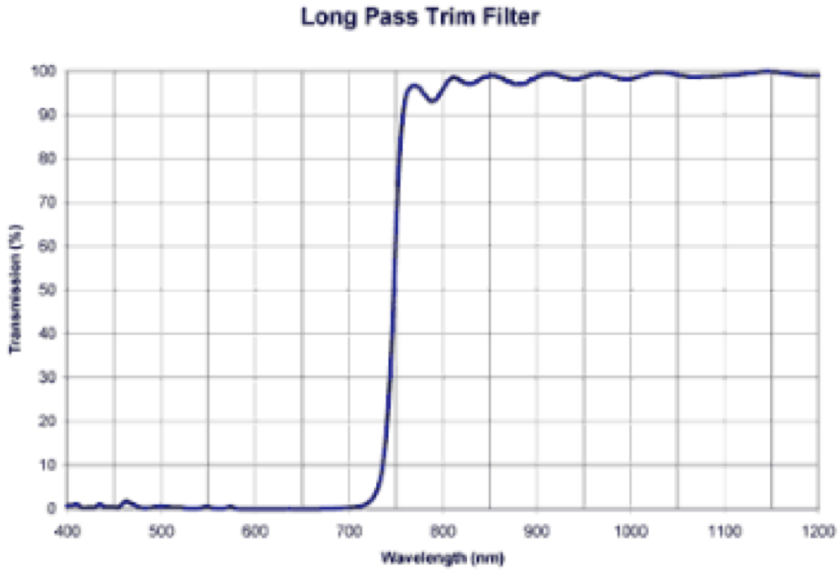
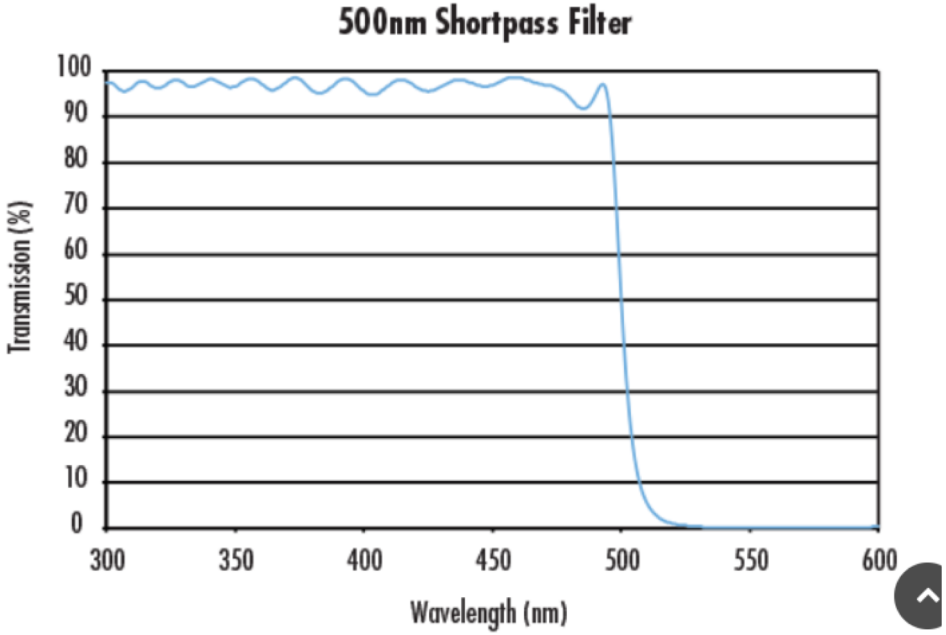
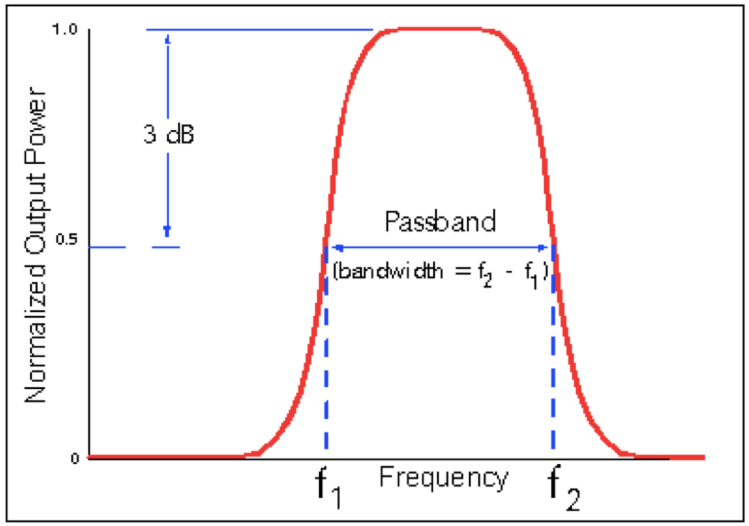
Técnicas fotônicas para diagnóstico - espectroscopia







bandpass filter

Espectroscopia de tempo de vida de Fl

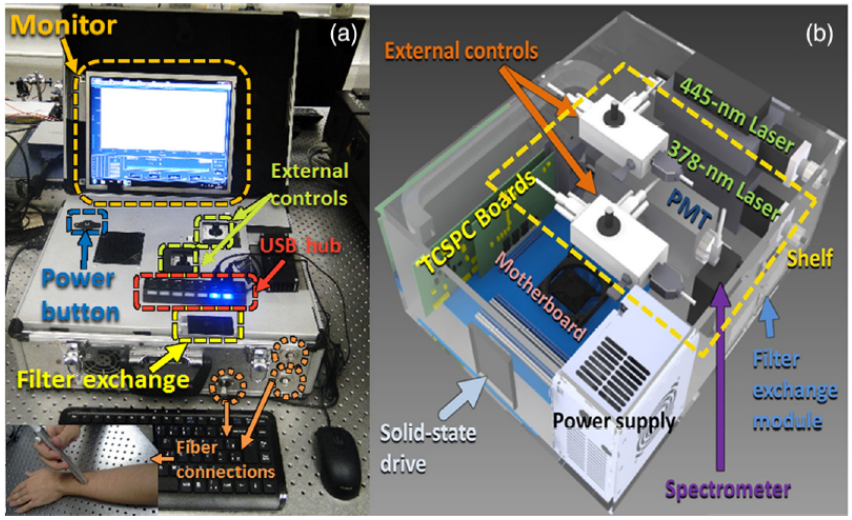


Fig. 3 (a) System assembly after its encasement provides protection for its components and a user-friendly design by exhibiting a control panel with access to external controls and the filter holder. The sample can be placed in any region around the system within the range of the optical fiber (2 m of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

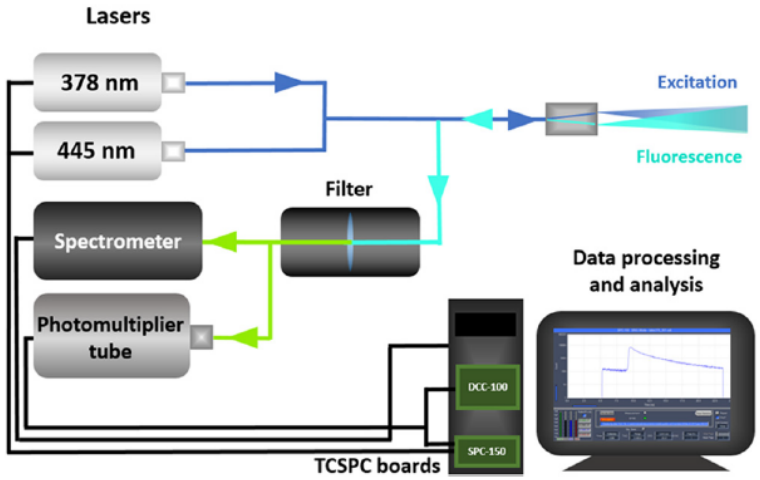
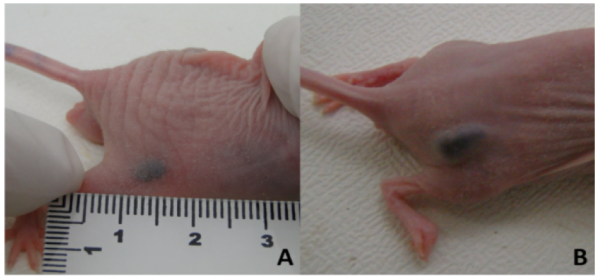


Fig. 1 Schematic drawing of the components for acquisition of fluorescence spectra and fluorescence lifetimes. The excitation light of one of the diode lasers (378 or 445 nm) is delivered to the sample through a bifurcated fiber optic probe. The fluorescence and backscattered light of this sample are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

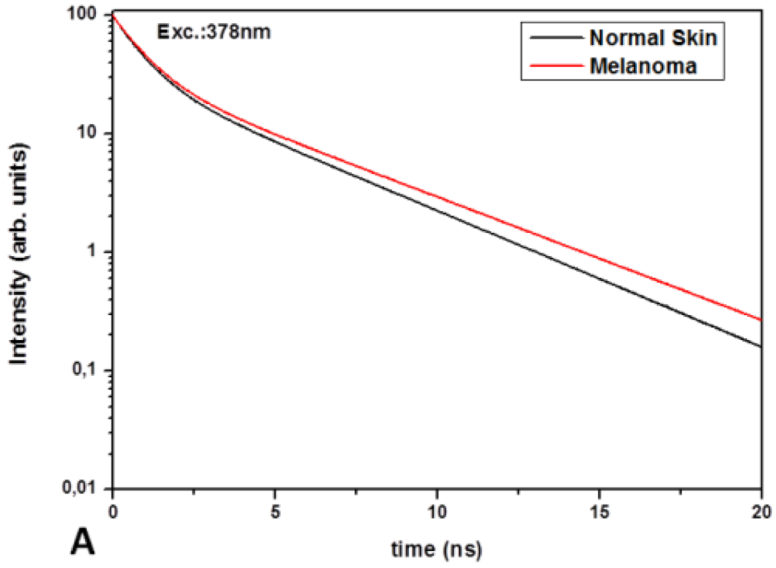
MS Nogueira et al., JBO 2017

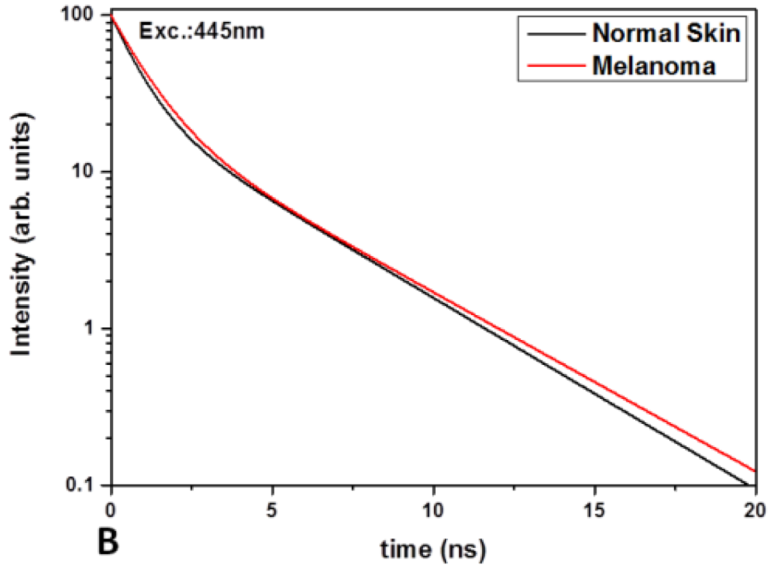
- **NADH molecules show a short lifetime component when it is free and a longer lifetime component when it is protein-bounded.**

- **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

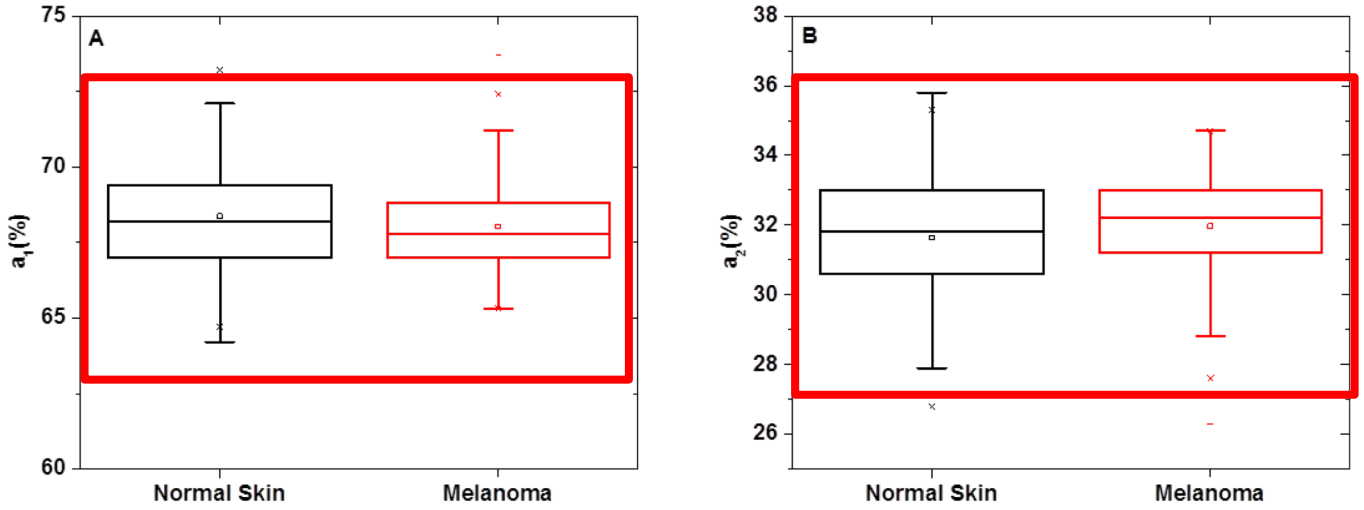




$$F(t) = a_1 . e^{\frac{t}{\tau_1}} + a_2 . e^{\frac{t}{\tau_2}}$$

L Pires et al., Biomed Opt Exp 2014

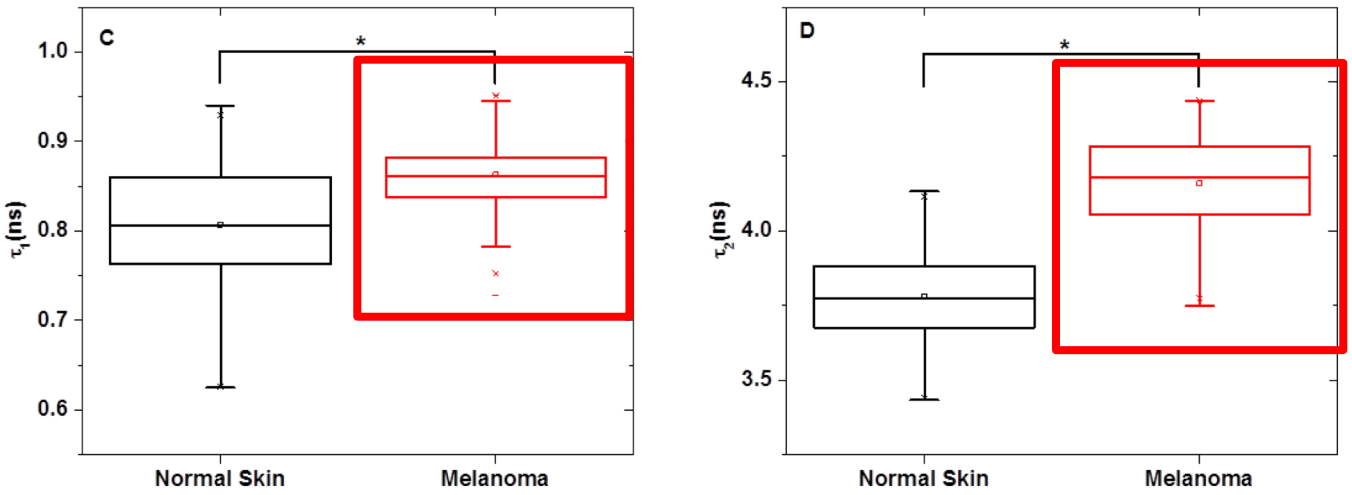
378nm excitation: NADH molecules



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



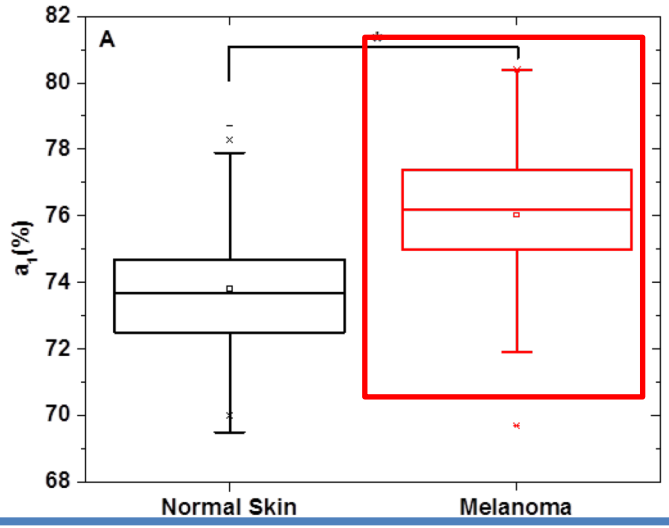
-Short and long lifetime components increased in melanoma;

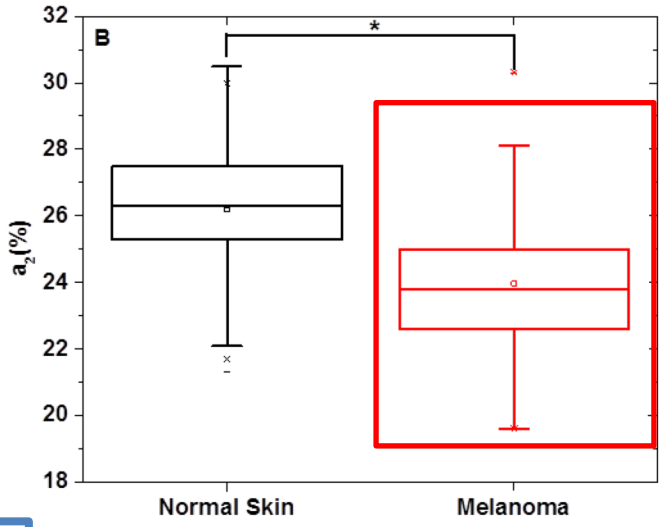
-Warburg effect: shorter lifetimes in melanoma due to the presence of high amounts of free NADH molecules (glycolysis respiration pathway);

- 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

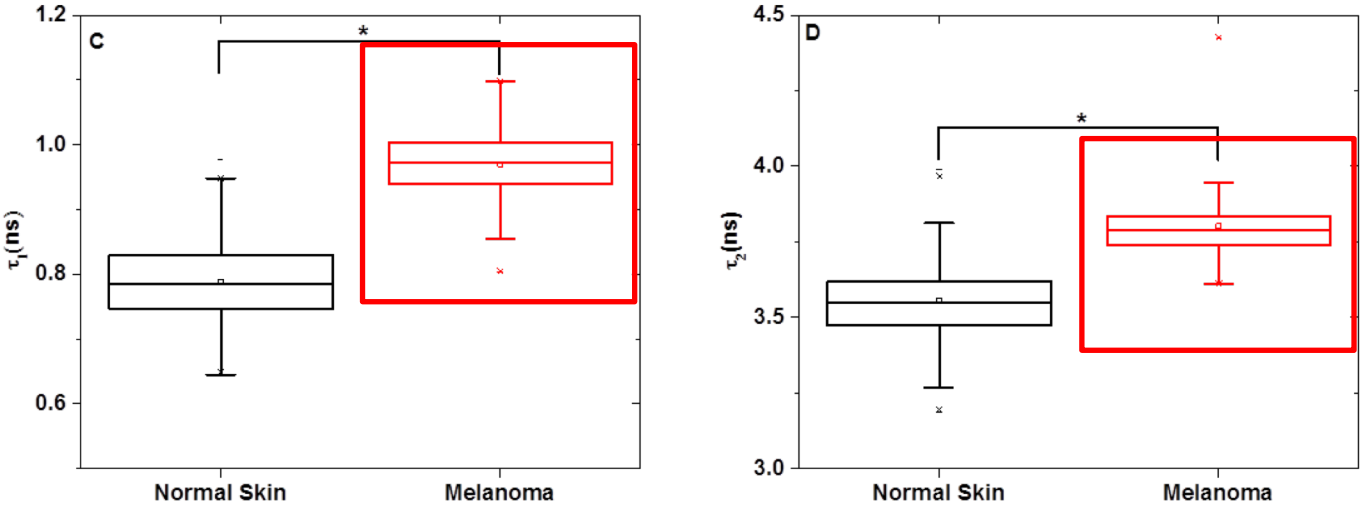
a1 – high amount of molecules with short lifetime;

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

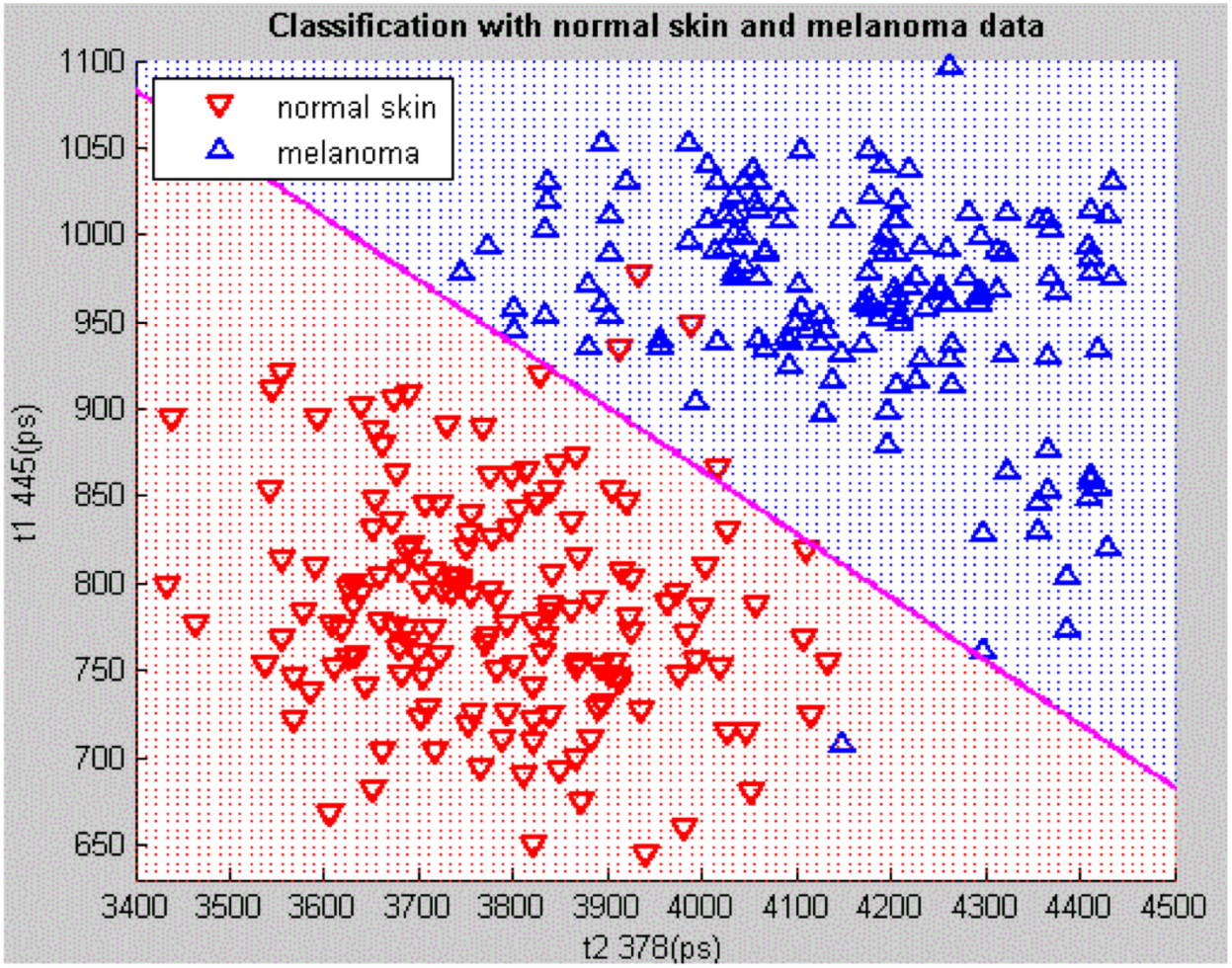
Short lifetime component of FAD
Long lifetime component of NADH

↓

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