

Acquisition Guide

Main dialogue box

Acquire	Acquire:	To acquire an image with the set parameters.
Acquire Image:	Exposure Time:	To set the exposure time manually. Exposure time manually set to keep a constant exposure condition for fluorescence quantification and densitometry.
AutoExpose Binning: 1 + V Autoscale	AutoExpose:	To meter light intensity and compute suitable exposure time.
Camera Area: → Full Chip Center Quad Use Active Region Show Live Live Bin: 1 ÷ Temp: ~ -30 c	Binning:	Acquisition binning factor. A higher binning results in higher sensitivity at the expense of lower resolution. Can be used to reduce exposure time to mainly viability of specimens and speed up acquisition.
Setting: Default Reset Display	Full Chip:	All pixels on the CCD are used for image acquisition.
Close Less <<	Center Quad:	Only pixels in the centre quadrant for image acquisition.
	Use ActiveRegio	n: Only pixels in the Active Region are used for image acquisition.
	Show Live:	To continuously acquire and display image.
	Live Bin:	Binning factor only for live mode. Can be set higher than acquisition binning factor to achieve a high live speed and brighter image to facilitate focusing.
	Temp:	Current CCD working temperature. To ascertain if CCD has reached good working condition and

for diagnosis in case cooling devices are faulty.

Acquire Acquire Acquired Save Image: Kave Line Save Lo: CXMM\ Vacquired0011.tif Set Save	Lo%:	% of pixel population to be clipped to black. Should be set to small number for transmitted light images.
Save w/Sequence Display Acquire Correct Annotate Special Exposure Time: Image Scaling: 50 * ms Lo % 0 * Hi % 0 * AutoExpose Image Scale Image Scale Image Scale	Hi%:	% of pixel population to be clipped to white in display. Does not saturate actual image. Should be set to extremely small number e.g. 0 to 0.1% for fluorescence images.
Camera Area: -> Full Chip Center Quad. Use Active Region Cham Line	Autoscale:	When activated stretch the display contrast to maximum. The image data are not changed. To minimise need for intervention when brightness changes from filed to field.
Show Live Image: Constraint of the state o	Scale within region:	Only grey levels in the active region are considered for contrast stretching.
Setting: Default Close Less << Setting: Load Save Save As	Image Gamma:	When Gamma>1, overall brightness is increased without changing the black and white points. With Gamma<1, image appears to be darker. When Gamma=1 is clicked, a linear relationship of display intensity to grey level is restored. Changing the Gamma does not modify the image data.

- Image histogram: Continuously display image histogram in live mode. As a diagnosis to ascertain if there is over - or under-exposure.
- Image histogram can be turned off to speed Off: up live image refreshing.

Display Tab



Acquire Tab

et Save	Target Intensity:	The grey level that the highlight will achieve with Auto-Exposure. The full scale grey levels for 12 bit camera is a grey level 4095.
	% of Max:	To set the target Intensity in percentage. This is easier as one does not have to note the current bit depth mode. Usually set to 75%-80% to prevent saturation.
•	Maximum Exposure:	To limit auto-exposure search in case of e.g. when camera port is not open.
nt	Shutter:	To select the shutter required to work in conjunction with acquisition. In this case, "Current Shutter" should be chosen.
Save As	Amount to adjust:	To set the step size when exposure time is incremented or decremented by arrows.

Zoom live image if binning is different:

With different binning factors, the image sizes are different. Checking this option allows the software to zoom the windows differentially to maintain a constant window size at different binnings.

Acquire		Background Subtrac
Acquire Acquire Save Image Save w/Sequence Exposure Time: 50 ÷ ms v AutoExpose Binning: 1 ÷ Camera Area: -> Full Chip Center Quad. Use Active Region Show Live Live Bin: 1 ÷ Temp: ~ -30 c	Image: <u>*</u> Acquired Save to: C:VMMVAcquired001.tif Set Save Display Acquire Correct Annotate Special Background Subtraction: © None © Constant © Region © Image Shading Correction: © None © Image	Background Subtrac
Default Close Less <<	Setting: Load Save Save As	

Correct Tab

Background Subtraction:

Usually to subtract a known background e.g. dark current for very long exposure times or background autofluorescence. Seldom used in microscopy.

То correct for uneven illumination, dirt on microscope or camera.

After "Image" is checked, one can acquire a background image with a blank slide. Future images will be divided by this reference image.

🗖 Acquire	
Acquire	Image: 📑 Acquired
Save Image	Save to: C:\MM\\Acquired001.tif Set Save
Exposure Time: 50	Automatic Image Annotation: Exposure Time Color Tab Special Tab Camera Area Temperature Camera Name Background Subtraction Shading Correction User Annotation:
Setting: Default	NOTE: Variables may be specified by enclosing the variable name between %'s (example: %X%).
Close Less <<	Setting: Load Save Save As

Annotate Tab

Camera settings can be stored in image header.

Illumination, magnification settings, precise acquisition time will be stored automatically too.

User comments can be typed in the "User Annotation" box.

Acquire	
Acquire	Image: 📑 Acquired
Save Image	Save to: C:\MM\\Acquired001.tif Set Save
🔲 Save w/Sequence	Display Acquire Correct Annotate Special
Exposure Time:	Sensor Mode: ALT_NORMAL
50 🗄 ms 💌	Digitizer: 20MHz
AutoExpose	Gain: Gain 1 (1x)
Binning: 1 📑	Camera Shutter: Open for Expose
Camera Area:	Clear Mode: CLEAR PRE EXP
Center Quad.	Clear Count: 2 🛫 🗾 Info
Use Active Region	Trigger Mode: Normal (TIMED)
Show Live	Show Focus Indicator
Live Bin: 1 🐳	
T emp: ~ -30 c	
Setting [Modified]:	
Close Less <<	Setting: Load Save Save As

Special Tab (differs from camera to camera)

- ALT_NORMAL is higher in sensitivity Sensor Mode: esp. for red and far red dyes. NORMAL allows overlapped exposure when exposure time is >96ms. Digitizer: Read out speed. 10MHz is lower in noise but slower. 20MHz is twice faster delivering up to 10 frames per second. Gain: Gain 2 reduces effective read noise slightly and thus more sensitive but reduces dynamic range to 1/4. Gain 1 allows full dynamic range. Camera Shutter: Always "Open for Exposure". Clear Mode: "Clear pre sequence" is recommended for highest speed performance. "Clear pre exposure" can be used when high speed acquisition is not required. Clear Count: 1 to 2
 - Trigger Mode: "Normal" unless a TTL trigger signal is used to trigger camera. Otherwise camera will not start.