# Chemical Crystallography and Structural Chemistry

(VO 270287)

Lecture 11

25th June 2020

Dr. Tim Grüne
Centre for X-ray Structure Analysis
Faculty of Chemistry
University of Vienna

tim.gruene@univie.ac.at

# Previous Lecture

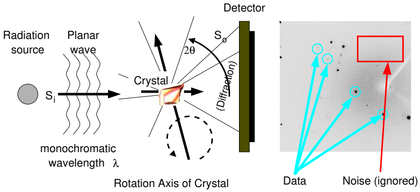
1. Absolute structure / Chirality
    - anomalous signal: breakdown of Friedel's law
    - Flack-Parameter, Parsons' Q-value
    - heavy atom method
2. Charge density refinement
    - limits of the independent atom model
    - multipole expansion
    - chemical features of electron density

# Today's Lecture

1. Structure Determination with Crystallography
2. Electron and X–rays
3. Applications for Electron Crystallography
4. Practical Aspects
5. Radiation Damage
6. Dynamic Scattering

# Structure Determination by Single Crystal Diffraction

# Data Collection for Single Crystal Structure Analysis



- Rotation (>1966): **Contiguous** recording of reciprocal space
- hybrid pixel detectors (>2002): shutterless, *i.e.* continuous data collection
- Spot position: wave type independent
- Intensity: wave type dependent
- Radiation source (wave): X-rays, electrons, or neutrons

# 3D Electron Crystallography

- $>$ late 1990s, as opposed to 2D electron crystallography
- confusingly many terms (ADT , RED, EDT, PEDT, MicroED, ...)
- Historical "dispute", Ute Kolb, Mainz University, $\approx$ 2007 (ADT), Xiaodong Zou & Sven Hovmøller, Stockholm University $\approx$ 2011 (RED)
- technical term: "3D Electron Diffraction", Enrico Mugnaioli (PSI 2017; IUCrJ (2019), 6, 178–188)
- "3D":
  1. Collection of 3D reciprocal space
  2. 3D crystals: $\geq 10-15$ unit cell in each direction; typically 200–1000nm per dimension

## Spot Position

- Spots positions according to Laue Conditions and orientation of Unit Cell:

$$
\begin{aligned}
(\vec{S}_o - \vec{S}_i).\vec{a} &= h \\
\textbf{and } (\vec{S}_o - \vec{S}_i).\vec{b} &= k \\
\textbf{and } (\vec{S}_o - \vec{S}_i).\vec{c} &= l
\end{aligned}
$$

- Monochhromatic wave: $\vec{S} = (\vec{S}_o - \vec{S}_i)$ depends on wavelength $\lambda$ and experimental geometry
- Spot **position** ⇔ Crystal lattice, independent from radiation type
- Resolution $d_{hkl}$ of a spot from position on detector *via* Bragg's law, $\lambda = 2d_{hkl}\sin(\theta)$

## Spot Intensity

- Spots **intensity** depends on physics of interaction

  **X–rays** interact with electrons, crystallographic map corresponds to electron density (number of electron per Volum, $e^-/A^3$).

  **Electrons** interact with electrostatic potential from electrons + nucleus $(\varphi(\vec{r}))$

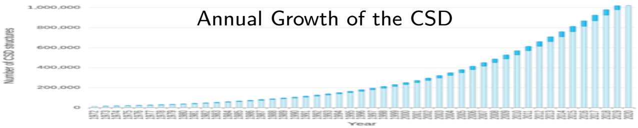
  **Neutrons** interact with nucleus *via* weak interaction, and magnetic moment.

- Spot intensity ⇔ Unit cell content: where are the atoms, what type of atoms are they

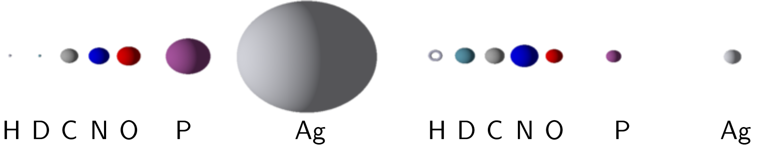
# Dominance of X-ray Crystallography

- most advanced (pipelines from data collection to structure refinement)
- typical wavelength: $\lambda$ =0.8–1.9Å
- standard structure determination

| | PDB (MX) | CSD (SX) |
|---|---|---|
| X-ray | $>$140,000 | $>$1,000,000 |
| neutron | 161 | 1,500 |
| electron | 111 | 10'ish |



## Neutron crystallography


X-ray scattering $f(2\theta = 0) = Z/\text{Å}$ Neutron scattering length $b_c$

1. visualisation of hydrogen atoms
2. adjacent elements (e.g. $K^+$ vs. $Cl^-$, $Zn^{2+}$ vs. $Cu^+$)
3. (virtually) no radiation damage
4. requires large crystals ($\geq 1\text{mm}^3$)

# 3D Electron Crystallography (3D ED)

- Electrons interact with electrostatic potential
- Electrons interact **much,much** stronger with matter than X-rays

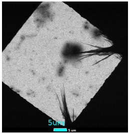
⇒ Much smaller crystals

⇒ problematic: dynamic diffraction, $|F| \neq \sqrt{I}$

- Electron optics enable some special applications and tiny beam (5 nm diameter)

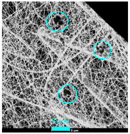
# 3D Electron Crystallography

# 3D ED: small crystals



organic compound

Silicalite–1 / ZSM–5 (Teng Li)



sucrose

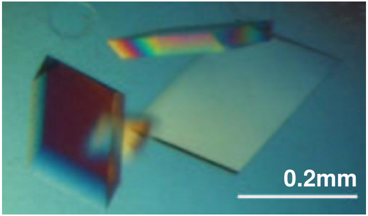
## Small Crystals

Main advantage for electron crystallography:

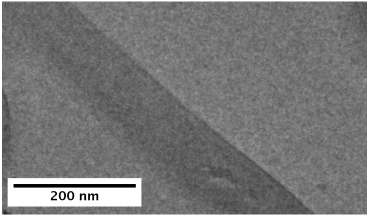
Diffraction from very small crystal ($< 1 \mu m$)

Some instruments provide 5–10 nm beam diameter

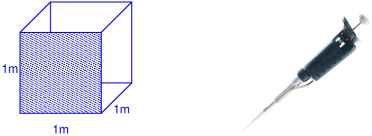
# How small is "nano"?



typical protein crystal size for X-rays



typical protein crystal size for electrons, 100x140x1,700 $nm^3$



volumes compare like $1m^3$ or 6 bath tubs of water *vs.* $10\mu l$

# Effects of Crystal Volume on Diffraction Data

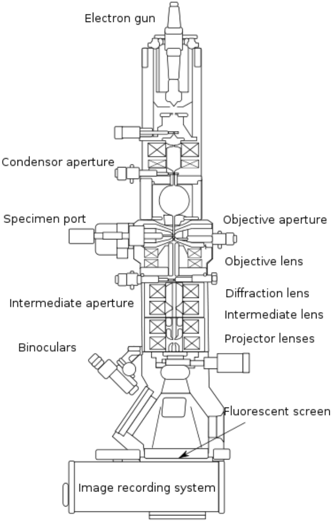
Reducing crystal volume reduces the resolution by (at least) two effects:

1. $I(hkl) \propto V_{\text{crystal}}$: 1/10 volume = 1/10 intensity
2. Henderson / Garman limit: maximum dose per volume before resolution is halved: 1/10 volume = 1/10 dose before radiation damage destroys crystal

From (1): In order to record the same quality diffraction pattern from a 10 times smaller crystal requires 10 times more intense beam.
From (1)+(2): This makes the crystal die 100 times faster

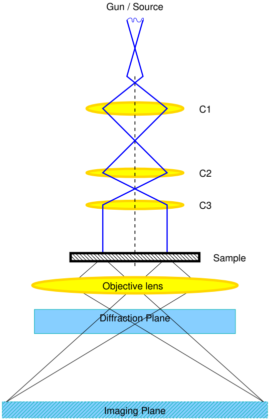
# Instrumentation for Electron Diffraction

# Electron Microscopes



(Wikipedia)

# The Lens System



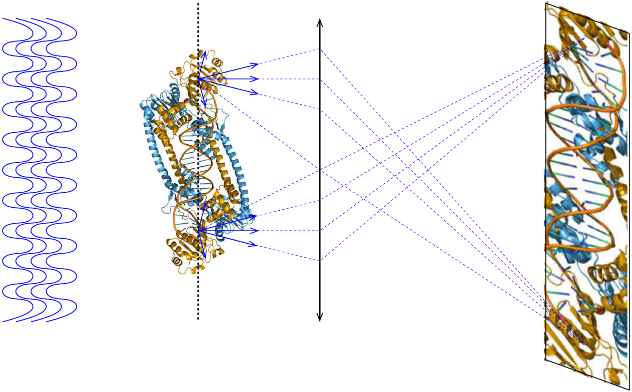
- Lenses C1–C3 shape beam
- Crystallography: Parallel beam
- Objective lens: sets effective detector distance to backfocal plane = diffraction mode
- C3 not present in all microscopes

**Lenses cause distortions.**

see *e.g.* Zuo & Spence, “Advanced Transmission Electron Microscopy”, Springer

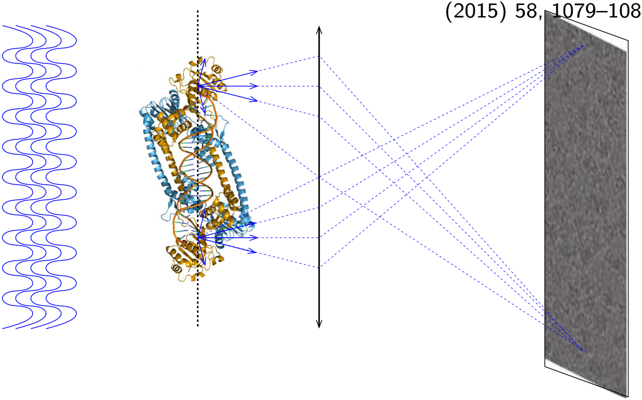
Carter & Williams, “Transmission Electron Microscopy”, Springer

# Electron Microscope: Imaging Mode



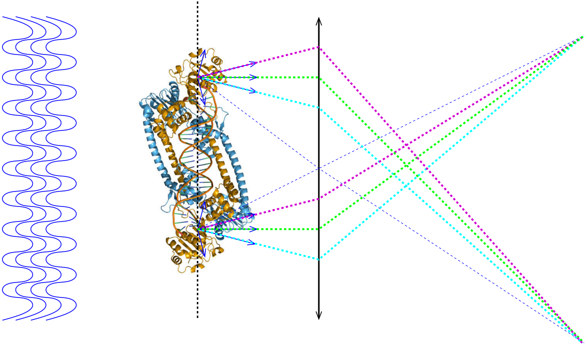
Plane Wave Object Lens Image Plane (Detector)
Rays of **equal origin** focus on detector

# Electron Microscope: Imaging Mode


Martinez-Rucobo *et al.* Molecular Cell (2015) 58, 1079–1089

Detector noise and radiation senstivity require low contrast images

# Electron Microscope: Diffraction Mode



Plane Wave Object Lens

# Electron Microscope: Diffraction Mode

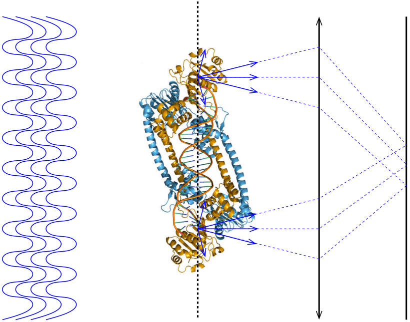


Image at Backfocal Plane = ||Fouriertransform of object||

when object is a crystal:

diffraction spots according to Laue conditions

Plane Wave Object Lens

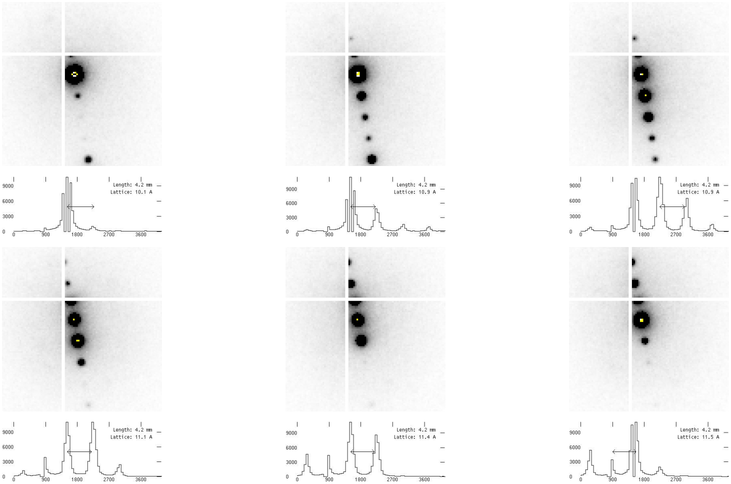
Backfocal Plane
Rays of **equal direction** focus on detector
“Image” = Fourier transform of object

## Dynamic Scattering

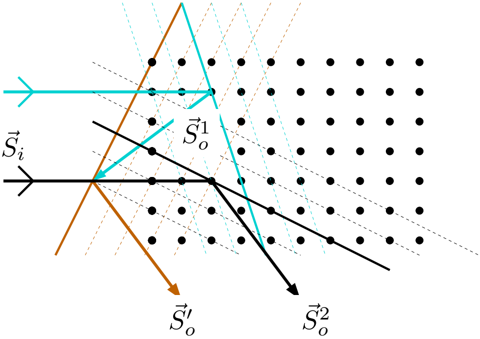
## Dynamic Scattering

- Kinematic Theory of Diffraction: Every photon / electron / neutron scatters once in the crystal
- $|F_{\text{ideal}}(hkl)| \propto \sqrt{I_{\text{exp}}(hkl)}$
- Dynamic Scattering: Multiple Scattering events occur
- Electron Diffraction: Multiple Scattering occurs even with nanocrystals

# Dynamic Scattering

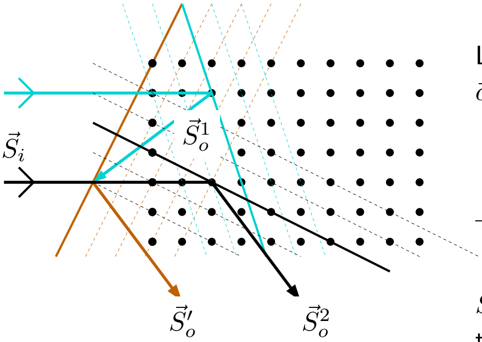


## Multiple (Dual) Scattering



- First reflection $\vec{S}_o^1$ acts as source of second reflection $\vec{S}_o'$.
- Second reflection $\vec{S}_o'$ overlaps with another reflection $\vec{S}_o^2$

## Multiple (Dual) Scattering



Laue Conditions (accordingly $\vec{b}$ and $\vec{c}$):

$$\begin{array}{c}(\vec{S}_o^1 - \vec{S}_i) \cdot \vec{a} = h_1 \\ (\vec{S}_o' - \vec{S}_o^1) \cdot \vec{a} = h' \\ \hline (\vec{S}_o' - \vec{S}_i) \cdot \vec{a} = h_1 + h' = h_2\end{array}$$

$\vec{S}_o' - \vec{S}_i$ fulfills the Laue conditions, hence the secondary reflection $\vec{S}_o' - \vec{S}_o^1$ overlaps with the "ordinary" reflection $\vec{S}_o' - \vec{S}_i$.

## Experimental Treatment of Dynamic Scattering for Organic Crystals

- Exact calculation very complicated
- Not feasible for complex molecules (more than a few atoms)
- Ignorance of dynamic scattering, i.e. assumption of kinematic scattering (as in X-rays) provides reliable structures
- Data statistics and model statistics poor, despite reliable structures

# Data Collection for Single Crystal Structure Analysis

- Rotation ($>$1966): **Contiguous** recording of reciprocal space
- hybrid pixel detectors ($>$2002): shutterless, *i.e.* continuous data collection
- Radiation source: X-ray, electron, or neutron crystallography
- Spot position: wave type independent
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## 3D Electron Crystallography

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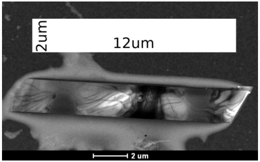
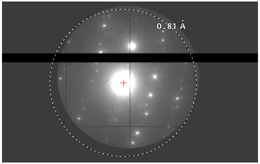
# Example Structures

# Single Crystal Structure from a Pharmacy Powder

Gruene *et al.*, Angew. Chemie. Int. Ed. (2018), 57, 16313–16317

# Grippostad®, STADA





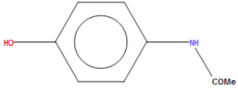
| **active compounds** | **non-active compounds** |
|---:|---:|
| paracetamol | gelatine |
| ascorbic acid | glycerol tristearate |
| caffeine | lactose monohydrate |
| chlorphenamine maleate | quinoline yellow (E104) |
| | erythrosine (E127) |
| | titanium dioxide (E171) |

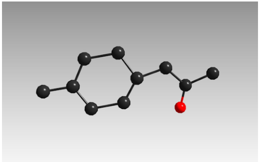
- powder from capsule deposited on sample grid
- Crystal dimensions $2\mu m \times 12\mu m \times \approx 300nm$
- $d_{min} < 0.8$Å

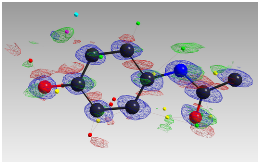
# Single Crystal Structure from a Pharmacy Powder

1. Data from single crystal: Completeness $< 40\%$
2. Cell parameters: $a = 6.9$, $b = 9.4$, $c = 11.6$, $\alpha = 90.6$, $\beta = 98.4$, $\gamma = 89.8$
   CSD search $a = 7.1$, $b = 9.3$, $c = 11.7$, $\alpha = 90.0$, $\beta = 97.7$, $\gamma = 90.0$

   CCDC HXACAN04, $P2_1/n$, Paracetamol,
3. SHELXT solves structure
4. Difference map reveals hydrogen atoms: data sensitivity





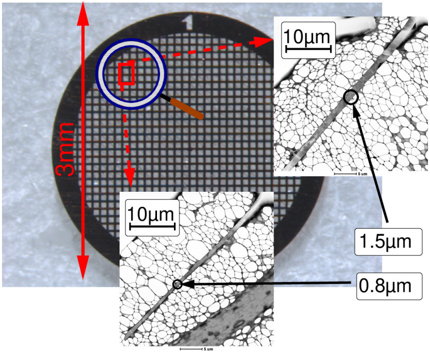


**Future: Complete crystallographic analysis from powder blends**

# Drug Design: Structure of a New Methylene Blue Derivative MBBF$_4$

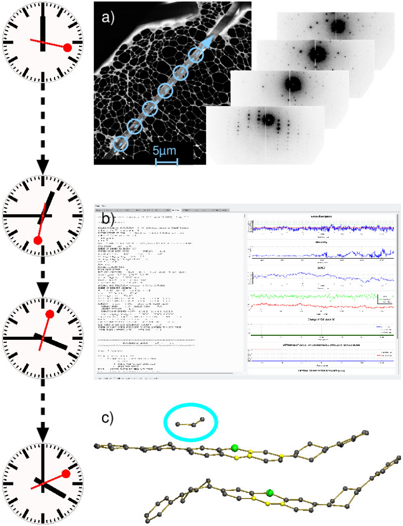
Collaboration Dr. J. Holstein & Prof. G. Clever, TU Dortmund
Gruene *et al.*, Angew. Chemie. Int. Ed. (2018), 57, 16313–16317

# MBBF$_4$-nanoCrystal (Holstein/Clever, TU Dortmund)



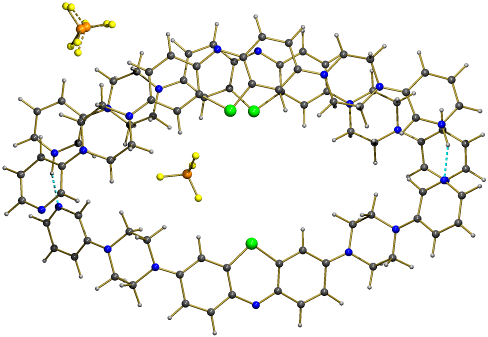
Tip of thin MBBF$_4$ needle on a TEM sample grid

# $\mathrm{MBBF_4}$ — EIGER and a TEM make a Synchrotron



- $60 - 120^\circ$ @ $3^\circ/s = 40\ s$ / data set
- 45 min for 16 data sets on both grids
- manual processing ≈ 4h to structure solution

# Structure of $MBBF_4$ (Refinement J. Holstein, TU Dortmund)

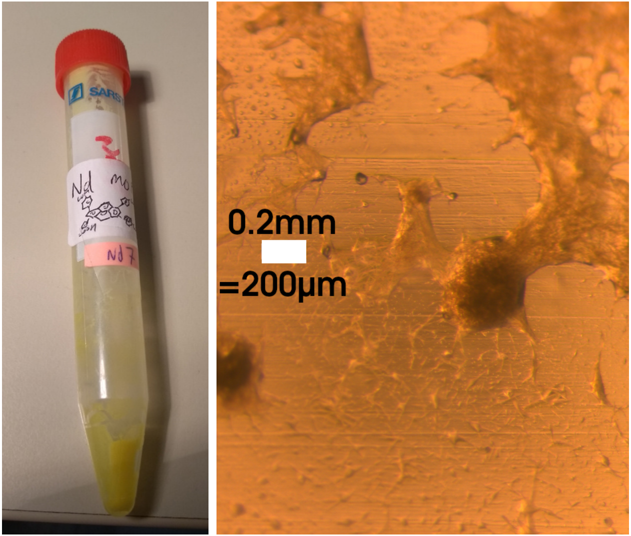


- $R1 = 22.7\% (2941 F_o > 4\sigma_F)$
- $R1 = 27.2\% (4832 F_o)$
- GooF $= 1.5$

# Nd-MOF

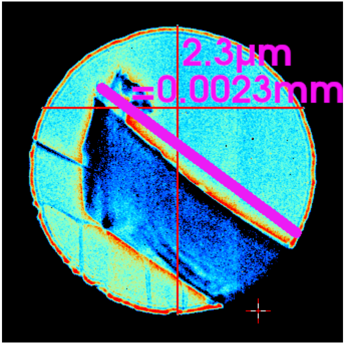
Prof. Jia Min Chin & Prof. Michael Reithofer, University of Vienna

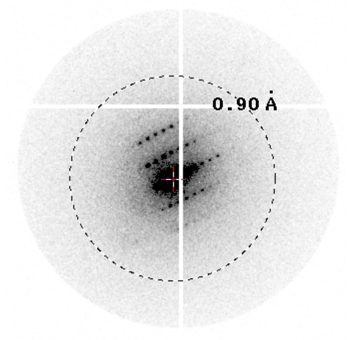
# Jewels in the mud



photographs courtesy Dipl.-WIng. A. Roller

# Powerful electron diffraction

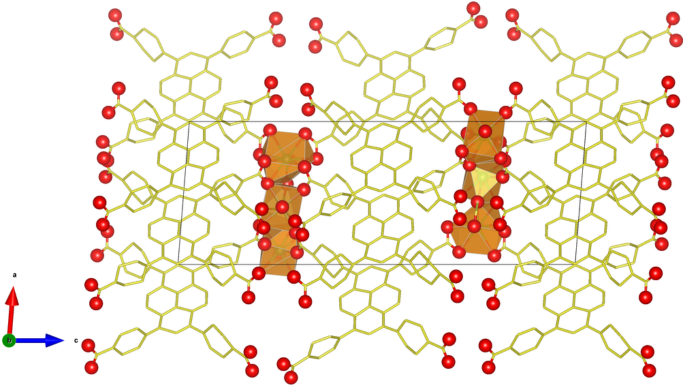




Sample preparation: A. Roller & N. Gajic

At DESY, the strongest X-ray source in the world, this crystal would probably not show any diffraction.

# Nd-MOF structure from 5 crystals



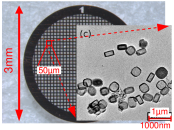
Courtesy Jia Min Chin & Michael Reithofer, unpublished data

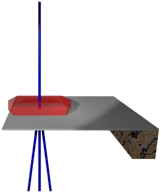
Room temperature measurement, under vacuum

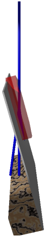
# Preferred Crystal orientation & the Missing Wedge Problem

Wennmacher *et al.*, Nat. Comm. (2019), 10, 3316; (Patent EP 18 202 868)

# Missing Wedge in Electron Diffraction

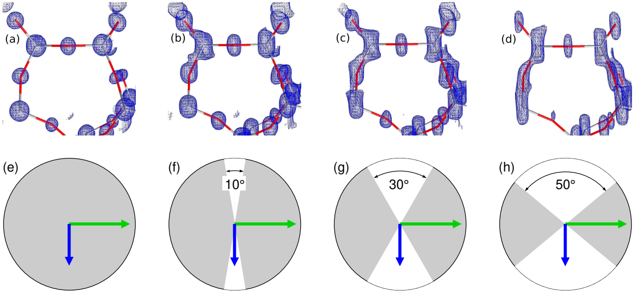






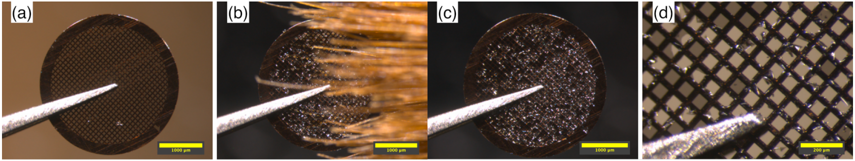
- Crystals very often have a **flat shape**: always the same orientation
- Sample support stabilised by Cu-grid
- Copper grid too thick: intransparent for electrons
- Limited rotation range
- Crystals typically have low symmetry space group

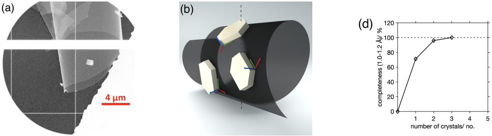
# Effect of Systematically Missing Data



- as little as $10^\circ$ degree of missing data lead to shearing of the experimental map
- Shearing of experimental map results in unreliable coordinates for structure

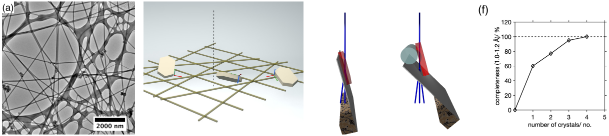
# Solution 1 — Coiled carbon grids





- Brush Stroke causes carbon layer to coil
- Visual selection of orientation from carbon curvature
- Complete data from 5'ish crystals

# Solution 2 — Nylon Fibres



- Nylon fibres ($\approx 100nm$ diameter) disturb preferred orientation
- Orientation less obvious from visual inspection — possibly more screening required
- Complete data from 5'ish crystals
- Nylon grids adaptable to sample size and shape

# Acknowledgements

- My colleagues at the X-ray Centre: A. Roller & N. Gajic
- My collaborators from the nanoArgovia project A3EDPI (*cf.* references)
- H. Stahlberg (C–CINA), N. Blanc (ScopeM), C. Schulze–Briese (DECTRIS Ltd.)
- B. Luethi, L. Wagner, L. Piazza, D. Mayani (all DECTRIS)
- B. Schmitt (PSI), E. Frojdh (PSI), G. Tinti (PSI), S. Vetter (all PSI detector group)
- R. Mezzenga (ETHZ), U. Shimanovich (Weizmann Inst.), I. Adrianssens Martiel (PSI), I. Schlichting (MPI Heidelberg), K. Diederichs (Uni Konstanz), J. Lübben (Bruker AXS), M. Clabbers (Uni Stockholm)
- J. Chin, M. Reithofer (both Uni Vienna)

DECTRIS QUADRO: to be installed at Vienna University

SNI SWISS NANOSCIENCE INSTITUTE
EINE INITIATIVE DER UNIVERSITÄT BASEL UND DES KANTONS AARGAU

FNSNF SWISS NATIONAL SCIENCE FOUNDATION

DECTRIS detecting the future

nanoArgovia A3EDPI SNF Project 169258