Biomedical Optics: In Vivo and In Vitro Applications

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Call it what you will: biomedical optics, biophotonics, optics in the life sciences, or lasers in medicine; light, lasers, and optics have played a tremendous role in biology and medicine over the last few decades, and this role is growing. This chapter covers activities on biomedical optics for in vivo and in vitro applications. Additional material on biomedical optics can be found in the chapter by Jim Wynne on LASIK.

Optical methods are used in medicine and biology for both diagnostics and therapeutics. Important aspects of optical methods for these applications include the ability to use multiple wavelengths to perform spectroscopy (i.e., detect or stimulate specific transitions to provide molecular information) or to perform multiplexing with multi-color probes, the ability to penetrate tissue (particularly in the near infrared), the ability to produce changes in molecules, and the potential to produce low-cost and portable instrumentation.

Clinical use of optical methods has a long history. Early methods relied on the observer's eye for imaging through human tissue, with reports of detection of hydrocephalus (accumulation of cerebrospinal fluid within the cranium, 1831) [1], hydrocele (accumulation of fluid around the testis, 1843) [2], and breast cancer (1929) [3]. The advent of the laser and microelectronics enabled applications such as retinal surgery using argon lasers in the 1960s [4] and pulse oximetry in the 1970s [5]. However, the largest growth in biomedical optics methods began in the 1990s, where advances in lasers, image sensors, and genetic modification led to the advent of many new biomedical optics methods, among them optical coherence tomography (OCT) [6], in vivo diffuse optical imaging, multi-photon microscopy [7], revival of coherent anti-Stokes Raman spectroscopy (CARS) microscopy [8], photoacoustic imaging, bioluminescence imaging [9], green fluorescence protein as a marker for gene expression [10], and bioimaging using quantum dots [11,12].

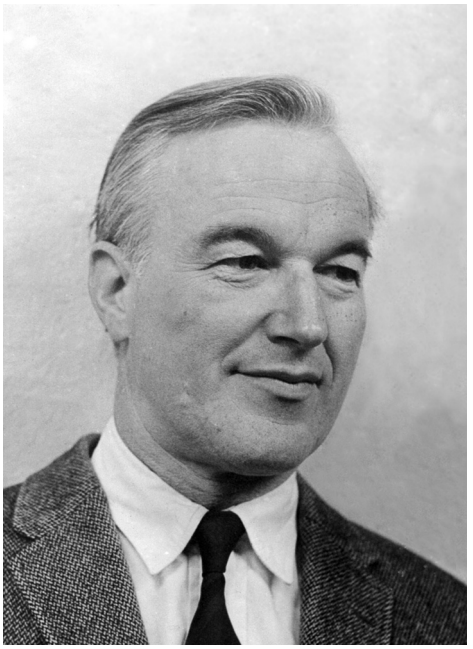
In Vivo Imaging and Spectroscopy

Optical imaging in tissue generally falls into two classes: those based on unscattered light ("ballistic" photons), which can provide very high spatial resolution (on the order of micrometers, i.e., the cellular level) but with limited tissue penetration (on the order of 1–2 mm), and those based on scattered light (diffuse imaging), which can provide good tissue penetration (many centimeters) at the expense of resolution (limited to on the order of 1 cm). Examples of high-resolution in vivo imaging include OCT, confocal imaging, and nonlinear microscopy. Examples of diffuse methods include diffuse optical tomography, tissue oximetry, and pulse oximetry.

In Vivo Molecular Probes and Image Contrast. The ability to perform molecular imaging or spectral multiplexing is one of the primary advantages of optical methods. For in vivo imaging, a range of targets is available with endogenous contrast. For absorption measurements, these include most notably oxyhemoglobin and deoxyhemoglobin (the basis for pulse oximetry, tissue oxygenation monitoring, optical brain monitoring and imaging, and diffuse optical tomography), as well as spectral variation of scattering, melanin, bilirubin, and

cytochrome oxidase. Endogenous fluorophores in vivo include nicotinamide adenine dinucleotide (NADPH), flavins, collagen, and elastin. Exogenous chromophores and fluorophores in clinical use include fluorescein for retinal angiography and corneal abnormalities, indocyanine green (ICG) for monitoring vasculature and perfusion, isosulfan blue for tracing the lymph system, and sensitizers for photodynamic therapy. More advanced chromophores and fluorophores are under development, including molecular beacons and nanoparticles. The latter can potentially combine diagnostic and therapeutic capabilities. A significant hurdle in the use of advanced chromophores in humans is regulatory approval, though the various advanced contrast agents are currently used in animal studies. There are several commercial systems available today for optical molecular imaging of small animals.

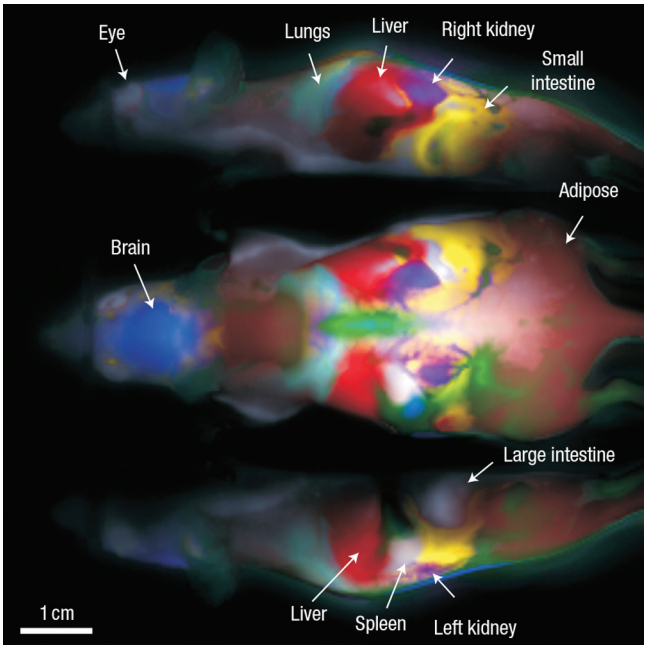
Diffuse optical imaging in vivo has been pioneered by Britton Chance (Fig. 1) and others. Significant application areas of diffuse optical imaging include small-animal imaging, brain monitoring and imaging, and cancer detection. In diffuse optical tomography, image reconstruction is used to produce two- or three-dimensional images from a set of absorption or fluorescence images. Dynamic or differential imaging can be used to enhance contrast from diffuse optical imaging. An example is shown in Fig. 2, which displays an image of internal organs in a mouse derived from the dynamics of dye uptake following injection.



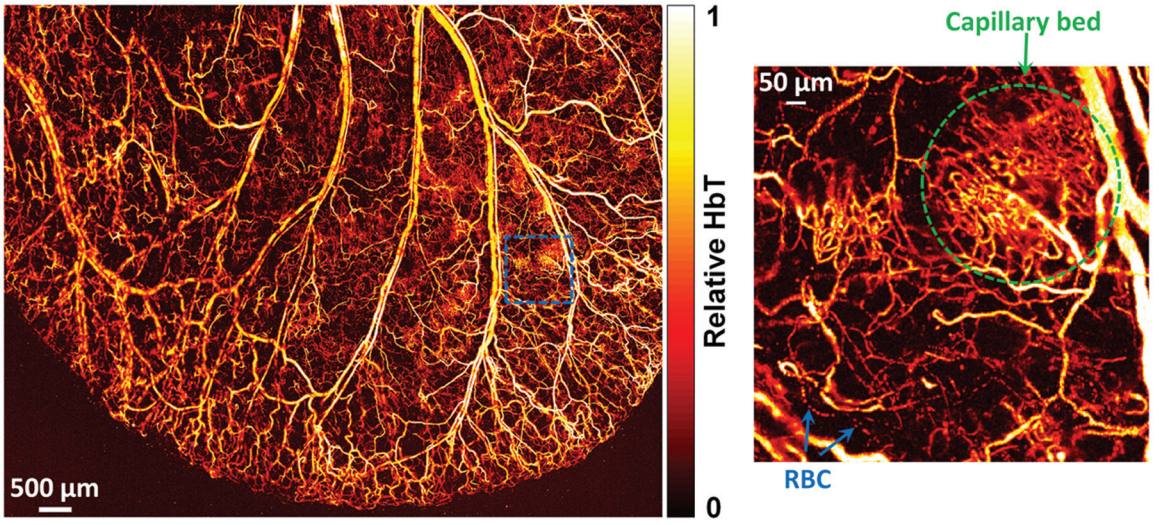
▲ **Fig. 1.** Britton Chance (The Optical Society [OSA]). (AIP Emilio Segre Visual Archives, Physics Today Collection.)

Photoacoustic imaging and spectroscopy combine the relative advantages of optical and acoustic methods. Absorption of a laser pulse produces an acoustic wave that is detected by an acoustic transducer. This method provides the molecular specificity of optical methods (e.g., localizing blood vessels through optical absorption of blood) with the spatial resolution of acoustic methods, which is superior to that of diffuse optics. An example of photoacoustic imaging of blood vessels with optical resolution in a mouse ear is shown in Fig. 3.

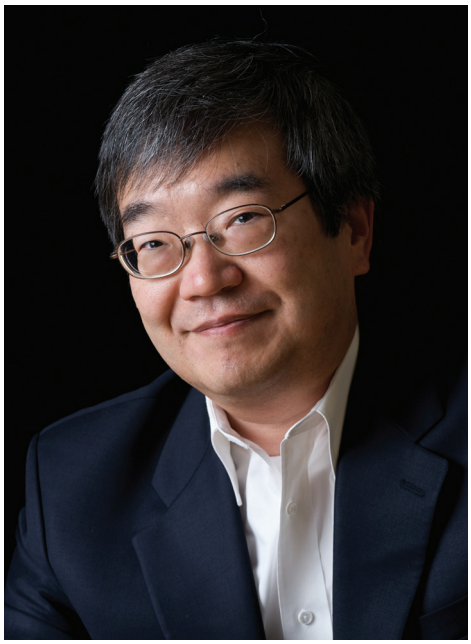
Optical coherence tomography (OCT). OCT, pioneered by James Fujimoto (Fig. 4) and others, is an interferometric method for reflectance in vivo microscopy providing high resolution (approximately a micron) at depths of approximately a millimeter in biological tissue. Early work on OCT was primarily performed in the time domain using very-short-coherence light sources [6]. More recently, spectral domain or Fourier domain



▲ **Fig. 2.** In vivo, non-invasive anatomical mapping of internal organs in a mouse derived from temporal response of ICG uptake following injection. Nine organ-specific regions are found from the different circulatory, uptake, and metabolic responses. (Copyright © 2007, Nature Publishing Group.)



▲ **Fig. 3.** Optical-resolution photoacoustic microscopy image of relative total hemoglobin in living mouse ear. Images show detailed vascular anatomy, including densely packed capillary bed and individual red blood cells traveling along a capillary in the inset at right [26].



▲ **Fig. 4.** James Fujimoto (OSA) (Photo by Greg Hren, courtesy of RLE at MIT.)

OCT [13] methods using tunable lasers or spectrometers have been widely adopted because these provide a better signal-to-noise ratio and faster scanning. OCT is widely used clinically in ophthalmology, with other applications to endoscopy for gastrointestinal or cardiovascular applications being evaluated.

Endoscopy and miniature imaging systems. As image sensors are produced in smaller sizes for applications such as smart phones, miniature imaging systems are being developed. This trend and the use of micro-electro-mechanical systems (MEMS) has allowed production of endoscopes with smaller sizes or with greater functionality such as higher resolution, better depth penetration, or molecular imaging capabilities. Miniaturization has enabled other applications such as swallowable pill cameras that can image the gastrointestinal system and miniaturized imaging systems for imaging brain activity in active animals [14].

In Vitro Methods

Microscopy. Although microscopy has been a well established method in the life sciences for hundreds of years, the development of lasers and low-noise image sensors has enabled several advances in microscopy in the last few decades. With ultrafast lasers, it has been possible to perform nonlinear microscopy with little or no damage to cells. A variety of nonlinear methods have been applied to microscopy including second and third harmonic generation microscopy, multiphoton excited fluorescence microscopy (pioneered by Watt Webb, Fig. 5, and others) [7], and nonlinear Raman spectroscopy (including CARS and stimulated Raman spectroscopy) [8,15]. Examples of images acquired using coherent Raman microscopy are shown in Fig. 6. Nonlinear microscopies have been performed in vivo with excitation wavelengths as long as 1700 nm, allowing imaging depths of over 1 mm [16].

A variety of methods have been applied to improve the resolution of microscopy beyond the diffraction limit. Superresolution (the subject of the 2014 Nobel Prize in Chemistry) has been achieved based on finding the centroid of intermittent dye emission [photoactivated localization microscopy (PALM) [17] and stochastic optical reconstruction microscopy (STORM) [18]] or through nonlinearities such as for stimulated emission (STED) [19] or saturated structured illumination microscopy [20]. Subwavelength information can also be obtained using light to monitor the proximity between fluorophores using Förster resonance energy transfer (FRET) or metal nanoparticles (molecular ruler) [21]. Lateral diffusion can be monitored using fluorescence recovery after photobleaching (FRAP). Digital holographic microscopy provides both amplitude and phase images and allows computational reconstruction at different imaging planes.

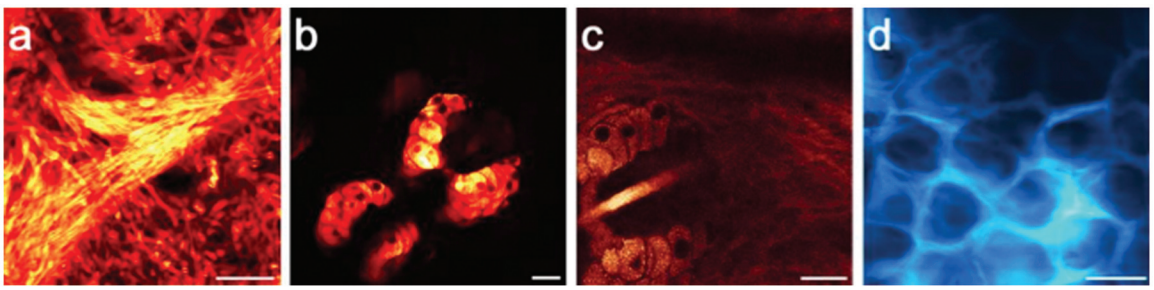
▲ **Fig. 5.** Watt Webb (OSA). (Photograph by Charles Harrington. Copyright Cornell University.)

Genetic modification and control. The DNA of cells or animals may be modified to produce optical signatures. For example, the green fluorescent protein (subject of the 2008 Nobel Prize in Chemistry) may be spliced into an organism to provide a fluorescent marker for gene expression. Bioluminescence such as that from the firefly can also be used to monitor gene expression. For example, insertion of the gene for luciferase into an animal allows imaging of gene expression by imaging yellow bioluminescence once the luciferin substrate is administered. For improved penetration in tissue, longer-wavelength versions of fluorescent proteins and bioluminescent substrates are being developed.

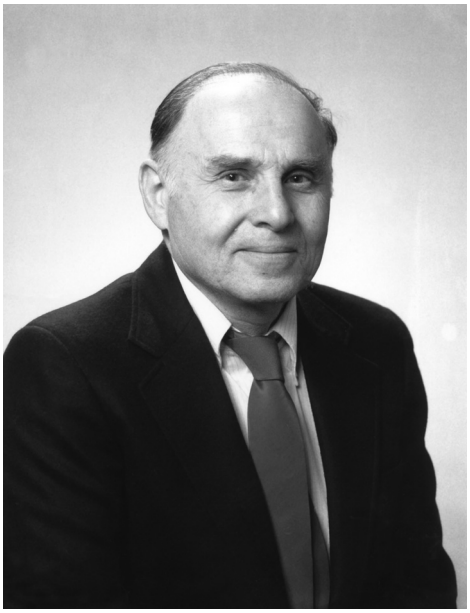
Single molecular detection. With the very small illumination volumes available with lasers and low-noise detectors it has been possible to image single molecules [11]. This allows probing variation in behavior of individual molecules rather than simply measuring ensemble averages of many molecules.

Optical tweezers or optical trapping (pioneered by Arthur Ashkin, Fig. 7, and others) [23] has allowed manipulation of cells or measurement of small forces for the study of molecular motors. Optical traps have enabled very precise studies of various molecular motors in cells. Recent developments include multiple optical traps produced using computer-generated holograms and cell stretching.

Microfluidics. Optics forms a natural pairing with microfluidics (optofluidics) because of the ability to remotely monitor conditions in microscopic volumes and the ability to use light to produce changes in the droplet contents or to manipulate or control microfluidic transport.



▲ **Fig. 6.** Label-free coherent Raman scattering microscopy showing (a) myelinated neurons in mouse brain, (b) sebaceous glands in mouse skin, (c), single frame of coherent anti-Stokes Raman movie acquired at 30 Hz, and (d) image of penetration trans-retinol in the stratum corneum. All scale bars are 25 μm [27].



▲ **Fig. 7.** Arthur Ashkin. (AIP Emilio Segre Visual Archives, Physics Today Collection.)

Other Applications. Optical methods have found other widespread uses in biomedicine. Examples include immunohistochemistry and fluorescence immunohistochemistry to label specific molecules on tissue sections in pathology, photolithography and fluorescence microscopy to map gene expression or genotype on DNA microarrays (gene chips), and matrix-assisted laser desorption/ionization (MALDI) for soft ionization of samples for mass spectroscopy.

Quantum dots are semiconductor nanoparticles for which quantum confinement leads to different colors based on the nanoparticle size and provides advantages for bioimaging [11,12]. Important qualities for quantum dots are the lack of photobleaching and wide range of colors that can be produced. Quantum dots are used for research including both in vitro and in vivo applications in animal studies.

Surface plasmon resonance. Surface plasmon resonance, particularly in noble metals, can be used in sensing and imaging. The resonance of the light field with the natural frequency of surface electrons at a gold layer is a powerful method for probing molecular interactions because of the high sensitivity, and no probe molecule is required. This method, commercialized notably by Biacore, is very widely used in biology laboratories. Surface plasmon resonance of single noble metal nanoparticles also allows detection of multiple colors using dark field microscopy.

Correlation methods and particle tracking. A number of other optical methods are well developed and commonly used in biomedical studies, such as dynamic light scattering and fluorescence correlation spectroscopy for monitoring the size and interactions of small particles such as proteins or micelles. For particles with stronger scattering, microscopic imaging can provide information on the cell's physical properties or intracellular interactions based on single particle tracking.

Therapeutics and Photomodification

One of the earliest applications of lasers in medicine was the use of argon ion lasers for retinal surgery. Other ophthalmic therapeutic applications include corrective surgeries such as LASIK and now ultrafast lasers for assistance in cataract surgery. Photodynamic therapy is used for treatment of certain cancers. Lasers are widely used for various cosmetic skin therapies including skin resurfacing, hair removal, vein treatment, acne scar treatment, tattoo removal, and treatment of port wine stain.

Cellular control and modification. Light may also be used to trigger changes in cells. For example, light may be used to turn on or off ion channels in vivo based on the proteins such as channelrhodopsin [24]. In this way light carried by optical fibers can activate different portions of the brain in awake animals. Ultrafast lasers are being used to perform nanosurgery and nanoporation on cells.

OSA's Role in Biomedical Optics

Throughout its history, OSA has played an active role in biomedical optics. The first issue of the *Journal of The Optical Society of America* in 1917 included articles titled "The nature of the visual receptor process" and "A photochemical theory of vision and photographic action," and this journal has been a significant publication for vision research since. As new journals were offered (*Applied Optics*, *Optics Letters*, and *Optics Express*) these, too, became important journals for

instrumentation and techniques in biomedical optics. In 2006, the Society created the *Virtual Journal for Biomedical Optics* to collect biomedical optics papers in a single place (Greg Faris, founding editor). In 2010, OSA initiated a journal dedicated to the field, *Biomedical Optics Express* (founding editor, Joe Izatt). This journal follows the open access, online format of *Optics Express*. OSA meetings, including the Annual Meeting (later Frontiers in Optics) and the Conference on Lasers and Electro-Optics (CLEO) have regularly had significant content in biomedical optics and vision. A topical meeting "Topics in Biomedical Optics" (BIOMED) with heavy emphasis on in vivo methods was launched in 1994, and OSA is the cosponsor of the European Conferences on Biomedical Optics (ECBO) together with SPIE. A second meeting, Optics in the Life Sciences, with particular focus on microscopy, optical trapping, and contrast methods was begun in 2009, occurring in alternate years with BIOMED.

References

1. R. Bright, "Diseases of the brain and nervous system," in *Reports of Medical Cases Selected with a View of Illustrating the Symptoms and Cure of Diseases by a Reference to Morbid Anatomy* (Longman, Rees, Orms, Brown and Green, 1831), Vol. II, Case CCV, p. 431.
2. T. B. Curling, "Simple hydrocele of the testis," in *A Practical Treatise on the Diseases of the Testis and of the Spermatic Cord and Scrotum* (Samuel Highley, 1843), pp. 125–181.
3. M. Cutler, "Transillumination as an aid in the diagnosis of breast lesions," Surg. Gynecol. Obstet. **48**, 721–729 (1929).
4. F. A. L'Esperance, Jr., "An opthalmic argon laser photocoagulation system: design, construction, and laboratory investigations," Trans. Am. Ophthalmol. Soc. **66**, 827–904 (1968).
5. J. W. Severinghaus and Y. Honda, "History of blood gas analysis. VII. Pulse oximetry," J. Clin. Monit. **3**, 135–138 (1987).
6. D. Huang, E. A. Swanson, C. P. Lin, J. S. Schuman, W. G. Stinson, W. Chang, M. R. Hee, T. Flotte, K. Gregory, C. A. Puliafito, and J. G. Fujimoto, "Optical coherence tomography," Science **254**, 1178–1181 (1991).
7. W. Denk, J. H. Strickler, and W. W. Webb, "Two-photon laser scanning fluorescence microscopy," Science **248**, 73–76 (1990).
8. A. Zumbusch, G. R. Holtom, and X. S. Xie, "Three-dimensional vibrational imaging by coherent anti-Stokes Raman scattering," Phys. Rev. Lett. **82**, 4142–4145 (1999).
9. C. H. Contag, P. R. Contag, J. I. Mullins, S. D. Spilman, D. K. Stevenson, and D. A. Benaron, "Photonic detection of bacterial pathogens in living hosts," Mol. Microbiol. **18**, 593–603 (1995).
10. M. Chalfie, Y. Tu, G. Euskirchen, W. W. Ward, and D. C. Prasher, "Green fluorescent protein as a marker for gene expression," Science **263**, 802–805 (1994).
11. W. C. W. Chan and S. Nie, "Quantum dot bioconjugates for ultrasensitive nonisotopic detection," Science **281**, 2016–2018 (1998).
12. M. Bruches, Jr., M. Moronne, P. Gin, S. Weiss, and A. P. Alivisatos, "Semiconductor nanocrystals as fluorescent biological labels," Science **281**, 2013–2016 (1998).
13. A. F. Fercher, C. K. Hitzenberger, G. Kamp, and S. Y. Elzaiat, "Measurement of intraocular distances by backscattering spectral interferometry," Opt. Commun. **117**, 43–48 (1995).
14. K. K. Ghosh, L. D. Burns, E. D. Cocker, A. Nimmerjahn, Y. Ziv, A. E. Gamal, and M. J. Schnitzer, "Miniaturized integration of a fluorescence microscope," Nat. Methods **8**, 871–878 (2011).
15. M. D. Duncan, J. Reintjes, and T. J. Manuccia, "Scanning coherent anti-Stokes Raman microscope," Opt. Lett. **7**, 350–352 (1982).
16. N. G. Horton, K. Wang, D. Kobat, F. Wise, and C. Xu, "In vivo three-photon microscopy of subcortical structures within an intact mouse brain," in 2012 Conference on Lasers and Electro-Optics (CLEO), OSA Techincal Digest (Optical Society of America, 2012).
17. E. Betzig, G. H. Patterson, R. Sougrat, O. W. Lindwasser, S. Olenych, J. S. Bonifacino, M. W. Davidson, J. Lippincott-Schwartz, and H. F. Hess, "Imaging intracellular fluorescent proteins at nanometer resolution," Science **313**, 1642–1645 (2006).
18. M. J. Rust, M. Bates, and X. Zhuang, "Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM)," Nat. Methods **3**, 793–795 (2006).

19. S. W. Hell and J. Wichmann, "Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy," Opt. Lett. **19**, 780–782 (1994).
20. M. G. Gustafsson, "Nonlinear structured-illumination microscopy: wide-field fluorescence imaging with theoretically unlimited resolution," Proc. Natl. Acad. Sci. U.S.A. **102**, 13081–13086 (2005).
21. C. Sonnichsen, B. M. Reinhard, J. Liphardt, and A. P. Alivisatos, "A molecular ruler based on plasmon coupling of single gold and silver nanoparticles," Nat. Biotechnol. **23**, 741–745 (2005).
22. W. E. Moerner and L. Kador, "Optical detection and spectroscopy of single molecules in a solid," Phys. Rev. Lett. **62**, 2535–2538 (1989).
23. A. Ashkin, J. M. Dziedzic, J. E. Bjorkholm, and S. Chu, "Observation of a single-beam gradient force optical trap for dielectric particles," Opt. Lett. **11**, 288–290 (1986).
24. E. S. Boyden, F. Zhang, E. Bamberg, G. Nagel, and K. Deisseroth, "Millisecond-timescale, genetically targeted optical control of neural activity," Nat. Neurosci. **8**, 1263–1268 (2005).
25. E. M. C. Hillman and A. Moore, "All-optical anatomical co-registration for molecular imaging of small animals using dynamic contrast," Nature Photon. **1**, 526–530 (2007).
26. S. Hu, K. Maslov, and L. V. Wang, "Second-generation optical-resolution photoacoustic microscopy with improved sensitivity and speed," Opt. Lett. **36**, 1134–1136 (2011).
27. K. Wang, C. W. Freudiger, J. H. Lee, B. G. Saar, X. S. Xie, and C. Xu, "Synchronized time-lens source for coherent Raman scattering microscopy," Opt. Express **18**, 24019–24024 (2010).