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FLIM Phasor Analysis for Time-Domain and Frequency-Domain Data

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The phasor analysis of FLIM images provides a fit free global view of molecular species and their interaction in cells and tissues. Different techniques are used to collect the original data either in the time domain or in the frequency domain. The ''phasor transformation'' which is based on the calculation of Fourier components should in principle make the phasor plot independent of the domain of data collection. However, technical differences between the modalities of data acquisition in various instruments result in slightly different phasor calculations. In this poster we discuss the origin of the variations between the different methods of data acquisition. In particular we compare data obtained with the classical analog frequency domain instrument, data obtained with the FLIMbox principle that is based on a digital equivalent of the frequency domain instrument and data obtained with the popular time-correlated single photon counting instrument. We discuss how to minimize these differences which could results in phasors plots that can be directly compared form data obtained with different instruments. We also discuss and compare methods of data filtering which can decrease the noise in the phasor plot without affecting the resolution of FLIM images. Finally we compare phasor plots obtained for different harmonics of the laser repetition frequency. We show that the phasor plot at high harmonics from autofluorescence tissue samples can distinguish between various extracellular components such as the weak fluorescence from collagen and elastin. Work supported in part by NIH-P41 P41-RRO3155, 8P41GM103540 and P50-GM076516