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TiltEM User Manual

August 2023

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U.S. DEPARTMENT OF ENERGY

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Overview

The ability to automate the scanning transmission electron microscope (S/TEM) is tantamount to addressing next generation artificial intelligence, machine learning, and materials modeling capabilities. Constant utilization of these high-end capital equipment purchases also serves the requirements of the Department of Energy to be fiscally responsible to the public. The development of an automated tilt series algorithm for darkfield/bright field S/TEM imaging has been achieved at PNNL.

This automated algorithm, TiltEM, allows users to collect oblique orientation tilt series (i.e., at any orientation with respect to the primary stage tilt axes) in the Gatan Microscopy Suite (GMS) with high fidelity through a range of tilt angles approaching ~±35°. These data provide a richer, more robust picture of the complex microstructures observed in additively manufactured samples, thereby allowing researchers a more comprehensive analysis of their work. The ability to operate in the GMS environment also makes this software package microscope agnostic. This lays the groundwork for future research that will include multimodal analysis, including energy dispersive spectroscopy (EDS) data.

This document covers the installation and operation of the TiltEM automated tilt series scripts. TiltEM is a set of Python scripts designed to run in the Gatan Digital Micrograph (DM) scripting environment. TiltEM is used to automatically produce a series of images on one or more detectors tilted around a user specified axis. TiltEM is supported for DM V3.5.3 or later.

Summary

As part of the US Department of Energy Office of Nuclear Energy (DOE-NE) Advanced Materials and Manufacturing Technology (AMMT) program this work focuses on creating an automated tilt series program for scanning transmission electron microscopy (STEM) in the Gatan Microscopy Suite (GMS) platform.

This program will be microscope agnostic as the GMS platform can be utilized on a wide range of STEM instruments.

The software interfaces with GMS to take user input as to desired location and orientation of the tilt series as well as required tilt increments for the series. Using an advanced algorithm, the program calculates precise, incremental steps in both alpha and beta tilts that input directly into the microscope controls. In-between sequential tilt steps the program utilizes a series of autofocusing routines to collect images that are utilized in aligning the sample to the previous images.

Once completed, the images can be stitched to create a movie which provides three-dimensional, microstructural information of the sample.

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User Manual

Operation Statement:

TiltEM controls the stage movements (including X,Y,Z, α, and β) as well as collects digital images from any number of cameras/detectors. Pacific Northwest National Laboratory assumes no liability to any damage to cameras/stage/hardware/software caused through the installation/use of TiltEM to operate any electron microscope. TiltEM is considered beta software and is not considered for commercial use. PNNL provides no performance guarantees with regards to stage precision.

Accessing TiltEM:

TiltEM is currently located on a Pacific Northwest National Laboratory (PNNL) GitLab repository. Access is granted to other national laboratories through assignment of a user account. This can be done by contacting the PNNL IT department.

Prerequisite Software:

TiltEM requires the installation of GMS 3.53 or later.

Gatan GMS software can be downloaded from Gatan.com.

This version of GMS is Python compatible and allows TiltEM to operate within the GMS environment. GMS versions prior to GMS 3.53 will not run TiltEM.

The GMS software **must have access and control** over a transmission electron microscope (TEM). This includes control of stage movements (including alpha, beta, X, Y, and Z) as well as capture of the digital signal from at least one STEM detector (e.g., annular darkfield).

Note: GMS software is typically installed by Gatan or microscope service engineers during the installation of a microscope. Updating of GMS software should be performed by a qualified service engineer, preferably under a service contract agreement. Updates to GMS have been known to be unstable in the past that have deleted preference files to hardware such as the GIF. PNNL assumes no liability for installation/updating of GMS software. Prior to installation it is recommended that a clone be made of the hard drive operating GMS software.

Installation:

Begin by making a clone of the TiltEM repo. This repo contains all script files and a copy of the modified Gatan venv.

TiltEM is a set of scripts developed by PNNL to run in the Digital Micrograph (DM) scripting environment. Modifications to the scripting environment are required.

First, all scripts used by the system must be in DM's default path.

The easiest way to support this is to copy the scripts to DM's executable path (C:\Program Files\Gatan). The files to be copied are:

- GUI.py
- TipTilt.py
- Tilt_utilities.py
- Gatan_utilities.py
- AlignEM.py
- StitchEM.py
- FocusEM.py
- Lookup.py

To support TiltEM, the Gatan venv (C:\ProgramData\Miniconda3\envs\GMS_VENV_PYTHON) needs to be modified. To do so, rename the existing venv to:

C:\ProgramData\Miniconda3\envs\GMS_VENV_PYTHON_original

<u>This step is important in order to have a backup in case of system failure or file corruption</u>.

Then, copy the modified venv from the GIT clone to the original's location.

Sample Loading Prerequisites:

The following instructions assume the microscopist has properly loaded a sample in the TEM and is in STEM mode. For the given imaging condition (e.g., brightfield or darkfield) the user will have selected the optimum conditions (e.g., mag, probe size, camera length) and has located a region of interest for performing an automated tilt series. This program assumes the alpha tilt axis of the microscope is vertical to the STEM image.

Step 1: Open and run GSM 3.53 by clicking on the GMS executable icon on the desktop (Figure 1)

Figure 1: Staring GMS 3.53 by clicking on the GMS desktop icon.

Step 2: Once GMS is running, open the folder containing GUI.py and double click the script (e.g. arrow in Figure 2).

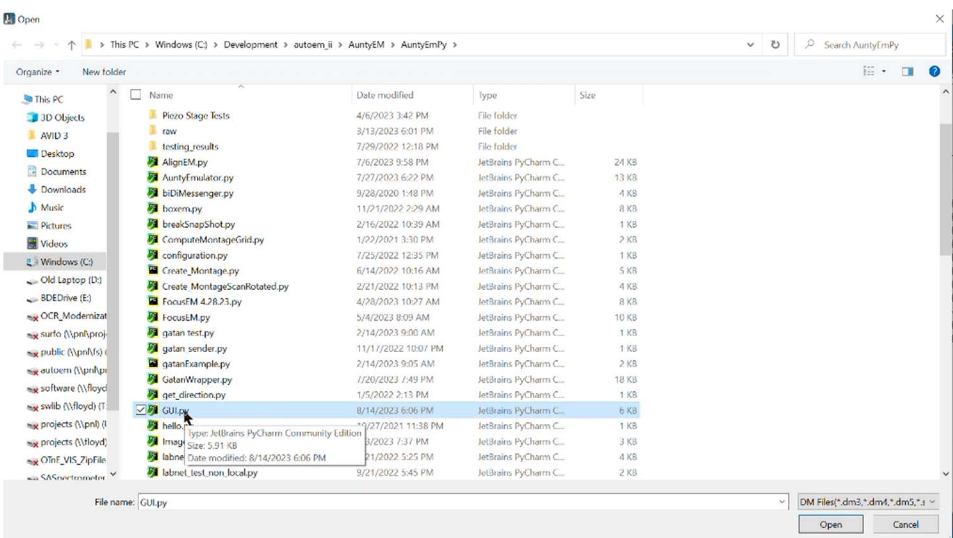

Figure 2: Windows screenshot showing the example location of the GUI.py script.

Step 3: The GUI prompt will start within GMS (Figure 3). Click on the Execute button in the lower left corner and select the root directory for the tilt data (e.g., c:\Tiltdata). All time stamped directories will be created under this directory as data is acquired.

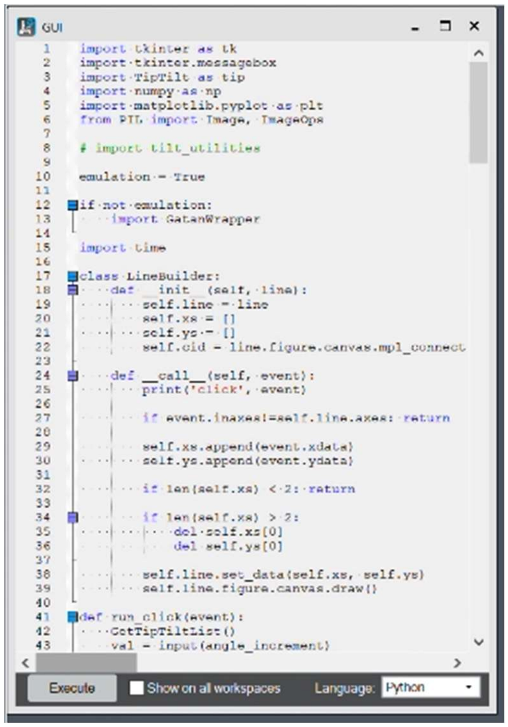

Figure 3: TiltEM GUI run screen. The program starts when the Execute button (lower left corner) is started.

Menus

An explanation of the various menu options is provided below. These menus will be utilized during the operation of TiltEM.

- File/Select Data Directory – selects the root directory for data. Individual tilt series directories will be created using a timestamp in this root.
- Tilt/Show Points – Shows a graph of the points that will be generated around the currently selected tilt axis. If Orthogonal flag is selected, the points will be orthogonal to the selected axis.
- Tilt/Select Axis:
  - Creates an image at the current location with the selected detector.
  - Selects current location/tilt as the center of the tilt series
  - User clicks on the image to define the tilt axis

Step 4: The user needs to find a desirable region of interest (ROI) within the sample (see Sample Loading Prerequisites) and then subsequently collect a focused image on the desired detector (e.g., JEOL DF detector).

The user is then directed to import the centered/focused image into TiltEM. Once the region of interest is selected (Figure 4) the user is directed to draw a line by clicking on two points (see blue line in inset of Figure 4). This will be the logical tilt axis by which the sample will tilt across. If the user is not satisfied with the drawn line, subsequent clicks can be performed to reset the line.

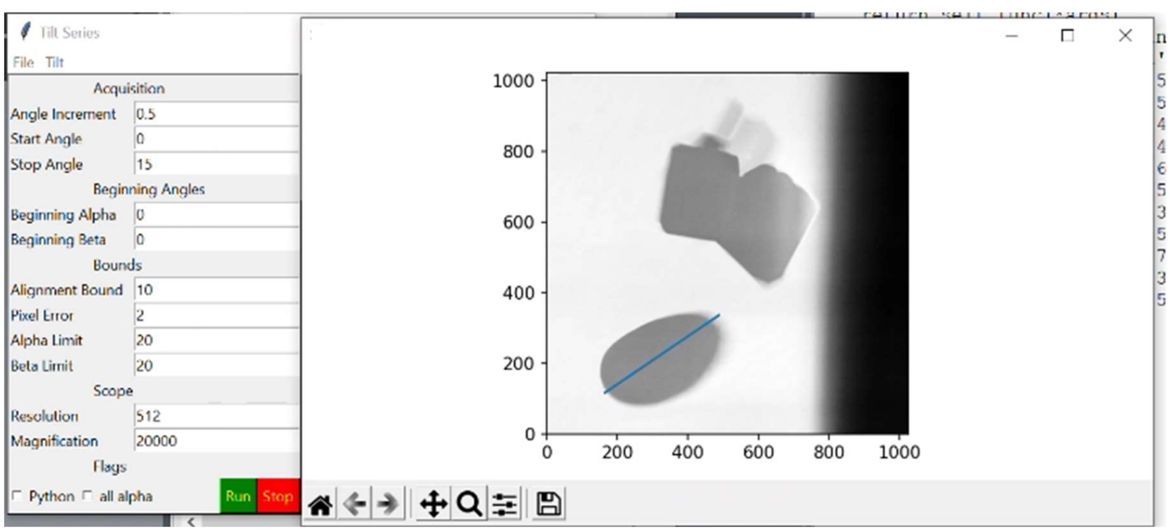

Figure 4: Tilt Series GUI with region of interest captured.

Step 5: Once the directionality of the tilt axis is defined, the user is then required to input the starting microscope parameters (e.g., beginning angles) and the conditions for the tilt series (e.g., acquisition, bounds).

Parameters:

An explanation of the parameters shown in Figure 4 are listed below.

- Acquisition
  - Angle Increment – (floating point) angle step increment around the selected tilt axis.
  - Start Angle – (floating point) used to generate a compound angle (alpha, beta) that will be the starting point of the tilt series.
  - Stop Angle – (floating point) used to generate a compound angle (alpha, beta) that will be the stopping point of the tilt series.
- Beginning Angles
  - Beginning Alpha – (floating point) Center point of the series in Alpha. Selected when the tilt axis is created.
  - Beginning Beta – (floating point) Center point of the series in Beta. Selected when the tilt axis is created.
- Bounds
  - Alignment Limit – (integer) maximum number of images to be tried when aligning to the reference image used for axis definition.
  - Pixel Error – (integer) absolute number of pixels in X and Y the alignment can be off before considering the current image aligned with the reference image. If this criteria is not met, the alignment process continues until it is met, or the Alignment Limit is met.
  - Alpha Limit – (floating point) Limit that the tilt stage can safely be set in Alpha. If a point generated for the tilt series exceeds this limit in the positive or negative direction, the user will be warned, and the series will not be attempted.
  - Beta Limit – See Alpha Limit
- Scope
  - Resolution – (integer) image resolution of the images to be acquired in pixels.
  - Magnification – (integer) Magnification of the scope
  - Detector – (string) detector to be used as reference for alignment and focus.
- Flags
  - Orthogonal = (Boolean) if checked, the tilt will be orthogonal to the selected tilt axis.

Step 6: Once all settings are inputted the user clicks the green Run button in the lower left-hand corner. This will start the automation routine.

Step 7: All images will be sent to the directory folder selected in Step 3. Users are then directed to take each image and enter them into an image processing software such as Photoshop or ImageJ to create stacks and subsequent movies.

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