different images and mouse-folders can be used in one run. However, the direction (to or from Allen space) and the resolution and interpolation parameter should be the same!

-For visualization of the warped images, see **Check Normalization** section.