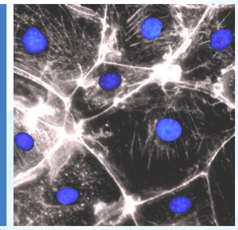
HORIBA

Fluorescence

Breaking the NIR Barrier: Characterizing World-Record-Holding Dyes for Advanced In-Vivo Imaging



Application Note
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Introduction

Probing light-matter interactions in the near infrared region (NIR) can unravel a plethora of research and innovation opportunities. Numerous technologies centered around NIR photon emission have attracted attention in the recent past, for example – photodynamic therapy and bioimaging. Improved NIR probes could transform medicine, enabling a new generation of diagnostic tools and targeted treatments that are less invasive and more effective, ultimately saving lives and improving quality of life for millions. However, a fundamental instrumentation challenge hinders the investigation of photoluminescence (PL) characteristics in the NIR region. Photon detectors used for visible-light detection offer poor sensitivity after 800 nm. Therefore, one must use dedicated NIR photon detectors to probe the PL characteristics of dyes and materials in the NIR region. In 2021, HORIBA introduced a new family of liquid nitrogen-cooled NIR solid-state detectors for the Fluorolog-QM spectrofluorometer. They include four types of InGaAs, one InAs, and one InSb diode.

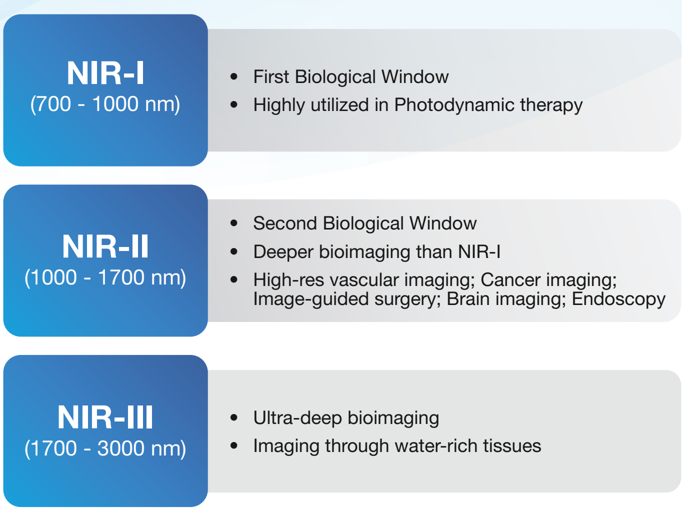

Figure 1. Subcategories of NIR regions and their corresponding applications

In this application note, we highlight a case study focused on the characterization of novel molecular dyes with PL emission in the NIR II (1000 – 1700 nm) and NIR III (1700 – 3000 nm) regions. The study utilized the Fluorolog-QM which is the fourth and latest generation of the HORIBA Fluorolog spectrofluorometer series, a QuantaMaster 8075-21 spectrofluorometer, and liquid nitrogen-cooled InAs and InGaAs NIR detectors.

Study Overview

This study[1] reports on three new xanthene-based organic molecular dyes with PL emission extending to 2,200 nm. At the time of publication, these dyes hold the world record for PL emission farthest in the NIR region. The dyes were eventually loaded in nanoemulsions and utilized to demonstrate in vivo bioimaging capabilities.

Methodology

Steady state spectra were acquired using the Fluorolog-QM spectrofluorometer. For excitation, a 980 nm diode-pumped solid-state (2 W) laser was mounted on the front panel of the highly modular spectrometer. A HORIBA liquid nitrogen-cooled InAs detector was used to detect the emission through a monochromator (350 mm focal length) with a ruled diffraction grating (600 grooves per mm, blazed at 1.25 µm), a scanning increment of 2 nm and an emission bandpass of 30 nm. Fluorescence quantum yield measurements were acquired using HORIBA's Fluorolog-QM and QuantaMaster 8075-21 spectrofluorometers. Liquid nitrogen-cooled InAs and InGaAs NIR detectors were used for photon detection.

Figure 2. HORIBA Fluorolog-QM used for steady state measurements of NIR dyes. Picture acquired from ESI.

Key Findings

Measuring PL emission in the NIR-II and -III region from small organic molecules is a challenging task due to low signal intensity. Nevertheless, the authors were able to acquire the PL emission spectrum of the dyes using the Fluorolog-QM configured with liquid nitrogen-cooled InAs NIR detector, as shown in Figure 3.

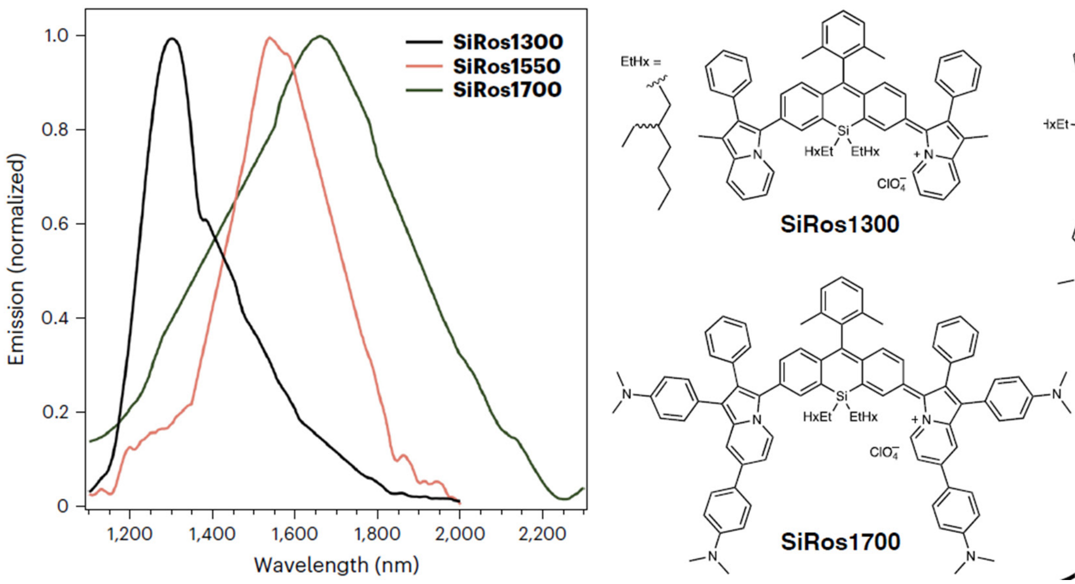

Figure 3. NIR emission spectra of the dyes reported in this study along with their molecular structures. The figures are excerpted from the paper.

The emission spectrum of the dyes peaks in the NIR-II region, 1300 nm, 1557 nm, and ~1700 nm for SiRos1300, SiRos1550, and SiRos1700 respectively, and extends to NIR-III region.

The fluorescence quantum yield (Φ_F) of the dyes was very low and measured relative to IR-1061, a standard NIR dye, as shown in Table 1.

Dye	Φ_F (%) in CH_2Cl_2	Φ_F (%) in D_2O
SiRos1300	0.0056 ± 0.0007	0.0068 ± 0.0009
SiRos1550	0.0025 ± 0.0003	0.0021 ± 0.0003
SiRos1700	0.0011 ± 0.0003	0.0007 ± 0.0001

Table 1. Relative fluorescence quantum yield of the dyes as reported in paper.

These results informed the authors of crucial photophysical characteristics of the dyes. They subsequently chose 2 dyes for NIR fluorescence imaging of the cardiovascular system in mice.

Conclusion

The authors synthesized 3 new small organic dyes for NIR-II and NIR-III fluorescence emission. The fluorescence emission spectrum and quantum yield were measured using Fluorolog-QM and QuantaMaster configured with InAs and InGaAs detectors respectively. The successful photophysical characterization of the dyes enabled the authors to utilize them for high resolution fluorescence imaging of the cardiovascular system in mice.

Availability

All data supporting the findings of this study, including experimental procedures, computational methods, and electronic supplementary information, are available within the manuscript and supplementary files.

[1] This application note is based on the following study: Meador, William E., et al. "Silicon-RosIndolizine fluorophores with shortwave infrared absorption and emission profiles enable in vivo fluorescence imaging." Nature chemistry 16.6 (2024): 970-978. https://doi.org/10.1038/s41557-024-01464-6

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