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Surface chemical analysis — Surface characterization — Measurement of the lateral resolution of a confocal fluorescence microscope

Analyse chimique des surfaces — Caractérisation des surfaces — Mesurage de la résolution latérale d'un microscope confocal à fluorescence

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Contents

Page

Foreword ... **iv**
Introduction ... **v**
1 Scope ... **1**
2 Terms and definitions ... **1**
3 Symbols and abbreviated terms ... **1**
4 General ... **1**
4.1 Background information ... 1
4.2 Types of CFM operation ... 2
4.2.1 General ... 2
4.2.2 Stage scanning CFM ... 2
4.2.3 Laser scanning CFM ... 2
4.2.4 Spinning disk CFM ... 2
4.3 Parameters that affect the lateral resolution of a CFM ... 3
4.3.1 General ... 3
4.3.2 Objective lens ... 3
4.3.3 Detection pinhole size and focal length of the tube lens ... 3
4.3.4 Collimation and purity of the laser illumination beam ... 3
4.3.5 Polarization of the laser illumination ... 3
4.3.6 Excitation and emission wavelengths ... 3
4.3.7 Image contrast ... 3
5 Measuring the lateral resolution by imaging a small object ... **3**
5.1 Background information ... 3
5.2 Selection of the sample and sample requirements ... 4
5.3 Setting the parameters prior to operating the instrument ... 4
5.4 Data collection and analysis ... 5
5.4.1 Selecting a proper spot ... 5
5.4.2 Extracting a line profile using band-average process ... 5
5.5 Recording the data ... 6
Annex A (informative) **Sample preparation and example of data and analysis** ... **7**
Bibliography ... **10**

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Foreword

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The committee responsible for this document is ISO/TC 201 *Surface chemical analysis*.

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Introduction

Confocal fluorescence microscopes (CFMs) are laser scanning confocal microscopes (LSCMs) operated in a fluorescence imaging mode so as to obtain a fluorescence image of a sample. Fluorescence is the light emitted by a molecule or solid lattice during relaxation after undergoing photon absorption and electronic excitation. The fluorescence wavelength, intensity and spectral shape are specific to the electronic structure of the material; therefore, fluorescence spectroscopy and imaging techniques are useful for chemical characterization and analysis. Among the optical imaging and spectroscopy tools, CFM yields a high spatial resolution that is advantageous for analysing nanomaterials and thin films. The spatial resolution is one of the most important performance factors for a CFM.

The spatial resolution of a technique refers to the maximum resolvability of two adjacent objects. This value is often characterized in different ways by the manufacturers. The spatial resolution of a CFM is characterized by both the lateral and axial resolution, which have different values and are not necessarily dependent on one another. In this International Standard, one convenient and effective method for measuring the lateral resolution of a CFM is presented. This method is suitable for use by non-expert operators.

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Surface chemical analysis — Surface characterization — Measurement of the lateral resolution of a confocal fluorescence microscope

1 Scope

This International Standard describes a method for determining the lateral resolution of a confocal fluorescence microscope (CFM) by imaging an object with a size much smaller than the expected resolution.

2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1
point spread function
response of an imaging system to a point source or point object

2.2
lateral resolution
distance measured either in the plane of the sample surface or in a plane at right angles to the axis of the image forming optics over which changes in composition can be separately established with confidence

Note 1 to entry: See Reference[1].

3 Symbols and abbreviated terms

OL	objective lens
APD	avalanche photodiode
FWHM	full width at half-maximum
CFM	confocal fluorescence microscope
NA	numerical aperture
PSF	point spread function
QD	quantum dot
PMT	photomultiplier tube

4 General

4.1 Background information

Laser scanning confocal microscopes (LSCMs) scan a tightly focused laser beam over a sample and record the optical intensity at each pixel to form a two-dimensional image. LSCMs have a pinhole in front of the photo detector or a spectrometer input slit at the conjugate focal plane of the laser focus at the sample. Light originating from the non-focal plane is largely prevented from reaching the photo detector. Confocal configurations significantly improve the contrast in an image, and the spatial resolution may be increased[2].

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