Principle idea of coherent diffractive imaging

**Principle:**
- record coherent diffraction pattern & solve structure by iterative phase retrieval

**Samples:**
- single biomolecules (ideally membrane proteins, viruses)
- inorganic and organic nano-crystals (structure & strain)
- cells (fully hydrated and living)

**Why XFEL?**
What is feasible at a future XFEL?

Biomolecules

Nano-Crystals

Cells

Today, we can image freeze-dried and unstained cells at 25 nm resolution

diffraction pattern with soft x-rays (750 eV, ALS), 65 sec
reconstruction

~ $q^4$

Today (@ SR or FLASH): 25 nm
Tomorrow (@ LCLS): < 1-5 nm

...and we know how to get them into the beam

(slides by H. Chapman)

- Single shot ~10 fs diffraction pattern recorded at FLASH (DESY) at a wavelength of 13.5 nm of a picoplankton organism.
- Injected into vacuum from solution, and shot through the beam at 100 m/s

J. Hajdu, I. Andersson, M. Svenda, M. Seibert (Uppsala)
S. Boutet (SLAC); M. Bogan, H. Benner, U. Rohner, H. Chapman (LLNL)

At the XFEL, we will be able to obtain snapshots (projections) of frozen-hydrated cells at a few nanometer resolution!
Coherent diffraction imaging of single biomolecules

One pulse, one measurement

Particle injection

XPFEL pulse

Noisy diffraction pattern

Combine $10^9$-$10^7$ measurements

Classification

Averaging

Orientation

Reconstruction


Email: franz.pfeiffer@psi.ch, Web: http://people.epfl.ch/franz.pfeiffer

Coherent diffraction imaging of single biomolecules

incident flux: $10^{12}$ photons/pulse
focus: ~100 nm ($10^4$ nm$^2$)
x-ray energy: 12.4 keV (1 Å wave length)
no vibrations (Debye-Waller factor)
detector to sample distance: 300 mm
detector pixel size: 200 μm x 200 μm
no. of detector pixels: 1024 x 1024

Email: franz.pfeiffer@psi.ch, Web: http://people.epfl.ch/franz.pfeiffer
Coherent diffraction imaging of single biomolecules

Coherent crystallography of protein nano-crystals?

- PX at X-ray synchrotron sources has solved many thousand structures!
- There is an opportunity with sub-micron protein Xtals
Expected x-ray diffraction patterns @ XFEL

10^{12} ph/pulse focused into 100 nm, X-ray energy 12.4 keV, 200 micron pixel size, 300 mm detector distance

Organic nano-crystals are important in nature!

Cypovirus polyhedra

several hundred crystals screened, final data set merged from ~ 10

F. Coulibaly et al., Nature 446, 97 (2007)
**2D Membrane Protein Crystallography @ XFEL sources**

- XFEL pulse
- KB optics
- TEM cryo holder with 2D membrane Xtals
- Detector # 1


---

**Sample preparation: Not easy, but not as hard as in 3D!**

- XFEL pulse
- KB optics
- TEM cryo holder with 2D membrane Xtals
- Detector # 1

L. Renault et al., JCAMD. 20, 519 (2006)
Expected coherent diffraction patterns for 2D protein crystals

10^{12} ph/pulse focused into 100 nm, X-ray energy 12.4 keV, 200 micron pixel size, 300 mm detector distance

From parallel beam to focused beam geometry

Plane wave approach:
\[ I(q) \sim \delta(q) + |F(\rho_{el}(r))|^2 \]

Including the complex incident wave:
\[ I(q) \sim |F\{P(r)O(r)\}|^2 \sim |F\{P(r)(1 + i\rho_{el}(r))\}|^2 \]

- we need to know the incident wave front precisely
- important to separate contributions from aberrations of focused beam from specimen diffraction pattern
More serious numerical feasibility study

- Protein Data Bank structure
- 3-D electron density map
- Iterative 3-D tomographic reconstruction
- Pychographic tilt-series data
  - 121 reconstructions
  - "N" diffraction patterns
- Reconstruction of 3D electron density
- Simulated XFEL experiment

Step 1: PDB structure > projected 2D electron density map

- Human AGP-1 2-D crystal, 81 x 41 unit cells, pixel width = 0.1 nm
- Projection at φ = 0°. Projected electron density = ρ(x,y)
**Step 2: Calculate exit wave**

**Exit wave**

= complex object transmission function

× randomly positioned Gaussian illumination (TE0 laser mode)

\[ \Psi(r) = P(r) O(r) \]

---

**included:**

- quantitative scattering cross-sections
- Poisson noise based on expected LCLS-XFEL flux values

**not (yet) included:**

- movements due to coulomb explosion
- Ewald sphere lift-off, …
Repeat all steps for +/- 60 deg tilt series

calculate exit waves and diffraction patterns for randomly positioned probes, and for +/-60 deg tilt series

Reconstruction of coherent 2D crystallography data sets

$I_m(r,\alpha,q)$

reconstruct projections

tilt series & tomography
Reconstruction of coherent 2D crystallography data sets

Equivalent:
Ptychographic reconstruction from diffraction patterns of overlapping illumination positions


Ptychographic phase retrieval


Dynamische Theorie der Kristallstrukturanalyse durch Elektronenbeugung im inhomogenen Primärstrahlwellenfeld

Some time ago a new principle was proposed for the registration of the complete information (amplitudes and phases) in a diffraction diagram, which does not—as does Holography—require the interference of the scattered waves with a single reference wave. The basis of the principle lies in the interference of neighboring scattered waves which result when the object function \( f(x, y) \) is multiplied by a generalized primary wave function \( p(x, y) \). The convolution of the Fourier transforms of these functions is the above mentioned interference necessary for the phase determination. It is obtained by suitable choice of the shape of \( p(x, y) \). To distinguish it from holography, this procedure is designated “ptychography” (\( \text{ptychography} \)). The procedure is applicable to periodic and aperiodic structures. The relationships are simplest for flat layers. In this paper, the theory is extended to space lattices both with and without consideration of the dynamic theory. The resulting effects are demonstrated using a practical example.
Current results/status of the work & future outlook

Achieved:
- Finished the first ‘full cycle’ simulation including forward calculation & reconstruction
- Experiment & reconstruction seems feasible, even if we include Poission statistics and expected XFEL flux values

Still to do:
- Influence of Coulomb explosion on reconstruction quality
- Effects of imperfections in the incoming XFEL wave front
- Ewald sphere lift-off,…

reconstructed 3D electron density with < 3 Å