XTEM

--Software for Complex Transmission Electron Microscopy—

Version 1.0

1. Introduction

XTEM is the software for complex microscopy on JEOL 3100 electron microscopes. The XTEM software consists of a suite of scripts and a Plug-In for use in Gatan's DigitalMicrograph 3.7, or later, for Windows platforms. The suite of scripts (XTEM LowDose scripts) assists users to acquire a pair of images respectively taken with and without a phase-plate using Gatan MultiScan (MSC)/BioScan cameras or a regular films. The XTEM software thus works with the LowDose software (LowDose for JEOL), which assists a user to acquire images or diffraction patterns with minimum electron doses to the beam-sensitive specimens. The XTEM plug-in supplies a suite of commands to process the pair of images for complex microscopy.

This XTEM User's Guide is written to provide information on the basic functions of the XTEM software, a procedure for installation of the Plug-In, some general tips on operation and troubleshooting. This Guide assumes the user is familiar with image acquisition and manipulation within DigitalMicrograph as well as the LowDose software, and only addresses those features specific to the XTEM software.

1.1 Features

1.1.1 Advanced flexible LowDose data acquisition

The complex microscopy requires two images taken with and without a phase-plate, respectively. Therefore, it is important to take these images under a minimum electron exposure. The XTEM software thus works with the LowDose software (LowDose for JEOL). Users can easily modify data acquisition procedure by changing the LowDoseScripts supplied with the XTEM (see **section 2.3**).

### 1.1.2 User friendly data processing

Data processing will be readily carried out step-by-step by using commands under the XTEM menu. Data processing conditions/parameters are easily changed on a XTEM setup dialog (see **section 2.1**).

1.2 XTEM LowDose Operation

The XTEM microscopy requires extra steps to manipulate the phase-plate under minimum electron exposure.   Therefore, the XTEM software works with the LowDose software that supports the four modes as shown below. The mode will be selected from the LowDose menu or the LowDose Tools.

Figure 1-1   LowDose Cycle
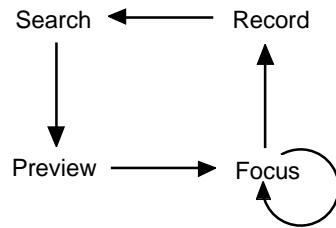
Currently the phase-plate is placed manually with respect to the optical axis. Therefore, XTEM microscopy requires at least one more LowDose cycle in addition to a normal Search-Focus-Record cycle.   One of the possible schemes to take a pair of XTEM images is as follows. Here, the Preview mode is set to be skipped.

Simplified off-plane three-mode 2-cycle scheme

	LD Mode	LD Function	EM Mode	PP	Blanking	Slit
1	Search	1. Specimen search	Defocused DIFF	Out	On -> Off	Out
	Focus	2. Focus setting	Mag for Focus	Out	On -> Off	Out
	Record	3. (Dummy function)	Mag for Record	-	-	-
2	Search	4. PP adjustment	Defocused DIFF	Center	On -> Off	Out
	Focus	5. (Dummy function)	Mag for Focus	-	-	-
	Record	6. PTEM acquisition	Mag for Record	Center	On/Exp/On	In
		7. PP retraction	Mag for Record	Out	On/On	In
		8. CTEM acquisition	Mag for Record	Out	On/Exp/On	In

In order to reduce adverse effects from hysteresis, the modes are changed always in the same sequence as shown here.   This is true, even when the user selects the mode that is not the next one on the LowDose cycle.

2. XTEM

The XTEM software consists of a suite of scripts (XTEM LowDose scripts), which works with the LowDose software (LowDose for JEOL). These scripts are called by corresponding LowDose commands, which has been described in details in **LowDose Section 2.2**.

Another important component of the XTEM software is a suite of commands to process a pair of XTEM images for complex microscopy. These commands for processing the complex wave reconstruction from a pair of XTEM images (a conventional image and a phase-plate images) will be explained in Section 2.2.

2.1 XTEM Preferences

The necessary commands for complex wave reconstruction are accessed through the XTEM menu added to the main DigitalMicrograph menus. The XTEM menu has selections shown below:

Figure 2-1   XTEM Menu

Various control parameters for XTEM will be specified through a window that will be opened by selecting Preferences… under the XTEM menu. This preferences setup window consists of four tabs as shown below. This Preferences dialog will appear at the beginning of each command, when the command is launched with the Alt-key down.

Figure 2-2   Preference window (Acquisition tab)
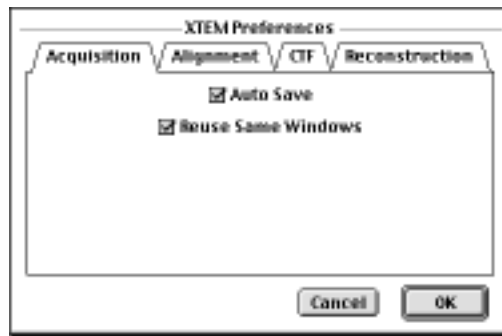
2.1.1 Acquisition tab

The front most tab has some options for image acquisition as shown above. These are optional controls for the LowDose data acquisition.

Auto Save: When checked, acquired images by using the CCD camera will be saved automatically after acquisition with a sample name and a serial number. Images taken with and without a phase-plate are identified with PTEM and CTEM extensions, respectively.   The sample name and the serial number are specified on the Open LowDose dialog (see LowDose manual).

Reuse Same Windows: When checked, the same windows will be reused to acquire XTEM images, instead of creating new image windows.   This will prevent the screen from being scattered with image windows.

2.1.2 Alignment tab

The next tab is designed for an alignment of XTEM image pairs, which controls a way of alignment of the XTEM image pair.

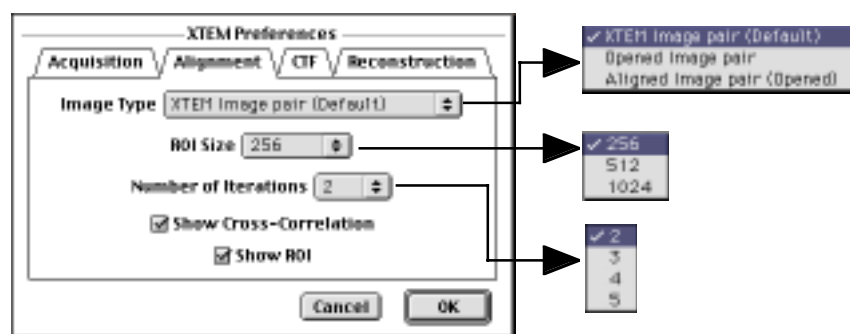Figure 2-3   Alignment tab

Image Type: Source image type for alignment will be selected from the list.
XTEM image pair: Images saved on the disk, which were acquired with the LowDose software by using CCD. The source image will be open through a regular file selection dialog.
Opened image pair: Source images already opened by DigitalMicrograph.
Aligned image pair: Previously aligned source images already opened by DigitalMicrograph.
ROI Size: ROI (Region Of Interest) size used for image alignment based on cross-correlation.
Number of Iterations: Maximum number of cycles for iterative displacement estimation.
Show Cross-Correlation: When checked, the final cross-correlation function between the displaced ROIs will be displayed.
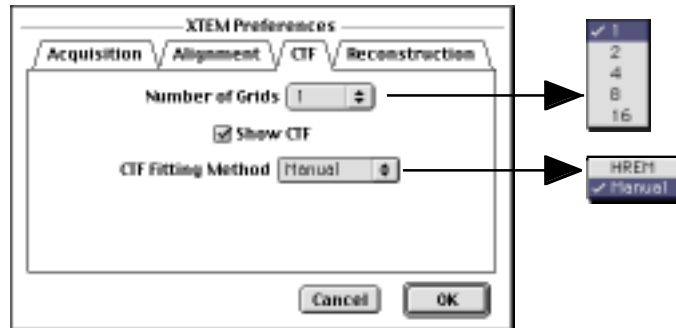**Show ROI**: When checked, ROIs of the same area of XTEM images will be displayed.

2.1.3 CTF tab

The next tab is used to control a way of calculating the Contrast Transfer Function (CTF). The CTF may be evaluated by averaging the CTFs calculated from the sub-areas (grids) of the original image, if Number of Grids is larger than 1.

Figure 2-4   CTF tab

Number of Grids: Number of sub-areas to calculate the CTF. Each edge is divided by this number.
Show CTF: When checked, an averaged CTF will be displayed.
CTF Fitting Method: CTF fitting method will be selected from the list.
HREM: CTF fitting will be carried out by using Gatan HREM plug-in.
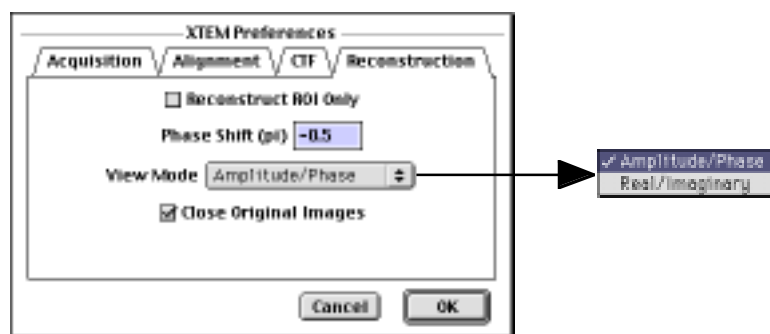Manual: Defocus and/or astigmatism will be estimated from a ring diameter(s) manually drawn by the user. Here, a ring number is also given by the user.

2.1.4 Reconstruction tab

The last tab is used to control a way of complex wave reconstruction and display.

Figure 2-5   Reconstruction tab

Reconstruct ROI Only: When checked, the only ROI is reconstructed.   Else a whole image is reconstructed.

Phase Shift: The amount of phase shift introduced by the phase plate is specified in pi.

View Mode: The way of display of the reconstructed complex image wave. A complex number image is also kept as a hidden image.

Close Original Images: When checked, the original images will be closed after the image reconstruction.

2.2 Image Reconstruction Menu

The necessary functions for complex wave reconstruction are accessed easily through the following XTEM menu.   The image reconstruction will be carried out sequentially from Setup Images to Reconstruct Image menus.   Note that the Preferences dialog will appear at the beginning of each command, when the command is launched with the Alt-key down.   Thus, you can change processing parameters just before the execution, when you changed your mind.

2.2.1 Setup Images

This is the first step of the image reconstruction.   Here, an XTEM image pair (CTEM and PTEM images) is selected and an interest area (ROI) is indicated with Mouse tool by the user.   After this step, image processing will be carried out on the images selected here.

2.2.2 Find Same Area

An image displacement is estimated by cross-correlation between the ROIs in the CTEM and PTEM images.

2.2.3 Get CTF

A CTF is obtained from the CTEM image.   If Number of Grids is larger than 1, the CTF is calculated by averaging the CTFs from sub-areas.

2.2.4 Estimate Aberration

An aberration function corresponding to the CTF obtained the previous step will be estimated automatically by the HREM software or from ring diameter(s) manually assigned by the user.   Here, the wave aberration includes only spherical aberration and defocus and/or astigmatism terms.

2.2.5 Reconstruct Image

A complex image wave is reconstructed from the XTEM image pair.

2.3 LowDoseScripts

The LowDose software works with a set of scripts called LowDoseScripts that is installed in the PlugIns folder.   These scripts classified into two groups: one to select a LowDose mode and the other to select a LowDose function.

Mode selection script:
- SelectSearchMode.s
- SelectPreviewMode.s
- SelectFocusMode.s
- SelectRecordMode.s

Function selection script:
- LowDoseView.s
- LowDosePreview.s
- LowDoseSetMark.s
- LowDoseRecord.s

An advanced user can easily modify these scripts to adapt them for his/her requirements.   For example, we can add a control of an energy filter slit state (insertion/retraction), a beam blanking control, and so on.   The following shows examples of the mode and function selection scripts.

2.3.1 Mode selection script: SelectREcordMode.s

The following shows the SelectRecordMode.s as an example of the mode selection scripts.

```
number n, skip, rc

LowDoseGetNumber("DataSetSelected", n)
if(n==0){
    OKDialog("No data set selected.")
    exit(1)
}

// **** Beam blanking ****
LowDoseBeamBlankingOn()                // you may comment off this line
//LowDoseBeamBlankingOff()             // you may comment off this line

// check MSC
try{
    MSCIsCameraIn()
}
catch{
    OKDialog("SSC Error")
    exit(1)
    break
}

number RecordOn = LowDoseGetNumber("Record:"+n+":RecordOn")

if(CAEMGetNumber("CCDType")==1 && RecordOn==0 && !MSCIsCameraIn()){
    MSCSetCameraIn(1)
    DelayInSecond(CAEMGetNumber("CCDDelayAfterInsert"))
}

if(RecordOn==0 && CAEMGetNumber("CCDPosition")==2)
    LowDoseScreenUp()
if(RecordOn==1){   //Record on Film
    LowDoseScreenUp()
    MSCSetCameraIn(1)
}
if(RecordOn==3) LowDoseScreenDown()        // Record on Screen
if(RecordOn==4 && CAEMGetNumber("TVPosition")==2)
    LowDoseScreenUp()        //   TV below the Screen

// Energy Slit Control
JFEFSetSlitIn(1)     // slit in

rc = SelectRecordMode()
//rc = !OKCancelDialog("Record mode setting")
if(!rc) LowDoseSetString("CurrentMode", "Record")

// **** Beam blanking ****
LowDoseBeamBlankingOn()                // you may comment off this line
//LowDoseBeamBlankingOff()             // you may comment off this line

exit(rc)
```

2.3.2 Function selection script: LowDoseRecord.s

The following shows the LowDoseRecord.s as an example of the function selection scripts.

```
number n
LowDoseGetNumber("DataSetSelected", n)
if(n==0){
    OKDialog("No data set selected.")
    exit(0)
}

// **** Beam blanking ****
LowDoseBeamBlankingOn() // you may comment off this line
//LowDoseBeamBlankingOff()            // you may comment off this line

string CurrentMode = LowDoseGetString("CurrentMode")
if(CurrentMode != "Record"){
    if(!TwoButtonDialog("Current mode is not RECORD.¥n" + ¥
      "Do you want to set the Record mode now?", "Yes", "No"))   exit(1)
    {
        number rc
        rc = LowDoseExecuteScriptFile("SelectRecordMode.s")
        if(rc > 0)   exit(rc)
    }
}

number RecordOn = LowDoseGetNumber("Record:"+n+":RecordOn")
if(RecordOn > 1) {
    OKDialog("Warning: Select ¥"CCD¥" or ¥"Film¥" for Recording media!")
    exit(0)
}

if(CAEMGetNumber("CCDType")==1 && RecordOn==0
      && !MSCIsCameraIn()) {
    MSCSetCameraIn(1)
    DelayInSecond(CAEMGetNumber("CCDDelayAfterInsert"))
}
if(RecordOn==0 && CAEMGetNumber("CCDPosition")==2)
    LowDoseScreenUp()        // Camera below the Screen

if(RecordOn==1){               //Record on Film
    LowDoseBeamBlankingOff()
    RecordOnFilm()
    LowDoseScreenUp()
}
else   RecordXTEMImages(0)

// **** Beam blanking ****
LowDoseBeamBlankingOn() // you may comment off this line
//LowDoseBeamBlankingOff()            // you may comment off this line

if(TwoButtonDialog("The first image has been captured.¥n" + ¥
    "Take out the phase plate, and then select ¥"Proceed¥" to take the second
    image.", "Proceed", "Skip")) {
    if(RecordOn==1){                       //Record on Film
        LowDoseBeamBlankingOff()
        RecordOnFilm()
        LowDoseScreenUp()
    }
    else   RecordXTEMImages(1)
}

// **** Beam blanking ****
LowDoseBeamBlankingOn() // you may comment off this line
//LowDoseBeamBlankingOff()            // you may comment off this line

LowDoseSelectNextModeAfterRecord()
```

3. Installation

This chapter describes hardware and software requirements to perform the XTEM experiment, and also describes installation procedure of the XTEM software.

3.1 Requirements

The XTEM runs under DigitalMicrograph environment, and the software and hardware requirements are similar to those for DigitalMicrograph. Since the XTEM works with the LowDose software for JEOL, the requirements for the LowDose software should also be satisfied. Note that the XTEM software is currently works only on a JEOL-3100 microscope, while the LowDose software works on almost all JEOL microscopes.

3.1.1 Hardware requirement

The following is a list of the hardware requirements necessary to run the XTEM:

1. **A network communication between the microscope and a computer running the DigitalMicrograph**.
2. **High-quality CCD camera**: A possible choice may be a Gatan MultiScan (MSC)/BioScan camera.
3. **TV-camera (Optional)**: Specimen search may be done on a focus screen.
4. **Windows machine with a minimum 17-inch monitor**.

Please note that *No hardware MDS option is required*.

3.1.2 Software requirement

The following is a list of the software requirements necessary to run XTEM:

- **DigitalMicrograph for Windows (version 3.6 or later)**.
- **Gatan Electron Microscope Control plug-in**.
- **Camera control plug-in shipped with an MSC/SSC camera**.
- **LowDose software for JEOL**

3.2 Installation

The following plug-ins and a script folder should be placed in the folder "PlugIns" on the same level of the DigitalMicrograph:

- **JEOLCOMPlugIn (for JEOL-3100)**
- **CAEMBasic**
- **CAEMDialog**
- **CAEMLibrary**
- **LowDoseLibrary**
- **LowDoseMenu**
- **LowDoseScripts folder** with script modules.
- **TrackerObject.dll**

The Packages folder should exist under a normal installation of the DigitalMicrograph and contain the MSC/SSC plug-in and the EM Control plug-in if you have a Gatan camera and a Gatan HREM or TEM AutoTuing extension. If you need to install the MSC/SSC plug-in and/or the EM Control plug-in, please consult relevant documents.

4. Getting Started…

This chapter explains the typical steps necessary to perform the XTEM experiment.   At first image acquisition is explained, and then image processing is illustrated.   Please install the LowDose software according to the **section 3** (**Installation**) before following the steps in this section.

4.1 XTEM Image Acquisition

An XTEM image pair will be acquired under a low dose exposure using the LowDose software.   Here, the process to take an XTEM image pair will be explained.   Please consult the LowDose software manual for its general features.

Before taking an XTEM image pair we have to setup the conditions for each LowDose mode (4.1.1).   Next, we have to establish a stable LowDose cycle by reducing any hysteresis (4.1.2).   Then, we can take an XTEM image pair from a region found in the Search mode (4.1.3).

4.1.1 Setup LowDose conditions

Each LowDose condition has to be set up according to the **LowDose section 4.2** (**Setup LowDose Conditions**) before starting the LowDose cycle for data acquisition.

In Short:
- LowDose -> Select Data Set to give a name to a set of new LowDose conditions.
- LowDose -> Read Conditions of -> each mode, after setting up microscope conditions,
- LowDose -> Read Conditions of -> Focus Position,

4.1.2 Refine LowDose cycle

For the LowDose experiment it is important that the same specimen position is imaged at the center for each mode during the LowDose cycle.   The refinement (hysteresis control) of the LowDose conditions has to be done according to the **LowDose section 4.5** (**Refine LowDose Cycle**). Use the LowDose Tools (colored buttons) to select each mode.

LowDose Tools

In Short:
1. LowDose -> Open LowDose session to select a LowDose data set.
2. Coarse alignment: The image center alignment will be done on the screen by using the Procedure 1 (in this case, the screen should be selected to observe an image for all modes at the previous step 1.).   If necessary, illumination conditions will be adjusted.
3. Fine alignment: LowDose conditions for each mode will be adjusted by looking images on the final viewing/recording means.
4. LowDose -> Close LowDose session to save the conditions onto the disk.

4.1.3 Data Acquisition

Now you are ready to investigate your sample and record an XTEM image pair under the low-dose condition.   In the following, the simplified off-plane three-mode scheme is assumed.   Use the LowDose Tools (colored buttons) to select each mode.   To record the image, you can directly push the "R" command button in the second row (note that the colored "R" button only set the mode to Record, and does not take images.).

LowDose Tools

In Short:
1. LowDose -> Open LowDose session to select a LowDose data set.
2. Find a good specimen position at Search mode (use colored button).
3. Adjust focus at Focus mode (use colored button).
4. Insert a phase-plate at Search mode (use colored button).
5. Take a pair of XTEM images by pushing the "R" command button.   A PTEM image is taken first, then a window appears prompting a use to retract the phase-plate. A CTEM image is taken when the user replies to the dialog after retracting the phase-plate.
6. Repeat the steps from Search to Record.
7. LowDose -> lose LowDose session

4.2 XTEM Image Processing

The necessary functions for complex wave reconstruction cab be accessed sequentially through the XTEM menu.

4.2.1 Setup Images

According to the Image Type selection (Figure 2-3), a regular open dialog to select a file on the disk or the following DM dialog will appear.
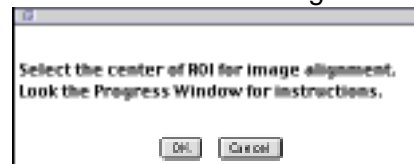
Figure 4-1   Image selection dialog for Open images
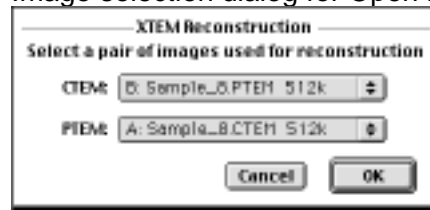
Then, a following modeless dialog will be appear prompting a user to select the center of ROI for the image alignment.

Figure 4-2   Select ROI center dialog

The ROI center is selected by using the mouse tool added into the standard tools.   Just select one point, and then click the "OK" button.

← Mouse Tool

4.2.2 Find Same Area

An image displacement is estimated by using the ROI, the center of which is specified the previous step.   The ROIs corresponding to the same area will be shown, when Show ROI is selected.

2.2.3 Get CTF

A CTF is obtained by averaging the CTFs from sub-areas.   The CTF will be shown, when Show CTF is selected.

2.2.4 Estimate Aberration

An aberration function corresponding to the CTF will be estimated automatically, when HREM is selected.   When Manually is selected, a following modeless dialog will appear prompting a user to draw a line or a pair of lines.

Figure 4-3   Draw line(s) dialog
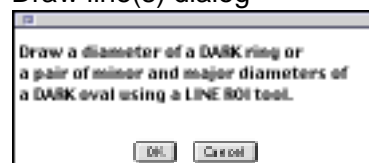
ROI Tools

Use the line ROI tool to draw a line spanning a dark ring. The ring number is entered in a dialog that appears when pressing the OK button, When an astigmatism is significant, the minor and major diameters of the dark ring could be specified,

TIPS: When drawing the second line, press SHIFT key at the beginning. Then, release the SHIFT key to free its direction. (If you don't press the SHIFT key, the previous line is deleted and you can not add the second line.)

2.2.5 Reconstruct Image

A complex image wave is reconstructed from the whole XTEM image pair or from the ROIs depending on your selection. The resulted complex function is displayed according to the View Mode. The reconstructed result of complex function is also kept as a hidden image, which can be selected from the Windows menu of the DigitalMicrograph. Then you can view the result in any form of the complex image.