

# Spectral Methods in Microscopy and Flow Cytometry. From spectral fingerprints to pattern classification.

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## Abstract

Hyperspectral imaging methods originated in remote sensing (satellite imagery), and over the years, spread into industrial imaging, computer vision, and biological microscopy. Some of the data processing approaches required to interpret the spectral information may feel familiar to cytometrists, as they are based on mathematical models of signal mixing employed in commonly used compensation. The spectral methods generated renewed interest recently owing to the introduction of commercial spectral cytometry systems. However, many researchers raise legitimate questions about whether the spectral techniques truly provide additional benefits and whether the spectral information can be interpreted and visualized in an accessible and approachable fashion. This presentation will explain the mathematical underpinnings and the physical constraints of the essential spectral-data analysis techniques commonly employed in the fields of multispectral microscopy and flow cytometry. We will reintroduce and review the concepts of spectral overlap, linear mixing model, compensation, and spectral unmixing. We will compare and contrast the basic idea of compensation with the more generalized notion of unmixing, focusing on how the implicit assumptions and compensation constraints may potentially produce sub-optimal results. Be prepared to hear about photonic noise, measurement uncertainty, unconstrained linear unmixing, blind unmixing, non-negative matrix factorization heuristics, principal component analysis, and manifold learning. This talk will not endorse any commercial spectral microscopy or cytometry platform and will not compare the pros and cons of the solutions marketed by different companies. Instead, we will focus on theory and try to bridge the mathematical description with an intuitive understanding of the spectral analysis process.

## Bio

**Dr. Bartek Rajwa** is a Research Associate Professor of Computational Life Sciences in the Bindley Bioscience Center at Purdue University where he conducts studies on the technologies of high-content imaging, biological image analysis, biological pattern recognition, and applications of statistical machine learning in cell biology, neuroscience and agriculture. Bartek is an Associate Editor of Cytometry Part A (the official journal of ISAC).