

TEM - TFS Titan Krios G3i - 2a: Collect Gain References for the K3

How to collect the gain Reference for the K3 camera

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INTRODUCTION

This guide from the Center for Microscopy and Image Analysis, UZH, Zurich, Switzerland shows how to prepare the gain reference for the K3 Gatan camera, at the transmission electron microscope Titan Krios G3i.

Please find more detailed information about the system here.

To be done once a week after every camera cycle, when weird stripes appear in your image, or before the data acquisition of your life.

Step 1 — Prepare the microscope

EF-CCD C	amera			*
1x 2x		Linear	Counted	*
	5760 x 4092 pixels			
View	Exposure	: <mark>0.0133 s</mark>	Ì	
Capture	Exposure	0.735 🔶 s	Correct Dri	ft
	Frames:	12	Save Stack	e

- Go to a void area of your grid (broken mesh/void).
- Select the linear mode of the K3 camera.
- Bring the electron dose to roughly 300 e-/pic/sec. Be sure to use the the same mag you would use for image acquisition. Usually, for such dose rate, you would need to use the C2 aperture of 150 um and a pretty low spot size (1-2).
- Be sure to be on the linear imaging mode, otherwise you could burn the K3 camera!!!!



Step 2 — Start the gain references collection

Select in the "Camera" Menu the "Prepare Gain Reference" option.

Step 3 — Collect the gain reference for the linear mode



- The software will ask you if you want to "Collect Gain Reference" for the Linear Mode -> choose this option.
- Wait Until the Dark reference is collected.
- Adjust the Beam intensity to the selected dose rate (1280 average counts). The measured dose rate is shown on the image, in red. When you are done, select done.

Step 4 — Collect the gain reference for the counting mode

DigitalMicrograph × Image: Collect reference images for Counted mode? Collect Gain Reference Skip	Collecting 10s dark image, please wait Abort	Call Beforeme Exposure Setup Prease specify nor desired does rate, operating does, and gain inference electron does. These settings will be used to calculate the appropriate microscope beam does. Hit the TAB hery to show updated time calculations. Call Ref Export Mode Calculation Does Rate Call Ref Export Call Ref Export Call Ref Export To abort gain reference mage collection, press the TESC key Cit Caucel
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- For the Gain reference in counting mode, you need a much lower electron dose!!!! Bring the screen down and increase the spot size to 4 or 5, before starting. Then you can lift the screen again.
- Collect the Gain reference for the counted mode.
- The Dark reference is automatically acquired.
- The software will ask you which parameter you would like to use for the gain reference collection in counted mode. Use the defaults.
- (i) You can change the parameters for the gain reference collection. I would suggests to use the defaults, and change them only if you know what you are doing.

Step 5 — Collect the gain reference for the counting mode



- Change the Beam intensity, so that you obtain the desired dose rate (in the picture, the default value of 15 e-/pix/sec is shown), and select done.
- Make sure that the Beam edge will not come into your image, otherwise it would be in your gain reference.
- When you are done, a window will pop up letting you know that the gain reference has been successfully signed and saved. Click "OK".

Step 6 — Check your gain reference



- Open the Gain reference for the counted mode (example in the picture). It is saved in:
 C:\ProgramData\Gatan\ReferenceIm ages . Does it look good?
- If the beam is coming in, repeat! This time use a larger beam.
- Copy your gain reference somewhere, you will need it for data processing.