Rotating Prism

Operating Manual

Manufactured by:
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Warranty

We guarantee the manufacture of the instrument and parts against faults for a period of twelve months from the invoice date.

If a fault should occur within this period then we undertake to either:

- Supply free of charge replacement parts for you to fit to the instrument.
- Upon return of the item at your expense, repair or replace (at our discretion) the instrument free of charge and return it to you at our expense.

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1. Installation

1.1 Fitting

To fix the Rotating Prism unit to the Microelectrophoresis Apparatus first remove the binocular head, then fit the prism unit in its place. Finally, replace the binocular head on top of the unit.

1.2 Electrical Connections

The unit should be earthed and connected to a mains plug as follows:

GREEN/YELLOW or GREEN	EARTH
BLUE	NEUTRAL
BROWN	LIVE

The box contains a 1 amp 1.25 inch fuse located at the rear. The prism is connected to the control unit via a seven-pin DIN socket at the rear. The prism should be connected before turning on.

2. Using the Rotating Prism

2.1 Initialisation

The control unit carries out an initialisation procedure when switched on which may last for up to twenty seconds. When switched on, the indicator should be illuminated and the display will momentarily flash four dots before becoming blank. The controller does not know where the prism is positioned and thus rotates anti-clockwise at full speed until a micro-switch, acting as an end stop, is contacted.

Once this end stop has been made, the initialisation is complete, the prism will start rotating at maximum speed and reversing direction automatically every thirty degrees (unless the command keys have been used to change the speed during initialisation).

2.2 Calibration

After switching on, the unit will need to be calibrated before displaying particle speed. Firstly, the prism should be stopped to allow focusing. This is done by using the red command key *Stop*. All command keys have a latching action (i.e. a single press causes the key to be latched on, then a further press causes that key to be cancelled). Pressing the *Stop* command key will cause the prism to stop rotating. This allows the Microelectrophoresis Apparatus to be focused onto something stationary (e.g. imperfections or dirt on the cell wall). The prism should now be restarted.

The apparent velocity caused by the prism rotating can be measured using the timer and a calibrated eyepiece graticule. Several timings should be made and an average speed in microns per second calculated. If the prism is travelling too fast to accurately time the speed, it can be slowed by pressing the *Slow* key, allowing the prism to slow until a more suitable speed is found. Pressing the *Slow* key again will return the prism to constant speed. Note the timing and entering of the calibrated speed must be done without changing the speed of the prism.

The calibrated speed is then entered as follows:

1. Press the *Cal.* key and enter a four digit number, two digits either side of the decimal point (e.g. 16.78), corresponding to the measured apparent speed. Leading zeroes must be entered (e.g. 2.57 is keyed in as 0257), no decimal point is required as this is assumed by the unit. When more than four digits are entered, the unit starts entering a new number, this can be used to correct an entry.

Key Press	Display
Cal.	<Blank>
3	3
7	37
6	376
2	3762
5	5762
9	5962

2. Now press the *Cal.* key again. The display should read slightly less than the calibrated speed just fed in and should have a decimal point between the second and third digit. The value displayed is lower than the entered value because the processor is unable to work fast enough to calculate the speed and to drive the motor. The processor only updates the display when the speed is altered. When a calibration is fed in, the processor automatically decreases the speed by one unit to enable the speed to be displayed instantly. If the *Fast* key is now pressed it will be noted that the next speed up is the calibrated speed.

Note the eyepiece graticule must be calibrated with the prism unit fitted, since the unit changes the magnification and thus the graticule calibration.

Once the unit has been calibrated, the *Fast* key should be pressed and the prism allowed to accelerate to maximum speed before the speed is noted. This speed can then be used to calibrate the unit after switching on (it will need to be calibrated every time it is switched on, after initialisation it is travelling at maximum speed thus the noted maximum speed can be fed straight in). If the display remains blank for several seconds after the calibrated speed has been keyed in and the *Cal.* key pressed for a second time, this is because the unit will accept a calibration speed but will not display an actual speed during initialisation.

2.3 Fast, Slow and Stop Keys

All the red command keys have a latching action and need to be pressed twice to return the unit to the original state. The only exceptions are the *Fast* and *Slow* keys. If one of these keys is pressed once and the prism is allowed to accelerate (or decelerate) to its maximum (or minimum) speed the key is automatically unlatched when that speed is reached.

2.3.1 Fast Key

Pressing the *Fast* key once will cause the prism to start accelerating to a faster speed until the key is pressed again (unless the prism is at its maximum speed or reaches its maximum speed before the key is pressed a second time).

Note that when the prism is accelerating the *Slow* key is ignored. The *Fast* key must be pressed again before the *Slow* key will have any effect.

2.3.2 Slow Key

The *Slow* key behaves as per the *Fast* key except that it causes the prism to decelerate and once latched causes the unit to ignore presses of the *Fast* key.

2.3.3 Stop Key

Pressing the *Stop* key once causes the prism to stop, pressing it again causes it to restart.

The *Stop* key can be used while any of the other three keys are latched but the following should be noted when the prism is stationary:

1. The unit will accept a calibration speed but will not display it when the *Cal.* key is pressed for the second time, but will be displayed as soon as the prism is restarted.
2. The *Fast* or *Slow* keys must be latched provided the other is not already latched but the speed is only altered while the prism is rotating.

2.4 Measuring Procedure

With the unit calibrated and rotating, the field is switched on and reversed. It is then switched to whichever polarity gives the slowest apparent particle speed. The speed of the prism should then be adjusted until the particles are approximately stationary (the field will need to be reversed when the prism reverses). The particles can then be made stationary by adjusting the electrode voltage. Note that care should be taken to keep the voltage within the field strength required for the run. When the particles appear stationary, their velocity can be read from the unit, and the electrode voltage also noted.

2.5 Cleaning the Prism

To clean the prism, stop it approximately at the midpoint of its travel then switch off and unplug the unit from the control unit.

Undo the four screws from the side where the socket is located and carefully remove the side. The motor and plate should now be slid out of the box enabling the prism to be cleaned with a lens tissue. The unit can then be reassembled. Note the prism unit must be connected to the control unit before switching on.

3. Operating Principle

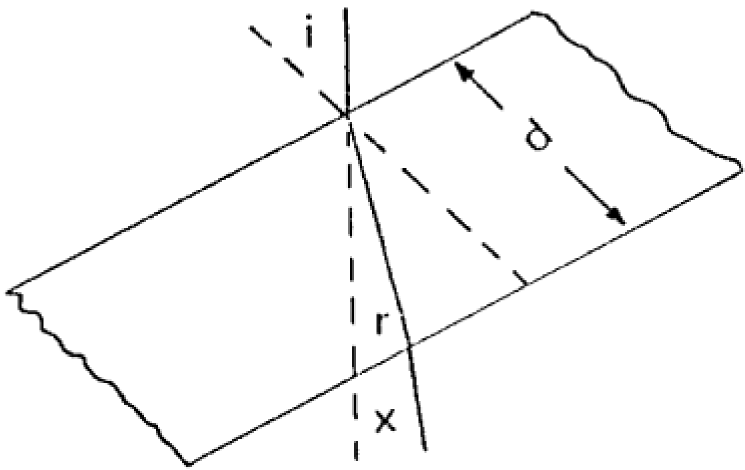

Figure 1 Glass "prism" refractive index n, thickness d.

When the glass prism is perpendicular to the incident light the beam is undeflected, in Figure 1 the angles i and r would be zero and the displacement x would vanish.

Thus if the prism is rotated from the perpendicular incidence position, a displacement x develops, and to an observer above, stationary objects below seem to be moving. Conversely, if the objects below are actually moving (e.g. particles in an electrophoresis cell) then rotation in the correct direction can make these particles appear stationary. Then, if the thickness refractive index and rate of rotation of the prism are known, the actual velocity of the apparently stationary particles can be calculated.

From the geometry of the above diagram it can be seen that:

$$\frac{x}{d} = \frac{\sin(i - r)}{\cos r}$$

and also we have, if the refractive index of the prism is n:

$$n = \frac{\sin i}{\sin r}$$

If the angles i and r are small (i.e. the prism has rotated through only a small angle from the perpendicular incidence position) then sin (i—r) = (i—r), cos r = 1 and n = i/r and the above two relations yield, with i in radians:

$$\frac{x}{d} = i \cdot \left(1 - \frac{1}{n}\right)$$

So that, if n is taken as 1.5:

$$\frac{dx}{dt} = \frac{d}{3}\frac{di}{dt}$$

Which relates the rotation rate to the apparent linear displacement rate at small angles. In practice, however, it is obviously much easier to calibrate the displacement rate at a known rotation rate against a stationary stage micrometer graticule than to attempt to calculate the relationship. This procedure is described in detail in section 2.2.

The expression:

$$\frac{x}{d} = i \cdot \left(1 - \frac{1}{n}\right)$$

is an approximation which refers to small angles about the perpendicular position. If the exact expression (involving the sin and cos functions) is used then the non-linearity is seen to be about 1% at a deflection of 10°. However, even this small non-linearity is mitigated by two other factors.

First the non-linearity is, of course, zero at i = 0 so that the average effect over a sweep is smaller, and secondly the rotation causes a small increase in optical path length which increases the magnification very slightly and operates in such a direction as to diminish the non-linearity effect.

It is because of this potential non-linearity effect that rotation of the prism in the instrument is limited to ±15°. Then in practice, determinations are made in the range ±10°.

Use of the rotating prism rather than the simple method of timing individual particles has the following advantages:

1. A large number of particles are, in effect, observed at once. The human eye can easily detect when (say) five particles are all stationary, or if four are stationary and one is not, even though the eye is unwilling to actually time more than one moving particle at once.
2. The tendency, when timing individual particles, to pick out the big bright ones is removed. Such large particles may not be typical of the total population.
3. A shorter time is needed to get an average based on a given number of particles.
4. If the particle mobilities are very heterogeneous then this is immediately obvious and steps can be taken.
5. If the dispersion is a mixture of two types of particle with different mobilities then not only is this immediately evident but the two types can be separately brought to the stationary state, and their mobility measured, in the presence of each other.

If the prism is not rotated then individual particle timings can be made in the usual way if desired.