TECHNICAL NOTE 02

TECHNICAL

Key sensitivity features of the MRC-1024

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Introduction

This Technical Note sets out to explain several important sensitivity enhancing features which are incorporated into the MRC-1024.

The features discussed here are:

- **Ultra-high reflectivity mirrors in the detection path**
- **Enhanced photomultiplier tubes**
- **Low signal acquisition mode**
- **Photon counting**

Ultra-high reflectivity mirrors

It is the case that there are several mirrors in the emission path of the MRC-1024 *(Fig 1)*. The use of these mirrors is essential to produce the extended optical path that gives several key advantages;

- Macroscopic, continuously variable apertures giving high opto-mechanical stability and great flexibility in multi-channel acquisition
- Parallel emission beam allowing exploitation of PMT enhancement as discussed in the following section
- Intrinsically achromatic excitation and emission optics

In order to realise all the above benefits and to ensure superb system sensitivity we have paid particular attention to the performance of all the optical elements in the emission path. Ultra-high sensitivity multi-layer coatings have been used wherever possible. The primary beamsplitter (BS) has been designed to allow a very high proportion of the emitted fluorescence to pass through and emission filters are optimised for maximum sensitivity whilst still retaining excellent detection specificity.

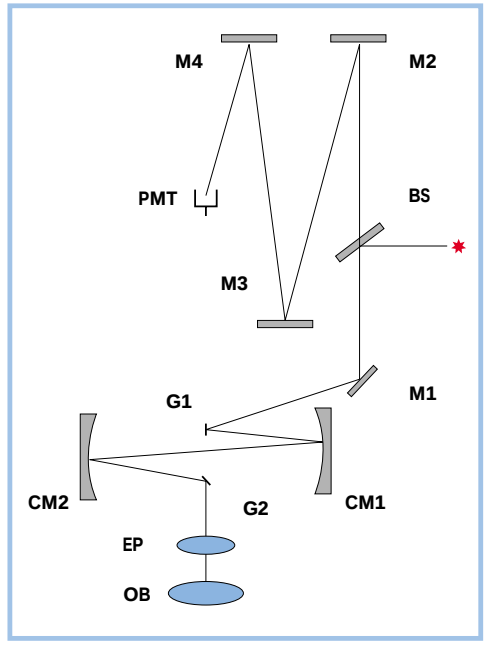

Fig 1 Optical schematic diagram for the MRC-1024

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The following table gives the key to the optical elements in the emission path.

Key in figure 1	Component
OB	Objective lens in microscope
EP	Eyepiece in scanhead
G2	Frame scanning galvanometer
CM2	Focusing concave mirror 2
CM1	Focusing concave mirror 1
G1	Line scanning galvanometer
M1	Plane reflector 1
BS	Primary beamsplitter
M2	Plane reflector 2
M3	Plane reflector 3
M4	Plane reflector 4

Enhanced photomultiplier tubes

It is undoubtedly the case that the Photomultiplier Tube (PMT) is the 'weak link' in all systems which use this type of detector (camera based systems are not so afflicted). It is for this reason that we have applied considerable development resource to this aspect of the system.

Using a well established technique which has been extensively used in the field of enhanced night vision we have developed an optical modification to our end-entry PMTs which results in an increase in quantum efficiency (QE) by more than a factor of two (at some wavelengths).

A reflective optical component *(Fig 2)* diverts the parallel light which passes through the confocal aperture through a particular angle and thence into the photocathode of the PMT. This angle is known as the 'critical angle' and results in all the photons in the beam undergoing 'total internal reflection'. In other words, each photon now has many chances to cause an electron to be emitted from the photocathode and to subsequently contribute to the electrical signal generated by the PMT. The QE of a PMT is in fact the ratio of the number of electrons generated to the number of photons incident upon the photocathode – by increasing the chance of each photon releasing an electron we increase the electrical current from the detector (for a given photon flux) and hence the sensitivity of the system.

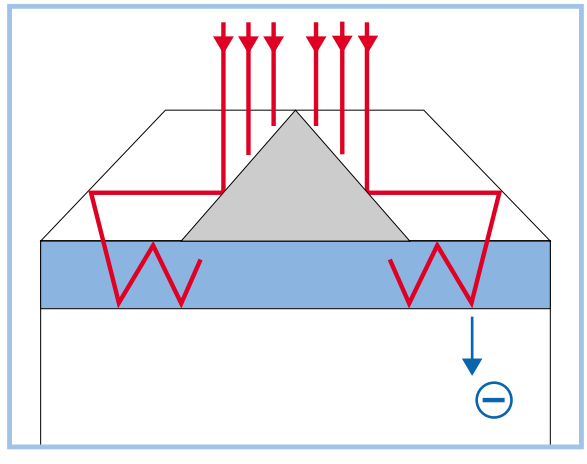

Fig 2 A reflective optical element diverts parallel light through a critical angle and gives multiple electron generation opportunities.

Measurements made on our own PMTs and data from other manufacturer's literature has been used to produce the following comparison chart *(Fig 3)*. This clearly shows a significant advantage in the spectral range from 350nm to 650nm, whereafter the Bio-Rad enhanced PMT (red) and the Hamamatsu 4457 (yellow) show almost identical performance.

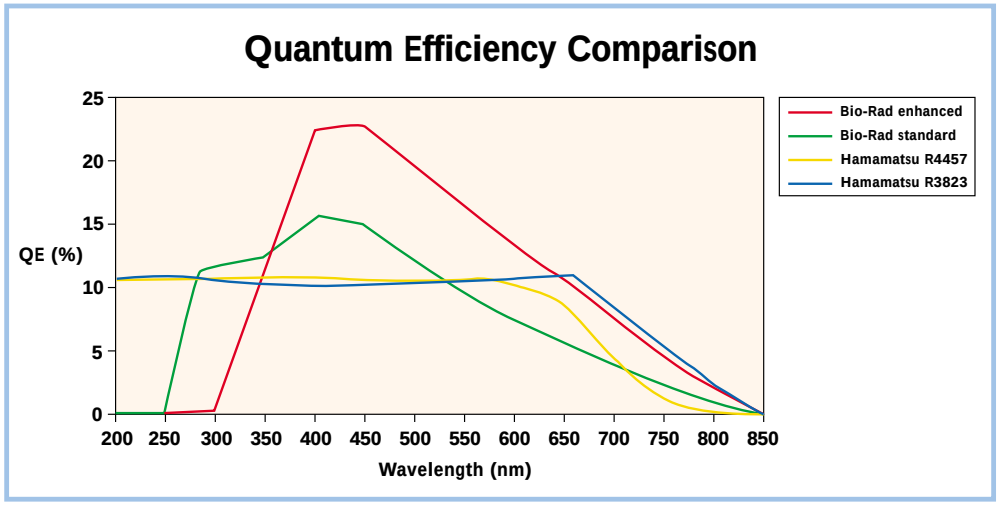

Fig 3 A comparison of the Quantum Efficiency of the PMTs used in Bio-Rad's MRC series of instruments and those used by other manufacturers.

Low signal acquisition mode

Digital signal processors in the Digital Mixer sample the analogue signal from the PMTs at a rate of 1.33 MHz. This means that during a typical pixel period of 2 microseconds (in the case of the Normal line scan rate of 500 Hz and 1024 pixels per line) the PMT signal is digitally sampled 2.59 times.

This oversampling can be used to optimise the image in two ways; The sample points can either be averaged (Low Sig OFF) to achieve a higher signal to noise ratio (S/N) or summed (Low Sig ON) to produce an image of greater intensity. The table below shows the approximate relative intensity levels for various box widths (in pixels per line) and scan speeds (Slow or Normal/Fast/Fastest) for a given signal level.

If the Low Sig mode is not selected then the S/N increases as the square root of the values shown in the table above.

* The Slow acquisition mode is not available at 128 x 128 box size.

One should note that once the analog signal from the PMT amplifiers has been digitally sampled all subsequent manipulation (addition, subtraction or division) is achieved using digital processors. Compared to analog processing systems this early digitisation and digital manipulation ensures retention of the signal to noise ratio rather than loss of signal/noise at every stage.

SPEED	BOX WIDTH					
	1280	1024	768	512	256	128
Normal	**1.0**	**1.2**	**1.67**	**2.5**	**5.0**	**10.0**
Slow	**3.0**	**3.6**	**5.0**	**7.5**	**15.0**	**NA ***

Photon counting

Photon counting is an effective technique used to detect very low level light in applications such as Raman spectroscopy, chemical or biological luminescence analysis and faint fluorescence.

The method of processing the output signal of a photomultiplier tube can broadly split between analog and digital modes, depending on the incident light intensity. Quoting Jonathan Art from the Handbook of Confocal Biological Imaging:

> ***At low light levels (≤10^8 photons/sec), direct photon-counting strategies have advantages over analog methods of light intensity estimation.***

As *Fig 4* shows, when light strikes the photocathode of a photomultiplier tube, photoelectrons are emitted.

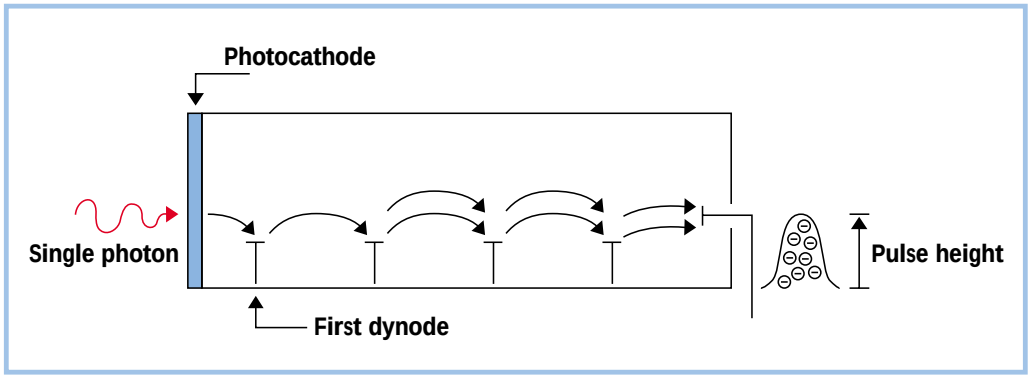

Fig 4 Photomultiplier tube operating in photon counting mode

These photoelectrons are multiplied by the cascade process of secondary emission through the dynodes and finally reach the anode from where they are fed into an output processing circuit.

If one were to observe the output signal of a photomultiplier tube with an oscilloscope as it was illuminated first with a high light level and then with a low light level, output pulses such as those shown in *Fig 5* would be seen.

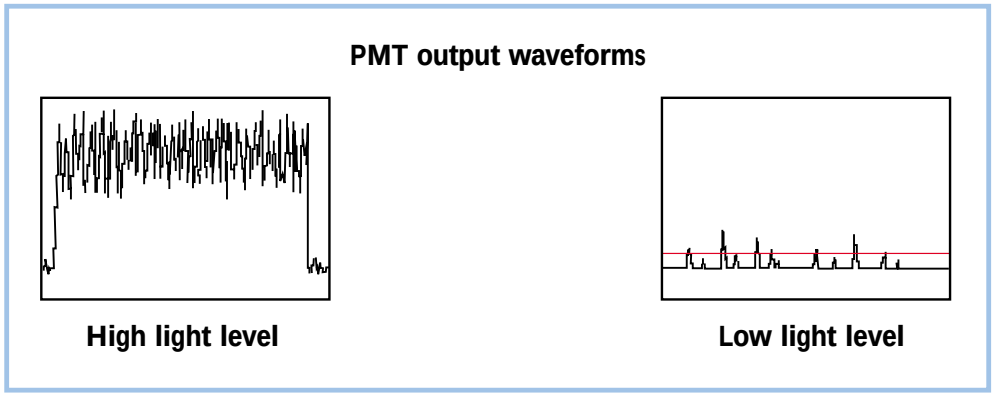

Fig 5 *Photomultiplier tube output waveforms observed at high and low light levels.*

At high light levels the output pulse intervals are so short that the pulses overlap each other thus producing an analog waveform. When the light level is reduced to a very low level (as in faint fluorescence emission) the output signal can be seen to comprise discrete pulses. By discriminating these pulses at an appropriate binary level the number of these pulses can be counted in a digital mode as shown in *Fig 6*. This is commonly known as photon counting and is precisely what happens in the MRC-1024.

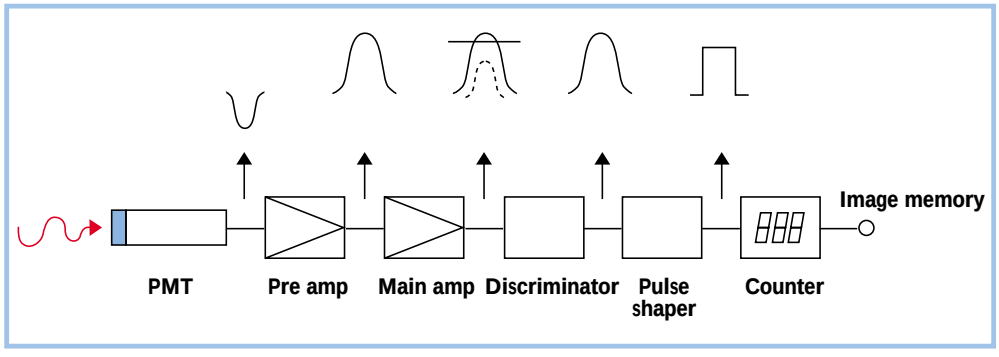

Fig 6 *Schematic diagram of the photon counting circuitry in the MRC-1024*

The benefits of photon counting are as follows:

- Increased S/N compared to the analog detection mode (higher contrast images)
- Very good intensity linearity (at low light levels)
- Extremely high operating stability (insensitive to variations in temperature or voltage supply to the PMT)
- Elimination of dark current counts from intermediate dynode surfaces (higher contrast images)
- Elimination of amplifier noise
- Elimination of DC leakage currents (higher contrast images)

In his summary (in the same publication as previously), Jonathan Art says:

> ***From the viewpoint of capturing a useful image, photon counting is to be preferred.***

Summary

The overall sensitivity of an imaging system is influenced by all aspects of signal collection and (electronic) processing. It is not enough to consider one aspect of a system's sensitivity in isolation, all components in the 'signal train' must be considered.

The truth of the matter is that the collection efficiency of confocal microscopes is low when compared to a CCD camera for instance – primarily due to the lower QE of the detector, and this is compounded by the intentional rejection of light by the confocal aperture. However, one must always remember that although the collection efficiency is low, the confocal microscope does produce images with exceptionally high 'signal to background'. That is, we see what we wish to see, with minimal interference from what we do not wish to see.

It is our belief and contention that by taking account of *all* the relevant issues, we have developed an extremely sensitive system which compares very favourably with offerings from any other manufacturer. This is borne out by the quality of the images which our systems consistently acquire.

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