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Optics and optical instruments — Microscopes — Reference system of polarized light microscopy

Optique et instruments d'optique — Microscopes — Système de référence en microscopie de polarisation

Reference number
ISO 8576:1996(E)

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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Optics and optical instruments — Microscopes — Reference system of polarized light microscopy

1 Scope

This International Standard establishes a reference system incorporating all calibrated motions of rotation and displacement on the microscope and its accessories so that the measuring procedures are uniform. Particular attention is given to the polarization parameters and measuring accessories such as rotary microscope stages, polarizing devices, and compensators.

2 Principles

The optical properties of an anisotropic, non-absorbing crystal of minimum symmetry under constant conditions of pressure, temperature and wavelength are described by a triaxial index ellipsoid. The lengths of the semi-axes are given by the principal refractive indices n_α and n_β and n_γ of the crystal. A random plane through the index ellipsoid containing the centre of the ellipsoid generally has the shape of an ellipse with the axes of length $n_{\alpha'}$ and $n_{\gamma'}$. By definition, the relationship $n_\alpha \leqslant n_{\alpha'} \leqslant n_\beta \leqslant n_{\gamma'} \leqslant n_\gamma$ is true.

All directions specified in observations in polarized light are referred to the direction of the highest refractive index n_γ.

NOTE — To emphasize that $|n_{\gamma'}| > |n_{\alpha'}|$ in an object, the subscripts γ and α are often used instead of γ′ and α′.

The index ellipsoid of uniaxial crystals is a rotation ellipsoid. This is characterized by two principal axes specified by n_ω and n_ε, where ω refers to the ordinary and ε refers to the extraordinary vibration direction. The latter is the direction of the rotation axis. The following definitions are true:

$n_\alpha = n_\beta = n_\omega \neq n_\gamma = n_\varepsilon$ (positive)

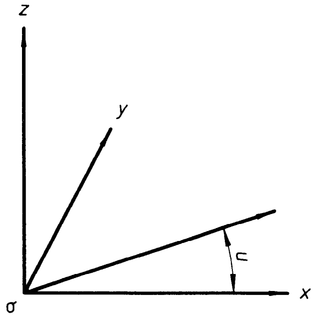
$n_\gamma = n_\beta = n_\omega \neq n_\alpha = n_\varepsilon$ (negative)

i.e. if n_ε is larger than n_ω the crystal is uniaxial and optically positive; if n_ω is larger than n_ε the crystal is uniaxial and optically negative.

3 Reference system for rotation directions and displacements (see figure 1)

3.1 General

Generally, a positive Cartesian reference coordinate system x, y, z is used as a basis whose z-direction is determined by the privileged direction of light propagation from the lamp towards the observer. Accordingly, in observation through the ocular, rising angles u in planes perpendicular to z are read off in a counterclockwise direction, in the mathematically positive sense. This is true for upright and inverted microscopes.



z: direction of light propagation
x: zero position

$\sphericalangle xy =$ 90°
$\sphericalangle xz =$ 90°
$\sphericalangle yz =$ 90°

Reference direction: west-east

Figure 1

3.2 Mechanical stage (see figure 2)

The mechanical stage is attached to the rotary microscope stage in order to move the object in coordinate directions x and y. In the zero rotation position of the microscope stage, the positive x-direction of the mechanical stage and the reference direction are thereby identical (u = 0°).

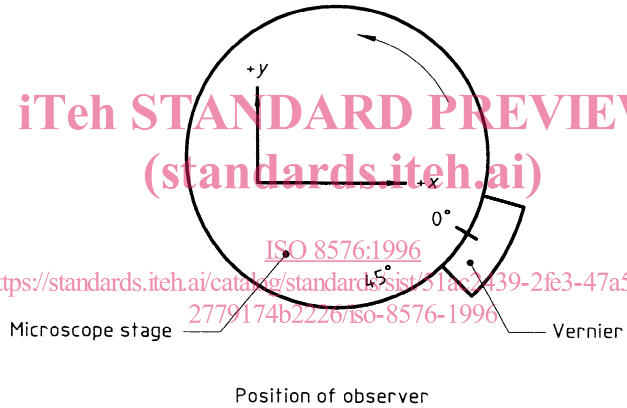


Figure 2

3.3 Rotation position of the microscope stage

The rotation position is zero when the x-direction of the mechanical stage is west-east, i.e. parallel to the vibration direction of light supplied by the polarizer, the latter being in v = 0° (see 4.2).

NOTE — If any direction other than west-east (u = 0°) is taken as the basis orientation of the polarizer, then it should be indicated on the microscope.

3.4 Rotation and tilting directions of the universal stage (see figures 3 and 4)

The universal stage is a device attached to the microscope stage, with which the object is turned towards any spatial direction. It has a system of rotary and tilting axes, specified by subscripts (according to Berek), as A_n, where n = 1, 2, 3...

The highest number specifies the rotation axis of the microscope stage. Odd numbers refer to axes which, in the zero position of the universal stage, are vertical, even numbers to axes which are horizontal. The movement of one axis changes the position of all axes with a smaller subscript number.

In the zero rotation position of the microscope stage, the scales for reading the tilting positions of the axes A_2 and A_4 are towards the right side of the observer so that the directions of the axes are defined as 180°. The working direction of A_2 is mostly perpendicular to the direction of A_4, i.e. 90°. When the universal stage is tilted around A_4, the direction 90° is that of the projection of A_2. Seen in the directions of A_2 and A_4, the tilting angles around A_2 and A_4 are defined as clockwise positive, starting at the horizontal position of the surface of the universal stage.

NOTE — The following designations of the axes of the universal stage are also used:

Berek (1924)	Nikitin-Duparc-Reinhardt	Reinhardt (1931)	R.C. Emmons
A_1	N (normal axis)	N (normal axis)	I.V.
A_2	H (horizontal axis)	H (horizontal axis)	N.S.
A_3	M (mobile axis)	A (auxiliary axis)	O.V.
A_4	I (immobile axis)	K (control axis)	O.E.W.
A_5	—	M (microscope axis)	M

Polarizer: v = 0°
Analyser: w = 90°
This means "crossed position".

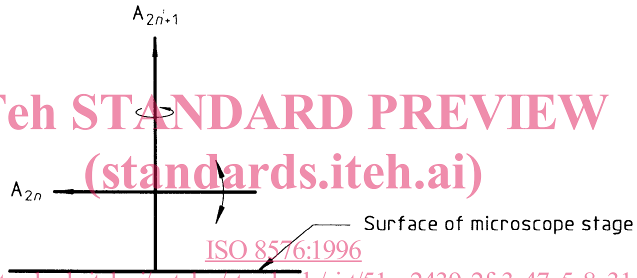


Figure 3

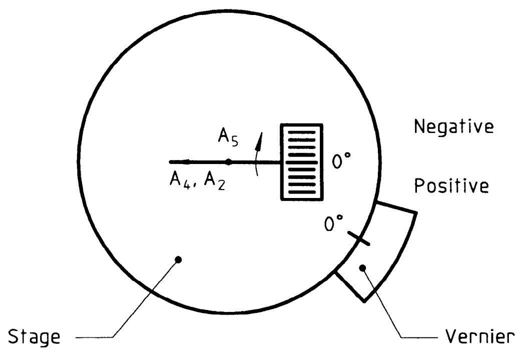


Figure 4

4 Adjusting the polarizing devices, optically anisotropic objects, compensators and auxiliary objects[1)]

4.1 Terminology

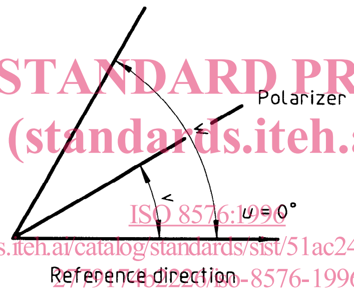
The polarizing devices transmit linearly polarized light of one vibration direction which is identical with the transmission direction of the device. Viewed in the direction of light propagation, the device in front of the object on the microscope stage is called a polarizer, and the one after the object is called an analyser. The latter is used to diagnose the stage of polarization produced by the object or by the combination of object and compensator.

Optically anisotropic objects are substances whose refractive indices vary with the direction of propagation and vibration of light.

Compensators and auxiliary objects are devices made of optically anisotropic materials; they serve to systematically increase or decrease path differences of polarized light waves. In this way the auxiliary objects exhibit the sign of the differences in the object under examination, the compensators indicate the magnitudes of differences.

4.2 Adjusting the polarizer and the analyser (see figure 5)

The transmission directions of both the polarizer and the analyser are referred to the zero direction west-east (u = 0°). In the counter-clockwise system of the microscope stage, the rotation angles of the polarizer and analyser are positive in counter-clockwise direction. The normal working rotation positions are as shown in figure 5.



Polarizer: v = 0°

Analyser: w = 90°

This means "crossed position".

Figure 5

4.3 Adjusting the optically anisotropic object on the microscope stage (see figures 6 and 7)

The extinction position is reached when the directions of the refractive indices $n_{\alpha'}$ and $n_{\gamma'}$ of the object lie in parallel with the transmission directions of the crossed polarizer and analyser.

The diagonal (or measuring) position is reached when the directions $n_{\alpha'}$ and $n_{\gamma'}$ are diagonal (at 45°) to the transmission directions.

1) The designations "auxiliary objects", "auxiliary specimens", "qualitative compensators" and "fixed compensators" are used synonymously in everyday language.

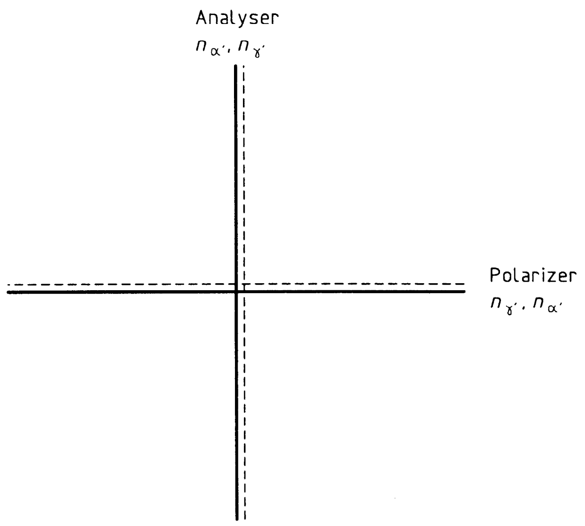


Figure 6

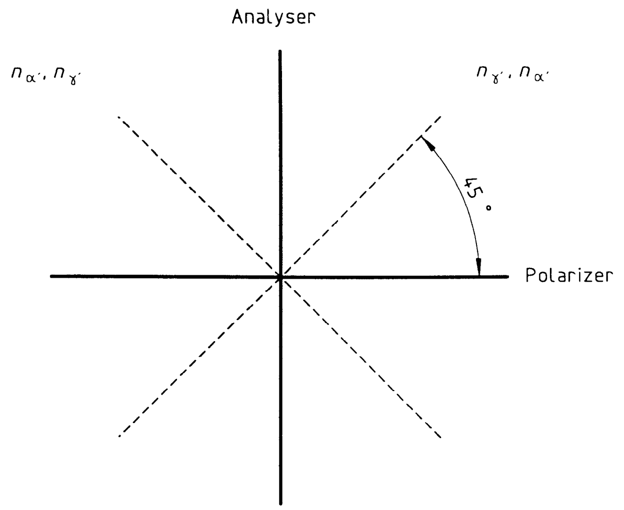


Figure 7

4.4 Adjusting the auxiliary objects and tilting compensators (see figure 8)

The auxiliary objects and compensators are inserted in the standardized tube slots. In this case the direction of the higher refractive indices of the compensators (see note in clause 2) forms a 45° angle with the reference direction. The direction of $n_{\gamma'}$ of the object on the microscope stage is then adjusted to the 135° (parallel to the n_{α} direction of the compensator), i.e. the subtraction position. Exceptions exist with certain compensators after Berek or Ehringhaus, where the n_{γ} direction of the compensator is set at the 135° position.

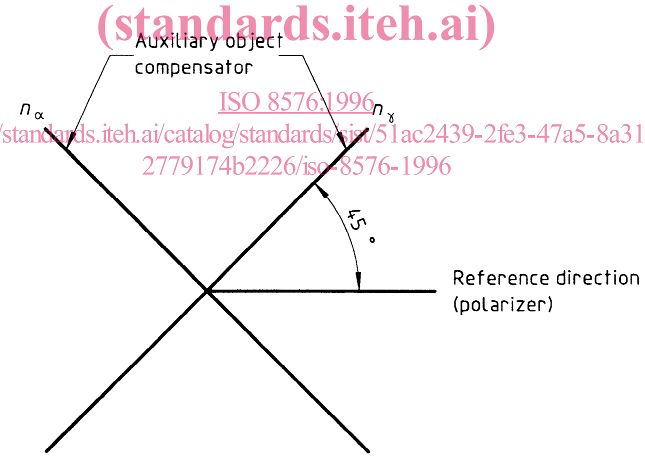


Figure 8

4.5 Adjusting the elliptic compensator after Brace-Köhler (see figure 9)

The elliptic compensator after Brace-Köhler is an azimuthally rotatable anisotropic plate with a known path difference Γ_{C} up to $\lambda/10$.

The rotation position of this plate is adjusted so that the n_{γ} direction is 90°, i.e. parallel with the transmission direction of the analyser

At the beginning of the measuring procedure the $n_{\gamma'}$ direction of the object on the microscope stage is adjusted to the 45° position. Then the compensator is rotated in a counter-clockwise direction until the object is extinguished. The rotation angle is β. The path difference of the object is calculated from the equation: $\Gamma = \Gamma_{\mathrm{C}} \sin 2\beta$.

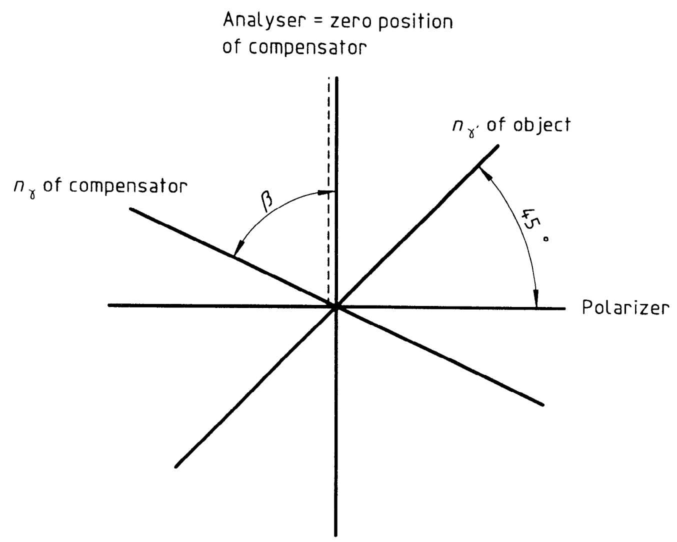


Figure 9

4.6 De Sénarmont compensation (see figure 10)

De Sénarmont compensation is achieved with a $\lambda/4$ plate adjusted to the wavelength of the monochromatic light used, which transforms the elliptical vibration emanating from the object into a linear vibration of distinct direction. The angle between this vibration direction and the transmission direction of the analyser is equal to half the phase difference which is the origin of the said elliptical polarization.

At the beginning of the measuring procedure the $n_{\gamma'}$ direction of the object on the microscope stage is adjusted at the 45° position. Then $\lambda/4$ is inserted in the standardized tube slot, its n_γ direction being parallel with the transmission direction of the polarizer. The analyser is rotated in the counterclockwise direction until the object is extinguished. The path difference Γ, in nanometers, of the object is calculated from the equation:

$$\Gamma = \Delta w \frac{\lambda}{180}$$

where

λ is the wavelength of the light, in nanometers, used for the measurement;

Δw is the rotation angle in degrees.

The range of path differences measured with de Sénarmont compensation should be restricted to $0 \leqslant \Gamma \leqslant \lambda$.

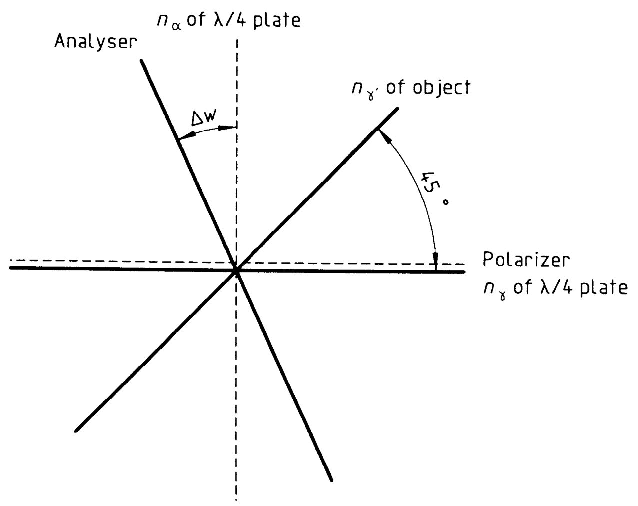


Figure 10

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