Advances in Large Field and High Resolution Electron Tomography

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University of California, San Diego
Banff Workshop on Emerging Modalities of Medical Imaging
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Credits

• *Software Development*
  – Sebastien Phan
  – Rajvikram Singh
  – Alex Kulungowski

• *Instrumentation*
  – James Bouwer

• *Technical Support*
  – Masako Terada
Supporting Clinical and Basic Biological Research

• Major activities in clinical Research:
  – Neurodegenerative disease
  – Cardiomyopathy
  – Diabetes
  – Stroke
  – Mental retardation
  – Cancer
  – Addiction
  – Autism

• Basic research:
  – Astrocyte structure
  – Computational modeling of synaptic function
  – Function of the node of Ranvier
  – Macromolecular and organellar dynamics
  – Structure and function of mitochondria
  – Adult neurogenesis

• Imaging sciences
• Instrumentation
• Computational sciences
The National Center for Microscopy and Imaging Research

**Mission:**

- Develop and deploy technologies to determine and reveal supramolecular details in their cellular and tissue contexts.
- Focus on the ‘Meso-scale’ - ~0.5nm to > 100um
Computational Challenges in the Biosciences

• Life is organized on many spatial scales
• Biochemistry is exceedingly complex
• Representations of physical structure grow exponentially in complexity as range of spatial scale widens
• Flood of data from microscopes challenges the capabilities of digital computers
• Three dimensional reconstruction of physical structure is already a supercomputer problem
• Subsequent modeling of dynamics even more so
Petascale Biology?

• From EM to light microscopy:
  • Scale Change: 100K
  • Volume Change: $1 \times 10^{15}$
  • Tremendous amplification factor in biological processes

• Which details are important?
• Which become statistical?
• Which become irrelevant?

• Biology is largely a descriptive science. We have to put together realistic structural descriptions before tackling the dynamics.

• Merely piecing together a structural description is a daunting computational task.

• Google Brain? A map of the brain down to molecular details—and in 3D.
Outline

• Large-field tomography—quick overview
• Instrument characteristics
• Inverse problems
• Ray transforms
• The reconstruction process
• TxBR
• New mathematical developments
Why Do EM Tomography?

• Eliminate shadowing effects

• Elucidate 3D structure

• Connect supramolecular structure with light microscopy
Electron Microscope Tomography

Electron Microscope Image

Section of Reconstruction

Electron Microscopic Tomography at a Glance

- Used for constructing 3D views of sectioned biological samples
- Sample is rotated around an axis and images are acquired for each ‘tilt’ angle
- Electron tomography enables high resolution views of cellular and neuronal structures.
- 3D reconstruction is a complex problem due to low signal-to-noise ratio, curvilinear electron path, sample deformation, scattering, magnetic lens aberrations...
Flock House Virus

4.1Kx Magnification

12.2 Å /pixel

995 frames
Flock House
Virus

4.1Kx
Magnification

12.2 Å /pixel

Six Fold Tilt
Series
Reconstruction

Single Z Section
of
Reconstruction
Tomography, in practice, requires many steps:

- Sample preparation
- Data collection
- Feature isolation and tracking
- Image alignment
- Image filtering
- Volume reconstruction
- Object segmentation

Each step carries its own set of problems.

Choice of methods on one step affects subsequent steps.
Technical Problems in EM Tomography

- Noise. Imaging is through electrons scattering out from beam.
- Limited data. Exponential decrease of flux thru sample at high angles.
- Positioning accuracy. Sub micron information required.
- Magnetic lenses. Electrons travel in helical paths in focusing fields.
- High energy electrons, Structure degradation. Number of angles exposure-limited.
- Sample mass loss. Sample warping.
Derivation from First Principles

- Dirac Equation
- Schrodinger Equation
- Paraxial Schrodinger Equation
- Paraxial Image Formation
- Classical Paraxial Equation
- Higher Order Corrections
Paraxial Equation

- Light waves in homogeneous media
- Classical charged particle in electromagnetic field
- Quantum mechanical electron in electromagnetic field

\[ \psi_{|xx} + \psi_{|yy} + \frac{2i}{\hbar} g(z)\psi_{|z} - \frac{g^2 r^2}{\hbar^2} F(z)\psi(x, y, z) = 0 \]

- This does not account for lens aberrations and diffraction effects.
Physics of Electron Lensing

Can be solved analytically through separation of variables in cylindrical coordinates to produce the equations of motion for the imaging electrons

\[ \omega_L = \frac{e}{2m_e} B_z(r, \theta, z) \]

- \( B_z \) = z-component of the magnetic field
- \( \omega_L \) = Larmor frequency
- \( e \) = charge of an electron
- \( m_e \) = rest mass of an electron

Bell Shaped Field:

\[ B_z = \frac{B_0}{1 + (z/a)^2} \]

* Image from L. Reimer, TEM 1993
Real Electron Trajectories in Rotating Reference Frame

Electron trajectories for electrons incident parallel to the optical axis for various B-field strengths

\[ k^2 = \frac{eB_0^2a^2}{8m_0U} \]

\[ \omega = \sqrt{1 + k^2} \]

* Image from L. Reimer, TEM 1993
The Electron Microscope

Iron Core

Sample

Cooling

Objective Aperture

1st condenser lens coil
2nd condenser lens coil
3rd condenser lens coil

Objective lens coil

Objective mini lens coil
1st intermediate lens coil
2nd intermediate lens coil
3rd intermediate lens coil
4th intermediate lens coil

Filter lens coil

1st projector lens coil
2nd projector lens coil

Electron gun 1st beam deflector coil
Electron gun 2nd beam deflector coil
Condenser lens stigmator coil
Condenser lens 1st beam deflector coil
Condenser lens 2nd beam deflector coil
Objective lens stigmator coil
1st image shift coil
2nd image shift coil
3rd image shift coil
4th image shift coil
Intermediate lens stigmator coil
Filter lens 1st stigmator coil
Filter lens 1st beam deflector coil
Filter lens 2nd beam deflector coil
Filter lens 2nd stigmator coil
Projector lens beam deflector coil
Focus is changed in steps so focal plane moves through object. Note effects due to Helical trajectories and differential magnification.
The Contrast Transfer Function (CTF)

\[ I(k) = O(k) \cdot CTF(k) \]

where:

\[ I(k) = \text{Image} \]
\[ O(k) = \text{Object} \]
\[ k = \text{spatial frequency} \]

and

CTF is also a function of position along optical axis
Wide-field Images of 60 degree tilted sample show a strong focus gradient (CTF gradient)
Large Field of View Requires CTF Reconstruction from a Thru Focus Series

Amplitude Contrast CTF on a JEM4000 at 5000nm Underfocus

2D => Fourier Transform
3D => Fourier Integral Operator

* Generated with CTF generator written by Wen Jiang and Wah Chiu
**Fringing Fields Affect Image Formation**

- Differential magnification
- Differential rotation
- More pronounced for large format images
- Rotation and magnification are troublesome for tomography

\[
\theta_L \propto B_z(r, z) \quad \text{(rotation)}
\]

\[
\frac{\Delta M_{ag}}{M_{ag}} = \frac{\Delta z}{f} \quad \text{(magnification)}
\]
Tilt Geometry Distortions

Differential Magnification

Differential Rotation

Differential Rot + Mag
Projector Lens Aberrations
Spherical Aberration Produces Spatial Distortion

Spherical aberration results from a change in focus in center vs outer edge of lens.

Virtual image produces barrel distortion.

Real image at projection produces pincushion distortion.
Largest Spatial Aberrations are S-type Distortions in the Projector Lens Optics

EM manufacturer Specs:

\[ \Delta < 1.5\% @ r = 5\text{cm} \]

For a 4k x 4k detector 6cm diameter
\[ \Delta \sim 40 \text{ pixels} \]

For a 8k x 8k detector 10cm diameter
\[ \Delta \sim 80 \text{ pixels} \]
Projection in Electron Microscope

\( \gamma(\mathbf{X}_0, \theta, S) \)

- \( \gamma \) = electron path
- \( S_i \) = point of entrance
- \( S_f \) = point of exit
- \( \theta \) = tilt angle
- \( \mathbf{X}_0 = (x, y) \)_{image}
- \( \mathbf{X} = (x, y, z) \)
- \( v \) = object density fn
- \( \hat{u} \) = image intensity

Linear “single scattering model”

\[
\hat{u}(\theta; \mathbf{X}_0) = u_0 \cdot e^{- \int_{s_i}^{s_f} v(\gamma(\theta; \mathbf{X}_0, s)) ds}
\]

\[
u = \ln(\hat{u}(\theta; \mathbf{X}_0)) = - \int_{s_i}^{s_f} v(\gamma(\theta; \mathbf{X}_0, s)) ds
\]
Backprojection Along Curvilinear Trajectories
Mathematical Model

\[ \Gamma = \{ \gamma(\theta;x,y) \} \]

\[ R_\Gamma u(\theta; x, y) = \int_{s=s_i}^{s=s_f} u(\gamma(\theta;x,y)(s)) \, ds \]

\[ R_\Gamma^* v(x, y, z) = \int R_\Gamma u(\theta; x, y) \, d\theta \]

Family of trajectories
Indexed by image point and sample angle.

Transform defined by integration of density along trajectories

Adjoint transform defined by integration over sample orientations
Operator Theory for Filters

\[ u + Tu = R^*_\Gamma FR_\Gamma u = R^*_\Gamma Fv \]

\[ \Psi = R^*_\Gamma R_\Gamma \]

\[ u = \Psi^{-1} R^*_\Gamma v \]

- In general, filtered backprojection works only up to an error term. For some special cases \( T \to 0 \).

- A well-known theorem states that the composition of a ray transform with its adjoint is an elliptic pseudo-differential operator.

- Heuristically, we would like to invert the operator, and compose with the adjoint ray transform.
Setting Up the Transform as a Fourier Integral Operator

\[ \Omega_\theta : (X, Y, Z) \rightarrow \gamma_{\theta; X, Y}(Z) \]
\[ \Pi_\theta^{aug}(X, Y, Z) = (\Pi_\theta(X, Y), Z) \]
\[ \Omega_\theta \Pi_\theta^{aug} = I \]
\[ A_\theta(X, Y, Z) = \Pi_\theta^{aug^{-1}} \Omega_0(\Pi_\theta^{aug}(X, Y, Z), Z) \]
\[ R_\Gamma u(\omega) = I_\Gamma \tilde{u}(\omega) = \]
\[ \frac{1}{(2\pi)^3} \int \int e^{i(x \cdot \xi - \omega \cdot A_\theta^{-1}(x))} \det(A_\theta^{-1}(x))\tilde{u}(\xi)dxd\xi \]
Tracks gold particles deposited on surface of sample through tilt series images.

- Accepts general set of sample orientations.
- Constructs series of geometrically nonlinear projections simultaneously with 3D model of gold particle positions via generalized bundle adjustment.
- Corrects projection maps to 9th order polynomials.
- Remaps (warp) tilt series images to align tracks to run orthogonally to projected tilt axis.
- Applies one of the common r-weighted filters to tilt series images.
- Backprojects via adjoint of curvilinear projection calculated in the bundle adjustment.
- Utilizes fast recursion, MPI code for backprojection.
Curvelet Noise Reduction and Quality Enhancement

Reconstruction from Original Tilt Series

Reconstruction after Curvelet Denoising of Tilt Series

- Curvelet algorithms are computationally costly
- Noise reduction is not always successful
- More research is needed—Combine with regularization?
Which is Better for Automatic Segmentation?

**Cardiac tissue reconstruction sections**

**TxBR**

**IMOD**
Tracking

- Correlation-based
- Marker based
- Feature based
- Extended structures
Multiple Axis Tomography
Alignment

- Electron trajectories are curvilinear
- This makes the alignment problem three dimensional
- Feature positions in object are calculated from tracks in images
- Feature positions and projection maps must be consistent with markers in images
- Intrinsic trajectory equations
  \[ P_\omega(\gamma_1^{x,\omega}(t), \gamma_2^{x,\omega}(t), \gamma_3^{x,\omega}(t)) = (x_1, x_2) \]
- Projection maps
  \[ P_\omega(X_1, X_2, X_3) = (x_1, x_2) \]
Alignment Models

• Projective model

\[ \lambda_\omega (X_1, X_2, X_3) \begin{bmatrix} x_1 \\ x_2 \\ 1 \end{bmatrix} = P_{orth} \begin{bmatrix} X_1 \\ X_2 \\ X_3 \\ 1 \end{bmatrix} \]

\[ \lambda_\omega (X_1, X_2, X_3) = 1 + \sum_i \lambda_i^i X_i \]

\[ \left[ G_\omega \mid t_\omega \right] = \begin{bmatrix} b_{11} & b_{12} & b_{13} & t_1 \\ b_{21} & b_{22} & b_{23} & t_2 \\ b_{31} & b_{32} & b_{33} & t_3 \\ 0 & 0 & 0 & 1 \end{bmatrix} \]

• Note that “projection” and “projective” are used in two different senses
General Alignment Model

• Projection model with features, projections, markers and tracks

\[ P_\omega = P_{\text{proj},\omega} + P_{\text{nonlin},\omega} \]

\[ T_{\omega,\rho} = \left\{ (x_{\omega,\rho_1}, x_{\omega,\rho_2}) \mid \omega \in \{\omega_1, \omega_2, \ldots, \omega_N\}, P_\omega \bar{X}_\rho = \bar{x}_{\omega,\rho} \right\} \]

• Error term for conjugate gradient optimization

\[ E = \sum_{\omega, \rho} \left\| P_\omega \left( \bar{X}_\rho \right) - \bar{x}_{\omega,\rho} \right\|^2 \]
“Remap the images so that the tracks are level”
A. Lawrence - The Tao of TxBR
Image Remapping Pre-alignment

Remaped Particle Tracks
3D TxBR Reconstruction of a Caulobacter Crescentus from 2kx2k electron micrograph

order 1: Reproj. Err.~0.95px

order 3: Reproj. Err.~0.3px
• Aligns on surface contours, fibers and point features; reconstructs surfaces as during alignment process
• Dewarping of objects distorted by mass loss
• Backprojection code runs on GPU boards
• Cross validation for elimination of limited angle artifact, discretization artifact and sampling bias.
Contour Alignment

- Spreading gold markers on the surface is a random process.
- No gold markers within a plastic section.
- Gold particles bring artifacts in the reconstructed volume.
- Living cells contain extensive membrane structures.
- Staining generally occurs along surfaces.
- Surfaces project to contours in images.
Possible Alignment Markers in Electron Tomography

• Point-like Markers (gold particle, ribosome...)

• Linear Markers (fibers, structure edges...)

• Surface Markers (membranes,...)
Projection Along Rays

• Surface $S_\rho$ of a 3D object $\rho$ is parameterized with $(t,u)$.

• We restrict $S_\rho$ to be small patches. Use of polynomial expressions for $S_\rho(t,u)$.

• Curvilinear rays tangent to surface. Use of a polynomial expression for the projection map $P_\omega$. Index $\omega$ represents a sample orientation.

• Contour in surface where $u_{\omega\rho}(t)$ projects to contour $C_{\omega\rho}$ in image $I_\omega$. 
Contour Alignment Model

- Contour Tracks:
  \[ C_{\omega \rho} = (x_{\omega q_1}(t), x_{\omega q_2}(t)) \]
  \[ x_{\omega q_i}(t) = \sum_{k=0,\ldots,N_1} c_{\omega q_i k} t^k \]

- Projection Map:
  \[ P_{\omega} (X_1, X_2, X_3) = (P_{\omega 1} (X_1, X_2, X_3), P_{\omega 2} (X_1, X_2, X_3)) \]
  \[ P_{\omega i} (X_1, X_2, X_3) = \sum_{N_2 \geq j, k, l \geq 0} b_{\omega i j k l} X_1^j X_2^k X_3^l \]

- Structure Patches:
  \[ S_{q}(t, u) = (S_{q 1} (t, u), S_{q 2} (t, u), S_{q 3} (t, u)) \]
  \[ S_{q i}(t, u) = \sum_{N_3 \geq k l \geq 0} a_{q i k l} t^k u^l \]

- Surface Contours:
  \[ u_{\omega q}(t) \cong \sum_{k=0,\ldots,N_4} d_{\omega q k} t^k \]
A Generalized Bundle Adjustment

Two Error Terms to minimize:

- A Projection Error:
  \[ P_{\omega}S_{\varrho}(t, u_{\omega i}(t)) = C_{\omega \varrho}^{(r)}(t) \]
  \[ E_{\omega \varrho}^{P} = \int_{t_0}^{t_1} \| P_{\omega}S_{\varrho}(t, u_{\omega \varrho}(t)) - C_{\omega \varrho}(t) \|^2 \, dt \]

- A Tangency Error:
  \[ P_{\omega i}(\gamma_{x,\omega}^{1}(t), \gamma_{x,\omega}^{2}(t), \gamma_{x,\omega}^{3}(t)) = x_{i} \quad \nabla P_{\omega i} \cdot \dot{\gamma}_{x,\omega} = 0 \]
  \[ E_{\omega \varrho}^{T} = \int_{t_0}^{t_1} \left\| \nabla P_{\omega 1} \times \nabla P_{\omega 2} \cdot \left( \frac{\partial S_{\varrho}}{\partial t} \times \frac{\partial S_{\varrho}}{\partial u} \right) \right\|^2 \, dt \]
Symbolic Calculation / Optimization

- Error functions are expanded and integration on contours performed prior to optimization.
- Symbolic calculation is implemented with GiNaC (GiNaC is Not a CAS). Use of the swiginac interface. Python libraries sympy and sympycore are too slow for calculations needed in the bundle adjustment process.
- Coefficients \( a_{ijkl}, b_{ijkl}, c_{ijkl}, d_{ijkl} \) are treated in a symmetric way. Code is built so it is easy to free or freeze variables during minimization, and also to easily add new variables.
- A linear combination of \( E^P_{\omega q} \) and \( E^T_{\omega q} \) is minimized with a Line-search Newton Conjugate Gradient algorithm
Structure Segmentation

Caulobacter Crescentus

Gia
Parameterization of the contours

What choice? For a tilt series:

- \( t \) parameterizes the projection of a surface point onto the camera plane.
- \( u \) parameterizes the tilt index

Simultaneous parameterization of tracks allows to assess optimal patch order to describe an object.

\[ \Rightarrow \text{Minimization is then implemented with independent parameterization for each contours.} \]
Re-projection Error at Minimum

Bundle adjustment applied on Caulobacter Crescentus dataset (500ptsx500pts) with parabolic patches
Pure Gold Markers case vs Combination of Gold and Surface Tracks case

91 Gold Markers

5 Gold Markers and 2 patches.

(X=262,Y=297,Z=110) Gold markers only on one side of the specimen.

Linear General Model - No other (orthogonal...) constraint.
Backprojection

• Backprojection requires evaluation of polynomial at each position of object

\[ x_{oi} = P_{oi}(X_1, X_2, X_3) = \sum_{n \geq i, j, k \geq 0} b_{ijkl} X_1^j X_2^k X_3^l \]

• This implies hundreds of calculations at each point for each summand in the backprojection
Recursion Scheme

• Along a line polynomial projection reduces to single variable

\[ q(X) = P_{\omega i} (X, N_1, N_2) \]

• Set up recursion scheme to evaluate polynomials

\[ q_0^n = q(n), q_1^n = q_0^n - q_0^{n-1}, q_2^n = q_1^n - q_1^{n-1}, etc \]

• If polynomial is of \textbf{nth} degree \textbf{nth} differences are constant
Implementing Recursion

• Reverse procedure which gives higher-order differences

\[ q_{m}^{n+1} = q_{m}^{n} + q_{m+1}^{n+1} \]

• Polynomial can be calculated from initial segment of difference table and nth order differences

• Evaluation of polynomial is reduced to a few additions at each point, linear increase of computations with increase of degree
• Development driven by the multi-billion dollar game industry
  – Bigger than Hollywood
• Need for physics, AI and complex lighting models
• Impressive Flops / dollar performance
  – Hardware has to be affordable
• Evolution speed surpasses Moore’s law
  – Performance doubling approximately 6 months
GPGPUs (General Purpose GPUs)

- A natural evolution of GPUs to support a wider range of applications
- Widely accepted by the scientific community
- Cheap high-performance GPGPUs are now available
  - It’s possible to buy a $500 card which can provide a TeraFlop of computing.
What Does it Mean for the Scientist?

• Desktop supercomputers are possible
  – No sharing
  – No network latency. Good for real-time applications

• Very efficient
  – Approx 200 Watts / Teraflop

• Turnaround time can be cut down by magnitudes.
  – Simulations can take several days
• Highly parallel architecture
  – SIMD
• Designed initially for efficient matrix operations and pixel manipulations pipelines
• Computing core is lot simpler
  – No memory management support
  – 32-bit native cores
  – Little or no cache
  – Largely single-precision support.
## TxBR Backprojection Speedup vs Polynomial Order of Approximation and Image Size

<table>
<thead>
<tr>
<th>Image Size</th>
<th>Order 1</th>
<th>Order 3</th>
<th>Order 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1K x 1K</td>
<td>3.7 X</td>
<td>4.6X</td>
<td>16.23 X</td>
</tr>
<tr>
<td>2K x 2K</td>
<td>4.57 X</td>
<td>6.16 X</td>
<td>44.9 X</td>
</tr>
<tr>
<td>4K x 4K</td>
<td>4.18 X</td>
<td>5.08 X</td>
<td>31.8 X</td>
</tr>
<tr>
<td>8K x 8K</td>
<td>4.78 X</td>
<td>6.84 X</td>
<td>45.64 X</td>
</tr>
</tbody>
</table>

- Fast recursion algorithm
- One thread per pixel row
- No modification of original MPI code
Some Cautions

• C-like language support
  – Missing support for function pointers, recursion, double precision not very accurate, no direct access to I/O
  – Cannot pass structures, unions

• Code has to be fairly simple and free of dependencies
  – Completely self contained in terms of data and variables.

• Speedups depend on efficient code
  – Programmers have to code the parallelism.
    • No magic spells available for download
  – Combining CPU and GPU code might be better in cases
• Performance is best for computation intensive apps.
  – Data intensive apps can be tricky.
• Bank conflicts hurt performance
• It’s a black-box with little support for runtime debugging.
• BUT...
• The technology is progressing rapidly
• Wider range of applications, and easier programming in the future
A 64 Mega Pixel Digital Detector for TEM
Montage Tomography

- stage montaging
- image shift
- montaging
Montaging Problem

- Thin slices become warped during sample sectioning, handling and data acquisition (beam induced mass loss and lens distortions)
- Difficulty in stacking volumes in a serial tomography
Serial Section Tomography

Two Possible Approaches

- Transform an already reconstructed volume
- Modify the projection maps during the bundle adjustment procedure
A shear based warping transformation

Z = f_1(X, Y)

Z = f_2(X, Y)

Orthogonal warping transformation

work by: Sebastien Phan, NCMIR-UCSD
Neuron Specimen

Shear based transformation
Continued Progress in Electron Optical Sectioning

- Collecting data to building accurate point-spread function models
- Working on techniques to deconvolve the sections
- Working with Angus Kirkland to explore techniques for controlling the stigmator coils to reduce aberrations

Dev Team: J. Bouwer, T. Molina, G. Yang, B. Smith, Y. Hakozaki
A Final Change of Topic

• Connecting with the physics

• Mathematics and electron microscopy

• Some research objectives

• Connections with hard analysis
“A glance at any image processing textbook reveals immediately that such works are more like cookery books than scientific treatises and that the vocabulary of the subject is quite different in the widely separated areas of application (microscopy, astronomy, medicine, geology, forensic science,…). In an attempt to harmonize all this work and to put it on a sound mathematical footing, an image algebra has been created, in terms of which the various image processing algorithms can be written compactly…

… But why should we stop there? It would be very satisfying if we could express the whole chain – image formation plus image processing – in terms of this algebra …”

Peter Hawkes, Recent advances in electron optics and electron microscopy, 2004.
Some Long-Term Objectives

• Automated tomography, with tracking, alignment, rebinning, filtering and reconstruction done while data is collected

• Explicit treatment of instrument physics and beam-sample interactions in tomographic reconstruction algorithms

• Advancement of mathematical treatment in algorithm development

• The biggest problem is the AI