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A Monochromatic, Aberration-Corrected, Dual-Beam Low Energy Electron Microscope for DNA Sequencing and Surface Analysis

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Low energy electron microscopy (LEEM) is a powerful surface imaging technique [1] that has been used extensively for the characterization of surfaces [2, 3] and extended to nanotechnology [4]. LEEM utilizes landing energies from 0 to 20 eV and images reflected rather than transmitted electrons. The main drawback of LEEM is its lateral resolution: in spite of the short deBroglie wavelength in the Å range, the resolution of conventional LEEM instruments is limited by lens aberrations to ~ 5 nm at 10 eV and sub-nm resolution has not been achieved yet. In addition, when a LEEM is used to image insulating specimens, the low landing energy exacerbates charging resulting in reduced image quality.

Recently, we proposed [5] to develop a novel monochromatic, aberration-corrected dual-beam LEEM (MAD-LEEM) capable of imaging nanostructures and surfaces at sub-nm resolution that utilizes electrons with landing energies in the range of 0 to a few 100 eV. A schematic layout of the MAD-LEEM column is shown in Fig. 1. The monochromator [6] reduces the energy spread of the illuminating electron beam to 0.1 eV or less, which significantly improves spectroscopic and spatial resolution. The mirror aberration corrector (MAC), based on the electron mirror approach first developed by Rempfer [7], is needed to improve the spatial resolution into the sub-nm regime. Dual flood illumination [3] eliminates charging generated when a LEEM is used to image insulating specimens.

The specialized software package MIRROR DA developed by MEBS, Ltd. has been used for the aberration analysis of both the objective lens and the MAC. This differential algebra-based software package computes aberrations of electron mirrors of any order with any symmetry and can handle combinations of electron mirrors and electron lenses in an unified way. We have carried out the aberration analysis of objective lens and MAC combinations for energies in the range from 1 to 200 eV, and the summary is shown in Fig. 2. It can be seen that with a tetrode MAC, the optical blur approaches 1 nm at 200 eV and can be extended into the sub-nm regime with a pentode MAC.

MAD-LEEM is in particular aimed at imaging of biological and insulating specimens, which are difficult to image with conventional TEM, Low-voltage SEM, and LEEM instruments. The low energy of electrons is critical for avoiding beam damage, as high energy electrons with keV kinetic energies used in SEMs and TEMs cause irreversible damage to many specimens, in particular biological materials. A potential application for MAD-LEEM is in DNA sequencing, which demands imaging techniques that enable sequencing at high resolution and speed, and low cost [5]. The key advantages of

the MAD-LEEM approach are long read length, the use of low electron energies, high throughput, and the absence of heavy-atom DNA labeling.

We have carried out preliminary experimental work on DNA samples in order to demonstrate that contrast mechanisms sensitive enough to distinguish individual nucleotides or pairs exist at low electron energies in the 1 - 500 eV range. We have studied three contrast mechanisms: electron reflectivity, X-ray photoelectron emission and Auger electron emission to distinguish bulk samples with oligomers containing only one of the four single DNA bases (A, C, G or T). We have carried out image contrast simulations (Fig. 2b, c) of the detectability of individual nucleotides in a DNA strand in order to refine the optics blur and nucleotide contrast requirements. All three contrast mechanisms can be readily obtained in our MAD-LEEM instrument, which in combination with the theoretical results on aberration correction hold promise for distinguishing individual nucleotides without labels and thus provide a path for sequencing by direct imaging.

[1] W. Telieps and E. Bauer, Ultramicroscopy **17**, (1985), p. 57.
[2] M. S. Altman, J. Phys.: Condens. Matter **22**, (2010), p. 084017.
[3] M. Mankos, V. Spasov and E. Munro, *Advances in Imaging and Electron Physics* **161**, edited by Peter W. Hawkes, (Elsevier, Ltd. 2010), p. 1.
[4] M. Mankos, H. F. Hess, D. L. Adler, and K. J. Bertsche, U.S. patent 6,870,172 (22 March 2005).
[5] M. Mankos *et al*, J. Vac. Sci. Technol. **B 30**(6), Nov/Dec 2012.
[6] M. Mankos, U.S. patent 8,183,526 (22 May 2012).
[7] G. F. Rempfer and M. S. Mauck, Optik **92**, (1992), p. 3.
[8] This project was supported by Grant Number R43HG006303 from the National Human Genome Research Institute (NHGRI). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NHGRI or the National Institutes of Health.

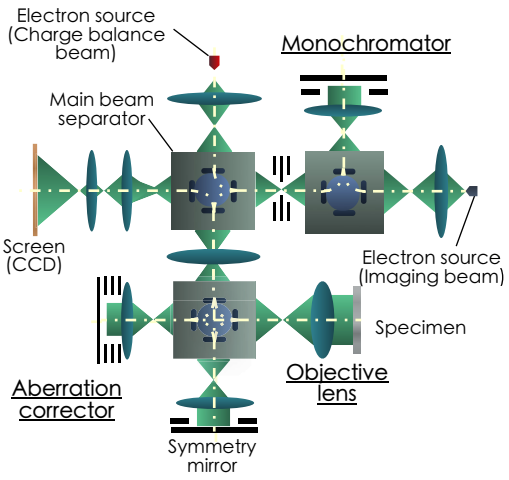

Figure 1. Layout of the MAD-LEEM column.

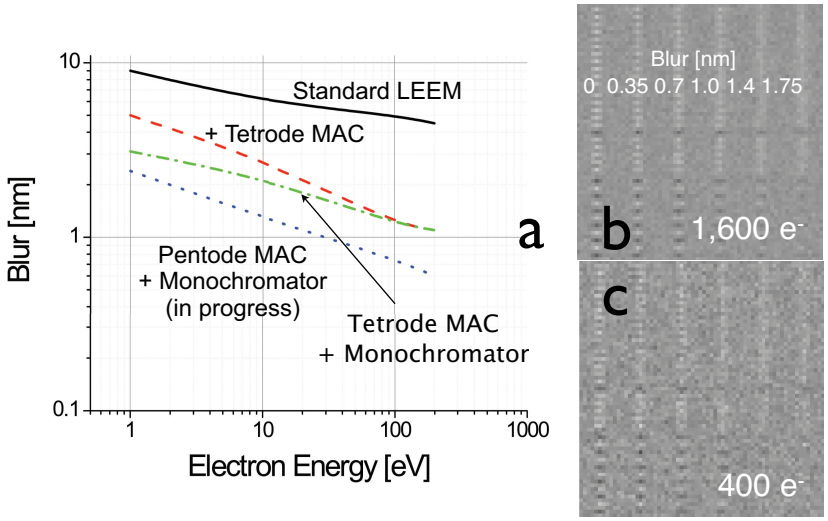

Figure 2. Blur vs. electron energy (a) for different correction schemes, and simulated images of a single DNA strand with a presumed +/- 20% base contrast for a range of optical blurs and 1600 (b), resp. 400 (c) electrons/pixel.

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