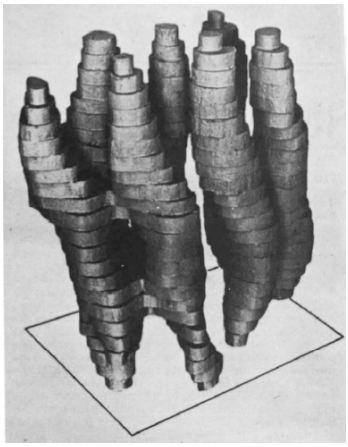
# MRC software for 2d crystal real-space image processing



Henderson & Unwin (1975) Nature 257, 28-32

- Introduction
- Image processing Workflow
  - Unbending
  - Extraction of amplitude /phase values of diffraction spots in FT
  - Boxing

## History

- Developed over 30 yrs by a number of people
- Written in Fortran
- image2000
  - image format compatible with ccp4
  - plots in postscript format


http://www.tonh.net/museum/plotter.html

## What it includes

- General processing
  - LABEL, FFTRANS, TWOFILE
- Two-dimensional crystals processing
  - Image analysis
  - Electron diffraction patterns
- Helical structures
- Rotational averaging and filtering
- General display
  - XIMDISP
- Automatic particle detection - sleuth

image2000/README; image2000/doc/improc/doc

## Documentation

- Most recent reference:
  - Crowther, Henderson and Smith (1996)

  J. Struct. Biol.116, 9-16
- Docs in the package: image2000/doc/
- Docs on 2dx.org
  - documentation from MRC (Crowther, Henderson and Smith)
  - image/FT formats
  - program source headers image2000/source/*.for
  - Electron crystallography-steps and program annotation (Unger and Cheng)
- Help Docs in 2dx applications
- Move to 2dx.org in the future?
  - Electron diffraction processing

http://www.scripps.edu/~acheng/publications/e-diffr_intro+annotation.pdf

# Example MRC Program Header

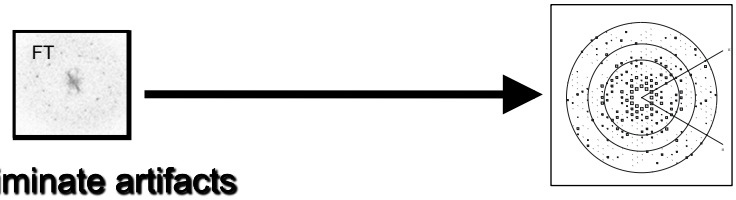
```
C LATLINPRESCAL : program to correct image amplitudes for effects of CTF and
C                                 to C calculate appropriate weights for output to LATLINE.
C
C vx 1.0 RH 18.9.93 original program fused from READBOTH + SCALIMAMP
C vx 1.1 RH 25.7.95 debug of WTFACTOR on move to Dec Alpha
C vx 1.2 AS 07.07.2003 changed IFILM to 10 digits
C*************************************************************************
C Control cards:
C
C          Card 1:   NSER,ZMIN,ZMAX (*)
C                                 serial number on ORIGTILT input
C                                and ZSTAR limits to be passed on to LATLINE.
C          Card 2:   IQMAX (*)
C                                 maximum value of IQ for spots to be used
C
C          INPUT file is raw file straight from ORIGTILT : on unit FOR001
C          OUTPUT has the format required by LATLINE : on unit FOR003
C
C*************************************************************************
```

http://www.rdrop.com/~jimw/j-hist.shtml#History

# Where to get MRC package

- Contact Judith Short (jms@mrc-lmb.cam.ac.uk) to get ftp download procedure

# Single Image Processing Workflow

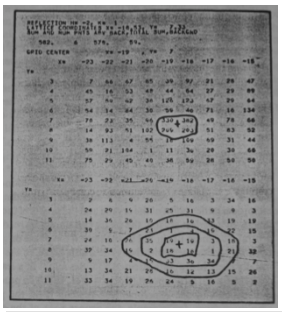



- Eliminate artifacts
- Determine the lattice
- Boost S/N of the repeating features in the image
  - Unbending
  - Boxing (Masking in the real space)
- Extract phases and amplitudes of the indexed spots in the Fourier space

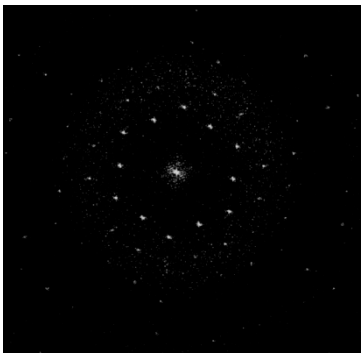
# Determine Amplitudes and Phases of Diffraction spots

## MMBOXA

- Fitting of the spots
- IQ values
- Results



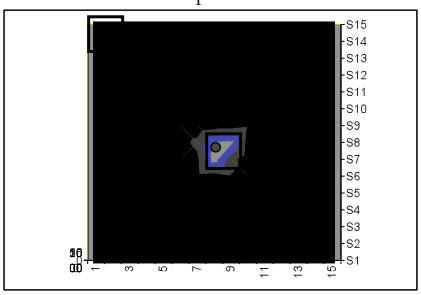
Amos, Henderson & Unwin (1982) Prog. Biophys. molec. Biol., 39, 183-231



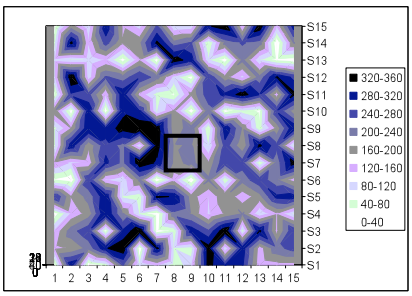
| h | k | Amp | Phase | IQ | Background |
|---|---|---|---|---|---|
| 0 | 1 | 434.9 | 34.2 | 3 | 156.8 |
| 0 | 2 | 7924.5 | 44.6 | 1 | 148.5 |
| 0 | 3 | 1373.7 | 210.6 | 1 | 158.3 |
| 0 | 4 | 1411.4 | 228.4 | 1 | 124.3 |
| 0 | 5 | 710.5 | 206.4 | 2 | 106.3 |
| 0 | 6 | 1870.8 | 126.0 | 1 | 121.1 |
| | | . | | | |
| | | . | | | |
| | | . | | | |


- 2D-sync function weighted vector sum of the center 2x2 pixels.
- Background RMS also sync weighted

Amplitude

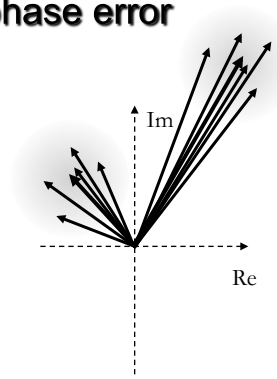


Phase




# IQ values

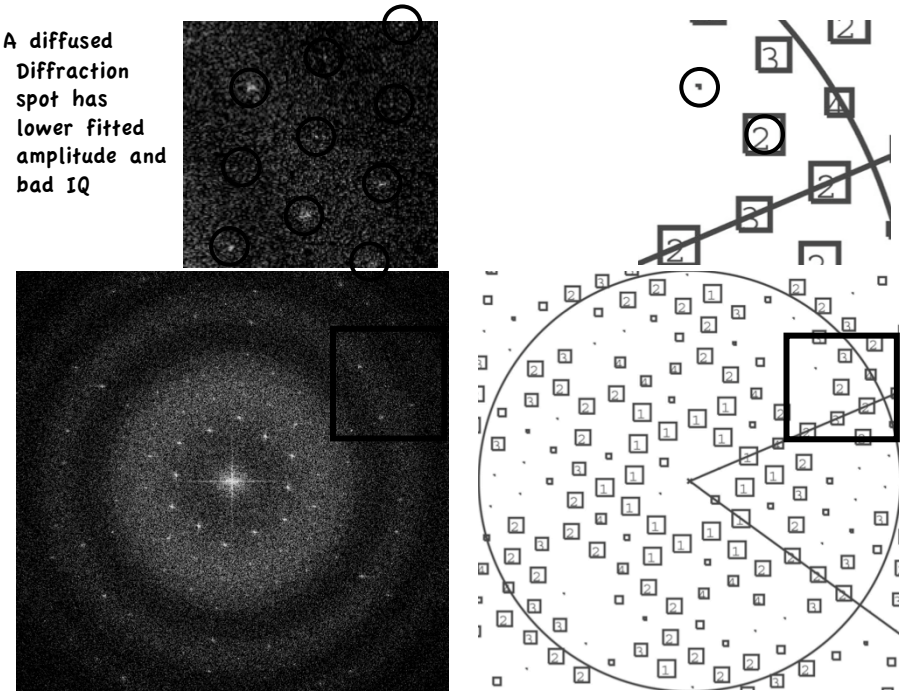
- determined by amplitudes
- Used as measure of phase error




| IQ | Amp/(RMS background) cutoff | Phase Error |
|---|---|---|
| 1 | 8.18 | 7 |
| 2 | 4.09 | 14 |
| 3 | 2.73 | 21 |
| 4 | 2.05 | 28 |
| 5 | 1.64 | 35 |
| 6 | 1.36 | 42 |
| 7 | 1.17 | 49 |
| 8 | 1.02 | 56 |

image2000/source/mmboxa.for

A diffused Diffraction spot has lower fitted amplitude and bad IQ



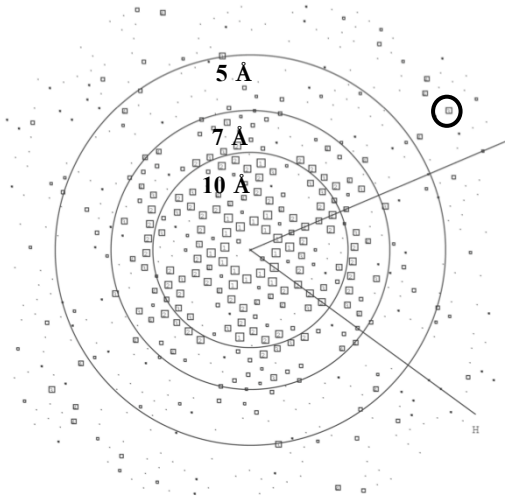
## Resolution of an image

8-6 A

| | | | | |
|---|---|---|---|---|
| 7 | 8 | 9 | 9 | 8 |
| 7 | 10 | 13 | 11 | 7 |
| 8 | 8 | 11 | 11 | 8 |
| 7 | 7 | 10 | 11 | 9 |
| 7 | 7 | 9 | 9 | 8 |

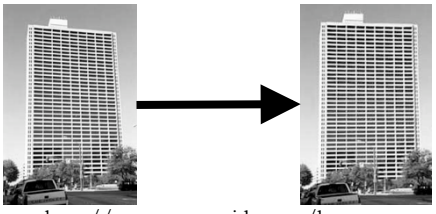
5-3.5 A

| | | | | |
|---|---|---|---|---|
| 7 | 7 | 7 | 7 | 7 |
| 8 | 7 | 6 | 7 | 7 |
| 7 | 7 | 7 | 7 | 6 |
| 7 | 8 | 7 | 7 | 7 |
| 7 | 7 | 7 | 7 | 7 |




Finding one or two good IQ spots does not mean that the resolution is extended that far

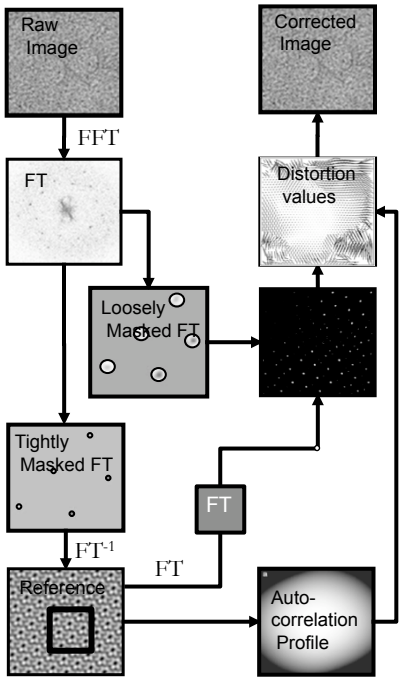
# Single Image Processing Workflow



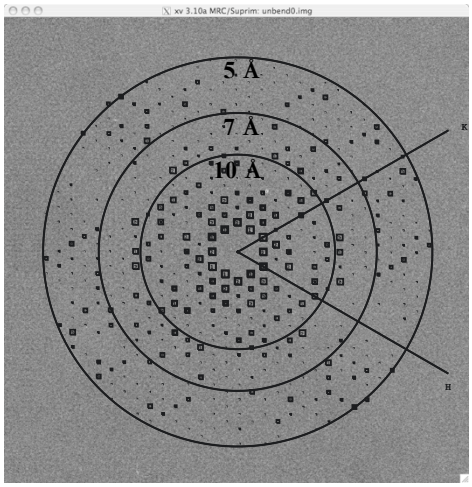
http://www.panoguide.com/howto/panoramas/distortion_correction.jsp

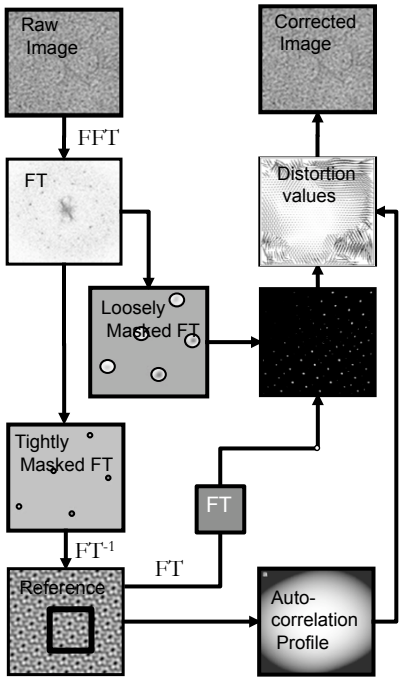
- Eliminate artifacts
- Determine the lattice
- Boost S/N of the repeating features in the image
  - Unbending
  - Boxing (Masking in the real space)

- Extract phases and amplitudes of the indexed spots in the Fourier space



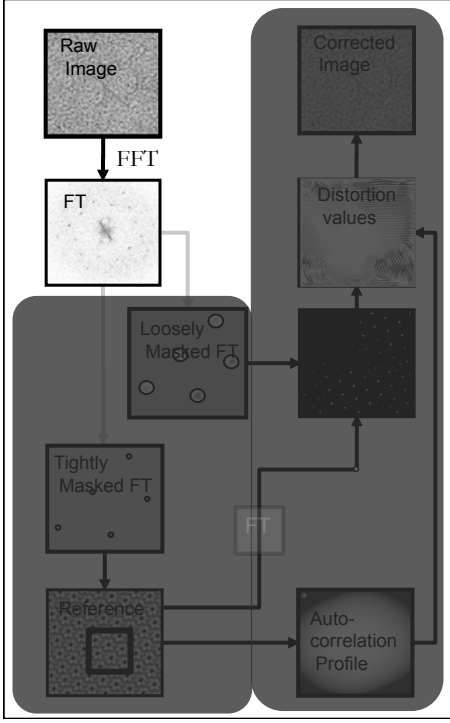

## Unbending





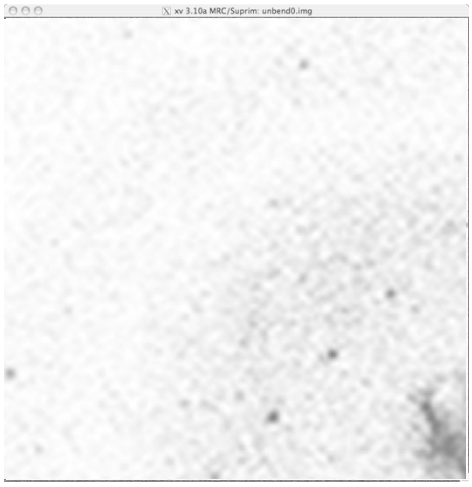

## Unbending

- Define a reference with good contrast
- Enhance the distortion at a similar contrast as the reference
- Generate Cross -Correlation map
- Create auto-correlation profile of the reference
- Quantify the distortion
- Make the correction

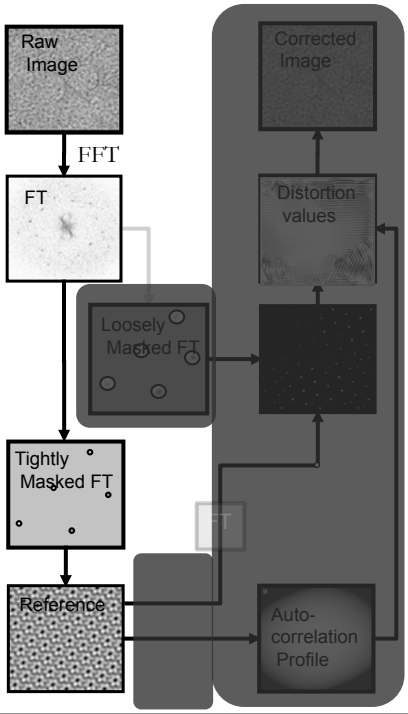



## Compute Fourier Transformation

FFTRANS

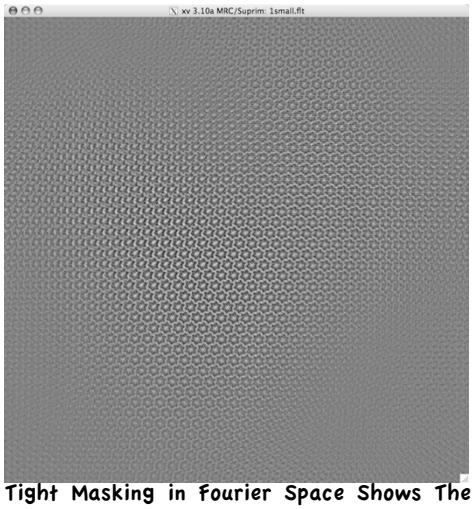



FT highlights periodical repeat

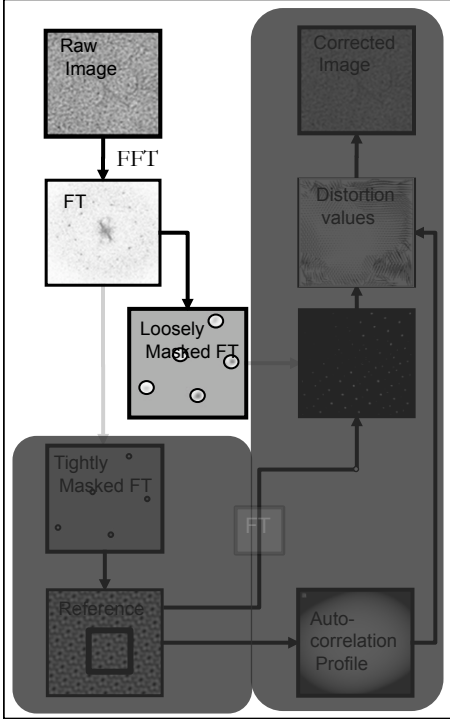



## Tight Fourier Space Masking

MASKTRAN

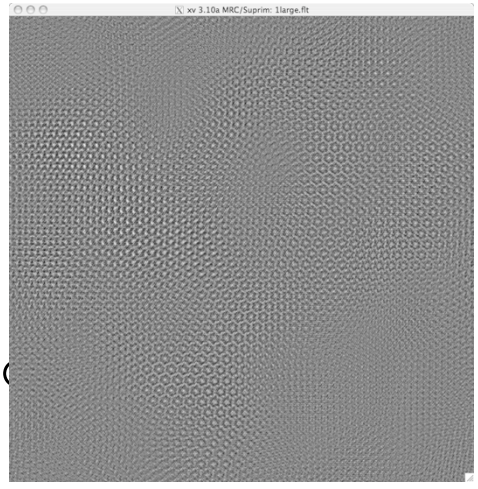



Tight Masking in Fourier Space Shows The Best area and the first glance of what the density looks like

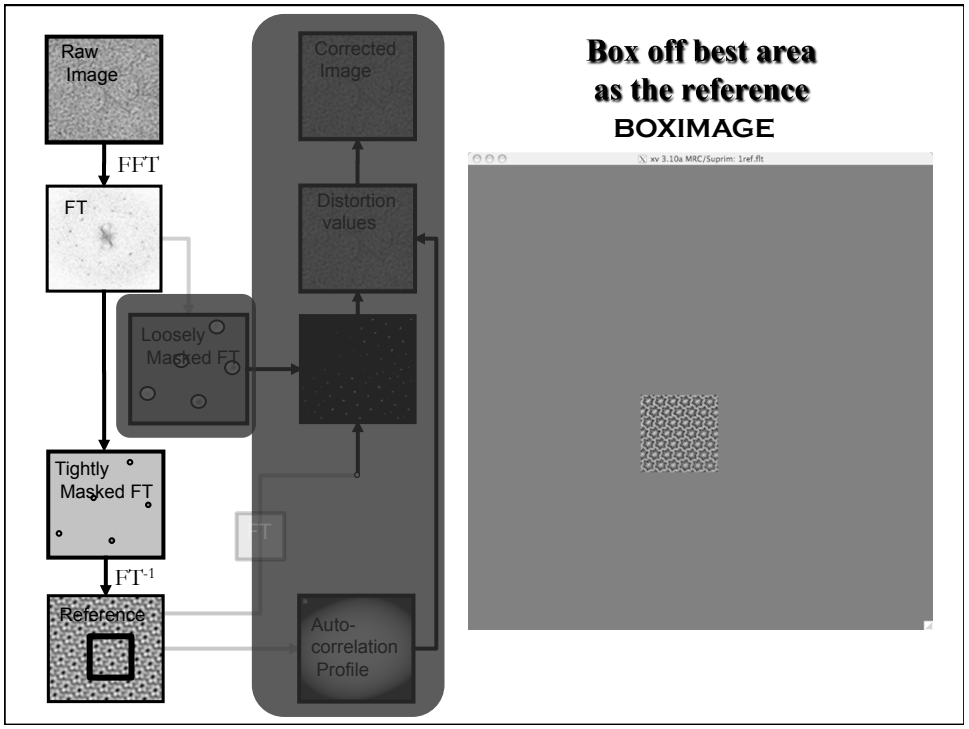


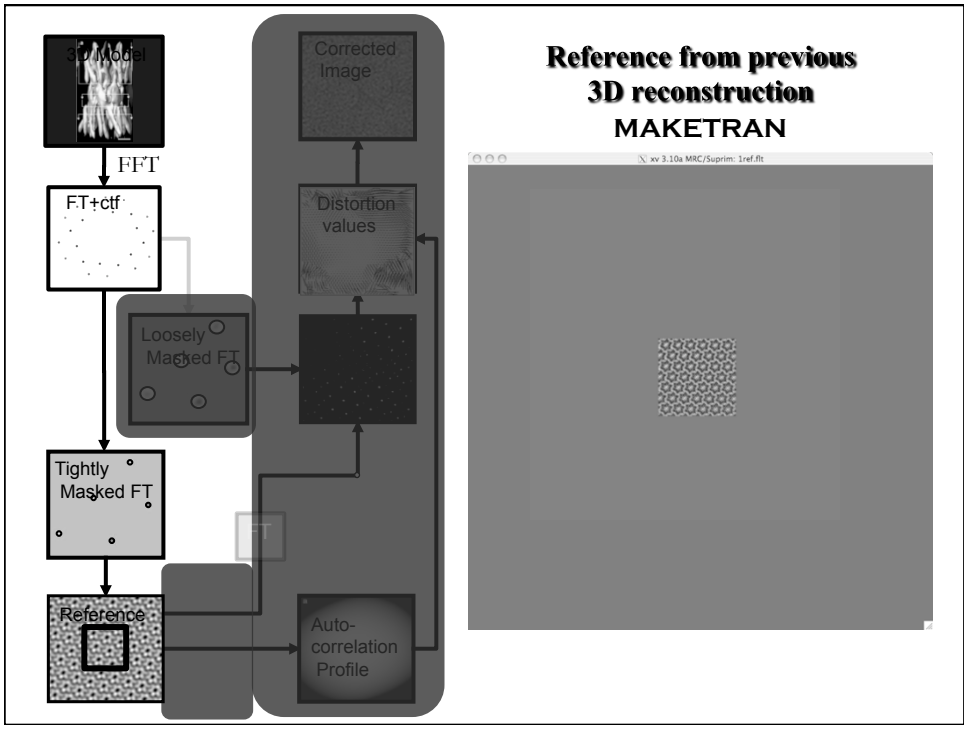

## Loose Fourier Space Masking

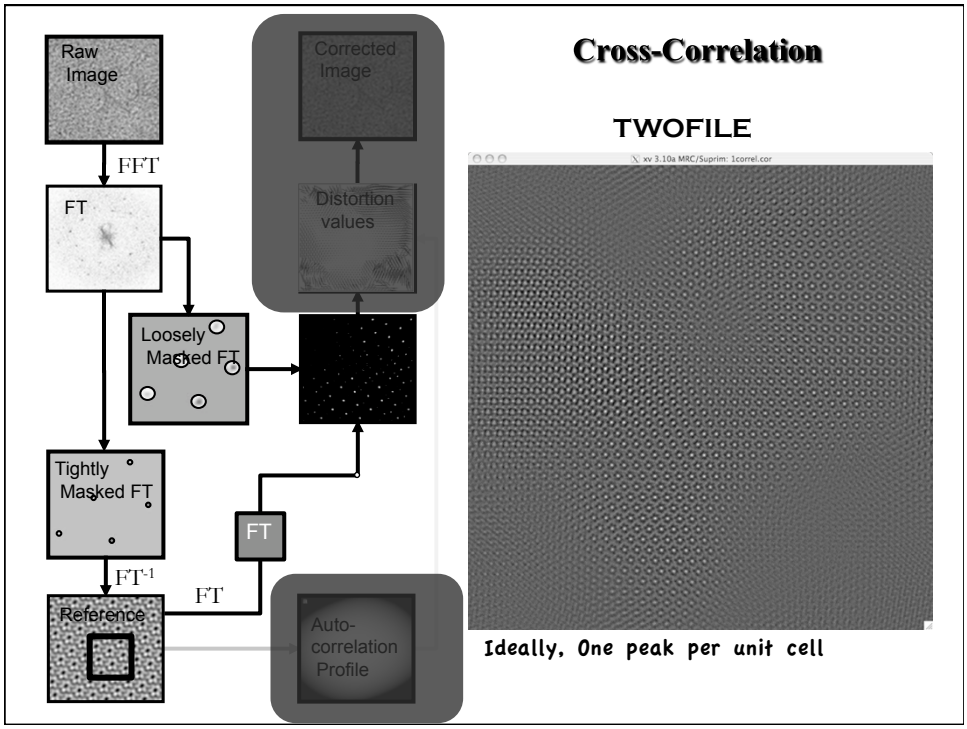
MASKTRANA

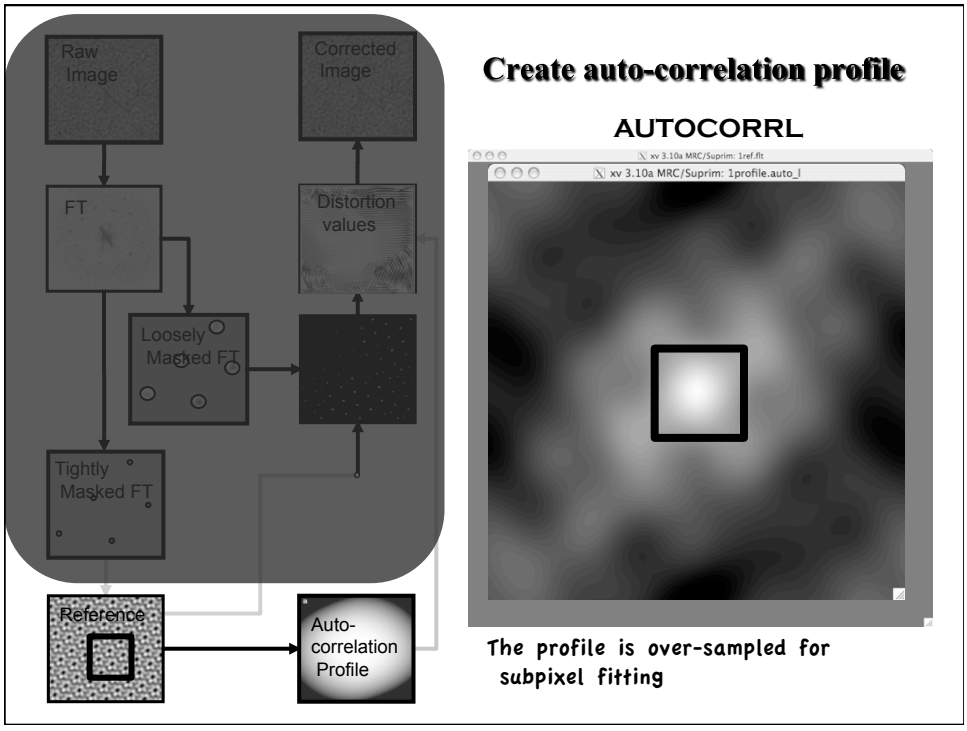


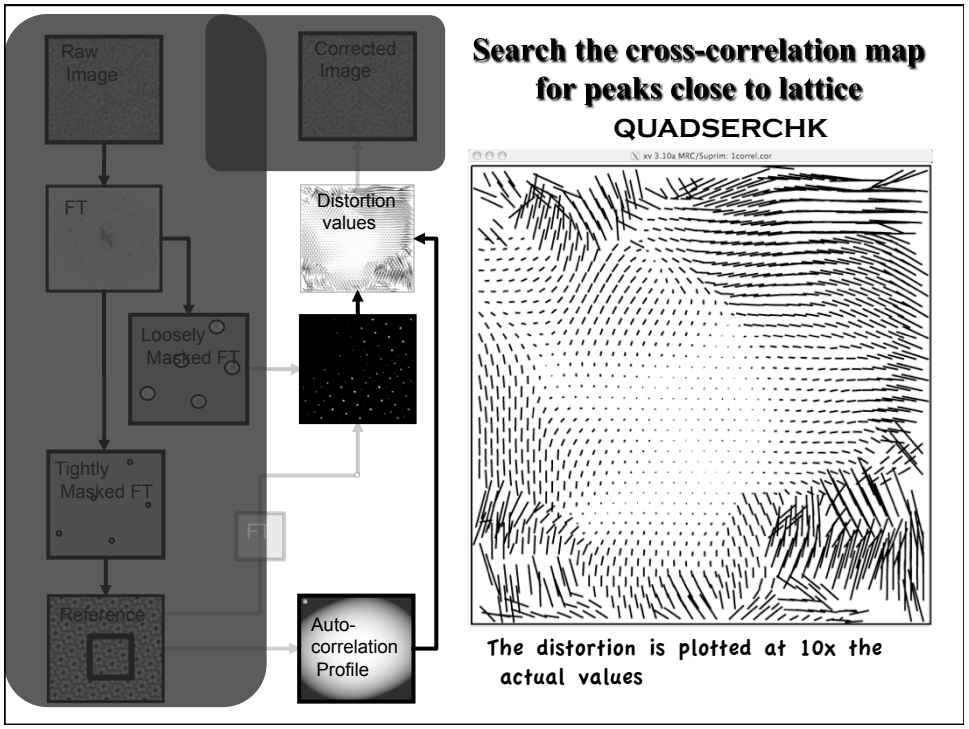

Loose Masking in Fourier Space preserves distortion

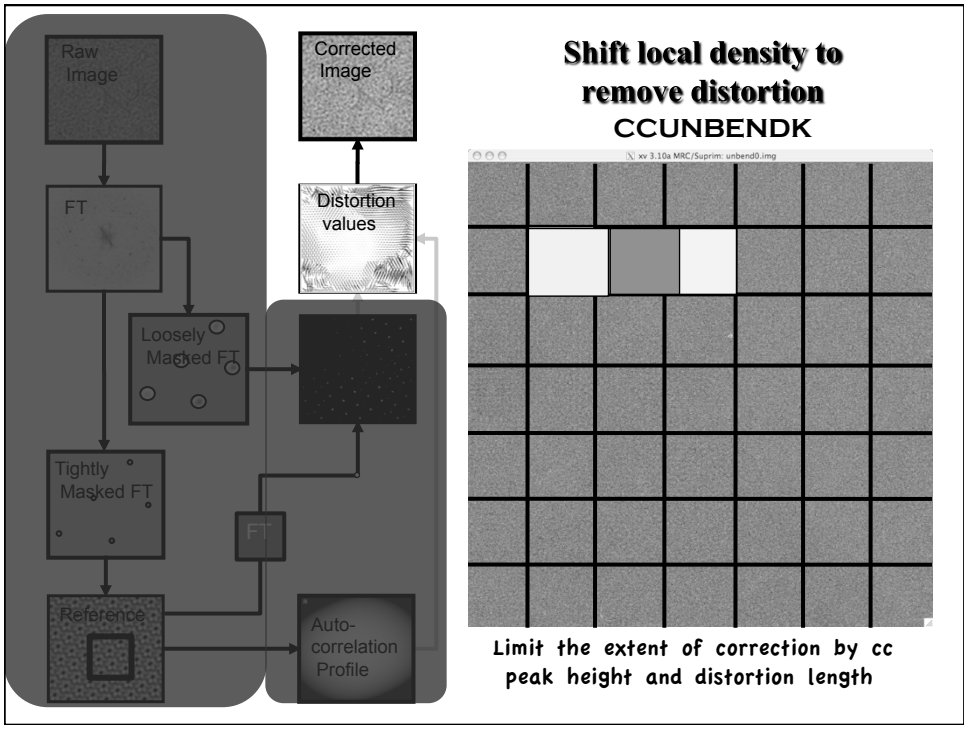

Raw Image
FFT
FT
Loosely Masked FT
Tightly Masked FT
FT-1
Reference
Corrected Image
Distortion values
FT
Auto-correlation Profile
Box off best area as the reference
BOXIMAGE
xv 3.10a MRC/Suprim: 1ref.flt

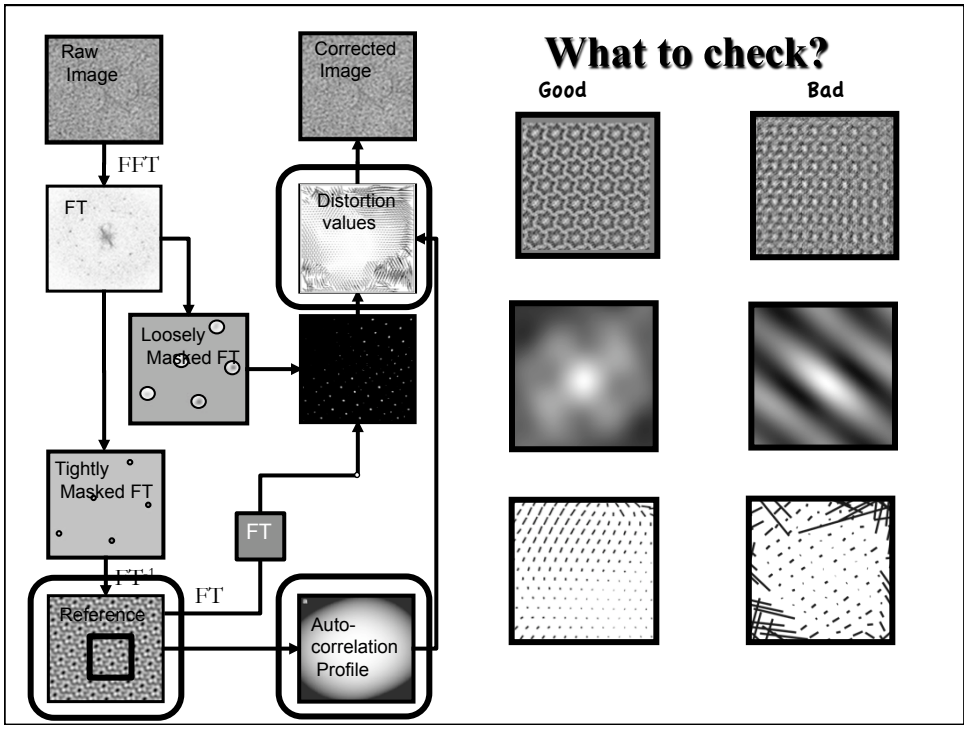

3D Model
FFT
FT+ctf
Loosely Masked FT
Tightly Masked FT
FT
Reference
Corrected Image
Distortion values
Auto-correlation Profile
Reference from previous 3D reconstruction
MAKETRAN
xv 3.10a MRC/Suprim: 1ref.flt


Raw Image
FFT
FT
Loosely Masked FT
Tightly Masked FT
FT
FT-1
FT
Reference
Corrected Image
Distortion values
Auto-correlation Profile
Cross-Correlation
TWOFILE
xv 3.10a MRC/Suprim: 1correl.cor
Ideally, One peak per unit cell


Raw Image
FT
Loosely Masked FT
Tightly Masked FT
Reference
Corrected Image
Distortion values
Auto-correlation Profile
Create auto-correlation profile
AUTOCORRL
xv 3.10a MRC/Suprim: 1ref.flt
xv 3.10a MRC/Suprim: 1profile.auto_l
The profile is over-sampled for subpixel fitting


Raw Image
Corrected Image
FT
Distortion values
Loosely Masked FT
Tightly Masked FT
FT
Reference
Auto-correlation Profile
Search the cross-correlation map for peaks close to lattice
QUADSERCHK
xv 3.10a MRC/Suprim: 1correl.cor
The distortion is plotted at 10x the actual values

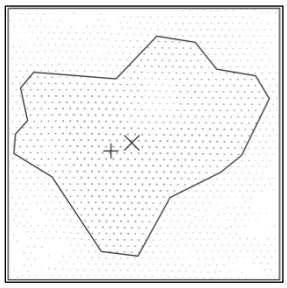

Raw Image
Corrected Image
FT
Distortion values
Loosely Masked FT
Tightly Masked FT
FT
Reference
Auto-correlation Profile
Shift local density to remove distortion
CCUNBENDK
xv 3.10a MRC/Suprim: unbend0.img
Limit the extent of correction by cc peak height and distortion length




# Single Image Processing Workflow


- Eliminate artifacts
- Determine the lattice
- Boost S/N of the repeating features in the image
  - Unbending
  - Boxing (Masking in the real space)
- Extract phases and amplitudes of the indexed spots in the Fourier space

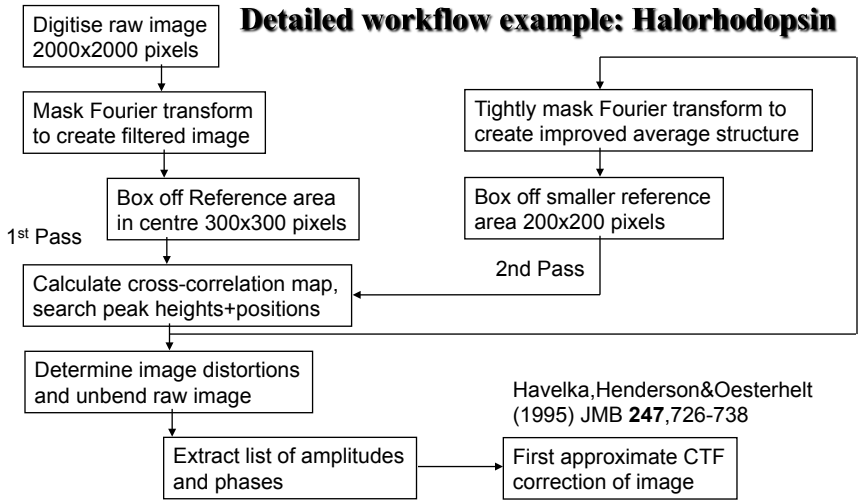
# Mask away non-crystalline region
# BOXIMAGE



| | Before Boxing | | | | | | After Boxing | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Resolution** | **# of spots at IQ** | | | | | **Peak** | **# of spots at IQ** | | | | | **Peak** |
| Å | 1 | 2 | 3 | 4 | < 8 | | 1 | 2 | 3 | 4 | < 8 | |
| 14 | 6 | 5 | 4 | 4 | 25 | 220 | 7 | 6 | 4 | 2 | 24 | 281 |
| 8 | 0 | 3 | 9 | 6 | 35 | 20 | 0 | 3 | 13 | 6 | 40 | 24 |
| 6 | 0 | 0 | 1 | 1 | 15 | 7 | 0 | 0 | 0 | 3 | 24 | 8 |

Simple description-
"The distortion of the lattice was corrected in two rounds of unbending using the best-ordered part of the crystal"
Baranova EA, Holt PJ, Sazanov LA. J Mol Biol. 2007 Feb 9;366(1):140-54.

## Detailed workflow example: Halorhodopsin

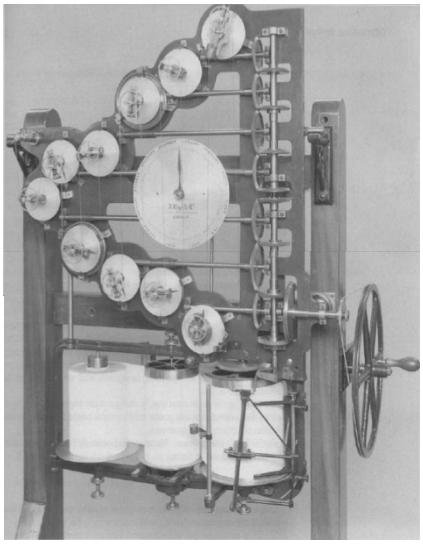



Havelka,Henderson&Oesterhelt (1995) JMB **247**,726-738

## Take this home:

- Computers have changed but the principle is the same.
- Modify and Refine the workflow for your own project.

Tide Predicting machine by Lord Kelvin
(William Thomson)
Science Museum, London



http://ed-thelen.org/comp-hist/CBC-Ch-05.pdf