onstrated the use of two LC SLM masks for both amplitude and phase control, and we have completely automated our pulse shaping system. The user need only specify the desired waveform to the computer, which determines the needed mask patterns and produces them through its interface with the SLM(s). Figure 2 illustrates examples of shaped waveforms generated from a 70-fs input pulse. Figure 2, A and B were produced with one SLM as a phase mask. Figure 2A is a 3.07-THz train of 70-fs pulses. Pulse trains with repetition rates ranging from 1.10 to 4.39 THz in 0.22-THz intervals were also easily produced. Figure 2B is a descending pulse train in which the heights of the consecutive pulses have been specified. Figure 2C is an 550-fs optical "square" pulse. Generation of such a waveform requires manipulation of both spectral amplitude and spectral phase and is accomplished with two SLMs.

Substantial improvements will be realized with "second-generation" commercial SLMs, which have many more pixels and much smaller gaps. In addition, techniques for rapid and complete characterization of amplitude and phase profiles have been demonstrated (*9*). Finally, preliminary results indicate that amplification of shaped waveforms in a titanium:sapphire regenerative amplifier introduces little or no loss of fidelity. This is crucial for achieving the high-power waveforms necessary in many "optical control" applications. Given current developments, we can anticipate an amplified pulse shaping system in which the amplified output is characterized and used in a feedback loop to refine the SLM masks iteratively, so that high-power, user-specified waveforms are generated.

Such waveforms will be used for control over molecular and material behavior. Spectroscopic applications that can be readily imagined include multiple-pulse vibrational spectroscopy within and beyond the harmonic oscillator (small-amplitude) regime, fluorescence-detected photon echoes, and optical analogs of multiple-pulse NMR techniques, such as dipolar decoupling, in which the electronic interactions of molecules with their neighbors are altered. For the most ambitious applications in material control, in which permanent structural changes are achieved, the entire experiment must be over in a single laser shot because the sample never returns to its initial state. Such a transformation could be monitored with recently developed real-time femtosecond probing techniques, by which the entire time-dependent response of a sample is recorded in a single shot (*10*).

In most optical-control applications, the optimal pulse sequence is not known in advance because the molecular or material properties, especially if they are far from equilibrium (on the way toward chemical or structural rearrangement), are not known precisely. Imperfect knowledge of a material Hamiltonian can, however, be the basis for a trial waveform, and the response of the material to this input can be analyzed to improve the waveform by iteration. Learning algorithms to realize this possibility have been proposed (*11*), raising the prospect that a material can actively participate in its own modification.

The development of femtosecond lasers and spectroscopic techniques has made the past decade one of remarkable advance in our ability to observe ultrafast molecular and material processes. Elementary molecular motions involved in chemical-bond breakage and elementary collective motions involved in structural phase transitions have been observed directly in the time domain. Automated generation of high-power femtosecond waveforms in the current decade will extend our capabilities from observation of, to control over, molecular and collective behavior, including chemical and structural rearrangements.

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Near-Field Optics: Imaging Single Molecules

Raoul Kopelman and Weihong Tan

Can we image a single molecule in vivo? Can we see it wiggle or react or break up? Can we simultaneously measure its energy dynamics? Can we monitor directly and in real time the ions or radicals released by a single ion gate or enzyme? More and more, these questions are being answered in the affirmative (*1*). Impressive evidence of progress is provided on page 1422 of this issue in the report by Betzig and Chichester on their success in imaging single molecules (*2*). Moreover, they do it in a way that reveals the orientation of each. At the same time, Betzig and Chichester have been able to turn the tables, using a single molecule to map the electric field distribution in the vicinity of a nanometer light source.

Traditionally, molecular structure and dynamics have been observed by averaging techniques, such as x-ray crystallography, electron diffraction, and various spectroscopies. On the other hand, electron microscopy and related methods do indeed image single molecules but at a heavy cost to their integrity—observing them in a vacuum or under highly perturbative conditions. Recent methods such as scanning tunneling microscopy (STM) and atomic force microscopy (AFM) come closer to the ideal, but the molecules are still exposed to perturbative electric fields or contact forces. These problems are particularly acute for the soft organic and biological molecules. Furthermore, it is impossible or nearly unfeasible to observe the molecular dynamics. Near-field optical (NFO) microscopy and spectroscopy (*3–6*) is a new tool providing hopes for highly improved imaging at a relatively low cost to the sample (and the researcher).

Observing the dynamics of a single molecule may have started with the direct patch-clamp–assisted observation of single sodium gates, and in particular the voltage jumps accompanying their opening and closing (*7*). Recent, very elegant single-molecule observations in the energy domain have been performed and reviewed (*8, 9*). In this case, single molecules move around in the very high resolution laser spectral domain in samples of dilute, mixed molecular crystals and polymers. Optical spectroscopy observations of single molecules have recently been made (*10,11*). Here, dye molecules are in a thin flow cell (*10*) or levitated microdroplet (*11*) and are fleetingly observed by laser fluorescence.

The report by Betzig and Chichester (*2*)

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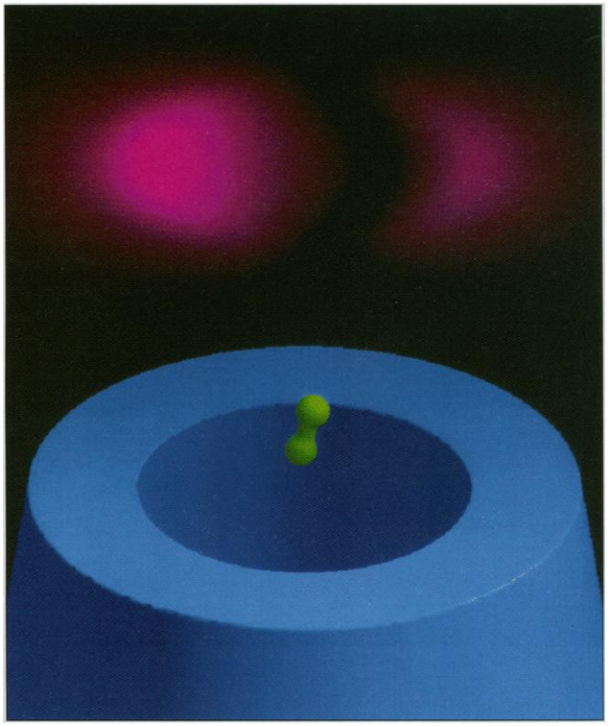
defines the state of the art concerning optical imaging. About two dozen isolated dye molecules are imaged within seconds. The imaging resolution is about 50 nm, and the molecular location is resolved within about 25 nm in the horizontal plane and 5 nm in the vertical direction. Furthermore, the much smaller molecular transition dipole is a point detector mapping out the electric field distribution of the near-field light source.

Most optical microscopies involve conventional optics (now called "far-field optics"), where the standard rules of interference and diffraction (*3–6*) lead to the Abbé diffraction limit on the resolution of optical microscopes. This limit is approximately $\lambda/2$, where λ is the wavelength. Even the elegant new confocal microscopes (*3*) cannot overcome the diffraction barrier. Electron and x-ray microscopy do not overcome it either, except that their λ is significantly shorter. This better absolute resolution comes, however, at a price of low penetration depth and high ionizing radiation, which causes severe damage to biosamples (not to mention the high monetary price of such instruments).

In near-field optics, the diffraction limit is overcome by the use of subwavelength light sources and samples positioned very close to them (that is, in the "near field"). In a gedanken experiment, we would use a tiny flash lamp, say only 100 nm. A 100-nm cover layer will stop the light, while a 50-nm cover layer will not. Imagine now a sample made of glass plate with aluminum spots on its front surface. If we scan this plate very close to the light source, we can image spots of the size of 100 nm or larger, with an effective resolution of about 100 nm. The resolution in the "green" (with wavelength around 500 nm) is now about $\lambda/5$. Furthermore, if we use an infrared light source with λ = 100 micrometers (but geometrical size still 100 nm), the resolution is about $\lambda/1000$.

What are the technical difficulties? One is that we need such a small subwavelength lamp with enough intensity. Another is that the sample has to be scanned closely and quickly. The latter requirement is not too difficult nowadays (magnetic disk drives are scanned extremely quickly at even closer distances). However, the first requirement of a tiny but intense light source has been a problem. Originally, tiny nanofabricated orifices were used as light sources (*3*, *6*). However, the photon throughput is very limited. Very recently, with the advent of active subwavelength light sources (*4*, *12*, *13*), one can get a throughput of 10^{12} photons/s or more (*4*, *13*). There are other problems, such as the necessity of a "feedback" mechanism to avoid physical contact and damage to the source. There are also wonderful recent solutions, like combined NFO and force, or combined NFO and STM operation (*3*, *4*, *14*). These further enrich the contrast mechanisms of near-field optics: refraction, reflection, polarization, luminescence, lateral force interactions, and so on.



Portrait of a single molecule. The subwavelength optical source is shown beneath a molecule, represented by the green dumbell (not to scale). The optical excitation results in the red fluorescence emission pattern shown at top. By comparison of the emission structures seen in the near-field scans, the orientation of individual molecules can be determined. [E. Betzig, AT&T Bell Laboratories]

The very same tip can be scanned over the same sample with an alternation of the contrast mechanisms (for example, fluorescence and shear force), yielding images with a high degree of fidelity, as well as additional information content. (This is like adding the natural color to a three-dimensional topographic map.) Very impressive images with about 50-nm resolution have been obtained by Betzig *et al.* (*1*) for cytoskeletal actin and cellular protrusions formed in the process of wound healing, as well as by Monson *et al.* (*1*) for blood cells and DNA. Such images of in-air or in vitro samples are, of course, of major interest to biology and medicine. However, organic surface images of Langmuir-Blodgett and polymeric films are also of much interest in chemistry and materials science. We note that not only is the resolution much improved compared with confocal microscopy, but the photobleaching problem is less severe in near-field scanning optical microscopy.

In addition to imaging individual dye molecules (see above), Betzig and Chichester (*2*) also obtained information on the orientation of these molecules (by means of polarization and transition dipole fitting). Another promising approach is to obtain spectra characteristic of a single molecule or molecular aggregate. Birnbaum *et al.* (*15*) have shown position-dependent fluorescence spectra that discriminate between single molecules and aggregates only 40 nm apart. Such work has also been shown to discriminate between submicroscopic nanocrystals (*5*). Near-field spectroscopy at low temperature has also been performed on quantum wells and on nanodots by Smith *et al.* (*16*), by Trautman *et al.* (*1*), and by Harris and Grober (*1*). Also, the mechanism of light-matter interaction may be different in the far- and near-field regimes, leading to different spectral selection rules and in particular to an enhanced cross section of light absorption (and thus fluorescence) (*5*). These phenomena are an extra bonus for near-field detection (*17*). In addition, good optical fiber tips do not affect light polarization (*2*, *4*), and not even cross correlation: Ultrafast (80-fs) nanometer light sources have been demonstrated by Smith *et al.* (*1*).

The reduced size of the light sources, together with the enhanced molecular-excitation cross section and the good spectral and time resolution have enabled another unexpected development: rugged, ultraslim, ultrasensitive, and ultrafast fiber-optic chemical sensors (*17*); sensors that require only attoliters of sample, zeptomoles (10^{-21} moles) of the unknown substance, and milliseconds or faster response times (*1*, *17*). In addition, the nanometer sensors are small enough (about 100 nm) to slip in and out of a cell's membrane without any damage or leakage. Such pH sensors have been used to investigate blood cells and, in particular, rat embryos (*17*). High-quality, nonperturbative, in vivo measurements by Tan *et al.* (*1*) have contributed to new information on organogenesis stages—such as anaerobic to aerobic transformation, and on physical and chemical insults—such as change in pH, removal of oxygen, or the addition of new drugs to the environment—during particular time windows.

Even smaller probes are in the making.

In principle, a nanometer light source can be as small as a single molecule or atom. At the same time, its position and scanning have to be defined in space as well as those of an STM tip (a randomly flying atom does not qualify). Existing designs for optical supertips (5) are based on the same principle as the green plant photosynthetic system. A submicrometer antenna collects the photons by absorption and transfers the excitation energy to a single active center. From there, the energy is either (i) radiated as a photon or (ii) transferred to the sample in an energy transfer process (Foerster-Dexter) (5). In either case, the result is generally affected by the nearby sample molecule: (i) the radiated excitation may be affected, for instance, by intermolecular spin-orbit coupling (Kasha effect) (5, *18*); (ii) the energy transfer results in a fluorescence or phosphorescence typical of the sample molecule. In the latter case, only virtual photons are produced by the supertip; this gives an excitation transfer tip ("exciton tip"), and only sample luminescence is detected. The world's largest ordered molecules, "dendrimers" (*19*), have been used or synthesized for this purpose. Such a single molecule exhibits a 125 Å antenna with an active center of 10 Å or less. So far, only tips with aggregates of sample molecules have been used [Tan *et al.* (*1*)].

Many technical problems still have to be solved, from understanding the contrast mechanism to the control of photobleaching (a standard problem in fluorescence microscopy). However, the future looks bright. Reversible bleaching (5) could be the basis for the highest density optical memories (with the "bit" occupying only a single molecule). More realistic in the near term would be pixels on the order of 100 Å. The necessary high scanning speeds are presently limited by the probe intensity, but we expect much higher photon outputs to become possible. Indeed, subwavelength probes have already been turned into high-flux lasers, as just reported by Betzig *et al.* (*1*). Analytical chemistry is being driven to the extreme of imaging single dye molecules and reaching absolute ion detection limits of zeptomoles or less. Complex polymeric samples may finally be characterized on the molecular level by nanospectroscopy. Maybe most importantly, biosamples, including living cells, could be imaged down to the molecular level and analyzed spectroscopically or by chemical sensors. The intracellular molecular dynamics of organogenesis, metabolism, splitting, and chemical damage could be followed in vivo and in real time. At the same time, DNA could be sequenced in situ (*18*) or even manipulated by the right probe at the right location. Although much of this might sound like science fiction, some of the above-mentioned achievements in near-field optics must have sounded like science fiction only a couple of years ago.

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Neutron Interferometry

Helmut Rauch

As quantum objects with both wave and particle properties, neutrons can exhibit familiar optical effects such as diffraction and interference. In the two decades since the first perfect-crystal neutron interferometer was tested by an Austrian-German group at our 250-kw TRIGA (Training Reactor, Isotopes General Atomic) reactor in Vienna (*1*), neutron interferometry has become a laboratory for fundamental tests of quantum mechanics. Neutrons are fermions of well-defined mass and are subject to strong electromagnetic and gravitational interactions, all of which cause measurable interference effects. When placed in a magnetic field, neutrons occupy two energy levels between which transitions can be induced by proper oscillating magnetic resonance fields; the existence of these energy levels increases the variety of quantum mechanical tests that can be performed.

To perform interferometry, separate but phase-coherent neutron beams are needed. Such beams can be produced by dynamical Laue-reflection of thermal neutrons in an appropriately shaped perfect silicon crystal. This is analogous to the Mach-Zehnder type of interferometer used in light optics and to the Bonse-Hart interferometers (*2*) developed for x-rays; electron interferometry (*3*) and the recently developed atom interferometery (*4*, *5*) use somewhat similar schemes. According to the complementarity principle of quantum mechanics, the neutron behaves purely as a wave inside the interferometer.

Symmetry dictates that the wavefunctions originating from beam paths I and II and composing the forward beam (0) behind the interferometer are equal in amplitude and phase because they are transmitted-reflected-reflected (TRR) and reflected-reflected-transmitted (RRT), respectively. Therefore, complete beam modulation is expected as a function of the phase shift between the beams:

$$I \propto |\psi_0^{I} + \psi_0^{II}|^2 \propto 1 + \cos\chi \qquad (1)$$

Different kinds of interactions can cause phase shifts between the coherent beams, which can be calculated as the path integral of the canonical momentum $\mathbf{k}_c$ along the interferometer loop, $\chi = \oint \mathbf{k}_c d\mathbf{s}$.

Neutron interference experiments belong to the domain of self-interference where, in nearly all cases, only one neutron is inside the interferometer, while the next one has yet to be born and is still contained

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