# Quality assessment of spatial transformation of locus coeruleus (LC) functional and structural magnetic resonance imaging (MRI) data

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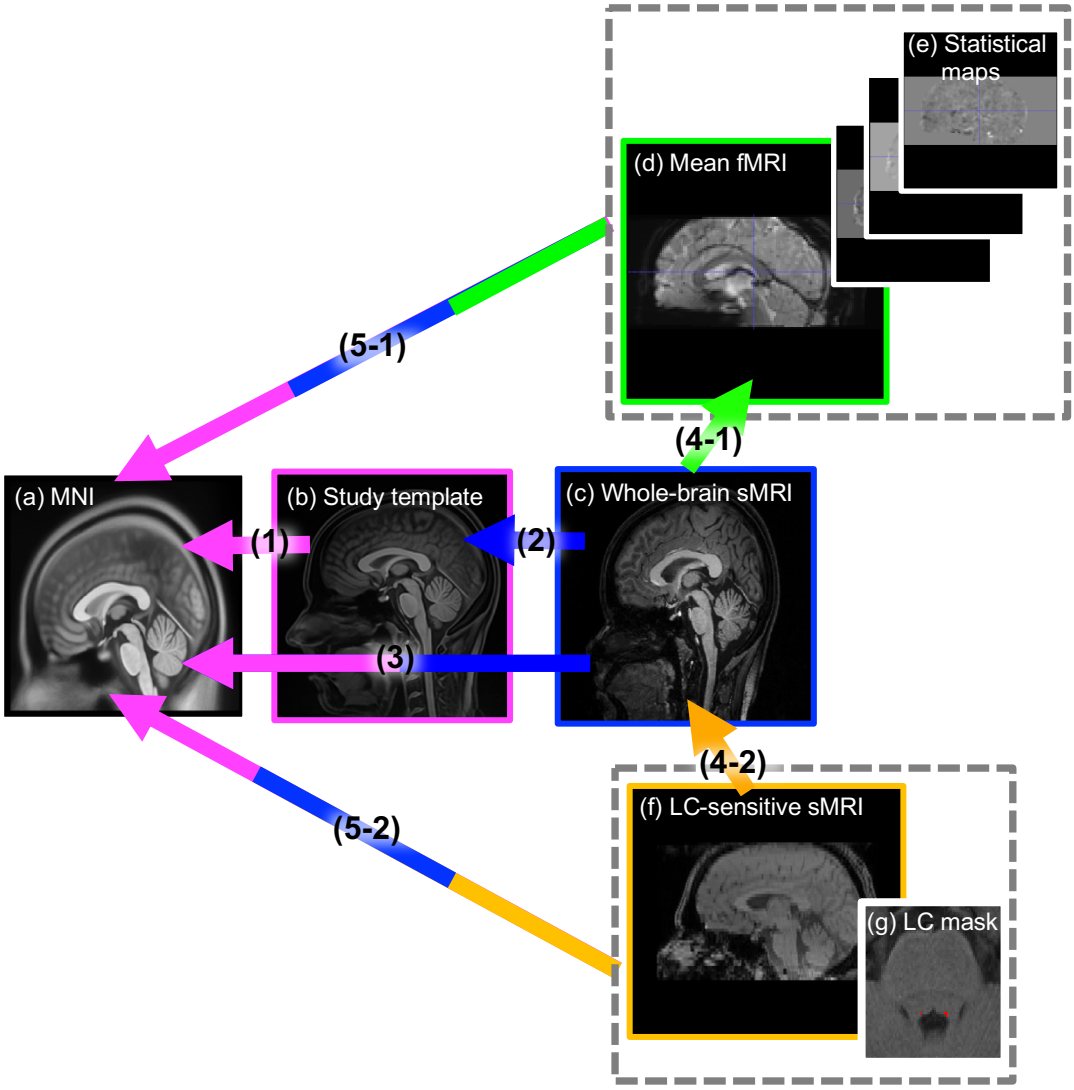
# 1. Introduction

## a. Summary of spatial transformation procedure




Previous to the landmark-based spatial transformation quality assessment, a series of transformations were executed on the mean functional images (d), whole-brain structural images (c), neuromelanin-sensitive structural images (f), and LC segmentations (g). As explained in detail in the section 2.5 of the main text, single-subject functional and structural images in native space (c-g) were transformed into MNI space (a). The code for the spatial transformation procedure described above is in the section 3.b of this manual.

### b. Overview of landmark assessment procedure

After all subject datasets have been processed, the quality of the transformation was examined. This examination was done by selecting the landmarks in the MNI space, drawing them on the individual transformed mean functional images, and calculating Euclidean distances between the MNI-defined landmarks and their single-subject counterparts. Additionally, prior to the landmark assessment, a video that takes single-subject transformed functional or structural images per frame can be made and inspected to quickly identify abnormal registration. More detailed procedures will be explained in the walkthrough section.

### c. The background and the goal of this procedure

The LC is so small that even a slight spatial deviation of a voxel size could significantly reduce the statistical power of group-level analyses. For more robust functional assessment of such structure, it is not only important to register and normalise each single subject functional image properly onto the group space, but also to control the quality of the transformed images and rectify any problems that arose from the spatial transformation. With the quality assessment protocol that are introduced in detail below, we aim to achieve more reliable and powerful voxel-wise analyses of the functional LC imaging data than existing approaches in the field.

## 2. Protocol

### a. Preparation

#### i. Required data

The files that are needed for the assessment are:

- the MNI space to which all images are transformed
- transformed mean functional images of each subject

The MNI space can also be a group space depending on the analysis that will be done with the transformed functional data. In the example dataset, an MNI space created by Fonov et al (2011) was used.

### ii. Required software

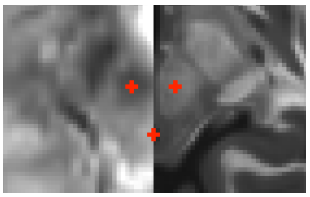
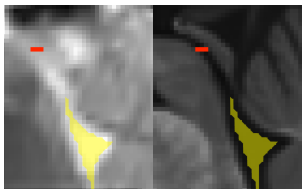
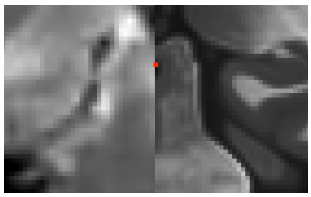
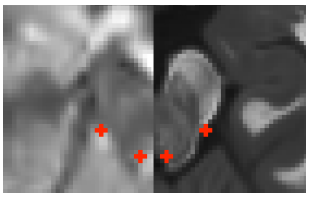
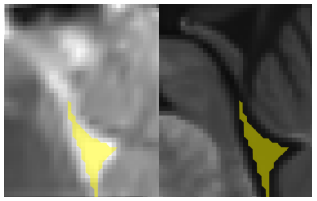
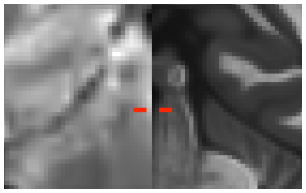
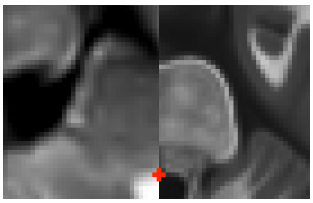
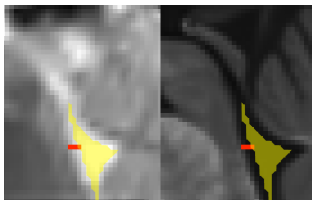
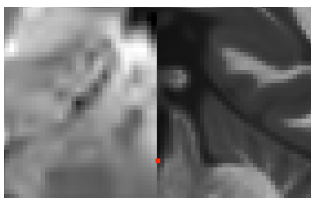
To draw the landmarks, we will use:

- ITK-SNAP (version later than 3.6.0-RC1; http://www.itksnap.org), or any segmentation-enabled brain image viewers
- MATLAB (version later than 2013b, Mathworks, Sherborn, MA, USA), or any computational programmes that can read Nifti images

The example MATLAB codes for generating the overlay video and statistical assessment is in section 4.

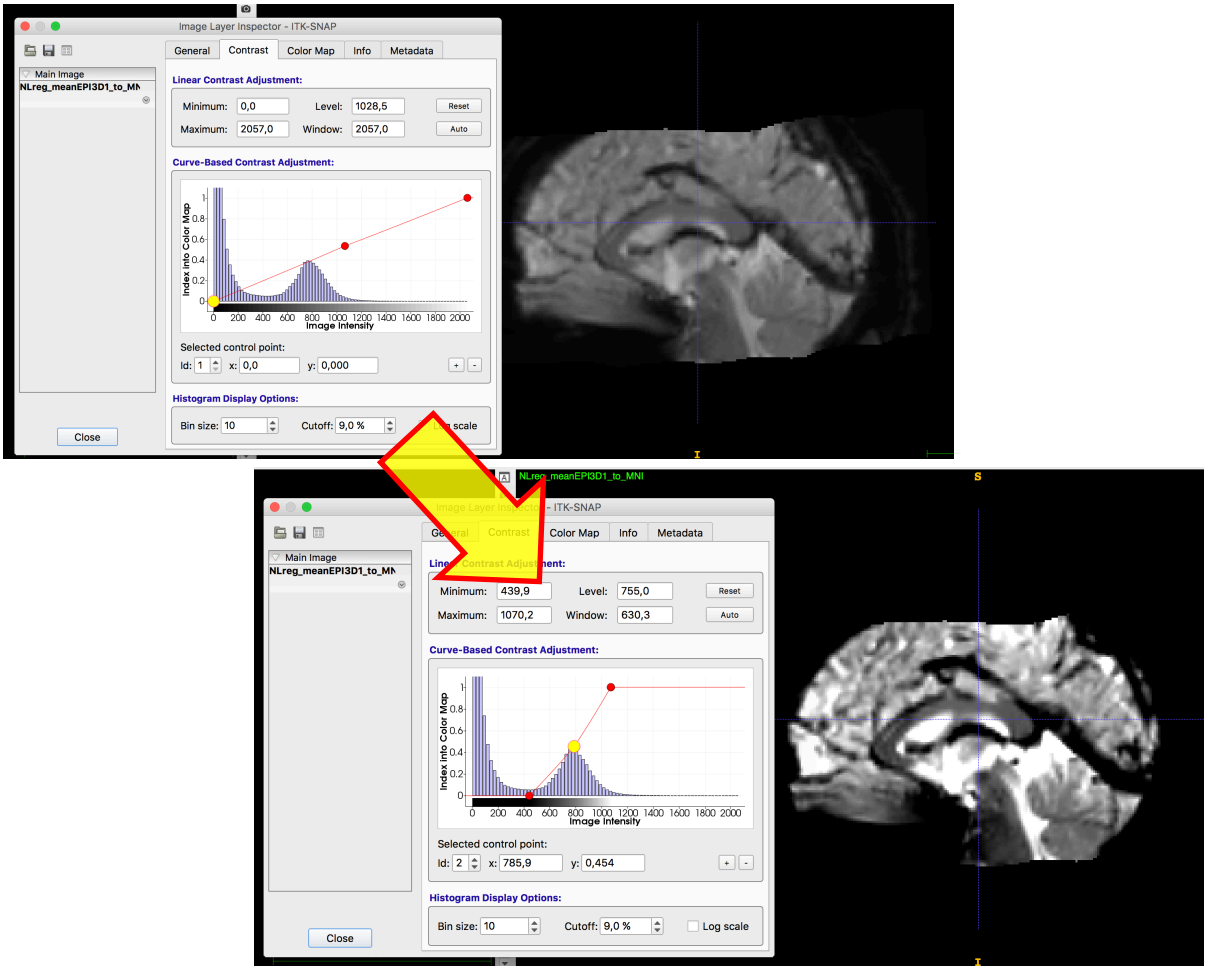
## b. Procedure

### i. Selecting landmarks

| | Axial view | Sagittal view | Coronal view |
|---|---|---|---|
| **Top of the brainstem and Nuclei Ruber (slice 71)** |  |  |  |
| **LC and the brainstem outline (slice 60)** |  |  |  |
| **Lower posterior border of brainstem (slice 50)** |  |  |  |

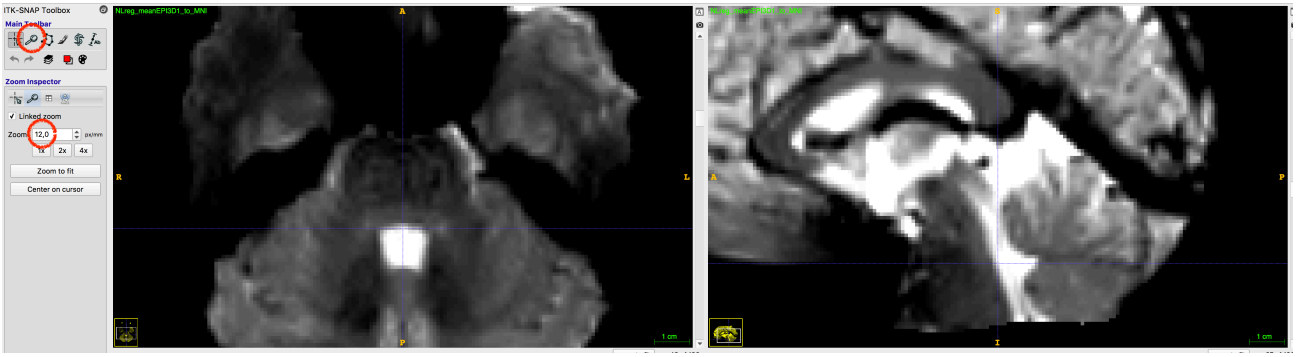
Selecting an appropriate set of landmarks depends on the region of interest (ROI). In this manual's ROI, LC, is located in the brainstem near the lateral floor of the fourth ventricle. Therefore, selecting landmarks in the upper brainstem that delineate the bounds of LC location is most helpful for the evaluation process. In addition, the landmarks should be clearly visible

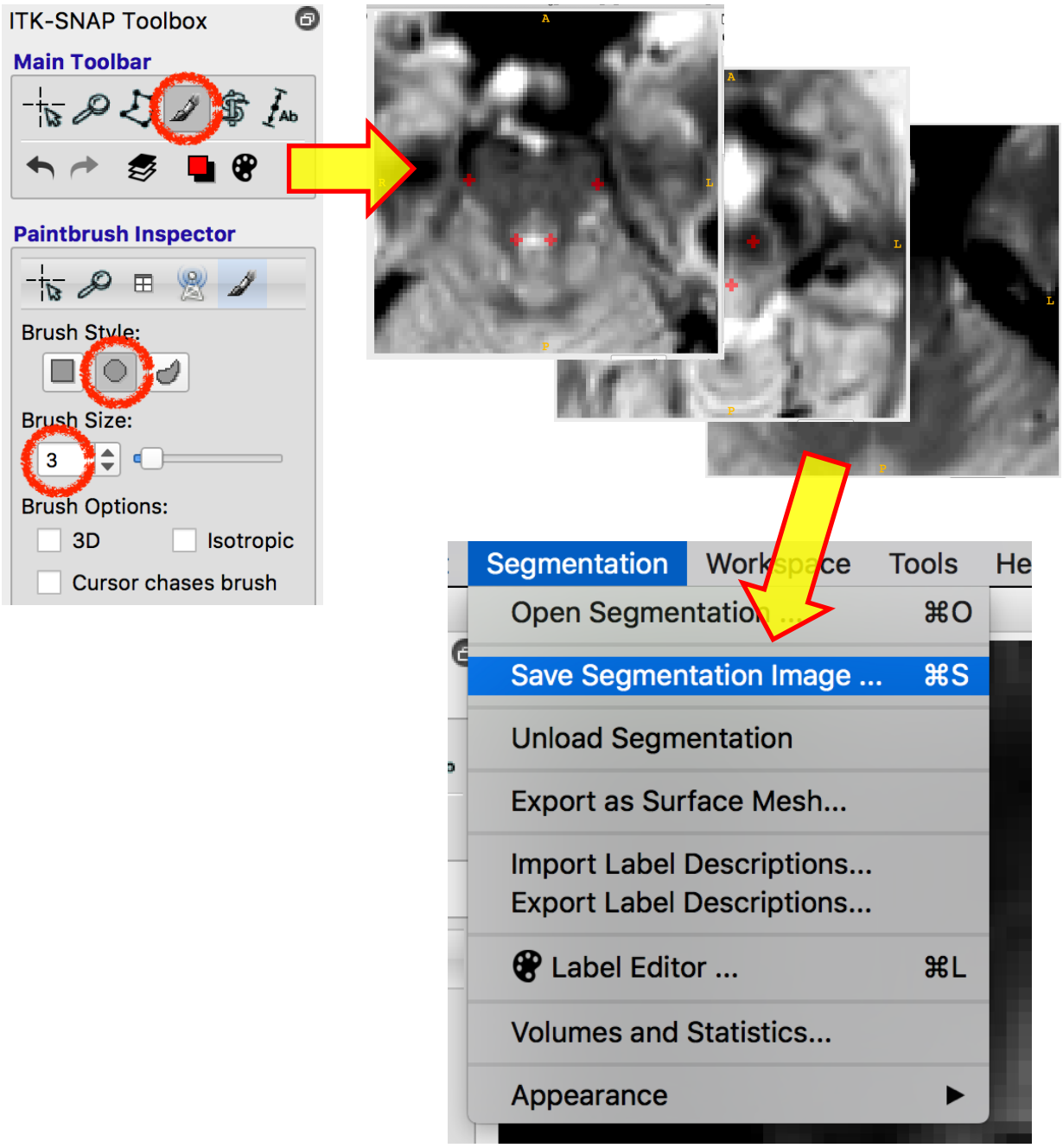
and anatomically distinguishable compared to the surrounding structures on the mean functional images, as these landmarks are drawn manually through visual inspection. Based on these criteria, the landmarks specified in the table above were selected in the main text. The top of the brainstem and nuclei Ruber were selected as a landmark to indicate the upper bound of the larger ROI area of the study, the brainstem. Also, Nucleus Ruber is distinctly visible in the functional images, which makes it an ideal candidate for a landmark. The LC and the outline of the brainstem was selected to delineate the bounds of LC activation. Lastly, the lower posterior border of brainstem was selected to indicate the lowest bound of possible LC location. Once the landmarks are selected, evaluators should familiarise themselves with them.



### ii. Drawing landmarks

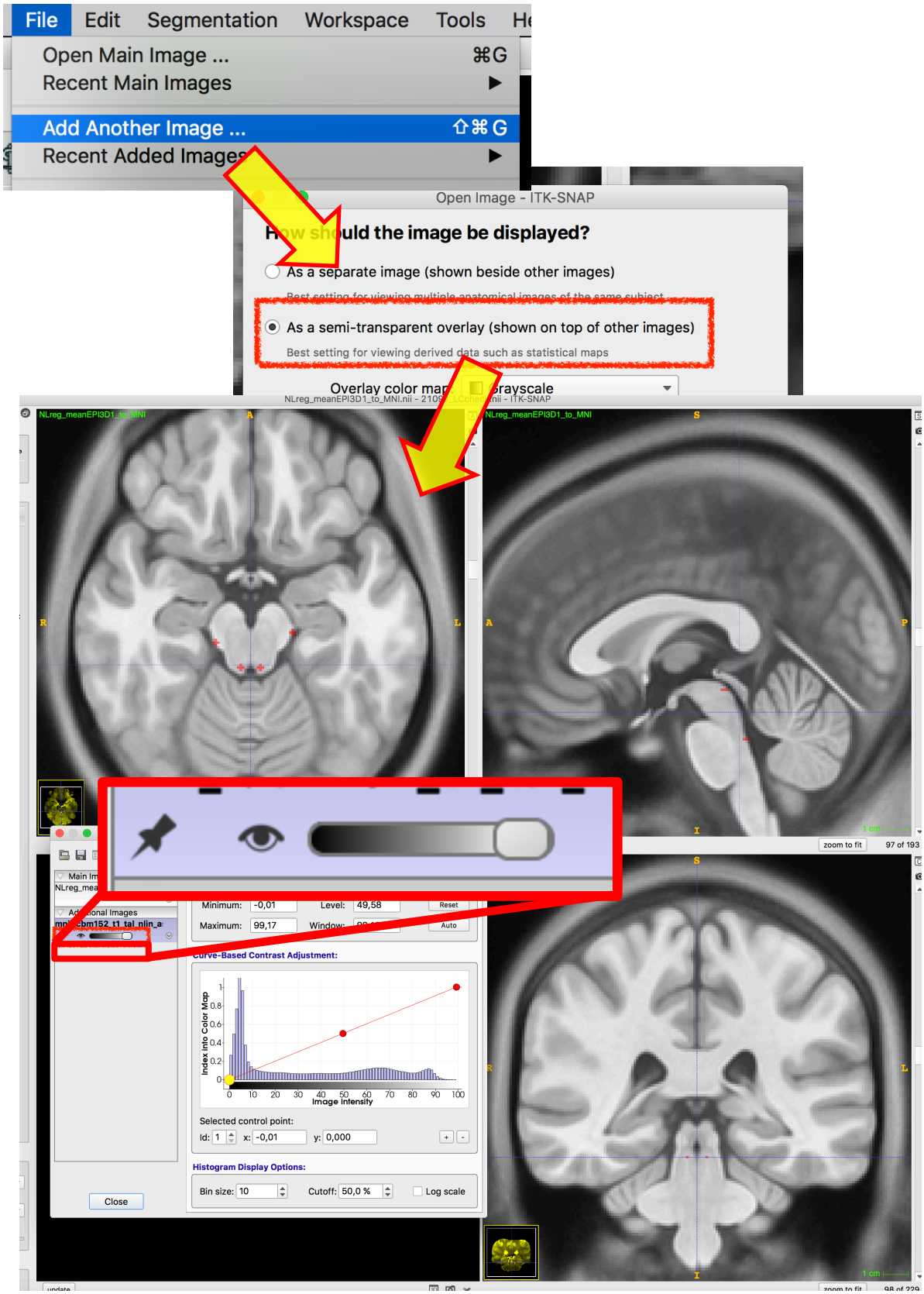
Next, evaluators inspect one of the transformed single-subject mean functional images. If necessary, adjust the contrast slider so that the fourth ventricle is as bright and distinguished as it can be. After adjusting the contrast of the image, magnify the image that you can draw the landmarks more precisely.






Then, using the size-3 round brush segmentation tool, place the landmarks on the transformed mean functional images in axial view. However, use the anatomical clues from other views to aid the landmark placement. For example, when drawing lower posterior border of the brainstem, the deepest groove of the fourth ventricle can be a guiding structure. When the assessment of the single-subject image is complete, save the landmarks as a segmentation image.

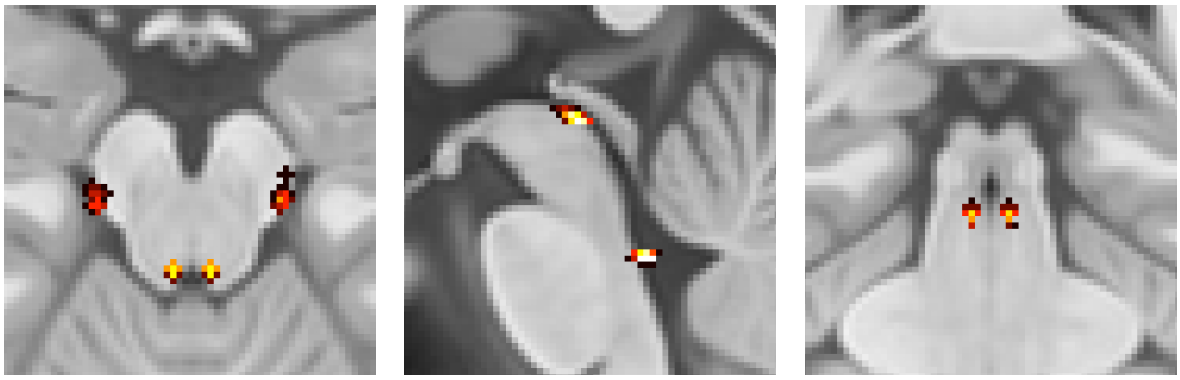
Optionally, after saving the image, evaluators could open the group space image used for transforming the mean functional images as a semi-transparent overlay. Once the image is overlaid, adjust the transparency setting to examine whether the landmark segmentations



are in the right place. Take note of the subject ID if the landmarks were badly placed. However, DO NOT change the landmark image you already saved, as it indicates the quality of the functional image transformation.

### iii. Aggregating single-subject landmarks for preliminary inspection

When the assessment of the whole dataset is complete, visual inspection of all landmarks aggregated in a same space is performed by executing the custom code from section 4 of this manual. By performing this step together with the frame-by-frame video of transformed images (the code to generate the video is in section 3-c, an example of this video is in the section 2.4 of the supplementary material), outliers are easily identified.



### iv. Statistical quality assessment of aggregated landmarks of transformed functional images

Once all faulty transformation issues are addressed and rectified, statistical analyses of the quality assessment is performed to quantify the transformation precision. An example code for the analyses is in the section 3.d. Through this step, the spatial transformation can be further refined. The detailed background and example results are described in the main text.

### v. Statistical quality assessment of transformed structural images

Additionally, the spatial transformation quality of the structural images can be assessed using the transformed LC segmentations. As specified in the section 2.5 of the main text and the section 1.a of this manual, the LC segmentations are transformed onto the MNI space using the transformation matrices and the deformation fields generated from warping neuromelanin-sensitive structural image (f) to the MNI space (step 5-2). The quantification of the assessment can be done by calculating Euclidean distance between the slice-wise centroids of the template LC mask in the MNI space, e.g. the meta LC mask by Dahl et al.

(2021) in this manual's case, and the transformed LC segmentations of each subject. An example code for the calculating the distance is in the section 3.e.

# 3. Codes

### a. The spatial transformation pipeline (a shell script)

```
#!/bin/bash

# subject ID
ID=1001

# set up folders and group space images
folder=/mnt/work/yyi/temp/ED_coreg/"${ID}"/
MNI=/mnt/work/yyi/temp/ED_coreg/mni_icbm152_t1_tal_nlin_asym_09c.nii
template=/mnt/work/yyi/temp/ED_coreg/pilot_template.nii.gz

# LC segmentation
mask=$(ls -t "${folder}"/data/LCmask_"${ID}".nii.gz)

# ---------- prepare images for trasnformation ---------- #

# bias field correct T1 and EPI
N4BiasFieldCorrection -d 3 -v 1 -r 0 -i "${folder}"data/T1mean.nii -o
"${folder}"data/T1mean_corrected.nii -s 2 -c [200x150x100x50,1e-6] -b 200
N4BiasFieldCorrection -d 3 -v 1 -r 0 -i "${folder}"data/meanEPI.nii -o
"${folder}"data/meanEPI_corrected.nii -s 2 -c [200x150x100x50,1e-6] -b 200

# make an EPI mask (FSL)
/usr/local/fsl/bin/bet "${folder}"data/meanEPI_corrected.nii "${folder}"data/meanEPI_brain -f
0.5 -g 0 -n -m

# resample t1slab to T1 resolution (FreeSurfer)
mri_convert -cs 1 -odt float -rl "${folder}"data/T1mean.nii -rt cubic "${folder}"data/t1slab.nii
"${folder}"data/t1slab_1mm.nii

# ---------------------------------------------------- #
```

```
# ---------- start the transformation ---------- #

# study template -> MNI
antsRegistrationSyN.sh -d 3 -t s -f "${MNI}" -m "${template}" -o
"${folder}"NLreg_template_to_MNI_

# T1 -> study template
antsRegistrationSyN.sh -d 3 -t s -f "${template}" -m "${folder}"data/T1mean_corrected.nii -o
"${folder}"data/NLreg_T1mean_to_template_

# T1 -> MNI
antsApplyTransforms -d 3 -v 1 -n BSpline[4] -t
"${folder}"NLreg_template_to_MNI_1Warp.nii.gz -t
"${folder}"NLreg_template_to_MNI_0GenericAffine.mat -t
"${folder}"data/NLreg_T1mean_to_template_1Warp.nii.gz -t
"${folder}"data/NLreg_T1mean_to_template_0GenericAffine.mat -i
"${folder}"data/T1mean_corrected.nii -r "${MNI}" -o
"${folder}"data/NLreg_T1mean_to_MNI.nii

# T1 -> EPI
antsRegistrationSyN.sh -d 3 -t r -m "${folder}"data/T1mean_corrected.nii -f
"${folder}"data/meanEPI_corrected.nii -x "${folder}"data/meanEPI_brain_mask.nii.gz -o
"${folder}"data/coreg_T1mean_to_meanEPI_

# t1slab(resampled) -> T1
antsRegistrationSyN.sh -d 3 -t r -m "${folder}"data/t1slab_1mm.nii -f
"${folder}"data/T1mean_corrected.nii -o "${folder}"data/coreg_t1slab_to_T1mean_

# T1 -> MNI
antsApplyTransforms -d 3 -v 1 -n BSpline[4] -t
"${folder}"NLreg_template_to_MNI_1Warp.nii.gz -t
"${folder}"NLreg_template_to_MNI_0GenericAffine.mat -t
"${folder}"data/NLreg_T1mean_to_template_1Warp.nii.gz -t
"${folder}"data/NLreg_T1mean_to_template_0GenericAffine.mat -i
"${folder}"data/T1mean_corrected.nii -r "${MNI}" -o
"${folder}"data/NLreg_T1mean_to_MNI.nii
```

```
# EPI -> MNI
antsApplyTransforms -d 3 -v 1 -n BSpline[4] -t
"${folder}"NLreg_template_to_MNI_1Warp.nii.gz -t
"${folder}"NLreg_template_to_MNI_0GenericAffine.mat -t
"${folder}"data/NLreg_T1mean_to_template_1Warp.nii.gz -t
"${folder}"data/NLreg_T1mean_to_template_0GenericAffine.mat -t
["${folder}"data/coreg_T1mean_to_meanEPI_0GenericAffine.mat, 1] -i
"${folder}"data/meanEPI_corrected.nii -r "${MNI}" -o
"${folder}"data/NLreg_meanEPI_to_MNI.nii

# LC segmentation -> MNI
antsApplyTransforms -d 3 -v 0 -n NearestNeighbor -t
"${folder}"NLreg_template_to_MNI_1Warp.nii.gz -t
"${folder}"/NLreg_template_to_MNI_0GenericAffine.mat -t
"${folder}"/data/NLreg_T1mean_to_template_1Warp.nii.gz -t
"${folder}"/data/NLreg_T1mean_to_template_0GenericAffine.mat -t
"${folder}"/data/coreg_t1slab_to_T1mean_0GenericAffine.mat -i "${mask}" -r "${MNI}" -o
"${folder}"/data/NLreg_LCmask_to_MNI.nii

# 1st-level stats images -> MNI : ! linear interpolation !
for I in {01..19}
do
        antsApplyTransforms -d 3 -v 1 -n Linear -t
"${folder}"NLreg_template_to_MNI_1Warp.nii.gz -t
"${folder}"NLreg_template_to_MNI_0GenericAffine.mat -t
"${folder}"data/NLreg_T1mean_to_template_1Warp.nii.gz -t
"${folder}"data/NLreg_T1mean_to_template_0GenericAffine.mat -t
["${folder}"data/coreg_T1mean_to_meanEPI_0GenericAffine.mat, 1] -i
"${folder}"data/con_00${I}.nii -r "${MNI}" -o "${folder}"data/con_00${I}_mni.nii
done

# ---------------------------------------------------- #
```

**b. Generate Nifti landmark overlay image (a MATLAB script)**

```
%% Check the coregistration with EPI landmarks
%  make a plot
```

```
clear;clc

%% preparation

% SET PATHS
path_root = '/path/to/your/landmark/images/';
path_save = '/path/to/the/folder/where/the/heatmap/will/be/saved/';

cd(path_save)

ids = [];% put your ID list

nid = length(ids);

%% make

cc = 0; % reset the counter
averplot = zeros(193,229,193); % pre-assign the dummy variable for plotting
the masks
figure; % open sketchbook
for i = 1:length(ids)

    averplotdum = zeros(193,229,193);
    name = num2str(ids(i));
    data = spm_read_vols(spm_vol([path_root name '/data/LCmask_check_' name
'.nii'])); % change here accordingly to your file name
    [x y z] = ndgrid(1:size(data,1), 1:size(data,2), 1:size(data,3));

    % get x/y/z level of LC masks
    ycoordz = y(find(data(:) == 1)); uycoordz = unique(ycoordz);
    ylevel = floor(median(uycoordz)); % take middle one

    xcoordz = x(find(data(:) == 1)); uxcoordz = unique(xcoordz);
    xlevel = ceil(median(uxcoordz)); % take middle one

    zcoordz = z(find(data(:) == 1)); uzcoordz = unique(zcoordz);
    zlevel = ceil(median(uzcoordz)); % take middle one
```

```
    % right now, z position, which is an axial view, seems to be the most
    % appropriate slice to check the coregistration. if you want to take a
    % look at the other views, please change the script accordingly
    dumz = squeeze(data(:,:,zlevel));
    imagesc(dumz);title([name ' ' num2str(i) ]) ;% middle slice
    cc = cc+1;
    zstore(cc) = zlevel;
    averplotdum(:,:,zlevel) = dumz; % average points and squeeze them in

%     dumy = squeeze(data(:,ylevel,:));
%     imagesc(dumy);title([name ' ' num2str(i) ]);% middle slice
%     cc = cc+1;
%     ystore(cc) = ylevel;
%     averplotdum(:,ylevel,:) = dumy;

%     dumx = squeeze(sum(data(:,(xlevel-1):(xlevel+1),:),2));
%     imagesc(dumx);title([name ' ' num2str(i) ]);% middle slice
%     cc = cc+1;
%     xstore(cc) = xlevel;
%     averplotdum(xlevel,:,:) = dumx;

    averplot = averplot + averplotdum; % record the points
    imagesc(squeeze(averplotdum(:,:,zlevel)));hold on % and plot

%     imagesc(squeeze(averplotdum(xlevel,:,:)));hold on % x position plot
%     imagesc(squeeze(averplotdum(:,ylevel,:)));hold on % y position plot

    clear dumx dumy dumz

end

averplot = averplot/cc; % get average

%% generate the overlay image in 3D space

% one mask where all voxel > 0 are 1
averplot_nifti = averplot; averplot_nifti(find(averplot_nifti(:)>0)) = 1;
hdr = spm_vol([path_root /data/landmarks.nii']); % pick just any header from
a file
hdr.fname = [path_save 'landmark_heatmap.nii'];
```

```
hdr.dim = size(averplot_nifti);
hdr = rmfield(hdr,'pinfo');
hdr.nii = spm_write_vol(hdr,averplot_nifti);
```

### c. Generate video of transformed mean functional or structural images (a MATLAB script)

```
%%  Visual check for coregistered images
%   make movie of epis to see whetehr OK aligned

%% preparation

clear;clc

% set paths
path_root = '/path/to/your/transformed/images/'; % where are the files?
path_save  =  '/path/where/the/video/will/be/saved/';  %  where  is  the  video
going to be saved?

% set variables
ids = [];

input_prompt  =  {'duration  per  frame  in  seconds  (default=0)';  'name  of  the
file';  'view(sagittal,  coronal,  axial)';  'which  slice  should  the  video
capture?'};
defaults     = {'0','null','sagittal','96'};
input_answer = inputdlg(input_prompt, 'specify the properties of the video',
1, defaults);

vidframerate = str2num(input_answer{1,1});
vidformat    = '.avi';
vidname      = [input_answer{2,1} vidformat];
vidview      = input_answer{3,1};
slicenum     = str2num(input_answer{4,1});

cd(path_save)
v            = VideoWriter(vidname);
if vidframerate ~= 0
    v.FrameRate  = 1/vidframerate;
elseif vidframerate == 0
    v.FrameRate  = 30; % it's super fast
end

%% start recording
```

```
open(v) % open the file

cc = 0; figure % open a sketchbook
for i = 1:length(ids)
        name = num2str(ids(i)); disp(num2str(ids(i)))
        data        =        spm_read_vols(spm_vol([path_root        name
'/data/transformed_image.nii']));

        % position the slice - dimensions are: (x=sagittal, y=coronal,
z=axial)
        if strcmpi(vidview,'sagittal')
            dum = squeeze(data(slicenum,:,:)); % sagittal
        elseif strcmpi(vidview,'coronal')
            dum = squeeze(data(:,slicenum,:)); % coronal view
        elseif strcmpi(vidview,'axial')
            dum = squeeze(data(:,:,slicenum)); % axial
        else
            error('check your view input')
        end

        dum = rot90(dum);% t1WB on template
%     dum = zscore(dum);

        imagesc(dum);
        title(num2str(ids(i)))
        cc = cc+1;
        % Store the frame
        M(cc)=getframe(gcf); % leaving gcf out crops the frame in the movie.

end

writeVideo(v,M) % export the video
close(v)
close all;
```

### d. Calculate the Euclidean distance of single-subject landmarks (a MATLAB script)

```
%% LANDMARK PLOTTING: create 3D plots of created heatmaps, and compare

clc;clear;close all

% set env
path_transformed = '/path/to/your/individual/landmarks/';
load('/path/to/your/subject/IDs/IDs.mat') % load IDs

% load individual landmark images
transformed_landmarks_single=[];
for subj=1:length(IDs)
    transformed_landmarks_single{subj,1}=spm_read_vols(spm_vol([
path_transformed 'landmark_' IDs{subj} '.nii']));
end; clear subj

% load the landmark image drawn on MNI
MNIlandmark =
spm_read_vols(spm_vol(['/path/to/the/MNI/landmark/MNI_landmark.nii'])); %
change here accordingly to your file name
MNIlandmark(find(MNIlandmark<1))=NaN;

% write down the coordinates of MNI landmarks
MNI.NucRuber=[];
MNI.NucRuber.left=[102 113 71];
MNI.NucRuber.right=[92 113 71];

MNI.TopBrainstem=[97 102 71];

MNI.OutlineBrainstem=[];
MNI.OutlineBrainstem.left=[109 103 60];
MNI.OutlineBrainstem.right=[85 103 60];

MNI.LC=[];
MNI.LC.left=[100 97 60];
MNI.LC.right=[94 97 60];

MNI.BottomBrainstem=[97 94 50];

disp('prep done')

%% identify median coords in the single landmarks

transformed_landmark_coords=[];

for subj=1:length(IDs)

    clear xs ys zs tmp idx2

    [xs,ys,zs] =
ind2sub(size(transformed_landmarks_single{subj}),find(transformed_landmarks
_single{subj}~=0));
    tmp = [xs ys zs];
    [~,idx2] = sort(tmp(:,3)); tmp_sorted = tmp(idx2,:);

    slices = unique(tmp(:,3));
    slices = slices(end:-1:1);
```

```
    %% the first slice, with three landmarks: top, NucRubs
    clear idx3 positionSlice positionX_sorted cluster1 cluster2 cluster3

    positionSlice = tmp_sorted(find(tmp_sorted(:,3)==slices(1)),:); % find
the points in the first slice
    [~,idx3] = sort(positionSlice(:,1)); positionX_sorted =
positionSlice(idx3,:);

    % ruber right
    cluster1 = min(positionX_sorted(:,1));
    cluster_med1 =
positionX_sorted(find(positionX_sorted(:,1)==cluster1+1),:);
    cluster_medpt{subj,1} = cluster_med1( find( cluster_med1(:,2)==ceil(
median(cluster_med1(:,2))) ),: );

    % top curve
    cluster2 = min(positionX_sorted(positionX_sorted(:,1)>cluster1+2,1));
    cluster_med2 =
positionX_sorted(find(positionX_sorted(:,1)==cluster2+1),:);
    cluster_medpt{subj,2} = cluster_med2( find( cluster_med2(:,2)==ceil(
median(cluster_med2(:,2))) ),: );

    % ruber left
    cluster3 = min(positionX_sorted(positionX_sorted(:,1)>cluster2+2,1));
    cluster_med3 =
positionX_sorted(find(positionX_sorted(:,1)==cluster3+1),:);
    cluster_medpt{subj,3} = cluster_med3( find( cluster_med3(:,2)==ceil(
median(cluster_med3(:,2))) ),: );

    transformed_landmark_coords{subj,1}.TopSlice = [];
    transformed_landmark_coords{subj,1}.TopSlice.NucRuber_left =
cluster_medpt{subj,3};
    transformed_landmark_coords{subj,1}.TopSlice.NucRuber_right =
cluster_medpt{subj,1};
    transformed_landmark_coords{subj,1}.TopSlice.TopBrainstem =
cluster_medpt{subj,2};
    clear cluster_medpt


    %% the second slice, with four landmarks: brainstem outline and LC

    clear idx3 positionY positionX_sorted cluster1 cluster2 cluster3

    positionSlice = tmp_sorted(find(tmp_sorted(:,3)==slices(2)),:);
    [~,idx3] = sort(positionSlice(:,1)); positionX_sorted =
positionSlice(idx3,:);

    % brainstem outline right
    cluster1 = min(positionX_sorted(:,1));
    cluster_med1 =
positionX_sorted(find(positionX_sorted(:,1)==cluster1+1),:);
    cluster_medpt{subj,1} = cluster_med1( find( cluster_med1(:,2)==ceil(
median(cluster_med1(:,2))) ),: );

    % LC right
    cluster2 = min(positionX_sorted(positionX_sorted(:,1)>(cluster1+2),1));
    cluster_med2 =
positionX_sorted(find(positionX_sorted(:,1)==cluster2+1),:);
```

```
    cluster_medpt{subj,2} = cluster_med2( find( cluster_med2(:,2)==ceil(
median(cluster_med2(:,2))) ),: );

    % LC left
    cluster3 = min(positionX_sorted(positionX_sorted(:,1)>cluster2+2,1));
    cluster_med3 =
positionX_sorted(find(positionX_sorted(:,1)==cluster3+1),:);
    cluster_medpt{subj,3} = cluster_med3( find( cluster_med3(:,2)==ceil(
median(cluster_med3(:,2))) ),: );

    % brainstem outline left
    cluster4 = min(positionX_sorted(positionX_sorted(:,1)>cluster3+2,1));
    cluster_med4 =
positionX_sorted(find(positionX_sorted(:,1)==cluster4+1),:);
    cluster_medpt{subj,4} = cluster_med4( find( cluster_med4(:,2)==ceil(
median(cluster_med4(:,2))) ),: );

    transformed_landmark_coords{subj,1}.MidSlice = [];
    transformed_landmark_coords{subj,1}.MidSlice.OutlineBrainstem_left =
cluster_medpt{subj,4};
    transformed_landmark_coords{subj,1}.MidSlice.OutlineBrainstem_right =
cluster_medpt{subj,1};
    transformed_landmark_coords{subj,1}.MidSlice.LC_left =
cluster_medpt{subj,3};
    transformed_landmark_coords{subj,1}.MidSlice.LC_right =
cluster_medpt{subj,2};
    clear cluster_medpt


    %% the third slice, with one landmark: bottom window brainstem

    clear idx3 positionY positionX_sorted cluster1 cluster2 cluster3
cluster4

    positionSlice = tmp_sorted(find(tmp_sorted(:,3)==slices(3)),:);
    [~,idx3] = sort(positionSlice(:,1)); positionX_sorted =
positionSlice(idx3,:);

    % brainstem bottom
    cluster1 = min(positionX_sorted(:,1));
    cluster_med1 =
positionX_sorted(find(positionX_sorted(:,1)==cluster1+1),:);
    cluster_medpt{subj,1} = cluster_med1( find( cluster_med1(:,2)==ceil(
median(cluster_med1(:,2))) ),: );

    transformed_landmark_coords{subj,1}.BottomSlice = [];
    transformed_landmark_coords{subj,1}.BottomSlice.BottomBrainstem =
cluster_medpt{subj,1}
    clear cluster_medpt


    fprintf('\n subject %s done\n',IDs{subj})

end;clear subj

disp('coordinates collected')

%% calculate euclidian distances in single subject level
```

```
varnames =
{'NucRuber_left';'NucRuber_right';'TopBrainstem';'OutlineBrainstem_left';'O
utlineBrainstem_right';...
    'LC_left';'LC_right';'BottomBrainstem'};
for v1=1:length(varnames)
   eval([varnames{v1} '=[];'])
end

EuclideanDistances_indv=[];
for subj=1:length(IDs)

    EuclideanDistances_indv{subj,1}.NucRuber=[];
    EuclideanDistances_indv{subj,1}.NucRuber.left =
sum((transformed_landmark_coords{subj,1}.TopSlice.NucRuber_left-
MNI.NucRuber.left).^2).^0.5;
    EuclideanDistances_indv{subj,1}.NucRuber.right =
sum((transformed_landmark_coords{subj,1}.TopSlice.NucRuber_right-
MNI.NucRuber.right).^2).^0.5;

    EuclideanDistances_indv{subj,1}.TopBrainstem =
sum((transformed_landmark_coords{subj,1}.TopSlice.TopBrainstem-
MNI.TopBrainstem).^2).^0.5;

    EuclideanDistances_indv{subj,1}.OutlineBrainstem=[];

EuclideanDistances_indv{subj,1}.OutlineBrainstem.left=sum((transformed_land
mark_coords{subj,1}.MidSlice.OutlineBrainstem_left-
MNI.OutlineBrainstem.left).^2).^0.5;

EuclideanDistances_indv{subj,1}.OutlineBrainstem.right=sum((transformed_lan
dmark_coords{subj,1}.MidSlice.OutlineBrainstem_right-
MNI.OutlineBrainstem.right).^2).^0.5;

    EuclideanDistances_indv{subj,1}.LC=[];

EuclideanDistances_indv{subj,1}.LC.left=sum((transformed_landmark_coords{su
bj,1}.MidSlice.LC_left-MNI.LC.left).^2).^0.5;

EuclideanDistances_indv{subj,1}.LC.right=sum((transformed_landmark_coords{s
ubj,1}.MidSlice.LC_right-MNI.LC.right).^2).^0.5;

    EuclideanDistances_indv{subj,1}.BottomBrainstem =
sum((transformed_landmark_coords{subj,1}.BottomSlice.BottomBrainstem-
MNI.BottomBrainstem).^2).^0.5;

    % write as a table
    NucRuber_left(subj,1)=EuclideanDistances_indv{subj,1}.NucRuber.left;
    NucRuber_right(subj,1)=EuclideanDistances_indv{subj,1}.NucRuber.right;
    TopBrainstem(subj,1)=EuclideanDistances_indv{subj,1}.TopBrainstem;

OutlineBrainstem_left(subj,1)=EuclideanDistances_indv{subj,1}.OutlineBrains
tem.left;

OutlineBrainstem_right(subj,1)=EuclideanDistances_indv{subj,1}.OutlineBrain
stem.right;
    LC_left(subj,1)=EuclideanDistances_indv{subj,1}.LC.left;
    LC_right(subj,1)=EuclideanDistances_indv{subj,1}.LC.right;

BottomBrainstem(subj,1)=EuclideanDistances_indv{subj,1}.BottomBrainstem;

end
```

```
spssID = cell2mat(cellfun(@str2num, IDs,'UniformOutput',false));

T=table(NucRuber_left,NucRuber_right,TopBrainstem,OutlineBrainstem_left,Out
lineBrainstem_right,...
    LC_left,LC_right,BottomBrainstem);
ED_export
=[spssID',NucRuber_left,NucRuber_right,TopBrainstem,OutlineBrainstem_left,O
utlineBrainstem_right,...
    LC_left,LC_right,BottomBrainstem];

save(['/path/where/the/folder/the/data/will/be/saved/EuclideanDistance_land
marks.mat'],'ED_export')

disp('ED calc done')
```

**e. Calculate the Euclidean distance between the slice-wise centroids of a template LC mask and single-subject transformed LC segmentations (a MATLAB script)**

```
%% calculate Euclidean distance: LC segmentations
clc;clear;close all
warning off

% paths
path_transformed = '/path/to/the/transformed/LCsegmentations/';
IDs=[]; % subject IDs

% load images
% aggregated LC segmentations, transformed & binarized
LC_tf_bin = spm_read_vols(spm_vol([path_transformed
'LCmask_heatmap_binary.nii'])); LC_tf_bin(find(LC_tf_bin==0))=NaN;
% template MNI LC mask
MNImask =
spm_read_vols(spm_vol(['/path/to/the/template/LCMask/mni_icbm152/mni_icbm15
2_LCmetaMask_MNI05_s01f_plus50_bin.nii'])); MNImask(MNImask==0)=0;

% identify coordinates of each voxel
[x_MNI,y_MNI,z_MNI] = ind2sub(size(MNImask),find(MNImask~=0));
[x_tf,y_tf,z_tf] = ind2sub(size(LC_tf_bin),find(~isnan(LC_tf_bin)));

% coordinates in double
positions_MNI = [x_MNI,y_MNI,z_MNI];
[~,idx1] = sort(positions_MNI(:,2));
slices_MNI_mask = unique(positions_MNI(:,3)); clear idx1


%% draw and check the transformed & aggregated LC segmentations in 3D space

close all

S1 = repmat([170],numel(x_tf),1);
S2 = repmat([200],numel(x_MNI),1);

hFig = figure();
axh = axes('Parent', hFig);
set(gca,'FontSize',25); hold on
hold(axh, 'all');
h2 = scatter3(x_MNI,y_MNI,z_MNI,S2,'d',...
    'MarkerEdgeColor','g',...
    'MarkerFaceColor',[0.4660 0.6740 0.1880]);
h1 = scatter3(x_tf,y_tf,z_tf,S1,'o',...
    'MarkerEdgeColor','r',...
    'MarkerFaceColor',[1 0.2 0.2],'MarkerFaceAlpha',.5);
xlabel('X','FontWeight','bold')
ylabel('Y','FontWeight','bold')
zlabel('Z','FontWeight','bold')
xlim([88 107])
ylim([91 99])
zlim([46 64])
view(axh, -33, 22);
grid(axh, 'on');
set(gca,'FontSize',18);
legend(axh, [h1,h2], {'Transformed Masks', 'MNI Meta LC mask'});

%% find centroid voxel
```

```
% left LC
dummybase = zeros(193,229,193); % make a blank canvas

tmp_MNI=[x_MNI,y_MNI,z_MNI]; % tidy up the xyz coordinates from the
original mask

indL = tmp_MNI(:,1)<mean(x_MNI); % identify coordinates of left and right
coordsL = tmp_MNI(indL,:,:);
indR = tmp_MNI(:,1)>mean(x_MNI);
coordsR = tmp_MNI(indR,:,:);

leftLC=dummybase;
leftLC(sub2ind(size(leftLC),coordsL(:,1),coordsL(:,2),coordsL(:,3))) = 1;
rightLC=dummybase;
rightLC(sub2ind(size(leftLC),coordsR(:,1),coordsR(:,2),coordsR(:,3))) = 1;

[x_Left,y_Left,z_Left]=ind2sub(size(leftLC),find(leftLC~=0));
[x_Right,y_Right,z_Right]=ind2sub(size(rightLC),find(rightLC~=0));

centroid_L_mni =
[round(mean(x_Left)),round(mean(y_Left)),round(mean(z_Left))];
centroid_R_mni =
[round(mean(x_Right)),round(mean(y_Right)),round(mean(z_Right))];
centroid_both_mni =
[round(mean(x_MNI)),round(mean(y_MNI)),round(mean(z_MNI))];

%% extract centroid of single-subject masks

centroid_L = []; centroid_R =[];
for id = 1:length(IDs)

    clear tmp leftLC rightLC indL indR coordsL coordsR xi_Left yi_Left
zi_Left ...
        xi_Right yi_Right zi_Right

    LCsegs_individual_binary{id,1} =
spm_read_vols(spm_vol([path_transformed 'threshold025_' IDs{id}
'_conjmask_mni.nii']));

    dummybase = zeros(193,229,193); % make a blank canvas

    [xi,yi,zi] =
ind2sub(size(LCsegs_individual_binary{id,1}),find(LCsegs_individual_binary{
id,1}~=0));
    tmp=[xi,yi,zi]; % tidy up the xyz coordinates from the original mask

    indR = tmp(:,1)>97;%mean(xi); % identify coordinates of
    coordsR = tmp(indR,:,:);
    indL = tmp(:,1)<97;%mean(xi);
    coordsL = tmp(indL,:,:);

    leftLC=dummybase;
    leftLC(sub2ind(size(leftLC),coordsL(:,1),coordsL(:,2),coordsL(:,3))) =
1;

    rightLC=dummybase;
    rightLC(sub2ind(size(leftLC),coordsR(:,1),coordsR(:,2),coordsR(:,3))) =
1;

    % tmp_keren=kerenmask;%[x_keren,y_keren,z_keren];
```

```
    % leftLC = tmp_keren;leftLC(x_keren<mean(x_keren),:,:)=0; % choose
everything that's on the left
    % rightLC = tmp_keren;rightLC(x_keren>mean(x_keren),:,:)=0; % choose
everything that's on the right

    [xi_Left,yi_Left,zi_Left]=ind2sub(size(leftLC),find(leftLC~=0));
    left_indivs{id,1} = [xi_Left,yi_Left,zi_Left];
    [xi_Right,yi_Right,zi_Right]=ind2sub(size(rightLC),find(rightLC~=0));
    right_indivs{id,1} = [xi_Right,yi_Right,zi_Right];

    centroid_L(id,:) =
[round(mean(xi_Left)),round(mean(yi_Left)),round(mean(zi_Left))];
    centroid_R(id,:) =
[round(mean(xi_Right)),round(mean(yi_Right)),round(mean(zi_Right))];
    centroid_both(id,:) =
[round(mean(xi)),round(mean(yi)),round(mean(zi))];
end



%% ED from the centroid point, slicewise analysis

% find centre of each slice
MNI_left = [x_Left,y_Left,z_Left];
MNI_right = [x_Right,y_Right,z_Right];
MNI_slices_L = unique(z_Left);
MNI_slices_R = unique(z_Right);

for slices = 1:length(MNI_slices_L)
    clear tmp zind
    % sort the left
    zind=z_Left==MNI_slices_L(slices);
    tmp = MNI_left(zind',:,:);
    if size(MNI_left(zind',:,:),1)==1
    Lcentrer_mni(slices,:) = [tmp(:,1:2) MNI_slices_L(slices)];
    else
    Lcentrer_mni(slices,:) = [mean(tmp(:,1:2)) MNI_slices_L(slices)];
    end
end

for slices = 1:length(MNI_slices_R)

    clear tmp zind
    % sort the left
    zind=z_Right==MNI_slices_R(slices);
    tmp = MNI_right(zind',:,:);
    if size(MNI_right(zind',:,:),1)==1
    Rcentrer_mni(slices,:) = [tmp(:,1:2) MNI_slices_R(slices)];
    else
    Rcentrer_mni(slices,:) = [mean(tmp(:,1:2)) MNI_slices_R(slices)];
    end

end



% now find centres of individual masks
Lcentre_indiv=[];Rcentre_indiv=[];
for id=1:length(IDs)

    clear maskL maskR slicesL slicesR sl
```

```
    % first, left
    maskL=right_indivs{id,1}; % there's something wrong with this left and
right
    slicesL=unique(maskL(:,3));
    for sl=1:length(slicesL)
        clear tmp zind
        % sort the left
        zind=maskL(:,3)==slicesL(sl);
        tmp = maskL(zind',:,:);
        if size(tmp,1)== 1
            Lcentre_indiv{id,1}(sl,:) = [tmp(:,1:2) slicesL(sl)];
        else
            Lcentre_indiv{id,1}(sl,:) = [mean(tmp(:,1:2)) slicesL(sl)];
        end
    end


    % then, right
    maskR=left_indivs{id,1}; % there's something wrong with this left and
right
    slicesR=unique(maskR(:,3));
    for sl=1:length(slicesR)
        clear tmp zind
        % sort the left
        zind=maskR(:,3)==slicesR(sl);
        tmp = maskR(zind',:,:);
        if size(tmp,1)== 1
            Rcentre_indiv{id,1}(sl,:) = [tmp(:,1:2) slicesR(sl)];
        else
            Rcentre_indiv{id,1}(sl,:) = [mean(tmp(:,1:2)) slicesR(sl)];
        end
    end

end

%% calculate Euclidian distance of centroids, slicewise

ED_slice_L=[]; ED_slice_R=[];
for id=1:length(IDs)

    % find the slices that matches
    clear Lslc_tmp A B
    Lslc_tmp = Lcentre_indiv{id,1}(:,3);
    indslc1=ismember(MNI_slices_L,Lslc_tmp,'rows');
    indslc2=ismember(Lslc_tmp,MNI_slices_L,'rows');

    A = Lcentrer_mni(indslc1,:);
    B = Lcentre_indiv{id,1}(indslc2,:);
    for k=1:size(B,1)
        clear v
        v    = B(k,:) - A(k,:);
        ED_slice_L{id,1}(k) = sqrt(nansum(v .^ 2));
    end

    % find the slices that matches
    clear Rslc_tmp A B
    Rslc_tmp = Rcentre_indiv{id,1}(:,3);
    indslc1=ismember(MNI_slices_R,Rslc_tmp,'rows');
    indslc2=ismember(Rslc_tmp, MNI_slices_R,'rows');
```

```
    A = Rcentrer_mni(indslc1,:);
    B = Rcentre_indiv{id,1}(indslc2,:);

    for k=1:size(A,1)
        clear v
        v    = B(k,:) - A(k,:);
        ED_slice_R{id,1}(k) = sqrt(nansum(v .^ 2));
    end

end


% additional processing
ED_slice_mean_L = cellfun(@nanmean, ED_slice_L);
ED_slice_mean_R = cellfun(@nanmean, ED_slice_R);

%% simple stats

means(1,1)=nanmean(ED_slice_mean_L);
means(1,2)=nanmean(ED_slice_mean_R);
stds(1,1)=nanstd(ED_slice_mean_L);
stds(1,2)=nanstd(ED_slice_mean_R);
medians(1,1)=nanmedian(ED_slice_mean_L);
medians(1,2)=nanmedian(ED_slice_mean_R);

fprintf('\n left LC mean: %1.4f, STD: %1.4f, median: %1.4f \n right LC
mean: %1.4f, STD: %1.4f, median: %1.4f \n', ...
    means(1,1), stds(1,1), medians(1,1), means(1,2), stds(1,2),
medians(1,2))
```