2014

# Retinal Imaging with Virtual Reality Stimulus for Studying Salticidae Retinas

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# Retinal imaging with virtual reality stimulus for studying *Salticidae* retinas

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## ABSTRACT

We present a 3-path optical system for studying the retinal movement of jumping spiders: a visible OLED virtual reality system presents stimulus, while NIR illumination and imaging systems observe retinal movement.

Keywords: retinal imaging, salticidae, stray light, NIR

## 1. INTRODUCTION

Salticids, spiders in the family *Salticidae (*commonly known as jumping spiders), have a unique visual system consisting of two principal eyes and six "side eyes" [1]. The principal eyes are responsible for most of the spiders' behavioral response to stimulus, while the side eyes orient the spider to view a stimulus with the principal eyes[1]. The principal eyes' relatively high visual acuity allows Salticids to accurately target and spring themselves onto prey, find potential mates, and avoid predators[2]. All of the lenses of these eyes are fixed to the carapace of the spider, but the retinas of the principal eyes are movable, allowing the spider to obtain a higher field of view with a smaller retina. Without this motion, the principal eyes' field of view would be limited to 0.8°-5° due to the angular view of the retina[3]. The retinas assist in pattern recognition by scanning across a target in the principal eyes' field of view, but precisely how different stimuli affect this retinal motion is not well known[4]. We present an optical system to study how various stimuli affect the motions of the retina: a stimulus (an image of predator, prey, or potential mate) is presented to the spider and the subsequent retinal motion is observed in real time with an imaging system that does not interfere with the stimulus. A similar optical system described by Canavesi et al. ("Generation I")[5] was the predecessor to this design ("Generation II"). Generation I (Gen I) built on an original design by a prior investigator that was analyzed and optimized by Canavesi et al., with the main goal to improve on the MTF of the retinal imaging path. While stray light was mitigated in part with beam dumps, it proved to remain insufficient. The purpose of Generation II (Gen II) is to further address the stay light inherent in the original and Gen I designs as a result of the in-line shared illumination and imaging paths. A goal remains to optimize the imaging path and to use off-the-shelf components to minimize overall costs.

## 2. OPTICAL DESIGN

An optical system was designed to present an active stimulus to the spider via a visible projection system and image its retina position in real time via a NIR illumination and imaging system. The stimulus image is generated by an OLED display that is then magnified and relayed to a virtual image 250 mm in front of the spider. The illumination is generated with a 738 nm LED fiber source that is relayed to a mask and then to the pupil of the spider. The NIR light enters the spider eye and a fraction scatters off the retina back into the optical system. The retina is conjugate to the CCD through the imaging path, so this scattered light from the retina forms an image. Figure 1 shows a spider on a ball allowing it to walk/move while being presented a stimulus.

International Optical Design Conference 2014, edited by Mariana Figueiro, Scott Lerner,
Julius Muschaweck, John Rogers, Proc. of SPIE-OSA Vol. 9293, 92931C · © 2014 SPIE
CCC code: 0277-786X/14/$18 · doi: 10.1117/12.2073271

Figure 1. Image of a jumping spider standing on a ball that allows it to "walk" while its retinas are being imaged.

With the main goal, the full reduction of stay light, cube splitters in the prior generations are now replaced with pellicles. The three sub-systems share a common path as shown below in Fig. 1. The three paths share two doublets near the spider and are split via pellicle beamsplitters; the imaging and projection paths share one 50/50 pellicle with the projection path folding 90°, and all three paths share a second pellicle with the illumination path folding 90°. The imaging path transmits through both pellicles and an additional filter, a NIR passband filter.

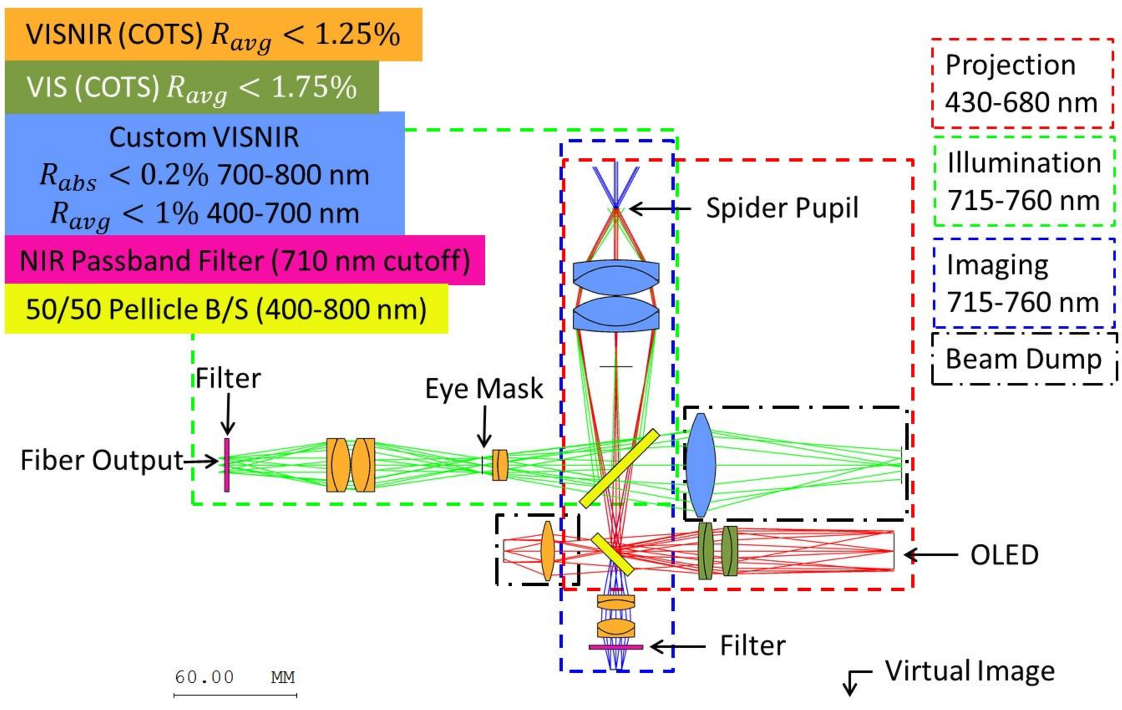


Figure 2. Optical layout showing the three sub-system paths: visible OLED projection (red), NIR illumination (green), NIR imaging (blue). The lenses, filters, and beamsplitters are colored according to their coatings. Beam dumps are boxed in black.

### 2.1 "Virtual reality" OLED projection path

The projection path relays an image or series of images of a stimulus (prey, predator, potential mate, etc.) from a visible OLED microdisplay to a virtual image 250 mm in front of the spider. The OLED is a full-color display with dimensions 15.36 x 12.29 mm and a 1.3MP resolution. The full OLED diagonal is mapped to 55° in the spider's field of view. The mapping is such that the size of an OLED pixel in virtual image space is smaller than the smallest resolution element of the spider's eye, as determined by the angular size of its photoreceptors. This ensures that the spider cannot resolve pixels in the virtual image. To achieve the required magnification of 13.2x, the projection path uses two off-the-shelf doublets in a back-to-back configuration in addition to the shared doublets. The projection path is folded 90° relative to the optical axis of the straight-through imaging path.

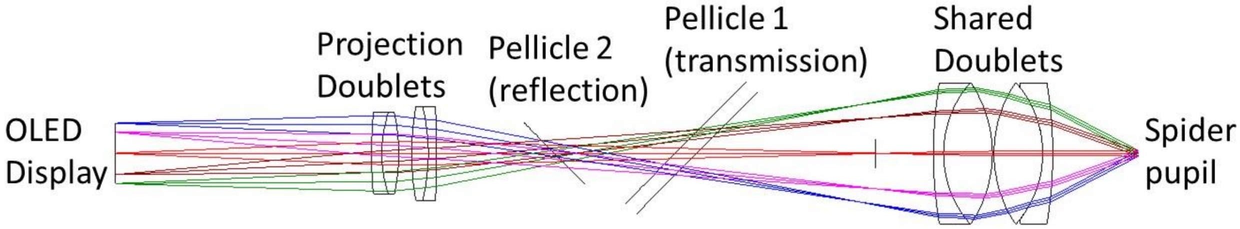


Figure 3. Optical layout of the stimulus projection sub-system showing how the OLED display is projected as a virtual image to the spider. The rays are diverging at the spider pupil so the object appears a finite distance away (250 mm) to the spider (virtual image surface not shown).

| 5/27/2014 | Projection Requirements & Specifications | | | |
|---|---|---|---|---|
| Item # | | Requirement/Goal | Generation I | Generation II |
| 1 | Entrance Pupil Diameter (spider pupil) (mm) | 1.6 | 1.57 | 1.6 |
| 2 | Full Field of View (spider) (degrees) | 60 | 52 | 55 |
| 3 | Virtual Image Distance (mm) | 250 | 255 | 250 |
| 4 | Spider-to-First Lens Clearance (mm) | >25.00 | 25.5 | 25.5 |
| 5 | Display Type | | LCD | AMOLED |
| 6 | Display Dimensions (mm) | | 71 x 53 | 15.36 x 12.29 |
| 7 | Used Display Semi-Diagonal (mm) | | 12.6 | 9.84 |
| 8 | Used Display Resolution (pixels) | | 158 x 117 | 1280 x 1024 |
| 9 | Pixel Pitch (at OLED) (µm) | | 64 | 12 |
| 10 | Magnification (OLED to Virtual Image) | Determined by used display semi-diagonal | 9.9 | 13.2 |
| 11 | Maximum Spatial Frequency at Virtual Image, determined by spider resolution (15 arc minute) (lp/mm) | 0.917 | same | same |
| 12 | Maximum Spatial Frequency at OLED (magnification X Spat. Freq. at Image) (lp/mm) | Depends on magnification | 9.1 | 12.1 |
| 13 | Wavelengths (nm, weight) | Visible, mimic spider spectral response | Photopic | |
| 14 | MTF @ Max spatial frequency (item 12) at OLED | > 0.3 | > 0.31 | >0.33 |
| 15 | Distortion (Max) (%) | 20 | 18 | 9.3 |

Figure 4. Specification table for the stimulus projection system, including the Gen I specifications for reference.

Figure 3 shows the unfolded projection path including the pellicle surfaces. The two projection doublets form an intermediate image of the OLED after passing through the two pellicles, and the shared doublets relay this image to the final virtual image. Figure 4 shows the specification table for the design of the Gen I and Gen II systems. The projection path was designed from long-to-short conjugates, which means from virtual image to OLED display, which is why the specifications are in OLED space. As seen in the table, the distortion is significantly reduced in the Gen II system while maintaining the MTF requirements.

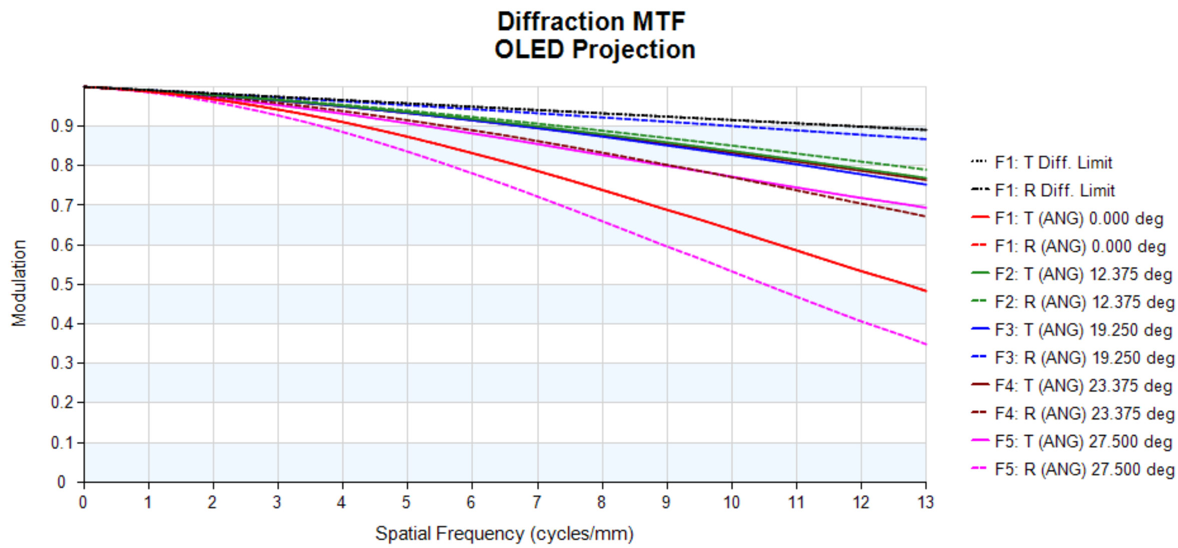


Figure 5. MTF of the stimuli projection system at 5 fields with Chebyshev spacing. The spatial frequency goal is greater than 30% modulation at 12.1 lp/mm.

## 2.2 NIR LED fiber illumination

An LED fiber source centered at 738 nm provides the illumination for the retinal imager. Since the spiders are not sensitive to wavelengths above 700 nm, the illumination will not interfere with the stimulus, as desired. As seen in figure 6, the illumination path is folded 90° using the pellicle closer to the spider. A mask is placed at an internal image of the fiber which is then relayed to the spider such that the fiber output, the mask, and the spider pupil are conjugate to each other, providing Kohler illumination of the retina. The mask is used to reduce reflections off the spider's face.

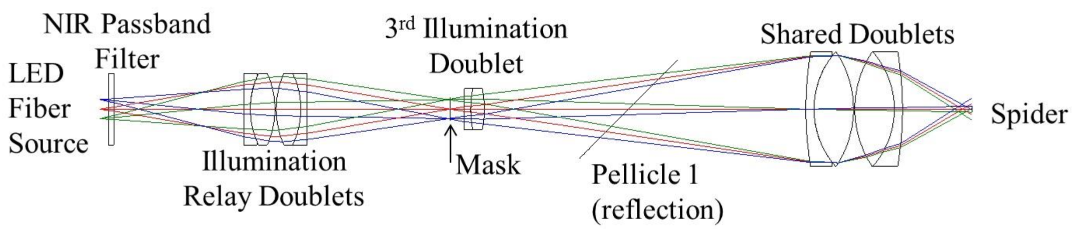


Figure 6. Unfolded layout of the illumination path showing the fiber output relayed to the mask and subsequently the spider pupil.

The original and Gen I systems had the illumination path sharing more of the path with the projection system, causing the mask to obstruct the stimulus path. By moving the illumination path folding closer to the spider, the mask is solely in the illumination path in Gen II. This image relay requires three off-the-shelf doublets in addition to the shared doublets: two in a back-to-back configuration, and one after the mask. The first two doublets relay the fiber output to the mask plane. The third doublet provides some power to de-magnify the mask down to the size of the spider face, since the two shared doublets are in a fixed configuration relative to the spider because of the imaging and projection paths. Figure 7 provides the specifications for both Gen I & II. Gen II improves primarily by using an NIR LED source instead of an incandescent illuminator. Figure 8 shows the relative spectra of the source, the filter transmission, and the pellicle transmission and reflections. The filter is used to eliminate the small amount of visible light from the LED source from interfering with the stimulus.

| 5/27/2014 | **Illumination Requirements & Specifications** | | | |
|---|---|---|---|---|
| Item # | | Requirement/Goal | Generation I | Generation II |
| 2 | Used Fiber bundle semi-diameter (mm) | 6.35 | 3.5 | 3.5 |
| 3 | Mask semi-diameter (mm) | 2.45 (from Cristina's slides) | 2.45 | 2.45 |
| 6 | Mask image semi-diameter on spider face (mm) | 0.8 | 0.8 | 0.8 |
| 7 | Magnification from source to mask | 0.551 | 1 | 1 |
| 8 | Magnification from mask to spider | 0.327 | 0.327 | 0.327 |
| 9 | Magnification from source to spider | 0.18 | 0.327 | 0.327 |
| 10 | Fiber Bundle NA | 0.55 | 0.55 | 0.55 |
| 11 | Used NA at source | - | 0.141 | 0.147 |
| 12 | Used NA at spider | >0.5 | 0.41 | 0.46 |
| 13 | Peak Wavelength (nm) | NIR | 650 | 738 |
| 14 | FWHM (nm) | - | N/A | 35 |
| 15 | Source Power | - | 1750 lumens* | 3900 mW |
| 16 | Filter (central wavelength, bandwidth) (nm) | Passes only 700-800 nm (not visible) | 795, 150 | 795, 150 |
| 19 | Wavelength (nm) | 700-800 , source spectrum | 700-800 | 716-761 (source spectrum) |

Figure 7. Illumination path specifications for both Gen. I and Gen. II systems.

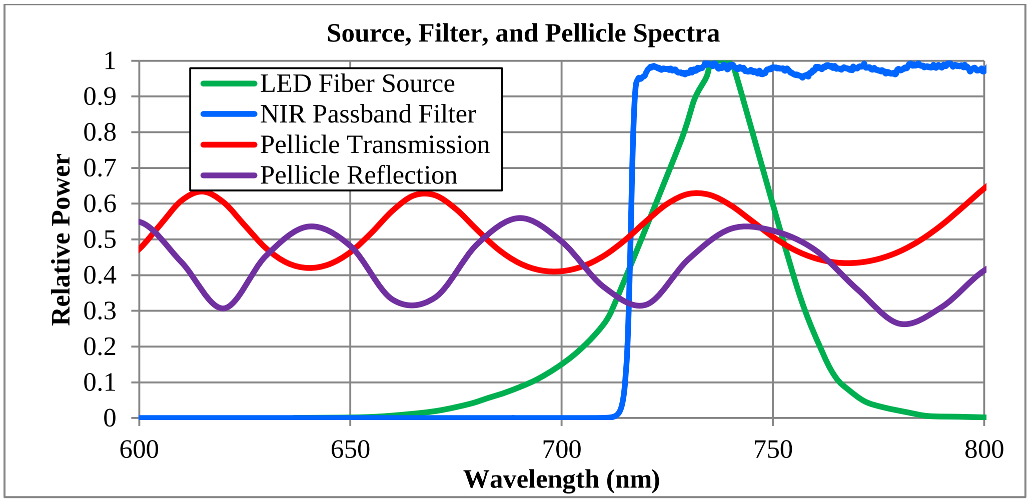


Figure 8. Spectra of the source, filter, and pellicle. Although the LED still emits some visible light, the filter removes it from interfering with the stimulus path.

### 2.3 NIR imaging path

The imaging path consists of two back-to-back off-the-shelf doublets in addition to the shared doublets. The system was designed using an object at optical infinity with the stop at the spider pupil, which places the spider retina conjugate to the 1.3 MP CCD. In order to track the retina, the position and rotation angle of the spider retina need to be resolved. This is accomplished by mapping the 1.3 MP CCD to a 55° field of view at the spider with a 0.5° resolution in object space (see figure 9). As figure 10 shows, the form for the imaging path is similar to the projection path, but the conjugates are slightly different, so they require separate designs.

| 5/27/2014 | **Imaging Requirements & Specifications** | | | | |
|---|---|---|---|---|---|
| | | Requirement/Goal | | Final Specification | |
| Item # | Specification | Generation I | Generation II | Generation I | Generation II |
| 1 | Entrance Pupil Diameter (spider pupil) (mm) | 1.6 | 1.6 | 1.57 | 1.6 |
| 2 | Full Field of View (degrees) | 60 | 60 | 53 | 55 |
| 3 | Image Semi-Diameter (detector height) (mm) | 2.66 | 2.72 | 2.47 | 2.72 |
| 4 | Focal Length ( h = f*tan(theta)) (mm) | 4.61 | 4.71 | 4.95 | 5.23 |
| 5 | Camera Sensor Dimensions (H x V) (mm) | 6.65 x 5.32 | 6.79 x 5.43 | Same | Same |
| 6 | Camera Sensor Resolution (H x V) | 1280 x 1024 | 1280 x 1024 | Same | Same |
| 7 | Pixel Size (H x V) (μm) | 5.2 x 5.2 | 5.3 x 5.3 | Same | Same |
| 8 | Sensor Nyquist Frequency (lp/mm) | 96.2 | 94.3 | Same | Same |
| 9 | Resolution needed in object space to track retina (degrees) | 0.5 | 0.5 | Same | Same |
| 10 | Max Spatial Frequency needed on detector to track retina (focal length dependent) (lp/mm) | 24.9 | 24.3 | 23.1 | 21.9 |
| 11 | Object Distance (mm) | Infinity | Infinity | Same | Same |
| 12 | Spider-to-First Lens Clearance (mm) | >25.00 | >25.00 | 25.5 | 25.5 |
| 13 | Wavelength (nm) | 700-800 , source spectrum | 700-800 , source spectrum | 700-800 | 716-761 |
| 14 | MTF @ Max Spat. Freq (item 10) | >0.3 | >0.3 | >0.25 | >0.47 |
| 15 | Max Distortion (%) | < 15 | < 15 | 18 | 11.7 |

Figure 9. Imaging path specifications for Gen I and Gen II. The distortion is reduced and MTF improved in Gen II over Gen I.

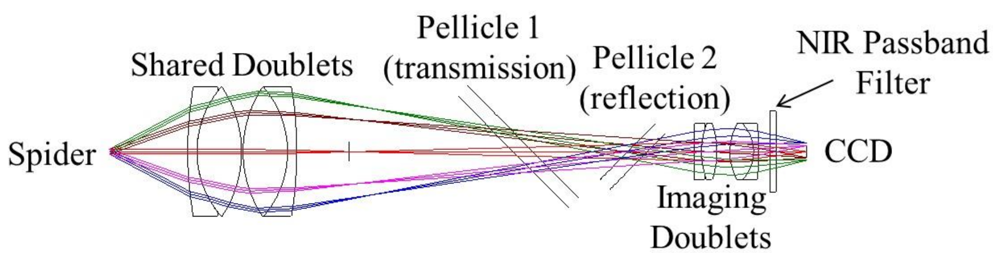


Figure 10. Layout of the imaging path. The scattered light from the retina passes through the spider eye and into the shared doublets, through both pellicles and the filter, and is imaged onto the CCD.

## 3. MINIMIZING STRAY LIGHT

The imaging path uses the scattered light off the retina to form an image, so back reflections off any elements in the shared path could significantly reduce the signal-to-noise ratio in the retinal image. Planar surfaces were avoided in the design, which is the reason for using pellicle beamsplitters over traditional cube beamsplitters. This was the main source of back reflections in Gen I, accounting for over 75% of back-reflected power hitting the detector, so simply removing these planar surfaces from the shared path significantly reduced back reflections. In addition, Gen I used a shared custom aspheric plate which had one planar surface, whereas Gen II avoids this altogether. Modeling both Gen I and Gen II in FRED Optical Engineering software showed that to further reduce the back reflections off the shared doublet elements to acceptable levels, a specialized anti-reflection coating was required. Figures 11-13 show the effect of back reflections on the visibility of the signal. A 0.2% AR coating was simulated on the shared elements to reduce the back reflections to significantly lower levels. Since the percentage of light reflecting off the retina is not directly known, it was estimated by varying that percentage in the Gen I simulation until it matched previous observations from Gen I. Using this analysis, approximately 10% of the NIR light entering the spider pupil is reflected by the retina. Using this model gives the results in figures 11-13 for Gen I and figures 14-16 for Gen II. The SNR is in Gen II is over 22, improved by over a factor of 40 from the Gen I SNR of 0.50.

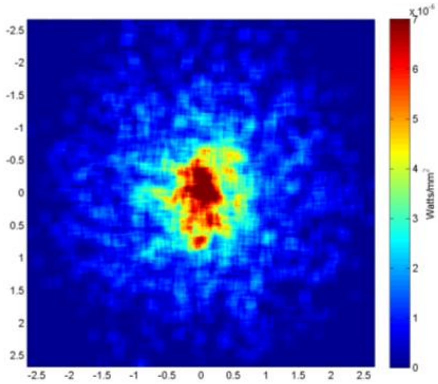

Figure 11. Simulated back reflections on CCD, Gen I

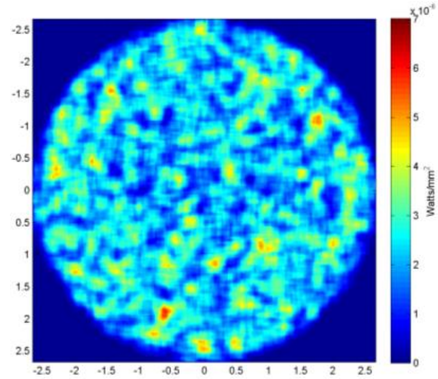

Figure 12. Simulated retina signal, Gen I

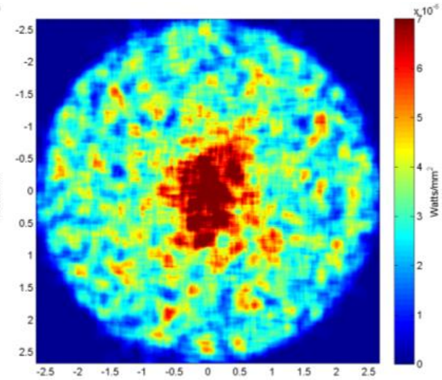

Figure 13. Simulated sum of back reflections and signal, Gen I

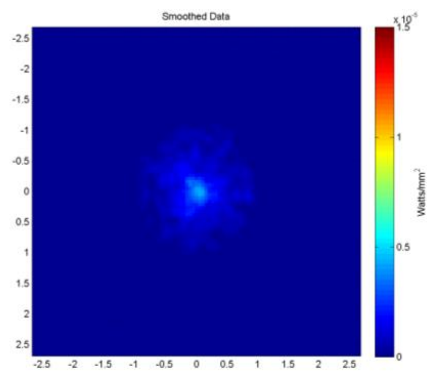


Figure 14. Simulated back reflections on CCD, Gen II

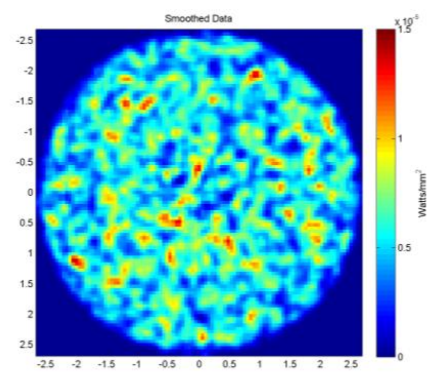


Figure 15. Simulated retina signal, Gen II

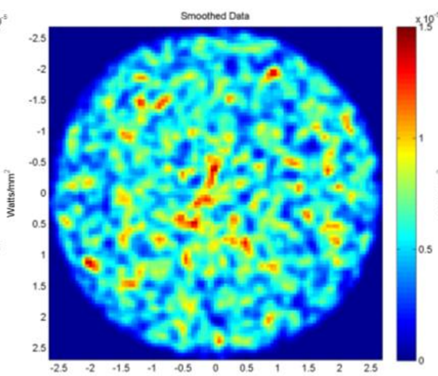


Figure 16. Simulated sum of back reflections and signal, Gen II

## 4. CONCLUSION

We have shown a design for Salticid retinal stimulus and tracking using a 3-path optical system with visible stimulus projection and NIR illumination/imaging. The design uses all off-the-shelf components and minimizes back reflections by avoiding planar surfaces and using a custom AR coating on the shared-path elements. With this system, it is possible to track the retina in real time and correlate the retinal motion with a certain stimulus, enabling further research into the ways stimuli and retinal motion influence Salticid behavioral response.

**Acknowledgement**: This work was funded by the National Science Foundation grants 0952822 and EECS-1002179, as well as by the ODALab (www.odalab-spectrum.org). Thanks to ORA for providing a CodeV student license and Photon Engineering for providing a FRED license.

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