## ABSTRACT

Many variants of microscopic techniques are the key methods for tissue structure examination in the research as well as in clinical practice. Since the vast majority of examined tissues are intrinsically opaque, it is difficult to reconstruct their 3D structure with light microscopy. However the spatial structure is inherently connected to the function of cells, tissues or whole organs. The most common approach is sample sectioning to the thin layers, which are subsequently examined or used for 3D structure reconstruction of the sample. A different approach is to use a series of chemical reagents able to clear the samples and make them accessible for imaging via microscopy without the need for physical sectioning.

Although this approach was first used more than a century ago, the widespread use of these methods came in the last few years. Since the resurgence of tissue clearing method a lot of new principles how to clear the samples were discovered as well as many new chemical compounds can be used for this purpose.. Clearing methods are becoming more and more sophisticated: the volume of samples which can be cleared is continuously growing, new methods are becoming faster and automated, the quality of after-cleared tissues is improving and compatibility with many stains including antibodies is becoming a common feature of many protocols. The development of clearing methods comes together with new approaches in field of fluorescence microscopy such as SPIM (single plane illumination microscopy). There can be seen a trend in many other methods towards the imaging of 3D structures of the sample for example in volume electron microscopy (EM) or magnetic resonance imaging (MRI) suitable for *in vivo* imaging. However together with the development of 3D imaging methods there come a few challenges and issues which should be solved including handling and statistical analysis of large amount of data.

The aim of this thesis is to describe and compare the state-of-the-art methods in tissue clearing to explain their principles and shortly describe other compatible methods for 3D imaging. The thesis will be focused on rodent model of central nervous system (CNS) with a special attention to the existing achievements to our knowledge of brain structure and function using 3D imaging. Major outcomes of these methods such as 3D mouse brain atlases will be addressed.

## KEYWORDS

3D models of brain, imaging of neurons and immune cells, tissue clearing techniques, microscopy techniques, confocal, SPIM, spinning-disc, MRI, EM