



# **Non-Invasive Detection of Unique Molecular Signatures in Laser-Induced Retinal Injuries: Future Battle Field Application**

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NASA Glenn Research Center

**AFOSR Program Review**

April 19, 2018 Arlington, VA



# Collaborative Translational Research Examples

NASA: Non-invasive Human Health  
Monitoring

NIH/NEI: Early diagnosis of Eye disease

USAF/DoD: Laser-induced Retinal injury

# NASA's Focus on Astronaut Health

- Enhance crew health and performance.
- Mitigate the risks associated with human spaceflight.

<https://www.nasa.gov/hhp/index.html>

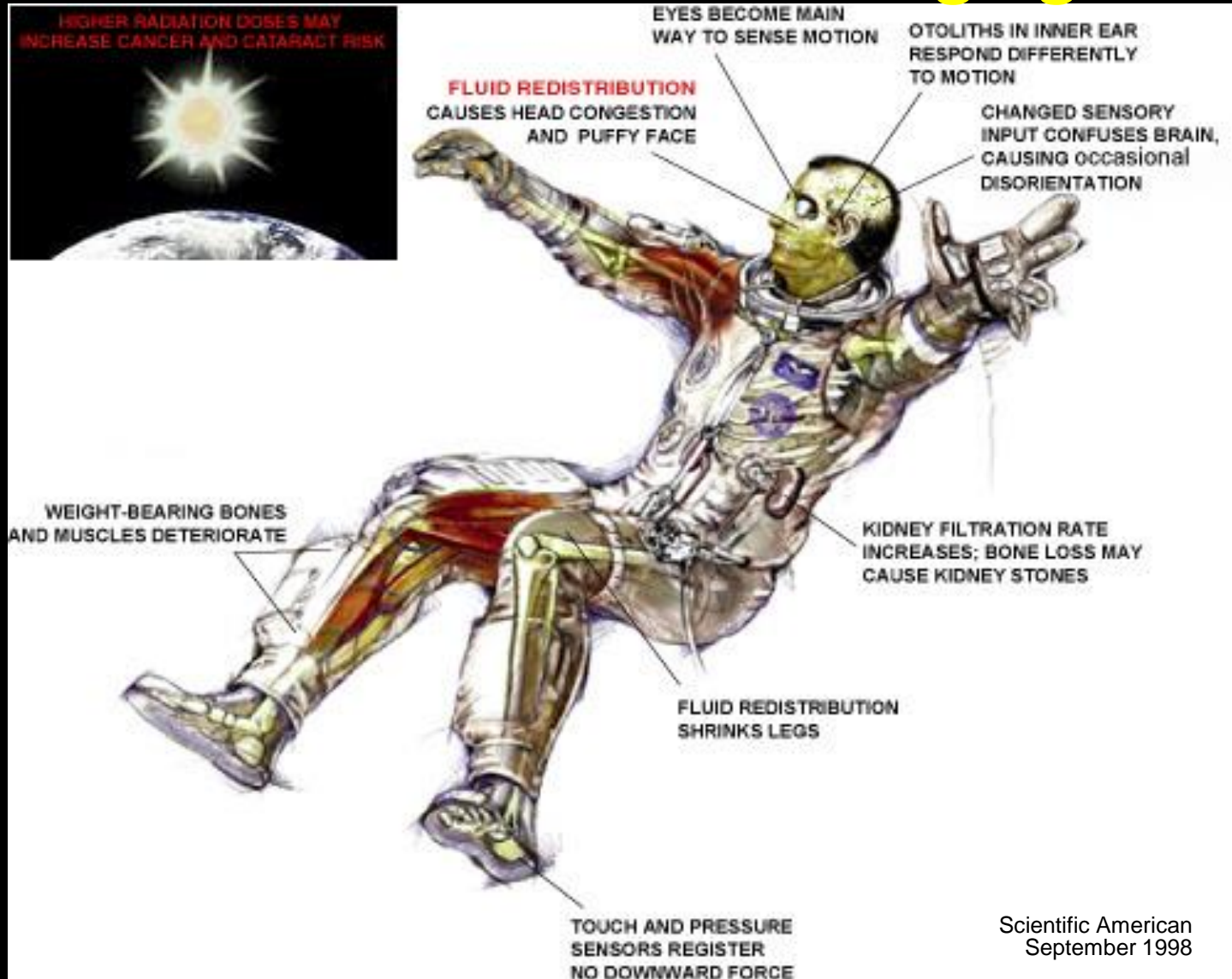
## Human Health and Performance Risks of Space Exploration Missions

Editors: Jancy C. McPhee, Ph.D.  
John B. Charles, Ph.D.



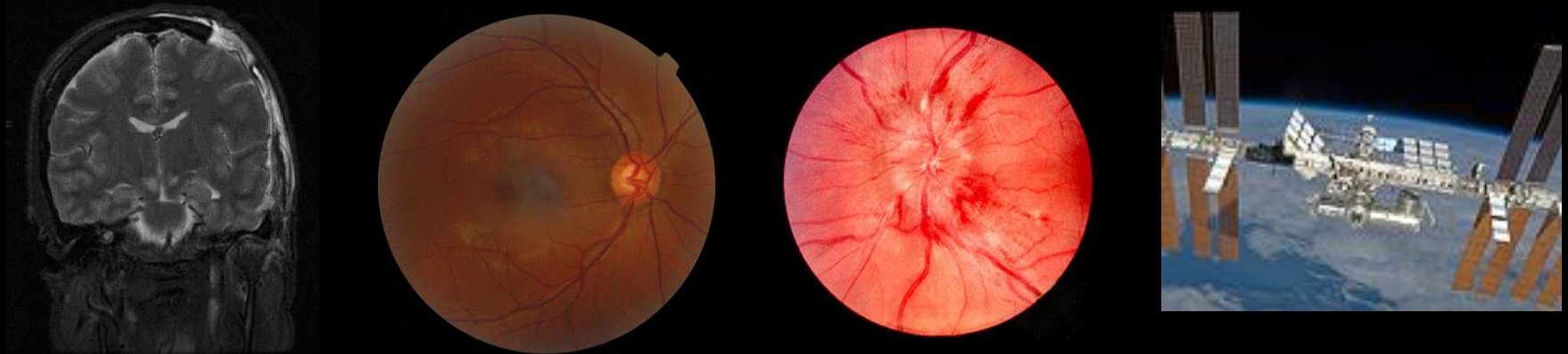
<http://humanresearchroadmap.nasa.gov/>

# Effects of Space Travel on the Human Body are Similar to that of Aging





# Increased ICP and Vision Problems in Astronauts



TH Mador et al, Optic Disc Edema, Globe Flattening, Choroidal Folds, and Hyperopic Shifts Observed in Astronauts after Long-duration Space Flight, Ophthalmology. 2011 ;118(10):2058-69

Major NASA Priority

# Significant Changes in Oxidative Biomarkers in astronauts are observed

Whole body cellular level injuries occur with oxidative stress due to reactive oxygen species (ROS)

**Oxidative Stress Leads to Aging and Disease**

**Space Travel Accelerates Aging**

# Holy Grail of Ophthalmology

## EYE "MICROCOSM OF THE BODY"

- Eye is built like a camera.
- Light from cornea to the retina traverses through tissues that are representative of nearly every tissue type in the body.
- **Cornea:** typical extra-cellular matrix composed primarily of collagen.
- **Aqueous:** an ultrafiltrate of blood, containing most of the molecules found in serum at concentrations that are reflective of serum levels.
- **Lens:** highly organized array of crystallin proteins.
- **Vitreous:** similar in nature to the articular cartilage and synovial fluid found in joints.
- **Retina and optic nerves** are in fact part of the central nervous system.
- Since eye is easily accessed by light, the optical technologies can be used for the evaluation of structure and physiology in health, aging, and disease.

## Early Detection and Prevention



## ABC NEWS Clip on Ansari's research



<https://www.youtube.com/watch?v=X4MwEg6ZgzY>

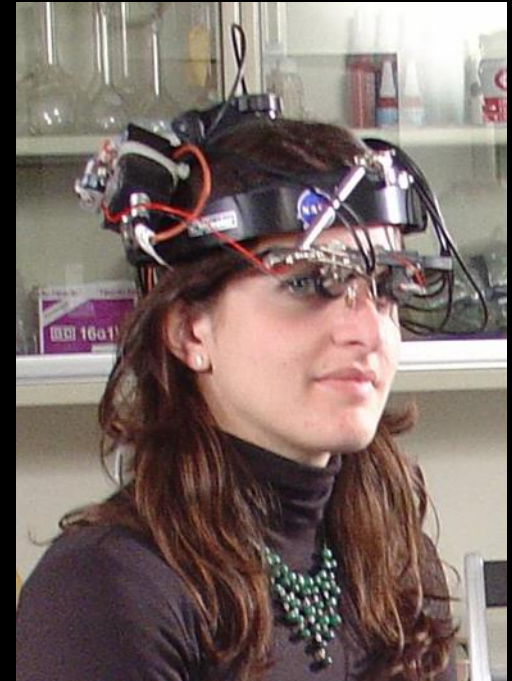
## NON-INVASIVE OPTICAL SENSORS and STUDIES

Technology Validation	Diseases/Studies
<b>Dynamic Light Scattering (DLS)</b> Animal Studies at FDA, NIH, Universities Clinical Studies at NIH & John Hopkins	<ul style="list-style-type: none"> <li>• Corneal Diseases and Wound Healing (LASIK), Lens Aging and Cataract, Uveitis, Glaucoma, Vitreous Aging and Lysis Studies, Cholesterol studies, Studies on Effects of Radiation, Hyperbaric Oxygen Diabetic lens studies and Vitreopathy, Oxidative Stress, Laser-Induced Retinal Injury Bio-Markers (USAF)</li> </ul>
<b>Corneal/Lens Autofluorescence</b> Clinical Studies by Collaborators in Europe	<ul style="list-style-type: none"> <li>• Radiation induced biological effects</li> <li>• Diabetic Retinopathy</li> </ul>
<b>Laser-Doppler Flowmetry (LDF)</b> Human Study in 0G Aircraft Glove-Box Astronaut Subject Study Premature Babies (UH)	<ul style="list-style-type: none"> <li>• Hemodynamic Response/Fingertip Study in astronauts</li> <li>• Choroidal blood circulation</li> <li>• AMD</li> <li>• Retinopathy of Prematurity</li> </ul>
<b>Raman Carotenoid Dispersion Analysis</b> Clinical Studies by Collaborators	<ul style="list-style-type: none"> <li>• Ocular Nutrition (Carotenoids)</li> <li>• AMD</li> </ul>
<b>Ocular Polarimetry</b> In Commercial Development	<ul style="list-style-type: none"> <li>• Blood-glucose Sensing/Diabetes</li> </ul>
<b>Tissue Oximetry</b> Flown on KC-135 and GA Airplane	<ul style="list-style-type: none"> <li>• Muscle Atrophy and Osteoporosis</li> <li>• Hyper-/Hypo-tension</li> <li>• Functional Imaging of Brain</li> <li>• Pilot Fatigue/Flicker Study</li> </ul>
<b>Wireless UV Sensor Goggles</b> Flown on GA Airplanes	<ul style="list-style-type: none"> <li>• UVA, UVB, UVC Simultaneous Dosimeters for Aviators, Astronauts, and others</li> </ul>
<b>Tele-Medicine</b> <b>Future Capability</b>	<ul style="list-style-type: none"> <li>• Non-Invasive and Early Detection of Various Ocular and Systemic diseases using "Eye and Skin as a window to the body"</li> </ul>



# Make *Invisible* - Visible

- *Beyond imaging  
Structural changes*
- *Early Detection of  
Ocular Disease at the  
molecular Scale long  
before the clinical  
symptoms appear*



“Paradigm Shifts in Ophthalmic Diagnostics”, Sebag et al., Trans Am Ophthalmol Soc, 114, 2016.

# Space Experiments and Eye Disease Studies using DLS

## Dynamic Light Scattering is a Unique Tool

- Non-invasive and quantitative
- Size range 3 nm to 3  $\mu\text{m}$
- Small sample volume
- Polydispersity and size distribution

## Other Names for DLS

- QELS: Quasi-Elastic Light Scattering
- PCS: Photon Correlation Spectroscopy
- IFS: Intensity Fluctuation Spectroscopy
- LBS: Light Beating Spectroscopy  
(Homodyne and Hetrodyne mixing)

For details:  
Laser Light Scattering  
by Chu  
Dynamic Light Scattering  
by Berne and Pecora



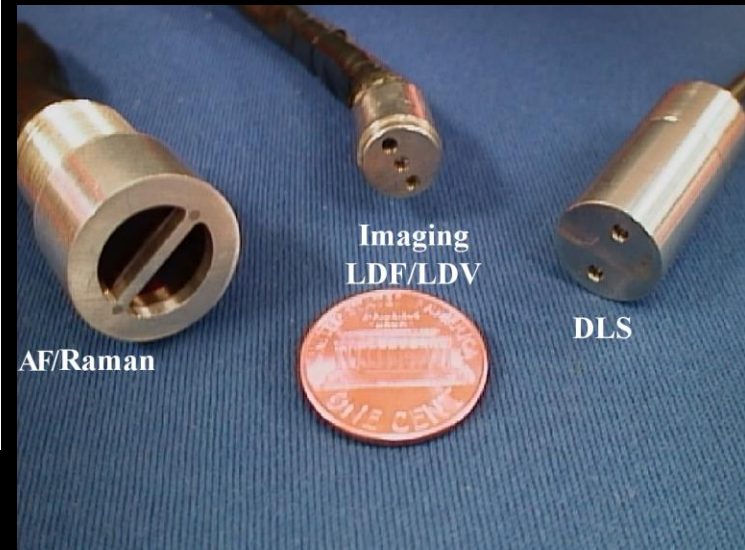
# Space (Microgravity) Experiments

## Laser Light Scattering in a Confined Space (Microgravity Glovebox)



Fluids Physics and Protein Crystal Growth  
Experiments

## Fiber-optic Probes



**Requirements:** Compact, Modular, Easy to Use,  
Power efficient, vibration and alignment free



# NIH/NEI-NASA Collaboration (Dynamic Light Scattering)

U.S. Department of Health & Human Services



Health Information Grants & Funding News & Events

Home » News & Events » News Releases

## NEWS RELEASES

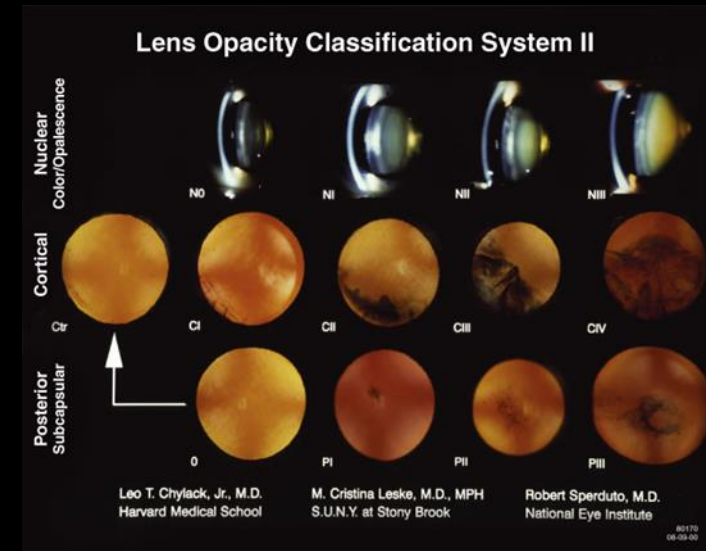
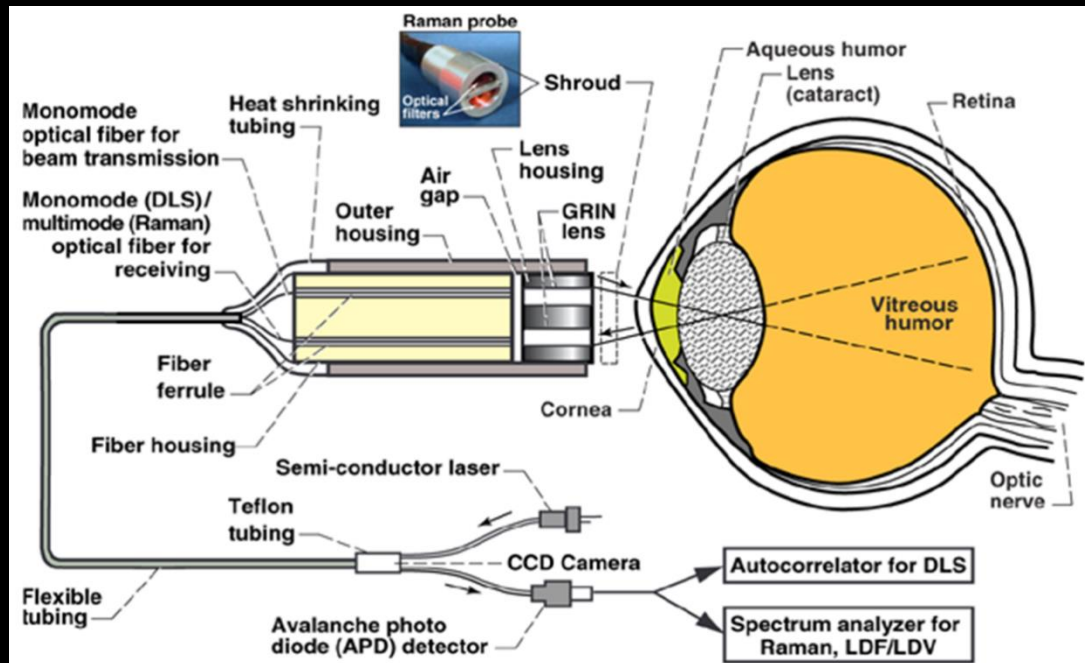
Thursday, January 8, 2009

### From Outer Space to the Eye Clinic: New Cataract Early Detection Technique

*Patients and Astronauts Benefit from NEI-NASA Collaboration*



# Fiber Optic (DLS) Imaging Probe



DLS: 2-3 orders of magnitude more sensitive  
over current imaging technologies

(In collaboration with NIH/NEI)

# LENS in Aging & Disease

- 51% of all blindness in the world is due to cataracts (UN/WHO Report)
- 19 M cataract surgeries done worldwide each year
- 3 Million cataract surgeries in US each year
- Most reimbursed procedure by Medicare
- 22 M Americans over 40 have cataracts, going up to 40M by 2030
- No medical cure
- Aviators and Astronauts have higher incidence of cataract

# Molecular Biology- Lens Proteins

- **35% of lens weight is protein** – highest concentration in any tissue in the body
- **Crystallins (alpha, beta and gamma)** make up 80-90% of total proteins; transparent, allowing transmission and focusing of light
- **Alpha Crystallins**- molecular chaperone- found to prevent other proteins from aggregating with each other: the built in **anti-cataract agent in the lens**

From: Hejtmancik JF & Datiles MB. Congenital and Hereditary Cataracts. In Duane's Clinical Ophthalmology (2009)

# DLS-Cross-Sectional Study:

Clinical Detection of Pre-cataractous Lens Protein Changes Using Dynamic Light Scattering

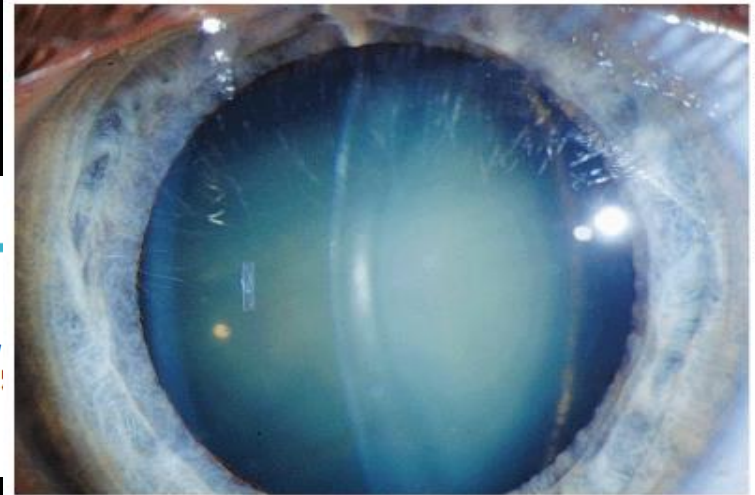
**ARCH OPHTHALMOL / VOL 126 (NO. 12), 2008**

Christopher Kent, Senior Editor

PUBLISHED 15 OCTOBER 2009

**Cracking the Cataract Code: New Technology  
New Hope**

**REVIEW<sup>®</sup>**  
of Ophthalmology



Manuel Datties, MD

**Alpha-crystallin protein appears to prevent other damaged proteins from forming an opacity, but is used up in the process. Once it drops below a critical level, a cataract can form.**



# Department of Human Health and Services (DHHS) Innovates Award 2011 NIH-NASA Team



L-R: Sam Zigler, Larry Tabak, Manuel Datiles (NEI/NIH),  
HHS Secretary Sebelius, Rafat Ansari (NASA), and  
Deputy Secretary HHS Bill Corr



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OF OPHTHALMOLOGY



CrossMark

# Longitudinal Study of Age-Related Cataract Using Dynamic Light Scattering

*Loss of  $\alpha$ -Crystallin Leads to Nuclear Cataract Development*

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Manuel B. Datiles III, MD,<sup>1</sup> Rafat R. Ansari, PhD,<sup>2</sup> Junko Yoshida, MD, PhD,<sup>3,4</sup> Holly Brown, COA,<sup>3</sup>  
Andrea I. Zambrano, MD,<sup>3</sup> Jing Tian, PhD,<sup>3</sup> Susan Vitale, PhD,<sup>1</sup> J. Samuel Zigler, Jr., PhD,<sup>3</sup>  
Frederick L. Ferris III, MD,<sup>1</sup> Sheila K. West, PhD,<sup>3</sup> Walter J. Stark, MD<sup>3</sup>

Ophthalmology. 2016 Feb;123(2):248-54.

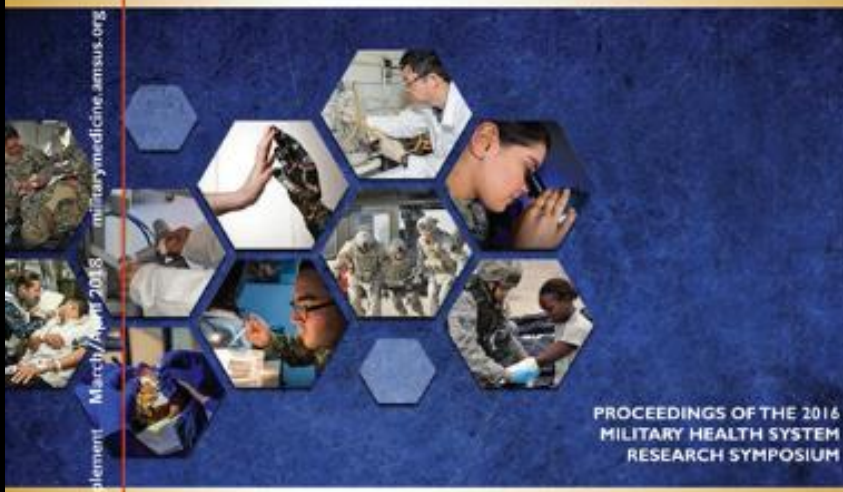
Another collaborative DLS study  
conducted in Japan is to be published

# Non-invasive Detection of Unique Molecular Signatures in Laser-Induced Retinal Injuries

*Melissa I. Naiman, PhD, PMP, EMT-B\*; Rachida Bouhenni, PhD†; Rafat R. Ansari, PhD‡; Jeffrey Dunmire, MST‡; Ying Liu, MD, PhD§; Qundeel Rafiq, MS§; Deepak Edward, MD§*

## MILITARY MEDICINE

International Journal of AMSUS



amsus.org  
militarymedicine.amsus.org  
March/April 2018  
Volume 183, Issue 3/4 Supplement

Col Patricia A. Reilly, USAF (RET), Ph.D.  
Ms. Teresa L. Hendrickson, MAT  
Guest Editors, 2016 MHSRS Supplement to  
the Journal of Military Medicine

**March-April 2018**

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Air Force Medical  
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Modernization  
Directorate  
AFMSA/SG5I

(Cooperative agreement USAF  
Q5, No. FA8650-13-2-6370)

# Retinal Injury Study Team



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Qundeel Rafiq, MS





# Retinal Injury Detection

- Determining whether a laser exposure event caused a retinal injury currently requires medical expertise and specialized equipment that is not always readily available in the battle field.



# **Laser-Induced Retinal Injuries in Military Settings**

- Laser-based devices are routinely used in a wide range of applications, including range determination, target designation, guidance, and as defensive counter measures.
- Laser dazzler misuse among US soldiers in Iraq caused 45 documented injuries between November 2008 and June of 2009.

# **Laser Exposures Incidents in Civilian Settings**

- In 2014 alone, FAA received 3894 reports of cockpit illumination in civilian aircraft in several instances, pilots were impaired to the extent that flight plans were altered or aborted.
- There are many case reports of children sustaining severe retinal damage from handheld laser devices.

# Laser-Induced Retinal Injuries

- Laser Exposure can cause damage to retinal pigment epithelial (RPE) cells and disruption of the outer blood-retinal barrier
- Protein Up-regulation in the Vitreous?

- DLS signal analysis can reveal significant changes in particle diameter and intensity in laser treated groups as compared to controls.
- Differences in protein profile in the vitreous of the laser treated eyes can be noted when compared to controls using Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS).

# Purpose and Study Design

- The purpose of this study was to test the feasibility of DLS to non-invasively detect laser retinal injuries through interrogation of the vitreous humor (VH).
- Three grades of retinal laser lesions were studied: mild (minimally visible lesions), moderate (Grade II) and severe (Grade III).
- A pre-post treatment design was used to collect DLS measurements *in vivo* at various time points, using a customized instrument.



# New Compact DLS Instrument in a Carry-on



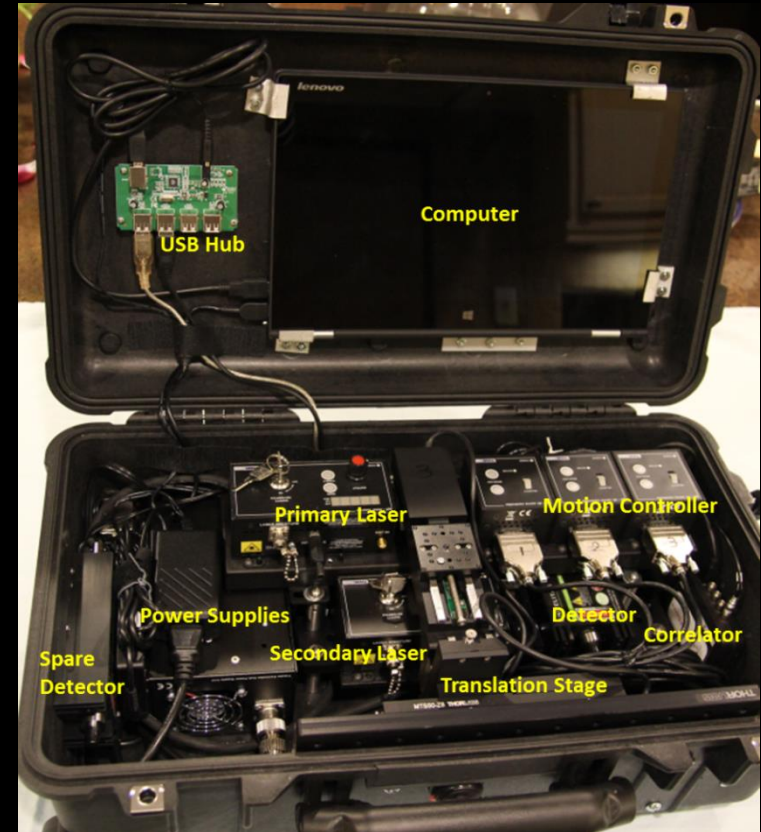
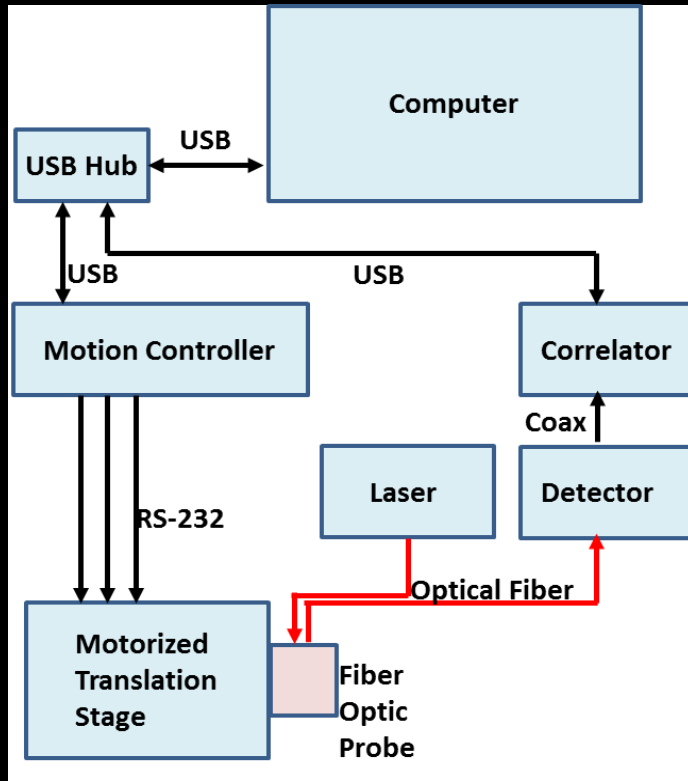
**Dimensions (L x W x D)**

Exterior: 22" x 13.81" x 9.0"

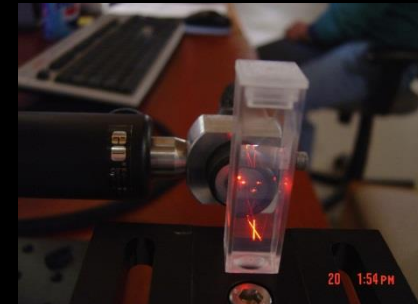
Interior: 19.75" x 11.0" x 7.6"

**Ansari and Suh, J. Lightwave Tech, Vol. 35,  
No. 16 (2017)**

# DLS Instrument System Lay-Out



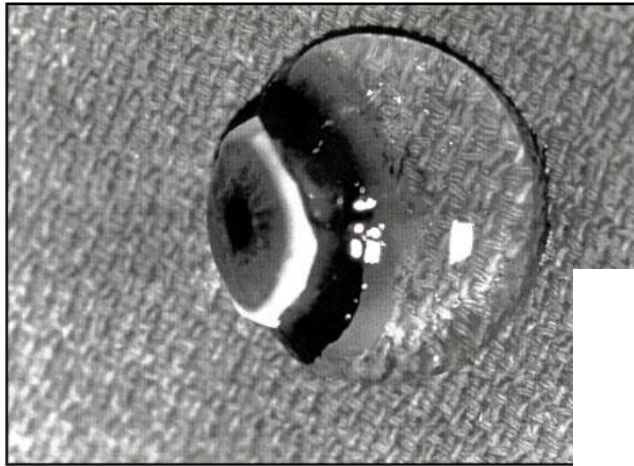
- Redundancy (2 lasers/2 detectors)
- 3-Axis Motorized control
- Easy (cross-beam) Alignment
- Built-in DAQ with handheld power meter



# What does DLS See in Normal Vitreous?

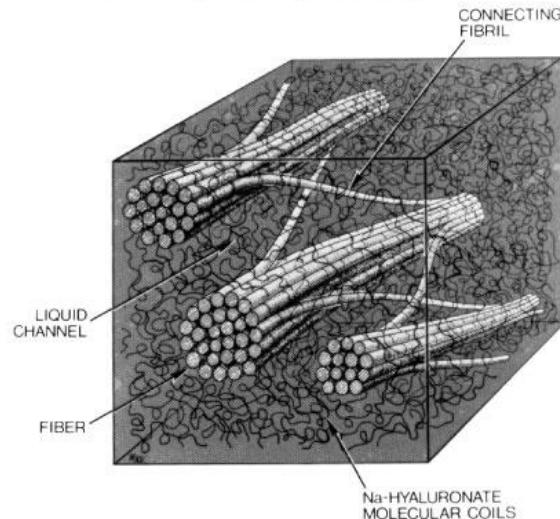
## Seeing the Invisible

Vitreous of a 9 Month Old Baby Boy



J. Sebag, Doheny E

The Vitreous  
J. Sebag, Springer-Verlag 1989



J. SEBAG  
EDITOR

## Vitreous

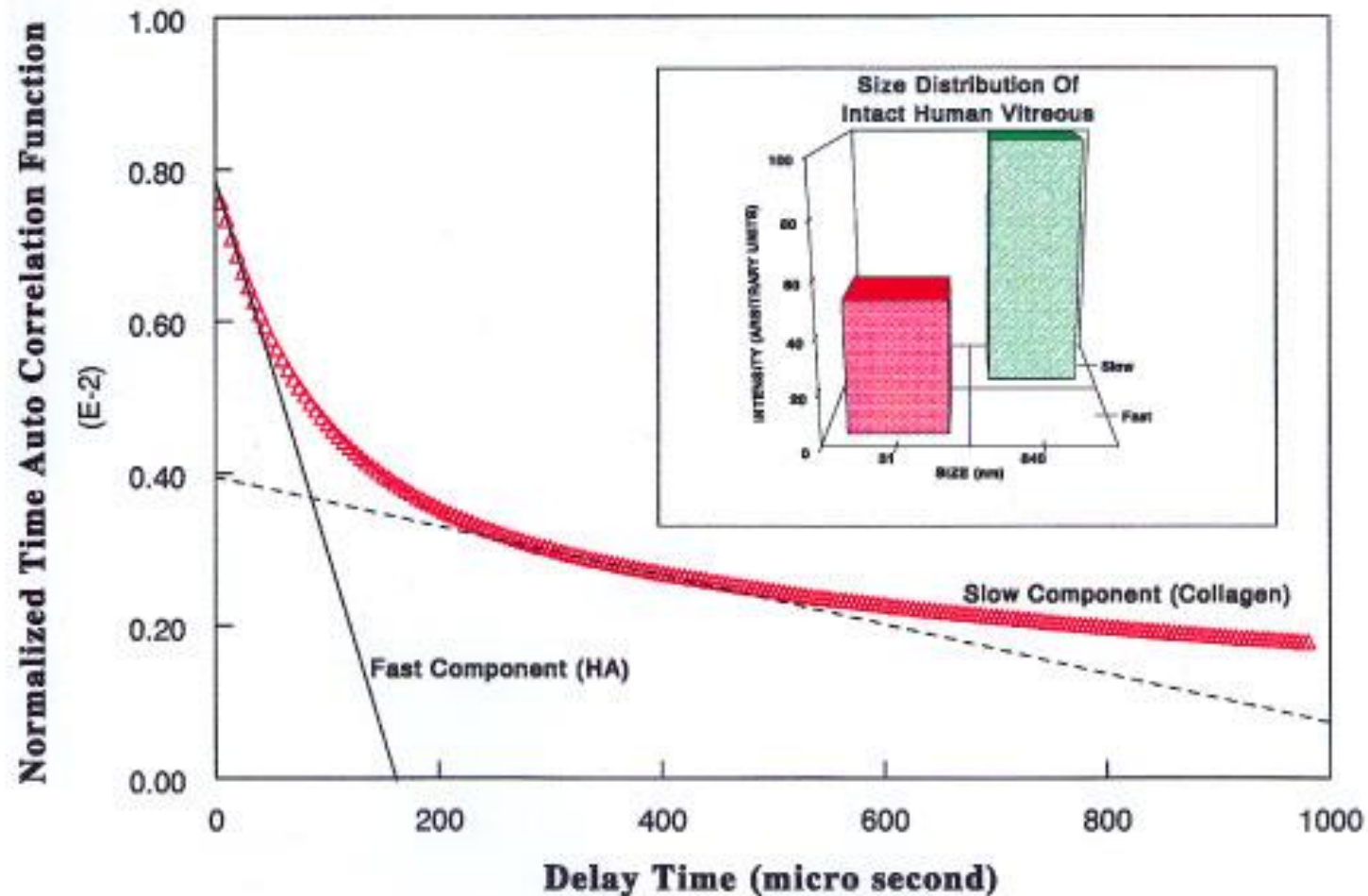
in Health and Disease

 Springer

J. Sebag, 2014

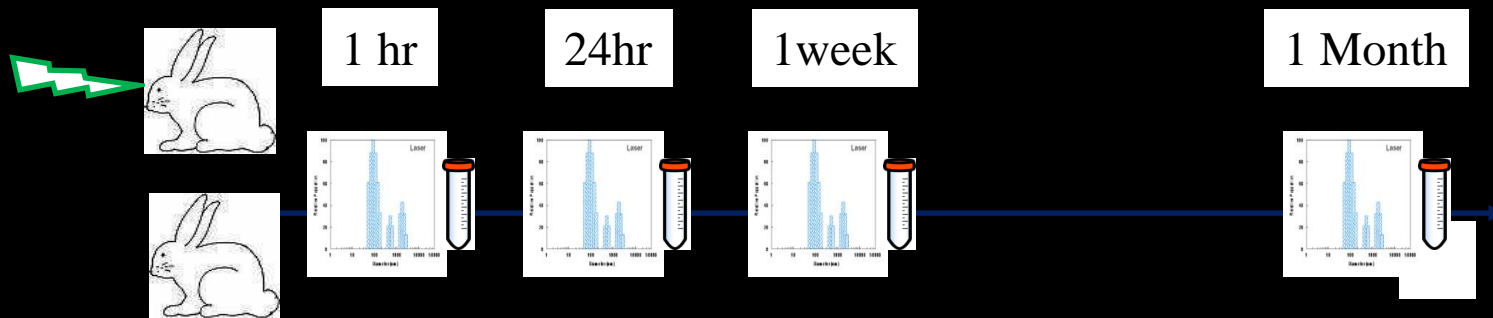


# Time Relaxation of Intact Human Vitreous Diffusive Motions of Hyaluronan/Collagen



# Experiment Design Summary

**152 Dutch-belted Rabbits were studied**



## Approvals:

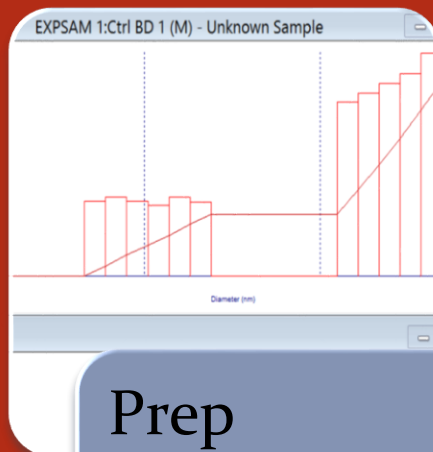
- USAF Sponsored Animal Research Programs (protocol no. AFDW-2013-003A)
- UIC Office of Animal Care and Institutional Biosafety (protocol no. 13–133)
- Institutional Animal Care and Use Committee at Johns Hopkins University (protocol no. RB13M210)

# Retinal Injury Study Workflow



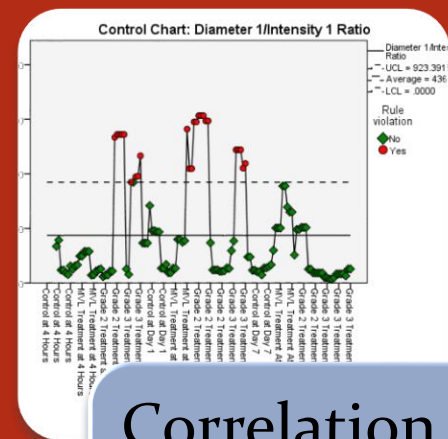
## In vivo

- Pre-Tx DLS
- Laser Tx
- Post-Tx DLS



## Prep

- DLS data processing
- Vitreous extraction



## Correlation

- Statistics
- Proteomics
- Joint interpretation



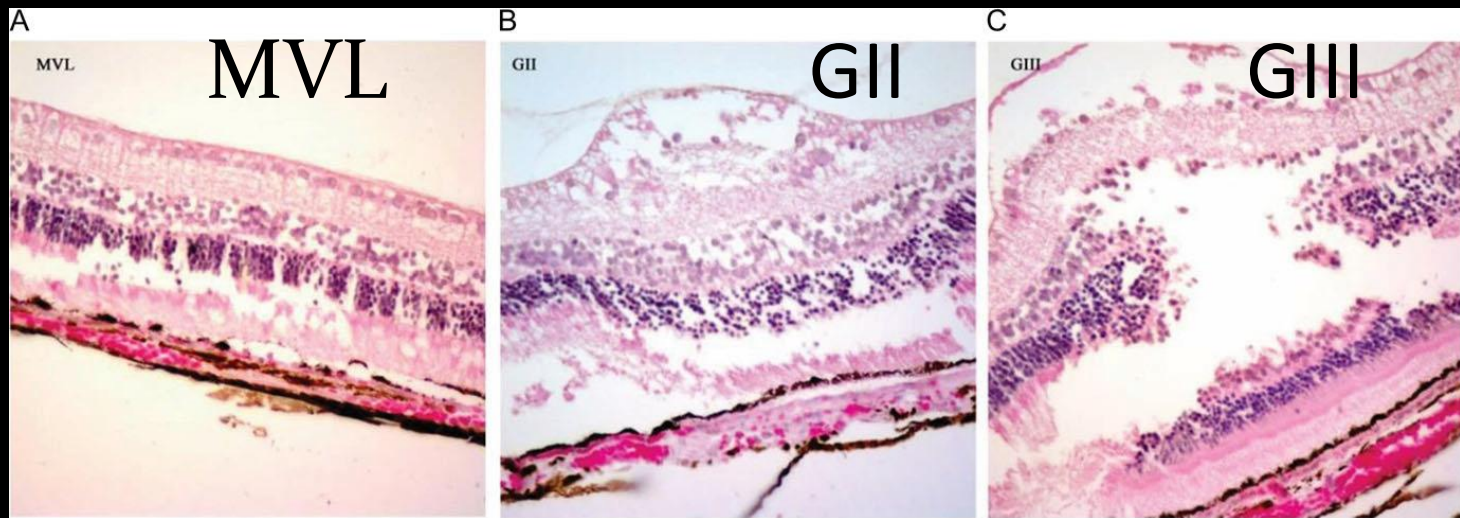
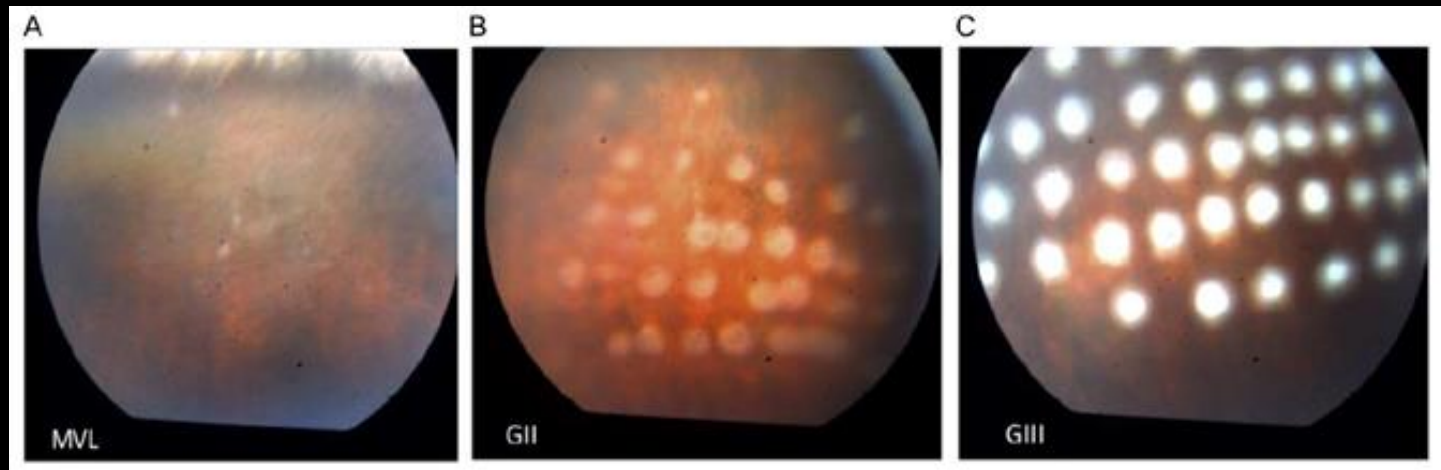
# Lesion Parameters

- Iridex/Iris Medical Oculight GL 532nm



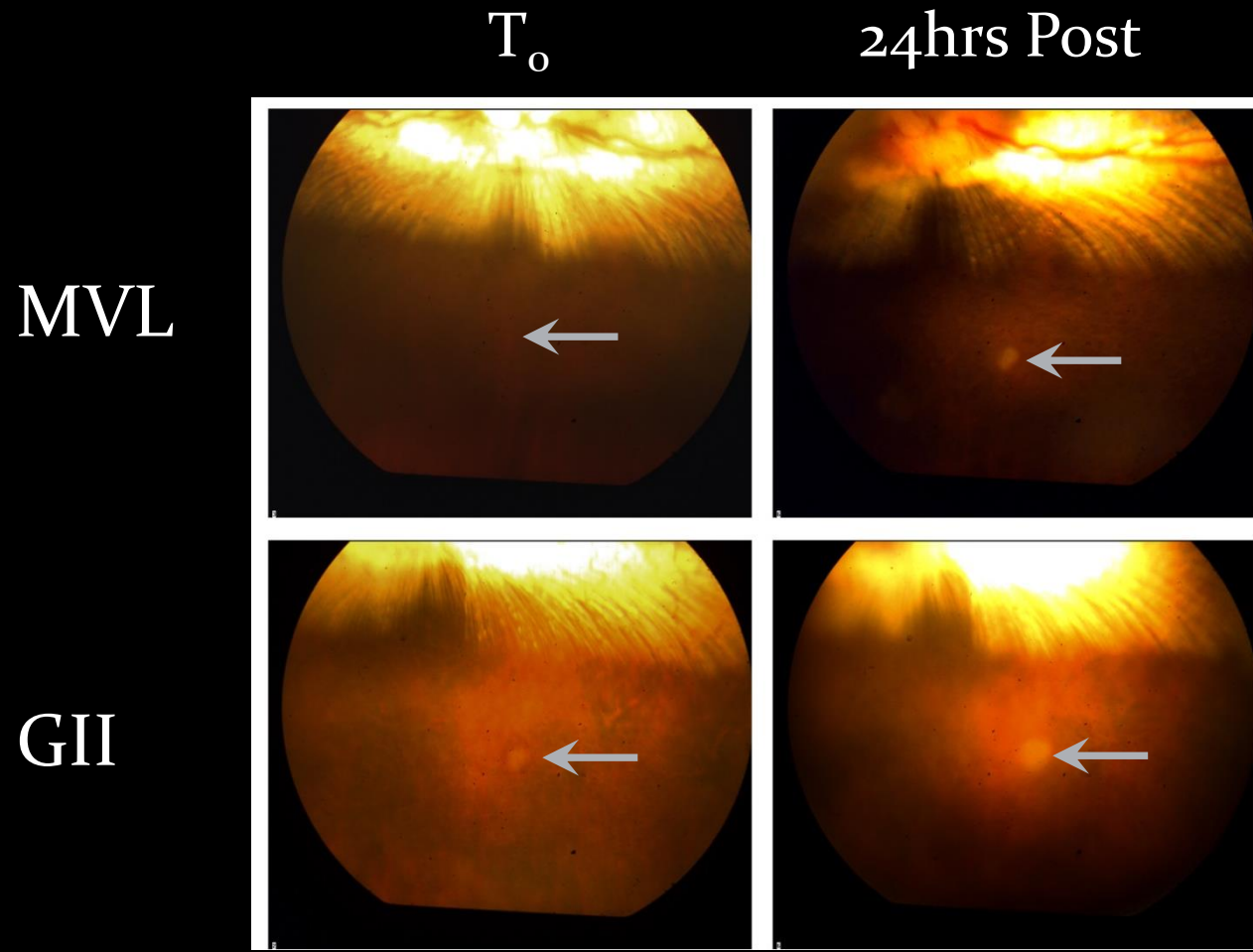
- MVL: 120~130mW, 100 ms, 500  $\mu$ m
- GII: 190mW, 100 ms, 500  $\mu$ m
- GIII: 250mW, 200 ms, 500  $\mu$ m

# Fundus Imaging/Lesion Severity

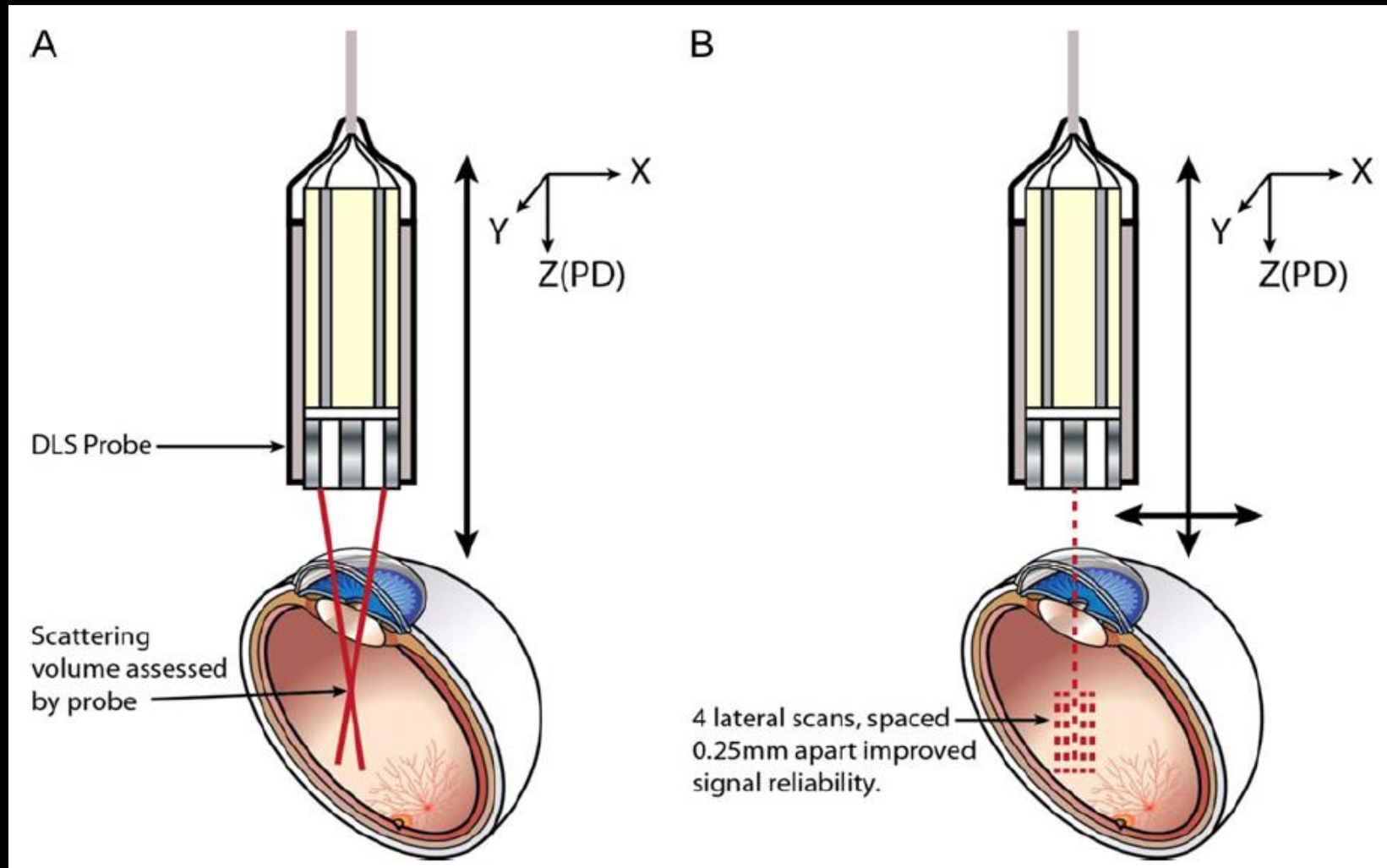


Histological Confirmation (H&E Staining)

# Fundus Images: 1 Lesion



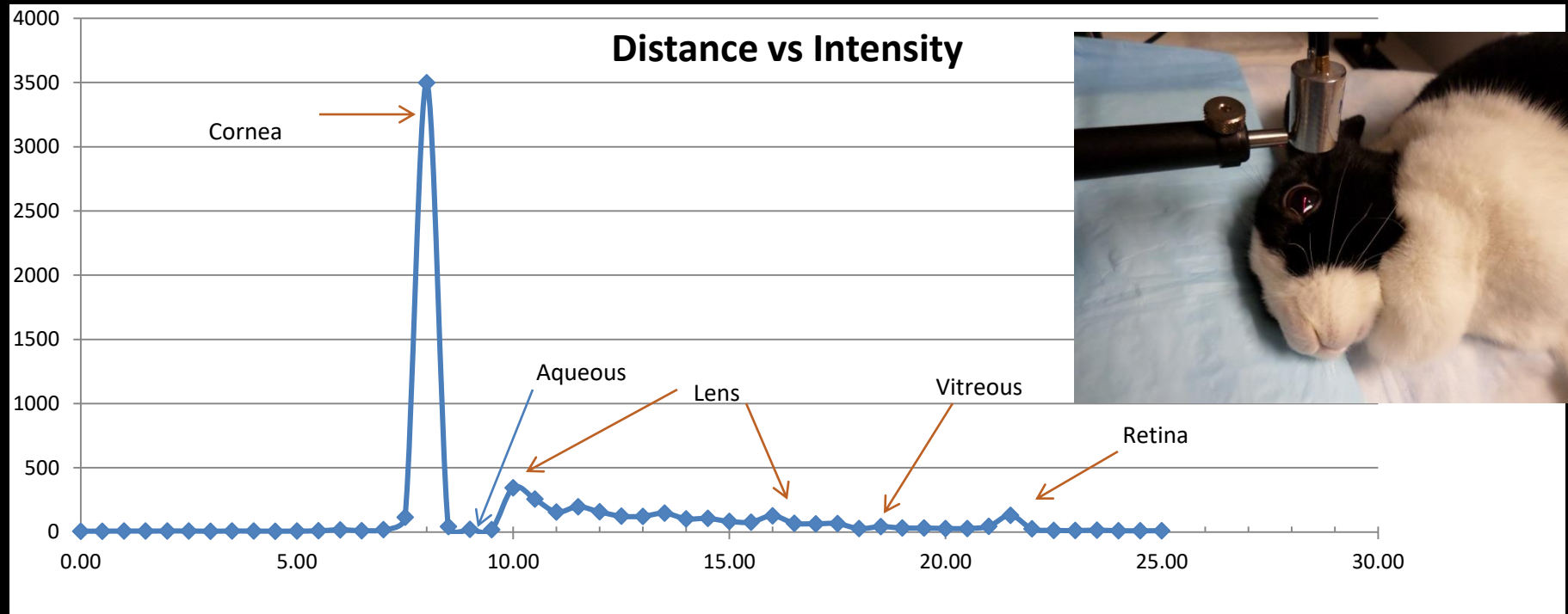
# DLS Scan Profile



(Incident Power: 100 microwatts)

# Static Scan: Landmarks and Interfaces

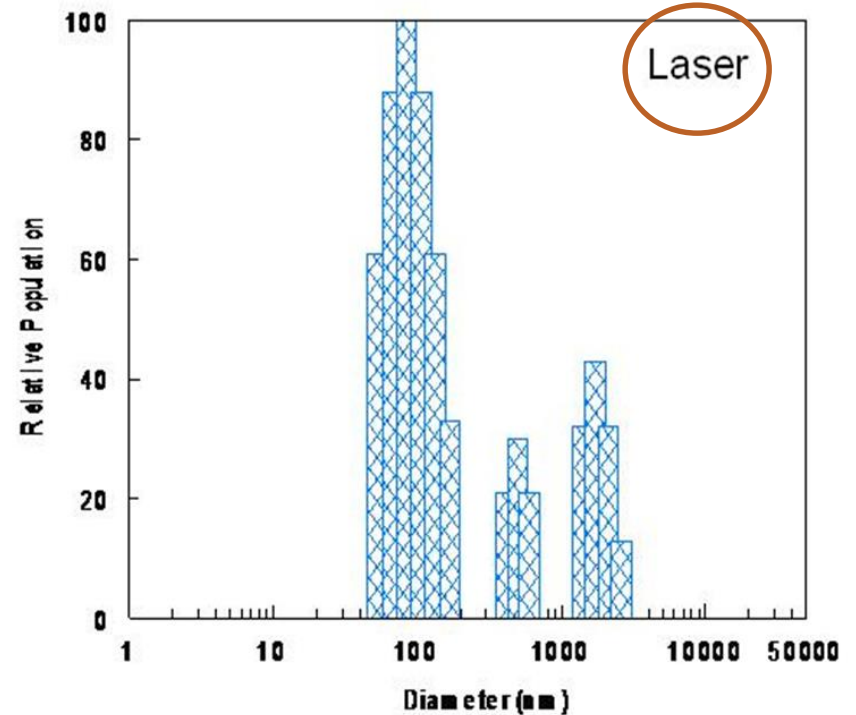
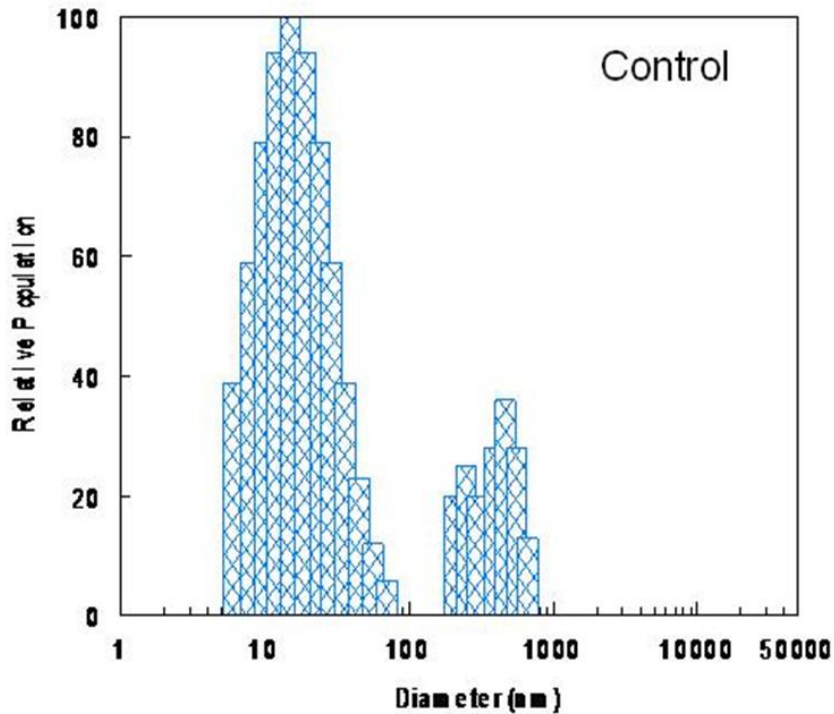
## Dutch-Belted Rabbit



Steps of 0.5 mm, laser power 100 microwatts; Intensity in Kilo counts/sec



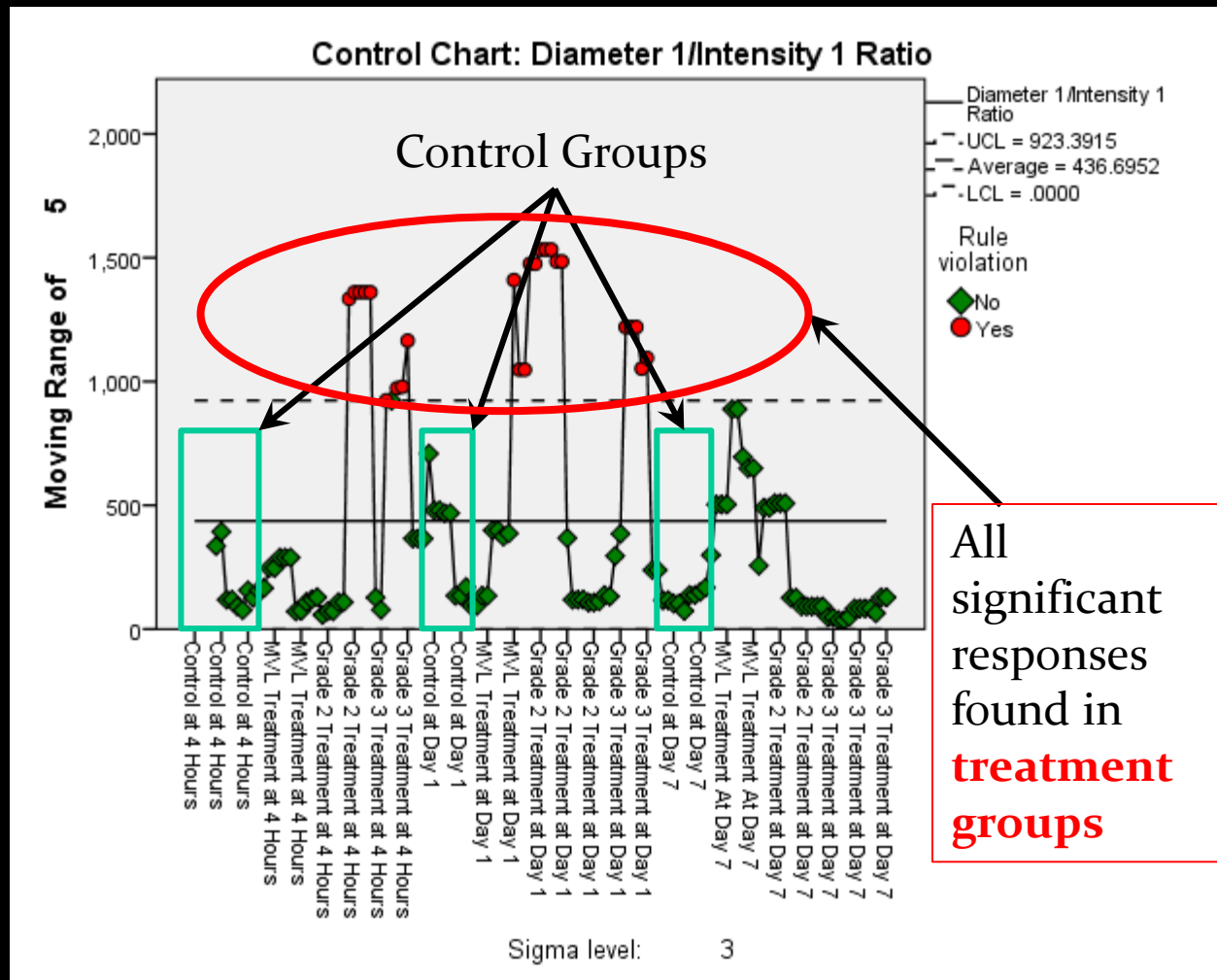
# Dynamic Scan



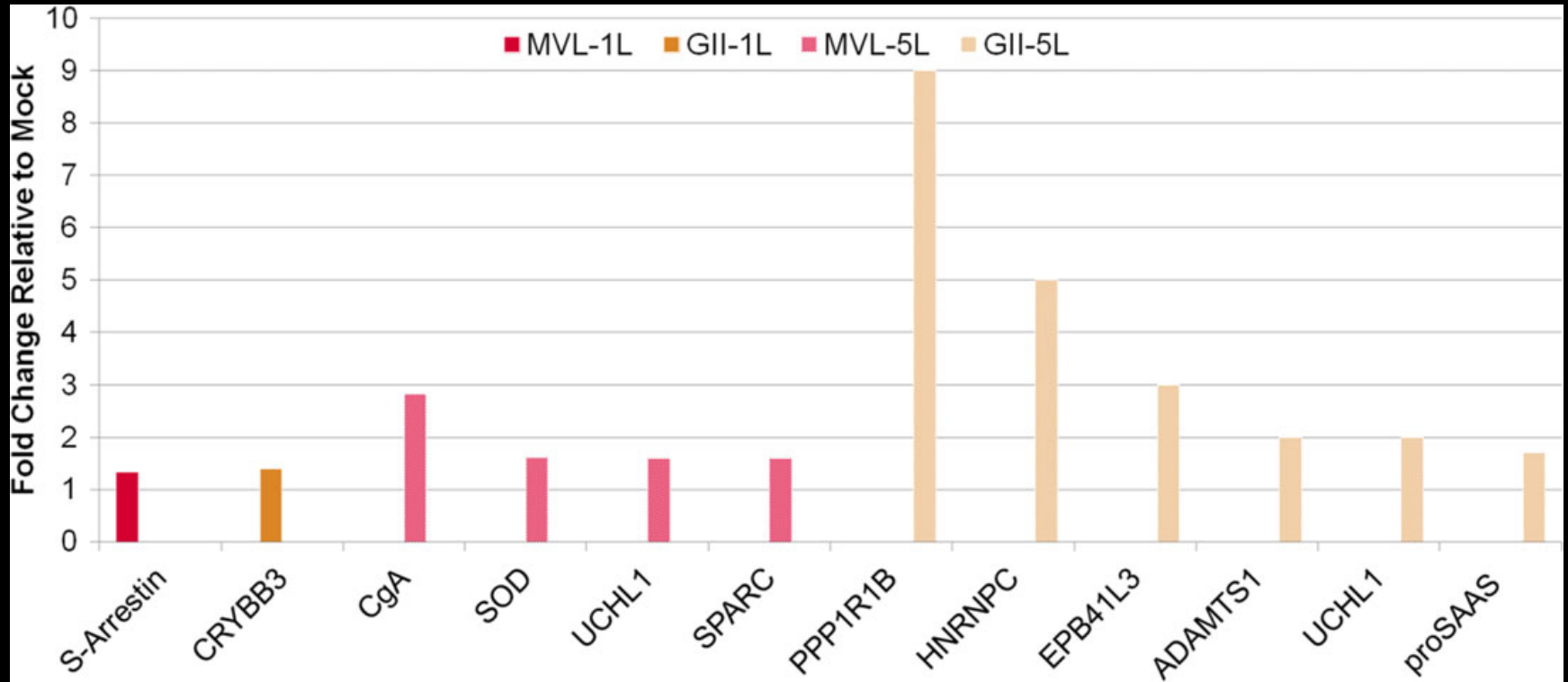
4 hours post laser treatment of MVL lesion showed a shift in particle size distribution



# DLS Results:



# Proteomic Results



Upregulated proteins in (MVL1 lesion, GII 1 lesion, MVL 5 lesions, and GII 5 lesions) relative to mock.



MILITARY MEDICINE, 183, 3/4:18, 2018

## Non-invasive Detection of Unique Molecular Signatures in Laser-Induced Retinal Injuries

Melissa I. Naiman, PhD, PMP, EMT-B\*; Rachida Bouhenni, PhD†; Rafat R. Ansari, PhD‡; Jeffrey Dunmire, MS†; Ying Liu, MD, PhD§; Qundeel Rafiq, MS§; Deepak Edward, MD§

## A Dual Wavelength Fiber-Guided Dynamic Light Scattering Instrument in a Compact Carry-On

Rafat R. Ansari and Kwang I. Suh

(Invited Paper)

JOURNAL OF LIGHTWAVE TECHNOLOGY, VOL. 35, NO. 16, AUGUST 15, 2017



# Conclusions:

- The outcomes of this study support the hypothesis that changes in the composition of the VH following laser injury to the retina alter its light-scattering characteristics sufficiently to be detected using DLS.
- Among the most promising findings is that the prototype DLS system could detect a single MVL or GII lesion within 4-24 hrs after injury.
- Overall, this study provided an important first step toward developing a non-invasive, field-deployable system to detect laser eye injury.



# Questions?



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