The Westmead Institute FOR MEDICAL RESEARCH

STANDARD OPERATING PROCEDURE

Olympus BX53\DP80 Fluorescence Microscope

PURPOSE

This document describes the operational setup and procedures for the Olympus BX53\DP80 fluorescence microscope (BX53). This document offers users, particularly beginners, a brief step-by-step instruction on how to operate the BX53 microscope and to take images with its acquisition software cellSens.

The BX53 is a standard wide-field upright epi-fluorescence microscope equipped with a DP80 dual chip CCD camera equipped with a wide range of objectives from 4x, 10x, 20x, 40x, 60x (Oil), to 100x (Oil) with most of them featuring phase contrast. Fluorescence filters are available for DAPI, FITC, TRITC, and Cy5 channels and therefore suitable for acquisition of multiple staining in tissue sections or fixed cells for both fluorescence and brightfield and providing fairly high quality images in both bright field (colour imaging) and fluorescence.

This system is used only for fixed slides and not for live cell imaging experiments.

SCOPE

The procedure applies to single-channel image acquisition for fixed samples on standard slides. The document covers operating the microscope, image acquisition, saving data, and basic post-acquisition image analysis.

Users are to refer to the full manual for cellSens provided by the vendor for more detailed operational instructions and other advanced data processing & analysis:
https://sydneyuni.atlassian.net/wiki/spaces/WIF/pages/768016621/Manuals+Protocols.

SAFETY

Safe operation of the instrument requires the awareness of risks e.g. glass cuts, exposure to mercury lamp explosion, fatigue and gesture discomfort/injury etc. Refer to the "WIMR-SWP-WHS-GEN-16.01 Use of fluorescence and laser microscopes" for details:
https://sydneyuni.atlassian.net/wiki/spaces/WIF/pages/768016621/Manuals+Protocols.

TRAINING / COMPETENCIES

All personnel require training prior to independent operation of the instrument. Training is conducted by facility staff with competency demonstration necessary before authorisation.

Competency is assessed via demonstration of independent instrument operation, in conjunction with verbal explanation of all aspects of operation and troubleshooting common faults. After training is carried out, a training competency quiz is to be completed and passed before instrument access is authorised by imaging staff. All instrument operation is to be conducted by trained operators.

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EQUIPMENT & SUPPLIES

The BX53 microscope is located in J2.08, Level 2 of WIMR.

PROCEDURE

Olympus BX53\DP80 Fluorescence Microscope

Microscope: Olympus BX53 Fluorescence microscope
Camera: Olympus DP 80 (dual chip for both colour and grey scale imaging)
Imaging Software: cellSens Standard (shortcut on the desktop)

1. Microscope operation

1.1 Choose the right objective

1.1.2 If you use an oil objective, apply a drop of oil onto the coverslip of your slide

1.1.3 For the 20X objective, make sure the work distance line aligns with “0.17” for standard 1.5# coverslip (this can be adjusted for better resolution to correct for different sized cover slips).

1.2 Eye-observation of your sample under microscope

1.2.1 For bright field (BF) observation

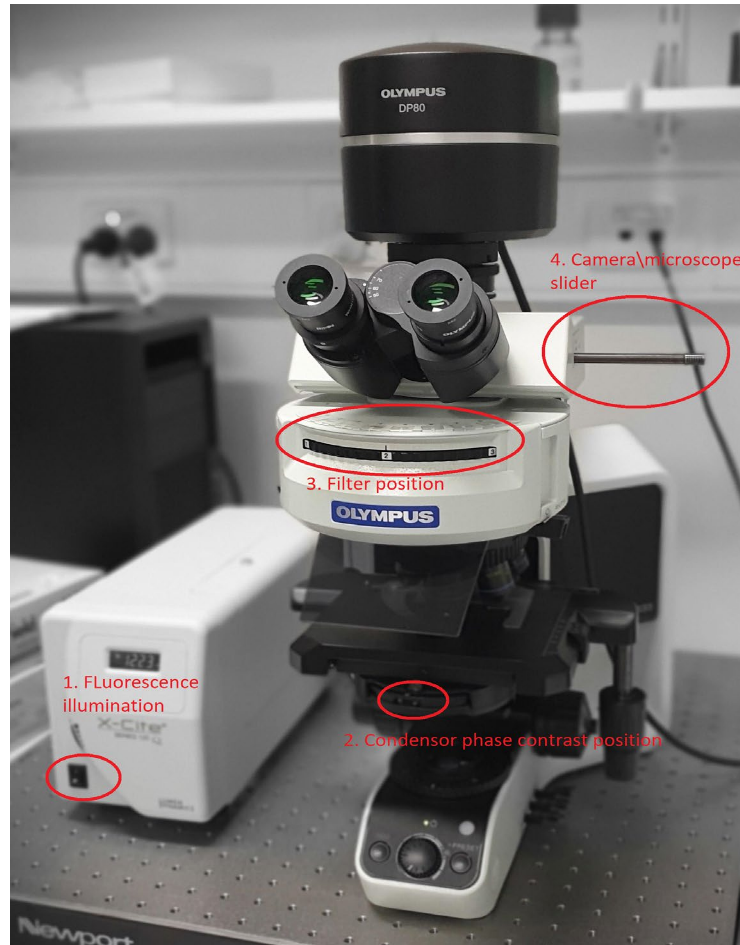
- Turn on the transmission lamp (switch it off when eye-observing or imaging fluorescence channel)
- Filter position: 1 (for BF)
- If you need phase contrast to view your sample better, make sure the condenser phase position (2) matches your objective requirement i.e. “Ph 2” for 20X lens.

1.2.2 For fluorescence observation

- Power up the X-cite unit to turn on the fluorescence illumination (1). **Note**: Let it cool down for 5 min at least before turning it back on when applicable.
- Filter position (3): 2 (for DAPI) or 3 (for FITC) or 3 (for TRITC)

1.3 Focus on your sample using either BF or fluorescence

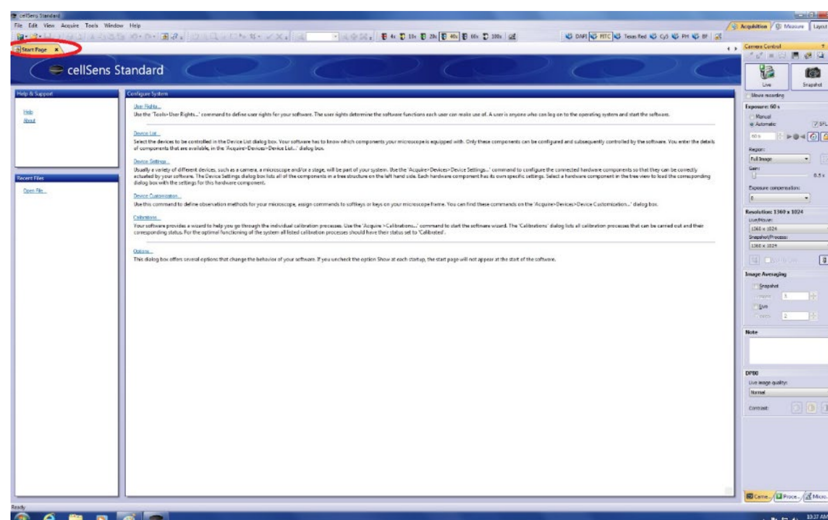
1.4 Pull the camera/microscope slider (4) all the way out to direct light to the camera.



2. Image acquisition

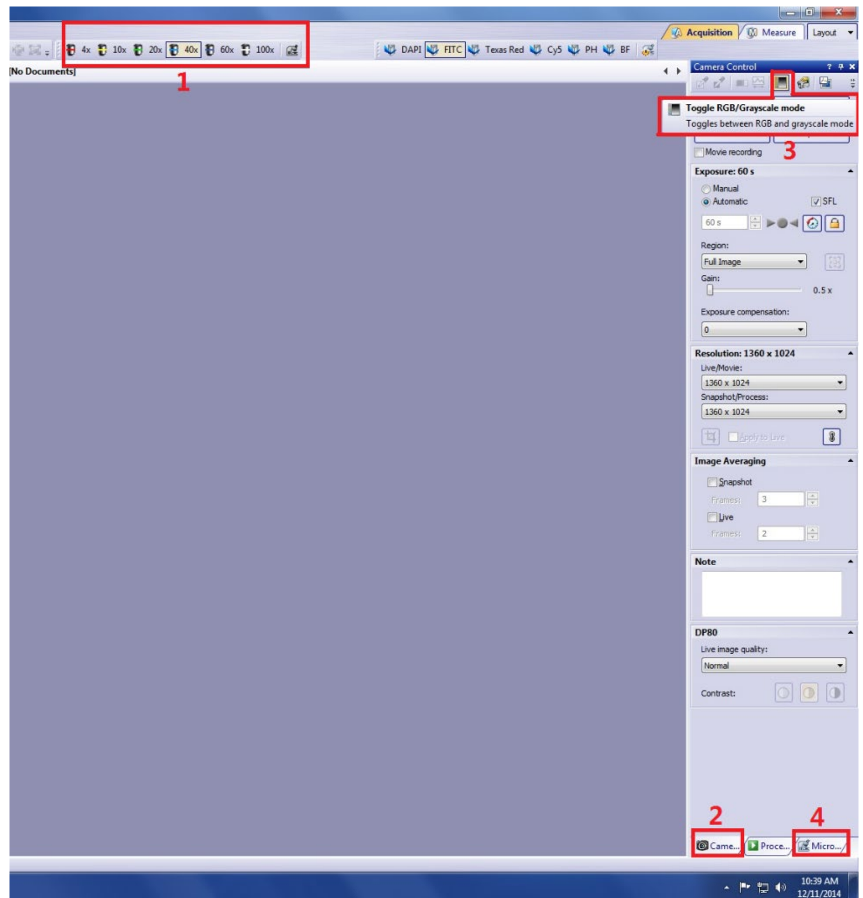
2.1 Opening cellSens

Double click the shortcut icon of "cellSens Standard" on the desktop, the "cellSens standard" window will pop up. Then close the "Start Page" window by clicking the "x" as shown below.



2.2 Acquiring an image

The current version of cellSens does not support multichannel imaging but only single image acquiring. You can merge images after acquisition (see later in Section 2.5).



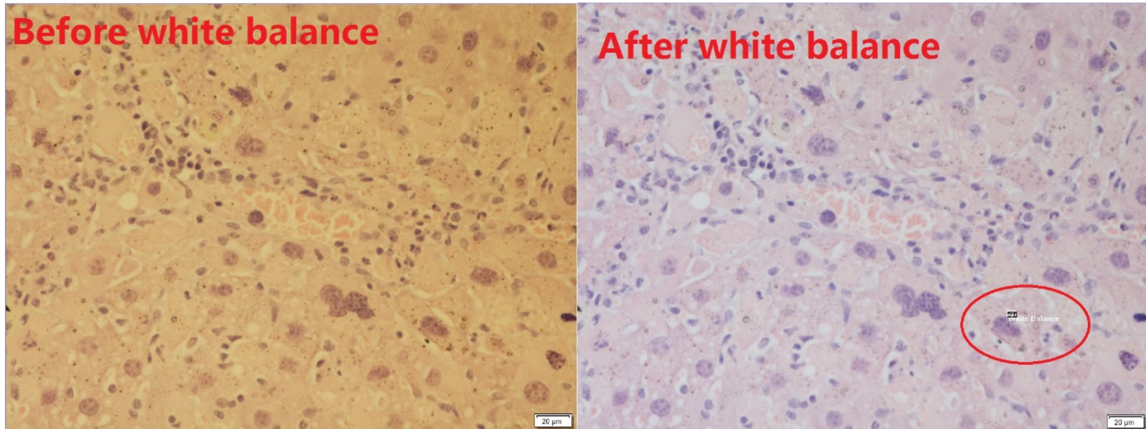
2.2.1 Choose the objective to ensure correct scaling: On the *Microscope Control* (1), click the button with the objective that you use for the image acquisition. This does not drive the objective on the microscope but more about getting the scaling right.

2.2.2 Choose the camera mode (greyscale or colour chip): click on "*Camera Control*" (2) to select either *Greyscale or RGB* (3).

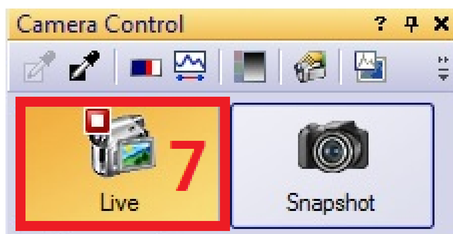
Greyscale mode is recommended for fluorescence imaging and resultant images will be of grey scale nature. Default colours for different channels can be loaded to the images after acquisition by choosing the right filter colours (4):

Note: it is not a problem to acquire fluorescent images using the colour camera chip and the resultant images will be of RGB nature.

RGB mode is recommended for colour BF imaging and *white balance* (5) is to be carried out to correct colour reproduction:



2.2.3 Turn on “*live window*” (7) to display the image on the monitor:



2.2.4 Taking a fluorescence image:

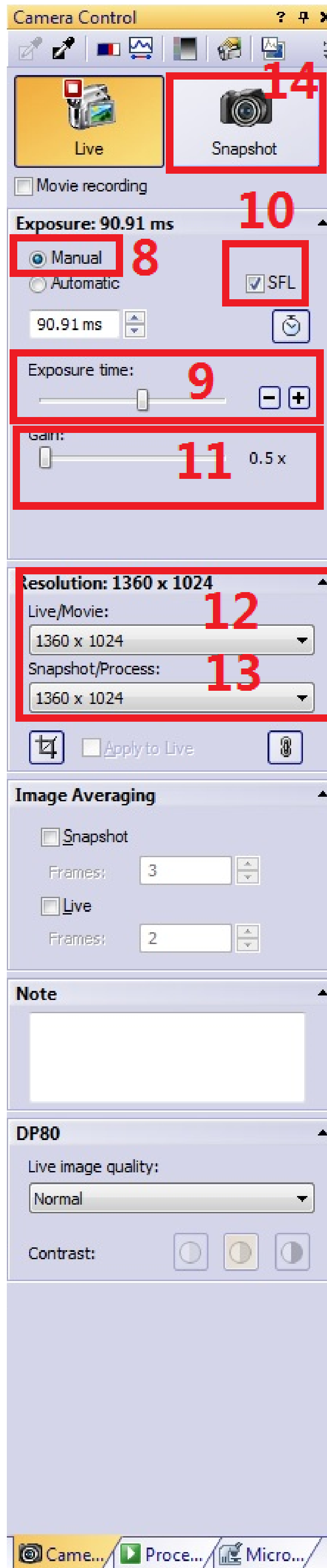
2.2.4.1 *Manual* (8) exposure mode: In this way, you adjust *exposure time* (9) manually.

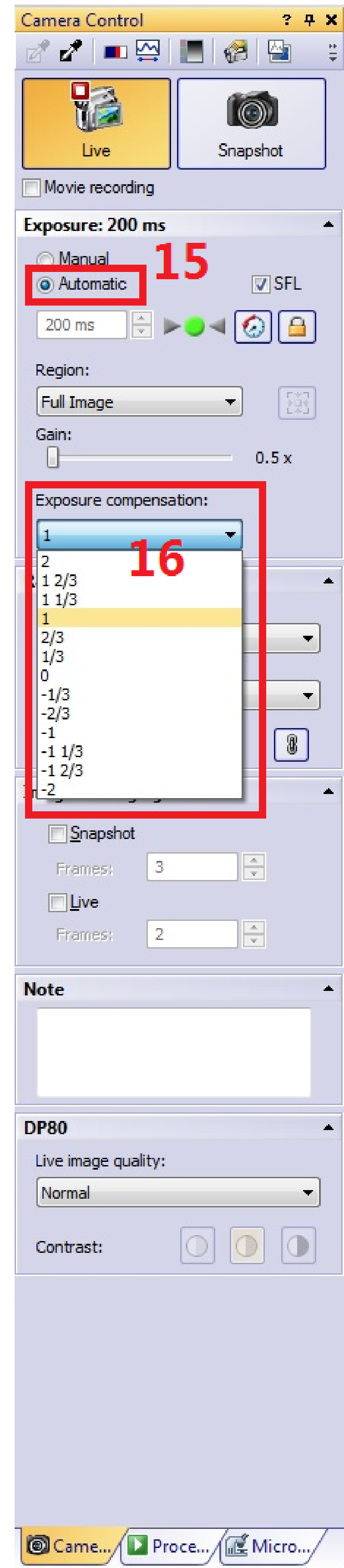
- *SFL (superfluorescence)* (10): turn it on when acquiring a fluorescence image.
- *Gain (11):* keep it lower than 1X if possible.
- *Live window resolution* (12):
- *Snapshot resolution* (13):
- Click "*Snapshot*" (14) to take an image.

2.2.4.2 *Automatic* (15) exposure mode: in this way, exposure time is automatically adjusted by the computer. However, should the live-image be too dark or bright, you can compensate the auto exposure time from "*exposure compensation*" list (16).

Other imaging conditions remain similar to manual exposure mode.

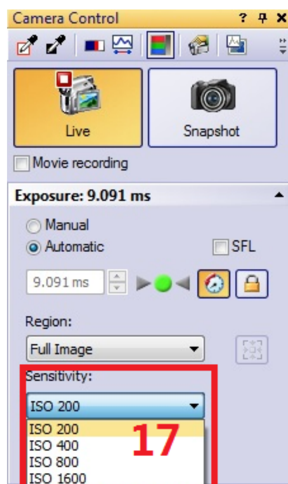
When using automatic exposure mode for BF imaging, "gain" is replaced with "sensitivity" (17).

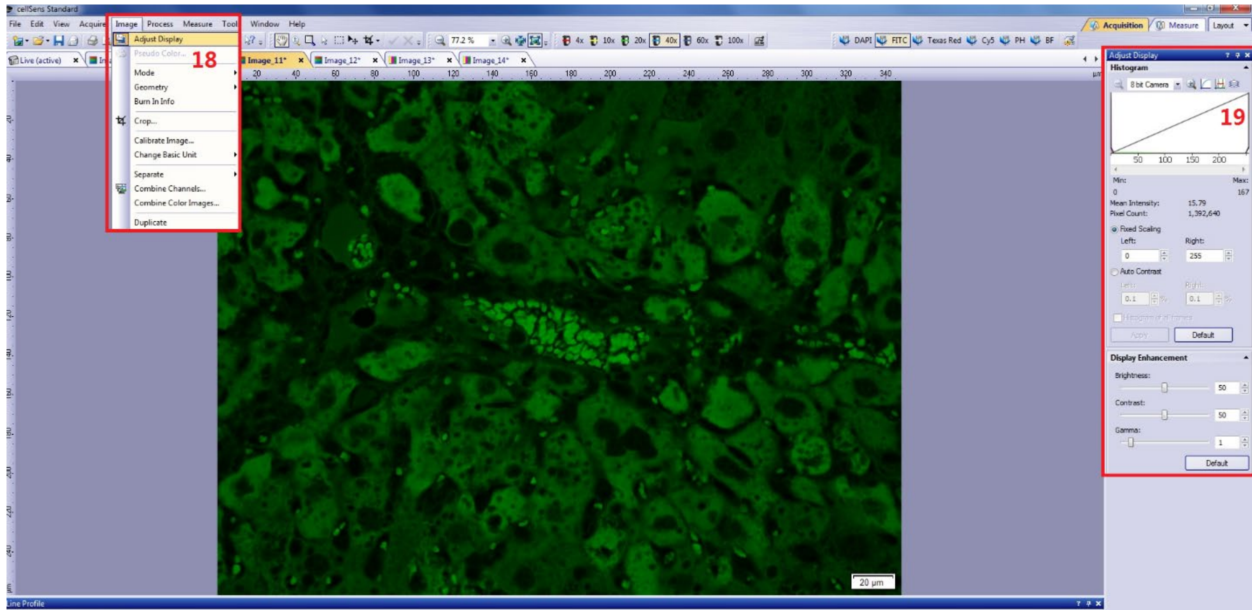




2.3 Basic post-acquisition analysis

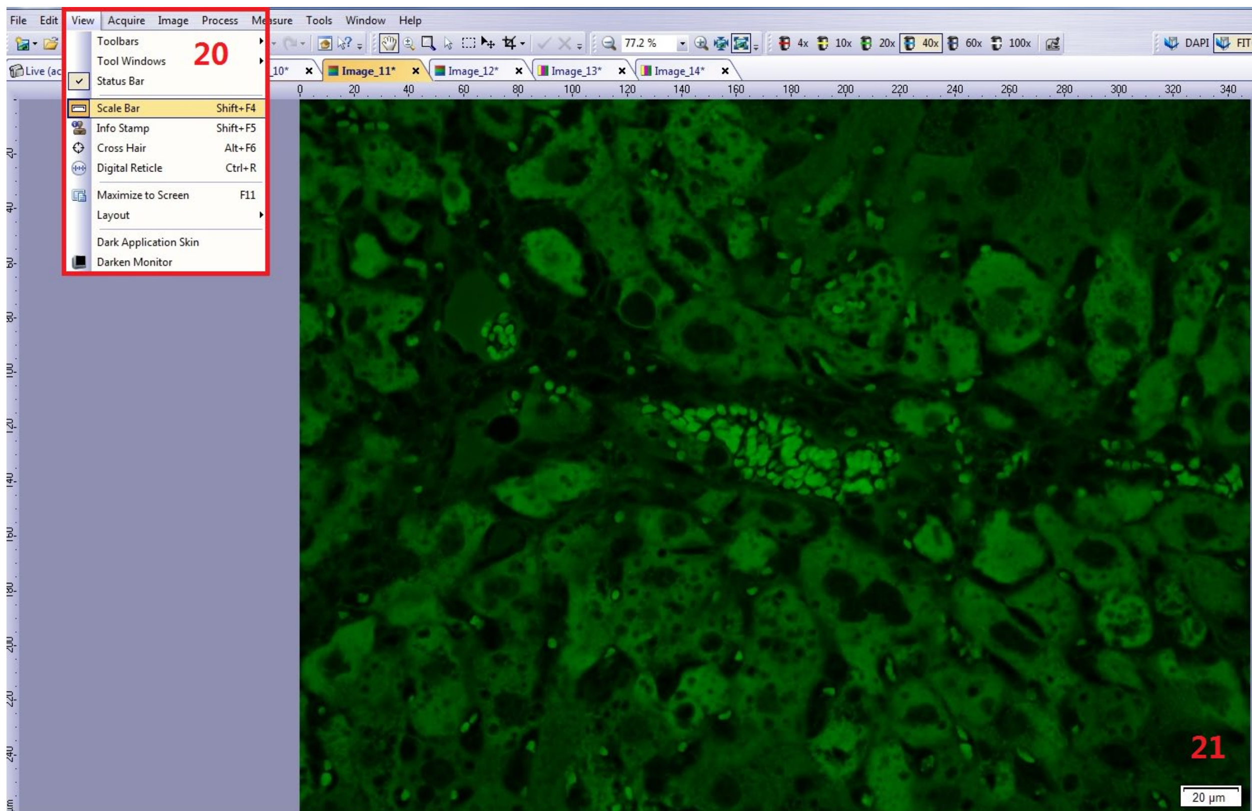
2.3.1 *Adjust display* (18): the *histogram* (19) will pop up after clicking "Edit"—"adjust display".





2.3.2 Adding a scale bar (20)

- lick “Edit”—“scale bar”, a bar will appear (21):



- The added scale bar can be edited: “tools” (22)—“options” (23)—“Format” (24)

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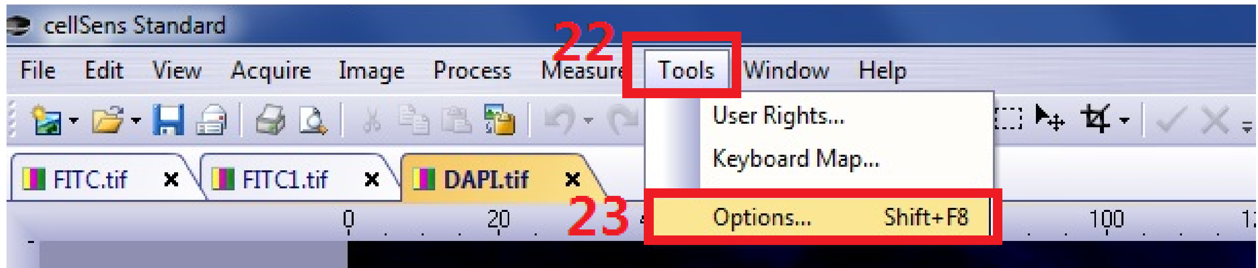
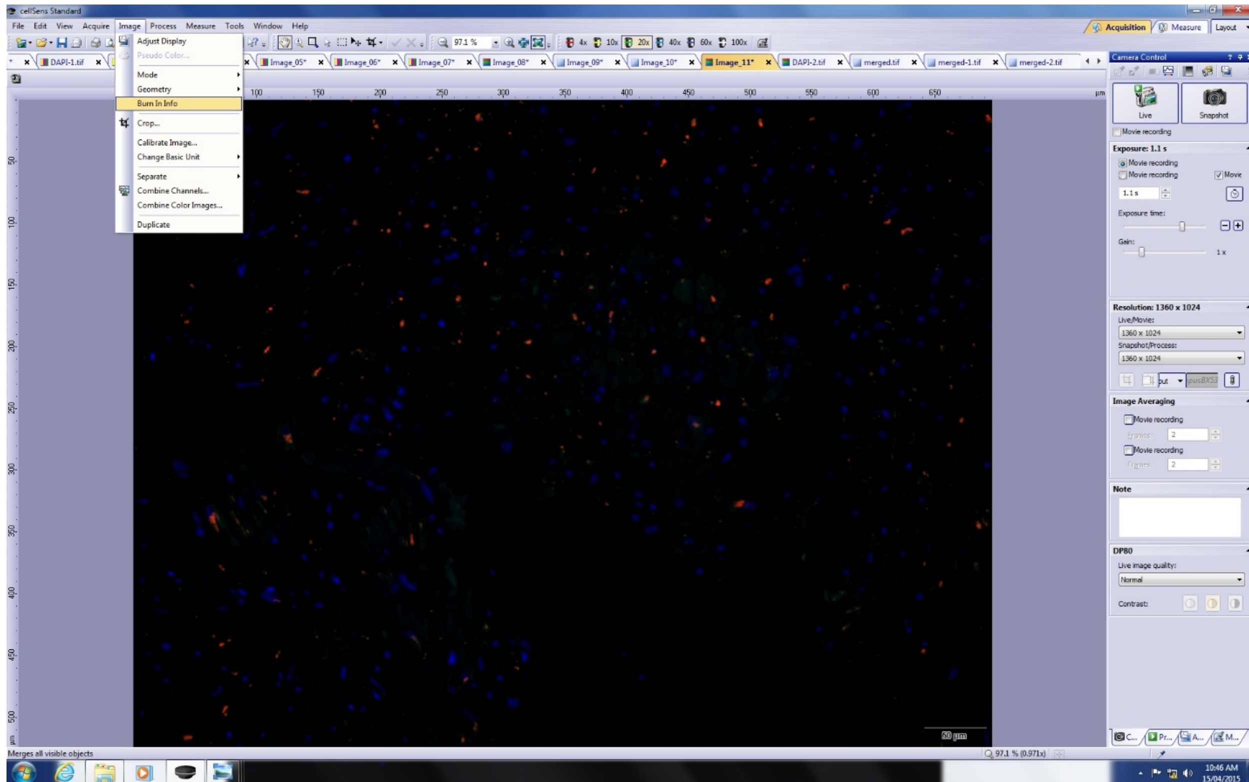


- Now stamp the scale bar to your image before saving it: “Image”—“Burn in info”:



2.4 Saving data

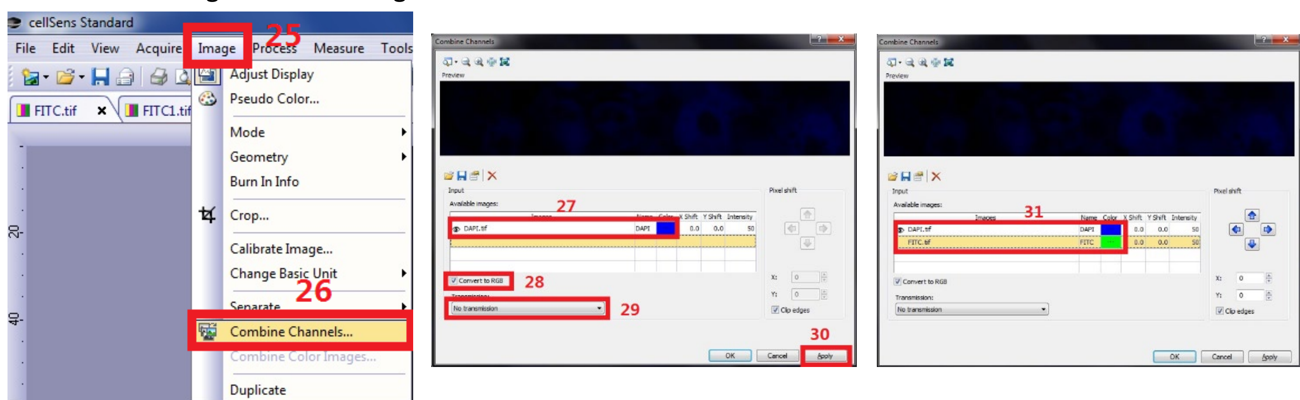
Data management policy: all data must be saved here (kept for 7 days before auto deleting): C:\ BX53 Users Data\“your folder”. Your data will be transferred to the server instantly: Scientific Platforms (\Cell Imaging\BX53\“your folder” (kept for 14 days before auto deleting).

2.4.1 Image format

- Greyscale: all images captured with monochrome chip camera are of grey scale even colour filters were used to show colours in live window (the colours are for display only). If you want to keep the filter colour for the grey scale images, you will need to convert them to RGB first.
- RGB: all images captured with RGB chip camera are of RGB mode. All RGB images can be saved directly and colours will be kept.

2.4.2 How to convert greyscale images to GRB mode

2.4.2.1 Single channel image

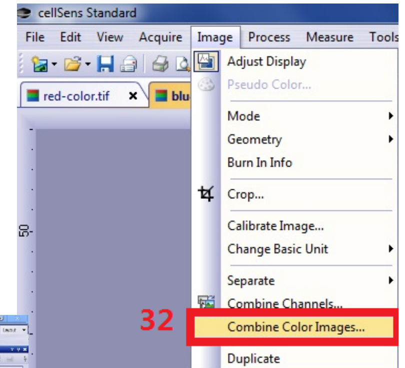


2.4.2.2 Merged multiple channel images: repeat Steps 25-26, then step 31 (others are the same as "single image")

2.5 How to merge images

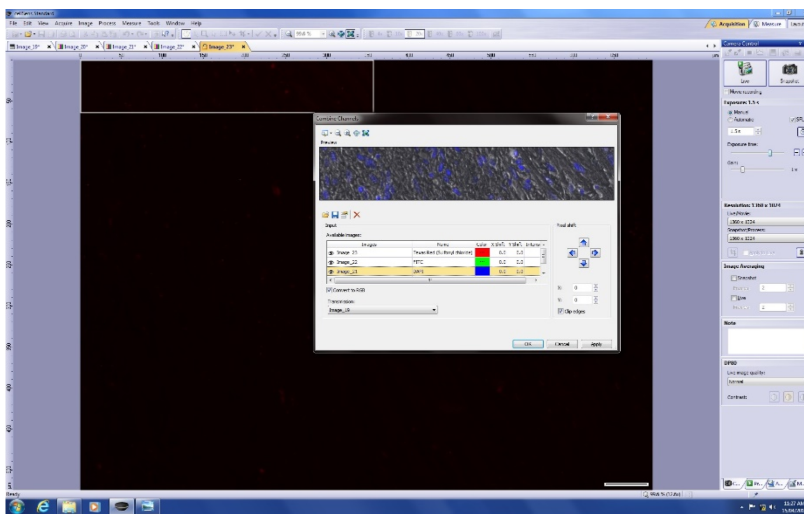
2.5.1 Greyscale fluorescence images. See step 25-26 and step 28-31.

2.5.2 RGB fluorescence images. Step 32 and then 30.

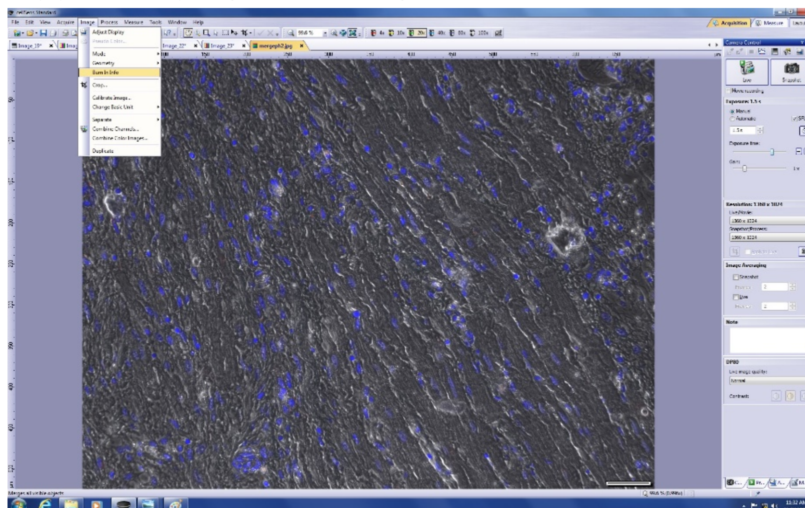


2.5.3 How to merge transmission image on top of fluorescence image (not applied to RGB images originally acquired from colour camera chip).

- Add transmission image
- Then stamp the phase contrast image on top of the fluorescence image:



- Say yes when you get this message:



DATA & RECORDS MANAGEMENT

All data must be saved here (kept for 7 days before auto deleting): C:\ BX53 Users Data\"your folder". Your data will be transferred to the server instantly: Scientific Platforms\Cell Imaging\BX53\"your folder" (kept for 14 days before auto deleting).

REFERENCES

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REVISION HISTORY

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