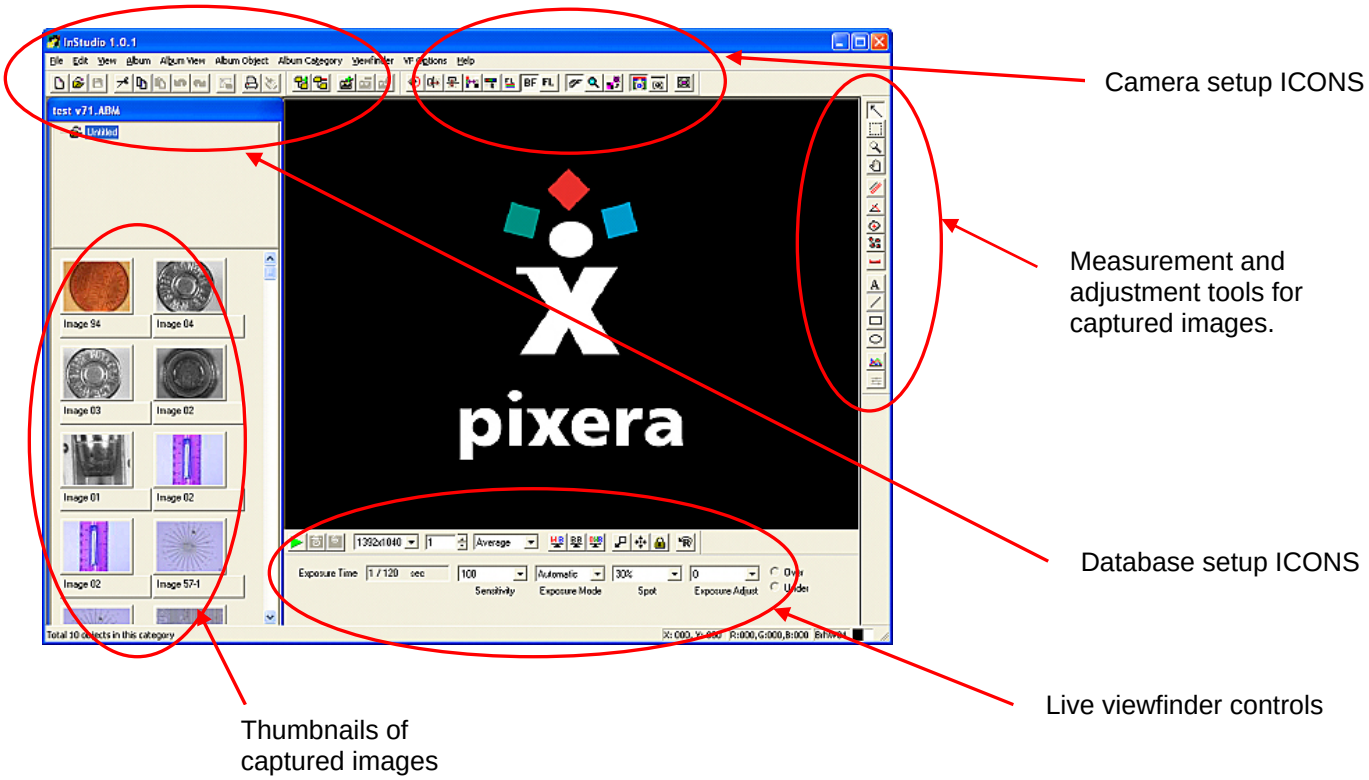
PIXERA InStudio Software Package

Background

InStudio is a comprehensive imaging capture and manipulation software program from Pixera. It combines the features of the original ViewfinderPro and StudioPro as well as many additional features into a single, easy to use program. These features include: image database with thumbnail views, measurement tools, image adjustment tools, intuitive camera control panel, fluorescent filters blend controls, and many more.



The above illustration shows the basic function areas of InStudio.

IMAGING System Setup

The camera, microscope and computer need to be setup correctly in order to achieve the best possible images. The following setup procedures will help you get started.

Computer setup:

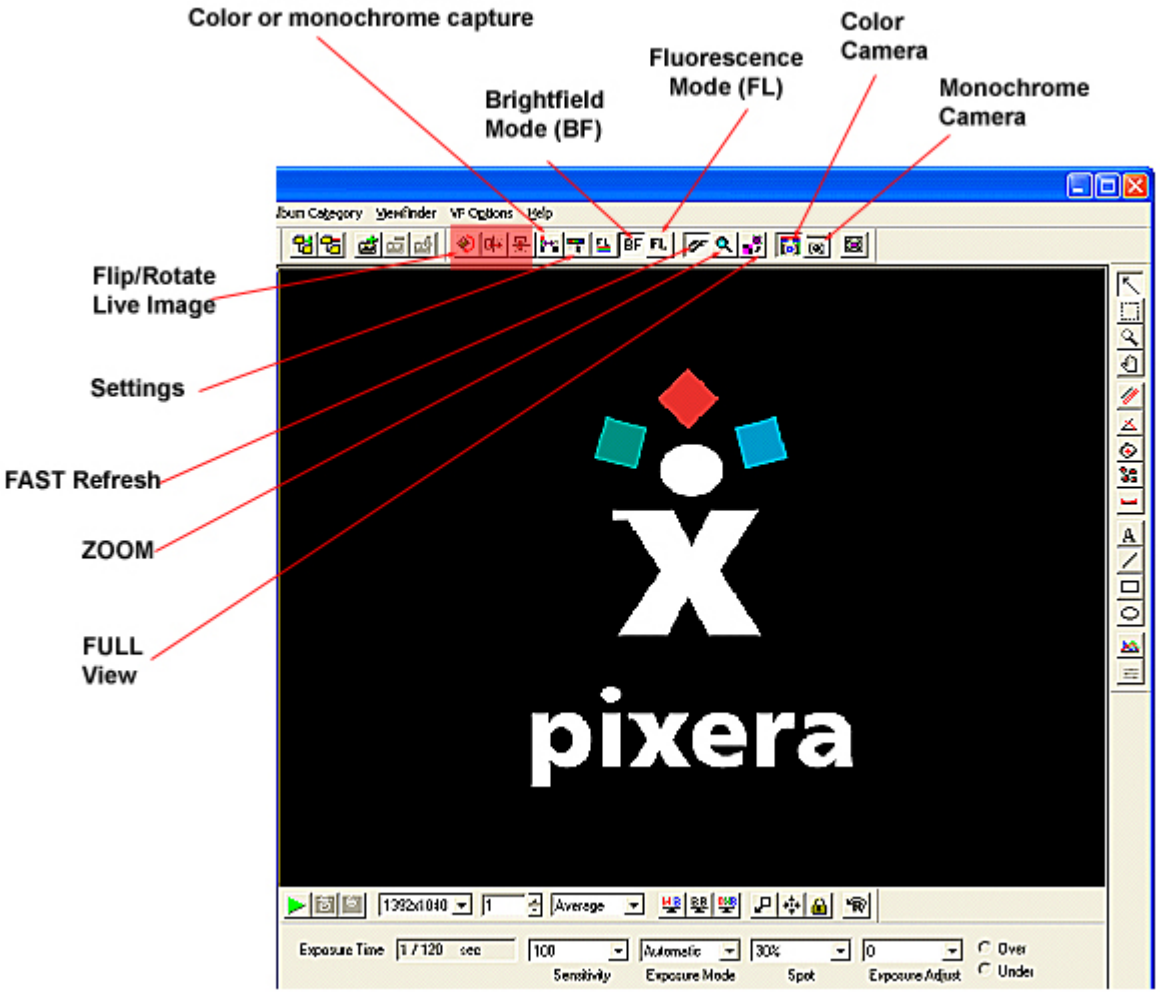
INSTALL the PCI interface card and software supplied with the camera setup the computer.

Set computer monitor to 'true color' mode preferably 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 still be complete. It may be necessary to increase the video memory (if possible) to gain full screen 'TRUE COLOR'.

Have adequate computer memory. Each full resolution capture with the 600 series will use >25MB of the available memory (twice as much if 16 bit capture mode is selected). Once the memory is

used up, the hard disk will be utilized which will substantially reduce the system performance. A minimum of 256MB is specified but much more is recommended for best performance. The realistic minimum recommended for Windows Xp is 1GB and Windows Vista should have 2GB.

INITIAL SETUP of the camera:



Controls for camera setup

Type of Camera

Select the type of camera you are using (Color or Monochrome Icon). The type of camera can only be selected when the camera in off (Camera on/off icon).

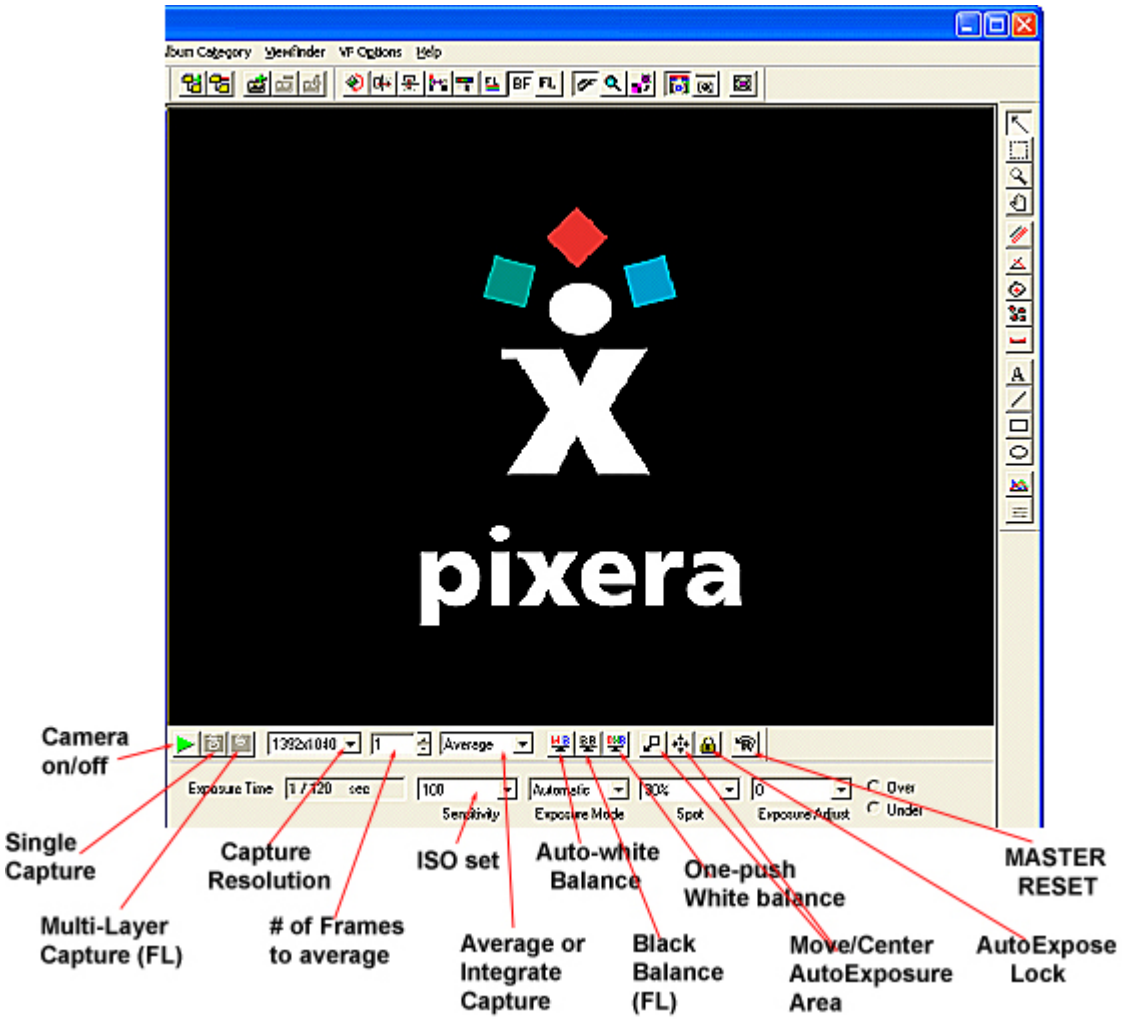
Type of microscopy to be done

BF (brightfield) mode:

This icon (BF) should be selected for most Brightfield, Darkfield, Phase Contrast, DIC Nomarski, and Hoffman Modulation microscopy in both transmitted and reflected light use.

FL (fluorescence) mode:

This icon (FL) should be selected for all fluorescence image capture. When selected, the proper software filters are set for fluorescence image capture.



LIVE view camera controls

POWERUP the camera:

Click on the Camera on/off icon to activate the live viewfinder.
Select the **FAST** refresh icon for the fastest delay-free viewing.

Use of the ZOOM or FULL modes slows down camera response so only use it to verify focus or to see image with all pixels. The FAST mode displays only about 25% of the available pixels. Regardless of the viewfinder mode chosen the captured image will be at the full resolution selected with 'Capture Resolution' pulldown menu.

Microscope setup:

Now that a live image is on the viewfinder the camera and microscope need to be set for parfocality. Parfocality is achieved when the eyepiece image and the image seen on the screen are both in focus (same focal plane). This is necessary to capture the highest quality images and makes capture at very low light levels much easier (particularly trace level fluorescence). The procedure is as follows:

- 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. Consult your camera sales engineer.

4) When properly setup, the camera image and the eyepiece images will both be in focus regardless of the objective lense in use.

For additional information see:
www.oem-optical.com/media/oem/pdf/digital_camera_parfocalizing_microscope.pdf

CAMERA Use:

All camera modes

RESOLUTION setting: The camera will usually default to the highest resolution available for your camera in use. You may find you can select a resolution higher than your camera actually has but this will not improve your cameras performance but actually provide a poorer quality picture. Only use the maximum (or less) resolution of your camera model (600series 2776 x 2048, 150series 1392 x 1040).

ISO setting: Default is ISO100 which is usually the best place to start. The ISO setting affects the camera sensitivity just as it does in a film camera. The higher the ISO number the grainier the image may be. With higher ISO settings FRAME AVERAGING can be used to reduce noise.

FRAME Averaging or Integration: When the # of frames to average is set to 1 (default) no frame averaging or integration occurs. Frame integration is a process where multiple captures are performed and the accumulated individual pixel data is divided by the # of frames to create an average value at each pixel location. This helps to eliminate random noise such as seen in high ISO settings. Experiment with this option as most users find that 2-3 frame averages can produce better quality images.

EXPOSURE mode: 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) for absolute exposure control.

'BF' CAMERA MODE (BRIGHTFIELD)

Set 'white balance' for accurate color.

- Accurate setting of white balance is critical for accurate color rendition.
- 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 WB 'auto-white balance' control. For reflected light operation a white sample can be put in the field and used to set white balance.
- The white balance is now set for your light source at the *current* lamp setting. If the intensity is changed, a white balance should be performed again to correct for the new intensity. Auto-white balance can only correct for small variations in lighting and the best color accuracy is always when WB is updated. As the light intensity is increased the images will take on a bluish tint, as it is reduced they will take on a reddish tint.

- The **OWB** setting is a one-push average of the entire viewfinder area. It is best suited for setting white balance without any specimen in place for transmitted light.

'FL' CAMERA MODE (FLUORESCENCE)

- Select the 'FL' 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

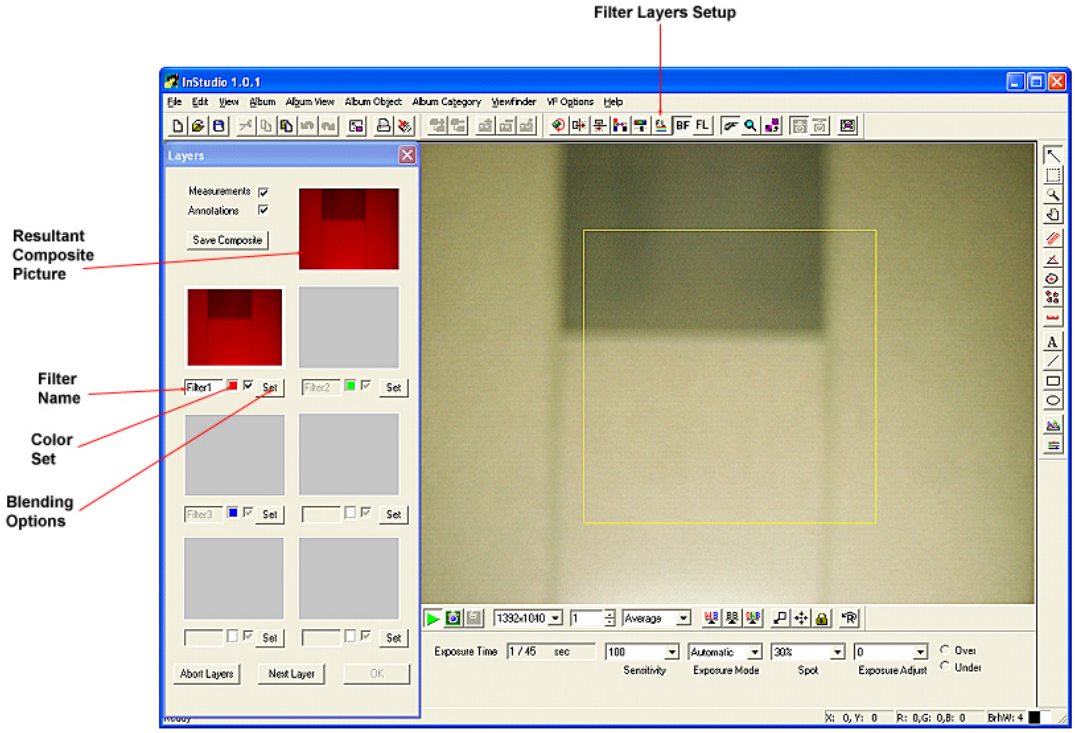
the black background in the captured image. Click on this area and hold the mouse button to draw a rectangular box. This represents the area which will set the black background levels. If you select an area that contains some of the specimen it will no longer be visible and will require resetting BB to fully see your sample.

Low/Trace Fluorescence:

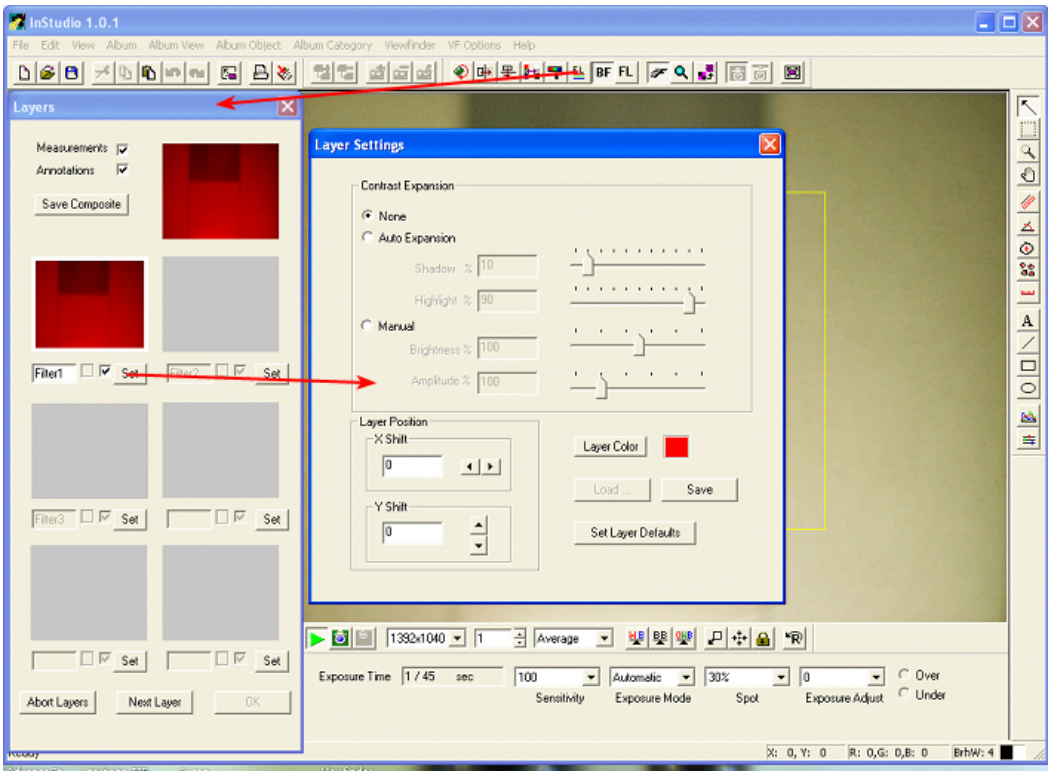
- Set as noted above. It may be necessary to use either the 'average' or 'integrate' functions of the camera to gather sufficient light for the final image. The number of captures to average or integrate can be from 2 to 256. Keep in mind that the time the specimen will be exposed to light (and resultant 'bleaching') will be determined by the number of captures selected. The sensitivity (50, 100, 200, 400) can also be varied to reduce exposure times.
- Most low level fluorescence should be done using a 'cooled' sensor camera such as the 600CL. Exposures requiring > 5 seconds should use a cooled camera. It is still possible to capture the fluorescence using the uncooled models but the image may have thermal noise pixels in them.

Multi-Layer Fluorescence Capture:

- The multiple capture icon will allow up to 6 separate capture of the same specimens and provide blending options and pseudo coloring capability. The filters can be specified with the filters icon or can be applied after the capture. You have a multitude of blending options for the layers which can be accessed by clicking the 'SET' button. Filter name and color assignment should also be performed. When the multi-layer button is selected the system will prompt you for the image capture by layer.



FL FILTERS SETUP



FL FILTERS BLENDING OPTIONS

MISC SETUP ISSUES:

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.

TROUBLESHOOTING:

- 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 condenser to prevent uneven field illumination.
- 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 ring lamp, it probably is a 60Hz design. Use of a high frequency version (30KHz+) is highly recommended for these applications to prevent the 'breathing' effect of the light (high/low illumination cycle).
- 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 (FL) 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 (fiber optic fan-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) are recommended for reduced thermal noise and greater sensitivity. See our website: www.oem-optical.com for camera options.
  - 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.

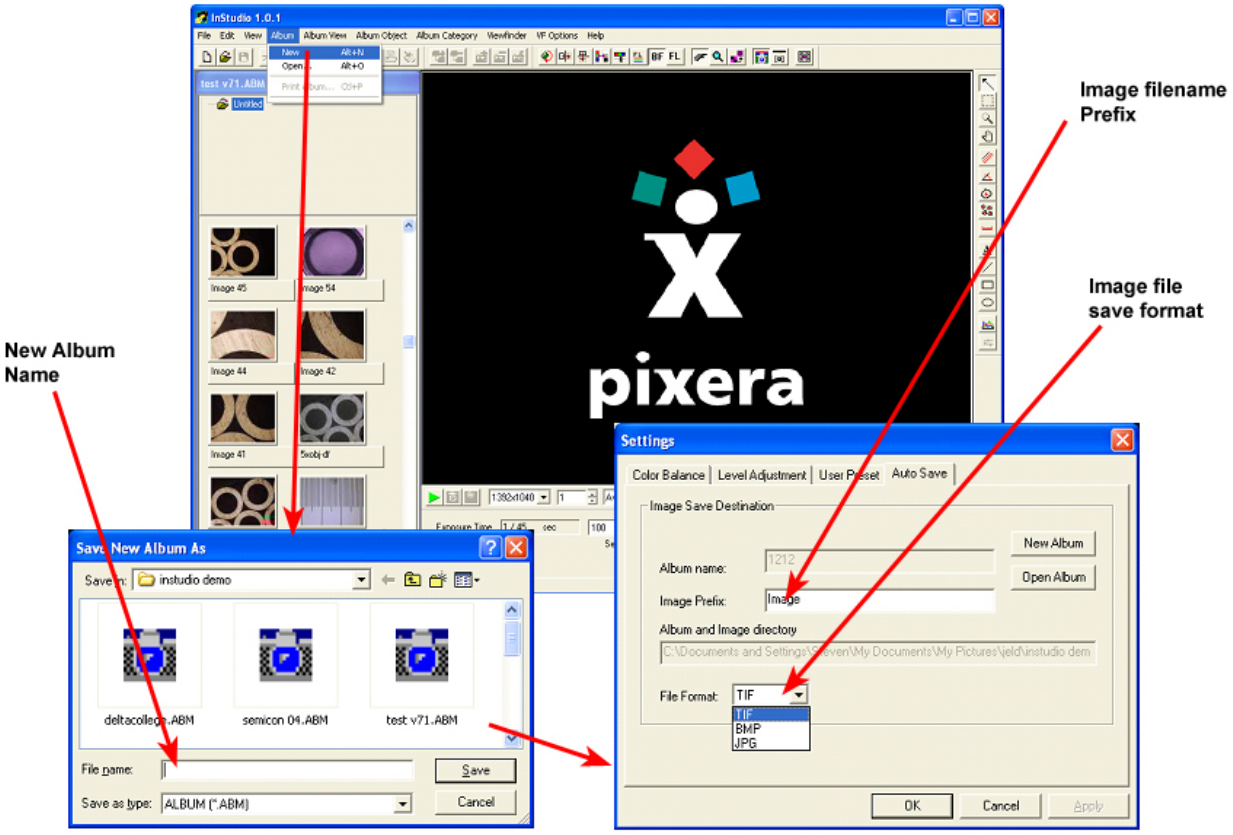
- If the image has uneven lighting, washed out or dark areas:
  - Most common cause is poor centering of the light source filament or arc. Center the light for best field coverage using centering adj.screws and condenser lense position (if so equipped). The camera tends to be more sensitive to even field 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 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)

TROUBLESHOOTING (Monochrome camera models specific):

- If the captured image appears to be out of focus and never achieves a sharp focus it is probably due to excessive IR (infra-red) light from the specimen illumination. Most color cameras have an IR block filter to prevent this problem but Pixera monochrome cameras typically do not have an IR blocking filter unless it was specifically ordered with one. It is important that the light source(s) filter all IR to prevent this issue
- The contrast in a monochrome image can be increased by using the Black Balance (BB) to set a reference black value. The area used to represent black should be as close to black as possible to prevent losing image information. To return to "normal", turn off black balance by using the SETTINGS icon or pulldown and select no black balance and no white balance.
- To increase subtle detail visibility the LEVELS control located in SETTINGS can be used. Sliding the highlight and shadow sliders to the edge of the actual live image information displayed will serve to expand the contrast of the captured image. The effects will be seen on the preview image. The gamma slider can also be adjusted to enhance midrange image detail. Remember to return the levels to normal by using the levels reset (in SETTINGS/LEVELS) to prevent losing image detail in future images.

KNOWN CONFLICTS :

- On systems having both the older ViewfinderPro/StudioPro software package as well as the InStudio package, trying to run both simultaneously will result in a resource conflict and may require restarting the computer to resolve. Using the task manager to terminate viewfinder.exe may correct to problem if InStudio was running first. The only way to prevent this problem is to make sure that the first program is terminated before starting the second.
- When using a switch box to allow multiple cameras to be run on the same computer it is important that the Viewfinder be turned OFF when switching between cameras. Failure to do so will usually result in the system locking up and may require a full reboot to clear. The best option is to terminate the program first than switch to the other camera and restart the program.



NEW ALBUM CREATION

InStudio DATABASE and TOOLS

InStudio database functions are fairly intuitive. When you first start InStudio it will ask for an album name or a new album. If the album exists it will be opened, if not, the Settings screen will open to allow for a new album setup. The picture above illustrates the same operation if done while already in Instudio.

ALBUM SETUP

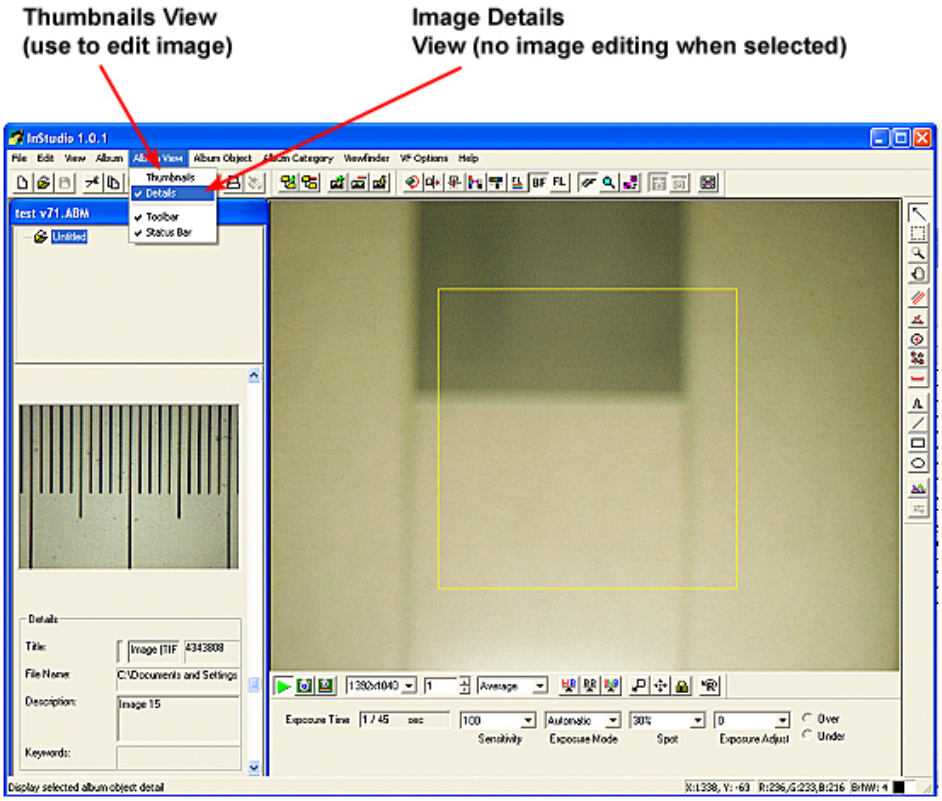
IMAGE Filename Prefix: This will be the label for all images contained in the album. The first will be 'prefix'-1, next is -2, etc.

IMAGE File Format: This is the form in which the image is saved on disk. The TIF format saves all data without any image compression which results in the largest file and uses up disk space faster. The JPG (jpeg) format compresses the image for a smaller resultant file size (typically 1/3 or less of TIF). The BMP format is primarily a Windows bit mapped format that also save all data…TIF is the preferred for uncompressed storage. If you plan on measuring features on the image than TIF will be the most accurate.

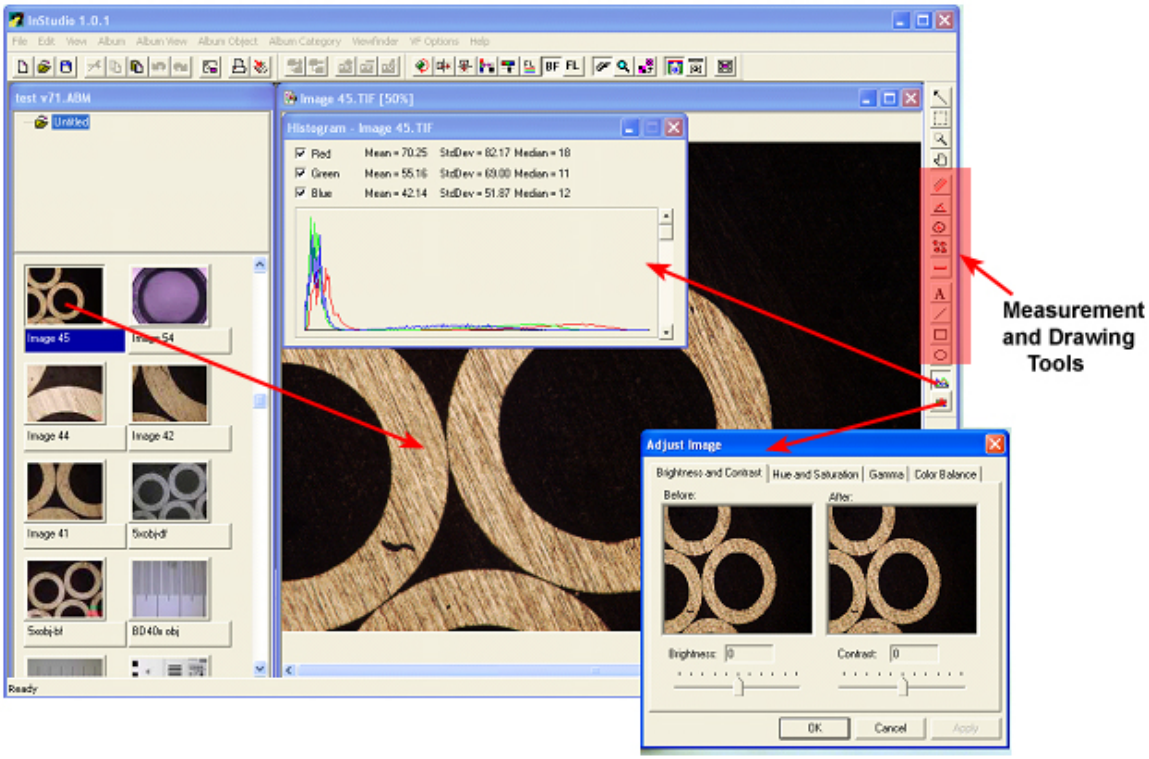
IMAGE TOOLS

To use the image tools (measurement, counting, adjustment) a captured image must be selected. The thumbnail view **must** be selected (Album View> Thumbnails). No editing is possible when in the Details view mode. Double clicking on a thumbnail will bring up the image in the viewfinder screen area. You can now use the tools for the various operations. The tools will not work on a live view (camera) image, only a captured one. Below are a few screen shots noting some of the operations and dialog boxes associated with them.

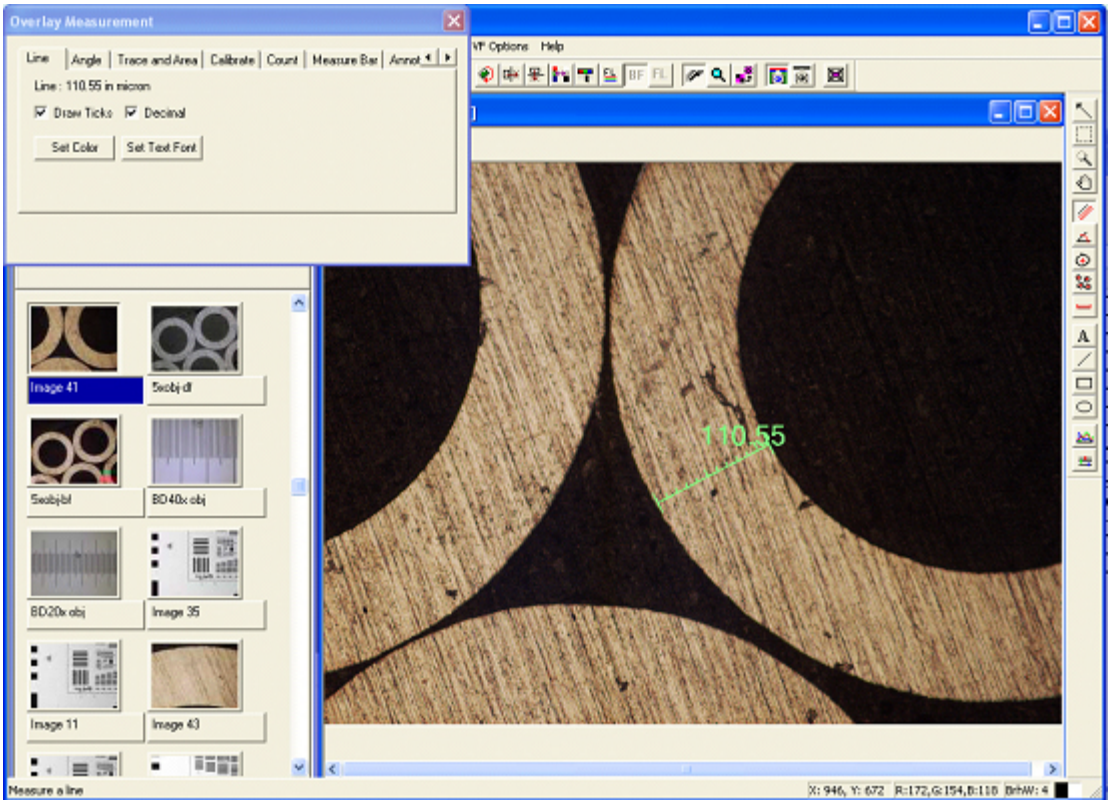
There are many other features not noted in the quick study of InStudio. This is only intended to get the user up and running as quickly as possible. Explore the InStudio features to fully appreciate all that is available. Most functions are intuitive using standard industry formats. Try it, you can always UNDO.



THUMBNAILS / DETAILS VIEW SELECT



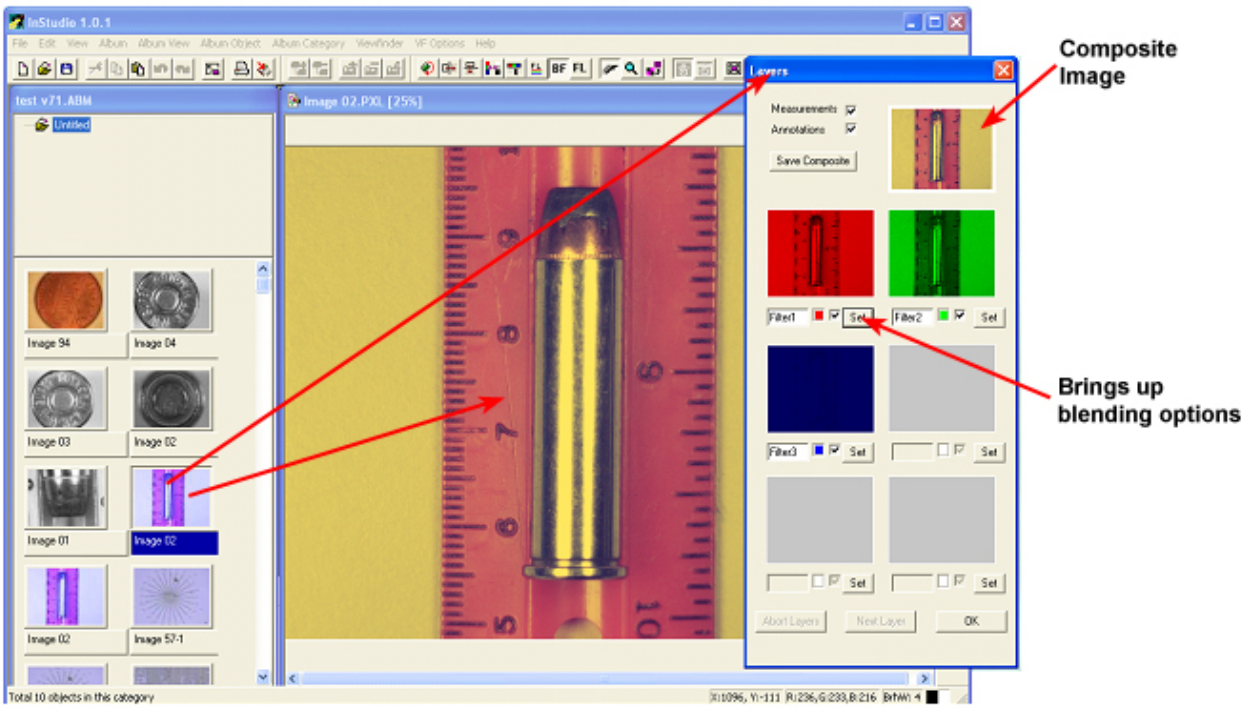
EDITING SEQUENCE DETAILS



MEASUREMENT TOOLS DIALOG

FLUORESCENCE FILTERS

Double clicking on an image that was captured using the multi-layer capture option will bring up all the layers as thumbnails. Clicking on the SET box in the Layers dialog box will bring up the blending options for each layer thumbnail. The blending options are the same as noted in the Viewfinder portion of the program.



FLUORESCENCE FILTERS DIALOG