

Visualising dental caries using fluorescence lifetime microscopy

Teeth are naturally fluorescent and changes in their composition, caused by decay for example, affect their fluorescence behaviour. The physical form and scattering nature of teeth can make simple measurements of fluorescence intensity problematic. The use of time-resolved fluorescence microscopy provides a quick and visual means of analysis, assisted by the fact that the fluorescence lifetime is an absolute measure. Fluorescence lifetime imaging (FLIM), providing both spatial and lifetime information, is well suited to characterise tooth structure and investigate tooth decay.

FLIM measurements on extracted teeth

The shape and scattering nature of the tooth can make it hard to obtain consistent intensity based fluorescence measurements. The advantage of the fluorescence lifetime is that it, unlike steady state which is relative, is an absolute measure. This means that it is well suited to be applied to study the intrinsic tooth fluorescence. The fluorescence decay behaviour of the tooth is multiexponential and has been found affected by things such as the aging process and the presence of dental caries. The ability to spatially visualise changes in the fluorescence using FLIM is therefore an attractive means by which to study tooth structure and monitor the causes of dental caries. The measurements presented here were performed using a *HORIBA Scientific DynaMyc* equipped with *DeltaDiode* laser excitation sources, shown below.

on the trinoc head. Such an image, seen through a x10 objective, is shown in Fig. 2.

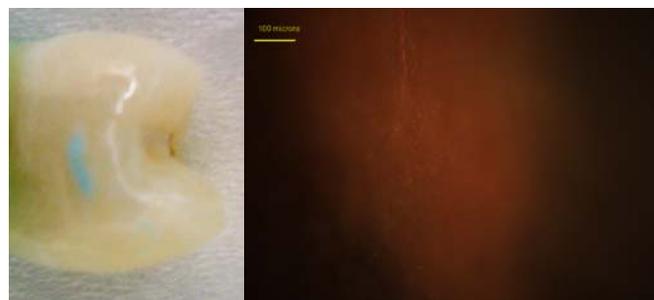


Fig. 2. Picture of tooth (caries marked) and microscope camera image around carious part (right), with the brighter central section showing the affected area



Fig. 1. DynaMyc – compact fluorescence lifetime microscope with fibre coupled DeltaDiode

The area of the tooth to be mapped, illuminated using the white light source and viewed using a beam splitter or filtercubes, can be selected using the CCD camera mounted

The time-resolved measurements are made by scanning the stage and the spatial resolution determined via the objective magnification and the size of the confocal pinhole. The measurement speed can be as short as 5ms per decay (with a maximum number of 10,000 decays), but in practice this will depend on the amount of fluorescence emitted by the sample. FLIM measurements, using an excitation wavelength of 450nm and emission at 520nm, were made at 5ms per decay and analysed using *DAS6* software. The decays were globally fitted to the sum of three exponentials and the recovered decay times were 0.6ns, 2.5ns and 10ns. The parameter map superimposed on the camera picture is shown in Fig. 3 for the average lifetime.

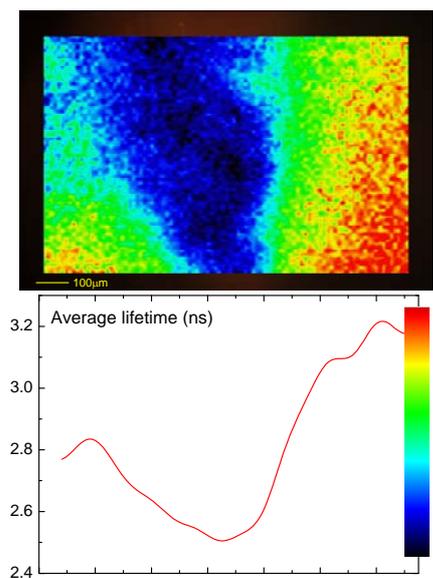
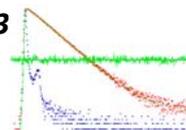


Fig 3. Average lifetime image (top) plus cross-section through central plane of image (bottom). The rainbow scale (right) relates to the lifetime values given left.

The composition of the fluorescence, in terms of the normalised pre-exponential factors can also be ascertained from the DAS6 analysis. These are shown in Fig. 4 for the three recovered lifetimes.

The results clearly show that in the carious region there is a decrease in the average lifetime and this relates to an increased prevalence of the shorter-lived fluorescence decay.

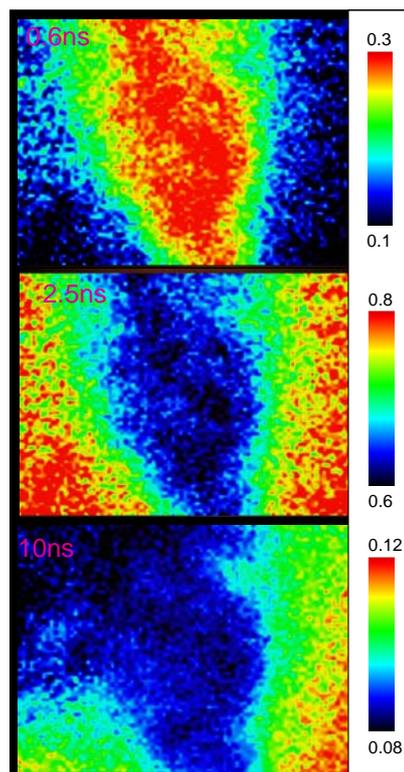
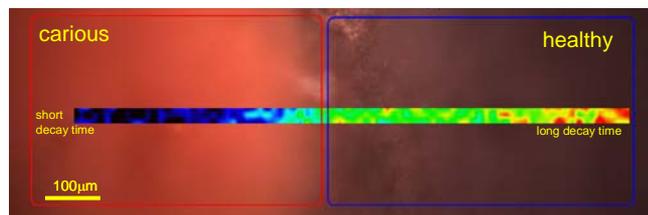


Fig 4. Plots of the normalised pre-exponential factors associated to the three lifetime components

Summary

FLIM measurements performed using the *DynaMyc* appear well suited in characterising and distinguishing healthy and carious regions in teeth. These observations are summarised visually below.



Acknowledgement

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