Holography with Low Energy Electrons: Principles and Applications

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Abstract
The concept of holography with low energy electrons is described in view of its applications in molecular biology. The challenges and difficulties associated with Gabor type holography are outlined and the differences between coherent electron beams of high and low kinetic energy are discussed. The properties of the coherent electron point source for low energy electrons are reviewed as well as its application in the lens-less holographic microscope. Investigations of in-situ manipulation of objects with nanometer sized dimension will be discussed. Those experiments have recently been applied to DNA molecules and it has been discovered that DNA molecules are in fact electrically conducting bio-polymers.

Basic Concepts of Holography

Fifty years ago, the idea of holography as "a new microscopy principle" was presented by Gabor [1] as a means to circumvent the inherent spherical aberrations of electron lenses. Gabor proposed to employ a divergent electron beam, propagating beyond the focus of an electron microscope, to illuminate an object placed in the path of the beam and to record the result of the interaction between the electrons and the object at a distant detector. The geometry of this set-up is illustrated in Figure 1. At first, the schematic appears like a simple projection set-up giving rise to a magnified image of the object at the distant detector and exhibiting a contrast which is given by the ability of the object to absorb some of the incoming electrons. However, if the phase space density of the electrons is high, which implies that their spread in location $\Delta(x_1,x_2,x_3)$ in real space and momentum $\Delta(p_1,p_2,p_3)$ is sufficiently small, the electrons are coherent and have to be considered as a wave with a de Broglie wave length λ=h/p. In such a situation, the divergent beam beyond the point focus of the electron microscope has to be regarded as a propagating coherent spherical wave which is impinging onto the object under study. Most of the wave will pass the object without interactions and arrive at the detector to form a coherent background. This part of the wave is referred to as the reference wave. A much smaller fraction of the primary coherent wave front is elastically scattered at the object. This gives rise to a phase shift in comparison with the primary wave. Since the elastic scattered wave is coherent with the reference wave, the two wave fields are able to interfere and form a fringe pattern at the detector. The intensity distribution of this record

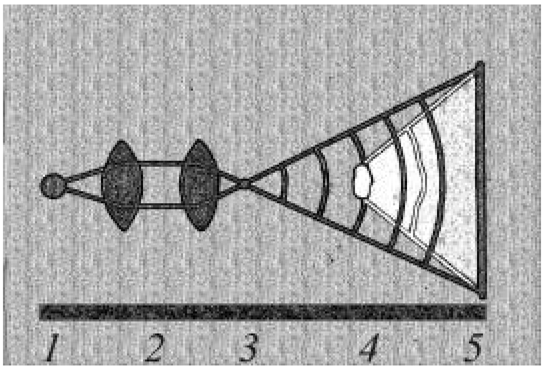

Figure 1: Schematic showing the set-up of an electron microscope to be employed for holography according to Gabor. 1: Electron Source. 2: Electron optical column. 3: The focus of the electron microscope is the de-magnified image of the primary electron source. 4: Object that scatters some of the coherent electrons. 5: Detector to record the hologram which arises from the interference between the coherent background and the part of the wave that experienced scattering by the object.

is determined by the constructive and destructive interference between reference and object wave. This justifies the term "holo" inasmuch as the "complete" information about the object wave, namely its amplitude and phase, is contained in this two-dimensional record. While the complete information about the object is available in the holographic record, it is however not in a configuration that allows to deduce structural information about the object by just looking at it. It is therefore necessary to implement a second step to retrieve the desired microscopy information about the object from the hologram. This is achieved based on fundamental optical principles of diffraction. Given an object, determined in its shape by an object function, the Huygens-Fresnel-Kirchhoff theory describes the hologram as a transformation applied to this object function. In order to derive the object function again from this interference pattern, the inverse transformation is applied to the holographic record. Physically, this corresponds to illuminate the hologram again with the same type of radiation as during the recording process of the hologram; however in the absence of the object. The waves arising from scattering at the hologram interfere in such a way as to reveal the shape of the wave front at the object. Another way to visualize the process of the hologram reconstruction is to regard all the elementary waves that form the hologram by interference as "back-propagating waves" that converge to form a real image of the object. The major handicap of in-line holography is the twin image problem, a second image of the object that appears in the reconstruction process. This twin image is a real image, located at twice the source-object distance opposite to the hologram plane. Since this two images are situated on the same axes, there is always the out of focus twin image superimposed on the primary image. A number of proposals and some attempts have been made to eliminate this disturbing background arising from the twin image. The easiest way to at least minimize the twin-image contribution is to arrange the geometry of the set-up accordingly. This can be accomplished by positioning the object at a distance from the source that is large compared to the size of the object under study. This arrangement ensures that the twin image is largely out of focus at the position where the primary image is evaluated and thus its background contribution will nearly be constant over the area covered by the object. This provision defines the field of sensible utilization of in-line holography to small objects that cover only part of the coherent wave front.

Early attempts of electron holography [2] following Gabor's insight did not lead to any practical applications due to the lack of sufficient bright electron sources at this time. Following the invention of the Laser, it was subsequently experimentally realized that Gabor's ideas proved to be correct. Furthermore, light optical holography became a tool with great practical applications. However, as mentioned already above, Gabor's original in-line holography concept proved to be limited to the study of small particles by employing pulsed laser beams. Laser-holograms of larger objects are exclusively taken in the off-axes geometry that was introduced by Leith and Upatnieks [3]. They came up with an effective way to eliminate the twin image problem by splitting the primary beam to physically separate the path of the object wave from that of the reference wave. The invention of the biprism for electrons by Möllenstedt [4] made the off-axes geometry also accessible to holography with electrons. As one can recognize by the contribution of H. Lichte [5] in this issue, off-axes electron holography has become an impressive domain of ultra high resolution electron microscopy.

Since the early efforts by Heine and Mulvey [2] to realize in-line holography experimentally with a conventional electron microscope, significant progress has been made over the decades to improve on the brightness of electron sources, which happened to be one of the major problems in the early days of electron holography. However, in-line electron holography with conventional electron microscopes still has its challenges and problems. They are primarily associated with the high kinetic energy of the electrons, which is in the 100 keV regime. They account for two major problems, the weak phase contrast and the radiation damage, that happens to be particular disturbing in studies of organic or biological objects. Despite all those complications, progress has been made by Tonomura et al. [6] in successfully employing high energy electron beams to perform Gabor type in-line holography.

An entirely different approach to electron holography has become possible after the invention of an electron point source which by itself provides a coherent electron ensemble without the need for lenses to optically de-magnify a more extended source.

The Electron Point Source

Field ion microscopy techniques [7] have been used to built an ultimately small pyramid terminated by just one individual atom that is supported by a cluster of three atoms [8]. From the field ion images, presented in Figure 2, one can assess that the electric field at the tip apex is only enhanced above an atomically small region, defined by the small protrusion created by three or ultimately just one atom. By reversing the polarity at this structure, field emission occurs by tunnelling of electrons from the metal into the vacuum. The energy spread of the emitted electrons is determined by the shape of the tunneling-barrier foldet with the density of states of the electrons near the Fermi energy.

This accounts for a fairly monochromatic electron beam with an energy distribution width around 200 meV. Inasmuch as the emission area is of atomic size and comparable to the de Broglie wave length of the electrons, we expect the emitted free electron ensemble to be highly coherent. This electron point source can consequently be used to carry out interference experiments in a conceptually simple set-up just as easy as with a Laser in light optics. If, for example, the electron point source is placed in front of two holes in a thin gold foil and a potential of 75 Volts is applied to emit electrons that impinge onto the foil structure, the well known Young double slit interference fringes can be detected some centimetres away from the foil, as displayed in Figure 2. In principle, coherent sources are simple to achieve. Even before the invention of the Laser a coherent light source could be made by just filtering light from an extended source and selecting a micrometer diameter aperture. However, the brightness of such a coherent light source is extremely low and it is therefore of little use compared to a Laser. Fortunately, our electron point source does not just directly deliver coherent electrons without the need for apertures and lenses, but it does so at a maximal rate which is of the order of 10^{15} electrons per second which relates to currents in the mA regime [9].

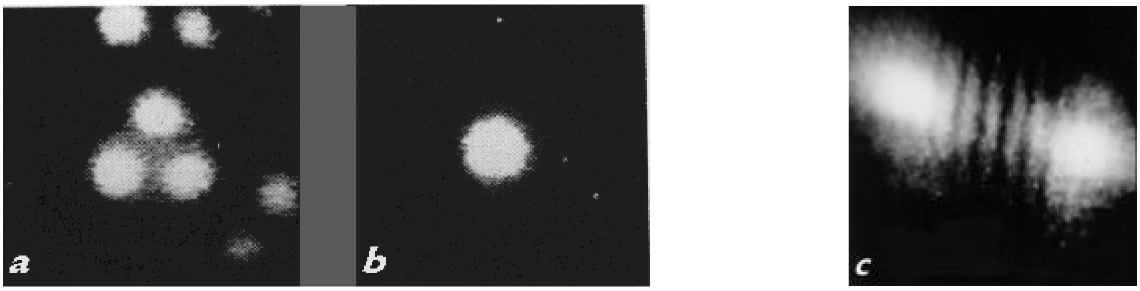

Figure 2: Helium field ion images taken from the top two layers of the ultimate smallest pyramid made up of tungsten atoms. A cluster of three atoms (a) support the individual atom that forms the top of the pyramid (b). The interference fringes observed behind two holes in a thin gold foil (c) show that the electrons with 75 eV kinetic energy originating from the point source are actually coherent.

Holography with Low Energy Electrons

With a bright and coherent electron source there is no need anymore to employ an electron optical column to perform holography [10]. After all, the main purpose of electron lenses in the classical electron holography set-up is to obtain a highly de-magnified image of the more extended primary source of the electron microscope. Even the electron guns of modern field emission electron microscopes employ sources that are at least three orders of magnitude larger than an atomic point source. We shall see that doing away with lenses does not just simplify the design and eliminate aberrations that are intrinsic to electron lenses, but it defines an entirely different interaction scenario between the electrons and the object due to the possibility to operate at low kinetic electron energies.
The schematic, following Gabor's ideas, as illustrated in Figure 1, reduces now essentially to the one shown in Figure 3 which contains only three basic elements. Element one: the source that prepares the coherent spherical electron wave front. Element two: the object that is to be examined. Element three: a detector that records the result of the interference between the scattered wave and the coherent background. Since the key to this microscope is the electron point source we used the term 'Low Energy Electron Point Source (LEEPS) - microscope' for this tool. The in-line hologram of a carbon fibre network is also shown in Figure 3. It displays the raw data taken with a photographic camera from the detector. The high contrast for light atoms such as carbon is due to the low kinetic energy of the electrons which amounts to 80 eV in this particular case. This image of a carbon sample does already clearly indicate the distinction of the LEEPS microscopy from conventional electron microscopy. High energy electron microscopy experiments frequently use carbon as a support to image heavier metal atoms like gold for example. Carbon with its low z-number is one of

the best, because almost transparent, supports for electrons in the 100 keV range. In contrast to this, the low energy of the electrons and the associated scattering mechanism produce high contrast even for atoms with a low z-number. As of today, no practical analogue to the transparent carbon films for high energy electron microscopy is known for the low energy regime, other than vacuum. This puts certain demands on the sample preparation inasmuch as the objects under study have to be surrounded by enough empty space. It turns out that this is not a severe constraint. Compact objects like clusters can be placed onto fibres that span holes, as for example in carbon films, and chain-like objects like polymers can be spanned over holes in a support film. In the latter case one might even argue that the molecule is in a more 'natural' state compared to being adsorbed onto a surface of a support film. In any event, the low energy of the electrons in the LEEPS-microscope connected with the high contrast that they provide, appears to be one of the major benefits of this tool in view of the examination of molecular species that are usually made up of light atoms. A second and equally important aspect associated with the low energy is the fact that scattering at the object is mainly elastic and does not lead to noticeable radiation damage. On the other hand, the low energy narrows the application range of LEEPS microscopy to small objects due to the low penetration length. Ultra low energies of only 7 eV at which the penetration of the electrons increases again have been realized in the LEEPS microscope by Morin [11]. However, even if it would be routinely possible to operate at just a few eV kinetic electron energy, one would have to sacrifice some of the resolution potential of this tool due to the increased electron wave length. At present the experimental lateral resolution, evaluated from the reconstructed images, is in the nanometer regime. In our present design the major experimental limitation is associated with the ability to detect high order interference fringes which is due to our present 8 bit detector dynamics. The use of modern CCD detectors should help to improve the experimental resolution limit. After all, the LEEPS microscope utilizes electrons with sub-Angstrom wave length and it does not suffer from lens aberrations. Apart from the contrast and the spatial resolution, another important aspect to characterize a microscope is its time resolution. Due to the parallel detection of the LEEPS technique and its bright electron source, an entire image containing some 10^8 electrons can be acquired in a time of only some 10 μs. This is important to carry out dynamical studies.

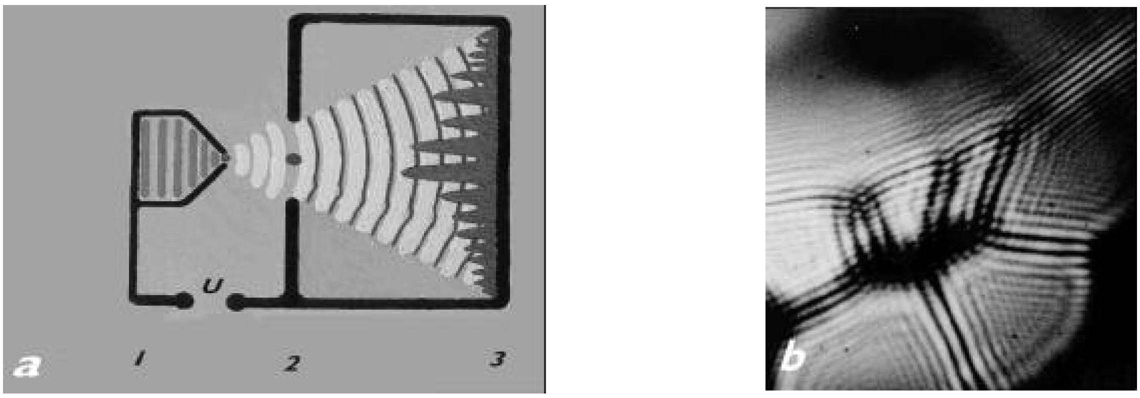

Figure 3: (a) Schematic of the Low Energy Electron Point Source (LEEPS) - microscope. 1: Electron point source. 2: Object supported on a partly transparent sample holder. 2: Detector to observe the in-line hologram. (b) A hologram of a carbon fibre network, taken with electrons of 80 eV energy, is shown.

Manipulation of Nanometer-sized Objects

Most microscopy tools are used to image objects in order to obtain information on their geometrical and electronic structures. Some tools are also able to observe dynamical changes of objects relative to their environment or within the object itself, like diffusion processes or conformational changes in clusters for example. Those observations are usually done in an ordinary thermal environment. Another challenge is to actually carry out an experiment on an object while observing it and its environment in-situ. With optical microscopes this is possible, for example by using Laser traps. Part of a biological cell can be modified or moved around while observing the entire cell at the same time.

The resolution is of course limited to that of red light which is used to not introduce radiation damage to the object. With the local probe methods, invented by G. Binnig and H. Rohrer [12], one can even manipulate samples on an atomic scale; D. Eigler [13] has shown us some time ago how to place atoms at will with an incredible accuracy. However, the sequential imaging mechanism inherent to all local probe methods allows to do only one thing at a time; that is to apply local forces at one time and to evaluate the changes on its environment at a later time. The possibilities of the LEEPS technology are at present somewhere in between that of the optical microscopy with its parallel detection and that of the scanning probe methods with its atomic resolution. With the LEEPS technology it is possible to introduce forces (mechanical or electrical) on a nanometer scale and observe the entire environment on a selected area, corresponding to a wide range of magnifications, at the same time. This includes experiments like bending or stretching objects in-situ to observe their mechanical responds while a force is acting. It also includes the application of electrical forces. An electrical potential can be applied to a certain part of a molecular structure and its response, that is generally distributed over a large area of the species, can be observed on a nanometer spatial- and 10 μs time-resolution scale.
A LEEPS microscope, in which an additional manipulation-tip has been incorporated between the sample plane and the detector, as illustrated in Figure 4, has been employed for this type of experiments. While observing the image, the manipulation-tip can be moved in a highly controlled fashion into the sample plane to contact a selected site. An example of such an experiment is also shown in Figure 4, in which the apex of a tungsten tip has been brought into contact with a carbon nanotube. The nanotube follows the motion of the tip in three dimensions while experiencing elastic deformations. The manipulation-tip can also be raised to a certain electrical potential. As a consequence, an electrical current flows through just this one selected nanotube and it can be monitored. It turns out that a single nanotube can carry some several 10 μA of current before it actually breaks. The part of the nanotube that remains attached to the manipulation tip is by itself a quite interesting object inasmuch as it can be employed as a coherent source for electrons [14].

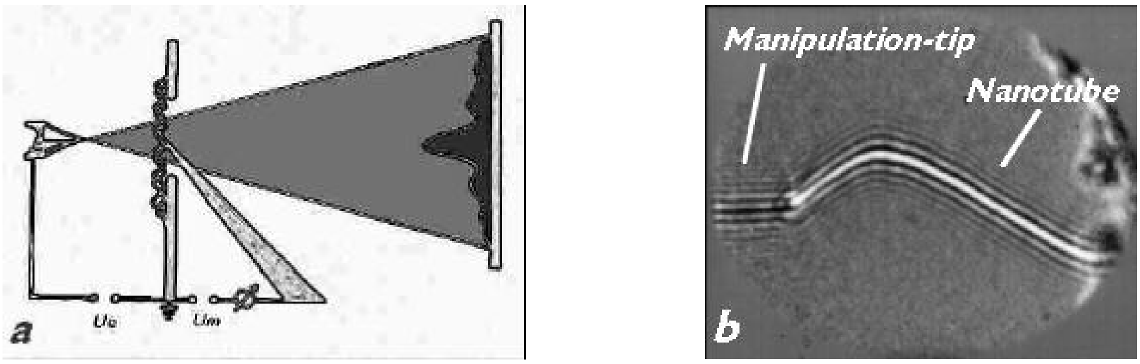

Figure 4: (a) Schematic showing the set-up to manipulate nanometer sized objects. A manipulation-tip is introduced into the LEEPS microscope to achieve mechanical contact to a selected object placed on a regular array of holes in a carbon film. An electrical potential applied to the manipulation-tip allows to probe the electrical conduction properties of the object under study. (b) A LEEPS image shows the manipulation-tip that has been guided to a nanotube that reaches over the rim of one hole of the sample holder.

Applications in Molecular Biology

The LEEPS microscope has been used to generate high contrast holograms of unstained DNA molecules, deposited on a dedicated sample holder fabricated by micromachining techniques. The numerical reconstruction of the DNA molecules reveals the structure of the molecules depicted as the magnitude of the electron object wave front [15].
Experiments to address the electrical conductivity of DNA molecules have recently been carried out by contacting individual DNA fibres by means of a manipulation-tip as described above [16]. The linear current vs. voltage curve for a 600nm long DNA fibre, shown in Figure 5, presents clear evidence for the fact the DNA molecules actually represent small conducting wires. The issue of

electrical conductivity in DNA molecules, important for example in the context of understanding repair mechanisms, was often debated but remained unsolved for a long time within the molecular biology community. Apart from the biological relevant aspects, the above mentioned results will open up the possibility to employ DNA molecules, whose chemistry happens to be extremely well understood, as tailored molecular one-dimensional conductors.

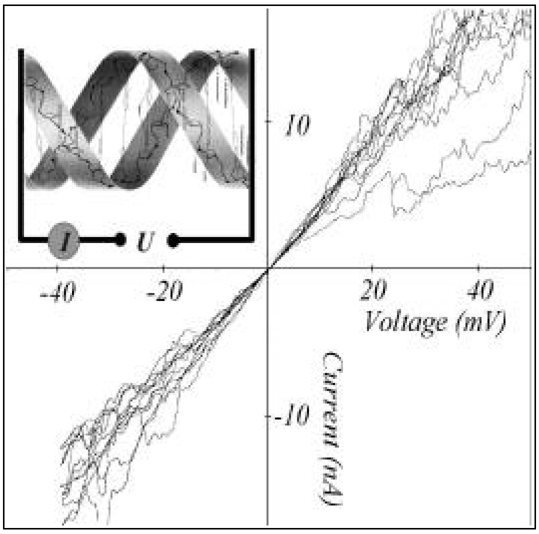

Figure 5: I-V characteristic of a 600 nm long DNA strand.

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