Multispectral Multidimensional Multiplexed Data: The More, the Merrier

Richard Levenson, Clifford Hoyt, Jim Mansfield and Kirk Gossage
CRI, CRI, 35B Cabot Rd., Woburn, MA 01801
rlevenson@cri-inc.com

Abstract
The ability to detect multiple molecular species at once is becoming increasingly important. Multispectral imaging systems can be used to capture multiplexed molecular signals, and can be applied to the analysis of chromogenically stained slides in brightfield mode and of samples stained with a variety of light-emitting dyes (from the visible to the NIR range) in fluorescence mode. Quantum dots make a particularly good match with this imaging technology, which is also extremely helpful for the identification and elimination of interfering autofluorescence. The ability to accurately determine the spectral qualities of dyes in-situ is also valuable. Multispectral imaging has proven to be useful for multicolor FISH, for resolving multiple species of GFP with overlapping emission spectra and for resolving red/brown double-labeled histopathology stains. The uses of spectral imaging in clinical pathology are still being explored and need to be matched to appropriate software tools. Appropriately constrained linear unmixing algorithms and novel automated tools have recently been developed to provide simple, accurate analysis procedures. Conventional hematoxylin-and-eosin- or Papanicolaou-stained pathology sections can have sufficient spectral content to allow the classification of cells of different lineage or to separate normal from neoplastic cells. Analysis of such specimens may succeed using spectral "signatures" and simple segmentation algorithms. The rich data sets also reward the use of more advanced analysis techniques. These can include a number of approaches pioneered for remote sensing purposes, such as spectral similarity mapping, automated clustering algorithms in n dimensions, principal component analysis, as well as other more sophisticated techniques.