Tomographic Diffractive Imaging

ASU Physics, LBL, LLL. DOE, ARO $.
First atomic-resolution image of a DWNT by any method.

Obtained by HiO. Aberration-free.

Gives ID, OD, Chiral vectors.


Fig. 2. (left) A section of the reconstructed DWNT image at 1-Å resolution and (right) a structural model constructed with the use of the chiral vectors of (35, 25) and (26, 24) that were determined from the image and diffraction pattern. The DWNT imaged here is one of many in our catalytic chemical vapor deposition–grown samples. Yellow and red lines mark the diameters of the inner and outer tubes, respectively. One side of walls is stronger than the other, which is because of the illumination. The DWNT is incommensurate. In projection, the structure has complex patterns showing both accidental coincidences and Moiré fringes, which are highlighted by hexagons and lines.
Why lensless imaging?

- A technique for 3D imaging of 0.5 – 20 \( \mu \text{m} \) isolated objects
- Too thick for EM.
- Too thick for zone-plate tomographic X-ray microscopy (depth of focus < 1 \( \mu \text{m} \) at 10 nm resolution for soft X-rays even if zone plates become available)

Goals

- \(<10 \text{ nm resolution (3D) in } 1 - 10 \mu \text{m size biological specimens}
   \)
  
  (small frozen hydrated cell, organelle; see macromolecular aggregates)
  
  Limitation: radiation damage!

- \(<2 \text{ nm resolution in less sensitive nanostructures}
   \)
  
  (Inclusions, porosity, clusters, composite nanostructures, aerosols…)
  
  eg: molecular sieves, catalysts, crack propagation, mesoporous structures
The basic idea - “oversampling” solves the phase problem. **1. Collecting all the information.**

Shannon’s theorem: All the info. in diffuse scattering is collected if it’s sampled at “half the Bragg angle”. Explain this……

![Diagram](Image)

**Bragg** sampling - from periodic repetition of object, width W. Collects all the information if amplitude and phase are recorded.

**Shannon** - at inverse of “bandlimit” 2W for diffracted INTENSITY. 2W is width of autocorrelation of object, which acts as “bandlimit”. This sampling collects all the info. if only intensity is detected. Phase is now “encoded” in the intensity.

Recall: Shannon sampling of a bandlimited function allows interpolation between samples to the value of the continuous function.

"collect all info" means **number of eqns = number of unknowns**
In practice....

Iterate between object and diffraction pattern, overwriting known info in each space.

1. Start with measured diffraction amplitudes, random phases.
2. Transform to real space. Set object to zero outside known boundary ("support constraint").
3. Make positive charge density zero. ("Sign constraint").
4. Transform to reciprocal space. Replace amplitudes with measured values. Keep old phases.
5. Go to 2.

With feedback, this is the “hybrid input-output” HiO algorithm of Feinup, Gerchberg, Saxton.

Also called oversampling (Sayre, Miao, Bates). Then **No. of eqns = No. of unknown phases**.

Diffract from region twice as big as object. Known half compensates for unknown phases.

*For details see Weierstall et al Ultramic. 90, p. 171 (2002).*
The basic idea - “oversampling” solves the phase problem. **2. Extracting the phases.**

Shannon sampling makes the number of Fourier eqns (one for each sample) equal to the number of unknown phases, hence inversion may be possible.

Are these eqns linear? Independent? Linear once estimated phases supplied.

In real-space, this “oversampling” (w.r.t Bragg sampling) surrounds the isolated object by a border of known, zero charge density. (cf padding out with zeros).

Phases are found using Fienup-Gerchberg-Saxton (HiO) iterative algorithm. Convergence properties have been studied in detail. (Err Red error cannot increase. Projections convex sets).

Information needed for convergence; 1. The Fourier modulii. 2. The sign of the charge density. 3. Approx boundary of the object (the support). (We have recently removed the need for this).

"Solves" **global optimization problem** (10^6 adjustable params!). Why does it work?
- Fourier modulus constraint is not convex, **knowledge of phase is convex**.

Is the inversion unique? (not in 1D. Yes in practice in 2D. Excellent in 3D).

Phase in physics. Bohr, Dirac, Squid, A/B effect, phase cooper-pair wavefunction?
The basic idea - “oversampling” solves the phase problem. 3. History of ideas.


Current research in several groups; Tomography, damage analysis for diffractive imaging, phase-extension to improve resolution of zone-plate microscopes, “shrink-wrap” to remove need for support, atomic resolution HiO images obtained with electron diffraction (single nanotube), application to cryomicroscopy, “thick” biological objects in ice etc. **
First atomic-resolution diffractive image reconstruction.

Double-walled Nanotube

Image reconstructed from electron-diffraction pattern by HiO

Requires image plate, not CCD.

Atomic-resolution diffractive imaging with electrons using a TEM.


Coherent, high-energy electron beam normal to page.
The competition from HREM and STEM

Tomography in STEM with 1nm diameter electron probe.

Catalyst particles. Red are Pt particles on Alumina crystal. Fringes are Moire.
Resolution about 1nm. HAAD. Like STXM. 100 keV. P.Midgely et al, 2002

Current best HREM resolution is 0.78 Angstroms.

Gate Oxide ~ 5 Si Atoms thick


Sb source turned on here
No Sb

Individual dopant atoms within Si, seen in projection
**Protein synthesis** ("Life itself") in the Ribosome: The ribosome structure determined to 1nm resolution by TEM (tomographic cryomicroscopy). J.Frank et al.

Experimental e-coli ribosome reconstruction from TEM images of non-crystallised mols in ice. mRNA bring 3-bit codons from DNA. tRNA "adaptors" (E,P,A) have plugs at one end to mRNA codon, at the other to an amino acid, which is added to the polypeptide chain as the ribo runs along the mRNA. Chain will fold to become a new protein. (also Baumeister).
C XDI experiments with soft X-rays at ALS LBL.

E = 588 eV
2.1nm wavelength

He, Howells, Weierrstall, Spence Chapman, Marchesini et al. Acta A59, 143. 03.
Soft X-ray transmission pattern at 588eV from cluster of gold balls on SiN. (Phase objects. Diam d=50nm). First minimum is at $\sin \theta / \lambda = 1.394 / d$. $\lambda = 2.1\text{nm}$.

“Airey’s disk” from one phase ball
Fringes due to interference between different balls

HiO algorithm requires boundary of isolated object to be approx known. Estimate this from autocorrelation fn.

Autocorrelation (Patterson) function showing two ellipses used to define the acentric support.

Phase shift is 0.36 Rad per 30nm thickness at 588 eV.
Successful reconstruction of image from soft X-ray speckle alone.

50nm diam. Gold Balls on transparent SiN membrane.

No “secondary image” was used - beyond "phase extension". Approximate object boundary obtained from autocorrelation fn, "by hand".

How to make an isolated object? Use AFM to remove unwanted balls. (No density outside the reconstructed area $L = \theta_{\text{min}}/\lambda$ must contribute to the diffraction pattern).

He, Howells, Weierstall, Spence Chapman, Marchesini et al. Phys Rev B67, 174114. 03.
Is it possible to reconstruct without any knowledge of support?

Get support of object from support of autocorrelation - then "shrink-wrap" to avoid previous subjective estimate of boundary.

Notes:
First estimate of boundary (support) is boundary (4% threshold) of autocorr.

Autocorr is centric, object not.

Boundary of autocorr shrinks as HiO improves estimate. Every 20 itns make new support at 20% threshold.

Inversion symm eventually lost!

Intensity obscured by beamstop "floats" during iterations.

Cf Solvent flattening.

**Shrink-wrap simulations.**

(Marchesini)

<table>
<thead>
<tr>
<th>Recovered Image</th>
<th>Recovered support</th>
<th>Original object.</th>
</tr>
</thead>
</table>

Greyscale bee.
(no "atomicity")

The noise level at which our algorithm fails to reconstruct occurs when the noise in real space becomes larger than the threshold used to update the support. Works better than HiO with loose support.

**Tomography**

-gold balls in 3D.

**Complex 2D object with complex focussed probe.**
Real part: - blue + red

Isolated by probe
Complex if large phase shifts, multiple scattering spatial variation of abs.

*QuickTime™ and a Planar RGB decompressor are needed to see this picture.*
A different data set, showing "heavy-atom" method.

**Autocorrelation from X-ray intensity**
- a map of all interball vectors.

Friedel symm is a test for a real object.

**SEM real-space image**

Note how balls at B are faithfully imaged in ball A in the autocorrelation function $\rho(r)\ast\rho(-r)$ at AB in figure (b).

This suggests using AFM to place single ball near unknown (eg cell). Autocorrelation then gives image and better support.

Support obtained from sem image.  

*Acta A.59, 143 (2003).*
Diffraction from protein monolayers.

Compact support normal to a slab.

Oversampling along the beam direction in transmission geometry.

Two dimensional xtal gives redundancy against damage.

Membrane proteins, important for drug delivery, are hard to xtalize.
2. Oversampling along beam direction for monolayer protein crystals - lysozome.

Current practice in TEM cryomicroscopy is to record an image and a diffraction pattern for every tilt to solve the phase problem. Very difficult and tedious, especially at high angles. Images are at <3 Ang resolution (resolves amino acids). Aberrations of lens must be deconvoluted. Can HiO help by reducing the number of images needed for 3D phasing?

Idea- Phase along rods with HiO. Link different rod phases with known phases from images.

Result: Compact support along z for thin film will not solve phase problem alone - need some known phases.

The progress of the HiO* algorithm toward convergence is seen here in this real-space movie for tetracyanoethelene. Known phases were supplied on three planes.

The result is independent of the random choice of initial phases.
Test for **lysozome protein** (egg white). About 8,000 atoms, 53,000 Bragg beams.

Isosurface map for the charge density of Lyzosome based on published atomic coordinates at 3 Angstrom resolution (identifies aminos). The four-fold screw axis is seen in projection down the c axis. Tetragonal, P 43 21 2  a=79.1=b, c=37.9 Ang. Amino acids sequenced.
HiO for TEM Cryomicroscopy. Solving proteins hard to crystallize.

Results for Lysozome using 3D Braggs to 3 Ang. plus phases from images to 15 degrees.

<table>
<thead>
<tr>
<th>Model Potential</th>
<th>HiO Reconstruction</th>
<th>HiO Recon. with noise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;15° tilts, 105 iterns</td>
<td>R=25% Same images</td>
</tr>
</tbody>
</table>

Corr Coeff=0.96       CC=0.95

54X54X78 voxels
To 3 Ang resolution
(Identifies amino acids)
1 sec per itern. 100 iterns. Conclusion: method works well.
Cross-correlation coefficient CC (upper curve) measuring agreement between model Lyzosome charge density and HiO estimate vs iteration number. No noise. HiO error $\varepsilon_k$ also shown (lower curve). Four images tilted at 30 degrees, and four at 20 degrees used with axial image. Error decreases to 0.006.

For unknown structure, only $\varepsilon_k$ is known, not CC ! (monotonic ?).

$$CC = \frac{\int \rho_t(r)\rho_e(r)dr}{\left[\int \rho_t(r)^2dr\int \rho_e(r)^2dr\right]^{1/2}}$$

$$\varepsilon_k = \left(\frac{\sum_{(x,y)\notin S} |\tilde{g}_k(x,y,z)|^2}{\sum_{(x,y)} |\tilde{g}_k(x,y,z)|^2}\right)^{1/2}$$

54X54X78 voxels
To 3 Ang resolution
(identifies amino acids)
1 sec per itern.100 itns.
(Mac,Matlab)
Correlation coefficient CC (upper curve) and rms error (lower curve) from HiO algorithm plotted against the angular range of reciprocal space, in degrees, within which known phases have been supplied to the algorithm.

What is the smallest range of tilts from which image phases are needed?
Summary

1. Proteins which cannot be crystallized are very important
   (eg hard-to crystallize membrane proteins for drug delivery)

2. These can often be crystallized in two dimensions (eg on liquid surface etc).

3. Radiation damage prevents diffraction patterns being obtained for single isolated proteins.

4. Diffraction from two-dimensional “hydrated” proteins can be done in 3 dims.
   by cryo-tomographic TEM. Eg in amorphous ice.

5. This leaves a phase problem. Traditionally this has been solved by taking one image
   for each plane in diffraction space. Commonly need image tilts up to 80°

6. By using the HiO iterative algorithm to oversample along the relps we can reduce the
   number of 0.3nm resolution images needed to phase the data from 100’s to a few (eg ±15°).
   (0.3 nm is sufficient to distinguish the 20 amino acids, whose atomic structure is known).

7. Additional constraints have to be explored - Histogram, Bond-lengths, Sequence.

Hilbert Transforms - incorporation into HiO for "phase extension".

E.g. Kramers-Kronig relations, dispersion relations, multiple scattering analysis etc.

For a "causal" function \( f(r) \) s.t. \( f(r < 0) = 0 \), there is a simple relationship between the real and imag parts of \( F(u) \).

Apply this to an object density which is zero for \( r < 0 \) for some origin.

Apply it to a crystal (Mishnev, Acta A52, p.629 (1996))

It then generates the half-order Bragg reflections (from known complex Braggs) which would result if the molecule was surrounded by a vacuum jacket in a doubled cell. This satisfies Shannon, solves phase problem.

But we don’t know the complex Braggs from the natural xtal !.

We have used this for phase extension. (Given image phases to some resolution, extend resolution by phasing additional higher-order Braggs). Mishnev obtains

\[
F(h' +0.5, k' +0.5, l' +0.5) = \frac{1}{i\pi^3} \sum_{h,k,l} F(h,k,l) \frac{1}{(h' - h + 0.5)(k' - k + 0.5)(l' - l + 0.5)}
\]

We now apply this a constraint in HiO, seeking the minimum number of low-resolution, known phases, needed for convergence.
Homometric structures.

One family of homometric structures (Acta Cryst 7, p. 237; Pauling's Bixbyite) may be generated using the result that.....

\[ \rho_1(r) = l(r) \ast m(r) \]

and

\[ \rho_2(r) = l(r) \ast m^*(-r) \]

have same Fourier modulus \(|R(u)|\), since

\[ R_1(u) = L(u)M(u) \quad \text{and} \quad R_2(u) = L(u)M^*(u) \]

If \( l(r) \) is a lattice and \( m(r) \) a molecule, then \( m, m^* \) are enants, but \( \rho_1, \rho_2 \) are not enants.

Example:

Note: Homo1 is not the inverse (enantiomorph) of Homo2.

Conclusion: HiO could not distinguish these unless tight support provided.
Coherence requirements for diffractive imaging.

Should spatial coherence width $X_c$ equal width of object $W$ or width of its autocorrelation function $2W$? (this changes exposure time by 4 in 2D for fixed source brightness).

Moving from source point $S_0$ to $S_1$ by $\alpha$ translates diffraction pattern by one detector pixel. These source points are statistically independent, so coherence needed for DI is then lost. (First-order Shannon sample excited by $S_0$ coincides with zero order from $S_1$).

Shannon sampling requires pixel spacing $\alpha = \lambda/(2W)$

Van-Cittert Zernike gives $X_c = \lambda/\theta_C$

Combining these with $\theta_C \leq \alpha$ gives $X_c > 2W$ (even for object in hole of diameter $W$!).

Summary: $\alpha$ defines source size, and cannot exceed first-order Shannon sample at $D_1$.
**Ptychography (greek "fold"). Periodic objects.**

**Experimental** coherent electron diffraction with overlapping orders (STEM, Tanaka, Terauchi)

- Intensity at midpoint is indep of aberrations!
- Overlap is shadow image of crystal planes!
- Overlap is Fourier Image, periodic in defocus.
- Midpoint intensity varies sinusoidally with probe coord.
- If probe defocussed, pattern is in-line hologram.
- Super-resolution beyond info. limit by stepping out.
  (need only stability of first-order period).

Set $d_p = d_{hkl}$ then $\theta_R = 1.2 \theta_B$

Conclude: Need 20% overlap of coherent orders to match probe size to xtal spacing.

Spence and Cowley, Optik, 50, p. 129. (1978)
Super-resolution through ptychography in STEM

Atomic columns in a thin slab of silicon projected along [110]


The scope was a VG501, 100 kV, Cs = 3.1 mm, thus **WPOA point resolution is 4.2 angstroms.** Phase retrieval using ptychography was performed for all diffracted orders up to the 004 reflection (1.36 angstrom), which involves phasing 12 beams, and the data inverted to give the real-space image shown. The greyscale for the magnitude plot spans the range 22 (black) to 60 (white) in arbitrary units. For the phase the greyscale spans -104 degrees (black) to +38 degrees (white).
Ptychography with soft 3.2 nm X-rays. STXM used to give microdiffraction patterns.

H. Chapman. SEM 1997

Coherent X-ray microdiffraction patterns from grating with overlap orders

Image of grating (amp, phase) reconstructed from patterns

Ptychography for non-periodic object (Latex balls. Scale is 0.5 microns).
**Relationship between Ptychography and Diffractive Imaging.**

Both depend on interference at "half-orders".

**HiO**

In HiO we multiply with something bigger than the object (the support) in real space to obtain interference at half-orders.

**Ptychography**

In Ptychog we multiply with something smaller (the probe) and get overlap, but the result depends on probe position.

Shannon sampling gives object period 2W (autocorr is repeated).

In HiO we multiply with something bigger than the object (the support) in real space to obtain interference at half-orders.

In Ptychog we multiply with something smaller (the probe) and get overlap, but the result depends on probe position.
Is atomic-resolution diffractive imaging possible with X-rays?

Consider 10nm nanoparticle, HAP. Spring-8.

5 kV Undulator, \( \lambda = 2 \) Ang, Si mono, zone-plate.

* After Si mono, flux is \( 10^{13} \) photons/sec.

* Rocking curve width for InSb nanodot is 12 millirads for 10nm thickness.

* Diffraction efficiency for 10nm thickness is \( \text{3.4} \times 10^{-4} \) photons/sec.

* Source size is 25 \( \times \) 200 microns FWHM, becoming 0.5 \( \times \) 10 microns.

* Horizontal divergence FWHM is 40 microradians, allowing X50 demagnification.

* Cannot fill this with zone-plate, which limits divergence to 2 mrad for 100nm outer zone.

* Rocking curve width for InSb nanodot is 12 millirads for 10nm thickness.

* After Si mono, flux is \( 10^{13} \) photons/sec.


How to take atomic resolution now….
Challenges:

1. Getting sufficient coherent photons

Analysis of recent ALS 9.0.1 experiment (588eV):
- Exposure that took 3 hours on 9.0.1, (10 nm resolution)
- Should have taken 5.27 min (with zone plate mono built to spec)
- Should take 0.31 min with current ALS,
  (U5.0 undulator, better beamline)
- should take 0.8 sec on upgraded ALS.

2. Choice of energy

- Reconstruction of real objects is easier.
- Electron density becomes more nearly real as energy increases.
- Exposure time scales as $E^4$. (Coherent flux for fixed brightness scales as $E^{-2}$, and cross section scales as $E^{-2}$)
- Best compromise specimen dependent 0.5 – 4 KeV for storage ring
- Damage is the key- see Howells talk tommorow!
DOSE and FLUX SCALING WITH RESOLUTION

M. Howells et al. calculated the coherent scattering cross section of a cubic voxel and thence the dose $D$ required to produce $P$ scattered x-rays into a detector with collection angle chosen for resolution $d$ with the following result

$$D = \frac{\mu P h \nu}{\varepsilon} \frac{1}{r_e^2 \lambda^2 |\rho|^2 d^4} \quad \text{Flux} = \frac{P}{r_e^2 \lambda^2 |\rho|^2 d^4}$$

$\mu = \text{the voxel intensity absorption coefficient}$
$h \nu = \text{the photon energy}$
$r_e = \text{the classical electron radius}$
$\lambda = \text{the photon wave length}$
$\rho = \text{the scattering strength of the voxel material in electrons per unit volume}$
$\varepsilon = \text{the density}$

The dose scales as the inverse fourth power of the resolution
**Summary - Coherent Diffractive Imaging (CDI)**

*M*Membrane proteins (important for drug delivery) are difficult to xtalize in 3D. 2D xtals give immunity to damage - ideal for CDI tomog (electrons , Xrays ?). Cryo gives "hydration"

*By using the HiO iterative algorithm to oversample along the relps we can reduce the number of 0.3nm resolution images needed to phase the data from 100’s to a few (eg ±30°). (0.3 nm distinguishes the 20 aminos, of known structure). Data collection reduced from months.

*Our large-angle diffraction camera with HiO data analysis forms a new type of **diffraction-limited, aberration-free tomographic** microscope. "Shrink-wrap" - no support.

*Large NA in CDI makes more efficient use of damaging radiation. Reconstruction from 3D data avoids depth-of-field limitation of zone-plate tomography. Focused illumination will allow selection of one or two-part (complex) objects from a field.

*Dose scales as (resolution)⁻⁴ . Measurements show that images of cells should be obtainable at 10 nm resolution, 0.5-10 µm thickness, by soft X-rays at 100 K.*

*Imaging by harder coherent X-rays of inorganic nanostructures (such as mesoporous materials, aerosols and catalysts) at perhaps 2 nm resolution or better can be expected.(Robinson..)

*Imaging with new radiations for which no lenses exist, and single molecule imaging with X-ray free-electron laser pulses remain to be explored (Chapman...).