

DPSS UV Lasers

Improve Mass Spectrometry and Laser Microdissection

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Diode-pumped solid-state UV lasers offer
several advantages over traditional gas lasers.

Nitrogen lasers have been used for more than 15 years and will no doubt continue to be important in many scientific and industrial applications. However, many applications are moving away from gas lasers to solid-state systems, which offer compactness, improved lifetime, lower cost of ownership, Gaussian beam properties, high repetition rates and increased pulse-to-pulse stability.

These characteristics are allowing pulsed diode-pumped solid-state (DPSS) ultraviolet lasers, in particular, to be used for some existing applications, and the technology will likely drive new applications in analytical chemistry, genomics, drug development and forensics. Matrix-assisted laser desorption/ionization (Maldi) mass spectrometry and laser microdis-

section already are benefiting from this technology.

In the Explorer DPSS ultraviolet laser, the output of the single-emitter laser diode is focused into the laser cavity to pump the Nd:YLF crystal (Figure 1). Except for the pump diode, all active and passive components are inside the cavity. Intracavity frequency-tripling offers intrinsic design advantages, such as high conversion efficiency, which leads to high pulse energy, short pulse widths and excellent pulse-to-pulse stability.

The fundamental wavelength of 1047 nm is frequency-doubled and then -tripled into the ultraviolet at 349 nm using non-linear crystals. The ultraviolet output has a Gaussian beam (TEM_{00}) with excellent beam properties and an M^2 of less than 1.3. The laser offers variable repetition

rates of up to 5 kHz and a pulse width shorter than 5 ns (Figure 2). The maximum average output power exceeds 120 mW. The standard pulse energy specification is either 60 or 120 μ J per pulse at a repetition rate of 1 kHz.

Lasers in Maldi

Maldi is a method used in analytical chemistry to determine the composition of unknown samples. The sample is vaporized and ionized using a pulsed ultraviolet laser. The molecular or fragment ions are separated in a time-of-flight (ToF) mass spectrometer based on the mass-to-charge ratio and analyzed to determine the concentration of the components in the original sample. In mass spectrometry, small molecules may be ionized directly, while larger molecules (proteins

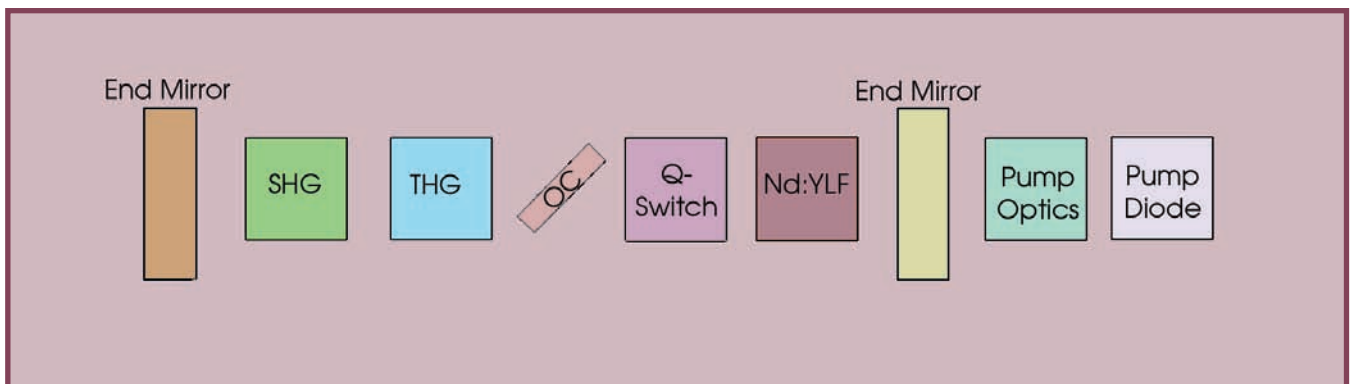


Figure 1. The laser cavity contains all active and passive components, except for the laser pump diode. (SHG = second-harmonic generator, THG = third-harmonic generator, OC = output coupler.)

and peptides) must be mixed with a matrix compound that aids the vaporization and ion formation process. Without this matrix, the number of ions generated would be too small for a conclusive analysis. Maldi traditionally uses nitrogen lasers at 337 nm, which are limited to a pulse repetition rate of less than 100 Hz. Demands for higher-throughput analysis have led users and systems suppliers to look to Q-switched DPSS lasers to increase the efficiency of Maldi-ToF mass spectrometry.

The higher repetition rate of DPSS lasers enables high-throughput analysis. For example, a variable repetition rate of up to 5 kHz means that the measurement time of the instrument is no longer limited by the laser but by the electronics of the analysis. Today, typical nitrogen laser repetition rates range between 10 and 60 Hz, and mass spectrometer technology can analyze up to 20 to 60 samples per second. But Bernhard Spengler of the Institute of Inorganic and Analytical Chemistry at Justus Liebig University

Giessen in Germany believes that the electronics can easily be adapted to accommodate 300 to 500 samples.

“High-throughput analysis is currently limited by electronics and mass analyzer technology, but we expect that in two or three years we will be able to do 500 or more mass spectra per second,” he said.

In addition, there are no power or wavelength issues when changing to a DPSS laser. The third harmonic of an Nd:YLF laser at 349 nm is close to the output wavelength of the nitrogen laser at 337 nm. The spectral absorption of the Maldi matrix is lower, but Spengler’s group has not seen a performance difference. The better focusability of DPSS lasers results in higher intensity at the ion formation threshold and greater possibility of multiphoton ionization.

“In our Maldi applications, we don’t see much difference in the ions generated per pulse,” Spengler said. He is working with a specific application of Maldi mass spectroscopy, called Maldi imaging, in which the mass analysis of the sample is performed pixel by pixel, enabling the creation of a high-resolution image of the sample mapping the local concentrations of components.

For this application, DPSS lasers are ideal because a high repetition rate allows the sample to be scanned at an appropriate speed, and good beam quality and focusability produce high lateral resolution. Spengler and his team focus the beam to 1 μm .¹ Most other Maldi imaging systems have a 50- μm or larger spot size.

The added stability of the DPSS laser also is important. “In order to get quantitative analysis in Maldi imaging, pulse-to-pulse stability is essential, since we need the differences we are seeing to correlate to the local concentrations, not to instabilities of the laser,” Spengler said. “We need high pulse-to-pulse stability over 100,000 shots or more for pixel-to-pixel analysis.” He has demonstrated that the better than 3 percent pulse-to-pulse stability of the Explorer laser is more than sufficient for this application.

The imaging technique can be used to optimize preparation techniques and to understand analytical processes. Spengler’s team prepared a color-coded concentration distribution of a mixture of three peptides from solution using a preparation technique commonly used for Maldi analyses.² The Maldi image of this preparation showed that, although the sample was homogeneously mixed in solution, the dried Maldi sample on the

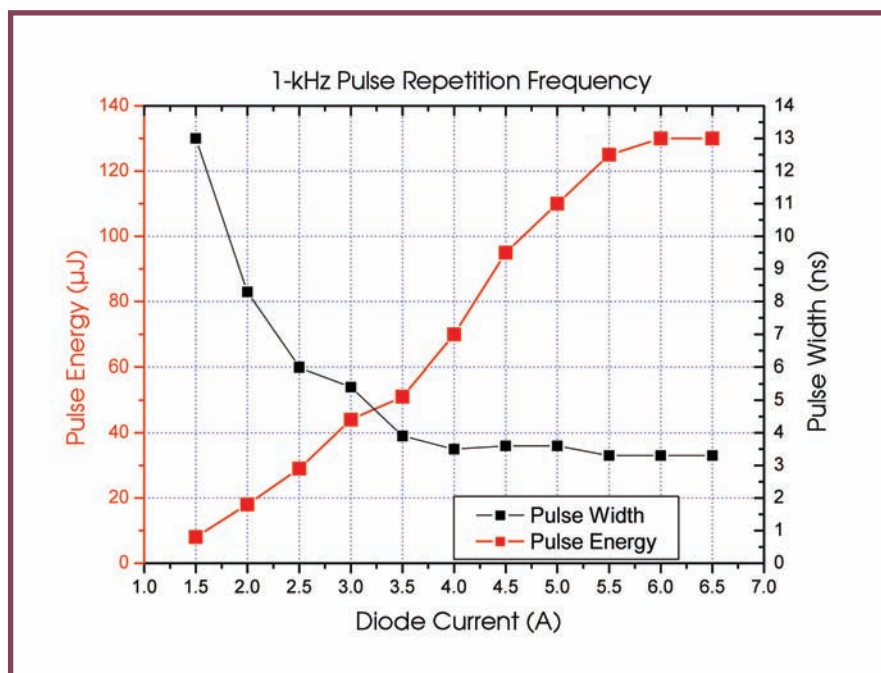


Figure 2. The laser provides variable pulse width and energy.

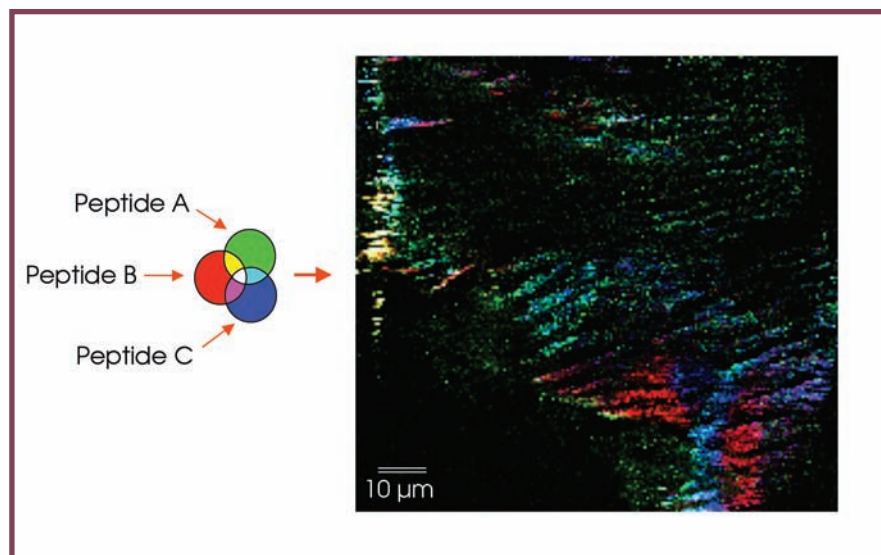


Figure 3. Maldi imaging can be used to optimize preparation techniques. This color-coded concentration distribution of a mixture of three peptides prepared from solution shows that the dried Maldi sample has demixed.

target was considerably demixed, or “segregated,” a tremendous problem for quantification studies (Figure 3). A perfectly mixed sample would have resulted in only white (=red+green+blue) pixels instead of red, green and blue areas.

DPSS lasers also offer an advantage for mobile environmental field analysis using Maldi-Tof mass spectrometry. Such analysis uses a small mobile mass spectrometer to analyze air particle samples to gain information on dust and haze in the atmosphere as well as bioaerosols. In the past, this analysis has been limited to inorganic compounds, but now laser-based mass spectrometry can analyze biological samples such as proteins and even bacteria and viruses. The compact, robust nature of the DPSS technology makes mobile mass spectrometry feasible, enabling potential applications in the areas of security and counterterrorism.

Laser microdissection

In laser microdissection, a laser is focused through a microscope to cut slide-mounted samples into smaller pieces, allowing cells to be removed. This type of removal is useful in areas such as oncology and microbiology and, when combined with DNA or RNA microarray analysis, it can study the genomic information of single cells.

Laser microdissection has been around since the mid-1970s. In its early years, it used nitrogen lasers whose repetition rates were on the order of 30 to 60 Hz and whose M^2 was close to 100. “The process was slow, due to the low pulse repetition rate of the nitrogen laser, and complicated by the poor beam quality of the laser,” according to Thomas M. Baer, adjunct professor of applied physics at Stanford University and founder of Arcturus Bioscience Inc. Ultraviolet lasers are used because they make the cleanest cuts. Now, pulsed ultraviolet DPSS lasers allow fast and precise cutting at the micron level. The beam quality, focusability and high repetition rate increases the speed of cutting and analysis, Baer said.

Pathology generally provides a large tissue sample that contains both normal and malignant cells. Laser microdissection can separate cancer cells from normal tissue for analysis. Dr. Dennis C. Sgroi

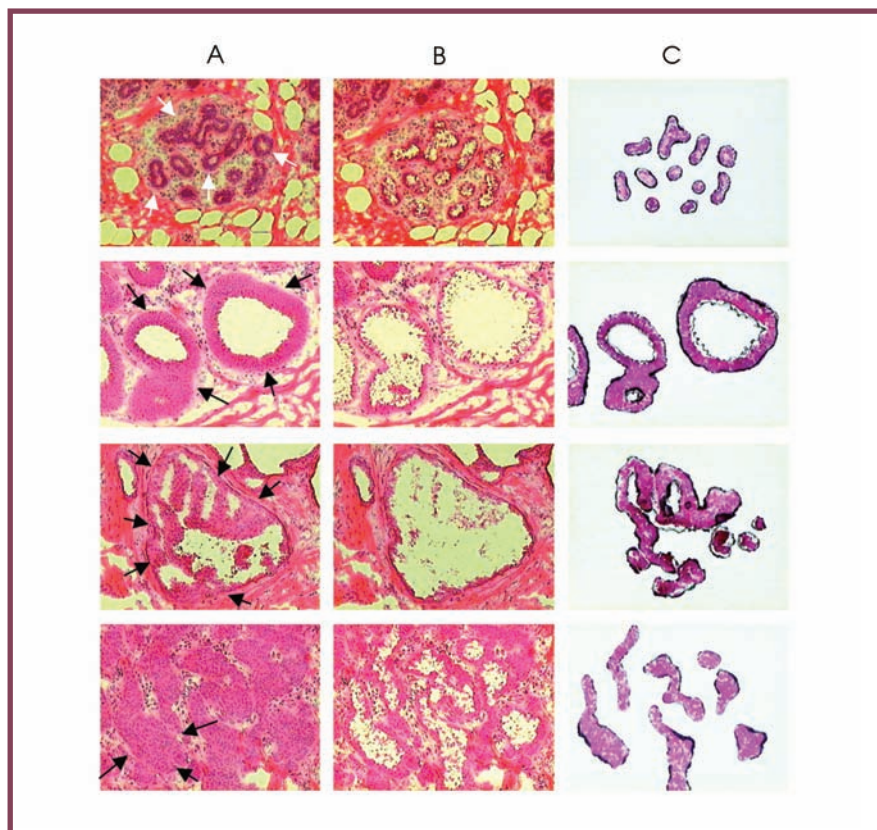


Figure 4. Microdissection was used to remove a single type of cancer cell. (A) shows the original samples, (B) shows them after the first laser microdissection, and (C) shows the removed cell.

of the department of pathology at Massachusetts General Hospital in Boston recently used laser microdissection and DNA microarray analysis to perform cellular-based gene expression profiles of breast cancer specimens to explore the changes associated with the stages of the disease (Figure 4).³ Microarray analysis of the dissected cells proved that there are three distinct stages of breast cancer and identified the gene transcription signature of each.

Although nitrogen lasers remain a valuable technology in the biomedical market for pulsed ultraviolet laser applications, Q-switched ultraviolet DPSS lasers, with their solid-state characteristics and higher performance parameters, will continue to drive improvements in traditional and emerging applications. Their beam quality, high repetition rates and improved pulse-to-pulse stability have enabled researchers to explore applications in bio-

chemical analysis and biomedical technologies. □

Meet the author

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References

1. B. Spengler et al (June 2002). Scanning microprobe matrix-assisted laser desorption ionization (SMALDI) mass spectrometry: Instrumentation for sub-micrometer resolved LDI and MALDI surface analysis. *J. AM. SOC. MASS. SPECTROM.*, pp. 735-748.
2. W. Bouschen, O. Schulz, B. Spengler, manuscript in preparation.
3. Xiao-Jun Ma et al (May 13, 2003). Gene expression profiles of human breast cancer progression. *PNAS*, pp. 5974-5979.



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Introducing the Spectra-Physics Explorer™ low power, actively Q-switched diode-pumped solid state UV laser at 349 nm delivering up to 120 μ J of pulse energy. This innovative new laser delivers the high pulse repetition rates and high peak power essential for meeting today's and future needs of demanding MALDI and Laser Microdissection applications. Designed for reliability and versatility, the Explorer offers many key advantages over traditional nitrogen and passively Q-switched UV DPSS lasers, including:

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- Pulse-to-pulse stability of <3% for improved signal-to-noise ratio
- Short pulse widths and high peak power

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