

# AreTomo User Manual

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**AreTomo**, an abbreviation for **A**lignment and **R**econstruction for **E**lectron **T**omography, is a GPU accelerated software package that provides an integrated solution to both fiducial-free alignment and reconstruction for cryoEM tomography. AreTomo strives to generate high-quality 3D reconstructions with its comprehensive corrections of various defects arising from the data collection including translational misalignment, in-plane rotation, tilt-angle offset, and anisotropic local motion due to beam induced motion. Furthermore, projection images, prior to 3D reconstruction, are dose weighted based upon their order of being acquired. Both SART (Simultaneous Algebraic Reconstruction) and WBP (Weighted Back Projection) provided for 3D reconstruction. The entire workflow is streamlined requiring no user intervention and yet easily configurable via command line parameters to suit various practical needs. While AreTomo is still under intensive development and testing, we release this version in the hope that it can benefit more people and promote the routine use of cryoEM tomography.

AreTomo is compiled on Centos 7 for CUDA 10.1, 10.2, 11.2, and 11.3. For running on other CUDA platforms, please contact the author for a customized build.

AreTomo is free for academic use only. For commercial use, please contact **David Agard** or **Yifan Cheng** for licensing:

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## 1. System Requirement and Installation

AreTomo is a GPU accelerated application compiled on Centos 7. Therefore, a Linux system equipped with an advanced NVidia GPU card is needed. The CPU memory allocated in AreTomo is roughly twice the size of the input tilt series calculated based upon 4 bytes per pixel. For a K3 tilt series containing 120 projection images of 5760x4092 pixels, 21 GB of CPU memory needs to be allocated. Therefore, a Linux system with 32 GB CPU RAM is deemed necessary. Since the current implementation has not optimized yet for GPU, any GPU card that is as good as GTX 1080 card or better is sufficient.

AreTomo is a single-program application. Once unzipped, no further installation is needed if Cuda driver and toolkit have been installed already.

## 2. Quick Start

AreTomo can be invoked from Linux command line. The version number and built date can be found by running the following command.

### **AreTomo --version**

Users can get a quick help information about the command line parameters by running the following command.

### **AreTomo --help**

When the above two commands can be executed, it is a good sign that the underlying libraries required by AreTomo are properly installed. The following is an example that takes an input tilt series and generated a 3D volume.

### **AreTomo -InMrc tiltSeries.mrc -OutMrc volume.mrc -VolZ 1200 -OutBin 4**

As can be seen, AreTomo requires all the projection images be stored in a single MRC file. In this example the input MRC file is **tiltSeries.mrc**. The output file, **volume.mrc**, stores the generated 3D volume. **-VolZ 1200** sets the volume height or the z dimension to 1200 unbinned voxels. **-OutBin 4** causes the generated volume to be 4x binned before it is saved to disk. Assuming 3838x3710 pixels per tilt image, the unbinned volume has 3838x3710x1200 voxels. **-OutBin 4** reduces the volume to 958x926x300 voxels.

It is important to note that in this example tilt angles are expected being stored as the first float in the extended headers of **tiltSeries.mrc**. If not, users must use other means described in the next section to pass tilt angles into AreTomo. Otherwise, the program will exit immediately.

## 3. Input of Tilt Angles

Tilt angles are required input parameters by AreTomo. When tilt angles are NOT stored in the extended headers, there are two ways for users to pass them into AreTomo.

### 3.1. -TiltRange

This option is best suited for a tilt series collected with a fixed angular step and tilt images in the MRC file are sorted according to their tilt angles. **-TiltRange** should be followed by two end angles as shown in the following example.

**AreTomo -InMrc tiltSeries.mrc -OutMrc volume.mrc -VolZ 1200 -OutBin 4 -TiltRange -60 60**

**-TiltRange -60 60** informs AreTomo that **tiltSeries.mrc** is collected in the range of  $[-60^\circ, 60^\circ]$  and the tilt images are arranged in ascending order of their tilt angles.

### 3.2. -AngFile

When a tilt series is not collected with a fixed angular step or its tilt images are not sorted, an angle file is needed that is a single-column text file with all the tilt angles listed in the same order as how the tilt images are stored in the input MRC file. Paired with **-AngFile** as in the following example, this angle file allows AreTomo to link each tilt image with its corresponding tilt angle.

**AreTomo -InMrc tiltSeries.mrc -OutMrc volume.mrc -VolZ 1200 -OutBin 4 -AngFile tiltAngles.txt**

An angle file can contain an **optional** second column that lists the accumulated dose on sample at each angular step. More details can be found in section 12 Dose Weighting.

## 4. -VolZ

### 4.1. Control volume thickness

The z axis refers to the one perpendicular to the projection images. As mentioned earlier, **-VolZ** provides a means for users to specify z height of the reconstructed volume. Ideally, we would like to choose a value such that the reconstructed volume just contains the entire structure with neither clipping nor too many empty voxels. In practice, users can estimate the z dimension based upon sample thickness and the pixel size of projection images and then add 200 voxels for precaution. Another option is to run AreTomo with an educational guess combined with a large binning for **-OutBin**, say 4 or even 8. Upon completion, review the x-z slice of the reconstruction and make corresponding adjustment based upon what you see. Note again the input unit for volume z height is unbinned voxel.

### 4.2. Generate aligned tilt series

Aligned tilt series will be saved in the output MRC file when **-VolZ 0** is included in the command. The aligned tilt images are sorted in ascending order of tilt angles in the output MRC file with tilt angles saved in MRC extended headers.

When dose weighting is enabled at the command line, the output is the dose-weighted aligned tilt series. If such a tilt series is intended to be reconstructed in the future, make sure to disable dose weighting at the command line. See section 12 for details.

An angle file is also created in the same directory along with the output MRC file. This file has the same name as the output MRC file but ended with ".tilt". This file can be edited to have a second column for accumulated dose should the aligned tilt series be reconstructed with dose weighting enabled. See section 12 for details.

### 4.3. Reconstruct aligned tilt series

If users want to reconstruct the aligned tilt series, it can be done by adding **-Align 0** and **-VolZ** followed by a positive integer in the command line. **-Align 0** prevents the alignment process from running on the already aligned tilt series.

## 5. Input tilt axis

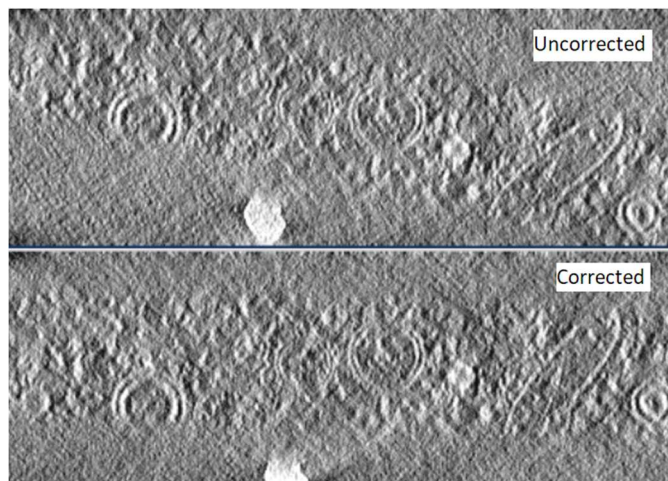
In general, AreTomo does not need to know the orientation of tilt axis. However, if there are several projection images bearing large translational shifts, the tilt axis may not be determined accurately. If you are not satisfied with the quality of the reconstructed volume, you may want to try to bootstrap AreTomo with **-TiltAxis** followed by the user provided value.

```
AreTomo -InMrc tiltSeries.mrc -OutMrc volume.mrc -VolZ 1200 -OutBin 4 -TiltRange -60 60 \
-TiltAxis 10.5
```

Note that the orientation of tilt axis is relative to the y-axis (vertical axis of tilt image) and rotates counter-clockwise.

## 6. Measure and correct tilt angle offset

Tilt offset refers to the difference between the actual tilt angles and those read from microscope during data collection. This single-value offset arises due to either sample mounting or milling angle. For a slanted sample, a larger z height than the sample thickness is needed for reconstruction without clipping the reconstructed structure, resulting a larger MRC file to store the bigger volume. See Fig. 1 for the effect of the correction of tilt offset.



**Fig. 1** (Top) Slanted sample reconstructed without the correction of tilt offset. (Bottom) Reconstruction with the correction of tilt offset.

Please note that the orientation of the missing wedge will be changed as a result of the correction of tilt offset. For subtomogram averaging, tomograms reconstructed from tilt series collected within the same tilt range may have different orientations of missing wedges.

**-TiltCor** can be followed by three values. **-TiltCor 1** results both the measurement and correction of tilt offset. **-TiltCor 0** only measures the tilt offset and is the default setting. The measured offset is corrected only for alignment but not for final reconstruction. **-TiltCor -1** disables the measurement and the correction. Occasionally, the measurement is erroneous and can impair the alignment accuracy.

In the case the tilt offset is not measured properly, an estimated tilt offset can be entered as the second parameter to **-TiltCor**. For example, **-TiltCor 0 10** adds 10° tilt offset to each tilt angle only for the

alignment. **-TiltCor 1 10** adds 10° tilt offset to each tilt angle for both alignment and reconstruction. The tilt offset can be estimated by performing a global only alignment and a quick reconstruction using 4x or even higher binning (**-OutBin 4**) followed by reviewing the xz slice.

## 7. Flip intensity: -FlipInt

By default, the reconstructed volume and the input tilt series use the same grayscale that makes dense structures dark. **-FlipInt 1** makes the dense structures white instead.

## 8. Flip volume: -FlipVol

By default, the x-z slices of the reconstructed volume are saved according to their y coordinates in the output MRC file. **-FlipVol 1** saves x-y slices instead according to their z coordinates.

## 9. Reconstruction schemes

By default, 3D volumes are computed by SART reconstruction. However, weighted back projection can be enabled by setting **-Wbp 1**.

SART is the default reconstruction with the number of iterations and the number of projections per subset set to 20 and 5, respectively. If users want to change these two settings, **-Sart** can be used for this purpose followed by the number of iterations and number of projections per subset.

## 10. Focused Alignment: -Roi

**-Roi** refers to region of interest (ROI). When it is not present in the command line, AreTomo assumes the region of interest at the center of 0° projection image. A circular mask is employed to down-weight the area outside ROI during the alignment. When the structures of interest are far away from the tilt axis, the angular error in the determination of tilt axis will significantly amplify the translational error. **-Roi** function can effectively improve the alignment accuracy for the distant structures. This function gets activated when **-Roi** is followed by a pair of x and y coordinates, representing the center of the region of interest. See the following example.

**AreTomo -InMrc tiltSeries.mrc -OutMrc volume.mrc -VolZ 1200 -OutBin 4 -Roi 50 100**

It should be pointed out that the region of interest should be selected from 0° projection image with the origin at the lower left corner. For your information, IMOD's Pixel View is a good tool to select the center of region of interest.

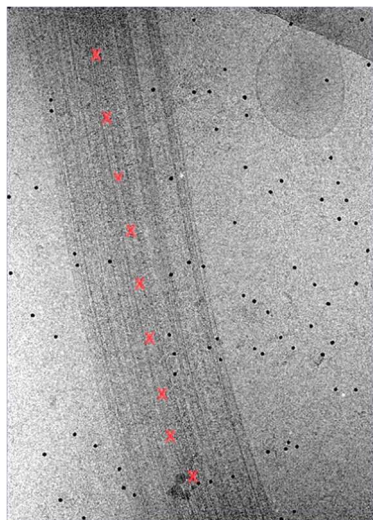
## 11. Local motion correction

Beam induced motion significantly complicates the situation in cryoEM tomography. Specimen movement results from not only stage tilting but also beam induced motion. Tomographic alignment premised only on single-axis rotation, regardless fiducial based or not, are not accurate enough for high-resolution tomography if beam induced local motion is left uncorrected. AreTomo provides two means to correct the local motion, one for isolated sample and the other for well distributed across the field of view.

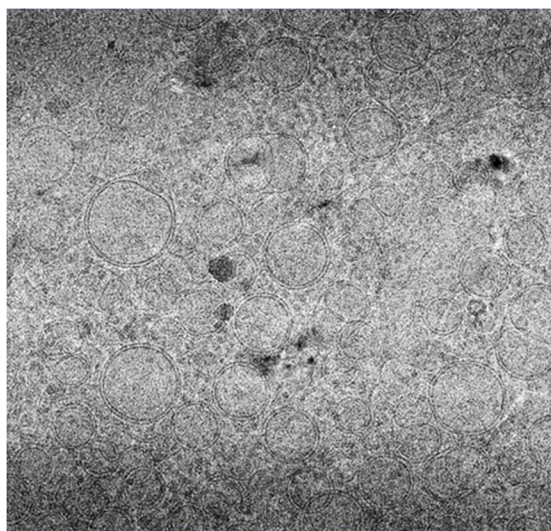


### 11.1. Isolated or Sparse Sample: -RoiFile

**-RoiFile** requires users to provide a list of x and y coordinates and put them in a two-column text file, one column for x and the other for y. Each pair defines a region of interest (ROI). AreTomo then performs focused alignment as described in section 11 on the structure in each of these ROIs. Local motion is then interpolated based upon a distance-based scheme and corrected at each pixel of each projection image before 3D reconstruction is performed. **Fig. 2** shows an example where nine targets were selected along the elongated structure. As mentioned before, the origin of the coordinate system is at the image's lower left corner.



**Fig. 2** Illustration of target selection along an elongated structure required to activate local motion correction.



### 11.2. Well-distributed Sample: -Patch

For well distributed samples, users can use **-Patch** option to activate the measurement and correction of local motion. **-Patch** is followed by two integers, e.g. **-Patch 5 4**, that specify number of patches in x and y directions, respectively. The focused alignment is performed at individual patches. As mentioned in section 11.1, local motion is calculated based upon distance-based scheme and then corrected at each pixel of each projection image. **Fig. 3** shows an example of what a well distributed sample looks like. As can be seen, a well distributed sample presents signals everywhere for the measurement of local motion.

**Fig. 3** An example of well-distributed sample suitable for patch based local motion correction.

It is worth noting that a problematic measurement on one patch can affect not only itself but also its neighboring patches. While the choice between **-RoiFile** and **-Patch** is left at users' discretion, **-RoiFile** may be a better choice if there is a significant amount of empty area in the projection images that makes some patches featureless.

## 12. Dose Weighting

Once the input tilt series is aligned, dose weighting can be applied to the aligned tilt series before it gets reconstructed. To activate dose weighting, high tension (**-Kv**), pixel size in angstrom (**-PixSize**), and the accumulated dose in  $e/A^2$  at each tilt angle are needed. AreTomo uses the angle file (**-AngFile**) described in section 3.2 to associate accumulative dose with tilt angle. Since the angle file lists all the tilt

angles of the images in a single column, we can add a second column for the accumulated dose to associate it with the corresponding tilt angle. The following is an example of dose augmented angle file. This column indicates that the tilt images in MRC file was sorted in ascending order of tilt angles from -60.0° to 36.0°. The second column shows that the first acquisition took place at -12.0°. An individual tilt image received a dose of 2e/A<sup>2</sup>.

-60.0	66.0
-57.0	64.0
-54.0	58.0
.....	.....
-15.0	8.0
-12.0	2.0
-9.0	4.0
.....	.....
30.0	54.0
33.0	60.0
36.0	52.0

The required parameters for dose weighting should be similar to the following example. Missing any of them will disable dose weighting. The second column is only needed for dose weighting. Otherwise it is optional.

**-Kv 300 -PixSize 3.5 -AngFile PathToAngFile/myAngFile.txt**

## 13. Alignment file

AreTomo automatically generates a text file containing both global and local alignment parameters. Named after the input MRC file, this file uses **aln** as file extension and is saved in the output directory containing the reconstructed volume. Should users decide to reconstruct the volume with a different setting, the corresponding aln file can be loaded by means of **-AlnFile** to bypass the lengthy alignment process, as is illustrated in the following example.

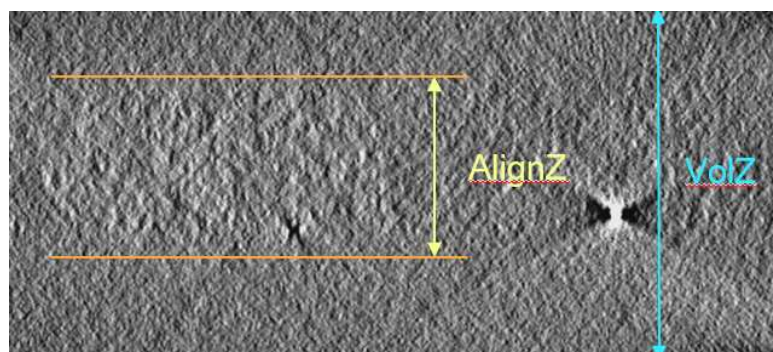
**AreTomo -InMrc tiltSeries.mrc -OutMrc ~/Temp/volume.mrc -AlnFile ~/Temp/tiltSeries.aln \**

**-VolZ 1200 -OutBin 1**



## 14. Volume z height for alignment: -AlignZ

This function specifies the z height of the temporary volume reconstructed for projection matching as part of the alignment process. This value plays an important role in alignment accuracy. The



default value is 800. This z height should be always smaller than that specified by **-VolZ**, as illustrated in Fig. 4. As can be seen, the z height for alignment is preferred to be close to the sample thickness whereas the z height for final reconstruction should be larger.

**Fig. 4** Illustration of **-AlignZ** and **-VolZ**

in a xz slice of a reconstructed volume.

In practice we can perform global only alignment and 3D reconstruction with a large binning. The sample thickness can then be measured in the xz slice of the volume.

## 15. Reconstruction without alignment: -Align 0

This option lets users reconstruct tomogram without aligning the input tilt series, which must be, therefore, pre-aligned either by AreTomo or some other software. One application of this option is that a raw tilt series is first aligned and then CTF-corrected. The aligned and CTF-corrected tilt series is finally input into AreTomo to reconstruct CTF-corrected tomogram.

## 16. Tilt axis determination: -TiltAxis initVal refineFlag

Tilt axis determination is a two-step processing in AreTomo. A single tilt axis is first calculated followed by the determination of how tilt axis varies over the entire tilt range. The “initVal” lets users enter their estimate and AreTomo refines the estimate in  $[-3^\circ, 3^\circ]$ . If users do not know, they can enter 0 instead and AreTomo will search an optimal value in full range. The “refineFlag” can be one of -1, 0, and 1. The -1 instructs AreTomo to the user input value without any refinement. The default setting is 0 and 1.

refineFlag	Meaning
-1	Use the input value without any refinement
0	Refine the input value and use the refined value for the entire tilt series
1	Refine the input value and calculate tilt axis at each tilt angle.

## 17. Output tlt file

When AreTomo is configured to generate an aligned tilt series with -VolZ 0, a tilt angle file will be created in the same directory where the aligned tilt series is saved. The angle file has the same file

name of the aligned tilt series but with file extension replaced with “.tlt”. The tilt angles in this file are in the same order as the projection images in the aligned tilt series.

An important application of this file is to reconstruct a tilt series that is both aligned and CTF-corrected as described in section 15. Although AreTomo saves the tilt angles in MRC extended headers, CTF correction by other software may strip the extended headers from the output MRC file. If this is the case, users should use -AngFile followed by the tlt file to pass tilt angles into AreTomo.

### 1.0.12 Release Note:

1. The most important change made in this version is that the handedness of the volume has been changed to the same as that in IMOD. The z axis of volumes is flipped 180° as opposed to those reconstructed by earlier versions.
2. Version 1.0.11 and 1.0.12 fixed a bug that causes random, although rare, crash.
3. Added a new function that allows users to input their own values of tilt axis and decide how they want to refine their values.

### Technical Support

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