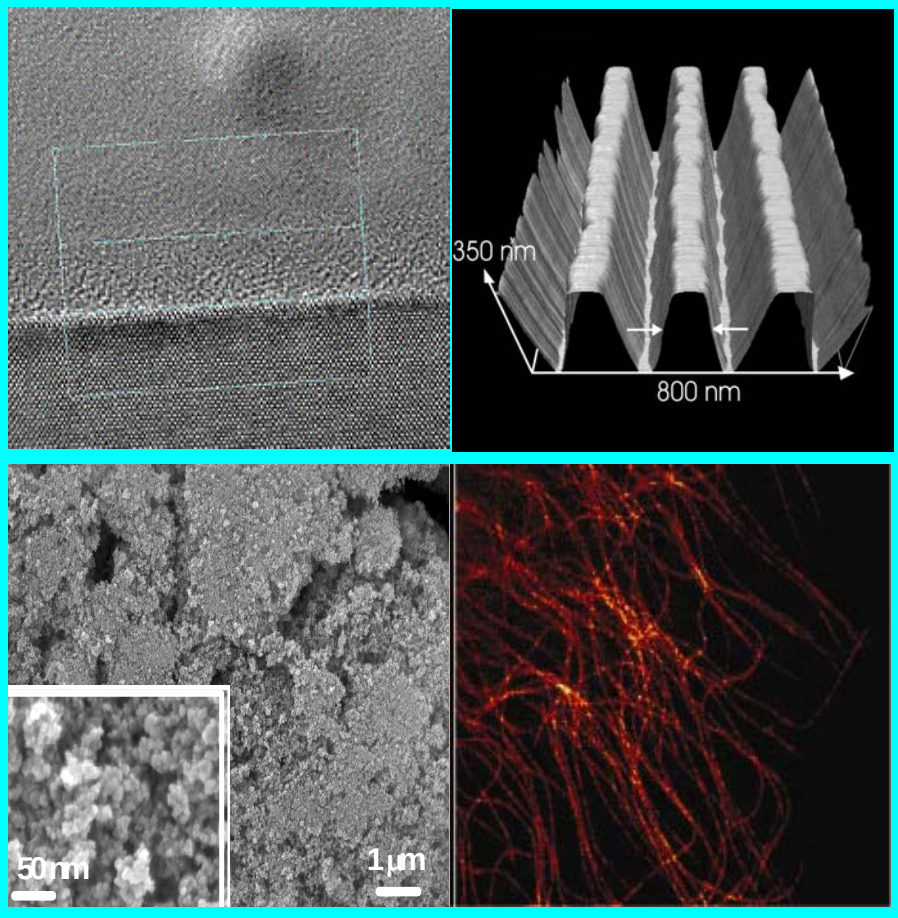
Introductory guide to nanometrology



Introductory guide to nanometrology

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Preface

Why should we care about nanometrology? For the same reason we care about metrology!
Metrology is necessary as the key discipline to enable the exchange of industrial products or components. To give a few examples, think of the pilot carefully observing his altitude, course, fuel consumption and speed, the food inspectorate measuring bacteria content, environmental engineers measuring air pollution, companies purchasing and selling their product using the same units. Goods and processes can be regulated and legislation can be implemented only because of measurements. Systematic measurement and results with known levels of uncertainty are cornerstones in science and industrial quality control.

Nanotechnology is associated with physical processes taking place at the atomic, molecular, meso- and microscopic levels where at least one dimension is below 100 nm. This dimensional definition makes nanotechnology an interdisciplinary science that covers a broad range of physics, chemistry and biology. New novel physical effects occur at such small scales that are important for the development of novel products. As an example it is now possible to produce new materials, called metamaterials, that have optical properties not known in nature. The ability to tailor-make these metamaterials makes it possible to break physical limits that we have accepted as laws of nature for centuries. The exploitation of such nanoscale effects thus helps to improve many products in a broad range of industries. Consequently, nanotechnology opens unprecedented engineering opportunities to specifically design the properties of materials for their particular application. Nanotechnology is thus expected to have a tremendous impact on future manufacturing in nearly all fields of industry.

This Guide introduces the reader to the science of measurements at the nanoscale, that is nanometrology. It is aimed at researchers in the nanotechnology area, for whom the metrology aspect is new, and at metrologists, interested in knowing about the specifics of metrology at the nanoscale. The Guide does not give an exhaustive review of the field. Rather it is intended to increase the general awareness of nanometrology, and its basic challenges.

In a first section, three main questions are addressed:

1. What is (nano)metrology?
2. Why is nanometrology important?
3. What are the main challenges for nanometrology?

The Guide continues with a section on the meaning of a number of generic metrology concepts.

In the third section, the Guide illustrates some of the identified nanometrological challenges with practical examples and case studies from three different application areas (thin films, surface structures and nanoparticles). A final subsection is devoted to the emerging issue of metrology for nanobiotechnology.

Acknowledgment

This "Introductory guide to nanometrology" is one of the products of the Co-Nanomet project ("Coordination of Nanometrology in Europe"), a project of the 7th Framework Programme, jointly financed by the European Commission and the participating consortium partners.

A first draft version of the Introductory Guide was written by Poul-Erik Hansen and Gert Roebben based mainly on discussion papers from four of the Co-Nanomet Action Groups (Critical Dimensions, Thin Films and Structural Analysis, Engineered Nanoparticles and Nanobiotechnology). The original discussion papers have been written by experts from the Co-Nanomet partners, as well as by external stakeholders. Based on the extensive and highly appreciated feedback to the first draft, this Guide was written. The people listed as contributing authors significantly contributed to the work, from the original discussion papers to the current finalised version of this Guide.

All Co-Nanomet partners, as well as the stakeholder organisations and individuals which have participated in the Co-Nanomet workshops in the course of 2009 and 2010, are acknowledged for their contribution to the success of Co-Nanomet and to the open exchange of views and opinions which have lead to this Introductory Guide. We hope the Guide can be an inspiration for everybody interested in the nanometrology field, coming from the metrology side or from the nanotechnology side, experienced researchers as well as young students. All feedback is welcome!

Contents

1 Introduction to Nanometrology: What is it? Why is it important? What are the main challenges?

Metrology is the science of measurements, and *nanometrology* is that part of metrology that relates to measurements at the nanoscale.

The importance of nanometrology is easily demonstrated: the ability to measure at the nanoscale has undoubtedly been a decisive factor in the development of *nanotechnology*.

One of the current challenges for nanometrology is the further development of methods to measure at the lower end of the nanoscale, thereby enabling a better scientific understanding of the phenomena that occur at the nanoscale and that make nanotechnology so interesting. An additional, newer challenge is the establishment of a reliable infrastructure (both conceptual and practical) that guarantees the reliability of nanoscale measurement results and of the results of measurements on nanomaterials and nanoproducts. This development must match the global race to invest in *nanotechnology* R&D, which is already creating new products and processes.

This introductory chapter will further elucidate the meaning of the terms metrology, nanotechnology and, ultimately, nanometrology.

1.1 Definitions and concepts

1.1.1 Metrology

The term **metrology** is used in different ways. It is sometimes used to refer to measurement instrumentation (as in "we need to develop new metrologies for measuring property X or Y…"). Sometimes, metrology is seen as a highly specialised activity that is exclusively performed by a limited number of experts, i.e. metrologists. The existence of these narrow views on metrology is a pity, since there actually is an agreed and clear definition, which is both more accurate and more generic than the given examples [1.1]:

> 'Metrology = science of measurement and its application
>
> Note: metrology includes all theoretical and practical aspects of measurement, whatever the measurement uncertainty and field of application.'

A first and obvious benefit of metrology is its potential to improve **scientific understanding**. This idea is captured in popular wisdom such as "to measure is to know" ("meten is weten" (Dutch); "att mäta är att veta" (Swedish); "Messen ist Wissen" (German)), or in quotes formulated by great scientists, such as "I often say that when you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind; it may be the beginning of knowledge, but you have scarcely, in your thoughts, advanced to the stage of science, whatever the matter may be" (Lord Kelvin, 1883[1]). The potential to improve scientific understanding is of course the most appealing part of metrology to scientists in the academic world, who are interested in developing and using new and advanced measurement methods.

[1] Lecture on "Electrical Units of Measurement" (3 May 1883), published in Popular Lectures Vol. I, p. 73;

A second, equally important, but less obvious benefit of metrology is much more practical. It is captured partly in another phrase attributed to Lord Kelvin "If you cannot measure it, you cannot improve it." In more general terms, metrology is closely linked to the concepts of **quality control** or **conformity assessment**, which means making a decision about whether a product or service conforms to specifications. Conformity assessment provides confidence for the consumer that requirements on products and services are met, it helps producers and suppliers to ensure product quality, and it is essential for reasons of fair trade and of public interest (public health, safety and order, protection of the environment and the consumer). The conformity assessment aspect of metrology turns science and innovation into economy and prosperity. Conformity assessment is especially relevant for nanotechnology, since a great deal of concern exists about the difficulty to turn its scientific developments into innovative products, in Europe. Therefore, the following section provides more details about conformity assessment.

1.1.2 Conformity assessment

Each measurement task for conformity assessment consists of a number of steps:

A. initial specification of the task in terms of the product or process requirements of the customer;

B. dialogue between customer and measurement specialist, to match measurement specifications to product or process specifications;

C. selection and use of measurement method, personnel, and equipment with a metrological performance appropriate for the task;

D. measurement and correct expression of the measurement results;

E. use of the measurement results by the customer for product conformity assessment.

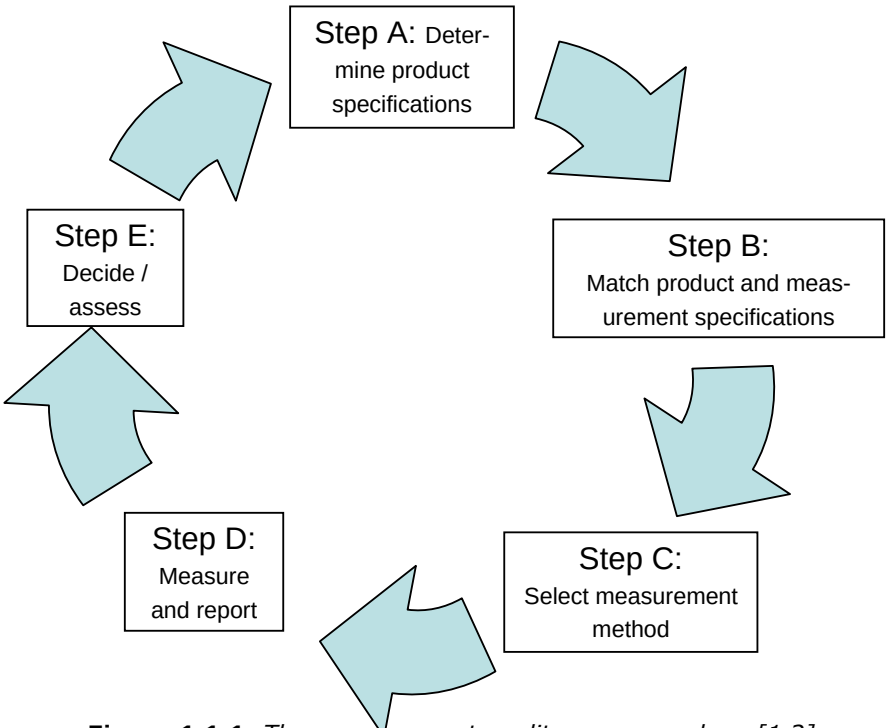

Figure 1.1.1: *The measurement quality assurance loop [1.2].*

The measurement quality assurance loop shown in Figure 1.1.1 is a special case of the Deming wheel or the plan-do-check-act approach, generally applicable to all production processes. The steps A – E will be exemplified below for the case of a product containing nanoparticles.

- *Step A: Product specification*

Step A specifies the quality characteristics of the product itself, by setting tolerances on the product, often with regard to the required function of the product. For example: a new product contains nanoparticles. To guarantee the functionality of the nanoparticles, their diameter is required to be between a lower specification limit of 20 nm and an upper specification limit of 80 nm.

- *Step B: Matching product and measurement specifications*

In order to assess the product specifications set in Step A, corresponding measurement specifications need to be set, for example the minimum required accuracy of the measurement results. The minimum required accuracy ensures that the measurement results are sufficiently accurate to reliably verify the product specifications. (Standardisation provides harmonised and unambiguous statements about these specifications [1.3].) Returning to our example: testing the product conformity with the specifications on the diameter of the nanoparticles will require a measurement system with an accuracy of better than, for example, 10 nm; this should be sufficient to confirm that the product is within the range of 20 nm to 80 nm.

- *Step C: Selection of the measurement method*

Step C in the measurement process will consist in selection of measurement method, personnel and equipment with a metrological performance appropriate for the task. Section 3.3 provides details about the particle size analysis methods relevant for the above example.

- *Step D: Measurement and reporting*

Step D consists of measuring and correctly expressing the measurement results: it is essential that both the measured value and an assessment of the measurement accuracy are reported. (Generic guidance on how to express measurement results in a harmonised and unambiguous way can be found for example in the publications of the Joint Committee on Guides in Metrology, including GUM [1.4] and VIM [1.1].) In our example: a first series of measurements of the diameter of the nanoparticles yield an average diameter of 72 nm ± 15 nm. The value '± 15 nm' is an expression of the accuracy of the measurement result. To be meaningful, the value must be stated together with its confidence level (for example: of about 95 %). The customer then knows that at a confidence level of 95 %, the true value is within the stated range (here [57 nm, 87 nm]). One observes that the measurement uncertainty exceeds the required accuracy (better than 10 nm). Measurements were, therefore, repeated with more care, resulting in a new value of the average diameter of 70 nm ± 10 nm.

- *Step E: Decision-making*

Based on the measurement results a decision has now to be made about whether the product satisfies requirements. The statistical nature of sampling (testing one or a limited number of products and extrapolating the measured properties to a larger number of products) and the confidence level of the measurement result, inevitably implies a risk that incorrect decisions of conformity of product may arise [1.5], particularly when a test result is close to a specification limit. This uncertainty can lead to:

- correctly conforming entities being incorrectly failed on inspection – supplier risk;
- non-conforming entities being incorrectly passed on inspection – consumer risk.

In our example: the test result of the product with respect to specifications on the diameter of the nanoparticles yielded an average diameter of 70 nm ± 10 nm (confidence level of measurement uncertainty is about 95 %). Product conformity assessment obliges the average diameter to lie in the tolerance interval 50 nm ± 30 nm. The test result is close to the upper specification limit of 80 nm. Assuming a symmetric probability distribution, the consumer risk is calculated to be 2.5 %. The cost of consumer risk associated with nanoparticles exceeding the upper specification limit is estimated to be 12500 €. This has to be compared with the cost of additional or improved testing (for example 2500 € at the actual measurement uncertainty of 10 nm).

1.1.3 Nanotechnology

Advances in metrology depend on many factors: improvements in scientific and technical knowledge, in instrumentation, in documentary standards (standard test methods), and in physical standards (as in reference materials). Evidently, choices have to be made, and the setting of priorities in the further development in nanometrology depends on a clear knowledge of the nanotechnology area and the future demands from the nanotechnology industry, its regulators and the society at large.

To understand the nanotechnology area, it has to be described first. A broad consensus-definition for the term **nanotechnology** has recently been released by the International Organization for Standardization (ISO) [1.6]:

> 'Nanotechnology = the application of scientific knowledge to manipulate and control matter in the nanoscale to make use of size- and structure-dependent properties and phenomena distinct from those associated with individual atoms or molecules or with bulk materials
>
> NOTE Manipulate and control includes material synthesis.'

The above definition of nanotechnology relies heavily on the ISO definition of the term **nanoscale** [1.7]:

> 'Nanoscale = size range from approximately 1 nm to 100 nm
>
> NOTE 1 Properties that are not extrapolations from a larger size will typically, but not exclusively, be exhibited in this size range. For such properties the size limits are considered approximate.
>
> NOTE 2 The lower limit in this definition (approximately 1 nm) is introduced to avoid single and small groups of atoms from being designated as nano-objects or elements of nanostructures, which might be implied by the absence of a lower limit.'

This definition implies that 'nanoscale' is actually short for 'nanometre scale'. Note 1 of the above definition is a reminder of the potential of nanotechnology, which does not stem from scale considerations alone, but from new properties. In the size range defined by the term nanoscale, novel physical effects occur that are decisive for the development of novel products. Nanotechnology presents unprecedented engineering opportunities to specifically design the properties of materials for particular applications. The exploitation of such nanoscale effects is helping to improve many products in a broad range of industries.

1.1.4 Nanometrology

There is an increasing use of the term "nanometrology", most often to indicate the metrology activities that relate to nanotechnology, nanomaterials and the nanoscale. In line with other 'nano'-definitions, such as those developed by ISO, this Guide will interpret the term nanometrology as 'the science of measurement at the nanoscale'; in that sense 'nanometrology' and 'metrology at the nanoscale' will become synonyms.

The adoption of a new term, "nanometrology", should not be misinterpreted: nanometrology is not a special "kind" of metrology. The fundamental issues and principles of metrology in general remain valid. Concepts such as metrological traceability and measurement uncertainty, method validation and calibration, accuracy (a combination of precision and trueness), do not have to be adapted to suit special needs of nanotechnology. It is of course true that measuring at the nanoscale is a technological and scientific challenge, and that is what makes metrology at the nanoscale special.

What are the challenges in metrology at the nanoscale? Compared to measurements in daily life, at normal dimensions, new or additional effects have to be taken into account. For example length measurement at the nanoscale is influenced by effects that are negligible at macro– and micro-scale. This is why all measurands known from the macro-scale have to be reconsidered when they are assessed at the nanoscale.

It is interesting to note that the various units of the International System of Units (SI) for the different measurement quantities are defined at a level where the actual measurement accuracy is highest. Thus for example the unit of mass is set at the one-kilogram level, since weighings at heavier and lighter levels are less accurate – heavier weights are more difficult to handle while smaller weights are more easily perturbed. Weighing machines similarly work best close to the kilogram level. These trends – where accuracy generally falls towards both shorter ranges and longer ranges – are similar for many measurement quantities. Measurements at the nanoscale (1 nm to 100 nm) are no exception. Because of this, it will be increasingly more challenging in nanometrology to establish metrological traceability and to reach target measurement uncertainties.

Examples of nanometrological challenges include:

- Longer chains of metrological traceability.
- Need for new reference materials and documentary standards.
- Need for new measurement instrumentation and test methods working at the nanoscale.
- Need to measure new characteristics unique to the nanoscale.
- Measurements in challenging environments, e.g., ultra-high vacuum or complex biological media.

1.2 Players in the field

1.2.1 Research centres

The invention of Scanning Tunneling Microscopy (STM) at the IBM Research Centre in 1981 is often quoted as the main contribution of research and development to the start of a new, nanometrology area. Their invention would win Binnig and Rohrer the Nobel Prize in physics in 1986. Interestingly, Binnig and Rohrer shared their Nobel Prize with Ruska, who developed the electron microscope decades earlier, at the Technical College in Berlin. Scanning and transmission electron microscopy are key tools for nanometrology.

Other pivotal discoveries were related to observations of mass spectrometry peaks corresponding to molecules with the exact mass of sixty or seventy carbon atoms. In 1985, Harold Kroto (then of the University of Sussex), James R. Heath, Sean O'Brien, Robert Curl and Richard Smalley, from Rice University, discovered C_{60}. Kroto, Curl, and Smalley were awarded the 1996 Nobel Prize in Chemistry for their roles in the discovery of this class of compounds, called 'fullerenes'. The discovery of a new class of materials is not per se a contribution to nanometrology. However, the use that is increasingly being made of carbon nanotubes, for example as AFM tips, shows the significance of this class of materials for nanometrology.

1.2.2 Industry

The aim of industry investments is to develop, improve and commercialise new, innovative products. Modern consumer products, including products from nanotechnology, are often assemblies of materials and components produced in different places and/or by different companies. This is only possible for components with compatible properties (e.g., size). The demonstration of this compatibility is a requirement for trade of these components and products, and is necessarily based on measurement results reported in material or product information sheets. This is why industry is an important player in the metrology field, both as a consumer, with measurement needs, as well as a provider of specific measurement solutions. It is noted that a lot of small and medium size enterprises are involved in the latter.

1.2.3 Regulatory bodies

Nanotechnology has enabled the large-scale production of nanomaterials. Governments around the world have invested in this technology with the expectation that these nanomaterials would contribute to the solution of a number of 'Grand Challenges', such as secure supply of water, energy and food. On the other hand, the potential release of engineered nanoparticles with special properties, and the fact that these particles have a size similar to the size of biological molecules, which determine all essential processes in the human body, has resulted in concern about the safety of nanomaterials, especially particulate nanomaterials.

As a result, both in terms of innovation expectations and of human health, governments and regulatory authorities have emphasised the importance of reliable measurements of the properties and characteristics of nanomaterials. This demand has already resulted in increased, dedicated funding for nanometrology.

1.2.4 Metrology institutes

A significant proportion of metrology research is performed at the national metrology institutes (NMIs), and their designated institutes, which have a pivotal role in the establishment of traceability of measurement results to the International System of Units (the SI-system). The NMI's calibration services realise the most direct link to the SI-units that form the basis of the international measurement system.

Most NMIs are not only concerned with traceability, but also with the development and realisation of measurements at the highest level of accuracy and with the smallest uncertainty. Their high accuracy measurement instrumentation constitutes the backbone of worldwide comparability of SI-traceable measurement results. International comparisons among the NMIs make sure that measurement results agree within their uncertainties worldwide. In order to ensure that industry and other stakeholders can optimally profit from the achievements at NMIs, most NMIs produce certified reference materials (CRMs) which can be used for calibration[2] and thereby serve as a bridge from the NMI to the end-user.

[2] More specific terms for CRMs used for calibration, are material measures, calibration artefacts, calibrators or calibrants. These alternative terms are typically used in specific measurement areas.

In terms of nanoscale measurements, the main and first NMI activities were related to dimensional measurements on surface structures ('critical dimensions'), often related to micro-electronics applications. NMIs have developed the tools and methods that allow a traceable calibration of scanning probe microscopes (e.g., STM, AFM). Consequently, this increased the accuracy of the measurement results obtained.

With time, different aspects of nanoscale measurements have entered the scope of the NMI activities, such as the characterisation of nanomaterials, and nanoparticles in particular. In other areas, e.g., biology, different instances played and still play an important metrological role. Illustrations of these activities and organisations will be provided at a later stage in this Guide.

1.2.5 Citizens

The ultimate ambition of metrology is to contribute to the well-being of citizens by improving the reliability of measurement results at an affordable cost. A direct effect on the well-being of citizens is that of increased confidence in the measurement data we are confronted with in our daily lives, such as the information on the labels of the food products we eat, or the effectiveness of the sun cream we apply on our children's skin. In a more indirect manner, improved measurement reliability increases the effectiveness of the way we use natural resources, and reduces the risks to which a globalised, job- and wealth-creating industry is exposed.

The ultimate ambition of metrology, stated above, is not different from the main aim of nanometrology. The focus of nanometrology on the developing and relatively young area of nanotechnology implies that there is still a great deal of uncertainty, part of which can only be resolved by more and more reliable measurements.

1.3 Structure of this Guide

In this introductory guide, we first provide the reader with a summary of the most elementary metrology terms and concepts (Section 2), which are needed to understand the later sections of the text. Section 3 consists of four subsections each of which provides case studies from different areas:[3]

- Metrology for dimensional analysis of thin films
- Metrology for dimensional analysis of structured surfaces
- Metrology for engineered nanoparticles
- Metrology for nanobiotechnology

Numbered references:

[1.1] ISO/IEC Guide 99:2007, International vocabulary of metrology – Basic and general concepts and associated terms (VIM), 2007; also known and available as JCGM 200:2008, JCGM 2008. www.bipm.org.

[1.2] Pendrill, L., http://metrology.wordpress.com/measurement-process-index/, 2008.

[1.3] Nanotechnology standards, eds. Murashov, V., Howard, J., Springer Science+Business Media, New York, NY, ISBN 978-1-4419-7852-3, 2011.

[3] These four areas reflect the structure of the action groups of the Co-Nanomet FP7 project (2009-2010).

[1.4] ISO/IEC Guide 98-3:2008, Uncertainty of measurement – part 3: Guide to the expression of uncertainty in measurement (GUM:1995), 2008; also known and available as JCGM 100:2008, JCGM 2008. www.bipm.org.

[1.5] ILAC Guide 8:03/2009, Guidelines on the Reporting of Compliance with Specification, 2009.

[1.6] ISO/TS 80004-1:2010, Nanotechnologies – Vocabulary – Part 1: Core terms.

[1.6] ISO/TS 27687:2008, Nanotechnologies – Terminology and definitions for nano-objects – nanoparticle, nanofibre and nanoplate, 2008.

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2 Metrology concepts and terminology

2.1 Measurand, measurement uncertainty and metrological traceability

The essence of metrology is captured in the following terms: **measurand**, measurement **uncertainty** and metrological **traceability**.

Measurand

'The measurand is the quantity intended to be measured' [2.1].

Typically, this quantity corresponds with a property (e.g., size, composition, temperature, strength) of a measurement object (a phenomenon, body or substance). This may seem obvious, but often it is not. Detailed examples will be given later in the text, such as that of particle size: a *particle size* is easy to ask for, but to give an answer is not so easy. Since particles are usually not regularly shaped, which is the size to be measured and reported? Particles come often in large groups: is an average size for all acceptable? The correct and full description of the measurand is therefore the first prerequisite for a successful measurement.

Measurement uncertainty

'Measurement uncertainty is a non-negative parameter characterising the dispersion of the quantity values being attributed to a measurand…' [2.1].

The value of a measurement result can only be appreciated in a meaningful way if the result is reported together with its uncertainty. Measurement uncertainty is needed to judge whether different measured values are equal, or not. It is also essential in assessing the risks of incorrect decisions of compliancy when comparing a test result with a specification limit, which is a key concern, for instance in trade or regulatory contexts.

Estimating the measurement uncertainty involves considering all known sources of uncertainty in the measurement process, and has to be done in accordance with 'ISO/IEC Guide 98-3:2008, Uncertainty of measurement – part 3: Guide to the expression of uncertainty in measurement' (GUM:1995)' [2.2]. Typically, the measurement uncertainty is reported as the standard uncertainty multiplied by a coverage factor k = 2, which for a normal distribution corresponds to a coverage probability of approximately 95 %, i.e., the correct value of the measurand is within the range [measured value ± expanded uncertainty] at a confidence level of about 95 %.

Metrological traceability

Metrological traceability is a property of a measurement result. A traceable result is a result that can be related to a metrological reference point through a documented and unbroken chain of calibrations, each contributing to the measurement uncertainty [2.1].

If measurement results, made at different times and different locations, are traceable to the same reference point, then they can be meaningfully compared. Where possible, the reference point should be one of the universal measurement references of the SI system, the guardians of which are the NMIs (see section 1.2.4). NMIs, and other institutes designated by the NMIs, regularly perform international key and supplemental comparisons to ensure that their national references are in agreement to those of other countries.

2.2 Origins and components of measurement uncertainty

2.2.1 Measurement errors, trueness, precision, accuracy

To illustrate measurement error and some related metrology concepts, Figure 2.2.1 shows the results of four archers aiming for the bull's eye on a target. In metrological terms, a measurement error is the difference between a measured value and a reference value [2.1]. The bull's eye in Figure 2.2.1 depicts the 'true' or 'reference' value, therefore the performance of the archers can be easily evaluated. Target 1) is used by an archer with a large scatter; he has an equal chance of hitting any part of the target. His performance is characterised by a large random measurement error [2.1]. On target 2) we have an archer with a low scatter who can consistently hit the central part of the target. In example 3) we have an archer who on average hits the target to the left of the bulls-eye: his performance is characterised by a systematic measurement error [2.1]. On target 4) the archer has most hits in the vicinity of bulls-eye but some are scattered.

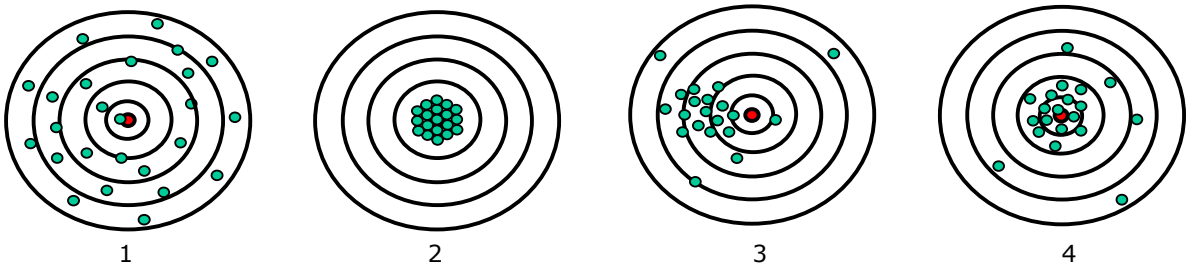

Figure 2.2.1 *Results of four archers shooting.*

Figure 2.3.1 shows the histograms corresponding with the four targets in Fig. 2.2.1. The histograms reveal the probability distributions of the archers' results. In Figure 2.3.1 the histograms are each overlaid with one of a number of probability distribution types. Archers 1 and 2 have produced histograms that correspond with rectangular distributions with large and small scatter respectively. Likewise it can be seen that archers 3 and 4 produced histograms that resemble normal (Gaussian) distributions, both with the same amount of scatter. However, the centre position of the normal distribution for archer 3 has a systematic offset relative to the bulls-eye.

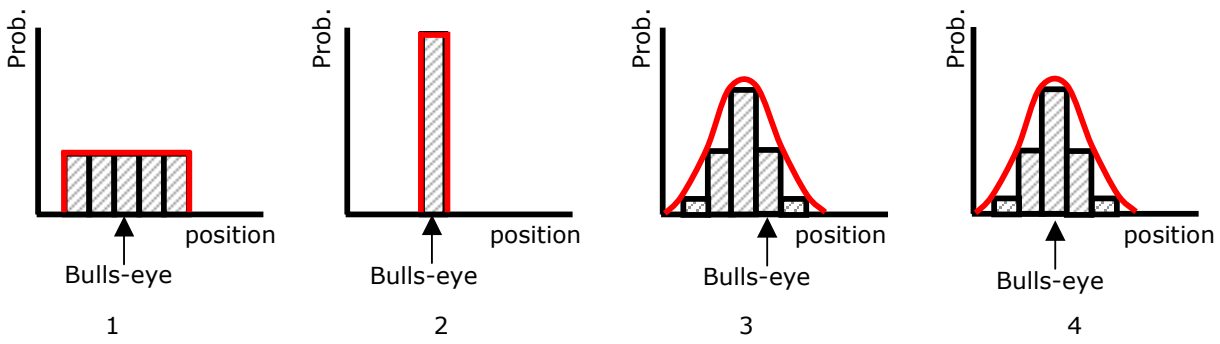

Figure 2.2.2 *Histogram (gray color) and probability distribution (red color) of the positions hit by the archers. Arrows indicate the position of the bulls-eye, here representing the true value.*

The metrology terms used to qualify the results in Figures 2.2.1 and 2.2.2 are trueness, precision and accuracy:

(Measurement) trueness is the closeness of agreement between the average value of an infinite number of replicate measured quantity values and a reference quantity value [2.1]. In this sense, archers 1, 2 and 4 each have performed equally well.

(Measurement) precision is the closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions [2.1]. In this sense, the performance of archer 2 is outstanding, the performances of archers 2 and 3 are comparable. Archer 1 has the lowest precision.

(Measurement) accuracy is the closeness of agreement between a measured quantity value and a true quantity value of a measurand. A measurement is said to be more accurate when it offers a smaller measurement error [2.1]. Accuracy requires both trueness and precision, and undoubtedly Archer 2 is the most accurate archer. Archer 4 comes closest, with the same trueness but a lower precision score than Archer 2. It is arguable which of the archers 3 or 1 are more accurate: Archer 1 scores best on trueness, Archer 3 scores best in precision.

2.2.2 From measurement error to measurement uncertainty

Archer 3, having a systematic deviation from bull's eye should be able to improve his performance by adjusting his aim. This corresponds with good metrological practice: determine measurement bias (= an estimate of systematic measurement error) and correct for it. For a real measurement, a bias might, for example, be associated with a measurement instrument. Calibration to traceable measurement standards will enable the instrument bias to be evaluated and then corrected for.

However, since time and financial resources do never allow to evaluate, quantify and control or correct for all measurement errors, some unquantified errors will remain and inevitable lead to uncertainty about the accuracy of the measurement result, or measurement uncertainty. Even if the underlying measurement errors are unquantified, it is possible, and required, to estimate measurement uncertainty. Several techniques for uncertainty estimation are known and described, for example in [2.2].

2.3 Estimation and combination of uncertainty contributions

One distinguishes two methods to assess contributions to measurement uncertainty: type A and type B.

A type A uncertainty estimate is deduced from the results of repeated experiments to assesses a random component of measurement uncertainty: the experiments determine how much the measurement results vary if the measurement is repeated.

Type B uncertainties are not determined by (repeating) experiments, but are estimates based on operator experience or taken from documents such as calibration certificates. Type B assessments are needed, for example, if a measurement is performed on an instrument that is calibrated with a calibrator[4]. The certified value of the calibrator is not without uncertainty. The uncertainty of the certified value of the calibrator is a type B uncertainty.

[4] A measurement standard, or etalon, is a realization of the definition of a given quantity, with stated quantity value and associated measurement uncertainty, used as a reference [2.1]; measurement standards used for calibration are called calibrator, calibration artefact, or calibrant, terms which will be used throughout this Introductory Guide.

In practice, most measurements are affected by a number of sources of uncertainty. Therefore, the total measurement uncertainty is usually calculated by combining several uncertainty contributions. In this calculation, one uses the following conventions:

- *s* is a standard deviation, obtained from a series of repeated experimental observations.
- *u* is a standard uncertainty (an uncertainty at a confidence level of about 68 %).
- *k* is a coverage factor, which is used to calculate a larger 'expanded' uncertainty, *U*, from the standard uncertainty u: $U = k \cdot u$. Expanded uncertainties are often used because the confidence level of a standard uncertainty is not satisfactory.
- If different probability distributions have to be combined, this is done at the level of standard uncertainties; the resulting combined standard uncertainty is converted to an expanded uncertainty.

Normal (Gaussian) and rectangular distributions are the most frequently encountered statistical distributions, so it is very important to have some understanding of these. Let's start with the normal distribution.

- The area below the curve is 1, corresponding to 100 % of the measurements.
- The standard uncertainty of the mean value $u = \frac{s}{\sqrt{n}}$, with *n* = number of (independent) replicates.
- Coverage factor *k* = 2 for about 95 % expanded uncertainty.

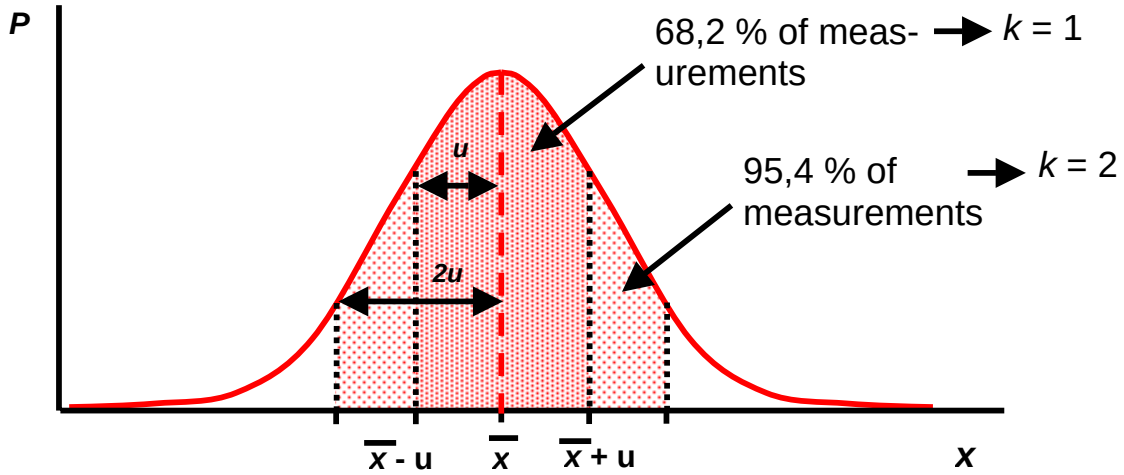

Figure 2.3.1 *Features of a normal probability distribution.*

For the rectangular probability distribution we have likewise:

- Area below curve is 1 corresponding to 100 % of the measurements
- Coverage factor $k = \sqrt{3}$ for about 95 % expanded uncertainty $U = k \cdot u$
- Standard uncertainty for a distribution of width *a*: $u = \frac{a}{2\sqrt{3}}$

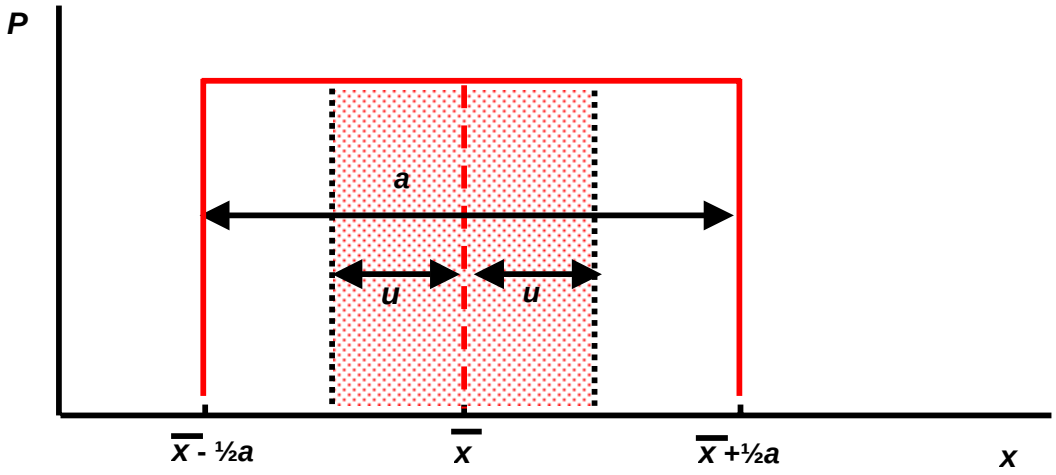

Figure 2.3.2 *Features of a rectangular probability distribution.*

2.4 Reference materials and certified reference materials

Reference materials are essential metrology tools and come in different classes and categories [2.3]. The minimum requirement for any reference material (RM) is to be sufficiently homogeneous and sufficiently stable with respect to a specified property, to be used for a specified step in the operation or verification of an instrument or measurement method. Traceability of a measurement result can be ensured by calibration of a measurement system with an appropriate calibrant, which must have the characteristics of a certified reference material (CRM). CRMs are characterised by a metrologically valid procedure, and come with a certificate, which summarizes the certified value, the corresponding uncertainty, a statement related to the metrological traceability and a reference to the used measurement method(s).

If we look at a CRM for thickness measurements (consisting, for example, of a SiO_2 layer on a Si substrate), the certificate states the certified thickness, a refractive index dispersion formula for a given wavelength range and the corresponding uncertainties. Furthermore the CRM may have been characterised with several techniques probing thickness and refractive index independently.

Numbered references:

[2.1] ISO/IEC Guide 99:2007, International vocabulary of metrology – Basic and general concepts and associated terms (VIM), 2007; also known and available as JCGM 200:2008, JCGM 2008. www.bipm.org.

[2.2] ISO/IEC Guide 98-3:2008, Uncertainty of measurement – part 3: Guide to the expression of uncertainty in measurement (GUM:1995), 2008; also known and available as JCGM 100:2008, JCGM 2008. www.bipm.org.

[2.3] Emons, H., The 'RM family' – Identification of all of its members, Accred. Qual. Assur., Vol. 10, p. 690-691, 2006.

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3 Metrology at the nanoscale: case studies

3.1 Metrology for dimensional analysis of thin films

Thin films and coatings often have a thickness, or internal features (e.g., sublayers) with critical dimensions less than 100 nm. The characterisation of these films and coatings is an important branch of nanometrology, with a large variety of measurement problems and tasks. Necessarily, this section has been focused on the particular area of *dimensional characterisation* of homogeneous or layered thin films, i.e. *films with an in-plane homogeneity*:

Films with in-plane homogeneity: more techniques are available for the characterisation of these specific films, because the lateral resolution of the measurement methods is less critical than the thickness. Examples of applications for this kind of films are:

- Semiconductors and dielectrics
- Optical components
- Wear resistant coatings
- Solar cells

The first step in obtaining the required functionality is the growth of a homogenous thin film by a layer formation process (e.g., physical vapour deposition, (atomic layer) chemical vapour deposition). Often, the layer formation process consists of a layer-by-layer method by which the first layer is deposited on top of the substrate and all other layers on top of the previous layers. Each film layer may have a different chemical mixture and thickness. A common method for layer formation is chemical vapour deposition. In chemical vapour deposition chemical reaction between gasses produces the layers on a substrate. For example, silicon dioxide layers can be manufactured by applying silane (SiH_4) and oxygen gases, which react and form silicon dioxide and hydrogen.

Dimensional characterisation of thin films: As more complex films are manufactured, a better control of all process parameters that may affect the final functionality, becomes more crucial. In parallel, the challenges for reliable measurements increase. Typical nanoscale measurement needs within this field are listed below.

- Thickness and density of the thin film
- Morphology of the thin film outer surface
- For multilayer films, the thickness, roughness and density of the individual sublayers
- Chemical compositions of the individual layers
- Uniformity of the individual layers
- Surface quality and material of the substrate
- Functional properties of the film (e.g., adhesion strength, hardness, friction coefficient, wear resistance)

It must be acknowledged that the quality of a thin film is often best assessed via a direct measurement of its desired functional property (wear resistance, friction coefficient). Nevertheless, it was chosen to limit the scope of this section to the dimensional (thickness) characterisation. For thickness measurements there are in general two classes of instruments: imaging and non-imaging. In the case of imaging techniques the dimensions can be obtained directly from the image by measuring the distance between marks on the image, and by comparing this distance with the image scale. In the case of thin film investigations, the image scale can be calibrated, for example by measuring the known lattice spacing of the substrate material as a built-in reference length scale. In non-imaging techniques one typically considers a mathematical model of the layers to represent the physical layers of the thin film. Each of these mathematical layers essentially is a set of equations and parameters to represent the corresponding physical layer. The dimensions are obtained by varying the mathematical parameters using a best-fit procedure between experimental data and calculated values. An important exception is the X-ray reflectometry (XRR). With this method the layer thickness can directly be obtained for a single layer system and periodic multilayer systems. For a single layer system the absolute layer thickness is found directly from the measured parameters wavelength and reflection angle via an analytical equation. Moreover, the use of the auto-correlation function (extracted from the derivative of density profiles obtained via a Fourier transform) can provide useful layer thickness measurements from samples that are too complex for a simple direct simulation and fitting approach. An overview of selected available methods is given in Table 3.1.1.

Case study: Measurement of ultrathin silicon oxide thickness

This case study is focused on thickness measurement, however, chemical composition and thickness cannot be separated at ultrathin thickness since the film is in a dynamical equilibrium with the surroundings. Furthermore, the thickness measured in this case study is at or close to the technical limits of the instruments. The case describes an international comparison of thickness measurements of ultrathin (1.5 nm to 8 nm, nominal) SiO_2 layers formed on Si wafers. Here it is to be noted that the industry requires not only accuracy, but also techniques able to measure a statistically significant number of sites or devices on multiple wafers within an economic timeframe.

Defining the problem: With the following example of thickness measurement at nanoscale, different aspects of analysing the requested task and staying below a required uncertainty and the specific features of different measurement technologies are illuminated at a practical job. A company has the contract to produce Si wafers with a thermal oxide layer on top with well-defined thickness. The thickness of this layer is expected to be approximately $d_{ox} \approx 4$ nm. Due to specifications of the client, a quality management system must be installed, including verification measurements of the oxide layer thickness with a relative standard uncertainty of 10 % of the mean value.

Measurement approach	Technique (abbreviation)	Depth calibration parameters & artefacts	Primary sensitivity
Cross-section image analysis	Scanning electron microscopy (SEM)	Grating period	Interface contrast
	Focused ion beam microscopy (FIB)	Grating period	Interface contrast
	Transmission electron microscopy (TEM)	Grating period or lattice parameter	Interface contrast
Tactile step-height analysis	Atomic force microscopy (AFM)	Height calibration standard	Surface
	Stylus profilometry (profilometry)	Height calibration standard	Surface
Optical techniques	X-ray reflectometry (XRR)	λ, angle	Interface density changes
	Optical reflectometry (OR)	λ, n, k, angle	Interface & film
	Spectroscopic ellipsometry (SE)	λ, n, k, angle, phase	Interface & film
	Optical (visible-IR) interferometry (OI)	λ, n, k, angle	Interface & film
Photon- or electron-spectroscopy	X-ray photoelectron spectroscopy (XPS)	Electron inelastic mean free path; density	Surface
	Auger electron spectroscopy (AES)	Electron mean free path; density,	Film
	Total reflection X-ray fluorescence analysis (TXRF)	Fluorescence yields; density	Film
	Electron beam X-ray spectrometry (EDX) / Electron Probe Micro-Analysis (EPMA)	Fluorescence yields; density	Film
	Fourier transform infrared spectroscopy (FTIR)	Absorption coefficient	Film
Ion beam spectroscopy	Rutherford backscattering spectrometry (RBS)	Energy scale; angle; density	Film
	Medium and Low energy ion scattering (MEIS / LEIS)	Energy loss scale; Angle; density	Film
	Secondary ion mass spectrometry (SIMS)	Measurement of crater depth after sputtering	Film

Table 3.1.1 *Overview of often-used techniques for thin film thickness measurement with sensitivity for the nanometric range [3.1.1].*

Since the client explicitly asks for accuracy (i.e., trueness and precision) and for SI-traceability[5] (i.e., with an unbroken chain of calibrations between the measurement result and the relevant SI unit), a careful method selection must be performed. The list of possible thickness measurement techniques at the nanoscale is very

[5] SI traceability in itself requires an unbroken chain of calibrations with their associated uncertainties. It does not require the final result to be either precise or accurate, just that it is known in traceably calibrated units with an associated uncertainty.

long and at the same time confusing [3.1.2]. In a first step a set of five methods has been preselected for a detailed assessment with respect to the specification list. These methods are:

i) X-ray reflectometry (XRR)

ii) Spectral ellipsometry (SE)

iii) X-ray photoelectron spectroscopy (XPS)

iv) Total reflection X-ray fluorescence analysis (TXRF)

iv) Transmission electron microscopy (TEM)

These five methods can be divided into three groups. The first two (XRR and SE) are utilising electromagnetic waves at different wavelength or energies respectively. They are both measuring directly the thickness of a thin film by an interferometric approach.

The next two (XPS and TXRF) both excite the atoms of the material with ionisation radiation. The electrons (XPS) or X-ray photons (TXRF) that are subsequently emitted by the excited atoms, are detected and analysed. In contrast to the first methods, XPS and TXRF are not length measuring methods. Only after an additional calculation, the thickness of a layer can be derived from the raw data, via the knowledge of the layer density. On the other hand, XPS and TXRF deliver additional information about the chemical composition of the oxide.

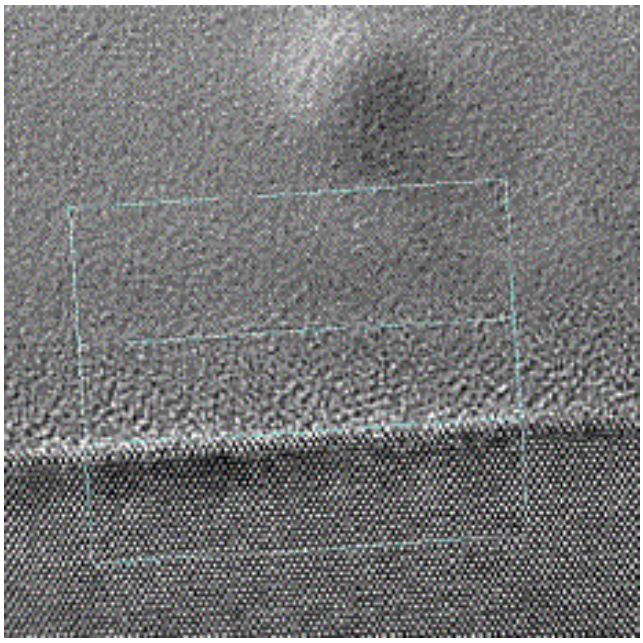

Figure 3.1.1 *TEM image of a cross-section of an amorphous SiO_x interface layer sandwiched between a crystalline Si substrate and a SiO_2 film [3.1.3]. The micrograph demonstrates the atomic resolution of TEM, as seen in the periodic structure of the Si grain, and the inherent problem for any thin film thickness measurement:How to identify exactly the substrate – oxide and oxide – capping material interfaces.. In imaging methods (such as TEM) the answer to the latter is highly depending on the experience of the TEM operator; in non-imaging methods the issue is equally critical, and depending on assumptions made in the treatment of the raw data of the method.*

TEM uses a completely different approach to the problem, since it is an imaging technique which can achieve atomic resolution (Figure 3.1.1). Because of this unique resolution, it is often assumed that TEM automatically has a very high accuracy. This assumption is, however, only valid if the image scale is correctly calibrated and if the image contrast between the thin film and the material surrounding it, is high enough. Furthermore, TEM has some additional complications, which will be described later on. Still, TEM can see the variability in the interface, whereas non-imaging technologies return an averaged value. In the initial stage of process development TEM pictures can therefore give important information on the quality of the oxide film formation and it can be used to justify the applied model in the non-imaging technologies.

Table 3.1.2 gives a survey of the assessment of the five methods with respect to the specification list given above. This assessment does not aim to identify a clear "winner". There is no "perfect solution" but at best good compromises. It is up to the expertise of the person entrusted with the measurement task, to find the best method, or, possibly, a combination of methods, to meet his customer's demands.

Specification	**XRR**	**SE**	**XPS**	**XRF**	**TEM**
Non destructive	yes	yes	yes	yes	no
Accuracy	very high	high	good[1)]	good	good
Directly applicable	yes	yes	yes	yes	no
Traceability	via calibration of goniometer	via artefact	via artefact	via artefact	via calibration of image scale with defined lattice parameter
Si/SiO_2 compatibility	(good)[2)]	very good	good	good	good
Measurement time (incl. sample preparation)	slow -moderate (hours)	very fast (10 seconds)	moderate (minutes)	moderate (minutes)	very slow (days)
Precision	high	very high	good	good	good

[1)] Only in reference geometry

[2)] For photon energies around the oxygen or silicon K absorption edge, accessible only with synchrotron radiation [3.1.4].

Table 3.1.2 *Overview of the selected instruments with respect to the presented specification list.*

In this example, XRR and TEM provide the shortest traceability chain. Looking closer to these two methods, and with the measurement task in mind (thickness measurement of an in-plane homogeneous thin film) the XRR is clearly the better option, since TEM is a destructive method that requires time-intensive sample preparation. As a result, XRR is preferred in this comparison. It must be reminded that for the investigation of the structure of a non-homogeneous, 3D structured film, only TEM offers the required lateral resolution.

Coming back to the remaining set of technologies, three of the methods are using X-ray radiation. This is an important topic for in-line measurement, because a special security environment and specially trained staff for the operation of ionisation devices is required. XPS in addition requires an ultra high vacuum during measurement. Therefore a special system of vacuum locks for loading the samples must also be installed in the manufacturing line.

On the other hand XPS has the benefit to give additional insights about binding states of the sample material. Due to the fact, that only the thickness is required and no additional information of the sample chemistry is requested XPS and XRF are excludes from the list.

The remaining two candidates (XRR and SE) show a balanced result in this assessment. The main benefits of SE are the very short measurement times and its very good in-line capability, as it can operate in ambient conditions. Furthermore, SE is a reliable technique in operation with relatively low operating costs, since it is used in the visible range of the electromagnetic spectrum.

On the other hand XRR provides the best accuracy and has a short traceability chain to the SI units, but it requires a higher effort, both in the set-up and in the operation. Additionally, the cost of this equipment is higher compared to SE.

To summarise: non of the both remaining techniques is a perfect solution to the pending problem of measuring the thickness of approximately 4 nm thick SiO_2 films on Si wafer. SE is clearly the best choice for in-line measurement (fast, precise, low price). On the other hand, XRR performs better in terms of traceability and accuracy, i.e. if well calibrated and operated, it guarantees the requested uncertainty.

To overcome this challenge and to establish the appropriate system for the measurement task would be a combination of both methods via calibration.

Intermission 3.1.1: Calibration

Typically the result of a measurement has a statistical component and often it has a systematic deviation from the true value. The aim of a calibration is, to estimate the value of x, for instance via a comparison of the values determined by two independent methods or laboratories on the same calibrator and with identical measurement conditions. The uncertainty of the calibration must be included into the overall uncertainty of the final result.

Fit-for-purpose calibrants, which can be purchased from CRM producers, help to create an unbroken chain of calibrations, establishing traceability of the measurement results to the SI system of units.

In the given example the final set-up for the thickness measurements would use SE for in-line measurements. This instrument must be periodically calibrated with an appropriate CRM (for example a Si wafer with a SiO_2 layer that has a certified thickness) that has been measured before, e.g., via XRR. CRMs for instrument calibration are typically available from different CRM producers and/or their authorised distributors. The CRMs must come with a certificate, which summarises the measurand, the certified value and the corresponding uncertainty, a statement related to the metrological traceability, and a reference to the used measurement method(s). Therefore the SE can be calibrated with a CRM, to establish traceable in-line thickness measurement. An expanded uncertainty (k = 2) of $U(d_{ox})$ = 0.4 nm can be established by the available equipment (SE) and calibration artefacts. The user can repeat the calibration every time it is required by the quality management system.

In conclusion of this example, three points are to be remembered:

1. A universal technique for thickness measurements at the nanoscale does not exist. Every measurement task needs a dedicated analysis, to identify and select the most appropriate measurement method, adjusted to the problem.
2. For the successful search of the appropriate set-up, one first needs to decide whether a precise (repeatable) determination of the thickness is sufficient, whether trueness is also important (requiring absence of bias from a well-chosen reference), and whether the chosen reference point must be the SI system of units (implying the need for SI-traceable results).
3. In many cases reference materials (calibrants or quality control materials) of CRM producers provide a valuable tool for use in a quality management system for the thickness measurement at the nanoscale.

Numbered references:

[3.1.1] Co-Nanomet Discussion papers, ISBN 978-0-9566809-0-7, 2010; www.co-nanomet.eu.

[3.1.2] M.P. Seah, W.E.S. Unger, Hai Wang, W. Jordaan, Th. Gross, J.A. Dura, Dae Won Moon, P. Totarong, M. Krumrey, R. Hauert and Mo Zhiqiang, *Ultra-thin SiO_2 on Si IX: Absolute measurements of the amount of silicon oxide as a thickness of SiO_2 on Si.,* Surf. Interface Anal. 41, 430 – 439 (2009)

[3.1.3] (TEM picture).

[3.1.4] M. Krumrey, M. Hoffmann, G. Ulm, K. Hasche and P. Thomsen-Schmidt, Thickness determination for SiO_2 films on Si with X-ray reflectometry at the Si K edge, Thin Solid Films 459, 241 – 244 (2004)

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ISO/IEC Guide 99:2007, International vocabulary of metrology – Basic and general concepts and associ-ated terms (VIM), also known and available as JCGM 200:2008, JCGM 2008. www.bipm.org

3.2 Metrology for dimensional analysis of structured surface

Samples with structured surface features are classified as being in the nanometrology domain if the smallest feature size, the critical dimension, is less than 100 nm. Such structures are nowadays part of many industrial devices and applications including:

- Semiconductors and integrated circuits
- Micro-electro-mechanical systems
- Biomedical devices
- Optical devices

In all cases the structure plays an important role in obtaining the desired functionality. One way to produce a structured sample can be summarised as follows:

1. The first process, called (etch) masking, involves the preparation of non-uniform layers of different materials stacked on top of a substrate, called an etch mask.
2. The second step is the etching step. This process typically uses a strong acid or a plasma to oxidise or sputter into the surface of the etch mask, thereby transferring the critical dimension (CD) of the mask to the target sample. The physico-chemical properties of the etch mask layers and the applied etching method are important factors which determine the final etch rate.

As more complex structures are manufactured, a tighter control of the dimensional parameters is needed and the challenges for measurements increase. Typical parameters to be measured within this field are height, width, angle, pitch (of periodic structures) and diameter (of e.g., particles).

The measurement quantities dealt with in this section are dimensional. The sizes of the structures or features define the physical behaviour of the whole system – even the material properties may change, if the structures are smaller than a certain limit. Furthermore, dimensional properties are needed as a reference for locating the position of any other measured (e.g., chemical) properties. Other aspects, such as chemical composition, are equally relevant but are not treated here.

The measurement ranges of several measurement methods, within dimensional metrology, are illustrated in Figure 3.2.1. The more an instrument reaches towards the lower left corner of the plot, the higher is its resolution. The figure also help to identify the needs for future activities, if nano-measurement instrumentation and methods are to reach the same degree of coverage with physical transfer standards and uniformly agreed measurement procedures as already established at larger scales.

While optical microscopes [3.2.1], like interference microscopes and to a lesser degree confocal microscopes [3.2.2], are limited in their lateral resolution by the diffraction limit, their much better vertical resolution capabilities make these well-established instruments interesting for nanometrology as long as a higher lateral resolution is not demanded. Their main advantage is that they are rather fast compared to scanning and tactile techniques and allow a large surface section to be investigated quickly.

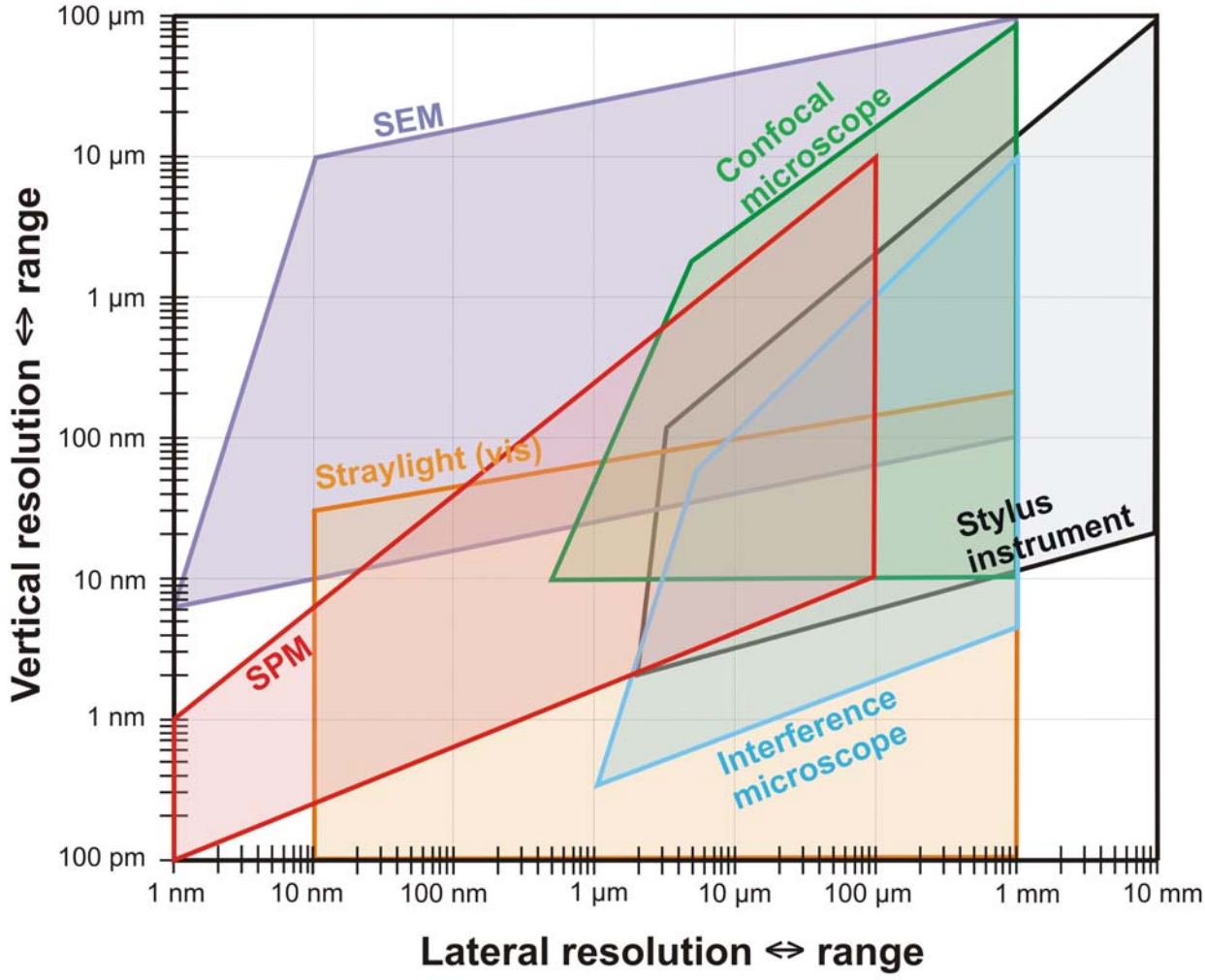


Figure 3.2.1 *Measurement techniques and their typical ranges in the lateral and vertical directions. The left and lower boundaries of the polygons show the typical resolution limits.*

On the other hand, light scattering techniques yield dimensional information about very small particles on surfaces [3.2.3]. A similar integral method, e. g., averaging over a surface area, is diffractometry and scatterometry. Both techniques offer fast and inexpensive ways to determine the mean pitch and dimensional parameters of periodic structures with lateral resolution of a few nanometres. Furthermore, scatterometry if calibrated has the additional advantage that it is a non-destructive technique capable of measuring very deep structures with a small pitch and periodic structure embedded in a material.[6] However, scatterometry is classified as a non-imaging technique that finds the dimensions by varying the parameters in a mathematical model of the structure using a best-fit procedure between data and calculated values (inverse method). This best-fit procedure requires powerful computer resources for complex structures. For this reason scatterometry is mainly applied to nanometrology of simple structures.

[6] This require that the materials above the structure are semitransparent to the applied wavelength(s)

The scanning electron microscope (SEM) is another versatile tool with a large dynamic range of magnifications [3.2.4]. The latest technology advances now permit imaging with lateral resolution into the sub-nm range resolution. Using an SEM in the secondary electron (SE) detection mode results in morphology contrast with a strong slope sensitivity. Thus while this is very well suited for 2D lateral dimensional metrology, access to sample height information is limited to cross-sectioning (i. e. a sample-destructive method, e.g., via focused ion beam technology) or viewing with different tilt angles. The ability of SEM to zoom from the millimetre scale to the nanoscale gives valuable information about the morphology over a very large dynamic range. Due to the difficulties in measuring height, SEM is primarily used for measuring the critical dimensions of lateral structures and its use within 3D dimensional metrology is limited.

The use of tactile techniques (techniques with mechanical probes like atomic force microscopy (AFM) and profilometry) within nanometrology depends largely on the size of the artefact being measured, the radius of the stylus or tip, and the force applied when scanning the surface [3.2.5]. Since profilometry requires a relatively large force and since it uses tips with radii of several or many micrometres, it cannot be used to measure nanostructures. AFM on the other hand is well suited for nanometrology since it applies a small force and since the apex of the tip is at the nanoscale (e.g., 15 nm). The dynamical range of AFMs is limited and they need an auxiliary optical microscope to locate the region of interest, as the scan ranges are mostly limited to microscopic dimensions. It is a popular quote to say that AFMs provide "2½D" information from the surface: information can be obtained from large X-Y-areas, but is limited in the third dimension, due to a limited vertical range of about 10 µm. AFM measurements are therefore restricted to nearly planar objects and their capability to study narrow spaced structures is furthermore limited by the tip shape. Within the dynamic range the AFM tip can scan the surface height with sub-nanometre resolution and thereby obtain topographic images of the surface. The dimensional parameters deduced from the images depend on the "effective tip shape", which is defined by the tip geometry but also by e.g., feedback control parameters and tip-surface interactions: the sample features observed on the AFM image are the result of a convolution of the tip shape and the shape of the actual sample feature modified by the probe-sample interaction. Much mathematical effort has been made to obtain the precise tip shape from scanning of reference samples and to allow tip deconvolution [3.2.6]. For measurements of features sufficiently larger than the tip radius, this allows correct dimensional parameters to be obtained from the image. For this reason AFM is the main workhorse within dimensional nanometrology for measuring structures not being too deep or too finely spaced.

Additionally, transmission electron microscopy (TEM) should be mentioned [3.2.7], as this remains the sole technique capable of Angstrom-level resolution of internal structures (e.g., layers and interfaces) and is thus a core nanometrology tool. The expense of high-resolution instruments, and the difficulty of preparing electron transparent sample foils from the regions of interest, can act as barriers to wider usage, though the information obtained, with achievement of atomic-level lattice imaging, is unparalleled. For these reasons it is not widely used.

To ensure comparability and stability of results obtained by different instruments it is necessary to link the measurement results to the SI system of units. The practical requirements to achieve SI-traceable results from diffractometry and interference microscopy are well established. Traceability for AFM results has only been addressed recently and has been studied in international comparisons of measurements results obtained with AFM, diffractometers and interference microscopes. It is important to point out that the direct traceability of AFM results can only be obtained using built-in interferometers, to calibrate the scan axis. Therefore, to have traceable 3D results, one needs to have an interferometer set-up on each scanning axis.

Still, it should be noted that this does not take into account any tip-sample interactions. The traceability chain for AFM results is shown in Figure 3.2.2 [3.2.8].

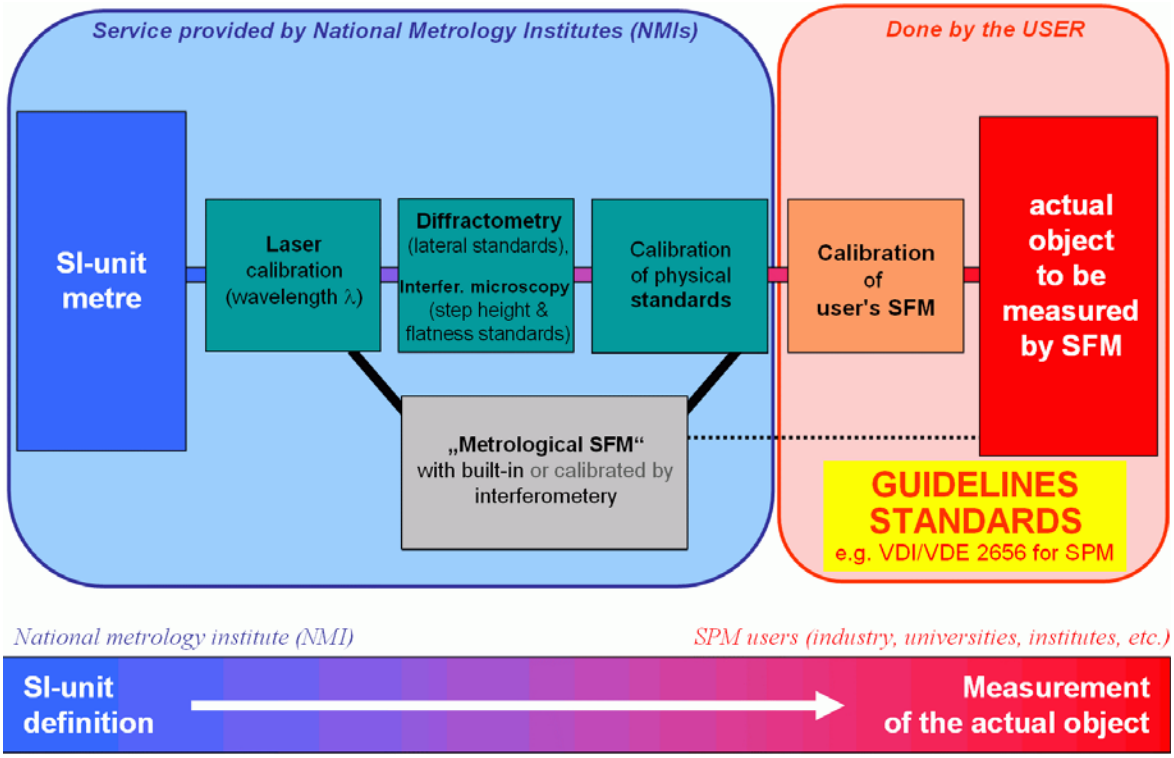


Figure 3.2.2 *Traceability chain for AFM using either diffractometry and interference microscopy or metrological AFM to establish SI-traceability; implicit assumption: no tip-sample interaction.*

The use of metrological AFM is important to directly obtaining SI-traceable results within areas not covered by the other techniques. This advanced set-up is, because of its complexity, used mainly by NMIs. Measurement results coming from non direct traceable instruments (conventional AFM, SEM/TEM, Scatterometry, Microscopy) can only be traceable to the SI metre, if the broken SI-traceability chain is re-joined, e.g., by calibration of the three measurement scale axes using calibration standards, called artefacts.

Table 3.2.1 summarises the main advantages and disadvantages of the above discussed methods. This assessment does not identify a clear "winner" for measurement tasks at the nanoscale. There is no "perfect solution" but at best good compromises. It is up to the experience of the user, to find the best method for their specific CD measurement task. However, if one is looking for a general-purpose instrument for precision measurements at the nanoscale, AFM is certainly a good choice.

In this guide we will consider AFM and optical diffraction techniques for finding the geometry and pitch of periodic test structures. Periodic test structures are widely applied in industry to study how manufacturing processes may change the geometry of the device to be manufactured. For this reason periodic test structures are placed next to the device. A widely used periodic test structure for measuring shrinkage in the lateral directions is made of two orthogonal periodic line structures.

Specification	Metrological AFM	Scatterometry	Microscopy[1)]	Diffractometry	SEM / TEM
Non destructive	yes	yes	yes	yes	no
Accuracy	very high	high	good	very high	high
Directly applicable	yes	yes	yes	yes	no
SI-traceability	via laser interferometers	via calibrator	via calibrator	via calibration of goniometer	via calibration of image scale with defined lattice parameter
Chemical information	(yes)[2)]	(yes)[3)]	(yes)[3)]	no	yes
Moderate structure height, width and spacing	yes	yes	yes	no	yes[5)]
Deep or narrow spaced structure	no	yes	no	no	yes[5)]
Embedded structure	no	yes	no	no	yes[5)]
Pitch	yes	yes[4)]	no	yes	yes
Measurement speed	hours	minutes	tens of seconds	minutes	SEM: hours, TEM: days
Precision	very high	high	good	very high	high
Other restrictions		only periodic structures		only periodic structures	

[1)] This column covers confocal and optical microscopes

[2)] Can detect material boundaries if the hardness is different

[3)] Can detect material boundaries if the refractive index is different

[4)] Only angular scatterometry

[5)] Cross sectional view

Table 3.2.1 *Overview of the main advantages and disadvantages of the discussed methods.*

Case study: Geometry and pitch measurement of periodic structures.

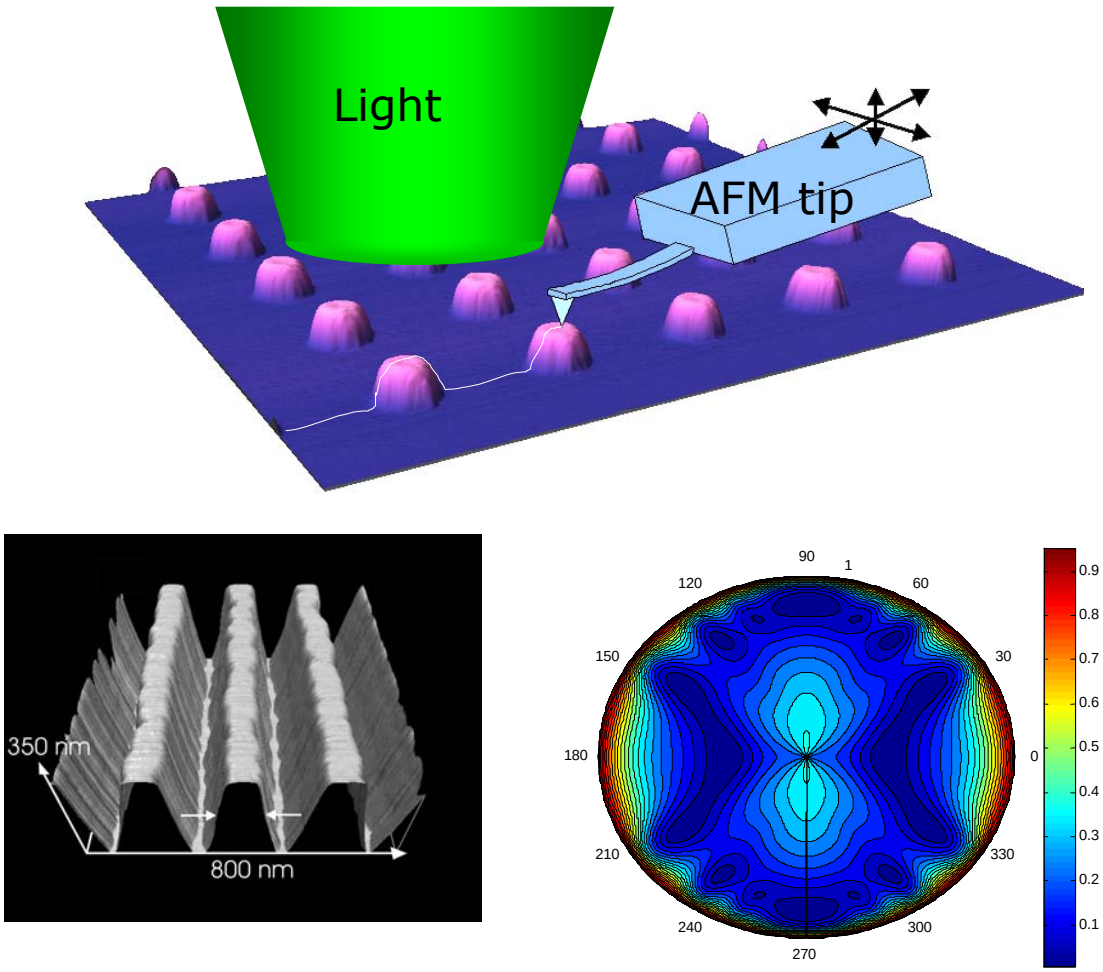


Figure 3.2.3*:Illustration of light and AFM interaction with a periodic surface structure (top). The figure clearly shows that the laser-light interaction is an integral characterisation technique investigating the surface region by region, whereas AFM is a local scanning technique, continuously probing the surface with a sharp tip. The bottom pictures show typical images of line scans obtained with AFM (left) and a reflectance simulation for single wavelength angular scatterometry (right). Note that in the scatterometry image we do not see any details of the structure only a collective response.*

This case compares global optical pitch measurements with the measurement of the local geometry of periodic structures called gratings. But before we start, we will consider how the two techniques interact with a surface structure. An AFM is a local probing technique which traces the topography of the sample surface with a tip. Optical methods are integral techniques in the sense that they collect information from larger areas than AFMs, see Figure 3.2.3. A pitch measurement comparison between optical methods and AFMs is thus only meaningful if one takes the average of many AFM measurements over many periods of the periodic structure, and if the measurements are performed in the same area(s).

Pitch measurements using diffractometry rely on the fact that a periodic structure emits all of the diffracted light at a number of discrete diffraction angles for a given angle of incident. Amongst the diffraction angles, there exists a special angle, termed the Littrow angle, for which the light reflects back along the same path as the incident light beam. This allows a very simple setup for accurate pitch determination as shown in Figure 3.2.4.

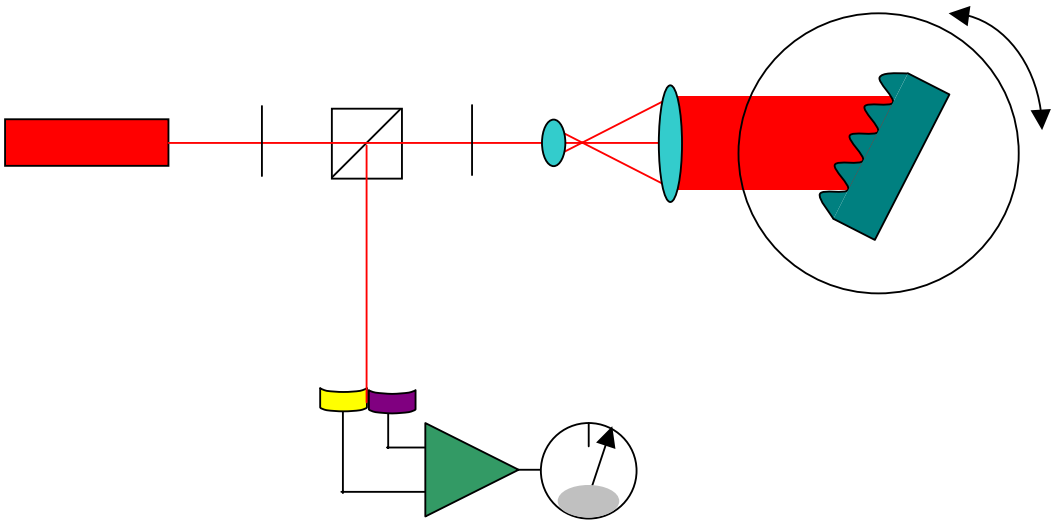


Figure 3.2.4: *Diffractometry setup for pitch measurement.*

A well-calibrated diffraction setup has its zero angle position determined by the collinear alignment of the incident and the reflected light from a reference sample[7]. In this position, the null detector in Figure 3.2.4 shows approximately zero. Switching to the grating sample we rotate and align the sample until the null detector shows approximately the same value again. This position marks the Littrow angle (θ). Rotating the grating in the opposite direction with respect to normal incident, we can find the mirrored Littrow angle. Taking the average of the angles we can determine the mean pitch p from the following equation.

$$p = \frac{m\lambda}{2n\sin(\theta)}\left(1+\alpha_s \Delta T\right)+e_m$$

Where m is the diffraction order, λ is the vacuum wavelength of the laser, n is the refractive index of air, α_s is the thermal expansion coefficient of the grating material, ΔT is the temperature deviation from 20 °C and e_m is the error due to the measurement repeatability. To control the number of unknowns variables, measurements are usually performed with a stabilised laser with known wavelength in a temperature, humidity and pressure controlled environment. This leaves us with one unknown n that can be determined from the modified Edlen equation. We are thus left with the following equation of known variables.

$$p = \frac{m\lambda}{2n\sin(\theta)}+e_m$$

[7] If an optical isolator is inserted after the laser no light is sent back into the laser.

In this equation, e_m represents the fact that even though we make an accurate measurement we do not get the same answer each time. If the measurement repeatability is perfectly random around the target value, e_m will not contribute to the average pitch measurements. However, it will contribute to its uncertainty. This point has been discussed in more detail in chapter 2. Each of these known variables has an associated uncertainty that will affect how precise we can determine the average pitch.

Intermission 3.2.1: Uncertainty contributions of diffractometry Major sources of uncertainty are the rotary table, and to a lesser degree the laser wavelength and the refractive index of air. To estimate the uncertainty from each of these contributors we need knowledge of the values and distribution to be used. This knowledge might be obtained from measurement results, literature or manufacture statements.

Rotary table: For the resolution we only have a manufacturer's statement for the width of the distribution, so we have to assume a rectangular distribution.

Wavelength: We have experimental observations that show that we can use a normal distribution.

Refractive index: We only have a mean value and a distribution width from literature, so we choose a rectangular distribution.

Measurement repeatability: We have experimental observations that show that we can use a normal distribution.

The values are inserted into Table 3.2.2

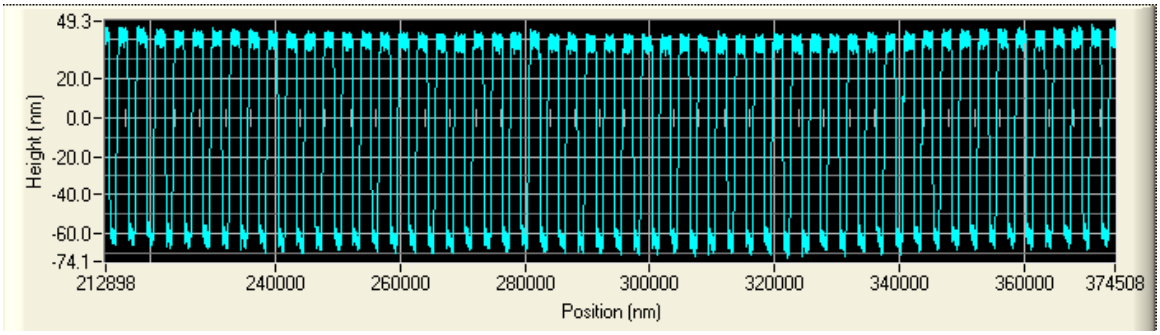
Using one of the available uncertainty calculators we are able to estimate the mean value and the uncertainty of our measurements: our effort has resulted in a mean pitch of 1822,0 nm with an expanded uncertainty of 1,2 nm (Table 3.2.2).

Quantity (unit)	Distribution	x_i	$u(x_i)$
Wavelength (nm)	Normal	632,8289	0,0005
Mean angle and uncertainties (rad)	Rectangular	0,1745	5,774E-05
Refractive index	Rectangular	1,000256	1,155E-06
Repeatability (nm)	Normal	0	0,0011
	Normal	1822,032068	0,5967123
	Conf. level =	95 %	k = 2
	Result =	1822,0 nm	U = 1,2 nm

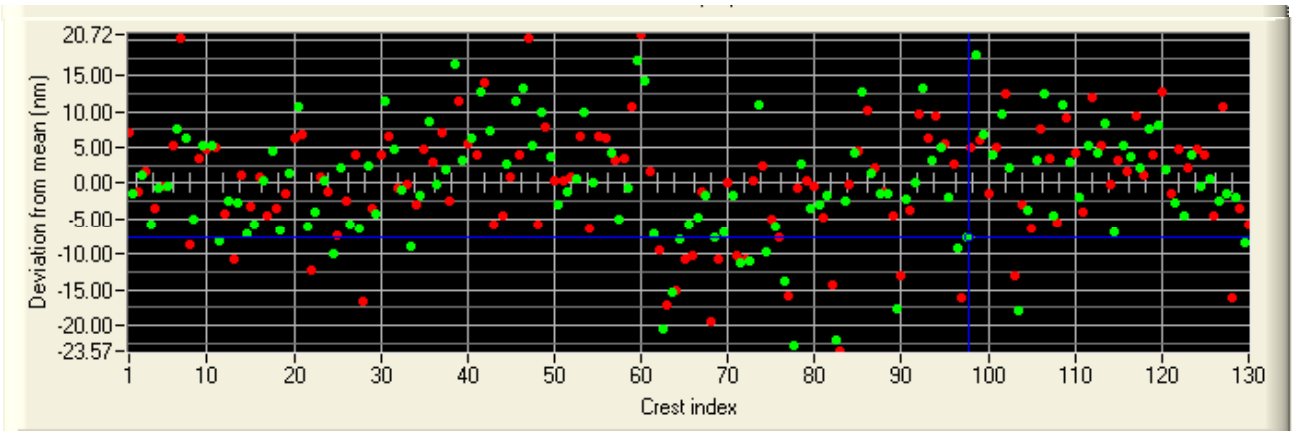
Table 3.2.2: *Result of uncertainty calculations.*

Pitch measurements using AFM: Figure 3.2.5 shows how AFM can be used to measure the pitch of periodic nanostructures. A grating similar to the one shown in Figure 3.2.3 was scanned, and a profile along a scanned line is taken (Figure 3.2.5). In order to determine the average pitch, the scanned data must first be corrected for probing artefacts such as non-linearities and slopes during the measurements. Secondly, one may choose to average several scan lines to minimise measurement noise. Thirdly the centre of gravity within each crest of the profile hill/valley of the averaged line scan is found. The mean pitch is then found as

the mean distance between the centres of gravity. The variations of the detected centres of gravity then allow the calculation of the uncertainty of the mean pitch. Fourier transformation of the periodic surface profile is an alternative method to measure the mean pitch. Comparing the diffration with the AFM shows directly that the AFM provides local information about the pitch of single lines.



A one-dimensional grating with a nominal pitch of approximately 3 µm was scanned over 161 µm with a long range AFM. The height of the grating is approximately 100 nm. The measured profile is plotted above. Subsequently, the position of each crest of the profile is determined by calculation of its centre of gravity.



Position deviation of the centres of gravity in the real grating compared to the ideally designed grating with a constant mean pitch. The red dots represent the deviations for the lines when scanning from left to right, green dots when scanning from right to left. The individual lines do not differ from the mean pitch by more than 20 nm.

Figure 3.2.5 : *Illustration of centre of gravity method used by AFM to measure the mean pitch.*

Profile measurements using scatterometry: Figure 3.2.3 shows how light interacts with a nanostructured area on a substrate. It is clear that the light cannot resolve the structure in a traditional optical microscope, but it is always possible to study the collective response from the nanostructures within the interaction area. This is what scatterometry is about: *scatterometry is the study of the collective amplitude and/or phase response from nanostructures*. Two common implementations of scatterometry are

angular scatterometry and spectroscopic scatterometry. In the first case one uses a fixed wavelength and fixed angle of incidence and one detects the signal from all the diffraction orders. In the second case one uses many wavelengths at a fixed angle of incidence and one detects the signal from one of the diffracted orders. Both implementations are examples of non-imaging techniques where one has a mathematical representation of the nanostructures. The nanostructure dimensions are obtained by varying the mathematical parameters using a best-fit procedure between data and calculated values [3.2.9]. Examples of the two methods are presented in Figure 3.2.6.

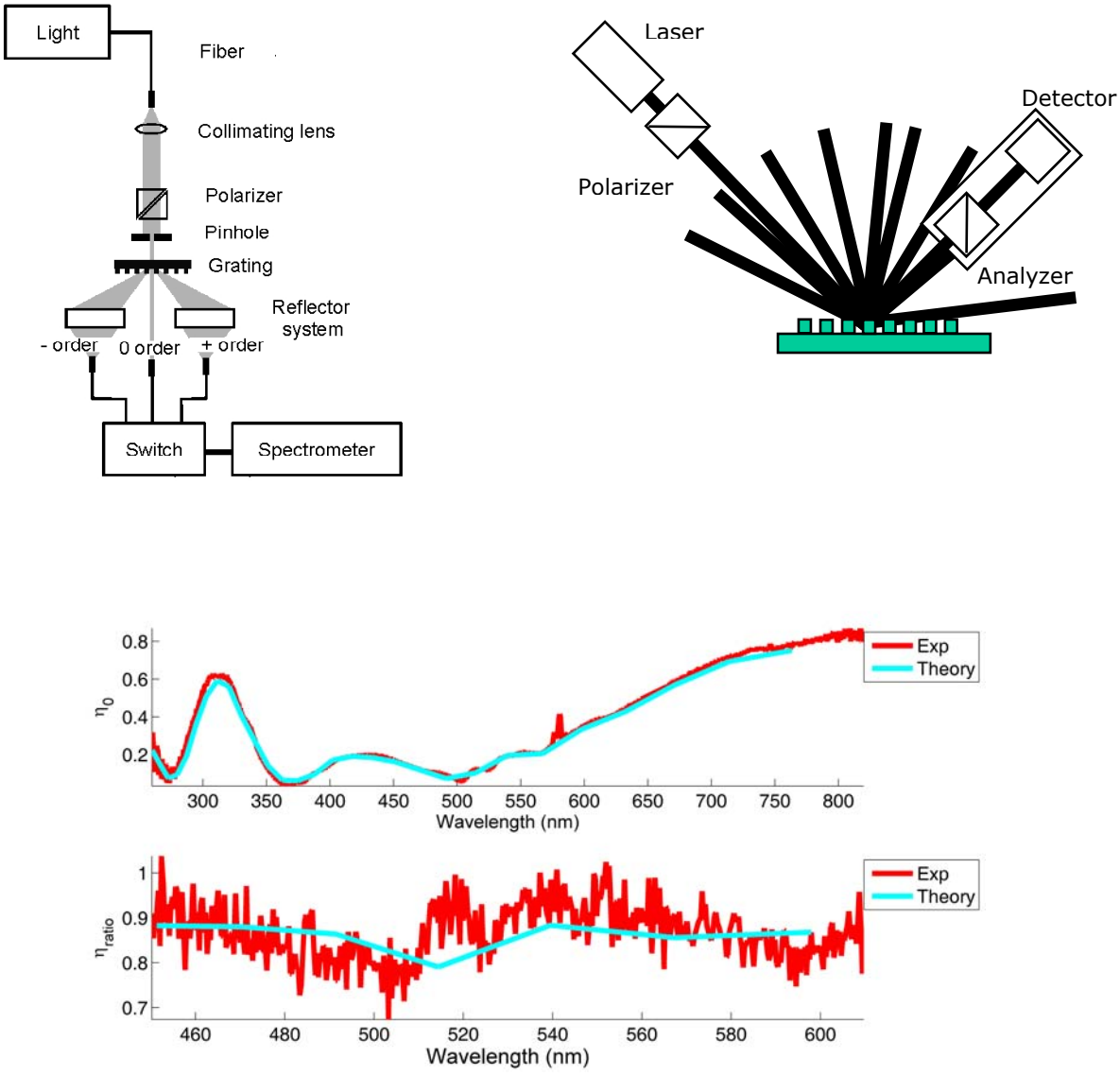


Figure 3.2.6: *The top pictures show experimental setups for spectroscopic scatterometry (left) and angular scatterometry (right). The lower picture shows spectroscopic scatterometry data points together with the best-fit curve found by varying the mathematical model.*

Profile measurements using atomic force microscopy: Figure 3.2.3 shows how AFM can be used to measure the pitch of periodic nanostructures by linewise scanning of the topography. It is clear that the tip does not measure the actual topography of the surface; its own shape is rather the limiting factor for the accuracy of the local spatial resolution. One can either use a sharper tip in order to better resolve smaller structures, or one can, with the help of an appropriate reference material, better estimate the shape of the tip for deconvolution purposes. Another approach to measure the curvature of steep structures consists in tilting of the sample and measuring one sidewall angle at the time. Figure 3.2.7 illustrates line scan profiles for tip movement both for a tilted and horizontally mounted surface. The tilted line curves (gray and black) allow in principle the reconstruction of the true shape of the profile if they can be patched correctly together. The drawing illustrates that the three profiles have a common line segment close to the top, which can be used to patch the line profiles together and obtain the profile. The method of sample tilting gives an accurate determination of the height and the sidewall angles since they are directly measurable. The determination of the width of the steps is less accurate, as it depends on the correct patching of the line scan profiles.

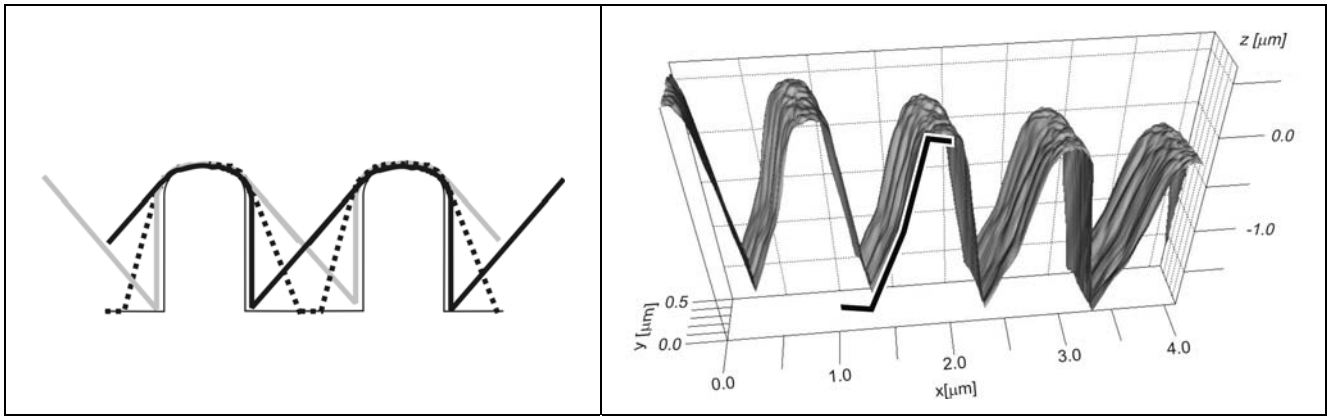


Figure 3.2.7*: The left picture shows the trace of the tip when it is scanned at different angles with respect to the surface. When scanning with the tip perpendicular to the surface, one will observe the dotted profile in the figure. This profile indicates the surface dilation caused by the tip geometry. The continuous gray and black lines are the line scan curves observed by tilting the samples in two directions. The right picture shows an experimental profile obtained by using the tilting technique. In addition to the experimental profile, an overlayed curve illustrates that the measured profile has different sidewall angles at the top and at the bottom of the profile.*

Putting it all together: We now turn to the profile comparison for a line grating with known pitch, 1013 nm, measured with conventional AFM and spectroscopic scatterometry. The experimental AFM and scatterometry data are presented in Figure 3.2.6 and Figure 3.2.7. However, we still need to explain how we turn the best-fit parameter from scatterometry and AFM line profiles into geometrical quantities such as height (*h*), width (*w*) and sidewall angles (γ_1 **to** γ_4 in Figure 3.2.8) of the line profile. For scatterometry these parameters are obtained directly from the best-fit procedure [3.2.9, 3.2.10], because each calculated set of data corresponds to a set of geometrical quantities. For AFM the height *h* is obtained by scanning the sample with an oscillating probe perpendicular to the sample surface; the sidewall angles are obtained by scanning a tilted sample. Finally the width is obtained by patching two tilted and one normal scan together [3.2.10]. The values obtained are presented in Figure 3.2.8.

	γ_1 [°]	γ_2 [°]	h [nm]	h_b [nm]	w [nm]
ODM	80 (0.8)	87 (0.7)	1945 (10)	855 (40)	652 (4)
Tilted AFM	80.9 (0.4)	88.3 (0.4)	1950 (15)	740 (45)	653 (15)

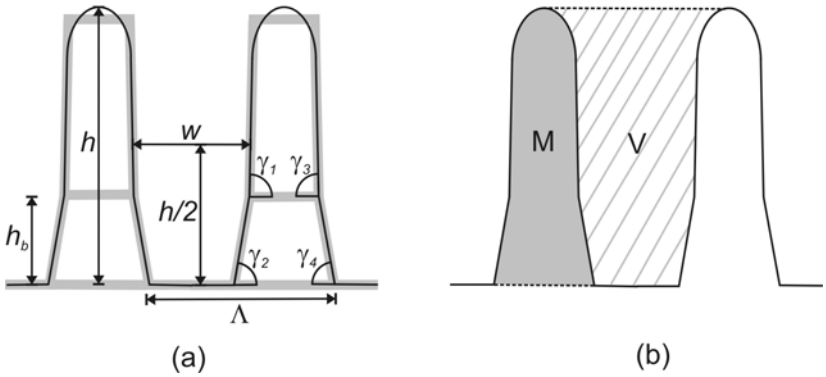


Figure 3.2.8: *(a) Shape and parameters that describe the profile of the grating. The wide gray line explains the trapezoid model profile estimated from the scatterometry and AFM measurements. The narrow black line shows the more complete model profile also estimated by AFM. (b) gives the definition of the material part of the grating, marked M, and the void part of the grating, marked V. The table shows the values for the parameters defined in (a) with uncertainties in brackets. The AFM values are obtained by averaging the profile over several* μm^2 *and the ODM values are averaged over a 0.5 mm*^{2}*. Only one set of sidewall angles is given since the profile is symmetric.*

The size range of the dimension presented in this case is larger than the nanoscale size range so one question remains to be answered. How do we extend the technology to the nanoscale size range?

For scatterometry and diffractometry the fundamental quantity is the pitch not the feature size. Nanoscale feature sizes can thus be studied in the described manner for pitch greater than 150 nm if the used wavelength is less or equal to the pitch. However, if the pitch is 150 nm or less one has to use extreme ultraviolet (EUV) wavelengths for studying the diffracted orders [3.2.11] or alternatively use an extended scatterometry approach called Mueller Polarimetry. In scatterometry only one polarisation property is measured which corresponds to one element of the response matrix. A Mueller polarimeter gives a complete description of the polarising action of the sample by measuring the full response matrix (16 elements) from the structure and gives therefore access to significantly more independent measurement data than conventional scatterometry [3.2.12]. For interferometric AFMs the fundamental quantities are set by the tip's ability to reach the groove bottom between neighbouring structures for tip scanning at different angles to the surface. The dynamical range along the z-axis furthermore limits non-interferometric AFMs. An alternative to these methods at the nanoscale is to use focused ion beam and scanning electron microscopy (FIB-SEM). This technique makes it

possible to combine conventional lateral information from top view SEM and z-axis information by making a cross sectional cut with the FIB. However, it is well known that observation at the nanoscale requires very high magnification that often results in unsharp pictures from which it are difficult to extract accurate dimensions.

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3.3 Metrology for engineered nanoparticles

3.3.1 What are nanoparticles?

Intuitively, everybody has an understanding of what is a *particle*; nevertheless, as testified by the more than 15 different definitions in ISO documents[8], the exact understanding of the term '*particle*' depends on the context in which the term is used. We will use the following definition: '*a particle is a minute piece of matter with defined physical boundaries*' from ISO/TS 27687:2008 [3.3.1].

The term '*nanoparticle*' is much younger, and there is little ambiguity about its definition: a nanoparticle is a particle '*…with all three external dimensions in the nanoscale…*' [3.3.1].[9]

The fact that the *term* nanoparticle is new does not imply that *nanoparticles* are new. Most of the particles that today can be called nanoparticle, are also described with the term *ultrafine particle*, defined as, for example, *'particle with an equivalent diameter less than 0,1 μm*' [3.3.2]. The difference between the definitions of nanoparticle and ultrafine particle is subtle.

It must be noted that, due to their high surface energy, nanoparticles most often occur as *agglomerates* or *aggregates*, which are groups of particles sticking together, either strongly (aggregates) or weakly (agglomerates) [3.3.1].

3.3.2 What are engineered nanoparticles?

One distinguishes two main categories of nanoparticles: natural nanoparticles and manmade (or '*anthropogenic*') nanoparticles. Nature produces nanoparticles, for example, through volcano eruptions or forest fires. Similarly, mankind produces nanoparticles unintentionally, creating 'incidental' nanoparticles, such as by burning candles or by driving combustion engine driven cars.

Even if the use of the term nanoparticles is relatively new, the intentional industrial production and application of particulate materials which are now called nanoparticles, such as carbon black or colloidal silica, is more than 100 years old. Modern carbon blacks are actually descendants of early 'lamp blacks' produced in China over 3500 years ago [3.3.3]. For these commercially produced nanoparticles, but also for other, more recently invented or created nanoparticles, which are not yet manufactured at a large scale, we will use in this text the term 'engineered nanoparticles' in the sense as defined by ISO: an engineered nanoparticle is a nanoparticle '… designed for specific purpose or function …' [3.3.4].

Volume-wise, the relevance of engineered nanoparticles is not increasing spectacularly. The main applications are well-established, such as the use of engineered nanoparticles as fillers or pigments in car tires or paints. However, the diversity of engineered nanoparticles is rapidly increasing, with a number of niche markets developing rapidly. Some of the new applications, such as the well-known use as functional UV-absorbing agents in sunscreens, lead to a higher potential for direct exposure and uptake of engineered nanoparticles by humans. This observation that is raising concern and is pushing scientists to develop methods to assess toxicity-relevant characteristics of engineered nanoparticles [3.3.5].

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[8] See http://cdb.iso.org; login as 'guest'

[9] Note that the term nanorod is preferred for elongated particles with 2 dimensions in the nanoscale and nanoplate for flat particles with 1 dimension in the nanoscale [3.3.1].

3.3.3 Particle metrology

As in other areas of 'metrology at the nanoscale' [see Sections 3.1 and 3.2], *metrology for nanoparticles* is a combination of issues related to fundamental, generic metrology (here: *particle metrology*), and a number of nano-specific aspects. Before addressing the nano-aspects, a few generic particle measurement issues are presented, focusing on the size measurement issue .

3.3.3.1 Particle size analysis

Particle sizing is about answering 2 main questions:

1) how *big* are the particles?

Often, the answer to this primary question is given in terms of an *equivalent diameter*, the value of which depends on the physical principle used in the measurement (examples: Stokes' diameter from sedimentation tests, hydrodynamic diameter from dynamic light scattering tests, …). An equivalent diameter is the diameter of a perfectly spherical particle which would create the same response (fluctuation of scattered light intensity, sedimentation time, …) as the response collected when actually measuring the particle; it is clear that a measured equivalent diameter can be very misleading if the actual particles are not spherical [see also section 3.3.3.2].

2) what is the *distribution* of particle sizes in the particle population?

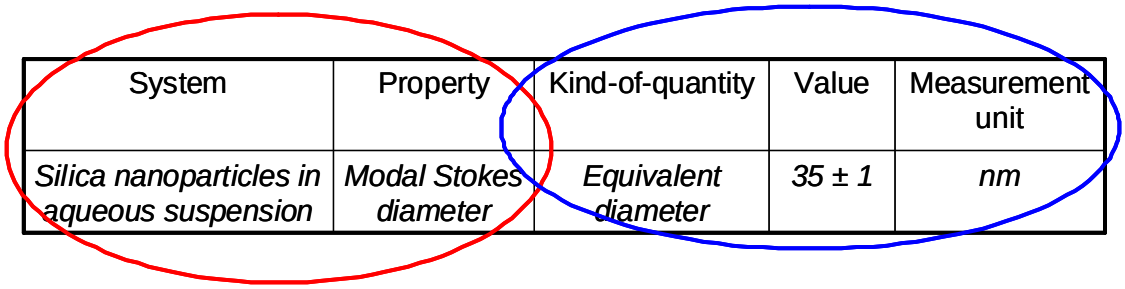
Particles, especially small particles, occur in large groups. This second question relates to the type of quantity used to express the amount of particles in each size-class; one can count the number of particles (number-based distribution), one can count the surface area they represent (surface-based distribution) or one can count their volume or mass (volume-based distribution). Moreover, one often makes distributions of particle sizes based on the intensity of a particular signal produced by an individual particle. This latter type of quantity can drastically change with the particle size within one measured distribution. For example, the often used light scattering intensity is severely particle size dependent [see section 3.3.3.3].

3.3.3.2 Traceability of method-defined measurands

An important metrology term is 'measurand', which is defined in ISO/IEC Guide 99:2007 [3.3.6] as "quantity intended to be measured". NOTE 1 of this definition states: 'the specification of a measurand requires knowledge of the kind of quantity, description of the state of the phenomenon, body, or substance carrying the quantity, including any relevant component, and the chemical entities involved'. Particle size analysis is a prime example of an area where the rigorous application of this definition could be helpful. Too often, the particle size or particle diameter is reported without a full description of the measurand, thereby neglecting that there are many different ways to measure and report a particle diameter, and these ways do not necessarily lead to comparable results of what nevertheless are results for the same *kind of quantity* 'particle diameter'.

One of the reasons for the difference between particle sizes measured with different methods is that the results are 'procedural, method- or operationally defined': the measured property is not intrinsic to the test object but is also determined by the way it is measured. It is well understood that, for example, if the shape of the particles is not perfectly spherical, this results in a bias of the equivalent particle diameter measured by centrifugal liquid sedimentation. In a sedimentation experiment, a non-spherical nanoparticle will align its long axis with the sedimentation direction; this will reduce the viscous drag and give the impression of a smaller equivalent particle diameter than the actual geometrically defined average diameter. Therefore, a most important element in the traceability statement of the measurement result is the reference to the measurement procedure.

A good example of how to report the result of a particle size measurement with a centrifugal liquid sedimentation test (providing a value of the modal Stokes' diameter), is given in Figure 3.3.1. The reported result necessarily comprises the explicit statement of the substance carrying the property, and of the measured property, including a reference to the used measurement procedure (in red ellipse). This information is additional to the more generally reported kind-of-quantity, numerical value, including the measurement uncertainty, and the measurement unit (in the blue ellipse) [3.3.7].



System	Property	Kind-of-quantity	Value	Measurement unit
Silica nanoparticles in aqueous suspension	*Modal Stokes diameter*	*Equivalent diameter*	*35 ± 1*	*nm*

Figure 3.3.1: *Example of how to fully report a particle size measurement result; inside the blue ellipse: the usual parts of a measurement result; in the red ellipse: the additional, explicit references to the measured system and the measurement method.*

3.3.3.3 Average values for particle populations

Often, particle sizing measurements are expected to capture in one (equivalent) diameter value (and its uncertainty) the shape and size of a (very) large number of particles. The complexity of this information can be captured in one diameter only if the test sample comprises a monodisperse population of regularly shaped particles. In any other case, the equivalent average diameter of the population of irregularly shaped particles depends on the physical principles of the chosen measurement method, and on the way the averaging was performed [3.3.8]. This average can be an arithmetic mean diameter, a modal diameter, or a diameter calculated with a more or less complex weighting function [3.3.9]. An excellent paper on the main particle size metrology issues is from Ehara and Sakurai [3.3.10].

3.3.4 Nano-specific aspects of particle metrology

The obvious additional complication of particle metrology when dealing with nanoparticles is the small size of the nanoparticles, which has two major consequences:

1) First of all, the effects of any substances adsorbed or absorbed on the particle surface, will become increasingly important with decreasing particle size. Actually, when a nanoparticle is suspended in a liquid, then the thickness of the electrical double layer around the particle is comparable with or even larger than the particle diameter, and the measurement of any of the particle's properties (size, composition, mass, …) will be severely affected by the substances collected in this layer around the particle.

2) Secondly, the use of imaging techniques to validate non-imaging methods becomes less evident. For example, the effect of an electron beam on the shape and size of the nanoparticles is often non-negligible, and the preparation of a TEM sample is likely to affect the particles' surface. This should be accounted for in the estimation of the measurement uncertainty. Also, the number of particles imaged in an electron microscope is never as large (normally less than 1000) as the billions of particles probed in a routine sedimentation or light scattering experiment. It must be noted that significant progress has been made and

continues to being made in this respect, with the development of cryogenic, low-pressure and scanning electron microscopy [3.3.11]. Atomic force microscopy overcomes some of the limitations of electron microscopy, by measuring the height of nanoparticles. No high-energy electron beam or vacuum system is required and the technique can be made directly traceable. On the other hand, AFM also is increasingly difficult when measuring smaller particles, because the effect of the particle-probe interactions has, in relative terms, a larger effect on the measured particle size.

3.3.5 Overview of important nanoparticle characteristics and properties

3.3.5.1 Basic particle characteristics relevant for environment, health and safety

Recent work on the methodology of environmental, health and safety (EHS) testing of nanoparticles has resulted in several reports of careful analysis of the most relevant characteristics of nanoparticles for toxicology [e.g., 3.3.12]. The basic properties of a nanoparticle (population) that, according to the relevant working group of ISO/TC 229, need to be characterized in order to describe the nanoparticles (population) and possibly predict their behaviour in an EHS study, are particle size/distribution, agglomeration/aggregation state, shape, specific surface area, composition, surface chemistry, surface charge, solubility, dispersibility.

ISO/TC 229 intends to provide lists of methods that are available for the measurement of the listed properties, and a number of the limitations of these methods. One important message is that for most of these nanoparticle properties, methods exist or can be imagined, but only few of the available methods are practiced in a manner that is metrologically reliable, leading to SI-traceable measurement results and meaningful measurement uncertainties.

3.3.5.2 Additional basic and functional particle properties

Not surprisingly, the above-mentioned basic particle characteristics are also important for the industrial applications of nanoparticles. A number of additional specific physico-chemical particle properties are needed for measurement purposes. For example, particle density and particle refractive index are properties needed to be able to correctly calculate particle sizes from sedimentation or light scattering measurements, respectively. It is actually a metrological challenge in itself to obtain reliable values for these properties. Very often, these physical properties of nanoparticles are taken from literature, where the property values are given for bulk materials with the same chemical composition as the nanoparticles.

In the new industrial applications, engineers want to exploit new physical properties that appear when the dimensions of the particles are reduced to the nanometre range. These new properties are leading to exciting new opportunities in electrical, optical, biological, mechanical, and chemical applications. It is then a necessity to characterise these physical properties precisely for reliable industrial and scientific applications. In addition, from the metrology point of view, they can open new windows for characterisation of nanoparticles. For example, the band gap of semiconductor particles increases with decreasing nanoparticle size in the nanometre range, resulting in a shift in the luminescence spectrum. A new measurement standard can be established utilising the colour of nanoparticles to determine their size. A similar approach can be used in the size estimation from Raman spectroscopy where the characteristic Raman signal exhibits measurable variations due the phonon confinement at nanoscale. The latter has already been used in size determination [3.3.13]. Some of the new physical properties appearing at the nanoscale are colour, enhanced atomic diffusion on the surface of smaller particles, reduced electrical conductivity, electrical tunneling between nanoparticles, phonon confinement effect, band gap changes leading to shifts in luminescence spectrum, surface effects in magnetic nanoparticles, giant magnetic resistance, ...

3.3.6 Case study: particle sizing

3.3.6.1 Setting the scene: the measurement problem

A nanoparticles producer wants to develop a new cerium oxide nanoparticulate material for the *chemical mechanical polishing* (CMP) of, for example, SiO_2 layers on silicon wafers.[10] CMP is performed with slurries of fine, abrasive particles [3.3.14]. The powder production unit provides the company's test lab with a series of nanopowder materials of different specific surface area. Specific surface area values are here used as a first indication of particle size. This is a prime example of the assumptions often made in the estimation of particle sizes: the specific surface area can only be calculated into an average particle size by assuming that all particles are spherical and of the same size.

Figure 3.3.2 shows typical images of candidate CMP materials, which reveal that the assumption of monodisperse and spherical nanoparticles is not correct. Nevertheless, BET measurements (measurements of the specific surface area of a solid powder following the BET method as described in, e.g, ISO 9277:2010 [3.3.15]) remain a very common method in industrial particle processing, since, for example, it does not require the suspension of the powder in a particular test medium.

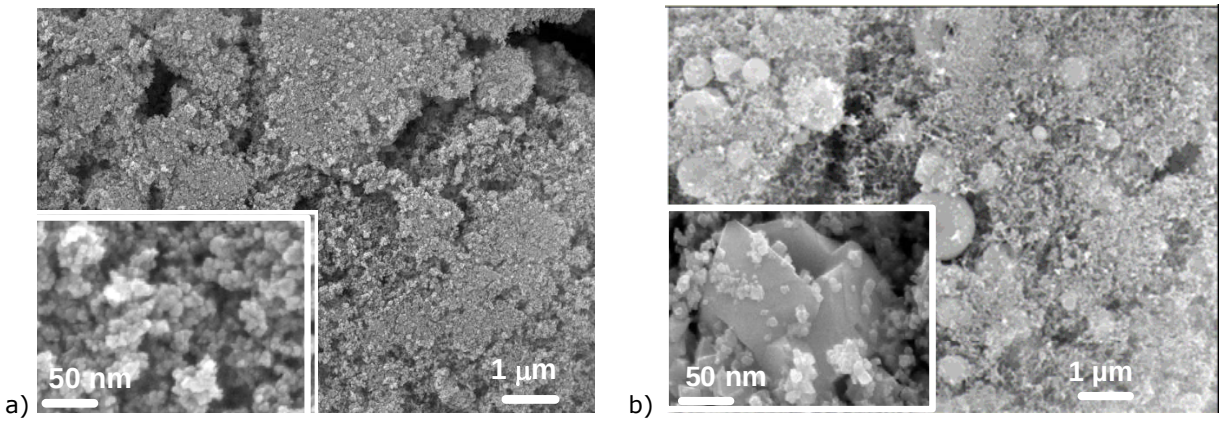


Figure 3.3.2: *Comparison of two cerium oxide powders with similar specific surface area but different particle size distribution [3.3.16].*

Using the powders, the test lab prepares ethanol-based CMP slurries. Comparing the results of a few CMP test runs, the lab confirms the expected correlation between specific surface area and the removal rate: lower specific surface areas (= higher average particle size) give higher removal rates. On the other hand, the number of defects also increases with increasing particle size (Figure 3.3.3). Based on the results in Figure 3.3.3, the test lab advises the production unit to prepare a series of powders consisting of nanoparticle agglomerates of a specific surface area of about 40 m^2/g. When the test lab receives a number of these batches, the CMP test runs do produce consistent results, but some slurries produce more polishing defects than others. The test lab then decides to check the particles with a method, which allows the assessment of the particle size distribution. At the lab's disposal is a battery of particle size analysis methods (see Intermission 3.3.1).

[10] This case study is loosely inspired by the presentation of S. Put at the Co-Nanomet workshop on Nanometrology, Braunschweig, November 2009.

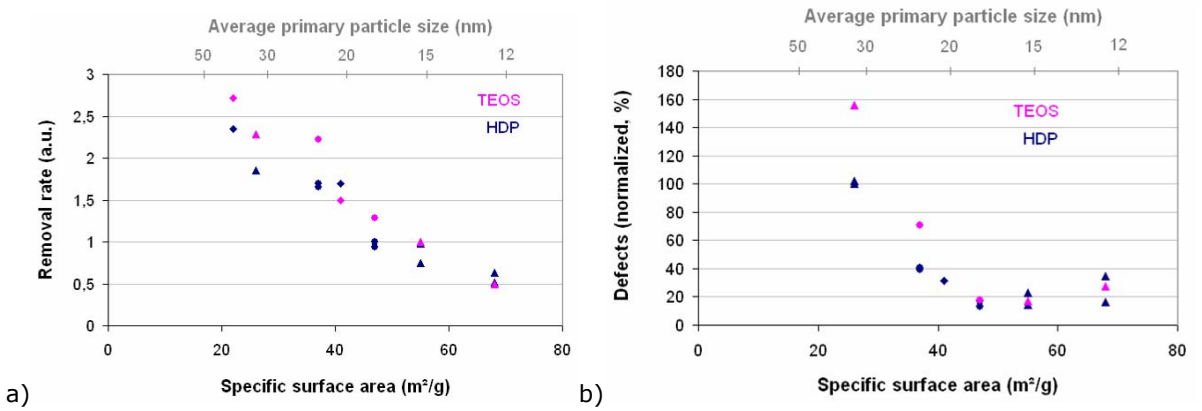


Figure 3.3.3: *Relation between the specific surface area of a cerium oxide nanoparticulate powder used to prepare the CMP slurry, and a) removal rate during CMP and b) number of defects after polishing [3.3.16].*

Intermission 3.3.1: Classification of particle size analysis methods

Particle size analysis methods are based on different physical principles. The methods can be classified as in Table 3.3.1, which shows two main categories: *counting methods* and *ensemble methods* [3.3.17].

Counting methods require the dilution of the nanoparticles population down to single particles in the observation zone. *Ensemble techniques* are either be classifying or non-classifying. *Classifying methods* separate out fractions of particle systems according to size-dependent properties, like settling velocity. *Non-classifying methods* analyse signal spectra of particle ensembles with different sizes together in the measuring zone in terms of their quantitative distribution. [11]

In general, *counting methods* enable the highest resolution of the particle quantity and size. Their sensitivity for the detection of a particle is independent from the presence of neighbouring particles. Unfortunately, the performance of counting methods is limited by the sample splitting and dilution needs that ensure only single particles pass through the measurement zone hence relatively low numbers of particles measured.

Non-classifying ensemble techniques can deliver ambiguous results, because numerical algorithms are necessary to deconvolute the contributions of different particles and different particle size ranges. These techniques do enable the characterisation of particles that are in a size class with reasonable signal intensities, but they may not detect well particles from less populated size classes, for example in the tails of the particle size distribution. On the other hand, they may also overemphasize size classes which contribute non-proportionally to the detected signal.

[11] Note that some counting and ensemble methods are based on the same physical phenomenon, e.g., light scattering can be used to detect individual particles, but also to analyse large groups of particles (as in photon correlation spectroscopy, also known as dynamic light scattering).

Counting methods	Ensemble methods	
	Classifying methods	*Non-classifying methods*
2D-image analysis	Sieving	Laser-diffraction
Tactile probe profile analysis (e.g., AFM)	Sedimentation	Ultrasonic spectroscopy
Single particle light scattering	Size exclusion chromatography	Electro-acoustic spectroscopy
Single particle electric impedance	Field-flow-fractionation	Dynamic light scattering
Single particle light extinction	Capillary hydrodynamic flow	Dynamic extinction spectroscopy
		Static light scattering
		Small-angle x-ray scattering

Table 3.3.1: *Classification of measuring methods for particle size distributions according to their physical principle.*

3.3.6.2 Selection of the relevant particle size analysis method

The suspicion of the test lab is that some of the particle slurries contain at least a few particles which are larger than average, and that these larger particles are responsible for the increased number of polishing defects. This kind of problem is not easily answered by a number of the candidate methods mentioned in Table 3.3.1: the counting methods and the non-classifying methods are – in principle – not so powerful when it comes to the detection of small numbers of particles in a large population. On the other hand, dynamic light scattering is very sensitive to the presence of a few isolated larger particles, and produce a severely deformed, unreliable particle size distribution measurement result [3.3.8].

The more obvious choices are the classifying methods, which will separate subpopulations of different size, before actually determining the particle size. An example of such methods is the centrifugal liquid sedimentation technique. This is a method in which the natural sedimentation process of suspended particles is accelerated by centrifugal forces. Centrifugation allows investigating also nanoparticles which, under normal gravitational circumstances, do not sediment out as they are kept in suspension through Brownian motion.

Intermission 3.3.2: measurement uncertainty in centrifugal liquid sedimentation

Centrifugal liquid sedimentation (CLS) determines the particle's Stokes' diameter, which is a particular kind of equivalent particle diameter. A special case of CLS is the line-start method or disc centrifugation, in which the particles are forced to sediment through a density gradient (ISO 13318-

2:2007 [3.3.18]). The measurement equation for centrifugal disc sedimentation is relatively straightforward:

$$x_{St} = \sqrt{\frac{18\eta \ln(M/S)}{(\rho_s - \rho_l)\omega^2 t}} \quad (1)$$

In Eq. 1, x_{St} is the Stokes' diameter, η is the (average) dynamic viscosity of the liquid in the density gradient, *M* and *S* are the outer and inner radius of the sedimentation path of the particles during the measurement. ρ_s and ρ_l are the effective particle and fluid density, respectively, ω is the rotational velocity, and *t* is the time it takes the nanoparticles to reach the detector, located at *M.*

Figure 3.3.4 demonstrates the first step in the establishment of an uncertainty budget: a (simplified) fishbone diagram, showing all parameters that can contribute to measurement uncertainty in centrifugal disc sedimentation, in this case for the sedimentation through a density gradient consisting of a solution of saccharose in water.

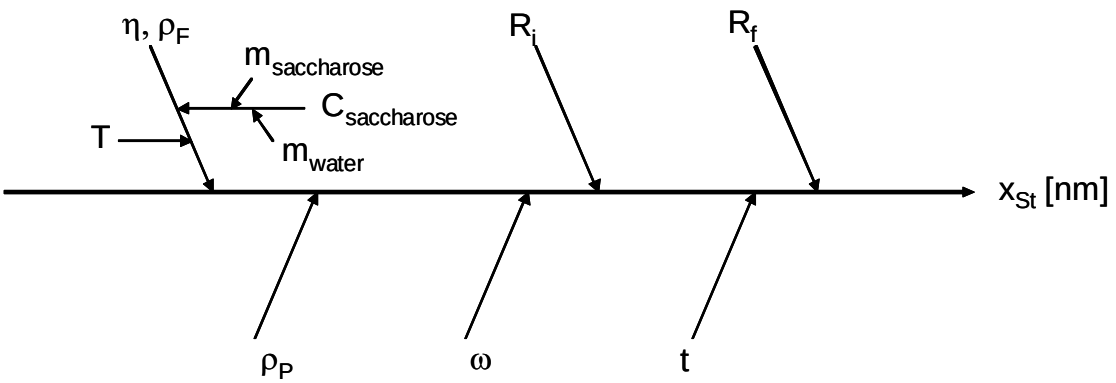


Figure 3.3.4: *Fishbone diagram showing the possible contributions from measurement parameters to the total measurement uncertainty [3.3.19].*

In theory, it is perfectly possible to estimate the values and associated uncertainties of all of the parameters mentioned in Figure 3.3.4; this approach is called the 'bottom-up' approach. Unfortunately, the estimate of the individual uncertainty contributions is often not straightforward, and the interrelation between the different uncertainty contributions and the combined uncertainty is complex. A commonly used alternative approach in daily laboratory practice is to calibrate the CLS instrument with a calibrant (suitable reference material of known particle size and density). This allows reducing the measurement equation to:

$$x_{St} = \frac{K(\rho_s)}{\sqrt{t}} \quad (2)$$

In Eq. 2, all system variables are captured in 1 parameter, *K*. The value of *K* changes with ρ_s hence the notation $K(\rho_s)$. If one knows the effective density of the particles to be tested after the calibration, and the time it takes for these particles to sediment, then one can calculate x_{St}. The uncertainty of this

method can be more easily performed through the so-called 'top-down' approach. The combined uncertainty corresponding with this approach can be spelled out as in Eq. 3:

$$u_c(x) = \sqrt{u^2(r) + u^2(ip) + u^2(cal) + \left(\frac{\partial x}{\partial \rho_s}\right)^2 u^2(\rho_s) + u^2(t)} \qquad (3)$$

Eq. 3 contains contributions from method repeatability (u(r)), intermediate precision (u(ip)), calibration (u(cal)), effective particle density (ρ_s) and trueness (u(t)). Most of these contributions can be determined by performing a suitably designed method validation with a reference material (to determine u(r) and u(ip)), by checking the certificate or other documented information about the calibrant (to find u(cal)), and by testing a certified reference material (to estimate u(t))). A numerical example of the application of this method is given in Table 3.3.2.

Type A standard uncertainty components	
Relative repeatability, u(r) (%) [1]	3.83
Relative intermediate precision, u(ip) (%) [1]	5.60
Relative trueness, u(t) (%)	2.18 [2]
Type B standard uncertainty components	
Relative standard uncertainty from PVC calibration RM, u(cal) (%)	3.42
Relative standard uncertainty from particle density, $u(\rho)$ (%)	1.20
Relative expanded uncertainty, $U(x)$ (%) [3]	**16**

[1.] From measurements on reference material IRMM-304; [2.] From measurements on polystyrene particle size RM; [3.] For a normal distribution of measured values x and a level of confidence of approximately 95 %, the combined uncertainty was multiplied by a coverage factor k = 2, to obtain the expanded uncertainty (U).

Table 3.3.2: *Uncertainty budget for particle sizing via centrifugal liquid sedimentation [3.3.19].*

3.3.6.3 Metrological traceability needs of the nanoparticles producer

At first sight, the traceability requirements of the nanoparticle producer, in this particular case study, may seem limited. The main interest of the producer is to detect whether there are particles of different sizes in the slurries he has prepared. This is not an absolute measurement, and traceability to the SI system is not a necessity: traceability is about comparing measurement results with results obtained at other places and/or times. One might even argue that the comparison of sedimentation times is sufficient for the integrated circuits (IC) producer to judge the quality of different batches of nanoparticle powders, and that therefore a calibration of the CLS instrument is not needed.

However, if the decision is taken to transpose the production of the powder to multiple plants, or if the production is outsourced (involving measurements at a supplier and incoming inspection measurements at the client, here the IC producer) then metrological traceability becomes an issue: measurement results can only be reasonably compared if they share a defined, common traceability end-point. In the case of CLS

measurements: if they are performed on different instruments these instruments need to be calibrated with a CRM with an SI-traceable certified value. In the absence of such CRM for calibration, at least a common reference material needs to be used in the different laboratories. The latter solution would lead to a traceability of the measurement results to the particle size of this reference material and measurement technique.

There is an common understanding that different aspects of particle sizing are best met by different methods, and most existing methods for nanoparticle size measurements (CLS, DLS, SAXS, image analysis, SMPS etc.) will continue to be routinely used. Therefore CRMs that can relate the measurements from one method to another are highly desirable.

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3.4 Metrology for nanobiotechnology

3.4.1 Nanobiotechnology and bionanotechnology

The saying goes that *'bio is nano by nature'* [e.g., 3.4.1]. The reasoning behind this simple statement is that many biological phenomena 'naturally' occur at the nanoscale: the nanoscale is the scale at which proteins take the specific shapes they need to assume their functions; it is also the scale that holds the width of DNA molecules and viruses, as well as the thickness of the membrane that forms the wall of cells. As a consequence, the fields of nanotechnology and biotechnology are closely related indeed.

One can distinguish nano-bio-technology (the application of nanotechnology to biology; for example magnetic nanoparticles used to destroy cancer cells) and bio-nano-technology (the application of biology to nanotechnology; for example: bacteriorhodopsin-based ultrahigh density optical memory). In the remainder of this text, and unless otherwise stated, we will use the term nanobiotechnology to cover both nano-bio- and bio-nano-technology.

In its broadest sense, nanobiotechnology includes a wide range of applications in different sectors such as health care, food, agriculture, and environment. The life sciences and health care sectors rely more and more on nanosciences and nanotechnologies to address their needs for technological advances [3.4.2]. Examples of needs are imaging techniques with higher spatial resolution and diagnostic techniques with better sensitivity, selectivity and speed. In the medical device industry, surface modification and coatings at the nanoscale are needed to tailor biological responses to materials used in e.g., implants. Modern pharmaceuticals are sensitive macromolecules that need to be supplied in special formulations where the nanoscale structure and chemical composition have been tailored for efficient delivery of the drug to its target. In food industry, nanotechnology contributes to better food packaging and storage materials, prolonging the shelf life of fresh food. Modern food processing is increasingly aiming at controlling the nanoscale structure of foods in order to achieve efficient nutrient delivery and improved bioavailability.

Nanotechnology is considered to hold great promise for the future development of areas related to bio- and life sciences. The impact of nanotechnology in some of these fields is, however, hindered by concerns related to the lack of knowledge about the potential effects and impacts of nano-sized materials on human health and the environment. The needs amongst end-users and industry, and the safety and potential hazard issues involved, means that the strong technology development that is currently taking place must be matched by developments with regard to metrology in the field, especially to ensure compliance with health and production related regulations. Reliable and valid measurement methods are key components for this.

3.4.2 Metrology in nanobiotechnology

The measurement requirements in nanobiotechnology cover a broad range and are strongly dependent on the application or product. Many of the requirements are not unique to the area, but in many respects similar to those of other areas of nanotechnology. What is unique to the area, however, is that it deals with biological systems. In this section we first present a number of metrology issues generic to biological systems, and some important actors in the field, before listing more specific nanoscale metrology issues.

3.4.2.1 Generic challenges associated with measurements in biological systems

Compared to synthetic materials, products or processes, biological systems are often characterised by a high degree of complexity and variability, which puts special requirements on the measurement techniques. Specific aspects of this complexity are, for example:

- Presence of a variety of substances with relevant amounts in the range of single molecules and upwards;

- Structures of relevant sizes from the scale of small molecules up to complete organisms;

- Interdependent and often not fully understood structure-function relationships and mechanisms that are underlying the observed biological phenomena;

- The importance of water for stabilizing biological structures and as the medium in which interactions take place. This is a restraint for classical measurement and characterisation techniques that are not operational or less easy to operate in liquid media.

3.4.2.2 Actors in the biology-metrology field

Traditionally, the metrology field has been focused on physical measurements (e.g., mass, time, length). With time, and recognising the importance of method validation and analytical quality assurance, also the chemistry domain has developed a prominent place in the formal metrology world. On the other hand, the field of biology has long been developed without the explicit involvement of the traditional metrology actors such as the International Committee for Weights and Measures (CIPM, after the french name Comité international des poids et mesures), and the NMIs. This is changing gradually, and, for example, a dedicated bio-analysis working group does exist since more than 5 years within the CIPM Consultative Committee for Quantity of Matter (CCQM), where work is focused on the chemistry aspects of biotechnology. Other important organisations have a longer history of activity than CCQM in the field of measurements for biology, such as the World Health Organization (WHO), the Food and Agricultural Organisation of the United Nations, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), and the national and regional Pharmacopoeias. An example of the collaboration between CIPM and these organisations is the Joint Committee on Traceability in Laboratory Medicine (JCTLM), which was developed to match for example the measurement quality requirements associated with a specific *in vitro* diagnostics regulatory initiative.

3.4.2.3 Particular measurement units for biology and medicine

When imaging biological structures, the interpretation of the biological implications of the images is of course dependent on a certain degree of dimensional accuracy of the images. This can be achieved by calibrations of the instruments used, and is usually done with non-biological calibrants. Organic and biological reference materials for calibration of dimensional scales are largely lacking, since their realisation is difficult due to the instability of biological matter. However, the interpretation of images of biological samples, for example of cells or tissues for diagnostic purposes is mainly relying on pattern recognition and the relative positions and shapes of the different structures observed, rather than on accurate dimensional scales. Only recently some of the nanotechnology tools such as scanning probe microscopy are being used to measure biological samples in physiological conditions more accurately.

A particular issue has to do with measurements in biology and medicine that are dealing with *amount of substance*. In pharmacology 'International Units' are the units of measurement for the amount of a substance. An International Unit (IU) is based on the measured biological activity or effect. The unit is used for analytes (e.g., vitamins, hormones, blood coagulation factors) which do not have a known elementar entity. To define an IU of a substance, the Committee on Biological Standardization of the WHO provides a reference preparation of the substance, arbitrarily sets the number of IUs contained in that preparation, and specifies a biological procedure to compare other preparations of that substance to the reference preparation. The goal in setting the standard is that different preparations with the same biological effect will contain the same number of IUs. The reference preparations are widely used by laboratories that are required to maintain a quality system, such as pharmaceutical industries and the clinical chemistry laboratories of hospitals. Despite its name, the IU is not part of the International System of Units. Nevertheless, basic metrological principles can and have to be used to improve the reliability of the values measured for properties with non-SI units. For example, the United States Pharmacopoeia (USP) has

recently started offering, in addition to their 'reference standards', certified reference materials (CRMs), which '*represent the next generation of exceptional quality USP reference standards. They have undergone additional metrologically based testing and statistical analysis to meet both USP's stringent criteria and guidelines established by ISO. The CRM provides a trueness of measurement that ensures traceability and provides a higher degree of confidence in the analytical measurement that leads to decisions regarding product specification and acceptance limits'.* Similarly, the Institute for Reference Materials and Measurements (IRMM) is producing reference materials for biological macromolecules that have been certified with a metrological approach in collaboration with the IFCC. This is one example of the successful cooperation between metrology institutes and the biomedical community.

3.4.2.4 Specific challenges for measurements at the nanoscale in nanobiotechnology

Nanobiotechnology deals with coatings and thin films, structured surfaces, and particles, and therefore the examples on dimensional analysis methods in the previous sections are also relevant for nanobiotechnology. For different nano-enabled devices and products, dimensions are indeed crucial for the function, or need to fulfil a given specification; this does require traceable measurement results. One example of this is the surface roughness of implants, which is known to play an important role for how tissue will respond to the implant. Despite its importance, no certified surface roughness (or surface texture) references exist for implant materials. This is also the case for thin coatings on implants, where film thickness and composition, and the functional properties that are derived from them, may be of crucial importance.

With respect to the additional complicating biological and biochemical aspects of nanobiotechnology, and in terms of metrological status, the nanobiotechnology area is, alike the biology area, in a much earlier phase of development than the other areas discussed in this Introductory Guide. A specific challenge is the fact that most of the techniques used in the area are relatively new. Many of the techniques are traditional biochemical techniques, but have been further developed using nanotechnology, while some techniques were originally developed for other applications than biological, but have found uses in biology after developments in instrumentation and/or procedures for sample preparation and analysis. To a large extent, this development has been carried out outside the NMIs, i.e. at universities and in specialised companies. With a few exceptions, metrology aspects (traceability and measurement uncertainty) are poorly developed, and sometimes completely lacking on the agenda.

In the next sections, first a few of the measurement techniques relevant to nanobiotechnology are presented. Then two examples are given of biomedical applications where nanometrology needs exist.

3.4.3 Examples of measurement techniques

The discussion paper of the Co-Nanomet action group on nanobiotechnology [3.4.3] lists and describes a wide range of measurement techniques that utilise nanotechnology or address nanoscale phenomena, and are used for nanoscale measurements on biological systems (Figure 3.4.1). The techniques described can somewhat arbitrarily be grouped into either of the two categories “Analysis and Diagnostics” or “Imaging”.

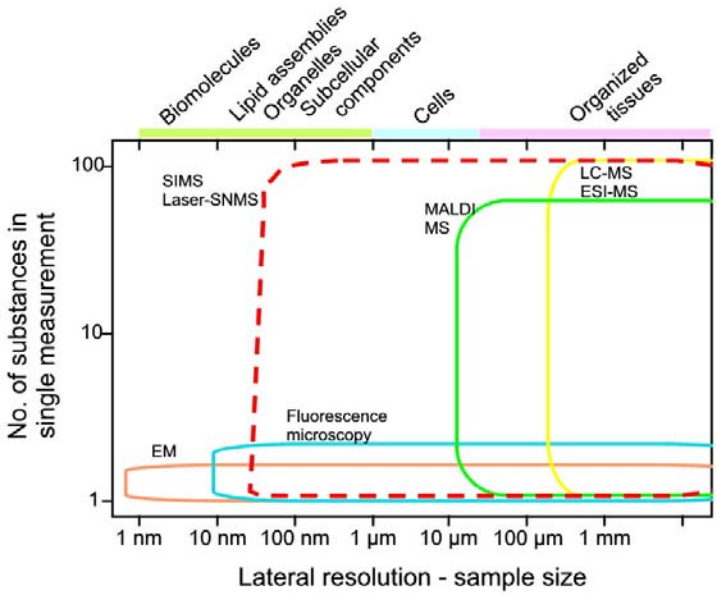

Figure 3.4.1: *Lateral resolution (indicative for the technique's smallest sample size) and number of (biological) substances that can be measured in a single measurement for some commonly used bio-analysis techniques. Electron microscopy and fluorescence microscopy offer lateral resolutions in the 1 nm to 10 nm range, but can only measure a few substances at a time, due to the necessity for labeling[12]) to create contrast in the image. Mass spectrometry (MS) methods on the other hand are label-free techniques. A few MS methods, most notably SIMS, also offer imaging capability at sub 100-nm lateral resolution for biological samples [3.4.4].*

3.4.3.1 Analysis and diagnostics

Techniques in the "Analysis and Diagnostics" category are used for identifying, detecting, and often also quantifying, specific substances in a biological sample. The sample can be e.g., a blood or urine sample, or a tissue sample from a biopsy. Most of the measurement methods require one or several steps of sample pre-treatment for purification or isolation before the measurement is made, and many of the commonly used techniques require some type of labelling of the specific substances of interest. Labeling means the use of antibodies or some other chemical reagent that bind specifically to the analyte of interest. To enable detection, the labels are tagged with fluorescent or other optically active groups for optical detection, with isotopes, or with colloidal particles with magnetic properties or acting as contrast media for electron microscopy. Several of the measurement techniques in the "Analysis and Diagnostics" category are not obvious nanoscale techniques, but have recently been or are currently being miniaturised by using nano- and microfabrication techniques to improve detection sensitivity and enable parallellisation.

3.4.3.2 Imaging

Techniques in the "Imaging" category are used for imaging the spatial distribution of substances in a biological sample, or for more general visualization of the morphology or structures of biological samples at ambient condition (e.g., Figure 3.4.2). They are typically used in cell biology research, pathology and for diagnostic purposes. The samples used consist of cell cultures or tissue samples taken from a biopsy. A key

[12] Labeling here is the selective addition of a molecule or ion to the substance of interest.

issue is the preparation of the biological sample, which should not alter or destroy the biological structures to be imaged. Some of the imaging techniques are non-invasive (e.g., techniques based on fluorescence), but require the use of labeling or contrast agents that are administered to the sample or patient prior to the measurement.

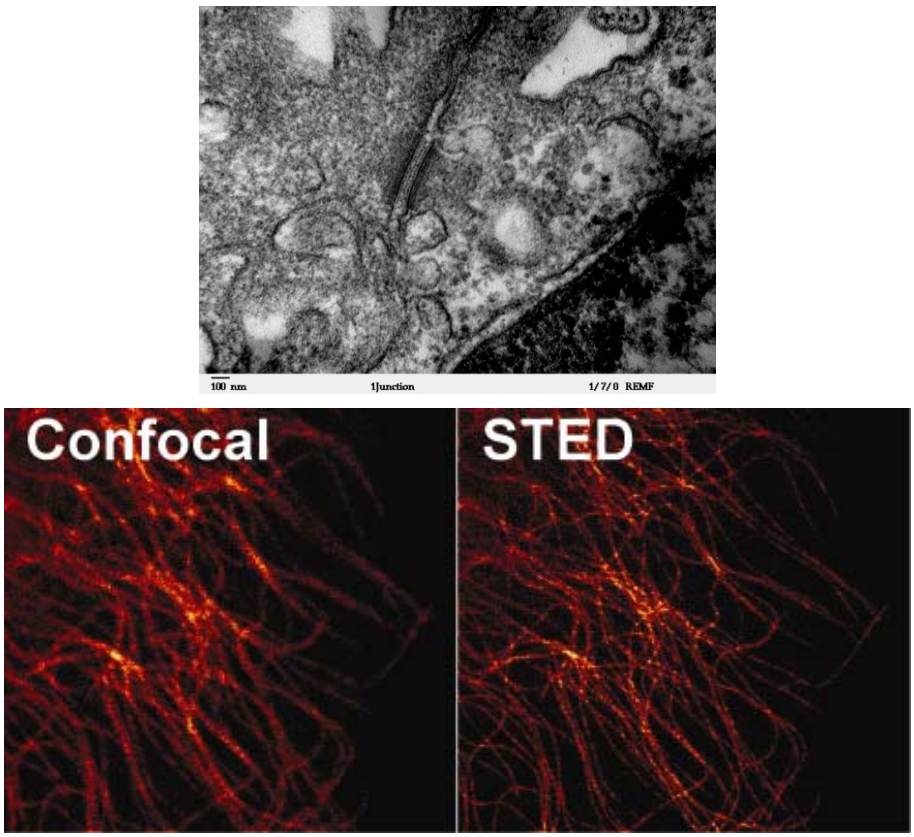

Figure 3.4.2: *Transmission electron microscopy image of lung tissue (upper). The sample consists of a thin section of chemically fixated tissue, in which particular structures have been chemically stained to enhance the image contrast (Courtesy of Louisa Howard, Dartmouth Electron Microscopy Facility, Dartmouth College, USA). Fluorescence microscopy images of live PtK2 cells (left and right). The left image was acquired with conventional confocal microscopy, where the lateral resolution is mainly limited by diffraction[13]. The right image shows the resolution improvement achieved with the recently developed stimulated emission depletion microscopy technique (STED), a particular fluorescence microscopy technique [3.4.5].*

3.4.4 Example 1: Magnetic biosensors for in-vitro diagnostics

Biosensors are instruments based on biochemical reactions, where the intensity of the reaction is converted into a measurable signal, such as current or light. The glucose sensor is probably the most important and most used biosensor, and it is used as an efficient tool in disease management. It is a prime example of what

[13] Microscopes have a diffraction limited lateral resolution of roughly half a wavelength whereas confocal microscope may have a lateral resolution up to 10 times better.

is called point-of-care diagnostics[14]. Modern versions of the glucose sensor are illustrations of how nanotechnology can bring progress, and are based on the general principles outlined below.

Biosensors use antibodies to detect and capture different kinds of substances of interest in a more or less complex (body-)fluid. For many years, ultrafine fluorescent particles (we would call them nanoparticles now) have been used as carriers for the antibodies which capture the target molecules. The nanoparticles which have captured the target analyte can bind to a specific zone in the sensor, which fixes an amount of nanoparticles that is related to the concentration of target analyte in the fluid. The amount of nanoparticles can be measured optically, thanks to their fluorescent nature. Since about a decade, one has been using modified particles, which contain small (5 nm to 15 nm diameter) Fe_2O_3 nanoparticles, which have superparamagnetic properties (they are only magnetised under a magnetic field). The magnetic properties of these particles enable them to be moved around in the sample liquid and to force their deposition at the end of the capturing stage on the detector's substrate, where the particles having collected the target analyte will bind [3.4.6]. Those particles that do not bind can be washed off from the substrate when switching off the magnetic field. The remaining nanoparticles are indicative of the amount of target analyte, and their amount can be deduced from the results of, for example, frustrated total internal reflection measurements.

The metrology aspects of the above described process are multiple. One easily recognizes the need for calibrating the optical response to the detected amount of target analyte. More broadly speaking, several method validation issues are at stake, such as the issues of robustness (does this work in the relevant liquid media, i.e. blood; is the method temperature sensitive, or sensitive to the amount of time given to the particles to capture the target analyte), selectivity (might this method also detect other species than the intended one?), repeatability and reproducibility, …

3.4.5 Example 2: Medical implants and regenerative medicine

Different types of medical implants are widely used in health care. They are used for repairing and restoring body functions that have been lost or impaired due to trauma or disease in, for example, the musculoskeletal system. Fracture fixation devices, artificial joints and dental implants are but just a few examples that have improved the quality of life for millions of patients. Key requirement of the materials that are used in implants is that they possess the necessary properties to ensure the efficacy of the device and most of all, the safety of the patient. The manufacturing and clinical use of implants is associated with a range of metrological issues, which can be illustrated using an artificial hip joint (total hip replacement, THR) as an example (Figure 3.4.3).

[14] diagnostic testing at or near the site of patient care

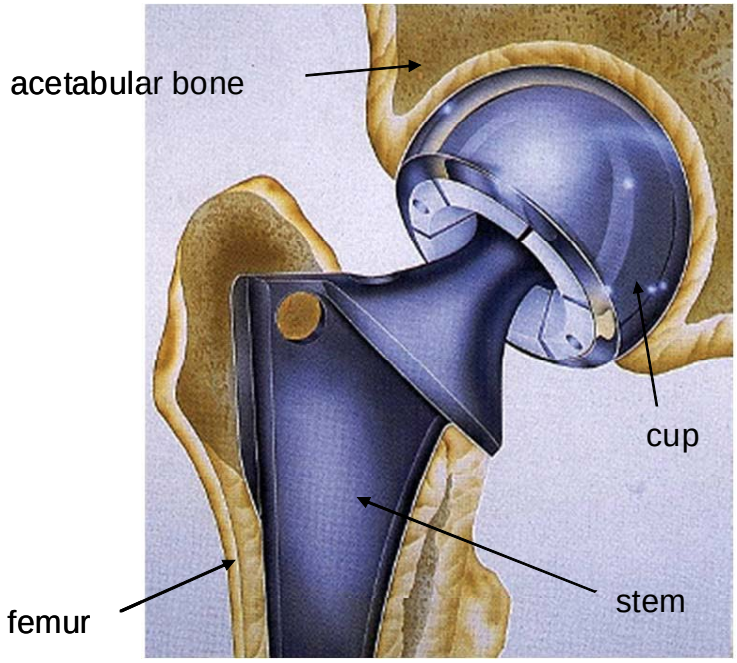

Figure 3.4.3: *Artificial hip joint (Courtesy SP, Sweden).*

An artificial hip joint implant consists of several key components; a first component is the stem which is placed in the femur of the patient. The stem is either directly anchored in the bone, or cemented in place using bone cement that is setting during the surgical procedure. The upper part of the stem is in the shape of a ball made from a wear resistant material with smooth surface finish. Fixated in the acetabular bone is a cup, usually consisting of a metal with an inner liner of a wear resistant ceramic or plastic. Key material requirements here are thus mechanical strength (the femoral stem), wear resistance of the articulating surfaces (ball and cup liner), and biocompatibility. The latter is a combination of properties that result in an appropriate tissue healing around the implant, without adverse reactions.

It is clear that the specification, production and clinical use of this type of device is associated with a wide range of measurement issues. Surface topography of the material on the scale from micrometres down to the nanoscale will influence the tissue response and the mechanical fixation of the device in the host tissue. It also influences the total area from which potentially harmful ions may be released from the material. For the articulating surfaces, the surface roughness on the nanometre scale will play an important role for the tribological aspects (friction, lubrication and wear). The surface topography of implants can be described with the same parameters as those described in the critical dimension and structural surface analysis case study. However, their measurement is relatively complicated due to the often relatively complex geometry of implants.

In addition to geometry and surface structure, the chemical composition of the materials involved will play an important role for the clinical function of the device. For stable (non-resorbable) materials, the biological response is determined by the chemical composition of the outermost few nanometres of the material surface. Medical implants are frequently surface modified or coated with thin films using different techniques. For example, synthetic hydroxyapatite (HA) - a ceramic material, similar to the natural HA component of natural bone – can be applied as a coating to enhance the bone healing around implants [3.4.7, 3.4.8, 3.4.9]. Characterisation of the modified surfaces or thin films can be done using the same methods as

described in section 3.2, but again the measurements are sometimes made more complicated by the geometry of the implants. The introduction of coatings with thickness in the nanometre range also leads to issues related to current documentary standards and guidelines. For example, according to current FDA (US Food and Drug Administration) guidelines for HA coatings the purity of a coating is expressed in terms of maximum allowed concentrations of impurities such as, rare earth elements. The rationale behind this is that in a worst-case situation the coating is fully degraded, whereby potentially toxic elements in the coating will all become bioavailable. For thick coatings in the 10-100 µm range, this means that impurities present in low concentrations (10^{-4} %) may reach amounts exceeding the limits where there is a risk for toxic effects. For coatings in the nanometre range, however, the same low concentrations will lead to total amounts far below established risk levels.

Another metrological issue especially for hip joint implants is the fact that the movement between the femoral head and the acetabular cup inevitably leads to the generation of wear particles, in sizes from nanometres up to several micrometres. The extent of wear particle generation, the particle size (and most likely also shape) distribution and the chemical composition of the particles, will have an effect on the tissues surrounding the device. Generation of wear particles is considered to be one of the factors that limit the functional life-time of these devices, manifested as an increase in the occurrence of implant loosening after 15-20 years. In the development of new and better material combinations for the articulating surfaces, there is thus a need for reproducible and traceable measurement results of the number and size of wear particles. Such methods need to be realistic, i.e., provide results that are relevant for the real clinical situation, which involves mechanical load cycles, geometries and chemical/lubricating conditions that are all relatively complex. Adding to this, the biological effects and possible toxicity of the wear particles needs to be assessed.

Finally some examples of more generic measurement needs in this area can be mentioned. A key issue in the development of new materials and implant devices is the relationship between material properties and cellular response. When it comes to the materials, the surface properties (e.g., wettability, roughness) are especially important, and the metrology aspects of characterisation are relatively well developed. In contrast, there is a great need for new methods for studying and characterising at the nano-level the interface between the coating of the implant material and the host tissue.

The complexity of this issue is increased by the fact that the material-tissue interface is a dynamic system, undergoing a continuous development from the time of implant insertion to periods that may extend to months and years. Addressing these questions will require a continuous development of new measurement and analytical technologies. The area thus offers ample challenges for the metrology community. Close collaboration with scientists from the biomedical community will be key to address the measurement questions in a successful way.

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4 Conclusions and outlook

4.1 Conclusions

This Introductory Guide has tried to introduce the reader to the science of measurements at the nanoscale, i.e. nanometrology. The term nanometrology has been specified as 'the science of measurement at the nanoscale'. Given the definition of nanoscale as the range between 1 nm and 100 nm, the term nanometrology is fairly precise. Nevertheless, nanometrology covers a vast area, due to the multidisciplinary nature of nanotechnology.

The importance of nanometrology has been illustrated with examples of major measurement breakthroughs, such as the invention of scanning probe microscopy, which contributed to the development of nanotechnology. Newly developed measurement techniques and instruments that enable measurements at increasingly small scale remain a crucial driver for the progress in nanotechnology. On the other hand, an additional contribution to the importance of nanometrology is recognized more and more, which is the demand for more reliable and comparable measurements. This increasing demand comes from both the industrial-commercial field, as well as from the regulatory-consumer area.

Among the main challenges for nanometrology, two main groups can be distinguished. A first group of challenges are generic, i.e., common to all metrology fields, such as the need for increased attention and metrological 'discipline' in the academic and industrial sectors. A broadly supported exercise in education on the proper use and implementation of some basic metrology concepts would be very beneficial, and this Guide hopes it can make a contribution. On the other hand, it is generally acknowledged that the number and possibilities of new nanoscale measurement methods and instruments is well ahead of the development of the specific measurement reference systems that are required to make the results from the new nanoscale methods and instruments, metrologically more robust, i.e. traceable and with reliable uncertainty estimates.

To illustrate the identified metrological challenges, practical examples and case studies from three different application areas (thin films, surface structures, and nanoparticles) have been shown. These examples have revealed how important it is to carefully analyse a measurement request, and how difficult it can be to select the proper measurement solution and conditions. With an ever increasing number of available techniques, this difficulty will remain a challenge in future as well.

The final subsection is devoted to the emerging issue of metrology for nanobiotechnology. Certainly, a number of the concepts developed in the thin film, nanoparticles or surface nanostructure areas, can be directly applied to specific nanobiotechnological applications, such as the geometrical analysis of articulating parts in biomedical implants. On the other hand, in those applications where physics, chemistry and biology each contribute to the complexity of the measurement need, it is acknowledged that there is a need to even start with the development of the required measurement reference systems.

4.2 Emerging challenges of nanometrology

The last sentence of the conclusions paragraph could have been the first sentence of this outlook paragraph: the *development of the required measurement reference systems* is needed. Developing new reference systems necessitates an improvement of the knowledge about the interaction between measurement probes and structures at the nanoscale. This is the area where substantial progress is expected from the (nano-)metrology community. While writing this outlook section, Co-Nanomet is developing a strategy proposal that will show what steps forward are needed to construct such reference systems.

A first step in the process will be to identify what are the relevant measurands ('What to measure?'), e.g., what are truly relevant parameters indicative for the desired functional properties (for example hydrophobicity) or for the unwanted toxicity of a nanomaterial? For every new identified and qualified measurement need, the appropriate reference system will have to be developed and agreed.

The reference systems to be developed will likely consist of netlike structures with reference methods, reference laboratories, and reference materials:

- Reference methods are fully validated, robust methods with adequate spatial resolution for measurements at the nanoscale. The dynamic components of nanotechnology, such as unstable and reactive nanoparticles or molecular interfacial structures, will require these methods to also have adequate time resolution. Reference methods will allow going beyond the temporary solution offered by standardization, where measurands are defined by the measurement procedure, leading to limited between-method comparability.

- Reference laboratories with proven competence and providing comparable measurement results will have to be established and prove their trustworthiness. The well-known process of interlaboratory comparisons will have to be used to support method validation and for laboratory proficiency assessment.

- The importance of reference materials for instrument calibration has been mentioned in several of the examples. Instrument suppliers do often provide reference materials to monitor the performance of their instruments. However, these reference materials can lack the traceability requirements needed to be able to compare the results with those obtained in other laboratories and with other methods. Only very few calibrators with the required characteristics (which are the characteristics required of a *certified* reference material) are currently available.

Lot's of work ahead, but *nil volentibus arduum…*[15]

[15] *Those who really want to achieve a particular thing will not experience the corresponding work as a burden.*