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The following are a few suggested techniques and setups to obtain quality biological image capture with the Pixera150/600 series cameras.

1) Computer setup:

- Set computer to 'true color' mode 24 or 32 bit or to the highest mode available on your system. Lower levels of display colors will result in poor appearance of the viewfinder and captured images, although the captured image data will be complete. It may be necessary to increase the video memory (if possible) to gain full screen 'TRUE COLOR'.
- Adequate memory. Each full resolution capture with the 600 series will use >17MB of the available memory. Once the memory is used up, the hard disk will be utilized which will substantially reduce the system performance (speed). A minimum of 128MB is specified but more is recommended for best performance.

2) Microscope setup:

- Set microscope eyepiece image and camera image to be 'parfocal'; both images in focus which will make high quality capture easier, especially in low level fluorescent conditions.
    - Parfocality procedure:
        1) Set eyepiece(s) to '0' reference diopter position. Use eyeglasses during this procedure if you use them normally.
        2) Select a specimen that has sharply defined edges (thin sections) and focus the microscope using a 20X or greater objective for the sharpest image.
        3) Look at the Pixera viewfinder window image. If it does not have sharp edges then the video coupler needs adjustment for parfocality. Most video couplers will have some method of setting the parfocality. See tutorial on setting parfocality of camera optical coupler.
        4) When properly setup the camera image and the eyepiece images will both be in regardless of the objective lense in use.

3) Camera use:

BRIGHTFIELD imaging:

- Select the 'Brightfield' icon to properly set the system filters.
- Set 'white balance' to correct for your light source at the current intensity.
    - Select the 'WB' white balance icon. Select an area in the viewfinder image that has only background lighting (no specimen). Click on this area and hold the mouse button to draw a rectangle. This area is used to set the white reference for the image capture when using the auto-white balance control.
    - Accurate setting of white balance is critical for accurate color rendition.
- Initially use the auto exposure mode to set the exposure time. The auto exposure area can be changed from 30% to 0.1% and relocated to the portion of the specimen where the light level is most critical. This exposure can be locked using the 'AE lock' if necessary or set to manual exposure mode (e.g.- to view sample degradation due to bleaching without camera auto-gain affecting the exposure results).

MEDIUM FLUORESCENCE:

- Select the 'Fluorescence' icon to properly set the camera filters.
- Make sure the system parfocality has been properly set as noted above since achieving accurate focus under low level fluorescence will be more difficult. The eyepieces can than be used to focus rather than the viewfinder image.
- Select the 'BB' black balance icon. Select an area on the viewfinder which should represent the black background in the captured image. Click on this area and hold the mouse button to draw a rectangular box. This represents the area which will set the black background levels.

LOW/TRACE FLUORESCENCE:

- Set as noted above. It may be necessary to use either the 'average' or 'integrate' functions of the camera to gather sufficient light for the final image. The number of captures to average or integrate can be from 2 to 256. Keep in mind that the time the specimen will be exposed to light (and resultant 'bleaching') will be determined by the number of captures selected. The sensitivity (50, 100, 200, 400) can also be varied to reduce exposure times.

FAST MOVING SPECIMENS:

Set to 1.5 Mpixel mode (600 series only)
Set sensitivity to 400 (ISO) setting.
Set light intensity to a level that allows comfortable observation but results in the shortest possible exposure time (as noted in the 'exposure time' box)
Set white balance using one-shot 'OWB' mode as follows:
- Remove specimen from light path...the camera should see only the light source
- Click on the 'OWB' icon. White balance is now set for the current light source at this intensity level. Any changes in light intensity will change the color temperature of the light source and affect final color

Set exposure and lock with either AE LOCK or put into manual mode.
Make sure that the capture accumulator value is set to '1'.
Pre-focus on a specimen in the field of view. Capture when a specimen is visible in the viewfinder using the mouse (not the remote control – if equipped). It may take a few attempts to obtain the best capture quality.

PHASE CONTRAST:

Occasionally, the color of the captured image will vary from the image in the live preview. This is partially due to the sampling nature of the live preview (rather than all pixels) in order to achieve the fastest frame rate. In order to match the capture color and the live color it may be necessary to use the 'FULL' mode in the live preview (selected by icon or the pulldown menu). The color can than be corrected in the settings menu (R,G,B as necessary) to achieve a color correct image. The 'FULL' mode closely represents the final image color and quality but has a very slow refresh rate so the 'FAST' mode should be selected once corrections are complete. The corrections can be saved as noted below to avoid repeating the process. Keep in mind that these color corrections are only accurate at the light intensity in use at the time. Any intensity changes will change the color temperature of the lamp and the overall color.

SAVING SETTINGS:

The camera setting used in captures can be saved in a file for future setup. This can be accessed by selecting the 'OPTIONS' pulldown menu and go to 'SETTINGS' (or select the 'Options' icon from the toolbar) and select the 'user' tab. Click on the 'SAVE SETTINGS' button and give the setup a name.

4) <u>TROUBLESHOOTING</u>:

- Problems in **brightfield** / phase contrast microscopy occur when the settings are incorrect:
  - Make sure to do the appropriate 'white balance' selection. It is suggested that the white balance frame mode be used as there will be less color variation between captures.
  - Frame averaging will increase pixel information presenting an image with more depth and reduced background noise.
  - Use the high resolution modes noted above for best results. The lower resolution mode can be useful when capturing moving specimens (specimens in solution, etc.) if the high resolution modes result in blurred images. (e.g 1.5Mpixel mode)
  - The enhanced capture modes (frame average, frame integration) may not be compatible with specimens which move as noted above.
  - Verify proper centering of light source and condensor as noted above.
- Inconsistent exposure may be caused by your light source varying or the 60Hz flicker.
  - Use of a DC power source usually resolves these issues.
  - If using a fluorescent ringlamp, it probably is a 60Hz design. Use of a high frequency version (30KHz+) is highly recommended for these applications.

- The majority of problems occur when trying to capture a **fluorescence** specimen. The trouble can usually be traced to a few areas:
  - Make sure you are selecting the fluorescence mode icon from the toolbar.
    - This will set the camera with the appropriate filters and black balance selection.
  - If the image appears out of focus:
    - Fine tune the parfocality adjustment on the video coupler. This is best achieved in brightfield mode using a specimen with well defined edges.
    - Parfocality with the eyepieces is essential in fluorescence due to the low viewfinder intensity which makes it difficult to determine best focus.
    - Make sure external vibration sources (fiberoptic cooled lights, poor rigidity bench tops) are properly isolated and damped. This is especially important in the higher magnification ranges (>50x objective) and in the 5.8Mpixel mode.
  - If the image is noisy or grainy:
    - Make sure you are using the high resolution modes (1.5Mpixel or 5.8 MP) in order to take advantage of the DiRactor™ capture technique. Lower resolution captures will result in background noise, even when using frame averaging.
    - If the specimen is very low in intensity, increase the amount of frame averages until all specimen detail is revealed and background noise canceled.
    - Make sure that the shutter control wasn't previously left closed down. Select the 'auto' exposure mode icon. If your specimen is too bright than select either a smaller auto exposure area and/or move the exposure window to the area of the specimen which is brightest. The manual mode can also be selected.
    - If exposure times are > 5 seconds the cooled version of the cameras (600CL or 150CL) may be necessary for reduced thermal noise and greater sensitivity. Contact your dealer regarding application.

TROUBLESHOOTING (cont.):

- If the image is overly bright or 'blooming':
  - Try decreasing the auto exposure window, window size, or camera sensitivity setting. Position the exposure window over the specimen area of concern.
  - Try manual mode to override the auto exposure system. Use the exposure slider to achieve the best possible image. The camera sensitivity may have to be reduced as noted above.
  - When moving the specimen to view other areas, the auto-exposure system will constantly re-adjust for the new light levels causing momentary 'blooming'. Setting to manual mode after the initial auto-exposure setting has been achieved will prevent this from happening. Resetting to auto mode once the area to be photographed has been located will prevent this distraction.
- If the image has uneven lighting, washed out or dark areas:
  - Most common cause is poor centering of the light source filament or arc. Center the light for best field coverage using centering adj.screws and condensor lense position (if so equipped). The camera tends to be more sensitive to even field lighting than your eyes. The goal is even light across the field without hotspots.
  - Make sure all microscope filters are fully positioned to detents and no obstructions are in the the light path. This applies to neutral density filters, POL filters, and excitation filters.
  - Make sure the objective lense is fully seated in the nosepiece detent.
  - Verify that the camera port is fully selected, not partially blocked.
  - Another cause of uneven lighting can be external light leakage from the eyepieces or other non-closed ports which will typically result in a lighter background or 'washout' in the center of the image. This is especially true on systems with a split image port which allows both to be used simultaneously (e.g. 40% / 60%). In these cases covering the eyepieces to block the light may be the only solution. (Some microscopes have an eyepiece shutoff setting)

5) SUGGESTED MINIMUM SYSTEM CONFIGURATION:

- P4/DuoCore processors of >1GHz provide frame refresh rates of 15 fps which makes it much easier to achieve proper focusing.
- 256MB memory (suggest 512MB for 600 series)
- 'TRUE' color 32 bit support with adequate memory
- Transfer media or network
  - DVD/CD-RW
- Large hard disk (>10GB)