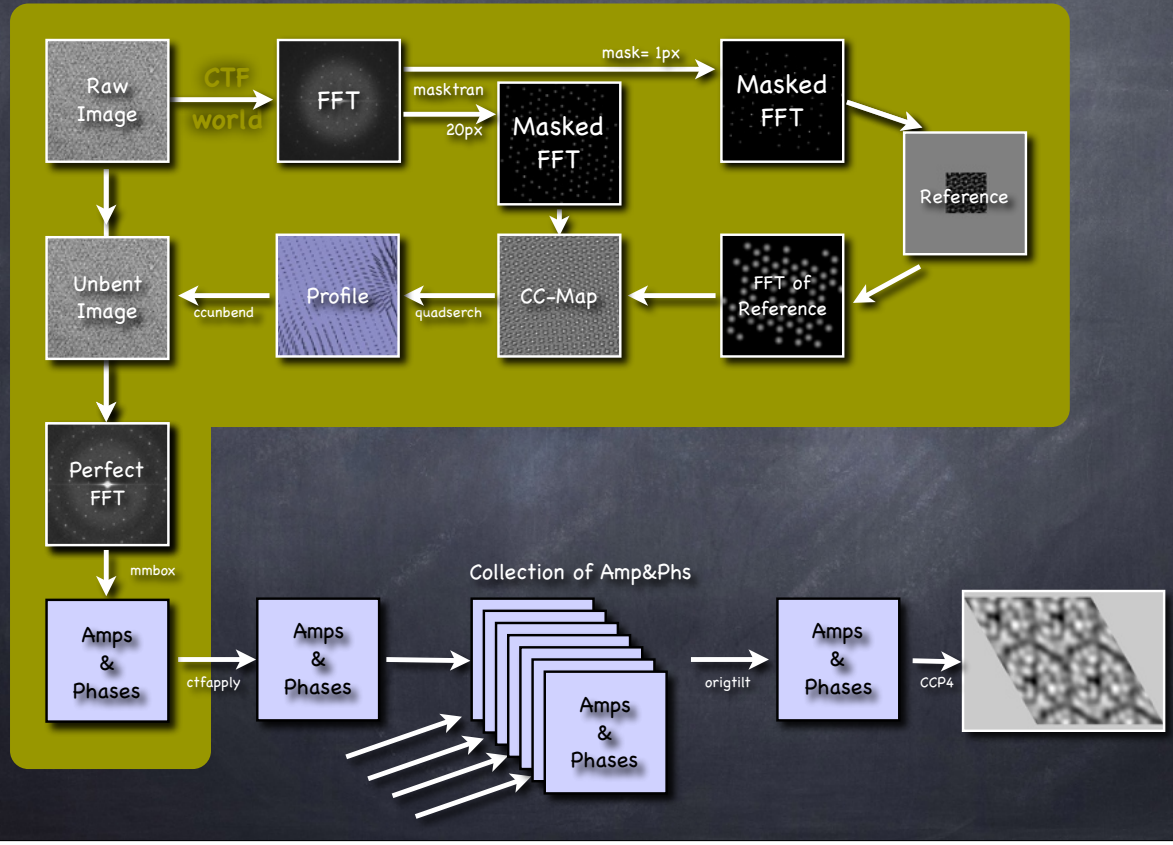
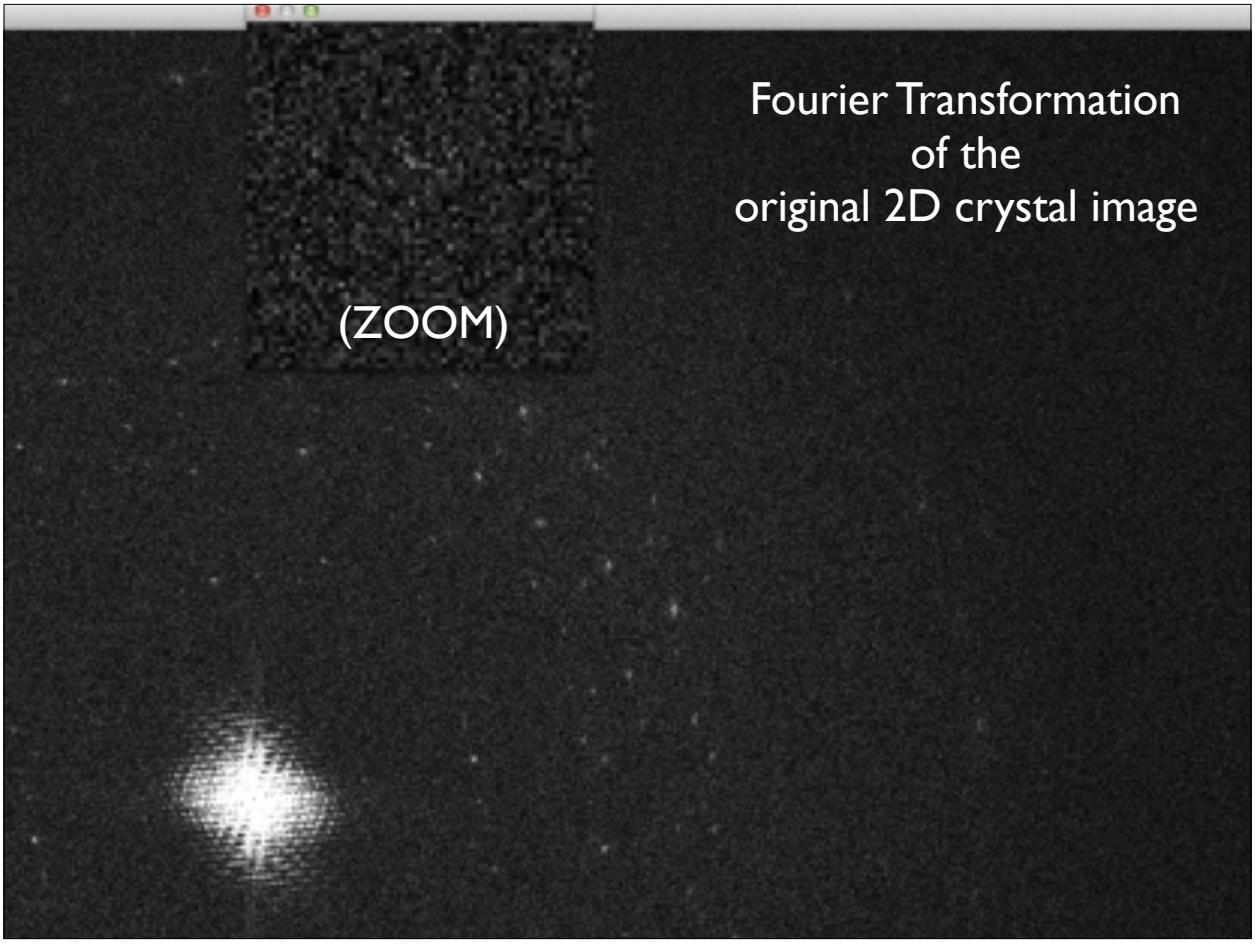
Unbending

Henning Stahlberg,
Biozentrum, Uni Basel, Switzerland
c-cina.org

2dx Workshop
Basel, August 23, 2016

Algorithm Non-tilted

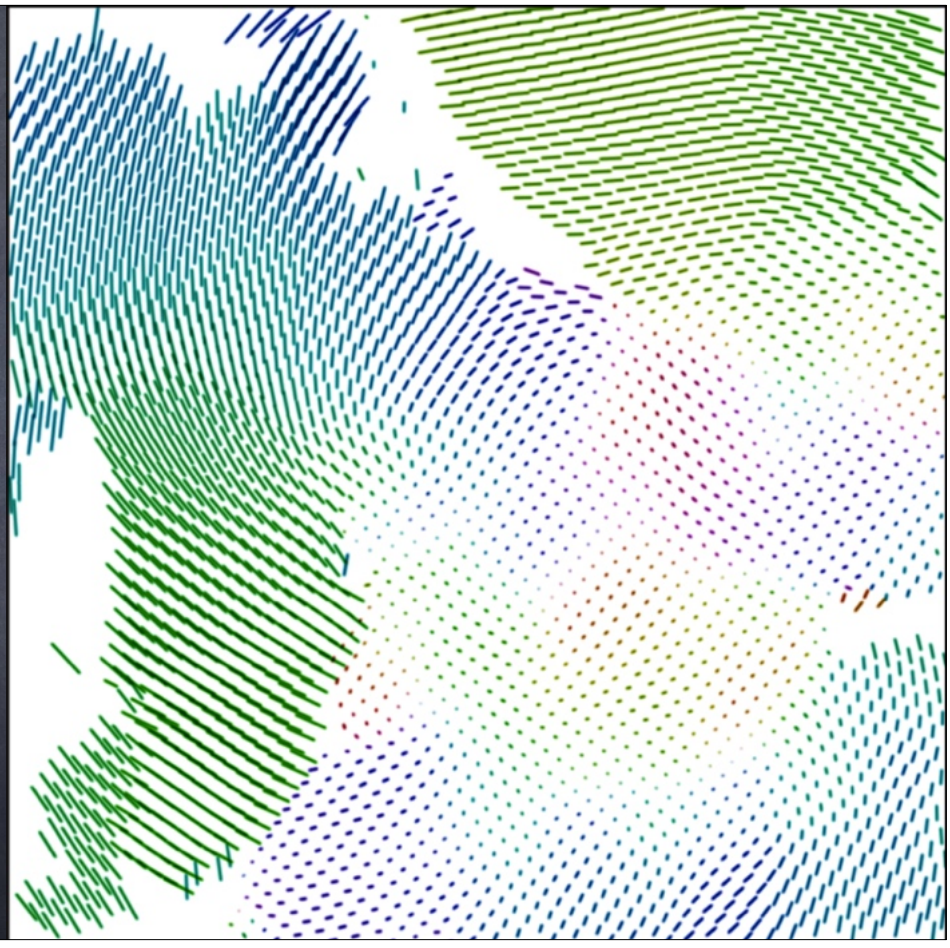


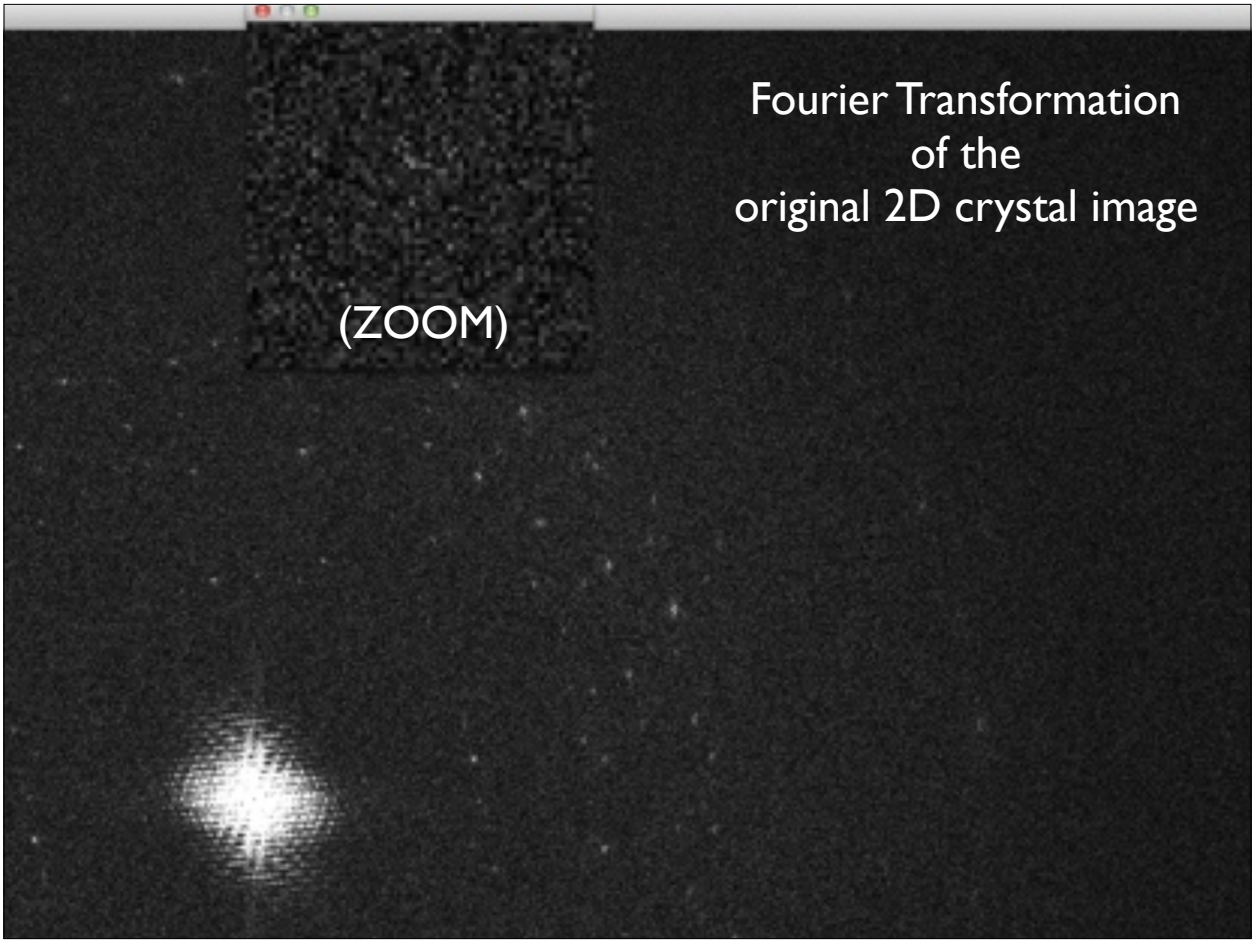


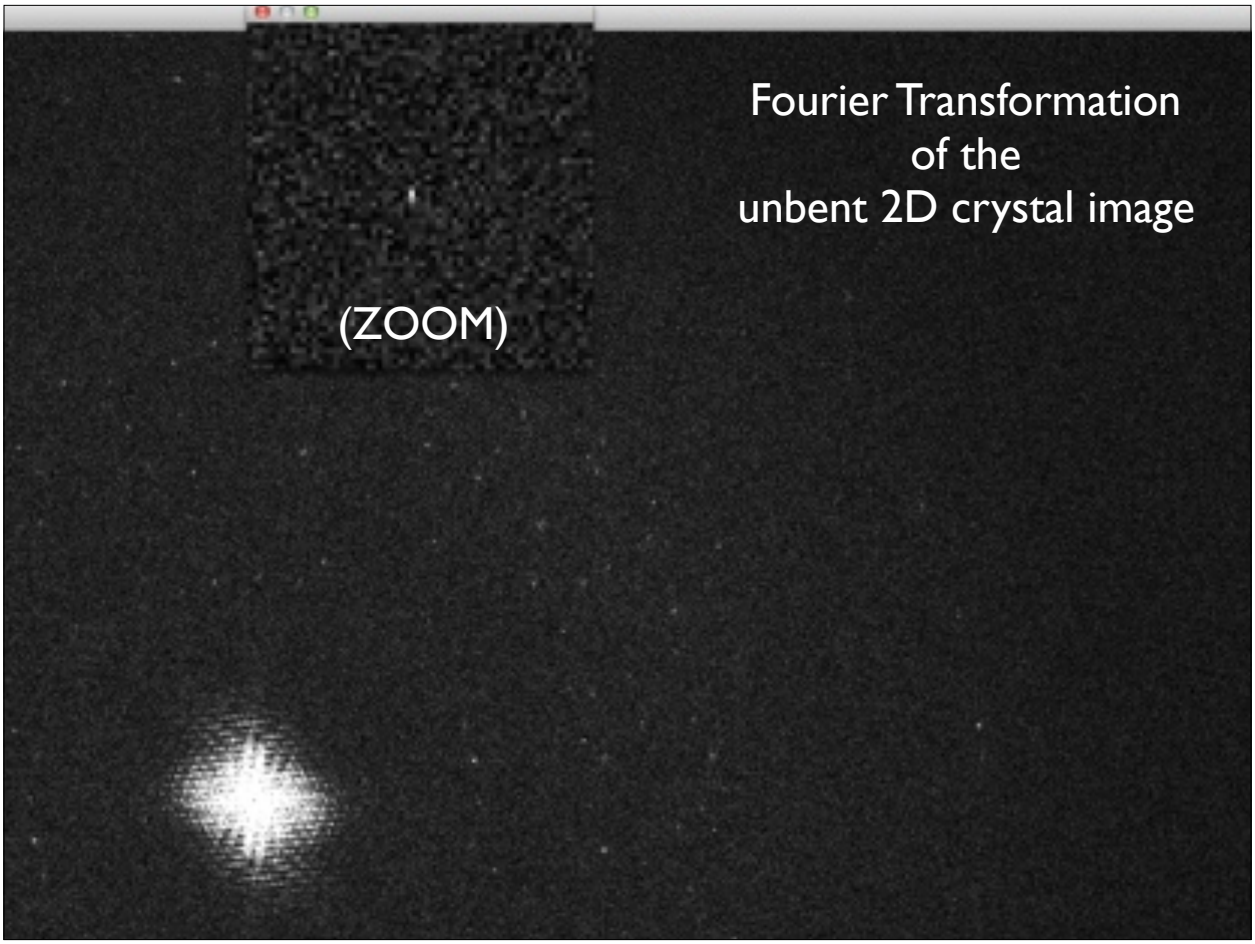
Unbending Profile

to correct crystal distortions

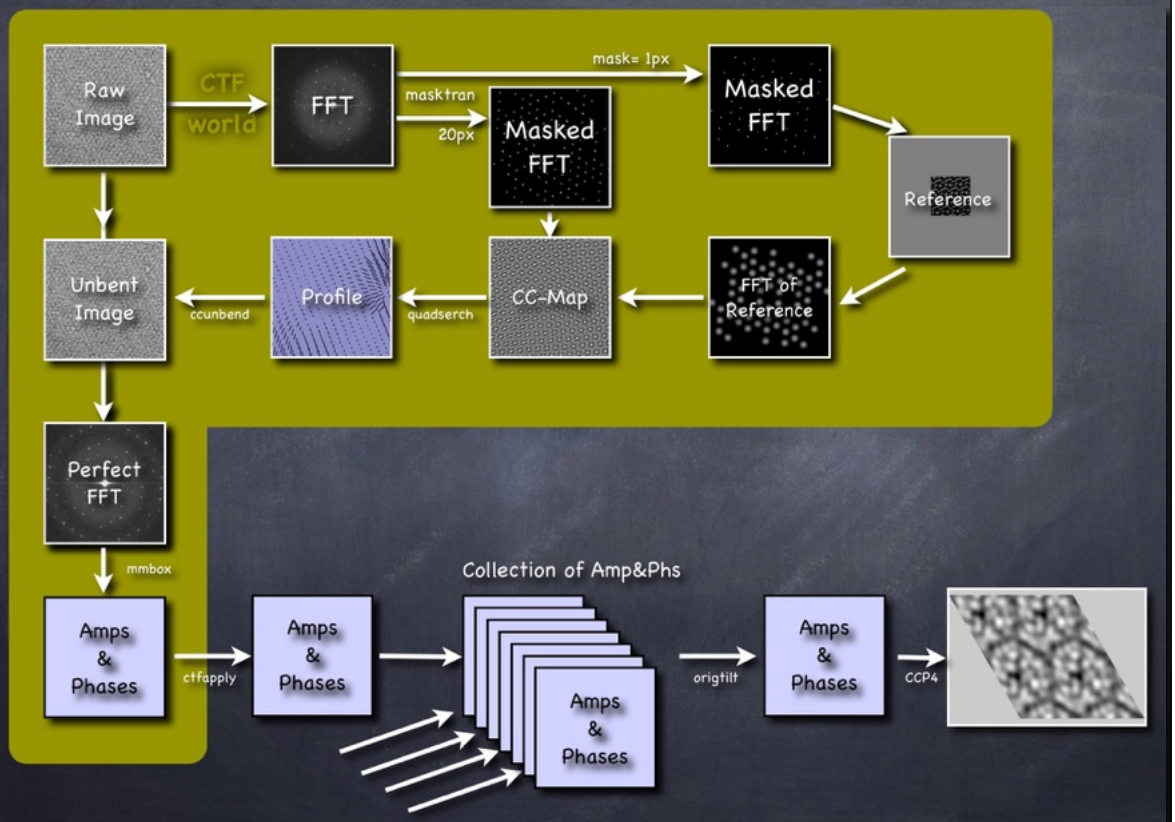
These lines show 10x exagerated vectors that indicate how specific areas of the image have to be "warped" to produce a perfect crystal image.




Fourier Transformation
of the
original 2D crystal image
(ZOOM)


Fourier Transformation
of the
unbent 2D crystal image
(ZOOM)

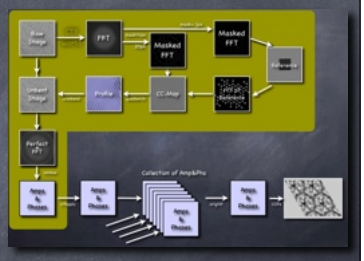
Algorithm Non-tilted



File Formats and Flow

mmbox => ctfapply => origtilt => avramphs => f2mtz

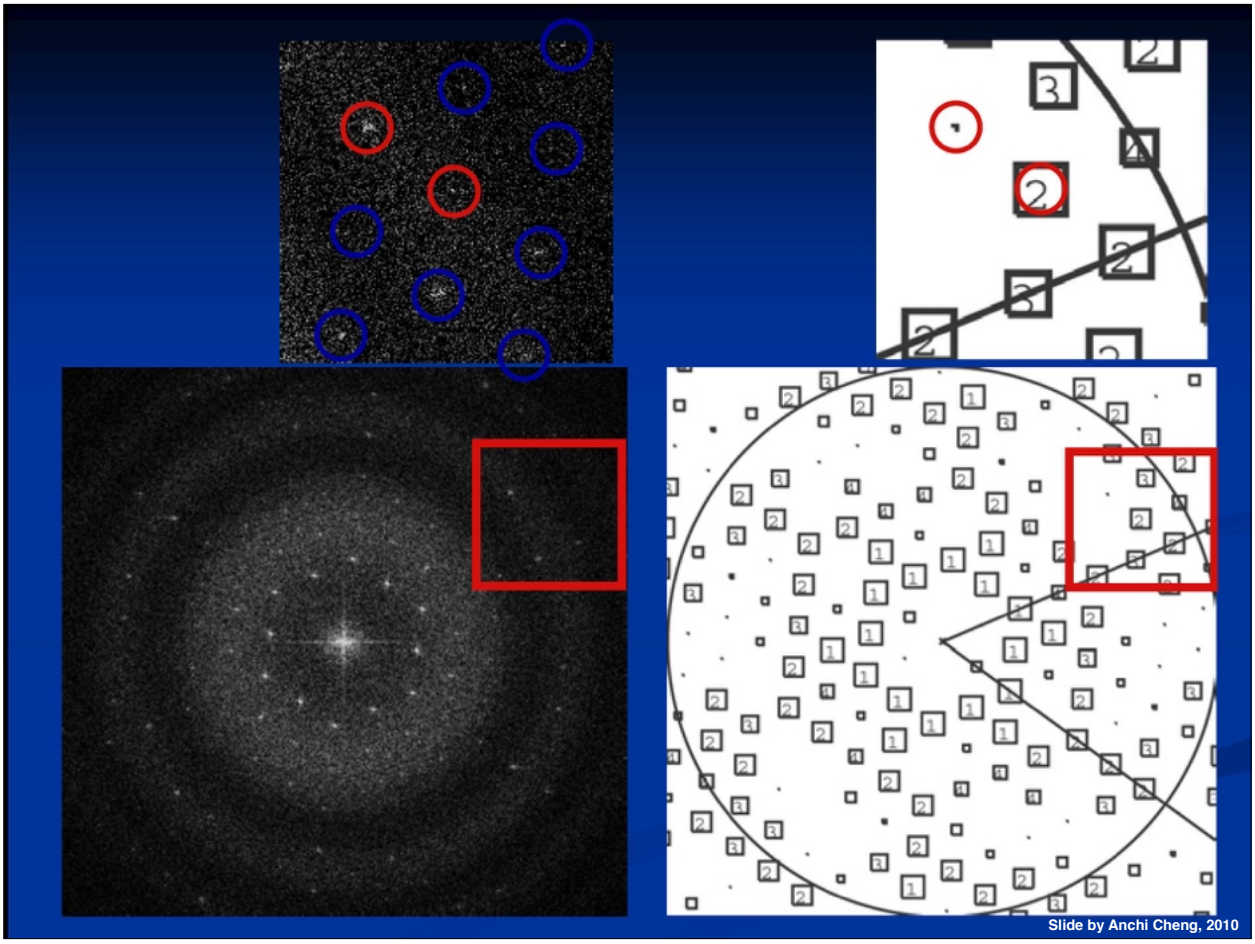
mmbox evaluates the Fourier transform of the unbent crystal image and produces a list of **Amplitudes**, **Phases**, and **Background Amplitudes**.



mmbox:

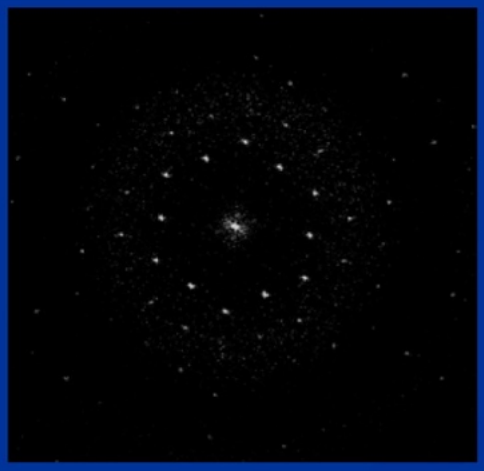
- takes FFTIR/cor${imagename}.fft.mrc
- generates APH/${imagename}.fou.nolimit.aph

```
Filename: APH/${imagename}.fou.nolimit.aph
1: Header
2:        H           K           AMP       PHS           IQ          BCK          CTF
Example:
    655201 gf06552, Unbend2, Mon Jun 16 00:33:23 CEST 2008
           0           1           0.0      60.9           9         72.2          0.0
           0           2         165.8     330.8           3         49.3          0.0
           0           3          17.1      52.3           8         39.3          0.0
           0           4         151.8     123.1           2         36.5          0.0
           0           5         411.8     127.5           2         50.9          0.0
```



Determine Amplitudes and Phases of Diffraction spots (MMBOXA)

- Fitting of the spots
- IQ values
- Phase gradient
- Evaluate results



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

.
.
.

Slide by Anchi Cheng, 2010

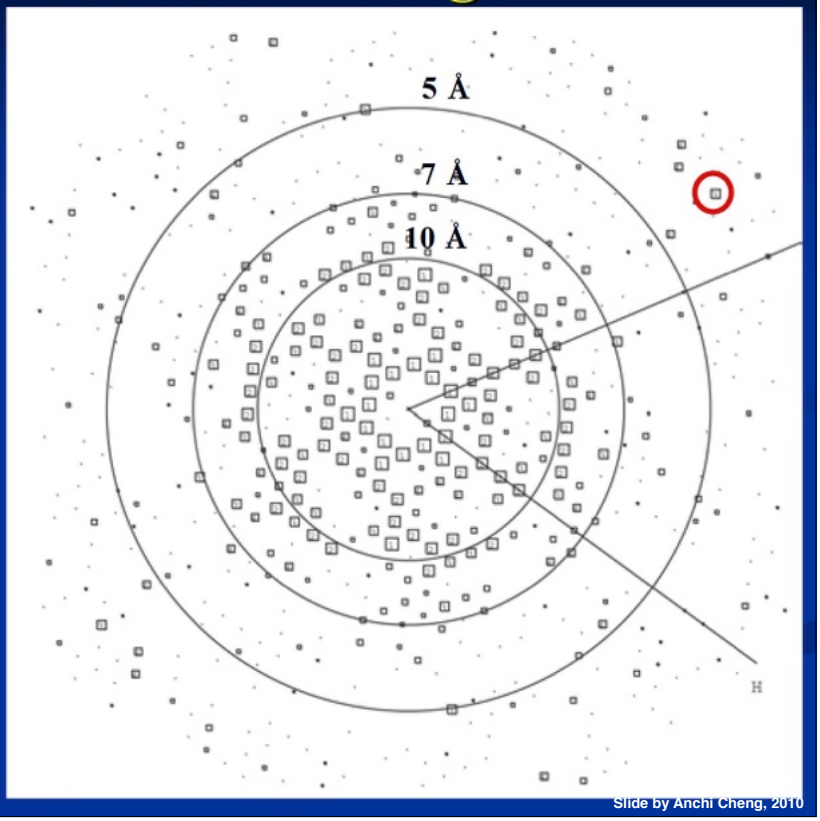
Resolution of an image

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

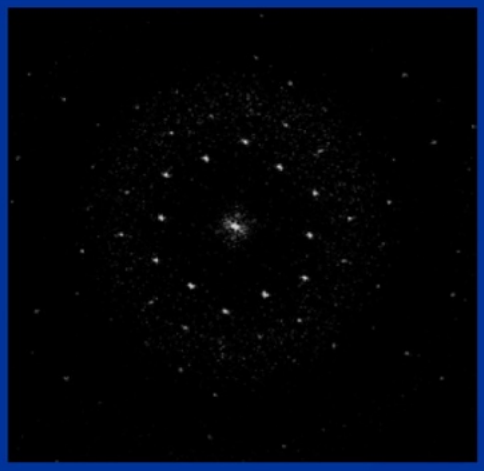
7	7	7	7	7
8	7	6	7	7
7	7	7	7	6
7	8	7	7	7
7	7	7	7	7



Slide by Anchi Cheng, 2010

Determine Amplitudes and Phases of Diffraction spots (MMBOXA)

- Fitting of the spots
- IQ values
- Phase gradient
- Evaluate results



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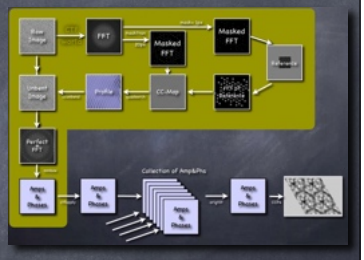
0	2	7924.5	44.6	1	148.5
0	3	1373.7	210.6	1	158.3
0	4	1411.4	228.4	1	124.3
0	5	710.5	206.4	2	106.3
0	6	1870.8	126.0	1	121.1

Slide by Anchi Cheng, 2010

File Formats and Flow

mmbox => ctfapply => origtilt => avramphs => f2mtz

mmbox evaluates the Fourier transform of the unbent crystal image and produces a list of **Amplitudes**, **Phases**, and **Background Amplitudes**.



mmbox:

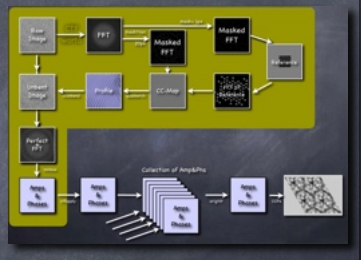
- takes FFTIR/cor${imagename}.fft.mrc
- generates APH/${imagename}.fou.nolimit.aph

```
Filename: APH/${imagename}.fou.nolimit.aph
1: Header
2:       H          K           AMP      PHS           IQ          BCK          CTF
Example:
    655201 gf06552, Unbend2, Mon Jun 16 00:33:23 CEST 2008
           0          1           0.0       60.9          9         72.2          0.0
           0          2         165.8      330.8          3         49.3          0.0
           0          3          17.1       52.3          8         39.3          0.0
           0          4         151.8      123.1          2         36.5          0.0
           0          5         411.8      127.5          2         50.9          0.0
```


File Formats and Flow

mmbox => **ctfapply** => origtilt => avramphs => f2mtz

ctfapply calculates the CTF correction factor for each reflection, and produces a CTF-corrected APH file, where only the phases are potentially "flipped".
This is one file for each image.



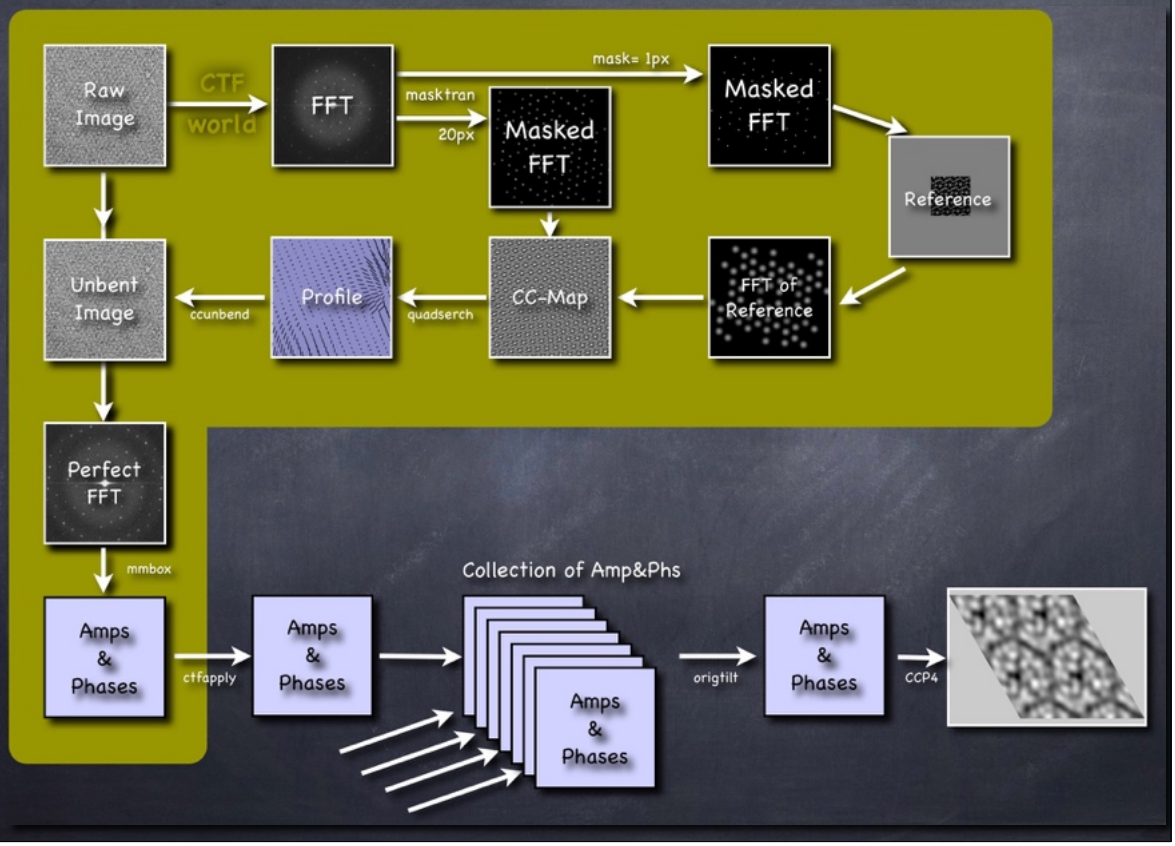
ctfapply:

- takes ${imagename}.fou.nolimit.aph
- generates ${imagename}.fou.ctf.nolimit.aph

```
Filename: ${imagename}.fou.ctf.nolimit.aph
1: Header
2:  H    K        AMP         PHS    IQ       BCK         CTF
                  AMP is not changed
                             PHS is already CTF-applied, possibly +180.
                                                         CTF lists the contrast.
                                                         Only phase (sign) was already applied.

Example:
    655201 gf06552, Thu Jul 3 22:49:19 PDT 2008
    0    1         0.0       240.9   9        72.2      -0.096
    0    2       165.8       150.8   3        49.3      -0.172
    0    3        17.1       232.3   8        39.3      -0.295
    0    4       151.8       303.1   2        36.5      -0.459
    0    5       411.8       307.5   2        50.9      -0.648
```

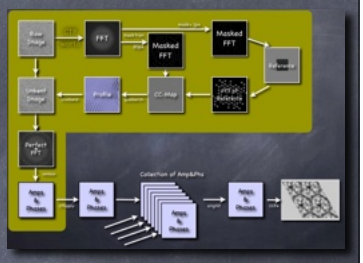
Algorithm Non-tilted



File Formats and Flow

mmbox => ctfapply => **origtilt** => avramphs => f2mtz

origtilt takes the several files from mmbox and combines them into one single large file merge.aph that contains AMP, PHS, original image number, and further data for each reflection.
This is one single file for the entire project.



origtilt:

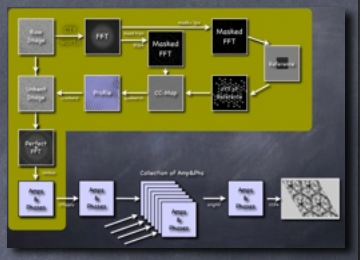
- takes several APH/${imagename}.fou.cor.aph
- generates APH/merge.aph, which now has for each spot potentially many entries

```
Filename: APH/merge.aph
1: Header
2:    H     K     Z*       AMP            PHS            NO     IQ  WEIGHT        BACK         CTF
                           AMP is the raw measured value, not yet changed
                                          PHS is CTF phase flipped, and has also the PhaseOrigin applied to it
Example:
      1001
      1     0  -0.0000     70.8670        -88.1100          65551 -6  0.078805     47.8734      -0.083
      1     0   0.0000     112.300        -21.6200           6543  6  0.192624     74.5000      -0.093
      1     0   0.0000     25.0594        -131.430          65551  8  0.027867     49.3105      -0.088
...
      2     0  -0.0000     120.801        -160.000           6557  4  0.192729     48.7613      -0.165
      2     0  -0.0000     156.540         169.480         665202 -2  0.276022     34.6150      -0.154
      2     0  -0.0000     202.253         170.080         655201 -3  0.375352     51.5399      -0.167
...
      2     0   0.0013     411.287        -178.300           7032  1  0.440457     50.2215      -0.383
      2     0   0.0013     313.198        -178.300           8032  2  0.307053     40.1020      -0.385
      2     0   0.0016     298.834        -161.900           5685  2  0.360523     61.4043      -0.198
```

File Formats and Flow

mmbox => ctfapply => origtilt => **avramphs** => f2mtz

avramphs takes the merge.aph file that contains for each reflection several measurements, and averages these into one AMPlitude and PHAse value for each reflection.
This is one single file for the entire project.



avramphs:

- uses zminmax = -0.025...0.025, to make sure only non-tilted data participate.
- takes APH/merge.aph
- generates APH/avrg2D.hkl

```
Filename: APH/avrg2D.hkl
1: merging number
2:   H     K     L        AMP            PHS            FOM
                       AMP is an average of several spots, and the values are now amplitude-CTF corrected (without inverting the sign).
                                PHS is an average of several spots, which are already CTF and phaseorigin corrected
                                                 FOM is between 0 and 100 and quantifies the reliability of the spots.

Example:
      1001
     1     0     0     196.833        -64.0629        96.9797
     1     1     0     1018.19        -6.10076        99.9556
     1     2     0     162.905        -177.142        98.7745
     1     3     0     262.806        -179.891        99.5678
...
     2     0     0     453.627        -177.303        99.8751
     2     1     0     109.832         4.30570        94.4169
     2     2     0     1278.94         177.975        99.9585
```

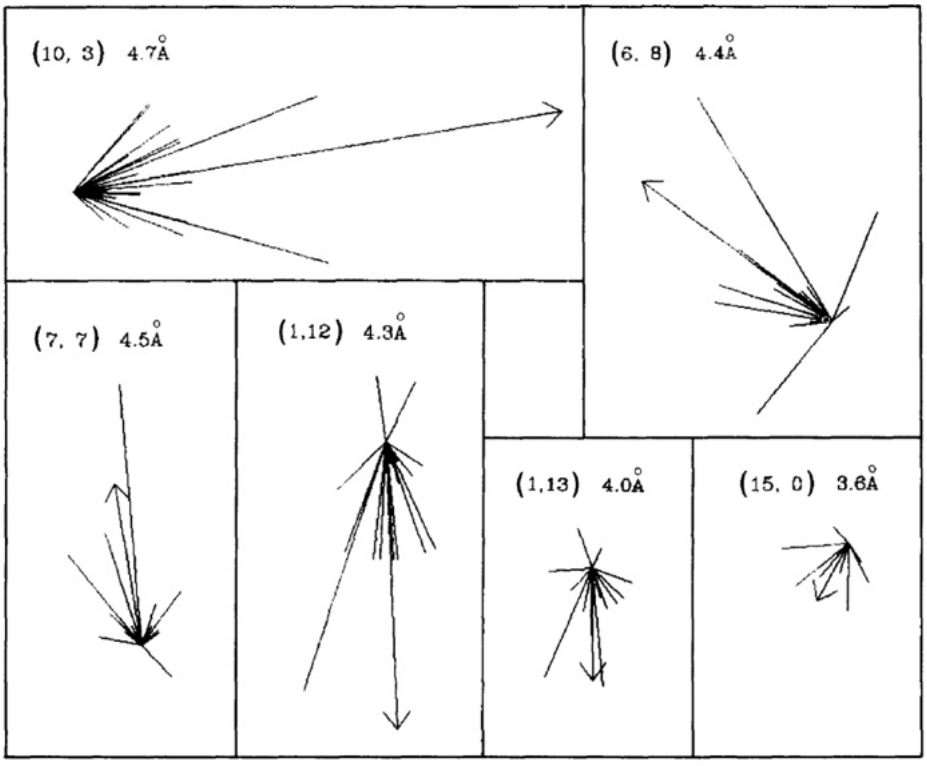



Fig. 7. Graphical comparison of all phases determined for six spots with resolution beyond 4.7 Å. Phases are plotted as vectors on a polar diagram with the length of each vector being proportional to $1/IQ^2$. Thus the strongest spots show up as longer vectors. The result of the summation of all the vectors is also shown as a vector, but this is plotted at 1/4 of its true length. The direction of this vector is our best estimate of the phase of the Fourier component in the structure, and its length can be used to provide an estimate of the error.

Averaging of data from different images in the MRC/2dx software is done in Fourier space by dealing with each reflection individually:

Amplitudes and Phases are averaged, taking $1/IQ^2$ as weight.

On the left, the Phase averaging is shown graphically.

```
Filename: APH/merge.aph
1: Header
2:    H     K     Z*        AMP            PHS            NO    IQ  WEIGHT        BACK         CTF
                             AMP is the raw measured value, not yet changed
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Example:
      1001
      1     0  -0.0000     70.8670        -88.1100          65551 -6  0.078805     47.8734      -0.083
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      2     0  -0.0000     202.253         170.080         655201 -3  0.375352     51.5399      -0.167
...
      2     0   0.0013     411.287        -178.300           7032  1  0.440457     50.2215      -0.383
      2     0   0.0013     313.198        -178.300           8032  2  0.307053     40.1020      -0.385
      2     0   0.0016     298.834        -161.900           5685  2  0.360523     61.4043      -0.198
```
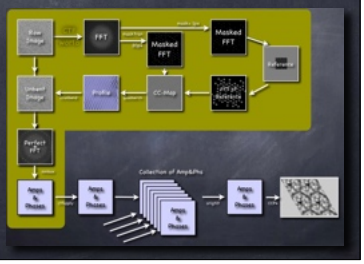

avramphs:

```
Filename: APH/avrg2D.hkl
1: merging number
2:   H     K     L        AMP            PHS            FOM
                          AMP is an average of several spots, and the values are now amplitude-CTF corrected (without inverting the sign).
                                  PHS is an average of several spots, which are already CTF and phaseorigin corrected
                                                FOM is between 0 and 100 and quantifies the reliability of the spots.

Example:
      1001
     1     0     0     196.833        -64.0629         96.9797
     1     1     0     1018.19        -6.10076         99.9556
     1     2     0     162.905        -177.142         98.7745
     1     3     0     262.806        -179.891         99.5678
...
     2     0     0     453.627        -177.303         99.8751
     2     1     0     109.832         4.30570         94.4169
     2     2     0     1278.94         177.975         99.9585
```



File Formats and Flow

mmbox => ctfapply => origtilt => avramphs => **f2mtz**

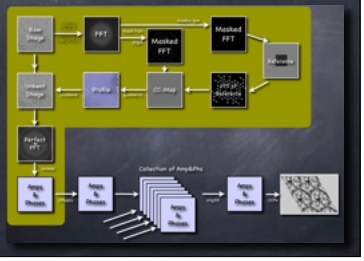
centric and **hklsym** apply boundary conditions to reflection values. Centric enforces for certain symmetries that reflections of non-tilted crystals must have phases of either 0º or 180º. Hklsym avearges symmetry-related reflections.

f2mtz transforms the AMP&PHS file into a binary MTZ file.

f2mtz:

- takes APH/sym2D.hkl
- generates merge2D.mtz

```
Filename: merge2D.mtz
SYMMETRY ${CCP4_SYM}
LABOUT H K L F PHI FOM
CTYPOUT H H H F P W
```



File Formats and Flow

mmbox => ctfapply => origtilt => avramphs => **f2mtz**

cent … **hklsym** … reflection values.
Cen… for c… …on-ti…
crystals must have phases of either 0º or 180º.
Hklsym avearges symmetry-related reflections.

f2mtz transforms the AMP&PHS file into a binary MTZ file.

f2mtz:

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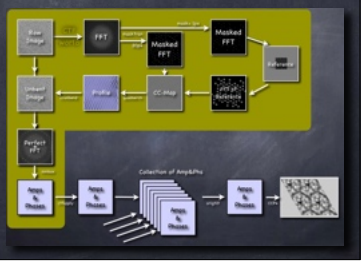
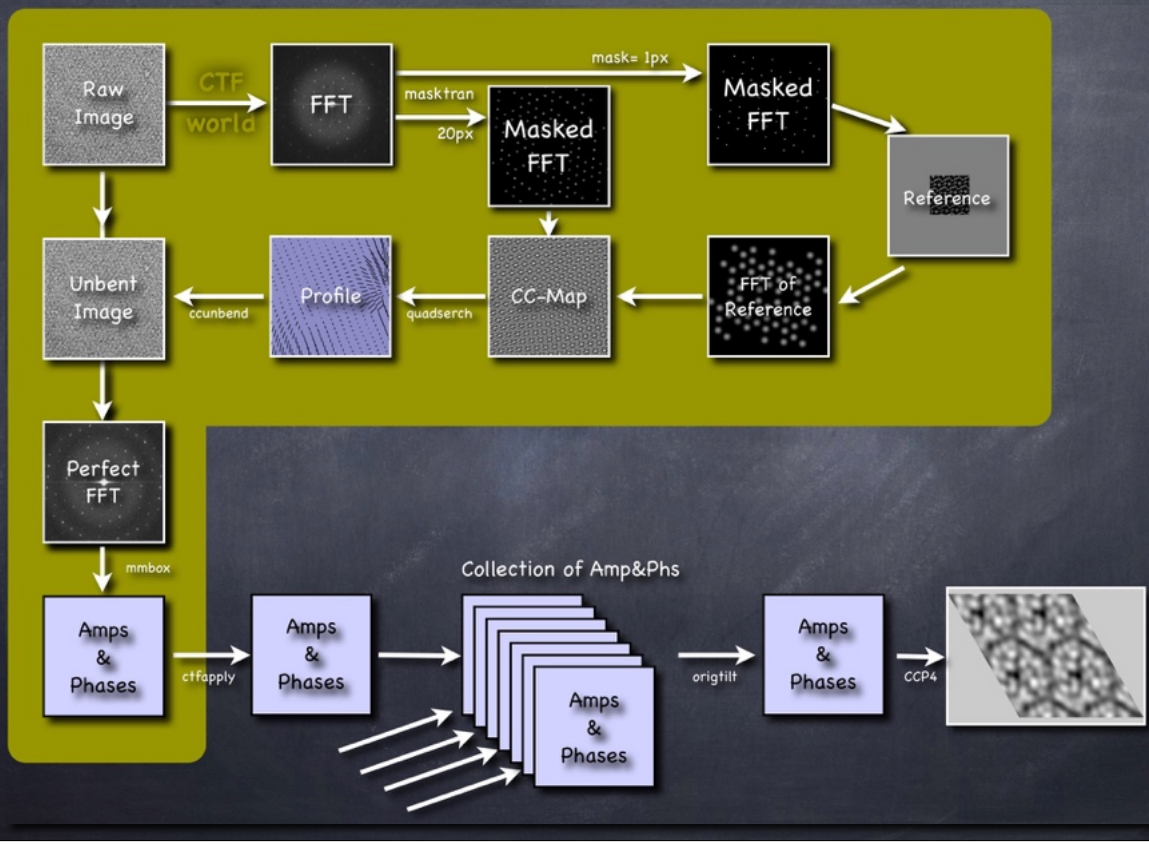
```
Filename: merge2D.mtz
SYMMETRY ${CCP4_SYM}
LABOUT H K L F PHI FOM
CTYPOUT H H H F P W
```

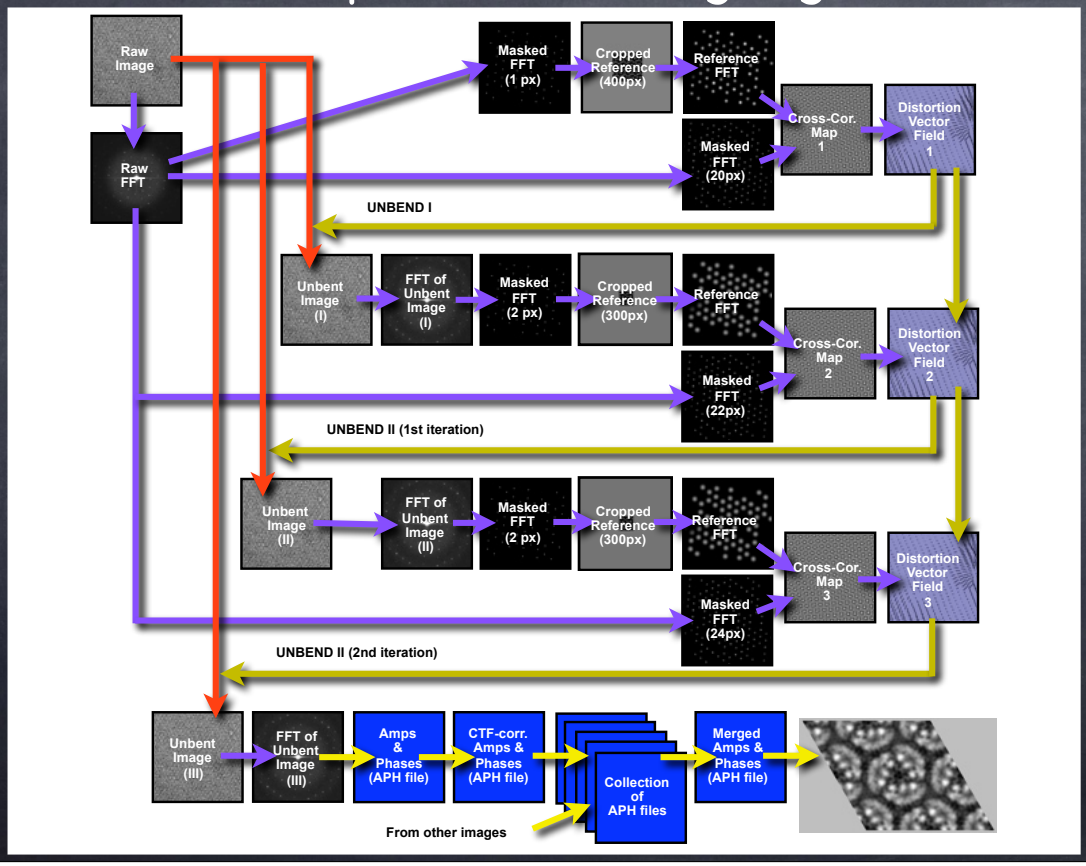


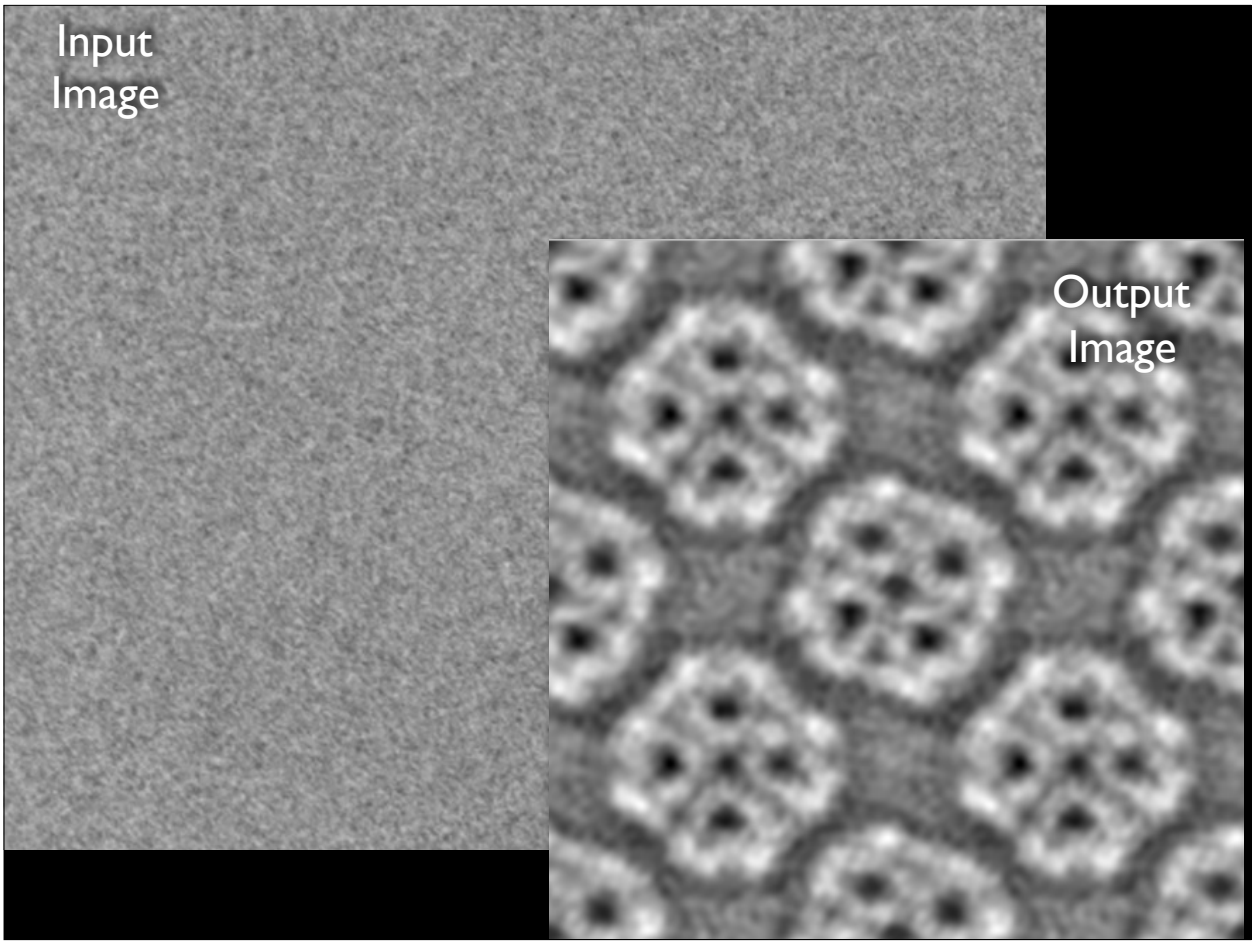
File Workflow is described on: 2dx.org

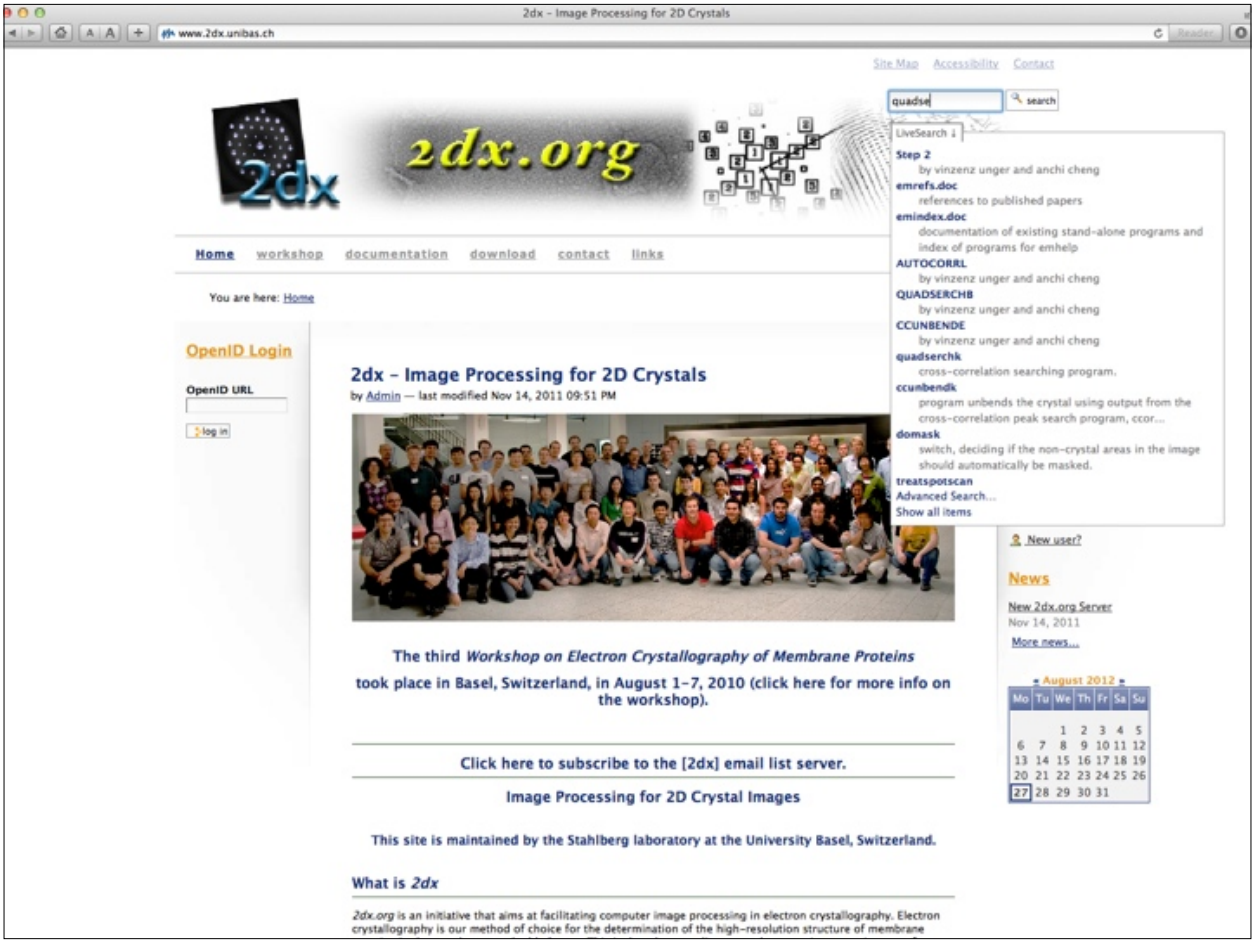
Algorithm Non-tilted



More Complete Unbending Algorithm






2dx - Image Processing for 2D Crystals
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quadserchk
cross-correlation searching program.
ccunbendk
program unbends the crystal using output from the cross-correlation peak search program, ccor...
domask
switch, deciding if the non-crystal areas in the image should automatically be masked.
treatspotscan
Advanced Search...
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log in
New user?
2dx - Image Processing for 2D Crystals
by Admin — last modified Nov 14, 2011 09:51 PM
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6 7 8 9 10 11 12
13 14 15 16 17 18 19
20 21 22 23 24 25 26
27 28 29 30 31
The third Workshop on Electron Crystallography of Membrane Proteins
took place in Basel, Switzerland, in August 1-7, 2010 (click here for more info on the workshop).
Click here to subscribe to the [2dx] email list server.
Image Processing for 2D Crystal Images
This site is maintained by the Stahlberg laboratory at the University Basel, Switzerland.
What is 2dx
2dx.org is an initiative that aims at facilitating computer image processing in electron crystallography. Electron crystallography is our method of choice for the determination of the high-resolution structure of membrane


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Processing an individual image
The Full-Screen Image Navigator
Refining a lattice
Refining the CTF
Spot Selection
Tilt Geometry Definition
ML algorithm applied to 2d crystals
Data Flow
Processing in 2dx_image of ONE image
Merging in 2dx_merge in 2D
Merging in 2dx_merge in 3D
Data Flow
by Admin — last modified Nov 14, 2011 11:40 AM
These pages describe the flow of data through the different programs. File names, formats and contents is listed. 2dx and the underlying MRC programs process and then evaluate images, and extract values for amplitudes and phases of Fourier spots. These are then handed over from one program to the next one, where each program does something to those values (e.g. correcting for the CTF, or merging several values together). Each program thereby unfortunately needs a different type of data that is therefore often in a different format, and the whole thing quickly gets very confusing. Most of these files are text files (ASCII), though, so that you can always open them with a text browser (vi, edit, SimpleText, or even Word (but don't save it back as "Word" file.doc file). We have here compiled a list of all the programs that are called in sequential order, and have listed the input and output files for these programs. For the output files we have then included a short description of the file format, followed by an example of how that should look like. This is only for reference purposes, in case somebody tries to follow the flow of data from one program to the next one.
Processing in 2dx_image of ONE image
This processing deals only with one single image and is therefore two-dimensional processing.
Read More...
Merging in 2dx_merge in 2D
This processing merges several images into one dataset. This here describes the merging of several non-tilted images into a two-dimensional dataset.
Read More...
Merging in 2dx_merge in 3D
This processing merges several images into one dataset. This here describes the merging of several tilted images into a three-dimensional dataset.
Read More...