



**International
Standard**

ISO 23124

**Surface chemical analysis —
Measurement of lateral and axial
resolutions of a Raman microscope**

*Analyse chimique des surfaces — Mesurage des résolutions
latérale et axiale d'un microscope Raman*

**First edition
2024-04**

STANDARDSISO.COM : Click to view the full PDF of ISO 23124:2024



COPYRIGHT PROTECTED DOCUMENT

© ISO 2024

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

Foreword	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 General information	1
4.1 Outline of the method	1
4.2 Lateral resolution	2
4.3 Axial resolution	2
5 Sample requirements	3
5.1 Selection of the sample and sample requirement to measure the lateral resolution	3
5.2 Selection of the sample and sample requirement to measure the axial resolution	3
6 Experimental parameters to be specified	3
6.1 Overview	3
6.2 Numerical aperture of objective lens	3
6.3 Size of confocal pinhole or the optics which works with similar function	4
6.4 Setting the parameters before the operation of the instrument	4
7 Data acquisition	5
7.1 Data collection and analysis for lateral resolution	5
7.2 Data collection and analysis for axial resolution	5
7.3 Recording of the data	5
Annex A (informative) Case study using dispersed carbon nanotubes and suspended graphene as samples	7
Bibliography	11

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).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 201, *Surface chemical analysis*.

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.

Introduction

The Raman microscope is usually built on an optical micro-spectroscopy platform and integrated with laser input, laser line filter and spectrometer output. The laser focus is scanned on the sample and the Raman-scattered photons are collected from each pixel to record the full spectrum. Raman spectral images contain a variety of spectral information, such as the intensity, peak position, or peak width of certain Raman bands.

Spatial resolution is one of the main specifications of the Raman microscope. However, the definition and the measurement procedures largely vary depending on the manufacturers of the Raman microscope, therefore the general assessment of the spatial resolution has been limited. In this document, we provide a standardized protocol that describes the measurement of the spatial resolution of a Raman microscope by performing simple measurements using specific standard specimens.

In the Raman microscope, spatial resolution includes the lateral resolution and axial resolution. For this evaluation, there are several methods, such as the straight edge method, narrow line method and grating method. This document describes only the narrow line method for evaluation of the spatial resolution for Raman measurement. A case study of the measurement is provided in [Annex A](#).

STANDARDSISO.COM : Click to view the full PDF of ISO 23124:2024

[STANDARDSISO.COM](https://standardsiso.com) : Click to view the full PDF of ISO 23124:2024

Surface chemical analysis — Measurement of lateral and axial resolutions of a Raman microscope

1 Scope

This document describes a method for measuring the spatial resolutions, lateral and axial, of the Raman microscope.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions 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 numerical aperture NA

number originally defined by Abbe for objectives and condensers, which is given by the expression $n\sin(u)$, where n is the refractive index of the medium between the lens and the object and u is half the angular aperture of the lens

[SOURCE: ISO 10934:2020, 3.1.10.4, modified — Note 1 to entry removed]

3.2 full width at half maximum FWHM

region of the axial response symmetrical to the maximum peak where the signal falls to one-half of the maximum peak signal

[SOURCE: ISO 25178-607:2019, 3.8]

3.3 signal-to-noise ratio

ratio of the signal intensity to a measure of the total noise in determining that signal

[SOURCE: ISO 18115-1:2023, 3.23, modified — Notes to entry have been removed]

4 General information

4.1 Outline of the method

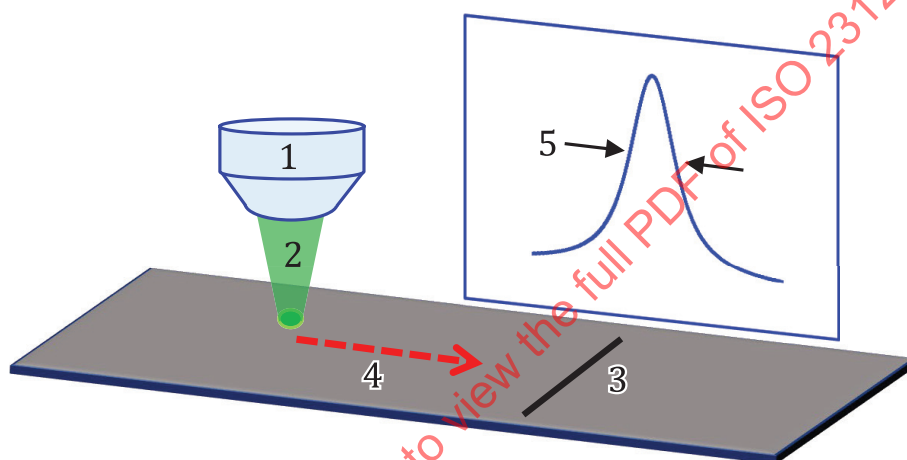
Spatial resolution is the capacity for imaging fine detail, which is determined by the minimum spatial resolution of two-point objects required for their observation as distinct objects. Various methods have

been proposed to determine the resolution, such as the straight edge, narrow line, and grating methods^[1]. This protocol describes a measurement based on the narrow line method.

In confocal microscopy, the resolution is described by the full width at half maximum (FWHM) of the confocal point spread function (PSF) using small objects^{[2][3]}. Besides FWHM of PSF, the FWHM of the line spread function (LSF) with line-shaped objects for lateral resolution and spread functions of ultra-thin or low reflective materials as well as LSF for axial resolutions are also applicable to determine spatial resolutions of confocal microscopy. In the narrow line method, these features are available to evaluate the lateral and axial resolution.

4.2 Lateral resolution

For measurement of lateral resolution, small objects, or line-shaped objects such as carbon nanotubes (CNTs) or semiconductor nanowires with widths of tens of nm that provide a strong Raman signal are recommended. The apparent size of small or diameter of line-shaped objects in a Raman map is a legitimate measure of the lateral resolution if the actual diameters of line-shaped objects are less than one-fourth of the lateral resolution^[2]. See [Figure 1](#).



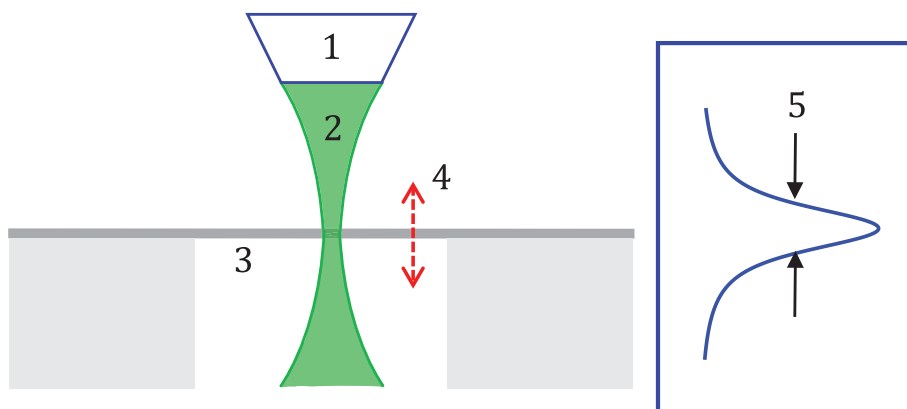
Key

- 1 objective lens
- 2 laser light
- 3 sample
- 4 scan direction
- 5 FWHM

Figure 1 — Scanning of laser focus over a line-shaped object for measurement of lateral resolution

4.3 Axial resolution

For measurement of axial resolution, small, line-shaped, (air-suspended) ultra-thin or low reflective materials are required as standard specimens. For small, line-shaped, ultra-thin or low reflective materials, the size, diameter, thickness, or penetration depth, respectively, shall be smaller than one-fourth of the axial resolution, respectively^[1]. See [Figure 2](#).

**Key**

- 1 objective lens
- 2 laser light
- 3 sample
- 4 scan direction
- 5 FWHM

Figure 2 — Scanning of laser focus through suspended ultra-thin materials for the measurement of axial resolution.

5 Sample requirements

5.1 Selection of the sample and sample requirement to measure the lateral resolution

It is known that an object size less than one-fourth of the PSF or LSF contributes to the size shown in the Raman image by less than 1 %^[2]. Considering the uncertainty of the whole measurement, this discrepancy may be regarded to be negligible. Therefore, the size of small objects or the diameter of the line-shaped objects for the measurements of lateral resolution shall be chosen to be less than one-fourth of the expected resolution value. The size or diameter of the reference sample should preferably be verified by independent traceable measurements, such as electron microscopy or atomic force microscopy. If the pixel size is larger than one fourth of the expected resolution, the evaluated resolution is no better than the one half of the pixel size.

5.2 Selection of the sample and sample requirement to measure the axial resolution

Ultra-thin sample with a thickness or sample with penetration depth less than one-fourth of the expected resolution can be chosen. The size, diameter, thickness of the sample shall be theoretically known or verified by appropriate measurement e.g., AFM, interferometry, or other methods.

6 Experimental parameters to be specified

6.1 Overview

The lateral and axial resolution in Raman spectral imaging depend on many instrumental parameters, therefore the instrument configuration shall be included in the recording of the spatial resolution.

6.2 Numerical aperture of objective lens

NA of the objective lens determines the tightness of the focus, which critically affects the spatial resolution of the Raman microscope.

6.3 Size of confocal pinhole or the optics which works with similar function

Most Raman microscopes run in a confocal scheme and the size of the confocal pinhole or the optics which work with similar function play a crucial role in the resolution and contrast of the Raman image. A smaller size of detection pinhole or the optics which work with similar function ensures strong rejection of light from outside the focal volume entering the spectrometer, resulting in high spatial resolution but is likely to cause the low level of detected light that can deteriorate the image contrast.

6.4 Setting the parameters before the operation of the instrument

For reliable lateral resolution, the preciseness of the xy-stage or laser scanning should be calibrated or checked before determination of the lateral resolution. Because the quality of the image obtained by the Raman microscope is dependent on the operator's skill and the experimental parameters, using what is thought to be exactly the same parameters can lead to different obtained resolution values if the instrument and the operator change. The extent to which a properly optimized experimental parameter set is achieved will depend on the operator. Therefore, this protocol does not impose specific values of experimental parameters in operating a Raman microscope, but the operator may choose the proper experimental parameters for optimized Raman imaging at their discretion. These include but are not limited to the spectral width of the incident collimated laser beam, polarization of the illumination, focusing of the microscope, and alignment of the optics.

The general procedure to obtain a Raman image is described below.

- a) The sample is to be placed on the microscope stage and the sample region can be confirmed by focusing white light through the objective lens of microscope. Then, a point light source such as laser light is focused onto the sample.
- b) Optimal alignment of the optical components ensures the best possible spatial resolution. General practice for alignment of objective lens (or the sample stage) and detection pinhole is to look for the maximum intensity of Raman signal from the sample or reflected laser light from the substrate while adjusting the positions of the objective lens (or the sample stage) and detection pinhole.

For lateral resolution measurement:

- The focus of objective lens and the position of detection pinhole shall be set.
- Integration time per point is to be chosen according to the Raman scattering efficiency of the sample, the laser power, and the specification of the spectrometer. A few tens or hundreds of ms/pixel are generally used. Minimum value of signal-to-noise ratio of the line profile is required. See [6.1](#) and [6.2](#).
- The laser focus is raster-scanned across the sample by scanning the sample stage or the laser focus and the Raman image is acquired; or a series of line profiles is obtained to provide the average line profile for the estimation of FWHM.

For axial resolution measurement:

- The laser focus is placed on a suspended specimen and vertically scanned by moving the sample stage or laser focus in the z-direction. Pixel step size shall be smaller than one-fourth of the expected axial resolution value.
- Integration time for each Raman spectrum and the step size are to be chosen in the same manner as measurement of lateral resolution described above.

7 Data acquisition

7.1 Data collection and analysis for lateral resolution

The FWHM of a cross-sectional profile across a selected feature on the Raman image is measured from the fitting of a single line-profile. The line-profile is taken with the Raman peak height in the Raman image.

- a) The total length of the line-profile shall be more than three times of the expected resolution value in narrow line method^[1].
- b) Signal-to-noise ratio (SNR) of the line profile shall be greater than 5. The signal is the peak of the fitting with the baseline subtraction to the line profile. To subtract a baseline from the profile, take an average of the baseline excluding the signal area (range of 1,5 times of FWHM of the fitting of the peak to each direction along the line profile). This average is subtracted as a constant baseline. Signal-to-noise ratio is the ratio of the peak signal to the standard deviation of the noise background of the line profile excluding the signal area.
- c) At least five measurements on five different points of Raman images are required to give the average and standard deviation.

7.2 Data collection and analysis for axial resolution

FWHM is measured from a line-profile through small, line-shaped, suspended ultra-thin or low reflective materials sample.

- a) The total length of the line-profile shall be more than three times the expected resolution value in the narrow line method^[1].
- b) SNR of the line profile shall be greater than 5.
- c) At least five measurements are required to give the average and standard deviation.

7.3 Recording of the data

The measured FWHM of the line profile is directly affected by the objective lens and the wavelengths of the laser excitation. Therefore, these experimental parameters shall be recorded to enable meaningful comparison between different instruments and experimenters.

For measurements of the lateral and axial resolutions, record the following.

- a) Specification of the objective lens: air, oil-immersion, water-immersion etc., and NA and magnification.
- b) The size of the detection pinhole with tube lens magnification (focal length of tube lens if provided). This information is optional because some manufacturers do not disclose this information.
- c) Excitation wavelength and detection wavelength.
- d) Spectral dispersion, spectral resolution, or grating and focal length information of spectrometer.
- e) Laser power density or laser power of the laser focused on the sample.

Only for the lateral resolution.

- f) Sample description: type of line-shaped objects, what it is made of dimension of the object such as diameter, shape (bend or not), and the length.
- g) The size of the image field of Raman image in μm .
- h) Number of pixels in the x and y directions or size of the pixel.
- i) Integration time per spectrum.

- j) The measured FWHM with the Gaussian, Lorentzian, Mixed, Voigt, etc. functions of the line profile obtained from the sample.

Only for the axial resolution,

- f) Sample description: e.g., thickness information, type of substrate
- g) Range of z-scan in μm .
- h) Number of pixels/ μm or size of pixel.
- i) Integration time per spectrum.
- j) The measured FWHM with the Gaussian, Lorentzian, Mixed, Voigt, etc. functions of the line profile obtained from z-scan.

STANDARDSISO.COM : Click to view the full PDF of ISO 23124:2024

Annex A (informative)

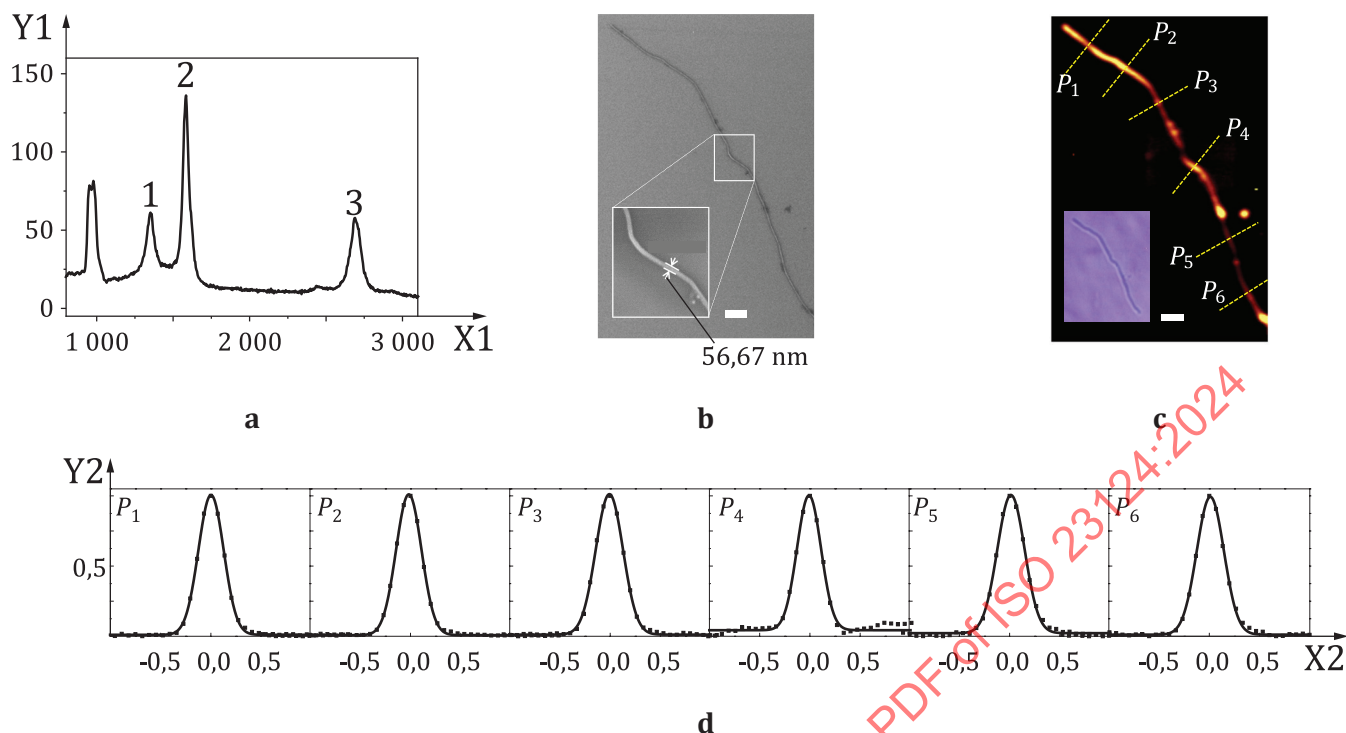
Case study using dispersed carbon nanotubes and suspended graphene as samples

A.1 General

For lateral resolution measurement, carbon nanotubes (CNTs) are dispersed on a Si substrate ([Figure A.1](#)) by spin-coating of suspended multi-wall CNTs^[4]. Specifically, a mass of 10 mg of multi-wall CNTs (Sigma Aldrich) was mixed with 10 mL of 1,2-dichloroethane (DCE) (Sigma Aldrich) in a 20 mL vial of glass and then sonicated (Wise clean, DH. WUC.A03H) for 4 h at a power of 100 W. The resultant suspension was further centrifuged for 30 min at 8 000 rpm, and the upper supernatant containing the well-dispersed CNTs was extracted. The extracted suspension of dispersed CNTs in DCE was further diluted 10 times using the same solvent and was spin-coated on a clean cover glass or silicon substrate. The substrates were cleaned beforehand using tri-chloroethylene, acetone, isopropanol, water, and ethanol, consecutively. Among the Raman modes detected from CNT, usually the G-band is observed the strongest at around at 1 590 cm⁻¹. From the spectra images, this G-band is integrated for each pixel and then a Raman image of the G-band is obtained. The line-profile obtained perpendicular to the length of the selected CNT is fitted with Voigt and FWHM of fitting function is measured for the lateral resolution. The detail experimental parameters of measurement of lateral resolution shows in [Table A.1](#).

Graphene suspended over holes on an Si substrate or grid for electron microscope could serve as sample ([Figure A.2](#)) because it has a strong Raman signal and can act as an approximation of a delta function for the convolution of the sample geometry with the system function. Suspended graphene can be commercially purchased or prepared manually. Following is an example of data analysis using obtained by axial scan through suspended graphene on a TEM grid with periodic hole of diameter 2 µm. Single layer thickness of the graphene was confirmed by the much higher Raman intensity of 2D peak than G peak. The FWHM is measured from the line profile, which represents the axial resolution of the Raman microscope. The axial scan was performed on the approximate centre of the hole and six points (holes) were measured and the average and standard deviation are provided. The detailed experimental parameters of measurement of axial resolution shows in [Table A.2](#).

A.2 Data and analysis



Key

X1	Raman shift with unit cm ⁻¹
X2	distance in μm
Y1	Raman intensity
Y2	normalized Raman intensity
1	D-band
2	G-band
3	2D-band
P_1 to P_6	line-profiles of Raman intensity of G peak

Figure A.1 — Extraction of a line profile from a Raman image of dispersed CNTs. (a) Representative Raman spectrum of CNT. (b) SEM image of CNT. (c) Raman intensity map of G peak. Six line-profiles were selected. The inset image is optical view of CNT. (d) Cross-sectional line profiles obtained from the Raman intensity map in (c).

Table A.1 — Experimental parameters of measurement of lateral resolution

Specification of the objective	Air objective. NA 0,90, 100×
Detection pinhole size	100 μm with 1× tube lens of $f=165\text{ mm}$
Laser wavelength, detection wavelength	532 nm, 581 nm (G peak)
Grating and focal length of spectrometer	600 grooves/mm, 30 cm
Spectral dispersion	5,4 nm/mm (for 600 grooves/mm)
Laser power	3 mW
Laser power density	$6,0 \times 10^5\text{ W/cm}^2$
Sample description (width or diameter of nanowires)	CNTs dispersed on Si/SiO ₂ substrate, Estimation of diameter by SEM is ~ 55 nm