A Novel Real-Time In-Vivo Mouse Retinal Imaging System

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Supplementary Materials

Historical Microscope Illumination Systems and Optical Constraints. All efforts to clearly monitor a trans-scleral, trans-choroidal subretinal injection using micro pulled glass needles were unsatisfactory using the commercial available non-contact illumination devices, due to the limited amount of light which was actually delivered to the retinal surface. Two different designs were evaluated including off-axis and on-axis illuminators. These devices are capable of visualizing the human retina, but the mouse eye has a number of unique characteristics making it a more difficult challenge. First, the volume of the adult mouse eye is approximately 375 times smaller than the volume of the adult human eye. Second, the dilated pupil is only one tenth the diameter of the dilated adult human pupil which makes it difficult to illuminate the interior contents as the area of the dilated mouse pupil is only about 1/100 that of the human pupil. Third, the steep curvatures of the cornea and the large spherical lens of the mouse create an eye with large optical power (~1000 diopters) and resultant spherical aberrations that further complicate retinal imaging. Microscopes that use off-axis illumination while viewing a small eye, like mouse, not only fail to deliver enough light to the retina but fail to direct the delivered light to the region of the retina being viewed due to angle of light path and the small pupil diameter (**Supp. Fig. 1A**). Although, Zeiss offers a coaxial system that directs the light beam emerging from a fiberoptic on-axis it makes no effort to couple the optical parameters of the light source and fiber into a shaped beam optimized for the small mouse eye. This results in extensive overfilling of the mirror and grossly overfilling the imaging plane where the eye is illuminated, such that little of the energy is delivered to the retinal surface which results in inadequate

illumination for high resolution imaging (**Supp. Fig. 1B**). These constraints and limitations sponsored the design requirements for the RIS which include a long working distance, delivering the illumination beam on-axis (coaxial) into the numerical aperture of the mouse pupil without overfilling the pupil, and maintaining the excellent field-of-view and field-of-depth of the standard Greenough type stereo Zeiss Stemi 2000-C microscope (**Supp. Fig. 1C**).

Fig. S1. Retinal Illumination Systems for Small Eyes. Schematic diagram illustrating difference between existing commercially available off-axis (**A**) and on-axis systems (**B**) used for retinal illumination and the RIS on-axis illuminator described herein (**C**). Off-axis illuminators (**A**) deliver only a small percentage of total light into the dilated mouse pupil due to poor optical coupling, angle of incidence, and long working distance. The commercially available on-axis illumination system (**B**) fails to properly couple the lamp and the fiber optic cable and results in extensive overfilling of the mirror and broad diffuse illumination of a large region of the stage such that a small proportion of the available energy enters the dilated pupil. The RIS delivers much of the available light energy into posterior segment of the mouse eye because it condenses a narrow beam on-axis into the dilated pupillary aperture of the small eye (**C**).

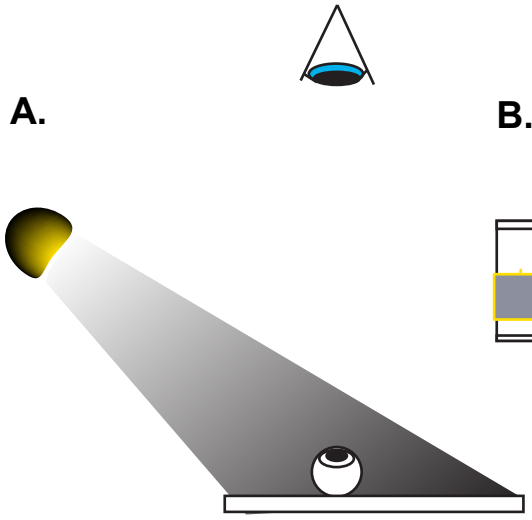
Fig. S2. Source Illuminator. Properties of the light source used for proof-of-principle design of the mouse retinal imaging platform. A Welch Allyn CL-100 fiber optic illuminator with a 100W Xenon lamp provides 350 Lumens of output when coupled to a small 1.4 mm core fiber with an NA of 0.52. SFI Microlink headlight fiber (1.4mm polymer fiber inside a teflon sheath

from Welch Allyn). Figure shows the absolute spectral output (Watts/cm^2) *vs.* wavelength. Excitation spectra for EGFP (**blue**) and %Transmission spectra for the far-red bandpass filter (**red**) are overlaid on the lamp output spectra (**black**). Data on the CL-100 output is courtesy of Welch Allyn. Data for the bandpass filter and EGFP absorption from Omega Optical /Nikon websites.

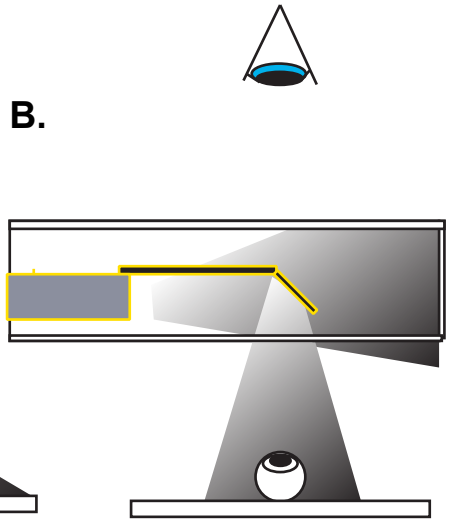
Fig. S3. Hruby lens principle. A Hruby lens is a plano concave or biconcave lens that compensates for the corneal curvature to improve retinal visibility (**A**). This optical effect can be simulated with a clear viscous gel and a thin flat glass coverslip (**B**).

Fig. S4. Comparison between Subretinal and Intravitreal injections. Sequential images of a subretinal and intravitreal injections were taken while injecting a Fluorescein Lite solution diluted to 10mg/mL with 1X PBS used to visualize the extent of the fluorescence spreading into the subretinal space (**bottom panel**) or into the vitreous (**top panel**) in real time. A twenty second injection (2.6 PSI at tip) captured in real time using 0.539 second exposure images taken every ~0.84 seconds over the duration of the entire injection. The final retinal detachment involves approximately 35% of total retina. Image was magnified to 1.61 and only the first 15 images are shown for each injection. The C1xBL/6 mouse retinal degeneration line was used (1.75 months of age).

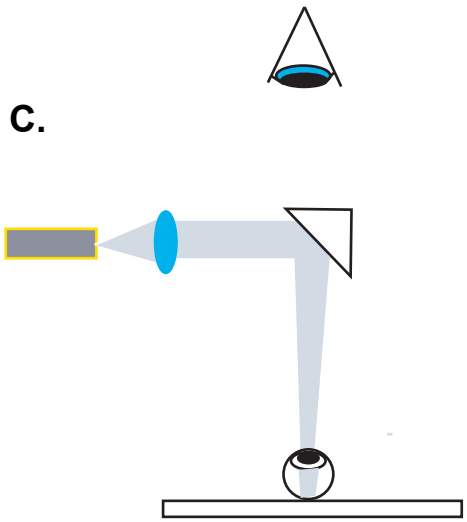
A.

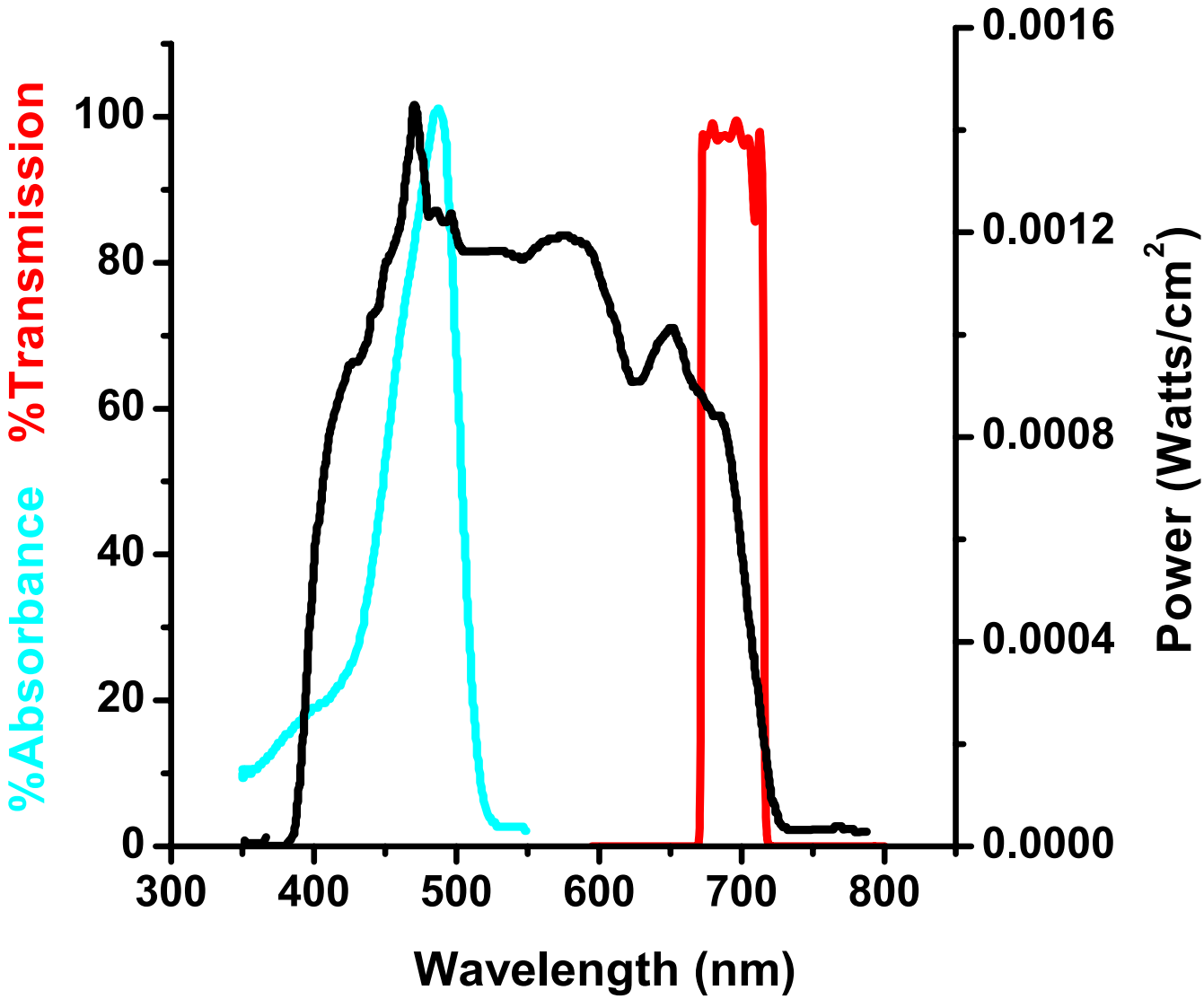


B.

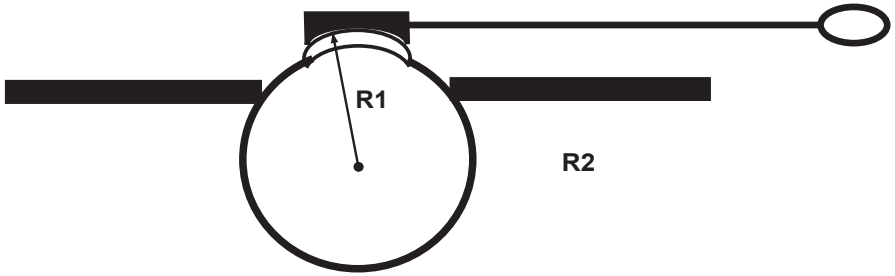


C.




%Absorbance
%Transmission
Power (Watts/cm^2)
Wavelength (nm)
0
20
40
60
80
100
0.0000
0.0004
0.0008
0.0012
0.0016
300
400
500
600
700
800

A.



B.