Progress Towards An Aberration-Corrected Low Energy Electron Microscope for DNA Sequencing and Surface Analysis


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Abstract

We are developing a novel aberration-corrected, low energy electron microscope (MAD-LEEM) armed with imaging DNA sequences as well as macromolecules, nanoparticles, and surfaces. The key advantages of this approach compared to current sequencing techniques are long reads, no need for DNA labeling, and imaging with low energy electrons. Longer reads reduce the complexity needed to assemble the sequences and minimize errors. The absence of heavy-atom labels, e.g. needed for proposed TEM-based sequencing techniques, simplifies sample preparation and improves accuracy. The use of low energy electrons ensures that radiation damage is minimized, i.e. high doses needed to achieve high throughput and low cost can be used. This novel approach promises to significantly improve the performance of a LEEM and extend its applications to a variety of samples that may benefit from high resolution imaging at low landing energies without charging effects, including biological samples, oxides, and catalysts.

Motivation

Electron Microscopy

Has potential to significantly extend individual read length and accuracy
- Transmission electron microscopy (TEM) techniques utilize the contrast from DNA bases labeled with heavy atoms (Honeycomb Molecule, Z5 Genomics)
- Main drawbacks
  - Need for labeling leads to read errors and complex sequence assembly
  - Radiation damage (alpha, x-rays) limits the total electron dose and throughput

Low Energy Electron Microscopy

Advantages
- Imaging nucleotide sequences of unlimited length, labels not needed
- High contrast for biological specimens at low energies, staining not needed
- Minimal radiation damage due to very low landing energy
- Monolayer sensitivity (small nuclear path; Δ-resolution in z)
- High throughput (projection, high dose) = affordable sequencing cost/gigabases

Challenges
- Lateral spatial resolution limited in today's LEEMS by aberrations to ~5 nm
- Charging on insulating layers
- Nucleotide contrast

MAD-LEEM

Key Features

Monochromator
- Energy spread reduced to < 0.05(eV) from 0.5-3(eV)

Aberration corrector
- Resolution improved to < c nm from 10xnm from (5nm)

Dual beam illumination
- 2 coaxial flood beams eliminate charging high pressure (e.g. ESEM) is not needed

Plan

Phase I (2011-2013)
- Electron Optics: Detailed column design and modeling of spatial resolution with aberration corrector and monochromator
- Experiments: Measurement and analysis of reflective/mesoscopic properties of individual DNA bases at low energies (0 to 1000 eV); Investigation of contrast

Phase II (2013-2015)
- Realization of MAD-LEEM prototype with resolution needed to distinguish individual nucleotides and capable of achieving throughput equivalent to reading one genome (30Gb) in < 8 hours with error rates < 10-4.

Optics Simulations

Objective Lens Analysis
- Investigated electrostatic and combined magnetic immersion objective lenses optimized for LEEM
- Computed 5th order analysis to understand resolution improvement prior to aberration correction
- 5th order aberrations are key to get sub-nm resolution

MAD-LEEM

Mirror Aberration Corrector (MAC) Analysis
- Completed analysis of tetrode MAC as proposed by Rose & Prekaskas up to 5th order for the differential algebra method (Mirror-DA software by MEIBS, Ltd.)
- MAC focuses electrons with larger aperture angles and lower energies less than electrons with smaller angles and larger energies, i.e. opposite to a conventional lens

MAC Principle
- Extended analysis to higher landing energies to improve resolution
- Fine-tuned tetrode MAC spherical and chromatic aberration coefficients to cancel aberrations of used objective lens for a range of electron energies

Tetrode MAC
- Resolution limited by 5th order geometric and 3rd & 4th rank chrom aberrations
- Monochromator needed to make 3rd & 4th rank chrom aberrations negligible
- Further improvement requires perpemt MAC to cancel 3rd order spherical aberration (design in progress)

Energy

100eV Standard LEEM with Tetrode MAC
- LEEM with Tetrode MAC and monochromator

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Electron Reflectivity Results

- Electron reflectivity spectra from Au substrates with and without immobilized DNA were acquired in a LEEM over a range of landing energies from 0-200eV
- Deposited DNA samples are easily visible over a range of landing energies
- Small change in landing energy has strong impact on achievable contrast
- Electron reflectivity spectra at low electron energies demonstrate the high contrast achievable for bulk DNA structures on a Au surface
- Early results indicate that immobilized strands with different bases (5'-SDTPA-C20mer vs. 5'-SDTPA-A20mer) produce different ‘signatures’ when compared to the underlying Au layer

Conclusions

- Detailed electron optical analysis of key MAD-LEEM components completed
- Tetrode MAC has widely tunable negative aberration coefficients
- Compensates the aberrations of the objective lens down to ~1nm resolution
- Monochromator is critical for further resolution improvement
- Perpend MAC with monochromator has potential for sub-nm resolution
- LEEM imaging and electron reflectivity spectra at low electron energies indicate that high contrast is achievable for DNA structures on a Au surface
- Monochromatic illumination, aberration correction and charge control opens a new opportunity for nm scale imaging in biology, nanotechnology, semiconductor, ceramics, ferroelectrics, dielectrics, polymers...

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