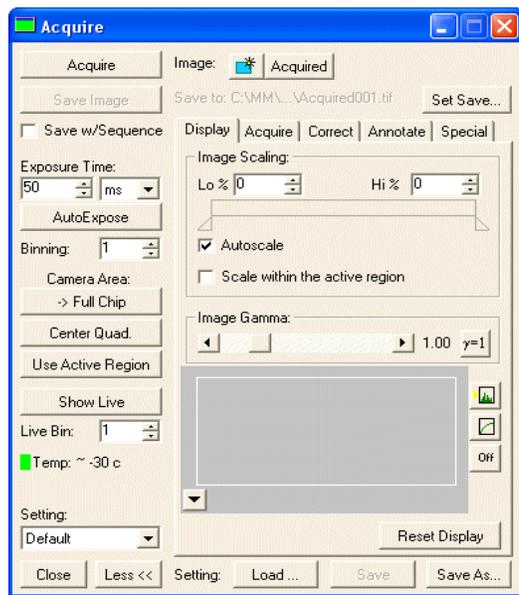




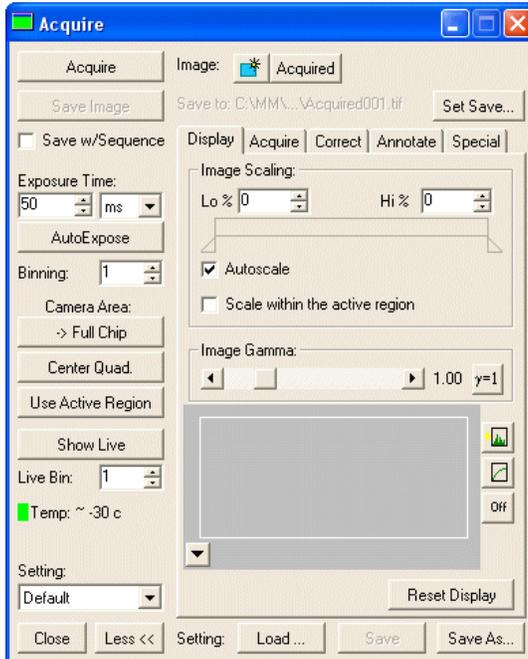
Acquisition Guide

Main dialogue box



- Acquire:** To acquire an image with the set parameters.
- Exposure Time:** To set the exposure time manually. Exposure time manually set to keep a constant exposure condition for fluorescence quantification and densitometry.
- AutoExpose:** To meter light intensity and compute suitable exposure time.
- 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.
- Full Chip:** All pixels on the CCD are used for image acquisition.
- Center Quad:** Only pixels in the centre quadrant for image acquisition.
- Use ActiveRegion:** 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.

Display Tab



Lo%: % of pixel population to be clipped to black. Should be set to small number for transmitted light images.

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.

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.

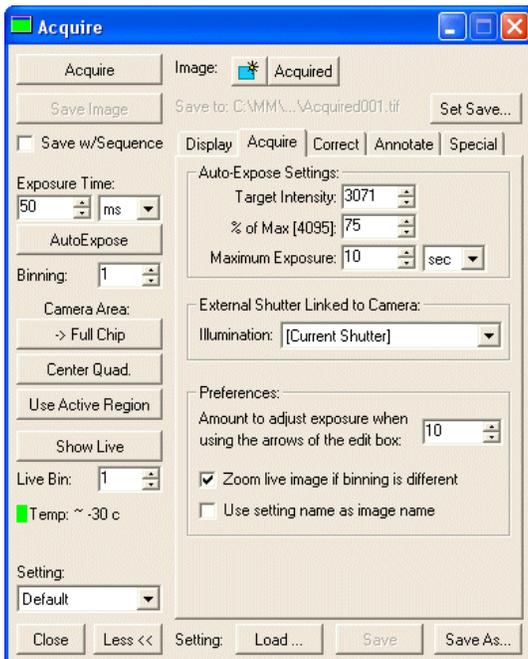
Scale within region: Only grey levels in the active region are considered for contrast stretching.

Image Gamma: When $\text{Gamma} > 1$, overall brightness is increased without changing the black and white points. With $\text{Gamma} < 1$, image appears to be darker. When $\text{Gamma} = 1$ 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.

Off: Image histogram can be turned off to speed up live image refreshing.

Acquire Tab



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.

Shutter:

To select the shutter required to work in conjunction with acquisition. In this case, "Current Shutter" should be chosen.

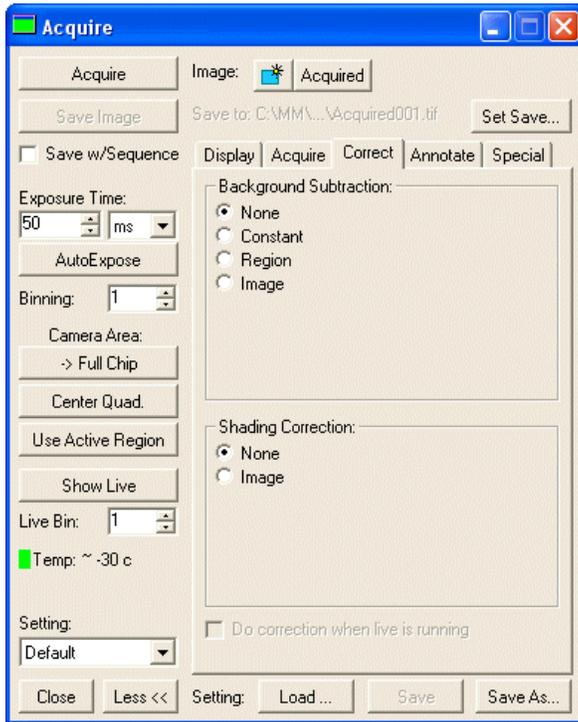
Amount to adjust:

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.

Correct Tab



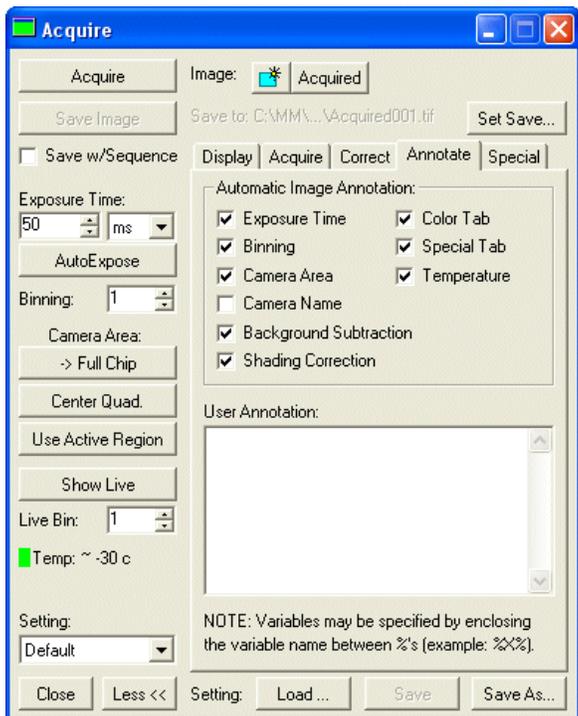
Background Subtraction:

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

Shading Correction:

To 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.

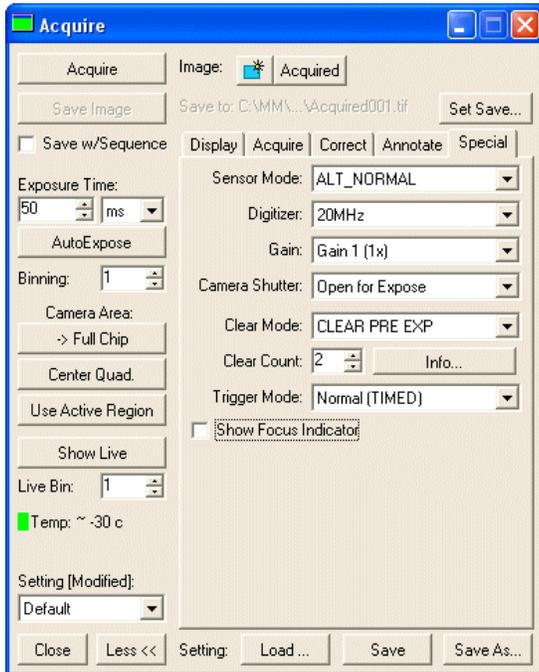


Annotate Tab

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.



Special Tab (differs from camera to camera)

Sensor Mode: ALT_NORMAL is higher in sensitivity 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.