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Optimization of Optical Tissue Clearing Protocols

Autor: Cinzia Brenna
Institut / Klinik: Zentrum für Medizinische Forschung
Doktorvater: Prof. Dr. N. Gretz

Optical Tissue Clearing (OTC) defines a large group of techniques, aiming to make the tissues optically transparent, in order to be microscopically analysed and to reconstruct in 3 dimensions (3D) physiologic and pathological processes at different levels, from whole animals, organs down to a subcellular level. The optical transparency is an important pre-requisite to achieve the 3D reconstruction. In fact, due to the presence of highly light-absorbing molecules, (i.e. blood molecules, bilayer cell membrane associated lipids), tissues appear ion a milky and opaque way. This does not make the 3D imaging effective and reproducible. Consequently, to reach the optical transparency and make the 3D imaging possible, the main principle of OTCs is to remove lipids from the sample. Furthermore, in order to decrease also the autofluorescence phenomenon, given by the blood molecules, perfusion is often required to remove them from the body of the animal. Concerning the 3D imaging, as OTCs are based on the use of specific clearing solutions, one of the most important parameter influencing its quality is the refractive index (RI) of the solutions used (clearing and mounting solutions). Taking into account all these aspects, the goal of this PhD project was to optimize and develop new approaches for visualizing and 3D-imaging tissues and whole organs, by optimizing OTC methodologies in combination to the use of Confocal and LightSheet Microscopies (CM, LSM). The core of this research was about the Ethyl Cinnamate-based OTC: starting from a recently published protocol, we worked in order to speed up and automate all the procedures, making it suitable for further studies. Starting from wild type mouse tissue, the protocol was, them. Extended also on pathological samples, as well as on human samples, for some preliminary studies. Additionally, we enhanced this methodology extending that to different scopes, as “re-vitalization” of old paraffin samples and 3D immunohistochemistry. In parallel, we also tried to figure out the best conditions for the use of both CM and LSM, in order to acquire 2D and 3D images for very large samples. Regarding the fluorescent dyes, although different molecules were tested, the most used staining was the Cy7-PEI, a dye previously synthesized at the ZMF department of the Universitäts Klinikum of Mannheim, Germany. This was injected during the perfusion, in order to fluorescently stain the inner components of different organs, helping, in this way, also to decrease the number of animals used for *in vivo* experiments.