Laser Confocal Scanning Microscopy
&
Ellipsometry

Confocal Laser Scanning Microscopy

In confocal microscopy, a laser beam is used to focus light of a specific wavelength onto the specimen.

The confocal scanning microscope has the ability to take optical sections at successive focal planes (known as a Z series).

Pinhole apertures are used so that only a small area of the specimen is focused at any given time.

Light from the plane of focus enters the detector, eliminating any scattered light, which has the tendency to blur images.

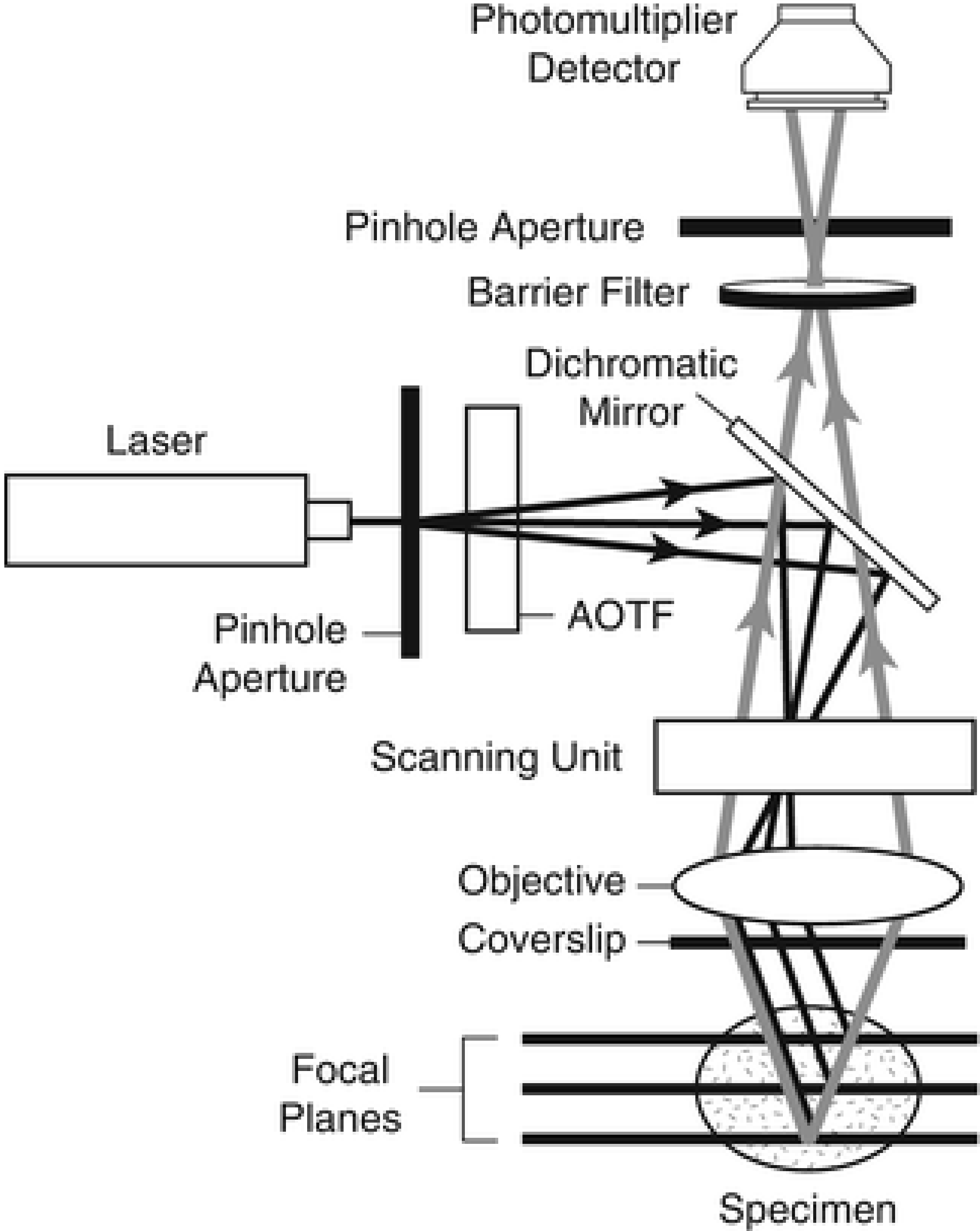
The focused light beam moves across the specimen, scanning it, which is required because only a small volume is illuminated at any given time, and a number of these small volumes must be collected for a complete specimen image.

The main components of a modern laser scanning confocal microscope- reflected light, upright version are shown in the figure

Light from one or more lasers passes through a pinhole, attenuated through an Acousto-Optic Tunable Filter (AOTF), bounces off a dichromatic mirror, and passes into the scanning unit.

A scanned beam enters the back focal plane of the objective lens, which focuses the light at a point in the specimen.

Any light coming back from the excitation of a fluorochrome at this point inside the specimen passes back through the objective lens and the scanning unit.



Acousto Optic Tunable Filter (AOTF)

AOTF uses acoustic waves to selectively diffract specific wavelengths of light.

It's a solid-state, electrically tunable filter that can be used to isolate or modulate individual wavelengths from a light source.

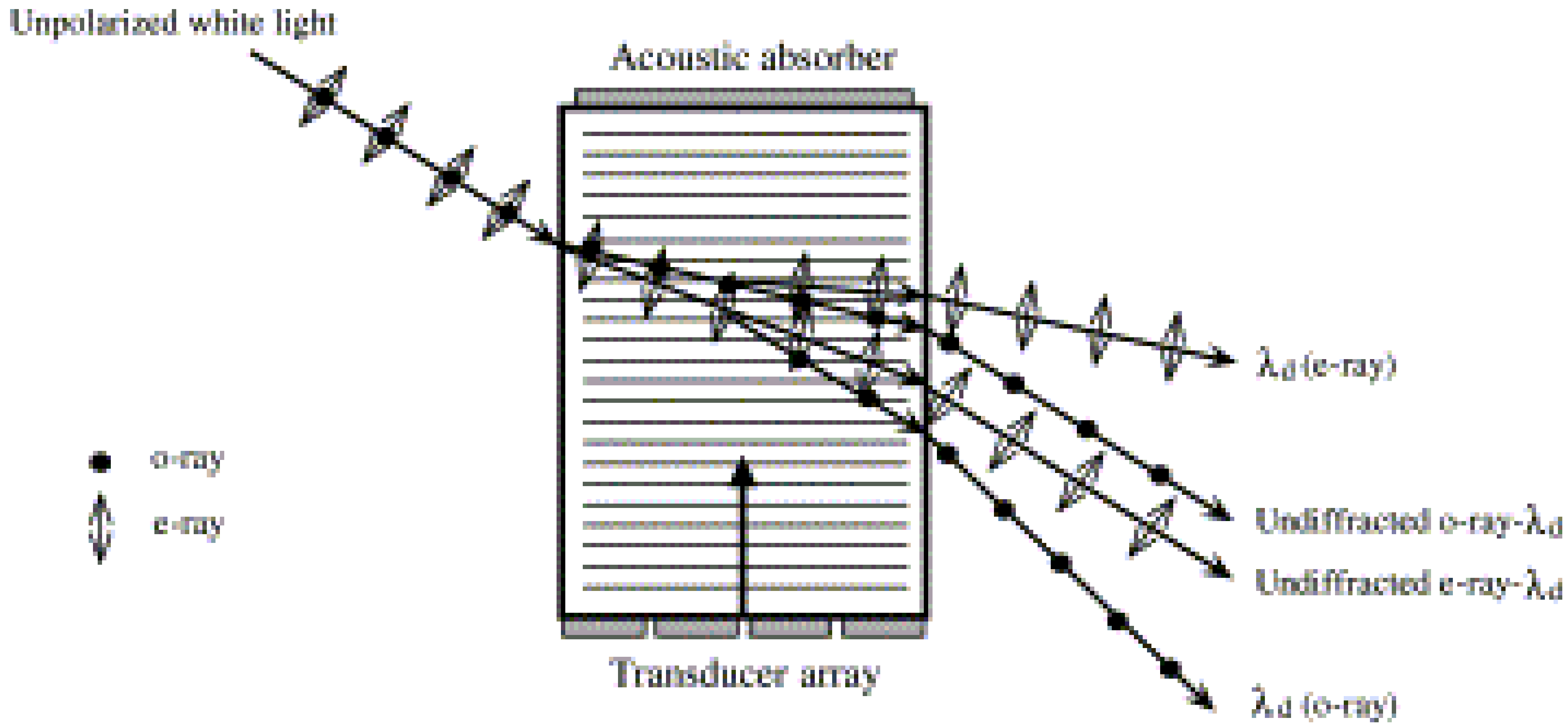
It consists of a piezoelectric transducer attached to a birefringent crystal.

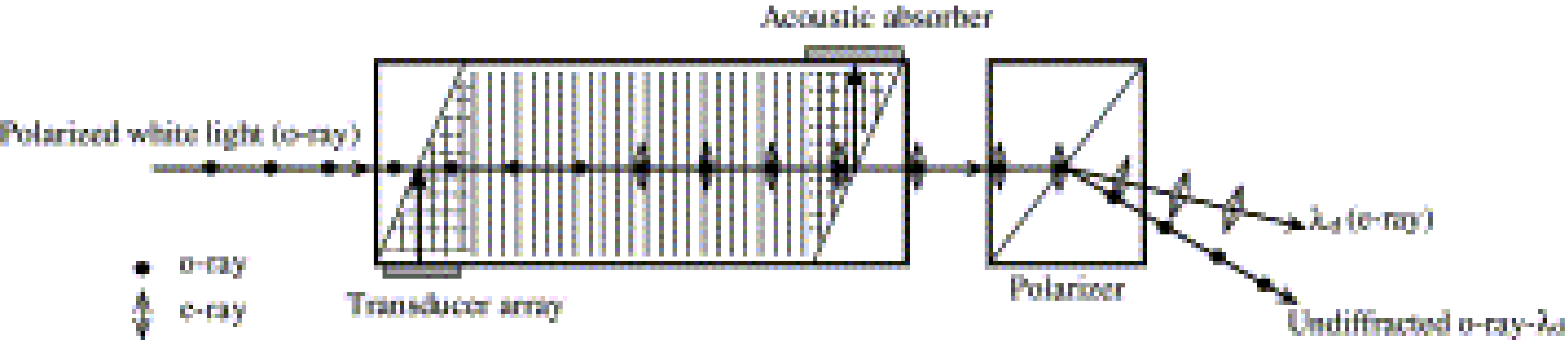
When an RF signal is applied to the transducer, it generates ultrasonic waves within the crystal.

These sound waves create a diffraction grating within the crystal, altering its refractive index.

This grating interacts with the incoming light, causing it to diffract. The wavelength of the diffracted light is determined by the frequency of the acoustic wave and the properties of the crystal.

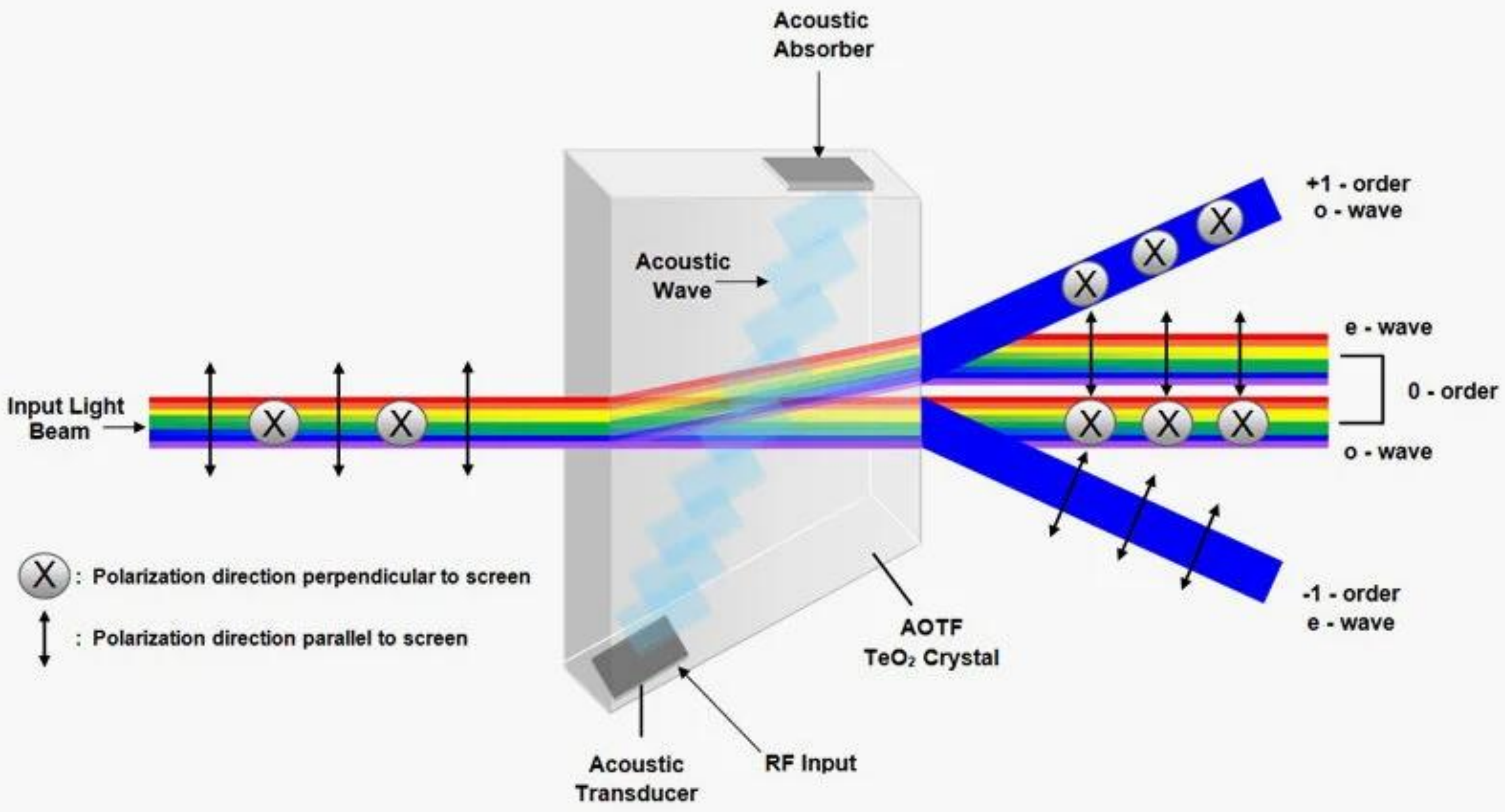
By changing the frequency of the RF signal applied to the transducer, the wavelength of the diffracted light can be tuned. This allows for the selection of different wavelengths from the light source.




Acoustic absorber
Polarized white light (o-ray)
o-ray
e-ray
Transducer array
Polarizer
λ_d (e-ray)
Undiffracted o-ray-λ_d


AOTFnC-VIS-TN 1002
MADE IN FRANCE
26793
A.A Sa 6 rue de Versailles
F-78470 St Rémy
Les Chevreuse
OUT


Gooch & Housego


Acoustic Absorber
Acoustic Wave
+1 - order
o - wave
e - wave
0 - order
o - wave
Input Light Beam
-1 - order
e - wave
: Polarization direction perpendicular to screen
: Polarization direction parallel to screen
AOTF
TeO_2 Crystal
Acoustic Transducer
RF Input

Since this light is of longer wavelength than the excitation light, it passes through the dichromatic mirror and is further cleaned up by a barrier filter (blocks shorter wavelength light and allows longer wavelength) and it is eventually focused at the second pinhole.

Any light that passes through the pinhole strikes a low noise photomultiplier detector, the signal from which subsequently passes to the computer imaging system of the confocal microscope.

The conventional light microscope is essential for efficiently finding the region of interest in the specimen by eye before scanning in the confocal mode.

This is extremely useful since one of the great strengths of the confocal microscope, i.e., the elimination of out-of-focus information, can make it extremely difficult to locate a region of interest in the specimen in the confocal mode.

This configuration is also very stable, especially when mounted on an anti-vibration air table. Any vibration results in a loss of resolution in the image, and can show up in the image as irregular horizontal lines.

The modern LSCM typically uses a laser rather than a lamp for a light source, acousto-optic tunable filters (AOTFs) for selecting specific excitation wavelengths, dichroics for multichannel emission discrimination, sensitive photomultiplier tube detectors (PMTs) and a computer to control the scanning mirrors and to facilitate the collection and display of the images.

Modern LSCMs can excite and detect multiple fluorophores simultaneously typically through the use of multiple lasers and multiple detectors for each channel.

Images are subsequently stored as digital image files and can be further analyzed using additional software.

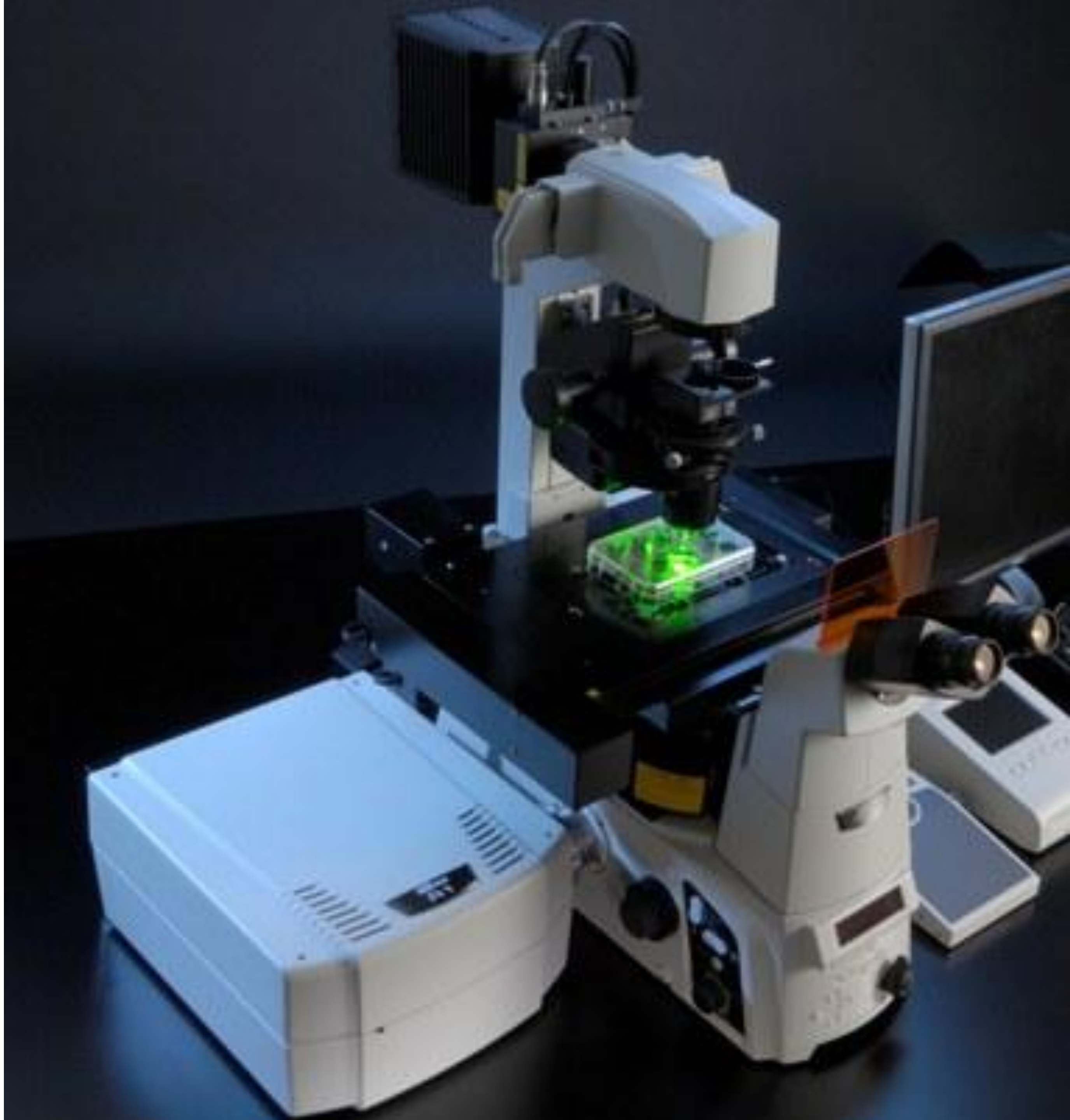
In the LSCM, illumination and detection are confined to a single, diffraction-limited, point in the specimen.

This point is focused by an objective lens, and scanned across it using some form of scanning device.

Points of light from the specimen are detected by a photomultiplier behind a pinhole, and the output from this is built into an image by the computer.

Specimens are usually labeled with one or more fluorescent probes (fluorescence mode).

Unstained specimens can be viewed using the light reflected back from the specimen (reflected light mode).



Ellipsometry

Ellipsometry is an optical technique for investigating the dielectric properties (complex refractive index or dielectric function) of thin films. Ellipsometry measures the change of polarization upon reflection or transmission and compares it to a model.

It can be used to characterize composition, roughness, thickness (depth), crystalline nature, doping concentration, electrical conductivity and other material properties.

It is very sensitive to the change in the optical response of incident radiation that interacts with the material being investigated.

The measured signal is the change in polarization as the incident radiation (in a known state) interacts with the material structure of interest (reflected, absorbed, scattered, or transmitted).

The polarization change is quantified by the amplitude ratio, Ψ, and the phase difference, Δ.

Because the signal depends on the thickness as well as the material properties, ellipsometry can be a universal tool for contact free determination of thickness and optical constants of films.

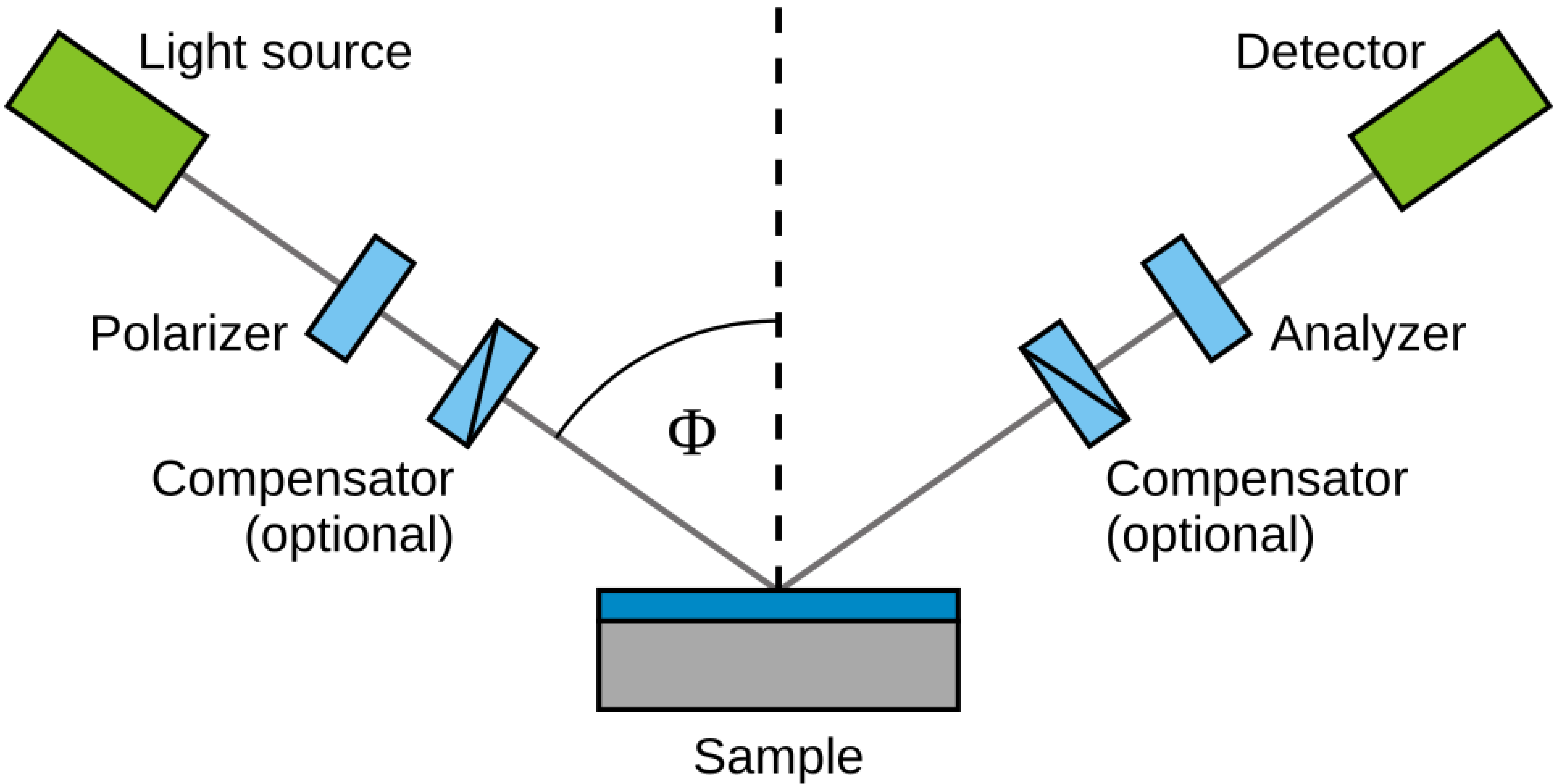
Upon the analysis of the change of polarization of light, ellipsometry can yield information about layers that are thinner than the wavelength of the probing light itself, even down to a single atomic layer.

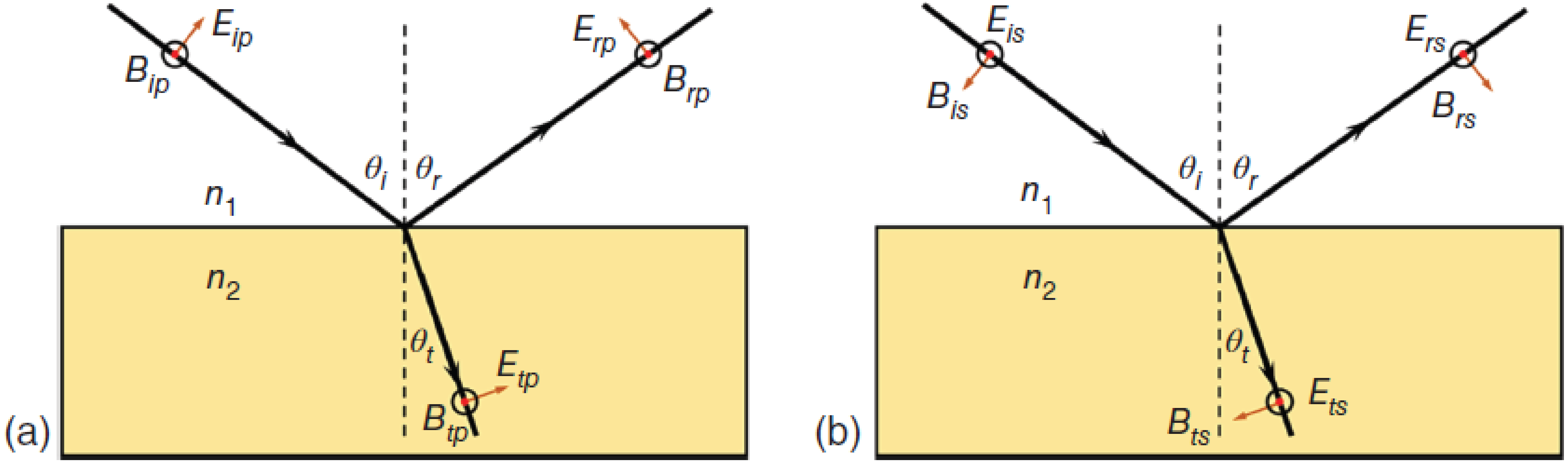
Ellipsometry can probe the complex refractive index or dielectric function tensor, which gives access to fundamental physical parameters.

It is used to characterize film thickness for single layers or complex multilayer stacks ranging from a few angstroms or tenths of a nanometer to several micrometers with an excellent accuracy.

Electromagnetic radiation is emitted by a light source and linearly polarized by a polarizer. It can pass through an optional compensator (retarder, quarter wave plate) and falls onto the sample.

After reflection the radiation passes a compensator (optional) and a second polarizer, which is called an analyzer, and falls into the detector. Instead of the compensators, some ellipsometers use a phase-modulator in the path of the incident light beam.





Electric and magnetic fields for (a) p-polarized and (b) s-polarized waves

$$\vec{E}(\vec{r},t) = \vec{E}_0 \exp[i(\vec{k}\cdot\vec{r} - \omega t + \delta)]$$

$$\vec{B}(\vec{r},t) = \vec{B}_0 \exp[i(\vec{k}\cdot\vec{r} - \omega t + \delta)]$$

where, $\vec{k}$ denotes the wave vector, ω denotes the angular frequency, and δ denotes the initial phase.

When light is reflected or transmitted through a sample/medium via an oblique angle, the electromagnetic wave can be resolved into two components – *p*-polarized (in-plane incidence) and *s*-polarized (perpendicular to incident plane) *E*-field components, respectively. (“p” refers to parallel and “s” refers to the German word “senkrecht which means perpendicular)

For a medium with refractive index *n*, based on Maxwell’s equations and boundary conditions, the amplitude of the reflection coefficient for the *p*-polarized light is expressed as

$$r_p \equiv \frac{E_{rp}}{E_{ip}} = \frac{n_t \cos\theta_i - n_i \cos\theta_t}{n_t \cos\theta_i + n_i \cos\theta_t}$$

Likewise, the amplitude of the transmission coefficient for the p-polarized light can be expressed as

$$t_p \equiv \frac{E_{tp}}{E_{ip}} = \frac{2n_i \cos\theta_i}{n_t \cos\theta_i + n_i \cos\theta_t}$$

whereas the *s*-polarized counterparts are expressed as

$$r_s \equiv \frac{E_{rs}}{E_{is}} = \frac{(n_i \cos\theta_i - n_t \cos\theta_t)}{(n_i \cos\theta_i + n_t \cos\theta_t)}$$

$$t_s \equiv \frac{E_{ts}}{E_{is}} = \frac{2n_i \cos\theta_i}{n_i \cos\theta_i + n_t \cos\theta_t}$$

These equations are known as the Fresnel equations.

When the refractive indices are complex, $\tilde{n}$ the Fresnel equations still hold. The complex dielectric function can be obtained via the expression

$$\widetilde{n}^2 \equiv \varepsilon$$

Based on Snell's law, the Fresnel equations for reflection can be further generalized as

$$r_p = \frac{\widetilde{n}_{ti}^2 \cos\theta_i - \left(\widetilde{n}_{ti}^2 - \sin^2\theta_i\right)^{\frac{1}{2}}}{\widetilde{n}_{ti}^2 \cos\theta_i + \left(\widetilde{n}_{ti}^2 - \sin^2\theta_i\right)^{\frac{1}{2}}} \qquad r_s = \frac{\cos\theta_i - \left(\widetilde{n}_{ti}^2 - \sin^2\theta_i\right)^{\frac{1}{2}}}{\cos\theta_i + \left(\widetilde{n}_{ti}^2 - \sin^2\theta_i\right)^{\frac{1}{2}}} \qquad \widetilde{n}_{ti} = \frac{\widetilde{n}_t}{\widetilde{n}_i}$$

The reflectances of the *p*- and *s*-polarized lights are expressed by

$$R_\mathrm{p} \equiv \frac{I_{rp}}{I_{ip}} = \left|\frac{E_{rp}}{E_{ip}}\right|^2 = |r_\mathrm{p}^2|$$

$$R_\mathrm{s} \equiv \frac{I_{rs}}{I_{is}} = \left|\frac{E_{rs}}{E_{is}}\right|^2 = |r_\mathrm{s}^2|$$

where the light intensity $I = n|E|^2$. Since the difference between r_p and r_s is maximized at the Brewster angle, ellipsometric measurements are usually performed at incident angles, θ_i, typically, in the range of $70\text{–}80°$ for the optical characterization of semiconducting systems.

In multilayered systems, the resultant amplitude of the reflection coefficients is expressed as the sum of individual components of the reflection and transmission coefficients at each interface. The phase differences of each wave are considered in the analysis.

When light is reflected or transmitted from a sample, the p- and s-polarized components of the incident light undergo changes to their amplitude and phase.

Ellipsometry measures the complex reflectance ratio ρ of a system, which may be parametrized by the amplitude component Ψ and the phase difference Δ.

The amplitudes of the s and p components, after reflection and normalized to their initial value, are denoted by r_s and r_p respectively.

The angle of incidence is chosen close to the Brewster angle of the sample to ensure a maximal difference in r_p and r_s.

Ellipsometry measures the complex reflectance ratio ρ (a complex quantity), which is the ratio of r_p over r_s.

$$\rho = \frac{r_p}{r_s} = \tan\Psi e^{i\Delta}$$

Thus, tan Ψ is the amplitude ratio upon reflection, and Δ is the phase shift.

$$\tan\Psi \exp i\Delta = \frac{r_p}{r_s} = \frac{E_{rp}/E_{ip}}{E_{rs}/E_{is}} = E_{rp}/E_{rs}$$

where, $E_{ip} = E_{is}$

Ellipsometry is an indirect method. The optical constants can not be obtained directly from the measured Ψ and Δ. Using a model analysis they have to be obtained.

Single-wavelength ellipsometry employs a monochromatic light source. This is usually a laser in the visible spectral region (HeNe laser with a wavelength of 632.8 nm).

The advantage of laser ellipsometry is that laser beams can be focused on a small spot size. They have a higher power than broad band light sources and hence laser ellipsometry can be used for imaging.

However, the experimental output is restricted to one set of Ψ and Δ values per measurement.

Spectroscopic Ellipsometry

Spectroscopic ellipsometry (SE) employs broad band light sources, which cover a certain spectral range in the infrared, visible or ultraviolet spectral region.

The complex refractive index or the dielectric function tensor in the corresponding spectral region can be obtained, which gives access to a large number of fundamental physical properties.

Infrared spectroscopic ellipsometry (IRSE) can probe lattice vibrational (phonon) and free charge carrier (plasmon) properties.

Spectroscopic ellipsometry in the near infrared, visible up to ultraviolet spectral region studies the refractive index in the transparency or below-band-gap region and electronic properties, for instance, band-to-band transitions or excitons.

Standard ellipsometry is applied for optical isotropic or optically uniaxial materials with their optic axis normal to the surface. For all other cases generalized ellipsometric principles are used.

Imaging ellipsometry

Ellipsometry can be used for providing real time contrast image of the sample by using a CCD camera as a detector which can be used to obtain information about film thickness and refractive index.

Advanced imaging ellipsometer technology operates on the principle of classical null ellipsometry and real-time ellipsometric contrast imaging.

The film under investigation is placed onto a reflective substrate. The film and the substrate have different refractive indexes.

In order to obtain data about film thickness, the light reflecting off of the substrate must be nulled. Nulling is achieved by adjusting the analyzer and polarizer so that all reflected light from the substrate is extinguished.

Due to the difference in refractive indexes, this will allow the sample to become very bright and clearly visible. The light source consists of a monochromatic laser of the desired wavelength. A common wavelength that is used is 532 nm green laser light.

A laser beam first passes through a linear polarizer (P) and then through a quarter wavelength compensator (C) which transforms the light into elliptically polarized light.

This elliptically polarized light then reflects off the sample (S), passes through the analyzer (A) and is imaged onto a CCD camera by a long working distance objective.

The analyzer is another polarizer identical to P which helps to quantify the change in polarization. This design is commonly referred to as a LPCSA configuration.

The orientation of the angles of P and C are chosen in such a way that the elliptically polarized light is completely linearly polarized after it is reflected off the sample.

For simplification of calculations, the compensator can be fixed at a $45°$ angle relative to the plane of incidence of the laser beam. This set up requires the rotation of the analyzer and polarizer in order to achieve null conditions.

The ellipsometric null condition is obtained when A is perpendicular with respect to the polarization axis of the reflected light achieving complete destructive interference.

In the null state the absolute minimum of light flux is detected at the CCD camera.

The angles of P, C, and A obtained are used to determine the Ψ and Δ values of the material.

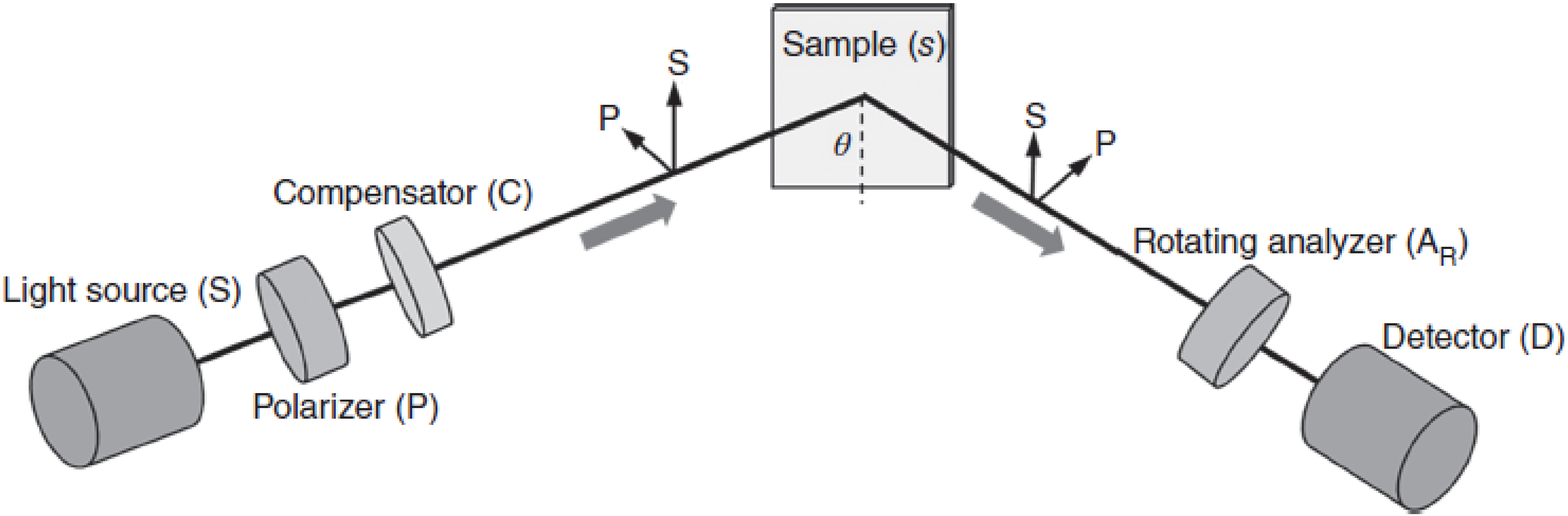
$$\Psi = A \text{ and } \Delta = 2P + \pi/2$$

Where, A and P are the angles of the analyzer and polarizer under null conditions respectively.

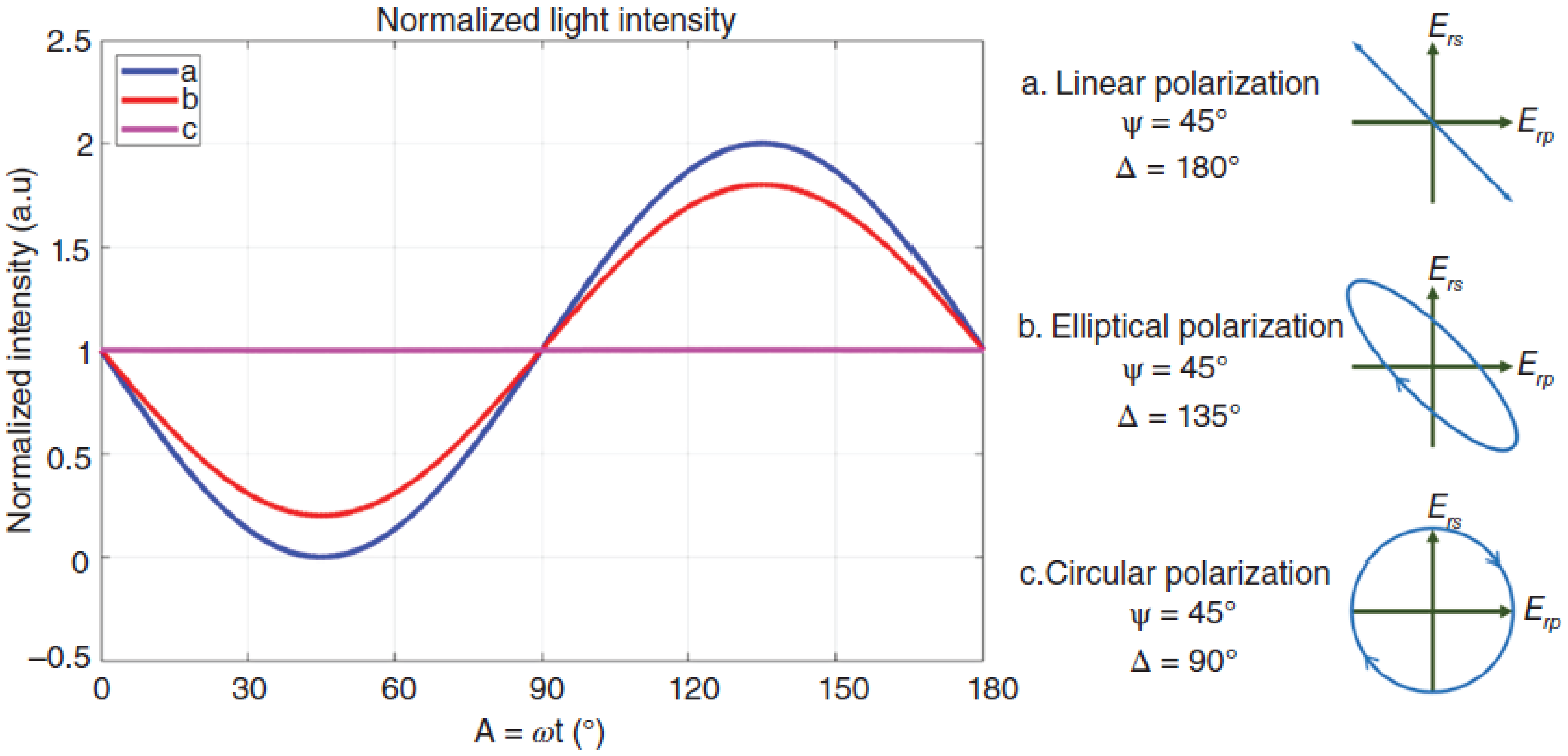
By rotating the analyzer and polarizer and measuring the change in intensities of light over the image, analysis of the measured data by use of computerized optical modeling can lead to a deduction of spatially resolved film thickness and complex refractive index.

As the imaging is done at an angle, only a small line of the entire field of view is actually in focus. The line in focus can be moved along the field of view by adjusting the focus.

To analyze the entire region of interest the focus must be incrementally moved along the region of interest with a photo taken at each position. All of the images are then compiled into a single, in focus image of the sample.



Sample (s)
S
P
θ
S
P
Compensator (C)
Rotating analyzer (A_R)
Light source (S)
Detector (D)
Polarizer (P)



Normalized intensity of linearly, elliptical, and circularly polarized light based on the rotating-analyzer configuration.