Capillary Electrophoresis

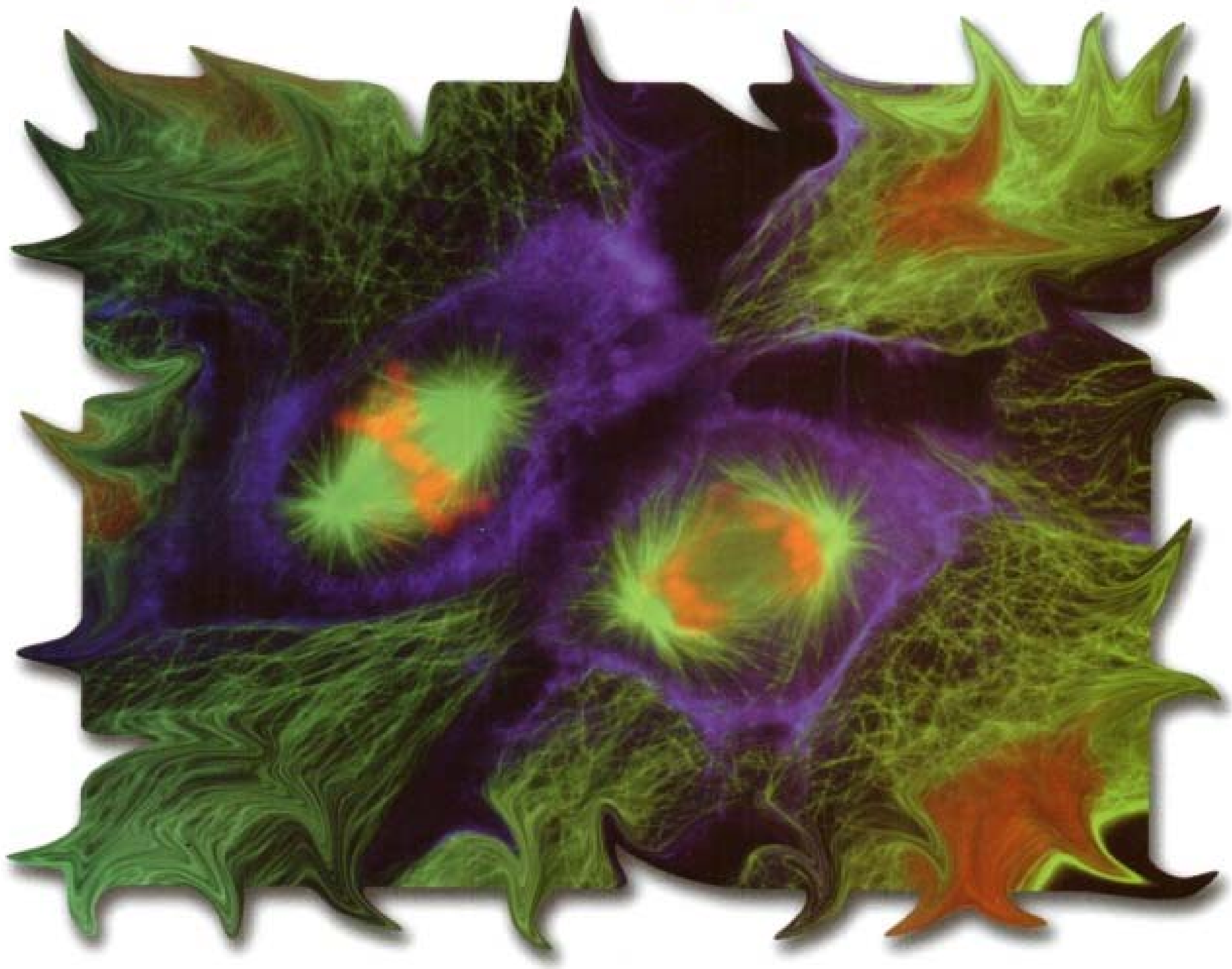
A Laurin Publication

BIOPHOTONICS INTERNATIONAL®

Photonic Solutions for Biotechnology and Medicine

August 2006

Core Microscopy Facilities



Spectroscopy

Novel femtosecond light source aids biosensing

Yb:KGW laser emits a second harmonic at ~520 nm and 180-fs pulses

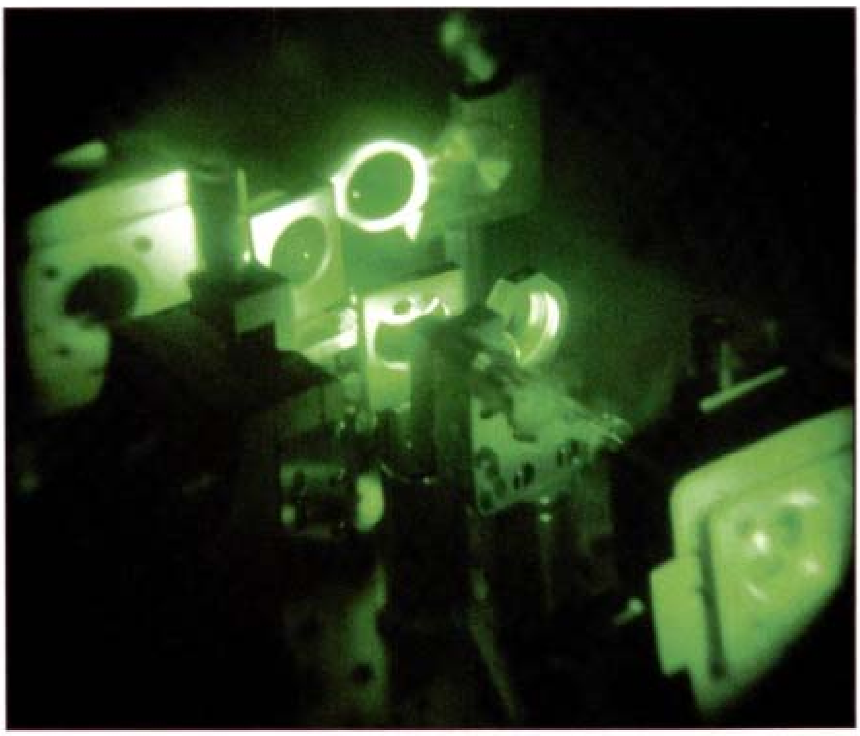
An ideal laser for fluorescence lifetime systems should have a repetition rate of several megahertz, a pulse duration much shorter than the fluorescence decay of the dye and the appropriate operating wavelength. That combination has proved challenging to achieve.

Now researchers at the University of Toronto and at the University of Toronto at Mississauga, both in Ontario, Canada, have developed a femtosecond laser — based on an ytterbium-doped potassium-gadolinium-tungstate crystal — that fits these requirements. The Yb:KGW laser could act as the source in tools for clinical diagnostics, environmental or food safety monitoring, biowarfare agent detection, veterinary testing or other applications.

"The ultimate goal is to use this technique as a platform for rapid, reusable, sensitive and selective nucleic acid diagnostics," said Paul A.E. Piunno of the University of Toronto at Mississauga.

Piunno and Sergei Musikhin of the Mississauga campus developed optical DNA biosensors in conjunction with the laser. The team also included postdoctoral researcher Arkady Major of the University of Toronto, who developed and characterized the laser system.

According to Major, the goal was to build a reliable and cost-effective ultrashort pulse laser that could provide green illumination. Those characteristics would enable the construction of a DNA biosensor based on the fluorescent label ethidium bromide, or any other dye that also absorbs in the green. The fluorophore has a fluorescence lifetime of ~1.5 to 2.0 ns when free in solution, but the lifetime climbs to ~21 ns when the dye is bound to double-stranded nucleic acid structures. That difference is easy to detect and acts as a signal.



Researchers have designed an Yb:KGW laser. An infrared image of its laser head is shown.

However, widely available lasers do not meet the illumination requirements. Dye lasers have repetition rates of a few hertz and nanosecond pulse durations, so they are too slow and the pulses too long for rapid and accurate fluorescence lifetime measurements. On the other hand, femtosecond lasers have short enough pulses, but they typically operate with repetition rates of 100 MHz. Rapidly repeating pulses can excite the dye and other molecules before they have a chance to relax to a ground state, thereby potentially throwing off the fluorescence lifetime determination.

Because ultrashort pulses can be frequency-doubled using a nonlinear crystal, the researchers wanted a laser with a fundamental wavelength of about 1 µm. They used Yb:KGW instead of perhaps better known alternatives because it is pumpable by telecom-grade laser diodes operating at 970 to 980 nm. Moreover, Yb:KGW supports 100-fs pulses at a wavelength of just over 1 µm.

The crystal selection led to a laser with significant manufacturing advantages. "Its design is very straightforward and takes advantage of commercially available long-lifetime telecom components and off-the-shelf high-quality laser optics. The construction was simple," Major said.

To decrease the repetition rate, the researchers extended the laser cavity, a technique that had the advantage of less complexity and lower cost than other methods.

To do this, they constructed two optical telescopes, using mirrors to fold their length. They extended the cavity up to 16 m per round-trip, resulting in a 15-MHz repetition rate. The 67 ns between two consecutive pulses were longer than the 20-ns fluorescence lifetime of bound ethidium bromide.

The pulses were about 180 fs in duration and centered around 1042 nm. The scientists sent them into a nonlinear crystal, producing a second harmonic at ~520 nm, which is the excitation peak of the fluorophore. The work is detailed in the June 12 issue of *Optics Express*.

To demonstrate in a preliminary feasibility study that the laser could be used as a source for optical detection of DNA, the researchers utilized a setup with focusing and collecting optics, a Hamamatsu photomultiplier and a Becker & Hickl single-photon counting device.

The frequency-doubled pulses entered a solution containing ethidium bromide and double-stranded 20-mer oligonucleotides in one case, and just the fluorophore in the other. The fluorescence lifetime in the bound case, the one with the DNA, was ~20 ns, while in the other case, the lifetime was ~2 ns.

With the feasibility shown, the researchers are working on an optical DNA sensor based on total internal reflection. In these studies, the sensor consists of a prism whose base has been functionalized with a DNA sequence; for example, one that could act as a diagnostic for a degenerative disease.

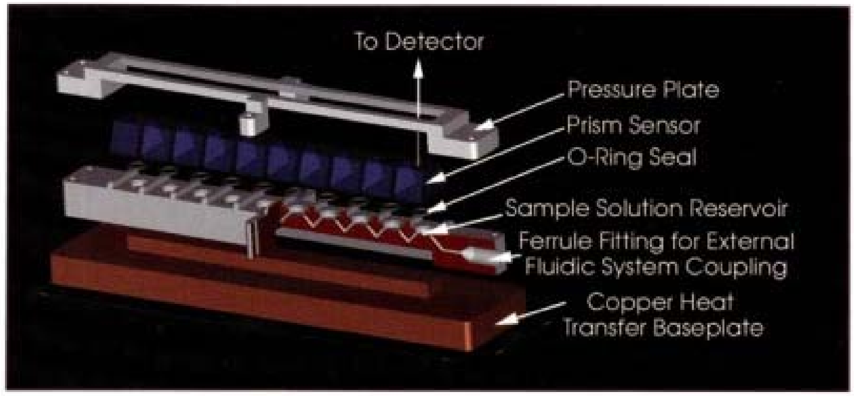
The sample DNA, which in the demonstration would be complementary to the oligonucleotides attached to the prism, would flow past this surface in a microfluidic device, along with an equivalent concentration of ethidium bromide. The change in fluorescence lifetime indicates whether the fluorophore is bound by the DNA or not, and that binding also can be switched off by heating the fluid using a temperature-controlled plate in the device. This work is ongoing.

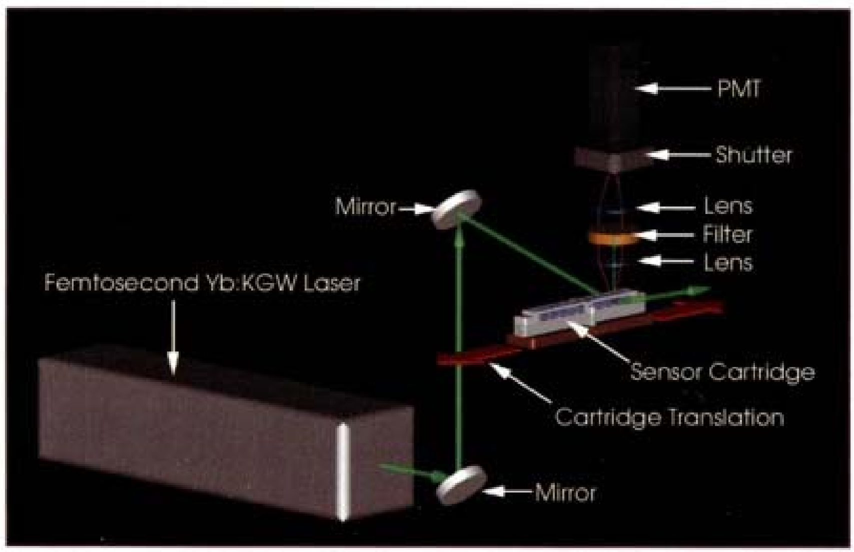
The group also is working on further improvements in which more miniaturization of the sensor elements is one of the goals, but Piunno reported that this was not the only goal. "A microfluidic construct is being explored while the optics of the system are being reworked so as to provide for higher-sensitivity detection," he said.

The laser also could be used in a variety of other applications. Major noted that the laser emits at 1 µm, which is longer than the 800 µm of Ti:sapphire femtosecond lasers. That longer wavelength reduces scattering by and increases penetration depth of biological tissue. It also produces a third harmonic that is not in the deep ultraviolet.

"[The Yb:KGW] is a good alternative to commonly used Ti:sapphire lasers," Major said. □

Hank Hogan





The Yb:KGW laser could be useful in biosensing. The design of the sensor cartridge is shown above and the layout of the experiment below.

Lymphatic origins in the optically clear zebra fish

Two-photon time-lapse imaging shows lineage of lymphatic cells

An important step in tumor progression is the incorporation of blood vessels to supply the tumor with blood from the host. Researchers are working to find a way to stop the growth and progression of tumors by interfering with that step. This requires understanding how vessels develop and grow. Because recent evidence shows that tumors use the lymphatic system to spread to secondary locations, knowing the lymphatic vessels' origin could help in the development of tumor-attacking drugs as well as in the promotion of healthy blood vessel growth.

The foundation of the lymphatic system has been difficult to study because available animal models, such as mice, cannot provide optical images with high resolution. Brant M. Weinstein and his