# Durable 3D test standards from mouse retina for performance testing of high-resolution optical coherence tomography systems

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## ABSTRACT

For performance testing of high-resolution label-free optical coherence tomography (OCT) systems, durable test standards with optical properties similar to native biological tissues are highly desirable. Therefore, we explored the establishment of *ex-vivo* 3D samples from dissected mouse retina. To achieve high stability, dissected tissue parts were embedded in EPON resin. Results from comparative investigations with OCT on murine retina in agarose demonstrate that by utilization of appropriate EPON embedding protocols durable samples with adequate refractive index properties can be created in which even tiny structures near the OCT resolution limits can be detected.

**Keywords:** Optical coherence tomography, retina, system performance testing, durable test standards, label-free imaging

## 1. INTRODUCTION

High resolution optical coherence tomography (OCT) [1,2] is a well-established technique for non-invasive label-free imaging in ophthalmology to aid the diagnosis of diseases such as macular degeneration, multiple sclerosis and glaucoma. However, there is still a high demand for more advanced imaging approaches. Improvement of screening and basic diagnostics can be expected by means of increased 3D resolution and stain-free contrast, e.g., by sophisticated ultra-high resolution (UHR) OCT systems. For example, in the diagnosis of glaucoma, which is a major cause of blindness throughout the world [3], novel UHR OCT systems may significantly contribute to a more precise determination of the thickness of the involved degenerating tiny inner retina layers. In this context, durable test standards with optical properties and layered structures similar to native biological tissues are highly desirable for reliable performance testing of such novel UHR OCT devices.

Murine retina tissue comprises thin layers of different cell types and tiny anatomic structures such as the optic nerve head. Thus, we explored the establishment of reliable *ex-vivo* 3D test samples from dissected mouse eyes for performance testing of high resolution OCT systems. To achieve a high temporal stability, dissected tissue parts were embedded in EPON resin. Moreover, a comparative characterization of the generated specimens with state of the art high resolution OCT and murine retina in agarose was performed. Our results demonstrate that, by utilization of appropriate EPON embedding protocols, durable 3D samples with adequate refractive index properties can be created, in which even tiny structures near the OCT resolution limits can be detected.

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## 2. MATERIALS AND METHODS

### 2.1 Preparation of OCT test standards from mouse retina

Murine eyes were isolated from healthy mice. After the extraction, in a first step, the whole eyes were fixed for 1 hour in 4% paraformaldehyde (PFA) solution in phosphate buffered saline (PBS) and then were pierced by a sharp scalpel at the limbus between the cornea and the sclera. The cornea was removed from the eyeball by cutting in a circular manner using Vannas scissors. Then, lens and vitreous humor were taken out while the retina, including the optical nerve head, remained within the eyecup. Subsequently, the size of the eyecup was reduced by cutting the sclera and the retina in parallel to the limbus approximately at the equator of the eye.

Two types of samples were prepared for investigations with optical coherence tomography. In order to preserve the native tissue properties, one batch was embedded in 1 % agarose in well chambers (µ-Slide, 8 well, ibidi GmbH, Munich, Germany). A second sample batch was further fixated in 1.25% glutaraldehyde and 2% formalin for several days, washed in tap water, then was dehydrated utilizing an ascending alcohol series, subsequently incubated with propylene oxide and finally embedded with EPON resin (casted in an embedding disposable, curing time: 24h at 60°C).

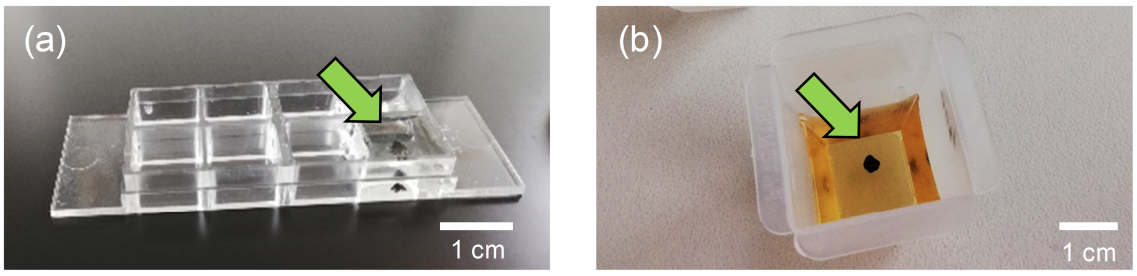


**Figure 1.** Representative images of established 3D test standards from dissected murine retina. (a) Control sample: PFA fixated mouse retina embedded in agarose. (b): PFA fixated mouse retina embedded in EPON resin within an embedding disposable. Green arrows indicate the sample within the embedding medium.

### 2.2 Setup for optical coherence tomography

Characterization of the established test standards (see Fig. 1) was performed utilizing a commercial state of the art high resolution spectral domain OCT system (Thorlabs Ganymede Series Spectral Domain system) with a central operation wavelength of 900 nm (axial resolution of the system in air is specified to ≈ 3 µm, lateral resolution: 8 µm).

## 3. RESULTS

Fig. 2 shows representative results from the characterization of the established 3D test standards shown in Fig. 1 with OCT. Fig. 2a depicts a B-scan of PFA fixated mouse retina in agarose which served as control sample, while Fig. 2b presents the corresponding B-scan of PFA fixated mouse retina embedded in EPON resin. Note that the two samples were prepared by using tissue from different animals.

In both images tiny tissue structures of the retina such including ganglion cell layer (GCL), the inner plexiform layer (IPL), the inner nuclear layer (INL), the outer plexiform layer (OPL), the outer nuclear layer (ONL), the inner and outer segments (IS and OS, respectively), and the retinal pigment epithelium (RPE) [3] as well as the optic nerve head can be clearly recognized with highly similar contrast. While the sample in agarose degenerated after several weeks, the EPON embedded tissue did not change any of its characteristics during the same observation period.

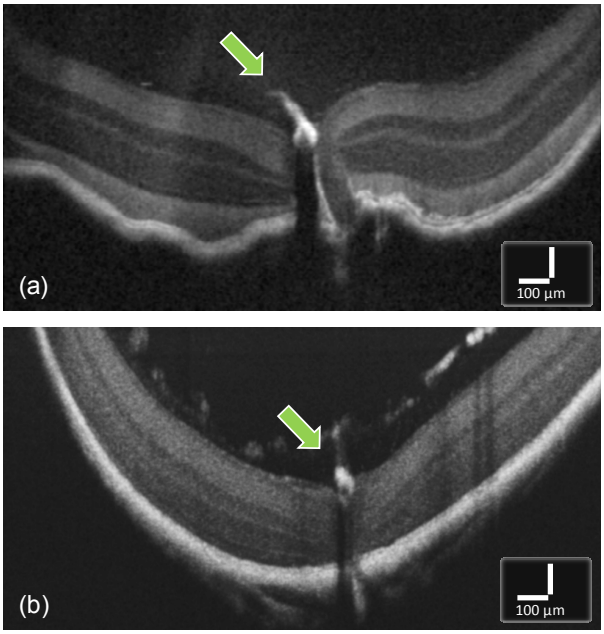


**Figure 2.** Representative B-scans. (a) Control sample (PFA fixated mouse retina embedded in agarose). (b) PFA fixated mouse retina embedded in EPON resin. Green arrow indicate the optical nerve head.

## 4. CONCLUSIONS

We have established durable 3D test standards with optical properties similar to native biological tissues from mouse retina by embedding of the sample in EPON resin. Results from comparative investigations with OCT on murine retina in agarose demonstrate that by utilization of appropriate EPON embedding protocols layered samples with adequate refractive index properties can be created in which even tiny structures near the OCT resolution limits can be detected. Current and future activities focus on suitable preparation protocol to achieve a further contrast enhancement as well as investigations with multispectral quantitative phase imaging [4,5] on the quantitation of the achieved spatial refractive index changes.

## ACKNOWLEDGEMENTS

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