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Deeply Cooled, Scientific InGaAs Cameras Facilitate NIR-II / SWIR Imaging for Drug Discovery / Small-Animal Research

*"The utilization of materials such as SWNTs, rare-earth–doped phosphors, and quantum dots in concert with deeply cooled, scientific InGaAs cameras holds great promise for the future of **in vivo** optical imaging applications in the NIR-II / SWIR range."*

Introduction

For decades, x-ray and UV-vis-NIR detection methods have been used in various scientific, military, and medical applications. Although generally employed to good success, these systems nonetheless have some limitations when utilized for such types of work.

Effectively leveraging the good sample penetration afforded by x-rays, for example, can prove difficult for personnel in the field owing to the need for a compact, high-power x-ray tube as well as the lack of system portability. Alternatively, UV and visible wavelengths are quite easily detectable using silicon-based CCD technologies but are unable to penetrate samples due to reflection and scattering of light.

CCD cameras can detect NIR-I wavelengths between 750 and 1060 nm, which provide slight penetration into samples, but above this threshold the silicon itself is transparent to light. Such CCD camera systems have therefore found limited use in military (e.g., surveillance), drug discovery, and commercial (e.g., inspection) applications.

The NIR-II window / short-wavelength infrared (SWIR) range, which extends to 1700 nm, achieves much deeper penetration. Spurred on by the development of SWIR-sensitive InGaAs and InSb detectors, it has opened up a new world of scientific, military, and medical applications.

Unfortunately, early NIR-II / SWIR detection systems still had key limitations for scientific research. Insufficiencies associated with camera system linearity, noise performance, and synchronization with external equipment, as well as the lack of sufficient flexibility to control exposure times all presented critical obstacles.

This application note will discuss newly developed, deeply cooled, scientific InGaAs cameras (see Figure 1) from Princeton Instruments that facilitate leading-edge methods of small-animal imaging for preclinical research in addition to other advanced research applications.

SWIR for Small-Animal Imaging

Figure 2 shows the difference between NIR-I and NIR-II / SWIR in terms of resolution and penetration depth. Notice that NIR-I wavelengths scatter, resulting in 'fuzzier' images. By comparison, the longer wavelengths of NIR-II / SWIR do not scatter, yielding 'crisper' images.

Figure 1.
Princeton Instruments' NIRvana® scientific cameras feature a thermoelectrically cooled InGaAs detector and can be operated at rates up to 110 full frames per second.

Figure 2.

NIR-I vs. NIR-II / SWIR: resolution and penetration depth. Images and graph courtesy of Dr. Dominik J. Naczynski, Stanford University, adapted from Lim, Y.T. et al. Mol Imaging. (2003) 2, 50.

NIR-I
(808 nm)

SWIR
(1525 nm)

3 mm
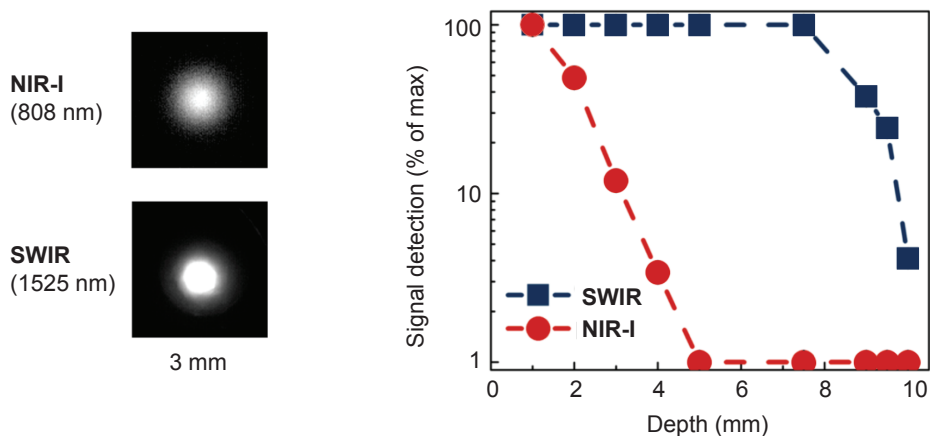
In recent years, research groups around the world have been developing different agents[1-7] to be utilized with the NIR-II / SWIR window in small-animal imaging for preclinical research, with the ultimate goal of earlier disease detection in human patients. Most of these approaches are based on one of three primary technologies: single-walled carbon nanotubes (SWNTs), rare-earth–doped phosphors, or quantum dots.

SWNTs, the most mature of the three approaches, demonstrate good emissivity in the NIR-II / SWIR range and can be PEGylated to reduce toxicity[1,2]. Low-toxicity, rare-earth–doped phosphors have also shown great promise, providing emissions that are tunable to different wavelengths, dependant on the size of the nanoparticle's undoped shell[3]. Similarly, quantum dots have demonstrated low toxicity and excellent emissivity that is tunable via particle size. (This application note focuses on the SWNT approach.)

Each approach utilizes a similar experimental setup, whose main elements are NIR-I illumination, a specimen (e.g., a mouse), and a camera system to detect emitted light in the wavelength range from ~1100 to ~1650 nm. See Figure 3.
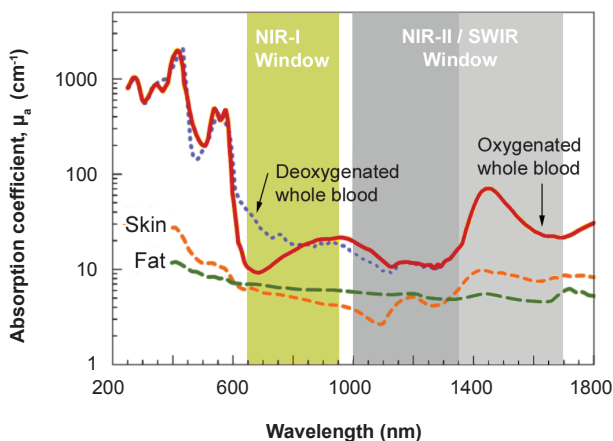
Figure 3.

Absorption chart. Courtesy of Dr. Dominik J. Naczynski, Stanford University, adapted from Smith, A.M. et al. Nat Nanotechnol. (2009) 4, 710.

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Figure 4.

(a) Upon excitation by a 785 nm laser, the SWNT-IRDye-800 conjugate emits at ~800 nm (NIR-I region) from the IRDye-800 dye and between 1100 and 1400 nm (NIR-II region) from the SWNT backbone. (b) Absorption spectrum of the SWNT–IRDye-800 conjugate (black dashed line), emission spectrum of the IRDye-800 dye (green line), and emission spectrum of the SWNTs (red line). (c) Imaging setup for simultaneous detection of both NIR-I and NIR-II / SWIR photons using silicon and InGaAs cameras, respectively. A zoomable lens set was used for adjustable magnifications. Schematics[1] and spectra[1] courtesy of Prof. Hongjie Dai, Stanford University.

Figure 5.

(a) NIR-I fluorescence image (top) and cross-sectional fluorescence intensity profile (bottom) along red dashed line of a mouse injected with the SWNT-IRDye-800 conjugates. Gaussian fit to the profile is shown in red dashed curve. (b) NIR-II / SWIR fluorescence image (top) and cross-sectional fluorescence intensity profile (bottom) along red dashed line of a mouse injected with the SWNT-IRDye-800 conjugates. Gaussian fit to the profile is shown in red dashed curve. Data[1] courtesy of Prof. Hongjie Dai, Stanford University.

Experimental Results

Prof. Hongjie Dai at Stanford University has performed NIR-I as well as NIR-II / SWIR fluorescence imaging of blood vessels in mice[1,2]. Biocompatible SWNT-IRDye-800 conjugates were utilized as dual-color imaging agents, where IRDye-800 was a commercial NIR-I fluorophore and high-pressure carbon monoxide conversion SWNTs were stably suspended by biocompatible surfactants[1]. See Figure 4 for experimental schematics and spectra.
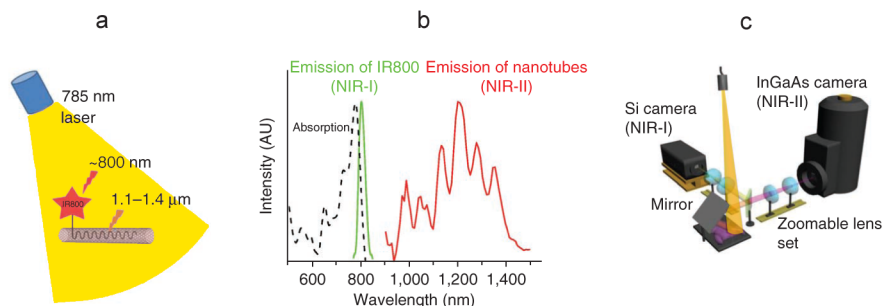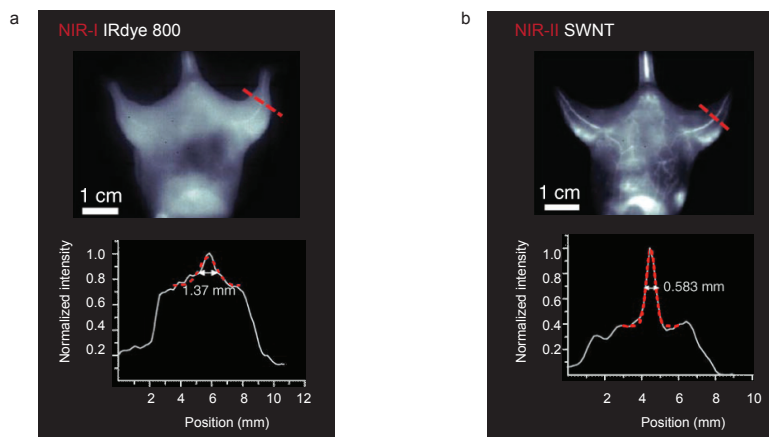

Figure 5 presents NIR-I and NIR-II / SWIR images acquired via the experimental setup shown in Figure 4c. The NIR-I image shown in Figure 5a was acquired using a scientific, silicon-based CCD camera. The NIR-II / SWIR image shown in Figure 5b was acquired using a scientific InGaAs camera. All images acquired in the NIR-I region employing IRDye-800 fluorescence showed indistinct vascular anatomy within the mouse; however, the detection of SWNT fluorescence in the NIR-II / SWIR window provided substantially improved spatial resolution of vessels in the same mouse at all magnifications[1].

New Technology

The NIRvana® family of InGaAs cameras from Princeton Instruments differentiates itself from other InGaAs cameras, which are typically designed for night vision applications, via a number of scientific performance features, including deep cooling, low dark noise, high linearity, low read noise, high frame rates, intelligent software, and precision control over integration times.

First and foremost, maintenance-free thermoelectric cooling (not liquid nitrogen) chills the NIRvana camera's InGaAs detector down as low as -85°C. This deep cooling is coupled with a proprietary cold shield design and vacuum technology to facilitate the lowest possible dark noise, which helps increase sensitivity as well as preserve signal-to-noise ratio (SNR) for long exposure times.

The NIRvana camera has the ability to expose for as short as 2 µs, up to many minutes. Ultra-low-noise readout electronics help ensure good SNR even when the camera is operated at its maximum rate of 110 full frames per second. Furthermore, excellent camera linearity means that the NIRvana is highly reliable for scientific research.

Princeton Instruments' 64-bit LightField® software, available as an option, provides a powerful yet easy-to-use interface that puts real-time online processing capabilities at the researcher's fingertips. The NIRvana camera can also be integrated into larger experiments using an available National Instruments LabVIEW® toolkit. Full triggering support is provided for synchronization with external equipment.

Summary

The extension of *in vivo* optical imaging for disease screening and image-guided surgical interventions requires brightly emitting, tissue-specific materials that optically transmit through living tissue and can be imaged with portable systems that display data in real-time[3]. Work performed by a number of research groups around the world has begun to demonstrate that fluorescence imaging in the NIR-II / SWIR range can provide appreciably greater *in vivo* sensitivity compared to fluorescence imaging in the NIR-I region[1–7].

The utilization of materials such as SWNTs, rare-earth–doped phosphors, and quantum dots in concert with deeply cooled, scientific InGaAs cameras like the new Princeton Instruments NIRvana holds great promise for the future of *in vivo* optical imaging applications in the NIR-II / SWIR range.

Resources

To learn more about the NIR-II / SWIR imaging techniques with SWNTs being developed by Hongjie Dai, please visit: **http://dailab.stanford.edu/**

To learn more about the NIR-II / SWIR imaging techniques with rare-earth–doped phosphors being developed by Dominik Naczynski, please visit:
https://med.stanford.edu/profiles/dominik-naczynski

For additional information about Princeton Instruments NIRvana cameras, please refer to:
www.princetoninstruments.com/products/imcam/nirvana

APPLICATION NOTE

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Visit the Princeton Instruments website to find a dealer in your area.

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APPLICATION NOTE

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