Photonics-Enabled Technologies

Lasers in Forensic Science and Homeland Security

OP-TEC

Optics and Photonics Series

Lasers in Forensic Science and Homeland Security

Photonics-Enabled Technologies

OPTICS AND PHOTONICS SERIES

STEP (Scientific and Technological Education in Photonics), an NSF ATE Project

National Center for Optics and Photonics Education

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PREFACE

This module is part of the STEP series on *photonics-enabled* technologies. The combined series on photonics-enabled technologies (comprising both STEP and OP-TEC materials) consists of modules in the areas of manufacturing, biomedicine, forensic science and homeland security, environmental monitoring, and optoelectronics, as listed below. (This list will expand as the OP-TEC series grows. For the most up-to-date list of modules, visit http://www.op-tec.org.)

Manufacturing

Laser Welding and Surface Treatment

Laser Material Removal: Drilling, Cutting, and Marking

Lasers in Testing and Measurement: Alignment Profiling and Position Sensing

Lasers in Testing and Measurement: Interferometric Methods and Nondestructive Testing

Environmental Monitoring

Basics of Spectroscopy

Spectroscopy and Remote Sensing

Spectroscopy and Pollution Monitoring

Biomedicine

Lasers in Medicine and Surgery

Therapeutic Applications of Lasers

Diagnostic Applications of Lasers

Forensic Science and Homeland Security

Lasers in Forensic Science and Homeland Security

Infrared Systems for Homeland Security

Imaging System Performance for Homeland Security Applications

Optoelectronics

Photonics in Nanotechnology

The modules pertaining to each technology can be used as a unit or independently, as long as prerequisites have been met.

For students who may need assistance with or review of relevant mathematics concepts, a review and study guide entitled *Mathematics for Photonics Education* (available from CORD) is highly recommended.

The original manuscript of this document was prepared by Raman Kolluri (Camden County College) and edited by Leno Pedrotti (CORD). Formatting and artwork were provided by Mark Whitney and Kathy Kral (CORD).

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Lasers in Forensic Science and Homeland Security

INTRODUCTION

Since September 11, 2001, national and international security has become a great priority for the safety and assets of people. Both crime prevention and detection techniques are given priority in national security. *Forensic science* plays a major role in this effort. It is a vast field in which many areas of science and technology contribute to the successful detection and prevention of crime. *Forensic pathology* examines body tissues and fluids; *toxicology* studies poisons (including drugs); *odontology* examines the teeth of deceased persons; and *anthropology* studies human beings. Within forensic science, the basic disciplines of biology, chemistry, and physics play significant roles.

The discovery of lasers in 1958 and their advances during the last decade has revolutionized many areas of science and technology. Forensic science is one of them. Laser-induced fluorescence for detection of latent fingerprints, laser ablation of minute quantities of samples, laser scanning microscopy, laser-assisted DNA sequencing, 3D imaging, portable lasers for discovery, and retrieval of trace evidence in crime scenes are some of the areas in which lasers are used extensively. This is a constantly improving science with advances in laser technology and forensic detection techniques. At this time there are no laser technicians *specifically* trained for homeland security, but that will undoubtedly change. This module discusses some of the important laser techniques used in forensic science.

PREREQUISITES

This module requires a basic understanding of high school algebra, geometry, trigonometry, general scientific nomenclature, the scientific processes, unit conversions and basic concepts in physics and chemistry. In addition, a study of Module 1-1: *Nature and Properties of Light,* Module 1-3: *Light Sources and Laser Safety,* and Module 1-6: *Principles of Lasers* form a basic foundation for a study of forensic science.

OBJECTIVES

A student completing this module will be able to:

- Describe the opportunities available to photonics technicians in forensic laboratories involved in Homeland Security.
- Describe the three states of matter.
- Describe the existence of energy levels in atoms.
- Define *luminescence, fluorescence,* and *phosphorescence*.
- Describe the decrease in light intensity as light passes through an absorbing medium.
- Give examples of absorption spectra.
- Distinguish between ordinary light and laser light.
- Describe the *cautions* associated with different classes of lasers.
- Understand the importance of *laser safety* and the need to use protective gear.
- Describe the lasers used in forensics investigations, their wavelengths and powers.
- Understand the fundamentals of laser use in fingerprint detection.
- Identify different fluorescent dyes used in fingerprint identification.
- Describe the use of film and digital photography.
- Understand the techniques of laser ablation in identifying minute crime-scene samples.
- Understand the fundamentals of laser-based DNA sequencing.
- Describe the use of microscopes in forensic science.
- Understand the basics of laser-based confocal microscopy.
- Understand the fundamentals of 3D image reconstruction.

SCENARIO

Rinaldo is a laser photonics technician at police headquarters in a major metropolitan city. He has several duties in his job. These include operating and servicing lasers and laser-based equipment, and assisting forensic experts. He uses dyes to detect latent fingerprints and then uses selected wavelengths from an appropriate laser to photograph the fluorescent fingerprints. He uses a computer to enhance, crop, and improve the contrast of fingerprint images taken form different surfaces. He records and shares these images with other laboratories involved in homeland security. He is a highly skilled laser technician and performs repair and maintenance of all the lasers in the laboratory. He assists forensic experts in selecting the correct dyes for the wavelengths selected and often measures the absorption and emission characteristics of various fluorescent dyes. He assists in the field by operating portable lasers. He sets up proper lighting to take pictures at crime scenes. He knows how to choose the proper lenses for a particular job

and uses the SLR camera with interchangeable lenses to take the correct pictures for recording evidence. He also operates lasers associated with advanced, laser-based equipment in the laboratory such as a laser confocal microscope and a laser-based, automatic DNA sequencer. He understands laser ablation techniques and assists forensic scientists in the ablation of minute samples retrieved at crime scenes for spectroscopic analysis. He works in close collaboration with other specialists in the forensic laboratory and feels greatly satisfied with his job and his part in homeland security.

A Review of Basics

Before we begin to examine how photonics and lasers affect forensic science and homeland security, we should revisit some basic ideas involving matters and energy. Much of the material that follows can be found in Module 1-1: *Nature and Properties of Light,* Module 1-3: *Light Sources and Laser Safety,* and Module 1-6: *Principles of Lasers,* each a part of Course 1: *Fundamentals of Light and Lasers*.

Matter and Energy

As we all have learned, the three kinds or states of matter are *gases, liquids,* and *solids.* When we look deeper into each one, we discover the basic building blocks—atoms and molecules. According to simple atomic theory, an atom contains a very tiny, positive *nucleus,* surrounded by a cloud of *electrons*. How many electrons there are in each atom determines whether the atom is hydrogen, iron, or uranium. And how fast the electrons are whizzing around their nucleus and how far away they are from the nucleus, tells us how much energy the atom has. Then, understanding something about the makeup of atoms, we learn that atoms can cluster together like hydrogen (H) and oxygen (O) to form molecules like water (H_2O), or atoms like sodium (Na) and chlorine (Cl) to form molecules of ordinary table salt (NaCl).

Atoms, by way of the energy of the electrons swarming around their nuclei, can absorb light incident on them and in turn emit light back outside of themselves. We find it helpful to think of the absorption and emission of light taking place with *photons* as the carriers of light energy. Our simple picture of a photon is that of a wave packet of electromagnetic energy E given by the formula $E = h\nu$, where h is the famous Planck constant and ν is the frequency of the light.

Absorption, Emission, and Energy Levels

To understand more clearly how atoms absorb or emit energy, we need to understand the idea of *energy levels* in atoms. To do this, we make use of the Bohr model of the atom. Below, in Figure 1a, we show a two-dimensional picture of what is really a three-dimensional space. The nucleus, the dark spot, is surrounded by circles that represent the paths of *orbits* that electrons in the cloud can trace out as they move around the nucleus. Even though we show them all in one plane, the orbits are really like *spherical* shells that surround the nucleus—in three dimensions. The shells containing the electrons are labeled K through O, as shown in the drawing to the left. The notation K(2) indicates that the K-shell is complete when it has 2 electrons. Similarly, L(8) indicates that 8 electrons complete the L-shell, and M(18) indicates that 18 electrons complete

the M-shell. Knowing how electrons fill these shells, in order, has led us to develop and understand the *Periodic Table of the Elements*, found in most chemistry texts.

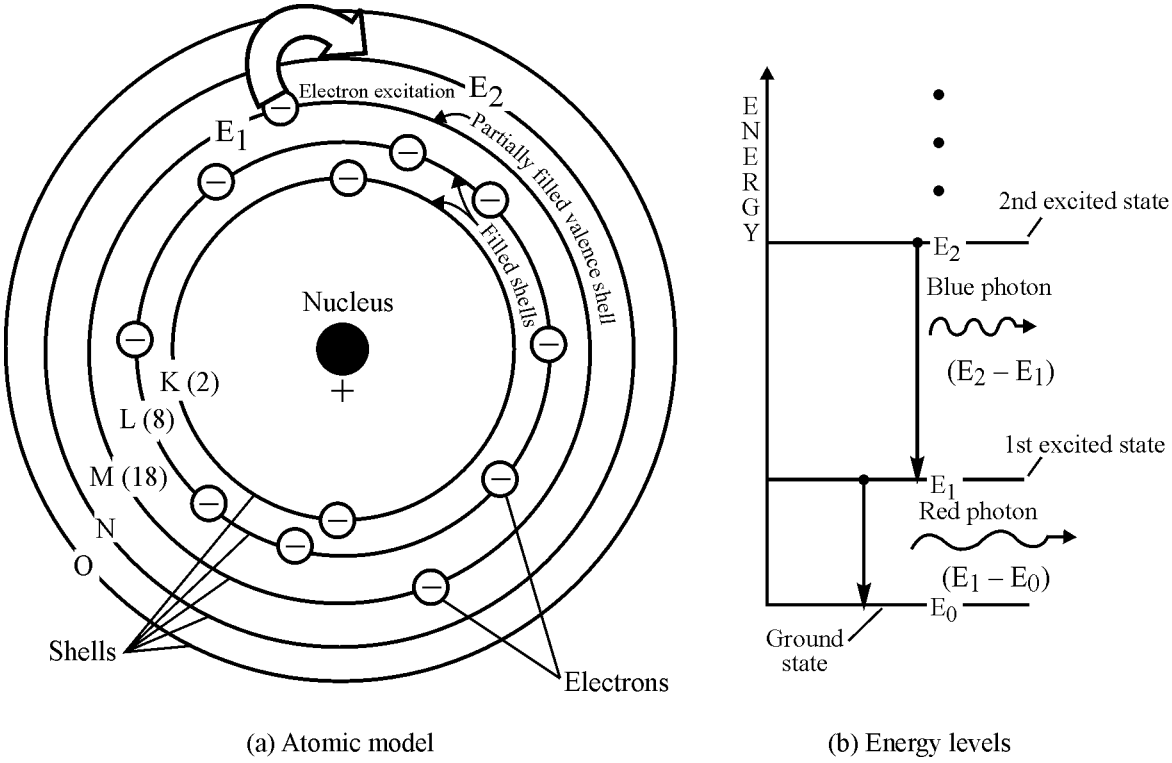


Figure 1 *Atomic model with energy levels*

As mentioned, in the Bohr model we treat the energy of an atom in terms of the positions of its electrons. When all the electrons are in an unexcited, or *ground,* state, the atom is assumed to be in its lowest energy state—or level. When the atom absorbs energy, electrons can be "excited" and moved into higher-energy states, or levels. As electrons move from one shell to another, unique amounts, or *quanta,* of energy are absorbed or emitted. A photon is such a quantum of energy, as we have mentioned earlier.

In Figure 1a, the atomic model shown has a total of 12 electrons—2 in the K shell, 8 in the L shell, and 2 in the M shell. Such an atom with 12 electrons around the nucleus represents the element *magnesium*. Note that the 2 electrons in the K shell fill that shell, and the 8 in the L shell fill that one. But in the M shell, where a total of 18 are required to fill that shell, only 2 electrons are present. Such electrons, in an outer, unfilled shell, are called *valence electrons*. Electrons in filled shells generally stay there. They are not involved in chemically bonding one atom to another, to form molecules. But valence electrons in unfilled shells, generally move to higher-lying shells in the same atom, or to unfilled shells in other atoms, to form molecules. Thus magnesium is a very *reactive* element, forming many different compounds such as magnesium oxide (MgO).

In Figure 1a we have shown the two valence electrons in the M shell, with the energy of the atom as E_1. The outer N and O shells are empty. Now if a photon of the correct energy is absorbed by the atom, it can move one of the valance electrons up to the N shell, giving the

atom an energy E_2. And later this electron can fall back to the M shell and give back the energy that was absorbed initially.

In Figure 1b we show the *energy levels* (horizontal bars) labeled E_0, E_1, and E_2. The level E_0 would correspond to all 12 electrons in magnesium located in the K, L, and M shells, as pictured. The level E_1 would correspond to an absorption of a photon to move a valence electron from the M shell to the N shell. The level E_2 would correspond to a movement of the valence electron to one notch higher, to the O shell, and so on. When the valence electron falls back to the M shell from the N shell, it gives up energy equal to $(E_1 - E_0)$. As it falls from the O shell to the N shell, on its way back to the M shell, it gives up energy equal to $(E_2 - E_1)$. Depending on how much energy is involved in the "fall backs," the photons emitted emerge with wavelengths in the red, blue, or some other part of the visible light spectrum. The totality of emitted photons—those emitted by an atom that has been "energized" through the absorption of energy from an outside electromagnetic energy source—leads to *spectra*. Such spectra will be used to identify unknown chemical toxics or hazards in forensic science, since each spectra is like a unique finger print.

Spectra of Light Sources

The sources of electromagnetic radiation are many. Usually sources are divided into two categories, natural and man-made. Examples of natural sources of radiation include the sun, observable stars, radio stars, lightning, and, in fact, any living body. Some of the man-made sources of radiation are incandescent and fluorescent lights, heaters, lasers, masers, radio and television antennas, radars, and X-ray tubes.

Two types of spectra are important in photonics: *emission* and *absorption* spectra. An emission spectrum is formed by light emitted from a source. An absorption spectrum is formed when light that passes through an optical medium is partially absorbed by that medium.

All materials with temperatures above absolute zero emit electromagnetic radiation. Every energy-emitting atom and molecule has its own characteristic set of spectral lines. The specific wavelengths and energies that produce a spectral "fingerprint" depend on the atomic and molecular structure of the material. The *line spectra* observed early in the scientific age led to a significant understanding of the structure of atoms and eventually to the development of modern quantum theory. This theory holds that light emitted by an atom or molecule has a discrete wavelength, corresponding to a specific energy-level change within the atom or molecule.

To observe *a line* or *band* spectrum, light is passed through a slit of a so-called *spectroscope*. The image of this slit is then refracted by a prism or diffracted by a grating and recorded on film—a spectrograph. The slit is imaged on the film at a different position and at a different intensity for each different wavelength of the light, thereby forming a "fingerprint" of that particular light. *Spectroscopy* is the science that analyzes line spectra and identifies the different chemical elements in the material that is emitting electromagnetic energy.

Emission spectra—Figure 2 represents a typical *emission spectrum* of a monatomic gas, such as helium or neon. This type of spectrum is produced by an electrical discharge passed

through a gas sample contained at low pressure. Each of the lines in the emission spectrum is produced by a single atomic transition, from one energy level to the other, and the *intensity* of each line produced is dependent on the probability of atoms making that particular transition. Stronger lines are the result of more probable transitions from energy states having short atomic lifetimes, that is, how long the atom remains in an upper energy level, or state, before falling back to a lower energy level. Weaker lines are the result of less probable transitions from states that have longer atomic lifetimes.

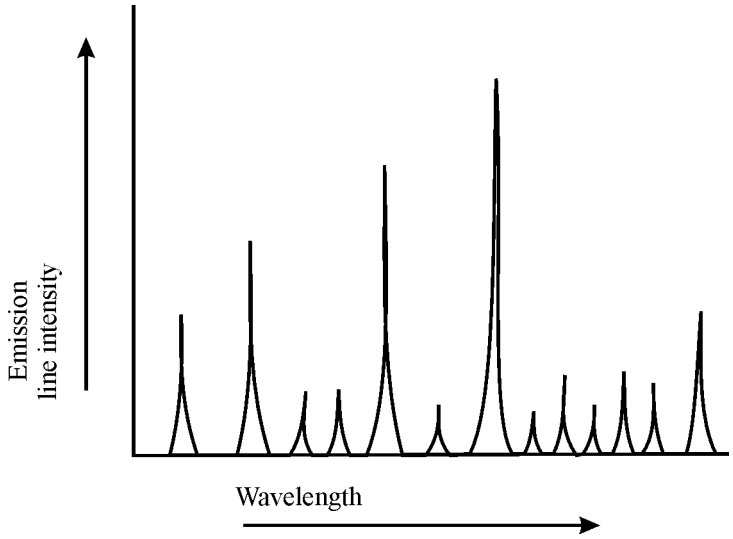


Figure 2 *Line spectrum of a monatomic element*

Absorption spectra—Absorption involves the transfer of energy from an incident electromagnetic wave, or photon, to the atoms or molecules in a material. Energy transferred to an atom, for example, excites electrons to higher energy states, as we have noted earlier. Thus absorption depends very much on the energy level structure of particular elements or compounds. The wavelength/intensity spectrum of light that passes through the material appears to have certain wavelengths removed (those of the absorbed light) and is called the *absorption spectrum. Selective absorption* explains why different objects that we see have different colors. For example, a red apple is "red" because the skin of the apple selectively absorbs all colors (wavelengths) incident on it except red, which it reflects to us to see as red light.

Figure 3 represents a typical absorption spectrum for a *solid* material. Unlike the comparatively narrow spikes of the *gaseous* spectrum seen in Figure 2, this spectrum consists of broad, irregularly spiked regions called *absorption bands*.

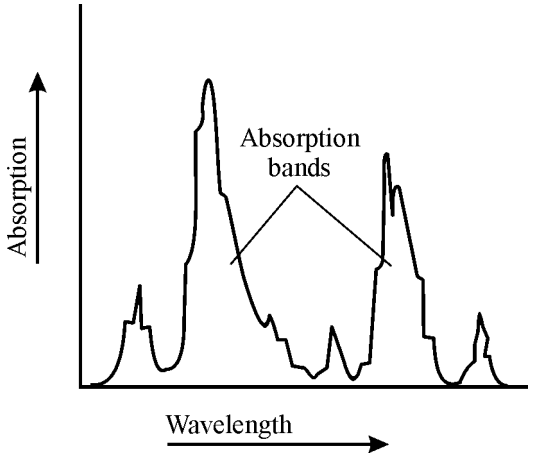


Figure 3 *Absorption spectrum of a solid*

Areas containing a closely spaced group of strong lines appear as an absorption band. In crystalline solids such as Nd:YAG (Neodymium atoms in an yttrium aluminum garnet host), these absorption bands consist of groups of closely spaced, sharp-edged lines. In solids such as Nd:glass that lack an ordered crystal structure, the bands are broader and less distinct. The definitions of these absorption bands are important in determining the type of electromagnetic radiation we should use to excite solid-state lasers.

Luminescence, Fluorescence, and Phosphorescence

Words that we shall meet in the broad topic of *emission* of light energy from excited atoms are *luminescence, fluorescence,* and *phosphorescence*.

Luminescence is defined as the light emitted by a substance over and above the normal radiation it emits due to its own "body" temperature. Luminescence in materials can be caused by the absorption of heat energy, electromagnetic energy, chemical energy, or mechanical energy (friction).

Fluorescence, a special case of luminescence, is the re-emission as electromagnetic energy, normally at wavelengths the same as, or longer than, the electromagnetic wavelengths involved in the absorption process. When the re-emitted energy comes out with less energy—at a longer wavelength—than the absorbed energy, the shift to a longer wavelength is referred to as the *Stokes shift.* In many ways, though, fluorescence is just the opposite of absorption, giving up a photon of the same energy and wavelength as the photon involved in the initial absorption event. After absorption, the fluorescent re-emission lasts for very short times of the order of nanoseconds (10^{-9} seconds) to microseconds (10^{-6} seconds).

Phosphorescence, another special case of luminescence, is the re-emission of absorbed electromagnetic energy at wavelengths longer (less energetic photons) than the wavelengths of the electromagnetic energy causing the initial absorption. This is again an example of a *Stokes shift.* After the initial absorption, the phosphorescence, unlike the much shorter fluorescence, lasts for intervals of a millisecond (10^{-3} seconds) to several seconds.

Ordinary Light and Laser Light

As discussed in Module 1-1, *Nature and Properties of Light,* electromagnetic radiation can be understood as consisting of waves traveling through space. Visible light contains different *wavelengths,* just as waves on the ocean vary in length. Wavelength is the distance between peaks on a light wave, as represented in Figure 4. The color of light depends on its wavelength. Violet light has the shortest wavelength of all visible colors, and red light has the longest, as seen in Figure 5a. White light is a simultaneous mixture of all visible colors or wavelengths.

Laser light, unlike ordinary light, is unique in that it usually consists of only a single color of light. The wavelengths that make up this single color, while not perfectly equal in length, occur within a very narrow range of values. **Monochromaticity** is the laser light's property of containing essentially only one pure color over a narrow range of wavelengths.

The light from an ordinary source—like a light bulb—radiates away from the source in all directions, as shown in Figure 5b. This spreading or divergence of ordinary light is what makes it so useful for lighting homes and workplaces.

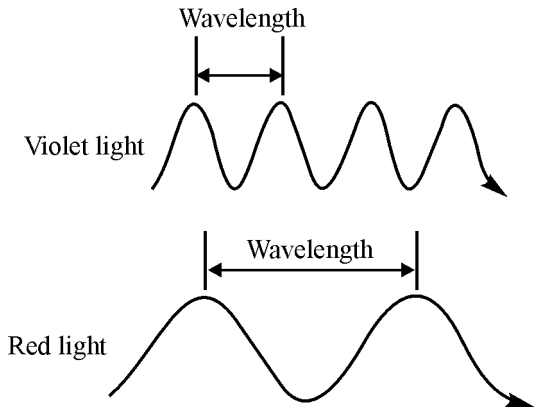


Figure 4 *Light waves*

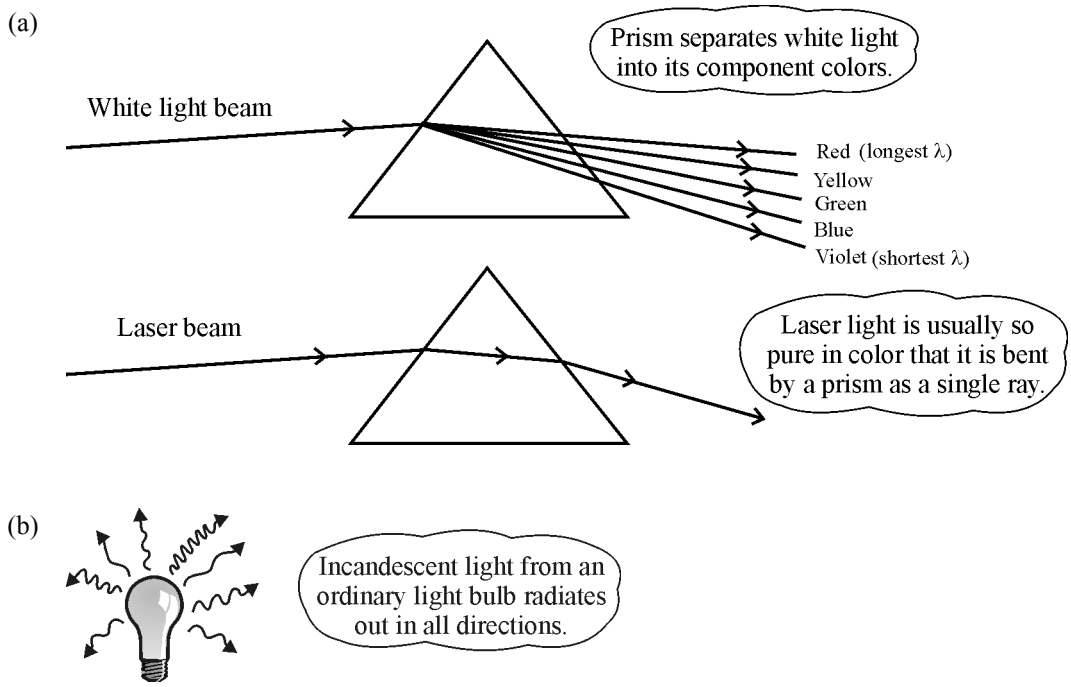


Figure 5 *White light, laser light, and light emitted by an incandescent bulb*

Laser light, by comparison, is very ***directional***. The light emitted by a laser diverges very little, as shown in Figure 6.

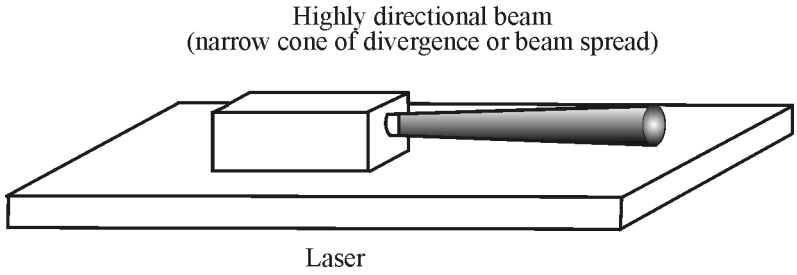
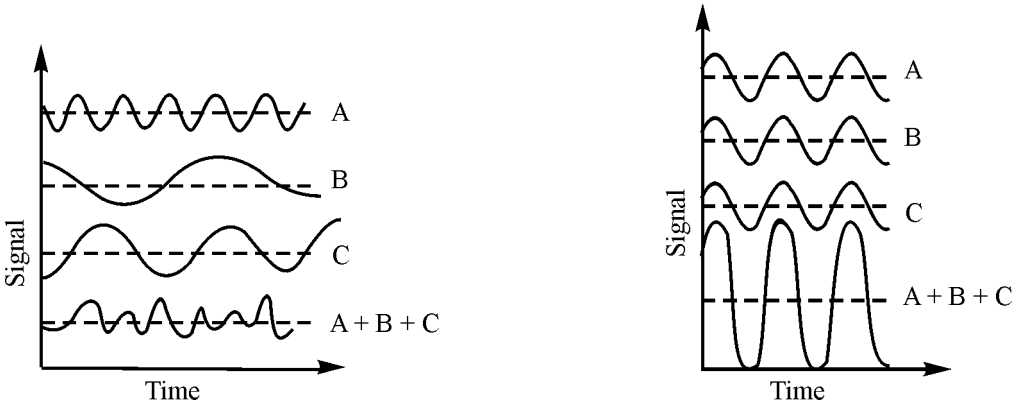


Figure 6 *Concentrated direction of laser light*

Out-of-step, noncoherent, light waves produced by ordinary sources don't form an orderly pattern. Their amplitudes combine in a *random* fashion and produce

a resultant wave that is no larger than any of the multiple single waves, as seen in Figure 7a. Such light is said to be *incoherent*. By contrast, the waves produced by a laser travel through space *in step* with one another. They're said to be "in phase" or ***coherent***. Since all the separate waves in the beam remain in step with one another, as shown in Figure 7b, the resultant wave is much stronger than any single wave. A very intense, coherent beam is generated.



(a) Incoherent light (b) Coherent light

Figure 7 *Adding coherent and incoherent light waves*

Absorption and Transmission of Light Energy

Figure 8 depicts a beam of light traveling through a piece of optical material or chemical solution. Some of the light energy is absorbed and some is transmitted.

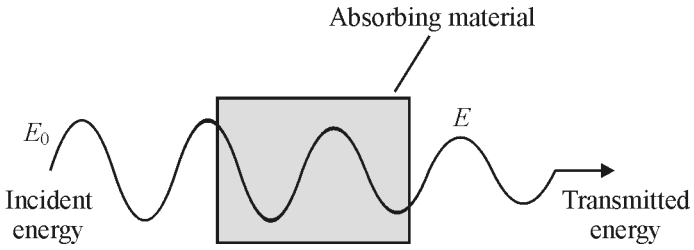


Figure 8 *Absorption of a light beam (Decrease in light irradiance is by absorption alone. Scattering and reflection, always present, are not considered here.)*

The transmission of the optical material is given by Equation 1.

$$T = \frac{E}{E_0} \qquad (1)$$

where T = Transmission, as a decimal less than one, or a percent
E_0 = Irradiance of light *incident* upon the material in units of power per unit area
E = Irradiance of light transmitted through the material in units of power per unit area.

Example 1 illustrates the use of this equation in solving a problem.

Example 1: Calculation of transmission

Given: The light incident upon the material in Figure 8 has an irradiance of 2 milliwatts/cm^2. The irradiance of the transmitted light is 0.50 milliwatts/cm^2.

Find: The transmission

Solution

$$T = \frac{E}{E_0}$$

$$= \frac{0.5 \text{ milliwatts/cm}^2}{2.5 \text{ milliwatts/cm}^2}$$

$$T = 0.20$$

Thus 20% of the light is transmitted.

If light is transmitted through a sample containing a chemical solution, as we might imagine in the arrangement of Figure 8, the incident light at a certain wavelength, is absorbed. Going beyond Equation 1, we can define the absorption in terms of the *optical density* (*OD*) of the solution in terms of Equations 2.

$$OD = \log_{10} \frac{E}{E_0} \qquad (2)$$

where E = Radiation transmitted by the solution in units of power/area
E_0 = Radiation incident on the solution in units of power/area
OD = Optical density, a pure number

The *extinction coefficient* (ε) of the chemical solution is related to the optical density (*OD*) of the solution by Equation 3.

$$\varepsilon = \frac{OD}{C\ell} \qquad (3)$$

where C = Concentration in molar units of moles/liter
ℓ = Thickness of the sample in centimeters
ε = Extinction coefficient in units cm^2/mole

The *absorbance* (*A*), a pure number, is related to the optical density (*OD*), and the extinction coefficient (ε) as shown in Equation 4.

$$\varepsilon = \frac{A}{C\ell} \qquad (4)$$

The use of Equation 4 to determine the extinction coefficient ε is illustrated in Example 2.

Example 2: Extinction coefficient

The molar concentration of cholesta-3, 5-diene in ethanol is 6×10^{-5} mole/liter. The path length of light in the liquid is 1 cm. The absorbance (A) determined with a spectrophotometer at wavelength 225 nm is 1.2. Calculate the extinction coefficient ε at this wavelength.

Solution

Making use of Equation 4, we have:

$$\varepsilon = \frac{A}{C\ell} = \frac{1.2}{6\times10^{-5}\times1} = 20{,}000\ \frac{\text{cm}^2}{\text{mole}}$$

Most organic liquids have high extinction coefficients such as that for cholsta-3, 5-diene in ethanol, as shown in Example 2. Thus the solutions of these organic liquids in ethanol, methanol, or water should be very dilute. Some substances such as the dye Rhodamine 6G, used in dye lasers, have very high extinction coefficients (of the order of 100,000 at certain wavelengths) and are often used in fluorescence studies.

Sources of Ordinary or Incoherent Light

A variety of light sources are available to illuminate a crime scene and also to produce fluorescence. Among these are simple, incandescent filament lamps, fluorescent low-pressure discharge lamps, high intensity and pressure discharge lamps, flash lamps, and light emitting diodes.

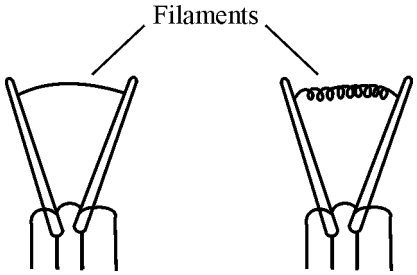


Figure 9 *Types of incandescent lamp filaments*

Incandescent lamps

These are filled with gas and contain filaments of different configurations (Figure 9). The gases used are generally mixtures of argon and nitrogen. Krypton is added in some cases for a longer lifetime. Tungsten halogen lamps contain a small amount of halogen gas such as bromine or chlorine. Tungsten halogen lamps do not form black coatings on the inner surface during long periods of operation. The outer envelopes of these lamps are made out of heat resistant glass or quartz. The filaments are usually coils that produce uniform intensity.

Fluorescent light sources

These are low-pressure discharge lamps with a fluorescent phosphor. Mercury discharge lamps emit 90% of their energy in the ultraviolet range (around 253.7 nm).These are very useful in producing fluorescence and are used frequently in forensic science. Fluorescent lamps can have either cold or hot cathode electrodes. Cold cathode electrodes are used when a rapid start is necessary. Hot cathode electrodes give greater luminescence efficiency

High-intensity discharge lamps (HID)

These lamps have mercury, sodium, or metal halides with quartz envelopes. The gas pressure is usually 2 to 4 atmospheres. High intensity discharge lamps usually have two envelopes for heat reduction. These lamps produce high amounts of ultraviolet radiation and will produce skin irritation and eye inflammation if unprotected. Schematic diagram of a mercury-HID lamp is shown in Figure 10.

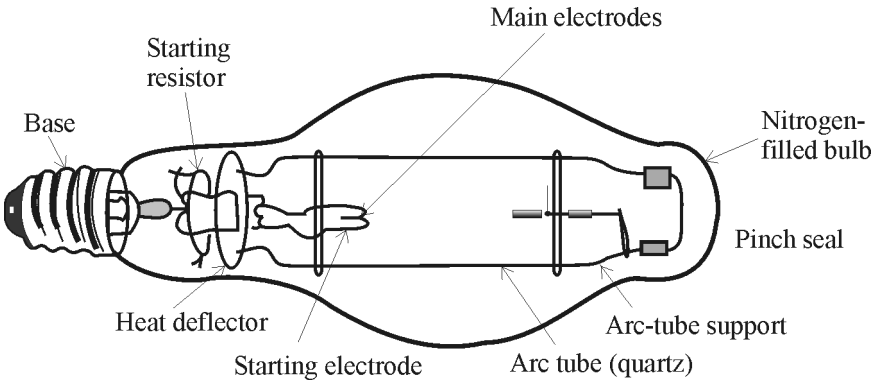


Figure 10 *Cutaway drawing showing the construction of an Hg-HID lamp*

Flash lamps

These are high-intensity discharge devices. They are often used for a high level of illumination in photography and laser technology. They usually contain gases such as xenon or krypton. Applying a high voltage across the discharge tube produces the flash or arc. This arc ionizes the gas and produces high-intensity light with maximum output in the visible and infrared regions. The quartz tube usually is surrounded by a cooling water jacket. A water-cooled flash lamp is shown in Figure 11.

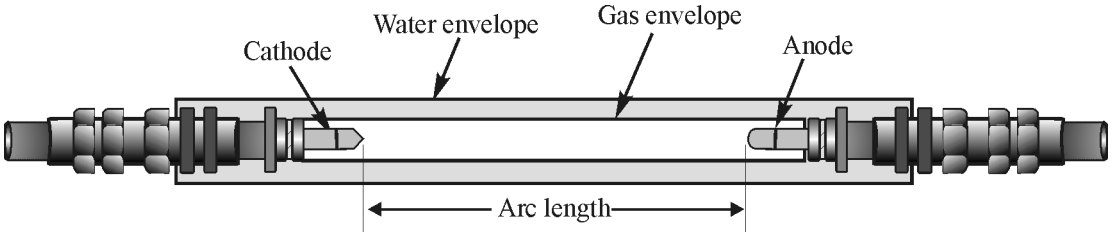


Figure 11 *Schematic diagram of a gas flash lamp with water cooling*

Laser Safety

Lasers are high-intensity radiation sources that can cause severe damage to various parts of the body unless protected by optical filters. The eye is most vulnerable to laser light since the intensity of the laser light is further increased by the optical magnification produced by the cornea and lens of the eye. When working with lasers, one must wear appropriate safety goggles. Since different types of lasers produce different output wavelengths and different intensities of light, the appropriate goggles have to be worn. A number of companies (such as Edmund Industrial Optics) supply goggles for specific lasers and powers.

The damage to skin tissue from laser light is also important. Reflection of the laser beam directly by skin must be totally avoided. The American National Standards Institute (ANSI) has classified lasers based upon their intensity as follows:

Class 1: Lasers in this class have powers less than 0.1 mW and do not emit hazardous radiation unless directly pointed into the eye for too long a period of time. **Never allow a laser beam from any laser to enter the eye directly.**

Class 2: Lasers in this class have powers of up to 1 mW and can cause damage to the eye if exposed directly to the eye for a long time.

Class 3a: Lasers in this class have powers between 1 mW and 5 mW. They can cause damage to the eye when exposed to the radiation but not when the beam crosses the eye momentarily.

Class 3b: Lasers in this class have powers between 5 mW and 500 mW. They always require protection to the eye.

Class 4: Lasers in this class have powers greater than 500 mW. Goggles **must** be worn.

For more details on laser safety, see Module 1-3, *Light Sources and Laser Safety,* in Course 1, *Fundamentals of Light and Lasers*.

Laser Types Used in Forensic Science

The following classifications and characteristics of lasers provide an overview of the topic of lasers used in the practice of forensic science.

Neutral atom lasers

These are neutral-atom lasers in which the active medium is electrically neutral. The atoms are ionized by initially passing a high voltage through the gas. An ionization current is produced with electrons moving towards positive electrode and the positive ions moving towards the negative electrode. Neon and cadmium are the well-known laser hosts in this category. Helium atoms are used to transfer electrons to the metastable states of neon through collision and non-radiative transfer. Stimulated photons are produced as electrons jump from the metastable state to the ground state. These photons are optically amplified. These lasers produce outputs in the visible (He-Ne) and ultraviolet (He-Cd) regions. He-Cd is often used in forensic science. These lasers use only a few hundred volts for operation and currents of the order of a few milliamperes. Beam divergence (angular spread of the beam) of these lasers is quite small. (See Table 1. This table is useful in noting the characteristics of the lasers that are described on the pages that follow.)

Ion lasers

The *most widely used laser* in forensic science is the *argon ion* (Ar^+) laser. The active medium of an ion laser consists of a singly-ionized inert gas such as argon or krypton. The gas is subjected to a low-voltage/high-current electrical power, and a single electron from each atom is removed. Ion lasers have an important distinction from neutral atom lasers. The terminal level for lasing in an ion laser is an ionized state itself—rather than an atomic state. This implies that the atoms have to be kept constantly in an ionized state and further excited to a higher state with a longer lifetime. The constant high current requires complicated current stabilization circuits and cooling systems. Ar^+ lasers produce wavelengths between 457 nm and 514 nm. The advantage of the ion laser is its ability to "tune" for different wavelengths. These lasers produce several wavelengths simultaneously and by using a prism in the cavity, any particular wavelength can be selected. Argon ion lasers can be used in the ultraviolet also (333.6 nm) by using UV mirrors. But the power output is very low. *Krypton ion* (Kr^+) lasers are similar to Ar^+ lasers and can be considered complementary to Ar^+ lasers. Portable mixed-gas lasers are now available in the market. Some of the wavelengths produced by Ar^+ and Kr^+ lasers are shown in Table 1. A schematic diagram of an Ar^+ ion laser is shown in Figure 12.

Table 1. A survey of some laser output parameters

Active medium	Wavelength (nm)	Power output TEM_{00} (W)	Beam Divergence (mrad)
Atomic			
He-Ne	632.8	80×10^{-3}	1.0
He-Cd	325.0	50×10^{-3}	0.5
Ion			
Ar^+	457–514	10.0	0.8
Kr^+	219,350,743	8.0	0.8
Molecular			
N_2	337.1	3×10^5	1×7
Excimer			
XeFl	351	125	4.5×1.5
XeCl	308	110	4.5×1.5
Dye			
R6G	540-630 (Ar^+ pumped)	1.0	2.0
Solid-state			
Ruby	694.3	5.7×10^3	5.0
Nd:YAG	1.06×10^3	5.0	5.0
Nd:glass	1.06×10^3	1.0×10^4	5.0
Ti.Sapphire	660–1100	1.8	2.0
Alexandrite	700–818	20.0	2.0
YLF	1.05×10^3	0.5	2.0
Semiconductor			
GaAs (single)	904	2.0	20×200
GaAs (array)	850	25.0	20×200

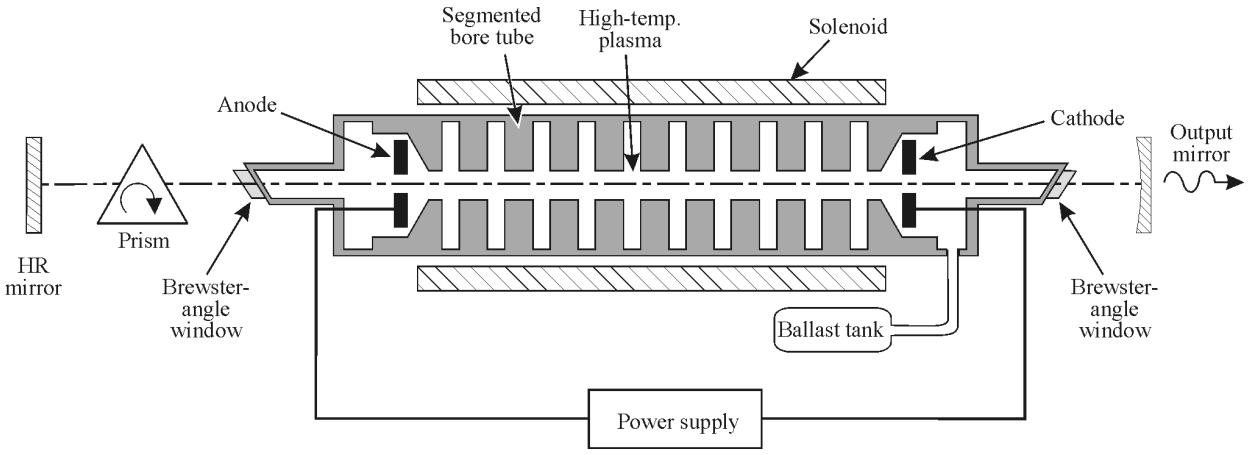


Figure 12 *Schematic diagram of an Ar^+ laser with tuning capabilities*

Molecular lasers

The nitrogen laser is an example of a molecular laser that operates in the ultraviolet region (337.1 nm). This wavelength is useful in fluorescence studies. These lasers can produce very large, peak power pulses (> 100 kW) at 100 pulses per second (pps). These lasers can work at low vacuum or even atmospheric pressure. The gain in these lasers is so high that they do not require mirrors! The quality of the gas required is not high and hence easy to operate. (See Table 1.)

Excimer lasers

Excimer lasers have active media that are made up only of molecules during a reaction. (*Excimer* stands for *excited state dimer*.) For example, Xenon is an inert gas atom and chlorine is a halogen gas atom. Normally, these two atoms do not combine to form a molecule. However, under high pressures and temperatures, they can be made to form a molecule XeCl. This molecule has an excited state from which lasing action can take place. Most of the excimer lasers produce wavelengths in the ultraviolet region and can produce pulses of peak power of the order of 10^9 W. In addition, these lasers can produce *ablation* of solid materials (direct conversion from solid to gas), and hence, are very useful in forensic studies. The excimer gases are toxic. Thus, great care must be taken in the waste disposal of these gases. (See Table 1.)

Solid-state lasers

Single crystals of transparent solids are used as the active medium in these lasers. A suitable *guest* material is *doped* in the host material while the single crystals are grown. The guest atoms have sharp electronic levels that function as the metastable states. Most often, these solids are pumped by flash lamps. In recent years, diode lasers are directly deposited on the rods and used as pumping sources. Both axial and transverse pumping is possible. In addition, most of the solid-state lasers can include cavity devices such as *Q-switches, mode lockers,* and *frequency doublers*. These enable a much better manipulation of the output beam. Solid-state lasers require very little maintenance, since the crystals remain unchanged for many years. Furthermore, the output remains stable, since the internal parameters of the crystals do not change. Solid-state lasers have, in general, a slightly larger beam divergence compared to gas lasers. In recent years, *vibronic* lasers have been developed. These lasers can be *tuned* very much as are the dye lasers. By using frequency multipliers and pumping by another laser, several *bands* of tunable wavelengths over wide wavelength ranges are obtained. A schematic diagram of a frequency-doubled Nd:YAG laser is shown in Figure 13. (See Table 1.)

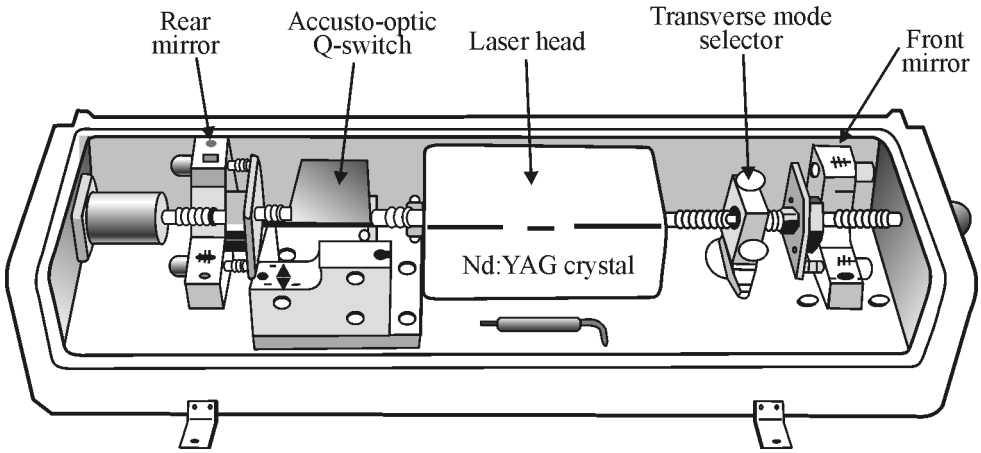


Figure 13 *Schematic diagram of an Nd:YAG laser with frequency doubler*

The most *well known solid-state laser* used in forensic studies is the *frequency-doubled Nd:YAG laser*. Here the output frequency of the laser in the near IR (1.06 microns) is doubled and hence the wavelength halved using a *nonlinear* crystal. Nd:glass, Ti:sapphire, Alexandrite (Al_2O_3), and YLF lasers also can be used. The last three are tunable lasers. These lasers can be operated in continuous as well as pulsed modes. (See Table 1.)

Dye lasers

Many organic dyes when dissolved in alcohol have wide bands of fluorescence and are suitable as amplifying media for tunable lasers. The most well known among these organic dyes are rhodamine 6G and rhodamine B. Tunable wavelengths of laser radiation, including practically the entire visible region, are obtainable with these and other dyes. These lasers can be pumped by Argon lasers. Dye degradation and turbulence are two factors that make these lasers unstable compared to the tunable solid-state lasers. These lasers can also be used in continuous and pulsed modes. A typical dye laser pumped by Ar^+ laser is shown in Figure 14. (See Table 1.)

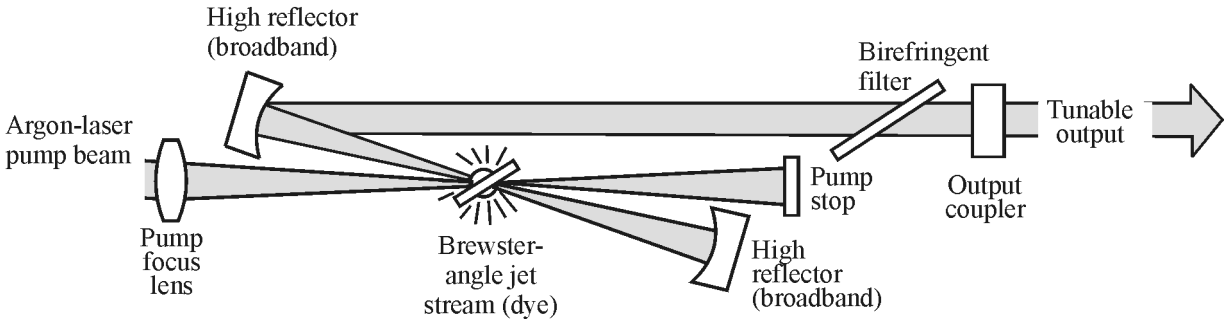


Figure 14 *Schematic diagram of an argon-laser-pumped dye laser*

Diode lasers

Semiconductor diode lasers are also solid-state lasers. However, the lasing action in them is different from that of the solid state lasers described above. Compound semiconductors such as gallenium arsenide or indium antimonide can be doped with certain materials to make them

behave like *extrinsic semiconductor*s. These materials conduct current when a small voltage is applied. A P-N junction is formed by making a junction of an N-type semiconductor (excess electrons) and a P-type semiconductor (excess holes). Many exciting phenomena take place in this junction if a positive or negative *bias* (voltage) is applied to this junction.

A junction will become unstable if both the P-side and the N-side are heavily doped. If a forward bias is applied to such a junction, electrons and holes rush from opposite sides of the junction to knock off electrons in the junction and produce photons. Under certain circumstances, these photons can be stimulated photons. Mirrors coated on either side of the junction can amplify these photons. In some cases, the semiconductor's cleaved faces themselves can work as a mirror. A typical PN junction laser is shown in Figure 15.

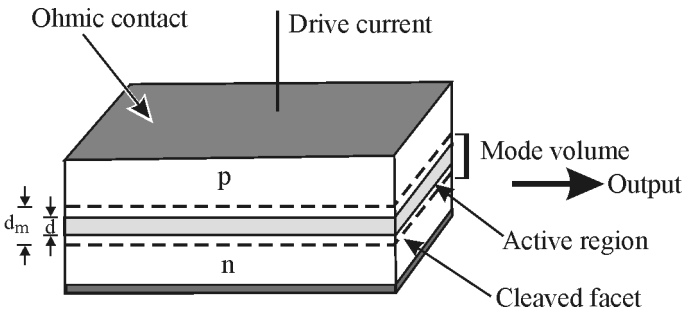


Figure 15 *Simplified structure of a diode laser*

The great advantage of the diode lasers is their small size and the low voltage needed to operate them. However, at this time, diode lasers in the blue-green region still have low powers. Until their power ratings improve, their use in forensic science is limited. (See Table 1.)

Laser Fingerprint Identification

Fingerprints

Identification of criminals based on fingerprinting is one of the most important parts of forensic science. William Herchel, a British civil servant working in India around 1800, was the first to use the fingerprints of natives as a means of identifying them. In 1880, Scottish physician Henry Fauld was the first to understand the importance of fingerprints in the identification of an individual. Fingerprints are uniquely characteristic to individuals and do not change with age, disease, or accident. The science of fingerprinting is called *dactyloscopy* and is used in identifying individuals to aid law enforcement.

Fingerprints are identified by carefully studying the *ridge* characteristics (also known as *minutiae*) on the different fingers. A typical fingerprint is shown in Figure 16. It has several identifiable characteristics such as *bifurcation, ridge ending, ridge crossing,* and *island*. These allow one to identify the person to whom they belong.

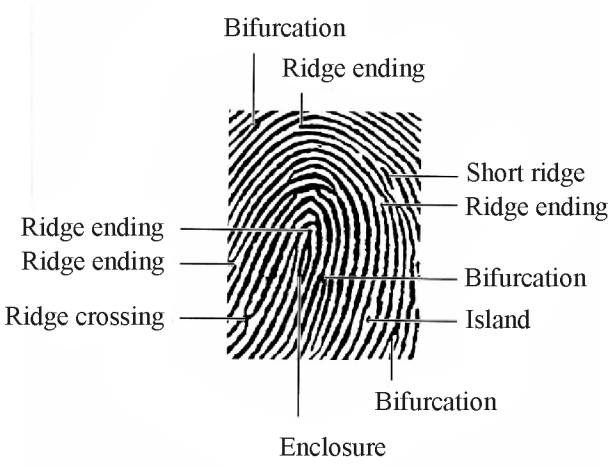


Figure 16 *Typical fingerprint showing the ridge characteristics (Courtesy Kemtec Educational Corporation, 877-536-8321)*

Ridges, bifurcations, enclosures, and islands are some of the important *ridge characteristics* in a fingerprint, as shown in Figure 16. Further identifying features in a fingerprint are *type lines, delta,* and *core.* Type lines are the diverging ridges surrounding the core (Figure 17). Fingerprints can be broadly divided into three categories: *whorls, arches,* and *loops* (Figure 17). About 65 percent of the population have loops, 30–35 percent have whorls, and about 5 percent have hair arches. When loops slope towards the little finger, they are called *unlar,* and when they slope towards the thumb, they are called *radical.* A whorl looks like a circular whirlpool with extended rings.

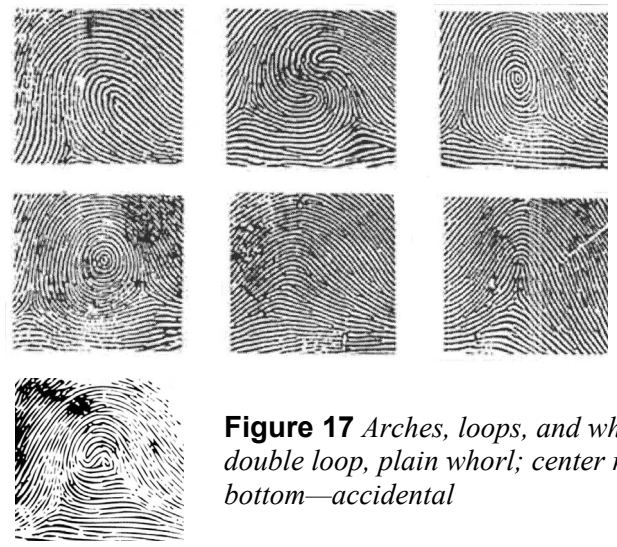


Figure 17 *Arches, loops, and whorls. From left to right: top row—loop with a delta, double loop, plain whorl; center row—central pocket loop, plain arch, tented arch; bottom—accidental*

Whorls are further subdivided into four distinct groups: plain whorl, central pocket loop, double loop, and accidental. A delta occurs between two type lines, and the core is the center of the loop. All loops have at least one delta. An accidental can be a combination of loop and plain whorl or a loop and tented arch. Arches are the least common among the fingerprints. These are divided into plain and tented types. Tented arches have a steep upward thrust at the center.

Identification of fingerprints

Because no two fingerprints have exactly the same ridge characteristics, a given fingerprint can often be traced to its origin by comparing it to a fingerprint database. Some fingerprints are easier to trace than others. This is because fingerprints fall into two general categories: (1) full, inked prints and (2) incidental prints. *Full, inked prints* include those taken as a security precaution, for example, when foreigners enter the country. Fingerprints taken in this manner can then be compared to databases of fingerprints of known criminals. Comparison of full, inked prints is easy and accurate because they are complete and are printed on surfaces and backgrounds designed to produce the clearest possible results. *Incidental prints,* on the other hand, are left accidentally, for example, at crime scenes. They are often unclear and/or incomplete and may be on any type of surface. In such cases, the task of tracing the print to the person who left it is much more complicated. Figure 18 shows matching fingerprints, one taken using an ink pad, the other lifted from a crime scene.

Full print Crime scene print

Figure 18 *Matching the ridges of two fingerprints—one from the inked impression of a suspect and the second from a crime scene. The one lifted from a crime scene will not be as good as the one taken on an ink pad.*

Even the print available may be on a surface on which it is not easily visible. It may be smudged or even obscured. Further, fingerprints depend on the nature of the fingers that made the prints (i.e., the organic and inorganic components of the sweat on the fingers, the environment to which the fingerprints are subjected, how much time has elapsed since the prints were made, and when they are studied. Hence, a perfect match between the fingerprints of the suspect and the fingerprints at the crime scene is difficult at best. Various techniques are used to improve the quality and contrast of the fingerprints. It is here that lasers play a major role. Identification and comparison of the fingerprints require expertise and experience. It is generally agreed that between 8 and 16 ridge comparisons are necessary before two prints can be identified as the same.

Perspiration through the pores of the skin on the friction ridges of the fingers consists mostly of water (98 percent) and a small amount of combinations of various organic and inorganic substances. The percentage of these latter substances depends on individuals. The list of organic and inorganic substances present in perspiration is given in Table 2. Among these substances, riboflavin, pyridoxine, and other organic compounds interacting with sodium are most important in terms of fingerprint identification. These compounds luminesce and will be visible under light.

Table 2. Organic and inorganic components of perspiration

A. Pyruvic Acid
B. Lactic Acid
C. Glycogen
D. Nitrogenous compounds
    1. Ammonia
    2. Urea
    3. Uric Acid
    4. Creatine
    5. Creatinine
    6. Amino acids
E. Lipids
F. Water soluble vitamins
    1. B Complex vitamins
        (a) Riboflavin
        (b) Pyridoxin
G. Eccrine sweat can be broken down into the twenty primary elements

1. Bromine	11. Magnesium
2. Calcium	12. Manganese
3. Carbon	13. Molybdenum
4. Chlorine	14. Nitrogen
5. Cobalt	15. Oxygen
6. Copper	16. Phosphorus
7. Fluorine	17. Potassium
8. Hydrogen	18. Sodium
9. Iodine	19. Sulfur
l0. Iron	20. Zinc (4)

However, these substances are not always present in every fingerprint. They vary with a person's condition when the prints were made. For instance, prints made by a very nervous person tend to show more luminescence than those made by a calm person. This is also complicated by the surface on which such prints are made. Prints made on soft materials like putty, tar, soap, or chocolate are very different from those made on hard surfaces such as glass, wood, plastic, or metal. The latter are usually *latent* and have to be brought out by dusting with fine aluminum powder. They can be *lifted off* with the help of adhesive tape and fixed on a card for further study. They can also be directly photographed. Latent prints on porous surfaces such as paper, cloth, and wood can be developed using iodine fumes or ninhydrin spray. Impressions on skin may be *visualized* using X-ray techniques. In some cases, direct examination of fingerprints using a laser can bring out latent prints. Low-powered lasers do not cause any damage to the prints, skin, or blood.

Fluorescence techniques in fingerprint identification

Preparation for examination of fingerprints—Fingerprints that are taken on an inkpad are generally clear and pose no problem for comparison. These prints do not require any preparation for observation. However, the prints left at a crime scene need *preparation* for observation based on the nature of the print. Some prints will be visible to the naked eye and can be directly photographed. Prints made after the finger touches a colored material like blood, paint, grease, or ink are included in this category. Prints left on soft materials such as wax, soap, or dusts are difficult to see directly and are called *latent fingerprints.* They require special treatment for observation. Hard and non-absorbent surfaces such as glass, tile, and some types of wood require an application of dusting or super glue (*cyanoacrylate ester*). Soft and porous surfaces such as paper, cloth, and cardboard require chemical treatment.

Commercially available fingerprint powders such as aluminum powder and black charcoal powder can be lightly dusted on a non-absorbent surface with a light brush. These powders will adhere to perspiration residue and make the prints visible. Magnetic sensitive powders are available that can be spread on the print with a *magnetic brush* which does not come into contact with the surface and will not damage the specimen. Some of the chemicals used for making the prints visible are iodine fuming, super-glue fuming, ninhydrin, and fluorescent dyes such as rhodamine 6G. The last one is extensively used in laser-induced fluorescence studies of fingerprints and will be discussed in greater detail. Iodine crystals sublimate when heated and the fumes react with water and fatty oils in the print to make it visible. Unfortunately, the prints begin to fade quickly. However, they can be fixed with a 1% solution of starch in water. This makes the prints turn blue and remain much longer. Fingerprints on non-porous surfaces like metals, leather, and plastic can be made visible by subjecting them to a vapor of *super glue*. It consists of 95% cyanoacrylate ester that reacts with the fingerprint and makes it visible. Super glue is treated with sodium hydroxide or heated to create fumes, and when these fumes come into contact with the fingerprint, it becomes white. Ninhydrin (triketohydrindene hydrate) forms a purple blue color (*Ruhemann's purple*) when it comes into contact with a fingerprint. It is useful when the fingerprints are on a porous surface. A solution of ninhydrin in water or 6% ethyl alcohol brings out the purple color in the fingerprints. Ninhydrin does not produce fluorescence. However, when aluminum chlorohydrate is sprayed on ninhydrin-treated fingerprints, fluorescence is produced and can be studied with the help of a laser. Sometimes the background of the fingerprint is such that the contrast may be low. In such cases, treating the surface with liquid hydrogen will increase the contrast.

Examination of fingerprints with lasers—Some fingerprints are inherently luminescent. The perspiration component of the fingerprint produces fluorescence in the presence of a shorter wavelength of light. Such a fingerprint can be examined with an argon ion laser. The advantage of an argon ion laser is that it can be tuned to a number of wavelengths (Table 1). Hence, the prints can be examined with different wavelengths, and depending on the composition of the fingerprints, the required luminescence can be observed. Typically, an argon ion laser beam can be made to spread out (diverge) with a negative lens and illuminate the print (Figure 19) or illuminate the print with a fiber optic probe.

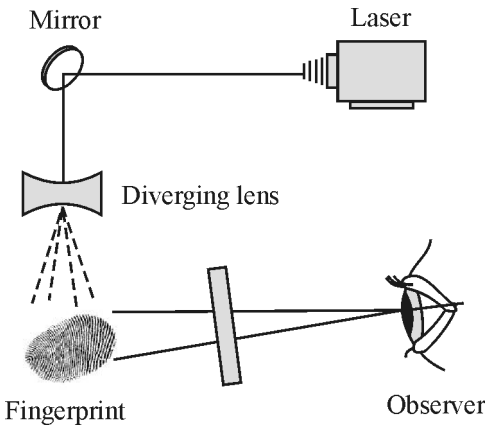


Figure 19 *Simple diagrammatic representation of observing laser-induced fluorescence in finger prints*

The fingerprint can then be photographed. Safety precautions associated with lasers—as given in Module 1-3, *Light Sources and Laser Safety,* of Course 1, *Fundamentals of Light and Lasers*—must be observed. In this case, goggles designed for the laser must be worn. The advantage of laser inspection is that it does not damage the fingerprint, or the surface on which the fingerprint is made. Also, if blood is present in the fingerprint, the laser does not harm the blood. The disadvantage of this method is that the small amount of perspiration component present in the fingerprint may not be sufficient to produce significant fluorescence. This can be overcome by chemically treating the prints to induce fluorescence.

Fluorescence is a three-step process that occurs in polyaromatic hydrocarbons or heterocycles. These substances are called *fluorescent dyes*. A number of such dyes are available for use with different laser output wavelengths. A photon with a discrete energy from a light source is absorbed by the dye, causing an electron to move to an excited energy state. The electron in the excited state has a very short lifetime (10^{-10} seconds). During this short lifetime, part of the energy is dissipated to the ground state as *fluorescence*.

Due to this energy dissipation, the energy of the emitted photon is smaller than the energy of the absorbed photon. Smaller energy means that the photon has a longer wavelength. The difference in wavelength between the absorbed and emitted photon is called the *Stokes shift*. Therefore, if the incident absorbed light of shorter wavelength is filtered by using the appropriate filter, the emitted light (fluorescence) can easily be observed. The great advantage lasers have over incoherent light sources is that lasers have all the energy concentrated in a very narrow wavelength region. Hence the energy per wavelength interval is extremely high. This makes the fluorescence several orders larger than the fluorescence produced by incoherent light. The fact that a fluorescent dye can generate thousands of detectable photons is important for high sensitivity of fluorescent detection techniques. Since most of the fluorescent dyes are polyatomic molecules, they give out fluorescence in a broad band rather than as a discrete line (Figure 20). Sensitivity is an important issue in fluorescence detection. Relatively small changes in ion concentration in living cells can have significant effects on the output. Concentration of less than 10^{-18}/mole can be detected by fluorescent techniques. Since the technique is noninvasive, it does not interfere with the sample. Many fluorescent instruments are now commercially available. Spectrofluorometers, fluorescence microscopes, and fluorescence scanners are some of them.

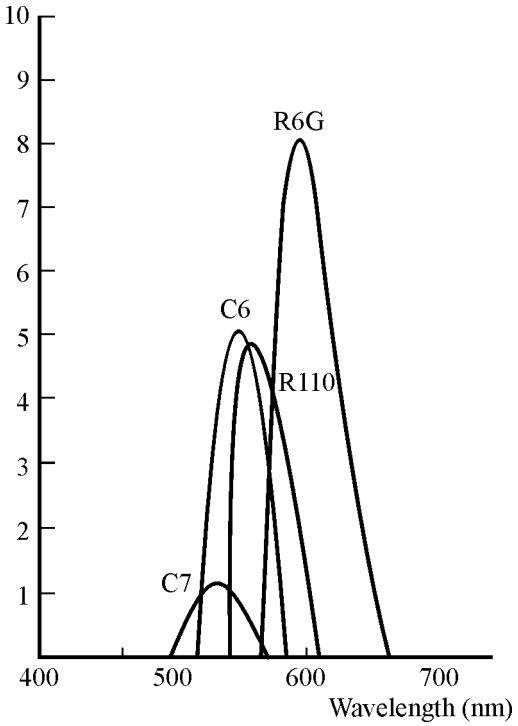


Figure 20 *Absorption bands and fluorescence of some dyes used in laser-induced fluorescence*

Dyes used for producing fluorescence with lasers—There are a number of chemical compounds that can be used to obtain fluorescence when excited with a laser of lower wavelength. For example, three dyes that show fluorescence between 500 nm and 700 nm when excited with lower wavelength lasers was shown previously in Figure 20. Rhodamine 6G has a fluorescence band between 550 nm and 650 nm when excited with an argon laser possessing a wavelength range between 458 nm and 514 nm. Rhodamine R110 shows fluorescence between 530 nm and 610 nm when pumped with an argon laser with a wavelength range of 458 nm to 514 nm. Coumarin 6 (C6) shows fluorescence between 520 nm and 580 nm when excited with an argon laser of 488 nm wavelength. Obviously, these compounds can also be excited with a frequency-doubled YAG. Ninhydrin and its derivatives show fluorescence when treated with zinc chloride dissolved in a solvent. Riboflavin mixed with conventional powders or magnetic powders also can be used for latent fingerprint detection.

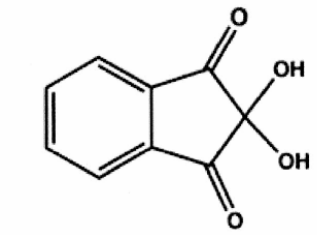
Photoluminescence-based chemical treatments

Rhodamine 6G—This is an organic molecule with the formula $C_{28}H_{31}Cl\ N_2O_3$ available in the form of red powder. This is easily soluble in organic solvents such as methanol, propanol, or water. There are a number of formulations for mixing rhodamine 6G. The simplest formula uses about 0.1 grams of the powder per 2.3 liters of solvent. With more intense lasers, a more dilute solution is preferable. On some types of surfaces, a methanol solution will be absorbed into the surface, and on other surfaces, water solution will be absorbed causing unacceptable situations. Experience and experimentation in each situation is desirable. Before applying the dye solution to the fingerprint, the print is often subjected to cyanoacrylate ester (super glue) fumes. This stabilizes the fingerprint so it will not be washed away by the dye that will be applied later. The dye solution is sprayed on the surface. This dye attaches itself to the cyanoacrylate-fumed

(super glue) fingerprint. Sometimes it will be necessary to clear the excess dye from the background by spraying methanol over the object until the excess dye is removed from the non-ridge areas. However, this has to be done carefully—removing only a little dye at a time. When using very dilute dyes, rinsing is not necessary. As stated earlier, rhodamine 6G has an intense fluorescence band between 550 nm and 650 nm when excited with Ar^+ 458 nm–516 nm. It can also be excited with a frequency-doubled YAG (530 nm) laser. While rhodamine 6G is not dangerous at low concentrations, it is advisable to use rubber gloves and safety glasses when it is involved. It should be mixed in the solvent under a fume hood. Super glue fuming and dye application works best on smooth surfaces such as metals. Photographing can be done in both black and white as well as color. The color photos show much better contrast. Recent advances in digital photography allow the use of both black and white and color without any waste of film. The images can be adjusted for brightness and contrast using any scanning software such as Adobe 6.0. Two fingerprints, one prepared with only super glue fuming, and the other with super glue fuming plus treatment with rhodamine 6G, are shown in Figure 21.

Figure 21 *Two fingerprints—one treated with only super glue (left) and the second (right) treated with super glue and rhodamine 6G*

Ninhydrin and analogs—Ninhydrin is an organic compound with the following structure (see inset). It comes in the form of a white powder that is water soluble. It is not a hazardous material. It is best suited for porous surfaces such as paper and is adaptable to laser enhancement. Ninhydrin- processed latent prints would not fluoresce under laser illumination. Occasionally, mild fluorescence is observed due to the byproducts in the print. Treating ninhydrin with aluminum chlorohydrate spray will make ninhydrin fluorescent. Lowering the temperature of the surface also enhances the fluorescence of the print and reduces the background fluorescence. Ninhydrin-based compound 5-methoxy-ninhydrin can also be used. This is prepared by first dissolving ninhydrin in methanol until saturation, and then adding trichlorotrifluoroethane. The later must be five times the volume of methanol used. To avoid the separation between methanol and trichlorotrifluoroethane, a few drops of acetic acid are added, and then shaken before spraying.



Several other analogs of ninhydrin are also used for laser-induced fluorescence in fingerprints. For example, ninhydrin treated with zinc chloride works very well with the output from the frequency-doubled Nd:YAG laser. 5-methoxyin-hydrin treated with zinc chloride produces intense fluorescence when used with the Ar^+ ion laser or copper vapor laser.

DFO—Another analog of ninhydrin, namely DFO (1,8-diazafluorin-9-one), produces a mild red colored product when it reacts with a fingerprint and is highly fluorescent when excited with either an Ar^+ or a frequency doubled Nd:YAG laser. DFO is produced by adding 0.05 g of the

powder to a mixture of 4 milliliters of methanol and 2 milliliters of acetic acid, and the solution is diluted to 100 milliliters with trichlorotrifluoroethane. The object with fingerprints is repeatedly dipped into this solution, drying for ten minutes between each submersion. This can be used on porous surfaces. It has an absorption maximum at 470 nm. The fluorescence of DFO is in the 570 nm region. Treatment with zinc nitrate will increase the fluorescence further, but is not very important.

5,6-dimethoxy-1,2-indenedione—Fluorescence produced by 1,2-indenedione is found to be better than DFO. Figure 22 shows a comparison of fingerprints obtained with DFO and one treated with 1,2-indenedione and viewed under a green light. This compound resembles ninhydrin except for the absence of one of the carbonyl groups. This solution is applied the same way as ninhydrin or DFO, and is treated with zinc nitrate. This reagent is dissolved in ethanol or methanol and diluted with Freon (1,1,2- trichlorotrifluoroethane) and sprayed on the fingerprint.

Figure 22 *Comparison of fluorescence in finger print treated with DFO (left) and 1,2-IND (right) viewed under green light (Courtesy-BVDA Corporation)*

Ninhydrin in heptane—Since 1970, Freon has been banned from use as a solvent because of the release of chlorofluorocarbons (CFC). Heptane (CH_3 $(CH_2)_5$ CH_3) is a suitable replacement for Freon. Ninhydrin heptane can be prepared by first dissolving 5 g of ninhydrin in 75 ml of ethanol, and then 25 ml of ethyl acetate and 3 ml of acetic acid are added. The resulting mixture is added to 1 liter of heptane. The solution works best when used within a few weeks. Since heptane is flammable, care must be taken to see that the solution and fumes are kept away from any source of ignition. This solution can be sprayed and is very effective in developing latent fingerprints. It can be used with an Ar^+ 488 nm–514 nm wavelength laser for excitation and fluorescence.

Coumarin 6—Coumarin 6 is another fluorescent dye extensively used in laser-based fingerprint detection. It has a chemical formula $C_{20}H_{18}N_2O_2S$ and has a strong absorption band between 350 nm and 500 nm. This dye is also used in the same manner as rhodamine 6G. The fingerprint is pre-stained with cyanoacrylate and sprayed with a solution of coumarin 6. Both the 457.9 nm, 488 nm, and 514.5 nm Ar^+ ion laser as well as the frequency-doubled Nd:YAG laser give excellent contrast in the fluorescent print produced with coumarin 6.

Silver nitrate—The use of silver nitrate is one of the oldest techniques for fingerprint detection on a porous surface. Silver nitrate reacts with the chloride component of a fingerprint deposit (eccrine secretion) to form light sensitive silver chloride. On exposure to light, it decomposes to silver metal and will produce a black image of the fingerprint. This technique is useful for detecting fresh prints. The fingerprint details are lost over time (one week) due to the diffusion of chlorides through the substrate.

DMAC (dimethylaminocinnamaldehyde)—Urea in the eccrine sweat reacts quickly with this reagent and produces a dark red image which will deteriorate in 770 hours. Its use is mainly limited for quick development of fresh marks on porous surfaces. It can be either sprayed or used as a fuming agent. The latter provides detailed visualization on a wide range of substrates. The DMAC fumes are produced by heating DMAC to 175°C. The samples are subjected to

these fumes for 24 hours at room temperature. The fingerprints can be observed with lasers of wavelengths 490 nm – 580 nm, and with a filter of 550 nm. The advantage of DMAC is that the samples can be subjected to ninhydrin or DFO after treatment with DMAC. The DMAC technique is highly successful on thermal paper, which is a problem with other techniques.

A flow chart useful for observing fluorescence on hard surfaces is shown in Figure 23 and that of porous surfaces is shown in Figure 24. The notation "Laser alone" at the top of each flow chart is meant to indicate that in some cases, the eccrine sweat contains a natural compound that fluoresces when the laser alone is shown on it. That is, application of the dyes listed in the rest of the flow chart may not be necessary.

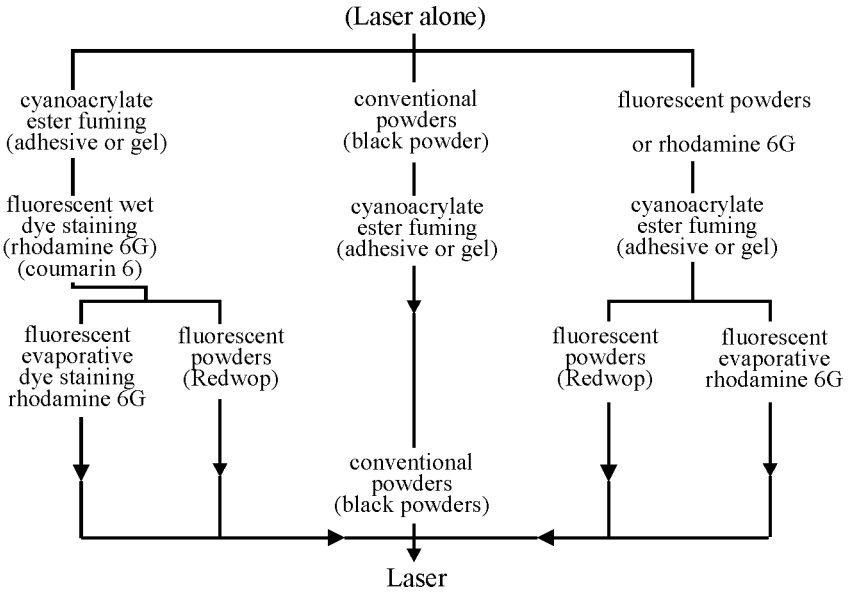


Figure 23 *Flow chart for observing fluorescence in fingerprints on hard surfaces (Redwop is available from Lightning Powder Co.)*

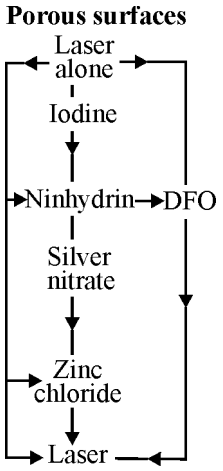


Figure 24 *Flow chart for observing fluorescence in fingerprints on porous surfaces*

Photographing Fingerprints

It is important to record the evidence in a crime scene for future comparison and possible use as evidence in court. At a crime scene, small items of evidence and fingerprints and the crime scene have to be photographed. Single lens reflex (SLR) cameras with interchangeable lenses, Polaroid cameras, and digital cameras are extensively used in forensic science. Digital SLR cameras such as the Nikon D70, or the Cannon EOS with interchangeable lenses, are most suitable in forensic science.

Film photography

Most of the film photography is done with SLR cameras like the Nikon N 2000 with interchangeable lenses. Interchangeable lenses give the opportunity to take pictures under different conditions. To get good depth of focus, F8 to F11 aperture settings are used. Teleconverter 2x macro is used for close up shots. Shutter speeds are adjusted to be compatible with the lighting and background. If laser illumination is used (as in the case of fluorescence-

induced fingerprints), an orange 056 filter has to be used. Black and white or color film can be used—depending on the object to be photographed. Choice between flash operation and separate light illumination has to be determined by trial and error. With the advent of digital cameras and their versatility, film cameras are slowly going out of favor.

Digital photography

There are many advantages in digital photography. Here, the information is stored as a digital file rather than on a film. This, perhaps, is the greatest advantage since the image can be seen immediately (as in a Polaroid camera). But, in addition, a digital photograph can be taken repeatedly with different camera settings and the best possible picture selected. Since photos are stored in digital format, a large amount of data can be stored in a very small space. The image can very quickly and easily be enhanced in terms of brightness, contrast, noise reduction, and sharpness. Digital information can easily be duplicated and shared worldwide. The handling of digital information is very reliable. Storage and retrieval is instantaneous. The problem of degradation of the quality of the picture with time is nonexistent. Distortions can be corrected using computers with available software. Resizing, cropping, and enlarging are very simple. With the availability of interchangeable lenses and all of the other features of conventional SLRs, *digital photography is vastly superior to conventional photography*.

In digital photography, the object information passes through the lens and is stored on extremely small squares called *pixels*. Each pixel in the image has a numerical value between 0 and 255 and is made up of three color channels. For example, a pixel can be 35 red, 78 green, and 135 blue. Depending on the capacity of the storage medium, several million possible combinations of colors are available for the recording chip.

Each type of camera has a specific *pixel count*. This refers to the number of individual pixels that go into making each image. This can go from 1 mega pixel to 14 megapixels. The larger the pixel count, the better the picture resolution will be. This is particularly important when the image has to be enlarged. Sometimes the words *effective megapixels* are used. This refers to the actual pixels used in the final image, dropping the image on the edges.

The *aspect ratio* of a digital camera is the ratio of the length to the width of the image. For example, a 36 mm film has a length of 36 mm and width of 24 mm. Hence, the aspect ratio is 36:24, which is expressed as 3:2. Digital SLR's have the same aspect ratio as regular SLR cameras. Digital video cameras usually have aspect ratios of 4:3.

The size of the sensor element in a digital camera is much smaller than 35 mm film. Most of the sensor elements are of the size 7 mm × 5 mm. For a given pixel count, the larger the sensor, the better the image quality and the lower the noise level. Cameras like the Cannon EOS 100 have very large sensor elements (10 mm × 30 mm) and have excellent image quality. Furthermore, the digital SLR's use a design known as *full frame.* The advantage of this is that the whole pixel area can be used to capture the image, while interline CCDs use part of each pixel to store the image.

With film cameras, the film has to be corrected for *color temperature.* This requires a correction for different light conditions. With digital cameras, there is an automatic setting for color balance. The digital SLR's also have much larger sensitivity settings (ISO 400 to ISO 3200) and are virtually noise free.

There is a distinction between *digital zoom* and *optical zoom*. The latter is the same as that of any zoom lens on a standard camera. The lens changes its focal length to enlarge the image. Image quality remains high through the zoom process. A digital zoom simply crops the image to a smaller size and then enlarges the cropped position to fill the frame. Digital zoom results in significant loss of picture quality.

There are a number (in fact, too many) ways memory is shared in a digital camera. Some cameras store the memory in the camera itself, while others store the memory on different (incompatible) memory cards. Most of these cards come with their own software which can be downloaded into a computer. In some cases, the camera can be connected to a computer through a USB port and the pictures downloaded, organized, and retrieved. In other cases, there are card readers—some incorporated into a computer, some stand-alone—that can be connected to a computer. In all cases, the software allows manipulation of the image in all the aspects mentioned above and also transfers the image onto a storage medium, which allows the information to be stored.

One of the most important aspects in taking a picture is the *depth of focus*. This relates to image sharpness and how long sharpness can be maintained with increased enlargement of the picture. This is extremely important in forensic science. As often is the case, the object to be photographed (like a fingerprint) is very small and has to be enlarged to see the details. In such a case, preservation of the sharpness of the image is vitally important. In digital cameras, small sensors require short focal length lenses (much smaller than normal SLR cameras). For example, normal SLRs use lenses of focal length 35 mm and corresponding digital cameras have 7 mm focal length lenses. This leads to a much smaller depth of field. This is not too bad if the whole picture is to be in focus (such as scenery). Conversely, however, this is not desirable if a small portion of the object has to be in focus and not the rest. This is not a problem in digital SLR cameras. The digital SLRs are very expensive but the advantages greatly outweigh the cost in terms of convenience and quality.

Laser use in examination of other physical evidence

Lasers are often used in the examination of other physical evidence such as semen stains, fibers, and glass fragments, and the examination of handwriting on documents. In many cases, simple illumination of the object and observation under magnification may be sufficient. In other cases, fluorescence from the objects may be observed. Since the luminescence from these objects may result from the use of a variety of wavelengths, it is advisable to use lasers that can produce many wavelengths, such as a tunable laser. Semen stains on objects such as clothing may be often difficult to observe normally. However, they can be seen well under fluorescence. A 488 nm or 514 nm wavelength Ar^+ laser is best suited for this. However, ultimately, they have to be chemically analyzed. Location of minute fiber fragments also can be accomplished by fluorescence techniques. Here, also, an argon laser can be used. Minute glass fragments may sometimes produce interference patterns due to reflection of the monochromatic beam of a laser from two adjoining surfaces. Interference patterns allow detection of these glass particles.

Laser Ablation and Analysis of Minute Samples

In a crime scene, very small items such as a smear of paint, hair strand, or drop of blood may be present. But often it may be too small for detection by conventional techniques. In such situations, *laser ablation mass spectrometry* can be used. This is an excellent tool for analyzing minute samples. It also can differentiate between samples with physical, chemical, and visual similarities. It can distinguish samples, which are chemically inert. This technique does not involve elaborate sample preparation or utilize hazardous substances. It only requires a minute sample.

The mass spectrometer

Laser ablation produces a gas stream that carries the sample vapor into a high-temperature plasma where it is ionized. Then it is introduced into a *mass spectrometer,* where the sample can be analyzed. A mass spectrometer measures the mass of individual atoms or molecules. The ionized atoms pass through a vacuum chamber containing a velocity selector. This device consists of uniform electric and magnetic fields perpendicular to each other and to the ion beam. Thus, one can select ions that enter the magnetic field by choosing the electric and magnetic fields properly. Charged particles in a magnetic field follow a curved path. The radii of these paths depend on the momentum (mass × velocity) of the charged particles. Ions having low mass (low momentum) will be deflected most by the magnetic field. Ions with large momentum will be deflected less. The two ions of different mass will hit the detector at two different points. They can be collected by an analyzer, and their mass-to-charge ratio can be determined by a computer. A typical mass spectrometer is shown in Figure 25.

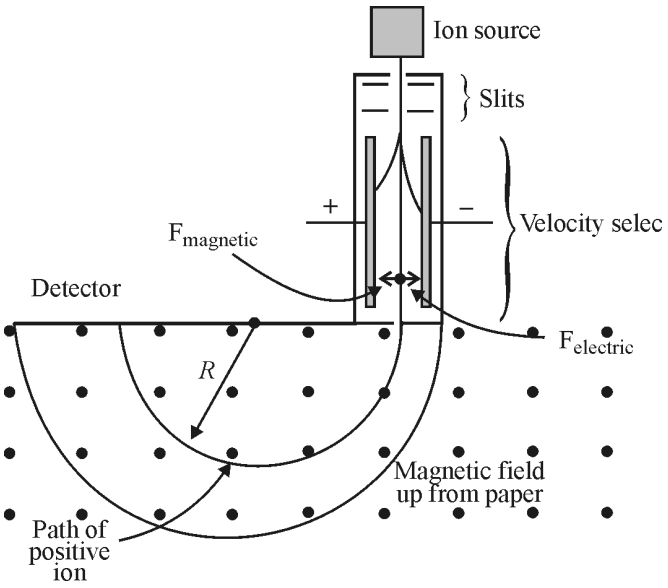


Figure 25 *Schematic diagram showing the mass spectrometer*

By varying the strength of the magnetic field, the mass-to-charge ratio of ions to be analyzed can be continuously varied. A typical mass spectrum of toluene is shown in Figure 26. The peak of the spectrum (labeled 92) is called the *base peak* and represents the stable benzyl cation. This cation undergoes a rearrangement to form a tropylium cation (m/*e* = 91). This rearrangement is a hallmark of compounds containing a benzyl unit. The minor peak at m/*e* = 65 represents a loss of neutral acetylene from the tropylium ion. The other minor peaks arise out of more complex fragmentation.

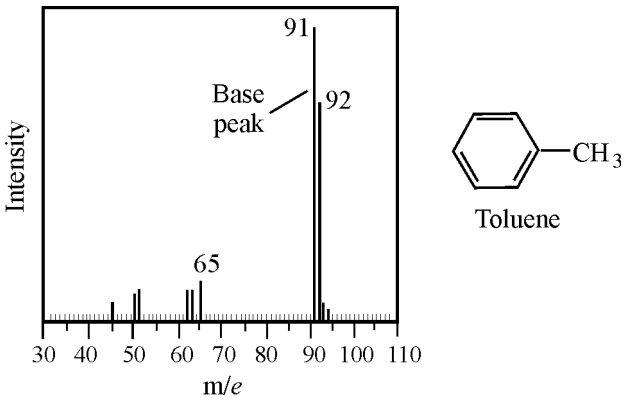


Figure 26 *Mass spectrum of Toluene showing a strong molecular ion at m/*e *= 92 and the base peak at m/*e *= 91*

Laser ablation

As mentioned above, laser ablation refers to material removal and vaporization with a laser beam. It can be accomplished by heat in metals, glasses, and crystals, or by photochemical changes as in polymers. A commonly used laser for ablation is a *pulsed excimer laser* with a relatively *low duty cycle.* (A *duty cycle* is defined as the ratio of the width—in time—of a single pulse to the width in time between successive pulses. Thus a low duty cycle laser emits narrow pulses spread out in time.) Another common laser is the frequency-tripled Nd:YAG lasers (213 nm). The low duty cycle makes the peak power of excimer lasers very large, since the energy is stored in a narrow peak (small time interval) and power is given by "energy divided by time."

There are several criteria to be considered in choosing a laser for ablation. The first is the selection of a wavelength with minimum absorption depth in the sample to be ablated. This results in high energy incidence over a small volume for rapid and complete ablation. The second consideration is the shortness of the pulse duration of the laser, which results in maximum peak power, and reduction of thermal conduction at the working surface. The third criterion is pulse repetition rate, that is, how many pulses can be produced per second. The pulse repetition rate should not be so slow as to allow the ablation zone to cool down. When more energy from the laser goes to the ablation region, there will be less energy lost to the surroundings. The fourth criterion is the beam quality, which is measured by the brightness, focusability, and homogeneity of the laser beam. In other words, the more coherent the beam and the less it spreads, the better the laser is for ablation. Finally, when dealing with ablation by evaporation, a plume (a plasma type of substance consisting of molecular fragments and neutral particles) will be generated just above the target and may absorb some of the incident laser energy. This, in turn, reduces ablation efficiency. Blowing an inert gas, such as argon, passed the ablation site, will reduce this undesirable effect.

Microscopy in Forensic Science

Compound microscopes

There are a variety of microscopes available for magnifying and resolving very small specimens. Microscopes, which use ordinary light, are *compound microscopes, stereomicroscopes, polarizing microscopes,* and *visible* and *infrared microspectrometers.* Microscopes using lasers are *laser confocal microscopes*.

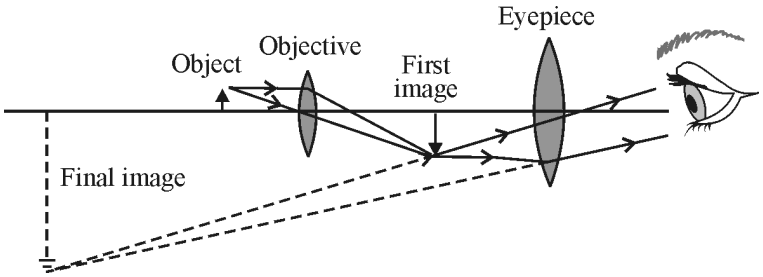


Figure 27 *Schematic diagram of a compound microscope*

A microscope consists of an *objective lens* and an *eyepiece lens*. The objective lens forms a real image of a small object at a distance from itself longer than its focal length. This image is located at a position nearer to the eyepiece than the focal length of the eyepiece. The final image, formed by the eyepiece, is a virtual and magnified image of the object. A schematic diagram of a compound microscope is shown in Figure 27.

The magnification of a lens is defined as the ratio of the image height to the object height. The total magnification obtained by an optical system is the product of the individual magnifications of the lenses involved. In a microscope, usually there will be three or four objective lenses (Figure 28) with different magnifications, each mounted on a rotating turret. By rotating the turret, any one objective lens can be used to view the specimen. Similarly, the eyepieces are also interchangeable.

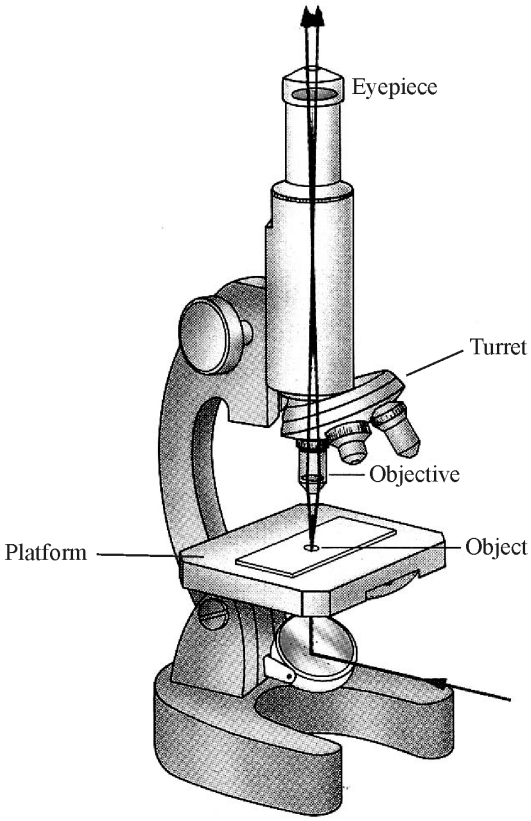


Figure 28 *Compound microscope with interchangeable objectives and eyepieces*

By a proper choice of objective and eyepiece, total magnifications of 100 to 500 can be obtained. However, by increasing the magnification, the *field of view* (available viewing area of the specimen on the platform) as well as *depth of focus* will be reduced. Depth of focus is the ability to see clearly the specimen throughout its extent. Nearer and further than this

extent, the image will be blurred. In addition, the resolving power of the objective lens also has to be taken into consideration. The resolving power is the ability of the objective lens to see two objects separately and clearly when they are very close to each other. The *numerical aperture* (N.A.) of the lens is closely related to the resolving power of the objective lens, and is given by Equation 5.

$$\text{N.A.} = \frac{1.22\, n\lambda}{x_{\text{min}}} \qquad (5)$$

Here x_{min} is the minimum separation of two objects just resolved by the objective lens, λ is the wavelength of the light used, and n is the refractive index of the medium between the objective lens and the object on the microscope platform. (Sometimes oil, which has a refractive index of 1.55, is used to increase the value of N.A. by a factor of 1.55 over air.) The higher the magnification of the objective lens, the higher the value of N.A. and the smaller is the separation distance x_{min}. For example, an objective lens of N.A. = 1.3 can separate details that are twice as close as can a lens of N.A. = 0.65.

The components of a microscope are (1) an illumination system suitable for either viewing a transparent object or an opaque object, (2) objective lens system, and (3) an eyepiece system which is either monocular or binocular. Some examples of microscopes are listed below:

Comparison microscope

In this, two microscopes are combined into one unit. Images from two separate objectives are combined to form side-by-side images in the same binocular piece. In one circular field, half will be from one objective and the other from the second objective. The optical characteristics of the objectives are perfectly matched such that both images have the same magnification. This type of microscope is most often used when comparing the markings of bullets. A photograph comparing markings for two bullets fired from the same gun is shown in Figure 29.

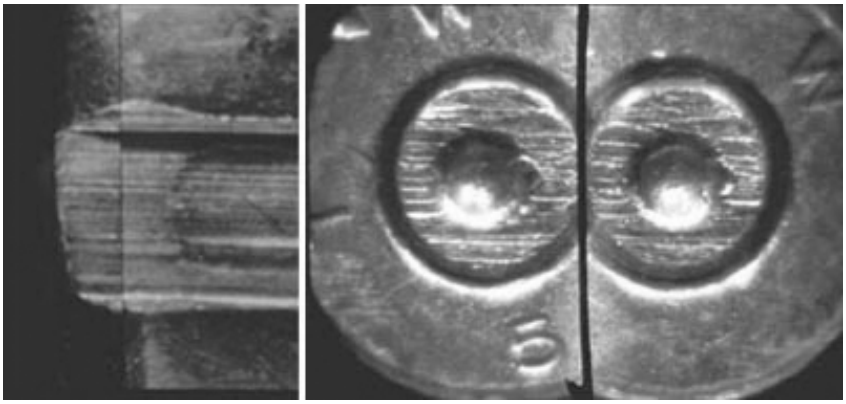


Figure 29 *Bullet and cartridge case identification through comparison microscope (Courtesy Wisconsin Department of Justice)*

Stereomicroscope

This type of microscope produces a three-dimensional image with a smaller magnification. It also provides an erect image compared to the inverted image produced by a compound

microscope. In this setup, two monocular compound microscopes are properly spaced and aligned to form a three-dimensional image. This microscope has a wide field of view and good depth of focus and, hence, is very useful in forensic examinations. The large distance between the objective lens and the specimen platform, as shown in Figure 30, allows examination of bulk samples.

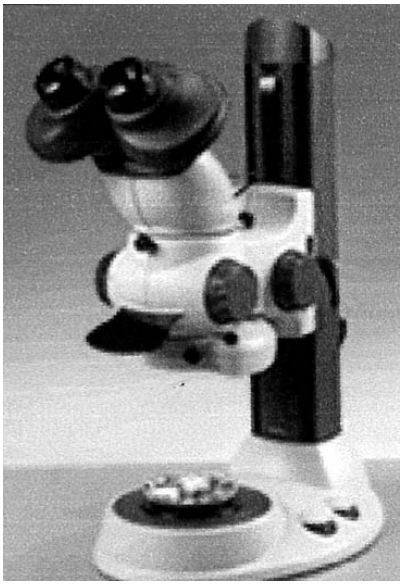


Figure 30 *A stereomicroscope consists of two individual microscopes with a common objective.*

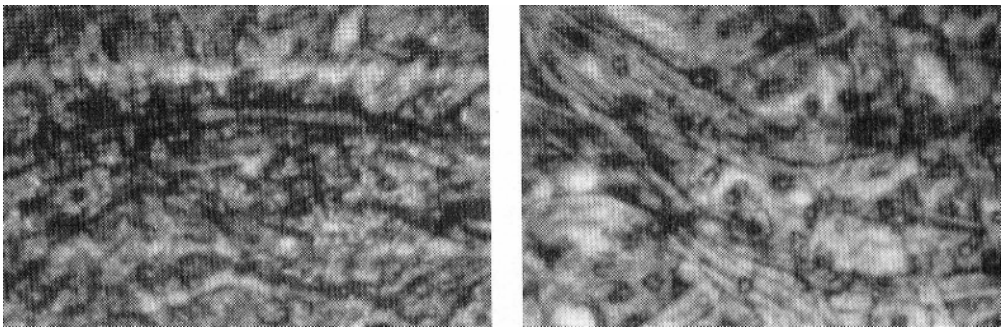
Polarizing microscope

This microscope uses two polaroids to create transmission or extinction portions of the specimen. Polaroids are devices which can cut off one of the two vectors of electromagnetic radiation. Two polaroids can be positioned to cut off both of the vectors (*crossed polaroids*) or positioned to allow only one vector of the light (*linearly polarized light*). Some of the specimens may be birefringent (e.g., minerals, synthetic fibers, etc., with two refractive indices) where light is split into two light beams with different speeds. The two beams are polarized perpendicularly to each other. One polaroid is placed above the specimen. Another polaroid is placed below the specimen but above the illuminating platform. They are in *crossed* positions. When viewed through the microscope without the specimen, no light will pass through the crossed polarizers. However, when the specimen is introduced between the polaroids, some regions of the object cause one of the planes of polarization to rotate, thereby enabling light to pass through and render the specimen visible in the microscope. This type of microscope is very useful with birefringent specimens.

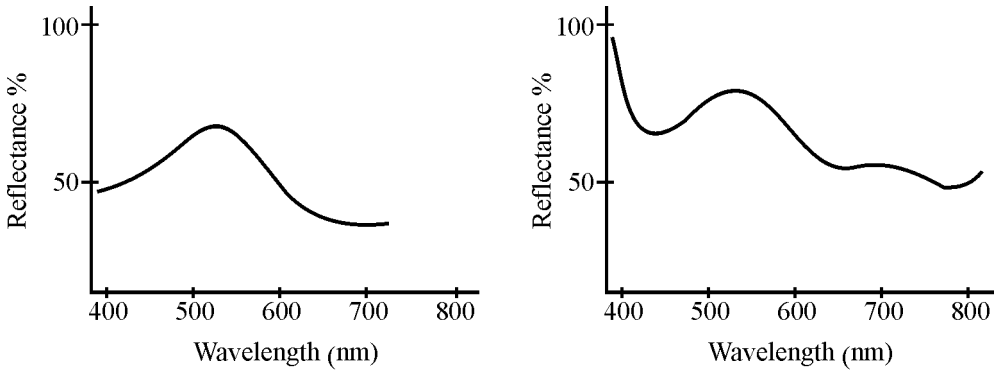
Microspectrophotometer

A *microscope* is sometimes combined with an *absorption spectrophotometer* to form an instrument known as a *microspectrophotometer*. In addition to the observation of the specimen with a microscope, the microspectrophotometer enables the light going through the specimen to go also through a grating spectrophotometer yielding an absorption spectrum. The absorption

spectrum of the specimen is characteristic of the composition of the subject. A comparison with a known object reveals the differences between the known object and the suspect object. For example, when a note of real currency and a note of counterfeit currency are viewed side by side in such a microspectrophotometer, the microscope reveals the structural differences in the paper and the absorption spectrum reveals the differences in the ink. Two microphotographs and their corresponding absorption curves are shown in Figure 31.



(a) Microphotographs of two currency notes



(b) Absorption spectra of currency notes pictured above

Figure 31 *Microphotographs and corresponding absorption spectra of two currency notes (Courtesy Peter W. Pfefferli, forensic scientist, Lausanne, Switzerland)*

Laser scanning confocal fluorescence microscopy (LSCM)

Confocal imaging refers to light reflected or emitted by a single plane of a specimen and then stacking such images from different planes to form a 3D image through digital signal processing. Obtaining pictures from each plane increases the contrast since the light from outside the focal plane is suppressed. Such a technique allows examination of layers of a specimen *without actually slicing* the specimen or making special preparations of the specimen. This is very useful in the forensic study of a specimen.

In this technique, a laser light beam is diverged and reflected by a beam splitter onto the objective lens. Through an *x-y* deflection mechanism, this beam can be converted to a scanning beam. The objective lens focuses the laser beam to a small spot on a specimen that has already been stained by a fluorescent dye. The reflected light (so-called out of focus light) is deviated by the beam splitter, while the fluorescent (in-focus) light passes through the beam splitter and then through a pin hole to be captured by the photomultiplier. The confocal aperture (pin hole)

rejects all light from the background and allows only the fluorescence from the single plane on which the objective lens has focused the laser beam. A schematic diagram of LSCM is shown in Figure 32.

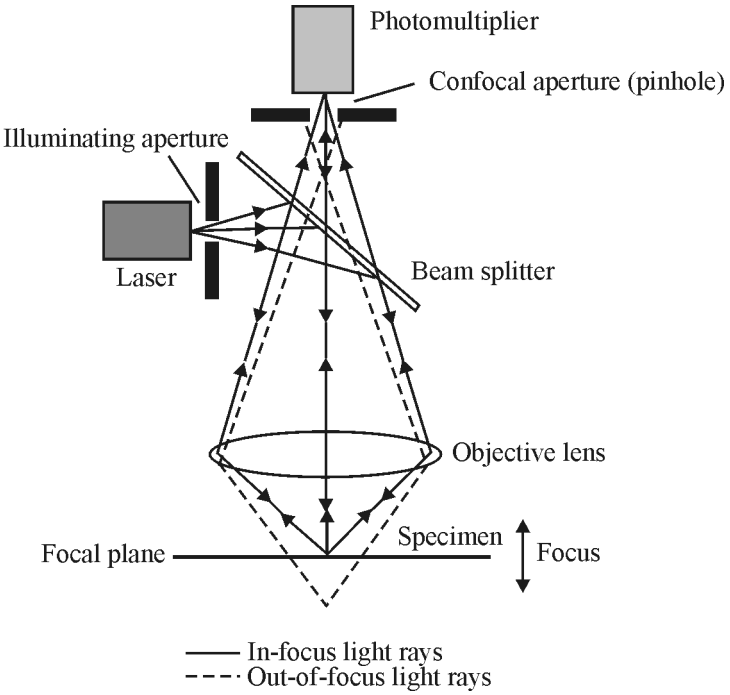


Figure 32 *Schematic diagram of a laser scanning confocal microscope*

By fast scanning of the specimen at the focal plane, a 2D image of a small portion of the specimen in the confocal plane is generated. The analog signal detected by the photomultiplier is converted to a digital signal that can be displayed on a computer monitor. The relative intensities of the pixels on the screen correspond to the fluorescence intensity of the spot in the focal plane. Moving the focal plane in the *z* direction with a computer-controlled stepper motor generates several such planes. The *x*-, *y*-, and *z*-motions can be coordinated to give a three-dimensional view of the interior of the specimen.

Several parameters control the image from an LSCM. The first is the choice of the wavelength(s) of the laser beam, and the corresponding fluorescence probe with which the specimen is labeled. The fluorescence probe is determined by the nature of the study—macromolecular structures (such as proteins, lipids, carbohydrates, and nucleic acids) or physiological ions (such as calcium and pH). The emission spectrum and quantum efficiency of fluorescence play a major role in the choice of the fluorophore. The larger the product of the two, the larger is the brightness of the fluorophore. Environmental factors such as pH also affect the efficiency of the fluorophore. Most of the commercially available LSCMs use lasers with wavelengths in the ultraviolet—less than 400 nm. Different fluorophores give fluorescence outputs at higher wavelengths compared to excitation wavelengths. *Neutral density filters* are used to reduce laser intensity and *narrow band filters* are used to select a single wavelength.

One of the drawbacks of this technique is that the laser scanning of the specimen bleaches the specimen. In this sense, the LSCM is a destructive technique. However, there are methods to reduce this destructive effect. One technique is to make several passes of the optical sections of

the stack using a lower laser power and then to average the optical sections obtained with each pass. Another method involves the addition of antifade reagents such as P-phenylenediamine (PPD), n-propyl gallate (NPG), or 1, 4-diazobicyclo[2,2,2]-octane (DABCO). These antifading agents slow down the fading process and allow longer observation times.

Signal-to-noise ratio (SNR) is another factor to be considered in acquiring the 3D image. To a great extent, this depends on the adjustment of the detector pinhole. If the SNR is low, then the size of the aperture can be increased with a resultant increased signal detection. The situation is similar to the aperture adjustment in a camera.

The 3D images received after processing the 2D images from the different planes can be improved by using *image filters*. These are mathematical algorithms implemented in the software. These filters allow sharpening and thereby smoothing of the images. Contrast and brightness can also be adjusted by changing the ramping of the grey scale values for the data set.

Segmutation is a process by which a desired object or objects in the image are separated from the background data. Techniques such as *thresholding* and *masking,* and more complex procedures such as *edge/boundary detection, region growing,* and *clustering algorithms* are used for this process. This can be done either by manual intervention or through software algorithms.

Laser-Assisted DNA Sequencing

DNA and replication

Inside every one-trillion cells in the body, there are strands of genetic material called chromosomes. Arranged along the chromosomes like beads on a thread, are nearly 30,000 genes. The *gene* is the fundamental unit of *heredity*. It is responsible for making proteins that, in turn, determine everything from the color of one's hair to one's susceptibility to diseases. DNA is the abbreviation for dioxyribonucleic acid, which is a *polymer* that carries the body's genetic information. A polymer is a long chain of molecule made by linking together a series of repetitive units. In DNA, the units are called *nucleotides*. This, in turn, is composed of a sugar molecule, (S), a phosphorous-containing group (P), and a nitrogen-containing molecule called *base.* The sugar component is joined to the phosphate group to form the backbone of the DNA strand. The bases connect two interwoven strands. This structure is shown in Figure 33. There are only four bases

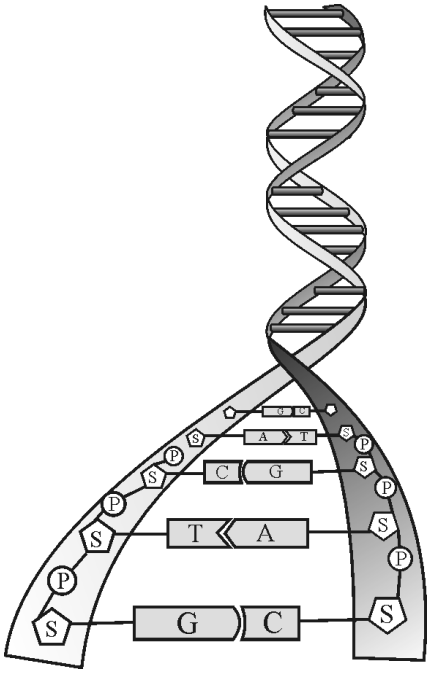


Figure 33 *Schematic representation of a DNA molecule (Saferstein, Richard E.,* Criminalistics: An Introduction to Forensic Science, 8th Edition, *© 2004. Reprinted by permission of Pearson Education, Inc., Upper Saddle River, NJ.)*

associated with DNA: adinine (A), cytosine (C), gaunine (G), and thymine (T). Millions of different combinations of these bases are attached to the double helix to form the DNA molecule.

The bases can only be aligned such that *G* is paired with *C,* and *A* is paired with *T* (*complementary base pairing*). However, there is no restriction as to how these can be sequenced. This implies that there can be millions of combinations of these sequences. An example of a simple sequencing is shown in Figure 34.

T A T T	*G T A A*	*G T C A*
| | | |	| | | |	| | | |
A T A A	*C A T T*	*C A G T*

Figure 34 *Possible pairings of bases to form a double helix formation in DNA*

DNA controls the production of complex molecules called *proteins*, which are made by linking a combination of amino acids. A slight variation of the sequence can lead to a significant difference. For example, Figure 35 shows two hemoglobins. The only difference between them is the presence of *valine* in the right protein chain instead of *glutamate* found in the left chain. This makes the single-cell hemoglobin "abnormal." This information is stored in the DNA and gives a clue to the genetic information.

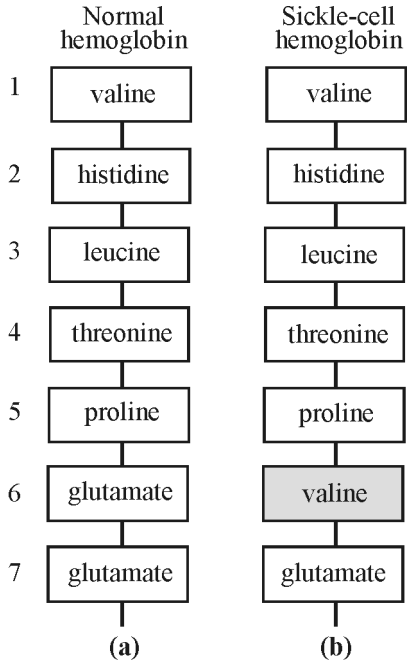


Figure 35 *Two protein chains (a) normal hemoglobin and (b) sickle-cell hemoglobin*

The genetic information that determines the amino acid sequence for every protein manufactured in a human body is stored in DNA in a genetic code. Typical coding of the amino acid chain is shown in Figure 36**.**

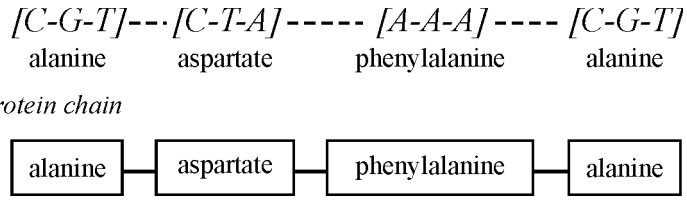


Figure 36 *Each amino acid is coded by a particular sequence of the bases, and they, in turn, form the protein chain.*

The DNA replicates itself by unwinding the strands in the double helix. Each strand then collects free nucleotides to form an identical double helix. *Polymerases* are enzymes that assemble the new DNA strand in the proper sequence. In forensic investigations, small quantities of DNA or broken pieces of DNA found at the crime scene can be copied by a technique called PCR (Polymerase chain reaction). It is possible to cut DNA into fragments with the help of *restriction enzymes* and later recombine the fragments. In this manner, it is possible to reproduce and control the segments that control protein synthesis. Portions of the DNA molecule contain sequences of letters that are repeated numerous times. This offers a means of distinguishing one individual from the other by means of *DNA typing*.

The polymerase chain reaction technique allows copying and multiplying of the DNA strands. An enzyme called *DNA polymerase* can be used to achieve this. This technique is useful when the available sample is small. A recently developed technique is called *short tandem repeats* (STR). STRs are locations (loci) on the chromosome that contain short sequence elements that repeat themselves in a DNA molecule. They can be used as markers since they are abundant in the human genome. STRs are ideal for multiplication by the PCR technique since extremely small quantities of DNA are sufficient for typing. The more STRs that can be classified, the smaller the percentage of population from which these STRs can emanate.

Automated DNA sequencing

The human cell consists of two types of DNA—nuclear and mitochondrial. The first constitutes the 23 pairs of chromosomes contained within the nuclei of our cells. Each parent contributes to the genetic makeup of these chromosomes. Mitochondrial DNA (mtDNA) is formed outside the nucleus of the cell and inherited solely from the mother. *Mitochondria* are cell structures that provide 90% of the energy needed for the body to function. A single mitochondrion contains several loops of DNA, and there are several thousands of mitochondria in each cell. This means that there are millions of mtDNA copies in a human cell. Thus, in forensic situations where nuclear DNA is either degraded or not present (such as in the charred remains of a body), an mtDNA can be obtained from a maternal relative. However, mtDNA analysis is more difficult, time consuming, and expensive. Nuclear DNA is composed of continuous, linear strands of nucleotides (A, T, G, C). Mitochondrial DNA is constructed in a circular loop configuration. Each loop contains large numbers of A, T, G, and C to comprise 37 genes. Two highly variable regions of mtDNA are found in human beings, those being the hyper variable region I (HVI) and the hyper variable region II (HVII). The process of generating many copies of HVI and HVII regions by PCR, and then determining the order of the A-T-G-C bases constituting these regions, is called *sequencing*. An mtDNA database containing these base sequences is now available. Thus, once the sequencing of these HVI and HVII regions from the crime case sample are completed, and the number of times these sequences appear in the mtDNA has been

determined, they then may be compared with the database maintained by the FBI. However, STR analysis is still more discriminating than mtDNA analysis.

Prior to 1990, DNA typing was done by adding of a radioactive DNA probe to the gel containing the DNA fragments. This technique is shown in Figure 37.

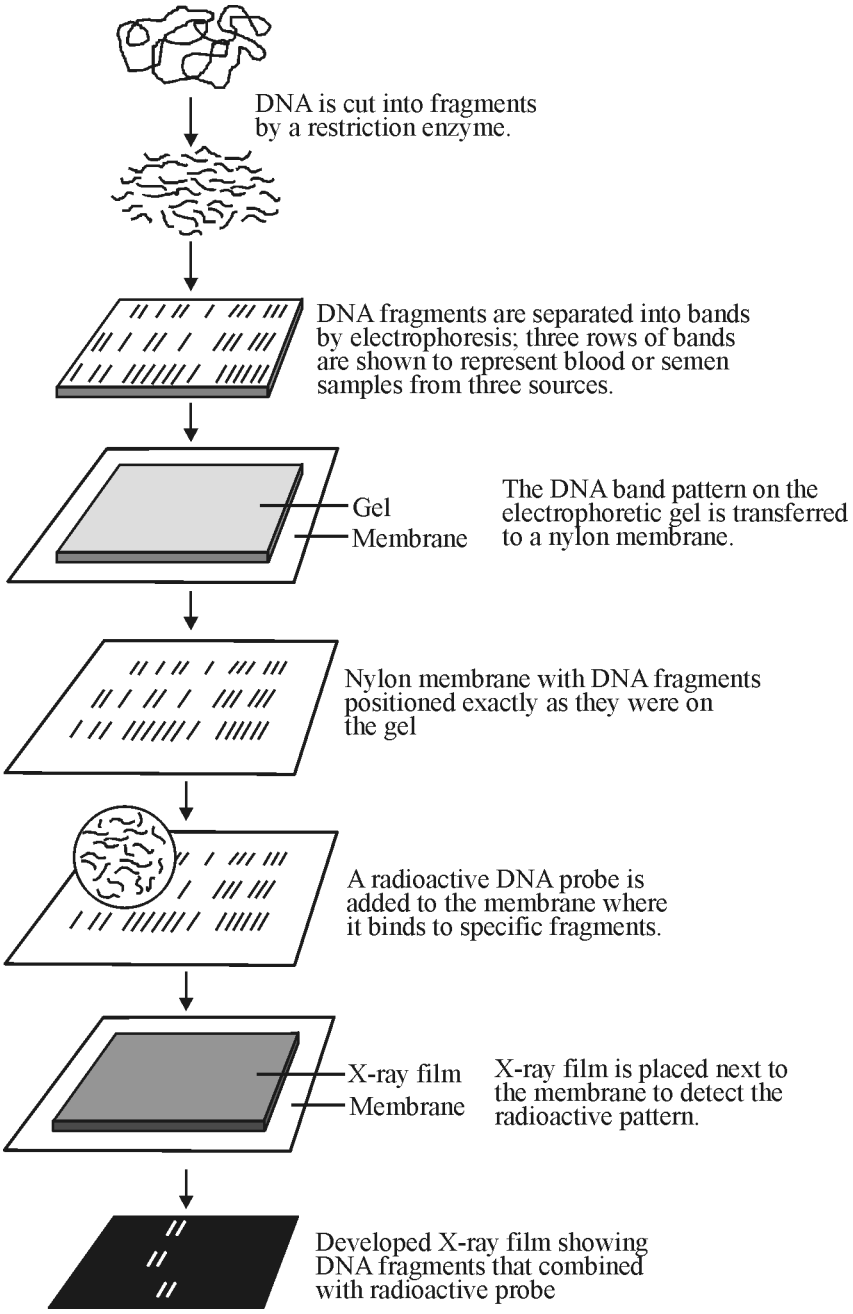


Figure 37 *DNA RLFP typing process (Saferstein, Richard E.,* Criminalistics: An Introduction to Forensic Science, 8th Edition, *© 2004. Reprinted by permission of Pearson Education, Inc., Upper Saddle River, NJ.)*

First, DNA is cut into fragments by a restriction enzyme. Then the DNA fragments are separated into bands by electrophoresis. Separate rows of bands are used for blood and semen

from different sources. The DNA band pattern on the electrophoretic gel is transferred to a nylon membrane keeping the bands intact. A radioactive DNA probe is added to the membrane where it binds to the specific fragments. An X-ray film placed next to the membrane is used to detect the radioactive pattern.

Laser-based fluorescence DNA sequencing

Laser-induced fluorescence techniques are developed as a substitute for radioactive detection. Three such techniques are shown here.

Side-entry laser—This technique uses a fluorescent-dye-treated-slab gel with DNA bands. A scanning laser beam is aimed at different positions on the slab sequentially. Repeated scans of irradiation positions allow the photo detectors to measure the fluorescence emission from multiple electrophoresisis migration paths in order of occurrence and thereby determine the base sequence of multiple DNA samples. By allowing the laser beam to scan from the side (Figure 38), it can simultaneously intersect all electrophoresis migration paths thereby increasing irradiation efficiency. A multi-pixel array CCD camera simultaneously detects fluorescence emissions from each migration path. Numerous samples can be studied at the same time.

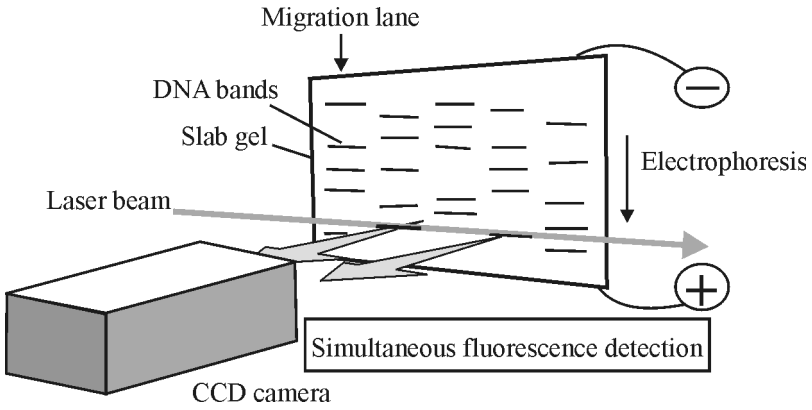


Figure 38 *Slab gel DNA sequencer using side-entry laser beam irradiation*

Sheath-flow technology—It is found that gel-filled capillaries dissipate heat much more effectively than conventional slab gels. This allows higher applied voltages to be used for accelerating electrophoresis. Each *capillary* represents one electrophoresis migration path and can be used for base reading one DNA sample. Two methods are described by two different groups of researchers: one from the University of California and the other from Iowa State University.

In the first method (Figure 39), a bundle of capillaries are placed on a flat surface and a laser beam is directed at one capillary at a time. By scanning the capillaries in sequence, the fluorescence emission is detected and multiple samples can be sequenced. One disadvantage of this technique is that the large number of capillaries shortens the time available to detect fluorescence emission from each capillary. This reduces the sensitivity of fluorescence detection. Further, maintaining scanning speeds in pace with faster electrophoresis migration speeds is difficult.

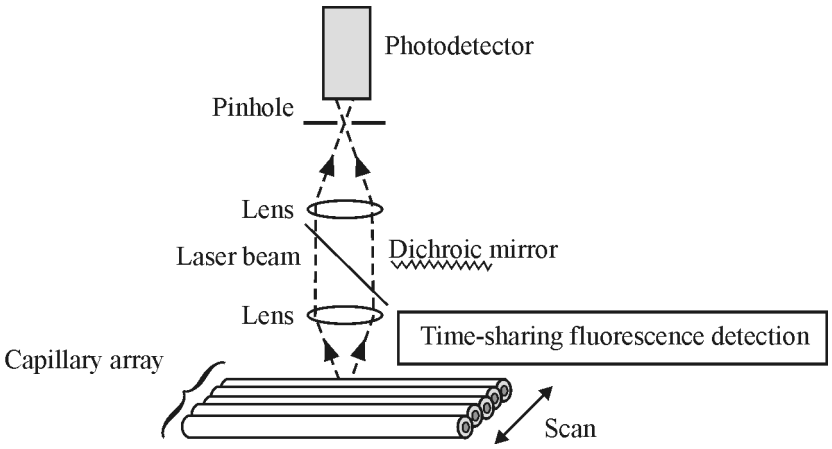


Figure 39 *Scanning laser capillary array DNA sequencer*

The second technique uses a cylindrical lens (Figure 40) to diverge the laser beam onto a number of capillaries. Again, capillaries are arranged in an array on a flat surface. A CCD camera is used to simultaneously detect fluorescence emission from all capillaries. Here, the intensity of the laser beam is reduced due to expansion of the beam. However, the intensity available for irradiation in each capillary is reduced, as in the corresponding emission for each DNA sample.

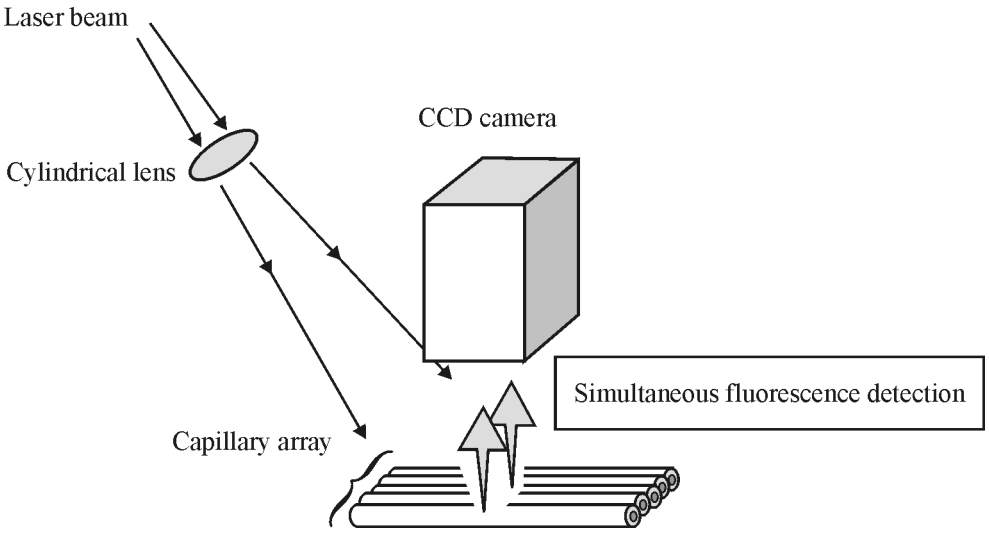


Figure 40 *Expanded laser beam capillary array DNA sequencer*

The lasers and the dyes that are used are the same as those used in all of the other laser-induced fluorescence studies.

Photonics in Homeland Security

Biometrics

Automated fingerprint identification—*Biometric techniques* are methods of identifying and authenticating the identity of individuals based on physiological or behavioral characteristics. These include identification through *fingerprints, eye patterns, hand-scan signature dynamics, facial features, voice identification,* and *keystroke dynamics.* Many of these techniques are already in use in homeland security. However, some of these techniques are more effective than others due to reasons of availability of equipment and cost, the number of manufacturers, non-friendliness intervention to subjects, and non-standardization. In general, all of the techniques have some features in common. Most of the devices are automated. They scan and capture an analog or digital image of the particular characteristic. The images are processed and compared with those in an existing data bank. Once a *match* is found between the scanned image and the one stored in the data bank, authentification takes place. This authentification confirms the identity of the individual and the relation of the particular scanned item to the individual. Sometimes the physiological and behavior characteristics may make the identification more complex. Physiological characteristics such as fingerprints, hand scans, iris patterns, or blood vessel patterns on the retina are relatively stable and unalterable under normal circumstances. Behavioral characteristics such as signatures or typing on a keyboard change significantly with time, and, hence, have to be updated more frequently for reliability. From this point of view, various techniques used in biometrics are complementary to each other and not one single technique can identify a person with one hundred percent certainty, although fingerprinting nearly does so.

Eye patterns—Two aspects of the eye—the pattern of flicks on the iris and blood vessel patterns on the retina of the eye—provide a unique base for identification. Figure 41 shows a schematic diagram of the cross-section of the eye.

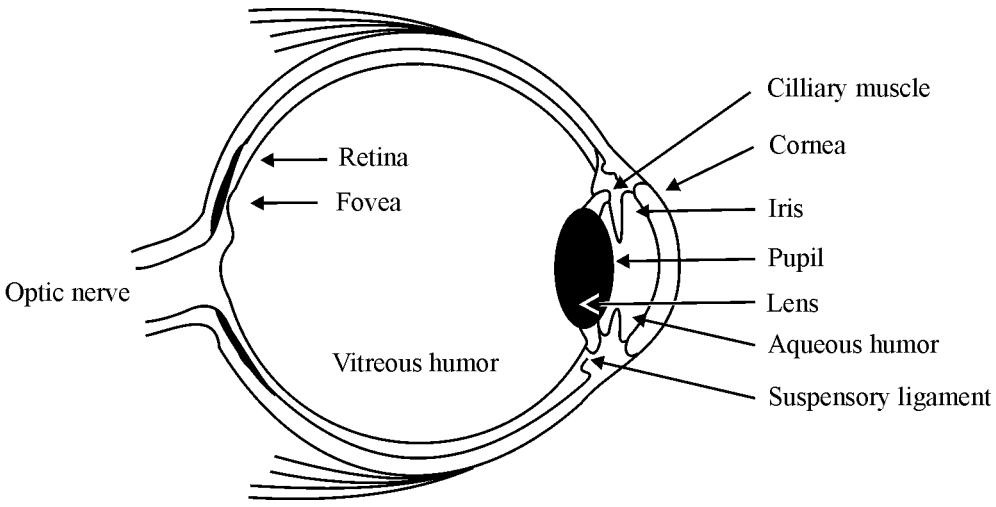


Figure 41 *Schematic diagram of the cross-section of the eye*

In the case of the iris scan, a video image can be taken from about three feet, relieving the subject from any direct interaction or discomfort. This is a great advantage since the device is subject friendly and does not lead to objections from the individual. Iris patterns (flicks) are

quite unique to individuals, and comparison with an image from the data bank is relatively easy to make. One company, Iris Scan, produces such a machine. Directing a low intensity, infrared light beam through the pupil and onto the back part of the eye constitutes a retinal scan. The retinal pattern is reflected back to a camera, which captures the unique pattern of the blood vessels on the retina. This is highly accurate and takes only 35 bytes of information space. *Retinal scans are one of the best biometric techniques with almost a zero false-acceptance rate.* At present, it is mostly used in the military and high-security facilities. However, the biggest hurdle to this technology is "user resistance." Since this technique is more intrusive, most people are afraid of the safety to their eyes. One company, Eye Deformity, manufactures a retinal scan machine.

Hand scans—Hands of individuals have their own unique features for identification. The top, sides, and palm of the hand have unique characteristics that can identify individuals. Of the three, only the palm patterns do not change much with time. However, the top and sides of the hand change with age. In particular, the top of the hand becomes wrinkled with age and has to be frequently updated. One system that uses this technology takes a video picture of both the top and sides of the hand and uses a comparison algorithm to compare with images in the data bank. Dirt and cuts on the hand detract from the viability, but the hand can easily be guided into correct position for scanning. This technique is employed at San Francisco International Airport to identify suspicious persons.

Facial features—This technique involves recognition of geometrical shapes of the face. 3D object recognition techniques allow features of face characteristics for comparison with existing data. Multimedia video technology allows such facial recognition. However, the major problems with this technique are the enormous amount of data base requirements with an increasing amount of individuals.

Signature verification—This technology is very widely used in supermarkets and department stores in conjunction with credit cards. The signature on a sensitive tablet is compared to one in the data bank. The key in signature dynamics is to differentiate between the parts of the signature that are habitual and those, which vary practically with each signing. However, the false rejection rates are still not low enough, and further development is needed to improve this technology.

Voice verification—This is the user-friendliest device for individual recognition. The technique used to recognize a person's voice is done by analysis with broadband speech processing technology. These are already available in communication applications. One of the serious problems with this technique is impersonation. However, speech patterns are formed by a combination of physiological and behavioral factors. Impersonators cannot do both accurately and can be identified. This technology is very useful in finding the whereabouts of parolees by voice verification to confirm that the person is present at a required place at a given time.

Keystroke recognition—This technology is still in the developing stage. It aims to analyze the way in which the user types at a terminal by monitoring the keyboard input. The subject does not know that he is being identified unless told. The better the user is at typing, the easier it is to make the identification. With an enormity of computer usage, the advantage of this biometric method is obvious. Further, since the input device is the existing keyboard, the cost of technology is less. However, differences in keyboards and communication protocol structures are still obstacles to the development of this technology.

3D imaging

Laser-assisted computerized 3D digitized image capture is finding a place in forensic science. One of the important areas where this is used is the *forensic facial reconstruction*. The purpose of forensic facial reconstruction is to produce an image from the skull of an unknown person, which then provides sufficient likeness to a living individual. From this, an identification of the dead person is possible. Plastic reconstruction methods alone do not provide enough information on the nose and mouth shapes. Furthermore, the size and build change with the age of the person and cannot be easily reconstructed. Computerized 3D reconstruction methods using lasers are now being employed more effectively. Computerized methods are repeatable, fast, and accurate.

The technique involves the use of a color laser scanner with a suitable graphics package to capture 3D images of the skull. The skull is placed on a rotating platform and a *wire frame* matrix is generated. Separately, large samples of tissue depth measurements are collected from different samples of live individuals with associated attributes of age, sex, build, and ethnic groups using computerized tomography (CT) scans. Digitized images of facial features which are not predicted by the skull contours (nose, eyes, and mouth) are added separately and a new wire frame face is generated. Color and texture are added to this. These four stages are shown in Figure 42. Sometimes, selected facial features from computerized image libraries can be added to the 3D image.

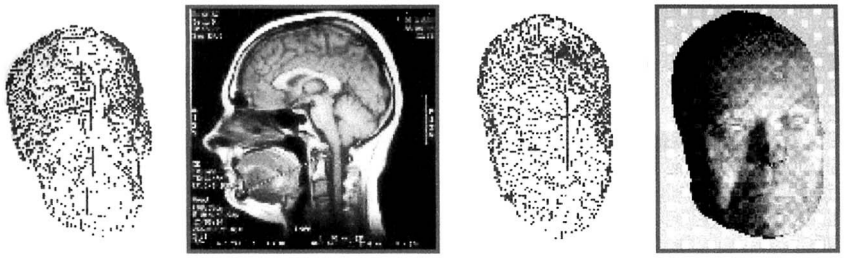


Figure 42 *From left to right—Wire frame matrix of a skull; computerized tomography (CT) scan; wire frame matrix of the skull with facial features such as nose, eyes and mouth; and image rendered facial reconstruction (Courtesy Martin P. Evison; see references.)*

Forensic facial reconstruction is, at best, a scientifically informed artistic recreation and interpretation. However, it is not, by itself, used conclusively to identify a person.

LABORATORY

This laboratory enables students to understand the following:

- The operation of Ar^+ and Kr^+ lasers using a prism cartridge
- Preparing the laser dye solution
- Applying the dye solution to a fingerprint
- Photographing the fingerprint
- Enhancing the photograph and obtaining the picture of the fingerprint

Equipment

Ar^+ and Kr^+ mixture ion laser (or at least Ar^+ laser) with prism cartridge

Glass plate with a fingerprint

Rhodamine 6G solution in methanol

Digital SLR camera

Computer with photo-editing software

Laser safety goggles

Sprayer

Wash bottle with ethanol

Photo-flow solution

Diverging lens with a stand

Procedure

In this experiment, the fingerprint is made on a glass plate, and the print is deliberately made in this manner so that the print will be clear and easy to observe. In real life this is not always the case since the fingerprint may be partial, the surface may be hard (like metal) or soft like paper, the background may be colored or may have its own luminescence and the contrast may be poor.

First, the fluorescent *dye solution* has to be prepared. (STOP: First read the *safety precautions* given at the end.) The dye solution is prepared by adding 1 gram of rhodamine 6G (dry powder) to one liter of methanol (isopropyl alcohol or water can be substituted) and then adding a few drops of photo-flow.

Using the sprayer, a few milliliters of the fluorescent dye are sprayed lightly on the fingerprint (preferably in a hood or spraying chamber). The dye is allowed to dry thoroughly. The print is then illuminated with the 515 nm line of the Ar^+ laser in a dark room. About 100 ml of the dye solution is placed in a beaker. The glass plate with the fingerprint is then dipped completely in the solution. It is allowed to remain there for 1 to 2 seconds and then allowed to drip dry for 10 to 15 seconds. The plate is then held over a 1000 ml beaker and the surface containing the fingerprint is rinsed with a stream of ethanol from a wash bottle. This plate is again allowed to dry thoroughly and is then viewed again by illuminating it with the 515 nm line of the Ar^+ laser. For best results, the Ar^+ beam is diffused with the help of a diverging lens. The fingerprint is then photographed using a digital SLR camera. The settings of the camera have to be adjusted based on the intensity of the fingerprint image. However, since the image can be seen on the camera screen surface, the image can be photographed by trial and error. It is recommended that an aperture setting of F8 or F11 be used and that an orange filter be used, if available. A close-up lens of 1x or 2x may be used for a good close up of the print. A shutter speed of 1 second or more may be required to get a good picture. The fingerprint is then described by type lines, delta, and loop to categorize the print. (See Figures 16 and 17.)

Safety precautions

- Fluorescent materials have to be handled very carefully. Gloves are essential when dealing with these materials.
- Every item to be used with fluorescent materials must be kept in a separate container (a Ziploc bag is preferable).
- All experiments with laser dyes must be made on a separate bench and not allowed to come into contact with anything else. It is extremely difficult to get rid of the dyes once they touch an object.
- A fume cupboard or a spraying chamber **must** be used when spraying the dye on the fingerprint. It must never be done in an open space.
- Each laser requires that specific goggles be used by the person(s) operating the laser. The proper goggles **must** always be used.
- Proper procedures have to be followed when disposing of the glass treated with the fluorescent dyes.

PROBLEM EXERCISES

1. How is fluorescence produced? What is the difference between luminescence, fluorescence, and phosphorescence?
2. State the different arch patterns that are recognizable in a fingerprint. Explain the terms delta, type line and core.
3. Identify the fingerprint ridge characteristics given in the text (Figure 16). Does a fingerprint ever change?
4. How does the fluorescence dyes help in detecting latent fingerprints. What are the available procedures for observing fingerprints on a) porous surfaces? and b) hard surfaces?
5. What are the advantages of digital photography compared to film photography?
6. Show how a small variation such as the type of hemoglobin can change by substitution of one amino acid.
7. Explain the components that make up a DNA molecule.
8. Explain the fundamentals of using lasers in DNA sequencing.
9. What are the types of microscopes that are used in forensic science? How does a laser assisted-confocal microscope differ from other microscopes?
11. What is biometrics? What are six different biometric techniques available for use in homeland security?
12. What is 3D imaging? How is it useful in reconstruction of objects?

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