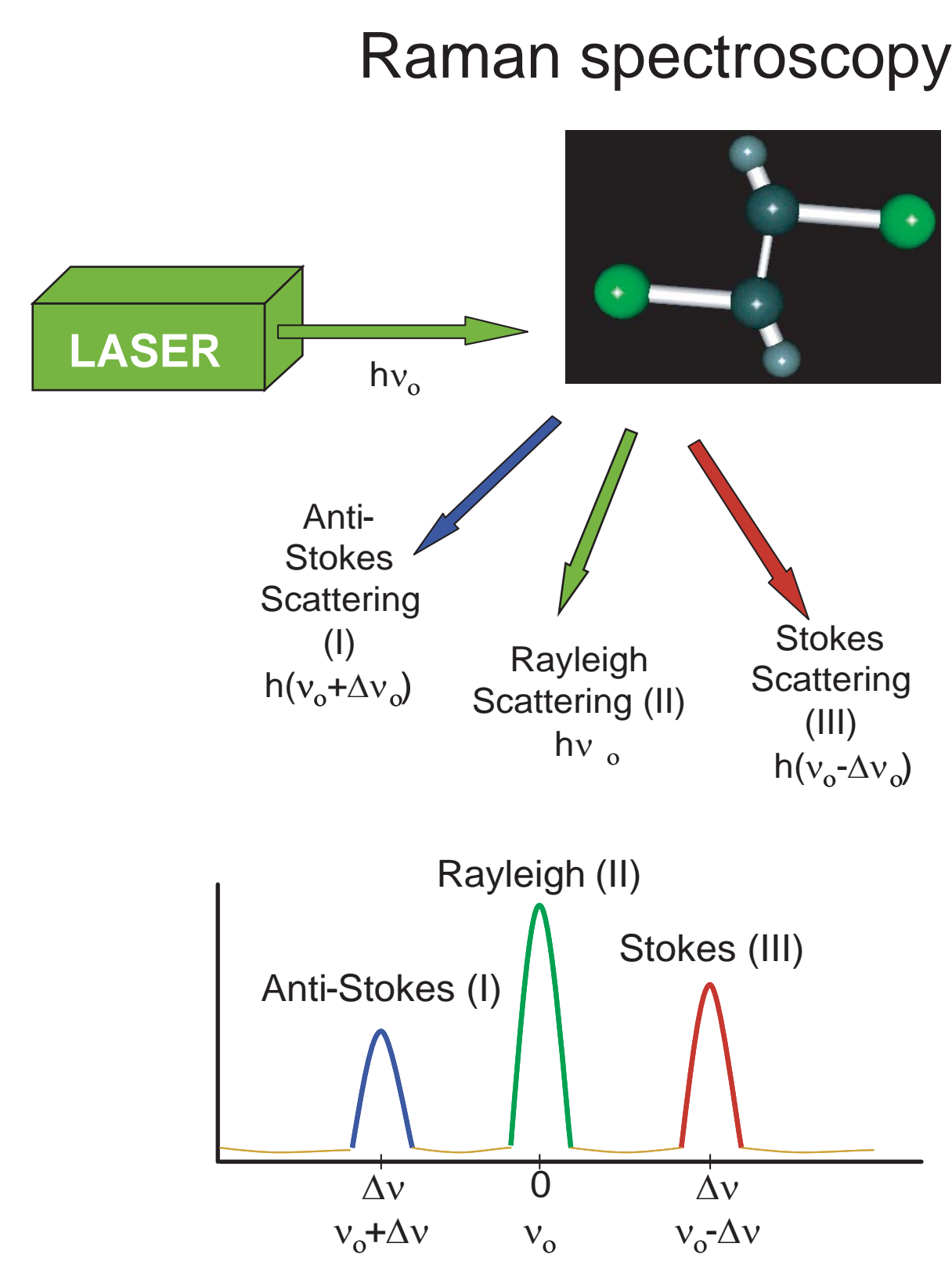
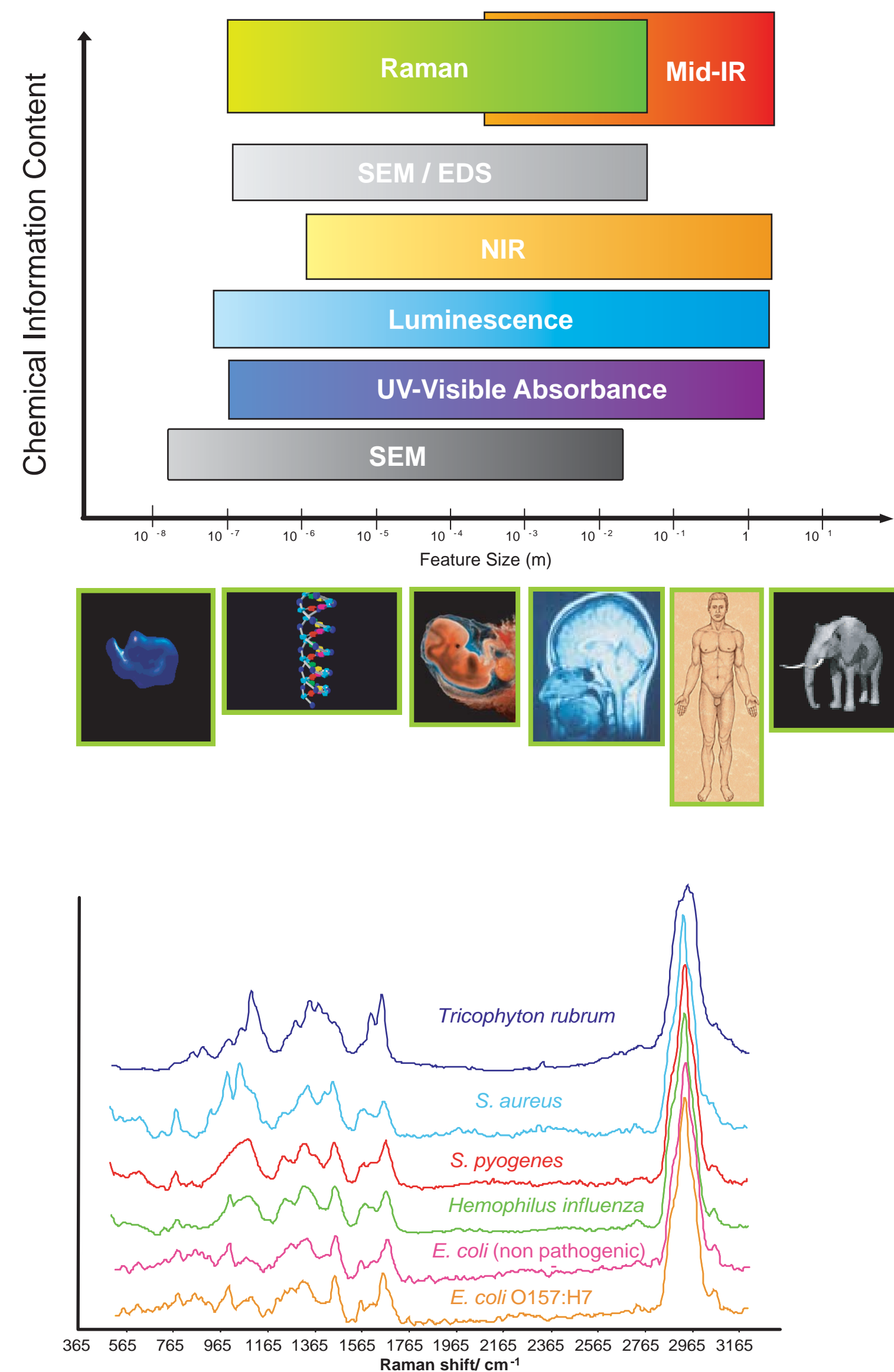




Raman Molecular Imaging: A Tool For Investigating Biological Systems

Authors: John Maier, Shona Stewart, David Tuschel, Amy Waligorski, Lindy McClelland

Chemical Imaging Techniques



- Raman Spectroscopy is Specific
- Inelastic scattering phenomenon
 - Laser based technique
 - Probes energy of molecular vibrations
 - Molecular "fingerprint"
 - Low light effect

- Chemical Imaging is a powerful tool combining digital imaging and molecular spectroscopy to yield a picture of the molecular specific components of a complex sample.

- Chemical Imaging is a rapid, nondestructive technique and can employ different methods of molecular spectroscopy such as Fluorescence and Raman.

- Raman Chemical Imaging is a quantitative imaging spectroscopy approach.

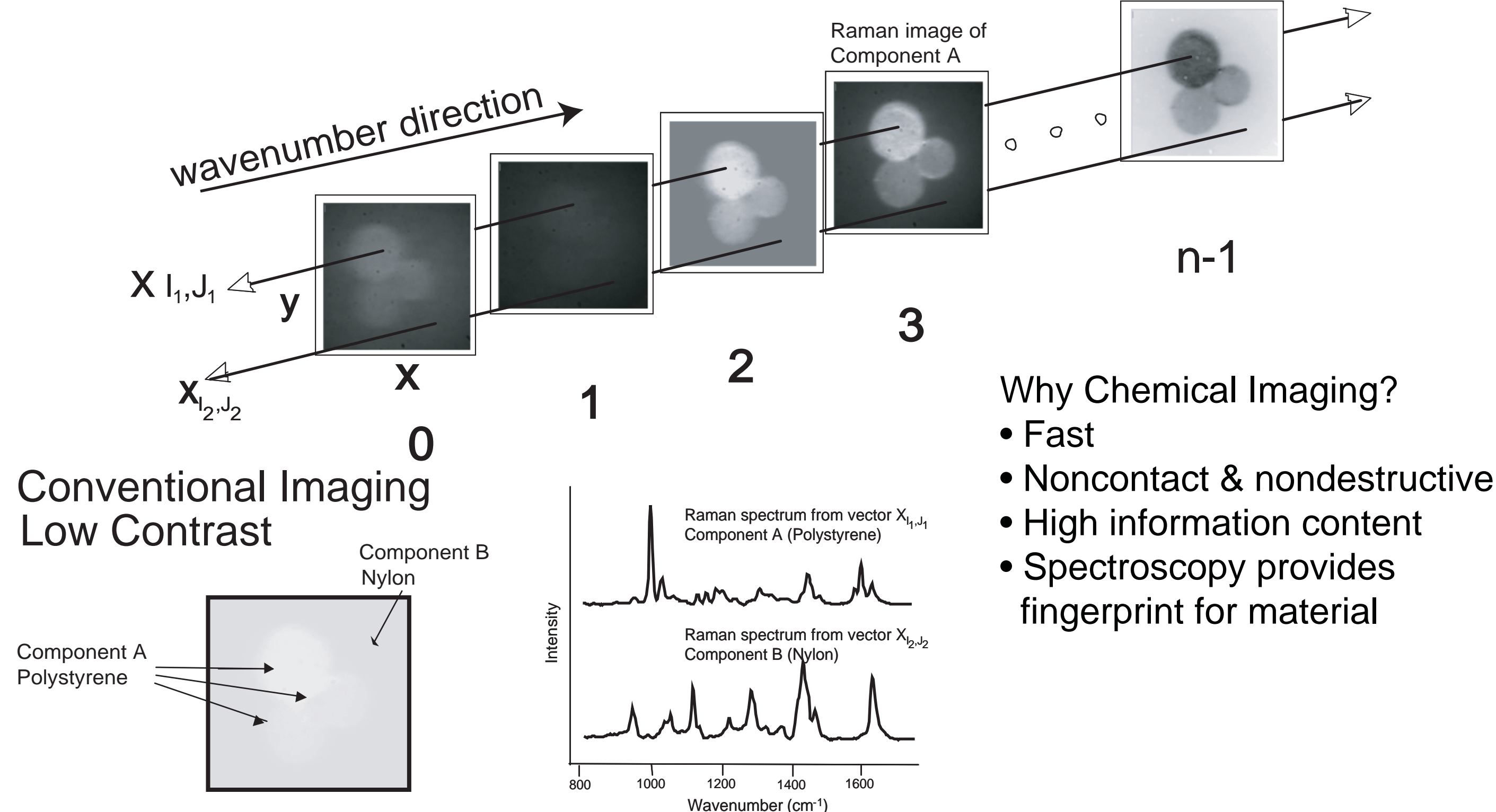
- Raman Chemical Imaging can be applied to cellular systems and can provide quantitative information about the chemical constituents of cells and tissues.

- Deconvolution techniques can be applied to Raman images to yield 3D images of chemical species within cells.

- 3D optical sectioning reveals the spatial arrangement of the sample components.

Raman Chemical Imaging

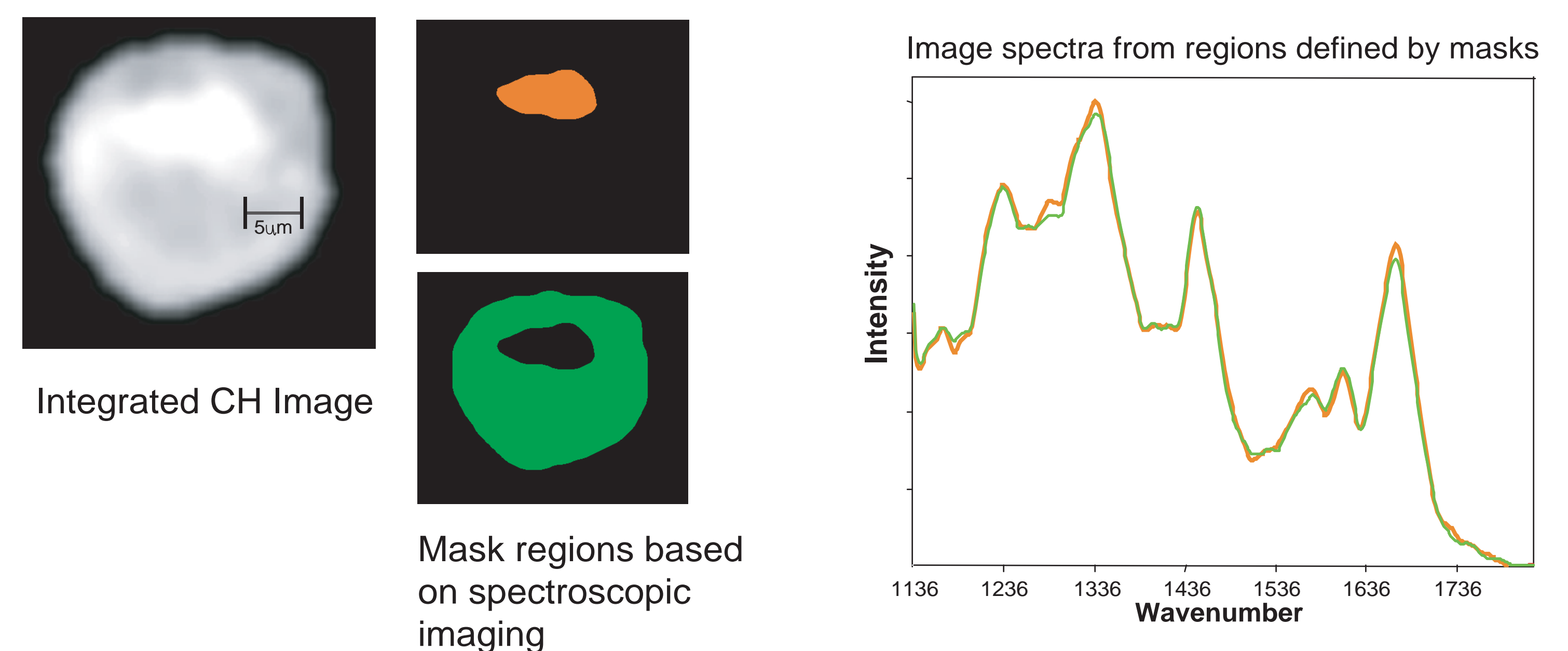
Massively Parallel Spectroscopy



- Why Chemical Imaging?
- Fast
 - Noncontact & nondestructive
 - High information content
 - Spectroscopy provides fingerprint for material

Raman Molecular Imaging of Breast Cancer Cell

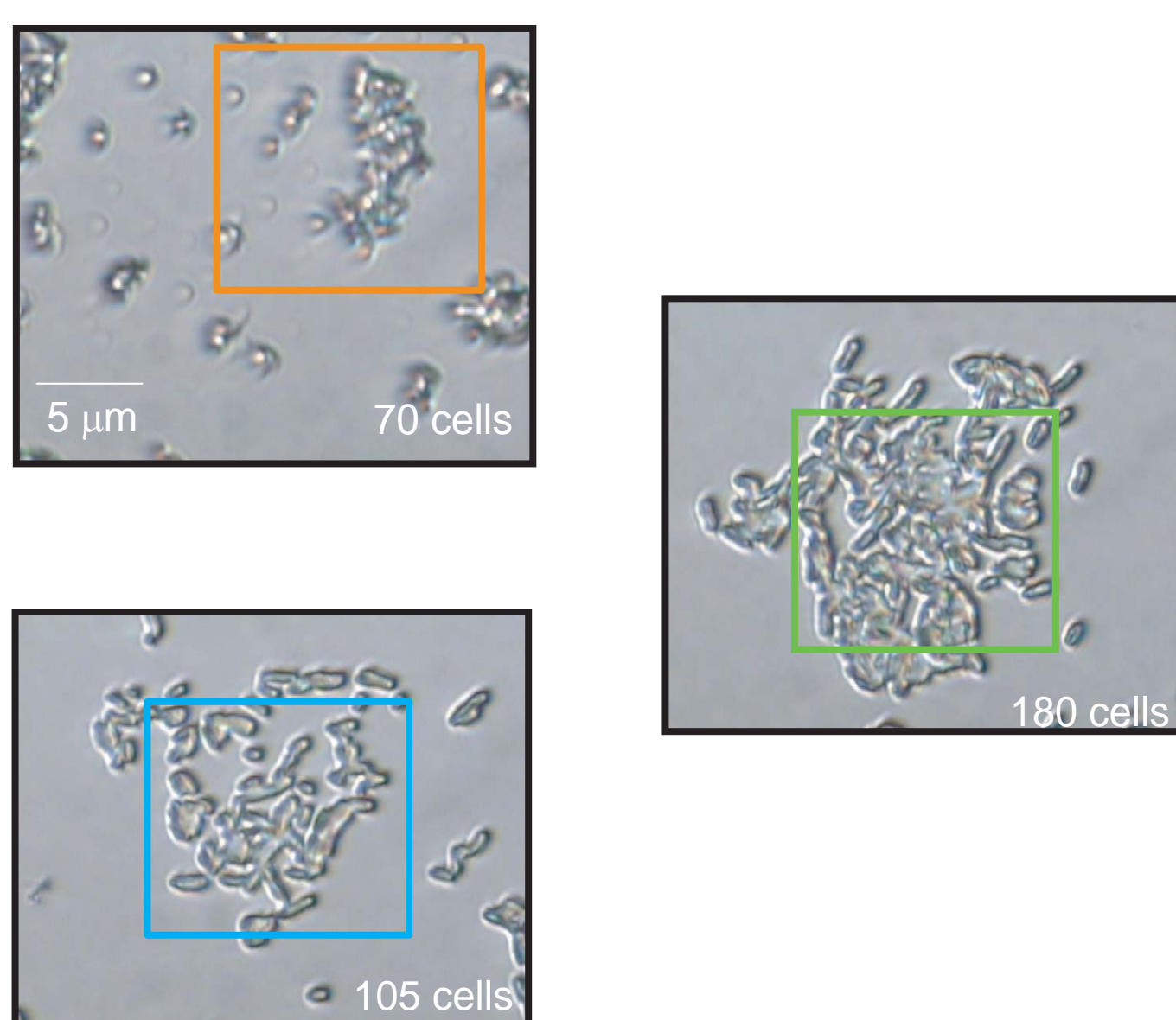
Imaging and spectroscopic identification of microorganisms



Raman Chemical Imaging : Quantification and Identification

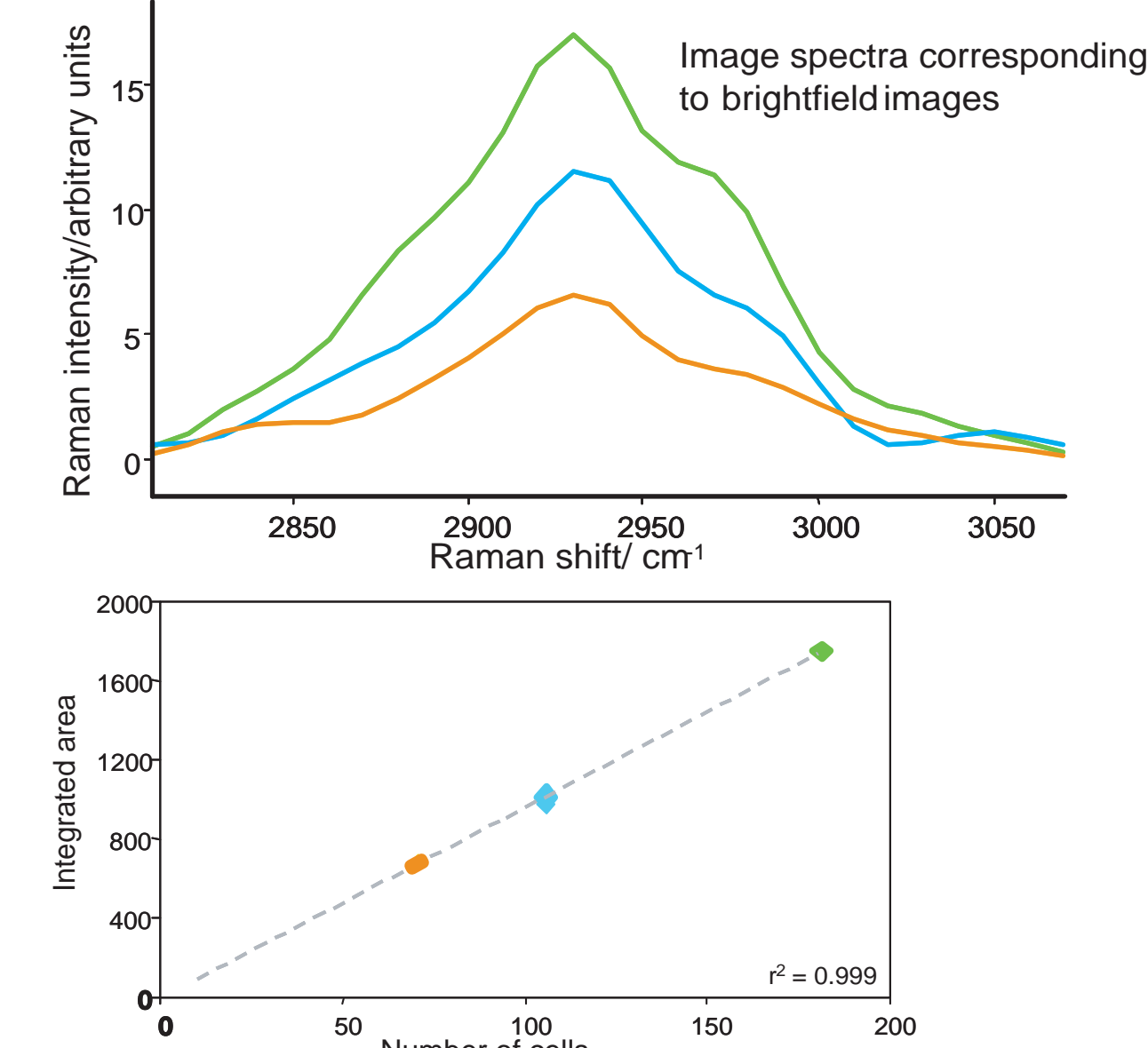
The specificity of Raman scattering enables detection and identification of samples as small as bacteria. The Raman Image of *E. coli* O157:H7 illustrates the spatial resolution which can be achieved using the FALCON II Raman Chemical Microscope. In addition, Raman imaging can be used to quantify particles such as *Staphylococcus epidermidis*. Fields-of-view containing different numbers of the bacteria exhibit Raman spectra of different signal intensities. The Raman intensities can, in turn, be used to estimate the number of bacteria in the field of view.

Brightfield images
100x magnification



Quantification of *S. epidermidis*

(In collaboration with J. M. vanBriesen and M. Escoriza, Carnegie Mellon University)



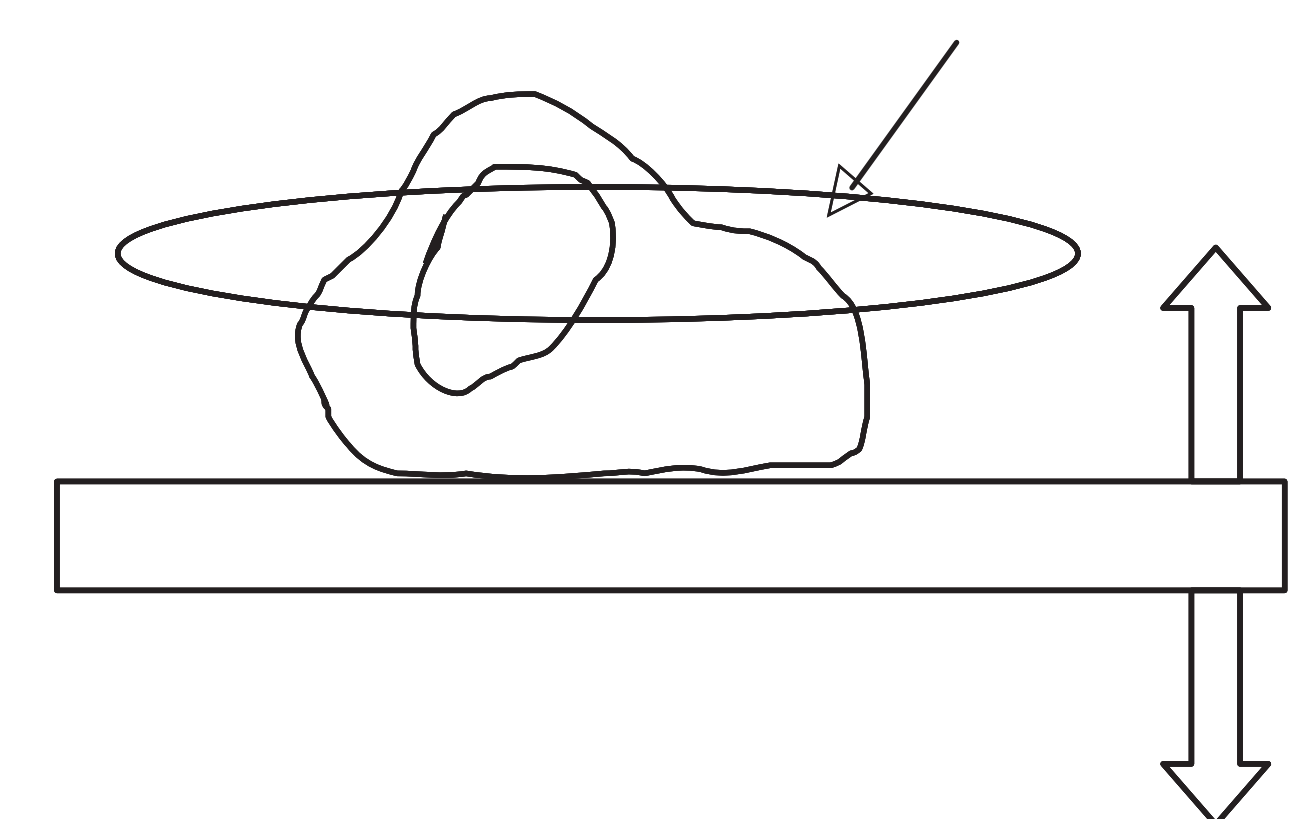
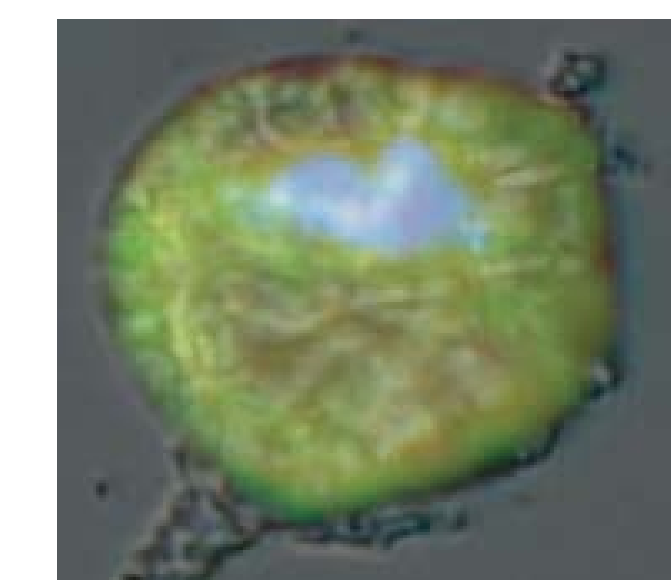
Imaging Subcellular Components

The inherent specificity of Raman scattering, in combination with the high spatial resolution of microscopy and data reduction techniques of chemometrics enable imaging of tissue cells and cellular components. Raman images of cells reveal elements including membrane, nucleus and cytoplasm. Detailed analyses indicate location and molecular structure of many other cellular components

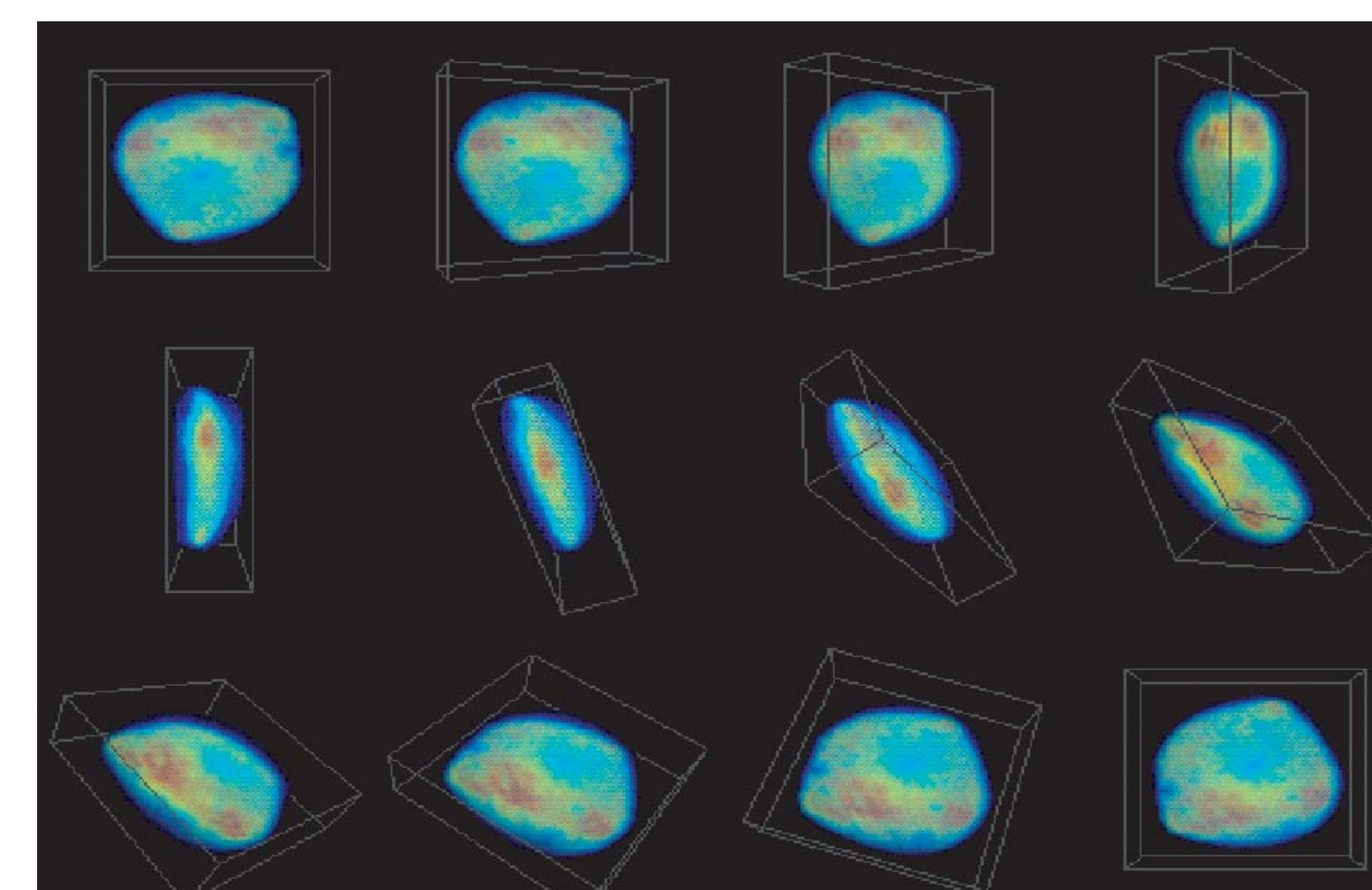
Brightfield Unstained

Raman Composite

Focal plane of Microscope



Views of 3D cell Raman Image from Different Angles



- Data is acquired by moving cell on stage relative to focal plane of instrument
- A Raman Chemical Image is acquired at each depth
- After correction for optical aberrations, a 3D image is constructed