

# Ultra High-Resolution 3D Anatomical MRI of the Ex Vivo Retina at 10x10x14µm

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**Introduction** The neural retina is characterized histologically into six distinct and highly stratified layers (1). From the vitreo-retinal interface, these layers are the ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), and inner+outer segment (IS+OS). The nerve fiber layer is on top of the GCL around the optic nerve head. Two flanking blood supplies nourish the retina (2). The *retinal* vasculature, located closest to the vitreous, exists predominantly within the ganglion cell layer but projects some capillaries deep into the IPL and INL. The *choroidal* vasculature, on the other hand, is external to the neural retina and is sandwiched between the retinal pigment epithelium (RPE) and the sclera. If considered with the six histologically defined layers, the *choroidal* vasculature (CH) constitutes an additional (seventh) layer.

In this study, we explored the use of ultra high-resolution 3D anatomical MRI to resolve different layers *ex vivo* rat retina at a nominal resolution of 10x10x14 µm. Multiple distinct bands of alternating signal intensities were consistently detected and carefully compared with similar histological sections. MRI and histological layer thicknesses were analyzed.

**Methods** Normal adult Sprague Dawley rats (250-300 g) were euthanized and eyes were enucleated. Extracted eyes were promptly injected with 10µl of a 20:1 solution of neutral buffered formalin to Gd-DTPA(0.5M Omniscan®) *via* a 30-gauge needle and a Hamilton syringe. Eyes were then immersed in 20:1 formalin:Gd-DTPA solution for 6hrs and then transferred to a 160:1 mixture for storage (at least 48hrs) (3). MRI experiments were performed on a Bruker 12-Tesla/16-cm scanner (Billerica, MA). A custom-built, small circular surface coil (id~1cm) was placed on the left eye. 3D FLASH (T<sub>2</sub>\*-weighted) MRI were acquired using TR = 39 ms, TE = 7.46 ms, data matrix = 720 x 720 x 512, and FOV = 7.3 x 7.3 x 7.3 mm, yielding an in-plane resolution of 10 x 10 x 14 µm. The total acquisition was 21 hrs. MRI layer thickness was quantified using an automated profile analysis (4). The layer thickness by MRI was taken at full-width half maximum. After MRI, the eyes were paraffin embedded and sectioned at 10 µm. H&E staining was performed. Each histological section was photographed and laminar thicknesses were measured. Histology slides were carefully matched with MRI for comparison.

## Results and Discussion

**Figure 1** shows a single slice from the 3D data along with the histology slide. MRI showed remarkable resolution and contrast around the optic nerve head. The characteristic convergences of various retinal layers are evident. MRI contrast and thickness corresponded well with other as tentative assigned. **Figure 2** shows the automated profile analysis and for the matching MRI and histological sections.

MRI and histological layer assignments and laminar thicknesses are summarized in **Table 1**. The overall thicknesses by MRI and histology are in good agreement.

## Conclusion

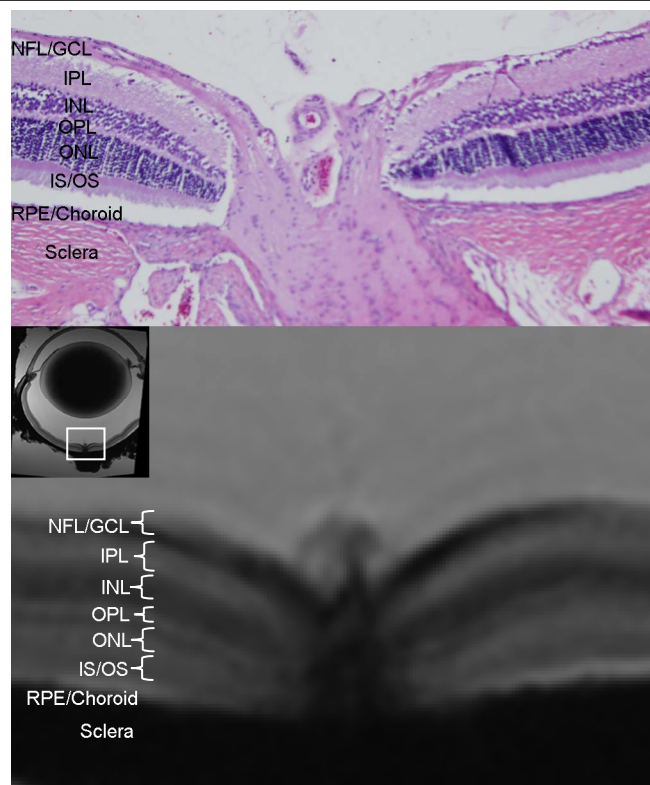
We developed and applied an ultra high-resolution 3D anatomical MR microscopy with manganese-enhanced MRI to study the *ex vivo* retina. This approach provides remarkable resolution and contrast, depicting multiple layers in the rat retina. These layers are consistent with histology. Future studies will apply this approach to image rat retinas *in vivo* and retinal disorders.

**References:** 1.H. Wassle, B. B. Boycott, *Physiol Rev* **1**, 447 (1991). 2. A.Harris, L. Kagemann, G. A. Cioffi, *Survey of Ophthalmol* **42**, 509 (1998). 3.Petiet AE *Proc Natl Acad Sci USA* **105**, 12331 (2008). 4.H. Cheng *et al.*, *Proc Natl Acad Sci USA* **103**, 17525 (2006).

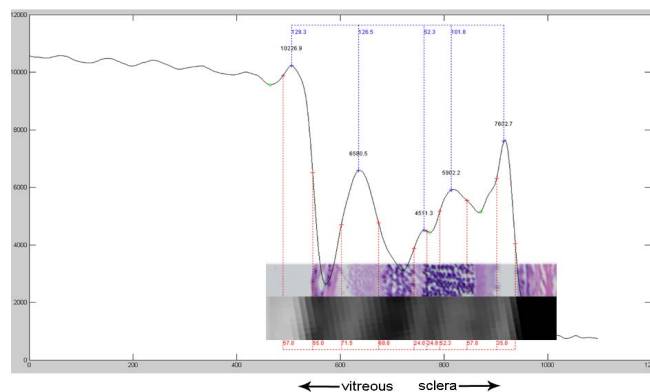
**TABLE 1.** The layer assignments and laminar thicknesses of normal retinas by MRI and histology (µm, mean±SD, n=5).

Band	MRI	Histology	Assignment
#1	48 ± 16	24 ± 5	GCL
#2	56 ± 17	54 ± 9	IPL
#3	61 ± 16	35 ± 12	INL
#4	29 ± 11	11 ± 3	OPL
#5, 6	66 ± 8*	70 ± 5	ONL
#7	51 ± 14	36 ± 4	IS OS
Total	311 ± 47	232 ± 16	

\*layer thickness includes 2 bands



**Figure 1.** A slice of the 3D MRI microscopy at a nominal resolution of 10x10x14 µm and a matching histological slide.



**Figure 2.** MRI layer thickness was quantified using automated analysis.