Potential ophthalmological uses of the FEL

Frank Lattanzio

Basic Science Director

T.R. Lee Center for Ocular Pharmacology

Eastern Virginia Medical School

Norfolk, VA 23501

lattanfa@evms.edu

FEL-multiple wavelengths on demand

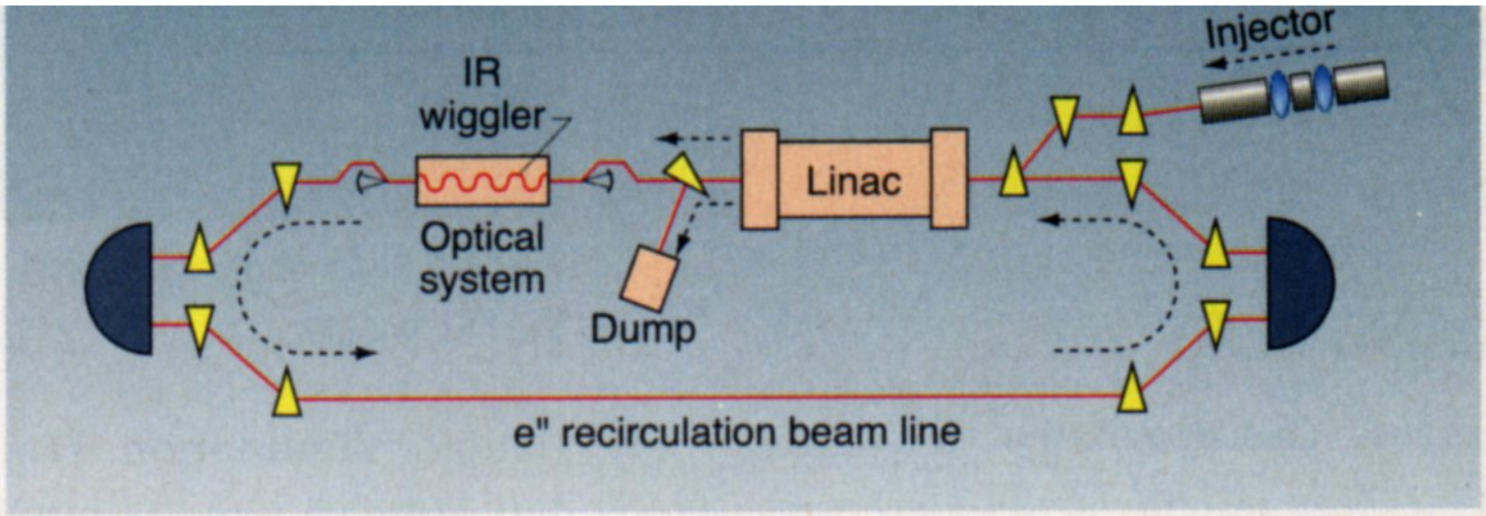


FIGURE 1. A superconducting radio-frequency linear accelerator drives Jefferson Lab's kilowatt-scale infrared free-electron laser. After electrons are injected into and accelerated by the linear accelerator, they transit a wiggler magnet—yielding kW-scale light in an optical cavity.

Laser Focus World. Sept., 2001, pg. 93

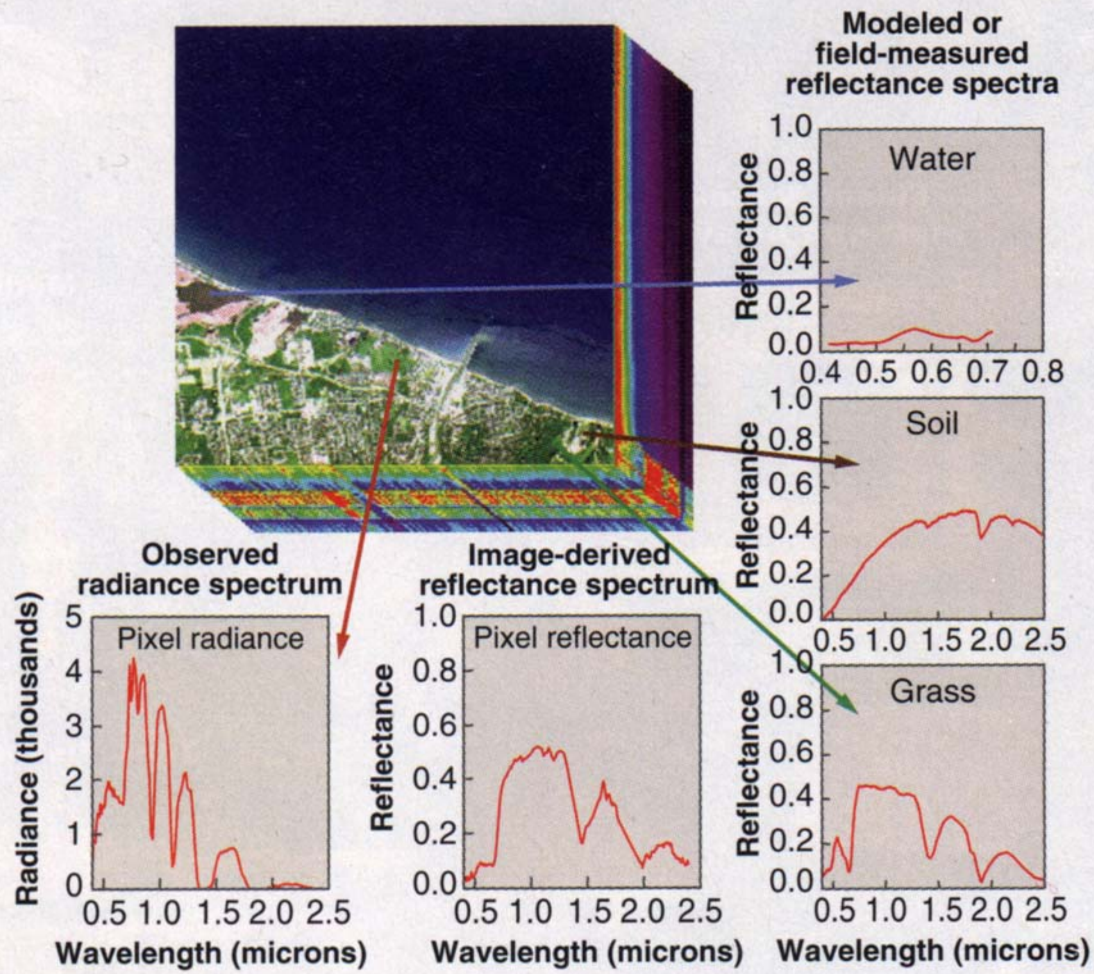


FIGURE 1. An image cube has two spatial dimensions and one spectral dimension. The curves along the right show spectral reflectance values associated with individual pixels. The two curves at the bottom show the spectral radiance reaching the sensor and the corresponding spectral reflectance for vegetation.

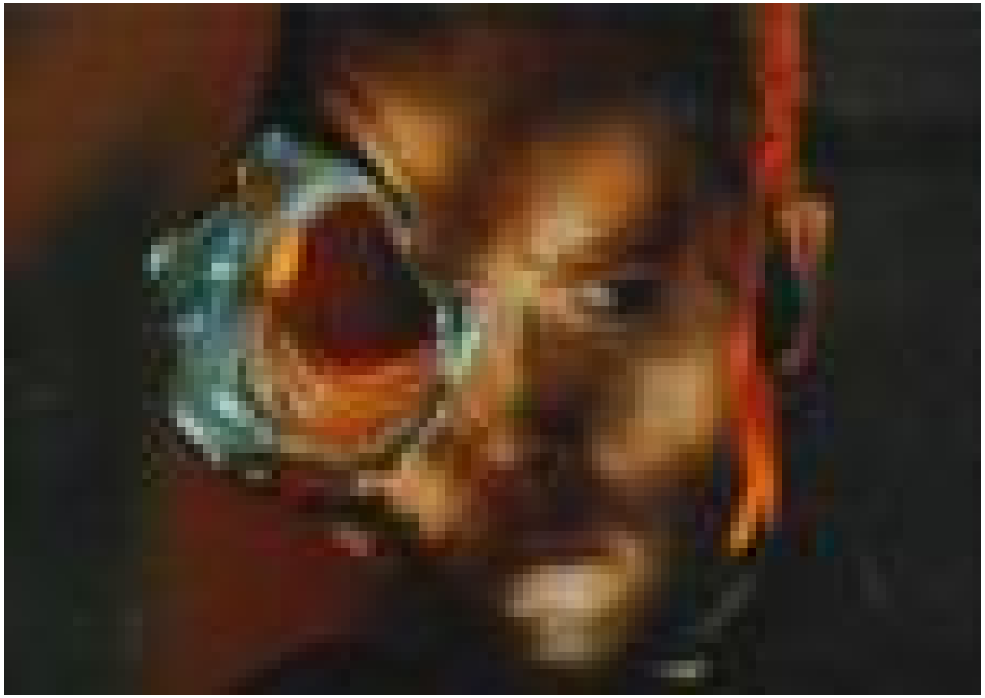
Transfer of satellite imaging technology

Laser Focus World., May 2004, pg 76

Benefits and limitations of the eye as a research model

- + Made to accommodate entry of light with many of its internal components visible and reasonably accessible
- + Demonstrates a wide variety of responses to local and systemic pathologies
- - Vital organ, susceptible to photodamage, ischemia, etc, with limited cellular regeneration
- - Certain aspects of ocular functionality not easily tested in tissue culture/animal models

Please don’t look at the laser with your remaining eye

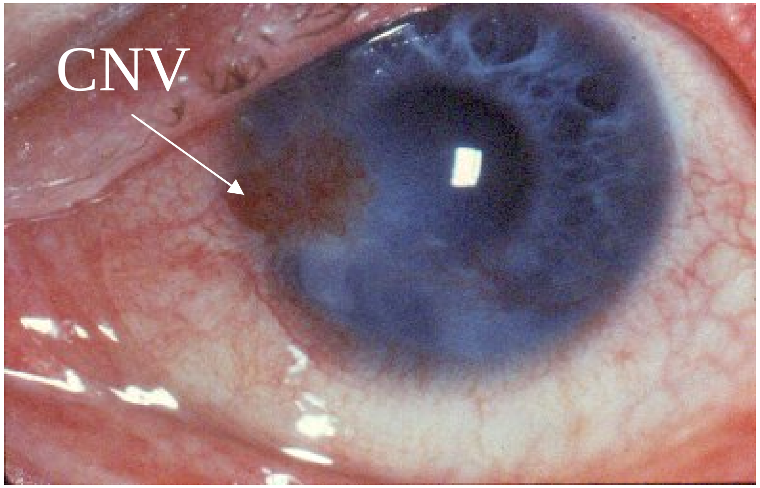


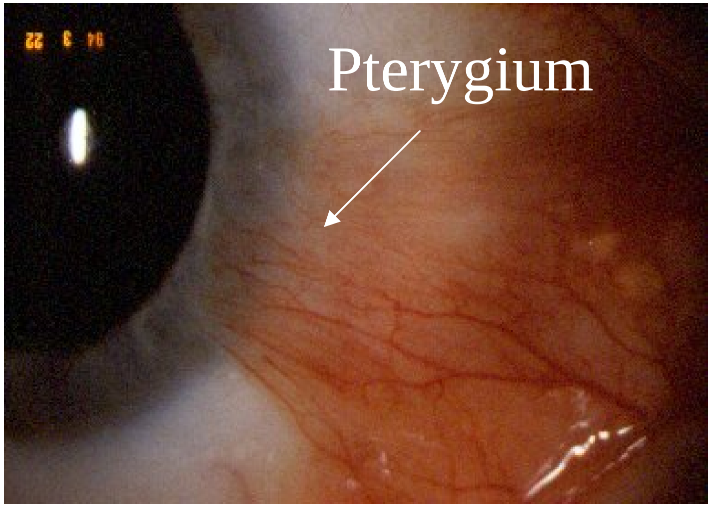
Free Electron Laser (FEL) Applications for Ophthalmology

- The T.R. Lee Center for Ocular Pharmacology is evaluating <u>topical</u> photodynamic (PDT) agents for treatment of pterygium, corneal neovascularization (CNV) and macular degeneration, as well as dermatological cancer models.

Free Electron Laser (FEL) Applications for Ophthalmology

- PDT agents permit ablation of blood vessels that compromise corneal clarity (pterygium, CNV) or retinal cell function (macular degeneration), as well as tumor cell disruption





Benefits of topical preparations of photodynamic agents

- Reduced systemic toxicity
- Improved patient compliance/recovery
- Reduced amounts of drug required

Potential uses of FEL

Macular degeneration, CNV, Pterygium, Cancer	Photodynamic therapy	Wide spectrum light source for testing PTD agents and regimens

Current photodynamic agents have been designed around existing lasers

- Development of novel PDT agents would be accelerated by the availability of non-standard laser light from UV to IR which can be provided by the FEL and its ability to irradiate large numbers of animals for PDT screening

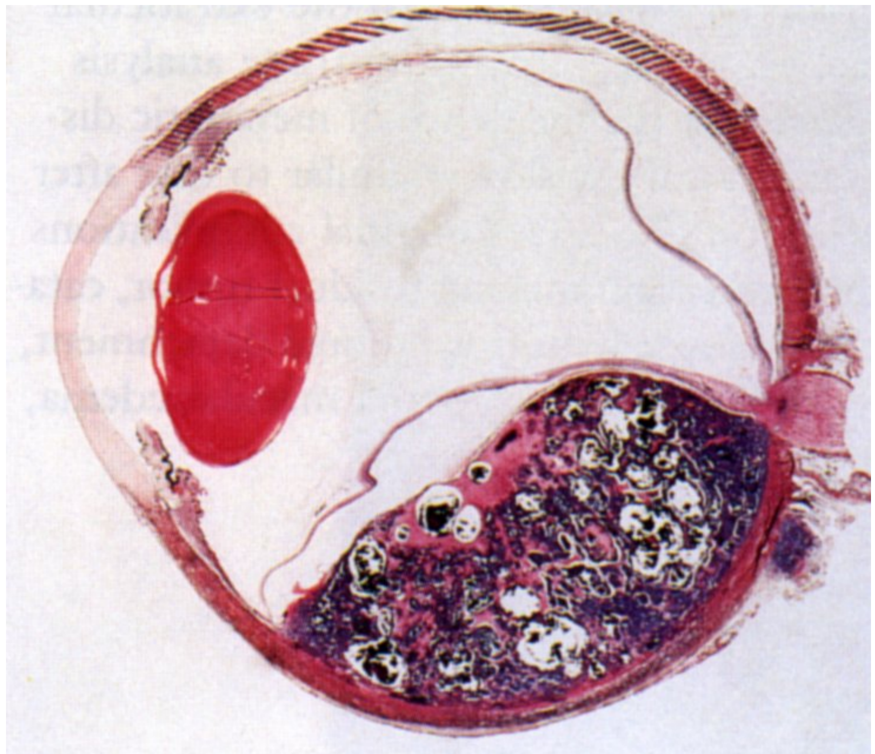
Test models

- Rabbit CNV
- Rat corneal transplant
- Primate/human corneal transplant
- In vitro, in vivo human

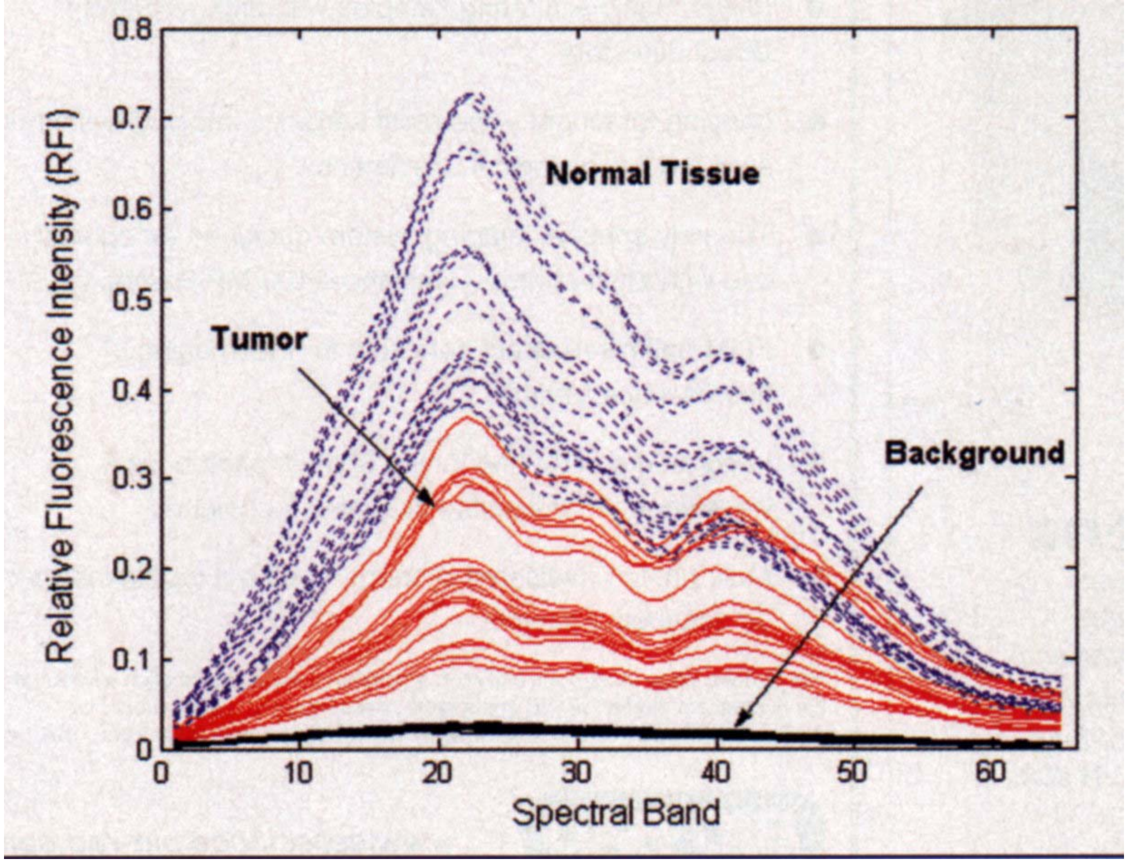
Potential uses of FEL

Uveal cancer	Detection	TeraHz, N(IR)
Corneal transplant	Detection of rejection	TeraHz, N(IR)

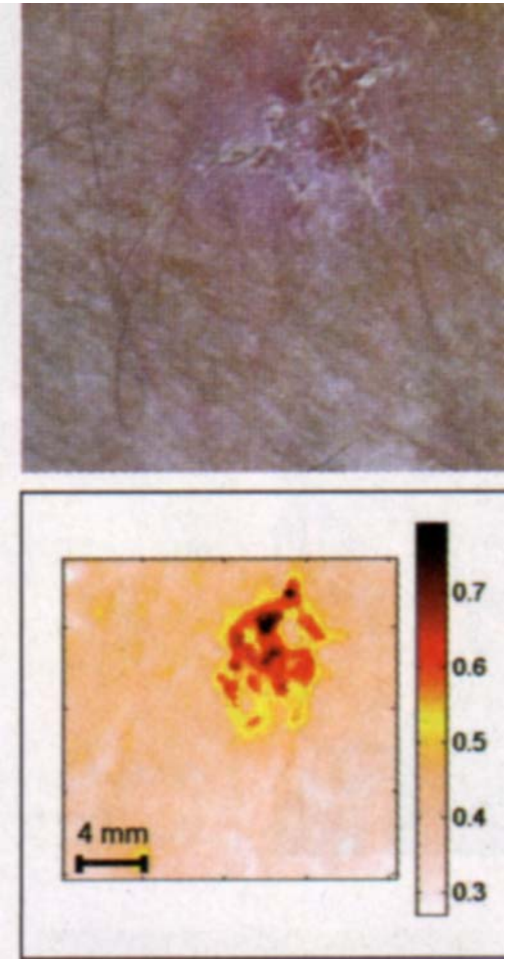
Uveal melanoma



Hyperspectral scan of chicken tumor



TeraHz imaging of basal cell tumor

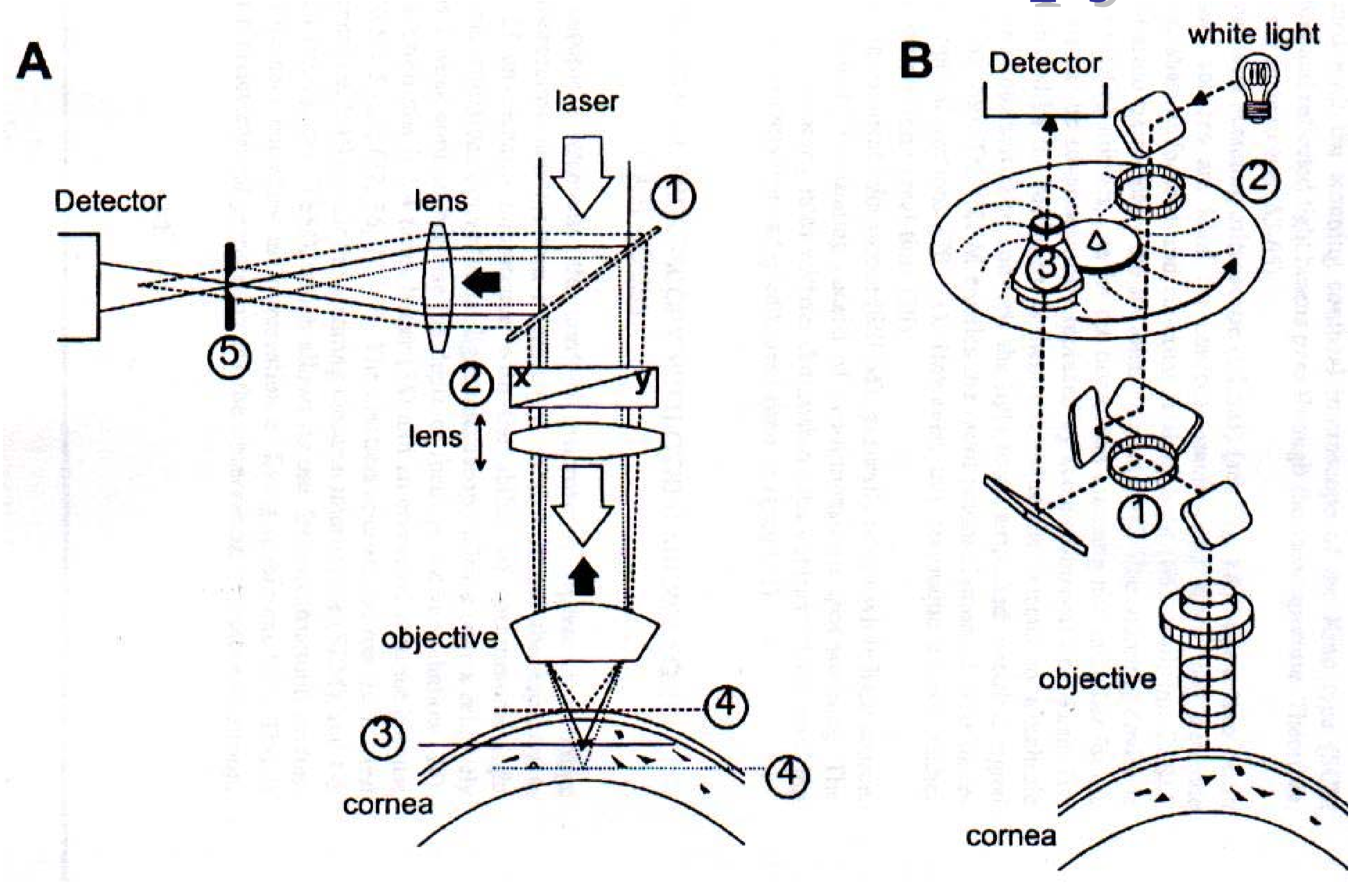


R&D, Sept. 2003, pg. 28

Confocal Microscope

- Uses spinning Nipkow disk to create a 5-7 um optical section
- Patient's eye is anesthetized and applanated
- Video data recorded on VCR or digital files (tif, jpeg, etc)

TSCM - *in vivo* Tandem Scanning Confocal Microscopy



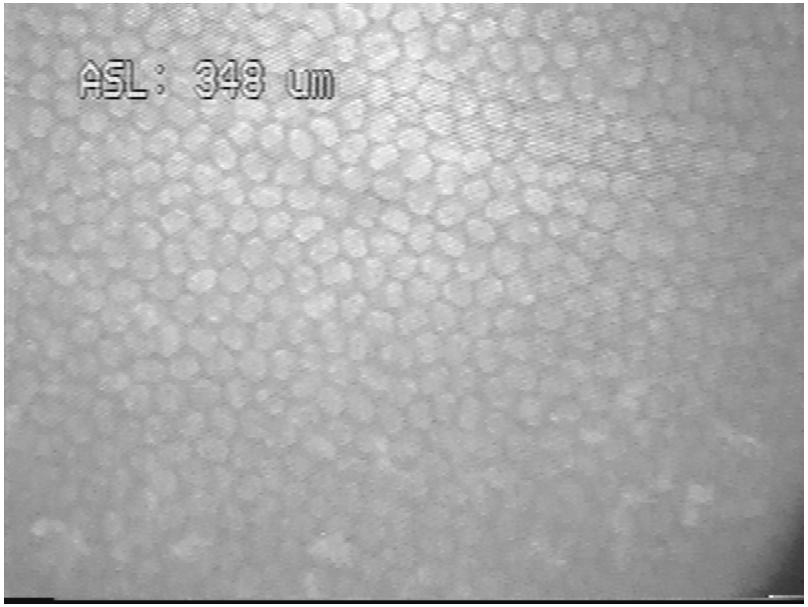
Furrer et al, J Ocular Pharm & Therapeutics, 1997; 13: 561

Cornea Transplant Rejection

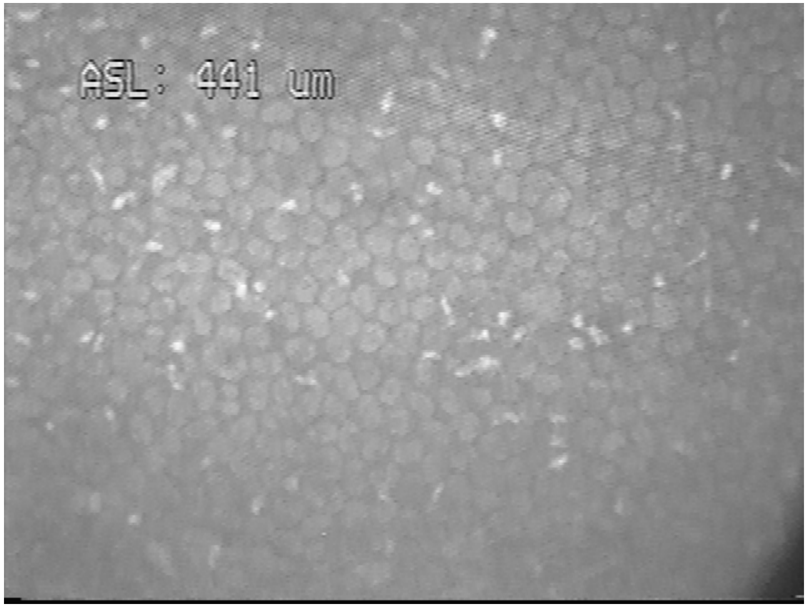
Normal Rat Corneal Stroma

3 Day Rat Corneal Transplant-Stroma

Uveitis

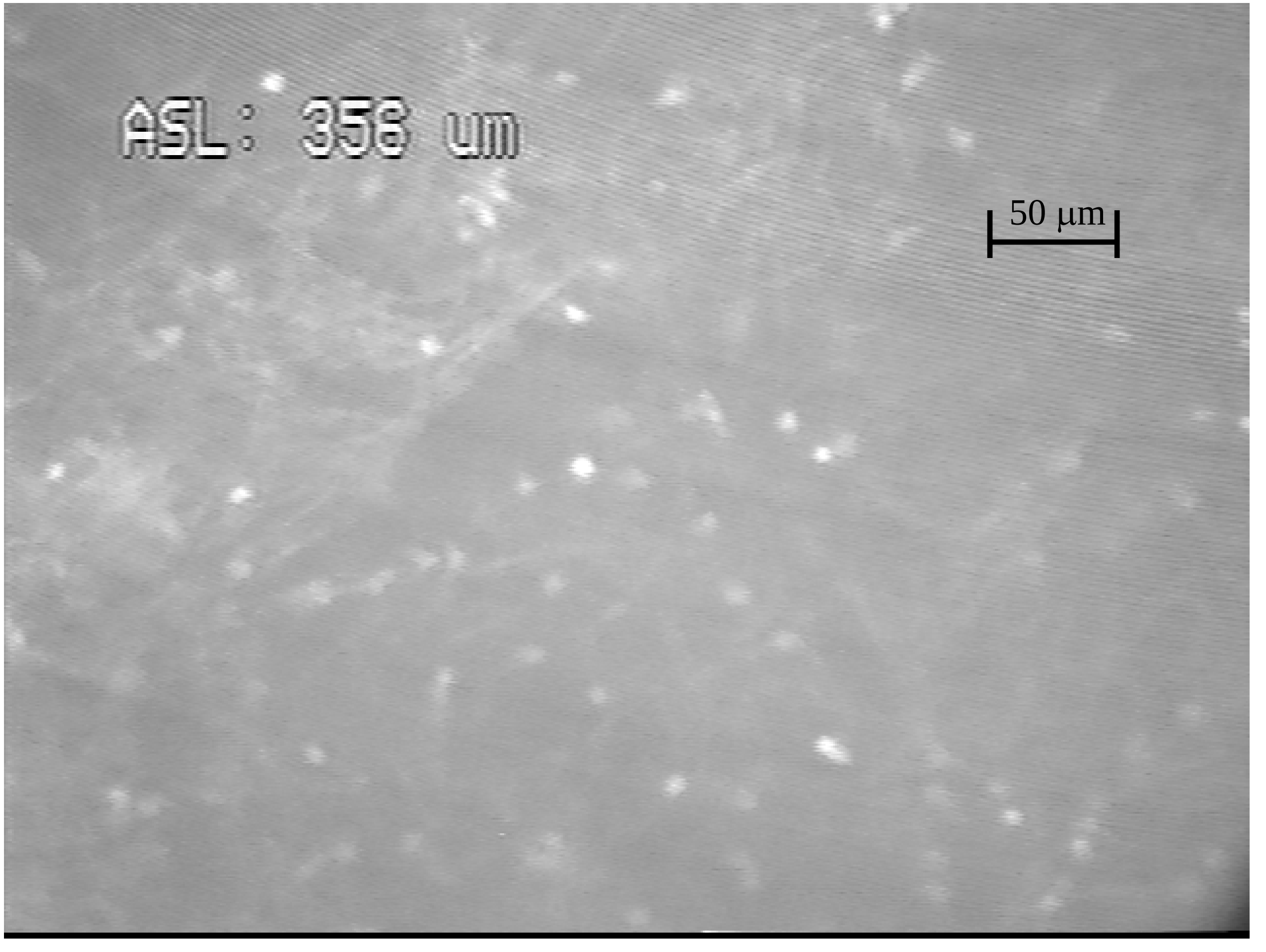


Normal Rabbit Endothelium



Uveitis-Rabbit Endothelium

AC Fibrin & Leukocytes



ICE Syndrome: Confocal

Thickened corneal endothelial cells impair vision in glaucoma patients

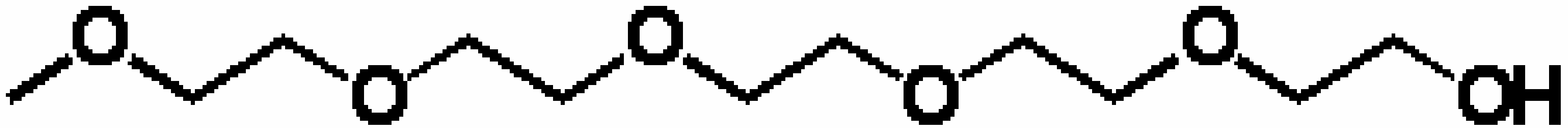
Free Electron Laser (FEL) Applications for Ophthalmology

- The T.R. Lee Center has also developed a novel group of free radical scavengers that are known radioprotectants.
- These agents have a low molecular weight methoxypolyethylene glycol (MPEG) backbone that allows entry of the compounds into cells.

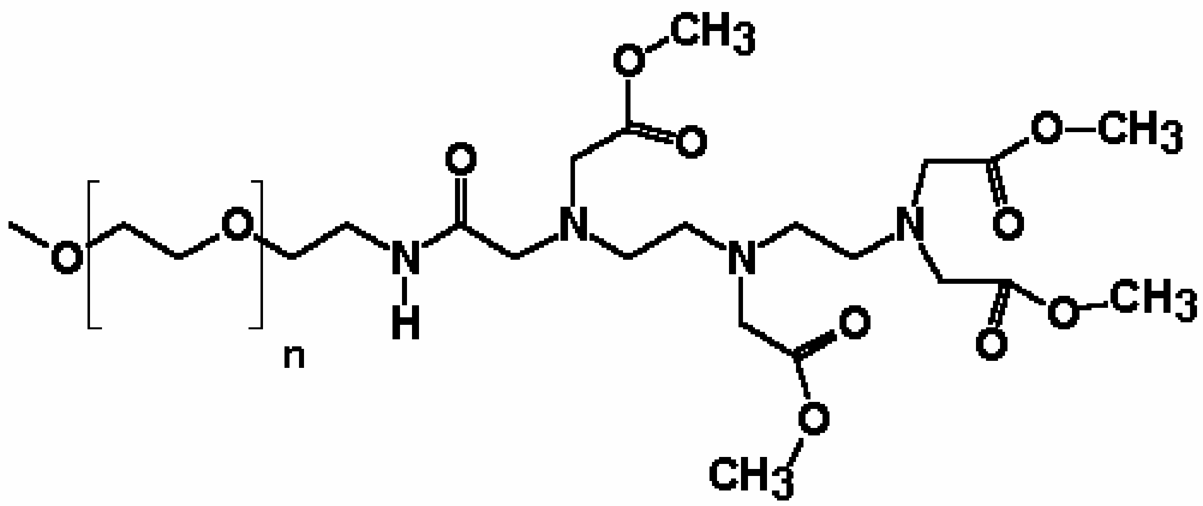
Free Electron Laser (FEL) Applications for Ophthalmology

- These agents can be formulated with different side groups, allowing them to chelate heavy metals responsible for free radical generation, as well as present reducing groups to sustain the cell against ischemic damage. Cellular penetration can be enhanced by the formation of MPEG esters.
- These agents have therefore potential for treating the free radical component of such ocular conditions as glaucoma, cataracts and macular degeneration, as well as heart attack, stroke and Alzheimer’s.

MPEG-methoxypolyethylene glycol)



Creation of esters to permit ready entry of MPEG derivatives into cells



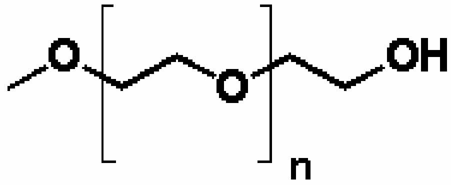
MPDTE n=7, MW ca 814 $C_{35}H_{66}N_4O_{17}$

Methoxypolyethylene glycol MW 350 amide of

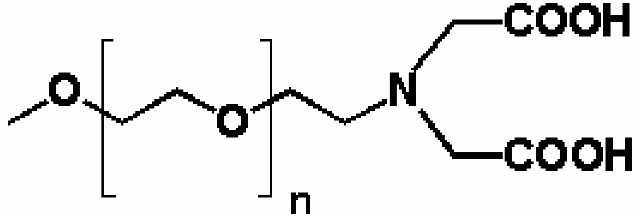
diethylenetriaminepentaacetic acid, methyl ester

Some MPEG based chelators

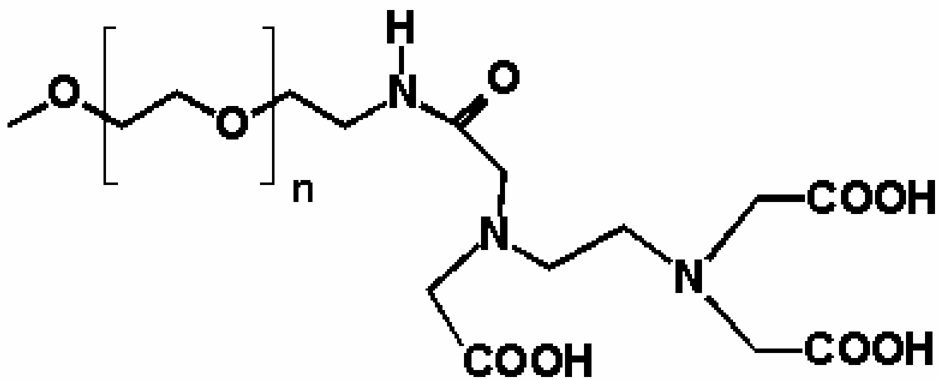
MPEG



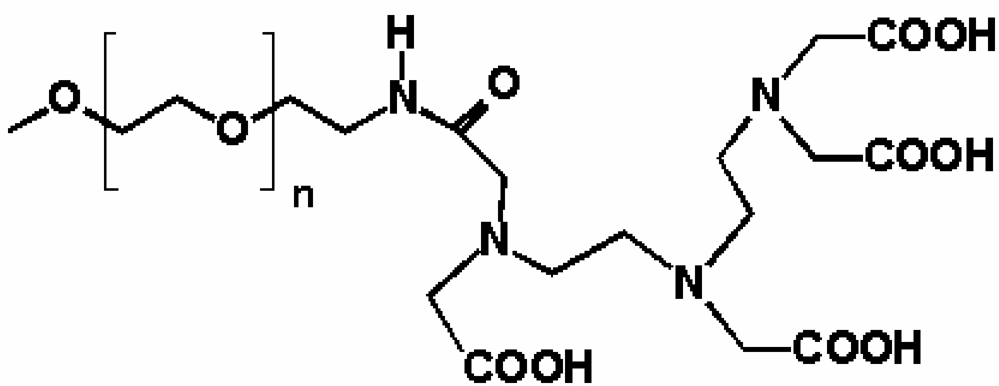
MPIDA



MPEDTA



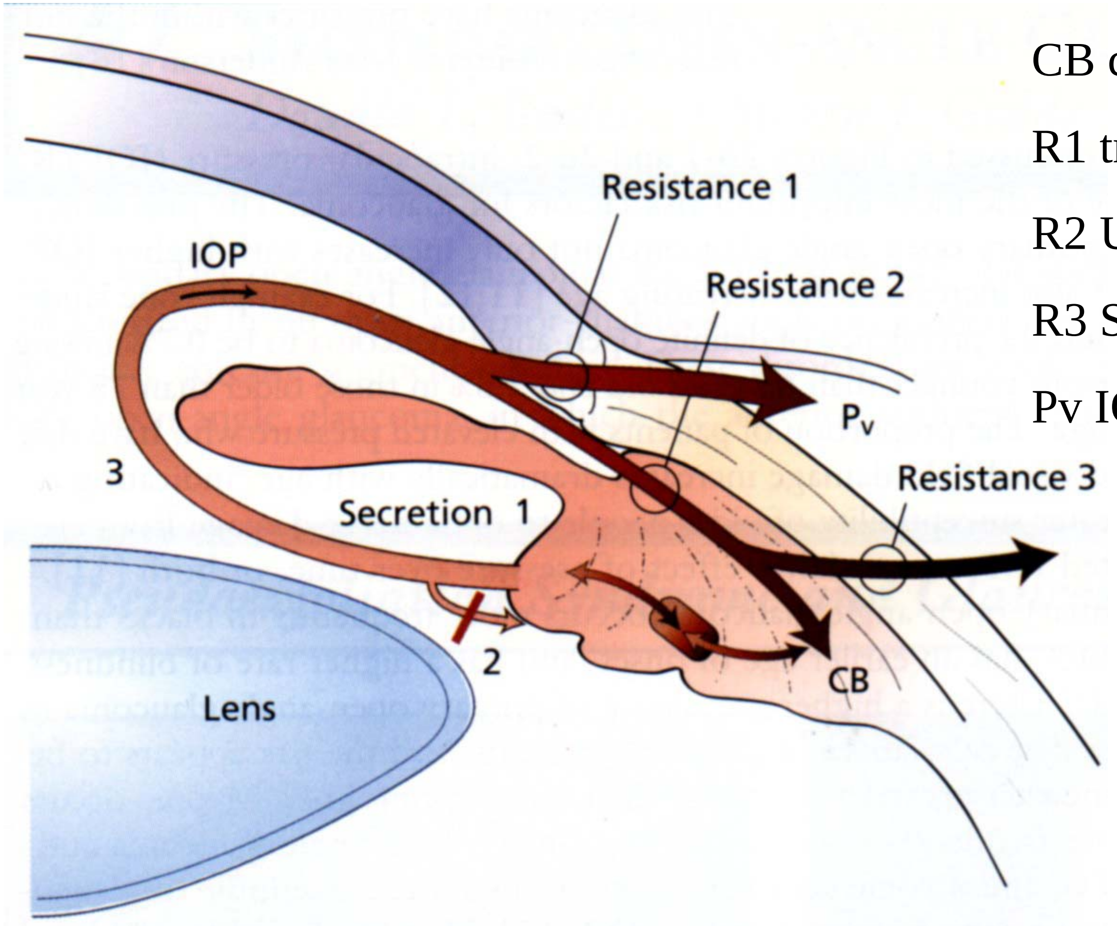
MPDTPA



MPEG attributes

- Ability to concentrate agent around cellular DNA
- Selectively protects against radiation damage to normal cells
- Reduces potential sources of free radicals, as well as detoxifying endogenous free radicals

Aqueous humor flow



CB cilary body

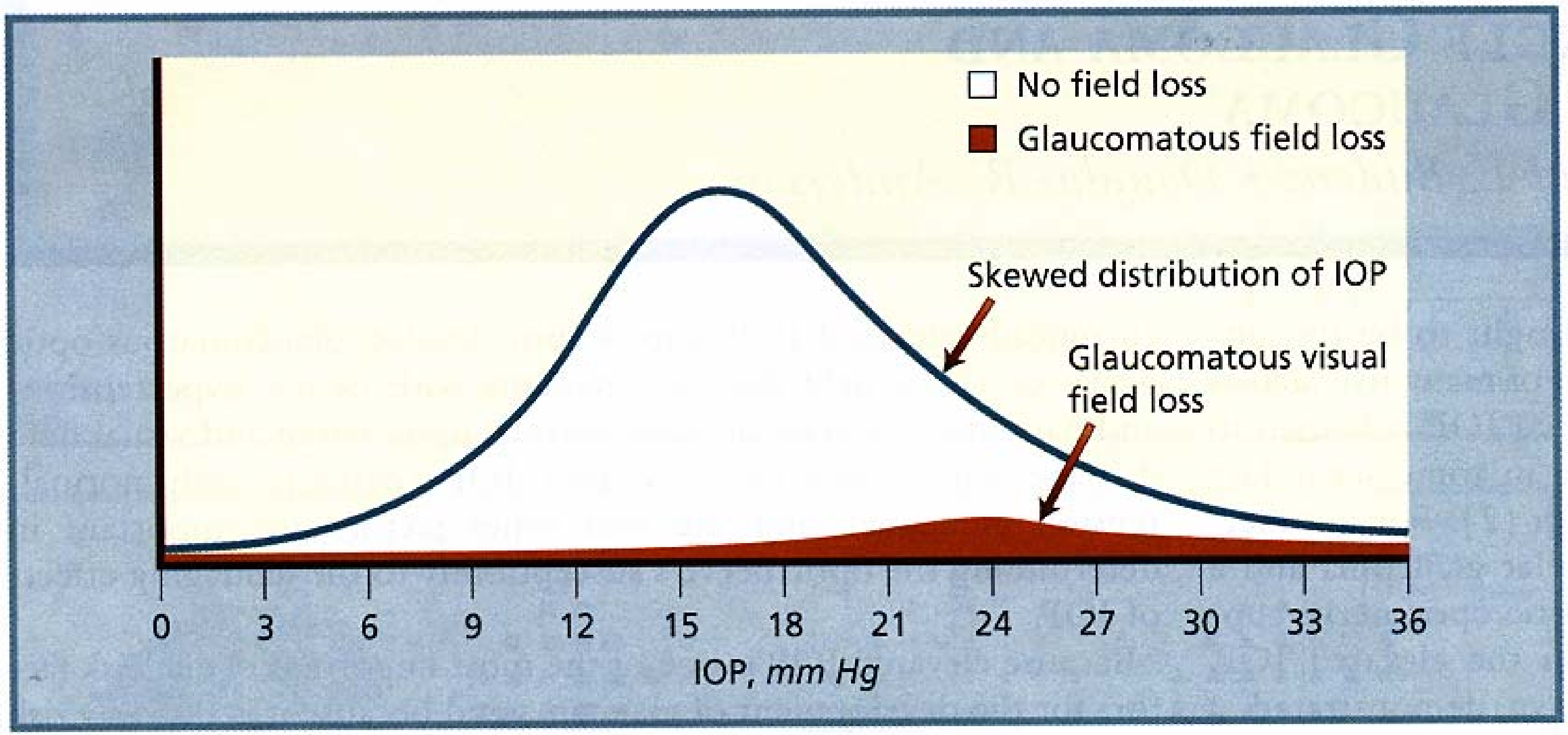
R1 trab/Schlemm's

R2 Uveoscleral

R3 Sclera

Pv IOP, episcleral vein

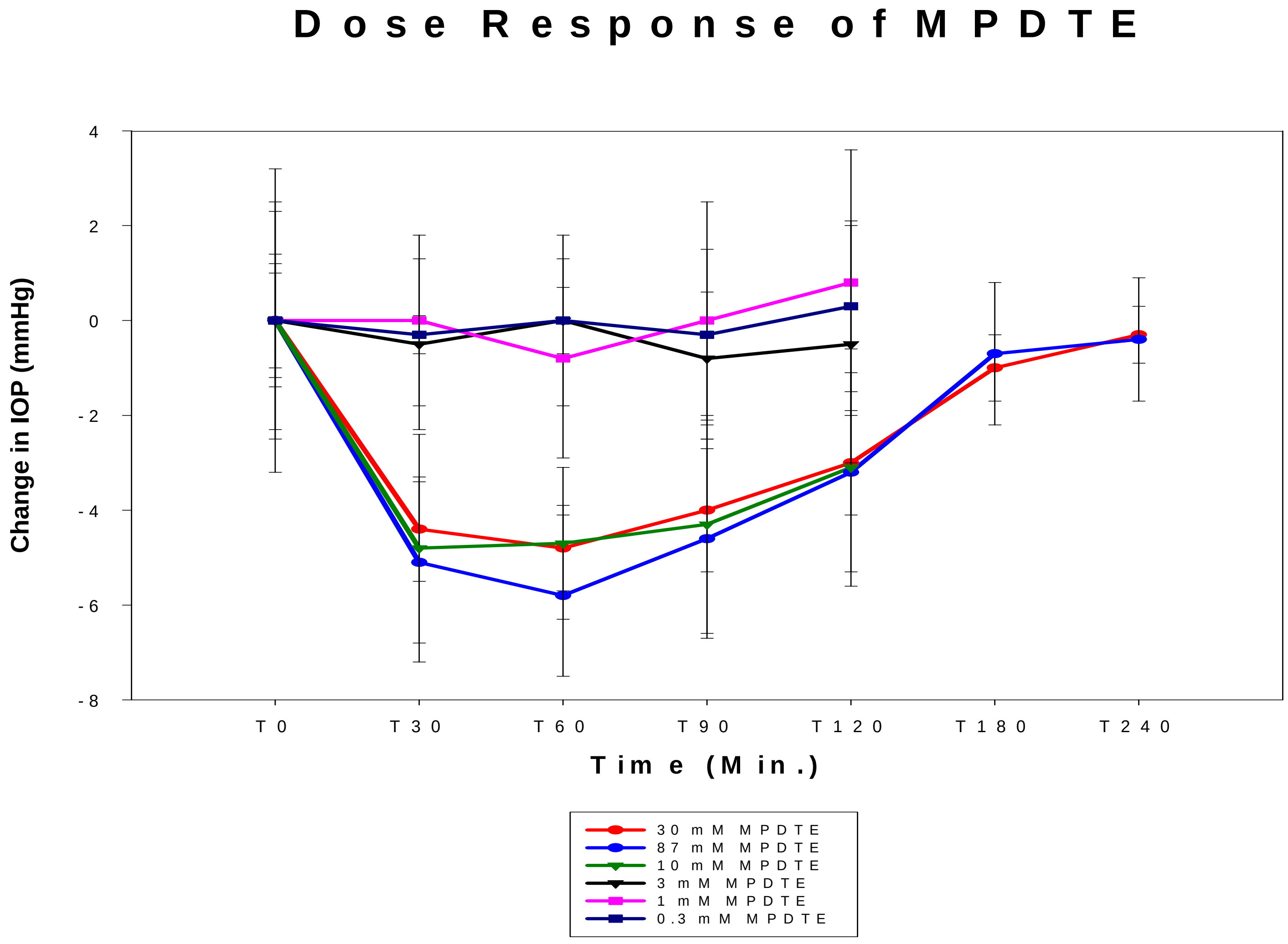
Glaucoma and IOP

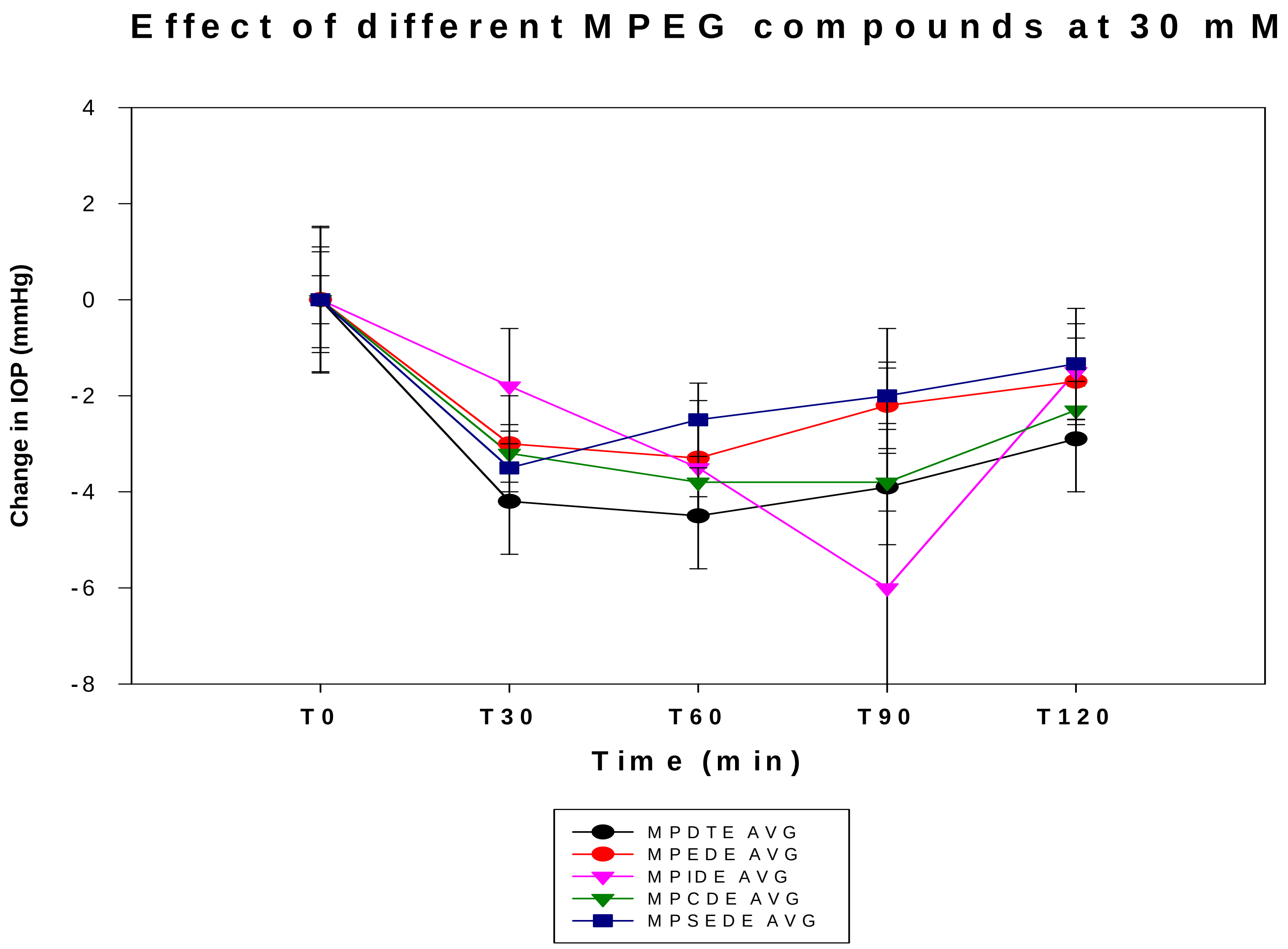


Pressure elevations generally undetectable to patients

Glaucoma Test models

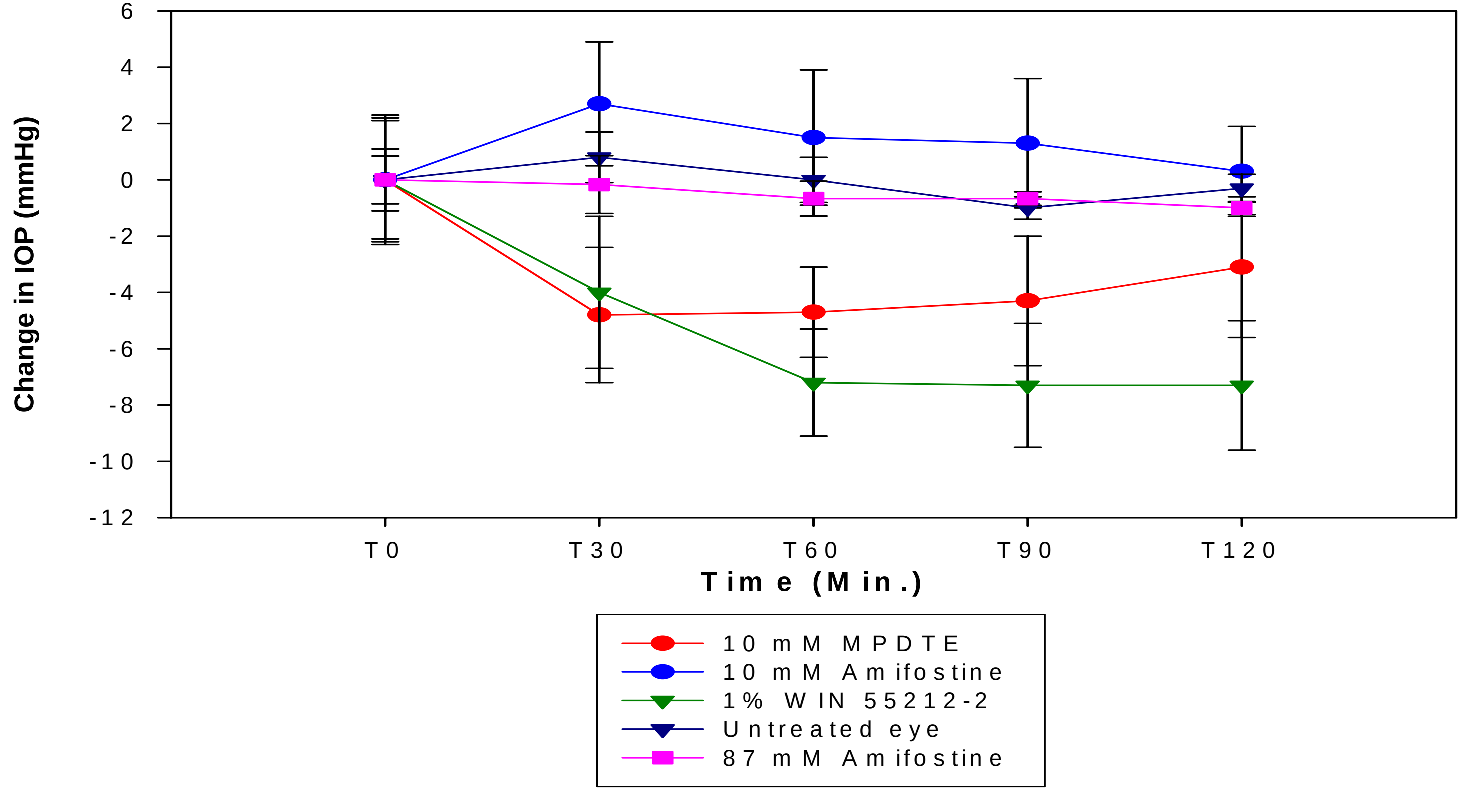
- Rabbit
- Rat episcleral vein ligation
- In vitro, in vivo human


Dose Response of MPDTE
Change in IOP (mmHg)
4
2
0
-2
-4
-6
-8
T0
T30
T60
T90
T120
T180
T240
Time (Min.)
30 mM MPDTE
87 mM MPDTE
10 mM MPDTE
3 mM MPDTE
1 mM MPDTE
0.3 mM MPDTE

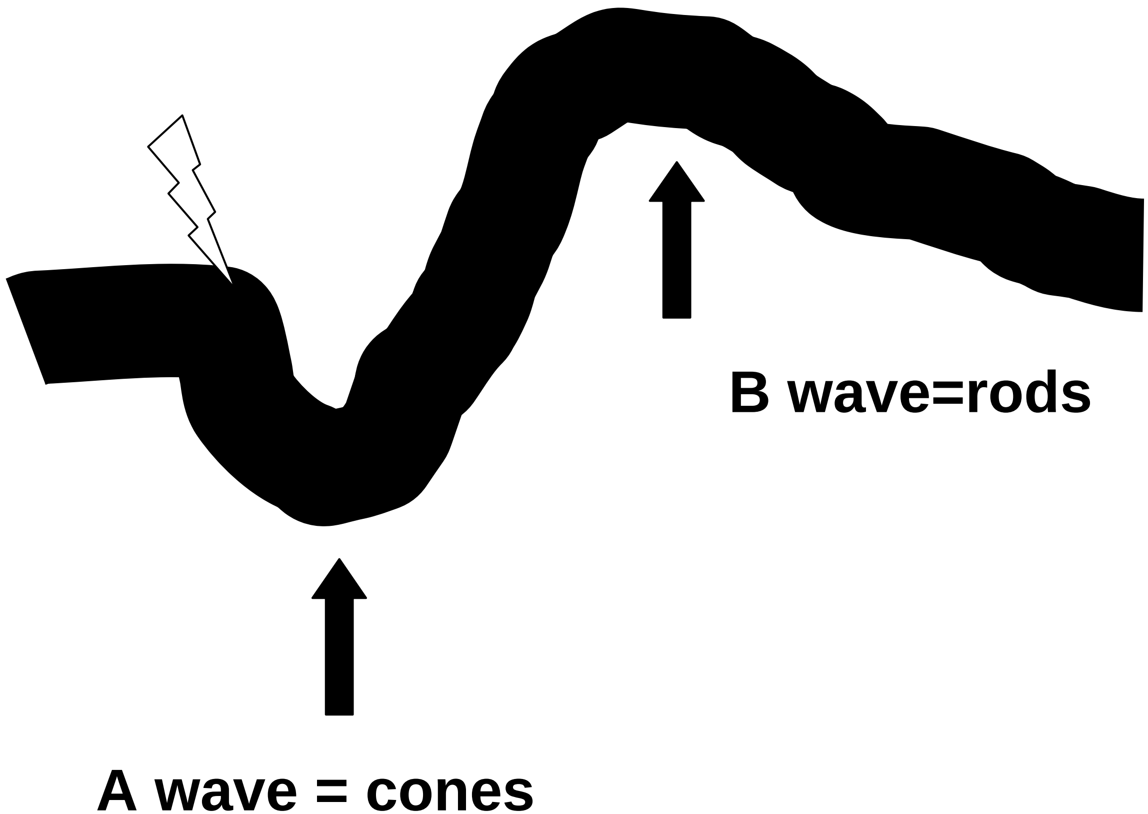


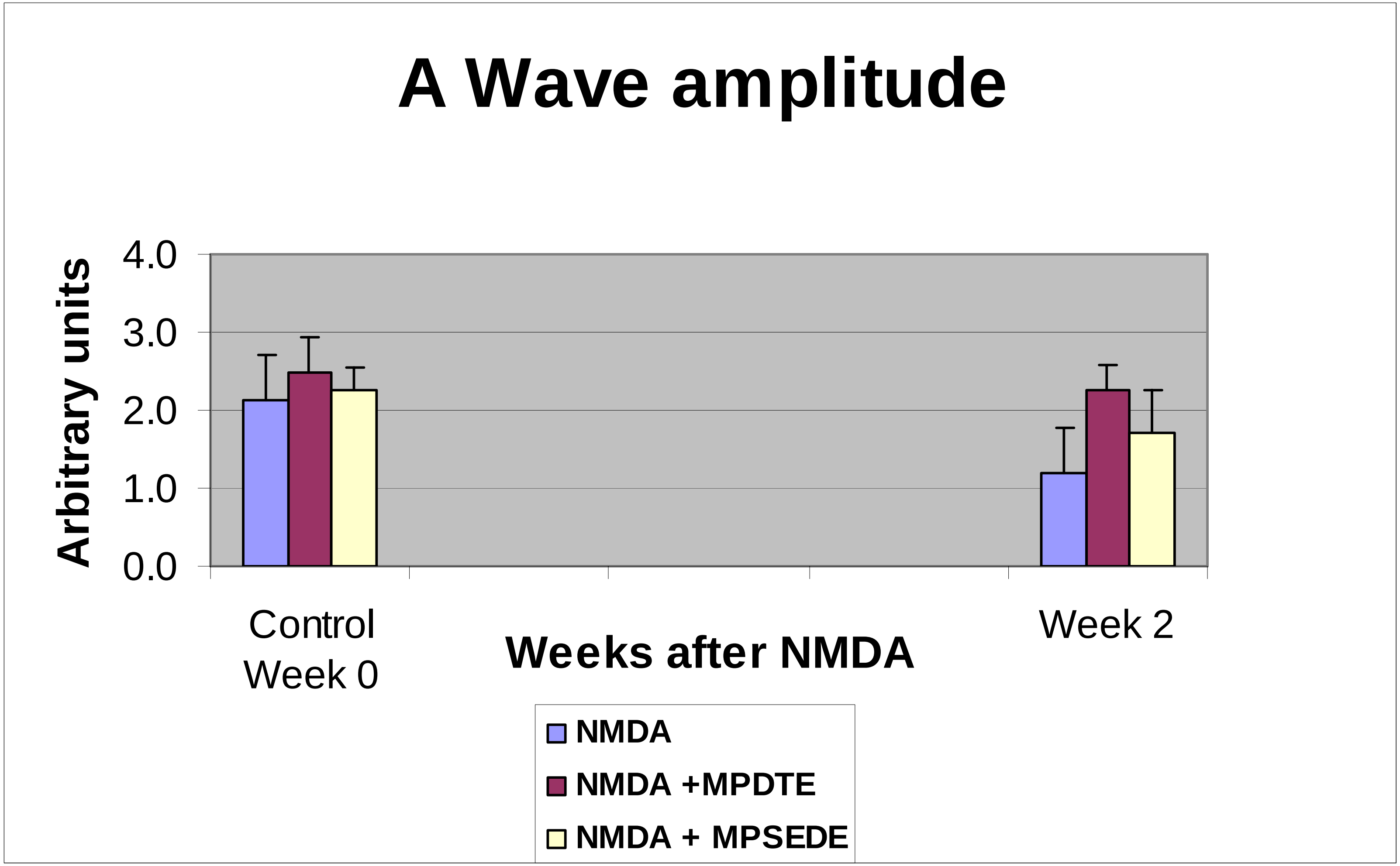
Effect of different MPEG compounds at 30 mM
Change in IOP (mmHg)
4
2
0
-2
-4
-6
-8
T0
T30
T60
T90
T120
Time (min)
MPDTE AVG
MPEDE AVG
MPIDE AVG
MPCDE AVG
MPSEDE AVG

Amifostine, a clinical effective free radical scavenger does not lower IOP

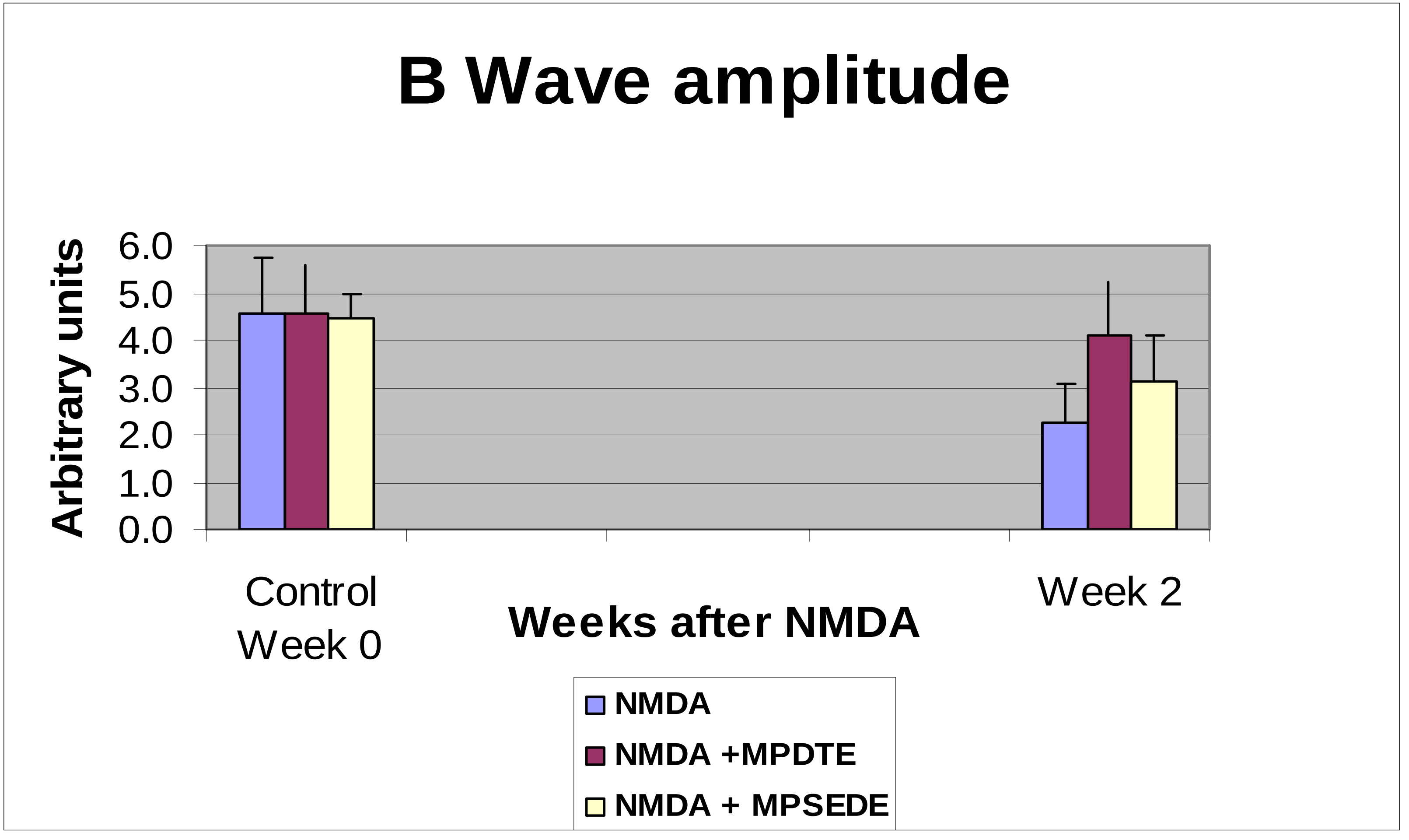


Electroretinogram basics





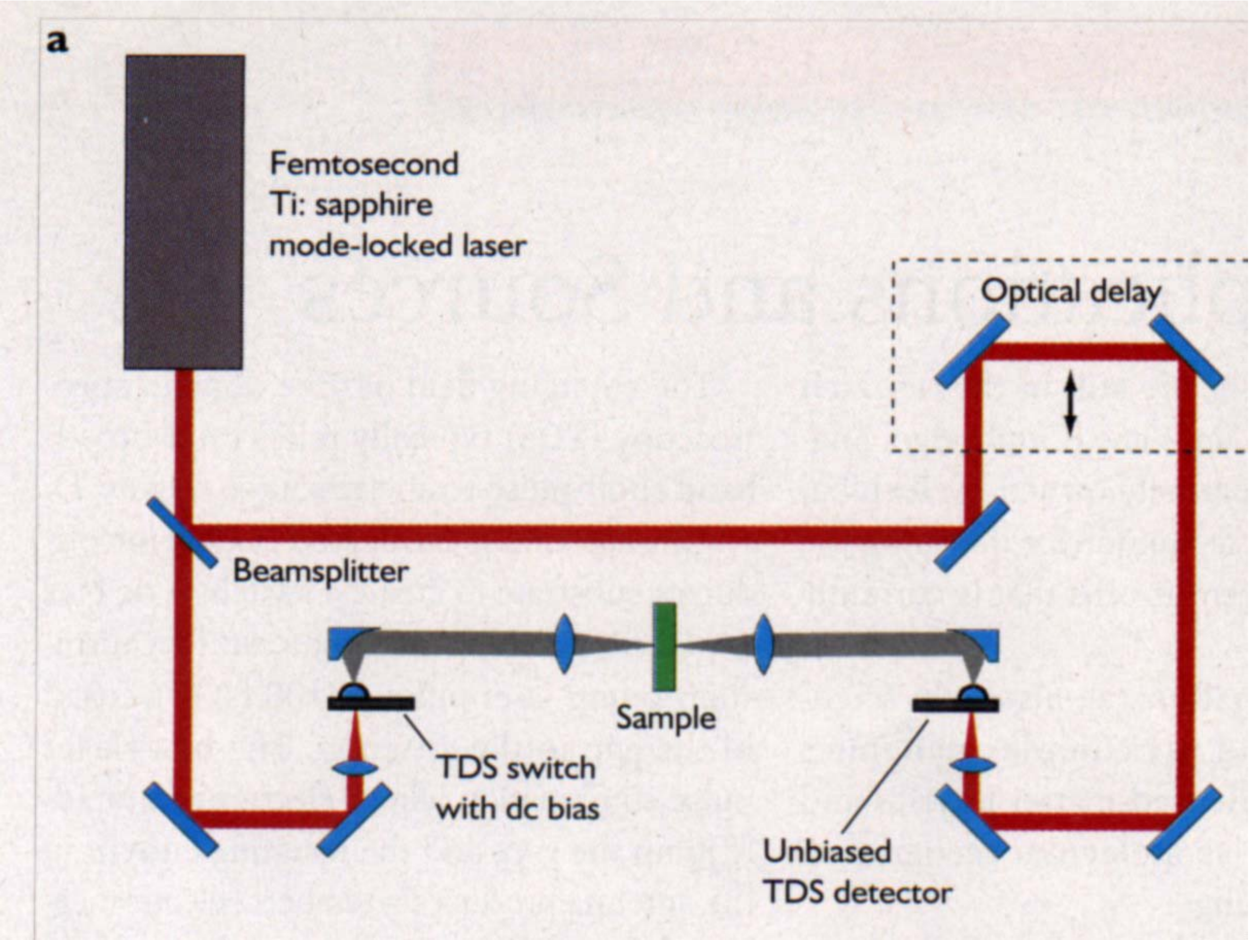
A Wave amplitude
Arbitrary units
4.0
3.0
2.0
1.0
0.0
Control
Week 0
Week 2
Weeks after NMDA
NMDA
NMDA +MPDTE
NMDA + MPSEDE


B Wave amplitude
Arbitrary units
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Control
Week 0
Week 2
Weeks after NMDA
NMDA
NMDA +MPDTE
NMDA + MPSEDE

Potential uses of FEL

Glaucoma	Shunt and valve function and integration into tissue	TeraHz
	Structural alterations affecting aqueous humor flow	TeraHz
	Drug depots and long term drug release	TeraHz, N(IR)
	Loss of retinal ganglionic cells, measurement of retinal pigments	TeraHz, N(IR)
	Metabolic changes, production of free radicals, ischemia	TeraHz, UV-N(IR)

TeraHz time domain spectroscopy



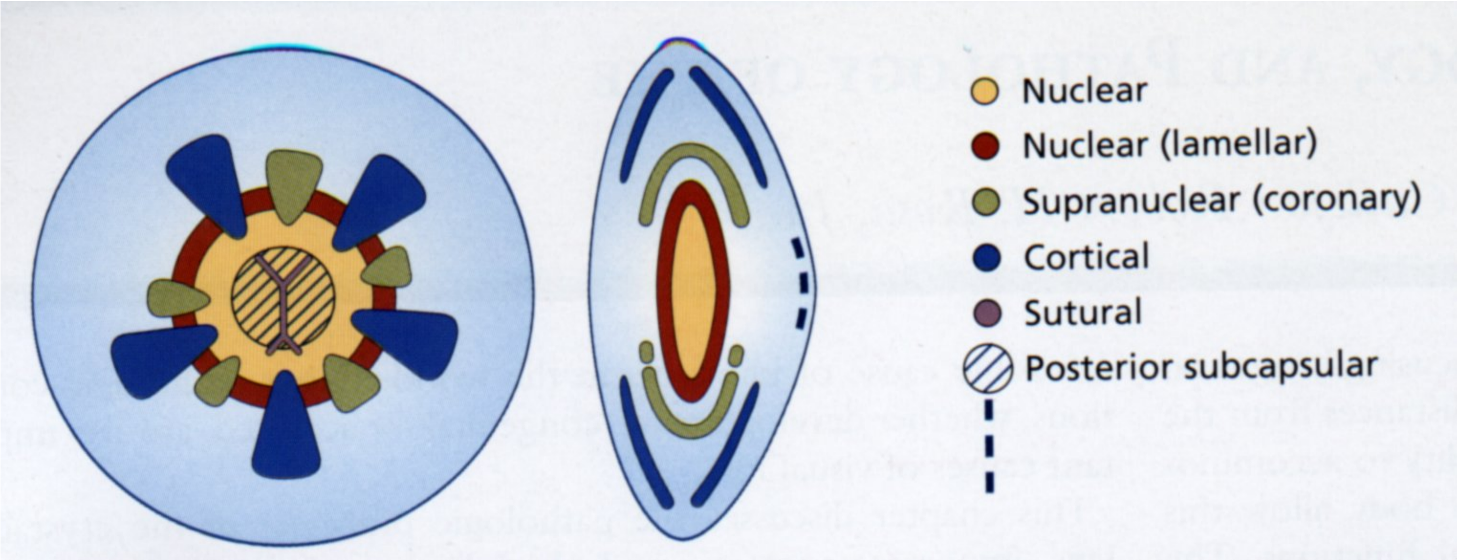
Industrial Phys.,
Aug/Sept. 2003, pg 28

TeraHz detection of tooth decay

Industrial Phys., Aug/Sept. 2003, pg 28

Cataract formation

- UV and cosmic radiation
- Drugs
- Trauma
- Diabetes, Down's, Glaucoma, etc.



Current limitations of cataract detection and treatment

- Slit lamp detects frank cataract formation often years after precipitating events
- Surgical treatment used to replace lens
- Necessity to follow early events to monitor efficacy of interventions as well as to track the pathology of disease process

Cataract Test models

- Mouse X-ray, UV, chemical
- Rat X-ray, UV, chemical
- In vitro, in vivo human

Free Electron Laser (FEL) Applications for Ophthalmology

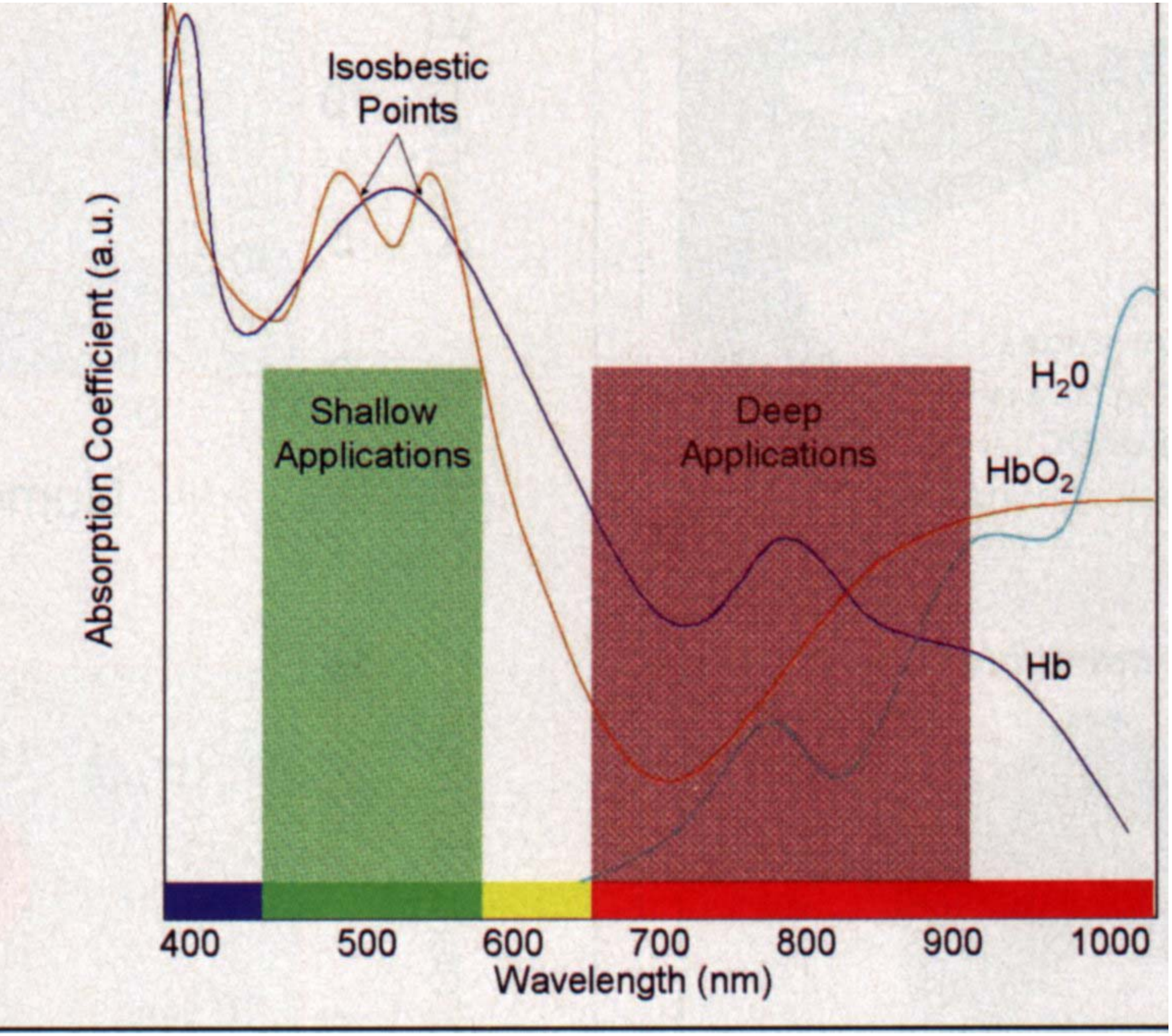
- The FEL can be used to generate ultraviolet radiation damage models to test the efficacy of MPEG agents to protect against cataract formation and UV skin damage.

Free Electron Laser (FEL) Applications for Ophthalmology

- The FEL can be used to develop (N)IR and terahertz radiation imaging technology which can be used to detect changes in the hydration of the lens that occur during cataract formation, as well as the presence of tumors in the skin and body, changes in retinal pigments that indicate retinal dysfunction and myloid plaques (Alzheimer's disease).

Potential uses of FEL

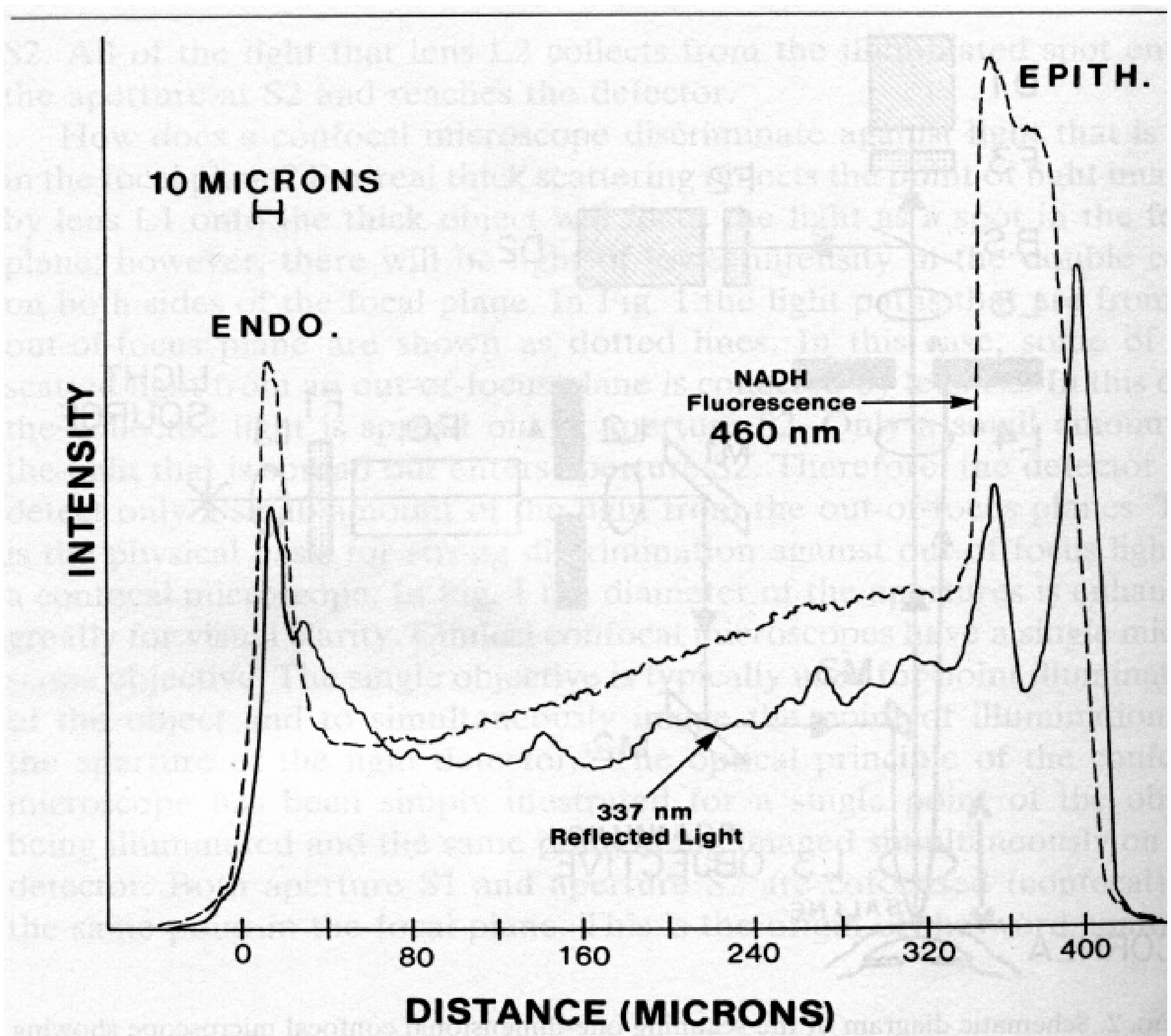
Cataracts	Creation of animal models	UVA, UVB radiation
	Early detection of capsule and lens changes	TeraHz, N(IR)
	Detection of IOL rejection	TeraHz, N(IR)



Ocular Hb oxygenation detection

Biophotonics Inter.., May 2004

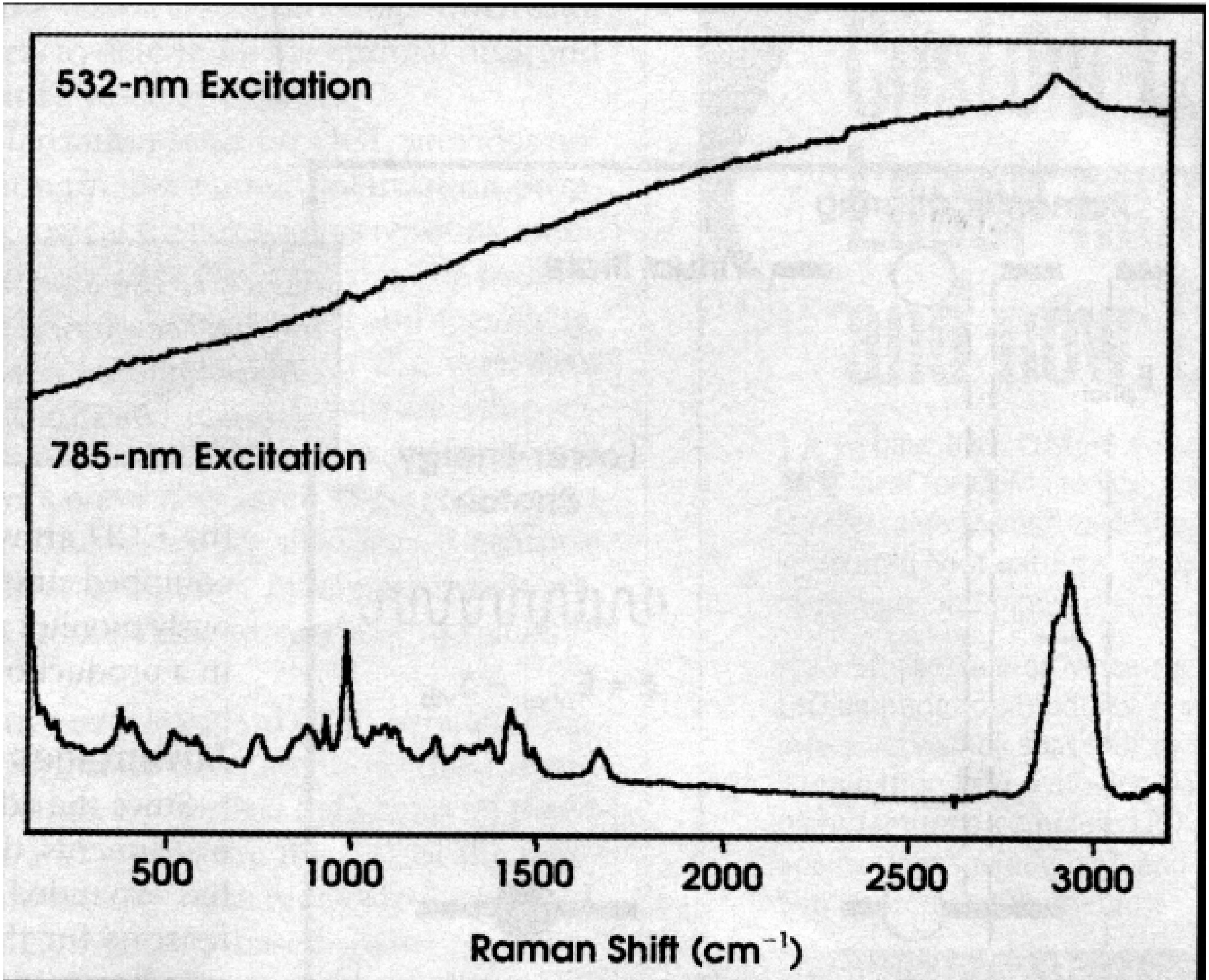
Measurement of Biochemical Parameters



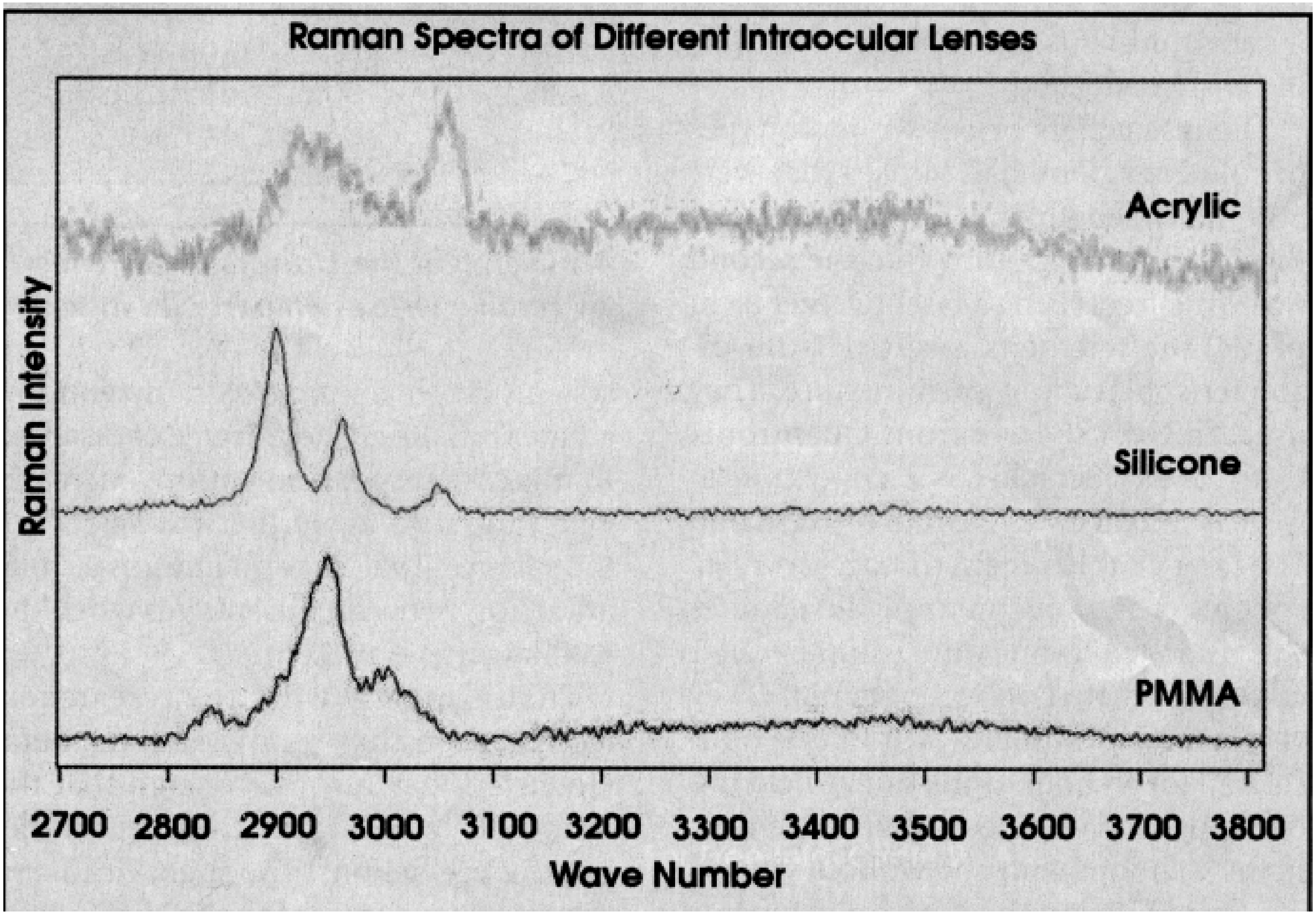
Conn, Methods in Enzymology, 1999;307: 540

Raman Spectroscopy

- Pharmacokinetic studies
- In vivo drug measurements and metabolite levels
- Localization of drugs – diffusion and compartmentalization

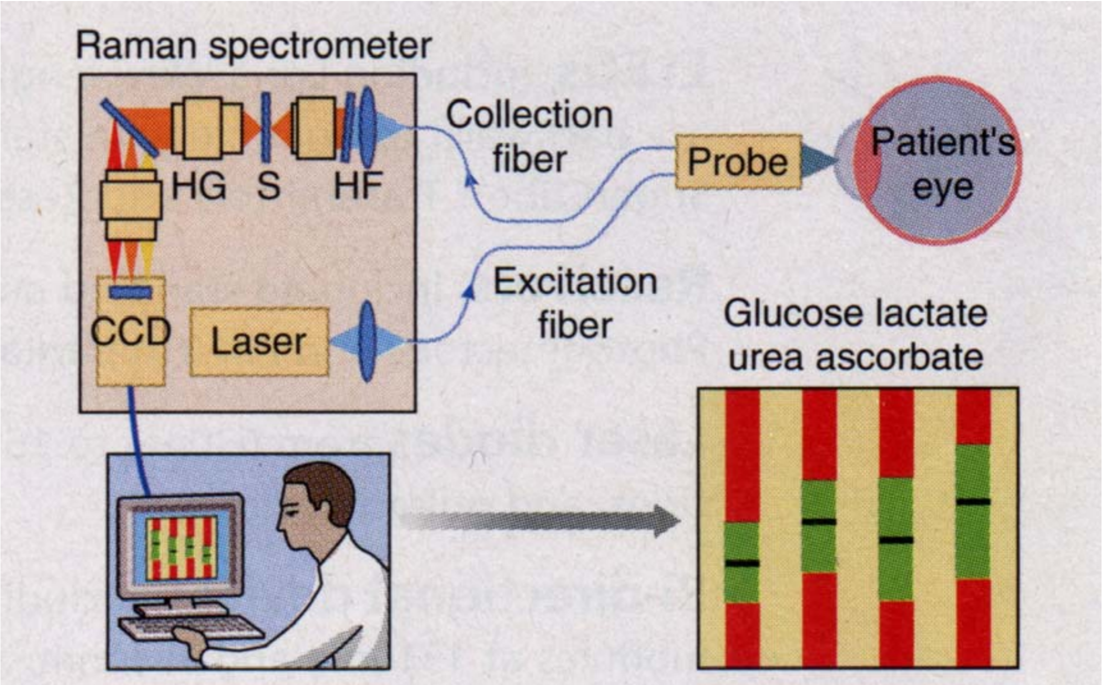


Biophotonics International, 2001: 48



Biophotonics News, 2001: 28

Raman Ocular Glucose detector



Blood-sugar levels are measured through the eye with a Raman spectrometer.

Confocal raman microscope

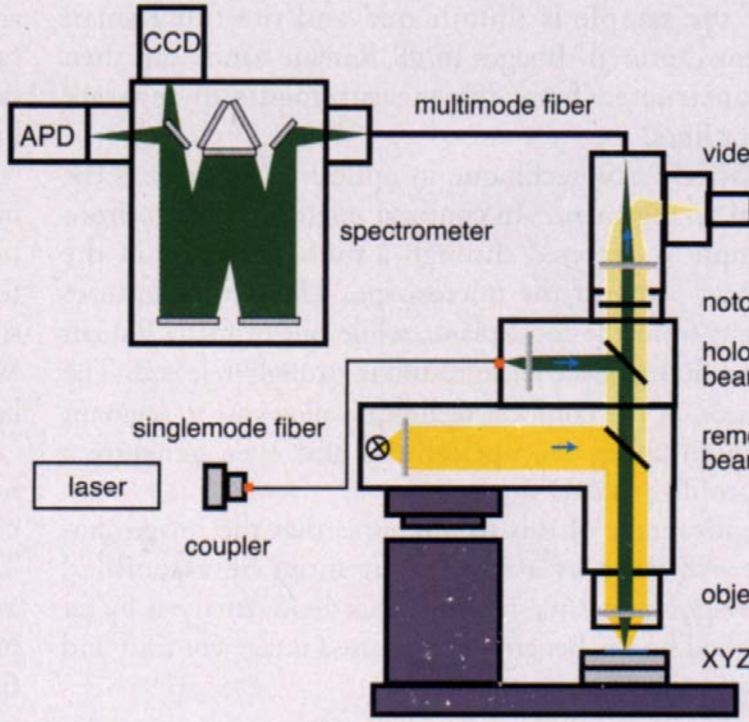
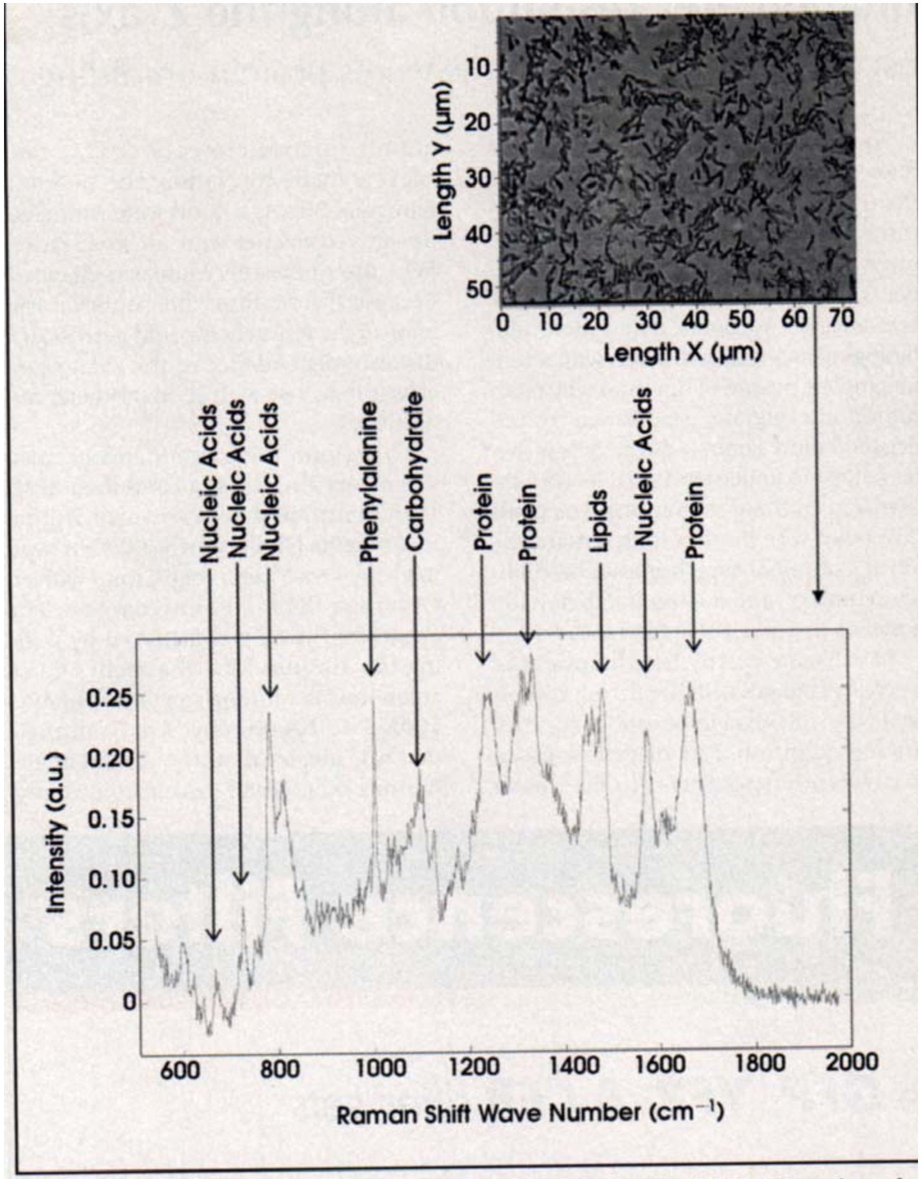


Figure 1 A confocal Raman microscope consists of a dual-output-port spectrometer integrated with a confocal microscope. A singlemode optical fiber provides point-source illumination and the core diameter of a multimode fiber provides the confocal pinhole in the focal plane.

Single cell Raman scan



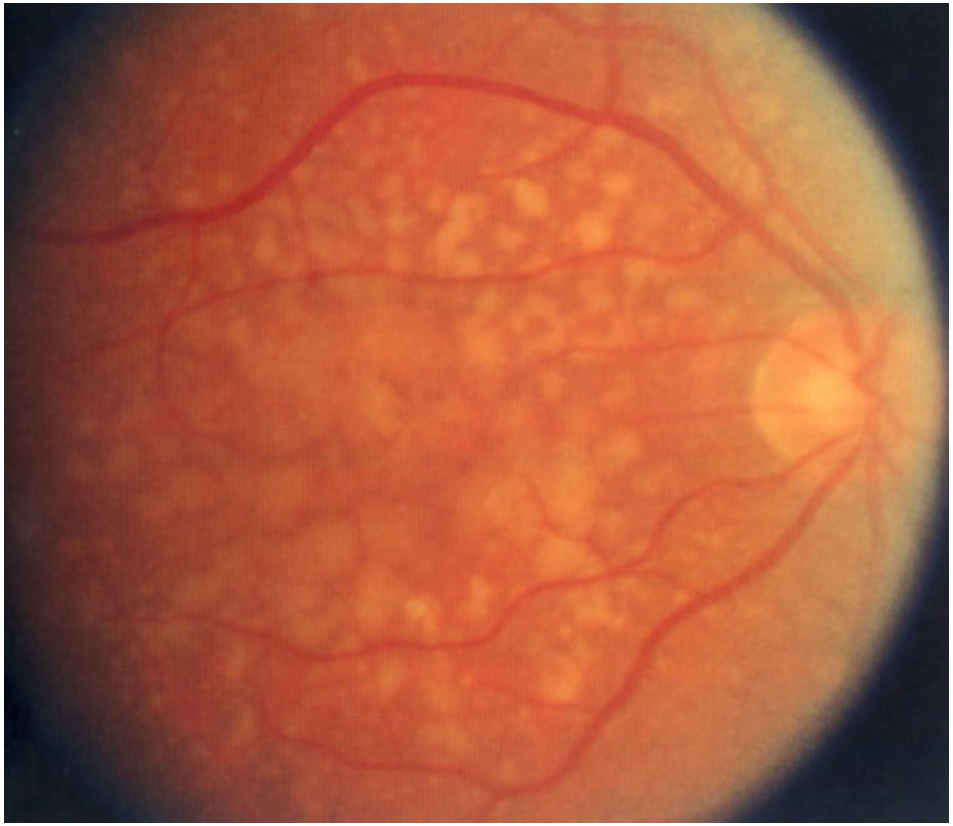
Researchers used Raman spectroscopy to identify single microbial cells such as the ones pictured in the top right. A typical Raman spectrum for a single cell is shown at the bottom. Reprinted with permission from Analytical Chemistry.

Biophotonics Intern., Oct. 2004.

Age related macular degeneration

- Major cause of vision loss
- Interventions include PDT, drugs, etc.
- Interventions have limited efficacy on this progressive disease
- Future interventions for retinal repair/replacement via stem cells, artificial retina

Dresen on retina of AMD patient



AMD, Diabetes, Dry eye test models

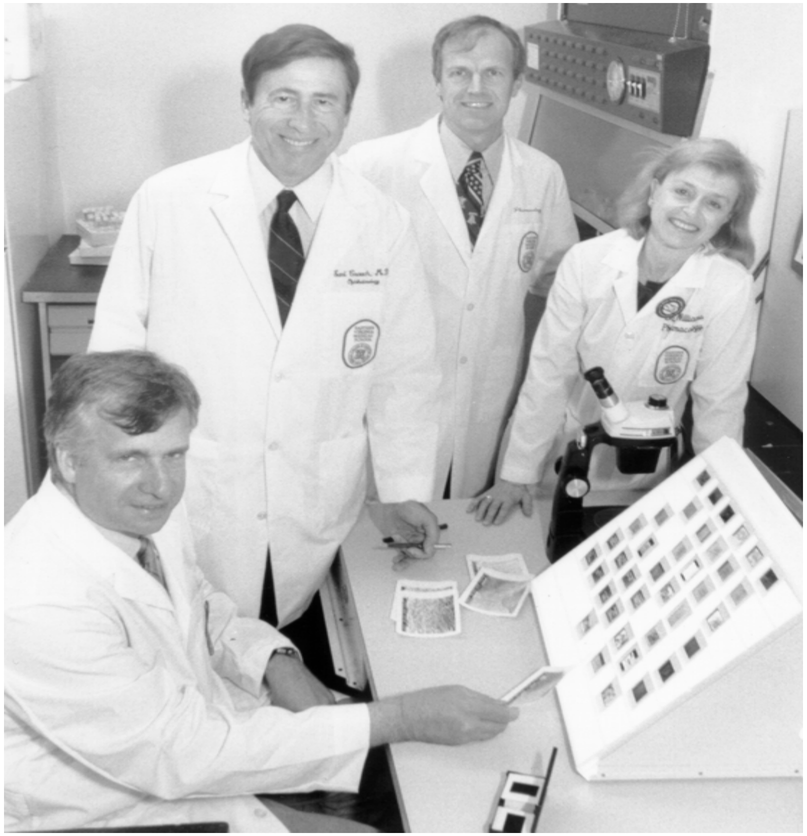
- Mouse
- Rat
- In vitro, in vivo human

Potential uses of FEL

Macular degeneration	Detection of dresen, retinal pigments, drug depot function	TeraHz, N(IR)
Dry eye	Progressive corneal surface changes	TeraHz
Diabetes	Detection of corneal and retinal changes	TeraHz, visible-N(IR)

T.R. Lee Center team

- Patricia Williams, PhD Director
- Earl Crouch, MD Chairman Ophthalmology
- John Sheppard, MD, MMSc Clinical Director
- Frank Lattanzio, PhD Basic Science Director



The Beginning

Schematic of imaging spectrometer

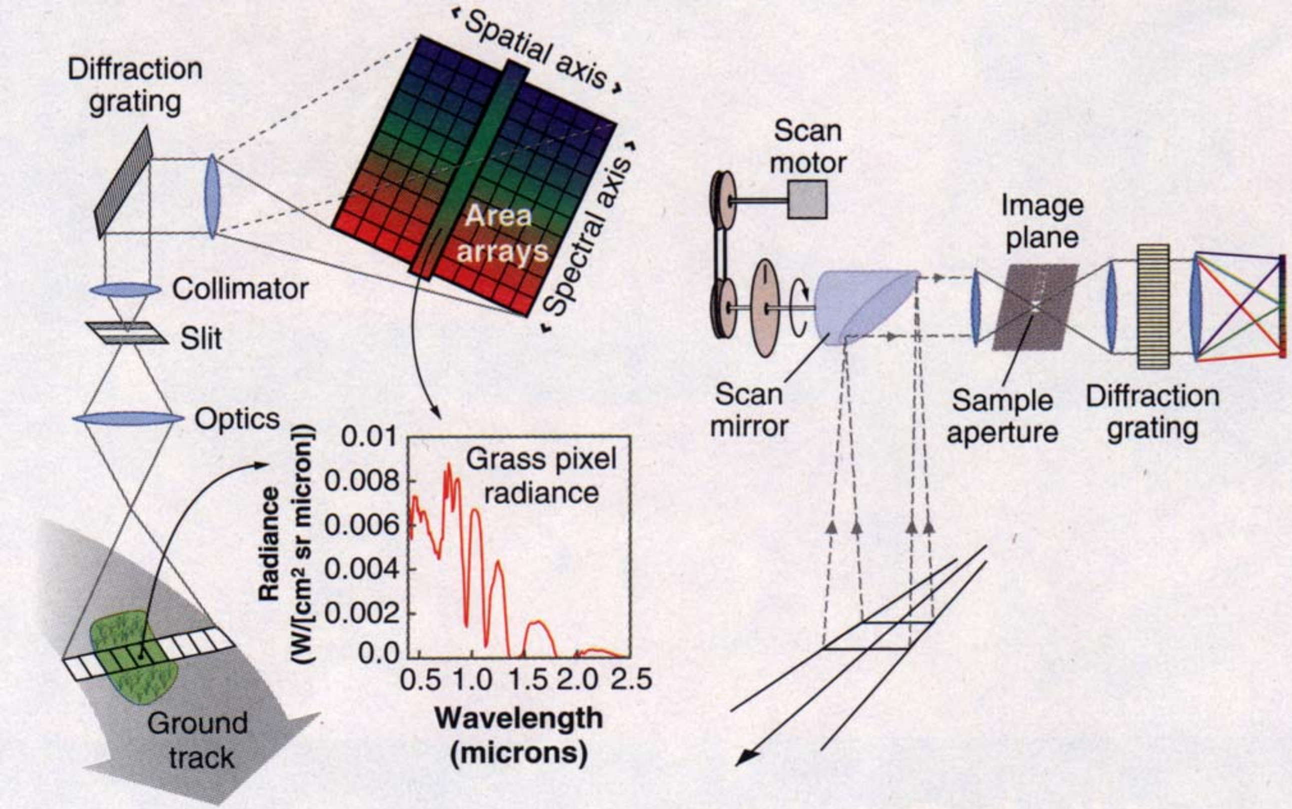


FIGURE 2. Two designs for an imaging spectrometer are a pushbroom design (left) and a line-scanner design (right).

Laser Focus World., May 2004, pg 77

IR cell chemical scan

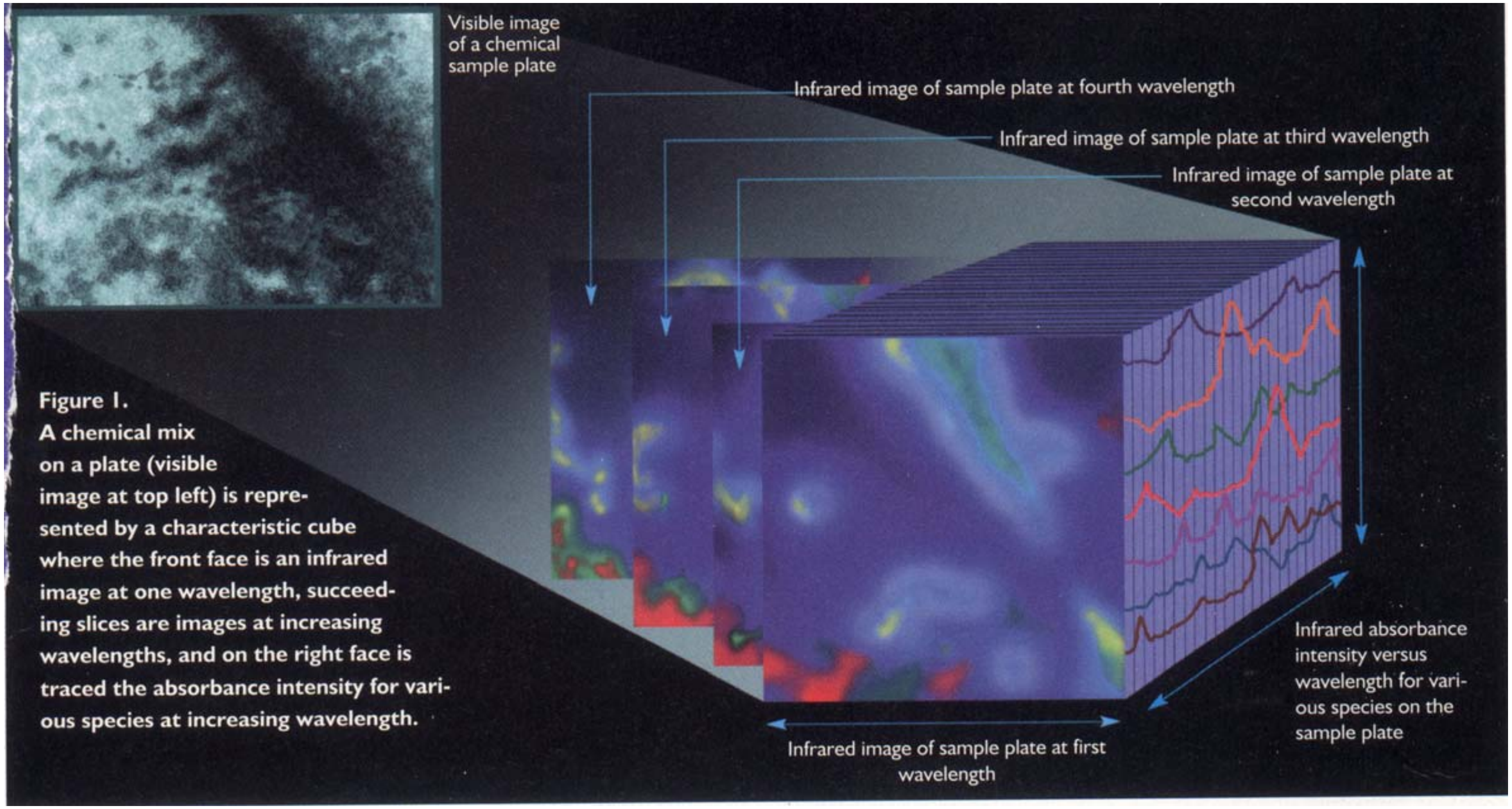
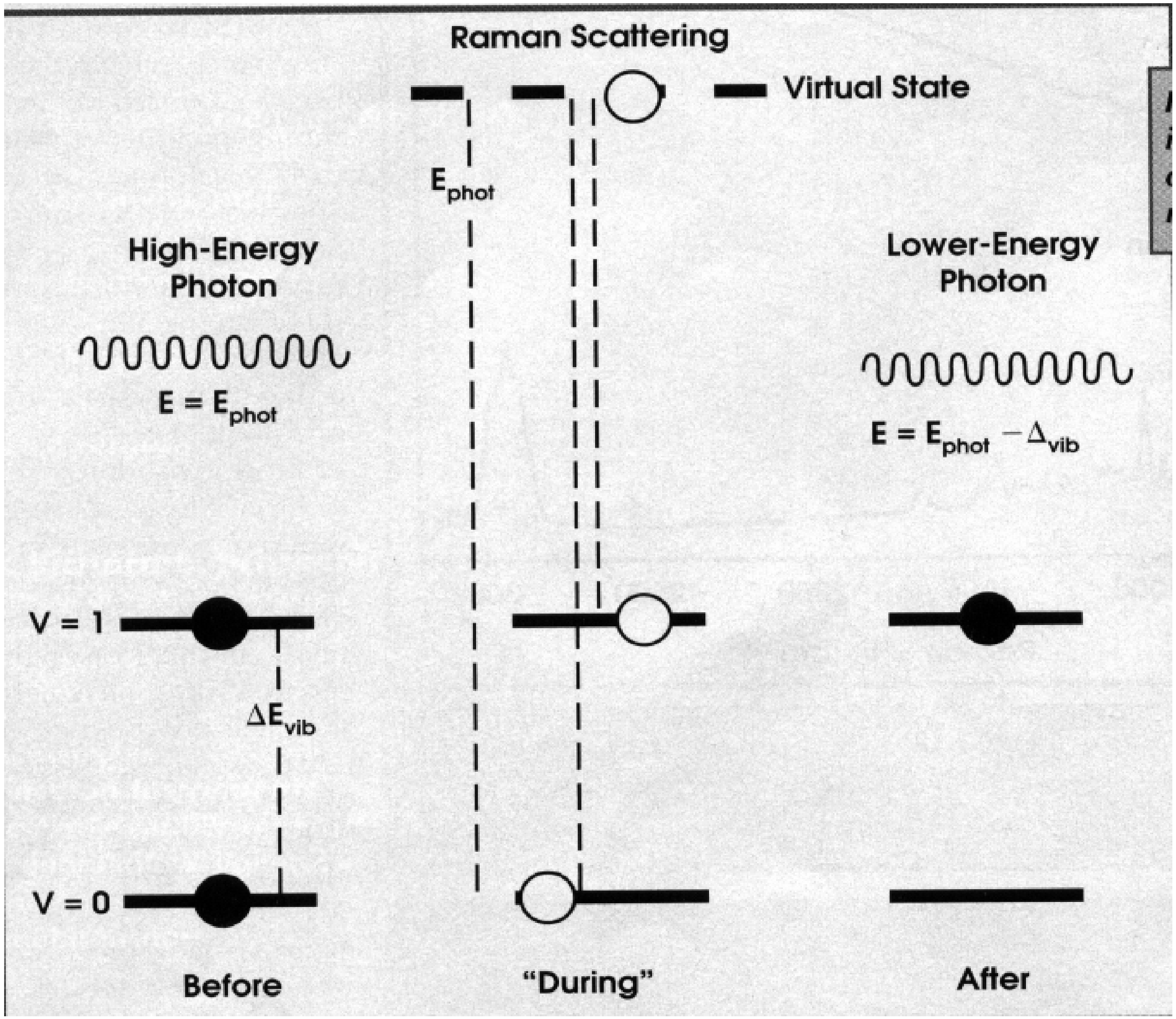


Figure 1. A chemical mix on a plate (visible image at top left) is represented by a characteristic cube where the front face is an infrared image at one wavelength, succeeding slices are images at increasing wavelengths, and on the right face is traced the absorbance intensity for various species at increasing wavelength.

Industrial Phys., Oct/Nov. 2003, pg 29



Biophotonics International, 2001: 47