TECHNICAL
SPECIFICATION

ISO/TS
10797

First edition
2012-06-01

Nanotechnologies — Characterization of single-wall carbon nanotubes using transmission electron microscopy

Nanotechnologies — Caractérisation des nanotubes de carbone monofeuillet par microscopie électronique à transmission

Reference number
ISO/TS 10797:2012(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

Published in Switzerland

Contents

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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of document:

- an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote;
- an ISO Technical Specification (ISO/TS) represents an agreement between the members of a technical committee and is accepted for publication if it is approved by 2/3 of the members of the committee casting a vote.

An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO/TS 10797 was prepared by Technical Committee ISO/TC 229, *Nanotechnologies*.

Introduction

Carbon nanotubes (CNTs) are nanomaterials composed of concentric layers of graphene sheets in the form of cylindrical tubes placed along the longitudinal fibre axis. Single-wall carbon nanotubes (SWCNTs) are seamless cylinders derived from the honeycomb lattice representing just a single atomic layer of graphene sheet. The transmission electron microscope (TEM), and especially its high-resolution version (HRTEM), were the first instruments that revealed the unique structural features of carbon nanotubes. TEM/HRTEM has played an essential role in the research and development of carbon nanotube materials. It has the advantage of being a “direct” technique that avoids the imposition of physical or mathematical assumptions. At the same time, it provides a variety of experimental results and information-rich images that make the investigation of a wide variety of samples possible. Beyond imaging, TEM, along with other techniques described in this Technical Specification, can provide qualitative purity assessment of SWCNT samples. In addition, it can also reveal detailed morphological and structural features of carbon nanotubes such as graphene wall structure, defects, diameter, length, bundle size and orientation, and the existence of materials and nanoparticles[8] besides SWCNTs. In other operational modes, it is also possible to study the chirality and thermal and mechanical characteristics of individual nanotubes. It is important to develop a systematic protocol for using TEM in order to acquire reliable and comprehensive information about a sample containing SWCNTs.

The transmission electron microscope operates on similar basic principles as the optical microscope but uses electrons instead of light. A beam of electrons is focused onto a thin, electron-transparent sample, allowing an enlarged version to appear on a fluorescent screen, a layer of photographic film, or on an array detector that is sensitive to electrons. Modern instruments are equipped with a computer-linked digital imaging system that can also record real-time images.

The HRTEM can investigate crystal structure by phase contrast imaging, where images are formed due to differences in the phase of electron waves scattered through a thin sample. Resolution of the TEM is limited by spherical and chromatic aberrations, but new generations of instruments with advanced electron-optical columns have significantly lowered these aberrations. Software correction of spherical aberration has allowed the production of meaningful images with sufficient resolution at magnifications of many millions times. The ability to determine the positions of atoms within materials has made the HRTEM an indispensable tool for nanotechnology research and development.

Nanotechnologies — Characterization of single-wall carbon nanotubes using transmission electron microscopy

1 Scope

This Technical Specification establishes methods for characterizing the morphology of single-wall carbon nanotubes (SWCNTs) and identifying the elemental composition of other materials in SWCNT samples, using transmission electron microscopy and chemical analysis by energy dispersive X-ray spectrometry.

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 22493, *Microbeam analysis — Scanning electron microscopy — Vocabulary*

ISO 29301, *Microbeam analysis — Analytical transmission electron microscopy — Methods for calibrating image magnification by using reference materials having periodic structures*

ISO/TS 80004-3, *Nanotechnologies — Vocabulary — Part 3: Carbon nano-objects*

ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 22493, ISO/TS 80004-3 and the following apply.

3.1
aggregate of nanotubes
particle comprising strongly bonded or fused particles of individual nanotubes and/or bundles of nanotubes

NOTE 1 This is often the form of as-produced SWCNT material. The forces holding an aggregate together are strong forces, for example, covalent bonds, or those resulting from sintering or complex physical entanglement.

NOTE 2 Aggregates are termed “secondary particles” and the original source particles are termed “primary particles”.

NOTE 3 Adapted from ISO/TS 27687:2008, definition 3.3.

3.2
bundle of nanotubes
single strand of two or more nanotubes held together by van der Waals forces

3.3
bright-field TEM
TEM technique of electron illumination and imaging in which the direct electron beam passes through the sample and the image is formed only by the transmitted wave, by selecting the wave using an objective aperture on the back focal plane

NOTE 1 Generally, the portions of the sample that are thicker or that have a higher atomic number (Z) appear darker against a brighter background. In this mode the contrast, when considered classically, is formed directly by occlusion and absorption of electrons in the sample. Thicker regions of the sample, or regions with a higher atomic number will appear dark, while regions with no sample in the beam path will appear bright, hence the term “bright-field”.

NOTE 2 This will be included in a vocabulary on analytical electron microscopy, which is under preparation by ISO/TC 202/SC 1.

3.4
dark-field TEM

TEM technique of electron illumination and imaging in which the direct electron beam passes through the sample and the image is formed only by diffracted wave, by selecting the wave using an objective aperture on the back focal plane

NOTE 1 Crystalline parts of the sample disperse the electrons of the direct beam into discrete locations in the back focal plane. By the placement of apertures in the back focal plane, i.e. the objective aperture, desired portions of the reflections can be selected, thus only those parts of the sample that are causing the electrons to scatter to the selected reflections will be imaged. If the selected reflections do not include the unscattered beam, then the image will appear dark wherever no sample scattering to the selected peak is present – hence the term “dark-field”.

NOTE 2 Modern TEMs are often equipped with sample holders that allow the user to tilt the sample to obtain specific diffraction conditions. The wave that caused scattering and reflection (for example, Bragg reflection) in a crystalline sample will form a dark-field image by selecting a specific diffraction wave through objective apertures placed on the back focal plane of objective lens.

NOTE 3 High-angle annular dark-field imaging (HAADF) is highly sensitive to variations in the atomic number of atoms in the sample and produces so-called Z-contrast images, which yield useful information on the presence of metals on nanotubes and catalyst residues, even when these small metal particles are imbedded within amorphous carbon or catalyst support while otherwise invisible in bright-field imaging mode.

NOTE 4 This will be included in a vocabulary on analytical electron microscopy, which is under preparation by ISO/TC 202/SC 1.

3.5
electron energy loss spectrum
EELS

energy spectrum of electrons from a nominally mono-energetic source emitted after inelastic interactions with the sample, often exhibiting peaks due to specific inelastic loss processes

NOTE The electron energy loss spectrum, measured with an incident electron beam, is a function of the beam energy, the angle of incidence of the beam, the angle of emission, and the electronic properties of the sample (see Reference [3]).

3.6
energy-dispersive X-ray spectrometer
EDS

device for determining X-ray intensity as a function of the energy of the radiation

[ISO 23833:2006, definition 3.6.4]

3.7
purity of SWCNT sample

indication of the amount of material other than SWCNTs in SWCNT samples

NOTE High purity refers to a low amount of (metal) catalyst residues and other usual by-products, such as multiwall carbon nanotubes, carbon nanofibres, fullerenes, amorphous carbon and graphite onions, which can also be assessed by TEM.

3.8
transmission electron microscope
TEM

instrument that produces magnified images or diffraction patterns of the sample by an electron beam which passes through the sample and interacts with it

NOTE Adapted from ISO 29301:2010, definition 3.37.

3.9
selected area electron diffraction
SAED
technique in electron microscopy in which the crystal structure of a sample area selected by an aperture is examined

NOTE Adapted from ISO 13794:1999, definition 2.38.

3.10
scanning transmission electron microscope
STEM
instrument that produces magnified images or diffraction patterns of the sample by an electron beam, which is focused into a narrow spot, scanned over the sample in a raster and which passes through the sample and interacts with it

4 General principles

4.1 TEM imaging and analysis

TEMs are valuable in very high-resolution imaging and in the analysis of the characteristics of carbon nanotubes, other forms of carbon, and other impurities in SWCNT samples. The diameters of SWCNTs generally range from less than 1 nm to more than 5 nm. Their lengths can vary considerably and can be greater than 10 mm. Due to the attractive forces between the tubes, SWCNT samples are entangled, i.e. large numbers of individual nanotubes form so-called bundles or ropes. The diameters and the lengths of the bundles are considerably larger than those of individual tubes. TEM can be used for fine structure measurements such as tube diameter, number of walls, chirality and defects, orientation, packing morphology and detailed structure of bundles. Length measurements in the TEM are limited.

4.2 EDS analysis

Another strength of the TEM is that, in combination with EDS analysis, it can be used to determine the elemental composition of non-carbonaceous constituents of CNT samples down to the nanometre-scale. All modern TEM/EDS systems can detect carbon and other impurities with good sensitivity and are capable of identifying various constituents and performing semi-quantitative analyses that are indispensable in SWCNT sample comparisons. The characteristic beam-sample interaction volume in TEM samples is smaller and the peak-to-background ratio of the TEM/EDS is significantly better than that of the typical samples of a scanning electron microscope (SEM)/EDS case. These allow for better sample differentiation, and comprehensive semi-quantitative point, line and area elemental analysis.

4.3 Additional characterization methods

Beyond common TEM imaging and TEM/EDS analysis, there are other TEM-based imaging and analytical techniques that can help in finding and characterizing various constituents of SWCNT samples. These can provide information on elemental composition, crystal structure, chemical bonding and doping, electronic state, and the various materials within the carbon nanotubes, etc. Among them are selected area electron diffraction and electron energy loss spectroscopy. It is expected that the use of these techniques will become more widespread as the number of applications of carbon nanotubes increases in the future.

4.4 Applicability to multiwall carbon nanotube analysis

The methods described in this Technical Specification for SWCNTs may also be applied to the analysis of multiwall carbon nanotubes (MWCNTs). MWCNTs consist of two or more concentric tubes of graphite. The interlayer distance in MWCNTs is close to the distance between graphene layers in graphite, approximately 0,34 nm. MWCNTs, depending on the number of graphene layers in their walls, have considerably larger outer diameters than SWCNTs. Double-wall carbon nanotubes are especially important because their morphology and properties are similar to SWCNTs but their resistance to chemicals is significantly better, which is important when functionalization is required (this means grafting of chemical functions at the surface of the nanotubes) to add new properties to the CNT. The sample preparation and dispersion methods are similar to those used for SWCNTs and all methods described here for the imaging, characterization and analysis of SWCNTs also apply to MWCNTs.

NOTE Additional information on MWCNT characterization can be found in ISO/TR 10929.

5 Sample preparation

5.1 General principles

Carbon nanotubes might be hazardous materials, therefore it is very important to observe relevant safety procedures related to the handling, preparation, use and disposal of SWCNT materials and samples. It is recommended that only trained scientific personnel handle carbon nanotube materials. Personal protective equipment should be used, including disposable gloves, safety glasses, laboratory coats, filter respirators, etc. Sample preparations should be carried out in a vented fume hood or glove box equipped with suitable air filters to avoid inhalation of SWCNT material (see References [2] and [10]).

Appropriate sample preparation is essential for reliable and reproducible characterization and measurement of SWCNT samples and establishes a uniform basis for comparative measurements. Fabrication, treatment and sample preparation methods might significantly affect the physical properties of SWCNTs. Therefore preparation methods that produce minimal alteration of the sample material should be used wherever possible.

TEM measurements are limited to thin samples. The shallow depth of penetration of electrons into solids forces the use of very thin samples, especially in high-resolution operating mode and with low primary electron energies. Depending upon the nature of the material and the desired resolution, the optimal sample thickness varies from 10 nm to 150 nm.

Commercially available SWCNT samples are usually in the form of dry powder or liquid suspensions. SWCNTs present specific challenges in the preparation of TEM samples. The nanotubes usually form bundles, which are difficult to disperse into individual nanotubes. Ultrasonic treatment can separate them, but can also damage their structure, even shortening the tube length, and alter the macroscopic morphology. Thus, it is generally recommended that mechanical treatment be minimized or not used in the preparation of thin carbon nanotube samples. Techniques such as ion milling, electro-polishing, grinding, replication/extraction and preferential chemical etching to create thin, electron-transparent films, are generally not recommended for preparing SWCNT samples.

At least three samples shall be prepared from the SWCNT material. The number of samples to prepare depends on the quality of the SWCNT material and the success of the sample preparation, which can be established with the examination of the three samples. If those show dissimilarity, more samples shall be prepared and measured. The key is to ensure relevant and reliable results and to avoid insufficient or excessive sampling.

The sample preparation procedures, including ultrasonic treatment conditions together with time (or visual end-point), as well as any pre-treatment or pre-washing of SWCNT samples, shall be reported in detail.

NOTE Various methods of preparing carbon nanotube samples for TEM analysis have been documented in pertinent literature[11][12][13].

5.2 Choice of TEM grid

The choice of TEM grid is important in sample preparation, imaging and measurements, as the type and suitability of the grid depend on the SWCNT sample and the intended use. A regular, 3,05 mm diameter copper grid with a 200 mesh size is recommended in general and for the purpose of purity analysis of carbon nanotubes. This type of grid has many 97 μm × 97 μm openings that, with successful sample preparation, allow for a large number of measurements. Other types of grids can be also suitable but their elemental composition should not interfere with the elemental analysis of the impurities of SWCNT material.

If special treatment such as heating is required, then molybdenum grids, or grids with silicon carbide membrane windows, are suitable, as these can work at high temperatures.

NOTE 1 An index grid provides index marks along each grid line, thus allowing for observations of easily identifiable locations and quantitative measurements or multiple examinations of the same features, even by different operators.

NOTE 2 Some materials can be self-supportive on a TEM grid without additional support film. The complex macroscopic morphology of carbon nanotubes usually requires a thin section in order to achieve satisfactory contrast and resolution, and makes self-support on a bare grid rather difficult without special processing. Both carbon and holey or lacey carbon films are suitable for supporting carbon nanotubes (thinner films are preferred). See B.1.1 for additional information.

5.3 Powder and film samples

5.3.1 Dry powder sample

In this case, dry SWCNTs are used directly for sample preparation.

a) Place a TEM grid into an appropriate holder.

b) Move the grid inside the fume hood or glove box, where the sample preparation will take place.

c) Select the SWCNT sample material and transfer it to the fume hood or glove box, and open its container.

d) Attach, by lightly pressing, a very small amount (approximately 0,01 mg) of SWCNT material with a sharp needle or a pair of narrow tweezers to the top surface of the TEM grid.

e) Remove loose sample material by gently knocking the holder against a hard surface. A clean, gentle nitrogen jet, briefly applied, may also be used.

f) Alternatively, place a droplet of organic solvent such as 2-propanol (isopropyl alcohol) or 2-butanol on top of a dry sample-loaded grid and allow it to dry. This can help “pull” the dry SWCNTs down to the grid surface as the solvent evaporates.

g) Transfer the grid sample directly to the TEM or into a storage box for later work.

NOTE 1 This method has the advantage of maintaining the original macroscopic morphology, but it also sometimes creates problems by leaving the sample too thick to allow transmission of electrons.

NOTE 2 Due to its poor adhesion, a loosely applied, excessive amount of SWCNT material can easily move away from the grid surface during pump down. This loose material might adhere to the strongly magnetic parts of the instrument, which can result in detrimental effects and should thus be avoided.

5.3.2 Dry film sample

In this case, dry SWCNTs are first suspended in a solvent and turned into a thin film, or a mat, which is then deposited onto a TEM grid. Use of a dry film can improve the adhesion of the SWCNTs to the TEM grid because many of the nanotubes are “locked” in place.

a) Place a TEM grid into an appropriate holder.

b) Move the grid inside the fume hood or glove box, where the sample preparation will take place.

c) Select the SWCNT sample material and transfer it to the fume hood or glove box, and open its container.

d) With a sharp needle, or pair of narrow tweezers, insert a very small amount (approximately 0,01 mg) of SWCNT material into a 20 ml vial and add 10 ml clean water or 2-propanol.

e) Once the SWCNT material is soaked completely, filter down a droplet onto a 0,2 µm to 0,5 µm pore size, hydrophilic polypropylene or polycarbonate membrane to form a mat.

f) Peel off a small portion of the dry mat and press it onto a clean TEM grid (without a film).

g) Transfer the grid sample directly to the TEM or into a clean, particle-free storage box for later work.

NOTE 1 It is unavoidable that many sections of the mat prepared by this method will be too thick for TEM work or contain too many SWCNTs.

NOTE 2 As the suspended SWCNTs dry, differences in the spatial distribution of carbon nanotubes and impurities develop, i.e. the original distribution of the various constituents of the SWCNT material becomes altered. Additionally, an inappropriate solvent can remove some forms of impurities, resulting in an inaccurate account for the purity of the material.

5.4 Liquid suspension sample

In this case, SWCNTs in liquid suspension or small aggregates of dry SWCNTs that were first suspended in a solvent are used for sample preparation. The SWCNTs are separated by ultrasonic treatment at room temperature before being deposited onto the TEM grid.

a) Place a TEM grid into an appropriate holder.

b) Move the grid inside the fume hood or glove box, where the sample preparation will take place.

c) Select the SWCNT sample material and transfer it to the fume hood or glove box, and open its container.

d) With a sharp needle, or a pair of narrow tweezers, insert a very small amount (approximately 0,01 mg) of SWCNT material into a 20 ml vial and add 10 ml 2-propanol (or chloroform, taking appropriate safety measures).

e) Transfer the vial to an ultrasonic bath and apply ultrasonic treatment to reach adequate separation of SWCNTs. With small-size laboratory ultrasonic apparatus, 5 min to 30 min treatment is usually sufficient.

f) Deposit a droplet of the liquid containing the SWCNT material onto a bare TEM grid.

g) Allow the sample to dry completely in air.

h) Remove loose sample material by gently knocking the holder against a hard surface. A clean, gentle nitrogen jet, briefly applied, may also be used.

i) Transfer the grid sample directly to the TEM or into a clean, particle-free storage facility for later imaging.

NOTE 1 The ultrasonic treatment should last for as short a time as possible. Ultrasonic treatment that is too long or too high energy will significantly alter the properties of the sample. The right ultrasonic treatment conditions depend on the sample and on the type of ultrasonic equipment and can be found by experimentation, starting with short treatment times and energies. Ultrasonic treatment in an ice water bath minimizes possible thermal damage to the SWCNTs.

NOTE 2 As the droplet of the suspended SWCNT sample dries, differences in the spatial distribution of carbon nanotubes and impurities develop, i.e. the original distribution of the various constituents of the SWCNT material becomes altered. Dispersion by ultrasonic treatment also has a similar effect. Additionally, an inappropriate solvent can remove some forms of impurities, resulting in an inaccurate account for the purity of the material.

5.5 Composite sample

In this case, a TEM sample of SWCNTs is prepared by embedding the nanotubes in epoxy or plastic matrix and then cutting thin slices of material with an ultra-microtome. This sample preparation method damages and removes some of the SWCNTs, but certain important SWCNT characteristics can still be investigated.

a) Place a TEM grid into an appropriate holder.

b) Move the grid inside the fume hood or glove box, where the sample preparation will take place.

c) Select the SWCNT sample material and transfer it to the fume hood or glove box, and open its container.

d) With a pair of narrow tweezers, add 0,1 g of SWCNT material to 10 g of epoxy with a premixed curing agent and mix them thoroughly.

e) Let the composite cure at room temperature for as long as is needed for complete curing.

f) Shape the cured block of nanotube-epoxy into a 3 mm rod and secure it in the ultra-microtome.

g) Cut 20 nm to 90 nm thin slices of the nanotube-epoxy sample (the thickness can be judged by the light transparency of the slice). Collect the slices from the water bath.

h) Deposit a nanotube-epoxy slice directly onto a bare TEM grid by applying light pressure.

i) Transfer the grid sample directly to the TEM or into a clean, particle-free storage facility for later imaging.

NOTE The significance of this method is that it is relevant to an important, practical use of carbon nanotubes, which is the production of composite materials with desired properties. Evaluation of various properties of embedded CNTs is more limited than that of freestanding CNTs. This method can also be applied to the investigation of nanotube-polymer composite materials. See B.1.2 for additional information.

6 Measurement procedures

6.1 TEM examination of an SWCNT sample

6.1.1 Large field-of-view (more than 1 μm) examination

After setting the correct instrument parameters and attaining the optimal beam conditions for high resolution, it is important to start the SWCNT sample investigation at low magnification, i.e. at large field-of-view. The largest field-of-view (minimum magnification) depends on the TEM's capabilities and the sample.

A quick survey of the sample can determine whether the quality of sample preparation is acceptable and whether the minimum of three samples provides sufficient results. Where it is advantageous, use additional dark-field imaging to expose the presence of catalyst particles or other impurities in the SWCNT material.

Large and small impurities, such as catalyst and metal particles, large carbon fibres, MWCNTs and other carbon material are visible on various field-of-view images; therefore the best field-of-view and pixel resolution, based on the characteristics of the SWCNT sample, and the TEM, can be determined. The key is to acquire images at as high a resolution as possible at the field-of-view that provides relevant information.

Table 1 summarizes the recommended settings for large and small fields-of-view and EDS analyses.

6.1.2 Small field-of-view (less than 1 μm) examination

The finest details of SWCNT samples, including individual SWCNTs and very small, nanometre-scale impurities, elemental composition, wall structure, defects, lattice information, etc., can be examined and measured only in small field-of-view images. The very small field-of-view range is where TEM instruments provide information that is not available with other instrumentation. At smaller than 100 nm field-of-view images, fine details of SWCNTs can be revealed. Where it is advantageous, use additional dark-field imaging to expose the presence of catalyst particles or other impurities in the SWCNT material.

The TEM image scale (magnification) in both x and y directions shall be calibrated using the procedures set out in ISO 29301 and a certified reference material (CRM), if available. CRMs may have accompanying instructions to assist proper calibration. Alternatively, reference samples with known parameters (e.g. crystal lattice parameters) can be used. It is important to recognize that the magnification, and hence the scale calibration, will change depending upon the placement of the sample within the microscope column. Therefore, reproducible sample positioning is essential to minimize this problem, for example, by positioning the sample at the so-called eucentric height. This is a repeatable sample position and thus helps to maintain a consistent sample location and scale calibration.

Table 1 summarizes the recommended settings for large and small fields-of-view imaging and EDS analysis.

The quality of the TEM images, especially at small fields-of-view, can vary considerably due to sample charging, electron-beam-induced contamination and other detrimental effects. Annex B provides essential information about the key elements of TEM investigations useful for SWCNT characterization. Additional information can be found in Annexes C and D.

6.2 EDS analysis of an SWCNT sample

TEM and STEM-based EDS analysis is capable of providing information at very small fields-of-view and can identify the elemental composition of even nanometre-sized particles. An additional advantage of TEM-based EDS is that the characteristic X-ray peaks of the constituents of the sample are on a small continuous background. Therefore, better sensitivity can be realized over areas that fall into the openings of the TEM grid.

The optimal beam current, accelerating voltage, acquisition time and energy range depend on the elemental composition of the SWCNT sample (see ISO 22309 for further information). Optimization of these parameters follows the discovery of material other than SWCNT by TEM imaging.

For EDS analysis, including elemental map acquisition, the recommended starting accelerating voltage is generally at the low end of the capabilities of the TEM or STEM instrument. The X-ray energy range for spectrum or map acquisition depends on the characteristic peaks of the SWCNT sample, though a 20 keV upper limit is generally sufficient. If unusual elements are detected, a different accelerating voltage and energy range can be necessary for optimal EDS analysis.

The EDS analysis shall be performed over a suitable area so that average compositions are obtained. Point analysis can determine the elemental composition of catalyst particles or impurities if these are present. To correct for sample drift during X-ray spectrum or map acquisition, a scan-control unit with drift correction software is recommended, especially for long acquisition times.

One EDS spectrum shall be recorded for each of the three different fields-of-views specified in Table 1 at three representative locations. These will be sufficient for pure SWCNT samples. The number of necessary EDS measurements depends on the results of the initial analyses. If the preliminary EDS analysis shows significant differences in the relative intensities of the peaks in different regions of the sample, then as many regions shall be examined in as much detail as necessary to obtain repeatable and relevant results.

For semi-quantitative analysis, it is necessary to average the results from the first three, different locations. Calculate the average and standard deviation. Report the data if the relative standard deviation, that is the standard deviation divided by the average, expressed as a percentage, is less than 20 %. If the variation is larger, analyse three more same-size field-of-view areas and, if needed, three additional areas in order to achieve the satisfactory statistical confidence in the measured results. If uniform values still cannot be achieved, then only report qualitative results (i.e. elements detected).

Using CRMs or reference materials, the EDS system shall be calibrated regularly according to the procedure the manufacturer and pertinent standards prescribe (see ISO 14595).

Table 1 — Recommended settings for TEM and EDS analyses

Large field-of-view TEM analysis	Small field-of-view TEM analysis	EDS analysis
0,5 mm	500 nm	50 µm
100 µm	100 nm	10 µm
50 µm	50 nm	1 µm
10 µm	20 nm	100 nm
5 µm		Point analysis
1 µm		

NOTE 1 Depending on the capabilities of the TEM, grid choice, the size of the grid openings and the type of examination, small deviations from these values or omissions of fields-of-view are permitted. The intention is to achieve consistency and to collect the appropriate amount of relevant information.

NOTE 2 In the presence of very large impurities, increase the field-of-view if necessary to capture full and relevant information. To obtain very large field-of-view images, suitable quality stitching of adjacent images is permitted.

NOTE 3 For large field-of-view analyses with a TEM equipped with a digital imaging system, acquire images at the highest pixel-resolution. For small field-of-view work, the pixel-resolution of digital imaging shall satisfy the Nyquist sampling criterion with an additional 50 % margin. Make sure that in the grey level histogram at least 80 % of the possible levels are used without pixels that are at minimum or maximum possible grey levels. With analogue image acquisition systems, record and digitize images so that these conditions are satisfied at the end of the imaging process.

NOTE 4 For EDS analysis, where possible use SWCNT sample areas that are in the windows of the supporting TEM grid.

7 Data analysis, interpretation and reporting of results

7.1 General principles

TEM and STEM images can provide information on nanotube diameter, length and defects, and on the characteristics of impurities and foreign objects of nanotube materials. The width or the diameter of the nanotubes can be calculated with high accuracy, provided the scale of the electron micrograph is calibrated properly. Visual analysis can give a qualitative estimate of the purity of a sample but it can be very difficult to yield any meaningful quantitative data because the TEM is not a bulk analytical technique. The results are largely dependent upon sampling and examination statistics. The measurement of the length of the nanotubes could also be difficult, especially with very long SWCNTs, because it would require the acquisition and stitching of many image frames.

A report that contains all pertinent images and measurement results of SWCNT samples shall be assembled in digital form using the instructions and the pertinent International Standard. Reporting of the results shall conform to ISO 17025. The evaluation of measurement data and calculation of uncertainty shall be carried out in accordance with ISO 29301. The images embedded in the report shall retain the resolution of the original images in order to facilitate digital magnification and to reveal all original details. If necessary, a duly marked, smaller-size report file can be additionally generated.

7.2 Data analysis and interpretation of TEM results

7.2.1 General

Insert into the report three representative images for each of the relevant fields-of-view, in landscape orientation. Include any additional images that were acquired to achieve fully representative information. The examination of these images shall allow the clear observation of features that are consistent with the presence of carbon nanotubes and possibly other materials of the SWCNT sample. Where it is found advantageous, include dark-field images exposing the presence of catalyst particles or other impurities in the SWCNT material as well. For easy comparisons, bright-field and dark-field image pairs shall be positioned next to each other. The imaging conditions, field-of-view and other pertinent information shall be unambiguously noted for each electron micrograph.

7.2.2 Assessment of purity of SWCNTs

Impurities present in a sample of SWCNTs consist of amorphous carbon, graphitic entities, including all carbonaceous materials exhibiting graphite lattice fringes, metals, metal oxides, and other possible elements such as sulfur, chlorine, etc. These, in very pure samples, can be rather small, truly nanometre-scale, so assessment of purity shall take place in both large and small field-of-view imaging. Figures A.1 and A.2 show characteristic impurities with various sizes and materials.

Report the findings by properly documenting the general characteristics of the purity of the SWCNT sample and the presence and morphology of sample components other than SWCNTs. Identify the location of all essential features in their corresponding images. Describe with their locations the accidental particles or other contaminations that ended up on the sample in preparation or storage.

7.2.3 Evaluation of impurities: metals, MWCNTs, amorphous carbon, and other materials in the SWCNT sample

In most cases, visual analysis can provide qualitative results on carbonaceous impurities. The presence of non-carbon impurities such as metals, catalyst materials and others can be revealed in dark-field imaging mode and easily identified by EDS or by the combination of EDS and EELS. Where possible, crystallography information shall be collected to further characterize the constituents of the SWCNT sample. Figures A.1 and A.2 show characteristic impurities with various sizes and materials.

Report the findings by properly documenting the presence, morphology and detailed characteristics of the impurities of the SWCNT sample, and of sample components other than SWCNTs. Identify the location of all essential features in their corresponding images. Disclose with their locations the accidental particles or other contaminations that ended up on the sample in preparation or storage.

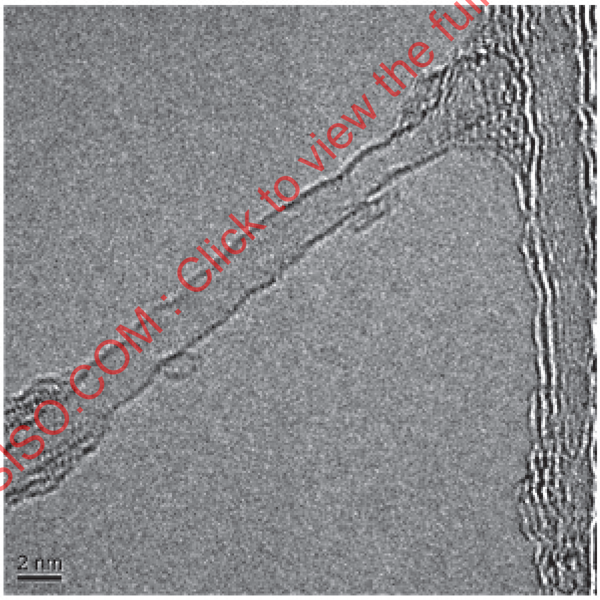
7.2.4 Examination of bundling, orientation and packing morphology

SWCNTs typically form bundles. A bundle of SWCNT can be identified as parallel dark lines with the distance between adjacent lines generally larger than the graphene spacing, e.g. 0,334 nm. Once the presence of SWCNTs in a bundle is confirmed, the measurement of bundle thickness becomes possible. Depending upon the orientation of the nanotube bundle and the packing morphology, nanotube density can be obtained, especially when the bundle is placed perpendicular to the objective plane of a microscope (see Figure A.4 for an example). On samples with thick bundles, STEM imaging at large fields-of-view can deliver faster results.

Report findings by properly documenting the bundling, orientation and packing morphology characteristics of the bundles of SWCNTs. Describe and identify the locations of all essential features on their corresponding images.

7.2.5 Examination of the quality of SWCNTs

Ideally, SWCNTs are composed of a single graphene sheet with seamless cylindrical morphology. In reality, point defects exist when nanotube growth is interrupted by any disturbance in the growth conditions. Under high magnification, one can inspect the structural feature of a given nanotube sample. A defect site is described as a discontinuation of graphite walls along the fibre axis. Such a discontinuation could be a “kink”, where the sidewalls are no longer straight (otherwise described as defect-free), or a missing portion indicating a “hole” on the wall. A kink is defined as the point where the chirality of the measured nanotube changes.



NOTE The field-of-view is 27 nm.

Figure 1 — A SWCNT in the centre with highly defective structure

Defective structural features are very difficult to examine unless debundled or individual nanotubes are present in the sample. Figure 1 shows a nanotube of a severely defective nature. Defects are visible in this regular TEM image. The walls are uneven, there are discontinuities at several locations and extra material is adhering to the tube at at least two locations (an elliptical feature in the left centre and a more structureless one further up on the lower wall of the SWCNT). It is not clearly visible, but likely that the chirality of the tube changes several times, i.e. there are several kinks along its length. It is also probable that there are holes, i.e. missing C atoms, at several locations. There are portions of the walls that look even and give good contrast where the tube wall is of good quality. Using a spherical-aberration-corrected HRTEM, it is possible to image carbon atoms in a SWCNT, making surface defects much easier to observe at the atomic level.

Report the findings by fully documenting the bundling, orientation and packing morphology characteristics of all representative types of SWCNTs. Describe and identify the locations of all essential features on their corresponding images.

7.2.6 Measurement of the diameter distribution of SWCNTs

The diameter distribution of SWCNTs is defined as the relationship between measured nanotube diameters and the numbers of nanotubes with those diameters.

A typical nanotube diameter distribution is shown in Figure 2, which is close to a normal distribution. There are features of the curve that could be interpreted as the diameter result. The “mode” is the size representative of the maximum of the distribution curve; the “median” is the size of which there are equal numbers of nanotubes smaller as there are larger. The “mean” diameter is generally considered as the true average diameter for a nanotube sample:

$$d_{\mathrm{m}} = \frac{1}{n}\sum_{1}^{n} d_i$$

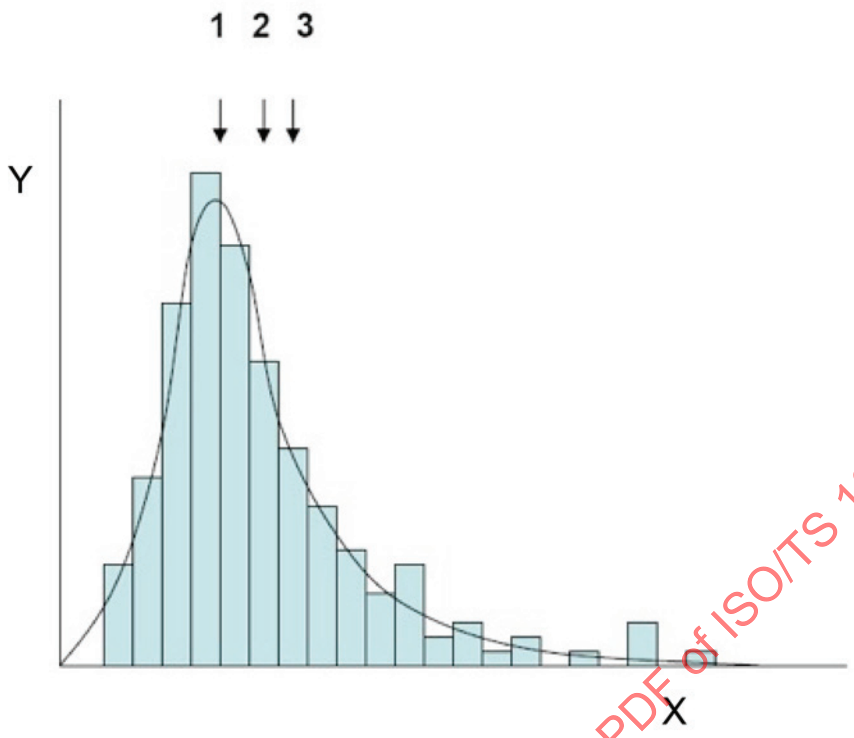
where

d_m is the mean diameter, expressed in nanometres;

n is the number;

d_i is the measured diameter of the individual SWCNTs, expressed in nanometres.

The average diameter obtained from TEM, STEM or SEM by visual analysis is an arithmetic mean value. The results of average diameter measurements depend upon the technique. If other techniques are applied, the average diameter may be area or volume weighted, sometimes resulting in very different values. When comparing these data with those acquired from TEM or SEM, one should be aware that the area- or volume-weighted average could be larger than the arithmetic mean if a broad distribution of nanotube diameters is observed.



Key

X diameter
Y frequency

1 mode
2 median
3 mean

Figure 2 — Typical nanotube diameter distribution

Bending and twisting commonly occur in SWCNT bundles. If the twisting is not too severe, the lattice parameter can be deduced from the periodicity of the fringes once the viewing direction is known. However, when the bundle is twisted around its axis, it induces a continuous change of the viewing direction and, therefore, a continuous change of the lattice fringe visible in the image. As indicated schematically in Figure C.1, the sequence of fringe spacings can be observed to follow a well defined sequence such as l_1, l_2. The lattice parameter a of the lattice formed by the bundle of tubes can be determined by measuring the spacing between different fringes. From those measurements, one can obtain the average diameter of nanotubes within the bundle, providing the inter-tube distance is known.

Measuring the end of the carbon nanotube, which is aligned parallel to the electron beam, is the most reliable measurement of the diameter of a SWCNT. Figure A.4 shows a micrograph with many tubes. Unfortunately, in most cases, the tubes form bundles and lie on the TEM grid surface. When the bundle of SWCNTs is aligned parallel to the electron beam, the TEM image shows a periodic array of dark circles having white dots in their centres. This image directly reveals the closely packed hexagonal arrangement of the tubes. Each dark circle is the image of an atomic layer of a SWCNT projected edge; the number of SWCNTs and the periodicity of their arrangement within the bundle can be readily observed. The one-to-one relation between the dark circle dimensions and the diameter of the tubes is valid only when the focus is close to the so-called Scherzer value. When the focus deviates from the Scherzer value, the image appears as an array of white dots located at the centres of the SWCNTs. A determination of the diameters of the nanotubes from such images is difficult if the exact focus is unknown.

To obtain relevant and representative results, it is recommended that tube diameters or widths be measured on a number of images and on many different SWCNTs selected at random. As the evaluation progresses, with the growing number of measured nanotube diameters, the uncertainty of the measurement diminishes.

Calculate the average and standard deviation, and continue evaluating additional tube diameters, unless the relative standard deviation, that is the standard deviation divided by the average, expressed as a percentage, is less than 10 %. The required number of independent measurements shall be determined as the number necessary to satisfy this relative standard deviation criterion. It is recommended that a number of image frames of different sample areas be used to avoid problems arising from inadequate sampling. Suitable image analytical methods help to achieve reliable results (see ISO 13322-1).

Report the results of SWCNT diameter or width measurements with their uncertainty estimates, and properly document the bundling, orientation and packing morphology characteristics of all representative types of SWCNTs. Describe and identify the locations of all nanotubes used in the calculations of the results on their corresponding images.

7.2.7 Examination of chirality of individual nanotubes by HRTEM and SAED

The chirality of the individual SWCNTs can be measured in electron micrographs acquired in high-resolution, spherical aberration-corrected TEM and with selected area electron diffraction. An HRTEM instrument can image the arrangement of C atoms in SWCNTs with the assistance of computer simulation. However, nanotubes in bundles or tubes having amorphous carbon coatings are usually difficult to image. When SWCNTs are packed closely in hexagonal arrangement, even if their diameters are the same, it is not known if the chirality of all tubes is the same. Many theoretical arguments and geometric analyses seem, however, to support such a case[14].

Selected area electron diffraction is another mode that can generate chirality data. It is common that reliable data can be acquired only from single and straight tubes. Bundling and curvature will cause overlap of multiple diffraction patterns in a single image, creating significant difficulty in discerning the patterns from individual tubes.

Future TEM developments, especially with regard to computer-assisted analysis, can help overcome these problems.

If one of the techniques just described is available, report the results of SWCNT chirality measurements of all representative types of SWCNTs. Describe and identify the locations of all nanotubes used in the calculation of the results on their corresponding images.

7.3 Data analysis and interpretation of EDS results

Report representative EDS spectra from 0 keV to 20 keV from the areas examined (see Table 1). List all the elements detected in addition to carbon. Identify any signal that can originate from the substrate. If feasible, include a summary table of the semi-quantitative EDS analysis data, identifying the mass fractions and amount-of-substance (i.e. atomic) fractions, expressed as percentages, of the detected elements, along with their average standard deviation and relative standard deviation values. If feasible, report elemental X-ray maps along with their corresponding STEM images. Describe and identify the locations of various elements present on their corresponding images and X-ray maps.

NOTE Additional information regarding EDS analysis of SWCNTs can be found in ISO/TS 10798.

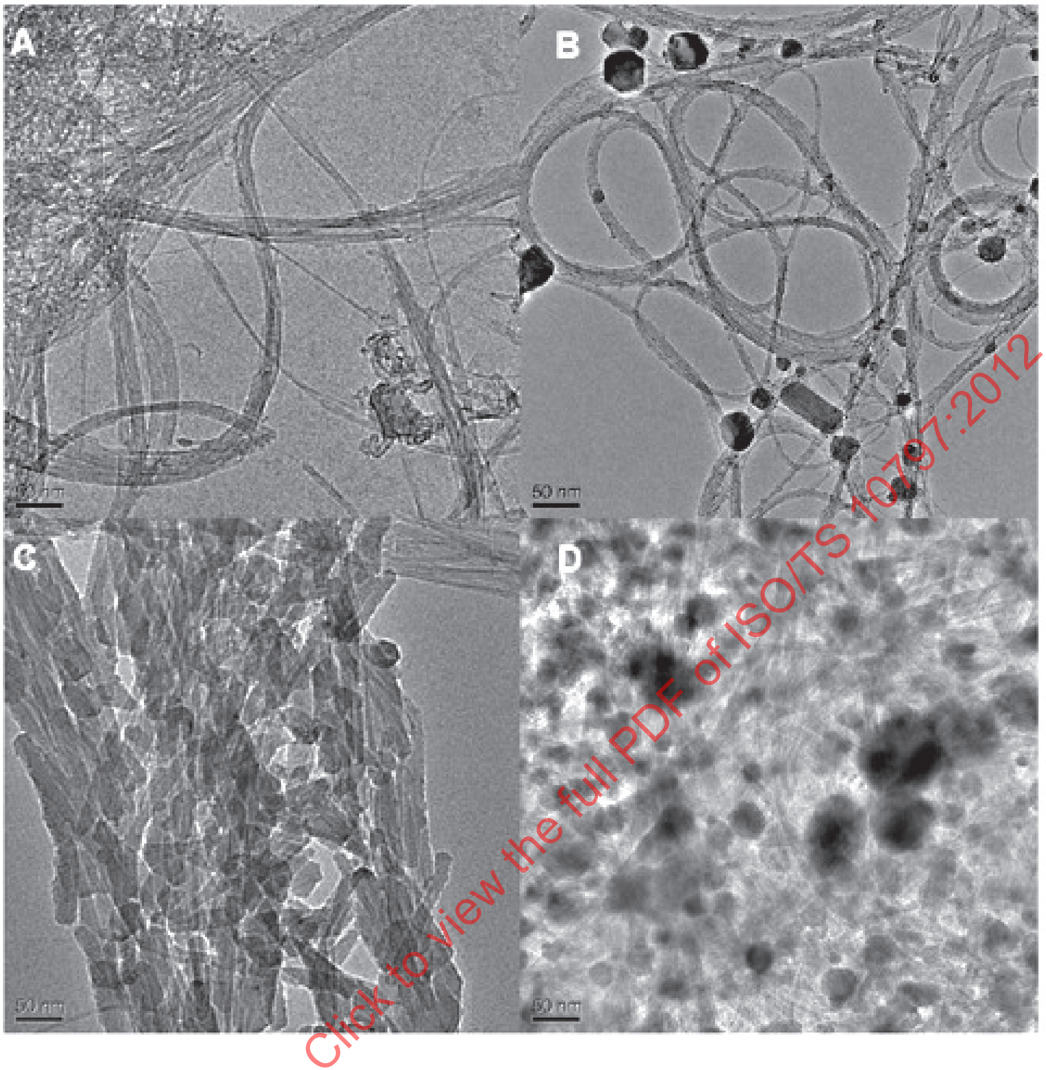
Annex A
(informative)

Case studies

A.1 Purity assessment

A raw SWCNT sample was weighed to be about 0,01 mg and placed in a vial. 10 ml of anhydrous 2-propanol was added to this sample. The sample vial was then treated in an ultrasonic clean bath for 30 s. A light grey suspension formed by the ultrasonic treatment, then a drop of the suspension was placed on a holey carbon/copper grid (200 mesh) and allowed to dry at room temperature.

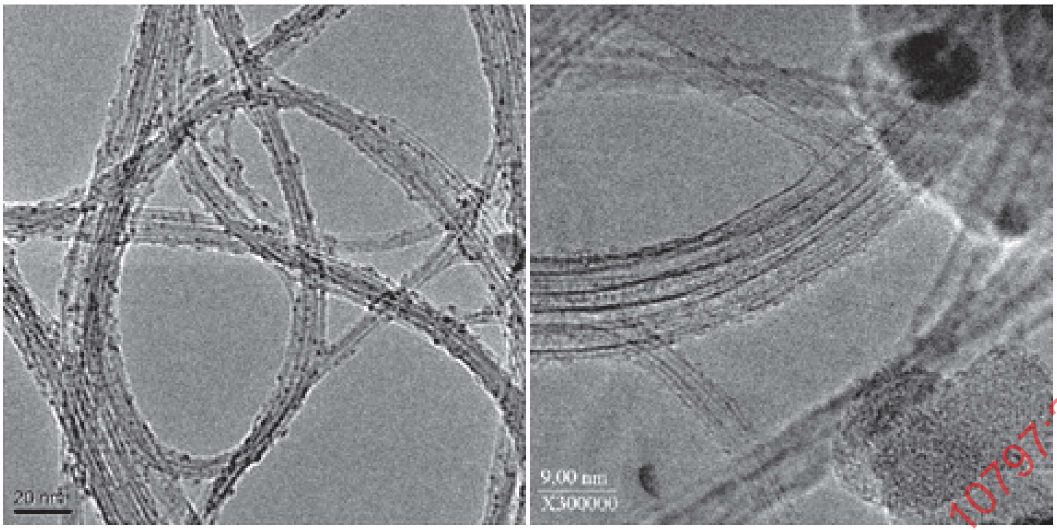
A TEM instrument equipped with an FE electron source was used. In this case, the accelerating voltage was set at 200 kV and the instrument was aligned properly (note that many times 80 kV or 60 kV, or even lower, accelerating voltages provide excellent results). The sample was then inserted in a pre-pumping station before being fully inserted into the instrument. Areas of interest were first established on large field-of-view images, and then the magnification was brought up to 100 000 to 150 000 times for higher-resolution examination. Various digital images were recorded as shown in Figure A.1. As seen from these images, fibre-like objects with embedded parallel lines indicated the presence of typical SWCNT bundles. In addition to this characteristic feature of SWCNTs, other objects were also observed. In images A and D of Figure A.1, large areas of amorphous matter, which can be either residual catalyst support or amorphous carbon material, were found overlapping carbon nanotube bundles. Large metal particles with regular hexagonal shape were present in many images, either on nanotube bundles or overlaid with the nanotube bundles and amorphous matter (see Figure A.1, image B). Graphite-based nanorod-like material was also observed in some areas, as shown in Figure A.1, image C. Generally, the bundle thickness is in the range of 5 nm to 40 nm. Individual nanotubes are observed, but in a very small fraction compared to the bundled form.



NOTE The field-of-view is 542 nm for all images.

Figure A.1 — Micrographs for large field-of-view purity assessment

On the small field-of-view images of Figure A.2, the fibre-like bundles were confirmed to be SWCNTs with diameters measured in the range of 1 nm to 2 nm. In addition, very fine particles of possible metal or metal carbide, which were otherwise invisible at lower magnifications, were observed on the surfaces of these bundles. This sample generally contains SWCNTs mostly in bundled form. Impurities such as metal particles, both large and small (possible carbides), amorphous matter and graphite rod-like materials were also found. EDS of various regions indicated the possible presence of iron, molybdenum, magnesium and copper, which can be attributed to the TEM grid material.



NOTE The field-of-view is 177 nm for the left image and 60 nm for the right image.

Figure A.2 — Micrographs for small field-of-view purity assessment

A.2 Diameter measurements

TEM is often used to measure nanotube diameters. However, nanotubes exist almost exclusively as bundles, which makes it nearly impossible to distinguish the walls of each nanotube in the bundle lying flat on the support film, in order to measure the diameter of each nanotube. Moreover, if the triangular lattice of a well ordered bundle is positioned in a certain way with respect to the electron beam, the line spacings (see Figure C.1), l, observed on the TEM image represent either l_1 or l_2, as defined in the following:

$$l_1 = \frac{\sqrt{3}}{2}\left(d_\mathrm{t} + d_\mathrm{i}\right)$$

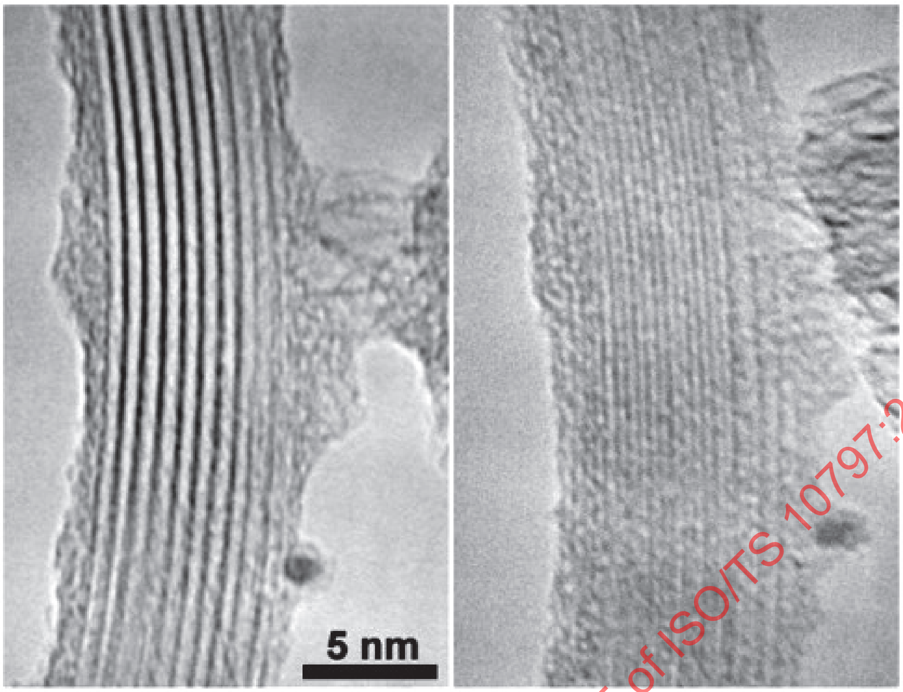
or

$$l_2 = \frac{1}{2}\left(d_\mathrm{t} + d_\mathrm{i}\right)$$

where

d_t is the nanotube diameter, in nanometres;

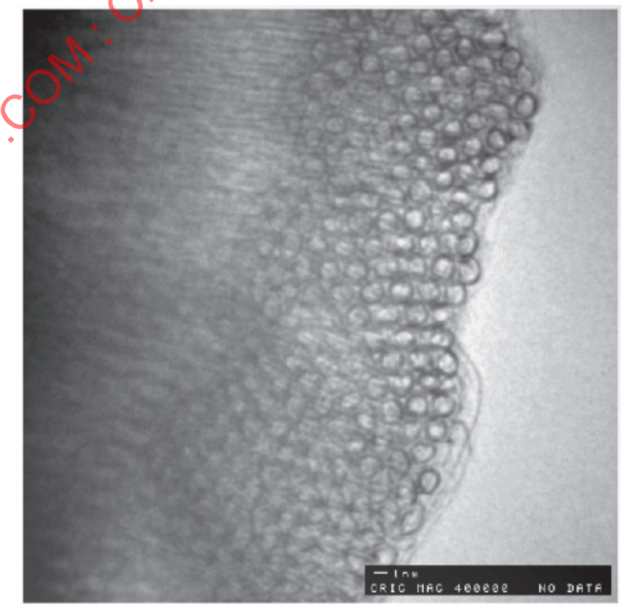
d_i is the van der Waals inter-wall spacing, in nanometres.



NOTE The field-of-view is 16 nm for both images.

Figure A.3 — Diameter measurement in SWCNT bundles

Figure A.3 shows the same bundle of nanotubes rotated 0° and 30° around its axis. The inter-line spacing follows the expression above[15]. So, unless the sample contains many, well separated individual nanotubes, the diameter measurements are complicated and prone to error, because the walls of the nanotubes cannot be seen clearly.

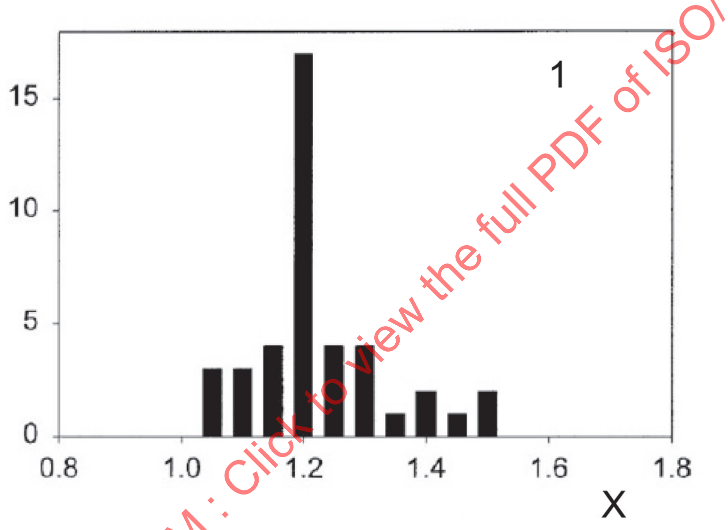


NOTE The field-of-view is 43 nm.

Figure A.4 — Micrograph of the cross-section of a SWCNT bundle for diameter measurements

If a bundle loops through the focal plane of the TEM so that nanotubes are parallel to the electron beam, it is possible to image the cross-section of the bundle with many nanotubes seen as circles (see Figure A.4)[16]. This makes it much easier to distinguish nanotubes and reliably measure a large number of diameters. To enhance the number of such cases, the sample being imaged can be prepared in the following fashion.

Several very small specks of material were separated from an aggregate of dry, as-grown carbon nanotubes. They were placed on a holey carbon TEM grid supported on a piece of tissue, and wetted with a drop of methanol to adhere to the grid. When treated in this way, nanotube particles become compressed by capillary forces as the solvent evaporates and stick to the support film. In the process, SWCNT bundles at the extremities of the particles fold onto themselves and create loops, most of which are in-plane or at some angle to the e-beam. A small fraction of the loops, however, usually ends up being parallel to the e-beam, which creates the cross-sectional images mentioned above. Otherwise, the sample can be tilted to achieve best results. The diameter measurements performed after this type of sample preparation are best accomplished by superimposing a circle over the nanotube cross-sectional image until best visual fit is achieved. A few software solutions also exist for this task. Successful applications of this diameter measurement technique have been documented in several publications[15][16][17][18][19]. An example of the diameter distribution of nanotubes made using the pulsed laser vaporization (PLV) process measured in this fashion is shown in Figure A.5[15].



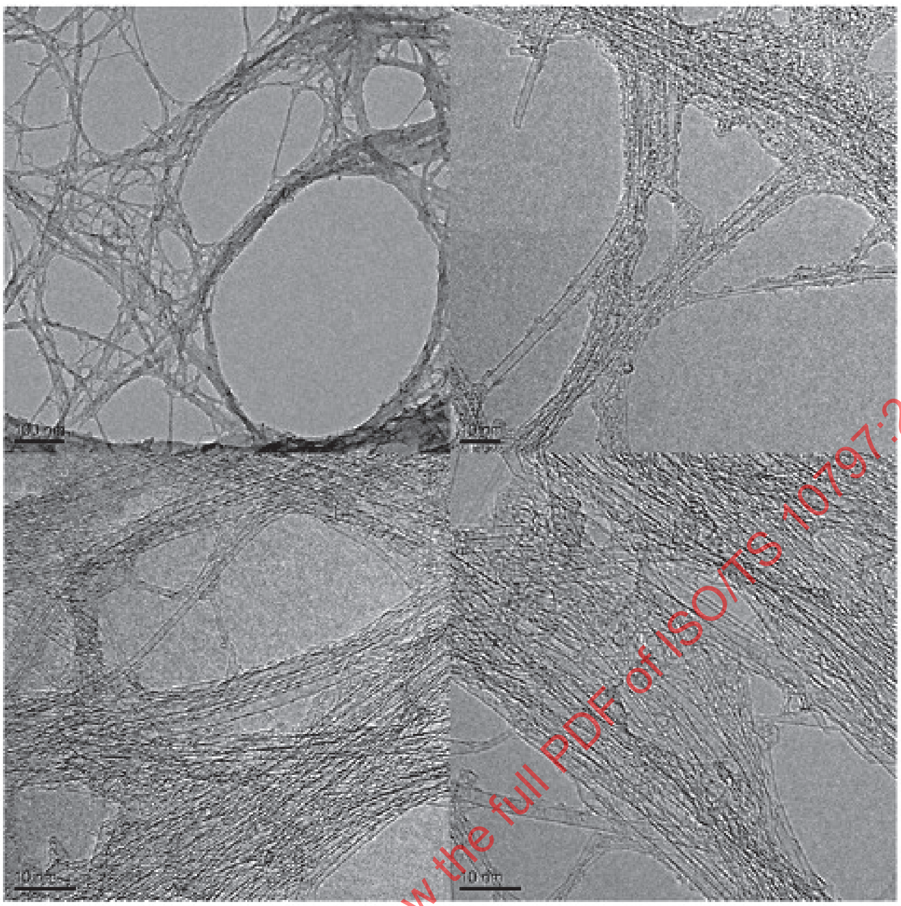
Key

X diameter, expressed in nanometres

1 10 cm material

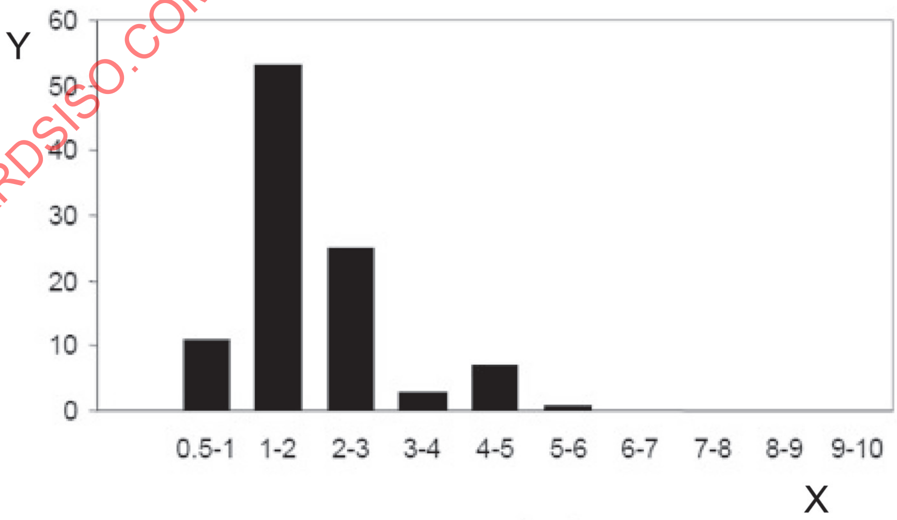
Figure A.5 — Diameter distribution of a SWCNT sample from the PLV process

While the above case showed a rather uniform diameter distribution for SWCNTs made by the VLD process, SWCNTs made by chemical vapour deposition (CVD) have different diameter distributions. In such cases, nanotubes are mostly in the form of individual tubes or small bundles instead of large (thick) bundles as shown in Figure A.6. This phenomenon can be caused by broad distributions of nanotube diameters as well as by differences in chirality. Since most tubes are individualized, the difficulty associated with overlapping or twisting inside a bundle is minimized. The diameter distribution of such a sample is measured and shown in Figure A.7.



NOTE The field-of-view is 1,8 µm for the upper left image, 109 nm for the upper right image and 145 nm for both lower images.

Figure A.6 — Micrographs for diameter measurements of SWCNTs made by the CVD process



Key

X diameter, expressed in nanometres

Y nanotubes, expressed as a percentage

Figure A.7 — Diameter distribution as a percentage of SWCNTs made via the CVD process

Annex B
(informative)

Additional information on sample preparation and experimental procedures

B.1 Sample preparation

B.1.1 Support materials on a TEM grid

The choice of TEM grid is important in sample preparation, imaging and measurements; the type and suitability of the grid depend on the SWCNT sample and the intended use. The advantage of using amorphous carbon film is to provide assistance in aligning the microscope and particularly to help with holding powder samples and correcting for astigmatism associated with the objective lens. The holes in holey or lacey carbon film provide “clean” areas where the sample can be observed without interference. Unfortunately, the drift of the TEM imaging system is never zero. Further, the carbon film is fragile, and holey and lacey versions are even more so. For most users, however, the drift rate is sufficiently small that it does not detract too much from the resolution in the resulting images. In addition, it is inevitable that some portion of a given sample will be located on the amorphous carbon surface, thus reducing the phase contrast against the background. Some inorganic film can be applied to improve phase contrast in high-resolution imaging.

B.1.2 Composite sample

An important practical use of carbon nanotubes is in forming composite materials that have advantageous properties. Evaluation of various properties of embedded CNTs is more limited than properties of freestanding CNTs, but some important characteristics can still be investigated. The main disadvantage of purposely embedding CNTs as a means of sample preparation is that this method alters the structure of the tubes to some extent and therefore is mostly useful when the information on the original structure is not important. The preparation of SWCNT composite samples involves cutting or deforming the carbon nanotubes, which makes it very difficult to elucidate the original state of aggregation as well as the original length distribution. To minimize the undesirable effects of sample preparation, an ultra-microtome with a diamond knife is generally required.

B.2 Theoretical and instrumental considerations

B.2.1 Electron source

There are thermionic and field emission (FE) types of electron sources used in TEM and STEM instruments. The electron “gun” is a heated filament for thermionic sources, whereas the field emission source generates electrons through extraction using an intense electric field. Electrons generated through field emission are more monoenergetic than those generated thermionically. The type of electron source defines the characteristics of the electron beam. The parameters of interest include work function, operating temperature, current density, crossover size, brightness, energy spread, emission current stability, vacuum requirement and source lifetime.

Tungsten filaments and properly shaped, heated LaB_6 crystals are the two common components used in thermionic electron sources associated with many conventional TEM instruments. Tungsten is economical, robust, easily replaceable and requires lower vacuum than LaB_6. A TEM instrument with a tungsten filament is excellent for routine TEM analysis. Preliminary purity assessment of carbon nanotubes can be carried out using a TEM instrument equipped with this type of electron source. LaB_6 has considerably higher current density capability, brightness, emitter lifetime and resolution and is a more powerful tool for nanotube purity analysis.

The FE electron source has very high current density, and its electron energy spread is small. For routine purity analysis of carbon nanotubes, an FE electron source is not ideal in all cases because the source current is smaller, thereby making it difficult to illuminate large areas of the sample with sufficient current density.

FE electron sources provide sufficient resolving power for HRTEM, as well as for special analytical working modes, such as STEM or electron holography. For spectroscopic examination of impurities by EELS, a field emission source has superior spatial and energy resolution compared to tungsten or LaB_6 electron sources.

B.2.2 Condenser lenses

The function of the condenser lens system, which is located between the electron gun and the object plane, is to focus the electron beam emerging from the electron gun onto the sample to permit optimal illuminating conditions for visualizing and recording the image. During the most common operations, the condenser lenses should be set to spread the electron beam (not focused at crossover) in order to prevent excessive heating.

B.2.3 Objective lens and sample stage

The optical enlarging system of an electron microscope consists of an objective lens followed by one or more projector lenses. The objective lens determines resolution and contrast in the image; all subsequent lenses bring the final image to a convenient magnification for observation and recording.

The objective lens is the most critical lens since it determines the resolving power of the instrument and performs the first stage of imaging. Aberrations in the image formed by the objective lens are subject to further magnification by the projector and photographic systems. The intermediate and projector lenses are used under conditions in which errors in these lenses do not interfere seriously with the imaging, except at low magnifications. The reason for this is that the angular aperture of the electron beam entering the projector lenses is so small that spherical aberration is negligible. Note, however, that this might not be true for imaging performed at low magnifications (less than 10 000 times) where pincushion, barrel and anisotropic distortions can occur, because a large portion of the intermediate image formed by the objective lens enters the projector lenses.

The objective forms, at a relatively great distance, an image of an object placed close to the front focal point. Since the object is practically at the first focal plane, rays leaving the same point are almost parallel on leaving the lens (neglecting spherical aberration) and unite at the image plane, a relatively great distance below the lens. In the absence of an objective aperture, electrons scattered through angles much greater than the effective aperture of the lens reach the image plane so far from their proper image point that they constitute a background “fog”. A region in the object with relatively high scattering power will therefore appear much darker than its surroundings in the image, even though there is negligible absorption. Capturing intensity scattered outside the aperture helps improve contrast in the image.

B.2.4 Projector lens

The projector lens, often used with a series of intermediate lenses, is responsible for transferring the initial image from the objective lens onto a view screen. The projector lens can further magnify the initial enlarged image of the illuminated portion of the sample.

B.2.5 Camera and viewing systems

The TEM viewing screen is usually coated with phosphorescent material such as zinc sulfide (ZnS) to create an image with green colour suitable for human eyes that are sensitive at approximately 550 nm wavelength. A TEM instrument records images onto an excellent grey-scale resolution photographic negative (a layer of photographic film or imaging plate). Modern instruments are equipped with an image sensor such as a CCD-based camera that produces digital images (data) that can be displayed on a computer screen and saved as an image file.

B.2.6 Lens aberrations and other non-ideal imaging properties

Glass, electrostatic and magnetic lenses all suffer to varying extents from the effects of five aberrations. These include spherical aberration, distortion, curvature of field, astigmatism and coma. Because of the rotation effect of magnetic fields on electrons, magnetic lenses also suffer from anisotropic distortion, anisotropic astigmatism and anisotropic coma. In addition, other factors that can result in image defects include chromatic aberration, rotational chromatic aberration and space-charge distortion.

Spherical aberration is one of the main factors that limit the resolution of the TEM instrument. It arises because peripheral rays are brought to focal points that are different from the focal points of rays that run nearer to the axis. As a result, it causes electron rays from one object point to not cross in a corresponding image point in the image space.

Distortion is another kind of spherical aberration. It mainly affects the projector lenses because their object is a magnified image (the intermediate image formed by the objective lens). Rays from each point in the “image” reunite at corresponding points in the image plane, but the magnification varies throughout the plane.

Chromatic aberration occurs when electrons of a different wavelength (velocity) leaving a point in object space are not brought to the same focus in image space. Similar to spherical aberration, chromatic aberration limits the resolution; however, thinner samples will suffer less impact from chromatic aberration. In the TEM instrument, for samples of “normal” thickness (less than 100 nm to 200 nm), the portion of the beam absorbed is minimal. When it is absorbed, the electron loses all its energy to the sample. Those electrons that give up part of their energy are said to be inelastically scattered, whereas those that give up none of their energy when scattered are said to be elastically scattered.

The amount of scattering that occurs at any particular sample point is dependent on the density and overall thickness and is relatively independent of the atomic number, chemical composition or other sample properties. The scattering power of a particular area of sample is directly proportional to its mass thickness in the form of mass per unit area. The mass thickness can be obtained by multiplication of bulk density and thickness. With increasing mass thickness, the probability of scattering increases. An electron passing close to a nucleus of an atom is attracted toward the positive charge. The electron travels a hyperbolic path near the nucleus and then travels a straight line, but at some angle with respect to the original trajectory. An electron passing closer to the nucleus is more strongly attracted by the positive charge and is therefore deflected through a larger angle. The momentum of the incident electrons is sufficient that they escape capture by the nucleus. Elastic scattering results in deflection of the incident electrons through angles up to, but not much greater than, 10^{-2} rad. Forces of repulsion determine the electron trajectories near the sample electrons. The closer the approach, the larger the deviation of the electron trajectory. In the case of electrostatic interactions and collisions between the beam electrons and the electrons surrounding the atomic nucleus, the deflected electrons are likely to undergo a loss of energy (i.e. a shift to longer wavelength and lower velocity). As stated above, scattering, in which the incident electrons lose some energy, is called inelastic scattering. The energy loss is generally 10 eV to 20 eV for thin samples (less than 100 nm), where there is generally only one scattering event as the electron passes through the sample. The beam electrons suffering such an energy loss are deflected through very small angles (approximately 10^{-4} rad), and thus nearly all of them pass through the objective aperture.

The proportional energy loss due to inelastic collisions corresponds to fluctuations in the accelerating voltage of one part in 10^4 to 10^5. Thus, the change in wavelength produced by a single electron scattering event is relatively insignificant. However, multiple scattering, which can occur in relatively thick samples, can be a serious source of chromatic aberration in the image.

Neither the homogeneity of available magnetic materials nor the accuracy of machining these metals into lens pole pieces is adequate for the direct production of lenses capable of displaying the theoretical resolving power established by the spherical aberration-diffraction limit. Asymmetry, resulting from lack of axial symmetry, has the effect of producing images in which the focal level varies with direction. The stigmator is the device used to compensate for asymmetrical lens fields.

B.2.7 Amplitude/phase contrast and their control

Contrast of images is determined by the nature and extent of interactions between electron beam and sample. Properties of both the sample (inherent contrast) and of the microscope system (instrumental contrast) are of importance. Contrast is defined as the relative difference in intensity between an image point and its surroundings.

Phase contrast imaging is often thought to be synonymous with HRTEM. In fact, phase contrast appears in most TEM images even at relatively low magnifications. It occurs whenever more than one beam contributes to an image. The image is directly related to the structural periodicity of a crystalline sample.

NOTE The so-called "Fresnel-contrast" image is caused by phase contrast. Fresnel fringes are a consequence of phase contrast, which arises from interference of the scattered electrons with the unscattered electrons. The image usually varies with small changes in the thickness, orientation or scattering factor of a given sample, and variations in the focus or astigmatism of the objective lens. These images can sometimes cause confusion in analysing a crystalline sample. Thus, extra caution is needed when dealing with thick sections or an out-of-focus area. Due to the higher coherence, the Fresnel fringes are more pronounced in a TEM instrument with a field emission electron source than with a thermionic source.

B.3 Additional working modes

B.3.1 Energy dispersive X-ray microanalysis

When high-velocity electrons interact with matter, electromagnetic radiation (in the form of X-rays and visible light) is also emitted. There are two sources of electron-induced electromagnetic radiation. The first is due to loss of energy from the incident electron to the outermost electron shells of the sample atoms as the electron slows down during its passage through the sample. This reappears as quanta of soft X-ray radiation. Since the slowing process is continuous and not stepwise, a continuous or "white" spectrum of soft X-rays is emitted. The wavelengths of the emitted X-rays are between 10 nm to 100 nm.

As the energy of the incident electrons increases above 1 keV, the electrons are able to interact with the inner shells of sample atoms. This causes an orbital electron to jump from a lower energy level shell to a higher energy shell. When the electron drops back, a quantum of energy in the form of a "hard" or short-wavelength X-ray is emitted. Electron transitions from one shell to another involve very precise energy quanta, so the emitted X-ray is of a very precise, characteristic wavelength or energy. Because the transition energies differ for each individual atomic species, the emitted wavelengths are characteristic of the atoms forming the sample. By measuring the wavelength or the energy of the emitted X-rays with an X-ray spectrometer, qualitative analysis of the atoms composing the sample is possible. If the relative intensity of the particular X-ray corresponding to a particular atomic species is measured, a quantitative analysis of the sample is also possible.

The energy dispersive X-ray spectrometer can be operated in point analysis or in scan mode. In point analysis, the X-ray spectrum from a small volume of the sample can be analysed. In scan mode, the detector is set to an X-ray spectral line or an energy corresponding to a selected element, and the probe beam is scanned in a raster across the desired area of the sample. A "map" of the sample in the "light" of the selected element is generated in a computerized acquisition system, on a synchronous display, and recorded photographically. The electron gun is then set to give a wide-angle flood beam, and the conventional TEM image is focused on the final screen and photographed in the usual way. The two photographs can subsequently be superimposed to show the distribution of the element of interest.

The limits of detection for analysing elements range from beryllium to uranium from a region of as small as 100 nm to 200 nm diameter, while the amount of the detected elements can be as small as 10^{-14} g to 10^{-19} g. Due principally to the lateral spread of the electron probe as it passes through the sample, X-rays are generated from a greater volume of the sample than would be expected simply from consideration of probe spot size. Scanning electron microscopy or scanning transmission electron microscopy can also carry EDS mode.

B.3.2 Dark-field TEM imaging

A bright-field image in electron microscopy is formed when the unscattered electrons of the incident beam combine with the scattered electrons as modified by passage through the objective aperture. Dark areas in the image arise from sample regions that scatter electrons widely and into the objective aperture. If the unscattered electrons are removed, the image is formed only from the scattered electrons (i.e. those that have interacted with the sample) and a dark-field image is produced. This imaging mode is called "dark-field," because the viewing screen is dark unless there is sample present to scatter electrons. Dark-field images typically have considerably higher contrast than bright-field images, although the intensity is greatly reduced, therefore requiring longer photographic exposures.

The advantage of dark-field imaging is that, for crystalline objects, specific diffraction spots can be selected in the back focal plane of the objective lens to form a dark-field image only from the electrons scattered by a chosen set of crystal planes. Therefore, it is a powerful tool to detect fine particles that are otherwise likely invisible under bright-field. However, it is also very difficult to focus and correct for astigmatism, since phase contrast is not present. The objective aperture transmits only a small fraction of the scattered beam; image brightness is very low, necessitating longer exposure times to obtain usable photographic images. Consequently, samples are subjected to greater levels of radiation damage.

When using annular dark-field (ADF) or high angle annular dark-field (HAADF) in STEM mode, the image contrast is based on atomic number differences. This technique has higher collection efficiency and will not miss small particles that are oriented such that they have only reflections outside the objective aperture in conventional dark-field TEM. Thus, this technique can yield very useful information on the presence of very fine metal particles, which otherwise are invisible using bright-field imaging.

B.3.3 Selected area electron diffraction (SAED)

The SAED method allows for the scale calibration of the TEM by using the diffraction pattern from materials with known crystal parameters. For this, the camera constant, $L\lambda$, is calculated (where L is the camera length expressed in millimetres, and λ is the electron wavelength, expressed in nanometres). Another parameter, R, expressed in millimetres, refers to the separation of the direct and diffracted beams as measured on the TEM screen or image. When the camera constant is known, by measuring R, the spacing, d, expressed in nanometres, in the crystal can be calculated. Note that, as the accelerating voltage decreases (and λ increases), the scale of the diffraction pattern increases.

$$\frac{R}{L} = \tan 2\theta \approx 2\theta$$

from the Bragg equation,

$$\frac{\lambda}{d} = 2\sin\theta = 2\theta \text{ .}$$

Thus,

$$Rd = \lambda L$$

where

L is the camera length, expressed in millimetres;

R is the separation of the direct and diffracted beams as measured on the screen, expressed in millimetres;

λ is the electron wavelength, expressed in nanometres;

d is the interplanar distance, expressed in nanometres;

θ is the diffraction angle, in degrees.

If the geometry of the system and the wavelength of the electron beam are known exactly, the camera constant can be derived and spacing in the sample can be calculated from measurements of the spots or rings in the diffraction pattern. It is difficult to determine accurately the wavelength by experiment, and the wavelength can change with time. Generally, it is impractical to measure the high voltage with sufficient accuracy to find the wavelength by calculation. The practical method to calibrate the instrument is using a diffracting substance of known structure. Since the instrument constant depends on lens currents and object position, ideally the calibration pattern should be taken at the same time as the unknown. This is commonly accomplished by evaporating the calibration substance on the grid before or after depositing the unknown substance.

The calibration is accomplished by taking a diffraction pattern of a sample whose d values are known, and measuring the radial distances of the spots produced by the diffracted beam of, for example, orders 1, 2, and 3, for a number of known d values, and then preparing a graph of distance R versus inter-planar spacing, d.

Evaporated films of gold, magnesium oxide and thallium chloride make excellent calibrating substances since they have d values accurately determined by X-ray diffraction.

B.3.4 Electron energy loss spectroscopy (EELS)

When an electron beam traverses a sample in the TEM, for every characteristic K-shell X-ray photon generated, there will be an electron, which has lost the characteristic energy, E_{kab}. By measuring the energy losses through a localized region of the sample, it is possible to infer the sample composition.

EELS is a technique confined to relatively thin transmission samples and is most usefully applied to light elements (e.g. sodium). “Thin” means thin enough for electrons to suffer only one inelastic collision. EELS imaging can also be used to investigate the core edge losses for high Z elements with a high degree of quantitative accuracy, and with excellent spatial resolution in energy filtered imaging mode. Today, only a limited number of microscopes are equipped to carry out EELS.

An energy analyser, fitted at the bottom of the TEM instrument column below the viewing screen, produces a spectrum of the energy distribution in the transmitted electrons relative to the primary beam energy. Most electrons in a spectrum are found in the initial zero loss peak and in loss peaks involving interactions with valence or conduction electrons, up to about 50 eV loss. Beyond this, a smoothly falling background has superimposed on it the ionization edges of atoms whose X-ray absorption energies are reached. These are the peaks used for analysis.

NOTE Information on chemical bonding, molecular structure and dielectric constant can be obtained from a detailed study of energy loss curves. The resolution of fine structure depends on the design of the spectrometer and on the energy spread of the primary electron beam. This is wider (1,5 eV) from a thermionic tungsten filament than from a cold field emitter such as a field emission gun (0,3 eV). The use of an energy filter to improve the energy spread of the primary electron beam can further improve resolution.

Annex C
(informative)

Additional information on observation of SWCNTs

C.1 High-resolution images

High-resolution TEM images are phase contrast images of the nanotube atomic structure, projected in the observation plane perpendicular to the electron beam. Different kinds of information can be extracted from these images, including the nature of self-organization of the tubes and the number and diameter of tubes. These characteristics can be easily and routinely determined and the examination becomes a straightforward task by following normal operating procedures adopted for transmission electron microscopes.