# thermoscientific

# EPU

## Versions 1.12 - 2.13 Release Notes

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### 1 Introduction

### 1.1 Purpose

This document describes the Thermo Scientific EPU software releases.

### 1.2 Audience and scope

These release notes are intended for:

- Those who manage the configuration of Thermo Scientific EPU installations.
- Users of the Thermo Scientific EPU software.

This document describes the content and dependencies of the most recent and historic EPU releases. This document does not describe the installation, licensing and use of the EPU software.

### 1.3 Hardware Requirements

The EPU software can be used on the Microscope PC of Thermo Scientific and FEI TEM systems that are equipped with a compatible camera. See the release specific chapters below for a specification of the supported TEM Server software versions and cameras.

### **1.4** System, software and configuration compatibility

The tables below show the compatible microscope software versions, the preferred EPU software versions per microscope software version, and the system configuration compatibility.

Although the EPU software is backward compatible with a limited range of microscope software versions, some of the new features and improvements may only be available for the most recent supported microscope software version(s).

#### 1.4.1 Preferred EPU Version per Microscope Software Version

Titan	Talos	Tecnai	EPU	Remarks
3.5 – 3.10	2.5 – 2.10	_	2.13	
3.4	2.4	_	2.12.1	
3.3	2.3	_	2.11	
3.2	2.2	_	2.10	
3.0 – 3.1	2.0 - 2.1	_	2.9	
2.15	1.15	_	2.12.1	
2.14	1.14	_	2.8.1	
2.13	1.13	_	2.7	
2.12	1.12		2.6.1	
2.6 – 2.11	1.6 – 1.11	5.6 - 5.7	1.12	Last release for Falcon II
2.0 - 2.5	1.1 – 1.5	_	1.7	
1.6	_	4.6.4	1.6	Last release for Windows XP

#### 1.4.2 Compatible Microscope Software Versions per EPU Version

EPU	Titan	Talos	Remarks
2.13	3.5 – 3.10	2.5 – 2.10	
2.12.1	2.15 3.4 – 3.9	1.15 2.4 – 2.9	<ul> <li>Preferred over EPU 2.12.0</li> <li>Most recent release that supports Windows 7</li> </ul>
2.12.0	2.15 3.4 – 3.9	1.15 2.4 – 2.9	
2.11	2.15 3.3 – 3.8	1.15 2.3 – 2.8	
2.10	2.15 3.2 – 3.7	1.15 2.2 – 2.7	
2.9	2.15 – 3.6	1.15 – 2.6	
2.8.1	2.14 - 3.5	1.14 – 2.5	Replaces EPU 2.8.0
2.8.0	2.14 – 3.5	1.14 – 2.5	EPU 2.8.0 is withdrawn. Do not install EPU 2.8.0
2.7	2.13 – 3.4	1.13 – 2.4	
2.6.1	2.12 - 3.3	1.12 – 2.3	Preferred over EPU 2.6.0
2.6.0	2.12 – 3.3	1.12 – 2.3	
2.5	2.12 - 3.2	1.12 – 2.2	
2.4	2.12 – 3.1	1.12 – 2.1	First release for Windows 10
2.3	2.12 – 2.15	1.12 – 1.15	
2.2	2.12 – 2.14	1.12 – 1.14	
2.1	2.12 – 2.13	1.12 – 1.13	
2.0	2.12	1.12	Released only to Beta customers

#### 1.4.3 Compatible Athena and EQM Versions per EPU Version

EPU	Athena	EQM	Remarks
2.13	1.14	1.7	
2.12	1.13	1.6	
2.11	1.12	1.5	
2.10	1.10	1.4	
2.9	1.9.2	1.3	
2.8	1.8.2	1.2	
2.7	1.8	1.1	<ul> <li>First EPU release with DMP integration for:</li> <li>Falcon 3EC and Falcon 4</li> <li>Gatan filters with Gatan K2 or K3 camera</li> </ul>
2.6.1 and earlier	_		Not supported

### 1.4.4 Compatible Cameras and Detectors for EPU

Camera	Supported	Remarks
Ceta	Yes	<ul><li>All Ceta Sensor Packages.</li><li>With and without Speed Enhancement (Ceta-2).</li></ul>
Falcon 4	Yes	EPU 2.6 and later.
Falcon 3EC	Yes	
Falcon I / II	No	EPU 1.12 and earlier.
Selectris / Selectris X	Yes	Requires Titan 3.6 and later, or Talos 2.6 and later.
Gatan Orius SC200 / SC1000	Yes	
Gatan OneView	Yes	
Gatan US1000 / 1000XP / 4000	Yes	
Gatan Enfinium SE / ER with US1000XP camera	Yes	

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Camera	Supported	Remarks
Gatan Quantum 963 / 964 / 965 / 966	Yes	
Gatan Quantum 967 with K2 camera	Yes	
Gatan BioQuantum 1967 with K3 camera	Yes	<ul> <li>EPU 2.3 and earlier: only in stand-alone configuration.</li> <li>EPU 2.4 and later:         <ul> <li>embedded configuration is supported with Titan 2.15.2/3 or 3.2 and later and with Talos 1.15.2/3 or 2.2 and later:</li> <li>all other Titan and Talos versions: only in stand-alone configuration.</li> </ul> </li> </ul>
Gatan BioContinuum 1069 with K3 camera	Yes	EPU 2.8 and later on systems with Titan 3.5 or later or Talos 2.5 or later.

Note The compatible cameras and/or filters may not be all be supported by the compatible microscope software versions. See the TEM Server Release Notes for a definitive list of supported cameras and filters.

The EPU 2.13 release brings significant throughput improvement due to multiple optimizations in various functionalities, including an increased AFIS range from 6  $\mu$ m to 12  $\mu$ m. This increases the cluster size, which reduces the number of stage moves and centering steps.

### 2.1 Mandatory and Breaking Changes

Only for systems with TEM Server 7.10 (Titan 3.10 / Talos 2.10) or later, and a connection to Athena on a DMP server.

The Storage Server functionality on the DMP server that is released alongside TEM Server 7.10 introduces Linux-managed data storage. For EPU 2.13, this means that the <code>RootStoragePath</code> address that is specified in the <code>DMP.json</code> file is no longer valid for all installations.

For detailed information and instructions, see the EPU Software Installation Manual and the EPU Software Upgrade Manual.

#### 2.2 New features

#### General

Significant throughput improvement due to multiple optimizations in various functionalities, including an increased AFIS range from 6  $\mu$ m to 12  $\mu$ m. This increases the cluster size, which reduces the number of stage moves and centering steps.

Additionally, TEM Server 7.10 software (Titan 3.10 / Talos 2.10) contains optimizations in the image acquisition functionalities, which increases the throughput even further.

#### 2.3 Improvements

#### General

- As of TEM Server 7.10 (Titan 3.10 / Talos 2.10), the TEM User Interface no longer has to be open to start and use EPU. The TEM User Interface is still needed to perform some of the calibrations.
- Traffic Light:

Depending on the TEM Server version, it is no longer necessary to use the TEM User Interface to revert HT changes and optics mode changes. These conditions can be recovered with the Traffic Light.

#### **Preparation > Acquisition and Optics Presets**

EPU now uses the dose that is reported by the camera, which is compensated for coincidence loss. This makes the dose that is displayed in EPU more accurate.

#### Atlas > Screening

• The TMP now only starts when a specimen exchange will take place.

• After the cassette is undocked, EPU now remembers the Slot Position from which the specimen was loaded that is currently on the stage. This makes it possible to continue the preparation of an automated acquisition run.

This information is only available until EPU is closed, or until a cassette is docked.

#### **Auto Functions**

The Calibration tasks and Auto-Functions tasks have switched. Calibrations are now below the Auto-Functions. The Auto Function calibrations are only available when logged in as a Supervisor or as a Thermo Fisher Scientific engineer.

#### Auto Functions > Auto-eucentric by stage tilt

Minimum cross correlation quality is replaced by Maximum Z-Height Deviation.

#### EPU > Automated Acquisition

In a *Faster acquisition* run, the AFIS range is increased from 6 µm to 12 µm. Because the clusters are now larger, fewer stage moves and centering steps are needed.

#### 2.4 Solved issues

Besides the Solved Issues that are listed below, numerous smaller issues have been solved that were not listed as Known Issues for preceding releases.

ID	Description	Remarks
EPU-7722	Autostigmate does not always stop after Stop is selected.	
EPU-7795	The Objective aperture mechanism is not re-inserted after Atlas alignment.	
EPU-7802	Atlas > Screening: after the specimen loading fails, EPU still executes the categorization routine.	
EPU-8176	Atlas > Screening: when a Slot Position Reset is cancelled, the image and checkmark still disappear.	

### 3 EPU 2.12.0 and EPU 2.12.1

The highlights of the EPU 2.12 release are:

- The Grid Square categorization and selection functionalities that are available for systems with an AutoLoader also become available for Side Entry systems.
- Dark reference acquisition for the Gatan K3 camera is now configurable.

### 3.1 Mandatory and Breaking Changes

None identified.

#### **3.2** New features

#### General

The **Traffic Light** can now switch on the C-FEG. *Requires TEM Server 7.9 or later.* 

#### Atlas > Screening

- Single Atlas acquisition for Side Entry systems is revised. The Side Entry loader is now presented as a single specimen loader slot. All Grid Square categorization and selection functionalities that were only available for systems with an AutoLoader are now also available for Side Entry systems.
- The Start Position can now be selected for the currently loaded specimen in a Screening acquisition, and for the Single Atlas acquisition.

#### **EPU > Automated Acquisition**:

Dark reference acquisition for the Gatan K3 camera is now configurable. A new ribbon bar is available with controls to enable/disable dark reference acquisition and to specify the acquisition interval (hrs).

#### 3.3 Improvements

#### EPU > Hole Selection and Area Selection

The Remove Holes/Areas Close To Grid Bar filter is improved. The filter is faster and reliable.

#### **EPU > Automated Acquisition**

- The Auto Zero Loss (AZL) function for Thermo Scientific Selectris filters is now faster.
- If the EPU Session Type is Automatic and the Atlas is re-aligned after the specimen is reloaded, then EPU re-centers each Grid Square just before the Auto Eucentric Height function is executed during the Automated Acquisition run.

### 3.4 Solved issues

Besides the Solved Issues that are listed below, numerous smaller issues have been solved that were not listed as Known Issues for preceding releases.

#### Solved in EPU 2.12

ID	Description	Remarks
EPU-5540	Remove Close To Grid Bar filter fails for UltraAuFoils (gold grid).	
EPU-7265	In some cases the <i>EPU</i> tab > <i>Area Selection</i> > <i>Spacing</i> entry field responds incorrectly to the entered value.	—
EPU-7509	Incorrect exposure rate after restarting EPU with an unfinished automated acquisition run.	_

#### Solved in EPU 2.12.1

ID	Description	Remarks
EPU-7370	Process keeps running when application is closed	

### 4 EPU 2.11 and EPU 2.11.1

Among multiple other new features and improvements, the EPU 2.11 release introduces the *Single Atlas* functionality. The Single Atlas functionality allows the acquisition of an Atlas for the specimen that is currently loaded on the stage. The Single Atlas acquisition functionality allows to prepare and execute an EPU run when no cassette is docked in the AutoLoader.

### 4.1 Mandatory and Breaking Changes

None identified.

#### 4.2 New features

#### General

EPU always uses the Pre-specimen shutter for Thermo Scientific cameras, regardless of the selected shutter settings in the TEM User Interface.

For Gatan cameras, the shutter settings must still be configured in GMS.

#### Atlas > Screening

When it is not known which specimen is currently loaded on the stage, then the *Single Atlas* functionality allows for the acquisition of an Atlas from the specimen that is currently loaded on the stage. The *Single Atlas* > *Acquire* button is only available when the status of the specimen on the stage is unknown, for example when there is no cassette in the AutoLoader or when the AutoLoader is not initialized yet.

#### **EPU > Square Selection**

- The new *Add new Grid Square here* function in the image display context menu creates a new Grid Square with its center at the mouse cursor coordinates. This allows users to add Grid Squares at locations that are interesting for acquisition, but that do not meet the selection criteria of the built-in Grid Square detection algorithm.
- Besides the X and Y position, the *Move to Grid Square* function now also sets the Z and A-tilt position.
- The Image Display shows an Atlas Alignment warning in the upper left corner if the specimen is reloaded after the Atlas has been acquired and the Atlas has been aligned.

#### EPU > Hole Selection

If the Column Valves are closed, then the Prepare All Squares function now requests to open the Column Valves before starting acquisition.

### 4.3 Improvements

#### General

• The User Interface styling is updated. In the User Manual, most screenshots are not updated yet.

- Resource usage is optimized. CPU and memory peaks are reduced to prevent (or at least further minimize) the impact of the EPU software on the performance of the system's TEM Server control software.
- The Traffic Light is now also available on Krios G3, Krios G3i and Krios G4 systems with TEM Server 7.8 (Titan 3.8) or later.
- Lens normalizations are optimized. After the optics settings have changed, only the changed lenses are normalized.

#### Atlas > Screening

EPU automatically starts the AutoLoader TMP when screening acquisition starts. After the screening acquisition is completed, EPU returns the AutoLoader TMP to its preceding state.

#### EPU > Square Selection

In the context menu, Add and Remove are replaced by Select and Unselect.

#### EPU > Hole Selection

After changing the Filter Ice Quality settings, the selected holes in the image display are updated much faster.

#### EPU > Area Selection

- To prevent overlapping Acquisition Areas, the minimum Spacing between Acquisition Areas is determined by the Illuminated Area (Titan) or Readout Area (Talos) in the Acquisition Preset.
- After changing the Filter Ice Quality settings, the selected areas in the image display are updated much faster.

#### **EPU > Template Definition**

The *Minimum stage settling time* replaces the *Delay after Stage Shift* timer. The difference between them is as follows:

- Delay after Stage Shift: the delay is applied always and in full. If the value was set to 10 seconds, then EPU would always insert a 10 second waiting time before acquiring an image.
   The countdown for the Delay after Stage Shift typically started a bit later than the actual completion of the stage move. Because the stage drift already decreased a bit when the countdown started, an optimized Delay after Stage Shift value would typically be slightly shorter than the actual stage settling time.
- *Minimum Stage Settling Time*: the countdown starts when the most recent stage move is completed. If the value is set to 10 seconds and the most recent stage move was completed 4 seconds ago, than EPU will insert only a 6 second waiting time before acquiring an image.

If the Minimum Stage Settling Time value is kept the same as the preceding Delay after Stage Shift value, then there may be a small amount of stage drift left at the end of the countdown. If some stage drift is noticed after the Minimum Stage Settling Time has ended, then slightly increase the value.

#### EPU > Automated Acquisition

The reference images for a Gatan K3 camera are now acquired once per hour instead of once per Grid Square.

### 4.4 Solved issues

Besides the Solved Issues that are listed below, numerous smaller issues have been solved that were not listed as Known Issues for preceding releases.

#### Solved in EPU 2.11

ID	Description	Remarks
EPU-5337	Image display shows black edges when rescaling the EPU window	
EPU-6689	Memory and CPU usage peaks during screening	_
EPU-6818	Slow navigation after creating a new EPU session	_
EPU-7076	EPU crashed when moving mouse over scalebar	_

#### Solved in EPU 2.11.1

ID	Description	Remarks
EPU-7348	On systems with a Thermo Scientific Selectris (X) Energy Filter or a Gatan filter, the slit is not automatically retracted at the start of an Atlas acquisition.	
EPU-7353	<ul> <li>Gatan K3 camera changes mode when EPU toggles between Acquisition and Optics Presets.</li> <li>Gatan K3 camera changes dose calculation when EPU changes mode from Super Resolution to Counted mode.</li> </ul>	

The EPU 2.10 release introduces a *traffic light* that monitors the system readiness for successful data acquisition.

This release also introduces the *EPU Multigrid* option, which enables the preparation and consecutive execution of multiple EPU Sessions for a selection of specimens from the AutoLoader.

# Note EPU Multigrid is a licensed option and may require a paid upgrade for your system. For Krios systems with Titan 3.X software (Windows 10), please contact your local service representative for a license key.

- For all other systems, please contact your sales representative.
- For all other questions, please contact UpdateEM@thermofisher.com for more information.

### 5.1 Mandatory and Breaking Changes

None identified.

#### 5.2 New features

#### General

• The **Traffic Light** functionality reports the system readiness status for a successful high quality data acquisition. If this *traffic light* is not green for all monitored parameters, then the **Recover** function automatically adjusts the parameters that are out-of-range.

The traffic light and recovery functions are only available on Krios systems with TEM Server 7.7 or later, and a Falcon 4 camera in bottom-mounted or post-filter position.

• The **EPU Multigrid** option enables the consecutive execution of multiple Automated Acquisition runs.

First, start and prepare individual EPU Sessions for multiple specimens that are present in the AutoLoader. The prepared EPU Sessions must have *Session Type*: *Automated* and are automatically added to a queue.

Then start the execution of the queue, so that the Automated Acquisition runs for all queued EPU Sessions are executed in a single automated procedure.

The EPU Multigrid option is only available for systems with TEM Server 7.X that have an AutoLoader.

Note EPU Multigrid is a licensed option and may require a paid upgrade for your system.
For Krios systems with Titan 3.X software (Windows 10), please contact your local service representative for a license key.

- For all other systems, please contact your sales representative.
- For all other questions, please contact UpdateEM@thermofisher.com for more information.

#### 5.3 Improvements

#### General

• For systems with TEM Server 7.7 or later, TIA is no longer a prerequisite for EPU. On these systems, the Startup Check no longer verifies if TIA is running.

• The stability of the Athena connection is improved.

#### Atlas > Load sample

The Atlas Alignment function is more robust, faster and has better accuracy.

#### EPU > Task Selection panel

- The tasks are grouped in collapsible Start, Preparation and Execution categories.
- The creation of a new EPU Session is split off from the Session Setup task and is now a separate task: Session Creation.

#### EPU > Hole Selection and Area Selection

In an Automated Preparation procedure, the Selection Brush remains active when moving to the next or the previous Grid Square.

#### **EPU > Automated Acquisition**

- The FEI2 Extension 2 MRC Header fields for *Detector Commercial Name* and *CFEG Flash Timestamp* are now filled in.
- The Start and Stop functions, and the Pause and Resume functions are now status-sensitive buttons:
  - When started, Start becomes Stop.
  - When paused, Pause become Resume.

### 5.4 Solved issues

Besides the Solved Issues that are listed below, numerous smaller issues have been solved that were not listed as Known Issues for preceding releases.

#### Solved in EPU 2.10

ID	Description	Remarks
EPU-6024	In the Screening task, the automatic Atlas alignment that is executed after Load sample can be inaccurate in case the objective aperture is inserted.	
EPU-6390	Low throughput with Gatan K2 camera.	_
EPU-6467	Failed to retrieve calibrated magnifications & insufficient memory.	—

Next to support for the *Thermo Scientific Selectris* and *Selectris X* energy filters, the EPU 2.9 release brings a variety of minor new features and improvements for a smoother user experience.

### 6.1 Mandatory and Breaking Changes

None identified.

#### 6.2 New features

#### General

The Thermo Scientific Selectris and Selectrix X energy filters are now supported. *Requires TEM Server 7.6 or later.* 

#### EPU > Hole Selection and Area Selection, and EPU > Automated Acquisition

In manual mode, the Alpha tilt angle at which each individual Grid Square is prepared is automatically applied again during the Automated Acquisition run.

#### EPU > Automated Acquisition

The MRC2014 FEI2 Extended Header image format is introduced.

Among other specification improvements, the FEI2 Extended Header specification contains more metadata fields that the FEI1 Extended Header. The FEI1 Extended Header format is now frozen, it will no longer be extended with new metadata.

Some of the new metadata fields in teh FEI Extended Header are not filled yet.

#### 6.3 Improvements

#### Installation

The Athena Connection settings have moved from the EPU.exe.config file to a new DMP.json file. The new file is not replaced during software installation. After the connection settings are verified once, it is no longer necessary to specify these settings at every EPU re-install or upgrade.

#### EPU > Preparation > Acquisition and Optics Settings

The default preset definitions are revised, so that they are require less adjustment and tuning for most experiments.

#### EPU > Session Setup

- It is now possible to set up a new Session for the specimen on the stage after the cassette from which the specimen was loaded is undocked.
- For *Type*: *Automatic* sessions, the Use Phase Plate option is automatically cleared and disabled.
- The Athena Settings Dataset selection is redesigned:
  - It is now possible to create a new Athena Dataset in EPU.
  - The Athena Portal can be accessed straight from the Dataset selection dialog.

 The Dose fraction output format options for the Gatan K3 camera are now also available for the Gatan K2 camera.
 Requires TEM Server 7.6 or later.

#### **EPU > Template Definition**

- The Template Definition task always shows the most recent Foil Hole image, regardless of the current stage position and regardless of the currently applied Acquisition and Optics Preset. This allows for easier adjustment of the Foil Hole Template during session setup and during the Automated Acquisition run.
- For *Session Setup > Acquisition Mode*: *Faster acquisition* sessions, the default Autofocus Area > Recurrence is After Centering

#### **EPU > Automated Acquisition**

The throughput for the Gatan K2 camera is improved. *Requires TEM Server 7.6 or later.* 

### 6.4 Solved issues

Besides the Solved Issues that are listed below, numerous smaller issues have been solved that were not listed as Known Issues for preceding releases.

#### Solved in EPU 2.9.0

ID	Description	Remarks
EPU-5818	Falcon 4 in linear mode still acquires images in counted mode.	
EPU-5683	I0 calibration warning popup during automatic execution of the Hole Selection / Area Selection task.	_
EPU-5865	Crosshair location on tilted samples is incorrect.	_

### 7 EPU 2.8.0 and EPU 2.8.1

The highlights for the EPU 2.8 release are:

- Atlas > Screening: Automatic Atlas Alignment of the existing Atlas after a specimen is loaded from the AutoLoader.
- EPU > Hole Selection and Area Selection: The Automated Preparation functions automatically prepare all selected Grid Squares according to the same selection and filter settings as the current manually prepared Grid Square.

### 7.1 Mandatory and Breaking Changes

None identified.

#### 7.2 New features

#### General

EPU now supports the Gatan BioContinuum filter with K3 camera Only on systems with TEM Server 7.5 or later (Titan 3.5 or later, Talos 2.5 or later).

#### Atlas > Screening

After Load Sample, the Automated Atlas Alignment functionality re-aligns a previously acquired Atlas with the physical position of the re-loaded specimen. This prevents the acquisition of a new Atlas after a specimen is (re-)loaded, while maintaining navigation accuracy.

#### EPU > Session Setup

The New Session function offers an option to create the new EPU Session with the same settings and parameter values as the current EPU Session. This is essentially a shortcut for saving the Preferences file for the current EPU Session, and then creating a new session from that Preferences file.

#### EPU > Hole Selection and Area Selection

The new Automated Preparation functions significantly decrease the time and effort that is needed to manually select the acquisition targets (Foil Holes or Acquisition Areas) for an Automated Acquisition run. After the suitable Grid Squares are selected and the first Grid Square is prepared, Automated Preparation offers the following functions:

- **Prepare All Squares**: EPU automatically performs the manual Hole Selection or Area Selection procedure in all selected Grid Squares:
  - Move to the next selected Grid Square.
  - Set the specimen to Eucentric height.
  - Acquire a Grid Square image.
  - Select Foil Holes or Acquisition Areas, using the selection parameters and filters of the current Grid Square.

If a Grid Square already contains a selection of Holes or Areas, then that existing selection remains '*as is*'.

• **Stop**: Aborts the automated preparation. The Eucentric Height values, Grid Square images and Hole or Area selections that are completed so far remain available.

If *Prepare All Squares* has been executed at least once in the EPU session, then the following functions can be used:

#### • Redo Preparation:

Use *Redo Preparation* after the parameters for Hole or Area selection have changed, for example after the Ice Filter or the Foil Hole diameter and interspacing is adjusted.

- In already prepared Grid Squares, *Redo Preparation* clears the Hole or Area selection, then creates a new selection. The Grid Square image and Eucentric Height value are maintained.
- For not yet prepared Grid Squares the *Redo Preparation* function performs the same actions as described above for the initial *Prepare All Squares* execution.
- Select **Prepare All Squares** again to prepare newly selected Grid Squares, or to complete an aborted preparation run. *Prepare All Squares* uses the current selection and filter values.
  - Already prepared Grid Squares remain as they are. If the selection and filter values have changed, then the existing selection is not adjusted.
  - For not yet prepared Grid Squares the *Prepare All Squares* function performs the same actions as described above for the initial execution.

#### EPU > Automated Acquisition

On systems with a CFEG, the tip is automatically flashed during the Automated Acquisition run to ensure a stable and optimal electron beam.

Only on systems with TEM Server 7.5 or later (Titan 3.5 or later).

### 7.3 Improvements

#### General

The Startup Check is extended with a Camera Offload Service (COS) version check. If Athena is available, then the Startup Check displays a warning if the COS software needs an update.

#### Preparation > Acquisition and Optics Settings

- The size of the Optics Settings > Get and Set buttons is increased to reduce the chance of unintentionally selecting the wrong function.
- In the Data Acquisition Preset for a Falcon 4 camera:
  - The Exposure Settings > Align option is now also available. Requires TEM Server 7.5 or later.
  - The Exposure Settings > Mode: Linear option is disabled to ensure that acquisitions are done in Counted or Electron Event Recording (EER) mode.
  - The limits for the *green area* in the **Dose Rate** are adjusted to better represent the capabilities of the Falcon 4 Sensor Package.
- On systems with a Gatan K3 camera, if CDS is enabled in Gatan GMS, then EPU will acquire images with CDS mode.
   Requires TEM Server 6.15.4, or TEM Server 7.5 or later.

#### Auto Functions > Auto Zero-Loss

The robustness of the Auto Zero-Loss function is improved.

#### Atlas > Screening and Atlas > Atlas Acquisition

- If the system does *not* have an AutoLoader, then the Screening task is not available. This is the same as preceding EPU releases.
- If the system has an AutoLoader, then the Atlas Acquisition task is no longer available. All Atlases are acquired in the *Screening* task. EPU automatically selects and aligns the Atlas for the specimen that is currently loaded on the Stage.
- The image display in the Screening task and in the Atlas Acquisition task now also offers the Color Enhancement option.

#### EPU > Session Setup

If the Storage folder is on the C: drive, then EPU shows a warning. The C: drive typically has not enough free disk space for the data volume that is generated in an Automated Acquisition run.

#### EPU > Hole Selection and Area Selection

If the Grid Square is set to Eucentric Height, then the Stage Z position in the lower left corner of the image display is green. If the Grid Square is not set to Eucentric Height, then the Stage Z value is white.

#### **EPU > Automated Acquisition**

For Faster Acquisition mode, the area in which the Foil Holes are collectively centered is reverted from 12  $\mu$ m back to a 6  $\mu$ m radius.

#### 7.4 Solved issues

Besides the Solved Issues that are listed below, numerous smaller issues have been solved that were not listed as Known Issues for preceding releases.

#### Solved in EPU 2.8.0

ID	Description	Remarks
EPU-3914	In the Hole Selection / Area Selection task, the warning for outdated I0 calibrations does not appear stay on top of the EPU main user interface.	
EPU-4460 TT44759	If the user does not have administrator privileges, then the Auto Functions calibrations cannot be stored.	The Auto Functions calibrations are no longer available for regular users.

#### Solved in EPU 2.8.1

ID	Description	Remarks
EPU-5795	Move stage to gridsquare sometimes inaccurate.	Introduced in EPU 2.8.0.
EPU-5776	LZW compression generates incorrect output file format.	Introduced in EPU 2.8.0.

The highlights for the EPU 2.7 release are:

- Introduction of the EPU Data Management Platform powered by Athena.
- Support for Falcon 4 Electron Event Registration (EER) acquisition.
- Multiple major and minor ease-of-use optimizations, including better streamlinedSquare Selection and Hole Selection tasks.

### 8.1 Mandatory and Breaking Changes

None identified.

#### 8.2 New features

General

EPU now supports the following Data Management Platform (DMP) functionalities:

- Athena for acquisition data management.
- EPU Quality Monitor (EQM) for live quality assessment of the acquired data.

#### Note EPU Quality Monitor (EQM) is a license-protected commercial option.

#### Preparation > Acquisition and Optics Settings > Camera Settings

For Falcon 4, EPU now supports the Electron Event Registration (EER) mode.

In EER mode, the camera records the individual electrons that hit the Sensor Package. The acquired data consists of an \*.eer file with a Gain Reference image, and an integrated image. The EER data requires special software for processing.

#### Preparation > Auto Functions > Auto Functions (TEM)

The new Auto Zero Loss function automatically maintains the proper alignment of the energy slit in the filter with the Zero Loss Peak.

#### EPU > Session Setup

The Athena Settings section is added to:

- Select a Dataset, Sample and Grid.
- Enable EPU Quality Monitor (EQM).

#### EPU > Square Selection and Hole Selection

Multiple new features and functions are added to significantly improve the ease-of-use of the Square and Hole Selection tasks. See the EPU User Manual for a detailed explanation of the streamlined workflow.

#### **EPU > Template Definition**

The **Defocus List** values can now be copied to all Acquisition Areas in the Template Definition at any time.

#### EPU > Automated Acquisition

- At the start of an Automated Acquisition run, EPU displays a notification if the AutoLoader TMP is running.
- For Gatan K3 cameras, the Dark Reference image is automatically refreshed at the start of each Grid Square.

Requires TEM Server 7.4 or later.

### 8.3 Improvements

#### Preparation: > Acquisitions and Optics Presets

The Image Display > Slider for the Falcon Electron Counting preview images is now cyclic.

#### **Preparation > Atlas Optics Alignment**

It is no longer mandatory to complete an ongoing Atlas Optics Alignment procedure. Select Stop to abort the alignment and revert to the current alignment.

#### Atlas > Screening

The Slot Positions user interface is simplified.

#### **EPU > Square Selection**

The Grid Square Detection algorithm is improved for Grid Squares with lower transparancy that have a smaller intensity contrast with the surrounding Grid Bars.

#### **EPU** > Hole Selection

- The Hole Detection algorithm and the Remove Holes Close to Grid Bar are improved for Gold Grid specimens (also known as UltrAuFoil™).
- The Remove Holes Close To Grid Bar filter is now a toggle that hides or shows the holes that meet the filter criteria.
  - It is no longer necessary to select Find Holes to undo the Remove Holes Close To The Grid Bar filter. If the result of the filter is not satisfactory, then select Remove Holes Close To Grid Bar again to revert the filter and automatically run the Find Holes function. The Ice Filter is automatically applied, but any manual selections are discarded.
  - Adjusting the Ice Filter values no longer clears the Remove Holes Close to Grid Bar filter.
- The responsiveness of the **Selection Brush** is improved.
- The **Calibrate I0** function is removed from the ribbon bar in the Hole Selection task.
  - The Calibrate I0 task is still available in the Preparation tab.
- The **Image Display** > Right-click > **Move Stage Here and Acquire** option is now only available when no foil holes have been selected yet. If the *Move Stage Here and Acquire* option were still available, then accidentally selecting it would overwrite the Hole Selection or Area Selection for the involved Grid Square.

#### EPU > Automated Acquisition

For Faster Acquisition mode:

- The area in which the Foil Holes are collectively centered is enlarged from a 6  $\mu m$  to a 12  $\mu m$  radius.
- The efficiency of the calibration that is executed at the start of the Automated Acquisition run is improved to decrease overhead.

### 8.4 Solved issues

Besides the Solved Issues that are listed below, numerous smaller issues have been solved that were not listed as Known Issues for preceding releases.

ID	Description	Remarks
EPU-986	EPU User Interface hangs for a while when starting the Automated Acquistion	
EPU-4319	Access denied could happen when creating a new folder when setting the output directory	

### 9 EPU 2.6.0 and EPU 2.6.1

### 9.1 Mandatory and Breaking Changes

None identified.

#### 9.2 New features

#### Preparation > Acquisition and Optics Presets

The Falcon 4 camera is now available.

#### Atlas > Screening

- Reset Selected reverts the selected Slot Position to its initial status and erases the Atlas. If desired, a new Atlas can be acquired.
- It is now possible to specify the number of tiles for the screening Atlases. The specified Number of tiles applies to all Slot Positions.

#### EPU > Square Selection

- The Image Display > Color Enhancement function applies a color mapping to the intensity values in a gray-scale image. This makes it easier to recognize intensity gradients and areas with similar intensity.
- If the Atlas is loaded from a Screening session, then:
  - The default selection no longer contains all detected Grid Squares, but only the selected Grid Square categories in the Screening Atlas.
  - The automatic Grid Square Suggestion function is active. Grid Square Suggestion is a
    machine learning algorithm that identifies and highlights multiple Grid Squares that are not
    included in the selection yet, but that have similar properties as the existing selection.
    With the Smart Extend function, the best match of the suggested Grid Squares is added to
    the existing selection.

#### EPU > Hole Selection

The Image Display > Color Enhancement function applies a color mapping to the intensity values in the image. This makes it easier to recognize intensity gradients and areas with similar intensity.

#### EPU > Automated Acquisition

• In Faster Acquisition mode, the Automated Acquisition run starts with an automatic Hole Position Correction calibration. This calibration acquires a limited number of Foil Hole images with image shift. This calibration can take up to 3 minutes.

The *Move stage to location* function also uses the corrected Foil Hole coordinates. Because the Hole Position Correction Calibration function does not acquire a new Grid Square image, the red crosshair in the Image Display may end up at a small offset from the Foil Hole center in the Grid Square image.

• To improve targeting accuracy, the Automated Acquisition procedure executes an automatic Hole Position Refinement function at the start of each Grid Square. This function updates the coordinates of the Foil Hole centers in the Grid Square.

#### 9.3 Improvements

#### General

- Responsiveness of the User Interface is improved. This is especially notable when switching between presets in Preparation > Acquisition Optics.
- The user friendliness of the Histogram side panel and the Filter Ice Quality side panel is improved. It is easier to relocate the left and right boundary and the Gamma curve.

#### Preparation > Acquisition and Optics > Presets

- The C2 Aperture for the Atlas Preset is no longer fixed at the largest aperture.
- The Import function ignores Presets that are not native to EPU.
- Measured Dose Rate values are now also reset when the energy filter parameters change, for example the slit width.
- The Auto Functions > Tasks > Calibrations section is collapsed by default to prevent accidentally starting a calibration instead of the stand-alone task execution.

#### Atlas > Screening

- If a Slot Position can not be scheduled, then a tooltip shows the reason why the Scheduling checkbox is disabled.
- The Column Valves are opened automatically at the start of a Screening acquisition.

#### EPU > Session Setup

Acquisition Mode > Faster Acquisition is no longer a Beta functionality.

#### **EPU > Square Selection**

The Gridsquare detection algorithm is significantly improved, which results in more reliable screening and automated acquisition.

#### EPU > Automated Acquisition

The Foil Hole positioning accuracy is improved.

### 9.4 Solved issues

Besides the Solved Issues that are listed below, numerous smaller issues have been solved that were not listed as Known Issues for preceding releases.

#### Solved in EPU 2.6.0

ID	Description	Remarks
EPU-1874	Incorrect imagebeamshift info in acquired data	Metadata in Gatan K2/K3 images

#### Solved in EPU 2.6.1

ID	Description	Remarks
EPU-4525	Not all GridSquares found in Atlas	Introduced in EPU 2.6.0

Chapter | EPU 2.6.0 and EPU 2.6.1

### **10.1 Mandatory and Breaking Changes**

None identified.

#### 10.2 New features

#### Preparations > Acquisition and Optics Presets

- The most recent acquired preview for each Preset remains available until a new Preview is acquired. On the preview image, the context menu provides navigation options.
- In the Data Acquisition Preset it is possible to select the C2 Aperture. The other Presets automatically use the same C2 Aperture, except for the Atlas Preset, which will always use the largest available C2 Aperture.

#### Atlas > Screening

For systems with Titan 3.X or Talos 2.X, when a new Screening Session is created, the slot descriptions are imported from the TEM User Interface > Autoloader control panel.

#### Atlas > Acquire Atlas

The Load from Screening function imports the Atlas for the loaded specimen from the Screening task. This way, no new Atlas needs to be acquired. If the specimen has rotated or has otherwise shifted, then it is still possible to acquire a new Atlas.

#### 10.3 Improvements

#### EPU > Template Definition:

- The Defocus List is simplified, so it is much more user-friendly to create, edit and remove defocus values, and to copy defocus lists to existing Acquisition Areas.
- The position of an Acquisition Area relative to the center of a Foil Hole is no longer specified in separate X and Y distances, but as the radial distance. This makes it easier to compare the offset with the maximum Image Shift.

#### EPU > Setup Session

The selection of values and settings that is supported by the Session Preferences is extended with Hole Selection, Area Selection and Automated Acquisition Settings (incl. Phase Plate settings). This makes it faster and easier to setup new EPU sessions.

#### EPU > Hole Selection and EPU > Automated Acquisition

The accuracy of the Find Holes function is significantly improved. This reduces the risk of acquiring images that are not usable for high-quality 3D reconstruction.

### 10.4 Solved issues

Besides the Solved Issues that are listed below, numerous smaller issues have been solved that were not listed as Known Issues for preceding releases.

ID	Description	Remarks
EPU-2089	Foil hole numbering goes out-of-sync after Skip Hole during a long run	
EPU-1076	Defocus list is not copied from selected Acquisition Area.	

EPU 2.4 is the first release that supports:

- Systems with a Windows 10 operating system. These are systems with Titan 3.X or Talos 2.X software.
- The Gatan BioQuantum 1967 filter with K3 camera.
   Features for the Gatan BioQuantum filter with K3 camera are only available on systems with a TEM Server version that supports an *embedded* Gatan BioQuantum filter with K3 camera.

### 11.1 Mandatory and Breaking Changes

None identified.

#### 11.2 New features

#### Atlas > Atlas Acquisition and Screening

The Objective aperture mechanism retracts automatically before the acquisition starts, and returns to its prior position after acquisition ends.

#### Atlas > Screening

The new Load to Stage function loads the cartridge from the highlighted slot.

#### EPU > Session Setup

- For the Gatan BioQuantum with K3 camera, the *TIFF LZW* image format is added. LZW is a lossless compression technique to decrease the file size of the images.
- The *Save preferences* function stores an EPU Session Preferences file. In EPU 2.4, this file contains:
  - All Acquisition and Optics Settings presets.
  - The results for the Image Shift calibration, I0 calibration, and the Atlas Optical Alignment.
  - The EPU Session Setup values, except for the session's name and description, the Storage folder, and the Email settings.
  - The Template Definition for Quantifoil specimens.

The EPU Session Preferences file contains all available information. It is not necessary to define all the above information before an EPU Session Preferences file can be stored. The contents may be extended for future releases.

• The *New from preferences* function creates a new EPU Session and loads the values from the selected EPU Session Preferences file. Any existing values are overwritten with the available values. If no value is available in the EPU Session Preferences file for a specific parameter, then the existing value is maintained.

It is not possible to load an EPU Session Preferences file into an existing EPU Session.

#### EPU > Automated Acquisition

- When the conditions for the *Faster Acquisiton* mode are met, then EPU automatically uses Faster Acquisition mode for Lacey Carbon specimens. For Quantifoil specimens it remains a user setting.
- The automated run stops automatically when the column valves are closed, for example due to vacuum or cooling issues. Depending on the *Session Setup > Email settings*, an email is sent when the run stops.

### 11.3 Improvements

#### Preparation > Acquisition and Optics Presets > Advanced Camera Settings

For Gatan BioQuantum with K3 camera, the Counted mode replaces the Linear mode for all presets. The Data Acquisition preset also offers the Counted / Super Resolution mode.

### 12.1 Mandatory and Breaking Changes

None identified.

#### 12.2 New features

#### EPU > Session Setup

For Quantifoil specimens, a new Faster Acquisition mode is added. In Faster Acquisition mode, EPU saves time by centering groups of Foil Holes instead of each individual Foil Hole. If the system configuration and optics conditions allow for it, EPU can save even more time by using Image/Beam Shift instead of Stage Shift to visit all Foil Holes in the centered group.

To use Image/Beam Shift:

- The system must not have an Image Corrector.
- Phase Plates must not be used.
- AFIS (Aberration Free Image Shift) must be calibrated. AFIS is available on systems with TEM 6.15 and later.

The conventional centering method is now named Accurate Hole Centering.

#### **EPU > Template Definition**

For Quantifoil specimens, an Export and Import functionality are added for the Foil Hole Template. The Template file contains all Template Areas, their positions and their settings. When a Template file is imported for a new Quantifoil specimen, EPU scales the Template Area positions depending on the Foil Hole diameter ratio between the source specimen and the target specimen.

#### 12.3 Improvements

#### General

The Zoom functionality in the image display is simplified. The slider has been removed. Also the [+] and [–] keys can no longer be used for zooming in and out. The scroll wheel of the mouse is the only remaining zoom control.

#### EPU > Session Setup

The Use Phase Plate checkbox has moved from the Automated Acquisition task to the Session Setup task.

#### **EPU > Template Definition**

It is no longer possible to schedule multiple acquisitions per Acquisition Area. The Defocus List is still available with the same functionality as before.

### **13.1 Mandatory and Breaking Changes**

None identified.

#### 13.2 New features

The Accelerated Phase Plate activation functionality supports the new generation of slow activating phase plates.

#### 13.3 Improvements

#### **Preparation > Acquisition and Optics Presets**

The Dose Measurement and Dose Fractions functionalities for the Falcon 3EC camera are improved.

### 14.1 Mandatory and Breaking Changes

None identified.

#### 14.2 New features

EPU 2.1 does not introduce new features. The main purpose of EPU 2.1 is to:

- Restore functionalities and performance levels of EPU 1.12 that could not be migrated from EPU 1.12 to EPU 2.0
- Solve issues that are reported by EPU 2.0 Beta customers.

### 14.3 Improvements

#### General

- Improved responsiveness of the User Interface.
- Improved image quality of the Image Display.
- The EPU User Manual is reworked from a high-level guide towards stepwise instructions, similar to the Cryo-EM SPA Workflow app.

#### EPU > Automated Acquisition

Increased throughput rate.

### 15.1 Mandatory and Breaking Changes

Note EPU 2.X does *not* support the Falcon II camera.

#### 15.2 New features

**Preparations** > **Acquisition and Optics Settings** The *Thon Ring* preset is added.

#### **Auto Functions**

- Autocoma (licensed functionality) is introduced.
- Autostigmate (licensed functionality) is introduced.

**Atlas > Screening** functionality is introduced.

### 15.3 Improvements

- Rebranding from FEI to Thermo Scientific is completed:
  - The User Interface is fully redesigned.
  - File locations have been aligned with the Thermo Scientific brand. (see details below)
- The Results Viewer is dropped. Install the Thermo Scientific Imaging Codec Pack to view images with built-in Windows image viewers.
- The Dose Fractions functionality is simplified. The detailed Fraction Advisor is dropped.
- The temporary Trial license is no longer available.

The new file locations are:

• Session files:

```
C:\Users\<user account>\AppData\Roaming\
Thermo Scientific EPU\Sessions\
```

• Parameters and setting files:

```
C:\Users\<user account>\AppData\Roaming\
```

Thermo Scientific EPU\Parameters\

The Parameters folder replaces the EPUShell directory that was used in EPU 1.X versions. When installing EPU 2.X for the first time, the contents of the EPUShell directory are migrated to the Parameters folder.

• Omp.config file: C:\ProgramData\Thermo Scientific EPU\Configuration

#### • Application and Installation log files:

C:\ProgramData\Thermo Scientific EPU\Log\ C:\ProgramData\Thermo Scientific EPU\Log\Installation\

### 16 EPU 1.12

EPU 1.12 is a compatibility release. As such, this release:

- Adds support for the most recent TEM Server versions on the supported microscopes.
- Does not introduce new features.
- Solves multiple functionality and performance issues.

#### Note EPU 1.12 is the last release that supports the Falcon II camera.

### 16.1 Mandatory and Breaking Changes

None identified.

### 16.2 New features

No (major) items.

#### 16.3 Improvements

No (major) items.

### 17 Known issues

A large amount of effort is spent on adding and improving workflows and on continuous quality improvements. The most recent release of EPU has the following Known Issues. Historic Known Issues that are solved in a released software version are not listed.

ID	Issue Description and Workaround
6334 After executing the template in <i>EPU</i> > <i>Template Execution</i> , there is acquired images. The acquired images are displayed during exect image is removed from the display as soon as a new image is acc	After executing the template in <i>EPU</i> > <i>Template Execution</i> , there is no way to inspect the acquired images. The acquired images are displayed during execution, but an acquired image is removed from the display as soon as a new image is acquired.
	Workaround: Not available

ID	Issue Description and Workaround
TT115829	Throughput issue when using K2 on BioQuantum. Calls to the energy filter can add significant delays. These can happen when switching between data acquisition and foil hole acquisition or between data acquisitions, depending on which function creates the problem.
	Workaround: Reset the camera and GMS. If that does not help, then reboot the Gatan PC.

ID	Issue Description and Workaround
TT121858	If the Atlas session folder is moved or renamed, then it is not possible to manually load an Atlas. In the EPU User Interface, a critical error appears that the Atlas.dm file may be corrupt.
	Workaround: Close EPU, restore the Atlas session folder path, start EPU, and try again.

ID	Issue Description and Workaround	
EPU-3040	On some systems, the displayed Dose is incorrect when Binning is not 1.	
	Renew the Counts-to-Electron calibration for Linear mode	

ID	Issue Description and Workaround	
EPU-4002 EPU-3693	Camera names and/or microscope settings are incorrect after a camera upgrade from Gatan K2 to Gatan K3.	
	Stop EPU     For all user accounts, delete the EFFEN Missions complete times, completions,	
	• For all user-accounts, delete the EFTEM_MicroscopeSettings.sxml life in the %appdata%\Thermo Scientific\EPU\Parameter\ folder.	

### 18 Copyright, Limited Rights and Revision History

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#### **Revision Table**

Revision	Date	Description of Changes
2.6	20-JAN-2020	<ul> <li>Revised the document structure.</li> <li>Scope is limited to: <ul> <li>EPU 1.12 (the final EPU 1.X release)</li> <li>all EPU 2.X releases</li> </ul> </li> <li>For an overview of changes in historic revisions, please see the Release Notes document that is available in EPU 2.5 and/or earlier releases.</li> </ul>
2.6.1	10-FEB-2020	Update for EPU 2.6.1
2.7	16-APR-2020	Update for EPU 2.7
2.8	25-JUN-2020	Update for EPU 2.8
2.8.1	03-SEP-2020	Update for EPU 2.8.1
2.9	13-OCT-2020	Update for EPU 2.9
2.10	19-JAN-2021	Update for EPU 2.10
2.11	14-APR-2021	Update for EPU 2.11
2.11a	29-APR-2021	Add known issue EPU-7348 and patch for EPU 2.11
2.11.1	31-MAY-2021	Update for EPU 2.11.1
2.12	01-JUL-2021	Update for EPU 2.12
2.12.1	04-AUG-2021	Update for EPU 2.12.1
2.13	12-OCT-2021	Update for EPU 2.13

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