TECHNICAL NOTE 01

# Obtaining the best performance from your Krypton Argon Laser

**By:** *Andy Sowerby and Graham Hogg,* Bio-Rad Microscopy Division

**The advantage of confocal microscopy is that it gives the user greater axial resolution and optical sectioning. To achieve this it requires focused illumination (e.g. points or lines) rather than wide field illumination as is the case in standard fluorescence microscopy.**

**A laser offers significant advantages for point illumination: high degree of mono-chromaticity, small beam divergence, high intensity and polarised emission. These combined features greatly improve the efficiency of a Confocal Laser Scanning Microscope (CLSM).**

The wavelengths of the emitted light from a laser are dependant upon the gas or the gas mixture within the tube. In the case of the 15 mW Krypton Argon (Kr/Ar) laser three discrete wavelengths are obtained 488, 568 and 647 nm with each line having a power in the range 3 to 5 mW. These individual wavelengths can then be separated or mixed as required using excitation filters to obtain the optimum illumination for single, double, or triple labelling experiments.

The advantages of Kr/Ar mixed gas lasers must be balanced against the disadvantage that the life times of Kr/Ar lasers are typically $^1/_3$ of an equivalent Argon laser. This is because the Krypton gas is more readily absorbed into the walls of the laser tube in comparison to Argon lasers, causing a depletion in the number of free Krypton atoms and a consequent reduction in the lifetime.

There are however a number of ways in which the performance of your laser can be assessed and lifetime potential improved.

Two different types of 15 mW Kr/Ar laser are currently in use, from different manufacturers; ILT and ALC. The performance checks are slightly different for each laser. We would therefore ask you to check carefully which laser you have before you carry out any tests.

The Kr/Ar laser output is continually measured during operation by a photodiode placed within the laser housing which adjusts the anode current of the laser to maintain a constant power output. This is termed “light control mode”. Typically during the lifetime of the laser the anode current will increase and if it goes above a certain level the laser will drop out of light control mode and become significantly more variable in output.

To check both the total laser power and the anode current requires a digital voltmeter that can measure DC voltages to a sensitivity of 0.01 mW. Before carrying out the performance checks the laser must have been switched on for at least 30 minutes to allow the laser to warm up and stabilise.

For all of these performance checks set the standby switch on the side of the laser from “Low” to “Norm”.

**a) Laser Power Determination**

Place the two leads from the voltmeter into the test jacks on the laser head marked “Laser Power” *(see fig 1)*. The output is determined by measuring the DC voltage. For the Kr/Ar laser 1 mW = 0.25 VDC and the total laser power for all of the lines can be determined by measuring the total voltage and then dividing this by 0.25. This should be in the range 12 to 15 mW.

This measurement applies equally for the ILT and ALC Kr/Ar lasers.

**b) Anode Current Determination**

The anode current is determined by placing the test jacks in to the laser head at the point labelled “Anode Current” *(see fig 1)*. Measure the voltage in mV. For the Kr/Ar laser.

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**I) ILT Lasers**

1 Ampere = 10 mV. Therefore to obtain the actual anode current divide the voltage by 10. If the anode current is above 10 Amps, the laser is outside the light control limit. This means that the laser power may fluctuate over time. In this instance contact you local Bio-Rad representative for readjustment or replacement of the laser head.

**II) ALC Lasers**

1 Ampere = 0.5V. Therefore to obtain the actual anode current multiply the voltage by 2. If the anode current is above 10 Amps, the laser is outside the light control limit. This means that the laser power may fluctuate over time. In this instance contact you local Bio-Rad representative for readjustment or replacement of the laser tube.

***Note: The laser contains no items that are user serviceable.***

***Fig 1*** Diagram showing the side panel of the ILT 15mW Kr/Ar laser.

**a) Standby mode**

Both of the lasers described here have a switch to control the laser power, in the absence of a Laser Standby kit which will automatically control the laser power during scanning, the following regime should be adopted.

When the laser is not being used for scanning it should be switched from the "Norm" position to the standby or "Low" position. It should be noted that the laser should not be used for confocal imaging in the "Low" position since the optical noise of the beam is increased. Switching the laser from "Low" to "Norm" before imaging results in no loss in performance since performance returns to optimal virtually instantaneously.

If your laser has been supplied or upgraded with the automatic switching mechanism it is not necessary to do it manually. In this case the power switch should be left in the Low position. If your laser does not have this capability please contact your local Bio-Rad representative to obtain information on how to obtain an upgrade kit to add this capability to your system.

**b) Free air flow**

To operate effectively the laser has a cooling fan. The fan should be placed in a position where the fan output is unobstructed and directed away from the laser head. Failure to do so may result in the laser tube becoming hotter than desirable. Note that the laser itself cannot overheat as it contains a temperature sensor which will switch the laser off if it goes above the maximum operating temperature. However increases in temperature of the laser above the norm will reduce laser life time. Finally the laser should be placed in an environment which is free of dust and moisture.

**c) Room Temperature**

Laser lifetime may be extended by maintenance of a constant temperature (+ 5°c) within the experimental area. Where possible we recommend that the confocal system is installed in an air conditioned room.

**d) Switching Off.**

If the laser will not be used for periods exceeding two hours it should be switched off by rotation of the key switch situated on the front panel of the laser power supply. DO NOT switch off at the mains as this will also turn off the cooling fan which should operate for several minutes after the key is switched to the off position. Failure to do so may result in serious damage to the laser. Note that before starting confocal imaging the laser should be switched on for at least 30 minutes to ensure maximum stability. The laser should not be switched repeatedly on and off over short periods as this will significantly reduce the tube life.

**e) Mechanical Shock**

The performance of the laser depends critically upon the alignment of the mirrors within the laser head. These will have been optimally adjusted during installation. It is therefore important that the laser be placed in a position where it will not be exposed to mechanical shocks, vibrations, or damage.

By following the suggestions listed above you will be able to obtain optimum performance and lifetimes from your Kr/Ar laser. If you have any queries relating to this article as a result of the performance checks please contact the local Bio-Rad subsidiary at the addresses listed below.


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***Microscopy Division***

*Molecular Bio-Science Group*

Bio-Rad Microscopy Division, Maylands Avenue, Hemel Hempstead, Herts HP2 7TD. Phone: (44) 1442 232552

**USA** (800) 4BIORAD • *California* (510) 741-1000 • *New York* (516) 756-2575 • **AUSTRALIA** 02-9914-2800 • **AUSTRIA** 01-877-8901
**BELGIUM** 09-385-5511 • **CANADA** 01-905-712-2771 • **CHINA** 010-2046622 • **DENMARK** 01-39-17-9947 • **FINLAND** 01-80-804-2200
**FRANCE** 1-4390 4690 • **GERMANY** 089 31884-120 • **HONG KONG** 27893300 • **INDIA** 011-46-10103 • **ITALY** 02-216091
**JAPAN** 035-811-6280 **NETHERLANDS** 0318-540666 • **NEW ZEALAND** 09-443-3099 • **SINGAPORE** 2729877 • **SPAIN** 91-6617085
**SWEDEN** Freephone 020 660 660 • **SWITZERLAND** 01-809-5555 • **UNITED KINGDOM** Freephone 0800-181134

For countries not listed. Please contact UK office, Microscopy Division • Phone: (44) 1442 232552 Fax: (44) 1442 234434



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