2015

Fully Mechanically Controlled Automated Electron Microscopic Tomography

Version 1.0
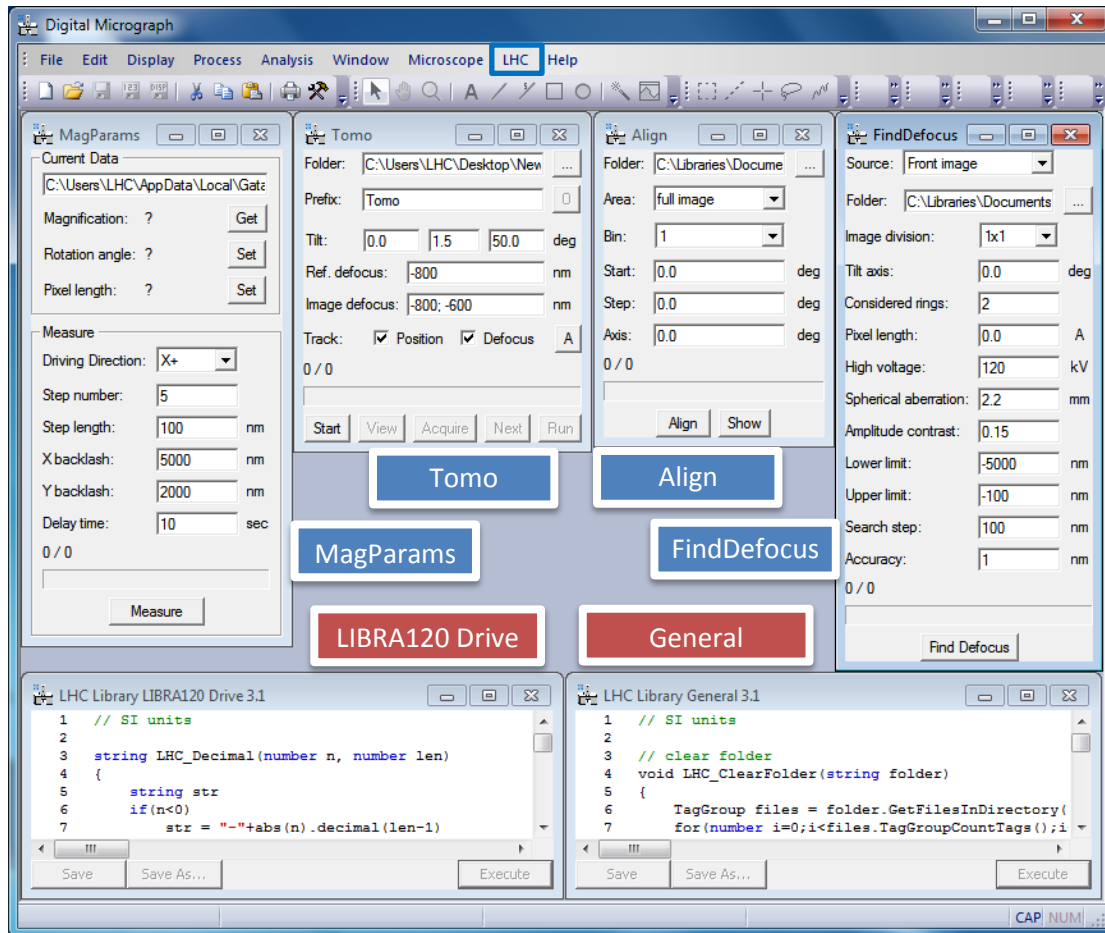
This User Manual is only applicable for the LIBRA 120 TEM, the Molecular Foundry, Lawrence Berkeley National Laboratory.

Contents

1. Architecture Overview

- **LIBRA120 Drive Library**

  Drive functions of LIBRA120 TEM (RS232 Protocol).

- **General Library**

  Functions including file operation, string processing, math, image processing, etc.

- **MagParams Dialog**

  Show or measure the actual magnification (angstrom/pixel) and image rotation angle.

- **Tomo Dialog**

  Automatic tomography data collection.

- **Align Dialog**

  Align a set of tomography images or a set of multiple exposure images.

- **FindDefocus Dialog**

  Find the defocus value of front image or a set of images.

2. LIBRA120 Drive Library

- string LHC_Decimal(number n, number len)
- number LHC_GetVoltage(void)
- number LHC_GetMag(void)
- number LHC_GetMagPointer(void)
- void LHC_SetMagPointer(number MagPointer)
- number LHC_GetIllAperture(void)
- void LHC_SetIllAperture(number ill)
- number LHC_GetFocusDistance(void)
- void LHC_SetFocusDistance(number FocusDistance)
- void LHC_GetGonPos(number &x, number &y, number &z, number &t)
- number LHC_GetGonX(void)
- number LHC_GetGonY(void)
- number LHC_GetGonZ(void)
- number LHC_GetGonT(void)
- void LHC_SetGonPos(number x, number y, number z, number t)
- void LHC_SetGonX(number x)
- void LHC_SetGonY(number x)
- void LHC_SetGonZ(number x)
- void LHC_SetGonT(number x)
- void LHC_MoveGonPos(number dx, number dy, number dz, number dt)
- void LHC_ResetBacklash(number xb, number yb, number zb, number tb)

3. General Library

- void LHC_ClearFolder(string folder)
- TagGroup LHC_CreateFileNameList(string folder, string suffix)
- TagGroup LHC_CreateFileNameList(string folder, string prefix, string suffix)
- number LHC_GetPixelLength(number mag)
- void LHC_SetPixelLength(number mag, number k)
- void LHC_DeletePixelLength(number mag)
- number LHC_GetRotationAngle(number mag)
- void LHC_SetRotationAngle(number mag, number a)
- void LHC_DeleteRotationAngle(number mag)
- image LHC_Val(string str, number &count)
- number LHC_STD(image img)
- number LHC_STD(image img1, image img2)
- void LHC_Rotate(number &x, number &y, number a)
- image LHC_Solve2(image A, image B)
- image LHC_Solve3(image A, image B)
- image LHC_Regress(image img, image weight, number &rx, number &ry, number &rc)
- image LHC_Regress(image img, image weight)
- image LHC_Regress(image img, number &rx, number &ry, number &rc)
- image LHC_Regress(image img)
- image LHC_RegressLimit2STD(image img, number &rx, number &ry, number &rc)
- image LHC_RegressLimit2STD(image img)
- image LHC_Resize(image img0, number w, number h)
- image LHC_Rotate(image img, number a, string m)
- image LHC_Stretch(image img, number a, number k)
- image LHC_Filter(image img, number f1, number f2)
- image LHC_Shift(image img, number sx, number sy)
- number LHC_CorrCoef(image img1, image img2)
- image LHC_CrossCorrelate(image img1, image img2)
- number LHC_MeasureShift(image img1, image img2, number &sx, number &sy)
- image LHC_CalculateCTF(image img)
- image LHC_CreateCTF(number n, number k, number V, number Cs, number AC, number defocus)
- image LHC_FindNode(image CTF, number nn)
- image LHC_CompareCTFs(image CTF1, image CTF2)
- number LHC_CompareCTFs(image CTF1, image CTF2, image nodes)

- number LHC_FitCTF(image CTF, number nr, number k, number V, number Cs, number AC, number defocus1, number defocus2, number accuracy)
- image LHC_FindDefocusMap(image img, number wDiv, number hDiv, number nr, number k, number V, number Cs, number AC, number DefocusMin, number DefocusMax, number DefocusStep, number DefocusAccuracy)

4. MagParams

DigitalMicrograph Menu -> LHC ->LHC Dialog MagParams


Current Data

Press "Get" to show current magnification, rotation angle and pixel length if the data was found. Press "Set" to input rotation angle or pixel length for the magnification. Rotation angle is the counterclockwise rotation angle from goniometer coordinate system to image coordinate system.

Measure

Press "Measure" to measure rotation angle and pixel length of current magnification. During the measuring process, goniometer moves sample along "Driving direction" with specified "Step length" and eliminates backlash according to "X backlash" and "Y backlash" for each step. Images are acquired after each step and "Delay time". Rotation angle and pixel length are calculated according to the accumulated image shift, "Step length" and "Step number".

5. Tomo

DigitalMicrograph Menu -> LHC -> LHC Dialog Tomo
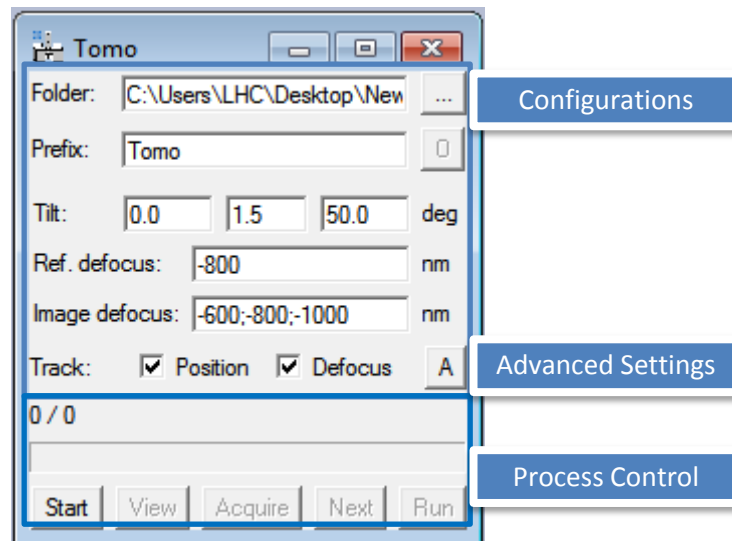
Configurations

- Folder:

    The save path of tomography images. Press "…" to browse for a new folder.

- Prefix:

    The prefix of tomography images. Press "O" to open acquired images when enabled.

- Tilt:

    The start, step and stop angles of tilting.

- Ref. defocus:

    Defines the defocus tracking target.

- Image defocus:

    Focus is adjusted to acquire multiple images for each tilt angle according to the distance between "Ref. defocus" and "Image defocus". Separate the numbers by ";" or space.

- Track:

    Check the corresponding boxes to track position and/or defocus. Press "A." to open advanced setting dialog.

Advanced Setting 1: Position Tracking

- Pointer color & diameter:

  Box and circle are two kinds of pointers. The box locates at the center of a frame. The circle locates on the tracking target. The default target is the center of the first image.

- Backlash compensation:

  Defines a reciprocating motion to compensate backlash after moving goniometer.

- Stabilization time:

  Defines a time period to stabilize the sample after moving goniometer.

- Position error integral coef.:

  This coefficient makes a tradeoff between random error and system error of position tracking. Set 0 to minimize the random error and set 1 to minimize the system error.

- Position error threshold:

  Exceeding the threshold triggers the checked actions in sequence.

Advanced Setting 2: Defocus Tracking

- CTF fitting parameters:

  A image is divided into subareas according to "Number of image divisions". The CTF of each subarea is calculated and compared with the CTF created using "Spherical aberration", "Amplitude contrast" and estimated defocus. This comparison only considers the first several rings specified by "Number of considered rings".

- Defocus finding parameters:

  Based on CTF fitting, the defocus of an image can be found in the range from "Lower limit of defocus" to "Upper limit of defocus". A large "Defocus search step" can speed up defocus finding but increases the risk of falling into a local optimum defocus value. "Defocus finding accuracy" specifies the final accuracy of the result.

- Defocus error integral coef.:

  This coefficient makes a tradeoff between random error and system error of defocus tracking. Set 0 to minimize the random error and set 1 to minimize the system error.

- Defocus error threshold:

  Exceeding the threshold triggers the checked actions in sequence.

Process Control

- Start:

  Press "Start" to tilt to start angle with position and defocus tracking if current tilt angle is different from the specified start angle, otherwise start tomography image collection after confirming the configurations. Reset backlash is necessary if you haven't done it. The user interface after starting is as:
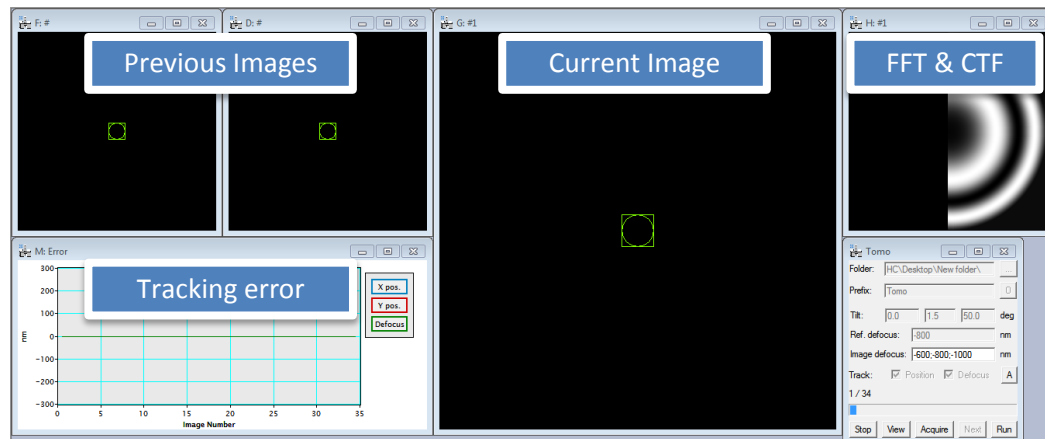
- Stop:

  The "Start" button turns to "Stop" after starting. Press "Stop" to abort current task.

- View:

  Press "View" to view the sample. Both position error and defocus error are calculated after viewing. Current image, circle and FFT & CTF image are updated after calculation.

- Acquire:

  Press "Acquire" to acquire and save images of different defocus values. Both position error and defocus error are calculated after acquiring. Current image, circle and FFT & CTF image are updated after calculation.

- Next:

  Press "Next" to trigger the corresponding actions checked in advanced settings if position error or defocus error exceeds its threshold, otherwise tilt to next angle.

- Run:

  Press "Run" to execute "View" and "Next" alternately when tilting to start angle, or execute "Acquire" and "Next" alternately when collecting tomography images.

Manual Operations

- Redefine position tracking target:

  Click on current image to define the clicked point as new position tracking target.

- Move image:

  Click on current image with "Ctrl" pressed to move the clicked point to frame center.

- Switch display mode of FFT & CTF:

Click on the left hand side of FFT & CTF image to switch between full FFT and center FFT modes. Click on the right hand side of FFT & CTF image to switch between target CTF and fitted CTF modes.
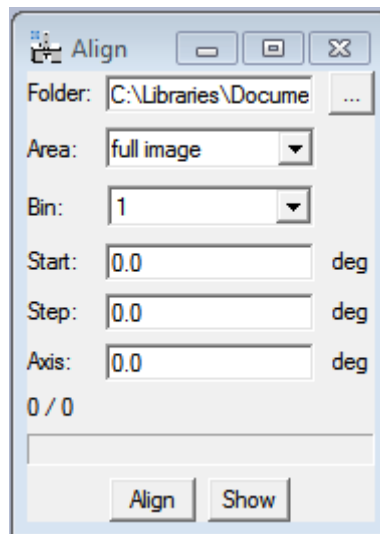
- Redefine found defocus:

  Click on the right hand side of FFT & CTF image with "Ctrl" pressed to input a new defocus value if the found defocus is not correct.

- Change defocus:

  Click on the left hand side of FFT & CTF image with "Ctrl" pressed to input an increment of focus distance to change the actual defocus.

6. Align

DigitalMicrograph Menu -> LHC -> LHC Dialog Align

- Folder:

    The folder of images that need to be aligned. Press "…" to browse for a new folder.

- Area:

    The subarea used to calculate shift.

- Bin:

    Select larger value to speed up the calculation and sacrifice accuracy.

- Start, Step and Axis:

    Specify start, step and tilting axis angles for tomography images to increase accuracy.

- Align:

    Press "Align" to start to align the images in the folder.

- Show:

    Press "Show" to display the images in the folder in sequence.

7. FindDefocus

DigitalMicrograph Menu -> LHC -> LHC Dialog FindDefocus
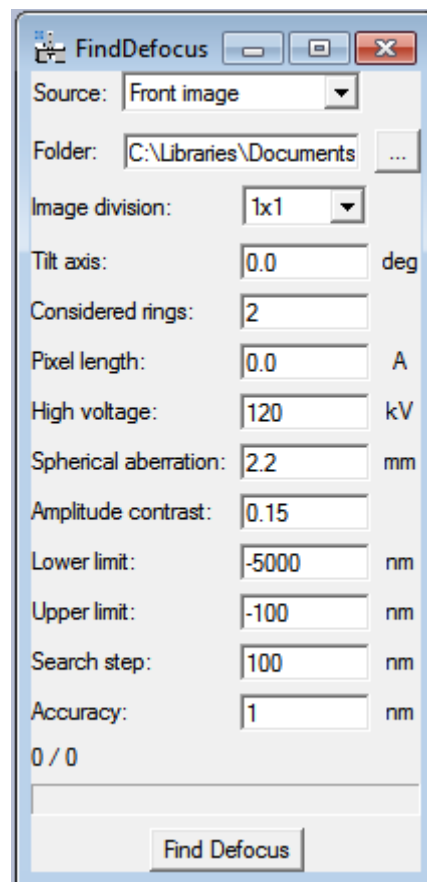
- Source & Folder:
  Find defocus of front image or all the images in the folder.
- Image division, Tilt axis, Considered rings, Pixel length, High voltage, Spherical aberration and Amplitude contrast:
  A image is divided into subareas according to "Image division" and "Tilt axis". The CTF of each subarea is calculated and compared with the CTF created using "Pixel length", "High voltage", "Spherical aberration", "Amplitude contrast" and estimated defocus. This comparison only considers the first several rings specified by "Considered rings".
- Lower limit, Upper limit, Search Step and Accuracy:
  Based on CTF fitting, the defocus of an image can be found in the range from "Lower limit" to "Upper limit". A large "Search step" can speed up defocus finding but increases the risk of falling into a local optimum defocus value. "Accuracy" specifies the final accuracy of the result.
- Find Defocus:
  Press "Find Defocus" to start calculation.