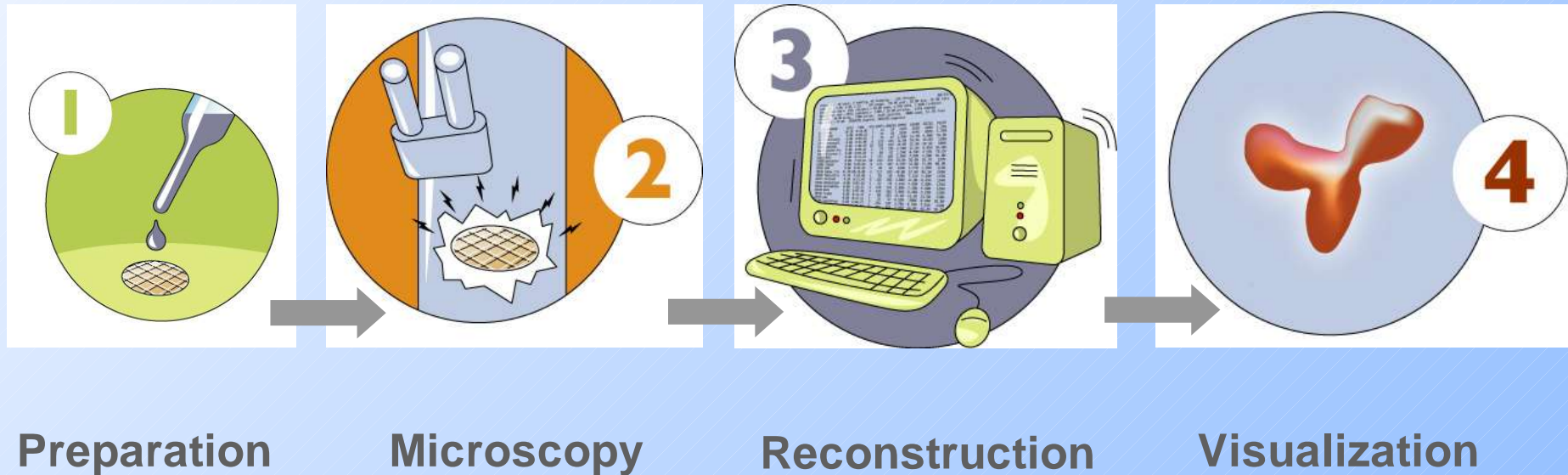

Refinement of 3D maps by Constrained Maximum Entropy Tomography

Lars-Göran Öfverstedt
Electron Tomography group,
Karolinska Institute,
Stockholm

Electron tomography process



Experimental setup

Equipment

- Philips CM200 FEG
- TVIPS 2k x 2k CCD camera

Specimens

- Cryo-sections, 70 – 100 nm thick, PVA-embedding (Tokuyasu) and light uranyl acetate staining
- Flash-frozen solutions on holey carbon, unstained

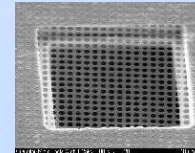
Single-axis tilt series

- Magnification 27000x, pixel size $\sim 5 \text{ \AA}$
- Tilt range $\pm 60^\circ$, every degree
- Dose per image 10 - 15 e/nm^2

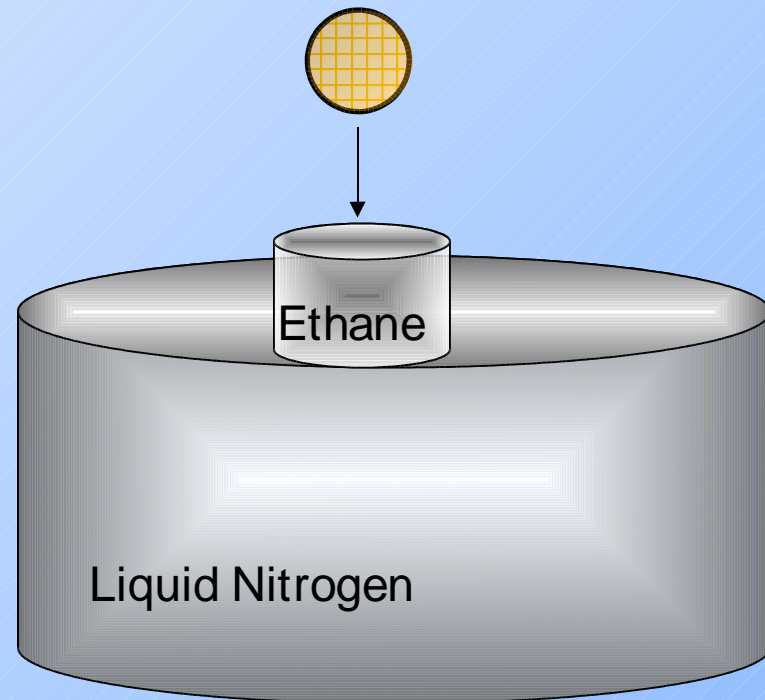


Specimen preparation

- Quantifoil holey carbon grid
- Plunge freezing into liquid ethane gives hydrated molecules in amorphous vitrified water
- No stain applied

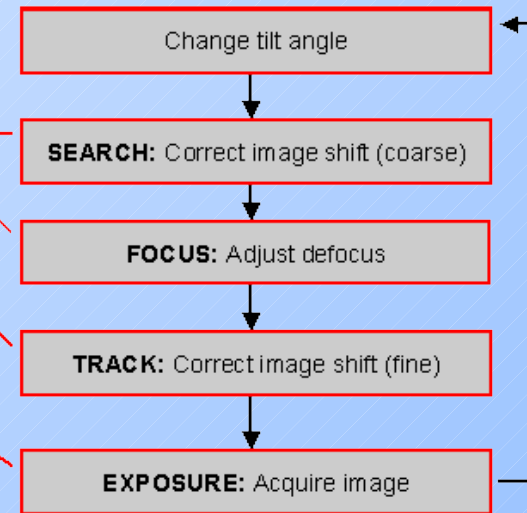
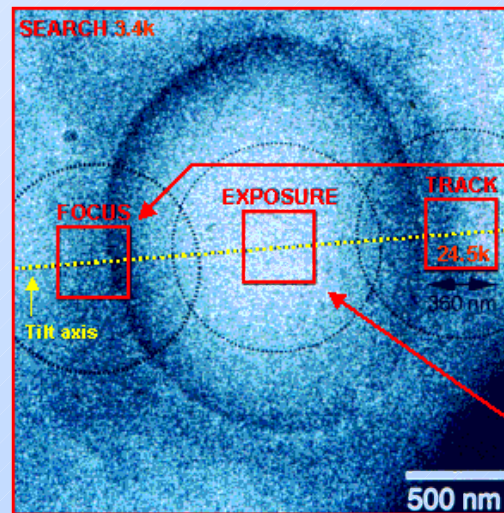


Quantifoil®



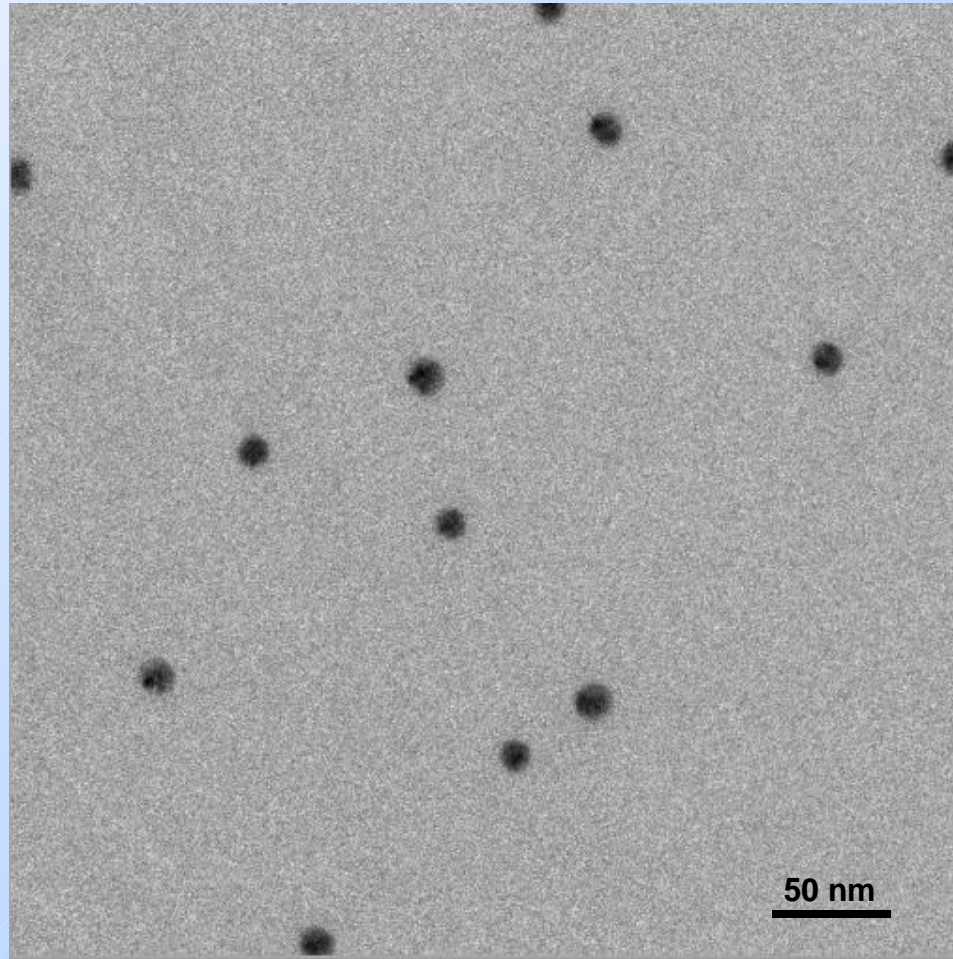
Cryo-electron microscopy

- Automated data collection
- Tilt -60° to $+60^{\circ}$
- Record 121 images
- Total dose $20 \text{ e}^{-}/\text{\AA}^2$

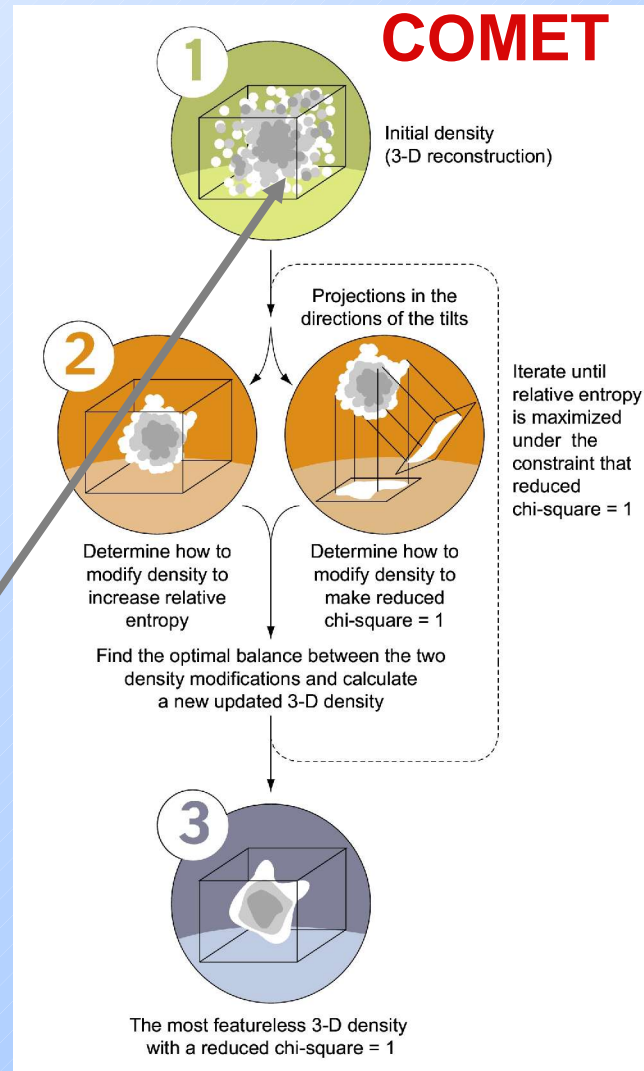
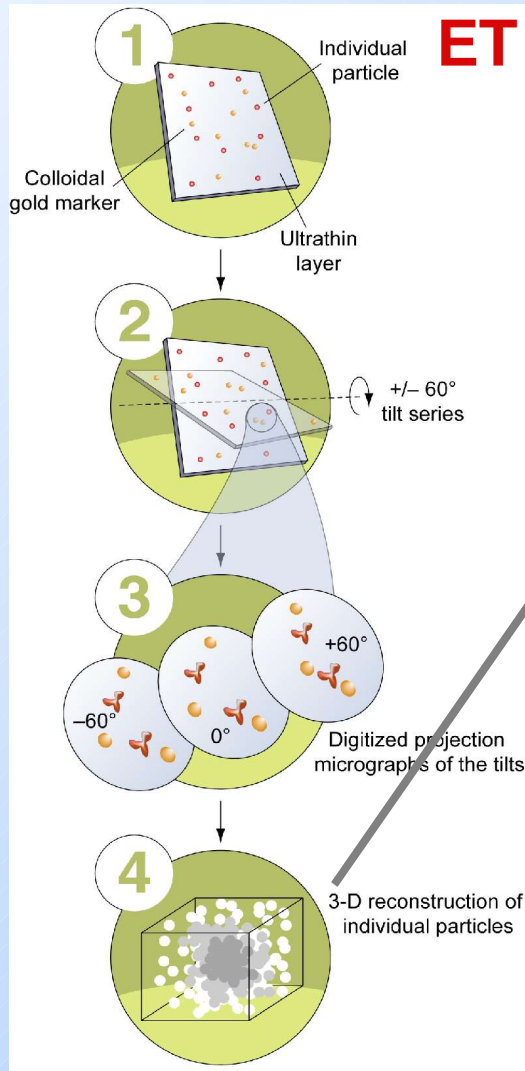


TVIPS®

Low-dose data (vitrified solution)



Electron Tomography and COMET



Basic equations

The **relative entropy** function is maximized:

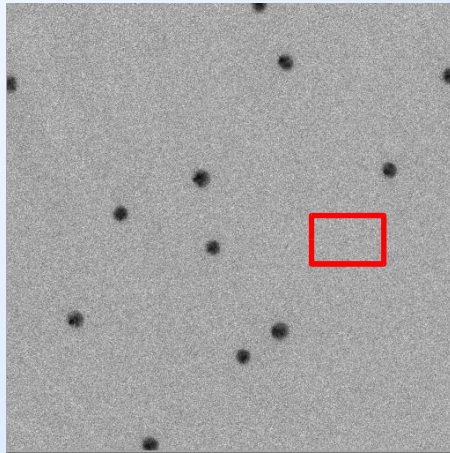
$$S = - \sum_{\vec{X}} \mathbf{F}(\vec{X}) \cdot \ln \left[\frac{\mathbf{F}(\vec{X})}{\mathbf{m}(\vec{X})} \right]$$

The **chi-square** merit function is minimized:

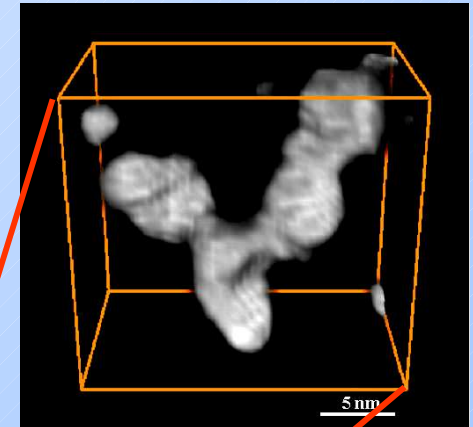
$$\chi_i^2 = \sum_{x^{(i)}} \frac{1}{\sigma_{x^{(i)}}^2} \left[b^{(i)} \cdot \mathbf{F}_{calc}^{(i)}(\vec{x}^{(i)}) - \mathbf{F}_{obs}^{(i)}(\vec{x}^{(i)}) \right]^2$$

The result is the most featureless density whose projections fit the observations within their variances.

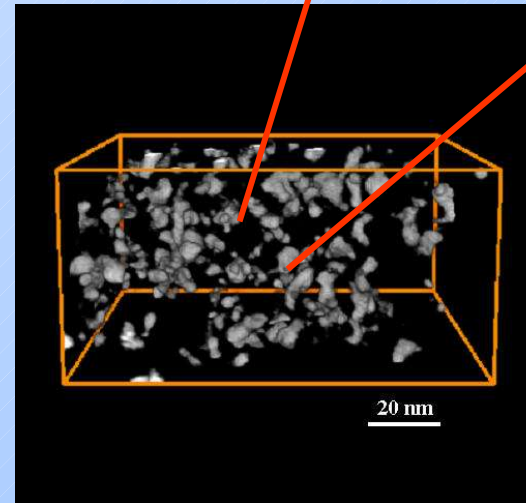
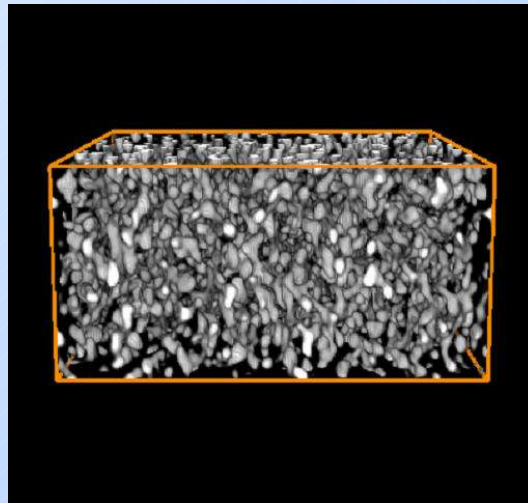
Reconstruction of macromolecule, IgG



Projection
micrograph



Conventional
ET

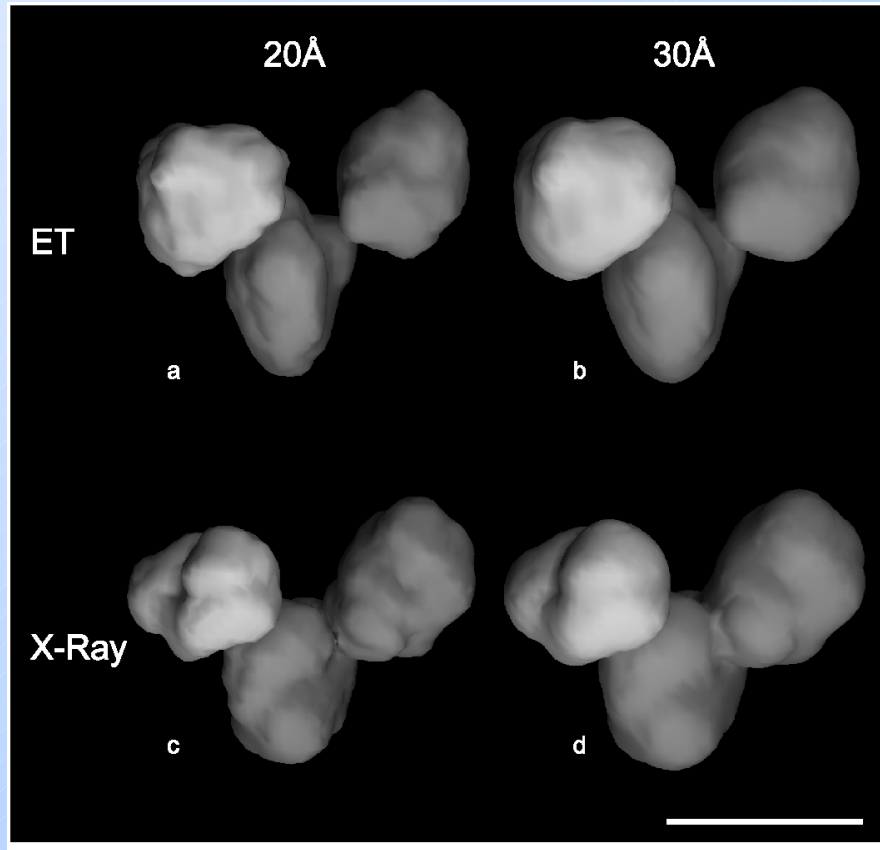


**COMET-
refined**

IgG

Volume-rendered IgG molecule in solution (top).

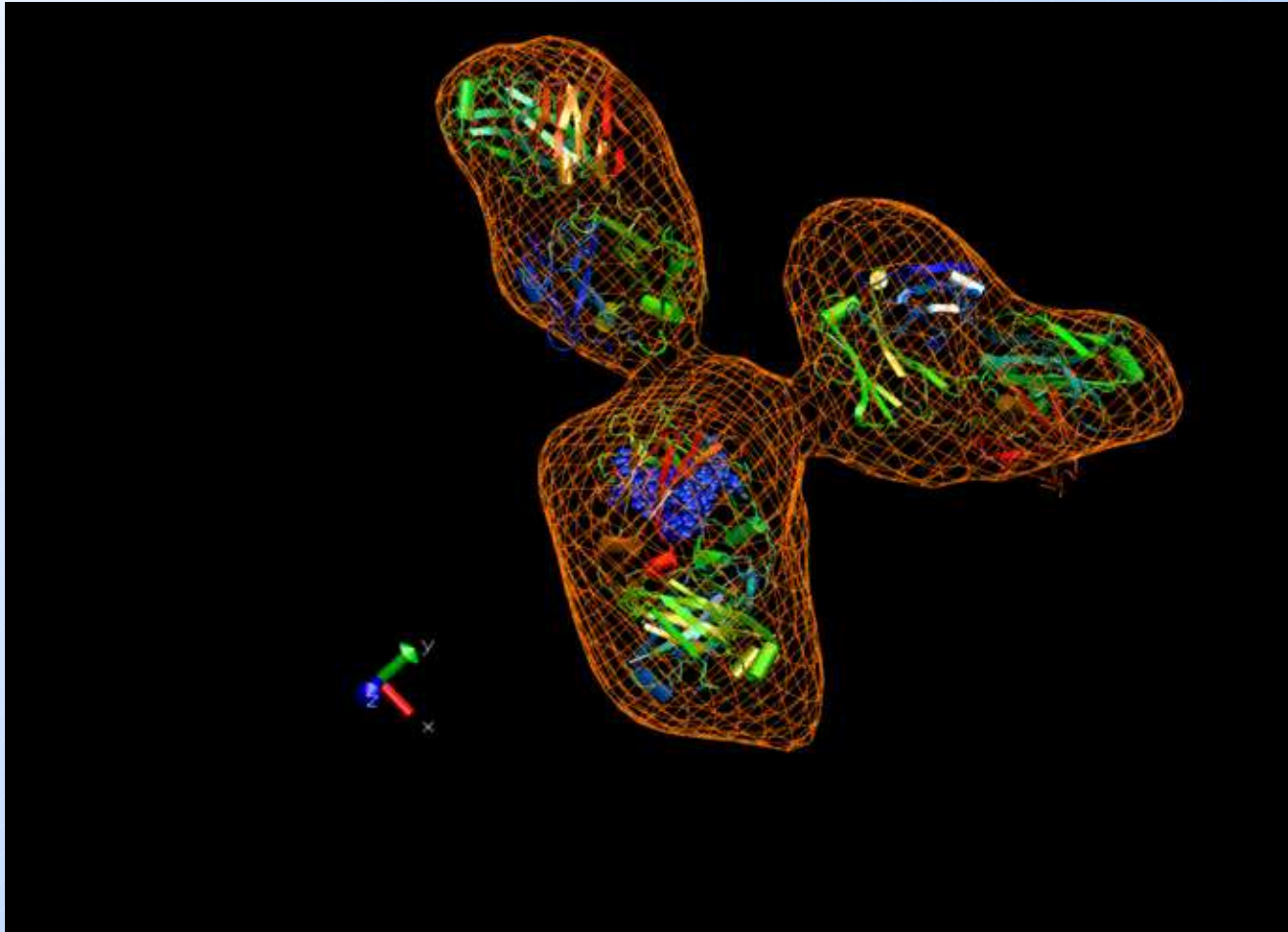
Atomic X-ray structure of IgG (bottom).



Sandin et al. (2004) Structure 12, 409-15

Bongini et al. (2004) PNAS 101, 6466-71

IgG2a + docked X-ray structure



Computer programs

Programs

- | | |
|---------------------|--------------------------|
| • XPIX | Pick marker coordinates |
| • REFINE | Geometry alignment |
| • IMAGE-EXTRACT | Data extraction |
| • BACKPROJ | Filtered back projection |
| • COMET | Post refinement |
| • BOB, AVS, VMD ... | Visualization |

Platforms

- Compaq OSF1
- Linux RedHat, SUsE
- Mac OSX

Parameter files

REFINE

Phi	Theta	Omega	Scale	Center_x	Center_y	Name
0.0000	-60.0000	0.2517	1.0012	1259.0000	964.6667	tilt_001.dat
0.0000	-59.0000	0.2252	1.0008	1246.0000	970.3334	tilt_002.dat
0.0000	-58.0000	0.2567	0.9993	1247.0000	991.0000	tilt_003.dat
0.0000	-57.0000	0.3031	0.9997	1250.0000	1021.6667	tilt_004.dat
0.0000	-56.0000	0.2518	1.0006	1252.3334	1052.3334	tilt_005.dat
0.0000	-55.0000	0.2646	1.0007	1249.6667	1090.3334	tilt_006.dat
...						

Tilt series geometry

IMAGE- EXTRACT

Phi	Theta	Omega	Scale	Center_x	Center_y	Name
0.0000	-60.0000	0.2517	1.0012	135.2970	190.9729	tilt_001.dat
0.0000	-59.0000	0.2252	1.0008	135.1556	190.1414	tilt_002.dat
0.0000	-58.0000	0.2567	0.9993	135.4915	190.6403	tilt_003.dat
0.0000	-57.0000	0.3031	0.9997	135.5295	190.3721	tilt_004.dat
0.0000	-56.0000	0.2518	1.0006	135.2792	190.4739	tilt_005.dat
0.0000	-55.0000	0.2646	1.0007	135.5951	190.2238	tilt_006.dat
...						

Extracts geometry

Magni	Post-mag	RUS(my)	Binning	Pixel(A)	Name
1.500E+4	1.781E+0	1.400E+1	1.000E+0	5.241E+0	tilt_001.dat
1.500E+4	1.781E+0	1.400E+1	1.000E+0	5.241E+0	tilt_002.dat
1.500E+4	1.781E+0	1.400E+1	1.000E+0	5.241E+0	tilt_003.dat
1.500E+4	1.781E+0	1.400E+1	1.000E+0	5.241E+0	tilt_004.dat
1.500E+4	1.781E+0	1.400E+1	1.000E+0	5.241E+0	tilt_005.dat
1.500E+4	1.781E+0	1.400E+1	1.000E+0	5.241E+0	tilt_006.dat
...					

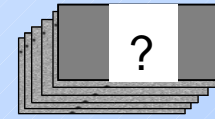
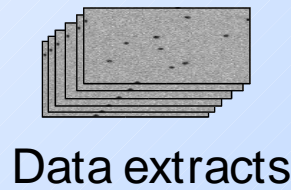
Image parameters

Acc_volt	Defocus	Sph.Abb	E.Decay	Famp	Name
2.000E+2	8.500E+3	2.000E+0	2.000E+1	1.000E-1	tilt_001.dat
2.000E+2	8.500E+3	2.000E+0	2.000E+1	1.000E-1	tilt_002.dat
2.000E+2	8.500E+3	2.000E+0	2.000E+1	1.000E-1	tilt_003.dat
2.000E+2	8.500E+3	2.000E+0	2.000E+1	1.000E-1	tilt_004.dat
2.000E+2	8.500E+3	2.000E+0	2.000E+1	1.000E-1	tilt_005.dat
2.000E+2	8.500E+3	2.000E+0	2.000E+1	1.000E-1	tilt_006.dat
...					

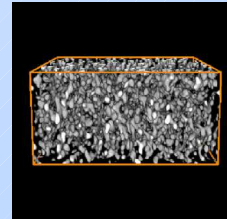
CTF function parameters

Binary files

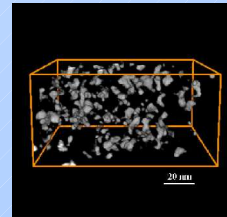
IMAGE-
EXTRACT



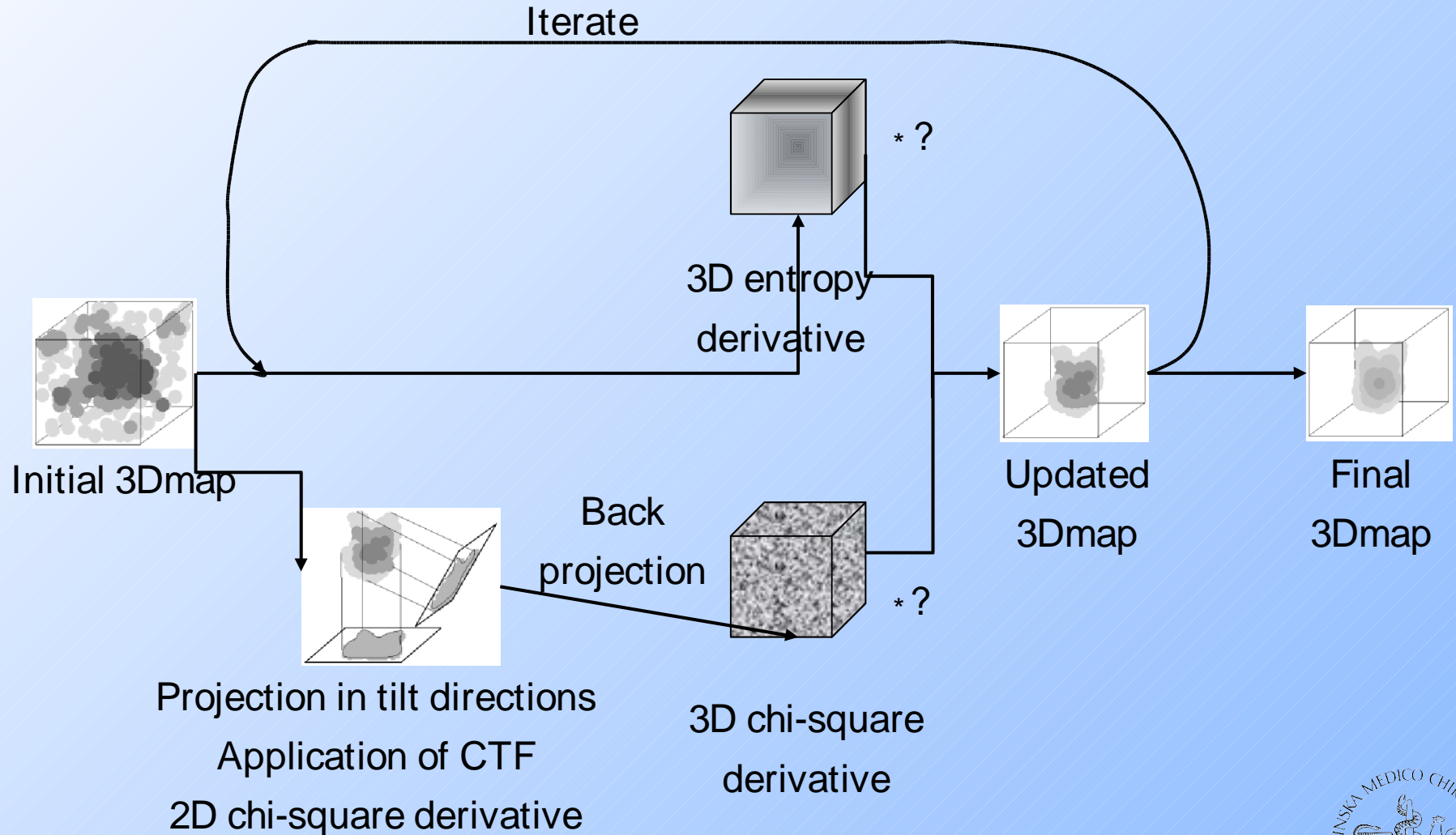
BACKPROJ



COMET



COMET



Project coworkers at KI

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Duccio Fanelli

Sara Sandin

Örjan Wrangé

Lars-Göran Öfverstedt