Optical Methods for Medical Imaging and Diagnosis

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UCLA, CASIT

Optical techniques play a major role in modern medical diagnostics through imaging and spectroscopy

Typical examples include endoscopic inspection of the GI tract and measurement of Oxygen concentration in the blood based on hemoglobin absorption

Recent advances in technology have improved both the optical diagnostic and imaging capabilities for a wide variety of applications

Near Infrared Spectroscopy (NIRS) – Promises and Pitfalls

Goals

1. Current Status of NIRS Devices.
2. Limitations in NIRS Monitoring Technologies.
3. Visible Light Approaches.

Neonatal
Oxygen Therapy

Problems Related to Oxygen Therapy

- Hyperoxia-induced Oxygen Toxicity

- *'Acute' Toxicity – "Bert Effect"*
  - Patient is exposed to very high concentrations of oxygen for short durations
    - Ex: CO poisoning, respiratory distress of newborns, etc.
    - Primarily CNS effects due to oxidation and polymerization of –SH groups of enzymes, resulting in cellular damage.

- *'Chronic' Toxicity – "Smith Effect"*
  - Low concentrations of oxygen are administered but for longer duration
    - Primarily has pulmonary effects
      - High oxygen conc. may damage the pulmonary epithelium, inactivate the surfactant and thus lead to atelectasis.

Problems Related to Oxygen Therapy

- Retinopathy of Prematurity (ROP)
- ROP, fka retrolental fibroplasia, is one of the single largest causes of blindness in childhood.
- Pathology: High oxygen concentration induces vasoconstriction, especially in the temporal portion of the retina.

Problems Related to Oxygen Therapy

- Retinopathy of Prematurity (ROP)

- Pathology (continued)
  - After the cessation of oxygen therapy, regeneration of the vessels occurs extending into the vitreous past the retina.

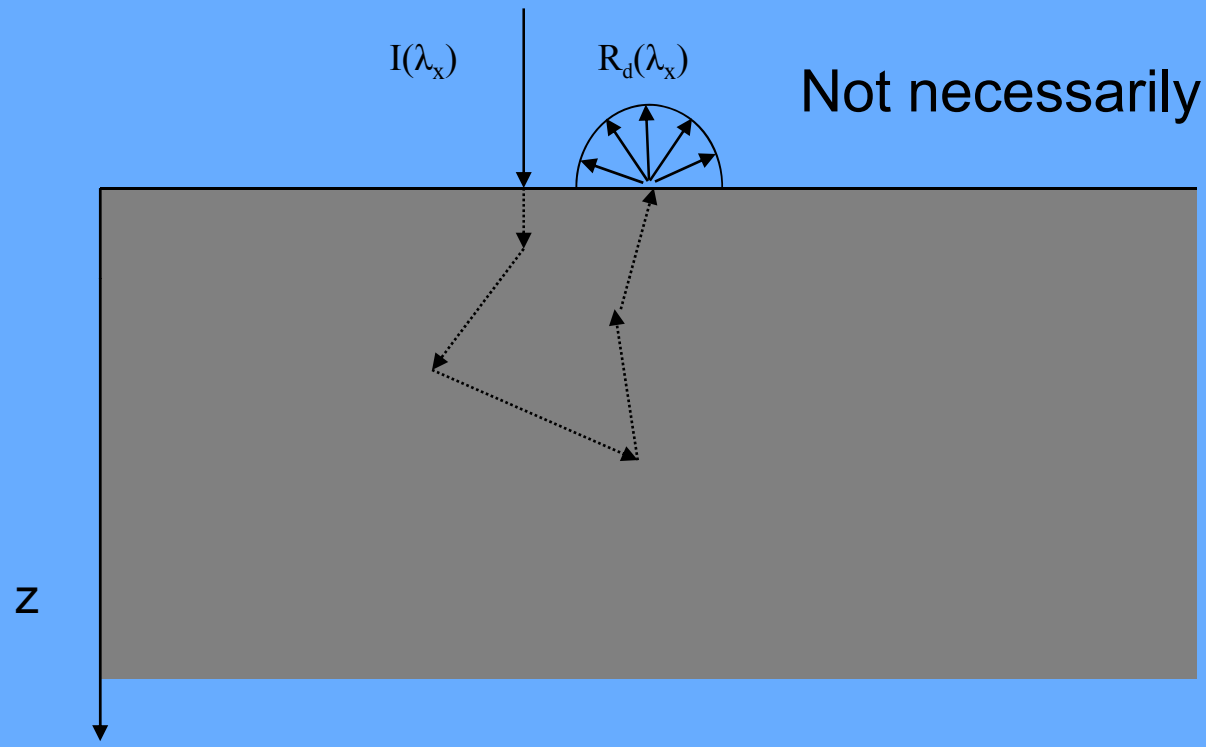
  - Dilation and rupture of these vessels can cause blindness due to:
    - Vitreous or retinal hemorrhage;
    - Fibrosis;
    - Adhesions leading to retinal detachment.

Possible Solutions to Problems Related to Oxygen Therapy

- Continuous Measurement of Cerebral Oxygen Saturation Levels.
- Continuous Measurement of SpO_2 Levels.
  - Use of Non-invasive Devices Preferred!

Theoretical Basis for Optical Measurements

Basics of Light Transfer

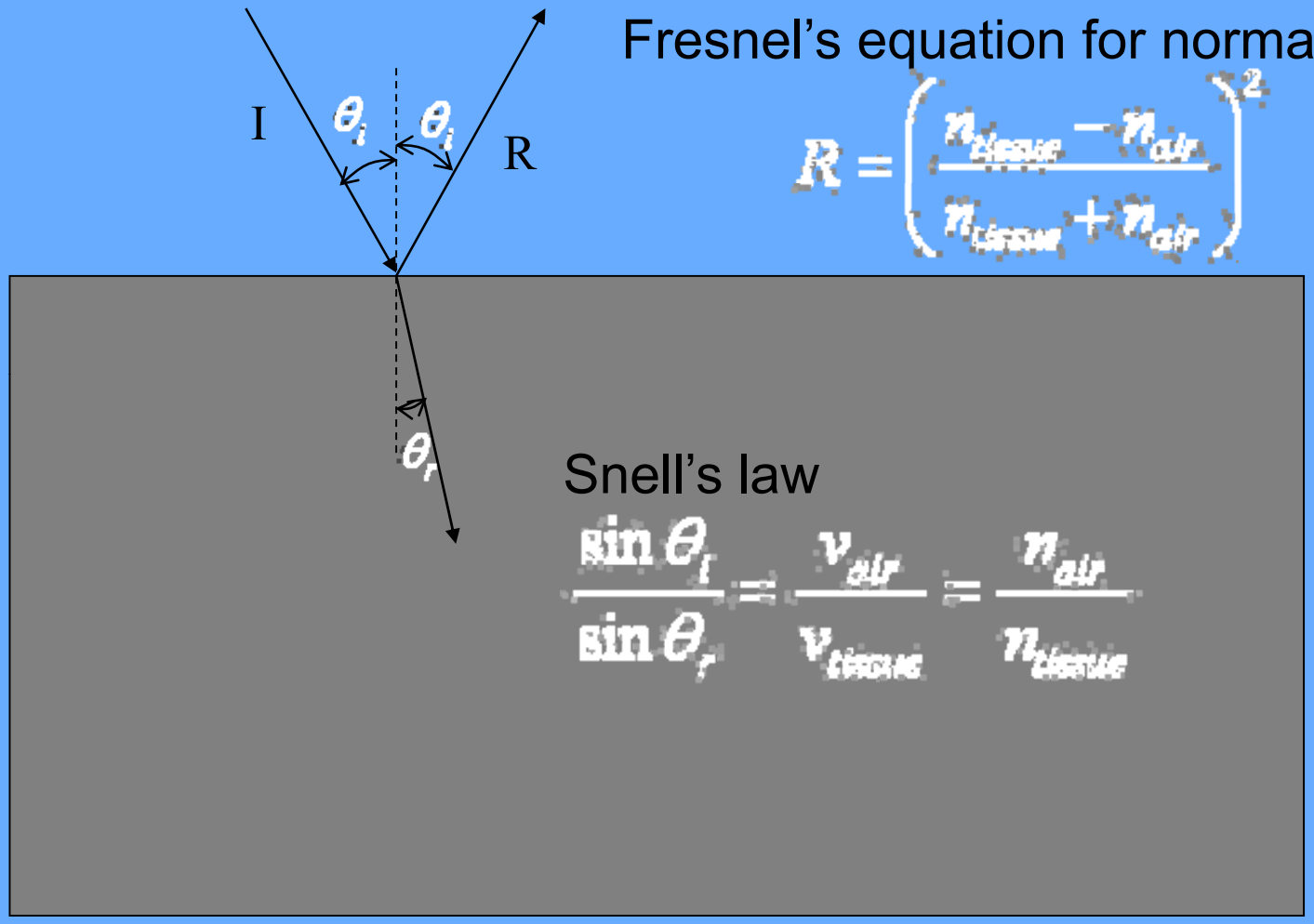


Not necessarily hemispherical!

Diffuse reflectance spectroscopy.

Given the reflected light, determine the optical properties of the medium.

Boundary Effects



Fresnel's equation for normal incidence

$$R = \left(\frac{n_{tissue} - n_{air}}{n_{tissue} + n_{air}} \right)^2$$

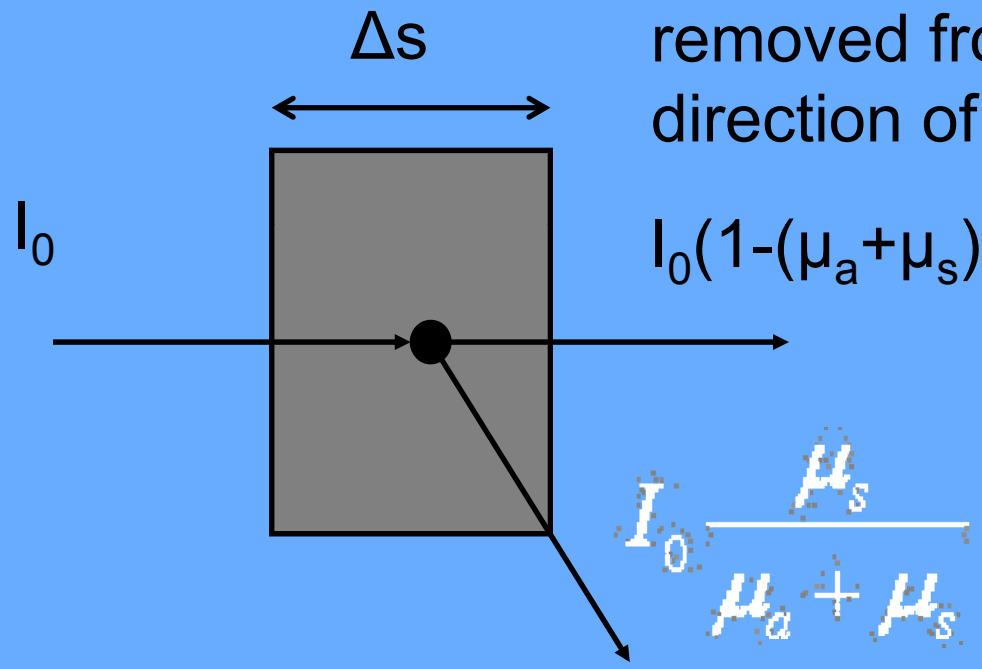
Snell's law

$$\frac{\sin\theta_i}{\sin\theta_t} = \frac{v_{air}}{v_{tissue}} = \frac{n_{air}}{n_{tissue}}$$

Specular reflectance, R, is usually removed with a polarized filter.

Source: *M.F. Modest.*

Absorption and Scattering



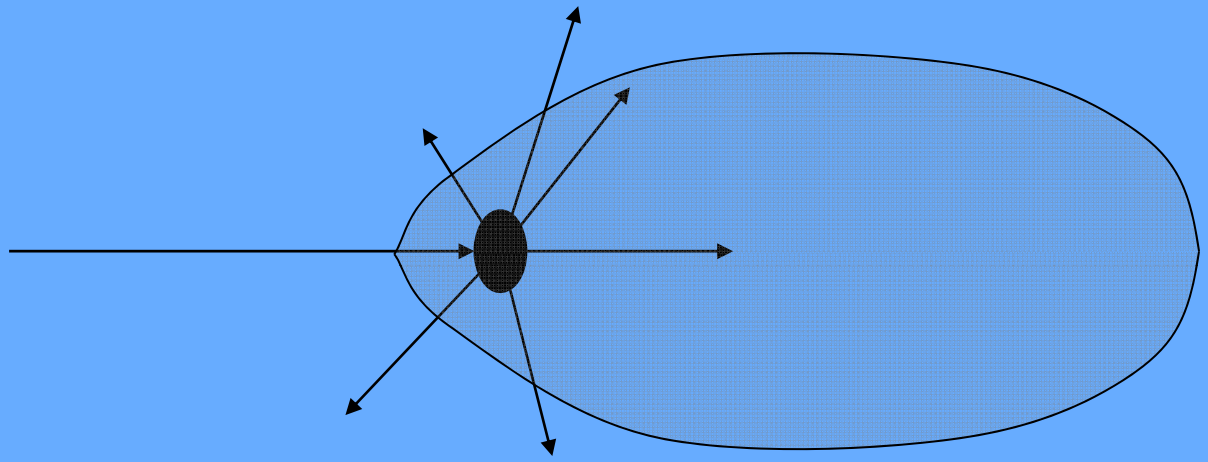
Amount of light removed from the direction of travel

$I_0(1-(\mu_a+\mu_s))\Delta s$

Idealized photon/media interaction

Source: *M.F. Modest.*

Scattering

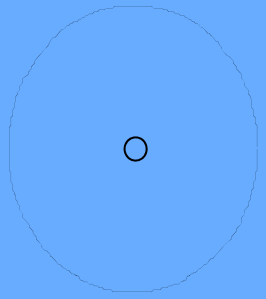


Scattering phase function determines the probability of scattering in a given direction.

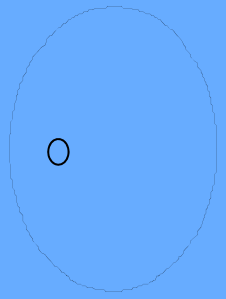
Source: *M.F. Modest.*

Henyey-Greenstein - Approximation

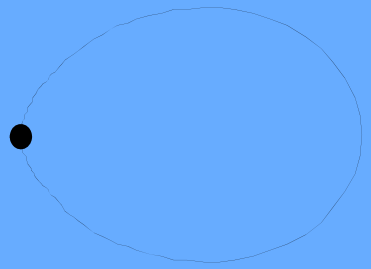
g = 0.00



g = 0.20



g = 0.90



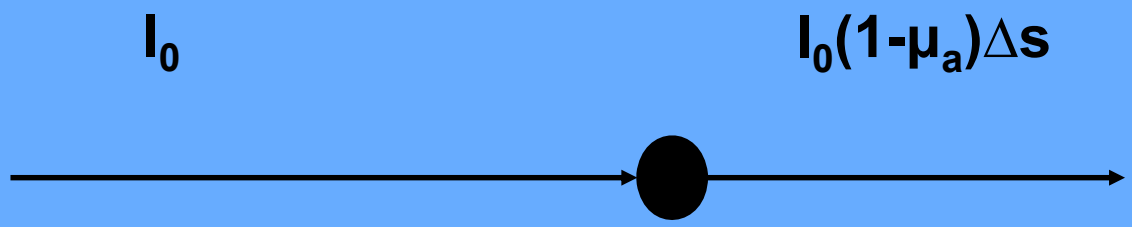
g = 1.00

$$p(\theta) = \frac{1}{4\pi} \frac{1-g^2}{\left(1+g^2-2g\cos(\theta)\right)^{3/2}}$$

Source: *Henyey, L.G., Greenstein, J.L.*

Reduced Scattering Coefficient

Incorporates the effects of anisotropy: $\mu_s' = \mu_s(1-g)$



Scattering plays no role

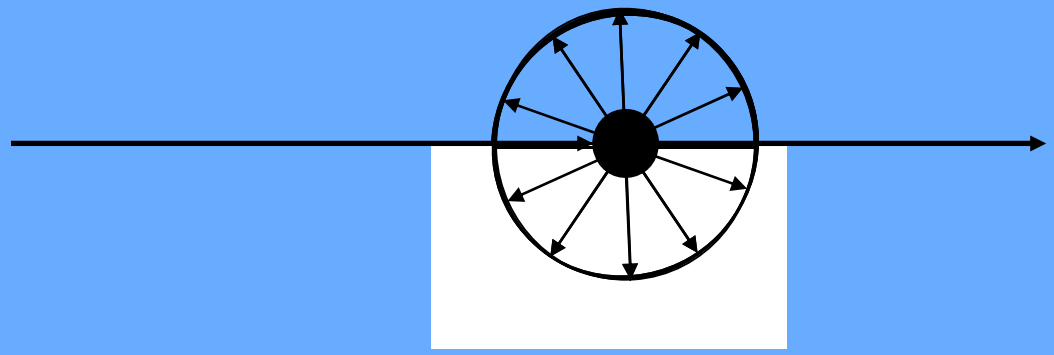
g = 1

Source: *G. Yoon et al.*

Reduced Scattering Coefficient

Incorporates the effects of anisotropy: $\mu_s' = \mu_s(1-g)$

I_0 $I_0(1-(\mu_a+\mu_s))\Delta s$



Scattering has maximum effect

g = 0

Summary of Optical Properties

Attenuation due to heat generation μ_a

Attenuation due to scattering accounting for anisotropy μ_s'

Attenuation due to scattering μ_s

1/cm

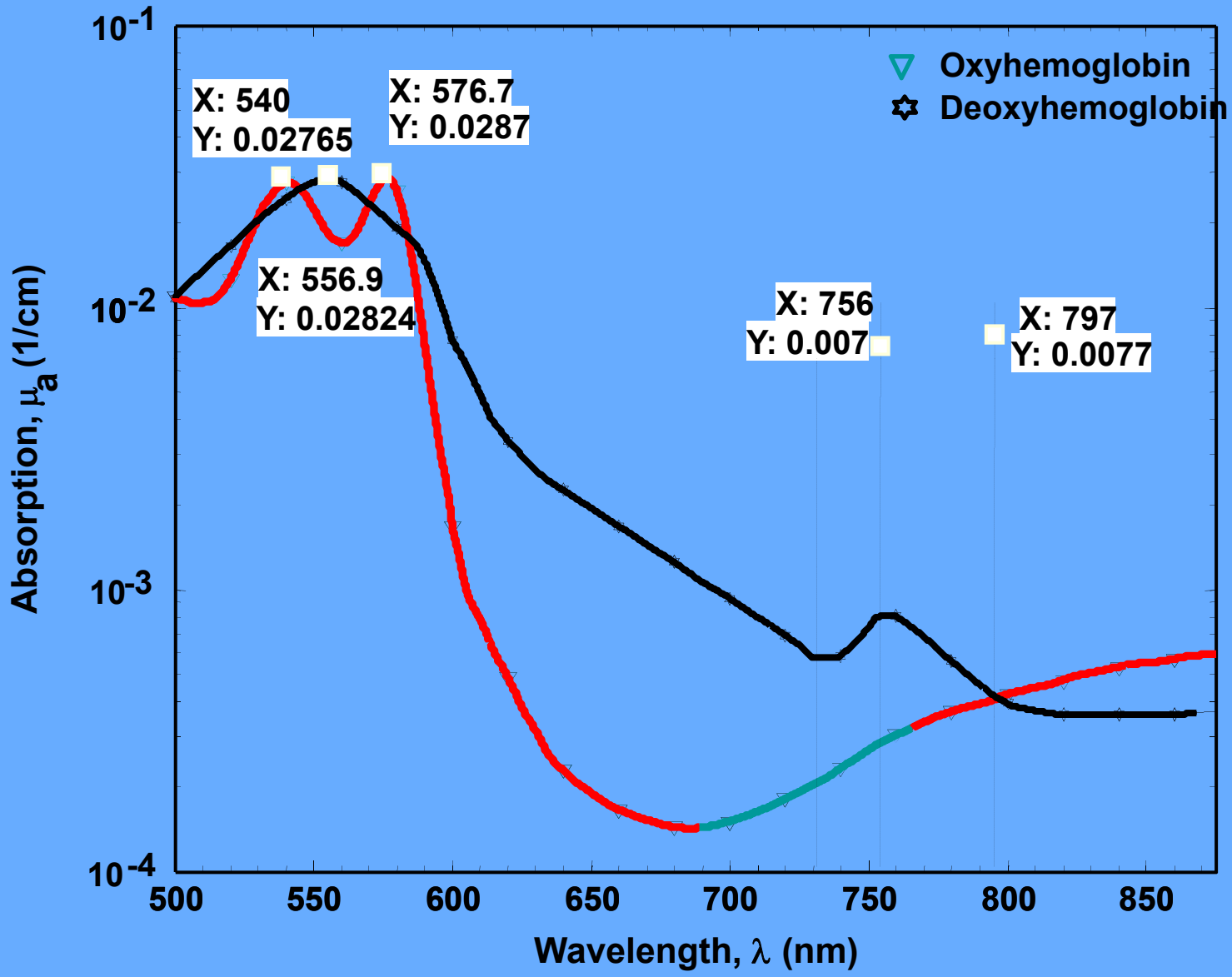
Single scattering albedo $\omega = \dfrac{\mu_s}{\mu_a + \mu_s}$

Total attenuation $\beta = \mu_a + \mu_s$

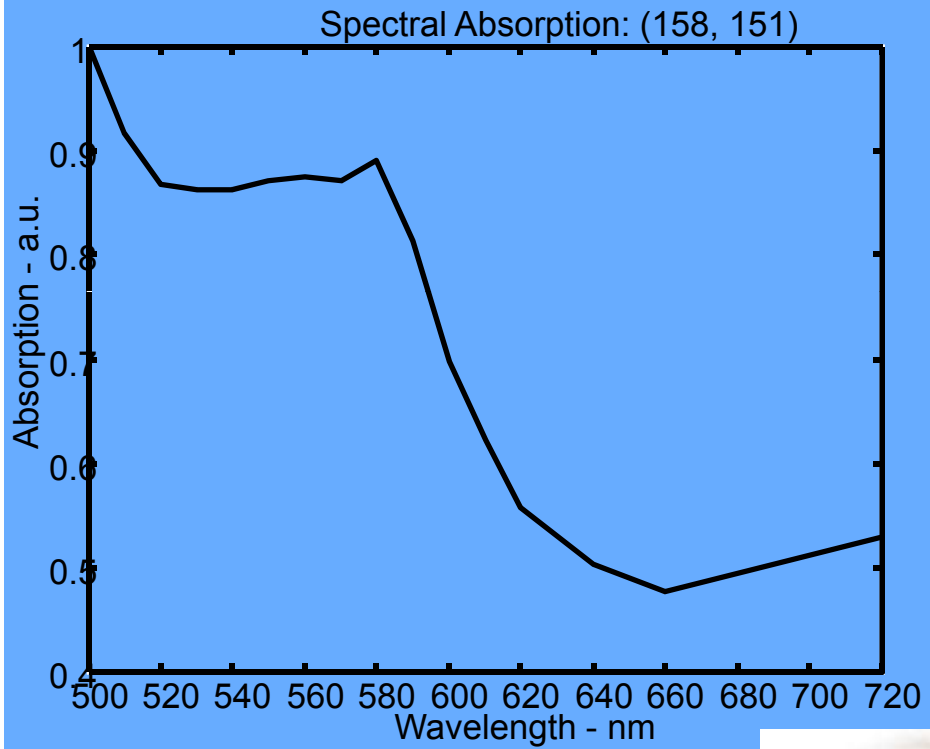
Anisotropy of scattering g

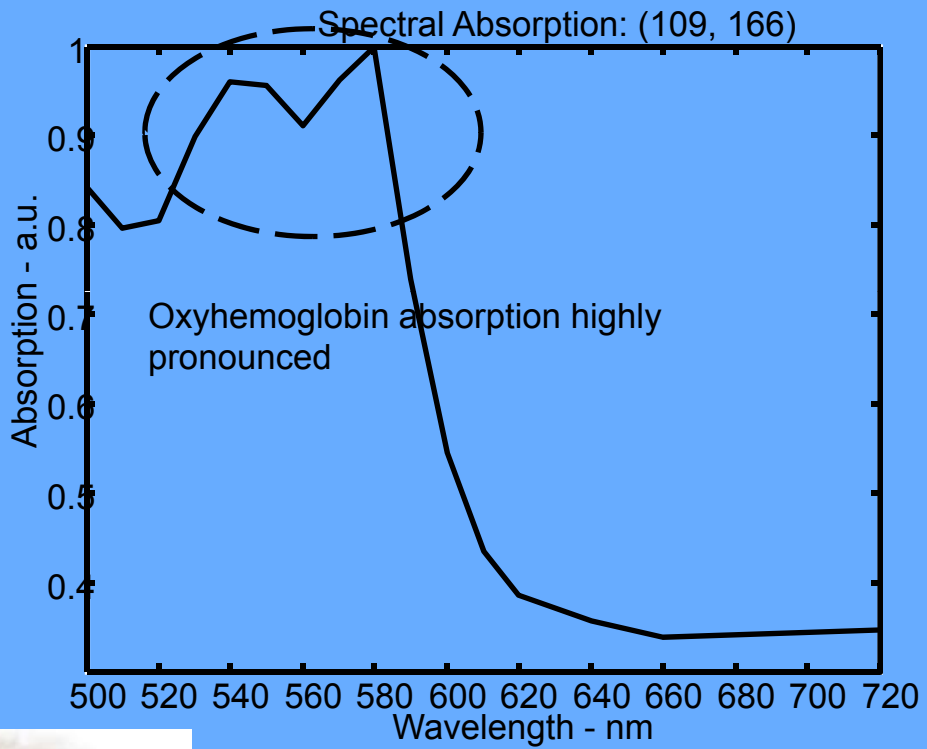
Index of refraction n

Absorption and Blood



Deferent Oxygen Saturations





Diffusion Approximation (DA)

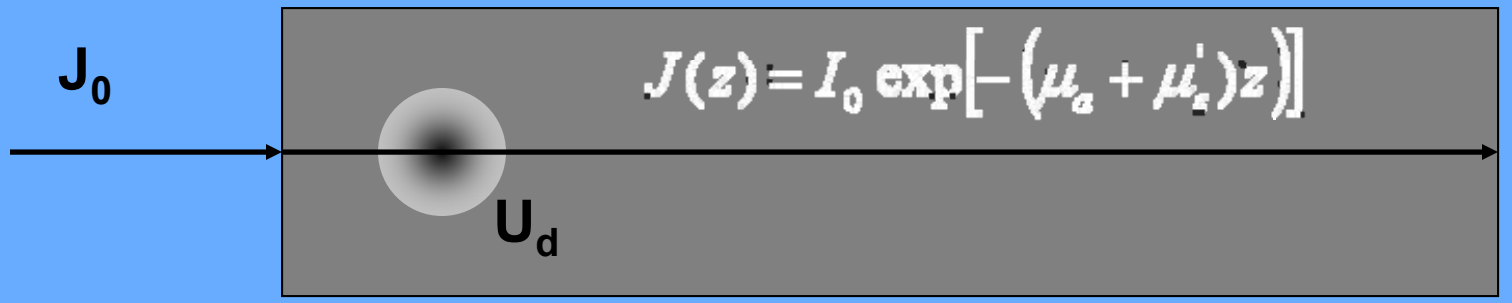
Effective diffuse flux

BL's law source term

$$\frac{d^2U_d(z)}{dz^2} - \kappa_d^2 U_d(z) = \frac{3}{4\pi}\frac{\mu_s'}{\mu_a + \mu_s'} J_0 \exp[-(\mu_a + \mu_s')z]$$

$$\kappa_d = 3\mu_a(m\mu_a + m\mu_s') \qquad \mu_s' = \mu_s(1-g)$$

Reduced scattering coefficient



Source: *Patterson, MS.*

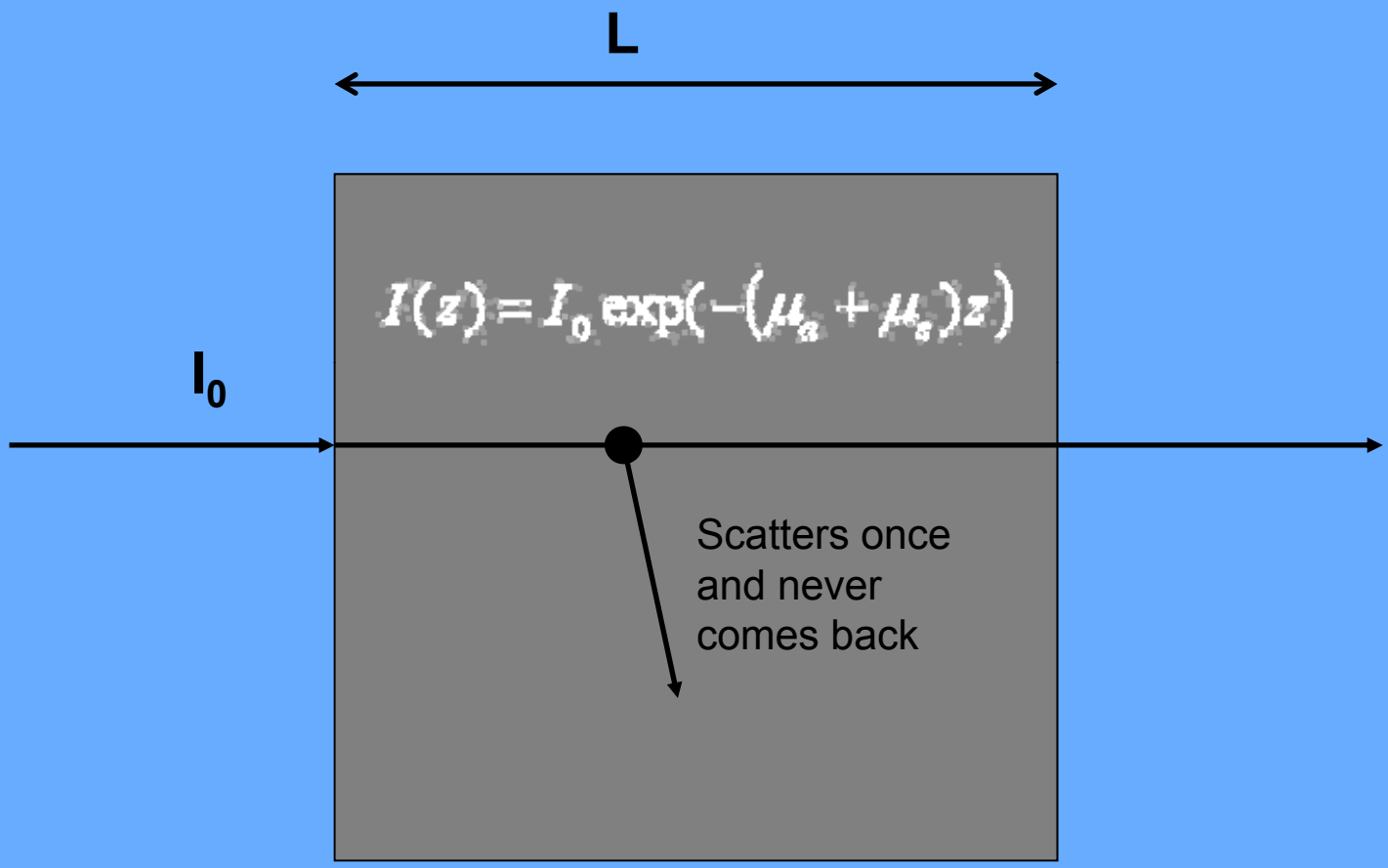
Application of DA

- Determine μ_a and μ'_s. Both carry biological significance.
- Absorption coefficient used to determine hemoglobin saturation.
- Scattering properties hold biological information about tissue structure.
- Time resolved formulation also exists.

Limitations of DA

- Model assumptions are not always valid.
- Difficult to formulate for multi-layer systems.
- Boundary conditions at interfaces complicate analysis.

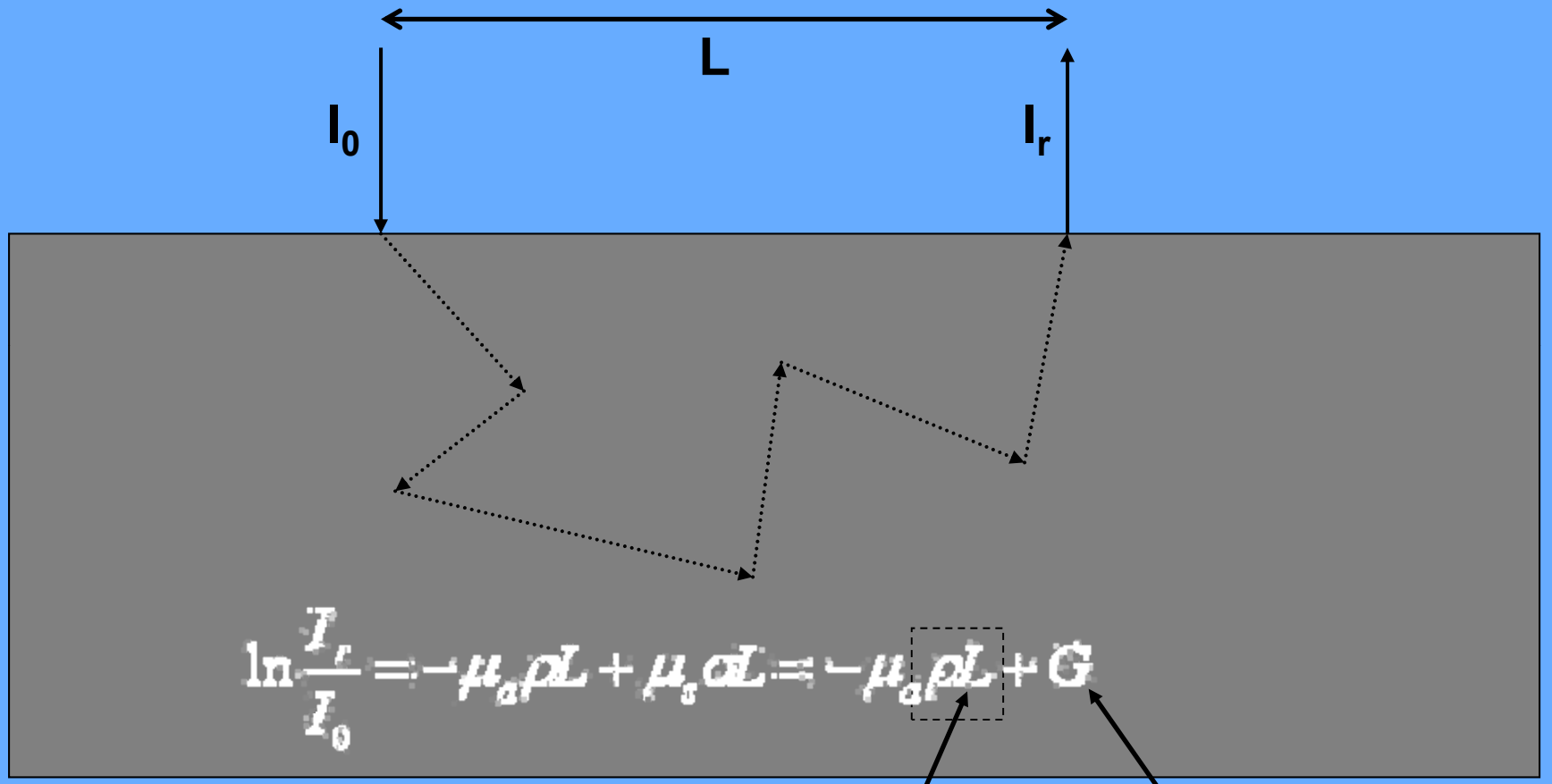
Beer-Lambert's (BL's) Law



Attenuation coefficient: $\ln \frac{I(L)}{I_0} = -(\mu_a + \mu_s)L$

Source: *M.F. Modest.*

Modified BL's Law



Source: *Kocsis, L.*

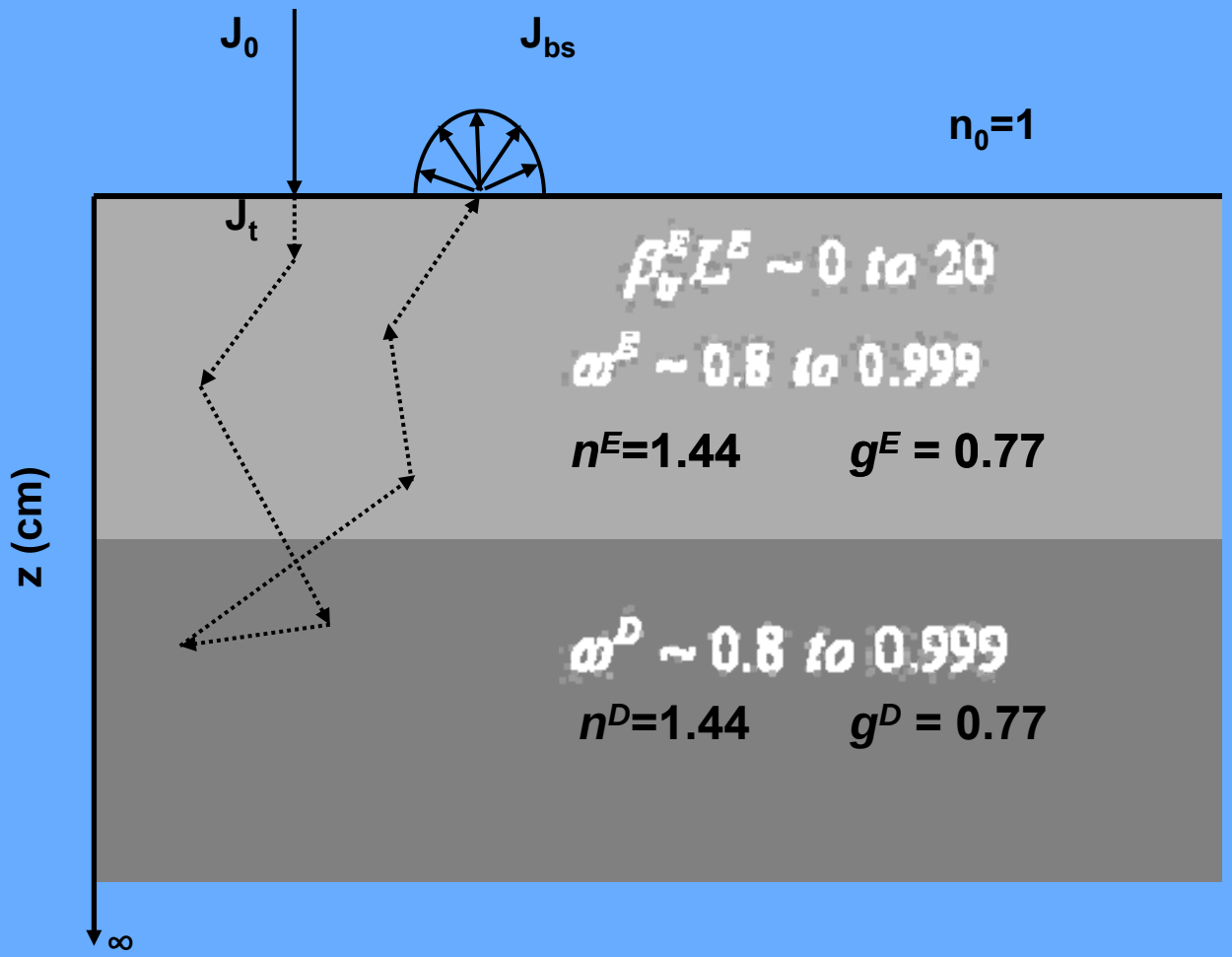
Application of Modified BL's Law

- Determine absorption coefficient at several wavelengths.
- Fit absorption spectra of hemoglobin to the observed.
- Thus determine hemoglobin saturation.

Limitations of Modified BL's Law

- Information about scattering is lost
  - Hard to interpret G in a physical manner;
  - Changes in blood volume cannot be observed;
  - Modified BL assumes a fairly constant optical system.
- Good for detecting change in oxy/deoxy levels.

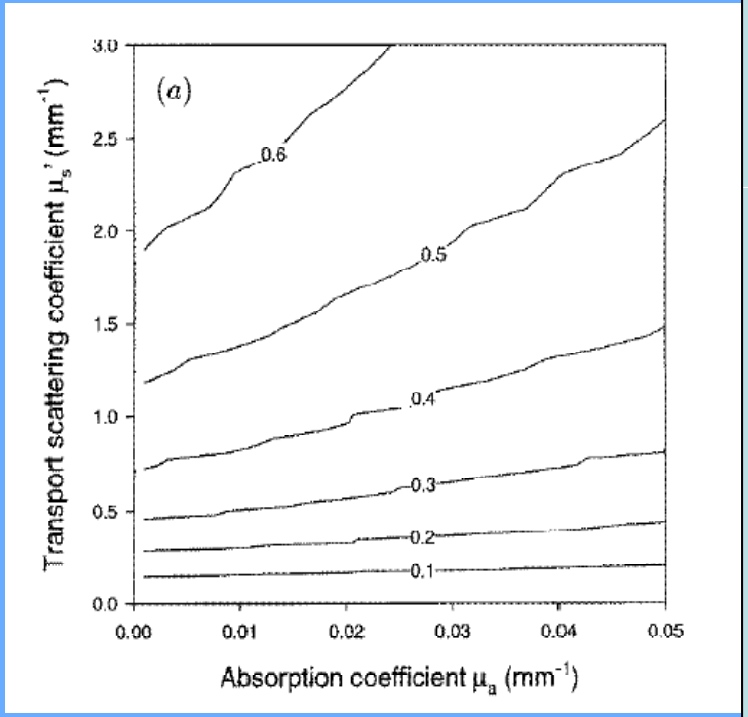
Monte Carlo (MC) Methods



Reduced optical thickness of epidermis: $\beta_{tr}^E L^E = L^E\left(\mu_a + \mu_s'\right)$

Monte Carlo Simulations

Perform multiple simulations of experimental setup with different samples.



Use this look up table to infer optical properties from measured data.

Source: *Simpson, CR.*

Monte Carlo Limitations

- Simulation is limited to particular g and n.
- Empirical method hard to interpret and modify without rerunning the experiment.
- Models generally single layer and insufficient for *in vivo* use.
- Current research is expanding MC

Summary of Theoretical Models

- Various predictive equations have been developed to extract information from optical signals.
- Measurement of scattering and absorption can provide quantitative information about Hb oxygenation and concentration.
- Theoretical models provide a framework for algorithm development and device operation.
- Continuing development of models has the potential to improve the accuracy and spatial resolution of optically derived information.

Current NIRS Devices for Cerebral Oxygen Monitoring in Neonates

NIRS Devices in Neonatal Oxygen Monitoring

- Definitions
  - Infrared (IR) – light with wavelength between 750nm and 1mm.
  - Near IR (NIR) – infrared light with wavelength between 750nm and 1400nm.
  - NIRS – near-infrared spectroscopy.
  - VV – veno-venous.
  - ECMO – extracorporeal membrane oxygenation.
  - $SctO_2$ – cerebral tissue oxygen saturation.
  - SvO_2 – cerebral venous oxygen saturation

NIRS Devices in Neonatal Oxygen Monitoring

- NIRS in Neonatal Patients:
  - Used to Study Cerebral Hemodynamics and Oxygenation.
  - Non-invasive, high temporal resolution, relatively easy to use.
- Types of Measurements:
  - Regional Cerebral Tissue Oxygen Saturation (rSO_2).
  - Absolute Cerebral Tissue Oxygen Saturation.

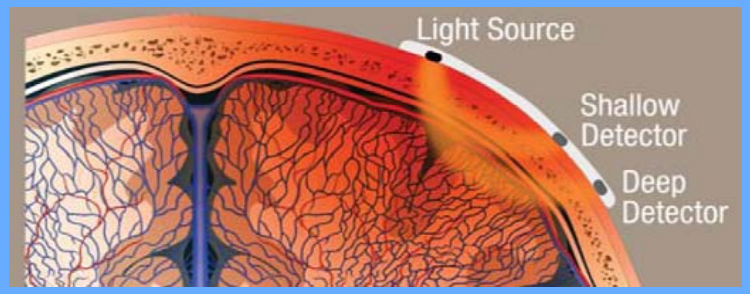
NIRS Devices in Neonatal Oxygen Monitoring

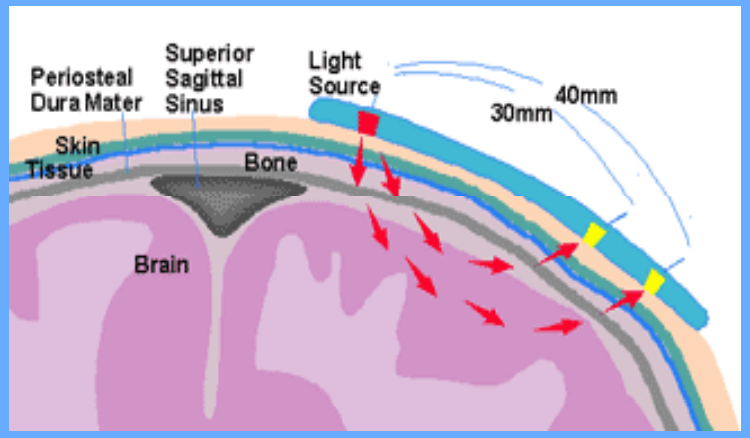
- **FDA-Approved Devices**
  - *INVOS® 5100C Cerebral Oximeter* (510k N^{O} K080769) by Somanetics® Corporation, Troy, Michigan.
  - *FORE-SIGHT® Cerebral Oximeter Monitor MC2000* (510k N^{O} K073036) by CASMED® Medical Systems, Inc., Branford, Connecticut.

Source: *www.fda.gov*

NIRS Devices in Neonatal Oxygen Monitoring

INVOS® 5100C Cerebral Oximeter



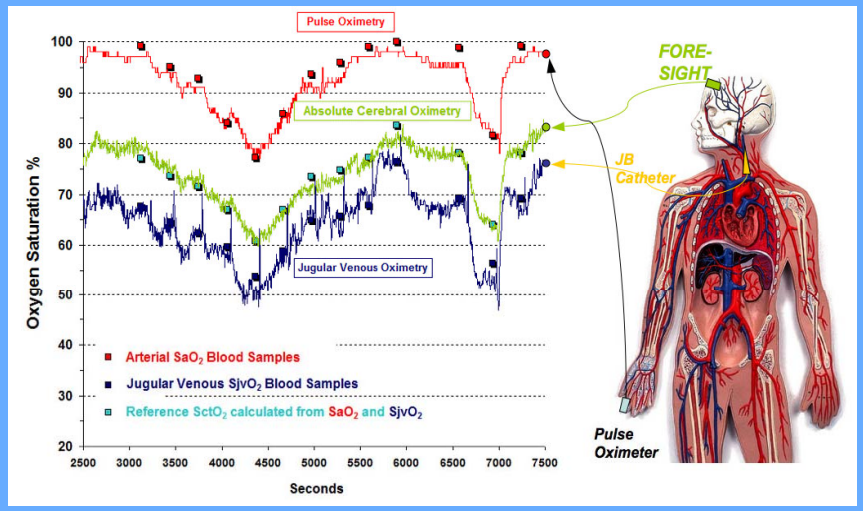


- Near-infrared light at wavelengths of 730 nm and 810 nm.
- Emitted from a source on one side of disposable sensor.
- The light gets scattered in biological tissue (mainly gray matter).
- The remaining light is detected by detectors on the other side of disposable sensor.

Source: *Somanetics Corporation (www.somanetics.com)*

NIRS Devices in Neonatal Oxygen Monitoring

FORE-SIGHT® MC2000 Cerebral Oximeter



- Light is projected into the brain in four precise wavelengths - 690, 780, 805, and 850 nm.
- It could reduces patient dependent variability (e.g. Melanin).
- More reliable measurements in low or zero perfusion states.

Source: *CASMED Medical Systems, Inc. (www.casmed.com)*

- No user calibration required.

Visible Light Oxygen Saturation Detection

Visible Light Oxygen Saturation Device

- T-STAT 303

- Manufacturer: Spectros, Portola Valley, CA
- Use visible light (470 nm to 600 nm range)
- Report capillary-weighted oxygen saturation due to the selection of the range of light emitted
- Hemoglobin has the strongest absorbance in 475 nm – 600 nm range. In capillaries, a large amount of light passed through capillary structures can be scattered back to the sensor.

T-Stat Comparison

Device	Spectros T-Stat	Somanetics Invos	Hutchinson InSpectra	CAS Medical Fore-Sight
Device Type	Visible Light	Near-Infrared	Near-Infrared	Near-Infrared
Ischemia Detection	Yes	Yes	No	No
Normal Range	Tight(±3%)	Wide(±9%)	Wide(±9%)	Wide(±9%)
Outcome changes?	Yes	Yes	No	No
Measured Site	Mucosal	Brain	Muscle	Brain

Near-Infrared (NIR) SpO_2 Monitoring – Peripheral/Arterial

Masimo Rad-57™

- Non-invasive
  - Finger pulse oximeter
- Continuous monitoring.
- Up to 72 hours of trending memory for SpCO, SpMet, SpO2, Pulse Rate and Perfusion Index.
- Light-weight and portable.

Source: *http://www.masimo.com/rad-57/*

Conclusions on Alternative Technologies

- Current NIRS technology suffers from motion artifact and measurement error.
- Capnographic measurements are effectively performed by currently approved devices.
- Peripheral SpO_2 measurements form the basis of current management
- Research objectives include improved spatial localization & measurement accuracy, and reduction of artifacts.

SEVERAL TECHNOLOGIES ARE DRAMATICALLY CHANGING THE FACE OF HEALTHCARE

ADVANCES IN IMAGING ARE THE FOUNDATION FOR THE DEVELOPMENT OF NEW MINIMALLY INVASIVE THERAPIES

Applications of Optical Technologies are expanding the capabiliteis of traditional medical imaging devices

New imaging modalities provide the physician with unique methods of identifying normal and diseased tissue.

Optical biopsy techniques may permit real time analysis of tissue without removing it from the body.

Current Methods include:

- direct insepction
- Rigid endocscopy
- Flexible endoscopy
- “Pill cam”

DEFINITIONS

The word ENDOSCOPY is derived from the Greek by combining the prefix "ENDO" = "WITHIN" and the verb "SKOPO" = "TO VIEW WITH A PURPOSE".

It is exactly this "observation with intent" which is the goal of Endoscopy. This intent can be diagnostic and/or therapeutic.

An ENDOSCOPE is an instrument for examining the "within", (bodily canals or hollow organs)

CLASSIFICATION OF ENDOSCOPES - 1

ACCORDING TO THEIR USE

INDUSTRIAL

Inspection of inaccessible or hazardous areas

MEDICAL

Diagnostic and/or therapeutic procedures

ACCORDING TO MECHANICAL RIGIDITY

RIGID

FLEXIBLE

HYBRID

Incorporate shafts with both rigid & flexible areas

ACCORDING TO DURABILITY

MULTIPLE USE

SINGLE USE: Disposables

LIMITED USE

CLASSIFICATION OF ENDOSCOPES - 2

ACCORDING TO ANATOMICAL SITE

GASTROINTESTINAL TRACT

Esophagoscope
Gastroscope
Duodenoscope
Proctoscope
Sigmoidoscope
Colonoscope

URINARY TRACT

Cystoscope
Urethroscope
Nephroscope

AIRWAY

Nasopharyngoscope
Laryngoscope
Bronchoscope

CLASSIFICATION OF ENDOSCOPES - 3

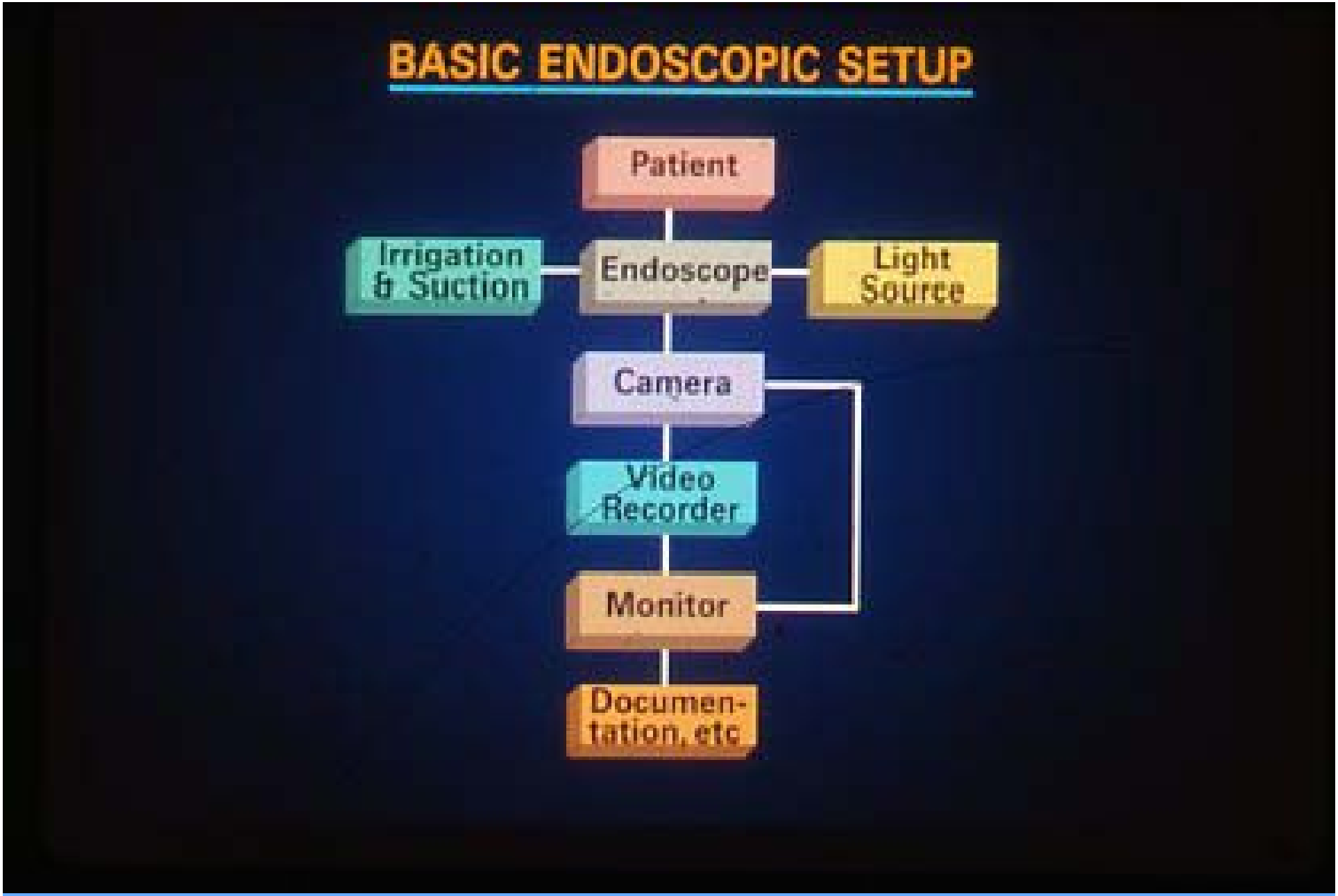
ACCORDING TO ANATOMICAL SITE

VASCULAR SYSTEM
Angioscope

SKELETAL SYSTEM
Arthroscope
Discoscope

OTHERS
Choledochoscope
Hysteroscope
Otoscope
Laparoscope

These names are for the most part conventional. The use of a particular endoscope depends on the anatomy to be accomodated, the skill and the imagination of the physician.


BASIC ENDOSCOPIC SETUP
Patient
Irrigation & Suction
Endoscope
Light Source
Camera
Video Recorder
Monitor
Documen-tation, etc

ENDOSCOPIC DESIGN REQUIREMENTS TECHNICAL

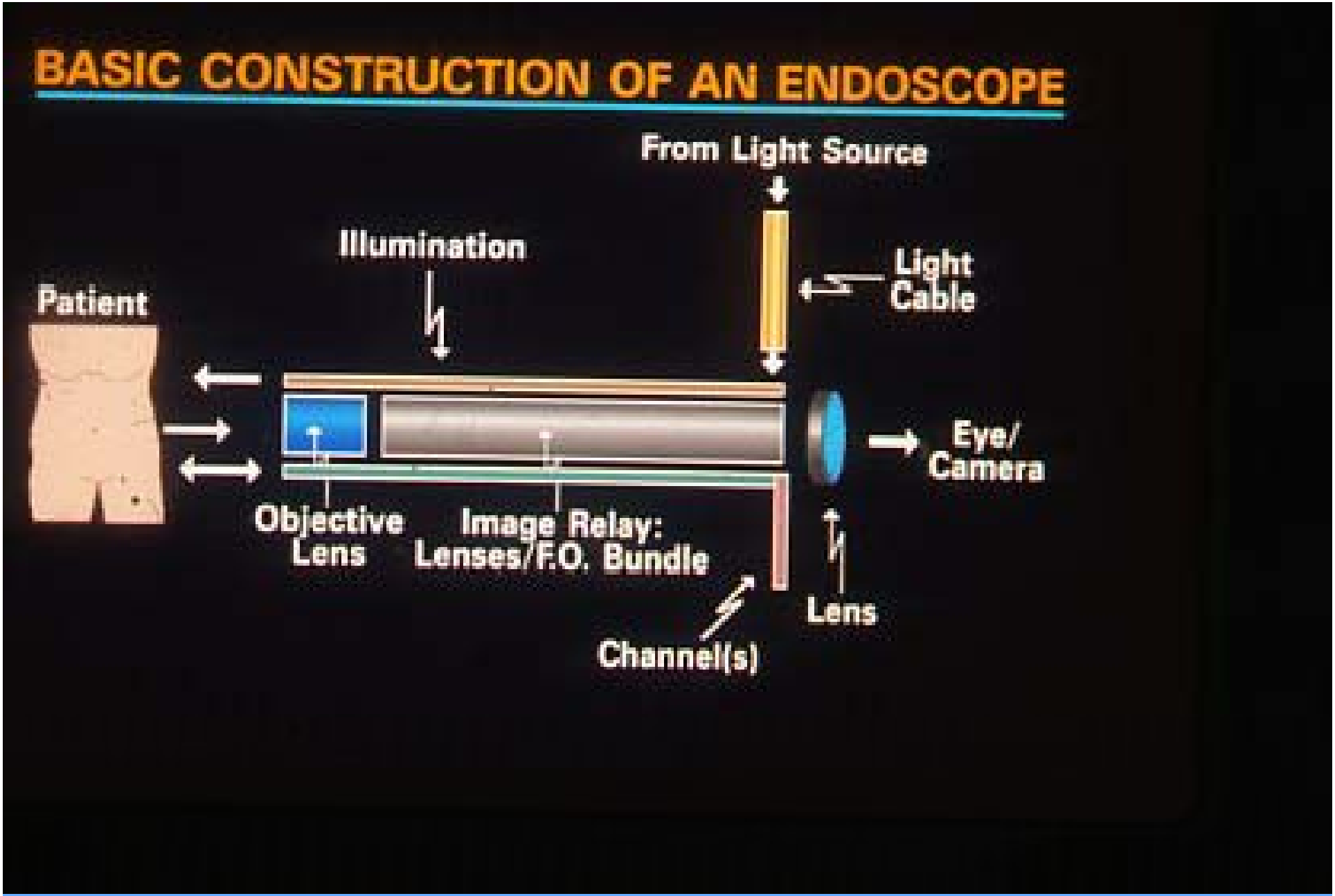
Optical Engineering (Lens Design)

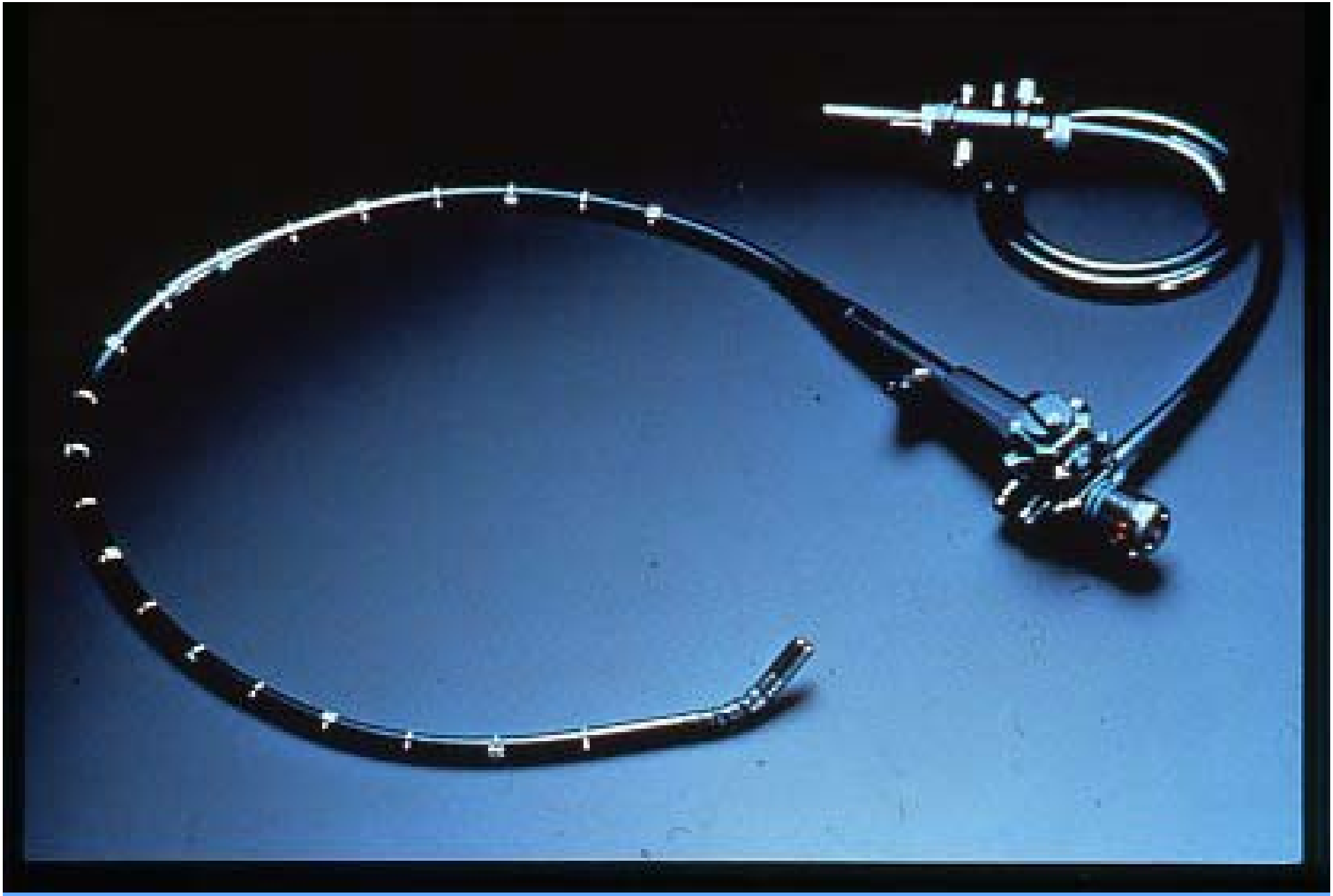
Mechanical Engineering

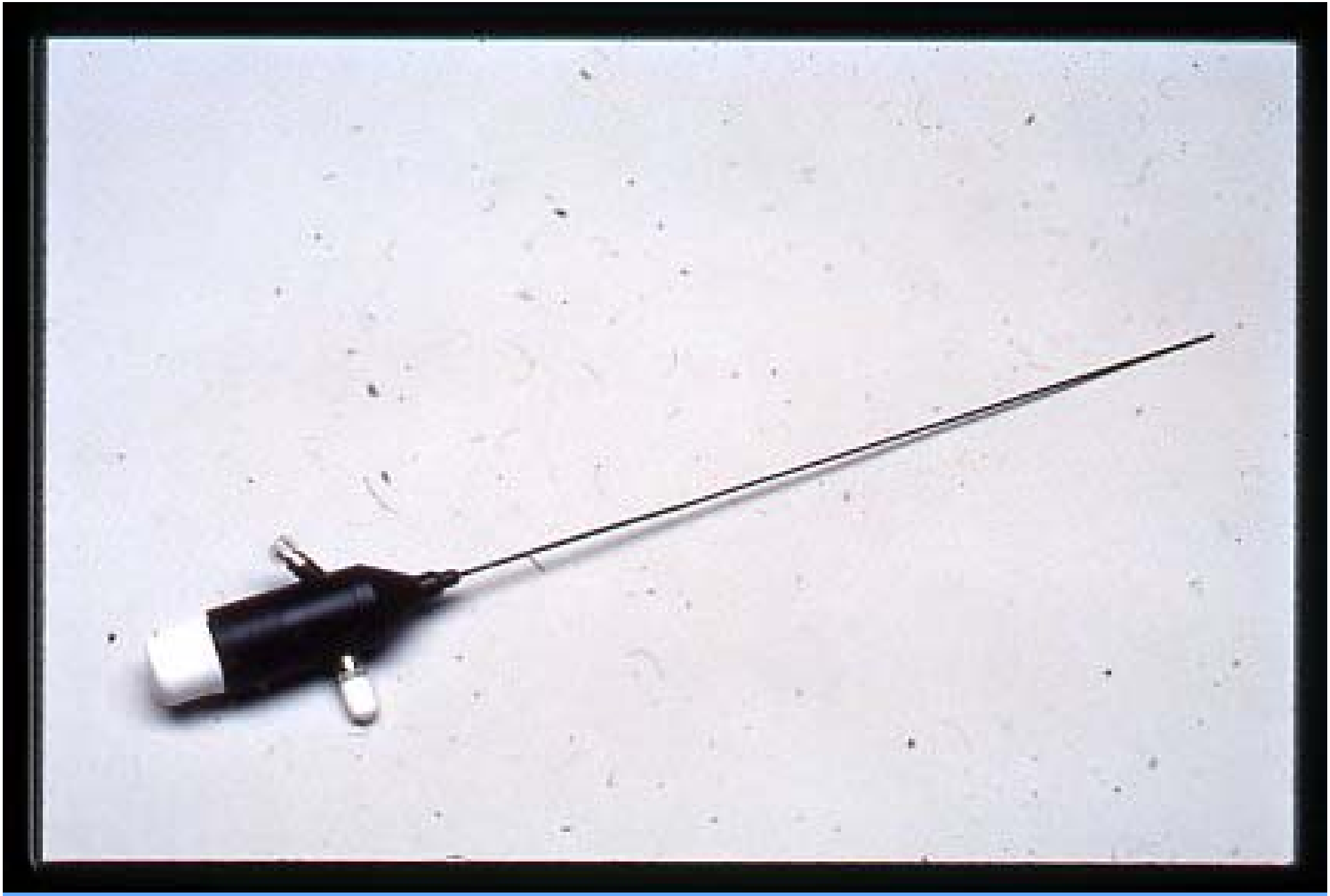
Material Science (glasses, semi-conductors, biocompatible materials)

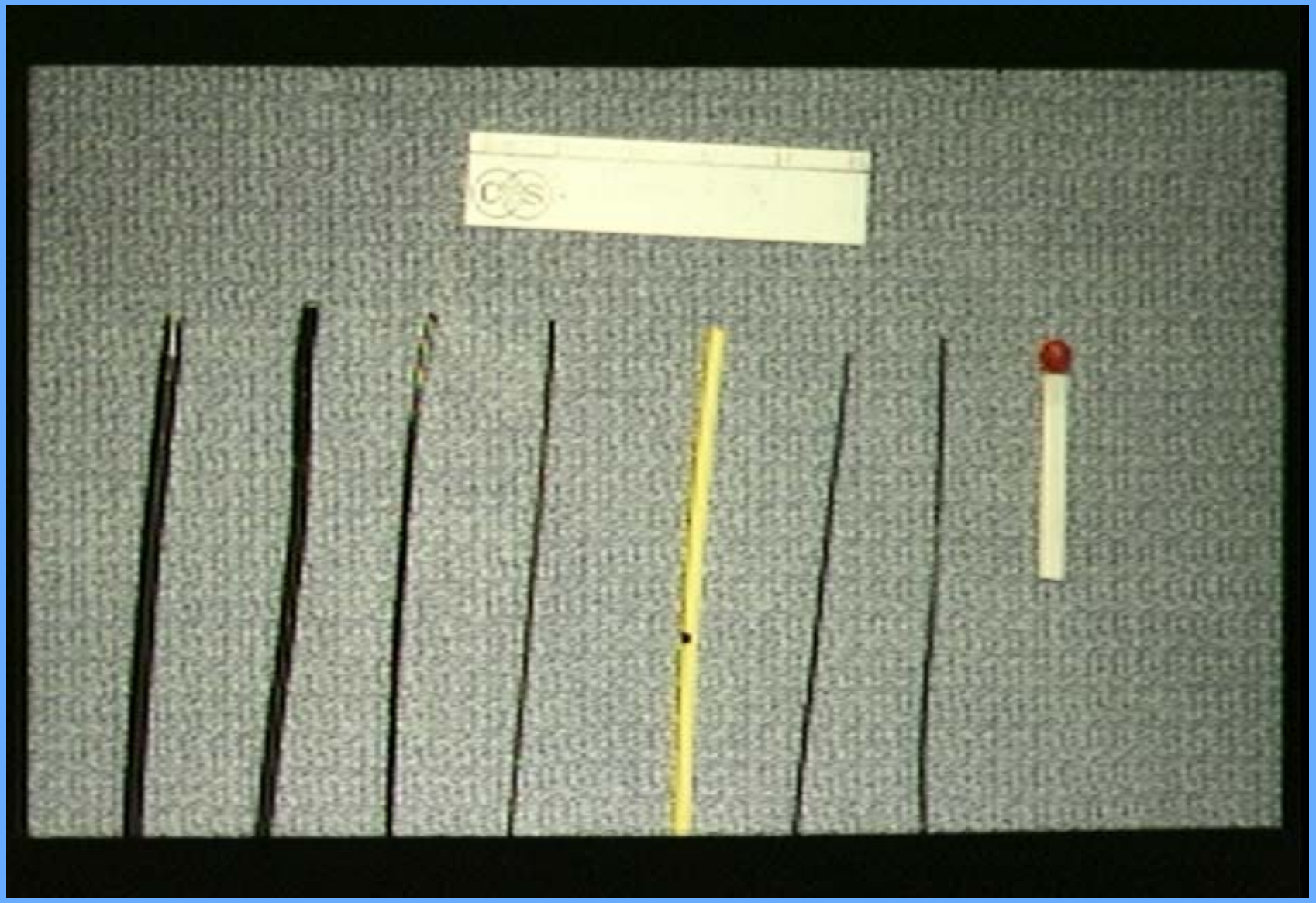
Video/Electronics Engineering (Camera design, documentation equipment)

Imaging Science


BASIC CONSTRUCTION OF AN ENDOSCOPE
From Light Source
Illumination
Light Cable
Patient
Eye/ Camera
Objective Lens
Image Relay: Lenses/F.O. Bundle
Lens
Channel(s)







THE EVOLUTION OF MINIMALLY INVASIVE SURGERY

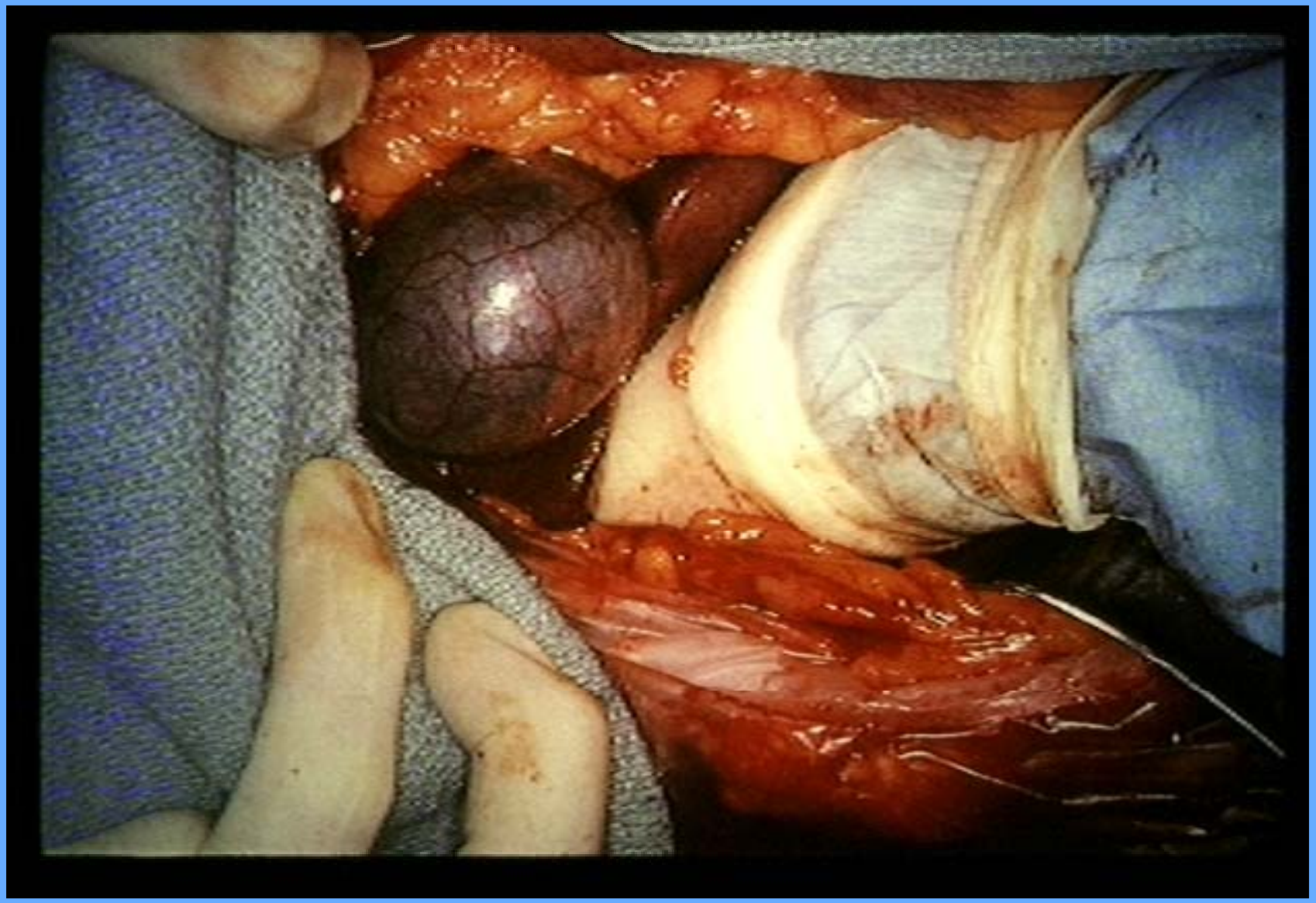
FOR MORE THAN 150 YEARS, LARGE INCISIONS INTO BODY CAVITIES WAS THE ONLY MEANS OF ACCESSING DISEASED ORGANS. IN THE LATE 1970'S, INTERVENTIONAL TECHNIQUES USING X-RAY IMAGING ALLOWED PHYSICIANS TO OPEN BLOCKED ARTERIES WITHOUT LARGE INCISIONS.

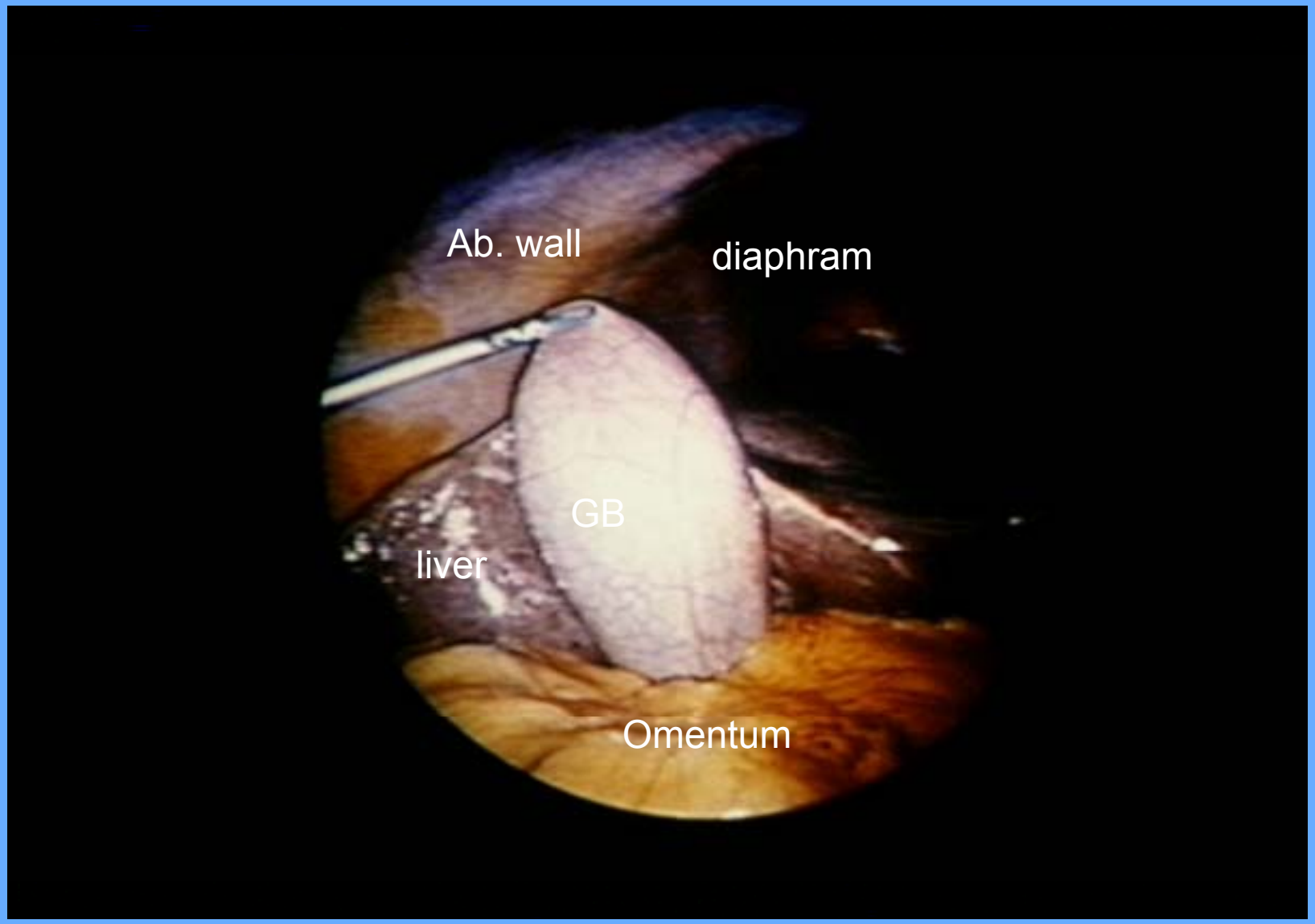
THE EVOLUTION OF MINIMALLY INVASIVE SURGERY

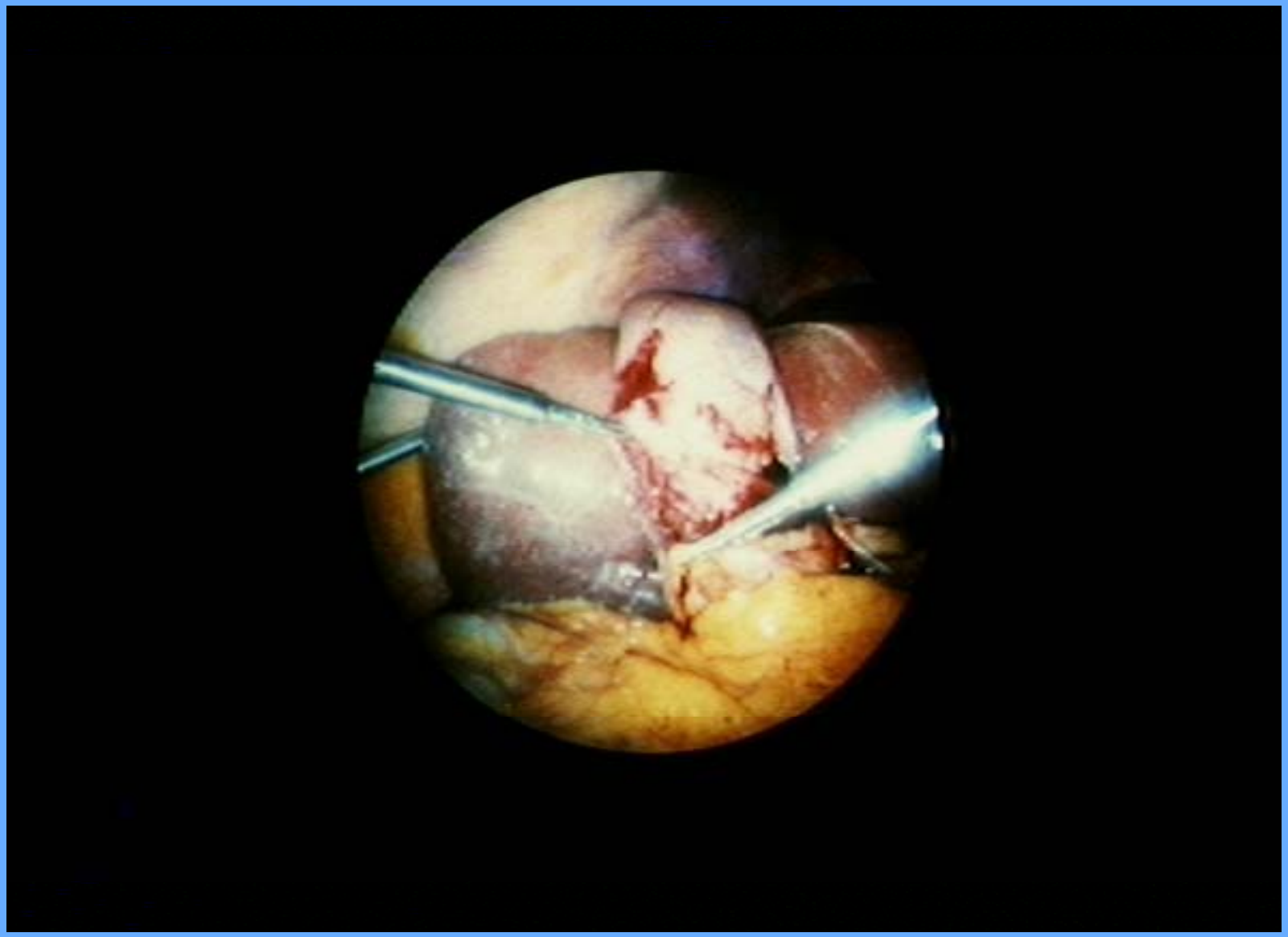
IN THE EARLY 1980'S, VIDEO IMAGING THROUGH ENDOSCOPES BECAME FEASIBLE, ALLOWING THE ENTIRE SURGICAL TEAM TO SEE INSIDE THE BODY.

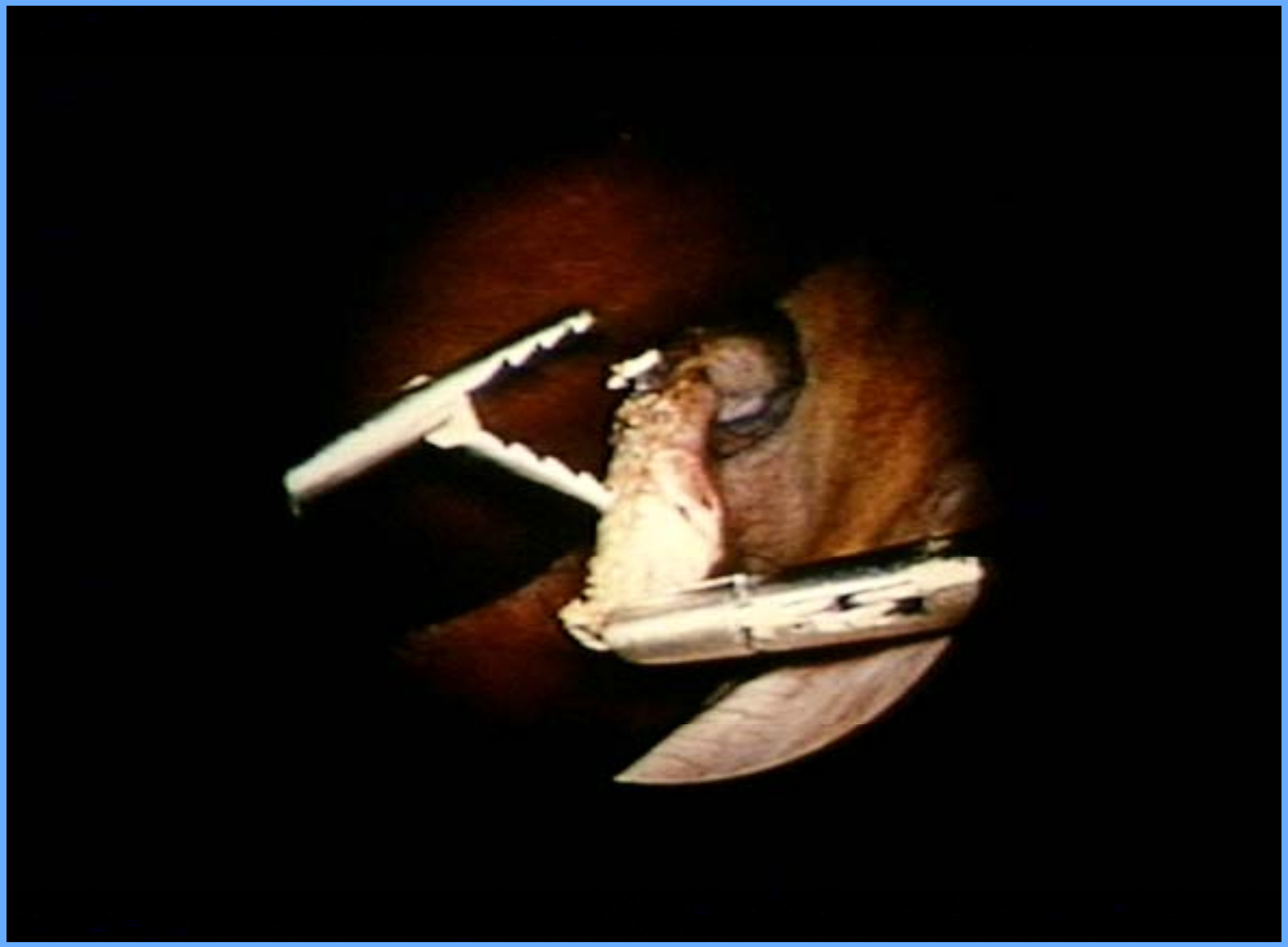
IN THE MID 1980'S, INSTRUMENT MAKERS BEGAN TO DEVELOP TOOLS TO WORK WITH THE OPTICAL IMAGING SYSTEMS AT OPERATIVE SITES WITHOUT LARGE INCISIONS.

OLYMPUS
POWER




Ab. wall
diaphram
GB
liver
Omentum





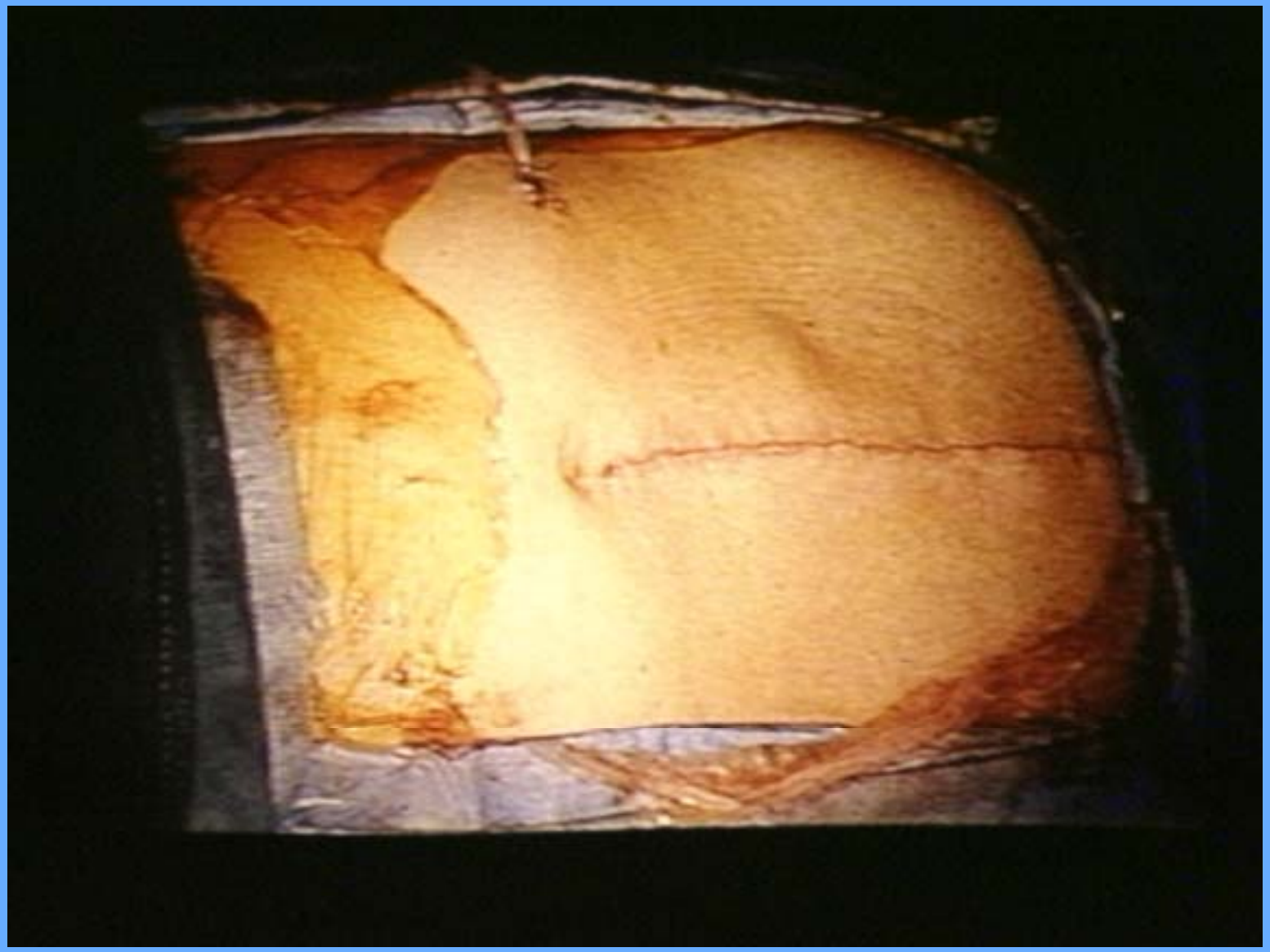
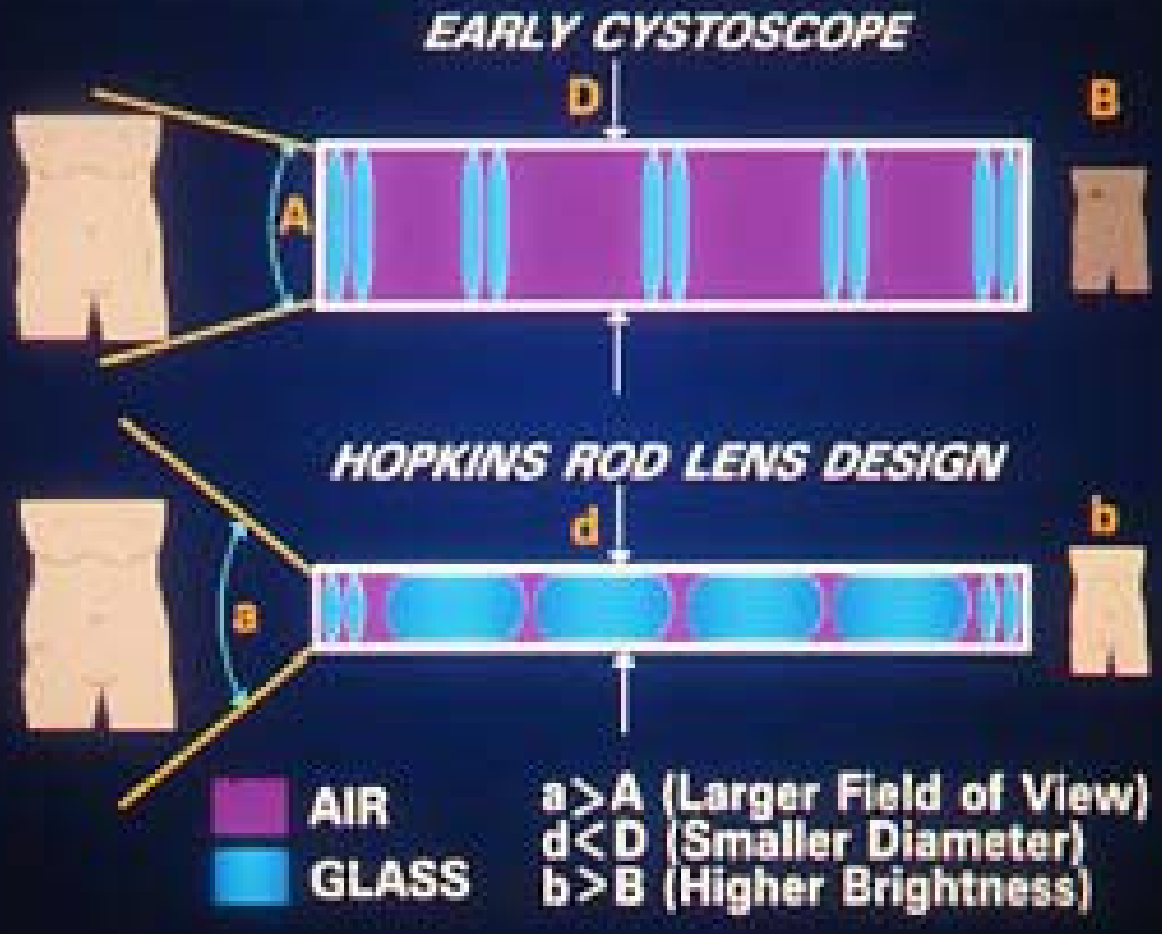


IMAGE GUIDED MINIMALLY INVASIVE SURGERY HAS REVOLUTIONIZED THE PRACTICE OF MEDICINE, IMPROVING THERAPEUTIC OUTCOMES, DECREASING PAIN, SHORTENING RECOVERY TIMES, ELIMINATING MILLIONS OF HOSPITAL BED DAYS AND DECREASING COSTS.

ADDITIONAL OPTICAL TECHNIQUES CAN EXPAND THE ROLE OF MIS IN THE DIAGNOSIS AND TREATMENT OF DISEASE.

Design of Endoscopes

OPTICAL DESIGN OF RIGID ENDOSCOPES



FIBEROPTICS

Core Refractive Index > Clad Refractive Index

Optical radiation is trapped in the cylindrical core due to Total Internal Reflection at the core clad interface.

Numerical Aperture (NA) is defined by the maximum allowable entrance angle, and indicates the light gathering efficiency of the fiber.

FIBER BUNDLES

SINGLE FIBERS

MULTI-FIBER BUNDLES

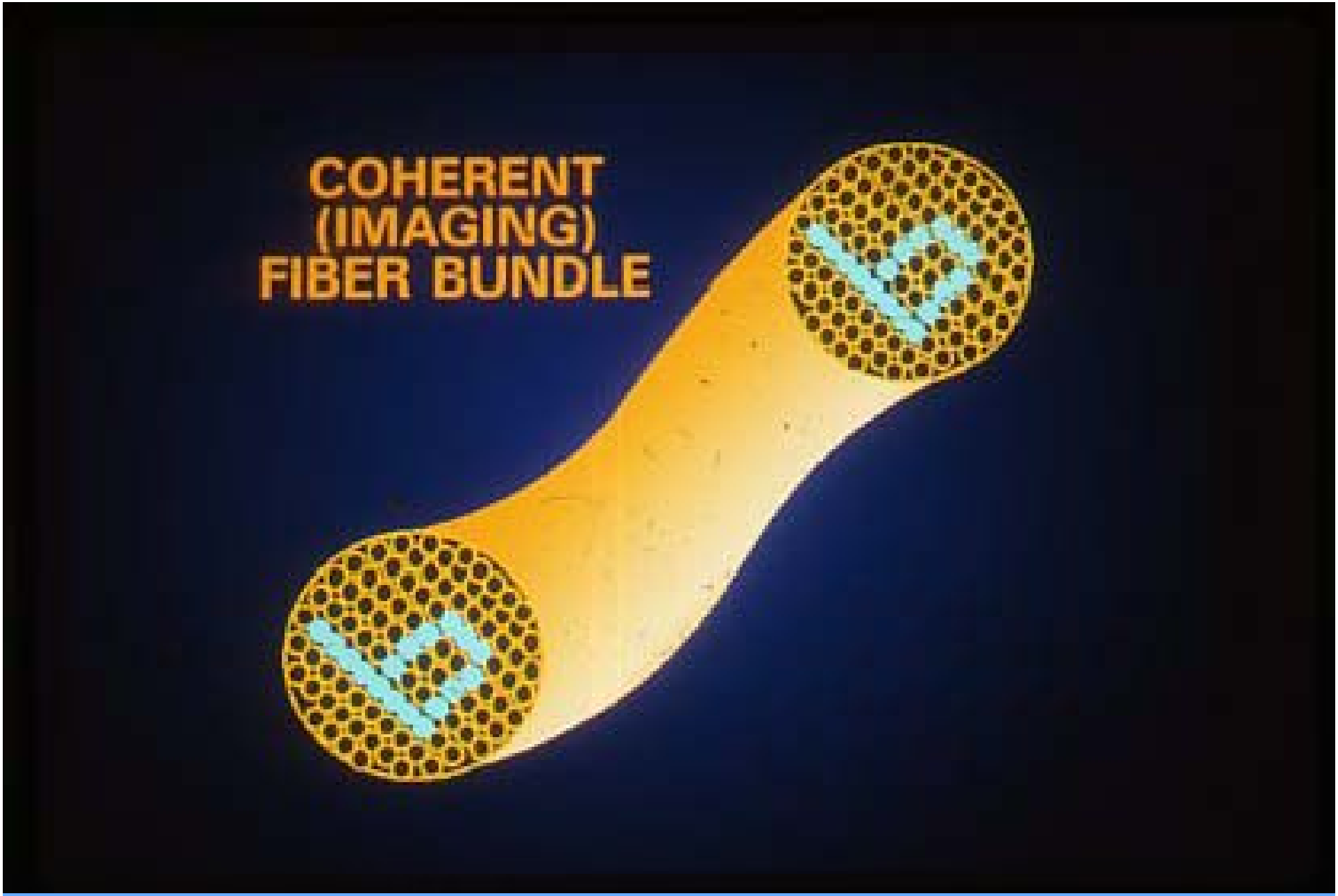
MULTI-MULTI-FIBER BUNDLES

Depending on the construction method they can be used for illumination or image conduits

Hexagonal (close) packed array fiber patterns provide maximum packing fraction

Number of pixels (i.e. individual fibers) ranges from 1,000 to 50,000

INCOHERENT
(ILLUMINATION)
FIBER BUNDLE


COHERENT
(IMAGING)
FIBER BUNDLE

FACTORS INFLUENCING THE QUALITY OF IMAGING CONDUITS

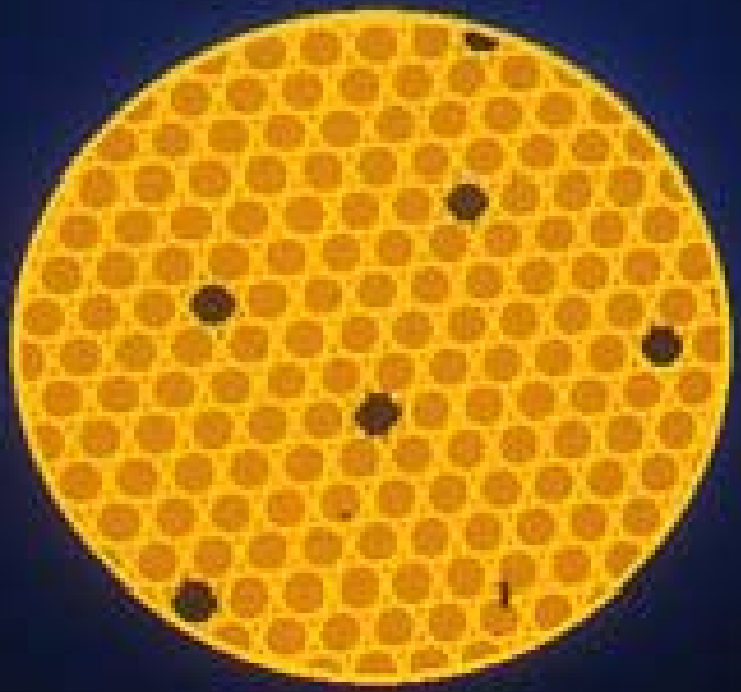
CROSSTALK due to light leakage from cladding modes or scattering from core impurities

BROKEN FIBERS

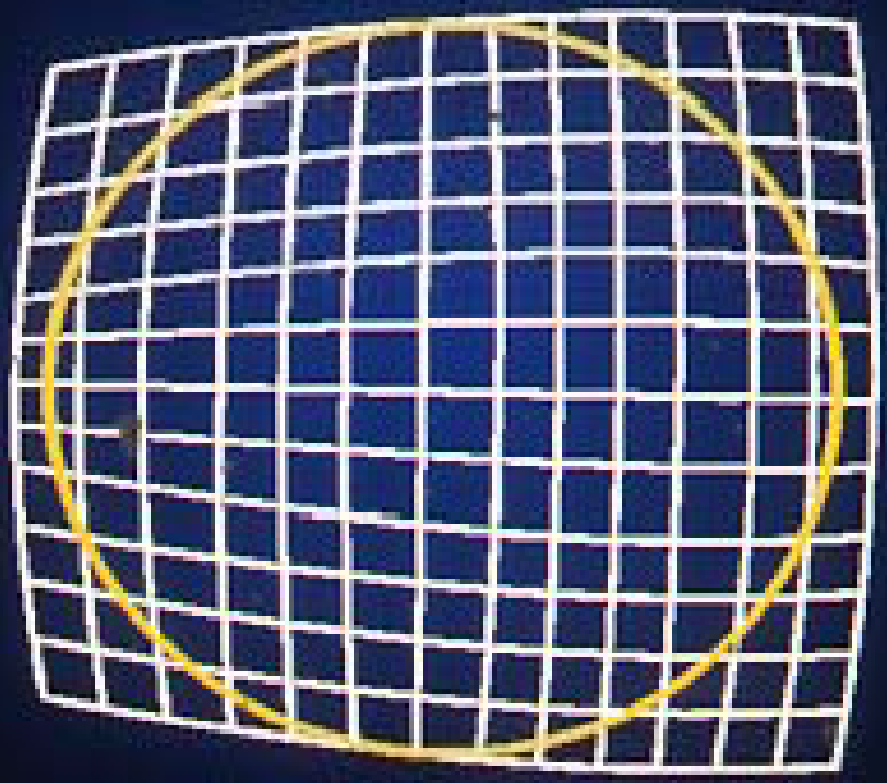
DISTORTED FIBER ARRAYS

INTERSTITIAL DUST/PARTICLES

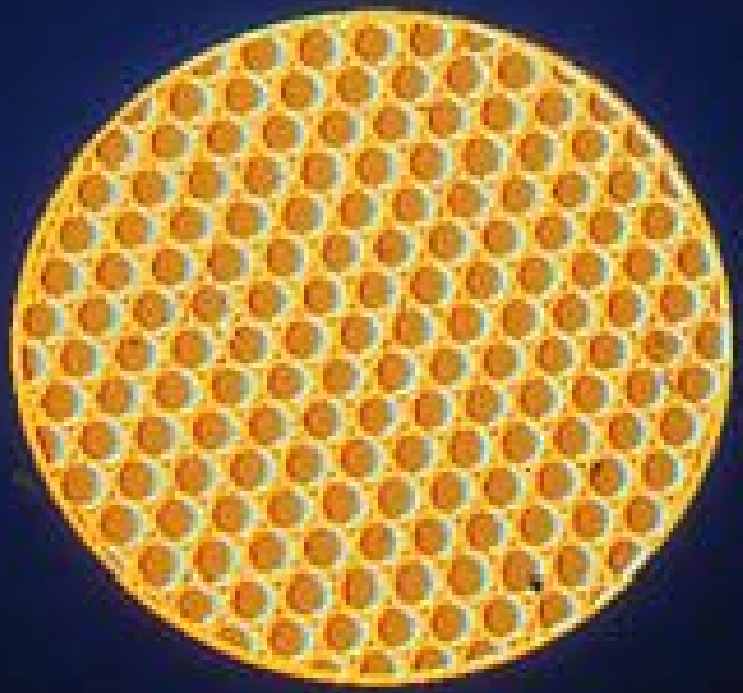
BROKEN FIBERS



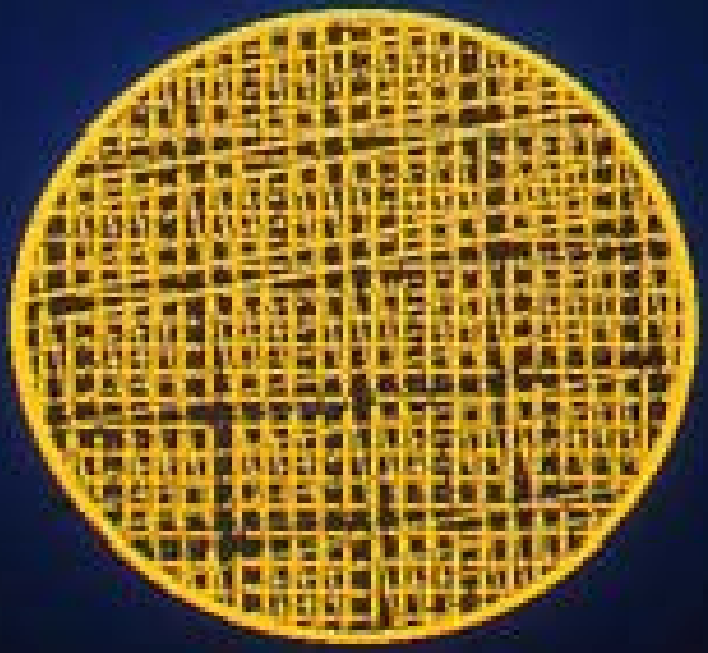
BARREL DISTORTION



CHROMATIC ABERRATION



MOIRE´ PATTERN



ILLUMINATION - 1

Xenon arc lamps and Tungsten Halogen lamps are the standard light sources used for endoscopy

Some sources employ feedback systems which adjust the light output according to the image brightness

The total light flux accepted by a certain endoscope (for fixed light source output) depends only on the object distance ($1/L^2$)

ILLUMINATION - 2

Light cables include (coherent) glass fiber bundles, or liquid guides, of typical sizes 3 to 5mm

The illumination channel is constructed of glass or plastic fibers or incoherent fiber bundles

The spectroscopic characteristics of the entire illumination chain is important for good color performance

Dual output sources or biffurcated light guides can be useful for cases requiring 2 scopes (mother-daughter), such as falloposcopy & CBD exploration

ENDOSCOPIC CAMERA

Solid State CCD Technology

Performs Sampling of the Image

Analog Device (accumulates/stores charges that are proportional to the reflected light

Typical number of pixels 350,000

Rectangular, representing the 4:3 Aspect Ratio of standard TV

Typical Formats: 1/2" (8mm), 2/3" (11mm)

Ease of operation/extended life/ small size/no lag/low power consumption

Filtered Line to eliminate RF Interference

INTEGRATED ENDOSCOPIC CAMERA

LIGHT WEIGHT

SMALL

STERILIZABLE

COMPOSITE, Y-C, RGB OUTPUTS

AUTO GAIN CONTROL (AGC)

CCD Camera vs. Fiber Optic Bundle

- CCD and CMOS chips are now small enough to fit at the working end of endoscopes
- A combination of LED and/or fiber optics is still required to deliver light to the tissue
- CCDs are more durable than fiber optics
- CCDs are difficult to package below 4 mm^2
- In smaller scopes, fiber optic bundles are still prefered.

ENDOSCOPIC PROCEDURES

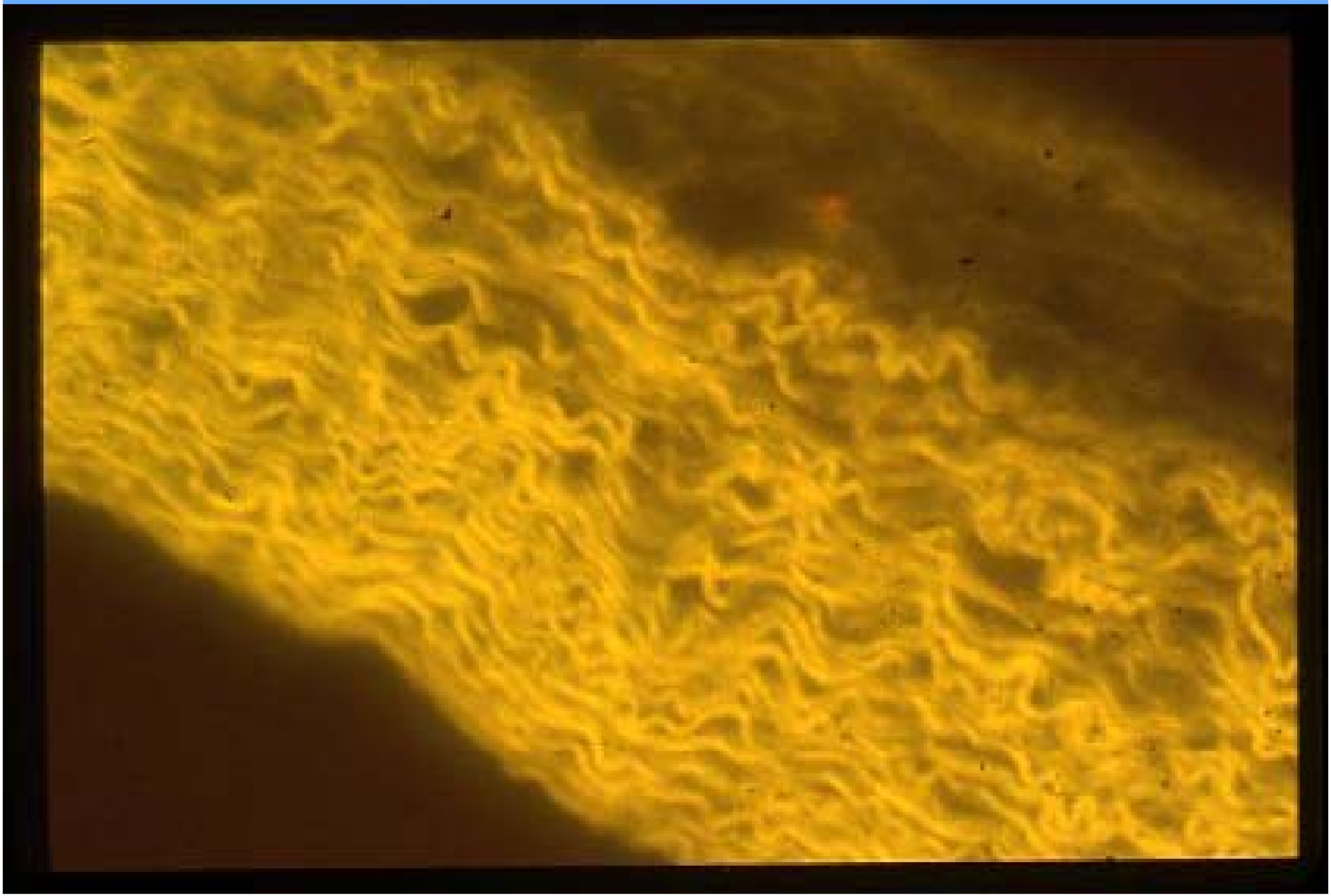
USING THE BODY'S OWN CHANNELS, PHYSICIANS CAN VIEW THE DISEASED SITE AND TREAT IT

BY ELIMINATING THE NEED FOR OPEN SURGERY, ENDOSCOPY REDUCES PAIN, IMPROVES HEALTHCARE & REDUCES COSTS

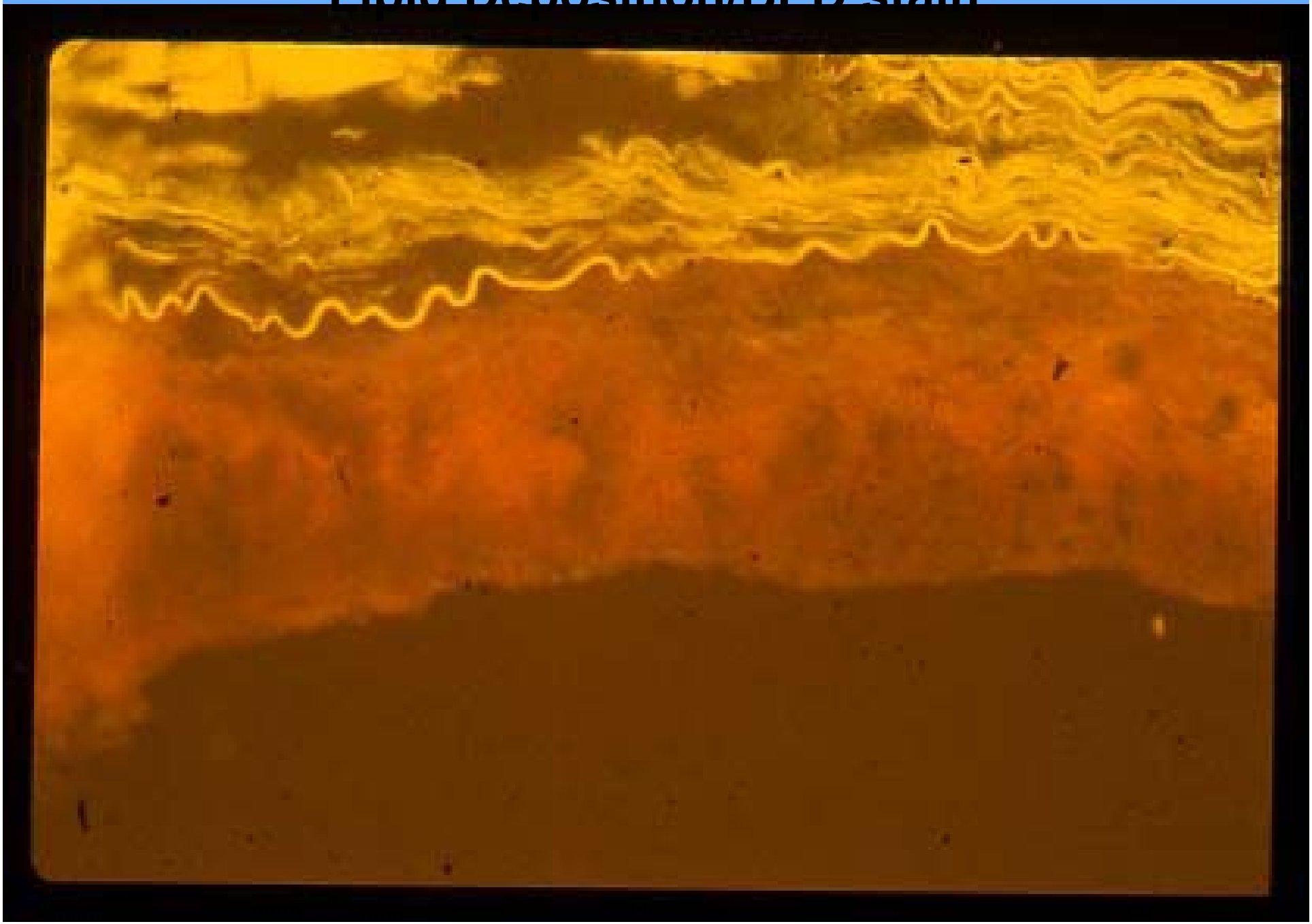
RECENT ADVANCES IN OPTICAL IMAGING TECHNIQUES PERMIT NEW METHODS FOR TISSUE CHARACTERIZATION

AS NEW MOLECULAR MARKERS & CHEMICAL DETECTION METHODS IMPROVE, THERE WILL BE A GREATER NEED TO LOCALIZE SMALL TUMORS

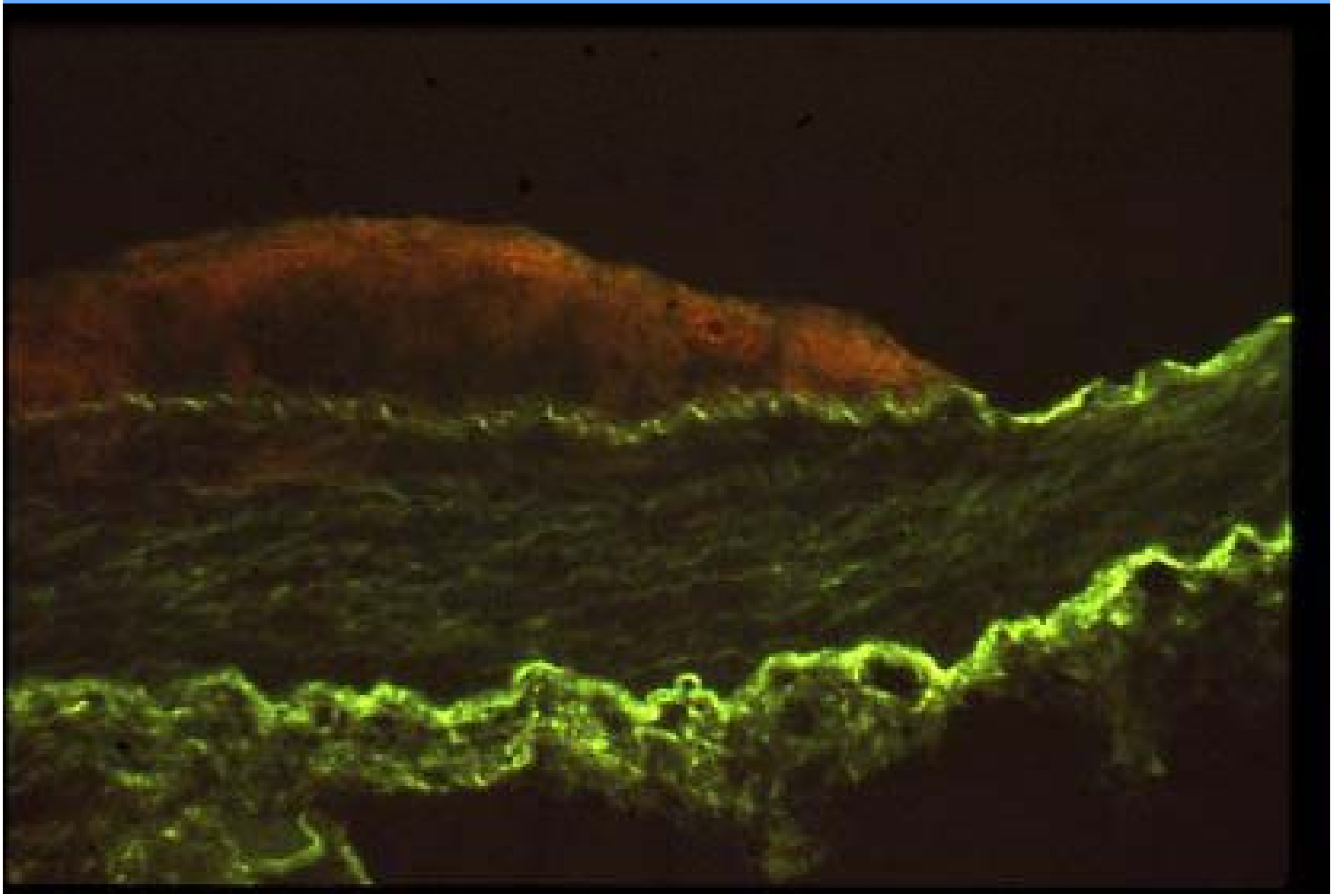
Elastin Fluorescence – artery wall



Lipid Deposition/BPD stain



Collagen Fluorescence



NEW METHODS FOR TISSUE CHARACTERIZATION

LASER INDUCED FLUORESCENCE SPECTROSCOPY (LIFS)

LASER INDUCED FLUORESCENCE ATTENUATION SPECTROSCOPY (LIFAS)

TIME RESOLVED SPECTROSCOPY (TRS)
NANOSECOND (nsec)
MICROSECOND (usec)
MILLISECOND (msec)
SECONDS (sec)

BIOLOGIC SPECTROSCOPY

TIME RESOLVED LASER INDUCED FLUORSCENCE SPECTROSCOPY (TRLIFS)

OPTICAL COHERENCE TOMOGRAPHY (OCT)

DIFFUSE PHOTON WAVE IMAGING (DPW)

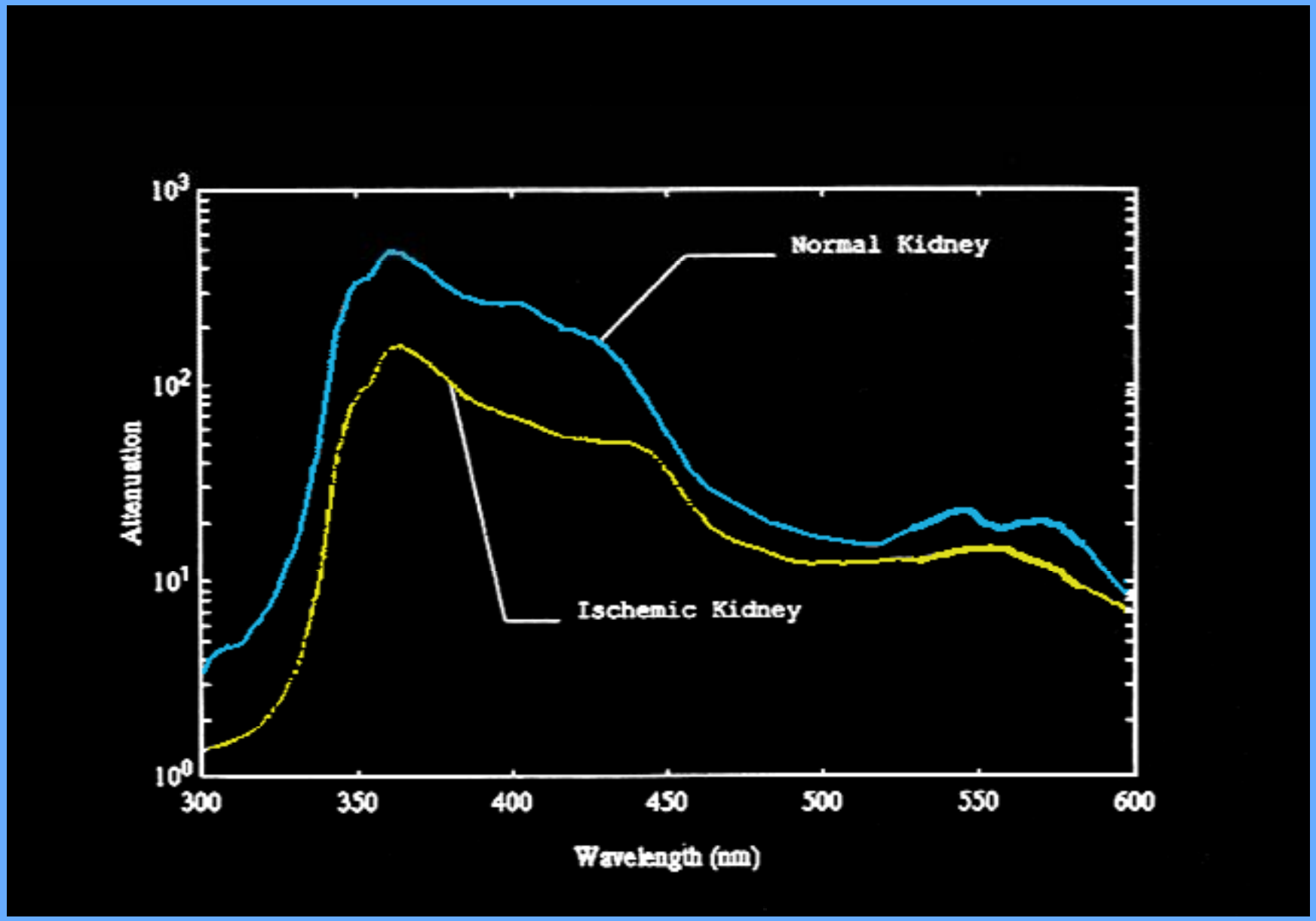
TWO PHOTON EXCITATION IMAGING (TPE)

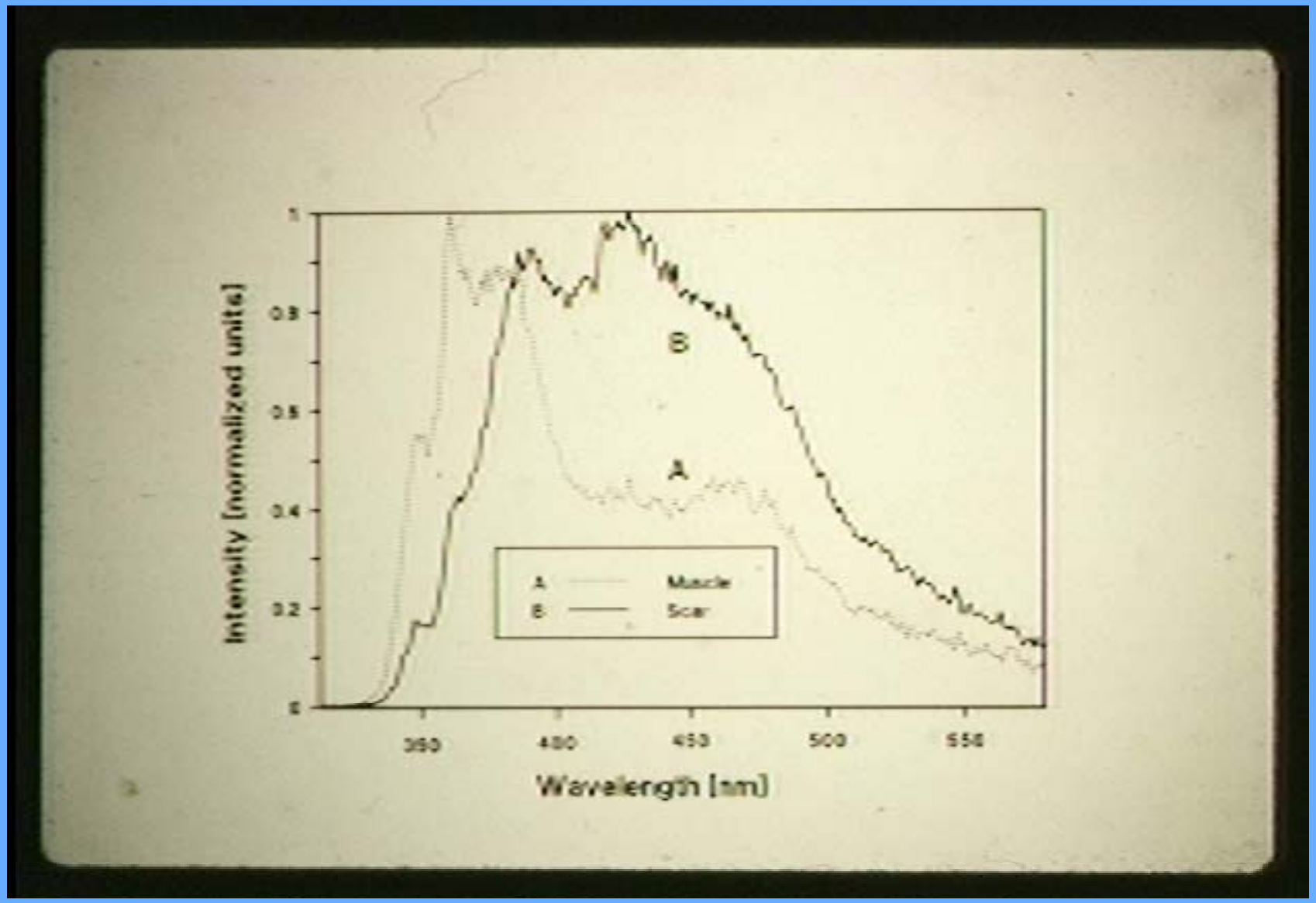
LASER INDUCED FLUORESCENCE SPECTROSCOPY (LIFS)

SHORT WAVELENGTH, HIGH INTENSITY LIGHT IS USED TO EXCITE ENDOGENOUS OR EXOGENOUS FLUOROPHORES WITHIN TISSUE

LASER INDUCED FLUORESCENCE ATTENUTATION SPECTROSCOPY (LIFAS)

SIMULTANEOUS RECORDING OF TWO FLUORESCENT SPECTRA FROM TWO DIFFERENT POINTS IN THE TISSUE PERMITS DETERMINATION OF THE ATTENUATION CHARACTERISTICS. SCATTERING AND ABSORPTION BOTH ATTENUATE OPTICAL SIGNALS. NORMAL AND MALIGNANT TISSUE MAY HAVE DIFFERENT OPTICAL PROPERTIES.


Normal Kidney
Ischemic Kidney
Attenuation
10^3
10^2
10^1
10^0
300
350
400
450
500
550
600
Wavelength (nm)


Intensity [normalized units]
1
0.8
0.6
0.4
0.2
0
B
A
A Muscle
B Scar
350
400
450
500
550
Wavelength [nm]

TIME RESOLVED SPECTROSCOPY (TRS)

WHEN LIGHT INTERACTS WITH TISSUE, THE PROCESSES OF REFLECTION, SCATTERING, ABSORPTION AND REEMISSION CAN OCCUR OVER VARIOUS TIME INTERVALS

TIME RESOLVED SPECTROSCOPY (TRS)

PHOTOBLEACHING

THE FLUORESCENT PROPERTIES OF TISSUE CAN CHANGE WITH EXPOSURE TO LIGHT. THIS PROCESS, KNOWN AS PHOTOBLEACHING, OCCURS OVER THE SECONDS TO MINUTES TIME SCALE AND IS AFFECTED BY THE CHEMICAL ENVIRONMENT OF THE FLUORO-PHORE.

INTRODUCTION 1

PHOTOBLEACHING OF FLUORESCENCE IS A DYNAMIC PROCESS IN WHICH FLUORESCENT MOLECULES UNDERGO CHEMICAL ALTERATION UPON EXPOSURE TO LIGHT, AND THUS LOSE THEIR ABILITY TO FLUORESCE.

IN GENERAL IT RELATES TO A NUMBER OF USUALLY-NONRADIATIVE DEEXCITATION PATHWAYS WHICH ARE AVAILABLE TO THE MOLECULES DURING THE EXCITATION-DEEXCITATION CYCLE.

INTRODUCTION 2

PHOTBLEACHING IS IMPLICATED TO A LESSER OR GREATER DEGREE IN ANY FLUORESCENCE BASED TECHNIQUE & PLAYS AN IMPORTANT ROLE IN A NUMBER OF BIOLOGICAL APPLICATIONS:

- QUANTITATIVE FLUORESCENCE MICROSCOPY
  - LOSS OF CONTRAST
  - LIMITED NUMBER OF IMAGES
- PHOTODYNAMIC THERAPY
  - DOSIMETRY
  - MONITORING
- FLUORESCENCE TISSUE IDENTIFICATION
  - RATIOMETRIC TECHNIQUES

MATERIALS AND METHODS 1

ANIMAL MODEL

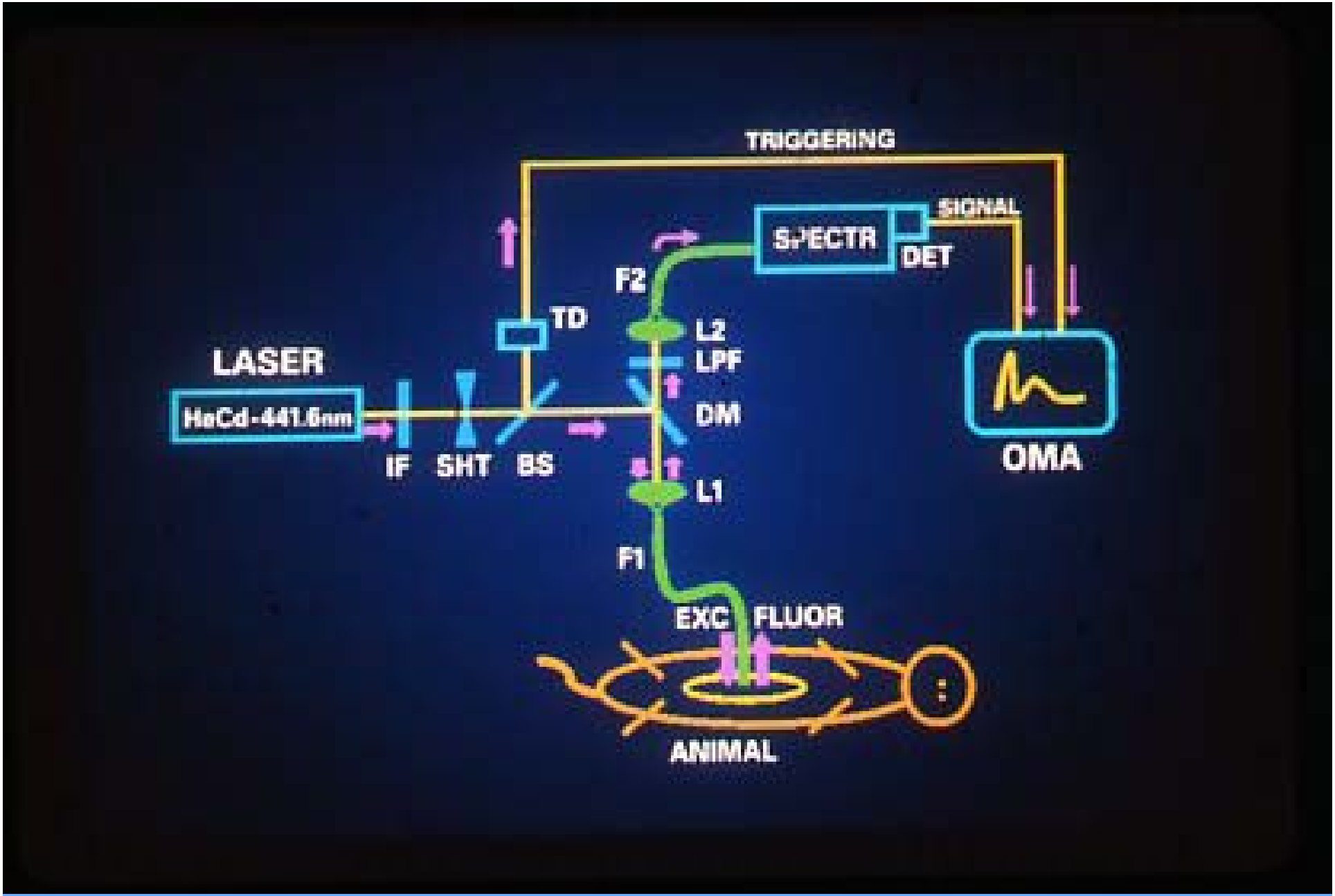
LOBUND WISTAR RATS (N-19)

INOCULATION OF 10^5 CELLS OF POLLARD RAT PROSTATIC ADENOCARCINOMA (PA-III),~40 DAYS PRIOR TO EXPERIMENTATION

INJECTION OF BPD-MA @ 2mg/KG ~4 HOURS PRIOR TO IRRADIATION

ANESTHESIA

LAPAROTOMY-ABDOMINAL EXPLORATION-PRIMARY TUMOR EXPOSURE


TRIGGERING
SIGNAL
SPECTR
DET
F2
TD
L2
LPF
LASER
HeCd-441.6nm
DM
IF
SHT
BS
OMA
L1
F1
EXC
FLUOR
ANIMAL

MATERIALS AND METHODS 2

LASER IRRADIATION

WAVELENGTH: 442nm, CW

LIGHT DELIVERY: MULTIMODE FIBER 600 µm

OUTPUT AT TISSUE: 10 mW

DURATION: 30 sec

LIGHT DOSE: ~105 J/cm^2

MATERIALS AND METHODS 3

ACQUISITION PROTOCOL

THREE SPECTRAL BANDS:
EXCITATION: (442 ± 1) nm
NATIVE: (530 ± 35) nm
BPD-MA: (693 ± 8) nm

DURATION: 30 sec@SAMPLING FREQUENCY: 20Hz

IRRADIATION SITES: RILN, LILN, MC-I, PRIMARY TUMOR

THREE SCANS PER SITE

MATERIALS AND METHODS 4

DATA ANALYSIS

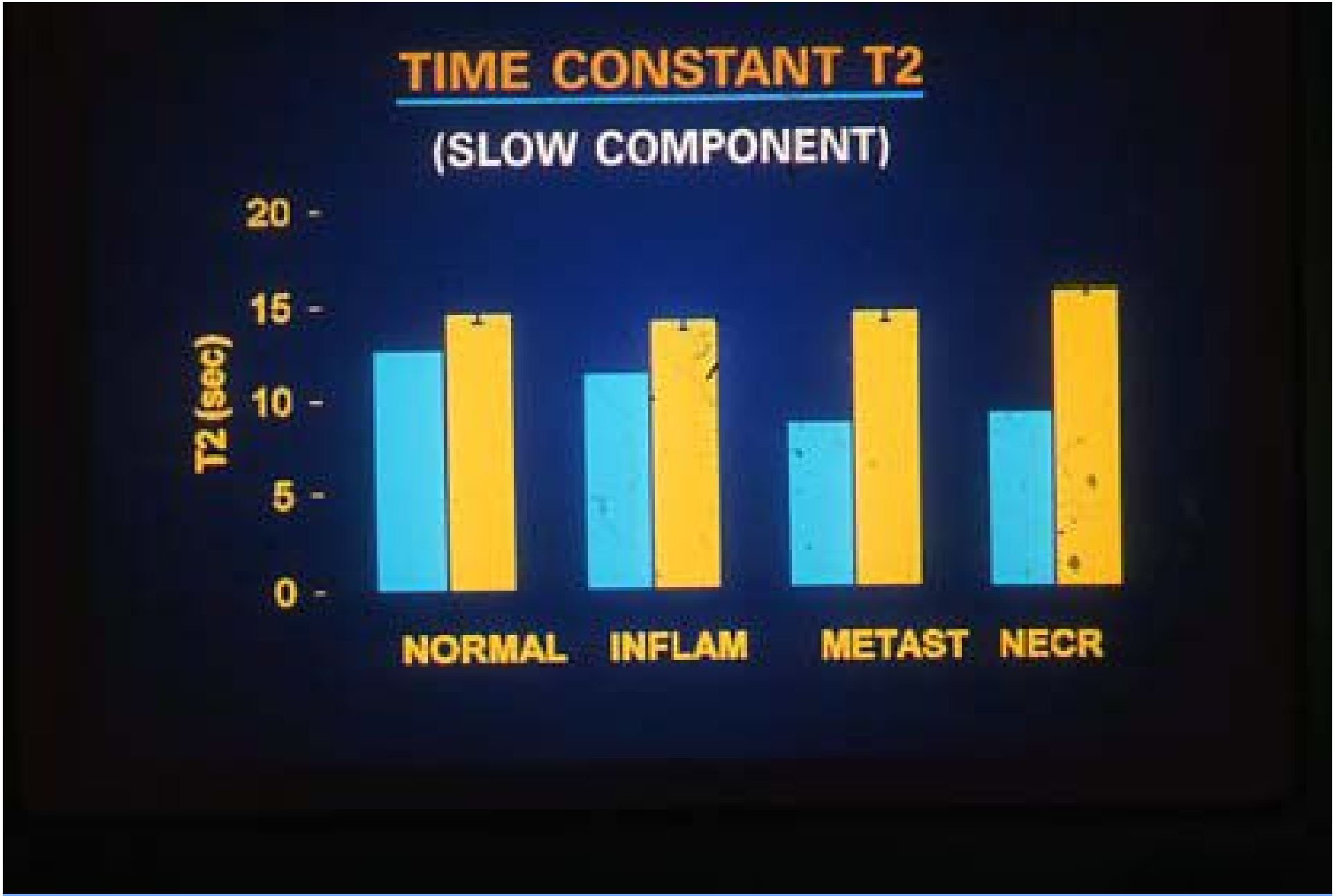
$$Y = A_0 + A_1 * \exp(-t/\tau_1) + A_2 * \exp(-t/\tau_2)$$

STATISTICAL ANALYSIS

ONE WAY ANOVA, SCHEFFE Post-Hoc TESTS

HISTOLOGY

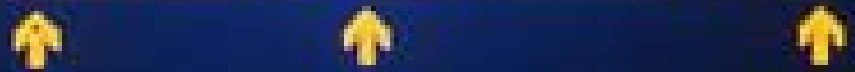
NORMAL
INFLAMMATORY (1^+, 2^+, 3^+)
METASTATIC-MOSTLY VIABLE(<50% NECROSIS)
METASTATIC-MOSTLY NECROTIC (>50% NECROSIS)


TIME CONSTANT T2
(SLOW COMPONENT)
T2 (sec)
20
15
10
5
0
NORMAL
INFLAM
METAST
NECR

RESULTS

AVERAGE DECAY PARAMETERS ACROSS ALL SITES

	A_0	A_1	A_2	τ_1 [SEC]	τ_2 [SEC]
NATIVE	0.55	0.27	0.18	0.96	10.4
BPD-MA	0.16	0.21	0.63	1.5	14.8



CONCLUSIONS

FLUORESCENCE PHOTOBLEACHING FOLLOWS SECOND ORDER DYNAMICS IN WHICH THE DECAY CONSTANTS DIFFER BY APPROXIMATELY AN ORDER OF MAGNITUDE FOR BOTH NATIVE AND BPD-MA FLUORESCENCE

CONCLUSIONS

OVERALL, THE AUTOFLUORESCENCE DECAYS FASTER THAN THE DYE FLUORESCENCE. HOWEVER, IT PHOTOBLEACHES LESS THAN DYE FOR THE SAME IRRADIATION PERIOD.

BIOLOGIC SPECTROSCOPY

THE LIGHT SCATTERING AND ABSORPTION PROPERTIES OF TISSUE CAN BE DETERMINED USING BROADBAND (WHITE LIGHT) ILLUMINATION. THE REFLECTED LIGHT IS COLLECTED, SPECTRALLY SORTED AND MEASURED AT MULTIPLE PIXELS WITHIN A GIVEN IMAGE.

BIOLOGIC SPECTROSCOPY

LIGHT INPUT EXCITES TISSUE

TISSUE EMITS LIGHT AT LONGER WAVELENGTHS

DIFFERENCES IN TISSUE TYPES PRODUCE DIFFERENT EMISSION PATTERNS

PATTERN RECOGNITION IS KEY STEP IN DEVELOPING SPECTROSCOPIC CONTROL SYSTEM

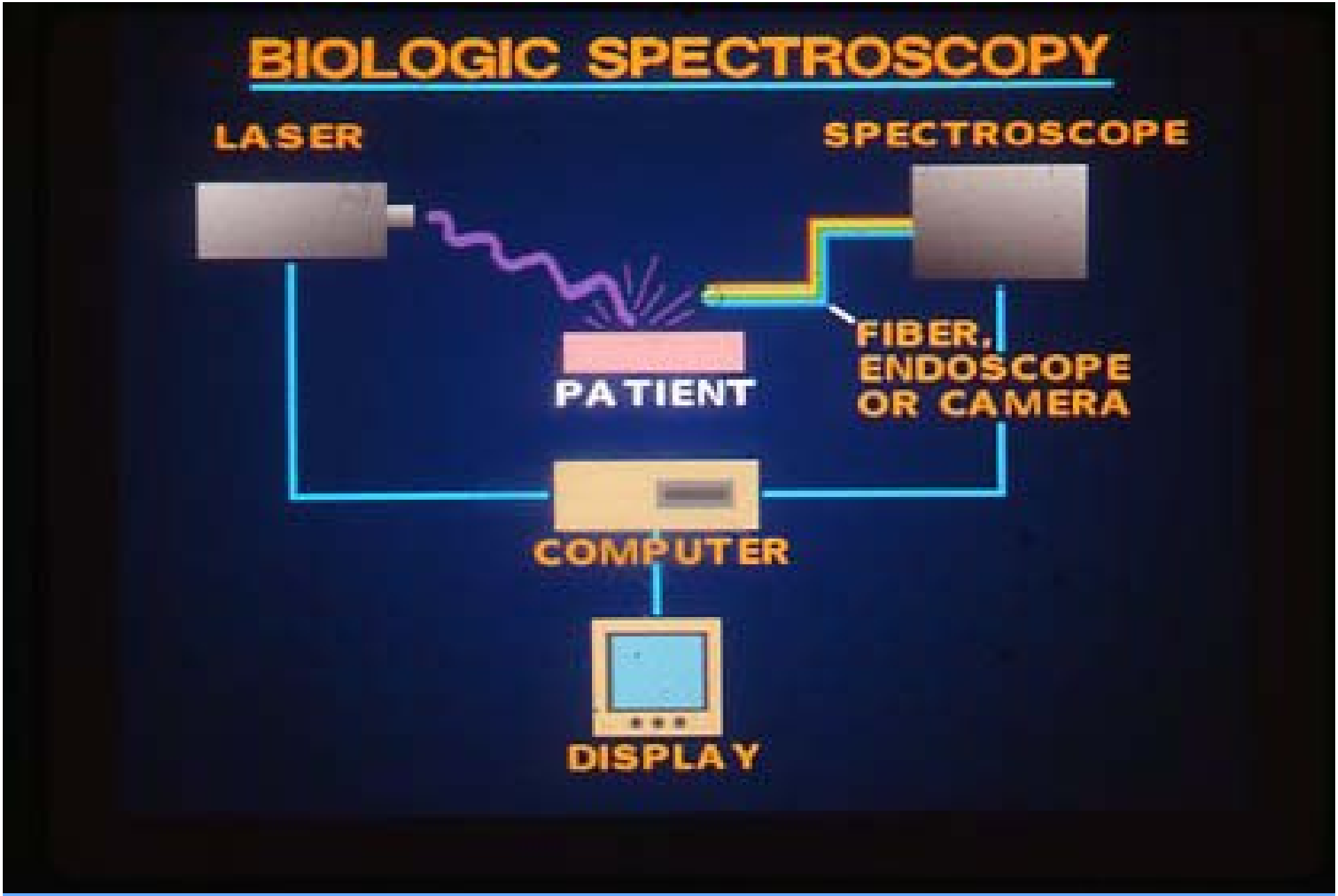
BIOLOGIC SPECTROSCOPY

DIFFERENCES IN TISSUE ABSORPTION CHARACTERISTICS PROVIDE MULTIPLE CONTRAST MECHANISMS WITHIN THE IMAGE. AREAS WITH SIMILAR SPECTRAL FEATURES CAN BE IDENTIFIED, SORTED AND DIFFERENTIATED FROM OTHERWISE NORMAL APPEARING TISSUE.

BIOLOGIC SPECTROSCOPY

HISTORICAL PERSPECTIVE

Pre 1900 - Development of the spectrometer
1940's - Development of modern spectrophotometer
1950's - Use of spectrometry in Biology
1960's - Identification of biologic compounds by spectral properties
1970's - Development of multichannel spectrometers
1980's - Recognition of the presence of characteristic spectral patterns in tissue
1990's - Development of real-time spectral imaging-spectral diagnostics as a useful medical tool


BIOLOGIC SPECTROSCOPY
LASER
SPECTROSCOPE
PATIENT
FIBER,
ENDOSCOPE
OR CAMERA
COMPUTER
DISPLAY

BIOLOGIC SPECTROSCOPY

CURRENT STATE-OF-THE-ART

USED PRIMARILY FOR RESEARCH

DEMONSTRATED ABILITY TO DETECT:
TISSUE ISCHEMIA
CERTAIN CANCERS
DRUGS

EXPENSIVE, COMPLEX
INSTRUMENTATION

REQUIRES HIGHLY SKILLED PERSONNEL

CURRENT EFFORTS

COLORECTAL CANCER - RATIO FLUORIMETRY BASED ON LASER INDUCED FLUORESCENCE SPECTROSCOPY (LIFS) WITH ENDOSCOPIC IMAGING

CERVICAL CANCER - LIFS AND SPECTRAL LINE MAPPERS DURING CULPOSCOPIC EXAMINATION

LUNG CANCER - MULTICOLOR FILTER WHEEL RATIO IMAGING DURING BRONCHOSCOPY AND PHOTO DYNAMIC DIAGNOSTICS (PDD)

RETINAL DISEASE - MULTI-SPECTRAL IMAGING FOR IDENTIFICATION OF ISCHEMIA AND DEEP CHOROIDAL VESSELS

IMAGING SPECTROSCOPY

ADVANCED SPECTRAL IMAGING HAS DEVELOPED A MULTI-SPECTRAL IMAGER BASED ON A SIGNAC INTERFEROMETER. THE DEVICE PERMITS ACQUISITION OF A COMPLETE SPECTRA FROM 370nm TO 1000nm FOR EVERY PIXEL IN THE CCD IMAGER. APPROXIMATELY 10 SECONDS IS REQUIRED PER IMAGE. SPECTRAL RESOLUTION IS 3nm AT 400nm AND 10nm AT 1000nm. SOFTWARE ALLOWS RAPID COMPARISON OF ANY SELECTED PIXEL WITHIN AN IMAGE & COMPARISONS TO OTHER IMAGES.

IMAGING SPECTROSCOPY

WILL IMPROVE THE ABILITY TO DETECT OCCULT MALIGNANCIES

WILL IMPROVE THE DIAGNOSIS OF VISCERAL ORGAN ESCHEMIA

WILL DRAMATICALLY ALTER THE CLASSIFICATION AND TREATMENT OF RETINAL DISEASES

OCT

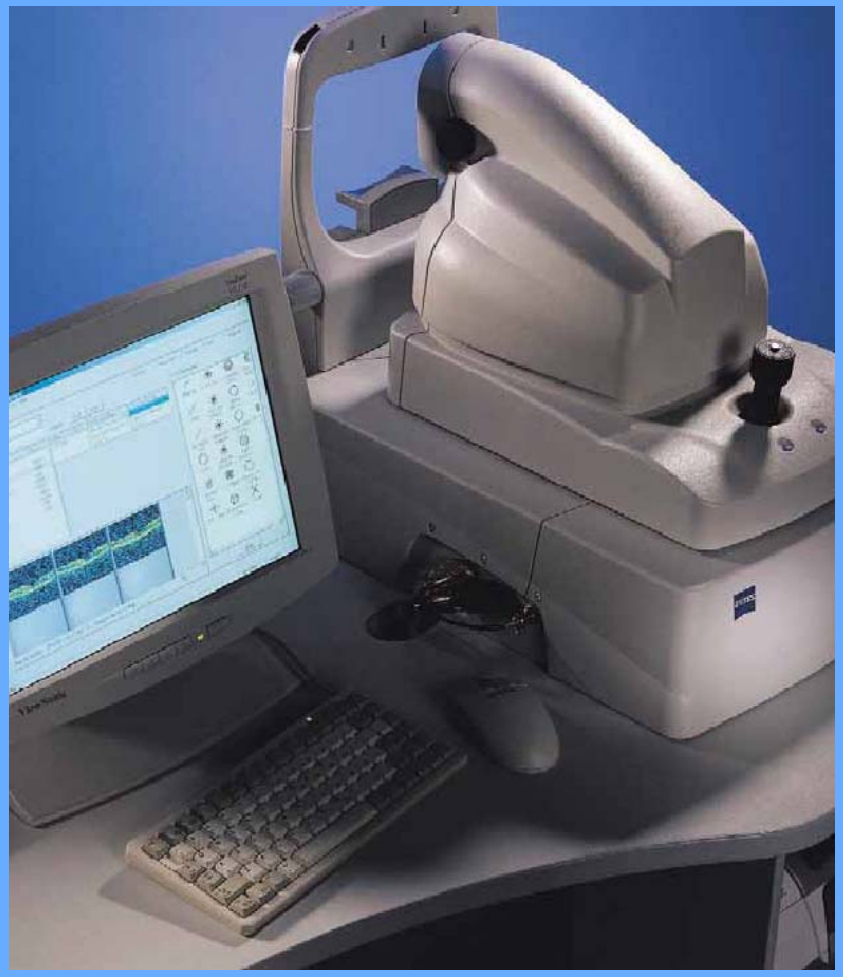
The development of ultrafast laser sources led to the invention of a new imaging modality called Optical Coherence Tomography (OCT).

It is the optical analogue of ultrasonic imaging. Ultra short (femtosecond) light pulses reflect off of layers with differential refractive indexes. The resulting interference patterns are processed to create an image

OPTICAL COHERENCE TOMOGRAPHY HAS BEEN PIONEERED BY FUJIMOTO, IZATT, TROMBERG, TIERNEY, BOUMA AND MANY OTHERS.

FEMTOSECOND LASER PULSES ARE USED TO INVESTIGATE TISSUE AND ARE COMBINED WITH A REFERENCE BEAM TO PRODUCE INTERFERENCE PATTERNS. THIS TECHNOLOGY PROVIDES HIGH RESOLUTION IMAGING OF TISSUE AT DEPTHS OF 300-500µ.

STRATUS OCT RETINAL IMAGER



COMMERCIALIZATION OF OCT TECHNOLOGY WAS PIONEERED BY ZEISS OPTICAL CORP. WHICH DEVELOPED THE FIRST CLINICAL OCT RETINAL IMAGER.

THIS DEVICE ALLOWS OPHTHALMOLOGISTS TO OBTAIN ACCURATE MEASUREMENTS OF THE THICKNESS OF VARIOUS RETINAL LAYERS FOR DIAGNOSTIC PURPOSES.

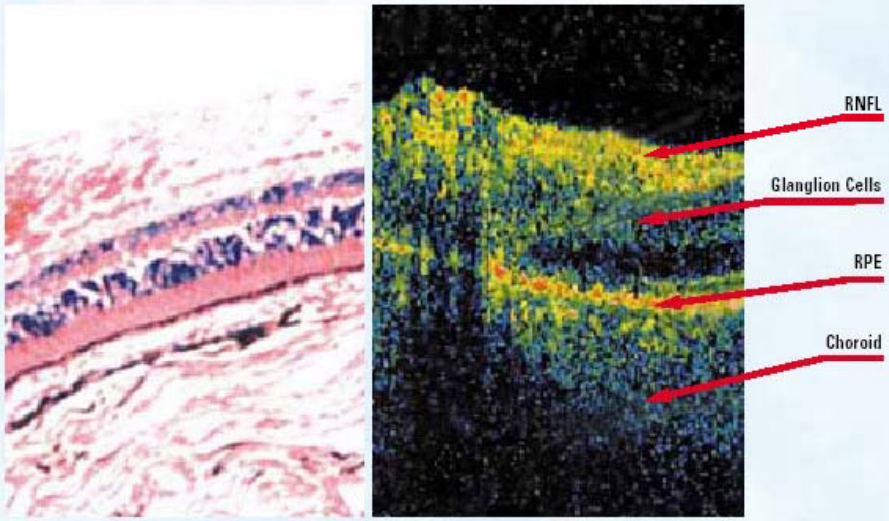
There has never been an optical diagnostic device with the total range of capabilities available in the Stratus$^{OCT™}$.

Stratus$^{OCT™}$ is the first advance of its kind to offer optical coherence tomography with in vivo diagnostic imaging, so practitioners can conduct ocular examinations for retinal disease and glaucoma at an unparalleled level of detail and accuracy. Stratus$^{OCT™}$ is the only device that measures RNFL, optic disk and retina. It's one remarkable innovation.

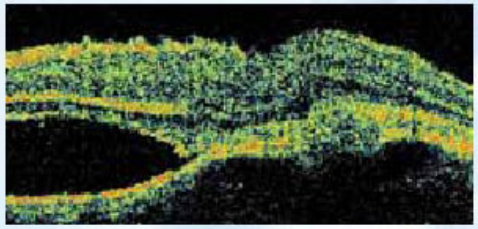
Advanced technology, only available for research . . . until now.

The Stratus$^{OCT™}$ delivers real-time, cross-sectional images of retinal tissue with an axial resolution of 10 microns or less. With the Stratus$^{OCT™}$, practitioners can avoid more invasive diagnostic procedures and literally see below the surface of the retina. This provides direct measurement of internal retinal structures as an aid in the diagnosis of glaucoma and retinal diseases.

Make clear, informed diagnoses with Stratus$^{OCT™}$.



Direct cross-sectional images of live tissue allow practitioners to see disease in vivo. More accurate histology means earlier detection and earlier, often presymptomatic, diagnosis of sight-threatening disease.

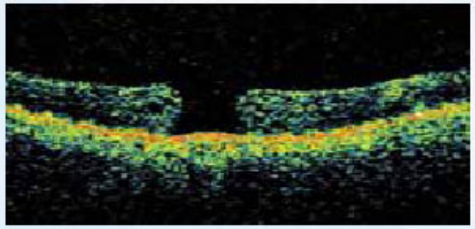


Cystoid Macular Edema (CME) with multiple cysts. No angiogram needed for diagnosis and follow-up. Cross-sectional confirmation of diagnosis, as seen only with Stratus$^{OCT™}$.

View the objective data and in vivo evidence of retinal disease.

Stratus$^{OCT™}$ allows practitioners to identify changes in the RNFL which can lead to early detection of glaucoma. And Stratus$^{OCT™}$ provides for RNFL thickness, bilateral analysis and serial analysis.

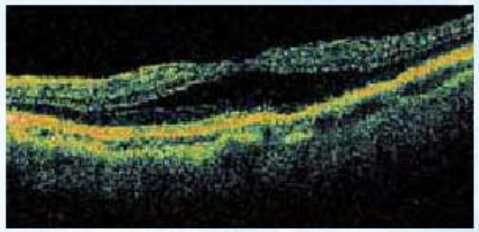
Stratus$^{OCT™}$ scans do not require dilation which increases patient compliance. Images and data for analysis are available instantly, in vivo, with no biohazard or blood-related risk.



Macular Hole. Measure the dimensions of macular hole. Cross-sectional confirmation of diagnosis.

Real-time, in vivo retinal images enhance your ability to diagnose.

Stratus$^{OCT™}$ allows practitioners to perform accurate diagnosis and measurement of CME, CSR and macular holes with cross-sectional scans of retinal thickness and in vivo histology of tissue. Diagnosis is further enabled by color-coded maps and retinal thickness in microns in nine map sectors.

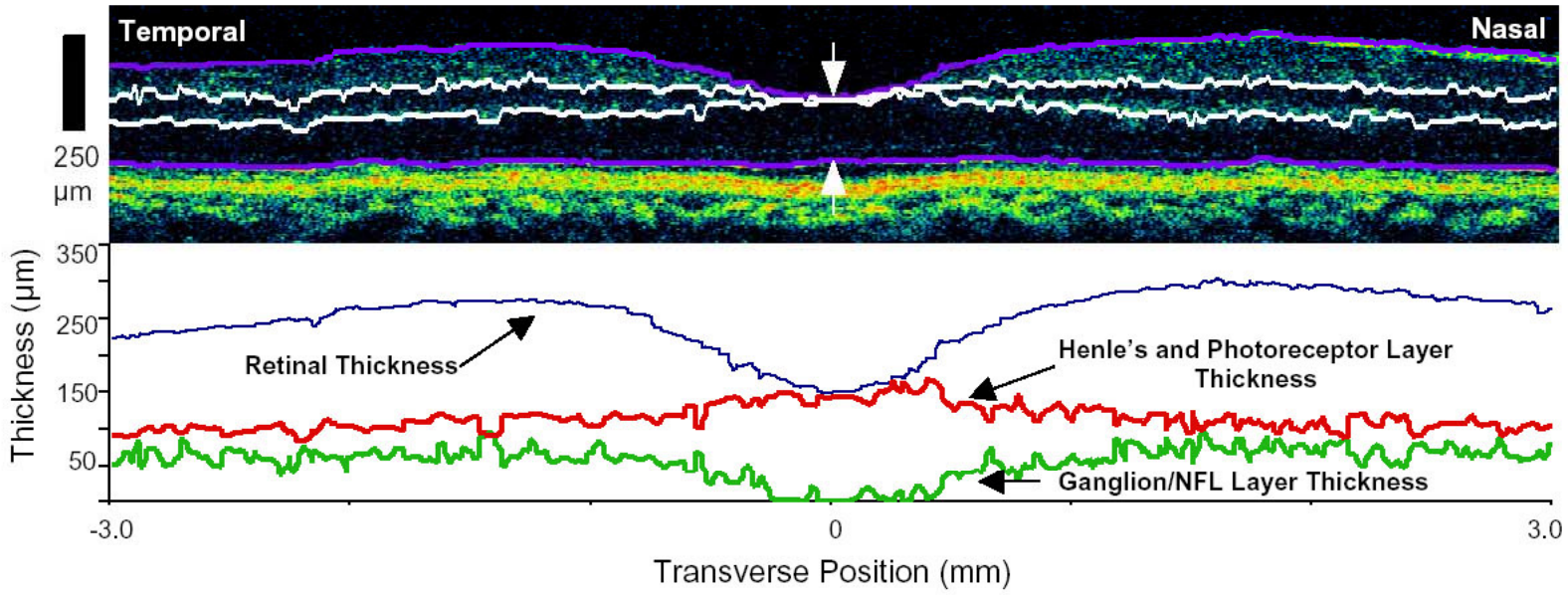


Central Serous Retinopathy Neurosensory detachment at the macula.

THE SUCCESSFUL INTRODUCTION OF RETINAL OCT HAS PROMOTED DEVELOPMENT OF THE TECHNOLOGY FOR MULTIPLE APPLICATIONS.

HIGH RESOLUTION CAN BE OBTAINED BY USING SHORTER PULSES, IMPROVED SCANNING TECHNIQUES AND MORE ACCURATE IMAGE RECONSTRUCTION ALGORITHMS.

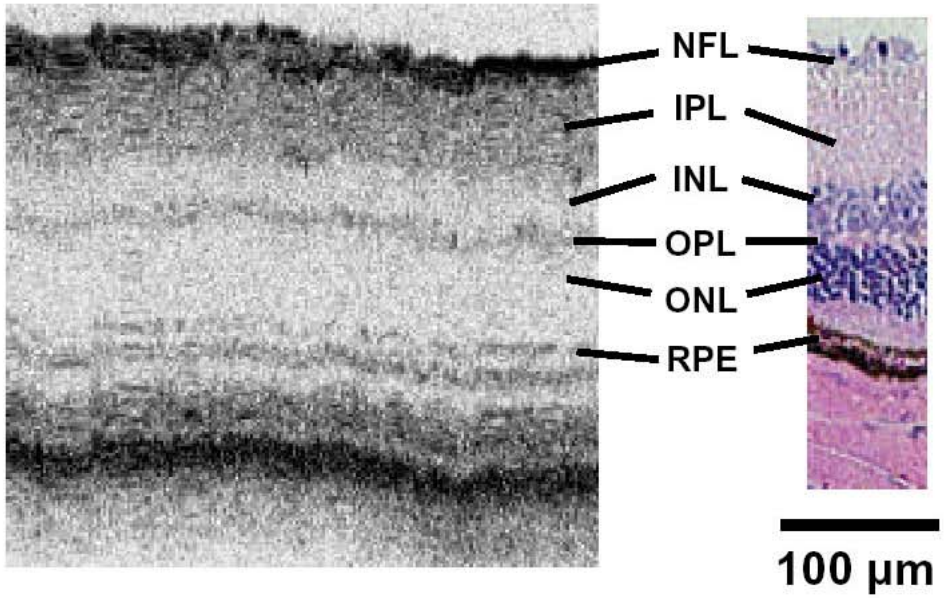
HIGH RESOLUTION OCT OF RETINA



Ultrahigh resolution allows for an unprecedented visualization of intraretinal structures which may be quantified to provide an objective measure of retinal disease.

FROM JAMES FUJIMOTO/MIT WEBSITE

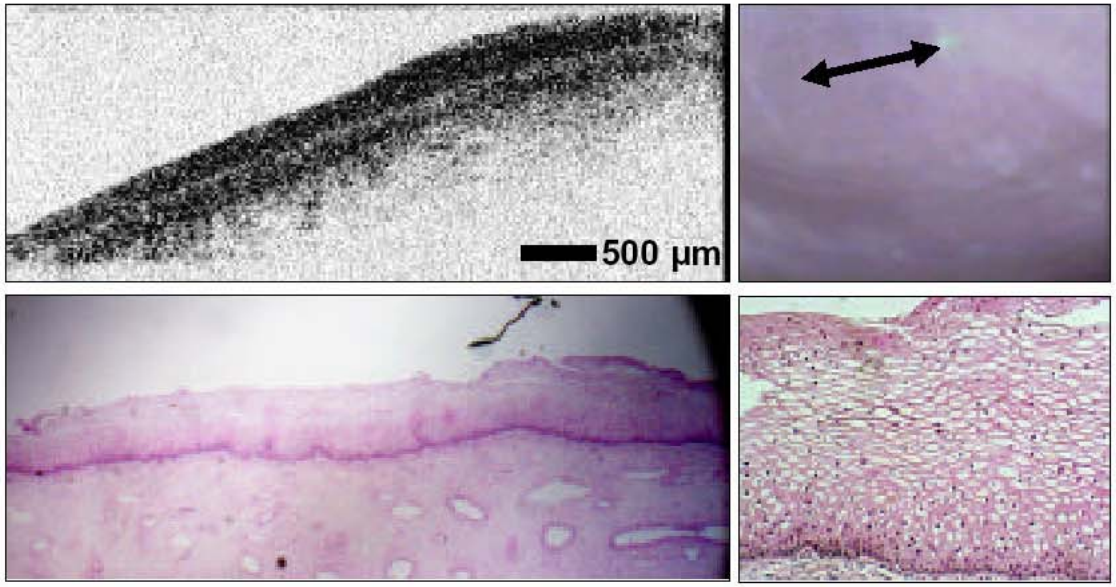
HIGH RESOLUTION OCT OF MOUSE RETINA



In vivo ultrahigh resolution OCT image of a normal mouse retina and corresponding histology. The layers identified in the OCT images correspond well with the layers identified in the histology.

FROM JAMES FUJIMOTO/MIT WEBSITE

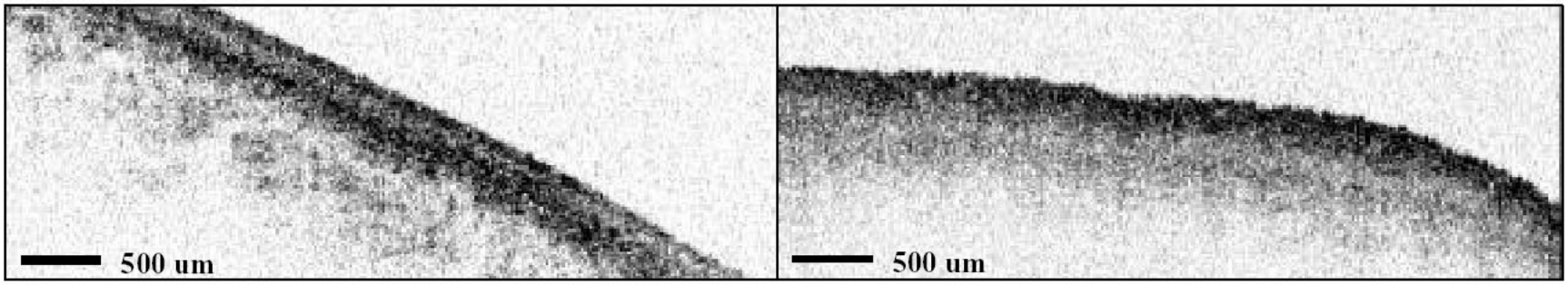
OCT AND HISTOLOGIC IMAGES OF CERVIX



OCT image of a normal cervix, a colposcopic view of the area scanned, and corresponding histology.

FROM JAMES FUJIMOTO/MIT WEBSITE

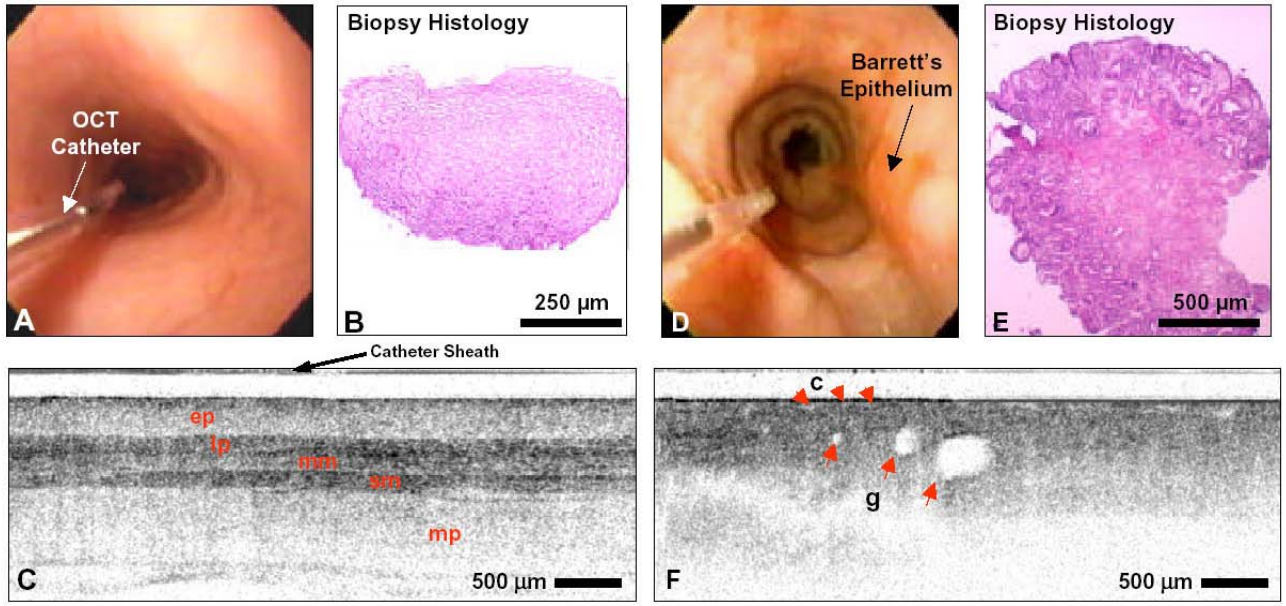
OCT OF CERVICAL DYSPLASIA



example of OCT images of a normal and severe squamous dysplasia from the same patient. In dysplasia, the epithelial layers were irregular with no clear borders. Higher backscattering intensity was also observed in areas with dysplasia or cancer. Further work is needed to determine whether OCT can accurately differentiate between high grade and low grade dysplasia.

FROM JAMES FUJIMOTO/MIT WEBSITE

OCT OF ESOPHAGUS



Clinical endoscopic OCT imaging of normal and Barrett's esophagus using linear scanning. (a) Endoscopic video image of normal region. (b) Biopsy histology of normal squamous epithelium. (c) OCT image of normal squamous epithelium with relatively uniform and distinct layered structures. (d) Endoscopic video image of region showing finger-like projection of Barrett's epithelium. (e) Biopsy histology of Barrett's esophagus showing characteristic specialized columnar epithelium. (f) OCT image of Barrett's epithelium with disruptions of layered morphology due to multiple crypt- and gland-like structures (arrows).

FROM JAMES FUJIMOTO/MIT WEBSITE

DIABETIC RETINOPATHY

AFFECTS 7 MILLION PATIENTS PER YEAR IN THE U.S.

CAUSES 12,000 NEW CASES OF BLINDNESS ANNUALLY

YEARLY RETINAL SCREENING IS RECOMMENDED SINCE LASER THERAPY CAN SLOW PROGRESSION OF DISEASE

DIABETIC RETINOPATHY

IN COMPARISON TO THE COST OF CARE FOR A BLIND PERSON, SCREENING FOR DIABETIC RETINOPATHY IS COST EFFECTIVE

CURRENT EXAMINATION METHODS OFTEN MISS EARLY SIGNS OF DIABETIC RETINAL DISEASE, THUS DELAYING POTENTIAL THERAPY

CURRENT TECHNIQUES DO NOT PERMIT ASSESSMENT OF PHYSIOLOGIC STATUS

FLUORESCEIN ANGIOGRAPHY

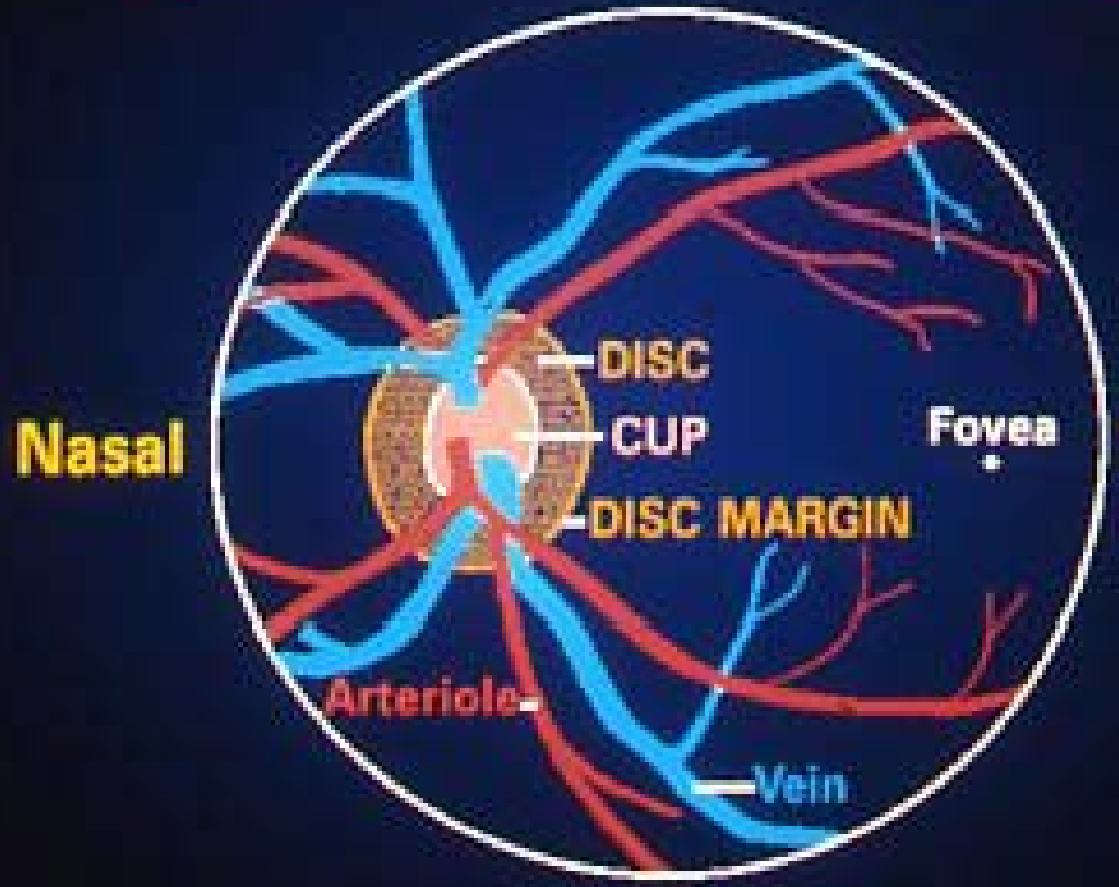
CURRENTLY THE BEST METHOD TO ASSESS RETINAL ISCHEMIA DUE TO DIABETIC RETINOPATHY

INSENSITIVE TO EARLY STAGES OF DISEASE

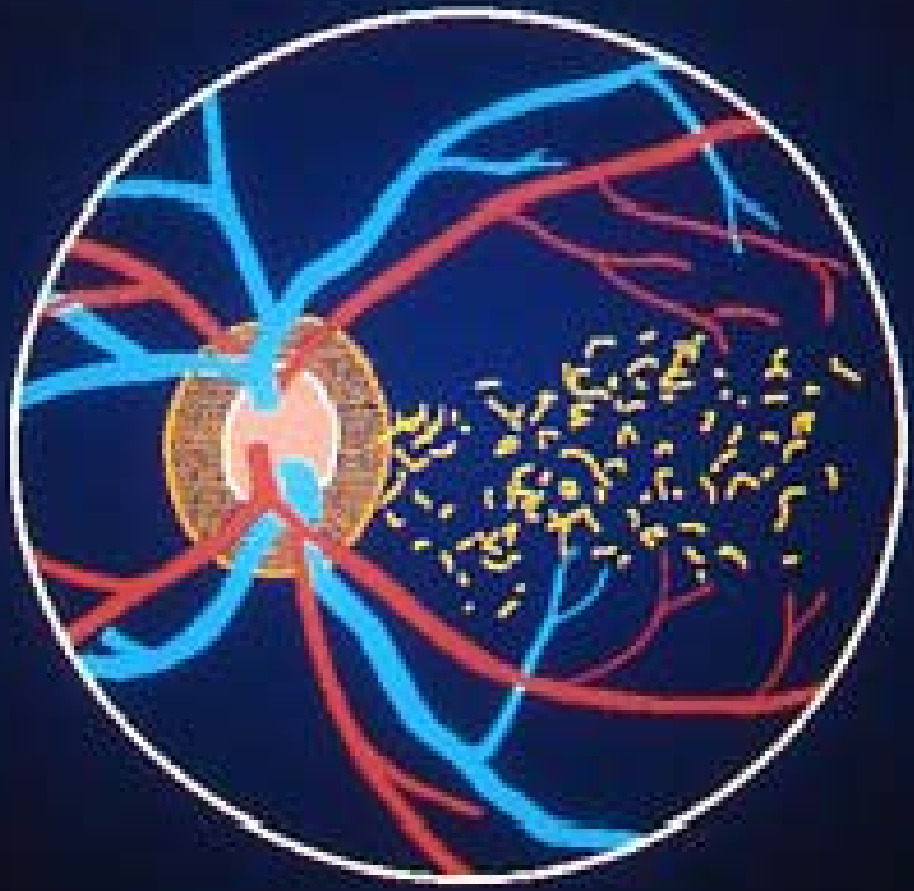
ONE PATIENT IN 2000 DEVELOPS SEVERE COMPLICATIONS

TIME CONSUMING

RETINAL VESSELS

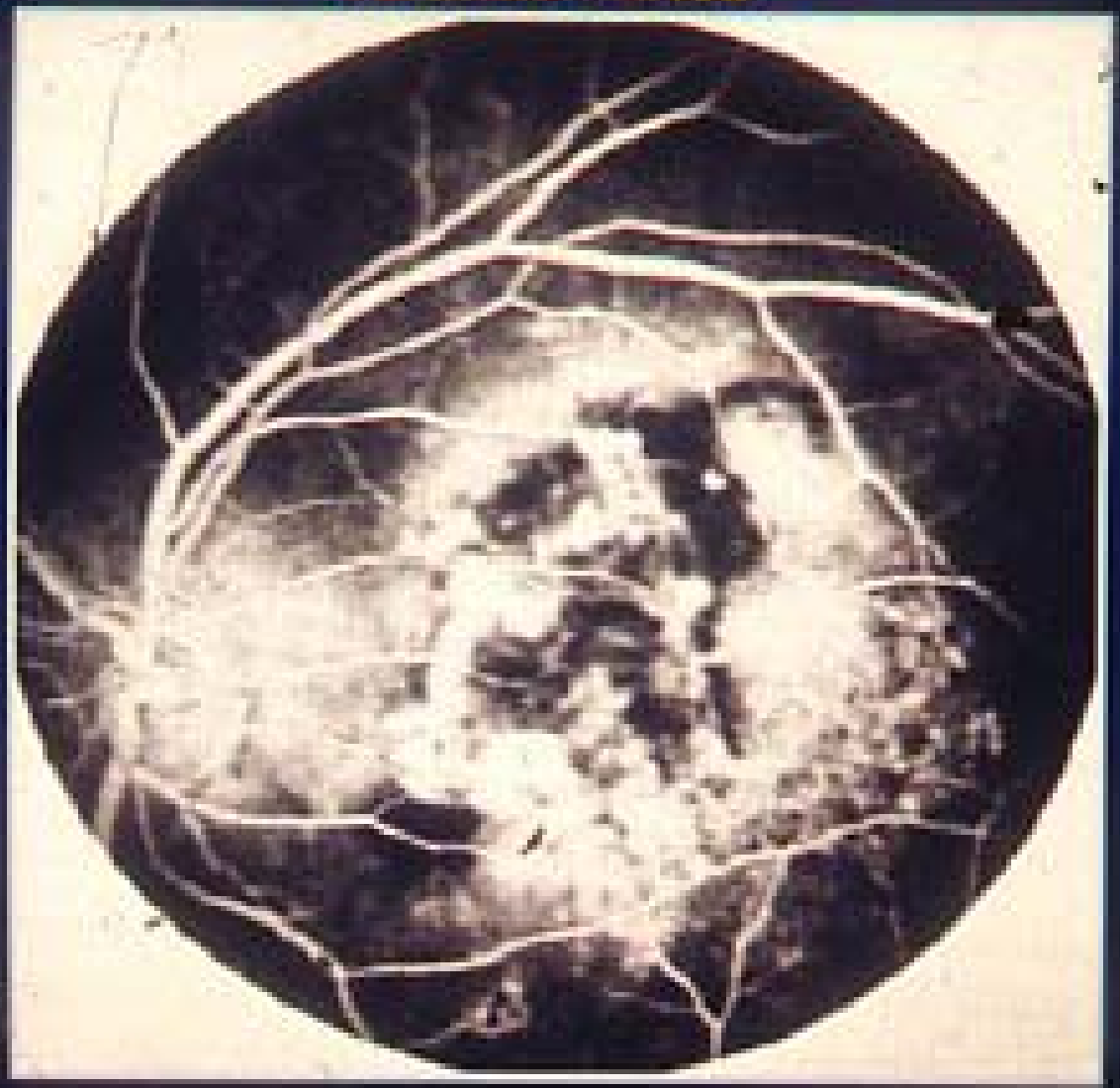


DIABETIC RETINOPATHY



FLUORESCEIN ANGIOGRAPHY

VENOUS PHASE



FLUORESCEIN ANGIOGRAPHY

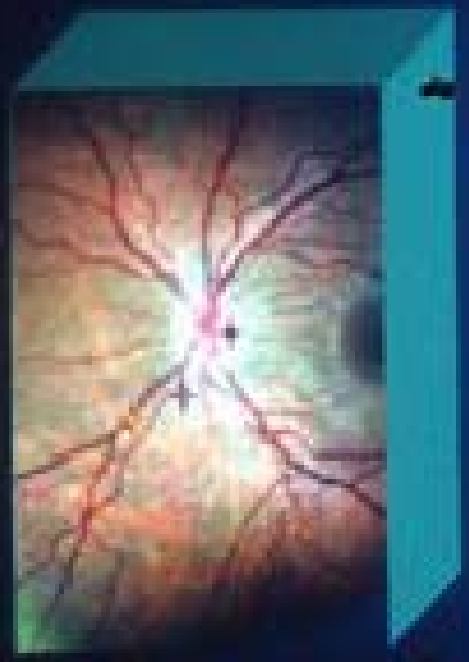
CURRENTLY THE BEST METHOD TO ASSESS RETINAL ISCHEMIA DUE TO DIABETIC RETINOPATHY

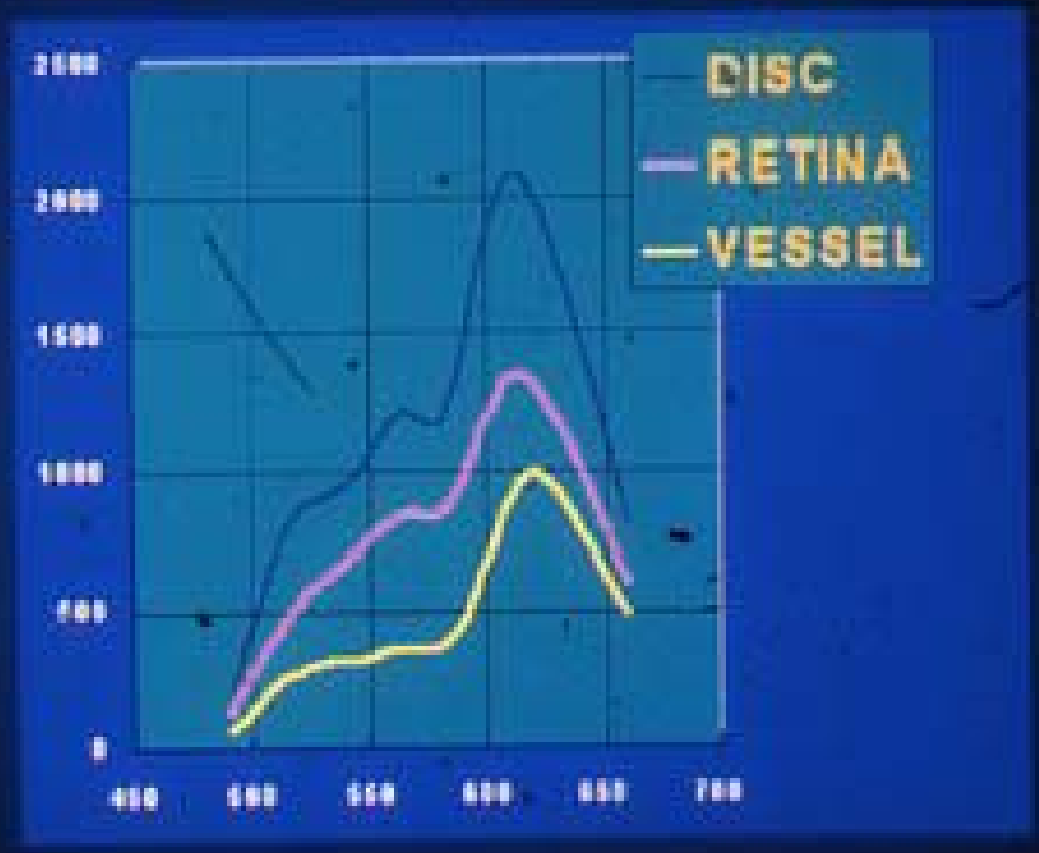
INSENSITIVE TO EARLY STAGES OF DISEASE

ONE PATIENT IN 2000 DEVELOPS SEVERE COMPLICATIONS

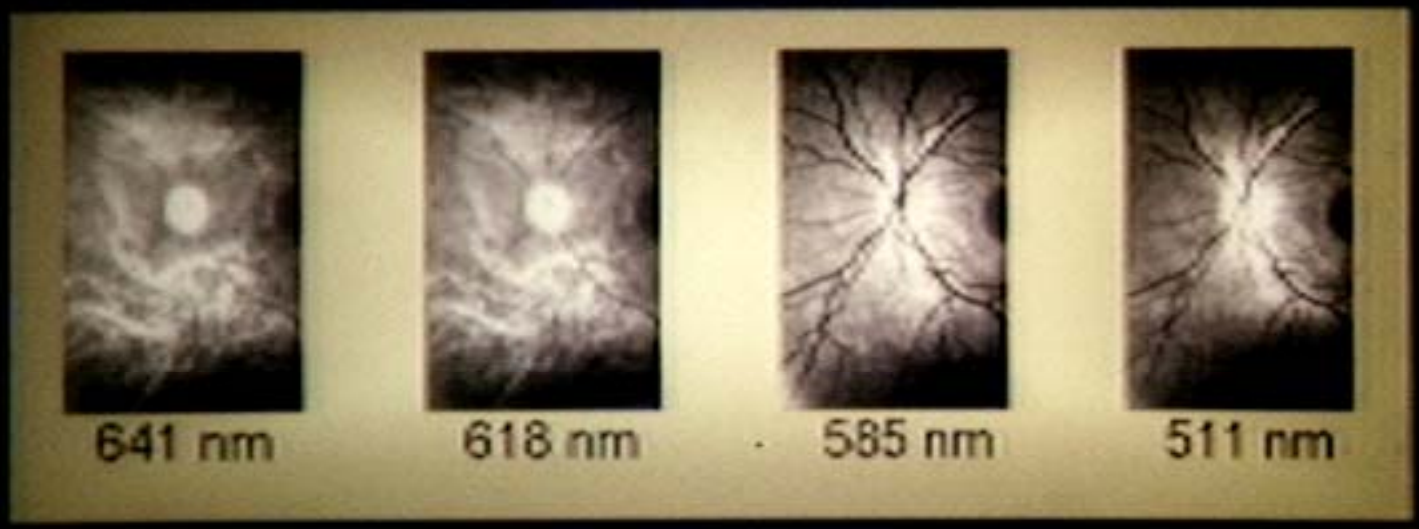
TIME CONSUMING

FROM CUBES TO POINT SPECTRA:



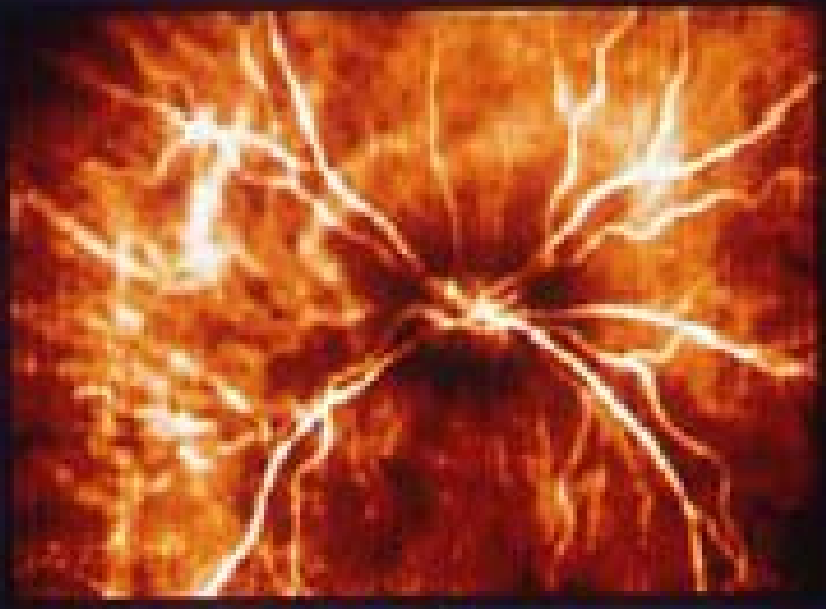


2D SPECTRAL INFORMATION



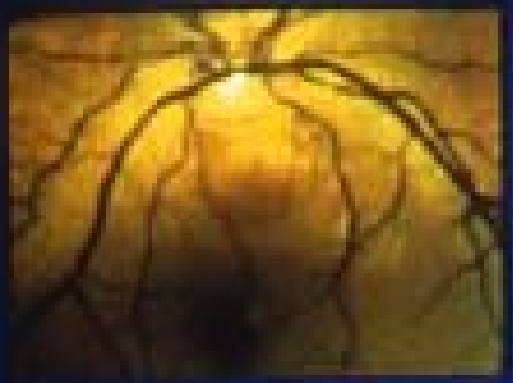
NORMAL FUNDUS

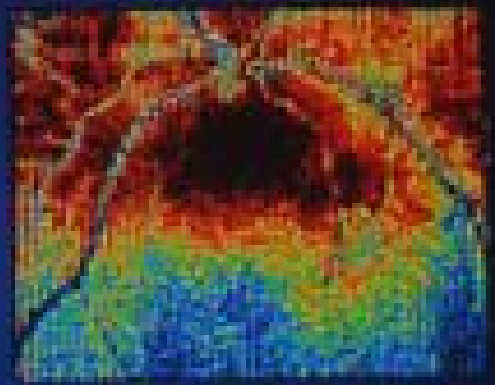
BAND RATIO IMAGE: 526 nm/652 nm



ENHANCED VIEW OF
CHOROIDAL VESSELS

HEMOGLOBIN ABSORPTION BANDS





RED: 540 nm
GREEN: 580 nm

INTENSITY-MAPPED BAND RATIO: 540nm/580nm

THE DATA PRESENTED IN THE PREVIOUS SLIDES WAS OBTAINED USED POINT SPECTROSCOPY. A SINGLE FIBER OPTIC CABLE WAS USED TO EXTRACT INFORMATION FROM A SMALL VOLUME OF TISSUE. WHILE ACCURATE, THIS TECHNOLOGY IS TOO SLOW FOR ENDOSCOPIC APPLICATIONS.

IT IS POSSIBLE TO DEVELOP A LIFETIME IMAGING SYSTEM WHICH USES LIFETIME AND WAVELENGTH DATA TO GENERATE CONTRAST MAPS FOR CANCER DETECTION.

HYPERSPECTRAL IMAGING CAN USE EITHER SINGLE OR MULTIPLE EXCITATION SOURCES TO STIMULATE FLUORESCENCE EMISSIONS FROM THE TISSUE.

THE EMITTED FLUORESCENCE IS CAPTURED AND ANALYZED FOR EVERY POINT IN THE IMAGE, YIELDING MAPS OF RELATIVE INTENSITIES. THESE DATA CAN THEN BE USED TO PREDICT THE PRESENCE OR ABSENCE OF CANCERS.

HYPERSPECTRAL IMAGING

Standard Laparoscopic View of Pelvis

Fused Laparoscopic and Hyperspectral Image

Fused Laparoscopic, LIFS and Hyperspectral Image

TRLIFS

Hyperspectral imaging to date has not achieved sufficient sensitivity and specificity to eliminate the need for tissue biopsy. In an effort to improve diagnostic accuracy multiple investigators have studied various forms of time resolved fluorescence spectroscopy in an effort to overcome these limitations.

TIME RESOLVED LASER INDUCED FLUORESCENCE SPECTROSCOPY (TRLIFS)

THE MEASUREMENT OF THE TIME EVOLUTION AND INTENSITY OF FLUORESCENCE SIGNALS. MULTIPLE TECHNIQUES ARE AVAILABLE FOR COLLECTION OF BOTH POINT AND IMAGING DATA. THE ENORMOUS QUANTITY OF DATA COLLECTED REQUIRES SUBSTANTIAL COMPUTING POWER AND ANALYSIS ALGORITHMS.

FLUORESCENCE MEASUREMENTS

μ_a	comp.
μ_s	λ
$\cos\langle\theta\rangle$	δ

- **Tissue fluorescence**

$$E_f = \int_{volume} (Exc)(Conv)(Esc)\,dV$$

$$E_f = \int_{volume} (E_0\phi_{ex})(\mu_a\Phi)(\phi_{em})\,dV$$

$$E_f = E_0 \varepsilon C \Phi \int_{volume} \phi_{ex}\phi_{em}\,dV$$

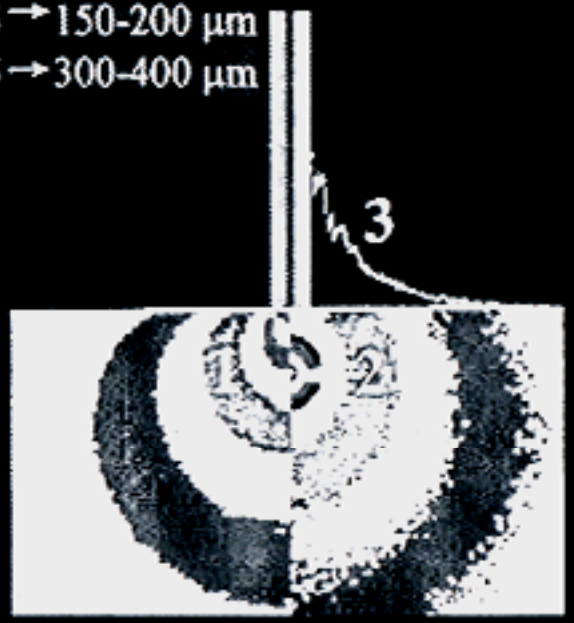
tissue optics

intrinsic fluorescence

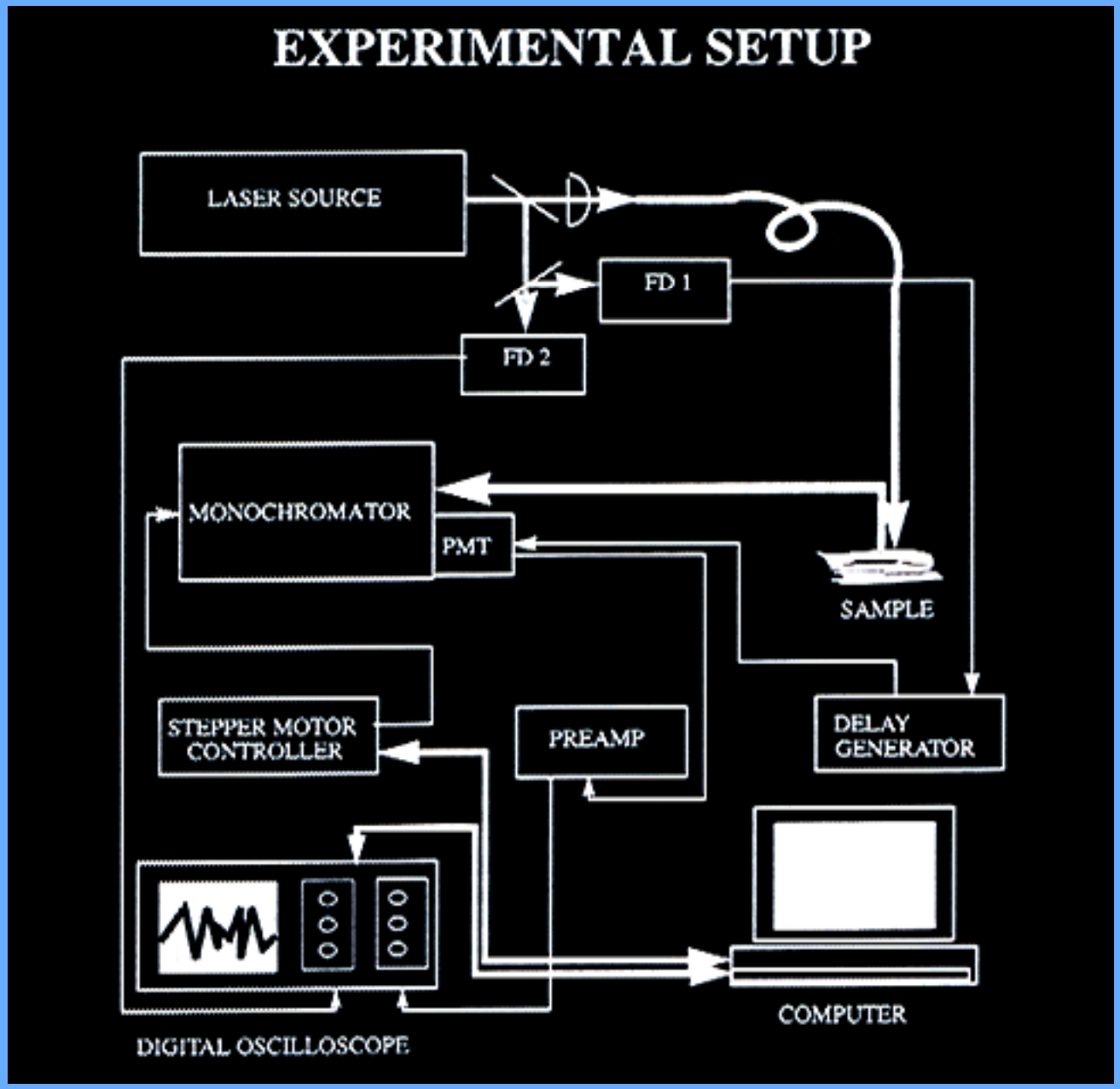
Exc. irradiance

Obs. fl. irradiance

308 → 80-100 μm
325 → 150-200 μm
475 → 300-400 μm



1. penetration of excitation light
2. absorption ➡ conversion to emission
3. escape of emission


EXPERIMENTAL SETUP
LASER SOURCE
FD 1
FD 2
MONOCHROMATOR
PMT
SAMPLE
STEPPER MOTOR CONTROLLER
PREAMP
DELAY GENERATOR
COMPUTER
DIGITAL OSCILLOSCOPE

DATA ANALYSIS

- **Data pre-processing**
  - background noise correction
  - laser energy fluctuations correction
- **Recover spectral emission from time-resoved spectra**
- **Fluorescence IRF deconvolution**
  - Fuorescence convolution integral

$$y(t) = \int_0^{\infty} I_f(\tau) x(t-\tau) d\tau$$

$$y(n) = \sum_{m} I_f(m) x(n-m) \qquad \text{Discrete-time}$$

- **Statistical analysis**
  - Analysis of variance (ANOVA)
  - Post-hoc comparison test (Student-Newman-Keuls)

DATA ANALYSIS

- **IRF deconvolution**
  - multiexponential decay (*a priori* postulated functional form)

$$I_f(t) = \sum_{i=1}^{n} a_i e^{-t/\tau_i}$$

  - expansion over the discrete-time Laguerre basis

$$I_f(m) = \sum_j c_j b_j(m,\alpha)$$

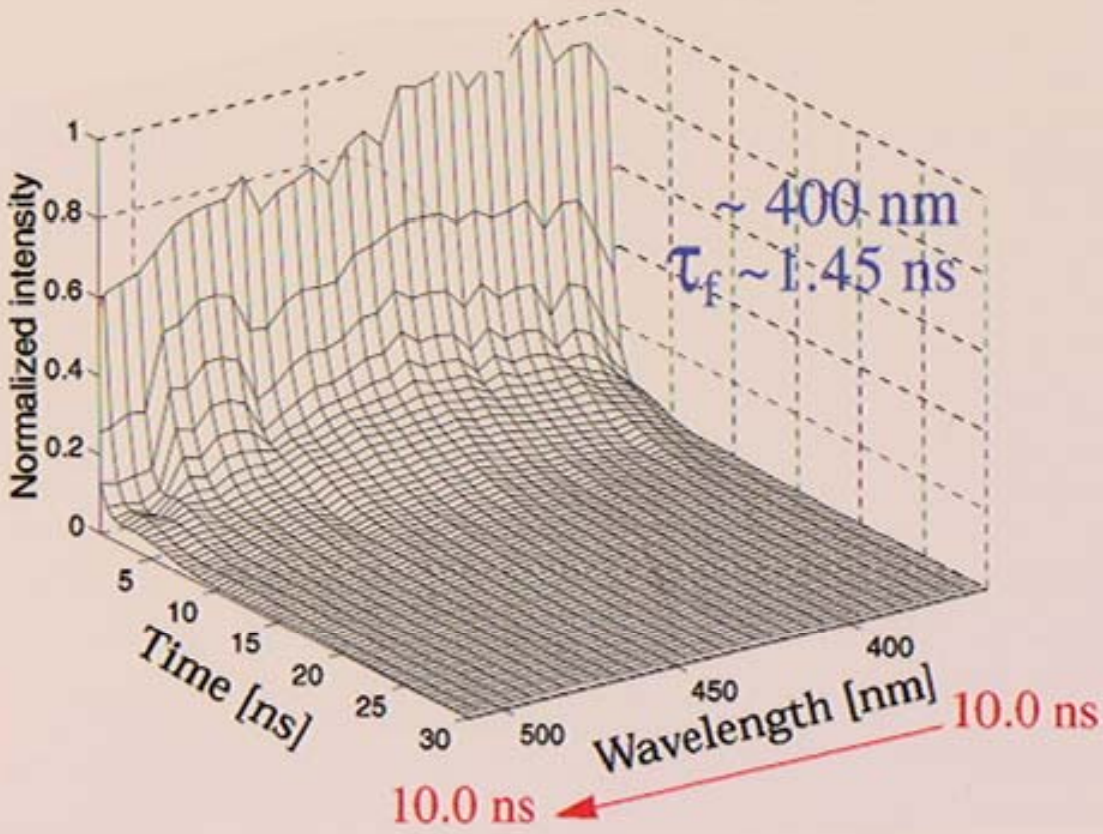
$$b_j(m) = \alpha^{\frac{(m-j)}{2}} (1-\alpha)^{\frac{1}{2}} \sum_{k=0}^{j} (-1)^k \binom{m}{k} \binom{j}{k} \alpha^{j-k} (1-\alpha)^k, (m \geq 0) \qquad j = 4$$

  - least-square iterative reconvolution technique

$$\chi^2 = \sum_{n=1}^{N} w_n [y_m(n) - y_c(n)]^2 \longrightarrow a_i, \tau_i \longrightarrow \alpha, c_j$$

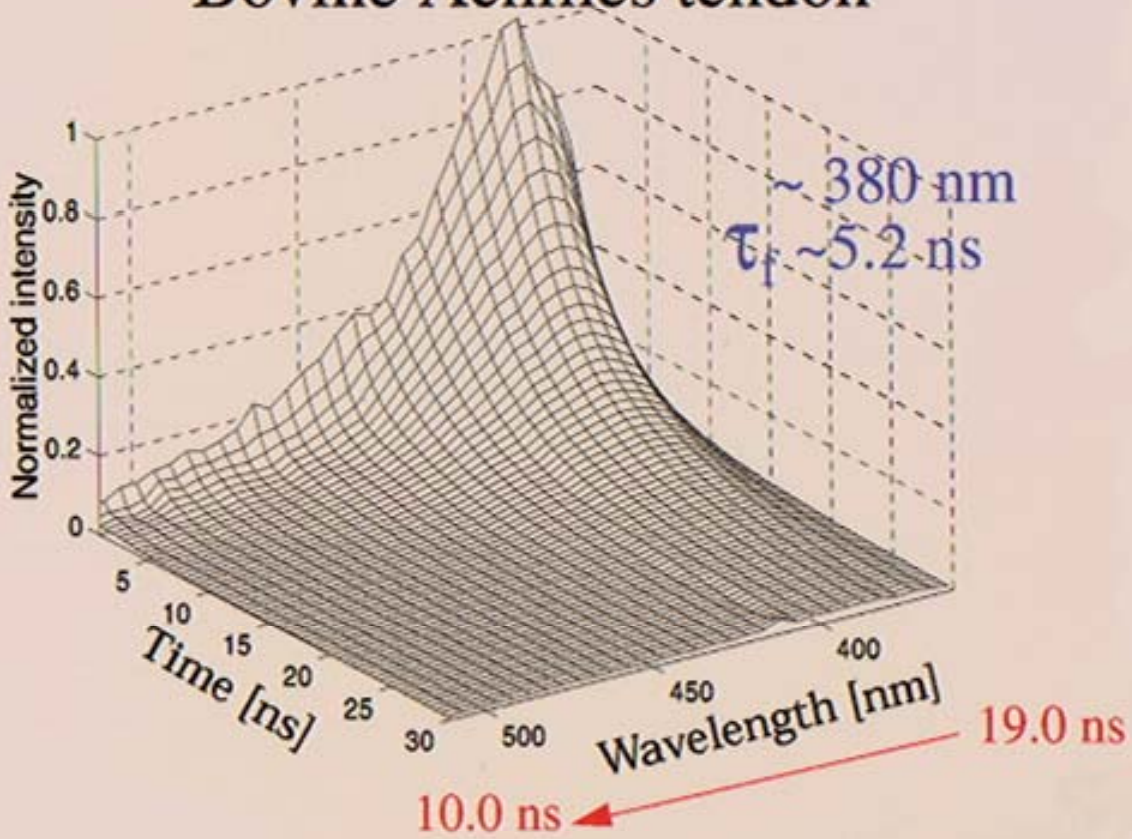
Type I Collagens

Rat tail

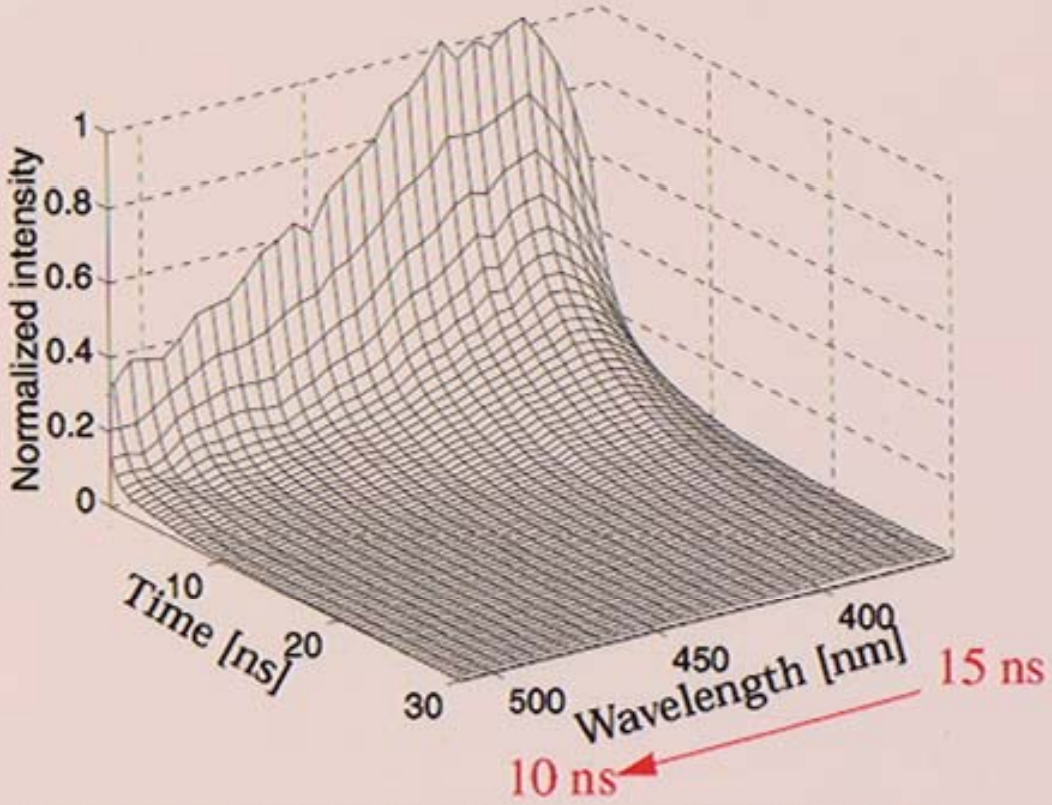


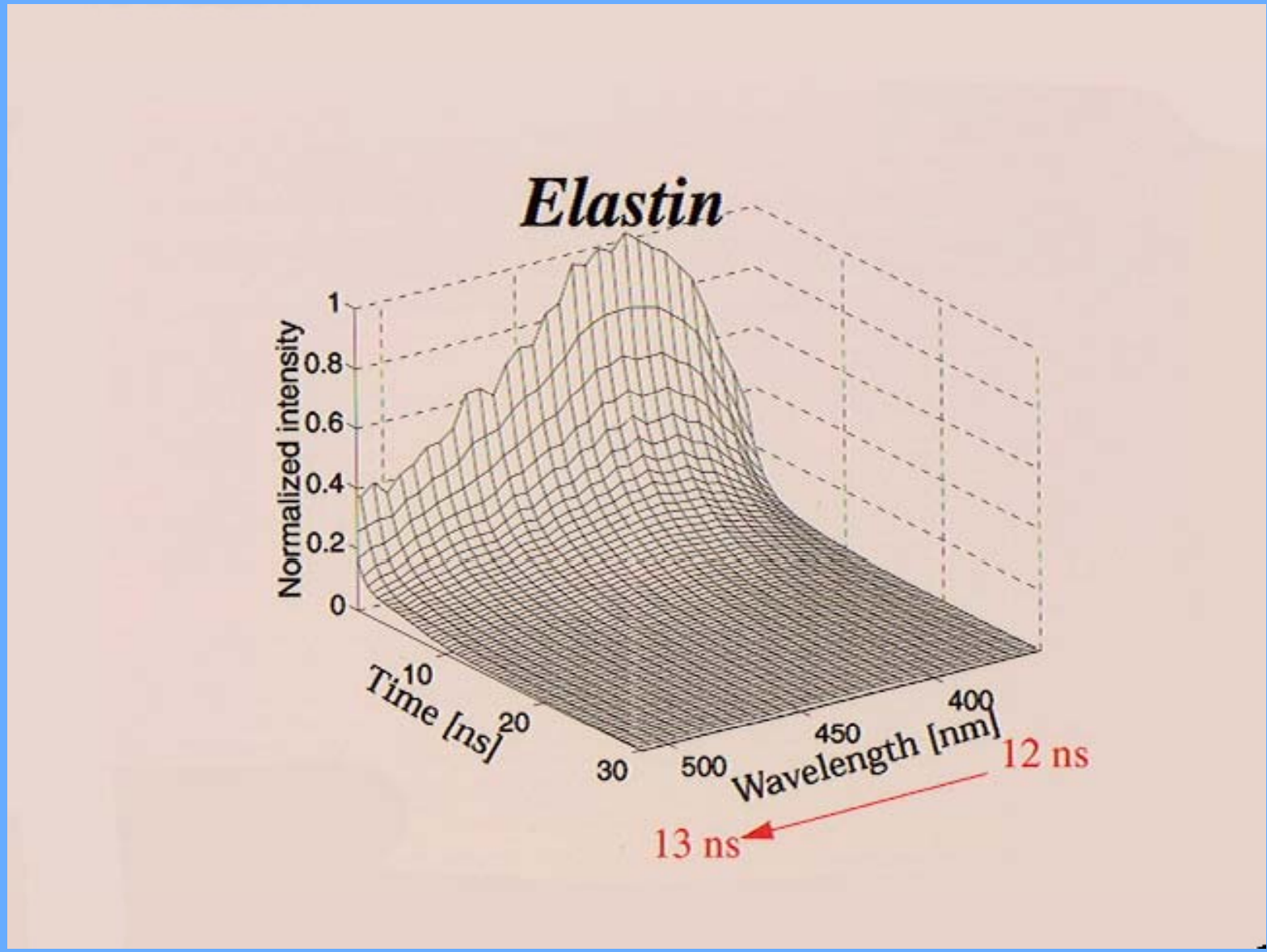
Type I Collagens

Bovine Achilles tendon



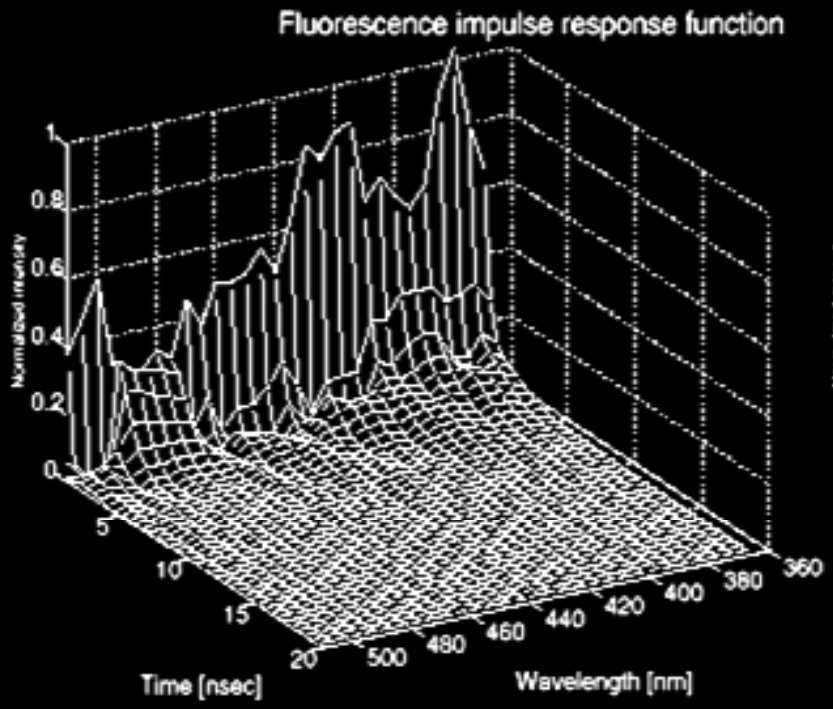
Collagen Type III



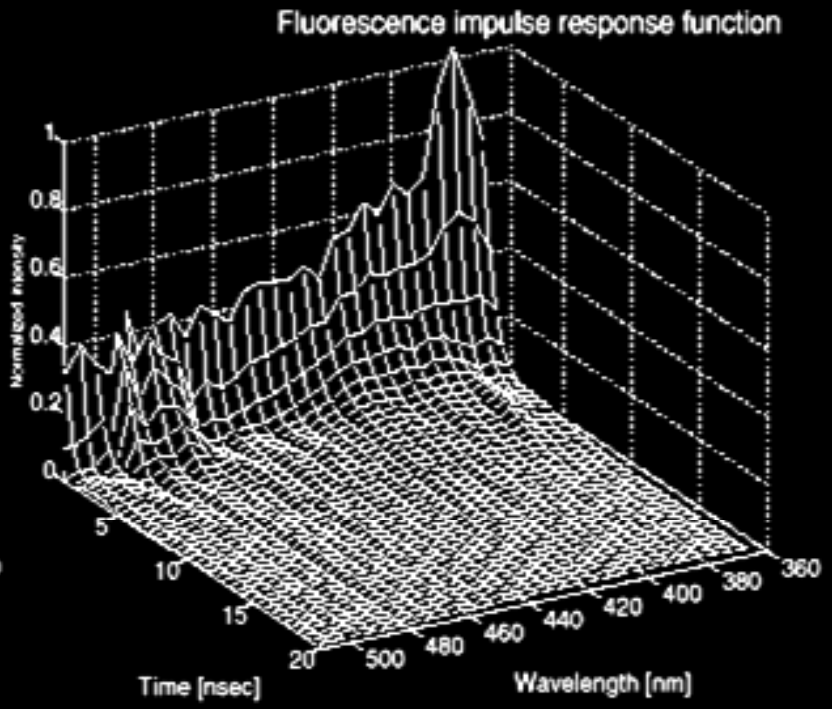

Elastin
Normalized intensity
1
0.8
0.6
0.4
0.2
0
Time [ns]
10
20
30
Wavelength [nm]
500
450
400
12 ns
13 ns

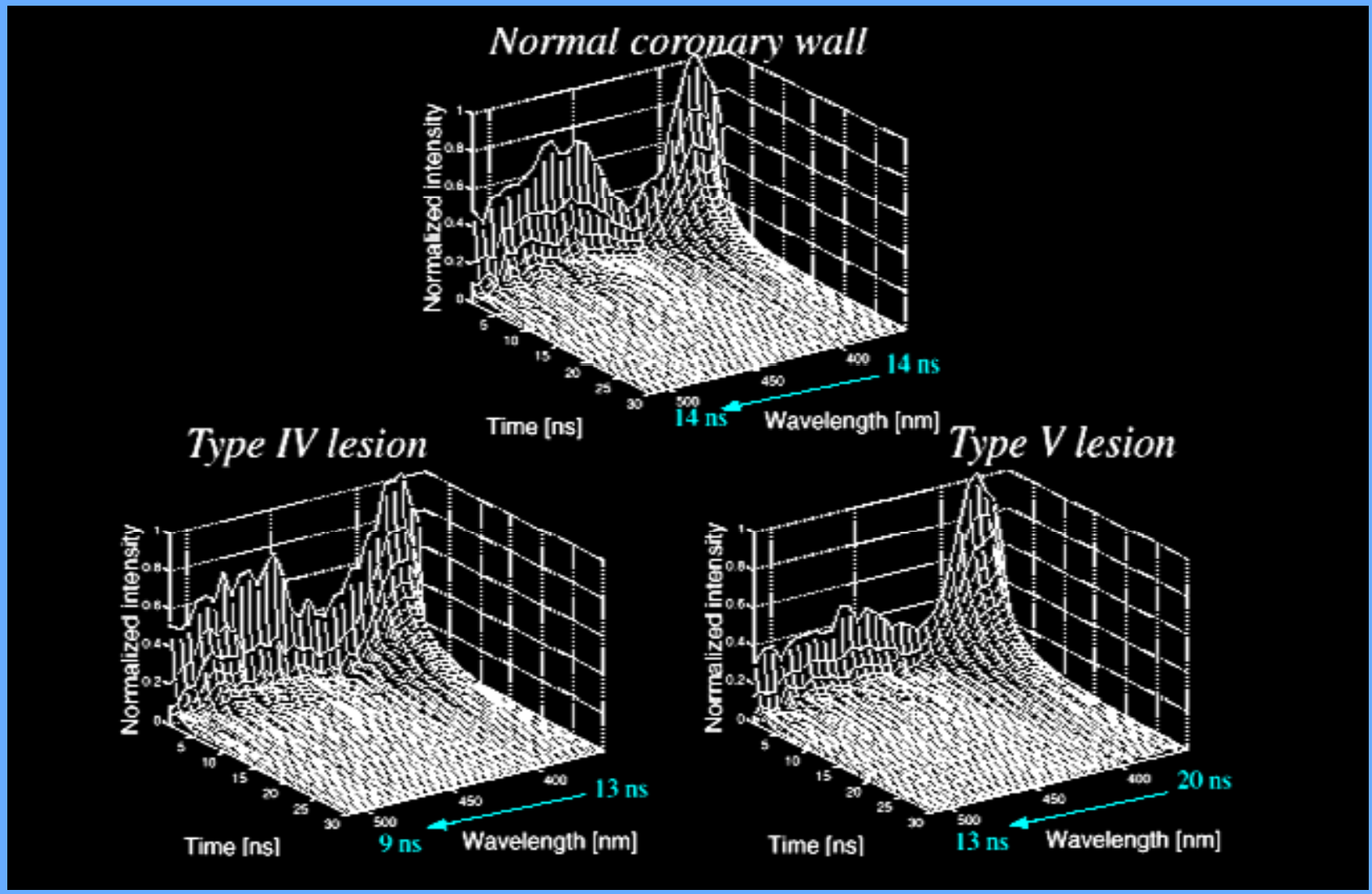
SPECTRO-TEMPORAL EMISSION

Cholesterol oleate



Cholesterol linoleate




Normal coronary wall
Normalized intensity
Time [ns]
Wavelength [nm]
14 ns
14 ns
Type IV lesion
Normalized intensity
Time [ns]
Wavelength [nm]
13 ns
9 ns
Type V lesion
Normalized intensity
Time [ns]
Wavelength [nm]
20 ns
13 ns

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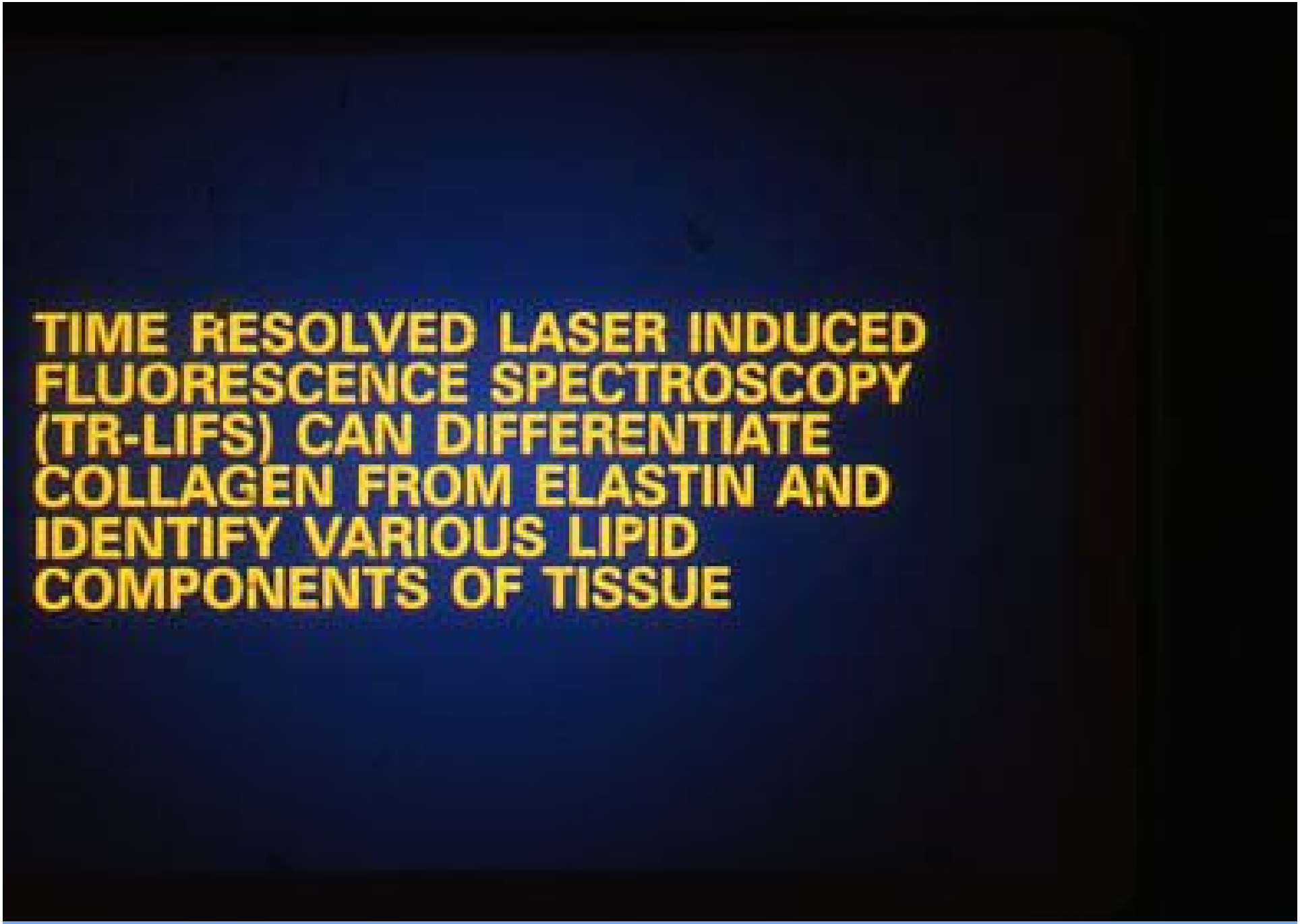
TIME RESOLVED LASER INDUCED
FLUORESCENCE SPECTROSCOPY
(TR-LIFS) CAN DIFFERENTIATE
COLLAGEN FROM ELASTIN AND
IDENTIFY VARIOUS LIPID
COMPONENTS OF TISSUE

USING UV EXCITATION, SAMPLE VOLUME IS SMALL AND THEREFORE, RESOLUTION IS HIGH. THE TECHNIQUE REQUIRES OPTICAL ILLUMINATION OF THE TARGET TISSUE, EITHER THROUGH A MICROSCOPE OR A FIBER OPTIC PROBE.

CONCLUSIONS

BIOLOGIC SPECTROSCOPY HAS BEEN SUCCESSFULLY APPLIED FOR OXYGEN SATURATION DETERMINATION & METABOLYTE ANALYSIS

NEWER TECHNIQUES, LIFS, TR-LIFS AND IMAGING SPECTROSCOPY ARE NOW AVAILABLE. CLINICAL TRIALS OF THESE MODALITIES ARE NOW UNDER WAY. THE VALUE OF THESE TECHNIQUES WILL DEPEND UPON THE COST OF THE INSTRUMENTATION, THE SENSITIVITY AND SPECIFICITY OF THE MEASURING SYSTEM AND ITS EASE OF USE.