Nanodevices

Nanophotonic Light Sources for Fluorescence Spectroscopy and Cellular Imaging**

Oliver Hayden and Christine K. Payne**

Dedicated to Professor Franz L. Dickert on the occasion of his 60th birthday

Herein we demonstrate a new approach to nanophotonics with the fabrication and application of nanoscale light-emitting diodes (nanoLEDs) for fluorescence spectroscopy and single-analyte detection, and as nearfield light sources for cellular imaging. The nanoLEDs have a final diameter of 50 nm and are easily integrated into microfluidic channels—a desirable property for future "lab-on-a-chip" systems. Nanophotonic devices have evolved by means of the bottom-up approach with quantum dots,[1] carbon nanotubes,[2] or semiconducting nanowires[3] serving as low-dimensional building blocks. Of these devices, semiconducting nanowires offer a number of advantages for the fabrication of electrically driven nanodevices as they allow properties such as the emission wavelength[4] of the device to be selected. Microfluidic assembly is then used to cross the semiconducting nanowires to form pn-junctions.[5] The resulting nanowire devices can be fabricated on a variety of unconventional substrates, such as flexible plastic.[6] We report here the first spectroscopic applications of nanoscale devices that use LEDs from crossed nanowire architectures as integrated light sources in microfluidic channels.

The nanoLEDs described here were fabricated by crossing 100-nm-diameter n-doped cadmium sulphide nanowires with 60-nm p-doped silicon nanowires (Figure 1). The nanowires have aspect ratios as high as 10^3. The smallest diodes were prepared from 50-nm cadmium sulphide and 20-nm silicon nanowires to give a final diameter of less than 20 nm. The snapshot in Figure 2a shows the emission from the nanoLED when pulsed at 10 Hz (see Supporting Informa-

[*] Dr. O. Hayden,[+] Dr. C. K. Payne
Department of Chemistry and Chemical Biology
Harvard University
12 Oxford Street, Cambridge, MA 02138 (USA)
E-mail: ckpayne@fas.harvard.edu

[+] Current address:
Institute of Analytical Chemistry
University of Vienna
Währingerstrasse 38, 1090 Vienna (Austria)
Fax: (+43) 1-4277-9523
E-mail: oliver.hayden@univie.ac.at

[**] O.H. is indebted to Prof. Charles M. Lieber for support of this project. C.K.P. is grateful to Prof. Xiaowei Zhuang and to a Ruth L. Kirschstein National Research Service Award (National Institutes of Health Postdoctoral Fellowship) for support. The authors would like to thank Dr. Richard Schalek and Dr. Laura Kaufman for helpful discussions.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

 DOI: 10.1002/ange.200462120

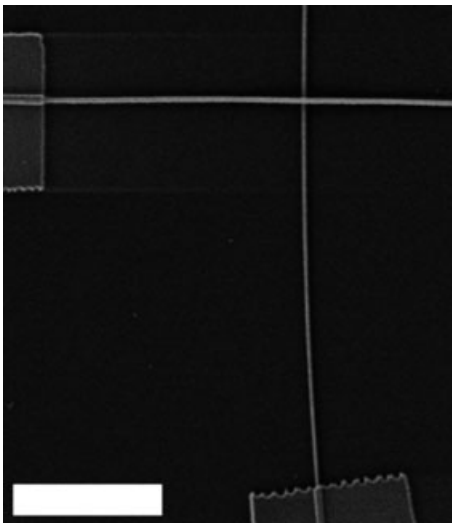

Figure 1. SEM image of a crossed nanowire device assembled from a CdS nanowire (horizontal) and a silicon nanowire (vertical). Scale bar: 2 μm.

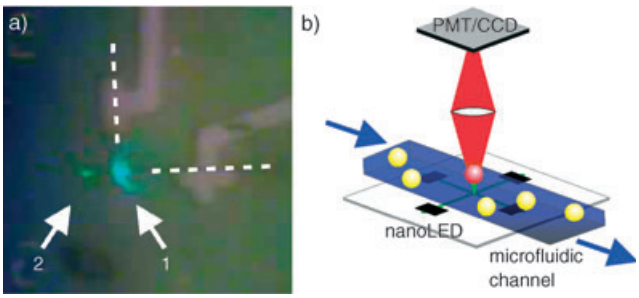

Figure 2. NanoLEDs with a crossed nanowire architecture as an integrated light source for microfluidics. a) The snapshot of a pulsed nanoLED ($13\times13\ \mu m^2$ micrograph) shows (1) green–blue emission at the crossing point of a 60-nm p-doped silicon nanowire (vertical) and a 100-nm n-doped CdS nanowire (horizontal) and (2) red-shifted green emission from the end of the cadmium sulphide nanowire owing to the cavity properties of the nanowire. The broken lines highlight the position of the crossing nanowires. b) Schematic of the integration of a nanoLED into a microfluidic channel. Labeled analytes flow in the microchannel and cross the beam path between the nanoLED and the CCD detector (PMT = photomultiplier tube). The fluorescence emission of single analytes is spatially and spectrally resolved.

tion) with emission at $\lambda = 514$ nm observed from the nanowire junction. End emission from the CdS nanowire can also be observed owing to the waveguide properties of this material. Leakage of light is not observed along the nanowire which indicates single-crystalline properties.

For integration in a microfluidic channel, the nanoLEDs were isolated with a 200-nm layer of Si_3N_4 (Figure 2b). Inlet and outlet connections to the microfluidic channels were formed by drilling holes into the silicon wafer substrate. The chip was then placed in either a homebuilt microscope for spectroscopy or in a laser scanning microscope. The crossed nanowire devices show the typical rectifying behavior of a diode at forward bias (Figure 3a). These LEDs emit light with a full width at half maximum (FWHM) of about 20 nm (Figure 3b), which is comparable to the photoluminescence spectrum (see Supporting Information). No emission at wavelengths larger than the bandgap of CdS was observed which indicates the absence of crystalline defects or impurities. This quality offers a large spectral window for the observation of fluorescence emission.

To test the use of nanowire light sources for fluorescence spectroscopy applications, a nanoLED was integrated into a microfluidic channel with a depth of 25 μm. This device was placed on a microscope, and a solution of quantum dots was passed into the microfluidic channel with a syringe pump. Fluorescence emission of the quantum dots from a wavelength-limited spot size was spatially resolved with a CCD. The photoluminescence

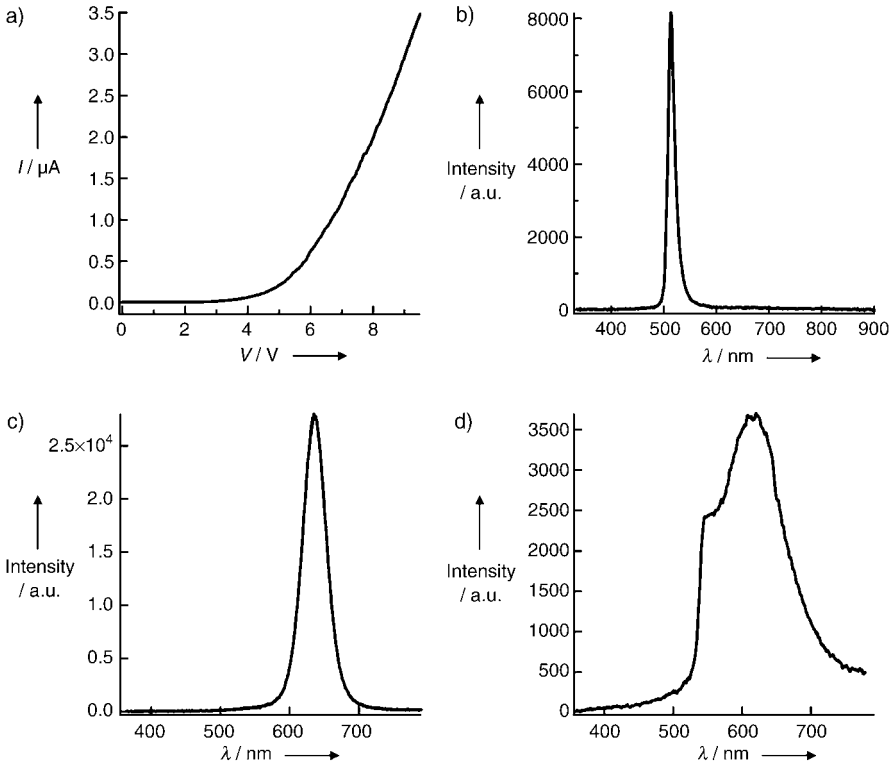

Figure 3. Physical characteristics and spectral properties of the nanoLEDs. a) The *I*–*V* curve of a Si/CdS crossed nanowire LED. The curve shows typical rectifying diode behavior. b) The electroluminescence spectrum from a crossed p-Si and n-CdS nanowire junction at room temperature. FWHM ≈ 20 nm for the emission peak maximum at $\lambda = 514$ nm. c) Photoluminescence of dried quantum dots on a glass slide. The fluorescence emission is red-shifted by 20 nm owing to the concentration of quantum dots (EviDots, $\lambda_{em} = 603$ nm). d) Fluorescence spectrum of the same quantum dots in a 25-μm microfluidic channel.

spectrum of the quantum dots recorded with external excitation is shown in Figure 3c for reference. The light from the nanoscale excitation source was filtered from the emission of the quantum dots with a 540-nm-long pass filter which allowed us to spectrally resolve the fluorescence emission of the quantum dots. (Figure 3d). This application demonstrates the unique nanoLED properties for highly confined fluorescence excitation.

A key challenge in modern microscopy is the excitation of a single molecule for the analysis of individual, rather than ensemble, behavior. Typically, single-molecule microscopy is carried out on a dilute solution with an excitation volume on the order of nanoliters, depending on the size of the focused laser beam. As an alternative method, an integrated nanoLED can be considered as a pointlike light source for single-analyte detection. By using a laser scanning confocal microscope, the performance of a single nanoLED as a light source for single-analyte detection was demonstrated, and movies of fluorescent beads, 1-μm diameter, flowed through a microfluidic channel were recorded.[7] Snapshots of the movies, which were taken by either laser scanning or by excitation with the nanoLED, are shown in Figure 4.[7] The

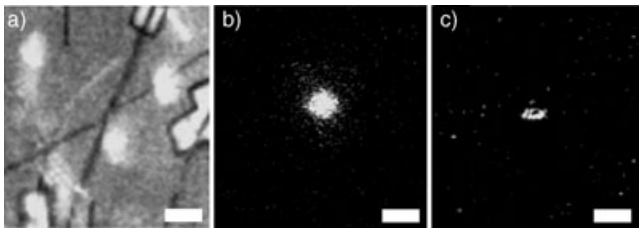

Figure 4. Single-particle detection with a nanoLED (scale bars: 2 μm).[7] a) Laser-scanned snapshot of 1-μm-diameter microspheres flowing in the microfluidic channel over a crossed nanowire device. b) The nanoLED emission is recorded at 4-V forward bias with the pinhole fully opened. c) Fluorescence from the microspheres is detected whenever microspheres cross the beam path between the nanoLED and the detector.

movement of single beads flowing in the microfluidic channel is shown in Figure 4a, and a crossed nanowire device can be seen in the center of the laser scanned image. The nanoLED is almost two orders of magnitude smaller in diameter than the 1-micron diameter of the fluorescent beads. The activated nanoLED is shown in Figure 4b, and the fluorescence emission of single beads upon excitation by the nanoLED is shown in Figure 4c. Fluorescence is only observed when the beads cross the beam path between the nanoLED and the detector. Furthermore, the intensity of the fluorescence depends on the distance between the nanoLED and the specimen. The light emission of the nanoLED is highly diffractive and is not a focused beam as in the case of an external light source. Also, the nanoLED excites only a small volume of the microfluidic channel, on the order of a few femtoliters ($\approx 10\ \mu m^3$), depending on the LED intensity and channel height. These devices should therefore be highly suitable as light sources for highly integrated nanoflow channels.

The 50-nm diameter of the nanoLEDs makes them potential light sources for the nearfield excitation of biological specimens. This application was demonstrated with the use of quantum dots (Qdots, CA) as cellular probes. Nearfield excitation was first tested by the direct deposition of approximately 25-nm-diameter quantum dots on the nanoLED. Given the 514-nm wavelength of light produced by the nanoLED, excitation within a distance less than this wavelength is defined as nearfield excitation. Results of the excitation by laser scanning and nanoLED are shown as Z-stacks (see Supporting Information). The strong fluorescence of the quantum dots along the CdS nanowire were recorded with a disk hole of 1.6 Airy and upon excitation at $\lambda = 543$ nm. The same result with spatially resolved excitation was demonstrated with the nanoLED at 1.6 Airy. Nearfield and farfield cellular imaging was subsequently performed with NIH3T3 murine fibroblast cells labeled with quantum dots. The cells were grown either directly on Si_3N_4-protected nanoLEDs or on glass coverslips, which were inverted on the nanoLEDs. Figure 5a shows a laser scanned image of cells cultivated on a chip. Quantum dots in a fibroblast cell at a distance as low as 500 nm from the nanoLED could be excited with this integrated nearfield light source as shown in Figure 5, b and c.

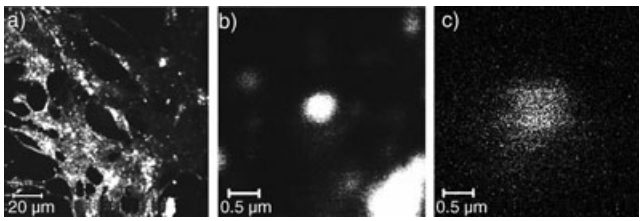

Figure 5. Comparison of cellular images collected by means of laser scanning confocal microscopy and with integrated nanoLEDs. a) Laser scanned image of fibroblast cells that are placed on top of a chip with nanoLEDs. The red spots correspond to intracellular quantum dots. b) Laser scanned image of the fluorescence from quantum dots in a fibroblast. c) NanoLED image of the same density of quantum dots within the fibroblast cell. In comparison to the laser scanned image, only quantum dots that are located on the nanoLED are excited with a vertical distance as low as approximately 500 nm. The use of a nanoLED eliminates excitation of neighboring quantum dots and reduces background fluorescence to provide an inherently confocal technique.

By using crossed Si and CdS nanowires, we have constructed a nanoLED with emission at 514 nm. These devices have been used to image single quantum dots both in solution and in cells. We believe that nanoscale optoelectronic devices could have wide applicability for integrated fluorescence spectroscopy by the use of future nanoarray-based chips as well as nearfield excitation sources for cell imaging. Furthermore, the spatially confined excitation of a few femtoliters could potentially be of use for single-molecule imaging.

Experimental Section

Nanowire synthesis: Si nanowires were synthesized catalytically by a VLS (vapor–liquid–solid) process using 60-nm gold colloids (Ted Pella) that were dispersed on an oxidized silicon substrate.[8] CdS

wires with diameters in the range of 100 nm were synthesized by pulsed laser deposition with gold clusters for catalytic VLS growth of the target according to a reported method.[9] *Integrated nanoLEDs and fluorescence spectroscopy*: Electrical contacts to crossed nanowires were defined by using electron-beam lithography (JEOL 6400). Electrode contacts of 100-nm Ti were deposited by using a homebuilt electron-beam evaporator system. The nanoLEDs were isolated with the deposition of 200-nm-thick Si_3N_4 by PECVD (plasma-enhanced chemical vapor deposition). Electrical transport measurements were made by using a computer-controlled homebuilt system. Room temperature electroluminescence was recorded with a homebuilt microscope (60× magnification, 0.6 NA (numerical aperture)), a liquid-nitrogen-cooled CCD (Princeton Instruments Spec-10), and a spectrometer equipped with a 1200-lines mm^{-1} grating blazed at 500 nm (Acton Scientific 300i). True-color videos of pulsed nanoLEDs were recorded on the same setup. Water-stabilized quantum dots with an emission at $\lambda = 603$ nm were purchased from Evident Technologies. The fluorescence emission was integrated for 1 s at a flow rate of 100 µl h^{-1} and a concentration of 25 µg mL^{-1}. The excitation of the single nanoLED was blocked with a 540-nm filter. *Single-particle detection and cell imaging*: A Zeiss LSM 510 microscope with a 60× water-immersion objective (1.2 NA) was used for confocal microscopy studies. TransFluospheres ($\lambda_{ex} = 488$, $\lambda_{em} = 605$ nm) with a diameter of 1 µm were purchased from Molecular Probes. The fluorescence of single particles was detected with a 560-nm-long pass filter in a microfluidic channel with a height of 25 µm and a flow rate of 1–5 µl h^{-1}. Movies were recorded with 10 frames s^{-1} (see Supporting Information). Glycerol was added to the aqueous solution to control the buoyancy of the microspheres. Labeling of the cells with quantum dots was performed with a QTracker Kit from the Quantum Dot Corporation ($\lambda_{em} = 655$ nm). *Cell cultures*: NIH3T3 cells were maintained in a 5% CO_2 environment in a medium of DMEM (Dulbecco's Modified Eagle's Medium, Invitrogen) with 10% calf bovine serum and were passaged every two to three days. For imaging, cells were cultured overnight in Petri dishes with removable coverslips or were grown directly on a nanoLED, which was protected with a 200-nm-thick layer of Si_3N_4. Prior to experiments, cells were fixed with 2% formaldehyde in PBS (phosphate buffered saline) solution and permeabilized in acetone. Cells on coverslips were placed on the nanoLEDs with fixed cells facing the nanoLEDs.

Received: September 27, 2004
Revised: October 20, 2004
Published online: January 20, 2005

Keywords: analytical methods · fluorescence spectroscopy · nanotechnology · semiconductors · single-molecule studies

[1] M. H. Baier, C. Constantin, E. Pelucchi, E. Kapon, *Appl. Phys. Lett.* **2004**, *84*, 1967.
[2] J. A. Misewich, R. Martel, Ph. Avouris, J. C. Tsang, S. Heinze, J. Tersoff, *Science* **2003**, *300*, 783.
[3] a) X. Duan, Y. Huang, Y. Cui, J. Wang, C. M. Lieber, *Nature* **2001**, *409*, 66; b) H.-M. Kim, T. W. Kang, K. S. Chung, *Adv. Mater.* **2003**, *15*, 567.
[4] Y. Xia, P. Yang, Y. Sun, Y. Wu, B. Mayers, B. Gates, Y. Yin, F. Kim, H. Yan, *Adv. Mater.* **2003**, *15*, 353.
[5] Y. Huang, X. Duan, Q. Wei, C. M. Lieber, *Science* **2001**, *291*, 630.
[6] M. C. McAlpine, R. S. Friedman, C. M. Lieber, *Nano Lett.* **2003**, *3*, 443.
[7] See Supporting Information for the movies that accompany Figure 4.
[8] Y. Cui, C. M. Lieber, *Science* **2001**, *291*, 851.
[9] X. Duan, Y. Huang, R. Agarwal, C. M. Lieber, *Nature* **2003**, *421*, 241.

