Metering systems to determine excitation intensities in fluorescence microscopy

Introduction

It is often advantageous to be able to monitor fluorescence excitation intensities when performing fluorescence microscopy. In computer-controlled instruments, it is particularly useful to monitor excitation power continuously. This allows for appropriate quality control in experiments performed over time, where lamp intensity or lamp alignment variations may occur. Another practical advantage is to eliminate the common and sometimes endless arguments about “the microscope is not working, I can’t see my fluorophore” and “the microscope is fine, it is your fluorophore which is weak”.

We describe here several approaches which can be used to monitor and measure fluorescence intensity. The first two approaches use built-in power meters, arranged to sample the excitation light just before it enters the objective. The third approach uses a simple photodiode and digital voltmeter, where the photodiode replaces the objective and is placed behind a circular aperture with diameter equal to that of the objective’s back aperture.

The built-in meter approaches have the obvious advantage of continuous monitoring, but have the disadvantage that sample excitation varies with objective back aperture, necessitating that all objectives must be calibrated and that the microscope’s intensity aperture diameter must be either constant or known. Two built-in meters are described, sharing a common mechanical arrangement; one has a linear response (and thus a somewhat limited dynamic range, ~4 decades), while the other has a logarithmic response, providing >6 decades of dynamic range.

The linear meter can be used with widefield (camera) imaging instruments, while the logarithmic device can be used with both widefield and laser scanning instruments. In scanning instruments, only a singe pixel is excited at one time (pixel dwell times are of the order of a few to a few tens of microseconds) and the detected power (4% of a few mW) is much lower than when the full field is excited, In our microscopes, we use both widefield and laser scanning operation, and hence a very wide dynamic range is essential. Although we have not used these devices with 2-photon excitation microscopes, they should be suitable, although the NIR filter discussed below should of course be removed.

1. Optical power meter mechanical and electrical interfaces

The built-in meters share common mechanical and electrical interfaces. Excitation light is sampled by a very simple beamsplitter fashioned from a standard microscope slide coverslip placed below the objective or turret at 45 degrees to the excitation light. Approximately 4% of the light striking the objective (but not necessarily going through it) is taken to a side port where it is diffused, attenuated and concentrated onto a small area photodiode with a plano-convex lens.

In our embodiment, we used components compatible with the Thorlabs (http://www.thorlabs.com) range of cage and tube systems, though of course other mechanical configurations are possible. The power meter modules are constructed in an SM05 tube and interface to the host logging system using I^2C protocol (application note AN10216, www.nxp.com). Figure 1 shows the sampling unit, Figure 2 the monitor housing and Figure 3 shows the assembled devices.

We have standardised on I^2C interfaces for microscope control applications because of their simplicity, ruggedness and ‘easy’ driver development. At the hardware system level, we use an in-house developed module based around FTDI devices (USB1.0) which may be connected to multiple I^2C slave devices; this is described separately. Address 0xD0 is the only one used for the analogue-digital converter.

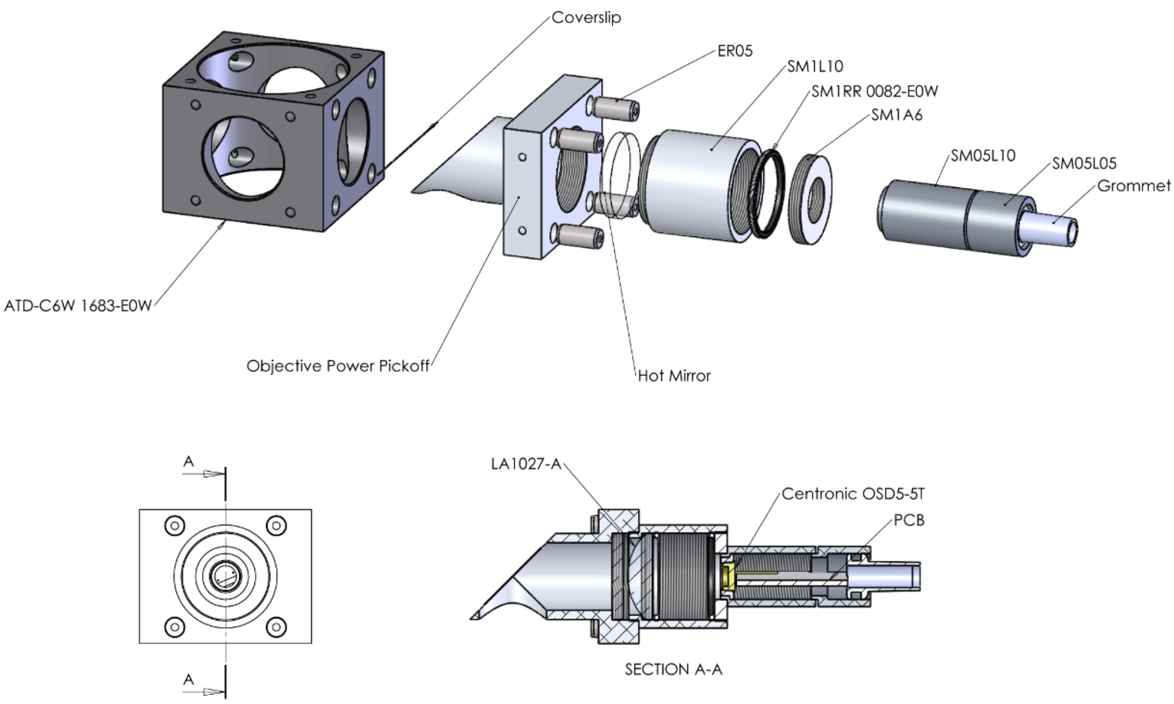


Figure 1: Drawing of the sampling unit. Part numbers refer to Thorlabs components. The sampling unit fits a Thorlabs C6W cage cube (top left), using short lengths of ER05 cage rods. An SM1 tube on the side allows an IR filter and, if required, attenuators to be inserted before the photodiode.

Figure 2: Assembled pick-off assembly. The coverslip can be seen on the left of the image, the SM1 optical components are to the right of the cage plate and the SM05 power meter assembly is on the right side. I^2C signals and power supplies are carried on a 6 way 'mouse and keyboard' style 6-way mini-DIN connector.

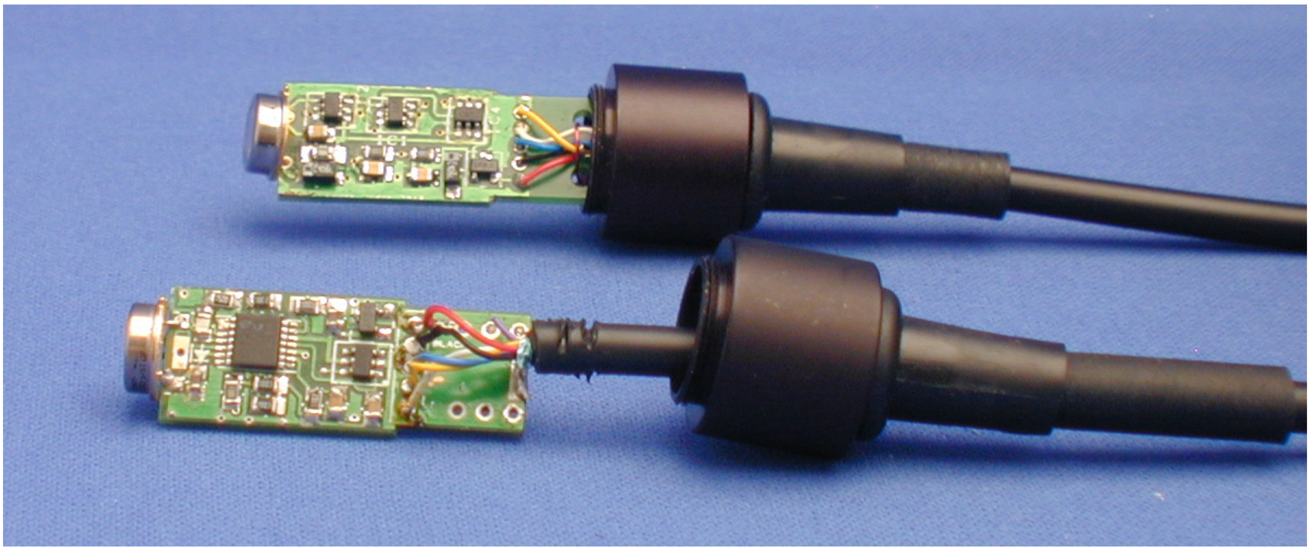


Figure 3: Partially and fully assembled power meters. Two's a company, but occasionally it is good to be alone…….

The components used in the pick-off assembly are listed below:

Beam sampler:	24 mm coverslip
Lens:	Thorlabs LA1027-A, plano-convex, 35 mm focal length
Hot Mirror:	Edmund Optics NT46-386
Mounting tube:	Thorlabs SM1L10
Clamping ring:	Thorlabs SM1RR
Tube adaptor	Thorlabs SM1A6
Mounting rods:	ER05

2. Photodiode sensor

We use a Centronics photodiode mounted in a TO-5 can, type OSD5-5T. Other photodiodes would doubtless be suitable but if the mechanical arrangement presented in Figure 1 is used, package dimensions should of course be compatible. The particular photodiode was chosen due to its easy availability (it is available both from Farnell and RS on the UK. Alternative devices are available from alternate suppliers (e.g. Hamamatsu S1223 or S1336-5BQ, lower dark current, but more costly) although the data presented here refer to the Centronic device. The OSD5-5T has a circular 5 mm^2 active area; 2.52 mm diameter and has the following characteristics:

Responsivity: 0.07 (350 nm) - 0.4 (650 nm) A/W.
Noise equivalent power at 436 nm: 3.3 x 10^{-14} W/$Hz^{1/2}$.
Capacitance @0 V bias: 130 pF Capacitance @12 V bias: 35 pF.
Shunt resistance 100-600 MΩ typical.
Photodiode usable current range 1 nA –10 mA (7 decades) typical when reverse-biased to 12 V or so, significantly greater when appropriate biasing used.

The response of the photodiode varies with wavelength and this can be taken into account by the acquisition and display software. The variation with photodiode responsivity with wavelength is shown in Figure 4.

Wavelength	Responsivity	Rel. output
350 nm	0.07	17.5%
375 nm	0.09	22.5%
400 nm	0.13	32.5%
425 nm	0.19	47.5%
450 nm	0.25	62.5%
475 nm	0.285	71.25%
500 nm	0.31	77.5%
525 nm	0.33	82.5%
550 nm	0.35	87.5%
575 nm	0.365	91.25%
600 nm	0.38	95%
625 nm	0.39	97.5
650 nm	0.4	100%

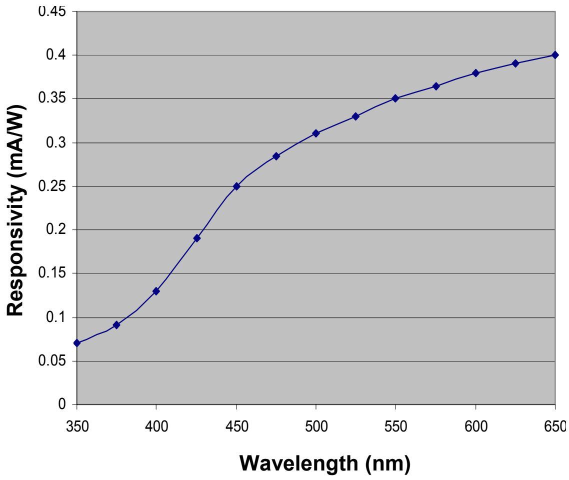


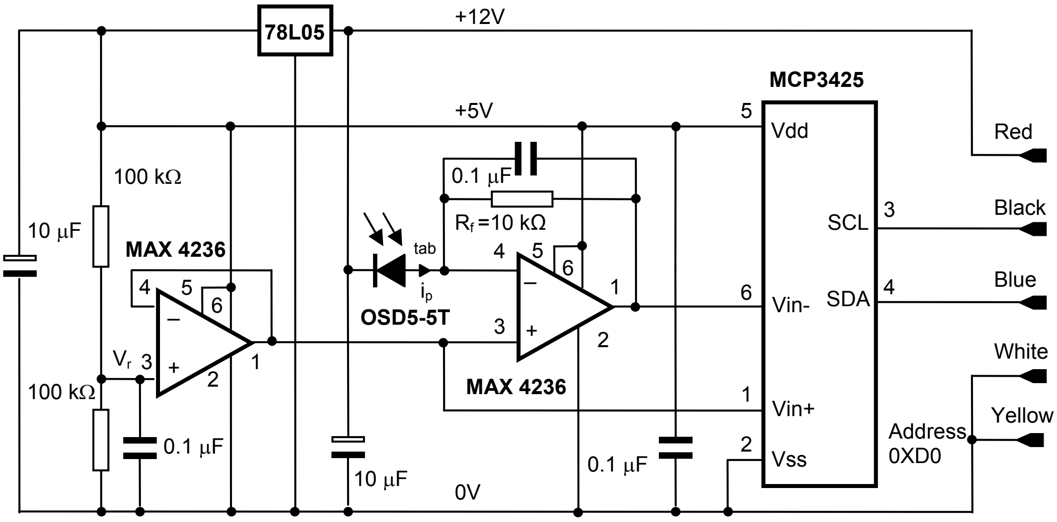
Figure 4: Variation of photodiode output current with wavelength.

A specific advantage of TO5 devices (apart from the relatively large area) is that they are mechanically compatible with the mounting arrangement which we use. The photodiode fits neatly inside an SM05 tube and is held in centred position with a small plastic ring spacer.

3. Optical power meter with linear output response

An optical sensor with a linear output response can be easily constructed by basing it around a current-to-voltage converter, which converts the output current (i_p)of a photodiode. The current-to-voltage converter is based around a MAX 4236 operational amplifier with it's non-inverting input biased to +2.5 V (V_r), so that the photodiode is reverse-biased by ~9.5V, as shown in Figure 5. The reason for lifting the MAX 4236 output to +2.5V is because we wished to use a differential input analogue-to-digital (a-d) converter to provide a serial data stream conforming to the I^2C communications format. This converter operates from a single supply and its 'low' input must be raised above 0V. The design shown in Figure 5 uses a 16 bit A-D converter, Microchip MCP3425, while the current-to-voltage converter's bias voltage is set by a further MAX 4236, providing +2.5 V derived from the regulated +5V power supply, regulated with a 78L05 three-terminal regulator.

The optical sensitivity of the monitor is set the current-to-voltage feedback resistor, R_f, as well as by the input sensitivity of the a-d converter, which includes a programmable gain amplifier (gain range x1-x8). The a-d converter includes a precision 2.048V reference voltage and its sensitivity (at x1 programmable gain) is ±2.048V. Since only a unidirectional input is provided, the output resolution is 15 bits (1 part in 32768, or 62.5 µV). Practical values of Rf range from 1-2 kΩ to >1 MΩ. The lowest value is set by the fact that the photodiode will become increasingly non-linear when delivering photocurrents close to 10 mA. The accessible full-scale photocurrent range is in the range 100 nA - 2000 µA, with appropriate choice of feedback resistor. A 100 nF (typical) feedback capacitor allows the circuit to be used with pulsed sources and reduces noise bandwidth.



$$Vin- = V_r - (i_p \times R_f)$$
$$Vin+ = V_r$$
$$D_{out} = Vin+ - Vin- = i_p \times R_f$$

Figure 5: Circuit of the linear response optical power meter

A dual operational amplifier could of course be used in the above circuit. We split the amplifiers purely for printed circuit board layout considerations, as it was much easier to design a relatively thin board by using separate amplifiers.

The printed circuit board layout for this device is shown in Figure 6 and the complete unit is shown in Figure 7

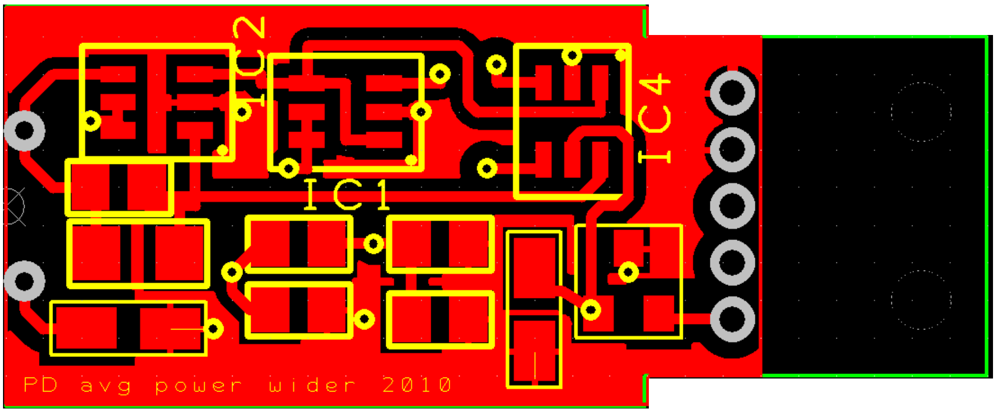


Figure 6: Printed circuit board layout of the linear response optical power meter. The area on the right side is intended to be available for cable clamping. Board dimensions are 1.3" x 0.496".

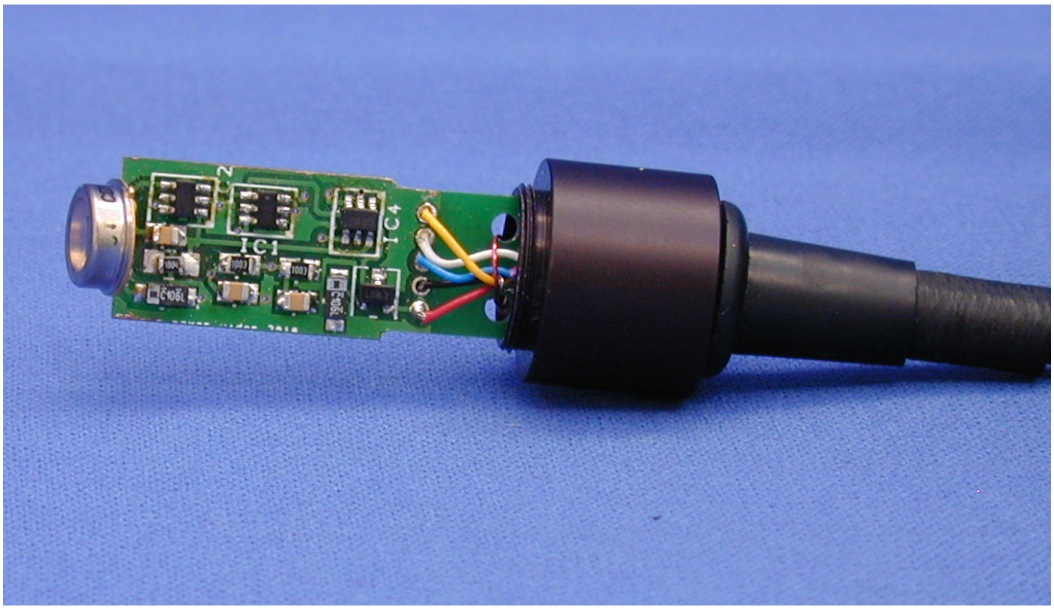


Figure 7: Assembled printed circuit board of the linear response optical power meter. The photodiode is 'side soldered' and can be seen on the left side. The operational amplifiers are parallel to each other, while the a-d converter is at right angles to them. Directly below the converter is the voltage regulator.

4. Optical power meter with logarithmic output response

An optical sensor with a logarithmic output response can be easily constructed by basing it around the Analog Devices AD8304 integrated circuit. We describe here a version of such a monitor where the output of the logarithmic amplifier is digitised with a Microchip MPC3425 analogue to digital converter, as described before, to provide a serial digital output stream arranged to conform to the I^2C communications format.

Once again, the photodiode sensor is a Centronic OS5-5T photodiode (2.5 mm diameter, 5 mm^2 area). This is operated in a reverse-biased configuration, with the bias voltage controlled by the AD8304, such that when low photocurrents are detected, the bias voltage is reduced so as to minimise the consequences of the detector's leakage current. The complete circuit is shown in Figure 8, and the reader is encouraged to read carefully the data sheets of the logarithmic converter and the analogue-digital (a-d) converter. The AD8304's uncommitted operational amplifier is configured as a Sallen & Key low pass filter. This reduces the detection bandwidth and hence noise and also provides additional amplification to compensate for the increase of the logarithmic intercept to 10 pA. The resulting positive-going 200 mV/decade output is applied directly to the a-d converter's input, arranged to operate with x1 internal gain.

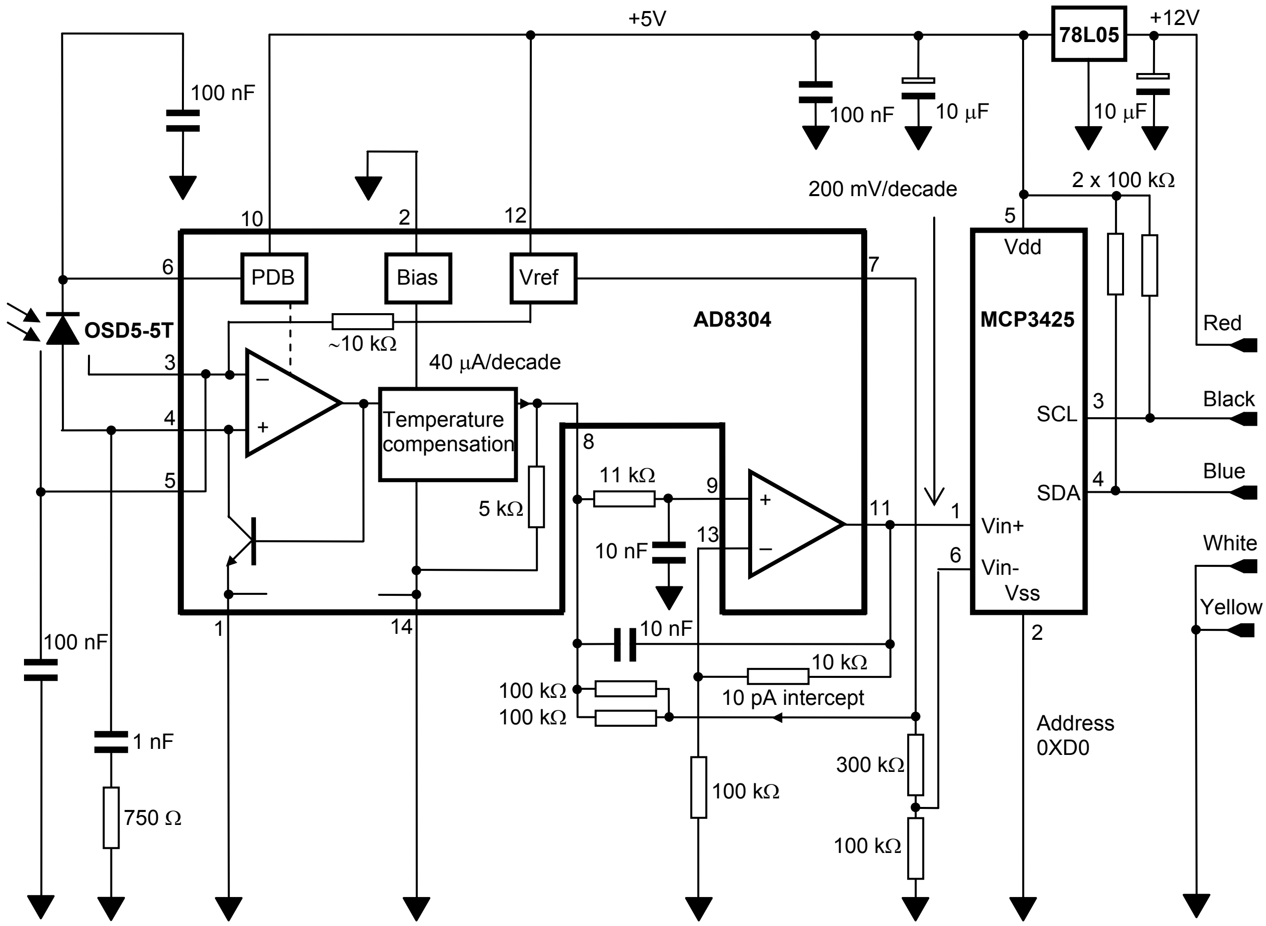


Figure 8 Circuit of the logarithmic response optical power meter

The printed circuit board layout for this device is shown in Figure 9 and the complete unit is shown in Figure 10.

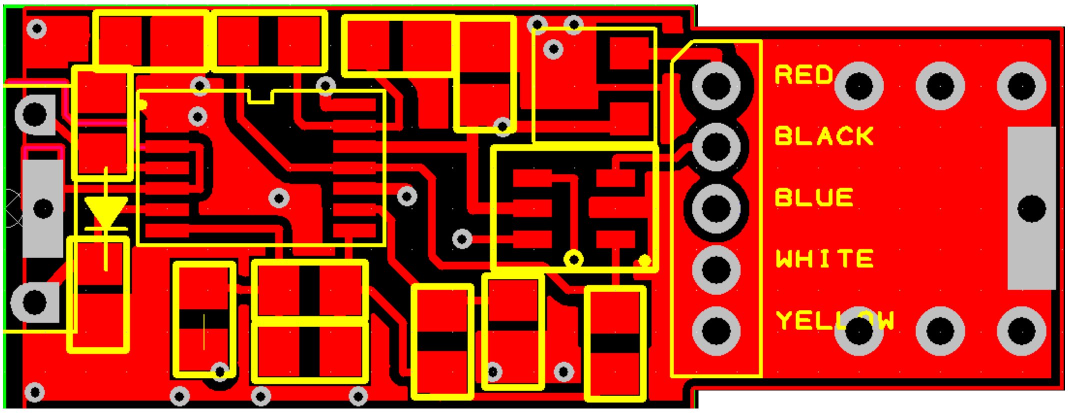


Figure 9: Printed circuit board layout of the logarithmic response optical power meter. It is clear that we 'learnt' from our layout of linear power meter: not only can we clamp the cable better, but we can read which wire goes where! Board dimensions are 1.3" x 0.496".

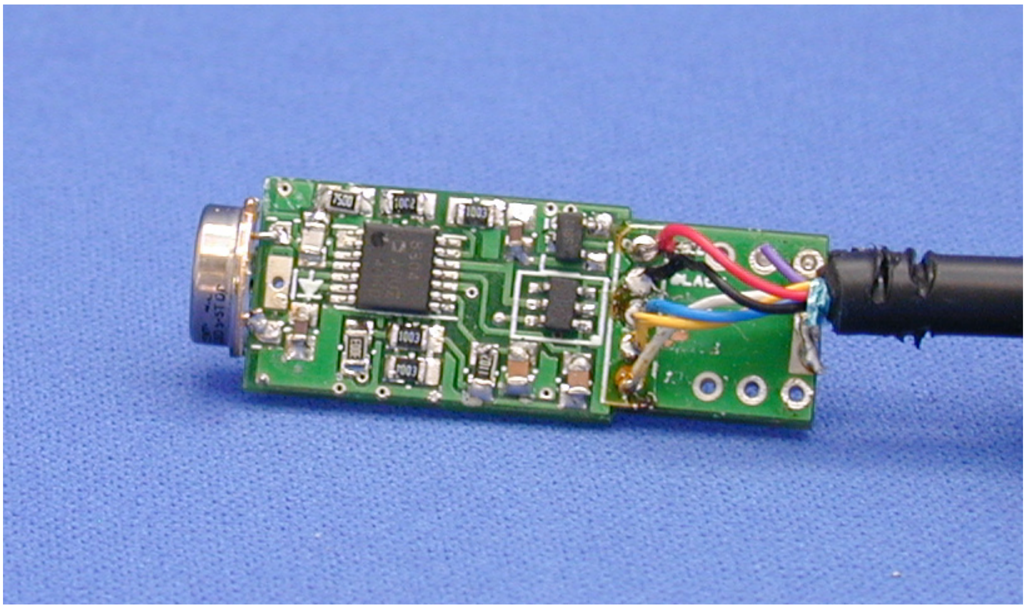


Figure 10: Assembled printed circuit board of the linear response optical power meter. The logarithmic converter is the larger of the surface-mount chips, the a-d converter is to the left of it and the regulator directly above the a-d converter.

We have found our logarithmic response system to work well with photodiode currents ranging from 100 pA to close to 10 mA, and we adjust the logarithmic intercept (I_Z) to be 10 pA. Hence at an input photodiode current of 10 pA, the logarithmic converter provides an output of 0V.

The output of the converter (V_{out}) is: $V_{out} = V_Y \times \log_{10}(I_{PD}/I_Z)$, where:

I_{PD} = photodiode current; V_Y = converter slope, set to 200 mV/decade, I_Z = intercept, set to 10 pA

We set the converter slope to be 200 mV/decade so as to best use the a-d converter dynamic range. V_{out} is digitised to 15 bit resolution (32768:1), such that the a-d converter provides a digital output of 16 counts per millivolt (0.0625 mV/count).
The photodiode current can thus be determined by applying the following:

Photodiode current (pA) = I_Z(pA) x antilog$_{10}$ (a-d reading / 16 x V_Y (mV)), i.e.
Photodiode current (pA) = 10 x antilog$_{10}$ (a-d reading / 3200)

Quantisation errors due to the conversion process are inevitable, but acceptably small, and do not exceed 0.5%. When the total error budget is taken into account (photodiode noise, logarithmic converter errors, a-d non-linearity etc.), we find that a ± 0.75% could occur. This level of performance was considered more than adequate in the current application. Table 1 below may be found helpful in interpreting the measured values. The shaded areas indicate 'safe' operation; below ~100 pA of photodiode current, leakage and dark current effects in the photodiode may become prominent and accurate operation is not guaranteed. However, we have not found any significant problems with four units which we have constructed. Nevertheless, careful cleaning and guarding of the photodiode is essential.

Table 1: Steps in deriving photodiode current from the digitised output of the logarithmic converter

Photodiode output current (I_{PD})	**I_{PD}/I_Z**	**$\log_{10}(I_{PD}/I_Z)$**	**Logarithmic amplifier output (mV)**	**a-d converter output count**	**15 bit digitised logarithmic output**	**Derived $\log_{10}(I_{PD}/I_Z)$**	**Derived photodiode current / 10 (derived I_{PD}/I_Z)**
10 pA	1	0	0.000	0	0.0000	0	0.000000
20 pA	2	0.30103	60.206	963	60.1875	0.3009375	1.9996
50 pA	5	0.69897	139.794	2237	139.8125	0.6990625	5.00106501
100 pA	1×10^{1}	1.00000	200.000	3200	200.000	1.0000000	10.000000
200 pA	2×10^{1}	1.30103	260.206	4163	260.1875	1.3009375	19.9957409
500 pA	5×10^{1}	1.69897	339.794	5437	339.8125	1.6990625	50.0106501
1 nA	1×10^{2}	2.00000	400.000	6400	400.000	2.0000000	100.00000
2 nA	2×10^{2}	2.30103	460.206	7363	460.1875	2.3009375	199.957409
5 nA	5×10^{2}	2.69897	539.794	8637	539.8125	2.6990625	500.106501
10 nA	1×10^{3}	3.00000	600.000	9600	600.000	3.0000000	1000.00000
20 nA	2×10^{3}	3.30103	660.206	10563	660.1875	3.3009375	1999.57409
50 nA	5×10^{3}	3.69897	739.794	11837	739.8125	3.6990625	5001.06501
100 nA	1×10^{4}	4.00000	800.000	12800	800.000	4.0000000	10000.000
200 nA	2×10^{4}	4.30103	860.206	13763	860.1875	4.3009375	19995.7409
500 nA	5×10^{4}	4.69897	939.794	15037	939.8125	4.6990625	50010.6501
1 µA	1×10^{5}	5.00000	1000.000	16000	1000.000	5.0000000	100000.000
2 µA	2×10^{5}	5.30103	1060.206	16963	1060.1875	5.3009375	199957.409
5 µA	5×10^{5}	5.69897	1139.794	18237	1139.8125	5.6990625	500106.501
10 µA	1×10^{6}	6.00000	1200.000	19200	1200.000	6.0000000	1000000.00
20 µA	2×10^{6}	6.30103	1260.206	20163	1260.1875	6.3009375	1999574.09
50 µA	5×10^{6}	6.69897	1339.794	21437	1339.8125	6.6990625	5001065.00
100 µA	1×10^{7}	7.00000	1400.000	22400	1400.000	7.0000000	10000000.00
200 µA	2×10^{7}	7.30103	1460.206	23363	1460.1875	7.3009375	19995740.87
500 µA	5×10^{7}	7.69897	1539.794	24637	1539.8125	7.6990625	50010650.09
1 mA	1×10^{8}	8.00000	1600.000	25600	1600.000	8.0000000	100000000.0
2 mA	2×10^{8}	8.30103	1560.206	24963	1560.1875	8.3009375	199957408.7
5 mA	5×10^{8}	9.69897	1739.794	27837	1739.8125	9.6990625	5001065009
10 mA	1×10^{9}	9.00000	1800.000	28800	1800.000	9.00000	1000000000

6. Parts and suppliers:

Most of the components used in the monitors described here are not critical and should be readily available from the usual electronic component suppliers. However, for completeness, we list below details of order numbers applicable to UK suppliers of some of the more specialised devices:

Centronic OS5-5T photodiode	Farnell 548748	RS 846-777
Analog devices AD8304	Farnell 1661040	RS 497-1316
Microchip MCP3425	Farnell 1578433	RS 669-6098
Maxim MAX 4236	Farnell 1609596	RS 732-8415
STMicroelectronics L78L05ABUTR	Farnell 1467762	RS 714-0675
Resistors	0805 size	
Capacitors	0805, apart from two, which are 1206 size	

Similarly, the mechanical and optical components required are listed below:

Front sensor tube:	Thorlabs SM05L10
Rear sensor tube:	Thorlabs SM1L05
Sleeved grommet:	Pro Power12468, Farnell 4326349
Cable	MiniDIN female socket cable assembly
Photodiode clamp ring:	In-house machined

7. Dynamic range and sensitivity

We compare here typical outputs obtained with the units and briefly discuss the reasons for the need of a high dynamic range. The figures presented are intended to illustrate the issues involved with dynamic range needs and should only be taken as a guide. The monitors will typically be used over the wavelength range 350-650 nm and thus a dynamic range of at least 6:1 (0.07-0.4 photodiode responsivity variation) is required. Variations in widefield excitation lamp intensities increase this to at least 60:1, probably more. Further, the intensity required depends very much on the fluorophore used, and it is reasonable to consider that a further order of magnitude in dynamic range is required. So we end up with a figure of 600:1 or so, or around 9 bits. When the linear power meter is used, we have 15 bits of range and it should thus be possible to achieve a 2% measurement quantisation even at the lowest light level. In practice, the a-d converter is extremely 'quiet' and its output can be further averaged in software.

In the case of a widefield operation, typical exposure times are of the order of 100 ms and typically 10^6 pixels are illuminated simultaneously, while for laser scanning operation, the exposure times are ~1-10 µs/pixel. Let's assume a really fast scanning system, operating at 1 µs per pixel. In order to acquire a 1 Mpixel image, the scanning system takes 10 times longer to acquire an image with the same signal-noise ratio (assuming comparable detector quantum efficiencies) and thus the excitation power per pixel is one tenth of that required per pixel in the case of the widefield image. Since there are 10^6 pixels, a dynamic range of 10^7 is required. When we fold in expected response variations with wavelength and other factors, it becomes clear that a 10^8 :1 or greater dynamic range is required to fully cover both laser-scanning and widefield imaging modes.

Of course, in practice, laser-scanned imaging resolution is often less than 10^6 pixels, and the imaged volume/pixel is much smaller than that used with widefield excitation, often requiring higher intensities in order to obtain similar signal-to-noise ratios. Nevertheless, it is clear that appropriate attenuators or filters should be included in order to allow the widest possible operating envelope.

7. Software interface

The a-d converter is arranged to run at 15 samples per second. A simple software interface can provide a 'live' feedback, running slowly (e.g. @ 0.5-1 Hz). When incorporated into a microscope with integrated control code, the value of the power meter is read during every camera image 'snap' and the corrected value inserted into the image metadata under the entry "*excitation level*". Example C code to get the value from the power meter can be found in Appendix A.

The recorded value is corrected for wavelength to account for responsivity of the photodiode. The value is multiplied by the average responsivity which is calculated as:

$$\bar{R} = \frac{\int_{\lambda_1}^{\lambda_2} R(\lambda) d\lambda}{\lambda_2 - \lambda_1} \qquad (1)$$

where λ_1 and λ_2 define the wavelength interval of the excitation light and $R(\lambda)$ is the responsivity curve. A small algorithm integrates by linear interpolation the data given in Figure 4 (see Appendix B).

The recorded value is corrected for apertures of the objective (diameter *a* mm) and the entrance to the power meter (diameter *m* mm). Assuming that the excitation beam diameter (*b* mm) has a top-hat intensity profile and $b \geq a$, $b \geq m$, the correction value is a^2/m^2. The aperture value *m* and an additional device specific calibration factor are held in a configuration file.

Figure 11 shows the user interface from a Gray Institute Open Microscope implementation of this device. The Minimum Exposure Time indicates the minimum shutter open time for an accurate measurement to be made.

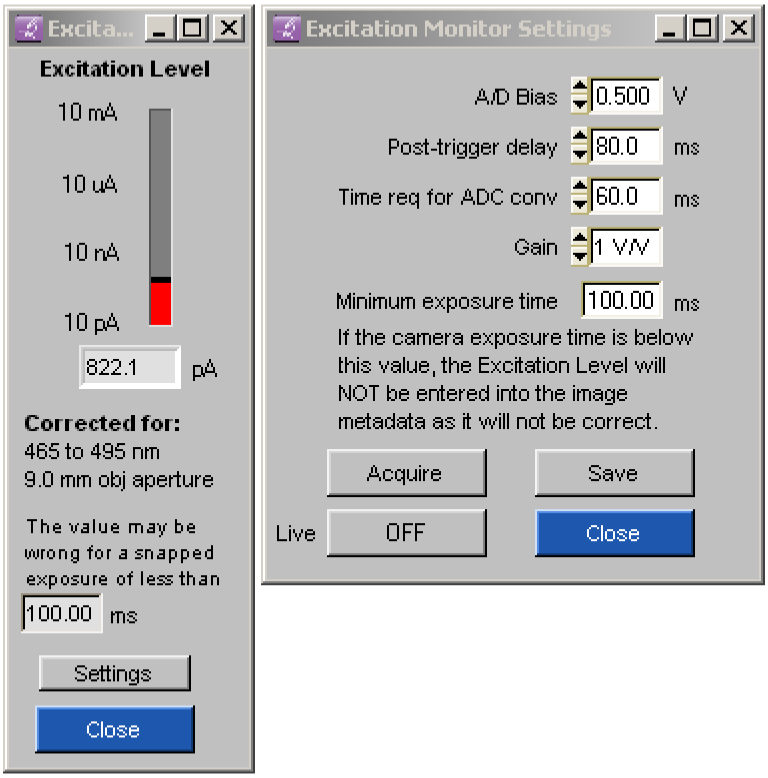


Figure 11: Typical user interface from a Gray Institute Open Microscope implementation of the power monitoring detectors.

8. Objective-replacing monitor meter

The devices described above of course have numerous uses as general-purpose optical power meters. When interfaced to computer software they are capable of providing a continuous readout of light energy impinging on the detector. However, in some instances, particularly when attempting to monitor the light intensity exciting a sample during fluorescence excitation microscopy imaging, it may be desirable to use a photodiode monitor is which screwed into the microscope nosepiece and simply 'replaces' the objective. Such a device is particularly useful when used in conjunction with widefield illumination, but does need to be calibrated using a power meter.

A reasonably accurate measurement can be made if the light reaching the detector is restricted to the same degree as does a given objective, i.e. if an aperture of diameter equal to that of the objective rear aperture is placed in front of the detector. An alternative would be to use a calibrated variable diameter aperture which could then be adjusted so that it may be used with a variety of objectives. In our laboratory we most often use Nikon CF160 objectives which can have very large rear apertures, in excess of 20 mm and which have a parfocal distance of 60 mm. A large photodiode is clearly desirable, but such large area devices are available only in large diameter housings, e.g. TO8, and the resulting assembly would be larger than the 30 mm or so diameter of modern objectives. Moreover, such a large area photodiode is quite costly. A convenient solution is to place a lens in front of a smaller diameter photodiode; in our case we use a 10 x 10 mm photodiode preceded by a 40 mm focal length, such that the photodiode appears significantly larger when viewed from the lens input side, as shown in Figure 12. As can be seen, both the 'blue' normal incidence rays and the 'red' rays coming in at angles comparable to those that the excitation light path takes are collected by the photodiode. In this instance, the ray colours are made different for clarity. All components used are available from Thorlabs, apart from the photodiode mounting disc. This is a small printed circuit board which is sandwiched between the end of a SM1L03 tube and a SM1RR clamping ring. The completed unit is shown in Figure 13.

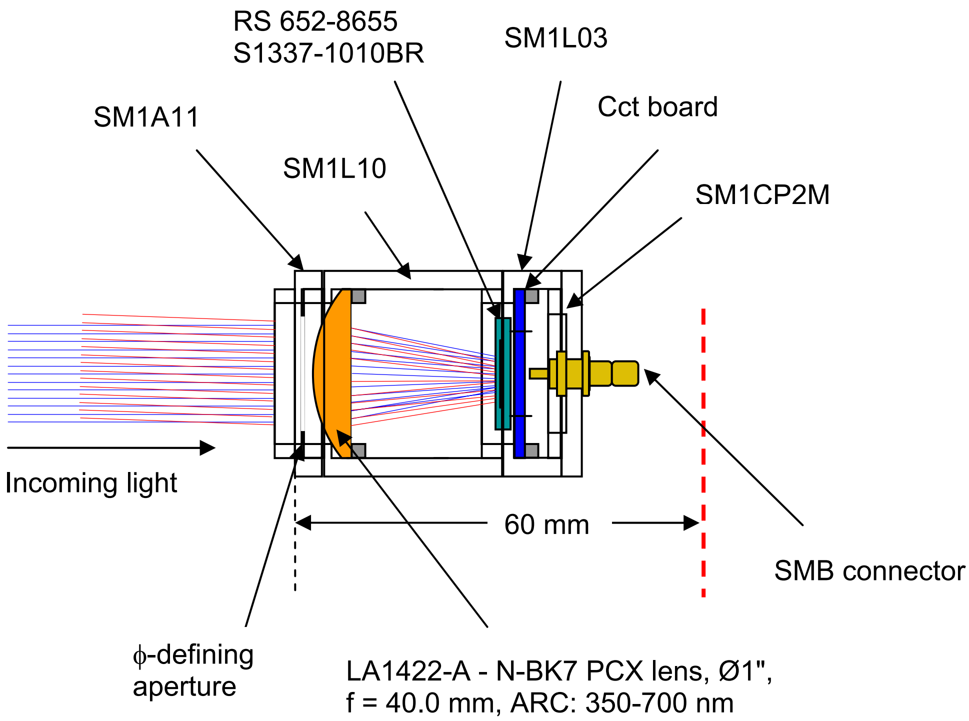


Figure 12: Construction of the photodiode sensor assembly which replaces the 60 mm parfocal distance objective. The overall length of the assembly is well below this maximum, so that even when a right-angle SMB connector is plugged in, the overall length is short enough to allow the system to replace an objective on an inverted microscope.

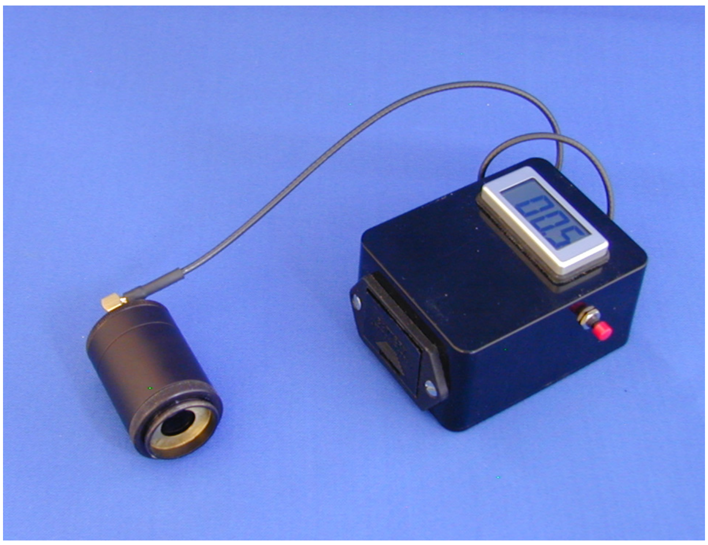
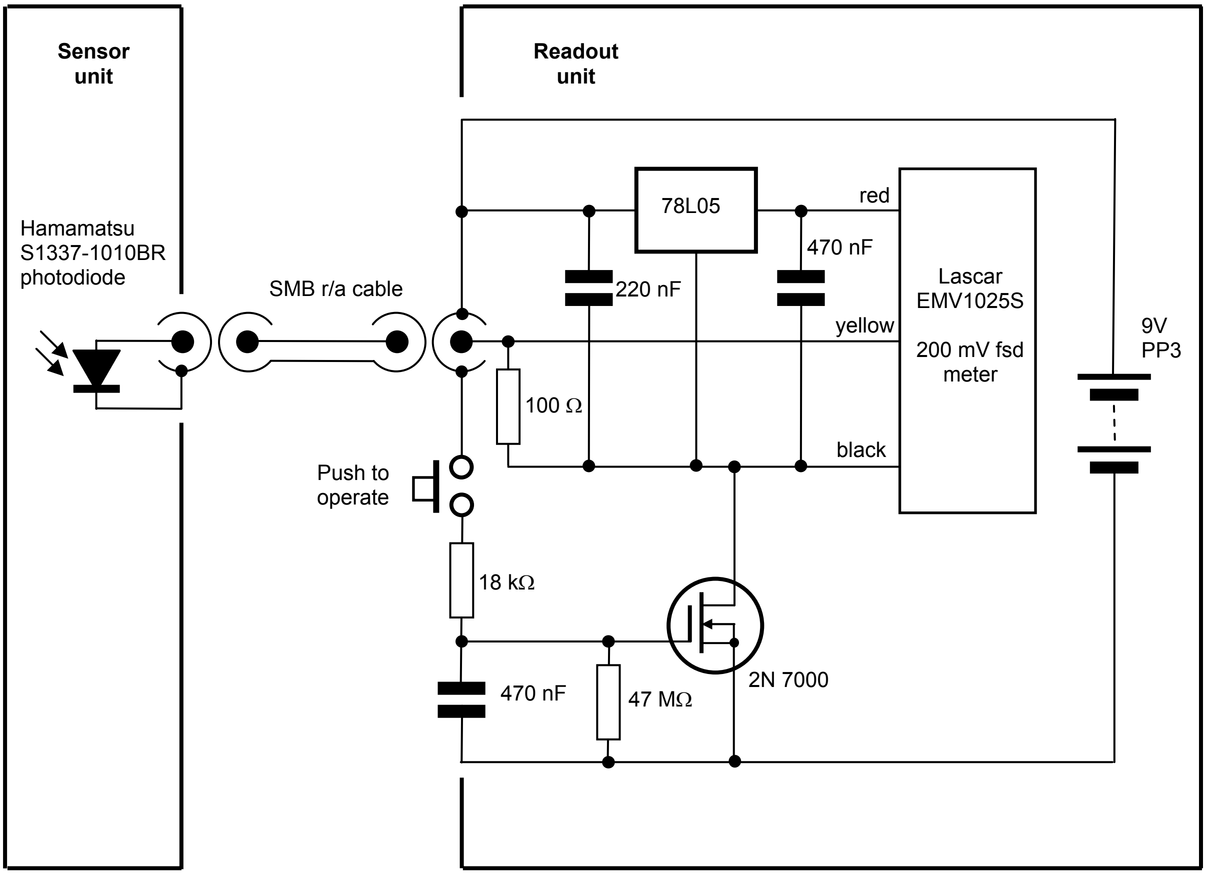


Figure 13: The completed sensor connected to the readout system through a short length of RG174 cable terminated in right-angle SMB coaxial connectors. The readout unit is housed in a plastic box which also contains a 9V PP3 battery. Battery life is extremely long since the unit is energised for only a short time (by pressing the red push-button on the side) after a time delay, battery current load is negligible. A non-zero meter reading is present because this image was acquired during one of the few UK sunny days!

The readout unit is extremely simple; its circuit is shown in Figure 14. When the 2N7000 MOSFET is turned on, the reverse-biased photodiode current passes through a 100 Ω resistor and develops a voltage which can be measured on a 200 mV 3.5 digit voltmeter. Since it is a well known fact that whenever a critical battery-powered unit is actually needed, it will have flat battery because someone's left it switched on, this unit's on-off switch is a push-button: this charges up the 470 nF gate capacitor and turns on the MOSFET; when it discharges through the 47 MΩ gate resistor, the MOSFET is turned off and a long battery life is ensured. The +5V regulator ensures that the digital voltmeter receives a stable supply. Table 2 lists the components used.



Item	Supplier	Stock#	Part #
Meter / Power Box			
Enclosure	Onecall	301-243	Multicomp - MB1 - BOX, ABS, BLACK
Battery holder	Onecall	118-4193	Bulgin - BX0023 - BATTERY HOLDER, 1XPP3
Voltmeter	Onecall	993-2844	Lascar - EMV 1025S-01 - 3.5 digit LCD Voltmeter 200 mV FSD
Push button switch	Rapid	78-0186	2P SPST off-(on) Mini Push switch RED
MOSFET switch	Onecall	146-7958	2N7000_D26Z MOSFET, N, TO-92
Connecting cable	Onecall	105-6197	Tyco Electronics 1337817-3 - RG174 Lead, SMB r/a, 1m
Voltage regulator	Onecall	101-4073	Fairchild semiconductor KA78L05AZ
Input connector	Onecall	419-4536	Radiall R114554000 SMB jack bulkhead rear mount
Photodiode head			
Photodiode	RS	652-8655	Hamamatsu Photonics S1337-1010BR
Output connector	Onecall	419-4536	Radiall R114554000 SMB jack bulkhead rear mount
Printed cct board	Gray	BS4C1B9E011BB44	PCB for PD.pcb
ϕ-defining aperture	Gray	Various	Depends on objective simulation
Objective thread adapter	Thorlabs	SM1A11	Adapter with external M25-0.75 and internal SM1
Field lens	Thorlabs	LA1422-A -N-BK7	PCX lens, Ø1", f = 40.0 mm, ARC: 350-700 nm
Lens tube	Thorlabs	SM1L10	SM1 Lens Tube, 1"
Lens tube	Thorlabs	SM1L03	SM1 Lens Tube, 0.3"
Connector end-cap	Thorlabs	SM1CP2M	SM1 Series end Cap for Machining

Figure 14: Circuit diagram of the objective-replacing monitor meter and list of components used.

Appendix A. C Code: Get Detector Value

This code snippet has been extracted from the Gray Institute Open Microscope codebase and is not self contained.

```
static int atd_laserpowermonitor_LOGP_get (LaserPowerMonitor*laserpower_mon, double *val)
{
    ATD_LaserPowerMonitor_LOGP* atd_laserpower_mon_LOGP = (ATD_LaserPowerMonitor_LOGP*) laserpower_mon;
    unsigned int msb, lsb;
    int reading, gain;
    double holdoff, delay, bias, PD_current;
    byte vals[10] = "";

    if (atd_laserpower_mon_LOGP == NULL)
      return HARDWARE_ERROR;

    // Get configuration values from the UI
    GetCtrlVal(laserpower_mon->_settings_panel_id, LSR_PR_SET_GAIN, &gain);
    GetCtrlVal(laserpower_mon->_settings_panel_id, LSR_PR_SET_HOLDOFF, &holdoff);
    GetCtrlVal(laserpower_mon->_settings_panel_id, LSR_PR_SET_DELAY, &delay);
    GetCtrlVal(laserpower_mon->_settings_panel_id, LSR_PR_SET_BIAS, &bias);

    // Apropriate delays so that reading occurs at correct point in the excitation cycle (e.g. when the FL shutter is open)
    Delay(holdoff/1000.0);      //Delay in seconds
    atd_laserpowermonitor_LOGP_set_gain(atd_laserpower_mon_LOGP, (LaserPowerverMonitorGainValue) gain);
    Delay(delay/1000.0);        //Delay in seconds

    // Read a value from the device
    if(ftdi_controller_i2c_read_bytes(atd_laserpower_mon_LOGP->_controller, atd_laserpower_mon_LOGP->_i2c_chip_address, 3, vals) !=
FT_OK) {
        return HARDWARE_ERROR;

    // decode the value from the least and most signifacant bytes
    msb = vals[0] & 0xff;
    lsb = vals[1] & 0xff;

    reading = (msb<<8 | lsb);

    // interpret as 16-bit signed int, in case value falls below zero
    if(reading>32767)
       reading=(reading-65536);

    // Calculate the photodiode current
    PD_current = 10 * pow (10,((reading/3200.0)+(5*bias)));   //Value in pA

    // correct for device specific calibration
    PD_current *= atd_laserpower_mon_LOGP->_calibration;

    // correct for wavelength range
    PD_current *= atd_laserpower_mon_LOGP->_wavelength_factor;

    // correct for objective back aperture
    PD_current *= atd_laserpower_mon_LOGP->_aperture_factor;

    *val = PD_current;

    return HARDWARE_SUCCESS;
}
```

Appendix B. C Code: Detector Response Wavelength Integral

```c
double detector_response_values[13]      = {0.07,0.09,0.13,0.19,0.25,0.285,0.31,0.33,0.35,0.365,0.38,0.39,0.4};
double detector_response_wavelengths[13] = {350.0,375.0,400.0,425.0,450.0,475.0,500.0,525.0,550.0,575.0,600.0,625.0,650.0};
const int detector_response_nValues = 13;
double detector_response_integral (double wl, int left_integral, int *left_index);


double get_detector_response (double min, double max)
{   // This function will return a number for the average detector response or responsivity over the wavelength range given
    // it uses the global arrays detector_response_values and detector_response_wavelengths

    int i=0, n = detector_response_nValues, i1, i2;
    double *wls = detector_response_wavelengths;
    double *vals = detector_response_values;
    double area=0.0, response=1.0;

    if (min<wls[0] || max<wls[0] || min>wls[n-1] || max>wls[n-1])
        return 1.0;  // cannot do it

    // get area from min wl to the nearest higher wl point
    area = detector_response_integral (min, 0, &i1);

    // add area from max wl to its nearest lower wl point
    area += detector_response_integral (max, 1, &i2);

    // add area of intervening points
    for (i=0; i<n; i++) {
        if (i>=i1 && (i+1)<i2) {  // whole region is between min and max
            area += (wls[i+1]-wls[i]) * (vals[i]+vals[i+1]) / 2.0;
        }
    }

    area /= (max-min);

    return area;
}

double detector_response_integral (double wl, int left_integral, int *left_index)
{  // Uses the detector_response_values global array
   // Finds the integral from the point at wl to the nearest defined pt lower (left_integral)
   // or higher (right integral)
   // returns the left index if needed

    int i=0, n = detector_response_nValues, i1, i2;
    double *wls = detector_response_wavelengths;
    double *vals = detector_response_values;
    double val, integral;

    // find values that are neighbours to wl
    for (i=0; i<n; i++){
        if (wls[i] > wl)
            break;
    }

    i2 = i;
    i1 = i-1;

    // find the linearly interpolated value
    val = vals[i1] + (vals[i2]-vals[i1]) * (wl-wls[i1]) / (wls[i2]-wls[i1]);

    if (left_integral)
        integral = (wl-wls[i1]) * (val+vals[i1]) / 2.0;
    else  // do right integral
        integral = (wls[i2]-wl) * (val+vals[i2]) / 2.0;

    if (left_index!=NULL)
        *left_index = i1;

    return integral;
}
```

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