TOMOCTF: A package for CTF determination and correction in electron cryotomography

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The package TOMOCTF contains the programs for CTF determination and correction in electron cryotomography (cryoET) as described in Ultramicroscopy 106:587-596, 2006.

The approach to CTF detection and defocus determination is based on strip-based periodogram averaging, extended throughout the tilt series to overcome the low contrast conditions found in cryoET, together with a spline-based strategy for background subtraction. The method of CTF correction deals with the defocus gradient in images of tilted specimens by decomposing the global restoration problem into multiple local spatially-invariant problems.

The package is structured into several programs. TOMOPS and TOMOCTFFIND are in charge of the determination of the average defocus (or, equivalently, CTF) from an input tilt series. The program CTFcorrect corrects individual images, from either tilted or untilted specimens, for the effects of the CTF. A new program, CTFphaseflipstack, has been added for phase correction of stacks via IMOD. An auxiliary program, TOMOCTFgrad, aims at determining the geometry of the acquisition-&-processing system so that a proper CTF correction can be carried out. Finally, another auxiliary program, TOMOCTFsim, helps simulate defocus gradients and compute the resolution ranges where the CTF is affecting.

Table of Contents

1 Installation

- Uncompress the ZIP file (`tomoctf_***.zip`).
- You will find these directories:
  - bin, where the executables are:
    `tomops.exe, tomoctffind.exe, CTFcorrect.exe, CTFphaseflipstack.exe, tomoctfgrad.exe, tomoctfsim.exe`
  - com, where examples of scripts are.
  - doc, where this documentation is.
  - examples, where some examples can be found.
- Set up your `PATH` environment variable to have direct access to these binaries.

Tested machines

So far, the package has been built and tested on a number of platforms. But we only provide binaries for these:

Linux (x86, 64 bits)
Intel-based Macs under Mac OS X (Snow Leopard).

In case of problems in these platforms or requests of the programs for other platforms, contact us and we'll try our best to help you.

2 Workflow

The very first step that any user should do is to determine the handedness of the system (hardware -tilt stage of the EM- and software -preprocessing and alignment programs-). It is mandatory that the handedness be compatible with the convention in TOMOCTF and IMOD. See Section 6 for details to determine it and how to deal with it in case the convention is not followed. This step is carried out only once. If the handedness of the system is known, this step is not required.

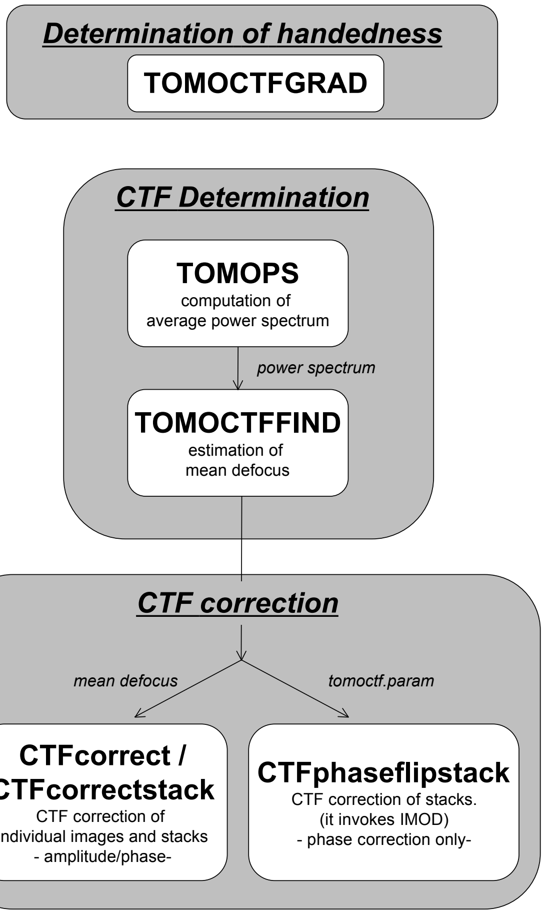

Figure 1: Sketch of the sequence of steps to follow for CTF determination and correction.

Determination of the CTF affecting a tilt-series is carried out by the sequence of programs TOMOPS and TOMOCTFFIND. The first one estimates the average power spectrum at the untilted plane. This information is then used by TOMOCTFFIND to estimate the mean defocus (i.e. that at the untilted plane). TOMOCTFFIND also produces several output files (CTFPROFILE, CTFPROFILE.ps) intended for users to check out the reliability of the estimated value. It also produces a file with the parameters of the CTF (tomoctf.param).

Correction for the CTF is to be applied to aligned tilt-series with the tilt-axis running along the Y-axis. The program CTFcorrect corrects for the CTF (Amplitudes/Phases) on individual images. In combination with the script CTFcorrectstack.csh, it can be used to correct a tilt-series. Alternatively, the speed of the program ctfphaseflip from IMOD (phase correction only) can be exploited. The program CTFphaseflipstack receives the file tomoctf.param generated by TOMOCTFFIND, and executes that IMOD program in parallel.

3 CTF Determination

CTF determination is carried out by strip-based periodogram averaging extended throughout the tilt series to compute an average power spectrum in the untilted plane, followed by a CTF fitting with an embedded spline-based strategy for background subtraction. The program TOMOPS performs the computation of the average power spectrum, that is then stored in an output MRC file, MODE 2. The fitting is then accomplished by the program TOMOCTFFIND which receives the output of TOMOPS as an input.

3.1 TOMOPS

TOMOPS estimates the average power spectrum in the untilted plane for an aligned tilt series in electron tomography. The tilt series must be either a set of projection images (as a result, for example, from the alignment with SPIDER) or a stack of projection images (as a result, for example, from the alignment with IMOD). The tilt axis must be located running along the Y-axis. The image format is MRC.

3.1.1 Methodology

This program estimates the average power spectrum in the tilt series by means of strip-based periodogram averaging. The method consists of the following steps:

1. Through the tilt series, it computes strips that are under similar CTF. The user has to specify the value of defocus difference (ΔD) under which the CTF can be assumed the same or with negligible difference.

2. It applies a tiling process over all the strips. The tiles are overlapping in order to have as many as possible. The overlapping area is half the tile size. For every tile, its spectrum is computed.

3. The spectrum in the untilted plane is estimated as the average of the spectra of all the tiles in the tilt series.

3.1.2 Parameters

On execution, the program asks the user for the following parameters, interactively. However, it is recommended the use of scripts to edit the parameters and launch the program. An example of script is provided below.

CARD 1: MODE.
CARD 2: Input file name for tilt series images.
CARD 3: Input file name for tilt angles list.
CARD 4: Output power spectrum file name.
CARD 5: Defocus Difference (ΔD) for strip extraction [Å]
CARD 6: XMAG, DStep[μm]
CARD 7: Tile size [pixels]

Description of the parameters

1. MODE: Must be 0 or 1.
   0 - the tilt series is given as a stack.
   1 - the tilt series is given as a set of images.

2. Input file name for tilt series images.
   If MODE=0, it must be the name of the stack (MRC format)
   If MODE=1, it must be the name of an ASCII file containing the file names of the projection images. There must be one file name per line.

3. Input file name for tilt angles list.
   It must be an ASCII file containing the tilt angles of the images in the tilt series. There must be one tilt angle name per line. The order of the tilt angles in this file must correspond to the order of the images in the input stack (if MODE 0) or input list (if MODE 1).

4. Output power spectrum file name.
   The output file where the power spectrum is stored.

5. Defocus Difference (ΔD) for strip extraction.
   This is the value of height under which negligible difference in CTF (defocus) is assumed. It is used for the stripping process. The larger this value is, the wider the strips are through the tilt series, and consequently the more tiles are. The units are Å.

6. Imaging parameters: XMAG, DStep

   XMAG: Magnification of original image

   DStep: Pixel size on scanner in microns

7. Tile size
   The program divides the strips into square tiles to calculate the average power spectrum. Tiles with a significatly higher or lower variance are excluded; these are parts of the images which are unlikely to contain useful information (beam edge, edges due to alignment of the tilt series, etc. IMPORTANT: Tile size must have even pixel dimensions.

3.1.3 Output

- Output power spectrum file.
  The program stores the average power spectrum in a MRC image for subsequent CTF fitting with the program TOMOCTFFIND.

3.1.4 Examples of UNIX scripts

Example of UNIX command file (1):

```
#!/bin/csh -f
#
# tomops.csh:  for a tilt-series in a stack
#
time tomops.exe << eof
0
stack.mrc
tiltangles.tlt
power.mrc
2000
24500,24
128
eof
#
```

Example of UNIX command file (2):

```
#!/bin/csh -f
#
# tomops.csh:  for a tilt-series in a set of images
#
time tomops.exe << eof
1
stack.lst
tiltangles.tlt
power.mrc
2000
24500,24
128
eof
#
```

3.1.5 Advices, tricks and tips

- The key parameters for TOMOPS are ΔD and the tile size.
  - ΔD has a direct influence on the width strip and the amount of averaging in power spectrum estimation. ΔD should have values larger than 1000-2000 Å to guarantee reliable estimates, as shown in the article in Ultramicroscopy. Setting ΔD to the approximate thickness of the sample is usually appropriate.
  - Typical values for tile size are in the range [100,150].
- For successful CTF detection, TOMOPS should be applied to the full-sized images. For huge images (4K$\times$4K or more) one binning factor might also be tolerable.
- The use of raw (i.e. unaligned) tilt-series to the couple TOMOPS/TOMOCTFFIND is possible, provided that the experimental tilt axis is around the Y axis (let's say $\pm$ 5 degrees around the Y axis).
- Sometimes, rather than the whole (aligned or not) stack, it is convenient to use a subset of the original raw low-tilt images (e.g. in the range $\pm$ 10 degrees) as an input to TOMOPS/TOMOCTFFIND. We have very good experience with this approach, in particular with data coming from Direct Electron Detectors (e.g. FEI Falcon, Gatan K2).
  Extraction of such a subset can be done with newstack command from IMOD. TOMOPS would require a new tiltangle file with entries for the images in that subset.

3.2 TOMOCTFFIND

TOMOCTFFIND determines the defocus in the untilted plane for an aligned tilt series of electron tomography. As an input, it receives the average power spectrum at the untilted plane that will have been previously computed by strip-based periodogram averaging implemented in TOMOPS.

3.2.1 Methodology

This program determines the defocus at the untilted plane of a tilt series by maximizing a correlation coefficient between a background-substracted average power spectrum and a square theoretical CTF. The method consists of the following steps:

1. The averaged power spectrum at the untilted plane is received from the TOMOPS program. As astigmatism is assumed negligible, rotational averaging is applied to further smooth the spectrum. As a consequence, the problem of CTF determination is reduced to 1D.

2. CTF fitting is carried out by optimizing a correlation coefficient between a background-substracted power spectrum and a square theoretical CTF. Background substraction is embedded in the optimization process itself.
   The background of the spectrum is estimated by least-squares cubic spline fitting to the set of samples of the power spectrum located at the positions of the zeros of the theoretical CTF.
   The correlation coefficient is computed in the restricted resolution range given by (see Figure 1):

   (a) the absolute resolution range provided by the user: [ResMin,ResMax]

   (b) and a low resolution limit that is located between the first peak and the first zero of the theoretical CTF. This limit is given by the user through the parameter CTFMin.

   The final low resolution limit is given by the maximum between ResMin and the resolution specified by CTFMin.

3. The defocus determination is refined by a Powell's optimization method.

3.2.2 Parameters

On execution, the program asks the user for the following parameters, interactively. However, it is recommended the use of scripts to edit the parameters and launch the program. An example of script is provided below.

CARD 1:	Input power spectrum coming from TOMOPS
CARD 2:	Output diagnostic file name
CARD 3:	CS[mm], HT[kV], AmpCnst, XMAG, DStep[μm]
CARD 4:	CTFMin[0,1], ResMin[Å], ResMax[Å]
CARD 5:	dFMin[Å], dFMax[Å], FStep[Å]

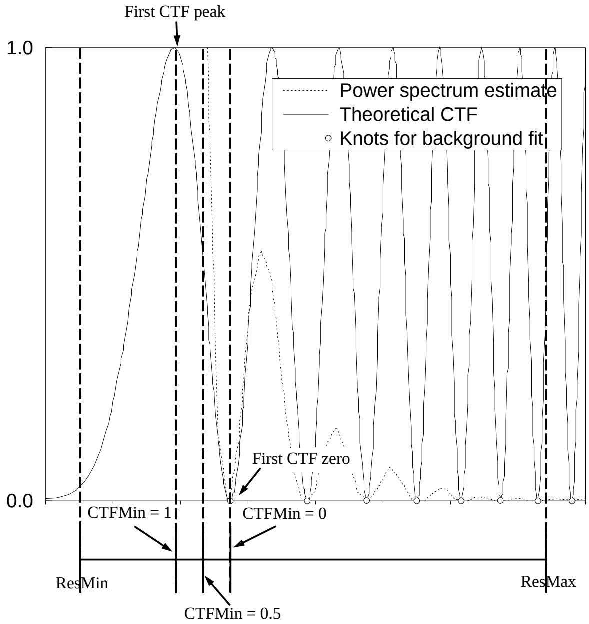

Figure 2: Resolution range for CTF fitting. The interval [ResMin,ResMax] define an absolute range. CTFMin imposes another low resolution limit. If CTFMin=0, the limit is located at the resolution of the firt CTF zero. If CTFMin=1, the limit is at the resolution of the firt CTF peak. Values in-between represent proportional locations between these two extreme values.

Description of the parameters

1. Input power spectrum coming from TOMOPS
   Average power spectrum computed with TOMOPS, in MRC format, MODE 2.

2. Output diagnostic file name
   The output diagnostic image file to check the result of the fitting shows the background-substracted average power spectrum at the untilted plane in one half, and the fitted squared CTF in the other half. The two halfs should agree very well for a successful fit.

3. Imaging parameters: CS, HT, AmpCnst, XMAG, DStep

   CS: Spherical aberration coefficient of the objective in mm.

   HT: Electron beam voltage in kV.

   AmpCnst: Amount of amplitude contrast (fraction).
   For ice images 0.07, for negative stain about 0.15.

   XMAG: Magnification of original image.

   DStep: Pixel size on scanner in microns.

4. Resolution parameters: CTFMin, ResMin, ResMax

   CTFMin: Defines a low resolution limit for the fitting. This parameter represents a location between the first peak and the first zero of the theoretical CTF. This parameter must have a real value in the range [0.0,1.0]:

   - 0 represents the location of the first zero of the CTF.
   - 1 represents the location of the first peak of the CTF.
   - values in-between represent proportional locations between these two extreme values.

   ResMin: Absolute low resolution end of data to be fitted.

   ResMaX: Absolute high resolution end of data to be fitted.

5. Defocus search parameters: dFMin, dFMax, FStep

   dFMin: Starting defocus value for grid search in Å. Positive values represent an underfocus. The program performs a systematic grid search of defocus values and astigmatism before fitting a CTF to machine precision.

   dFMax: End defocus value for grid search in Å.

   FStep: Step width for grid search in Å.

3.2.3 Output

- Fitted Defocus.
  The program shows the defocus value at the untilted plane in the standard output (console or a file where the output is redirected).

- Output diagnostic file.
  The program generates an image in MRC format to check the result of the fitting. On the right half, it shows the scaled background-substracted average power spectrum at the untilted plane, and on the left half the fitted squared CTF. The two halfs should agree very well for a successful fit.

- Output text file: CTFPROFILE
  The program writes an ASCII table with the data of the profile of the estimated power spectrum and the squared CTF fitted. The name of this output file is: CTFPROFILE
  The content of this file is structured into 6 columns

  1. Index of Bin
  2. Resolution [1/Å]
  3. Estimated scaled background-substrated power spectrum.
  4. Square CTF fitted
  5. Rotational profile of the average power spectrum at the untilted plane.
  6. Background estimate corresponding to the CTF found.

  This table can be dealt with many graphical software.

- Output Postscript file: CTFPROFILE.ps
  The program generates a Postscript file with the CTF profiles (i.e. columns #3 and #4 from the previous table), provided that the program `gnuplot` is installed in the system and it is in the shell's path. It is not in the path, it can be set up by means of the environment variable `GNUPLOTPATH` The name of this postscript file is CTFPROFILE.ps If gnuplot is not available or reachable, the postscript file is not generated.

- Output Parameter file: tomoctf.param
  The program produces an ASCII file with the main parameters required for CTF correction. In general, this file uses the keywords as defined by IMOD for the CTF parameters (`http://bio3d.colorado.edu/imod/doc/man/ctfphaseflip.html`).

  The following keywords and parameters are stored in this file:

SphericalAberration	Value of Cs (in mm).
Voltage	Voltage (in kV).
AmplitudeContrast	The fraction of amplitude contrast.
PixelSize	Pixel size in nm.
defFn	Defocus Found by TOMOCTFFIND in nm.

  Note that the pixel size is given in nanometers, and it refers to the pixel size of the tilt-series (or subset of images) used for CTF determination. Bear this in mind in case the CTF correction is planned for a binned tilt-series.

  Note that the keyword 'defFn' is used in 'tomoctf.param' to store the defocus found by TOMOCTFFIND. This does not follow the convention used by IMOD, so this file could not be entered directly through IMOD unless some trick is used (see below, description of program CTFphaseflipstack).

3.2.4 Example of UNIX scripts

Example of UNIX command file:

```
#!/bin/csh -f
#
# tomoctffind.csh
#
setenv GNUPLOTPATH /usr/local/bin
time tomoctffind.exe << eof
power.mrc
diagnostic.mrc
2.0,300.0,0.07,24500,24
0.5,10000.0,22
100000.0,300000.0,1000.0
eof
mv CTFPROFILE CTFprof.txt
mv CTFPROFILE.ps CTFprof.ps
mv tomoctf.param mytomoctf.param
#
```

3.2.5 Advices, tricks and tips

The key parameters for TOMOCTFFIND are those related to the resolution range where the fitting is focused (CTFMin, ResMin, ResMax), and the range of defocus (dFMin,dFMax) to test. Here, some advices are provided.

- CTFMin sets up a low resolution limit for every theoretical CTF tested. It should have values in the range [0,1] to specify a spatial frequency located between the first peak and the first zero of the CTF (see Figure and explanation above).

  Background fitting works well beyond the position of the first CTF zero. The background fitting in the region between the first peak and the first zero of the CTF is not 100% reliable, specially in the areas near the peak.

  In order to avoid contributions from these not-so-reliable areas in the CTF fitting, CTFMin should have values around [0,0.5]. These values usually work very well with a wide range of defoci.

  In some particular cases, when data is acquired with extremely low underfocus and there are just a few CTF zeros in the spectrum, it might be useful to set up CTFMin with higher values (i.e., around [0.5,1.0] to avoid local minima in the CTF fitting.

- ResMin and ResMax define an absolute resolution range where the correlation function is computed for fitting. ResMin will be 'overwritten' by CTFMin when the latter is greater. ResMax represents the maximum resolution considered in the fitting. Typically, ResMax should have values around 80%-90% of the Nyquist frequency to discard the high resolution region where the noise is predominant.

- dFMin and dFMax define the range of defocus to test in the CTF fitting. The correlation function has a Gaussian-like shape around the global minimum (see the article in Ultramicroscopy). However, at locations very far away from the global minimum (say, farther than 10-20μm), local minima may arise. To avoid these local minima, the range of defocus given by dFMin and dFMax should have plausible values, for instance 10μm around the nominal defocus used in the acquisition.

  As the defocus found is finally refined by means of Powell's minimization, valid values for the parameter Fstep should be around 500-1000 Å.

- Don't be blindly confident on TOMOCTFFIND. You should always take a look at the output diagnostic image as well as the output profiles, to be sure that the fitting really worked well. Use different sets of parameters (CTFMin, ResMax, dfMin, dfMax), and compare the results, to confirm the validity of the defocus found.

4 CTF Correction

There are two ways to perform CTF correction within TOMOCTF. Both of them require that the images or stack to be corrected be aligned and with the tilt axis running along the Y axis.

- The first one is the originally shipped with the package and it works exactly as described in our article published in Ultramicroscopy 106:587-596, 2006. Here, CTF correction (Amplitude/Phase) is carried out by a single program, CTFcorrect, that corrects single individual images, from either tilted or untilted specimens. Correction of stacked tilt series requires previous de-stacking, correction of individual images, and re-stacking to produce the new corrected stack. A script is provided below to correct stacked tilt series. A program to directly correct tilt-series is planned for the next version of the package.

- The second one consists of using the program ctfphaseflip available in IMOD / eTomo (`http://bio3d.colorado.edu/imod/doc/man/ctfphaseflip.html`). Here the CTF correction (just phase; no amplitude correction is available) is only performed on a number of strips (typically a number resulting from the division of the X dimension of the images by 20). The final values of the pixels are determined by interpolation of the two nearest neighbour corrected strips. A description of this approach is available in the work by Xiong et al. J. Struct. Biol. 168:378–387, 2009. This approach yields an approximation of the corrected tilt-series, but it is much faster than ours. Furthermore, the program ctfphaseflip may be convenient because it directly deals with tilt-series, not individual images. For that reason, we felt it would be useful to link TOMOCTF with ctfphaseflip.

In the following, these two ways to perform CTF correction and the corresponding programs are described.

4.1 CTFcorrect

CTFcorrect corrects an image from an electron tomography tilt series for the effects of the CTF. Phase flipping and amplitude correction by a Wiener-like filter are available. The program corrects images of tilted and untilted specimens. In the tilted case, the tilt axis must be located running along the Y-axis (i.e. the convention used by IMOD). The image format is MRC, MODE 2 (float).

4.1.1 Methodology

This program corrects images of tilted specimens for the effects of the CTF by exploting a similar concept of strip as used in TOMOPS. Around any x-line of the image, a strip can be extracted where a single constant defocus values can be considered. CTF correction for a strip can be then carried out assuming a single CTF value. Essentially, this approach decomposes the global spatially-variant restoration problem into multiple local spatially-invariant problems that can be solved by conventional CTF correction. The defocus at the untilted plane will have been computed by TOMOCTFFIND.

The method consists of the following steps:

For each x-line:

1. Extract a strip around this x-line of the original image. The user has to specify the value of defocus difference (ΔD) to determine the strip width.
2. Compute the Fourier transform of the strip.
3. Correct for the CTF assuming a single defocus. The defocus for the specific x-line is computed from the defocus at the untilted plane determined by TOMOCTFFIND, and from the tilt angle and pixel size.
4. Compute the inverse Fourier transform.
5. Extract the corrected x-line from the corrected strip and store it into the x-line of the output corrected image.

Step 3 is the conventional CTF correction. Amplitude and phase correction can be applied. For amplitude correction, a new Wiener-like filter is provided.

4.1.2 Parameters

This program requires parameters through the command line (as usual in C) and through CARDs (as usual in FORTRAN programs). The parameters through CARDs can be input via scripts, or the user is asked at the beginning of the program.

Usage and command line parameters:

CTFcorrect.exe Input_image Output_image Tiltangle

with :

- Input_image: File name of the input image. The image must be in MRC format.
- Output_image: File name of the output CTF-corrected image. MRC format, (same mode as input image).
- Tiltangle: Tilt angle at which the image was taken.

CARD parameters:

CARD 1:	Defocus at the untilted plane [Å]
CARD 2:	Defocus Difference (Delta D) for strip extraction [Å]
CARD 3:	CS[mm], HT[kV], AmpCnst, XMAG, DStep[μm]
CARD 4:	Amplitude Correction? [Y|N]
CARD 4a:	Wiener filter parameters: w1, w2 Only required if CARD 4 (Amplitude Correction) is Y.

Description of the CARD parameters

1. Defocus at the untilted plane
   This is the average defocus found at the untilted plane of the tilt series. This is the value obtained by TOMOPS.

2. Defocus Difference (ΔD) for strip extraction
   This is the value of height under which negligible difference in CTF (defocus) is assumed. It is used for the stripping process. The units are Å.

   A value of 0.0 will make the program apply CTF correction in a line-by-line basis as in Henderson et al, JMB 213:899-929, 1990. That is, every single x-line of the image will be corrected assumming a 1D CTF.

3. Imaging parameters: CS, HT, AmpCnst, XMAG, DStep

   CS: Spherical aberration coefficient of the objective in mm.

   HT: Electron beam voltage in kV.

   AmpCnst: Amount of amplitude contrast (fraction).
   For ice images 0.07, for negative stain about 0.15.

   XMAG: Magnification of original image.

   DStep: Pixel size on scanner in microns.

4. Amplitude Correction? [Y|N]
   The user is asked if the CTF correction must include amplitude correction [answer Y] or if only phase-flipping is to be applied [answer N].

5. (CARD 4a) Wiener filter parameters: w1, w2
   If Amplitude Correction is included (answer Y in the previous CARD), the user must input the parameters of the Wiener-like filter: w1 and w2.

   w1 defines the minimum value of CTF from which restoration by inverse filter (i.e. division by the CTF) is to be applied. This parameter should be in [0.5,1.0] to avoid noise-overshooting.

   w2 defines the maximum value of CTF below which the standard Wiener filter used in 3DEM is to be applied. In this Wiener filter, w2 is also applied as a factor to avoid noise-overshooting. Typical values for this parameter should be in the interval [0,0.25].

4.1.3 Output

- The output of the program is the CTF-corrected image stored in a file in MRC format, with the same mode (0 - unsigned bytes; 1 - signed shorts; 2 - floats) as the input image. The file name should have been introduced through the command line parameters.

4.1.4 Example of UNIX scripts

Example of UNIX command file:

```
#!/bin/csh -f
#
# CTFcorrect.csh:  script to launch CTFcorrect.exe
#
# Usage:
# CTFcorrect.csh Input_image Output_image Tiltangle
#
#--------------------
if($#argv != 3) then
echo Usage:  CTFcorrect.csh Input_image Output_image Tiltangle
exit
endif
#--------------------
time CTFcorrect.exe $1 $2 $3 << eof
266500
2000
2 300 0.07 24500 48
Y
0.5, 0.25
eof
#
```

This script requires the same command line parameters as CTFcorrect.exe. An example of launching the script is:

```
CTFcorrect.csh input_image.mrc output_image 60
```

4.1.5 Example of UNIX scripts for correcting a tilt series

To correct tilt-series given as stacks (e.g. IMOD), it is necessary to implement a loop where individual images are de-stacked from the tilt-series, corrected for the CTF, and stacked into the new corrected stack. In the directory `com` we provide a script called 'CTFcorrectstack.csh' which performs these tasks in parallel fashion. The usage of this script is the following:

CTFcorrectstack.csh Input_stack Output_stack Tiltfile [Nproc]

with :

- Input_stack : the input aligned tilt-series.
- Output_stack : the output aligned, CTF-corrected tilt-series.
- Tiltfile : the tilt angle file (typically .rawtlt or .tlt).
- Nproc : It is an optional parameter to specify the number of processors to use for exploiting parallel computing. Typically, this parameter should be given a value equal to the number of cores of the computer where the script is to be executed on.

This script internally uses IMOD commands (header, newstack). So, IMOD is required to be installed and running, with direct access to its programs via the proper PATH update.

This script also requires CTFcorrect.csh (see Section 4.1.4) be available because it is called to make the correction of individual images de-stacked from the input tilt-series.

4.1.6 Advices, tricks and tips

The key parameters for CTFcorrect are ΔD and the parameters for amplitude correction.

- ΔD should have a value consistent with the one used in TOMOPS. Values lower than that are also acceptable.

  Setting up ΔD to 0 would make the correction based on 1D CTF. However, this restoration is parcial, as the CTF and the PSF are 2D by definition, and as a result the effects of the correction could be hardly observed.

- The parameters w1 and w2 define the Wiener filter for amplitude correction. w1 should be in [0.5,1.0] to avoid noise-overshooting. w2 should be in the interval [0,0.25].

4.2 CTF correction using IMOD

CTF correction through this way implements a pipeline TOMOCTF → IMOD / Etomo. The programs TOMOPS/TOMOCTFFIND are used to determine the mean defocus of the tilt-series. Then, ctfphaseflip from IMOD is used for the actual correction. The methodology is basically the same as described in Section 4.1.1, except by the fact that the correction is actually applied to a number of strips much lower (typically by a factor 20x) and the final values of the pixels are determined by interpolation of the two nearest neighbour corrected strips (Xiong et al. J. Struct. Biol. 168:378–387, 2009). Another important difference is that only phase correction is available. As ctfphaseflip is a convenient program because it directly deals with tilt-series and is fast, the pipeline TOMOCTF → IMOD / Etomo may be helpful for users.

As a result of the execution of TOMOPS/TOMOCTFFIND we will have the value of the mean defocus of the tilt series, and the file 'tomoctf.param' generated by TOMOCTFFIND. To perform CTF correction with IMOD, we can follow one of these alternatives:

- With eTomo:
  Go to the 'Correct CTF' panel within the 'Final Aligned Stack' step in Etomo. Input the mean defocus value found by TOMOCTFFIND into the 'Expected defocus' field. Bear in mind that TOMOCTFFIND gives you the defocus in Angstroms while ETomo requires it in **nanometers**!!. Then , toggle on the option to 'Use expected defocus instead of ctfplotter output' in 'CTF Phase Flip'. See Figure 3 below for more details.

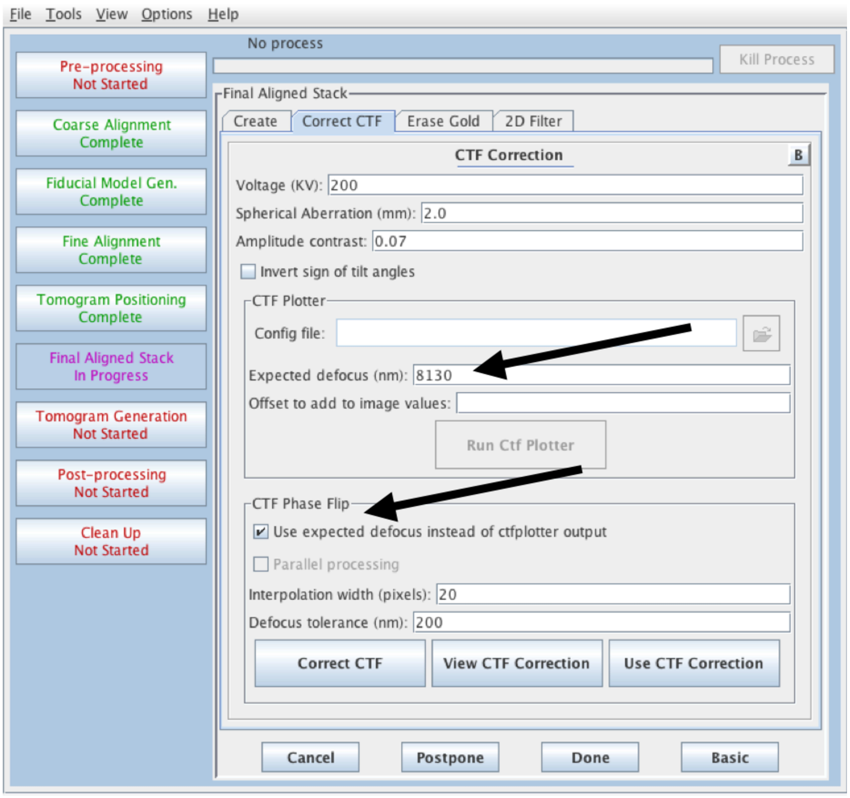

Figure 3: Input the mean Defocus value found by TOMOCTFFIND into ETomo (conversion from Angstroms to nanometers is required!!) and toggle on the use of this value instead of the ctfplotter output.

- With the program CTFphaseflipstack:
  The program CTFphaseflipstack has been developed to invoke the command ctfphaseflip from IMOD automatically using the parameters found by TOMOCTF. It also uses parallel computing to exploit all computing cores available in the computer.

  This is the usage of the program:

  > CTFphaseflipstack.exe Input_stack Output_stack Tiltfile tomoctf.param_file
  > [-defTol nm] [-iWidth pixels] [-pixelSize nm] [-Nprocs value]

  Mandatory parameters:

  – Input_stack : the input aligned tilt-series.
  – Output_stack : the output aligned, CTF-corrected tilt-series.
  – Tiltfile : the tilt angle file (typically .rawtlt or .tlt).
  – tomoctf.param_file : The 'tomoctf.param' file output of TOMOCTFFIND.

  Options:

-defTol nm	: By default, the program assumes a Defocus Difference (ΔD) of 200 nm. This option allows the user to change this value (it must be in nanometers!!). (see also the 'Defocus Tolerance' field in eTomo, in Figure 3).
-iWidth pixels	By default, the program assumes an interval of 20 pixels of separation between consecutive strips actually corrected. This option allows the user to change this value. Note that a value of 1 pixel would essentially implement the approach in TOMOCTF (Section 4.1.1). (see also the 'Interpolation Width' field in eTomo, in Figure 3)
-pixelSize nm	By default, the program takes the pixel size (in nanometers!) from the input 'tomoctf.param' file. Bear in mind that this value refers to the images/tilt-series from which the defocus has been determined. If the CTF correction is to be applied to a binned version of the tilt-series used for determination, this option must be used to input the proper pixel size (in nanometers!!). Alternatively, it would be possible to change it in the input 'tomoctf.param'.
-Nproc value	By default, the program automatically detects the number of cores available in the computer and creates as many processes as cores, which will collaborate in the CTF correction process. This option allows the user to specify a particular number of processes instead of using the default value. In that case, the user should bear in mind that the maximum number of processes should not be higher than the number of cores in the computer, so for a computer with a quad-core, 4 should be the maximum number of threads. Anyway, the processes simultaneously write onto the output file, so it is suggested that no high values for Nproc is used (typically 4–8 at most).

On the execution, CTFphaseflipstack shows on console the actual IMOD command (ctf-phaseflip) that is being launched, just in case the user wants to explore with it.

Requirements:

CTFphaseflipstack requires IMOD be installed and accessible (i.e. the PATH environment variable properly set).

Advices, tricks and tips

The processes created by CTFphaseflipstack to perform CTF correction in parallel are all writing the output file concurrently. As a consequence, the statistics value (min, max, mean) stored in the header of the output file might be close, but not exact. So, we advice to run the 'alterheader' command, option MMM, from IMOD to recompute the proper values and update the header of the output file.

5 CTF determination and correction: a full example

Let's assume we have a tilt-series of 201 images of 4Kx4K, acquired with a FEI Falcon Direct Electron Detector and with a pixel size of 6 angstroms. The microscope was a 300 kV, with Cs 2.7, and the specimen was prepared under cryomicroscopy conditions (i.e. amplitude contrast 0.07). The name of the aligned tilt-series is 'stack.mrc' and the tiltangle file is 'tiltangles.tlt'.

CTF determination

The first step is to compute the power spectrum with TOMOPS. This script is helpful for this task:

```
#!/bin/csh -f
#
# tomops.csh:  for a tilt-series in a stack
#
time tomops.exe << eof
0
stack.mrc
tiltangles.tlt
power.mrc
2000
10000,6
128
eof
#
```

The file 'power.mrc' then contains the power spectrum stored in an MRC, MODE 2, file. If you try to visualize it, you will get nothing understandable. The high value at the origin of the Fourier space obscure all the information in a standard visualizer (e.g. 3dmod).

The next step consists of defocus estimation from the power spectrum. The following script invokes TOMOCTFFIND with the proper parameters of the EM CTF. Assuming a nominal defocus of around 7-8 μm, we used a search range of [4,10] μm=[40000,100000] Å. For the computation, the maximum spatial frequency would be the Nyquist frequency (2 $\times$ pixelsize = 12 Å). Here we used 14 Å.

```
#!/bin/csh -f
#
# tomoctffind.csh
#
setenv GNUPLOTPATH /usr/local/bin
time tomoctffind.exe << eof
power.mrc
diagnostic.mrc
2.7,300.0,0.07,10000,6
0.5,10000.0,14
40000.0,100000.0,1000.0
eof
mv CTFPROFILE CTFprof.txt
mv CTFPROFILE.ps CTFprof.ps
mv tomoctf.param mytomoctf.param
#
```

The output of TOMOCTFFIND yielded a mean defocus of 8130.44 nanometers, as shown on console (Note that TOMOCTFFIND gives the defocus in Angstroms):

```
====================================================================
            Defocus found by TOMOCTFFIND: 81304.35 Angs
====================================================================
```

The output diagnostic image (diagnostic.mrc, Figure 4) and output profile (CTFprof.ps, Figure 5) show a reliable fit of theoretical CTF model with the background-substrated experimental profile of the power spectrum.

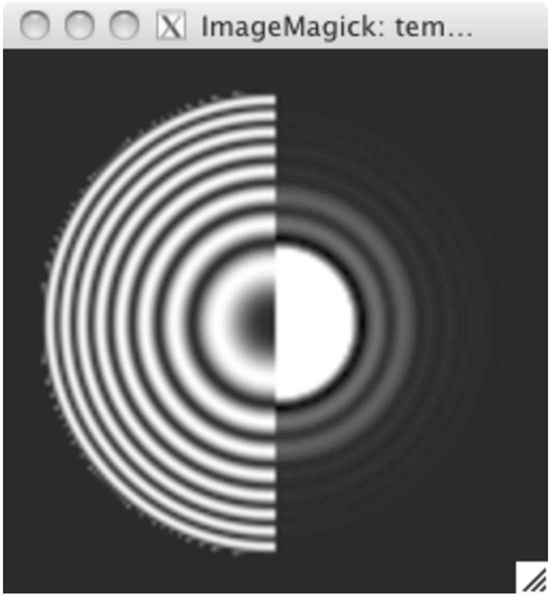

Figure 4: Output diagnostic image.

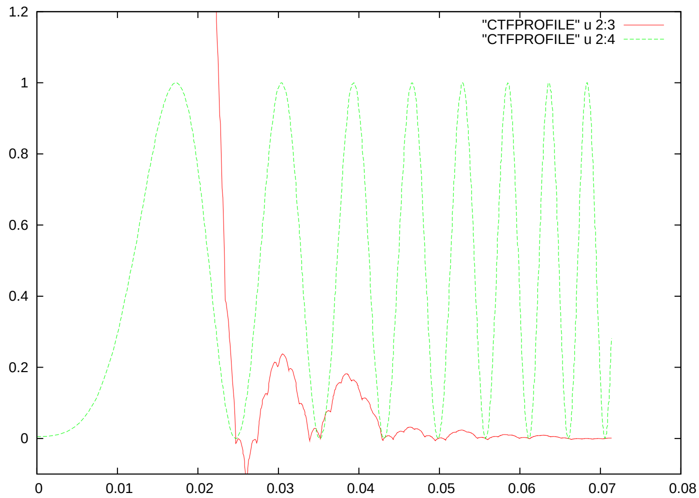

Figure 5: Output CTFprof.ps showing the fitted CTF and the background-substrated experimental profile of the power spectrum.

The output ’tomoctf.param’ file generated by TOMOCTFFIND has the following content. Note that the pixel size and the defocus are given in **nanometers**:

SphericalAberration	2.70
Voltage	300
AmplitudeContrast	0.07
PixelSize	0.60
defFn	8130.44

CTF correction

CTF correction by means of the procedure described in our article in Ultramicroscopy (2006) requires copying of the scripts CTFcorrect.csh and CTFcorrectstack.csh to the directory where the tiltseries is. Then, proper editing of CTFcorrect.csh to set the CTF parameters needs to be done. Note that here we decided to apply only phase correction (4th line, with ’N’):

```
#!/bin/csh -f
#
# CTFcorrect.csh:  script to launch CTFcorrect.exe
#
#--------------------
if($#argv != 3) then
echo Usage:  CTFcorrect.csh Input_image Output_image Tiltangle
exit
endif
#--------------------
time CTFcorrect.exe $1 $2 $3 << eof
81304.35
2000
2.7 300 0.07 10000 6
N
0.5, 0.25
eof
#
```

Next, the script CTFcorrectstack.csh is invoked. Assuming we have a computer based on a quad-core processor, we can exploit the four cores by specifying ’4’ as the last parameter:

```
CTFcorrectstack.csh  stack.mrc  corrected_stack.mrc  tiltangles.tlt  4
```

During the execution, the script informs you about the progress of the correction by showing the index of image(s) being corrected:

```
                         . . .
Correcting image XXXX for the CTF, tilt angle = JJJJ
Correcting image YYYY for the CTF, tilt angle = KKKK
                         . . .
```

A LOG file is created with the name of the input tiltseries and the extension ’.log’ (i.e. in this example ’stack.mrc.log’). In this log file, it is possible to analyze the actual defocus values corrected for in each image of the tilt-series.

CTF correction with IMOD

To correct for the CTF with IMOD, one option would be within eTomo, as described previously (see Figure 3).

The second option is by means of the program CTFphaseflipstack, which should be used this way:

```
CTFphaseflipstack.exe  stack.mrc  corrected_stack.mrc  tiltangles.tlt  mytomoctf.param
```

On the quad-core computer, this program would create four processes collaborating in the correction. The program shows this report on console:

```
   CTF correction - phase flipping with IMOD, V1.0 (Oct-2012)
===========================================================


======================================================================
CTF correction is done with IMOD using the following command:

ctfphaseflip -input stack.mrc -AngleFile tiltangles.tlt
-output corrected_stack.mrc -cs 2.70 -volt 300 -ampContrast 0.07
-pixelSize 0.600 -defTol 200 -iWidth 20 -DefocusFile defocus.imod

with the file 'defocus.imod' containing '1 1 0.  0.  8130.44'
This temporary file is automatically removed.
======================================================================
CTF correction is carried out in parallel using 4 processes

Launching CTF correction of views 1 to 51
Launching CTF correction of views 52 to 101
Launching CTF correction of views 102 to 151
Launching CTF correction of views 152 to 201
```

When finished, a series of log files (one per process) would be available, in case the user in interested in seeing what it actually being executed:

```
ctfphaseflip_001_051.log
ctfphaseflip_052_101.log
ctfphaseflip_102_151.log
ctfphaseflip_152_201.log
```

Note that CTFphaseflipstack provides the actual IMOD command to execute (see the lines in blue color above). It is EXTREMELY IMPORTANT to note that the pixel size to be used in the ctfphaseflip command refers to that of the tilt-series to correct. However, the value of the pixel size stored in the file 'tomoctf.param' corresponds to the tilt-series that was used for CTF determination. Bear this in mind if CTF correction is to be done on a binned version of the tilt-series used for CTF determination.

6 Determination of the defocus gradient direction

Before CTF correction, it is important to determine the direction of the defocus gradient in tilted images. In other words, for a tilted image at, say, $+50^o$ ... which part of the image (left or right?) is closer to focus (i.e. lower underfocus magnitude) and which is farther from focus (i.e. higher underfocus magnitude) ?. With TOMOPS and TOMOCTFFIND, the average defocus at the untilted plane is found out. However, the direction of the gradient is not determined because of the poor signal to noise ratio (SNR).

The convention used in TOMOCTF (as stated in Ultramicroscopy 106:587-596, 2006) is the following: in tilted images with positive tilt angle, the underfocus magnitude increases with the X axis (from left to right). And the opposite for negative tilt angles. This is clearly reported as images are corrected with CTFcorrect, as shown in the output on console.

It is important to determine whether your electron tomography data acquisition system is consistent with the convention in TOMOCTF. If not, the sign of the tilt angles should be changed to make them consistent with the correction carried out by CTFcorrect.

The program TOMOCTFgrad has been developed for that purpose. However, to obtain reliable results, it is important to provide datasets with high contrast and SNR so that a single image can provide reliable CTF determination. The following protocol can help you determine the geometry of your acquisition system.

6.1 Preparation of datasets suitable for finding out the gradient direction

- Prepare a sample specially suited to provide high contrast and SNR when imaged in the electron microscope:
  - negative staining.
  - carbon.
  - avoid low electron doses.
- Obtain a few tilted images of the sample, with tilt angles higher than 10^o:
  -50^o, -40^o, -30^o, -20^o, -10^o, $+10^o$, $+20^o$, $+30^o$, $+40^o$, $+50^o$.
  You need not acquire 10 or more images. But at least, acquiring one image with positive tilt angle and another one with negative tilt angle is advisable. A single tilted image, at -40^o or $+40^o$, would also be useful.
- Stack the acquired images in order to create a tilt-series in IMOD format. In addition, create a tilt-angle file with the tilt angles, as used with IMOD.
- It is extremelly important to acquire and stack the images **the same way you normally do in your experimental works**. It is also important to have the tilt axis running along the Y axis (or slightly deviated, say 2-5^o). In principle, you need not align the images in the tilt-series, just use them directly as acquired.
- Run TOMOCTFgrad.
  $+$ If the defocus gradients are consistent with the convention in TOMOCTF
  $\rightarrow$ you can proceed with your experimental datasets as you normally do.
  $+$ If the gradients are NOT consistent with TOMOCTF
  $\rightarrow$ you always must change the sign of the tilt angles of your datasets before CTF correction.

6.2 TOMOCTFgrad

TOMOCTFgrad determines the direction of the defocus gradient and analyzes whether the tilt angles are consistent with the direction found. If not, the user is notified to make the proper corrections: change the sign of the tilt angles before CTF correction. The program receives a tilt series as input, with the tilt axis running along the Y axis and a file with the tilt angles (i.e. both as in IMOD), along with the several imaging parameters.

6.2.1 Methodology

For each image in the input tilt series, the program applies periodogram averaging for different strips extracted along the X axis. This process thus supplies estimates of the power spectrum as a function of X, for each image. Then, the same procedure used in TOMOCTFFIND to determine the defocus is used here for each of the power spectrum estimates. As a result, the defocus variation along X is obtained, for each image in the tilt series. By comparison of the defocus gradients of all the images with the input tilt angles, the program can determine whether the acquisition system is using the same convention as in TOMOCTF or not.

6.2.2 Parameters

On execution, the program asks the user for the following parameters, interactively. However, it is recommended the use of scripts to edit the parameters and launch the program. An example of script is provided below. The parameters are similar to those required for TOMOPS and/or TOMOCTFFIND.

CARD 1: Input file name for tilt series.
CARD 2: Input file name for tilt angles.
CARD 3: Output diagnostic file name
CARD 4: Tile size [pixels]
CARD 5: CS[mm], HT[kV], AmpCnst, XMAG, DStep[μm]
CARD 6: CTFMin[0,1], ResMin[Å], ResMax[Å]
CARD 7: dFMin[Å], dFMax[Å], FStep[Å]

Description of the parameters

1. Input file name for tilt series.
   Stack (MRC format, MODE 0, 1 or 2) containing the input tilt series.

2. Input file name for tilt angles.
   It must be an ASCII file containing the tilt angles of the images in the tilt series. There must be one tilt angle name per line. The order of the tilt angles in this file must correspond to the order of the images in the input stack.

3. Output diagnostic file name
   The output diagnostic image file helps check the result of the fitting. In order to represent the variation of the CTF along the X axis, for each image in the tilt series it actually shows three diagnostic results: the result of the fitting at the left extreme, at the center, and at

the right extreme of the image. Each diagnostic result shows the background-substracted average power spectrum in one half, and the fitted squared CTF in the other half. The two halfs should agree very well for a successful fit.

4. Tile size (as in TOMOPS)
   The program divides the strips into square tiles to calculate the average power spectrum. Tiles with a significatly higher or lower variance are excluded; these are parts of the images which are unlikely to contain useful information (beam edge, edges due to alignment of the tilt series, etc.) IMPORTANT: Tile size must have even pixel dimensions.

5. Imaging parameters: CS, HT, AmpCnst, XMAG, DStep (as in TOMOCTFFIND)

   CS: Spherical aberration coefficient of the objective in mm.

   HT: Electron beam voltage in kV.

   AmpCnst: Amount of amplitude contrast (fraction).
   For ice images 0.07, for negative stain about 0.15.

   XMAG: Magnification of original image.

   DStep: Pixel size on scanner in microns.

6. Resolution parameters: CTFMin, ResMin, ResMax (as in TOMOCTFFIND)

   CTFMin: Defines a low resolution limit for the fitting. This parameter represents a location between the first peak and the first zero of the theoretical CTF. This parameter must have a real value in the range [0.0,1.0]:

   - 0 represents the location of the first zero of the CTF.
   - 1 represents the location of the first peak of the CTF.
   - values in-between represent proportional locations between these two extreme values.

   ResMin: Absolute low resolution end of data to be fitted.

   ResMaX: Absolute high resolution end of data to be fitted.

7. Defocus search parameters: dFMin, dFMax, FStep (as in TOMOCTFFIND)

   dFMin: Starting defocus value for grid search in Å. Positive values represent an underfocus. The program performs a systematic grid search of defocus values and astigmatism.

   dFMax: End defocus value for grid search in Å.

   FStep: Step width for grid search in Å.

6.2.3 Output

- Defocus gradients.
  The program shows in the standard output (console or a file where the output is redirected) the defocus gradients found for each image in the input tilt series. Three defocus values are shown for each image: the defocus found near the left extreme, around the center of the image, and near the right extreme.

- Gradient direction.
  The output report ends with the analysis of the global defocus gradient and its consistency with the convention in TOMOCTF. If the results are consistent, the following message in shown on console:

  Tilt angles are according to the convention used in TOMOCTF

  Otherwise, it is shown:

  Tilt angles NOT according to the convention used in TOMOCTF:
  Please, change the sign of tilt angles to follow the convention

- Output diagnostic file.
  The program generates an image in MRC format to check the result of the fittings. For each image in the tilt series it actually shows three diagnostic results: the fitting at the left extreme, at the center, and at the right extreme of the image. These diagnostic results correspond to the values of defocus gradients reported on console (see above). This helps the user analyse the variation of the CTF along the X axis for all input images.
  Each diagnostic result shows, on the right half, the scaled background-substracted average power spectrum and, on the left half, the fitted squared CTF. The two halfs should agree very well for a successful fit.

6.2.4 Example of UNIX scripts

Example of UNIX command file:

```
#!/bin/csh -f
#
# tomoctfgrad.csh:  Script for launching tomoctfgrad.exe
#
time tomoctfgrad.exe << eof
GradientSeries.mrc
GradientSeries.tlt
diagnostic.mrc
128
2.26 200.0 0.15 32500.0 24.0
0.5 10000 15
30000.0 60000.0 500.0
eof
#
```

6.2.5 Example

An example of determination of the gradient defocus is included in the directory examples:

- extiltimg.mrc. Input tilt-series, comprising only one image taken from a sample (negative staining, carbon) tilted at -40^{o}.
- extiltimg.tlt: The tilt angle file.
- tomoctfgrad.csh: Script to run TOMOCTFgrad
- tomoctfgrad.log: Output resulting from the execution of tomoctfgrad.csh, showing the defocus gradient for the input image. For the input image, the program finds that the defocus on the left, center, and right is 4.45, 4.15 and 3.85μm, respectively.
- diagnostic.mrc: output diagnostic showing the defocus gradient resulting from the fitting.

7 Simulation of the defocus gradient

The program TOMOCTFsim helps compute the defocus gradient of a tilted image, given some imaging parameters. This can also be useful to assess the quality of the fitting carried out by TOMOCTFgrad.

More importantly, this program allows the user to find out the maximum resolution up to which the information in the dataset is reliable, if CTF correction is not to be applied. Therefore, it can be used to analyze the imaging conditions (defocus) that should be used to avoid CTF correction, or the resolution range where CTF artefacts will begin to be present.

7.1 TOMOCTFsim

7.1.1 Parameters

As with the other programs in the package, the program interactively asks the user for a number of parameters. It is recommended the use of scripts to edit the parameters and launch the program. An example of script is provided below.

CARD 1:	Output text file name.
CARD 2:	Output postscript file name.
CARD 3:	CS[mm], HT[kV], AmpCnst, XMAG, DStep[μm]
CARD 4:	Input Defocus[A]
CARD 5:	Tilt angle[deg] and Image size[pixels]

Description of the parameters

1. Output text file name.
   The program writes an ASCII table with the curves of the CTF for the left extreme, for the center, and for the right extreme of the image, given the image parameters supplied by the user.

2. Output postscript file name.
   The program generates a Postscript file with the CTF curves (i.e. columns #3, #4 and #5 from the previous table), provided that the program `gnuplot` is installed in the system and it is in the shell's path. It is not in the path, it can be set up by means of the environment variable `GNUPLOTPATH`

3. Imaging parameters: CS, HT, AmpCnst, XMAG, DStep
   (as in TOMOCTFFIND and TOMOCTFgrad)

4. Input Defocus.
   The value represents the average defocus at the center of the image.

5. Tilt angle[deg] and Image size[pixels]
   These parameters represent the tilt angle at which the image would be obtained and the dimension in the X axis (typically, 1024, 2048, 4096).

7.1.2 Output

- Output on console.
  The program reports the defocus at the left and right extremes of the image. The defocus at the center matches the value introduced by the user.

- Output text file with the CTF curves.
  This is an ASCII table with the curves of the CTF corresponding to the three defoci reported by the program.
  The content of this file is structured into 5 columns

  1. Index of Bin
  2. Resolution [1/Å]
  3. CTF for the left extreme of the image.
  4. CTF for the of the image.
  5. CTF for the right extreme of the image.

  This table can be dealt with many graphical software.

- Output postscript file name.
  The postscript file represents the three curves in the previous table. The first zero-crossing of the CTF curve corresponding to the most underfocused area represents the resolution from which the data are affected by the CTF (by the phase reversals of the CTF). The data corresponding to poorer resolution are not affected by phase reversals, though they are affected by the CTF amplitude.

7.1.3 Example of UNIX scripts

Example of UNIX command file:

```
#!/bin/csh -f
#
# tomoctfsim.csh:  Script for launching tomoctfsim.exe
#
setenv GNUPLOTPATH /usr/local/bin
time tomoctfsim.exe << eof
CTFsim.txt
CTFsim.ps
2.0 160.0 0.07 25000.0 14
30000.0
60 2048
eof
#
```

7.1.4 Example: Evaluation of the result obtained by TOMOCTFgrad

In the example shown in the section for TOMOCTFgrad, the defocus found at the left, center, and right parts of the image was 4.45, 4.15 and 3.85μm, respectively. If TOMOCTFsim is run using the same imaging conditions, with 4.15μm as the input defocus, the program TOMOCTFsim yields: 4.47,4.15 and 3.83μm, respectively. These results confirm the conclusions drawn by TOMOCTFgrad on an experimental dataset.

7.1.5 Example: Determination of the resolution range 'free' of CTF artefacts.

Let us assume we are working with a 160 kV electron microscope under the imaging conditions shown in the previous script (Cs=2.0, cryomicroscopy, pixel size of 5.6Å and defocus of 3μm). Let us assume that the tilt series contains images up to 60^o tilt, with size of 2Kx2K, as indicated in the script shown above. The program yields that the image at $+60^o$ would have the following defocus gradient:

Defocus at the left extreme of the image:	20067.7
Defocus at the center of the image:	30000.0
Defocus at the right extreme of the image:	39932.3

The postscript file shows the CTF curves:

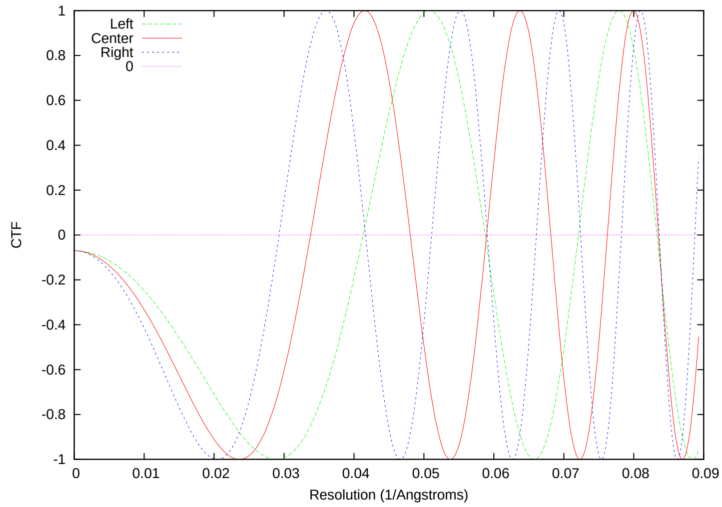

Figure 6: CTFs for the left, center and right parts of a 2Kx2K image taken at $+60^o$ tilt, under the following imaging conditions: 160 kV, Cs=2.0, cryomicroscopy, pixel size 5.6Å and defocus of 3μm. The first zero-crossing is around 3.4nm (0.029$Å^{-1}$), and comes from the more underfocused right part of the image (defocus $\sim 2\mu$m). In the case of an image taken at -60^o tilt, the result would be equivalent, but the more underfocused area would come from the left area.

The figure clearly shows that, under those imaging conditions and using a defocus 3μm, a tilt series in the tilt range [-60^o,$+60^o$] would begin to be affected by the artefacts of the CTF (phase reversals) from $\sim$ 3.4nm. Therefore, that would be the maximum resolution achievable without CTF correction.

This figure also shows that the CTF amplitude modulation begins to affect from $\sim$ 5.0nm ($\sim$ 0.02$Å^{-1}$). Correction for this amplitude modulation could partially restore those frequency terms, as described in Ultramicroscopy 106:587-596, 2006.

8 History

V. June 2006

- Original package containing TOMOPS, TOMOCTFFIND and CTFcorrect, as described in Ultramicroscopy 106:587-596, 2006.

V. December 2009

- The auxiliar programs TOMOCTFgrad and TOMOCTFsim are included.
- CTFcorrect can now work with images in modes 0 (unsigned bytes), 1 (signed shorts) and 2 (floats) of the MRC format.
- TOMOPS and TOMOCTFFIND remain the same.

V. October 2012

- The I/O MRC library has been modified so that TOMOPS is able to read huge tilt-series (e.g. stacks of 200 images of 4Kx4K).
- The program TOMOCTFFIND now produces an output file called 'tomoctf.param' with the parameters of the CTF, including the defocus found.
- Additional advices and tricks are provided for CTF determination with TOMOPS/TOMOCTFFIND.
- The program CTFphaseflipstack has been included. It applies phase correction by invoking the program ctfphaseflip in IMOD. Abilities to work in parallel have been added.
- CTFcorrect now writes the output corrected image in the same mode (0 - unsigned bytes; 1 - signed shorts; 2 - floats) as the input image.
- A bug in CTFcorrect that sometimes appeared with high tilt images has been sorted out.
- The script CTFcorrectstack has been modified to only invoke IMOD commands. Abilities to work in parallel have been added.
- TOMOCTFGRAD and TOMOCTFSIM remain the same.
- Statically linked binaries are provided for linux (64 bits). For OSX, only the system library is required.

V. July 2013

- Bug fixed in the program CTFcorrect.

9 References

• of special interest and closely related to the approach in TomoCTF.

Reference describing the method implemented in TOMOCTF:

- J.J. Fernandez, S. Li, R.A. Crowther. CTF determination and correction in electron cryotomography. *Ultramicroscopy* Vol. 106, pp. 587-596, 2006. http://dx/doi.og/10.1016/j.ultramic.2006.02.004

References on CTF in electron tomography:

H. Winkler and K. A. Taylor. Focus gradient correction applied to tilt series image data used in electron tomography. *J. Struct. Biol.* 143:24–32, 2003.

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