

## **Fault Finding on Spectrophotometers**

Should you experience “wrong” or “inconsistent” results with your Biochrom spectrophotometer the following procedure should be followed to enable the Biochrom Support team to assist you in solving your problem.

1. All tests should be carried out and the results documented by measuring Absorbance values – reporting Ratio or concentration measurements can mask the cause of the problem.
2. For instruments fitted with an automatic cell changer this should be switched off within the software( see user manual)
3. If they are available Certified Reference Materials should be used to check the performance of the instrument to it’s published specification.
4. If they are not available the following procedure should be followed with the tests run in order:

### **Test 1**

- Set the instrument to the wavelength of interest
- Zero the instrument with no cell in the beam
- Press Run 5 times noting the reading each time

The above test is checking the actual reproducibility of the instrument, the results should be 0.000A +/- a small change depending upon instrument specification but typically 0.002A.

### **Test 2**

- Put the cell normally used for analysis filled with reference solution in the beam
- Press Run 5 times noting the reading each time

The above test is checking the actual Absorbance value of the Reference solution and cell. The reading obtained should be less than approx 1.00A, a higher value could indicate the wrong type of low volume cell being used( wrong beam height), wrong cell material for the wavelength( glass cell in UV region) or too highly concentrated reference solution.



### **Test 3**

- Put the cell normally used for analysis filled with sample solution in the beam
- Press Run 5 times noting the reading each time

The above test is checking the Absolute Absorbance of the solution being run which looks for similar problems as above. The reading obtained and reproducibility depends upon instrument specification

### **Test 4**

- Put the cell normally used for analysis filled with sample solution in the beam
- Press Run noting the reading
- Remove cell then replace in cell holder
- Press Run noting the reading
- Repeat 5 times

The above test is checking the reproducibility of the customers cell in the holder

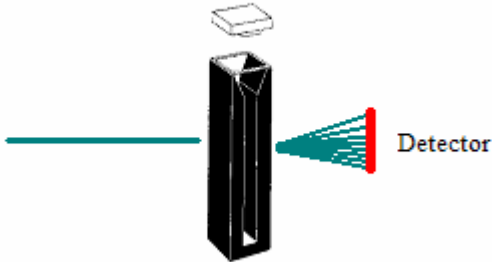
All of the above results should be e-mailed to [support@biochrom.co.uk](mailto:support@biochrom.co.uk) along with full details of the instrument, the method being run and the “wrong” results originally obtained.

### **Cell Density Measurements**

A common use for photometers is to measure the growth rate of bacteria by measuring the optical density/absorption of a sample at 595nm or 600nm. This often leads to confusion as the same sample will give different readings on different models of spectrophotometer. As bacterial samples are cloudy, they mainly scatter light rather than absorb it. This means that the actual reading obtained is very dependent on the collecting area of the detector after the sample. The collecting area used depends entirely upon the optical configuration of the instrument, so differences in readings between types of spectrophotometers are to be expected.

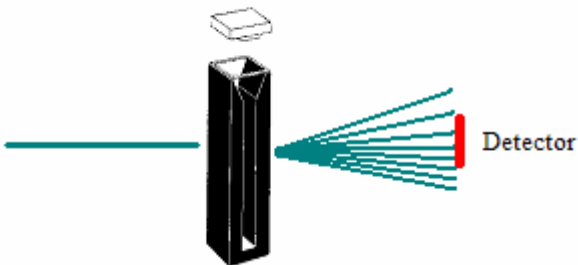


### Instrument 1



All scattered light hits detector

### Instrument 2



Not all scattered light hits detector therefore higher reading

Most codes of practice for measuring Bacterial Growth Rates (BGR) require the instrument to be calibrated for optical density/absorbance against the dry weight in grams/litre for the species under study, but this is commonly not done due to the time involved.

