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# Microscopes — Designation of microscope objectives —

## Part 4: Polarization characteristics

*Microscopes — Désignation des objectifs de microscope —*

*Partie 4: Caractéristiques de polarisation*

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## 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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see [www.iso.org/directives](http://www.iso.org/directives)).

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This document was prepared by Technical Committee ISO/TC 172 *Optics and photonics*, Subcommittee SC 5 *Microscopes and endoscopes*.

A list of all parts in the ISO 19012 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at [www.iso.org/members.html](http://www.iso.org/members.html).

## Introduction

The polarization characteristics of microscope objectives are important parameters that allow users to choose the appropriate product for a given application. This document shows the requirement(s) for the marking of polarizing objective lenses along with the measurement procedure, environmental conditions, and microscope settings to be carried out correctly in order to arrive at the stated performance characteristics.

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# Microscopes — Designation of microscope objectives —

## Part 4: Polarization characteristics

### 1 Scope

This document specifies the use of the marking on objectives for polarization microscopes and defines measurands and measurement procedures of polarization characteristics of the objectives for qualitative polarization imaging. These measurements are defined on the image plane of the objective lens.

This marking is consistent with ISO 8578.

NOTE This document does not apply to objectives exclusively used on stereomicroscopes.

### 2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 10934, *Microscopes — Vocabulary for light microscopy*

### 3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 10934 and the following apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

#### 3.1

##### extinction ratio

ratio of transmitted intensity through parallel polars to the transmitted intensity through crossed polars

### 4 Requirements

#### 4.1 General

For quantitative polarization observation, it is necessary to use an objective lens that has little influence on the observation. The level of influence can be confirmed by measuring the value of the extinction ratio of the objective lens. The objectives named "POL", "PO" or "P" shall satisfy the requirements in 4.3.

#### 4.2 Extinction ratio

Extinction ratio shall be obtained by following [Formula \(1\)](#):

$$r_e = P_{\text{parallel}}/P_{\text{cross}} \quad (1)$$

where

- $r_e$  is the value of extinction ratio;
- $P_{\text{parallel}}$  transmitted intensity through parallel polars;
- $P_{\text{cross}}$  transmitted intensity through crossed polars.

#### 4.3 Definition of objectives named POL, PO or P

Mandatory extinction ratio shall be defined below. Measurement shall be performed at 25 % of the numerical aperture of the objective lens.

- numerical aperture < 0,5:  $r_e \geq 12,000$
- $0,5 \leq \text{numerical aperture} < 0,75$ :  $r_e \geq 10,000$
- $0,75 \leq \text{numerical aperture} < 1,0$ :  $r_e \geq 5,000$
- $1,0 \leq \text{numerical aperture}$ :  $r_e \geq 2,500$

#### 4.4 Measurement procedure

##### 4.4.1 General

In addition to defining the measurement geometry and procedure, it is necessary to describe essential settings of the microscope in order to eliminate their influence on the measurement of polarization characteristics.

##### 4.4.2 Measurement environment

The measurement shall be performed under the prescribed conditions of 23 °C, relative humidity 50 % and atmospheric pressure 86 kPa to 106 kPa. This is consistent with ISO 554.

The microscope system shall be placed in a darkroom, and careful attention shall be paid so that, for example, sun light and room light do not affect the measurands. The room and all optical components shall be kept clean enough to prevent dust from influencing the measurands.

##### 4.4.3 Microscope settings

- a) Light source and measurement wavelength.

The light source should have sufficient intensity to measure the extinction ratio.

The measurement wavelength shall be 546 nm ± 5 nm.

- b) Field diaphragm.

The field diaphragm of the illumination systems shall be limited to an approximately circular diameter of approximately 10 mm in the image plane.

- c) Aperture diaphragm.

An approximate circular aperture shall be used for the aperture stop. Measurement shall be performed at 25 % of the numerical aperture of the objective lens.

- d) Polarizer and analyser.

A polarizer and an analyser having an effective area larger than the maximum diameter of the aperture stop and also having an extinction ratio of 20,000 or more shall be used.

e) Detector.

Detectors which have sufficient sensitivity necessary for measurement shall be used. The dynamic range shall be higher than the limit of extinction ratio.

An IR cut filter should be placed in front of the detector, in case IR light from the light source affects the measurands.

f) Uniformity in the pupil plane.

The illumination uniformity of the exit pupil plane of objectives limited by the aperture diaphragm size for the measurement as described in [4.4.3 c\)](#) shall be at least 90 % without polarizer and analyser.

g) Adjustment of samples and focusing for diaphragms.

The focus, the field diaphragm, and the aperture diaphragm shall be adjusted using a glass slide with a cover glass or no cover glass, depending on the design cover glass thickness of the objective. The extinction measurement shall be performed without samples.

h) Condenser lens.

A condenser lens which has a sufficient extinction ratio shall be used. In addition, a condenser lens should be made of glass material and strain-free to be optimized for polarization observation.

i) Temporal stability.

For the measurement of extinction ratio the illumination shall be warmed-up according to the manufacturer's specifications. If the manufacturer does not specify a warm-up time, the illumination shall be warmed-up for at least 30 min prior to measurement.

#### 4.4.4 Measurement of extinction ratio

Extinction ratio shall be measured by the following method and procedure.

Microscope system shall be set as described in [4.4.3](#) and the light intensity measurement detector shall be positioned to optimally capture all the light at the image plane of the objective. It is shown as [Figure 1](#).

The condenser illumination numerical aperture (NA) shall be set to equal the prescribed measurement criteria in [4.4.3 c\)](#) of the objective to be measured, namely the measurement shall be performed at 25 % of the numerical aperture of the objective lens. When the measurement criteria have been met, two measurements shall be taken:

- a) the amount of light intensity shall be measured for the condition of parallel polars (polarizer and analyser are parallel);
- b) crossed polars (polarizer and analyser are mutually perpendicular).

Each value is taken as  $P_{\text{parallel}}$ ,  $P_{\text{cross}}$ .

In measurement a) when polarizer and analyser are parallel, the objective should be rotated until the maximum amount of light intensity is achieved.