**Image Analysis 101**

**A note on diffusion imaging:** The primary type of image analysis performed in this lab is dMRI analysis. dMRI is concerned with the movement of water molecules in the brain. You can read more about the details of Diffusion Tensor Image (DTI) [here](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2041910/) and Diffusion Kurtosis Imaging (DKI) [here](http://mriquestions.com/diffusion-kurtosis.html).

It is also highly recommended to check out **Introduction to Neuroimaging Analysis** by Jenkinson and Chappell (there are several lab copies available).

**There are numerous pieces of software that are essential and/or beneficial for image analysis**:

[Filezilla](https://filezilla-project.org/) – An FTP software used to access CBIHome to obtain raw image data

[Horos](https://horosproject.org/) – A program that allows viewing of dicom files (Mac only)

[Radiant](https://www.radiantviewer.com/) - A program that allows viewing of dicom files (Windows only)

[dicomsort](https://github.com/TheJaeger/dicomSort) – An in-house Matlab plugin for sorting dicoms into sequence folders

[MRIcroGL](https://www.nitrc.org/projects/mricrogl) – An image viewing package that includes the necessary tool dcm2niix

[PyDesigner](https://pydesigner.readthedocs.io/en/stable/) – An in-house software that is used to remove image artifacts from raw diffusion data and calculate parametric maps

[FSL](https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSL) – A toolbox that allow for a variety of image manipulations\*

[MRtrix3](https://www.mrtrix.org/) - A toolbox that allow for a variety of image manipulations\*

[FSLeyes](https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLeyes) – An image viewer included as a part of FSL; pointed out specifically due to its utility and uniqueness among other FSL tools

[ImageJ](https://imagej.nih.gov/ij/) - A program that allows for image viewing, processing, and analysis

[MRIcron](https://www.nitrc.org/projects/mricron) – Another image viewer with a slightly different interface than FSLeyes; optional but useful

[Matlab](https://matlabacademy.mathworks.com/) – A programming environment used for certain types of analysis

[SPM](https://www.fil.ion.ucl.ac.uk/spm/) – A Matlab-compatible software package that allows for a variety of types of image analysis

[Anaconda](https://www.anaconda.com/) – A data science platform that gives you access to Python, R, Jupyter Notebook, and various other useful programming shells and programs.

MS Teams – The messaging service we use in our lab; a great place to connect and ask questions.

\*Note: FSL and MRTrix3 are very similar; however, they are both ubiquitous in this field and are often required so that other pieces of software will function. Despite their similarities, it is essential to have both.

**A typical project lifecycle will go as follows**:

1. **Scan Data Acquisition** - You may need to access CBIHome in order to retrieve raw data from the main MRI server. I recommend Filezilla as it is straightforward and user-friendly.
   1. To log into the server, you will need the address, your NetID, and PW.
2. **File Organization** - It is recommended to begin every project by organizing your data.
   1. A general recommended folder/file organization structure can be seen in the folder **/01\_Basics/File\_Organization\_Example/**
   2. Keep a record of your subjects, any scan/study/demographic information you may need, and any processing steps you’ve completed in a spreadsheet or other organization tool of your choice.
      1. An example of this documentation can be seen here:
      2. **/01\_Basics/File\_Organization\_Example/04\_Summary/ProcessingLog.xlsx**
   3. For more on file organization, see **03\_Project\_Organization.docx**
3. **Raw Data QC** - QCing raw data is a crucial step before doing any image manipulation. See **02\_Image\_QC.docx** for more details.
4. **Dicom sorting and conversion to nifti**
   1. dicomsort is a tool that will sort your dicoms into more manageable folders based on MRI sequence.
      1. It is a Matlab plugin, so you will call it through Matlab by simply using the following command:
      2. dicomSort(/path/to/folder);
   2. dcm2niix is a tool that comes packaged with MRIcroGL. While it is not the only way to convert dicoms to niftis, it is an effective, simple, and user-friendly way of doing so.
      1. Once dcm2niix is set up on your system, it should only be necessary to run the following command in your terminal:
      2. dcm2niix -f %p [path/to/your/data]
5. **Preprocessing** - Preprocessing raw data is necessary to remove image articles. PyDesigner includes both artifact removal and tensor calculation to derive parametric maps. Preprocessed data should also be QC’d to ensure that preprocessing was completed as expected. See **02\_Image\_QC.docx** for more details.
   1. Using PyDesigner is quite simple, though it does have some requirements and best practices:
      1. It is helpful to have your data organized consistently for each subject.
      2. PyDesigner requires a corresponding .json, .bvec, and .bval file for each .nii file you give it. These are generally created by dcm2niix.
      3. A typical file setup will look like this:
      4. A close up of text on a black background

         Description automatically generated
      5. Where the “nifti” folder contains all of your raw data and the “pydesigner” folder is the output folder where your processed data will go.
      6. A typical command using the folder explain above will look like this:
      7. pydesigner --denoise --degibbs --mask -w --force /desktop/user/PyDesigner-Example/Subj1/nifti/DKI.nii, /desktop/user/PyDesigner-Example/Subj1/nifti/B0.nii -o /desktop/user/PyDesigner-Example/Subj1/pydesigner
      8. (Notes: In this example we have a DKI sequence and an additional B0 sequence, which is common. Both sequences can be given to PyDesigner by just putting a comma between the file paths as you can see in the example command. Also note the “-o” command which specifies where files will be output. Other commands such as “--denoise” and “--degibbs” are described in the PyDesigner documentation linked at the top of this document.)
6. After preprocessing, next steps will depends on the PI’s desired analyses.
   1. Further reading on analysis techniques can be found throughout the rest of this documentation collective, most of which are located in 02\_Extras/Image\_Analysis\_Guides
7. Once all analyses are complete, it is a good idea to organize your project folder and store/backup/archive however your PI would like you to do so.
   1. See **03\_Post\_Project\_Data\_Storage.docx** for more info.

**Common Terms:**

* **B-value** – Indicative of the timing/strength of gradients in diffusion MRI scans; higher b-values capture more information about diffusion at the cost of a higher signal-to-noise ratio; two b-vals (b1000 and b2000) are required for DKI
* **dicom** – the format in which MRI data is stored initially after acquisition
* **Diffusion metrics** –
  + FA – fractional anisotropy; describes the degree to which diffusion within a voxel is isotropic (free diffusion in all directions) or anisotropic (highly aligned structures driving diffusion in a specific direction); 0 = completely isotropic, 1 = complete anisotropic
  + MD – mean diffusivity; describes the magnitude of diffusion
  + RD – radial diffusivity; describes the magnitude of diffusion perpendicular to a fiber tract
  + AD – axial diffusivity; describes the magnitude of diffusion parallel to a fiber tract
* **Hyperintensity/hypointensity** – a cluster of voxels at a markedly higher/lower intensity (appearing brighter) than those around it
* **Intensity** – the measure of tissues as reflected in the brightness or darkness of each voxel
* **Kurtosis metrics** – MK (mean kurtosis), AK (axial kurtosis), RK (radial kurtosis); describe non-Gaussianity
* **nifti** – an MRI data format that compiles all dicoms in a sequence into a viewable 3D image
* Normalization – The process of warping an image or many images into a common space.
  + This may involve warping all images into an average space or, more often, warping all images into a standard space (such as MNI space)
* **Preprocessing** – the process of removing image artifacts and performing necessary corrections to raw MRI data; types of corrections performed during preprocessing
  + **Denoising** –
  + **Unringing** –
  + **EPI Distortion correction** –
* **ROI** – Region of interest; a specific part of the brain of interest to a specific analysis pipeline.
* **Registration** – The process of spatially aligning 2+ images
* **Sequence** – The specific type of scan that was performed when the patient/participant was in the MRI scanner; eg. Diffusion weighted (DKI, DTI, FBI, etc), T1MPRAGE, T2 FLAIR, proton density (PD), MR spectroscopy (MRS), etc.
* **T1 Weighted (T1W/MPRAGE)** – high res anatomical image included is basically every scan ; white matter appears more light, grey matter appears more mid-ranged grey, CSF appears dark grey or black
* **T2 Weighted (T2W/FLAIR)**
* **Warping** – The process of manipulating an image; this can be done directly or by applying a transformation matrix derived from a previous warp
  + This term can sometimes be interchangeable with “registration” for the purposes of MR image manipulation; the important distinction is that “warping” denotes any manipulation in the dimensions or shape of an image whereas “registration” denotes warping an image from its original space to the space of another image.
  + An analogy would be that registration is like traveling from one town to another via car and warping is like driving a car.