

Técnicas fotônicas para diagnóstico - espectroscopia

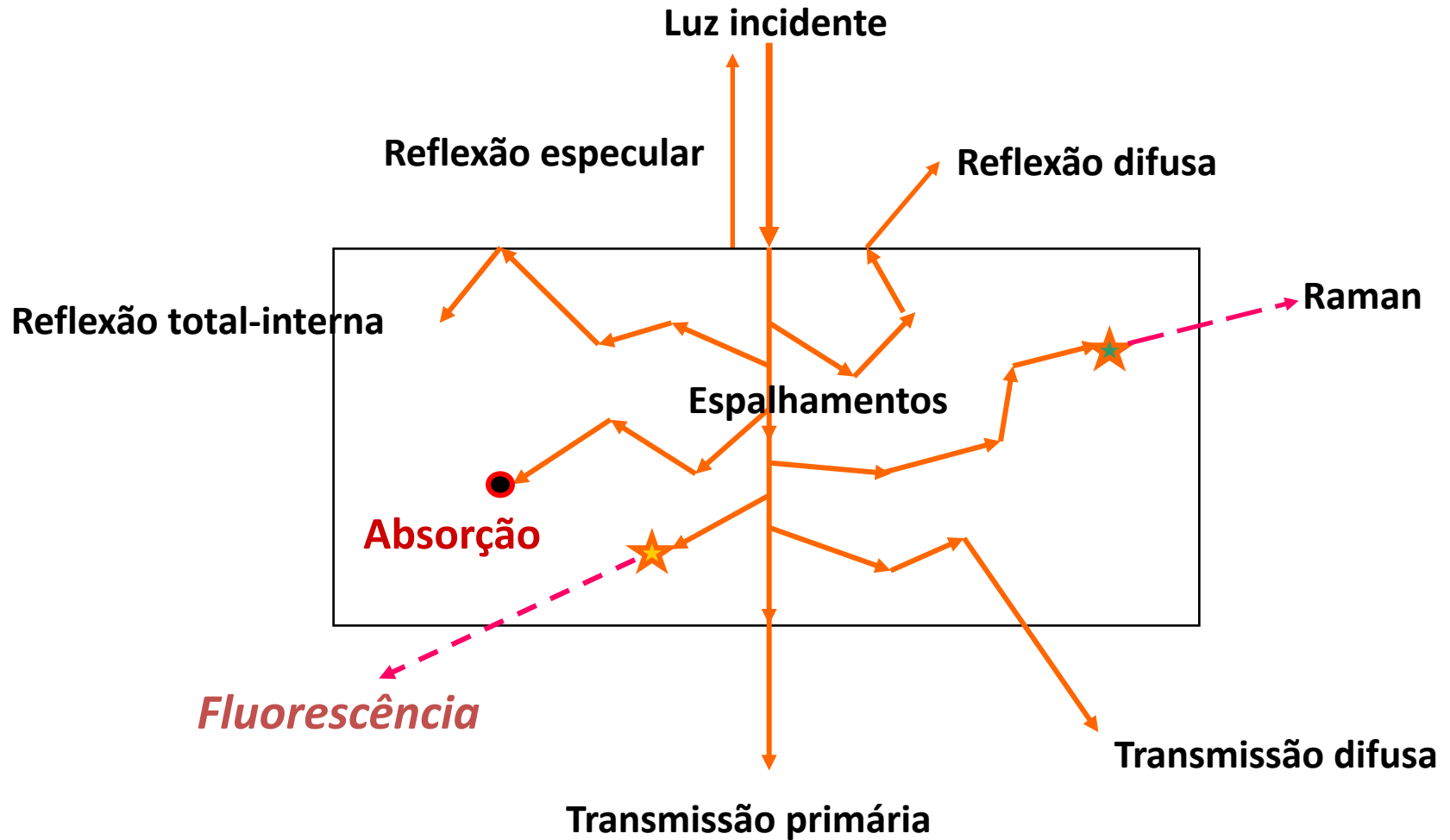
Diagnóstico de lesões

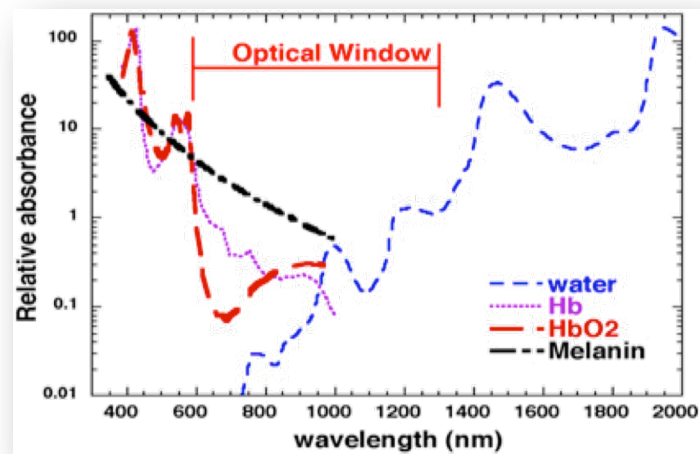
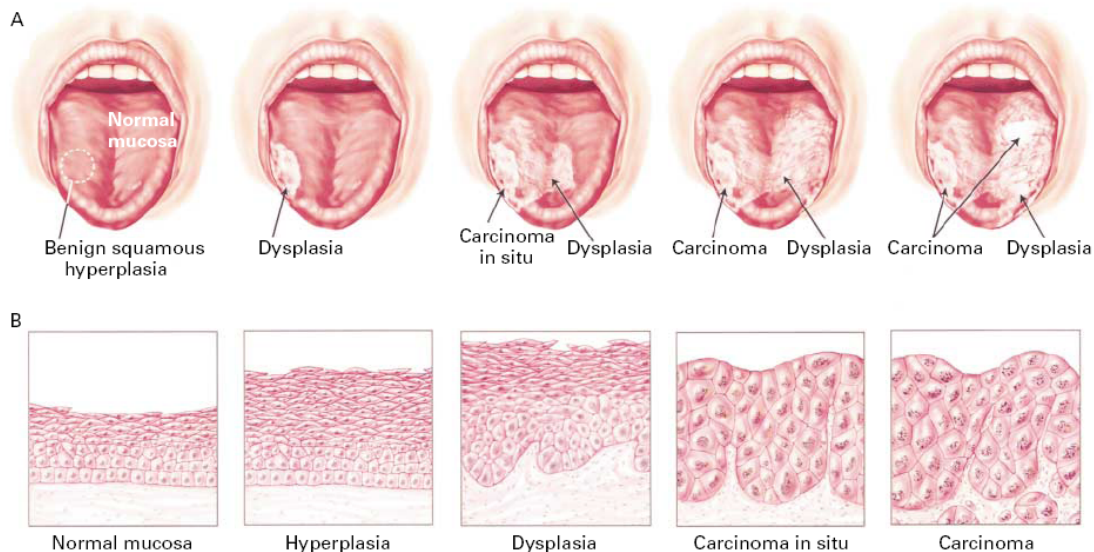
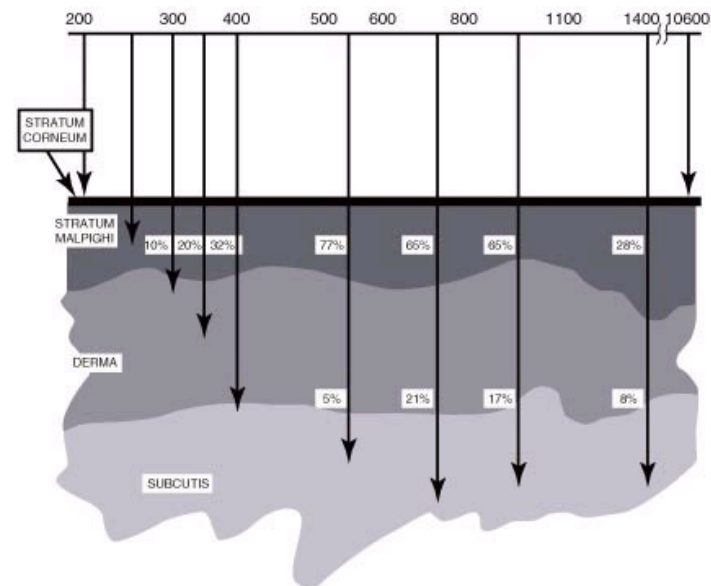
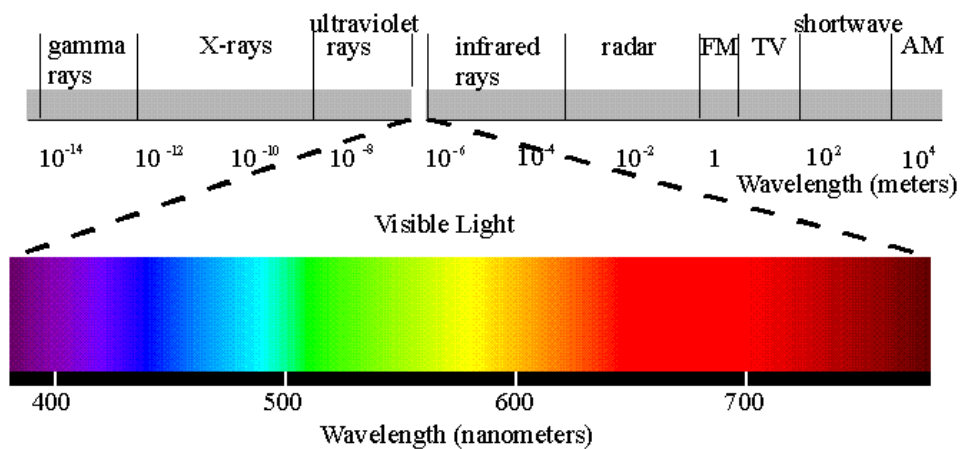
- Anamnese
- Exame clínico
- Biópsia convencional
 - Avaliação histopatológica

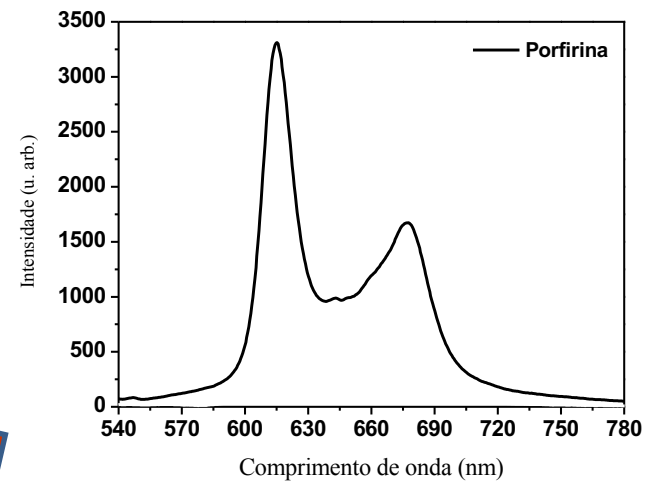
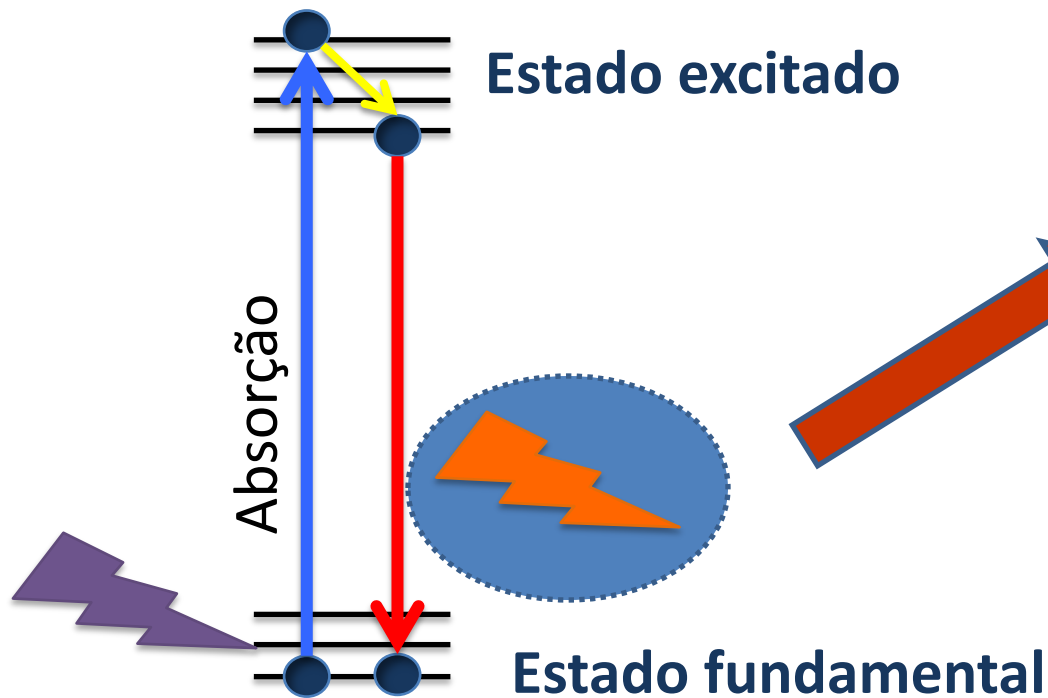
Diagnóstico óptico



Interação da luz com a matéria



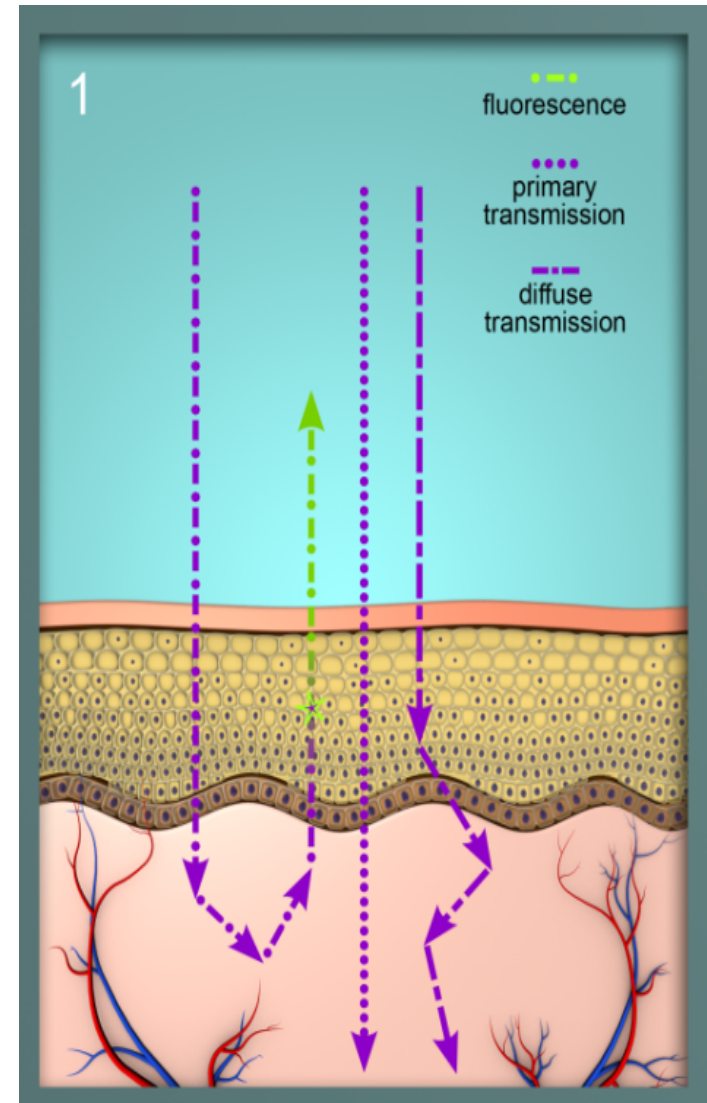
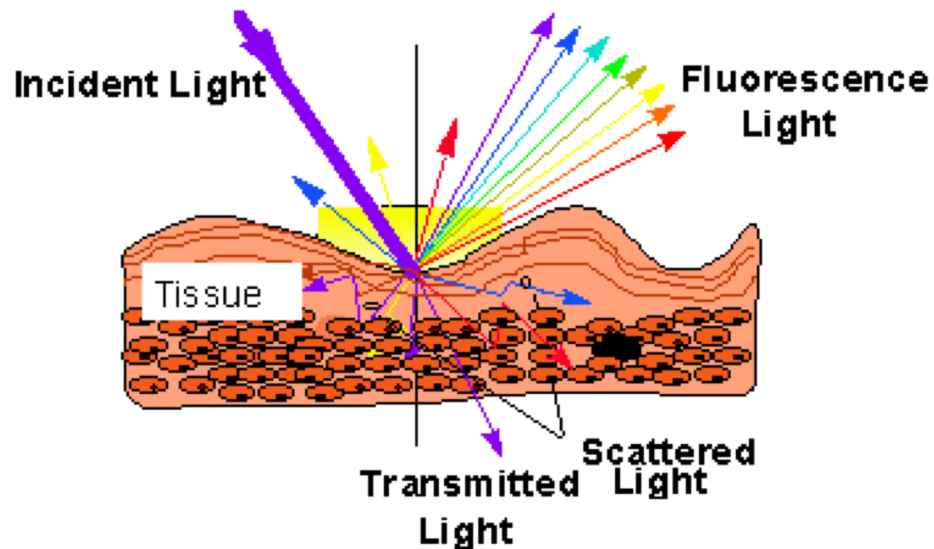


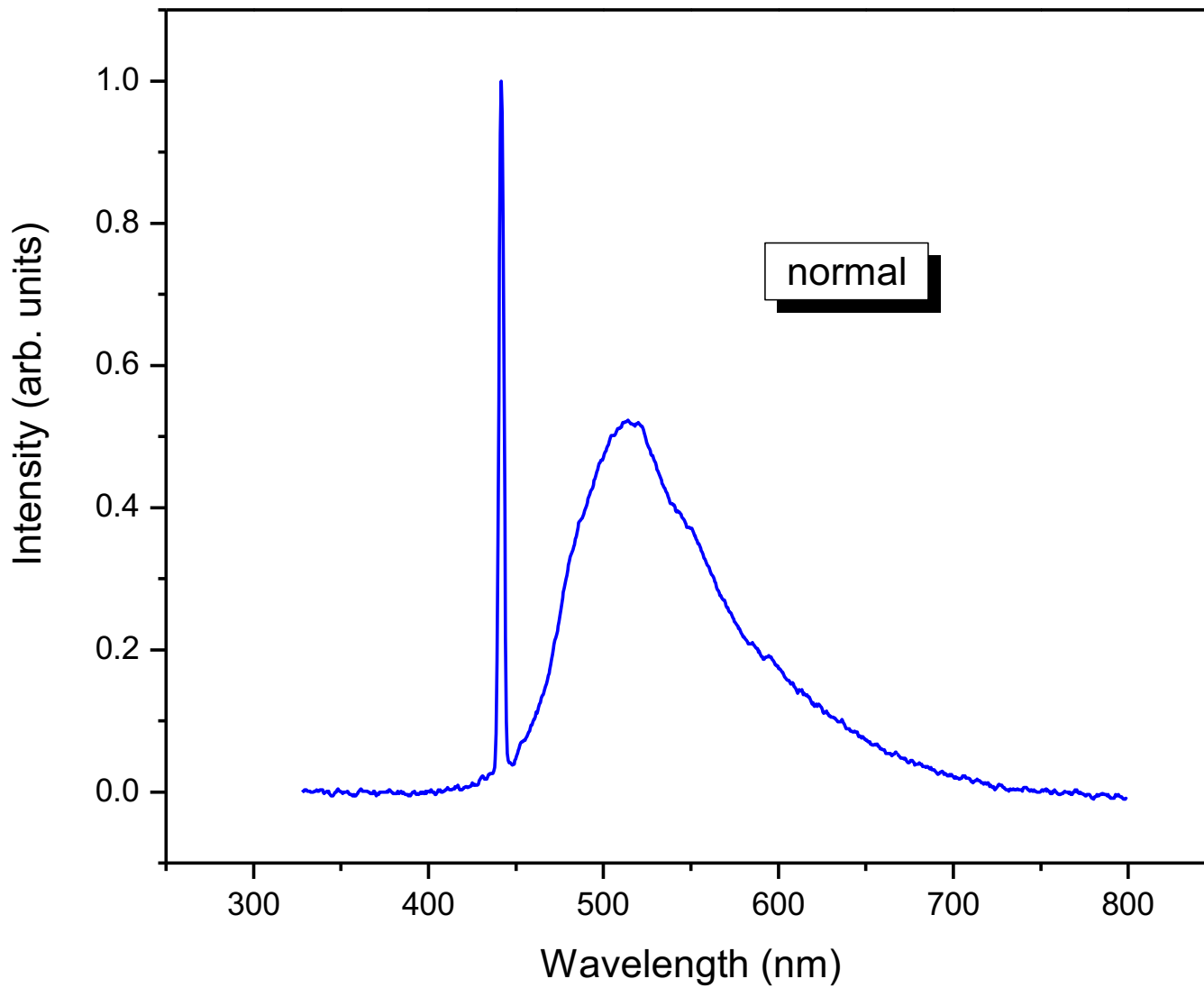


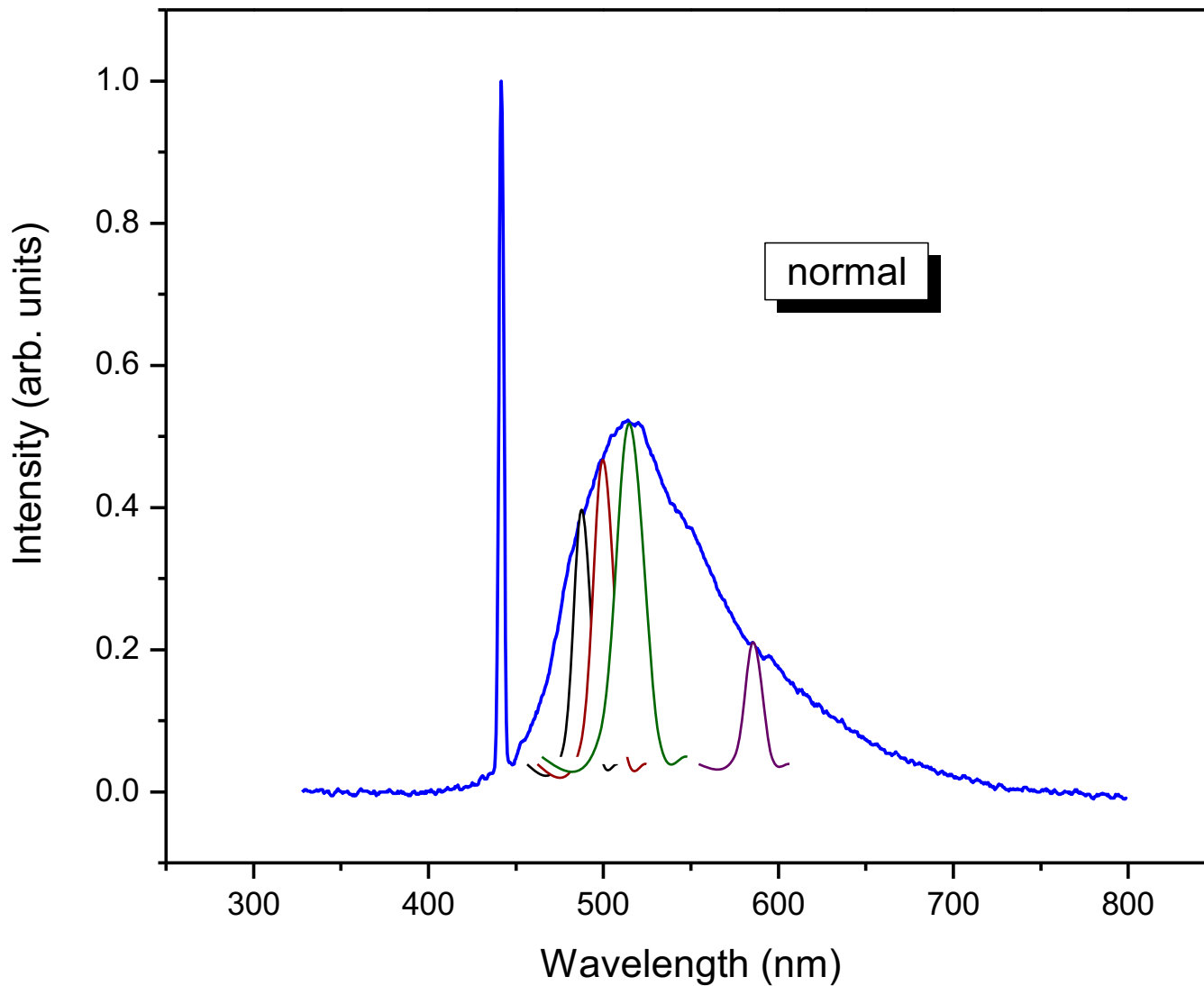
FLUOROPHORES	BIOMOLECULES / CELL LOCALIZATION	EXCITATION Peak position range	EMISSION Peak position range
Aromatic AminoAcid Residues	Proteins A= Phe+Tyr; B= Trp+Phe+Tyr	240 – 280 nm	280 – 350 nm
Collagen Elastin Cytokeratins	Extracellular matrix Connective tissue Epithelia	330 – 340 nm 350, 420 nm 280, 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 & Precursors	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. Bottirolì 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







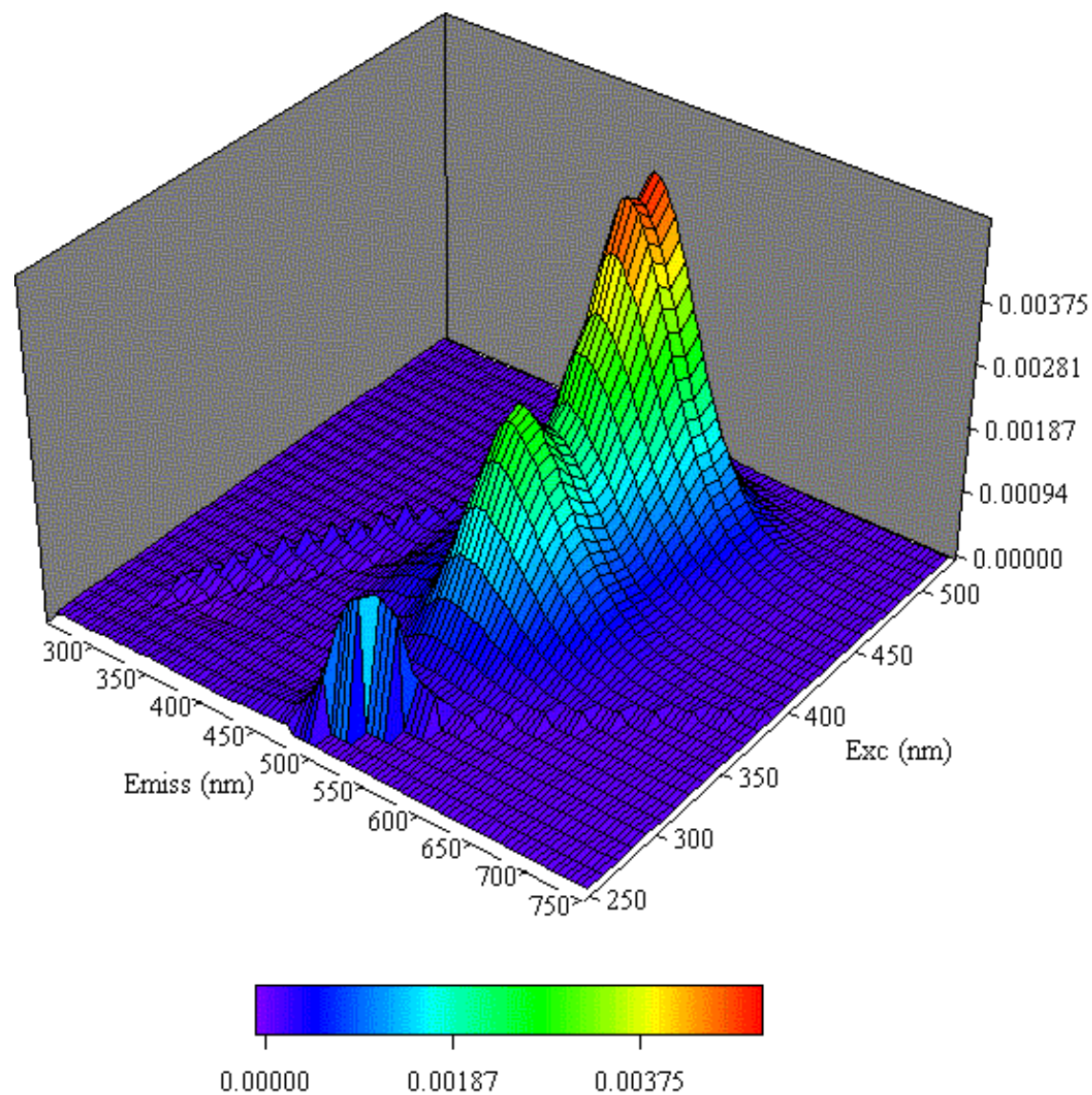
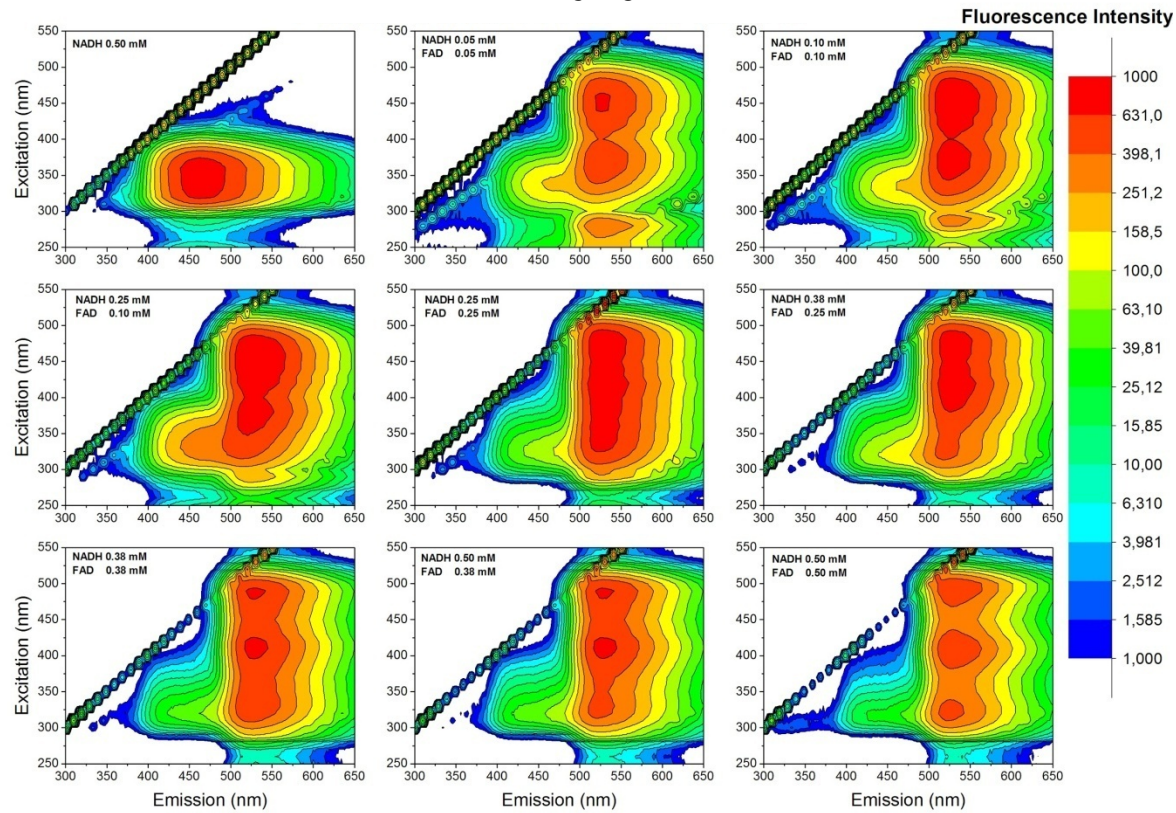
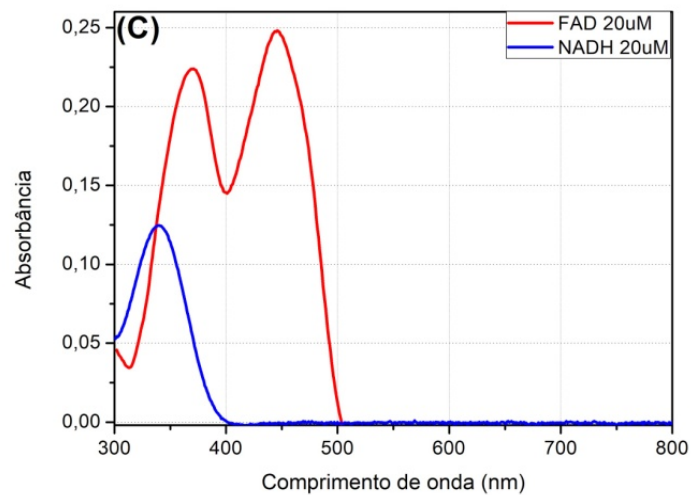


Figure 3: A plotted FAD EEM matrix. (Reproduced from DaCosta et al.)

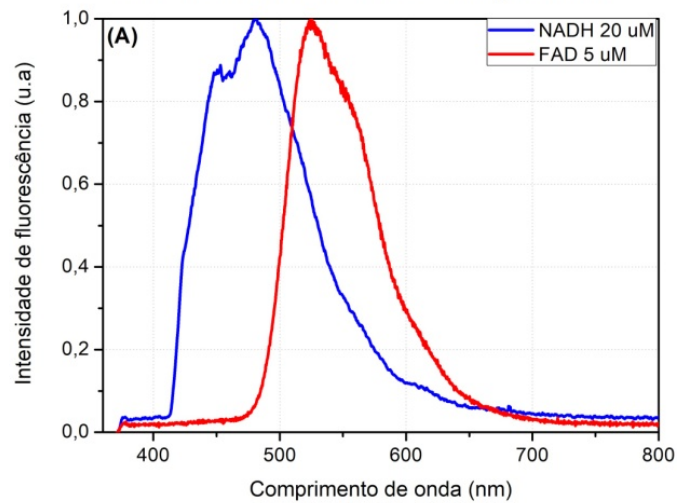
EEM – NADH and FAD



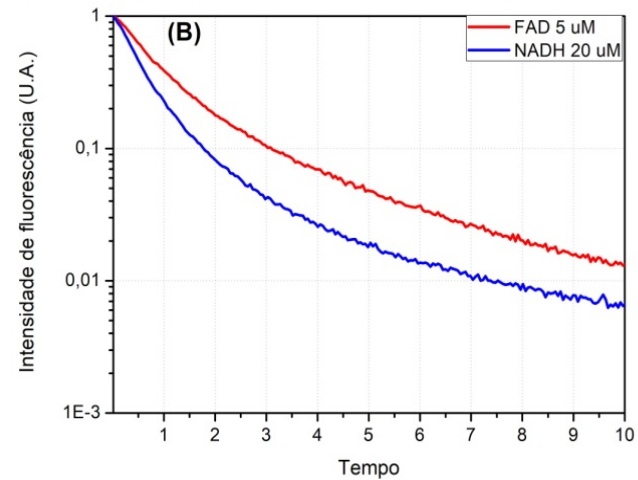
Absorbância NADH e FAD



Fluorescência NADH e FAD - excitação 378nm

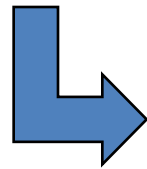
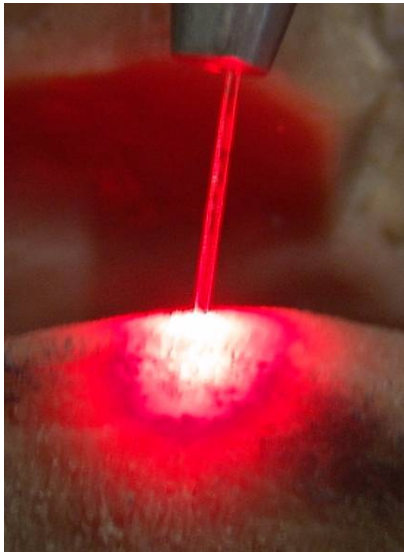


Tempo de vida de fluorescência NADH e FAD - excitação em 378nm



Fundamentos básicos

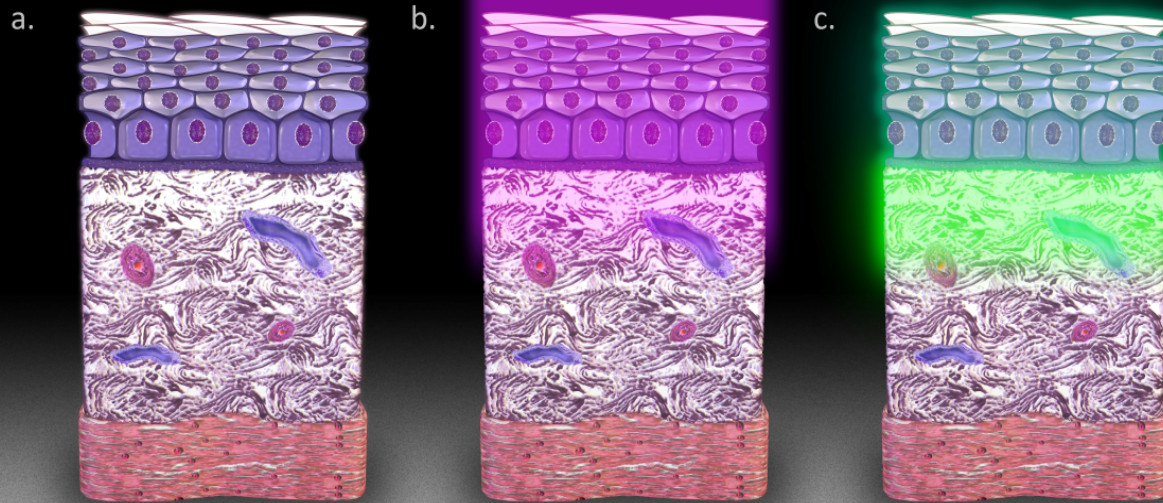
- Interação luz/tecido biológico
- Absorção – espalhamento
- **Fluorescência**
 - Fluoróforos biológicos (tipos e quantidades)
 - Dependência espectral: λ_{exc} , microambiente



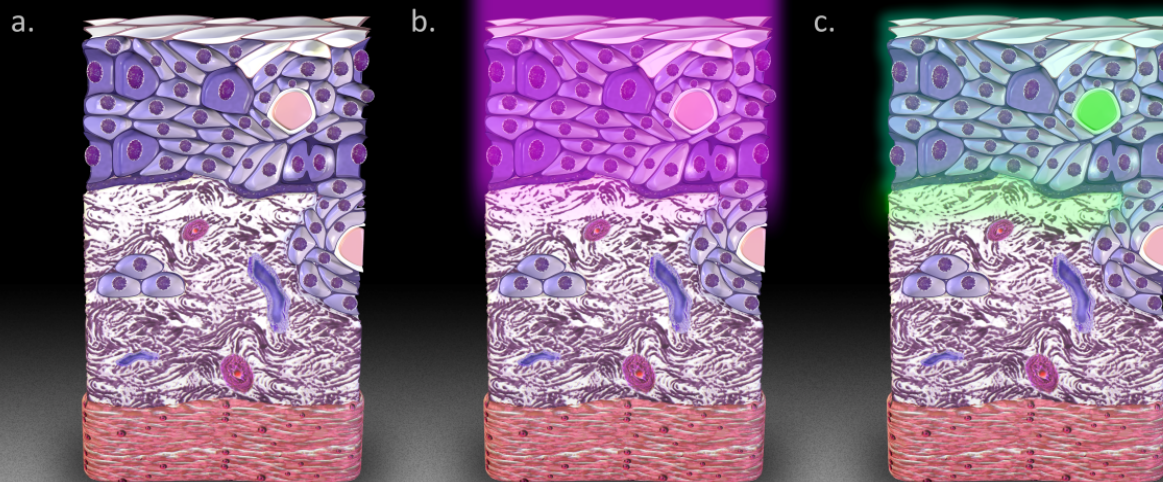
Diferenciação de tipos teciduais ou de alterações químicas

➤ Caracterização tecidual bioquímica e estrutural (arquitetura)

Normal oral mucosa



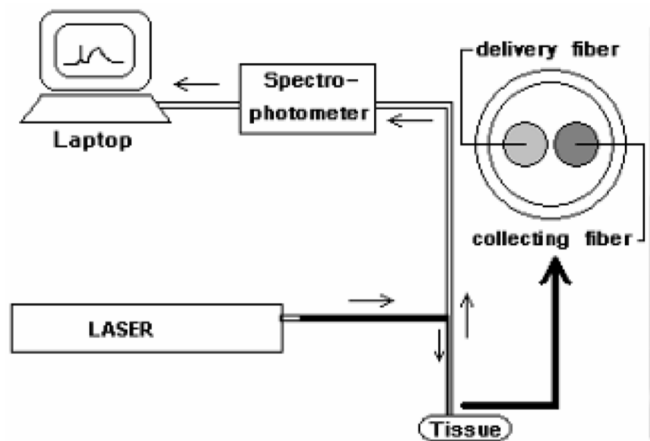
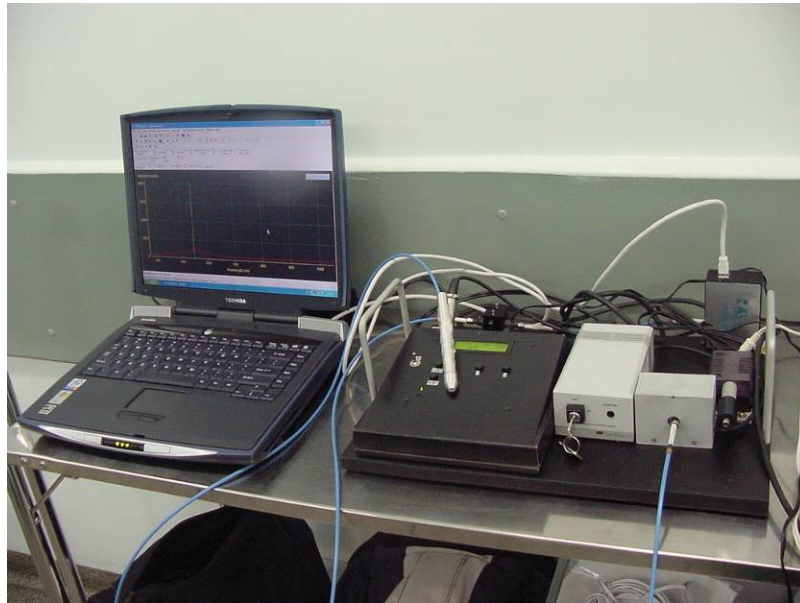
Oral carcinoma

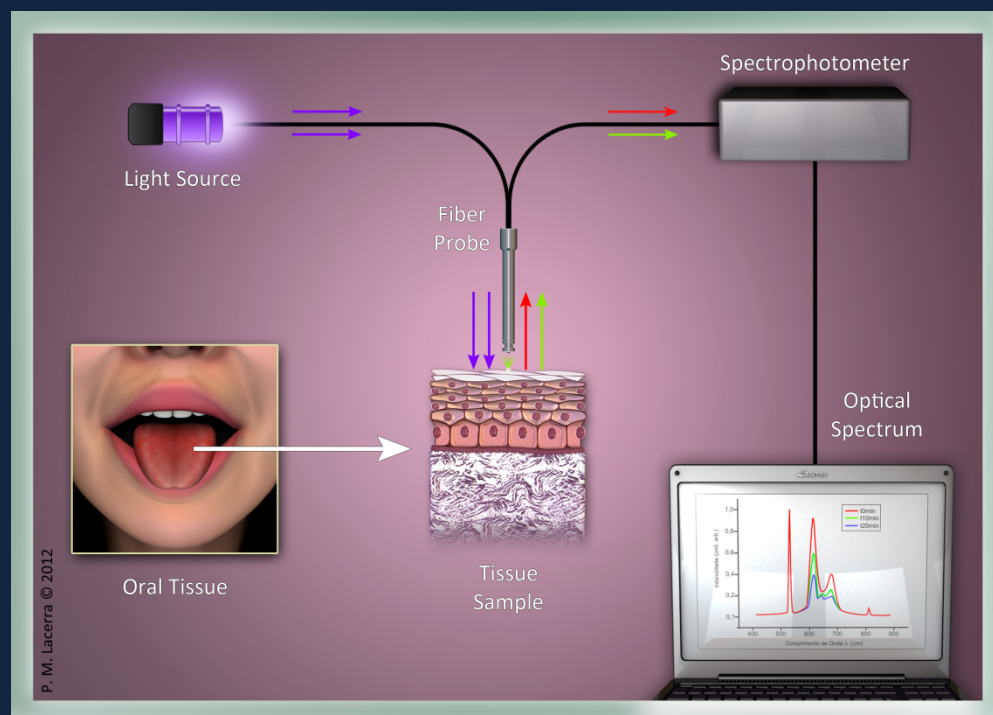
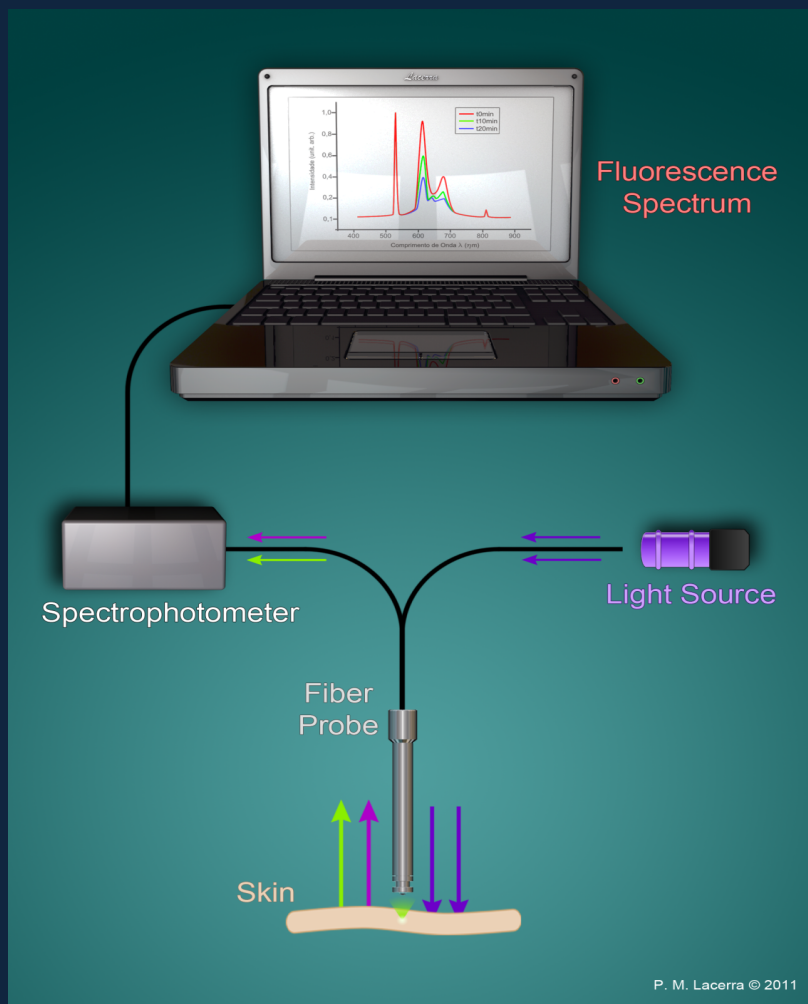


Espectroscopia de fluorescência

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





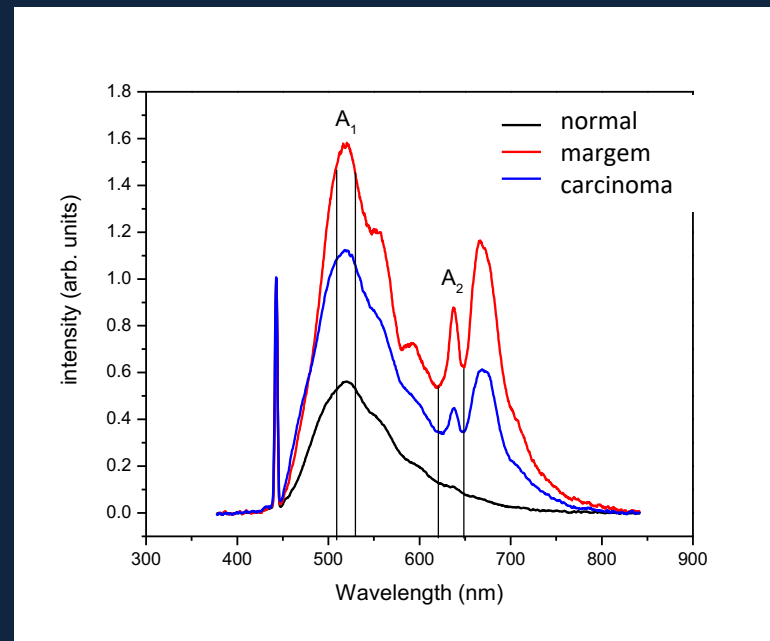
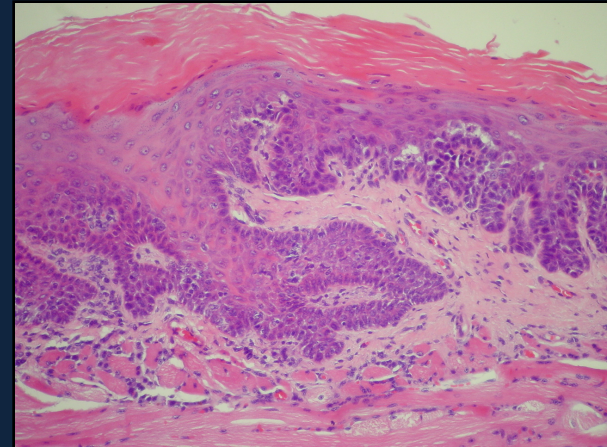


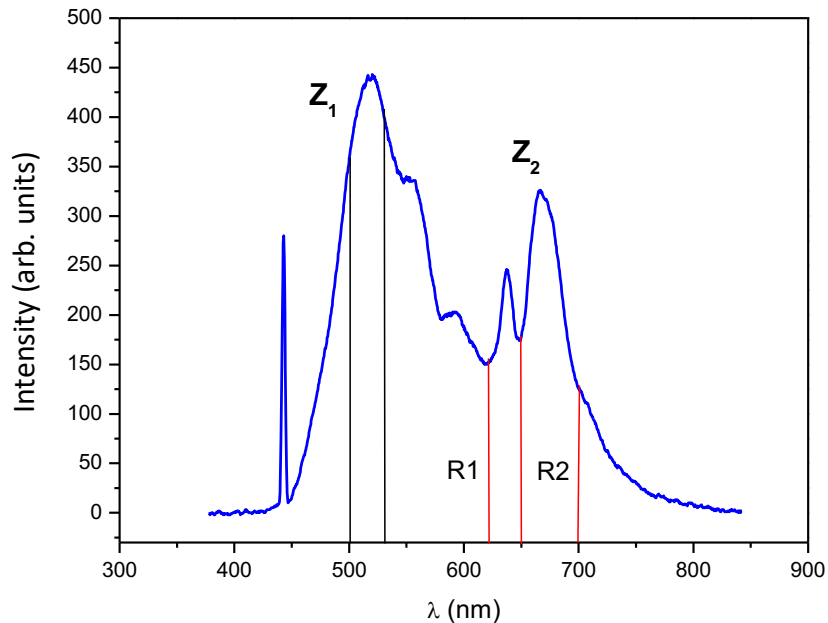
Espectroscopia de fluorescência – CEC de língua

Padrão espectral
("assinatura espectral")



Características
histopatológicas





Processamento espectral

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

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

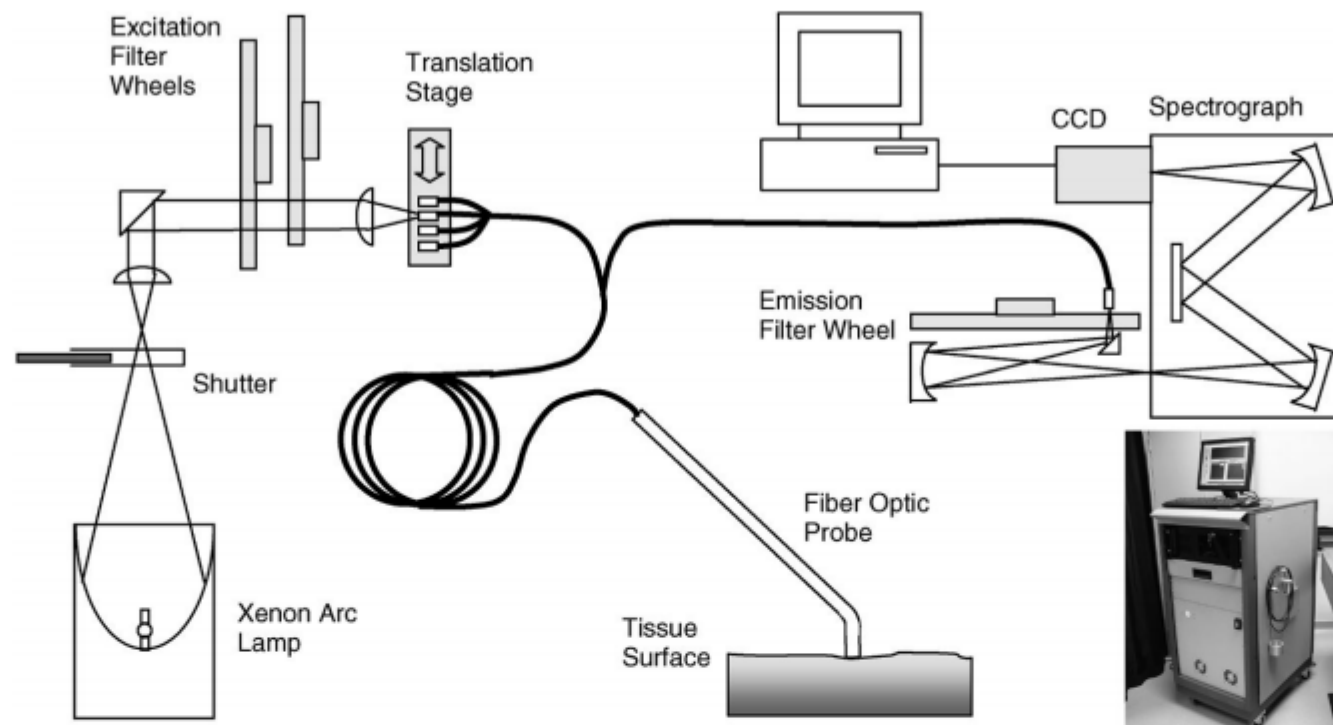
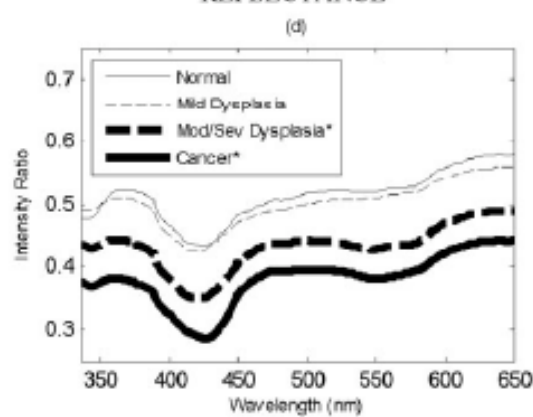
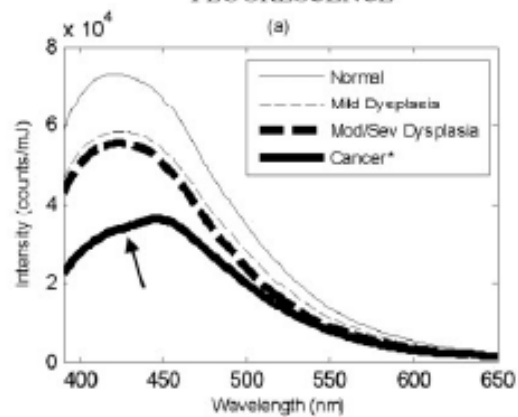


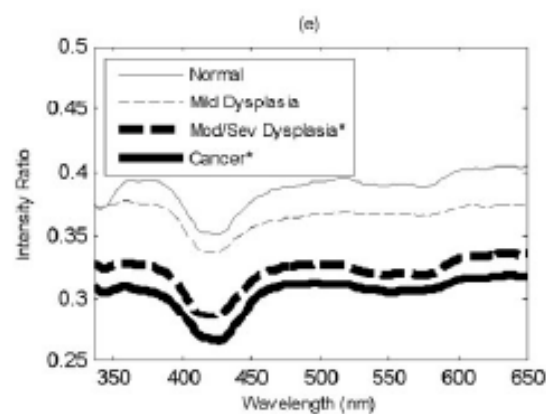
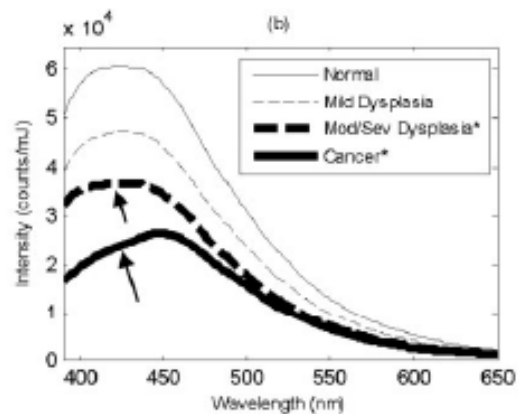
Fig. 1.
Diagram and photograph of the clinical spectroscopy system.

FLUORESCENCE

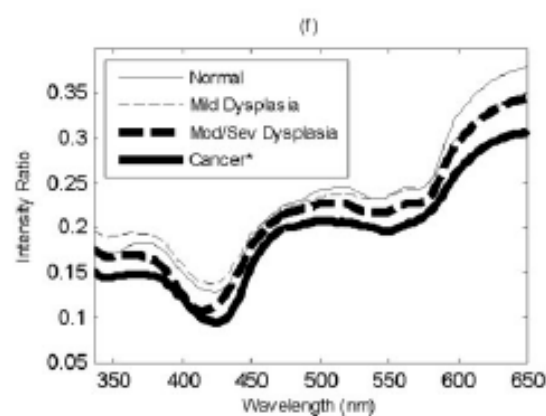
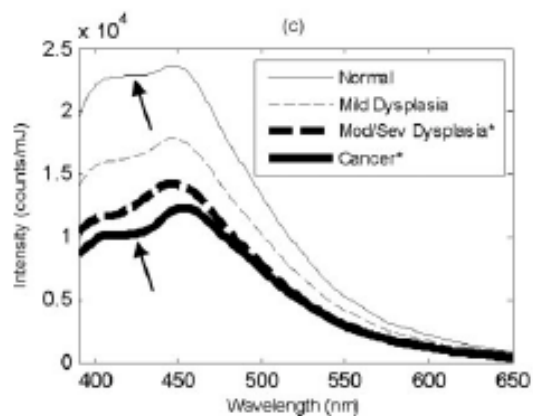
REFLECTANCE



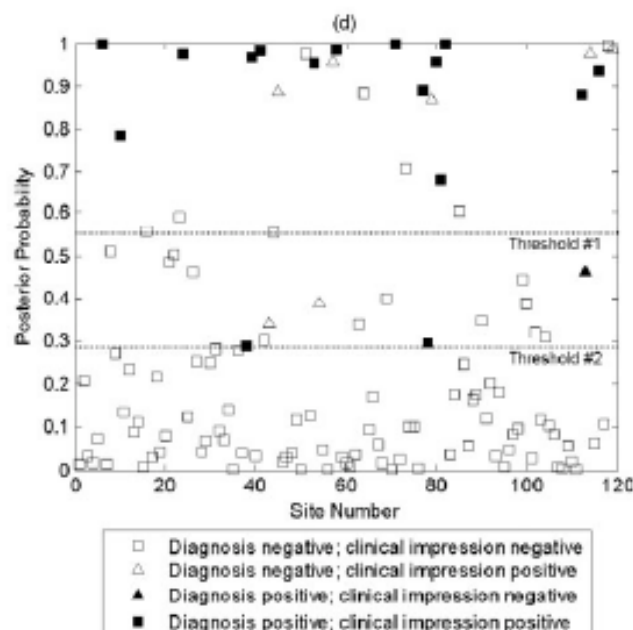
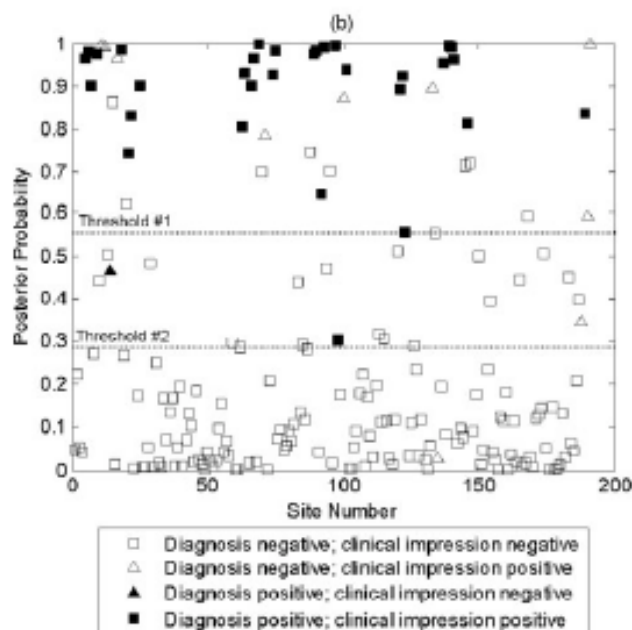
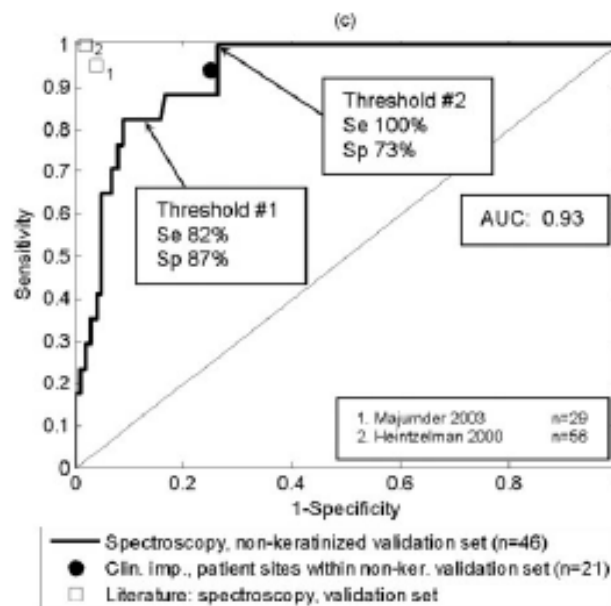
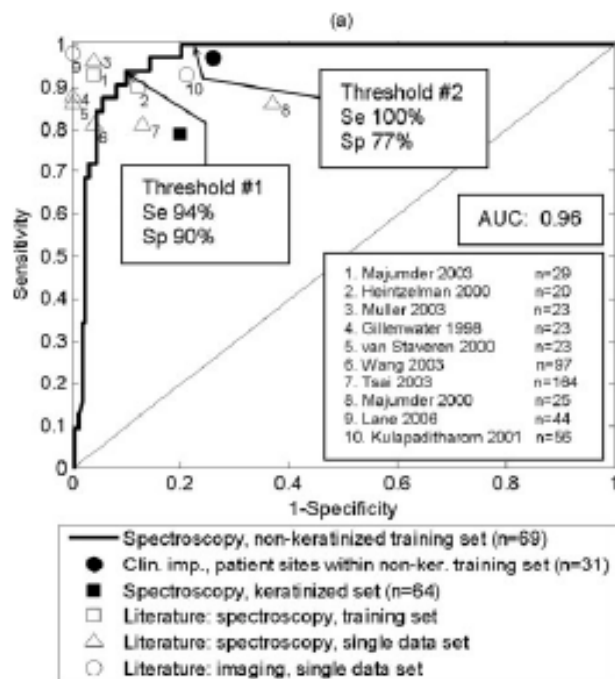
SHALLOW



MEDIUM



DEEP



ESPECTROSCOPIA DE FL



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



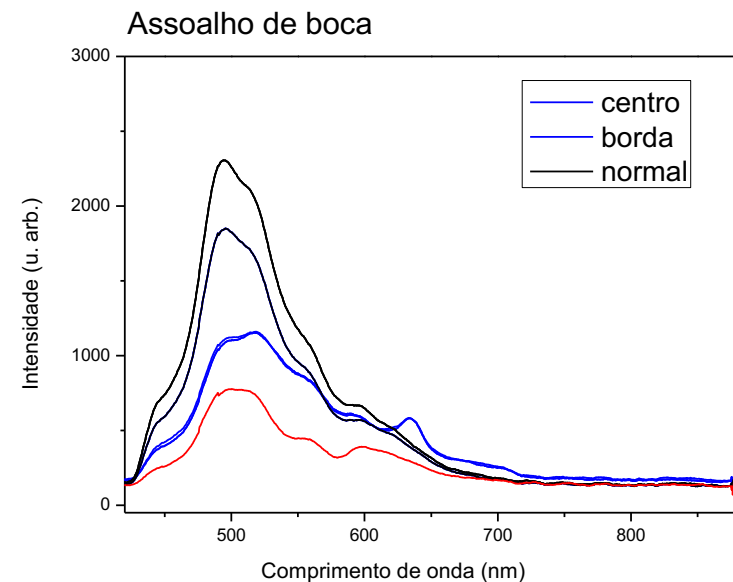
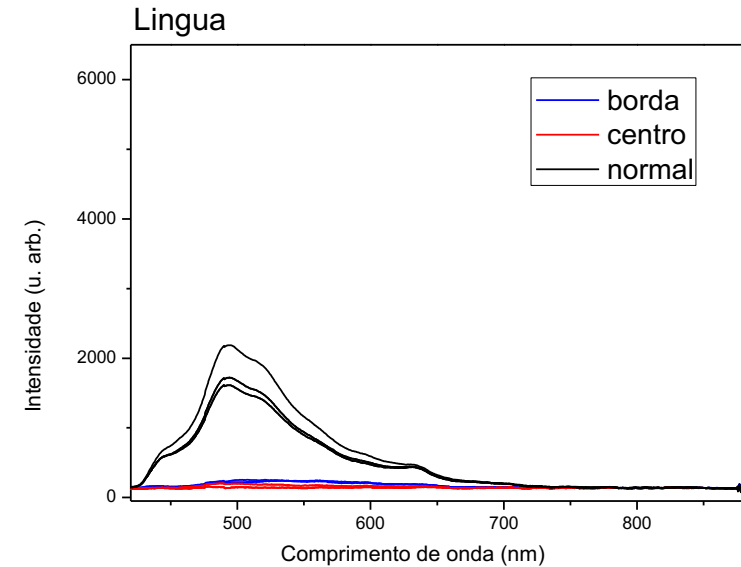
Espectroscopia de fluorescência – CEC de boca



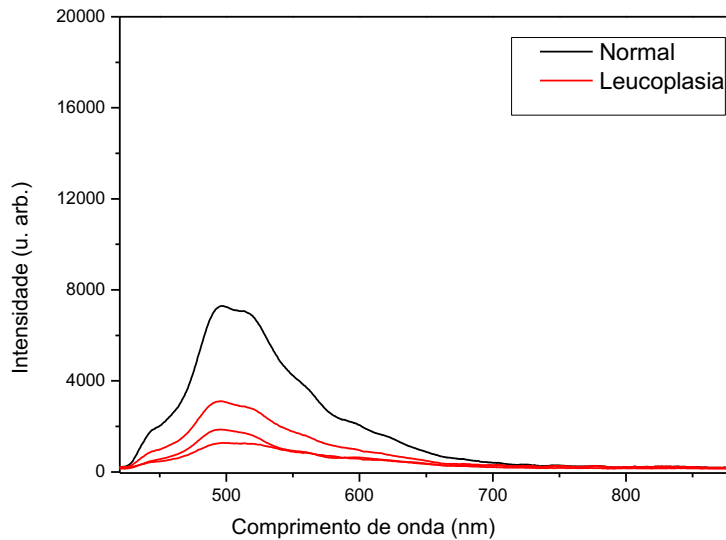
Padrão espectral
("assinatura espectral")



Características
histopatológicas



Assoalho bucal

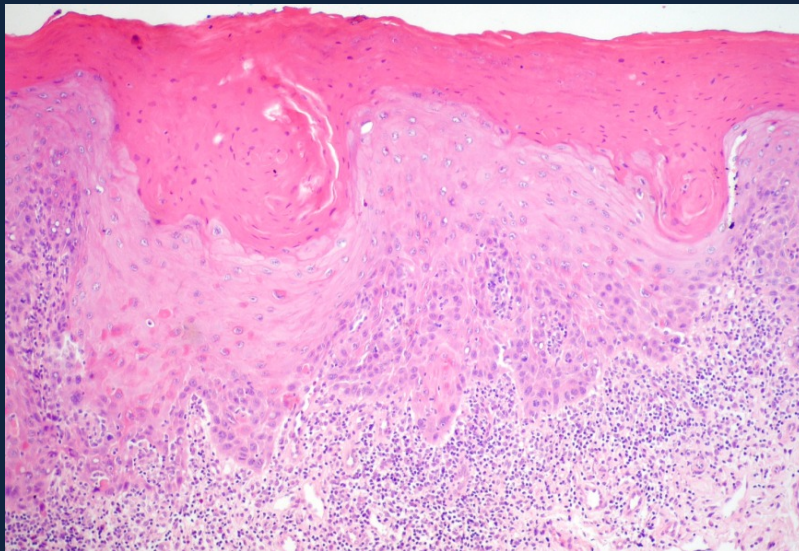


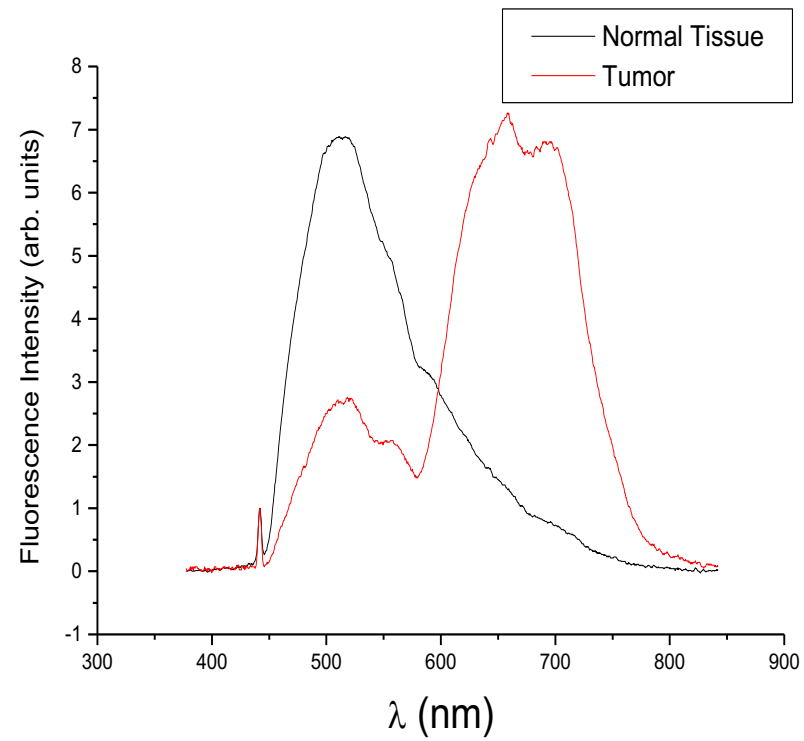
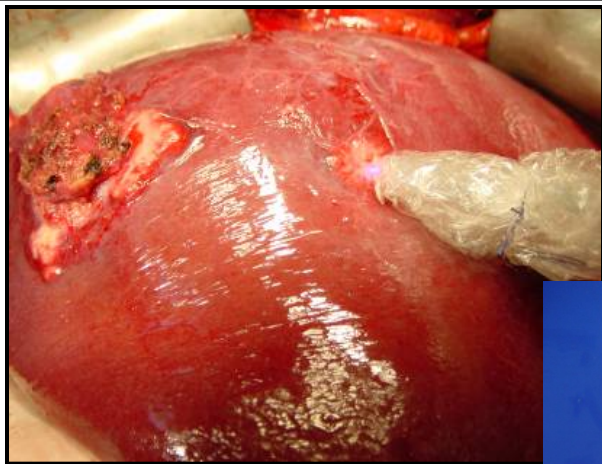
Processamento espectral

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



Acurácia e precisão ~85%

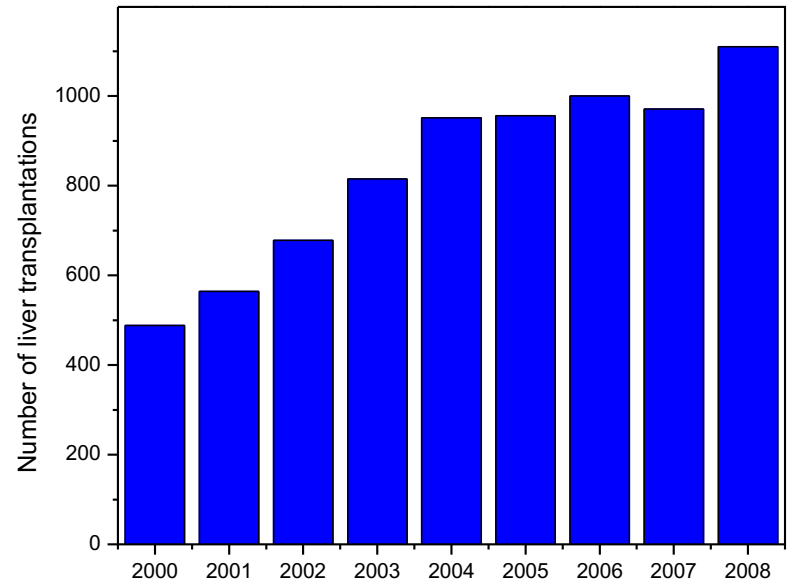




Liver transplantation

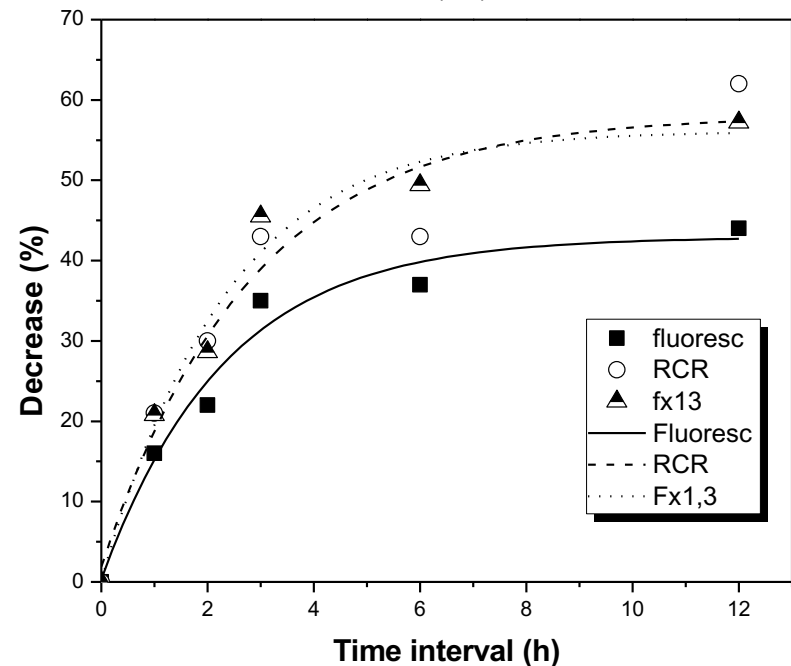
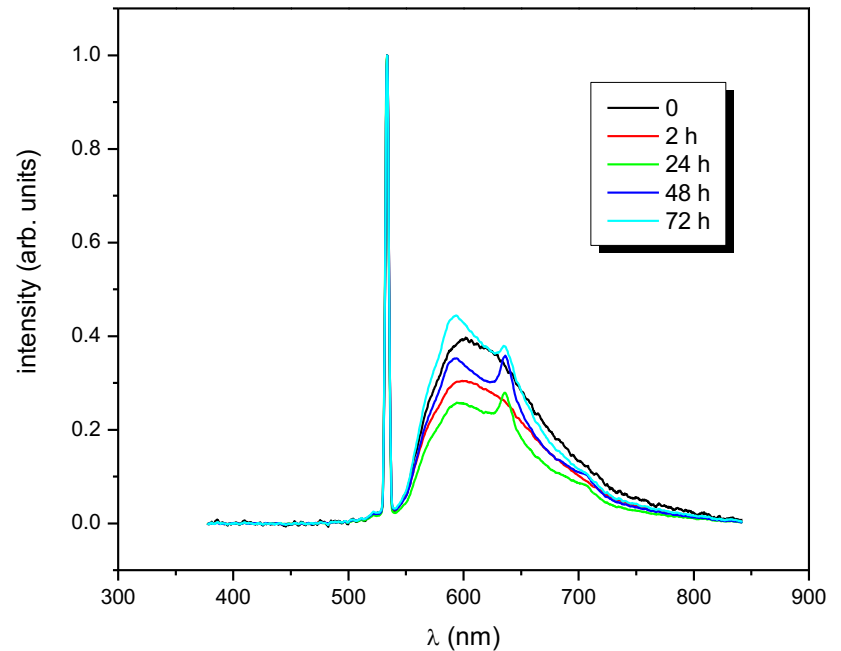
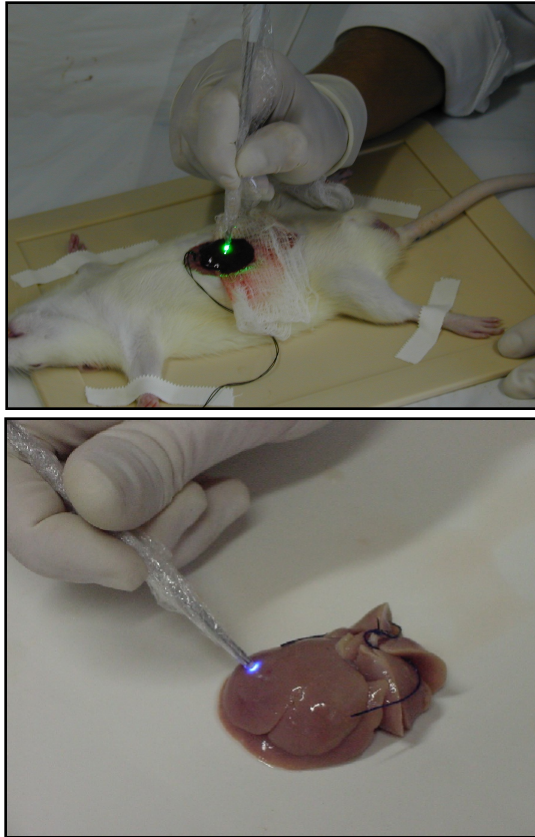
2005: Brazilian data

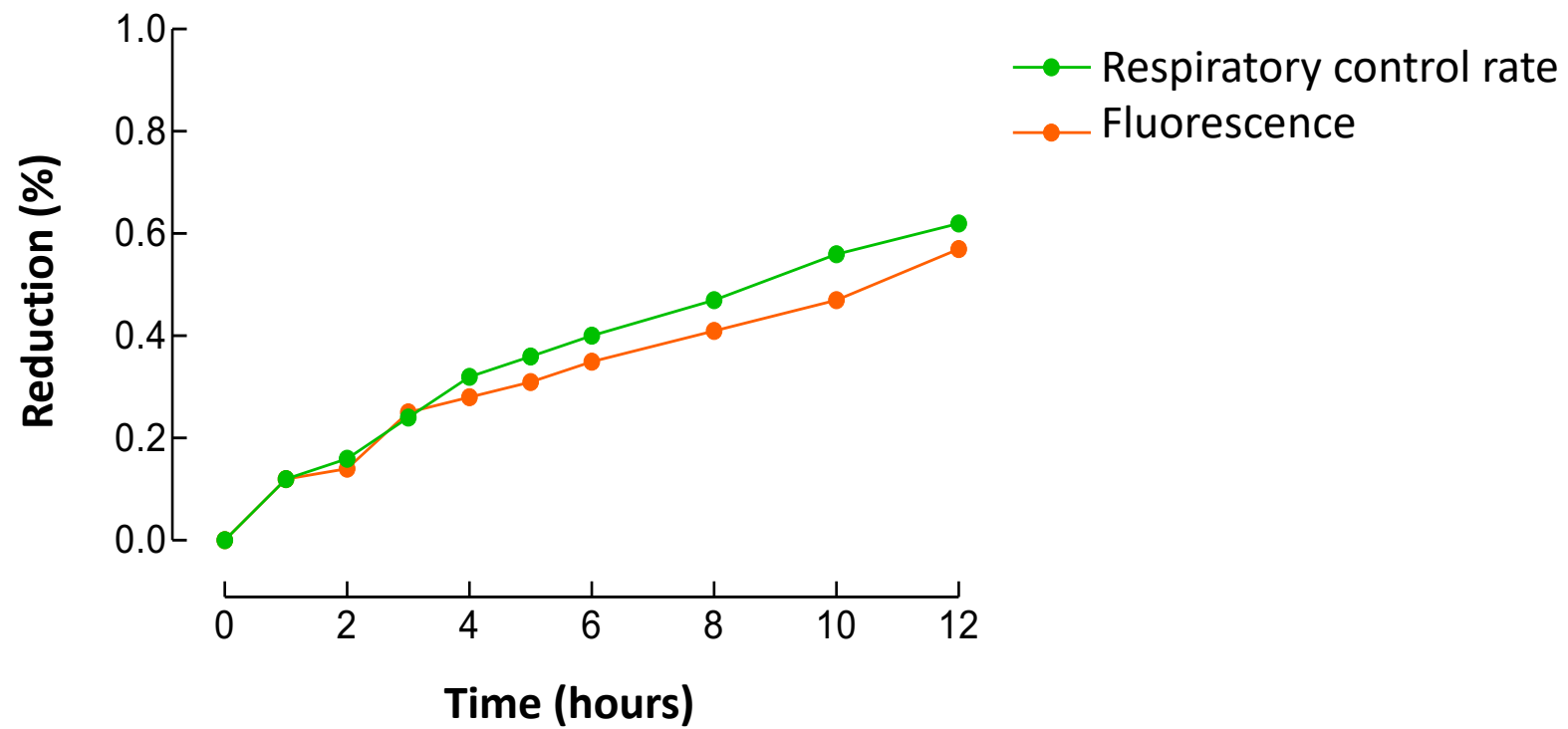
- 956 transplantations
 - 6,288 patients at the waiting list
- 20% mortality rate of patients at the waiting list



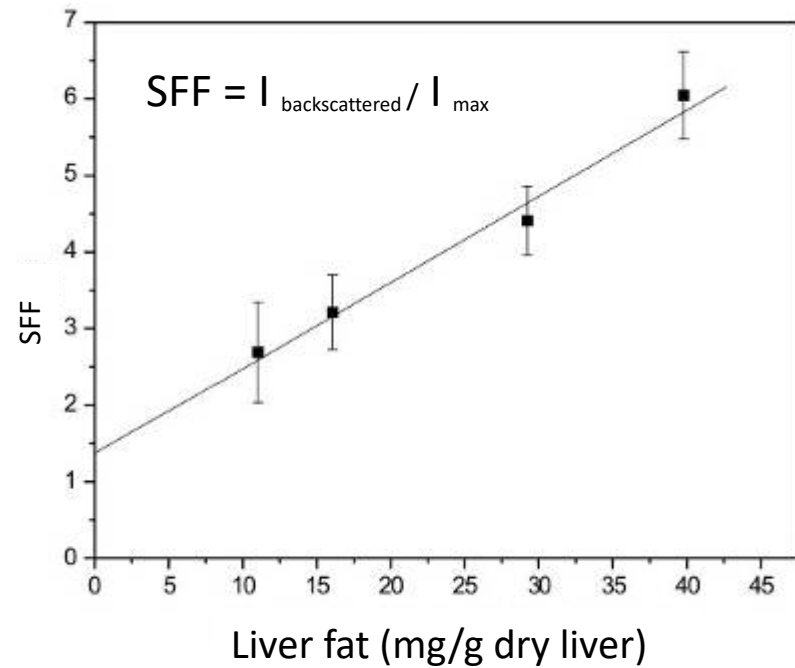
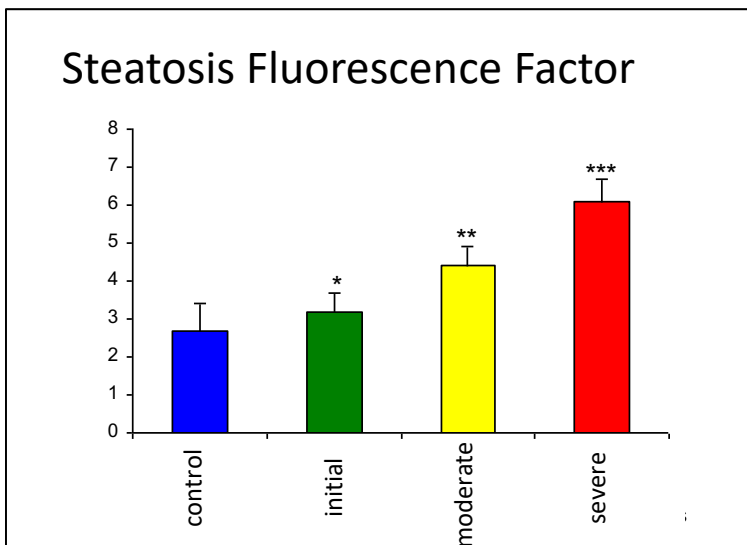
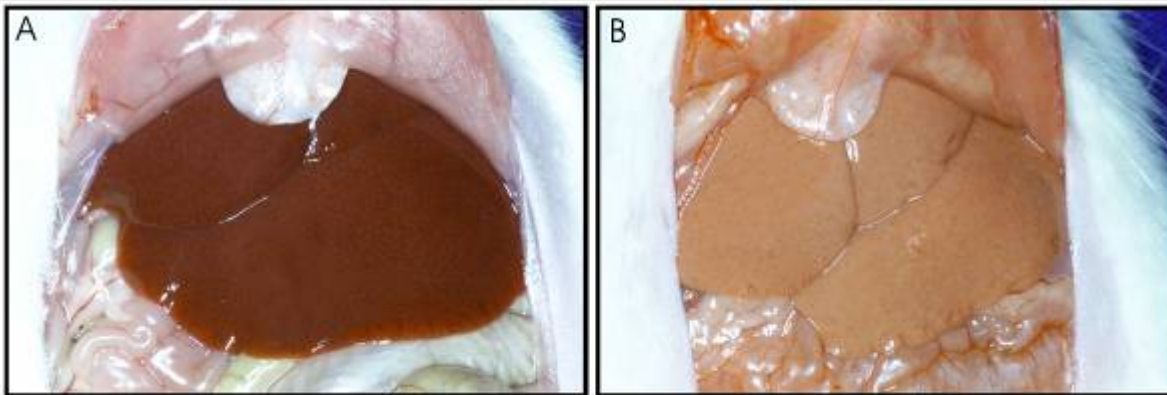
Evaluation of liver graft status (transplantation)

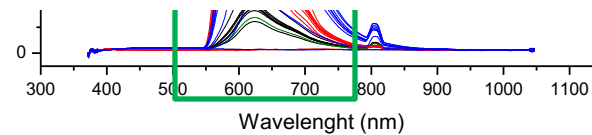
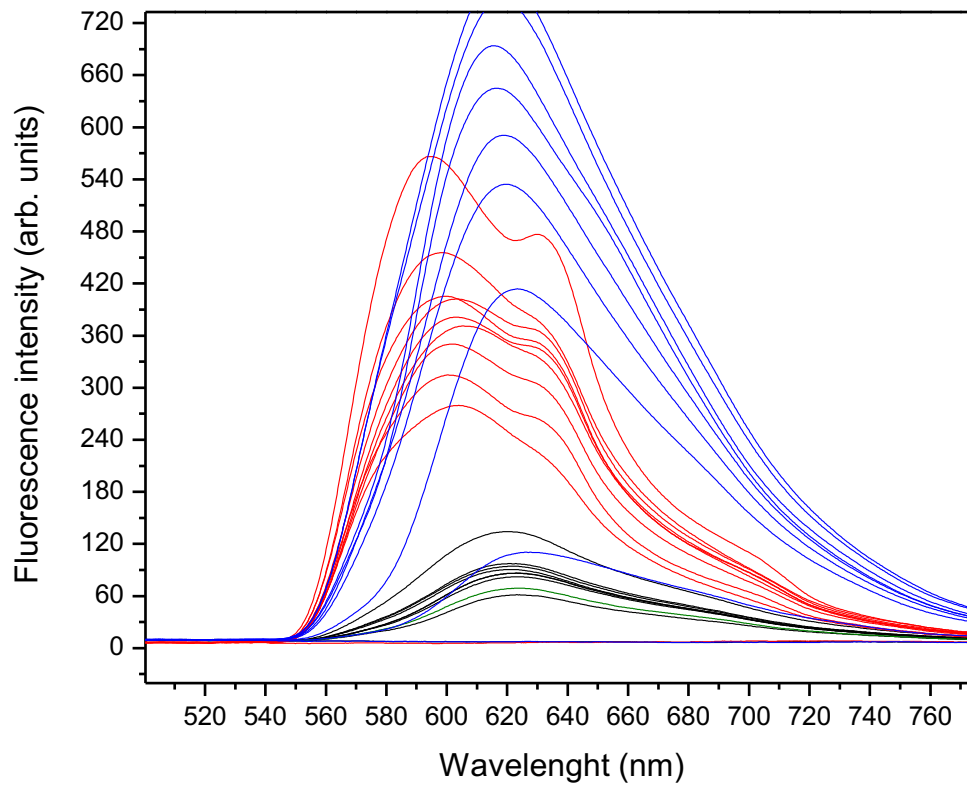
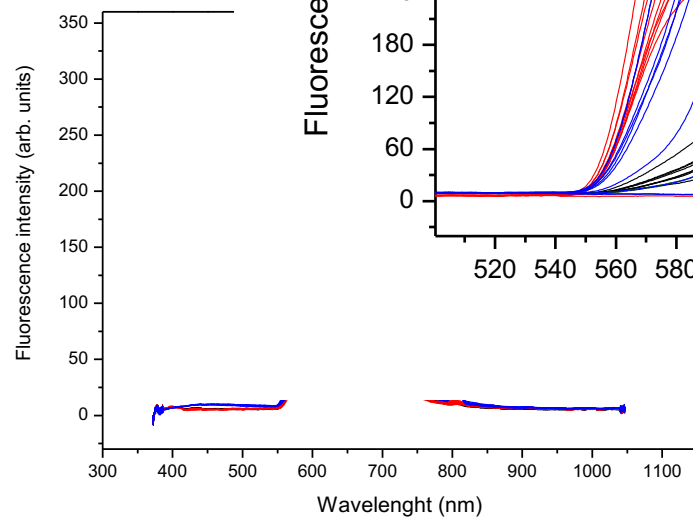
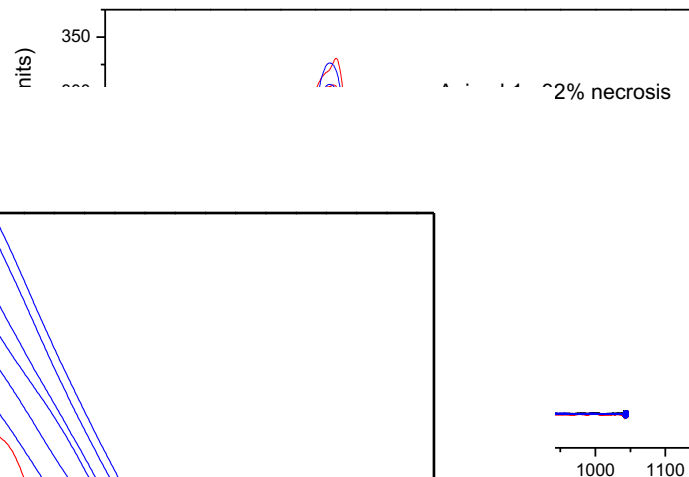
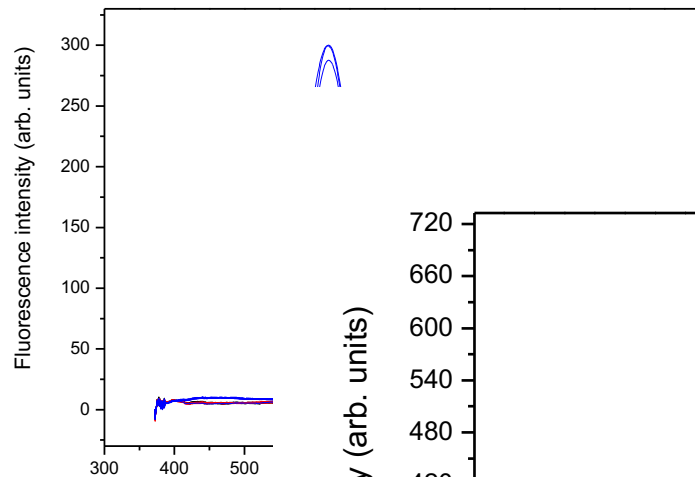
- Comparison with the mitochondrial respiratory rate





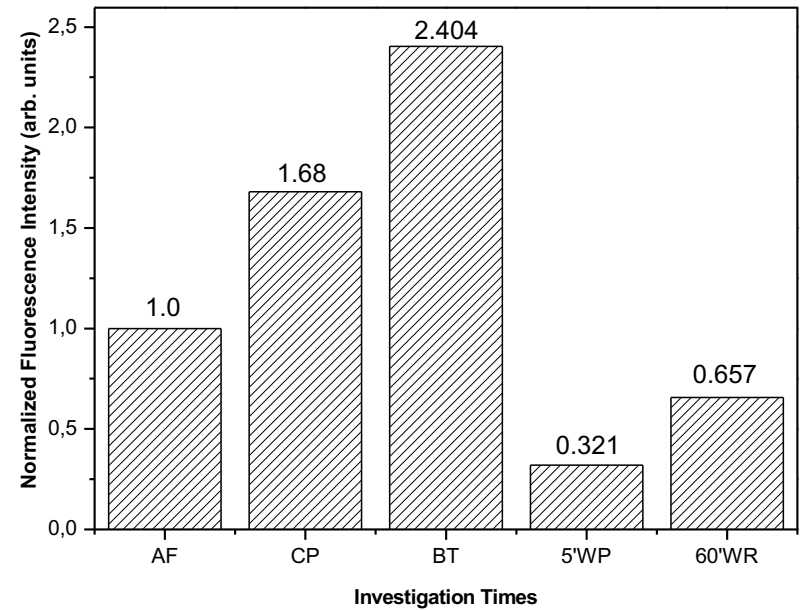
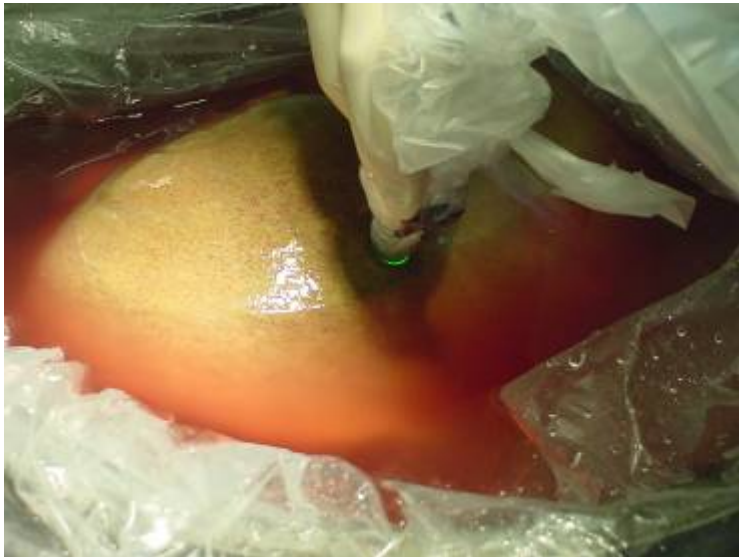
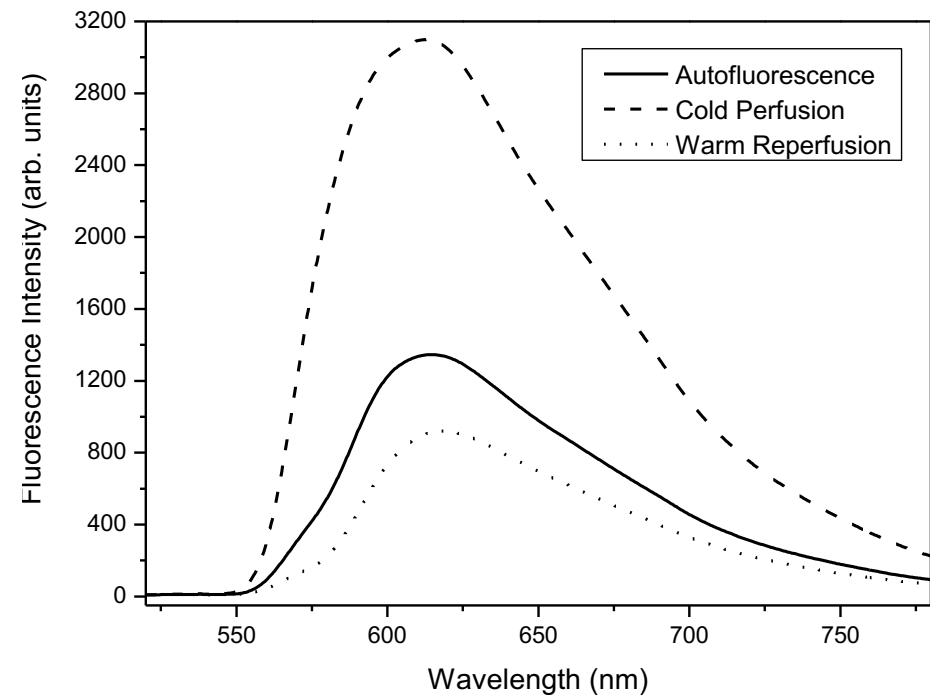
Liver steatosis







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



Determinação do intervalo post-mortem usando a espectroscopia de fluorescência



Medicina forense

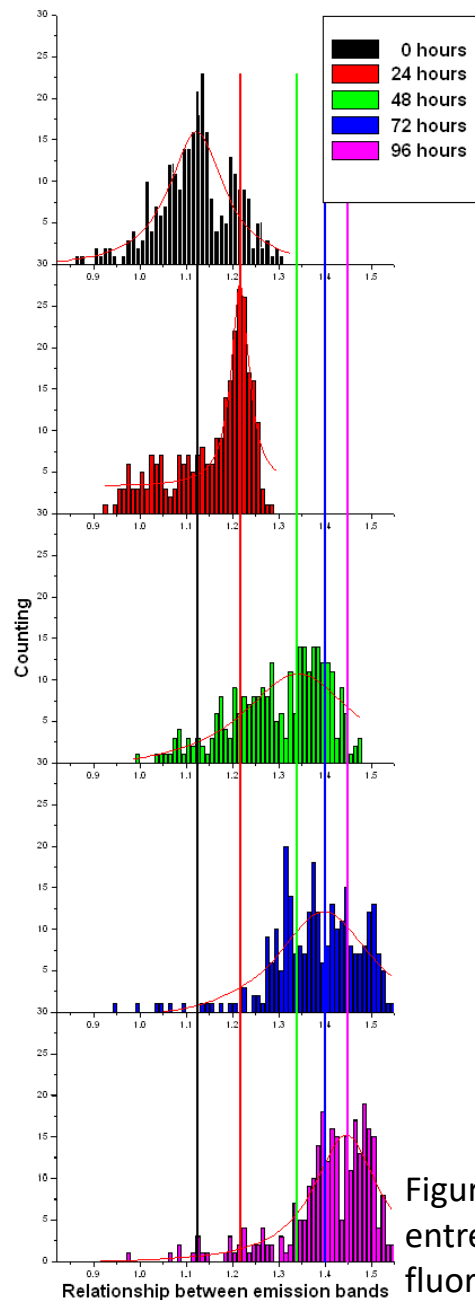


Figura 8: Distribuição da razão entre os picos de emissão de fluorescência

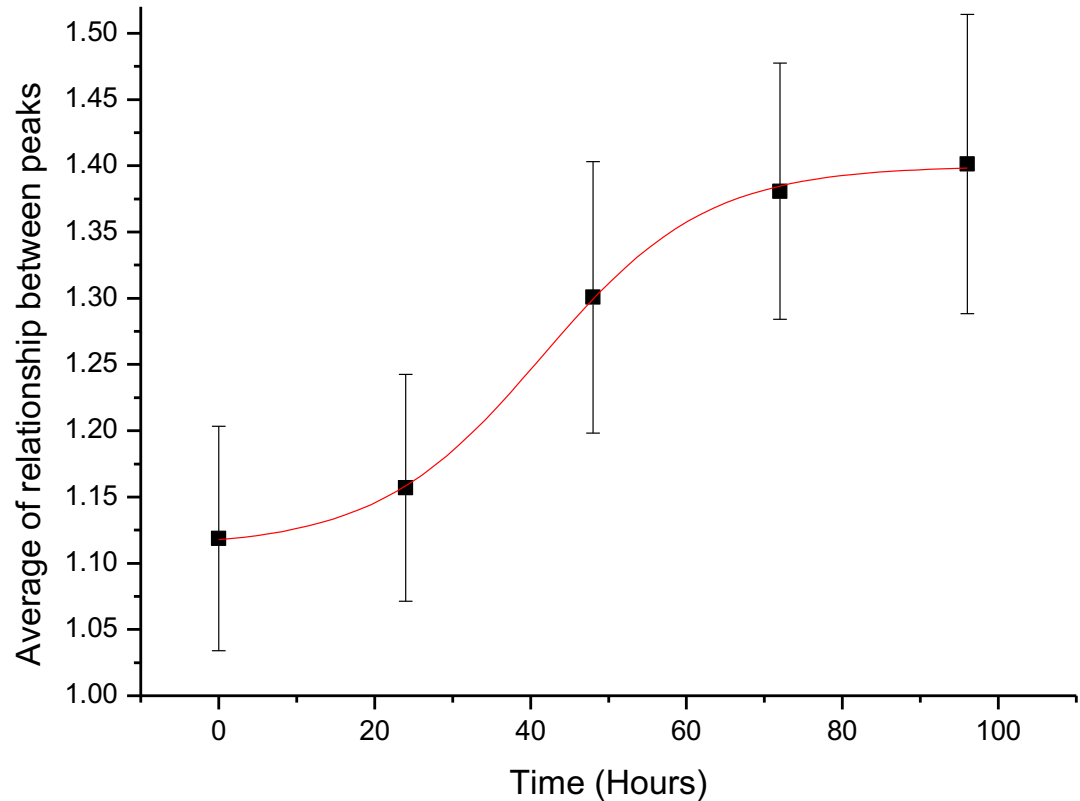


Figura 9: Evolução temporal do valor médio da razão entre os picos de fluorescência

Excitação em 408nm - Normalização em 488nm

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

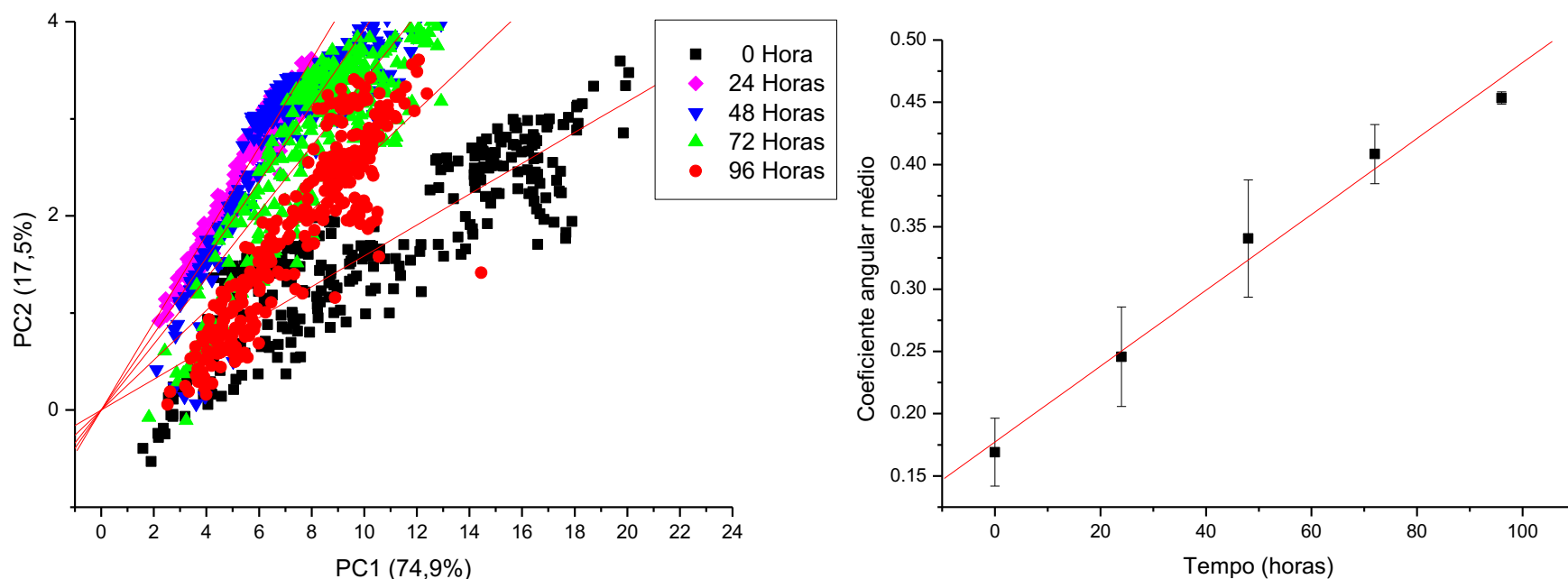


Figura 15: PC1 *versus* PC2 dos espectros no intervalo de 0 à 96 horas pós-morte (esquerda), variação temporal do coeficiente angular médio (direita) ;

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

Espectroscopia de tempo de vida de FI

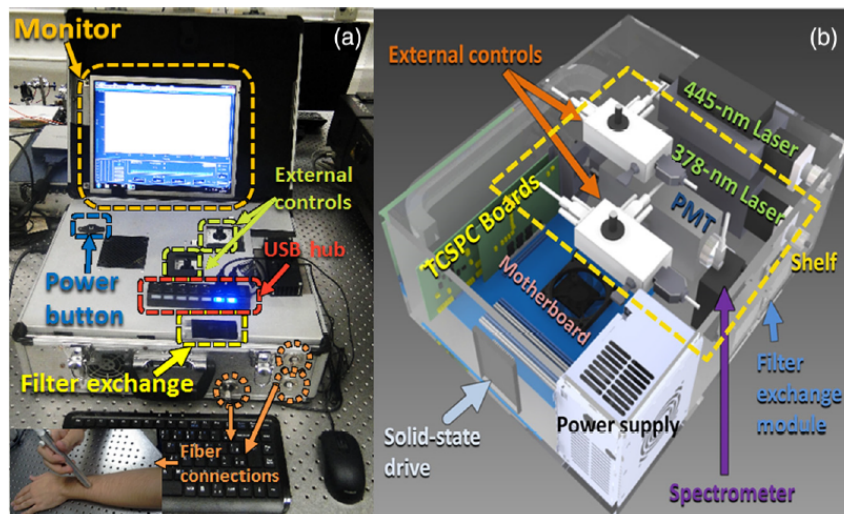


Fig. 3 (a) System assembly after its encasement provides protection for its components and a user-friendly design by exhibiting a control panel with access to external controls and the filter holder. The sample can be placed in any region around the system within the range of the optical fiber (2 m of 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.

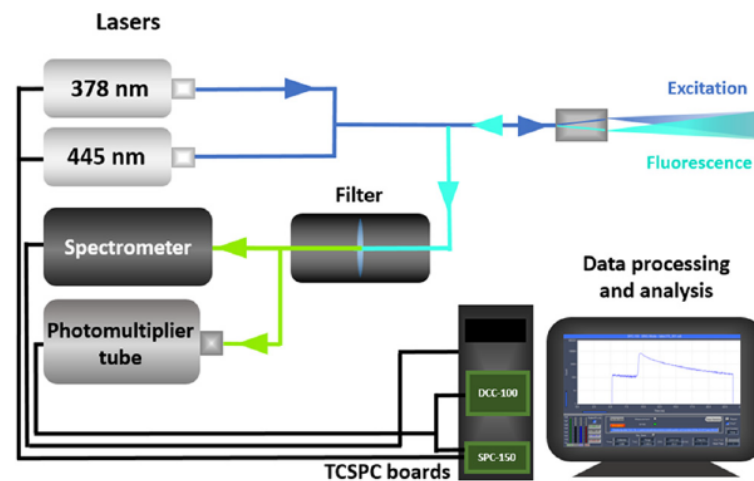
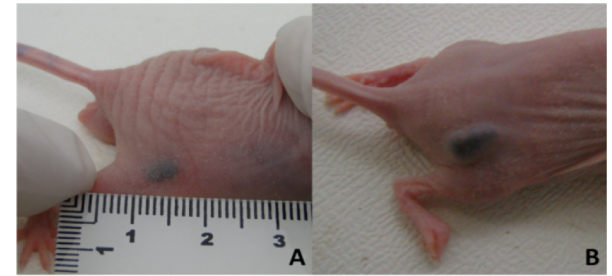


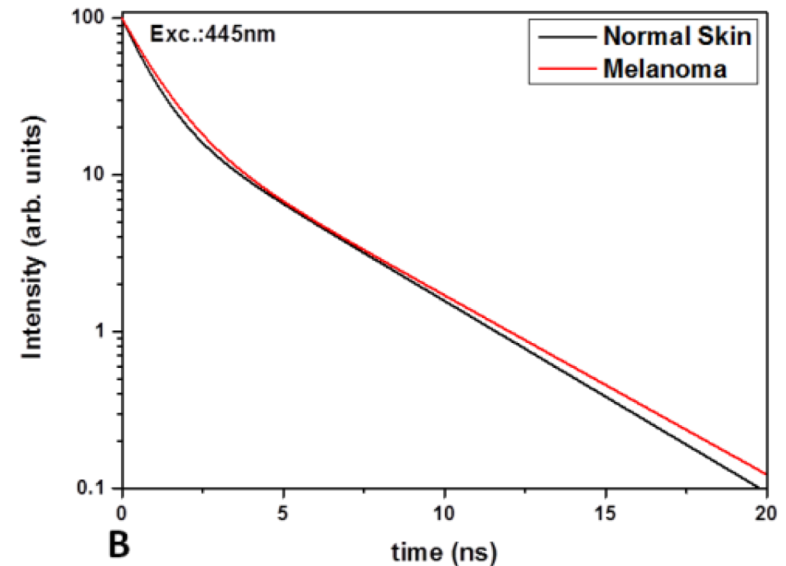
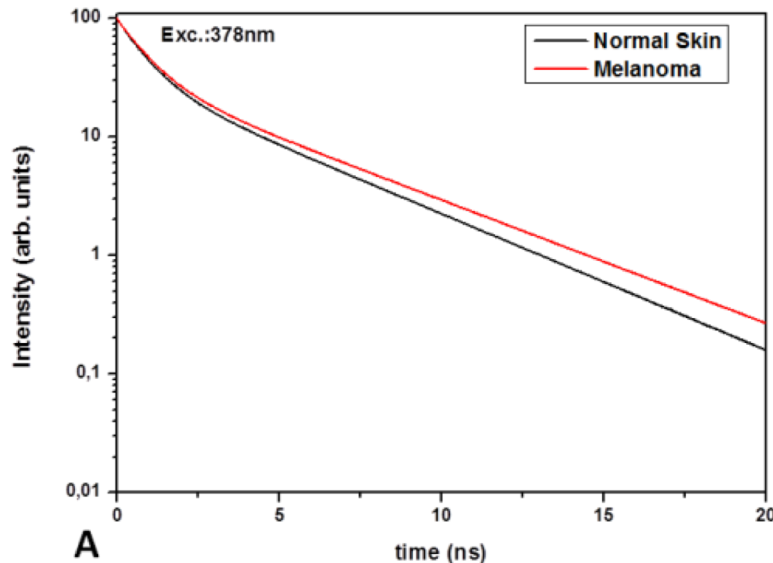
Fig. 1 Schematic drawing of the components for acquisition of fluorescence spectra and fluorescence lifetimes. The excitation light of one of the diode lasers (378 or 445 nm) is delivered to the sample through a bifurcated fiber optic probe. The fluorescence and back-scattered light of this sample are 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).

- 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.

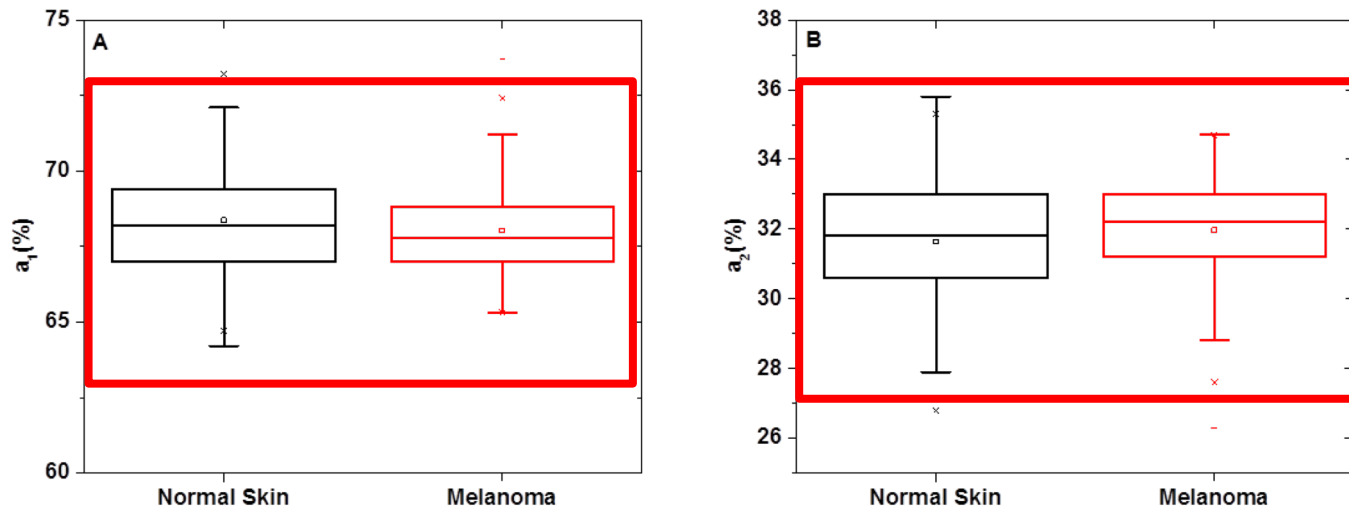


Camundongo nude BALB c (atímico)
 10^7 melanoma cells



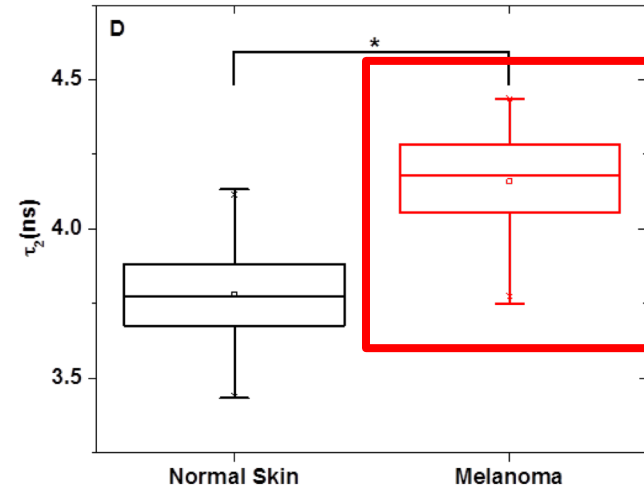
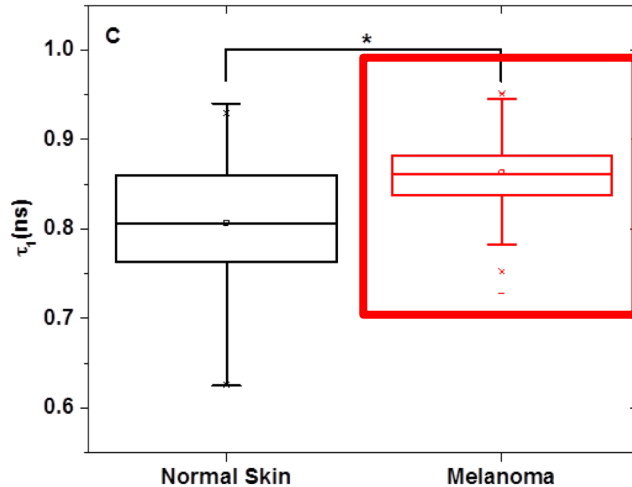
$$F(t) = a_1 \cdot e^{-\frac{t}{\tau_1}} + a_2 \cdot e^{-\frac{t}{\tau_2}}$$

378nm excitation: NADH molecules



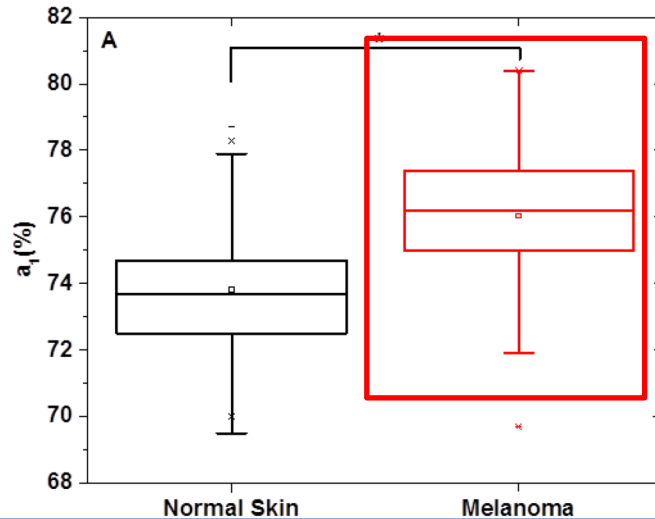
It was not observed difference between the a_1 and a_2 values obtained for melanoma and normal skin, probably due to the thin normal epidermis layer present over the experimental lesion. In addition to its contribution to the collected fluorescence signal, this thin skin layer also reduces the laser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

378nm excitation: NADH molecules



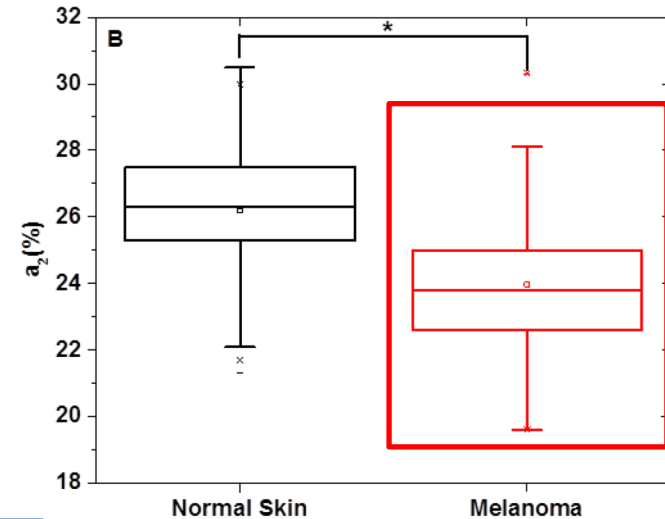
- Short and long lifetime components increased in melanoma;
- Warburg effect: shorter lifetimes in melanoma due to the presence of high amounts of free NADH molecules (glycolysis respiration pathway);
- keratin, collagen and melanin – may contribute for the lifetime changing;

445nm excitation: FAD molecules



Normal skin

a_1 – low amount of molecules with short lifetime;
 a_2 – high amount of molecules with long lifetime.

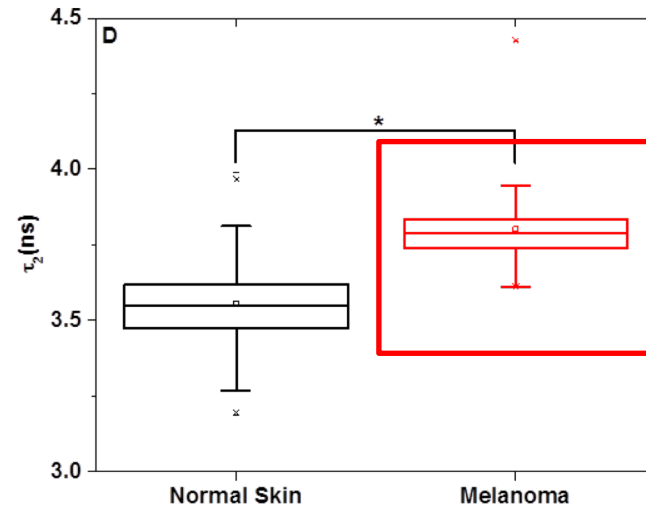
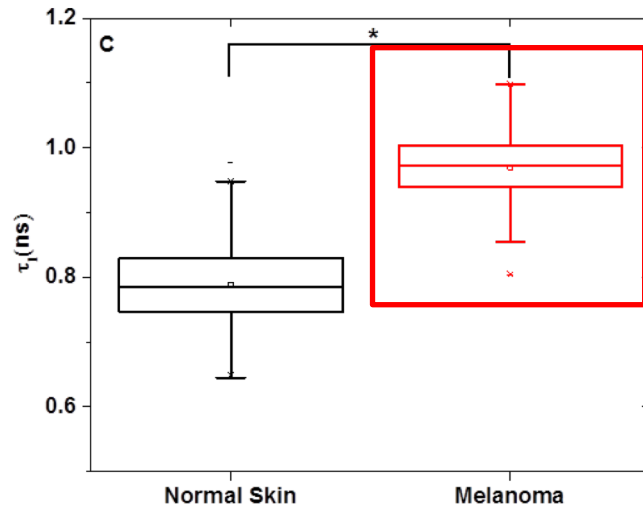


Melanoma

a_1 – high amount of molecules with short lifetime;
 a_2 – 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.

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

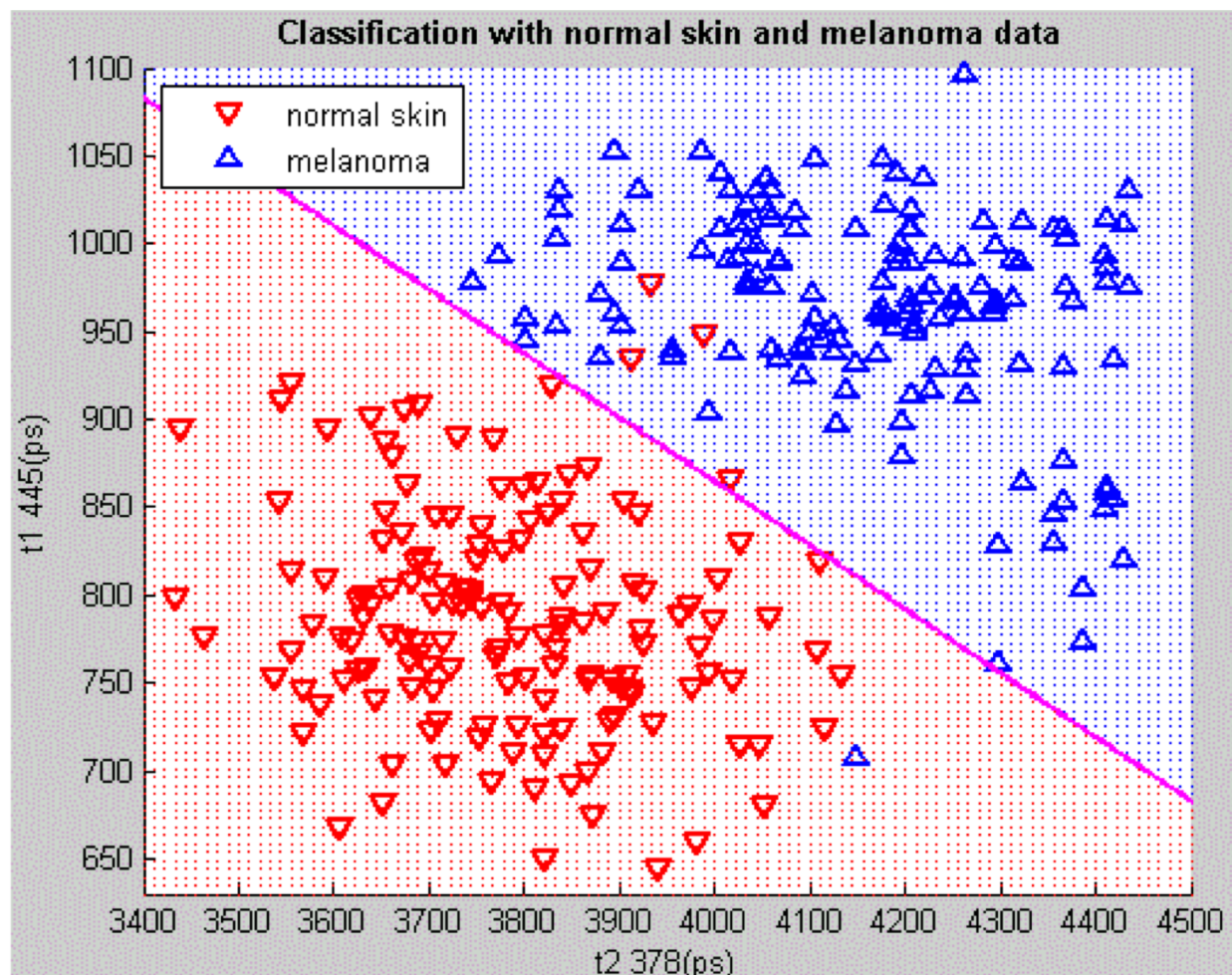
Melanoma

- Longer lifetime than normal skin
- Warburg effect: glycolysis

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%

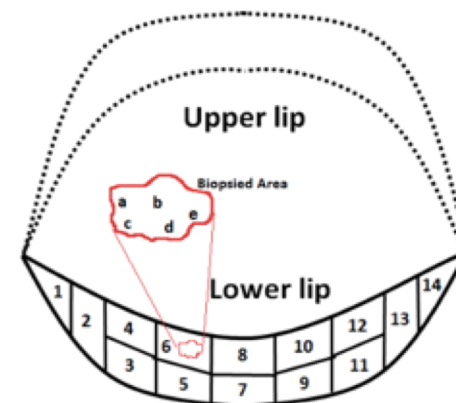
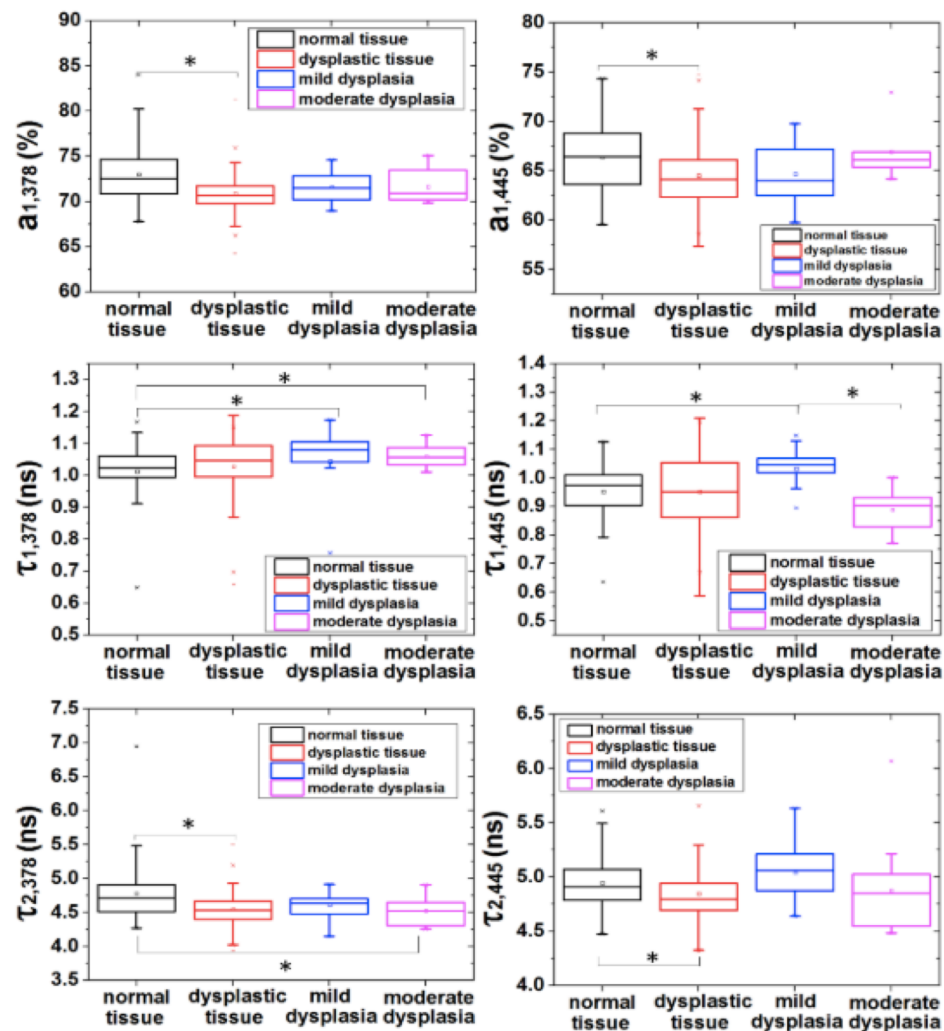


Tabela 4. Matriz de confusão para a classificação pelo algoritmo da LFS *versus* o diagnóstico histopatológico. Adaptado de ⁴².

	Normal (KNN)	Displasia leve (KNN)	Displasia moderada (KNN)
Normal (histologia)	52	3	1
Displasia leve (histologia)	3	27	0
Displasia moderada (histologia)	2	1	12

As células sombreadas correspondem aos verdadeiros positivos obtidos pelo KNN, resultando em valores de sensibilidade de 92,9%, 90% e 80% para a detecção de normal, displasia leve e displasia moderada, respectivamente. A taxa de especificidade obtida foi de 88,8%.