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Serial Sectioning for Examination of Photoreceptor Cell Architecture by Focused Ion Beam Technology

D. Mustafi*, A. Avishai**, N. Avishai**, A. Engel*, A. H. Heuer**, and K. Palczewski*

*Department of Pharmacology, Case Western Reserve University, Cleveland, OH, 44106-4965
**Department of Materials Science and Engineering, Case Western Reserve University, Cleveland OH, 44106-4956

The neural tissue of the eye, the retina, mediates normal vision and is composed of two types of light-sensitive neurons: rod and cone photoreceptors. With photoreceptor diameters on the micrometer scale and internal disc element structures even smaller, high resolution techniques are required to study these fine structures. Furthermore, with retinal dystrophies caused by photoreceptor degeneration as the major cause of familial blindness [1], it is of great importance to understand the structural architecture of photoreceptors, and the changes that accompany disease. Cryoelectron tomography elucidated the three-dimensional structure of the vitrified murine rod photoreceptor [2], but this was restricted by the size limit of the method, thus necessitating development of methods with a viewing area large enough to visualize photoreceptors at high resolution to decipher their internal architecture.

Techniques relying on light microscopy [3] in most cases cannot resolve neural network components that have diameters that are substantially below the wavelength of light. Electron microscopy, on the other hand, provides the resolution required to visualize and structurally characterize neural networks [4]. Transmission electron microscopy (TEM) has high lateral resolution, but is restricted by poorer resolution in the z-direction, thus limiting the reliability of reconstructions resulting from serial sectioning. Scanning electron microscopy (SEM) coupled with focused ion beam (FIB) technology [5] alleviates the distortion that accompanies TEM serial sectioning, because each tissue slice is imaged before sectioning. The ion source removes material from the surface of the specimen uniformly, exposing the internal architecture for serial imaging by the scanning electron beam for ultimate three dimensional reconstructions.

Murine models were used for photoreceptor structural analysis. *Wt* C57/BL6 strain mice and *Nrl*-deficient mice [6] at age 4 weeks were sacrificed and eye cups dissected under a surgical microscope. Tissues were then fixed in a solution of 2.5% glutaraldehyde, 0.1 M cacodylate buffer, 2% sucrose, pH 7.4, for 24 h at 4 °C, washed, and subsequently fixed in 1% OsO_4 in 0.06 M cacodylate buffer, 5% sucrose, pH 7.4, for 2 h. The samples were then washed and dehydrated with ethanol and dried by a critical point drying method [7]. The retina was mounted on standard SEM stubs with carbon tape and the edges covered with silver paste as needed and stored overnight at 30 °C. The cured samples were finally sputter-coated with 5-10 nm thick gold.

The prepared retina was placed inside an xT Nova Nanolab 200 or Helios Nanolab 650 (FEI) dual beam microscope. Once the area of interest was identified, a layer of platinum (Pt) was deposited by a gas injection system. Ion beam parameters were set to mill layers from the region of interest, i.e., 35-50 nm thick for these experiments. After each layer was removed, a secondary electron image was collected in the immersion lens mode. Images were collected with a dwell time of 10-30 μs/pixel at a resolution of 4096 x 3536 pixels. Acquired SEM images were

registered with Fiji-win32 by using a rigid feature extraction model. Individual photoreceptors and their disc elements were then mapped in Reconstruct.

Our results highlight the applicability of FIB-SEM to visual science research. FIB-SEM analysis of wild-type (*wt*) retinal tissue at various magnification and ablation conditions allowed sufficient field of view and resolution to reveal the larger scale packing architecture of rod photoreceptors and also the more detailed packing of internal photoreceptor disc elements. When looking at overall photoreceptor architecture our results for photoreceptor diameters and disc structure distances for *wt* mouse rod cells agree well with previously published data, and even with those that used non-fixed, vitrified photoreceptor samples [2]. Examination and analysis of retinal tissue from a mouse ($Nrl^{-/-}$) with phenotypic changes seen in the human retinopathy enhanced S-cone syndrome (ESCS) revealed the structural disruptions that accompany this disease. Reconstructions of individual ESCS photoreceptors revealed that there was considerable disruption in the normal packing of internal photoreceptor discs, with abnormal structures collected at the head of the outer segments at the retinal pigmented epithelium interface.

The methodology in this work presents a new way to study the neuronal cells of the eye and can be extended to other neuronal tissues for more detailed structural characterizations, especially those changes that occur in the face of disease.

References

1. A. I. den Hollander et al., *J Clin Invest*. 120 (2008) 3042.
2. S. Nickell et al., *J Cell Biol* 177 (2007) 917.
3. J. Livet et al., *Nature* 450 (2007) 56.
4. K. M. Harris et al., *J Neuroscience* 26 (2006) 12101.
5. S. Reyntjens and R. Puers *J Micromech Microeng* 11 (2001) 287.
6. A. J. Mears et al., *Nature Genetics* 29 (2001) 447.
7. D. F. Bray et al., *Microsc Res Tech* 26 (1993) 489.

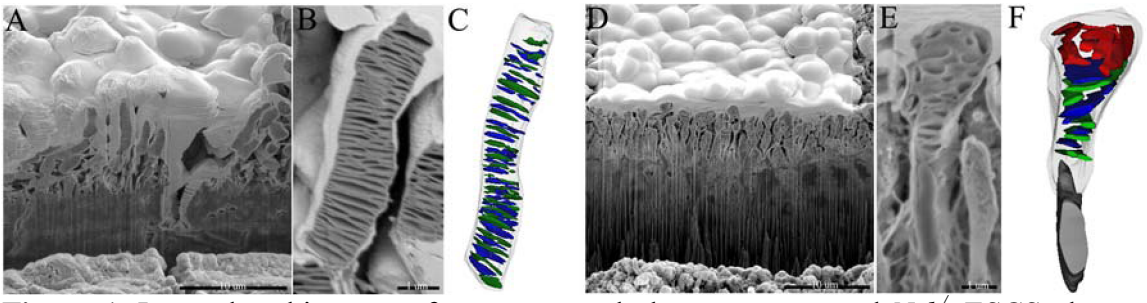

Figure 1. Internal architecture of *wt* mouse rod photoreceptors and $Nrl^{-/-}$ ESCS photoreceptors. (A) FIB milling allows visualization of the internal architecture with (B) a zoomed image of a single rod photoreceptor used for (C) the reconstruction shown. The rod cell outer segment membrane is outlined in a transparent gray color with the internal discs shown in blue and green colors. (D) FIB milling allows visualization of the internal architecture with (E) a zoomed image of a single ESCS photoreceptor used for (F) the reconstruction shown. The ESCS photoreceptor inner segment is shown in gray and the outer segment in transparent gray. Disc elements inside the outer segment are shown in blue and green with the disrupted elements at the head in red. Scale bars in panels A, B, D and E are 10, 1, 10, and 1 μm, respectively.

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