Confocal microscopy of light-induced holes deep in compound eyes of white-eyed *Drosophila*.

Anderson, Katelyn[1], and William S. Stark[2]. [1]Medical School, [2]Department of Biology, Saint Louis University, St. Louis, MO 63103. e-mail starkws@slu.edu

For two decades, this laboratory has utilized confocal microscopy and optical ("pseudopupil") techniques (Stark and Thomas, 2004) to investigate rhodopsin levels and retinal degeneration in the *Drosophila* compound eye (Zinkl, *et al*., 1990). All the time, strikingly clear images of tips of the rhodopsin-containing organelles (rhabdomeres) were seen in a narrow band delimited by the small "optical section", which is the hallmark of confocal microscopy, especially when green fluorescent protein (GFP) labeled the rhodopsin (Shah, *et al*., 2011).

With newer techniques (Selimovic *et al.,* 2010), we have appreciated our rediscovery of Zinkl *et al.*'s findings that (1) white-eyed flies reared on a day-night cycle of room lighting have less rhodopsin than those maintained in the dark, and (2) if kept in constant room light, they have receptor degeneration.

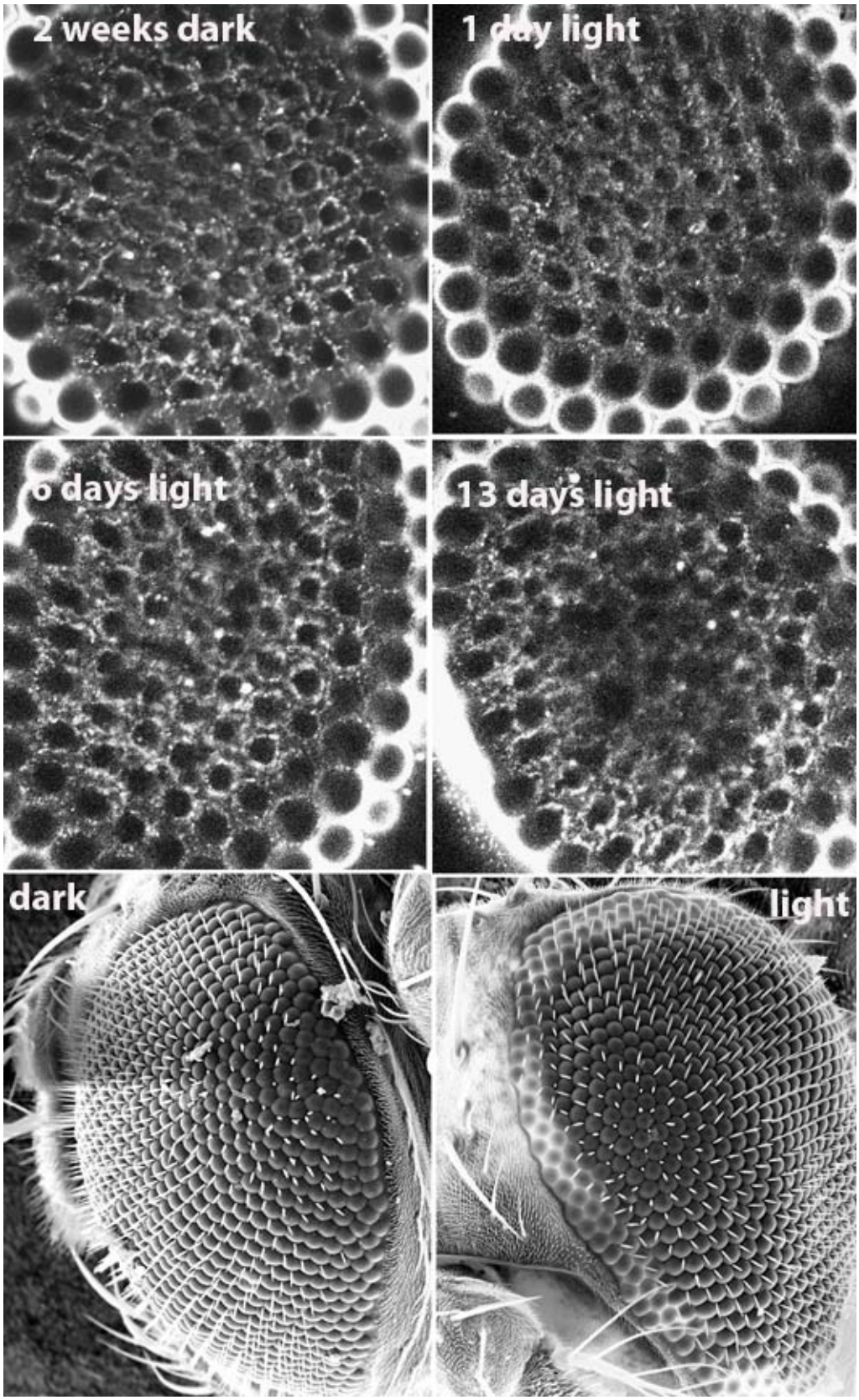
Never, until recently, did we think that there was useful information in confocal images at a deeper plane of section than that where the rhabdomere tips were seen. Here we show that "holes" or "islands" of ommatidial irregularity show up deeper in the retina as light-induced degeneration progresses. The accompanying Figure (top four images) presents confocal micrographs; most of the fluorescence is from the cornea, seen around the perimeter, and the unpigmented pigment granules of these white-eyed flies, revealing the ommatidial array. Notice the regularity in the ommatidial array in flies that had been maintained in the dark for two weeks; the dim autofluorescence of rhodopsin (the R1-6 rhabdomeres) can even be seen. Structure is still intact for flies that had been moved from dark to light for 1 day; as expected, rhabdomere autofluorescence is diminished since there is less rhodopsin. However, by 6 and 13 days in constant light, holes become apparent. These are, of course, selected images representative of a substantial body of data. We thought, while making these observations, that there might be more "misbehaviors" in the interfacetal bristles which should be at every other corner of each hexagon. However, scanning electron microscopy failed to reveal any difference between light- *vs* dark-reared flies.

The conclusions relevant to our research focus are that visual pigment decreases, then structural disintegration, can be seen non-invasively in the eyes of living white-eyed flies. In addition, other researchers may want to take advantage of the knowledge that they can see deeper than the surface into compound eyes using confocal microscopy.

Acknowledgments: Funding was from SLU's Beaumont and Presidential funds. This study was a follow-up from a serendipitous observation by George Denny (now in Washington University Medical School) when he used to work in this laboratory. We thank Prof. Jan Ryerse of Saint Louis University's Research Microscopy Core for help with the scanning electron microscopy.

References: Selimovic, A., *et al*., 2010, Dros. Inf. Serv. 93: 1-2; Shah, C., *et al.,* 2011, Dros. Inf. Serv. 94: 80-82; Stark, W.S., and C.F. Thomas 2004, Molec. Vision 10: 943-955 (on line at http://www.molvis.org/molvis/v10/a113); Zinkl, G., *et al.,* 1990, Vis. Neurosci. 5: 429-439.

Figure 1. See next page.


2 weeks dark
1 day light
o days light
13 days light
dark
light