Femtosecond Pulses:
Control Is Key to New Discoveries

From microscopy to micromanipulation, femtosecond pulses are broadening their reach throughout the photonics research world. To fully realize their potential, precise pulse control is critical.

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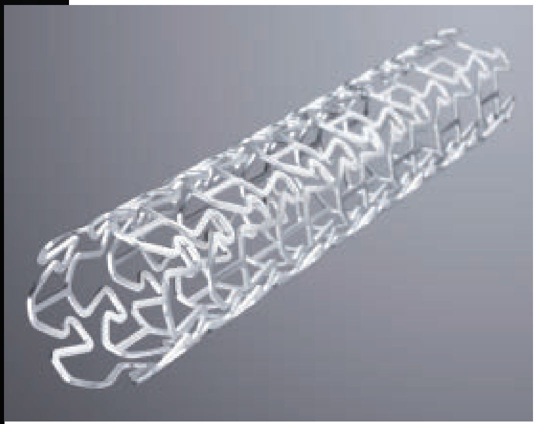
Ultrafast lasers have transformed the worlds of clinical research by revealing biological mechanisms in greater detail than previously possible. Femtosecond lasers are the critically enabling tools in multiphoton microscopy, where shorter pulses generally enable brighter images.

Short pulses deliver high peak intensity with relatively low pulse energy, making femtosecond pulses the ideal choice for imaging living tissue while limiting potential damage. Precise control of the pulse's width, duration, shape, repetition rate and propagation ensures that desired results are enhanced and unwanted effects are minimized.

Beyond imaging, ultrafast pulses are also finding new applications in microsurgery and micromanipulation, as demonstrated by the growing treatments in eye surgery, dentistry, and the fabrication of precise surgical tools and biomedical equipment, such as stents.

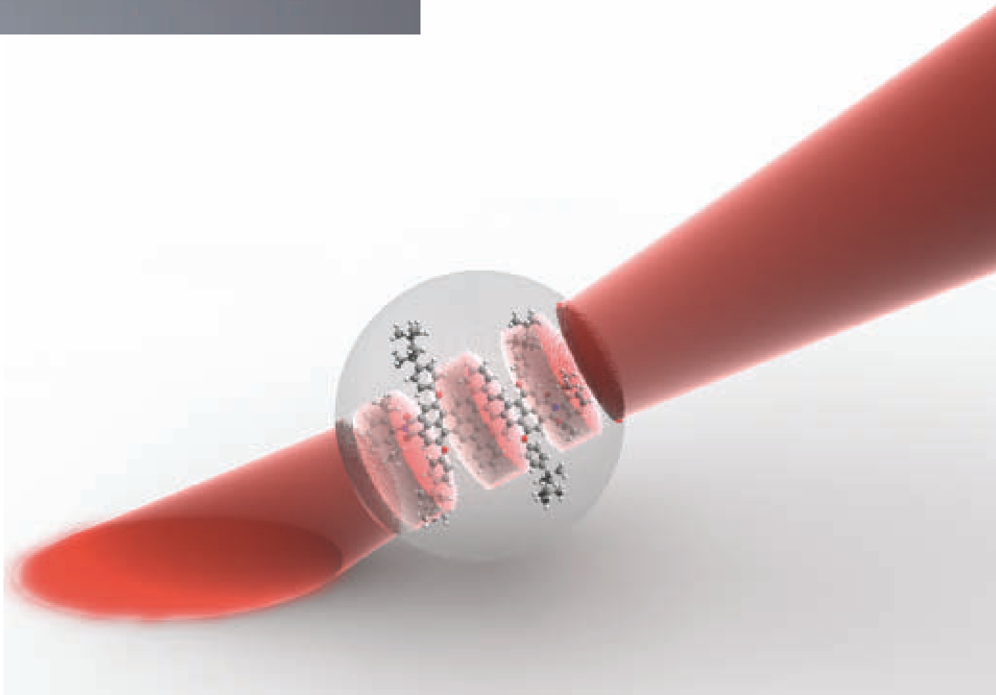
Optogenetics is a particularly hot area in neuroscience right now where light is used to activate or "silence" neurons in



Laser-cut stents embody the precise micromachining made possible by carefully controlled pulses from femtosecond lasers. Courtesy of Trumpf GmbH.

A laser-cut nitinol stent. Courtesy of Trumpf GmbH.

Cold laser material processing of a matchstick head with the TruMicro Series 5000. Courtesy of Trumpf GmbH.



Shaped laser pulses excite molecules to specific excited states. Courtesy of ICFO (The Institute of Photonic Sciences).

the brain of a lab animal. Although many of these experiments do not need lasers, multiphoton excitation with femtosecond lasers is the preferred method when it comes to investigating the optogenetics in living animals with in-depth, single-neuron resolution.

While femtosecond lasers and pulse shapers are relatively advanced and are most commonly applied in the laboratory, mastering their control in biological environments proves much more difficult.

"Broadband laser systems are developing rapidly into user-friendly turnkey system[s]; while also pulse shapers are moving towards more accessible systems," said Dr. Niek van Hulst, professor of molecular nanophotonics at The Institute of Photonic Sciences in Barcelona, Spain. "Still, the tools are expensive and specialized, which is an obstacle for broader applications."

Pulse duration and pulse width

Temporal control is used for obtaining brighter multiphoton, second-harmonic and third-harmonic images, which has improved the imaging of highly pigmented tissues, such as melanoma and red blood cells.

A shorter pulse width at the sample translates directly into brighter images. This is because the excitation efficiency in any multiphoton method has a highly nonlinear dependence on peak intensity and, hence, peak power.

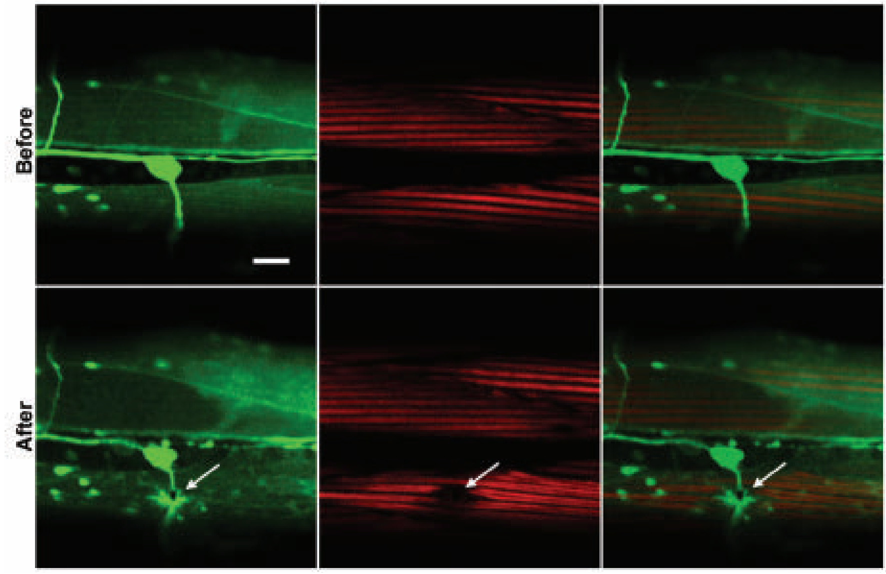
"A technical complication of broadband short-pulse excitation in microscopy is dispersion," van Hulst said. "The high-

NA [numerical aperture] objective with a centimeter of glass does disperse the incident pulse, such that the pulse gets stretched: The pulse is 'chirped' with different colors passing at different moments. Correction by pulse compensation and compression is crucial in multiphoton microscopy."

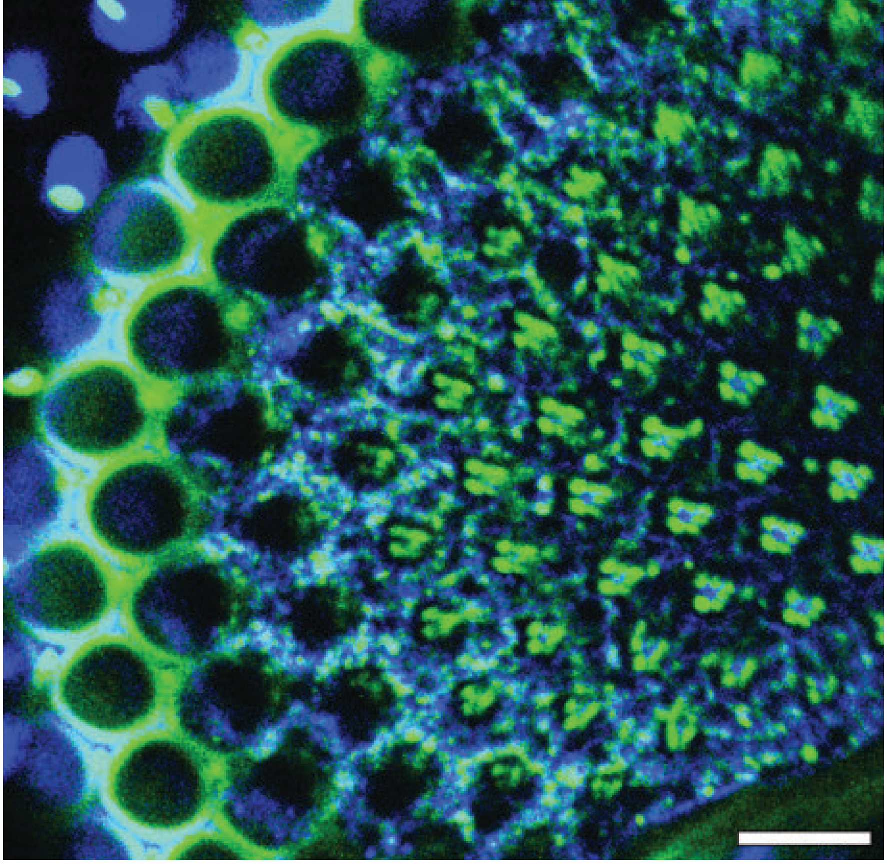
"Adaptive temporal control is used to make sure the pulse duration is shortest at the focal plane," said Marcos Dantus, founder and CTO of Biophotonic Solutions Inc., and who also is a professor of chemistry and physics at Michigan State University. "Given that microscope objectives have very high dispersion characteristics, temporal shaping can also make improvements over one order of magnitude."

The peak intensity of the pulse is intimately related to the pulse duration. For ultrashort laser pulses with extremely high peak intensities, nonlinear effects come into play that define what kind of process occurs.

Although shorter pulse widths yield brighter images, they also increase the



Two-photon fluorescence, second harmonic and merged images of *C. elegans* worm muscles; before (top) and after (bottom) cutting with a laser at position of the arrow. Scale bar is 10 μm. Courtesy of ICFO (The Institute of Photonic Sciences). Reference: S. Santos et al (2013). *PLOS ONE*, 8(3), p. e58600. doi: 10.1371/journal.pone.0058600.g006.



Multimodal microscopy image of an adult *Drosophila melanogaster* unstained eye. Cross section by the imaging plane through the eye elements shows their contents with high optical resolution. Excitation source: sub-50-fs pulses from a Yb fiber laser oscillator with spectrum centered at 1060 nm, built at the Dantus Research Group using adaptive pulse compression. Scale bar is 20 μm. Courtesy of Ilyas Saytashev, Dantus Research Group, Michigan State University.

possibility of photobleaching or otherwise damaging the sample – the shortest pulse widths are not always optimum for every application.

Given a certain bandwidth and pulse width produced by the laser, delivered pulse width can be adjusted by introducing the appropriate level of chirp. In some applications, those having a lot of downstream optics, particularly transmissive components, such as acousto-optic modulators (AOMs), can cause undesirable stretching of the pulse through the material. This is known as positive group velocity dispersion (GVD).

"So the laser pulses are often prechirped with negative GVD before they leave the laser head, in order to offset this downstream stretching and minimize pulse width at the actual sample or experiment," said Marco Arrigoni, director of marketing for scientific lasers at Coherent Inc. in Santa Clara, Calif. "Providing adjustable prechirp enables the researcher to experiment for themselves, and to empirically find the optimum pulse width based on image brightness and any observed trade-off in photodamage."

In some of Coherent's products, prechirp can be set by a user either by adjusting a pair of gratings in the laser head (as in the company's Fidelity ytterbium fiber laser) or via a software program that automatically adjusts spacing and positioning of a set of prisms, according to the desired wavelength and prechirp value (as in Coherent's Chameleon product line).

"In our Vitara lasers, we use an external chirped mirror-based accessory to deliver an adjustable amount of prechirp," said Arrigoni. "Here multiple bounces of the output beam on the mirrors result in the desired amount of prechirp, adjusted by selecting an appropriate number of bounces of the fixed GVD mirrors."

Pulse shape

Advances in pulse shaping and coherent control of broadband pulses have been key to some of the recent achievements in ultrafast spectroscopy on biomolecular complexes.

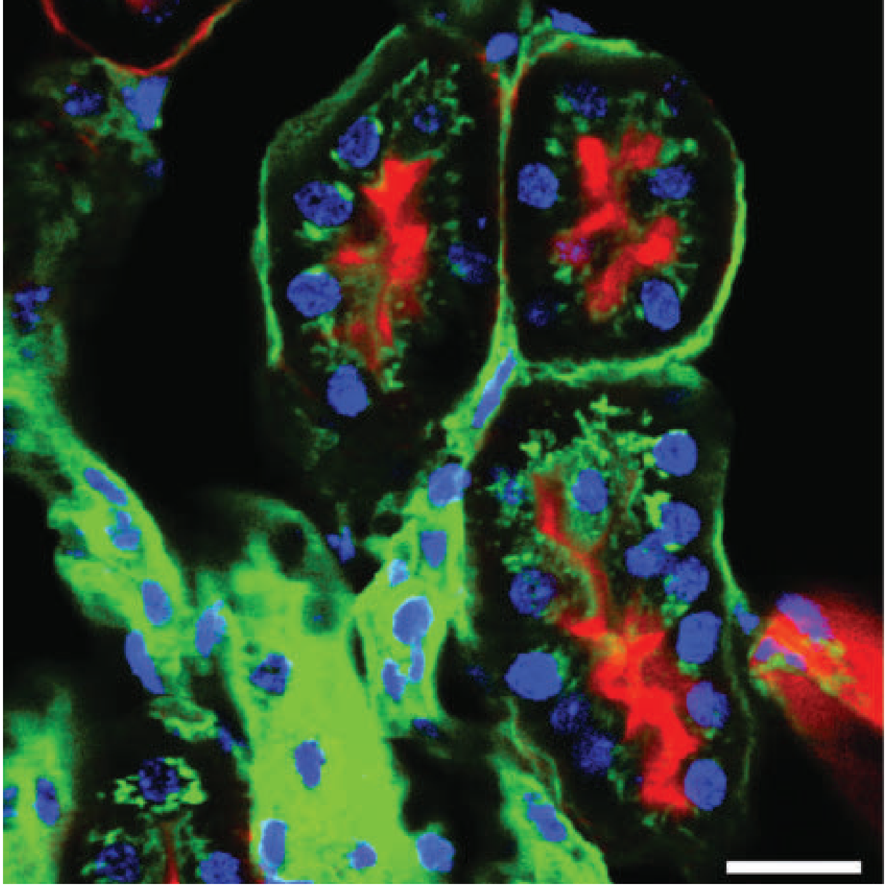
"For nonlinear multiphoton microscopy, peak power is important; thus pulses need to be Fourier-limited in the focus, which requires optimized dispersion control," van Hulst said. "For STED [stimulated emission depletion], control of both spatial and temporal overlap is mandatory."

Adaptive optics can be used to enhance peak intensity by shaping the spatial, temporal and spectral characteristics of the pulse, revealing never-before-seen images, as well as achieving ultimate precision manufacturing.

"With femtosecond pulses, we enter the next order of magnitude in terms of precision at microprocessing," said Dr. Max Kahmann, product manager of TruMicro at Trumpf GmbH in Ditzingen, Germany. "Especially processes where any kind of thermal influence is extremely critical, femtosecond pulses open the field. Some materials (e.g., nitinol) are very sensitive in terms of achieved quality and precision regarding the pulse duration."

While peak intensity depends on the pulse shape, results are further complicated by the type of material in which the beam is incident, as well as by the application. "The results may also depend on how steep the intensity distribution over time is, especially in the beginning of the pulse," Kahmann said.

Proper spatial control allows users to retrieve ultrafast dynamics of biological systems, as well as to enable better imaging through scattering media – a technique that has, for example, greatly improved retinal imaging. However, leaked power – unwanted energy applied outside of the pulses – poses a significant challenge. This can be a problem, and it is important to suppress.



Two-photon excited fluorescence (TPEF) microscopy image of a mouse kidney section, stained with Alexa Fluor 488 WGA, Alexa Fluor 568 phalloidins and DAPI. Excitation source: sub-10-fs pulses from a Ti:sapphire laser oscillator and adaptive pulse compression. TPEF signals were detected in epi-direction by a spectrally resolved multichannel photomultiplier tube. Scale bar is 20 μm. Courtesy of Ilyas Saytashev, Dantus Research Group, Michigan State University.

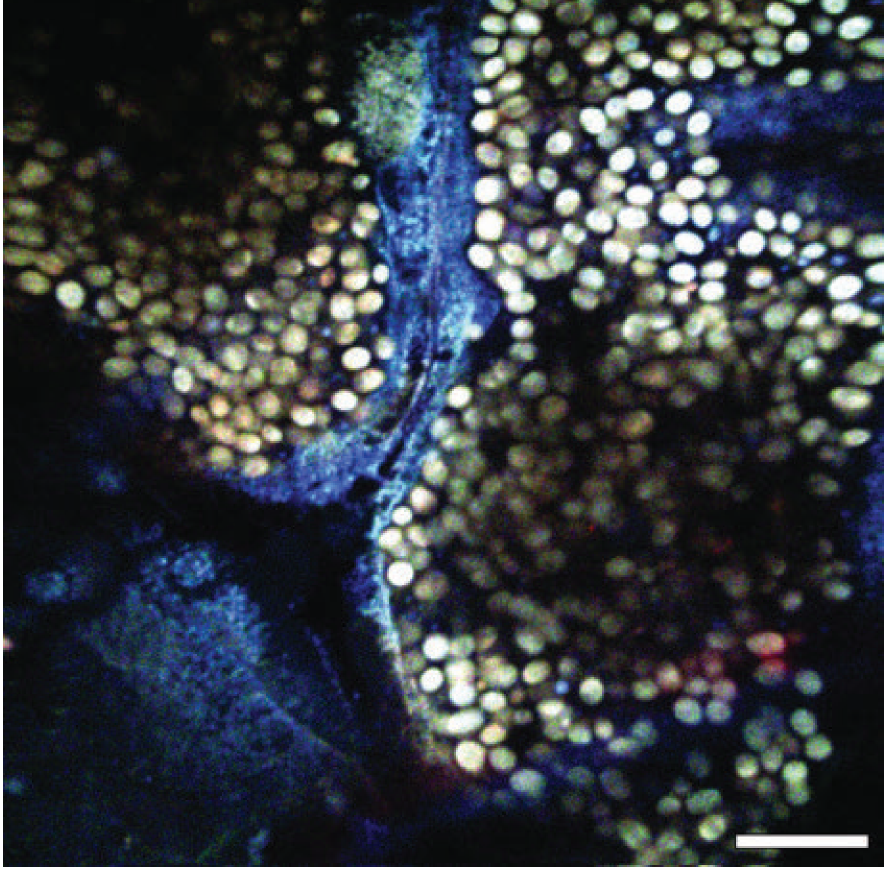
Wavelength

While wavelength is not necessarily a parameter that should be controlled, it is important to choose the correct wavelength according to the application.

"The wavelength gives the Rayleigh length, as well as the smallest possible beam diameter size. Further, the absorption behavior might depend on the wavelength for different materials," Kahmann said.

While continuous-wave sources lase at a single wavelength, modern pulsed lasers are generally mode-locked, meaning they lase over a broader band of colors with a range of wavelengths simultaneously circling the laser cavity. The modes of all these colors are in phase (i.e., mode-locked) and, as a result, in the time domain the laser is pulsed.

For example, the titanium-sapphire laser operates within the 10- to 20-nm bandwidth with around 100-fs pulses. Modern broadband lasers operate within



Multimodal microscopy image of an unstained, undissected *Drosophila melanogaster* RFP mutant larva. TPEF from fat cells pseudocolored in yellow; second-harmonic generation (SHG) from fibrous tissue signal shown in blue. Excitation source: sub-10-fs pulses from a Ti:sapphire laser oscillator and adaptive pulse compression. Scale bar is 20 µm. Courtesy of Ilyas Saytashev, Dantus Research Group, Michigan State University.

the 100- to 200-nm bandwidth with around 10-fs pulses.

"First, such pulsed lasers typically have ~10^5 times higher peak power for the same average ("CW") [continuous wave] power, which is important for nonlinear applications: multiphoton microscopy, optical sectioning, etc.," van Hulst said. "Second, the short pulses allow [users] to study dynamic processes on [the] femtosecond-nanosecond timescale: energy transfer, quenching, lifetime. Finally, the broad bandwidth allows coherent control of the excitation and thus certain energy pathways."

When it comes to imaging, spectral control is used for functional imaging and enhancing contrast; it currently is being used to determine the margins of cancerous tumors.

"Adaptive spectral control is used with broadband femtosecond laser pulses to selectively excite some chromophores but not others," Dantus said. "Spectral control is used to gain 'chemical' selectivity via multiphoton transitions or via vibrational excitation."

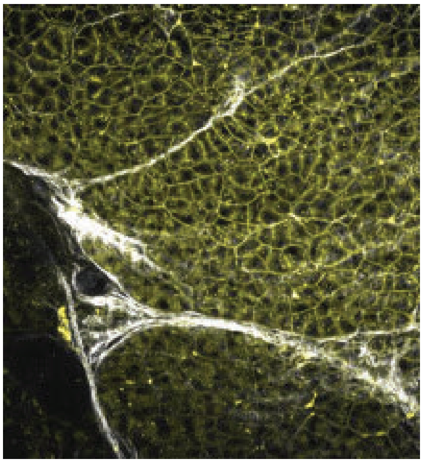
Other controllable laser parameters

Beam propagation – Using diffractive optical elements (DOEs), the intensity distribution in the focus plane, as well as along the beam axis, can be fitted to a specific application (e.g., creating multispot in the focus plane).

Repetition rate – The possibility of using only every second or third pulse can be important for cutting materials. Once the cutting edge radius gets smaller, the scanning speed of the laser has to be reduced to avoid overlap from one pulse to the next, which could cause adverse thermal heating effects.

While repetition rate and pulse energy can be controlled from pulse to pulse by external modulators, pulse duration and pulse shape can only stay constant if the right laser technology is selected (e.g., fiber-amplified lasers, disk-amplified lasers).

"Other solid-state laser techniques (e.g., Q-switched slab lasers) allow [users] to adjust the repetition rate without an external modulator, but a change of the repetition rate changes the pulse duration and the pulse shape and thus the process parameters, like the intensity, and it further does not enable you to control the pulse energy (without an external modulator)," Kahmann said.



An example of the benefits of next-generation dual-wavelength excitation lasers. Pancreas tissue was imaged using SHG at 1040 nm to show collagen surfaces and a Raichu-Rac biosensor expressed as a FRET probe, revealing cell crypts at 830 nm. Courtesy of Ewan McGhee and Kurt Anderson of Beatson Institute, Glasgow, Scotland.

Sophisticated pulse shaping of the future

The number of adjustable parameters, combined with the huge number of possible materials and applications for which ultrafast lasers may be used, leaves researchers with a mind-blowing number of options to explore. Currently, they are only at the beginning stages of this pursuit.

Highly sophisticated forms of pulse control, such as spectral phase and amplitude shaping, are being reported in a number of scientific journals. According to Coherent's Arrigoni, though, this makes more sense in conjunction with very short, broadband pulses (i.e., $<$20 fs). "At this stage, most biological applications of femtosecond lasers still rely on pulses longer than 50 femtoseconds, so sophisticated pulse shaping is not widely adopted as of now," he said.

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