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Laser Sources for Confocal Microscopy

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INTRODUCTION

Laser assisted confocal microscopy has made a lot of progress over the past few years. Laser systems have become more modular and compact. There is an ever-increasing number of available laser excitation lines as well as an improvement in user friendliness and ease of use. At the same time, expansion of web resources has provided easy access to a wealth of information. Our goal is both to aid the experienced and novice microscopist in quickly locating and sorting through the relevant laser information and to provide a means of avoiding common problems and pitfalls in the use of laser excitation in the various fluorescence techniques such as fluorescence correlation spectroscopy (FCS), fluorescence lifetime imaging microscopy (FLIM), fluorescence loss in photobleaching (FLIP), fluorescence recovery after photobleaching (FRAP), optical coherence tomography (OCT), second harmonic generation (SHG), single molecule detection (SMD), and single particle tracking (SPT). In this chapter we describe the characteristic properties of a number of lasers commonly used in fluorescence microscopy. We concentrate on the characteristics of lasers in relation to their use as an illumination source for microscopy. Compared to other sources emitting electro-magnetic radiation, such as hot filaments, arc lamps, and light-emitting diodes (LEDs), lasers have a number of unique properties, which make them an almost ideal light source for use in confocal microscopy. These properties are:

- high degree of monochromaticity
- small divergence angle
- high brightness
- high degree of spatial and temporal coherence
- plane polarized emission (for many types)
- a Gaussian beam profile (in some cases this requires special optics).

In the 40 years since the realization of the first experimental laser, a wide and still rapidly expanding variety of lasers has been developed. Currently very rapid development of miniaturized, easy-to-use, tunable “pocket” lasers is taking place. These small convenient lasers are in the process of replacing many of the large laser systems still in use.

Available laser systems cover an extremely wide range, differing from each other in physical size, principle of operation, and optical, temporal, and mechanical properties, such as beam diver-

gence, output power, polarization properties, duty cycle, stability of the beam, and vibration sensitivity. These characteristics are related to the mechanical design, emission wavelengths and tunability, ease of operation, maintenance costs, reliability, and safety aspects. This chapter introduces the microscopist to the operation of the laser, the most important laser parameters, their influence on the quality of the confocal image, and methods to create wavelength-tunable light sources. In addition, laser systems for second harmonic generation and optical tweezers are described.

LASER POWER REQUIREMENTS

First, we need an order of magnitude estimate of the emission intensity that can be obtained in fluorescence microscopy using 1 mW of input light. The amount of laser power needed depends crucially on the quantum efficiency of the contrast medium being studied. The most common contrast factors are sample fluorescence and backscatter.

It is convenient to express the quantities in terms of photons/(s * pixel * mW) of incident light at a given wavelength because the intrinsic dark noise of modern detectors is often specified in similar units. Also, expressing the flux per pixel provides a quantity that is independent of the illuminated area. The following are useful relationships:

- Energy of one photon: $h\nu = hc/\lambda = 4 \times 10^{-19} \text{ J}$ at $\lambda = 500 \text{ nm}$
- 1 mW of light intensity at 500 nm represents 2×10^{15} photons/s

On a widefield image of 1000×1000 pixels, 1 mW of incident light, uniformly distributed, is equivalent to

- flux per pixel = 2×10^9 photons/(s * pixel * mW) at 500 nm

There are two considerations. First, how many photons will be emitted per pixel? Secondly, how many photons can be tolerated per pixel before saturation of the fluorescent molecules occurs?

Let us analyze the first question. Using fluorescein, one of the most common probes, the molar extinction coefficient is about 100,000/cm of optical path. Assuming an effective optical path of about $1 \mu\text{m}$ (the depth of field), the molar extinction is about 10. The local concentration of fluorescein can vary, depending on the spatial location and the degree of labeling. Assuming that a

concentration of $10^{-5}M$ is reasonable, the optical density (OD) of a $1\mu\text{m}$ path length is $\approx 10^{-4}$. The number of photons absorbed is then

$$\text{photons absorbed} = (\text{flux per pixel}) \times (\text{OD}) = 2 \times 10^5 / (\text{s} * \text{pixel} * \text{mW}) \text{ at } 500\text{nm}.$$

Assuming a quantum yield of 0.8 and a collection efficiency of 10%, the detector receives

$$\text{photons at the detector} = 1.6 \times 10^4 / (\text{s} * \text{pixel} * \text{mW of incident light}).$$

Given the quantum efficiency for a good detector (10% at 500nm), the final detected photon flux should be about

$$\text{flux detected} = 1600 \text{ photons} / (\text{s} * \text{pixel} * \text{mW of light}).$$

This flux should be compared with the dark noise of most detectors, which can vary between 10 and 100 equivalent photons/(s * pixel). In our estimation, the only quantities that can vary over a wide range are the power of the laser and the effective concentration of the probe. Lasers can have up to watts of power and the concentration of the probe can be higher than we have assumed. The efficiency of detection is usually smaller than we estimate and the noise can be larger. The purpose of our calculation is to give a rough idea of the kind of power that a laser must furnish to be usable for fluorescence detection in confocal laser scanning microscopy (CLSM). Tsien and Waggoner (Chapter 16, *this volume*) find an optimal power with the best signal-to-noise ratio (S/N) with respect to autofluorescence and elastic and inelastic scattering of $76\mu\text{W}$ at 488nm and $590\mu\text{W}$, as long as triplet formation is neglected. Therefore, a laser power of 1 to 2mW spread over 10^6 pixels at the specimen position should be more than sufficient for most applications. Effectively, 10 to $100\mu\text{W}$ is common in confocal. Assuming a 10% optical path efficiency a laserhead output power of $>\sim 1\text{mW}$ suffices.

There are two different types of saturation effects. One is related to the number of molecules that can absorb light in a given area for a certain incident flux. In a given pixel, assuming a volume of $1\mu\text{m}^3$, the volume is 10^{-15}L . At a molar concentration of 10^{-5} , we should have approximately 6000 molecules/pixel. Since the number of photons absorbed per milliwatt of incident light is about $2.5 \times 10^{15}/\text{s}$ on a single pixel in widefield, each molecule is excited about 40 times per second. From the photophysical point of view, the decay of fluorescein (and in general any singlet single state decay) is very fast ($4 \times 10^{-9}\text{s}$), so that the ground state should be repopulated very rapidly. However, in the confocal microscope for a pixel dwell time of about $1\mu\text{s}$, the $40 \times 4\text{ns} = 160\text{ns}$ dead time represents 16% of the pixel period.

There are many possible photochemical processes that are either irreversible or have a cycle time of several milliseconds to seconds. In this latter case, even if the quantum yield for these effects is very low (below 0.001), and the exposure time is on the order of seconds, molecules lost to the long-lived state will severely limit the overall peak excitation intensity that can be used before the output loses its linear relationship with the input. For quantitative microscopy this is the most important limitation. Hess and Webb (2002) found that their FCS data implied a non-Gaussian three-dimensional (3D) volume and distortion of the calibration of the excitation volume at a power level of 10 to $100\mu\text{W}$ at 488nm, for one photon Rhodamine Green excitation and 5 to 10mW at 980nm, for the two-photon case (Rhodamine Green or Alexa 488, Molecular Probes).

Having discussed the power requirements, we continue with a concise description of the basic elements of a laser, its principle

of operation, and other important practical aspects, such as heat removal and mechanical and optical stability.

In general, confocal microscopes work best at $<1\text{mW}$ of continuous wave (CW) beam power at the specimen and useful images of living cells have been made with $<100\text{nW}$ (see Chapter 19, *this volume*). When pulsed lasers are used for two-photon excitation it is important for the pulse frequency to be high enough (80–100MHz) so that many pulses occur during the 1- to 4- μs pixel dwell time. The intensity instability of this light **when it reaches the specimen** must be small compared to the statistical uncertainty that will occur when the signal photons are detected; if the signal level is only 100 photons/pixel, then Poisson statistics will limit accuracy to 10%, while 10^4 detected photons will yield 1% accuracy.

THE BASIC LASER

The acronym *laser* stands for **light amplification by stimulated emission of radiation**. Laser action, that is, the emission of coherent radiation, has been observed for a large number of different media, but all lasers have several features in common (see Fig. 5.1).

- **Active laser medium:** Atoms, molecules, and atomic ions in pure or mixed gases, vapors, liquids, or solids, confined to the gain medium volume where they can be excited by an external source of energy.
- **Excitation source:** An external source of energy used to pump the laser medium. This can be another laser, arc lamp or flash lamp, electron beam, proton beam, electrical current, electrical discharge or radio frequency (RF) excitation, etc. The choice of pump source is determined by the optical, thermal, and mechanical properties of the active medium and the wavelength region of interest.
- **Optical resonator:** The laser medium is enclosed between two parallel mirrors, which form a Fabry–Perot interferometer. The mirrors are placed at each end of the laser medium. One mirror, the high reflector (HR), totally reflects ($R = 99.9\%$); the other mirror, the output coupler (OC), only partially reflects. To a first approximation, the ratio of these two reflectivities is the gain of the cavity that they form. For instance, if the reflectivity of the OC is 95%, on average a given photon will pass through it 1 time in 20 and, in the absence of other loss mechanisms, the cavity gain will be 20. In small semiconductor lasers the physical polished sides of the devices may act like

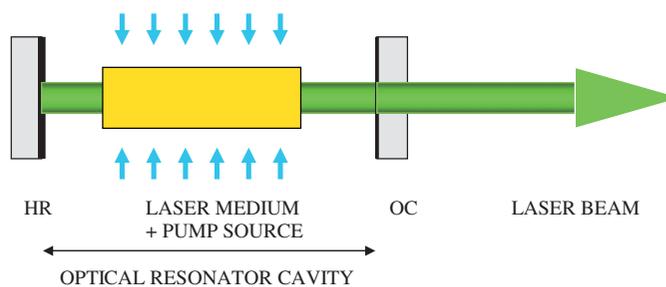


FIGURE 5.1. An optical resonator is formed between a highly reflective mirror (high reflector, $R = 99.99\%$), HR, and a mirror with a reflectivity of, for example, 80%, the output coupler (OC). Within this resonator we find the active lasing medium (a crystal, semiconductor, liquid or gas) and its energy supply: the pump source, an arc lamp or flash lamp, another laser.

effective mirrors. Laser emission generated by electronic transitions that are relatively rare require cavities with higher gain.

Principle of Operation

A particle of the active laser gain medium becomes excited when it absorbs pump energy and goes to an excited level [Fig. 5.2(A)]. It then returns to the ground state via non-radiative relaxation processes and also by emission of radiation [Fig. 5.2(B)]. Under normal conditions, a Boltzmann equilibrium describes the population of the various energy levels: the higher the energy level the lower the population of that level. When an excited, metastable level with a long lifetime exists in the laser medium, energy will accumulate in this level. If the excitation is intense enough, the Boltzmann distribution normally present will “invert” for the population of this metastable state (i.e., there will be many more electrons in the excited state than Boltzmann would predict). In the laser cavity, photons emitted from this energy level will strongly interact with the population of the metastable level [Fig. 5.2(C)], forcing it to release energy and return back to the lower level [Fig. 5.2(D)]. This process is called stimulated emission of radiation, that is, the interaction of the light with the excited particle increases the likelihood that the particle will return to the ground state. The stimulated emitted light has a high degree of monochromaticity, because emission occurs from a well-defined transition. In addition, the photon that results from this stimulated de-excitation process is in phase with the electromagnetic wave traveling in the laser medium. As a result, the emitted radiation has excellent spatial and temporal coherence and is highly directional.

A convenient way to let the electromagnetic radiation interact with the laser medium is a resonant cavity (Fig. 5.1). At optical wavelengths, this is achieved by a Fabry–Perot type interferometer. Two plane-parallel mirrors, one highly reflective, the other semi-transparent, are separated by a distance equal to an integral multiple of half the lasing wavelength. Because the electromagnetic radiation interacts repeatedly with the laser medium, this resonant cavity increases the probability of stimulating de-excitation. It also provides the necessary feedback to make the emission grow coherently. Because the emission of the first photon going in precisely the correct direction to reflect back and forth in the cavity is a low probability event, getting the laser to fire is a chaotic phenomenon that exhibits a threshold effect; lasers won’t start or work stably below a certain output power level.

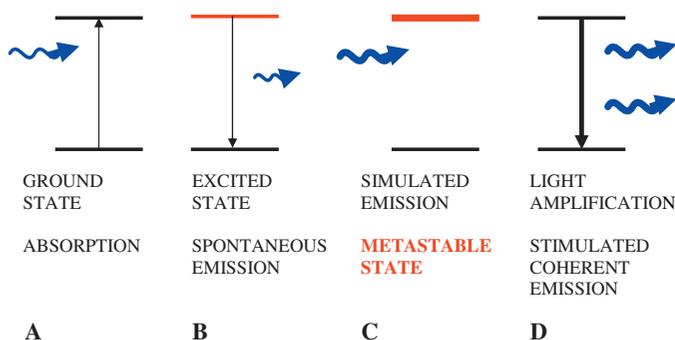


FIGURE 5.2. Optical (de)excitation processes. (A) Transition from ground state to excited state upon absorption of a photon, an electron moves to a more outward shell, timescale < femtoseconds. (B) Relaxation to a lower level, for example, ground state under spontaneous emission of a photon, electron returns to a lower level, timescale nanoseconds. (C) Light driven interaction inside laser gain medium of a photon with electrons in excited metastable state with a long lifetime (D) Stimulated coherent emission and light amplification.

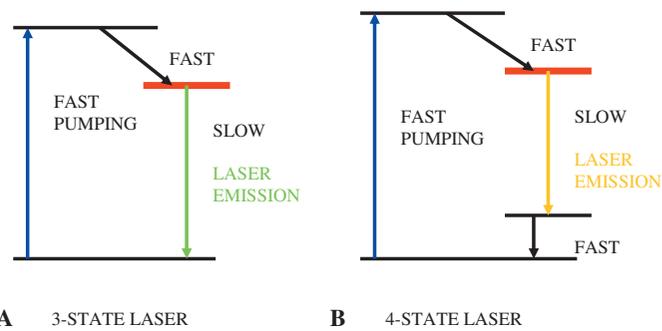


FIGURE 5.3. Fast non-radiative relaxation processes couple an excited state with a lower metastable energy level. Laser emission occurs in the gain medium from the metastable state. Dependent on the gain medium properties this laser process is described by a three-level or four-level lasing scheme.

So far we have only discussed a three-level laser, that is, ground-state, upper excited-state, and lower excited-state [Fig. 5.3(A)]. In a four-level laser [Fig. 5.3(B)], as in the helium-neon (He-Ne) laser, the population inversion can be obtained more easily (Arecchi and Schultz-Dubois, 1972). The titanium–sapphire (Ti:Sa) vibronic laser is effectively also a four-level laser due to its broad energy bands (see later section). Other improvements relate to the replacement of the Fabry–Perot mirrors by corner cube reflectors or crossed-roof prisms to increase mechanical and thermal stability. For further information, see Arecchi and Schultz-Dubois, 1972; Stitch, 1979; Bertolotti, 1983; Bass and Stitch, 1985; Kudryashov and Weber, 1999; Webb and Jones, 2004; Hodgson and Weber, 2005.

An interesting approach is the increasing use of optical fibers that act as both lasing medium and cavity at the same time; a very compact design is described later in this chapter.

Pumping Power Requirements

In order to sustain laser action, the gain of the optical resonator needs to be larger than the losses due to resonator walls and other optical elements. The minimum necessary pumping power, P , is proportional to ν^3 . This means that, as one shifts from the infrared (IR) through the visible (VIS) towards the ultra violet (UV), an ever increasing amount of energy is needed to obtain laser action. This limits the possible pumping mechanisms.

Laser Modes: Longitudinal (Axial) and Transverse

- **Axial or longitudinal modes:** Separated by a distance, L , the two plane-parallel mirrors of the Fabry–Perot interferometer cavity form an optical resonator. This separation distance can be long (meters, as in big frame ion lasers) or very small (micrometers, as in very compact diode lasers). A number of standing wave patterns each consisting of an integer multiple, m , of half wavelengths, $\lambda/2$, exists in an optical cavity of length L : $m = L/(\lambda/2)$. The frequency, ν , for the m th vibration along the long axis of the laser is, therefore, $\nu = mc/2L$, where c is the speed of light in the laser cavity. The frequency spacing between adjacent longitudinal modes is $c/2L$, that is, the inverse of the laser cavity round trip time. A very large number of longitudinal modes can exist in the laser cavity unless bandwidth-limiting devices such as Fabry–Perot etalons are installed in the cavity.

- **Transverse modes:** These modes vibrate perpendicular to the long axis of the laser and are known as transverse electromagnetic modes, TEM_{mn} , where m and n are integers describing the nodal points in the two directions perpendicular to the laser axis. For each transverse mode, many longitudinal modes can exist. (For an in-depth derivation, see laser handbooks by Arecchi and Schultz-Dubois, 1972; Stütz, 1979; Demtröder; 1996; Bass and Stütz; 1985; Hecht and Zajac, 2003; Silfvast, 2004). An adjustable diaphragm or pinhole inside the laser cavity is sometimes used to select the TEM_{00} lasing mode and control its intensity. No transverse modes exist in single (longitudinal) mode fiber lasers.

The TEM_{00} mode is desired for most light microscopy experiments. It has a Gaussian beam profile with no phase shifts across the beam. The maximum intensity is in the center of the beam, it has complete spatial coherence, and the smallest possible beam divergence. Only this mode can be focused to a diffraction-limited spot. Donut-shaped transverse modes TEM_{01} (TEM_{10} in overlap with TEM_{01}), such as produced by some helium-cadmium (He-Cd) lasers operating at 325 nm, are not desirable because they possess no intensity in the center of the beam. In this case, spatial filtering is necessary (see later section).

Polarization

The output of many lasers is linearly polarized with the polarization vector vertical.

- **Lasers with Brewster surfaces:** A convenient and inexpensive way to minimize reflection losses and to generate linearly polarized light is the installation of a Brewster surface (i.e., one that is tilted so that the normal of the plane and the incoming beam form a specific angle, θ_{Brewster}) at the end of the resonator. Horizontally polarized light incident on such a plane-parallel plate will be completely reflected. Vertically polarized light will be completely transmitted without any reflection losses (Fig. 5.4). In laser resonator cavities, this plate is usually a quartz or a fused-silica plate. In solid-state lasers, the semiconductor rod itself is sometimes cut at the Brewster angle to minimize reflection losses. In gas lasers, the exit

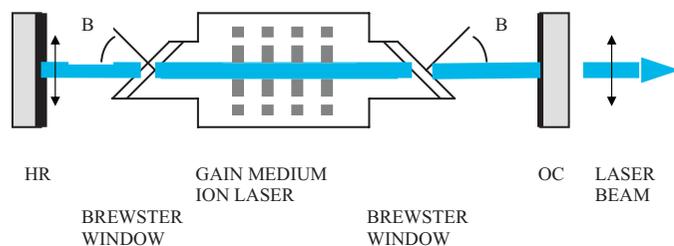


FIGURE 5.4. Emission of linearly (vertically) polarized laser light from an ion laser resonator cavity equipped with Brewster angle plasma-tube windows. Only vertically polarized light is amplified. It experiences no reflection losses at the Brewster angle: $B_{\text{Brewster}} = \tan^{-1}n$, with n the refractive index of the pure crystalline quartz window. Horizontally polarized light suffers reflection losses and stays below the lasing threshold. Windows normal to the optical axis of the laser would introduce reflection losses and could prevent the laser from operating at all or introduce a set of reduced length resonator cavities. Tungsten disks or BeO tubes with a central bore with or without focusing magnetic fields keep the lasing plasma volume centered in the cavity. Gas return paths are also provided in the disks. HR, highly reflecting mirror; OC, output coupling mirror.

windows are usually oriented at the Brewster angle to obtain vertically polarized light. Dust often collects on Brewster windows that point upward, damaging the coating. To operate properly, they must be kept absolutely clean using a dust cover and a slightly positive dry air or nitrogen pressure in the laser head. In the jet-stream dye laser, the jet is placed at Brewster angle in order to minimize losses due to reflection of the pumping beam, thereby maximizing the pumping efficiency. For the same reason, the tuning element of the dye laser is also placed at Brewster angle. Brewster surfaces can also be the origin of very dangerous reflections. Eye protection should always be worn when protective covers are removed from a laser.

- **Lasers built without Brewster windows** will still show some polarized output due to birefringence within several optical components. However, the plane of polarization may change with time and, even though the total intensity stays constant, should this light pass through a polarizer, large output intensity fluctuations may be observed.

Randomly polarized beams contain two orthogonal components but the phase and ratio of the two components vary rapidly in time, creating polarization noise. Dichroic mirrors, and, in fact, any mirror or lens surface, not at right angles with the incoming radiation will reflect vertically and horizontally polarized light differently (directions are taken with respect to the plane of incidence formed by the reflected and transmitted beams).

A convenient way to depolarize laser emission is to install a polarizer or polarizing beam-splitter and a 1/4-wave plate. When placed at an angle of 45° with respect to the incoming linear polarization, this 1/4-wave plate converts linearly polarized light into circularly polarized light. These waveplates are usually designed for a specific wavelength. Achromatic retarders, such as Fresnel rhombs, are sometimes preferred, but are quite expensive (Driscoll and Vaughan, 1977; Hecht and Zajac, 2003). Another advantage of this arrangement in optical microscopy is that it prevents reflected or backscattered light from reaching the detector. The reflected light passes through the 1/4-wave plate in such a way that, on return, it is blocked by the input polarizer because its plane polarization is now orthogonal to the main transmission direction of the input polarizer. Phase randomizing will briefly be discussed in a later section. For an in depth treatment see Chapter 31 by Harris and Delaney. In fiber lasers, optical isolators are inserted for the same reason, that is, to prevent backreflections. The polarization-independent isolator consists of a small cylindrical package containing a fiber pigtail, non-spherical collimating lenslet, a slightly wedged birefringent platelet, a magneto-optic Faraday rotator at 45° , exit birefringent platelet also at 45° , and refocusing lenslet followed by another fiber pigtail.

Coherent Properties of Laser Light

Laser beams illuminate objects coherently. The process of stimulated emission imposes coherence effects on the emitted laser light waves. All parts of the electromagnetic wave reach a crest that is, are in phase, at a given point in space at the same time. Spatial and temporal coherence is present. This coherence stretches for a certain distance and time and depends on the spectral width or pulse duration of the laser light. Beyond a certain distance or time interval synchronization differences arise. When a choice exists, a short coherence length laser should be used for CLSM or scrambling devices should be inserted into the optical path.

- **Temporal coherence:** The coherence time is the time interval during which light traveling over a certain distance maintains a phase difference of less than π (or 180°), $\tau_{\text{coh.}} = 1/\Delta\nu$.
- **Coherence length:** The path traveled by the wave during the coherence time is called the coherence length, $L_c \sim c/\Delta\omega$, where $\Delta\omega$ is the spectral width of the laser. Longitudinal coherence length $L_{\text{coh.}} = c/\Delta\nu$. Lateral coherence length, $l_{\text{coh.}} = \lambda/\phi$, with ϕ the beam divergence. For a typical gas laser, for example, a He-Ne laser with a bandwidth of 1.5 GHz, this leads to $L_{\text{coh.}}$ of ~ 20 cm. In single-mode operation with $\Delta\omega \sim 1$ MHz, $L_{\text{coh.}}$ is ~ 50 m. A diode laser with a spectral width of 0.1 nm at 780 nm possesses a coherence length of 1 mm. Dye lasers equipped with tuning elements, diode lasers, and other lasers such as the Ti:Sa systems, usually have bandwidths on the order of tens of gigahertz and an $L_{\text{coh.}}$ of a few millimeters or less. Depending on the type of line narrowing element installed, $L_{\text{coh.}}$ can be increased by a factor 10^3 to 10^6 . For practical confocal microscopy, a shorter coherence length is preferred to eliminate the influence from out-of-focus defects. For example, dust on semi-transparent surfaces, lenses and mirrors creates interference fringes when a laser with long coherence length is used (Hell *et al.*, 1991). (See Tables 6.1 and 6.2 which contain columns with spectral width and pulse length, from which the coherence length follows.)
- **Spatial coherence:** Spatial coherence occurs when a constant, time-independent, phase difference exists for the optical field amplitude over a given distance at two different points in space.
- **Coherence surface:** The coherence surface is the region of space for which the absolute phase difference of the optical field is less than π . The well-known "speckle" pattern consisting of a pattern of darker and brighter light spots is visible when a matte wall surface is illuminated by a distant He-Ne laser. The interference effects caused by irregularities in the surface will be noticeable.
- **Coherence volume:** The coherence volume is the product of coherence length and the coherence surface. Interference between superimposed coherent waves will only be visible within this volume.

Phase Randomization: Scrambling the Coherence Properties of Laser Light

A long coherence length of lasers will cause laser speckles (i.e., interference effects) and scatter from out-of-focus particles and these will interfere with the image. Normally in a fluorescence CLSM, this scattered light is removed by the dichroic mirror as the emitted fluorescence has a different wavelength than the illuminating light source. However, a polarizing beam-splitter and 1/4-wave retardation plate can be equally effective for the removal of light backscattered by dust or optical surfaces. The angular position of this 1/4-wave plate and the polarizer is important (Szarowski *et al.*, 1992). For generating circularly polarized light, it is only necessary that the incoming plane of polarization and the principal axis of the 1/4-wave plate be at a 45° angle. Light reflected or scattered from an illuminated dielectric surface has its direction of rotation reversed. On its return passage through the plate, the light becomes linearly polarized again, but its plane of polarization is now rotated by 90° and it is removed from the optical path by the polarizing beam-splitter.

Another method to reduce speckle comes from color confocal microscopy where one uses several lasers. As the coherence prop-

erties of the various laser light sources are not the same, averaging over the three wavelengths and over several planes will reduce the effect of the laser speckle (Cogwell *et al.*, 1992b).

Measures to Reduce the Coherence Length of Laser Light

Many applications work better and image quality improves if the coherence of the laser light is reduced. One way to achieve this is to place a rotating diffuser wedge in front of the beam expander (Hard *et al.*, 1977). Rotation or vibration of this wedge is essential to average out local diffuser properties. Alternatively, focusing the laser beam into a multi-mode optical fiber bounces the light around inside the fiber and mixes the various propagation modes to scramble the Gaussian intensity pattern (TEM_{00}) to a multi-mode pattern with a homogeneous spot intensity. This eliminates or reduces the coherence surface. A short focal length lens or graded index lens (GRIN) recollimates the light at the fiber exit.

To eliminate the speckle pattern due to temporal coherence properties, a piezo-electric driver can be used to induce vibrations in a section of the fiber (Ellis, 1979). In the context of medical imaging, Connor, Davenport and Gmitro (1992) used a rotating diffuser to eliminate the speckle pattern by coupling the laser beam into a bundle of small, multi-mode fibers. The latest method in this area is the introduction of ultra-thin single-mode image fibers. A fiber-optic bundle with a total diameter of 200 to 500 μm consisting of 2000 to 5000 micro image fibers, each with a core size of 1.0 to 1.4 μm (Kiat *et al.*, 1992), can be used as a phase randomizer in conjunction with a rotating diffuser. Although these ultra-thin fiber bundles propagate shorter wavelengths much more strongly than longer, leaky ones, this is not a problem as long as monochromatic laser light is used.

As light is scattered in all directions by most diffusers, they reduce the intensity of the laser light. However, holographic diffusers have been designed to carry strong forward intensity lobes. Among others, Lasiris, Inc. and Physical Optics Corp. (see Table 5.3) produce holographic laser beam homogenizers, having a conversion efficiency of 80% to 90% and low backscatter (Bains, 1993).

Laser coherence effects can also be eliminated by spot scanning the field of view with a tiny, focused, single-mode laser beam. Alternatively, the condenser plane can be scanned with the same tiny, focused, single-mode laser beam while illuminating the whole field of view. Coherence scrambling occurs because the laser spot rapidly scans the back-focal plane and the field is continuously illuminated with a mix of beam angles (Ellis, 1988).

Heat Removal

Most of the laser excitation energy is converted into heat. This must be removed to prevent thermal destruction of the active laser medium. Small laser systems can use convective air cooling, but larger laser systems need forced-air or water cooling. Especially for the largest systems, fans and turbulent water-flows may introduce vibrations in the system (microphonics). These unwanted mechanical vibrations are inevitably coupled to the mirror mounts of the resonator cavity, causing increased noise in the optical output. Pumps that recycle liquid laser-media are another source for vibration. In this case, the vibration is transferred via hoses to the active medium in the resonator. To minimize these effects, the hoses should be clamped or fastened to the support table as near as possible to the laser head.

The heat generated will also put thermal stress on the mechanical parts of the resonator. Poorly designed laser systems quickly lose their proper alignment or may need an unacceptably long warm-up time. The installation of a laser system in a room with large daily or annual temperature fluctuations or a ceiling fan continuously blowing cold, dusty, air directly onto a laser will also hamper operation and cause performance to deteriorate. The use of thermo-electrically (TEC) cooled diode lasers that are in thermal contact with their enclosures may create hot surfaces that should be labeled as such. Diode arrays may require water cooling. Because of their monolithic design, they are less susceptible to mechanical instabilities but thermal stress has to be avoided at all cost. The same is true for fiber lasers, which, due to the fiber length, are sensitive to temperature fluctuations unless counter measures such as negative temperature coefficient materials are used.

Other Installation Requirements

Manufacturers usually describe the electrical power requirements and flow rates for cooling water (Gibson, 1988, 1989; Rapp, 1988). Before installation of a laser system, adequate control of room temperature and air conditioning should be available. Walls should be painted to eliminate concrete dust from settling on delicate optical surfaces. For emergency situations, a master switch for the laser power should be installed with easy access at eye height and labeled as such. Heat from large fan-cooled laser power supplies can sometimes be plumbed directly into the intake of the air conditioning system, with backflow protection, if this is allowed. Similarly, acoustic noise can be very tiring and noisy power supplies should be placed outside the experiment room.

An exhaust for noxious fumes such as ozone or dye vapor should exist and backup systems should be established to prevent interruptions of power or coolant flow. Mechanical vibrations due to nearby traffic can be eliminated by installing the system on commercially available vibration-free, air-cushioned laser tables or on tables isolated using sand-filled containers, tennis balls, or motorcycle inner tubes. When equipment used for radio-frequency crystal-growth, nuclear magnetic resonance (NMR) equipment, building air-conditioning machines, large elevators, or other large laser systems are present, it is often wise to equip each laser with its own stabilized power line to prevent large voltage spikes from reaching laser power supplies. Hidden cables drawing a large current (for elevators, for example) in ducts near your facility may ruin a carefully planned sensitive system by disruptive induction currents. Stray magnetic fields may affect the flow of ions in gas lasers.

Movement of fiber-optic components changes the beam propagation properties of the fiber (bundle) and should be prevented for best stability (unless the device is used intentionally to scramble the laser light polarization and reduce the coherence length).

Attenuation of Laser Beams

Laser damage levels for materials are usually given in mW/cm^2 while laser output is given as total average power (mW) for a small, for example, 2-mm diameter beam. Although intensities in the milliwatt range can be attenuated with neutral density filters, high-power lasers will easily destroy this type of filter. The absorbed light may even bleach or heat them so much that they fracture or explode. Polaroid material can be used only for low light intensities and but this material usually has a peak transmis-

sion of only 30% to 50%. For intensities above about 10 mW, better attenuators are Glan-Thompson polarizers. When a laser emits only polarized light, one rotatable polarizer, set at a proper angle will suffice. For randomly polarized lasers a set of two polarizers can be used. The first one passes 50% of the total intensity. The second polarizing analyzer transmits a continuously adjustable amount of light depending on its orientation angle. In all these cases one must be careful to ensure that light reflected from the various crystal surfaces is also absorbed by beam stops. Initial alignment should be done with only the lowest laser power available. If a polarizer is used to attenuate an initially randomly-polarized laser beam, fluctuations in the intensity may be observed. These are due the varying nature of the interaction between the cavity modes and therefore the polarization state inside the laser.

Glan-Thompson polarizers contain Canada balsam to hold them together. This material may absorb slightly, specially in the UV. Power levels above several watts (power = energy/time = area of pulse) will destroy this type of polarizer. For the highest expected power levels, air-spaced Glan-Taylor polarizers are strongly recommended. A new type of polarizing element is the Microwire™ polarizer commercialized by Moxtek Inc. (see Table 5.3). It fits in very tight spaces but is somewhat more fragile.

STABILIZATION OF INTENSITY, WAVELENGTH, AND BEAM POSITION IN LASERS

Sources of Noise in Lasers

Stability, both temporal and positional (beam pointing), is a very important aspect of any CLSM laser light source. As beam pointing affects the amount of light coupled into the delivery fiber, pointing instability can cause intensity instability. Other intensity instabilities are related to backreflections from the fiber that can be avoided (Cogwell *et al.*, 1992a) by employing a wedged fiber input (e.g., Point Source, see Table 5.3). Figure 5.5 gives examples of noise in the output of several laser systems. The most stable are the diode laser and the forced-air cooled (fan) small-frame argon-ion laser. The argon-ion laser-pumped titanium:sapphire laser output was coupled into a fiber-optic attached to the detector. The average relative intensity fluctuations are defined as $N^{-1} \sum_i (I_i - \langle I \rangle) / \langle I \rangle$, where N is the number of points. For the diode laser this quantity is about 0.1% (Franceschini *et al.*, 1994), and for the Ti:Sapphire laser at the fiber output, about 2%. Both would be acceptable for microscopical applications in which less than 50 photons were detected per pixel.

It is imperative to keep lasers well-aligned with very clean windows. Misaligned mirrors, a half-illuminated reference monitor diode, or a dust speck may create a very unstable output. As misalignment can cause unusual modes to appear, these laser lines will fluctuate independently, and the total output becomes unstable. For instance, the two UV lines of an old water-cooled argon-ion laser were observed to alternate between bright and dim with a period of a few seconds to a minute. Realignment helped but the tube had to be replaced. Transverse mode swapping is seen in He-Cd tubes that need replacement.

Noise in the optical output of a laser can be created by a variety of phenomena. Several factors produce high-frequency (noise) or slow variations (drift) in beam power. These include power-supply variations, thermal drift of the cavity, and mode competition. Examples include:

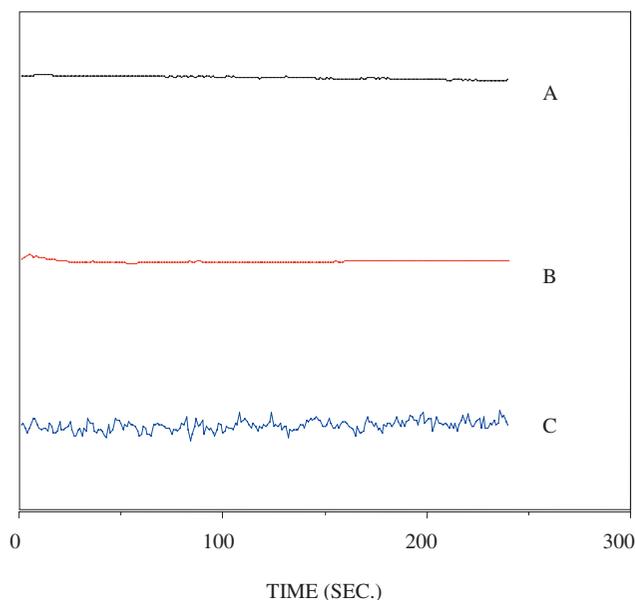


FIGURE 5.5. Measured laser emission stability (arbitrary units). Normalized and shifted intensities for (A) a diode laser (Sony SLD104AV, $\lambda = 780$ nm; *top* curve); (B) a forced-air cooled small frame argon-ion laser, Omnichrome model 532, running at 488 nm and 7 mW power (*center* curve); (C) Mira™ (Coherent) Ti:Sa laser running at 780 nm and 1.2 W (*bottom* curve). The normalized noise for the diode laser is 0.1% and for the Ti:Sa laser about 2%. The attenuated Ti:Sa laser was focused into a fiber-optic.

- **Dye lasers:** Noise and drift in dye lasers can be caused by air bubbles and inhomogeneity in the dye, the dye pump, and by the laser pump source.
- **Gas lasers:** A major source of noise comes from plasma oscillations and from microphonics generated by cooling water turbulence. Forced-air cooling for smaller lasers can avoid most microphonics, but the fan may introduce some new noise. Commercial confocals often mount the lasers on a heavy stone slab (Zeiss) or place them in a separate, shielded drawer (Leica). Also any optical pump source can introduce noise.
- **He-Cd lasers:** Strong plasma oscillation between 225 and 400 kHz may create an output variation of up to 12%.
- **Semiconductor or diode lasers:** Semiconductor laser diodes require a current source with the highest stability and the lowest electronic noise (Malzahn, 2004). Furthermore, temperature stability is imperative for adequate intensity and wavelength stability (Hodgson, 1994). Forward-biased semiconductor junctions are inherently thermally unstable; an increase in current increases the temperature, which, in turn, increases the current even more. Therefore, current supply design is very important and must be suited to the specific laser. Internal damage in laser diode junctions due to heat buildup ages the device, resulting in a reduced output and slope efficiency. $1/f$ noise is due to trapping of carriers in the device (Mooradian, 1993). Drive-current fluctuations also degrade the performance and will modulate the beam intensity. Mistreated fiber-optics can also increase the amount of noise. On the other hand, an imposed high frequency (MHz) modulation allows the laser to be used for time-resolved and frequency-domain imaging applications.

- **Solid-state lasers:** The important noise sources are microphonic noise and $1/f$ noise related to thermal fluctuations in the lasing rod. Furthermore the Ti:Sa rod temperature bath should be set at the correct temperature, for example, 18°C. Failing to do so creates condensation droplets on the pump surface. Fiber lasers should be kept in a stable temperature environment.

All lasers suffer from noise introduced by their powersupplies. Switching-mode powersupplies introduce switching ripple, typically at tens of kilohertz. This type of power supply was developed to take advantage of better transformer efficiencies and smaller physical sizes (Forrester, 1994a,b).

Planar optical elements and filters should be inserted at a small angle with respect to the optical axis to prevent beam reflections from returning to the laser cavity where they might increase the noise level of the laser (Cogswell *et al.*, 1992a).

Images created on commercial CLSM instruments may show a fine pattern of irregular vertical lines. Possible sources include resonances in the scanning mirror systems and high frequency ripple in the switching power supplies. However, sometimes the problem is not the light source but the detector system (French and Gratton, personal communication). Last but not least, the main laboratory power supply lines may be unstable.

Other sources of noise are caused by external influences: traffic vibrations, etc. A simple means to reduce its effect is a well-damped, stable support platform (see above).

Laser Beam Intensity Stabilization in Current- or Power-Control Mode

Continuous wave gas lasers can easily be intensity stabilized using either tube current stabilization or external modulation of the light intensity (Miller and Hoyt, 1986).

- **Constant-current mode:** An electronic feedback loop directly controls the tube current and minimizes current drift. As this system takes no account of how temperature may affect cavity gain, best results are obtained after the laser temperature stabilizes.
- **Constant output power mode:** The drive current is controlled via a signal obtained from a built-in monitor diode. The stabilization circuit typically consists of a beam-splitter-photodiode assembly mounted behind the output coupler. The monitor diode picks off a small amount of laser intensity. Changes in intensity are compensated for by opposing changes in the drive current. When not properly aligned, large intensity fluctuations may result. Multi-line lasing depends on an interplay of different gain and mode patterns specially when the bore holes in the internal BeO laser disks become larger with time (Fig. 5.4). A constant output power supply in combination with an aging plasma tube will increase in tube voltage and current supplied, damaging the tube further and even more rapidly. Both effects are even stronger in mixed gas lasers.

Even if the laser beam is stable, external noise can be introduced to the system by room dust, traffic vibrations, or movement of fiber-optic components and may still adversely affect intensity stability. The long-term stability of the largest argon-ion models is better than 1% in this control mode. But this light control mode does not work so well when multiple emission lines are monitored simultaneously (Brelje *et al.*, 1993).

Laser Beam Intensity Stabilization with External Pockels Cell Modulator

Some manufacturers provide special accessories to improve the intensity stability of CW lasers. These devices are external modulators incorporating a fast feedback system (Miller and Hoyt, 1986; Miller, 1991; see Fig. 5.6), and, of course, they reduce the total output power. Intensity fluctuations of up to 50% of the maximum power can be corrected in this way, but only with a 50% reduction in the total available power. Laser intensity stabilizers can regulate the output of CW and mode-locked lasers to within 0.025%, with a noise attenuation of 400:1 and a bandwidth from direct current (DC) to several megahertz in some systems.

Depending on the polarization state of the laser light, up to 80% transmission is obtained. In this system (Fig. 5.6), a photodiode is illuminated with a fraction of the laser light deflected by a beam-splitter. A servo-control unit compares the detector signal with a user-selected set point. The difference signal is amplified and drives an electro-optic (Pockels cell) modulator. The amplified electrical signal causes a rotation of the plane of polarization. Depending on the voltage applied, more or less light can pass through the modulator creating a variable beam attenuator. Ideally the laser beam should be stabilized as close as possible to the sample position rather than at the exit of the laser head. Otherwise, vibrations or dust may alter the stability of the beam after it leaves the source.

Stabilization units should be able to operate at a variety of wavelengths with UV capability when necessary. They should be protected against driver signals beyond a certain maximum, so that a runaway situation does not occur, for example, if the beam is blocked or temporarily interrupted. When the stabilizer is driven by an external control signal, complex intensity sequences may illuminate the sample. Commercial laser power controller (LPC) stabilizers can be obtained from BEOC and several other manufacturers (see Table 5.3).

When randomly-polarized laser light has to be stabilized, the external Pockels cell modulator must be placed between two crossed polarizers. Unfortunately, such a modulator can not work with low-repetition-rate pulsed lasers. Another simple way to remove laser noise is similar to that typically used in steady-state fluorescence spectroscopy. The effect of source fluctuations is cancelled by dividing the detected signal from the sample by a signal derived from the laser sampling monitor detector.

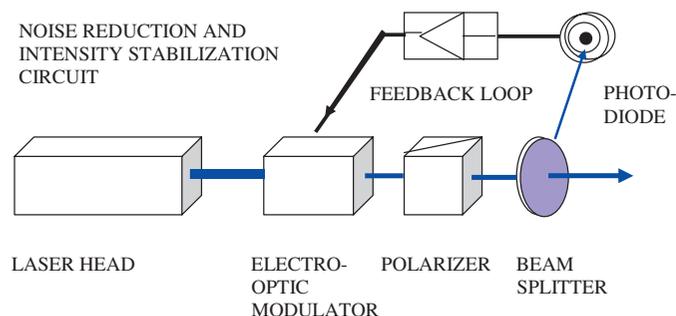


FIGURE 5.6. Example of a laser beam intensity stabilizer and laser noise suppressor. A beam-splitter delivers a fraction of the laser output beam to a fast photodiode detector. Electronic feedback loops for low- and high-frequency signals control the electro-optic modulator (EOM) intensity regulator. The rotated plane of polarization exiting from the EOM passes through an angle-adjustable Glan-Taylor polarizer. It defines a single plane of linear polarization. A detected intensity fluctuation alters the drive voltage to the crystal.

Diode Laser Intensity and Wavelength Stabilization

Incorporating a Fiber Bragg Reflector (FBR) or Grating (FBG) in a (pump) diode laser stabilizes wavelength, improves coupling efficiency, reduces unwanted reflections and the effects of temperature and drive current fluctuations, as well as simplifying manufacturing (Guy and Painchaud, 2004). The FBR consists of a stack of thin coating layers forming a bandpass reflectivity filter at either the high reflector or output coupler end. Even simpler is lens coupling the diode-laser output into a section of FBG fiber pigtail. A periodic or non-periodic (chirped) refractive index variation of the Bragg grating can be present in its core, creating a narrow band low-reflectivity filter that reflects a small fraction of the light from the diode laser back into it. However, due to interference effects, only a certain wavelength — the designed lasing wavelength — is actually fed back into the laser diode. All other wavelengths are out of phase and die off. In this way, the attachment of the proper FBG fiber pigtail creates a diode laser that is stabilized in wavelength and intensity. Naturally, if the FBG temperature or internal strain changes, the lasing wavelength will vary slightly. A typical temperature coefficient is 0.012 nm/°C. A temperature increase from 18°C to 40°C will cause the lasing wavelength to shift 0.26 nm, but in practice it may well be 5-fold larger. With temperature compensating packaging and a strain-inducing material with a negative temperature coefficient, this effect can be reduced to 0.001 nm/°C.

Beam Pointing Improvement Via Active Laser Cavity Stabilization

The mirror orientation changes when the laser cavity warms up. This causes the laser beam to wander and the beam intensity to vary. Active cavity stabilization corrects for misalignment of the resonator via a feedback mechanism (Peuse, 1988). The advantage is that the resonator structure becomes independent of changes in the environment, for example, temperature, and as a result provides an extremely short warm-up time (seconds), enabling hands-off operation. In addition, there is less chance that setting the power supply for a constant optical output will provide damaging power levels to a tube that has low gain because it is misaligned. Large frame ion lasers currently come standard with active resonator stabilization systems such as PowerTrack™, ModeTrack™ and ModeTune™ (Coherent) or BeamLok™ Z-Lok™ (for mode-hop-free single-frequency operation) and J-Lok™ (for jitter reduction; Spectra Physics). These systems result in 1-min warm-up times, hands-off operation, and long-term intensity stability of better than 1%. Beam-pointing stability is obtained using a quadrant photodiode detector.

The horizontal and vertical positions of the high reflecting mirror are continuously tuned for optimum laser power with independent (magnetic or piezo) actuators. A small oscillation (dither signal) is superimposed on both actuators. A sensor photodiode signal reflects the power level of the beam. The filtered dither signal (lock-in detection) creates vertical and horizontal error signals. This oscillation is compared with a microprocessor-stored reference. The horizontal and vertical difference signals drive the mirror positions independently to obtain maximum power. The minimum detected oscillation (dither) signal corresponds with optimum alignment (Miller, 1991).

Beam Delivery and Positioning Fiber-optic Coupling

Continuous wave laser beams can be delivered to the scan head or microscope frame via single-mode, polarization-preserving, dual-

circular stress-rod design, fiber-optic (the so-called “Panda-eye pattern type,” see Chapter 26, *this volume*). One should use fiber-optics whenever laser light has to be transported, eye-safe, over large distances, especially when the laser is located in a dusty environment (i.e., not a clean room). A single-mode fiber core typically has a diameter of 3 to 6 μm for VIS wavelengths. This creates a point-like excitation spot for confocal microscopy. Multi-mode fibers with core diameters ranging from 0.1 to 1 mm may also be used at times but beam characteristics such as beam width and polarization state are altered. However, fibers have been found less suitable for carrying UV light or for preserving the pulse width of femtosecond pulsed lasers. For femtosecond pulse lasers, the beam should be enclosed in a dust-free tube and reflected from dispersion controlling mirrors (e.g., from Newport Corp). Picosecond systems are less affected (see Chapter 26, *this volume*). Handy beam couplers are available from many suppliers (OZ Optics Ltd., see Table 5.3). The Cell-viZio from Mauna Kea Technologies (see Table 5.3) uses a bundle of 30,000 fibers of 2 μm each.

Ultrafast pulses can currently be delivered by fiber-optic without dispersion using two methods: by pre-compensation or by photonic-bandgap fibers. Ultrafast pulses create non-linear effects in glass fibers and the fibers suffer from dispersion effects. Even at low pulse energies, a non-linear Brillouin and Raman background is generated. Pulse trains can only be transported without detrimental effects from chromatic dispersion under special conditions. By precompensating for the expected fiber pulse chirp with a dual prism or grating configuration (Fork *et al.*, 1984; Zeek *et al.*, 1999), the initial shape of the pulse can be preserved after it exits the fiber, but this adds to the complexity of the system and creates power loss. This precompensation uses the fact that a short pulse consists of many frequencies described by a Fourier sum, with proper amplitudes and phases.

In holey fiber coupling, single-mode, hollow-core (= air-filled) photonic-bandgap or crystal fibers (PCF), non-linear effects occur at a much higher threshold than in glass. As long as the zero-dispersion region of $\sim 812\text{nm}$ for the waveguide matches the laser wavelength, a perfect femtosecond pulse is transmitted without distortion (Tai *et al.*, 2004). Tuning outside this range gives pulse broadening again (Hitz, 2004c). The spectral width of the 0 to 350 mW Ti:Sa laser stays unchanged at 812 nm after passing through a 1.5-m length of HC-800-01 bandgap fiber (Crystal Fibre A/S, see Table 5.3). Dunsby and colleagues (2004) describe an electronically tunable white light continuum, 435 to 1150 nm, generated by injecting 80 MHz 120 femtosecond light into a micro-structured fiber.

Direct Mirror Coupling and Pulse Width and Pulse Shape Control

Combinations of CW Lasers All commercial confocal systems these days offer facilities to combine the output of several lasers using dichroic mirrors and intensity-balancing neutral-density filters. A device for selecting wavelength and intensity, such as an acousto-optic tuning filter (AOTF) or acousto-optic beam splitter (AOBS), is then added. One of the advantages of having many separate lasers is that the intensity of each line is servo-controlled independently. In the competing Kr/Ar ion laser, it is only the total optical output that is stabilized. The portion of this total associated with each individual line can vary widely with time. For example, Cogswell and colleagues (1992a,b) combined a 632.8 nm He-Ne with a 532 nm frequency-doubled Nd-YAG laser and the 442-nm line from a He-Cd laser for true-color confocal reflection microscopy. Issues that may arise are spectral purity and polarization of the excitation.

Ultrafast Lasers Assuming the proper coatings, direct mirror coupling into the scan head can maintain the femtosecond pulse width and multi-photon intensity for ultrafast lasers such as Ti:Sa systems (Fermann *et al.*, 2002). On the other hand the dispersion material used in acousto-optic modulator (AOM) or acousto-optic deflector (AOD) devices or the glass in thick objectives may cause inappropriate pulse broadening due to group velocity dispersion (GVD). A GVD compensating prism or grating pair or a Gires-Tournois interferometer (GTI) can control the GVD and compensate the widening to some extent. Instead of prism and grating pairs, chirped mirrors could be used. These limit tunability but improve stability against environmental effects such as temperature changes and reduce high-frequency noise. In a chirped mirror, the Bragg wavelength increases with increasing penetration depth. Double-chirped mirrors that produce an additional chirp in the coupling of the incoming and reflected wave reach almost perfect dispersion compensation.

Multi-photon ultrafast (100 fs) lasers possess a phase that depends on the spectral region. To match the slightly different two-photon absorption properties of fluorophores in different environments one could tune the laser but this changes the beam properties such as intensity, phase distortions, etc. A better approach is to use a charge-coupled device (CCD) computer-assisted pulse shaper either to create a transform-limited flat phase across the spectrum or to modify the phases within the pulse via multi-photon intra-pulse interference (MII), making selective excitation possible (Dantus, 2003; Dela Cruz, 2004). MII can also limit the amount of three-photon processes, thereby limiting deep UV damage to living cells.

Wavelength Selection and Intensity Control

Wavelength Selection

In commercial confocal systems, several laser beams are often combined via dichroic mirrors onto the same optical path leading towards an AOTF or AOBS (Chapter 3, *this volume*). Both allow very fast wavelength and intensity control on a microsecond timescale. We found, however, that the spectral purity of the AOTF-fiber combination is sometimes insufficient. When viewing mirror-like solid surfaces covered with labeled biosensor molecules excited at 488 nm, a little bit of 514-nm laser light was observed passing through the fiber-optic and masquerading as fluorescein isothiocyanate (FITC) 509 nm emission. A thin 488 nm interference filter placed in front of the fiber-optic entrance in the AOTF module remedies this problem.

Intensity Control

Continuous wave laser beams can be deflected with acousto-optic devices/deflectors/modulators (AOD, AOM) for intensity and position control. The functioning and practical use of these devices has been discussed in great detail in Art and Goodman (1993). Draaijer and Houpt (1988) used these devices for video-rate scanning in confocal microscopy and their system was commercially available through Noran and still supported by Visitech Intl. Ltd. and by Prairie Technologies, Inc. (see Table 5.3). Intensity control via an electro-optic modulator is described in Chapter 3. For use of polarizers to control laser intensity, see above under Attenuation of Laser Beams.

Polarization of the Laser Light

We measured a degree of polarization of the light entering the Zeiss AOFT at better than 1000:1. Using a compact home-built polarization sensitive power meter at the sample position the laser

light was still very well polarized, better than 200:1 with the principal axis 5° counterclockwise from the positive x -axis of the stage (i.e., left to right direction).

SPATIAL BEAM CHARACTERISTICS

Although lasers provide very small beam divergence, the uniformity of the intensity across the beam may be relatively poor. Fortunately, this is generally not a serious problem because the uniformity of illumination can be easily improved by spatial filtering. Filtering is very efficient when there is good spatial coherence of the beam.

Several devices are available for spatial filtering; the most common is the Gaussian spatial filter (Melles Griot, see Table 5.3), which consists of a focusing lens that converges the beam toward a very small pinhole. After this pinhole, a second lens, one focal length away, is used to regenerate a parallel beam. By choosing the focal lengths of the two lenses carefully, this configuration can also be used to expand or decrease the beam diameter but a pinhole is always essential for spatial filtering. Spatial filtering takes place because diffraction effects at the pinhole produce an out-going wavefront, which is the Fourier transform of the pinhole and is not affected by the spatial properties of the light impinging on the pinhole (Fig. 5.7). It should be noted that dust particles moving in the vicinity of the pinhole can modulate the transmitted intensity, so great care must be taken to ensure that this does not occur. For most practical purposes, a single-mode optical fiber functions as a spatial filter.

For large size beams from, for example, excimer lasers, an external beam homogenizer consisting of a prism/lens combination can be used. The optics recombine sections of the beam to produce a homogeneous rectangular shape (Austin *et al.*, 1989).

Edge-emitting diode lasers typically require extensive astigmatic and anamorphic corrective optics to obtain a circular, parallel beam. Commercial packages are available to implement this (Ingeneric GmbH, see Table 5.3). However, the use of a spatial filter, a single-mode fiber-optic, or an overfilled back aperture plane can also produce a symmetric beam.

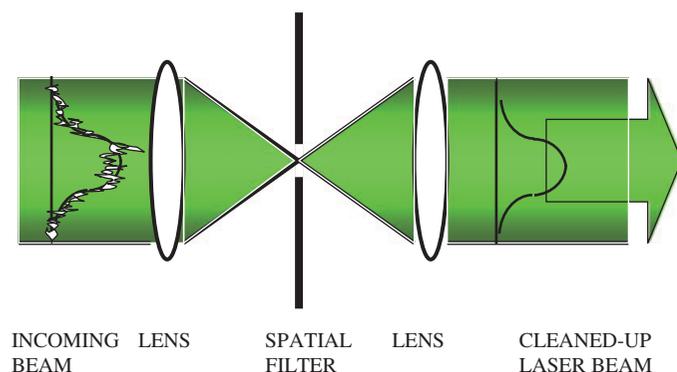


FIGURE 5.7. Diagram of a spatial filter for by a pair of lenses and a small pinhole. High frequency noise is removed from the laser beam. The spatial intensity is cleaned up.

LASER REQUIREMENTS FOR BIOLOGICAL CLSM-RELATED TECHNIQUES

Optical Tweezers

Single-cell manipulation is feasible by trapping the cell in an optical box formed by one or more focused laser beams. Each focal spot has a diameter of about 1 μm . Taken to the extreme, optical trapping can take place in a completely enclosed environment. Ashkin and colleagues (1987) reported the optical trapping of particles and viruses although the technique was also used decades earlier for levitation of particles in light-scattering experiments. A simple design is given, for example, in Smith and colleagues (1999). A beam power of 20 to 100 mW TEM₀₀ at the specimen is sufficient. For biological specimens infrared light should be used. A careful choice of the trapping wavelength is essential to reduce optical damage to biological samples as much as possible. Shorter wavelengths are absorbed by the specimen, longer wavelengths by water. Therefore, traps commonly use a Nd-YAG (Hoffmann *et al.*, 2000; Reichle *et al.*, 2001) or a Nd-YLF 100 mW laser operating at 1064 nm. Other useful lasers may be a 632.8 nm, 25 mW He-Ne laser, or a diode laser beam. An improved design is given by Bechhoefer and Wilson (2002). Fiber lasers with their diffraction-limited output and excellent beam quality are also very suitable for laser tweezers applications (Woods, 2003).

Optical trapping of particles larger than the illumination wavelength can be explained with geometric optics. When a transparent spherical particle, having a refractive index larger than the surrounding medium, refracts a ray of light, a change in momentum of the refracted photon occurs. Due to the principle of conservation of momentum, the particle receives an equal but opposite change in momentum. This force pushes the particle away from the direction of the refracted photon.

When a particle, somewhat smaller than the focal spot of the laser beam, is suspended in the intensity gradient of a Gaussian beam profile, then the intensity of the light on one side of the particle is larger than on its opposite side. This makes the particle move to the side with the higher intensity (i.e., the center of the laser beam) and it seems as if the laser beam attracts the particle.

Heating and Damage Effects in the Cellular Environment

Cells attached to biosensor surfaces may suffer additional heating effects because of one-sided contact with a buffer solution and close contact with coated or absorbing semiconductor surfaces. Objects caught in an optical trap are also subjected to more severe heat stress. For instance, a 100 mW Nd-YVO₄ laser focused into watery media heats it by 0.8 K/100 mW, but, add a polystyrene bead in glycerol, and it jumps to 4 K/100 mW. Even when this amount of heat is not damaging to the cell, it influences the calibration of the trap, that is, the force-displacement relationship (Boas, 2003; Peterman, 2003). Several studies report cell damage conditions (König *et al.*, 1996) of NIR multi-mode optical traps. Neumann (1999) shows that for the spectral region from 790 to 1064 nm, an oxygen-mediated, one-photon process is causing the damage. Wavelength and power level effects monitored over time by Leitz and colleagues (2002), indicate that the 700 to 760 nm spectral region is unsuitable due to induced photochemical effects, but that 810 nm is better because only photothermal effects occur. These can be controlled by reducing the trap energy to 36 mW. Cellular response to NIR light with varying pulse length is reported by König and colleagues (1999 and Chapter 38, *this volume*).

Zhang and colleagues (1999) look at cell viability in CW Al:GaAs diode laser traps.

Total Internal Reflection Microscopy

For imaging cell adhesion and other biological processes such as vesicle transport and observing thin self-assembled layers for biosensor applications, total internal reflection provides a background-free fluorescent imaging technique. Penetration of the evanescent wave beyond the boundary plane is limited to roughly 100 nm. Objects farther from the interface are not excited and stay dark. Two methods exist to create total internal reflection fluorescence (TIRF) conditions. One is to inject the light through a very high, ≥ 1.45 , numerical aperture (NA) objective lens and the other utilizes an external prism coupling to the slide on the far side of the specimen. The through-the-objective lens method requires only 40 μ W CW at 488 nm for green fluorescent protein (GFP) imaging with reduced bleaching effects. For the prism coupling technique, 20 mW CW was barely sufficient and would have benefitted from higher power. Other suitable lasers include 50 mW green 532 nm; 70 mW He-Ne laser, or 200 mW 488 water-cooled argon-ion lasers. It must be noted that, at these power levels, some published configurations of the external-beam prism method can be very hazardous to the eye.

Confocal Raman CLSM for Chemical Imaging

Raman spectroscopy and imaging is used in research on immobilized molecules and biosensor interfaces. It requires a mode-hop-free tuning range of several gigahertz, a narrow line width of 1 MHz, and output power of several hundred milliwatts. Cellular autofluorescence can present a large background. In order to reduce its influence, red excitation from a diode laser is a good choice. Stry and colleagues (2004) utilized a Tiger ECDL laser system (see Table 5.2), with grating tuning from 775 to 785 nm, and also from 730 to 1085 nm with 1 W output power.

Coherent anti-stokes Raman scattering (CARS), complementary to CLSM, maps via vibrational contrast the intracellular water distribution. It uses the beat frequency of two lasers, preferably operating in the IR to avoid damage to cells as much as possible. Multi-photon lasers emitting picosecond pulses with widths of 2 to 5 ps are better for CARS because their spectral linewidth matches the typical Raman bandwidth of $\sim 20 \text{ cm}^{-1}$ much better than femtosecond pulses (Nan *et al.*, 2004). They were able to visualize the CH_2 stretch vibrations from lipid tails (see also Chapter 33, *this volume*).

Non-Linear Confocal Microscopy

Second harmonic imaging is a very good method to probe external membranes of living cells. Laser system considerations and characteristics are given below under Wavelength Expansion Techniques. The SHG images show little speckle background and can be created with about the same laser intensity as used for fluorescence imaging. One can track microtubules with SHG and intracellular inhomogeneities with THG (Hogan, 2004). Yang and Mertz (2002) used, for example, a mode-locked 860 nm Ti:Sa laser (see below) with a $\sim 100 \text{ fs.}$, 82 MHz optical pulse train that delivered 10 mW at the sample (see also Chapter 40, *this volume*).

Nanosurgery and Microdissection

Multi-photon imaging systems can be used for multi-photon ablation of cellular structures and tissue. It requires high (kHz) repeti-

tion rate lasers with 1 to 10 μ J pulse energies (Arrigoni, 2004a; also see Chapter 38, *this volume*).

TYPES OF LASERS

The earliest lasers were solid-state lasers using ruby as the active laser medium (Bertolotti, 1983). Subsequently, a wide variety of lasers were developed (Weast and Tuve, 1971; Arecchi, 1972; Brown, 1981; Bass and Stitch, 1985; Eden, 1988; Fermann *et al.*, 2002; Silfvast, 2004; Webb and Jones, 2004). Essentially, all CW gas lasers (Bloom, 1968) and some solid-state lasers with emission in the visible part of the electromagnetic spectrum, meet the minimum intensity requirements estimated above for fluorescence microscopy. The list of available wavelengths continues to expand (Weber, 1999). For trapping and label-free SHG microscopy, IR wavelengths are the most suitable. Some other important parameters are the output power at each wavelength, efficiency, and stability. Table 5.1 lists the major types of CW lasers. Table 5.2 lists the major options for pulsed-laser systems.

CONTINUOUS WAVE (CW) LASERS

Continuous wave (CW) lasers can be divided into several classes:

- gas lasers
- dye lasers
- solid-state lasers.

Gas Lasers

Three major types of CW gas lasers are available (Bloom, 1968):

- argon-ion, krypton-ion, and a mixture of argon and krypton (mixed gas)
- helium–neon
- helium–cadmium
- alkali metallic vapor lasers.

The CO and CO₂ lasers that emit around 5 μ m and at 10.6 μ m are not discussed here. Commercially available, but not widely used and therefore also omitted from further discussion, are the neon laser that can emit 1 W of multi-line UV (CW) at 339.2 (0.3 W), 337.8 (0.2 W), and 332.4 nm (0.5 W) and the xenon laser emitting between 488 and 540 nm but usually only operated in pulsed mode. In general, krypton and helium–neon lasers are used only if red excitation is necessary, but the latter also now provide lines in yellow, green (GreNe), and orange (594 nm). With the increasing availability of red-absorbing dyes, their importance is growing. Several leading confocal microscope manufacturers still equip their systems with red and green helium–neon lasers emitting a few milliwatts and air-cooled argon-ion lasers. Argon–krypton mixed-gas ion lasers often form a cheaper alternative to the purchase of separate argon and krypton systems plus the additional requirements for power and beam-steering optics. It is worth realizing however, that the lifetime of a specific red laser line in a mixed gas laser may be much less than the stated lifetime of the strongest laser line (green, 514 nm).

Argon-ion

Because its emission wavelength matches the absorption peak of fluorescein and other popular dyes, the argon-ion laser is still by far the most common laser in microscopy. Lately, however, highly efficient 488 nm small-footprint, solid-state lasers have appeared.

TABLE 5.1. Continuous Wave Lasers

Tunable CW Dye lasers													
Manufactory & Model	Pump Power mJ	Wavel. Range nm	Spectral Width mW	CW Av. Power	Noise rms, % 1/3-day	Abs. Acc. nm	Wavel. Reset. nm	ASE %	Conv. Eff. %	Beam Parameters			
										Freq. Stab. MHz/b	Diam. mm	Div. mrad	Mode
Coherent 599 ¹		390–930	20–401 60–100 500GHz	200–1200						0.6	1.5	TEM ₀₀	V
Radiant Dyes & CW Dye L.		560–650			2/h				<25	<1.3	<1.5		
Coherent 899 ²	5–15 W	370–1100	40–200 GHz	10–2000		0.1	0.1						
Radiant Dyes ³ & Dye Ti:Sa		400–850 700–950	1/20MHz 2 GHz		2.5	<0.03	<0.005	<0.5	12–32	100 MHz/h	<0.5	TEM ₀₀	V, >98

Legends: Wavel(ength); CW: Continuous Wave; Av(erage), Power; Abs(olute) Acc(uracy); Wavel(ength) Reset(tability); ASE: Amplified Spontaneous Emission; Conv(ersion) Eff(iciency); Freq(ueency) Stab(ility); Reset(ability); Beam Parameters Diam(eter) (e⁻² = 13.5% intensity level), Div(ergence) (full angle), Mode, Pol(arization).
¹ Standing wave 3 mirror cavity dye laser. Invar bar temperature stabilized, Argon-ion and Verdi DPSS pump power 5 W UV or R, B, G, pump wavelength and output power depend on dye. 20–40, 60–100 and 500 GHz 1, 2 and 3 plate birefringent tuning, noise rated between 10 Hz and 100 kHz.
² Combined dye + Ti:Sa resonator design, single frequency ring dye laser, accommodates both low (<8 W) and high power (>10 W) pump lasers, LiO₂ and KTP frequency doubling extends range down to 270 nm. Autoscan II complete automation. Example of costs for a second hand 899 system, without Ti:Sa: ~ 12,000 USD.
³ Ti:Sa/Dye ring laser: Pumped with 308 nm excimer or 532 nm Nd:YAG; invar bar resonator, Linewidth for active/passive single frequency operation 1/20 MHz, broadband 3 plate birefringent filter 2 GHz, noise between 10 Hz and 1 MHz, Labview and C++ software control.

(Continued)

TABLE 5.1.2. Continuous Wave Lasers

Manufactory & Model	Life & Power Cons. (h) & (W)	Wave-length (nm)	CW		CW		Wave-length (nm)	CW		Noise & Stabil. (rms, %)	Beam Parameters			
			Av. Pwr (mW)	Wave-length (nm)	Av. Pwr (mW)	Wave-length (nm)		Av. Pwr (mW)	Wave-length (nm)		Diam. (mm)	Div. mrad	Mode	Pol.
Gas lasers: Helium-Cadmium (He-Cd), Green/Yellow/Orange/Red Helium-Neon (He-Ne)⁷														
Cooke Corp. & He-Cd, White+ Black Knight ⁸	n.a. & <600-700	RGB 325, 441.6 RG, UV	20-50	20-50	20-50	20-50	30			<0.5	1-2	0.5-1	Single/Multi	H 100:1
Melles Griot & Green HeNe	>50,000	543.5	1							<0.5	0.88	0.81	V 500:1	
Yellow HeNe		591.1	1								0.75	0.92	random	
Orange HeNe		611.9	1								0.88	2.2		
Lasos Lasert & Red HeNe	>20,000	632.8	>10							0.5%	0.7	<1.4	TEM ₀₀ 500:1	<1.2

Legends: Lftm. (Lifetime); Pwr = Power; Cons(umption); FWHM (Full Width Half Maximum); Beam Parameters Diam(eter) (e⁻² = 13.5% intensity level), Div(ergence) (full angle), Mode, Pol(arization); Qual(ity); ph(ase); ML (Multiline; MM = Multi Mode; pp; peak-to-peak; regulation), RGB: Red Green Blue, TBM: Tunable Bragg Mirror.

¹Spectra-Physics produces similar water-cooled on lasers both in pure and mixed Argon/Krypton with Z-Lok automatic single frequency stabilization and J-Lok jitter reduction. Examples are the BeamLok 2060 and 2080 pure gas models and the Stabilite models 2017 and 2018-RM, as well as BeamLok 2060 mixed gas lasers.

²Innova Series V plasma tube, SuperInvar resonator, Model 70C: 3 phase with <40 A/phase, 8.5 l cooling water flow rate/min., Model 300C: 3 phase with <55 A/phase, 9.6 l/min. cooling water, sealed mirrors, with PowerTrack, ModeTune, ModeHop-free operation with etalon.

³3 phase with <70 A/phase, SuperInvar stable resonator, with Sentry system management complete automation for wavelength selection, mode-control, search and tune, 5 min warm-up, NuTrack active cavity length stabilization for single frequency operation, Sabre Purelight Low Divergence beam diameter and beam divergence, noise level between 10 Hz and 2 MHz.

⁴Single phase and <31 A, SuperInvar resonator, 8 l/min. cooling water, Series V plasma tube, Single line and UV emitting systems also, Innova: Series V plasma tube, SuperInvar resonator, Model 70C: 3 phase with <40 A/phase, 8.5 l cooling water flow rate/min., Model 300C: 3 phase with <55 A/phase, 9.6 l/min. cooling water, sealed mirrors, noise level with PowerTrack, ModeTune; mode-hop-free with etalon.

⁵Warm-up time 15 min., stability <1% over 2h., noise between 20 Hz and 2 MHz, beam pointing stability <30 μrad/3K.

⁶Advantage Model 163C-A 02, Noise level from 10 Hz-3 kHz, 0.3% from 10 Hz to 2 MHz.

⁷Many He-Ne suppliers exist. He-Cd are made by The Cooke Corporation, Melles Griot and Kimmon Electric, Vapor lasers. HeNe: Green 543 nm; Orange 594 nm; Red 633 nm Model LGK-7654-8 20 min warm-up time, noise between 30 Hz and 10 MHz, 5% power stability over 8h.

⁸Black + White Knight RGB-450M, RGB 441.5/537.8/635.5 nm, other non-RGB version as indicated. Warm-up time 20 min., noise between 10 Hz and 10 MHz, stability 3%/2h. (Continued)

TABLE 5.1. (Continued)

Diode lasers: Continuous Wave															
Manufacture & Model	Lifetime h	Power Cons. W	Wavel. & Spectr W. nm	Av. Power mW	Noise rms, %	Pointing Stab. μ rad/ $^{\circ}$ C	Power Stab. %	Mod. Freq. MHz	Pulse Mod MHz	Rise time msec	Beam Parameters			Qual. M ²	
											Diam. mm	Div. mrad	Mode		Pol.
Blue Sky Res & Chroma- Lase 488 ¹		<12	488 \pm 0.5 & 0.1	25	<1%	<4	<2				0.7	<1	H 100:1		
Coherent ² & Radium 375-8		<15	375 \pm 5 405 \pm 5	8 50	<1	<0.6 <0.6	<5 <5	<5 Hz <5 Hz		<200 <200	1 \times 3 4.7 \times 1.6	0.6 \times 1 0.7 \times 0.3	TEM ₀₀ TEM ₀₀	100:1 100:1	
Radium 405-50 Sapphire 460	>10k		408 460 \pm 2	25 200	<0.5	<30	<2			<5	0.7	<1.2	TEM ₀₀	V100:1 <1.1	
488-200 ³ Radium 440-16	>20k	<15	488 \pm 2 440 \pm 5	200 16	<0.5 <0.5	<30 <0.6	<2 <5	<5 Hz <100 Hz		<200 <5	0.7 1 \times 3	<1.2 0.7 \times 0.3	TEM ₀₀ TEM ₀₀	V100:1 100:1	<1.1 <1.1
Radium 635-25 Microlaser & Lepton series			635 \pm 7 375 \pm 5 408 \pm 10	25 6 36	<0.5	<0.6	<2				6 stand. or 1/2/4/	<0.7 <0.7 <0.7	TEM ₀₀ TEM ₀₀ TEM ₀₀	100:1 >100:1 >100:1	
L4 xxx-xx-TE/ Novalux Inc. ⁴ & Protera	Twice that of argonion	<20 <20 <20	460 \pm 2 488 \pm 2 532 \pm 2	5 5-20 5	<0.2 <0.2 0.2	<30 <30 <30	<2 <2				0.7 0.7 0.7	<1 <1.2 <0.8	TEM ₀₀ TEM ₀₀ TEM ₀₀	>100:1 >100:1 >100:1	<1.2 <1.2 <1.2
xxx-x Power Technology Inc. ⁵ & IQ series			375 \pm 5 405 \pm 10 440 \pm 10	8 50 16				20-100	100 MHz dig. TTL		2.5 \times 4 1.3 2.5 \times 4	<0.5 <0.5 <0.5	TEM ₀₀	100:1	
Toplica & PVL5 500 PVL5 3000	>5000 >5000		473 \pm 5 405 \pm 10	4 3.5 50							2.5 \times 4 2.1-4.3	<0.5 <0.2 <0.2	TEM ₀₀	100:1	
Fiber Lasers															
Guided Color ⁶ & Fiber laser			491	10	<1	<25								50:1 or random	<1.3
Lumics GmbH ⁷ National Laser & LasNOVA ⁸		<15	490 \pm 3 490	>5 10	<0.5 <0.5	<30	<2 <2				0.7	<1.2	TEM ₀₀ TEM ₀₀	random random	<1.1 <1.4
Unique-m.o.d.e AG ⁹ & UMFL491	>10,000	5	491.5 & <1	5-10	V 1 <0.3		<2				0.75	<1.2	TEM ₀₀	random	<1.1

Continuous Wave DPSS Lasers

Cobolt AB & Blues ¹⁰	<20	473	50	<0.5	3 (3h)	0.1	TEM ₀₀	100:1	<1.2
Samba	<20	532	100	<0.5	3 (3h)	0.1	TEM ₀₀		<6
INNOLIGHT ¹¹ & Mephisto QTL		947	500	<0.1			TEM ₀₀		<1.1
Mephisto Mephisto		1064	2000	<0.1			TEM ₀₀		<1.1
YLF Mephisto E		1319	500	<0.1			TEM ₀₀		<1.1
		1444 & 1 kHz	200	<0.1			TEM ₀₀		<1.1
LaserQuantum & Torus 532		532	50–300	<1	<5	2.5	TEM ₀₀	V 100:1	<1.1
Melles Griot ¹² & 85 BTA		442 ± 3	5/8/10	<2 pp	~2.5	0.65	TEM ₀₀	V100:1	<1.2
85 BTC		442	45						
85 BCA ¹³	<100	473 ± 5	5/10/15	<10 /h	~2.5	0.67	TEM ₀₀	V100:1	<1.2
85 YCA	<60	561	>10	<2 pp		0.67	TEM ₀₀		<1.2
				<0.5					
				<3 pp					
Rainbow Ph. ¹⁴ & BluePoint430	<60	430 ± 1	10	<0.5	<30	0.4 × 0.3	TEM ₀₀	V100:1	<1.1
473 laser	<15	473	5–40	<1	<20	0.25	TEM ₀₀		<1.2
BluePoint488	<60	488 ± 1	5	<0.5	<30	0.4 × 0.3	TEM ₀₀		<1.1
Topica ¹⁵ & Eksel 110		605	50						
		evaluation							
TorsanaLas. ¹⁶ & Starbright 488	<40	488 ± 0.3 & 0.1		<0.75	<40	0.7		V	

DPSS Kits

ALPHALAS		1064	350						
DPSS ¹⁷		532	15						

Legends: Pwr = power; Cons(umption); Wave(Length); Spectral Width given as FWHM (Full Width Half Maximum); CW: Continuous Wave; Av(erage) Power; Pointing & Power Stab(ility); Mod(ulation) Freq(ueency); Beam Parameters Diam(eter) (e⁻² = 13.5% intensity level), Div(ergence)(full angle), Mode, Pol(arization), Beam Qual(ity). DPSS: Diode Pumped Solid State lasers.

¹Noise level between 20 Hz and 20 MHz, power stability over 8 h.

²Several models with varying output available. Model 375-8 CDRH; Model 405, noise between 210 Hz and 10 MHz, modulation may save tube life.

³Noise between 20 Hz and 2 MHz, power stability after 2 hours and ±3°C.

⁴Model 460-5; Model 488-15; Warm-up time <5 min, Spectral width <0.01 nm, Noise between 20 Hz and 2 MHz typically only 0.03%, IR emission <0.1 mW, warm-up time 2 min.

⁵IQ series; Instrument Quality, beam circularization available as well as analog and digital modulation.

⁶Fiber Laser (Guided Color Technologies GmbH); noise level when stabilized otherwise >10%.

⁷Lumics LU0490F005.

⁸Noise level between 30 Hz and 100 MHz.

⁹Warm-up time <10 min, noise between 10 Hz and 10 MHz, optional single-mode fiber coupling.

¹⁰Noise level between 10 Hz–100 kHz pp, <3% between 100 kHz–5 MHz, <0.5% between 5–100 MHz, 3% long term (3 h) stability.

¹¹Ring geometry DPSS, single frequency (1 kHz) with PZT over 20 GHz tuning range, noise eater option.

¹²Warm-up time 3 min, to beam-pointing stability 10–15 min, noise between 20 Hz and 10 MHz, TEC cooled.

¹³Warm-up time <3 min, Noise between 20 Hz and 10 MHz, pointing stability for ±2°C.

¹⁴Noise between 10 Hz and 10 MHz, power and pointing stability after 2 h, 473 nm laser data from CrystalLaser Inc., warm-up time 1 min.

¹⁵Evaluation kit for GaAsSb 5 W 808 nm pumped DPSS laser.

¹⁶Warm-up time <1 min, noise level between 20 Hz and 20 kHz.

¹⁷Kit comes complete with Nd:YVO4 or Nd:YAG crystal, pump diode, mirror sets, KTP doubling crystal, 670 nm alignment laser diode, IR — VIS converter.

TABLE 5.2. Pulsed Lasers

Manufactory & Model	Pump Power mJ	Wavel. Range nm	Spectral Width mW	CW Av. Power mW	Noise (rms) (%)	Abs. Accur. nm	Wavel. Reset. nm	ASE %	Conv. Eff. %	Freq. Stab. cm ⁻¹ /°C	Beam Parameters			
											Diam. mm	Div. mrad	Pol. %	Qual. M ²
Pulsed dye lasers														
LambdaPhysik & ScanMatePro ¹	<65@355 <150@53 2 @ 40Hz	198–320 850	0.03, 0.07 –0.15 cm ⁻¹		(rms) (%)			<0.5	28		0.5			
SpectraPhys. ² & Duo	@ 1–30Hz	360–950		1.5	6 pp						2 × 3	4	<1.1	
SpectraPhys. ³ & Cobra	5–35 (gr) 5–80 (pr)	330–780	4 / 0.04 and		<0.5	<0.03	<0.005	<0.5	<20	<0.01	<1.5	<1	>98	
Cobra Stretch	8–150 (gr) 8–230 (pr)		0.02 cm ⁻¹		<0.5	<0.03	<0.005	<0.5	<28	<0.01	<1	<1	>98	
PrecisionScan	50–650 (gr) @ <100Hz				<0.5	<0.03	<0.005	<0.5	<28	<0.01	<0.5	<0.5	>98	
Pulsed nitrogen lasers														
Manufactory & Model	Lifetime h	Power Cons. W	Wavel. & Spectr W nm & Mhz	CW Av. Power mW	Noise rms, %	Pointing Stab. μrad/C	Pk-Pk Stab. %	Rep. Rate MHz	Pulse Width ps	Pk Pwr & Pk E. mW & μJ	Diam. mm	Div. mrad	Pol. %	Qual. M ²
SpectraPhysics ⁴ & VSL337		337.1	0.1				<4 pp	1–20Hz	4 k	30 kW & >120	3 × 7	3 × 8		
Pulsed excimer lasers														
Lambda-Physik & LPXPro		308 351		38 k 28 k			<0.0001 <0.0001		n.a. & 400 320					
Tuulaser AG & Excistar ⁵		308 351		3.5 k 3.5 k			<2 <2	10,000 10,000	n.a. & 8 8	3 × 6 3 × 6	<1 × 2 <1 × 2			
Pulsed diode lasers														
Becker-Hickl ⁶ & BDH series			375, 405, 475, 635, 680, 780	0.8, 2.4 0.9, 0.5, 10			<2	20 50 80	60, 60, 50, 50, 80	100–500				
Hamamatsu ⁷ & PLP-10		90	375, 405, 440, 470, 635, 650, 670, 780, 850 All ±10	70, 50, 20, 20, 30, 30, 30, 30, 50 pk power				2 Hz–100	100				15–34, 13–22, 10–35, 10–30, 12–32	

Pulsed diode laser		0.1–1500			
PicoQuant ⁸ & MDL 300	400, 635				
PicoQuant & PDL LDH	670, 805 375, 405, 440, 470 & ±3	<1 3 pp	<70, 70, 90, 90 narrow	>90 >90	
PicoQuant ⁹ & PDL LDH xxx	635, 637, 655, 660, 665, 670, 690, 735, 757, 785, 806, 830, & ±7	1.0, 1.0, 1.0, 1.0 (wide) 3.5, 8.0, 6.0, 8.0, 4.0, 2.0, 8.0, 3.5, 6.0, 8.0, 10, 4.0 (wide)	<1% 40–80 3%pp	>90 >90 >90 >90 >90 >90 >90	
PicoQuant ¹⁰ & PicoTA	530 (SHG) 780–790, 1060.1065	600 160 150	narrow <50 <90 <80	600 7000 6000	

Pulsed vapor lasers

Oxford Lasers ¹¹ & LS 10-10		2.5 k to 3.3			
LS 20-10	510.6 578.2	<3 k <3 k 20 k	<5 <5 0.01 0.01	40 80 80	4 4 4
LS 20-50		<3 k	<2 0.01	80 140	4 4

Legends: Power Consumption; Spectral Width; CW: Continuous Wave; Average Power; Absorbance Accuracy; Wavelength; Resonator; ASE: Asynchronous Spontaneous Emission; Conversion Efficiency; Frequency; Pointing, Power and Peak-to-Peak (pk-pk & pp) Stability; Repetition Rate; Peak Power (Pk Pwr) & Pulse Energy; Beam Parameters Diameter (e² = 13.5% intensity level), Divergence (full angle), Mode, Polarization, (Beam) Quality.

¹Model ScanMatePro 2 pumped by both <40Hz 308nm Excimer and <50Hz 355 as well as 532nm Nd-Yag, 28% pump efficiency at 560nm with Rhodamine 6G, frequency extension to 198nm.

²Nitrogen laser pumped, pulse width >4ns.

³Cobra series and PrecisionScan pumped by 355 nm and/or 532 nm at 10–100Hz with Nd-Yag laser, LambdaLok (LLOck) frequency stability <0.01 cm⁻¹ in Table LLock for PrecisionScan conversion efficiency at 570nm, Labview and Sirah control software, SHG and THG packages available.

⁴Air-cooled VSL-337, int. and ext. trigger capabilities.

⁵Excistar S-500 suitable as pump source for cuvette type dye laser.

⁶Warm-up time 3 min., average power at 80 MHz pulse repetition rate, pulse width at 50MHz @ 1 mW power, more red wavelengths available.

⁷Output peak power after fiber pigtail, wide pulse (listed) and narrow pulse operation.

⁸MDL: Modulated diode lasers, modulation frequency range 100kHz–1.5GHz.

⁹Repetition rate 80 MHz but other frequencies possible, pulse width with narrow pulse listed; with high power adjust pulse width is ~<300ps for wavelengths below 470nm; above 470nm pulse width in high power adjust mode <400 ps (wide pulse listed), selected 50 ps devices, narrow distributed feedback bandwidth available, 440nm also available with 3 mW average output.

¹⁰80, 40, 20, 10 and 5 MHz pulse repetition rates and adjustable rates, ASE <50 mW @ 780 nm, <60 mW @ 1060 nm, fiber coupling losses <5% IR in multimode and <0.1% for single-mode fiber-optic, spectral linewidth 4 nm or 0.1 nm (narrow).

¹¹Water consumption 2l/min, 80% of plane-plane cavity output within 0.6 mrad, Timing jitter (ps): LS10-10/20-10: 5 ps, LS 20-50: 2 ps, LS 35: 3 ps.

(Continued)

TABLE 5.2. Pulsed Lasers (Continued)

Manufactory & Model	Lifetime h	Power Cons. W	Wavel. & Spect W nm/Mhz	Peak Energy mJ	Jitter/ Noise, rms ns / %	Pointing Stab. µrad/C	Power Stab. %	Mode lock Freq. MHz	Pulse Width ps	Pulse Width mW & µJ	Beam Parameters		
											Diam. Mm	Div. mrad	Pol. Dir/%
Pulse lasers: Ultrafast kits													
Avesta Project & TIF-kit-xx			740–840 720–980	<250 <1k	n.a. & <2				<20 <100		<2	TEM ₀₀	H
CrF-kit-65P			1,240–1,270	<250					<65 s				
DelMarVentures FemtoStart ¹⁸			780–840, 740–950,	250–1 k				<5	70–140		2–3	TEM ₀₀	H
TISSA-20-100 ¹⁹			710–950						20–200				
Kapteyn- Murnane Labs. & TS ²⁰			780–810 750–840 & >60 nm	400–450	n.a & 4.4–6.1				<15 fs <25 fs		~1	~ TEM ₀₀	H

Legends: Power Consumption; Wavelength; Spectral Width at Half Maximum (FWHM); Average Power; Timing Jitter; Pointing and Energy Stability; Repetition Rate; Peak Power (Pk Pwr) & Pulse Energy; Beam Parameters Diameter, Divergence, Mode, Polarization, Beam Quality; Warm-up time 3 min., average power at 80 MHz pulse repetition rate, pulse width at 50 MHz @ 0.5 mW power, more red wavelengths available.

²Ti:Sa pump laser, pulse repetition rate 1–10 kHz, Peak energy at 1 kHz pulse repetition rate, noise over 8 hours < 1% (rms).

³Noise level defined between 10 Hz to 1 GHz, consumes 600 W, linewidth without etalon < 1 GHz, birefringent tuner for 1000–1060 nm region.

⁴Single-mode Nd:YVO₄ ring laser.

⁵Nd:YLF pump laser for ultrafast amplifiers.

⁶Ti:Sa pump laser, noise between 10 Hz and 100 MHz, Quiet Multi-Axial Mode doubling (QMAD), laserhead closed chiller cooled for 5, 6, 8 an 10 W versions.

⁷Model Vanguard 2000-HM-32, Nd:YVO₄ lasing crystal, warm-up time 30 min, to full specifications 1 h, cooling air flow 300 cfm.

⁸Nd:YVO₄ crystal, non-linear crystal, set of mirrors, Cr:YAG passive Q-switch, Glan-Taylor prism.

⁹Pumped by Verdi lasers, with Power Pulse automatic pulse width optimization, and Power Track active alignment, with MRU miniature air re-circulator unit for easy access to red wavelengths.

¹⁰Verdi model V8, Innova Argon-ion model 310-8 or V10 pump or Sabre ion laser 14 W pump laser, Optima version provides complete automated optimization, version S (imple) has manual starter, external Mira model 9300 harmonic generator with LBO crystal doubler with BBO frequency mixing crystal.

¹¹Ti:Sa oscillator with built-in Verdi pump laser, noise between 10 Hz and 10 MHz.

¹²(FemtoSource) Scientific SL.

¹³Ti:Sa model IC-xxx-400 many options w.r.t. crystals, pulse width, repetition rate, and PicoTrain, Nd:YVO₄ crystal, output stability over 24 h.

¹⁴Pump source for OPA, includes Nd:YLF pump laser, stretcher, seed oscillator with multi-pass regenerative amplifier, compressor.

¹⁵Peak power at 800 nm, closed loop chiller, StabiLok beam pointing stability.

¹⁶Millennia V and X pump lasers, broadband optics, extra mirror set for wavelength range 970–1080 nm, average power at 970 nm, noise between 10 Hz and 2 MHz.

¹⁷Birefringent tuner.

¹⁸5 W DPSS pump recommended, KLM mode-locking.

¹⁹3–10 W DPSS or ion laser pump power.

²⁰Pump power 6–10 W Ytterbium fiber pump, options available w.r.t. emission center, bandwidth and average power; FemtoRainbow 100 OPO KTP based delivers ~ 100 fs pulses.

Ultrafast fiber lasers

Manufactory & Model	Wavelength nm	CW Av. & Pk Power mW & kW	Time-jitter & Noise rms, ns/ %	Rep Rate ML MHz	Pulse E & Width (FWHM)			Beam Parameters					
					ML mJ & ps	Q-sw μ J & nsec	ML & Q-sw mJ & nsec	Diam. mm	Div. mrad	Mode	Pol. Dir/%	Qual. M ²	
IMRA & FemtoLiteA25	780	25		50	180 fs								
FemtoLiteFX-10	800	10		50	120 fs								
Topical ¹ & FFS-F-C Sys	1,150–1,400	100			100 \pm 10 ps						TEM ₀₀	H	

Optical parametric oscillators (OPO)

Coherent Mira-OPO ²	525–665 KTP-ring-SHG 1050–1330 KTP linear/ ring-IR 1350–1600	>200–240 high power version	n.a. & <2 rates	76 MHz or other rep.	<1.2 ps <200		<1.25	<1.30	TEM ₀₀	H >99		
Continuum & Sunlite EX ³	445–1750 & <0.075 870–1550 (s) 1570–5600 (i)	200(s)	n.a. & \pm 10	10 Hz	350 mJ @ 355 nm	50 mJ & 3–6 ns n.a. & <250	~4–6	<1.5		V 99%		
GWU-Lasertechnik & Synchro fs ⁴	1050–1510(s) 1590–3100(i)	250(s)				n.a. & <2k						
Spectra-Physics ⁵ & OPAL 1.3	1100–1250	250	<5 stability	80 MHz	n.a. & <130 ps		<2.0	<1.0	TEM ₀₀	H >99		
OPAL 1.5	1350–1600	200	<0.2		<130 ps							

Optical parametric amplifiers (OPA)

Coherent ⁶ & OPA 9800/9850	1150–1600 (s) 1600–2630 (i) 400–1150 (SHG) 300–400 (FHG)		<2 (8h)		>1 @ 800nm 1 kHz	120 μ J @ 1.3 μ m & <130 <130 f				H(s) V (i)		
Quantronix ⁸ & TopasPlus Hep	417–2400 245–1700				n.a. & 5–50 ps 80–150 ps	10–50 10–50						
Topas White	1048	4k		<7	n.a.							99.9
Spectra-Physics & Eclipse	524	1.5 k			<500 ps							

Legends: Av(erage) & Peak (Pk) Power; Rep(ition) rate; ML: Mode-Locked; Q-switched; Pulse E(nergy); Beam Parameters Diam(eter) (e⁻² = 13.5% intensity level), Div(ergence) (full angle), Mode, Pol(arization), (Beam Qual(ality); i: idler; n.a.: not applicable; s: signal).

¹ FFS-F-C laser system: FemtoSecond Scientific Er doped fiber laser.

² 500 mW Mira 900 pumping threshold, ring cavity version gives more output power, active length stabilization, sealed and purgeable, intracavity doubling for VIS range, NCPM crystal.

³ Precision II models 355 nm pump laser, temperature stabilized, narrow bandwidth related replacement for a dye laser.

⁴ 720–800 nm 1.2 W 100 fs pump at 1200 nm.

⁵ Millennia X pump, LBO crystal, active stabilization, frequency doubler for visible, automated.

⁶ OPA 9400/9450 and 9800/9850: RegA wavelength extension for Vis (blue) – NIR; RegA wavelength extension for NIR – MIR. RegA 9000/9500: High repetition rate, < 260 kHz, ultrafast amplifiers, high spatial beam quality, one RegA can pump two OPAs. Other systems are: Hidra: custom high energy; Legend: Flexible Ti:Sa regenerative amplifier; Libra: One box, ultrafast Ti:Sa amplifier.

⁷ gap free tuning from UV < 300 nm — MIR for wavelength extension of Legend and Libra, fs, lowest optical noise, automated, white light initiated OPA.

⁸ Traveling Wave Optical Parametric Amplifier: femtosecond (listed) and picosecond pumped at e.g. 800 and 355 nm as well as 400 nm.

Their contrasting characteristics are described near the end of this section. As a pump source for Ti:Sa lasers, the argon-ion laser has been completely replaced by solid-state lasers.

In general, argon-ion laser stability is better than that of a krypton laser, with less gas slushing. The output power of krypton is at best about 30% that of argon under identical lasing conditions. Commercial systems provide a large variety of emission wavelengths and output powers (Fig. 5.8; Ginouves, 2002).

Fragile quartz plasma tubes have been replaced by versions with rugged metal-ceramic (beryllium oxide, BeO) envelopes for the small- and medium-frame lasers, which usually do not use a magnet to confine the plasma to the central core of the tube. External copper disks remove the heat.

Large systems with high output power use alumina (Al_2O_3) ceramic tubes with brazed tungsten/copper disks inside, crystalline quartz-coated windows, and quartz-metal hard seals (Fig. 5.4). This significantly increases their reliability. Other systems use rugged metal/ceramic BeO tubes. The central bore defines the laser beam. The plasma discharge pumps ions towards the cathode end of the tube, creating a pressure gradient between the anode and cathode. Return holes around the outer edge of the disks form an internal gas return path to maintain a uniform gas pressure along the length of the plasma tube. Typical gas pressure inside a gas laser is about 1 Torr.

The formation of color centers in the Brewster windows has been reduced by the use of better quality window materials, increasing the lifespan of this type of laser to 3000 to 10,000 h. However, tube lifetime may only reflect the life of the strongest laser line and not that of the weaker lines needed on a daily basis. The stability of these lasers, especially the small- and medium-sized models, is good because they can be cooled by convection (passive cooling) or forced air and their electric power requirements are modest. Some models are portable and easy to use. The larger frame lasers provide active resonator stabilization.

The wall-plug efficiency of large argon-ion models is very low (0.1% typical) and the heat generated must be removed with a large amount of cooling water. The turbulent flow causes some vibration in these systems while insufficient water flow may cause the cooling water to boil and lead to the destruction of the plasma tube. The large amount of heat generated also puts more strain on the

resonator cavity. Longer warm-up times are necessary when no active stabilization is incorporated in the design. The operational lifetime of the higher power laser tubes is now well above 2000 h.

Using proper interference filters (inserted with their shiny side towards the laser head to prevent damage) or emission line selection by means of an AOTF or AOBS, single, strong laser lines can be selected. Dual line emitting argon-ion lasers simultaneously emitting at 454 to 457 nm and 514 nm are also offered (Table 5.1).

When the experiment requires a reliable, stable source of deep UV radiation at power levels of tens to hundreds of milliwatts, these ion laser systems provide a better alternative than any current solid-state UV laser system, as these require more steps to produce UV light. The area where the argon-ion laser was very valuable, as a 5 to 10 W pump source for solid-state Ti:Sa lasers, has been completely overtaken by solid-state lasers.

Argon-ion Laser Lines in the UV

Commercially available argon lasers have emission wavelengths extending from 275 to 528 nm (Hecht, 1992). Higher discharge currents are necessary to populate the doubly-ionized argon levels sufficiently for deep UV emission. Wavelength selection is obtained by installation of an angle-tuned quartz prism or other appropriate optical elements. The wavelength range is extended down to 229 nm by inserting a frequency-doubling crystal in the laser cavity and thereby producing a CW intra-cavity frequency-doubled argon-ion laser system (FreD™, Coherent, see Tables 5.1, 5.3). Reported power levels range from 40 mW at 228.9 nm to 1 W at 257 nm for the model Innova Sabre MotoFreD™.

Argon-Ion Laser Lines in the Visible Region of the Spectrum

The most common spectral region for lasers in microscopy is between 488 and 514 nm, although argon-ion laser emission can be obtained at 454.5, 457.9, 465.8, 472.7, 476.5, 488.0, 496.5, 503.7, 514.5, and 528.7 nm.

Krypton-ion

When strong red emission at 647.1 nm is needed to expand the spectral coverage of an argon-ion laser, a krypton laser is the system of choice (Brelje *et al.*, 1993). Its stability is slightly less than that of a comparably sized argon-ion system and the gas retention in the graphite disks is slightly larger. However, with active stabilization now available and with ceramic tube technology firmly in place, both drawbacks have now been largely overcome. Based on the FreD™ argon-ion laser, see above, we may see the introduction of an intracavity frequency-doubled krypton-ion laser as a source for UV light at 376, 338, 323, 284, and 266 nm.

Mixed Gas Argon-Krypton

Combining the best properties of the argon and krypton ion lasers creates a reliable laser with stable output and the broad spectral coverage needed for multicolor CLSMs. Brelje and colleagues (1993) used an air-cooled 15 mW Kr-Ar ion laser for multicolor immunofluorescence microscopy and described a range of applications. Several companies produce systems delivering several tens of milliwatts, for example, Melles Griot (Table 5.3).

Helium-Neon

The use of these stable, inexpensive lasers in microscopy has been somewhat limited by their relatively low intensity and predominantly 632.8 nm red emission. Several manufacturers have recently

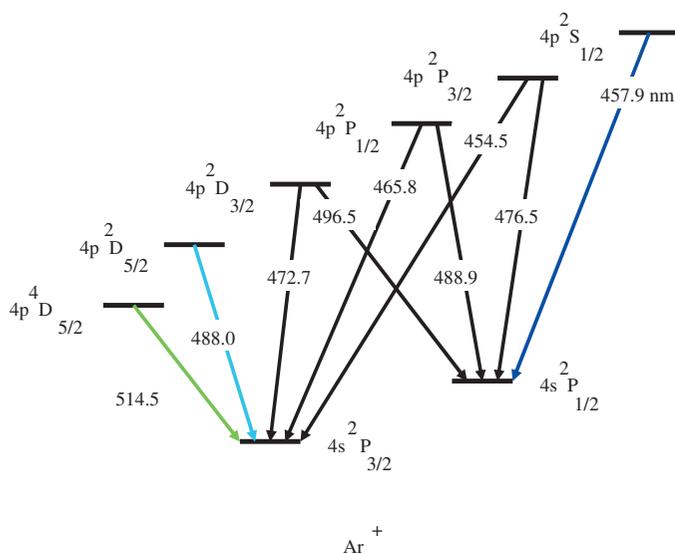


FIGURE 5.8. Schematic energy diagram for an argon-ion laser.

introduced He-Ne lasers with emission at 534, 594, 612, and 632.8 nm and lines in the infrared, for example, 1152 nm (Fig. 5.9). To better excite Texas Red, Alexa 594, and several other dyes, the red He-Ne at 632.8 nm and the green He-Ne (GreNe) at 543 nm have been joined by the orange He-Ne at 594 nm. Typical powers for the 632.8 nm line range from 0.5 to 10 mW with a maximum of 75 mW. Although increased competition is expected from solid-state, semiconductor, and diode lasers, the He-Ne laser still competes well thanks to its good beam quality, shorter wavelengths, and long lifetime. Invar-stabilized He-Ne frames are now available from Research Electro-Optics Inc. (Table 5.3). The 632.8 nm can be used to create an autofocus feedback circuitry that automatically adjusts for specimen vertical movement (ASI, Zeiss, Table 5.3).

Helium–Cadmium

Helium–cadmium (He–Cd) lasers have found several applications in microscopy. Usually they operate at three emission wavelengths: 325, 354, and 442 nm (Fig. 5.10), with power levels up to several hundred milliwatts (Hecht, 1993c). The shortest wavelength requires special optics and is very rarely used in fluorescence microscopy. Membrane probes such as Indo-1 and Fura-2 can be conveniently excited with the 354 nm line. The emission at 442 nm is ideal for excitation of cyan fluorescent protein, flavins, and other fluorescent molecules. The power available at 325 and 354 nm is generally on the order of a few to tens of milliwatts, while at 442 nm up to 150 mW is now available.

The stability of these lasers is substantially less than that of the argon-ion lasers. In the 325 nm region, intensity fluctuations of 10% to 20% are not uncommon but the emission tends to be more stable at 442 nm. Although most He–Cd systems had an operational life of a 1 to 1.5 years (1000 to 2000 h), a new mirror technology has increased the lifetime of the coatings to > 5000 h for 325 nm mirrors. The less powerful, smaller frame lasers, operating at 325 nm, may still have a limited life span. One manufacturer now supplies a sealed, multiple-wavelength version with up to 10,000 h lifetime (see Table 5.2).

The He–Cd laser experiences heavy competition from the numerous blue diode lasers coming to market in the wavelength region spanning roughly 400 to 450 nm.

Alkali Vapor Lasers

Diode-pumped, alkali lasers (DPAL) can generate blue laser emission by a two step up-conversion scheme (Beach et al., 2004; Krupke, 2004). Cesium and rubidium vapor CW lasers pumped by diode lasers either at 852 or 859 or with 876 or 921 nm (Cs) or at 780 or 795 or with 761 or 776 nm (Rb), emit at 455 or 459 (Cs) and 420 or 422 nm (Rb). With their low cost and compact design, potential 50% efficiency, high average power (>100 mW), easy scalability, and good beam quality, they will be competition for thin disk and fiber lasers and show a great potential.

Dye Lasers

As all dye lasers for confocal microscopy are optically pumped by other powerful lasers, they are not so much lasers as wavelength shifters. Suitable pump sources are excimer, ion, neodymium–yttrium aluminum garnet (Nd–YAG), neodymium–yttrium lithium fluoride (Nd–YLF), and metal vapor lasers. For CW operation, the dye is circulated to prevent heating and bleaching, to reduce the competing triplet-state population, and to remove dye molecule aggregates. Compared with gas lasers, the emission spectra are quite broad and this permits them to be tuned to lase over a fairly

broad wavelength band. This band can be extended by changing dyes. Tuning of the laser is accomplished by a prism, a diffraction grating, and a stack of birefringent plates inserted at the Brewster angle or by etalons (Demtröder, 1996; Mollenauer and White, 1987). The laser medium is easy to handle, inexpensive, and consists of a fluorescent dye dissolved in a solvent. Because of so-called “forbidden transitions,” the molecules tend to pile up in the first excited triplet state and thereby become unavailable for lasing. Rapid pumping or the use of a dye-jet stream reduces the population of this triplet state. In order to keep the quantum yield high, the dye is usually cooled to minimize competing processes, such as collisional and vibrational de-excitation. Intrinsic optical efficiencies as high as 20% or 30% can be obtained (not counting the efficiency of the pump laser) reaching an output power of several hundred milliwatts.

Operating a dye laser is sometimes rather cumbersome, involving bottles of spent laser dye and waste materials, dye spillage, and often large quantities of dye solvent that must be stored in a safe storage area. Cuvettes holding laser dye have to be cleaned regularly because the pumping beam bakes dye molecules onto the optical surfaces. Although solid-state lasers have largely replaced the dye laser, they still can fill several spectral gaps between 390 nm and the IR.

The intensity stability of dye lasers is lower than that of its lamp or laser pump source. Two percent to 5% intensity stability is considered good. Beam pointing stability is heavily influenced by the regular tuning done on these systems. Distances should be kept as short as possible or fiber-optic beam transport should be considered.

The spectral regions covered, tuning curves, and the output power offered can be found in the manufacturer’s documentation.

Solid-State Lasers

An ever-growing number of power-efficient solid-state lasers is being offered for use in confocal microscopy. They are particularly successful in replacing inherently inefficient gas lasers. Their versatility and ease of operation either for use as CW or pulsed direct light sources or for use as pump sources for other lasers guarantees continued rapid development. There is a trend towards smaller more efficient all-in-one-box designs and a drive to increase total average power by creating laser arrays.

- **Semiconductor lasers:** Most are based on gallium mixtures, emit a single line in the red or NIR and can be pumped electrically with very high efficiency. SHG generation in an appropriate crystal creates UV, blue, and green emission. The number and versatility of these lasers increases daily.
- **Non-tunable solid-state laser:** Another class of laser is formed by the Nd–YAG, Nd–YLF, and neodymium–yttrium ortho vanadate (Nd–YVO₄) systems operating at 1064, 1047, and 1064 nm, respectively. These can be used as a stand-alone light source or serve as a pump for dye-lasers and titanium:sapphire amplifier systems.
- **Tunable solid-state ultrafast lasers:** These are epitomized by the titanium:sapphire laser systems generating very short pulses down to a few to tens of femtosecond. They provide a very broad tuning range, comparable with that of dye lasers, but they are much easier to operate, generate a comparable or higher output, provide significantly better stability, and can be built in a range of configurations. They can also generate stable, high-power picosecond and femtosecond pulses as we will discuss later.

TABLE 5.3. Sample Listing of Manufacturers

ALPHALAS GmbH, Göttingne, Germany, +49-(0)551-770-6146, <http://www.alphalas.com/>
 Amplitude Systemes, Talenc, France, +33-(0)5-4000-3447, <http://www.amplitude-systemes.com>
 AVESTA PROJECTS Ltd., Troitsk/Moscow region, Russia, +7-(0)95-334-0078, <http://www.avesta.ru/>
 ASI, Applied Scientific Instrum., Eugene, OR, (800)-706-2284, <http://www.ASIimaging.com>
 BATOP GmbH, Weimar, Germany, +49-(0)172-3520029, <http://www.batop.com/index.html>
 BEOC, Brocton Electro-Optics Corp., Brockton, MA, (508)-559-9958, <http://www.brocktoneo.com/services.htm>
 Blue Sky Research, Milpitas, CA, (408) 941-6068, <http://www.blueskyresearch.com/index.htm>
 Bromba GmbH, München, Germany, +49-(0)89-3266-8930, <http://www.bromba.com/indicard.htm>
 Cambridge Laser Laboratories, Fremont, CA, (510)-651-0110, <http://www.cambridgelasers.com/>
 CDP Inc. or Systems, represented by Edinburgh Instrum. Ltd. Inc, <http://www.edinst.com/agencylaser.htm> and PicoQuant GmbH.
 Clark-MXR, Inc., Dexter, MI, (734)-426-2803, <http://www.cmxr.com/gate.htm>
 Cobolt AB, Sticckholm, Sweden, +46-(0)8-545-91-230, <http://www.cobolt.se/>
 Coherent Inc., Laser Group, Santa Clara, CA, (800)-527-3786, <http://www.cohr.com/> and <http://search.coherentinc.com/cgi-bin/MsmFind.exe?RESMASK=MsRes.msk&CFGNAME=MsFind.cfg&QUERY=brochures>
 Continuum, Santa Clara, CA, A division of Excel Technology Comp., (888)-532-1064 <http://www.continuumlasers.com/mainswf.html>
 The Cooke Corp., Auburn Hills, MI, (248)-276-8820, <http://www.cookecorp.com/>
 CSK Optronics, Torrance, CA, (310)-574-8181, csk@usa.com
 Crystal Fibre, A/S, Birkerød, Denmark, +45-(0)4348-2820, <http://www.crystal-fibre.com/>
 CrystaLaser Inc., Reno, NV, (775)-348-4820, <http://www.crystalaser.com/homenew.html>
 Del Mar Ventures, San Diego, CA, (858)-481-9523, <http://www.femtosecondsystems.com/index.php>
 Edmund Optics Inc., Barrington, NJ, (800)-363-1992, <http://www.edmundoptics.com/>
 Edmund Scientific, Tonawanda, NY, (800)-728-6999, <http://scientificsonline.com/Default.asp?bhcd2=1099033491>
 Elektronik Laser Systems GmbH, <http://www.els.de/Products/vdisk.html>
 Electro Optical Components, Inc., Santa Rosa, CA, (707)-568-1642, http://www.eoc-inc.com/ir_sensor_cards.htm
 Evergreen Laser Corp., Durham, CT, (860)-349-1797, <http://www.evergreenlaser.com/Default.htm>
 Excel/Quantronix Corp., East Setauket, NY, (631)-784-6100, <http://www.quantron.com/>
 Femtolasers Productions GmbH, Vienna, Austria, +43-(0)1-503-70020, and Femtolasers Inc., Harvard, MA, (978)-456-9920, <http://www.femtolasers.com/>
 Fianium Ltd., Southampton, UK, +44-(0)2380-458776, <http://www.fianium.com>
 Guided Color Technologies GmbH, Jena, Germany, +49-(0)3641-675350, <http://www.gc-tec.com/>
 GWU-Lasertechnik mbH, Erfstadt, Germany, +49-(0)2235-955220, <http://www.gwu-group.de/laser/index.html>
 High Q Laser, Hohenems, Austria, +43 (0)5576-43040, and High Q Laser (US) Inc., Watertown, MA, (617)-924-1441, <http://www.highqlaser.at/> and <http://www.HIGHQ-US.com/>
 Holo-Spectra Inc., Van Nuys, CA, (818)-994-9577, <http://www.lasershs.com/index.html>
 IMRA America, Inc. (IMRA: Institut Minoru de Recherche Avancee, now IMRA Europe S.A.), Ann Arbor, MI, (734)-930-2590, <http://www.imra.com/>
 Ingeneric GmbH, Aachen, Germany, +49-(0)241-963-1343, <http://www.ingeneric.com/>
 INNOLIGHT GmbH, Hannover, Germany, +49-(0)511-760-7270, <http://www.innolight.de/products/start.htm>
 Kapteyn-Murnane Laboratories, Inc., Boulder, CO, (303)-544-9068, <http://www.kmlabs.com/>
 Kimmon Electric USA., Englewood, CO, (303)-754-0401, Edinburgh Instrum. Ltd. rep <http://www.edinst.com/agencylaser.htm>
 Lambda Physik AG (sub. of Coherent Inc.), Göttingen, Germany, +49-(0)551-69380, <http://www.lambdaphysik.com/>
 Laser Diode, Inc. (part of Tyco Electronics Co.), Edison, NJ, (732)-549-9001, <http://www.laserdiode.com/>
 Laser Innovations, Santa Paula, CA, (805)-933-0015, http://www.laserinnovations.com/coherent_lasers.htm
 Laser Quantum Ltd, Stockport, Cheshire, UK, +44-(0)161-975-5300, <http://www.laserquantum.com/>
 Laser Resale Inc., Sudbury, MA, (978)-443-8484, <http://www.laserresale.com/>
 LaserMax Inc., Rochester, NY, (585)-272-5420, <http://www.lasermax-inc.com/>
 Lasermet Ltd., Laser Safety Solutions, Bournemouth, UK, +44-(0)1202-770740, <http://www.lasermet.com/>
 Lasiris Inc. (Div. of StockerYale), StockerYale Canada, Montreal, Quebec, Canada, (800)-814-9552
 National Laser Corp., Salt Lake City, UT, (801)-467-3391, <http://www.nationallaser.com>
 Lasos Laser Technik GmbH, Jena, Germany, +49-(0)3641-29440, <http://www.lasos.com/index.htm>
 Lexel Laser Inc. (div. of Cambridge Laser Laboratories), Fremont, CA, (510)-651-0110, <http://www.lexellaser.com/>
 LFW, Laser Focus World Buyers Guide, PennWell Corp., 2004, Nashua, NH, (603)-819-0123, <http://lfw.pennnet.com/home.cfm>
 LG Laser Technologies GmbH, Kleinostheim, Germany, +49-(0)6027-46620, <http://www.lg-lasertechnologies.com/>
 LIA, Laser Institute of America, Orlando, FL, (800)-345-2737, <http://www.laserinstitute.org/>
 Limo Lissotschenko Mikrooptik GmbH, Dortmund, Germany, +49-(0)231-222410, <http://www.limo.de/>
 LUMERA LASER GmbH, Kaiserslautern, Germany, +49-(0)6301-703-181, <http://www.lumera-laser.de/index2.html>
 Lumics GmbH, Berlin, Germany, +49-(0)30-6780-6760, <http://www.lumics.de/>
 Lumitek Intl. Inc., Ijamsville, MD, (301)-831-1001, <http://www.lumitek.com/Sensorcatalog.PDF>
 Mauna Kea Technologies, Paris, France, +33-(0)1-4824-0345, <http://www.maunakeatech.com/>
 Melles Griot, Laser Div., Carlsbad, CA, (800)-645-2737, <http://www.mellesgriot.com/contactus/default.asp>
 Micro Laser Systems, Garden Grove, CA, (714)-898-6001, <http://www.microlaser.com/blueConfocal.html>
 MWK Industries, Corona, CA, (909)-278-0563, <http://www.mwkindustries.com/>
 Newport Corp., Irvine, CA, (800)-222-6440, <http://www.newport.com/>
 New Focus, Inc. (a division of Bookham), San Jose, CA, (408)-919-1500, <http://www.newfocus.com/>
 Noran Instruments Inc., see Prairie Technologies, Inc.
 Novalux Inc., Sunnyvale, CA, (408)-730-3800, <http://www.novalux.com/contact.html>
 Oxford Lasers Inc., Littleton, MA, (978)-742-9000, <http://www.oxfordlasers.com/>
 Optronics Technologies S.A., Athens, Greece, +30-(0)210-983-7121, <http://www.optronics.gr/>
 OZ Optics, Ltd., Carp, Ontario, Canada, (800)-361-5415, <http://www.ozoptics.com/>

TABLE 5.3. (Continued)

P.A.L.M Microlaser Technologies AG, Bernried, Germany, +49-(0)8158)-99710, <http://www.palm-microlaser.com/>
 Photonic Products Ltd., Bishops Stortford, Hertfordshire, UK, +44-(0)1279-719190, http://www.photonic-products.com/company_ov.htm
 Picoquant GmbH, Berlin, Germany, +49-(0)30-6392-6560, <http://www.tcspe.com/>
 POC, Physical Optics Corp., Torrance, CA, (310)-320-3088, <http://www.poc.com/>
 Point Source, Hamble, UK, +44-23-80-744500, <http://www.point-source.com/products.asp>
 Power Technology Inc., Little Rock, AR, (501)-407-0712, <http://www.powertechnology.com/pti/index.asp>
 Prairie Technologies Inc., services Noran microscopes, Middleton, WI, (608)-662-0022, http://www.prairie-technologies.com/index_files/page0005.htm
 Quantronix (see Excel/Quantronix)
 R&D Ultrafast Lasers Kft., Budapest, Hungary, +36-(0)1-392-2582, <http://www.szipocs.com/products.php>
 Rainbow Photonics AG, Zürich, Switzerland, +41-0)1-445-2030, <http://www.rainbowphotonics.com/>
 Research Electro-Optics, Boulder, CO, (303)-938-1960, <http://www.reoinc.com/>
 Rockwell Laser Industries, Inc., Cincinnati, OH, (800)-945-2737, <http://www.rli.com/>
 Roithner LaserTechnik, Vienna, Austria, +43-(0)1-586-52430, <http://www.roithner-laser.com/>
 Sacher Lasertechnik Group, Marburg, Germany, +49-(0)6421-3040, <http://www.sacher-laser.com/>
 Spectra-Physics Lasers and Photonics, Inc., Mountain View, CA, (800)-775-5273, <http://www.spectra-physics.com/> and Darmstadt, Germany, +49-(0)6151-7080
 StockerYale, Inc., Salem, NH, (603)-893-8778, <http://www.stockeryale.com/i/lasers/index.htm>
 Teckhnoscan Laser Systems, Novosibirsk, Russia, +7 (0)3832-397224, <http://www.tekhnoscan.com/english/index.htm>
 Toptica Photonics AG., Martinsried, Germany, +49-(0)8989-99690, <http://www.toptica.com/index.php>
 Torsana Laser Technologies A/S, Skodborg, Denmark, +45-(0)4556-0056, <http://www.torsanalaser.com/index.html>
 Time-Bandwidth Products, Inc. Zürich, Switzerland, +41 (0)44-445-3120, http://www.tbwp.com/Time_Bandwidth/Home/FrameSetHome.htm
 TuiLaser AG, Germering/München, Germany, +49-(0)89-894070, <http://www.tuilaser.com/products/lasers/index.htm>
 Uvex, Smithfield, RI, (800)-343-3411, <http://www.uvex.com/>
 Visitech International Ltd., Sunderland, UK, +44-(0)191-516-6255, <http://www.visitech.co.uk/index.html>

Semiconductor or Diode Injection Lasers

Charge carriers in a semiconductor material can be pumped either optically or by an electric current (Fig. 5.11). Electron-beam-pumped semiconductor lasers can emit 3 to 5 W in the 480 to 650 nm range (Hobbs, 1993), but the most common excitation method simply starts with a diode not unlike that in a light-emitting diode (LED) and uses an externally applied current in the forward-biased direction. This process can have an efficiency as high as 80%. To do so, the electrons must be confined not to a bulk

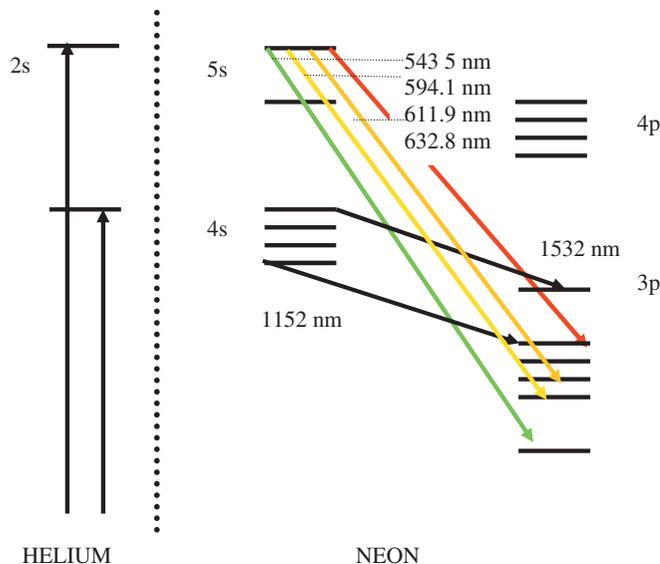


FIGURE 5.9. Schematic energy diagram for a helium–neon laser. Helium functions as a pump energy transferring buffer for neon.

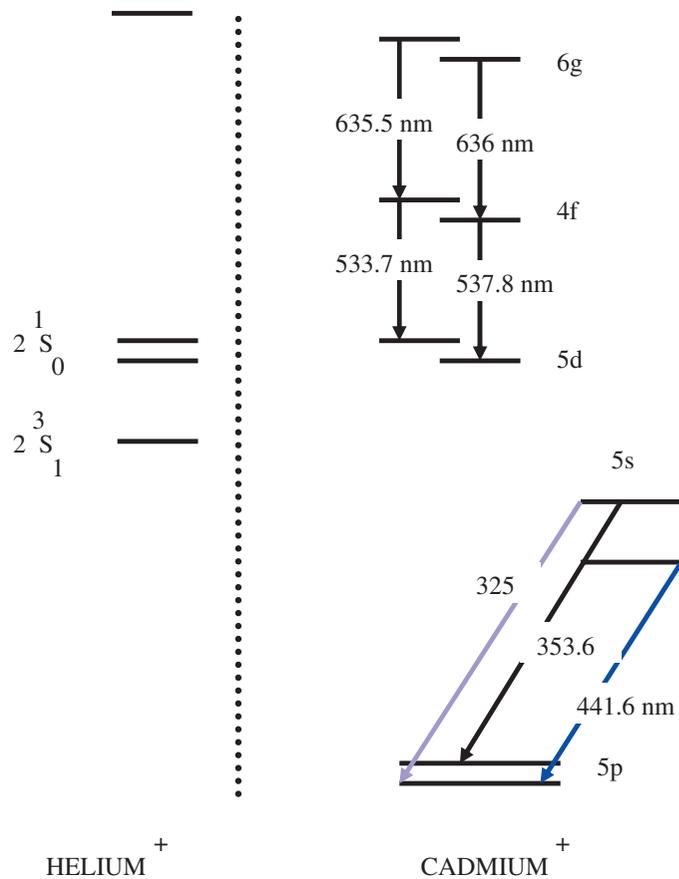
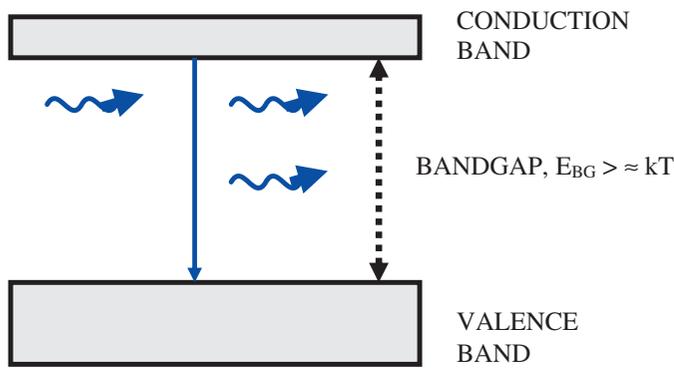


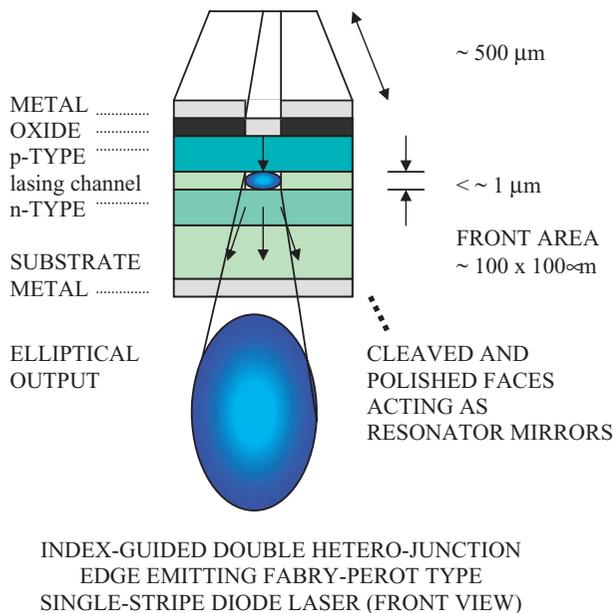
FIGURE 5.10. Schematic energy diagram for a helium–cadmium laser. Green and red emission is also possible but not commercialized.



STIMULATED EMISSION

FIGURE 5.11. Schematic energy diagram for a semiconductor laser. It is based on laser emission from a forward biased p - n junction when driven by a well-regulated drive current.

volume (Fig. 5.12), but to a stack of very thin layers forming multiple quantum wells (MQW), or planes of quantum wires or dots (super lattice). Due to the small confined volume a high radiative efficiency exists as well as a low lasing threshold. Most devices still operate in NIR but the trend is to develop diode lasers using wide bandgap materials that have an output below the red. Blue diode lasers based either on ZnSe or doubling 860 nm light, emit at around 430 nm and are about to enter the commercial market. At higher power levels (Figuroa, 2002), direct frequency-doubling in the diode laser forms an alternative route to the blue region. For example, the D^3 TM (Direct Doubled Diode laser, Coherent) delivers 10 mW at 430 nm. When selecting a drive current source, it is important to select one with a low noise, good stability, and including a temperature controller on the diode laser head.



INDEX-GUIDED DOUBLE HETERO-JUNCTION
EDGE EMITTING FABRY-PEROT TYPE
SINGLE-STRIPE DIODE LASER (FRONT VIEW)

FIGURE 5.12. Dimensions and cross-section through an edge emitting diode laser. The polished sides act as highly reflecting mirror and output coupling mirror. Thermo-electric cooling to remove heat required.

Diode lasers can change their emission wavelength over a limited range (10–20 nm) by varying their drive current and junction temperature (Hodgson, 1994). The approximate tuning rate is about 0.1 nm / °C. Cooling brings the lasing wavelength down. Beam quality suffers from astigmatism that has to be optically corrected (Snyder and Cable, 1993). Edge emitters now provide high power, up to several watts CW in NIR.

Semiconductor lasers are very appealing because they are small [Fig. 5.13(B)], highly efficient, easy to use, and relatively cheap. Integrated fiber-optic output is another feature available from many manufacturers [Fig. 5.13(A)]. However, it should be stressed that this small package comes with important special requirements. The devices can be rapidly destroyed if both current transients or nanosecond current spikes at start-up and internal heating are not kept under control by the power supply electronics. Static discharges (SD) from a person or an ungrounded soldering iron, or the use of solder that is too hot or remains in contact for too long may instantly destroy the laser. A mechanical shunting device (Unger, 1994) may prevent SD damage during handling. Alternating current (AC) line filters are recommended (Hodgson, 1994). This market is strongly driven by the digital video disk (DVD) and audio compact disk (CD) industry where the goal is to increase information storage densities. Most, if not all, of the following examples are also wavelength stabilized by stacks of multi-layer coatings usually deposited at the HR side and producing bandpass filter reflectivity only for the lasing wavelength [Fig. 5.14(B,C)]. Combining a proper OC coating with a fiber pigtail having an inscribed Bragg grating has the same stabilizing effect.

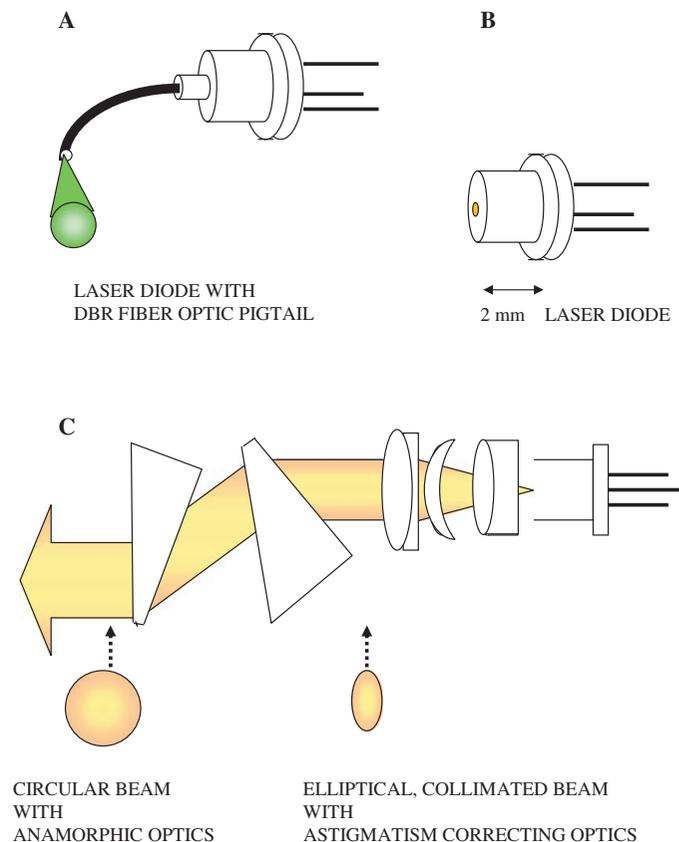


FIGURE 5.13. Example of (A) optional pig-tail fiber-optics, (B) physical dimensions of a diode laser, and (C) corrective optics to create a circular beam profile.

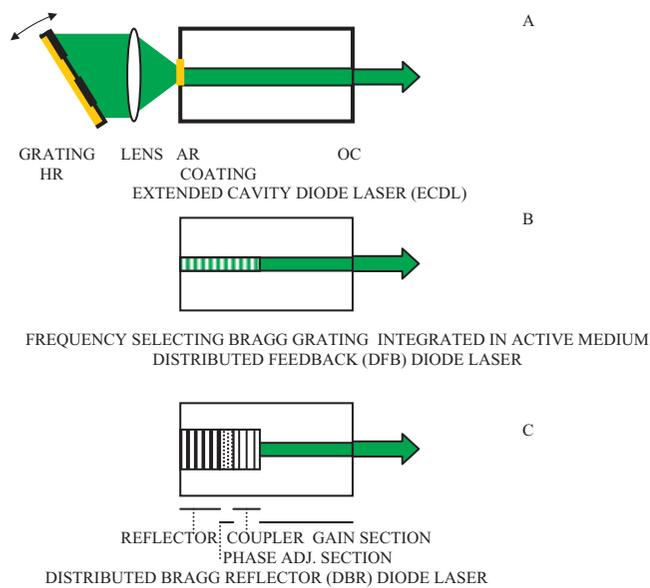


FIGURE 5.14. Diode lasers with wavelength tunability via an angle-adjustable grating in an extended cavity configuration (A); improved mechanical and thermal stability as well as wavelength and intensity control via a bandpass reflective filter formed by a stack of thin coating layers (Bragg grating). When the grating is incorporated into the gain medium the device is a distributed feedback (DFB) diode laser (B), otherwise it is a distributed Bragg reflector (DBR) diode laser. For ultrafast pulsed operation semiconductor saturable absorber (SESAM) layers can be added to the high reflecting mirror stack or to the saturable output coupler (SOC) to the output coupling face. Edge emitting diodes require corrective optics because the emission is elliptic.

Violet and Deep Blue Diode Lasers

Because these lasers produce 10 mW of deep blue emission (395–440 nm) from an input power of less than 10 W, they do not require water cooling. Compare this with an argon-ion laser producing the same 10 mW from 1 kW of electrical input, gallons of cooling water per minute, and filling a good part of a laser table. Most of the diode lasers have a single fixed-wavelength, which may vary somewhat (see above under FBG Wavelength Stabilization). Violet 405 nm (Photonic Products Ltd., Table 5.3) lasers efficiently couple into 3 μm core single-mode fibers. Diode lasers presently operate at 395 to 440 nm (LG Laser Technologies GmbH, Coherent; Table 5.3), 415 and 430 nm (Crystal Laser Inc.), 440 nm (PicoQuant model LDH 440, Power Technology Inc.), 457 nm (Melles Griot), and 473 nm (Crystal Laser Inc., Power Technology Inc.). But they are not nearly as cheap as laser-pointers! Prices range from a few thousand to US\$25,000, depending on complexity and features such as thermo-electric cooling.

Visible and Red Diode Lasers

488 nm Diode Lasers An alternative to the argon-ion laser has appeared in the form of the Protera™ 488 system from Novalux Inc. (Table 5.3). Listed advantages over the argon-ion laser include twice the life expectancy (20,000 h), 1% of the power consumption, up to 20 mW output power, 2% of the size, 0.2% rms noise level, and built-in thermo-electric Peltier cooling with conductive heat removal via the enclosure. The ChromaLase 488™ from Blue Sky Research has a typical power consumption of 2 W and output power from 1 to 25 mW output and creates 488 nm laser light by

SHG doubling of ~ 980 nm IR light. Similar specifications are found for the Starbright 488, 10 mW pumped at 40 W (Torsana Laser Technologies A/S); the BluePoint 488 at 2 to 5 mW from Rainbow Photonics AG; and the tunable 488 nm laser from Optica Photonics AG. Coherent introduced the Sapphire (NOT to be confused with a Ti:Sa system). It is a VECSEL GaAs diode laser consuming 30 to 60 W (mostly in the thermo-electric cooler), optically pumped at 976 nm, and intra-cavity frequency-doubled to provide 20 mW of electrical input.

The Novalux Inc. Protera is based on an ECSEL design with a three-mirror folded cavity for better mode control and uses a KNbO_3 doubling crystal inside the laser cavity.

491 nm Diode Lasers Some designs are based on up-conversion of a fiber laser to produce 10 mW at 491 nm (Guided Color Technologies and Lumics GmbH). Others use a different principle. The DPSS Dual Calypso laser (Nordborg and Karlsson, 2004) from Cobolt AB (Table 5.3) offers 491 nm at 20 mW and 532 nm at 50 mW simultaneously. It uses a periodically poled potassium titanium oxide phosphate (KTP) crystal, partly inside the laser cavity, for non-linear optical frequency conversion. The 491 nm line stems from sum frequency mixing of Nd:YVO₄ with the 1064 nm line from the Nd:YAG laser. Four hundred fifty-seven and 1340 nm lines can be added using similar methods. This laser has a noise level of <0.3% and can directly replace the argon-ion laser operating at 488 and 514 nm.

606 and 635 nm Diode Lasers The He-Ne 594 nm orange and the red 632.8 nm lines can be replaced by the 606 nm (orange) VECSEL Eksel 110 diode laser (Toptica Photonics AG; Häring and Gerster, 2003) and the ChromaLase 635 25 mW (Blue Sky Research). The Radius 635 nm CW 25 mW (Coherent) has a 16-h beam pointing stability of about 60 mrad compared with ~ 160 mrad for a 17 mW He-Ne. A 637 nm red diode laser He-Ne laser substitute has been incorporated into the Bio-Rad Radianc2100™ CLSM (currently Carl Zeiss CellScience Ltd).

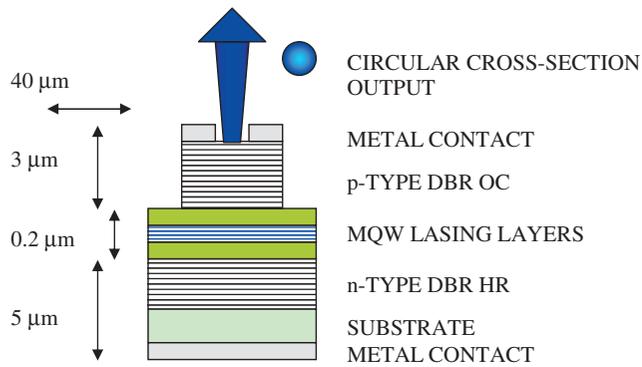
685 nm Diode Laser A 685 nm 30 mW CW diode laser is offered by Lasos Laser Technik GmbH.

Recent Developments in Diode Lasers

In this expanding field, many features are rapidly improving to the benefit of the user.

Emerging Tunability By incorporating a diode laser into an external cavity equipped with a tunable grating (a Littman-Metcalf cavity) wavelength tunability from 632.5 to 1630 nm within a 50–150 GHz bandwidth is now available (New Focus; Table 5.3; Scifres, 1994). These devices are listed as external or extended cavity diode lasers [ECDL; Fig. 5.14(A)]. A narrow-line width version has been described in Day and Dessau (1994). Although for the moment manufacturers seem to focus on single wavelength devices, mechanical and thermal stability should not be compromised.

Beam Quality and Delivery Diode lasers typically require extensive astigmatic and anamorphic corrective optics to obtain a circular, parallel beam [Fig. 5.13(C)]. Other methods include using a spatial filter or a single-mode fiber-optic as well as beam homogenizers, such as the monolithic V-step design by Ingeneric GmbH (Table 5.3), arrays of symmetrically arranged lenslets, the so-called “fly’s-eye” lenses or light integrators (Homburg *et al.*, 2003), available, for example, from Limo Lissotschenko Mikroop-



MULTI-QUANTUM WELL (MQW)
VERTICAL CAVITY SURFACE EMITTING LASER DIODE (VCSEL)

FIGURE 5.15. Dimensions and layout of a vertical cavity semiconductor diode laser (VCSEL). Stacks of thin coating layers form the distributed Bragg reflector (DBR) highly reflective mirror (HR) and the output coupling mirror (OC). The emission pattern is circular and can be near-Gaussian.

tik GmbH (Table 5.3). Optical elements, called graded index (GRIN) lenses, have also been introduced to eliminate spherical aberration in an elegant way (Carts, 1994). The vertical-cavity, surface-emitting semiconductor laser (VCSEL; Fig. 5.15), a semiconductor laser with good circular-pattern beam quality (Cunningham, 1993). Power levels are now reaching 100 mW with a wall-plug efficiency of 20%, high temperature stability, and good beam quality.

Output Power and Cooling Output power has been increased by building diode lasers as banks or arrays but these often require additional water cooling that adds to their complexity. Quasi-CW output levels can reach 300 mJ and 1.5 kW peak power. Tens to 100 s of watts of CW power and long lifetimes are becoming common. Cooling requirements depend on the output power generated and they range from passive air cooling via thermo-electric (Peltier) cooling to water cooling, the latter often using microchannels for optimum control of the junction temperature.

Temperature Tuning There is no longer a need to have a large number of different diode lasers nor a large collection of emission filters for CLSM. Siviers and colleagues (2004) showed that, with a 3 mW low-power superlattice (multi-quantum well) 635 nm diode laser attached to a Bio-Rad MRC600 CLSM, many cell stains that absorb in a broad range around 640 nm can be reached by cryogenically cooling the laser down to -196°C . This caused the emission wavelength to shift linearly ~ 25 nm down, while the output power went up about five times. For equal fluorescence signal, the noise level in the image caused by reflected laser light decreased five times because it was possible to optimize the laser wavelength to the optical filters more precisely.

Output Modulation Another important feature of semiconductor lasers is that their output can easily be modulated to well above 100 MHz and some can reach tens of gigahertz. This makes them prime candidates for use in frequency-domain fluorescence lifetime imaging microscopy (FLIM; see Chapter 27, *this volume*).

Diode- and Lamp-Pumped Solid-State Lasers (DPL and DPSS)

A second class of solid-state lasers includes those using neodymium yttrium aluminum garnet (Nd-YAG) and lasing in the IR at 1064.2 nm with very high fracture resistivity and good thermal conductance (Fig. 5.16); Yttrium lithium fluoride (Nd-YLF) with low thermal lensing (Arrigoni, 2004b); Nd-BaYF with better subpicosecond generation and amplification properties; yttrium orthovanadate lasing at 1064.3 nm having a lower lasing threshold than Nd-YAG (Kaminskii, 1981); GdVO_4 lasers and Nd:LuVO_4 , also having a lower lasing threshold. All of the materials listed above are pumped by a FBG diode lasers [Fig. 5.14(C)]. Nd-YAG and Nd-YLF provide high overall efficiency and the good beam quality necessary for frequency doubling to the visible producing lines at 532 and 523 nm. Microchip lasers are formed by sandwiching laser and frequency doubling crystals into a millimeter size compact structure. Due to their small size they benefit from ceramic laser materials with high thermal conductivity (Wisdom and Dignonnet, 2004).

A simpler design is possible with ytterbium tungstate (Yb:KGW) absorbing between 900 and 1000 nm that can be directly pumped by a red diode laser.

Early models used flash lamp or CW krypton lamp pump sources but the latest models tend to be equipped with a diode laser [diode-pumped solid-state lasers (DPSS); Hobbs, 1994]. The advantages of diode lasers over conventional pump sources, such as lamps, are reduced cooling requirements because of higher efficiencies due to a collimated and focused output, a perfect match of pump-source emission wavelength with the absorption spectrum of the lasing medium, and enhanced frequency stability (Baer, 1986). A typical improvement in electrical-optical conversion efficiency is from 0.5% (TEM_{00} mode) to 6%.

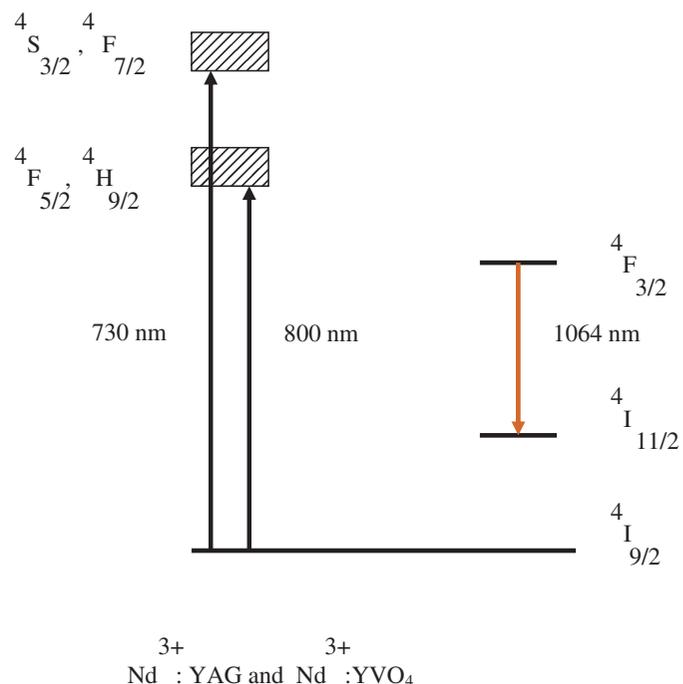


FIGURE 5.16. Schematic energy diagram for a neodymium-yttrium-aluminum garnet (Nd-YAG) laser and a neodymium-yttrium orthovanadate (Nd:YVO₄) laser.

The infrared output at 1064 (Nd-YAG) or 1054 nm (Nd-YLF) is easily frequency-doubled, tripled, or quadrupled with appropriate non-linear crystals such as lithium triborate (LBO) and beta barium borate (BBO) to provide radiation at 532 and 527 nm (SHG), 355 and 349 (THG), 266 and 262 nm [fourth harmonic generation (FHG)], respectively. BBO even allows generation of the fifth harmonic at 213 nm. The stability of these lasers is very good and the green emission wavelength is ideal for the excitation of rhodamine dyes or for pumping the *tunable* solid-state lasers described below. Nd-YAG also has lower-gain wavelengths at 1440, 1320, and 946 nm, which when doubled provide 720, 660, and 473 nm. Power levels sufficient to obtain frequency doubling create a blue laser suitable to replace the ion laser lines. Argon-ion 514 nm emission can be replaced with a frequency-doubled 532 nm DPSS design when the mode and beam characteristics are optimized. A 473 nm DPSS laser (CrystaLaser Inc. and National Laser Corp. with 5–10 mW, Table 5.3) is created by intracavity-KNbO₃ doubling the 946 nm IR light of a diode-pumped Nd:YAG laser. Typical cost is about US\$8000.

As a pump source, the 514 nm line of a large frame argon-ion lasers may well be replaced with a frequency doubled VersaDisk-515 laser from Electronik Laser Systems GmbH (Table 5.3) emitting, depending on the model, 2.5 to 15 W of 515 nm light in a small foot print without water cooling, and improving the Ti:Sa pump efficiency by 20% compared with the more common 532 nm DPSS pump source. Several companies now offer DPSS systems in kit form. Examples are the DPSS educational kit from Optronics Technologies S.A. and the Nd:YVO₄ and Nd:YAG kits with Cr:YAG SESAM from ALPHALAS GmbH. These lasers are extremely compact, stable, and efficient with good beam quality and offer turn-key operation. Electrical power requirements are low: tens of watts. Beam quality can be improved with Gaussian resonator mirrors and phase plates (Casperson, 1994).

Thin Disk Lasers

Very intense lines with much reduced thermal lensing and birefringence can be generated by thin disk lasers. Radial thermal effects are most severe in the rod-type lasing materials used in the original Nd-YAG designs. Slab-type designs largely lift this thermal problem. Scientific designs are profiting from Yb:YAG or similar based thin disk systems such as the VersaDisc by Electronik Laser Systems GmbH, which can attain tens to hundreds of watts of output power with minimal heating effects. It is based on a 100 μm thin disk of Nd:YAG, Yb:YAG, or other material bonded to a heat sink and optically pumped on the opposite side. Because the heat gradient is almost perfectly planar (i.e., parallel to the bonding surface), the thermal lensing strongly present in high-power, rod-type media is drastically reduced along with the amount of birefringence. Optical aberrations are not introduced. As the disk is small and thin, a special mechanical mirror arrangement makes the pump light impinge on it many times. Lasers such as the VersaDisk currently deliver up to about 100 W CW at 1030 nm and about 15 W at 515 nm (Hitz, 2004a).

Tunable All Solid-State Laser

The release of the CW titanium-sapphire laser was an important event (Hammerling *et al.*, 1985). These lasers can be pumped by a CW ion, Nd-YLF, Nd-YAG, or diode laser. Because their vibrational-electronic levels are spread in a broad band, laser transitions can take place over a wide range, and, given the right mirrors, the tuning range extends from 700 to 1000 nm (Fig. 5.17). Power output is 3.5 W at 790 nm when pumped with an 18 W Nd-

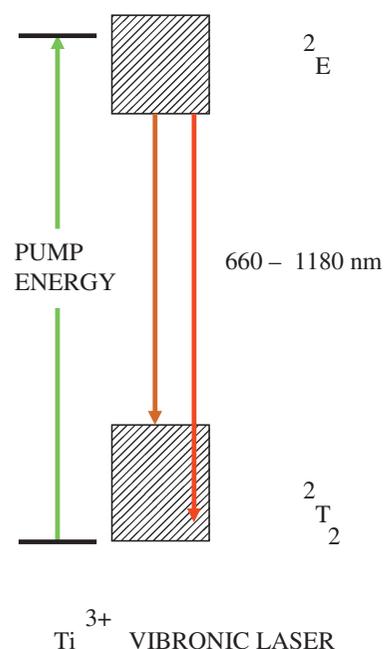


FIGURE 5.17. Schematic energy diagram for the Ti:Sa four-level vibronic laser.

YVO₄ or 20 W all-line argon-ion laser. The Ti:Sa laser has virtually replaced the IR dye lasers; it is much easier to operate, no dyes have to be changed, and the long-term stability is better than 3% due to the elimination of flowing dye. The RMS noise between 10 Hz and 2 MHz is less than 2%, a factor of about 10 less than for dye lasers. The main characteristics of these systems are the generation of short pulses of about 100 fs (FWHM), and high peak power for frequency-doubling to the blue and near-UV spectral range and for use in two-photon CLSM. Several configurations have appeared resembling either CW ring or standing-wave dye lasers. To cover the complete spectral range of this laser special mirror sets are required.

A similar wide-tuning range and high average power is offered by the Alexandrite laser (Cr³⁺ in BeAl₂O₄ host), which covers the 730 to 826 nm region when pumped CW at room temperature. This laser can also be flash lamp pumped. The Forsterite laser (Cr⁴⁺ ions in a Mg₂SiO₄ host material) emits between 1167 and 1345 nm and can be used in the 1200 to 1250 nm tissue penetration window. Its three main pumping bands are centered around 350 to 550, 600 to 850, and 850 to 1200 nm. Both CW and pulsed models are available. Its second harmonic is tunable from 600 to 650 nm (Mortensen, 1994). Liu and colleagues (2001) report multi-photon femtosecond excitation of plant tissue at about 1250 nm with a Cr:forsterite laser (see also Chapter 21, *this volume*). Potential members of this family of tunable solid-state lasers are the LiSAF laser (Cr in LiSrAlF₆) with a tuning range from 780 to 1060 nm and a peak emission at 825 nm (Perry *et al.*, 1993), and the LiCAF laser (Cr in LiCaAlF₆) tunable from 720 to 840 nm. Tunable UV could be created by Ce:LiSAF (Anderson, 1994b).

CW Fiber Lasers and Up-Conversion

Confusingly, fiber-coupled lasers in which a fiber-optic merely guides the emission are also called fiber lasers. Although also capable of generating a white continuum, micro-structured photonic crystal fiber (PCF) laser delivery systems should not be classified as fiber lasers. Unlike single-mode fibers, highly multi-mode

core fibers are insensitive to twisting and bending but otherwise behave like single-mode fibers. PCF fibers designed to have better higher order dispersion compensation include the crystal fiber large mode area (LMA) PCF.

We will only use the term *fiber laser* for systems where the laser gain medium is the fiber itself. IMRA America (Table 5.3) shows that the design of a fiber laser can be very simple: Two resonator mirrors with the fiber-optic in between (Fig. 5.18). Double-clad fibers are preferred for fiber lasers because the pump light can be efficiently coupled into the high NA, ≥ 0.46 , inner cladding while only a single, transverse-mode-doped core, having a low NA of about 0.06, is excited. They also possess excellent heat dissipation (Limpert *et al.*, 2004). Furthermore, using LMA double-clad fiber (for example, 20/400 μm core/outer cladding) reduces non-linear Brillouin and Raman effects (Galvanauskas and Samson, 2004). PCF fibers can accommodate high powers (Folkenberg and Broeng, 2004), with 260 W CW output from a short piece of 4 μm double-clad fiber, thereby also limiting non-linear effects.

Up-Conversion Fiber Lasers

These devices are based on excited state absorption of rare earth ions in fluoride “ZBLAN” glass fibers (Fig. 5.18). ZBLAN stands for zirconium, barium, lanthanum, aluminum, sodium fluoride. When pumped by a pair of diode lasers, each operating at a different red wavelength, several milliwatts of green light can be created (Piehler, 1993). In this typical up-conversion scheme, low-energy photons are sequentially absorbed and create a population inversion in the excited state (Chenard, 1994). By doping the fiber with more than one rare-earth material (Fig. 5.19), the spectral region longer than $\sim 500\text{ nm}$ can be covered. Examples include doping with Er(bium) for 546 nm, with Pr(aseodymium) to obtain 491, 520, 605, 635, and 720 nm emission at 10 to 40 mW (Guided Color Technologies, GmbH; Table 5.3), with PrYb for 840 nm, with T(hulium)m for 482 and 800 nm as well as 470 nm and $1735 \pm 1\text{ nm}$ from a single-frequency Tm-doped DFB fiber laser (Hitz, 2004b), with Tm for 980 nm, and with Yb for 1060 nm. One more example is the 700 blue fiber laser series from LasNova (Table 5.3).

Lumics GmbH has developed a fluorozirconate (ZBLAN glass) fiber laser that is a potential replacement for argon-ion lasers. The spectral linewidth of a fiber laser is typically very small, about 0.02 nm (Gabler, 2004). One notes that the exact wavelengths at times differ by 1 to 2 nm between various designs, a fact which may be related to doping and operating conditions.

In the IR, fluoride glass fiber can be doped with rare-earth ions, such as erbium, which emits at 1540 nm (Hecht, 1993a). The

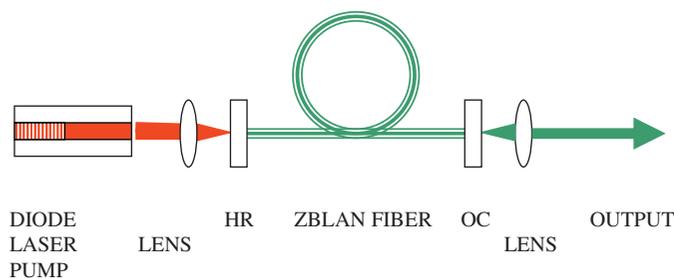


FIGURE 5.18. Schematic diagram of a dual ion doped CW ZBLAN up-conversion fiber laser. Pumped by a distributed feedback (DFB) diode laser is emits visible laser light. The connectors contain the high reflecting mirror (HR) and the output coupling mirror (OC).

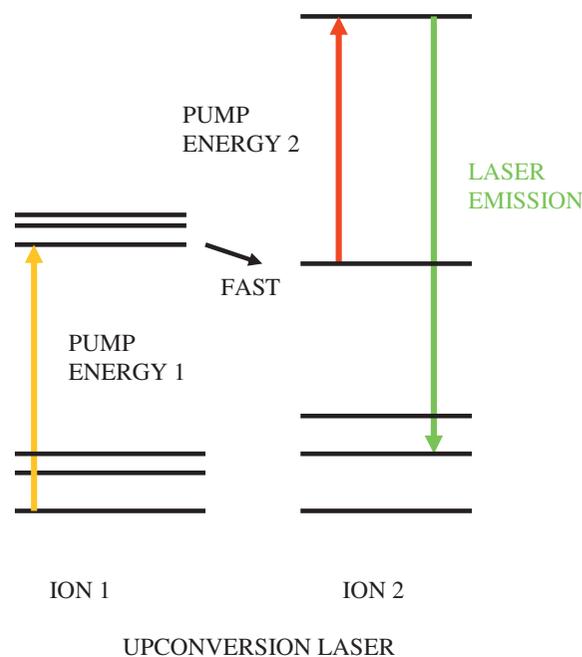


FIGURE 5.19. Schematic diagram for a dual ion doped up-conversion laser.

energy stored in the single-mode laser is about 500 μJ and, in a multi-mode laser, 10 mJ. The maximum optical peak power amounts to about 100 kW. Although initial tests were apparently carried out with an IMRA 775 nm 100 fs laser on a Zeiss microscope, no commercial system has yet been offered by Zeiss.

In a single-longitudinal-mode fiber laser with a fiber core of 2 to 4 μm , the interaction area is very small (a few square micrometers). The interaction length is very long, from millimeters to even kilometers. This results in a very high single-pass optical gain at very low threshold (sub-mW) pumping power levels with pumping efficiencies to 75%. Fibers consist of amorphous material that give very broad absorption bands (Chenard, 1994). The single-pass gain is practically unity. Output is always diffraction limited with excellent beam quality. In combination with an easily attainable average power level of a few watts this makes them very suitable for laser-tweezer applications. These advantages of a fiber laser were recognized as early as 1964. However, at that time there was little capacity to produce good quality, low-loss, single-axial-mode fiber.

PULSED LASERS

Why are pulsed lasers useful? The simple answer is higher peak power. Sufficiently powerful CW pump sources are often not available. Often the wavelength and intensity offered by available lasers are not appropriate. Therefore, wavelength conversion schemes have been developed and many of them are based on non-linear phenomena that occur only at very high intensity. Because of their high peak power, pulsed systems provide efficient wavelength conversion and tuning capabilities. In general terms, the peak intensity is greater than the average intensity by a factor that is the inverse of the duty cycle. The duty cycle is the ratio of pulse width to pulse interval and can often be 10^{-5} .

It is important to remember that pulsed lasers are only useful in scanned fluorescence microscopy if the pulses occur at a high

enough rate that many pulses occur in each digitizing period and intensity integration over the pixel period can provide a performance similar to a CW laser. Pulsed lasers are characterized by the high peak power (see Table 5.2).

Classification of Pulsed Laser Systems

A logical way to classify pulsed laser systems is according to their temporal pulse behavior. Four methods for pulsed operation exist: normal mode, Q-switching, mode-locking, and cavity-dumping.

- **Normal mode, free-running:** When observed with a fast diode and an oscilloscope, solid-state lasers generate a continuous train of random spikes with varying intensity caused by the interaction of various modes in their very small cavities. This type of operation is not very useful.
- **Q-switched lasers:** The term *Q-switched* is derived from the radio and microwave terminology, and it relates to a forced change in the quality factor, Q , of the resonant cavity (i.e., a bell is “high- Q ,” a lump of wet clay is “low Q ”). A low Q resonator is one which hardly supports laser action. What is needed is a method of changing the gain, or resonance, of the laser cavity. A simple implementation of a Q-switching device is the installation of an optical shutter in the resonator. The sudden opening of the shutter releases all the energy stored in the excited electrons of the medium in one giant optical pulse.
- **Active, mode-locked:** In a resonant cavity, many simultaneously oscillating optical modes are present at the same time (Arecchi, 1972; Mollenauer and White, 1987). Usually these modes interact with each other in a random way and no phase relationship exists between them. The output power fluctuates randomly according to a time-dependent interaction between the various modes. Installation of an intra-cavity modulator (i.e., a high-speed shutter) with a resonance frequency exactly matching the round trip time, $2L/c$, in the laser resonator will lock all modes to that particular resonance frequency. A certain phase relationship is maintained. Throughout the cavity, a single, intra-cavity pulse is created at a pulse rate of 70 to 100 MHz. Mode-locking frequencies can be changed by using a crystal with different mode-locking frequency and simultaneously adjusting the laser cavity length. Thermal stability has to be excellent. Folded compact cavity design, further miniaturization, a one-box design strategy, direct IR diode pumping using ytterbium tungstate, and standard thermo-electric Peltier cooling help maintain proper mode-locking. For fiber lasers, smaller size LiNbO₃ Mach-Zehnder modulators are used because they possess a lower insertion loss and better control of chirp.
- **Passively mode-locked lasers:** In passive mode-locking a very high loss is introduced to interrupt CW operation.

Kerr Lens Mode-Locking (KLM)

One way to generate short pulses from a solid-state Ti:Sa laser is using Kerr lens mode-locking. The Kerr effect refers to the quadratic non-linear dependence of the refractive index of a non-linear material on the applied electrical field. The intensity dependent refractive index induces a linear frequency sweep on any short optical pulse leading to spectral broadening. This effect can be compensated by sending the pulse through a few millimeters of glass or short piece of fiber-optic. High intensity optical pulses exert strong forces on the valence electrons of the laser medium. The transverse gradient in a Gaussian beam profile delivers the highest intensity near the center of the beam, less so near

the perimeter. Kerr-induced refractive index effects cause the beam to contract producing self-focusing. This regular focusing pattern in the gain medium imposes a specific set of modes in the Ti:Sa laser and eliminates other non-matching modes. An important consequence is that the gain medium works like a master oscillator with its own set frequency. This method requires an intracavity slit or pinhole to suppress CW contributions and needs an active starter such as a noise spike created by tapping a mirror mount and otherwise perturbing the cavity.

Saturable Bragg Reflector (SBR) and Semiconductor Saturable Absorber Mirrors (SESAMs)

Creation of self-starting intense optical pulse trains in, for example, a Nd:YVO₄ or fiber laser running at a high frequency of, for example, 80 MHz, is implemented by introducing into the cavity a low-loss, super-lattice, saturable-crystal absorber such as InGaAs. The material possesses a low reflectance for weak optical signals (such as noise) and a high reflectance for high power signals (such as optical pulses) and has a very short recovery time (saturable absorber mirror, SAM). It is incorporated into a high-reflectivity Bragg mirror. Quantum wells can be used in place of the InGaAs. The wider the spectral bandwidth of the gain medium, the shorter the optical pulse generated and, in addition, shorter pulses are created when a shorter rod is used because dispersion can be better compensated. For normal dispersion, red is ahead of blue light. For anomalous dispersion the opposite is true. Transparent materials usually possess normal dispersion. Because a Ti:Sa rod introduces normal dispersion, it must be compensated by inserting anomalous dispersion components such as prisms, grating pairs, or chirped mirrors into the cavity. In ultrafast Ti:Sa, Cr:LiSAF, or fiber lasers, pulse durations of a few tens of femtosecond can readily be created. Mode-locked oscillators such as an ultrafast Ti:Sa laser create nanojoule pulse energies.

The latest oscillator addition, Yb:KGW (tungstate), is directly pumped by two fiber-delivered high-power 940 or 980 nm IR diode pumps (Eclipse, Spectra Physics). No prism pair is required for compensation of the positive GVD because the cavity contains dispersion-compensating coatings. In addition, an SBR Bragg high reflector provides self-starting and passive mode-locking. At 1048 nm, 100 mW average power is generated with a repetition rate of 80 MHz and a pulse width of 150 fs. Components are readily available (BATOP GmbH; Table 5.3). Similarly, a microchip Nd:YAG/Cr:YAG gain medium/absorber can be designed (Kraft, 2004).

Saturable Output Coupler (SOC)

The SAM can be replaced by a saturable output coupler (SOC) incorporated into the cavity. A passively mode-locked DPSS laser creates a very simple design because the pump power can enter through the high reflector into the laser medium.

Cavity-Dumped Lasers

Cavity dumping is a common technique for reducing the laser repetition rate. For example, for frequency-domain fluorescence lifetime imaging microscopy (FLIM) the 80 or 76 MHz laser pulse repetition rate is too high. The repetition rate can be brought down to 2 or 4 MHz by applying a momentary radiofrequency (RF) signal to an appropriate modulator crystal. This allows more harmonics to be used for FLIM imaging and optimizes the system for the nanosecond or picosecond lifetimes encountered.

Nitrogen Lasers

Among the more common pulsed lasers are nitrogen lasers. Because of their low pulse repetition rate (20–100 pps), nitrogen lasers are only used in microscopy for intentionally wounding cells (Burgess, 2004). The average power is between 1 mW to 1 W, and the emission wavelength is 337.1 nm (Hecht, 1993b). The pulse width of these lasers varies between a fraction of a microsecond to less than a nanosecond. The time characteristics of the pulsed nitrogen laser emission at 337 nm was exploited in fluorescence microscopy by Wang and colleagues (Wang, 1990; Wang *et al.*, 1990, 1992). Their conclusion was, however, that the pulse-to-pulse intensity stability of this type of laser (500 ps FWHM pulse at 25 Hz) is not presently sufficient and long integration times were necessary. For this reason the nitrogen laser is unlikely to be useful in microscopy, particularly now that deep UV diode lasers (Crystal Laser Inc.; Table 5.3) possess much more favorable lasing characteristics. Bioeffects on living cells and tissues of UV laser irradiation can be severe but for microdissection applications the nitrogen 337 nm line is very suitable (Burgess, 2004).

Excimer Lasers

Although the vacuum-UV and UV output of these excimer lasers has given them a strong foothold in tissue ablation, this type of laser is not often used for CLSM. Air-cooled, compact models are available, such as LEXtra and COMpex models (Lambda Physik; Table 5.3). This company also introduced a metal–ceramic based NovaTube™ design, which lasts for 7×10^8 pulses on a single gas fill.

Metal Vapor Lasers

Metal vapor lasers (Lewis *et al.*, 1988; Hecht, 1993d) are also extremely powerful and can be operated at much higher repetition rates than excimer lasers. The average power can exceed 100 W and the repetition rate can be as high as 20 to 50 kHz, which is still a bit slow for most microscope applications. Wall plug efficiencies reach 1%. Their characteristic emission wavelengths are also reported in Table 5.2.

Dye Lasers

In cancer treatment, high fluency rates are sometimes required. Real-time CLSM following the effects of high-energy 7.5 J/cm^2 585 nm pulsed dye laser irradiation on hyperplasia *in vivo* was reported by Aghassi and colleagues (2000). Otherwise, the need today for stable well-characterized tunable sources is largely filled by optical parametric oscillators (OPO) and optical parametric amplifiers (OPA; see below) in combination with solid-state pumping lasers. Not only is this technology far less messy than dye laser technology, it also adds IR coverage via difference frequency mixing (Radunsky, 2002).

Dye Laser with Intra-Cavity Absorber

Synchronously-pumped dye lasers equipped with saturable absorbers have been used extensively in the past to obtain short pulses to cover the gap between 525 and 700 nm, which exists for Ti:Sa lasers (see below; Herrmann and Wilhelmi, 1987; Muckenheim *et al.*, 1988).

Colliding-Pulse Dye Laser

Colliding-pulse mode-locked dye lasers can emit optical pulses of <100 fs pulse width at repetition rates of 100 MHz. They can produce an average power of 15 mW at 630 nm when pumped with at least a 4 W all-line argon-ion pump. Noise is less than 1% but this figure depends critically on pump laser performance. This type of laser was used by Denk and colleagues (1990) and Piston and colleagues (1992, 1994) for their first two-photon experiments. It has rather limited tunability. Thermal equilibrium is reached within 1 h after turning the system on. Ti:Sa lasers, which produce 100 fs pulses at 100× higher power, have replaced colliding-pulse mode-locked dye lasers.

Modulated Diode Lasers

A small current change makes a large change in the output intensity of the diode laser, allowing most CW diode lasers to be operated in pulsed mode. When the internal diode laser processes are fast enough and stray capacitance from leads, etc., is kept low, pulse repetition rates of several hundred megahertz can be obtained as long as the required average output power is moderate. To generate a crisp pulse train, the cut-on and cut-off slopes must be steep. A recent review by Landgraf (2001, 2003) lists a variety of diode lasers and their upper frequencies. Selected laser diodes have reached ~700 MHz, for use in determining fluorescence lifetime. With a trigger circuit, these devices could act as a poor-man's Ti:Sa for FLIM.

DPSS Laser in Pulsed Mode

Doubled or tripled output could be used in tissue ablation but otherwise the pulse repetition rate is too low and the high output power a waste for CLSM. Used as Ti:Sa pump laser, a dual rod, tandem cavity DPSS laser can now produce up to 90 W at 527 nm. An example of these systems producing 1 to 10 kHz optical pulse trains with a width of a few hundred nanoseconds are the Q-switched Evolution Nd:YLF family from Coherent Inc. (Arrigoni, 2004b) and the 30 W Empower series from Spectra Physics.

Ultrafast DPSS Lasers

Many one-box compact and flexible solutions are appearing. Examples are the Pallas and Tiger compact picosecond/femtosecond laser systems from Time-Bandwidth (Table 5.3). Direct diode-pumped, passively mode-locked DPSS Nd:YLF lasers can make 70 fs pulses (Tiger-picosecond from High Q Laser Production) and 100 ps (Pallas) covering 780 to 860 nm, with a repetition rate of 50 to 200 MHz and an average output power of 500 mW.

Titanium-Sapphire and Related Ultrafast Lasers

This type of laser covers 660 (18 W pump) or 700 to 1100 nm (Duling, 1995; Fermann *et al.*, 2002). It is an ideal source for two- and multi-photon microscopy. The long wavelength reduces scattering and allows it to penetrate farther into tissues than visible lasers. The fact that excitation occurs only at the focus plane improves the signal-to-background ratio and reduces phototoxicity. Extensive overviews of multi-photon fluorescence microscopy and applications can be found at the excellent Molecular Expressions site (Florida University) and the Cornell site of DRBIO as well as in Chapters 28 and 37.

To cover the entire wavelength range, several sets of mirrors may be necessary. Most Ti:Sa systems are now pumped with low-

maintenance, highly efficient 5 to 10 W solid-state diode lasers. The self mode-locking Kerr effect of the Ti:Sa lasing rod delivers 100 fs pulses at 80 to 100 MHz repetition rate. Modifications in design may lead to pulses as short as 20 fs or even 5 fs.¹ These very short pulses exhibit extreme pulse broadening as they pass through dispersive media. Measures to reduce or eliminate this effect are given above under beam delivery. The high average power combined with the short optical pulse width makes the Ti:Sa laser almost ideal for frequency doubling and tripling to the visible and 350 to 525 nm spectral regions. Following user demand, completely automated and tunable systems are now offered. These provide a somewhat limited tuning range to stay away from the edges of the gain curve. Examples are the Mai-Tai family from Spectra Physics and the Chameleon products from Coherent (Table 5.3). Gaussian profile and broadband mirrors are standard features.

On the other hand, several companies now offer kits to allow users to build their own ultrafast or CW Ti:Sa laser. These kits are much less expensive and also less complex. Still further simplification is underway by having all components on a small footprint in an all-in-one-box design. First, the Nd:YVO₄ green pump laser was upgraded with stronger pump diodes and higher efficiency doubling crystals. It now emits CW 18 W of 532 nm with low noise and in TEM₀₀ mode. One pump diode suffices, halving replacement costs. Not only can the 532 nm output pump both the Ti:Sa oscillator and a regenerative Nd:YLF-based pulse amplifier, halving the number of green pump sources, but also the edges of the gain curve tuning range are more easily accessible. Thermal lensing gets worse with increased pump power but has been overcome by adjusting the focal length of the cavity mirrors. Nevertheless cryogenic cooling may be required if systems are scaled up further (Arrigoni, 2004b).

Heat dissipation and efficiency are areas where fiber and thin disk lasers have a clear edge. A further simplification is underway by using ytterbium oscillators, which can be pumped directly with IR diode lasers, bypassing the green pump step, but their pulse width, etc., is still not as short. Table 5.2 lists the specifications of several commercially-available examples also in kit form. Most, if not all, use semiconductor saturable-absorber mirrors (SAMs or SESAMs, see above under mode-locking) to generate a self-starting, high-repetition-rate femtosecond or picosecond pulse train (Hogan, 2004).

White Light Continuum Lasers

A rather inexpensive source of quasi-white light is the CW He-Cd laser, which seems to the eye to emit white light. Some with a positive column discharge actually emit only three wavelengths simultaneously (325, 354, and 442 nm), while the hollow cathode design emits from UV to NIR. Selection of five lines creates an RGB white light laser emission (White Knight RGB Series, The Cooke Corp.; Table 5.3).

An elegant and wider supercontinuum white light source, with generation stretching from below 450 nm to above 950 nm, is feasible using a 1 m long photonic crystal fiber (PCF) with a 1.7 μm core diameter illuminated by the 3 to 5 ps 800 nm pulses from a 5 kW Ti:Sa laser (Espinase, 2004). This is an important step towards a white light femtosecond light source. A similar PCF white continuum source with a length of 38 cm was used by

McConnell (2004) on a Bio-Rad 1024ES scan head. Coherent introduced a CW-pumped, cavity-dumped, regenerative amplifier with white light continuum generation at repetition rates up to 250 kHz and 100 fs pulses. However, even this much improved repetition rate is too slow for the usual CLSM applications. This problem is elegantly solved by a cavity dumped OPO, pumped by the second harmonic of a Ti:Sa laser. It delivers 30 fs pulses with a repetition rate of up to 4 MHz between 570 and 660 nm with a pulse energy of 13 nJ (Potma *et al.*, 1998).

Ultrafast Fiber Lasers

To generate short pulses with high peak power, a ring cavity can extract more energy from the pump. Bragg feedback (Friebele and Kersey, 1994) stabilizes the laser wavelength. Mode-locked single-mode fibers produce reliable, high repetition rate, short pulses with a small wavelength tuning capability (Duling, 1993; Smith and Lucek, 1993). Diode pumping and proper optics are necessary for best performance. The pump DFB diode laser, output, and amplifier stages are simply coupled by splicing devices (Fig. 5.20). This type of laser creates a very compact, efficient, and cheap alternative source for femtosecond pulses at gigahertz repetition rates and may soon replace the dye laser and some Ti:Sa systems. Hitz (2004d) reports a diode-laser-pumped Yb ultrafast fiber laser oscillator-amplifier system equipped with external grating compressor and generating 62 MHz, 100 fs with 25 W average power. Stability has to be improved against environmental changes caused by mechanical stress-induced birefringence and temperature variations. At the moment, a fiber laser is one fifth the size, has the same initial cost, but only one third the operational costs of a Nd-YAG or Nd-YVO₄ ultrafast system. The FemtoMaster1060, a passively mode-locked soliton fiber laser is offered by Fianium-

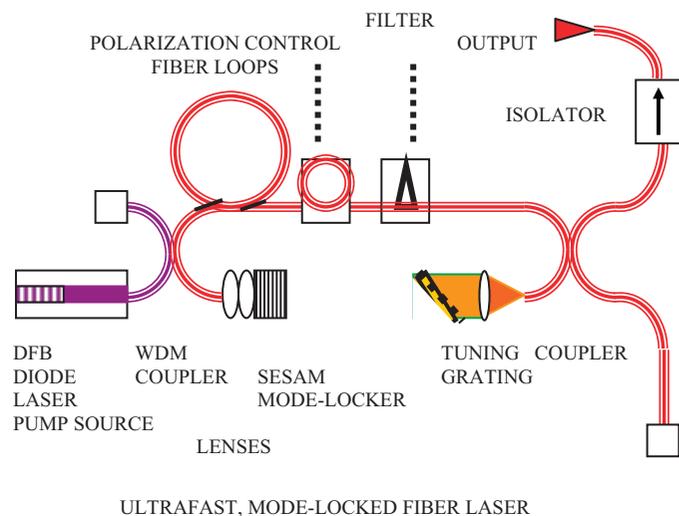


FIGURE 5.20. Schematic diagram for a femtosecond pulse mode-locked ultrafast diode laser. The emission of the fiber pigtailed distributed feedback (DFB) diode laser is coupled to the main laser gain medium fiber via a wavelength division multiplexer (WDM). Laser output exits from the laser resonator cavity in a similar way. The resonator consists of a lens-coupled high-reflecting mirror and a semiconductor saturable absorber mirror (SESAM). The other end of the cavity contains the grating tuning element. For polarization, pulse width and dispersion control, extra elements are spliced in. Coupling the SESAM in a different way and lens coupling both gain medium fiber ends to the tuning grating would create a fiber-ring laser. In a flexible and compact way, diode-laser-pumped amplifier stages, pulse-width compressors, and frequency-doubling stages can be added.

¹ One can predict from the relationship $\Delta t \cdot \Delta \nu > 1$ that for a spectral width of 200 nm one obtains a 10 fs pulse at 800 nm.

New Optics, Ltd., a company that has also developed mode-locked, tunable Yb and Nd fiber lasers. Typical specifications are tuning from 1040 to 1120 nm, 3 ps pulse width and 30 to 100 MHz repetition rate. With some further development, it may become a competitor for the Ti:Sa laser (Okhotnikov *et al.*, 2003; Rusu *et al.*, 2004). Pumped by a pig-tailed diode laser, the mode-locked ultrafast Er fiber laser FemtoFiber Scientific (FFS) from Toptica Photonics AG (Table 5.3) produces pulses with a width of <100 fs at 100 ± 10 MHz, optional tunability over 1150 to 1400 nm and SHG doubling to 575 to 700 nm, 1550 nm is standard with 200 mW average and 18 kW peak power; the Femtolite family Er fiber laser and Yb-fiber based chirped pulsed amplifier FCPA μ Jewel series from IMRA America Inc.

WAVELENGTH EXPANSION THROUGH NON-LINEAR TECHNIQUES

The spectroscopist wishing to excite with laser light finds several gaps in the table of available wavelengths. Although dye lasers cover an extended portion of the spectrum, they require too much maintenance to be useful in standard confocal microscopy. Optically anisotropic crystals provide a good alternative towards tunable light as long as they are pumped with lasers having high (pulsed) peak power at a high repetition frequency.

Second and Higher Harmonic Generation: SHG, THG, FHG Label-Free Microscopy

Non-linear optical (NLO) effects occur in certain classes of optically anisotropic crystals (Lin and Chen, 1987; Tebo, 1988). Focusing intense laser light into these crystals generates radiation at half the wavelength or double the frequency. This process, therefore, is known as “frequency doubling” or “second harmonic generation” (e.g., Huth and Kuizenga, 1987; Higgins, 1992). The intensity of the frequency-doubled light output is proportional to the square of the incoming light intensity. A Cr:forsterite laser running at 1230 nm was used to create a very elegant second harmonic generation (SHG) and third harmonic generation (THG) system operating at 615 and 410 nm (respectively) with a pulse repetition rate of 110 MHz and a pulse width of 140 ps (Hogan, 2004; see also Chapter 40, *this volume*). When the incoming beam is very intense, even third, fourth, or fifth harmonics can be generated. However, the efficiency of harmonic generation decreases at higher orders. Most doubling crystals can be angle or temperature tuned. THG is very interesting for label-free monitoring of inhomogeneities at interfaces, for example, cell membranes (Moreaux *et al.*, 2000). Yelin (2002) and Brocas and colleagues (2004) compare the THG performance of several systems: the Spectra Physics Tsunami-OPAL synchronously pumped OPO, which emits a 80 MHz 150 fs pulsetrain at around 1500 nm with an average power of 350 mW; and the T-pulse laser (Amplitude Systèmes) emitting 50 MHz 200 fs pulses at 1030 nm with 1.1 W of average power. THG reaches 500 and 343 nm, respectively, so UV transmitting optics may be required. A not-so-pleasant consequence of the doubling process is the doubling of the noise level. Pump lasers with excellent stability are a must. Higher peak powers and new, low-threshold materials require a careful reconsideration of design strategies (Beausoleil, 1992). CSK Optronics (Table 5.3) sells a line of compact doublers and triplers for Ti:Sa lasers. An important issue in this type of experiment is the amount of optical damage done to living cells and tissue by these power-

ful femtosecond pulses (Chu *et al.*, 2003; and also see Chapter 38, *this volume*). THG peak intensities of 100 to 300 GW/cm² illumination (i.e., nJ/pulse) are not damaging.

Presently, it is also possible to combine less powerful tunable dye or semiconductor lasers to cover the wavelength range from 170 nm to 18 μ m almost continuously.

Sum or Difference Mixing

Another technique for generating different wavelengths from certain basic laser wavelengths is sum or difference mixing. When two laser beams of high and low intensity and of frequency ω_1 and ω_2 , respectively, are simultaneously focused into a non-linear optical crystal, a sum signal is generated. The intensity of the sum signal, $I_{(\omega_1+\omega_2)}$ is proportional to the product $I_{(\omega_1)} \times I_{(\omega_2)}$. The higher-intensity ω_1 laser helps in generating enhanced UV output $I(\omega_1 + \omega_2)$ with an extended tuning range. In a similar way, difference mixing, $I_{(\omega_1-\omega_2)}$ leads to a tunable IR laser. (For examples, see Dunning, 1978; Adhav, 1986; Herrmann and Wilhelmi, 1987; Kaiser, 1988; Marshall, 1994; Demtröder, 1996)

Optical Parametric Oscillators (OPO) and Amplifiers (OPA)

The highest repetition rate that can be obtained depends on pump energy storage. Potma and colleagues (1998) developed a cavity-dumped OPO with 13 nJ pulses that reached a repetition rate of 4 MHz. These devices can generate a continuously-tunable output by non-linear conversion of fixed wavelength high-intensity CW or pulsed laser light (Butcher, 1994; Radunsky, 2002). OPOs are a reliable and easy-to-use means to cover spectral gaps left by dye or Ti:Sa lasers. Between the Ti:Sa fundamental, covering 700 to 1000 nm, and the second harmonic, covering 350 to 500 nm, a spectral gap also exists from 250 to 350 nm. This means that neither tryptophan, tyrosine, coumarin, or naphthalene nor the caged probes can be covered by a Ti:Sa. OPO efficiencies easily reach 40% to 60%, (Anderson, 1993) and practical systems are available from many companies (Table 5.2). A continuously tunable output stretching from UV to IR is generated by non-linear conversion, that is, optical frequency mixing. Parametric frequency conversion creates two lower-energy photons (signal and idler) from each incoming photon. This process is the opposite of sum mixing.

There are two methods to overcome the low efficiency of the parametric generation: Method 1 — Parametric amplification in a non-linear BBO crystal pumped by the second harmonic of the Ti:Sa followed by pulse compression. Parametric down-conversion is incompatible with long pixel integration times, and high pulse energies may possibly be damaging, and instrumentally, it is quite involved. Method 2 — Cavity-dumping a synchronously pumped OPO can produce pulse repetition rates up to 100 MHz. This allows FLIM imaging and reduces somewhat the average power delivered and cell damage. In addition, residual pump beams at 400 and 800 nm are available. Synchronous pumping means that the cavity lengths and, therefore, the pulse round-trip times are equal in both pump source and OPO (Potma *et al.*, 1998).

Non-critical phase-matching (NCPM) in a crystal is preferred because it allows tight focusing of the pump beam and long interaction lengths, resulting in low OPO thresholds. Efficiency can be further increased by using periodically poled (PP) waveguides, which do not diffract a tightly focused beam as does bulk material, while at the same time they allow long interaction lengths with a good mode profile. For example, green output from a Q-switched diode-pumped, frequency-doubled Nd-YAG laser pumps a non-

linear crystal creating a broad tuning range and narrow bandwidth with 15 mW average power at around 670 nm. Typical oscillator and amplifier crystal combinations cover various spectral regions. Angle tuning a proper crystal will give a 405 to 2700 nm tuning range with 45% total (signal + idler) efficiency (QWU-Lasertechnik mbH; Table 5.3). Continuum (Table 5.3) introduced the Sunlite OPO (Anderson, 1994a), which is currently the Sunlite EX OPO, with a very broad tuning range stretching from 222.5 to 1750 nm without a gap and providing line widths of 0.075 cm^{-1} . It is pumped with 100 to 160 mJ pulses at 355 nm from a Q-switched Nd:YAG. Such a system is a good replacement for pulsed dye lasers.

As mentioned above, there is a trend to create easy-to-operate automated all-in-one-box solutions. Spectra-Physics provides the LBO-based femtosecond synchronously pumped OPAL. Near the peak of the OPO tuning curve, it generates 200 to 250 mW and covers a wavelength range from 1100 to 2260 nm with pulse widths <130 fs. By frequency doubling, one covers the 550 nm gap left by the Ti:Sa systems. A further improvement with higher average power and repetition rates is obtained with ytterbium tungstate (Yb:KGW) employed in the Eclipse fs OPA (Spectra-Physics; Krueger and Féru, 2004).

Several OPO classes exist depending on the bandwidth narrowing optics used. The narrower the bandwidth, the less the gain. In addition, the number of passes through the crystal influences the gain. A single-pass device creates little gain for a 5 ns, 355 nm, 100 mJ pulse. Enclosure of the non-linear material in a resonant cavity gives optical feedback and adding a pulsed pump source creates extra gain. The complete design forms an optical parametric amplifier (OPA). The frequency stability of an OPO depends on the pump source stability and, with enough stability, single-resonant OPO (SRO), with feedback for idler or signal, or a double-resonant OPO (DRO) with feedback for signal and idler, can be built (Radunsky, 2002). OPOs as well as tunable CW dye lasers are often used as narrow bandwidth seed sources for OPAs. For example, by SHG doubling the 820 nm output from a Ti:Sa regenerative amplifier in a BBO crystal, one obtains 410 nm. This 410 nm pumps a second BBO crystal, the OPA. An 80-fold amplification is obtained resulting in 100 mJ pulses with narrow bandwidth. Changing the crystal angle (angle tuning) covers the wavelength range from 450 to 700 nm. These techniques can be adapted and implemented into fiber-optic OPO and OPA designs as well.

Pulse Length Measurement

Continuous on-line monitoring of the picosecond or femtosecond pulse shape immediately provides the feedback needed to align the cavity and perform other adjustments. Thermal drift may require cavity length retuning twice a day, even in a very large air-conditioned dedicated laboratory space. In a smaller space this may be required far more often. A pick-off signal from the monitoring diode is fed into an auto-correlator unit attached to a 400 MHz digital oscilloscope. Several designs exist but a computer-assisted background-free design is favored. A website explaining the operation of a background-free correlator is http://linac.ikp.physik.tu-darmstadt.de/fel/optical_pulse.html. Both time-dependent amplitude and phase must be known for many experimental procedures. Frequency-resolved optical gating (FROG) creates a two-dimensional function of the spectrally resolved autocorrelation at a range of correlation delays. Second harmonic-FROG in a non-collinear configuration is background free. Avesta (Del Mar Ventures; Table 5.3) offers a semiconductor-based design eliminating the non-linear crystal and the PMT detector. Created from a single pulse, the overlap of two identical, time-delayed pulses

with different center frequencies generates a spectral interference pattern in spectral phase interferometry for direct electric-field reconstruction (SPIDER). Pulse spectrum and cross-correlations of a pulse with a pulse copy that has been altered by a dispersive plate or attenuator in one arm of the correlator are used for phase and intensity determination from correlation and spectrum only (PICASO).

MAINTENANCE

On delivery, most laser systems perform well above the guaranteed specifications given by the manufacturer. The consumable parts (i.e., laser tubes, lamps, dyes, and amplifier optics in solid-state lasers and attached fiber-optics) are usually not covered by the warranty. Depending on the type of laser used, these costs can be substantial.

A factor usually forgotten at the time of an initial laser purchase is the need to acquire peripheral equipment for proper maintenance. Extra dyes, filters, power meters, sampling oscilloscopes and sampling units, fast diodes, infrared viewers, spectrum analyzers, beam-dumps, radiation shields, warning signs, covers, laser goggles, explosion- and fire-proof cabinets for solvent storage, and hazardous waste disposal, etc., are often not included.

Maintenance of Active Laser Media

Laser Tubes

The plasma tubes in ion lasers must be replaced when the cathode sags or the bore of the tungsten disks or BeO tube corrodes to become too big. The “getter,” always installed in the laser system to remove impurities in the gas, may become saturated. Cracks that form in cooling tubes usually lead to its immediate failure. The introduction of beryllium-oxide (BeO) tubes with superb heat conduction characteristics has significantly reduced the fragility of these laser systems and increased their reliability. Medium-power systems should last for 3000 to 5000 h (see Tables 5.1 and 5.2) and high-power systems for 2000 h.

Most laser systems come with 18-month warranties, but some self-contained, sealed systems carry a warranty for up to 5 years. The technology has significantly improved over the last few years to reduce the deposition of disk material and other contaminants on the inner surface of the Brewster windows. For the largest ion lasers, the aluminum oxide ceramic tube technology with brazed tungsten/copper disks has revolutionized deep UV output power levels and reliability. For He-Cd lasers, regular tube replacements are necessary after 1000 h of operation.

Tube Replacement and the Secondhand Laser Market

Several companies repair and resell worn laser tubes. Always ask for warranties and try-out. Old BeO tubes are considered hazardous material (HazMat) and can for a nominal amount be returned to the manufacturer.

What the User Can Do

To prolong the life of a tube, check mirror alignment so that the stabilized output can be attained with minimum excitation power to minimize tube component degradation. Check the water flow around the laser head for bad connections and look for internal deposits at least once a year. Keep dust out of laser systems; the laser should be connected to a permanent supply of clean, dry, room-temperature, oil-free air or nitrogen gas. A slight over-

pressure helps to prevent dust from entering the laser head. Commercial air filters that might introduce dry powders into the laser system should never be used. Synchronously-pumped systems perform markedly better and the intensity stability improves when dust is absent. Do not run at the highest power unless necessary, keep a user log, and inform users on the cost per hour to prevent idling.

Dyes

Dye lasers pose a number of hidden costs. Rhodamine 6G is one of the least expensive dyes, and is the most stable with a lifetime of 2000Wh for a typical 1-L reservoir. (i.e., the output power of the dye is reduced to 50% when it is pumped for 1000h at 2 W or 400h when pumped at 5 W). Dye aggregates can be filtered out but evaporation of the solvents necessitates periodic refilling. When the dye pump inadvertently starts to suck in air, air bubbles are generated and cause immediate contamination of the optical mirror surfaces. This problem can be prevented by careful start-up. Any faint, sharp clicks at the nozzle position indicate air bubbles passing the nozzle opening. Operating the dye laser with the wrong solvent, for example, incorrect viscosity, may cause the dye to foam, which will quickly clog the system. In order to reduce downtime and contamination of dyes, it is recommended that every dye is run with its own pump module. This is more expensive, but results in a much faster hook-up. Used dye and leftover solvent should be discarded in a manner that does not pose a threat to humans or the environment. Dye nozzles should be cleaned regularly in a sonicator, especially when the system has not been used for a while. Non-laminar flow from the jet nozzle is an indication of nozzle problems.

Gases

Excimer lasers need regular gas changes. Ion lasers refill their gas from a ballast tank via a microcomputer-controlled valve system. This reduces the risk of overfilling the plasma tube and degrading the performance or losing laser power altogether.

Laser and Amplifier Rods

Solid-state media, at least for CW lasers, are quite robust. However, pulsed systems need a very good Gaussian beam quality, otherwise the rod coating will be damaged. Often one can hear this as sharp clicking sounds that indicate that the laser needs immediate attention. Improper Ti:Sa rod cooling leads to water condensation on the pump surface and produces instabilities. In the worst case, continued operation may damage the bulk of the laser rod (Soileau, 1987). Degradation of the surface layers of the medium, caused by the intense illumination from the pumping system and is referred to as solarization, is less of a problem.

Diode Lasers

These devices are quite sensitive and can easily be damaged or destroyed. Damaged systems may show very weak output, a shift in emission wavelength, or a change in divergence or beam shape. Pig-tail fiber-optics, normally attached in front of the laser diode, also are suspect when the output power drops significantly but the drive electronics should be checked as well (as we recently experienced!). The fiber-optics may have been pulled out of its socket or it may have broken. At high power levels damage to the fiber surface may also occur, especially with plastic fiber-optics. As discussed above, current transients (spikes) and electric discharges should be prevented at all times. Forward-biased semiconductor junctions are inherently thermally unstable; an increase in current increases the temperature, which, in turn, increases the current

even more. Therefore, current supply design is important and must be suited to the specific laser.

Maintenance of Pumping Media

For CW or pulsed lasers, which are pumped by arc lamps, the lamp(s) must be replaced when the output power of the laser decreases. On the inside of the arc lamp, dark deposits may develop, which, over time, will cause an increase in absorption and catastrophic failure due to local heating. Defective lamps usually fail within the first 10 to 20h after start-up (Smith, 1986; Littlechild and Mossler, 1988). Optical amplifier components can be shielded from damaging UV radiation by doping the quartz lamp envelope with cerium. Electrode sputtering can be reduced by using sintered tungsten doped material with a low-work function to create a more uniform cathode current and lower temperatures (Erlandson and Powell, 1991). In addition, longer flashlamp lifetime can be obtained by using mushroom-shaped cathodes.

Maintenance of the Optical Resonator

Dust is one of the major enemies of the laser and tobacco smoke should be kept out of any laser laboratory. Dust covers should be in place at all times and any dust on optical surfaces should be removed as soon as possible, for example, on wavelength tuning prisms, birefringent plates, mode locker or Pockels cell surfaces, or mirrors. As using the wrong solvent to clean optics can destroy the optical coating, use only the ultra-pure solvents recommended by the manufacturer and follow the proper procedure. When in doubt, first try it out on a test surface. Most laser manuals describe in detail the proper cleaning methods. Never use the same side of a cleaning wipe for more than one pass. A patterned movement should be used when cleaning optical surfaces.

The optical coatings on laser cavity mirrors and external mirrors or fibers cannot withstand prolonged exposure to UV radiation. Colored rings will appear on the coating surfaces or they may look hazy or foggy. The formation of color centers, which cause increased absorption, occurs in fused quartz materials: lenses, optical fibers, etc. Color centers are usually caused by included impurities. Resulting lattice defects are mostly single electrons bound to negative-ion vacancy sites: F-centers. Free electrons created by intense radiation become trapped in these sites. Excimer lasers with their high UV peak powers are particularly prone to creating color centers. At high power, too tight focusing, or self-focusing, conditions can produce mechanical damage (remember, lasers are used for machining!; Günther, 1993). Damaged parts must be replaced immediately to prevent the deteriorated beam from damaging other components. This is especially true for pulsed systems. Frequency-doubling elements should be inspected regularly or whenever laser intensity fluctuations occur. A microscope may be necessary to see damage to the coating. Heating of some crystals (e.g., KTP) seems to reduce these effects. Frequency-doubling and other hydroscopic crystals should be kept under optimum dry conditions at all times.

Maintenance of Other System Components

Cooling Water

A gradual decrease in laser power can be caused by the growth of algae in the cooling water. A small addition of sodium azide (NaN_3) prevents a reoccurrence of the growth. An annual check of the rubber rings in the cooling hoses is recommended. At least once a year the cooling circuitry should be checked for galvanic and other

types of corrosion (Schneider and Williams, 1993). Do not use tap water for cooling. Many laser and temperature bath manufacturers now offer closed-loop laser cooling systems. Heat removal with air-to-liquid cooling is also possible (Goldman, 1993). For proper operation of a Nd-YAG laser, the resistivity of the cooling water should be in the range recommended by the manufacturer. When the resistivity is too low, the lamps will not start. If it is too high, the plating on the inside of the elliptical resonator will dissolve and cause a decrease in laser power. Water filters and de-ionizing filters should be replaced regularly as demanded by the performance of the laser. Stacked diode laser arrays, small in size but with several tens of watts of optical output power, require liquid cooling with micro-channel technology or thermo-electric (Peltier) cooling.

External Optics

All optical surfaces should be kept as clean as possible. Mirror surfaces exposed to UV radiation will have to be replaced regularly, depending on the impinging power density. Coatings may peel off. Apertures in spatial filters should be inspected and replaced when damage (burn) occurs. Fiber-optics, especially the ones exposed to high-intensity UV light, may develop color centers, which result in increased absorption and, in the end, failure of the entrance section. High-intensity visible or infrared light may lead to overheating at the input fiber tip. Fortunately, the length of most fibers is sufficient to allow them to be cut back, repolished, and reconnectorized, though sometimes at substantial cost and time delays. If they are bent too tightly, fibers will break, causing a sudden loss of output power. Pig-tail fibers attached to the main body of a laser diode may dislodge themselves. Due to the automated fabrication process, which includes angular positioning to optimize the output and use the most intense emission spot, manual reattachment usually does not re-establish the original power level. Laser damage thresholds for dielectric mirrors and anti-reflective coatings are roughly 250 MW/cm^2 at 532 nm and 500 MW/cm^2 at 1064 nm, assuming surfaces are spotless and clean (Aubourg, 2002).

TROUBLESHOOTING

A very extensive and instructive body of information for gas, dye, and diode laser maintenance, including optics cleaning and repair, exists in Sam's Laser FAQ (see website listings).

SAFETY PRECAUTIONS

Laser hazards include thermal and fire, acoustic shockwave, and strongly wavelength-dependent photochemical damage. Types of beam exposure are direct exposure intrabeam, specular, and diffusion reflection effects. Eyes and skin are most commonly affected. Non-beam-related hazards include electrical shock, capacitors, hose leakage, water vapor condensation, air contaminants, fumes, aerosols with biological agents, cadmium and zinc telluride (that burns in the presence of high laser intensity and oxygen), radiation damage, fire, compressed gases, gas cylinders, earthquake damage, excimer gases, and laser dyes.

All lasers are generally divided into four classes:

Class 1. Embedded lasers and laser systems

Laser completely enclosed, radiation not accessible during use.

Class 1M. Lasers and laser systems:

CW = $<40 \mu\text{W}$ blue and $<400 \mu\text{W}$ red.

Very low power: Safe for long-term intrabeam viewing.

Class 2. Low power visible lasers and laser systems:

CW = $<1 \text{ mW}$

Low power level. Safe for brief accidental naked eye direct exposure with blink and aversion response active.

Class 2M. Low-power visible lasers and laser systems.

Low-power visible collimated or divergent large beam diameters. Potential hazard with magnifiers

Class 3R. Visible. Low-power lasers and laser systems.

Accidental exposure usually not hazardous but eye injury possible upon intentional long-term viewing. Training required. Equivalent to Class IIIA "danger" [Center for Devices and Radiological Health (CDRH)] and ANSI 3a (USA).

Class 3R: Invisible. Low-power lasers and laser systems.

Wavelength dependent, limits are five times those of Class 1.

Class 3B. Medium-power lasers and laser systems:

CW = $<500 \text{ mW}$.

Serious eye injury even for brief accidental exposure to direct beams possible. Training required.

Class 4. High-power lasers and laser systems.

Even diffuse and certainly direct-eye exposure will lead to serious eye and skin injury. Poses fire hazard as well. Training required. with M: magnifying instruments and R: relaxed requirements. Limiting values for small point-like lasers with angular retinal spot size smaller than 1.5 mrad.

General safety precautions, that become more stringent with increasing classification, must be followed when operating a laser [International Commission of Non-Ionizing Radiation Protection (ICNIRP) and International Electrotechnical Commission (IEC) 60825-1 guidelines, see revised ANSI-Z-136.3 1996 classification, safe use of lasers; Tozer, 2001; Schulmeister, 2003; Sliney, 1994; LIA and Rockwell Laser Industries Inc.; Table 5.3]. Separate photochemical and thermal retinal exposure limits, including limits for damage from ultrashort, $<1 \text{ ns}$ pulses, have been added as well as the *.M classes. Every laboratory should have a Laser Safety Officer (LSO) who should be consulted when necessary. This person should be responsible for the proper training of users of laser-assisted equipment. Safety training videos are available from LIA, OSHA, and Rockland Laser Industries (Table 5.3) and often also from your own local university. Outside the laser laboratory, a "laser in use" warning sign must be posted and a red warning light should be positioned at the entrance to the laser room.

Inside the laser laboratory, safety precautions are necessary even for users of enclosed systems attached to CLSMs whenever the system is opened or when the fiber-optics are aligned and exposure to a laser beam becomes a possibility.

A brief "Do and Don't" list:

- Inform everybody near the laser set-up to be worked on. Check the sign-up agenda.
- Check and put all required accessory equipment on the ready.
- Have the entrance warning red light on when any laser is on.
- Remove everybody from the laser site whose presence is not required.
- Close the access doors.
- Close curtains.
- Remove rings, watches, and ties.
- Put safety goggles on.
- Never look directly into any laser beam.

- Align open laser heads with minimum power.
- Check for stray light and reflections with a hole-punched business or IR indicator card.
- Block beams not being attended.
- Disconnect electrical connections.
- Discharge large capacitors with a grounded large screwdriver with an insulated handle.

Beam Stops

Beam stops should be made out of anodized, flat black aluminum and positioned in such a way that no radiation is reflected back into the room. Stray reflections must be prevented; powerful IR or visible beams may easily start a fire on electrical cables or other flammable materials.

Curtains

Curtains should be made of special non-combustible material, preferably black in color. When a spot of 1 mm² is irradiated with approximately 1 MW/m² or more, the black side should emit a non-toxic smoke (that nevertheless may form damaging deposits on critical optical surfaces!) and may glow. See, for example, the Rockwell Laser Industries, Inc. and Lasermet Ltd. websites. Curtains are designed for low-power, 100 W/cm² or up to 1.2 kW/cm² lasers (MacMullin, 2004). Curtain shielding will prevent the accidental illumination of other people while aligning a laser or beam-splitter.

If a visual control is necessary from outside the room, a radiation-absorbing plastic window should be mounted in the door or wall. Tape should **not** be used to hold curtains together because this could allow the build-up of poisonous gases. Clothing is another possible source of trouble that is often disregarded. Famous are the stories of halved neckties.

Laser Goggles

A large range of eye protection devices is available (e.g., from LFW and Uvex; Table 5.3). A telling Ti:Sa fovea burn track is shown in Robinson (1998). It is important to realize that a 1 W/cm² irradiance level at the cornea can become a focused 100 kW/cm² retina spot of about 20 μm diameter.

Screens

Where possible, anodized, flat black aluminum pipes should be used to enclose all laser beams. These not only protect the operator, they also reduce dust in the beam line.

Exposure Effects, Warning Signs, and Interlocks

Each laser system must be equipped with the proper warning signs and interlocks. The listed references (Sliney and Wolbarsht, 1980; Rockwell, 1983, 1986; Winburn, 1985; Sliney, 1986) describe in detail electrical hazards and detrimental reflections from rings, watches, etc. Biological effects are also extensively covered, that is, thermal and photochemical effects upon exposure to CW and pulsed laser radiation, and the maximum permissible exposure to eyes and skin.

Chemical hazards may be caused by laser dyes, some of which are mutagenic. Dye solvents and laser gases must be properly handled. Basic protective measures include laser goggles (Burgin,

1988), cleanliness, and proper handling of chemicals. A sufficient number of eye protection devices should always be at hand when laser system components have to be repositioned or realigned. Naturally, one should reduce the power level as much as possible during alignment. Periodic training meetings and medical check-ups are recommended and can be used as a certificate of good laser usage conduct.

Infrared Paper

To safely find infrared beams, one can rely on infrared viewers but these are fairly expensive. An alternative is the use of IR indicator cards. These emit a visible luminescence via electron-trapped up-conversion. Suppliers include Bromba GmbH, Edmund Scientifics, Electro Optical Components, and Lumitek Intl. Inc. (Table 5.3).

CONCLUSION

In the previous sections, we have described the large number of lasers currently available for CLSM. These lasers vary widely in characteristic wavelengths, tunability, stability, output power, ease of use, and price. Rapidly expanding areas such as diode lasers, optical parametric amplification, and direct-doubling diode lasers would require an update every three months. Solid-state laser systems with wide tunability through the incorporation of optical parametric techniques and fiber lasers are areas showing rapid development.

Brelje and colleagues (1993) show a figure displaying the excitation wavelengths of various popular fluorophores with matching ion laser lines. This picture has certainly changed in the past years with the increase in power of solid-state and fiber lasers and the expansion of their range of tunability. For the moment we summarize several of the most often used lasers in Table 5.4. It is clear from this table that the ion lasers are being pushed out of the door.

- For CLSM, a stable, TEM₀₀ mode, easy-to-use laser with short coherence length is imperative.
- Wavelength tunable lasers require pulsed pump lasers with high peak power, good mode quality, and the best possible stability to reduce noise in the subsequent wavelength conversion and amplifier stages, as well as OPO, OPA, or master oscillator parametric amplifier (MOPA) optics. The best source is currently the tunable Ti:Sa laser. Excimer, nitrogen, and similar pulsed lasers and mode-locked solid-state lasers are still useful pump sources for tunable dye lasers if pulse repetition rates are sufficient for your purpose. The goal should be to each have a US\$6000 modular, personal 2-photon source!
- Miniaturization and ease-of-operation via efficient, low-cost, low-noise, tunable semiconductor and fiber lasers is a reality now and these lasers are replacing big CW and pulsed laser systems. A trend exists towards modularity, efficiency, and small footprint.
- With the advent of completely automated, self-contained laser systems (Mai-Tai and Chameleon) the ideal turnkey system is almost present. Older, bigger, flexible laser systems cannot be considered turnkey systems and require constant attention from well-trained, qualified persons.
- Laser safety training is a must also for CLSM users and eye protection and other safety measures should be at hand as required.

TABLE 5.4. (Continued)

Laser Type	Manufacturer and CLSM system						
	Visitec international Vt-eye and Vt-Infinity	Leica TCP-SP2-AOBS SP2	Nikon Digital Eclipse C1 Plus	Olympus Fluoview FV1000 & FV500	Perkin Elmer Ultraview	WITEC CRM200	Zeiss Axiovert 200M LSM 510 META
Broad-band tunable pulsed lasers							
Dye laser							
630 nm						3 mW	
Ti:Sa-Chameleon		1000 mW					1000 mW
720–930 nm		>1 ps					<100 fs
Ti:Sa-Mai-Tai		<1000 mW					<1000 mW
750–850 or 710–920 nm		>1 ps					<100 fs
Ti:Sa-Mira		100–700 mW					100–700 mW
690–1000 nm		>1 ps					<100 fs
Ti:Sa-Tsunami		100–700 mW					100–700 mW
680–1050 nm		>1 ps					<100–130 fs
NIR laser			1.2 ps, 1000 mW				
700–900 nm			720–1000 nm				

Mod.: modulated

Pp peak-to-peak

Visitech International, Inc, Sunderland, UK +44 (0)191 516 6255 <http://www.visitech.co.uk/>Leica Microsystems, Mannheim, Germany, Ph. +49-(0)621-70280 <http://www.leica-microsystems.com/website/lms.nsf>Nikon Inc., Melville, NY. Ph. (800)-526-4566, <http://www.nikonusa.com/>, <http://www.nikon-instruments.jp/eng/>Olympus America, Melville, NY. Ph (631)-844-5000, <http://microscope.olympus.com/>, http://www.olympusamerica.com/seg_section/seg_home.asp andPerkin-Elmer, Boston, MA, Ph. (800)-762-4000, <http://las.perkinelmer.com/content/livecellimaging/index.asp>Carl Zeiss AG, Oberkochen, Germany, Ph. +49-(0)7364-200 <http://www.zeiss.com/>WITec GmbH, Ulm, Germany, Ph. +49-(0)700-948-32366, <http://www.WITec.de> Confocal Raman microscope

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APPENDIX

LIST OF USEFUL WEBSITES AND TUTORIALS (NOT EXHAUSTIVE)

Books: Lists of General Reference Books on Lasers and Applications

<http://micro.magnet.fsu.edu/primer/lightandcolor/lasersreferences.html>
Spring, K.R., Parry-Hill, M.J., et al., *Molecular Expressions*, Florida State University, Tallahassee, Florida.
Dr. Rami Arieli, <http://www.phys.ksu.edu/perg/vqm/laserweb/>

Buyer's Guides

Photonics Laser Buyer's Guide, Laurin Publishing, <http://www.photonics.com>
Institute of Physics (IOP) Publishing Ltd., Temple Back, Bristol, UK, Ph. +44-(0)117-9297481- <http://optics.org/buyers/>

General References: Company Listings and Buyer's Guides

General

<http://www.photonics.com/directory/bg/XQ/ASP/QX/index.htm>
Laurin Publishing, 2002, *Photonics Directory, Buyer's Guide*. Brief, instructive chapters on optics, optical components, and comparison of developments for the various laser types in Book 3, *The Photonics Design and Applications Handbook*.

More Specific

Samual M. Goldwasser. Laser sales and services, <http://www.repairfaq.org/sam/laserlps.htm#lpslss>
Dr. Alexander N. Cartwright, <http://www.ece.buffalo.edu/faculty/cartwright/links.html>

Laser Information: Cleaning of Laser Optics

<http://www.repairfaq.org/sam/laseratr.htm#atrcln>

General Information with Many Links

<http://resonator.physics.sunysb.edu/laser/laserlinks>

History of the Laser

<http://www.bell-labs.com/history/laser/>
Schawlow and Townes biographies

Titanium-Sapphire Laser

http://www.drbio.cornell.edu/Infrastructure/Apparatus_WWW/Ti%20Sapphire%20Lasers.html
Evolution of Ti:Sa laser systems

Pulse Length Measurement by Optical Means

http://linac.ikp.physik.tu-darmstadt.de/fel/optical_pulse.html

Safety Issues

The health and safety committee at educational institutions should have Standard Operating Procedures (SOP) for laser operation and maintenance available. As examples may serve:

<http://phantom.ehs.uiuc.edu/~rad/laser/>

<http://www2.umdj.edu/eohssweb/aiha/technical/lasers.htm>

<http://web.princeton.edu/sites/ehs/laserguide/appendixC.htm>

Laser safety self-audit checklist, <http://www.microscopyu.com/articles/fluorescence/lasersafety.html>

Spring, K.R., Parry-Hill, M.J., *et al.*, *Molecular Expressions*, Florida State University, Tallahassee, Florida. Laser classifications, hazards, and bioeffects of various laser types, a brief and concise overview worth your attention.

<http://stwi.weizmann.ac.il/Lasers/laserweb/> <http://www.phys.ksu.edu/perg/vqm/laserweb/Misc/Appendix.htm>

Dr. Rami Arieli, The Laser Adventure, Virtual school

Rockwell Laser Industries. Safety materials, regulations: laser safety standards organizations and products, e.g., Z136.1 ANSI document, laser accident database, access control measures, and courses, <http://www.rli.com/>

Rockwell Laser Industries safety issues and links laser bioeffects, manufacturers, standards, and laser user institutes and laboratories, <http://www.rli.com/resources/links.asp?Link=3>

<http://repairfaq.ece.drexel.edu/sam/CORD/leot/>

Laser Electro-Optics Technology (LEOT), Center For Occupational Research and Development (CORD), Waco, Texas, electrical safety issues.

Standards, hazards, and solutions, <http://www.osha.gov/SLTC/laserhazards>

Specific Lasers

Argon-Ion Laser

One page argon-ion laser basic construction and emission properties, one page overview of laser types and applications, basic level, <http://www.rli.com/resources/argon.asp>

Basic argon-ion laser, graph of relative laser line intensities, <http://www.rli.com/resources/argon.asp>

Advanced Level

Ionized gas spectra, construction characteristics, http://www.lexellaser.com/techinfo_gas-ion.htm

Laser wavelengths chart, http://www.lexellaser.com/techinfo_wavelengths.htm

Dye Laser

Commercial laser dye as well as CLSM companies have colorful wall charts and list dyes and matching laser light sources on their sites.

Advanced Level

Dr. R. Alexander, http://members.aol.com/kpublish/Laser/Dye_Laser.html

Dr. Ladic site with excitation and emission spectrum maxima for dyes useful for CLSM, <http://www.es.ubc.ca/spider/ladic/images/fluoro.gif>

Expert Level

Instructive site from LEOT, http://repairfaq.ece.drexel.edu/sam/CORD/leot/course03_mod10/mod03-10.html

Colliding Pulse Dye Laser

Expert Level

<http://utol.ecen.ceat.okstate.edu/thz%20interactive%20tour/cpmlaser.htm>

Fiber Lasers Tutorial

Construction, wavelengths, and optical characteristics, <http://www.imra.com/lasers-tech-tut-frame-pdf.html>

Titanium-Sapphire Laser

Basic Level

Evolution of Ti:Sa laser systems, http://www.drbio.cornell.edu/Infrastructure/Apparatus_WWW/Ti%20Sapphire%20Lasers.html

Advanced Level

<http://micro.magnet.fsu.edu/primer/techniques/microscopylasers.html>

Spring, K.R., Parry-Hill, M.J., *et al.*, *Molecular Expressions*, Florida State University, Tallahassee, Florida. Beam divergence, beam expander, fiber-optic coupling (*), single and multiple mode fiber-optic, laser types.

Expert Level

Dr. Gavin D. Reid, Leeds University, and Klaas Wynne, University of Strathclyde, Glasgow, UK, Introduction to ultrafast laser systems and optical characterization, dispersion and its control, pulse broadening, amplification, optical Kerr effect, white light generation, OPA, auto and cross correlation, <http://phys.strath.ac.uk/12-503B2/introduction/introduction.html>
Construction of a fs mode-locked laser with a nice Ti:sa energy diagram, <http://www.df.unipi.it/~fisapp/Gruppi/Metrologia/spiegazioni/boris.pdf>

Troubleshooting

<http://www.repairfaq.org/sam/laseratr.htm#atrcln>

Tutorial Sites

Classification of sites:

- Basic level indicates many images, applets but no formulae.
- Advanced level with more thorough description of the issues supported often with applets.
- Expert level with equations and examples.

Basic Level

John Gormalli, stimulated emission explained, Web Science Resources Net (WSRNet), <http://members.aol.com/WSRNet/tut/t1.htm>

Stimulated emission explained, laser theory quiz, <http://home.achilles.net/~ypvsj/quiz/index.html>

Laser theory and applet on stimulated emission, <http://www.point-source.com/LaserTheory.asp>

Basic atom model, general laser classification and safety issues, <http://www.howstuffworks.com/laser.htm>

One page, ruby laser design and applications, <http://home.achilles.net/~ypvsj/glossary/laser.html>

Fraunhofer Institute's laser tutorial, <http://www.ilt.fhg.de/ger/lasertutorial.html>

Advanced Level

<http://micro.magnet.fsu.edu/primer/lightandcolor/laserhome.html>

Spring, K.R., Parry-Hill, M.J., *et al.*, *Molecular Expressions*, Florida State University, Tallahassee, Florida. Laser fundamentals, safety issues, microscopy and lasers used, intensity modulation, references, many instructive applets worthwhile to visit.

<http://www.olympusmicro.com/primer/lightandcolor/lasersintro.html>

Samual M. Goldwasser's site, "A Practical Guide to Lasers for Experimenters and Hobbyists," Version 6.60, very extensive with safety issues, reference books and materials, schematics, construction, safety, repair issues, <http://www.repairfaq.org/sam/laserfaq.htm>

Dr. Alexander N. Cartwright, University of Buffalo, laser gain and pumping schemes, photonics educational applets, <http://www.ece.buffalo.edu/faculty/cartwright/photonics/rateequations.html>

Expert Level

Online educational resources for physics teachers
<http://www.ba.infn.it/www/didattica.html>

Dr. Rami Arieli, The Laser Adventure, virtual school about lasers and their applications. Optics aspects, basic laser theory, laser cavities, lasing modes, fluorescence, laser line shape and width, laser beam properties, power, applets, applications, laser safety issues, questions, reference books, very extensive and useful also for course development, <http://www.phys.ksu.edu/perg/vqm/laserweb/>

Laser Electro-Optics Technology (LEOT), Center For Occupational Research and Development (CORD), Waco, Texas. Introduction to laser optics, electronics, components, systems, electrical safety issues, <http://repairfaq.ece.drexel.edu/sam/CORD/leot/>

Dr. P.L. Cross, Optical concepts, laser cavity design, laser operation, database of spectral properties of lasing materials, absorption and emission spectra, http://aesd.larc.nasa.gov/GL/tutorial/laser/las_out.htm

List Servers

<http://www.msa.microscopy.com/MicroscopyListserver/MicroscopyArchives.html>

Confocal microscopy list server for maintenance and trouble shooting. When there is a problem often it helps to look for similar experiences and solutions on the web. A good site to start: <http://listserv.acsu.buffalo.edu/archives/confocal.html>

Microscopy Laboratory Sites

Dr. W. Webb, DRBIO, Cornell University, Ithaca, New York. Multi-photon excitation, physical principles, excitation cross-sections, fluorophore

photostability, fluorescence techniques, e.g., FP(photobleaching)R (ecovery), FCS, SPT, cell viability, resolution with an underfilled objective, real-time image acquisition. A lot of specialized information together for live cell imaging and spectroscopy, <http://www.drbio.cornell.edu/Infrastructure/Infrastructure%20Index.html>

Dr. Bruce Jenks, Nijmegen University, The Netherlands. Laser principle, optical scanner principle, optical sectioning, single and multi-photon illumination (dis)advantages, <http://www.celanphy.sci.kun.nl/Bruce%20web/scanning%20microscopy.htm>

Optics

Ultrafast optics, <http://www.newport.com/Support/Tutorials/Optics/o4.asp>

LIST OF ABBREVIATIONS

AC	Alternating Current	LED	Light-Emitting Diode
ASE	Amplified Spontaneous Emission	LMA	Large Mode Area
AOTF	Acousto-Optic Tuning Filter	LPC	Laser Power Controller
AOBS	Acousto-Optic Beam Splitter	MII	Multi-Photon Intrapulse Interference
AOD	Acousto-Optic Deflector	MOPA	Master Oscillator Parametric Amplifier
AOM	Acousto-Optic Modulator	NA	Numerical Aperture
BBO	Beta Barium Borate	NCPM	Non Critical Phase Matching
CCD	Charge-coupled Device	Nd-YAG	Neodymium–Yttrium–Aluminum Garnet
CDRH	Center for Devices and Radiological Health	Nd-YLF	Neodymium–Yttrium Lithium Fluoride
CLSM	Confocal Laser Scanning Microscopy	Nd-YVO ₄	Neodymium–Yttrium Ortho Vanadate
CTA	Cesium Titanyl Arsenate	NLO	Non-Linear Optical
CW	Continuous Wave	NMR	Nuclear Magnetic Resonance
DC	Direct Current	OC	Output Coupler Mirror
FBG	Fiber Bragg Grating	OCT	Optical Coherence Tomography
FCS	Fluorescence Correlation Spectroscopy	OD	Optical Density
FHG	Fourth Harmonic Generation	OPA	Optical Parametric Amplifier
FITC	Fluorescein IsoThioCyanate	OPO	Optical Paramagnetic Oscillator
FLIM	Fluorescence Lifetime Imaging Microscopy	PCF	Photonic Crystal Fiber
FLIP	Fluorescence Loss in Photobleaching	PICASO	Phase and Intensity from Correlation and Spectrum Only
FRAP	Fluorescence Recovery After Photobleaching	PZT	Piezo Tuning
FROG	Frequency-Resolved Optical Gating	Q-switch	Quality (of the laser resonator) switch
GFP	Green Fluorescent Protein	RF	Radio Frequency
GreNe	Green Helium-Neon	SAM	Saturable Absorber Mirror
GRIN	Graded Index Lens	SBR	Saturable Bragg Reflector
GTI	Gires-Tournois Interferometer	SD	Static Discharge
GVD	Group Velocity Dispersion	SESAM	Semiconductor Saturable Absorber Mirror
He-Cd	Helium–Cadmium	SHG	Second Harmonic Generation
He-Ne	Helium–Neon	SMD	Single Molecule Detection
HR	High Reflector Mirror	SOC	Saturable Output Coupler
ICNIRP	International Commission on Non-Ionizing Radiation Protection	SPIDER	spectral phase interferometry for direct electric-field reconstruction
IEC	International Electrotechnical Commission	SPT	Single Particle Tracking
IR	Infrared	TEC	Thermo-Electrically Cooled
KLM	Kerr-Lens Mode-Locking	TEM	Transverse electromagnetic
KTP	Potassium Titanium Oxide Phosphate	THG	Third Harmonic Generation
LASER	Light Amplification by Stimulated Emission of Radiation	TIRFM	Total Internal Reflection Fluorescence Microscopy
LBO	Lithium TriBorate	UV	Ultraviolet
		VECSEL	Vertical External Cavity Surface Emitting Laser
		VIS	Visible