Millimeter-Deep Detection of Single Shortwave-Infrared-Emitting Polymer Dots through Turbid Media

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Abstract

Fluorescence imaging at longer wavelengths, especially in the shortwave-infrared (SWIR: 1000–1700 nm) region, leads to a substantial decrease in light attenuation, scattering, and background autofluorescence, thereby enabling enhanced penetration into biological tissues. The limited selection of fluorescent probes is a major bottleneck in SWIR fluorescence imaging. Here, we develop SWIR-emitting nanoparticles composed of donor–acceptor-type conjugated polymers. The bright SWIR fluorescence of the polymer dots (primarily attributable to their large absorption cross-section and high fluorescence saturation intensity (as high as $113 \text{ kW} \cdot \text{cm}^{-2}$)) enables the unprecedented detection of single particles as small as 14 nm through millimeter-thick turbid media. Unlike most SWIR-emitting nanomaterials, which have an excited-state lifetime in the range of microseconds to milliseconds, our polymer dots exhibit a subnanosecond excited-state lifetime. These characteristics enable us to demonstrate new time-gated single-particle imaging with a high signal-to-background ratio. These findings expand the range of potential applications of single-particle deep-tissue imaging.

KEYWORDS: shortwave infrared, conjugated polymer nanoparticles, fluorescent probes, fluorescence imaging, single-molecule, tissue phantoms, time-gated fluorescence detection

Over the past decades, fluorescence microscopy has become an essential noninvasive tool for life-science researchers, enabling the visualization of structures and functions of biological specimens with sizes ranging from submicrometers to millimeters at nanometer spatial resolution and with single-molecule sensitivity.¹ However, the strong absorption and scattering of visible light by biological samples (e.g., skin, tissue, and blood) limit the applicability of fluorescence imaging for large biological specimens (e.g., tissues, organs, and embryos).² As the focus of biomedical researchers has shifted from cellular-level to organism-level biological systems, interest in the development of new imaging modalities that enable the visualization of structures and functions of entire mesoscopic (i.e., millimeter to centimeter) biological specimens has increased. Multiphoton excitation fluorescence microscopy has been the primary tool for deep-tissue fluorescence imaging. Single-particle imaging through submillimeter tissues has been achieved in some cases using dye-doped organic nanoparticles (NPs).³ Recent studies suggest that fluorescence imaging in the shortwave-infrared (SWIR, 1000–1700 nm) spectral region is a promising alternative approach for obtaining high-quality images through thick biological samples because both absorption and scattering of light by biological specimens are highly suppressed in this wavelength region.⁴ SWIR fluorescence imaging has been used for deep-tissue imaging, providing high-quality fluorescence images of targeted molecules and structures located deep in the body (e.g., brain tissues).⁵⁻⁸ Although infrared detection technology has rapidly developed, the lack of fluorescent probes that function in this wavelength range poses a major obstacle to the widespread application of SWIR imaging.⁹

Various light-emitting nanomaterials have been developed as SWIR-emitting fluorophores, including single-walled carbon nanotubes (SWNTs), semiconductor quantum dots (QDs), and rare-earth-doped nanoparticles (RENPs).^{8, 10-13} Although these materials have been used

successfully in fluorescence microscopy imaging experiments, they are not always ideal fluorophores for bioimaging applications. Fluorophores that are large (e.g., submicrometer-sized SWNTs) and/or highly toxic (e.g., QDs with heavy metals) are unsuitable for use in a wide range of biological specimens.¹⁴⁻¹⁸ Whereas a long excited-state lifetime (e.g., in the case of QDs and RENPs) of fluorophores is beneficial for ensemble-level time-gated imaging, detecting a sufficient number of photons from individual fluorophores within an integration time of milliseconds, which is required for single-particle-level time-gated imaging, is difficult. Thus far, SWIR fluorescence-based single-particle deep-tissue imaging has been limited to approximately 100- μ m-thick specimens using SWNTs and QDs.^{19, 20} A few small organic SWIR-emitting dyes (e.g., polymethine cyanine dyes and donor–acceptor–donor (DAD)-type dyes) with low cytotoxicity have been developed for potential clinical applications.²¹⁻²⁴ Although their small size and low cytotoxicity make organic dyes suitable for most applications, they often exhibit relatively dark fluorescence compared with fluorescent NPs. In addition, small organic SWIR-emitting dyes often exhibit a substantial portion of fluorescence emission outside the SWIR spectral region.²¹ As such, single-molecule fluorescence imaging in the SWIR region using small organic dyes has not been reported yet.

Recent studies have shown that organic NPs are promising fluorophores for SWIR fluorescence imaging. Organic NPs doped with small dyes have been reported; these exhibit aggregation-induced emission²⁵ and J-aggregate formation.²⁶ These NPs are characterized by high biocompatibility without compromising fluorescence brightness. Conjugated polymer nanoparticles (Pdots) composed of DAD-type conjugated polymers (CPs) have also emerged as new SWIR probes.²⁷⁻³¹ In addition to their high biocompatibility,³² Pdots can be reliably prepared as ultrasmall (<5 nm) NPs using proper protocols.^{33, 34} Thus, Pdots are promising fluorescent NPs

for single-particle SWIR imaging. Researchers have developed small-bandgap CPs with a large extinction coefficient by designing DAD-type CPs that exhibit strong intramolecular charge transfer absorption and fluorescence.³⁵ In addition, the fluorescence properties of Pdots can be fine-tuned through the selection of appropriate DAD units and through control of the spatial packing of the CPs inside the Pdots.³³ Despite the strong potential of Pdots as fluorophores for SWIR fluorescence imaging, only a few studies on SWIR-emitting Pdots have been reported.³⁰ We recently reported a new generalized strategy for obtaining Pdots that exhibit bright SWIR fluorescence by integrating the rational design and synthesis of CPs; we also optimized the fabrication protocol for Pdots.³⁴

Here, we report single-particle fluorescence imaging of SWIR-emitting Pdots. We show that ultrasmall (as small as 3.5 nm) SWIR-emitting Pdots exhibit much brighter (5× brighter) fluorescence compared with frequently used semiconductor quantum dots (e.g., PbS QDs) under typical experimental conditions for single-molecule imaging. The SWIR-emitting Pdots enable unprecedented millimeter-deep single-particle fluorescence imaging through tissue phantoms. In addition, the characteristic spectroscopic properties of the SWIR-emitting Pdots enabled us to develop a new mode of time-gated single-particle SWIR imaging.

Fabrication of SWIR-emitting Pdots with controlled brightness

We used two different DAD-type SWIR-emitting CPs—poly(dithiazolfluorene-*alt*-thiadiazolobenzotriazole) (PSN) (Figure 1a) and poly(diethynylcarbazole-*alt*-dithienylbenzobisthiadiazole) BBT (Figure 1b)—for the fabrication of Pdots. They display peak absorption in the near-infrared-I (NIR-I) spectral region (741 nm and 835 nm for PSN and BBT, respectively) and fluorescence emission in the SWIR region, peaking at 924 and 1001 nm for PSN

and BBT, respectively, in tetrahydrofuran (THF) (Figure S1). Pdots were fabricated using a nanoprecipitation method, which involved rapid mixing of a dilute solution of CPs dissolved in an organic solvent (THF in the present study) with excess water. We could not use a standard method (i.e., changing the starting concentration of CPs in the organic phase³⁶) to control the size of Pdots because of the low solubility of our CPs in THF. Thus, we performed nanoprecipitation using a saturated solution of the CPs in THF and varied the organic-to-water-phase mixing volume ratio (1:5, 1:1, and 2:1) to obtain a series of Pdots with different sizes. Using this protocol, we obtained PSN Pdots with mean diameters of 3.5, 14.3, and 31.9 nm and relatively narrow size distributions (Figure 1e, 1f, 1g, 1i). The absorption and fluorescence spectra of the PSN Pdots with different sizes dispersed in water were nearly identical, with peaks at 736 and 1040 nm, respectively (Figure 1c). In addition, the fluorescence quantum yield (QY) of the PSN Pdots was size independent (QY = 0.31%).³⁴ The 3.6-nm BBT Pdots fabricated in a similar manner as the 3.5-nm PSN Pdots displayed absorption and fluorescence peaks at 835 and 1035 nm, respectively, with a QY of 0.29% (Figure 1d, 1h, 1i). The fluorescence characteristics of the PSN Pdots were not affected by the nanoprecipitation conditions, whereas the fluorescence properties of the BBT Pdots were strongly dependent on the fabrication protocol.

Single-particle fluorescence microscopy of the SWIR-emitting Pdots

To evaluate the applicability of our SWIR-emitting Pdots for SWIR fluorescence imaging at the single-particle level, we constructed a customized multispectral confocal fluorescence microscopy setup equipped with both silicon and InGaAs single-photon avalanche photodiodes (SPADs) for detecting visible/near-infrared-I (VIS/NIR-I: <950 nm) and SWIR (>950 nm) fluorescence, respectively (Figure 2a). We confirmed the single-molecule (particle) sensitivity of our setup in the SWIR wavelength region using PbS QDs (Figure S2). The fluorescence from individual 3.5-

nm PSN and 3.6-nm BBT Pdots was captured on both detectors (Figure 2b and Figure S3a). The fluorescence spots that appeared on the SWIR channel spatially overlapped perfectly with those that appeared on the VIS/NIR-I channel (Figure 2f, Figure S3b, and Figure S4), demonstrating that the fluorescence of our SWIR-emitting Pdots can be detected at the single-particle level in the SWIR spectral region. Fluorescence intensity trajectories of single PSN and BBT Pdots captured using the two detectors exhibited nearly constant intensities over time without the blinking that is typically observed for QDs (Figure 2c, Figure S5a, and Figure S5b). The fluorescence brightness of the Pdots increased with increasing particle size (Figure 2d); this is beneficial for single-particle imaging experiments because an optimum fluorescence brightness can be attained by simply controlling their size. We found that the 3.5-nm PSN Pdots displayed approximately $5\times$ brighter fluorescence than the PbS QDs, although the particles were similar in size (Figure 2d). The 30-nm PSN Pdots exhibited fluorescence intensity similar to that of 200-nm polystyrene NPs doped with SWIR-emitting dyes (IR-E1050) (Figure 2d, Figure S6). The fluorescence brightness of the SWIR-emitting Pdots was further demonstrated by their fluorescence intensity per unit volume, which revealed that the 3.5-nm PSN Pdots showed $5\times$ and four orders of magnitude greater fluorescence brightness per unit volume than the PbS QDs and the IR-E1050-doped NPs, respectively (Figure 2e). All these results demonstrate the superior fluorescence brightness of the SWIR-emitting Pdots.

Fluorescence intensity saturation of the SWIR-emitting Pdots

Fluorescence intensity saturation is one of the greatest limitations in single-molecule fluorescence imaging, which typically requires a high illumination intensity from a focused laser through a high-numerical-aperture (NA) objective because the saturation leads to low signal-to-background ratio (SBR), which results in deteriorated images.³⁷ To investigate the fluorescence intensity saturation behavior of our Pdots, we measured the integrated fluorescence intensity of

the PSN and BBT Pdots at different excitation powers and compared them with the saturation behavior observed for the PbS QDs and dye-doped NPs (Figure 3). The intensity saturation behavior was characterized using a simple two-state model using Eq. 1:

$$S = S_{\infty} \frac{I}{I + I_S} \quad (1)$$

where I is the excitation intensity, I_S is the saturation intensity, and S_{∞} is the saturation count rate (i.e., the maximum fluorescence count rate). This analysis revealed that the SWIR-emitting Pdots exhibit much higher fluorescence saturation intensities ($39 \leq I_S \leq 113$ and $183.6 \text{ kW} \cdot \text{cm}^{-2}$ for the PSN and BBT Pdots, respectively) (Figure 3b, 3d, 3e, 3f, 3g) than other frequently used SWIR-emitting NPs ($I_S = 25$ and $3.9 \text{ kW} \cdot \text{cm}^{-2}$ for the IR-E1050-doped NPs and PbS QDs, respectively) (Figure 3a, 3c, 3g). In addition, a higher I_S was observed for the smaller PSN Pdots ($I_S = 113, 79$, and $39 \text{ kW} \cdot \text{cm}^{-2}$ for the 3.5-, 14.3-, and 31.9-nm Pdots, respectively; Figure 3d, 3e, 3f, 3g). At the moment, we can only speculate about the reason for the higher I_S of the SWIR-emitting Pdots and their dependence on size. Given the high density of the emitting species inside the particles and the expected energy migration and resultant excited-state annihilation, the higher I_S might be due to relatively inefficient intraparticle resonance energy transfer. The fact that highly efficient energy transfer between doped dyes was observed for the IR-E1050-doped NPs supports this hypothesis. The relatively low I_S observed for the PbS QDs is attributed to its long excited-state lifetime. Because of the very high I_S values, the 31.9-nm PSN Pdots show a $2.5\times$ higher calculated maximum fluorescence intensity ($I_{\infty} = 40.8 \text{ kcnts} \cdot \text{s}^{-1}$) compared with the 200-nm IR-E1050-doped NPs ($I_{\infty} = 16.4 \text{ kcnts} \cdot \text{s}^{-1}$, Figure 3h). Even the 14.3-nm PSN Pdots are expected to exhibit maximum fluorescence intensity ($I_{\infty} = 13.2 \text{ kcnts} \cdot \text{s}^{-1}$) close to that of the 200-nm IR-E1050-doped NPs (Figure 3h). All these results further suggest that the SWIR-emitting Pdots are promising

fluorophores for single-molecule (particle)-level fluorescence imaging of thick biological specimens that require high excitation intensity.

Single-particle fluorescence imaging through tissue phantoms

Encouraged by these findings, we tested the applicability of our SWIR-emitting Pdots for single-particle fluorescence imaging through tissue phantoms, which mimic the optical properties (i.e., absorption and scattering of light) of biological tissues (Figure 4a). We used polydimethylsiloxane (PDMS)-based tissue phantoms containing absorbing agents (coffee, nigrosine dye; Figure 4c) and a scattering agent (TiO₂ particles; Figure 4d) that mimic the optical properties of skin tissue (e.g., dermis).^{38, 39} Examples of tissue phantoms with varying thickness are shown in Figure 4b. We detected fluorescence from the PSN Pdots deposited onto the tissue phantoms through the phantoms using a continuous wave (CW) 785-nm laser at 44 kW·cm⁻² excitation power and long working distance (WD) high-NA objective lenses (water immersion (NA = 1.1, WD = 1.5 mm) or silicone immersion (NA = 1.05, WD = 0.8 mm)) (Figure 4a). We used only the PSN Pdots in these experiments because the PSN Pdots exhibit better long-term stability than the BBT Pdots. We detected fluorescence from individual 14.3- and 31.9-nm PSN Pdots through 0.80 and 1.27 tissue phantoms (Figure 4e and 4f) on both VIS/NIR-I and SWIR channels. Notably, the SWIR fluorescence image of the 31.9-nm PSN Pdots deposited onto a coverslip shows a higher background signal because of the saturation of the detector. The fluorescence brightness obtained from individual PSN Pdots gradually decreases with increasing phantom thickness, mainly because scattering of the excitation light leads to intensity attenuation at greater depths (Figure 4g). This attenuation is due to the reduced amplitude of both excitation light and fluorescence emission by light absorption and scattering. The effect of light scattering is also evident in the fluorescence spot size (i.e., our instrumental point spread function), which gradually increased

from 680 nm without the tissue phantoms to 1025 nm with the 1.27-mm-thick dermis tissue phantom (Figure 4h). Both effects strongly influence the SBR obtained in the through-tissue-phantom imaging experiments (Figure 4i). The SBR gradually decreases with increasing thickness of the tissue phantom from 33:1 without the tissue phantom to 4.5:1 for the 1.27-mm-thick phantom (Figure 4i). Importantly, our results show that better SBRs are obtained for the SWIR detector compared with the Vis/NIR-I detector for the thicker samples, highlighting the effectiveness of using the SWIR spectral region for single-particle fluorescence imaging through light absorbing/scattering media. Notably, even at the maximum depth, which is determined by the WD of the objective lens, we observed a relatively high SBR (4.5:1) because of the very bright fluorescence signal obtained from the SWIR-emitting Pdots. This result indicates that the maximum depth that could be reached in these experiments was limited not by the fluorescence intensity obtained from each Pdot or the obtained SBRs but by the WD of the objective lens.

Time-gated SWIR fluorescence imaging

Biological tissues exhibit weak light absorption in the SWIR spectral region. However, even in this spectral range, the scattering of light by biological tissues remains a major issue in fluorescence imaging. The scattering of the excitation light, which causes a strong attenuation of light intensity that adversely affects image quality, is one of the most critical issues in deep-tissue fluorescence imaging. The quality of the recorded fluorescence images can be dramatically improved by suppressing this effect. Thus, a time-gated detection of the fluorescence signal is an effective approach for obtaining fluorescence images of biological specimens with minimal background signal. Because elastic scattering is a nearly instantaneous optical process, the scattering signal caused by pulsed excitation light can be easily removed by applying a time gate if the fluorescence lifetime of the fluorophore is longer than the pulse width. In previous studies,

time-gate fluorescence imaging has been widely implemented using fluorophores with very long excited-state lifetimes (e.g., RENPs with an excited-state lifetime longer than milliseconds).⁴⁰ By exciting the fluorophores with microsecond-pulsed light and applying a time gate of milliseconds (i.e., a repetition rate less than 1 kHz), a time-gated fluorescence image can be captured using a single shot of the pulsed excitation and image acquisition. Although this popular acquisition mode allows capturing nearly background-free images at the ensemble level, single-molecule (particle) time-gated imaging does not work in this mode. Because a low repetition rate of the excitation pulse greatly restricts the number of fluorescence photons detected from individual particles, this mode of time-gated imaging is precluded for single-molecule (particle) imaging.

The short nanosecond-scale excited-state lifetime of our SWIR-emitting Pdots enables us to design an alternative mode of time-gated imaging. We used a pulsed light with a high repetition rate (repetition rate = 80 MHz, pulse width = 100 fs, wavelength = 790 nm) as an excitation light source and detected the SWIR fluorescence emission by applying a subnanosecond time gate to the SPAD photodetector (Figure 5a). We tested the performance of this mode of time-gated imaging using the IR-E1050-doped NPs deposited onto a 60- μm -thick poly(butyl methacrylate-*co*-methyl methacrylate) (PBMA)-based tissue phantom with TiO_2 NPs incorporated as a scattering agent (Figure 5b). The SBR of the image increased dramatically when a time gate was applied—from 2:1 without a time gate to 9:1–32:1 with the time gates (Figure 5d). The SBR increased when narrower time gates were applied, thus demonstrating that the observed improvement in the SBR was a result of the effective removal of the background scattering.

The same trend was observed for the 3.5-nm PSN Pdots, whereas the SBRs at each time-gate condition differed from those observed for the IR-E1050-doped NPs (Figure 5c, 5e). A greater improvement of the SBR—from 6:1 without the time gate to 172:1 with the maximum gate delay

(i.e., a $29\times$ improvement in the SBR)—was observed for the PSN Pdots than for the IR-E1050-doped NPs, which exhibited a maximum $16\times$ improvement in the SBR when the time gate was applied (Figure 5d, 5e). This difference is due to the fluorescence brightness and saturation intensity of the NPs. The results demonstrate that time-gated imaging enables the acquisition of single-particle fluorescence images through turbid media without compromising photon counting throughput by selectively removing the scattering of the excitation light. Our findings also suggest that this mode of time-gated fluorescence imaging could be used as a general approach for improving the SBR in single-particle fluorescence imaging in the NIR/SWIR wavelength region.

Importantly, the improvement in the SBR through this mode of time-gated imaging is achieved by the reduction of the background without substantially affecting the detection efficiency of the emitted fluorescence photon. In contrast, in other imaging modes with enhanced SBR (e.g., afterglow fluorescence imaging), the improved SBR is achieved at the expense of fluorescence brightness, which limits the application of the imaging mode to single-particle imaging.

We stress that our time-gated imaging is compatible only with fluorophores with an excited-state lifetime substantially shorter than the pulse interval (i.e., tens of nanoseconds). Thus, most other SWIR-emitting fluorophores (e.g., RENPs and QDs) are not compatible with our imaging mode because their excited-state lifetimes are of the order of microseconds to milliseconds. Thus, the unique fluorescent characteristics (i.e., very bright SWIR fluorescence emission together with a nanosecond excited-state lifetime) of our SWIR-emitting Pdots are critical for this single-particle time-gated imaging.

Conclusion

In conclusion, we demonstrated that the SWIR-emitting Pdots developed in this study exhibit exceedingly bright SWIR fluorescence partly due to the high fluorescence saturation intensity; this enabled us to detect the fluorescence from individual Pdots through millimeter-thick tissue phantoms using customized single-molecule SWIR microscopy setup equipped with both silicon and InGaAs photodetectors. Currently, deep-tissue fluorescence imaging at the single-particle level is conducted at a maximum specimen thickness of approximately 100 μm using nonideal fluorophores (e.g., large fluorophores based on SWNTs or toxic fluorophores based on QDs). Thus, its application is limited to specimens with a large free volume (e.g., visualizing blood flow, characterizing motion of molecules in extracellular matrix) and which do not require low cytotoxicity. Given the size of the biological tissues used in biomedical research (e.g., mouse brain is several millimeters in size), the millimeter-deep single-particle detection is a significant step toward imaging entire biological tissues at the single-particle level. In addition, the new single-particle time-gated imaging that we demonstrated herein could, in principle, be extended to real-time imaging using a gated camera. This study could, therefore, have far-reaching impacts in relevant fields and expand the applicability of SWIR fluorescence imaging to a wide range of fields.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available. Materials and Methods, Supporting Note, and Figures S1 – S6. This material is available free of charge via the internet at <http://pubs.acs.org>.

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Author Contributions

S.H. and H.P. conceived the project. H.P and S.H. designed the experiments. H.P. conducted the experiments and analyzed the data. Y.W., W.L., and T.M. synthesized the polymer. H.P. and S.H. wrote the manuscript. All authors discussed the results.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

This study was supported by King Abdullah University of Science and Technology (KAUST) and the KAUST Office of Sponsored Research (OSR) under Award No. OSR-2016-2967-CRG5.

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Figures

Figure 1. Chemical structures of (a) PSN and (b) BBT. (c) Steady-state absorption (solid lines) and fluorescence (dashed lines) spectra of the PSN Pdots with three different sizes dispersed in water. (d) Steady-state absorption (solid line) and fluorescence (dashed line) spectra of the 3.6-nm BBT Pdots dispersed in water. Transmission electron microscopy images of the (e) 3.5-nm PSN Pdots, (f) 14.3-nm PSN Pdots, (g) 31.9-nm PSN Pdots, and (h) 3.6-nm BBT Pdots. (i) Size distribution of the 3.6-nm BBT Pdots (B3.6), 3.5-nm PSN Pdots (P3.5), 14.3-nm PSN Pdots (P14.3), and 31.9-nm PSN Pdots (P31.9). Error bars show standard deviations determined from 83, 210, 89, and 116 particles for B3.6, P3.5, P14.3, and P31.9, respectively.

Figure 2. Single-particle SWIR fluorescence microscopy experiment. (a) Schematic of custom-built single-molecule SWIR fluorescence microscopy setup. (b) Absorption (blue line) and fluorescence (red line) spectra of the PSN Pdots. The fluorescence of the PSN Pdots detected using the VIS/NIR-I detector and SWIR detector are shaded blue and red, respectively. (c) Fluorescence intensity time trajectories of a single 3.5-nm PSN Pdot deposited onto a glass coverslip, as captured by the VIS/NIR-I detector (top) and SWIR detector (bottom). (d) Integrated fluorescence intensity and (e) fluorescence brightness per unit volume obtained from individual PbS Qdots (QDs), 3.6-nm BBT Pdots (B3.6), 3.5-nm PSN Pdots (P3.5), 14.3-nm PSN Pdots (P14.3), 31.9-nm PSN Pdots (P31.9), and IR-E1050 dye-doped NPs (IRNPs). Error bars show standard deviations determined from 2231, 483, 1966, 691, 1935, and 444 particles for QDs, B3.6, P3.5, P14.3, P31.9, and IRNPs, respectively. (f) Fluorescence images obtained from individual 3.5-nm (top), 14.3-nm (middle), and 31.9-nm (bottom) PSN Pdots deposited onto a glass coverslip, as captured by the VIS/NIR-I

detector (left) and SWIR detector (right) with 2.5-ms dwell time. The samples were excited using a CW laser beam centered at 785 nm and with an average power of $29 \text{ kW} \cdot \text{cm}^{-2}$.

Figure 3. Excitation power dependence of the fluorescence intensity obtained for individual (a) PbS QDs (QDs), (b) 3.6-nm BBT Pdots (B3.6), (c) IR-E1050 dye-doped NPs (IRNPs), (d) 3.5-nm PSN Pdots (P3.5), (e) 14.3-nm PSN Pdots (P14.3), and (f) 31.9-nm PSN Pdots (P31.9) deposited onto a glass coverslip. The solid lines show fitting of the data to Eq. 1. (g) Saturation intensities and (h) maximum fluorescence count rates obtained for the SWIR-emitting fluorophores determined by fitting the excitation power-dependent fluorescence intensity displayed in (a–f) to Eq. 1. The shade in (g) shows the range of the excitation power used in the imaging experiments in Figures 2 and 4.

Figure 4. Single-particle fluorescence imaging of the PSN Pdots through tissue phantoms. (a) Schematic describing experimental configuration. (b) Photograph of the tissue phantoms with varying thickness used in the experiment. (c) Absorption and (d) reduced scattering spectra of the tissue phantoms used in the experiment. Fluorescence images of individual (e) 14.3-nm PSN Pdots and (f) 31.9-nm PSN Pdots deposited onto the tissue phantoms with varying thickness (0.00–1.27 mm), as captured by the VIS/NIR-I detector (left) and SWIR detector (right) with a 1-ms dwell time. The samples were excited using a CW laser beam centered at 785 nm and operated at an average power of $44 \text{ kW} \cdot \text{cm}^{-2}$. (g) Tissue phantom thickness-dependent average fluorescence intensities obtained from individual 31.9-nm (P31.9) and 14.3-nm (P14.3) PSN Pdots. Error bars show standard deviations determined from 687 and 427 particles for P31.9 and P14.3, respectively.

Tissue phantom thickness-dependent (h) fluorescence spot sizes and (i) signal-to-background ratio (SBR) of the 31.9-nm PSN Pdots.

Figure 5. Single-particle time-gated SWIR fluorescence imaging. (a) Schematic of the custom-built time-gated single-molecule time-gated SWIR fluorescence imaging setup. Fluorescence decay curves of the (b) IR-E1050-doped NPs and (c) 3.5-nm PSN Pdots (red) with the instrument response function (green). The gray shaded regions correspond to the applied time delay of the detection. The blue arrows show the applied time gate of the detection. Fluorescence images of individual (d) IR-E1050-doped NPs and (e) 3.5-nm PSN Pdots deposited onto 40- μm -thick

PBMA-TiO₂ tissue phantom captured at different time gates. The samples were excited using a femtosecond laser pulses centered at 790 nm and operated at an average power of 246 W·cm⁻².

TOC Graphic