TECHNICAL NOTE 13

BIORAD

TECHNICAL

Bio-Rad Signal Enhancing Lens System (SELS) – *turbo mode for confocal*

A new method* for increasing the sensitivity of a confocal microscope while retaining confocal optical sectioning

Written by: W.B. Amos and S. Reichelt, MRC Laboratory of Molecular Biology, Hills Road, Cambridge, UK.

A key step in the development of confocal microscope systems for biology was the realization that if the diameter of the detector iris was increased above the size needed for optimum confocal function, the image brightness could be increased and lower laser powers used (White *et al.*, Wells *et al.*). The variable iris, introduced by Bio-Rad in 1986, was eventually adopted for this purpose by all manufacturers. In the Radiance series it allows the iris diameter to be increased approximately 24 times above the optimum confocal size for high-power high-N.A. objectives.

The new **Bio-Rad signal-enhancing lens system (SELS)** in combination with the Radiance confocal systems is optically equivalent to raising the upper iris diameter to 500 times the confocal optimum: higher than is available in any other confocal system. The level of signal enhancement depends on the thickness of the specimen but in many cases, such as live embryos, the intensity is increased by a factor of five or more. This makes it possible to image with a great reduction in laser power, with dramatic improvements in the viability of living cells in the scanning beam.

SELS (*Fig 1*) works by reducing the magnification of the image at the level of the detector iris by a factor of 20. The detector is thus able to pick up light from a larger volume of the specimen. *Fig 2a–c* shows how the SELS increases the signal strength, particularly when used in combination with an increased detector iris size. A precision motorized mount allows the SELS to be inserted or removed from the imaging pathway of the Radiance under computer control at any time during imaging.

Since there is no disturbance of the confocal telescope optics it is possible to switch in less than a second from high-signal to optimised confocal operation.

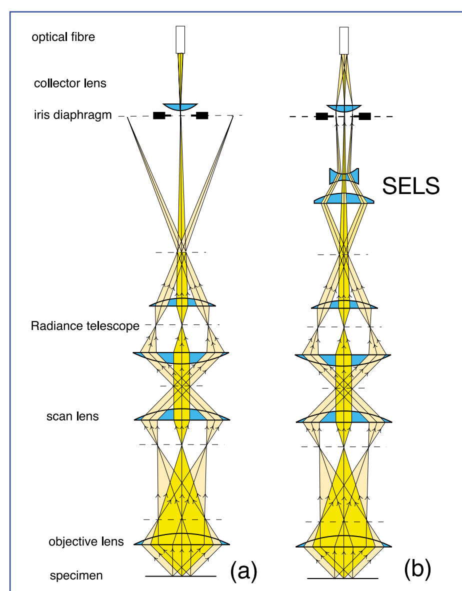


Figure 1 (*1a*) Optical system of the Radiance confocal microscope. The scanning system is omitted for simplicity. Light is traced from three points in the specimen plane, one on axis and the others equidistant from the axis. Note that the light from the peripheral points does not enter the detector. (*1b*) The same optics with the SELS inserted. The light from the peripheral points is now included in that collected by the detector. The SELS is in reality a four-element coated lens system optimised for light collection efficiency.

*Patent Pending

BIO-RAD

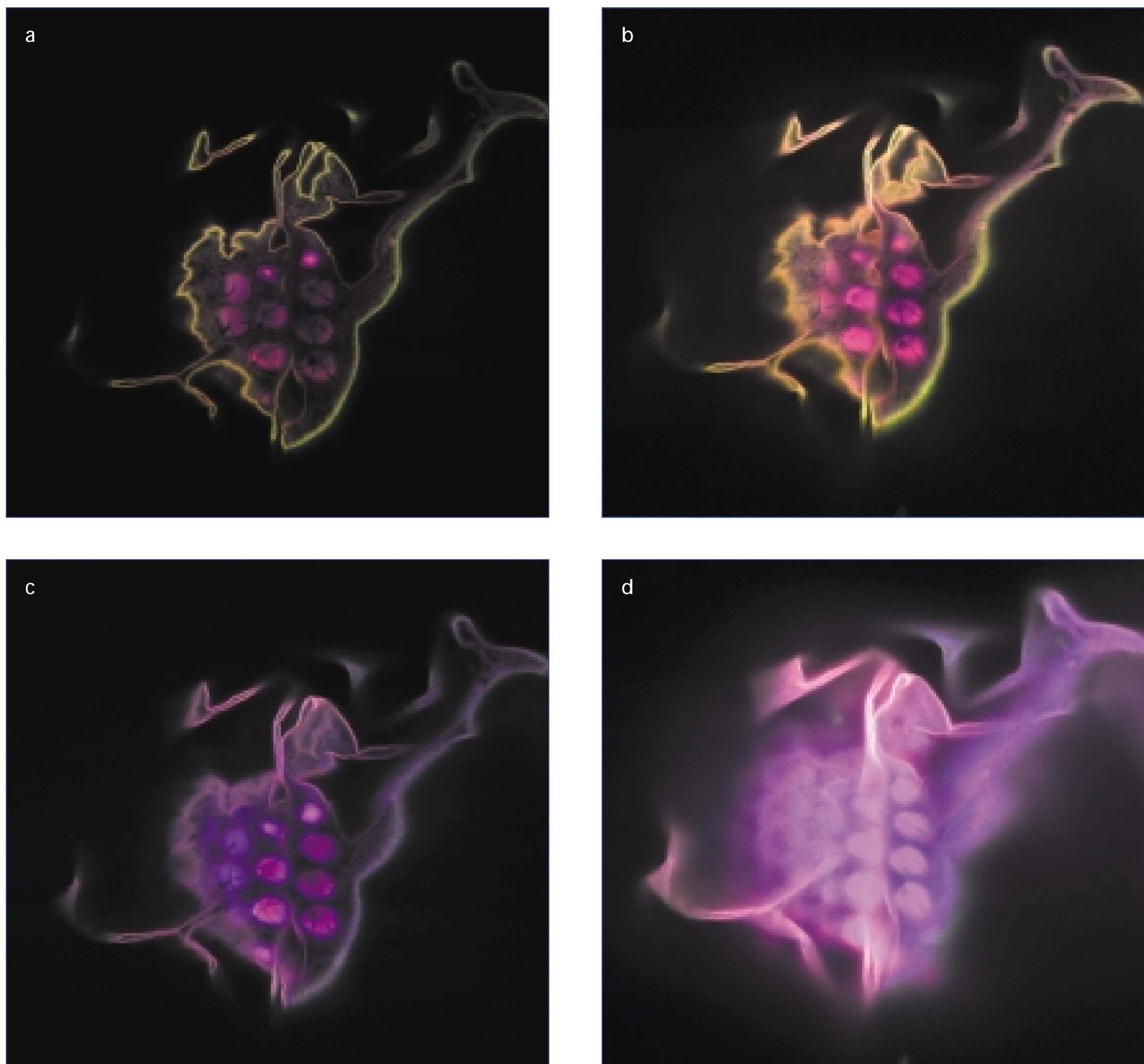
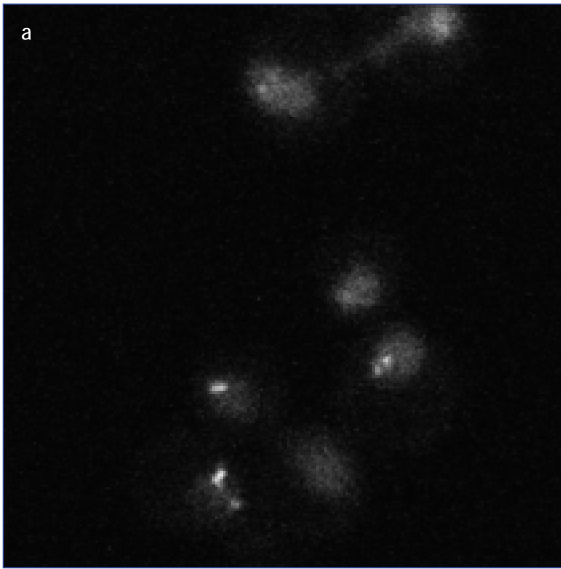


Figure 2 Eye region of the waterflea *Polyphemus*, stained metachromatically with eosin and viewed with the Radiance Method for simultaneous imaging of red, green and infra-red fluorescence. (*2a*) ideal confocal settings, with the iris at 1.3 mm diameter; (*2b*) with SELS inserted into the optical path and iris still at 1.3mm; (*2c*) SELS removed, and iris diameter increased fully to 13mm; (*2d*) iris opened to 13mm and SELS system inserted: note the dramatic increase in intensity in *2d*. Laser power and gain were kept constant throughout. Nikon E800 with 40x Fluor objective, N.A. 1.4. The specimen was dehydrated and mounted in Fluoromount (BDH).

Embryos, thick tissue specimens and whole mounts of organisms (*Fig 2*) can be imaged with particular advantage by SELS. However, thin specimens such as yeast can also be imaged with lower laser powers and a useful increase in depth of focus, without total loss of optical sectioning ability (*Fig 3*). Vertical optical sections through 210 nm fluorescent beads (*Fig 4*) show that the lateral resolution is very little affected by the use of SELS.

The cost of the increased sensitivity to fluorescence in thick specimens is, inevitably, some reduction in resolution along the instrument axis (z resolution). In order to explore this, the boundary between a non-fluorescent medium (glass) and a solution of a fluorochrome in a medium, which matches the glass in refractive index (Nile Red in Fluoromount, BDH) was examined in xz section (*Fig 5*). This boundary, which is undetectable in a conventional microscope, is sharply defined in the confocal image with the detector iris closed, less so with it open and even less with SELS inserted. The intensity profiles (*Fig 6*) show that although the resolution is lessened with SELS, the SELS image is not equivalent to conventional one: there is still a useful degree of optical sectioning occurring.



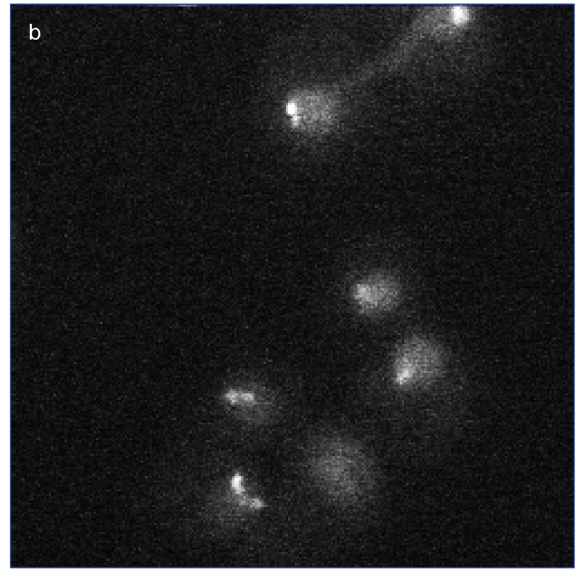
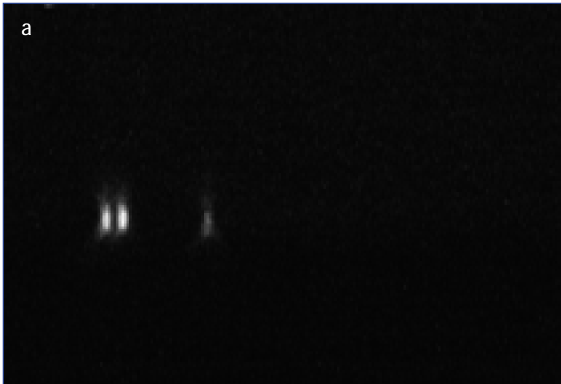


Figure 3 shows results with a thin specimen where resolution is critical (GFP-labelled spindle pole bodies in the yeast *Saccharomyces cerevisae*). (*3a*) without SELS, (*3b*) with SELS. As expected, the gain in brightness is a factor of only two: less than with the thick specimen of *Fig 2*. However, the increased depth of focus makes possible the visualization of more SPBs at one time (Specimen by courtesy of Dr J.V. Kilmartin, MRC-LMB, Cambridge).



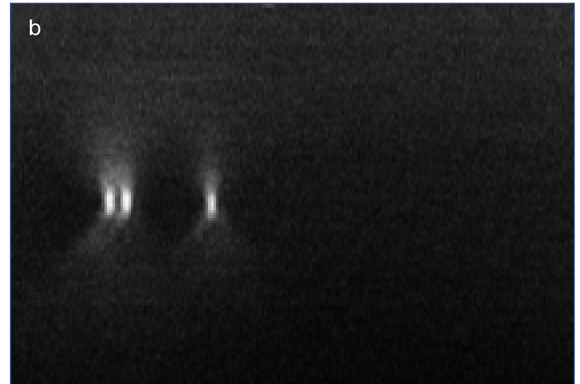
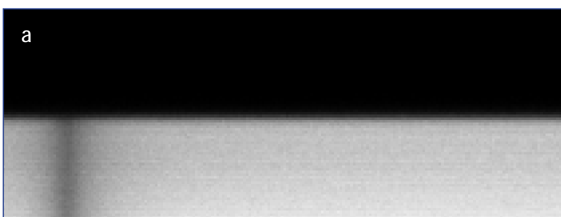


Figure 4 compares xz sections of 210nm Fluoresbrite (Polysciences) beads. The beads were restricted to a single z level by attachment to the glass coverslip. Without SELS (*4a*) the normal confocal xz section is obtained, with a vertically-elongated spot corresponding to a convolution of the bead structure with the point spread function of the objective lens. With SELS (*4b*), the vertical extent of the spot is increased (lower z resolution) and the total signal increased. Note the appearance of low but significant intensity in z regions distant from the maxima: this results from the summation of contributions from many beads, including some not in the plane of the xz section. Note that although the z-resolution falls, the xy resolution is affected much less.



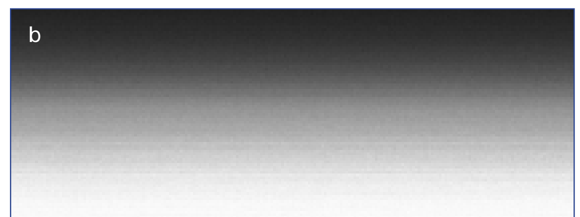


Figure 5 Boundary between glass coverslip and fluorescent medium with optimum confocal settings (*5a*) and with SELS and maximally opened iris (*5b*).

Conclusions

- SELS greatly enhances signal strength from thick fluorescent specimens
- This allows imaging with less laser power e.g. for more prolonged or faster time sequences of living cells without photodamage.
- Even with thin specimens such as yeast the increase in optical section thickness is useful and may even result in the capture of the entire volume of interest in a single image.
- SELS increases axial section thickness without a proportional decrease in xy resolution.
- SELS can be switched rapidly out of the light path at any stage in imaging if maximum confocal performance is required. In this respect it makes the Radiance superior to Nipkow-disk-based quasi-confocal systems, where confocality is permanently reduced by cross-talk between the apertures.
- The increased depth of focus with SELS facilitates searching, which is difficult under strict confocal conditions.

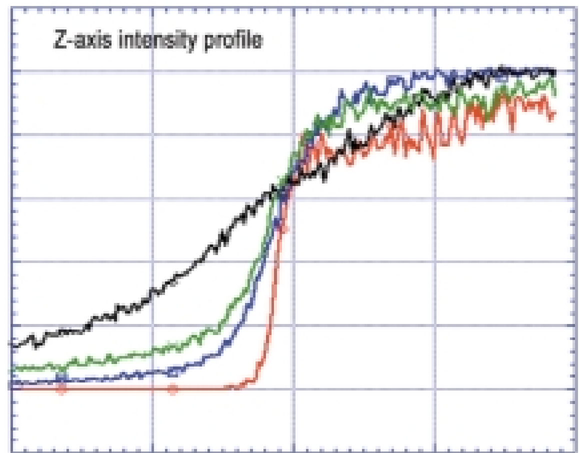


Figure 6 Intensity profiles through images as in *Fig 5*. Red curve, maximally confocal. Black curve, iris open and SELS inserted. Other curves intermediate settings of iris.

References

White, J.G., Amos, W.B. and Fordham, J.M. (1987) J.Cell Biol. **105**, 41-48.

Wells,K.S., Sandison,D.R., Strickler, J. & Webb, W.W. (1989) *The Handbook of Biological Confocal Microscopy* Ist Edn. Ed. J.Pawley

IMR Press Madison

Visit our main website at **microscopy.bio-rad.com**

ISO 9001 registered www.bio-rad.com

BIO-RAD *Bio-Rad Laboratories*

Microscopy Division

U.S. (800) 4BIORAD **California** (510) 7411000 **Australia** +61 (2) 99142800 **Austria** +43 (1) 8778901
Belgium 0800 75489 **Canada** +1 (905) 7122771 **China** +86 (10) 62051850 **Denmark** +45 (39) 179947
Finland 0800 118301 **France** 0800 909190 **Germany** 0800 1800374 **Hong Kong** +852 (2789) 3300
India +91 (11) 4610103 **Israel** +972 (3) 9617580 **Italy** +39 (2) 216091 **Japan** +81 (35) 8116290 **Korea** +82 234734460
The Netherlands 0800 0226048 **New Zealand** +64 (9) 4433099 **Singapore** +65 (272) 9877 **Spain** +34 (91) 6617085
Sweden 020 790360 **Switzerland** 0800 836869 **United Kingdom** 0800 0284015

9MRC50TN27