Kentech Instruments Ltd.

Introduction to Fluorescence Lifetime Imaging [FLIM]

Imagers suitable for FLIM

Introduction

Kentech is collaborating with the Photonics Group at Imperial College who are using FLIM for Biophotonics imaging experiments see the publications list below.

By using a Kentech High Rate Imager (HRI) the group is able to produce near real time FLIM image maps with around 20ps time resolution. It is hoped that improved equipment timing will be able to extend the time resolution.

Kentech has also worked with LA Vision in Germany on Fluorescence Lifetime Imaging Systems

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Optical imaging in the visible/near infrared spectral region has previously been investigated as an alternative to traditional medical diagnostic techniques. However, optical imaging is limited by the high scattering cross section of biological tissue, which affects the ability to detect variations in the optical properties of the tissue under investigation. To increase the contrast between tissue types one may use fluorescent marker dyes (fluorophores). These can be designed so they are selectively absorbed in the specific area of tissue under investigation. In medical diagnostic techniques the presence of a particular tissue type (e.g. cancerous tissue) can be established by detecting the emission-wavelength signature, of the fluorophore (which will not be present if the tissue of interest is not present). This spectroscopic technique can be combined with optical imaging techniques to produce a "map" of the localisation of the fluorophore and hence a map of the tissue under investigation. However, traditional fluorescence imaging techniques, which rely on quantitative intensity measurements, become increasingly difficult when imaging into greater tissue depths, due to scattering. An alternative method is fluorescence lifetime imaging (FLIM) where the lifetime of the fluorescence signal, rather than its intensity, is measured. Fluorescence lifetime is a signature of a fluorophore which is only weakly affected by the increase in photon propagation times due to scattering in tissue, making it possible for measurements to be made through greater tissue depths.

In biochemical applications of fluorescence imaging the effect of environment on the process of fluorescence is used to map chemical or physical changes within a sample. The quantum efficiency of fluorescence is a function of the radiative and non-radiative decay rates. The radiative decay rate is considered constant for a given fluorophore, while the non-radiative decay rate can vary with environment. Unfortunately the quantum efficiency is not easy to determine as it is difficult to measure the exact quantity of fluorophore in a particular region, and to quantify how much pump-light is absorbed. However, fluorescence lifetime is also a function of fluorophore environment.1 Since determination of fluorescence lifetime requires only relative intensity measurements, knowledge of the fluorophore concentration or excitation flux in the sample is no longer required. Thus imaging using fluorescence lifetimes may provide functional data about a tissue, sample under investigation. Fluorescence Lifetime probes already exist for the measurement of e.g. Ca2+ concentration, oxygen concentration and pH. Non-biomedical applications also exist for FLIM, such as determination of impurities in metal samples for nuclear process control,2 and in combustion related studies.3

Selected FLIM Publications

2019

Characterization of Protoporphyrin IX Species in Vitro Using Fluorescence Spectroscopy and Polar Plot Analysis
Kai Wen Teng and Sang Hak Lee
J Phys Chem B. 2019 Jul 11;123(27):5832-5840. doi: 10.1021/acs.jpcb.9b01913. Epub 2019 Jun 26.