Breast Cancer Probed by Fluorescence and Raman Spectroscopy

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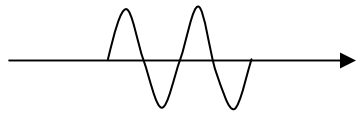
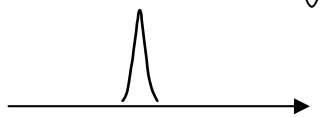
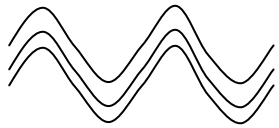
Properties and Processes of Light

Salient properties

- Wavelength (color)
- Time
- Polarization
- Coherence







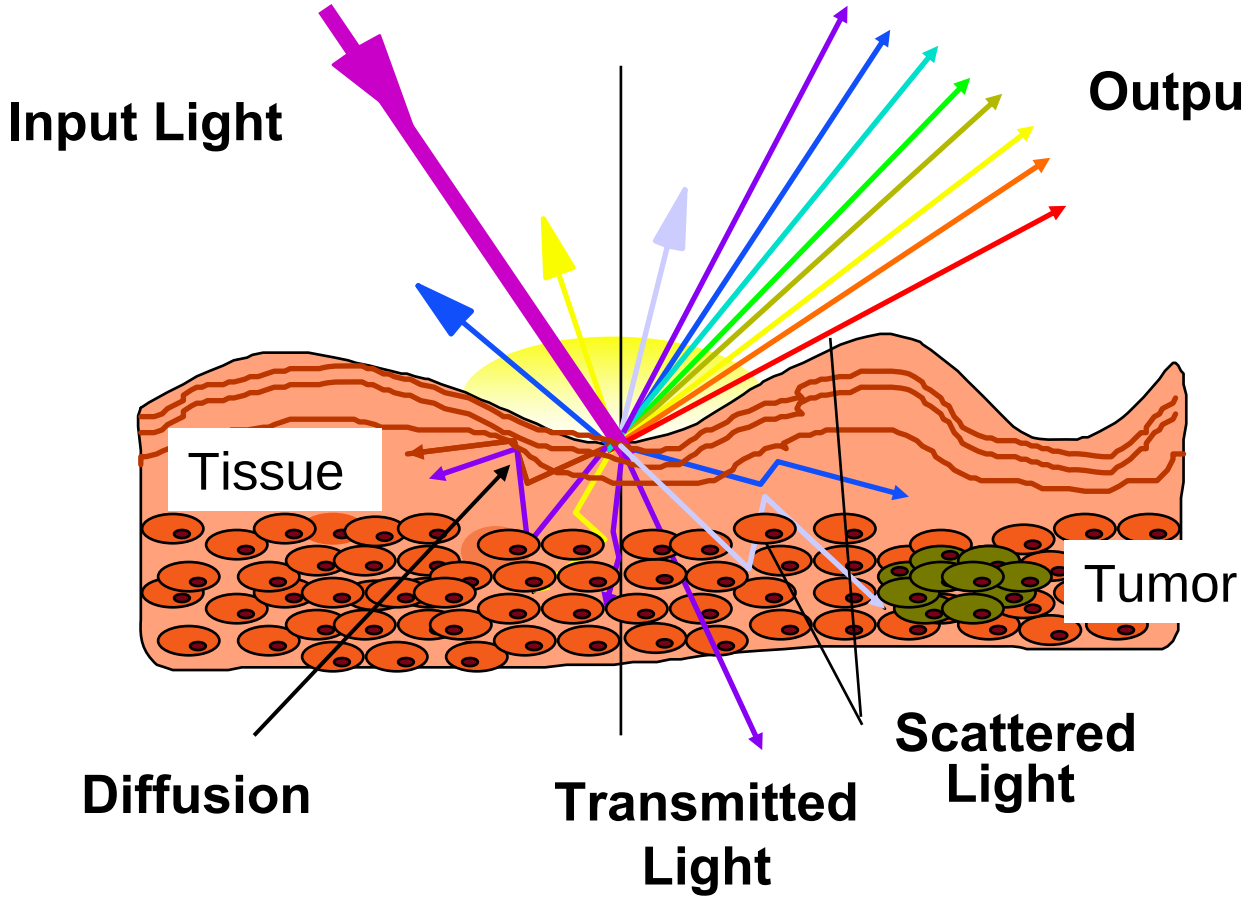
Processes – Interaction with matter

- Emitted – Fluorescence spectroscopy
- Absorbed – Excitation spectroscopy
- Scattered – Raman and elastic

Motivation

- Use optical spectroscopic techniques for noninvasive detection of Breast and other disease (Optical Biopsy)
  - Fluorescence
  - Stokes Shift
  - Raman
- Develop compact instrumentation and techniques, called Photonic Spectral Explorers (PSE), suitable for use in a clinical setting (surgical theater, doctor's office, civilian and military field deployment)

Optical Biopsy



Started in 1984, QE IEEE 24, 1507

Advantages of Optical Biopsy:

- **Noninvasive**
- **it is rapid -** **real time diagnosis: Image, component, composition changes**
- **it requires** ***no tissue removal***
- **it can be performed with**

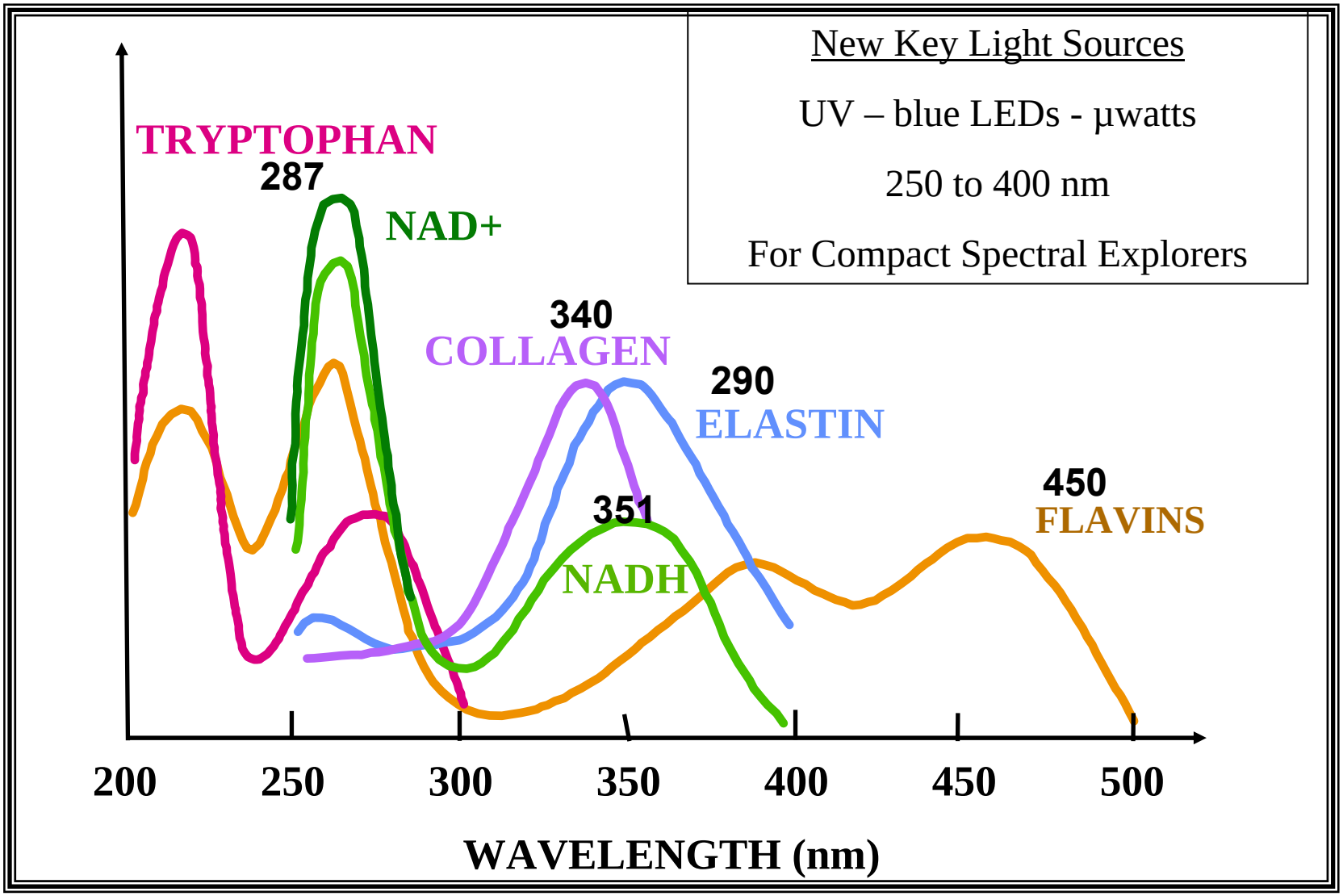
  endoscopes, needles, and fibers

Fluorescence Detection

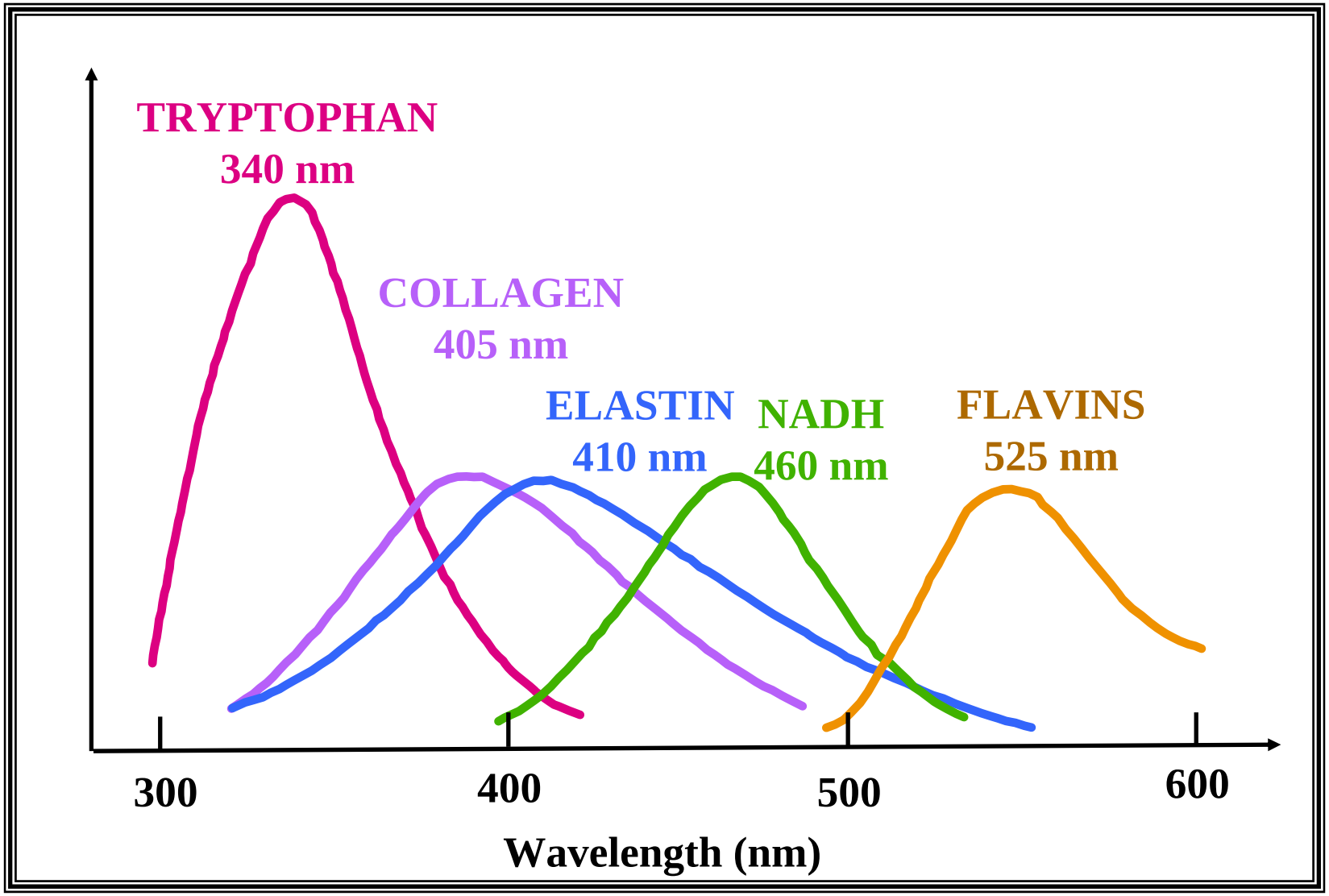
- Key native tissue fluorophores (no dyes)
  - Collagen
  - Elastin
  - Tryptophan
  - NADH
  - Flavins
  - Porphyrins

- Structural and content changes
  - Thickening of basal cell layer
  - Increased vascularity

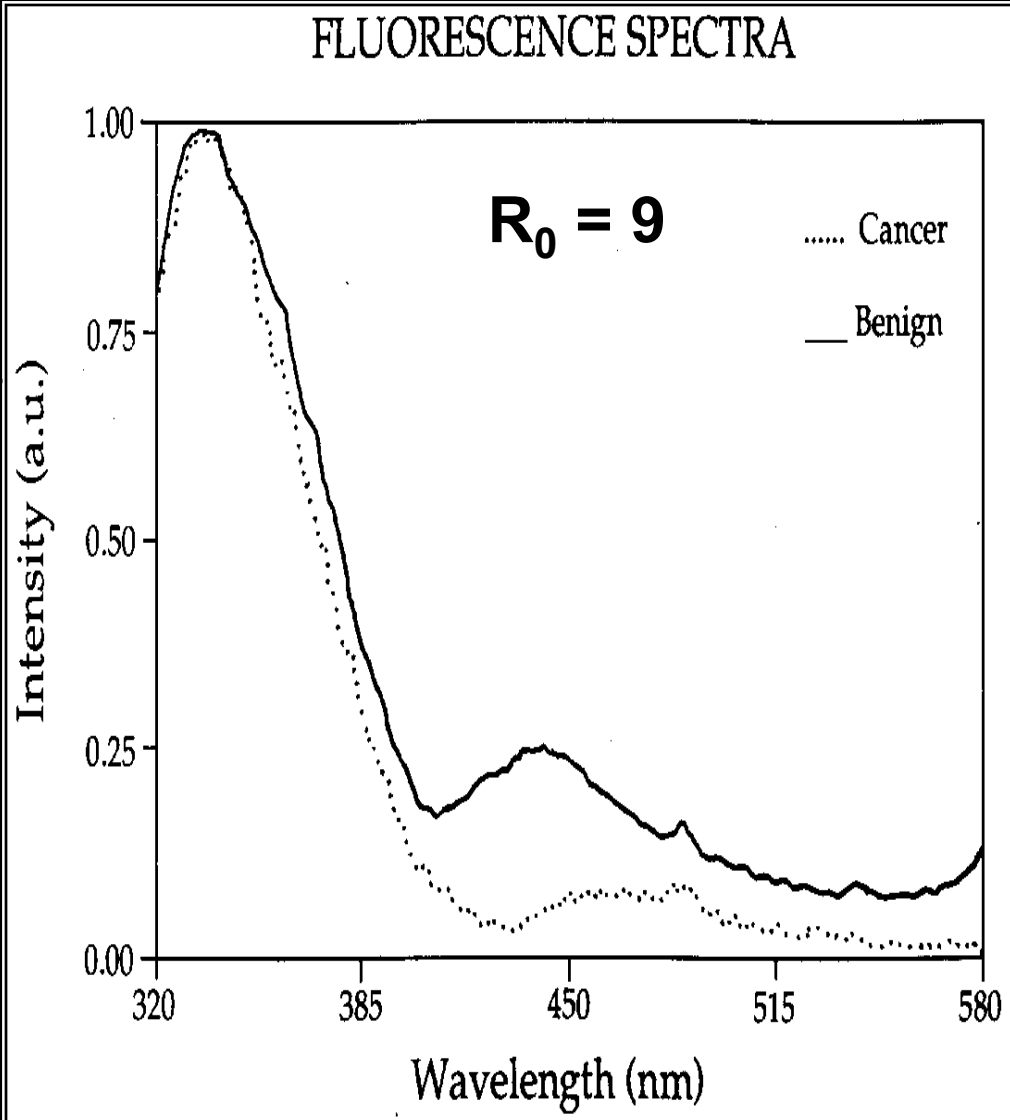
Key components in Absorption Spectra

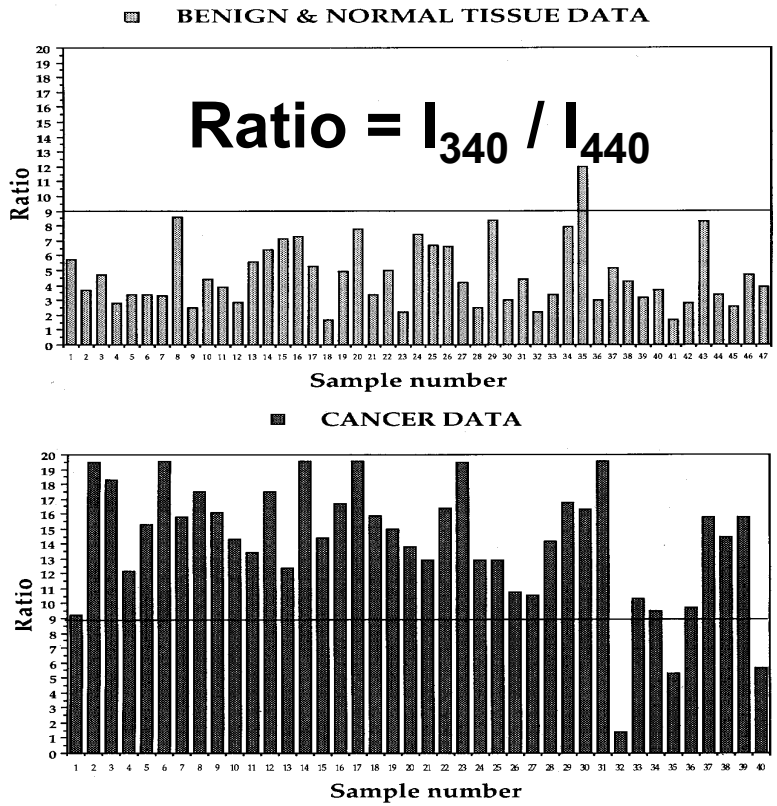


Key Components in Emission Spectra



Optical Biopsy (1) - Fluorescence Data





Parameter	Cancer	Normal or benign
No. of samples	40	47
Sensitivity	37/40 = 92.5%	-
False negative	3/40 = 7.5%	-
Specificity	-	46/47 = 98%
False positive	-	1/47 = 2%

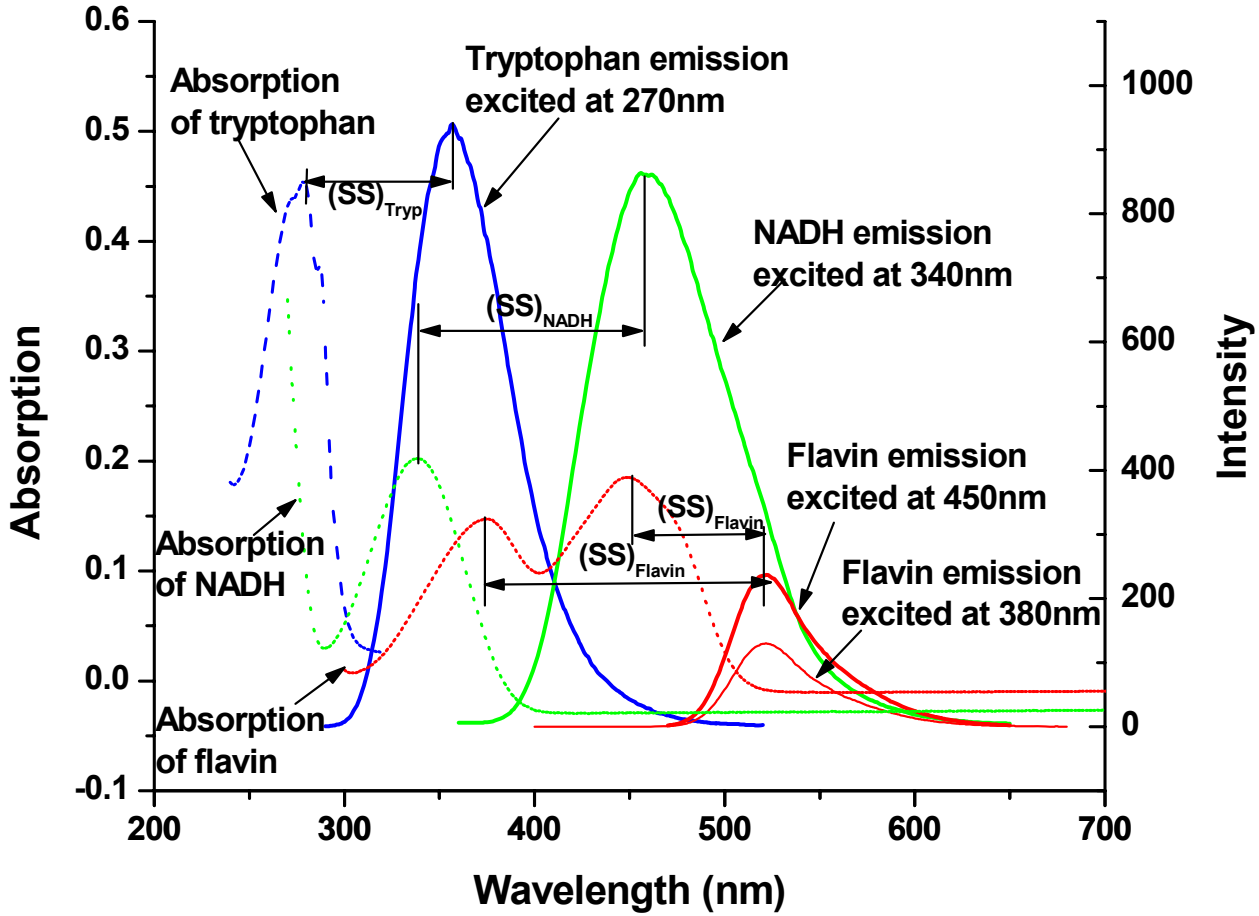
Sensitivity and Specificity

Tissue Type *(in vitro)*	Number of Samples	Pathology	Sensitivity	Specificity	Year
Breast	16	Cancer	87.5%		1988
	15	Normal		87%	
Breast	40	Cancer	92.5%		1988
	47	Benign and Normal		98%	
GYN	22	Malignant	95%		1992
	10	Non-Malignant		100%	
GYN	65	Cancer	97%		1994
	24	Normal		87.5%	
Colon	35	Cancer	94%		1995
	39	Normal		92%	
Breast	99	Cancer	90%		1995
	67	Normal		90%	

Sensitivity and Specificity
(Continued)

Tissue Type *(in vitro)*	Number of Samples	Pathology	Sensitivity	Specificity	Year
Breast	97	Cancer	95%		1996
	127	Normal		93%	
Breast	103	Cancer	90%		1998
	63	Normal		90%	
Esophagus (Barrett's)	31	Cancer	93%		1998
	33	Normal		93%	
Colon	11	Cancer	95%		1999
	11	Normal		95%	

Optical Biopsy (2) - Stokes Shift Spectral Technique

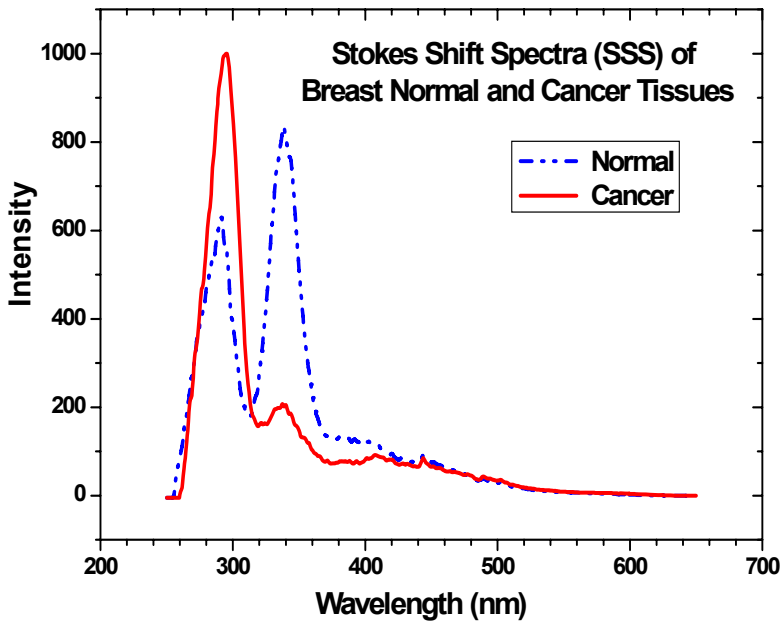


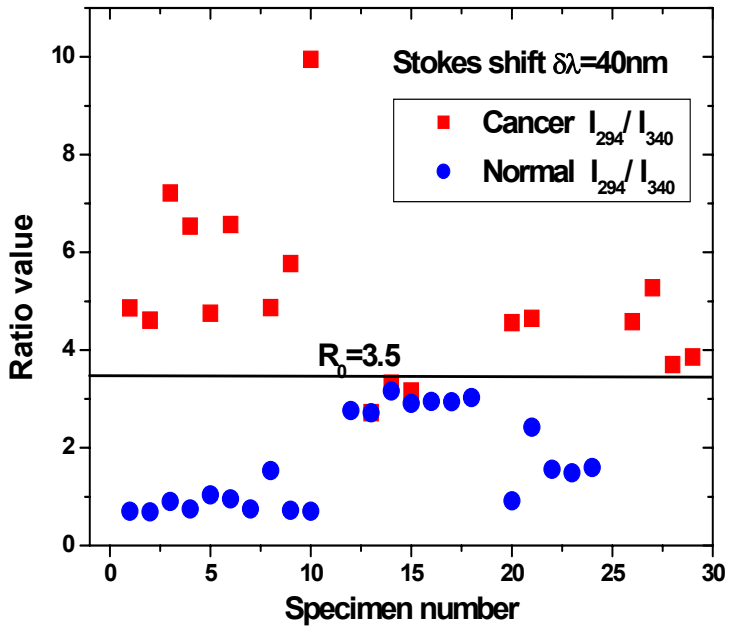
Optical Biopsy - Stokes Shift Spectral Data - 1

Absorption and Emission Peaks, the Stokes Shift of Key Biomolecules obtained from their Fluorescence and Absorption Spectra

Molecular	Absorption peak (nm)	Emission peak (nm)	Stokes shift (nm)
Tryptophan (T)	280	356	76
Collagen (C)	340	380	40
NADH (N)	340	460	120
Flavin (F)	375	520	145
	450	520	70

Optical Biopsy - Stokes Shift Spectral Data - 2

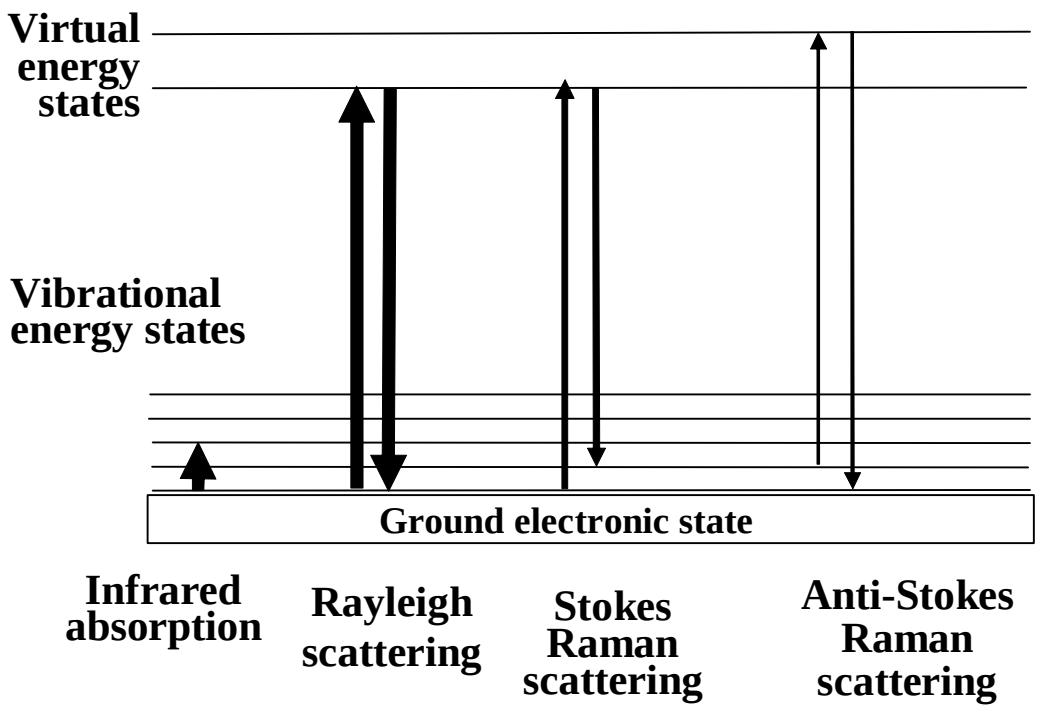




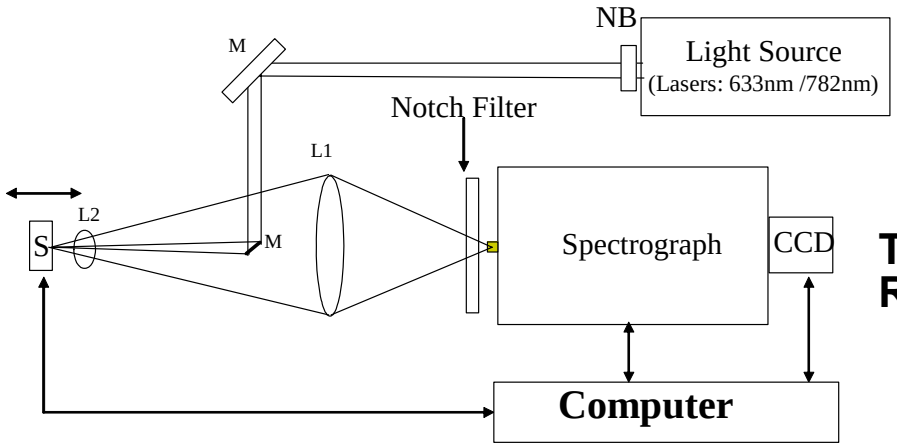
Optical Biopsy – (3)
Raman spectral technique

- The Raman bands are more sharper in biological macromolecules
- Noninvasive, and independent of the size or shape of tissue
- Raman spectra can be obtained for components in various phases: crystal, powder, aqueous solution, gel forms with sub mm accuracy
- Raman frequencies arise from changes in the electronic polarizability associated with nuclear vibrational displacements
- By analyzing the Raman intensity changes and frequency shift, one can exam the molecular properties of a compound
- it is rapid - real time diagnosis changes in structure and components with BBCA
- it can be performed with *endoscopes, needles, and fibers*

Energy level diagram showing the states involved in Raman signal



Raman Spectral System



The Experimental Setup used for Raman Spectral Measurements

The Raman Systems R-3000 and

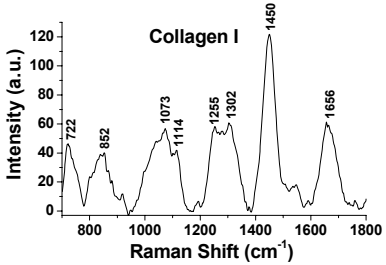
Fiber optic probes for Raman spectroscopy

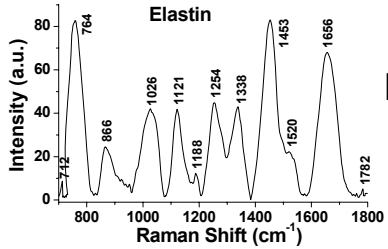
Raman Spectroscopy Detection key biochemical molecular changes

- Proteins: Collagen, Elastin
- Amino acids- Tyr. Tryp. Phen. Glycine
- Lipids- cholesterol, fat
- Enzymes & Coenzymes- NADH, Flavin
- Vitamins: Beta Carotene
- Structural and content changes:
  - Thickening of basal cell layer
  - Increased vascularity

Raman spectra of five key basis biochemical components collagen types I, III, V, elastin and cholesterol

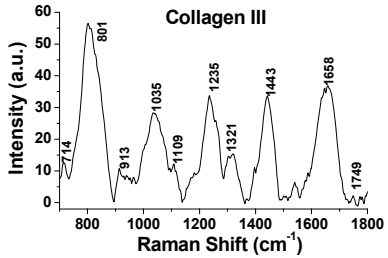
Collagen Type I

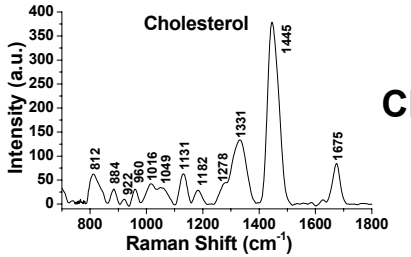




Elastin

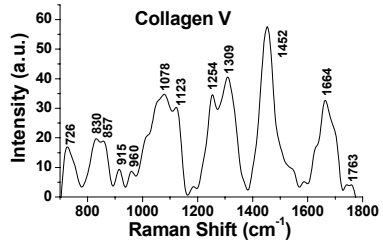
Collagen Type III



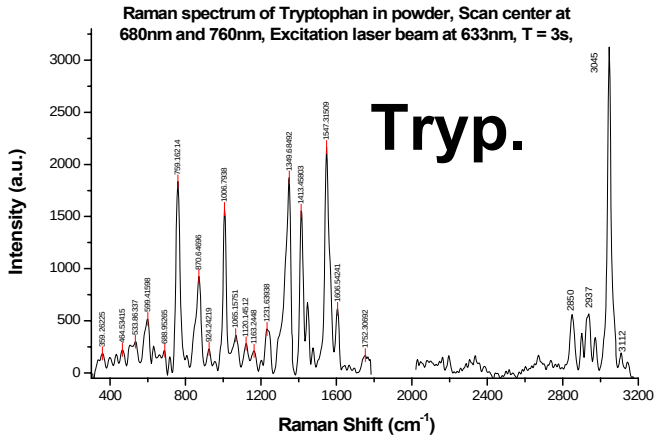


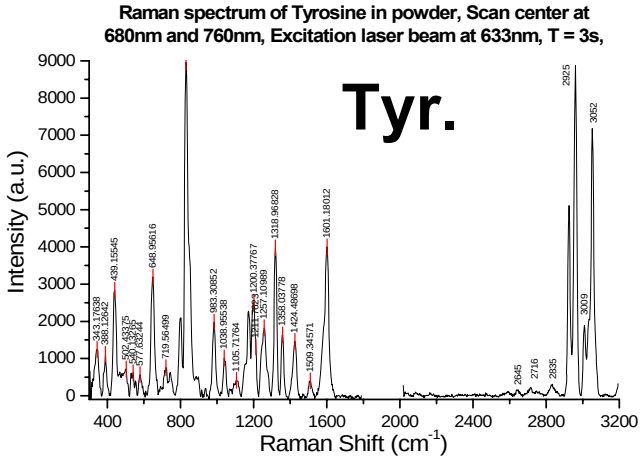
Cholesterol

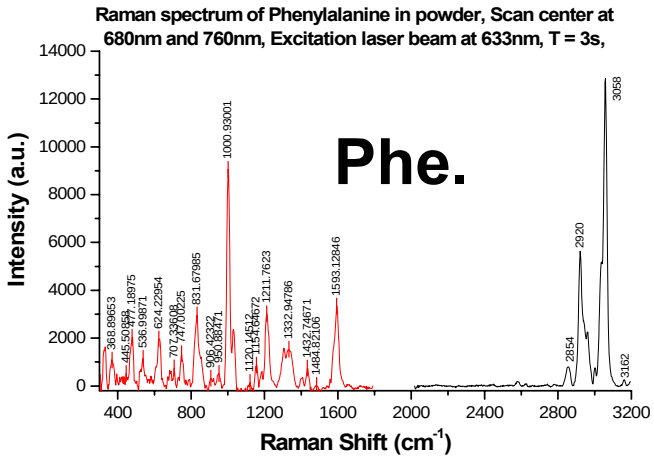
Collagen Type V

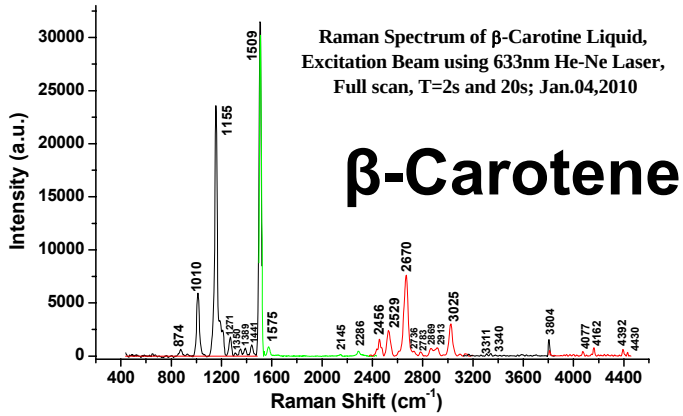


Raman spectra of key basis biochemical components Tyrosine, Tryptophan, Phenylalanine, β -Carotene

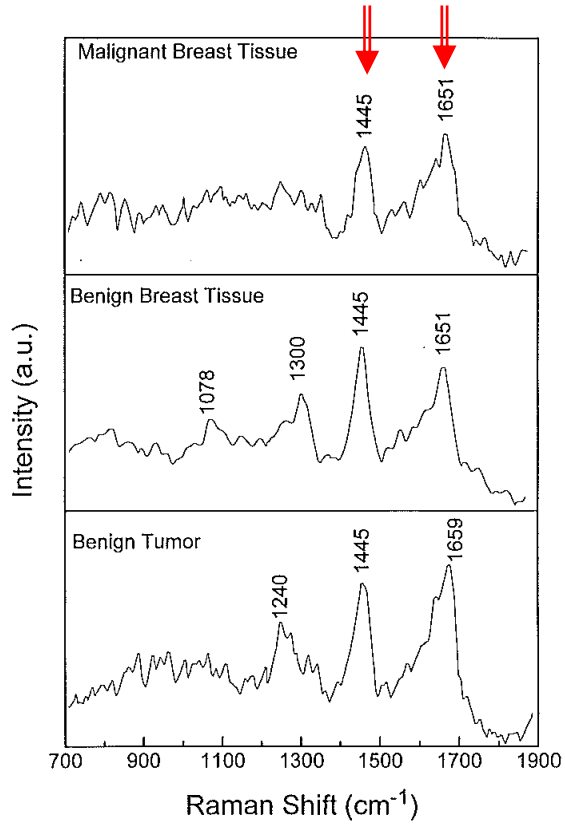








Raman Spectra of Breast Tissues (1)



FT Raman spectrum from breast malignant tumor tissue

FT Raman spectrum from benign breast tissue

FT Raman spectrum from breast benign tumor tissue

The first Raman spectroscopy study

Started in 1991, Lasers in Life Sciences 4(1), p23.

$I_{1445} < I_{1657}$ **- cancer**

Raman Spectra of Breast Tissues (2)

Differences in NIR-FT Raman spectra for benign, benign tumor and malignant tumor of breast tissue between 700cm-1 to 1900cm-1

Tissue	Benign tissue	Benign tumor	Malignant tissue	Tentative assignment
Raman frequency	1651	1659	1651	Protein: Amide I, Collagen and Lipid, C=O stretching;C=C stretching from hydrocarbon region
	1445	1445	1445	Protein/Lipid, CH_2 scissoring deformation
	1300-1240			Protein/Lipid, CH_2,CH_3, twisting
		1240		Protein: Amide III
	1079			Lipid: C-C stretch
Relative intensity	$I_{1445}>I_{1651}I_{1079}<$ $I_{1300}<I_{1445}<I_{1651}$	$I_{1445}<I_{1659}$	$I_{1445}<I_{1651}$	
Intensity ratio	1.25 (0.09)	0.93 (0.03)	0.87 (0.05)	

Raman Spectroscopy of GYN Tissues

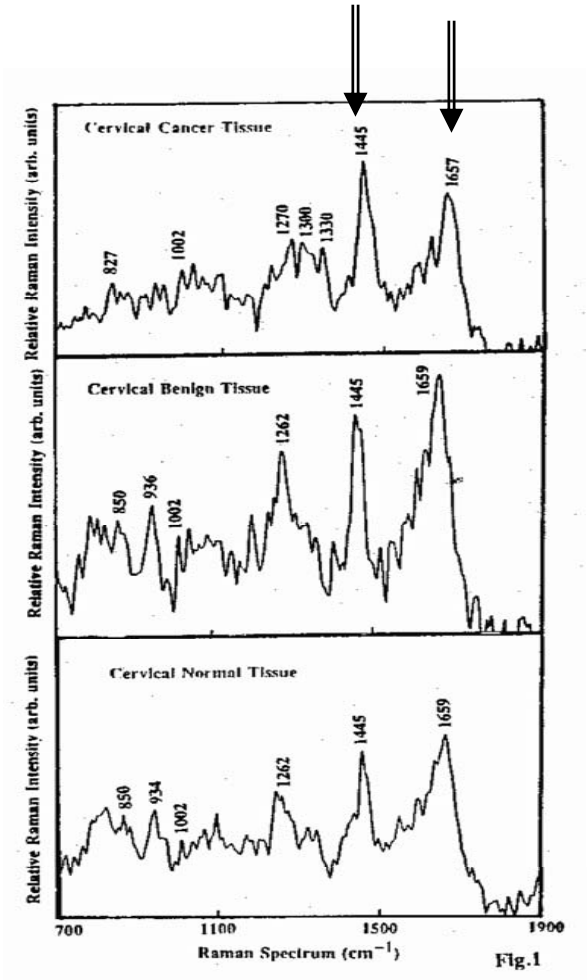


Fig.1

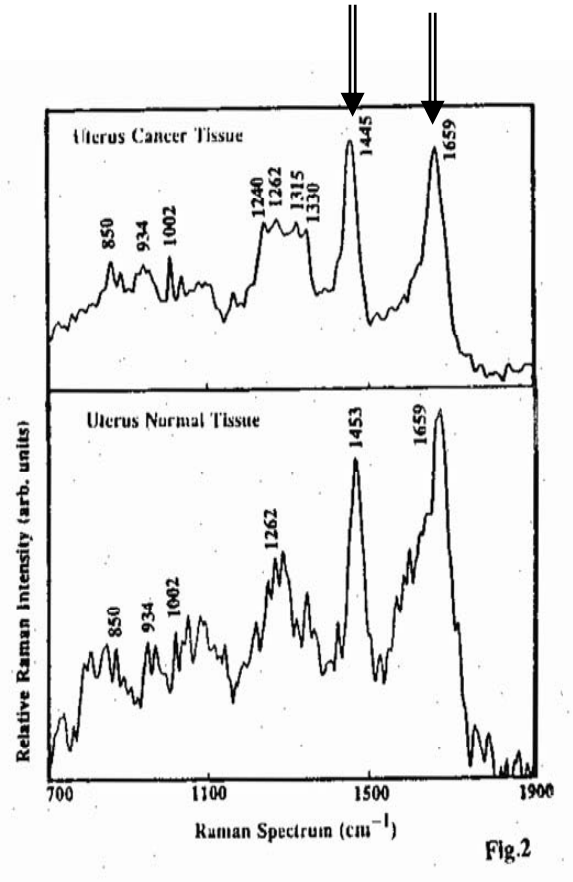


Fig.2

$I_{1445} > I_{1657}$ - cancer

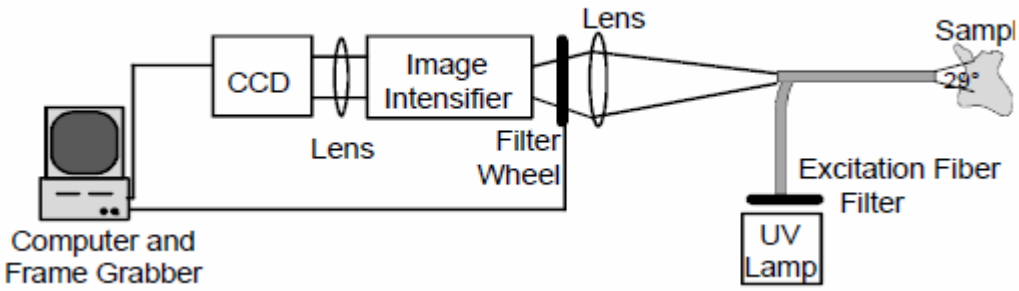
Raman Spectroscopy of GYN Tissues

TABLE: NIR-FT Raman Spectra for Gynecological Tract Tissues

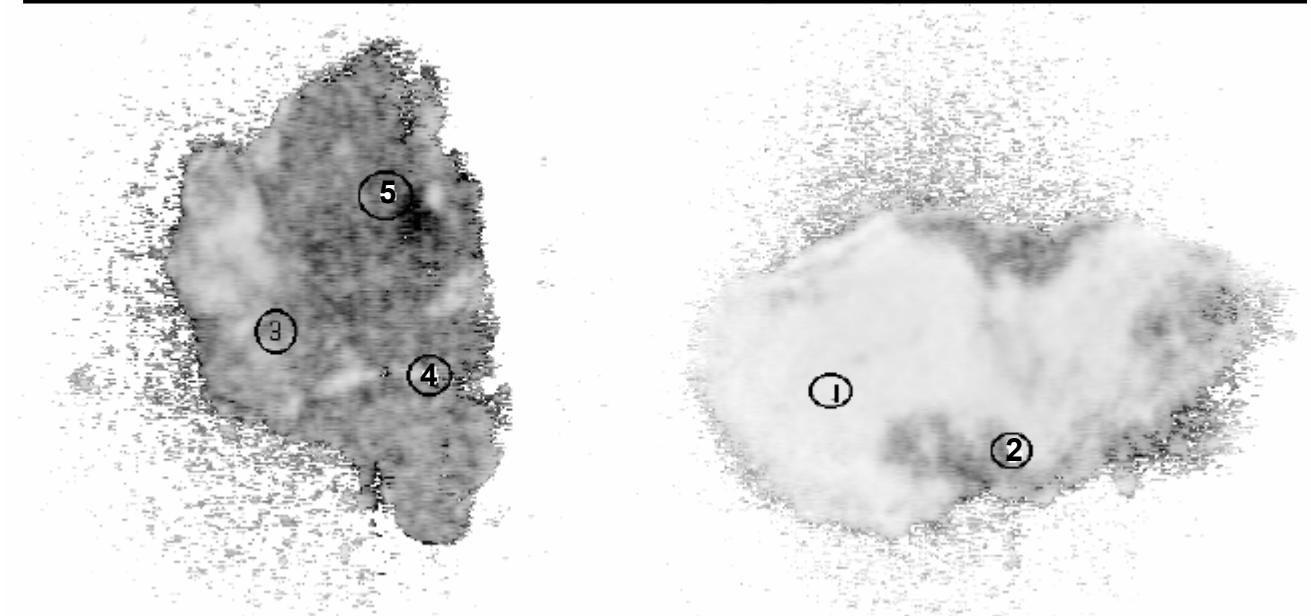
Tissues	Cancer		Benign or Normal		Assignment
	Cervix	Uterus	Cervix	Uterus	
Test No.	6	9	3	7	Amide I C=O Stretching
Raman Frequency	1657 VS	1659 VS	1659 VS	1659 VS	
	1445 VS	1445 VS	1445 VS	1453 VS	$\delta_{(CH)2}$, $\delta_{(CH)3}$ Bending
	1270 -- W 1330 W	1270 -- W 1330 W	1262 S	1262 VS	Amide III C-N Open Stretching ν (C-C) Stretching Tryptophane Mode
Relative Intensity	$I_{1657} < I_{1445}$		$I_{1659} > I_{1445}$		

1657 = Amide I; C=O, C-N, NH, collagen
1445 = CH_2, CH_3
1270 = Amide 3, C-N, collagen
1326 = elastin

Optical Biopsy
3rd Generation of the CD-Map (3)

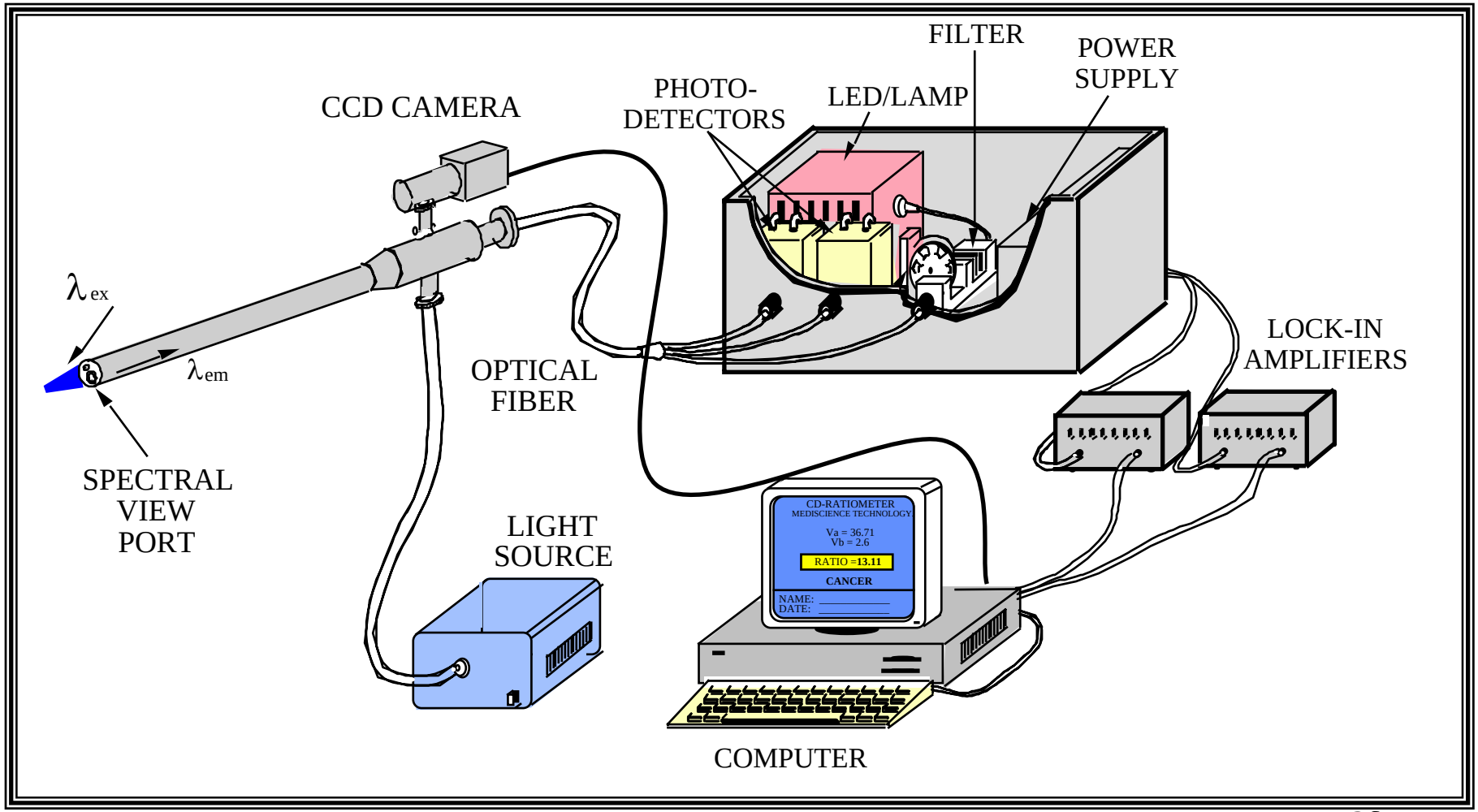


Fluorescence ratio mapping system in schematically



Malignant Breast Tissue **Normal Breast Tissue**

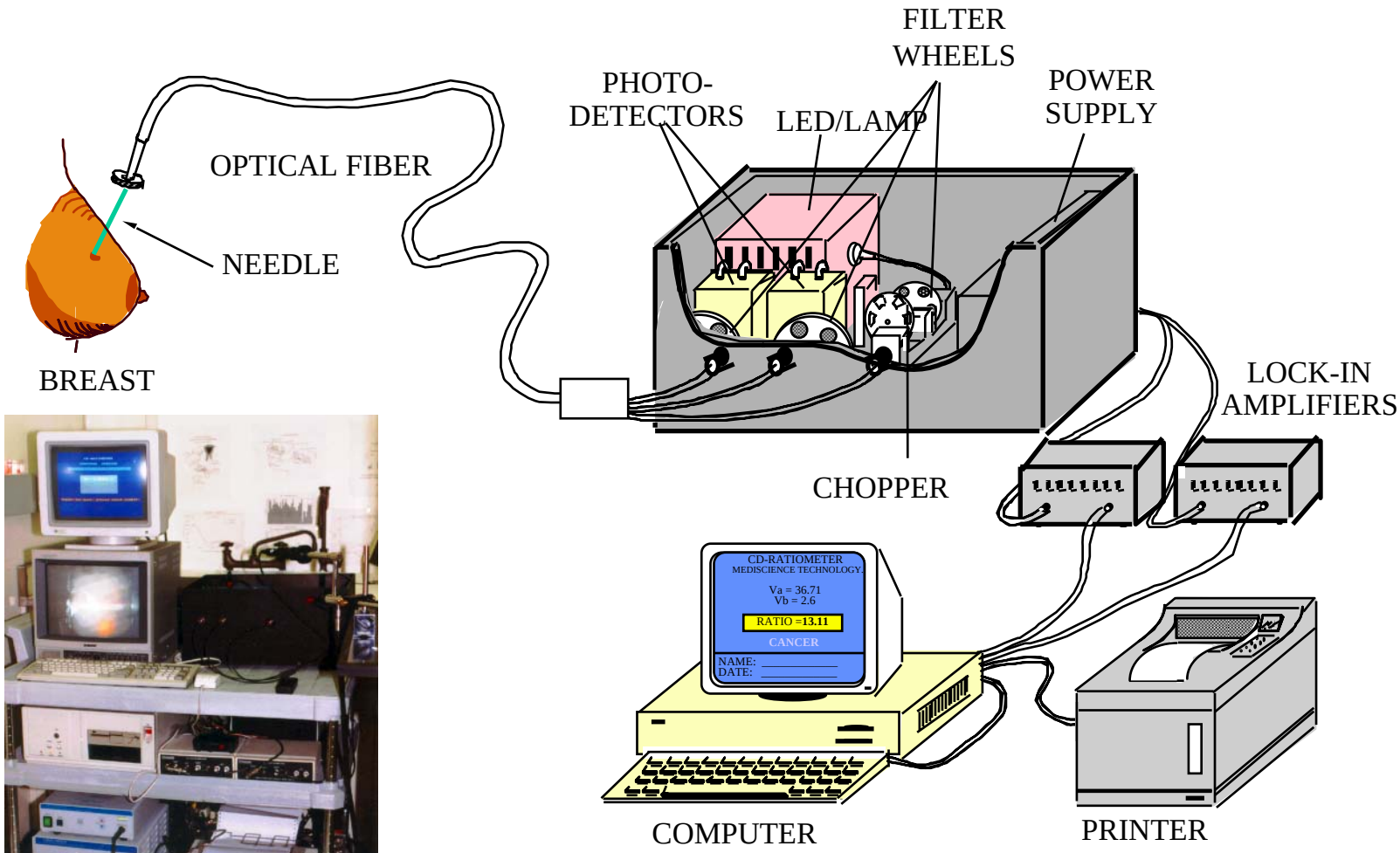
Optical Biopsy - 3rd Generation (PSE)
Compact Photonic Spectral Explorer - Ratiometer



PSE - Ratiometer

- Dual Channel Detection
- Excitation and Emission Ratios – Fast Data Collection
- Multiple Wavelength Combination
- High UV Sensitivity
- Probe Integrates into Endoscope or Laparoscope
- Optical Needle Probe

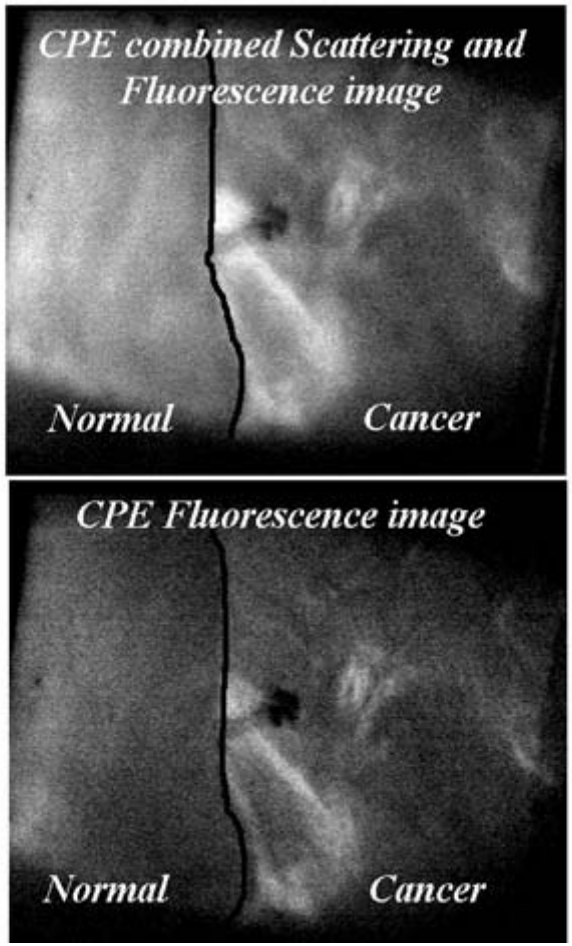
PSE - Ratiometer - Needle Version



U.S. Patent 5,983,125

Compact Spectral Photonic Explorer (MAP)

Cancerous Breast Tissue Fluorescence Imaging



Comparison of Fluorescence Imaging, using the CSPE, between normal and cancerous breast tissue section

LED Excitation: 365nm

LP400 nm filter

It is safe!

Photonic Explorers Safe from UV

For skin change = 1/3 MED ~ 10 mJ/cm^2

Photonic Explorer units operate at 333 µJ/cm^2 at 300 nm (30 times less than threshold for skin change ~ 10 mJ/cm^2)

(UVC) Setlow weighted – action spectra for DNA damage

(Proc. National Academy 71, 3363 (1974))

(UVB) Diffey Weight – action spectra weight for erythemal

(Human Exposure, Bosnajakovic, editor 1987, p. 83 -)

(UVA) – melanoma (surprise in fish)

Examples of Photonic Explorers Safety Light Levels

Lamp, LED: 2.4 µW (300 nm), 114 µW/cm^2 (spot size 2.1 mm^2)

Weighted Setlow 3.8 µW/cm^2

Weighted Diffey 7.4 µW/cm^2

ACGIH spectral 20 µW/cm^2

PSE (20 sec operation exposure):

Weight patient exposure

76 µJ/cm^2 (Setlow)

1480 µJ/cm^2 (Diffey)

400 µJ/cm^2 (ACGIH)

Taking maximum permissible exposure limit over 8 hours:

~3 mJ/cm^2 – 10 mJ/cm^2

Then PSE energy/cm^2 exposure is safer by

33 times (Setlow) DNA

2 times (Diffey) Erythema

7 times (ACGIH)

Conclusion

- Fluorescence and Raman spectroscopy can be an important tool for detection of cancer and pre-cancer.
- High sensitivity and specificity (87%-95%) are obtainable in wide spectral region- from ultraviolet to infrared .
- Algorithms based on intensity ratios can reduce data collection time in clinical applications.
- A variety of fiber optic probes can be used to access multiple organ sites.
- Is it safe at μW levels / cm^2? Yes

Breast Cancer Probed by Fluorescence and Raman Spectroscopy

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