



Microspectrophotometry

Calibration

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Calibration ensures that the instrument is functioning to an acceptable level.

Before carrying out calibration the instrument should be turned on and allowed to warm up/stabilise according to the manufacturers specifications.

The microscope should be set up for Kohler Illumination every time it is used.

Calibration continued



Calibration Standards are used for the following:

- **Wavelength Registration – to measure the accuracy of the peak positions.**
- **Photometric Accuracy – to check the intensity at each wavelength.**

Calibration continued



- **Examples of Calibration Standards**
 - **Wavelength Registration**
 - **Visible wavelength range – Holmium Oxide and Didymium glass.**
 - **UV wavelength range – Holmium Oxide.**

Calibration continued



- **Examples of Calibration Standards**

- **Photometric Accuracy**

- **Visible wavelength range – Neutral Density Filters (NDF).**
- **UV wavelength range – A suitable standard is difficult to find.**

NDFs are glass and do not transmit light in the UV region. However if the reference point is within the set tolerance for the visible range it means that the UV range is also functioning correctly.

Calibration continued



- **An annual service should be carried out by a qualified engineer.**
 - **Wavelength registration and photometric accuracy should be checked on a regular basis e.g. photometric accuracy at least once a week and wavelength registration at least on a monthly basis.**
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