COMPARISON OF DIFFERENT METHODS OF MELANIN DETECTION BASED UPON TIME-RESOLVED TWO-PHOTON EXCITED FLUORESCENCE

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Up to now, fast in situ 3D non-invasive melanin detection is a still challenging objective. In the past years, different methods based on time-resolved two-photon excited fluorescence were proposed. These methods take advantage of the specific melanin fluorescence lifetime properties and use multiphoton FLIM imaging (Fluorescence-Lifetime Imaging Microscopy) to acquire specific signals.

A common way to process the data uses multi exponential fitting to estimate the two coefficients $(a_i\text{ and } \tau_i)$ of each exponential component expressed as : $C_i(t) = a_i \exp(-t/\tau_i)$. Their accurate estimation requires enough photons inside each time channel, but this comes almost impossible in 3D imaging approaches that require reasonably short acquisition times. Therefore, in practice, FLIM imaging is limited to selected 2D slices, obtained at a depth chosen by the operator. In the past, for in vivo applications¹, we proposed a fast melanin detection method, called “Pseudo-FLIM”, based upon temporal binning of time channels (2 ns/channel instead of a few ps) and estimation of the log-slope². This “Pseudo-FLIM” requires only a few photons per pixel and is therefore compatible with in vivo 3D imaging.

This paper aims at comparing these methods to integrate their specific advantages and establish their limitations for detecting melanin. Accordingly, a pigmented cell model, containing normal human keratinocytes and melanocytes that form a 3D epidermal sheet with a thickness of about 40 µm was chosen. Multiphoton imaging was performed using a LEICA TCS SP8 microscope at 760 nm with a 40x/1.1NA W objective and a Picoquant Picoharp 300 TCSPC module. Different acquisition protocols were used to collect data available either for FLIM or “Pseudo-FLIM” analysis. A third method was compared, called “Fast FLIM”, recently available in the Symphotime software (Picoquant, Germany) that estimates an average lifetime per pixel as the mean arrival time of the fluorescence photons. Results obtained with these different methods will be shown and discussed.

We demonstrate in this work that the combination of multiphoton microscopy with “Pseudo-FLIM” offers the best compromise for fast melanin retrieval from time-resolved multiphoton fluorescence data acquired with an acceptable acquisition time compatible with 3D imaging.