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Olympus BX51 Microscope & DP70 Digital Camera System

The BX51 was voted Microscope Winner by Scientific Computing & Instrumentation magazine as the 2002 Readers' Choice Award. July 2002.

Olympus BX51 Research Microscope

The BX51 offers increased rigidity coupled with easy-to-operate front controls on a compact space-efficient frame. The new research microscope is a union of the expanded range of Olympus infinity corrected optical systems (UIS2 - universal infinity system) and advanced fluorescence or differential interference contrast <u>DIC</u> (Nomarski) technology. Based around an enhanced Y-shaped frame, an extended range of accessories and objectives can be used. The rigid design accepts a variety of heavy attachments required for today's discerning microscopists and their various techniques and accessories.

- Bright 12V/100W halogen illumination source is ideal for transmitted light viewing
- The frame has two built-in neutral density filters (ND6, ND25), one daylight balancing filter, and one empty slot for an optional filter-fluorescence illuminator optional
- A light intensity, preset switch ensures reproducible lighting for photomicrography
- Ergonomic Y-shaped frame for maximum stability and comfort, with low position focus control knobs located on both the left and right sides of the frame
- Modular design and infinity optics system allows easy attachment of accessories without any compromise in image
- Large 22mm field of view reduces scanning time (a superwide trinocular head and eyepiece combination with a 26.5mm field of view is available as an option)
- UIS2 optics deliver bright, sharp, high-contrast images
- The 8-position universal condenser, with either a dry or oil top lens, enables you to perform brightfield, <u>darkfield</u>, Phase Contrast, DIC, polarization, and<u>fluorescence</u> with all objectives from 1.25x to 100x

Flexible System Format

- Modular design allows easy attachment of accessories without any compromise in image
- Accepts a variety of video attachments and film cameras, plus the BX-URA2 or BX-RFA fluorescence attachment
- 100W Halogen for transmitted or reflected light, 100W HBO* (Mercury) and 75W XBO (Xenon) for reflected light and fluorescence.
- Ceramic-coated stage with left-hand or right-hand low drive control, rotating mechanism and torque adjustment mechanism, optional rubber grips (non-stick grooved coaxial, plain, rotatable stages and others are also available)

Modularity & Versatility Meet Growing Research Needs

The modular design of the BX51 allows researchers to fully expand the design as their needs grow. This ensures a flexible and modular configuration even for special high-end research applications. New enhancements are a 6-position cube turret, 7-position nosepiece and 8-position universal condenser-not to mention REXBA capability, all-new mercury* and xenon lamp houses, and a total of three series of DIC prisms.

All components - from the stage, to the eyepieces, to the lightsource - can be customized and mounted onto the BX51, making it one of Olympus' most versatile microscopes. The standard UIS2 optics system means there's no compromise when adding intermediate attachments within the viewing path. For added versatility and flexibility, brightfield, darkfield, fluorescence, and Nomarski DIC observation all can be performed using a single set of objectives (U Plan Apochromat or U Plan Fluorite).

Ergonomic Comfort

The six-cube turret rotates in either direction for user convenience and filter set identification markers are clearly located directly in front of the user. The fluorescence shutter, illumination controls, and filter slots are all conveniently located within easy reach.

Advanced Fluorescence Technology Doubles Brightness

<u>Fluorescence</u> applications can be done by attaching a fluorescence illuminator that results in greater mechanical and thermal stability. The accompanying fluorescence illuminator, BX-URA2, incorporates a six-cube filter turret including front shutter control. The newly-developed aspherical lens within the lamp houses allows fluorescence illumination of up to double the brightness of existing models. To restrict the illumination to the need of any camera type, the BX2-RFA illuminator allows the substitution of the conventional circular field stop by a rectangular field diaphragm. As an Olympus accessory, two excitation balancers can be attached to the BX2-RFA illuminator. Thereby, improved differentiation of multi-labeled fluorescence specimens and better visibility of details is achieved.

Optimized DIC Capability Increases Resolution or Contrast

New DIC (Nomarski) units provide combinations for all specimens and magnifications with increased contrast or resolution. There are three series of DIC prisms to generate three levels of contrast/resolution. In addition to the current set of standard prisms, a series of high contrast prisms and high resolution prisms have been developed to produce high-quality images for your most demanding requirements. Within the new universal condenser, up to 8 optical components can be easily selected.

* Lamp contains mercury. Dispose according to local, state or Federal laws. Click <u>here</u>for important information.

BX51 Specifications		
Frame	Optical system	UIS2 optical system
	Focus	Vertical stage movement: 25mm stage stroke with coarse adjustment limit stop Torque adjustment for coarse adjustment knobs Stage mounting position variable High-sensitivity fine focusing knob (adjustment gradations: 1 � m)
	Illuminator	Built-in Koehler illumination for transmitted light 12V100W halogen bulb (pre-centered) Light preset switch Light intensity LED indicator Built-in filters (LBD-IF, ND6, ND25 optional)
Revolving nosepiece		Interchangeable reversed quintuple/sextuple/septuple nosepiece
Observation tube	Widefield (F.N. 22)	Widefield binocular, inclined 30� Widefield tilting binocular, inclined 5� - 35� Widefield trinocular, inclined 30� Widefield ergo binocular, inclined 0� -25�
	Super widefield (F.N. 26.5)	Super widefield trinocular, inclined 24 �
Stage		Ceramic-coated coaxial stage with left or right hand low drive control; with rotating mechanism

	and torque adjustment mechanism, optional rubber grips available (non-stick grooved coaxial, plain, rotatable stages are also available)
Condenser	Abbe (N.A. 1.1), 4x-100x Swing out Achromatic (N.A. 0.9), 1.25x-100x (swing out: 1.25x-4x) Achromatic Aplanatic (N.A. 1.4) 10x-100x Universal (N.A. 1.4/0.9), 2x-100x (swing-out: 2x- 4x, with oil top lens: 20x-100x)

BH51 Optics

The BX51 will accept all UIS optics:

- Achromats 10x, 20x, 40x, 60x, and 100x oil
- Plan Achromats 2x, 4x, 10x, 20x, 40x, 50x oil, and 100x oil
- U Plan Fluorites 4x, 10x, 20x, 40x, 60x oil, 100x oil or dry
- U Plan Apochromats 1.25x, 2x, 4x, 10x, 20x oil or dry, 40x oil or dry, 60x oil or dry, 100x oil
- A complete line of Phase Contrast objectives is also available

Specialized objectives include a range optimized for 340nm operation, water immersion objectives with high numerical aperture (NA), objectives designed for high NA observation of living cells, and a water immersion objective (60x) corrected for apochromatic performance from 450-1100nm.

Other Special Purpose Objectives:

- Pathology: 60x Achromat for high magnification and convenient oil-free use
- Pathology: 2x Plan Achromat for large field scanning and imaging

Olympus DP 70

The latest generation of digital cameras designed for wide-ranging applications in optical microscopy combine excellent resolution, high sensitivity, and rapid data transfer to a host computer. The Olympus DP70 is a 12.5 million-pixel cooled digital color camera system that incorporates the latest innovations in imaging technology to enable the capture of superb images in the most demanding current microscopy applications, including differential interference contrast (**DIC**), darkfield, phase contrast, polarized light, and most widefield fluorescence techniques.

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The control software for the DP70 camera system was developed to provide a multifunction

photomicrography interface (incorporating several features from traditional film camera exposure

monitors) that facilitates control of real-time image acquisition, as well as subsequent image management. In addition to the image acquisition user interface displayed in the tutorial window above, the DP70 software package includes an image management program through which stored images, displayed as thumbnails, may be easily viewed and manipulated by drag and drop procedures. A six-channel image merging function is provided that enables individual fluorescence channels and differential interference contrast optical sections, for example, to be merged into a single multi-color image. This tutorial demonstrates the functionality of the DP70 digital camera image capture interface. Many, but not all, of the operational features of the actual camera control software are enabled in the tutorial, and may be explored by the viewer. Upon initialization, the image window displays a specimen image randomly chosen from a group of digital images captured utilizing a variety of optical microscopy techniques.

A different specimen can be manually chosen by selecting it from the **Choose A Specimen** pull-down menu located beneath the camera control window. To the left of this menu is a **Focus Adjustment** slider that allows optimum focus of the image to be obtained by dragging the slider with the computer mouse. Each specimen name in the pull-down menu includes, in parentheses, an abbreviation designating the contrast mechanism employed in obtaining the image. The following nomenclature is used: (**FL**), fluorescence; (**BF**), brightfield; (**DF**), darkfield; (**PC**), phase contrast; (**DIC**), differential interference contrast (Nomarski); and (**POL**), polarized light.

The DP70 camera (illustrated in Figure 1) employs a single-chip charge-coupled device (**CCD**) sensor with Bayer RGB primary color filtration. The two-thirds-inch CCD chip incorporates 1.45 million effective pixels, which can be piezo-shifted during image acquisition to obtain a maximum effective resolution of 12.5 million pixels. Images may be acquired at the ultra-high resolution of 4080 x 3072 pixels or at any of three lower-resolution settings, as dictated by the optical system capabilities or the intended image use.

With the **Capture** tab selected from the group appearing below the image window (the default tab

selection), a **Still-Image** control panel provides a **Size:** pull-down menu for choosing the image resolution. When high numerical-aperture microscope optics are employed, the camera's maximum resolution is capable of exploiting the full range of the optics. The DP70 is a 12-bit camera, producing images with 36- bit RGB color depth, which are saved as 48-bit image files. This degree of color fidelity, coupled with high sensitivity enhanced by Peltier cooling of the chip, produces images with outstanding detail and color, which are created and transferred in approximately 3 seconds.



Olympus DP70 Digital Camera System

Displayed within the image capture window is a yellow-bounded square, which identifies the area utilized by the exposure metering system. With either **Auto** or **SFL-Auto** selected in the **Exposure Mode** control area, "spot" metering is enabled within the area defined by the yellow box. The box may be adjusted, as appropriate for the image characteristics, to 30 percent, 1 percent, or 0.1 percent of the image area by cursor selection of the corresponding button in the **Spot** panel of the control window. The metering area box can be positioned by dragging with the mouse cursor to any location within the image window, so that an image area appropriate for exposure metering can be chosen. The large button appearing to the immediate right of the **Spot** radio buttons recenters the yellow box in the image window when clicked with the cursor.

The **Exposure Mode** control panel provides two other choices in addition to the default **Auto** mode, which employs an algorithm that is most suitable for brightfield imaging of conventional stained specimens. The **SFL-Auto** (Super FL) mode is designed for simplified automatic exposure adjustment during fluorescence image acquisition, and determines exposure time utilizing metering parameters developed specifically from fluorescence imaging experience. Selecting **Manual** mode disables automatic exposure adjustment and allows the preferred image brightness to be set by clicking and dragging the slider positioned in the **Exposure Time** panel. The image brightness in the display window will reflect changes in the slider position, and the corresponding exposure time is displayed in the window above the left-hand boundary of

the slider scale. The camera's exposure time range is from one-44,000ths to sixty seconds. A pull-down menu labeled **Adjust**, to the right of the exposure time indication,

is enabled in either of the auto exposure modes, and allows the metered exposure value to be compensated, or offset, by a fixed amount ranging from 2 exposure value (**EV**) units less (-2) to 2 EV units more (+2) exposure. The compensation chosen is reflected in the exposure time displayed.

The high-sensitivity CCD was developed specifically for the DP70 camera system, and with the benefit of Peltier cooling, achieves low-noise performance at sensitivity settings as high as the equivalent of International Standards Organization designation of ISO 1600. Settings ranging from ISO 200 to ISO 1600 are selected in the tutorial by clicking the corresponding radio button in the Sensitivity area of the control panel. High sensitivity is particularly beneficial in that it allows higher frame rates during image preview, facilitating focusing and framing prior to image capture, even when very faint fluorescence signals are being collected. The effective chip sensitivity can be further extended by utilizing pixel binning during image preview. This function can be explored by selecting the **Preview** panel tab beneath the image window. The DP70 provides both 2x2 and 4x4 binning options, selectable by clicking the appropriate radio button in the **Binning** control panel. When pixels are combined in this fashion, the camera allows live lower-resolution images (680 x 512 pixels) to be displayed at a rapid frame rate of up to 15 frames per second (depending upon the computer configuration) for preview purposes of focusing and framing. A frame-averaging feature can be selected by clicking the 2 frame radio button in the Real-time frame

average panel to the right of the binning controls.

Several additional settings panel tabs located beneath the image display window provide further control possibilities. Selection of the Color balance tab provides several mechanisms for setting white balance or black balance. Within the White Balance control panel, two buttons are provided that enable automatic white balancing of the image, a function primarily employed in brightfield observation to provide accurate color rendition regardless of illumination variations. Selecting the button labeled **One Push** adjusts the image sensor response so that the entire image area is white. In practice, this setting would be performed with the slide removed from the light path in order to set a white background. Upon execution, the Manual radio button is enabled and the sliders for the **Red**, **Green**, and **Blue** color channels can be utilized to make any necessary manual adjustments to the color balance. For many specimens, better white balance adjustment may be achieved by manually selecting a white or neutral gray area for reference within the specimen image area, and the **One Touch** button can be used to enable pixel selection by mouse cursor. When the button is selected by mouse, an evedropper icon (a white balance setting pointer) replaces the mouse cursor when it is positioned within the image area. Clicking the icon on a suitable reference area for white balance adjustment will result in the appropriate color adjustments being conducted, and the manual adjustment sliders will be enabled for further fine-tuning of the color balance. In the actual camera software, the cursor may be used to designate a rectangular marguis area by clicking and dragging from the upper left corner to the lower right corner of the region to be rendered as white. After either method of automatic white balancing has been employed, the adjustment made can be cancelled, returning to the

original image, by selecting the **Off** radio button in the white balance control area.

A **Black Balance** control panel has functional features similar to the white balance controls, except a single level slider is provided to adjust the image brightness (offset), instead of the individual color channel sliders. Black balance adjustment is used primarily in fluorescence observation, in which the background area is dark, and white balance adjustment would not be appropriate. The **One Touch** button is utilized in the same manner as for white balance adjustment, although the eyedropper tool is used to select a black or dark area in the image for black balance reference. **One Push** setting is carried out on the black background with no specimen in place, and adjusts the brightness so that the entire image area appears black. In either white or black balance adjustment, the automatic-setting step can be skipped when fully manual control is desired, simply by selecting the appropriate **Manual** radio button as the initial step, followed by manipulation of the corresponding slider(s).

The tab labeled Level adjust may be selected to display histograms for each of the red, green, and blue color channels, allowing precise adjustment of the image sensor's response characteristics. This function allows the contrast and gamma of the preview image to be altered in order to improve both live visualization of the specimen and the quality of the captured image. Images captured with the DP70 camera will reflect changes made to the preview image through the level adjustment function. Input and output intensity values can be altered for the combined **RGB** signal, or for the individual color channels, by selection from the Channel pull-down menu. The upper slider appearing beneath the histogram window enables adjustment of input intensities, and the lower slider serves the same function for output levels. Modifications are made by dragging and dropping the set-point triangles along the sliders. Numerical values are displayed in the **Input** and **Output** windows as the setting marks are repositioned, or alternatively, the desired numerical values may be entered directly through the keyboard. The left-hand triangle on each slider corresponds to the shadow intensity level, while the right-hand marker controls the highlight intensity. The center triangle on the input slider adjusts the gray level, and functions effectively as a gamma adjustment, which should be varied as necessary by observing the histogram and the effect on the preview image. The level values for the image may be reset to the default condition by clicking on the Reset button.

Selection of the **Scale** tab enables the user to establish up a scale bar that is superimposed on the image as a reference for making approximate size measurements during specimen observation. The appropriate scale bar is generated by the DP70 software based on the objective and relay magnification values entered in the **Objective Lens** text box and selected from the **Adapter Lens** pull-down menu. In the tutorial, a sample scale bar is displayed, but does not change in response to values entered in the numerical fields, nor does the displayed objective magnification change in response to clicking the objective selection buttons. The **Display** area of the panel contains a check box labeled **Show Scale** that determines whether the scale is displayed or is turned off. A simple time-lapse function is provided in the DP70 capture software, and is accessed by clicking the **Timelapse** tab beneath the image window. Although not enabled in the tutorial, this feature of the camera user interface allows the time and interval variables to be entered, and a series of images to be captured and converted to an **AVI** file for immediate playback.



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