Field, F. Holtzberg, *Phys. Rev. Lett.* **62**, 827 (1989).
6. H. W. Zandbergen *et al.*, *Nature* **331**, 596 (1988).
7. A. Ourmazd *et al.*, *ibid.* **327**, 308 (1987).
8. MacTempas is a program for the simulation of HRTEM images and is a registered trademark of R. Kilaas, c/o Total Resolution, Berkeley, CA.
9. A. F. Marshall *et al.*, *Phys. Rev. B* **37**, 9353 (1988).
10. We acknowledge stimulating discussions with and the support of P. L. Key, J. H. Wernick, J. M. Tarascon, M. J. Bowden, P. F. Liao, and J. M. Rowell. The assistance of the staff of the National Center for Electron Microscopy is greatly appreciated. The work at Lawrence Berkeley Laboratory is supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Materials Sciences Division of the U.S. Department of Energy under contract DE-AC03-76SF00098. The work at Stanford University is supported in part by the Center for Materials Research under the National Science Foundation Materials Research Laboratories program.

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A Light Source Smaller Than the Optical Wavelength

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A method has been developed for the efficient emission of light from subwavelength dimensions. It is based on packaging photons as molecular excitons, effectively reducing the volume of the light beam by 10^9 and making possible propagation through dimensions of 1 nanometer. Molecular microcrystals are grown in the tips of micropipettes that have inner diameters of 100 nanometers or less. Measurements are presented that demonstrate this improvement in transmission for pipettes of various diameters. The ultrasmall dimensions of these light sources, the wavelength range (ultraviolet to red) of their emission, their ease of production, and their expected unique abilities for high efficiency excitation-imaging of surfaces portend significant applications for this methodology.

RECENTLY THERE HAS BEEN considerable interest in the development of schemes for subwavelength illumination (*1–4*). The aim of most of this research has been to advance near-field light microscopic imaging of surfaces to the resolution of a scanning electron microscope (*2–4*). Indeed, such superresolution light microscopy has enabled researchers to optically examine a variety of specimens without being limited by the diffraction properties of visible light. The basis of this near-field imaging technique is the following. As an electromagnetic wave emerges from an aperture, it is at first highly collimated to the aperture dimension. It is only after the wave has propagated a finite distance from the aperture that the diffraction that limits classical optical imaging takes effect. Thus, in the near-field region, a beam of light is present that is largely independent of the wavelength and is determined solely by the size and shape of the aperture (*5*). A critical factor in such near-field imaging systems is the nature of the light probe that accomplishes the subwavelength illumination. In this report we present an approach for producing sources of light with subwavelength dimensions and with significant improvement over previous approaches. Our method involves aiding the transmission of light through subwavelength dimensions with the introduction of crystals that store light energy as excitons, propagate these excitons in dimensions that are less than 10 nm, and then emit the light in a highly local region of a surface.

Several approaches have been used for producing subwavelength illumination, all of which involve the stopping down of a larger light source with some form of aperture. In one of the more successful methods for producing such subwavelength apertures, a metal-coated glass micropipette has been used that can be produced readily with inner diameters (at the tip) of less than 50 nm (*6, 7*). The details of pipette fabrication have been described elsewhere (*6, 7*). Even though the dimension of these pipettes can be reduced significantly below 50 nm, it has been argued that it would be difficult to reduce the spot size of such a light probe below this diameter. The essence of the argument is that the spot size of such an aperture depends ultimately on the finite conductivity of the metallic coating around the pipette. In order to appreciate this point, one has to remember that there are no propagating electromagnetic modes in a subwavelength cylindrical metallic wave guide such as a metal-coated pipette. The least attenuated mode for a round aperture has been found to be the TE_{11} mode, for which the energy decays at a rate of

$$E = E_0 \exp[-2 \times 1.81(\ell/a)] \quad (1)$$

where ℓ is the length of the aperture and a is the radius (*5*). With a sufficiently rapid tapering of the pipette, however, this evanescent region can be kept short enough to obtain a fairly large throughput of light. In addition to this effect, the electromagnetic wave penetrates the metallic coating and decays within it at a finite rate given by

$$E = E_0 \exp(-d/c) \quad (2)$$

where d is the depth of penetration and c is the extinction length of the metal. When the attenuation due to the wave-guide effect exceeds the attenuation in the metal, the contrast between the aperture and the surrounding medium becomes insufficient for superresolution applications. The metal with the largest opacity in the visible region is aluminum, for which $c = 6.5$ nm when the wavelength is 500 nm. This yields a minimum usable aperture of about 50 nm. In practice, multilayered coatings are necessary to obtain good adhesion and a practical aperture is expected to perform with reduced efficiency.

Our solution makes use of the energy-packaging capabilities of certain materials to circumvent the boundary problem of the edge of the aperture. According to our present understanding of energy propagation in materials, excitations can be confined to molecular and atomic dimensions under appropriate conditions (*8–16*). We have used this property of materials in our development of a subwavelength light source by growing a suitable energy-confining crystal within the subwavelength confines of a pipette. With this approach, energy can be guided directly to the aperture at the pipette tip instead of being allowed to propagate freely in the form of an electromagnetic wave through a region of the pipette whose dimension cannot support a propagating mode. Such energy-confining materials can be excited through either electrical or radiative processes to produce an abundance of excitons that allow light to be effectively transmitted through the "bottleneck" created by the subwavelength dimensions of the tip near the aperture. The excitons can be produced directly at the tip, or they can be generated within the bulk of the material and allowed to diffuse to the tip by an electric dipole (Forster) transfer mechanism (*8–12*). In either case, these excitons will then undergo a radiative decay, producing a tiny source of light at the very tip of the pipette.

The proximity of the metal coating along the outer surface of the pipette does not interfere with the exciton transfer and should actually help to increase the throughput, because the only allowed radiative transition will be for photons emitted along the

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axis of the pipette. The throughput is basically independent of the wavelength and is a linear function of the aperture cross section.

To demonstrate the feasibility and usefulness of the method, we chose to work with crystals of molecular anthracene because of its well-characterized electrical and radiative properties. Crystals of anthracene were grown inside the tip of a pipette from a benzene solution. By varying the concentration of the solution, we could accurately control the size of the deposited crystal.

The source of excitation for our experiments was the 363.8-nm line of an argon ion laser. Anthracene exhibits a very strong fluorescence in the blue with a quantum efficiency that approaches unity when it is illuminated in the near ultraviolet. One can illuminate the crystal either by directing the light through the pipette, as in the standard aperturing method, or by having an external beam incident on the crystal at the tip of the pipette. With the second method large amounts of energy can be brought to bear directly on the spot where the illumination is desired, with the upper limit imposed only by the onset of photochemical bleaching. In terms of this photobleaching, anthracene may not be the best material for such a light source because it undergoes a photochemical oxidation that does not occur in other materials. These materials and others can also tune the emission of the light source to a wide spectrum of wavelengths. Figure 1 is a picture of the pipette with a fairly large anthracene crystal of approximately 1 μm extending past the tip of the pipette and illuminated, head on, with several milliwatts of energy at 363.8 nm. The spot of light thus produced was clearly visible to the naked eye under room lighting and is shown here magnified about 1000 times.

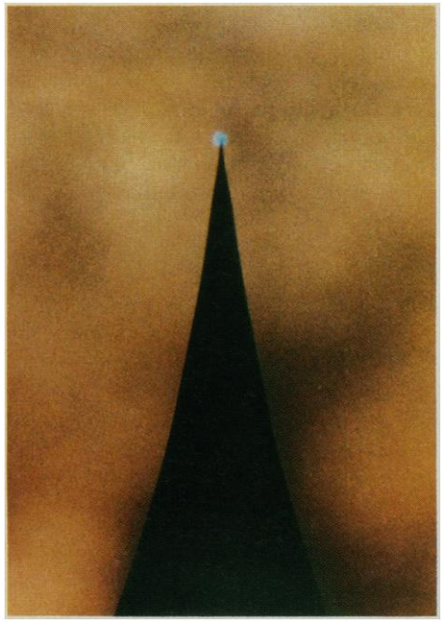

Fig. 1. A pipette exciton light source excited with 5 mW of 363.8-nm ultraviolet light from an argon ion laser. The tip inner diameter is about 100 nm. Magnification is roughly $\times 10^3$. The blue dot is exaggerated significantly by the photographic copying process.

To show that the anthracene does indeed aid in guiding light through a pipette, we measured the throughput for an empty pipette and compared it to that for the same pipette with a crystal in it. We illuminated the pipette by inserting a 0.21-mm fiber light guide up to the very tip. The light emerging from the pipette was collected with a photomultiplier and a photon counting system. The pipette was then removed from the system, a small crystal was grown inside the tip, and this crystal was replaced in exactly the same position. Both the ultraviolet light and the blue fluorescence were collected, although most of the light was converted to the visible. The experiment was repeated for a large number of pipettes of different dimensions and different illumination intensities. We did not correct for glass (pipette) fluorescence (which was approximately 30% of the signal observed in the empty pipette) and nonlinearity in the photomultiplier.

Improved throughput was seen in all the pipettes tested with an inner diameter below several micrometers. The results of several measurements are summarized in Table 1. The largest improvement observed was a transmission of 2.9 times that of an empty pipette. Correcting for fluorescence from the pipette and phototube nonlinearity can only increase these values. Furthermore, these improvements are low by at least a factor of 3 since the readings were taken after initial photobleaching of the anthracene (to achieve stabilization). The actual throughput was dependent chiefly on the dimension of the pipette, but we could easily control the throughput by adjusting the alignment of the optical delivery system. Counts of several hundred thousand photons per second could be achieved for moderately small (0.25-μm) pipettes, although lower intensities were preferable so as not to saturate the photomultiplier.

The smaller the dimensions of the pipette, the greater the improvement in transmission should be, although the absolute value of the signal will decrease. This was generally observed to be the case, although quantitative data, with appropriate error bars, were difficult to obtain because of the difficulty in characterizing the precise profile of the pipette. In addition, because the crystals do not grow uniformly, the size and shape vary from pipette to pipette with a corresponding change in the gain. The crystal also aids in transmitting light through pipettes larger than half a wavelength because much of the energy exiting the fiber is in higher order modes than TE_{11} and has correspondingly larger cutoff diameters. Geometric factors may also increase the throughput in anthracene-filled pipettes, even when the diameter is larger than the cutoff, because part of the light incident on the inner wall, which otherwise would have been lost, will be reemitted from the crystal coating the wall along the axis of the pipette and will reach the aperture. It was only when the pipette was broken at the tip to obtain a very large aperture that we saw a decrease in transmission due to the crystal blocking the propagation of the light.

Table 1. Amplification with pipettes of different diameters.

Empty pipette (counts per second)	Pipette with crystal (counts per second)	Improvement	Approximate inner diameter (μm)
170,000	500,000	2.94	0.5
61,000	147,000	2.4	0.1
90,000	150,000	1.7	0.7
6,000*	700	0.12	14

*Lower light input than in other pipettes.

Electroluminescence in anthracene has been reported (*11, 17*), and this method should provide an ideal compact source of illumination that requires no external source of excitation such as a laser. The external metal coating on the pipette would provide one contact, and an electrolytic solution can be injected into the pipette to provide the other. Electron-injecting contacts in anthracene require a metal with a low work function such as a sodium alloy or solution, with all the accompanying environmental problems, but the feasibility of this method is currently being investigated.

The importance and applicability of such light sources for subwavelength imaging based on the methodologies of near-field microscopy are obvious. However, the availability of such a source of excitons in a confined crystal that can be brought close to a surface, with the micromovement capabilities developed for scanned tip microscopies (*18*), could have important implications both for imaging with light and for molecular sensing with light. First, if one were to bring such a crystal within a few nanometers of a surface containing acceptors for its excitons, the excitation of such a surface would be many orders of magnitude more efficient than direct excitation of the same surface with freely propagating light. For example, for a dye such as rhodamine, based

on the absorption cross section, a single molecule requires 10^9 incident photons for one photon to be absorbed, but the presence of a single exciton of the appropriate characteristics is capable of causing this same effect. Second, the combination of single-molecule excitation with an exciton source and a pipette that can be scanned with high resolution across a surface leads to a new way of imaging with light that surpasses any presently available methodologies. Specifically, if one scans such an exciton source across a surface, exciton transfer to the surface will occur only when a point in the crystal is within about 8 nm of an acceptor in the surface. Thus, by detecting the acceptor's emission or a reduction in the intensity of the donor's emission as a function of the position of the exciton source, an image with molecular resolution should be obtainable with light. This form of imaging, which we call molecular exciton microscopy (MEM), should have wide applicability to nondestructive imaging of biological membranes and other molecular surfaces at ambient conditions.

Such sources should also be important as an analytical chemical tool for microanalysis and sensing with light that bypasses absorption path-length requirements. A specific example of such an application for these exciton sources is capillary zone electrophoresis (*19*). In this technique molecules are separated by electric fields along a glass capillary. In principle, the smaller the dimension of the capillary, the better the separation. However, part of the limitations encountered by this technique are absorption path-length requirements for the detection of the separated species. Exciton sources and the methodology of exciton excitation outlined here conceivably could alleviate some of these problems.

Finally, it is instructive to compare the dimensionalities, the ease of fabrication, and the approach to light excitation described here to those presently in use in integrated optics. The smallest light sources available today for integrated optics are light-emitting diodes and diode lasers, which have the potential to be used as light sources, switches, modulators, and detectors. These devices are fabricated by means of sophisticated molecular beam epitaxy techniques that involve multimillion-dollar instrumentation. Even with such instrumentation, the typical devices constructed have wavelengths beyond 670 nm and have rectangular rather than cylindrical symmetry, with initial spot sizes of 2 by 5 μm (*20*). This rectangular structure causes significant astigmatism with parallel and perpendicular parts of the beam acting as if they come from two different sources separated by 40 μm (*20*). These beam characteristics of diodes waste much light when coupled to the circular entrance of an optical fiber. In contrast, our exciton sources have circular beams and the fact that the crystals used in such sources have been made to lase is also important for possible applications in integrated optics. Because of the high efficiency of exciton generation, electrical excitation of such crystals may make it possible to achieve laser action in the confined space of a pipette (which has a structure that could be ideal for laser action). The methodologies described in this report have much potential for use in molecular exciton microscopy, molecular sensing, or as a cheap and compact short wavelength light source.

REFERENCES AND NOTES

1. U. Ch. Fischer and H. P. Zingsheim, *J. Vac. Sci. Technol.* **19**, 881 (1981).
2. A. Lewis, M. Isaacson, A. Muray, A. Harootunian, *Biophys. J.* **41**, 405a (1983); A. Lewis, M. Isaacson, A. Harootunian, A. Muray, *Ultramicroscopy* **13**, 227 (1984).
3. D. W. Pohl, W. Denk, M. Lanz, *Appl. Phys. Lett.* **44**, 651 (1984).
4. U. Ch. Fischer, *J. Vac. Sci. Technol. B* **3**, 386 (1985).
5. E. Betzig, A. Harootunian, A. Lewis, M. Isaacson, *Appl. Opt.* **25**, 1890 (1986).
6. E. Betzig, A. Lewis, A. Harootunian, M. Isaacson, E. Kratschmer, *Biophys. J.* **49**, 269 (1986).
7. A. Harootunian, E. Betzig, M. Isaacson, A. Lewis, *Appl. Phys. Lett.* **49**, 674 (1986).
8. R. Kopelman, *Rec. Chem. Prog.* **31**, 211 (1970).
9. H.-K. Hong and R. Kopelman, *Phys. Rev. Lett.* **25**, 1030 (1970).
10. H. Kuhn, *J. Chem. Phys.* **53**, 101 (1970).
11. M. Pope and E. Swenberg, *Electronic Processes in Organic Crystals* (Oxford Univ. Press, New York, 1982).
12. A. H. Francis and R. Kopelman, in *Topics in Applied Physics*, vol. 49, *Laser Spectroscopy of Solids*, W. M. Yen and P. M. Selzer, Eds. (Springer, Berlin, ed. 2, 1986), p. 241.
13. J. Prasad and R. Kopelman, *Phys. Rev. Lett.* **59**, 2103 (1987).
14. R. Kopelman, *Science* **241**, 1620 (1988).
15. E. B. Fauman and R. Kopelman, *Comments Mol. Cell. Biophys.* **6**, 47 (1989).
16. C. S. Li and R. Kopelman, *Macromolecules*, in press.
17. W. Mehl and B. Funk, *Phys. Lett. A* **25**, 364 (1967).
18. P. K. Hansma, V. B. Elings, O. Marti, C. E. Bracker, *Science* **242**, 209 (1988).
19. M. J. Gordon, X. Huang, S. L. Pentoney, Jr., R. N. Zare, *ibid.*, p. 224.
20. J. Zeman, *Laser Focus World* **25**, 69 (1989).

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Evidence for a Novel Thioredoxin-Like Catalytic Property of Gonadotropic Hormones

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It has been proposed that dithiol-disulfide interchange and oxidation-reduction reactions may play a role in hormone-induced receptor activation. Inspection of the sequences of the gonadotropic hormones revealed a homologous tetrapeptide (Cys-Gly-Pro-Cys) between the β subunit of lutropin (LH) and the active site of thioredoxin (TD). The β subunit of follitropin (FSH) has a similar sequence (Cys-Gly-Lys-Cys). Thioredoxin is a ubiquitous protein serving as an electron donor for ribonucleotide reductase, but it also exhibits disulfide isomerase activity. The catalytic activity of TD was assayed by its ability to reactivate reduced and denatured ribonuclease. In this assay, the purified ovine FSH and bovine LH preparations tested were ~60 and ~300 times, respectively, as active as TD on a molar basis. This heretofore unsuspected catalytic property of FSH and LH may be important in understanding their mechanism of receptor activation and signal transduction.

FOLLITROPIN (FSH) AND LUTROPIN (LH) are complex, heterodimeric glycoprotein hormones [relative molecular mass (M_r) of ~35,000] secreted by the anterior pituitary gland and are responsible for normal gonadal development and function (*1–3*). These disulfide-rich hormones share a common α subunit, with biological specificity conferred by their β subunits (*1–3*). Recently, the LH receptor from porcine testis (*4*) and rat ovaries (*5*) has been cloned, and the FSH receptor has been purified from bovine calf testis (*6*). Signal transduction for FSH and LH occurs via the adenosine 3′,5′-monophosphate (cAMP) second-messenger system (*1–3*), and the nature of the hormone-receptor interaction has been the subject of intense study (*3*). Nevertheless, the molecular events leading to the activation of gonadotropic hormone receptors after interaction with FSH or LH remain obscure.

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