in capabilities between the two instruments, essentially, each instrument is equipped with an energy source for irradiation of sample (an argon laser, light emitting diodes, etc.) in a microtiter plate (96, 384 well) and a charge-coupled device camera capable of collecting data from all plate wells simultaneously. Multiple images of the assay plate are recorded through the course of a single experiment so that changes in fluorescent intensity can be monitored and interpreted by software that drives the instruments. Plate images can be captured in rapid succession at intervals of less than 1 s per image, making these systems well suited toward the examination of transient cellular events that would be otherwise impossible to monitor (Figure 4.32).

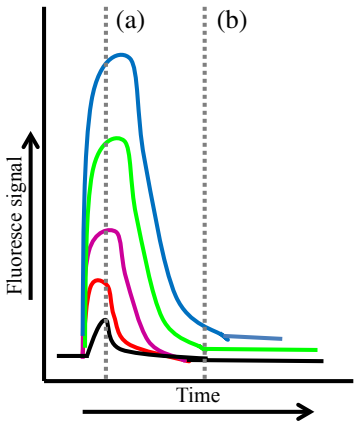

FIGURE 4.32 Kinetic assay systems are capable of monitoring changes in fluorescent intensity over time. Each color represents the impact of increasing concentrations of a test compound on the fluorescent intensity. The importance of this technology becomes apparent when one considers the difference in observed potency at time points (a) and (b). If only data from time point (b) were available, this test compound would appear to lack potency, even though there is a potent effect at an early time point.

Both of these systems are capable of supporting a wide range of fluorescent assay systems (FRET, TRFRET, AlphaScreen, etc.) and have been successfully employed to study a wide range of transient cellular events.

LABEL-FREE ASSAY SYSTEMS

The overwhelming majority of *in vitro* assay screening systems employed in modern drug discovery research rely on fluorescent labeling or radiolabeling of small molecules, proteins, DNA, antibodies, or related materials. While labeling techniques have clearly been successfully used to develop sophisticated high throughput screening assays capable of driving drug discovery programs, it is important to understand that the labels themselves have the potential to influence screening results. Attaching a label to a biologically relevant compound can alter its conformation, its overall molecular properties, and its ability to interact with biological systems necessary for functional activity. Fluorescent labeling of an enzyme's natural substrate, for example, can have