In the last years post-mortem imaging has emerged as a rapidly evolving research field providing unique insight into anatomical features in the human brain yet not easily observable in vivo (among many studies:) [1-3]. The post-mortem brain provides a unique anatomical environment that is as complex as in vivo therefore suitable for the validation of advanced biophysical modeling techniques within diffusion MRI [4,5]. However, ensuring the quality of the fixed tissue including preparation before MR scanning as well as tuning scanning parameters to ex vivo imaging can be challenging. Many of the challenges can be handled/considered with insight into the fixation processes and by knowing a few practical tricks for the ex vivo imaging setup as described in [6,7].

First of all, the post mortem tissue immediately starts a self-degenerating process. With time (hours) the degree of degeneration gets so severe that it is reflected in diffusion MRI hence the tissue cannot be used as a valid model of the in vivo situation. This has been demonstrated for tractography [8] and been observed with histology. Fixation procedures using e.g. the fixative formaldehyde stop tissue degeneration. Two such procedures exist; immersion fixation where the (whole) postmortem brain is immersed in a fixative whereas with perfusion fixation the fixative is flushed via the vascular system within the whole brain (only for animal models). The latter ensures an immediate and very homogeneous preservation of whole brain tissue, but the first fixation method does not [9]. For well-fixed tissue the diffusivity is decreased to about one third of the in vivo situation, however the structural integrity reflected by fractional anisotropy (FA) is preserved and corresponds to the in vivo situation [10]. Moreover, well-fixed tissue can be rescanned and stored for long time (years) [6]. Scan parameters (the b-value) need to be adjusted according to the decreased diffusivity ex vivo [1,6,7]. In addition, the fixative lowers T2 and hence the SNR, but when washed out T2 can be regained [11]. When preparing tissue for ex vivo diffusion MRI, one needs to ensure that the temperature of the tissue is similar to that within the bore of the magnet. Importantly, starting to acquire diffusion MRI immediately after any physical handling of the brain tissue can introduce severe short-term instabilities [6] in the dataset which cannot easily be post-processed. Physical handling includes for example the mechanical placement and stabilization of the tissue within a RF coil. Short-term instabilities can be minimized simply by preparing the ex vivo setup hours before the planned scanning session.

**Overall learning objectives:**

- Understand the potential of ex vivo diffusion MRI for structural analysis, and its limitations.
- Be able to describe the differences between in vivo and fixed tissue, fixation strategies and their impact on tissue and imaging.
- Know how to prepare and setup an ex vivo diffusion MRI experiment for acquiring high-quality datasets.

**References:**