Preventing Photobleaching by Intensity Modulation of Continuous Wave Laser
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Light microscopy is non-destructive and preferred over electron microscopy for imaging biological samples. Fluorescence light microscopy is particularly useful for tracking individual molecules or staining certain organelles, which may not be clearly visible during bright-field observation. Fluorescence signals are often limited by the effect of photobleaching, which reduces the signal intensity over time. Attempting to increase the signal by increasing the intensity of the excitation laser only adds to the photobleaching, and is not effective. Molecules undergo photobleaching from the triplet state, which has a theoretical lifetime of a few $\mu$s in fluorescence molecules such as Rhodamine 6G (R6G), used in this study. When molecules are continuously excited further from the triplet state, the risk of photobleaching increases. If the molecules can first relax to the triplet state, they can then be safely excited again to release more photons, which results in a gain in fluorescence signal without photobleaching. In this study, we modulate the intensity of a continuous wave (CW) laser with an acousto-optic modulator (AOM) at a frequency range of 0.1 – 10 MHz. Preliminary results suggest that amplitude modulation causes a decrease in fluorescence decay and increased fluorescence signal in R6G thin film samples compared to CW laser excitation. Decay rate and initial fluorescence signal were found to be correlated with excitation intensity. We find that manipulating the modulation parameters gives a frequency and wave shape that together reduce photobleaching in fluorescent molecules. Incorporating this modulation scheme into fluorescence microscopy should significantly improve fluorescence imaging for biological and other applications.
Abstract

Incorporating this modulation scheme into fluorescence microscopy should result in a gain in fluorescence signal without increasing photobleaching. Fluorescence microscopy is particularly useful for tracking individual molecules or staining certain organelles, which may not be clearly visible due to bright-field illumination. Study a theoretical lifetime of a few ms in fluorescence molecules such as Rhodamine 6G (R6G), used in this study. When molecules are continuously excited further from the triplet state, they can then be safely excited again to release more photons, which results in a gain in fluorescence signal without photobleaching. Preliminary results suggest that amplitudes modulation causes a decrease in fluorescence decay and increased fluorescence signal in R6G thin film samples compared to CW laser excitation. Decay rate and initial fluorescence signal were found to be correlated with excitation intensity. We find that manipulating the modulation parameters gives a frequency and wave shape that together reduce photobleaching in fluorescent molecules. Incorporating this modulation scheme into fluorescence microscopy should significantly improve fluorescence imaging for biological and other applications.

Purpose

• Fluorescence microscopy
  • Useful for imaging biological samples
  • Tracking individual molecules or staining certain organelles or proteins with fluorescent dyes
  • Limited by decrease in signal due to photobleaching
• Goal: reduce photobleaching to reduce fluorescence decay and increase fluorescence yield

Sample Preparation

Samples prepared using:

• Fluorescent Molecule: Rhodamine 6G
• Polymer Matrix: PMMA
• Solvent: Chloroform
• Method: Spin coating
• Concentrations: 10 μM, 5 μM, 2 μM, 1 μM

Results

(Left) Fluorescence vs. time graphs for 4 modulation conditions.
(A) CW no modulation.
(B) 0.3 MHz sine modulation.
(C) 1 MHz sine modulation.
(D) 10 MHz sine modulation. Comparing (A) and (C) shows that sine modulation at 1 MHz gives a slower decay rate when compared to no modulation case.

Future Research

1. Determine exactly which modulation parameters are most effective in preventing photobleaching.
2. Examine modulation effect in different film concentrations.
3. Repeat pulsed and square waves with a faster AOM and detector.
4. Incorporate modulation into imaging microscope to try with biological samples.

References


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