WHERE DID MY PHOTONS GO? – ANALYZING THE MEASUREMENT PRECISION OF FLIM

Qiaole Zhao[1], Ian Ted Young[1], Jan Geert Sander de Jong[2]
[1]Department of Imaging Science and Technology,
Delft University of Technology, The Netherlands
[2]Lambert Instruments, The Netherlands
E-mail: qiaole.zhao@tudelft.nl

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1. MODEL FOR PHOTON EFFICIENCY

A mathematical model has been constructed to analyze the photon efficiency of frequency-domain fluorescent lifetime imaging microscope (FLIM) systems [1,2]. As shown in Equations 1 and 2, the power of the light source needed and the signal to noise ratio (SNR) expected at the detector can be calculated. Figure 1 shows the model at the system level. The SNR analysis assumes Poisson noise and has been validated in experiments. Moreover, experiments using samples in differing concentrations have been carried out to validate other aspects of the model.

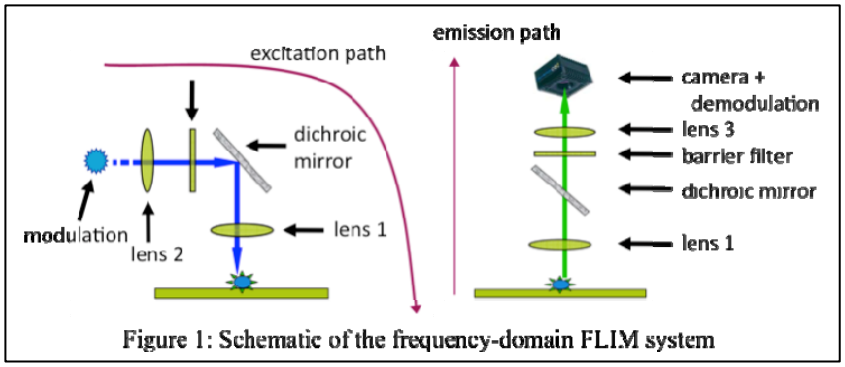

Figure 1: Schematic of the frequency-domain FLIM system

Eq.1: $$n_{emit} = \frac{WR_D\tau_{EF}\tau_{lens2}rT\left(1-10^{-\frac{\varepsilon m M^2}{N_A b^2}}\right)}{a^2 m E_{photon}}$$

Eq.2: $$SNR = \sqrt{(\tau_{lens1}\tau_D\tau_B\tau_{lens3}\tau_w F\eta)\left(\frac{n_{emit}}{\pi}\right)\arcsin\left(\frac{NA}{n}\right)}$$

2. MEASUREMENT PRECISION IN FLIM

Using this (validated) model, simulations in Matlab have been carried out to predict the precision of the lifetime measurement in frequency-domain FLIM. Various factors such as signal length, sampling frequency, noise, and the form of the demodulation signal have been studied to determine their influence on the lifetime estimation in a single lifetime system. This simulation package is also being used to study a two-lifetime system. Based upon the Poisson noise assumption, the precision of a fluorescence lifetime estimation of τ = 2 ns using a modulation frequency 80 MHz is better than 1%.

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