

## Perfecting and extending the near-infrared imaging window

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The propagation of excited ballistic and diffused emission photons in the biotissue with small (left) and moderate (right) light absorption and the resulting signal to background ratios (SBRs) of fluorescence imaging. Credit: Zhe Feng, Tao Tang, Tianxiang Wu, Xiaoming Yu, Yuhuang Zhang, Meng Wang, Junyan Zheng, Yanyun Ying, Siyi Chen, Jing Zhou, Xiaoxiao Fan, Dan Zhang, Shengliang Li, Mingxi Zhang and Jun Qian

The deep-rooted beliefs that light absorption and scattering are totally harmful to fluorescence catching urge most researchers to chase a



perfect window with minimal photon absorption and scattering for bioimaging. Due to the generally accepted less photon scattering, the fluorescence bioimaging in the second near-infrared window (NIR-II) gives admirable image quality, especially when deciphering the deepburied signals in vivo. Nowadays, NIR-II fluorescence imaging has already guided complicated liver-tumor surgery in clinic. However, the constructive role of light absorption, to some extent, seems to be ignored.

The final presentation of high-quality images even makes the overstated positive effect of scattering suppression by lengthening wavelength more convincing since the absorption simultaneously is considered to attenuate the signals. As a matter of fact, some works have revealed absorption-induced resolution enhancement in the scattering media due to the depressing of long-optical-path background signals. Yet how to take full advantage of light absorption to select a suitable <u>fluorescence</u> imaging window remains unspecified.

In a new paper published in *Light Science & Application*, a team of scientists, led by Professor Jun Qian from State Key Laboratory of Modern Optical Instrumentations, Centre for Optical and Electromagnetic Research, College of Optical Science and Engineering, International Research Center for Advanced Photonics and co-workers have perfected the mechanism accounting for the excellent performance of NIR-II fluorescence imaging. By simulating the NIR photon propagation in bio-tissue, they innovatively proposed the well-performance imaging in 1400-1500 nm, 1700-1880 nm, and 2080-2340 nm, which were defined as NIR-IIx, NIR-IIc, and the third near-infrared (NIR-III) window, respectively.

The designed PbS/CdS core-shell quantum dots (CSQDs) with peak emission wavelength at ~1100 nm, ~1300 nm, and ~1450 nm were used as the imaging probes, and they found the detection regions around the



absorption peaks of water always provide vastly improved image quality, and thus the definition of the NIR-II window was further perfected as 900-1880 nm. The NIR-IIx region was proved to provide more superior fluorescence images than the NIR-IIb region. With the help of light absorption, wide-field micro- and macro- fluorescence imaging with excellent imaging quality were performed.

General cognition of the NIR-II window guides us to emphasize the scattering depression with the increase of wavelength but underestimate the constructive effect of absorption. As a matter of fact, the light absorbers would preferentially deplete the multiply scattered photons in propagation in theory since scattered photons have longer path lengths through the biological medium than ballistic photons (see Figure 1).





a, The light absorption spectra of water within 700-2500 nm (Appl. Opt. 32, 3531-3540, 1993) and the definition of the NIR imaging windows. b-g, Equivalent images of a line source through a bio-tissue of 1 mm thickness in (b) 1300-1400 nm, (c) 1400-1500 nm, (d) 1500-1700 nm, (e) 1700-1880 nm, (f) 1880-2080 nm and (g) 2080-2340 nm after the simulation via the Monte Carlo method. Credit: Zhe Feng, Tao Tang, Tianxiang Wu, Xiaoming Yu, Yuhuang Zhang, Meng Wang, Junyan Zheng, Yanyun Ying, Siyi Chen, Jing Zhou,



Xiaoxiao Fan, Dan Zhang, Shengliang Li, Mingxi Zhang and Jun Qian

Water is the most important component of organisms, whose light absorption spectrum within 700-2500 nm (data from *Appl. Opt.* 32, 3531-3540, 1993) is shown in Figure 2a. Because of the absorption peak at ~980 nm, 900-1000 nm should not be excluded from the NIR-II window for bio-imaging. The imaging in 1400-1500 nm has not long been recognized, but the high light absorption within this band, which is called as NIR-IIx region here, is no longer the barrier in the NIR-II region, as long as the fluorescent probes possess enough brightness to resist the attenuation by water.

At present, the photoresponse of the classic InGaAs detector limits the optical imaging beyond 1700 nm, thus the NIR-II window is defined as no more than 1700 nm. Because of the similar absorption and scattering properties, they believe that 1700-1880 nm possessed comparable imaging quality with the NIR-IIb imaging and define 1700-1880 nm as the NIR-IIc region. Over the absorption "mountain" peaking at ~1930 nm, the region of 2080-2340 nm, which is considered as the third nearinfrared (NIR-III) region, becomes the last high-potential bio-window in general since the water absorption of light beyond 2340 nm keeps stubbornly high. Furthermore, the photon propagation in 1300-1400 nm (NIR-IIa), 1400-1500 nm (NIR-IIx), 1500-1700 nm (NIR-IIb), 1700-1880 nm (NIR-IIc), 1880-2080 nm and 2080-2340 nm (NIR-III) window were simulated, considering the absorption spectrum of water and the scattering property of skin. As shown in Figure 2b-g, except for the extremely intense depletion in 1880-2080 nm (Figure 2f), rising light <u>absorption</u> and falling photon scattering both make positive contributions to the precise imaging. The NIR-IIx and NIR-III imaging show superior background attenuation strength.



The intravital imaging in mice was conducted to objectively evaluate the fluorescence imaging with collection around 1450 nm. It could be seen in Figure 3a-d that, the closer the imaging window is to the peak absorption, the lower the imaging background. The measured SBRs shown in Figure 3e-h further confirm the positive contribution of the absorption. User-friendly fluorescence wide-field microscopy, as a classical technique, is often utilized for cell or tissue slice imaging. However, despite the large imaging depth, the scattering photons and the signal photons outside the focal plane induced background keep the details hidden beneath a veil of "mist". The results shown in Figure 3i-p, with excellent background attenuation, wide-field microscopy around the NIR-IIx region possesses excellent performance.

NIR-IIb fluorescence imaging has long been regarded as the most promising NIR-II fluorescence imaging technique due to the suppressed photon scattering until then, but the new results prove greater contribution of rising absorption than the decrescent scattering and the NIR-IIx fluorescence imaging proposed owned optimum performance, even exceeding the NIR-IIb fluorescence imaging.





a-d, the hind limb imaging of the same mouse in (a) 1400-1550 nm, (b) 1425-1475 nm, (c) 1500-1700 nm and (d) 1550-1700 nm. e-h, cross-sectional fluorescence intensity profiles along the indigo lines of the blood vessel in (a-d). Scale bar, 10 mm. i-l, the 5× microscopic imaging of cerebral vasculature in the same mouse in (i) 1400-1550 nm, (j) 1425-1475 nm, (k) 1500-1700 nm and (l) 1550-1700 nm. m-p, cross-sectional fluorescence intensity profiles along the indigo lines of the blood vessel in i-l. Scale bar, 300  $\mu$ m. The numbers show the SBRs. Credit: Zhe Feng, Tao Tang, Tianxiang Wu, Xiaoming Yu, Yuhuang Zhang, Meng Wang, Junyan Zheng, Yanyun Ying, Siyi Chen, Jing Zhou, Xiaoxiao Fan, Dan Zhang, Shengliang Li, Mingxi Zhang and Jun Qian



These scientists summarize their discovery:

"NIR-IIb fluorescence imaging has long been regarded as the most promising NIR-II fluorescence imaging technique due to the suppressed photon scattering until then, but our results now proved greater contribution of rising <u>absorption</u> than the decrescent scattering and the NIR-IIx fluorescence imaging proposed in this work owned optimum performance, even exceeding the NIR-IIb fluorescence imaging"

"We believed these results are pretty crucial for the further development of NIR fluorescence imaging."

**More information:** Zhe Feng et al, Perfecting and extending the nearinfrared imaging window, *Light: Science & Applications* (2021). DOI: <u>10.1038/s41377-021-00628-0</u>

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