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Image Analysis Enhancement of the Laser Scanning Cytometer

Gerrit Woltmann, Andrew J. Wardlaw, and David A. Rew*

University Department of Respiratory Medicine, University Department of Surgery, and the Advanced Laser Cytometry Unit, University of Leicester, Leicester, United Kingdom

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The laser scanning cytometer offers a range of novel applications and the capacity for direct visual validation of experiments through sample analysis on a microscope slide. Linkage of the instrument to an image analysis system through standard connections and software enhances the capabilities of the instrument in image capture and manipulation. In this technical note, we describe a simple linkage between the LSC and the Kontron KS100 Image Analysis System, an example of a standard commercial image processing instrument. Cytometry 33:362–365, 1998. © 1998 Wiley-Liss, Inc.

Key terms: laser scanning cytometer; image analysis system

The laser scanning cytometer (LSC) is a new tool for the analysis and quantitation of fluorescence in monodispersed cell and disaggregated tissue samples (1–6). Although it has evolved from flow cytometry and shares many components and functions, laser scanning cytometry offers the key advantage of sample study on a microscope slide. The laser beam is scanned across a microscope slide on a conventional microscope with an integral epifluorescence system. After laser excitation, fluorescence emissions from labeled cells are returned through the microscope optics to the photomultiplier tubes housed in the main assembly unit through a beam splitting mirror. This also transmits light to two camera ports above the microscope. The conventional instrument is supplied with a monochrome camera and monitor to observe and locate cells during standard analysis. Grey scale images are thus displayed, and are also transmitted to the computer monitor for further manipulation, including, for example, the drawing of threshold contours around individual cells.

The power to visualise, recall, and relocate cells which have been excited and measured in laser light represents a significant advance over flow cytometry in many applications. Viscid samples such as sputum, paucicellular samples such as fine needle aspirates, and cytospins can be conveniently analysed using this system. Direct visualisation of selected cells also introduces the capacity for certainty in cytometric assays through optical validation of cells of interest identified from dot plot and histogram data.

The basic, first-generation commercial laser scanning cytometry instrument is nevertheless limited in its capabilities for colour image capture, analysis, manipulation, and presentation. These features are intrinsic to modern image analysers (7,8), a conventional example of which is the Kontron KS100 system (Kontron GmbH, FRG). This comprises a Pentium-based computer (75 MHz processor, 16Mb RAM) with proprietary software and a separate microscope mounting a CCD camera. We thus hypothesised that the camera port in the direct optical line could be used to capture and digitise video signals and sample images for offline processing and archiving.

METHODS

Using components common to both instruments, we linked our laser scanning cytometer to a KS100 by the simple manoeuvre of placing the CCD colour video digitising camera (JVC TK-1270) from the KS100 on the anterior universal mounting (the C mount) on the Olympus microscope, in the direct optical line of the microscope. The black and white camera was placed on the posterior universal port above the microscope to maintain the gray scale feed to the analog video monitor (Sony PVM-97, Sony Corp.) (Figs. 1, 2). No other modifications were required to the laser scanning cytometer.

By this simple adaptation, the image analyser could be utilised to capture and digitise still video signals and sample images, for further study and for export to other offline systems. Initial image acquisition and digital storage

*Correspondence to: D.A. Rew, Clinical Sciences Wing, Glenfield Hospital, Leicester LE3 9QP, United Kingdom.
E-mail: dar2@le.ac.uk

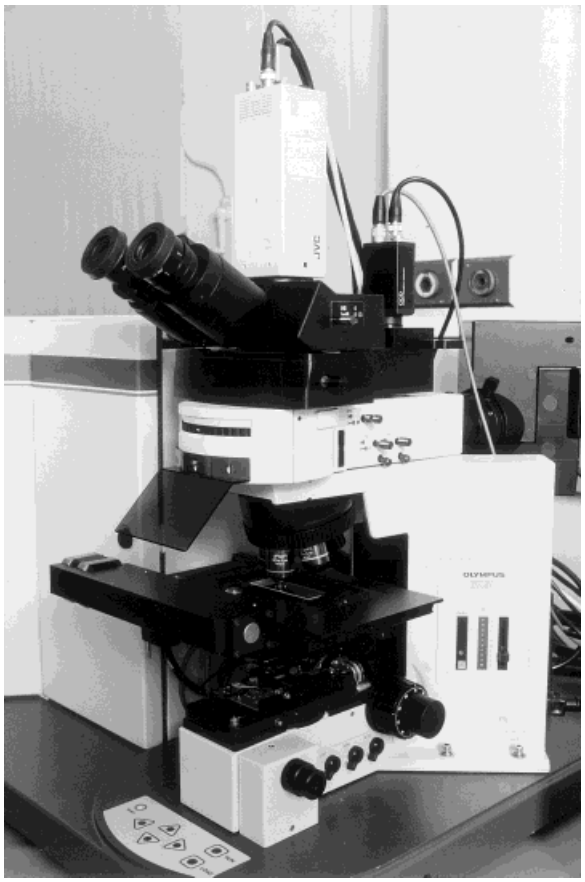

FIG. 1. Photograph of the CCD colour video digitising camera, installed on the front camera mount of laser scanning cytometer, with top mounted cable leading to the image analyser. The black and white camera integral to the LSC is remounted on the posterior camera port.

was assessed in a variety of compressed and uncompressed graphics data formats through the Kontron system.

The Kontron computer system was further enhanced with freely available image processing software from the Internet, including Scion Image (Scion Corporation, MD); Image Tool 1.27 (UTHSA, San Antonio, TX); Paint Shop Pro Ver. 4.1 (JASC Inc,); LView Pro (MMedia Research Corp.), and image cataloguing software, ThumbsPlus 3.0 (Cerious Software Inc.).

RESULTS

Enhanced facilities obtained from the linked LSC-KS100 system included time efficient colour image capture, manipulation and display, and the improved archiving of digital images. In addition to relocated and rescanned black and white images, we could obtain epifluorescent and brightfield colour images of any scanned cell or feature of interest.

We found that image acquisition can be performed independently in time of the laser scanning process. For example, regions of interest can be visualised at low or high magnification and captured under epifluorescence immediately after scanning. We also found that brightfield microscopy and image capture can be performed after a time interval. An example of this application is to visualise the effects of chromatic staining in the same cell subset.

For example, when analysing cytospins of sputum from asthmatic subjects, we were able to detect and characterise eosinophils with the LSC using fluorescently labeled antibodies against major basic protein (MBP; Fig. 3A,B). Relocation to cells with bright green fluorescence was possible immediately after the scanning process, thus

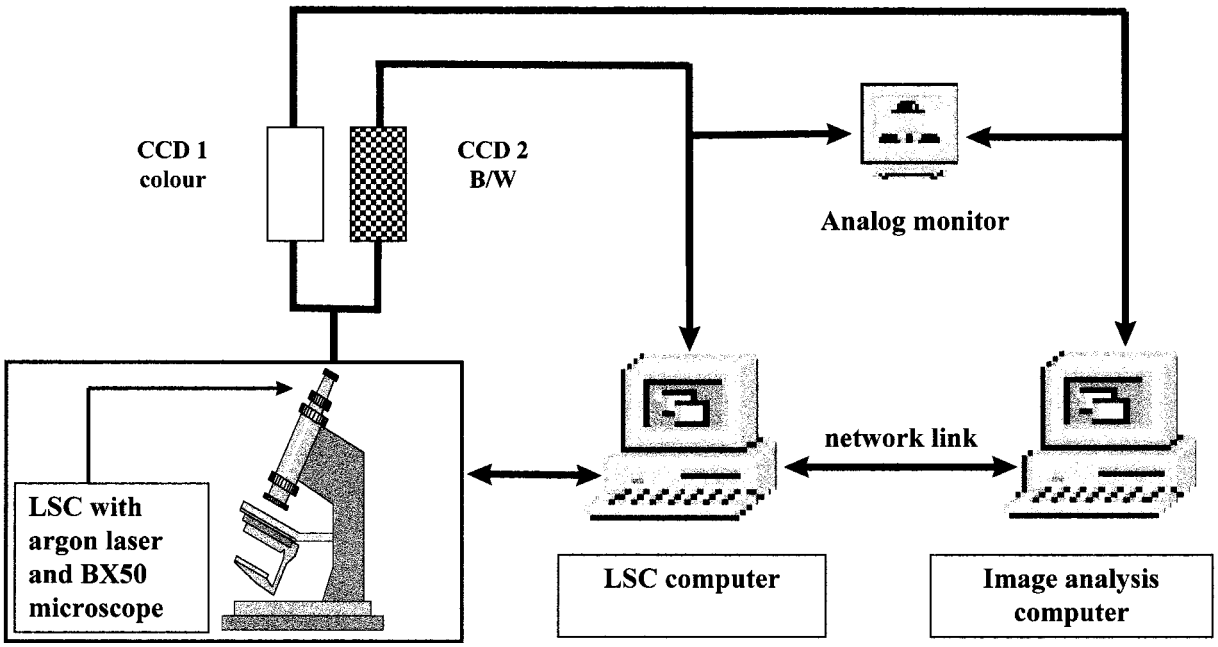

FIG. 2. Linkage of laser scanning cytometry and image analyser components.

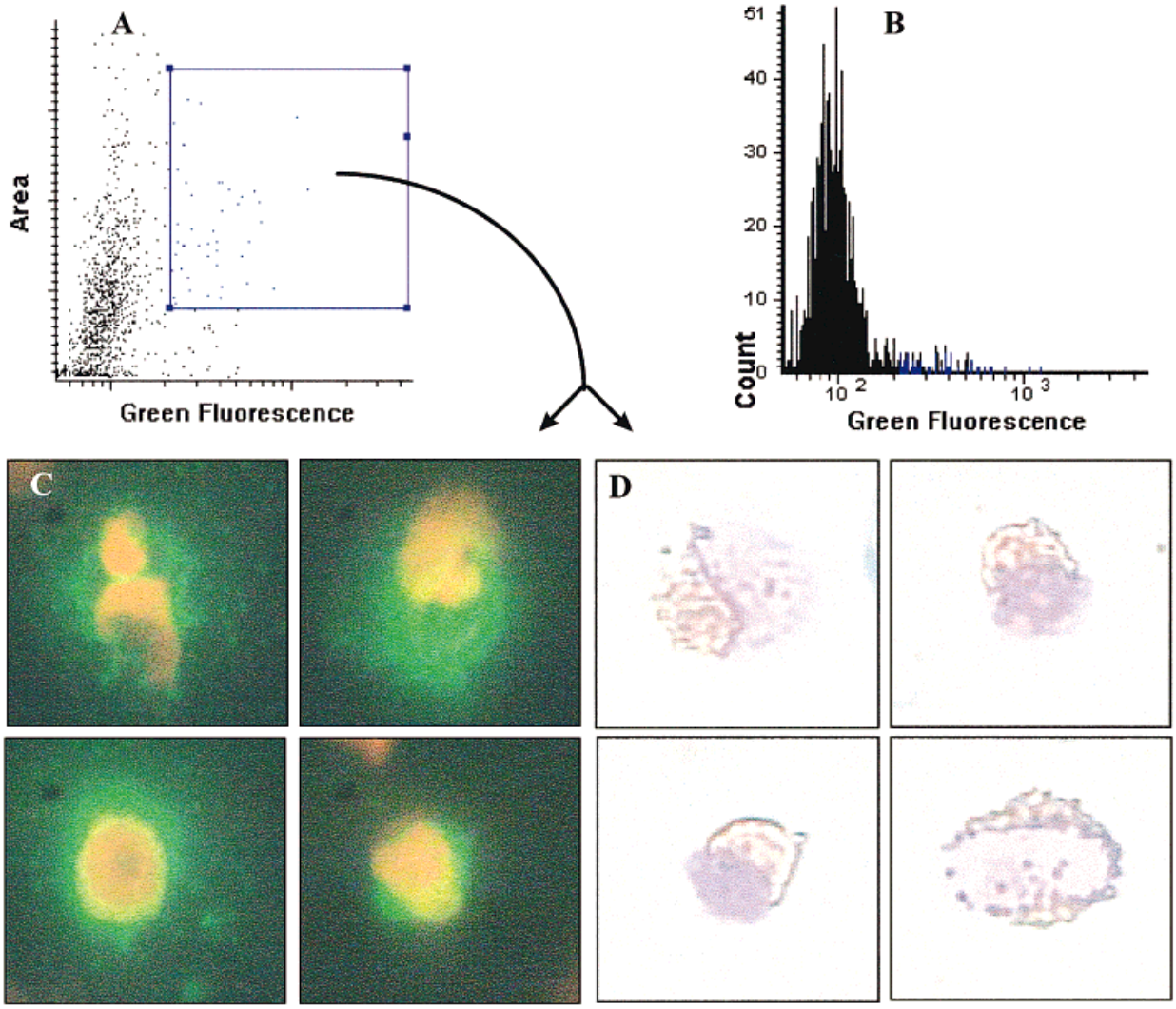

FIG. 3. Composite graphic of a population of eosinophils analysed within a sample of human asthmatic sputum, as described in the text. (The images of the cells have not been matched in this illustration.) **A**: Gated dot plot of cell size versus fluorescence of FITC labelled major basic protein antibody. **B**: Histogram of the green fluorescence profile of a sputum sample labelled with FITC-MBP antibody. Eosinophils are identified by their high fluorescence. **C**: Epifluorescent image of MBP-labelled eosinophils following relocalisation on the LSC. **D**: Same slide restained with eosinophil-specific chromotype 2R and imaged under brightfield microscopy.

allowing us to document the morphology of these cells under epifluorescence (Fig. 3C). After restaining the same slides with the conventional eosinophil specific stain Chromotrope 2R, we were able to confirm that cells within the MBP-positive region of interest were definitely eosinophils by traditional morphological criteria under brightfield microscopy (Fig. 3D).

False colour could also be applied to laser scan images obtained from up to three photomultiplier tubes, and false colour assigned to the laser scatter sensor. For example, three gray scale images of cell fluorescence or scatter signals, each of eight bits, could be combined into a 24 bit RGB colour image. Examples of images obtained from the combined system are shown. Cytometry datasets were thus combined with detailed, digital morphological information obtained by a variety of techniques.

DISCUSSION

The use of common components, adaptors, and compatible software in modern laboratory instrumentation allows interconnectivity and enhanced utility in novel ways. The combination of two such instruments with synergistic capabilities has added to the utility and effectiveness of both instruments.

Standard computers and software allow a range of additional modifications. For example, our system has been further enhanced by installation of ethernet cards

(3COM) into each computer. This allows networking to a departmental or university LAN, and to the Internet. Data and protocol files can thus be freely exchanged locally and with researchers in other laboratories. We can conceive of online diagnostics and upgrades to further enhance the efficiency and cost effective use of the equipments. We thus commend the use of common components and systems by manufacturers and designers wherever possible, to enhance the utility, the communicability, and the interoperability of diverse electronic laboratory instruments.

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