

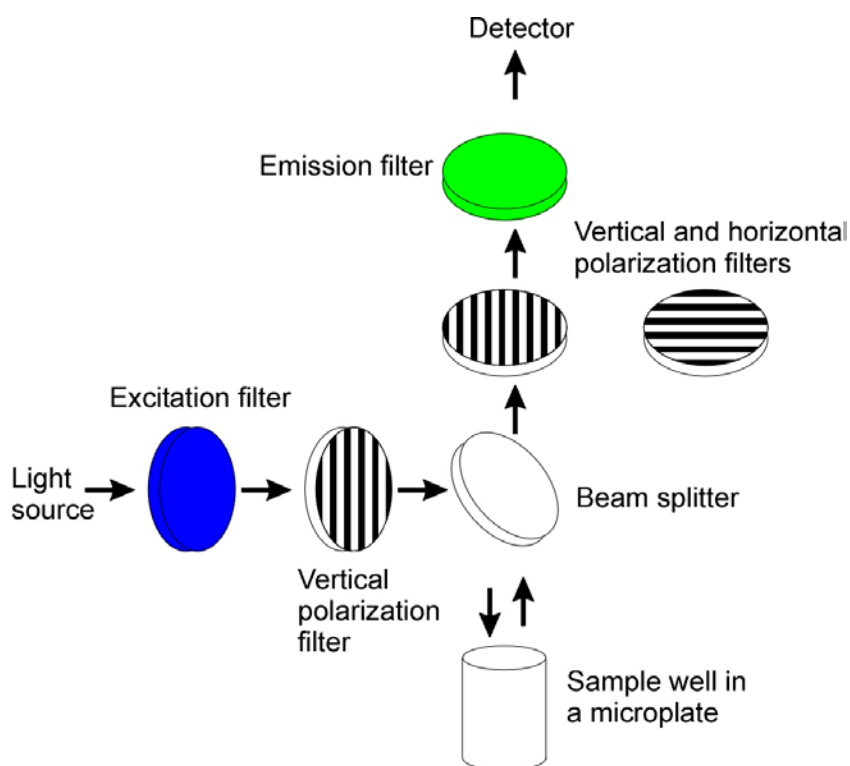
## Measuring Fluorescence Polarization with Plate CHAMELEON™

### The principles of fluorescence polarization

Fluorophores undergo significant molecular motion under the Fluorescence lifetime. Fluorescence life-time is the time between absorption of the excitation photon and the emission of a photon through the fluorescence phenomenon.

The molecular movement causes a difference in the polarization planes of the excitation and emission light. When the fluorophores are bound to a significantly larger molecule the movement slows down. This creates a difference between the polarization of bound and unbound fluorophore.

The principle of the fluorescence measurement in Plate Chameleon is described in figure 1. The figure also shows the polarization filters.



**Figure 1 shows a diagram of the optical system in Plate CHAMELEON for fluorescence polarization measurements.**

Fluorescence polarization is measured in millipolarizations [mP]. mP is defined as

$$mP = 1000 \times \left[ \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}} \right] \quad (1),$$

where  $I_{\parallel}$  is fluorescence intensity measured with vertical excitation and vertical emission polarization filters what is called parallel intensity and  $I_{\perp}$  is fluorescence intensity measured with vertical excitation and horizontal emission filters what is called perpendicular intensity.

It is important to notice that mP is a ratio of fluorescence intensities. This means that mP is independent of concentration of the fluorophore.

### **Background correction**

When the concentration of the fluorophore is in the nanomolar range it is advisable to correct the intensities for background. A plate should always blank wells which contains all other assay components but the tracer. For a very low concentration the difference between the sample and blank may not be high. This affects the assay window and the difference between bound and unbound samples is not clearly seen.

### **G-factor**

In practice the optical components of an instrument affect the passage of light depending on its polarization plane. The G-factor is used to correct for the errors caused by this. Equation 1 comes

$$mP = 1000 \times \left[ \frac{I_{//} - G \cdot I_{\perp}}{I_{//} + G \cdot I_{\perp}} \right] (2).$$

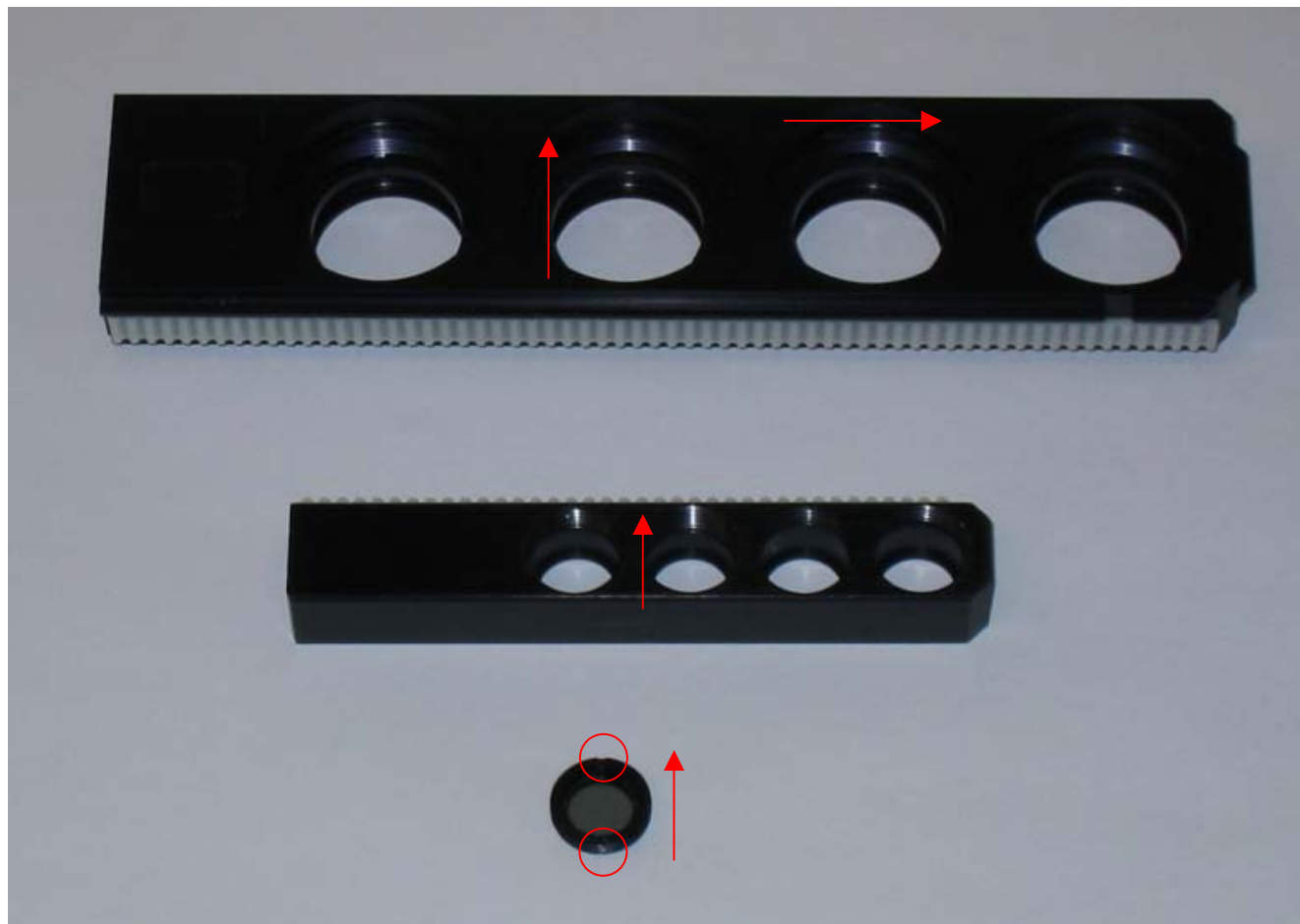
The G-factor can be defined by pipetting a dilution series of the used fluorophore. Measured mP values should be between 20 and 30 mP with standard deviation of less than 5 mP.

### ***Measuring FP with Plate Chameleon***

#### **Setting up your filters**

Normally the polarization filters are setup in the factory so you don't have to changes the alignment in the filter slide. If they positions however are changed check the filter alignments. In figure 2 both filter slides are shown. Red arrows point the directions how the polarization filters should line up in a correct setup.

A Plate CHAMELEON fluorescence polarizations protocol would then be Excitation filter pos. 2 Parallel emission filter pos. 2 and perpendicular pos 3. In a normal factory setup we also have in positions 1 a regular fluorescence filters for both excitation and emission wavelengths.



**Figure 2 shows the excitation and emission filter slides and example of excitation polarizer. The red circles show small slits the polarization plane is always set according to these slits. The arrows point the directions of the polarization planes.**

### **Define GAIN**

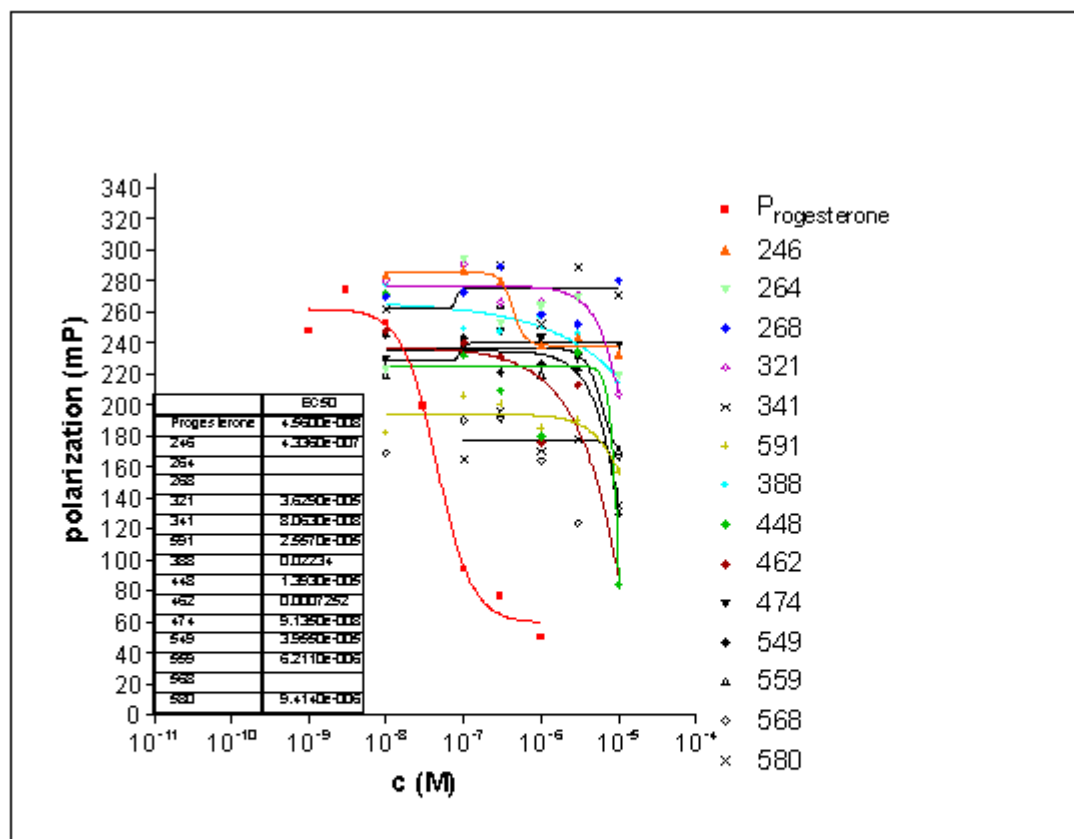
Measure a dilution series of your fluorophore with an FP protocol and set the gain so that your Fluorescence intensity readings are between 10 and 200000.

### **Define the G-factor**

Measure the same dilution series and set the G-factor so that you receive mP values of 20 to 30 mP. The G-factor is unique to all instruments and optical components so they may vary between different filters. Normally the G-factor is between 1 to 1.3.

### **Measure your samples**

The Plate Chameleon automatically calculates the mP values, but it doesn't take into account the background subtraction. You can either define a level of background intensity in the protocol or calculate the mP values from intensity levels using your data analysis software.

**Example of an Fluorescence Polarization assay on Plate CHAMELEON**


**Figure 3.** Steroid competition with progesterone labelled with a fluorophore.

*Progesterone Receptor Competitor Assay (PanVera).*