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A novel low energy electron microscope for DNA sequencing and surface analysis

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ABSTRACT

Monochromatic, aberration-corrected, dual-beam low energy electron microscopy (MAD-LEEM) is a novel technique that is directed towards imaging nanostructures and surfaces with sub-nanometer resolution. The technique combines a monochromator, a mirror aberration corrector, an energy filter, and dual beam illumination in a single instrument. The monochromator reduces the energy spread of the illuminating electron beam, which significantly improves spectroscopic and spatial resolution. Simulation results predict that the novel aberration corrector design will eliminate the second rank chromatic and third and fifth order spherical aberrations, thereby improving the resolution into the sub-nanometer regime at landing energies as low as one hundred electron-Volts. The energy filter produces a beam that can extract detailed information about the chemical composition and local electronic states of non-periodic objects such as nanoparticles, interfaces, defects, and macromolecules. The dual flood illumination eliminates charging effects that are generated when a conventional LEEM is used to image insulating specimens. A potential application for MAD-LEEM is in DNA sequencing, which requires high resolution to distinguish the individual bases and high speed to reduce the cost. The MAD-LEEM approach images the DNA with low electron impact energies, which provides nucleobase contrast mechanisms without organometallic labels. Furthermore, the micron-size field of view when combined with imaging on the fly provides long read lengths, thereby reducing the demand on assembling the sequence. Experimental results from bulk specimens with immobilized single-base oligonucleotides demonstrate that base specific contrast is available with reflected, photo-emitted, and Auger electrons. Image contrast simulations of model rectangular features mimicking the individual nucleotides in a DNA strand have been developed to translate measurements of contrast on bulk DNA to the detectability of individual DNA bases in a sequence.

1. Introduction

Significant demand exists for the development of novel techniques capable of imaging nanostructures, macromolecules, and surfaces to provide analytical capabilities with sub-nanometer resolution. Many specimens of interest, including biological macromolecules, sub-cellular structures, and protein crystals are difficult to image in standard (scanning) transmission electron microscopes ((S)TEM) as well as in scanning electron microscopes (SEM) because of a lack of contrast, radiation damage, and charging effects induced by the illuminating electron beam. Recently, more 'gentle' low-voltage (S) TEMs have been developed that reduce the beam energy to 20 keV [1,2]. However, the application of these instruments becomes limited to extremely thin samples, while the damage to biological specimens remains significant. Electron landing energies have been reduced to 1 keV or less in low-voltage SEMs; however the lack of resolution and the resulting, shallow deposition of charge on the sample enhances charge induced beam distortion and blur, which limits the utility of these microscopes. Environmental SEMs [3] mitigate charging effects by introducing gas at relatively high pressures; however, the scattering of the electron beam by the gas molecules degrades the resolution to inadequate values, particularly at low beam energies.

Low energy electron microscopy (LEEM) is a technique that was developed in the 1980s by professor E. Bauer's group [4]. Similar to a SEM, a LEEM detects electrons emitted, scattered, or reflected from the surface of a specimen. However, rather than scanning a finely focused beam across the specimen, the microscope illuminates the sample surface with a broad beam of electrons, like a TEM. The illumination and projection optics are separated by a beam separator, which is a magnetic prism array that deflects the

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electron beam towards the axis of the objective lens. The objective lens is an immersion cathode lens, which decelerates the electrons from the column transport energy, typically of order 10–25 keV, to a landing energy ranging from 0 eV to a few 100 eV. In the opposite direction, moving away from the specimen, the objective lens simultaneously accelerates the reflected and emitted electrons and forms an image that is magnified by the projection optics on a scintillating screen.

The extremely low energy of the illuminating electrons makes LEEM an exquisitely sensitive surface imaging technique, capable of imaging single atomic layers with high contrast [5]. Furthermore, the low electron impact energies prevent radiation damage to delicate samples such as biological molecules. The main drawbacks of LEEM are its increased susceptibility to chromatic aberrations and charging effects. In spite of the short deBroglie wavelength, which is in the range of 4 Å for 10 eV electrons, the lateral resolution of conventional LEEM instruments is limited to a few nanometers. In addition, when a conventional LEEM is used to image insulating specimens, sample charging adversely impacts the low energy electron beam, blurring and distorting the image.

This paper describes the results to date of a phase I study for sequencing DNA with a MAD-LEEM instrument. This work has been focused on the simulation of the achievable spatial resolution of this microscope as well as on the experimental characterization of nucleotide-specific contrast of bulk homo-polymers. The ultimate goal of this work is to produce a microscope that is capable of imaging label-free bases in single- and double-stranded DNA sequences of practically unlimited length, with sub-nanometer resolution, at an affordable cost, and with no radiation damage. To this end, the second phase will be devoted to building a MAD-LEEM prototype, preparing substrates with linearized DNA, and validating the resolving power of the instrument by imaging selected sequences of DNA.

MAD-LEEM is a novel instrument that aims to overcome the aforementioned drawbacks of current LEEMs. The instrument utilizes an energy filtering mechanism to reduce the energy spread of the electrons scattered (or emitted) by the sample to a few tens of meV. The same mechanism is also used by the monochromator to reduce the energy spread of the illuminating electrons. It also employs electron mirrors as aberration correcting elements to achieve sub-nanometer resolution over a relatively large field of view. Last, it illuminates the sample with a second low energy, overlapping electron beam with a tunable landing energy to neutralize the charge deposited by the imaging beam, thereby eliminating surface charging.

2. MAD-LEEM electron optics

Fig. 1 shows a schematic layout of the MAD-LEEM electron-optical column, containing two independent illumination beams, a monochromator, an aberration corrector, and an energy filter, which are integrated into a single column by three beam separators. The basic features of MADLEEM and its simulated performance have been reported [8,16]. The key new features of this electron-optical design are the pentode mirror aberration corrector, the knife-edge based imaging energy filter, the addition of the photoemission electron microscopy (PEEM) imaging mode and the modified and improved objective lens. Detailed simulations of the system performance based on these new features have been undertaken showing significant improvements.

The beam separators are based on compact, double-focusing magnetic prism arrays composed of uniform magnetic fields of different strengths and lengths. Each separator quadrant deflects the beam by 90° and transfers stigmatically two planes, the diffraction (slit) and (achromatic) image planes, with unit magnification. The excitations of the coils are chosen so that the prism behaves as a thick, round field lens along the curved axis. The contribution of the individual beam separators to the overall aberration of the system is minimized by placing a highly-magnified image at the achromatic plane, at the center of this field lens.

The monochromator and energy filter are needed to reduce the energy spread of the beam to 100 meV or less, which is a prerequisite for obtaining detailed information about the chemical composition, interatomic bonding, and local electronic states of macromolecules [6]. The aberration corrector utilizes an electron mirror that can compensate one or more aberrations of the objective lens. The dual beam approach eliminates specimen charging by illuminating the sample specimen with two superimposed flood beams, where the landing energy of the secondary beam is tuned to remove the charge deposited by the first [7].

2.1. Illumination optics

The illumination configuration shown in Fig. 1a has two branches, one for the higher energy imaging beam (solid lines) and one for the mirror, charge balance beam (dash lines). Both beams are recombined by the main beam separator.

2.1.1. Monochromator

The MAD-LEEM illumination optics includes a monochromator for the imaging beam that can reduce the electron energy spread of commonly used electron sources (e.g. thermionic and thermally assisted field emitters) from the range of 0.5–2 eV to less than a few tens of meV. It utilizes the combination of a second energy-dispersive beam separator and an electron mirror [8,9] to filter electrons with energies that are lower or higher than the selected nominal energy, E_0. The electron source image is transferred via the slit planes of the main and second beam separators onto the objective lens back-focal plane, while its angular distribution is transferred via the beam separator achromatic planes and used to illuminate the specimen. The practical realization of the monochromator incorporates several transfer lenses with net zero rotation and an electron mirror to account for the residual uncertainties in the optical parameters of the separator and mirror [8]. This arrangement uses a knife edge as the energy-selecting device, a much simpler and more reliable design when compared to the narrow, often sub-micrometer slits needed in typical monochromator applications. Furthermore, the symmetry introduced by the double pass through the monochromator eliminates the energy dispersion of the beam separator.

The effect of the reduction in energy spread of the incident electron beam to a few tens of meV is twofold: first, it can improve the energy resolution in spectroscopic imaging modes, and second, it reduces the higher rank chromatic aberrations remaining after the correction of the primary chromatic aberration, in particular at low landing energies. The impact of the monochromator is less noticeable at higher electron energies as the relative contribution of chromatic aberrations is reduced.

2.1.2. The charge balance beam

After exiting the monochromator, the nearly monochromatic imaging beam passes through transfer lenses and an Einzel lens, a three-electrode electrostatic lens with both outer electrodes at the ground potential and with the central electrode biased at a high negative potential. The beam passes through this Einzel lens and enters the main beam separator, which deflects the beam into a third beam separator. This separator deflects the beam by another 90° into the objective lens and, in the process, cancels the dispersion introduced by the main beam separator.

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