Sectioning Formalin-Fixed Brain Samples with ROWIAK TissueSurgeon

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Introduction

The delicate and fragile nature of brain tissue can cause a number of problems when processed with standard histological techniques. Especially artefacts introduced during sectioning can interfere with and even limit histological assessment of specimen. Here we describe a new approach to sectioning formalin-fixed brain samples using the femtosecond laser-technique of ROWIAK TissueSurgeon.

Material and Methods

Porcine brain tissue was obtained from the local slaughterhouse and fixed in 20% formal saline, cleared with and then stored in tap water at 4°C. Samples derived from different parts of the brain were sectioned at a thickness of 12µm to 15µm. Sections were removed with the aid of gelatin- coated slides (10%; bloom strength 300), stained with H&E and cover slipped with a water-based mounting medium.

Results

Sections cut with ROWIAK TissueSurgeon showed no difference between grey (Fig. 1 and 2) and white matter (Fig. 3) in terms of quality. Both were equally well sectioned and stained. Gelatin coating did not interfere with staining. Artefacts (e.g. folds, tears) usually linked with conventional microtomy were absent in sections obtained.

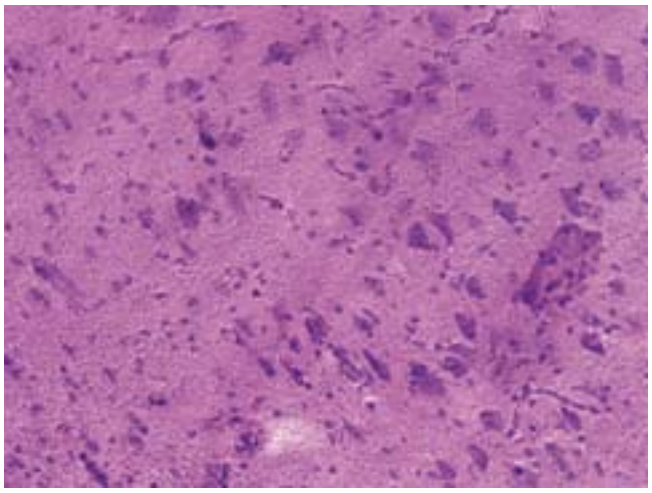

Fig. 1: Porcine brain tissue, grey matter; 12µm, H&E, 20x

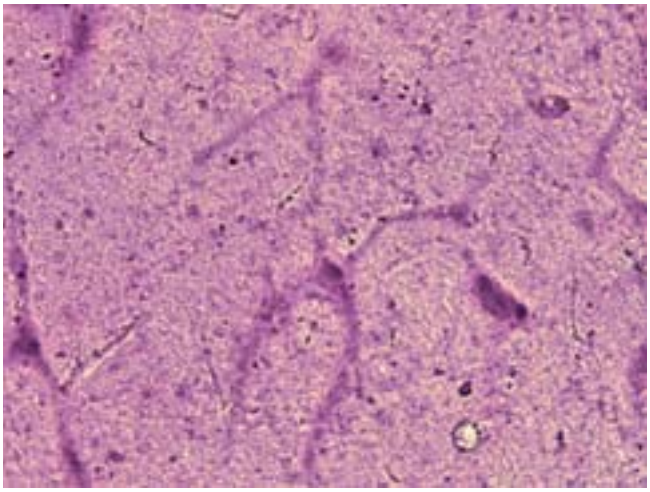

Fig. 2: Porcine brain tissue, grey matter; 12µm, H&E, 40x

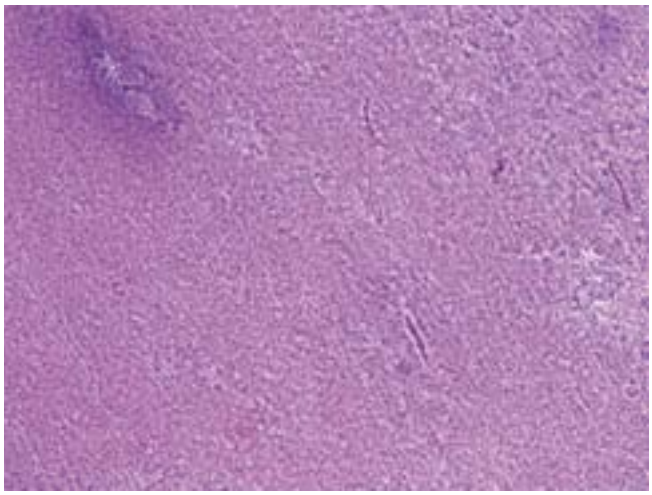

Fig. 3: Porcine brain tissue, white matter; 12µm, H&E, 20x

New Approach in Sectioning Native Brain Samples with High Power ROWIAK TissueSurgeon

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Introduction

Sectioning brain for histology and physiological tests with common methods are either impossible or generate a lot of artefacts. Earlier results in sectioning brain with the ROWIAK TissueSurgeon could only be performed with fixed samples. Especially section thickness was limited to 40 µm. Here we describe a new approach in sectioning brain samples in its native state using a femtosecond laser with 20% enhanced power with the ROWIAK TissueSurgeon. To preserve the structure of native brain during sectioning, the brain was stored in a self-developed chamber filled with Ringer solution during sectioning.

Fig. 1: Microscope slide with chamber to keep tissue in Ringer solution during sectioning

Material and Methods

Mouse brain tissue was obtained from the local pet shop. After extraction, it was transferred into Ringer solution (VWR, Darmstadt, Germany) and stored at 4°C. Brain was pre-sectioned with a scalpel and transferred into a chamber, mounted on a microscope slide (Fig. 1), with the sectioned surface facing the glass surface. The sample was loaded with a light weight. Sections were performed at thicknesses between 15 and 100 µm and monitored via Optical Coherent Tomography (OCT). The sections were removed with the aid of gelatin- coated slides (10%; bloom strength 300).

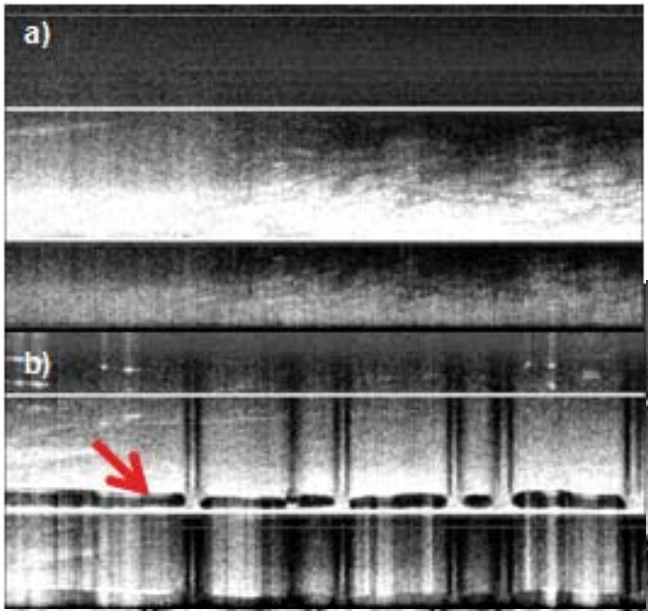

Fig. 2: OCT-image of murine brain a) before sectioning, b) after successful sectioning (arrow, 15 µm)

Results

Sections of native brain cut with the ROWIAK TissueSurgeon were performed at different thicknesses (15 - 100 µm). OCT-imaging shows successful sections at 15 µm (Fig. 2) and 100 µm (Fig. 3), indicated by big bubbles after sectioning. For the first time, contact-free sections of native brain were performed. Thin sections can be used for common histology or further analysis, thick sections were performed for physiological tests of native brain neurons.

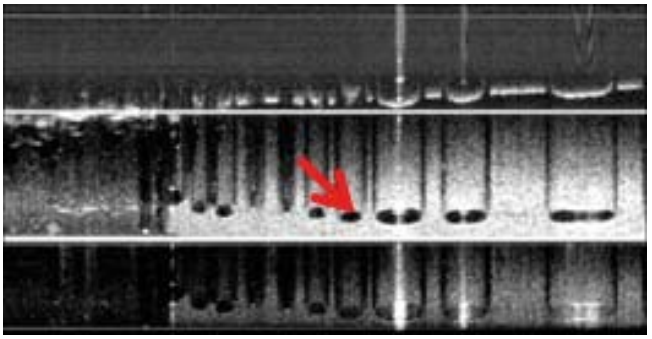

Fig. 3: OCT-image of murine brain after sectioning (arrow, 100 µm)