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Far field optical nanoscopy: How far can you go in nanometric characterization without resolving all the details?

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ABSTRACT

In the development of nanomaterials and biomaterials, new characterization techniques are required that overcome the challenges presented by the increasing dimensional ratio between the different entities to be studied and the growing complexity introduced by the use of heterogeneous materials and technologies. Diffraction limited far field optical nanoscopy techniques are receiving growing interest because of their ability to detect nanometer structures over very large fields and at high speed. We present a classification scheme of the different types of optical nanoscopy techniques. In particular, we highlight four categories of far field diffraction limited techniques based on increasing the contrast, measuring the phase, using deconvolution and using nano-markers. We demonstrate that by increasing the power of detectability, observability or measurability, a wealth of information concerning nanometric structures becomes available even though all the lateral details may not be resolved. For example, it is possible to determine the presence, the structure and orientation of nanostructures, to measure their density, position and 2D and 3D distribution and to measure nanometric surface roughness in bulk materials, surfaces, nano-layers, soft matter and cells. These techniques conserve all the advantages associated with classical imaging such as real time imaging, non-invasiveness, non-destructiveness and ease of use.

1. Introduction

Two of the challenges facing characterization in the development of new nano- and bio-materials and structures are the dimensional ratio between the basic nano-structures and the finished system and the growing complexity due to the use of inhomogeneous materials and different technologies. In the case of biomaterials, for example in layers of hydroxyapatite, the mineral part of bones and teeth, morphological, chemical, optical and mechanical information is necessary at several scales, from the nm level of the basic crystals to the millimetre level for the functional material [1]. Such materials may also be incorporated with polymers or metal alloys for implants or on microelectronic circuits for biochips.

For high resolution imaging of the basic elements at the nanometre level, electron microscopy and near field microscopy are the techniques of choice. But as with all techniques, while being well adapted to certain applications, their limitations make them unsuitable for others. For example, in electron microscopy, the electron beam and the vacuum or near-vacuum conditions can be destructive for many types of samples. In near field microscopy, point by point scanning limits the measurement bandwidth and the field of measurement. The physical presence of the tip also leads to measurement biases and a restriction to surface or near surface characterization.

Far field optical microscopy has received renewed interest in recent years for several reasons, not the least being because of the availability of high resolution and high speed cameras and image processing that allow quantitative and real time analysis. In addition, the development of new super-resolution techniques makes it possible to go well beyond the limits of diffraction. Finally, there is the realization that the measurements made with far field imaging can contribute significantly to the gaining of a deeper understanding of the structural, physical and chemical properties of nano-materials, sensors and systems without having to resolve all the details.

The different approaches mentioned concerning near field, far field and super-resolution optical techniques generally fall under the term of nanoscopy. One thing that can be remarked when approaching this new field of nanoscopy is that the large number of different techniques that exist can make it quite confusing when considering their performance and limitations. There are several different ways of classifying nanoscopy techniques. In Fig. 1 we propose one particular classification scheme that although not exhaustive, can help in better distinguishing between the different families of techniques that exist. A first level of classification concerns the distance at which the optical information is obtained, in the far field, with an imaging objective, or in the near field with a

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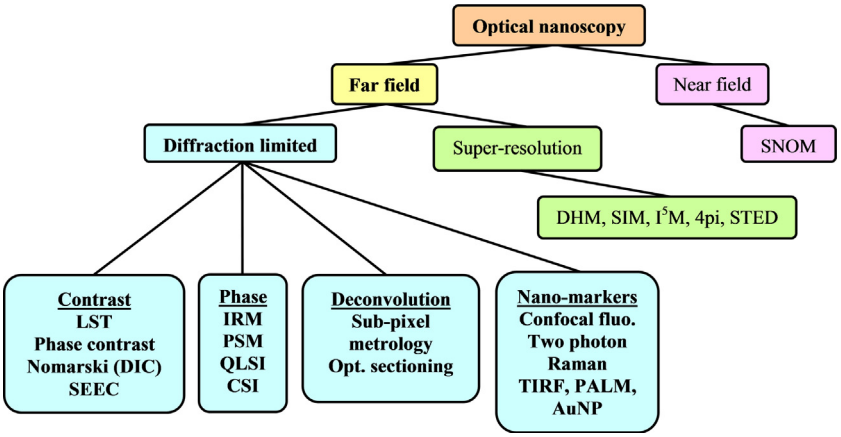

Fig. 1. The four sub-divisions of diffraction limited far field techniques (marked in blue) in the context of a global classification scheme for optical nanoscopy techniques. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

physical probe placed in the nanometric vicinity of the surface such as SNOM (scanning near-field optical microscopy).

Amongst the far field techniques, a second level of classification can be made concerning the lateral resolution attained, either limited by diffraction or by the super resolution technique used to go beyond such limits. Resolving lateral details below the wavelength of the light used while remaining in far field conditions, can be achieved by diffraction tomography (or DHM, digital holographic microscopy), SIM (structured illumination microscopy), I^5M, 4pi or STED (stimulated emission depletion).

The latter 3 techniques give the highest resolutions, from 100 nm down to 30 nm. In I^5M and 4Pi fluorescence microscopy [2], super-resolution is attained using the coherent addition of spherical wavefronts from two high numerical aperture objectives placed on opposite sides of the sample. In both cases, the central spot of the point spread function (PSF) is sharpened, leading to a 5–7-fold improvement in the axial resolution of ~100 nm. The characteristics of I^5M [3] are the use of incoherent illumination, wide field detection and a stronger signal leading to faster image acquisition but with the presence of more artefacts due to the stronger sidelobes in the PSF.

In 4Pi microscopy on the other hand, the use of coherent illumination and pin-hole detection leads to less artefacts due to the smaller sidelobes along the optical axis of the PSF, but is slower due to the need for point scanning. A further improvement in resolution can be obtained in 4Pi microscopy by means of stimulated emission depletion (STED) [4] using a saturated depletion of the fluorescent state of marker molecules. One wavelength is used to create fluorescence over a small diffraction spot and then a doughnut shaped ring at another wavelength is used to deplete specific regions of the sample so as to leave a much smaller central focal spot, effectively reducing in width the central spot of the PSF and resulting in a lateral resolution of 30 nm. It should be noted nonetheless, that the best resolutions are only attainable in fluorescence microscopy.

The second category of far field techniques concerns those that are diffraction limited and that can be sub-divided into four categories according to the method used to give the nanometric sensitivity:

1. Increasing the contrast by means of the illumination, the phase or the polarization.
2. Measuring the phase by interferometry.
3. Using deconvolution in sub-pixel metrology techniques.
4. Using nano-markers such as fluorochromes or gold nanoparticles.

It is this particular category of far field, diffraction limited techniques (marked in blue/grey boxes in Fig. 1) that is the subject of this paper. We provide some of the answers to the question of just how far it is possible to go in nano-characterization without resolving all the details. While being diffraction limited, these techniques can nonetheless be used to obtain important information from nano-structures while conserving all the advantages of classical imaging [5]. For example, characterization can be performed over wide fields of hundreds of micrometres and even millimetres and at a very high rate that often allows real time measurement. The use of an optical probe is also non-invasive, non-destructive and non-toxic for living organisms. We pointed out the advantages of such far field nanoscopy techniques in several papers at the beginning of the 1990s [5–7] but it is only in more recent years that the idea has become more popular. Such a review and identification of the basic principles involved in far field nanoscopy in the context of today's nano- and biomaterials could be useful to stimulate the development of new, powerful instrumentation. In the four sections that follow, we therefore describe a selection of far field, diffraction limited nanoscopy techniques using contrast, phase measurement, deconvolution and nano-markers to illustrate some of the principles behind them for performing nano-characterization.

2. Nanoscopy using high contrast for detection of nm structures

When the size of an object under a conventional optical microscope is smaller than the Rayleigh limit, the intensity decreases rapidly to the point of the object becoming no longer visible. This can be described in terms of Rayleigh scattering; when the object size $d \ll \lambda$, the intensity of the scattered light decreases as a function of d^6. But since scattering is uniform in all directions, one way of making nano-particles visible in the far field is simply to increase the contrast. For images containing small features that are present on a large uniform background, the contrast is given by the Weber contrast, C_W:

$$C_W = \frac{I_{Max} - I_{Min}}{I_{Min}} \quad (1)$$

where I_{Max} is the maximum intensity (intensity of the features) and I_{Min} is the minimum intensity (background intensity). For an 8 bit image depth, C_W therefore varies from 1 to 255. In images having structures containing equivalent bright and dark features,