light from the substance in question stops almost immediately after the incident radiation is terminated, and the level of fluorescence can be measured and quantified over time.

Fluorescence intensity assays are the simplest systems designed to take advantage of fluorescent properties for the purposes of monitoring biological systems and examining test compounds. There are two basic types. Assay systems can be designed to measure either the increasing or decreasing levels of fluorescence over time to monitor biological activity. Consider, for example, a fluorogenic assays in which an enzyme converts a non-fluorescent substrate into a fluorescent product. Compounds that inhibit the enzyme will slow the rate of formation of the product in a quantifiable manner, and this information can be used to determine the IC_{50} of test compounds. Alternatively, an enzyme could convert a fluorescent substrate into a non-fluorescent substrate. In this case, the biological activity of test compounds would be determined by monitoring the loss of fluorescence, as enzyme inhibitors would slow the conversion of the fluorescent substrate into the non-fluorescent material. In either case, it is important to be aware that readouts from a fluorescence intensity assay are potentially subject to interference created by natural fluorescence of test compounds, colored compounds, and test compounds that are also fluorescence quenchers on their own. These issues can create both false positive and false negative readouts in fluorescent intensity assays and should be considered when assay data are reviewed.

Fluorescence Polarization (FP)

Simple fluorescence intensity assays have proven to be very valuable tool for scientists in a number of fields, and it should come as no surprise that more advanced fluorescence assays have been developed in order to take advantage of the properties of light. Fluorescence polarization technology relies on differences in polarization of light created by changes in molecular size. Polarized light was first described by Eitenne-Louise Malus in 1808,[28] well before the appearance of organized drug discovery programs. Over 100 years later, Perrin[29] and Weigert[30] separately described the relationship between molecular size, polarization of light, and fluorescence, and how these properties could be used to study molecular interactions. Instrumentation and homogeneous assay were later developed by Weber[31] and Dandliker[32] respectively. Their work forms the basis of screening technologies that are widely used in modern high throughput screening laboratories.

As previously described, excitation of a fluorophore leads to emission of light at a lower wavelength in a predictable and quantifiable manner.