Darkfield 360 Toolkit

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# System Requirements

Tested with Gatan Digital Micrograph version 1.8 / 2.3 using Windows XP / Windows 8

# Toolkit Information

# Running and Installing the Toolkit

The toolkit does not require any additional software other than Digital Micrograph and does not require root/admin access to the computer in order to be run. The simplest way to run the toolkit is to open the script file (DF360Toolkit.s) in Digital Micrograph and execute the code each time you wish to use it. This is shown in Figure 1 (a).

Figure 1: (a) Script ready to be executed (b) Install Script dialog

The toolkit can also be installed on the Menu bar at the top of the screen so a user can start the toolkit simply by selecting it. This is achieved by selecting the script file after choosing File → Install Script File. Digital Micrograph will then open a dialog shown in Figure 1 (b) for the user to customize. The name of the command will appear in the menu, so it should be named something descriptive such as “Run Darkfield 360 Toolkit”. The “Which Menu?” field allows the user to place the command inside a sub-menu from the menu bar if desired. It is recommended to put the script inside a menu called “DF360”. The “Optional sub-menu” field allows the user to name an additional sub-menu inside the previously defined menu. This is not recommended for the toolkit. The final option is to install the script for all users or just the current one. It is recommended that the script be installed for all users, but this will depend on the access rights and configuration of the computer system.

# The Toolkit Interface

Figure 2: The toolkit interface components (a) Live View Window (b) Toolkit control (c) Results Window

(b)

(c)

(a)

The DF360 Toolkit is made up of three interactive components shown in Figure 1. The Live View Window (a) is created whenever the digital camera is producing a live image and should be familiar to anyone using the Digital Micrograph software. The Results window (c) is also a part of Digital Micrograph and can be accessed at any time from Window → Show Output Window. Text will appear in the results window to provide feedback to the user. Feedback includes function progress, file names, toolkit settings and other potentially helpful information. The Toolkit is controlled from the control window (b) by pressing the buttons with the mouse and entering values into text fields. The layout and available functions in the toolkit are discussed below.

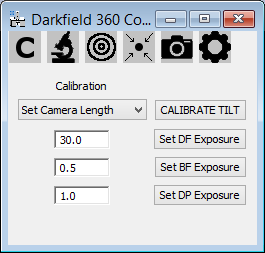
## Toolkit Sections

Figure 3: Buttons to access toolkit sections

Each section of the toolkit is accessed by pressing one of the buttons that are always present at the top of the toolkit window, as shown in Figure 2. The features of each section are described below.

### Calibration



The calibration section controls how the toolkit works with the microscope’s digital camera and tilt system. If there is no microscope present you can still set the camera length for working with stored images to correct their scales.

**Set Camera Length:** Choose the camera length setting used to display the diffraction pattern. **This should be the first thing the user does when using the toolkit**. The scale factor displayed on the Digital Micrograph Live View screen may or may not be the correct scale when operating in the diffraction mode. The correct scales have been calculated and recorded in the toolkit, so when looking at the live view screen or previously saved images the functions such as image tilting and ring measuring will be the correct value, independent of the default scale used by the microscope.

**Set DF Exposure:** This is the length of exposure time used when taking a Dark Field image. It is in units of seconds. For weak beam conditions the signal intensity can be relatively low, so taking an image over 10-30 seconds is recommended.

**Set BF Exposure:** This is the length of exposure time used when taking a Bright Field image. It is in units of seconds. The optimum exposure time will change with the brightness of the central beam, but it is usually much less than for taking dark field images.

**Set DP Exposure:** This is the length of exposure time used when imaging a diffraction pattern. It is in units of seconds. Generally the brightest spots are clear with less than a second of exposure, but the dimmest diffraction spots may take longer.

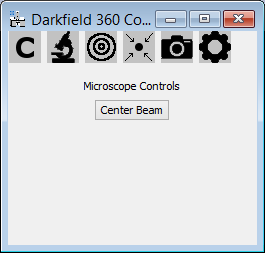
**CALIBRATE TILT:** Pressing this button will begin the tilt calibration process. **Make sure that either Bright Tilt or Dark Tilt mode are selected** (CLA on the alignment control dialog on the control computer) The first step is to use the Def/Stig dials to centre the diffraction pattern and then record the X/Y tilt values that correspond to this default position. The second step is to calibrate the X-Tilt scale by moving the diffraction pattern to approximately the d-spacing you will be working at, using **only** the X-tilt control. The change in X-tilt and the distance moved by the diffraction pattern will then be recorded. The third step is the same as step two but uses only the Y-Tilt control. These values are used by the toolkit to tilt the beam the correct amount, so if the calibration is not performed correctly it will reduce the accuracy of the system.

Note: The diffraction pattern is likely to drift during a TEM session and should be re-calibrated to keep it centred. When performing a repeat calibration to adjust the centre point the second and third steps can be omitted by selecting “Yes” when asked “Use existing tilt values?” Any points targeted *before* the pattern drifted will still be accurate because they are recorded relative to the central point, not just as absolute values. If the X/Y tilt settings are changed then any targets stored previously may no longer be accurate.

### Microscope Controls



The Microscope Controls section contains buttons to control how the microscope itself behaves.

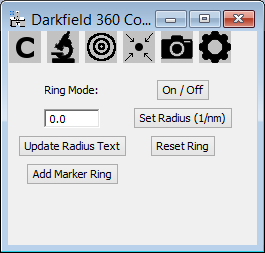


**Center Beam:** Pressing this button moves the diffraction pattern back to its central position. If the diffraction pattern has drifted since the central position was set then you can run the “CALIBRATE TILT” process in the Calibration section.

### Ring Controls



The Ring Controls section allows the toolkit to measure the d-spacing of diffraction rings, target an entire diffraction ring for dark field imaging and to add different coloured rings onto images to provide a visual reference.



**Ring Mode:** When ring mode is ‘On’ the red marker ring will appear on the live view image. Turning ring mode off makes the marker ring and its radius measurement invisible.

**Set Radius:** The marker ring can be set to a radius of your choosing by entering the radius (in units of 1/nm) in the field and then pressing the button. The marker ring will change radius and update its measurement reading.

**Update Radius Text:** The marker ring radius is displayed in units of 1/nm and Angstroms on the live view image. However, this value will not automatically change when the marker ring is manually re-sized by clicking and dragging it. Pressing this button forces the text read-out to update to the new measurement.

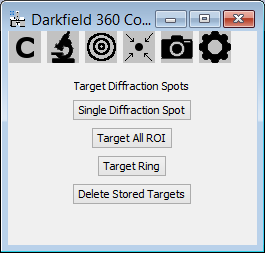
**Reset Ring:** Pressing this button will force the marker ring to become circular. This button can be useful if you manually adjust the marker ring and accidentally leave it as an ellipse.

**Add Marker Ring:** Rings of different colours and radii can be added to any open image. These rings are purely for visual reference and do not control any other functions. Once added, the rings can be re-sized, moved or edited in any way. However, their corresponding radius measurement will not be updated.

### Diffraction Spot Targeting Controls



The Diffraction Spot Targeting controls are used to store the X/Y tilts that bring the desired diffraction spots to the centre of the screen for dark field imaging. There are three different ways to enter these values into the toolkit memory.



**Single Diffraction Spot:** Pressing this button will record the current tilt settings for future imaging. You can manually centre points of interest and record them this way in order to be certain of the tilt values used. However, this can be time consuming for significant numbers of points.

**Target All ROI:** Regions of Interest can be added to the live view window in the Digital Micrograph software. Add **Points** of interest to the live view image in order to mark the diffraction spots you wish to image, then press this button to have the toolkit calculate the tilt values needed to centre them. If the toolkit is correctly calibrated then these values will be reliable, but inaccuracies can occur if there was a problem with the calibration stage.

Note: Hold Shift when adding a point of interest to the image to prevent the other points being erased.

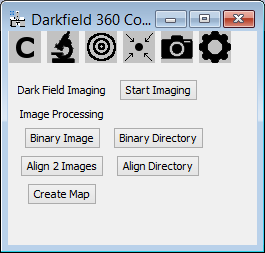
**Target Ring:** The red marker ring is used to target a specific d-spacing. The toolkit will calculate the tilt values needed to dark field image any number of points around the marker ring. This technique is intended to catch diffraction spots that are not very bright, diffraction rings created by amorphous material or rings formed when there are too many individual spots present to select each one. When the calculations are performed the user is asked for the number of points they wish to image and the length of time this will take is calculated before accepting.

**Delete Stored Targets:** Pressing this button will delete the recorded diffraction spot locations and can also remove the tilt calibration values.

### Imaging & Image Processing Controls



**Start Imaging:** Press this button to begin the dark field imaging process. The toolkit will move the diffraction pattern to your recorded values and then take an exposure with the digital camera. Before imaging begins you must insert the Objective Aperture and make sure that it is centred. Switch to **SAMAG** mode to see what the bright field image of the area looks like and adjust the stage position and aperture position as required to get a good image.



*Integrated Images:* Use integrated images when all of your points are for the same d-spacing and you do not need them to be in separate images. This is primarily used when imaging a selected D-spacing with the marker ring, because hundreds of images would be produced, need to be aligned and their contrast levels adjusted. An integrated image is simply a number of exposures (selected by the user) added together. These will still show the location of materials with the desired d-spacing/s, but will not record which diffraction spot the image was made by.

**Binary Image:** An open image can be processed to create a binary image showing only the brightest parts pixels. The user controls the proportion of pixels that will be ignored based on their brightness and then the toolkit creates a single binary image from this information. Binary images are used to create maps.

**Binary Directory:** Performs the same process defined in ‘Binary Image’ but for an entire set of dark field images. This option asks the user several questions to figure out exactly what must be done

* *Make binaries of ALL images in directory?*

If the user selects ‘Yes’ then every image file in the directory will have a binary version of itself created using the same settings. This includes images files of diffraction patterns, bright field images, etc. if any are present.

* *Export Binaries as Gifs?*

If the user selects ‘Yes’ then binary images will also be created in .gif format. These are much smaller than DM3 format images and do not lose any resolution because binary images contain only 1’s and 0’s. Note: Gifs do not store any other information, such as image scales, microscope details or equipment settings. Make sure these values are stored somewhere in case you need them later.

* *Save ALL thresholded binaries as Gifs?*

If the user selects ‘yes’ then in addition to the final binary images the toolkit will also save a number of intermediate images. This is only really useful for debugging and is not normally used.

* *Save useful Binaries in Gatan DM3 format?*

If the user selects ‘yes’ then the binaries will be saved in the dm3 format, which takes up a lot of space but includes a lot more information about the microscope settings.

* *Save ALL thresholded binaries in Gatan DM3 format?*

If the user selects ‘yes’ then in addition to the final binary images the toolkit will also save a number of intermediate images. This is only really useful for debugging and is not normally used.

* *Would you like to use Shadowing?*

Shadowing is explained in another section of these instructions. If the selected directory contains shadowing images then selecting ‘yes’ will use them. If ‘no’ is chosen the toolkit will only use the ‘middle’ images to create binaries.

* *Are the images binary Images?*

If the files in a directory have already been converted into binaries then select ‘yes’ to skip that processing step and simply create a single binary image made by adding up all of the binaries.

* *Open images for viewing*

If the user selects ‘yes’ then all of the binaries will be opened in Digital Micrograph at the end of the process.

**Align 2 Images:** Allows the user to select two open images and then opens them in the alignment tool, described in a future section.

**Align Directory:** Loads an entire directory of images in sequence into the alignment tool. One image is designated the ‘base’ image which all other images are compared to. By aligning an entire directory the user will create images that are aligned with each other and are the exact same size. These aligned images can then all be used in the creation of an EBSD-like map.

**Create Map:** A directory of images will be turned into an EBSD-like map. The directory should contain the following images:

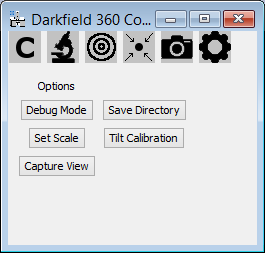
* A bright field image to act as the ‘base’ layer of the map
* One or more binary images (.dm3 or .gif) highlighting the regions to add to the map

This tool is described in more detail in a future section.

### Options



The options panel allows for a number of toolkit settings to be changed. Under ideal conditions the user will not need to use this panel but the various functions activated by these buttons may be helpful in some circumstances.



**Debug Mode:** This button toggles the debug mode on and off. Debug mode should only be activated to diagnose a problem because it provides a lot of feedback and displays (but does not automatically save) any images created.

**Save Directory:** This function prompts the user to select a new save directory for auto-saving images with the toolkit.

**Set Scale:** Prompts the user to enter a new scale for unbinned images taken by the camera. The scale is in units of (1/nm) per pixel. The scale is normally selected automatically when the camera length is chosen.

**Tilt Calibration:** Prompts the user to enter values normally derived from the CALIBRATE TILT function on the calibration controls panel. The values requested are the number of pixels moved in the X/Y directions when the X and Y tilt controls are changed by 1 arbitrary unit.

**Capture View:** Pressing this button causes the toolkit to search for a Live View window and activates the control functions of the toolkit. This is used if the toolkit is started before the Live View Window is active or if the window is closed while the toolkit is running.

# Alignment Tool

Figure 4: The alignment tool being used

The alignment tool is used to correct for sample and beam drift in a set of images. Two images are shown in the Manual Alignment Window (see Figure 3) either simultaneously, as a sum of both images, or alternating between the two at an adjustable rate. The arrow buttons are then used to move the images relative to one another until they are aligned. This alignment method is lossless because it does not delete any of the images to make them the same size. Instead, any additional space is filled with black, which is ignored in any future image processing such as creating binaries and EBSD-like maps.

## Alignment Controls

**Contrast A/B:** The contrast settings control the appearance of the images being aligned. By default these values are set to the current contrast settings of the image displays. The values can be changed to improve the image contrast or make certain features stand out more for alignment. These values do not change the images in any way, they only control their appearance on the screen.

**Start Alignment:** Press this button to begin the alignment process and make the Manual Alignment Window appear.

**Alternate / Simultaneous:** Selects one of the two available alignment modes. Alternate will cause the two images to be swapped at a regular interval to compare them. Simultaneous will add both images together and display the result.

**Delay +/-:** Controls the speed at which the images will alternate if the Alternate mode is selected.

**Arrow Buttons:** Moves the images relative to each other.

**Sensitivity +/-:** Controls the distance moved by each press of the arrow buttons. Each setting is double the previous setting.

**OK:** Confirms the alignment and closes the alignment tool. If the user is aligning only two images then the two new aligned images will be displayed in Digital Micrograph. If the user is aligning an entire directory then nothing will be shown until all of the images have been aligned.

**Cancel:** Stops the alignment process and closes the alignment tool. This will also cancel the alignment of an entire directory, so if a mistake is made it is often better to just select OK rather than lose the previous alignments.

# Workflow for darkfield imaging

* Align TEM and find a site of interest
* Ensure the camera can observe both the image and the diffraction pattern for the site of interest. The fewer values that need to be changed to switch between imaging / diffraction modes the better.
* Start the toolkit. If the Live View window is not present when the toolkit is started it can be ‘captured’ using the appropriate button in the options menu.
* Select the camera length to set the scale being used.
* Calibrate the Toolkit with the CALIBRATE TILT button. Follow the onscreen instructions to allow the toolkit to move the diffraction pattern.
* Select diffraction spots that you wish to DF Image by one of the following methods:

1. Use the TEM beam tilt controls to centre a spot on the screen and then select "Record Diffraction Spot"
2. Position a (point) region of interest marker over them. Hold shift when clicking to place multiple ROI points in Digitalmicrograph.

* When all the ROI have been placed selected the "Store ROI" button to move the ROIs to the centre of the screen and take a diffraction pattern (to record which spot is used for each DF image).
* An image will be saved of the diffraction pattern centred on each ROI for future reference.

1. To build a complete map for a single D-spacing the 'ring' system is used.

* Turn on the Marker Ring and move it to the desired d-spacing. The ring is then stored as a series of individual tilt coordinates, just as with the ROI storing function. Note: A complete d-spacing map will require a lot of images.
* When the reference images are stored you can centre the beam and insert your desired objective aperture. Check that the bright field image formed is in focus, bright enough and centered.
* Select “Begin Darkfield Imaging” to start the imaging process.
* The Toolkit will use the beam tilt controls to move the diffraction spots into the centre of the field of view. As long as the objective aperture is aligned with the crosshair the spots should be in the correct position and not require any further adjustments.
* If 'Shadowing' is used then 2 additional darkfield images will be taken for each spot. One at a greater distance from the central beam and one at a lesser distance. Shadowing is intended to reduce the problem of inaccurate tilt calibration or to resolve diffraction spots that are closer together on the DP than the objective aperture can isolate.
* The Toolkit can produce images as quickly as the camera can perform exposures, so be careful to have a lot of hard drive space available.
* The images are saved to the selected directory and can then be processed in many ways. Make sure that image sets are kept in their own directories and are not mixed together.