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 microdissection 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 nonlinear 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 µJ 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...
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. Most other Maldi imaging systems have a 50-µm or larger spot size.

The imaging technique can be used to optimize Maldi 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. The Maldi image of this preparation showed that, although the sample was homogeneously mixed in solution, the dried Maldi sample on the
target was considerably demixed, or “seg-
regated,” a tremendous problem for quan-
tification 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 analy-
sis uses a small mobile mass spectrome-
ter to analyze air particle samples to gain
information on dust and haze in the at-
mosphere as well as bioaerosols. In the
past, this analysis has been limited to in-
organic compounds, but now laser-based
mass spectrometry can analyze biologi-
cal samples such as proteins and even bac-
teria and viruses. The compact, robust
nature of the DPSS technology makes
mobile mass spectrometry feasible, en-
abling potential applications in the areas
of security and counterterrorism.

Laser microdissection

In laser microdissection, a laser is fo-
cused through a microscope to cut slide-
mounted samples into smaller pieces, al-
lowing cells to be removed. This type of
removal is useful in areas such as oncol-
ogy and microbiology and, when com-
bined with DNA or RNA microarray analy-
sis, 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 cut-
ting and analysis, Baer said.

Pathology generally provides a large tis-
tue sample that contains both normal
and malignant cells. Laser microdissec-
tion can separate cancer cells from nor-
mal tissue for analysis. Dr. Dennis C. Sgroi
of the department of pathology at
Massachusetts General Hospital in Boston
recently used laser microdissection and
DNA microarray analysis to perform cel-

lar-based gene expression profiles of breast cancer specimens to explore the
changes associated with the stages of the
disease (Figure 4). Microarray analysis of
the dissected cells proved that there are
three distinct stages of breast cancer and
identified the gene transcription signa-
ture of each.

Although nitrogen lasers remain a valu-
able 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 qual-
ity, high repetition rates and improved
pulse-to-pulse stability have enabled re-
searchers to explore applications in bio-
chemical analysis and biomedical tech-
nologies.

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Boost Your Throughput

Introducing the Spectra-Physics Explorer™ low power, actively Q-switched diode-pumped solid state UV laser at 349 nm delivering up to 120 μJ 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:

- Pulse repetition frequencies up to 5 kHz for higher throughput
- Beam properties optimized for high sensitivity and high spatial resolution
- Pulse-to-pulse stability of ~3% for improved signal-to-noise ratio
- Short pulse widths and high peak power

And, the Explorer’s compact laser head and flexible power supply ensure straightforward integration, allowing you to directly apply the standard triggering schemes for nitrogen lasers and more. All in all, the Explorer is a high performance drop-in alternative to nitrogen lasers in your biomedical application.

To learn more about the Explorer, download a datasheet at www.newport.com/explorer or call 1-800-775-5273.