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BSI Standards Publication

Surface chemical analysis — Scanning-probe microscopy — Definition and calibration of the lateral resolution of a near-field optical microscope

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National foreword

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Foreword

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ISO 27911 was prepared by Technical Committee ISO/TC 201, *Surface chemical analysis*, Subcommittee SC 9, *Scanning probe microscopy*.

Introduction

The near-field scanning optical microscope (NSOM or SNOM) is a form of scanning-probe microscope (SPM) that uses an optical source but achieves, through the use of the near field, a spatial resolution significantly superior to that defined by the Abbe diffraction limit. NSOM instruments are mainly either apertured, when the resolution is governed by the aperture size, or apertureless, when the resolution is more complex. In apertureless NSOMs, a very sharp scannable tip is used to probe the surface, or molecules on the surface, through local scattering of light from the test specimen surface or the tip apex. The spatial resolution for scattering NSOMs is a complex phenomenon and is less easily characterized in terms of an instrumental property, and so this International Standard focuses on, and is limited to, the lateral spatial resolution of apertured NSOM instruments.

Although the term spatial resolution has a clear meaning, it is often characterized in different ways. In this International Standard, one convenient and effective method for measuring the spatial resolution of an apertured NSOM instrument is presented, suitable for use by non-expert operators.

INTERNATIONAL STANDARD **ISO 27911:2011(E)**

Surface chemical analysis — Scanning-probe microscopy — Definition and calibration of the lateral resolution of a near-field optical microscope

1 Scope

This International Standard describes a method for determining the spatial (lateral) resolution of an apertured near-field scanning optical microscope (NSOM) by imaging an object with a size much smaller than the expected resolution. It is applicable to aperture-type NSOMs operated in the transmission, reflection, collection or illumination/collection mode.

2 Normative references

The following referenced documents are indispensable for the application 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 18115-2, *Surface chemical analysis — Vocabulary — Part 2: Terms used in scanning-probe microscopy*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 18115-2 and the following apply.

3.1
far field
electromagnetic field at a distance from a light source significantly greater than the wavelength of the light

3.2
point spread function
response of an imaging system to a point source or point object

4 Symbols and abbreviated terms

APD avalanche photodiode

FWHM full width at half maximum

NA numerical aperture

PMT photomultiplier tube

PSF point spread function

QD quantum dot

δ FWHM of the PSF of the NSOM, i.e. the lateral resolution of the NSOM instrument

5 General information

5.1 Background information

The NSOM is a form of scanning-probe microscope with a probe that has an optical aperture that can illuminate, and/or collect the light from, the surface of a test specimen in the distance within a fraction of the wavelength of the light, this region being called the near field. A two-dimensional NSOM image consists of pixels that contain optical information (normally, the light intensity or photon counts obtained at each pixel position). For an apertured NSOM, an open optical aperture of subwavelength diameter is located at the apex of a sharp probe, and light is emitted and/or collected by it. The NSOM probe is scanned over the specimen surface in the near field. Because the aperture is so close to the surface, the size of the spot illuminated on the surface (or from which light is collected) is determined not by the light wavelength but mostly by the aperture size. Since the aperture size can be made as small as a few tens of nanometres, spatial resolution far better than the theoretical resolution limit of the conventional far-field optical microscope can be achieved by an NSOM. The spatial resolution achievable by reducing the size of the aperture is limited by the skin depth of the metal coating of the NSOM probe, which defines the aperture, and by the fact that optical throughput decreases rapidly with decreasing aperture diameter, going beyond the limits of practical detection.

5.2 Types of NSOM operation

5.2.1 General

Below we describe different modes of NSOM operation. This International Standard is concerned with apertured NSOMs operated in the illumination, collection or illumination/collection mode. Control of the gap between the specimen and the probe is achieved by shear-force detection using optical or electrical transduction for a straight-fibre probe, and by cantilever deflection using optical transduction for a bent or cantilevered probe.

5.2.2 Classification

5.2.2.1 NSOMs can be classified on the basis of how the light is transmitted to/collected from the specimen:

a) Illumination mode: The light emanates from the aperture and is collected with a lens in the far field.

b) Collection mode: The specimen is illuminated by light from a far-field source or excited to emit light by another means and light is detected (collected) using the NSOM aperture.

c) Illumination/collection mode: The NSOM aperture is used for both illumination and collection.

5.2.2.2 NSOMs can also be classified on the basis of the position of the collection optics with respect to the illumination optics:

a) Reflection mode: Both illumination and collection are carried out on the same side of the specimen in any of the three modes defined above.

b) Transmission mode: The collection and the illumination optics are located on opposite sides of the specimen. In most cases, including the reflection mode a) above, a high-NA lens is used for high collection efficiency.

5.2.3 Control of gap between probe and specimen surface

The gap between the NSOM probe and the surface is typically controlled in one of two ways, depending on the type of probe:

a) Shear-force detection type: The NSOM probe is attached to a piezo tube or tuning fork and vibrated laterally to the surface with an amplitude of a few nanometres. Feedback is provided to keep the amplitude, phase or frequency of the vibration constant. For homogeneous surfaces, this would provide a constant gap; for most materials with a structured surface, the situation is more complicated, but often the constant-gap approximation holds.

b) Cantilever type: The NSOM probe is cantilevered so that various ways of controlling atomic-force microscope tips can be used. In particular, the deflection of a laser beam off the end of the cantilever can be used to sense the surface topography and maintain a constant gap distance.

NOTE Care is required to ensure the correct way of doing this.[1]

5.3 Methods of measuring the lateral resolution of an NSOM

The spatial resolution of an NSOM is mainly determined by the size of the aperture probe, its distance from the surface, and the contrast mechanism. In addition, the nature of the specimen, pixilation and signal-to-noise issues can affect resolution. Therefore the spatial resolution of the NSOM can be defined only for a particular instrument and a particular specimen and, accordingly, any claim of spatial resolution should specify the details of the experimental conditions[2], such as the properties of the specimen, the type of imaging mode, the height regulation mechanism, the type of NSOM probe and other factors that could affect the measurement of the spatial resolution.

Measurement of the spatial resolution of an NSOM instrument has been estimated by several methods, including measurement of the size of the smallest feature appearing in the NSOM image[3], imaging small objects in a fluorescent mode[4] to [7] and imaging a specimen having an abrupt optical-contrast edge[8].

The method chosen here is the imaging of a small object. It is based on the concept of the PSF[9], which is a critical concept that determines the spatial resolution of an optical microscope. In using this method, the following limitations of the method should be noted:

a) It is recognized that, with NSOMs, the resolution is a result of near-field interactions between a specimen and a probe. The intensity profile in the near field of an aperture, even for the simplest possible case of an aperture in an infinite plane, and in the absence of interactions with a specimen, is not a simple Gaussian one[10]. In general, the field shape varies with the aperture shape, the condition of the outer metal coating and the polarization of the input light, etc.

b) Topography-induced artefacts that appear in the optical images produced by NSOMs are sometimes mistaken for optical contrast[11]. If the optical contrast of the specimen is low compared to the background signal, which is not specific to the optical characteristics of the specimen, the contrast appearing in the NSOM optical image could originate totally or in part from topographic change in the specimen surface. To minimize the influence of topographic change on the NSOM optical image, this International Standard describes fluorescence mode NSOM, and the topographic heights of the objects to be imaged are limited to one-tenth of the expected value of the lateral resolution.

5.4 Parameters that affect the lateral resolution

5.4.1 General

The measurement of lateral resolution can depend upon a number of experimental factors, including the physical properties of the NSOM aperture, the specimen, the contrast mode, the feedback conditions, the relative positions of the source and detector, and the instrumental noise. Improperly formed images suffering from the effects of pixilation can also affect resolution, but do not present any fundamental limitations and are easily eliminated.

5.4.2 Aperture size of NSOM probe

The aperture size of the NSOM probe is of primary importance. A smaller aperture size results in a better resolution. There is a trade-off between aperture size and the signal-to-noise ratio: smaller apertures have a lower throughput, which results in a poorer signal-to-noise ratio. Apertures are produced in different ways. Coating the outside of the probe with a metal is the most popular method.

5.4.3 Condition of outer metal coating

For metal-coated probes, it is crucial that they do not have pinholes in the coating, as pinholes greatly compromise both resolution and contrast. The method of formation of the aperture in the metal coating is also important. For example, if it was by focused ion beam milling[12] or by the pounding method[13], it will produce a rather blunt-ended probe shape but a well-defined aperture, whilst the shadowing method[14] will usually result in a sharper probe end but the aperture boundary might not be so clearly defined. In general, coatings that are smooth and homogeneous also offer electromagnetic fields that are more predictable and better confined and therefore provide better imaging qualities.

5.4.4 Vertical size of the specimen

This is particularly important because topographic change in the specimen surface has been known to contribute to the optical contrast of the NSOM image. This phenomenon, caused by an effective crosstalk between the topographic-signal channel and the optical-signal channel, results in topographic artefacts in the optical image[11]. Any change in the topography signal might induce modulation in the optical signal, so that one ends up with similar features in both the optical image and the topography image, even though no corresponding optical contrast exists in the specimen. This effect is more often observed if there is a strong background signal in the optical-signal channel that can be perturbed by the modulation of the topography signal. Therefore, transmission or reflection images that have an intrinsic background optical signal throughout the whole area of the image are especially subject to this topographic artefact. The effect is also more likely when a blunt probe is used to image a surface with a rapidly varying topography. In this case, the variation (with changing tip-specimen separation) of the optical coupling between the NSOM probe and the specimen surface results in topographic artefacts.

In order to avoid the above artefacts, one should eliminate both topographic changes in the specimen and the optical background signal as far as possible. A test specimen that is topography-free but still provides optical contrast is a good way of eliminating topography changes in the specimen surface, even though the preparation of such a specimen could be technically challenging.[3] The optical background could be effectively eliminated by using fluorescence imaging where Stokes-shifted luminescence is detected while incident laser light is blocked. This way, one can isolate the true optical signal from the background signal that can be affected by the crosstalk, because the background signal and the fluorescence signal are spectrally separated.[4] However, even using fluorescence imaging, if the whole or part of the surface to be imaged has a background fluorescence signal, any optical contrast riding on this background signal could be due to the topographic artefacts. Therefore, isolated fluorescent objects of nano-size on a non-luminescent substrate would be desirable as the test specimen.[4]

5.4.5 Lateral size of the specimen

This is also important in measuring the lateral resolution of an NSOM. When imaging a small object, the recorded NSOM image is the convolution of the object and the PSF of the NSOM. Therefore, the apparent size of the object is larger than the PSF of the NSOM. The PSF of the NSOM cannot be regarded as a simple Gaussian or Lorentzian distribution, but rather as having a complicated structure that depends on the aperture shape, the condition of the outer metal coating, the input polarization, the light-coupling conditions, etc., and it is virtually impossible to de-convolve the true PSF of the NSOM from the observed profile of the small object in the NSOM image. Therefore, it is recommended that the effect of the object size be minimized by using objects as small as possible, so that one can regard the FWHM of the observed profile of the small object, to a reasonable approximation, as the FWHM of the NSOM PSF (see 6.2).

5.4.6 Polarization of illumination light

Different polarizations can produce different NSOM images of the same specimen in otherwise the same experimental conditions; therefore, one should take care in selecting the input polarization of the incident light. The polarization strongly affects the field distribution around the aperture of the NSOM probe both when the aperture of the NSOM probe is isotropic and when it is not. This will also affect the PSF of the NSOM instrument and thus the estimation of the lateral resolution. In cases where the NSOM image shows an apparent influence on the measurement of the lateral resolution due to a certain polarization status, it is suggested that the polarization of input light be scrambled.

There also exist certain polarization-sensitive specimens with anisotropic absorbance or fluorescence[16] which will give a different optical contrast in the resultant NSOM images, depending on the polarization of the input light. Therefore, these kinds of specimen should be avoided as test specimens for NSOMs.

5.4.7 Gap between the probe and the specimen surface

The gap between the NSOM probe and the specimen surface has a direct influence on the resultant NSOM image. The near-field distribution on the specimen surface is dependent upon the distance from the specimen surface and, as the distance between the probe and the surface increases, the spatial resolution and the optical intensity rapidly decrease. Therefore, the gap distance shall be regulated so that it has no influence on the NSOM image. In theory, the smallest gap would produce the best resolution; however, an optimum gap distance should be chosen for the stable operation of the NSOM.

5.4.8 Collection optics

For good contrast, it is generally best to have high-NA collection optics. Better contrast often translates into better resolution through better signal-to-noise (S/N) ratios, enabling the use of smaller apertures. A confocal pinhole in front of the photodetector could enhance the contrast by excluding background light.

5.4.9 Photodetector

The light from, or collected by, the NSOM probe is relatively weak, sometimes only a few picowatts. The use of highly sensitive photodetectors, such as PMTs or APDs, is recommended to improve the S/N ratio and help the use of smaller-apertured NSOM probes.

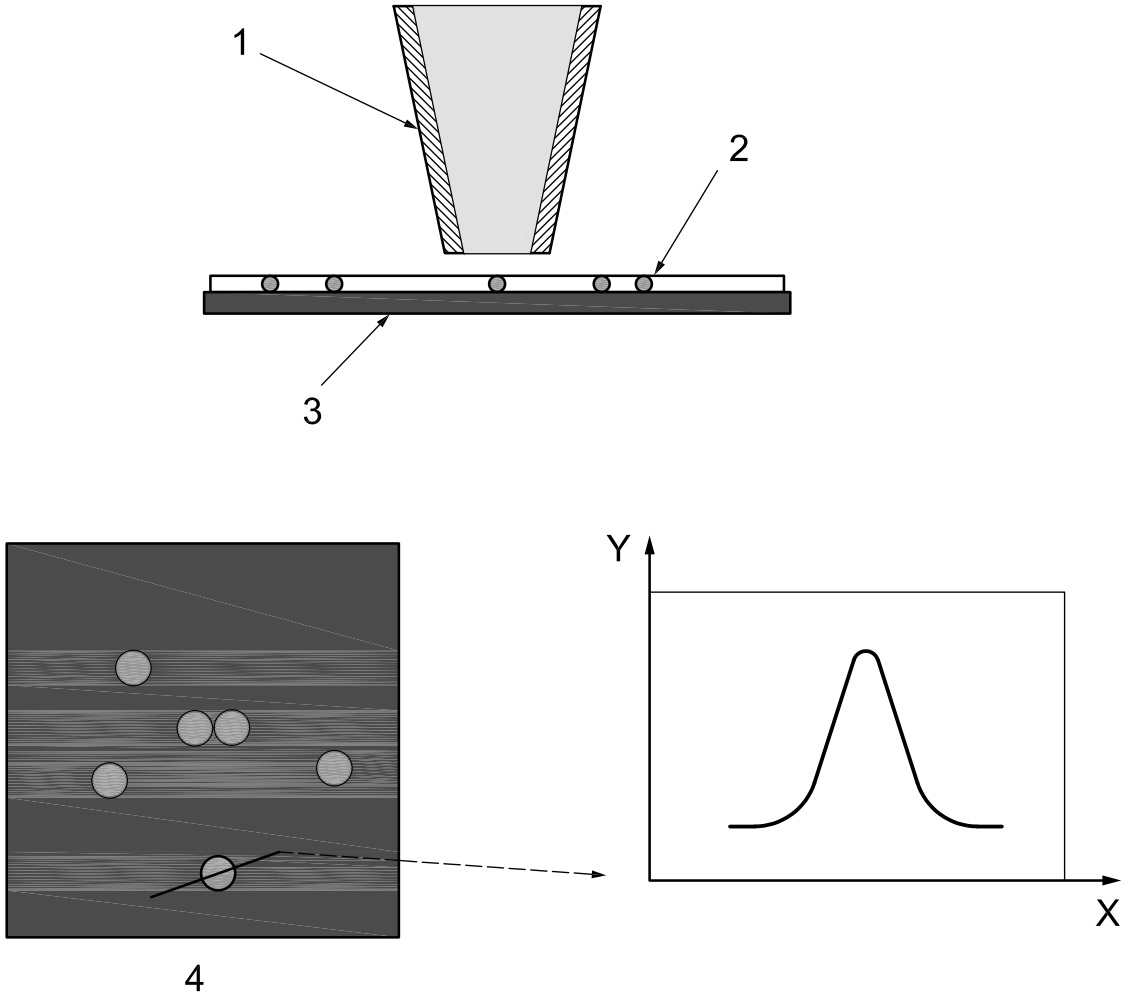
5.4.10 Contrast mode

Depending on what contrast mode is used, one might obtain a different value of the lateral resolution even using the same specimen. In this International Standard, only the fluorescence mode is described to determine the lateral resolution of an NSOM.

6 Measurement of lateral resolution by imaging a very small object

6.1 Background information

Imaging of a very small object to estimate the lateral resolution has the advantage that one can obtain a two-dimensional profile containing information on the PSF of the NSOM from a single NSOM image (see Figure 1). The lateral resolution can be defined as the FWHM of the PSF. Because the finite size of the small object contributes to the observed size of the object in the NSOM image, one should understand that the observed image of the object is the convolution of the PSF and the spatial distribution of the small object. There is a wide range of specimens that can be used in this method, and nanoparticles such as nano-scale polymer beads[4], QDs[5] and single molecules[6],[7] have been used. To minimize the possible effect of topography-induced contrast, Stokes-shifted photoluminescence imaging should be used to reduce the background optical signal, and the particle size shall be small compared to the expected resolution of the NSOM (see 6.2).



Key

X spatial distance

Y luminescence intensity

1 NSOM probe

2 nanoparticles embedded in polymer film

3 substrate

4 NSOM image

Figure 1 — Obtaining an NSOM image of nanoparticles or QDs (the FWHM of a cross-sectional profile is used to estimate the lateral resolution of the NSOM instrument)

6.2 Selection of the specimen and specimen requirements

In this method, any kind of small object that emits fluorescence may be used as the specimen. In the case of nanoparticles, dispersion on a substrate is required so that isolated single nanoparticles are found.

It is known that the effect on the resultant FWHM is as small as 1 % and 3 % of the FWHM for a Gaussian beam and a Lorentzian beam, respectively, when the size of the object is one-fourth of the lateral beam size[15]. In this International Standard, the lateral dimension of the object shall be less than one-fourth of the expected lateral resolution and, in order to minimize topographic artefacts, the topographic height of the objects to be imaged shall be less than one-tenth of the expected lateral resolution.

6.3 Setting the parameters before operation of the instrument

Because the NSOM image is very dependent on the operator's skill and the experimental parameters, a very different NSOM resolution value might be obtained, even using what are thought to be exactly the same parameters, if the instrument and the operator are different. The extent to which a properly optimized experimental parameter set is achieved will depend on the operator. Therefore, this International Standard does not impose specific values of experimental parameters in operating an NSOM instrument, but operators may choose the proper experimental parameters for optimized NSOM imaging at their discretion. These include, but are not limited to, polarization of input light, gap distance between the probe and the specimen, any electronic settings, selection of the NSOM probe, and the photodetector type. Reference to 5.4 might be helpful in taking most advantage of the NSOM instrument. Furthermore, the size of one pixel in the image shall be less than one-fifth of the expected lateral resolution, i.e. at least five pixels shall be present in the range of the lateral resolution.[15]

6.4 Data collection and analysis

An NSOM image is obtained by recording Stoke-shifted fluorescence and, in the NSOM image, one bright spot that is expected to represent a single nanoparticle is selected for measurement. The FWHM is measured from a single line-profile or an average of multiple line-profiles.

a) The bright spot shall be isolated, without the presence of overlapping adjacent spots.

b) The observed bright spot should preferably be circular in shape. If the spot is not circular, the analysis shall be carried out along the longest direction.

   In some preparations, agglomeration of the particles might occasionally occur, so that two or more particles are being analysed. Occasional larger or asymmetric spots may be ignored.

   It is important to use a single QD, or at least a very small cluster of QDs, for the measurement of the lateral resolution. For this reason, the QD used for the measurement shall be verified to be a single QD, or a sufficiently small cluster, by observing the blinking behaviour or single-step photo-bleaching.[17]

c) The total length of the line-profile shall be at least five times the expected resolution[15].

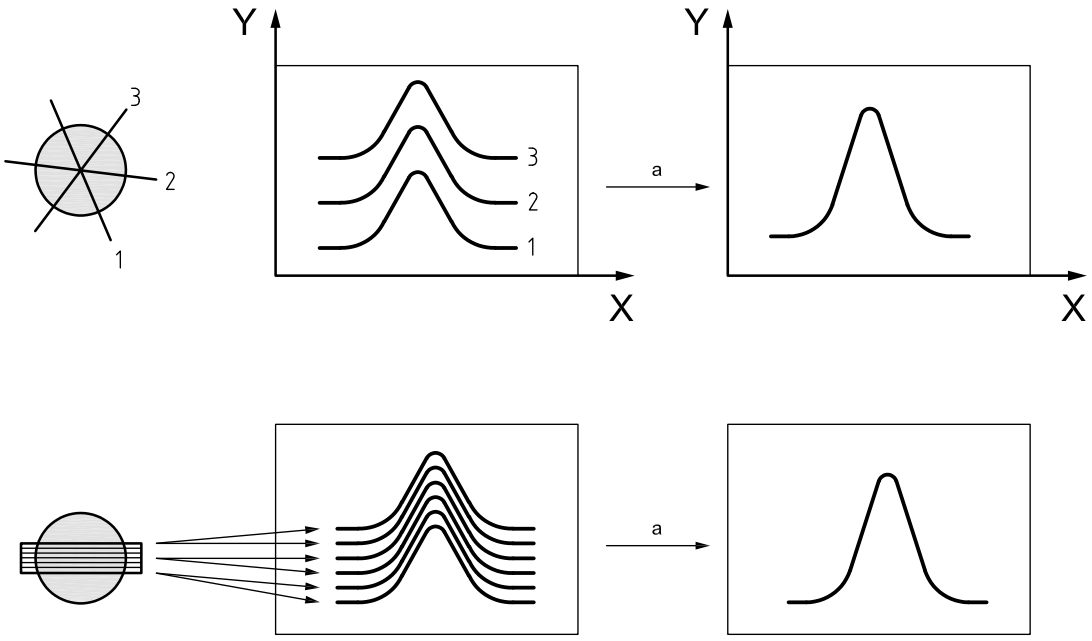
d) The S/N ratio of the line-profile used for measurement of the lateral resolution shall be higher than 5:1. The S/N ratio is the ratio between the central peak height above the baseline of the profile and the maximum peak-to-peak value of the cross-sectional profile used to estimate the lateral resolution. To increase the S/N ratio, a number of line-profiles may be averaged into one line-profile. Two possible ways are suggested to average multiple line-profiles obtained from one single dot-profile:

   1) Averaging of a number of independent line-profiles: Multiple line-profiles that go across the centre of a single spot are arbitrarily selected and averaged to give a single line-profile.

   2) Band-averaging: A rectangular region consisting of multiple adjacent lines is selected over a spot appearing as a single fluorescent object. The average line-profile is obtained from these lines constituting the rectangular region.

   Quantum dots can exhibit a blinking behaviour. This can lead to random fluctuations in intensity in line-scans along the fast-scan direction. Care should be taken to ensure that sufficient data are recorded to make certain that an adequate S/N ratio is obtained.

A schematic diagram for each method is given in Figure 2.



Key

X spatial distance

Y intensity

[a] Average.

Figure 2 — Two methods of extracting multiple line-profiles from one bright spot and averaging

6.5 Recording of data

The following data shall be recorded:

a) the type and mode of NSOM operation (transmission, reflection, collection or illumination/collection mode);

b) a description of the probe used (such as pulled or etched fibre), the thickness and material of the outer coating, the method of formation of the aperture (such as shadow evaporation, pounding or focused ion beam milling) and the expected value of the aperture size;

c) the excitation wavelength and the detection wavelength;

d) the polarization state of the input light;

e) the laser power coupled to the NSOM probe;

f) a brief description of the specimen, including the known size of the nanoparticle or QD used for imaging, its lateral diameter and its height above the surface of the background layer or substrate;

g) the size of the image field, in μm;

h) the number of pixels in the X and Y directions;

i) the fast-scanning direction;

j) the S/N ratio of the line-profile selected for measurement of the lateral resolution;

k) the FWHM of the single particle appearing in the NSOM image.

Annex A
(informative)

Examples using a line-profile and a CdSe/ZnS quantum dot as specimen

A.1 Case study 1

A.1.1 General

This case study is an example of the measurement of the lateral resolution of an NSOM using a CdSe/ZnS quantum dot as the test specimen.

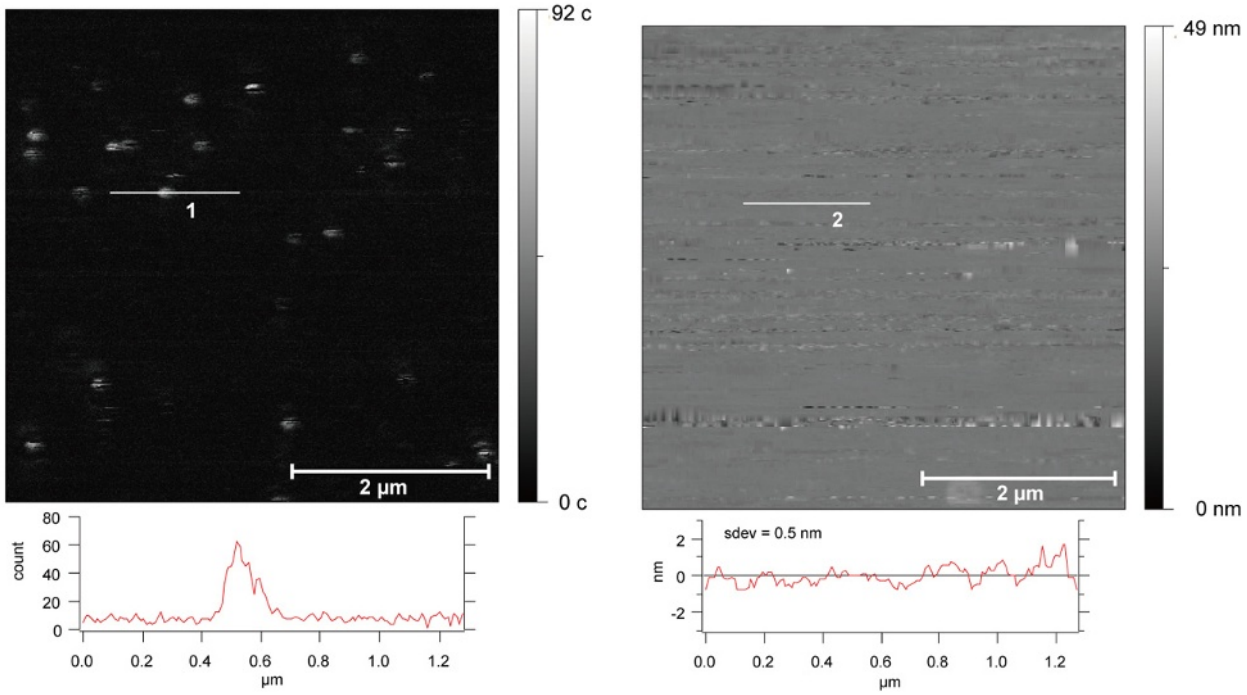
Any of the nanoparticles spread onto a flat substrate can be used to determine the point spread function because they can be regarded as a point light source. This has the advantage that the defined resolution becomes two-dimensional. Provided the size of the nanoparticle is no bigger than one-fourth of the expected resolution (see 6.2), there will be no problems of topographical artefacts. Spreading individual nanoparticles is not easy, however, and an example is given below of the specimen fabrication procedure and obtaining the results, in order to avoid difficulties.

The specimen used consisted of CdSe/ZnS QDs embedded in an ultrathin poly(vinyl alcohol) (PVAL) film. The QDs were dissolved in an aqueous solution of PVAL. Then, the solution was spin-coated onto a glass substrate. The QDs were 5 nm to 6 nm in diameter and the thickness of the PVAL film was about 10 nm. Both values were much less than the required values. The surface roughness of the glass measured by the NSOM probe was less than 1 nm, which was much less than the required value and therefore did not cause any topographic artefacts. Aperture-type, illumination, transmission mode NSOM was used. The lateral resolution was estimated in only one direction in this particular case.

NOTE All data courtesy of Prof. Hiroyuki Aoki, Kyoto University, Japan.

A.1.2 Raw data

The topography image and optical image used for measurement of the lateral resolution are shown in Figure A.1.



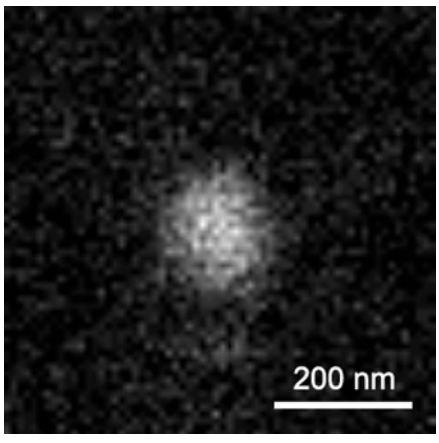
a) Fluorescence image **b) Topography image**

Figure A.1 — Fluorescence and topographic aperture NSOM images of a 5 µm by 5 µm region of CdSe/ZnS QDs embedded in ultrathin PVAL film

The fluorescence image in Figure A.1a) was recorded for 5,0 µm × 5,0 µm with 512 × 512 pixels. A 64 × 64 pixel part was cropped from the image to show a single QD for the data analysis.

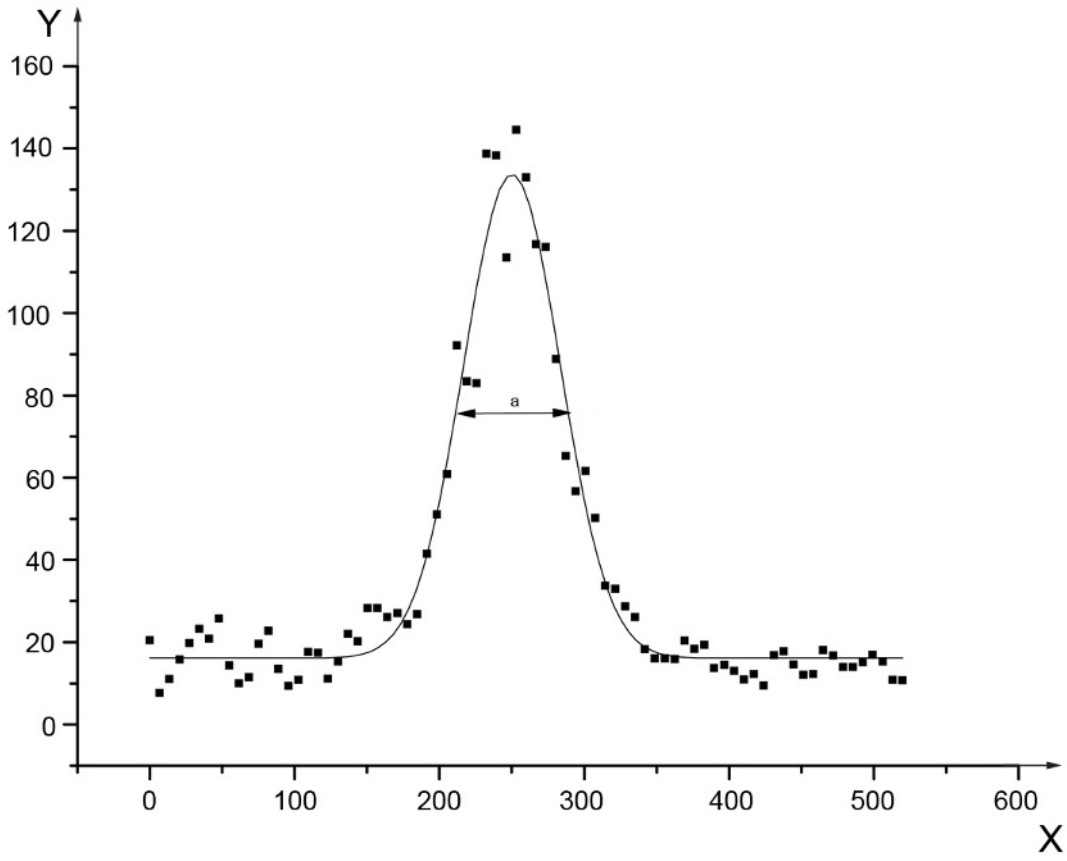
A.1.3 Data analysis

See Figure A.2.



a) Magnified fluorescence image

Figure A.2 — (*continued on next page*)



Key

X spatial position (nm)

Y photoluminescence intensity (arbitrary units)

[a] FWHM (78,6 ± 2,4) nm.

b) Linescan with Gaussian fit

Figure A.2 — A single QD from Figure A.1 and a linescan with a Gaussian fit

A.1.4 Data recorded

NSOM mode	Illumination from an aperture, transmission detection
Probe type	60 nm aperture at the apex of a pyramidal tip on a cantilever
Laser wavelength	532 nm
Input polarization	Scrambled
Laser power	5 mW coupled to the NSOM fibre probe
Specimen description	Qtracker® 565, purchased from Invitrogen,[a] was dispersed on a glass plate by spin-casting from a mixed aqueous solution of Qtracker and poly(vinyl alcohol)
Size of nanoparticle or QD	~5 nm
Image size	625 nm
Pixel number	64×64 pixels (the image was recorded for 5 µm × 5 µm with 512×512 pixel resolution and cropped to show a single dot)
Fast-scan direction	X-axis
Speed of line scanning	2 500 nm/s (0,5 Hz for 5 µm range)
S/N ratio	~8
FWHM of a single particle appearing in the NSOM image	(78,6 ± 2,4) nm

[a] This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

A.2 Case study 2

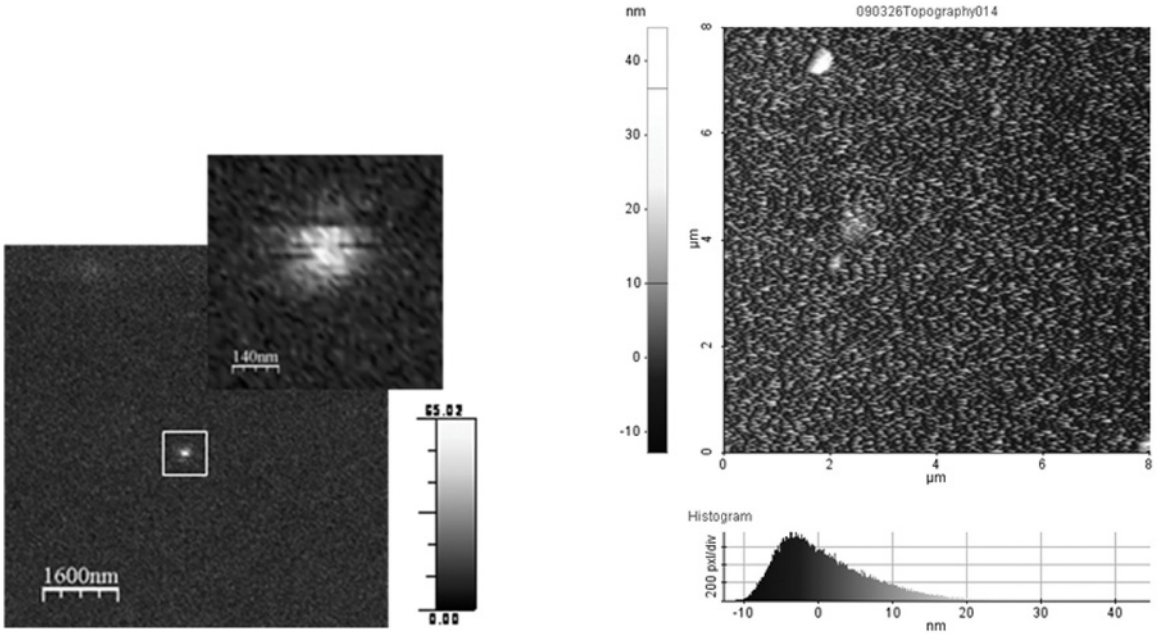
A.2.1 General

This case study is an example of the measurement of the lateral resolution of an NSOM using a band-average profile.

QDs were spread on a coverglass for NSOM imaging. To prevent the QDs moving during the scanning of the NSOM probe over the specimen surface, the QDs were prepared in a polymer solution before spin-coating. The QDs used were CdSe nanospheres with a specified mean diameter of 31 nm. However, there were biomolecule layers coated on the QDs, and the actual diameter of the CdSe QDs was less than 20 nm. The wavelength of maximum fluorescence emission was 655 nm. An aqueous solution of PVAL (0,1 molecular mass percent) was mixed with the QDs in the ratio 10 000:1. Then 20 µl of the resulting solution was spin-coated onto the coverglass at 5 000 rpm for 150 s. The thickness of the PVAL film was determined as ~30 nm by making a scratch in the film with a sharp knife and measuring the thickness with an atomic force microscope (AFM). The 514,5 nm line from an argon ion laser was used as fluorescence excitation.

A.2.2 Raw data

An (8×8) µm NSOM fluorescence image and a simultaneously obtained topography image are shown in Figure A.3. The topography image shows a flat surface with an average roughness of 4,9 nm. In view of the diameters of the QDs, it is believed that most of them were embedded in the transparent PVAL film. A square area measuring (2×2) µm that included a QD at the centre was re-imaged and part of this area, measuring $(0{,}7 \times 0{,}7)$ µm (45×45 pixels), was cropped and is shown in the inset.

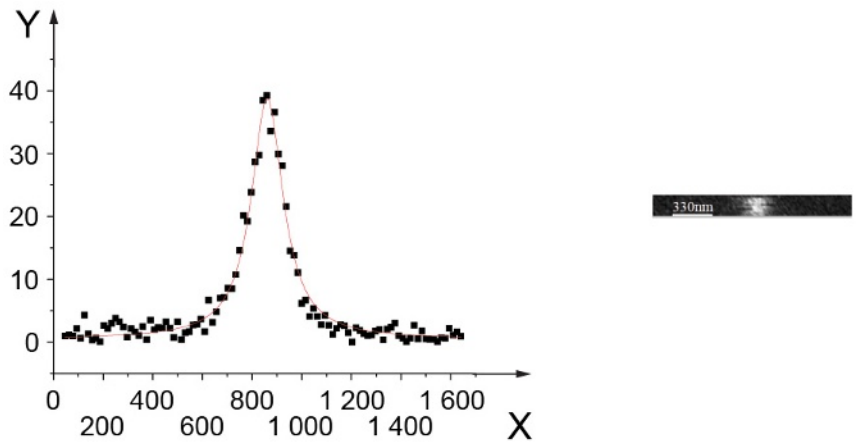


a) Fluorescence image **b) Topography image**

Figure A.3 — Fluorescence and topographic aperture NSOM images of an 8 µm by 8 µm region of CdSe nanospheres embedded in ultrathin PVAL film [also shown are a (0,7 × 0,7) µm area of the fluorescence image (see inset) and the height spectrum of the topography image]

A.2.3 Data analysis

From the recorded (2 × 2) µm NSOM image, a band consisting of 12 line-profiles crossing the QD in the X-direction was selected and averaged to give an average profile, as shown in Figure A.4[21]. The solid line represents the Lorentzian fit. The FWHM of the line-profile was estimated to be (155 ± 5) nm.



Key

X spatial position (nm)

Y photoluminescence intensity (arbitrary units)

Figure A.4 — Line-profile for a single QD (inset shows the band chosen for the band-average of 12 line-profiles)

A.2.4 Data recorded

NSOM mode	Illumination mode and fluorescence mode
Probe type	Etched optical fibre coated with Al
Laser wavelength and detection wavelengths	514,5 nm for excitation; longer than 637 nm detected
Input polarization	Scrambled
Laser power	330 μW coupled into the fibre
Specimen description	QDs embedded in ~30 nm thick PVAL film (thickness of PVAL film measured with an AFM after making a scratch in the film with a knife)
Size of nanoparticle or QD	Less than 20 nm, specified by the manufacturer
Image size	(2×2) μm
Number of pixels per line	128×128
Fast-scan direction	X-axis
Speed of line scanning	0,8 Hz line scan
S/N ratio	Better than 5:1
FWHM of a single particle appearing in the NSOM image	(155 ± 5) nm

Annex B
(informative)

Example of a procedure for preparing standard NSOM specimens

NOTE The information concerning specific products in this annex is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of any of the products.

B.1 Apparatus and reagents

B.1.1 Quantum dots

Qdot® 655 ITK™ Amino (PEG) Quantum Dots, made by Invitrogen Corporation (Catalogue No.Q21521MP)

Concentration: 8 μM

Unit size: 250 μl

B.1.2 PVAL

Poly(vinyl alcohol) for absorbance analysis, made by Wako Pure Chemical Industries, Ltd.

Unit size: 500 g (powder)

Saponification value: 78~82 mol %

Degree of polymerization: 2 000

B.1.3 Cover glass

Area: (18 × 18) mm

Thickness: about 0,12 mm to 0,17 mm

Material: borosilicate glass

B.1.4 Glass micro-tube

Internal diameter: 6 mm

B.1.5 Micropipette

B.2 Procedure

Carry out the following steps (see also Figure B.1):

1) Weigh 0,02 g of PVAL into a glass micro-tube.

2) Add sufficient distilled water to give 1 ml of solution.

3) Using a micropipette, introduce 1 µl of a colloidal suspension of Qdot® 655[1)] into the micro-tube. Disperse the quantum dots in the solution.

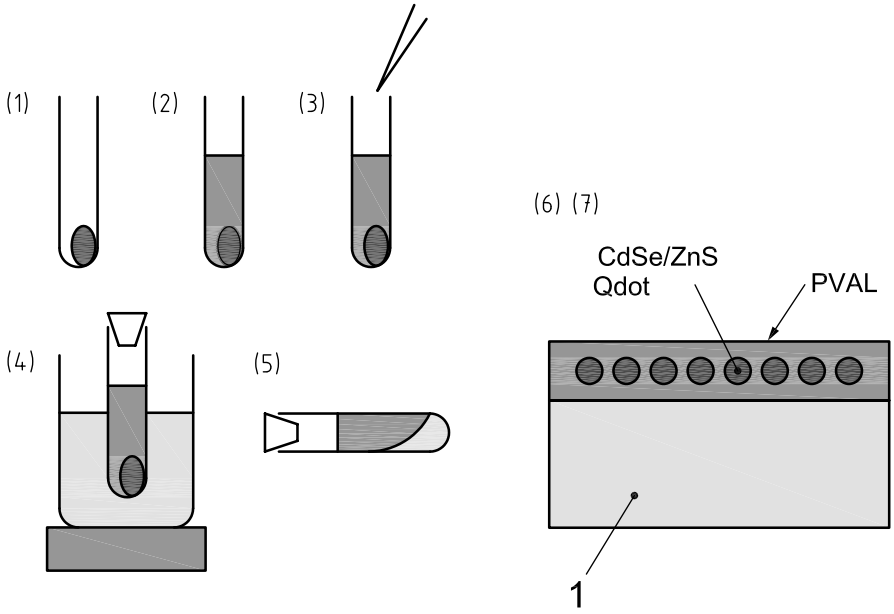
4) Heat the micro-tube in a water bath (~80 °C) to dissolve the PVAL. Allow the micro-tube to cool and stopper it.

5) Leave the micro-tube on its side overnight.

6) Prepare a cover glass by cleaning it thoroughly. An example of a suitable cleaning method is the use of Piranha solution[18] or 1-molar KOH solution[19] in an ultrasonic bath, followed by rinsing with water. Plasma cleaning has also been found effective[20].

7) Spread the PVAL solution on the clean cover glass using a spin-coater (1st step: 500 rpm for 60 s, 2nd step: 5 000 rpm for 180 s).

8) Dry at 150 °C for 5 min.



Key

1 cover glass

Figure B.1 — Steps in procedure

1) This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

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