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### SPECIFICATIONS

<b>Scan RDI Specifications</b>	
<b>System Components</b>	Main analytical console Laser Module CRT Printer Data Manager Motorized stage (option)
<b>Analytical Performance</b> (Main console)	Throughput rate : 15-20 membranes per hour Scan Time : 3-3.5 minutes max. Sensitivity level : sub micron, to approx.0.3µm (dependent on labeling quality).
<b>Samples</b>	Membrane size : 25mm Sample type : Filterable
<b>Sample compartment</b>	Light-tight compartment, with safety interlocks for Laser control Temperature regulated support mechanism, with positive focus alignment. Membrane support location accuracy : 100µm. Membrane Support Temperature : 4-8°C with Peltier cooling Motorized drawer.
<b>Detection System</b>	Scan Area : 25mm (max) Scan Speed: 2 metre/sec. Scan line spacing 2.2µm (resolution) Laser type : Argon Ion (air cooled) 488nm. Laser Power range : 5-30 mW (software controlled), coupled by fibre-optic Laser spot size : 6µm Detection wavelength: 500-530 & 540-585 nm.
<b>Computer Process &amp; Software</b>	Data acquisition and processing by Intel Pentium Pro PC Data capture : High performance DSP technology (20 million instr./sec.) with high speed 12 bit analogue-digital converters. Mass Storage : 2.0Gb hard disk. Data storage : 10000 scans (typical) with full sample details. Operating system is Windows NT 4.0, with the applications programs in C++; all real-time applications are in assembly or DSP code.
<b>CRT and Printer specifications</b>	CRT size : 35.5 cm (14 inch) diagonal CRT color SVGA resolution Printer : system supports one parallel interfaced printer, Hewlett Packard inkjet. Interfaced cable length : standard 1-1.2m (4ft).

<b>Microscope</b>	Epifluorescence microscope with full drive control for <b>(automated table drive)</b> positive XY location to any membrane spot corresponding to Scan RDI detection. Accuracy of location : within 100 micron
<b>Environment requirements</b>	Ventilation sufficient to remove heat below 30°C with 80% relative humidity; assuming total system load is 11 A at 230V. Temperature operating : 5 to 30°C Humidity operating : 5 to 80% Altitude 0-2000 meter
<b>Electrical requirements</b>	Scan RDI operates on single-phase grounded power line at 110V / 230V at 50Hz / 60Hz. Voltage variations of +/- 10% can be tolerated. The laser module has a main connection to the main console and can be located up to 2 meters from the console. Laser presents a load of 6-8A at 110V / 230V – 50Hz / 60Hz. Fuses : Fuse FS1 : T5A - H 500V ; Fuse FS2 : T10A - H 500V.
<b>Dimensions</b>	<p>Analytical console:      55,7 cm deep    42,5 cm high    80,1 cm wide    Weight 50kgs</p> <p>Laser module:              59,3 cm deep    42.5 cm high    31,8 cm wide    Weight 20kgs</p> <p>CRT (typical) : 35 cm wide    46 cm deep    35 cm high</p> <p>Keyboard:                    20 cm deep    46 cm wide    7,5 cm high</p>
<b>Regulatory requirements</b>	Safety: Scan RDI is designed to meet the CE standards RFI/EMC: TBA

The Scan RDI Unit is a machine that is capable of the rapid detection and enumeration of very low numbers of micro-organisms. Samples are collected on a solid support, labelled with a fluorescent marker and laser scanned.

Liquid samples are taken and filtered using a 25mm diameter membrane which retains microbial cells on its surface. The retained cells are directly labelled in situ with viability markers in order to obtain a total viable count reading.

The membrane is then manually transferred to the analyser which laser scans the entire surface of the membrane and analyses the resulting data. The laser scanning facility is fully overlapping to ensure that any cell fluorescence on the membrane will be detected by the system's multiple detection channels.

To ensure reliable results, the Scan RDI Unit incorporates advanced proprietary discrimination parameters. Thus the system can differentiate between valid signal (labelled micro organisms)

and background (electronic, optical, and auto-fluorescent particles). The resulting data is then transferred to a computer memory.

The data is available as a scan map which shows the location of each detected micro-organism. With the aid of a link to a motor-driven microscope stage, each micro organism that has been detected can be visually confirmed on the membrane if necessary under the microscope.



The Scan RDI Unit comprises the following main items of equipment :

- Membrane Scanning Support
- Laser Scanning System
- Optical Detection System
- Digital Signal Processor / Instrument Personal Computer
- General Framework And Cover

### **Olympus DP70 Digital Camera System**

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.

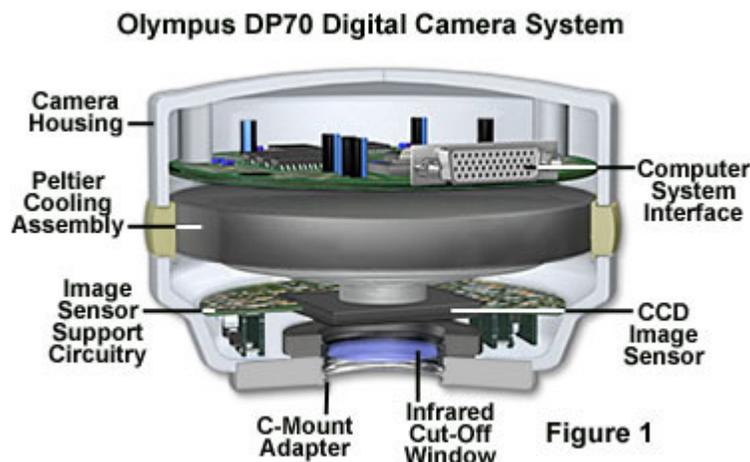
The latest generation of digital cameras designed for wide-ranging applications in optical microscopy combine excellent resolution, high sensitivity, and rapid data transfer to a host computer. The Olympus DP70 is a 12.5 million-pixel cooled digital color camera system that incorporates the latest innovations 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.

The control software for the DP70 camera system was developed to provide a multi-function 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.



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 re-centers 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 eyedropper 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 marquis 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.

### **Contributing Authors**

**Thomas J. Fellers** and **Michael W. Davidson** - National High Magnetic Field Laboratory, 1800 East Paul Dirac Dr., The Florida State University, Tallahassee, Florida, 32310.



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