

Comparison of Spectral Imaging Modalities and Quantitative Data Analysis Techniques

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Spectral imaging combines two methodologies, spectroscopy and imaging, to yield data that contains spectral and spatial information. Spectral segmentation of this data allows for quantitative analysis of different entities in organic and inorganic samples, with a wide range of applications in biological and material sciences. This imaging modality is particularly beneficial for multiplex labeling methods where fluorochromes of similar or overlapping spectra are used, as well as identification and removal of artifacts such as autofluorescence. Spectral imaging may be achieved with multiple types of equipment, including wide-field and confocal microscopy, as well as analyzed using a variety of software. Here, we compare several confocal imaging methods and the Mantra Quantitative Pathology Imaging Workstation (PerkinElmer, USA), as well as three different image analysis software packages: InForm (PerkinElmer), FIJI (An open-source platform for biological-image analysis) and Volocity (PerkinElmer). Sample preparation, imaging techniques and data analysis algorithms will be discussed and example data sets from the different modalities will be presented. The aim of this comparative overview is to provide researchers with a broad understanding of the technique, potential applications and the types of information which may be gathered for materials and biological studies.