Hand-on session FFYS7086 23.5.2022
Practical work on MRI signal modelling and processing 13:00-17:00

Harri Merisaari, haanme@utu.fi

Learning objectives:

WORK1
- Basic **ITK-SNAP** use for visualization and **quality control** of **3D** and **4D** medical imaging data
- Basic T1W brain processing with **Freesurfer** for **volymetry** and **cortex surface measures**
- Basic use for Freesurfer's **freeview** tool for visualization and **quality control**

WORK2
- Basic DTI processing with **fsl** toolbox for **DTI scalar measurements**
- Basic DTI processing with **DSIstudio** for **tracktography**

Data:
- **Hands_on_data_v1** https://filesender.funet.fi/?s=download&token=a05ee396-dc1d-4e27-a8db-bec58f147246
- The original data used in the session is freely available from https://www.nitrc.org/projects/multimodal

Platform:
- The exercises are designed to be run at UTU Quantum 109 classroom Windows machines, with VMware virtual desktop
    o Search with name **VMware Workstation 16 Player** in a classroom workstation
- Optionally, you can do all of the excercises in you own computer, if it can run VMware virtual machine images
- Optionally, you can install the used software natively. For this, please install required software for **WORK1** and **WORK2**, respectively
    o Please note that some of the software is not natively supported in all main OS.

WORK1: Instructions for processing anatomical T1W (3D) image:

1. Download and extract virtual machine image:
   https://filesender.funet.fi/?s=download&token=ffbc5e91-3804-4c2f-9173-b87acb22478c
- Requires software installations: ITK-SNAP (supported in Win, OS X, Linux), Freesurfer (supported in OS X, Linux)

2. Start **VMware Workstation 16 Player**

3. Start virtual the machine (Ubuntu 20.04 LTS)

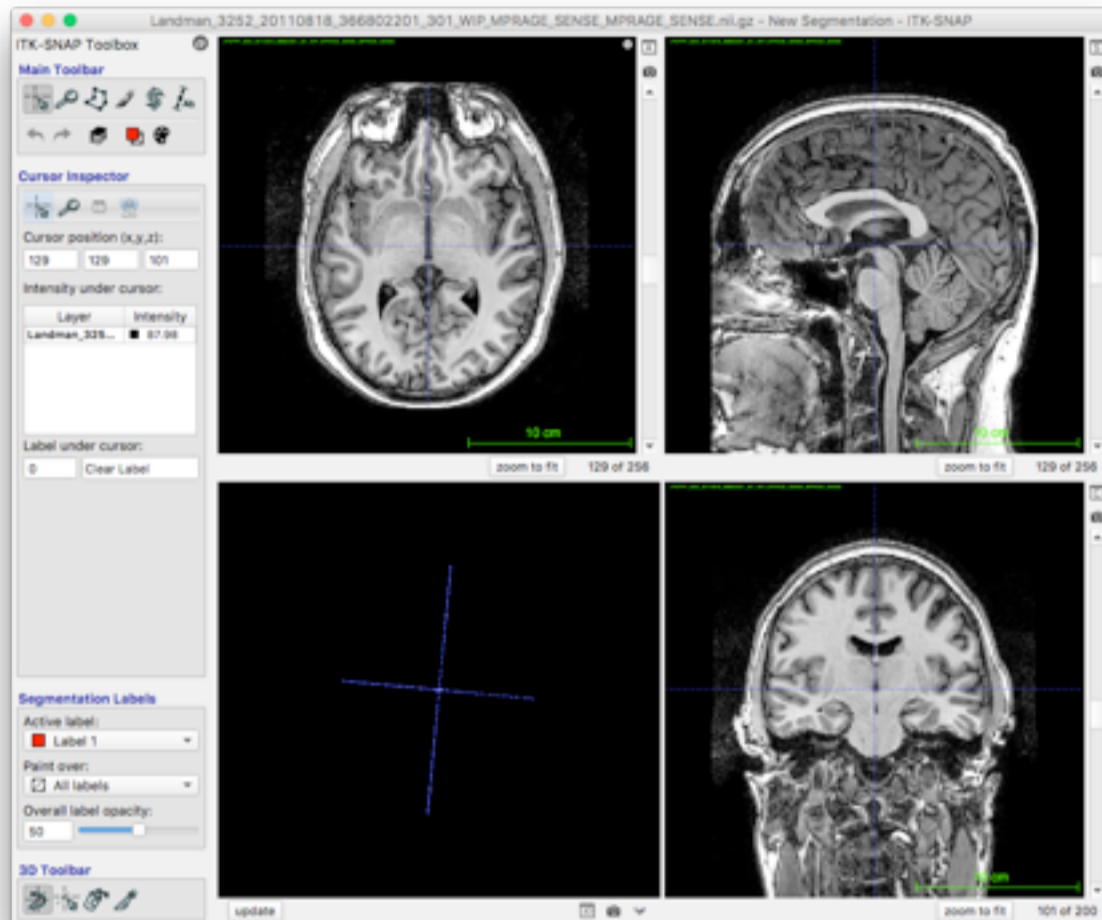4. Locate pre-downloaded data folder **Hands_on_data_v1** at the **Desktop**

5. (Quality Control) with ITK-SNAP (start from command line by running command **itksnap**)
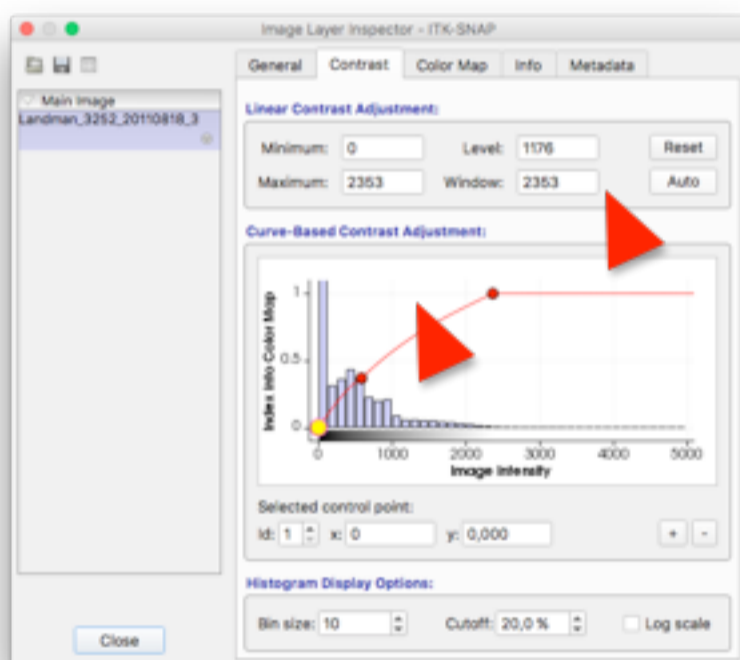
1. Open (File > Open Main Image …) T1W:
   **MMRR-3T7T-2-1_multimodal/3T/Landman_3252_20110818_366802201_301_WIP_MPRAGE_SENS**

2. Navigate through 3D data) Scroll through 3D image along transaxial, sagittal and coronal slices

3. (Adjust visualization contrast) Select 'Tools > Layer Inspector...', then 'Contrast' tab

4. (Detect basic properties of the image) Adjust image contrast so that you can see variation in the brain white matter and thalamus (http://en.wikipedia.org/wiki/Thalamus) more clearly
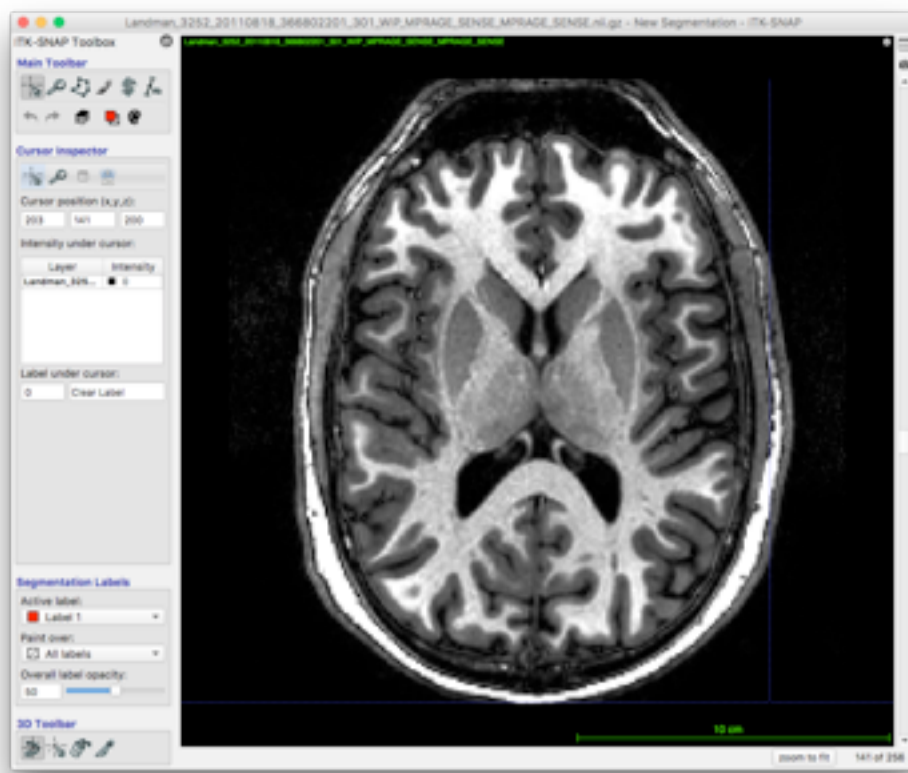
5. Is there inhomogeneity (white matter being brighter/darker in lobes of the brain) in the white matter? If yes, where?

6. Is contrast good enough to recognize thalamus region in this image? With this data, is it possible to see medial, anterior and lateral subnuclei (https://en.wikipedia.org/wiki/Thalamus#/media/File:Thalmus.png) of the thalamus?

6. (Image co-registration):

   1. Add another repetition of the same data:
      MMRR-3T7T-2-1_multimodal/3T/Landman_3252_20110818_366802201_801_WIP_MPRAGE_SENS

   2. Click on regions of image, notice that the T1W intensity value varies. This difference can be used to calculate test-retest repeatability, standard error of measurement etc. at voxel level.

   3. Run co-registration: **Tools > Registration ... > Run Registration**
      · After registration finishes verify the results, e g select between images and look at transaxial, sagittal and coronal views for difference in orientation

   4. Try different parameters.
      · Goodness-of-Fit criteria: Mutual Information, Cross-Correlation, Intensity difference
      · Coarse scale and fine scale smoothing ratio (1x is no smoothing, bigger number means more smoothing i e more coarse scale coregistration)
      · Also manual alignment is an option

   5. Can you manage to create an adequate aligment between the images, based on visual examination?

7. (Gray matter and subcortical regions segmentation with **Freesurfer**) with command line:

   1. Goto data folder: **cd Desktop/Hands_on_data_v1**

   2. Create output folder: **mkdir Freesurfer_output**

   3. Set environmental variable for Freesurfer output:

export SUBJECTS_DIR=/home/ubuntu/Desktop/Hands_on_data_v1

4. Go to data subfolder: **cd MMRR-3T7T-2-1_multimodal/3T**

5. Start segmentation (1st portion with **-autorecon1**, all steps can be run with **-all**):

   recon-all –autorecon1 –i Landman_3252_20110818_366802201_301_WIP_MPRAGE_SENSE_MPRAGE_SENSE.r –subjid Landman_T1W
   - At classroom Quantum 109 machine with VMware virtual machine, do not run the command (takes some 6 hours to finish), instead use pre-calculated results in folder **Freesurfer_output_precalculated** and go directly to next item 8 (quality Control for segmentation)

6. Verify that the segmentation starts properly. This step runs through <u>various steps</u>, creating segmentation for cortex.

```
[root@26ba22931e9d 3T]# recon-all –all –i Landman_3252_20110818_366802201_301_WIP_MPRAGE_SENSE_MPRAGE_SENSE.nii.gz –subjid Landman_T1W
Subject Stamp: freesurfer-linux-centos7_x86_64-7.2.0-20210720-aa8f76b
Current Stamp: freesurfer-linux-centos7_x86_64-7.2.0-20210720-aa8f76b
INFO: SUBJECTS_DIR is /data/Freesurfer_output
Actual FREESURFER_HOME /usr/local/freesurfer
Linux 26ba22931e9d 5.10.25-linuxkit #1 SMP Tue Mar 23 09:27:39 UTC 2021 x86_64 x86_64 x86_64 GNU/Linux
/data/Freesurfer_output/Landman_T1W

 mri_convert /data/MMRR-3T7T-2-1_multimodal/3T/Landman_3252_20110818_366802201_301_WIP_MPRAGE_SENSE_MPRAGE_SENSE.nii.gz /data/Freesurfer_output/Landman_T1W/mri

 mri_convert /data/MMRR-3T7T-2-1_multimodal/3T/Landman_3252_20110818_366802201_301_WIP_MPRAGE_SENSE_MPRAGE_SENSE.nii.gz /data/Freesurfer_output/Landman_T1W/mri/
INFO: using NIfTI-1 qform
reading from /data/MMRR-3T7T-2-1_multimodal/3T/Landman_3252_20110818_366802201_301_WIP_MPRAGE_SENSE_MPRAGE_SENSE.nii.gz...
TR=0.00, TE=0.00, TI=0.00, flip angle=0.00
i_ras = (-0.999793, 0.0201965, -0.00262871)
j_ras = (-0.00449651, -0.0930006, 0.995656)
k_ras = (-0.0198643, -0.995461, -0.0930721)
writing to /data/Freesurfer_output/Landman_T1W/mri/orig/001.mgz...
#--------------------------------------------
#@# MotionCor Fri Apr 29 14:45:12 UTC 2022
Found 1 runs
/data/Freesurfer_output/Landman_T1W/mri/orig/001.mgz
Checking for (invalid) multi-frame inputs...
Only one run found so motion
correction will not be performed. I'll
copy the run to rawavg and continue.
```

7. Wait for the segmentation to finish OR alternatively

8. (Quality Control for segmentation) with **Freeview**, run (from command line):

   1. Start from command line: **freeview**

   2. Open T1W (File > Load Volume… > Select volume file):
      Freesurfer_output_precalculated/Landman_T1W/mri/T1.mgz (Freesurfer compressed image format)

   3. Open segmentation results atlas at individual space (File > Load Volume… > Select volume file):
      Freesurfer_output_precalculated/Landman_T1W/mri/aparc+aseg.mgz

   4. Open segmentation results (left hemisphere WM/GM) surface (File > Load Volume… > Load Surface…):
      Freesurfer_output_precalculated/Landman_T1W/surf/lh.white
      Freesurfer_output_precalculated/Landman_T1W/surf/rh.white
      Freesurfer_output_precalculated/Landman_T1W/surf/lh.pial.T1
      Freesurfer_output_precalculated/Landman_T1W/surf/rh.pial.T1

   5. Open label file for corrected defects (File > Load Volume… > Select volume file):
      Freesurfer_output_precalculated/Landman_T1W/mri/surface.defects.mgz
      · These labels give hints of regions where the segmentation for boundary surfaces had difficulties, and problem in segmentation may be found near or at them

6. Can you detect defects in GM/WM segmentation and/or in GM/brain segmentation surface? If yes, where?
    · Try to observe the image in all three orthogonal views simultaneously, as individual 2D view may sometimes give wrong impression of where the surface is located in 3D space

9. (Contrast-to-Noise Ratio, explore effect of inhomogeneity correction) with command line

    1. Calculate Contrast-to-Noise Ratio (CNR, noise vs brain gray/white matter difference) of original converted T1W image:

       mri_cnr Freesurfer_output_precalculated/Landman_T1W/surf Freesurfer_output_precalculated/Landman_T1W/mri/orig.mgz

   2. Calculate Contrast-to-Noise Ratio (CNR) of original inhomogeneity-corrected T1W image:

       mri_cnr Freesurfer_output_precalculated/Landman_T1W/surf Freesurfer_output_precalculated/Landman_T1W/mri/orig_nu.mgz

    3. Start from command line: **freeview**

    4. Visualize and compare the two images

    5. Has the inhomogeneity correction improved CNR (i e in **mri_cnr** output, MPRAGE T1W CNR should be some where 2.0-5.0)? If yes, can it be seen in visual inspection?

At this point, you have successful segmentation of GM/WM and subregions, and parcellations for Region-of-Interest (ROI) level analysis. The ROI mean values, and radiomic feature extraction can be further extracted using these ROIs.

WORK2: Instructions for processing Diffusion Tensor Image (DTI, 4D image):

1. Download and extract virtual machine image:
   https://filesender.funet.fi/?s=download&token=a6f7d406-c4e4-4dd5-a103-4b5c77e962cd
   - Requires software installations: fsl (OS X, Linux), DSIstudio (Windows, Linux), docker (Windows, OS X, Linux) or mrtrix 3 as native installation
   -
2. Start the virtual machine **(Ubuntu 20.04 LTS)**

3. (Quality control) With ITK-SNAP (run **itksnap** from command line):
   - Open DWI image:
     Landman_3252_20110818_366802201_501_WIP_DTI_2_2_iso_SENSE_DTI_2_2_iso.ni
   - Select 'Tools > Layer Inspector…', then 'General' tab
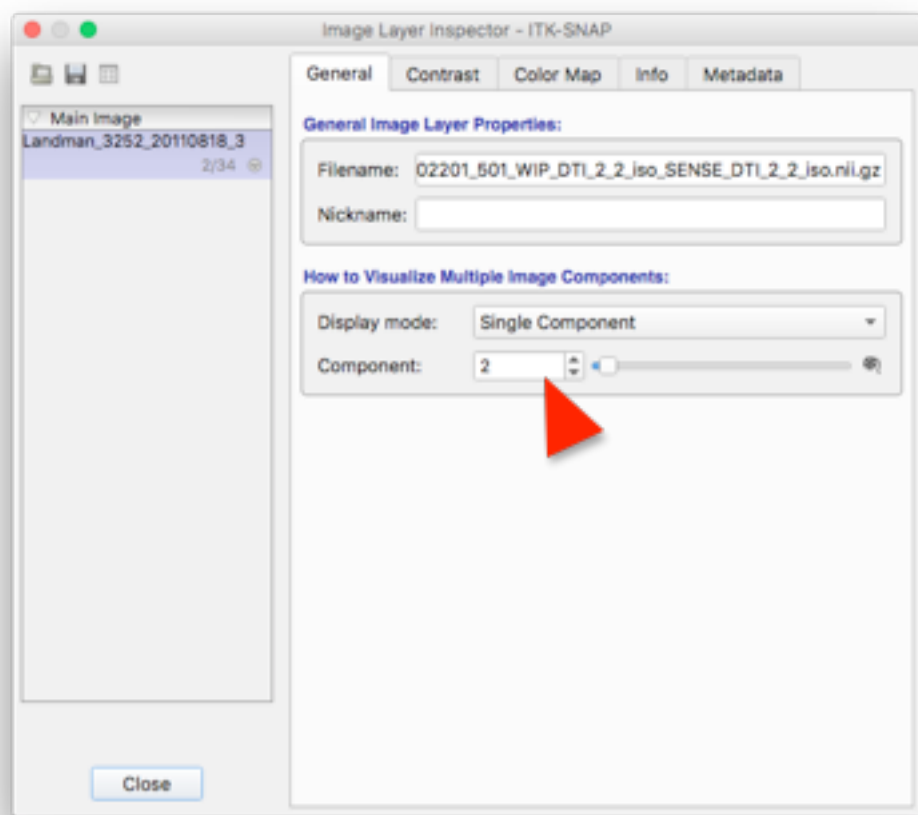
- Scroll through the diffusion encoding direction volumes. Find locations and index number of all B0 images (without diffusion weighting) in the 4D image. They are recognized by being more bright than diffusion weighted images, which have lower intensity due to signal loss from water motion.

- Re-adjust display color range to see B0 volumes and diffusion enconding direction volumes clearly

- Which component numbers are B0s (i e no diffusion weighting)?

- Browse through direction images, look for motion and other artefacts

- Was there detectable inter-volume motion in this image? If yes, between which volumes there was detectable motion occurring?

4. (Fusion imaging for quality control purposes):

- Open DWI image:
  Landman_3252_20110818_366802201_501_WIP_DTI_2_2_iso_SENSE_DTI_2_2_iso.ni

- Add T1W image with ITK-SNAP (File > Add Another Image …):
  Landman_3252_20110818_366802201_301_WIP_MPRAGE_SENSE_MPRAGE_SENSE.n

- Adjust overlay for visualization back and forth to see DWI deviating from anatomical reference in T1W at certain locations (Tools > Layer Inspector… > General > Additional Images > slide bar)

- Can you detect DWI deformations from normal brain anatomy, intensity anomalies? If yes, where?

5. (Artefact removal, using **mrtrix** tools inside **docker** container) With command line:

- Goto data folder **Hands_on_data_v1**

- Start docker with command:

  sudo docker run –it –v $(pwd):/data haanme/mrtrix:3.0.2

- If you have trouble starting the container, try pulling it first **sudo docker pull haanme/mrtrix:3.0.2**
- This starts the container **mrtrix** version **3.0.2** in **haanme** repository
- Starting location (which should be **Desktop/Hands_on_data_v1**) is mounted to **/data** directory (i e all content is accessed from there)

- Goto data folder inside **mrtrix:3.0.2** Linux container:

  cd /data

- Run Marchenko-Pastur theorem (for signal-noise threshold) based PCA (Principal Component Analysis) denoising with command:

  dwidenoise MMRR-3T7T-2-1_multimodal/3T/Landman_3252_20110818_366802201_501_WIP_DTI_2_2_iso_SE Landman_501_DTI_denoised.nii.gz

- Run Gibbs artefact removal with command:

  mrdegibbs MMRR-3T7T-2-1_multimodal/3T/Landman_501_DTI_denoised.nii.gz Landman_501_DTI_denoised_dg.nii.gz

- Exit from docker with command: **exit**

6. (Distortion correction with anatomical reference, because we do not have blip direction image with this data) With command line:

   - Go to data folder **Hands_on_data_v1**

   - Start docker with command:

     sudo docker run –it –v $(pwd):/data haanme/mrtrix:3.0.2

   - Go to data folder inside container: **cd /data**

   - Create working directory: **mkdir SynB0**

   - Extract 1st volume (which is B0 image without diffusion weighting) from DWI image to working directory:
     fslroi Landman_501_DTI_denoised_dg.nii.gz SynB0/b0.nii.gz 0 1
     - 0 means starting index, 1 means 1 consecutive volumes extracted, i e only the 1st volume

   - Copy T1W image to working directory:
     cp MMRR-3T7T-2-1_multimodal/3T/Landman_3252_20110818_366802201_301_WIP_MPRAGE_SENS SynB0/T1.nii.gz

   - Generate acquisition parameters for TOPUP distortion correction: **printf "0 -1 0 0.01\n0 1 0 0.01" > SynB0/acqparams.txt**
     - You can also use text editor to create the file

- Exit from docker with command: **exit**

- Verify that the created files are properly created into **Hands_on_data_v1/SynB0**:
    - **b0.nii.gz**, b0 image with distortion
    - **T1.nii.gz**, T1-weighted anatomical image, used as reference to estimate the amount of distortion in **b0.nii.gz**
    - **acqparams.txt**, ASCII file containing correction parameters

- Continue in Linux command line. At data folder, start **SynB0** distortion estimation with command:
  sudo docker run --rm -v $(pwd)/SynB0:/INPUTS/ -v $(pwd)/SynB0:/OUTPUTS/ -v $(pwd)/license.txt:/extra/freesurfer/license.txt -user $(id -u):$(id -g) hansencb/synb0:v1.1
    - At classroom Quantum 109 machine with VMware virtual machine, do not run the command (takes some 3 hours to finish), instead use pre-calculated results in folder **SynB0_precalculated** and go directly to next item 7 (Visualization of correction effect)
    - **SynB0** folder should be in the execution directory
    - Input and output folders for the script are mounted as the same: **SynB0**, input is read from **/INPUT** folder inside the container
    - The container is **synb0** from **hansencb** repository
    - Script uses Freesurfer for which license file need to be given explicitly: **license.txt**

- Wait for task to end OR use precalculated files in **SynB0_precalculated**

7. (Visualization of correction effect) Inspect preprocessed b0 image with **ITK-SNAP**

- Open original distorted image: **SynB0/b0_all.nii.gz**

- Add preprocessed image: **SynB0/b0_all_topup.nii.gz**

- Add anatomical reference: **Landman_3252_20110818_366802201_301_WIP_MPRAGE_SENSE_MPRAGE_SENSE.n**

- Are there improvements after corrections? Can you find inaccuracies left in the distortion corrected image?

8. (Eddy current and motion correction, DTI modelling) With command line:

- Go to data folder: **Hands_on_data_v1**

- Start docker with command:

  sudo docker run -it -v $(pwd):/data haanme/mrtrix:3.0.2

- Goto data folder inside Linux container: **cd /data**

- Create brain mask image with brain extraction tool **bet2**:
    - Extract corrected b0 image:
      fslroi SynB0/b0_all_topup.nii.gz b0_1st_topup 0 1
    - Create brain mask:
      bet2 SynB0/b0_1t_topup.nii.gz b0_1st_topup_brain -m

- Make copy of b-vector file and b-value file
    - **cp MMRR-3T7T-2-1_multimodal/3T/Landman_3252_20110818_366802201_501_WIP_DTI_2_2_i bvecs**
    - **cp MMRR-3T7T-2-1_multimodal/3T/Landman_3252_20110818_366802201_501_WIP_DTI_2_2_i bvals**

- Remove last frame with motion from the DWI
    - Open the **bvecs** and **bvals** file with text editor, then remove last frame from them (number 34).
        - **bvecs** file has three rows, the diffusion encoding directions are in the columns
        - **bvals** file has one row, the used b-values for b=0 s/mm^2 and b=700 s/mm^2 volumes in bvals file, and corresponding vectors in bvec file
        - Use **nano** at command line or other text editor
    - Create **dwi.nii.gz** Nifti image without last volume 34:
      fslroi MMRR-3T7T-2-1_multimodal/3T/Landman_3252_20110818_366802201_501_WIP_DTI_2_2_i dwi.nii.gz 0 33

- Create index parameter file for **fsl eddy** tool (33 is now the number of volumes in the 4D DWI image):
    - At command line:
      for ((i=1; i<=33; i+=1)); do indx="$indx 1"; done
      echo $indx > index.txt
    - You can also create the index.txt file with text editor (one row with 33 '1' separated by spaces)

- Apply joint eddy current and motion correction step with command:
  eddy_openmp --imain=/data/dwi.nii.gz --index=/data/index.txt --mask=/data/b0_1st_topup_brain_mask.nii.gz --acqp=/data/SynB0/acqparams.txt --bvecs=/data/bvecs --bvals=/data/bvals --topup=/data/SynB0/topup --out=/data/eddy_outputs
    - At classroom Quantum 109 machine with VMware virtual machine, do not run the command (takes some 3 hours to finish), instead of eddy_outputs folder, use pre-calculated results in folder **eddy_output_precalculated** and go directly to next item 9 (Visualization of correction effect)

- Wait for the step to finish OR use results in folder **eddy_output_precalculated**

- Exit from docker with command: **exit**

9. (Visualization of correction effect) Inspect effect of distortion/eddy/motion correction with **ITK-SNAP**

    - Open original distorted image: **dwi.nii.gz**

    - Open preprocessed image (File > Add another Image ..): **eddy_outputs.nii.gz**

    - Can you detect effects of correction? If yes, where?

10. Apply fsl toolkit's DTI model fitting with command line:

- Go to data folder: **Hands_on_data_v1**

- Start docker with command: **docker run –it –v $(pwd):/data haanme/mrtrix:3.0.2**

- Goto data folder inside Linux container: **cd /data**

- Fit Diffusion Tensor model to the DWI data (note to use rotated b-vectors because data is rotated):
  dtifit –k eddy_outputs.nii.gz –r eddy_outputs.eddy_rotated_bvecs –b bvals –m SynB0/b0_1t_topup_brain_mask.nii.gz –o Landman_501
    - Using corrected image **eddy_outputs.nii.gz**
    - Using rotated diffuion encoding direction vectors **eddy_outputs.eddy_rotated_bvecs**
    - This created DTI scalar maps (**FA, MD**) eigenvectors (**V1, V2, V3**) an eigenvalues (**L1, L2, L3**)

- Calculate **Radial Diffusivity (RD)** as average of 2nd and 3rd eigenvalues of modelled tensors:
  fslmaths Landman_501_L2.nii.gz –add Landman_501_L3.nii.gz –div 2.0 Landman_501_RD.nii.gz
    - This calculates **RD** = (**L2 + L3**)/2.0

- Exit from docker with command: **exit**

11. (Visualize DTI scalar maps) With **ITK-SNAP**:

- Open Fractional Anisotropy scalar map: **Landman_501_FA.nii.gz**
    - Is the axonal integrity high in the brain white matter (i e FA values are > 0.3)?
    - Can you find clear artefacts, i e exceptionally low/high FA values from what is expected by anatomy? If yes, where?

- Open Mean Diffusivity map (File > Add another Image ..): **Landman_501_MD.nii.gz**
    - Cerebropinal fluid (CSF) characteristics are close to free water in terms of diffusion. Are the CSF values a expected, i e near 3.0×10^{-3} mm^2/s?

- Open Axial Diffusivity (AD, i e the 1st eigenvalue) scalar map (File > Add another Image ..): **Landman_501_L1.nii.gz**

- Open Radial Diffuivity scalar map (File > Add another Image ..): **Landman_501_RD.nii.gz**

- Observe FA, MD, AD, RD together visually. Do you see obvious artefacts/noise in the images?

- Observe DTI scalar values in Corpus Callosum (https://en.wikipedia.org/wiki/Corpus_callosum), where all axons (and therefore diffusion to large extent) are expected to be in one direction only (along left-right axis). Are the values as expected? If not, where?

12. (Quality Control summary of distortion/eddy/motion corrections) with command line:
   - Go to data folder: **Hands_on_data_v1**
   - Start docker with command: **docker run –it –v $(pwd):/data haanme/mrtrix:3.0.2**
   - Goto data folder inside Linux container: **cd /data/eddy_outputs_precalculated**
   - Run fsl's quality control tool for eddy outputs: **eddy_quad eddy_outputs –idx ../index.txt –par ../acqparams.txt –m SynB0/b0_1st_topup_brain_mask.nii.gz –b bvals –g bvecs –f SynB0/b0_1st_topup.nii.gz**
      - The output should say that you have 1 b0 and 32 diffusion encoding directions
   - Verify adequate quality of processed data: **eddy_outputs.qc/qc.pdf**
      - Is the observed motion (estimate from correction parameters) to any direction bigger than voxel size?
      - Is the motion in degrees less than 5 deg?
      - How is the motion (Translation/Rotation/Displacement) distributed across acquisition time (noting that the volumes can be here considered as time points)?
         - Regular/irregular motion?
         - Constant gradual motion to certain direction?
         - Specific time points where subject moved more/exceptionally much?
      - In last figure of the QC file, is there specific volume(s) (depicted by columns) which have a lot of slice deviating from average slice signal intensity (i e indicating signal dropout likely due to motion). If yes, do you see corresponding motion in the motion graphs above?
   - Exit from docker with command: **exit**

13. (Tracktography) DSIstudio:

   1. Start DSIstudio with command:
      dsi_studio

   2. Open pre-processed image (select **Step T1: Open Source Images**) with file:
      Landman_3252_20110818_366802201_501_WIP_DTI_2_2_iso_SENSE_DTI_2_2_iso.ni
      - Load b-values (B-table window, Files > Open bval…):
        Landman_3252_20110818_366802201_501_WIP_DTI_2_2_iso_SENSE_DTI_2_2_iso.bv
      - Load b-vectors (B-table window, Files > Open bvec…):
        Landman_3252_20110818_366802201_501_WIP_DTI_2_2_iso_SENSE_DTI_2_2_iso.bv

   3. Reconstruct (select **Step T2: reconstruction**) with file:
      Landman_3252_20110818_366802201_501_WIP_DTI_2_2_iso_SENSE_DTI_2_2_iso.ni
      - Select DTI (Diffusion Tensor Imaging) radiobutton below the displayed images instead of GQI (Q-ball Imaging)
      - Start reconstruction by clicking **Run Reconstruction**
      - Close the window

   4. Fiber tracking (select **Step T3: Fiber Tracking & Visualization**) with file:
      Landman_3252_20110818_366802201_501_WIP_DTI_2_2_iso_SENSE_DTI_2_2_iso.ni

   5. Perform deterministic trackography
      - Change these parameters from default, to avoid overloading of the platform
         - '**Tracking Threshold**': 0.3 (streamlines do not pass through voxel having less than 0.3 FA in them, e g in CerebroSpinal Fluid)
         - '**Angular Threshold**': 60 (streamlines do not continue if their local curvature would go beyon 60 degrees)
         - '**Min Length(mm)**': 50.0 (streamline are not accepted if their length is less

than 50 mm)
- - **'Terminate if':5000'**, 'Tracts' (maximum number of streamlines)
- · Start streamlines algorithm by clicking **Fiber Tracking**
- - Note that this is very demanding task and the software/VM may crash
- - If that happens, re-start and reduce the number of tracks
- · Save screenshot(s) in orthogonal views
- · Save basic tractogram statistics (Tracts > Statistics > Save)

14. (Tracktography with correcte DTI dataset) With **DSIstudio**:
   1. Create tractogram as in the previous step (if you changed the tracking parameters, use the same ones here for consistency), but with the corrected image:
      Hands_on_data_v1/eddy_outputs_precalculated/eddy_outputs.nii.gz
      Hands_on_data_v1/bvals
      Hands_on_data_v1/eddy_outputs_precalculated/eddy_outputs.eddy_rotated_bvecs
   2. As in previous step, save screenshot(s) in orthogonal views, and tractogram statistics

15. (Comparison of DTI tracktography before and after corrections)
   - Looking at screenshots and statistics side-by-side, do you see any major differences (e g more than 10% difference in metrics)?

At this point, you have gone through DTI image processing, including quality control for individual case. Optionally, the hands-on data contains another repetition of the exact same case, the test-retest repeatability can be calculated by running the process with that data as well, and comparing the results (the numbers should be the same in absolute sense since they come from the same scanner with the same subject with negligible timespan between scans).