Single Molecule Microscope Adapted to Provide FLIM Images in the Visible and NIR

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INTRODUCTION. Herein we describe the development of a time-resolved NIR phosphorescence confocal microscope that permitted sample scanning and construction of intensity and lifetime image profiles of visible and NIR emission, allowing for analysis of FLIM images of natural and synthetic photosensitizers, as well as of singlet oxygen luminescence. **OBJECTIVES.** To measure phosphorescence decays in the NIR, we adapted a single molecule lifetime confocal microscope (Picoquant's Microtime 200). **MATERIAL AND METHODS.** Excitation light was provided by a cycle of 200 picosecond pulses delivered by diode lasers (80 MHz frequency), followed by a microsecond detection period. After reflection by the major dichroic mirror, light beam reaches the sample that is laid at an inverted microscope bearing a silicon-immersion NIR objective mounted on a piezo scanner. Light is transmitted by the major dichroic and go through the pinhole. **DISCUSSION AND RESULTS.** Light was selected through long-pass filters, and reaches a NIR cooled PMT for singlet oxygen emission. For fluorescence emission in the visible, high efficiency APDs were used. Data analysis required time-gating to eliminate the time length coinciding with light excitation. **CONCLUSION.** Images of polymers beads and HaCaT cells containing synthetic (rose bengal and methylene blue) and natural photosensitizers (flavins and tioguanine), respectively, showed the potential of such equipment for studies in cell biology and biochemistry.

Key words: Fluorescence Microscopy, FLIM, NIR, singlet oxygen.
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