

Near infrared fluorescence lifetime imaging of biomolecules with carbon nanotubes

*Linda Sistemich¹, Phillip Galonska¹, Jan Stegemann^{1,2}, Sebastian Kruss^{*1,2}*

¹Department of Chemistry, Ruhr University Bochum, 44801 Bochum, Germany

²Biomedical Nanosensors, Fraunhofer Institute for Microelectronic Circuits and Systems, 47057 Duisburg, Germany

Nanosensors, fluorescence lifetime, confocal microscopy, single wall carbon nanotubes, FLIM, TCSPC, dopamine

Abstract

Single wall carbon nanotubes (SWCNTs) are versatile building blocks for biosensors. Their near infrared (NIR) fluorescence enables detection of biomolecules in the optical tissue transparency window. The fluorescence intensity of SWCNTs changes in response to an analyte and this interaction can be chemical tailored by the surface chemistry. However, optical signals based on intensity are affected by external factors such as sample movement or fluctuations in excitation light. Here, we demonstrate fluorescence lifetime imaging microscopy (FLIM) of SWCNT-based sensors in the NIR as calibration-free method.

For this purpose, we tailored a confocal laser scanning microscope (CLSM) for NIR signals (>800 nm) and employed time correlated single photon counting (TCSPC). (GT)₁₀-DNA functionalized SWCNTs are then used as sensors because they increase their fluorescence (995 nm) in response to the important neurotransmitter dopamine. Their fluorescence lifetime (> 900 ns) follows a biexponential decay and the longer lifetime component (370 ps) changes with dopamine concentration. It increases by up to 25 % with detection limits in the nM range. These sensors serve as paint to cover cells and report extracellular dopamine in 3D via FLIM. We therefore show the potential of using fluorescence lifetime in combination with confocal microscopy as readout for SWCNT-based sensors.

Single wall carbon nanotubes (SWCNTs) are a 1D material with beneficial optoelectrical properties. In contrast to organic dyes or quantum dots, SWCNTs do not blink or bleach and fluoresce in the near Infrared (NIR) region of the electromagnetic spectrum ¹⁻³. The NIR (> 800 nm) is especially beneficial for biological applications as it falls into the tissue transparency window where light absorption, scattering as well as autofluorescence are minimal ⁴⁵⁻⁷. Light absorption by SWCNTs leads to excitons that travel along the SWCNT axis for around 100 nm ⁸⁻¹⁵. The SWCNT's organic corona influences the exciton, making the SWCNT itself highly sensitive for chemical changes around it. Surface chemistry is also crucial for colloidal stability because pristine SWCNTs are hydrophobic and cannot be suspended in polar solvents like physiological buffers and media.

Due to their properties SWCNTs have emerged as versatile building blocks for chemical sensing and imaging ^{1,7,16,17}. Functionalization of the SWCNT's with DNA ¹⁸, peptides ¹⁹ or polymers ²⁰, does not only result in solubility, but renders them chemically selective for detection of different analytes like reactive oxygen species ²¹⁻²⁶, small molecules ²⁷⁻³², sugars ³³ and larger biomolecules like peptides, proteins and nucleic acids ³⁴⁻⁴¹ or neurotransmitters like dopamine ⁴²⁻⁴⁵ or serotonin ^{46,47}.

Neurotransmitter play an essential role in the human body, mainly in chemical signaling in the neuronal network of the brain. A key player is the neurotransmitter dopamine, regulating motor function as well as reward and motivation ^{48,49}. Detection of dopamine and other neurotransmitters with a high spatiotemporal resolution is therefore a major but challenging goal to gain deeper insights in intercellular communication. In contrast to other approaches such as electrochemical or genetically encoded sensors ⁵⁰⁻⁵⁸ imaging of neurotransmitter with SWCNT-based sensors offers very high spatial and temporal resolution ^{42,46,59-61}.

First surface chemistry screening approaches showed that SWCNTs coated with single stranded (ss)DNA respond to dopamine and other catecholamines by a reversible increase in fluorescence intensity⁴³. MD simulations revealed that an interaction between polar groups from dopamine and the phosphate backbone of the DNA moves the backbone closer to the SWCNT resulting in an altered electrostatic potential at the SWCNT surface^{59,62,63}. Coating of SWCNTs with guanine (G) containing ssDNA sequences such as (GT)_x-SWCNTs showed the best results and these sensors were used for biological studies^{42,59,61}. Additional characterization of (GT)_x-SWCNTs rendered a length of 10 bases ((GT)₁₀) to be most selective and sensitive⁴⁴. Imaging of dopamine in a cellular context⁶⁴ using such sensors was first performed by coating the glass surface before seeding neuroprogenitor cells⁵⁹. This approach provided a good spatiotemporal resolution to observe dopamine release and was also applied to the detection of serotonin⁴⁶. Furthermore, by coating cells with sensors similar to a paint (*AndromeDA*) dopamine detection at axonal varicosities of primary neurons was achieved⁴².

NIR imaging of fluorescence intensities is a quickly advancing field, ranging from widefield microscopy⁶⁵ over laser scanning confocal microscopy⁶⁶ to spinning-disc confocal microscopy⁶⁷. However, fluorescence signals contain much more information than the mere intensity. Fluorescence anisotropy as well as fluorescence lifetimes contain valuable additional and unique information. Their advantage is that they are absolute measures or in other words internally calibrated. It means that e.g. the fluorescence lifetime does not change when the focus in a microscope changes or the light source fluctuates. This is an important advantage especially for biological samples. Fluorescence lifetimes depend on intrinsic dye properties, micro surrounding and molecular interactions but is independent of fluorophore concentration, photobleaching, sample thickness and excitation intensity⁶⁸. Fluorescence lifetime imaging microscopy (FLIM)

offers the opportunity to assess and image changes of the molecular environment in the direct vicinity of the fluorophore leading to changes in the fluorescence lifetime. FLIM is typically performed by time correlated single photon counting (TCSPC) in a confocal laser scanning microscopy (CLSM). A challenge in FLIM is to find the sweet spot of sufficient photon counts and a minimal acquisition time to obtain also a high temporal resolution of changes in the fluorescence lifetime. Therefore, short pulsed lasers with high repetition rates, a CLSM, suitable detectors like single photon avalanche diodes (SPAD) and the respective electronics to record TCSPC traces are required.

The fluorescence lifetime of SWCNTs depends on the type of functionalization of the corona, the solvent and the temperature ⁶⁹⁻⁷¹. It lies in the order of hundreds of picoseconds and the measurements are therefore more challenging compared to lifetimes in the nanosecond regime e.g. of organic fluorophores. Lower temperatures ^{72,73}, organic solvents ⁷⁴ and for example guanine quantum defects in (GT)₁₀ coated SWCNTs ⁷⁵ prolong the fluorescence lifetime, simplifying their detection.

In this work, we exploit for the first time the fluorescence lifetime of SWCNT to detect biomolecules in the NIR above 900 nm. Combining time correlated single photon counting and confocal fluorescence microscopy we show that fluorescence lifetime acts as sensitive measure for the neurotransmitter dopamine and confocal microscopy allows for scanning in 3D around cells (Figure 1).

ssDNA coated SWCNTs are known to be sensitive to several analytes. The sequence (GT)₁₀ has been reported to make SWCNTs selective and sensitive in the nM range for the important neurotransmitter dopamine ⁴⁴. Therefore, (6,5)-chirality enriched SWCNTs were functionalized

with (GT)₁₀-ssDNA. Fluorescence emission spectra of 0.1 nM (GT)₁₀-SWCNTs in PBS showed a strong increase in fluorescence intensity upon addition of 100 μ M dopamine at around 995 nm, corresponding to the emission maximum of the (6,5)-chirality (Figure 2A). We hypothesized that a fluorescence increase is correlated with an increase of fluorescence lifetime. This makes also sense given that the quantum yield depends on the fluorescence lifetime⁷⁶. (6,5) SWCNTs have their excitation maximum at around 560 nm but in our optical setup only a pulsed 481 nm laser was available. The excitation-emission spectrum (Figure 2B, S1) shows that even though less efficient other wavelengths can be used for excitation as well. Therefore, we tailored a standard CLSM (MicroTime200 PicoQuant) for the NIR. It consists of a confocal fluorescence microscope unit with a pulsed laser and single photon detection unit controlled by electronics that allows time correlated single photon counting (TCSPC) (Figure 1). The optical setup was optimized for the NIR by replacing the general dichroic mirror and adjusting the focal length for NIR signals. Detection of fluorescence signals in the NIR can be challenging and the optical setup was optimized to obtain best results. A cut-on dichroic mirror, allowing a high transmission of (N)IR light up (800-2500 nm), was compared to a Quad Line dichroic mirror which is regularly used to detect fluorescence up to 1200 nm. In the relevant wavelength range (900-1200 nm) transmission is comparable but the Quad Line dichroic allowed additional detection of the membrane stain in cell experiments. Furthermore, we found that red-light sensitive Si-based single photon avalanche diodes (SPAD, Excelitas Technologies) are better suited than a NIR-photomultiplier tube (Hamamatsu).

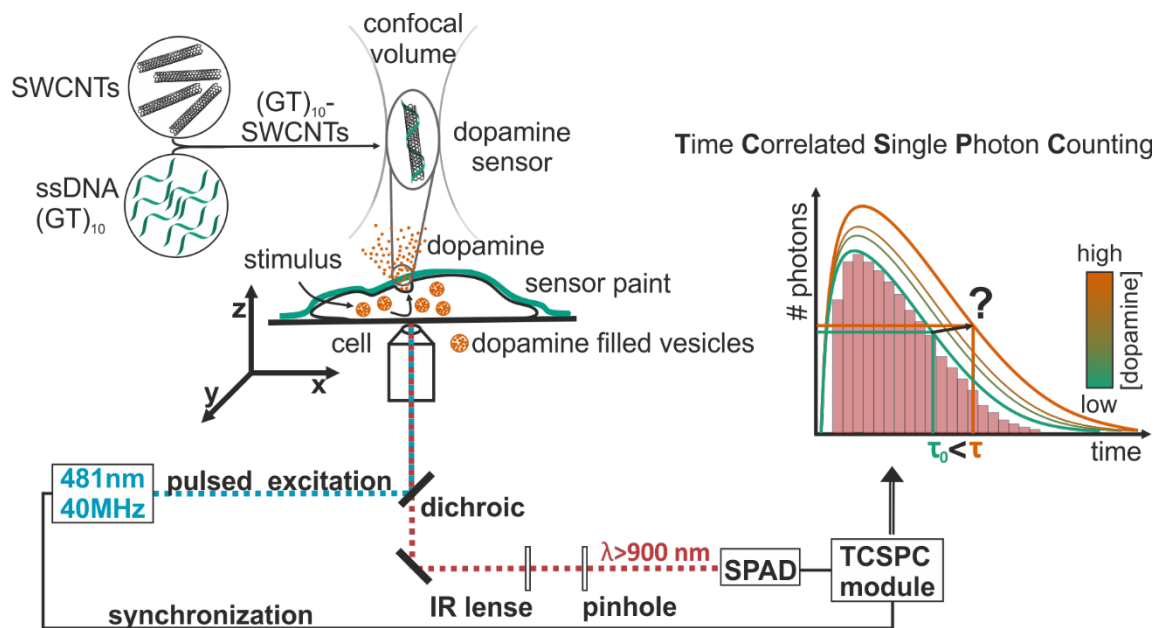


Figure 1. Near infrared fluorescence lifetime imaging of nanosensors. (GT)₁₀-SWCNTs serve as sensors for the neurotransmitter dopamine. Pulsed excitation and time correlated single photon counting is used to measure changes in the fluorescence intensity and lifetime of the nanotubes dependent on the dopamine concentration. It allows to perform fluorescence lifetime imaging in a confocal microscope and study neurotransmitter release by cells coated with these nanosensors.

In the described setup 1 nM (GT)₁₀-SWCNTs were excited with a pulsed laser (481 nm, 40 MHz) to obtain Time Correlated Single Photon Counting (TCSPC) histograms (Figure 2C), containing information about the fluorescence intensity and lifetime. Both detectors revealed an increase in fluorescence intensity in the presence of 100 μ M dopamine (Figure S2). Nevertheless, only the SPAD detector was able to report the fluorescence lifetime of our dopamine sensor as the temporal resolution of the PMT was too slow (400 ps) to allow detection of photons with a lifetime below this limit. In Figure 2C the TCSPC histogram in absence and presence of dopamine, recorded by the SPAD is shown. For fluorescence intensity (Figure 2C) as well as for fluorescence lifetime (Figure S3) an increase was detected upon addition of 100 μ M dopamine. For further

application in a cellular context, possible side effects on the fluorescence lifetime were ruled out. For this purpose, ensemble measurements of the sensors were performed in cell culture medium. While addition of dopamine to SWCNTs in RPMI resulted in a comparable increase in fluorescence lifetime as in 1x PBS, the sensors did not respond to high concentrations of K^+ ions, which is relevant because they have been used later to stimulate exocytosis of dopamine filled vesicles from neural progenitor cells (Figure S3).

The short fluorescence lifetimes are challenging and at the limit of the optical setup (Figure 2C) as can be seen by the small difference between the instrument response function (IRF, gray) and the TCSPC histogram (cyan) of the dopamine sensor in absence of dopamine. To fit the TCSPC histograms we made use of the IRF, which was calculated by the Software (SymPhoTime64, PicoQuant). The instrument response function can also be determined experimentally by recording the TCSPC histogram of a quenched fluorophore. The fluorescence lifetime of SWCNTs is already very short and close to the time resolution of the detector and the IRF. Further shortening of the SWCNT's lifetime should be difficult with the used electronics. Nevertheless, we tested riboflavin as a known quencher of SWCNT fluorescence⁶³ but detected predominantly the lifetime of leaking photons from riboflavin and therefore this method was not applicable for us. The obtained TCSPC histograms showed a biexponential decay from which a shorter and a longer lifetime was obtained. While the shorter lifetime component, in the range of 85 ps, is below the resolution limit of the SPAD detector, the longer lifetime can be resolved with the used optics (SI, Table 1). The determined longer lifetime component was in the range of 350–460 ps and comparable to published lifetimes of (GT)₁₀-SWCNTs in water⁷⁵. Differences in the lifetimes can be accounted by different lasers and detectors and purity of the SWCNTs. Most importantly, the longer lifetime component increased in response to dopamine (Figure 2C). Therefore, we focused on the reliable changes of

the longer lifetime component as depicted in Figure S3 and Figure 3A rather than the absolute lifetime values (SI, Table 1).

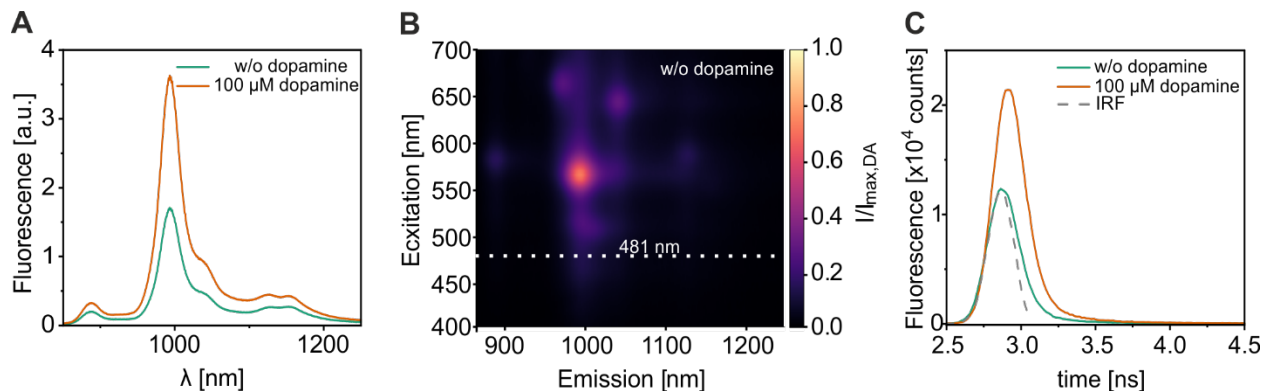


Figure 2. Characterization of the fluorescence lifetime sensor. A) Fluorescence spectrum of SWCNTs without (cyan) and with (orange) 100 μ M dopamine excited at 561 nm. B) 2D excitation-emission plot of 2 nM (GT)₁₀-SWCNTs in the absence of 100 μ M dopamine. C) TCSPC histogram of SWCNTs fluorescence in the absence without (cyan) and with (orange) 100 μ M dopamine excited at 481 nm. The instrument response function (IRF) is depicted in gray.

The change in fluorescence intensity as a readout for dopamine concentration is associated with dissociation constants in the nM regime ⁴⁴. To obtain a dose-response curve for the relative change in fluorescence lifetime as a function of dopamine concentration 1 nM (GT)₁₀-SWCNTs were mixed in physiological buffer with increasing dopamine concentrations (1 nM-100 μ M) and TCSPC histograms were recorded by collecting all photons > 900 nm. The obtained relative changes in fluorescence intensity and fluorescence lifetime are depicted in Figure 3A. Fluorescence intensities increased up to 60 % and fluorescence lifetimes increased by up to 25 % with increasing dopamine concentration (Figure 3A). These results show that fluorescence lifetimes can be used as measure for the dopamine concentration.

To rule out, that the determined lifetimes are not biased by the IRF and the fitting procedure, a phasor analysis was performed for the obtained concentration dependent TCSPC histograms. This fit-free analysis approach revealed that the relative changes in the longer lifetime component obtained by a biexponential fit are real and distinct lifetimes can be differentiated (Figure 3B,C, S4). The increase of absolute (mean) lifetimes is depicted in Figure 3C, showing an increase from 121 ps, in the absence of dopamine, to 145 ps in the presence of 100 μ M dopamine (SI Table 2). These lifetimes are in good agreement with already published lifetimes of (GT)₁₀ coated (and additionally guanine defected) SWCNTs by Zheng et al.⁷⁵ revealing a lifetime of ca. 170 ps for (GT)₁₀-SWCNTs. It is important to note that the lifetime values are not on the hemisphere in the phasor plot because of the biexponential decay. Furthermore, these numbers are different from the long lifetime component (Figure 3A) because the data includes as well the non-changing short component.

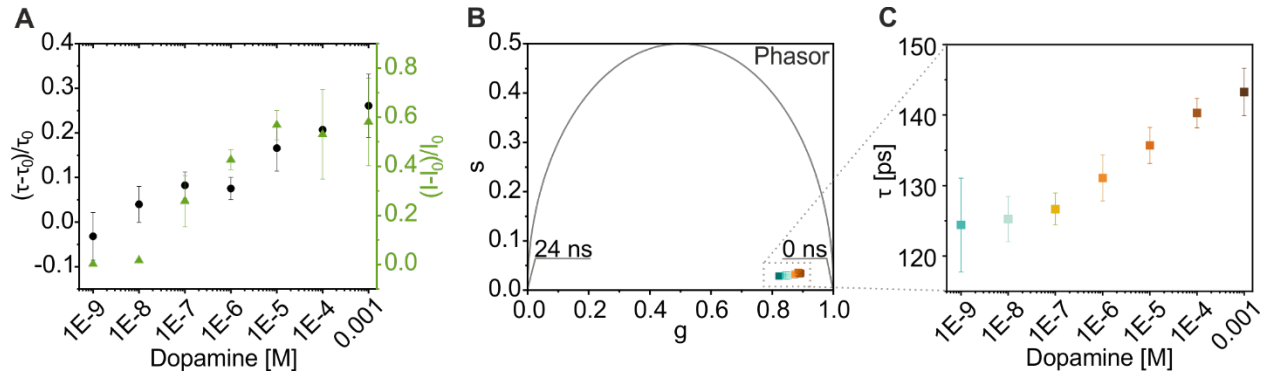


Figure 3. Fluorescence lifetime increases with dopamine concentration. A) Relative change in the longer-lived fluorescence lifetime component (black) and fluorescence intensity (green) of 1 nM (GT)₁₀-SWCNTs as function of the dopamine concentration. $N=3$, mean $\pm$ SD. B) Phasor plot of fluorescence lifetimes of dopamine concentration dependent TCSPC. Note that the data points look squeezed because the whole plot covers a much longer time scale. C) Absolute lifetimes from fit-free phasor analysis (B). $N=3$, mean $\pm$ SD.

The neurotransmitter dopamine plays an essential role in intercellular communication in the brain. Detection of the extracellular dopamine levels is important to better understand this type of chemical communication, which is based on release and uptake events. To test the lifetime sensor in this biological context we used the recently published sensing approach called *AndromeDA*⁴². Here, the SWCNTs are used as a sensor paint that covers both, glass surface between the cells as well as the cells itself⁴² instead of placing the sensor beneath the cells as it was state of the art^{46,59,61}. To find out if FLIM with SWCNT-based sensors is possible in a complex environment in cell medium surrounded by cells, first a more general approach was tested. For this purpose, HeLa cells were seeded and coated with the sensors. Z-stack imaging of sensor coated HeLa cells showed, that the sensors distributed around and on top of the cells but were not located beneath the cells and additionally they were not taken up within the time scale of the experiment (20 min) (Figure 4A). FLIM is a sensitive tool to distinguish between environmental changes and the lifetime is a robust readout. A drawback of CLSM-based FLIM is that scanning takes time. To show that fast (non-parallel) 3D measurements are possible we placed the confocal spot on a region of interest close on the cells and tracked changes in the fluorescence intensity and fluorescence lifetime to monitor temporal changes in fluorescence by artificially added dopamine (100 μ M). The obtained time traces of fluorescence intensity show an increase in intensity upon dopamine addition ($t=0$ s) as shown exemplarily for one cell in Figure 4C (upper panel). Similarly, the TCSPC histograms before and after addition of dopamine reveal an increase in lifetime indicating the increased concentration of dopamine (Figure 4D). This shows that even around HeLa cells the lifetime sensor is able to report dopamine concentrations.

Next, we observed the sensors on and around the neural progenitor cell line PC12, which is known to release dopamine *via* exocytosis upon stimulation^{59,77,78}. Again, the PC12 cells were

seeded, and the sensors were used as (physisorbed) paint to cover the cells (Figure 4B). A Z-stack was obtained prior to the time resolved fluorescence detection to identify an interesting point of interest for measuring the local dopamine concentration. To activate exocytosis of dopamine-filled intracellular vesicles, PC12 cells were triggered by addition of KCl, while recording a fluorescence time trace. The FLIM measurements before and after triggering the cells as well as the time resolved fluorescence in a defined confocal volume revealed an increase in fluorescence intensity (Figure 4B,C). Additionally, dissection of the fluorescence time trace before and after KCl addition and respective lifetime analysis showed again an increase in fluorescence lifetime (Figure 4D). All together we thus demonstrate NIR FLIM of biomolecules around cells.

Fluorescence traces can easily be affected by the addition/pipetting of dopamine or KCl because the focus slightly shifts, which is a common problem of such experiments (Figure S5). Nevertheless, when extracting the fluorescence lifetime from the recorded traces an increase in the fluorescence lifetime after dopamine addition or release was still revealed. This observation perfectly demonstrates the advantage of an internally calibrated readout (fluorescence lifetime) compared to the easily biased fluorescence intensity.

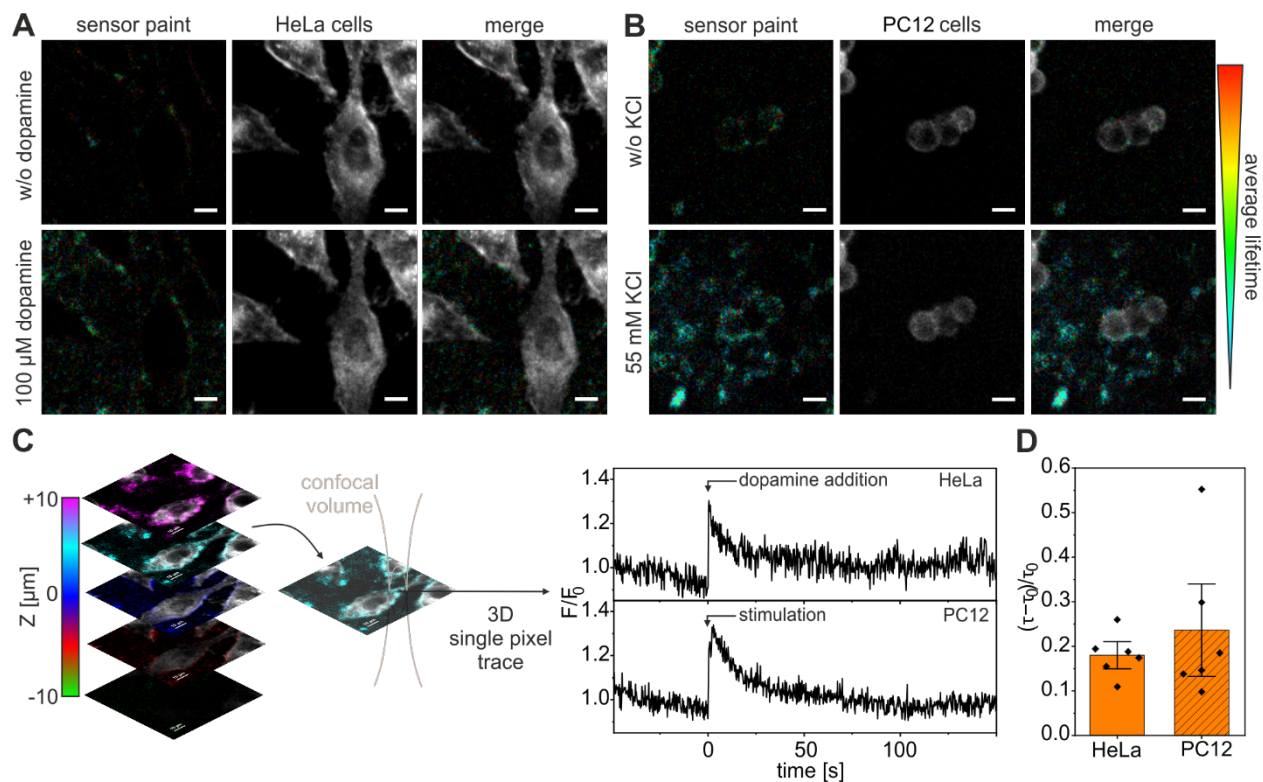


Figure 4. Confocal fluorescence lifetime imaging (FLIM) and single pixel tracing of dopamine around cells. A) FLIM of (GT)₁₀-SWCNTs (left panel) surrounding HeLa cells (middle panel) in the absence (upper row) and presence (lower row) of 100 μM dopamine. B) FLIM of (GT)₁₀-SWCNTs (left panel) covering PC12 neuroprogenitor cells (middle panel) sensing dopamine release triggered by KCl (lower row). Scale bars equal 10 μm. C) Time resolved dopamine sensing in a defined small volume. Exemplary fluorescence intensity traces before and after addition of dopamine (HeLa, upper row) or KCl (PC12, lower row) at $t=0$ s. D) The increase in lifetimes of (GT)₁₀-SWCNTs show dopamine presence around HeLa cells or released by PC12 cells. $N=6$, mean $\pm$ SD).

Chemical signaling by cells is typically very fast, which means that fast imaging is required. This is especially true for release of neurotransmitters⁷⁹. The current limitations in temporal resolution because of the confocal scanning process could be further increased by several technical

improvements. First of all, the laser excitation (Figure 2B) that we used in our optical setup was not resonant. For (6,5)-SWCNTs resonant excitation at 560 nm would increase the signal by a factor of around 10. Additionally, one could use monochiral samples to improve the resonant excitation further^{17,80}. In this context, (6,4)-SWCNTs that emit closer to the visible range (870 nm) could further increase the signal because the quantum yield of the Si-based detectors significantly increases compared to the 995 nm light detected by us. Nevertheless, our results show the potential of FLIM with SWCNT-based sensors and technical improvements could further increase temporal resolution etc.

In conclusion, we demonstrate for the first time that the NIR fluorescence lifetime of SWCNTs can be used for detection of molecules. It is possible in simple aqueous solutions as well as in cell experiments. This combination of lifetime imaging and confocal microscopy of nanosensors allows to probe biomolecule concentrations in well-defined locations around cells. In the future this approach will help to gain deeper understanding of chemical signaling between cells.

Supporting Information

Experimental details, characterization figures, additional sensor response data

Corresponding Author

Sebastian Kruss, Department of Chemistry, Ruhr University Bochum, 44801 Bochum, Germany, Sebastian.kruss@ruhr-uni-bochum.de, +49-234-32-29946

Author Contributions

SK and LS designed and conceived the research. LS performed lifetime measurements. PG functionalized SWCNTs and contributed to sensor characterization. JS performed the phasor analysis. LS, PG, JS and SK analyzed data. LS and SK wrote the manuscript with inputs from all authors.

Funding Sources

Funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy 3 EXC 2033 3 390677874 3 RESOLV. This work is supported by the >Center for Solvation Science ZEMOS< funded by the German Federal Ministry of Education and Research BMBF and by the Ministry of Culture and Research of Nord Rhine- Westphalia. Funded by the VW foundation. This work was supported by the Fraunhofer Internal Programs under Grant No. Attract 038 - 610097.

HeLa CCL-2 cells were a kind gift from Prof. Dr. Jörg Tatzelt (Department Biochemistry of Neurodegenerative Diseases, Institute of Biochemistry and Pathobiochemistry, Ruhr-University Bochum, Bochum, Germany). We thank Roman Tsukanov for fruitful discussions about phasor analysis.

AndromeDA, adsorbed nanosensors detecting release of dopamine; CLSM, confocal laser scanning microscopy; FLIM, fluorescence lifetime imaging microscopy; SWCNT, single wall carbon nanotube; TCSPC, time correlated single photon counting;

References

- (1) Ackermann, J.; Metternich, J. T.; Herbertz, S.; Kruss, S. Biosensing with Fluorescent Carbon Nanotubes. *Angew. Chemie - Int. Ed.* **2022**. <https://doi.org/10.1002/anie.202112372>.
- (2) Hong, G.; Antaris, A. L.; Dai, H. Near-Infrared Fluorophores for Biomedical Imaging. *Nat. Biomed. Eng.* **2017**, *1* (1), 0010. <https://doi.org/10.1038/s41551-016-0010>.
- (3) Hong, G.; Diao, S.; Antaris, A. L.; Dai, H. Carbon Nanomaterials for Biological Imaging and Nanomedicinal Therapy. *Chem. Rev.* **2015**, *115* (19), 10816–10906. <https://doi.org/10.1021/acs.chemrev.5b00008>.
- (4) Farrera, C.; Torres Andón, F.; Feliu, N. Carbon Nanotubes as Optical Sensors in Biomedicine. *ACS Nano* **2017**, *11* (11), 10637–10643. <https://doi.org/10.1021/acsnano.7b06701>.
- (5) O'Connell, M. J.; Bachilo, S. H.; Huffman, C. B.; Moore, V. C.; Strano, M. S.; Haroz, E. H.; Rialon, K. L.; Boul, P. J.; Noon, W. H.; Kittrell, C.; Ma, J.; Hauge, R. H.; Weisman, R. B.; Smalley, R. E. Band Gap Fluorescence from Individual Single-Walled Carbon Nanotubes. *Science* (80-.). **2002**, *297* (5581), 593–596. <https://doi.org/10.1126/science.1072631>.

- (6) Bachilo, S. M.; Strano, M. S.; Kittrell, C.; Hauge, R. H.; Smalley, R. E.; Weisman, R. B. Structure-Assigned Optical Spectra of Single-Walled Carbon Nanotubes. *Science* (80-.). **2002**, 298 (5602), 2361–2366. <https://doi.org/10.1126/science.1078727>.
- (7) Kruss, S.; Hilmer, A. J.; Zhang, J.; Reuel, N. F.; Mu, B.; Strano, M. S. Carbon Nanotubes as Optical Biomedical Sensors. *Adv. Drug Deliv. Rev.* **2013**, 65 (15), 1933–1950. <https://doi.org/10.1016/j.addr.2013.07.015>.
- (8) Zhao, H.; Mazumdar, S. Electron-Electron Interaction Effects on the Optical Excitations of Semiconducting Single-Walled Carbon Nanotubes. *Phys. Rev. Lett.* **2004**, 93 (15), 8–11. <https://doi.org/10.1103/PhysRevLett.93.157402>.
- (9) Ando, T. Effects of Valley Mixing and Exchange on Excitons in Carbon Nanotubes with Aharonov-Bohm Flux. *J. Phys. Soc. Japan* **2006**, 75 (2), 1–12. <https://doi.org/10.1143/JPSJ.75.024707>.
- (10) Spataru, C. D.; Ismail-Beigi, S.; Capaz, R. B.; Louie, S. G. Theory and Ab Initio Calculation of Radiative Lifetime of Excitons in Semiconducting Carbon Nanotubes. *Phys. Rev. Lett.* **2005**, 95 (24), 1–4. <https://doi.org/10.1103/PhysRevLett.95.247402>.
- (11) Berciaud, S.; Cognet, L.; Lounis, B. Luminescence Decay and the Absorption Cross Section of Individual Single-Walled Carbon Nanotubes. *Phys. Rev. Lett.* **2008**, 101 (7), 15–18. <https://doi.org/10.1103/PhysRevLett.101.077402>.
- (12) Srivastava, A.; Htoon, H.; Klimov, V. I.; Kono, J. Direct Observation of Dark Excitons in Individual Carbon Nanotubes: Inhomogeneity in the Exchange Splitting. *Phys. Rev. Lett.* **2008**, 101 (8), 1–4. <https://doi.org/10.1103/PhysRevLett.101.087402>.

- (13) Lüer, L.; Hoseinkhani, S.; Polli, D.; Crochet, J.; Hertel, T.; Lanzani, G. Size and Mobility of Excitons in (6, 5) Carbonnanotubes. *Nat. Phys.* **2009**, *5* (1), 54–58. <https://doi.org/10.1038/nphys1149>.
- (14) Cognet, L.; Tsybouski, D. A.; Rocha, J.-D.; Doyle, C. D.; Tour, J. M.; Weisman, B. R. Stepwise Quenching of Exciton Fluorescence in Carbon Nanotubes by Single-Molecule Reactions. *Science* (80-.). **2007**, *316* (8), 1465–1469. <https://doi.org/10.1126/science.1141316>.
- (15) Siitonen, A. J.; Tsybouski, D. A.; Bachilo, S. M.; Weisman, R. B. Surfactant-Dependent Exciton Mobility in Single-Walled Carbon Nanotubes Studied by Single-Molecule Reactions. *Nano Lett.* **2010**, *10* (5), 1595–1599. <https://doi.org/10.1021/nl9039845>.
- (16) Prato, M.; Kostarelos, K.; Bianco, A. Functionalized Carbon Nanotubes in Drug Design and Discovery. *Acc. Chem. Res.* **2008**, *41* (1), 60–68. <https://doi.org/10.1021/ar700089b>.
- (17) Nißler, R.; Ackermann, J.; Ma, C.; Kruss, S. Prospects of Fluorescent Single-Chirality Carbon Nanotube-Based Biosensors. *Anal. Chem.* **2022**, *94* (28), 9941–9951. <https://doi.org/10.1021/acs.analchem.2c01321>.
- (18) Amoroso, G.; Ye, Q.; Cervantes-Salguero, K.; Fernández, G.; Cecconello, A.; Palma, M. DNA-Powered Stimuli-Responsive Single-Walled Carbon Nanotube Junctions. *Chem. Mater.* **2019**, *31* (5), 1537–1542. <https://doi.org/10.1021/acs.chemmater.8b04483>.
- (19) Mann, F. A.; Horlebein, J.; Meyer, N. F.; Meyer, D.; Thomas, F.; Kruss, S. Carbon Nanotubes Encapsulated in Coiled-Coil Peptide Barrels. *Chem. - A Eur. J.* **2018**, *24* (47), 12241–12245. <https://doi.org/10.1002/chem.201800993>.

- (20) Giacalone, F.; Campisciano, V.; Calabrese, C.; La Parola, V.; Syrgiannis, Z.; Prato, M.; Gruttadauria, M. Single-Walled Carbon Nanotube-Polyamidoamine Dendrimer Hybrids for Heterogeneous Catalysis. *ACS Nano* **2016**, *10* (4), 4627–4636. <https://doi.org/10.1021/acsnano.6b00936>.
- (21) Wu, H.; Nißler, R.; Morris, V.; Herrmann, N.; Hu, P.; Jeon, S. J.; Kruss, S.; Giraldo, J. P. Monitoring Plant Health with Near-Infrared Fluorescent H₂O₂ Nanosensors. *Nano Lett.* **2020**, *20* (4), 2432–2442. <https://doi.org/10.1021/acs.nanolett.9b05159>.
- (22) Lew, T. T. S.; Koman, V. B.; Silmore, K. S.; Seo, J. S.; Gordichuk, P.; Kwak, S. Y.; Park, M.; Ang, M. C. Y.; Khong, D. T.; Lee, M. A.; Chan-Park, M. B.; Chua, N. H.; Strano, M. S. Real-Time Detection of Wound-Induced H₂O₂ Signalling Waves in Plants with Optical Nanosensors. *Nat. Plants* **2020**, *6* (4), 404–415. <https://doi.org/10.1038/s41477-020-0632-4>.
- (23) Giraldo, J. P.; Landry, M. P.; Kwak, S. Y.; Jain, R. M.; Wong, M. H.; Iverson, N. M.; Ben-Naim, M.; Strano, M. S. A Ratiometric Sensor Using Single Chirality Near-Infrared Fluorescent Carbon Nanotubes: Application to in Vivo Monitoring. *Small* **2015**, *11* (32), 3973–3984. <https://doi.org/10.1002/smll.201403276>.
- (24) Meier, J.; Stapleton, J.; Hofferber, E.; Haworth, A.; Kachman, S.; Iverson, N. M. Quantification of Nitric Oxide Concentration Using Single-Walled Carbon Nanotube Sensors. *Nanomaterials* **2021**, *11* (1), 1–9. <https://doi.org/10.3390/nano11010243>.
- (25) Jin, H.; Heller, D. A.; Kalbacova, M.; Kim, J.-H.; Zhang, J.; Boghossian, A. A.; Maheshri, N.; Strano, M. S. Detection of Single-Molecule H₂ O₂ Signaling from Epidermal Growth

- Factor Receptor Using Fluorescent Single-Walled Carbon Nanotubes. *Nat. Nanotechnol.* **2010**, 5 (4), 302–309. <https://doi.org/10.1038/nnano.2010.24>.
- (26) Yaari, Z.; Cheung, J. M.; Baker, H. A.; Frederiksen, R. S.; Jena, P. V.; Horoszkó, C. P.; Jiao, F.; Scheuring, S.; Luo, M.; Heller, D. A. Nanoreporter of an Enzymatic Suicide Inactivation Pathway. *Nano Lett.* **2020**, 20 (11), 7819–7827. <https://doi.org/10.1021/acs.nanolett.0c01858>.
- (27) Heller, D. A.; Pratt, G. W.; Zhang, J.; Nair, N.; Hansborough, A. J.; Boghossian, A. A.; Reuel, N. F.; Barone, P. W.; Strano, M. S. Peptide Secondary Structure Modulates Single-Walled Carbon Nanotube Fluorescence as a Chaperone Sensor for Nitroaromatics. *Proc. Natl. Acad. Sci.* **2011**, 108 (21), 8544–8549. <https://doi.org/10.1073/pnas.1005512108>.
- (28) Wong, M. H.; Giraldo, J. P.; Kwak, S. Y.; Koman, V. B.; Sinclair, R.; Lew, T. T. S.; Bisker, G.; Liu, P.; Strano, M. S. Nitroaromatic Detection and Infrared Communication from Wild-Type Plants Using Plant Nanobionics. *Nat. Mater.* **2017**, 16 (2), 264–272. <https://doi.org/10.1038/nmat4771>.
- (29) Zhang, J.; Boghossian, A. A.; Barone, P. W.; Rwei, A.; Kim, J. H.; Lin, D.; Heller, D. A.; Hilmer, A. J.; Nair, N.; Reuel, N. F.; Strano, M. S. Single Molecule Detection of Nitric Oxide Enabled by d(AT)₁₅ DNA Adsorbed to near Infrared Fluorescent Single-Walled Carbon Nanotubes. *J. Am. Chem. Soc.* **2011**, 133 (3), 567–581. <https://doi.org/10.1021/ja1084942>.
- (30) Kim, J. H.; Patra, C. R.; Arkalgud, J. R.; Boghossian, A. A.; Zhang, J.; Han, J. H.; Reuel, N. F.; Ahn, J. H.; Mukhopadhyay, D.; Strano, M. S. Single-Molecule Detection of H₂O₂

- Mediating Angiogenic Redox Signaling on Fluorescent Single-Walled Carbon Nanotube Array. *ACS Nano* **2011**, 5 (10), 7848–7857. <https://doi.org/10.1021/nn201904t>.
- (31) Shumeiko, V.; Malach, E.; Helman, Y.; Paltiel, Y.; Bisker, G.; Hayouka, Z.; Shoseyov, O. A Nanoscale Optical Biosensor Based on Peptide Encapsulated SWCNTs for Detection of Acetic Acid in the Gaseous Phase. *Sensors Actuators, B Chem.* **2021**, 327. <https://doi.org/10.1016/j.snb.2020.128832>.
- (32) Shumeiko, V.; Paltiel, Y.; Bisker, G.; Hayouka, Z.; Shoseyov, O. A Nanoscale Paper-Based near-Infrared Optical Nose (NIRON). *Biosens. Bioelectron.* **2021**, 172. <https://doi.org/https://doi.org/10.1016/j.bios.2020.112763>.
- (33) Zubkovs, V.; Schuergers, N.; Lambert, B.; Ahunbay, E.; Boghossian, A. A. Mediatorless, Reversible Optical Nanosensor Enabled through Enzymatic Pocket Doping. *Small* **2017**, 13 (42), 1–10. <https://doi.org/10.1002/sml.201701654>.
- (34) Harvey, J. D.; Jena, P. V.; Baker, H. A.; Zerbe, G. H.; Williams, R. M.; Galassi, T. V.; Roxbury, D.; Mittal, J.; Heller, D. A. A Carbon Nanotube Reporter of MiRNA Hybridization Events In Vivo. *Nat. Biomed. Eng.* **2017**, 1. <https://doi.org/doi:10.1038/s41551-017-0041>.
- (35) Bisker, G.; Dong, J.; Park, H. D.; Iverson, N. M.; Ahn, J.; Nelson, J. T.; Landry, M. P.; Kruss, S.; Strano, M. S. Protein-Targeted Corona Phase Molecular Recognition. *Nat. Commun.* **2016**, 7, 1–14. <https://doi.org/10.1038/ncomms10241>.
- (36) Ehrlich, R.; Hendler-Neumark, A.; Wulf, V.; Amir, D.; Bisker, G. Optical Nanosensors for Real-Time Feedback on Insulin Secretion by β -Cells. *Small* **2021**, 17 (30), 1–11.

<https://doi.org/10.1002/sml.202101660>.

- (37) Williams, R. M.; Lee, C.; Heller, D. A. A Fluorescent Carbon Nanotube Sensor Detects the Metastatic Prostate Cancer Biomarker UPA. *ACS Sensors* **2018**, *3* (9), 1838–1845. <https://doi.org/10.1021/acssensors.8b00631>.
- (38) Tu, X.; Manohar, S.; Jagota, A.; Zheng, M. DNA Sequence Motifs for Structure-Specific Recognition and Separation of Carbon Nanotubes. *Nature* **2009**, *460* (7252), 250–253. <https://doi.org/10.1038/nature08116>.
- (39) Reuel, N. F.; Grassbaugh, B.; Kruss, S.; Mundy, J. Z.; Opel, C.; Ogunniyi, A. O.; Egodage, K.; Wahl, R.; Helk, B.; Zhang, J.; Kalcioglu, Z. I.; Tvrđy, K.; Bellisario, D. O.; Mu, B.; Blake, S. S.; Van Vliet, K. J.; Love, J. C.; Wittrup, K. D.; Strano, M. S. Emergent Properties of Nanosensor Arrays: Applications for Monitoring IgG Affinity Distributions, Weakly Affined Hypermannosylation, and Colony Selection for Biomanufacturing. *ACS Nano* **2013**, *7* (9), 7472–7482. <https://doi.org/10.1021/nn403215e>.
- (40) Antonucci, A.; Kupis-Rozmysłowicz, J.; Boghossian, A. A. Noncovalent Protein and Peptide Functionalization of Single-Walled Carbon Nanotubes for Biodelivery and Optical Sensing Applications. *ACS Appl. Mater. Interfaces* **2017**, *9* (13), 11321–11331. <https://doi.org/10.1021/acsami.7b00810>.
- (41) Yaari, Z.; Yang, Y.; Apfelbaum, E.; Cupo, C.; Settle, A. H.; Cullen, Q.; Cai, W.; Roche, K. L.; Levine, D. A.; Fleisher, M.; Ramanathan, L.; Zheng, M.; Jagota, A.; Heller, D. A. A Perception-Based Nanosensor Platform to Detect Cancer Biomarkers. *Sci. Adv.* **2021**, *7* (47), 1–10. <https://doi.org/10.1126/sciadv.abj0852>.

- (42) Elizarova, S.; Chouaib, A.; Alrahman Shaiba, A.; Hill, Björn; Mann, F.; Brose, N.; Kruss, S.; Daniel, J. A. A Fluorescent Nanosensor Paint Detects Dopamine Release at Axonal Varicosities with High Spatiotemporal Resolution. *Proc. Natl. Acad. Sci.* **2022**, *119* (22), e2202842119. [https://doi.org/10.1073/pnas.2202842119/-](https://doi.org/10.1073/pnas.2202842119/-/DCSupplemental) /DCSupplemental.Published.
- (43) Kruss, S.; Landry, M. P.; Vander Ende, E.; Lima, B. M. A.; Reuel, N. F.; Zhang, J.; Nelson, J.; Mu, B.; Hilmer, A.; Strano, M. Neurotransmitter Detection Using Corona Phase Molecular Recognition on Fluorescent Single-Walled Carbon Nanotube Sensors. *J. Am. Chem. Soc.* **2014**, *136* (2), 713–724. <https://doi.org/10.1021/ja410433b>.
- (44) Mann, F. A.; Herrmann, N.; Meyer, D.; Kruss, S. Tuning Selectivity of Fluorescent Carbon Nanotube-Based Neurotransmitter Sensors. *Sensors (Switzerland)* **2017**, *17* (7). <https://doi.org/10.3390/s17071521>.
- (45) Beyene, A. G.; Delevich, K.; Del Bonis-O'Donnell, J. T.; Piekarski, D. J.; Lin, W. C.; Wren Thomas, A.; Yang, S. J.; Kosillo, P.; Yang, D.; Prounis, G. S.; Wilbrecht, L.; Landry, M. P. Imaging Striatal Dopamine Release Using a Nongenetically Encoded near Infrared Fluorescent Catecholamine Nanosensor. *Sci. Adv.* **2019**, *5* (7), 1–11. <https://doi.org/10.1126/sciadv.aaw3108>.
- (46) Dinarvand, M.; Neubert, E.; Meyer, D.; Selvaggio, G.; Mann, F. A.; Erpenbeck, L.; Kruss, S. Near-Infrared Imaging of Serotonin Release from Cells with Fluorescent Nanosensors. *Nano Lett.* **2019**, *19* (9), 6604–6611. <https://doi.org/10.1021/acs.nanolett.9b02865>.
- (47) Jeong, S.; Yang, D.; Beyene, A. G.; Del Bonis-O'Donnell, J. T.; Gest, A. M. M.; Navarro,

- N.; Sun, X.; Landry, M. P. High-Throughput Evolution of near-Infrared Serotonin Nanosensors. *Sci. Adv.* **2019**, *5* (12), 1–13. <https://doi.org/10.1126/sciadv.aay3771>.
- (48) Schultz, W. Multiple Dopamine Functions at Different Time Courses. *Annu. Rev. Neurosci.* **2007**, *30*, 259–288. <https://doi.org/10.1146/annurev.neuro.28.061604.135722>.
- (49) Baik, J. H. Dopamine Signaling in Reward-Related Behaviors. *Front. Neural Circuits* **2013**, *7*, 1–16. <https://doi.org/10.3389/fncir.2013.00152>.
- (50) Lee, D.; Creed, M.; Jung, K.; Stefanelli, T.; Wendler, D. J.; Oh, W. C.; Mignocchi, N. L.; Lüscher, C.; Kwon, H. B. Temporally Precise Labeling and Control of Neuromodulatory Circuits in the Mammalian Brain. *Nat. Methods* **2017**, *14* (5), 495–503. <https://doi.org/10.1038/nmeth.4234>.
- (51) Patriarchi, T.; Cho, J. R.; Merten, K.; Howe, M. W.; Xiong, W.; Folk, R. W.; Broussard, G. J.; Liang, R.; Jang, J.; Zhong, H.; Dombeck, D.; Zastrow, M. Von; Nimmerjahn, A.; Gradinaru, V.; Williams, J. T.; Tian, L. Genetically Encoded Sensors. *Science (80-.)*. **2018**, *360* (6396), 1–22. <https://doi.org/10.1126/science.aat4422>.
- (52) Sun, F.; Zeng, J.; Jing, M.; Zhou, J.; Feng, J.; Owen, S. F.; Luo, Y.; Li, F.; Wang, H.; Yamaguchi, T.; Yong, Z.; Gao, Y.; Peng, W.; Wang, L.; Zhang, S.; Du, J.; Lin, D.; Xu, M.; Kreitzer, A. C.; Cui, G.; Li, Y. A Genetically Encoded Fluorescent Sensor Enables Rapid and Specific Detection of Dopamine in Flies, Fish, and Mice. *Cell* **2018**, *174* (2), 481–496.e19. <https://doi.org/10.1016/j.cell.2018.06.042>.
- (53) Muller, A.; Joseph, V.; Slesinger, P. A.; Kleinfeld, D. Cell-Based Reporters Reveal in Vivo Dynamics of Dopamine and Norepinephrine Release in Murine Cortex. *Nat. Methods* **2014**,

- 11 (12), 1245–1252. <https://doi.org/10.1038/nmeth.3151>.
- (54) Tomagra, G.; Picollo, F.; Battiato, A.; Picconi, B.; De Marchis, S.; Pasquarelli, A.; Olivero, P.; Marcantoni, A.; Calabresi, P.; Carbone, E.; Carabelli, V. Quantal Release of Dopamine and Action Potential Firing Detected in Midbrain Neurons by Multifunctional Diamond-Based Microarrays. *Front. Neurosci.* **2019**, *13* (April), 1–13. <https://doi.org/10.3389/fnins.2019.00288>.
- (55) Robinson, D. L.; Hermans, A.; Seipel, A. T.; Wightman, R. M. Monitoring Rapid Chemical Communication in the Brain. *Chem. Rev.* **7544BC**, *108* (7), 2554–2584. <https://doi.org/10.1021/cr068081q>.
- (56) Jaquins-Gerstl, A.; Michael, A. C. A Review of the Effects of FSCV and Microdialysis Measurements on Dopamine Release in the Surrounding Tissue. *Analyst* **2015**, *140* (11), 3696–3708. <https://doi.org/10.1039/c4an02065k.A>.
- (57) Goyal, R. N.; Oyama, M.; Gupta, V. K.; Singh, S. P.; Sharma, R. A. Sensors for 5-Hydroxytryptamine and 5-Hydroxyindole Acetic Acid Based on Nanomaterial Modified Electrodes. *Sensors Actuators, B Chem.* **2008**, *134* (2), 816–821. <https://doi.org/10.1016/j.snb.2008.06.027>.
- (58) Han, H. S.; You, J. M.; Jeong, H.; Jeon, S. Synthesis of Graphene Oxide Grafted Poly(Lactic Acid) with Palladium Nanoparticles and Its Application to Serotonin Sensing. *Appl. Surf. Sci.* **2013**, *284*, 438–445. <https://doi.org/10.1016/j.apsusc.2013.07.116>.
- (59) Kruss, S.; Salem, D. P.; Vuković, L.; Lima, B.; Ende, E. Vander; Boyden, E. S.; Strano, M. S. High-Resolution Imaging of Cellular Dopamine Efflux Using a Fluorescent Nanosensor

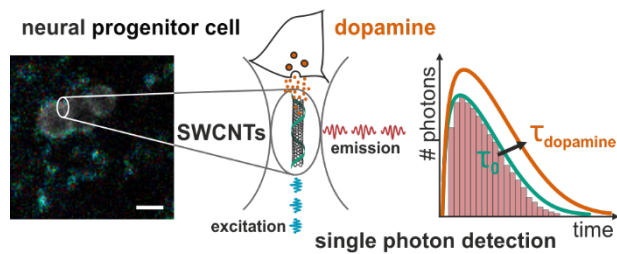
- Array. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114* (8), 1789–1794.
<https://doi.org/10.1073/pnas.1613541114>.
- (60) Paviolo, C.; Ferreira, J. S.; Lee, A.; Hunter, D.; Calaresu, I.; Nandi, S.; Groc, L.; Cognet, L. Near-Infrared Carbon Nanotube Tracking Reveals the Nanoscale Extracellular Space around Synapses. *Nano Lett.* **2022**, *22* (17), 6849–6856.
<https://doi.org/10.1021/acs.nanolett.1c04259>.
- (61) Ackermann, J.; Stegemann, J.; Smola, T.; Reger, E.; Jung, S.; Schmitz, A.; Herbertz, S.; Erpenbeck, L.; Seidl, K.; Kruss, S. High Sensitivity Near-Infrared Imaging of Fluorescent Nanosensors. *Small* **2022**.
- (62) Nißler, R.; Mann, F. A.; Chaturvedi, P.; Horlebein, J.; Meyer, D.; Vuković, L.; Kruss, S. Quantification of the Number of Adsorbed DNA Molecules on Single-Walled Carbon Nanotubes. *J. Phys. Chem. C* **2019**, *123* (8), 4837–4847.
<https://doi.org/10.1021/acs.jpcc.8b11058>.
- (63) Polo, E.; Kruss, S. Impact of Redox-Active Molecules on the Fluorescence of Polymer-Wrapped Carbon Nanotubes. *J. Phys. Chem. C* **2016**, *120* (5), 3061–3070.
<https://doi.org/10.1021/acs.jpcc.5b12183>.
- (64) Dinarvand, M.; Elizarova, S.; Daniel, J.; Kruss, S. Imaging of Monoamine Neurotransmitters with Fluorescent Nanoscale Sensors. *Chempluschem* **2020**, *85* (7), 1465–1480. <https://doi.org/10.1002/cplu.202000248>.
- (65) Roxbury, D.; Jena, P. V.; Williams, R. M.; Enyedi, B.; Niethammer, P.; Marcet, S.; Verhaegen, M.; Blais-Ouellette, S.; Heller, D. A. Hyperspectral Microscopy of Near-

- Infrared Fluorescence Enables 17-Chirality Carbon Nanotube Imaging. *Sci. Rep.* **2015**, *5* (September), 1–6. <https://doi.org/10.1038/srep14167>.
- (66) Sun, C.; Wang, Y.; Zhang, H.; Qian, J. Near-Infrared Laser Scanning Confocal Microscopy and Its Application in Bioimaging. *Opt. Quantum Electron.* **2018**, *50* (1), 1–9. <https://doi.org/10.1007/s11082-017-1309-8>.
- (67) Zubkovs, V.; Antonucci, A.; Schuergers, N.; Lambert, B.; Latini, A.; Ceccarelli, R.; Santinelli, A.; Rogov, A.; Ciepielewski, D.; Boghossian, A. A. Spinning-Disc Confocal Microscopy in the Second near-Infrared Window (NIR-II). *Sci. Rep.* **2018**, *8* (1), 1–10. <https://doi.org/10.1038/s41598-018-31928-y>.
- (68) Datta, R.; Heaster, T. M.; Sharick, J. T.; Gillette, A. A.; Skala, M. C. Fluorescence Lifetime Imaging Microscopy: Fundamentals and Advances in Instrumentation, Analysis, and Applications. *J. Biomed. Opt.* **2020**, *25* (07), 1. <https://doi.org/10.1117/1.jbo.25.7.071203>.
- (69) Berger, F. J.; De Sousa, J. A.; Zhao, S.; Zorn, N. F.; El Yumin, A. A.; Quintana García, A.; Settele, S.; Högele, A.; Crivillers, N.; Zaumseil, J. Interaction of Luminescent Defects in Carbon Nanotubes with Covalently Attached Stable Organic Radicals. *ACS Nano* **2021**, *15* (3), 5147–5157. <https://doi.org/10.1021/acsnano.0c10341>.
- (70) Berger, F. J.; Lüttgens, J.; Nowack, T.; Kutsch, T.; Lindenthal, S.; Kistner, L.; Müller, C. C.; Bongartz, L. M.; Lumsargis, V. A.; Zakharko, Y.; Zaumseil, J. Brightening of Long, Polymer-Wrapped Carbon Nanotubes by Sp³ Functionalization in Organic Solvents. *ACS Nano* **2019**, *13* (8), 9259–9269. <https://doi.org/10.1021/acsnano.9b03792>.
- (71) Hartmann, N. F.; Velizhanin, K. A.; Haroz, E. H.; Kim, M.; Ma, X.; Wang, Y.; Htoon, H.;

- Doorn, S. K. Photoluminescence Dynamics of Aryl Sp³ Defect States in Single-Walled Carbon Nanotubes. *ACS Nano* **2016**, *10* (9), 8355–8365. <https://doi.org/10.1021/acsnano.6b02986>.
- (72) Zheng, Y.; Kim, Y.; Jones, A. C.; Olinger, G.; Bittner, E. R.; Bachilo, S. M.; Doorn, S. K.; Weisman, R. B.; Piryatinski, A.; Htoon, H. Quantum Light Emission from Coupled Defect States in DNA-Functionalized Carbon Nanotubes. *ACS Nano* **2021**, *15* (6), 10406–10414. <https://doi.org/10.1021/acsnano.1c02709>.
- (73) Younghee, K.; Velizhanin, K. A.; He, X.; Sarkpkaya, I.; Yomogida, Y.; Tanaka, T.; Kataura, H.; Doorn, S. K.; Htoon, H. Photoluminescence Intensity Fluctuations and Temperature-Dependent Decay Dynamics of Individual Carbon Nanotube Sp³ Defects. *J. Phys. Chem. Lett.* **2019**, *10*, 1423–1430. <https://doi.org/10.1021/acs.jpcclett.8b03732>.
- (74) He, X.; Velizhanin, K. A.; Bullard, G.; Bai, Y.; Olivier, J. H.; Hartmann, N. F.; Gifford, B. J.; Kilina, S.; Tretiak, S.; Htoon, H.; Therien, M. J.; Doorn, S. K. Solvent- and Wavelength-Dependent Photoluminescence Relaxation Dynamics of Carbon Nanotube Sp³ Defect States. *ACS Nano* **2018**, *12* (8), 8060–8070. <https://doi.org/10.1021/acsnano.8b02909>.
- (75) Zheng, Y.; Weight, B. M.; Jones, A. C.; Chandrasekaran, V.; Gifford, B. J.; Tretiak, S.; Doorn, S. K.; Htoon, H. Photoluminescence Dynamics Defined by Exciton Trapping Potential of Coupled Defect States in DNA-Functionalized Carbon Nanotubes. *ACS Nano* **2021**, *15* (1), 923–933. <https://doi.org/10.1021/acsnano.0c07544>.
- (76) Spreinat, A.; Dohmen, M. M.; Lüttgens, J.; Herrmann, N.; Klepzig, L. F.; Nißler, R.; Weber, S.; Mann, F. A.; Lauth, J.; Kruss, S. Quantum Defects in Fluorescent Carbon Nanotubes for

- Sensing and Mechanistic Studies. *J. Phys. Chem. C* **2021**, *125* (33), 18341–18351.
<https://doi.org/10.1021/acs.jpcc.1c05432>.
- (77) Rodriguez, P. C.; Pereira, D. B.; Borgkvist, A.; Wong, M. Y.; Barnard, C.; Sonders, M. S.; Zhang, H.; Sames, D.; Sulzer, D. Fluorescent Dopamine Tracer Resolves Individual Dopaminergic Synapses and Their Activity in the Brain. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110* (3), 870–875. <https://doi.org/10.1073/pnas.1213569110>.
- (78) Gubernator, N. G.; Zhang, H.; Staal, R. G. W.; Mosharov, E. V.; Pereira, B.; Yue, M.; Balsanek, V.; Vadola, P. A.; Mukherjee, B.; Edwards, H.; Sulzer, D.; Sames, D. Fluorescent False Neurotransmitters Visualize Dopamine Release from Individual Presynaptic Terminals. *Science* (80-.). **2009**, *324* (5933), 1441–1444.
<https://doi.org/10.1126/science.1172278>.
- (79) Meyer, D.; Hagemann, A.; Kruss, S. Kinetic Requirements for Spatiotemporal Chemical Imaging with Fluorescent Nanosensors. *ACS Nano* **2017**, *11* (4), 4017–4027.
<https://doi.org/10.1021/acsnano.7b00569>.
- (80) Nißler, R.; Kurth, L.; Li, H.; Spreinat, A.; Kuhleemann, I.; Flavel, B. S.; Kruss, S. Sensing with Chirality-Pure Near-Infrared Fluorescent Carbon Nanotubes. *Anal. Chem.* **2021**, *93* (16), 6446–6455. <https://doi.org/10.1021/acs.analchem.1c00168>.

For Table of Contents Only



DNA functionalized SWCNTs are fluorescent sensors in the near infrared for important biomolecules such as the neurotransmitter dopamine. These sensors change their fluorescence lifetime, which can be observed by pulsed excitation and single photon counting. Lifetimes are sensitive to the concentration of dopamine, which can be used for imaging dopamine release by cells.