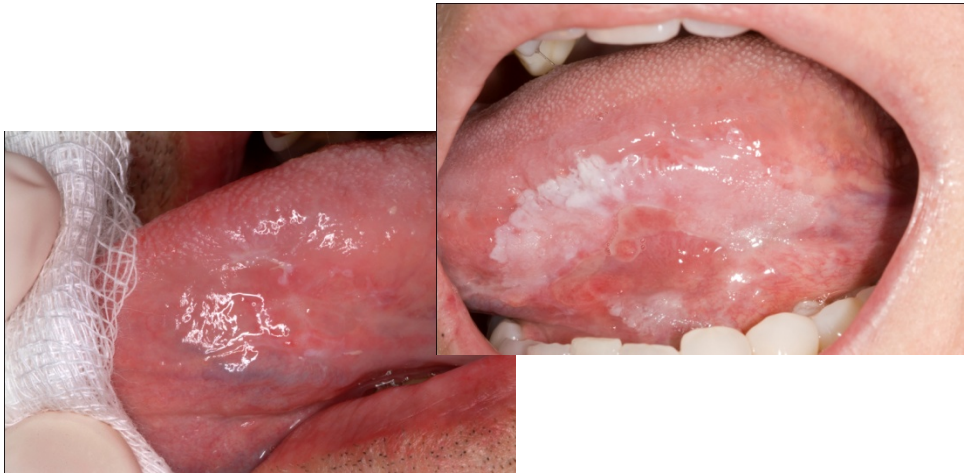
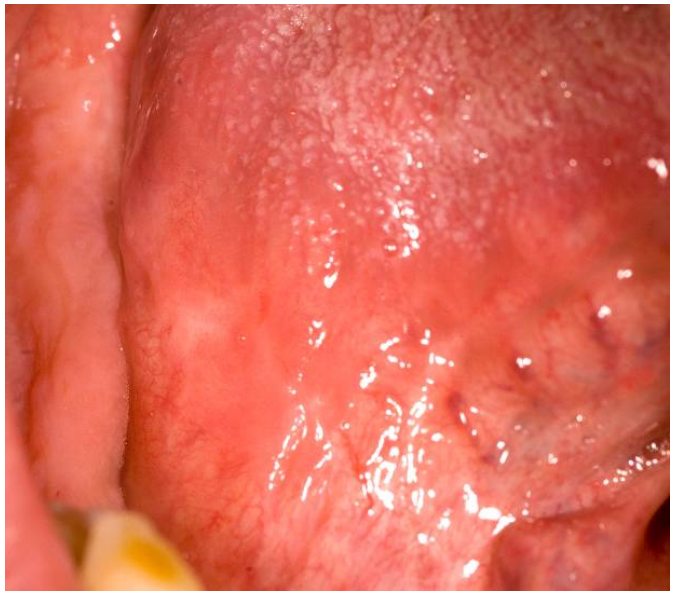
Técnicas fotônicas para diagnóstico - espectroscopia

Diagnóstico de lesões

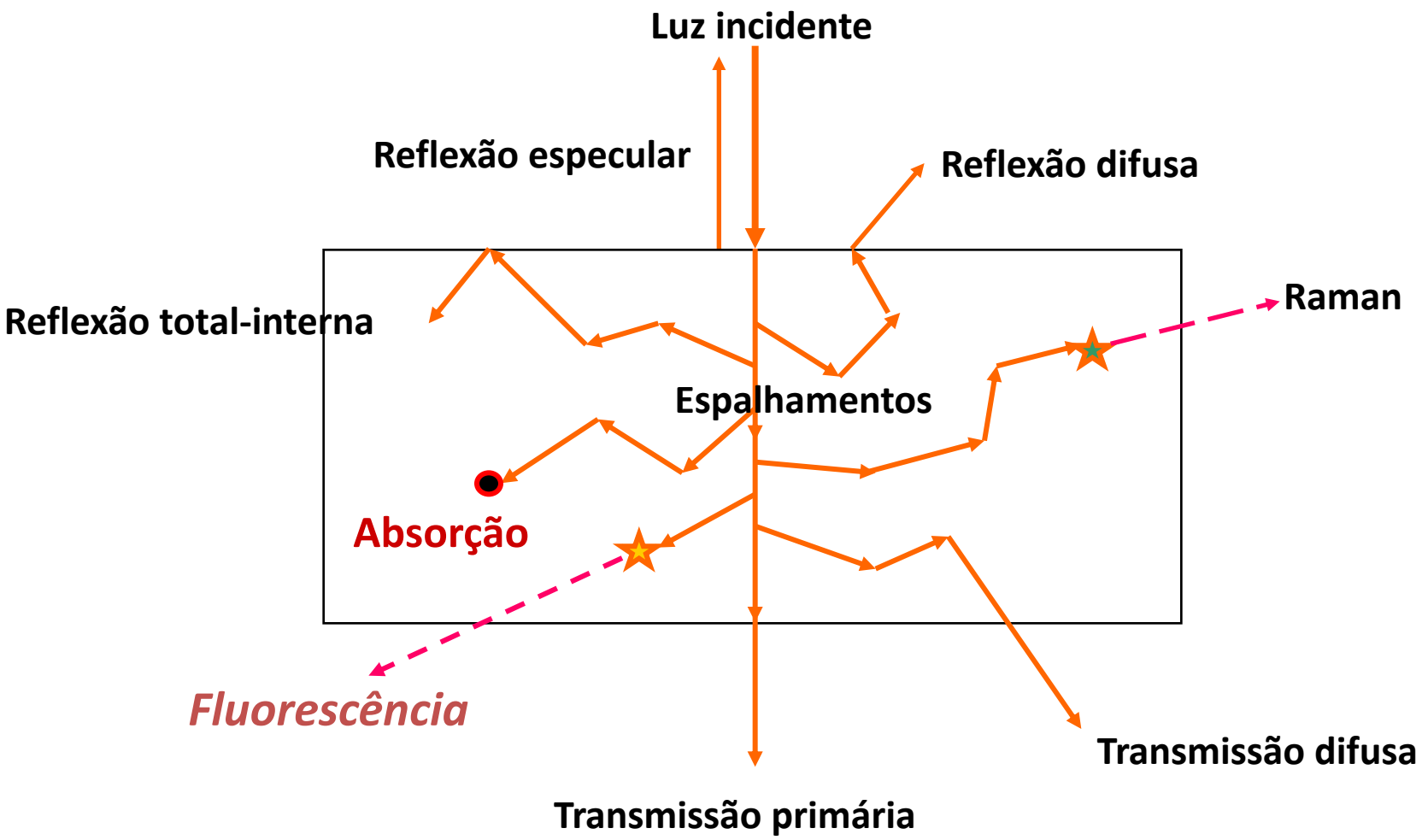
- Anamnese
- Exame clínico
- Biópsia convencional
- Avaliação histopatológica

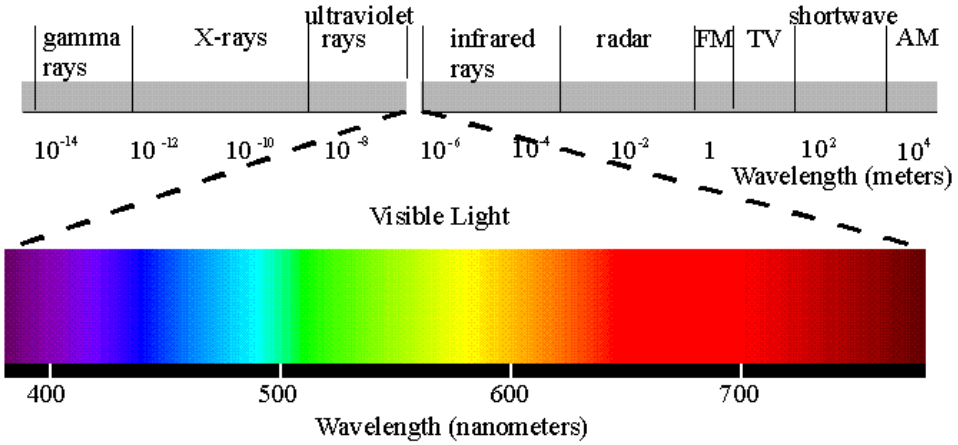


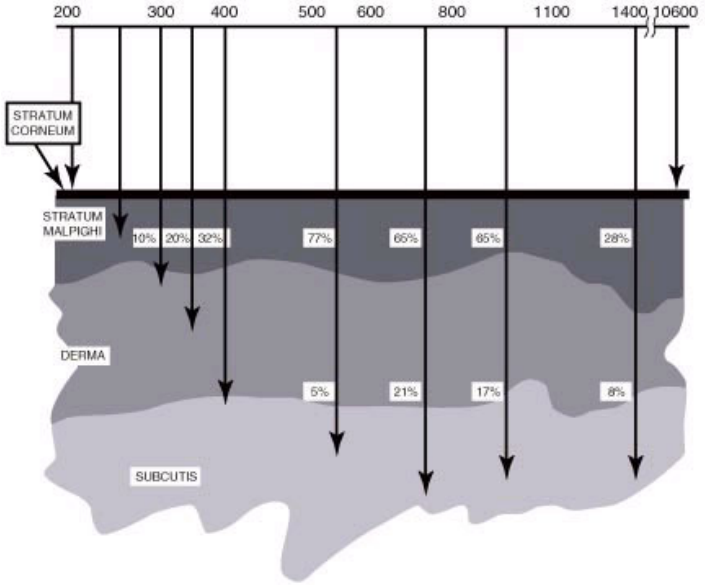
Diagnóstico óptico

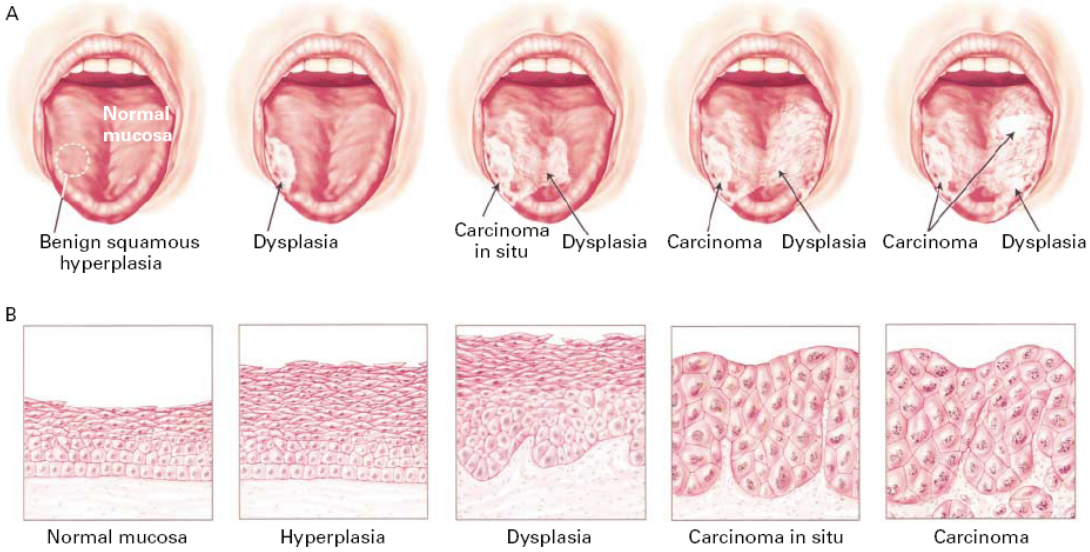


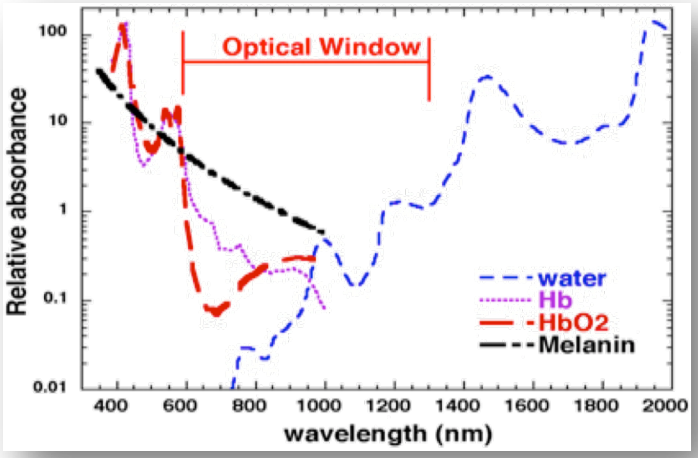
Interação da luz com a matéria

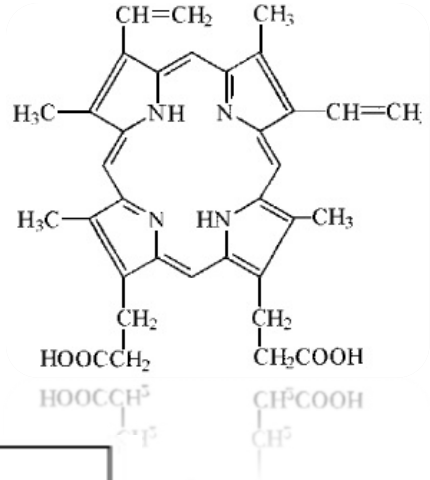


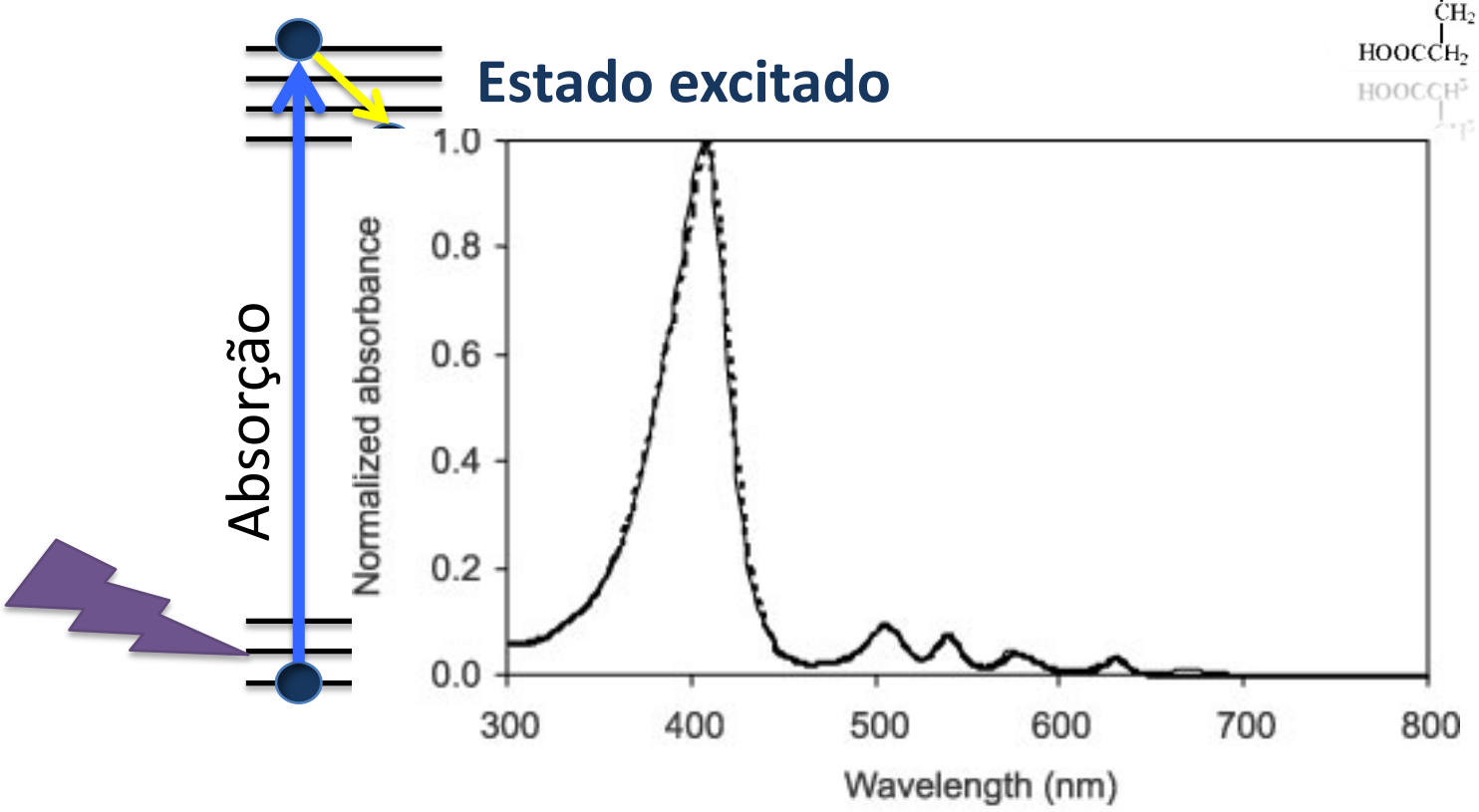

gamma rays
X-rays
ultraviolet rays
infrared rays
radar
FM
TV
shortwave
AM
10^{-14} 10^{-12} 10^{-10} 10^{-8} 10^{-6} 10^{-4} 10^{-2} 1 10^{2} 10^{4}
Wavelength (meters)
Visible Light
400 500 600 700
Wavelength (nanometers)

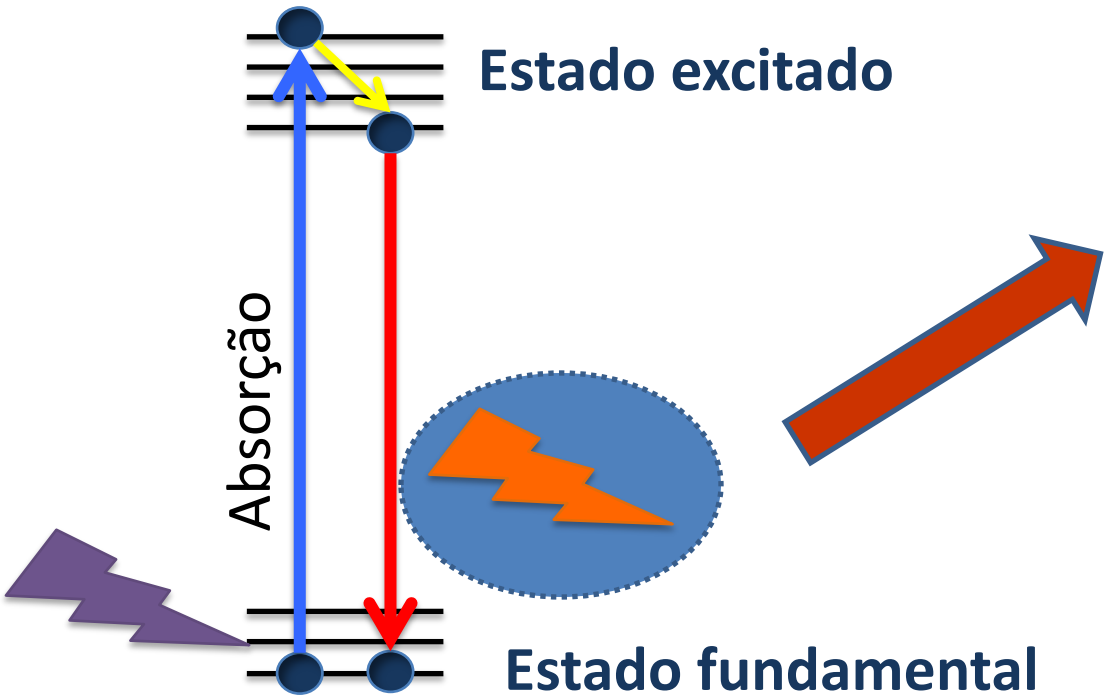

200 300 400 500 600 800 1100 1400 10600
STRATUM CORNEUM
STRATUM MALPIGHI
10% 20% 32% 77% 65% 65% 28%
DERMA
5% 21% 17% 8%
SUBCUTIS

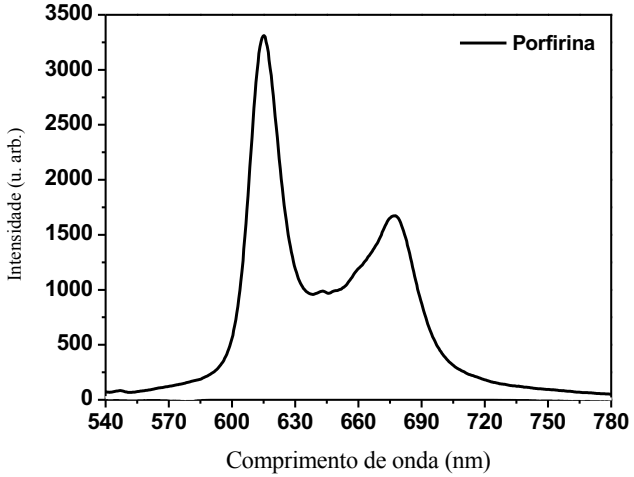

A
Normal mucosa
Benign squamous hyperplasia
Dysplasia
Carcinoma in situ
Dysplasia
Carcinoma
Dysplasia
Carcinoma
Dysplasia
B
Normal mucosa
Hyperplasia
Dysplasia
Carcinoma in situ
Carcinoma


Optical Window
Relative absorbance
100
10
1
0.1
0.01
400 600 800 1000 1200 1400 1600 1800 2000
wavelength (nm)
water
Hb
HbO2
Melanin


CH=CH2
CH3
H3C
NH
N
CH=CH2
H3C
N
HN
CH3
CH2
CH2
HOOCCH2
CH2COOH


Estado excitado
Absorção
Normalized absorbance
1.0
0.8
0.6
0.4
0.2
0.0
300
400
500
600
700
800
Wavelength (nm)

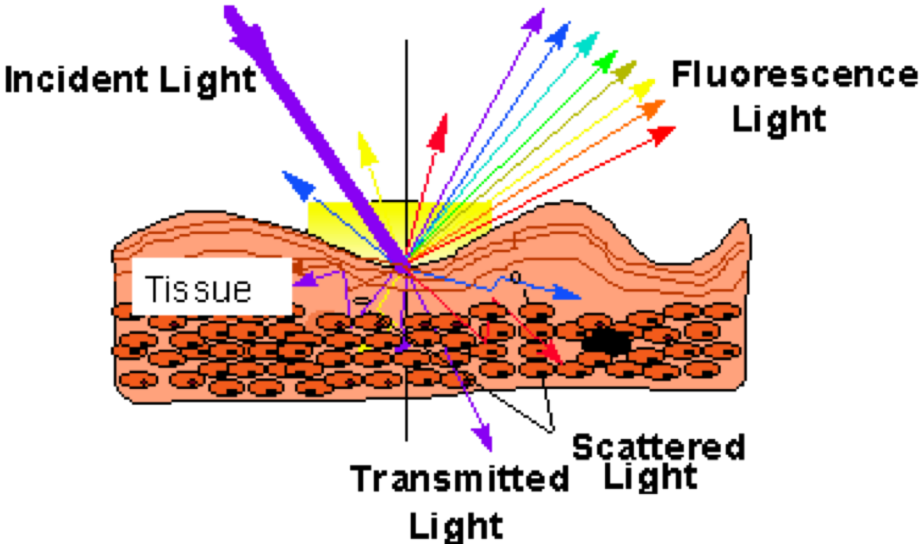

Estado excitado
Absorção
Estado fundamental

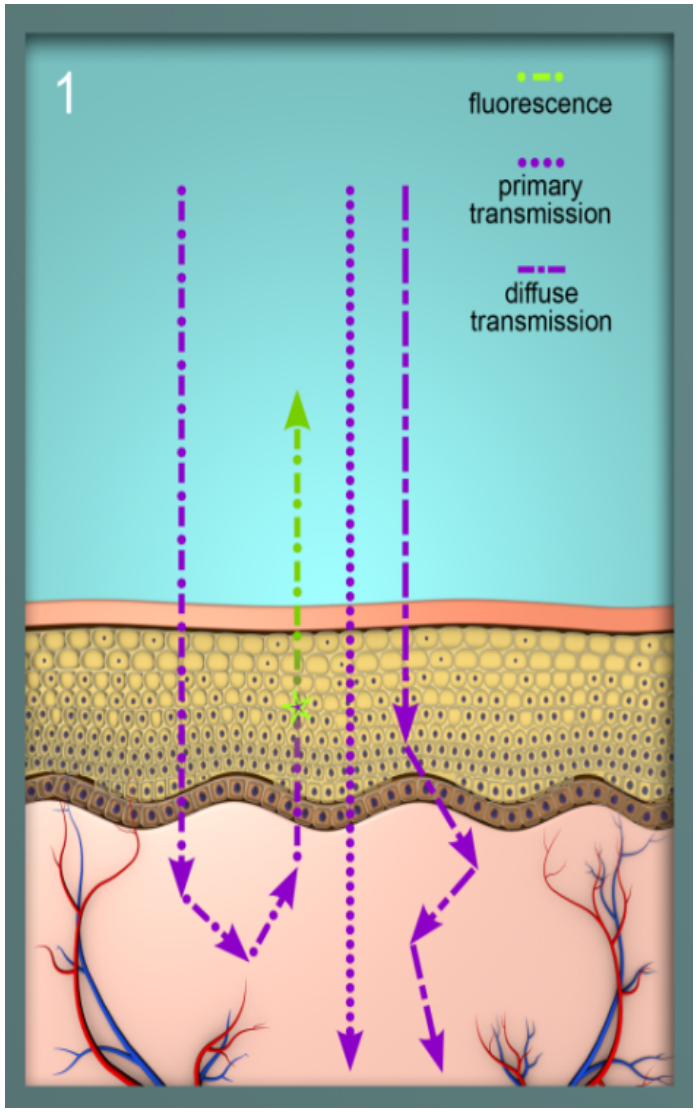

Porfirina
Intensidade (u. arb.)
3500
3000
2500
2000
1500
1000
500
0
540
570
600
630
660
690
720
750
780
Comprimento de onda (nm)

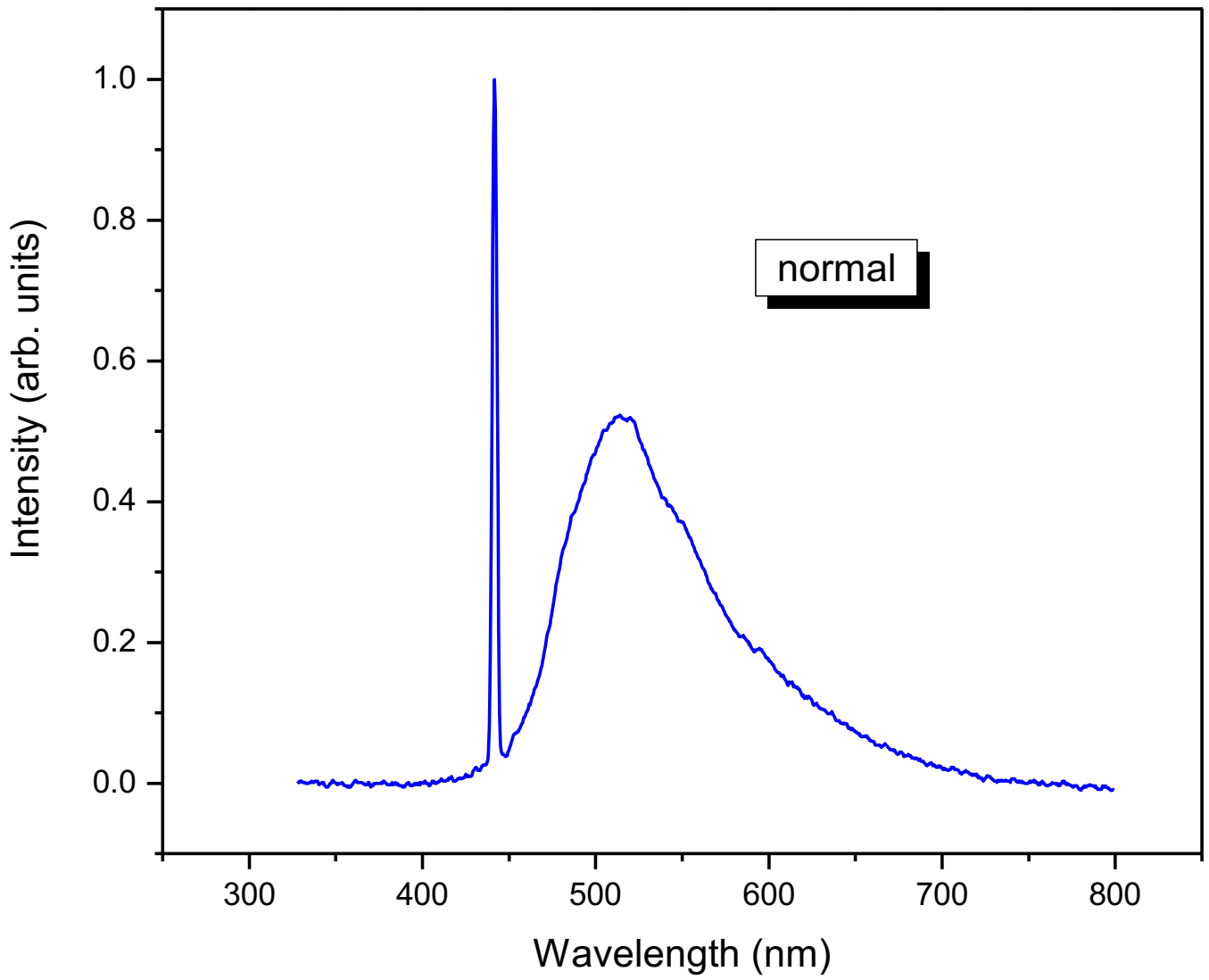
FLUOROPHORES	BIOMOLECULES / CELL LOCALIZATION	EXCITATION Peak position range	EMISSION Peak position range
Aromatic AminoAcid Residues	Proteins A= Phe+Tyr; B= Trp+Phe+Tyr	240 – 280 nm	280 – 350 nm
Collagen Elastin Cytokeratins	Extracellular matrix Connective tissue Epithelia	330 – 340 nm 350, 420 nm 280, 325 nm	400 - 410 nm 420, 510 nm 495, 525 nm
Reduced Pyridine Nucleotides	NAD(P)H (Cofactors in metabolism) mitochondria / cytoplasm	330 – 380 nm	440 nm (bound) 462 nm (free)
Flavins Flavin Nucleotides	Riboflavin, FMN, FAD (Coenzymes of Flavoproteins) mitochondria / cytoplasm	350 – 370 nm 440 – 450 nm	480 – 540 nm
Porphyrins (Zinc-Protoporphyrin)	Prosthetic group of proteins Hemoglobin Myoglobin Cytochrome Erythroid cells	405 nm 500 – 600 nm	630, 670 nm
Lipofuscins Lipopigments	Pigments (cell catabolism / cell age) cytoplasm	UV 400 – 500 nm	> 540 nm
Vitamins	Vitamin A Vitamin B6 & Precurors	370 – 380 nm 290 – 310 nm / 375 – 395 nm	490 – 510 nm 375 – 395 nm / 400 – 500 nm
Lipids	Arachidonic Acid Phospholipids	330, 350 nm 430 - 440 nm	470 – 480 nm 520 – 570 nm
Catecholamines Serotonin	Neurotransmitters	280 – 290 nm 305, 360 (dimer), 420 (trimer) nm	320 – 340 nm 350, 440 (dimer), 520 (trimer) nm

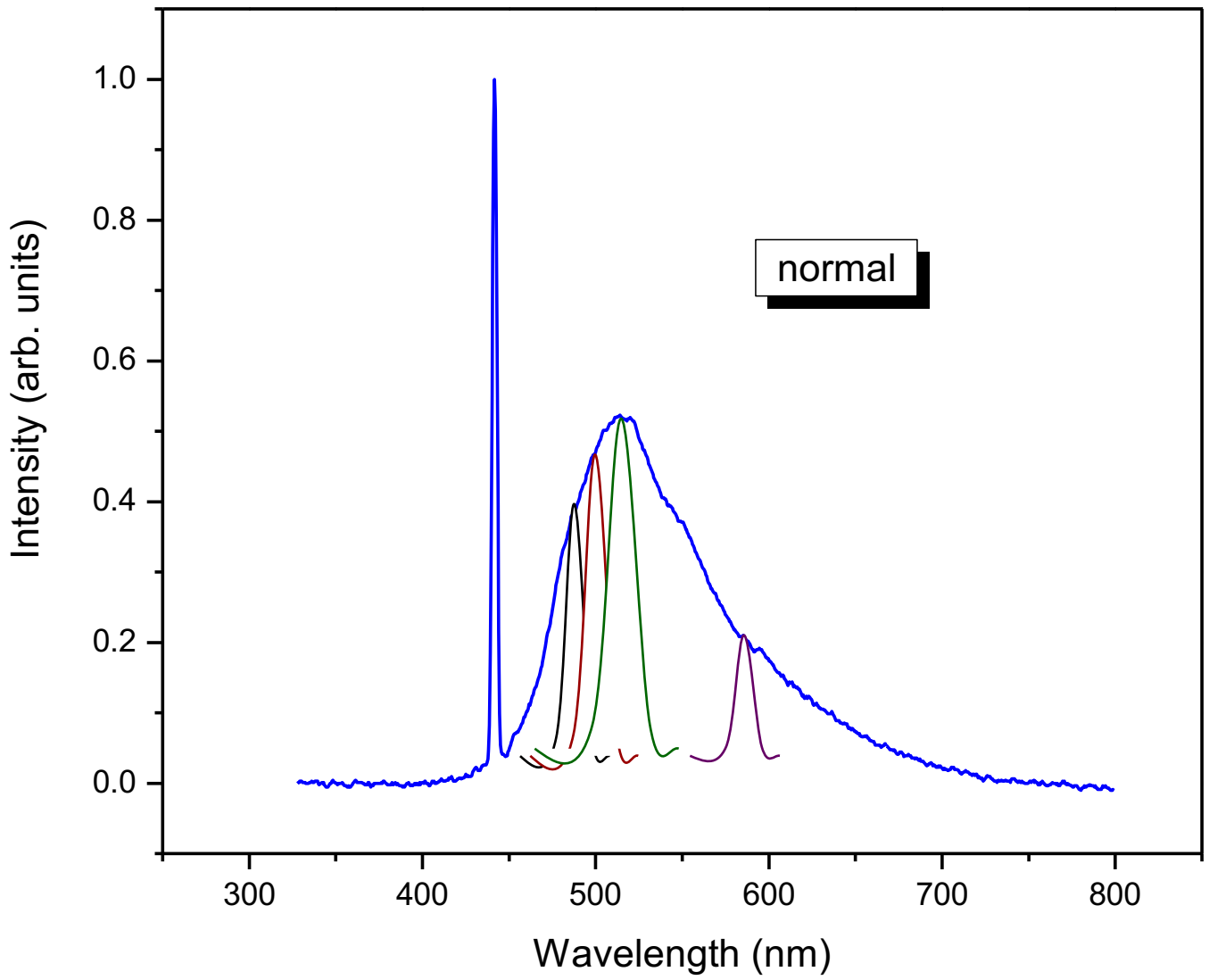
G. Bottiroli and AC. Croce – The autofluorescence spectroscopy of cells and tissues as a tool for biomedical diagnosis. In: *Lasers and current optical techniques in biology*. G. Palumbo and R. Pratesi Eds. Comprehensive Series in Photosciences, vol. 5. The Royal Chemical Society, London. 2004.

Diagnóstico Óptico






Intensity (arb. units)
1.0
0.8
0.6
0.4
0.2
0.0
normal
300
400
500
600
700
800
Wavelength (nm)


normal
Intensity (arb. units)
Wavelength (nm)
0.0
0.2
0.4
0.6
0.8
1.0
300
400
500
600
700
800

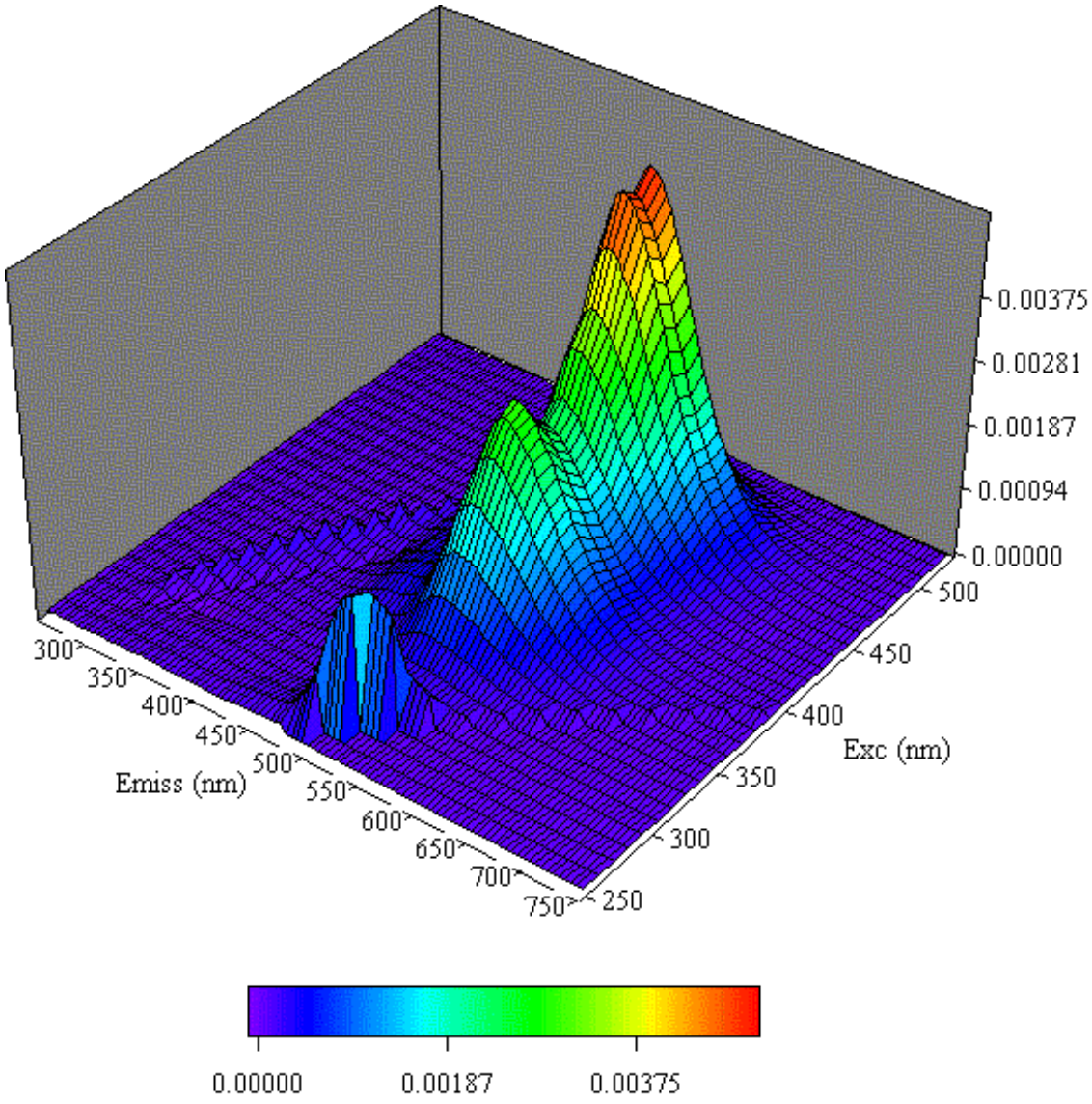
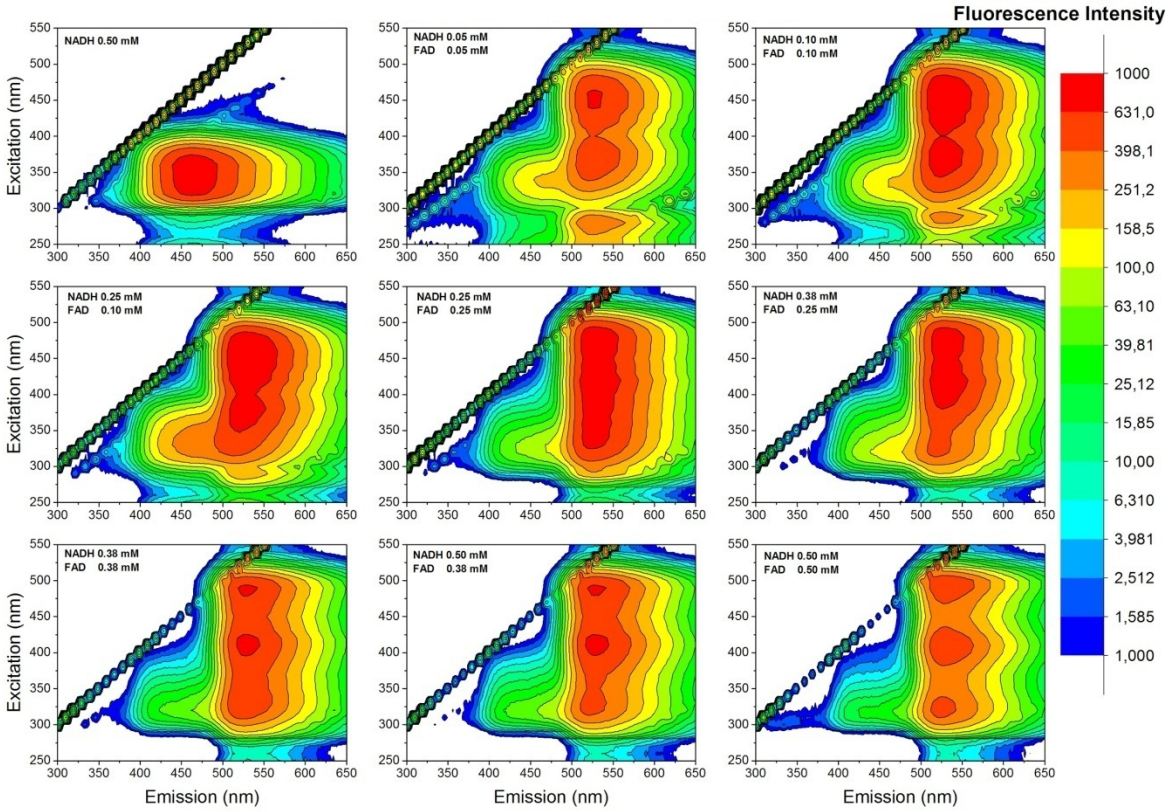
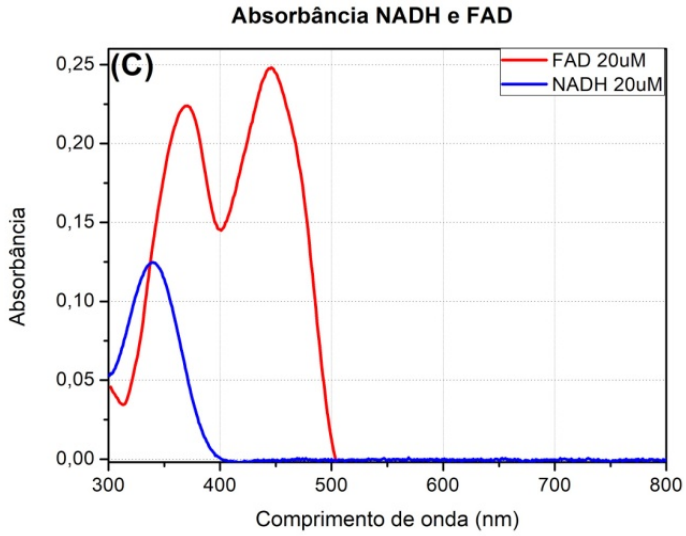
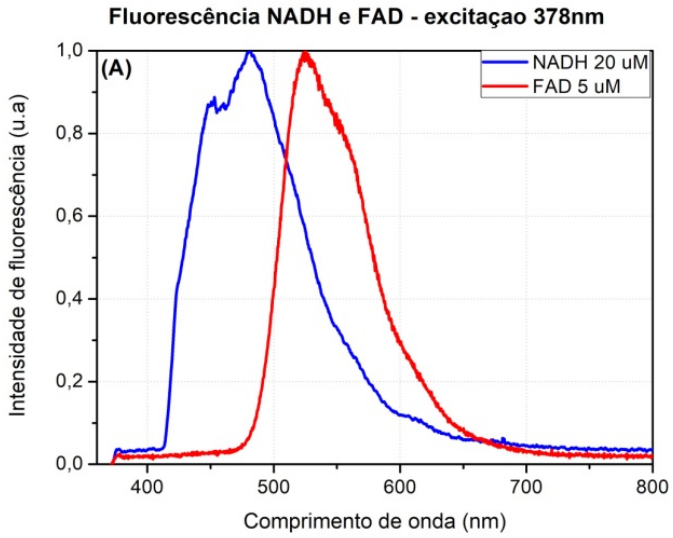


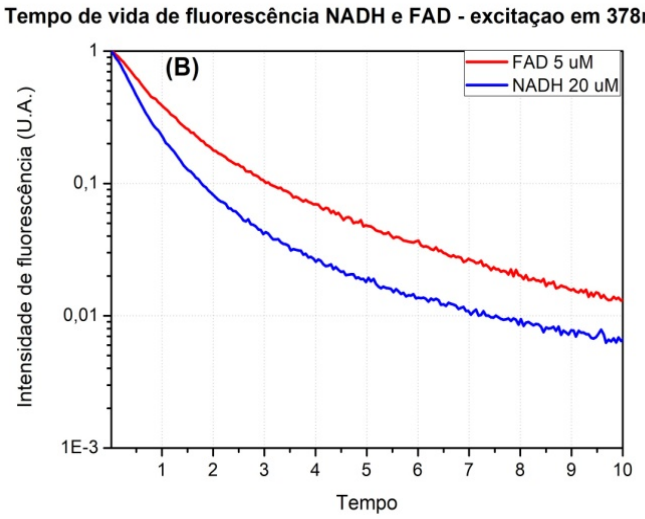
Figure 3: ***A plotted*** **FAD** ***EEM matrix. (Re*****produced from DaCosta et al.)**

EEM – NADH and FAD



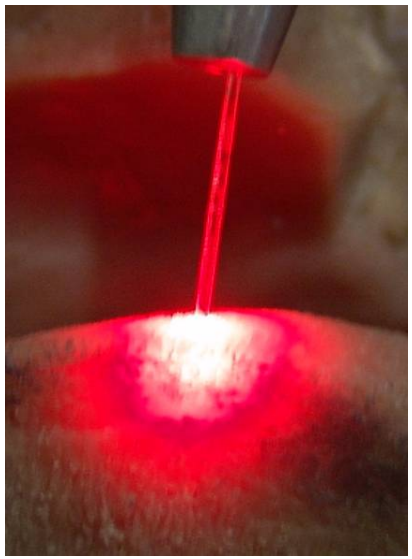

Absorbância NADH e FAD
(C)
FAD 20uM
NADH 20uM
Absorbância
0,25
0,20
0,15
0,10
0,05
0,00
300
400
500
600
700
800
Comprimento de onda (nm)


Fluorescência NADH e FAD - excitaçao 378nm
(A)
NADH 20 uM
FAD 5 uM
Intensidade de fluorescência (u.a)
1,0
0,8
0,6
0,4
0,2
0,0
400
500
600
700
800
Comprimento de onda (nm)


Tempo de vida de fluorescência NADH e FAD - excitaçao em 378nm
(B)
FAD 5 uM
NADH 20 uM
Intensidade de fluorescência (U.A.)
1
0,1
0,01
1E-3
1
2
3
4
5
6
7
8
9
10
Tempo

Fundamentos básicos

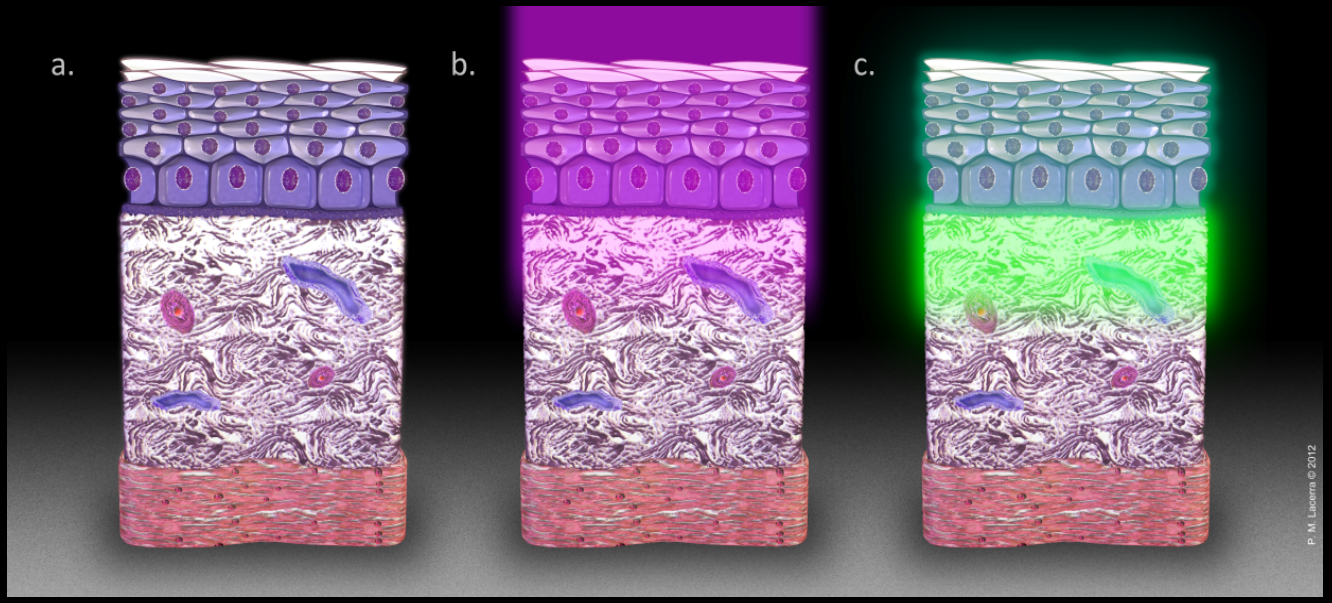
- Interação luz/tecido biológico
- Absorção – espalhamento
- Fluorescência
  - Fluoróforos biológicos (tipos e quantidades)
  - Dependência espectral: λ_{exc}, microambiente



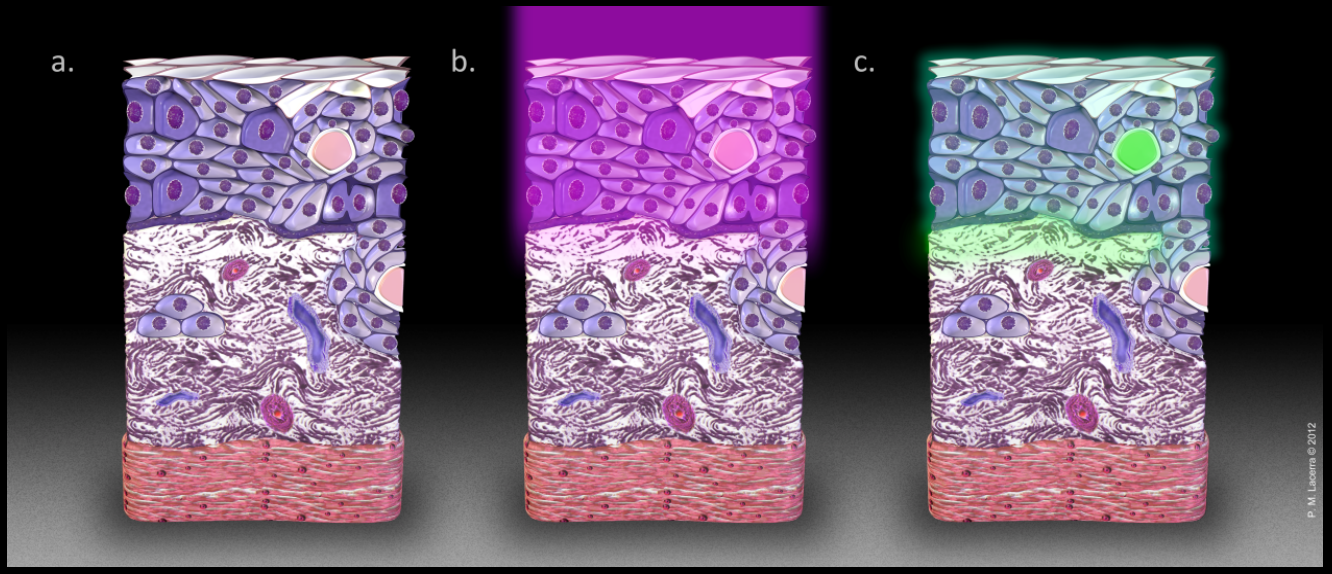
Diferenciação de tipos teciduais ou de alterações químicas

➢Caracterização tecidual bioquímica e estrutural (arquitetura)

Normal oral mucosa

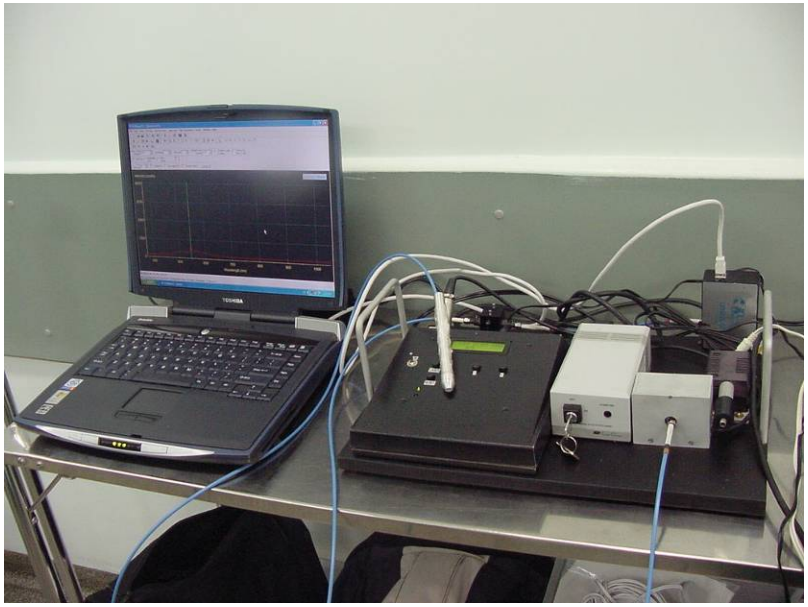


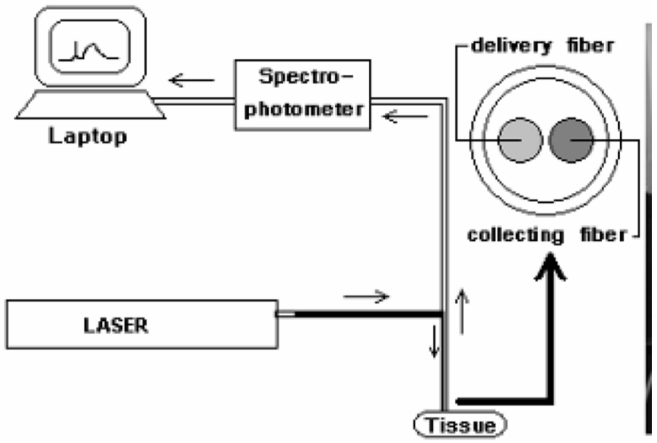
Oral carcinoma



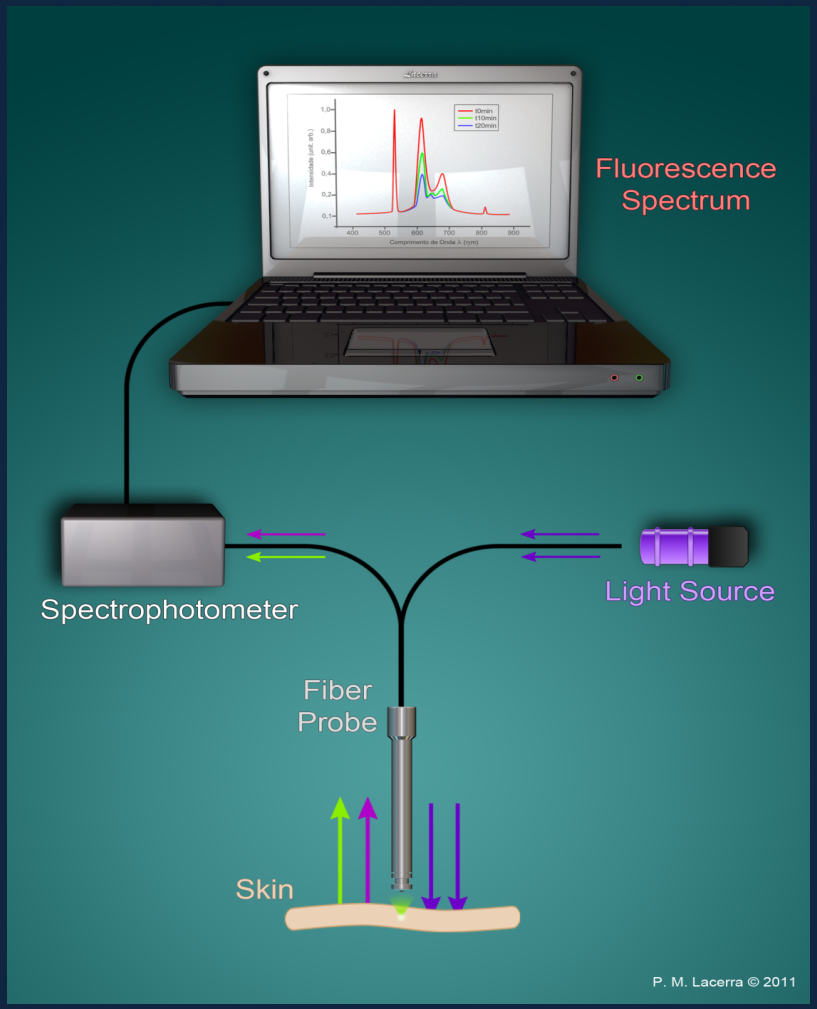
Espectroscopia de fluorescência

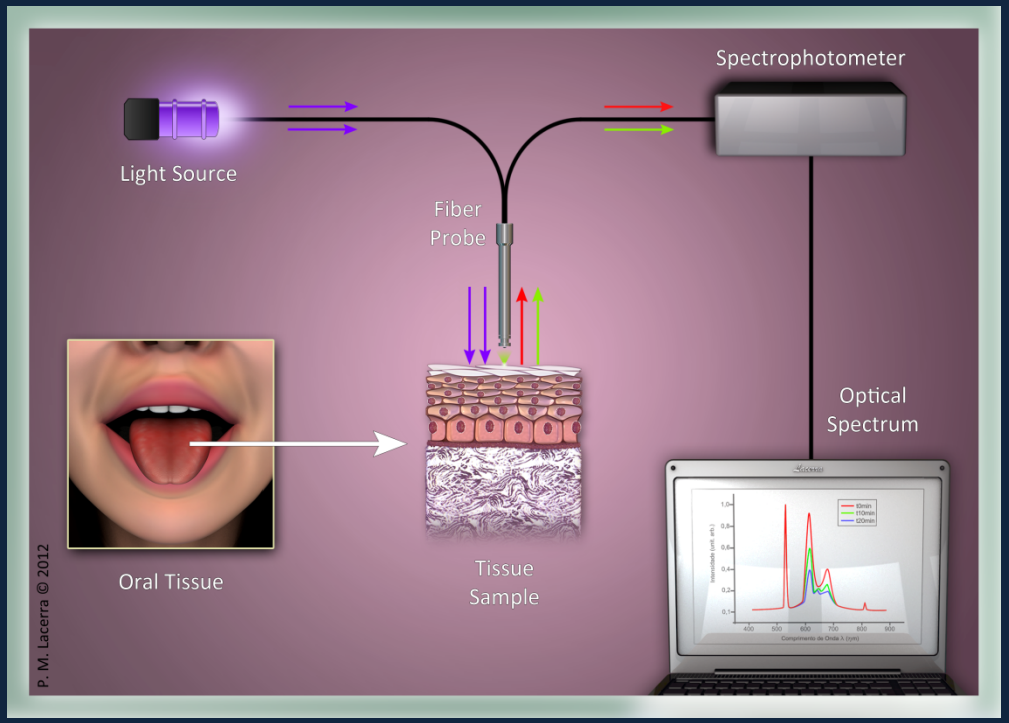
- Pontual
- Não-invasiva
- Não-destrutiva
- Resposta em tempo real
- Quantificação relativa



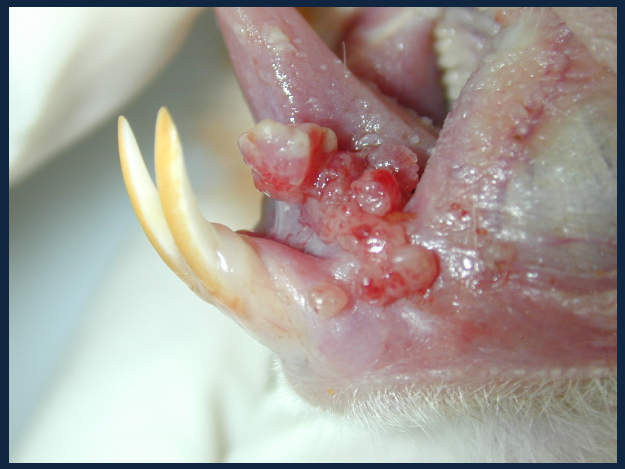

Laptop
Spectro-
photometer
delivery fiber
collecting fiber
LASER
Tissue

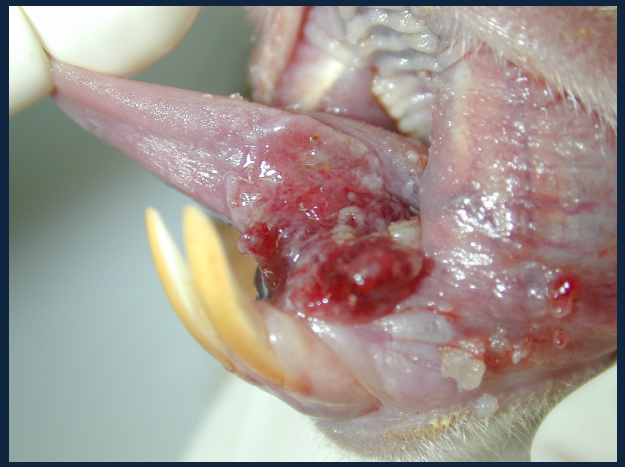

PROTÓTIPO
LAT - Laboratório
de Apoio Tecnológico
408nm


Fluorescence Spectrum
Spectrophotometer
Light Source
Fiber Probe
Skin
P. M. Lacerra © 2011

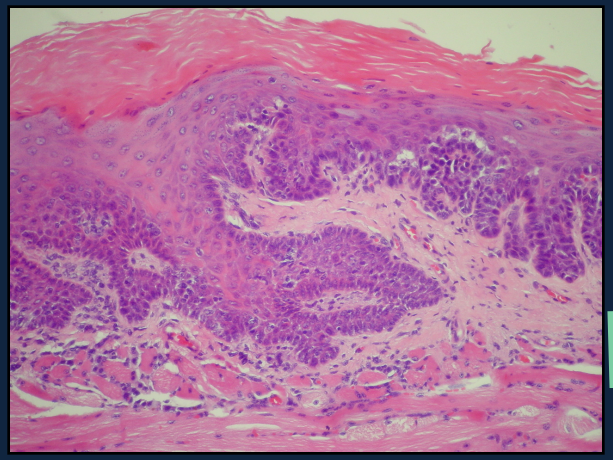

Spectrophotometer
Light Source
Fiber Probe
Optical Spectrum
Oral Tissue
Tissue Sample
P. M. Lacerra © 2012

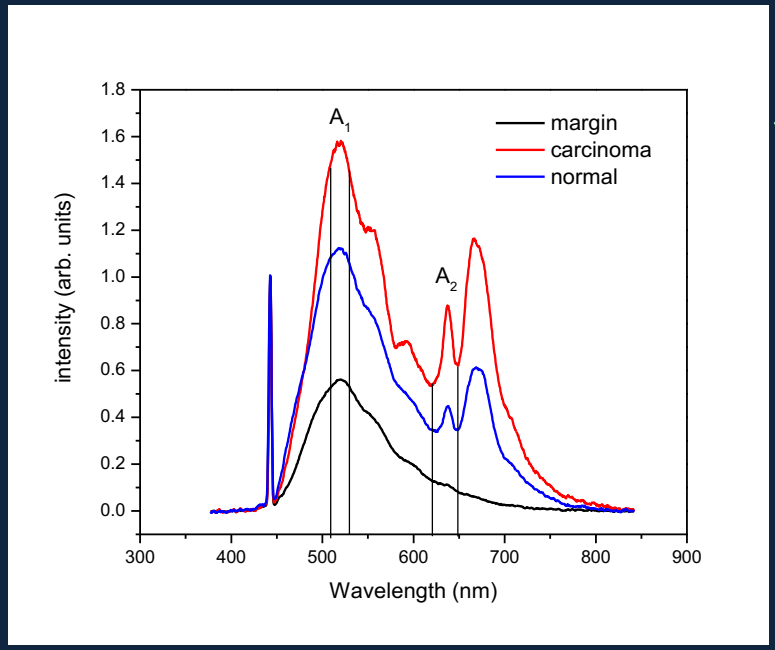
Espectroscopia de fluorescência – CEC de língua

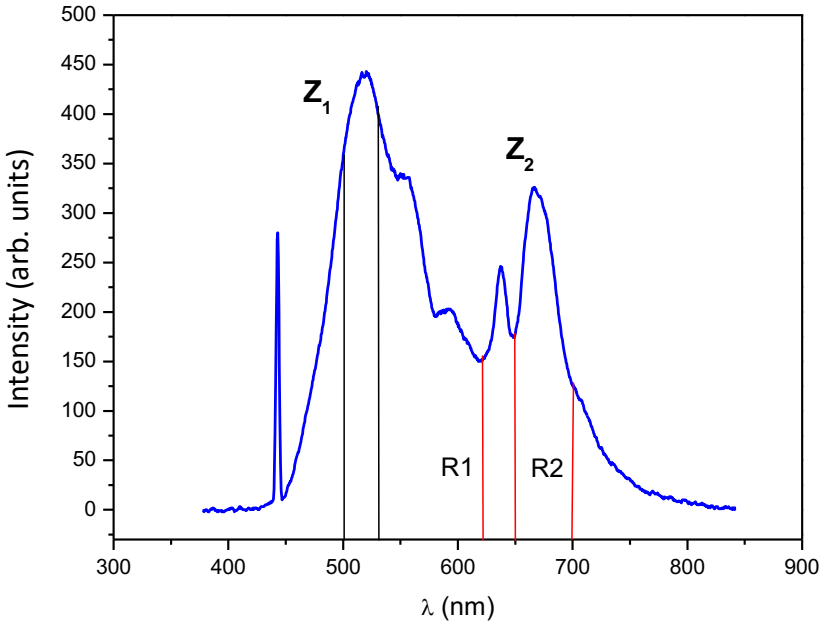




Padrão espectral
("assinatura espectral")
⇓
Características
histopatológicas







Processamento espectral

- análise intra-espectral (razão entre bandas de emissão)
- Kth nearest neighboor

Análise	Sensibilidade	Especificidade
Coeficiente A_3	90.9%	81.5%
KNN (442 nm)	95.7%	91.6%

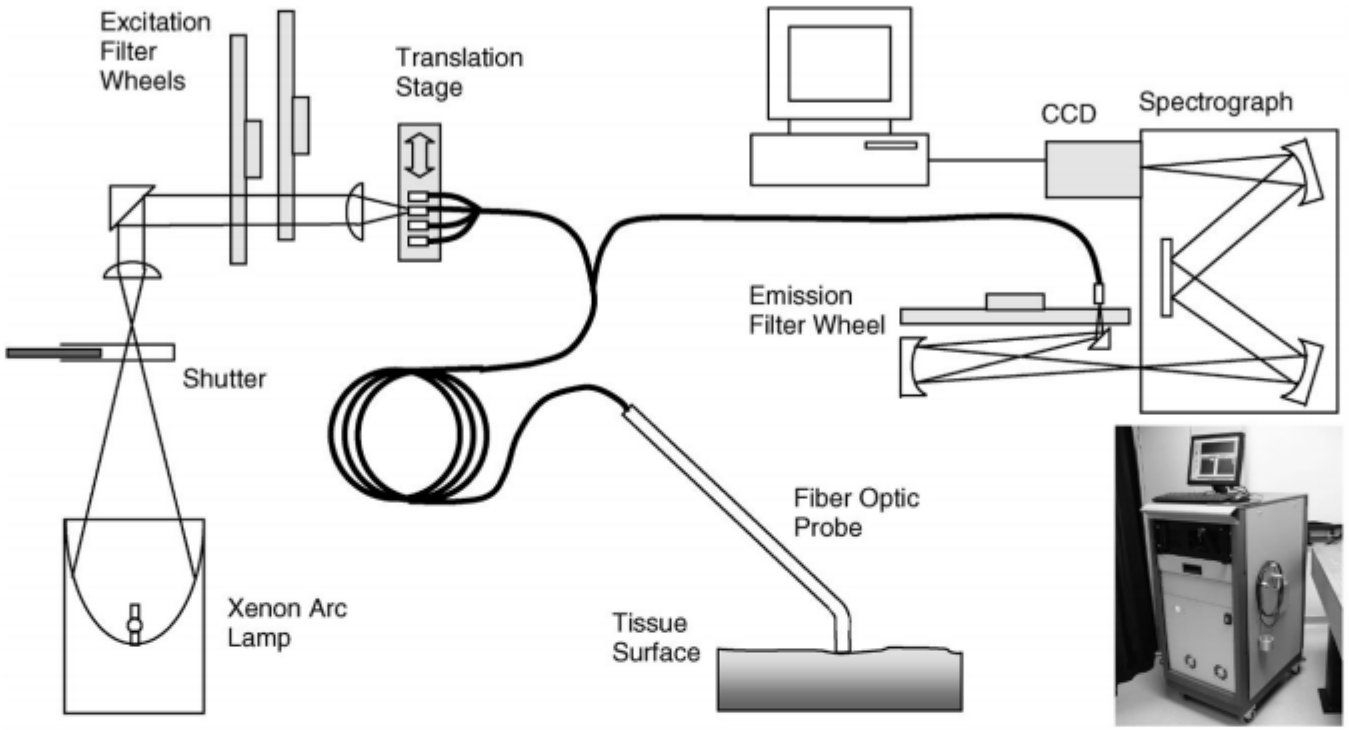
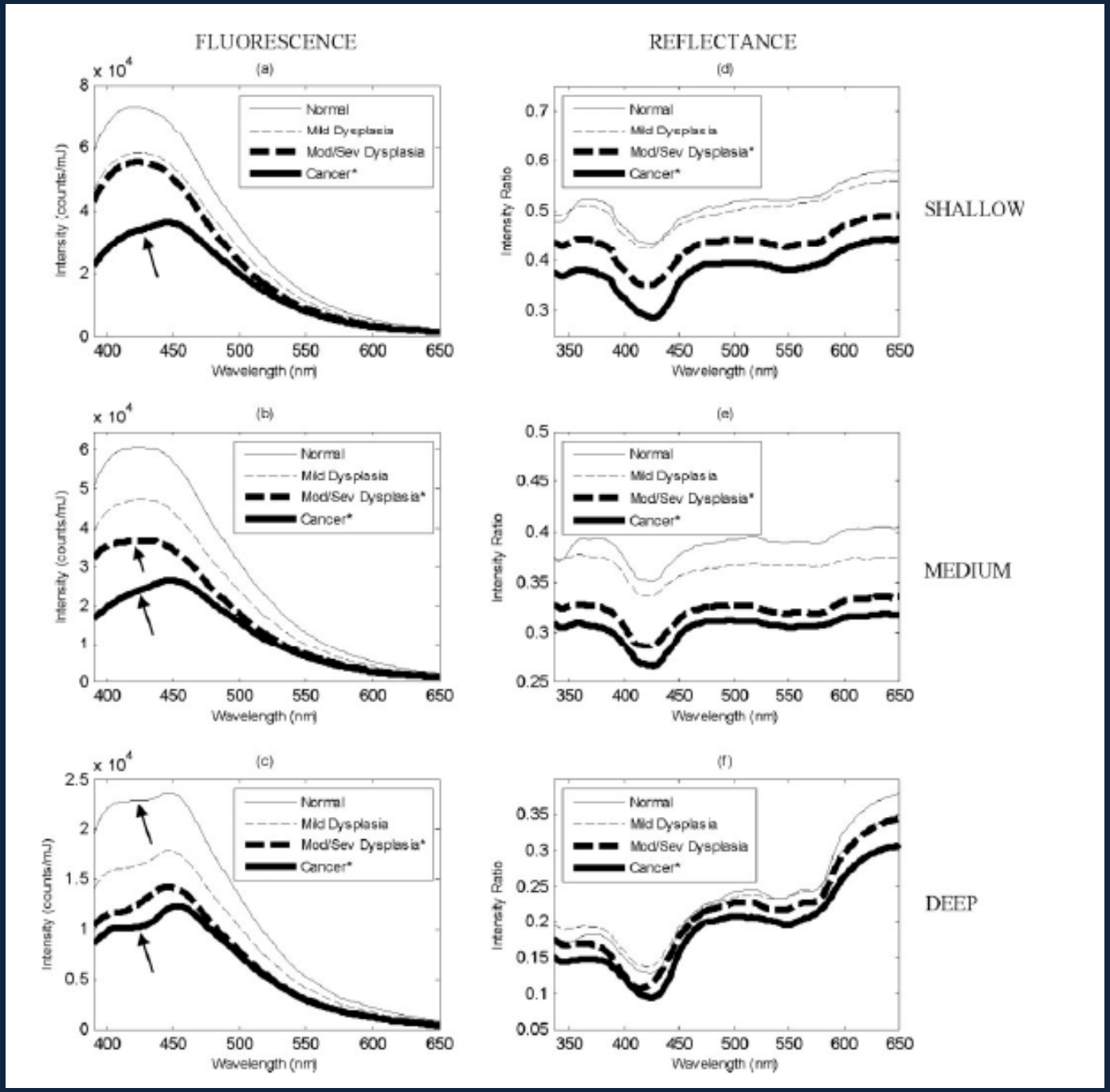
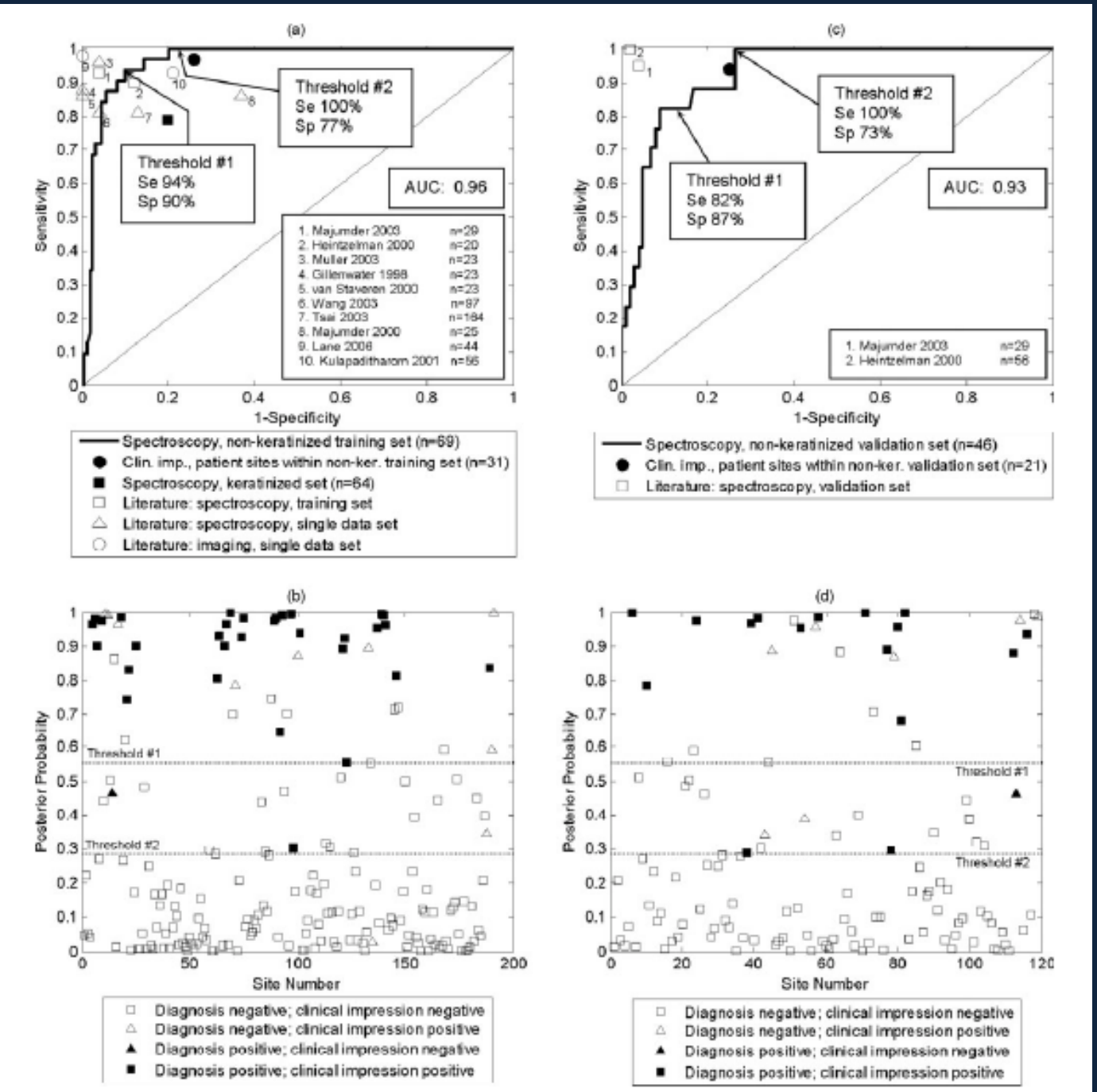


Fig. 1.
Diagram and photograph of the clinical spectroscopy system.

Schwarz et al., Appl Opt 2008

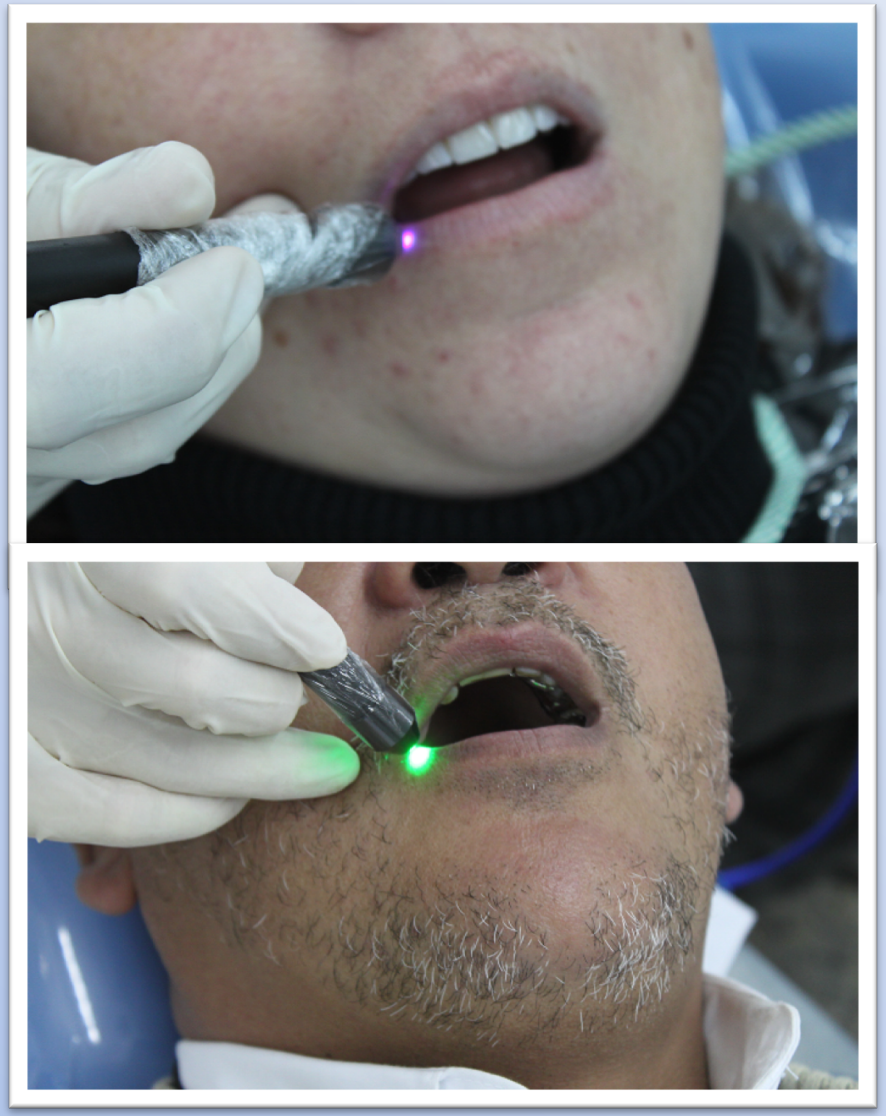


Schwarz et al., Cancer 2009



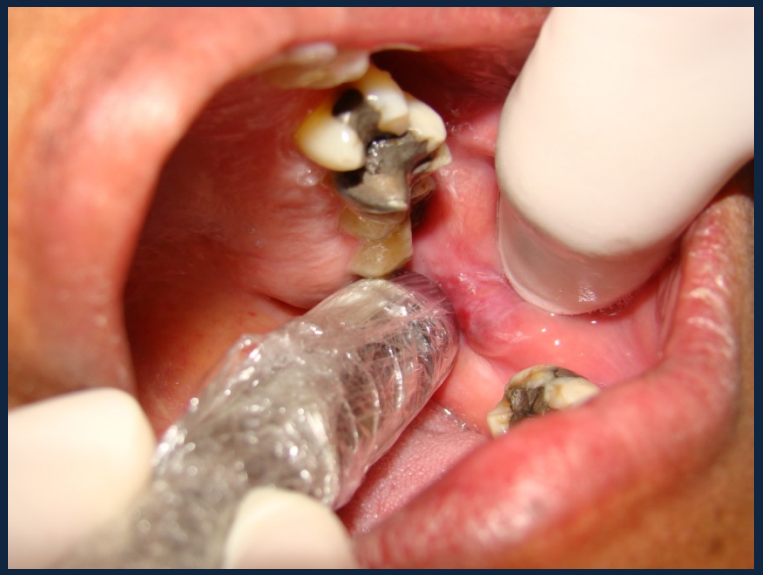
Schwarz et al., Cancer 2009

ESPECTROSCOPIA DE FL

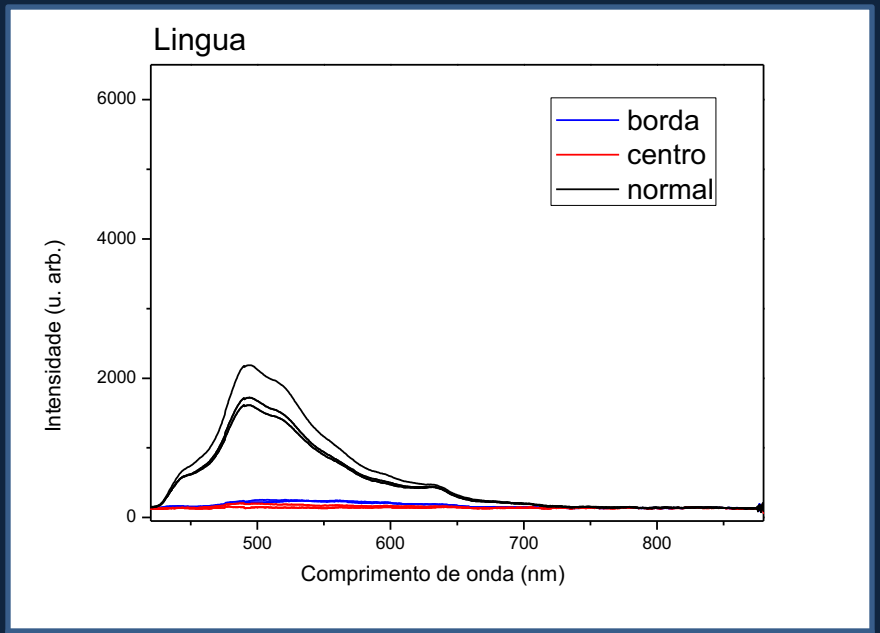


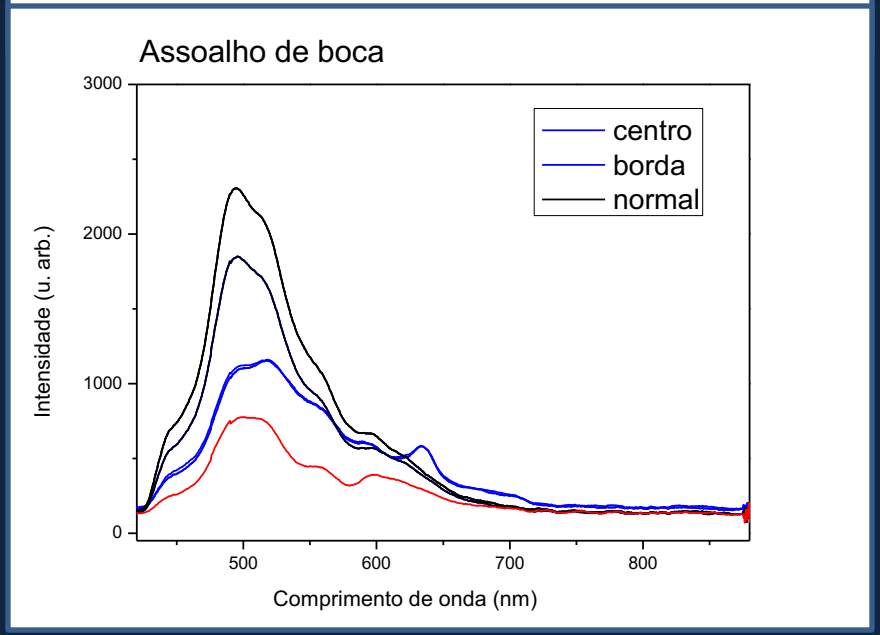
-Excitação a laser: 405 and 532 nm
- espectrômetro USB2000 (Ocean Optics)
- sonda tipo Y (duas fibras de 600 µm)
- laptop

Espectroscopia de fluorescência – CEC de boca

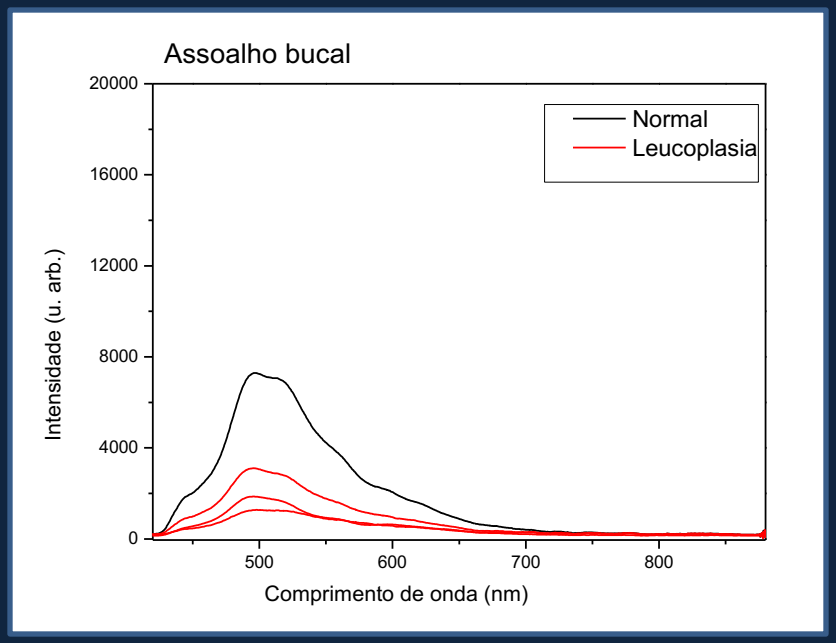


Padrão espectral
("assinatura espectral")
⇓
Características
histopatológicas

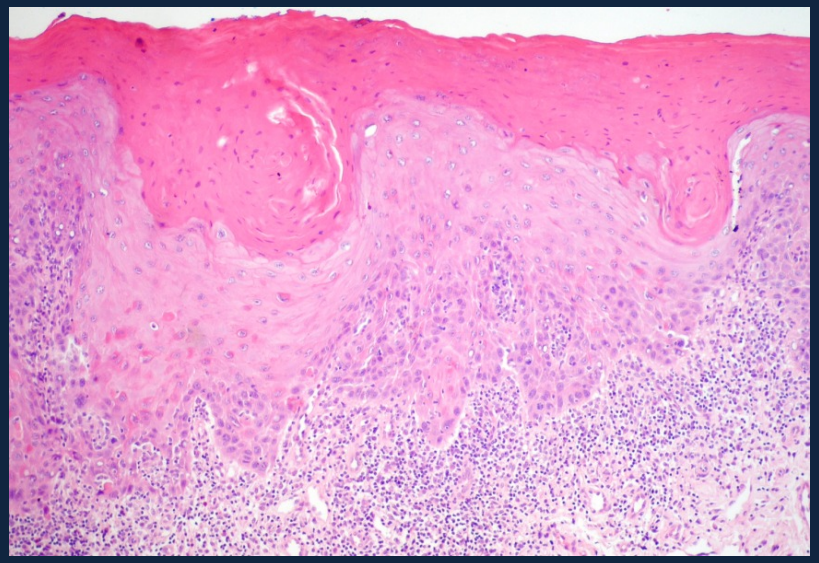




ALN Francisco et al., Las Phys and Oral Oncol 2014



?

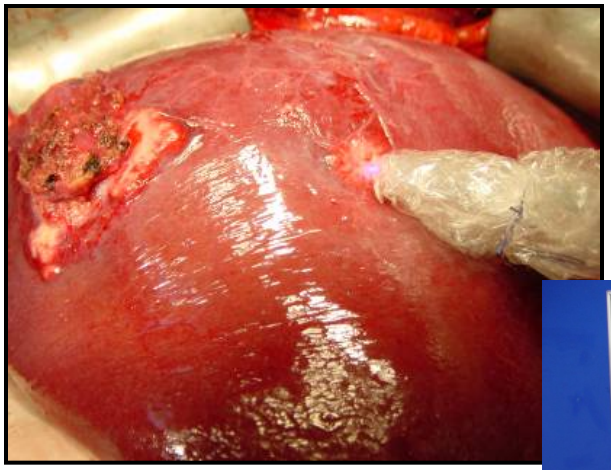


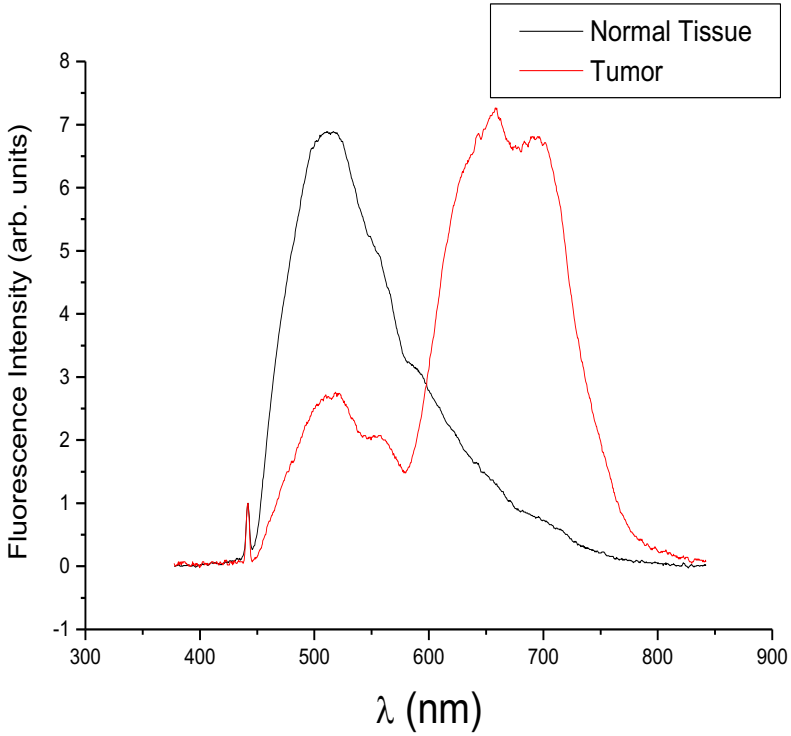
Processamento espectral

- análise intra-paciente
- análise global (sítio/histologia)
- Kth nearest neighboor
- J48; reliefF(10%)

Acurácia e precisão ~85%

ALN Francisco et al., Las Phys and Oral Oncol 2014



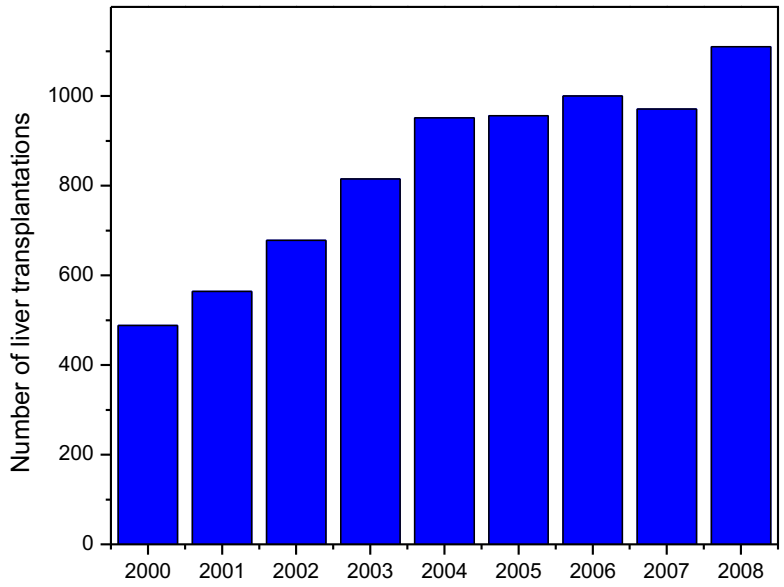

Normal Tissue
Tumor
Fluorescence Intensity (arb. units)
8
7
6
5
4
3
2
1
0
-1
300
400
500
600
700
800
900
λ (nm)

Liver transplantation

2005: Brazilian data

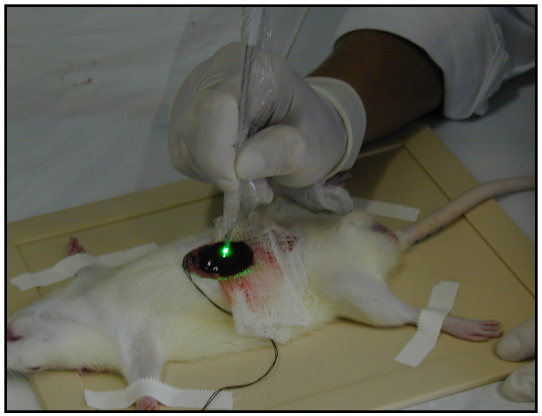
- 956 transplantations
- 6,288 pacients at the waiting list

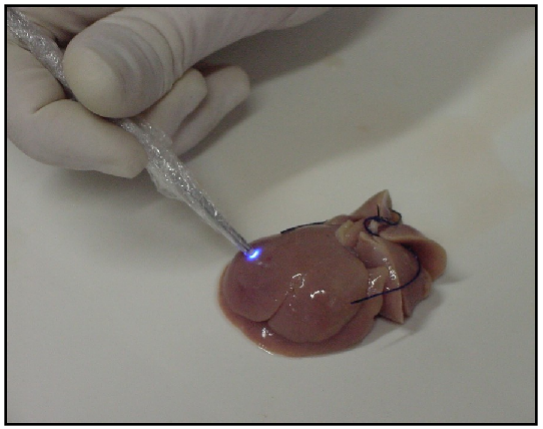
➢ 20% mortality rate of patients at the waiting list

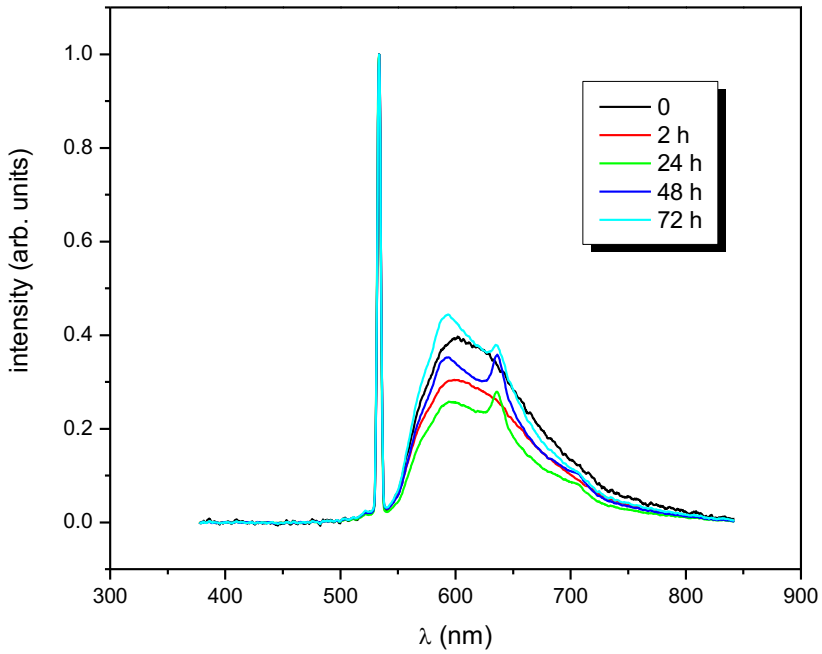


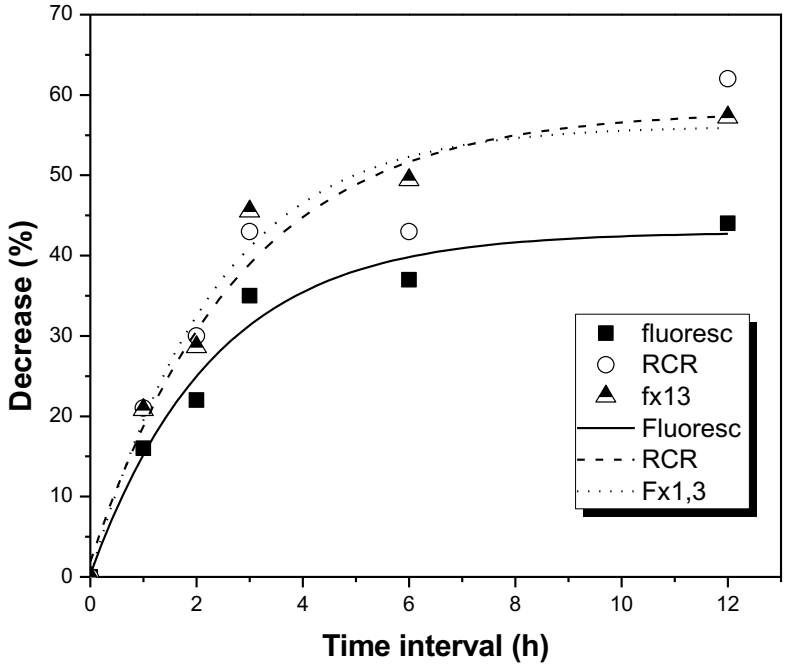
Evaluation of liver graft status (transplantation)

- Comparison with the mitochondrial respiratory rate

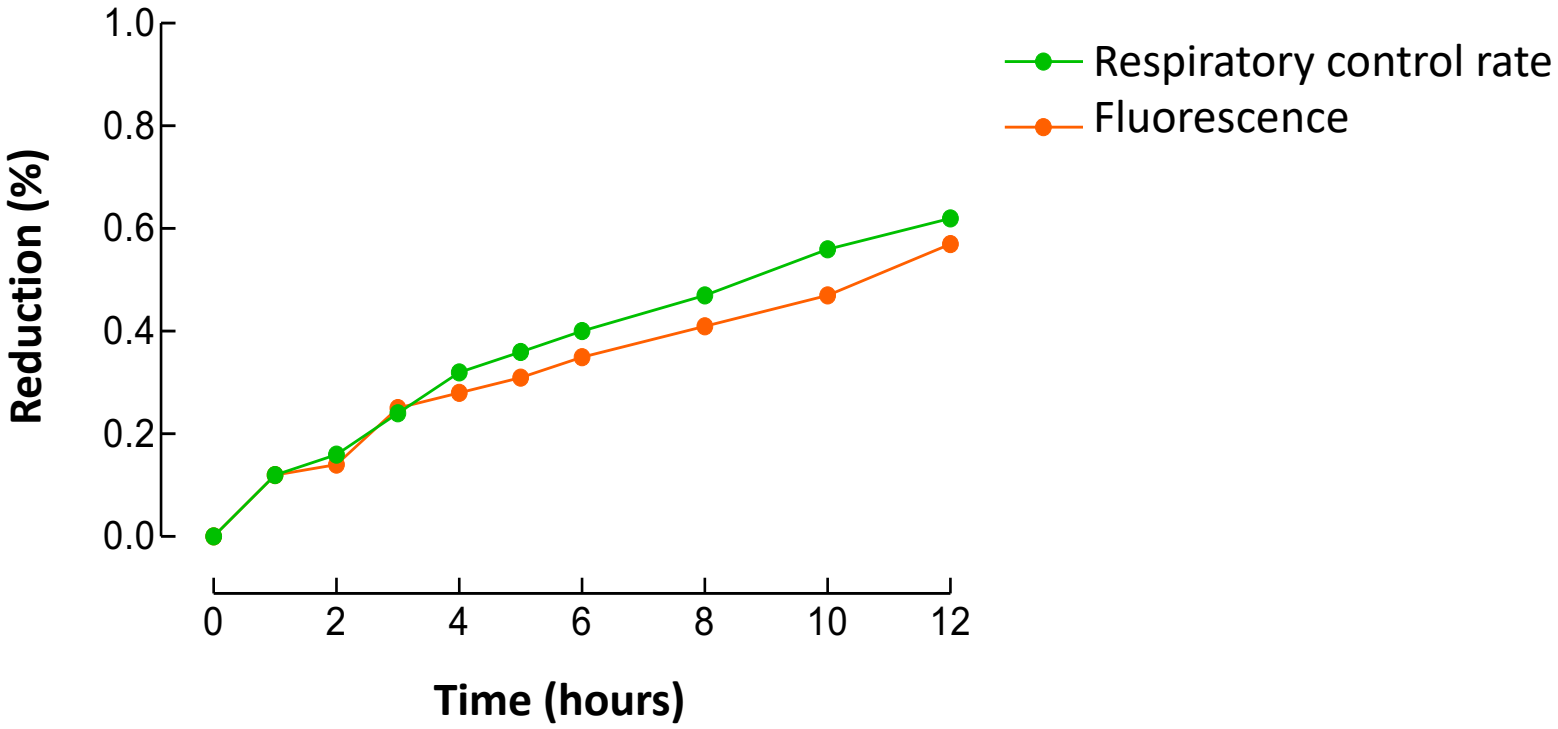




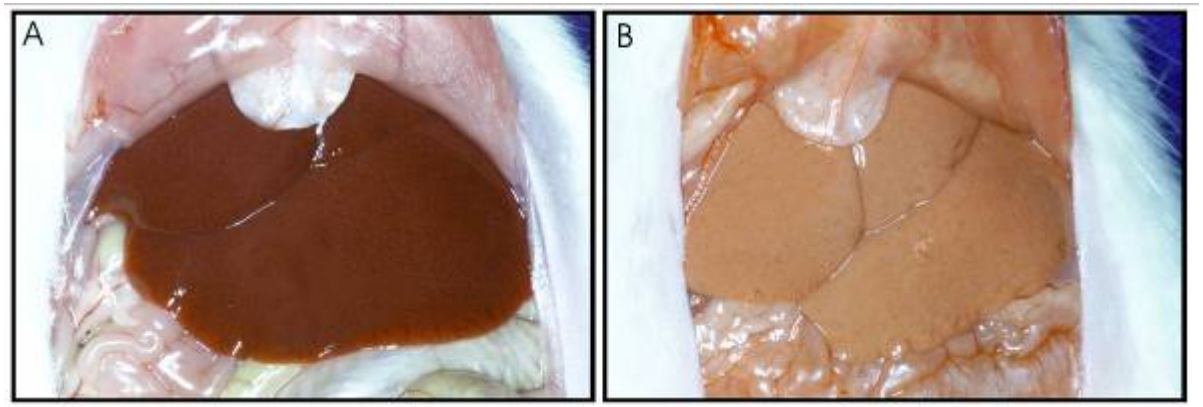


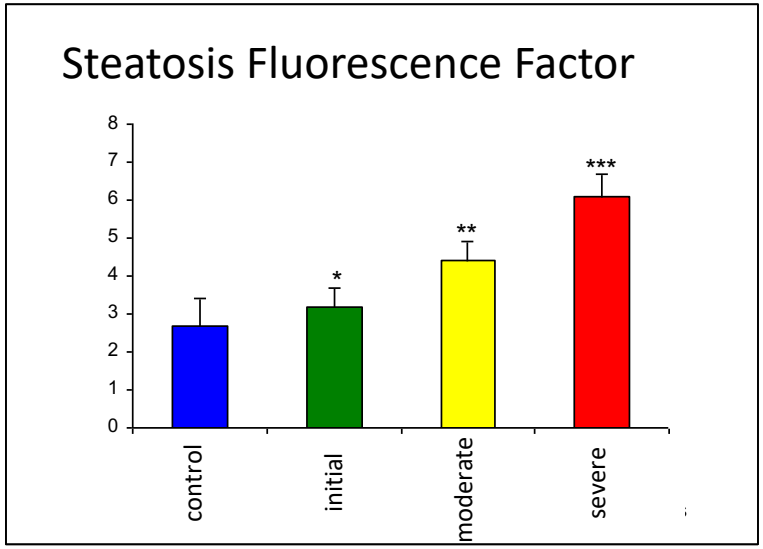


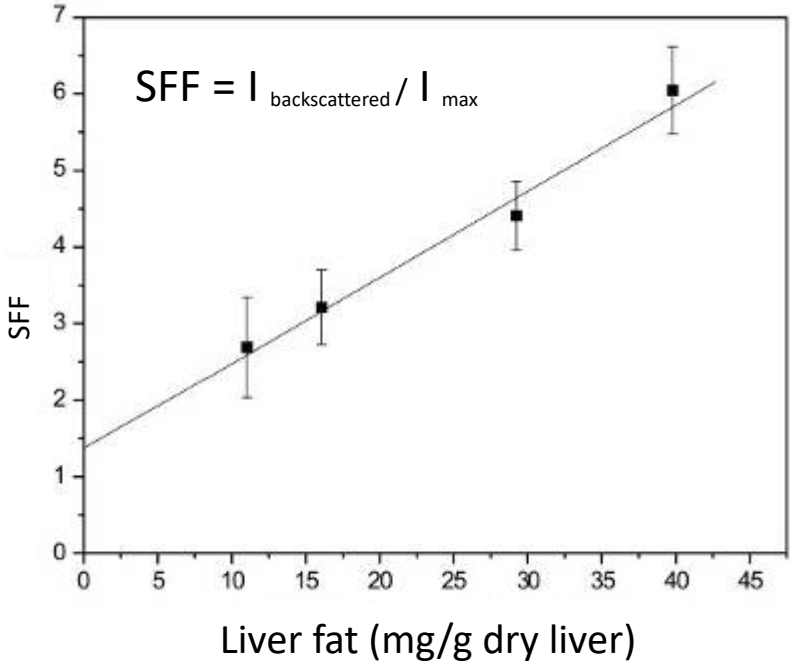
Sankarankutty et al., *Laser Phys. Lett.* 3(11): 539–545 (2006)


1.0
0.8
0.6
0.4
0.2
0.0
Reduction (%)
0
2
4
6
8
10
12
Time (hours)
Respiratory control rate
Fluorescence

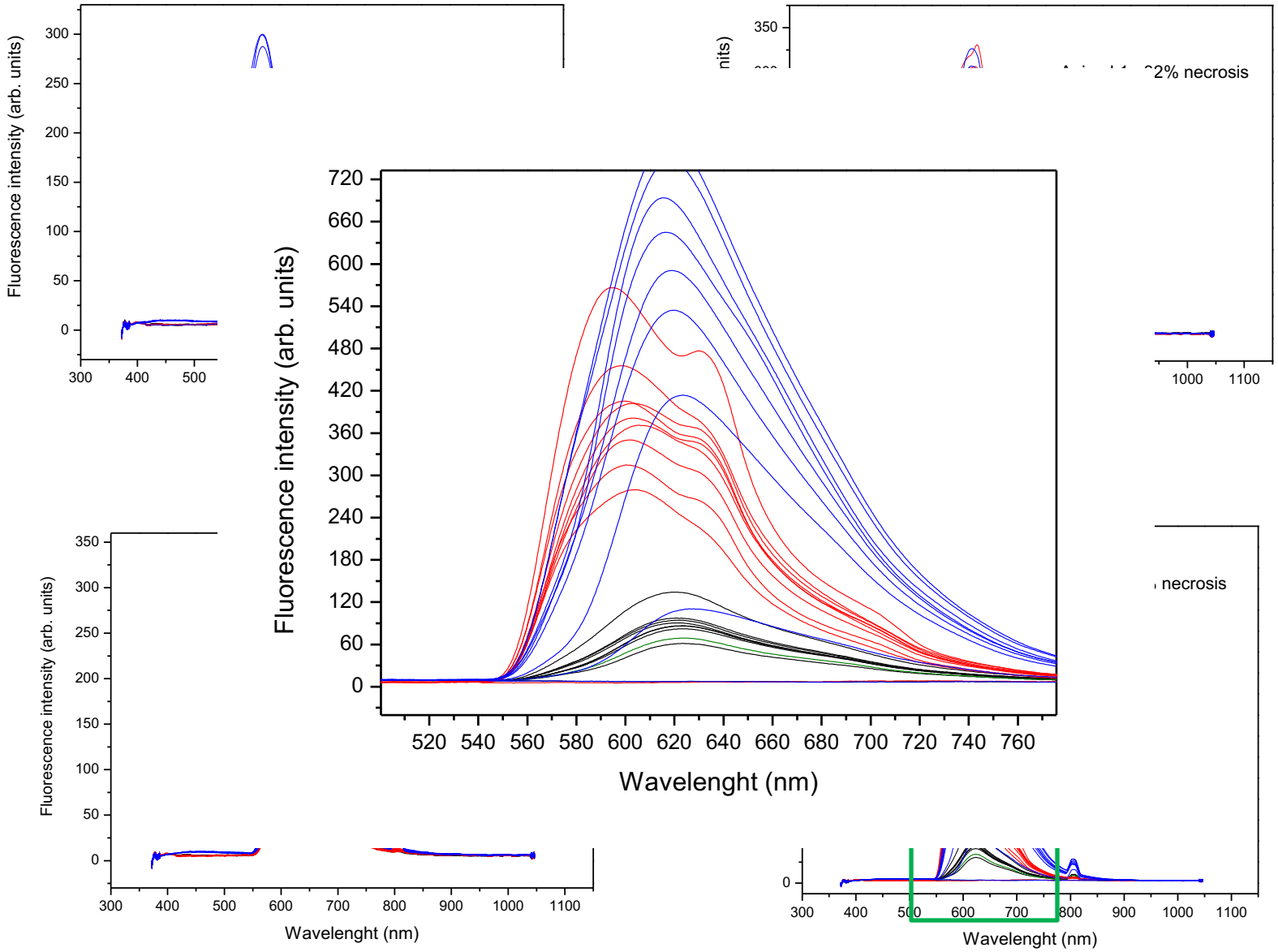
Liver steatosis

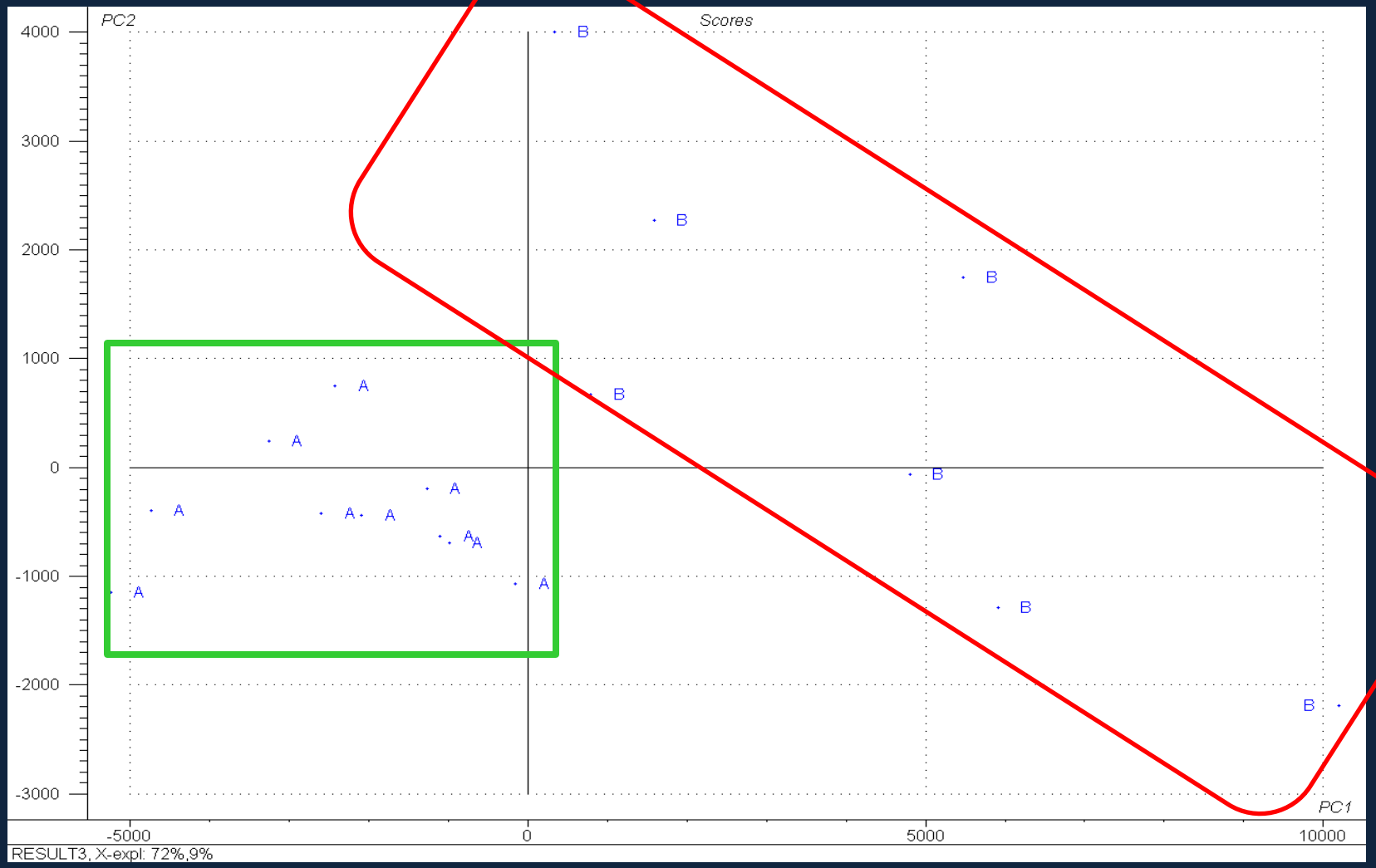




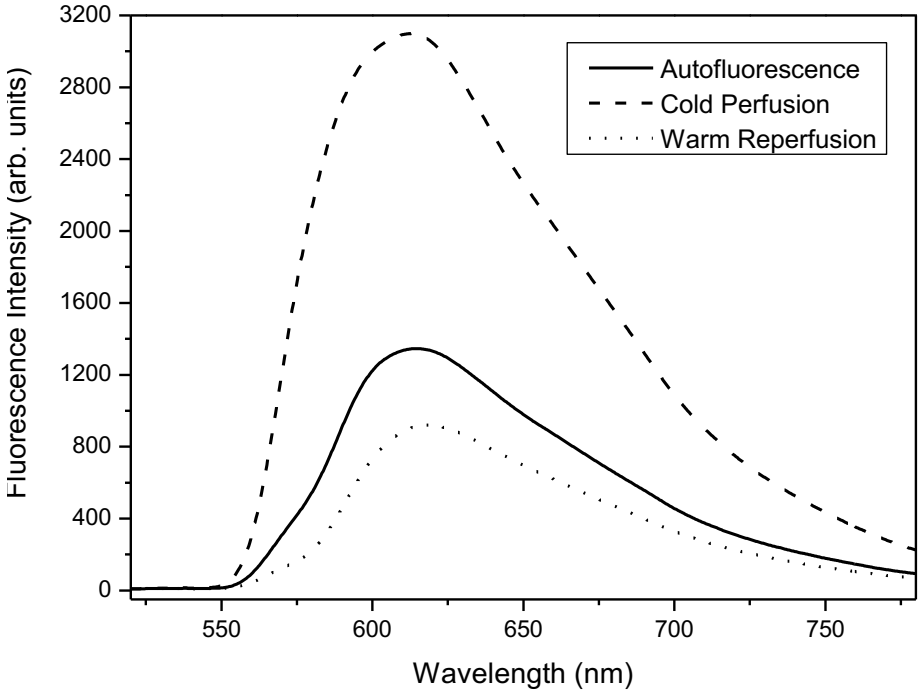


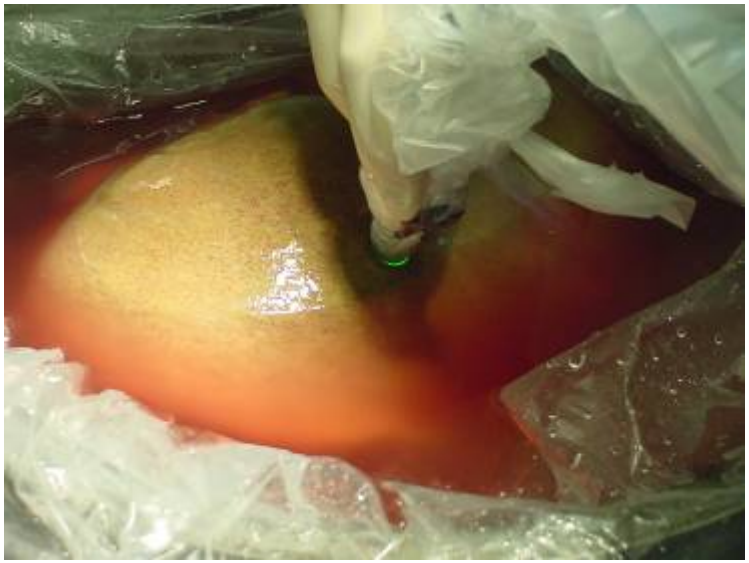
Oliveira et al., *Liver International* 29(3): 331-336 (2009)

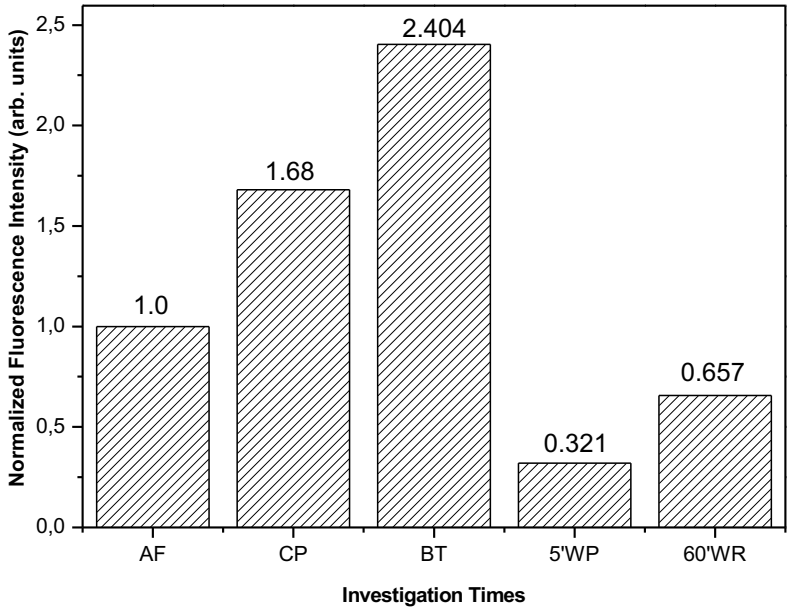

Fluorescence intensity (arb. units)
Wavelenght (nm)
2% necrosis
necrosis



Fluorescence spectra (after ischaemia and reperfusion)/tissue morphometry
A (necrosis < 85%) and B (necrosis ≥ 85%)







Castro-e-Silva et al., *Transplantation Proceedings, 40, 722–725* (2008)

Determinação do intervalo post-mortem usando a espectroscopia de fluorescência

Medicina forense

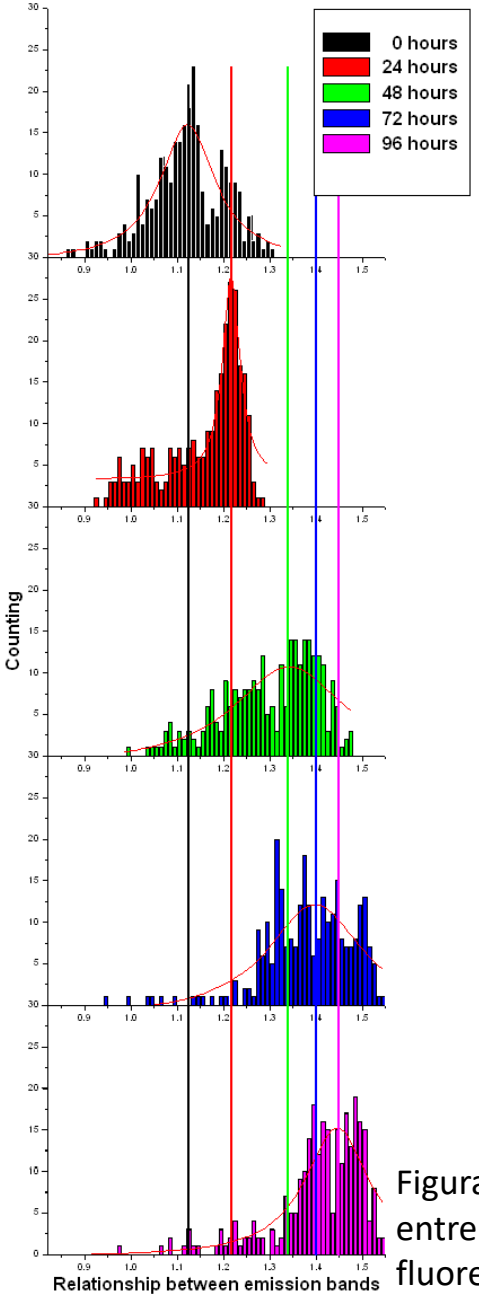


Figura 8: Distribuição da razão entre os picos de emissão de fluorescência

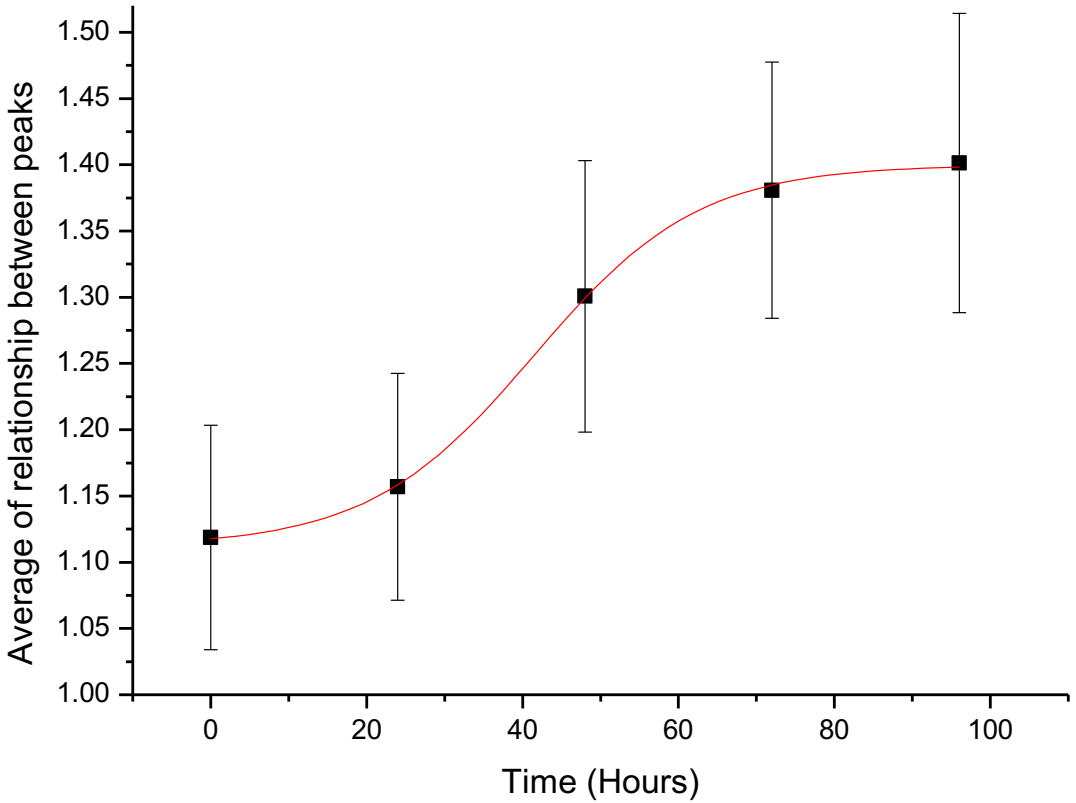


Figura 9: Evolução temporal do valor médio da razão entre os picos de fluorescência

ES Estracanholi et al., Opt Exp 2009

Excitação em 408nm - Normalização em 488nm

Determinação do IPM pelo coeficiente angular das retas obtidas das componentes principais

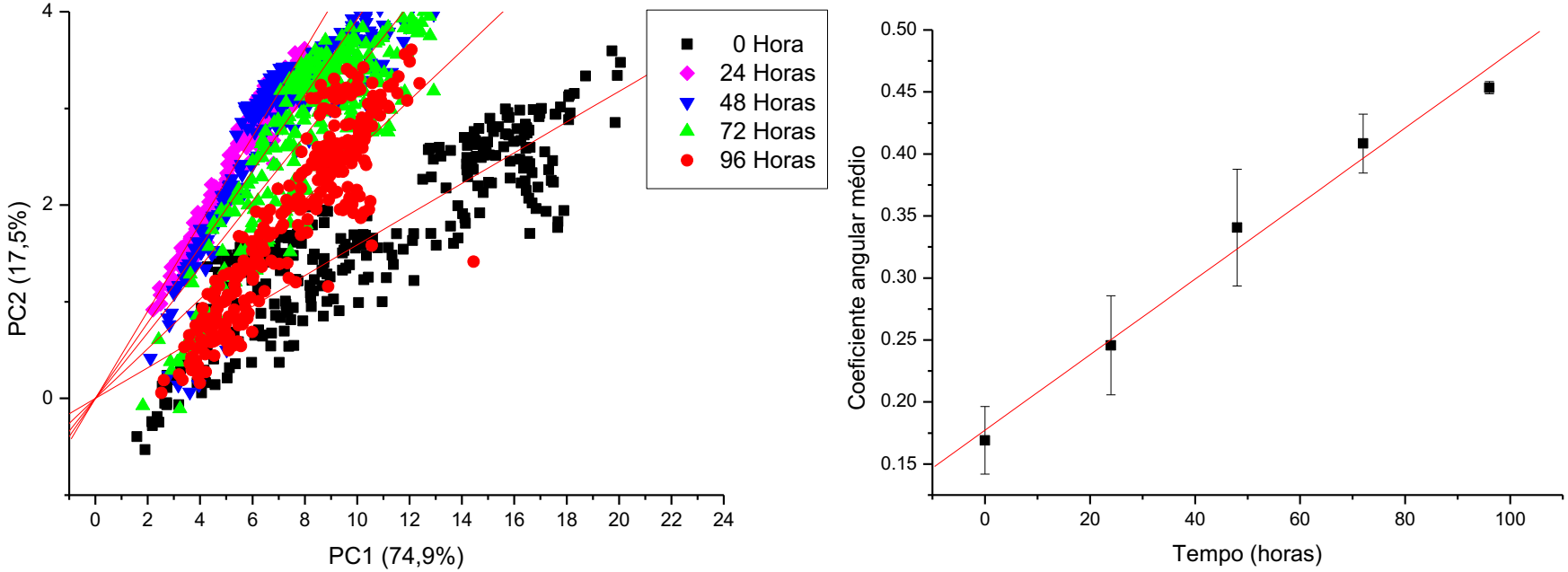


Figura 15: PC1 *versus* PC2 dos espectros no intervalo de 0 à 96 horas pós-morte (esquerda), variação temporal do coeficiente angular médio (direita) ;

ES Estracanholi et al., Opt Exp 2009

Comparação com alguns métodos atuais

Método	Modelo Animal	IPM Analisado (h)	*Desvio (h)
Coloração de hipóstases	Humano	0 a 30	16
		0 a 72	24
Concentração de H-MRS no cérebro	Ovino	50 a 100	10
		100 a 200	50
Degradação protéica (Troponin I)	Bovino	0 a 10	9
		0 a 70	20
Degradação DNA	Humano	3 a 56	15
Integridade do RNA	Humano	0 a 40	10
Concentração de K^+ no humor vítreo**	Humano	0 a 30	10
Concentração de K^+ no humor vítreo**	Humano	0 a 133	23
Variação da impedância da pele**	Ratos	0 a 120	27
Variação da impedância da pele**	Ratos	0 a 504	240

Quadro 5: Métodos cronotanatognóticos atuais. * Resultados obtidos da interpretação dos dados fornecidos nos trabalhos publicados. ** Resultados obtidos de distintos trabalhos publicados

ES Estracanholi et al., Opt Exp 2009

Espectroscopia de tempo de vida de Fl

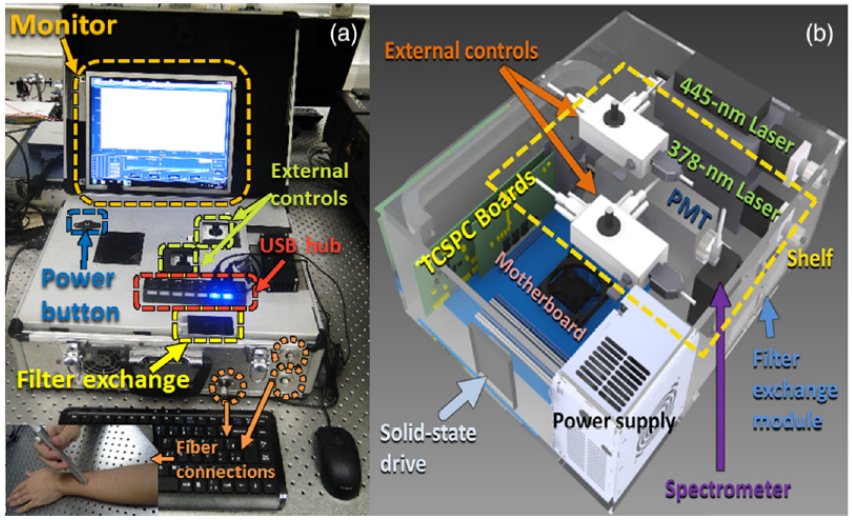


Fig. 3 (a) System assembly after its encasement provides protection for its components and a user-friendly design by exhibiting a control panel with access to external controls and the filter holder. The sample can be placed in any region around the system within the range of the optical fiber (2 m of length). (b) Top view schematic showing the position of each of the system components inside the suitcase. Parts in the upper part of the suitcase, such as 445-nm laser and external controls, are supported by a shelf, which holds the some of the components of the front panel.

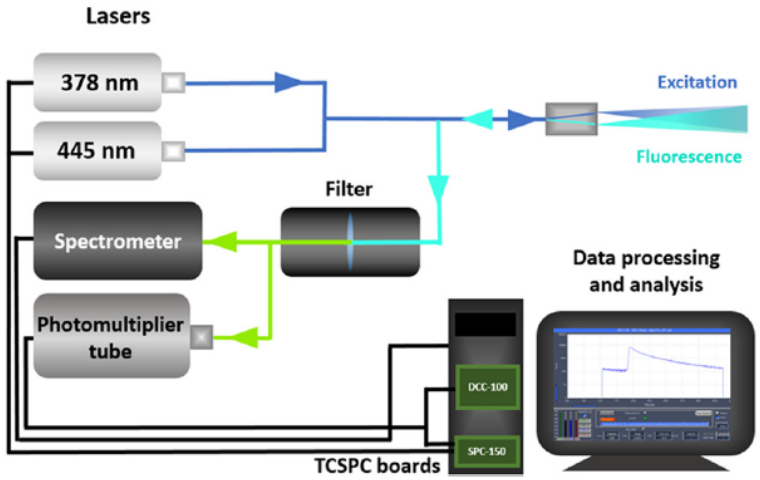
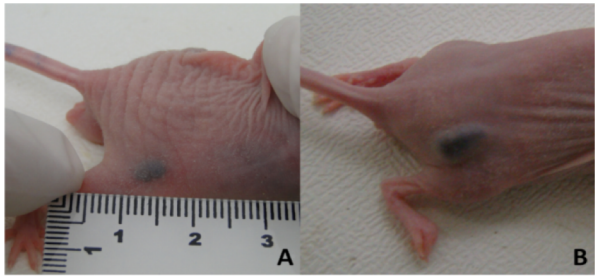


Fig. 1 Schematic drawing of the components for acquisition of fluorescence spectra and fluorescence lifetimes. The excitation light of one of the diode lasers (378 or 445 nm) is delivered to the sample through a bifurcated fiber optic probe. The fluorescence and backscattered light of this sample are collected using the same probe, which will send the light to the filter holder. A combination of four possible filters can be used to remove the backscattered light and acquire the spectral region of interest: bandpass filters at 440 ± 20 nm or at 514 ± 15 nm and 405- or 475-nm longpass filters. The fluorescence goes to a spectrometer or a hybrid PMT, and, then, the fluorescence spectrum and fluorescence decay curve are measured. The PMT is connected to two TCSPC boards: the detector control module (DCC-100) and the time-correlated single photon counting module (SPC-150).

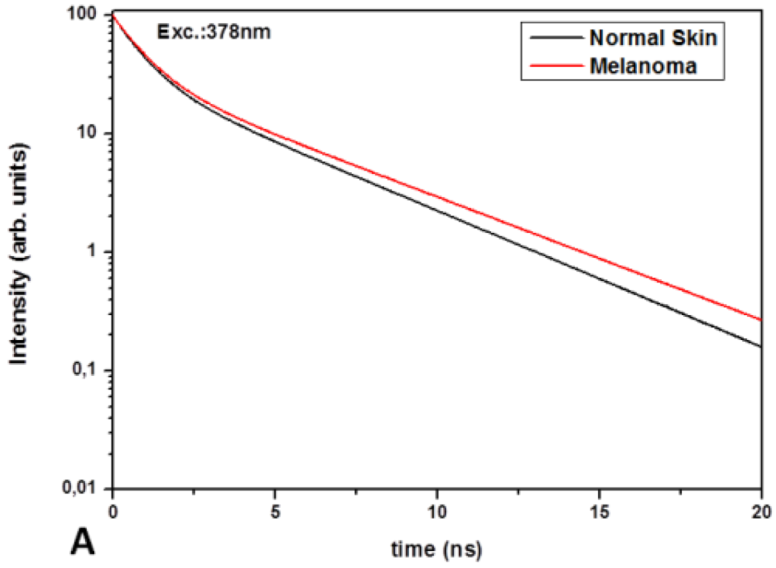
MS Nogueira et al., JBO 2017

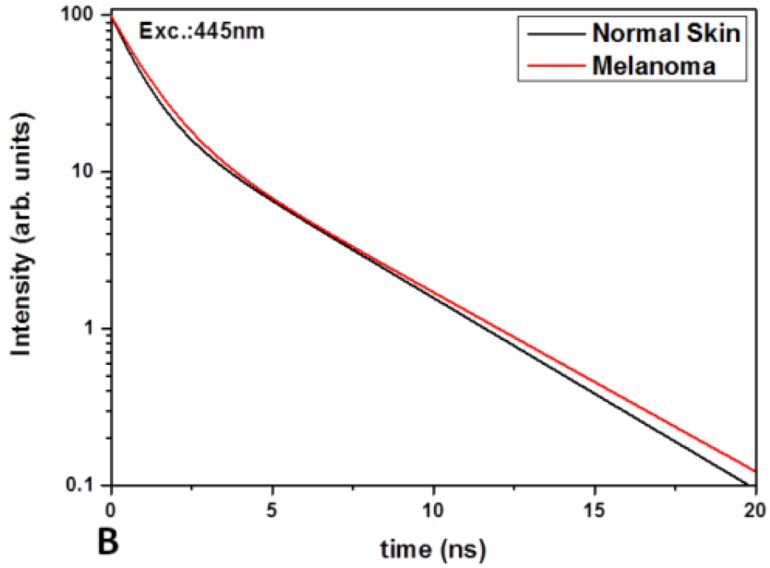
- **NADH molecules show a short lifetime component when it is free and a longer lifetime component when it is protein-bounded.**

- **FAD molecules, short lifetime component is present for protein-bounded and longer lifetime component for its free state.**



Camundongo nude BALB c (atímico)
10^7 melanoma cells

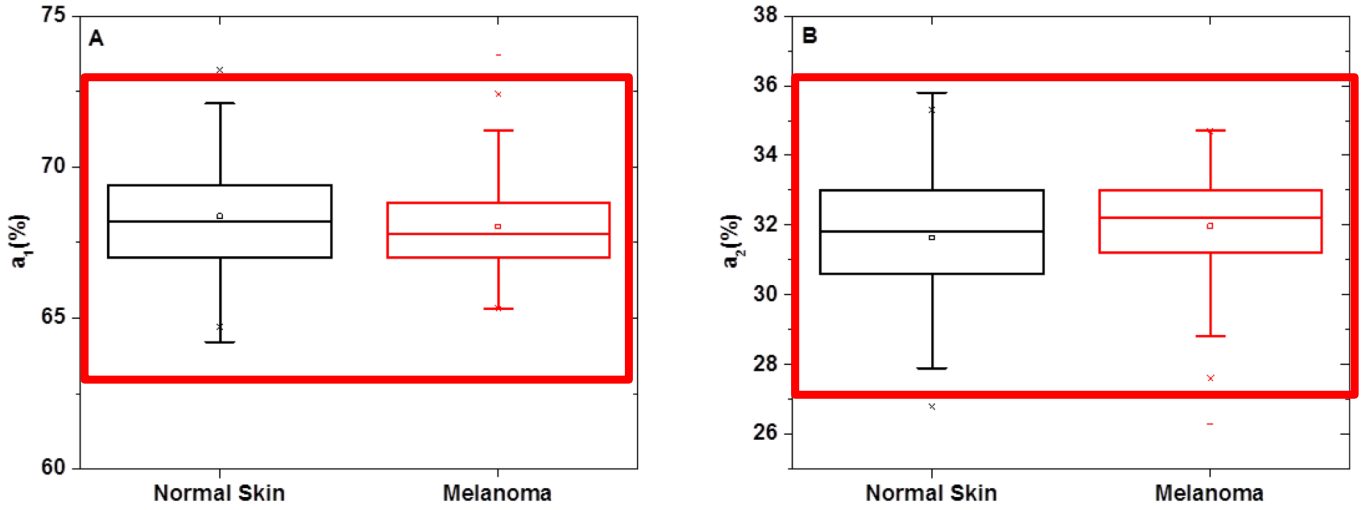




$$F(t) = a_1 . e^{\frac{t}{\tau_1}} + a_2 . e^{\frac{t}{\tau_2}}$$

L Pires et al., Biomed Opt Exp 2014

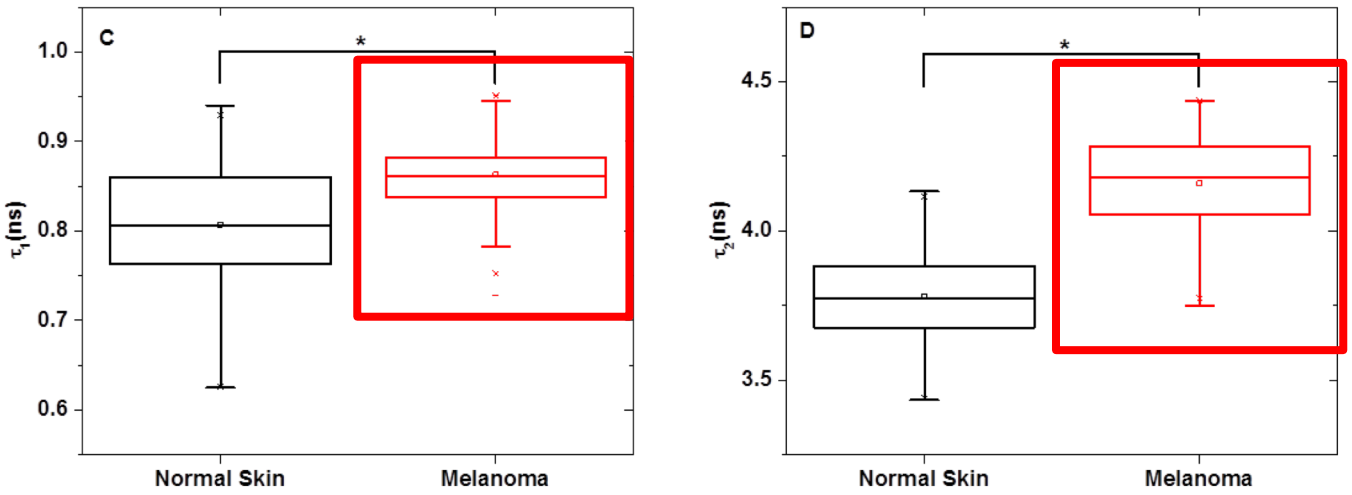
378nm excitation: NADH molecules



It was not observed difference between the a_1 and a_2 values obtained for melanoma and normal skin, probably due to the thin normal epidermis layer present over the experimental lesion. In addition to its contribution to the collected fluorescence signal, this thin skin layer also reduces the laser penetration and resulted excitation of the target tissue.

L Pires et al., Biomed Opt Exp 2014

378nm excitation: NADH molecules



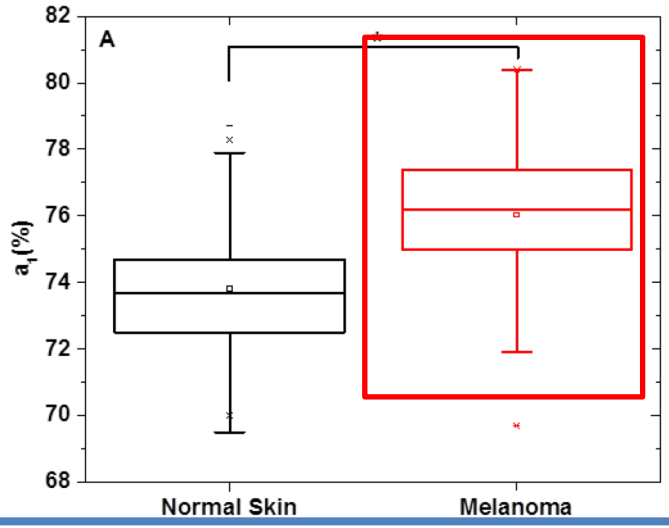
-Short and long lifetime components increased in melanoma;

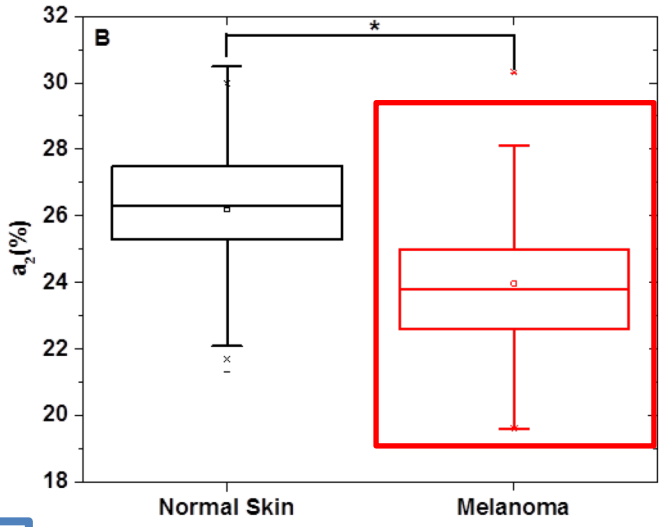
-Warburg effect: shorter lifetimes in melanoma due to the presence of high amounts of free NADH molecules (glycolysis respiration pathway);

- keratin, collagen and melanin – may contribute for the lifetime changing;

L Pires et al., Biomed Opt Exp 2014

445nm excitation: FAD molecules





Normal skin

a1 – low amount of molecules with short lifetime;

a2 – high amount of molecules with long lifetime.

Melanoma

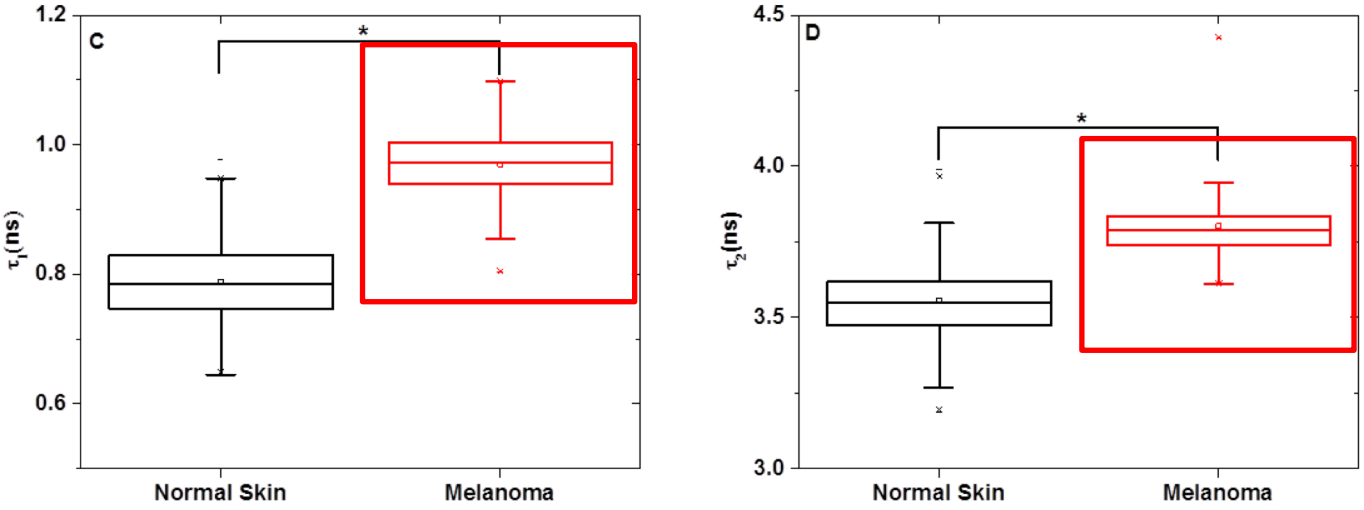
a1 – high amount of molecules with short lifetime;

a2 – low amount of molecules with long lifetime.

It does not agree with Warburg effect, maybe due to the presence of others molecules such as collagen and melanin.

L Pires et al., Biomed Opt Exp 2014

445nm excitation: FAD molecules



- Small lifetime component: protein-bonded FAD (oxidative phosphorylation metabolism);
- Long lifetime component: free FAD

Normal skin

-Smaller lifetime than melanoma

- Pasteur effect: oxidative phosphorylation

Melanoma

-Longer lifetime than normal skin

- Warburg effect: glycolysis

L Pires et al., Biomed Opt Exp 2014

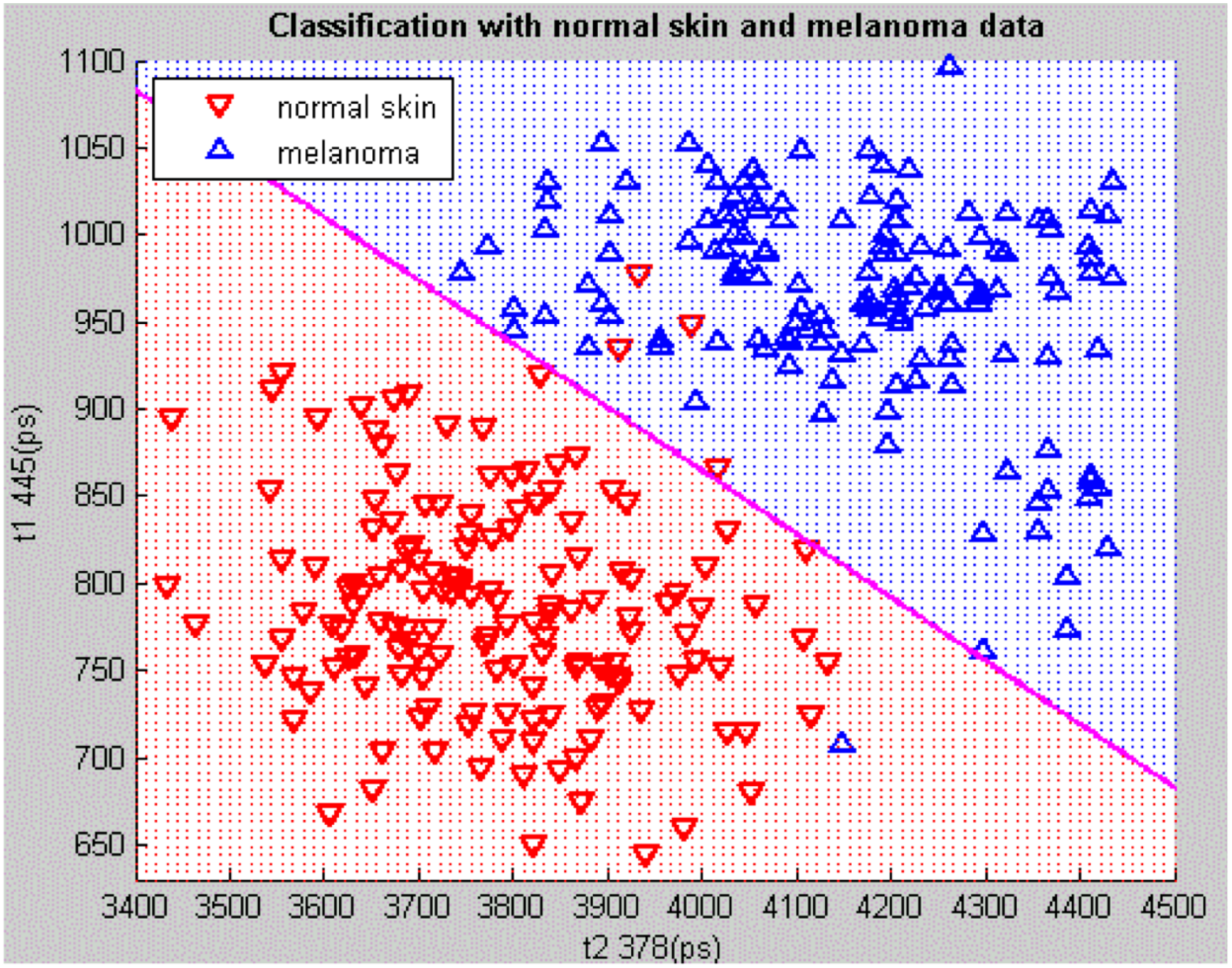
Short lifetime component of FAD
Long lifetime component of NADH

↓

Oxidative phosphorylation pathway

↓

Aerobic metabolism present in both tissue – Pasteur effect



Sensitivity: 99.4%

Specificity: 97.4%

Accuracy: 98.4%

L Pires et al., Biomed Opt Exp 2014