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## 8.8 Microscopy Terminology

**Anisotropic:** An object that has properties that differ according to the direction of measurement when viewed in polarized light.

**Barrier Filter:** A filter used in fluorescence microscopy that suppresses unnecessary excitation light that has not been absorbed by the specimen and selectively transmits only the fluorescence.

**Becke Line:** The bright halo near the boundary of a specimen that moves with respect to that boundary as the microscope is focused through best focus.

**Becke Line Method:** A method for determining the refractive index of a specimen relative to its mountant by noting the direction in which the Becke line moves when the focus is changed. The Becke line will always move toward the higher refractive index medium (specimen or mountant) when focus is raised and will move toward the lower refractive index medium when focus is lowered.

**Birefringence:** The numerical difference in refractive indices for a specimen, given by the formula:  $n_{ll} - n$ . Birefringence can be calculated by determining the retardation (r) and thickness (T) at a particular point in a specimen and by using the formula:  $B = r (nm) / 1,000 T (m)$ .

**Comparison Microscope:** A system of two microscopes positioned side by side and connected via an optical bridge in which specimens are examined simultaneously in either transmitted or reflected light.

**Compensator:** Any variety of optical devices that can be placed in the light path of a polarizing microscope to introduce fixed or variable retardation comparable with that exhibited by the specimen. The retardation and sign of elongation of the specimen is then determined. Compensators can employ a fixed mineral plate of constant or varying thickness or a mineral plate that is rotated to alter the thickness presented to the optical path (and retardation introduced) by a set amount.

**Compensator, Full Wave (Red Plate):** A compensator using a plate of gypsum, selenite, or quartz, which introduces a fixed retardation between 530-550 nm (approximately the retardation of the first order red color on the Michel-Lévy chart).

**Compensator, Quarter Wave:** A compensator, usually with a mica plate, which introduces a fixed retardation between 125-150 nm.

**Compensator, Quartz Wedge:** A wedge, cut from quartz, having continuously variable retardation extending over several orders of interference colors (usually 3-7).

**Compensator, Sénarmont:** A quarter-wave plate inserted above the specimen in the parallel 0° position with a rotating calibrated analyzer. Measures low retardation and requires the use of monochromatic light.

**Compensator, Tilting (Berek):** A compensator typically containing a plate of calcite or quartz, which can be rotated by means of a calibrated drum to introduce variable retardation up to about ten orders.

**Dichroism:** The property of exhibiting different colors, especially two different colors, when viewed in polarized light along different axes.

**Dispersion of Birefringence:** The variation of birefringence with wavelength of light.

**Dispersion Staining:** A technique for refractive index determination that employs central or annular stops placed in the objective back focal plane of a microscope. Using an annular stop with the substage iris

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closed, a specimen mounted in a high-dispersion medium will show a colored boundary of a wavelength where the specimen and the medium match in refractive index. Using a central stop, the specimen will show colors complimentary to those seen with an annular stop.

**Excitation Filter:** A filter used in fluorescence microscopy that transmits specific bands or wavelengths of light capable of inducing visible fluorescence in various substrates.

**Interference Colors:** Colors produced by the interference of two out-of-phase rays of white light when a birefringent material is observed at a nonextinction position between crossed polars. The retardation at a particular point in a specimen is determined by comparing the observed interference color to the Michel-Lévy chart.

**Isotropic:** An object that is identical in all directions and invariant with respect to direction.

**Light Microscope:** A microscope that employs light in the visible or near-visible portion of the electromagnetic spectrum.

**Michel-Lévy Chart:** A chart relating thickness, birefringence, and retardation so that any one of these variables can be determined for an anisotropic specimen when the other two are known.

**Microscopical:** Concerning a microscope or the use of a microscope.

**Plane Polarized Light:** Light that is vibrating in one plane.

**Pleochroism:** The property of exhibiting different colors, especially three different colors, when viewed in polarized light along different axes.

**Polarized Light:** A bundle of light rays with a single propagation direction and a single vibration direction. The vibration direction is always perpendicular to the propagation direction. It is produced by use of a polarizing filter, from ordinary light by reflection, or double refraction in a suitable pleochroic substance.

**Polarized Light Microscope:** A microscope equipped with two polarizing filters, one below the stage (the polarizer) and one above the stage (the analyzer).

**Privileged Direction (of a Polarizer):** The direction of vibration to which light emerging from a polarizer has been restricted.

**Refractive Index:** For a particular transparent medium, the ratio of the speed of light in a vacuum to the speed of light in that medium.

**Relative Refractive Index:** The estimate of the refractive index of a specimen in relation to the index of its surrounding medium.

**Retardation (r):** The actual distance of one of the doubly refracted rays behind the other as they emerge from an anisotropic specimen. Dependent upon the difference in the two refractive indices,  $n_2 - n_1$ , and the thickness of the specimen.

**Sign of Elongation:** Referring to the elongation of a specimen in relation to refractive indices. If elongated in the direction of the high refractive index, the specimen is said to be positive, and if elongated in the direction of the low refractive index, it is said to be negative.

**Stereomicroscope:** A microscope containing two separate optical systems, one for each eye, giving a stereoscopic view of a specimen.

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**Thickness (T):** The optical path through the specimen used for the calculation of birefringence, typically measured in micrometers.