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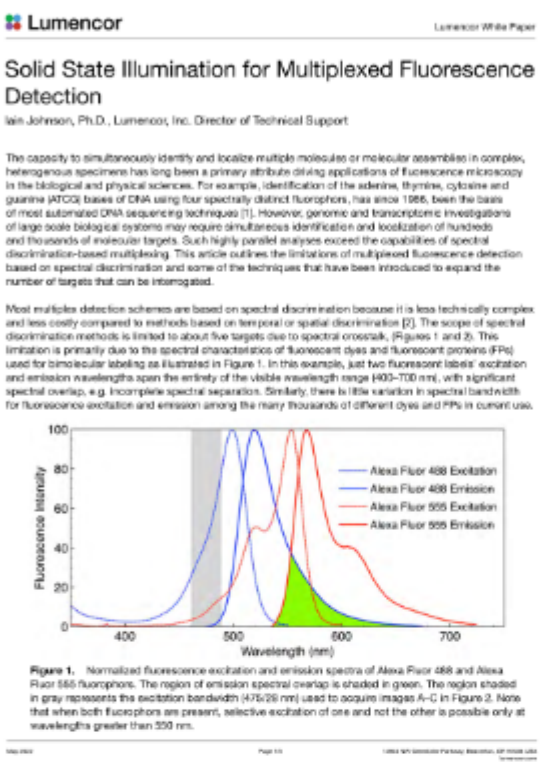
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Lumencor White Paper

Solid State Illumination for Multiplexed Fluorescence Detection

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The capacity to simultaneously identify and localize multiple molecules or molecular assemblies in complex, heterogenous specimens has long been a primary attribute driving applications of fluorescence microscopy in the biological and physical sciences. For example, identification of the adenine, thymine, cytosine and guanine (ATCG) bases of DNA using four spectrally distinct fluorophors, has since 1986, been the basis of most automated DNA sequencing techniques [1]. However, genomic and transcriptomic investigations of large scale biological systems may require simultaneous identification and localization of hundreds and thousands of molecular targets. Such highly parallel analyses exceed the capabilities of spectral discrimination-based multiplexing. This article outlines the limitations of multiplexed fluorescence detection based on spectral discrimination and some of the techniques that have been introduced to expand the number of targets that can be interrogated.

Most multiplex detection schemes are based on spectral discrimination because it is less technically complex and less costly compared to methods based on temporal or spatial discrimination [2]. The scope of spectral discrimination methods is limited to about five targets due to spectral crosstalk, (Figures 1 and 3). This limitation is primarily due to the spectral characteristics of fluorescent dyes and fluorescent proteins (FPs) used for biomolecular labeling as illustrated in Figure 1. In this example, just two fluorescent labels' excitation and emission wavelengths span the entirety of the visible wavelength range (400–700 nm), with significant spectral overlap, e.g. incomplete spectral separation. Similarly, there is little variation in spectral bandwidth for fluorescence excitation and emission among the many thousands of different dyes and FPs in current use.

Figure 1. Normalized fluorescence excitation and emission spectra of Alexa Fluor 488 and Alexa Fluor 555 fluorophors. The region of emission spectral overlap is shaded in green. The region shaded in gray represents the excitation bandwidth (475±28 nm) used to acquire images A–C in Figure 2. Note that when both fluorophors are present, selective excitation of one and not the other is possible only at wavelengths greater than 550 nm.

Solid-State Illumination for Multiplexed Fluorescence Detection

The capacity to simultaneously identify and localize multiple molecules or molecular assemblies in complex, heterogenous specimens has long been a primary attribute driving applications of fluorescence microscopy in the biological and physical sciences. However, genomic and transcriptomic investigations of large scale biological systems may require simultaneous identification and localization of hundreds and thousands of molecular targets. Such highly parallel analyses exceed the capabilities of spectral discrimination-based multiplexing. This article outlines the limitations of multiplexed fluorescence detection based on spectral discrimination and some of the techniques that have been introduced to expand the number of targets that can be interrogated.

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