A compact, low-power, high repetition rate (10 kHz) laser has been tested for use in MALDI in a conventional linear time-of-flight mass spectrometer (axial-TOF) and in an orthogonal-injection TOF instrument (QqTOF [1]). The diode-pumped, passively-Q-switched Nd:YAG laser (NanoUV-355 from Uniphase) produces about 0.25 $\mu$J per pulse at a wavelength of 355 nm. The pulse width is less than 700 ps, and the laser operates at a frequency of about 10 kHz. The laser head is about 20 cm long by 4 cm square.

The most common laser used for MALDI is a nitrogen laser (Laser Science ND337) with about one thousand times more energy per pulse, and with a controlled repetition rate up to 20 Hz. Under typical conditions, this $\text{N}_2$ laser produces $>10^5$ ions per laser shot so that analog detection with a transient recorder (TR) is necessary; with MCP detectors this data rate produces detector saturation. The use of a lower power laser focused to a smaller spot may potentially produce fewer ions per shot and may introduce the possibility of using a multi-anode detector with a TDC to reduce noise level and improve resolution. Operation at high repetition rate offers compensation for reduced signal intensity from individual shots. Furthermore, some applications, particularly post-source decay (PSD) MALDI, require thousands of laser shots to produce useful spectra. In such cases, a high repetition rate is clearly desirable.

To reach an acceptable fluence for MALDI, the laser was focused outside the spectrometer with a 10 mm focal-length lens. Two 100 mm focal-length lenses, arranged in infinite conjugation, were used to image the focused light onto the target. The resulting spot size on the target was approximately 30 $\mu$m in diameter, and the fluence per pulse was on the order of 100 J/m$^2$.

Pulse-counting with a single channel TDC was performed on an axial TOF instrument to determine if the number of ions per pulse is compatible with such a system, but useful spectra of peptides and proteins were not obtained. Although the average number of ions per pulse is quite small, it is likely that most of the ions were produced in a small fraction of the laser pulses. A single-anode detector is therefore not suitable for pulse-counting methods with this laser, but the possibility of using a multi-anode detector with a TDC has not been excluded.

Better results were obtained with an integrating transient recorder (TR), but even this required considerable trial and error, because at 10 kHz the sample was quickly exhausted. Fig. 1 shows a spectrum acquired in one second although in principle much less time is required since a only small fraction of the many recorded shots contribute useful data. The unusual spectrum of cytochrome C shown in Fig. 2 illustrates the problem of including many shots which contain only noise; the spectrum is shown with a large constant background subtracted. The heaviest protein observed with this technique (using 15 kV acceleration) was Lysozyme (~14 kDa).

Much more promising results were obtained in the QqTOF instrument. The injection pulse rate in this instrument is similar to the laser repetition rate, so ideally the average number of ions would be similar to the data obtained with axial TOF. But the QqTOF introduces considerable attenuation and perhaps more importantly smooths out the variation in the number of ions per pulse and allows a 4-channel TDC to be used. Fig. 3 shows the spectrum of a mixture of
peptides, which was acquired in approximately 30 seconds with comparable ease and reproducibility to that of the 20 Hz nitrogen laser and with comparable count rate and sensitivity. Fig 4 shows a spectrum of cytochrome C without background subtracted; the highest protein observed was trypsinogen (~ 24 kDa). In this mass range the nitrogen laser performs somewhat better, but for both lasers the performance is limited by the low 10 kV acceleration.

The nanolaser in its present form is already competitive with the usual nitrogen laser in the orthogonal geometry. However, a modest increase in power would provide significantly higher count rates than are possible with nitrogen lasers, and for significantly less expense than conventional high repetition rate Nd:YAG lasers. Acquisition times for MS/MS measurements in the QqTOF instrument, while significantly shorter than PSD measurements in the axial geometry, are still limited by the rate of ion production. For weak precursor ions, a higher count rate could reduce acquisition times from minutes to seconds.

The nanolaser also has potential advantages for imaging applications because a microscopic beam spot can be produced, while maintaining the same count rate. Even in an axial TOF instrument, the faster spectrum acquisition may be exploited if higher power and better control of the repetition rate is achieved.

References