

## Automated segmentation of cellular ultrastructure in large scale ColorEM

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### Abstract

Large-scale electron microscopy (EM), or nanotomy ([www.nanotomy.org](http://www.nanotomy.org)), is a powerful technique that gives the opportunity to view biology at the nanoscale while retaining tissue context. These greyscale projections of cellular ultrastructure offer a wealth of information that becomes increasingly difficult to analyze as its size grows. ColorEM, hyperspectral imaging through elemental dispersive X-ray (EDX) imaging, maps the presence of elements in the sample during EM acquisition. We implement ColorEM in the nanotomy pipeline, which allows color-based identification and localization of varying structures at nanoscale based on the elemental fingerprint. However, only few elements can be visualized with false color representations, and a large portion of the hyperspectral image remains unused. A data-driven pipeline ('IDENTIFY') identifies spectral fingerprints from the hyperspectral image and visualizes their presence and abundance. This pipeline highlights varying ultrastructural features based not only on the electron density but also on the relative elemental content. While we currently present only a single tissue, the newly created IDENTIFY pipeline is applicable to all human tissues and will aid in automated analysis of nanotomy and volume EM datasets in general.