

Chalmers University of Technology



Annika Enejder

Associate Professor, group leader of Molecular Microscopy

Develops microscopy techniques (CARS, SRS, SHG, and Raman) where inherent molecular vibrations are probed to 3D images



Juris Kiskis

PhD student in the Group of Molecular microscopy

Juris works on combining non-linear optical microscopy with scanning probe microscopy to achieve molecular specific nano-scale imaging. The goal is to study the formation of beta-amyloid aggregates and the role of lipids in neurodegenerative disorders without the need for staining and at spatial resolutions significantly higher than that achieved by optical microscopy alone.



CHALMERS
UNIVERSITY OF TECHNOLOGY

Non-linear Raman scattering microscopy techniques

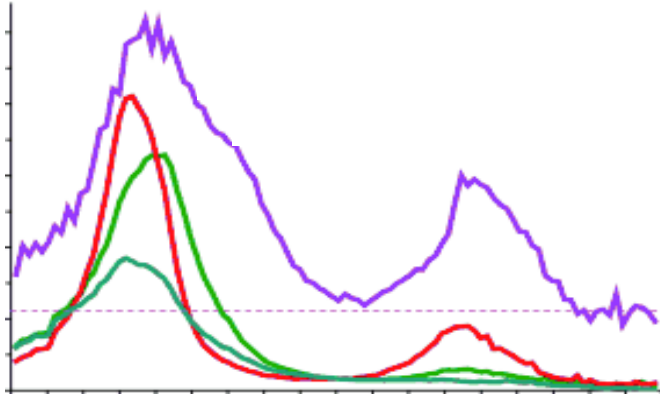
Jūris Kiškis

Group of Molecular Microscopy

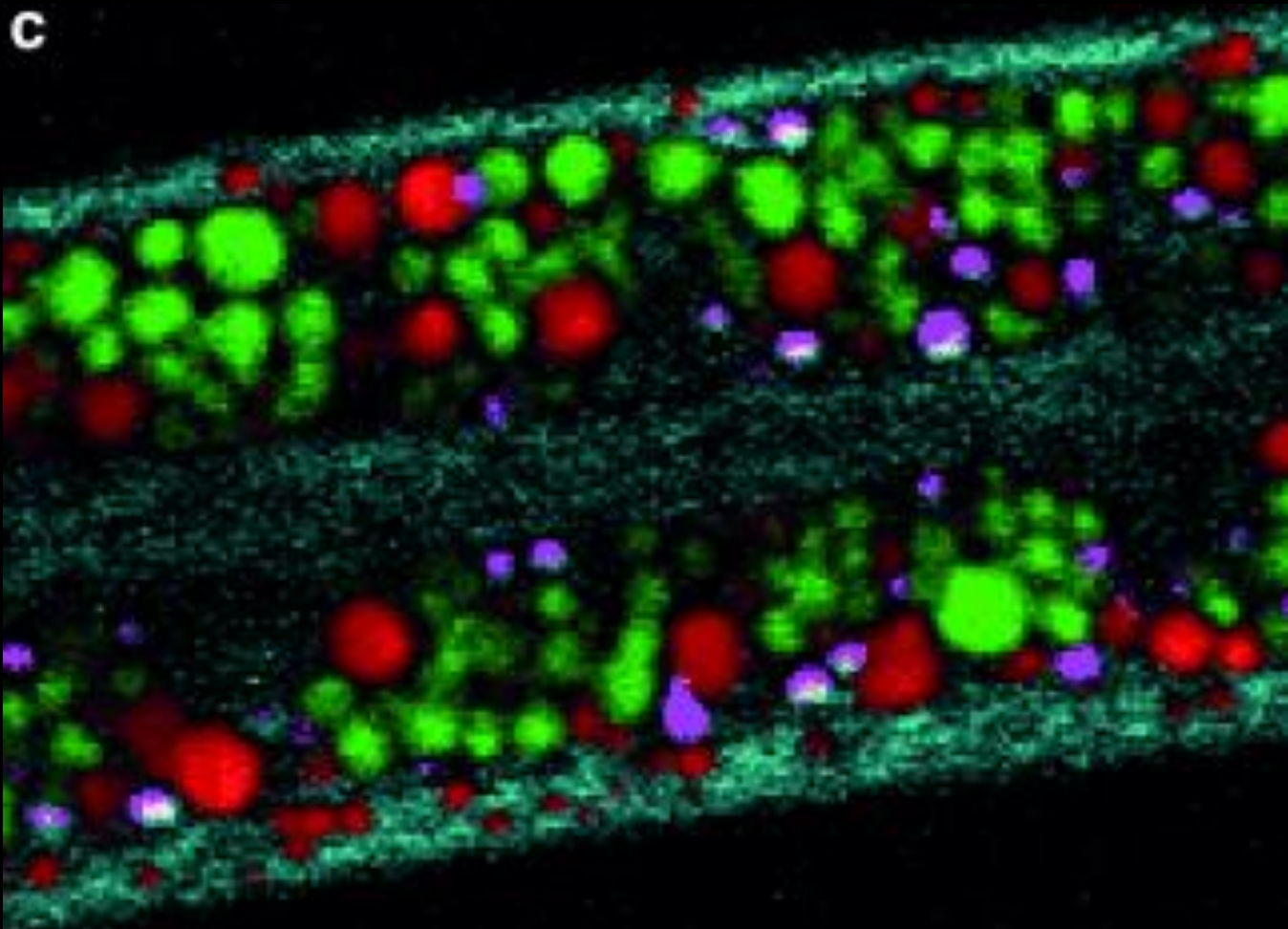
Department of Chemical and Biological Engineering

Chalmers University of Technology

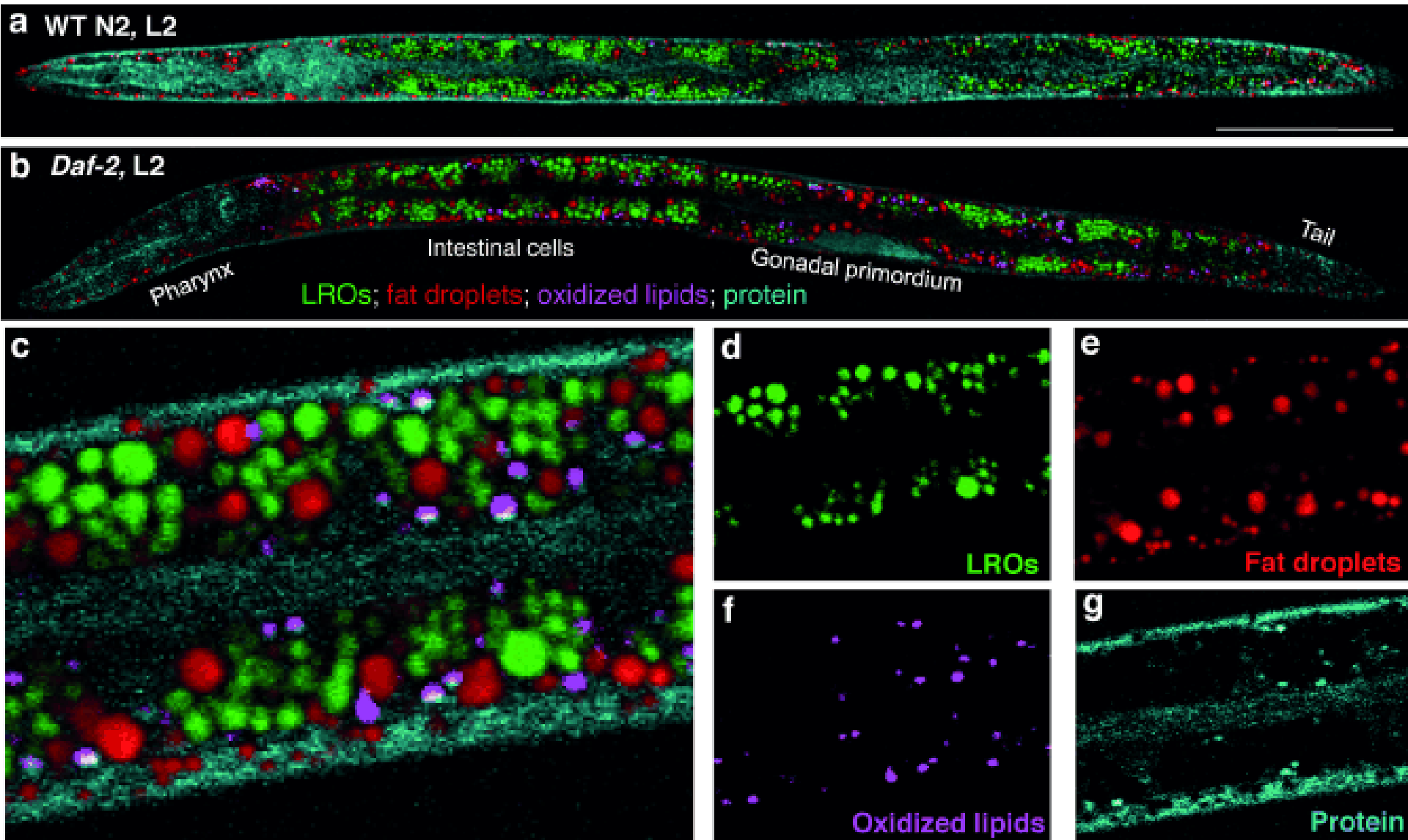
Göteborg



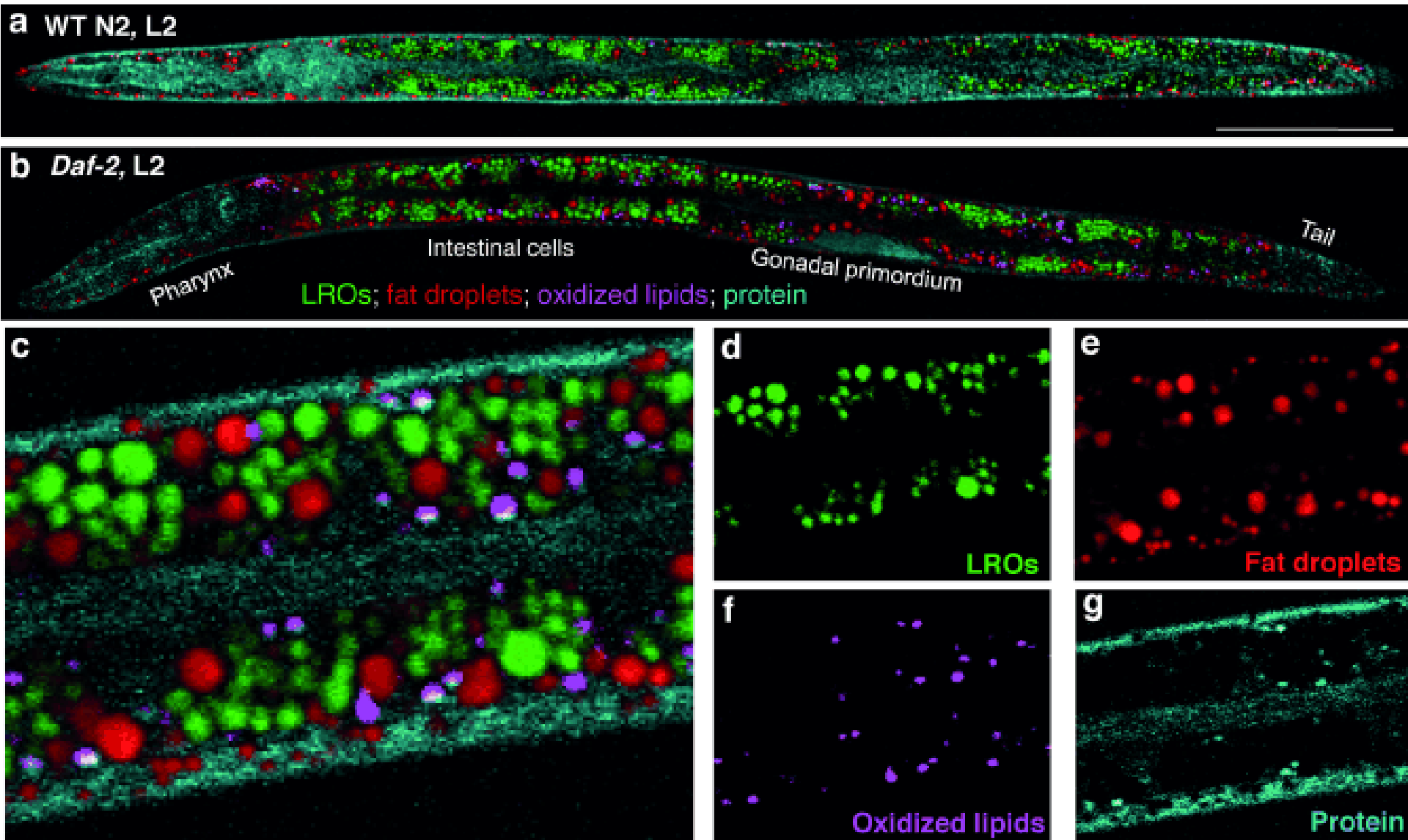
?



Imaging lipid metabolism in live *Caenorhabditis elegans* using fingerprint vibrations

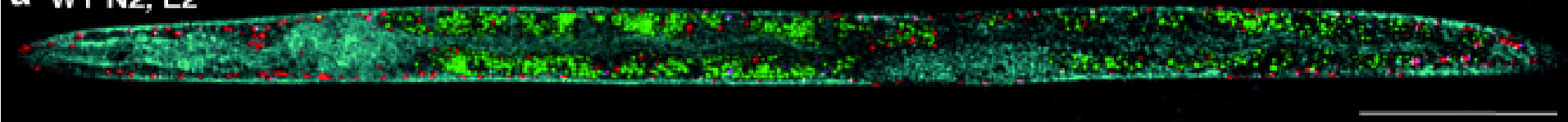


Imaging lipid metabolism in live *Caenorhabditis elegans* using fingerprint vibrations



Imaging using fingerprint vibrations

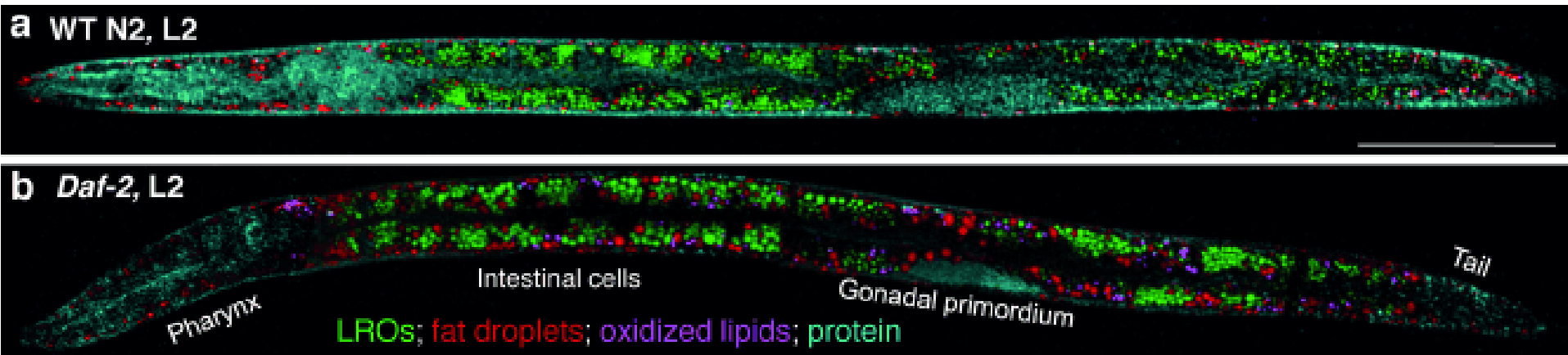
a WT N2, L2



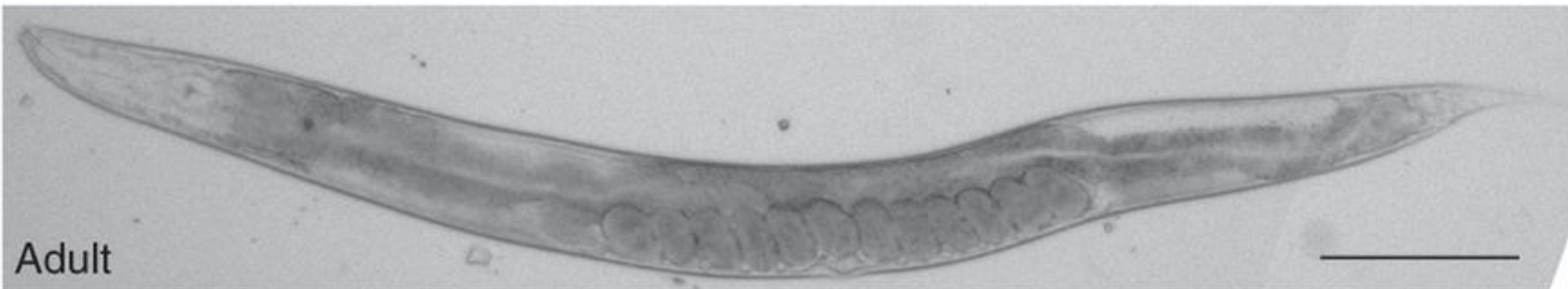
b *Daf-2*, L2



Imaging using fingerprint vibrations



When I look at *c. elegans*, I see this



ROBERT THOM



Von Leeuwenhoek Microscope (circa Late 1600s)

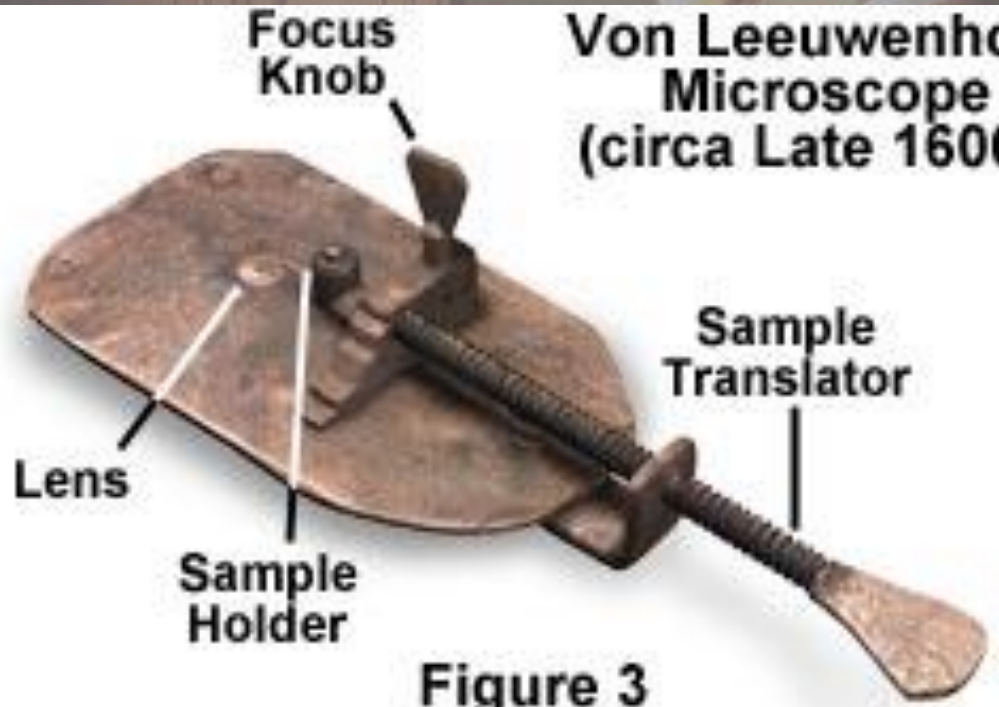


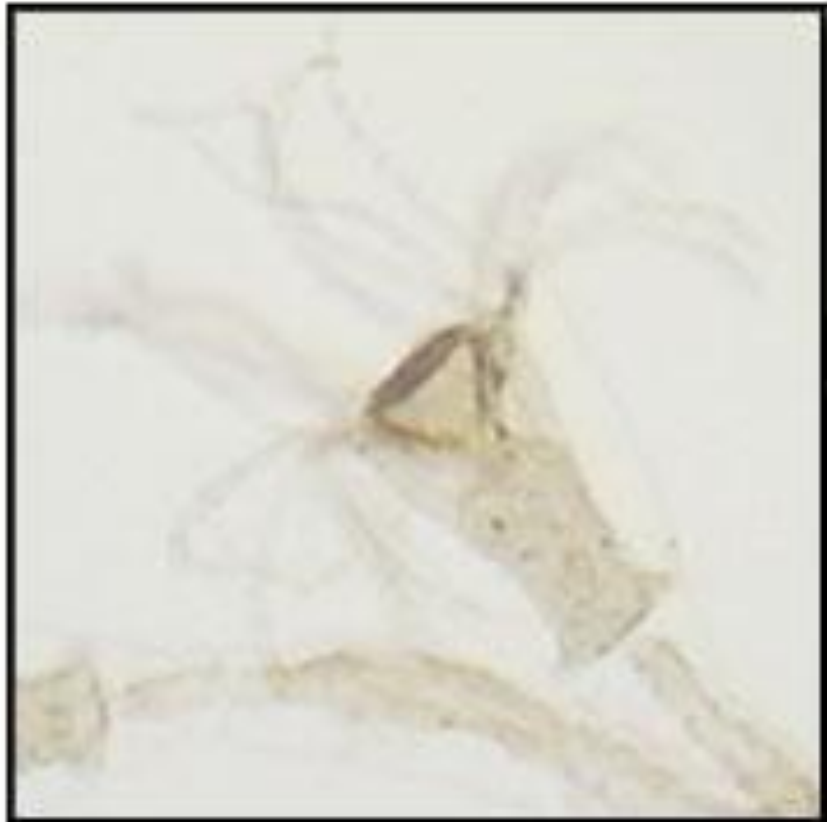
Figure 3

Objectives of the lecture

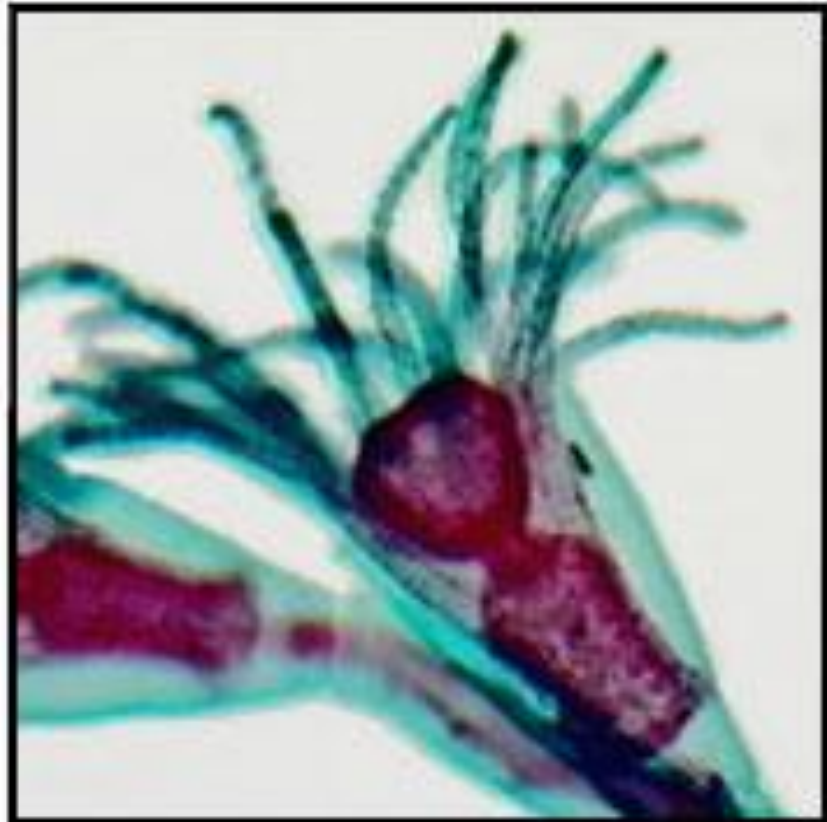
After the lecture I hope that you will be able to:

- explain how different light-molecule interactions can be used in microscopy and what information about molecule can be extracted using energy diagram of the molecule .
- Make a sketch of spontaneous, stimulated and coherent anti-Stokes Raman scattering using energy diagram of the molecule and explain how Raman spectra are measured.
- Compare labeling microscopy with coherent Raman scattering microscopy in terms of chemical specificity, resolution and invasiveness of the method.

Unstained and Stained Specimens in Brightfield Illumination



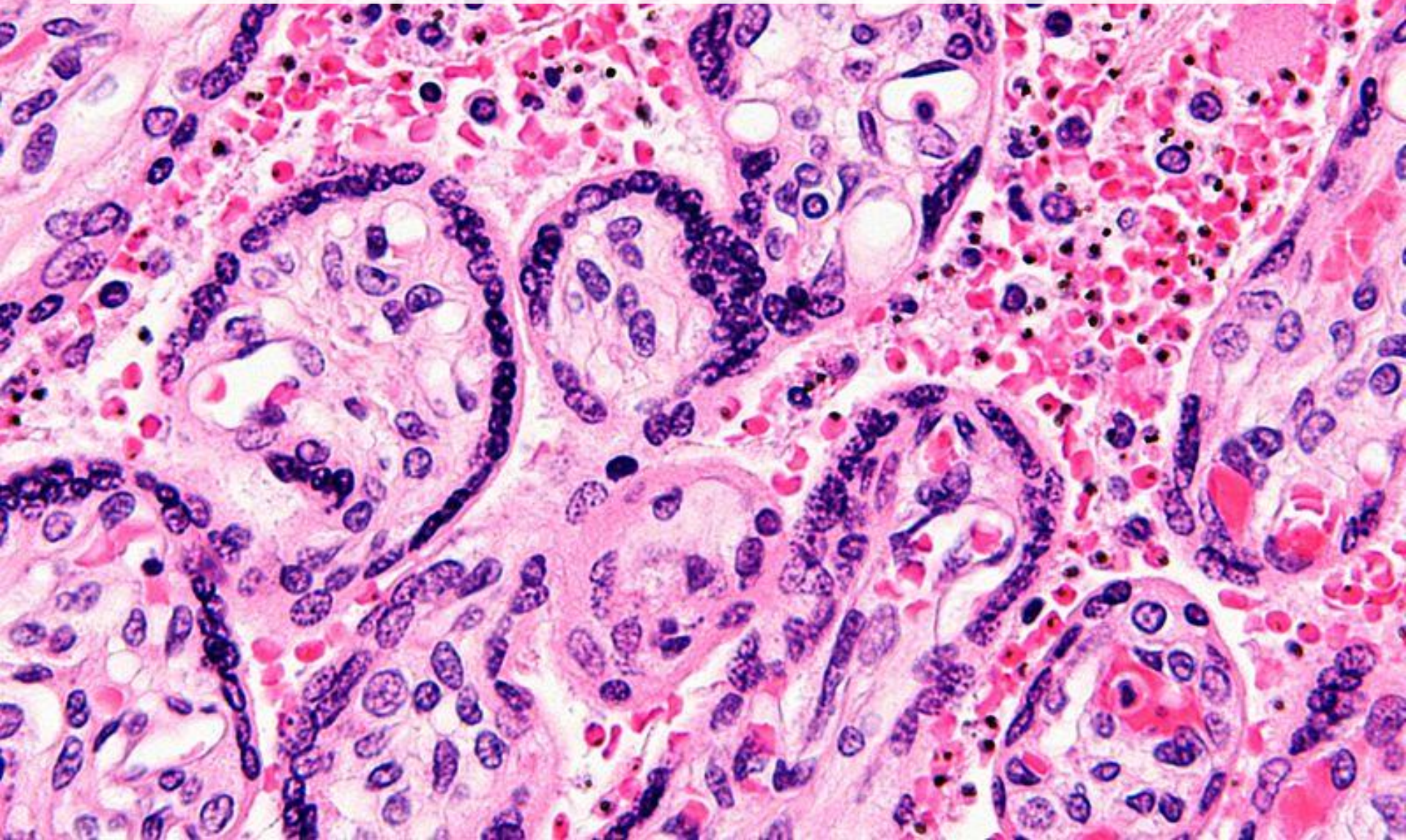
(a)



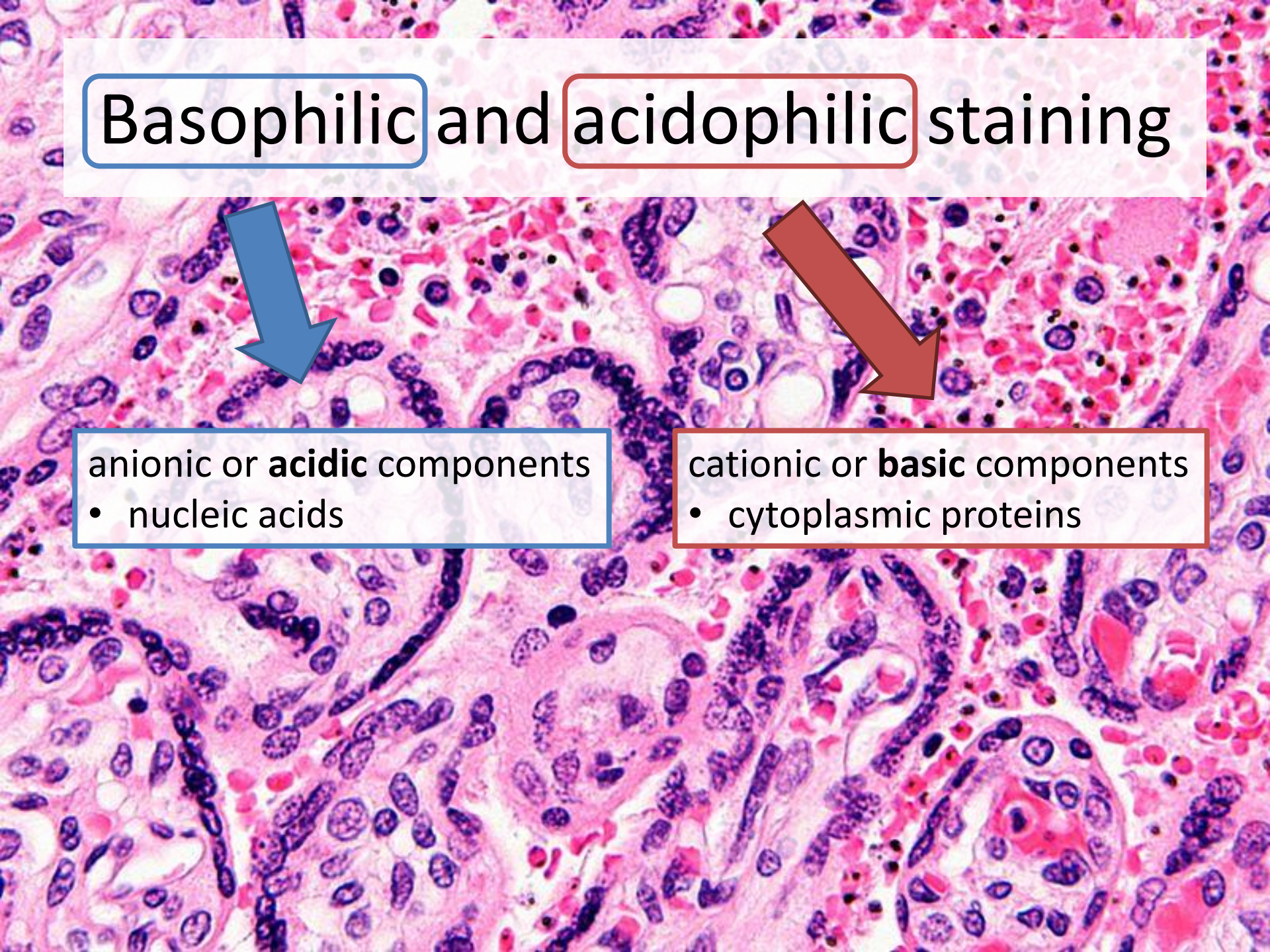
(b)

Figure 2

Basophilic and acidophilic staining



Basophilic and acidophilic staining



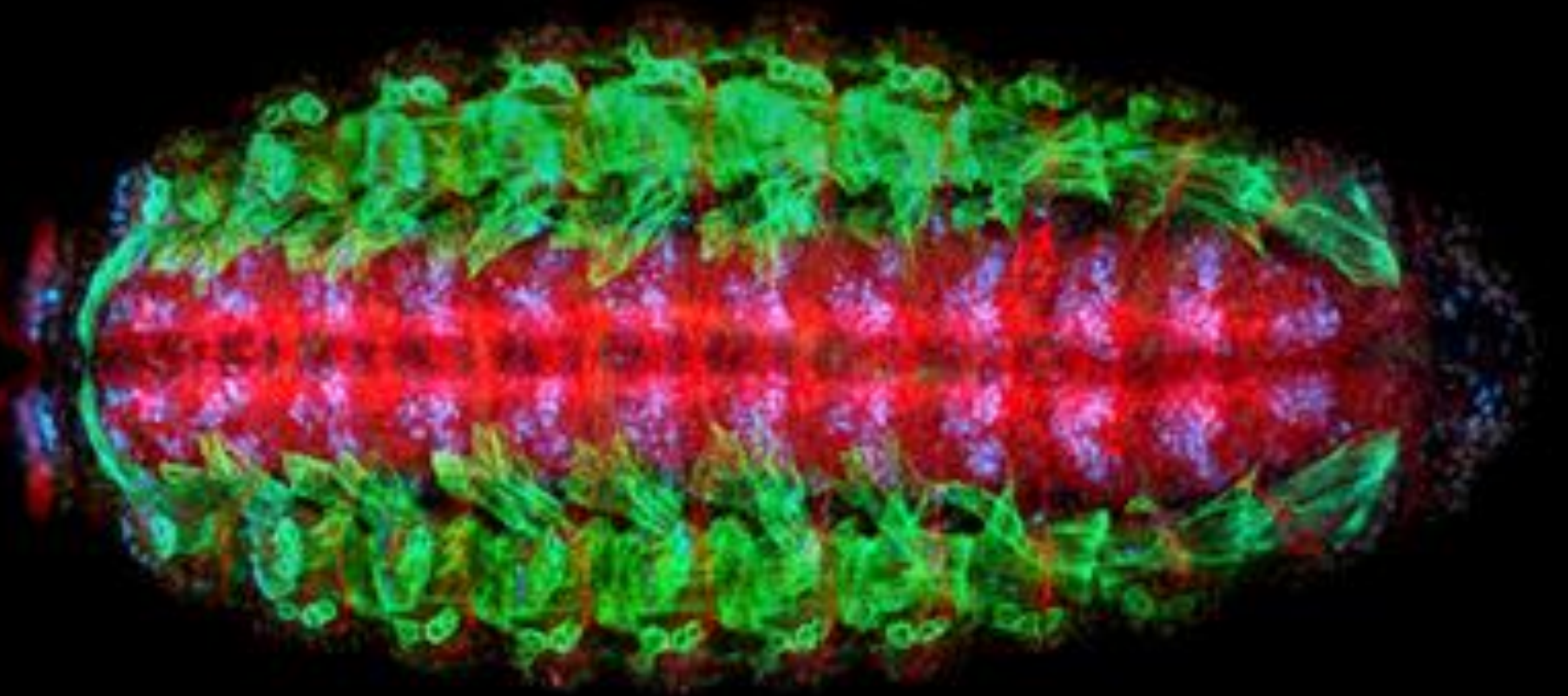
anionic or **acidic** components

- nucleic acids

cationic or **basic** components

- cytoplasmic proteins

Immunohistochemistry

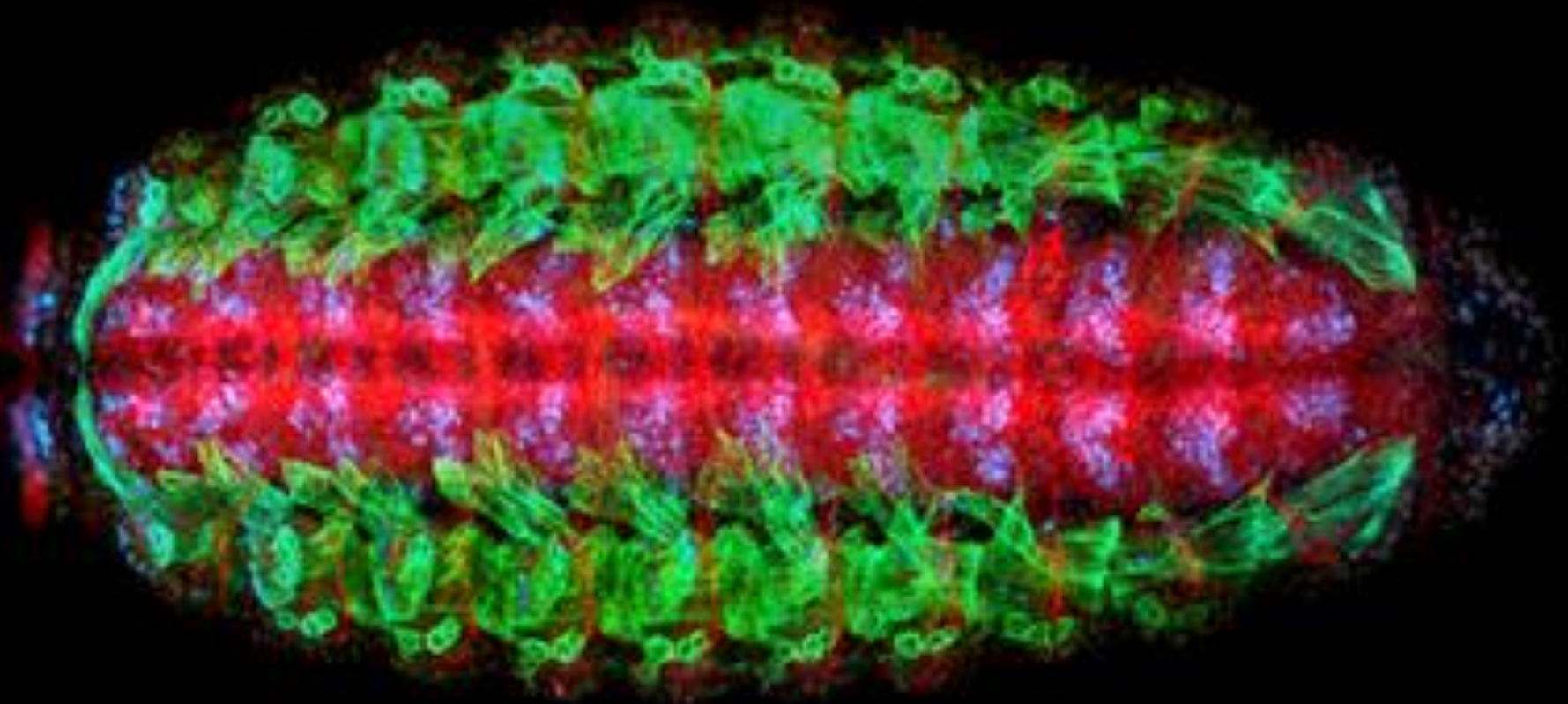


Immunohistochemistry

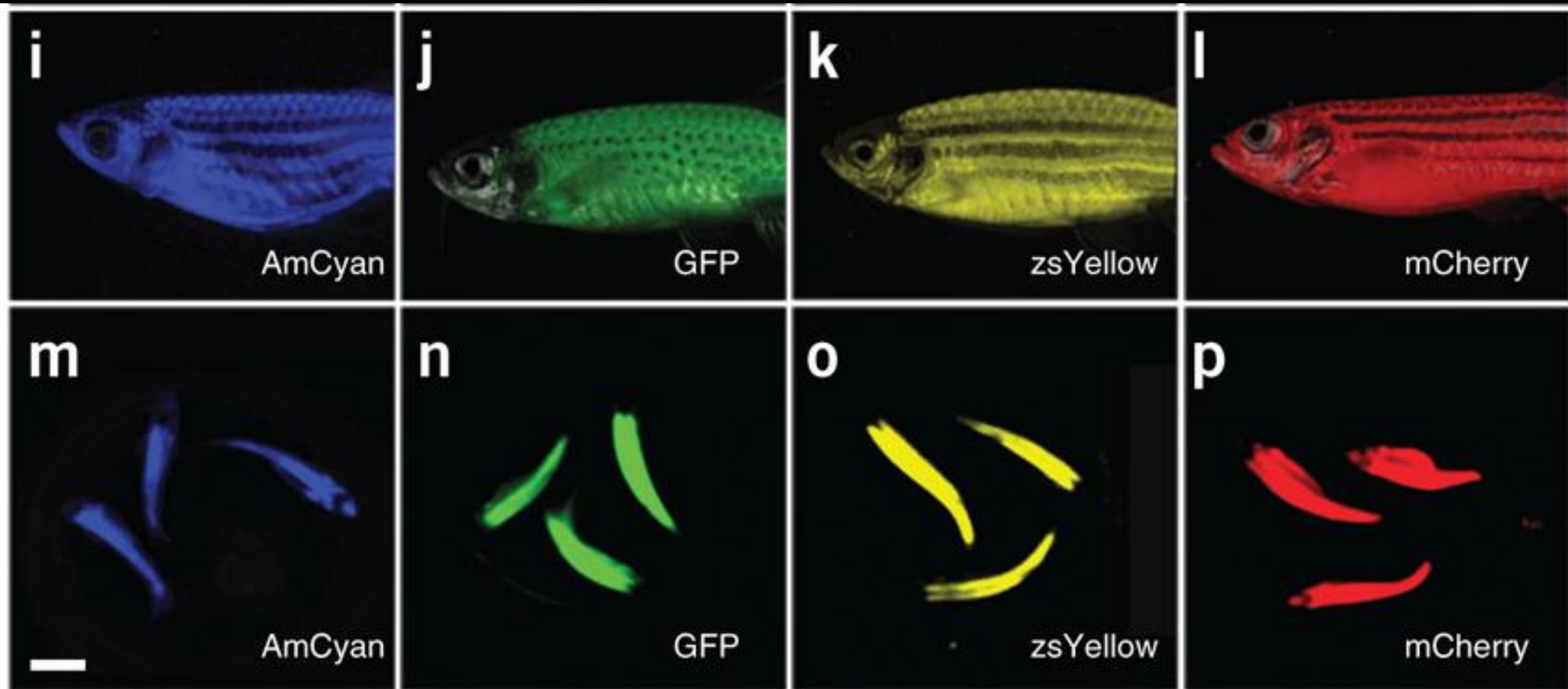
Targeting antigens

with specific antibodies

tagged with a visible label

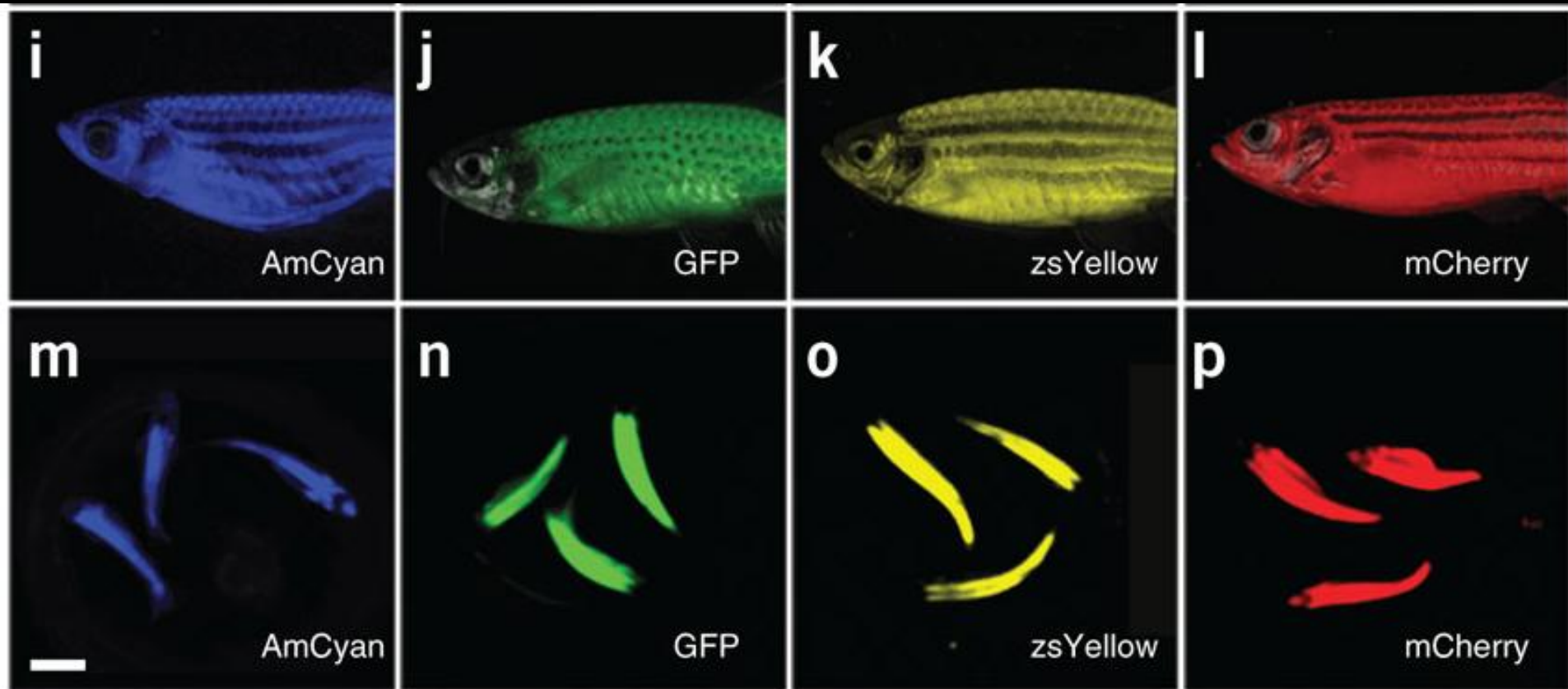



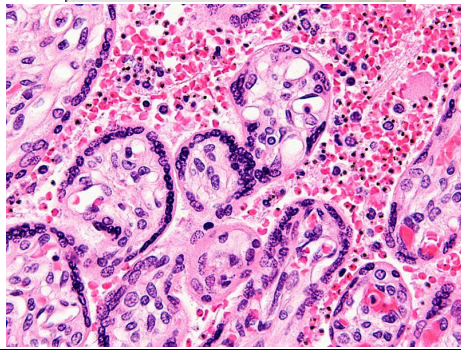
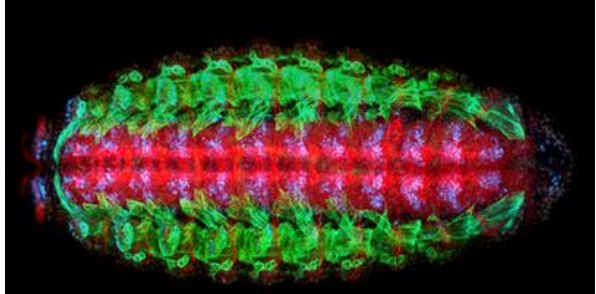
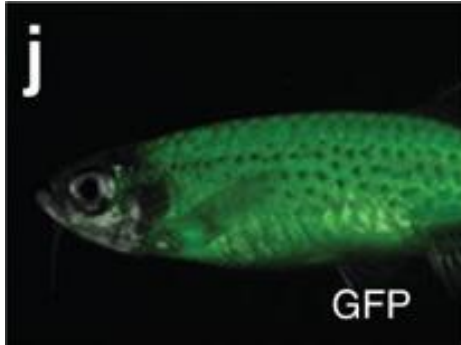
Fluorescent protein labeling



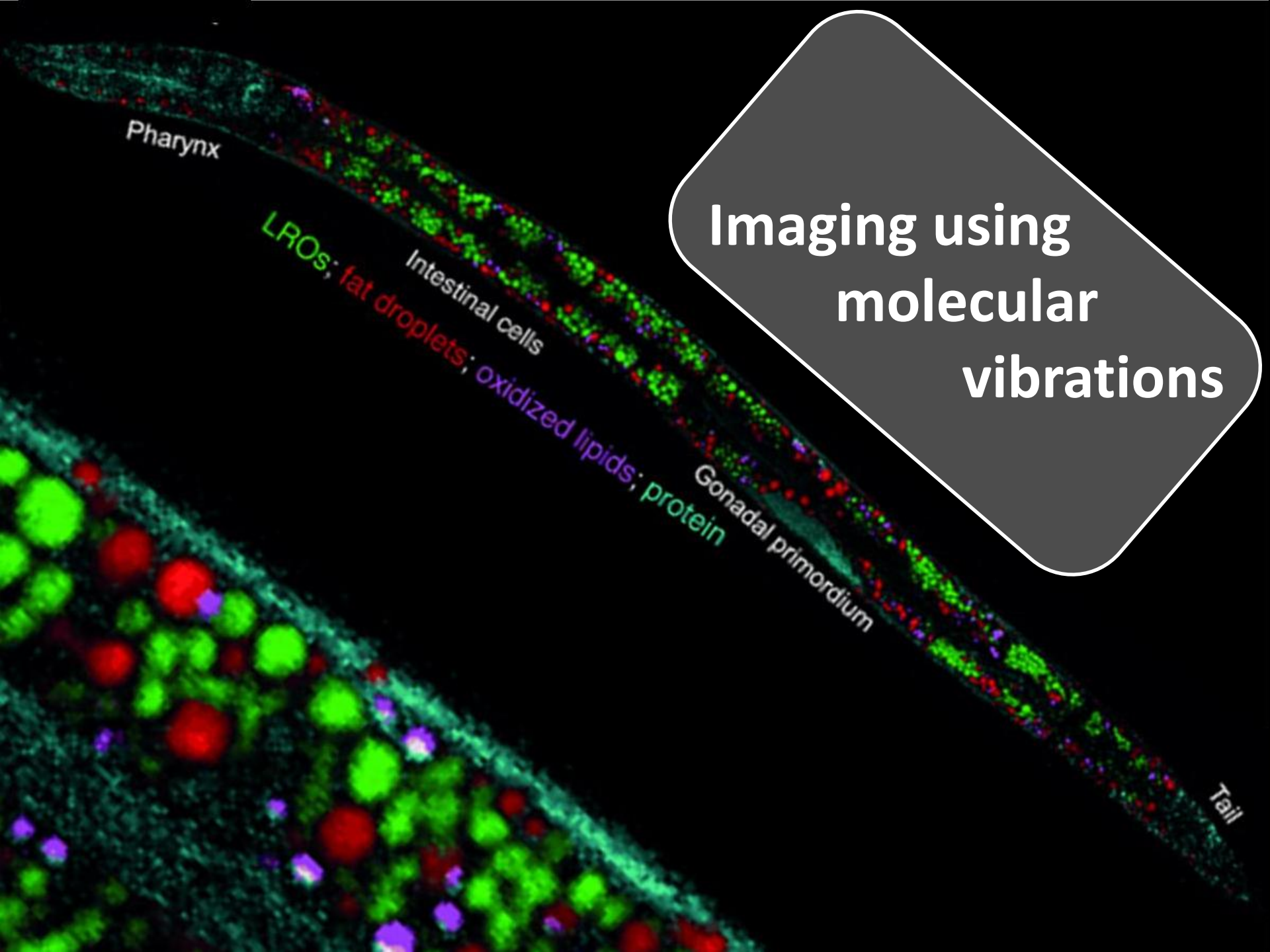
Fluorescent protein labeling

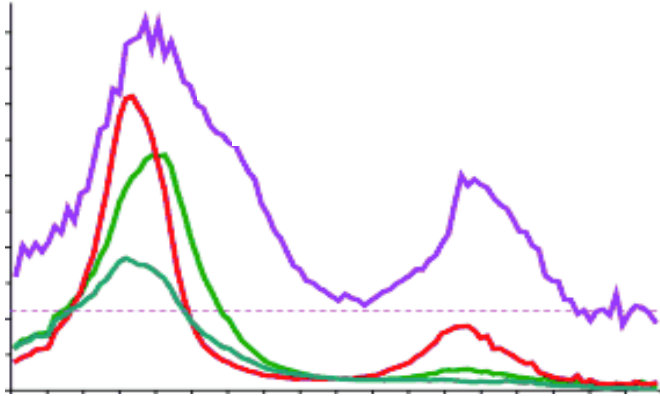
Genetically modifying original protein to include a sequence of fluorescent protein



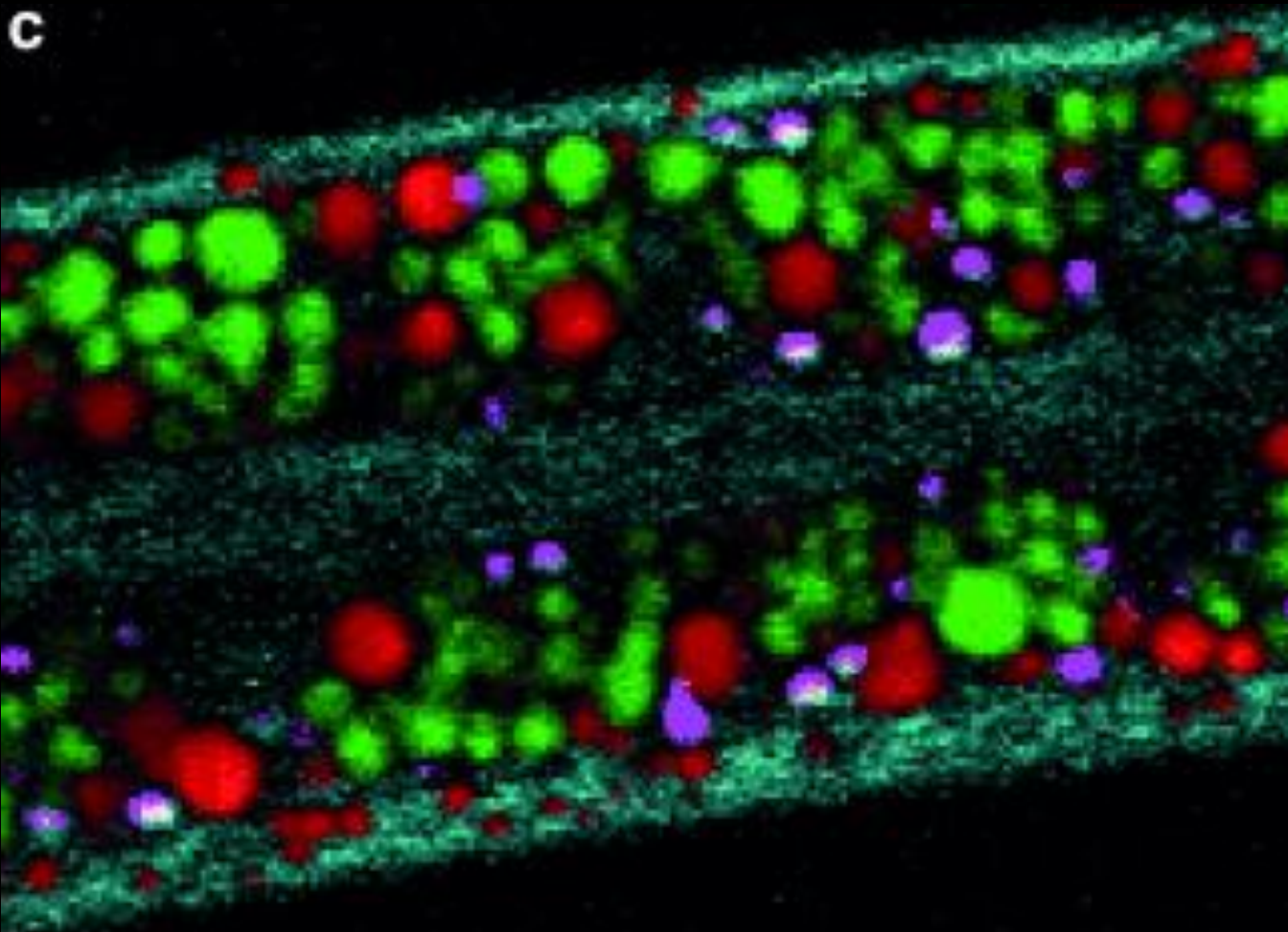
Labeling	Selectivity	Sample image
No labeling	No selectivity/ Natural pigments	
Basophilic and acidophilic staining	Acidic/Basic components	
Immuno-histochemistry	What antibodies bind	
Fluorescent protein labeling	Expressed genetically modified proteins	 <p data-bbox="1400 1106 1439 1185">j</p> <p data-bbox="1709 1363 1806 1406">GFP</p>

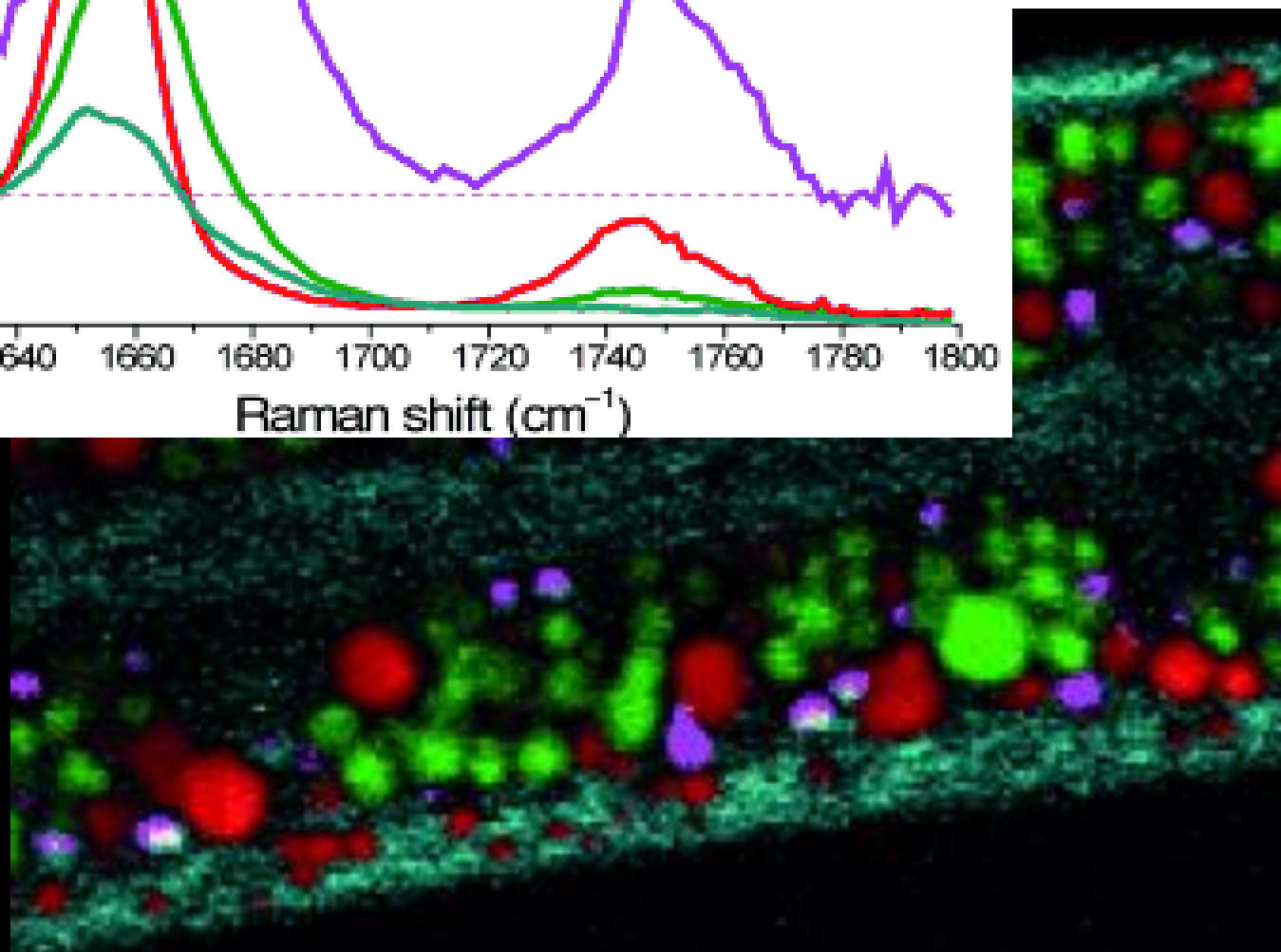
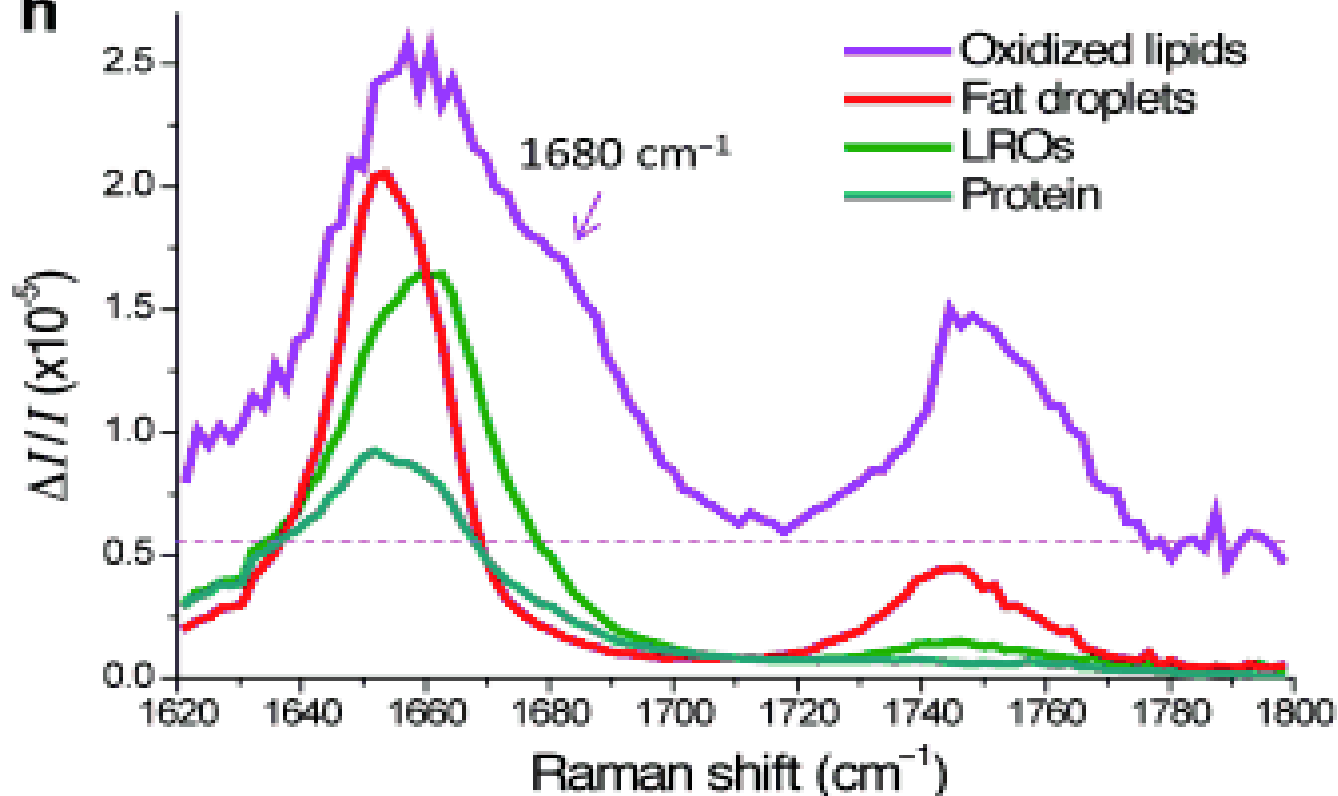
Imaging using molecular vibrations

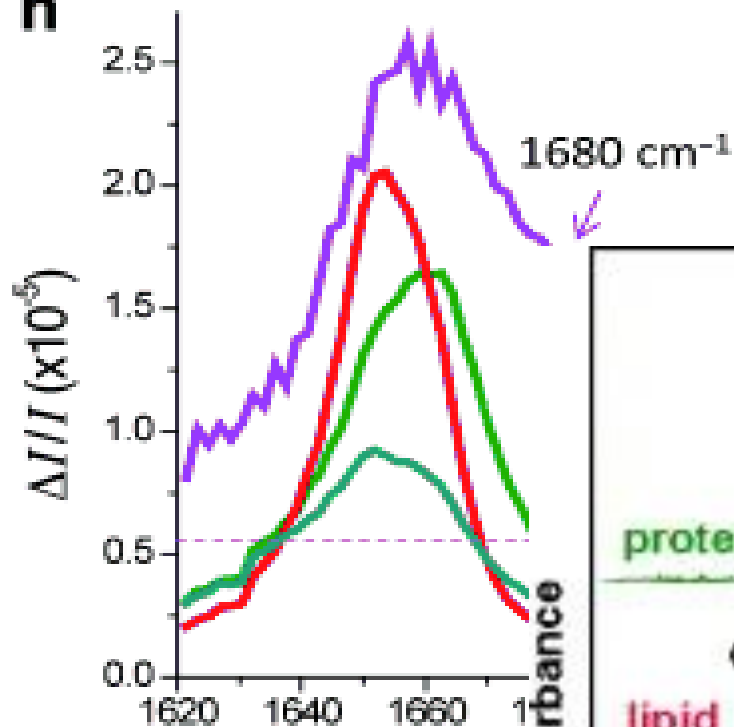




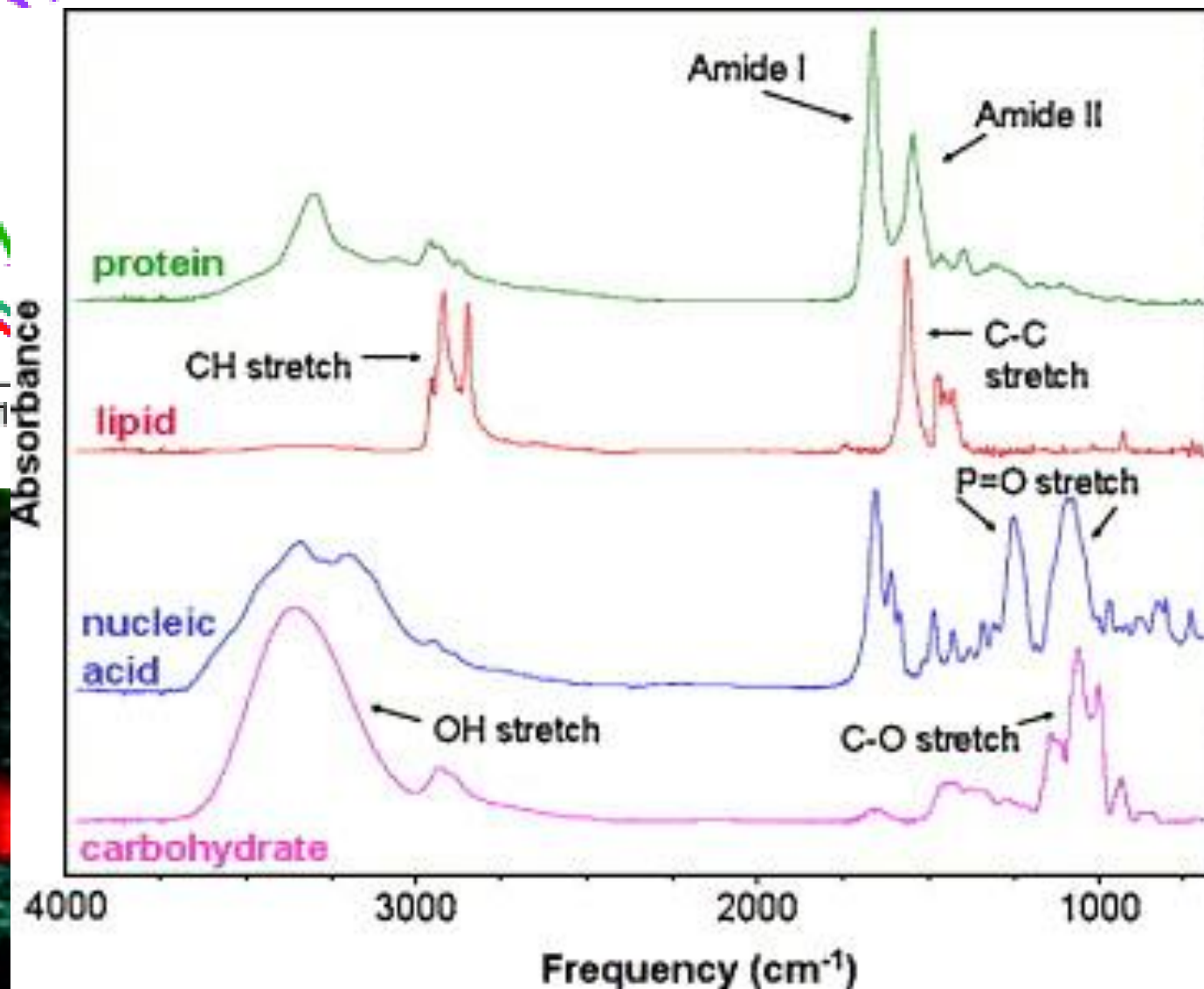
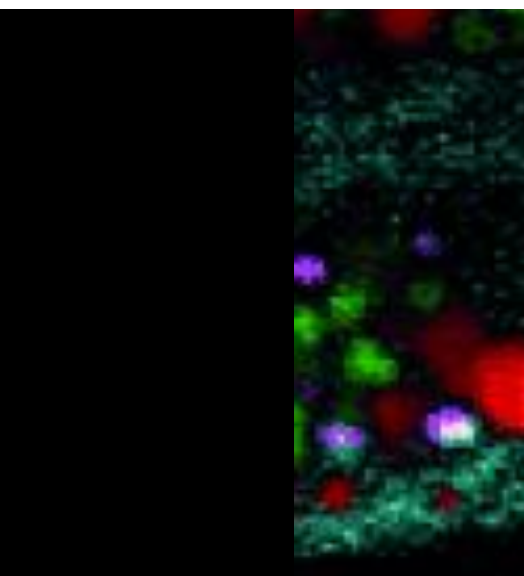
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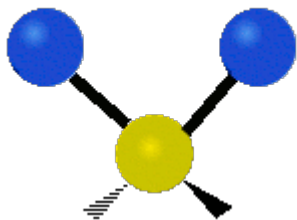
B

5

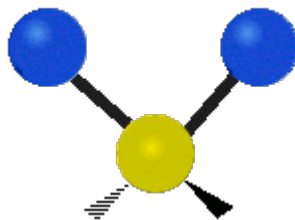
- Oxidized lipids
- Fat droplets
- LROs
- Protein



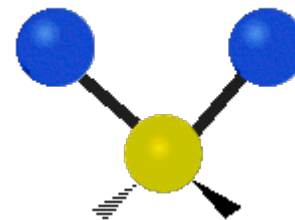
Vibrations of a methylene group (-CH₂-)



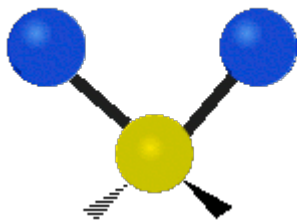
Symmetrical
stretching



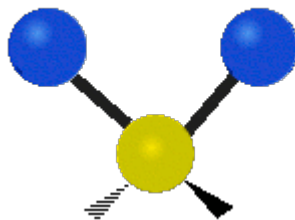
Asymmetrical
stretching



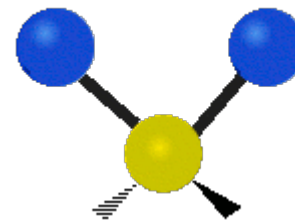
Scissoring
(Bending)



Rocking

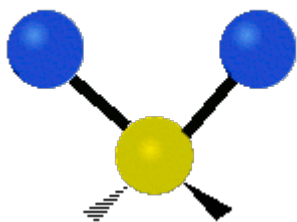


Wagging

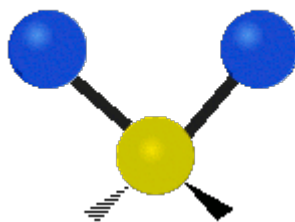


Twisting

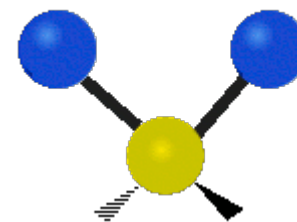
Vibrations of a methylene group (-CH₂-)



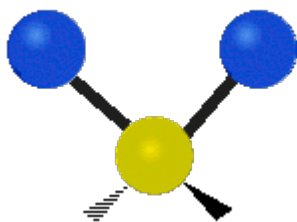
Symmetrical
stretching
~2845 cm⁻¹



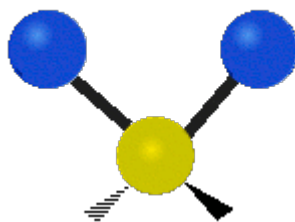
Asymmetrical
stretching
~2880 cm⁻¹



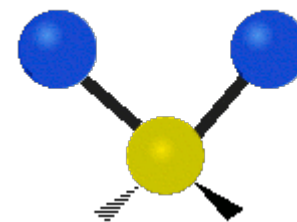
Scissoring
(Bending)



Rocking

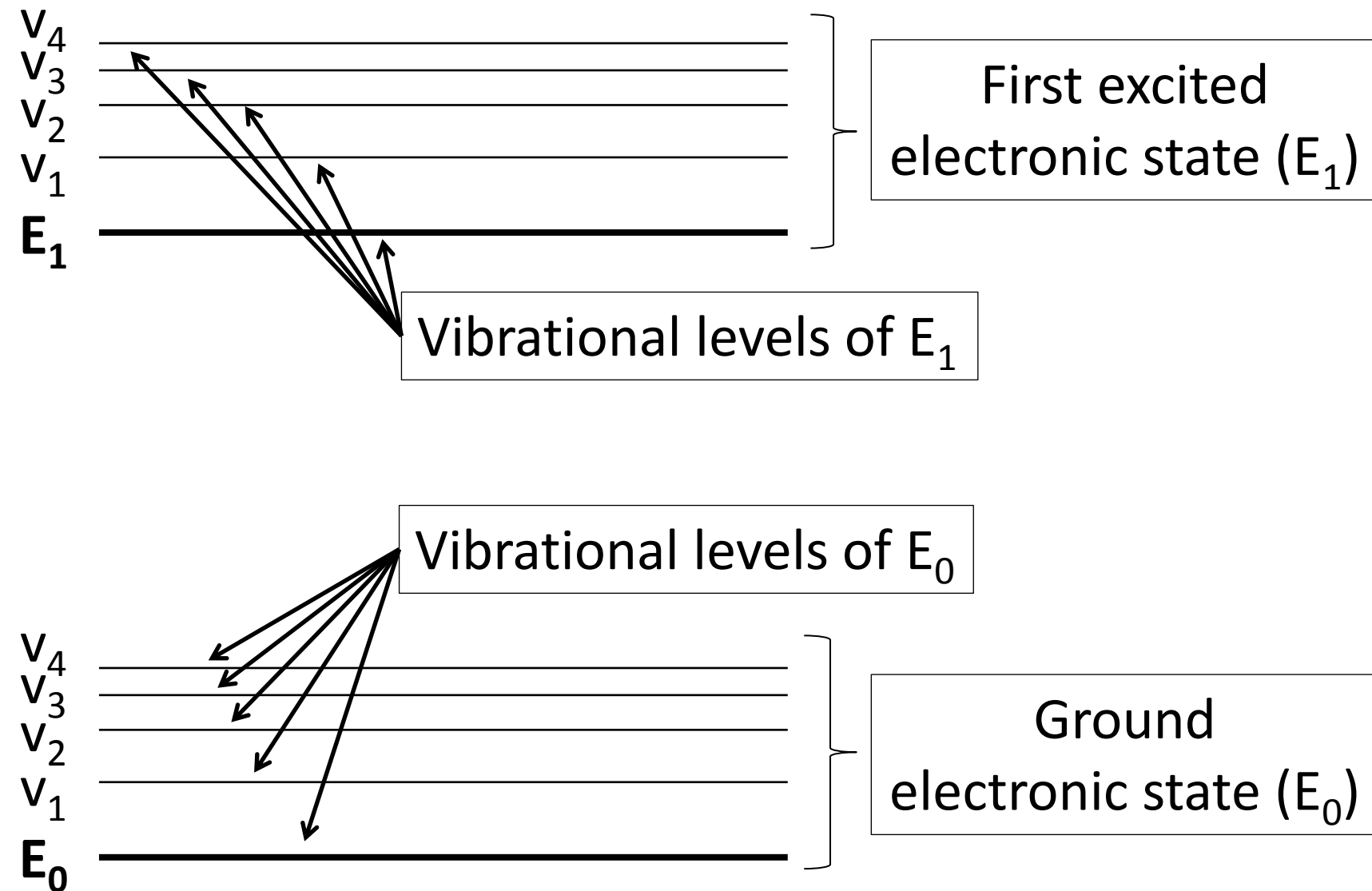


Wagging

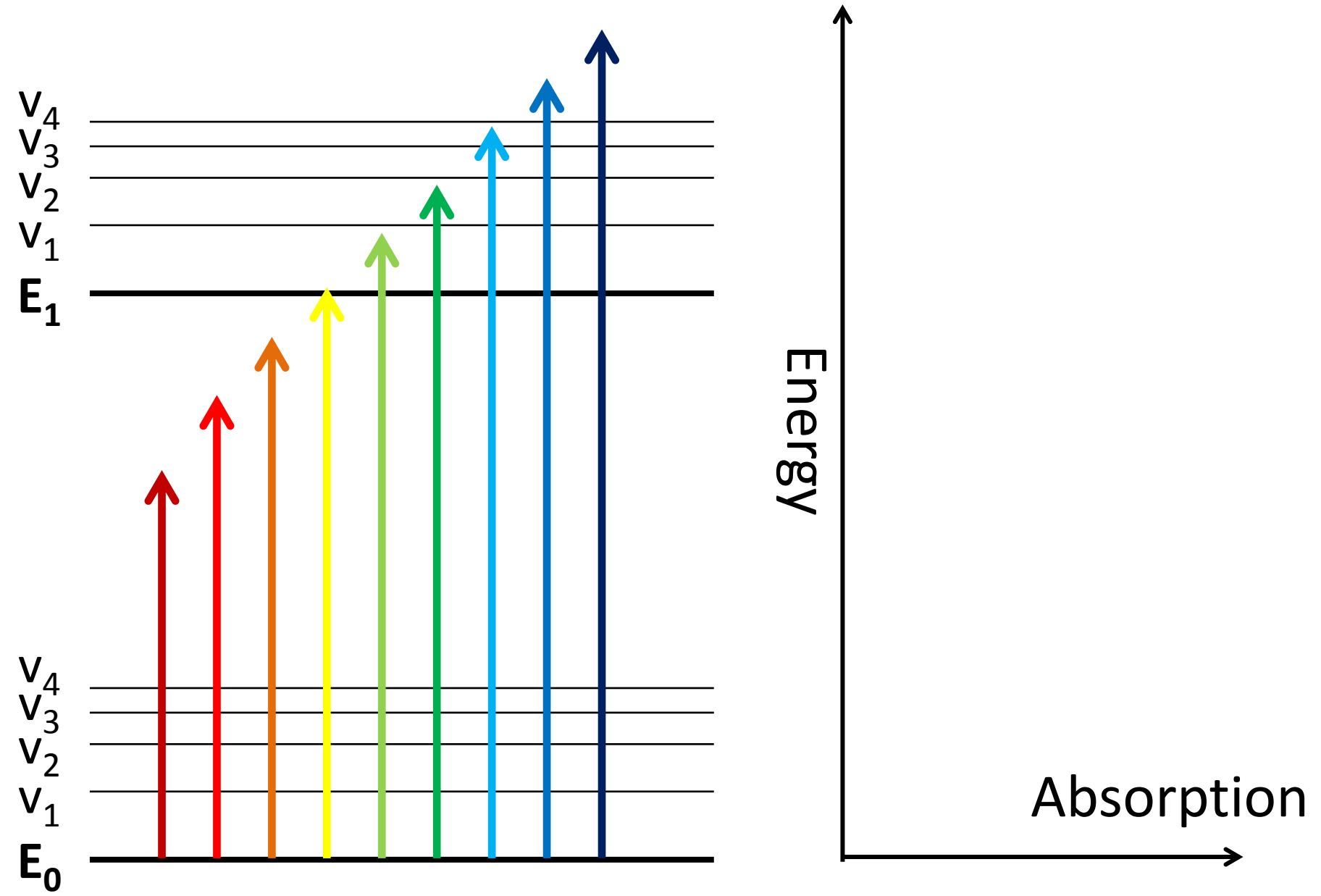


Twisting

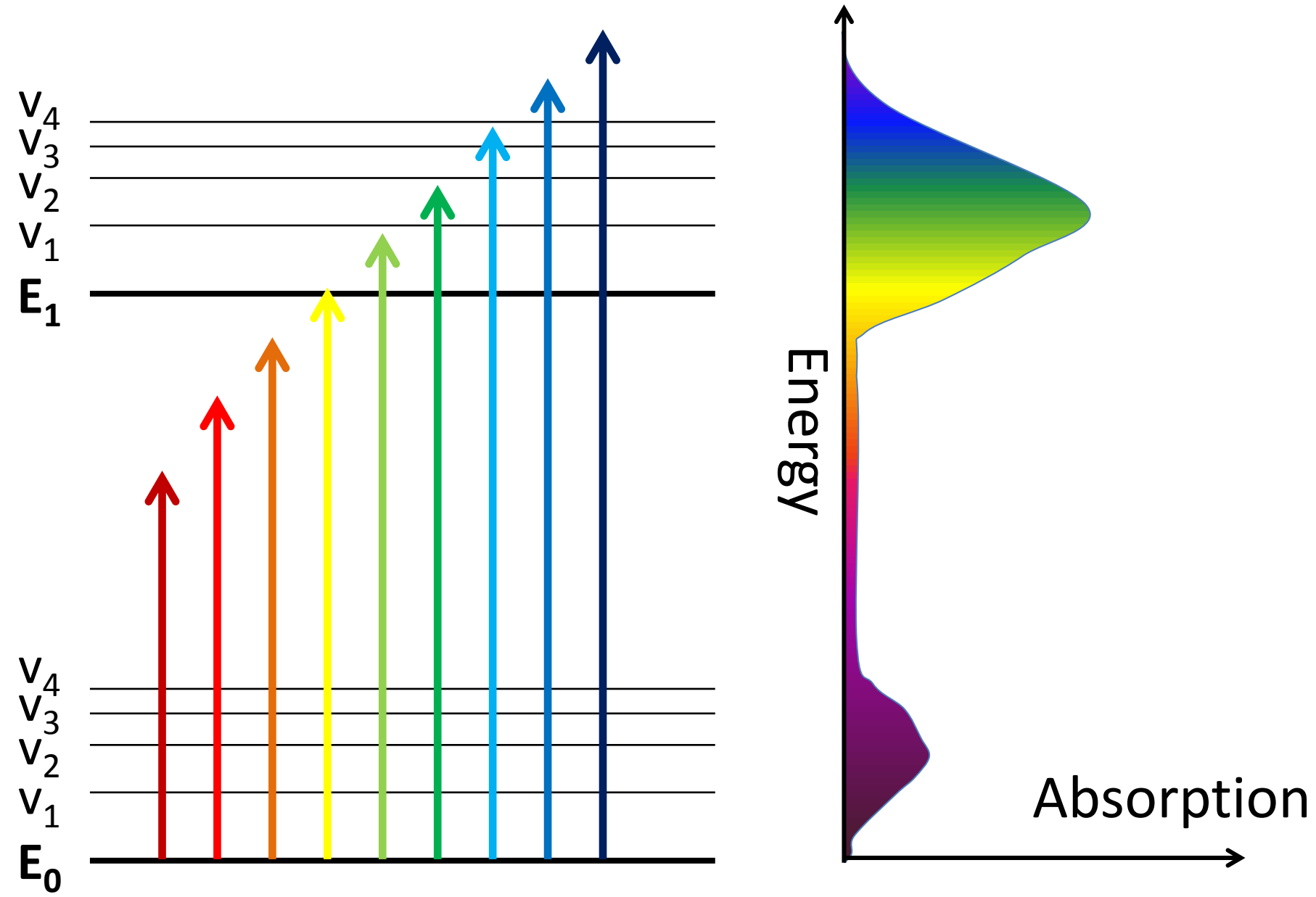
Energy levels of the molecule



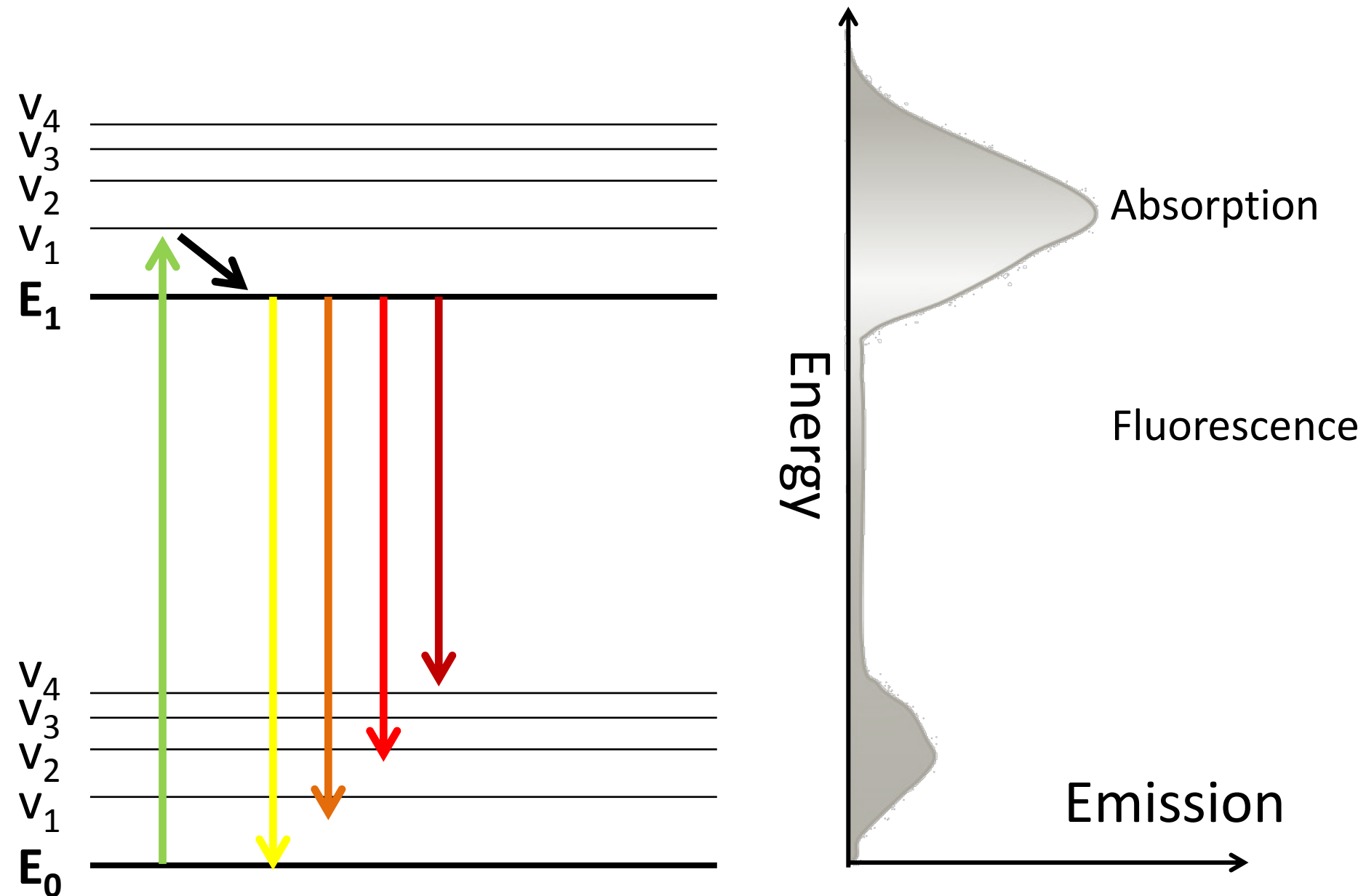
Absorption (VIS)



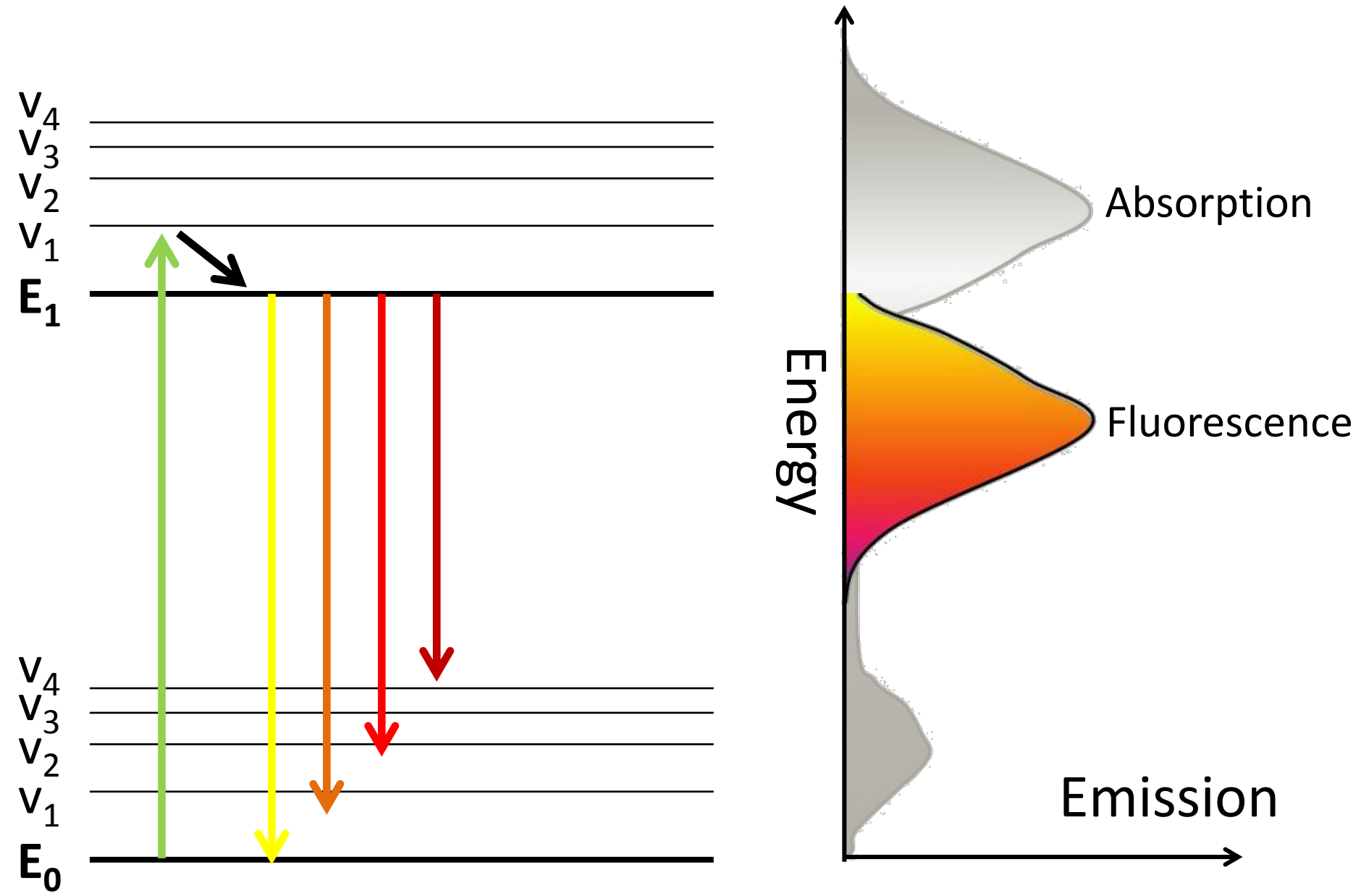
Absorption (VIS)



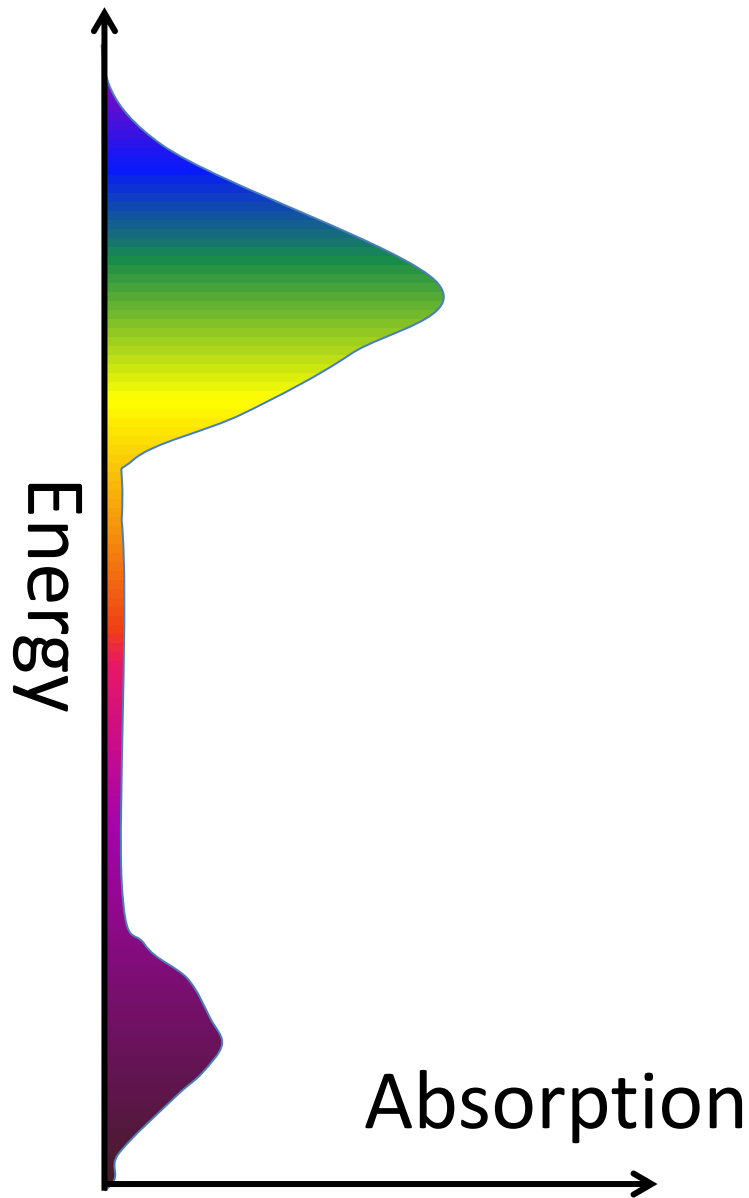
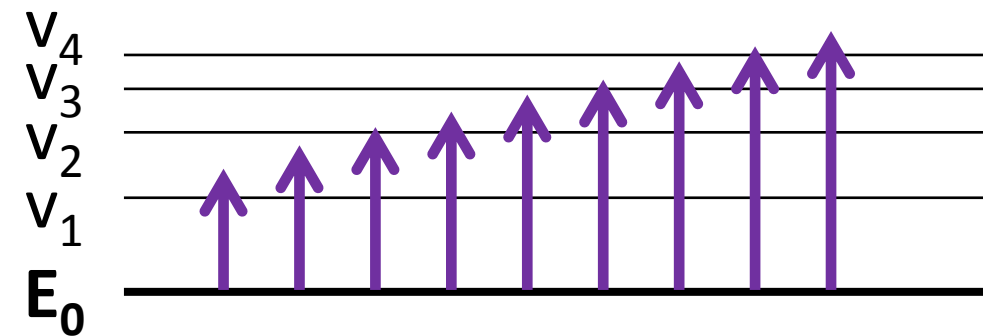
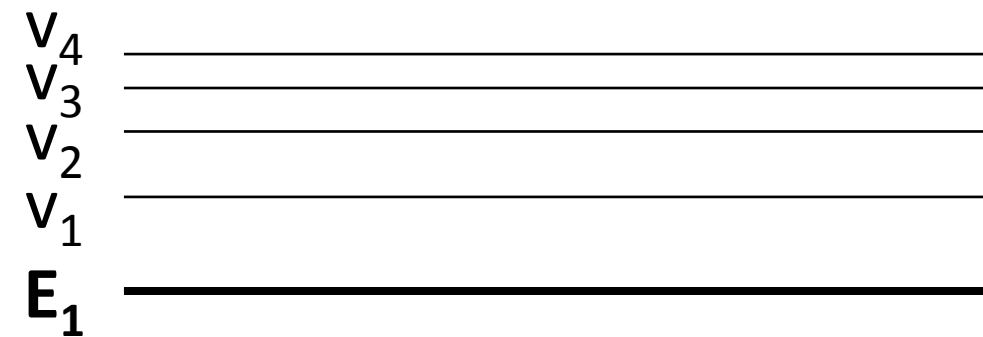
Fluorescence



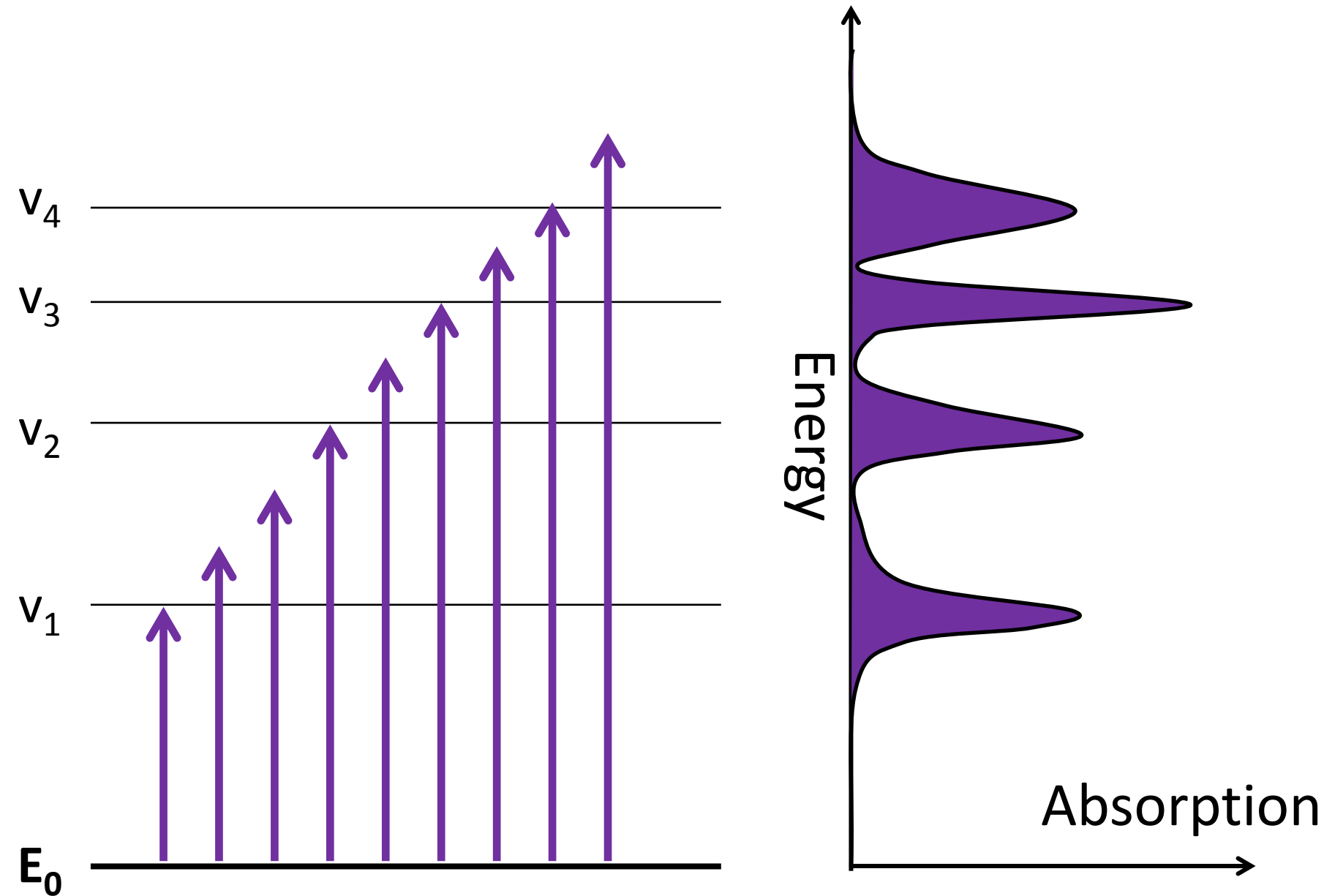
Fluorescence



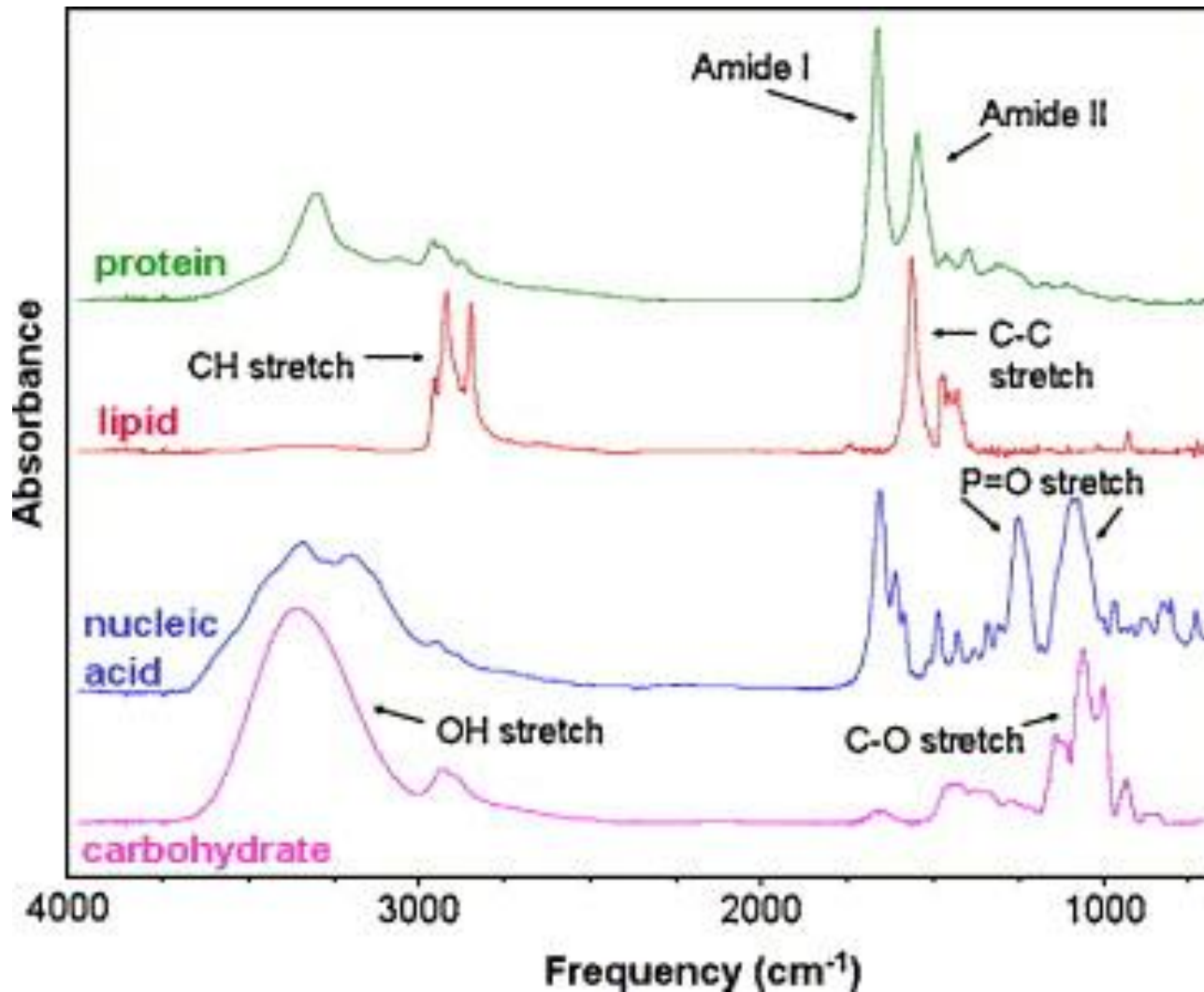
Absorption (mid-IR)



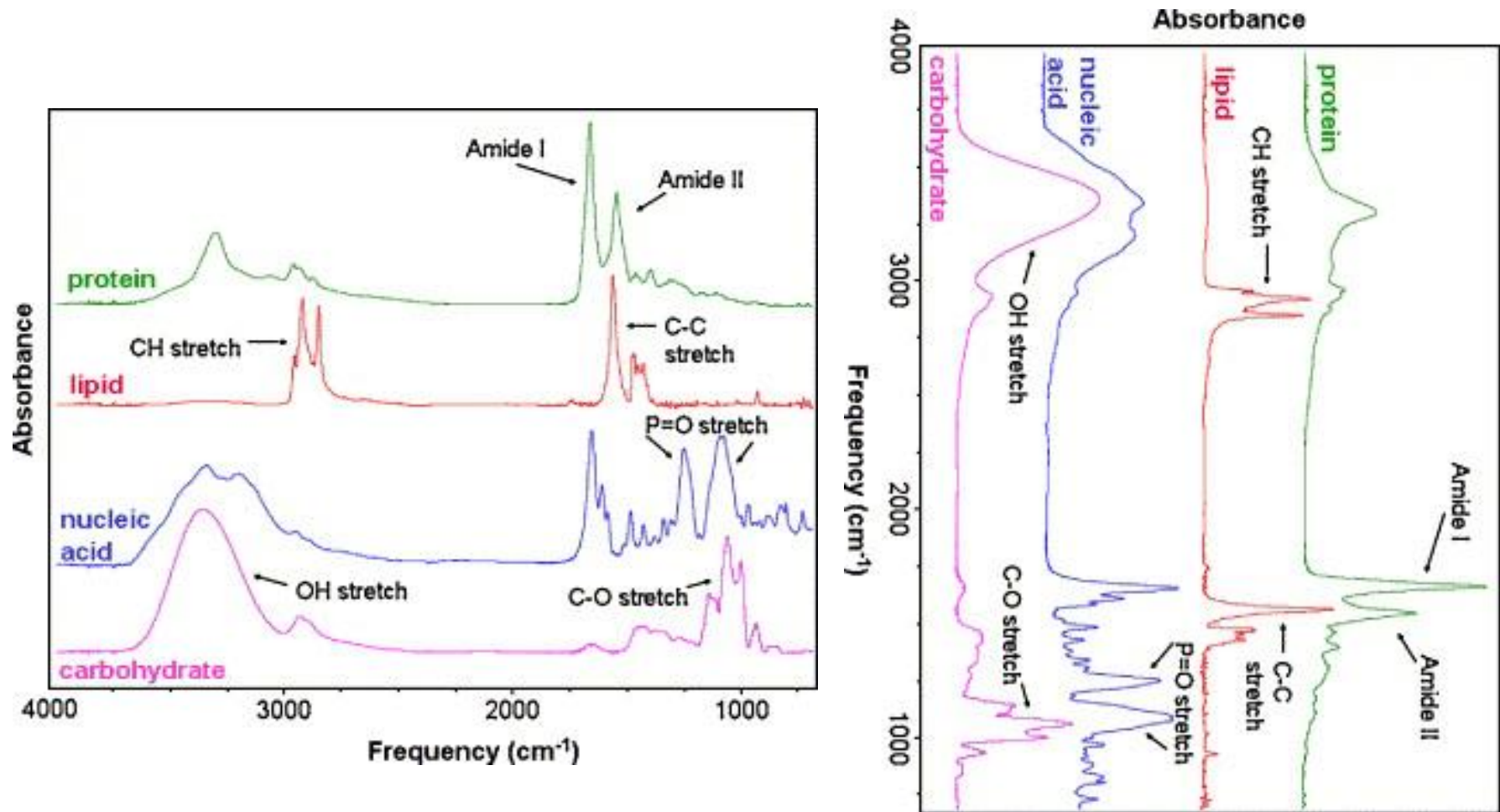
Absorption (mid-IR)



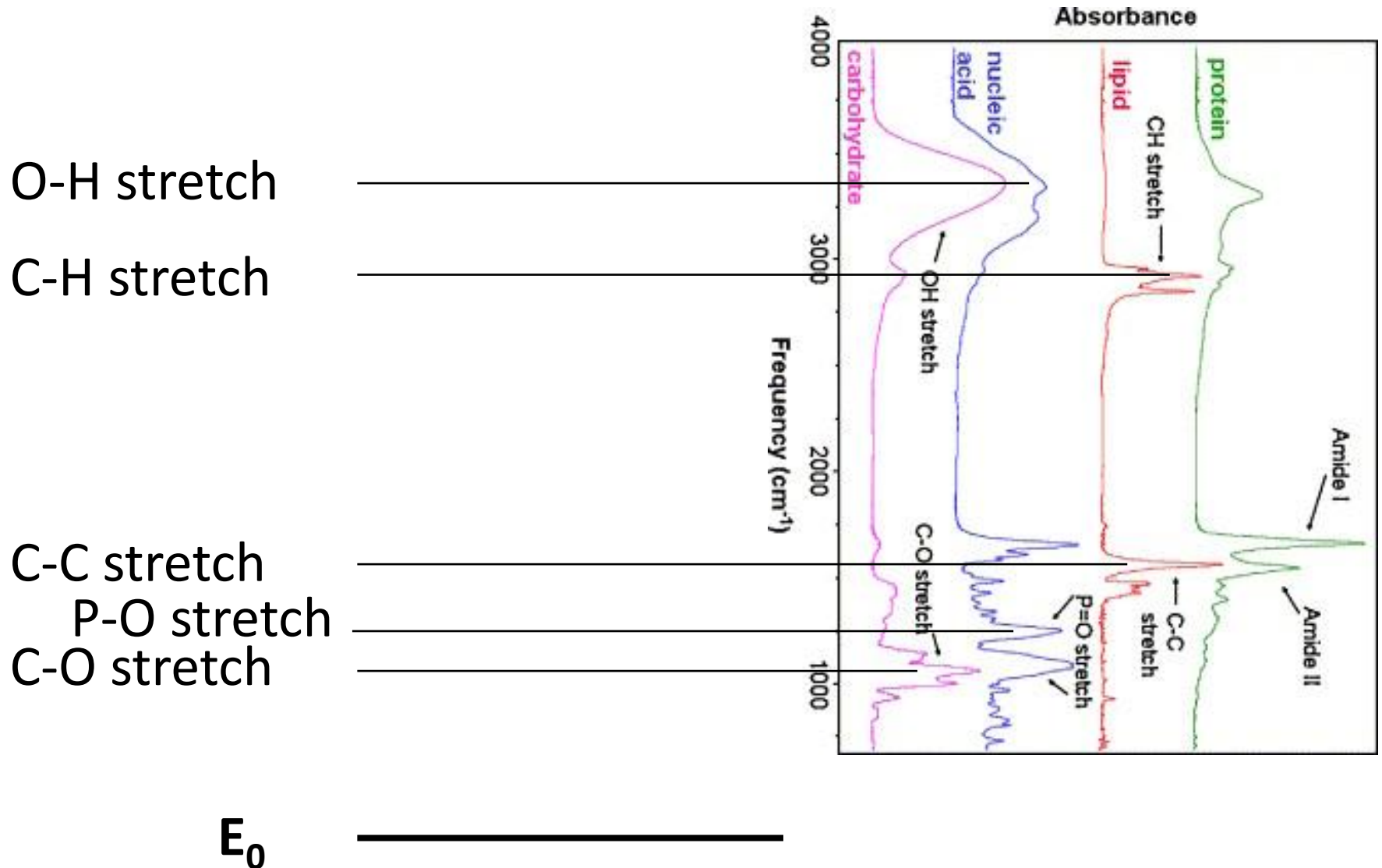
Energy levels of the molecule



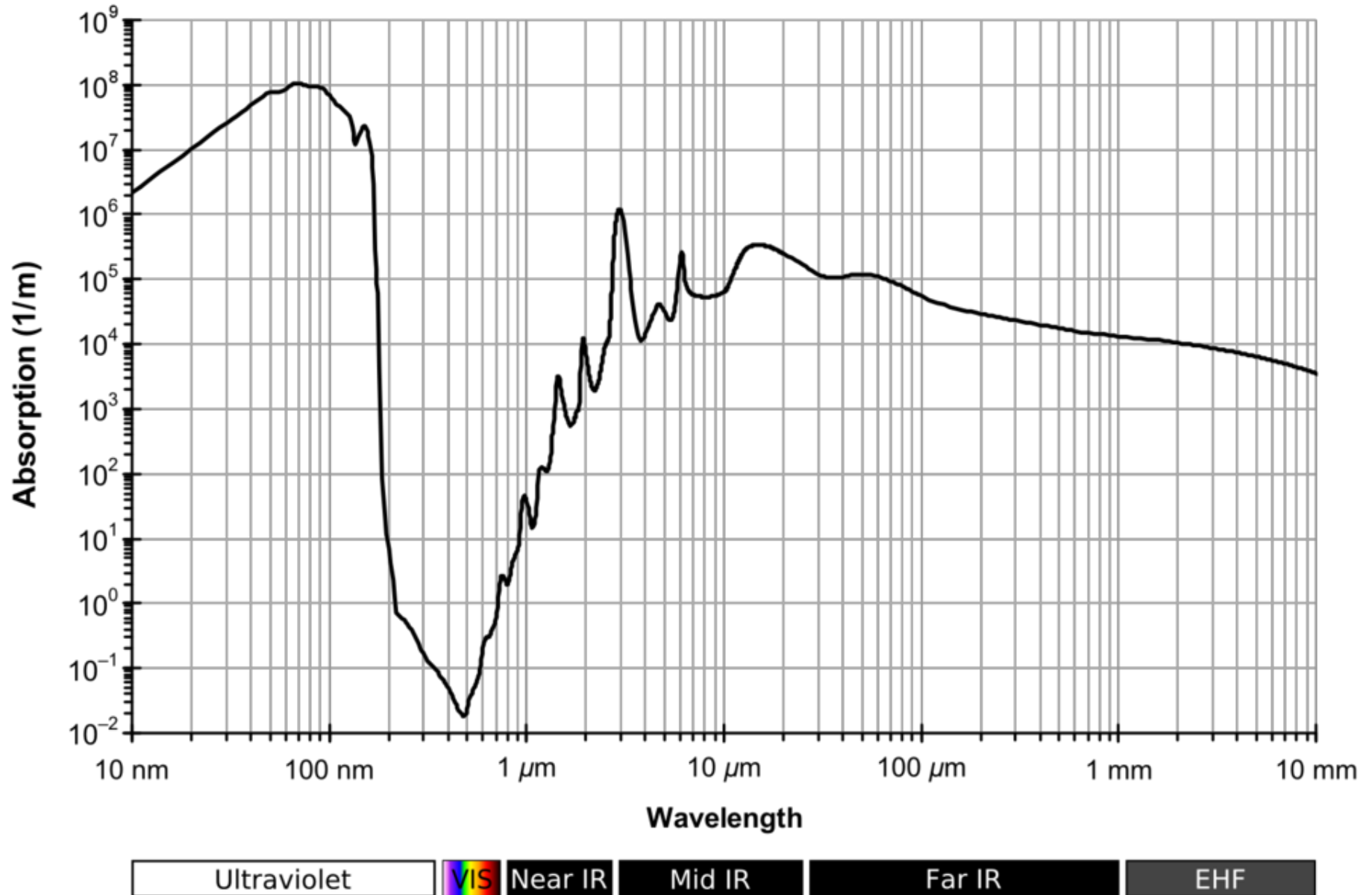
Energy levels of the molecule



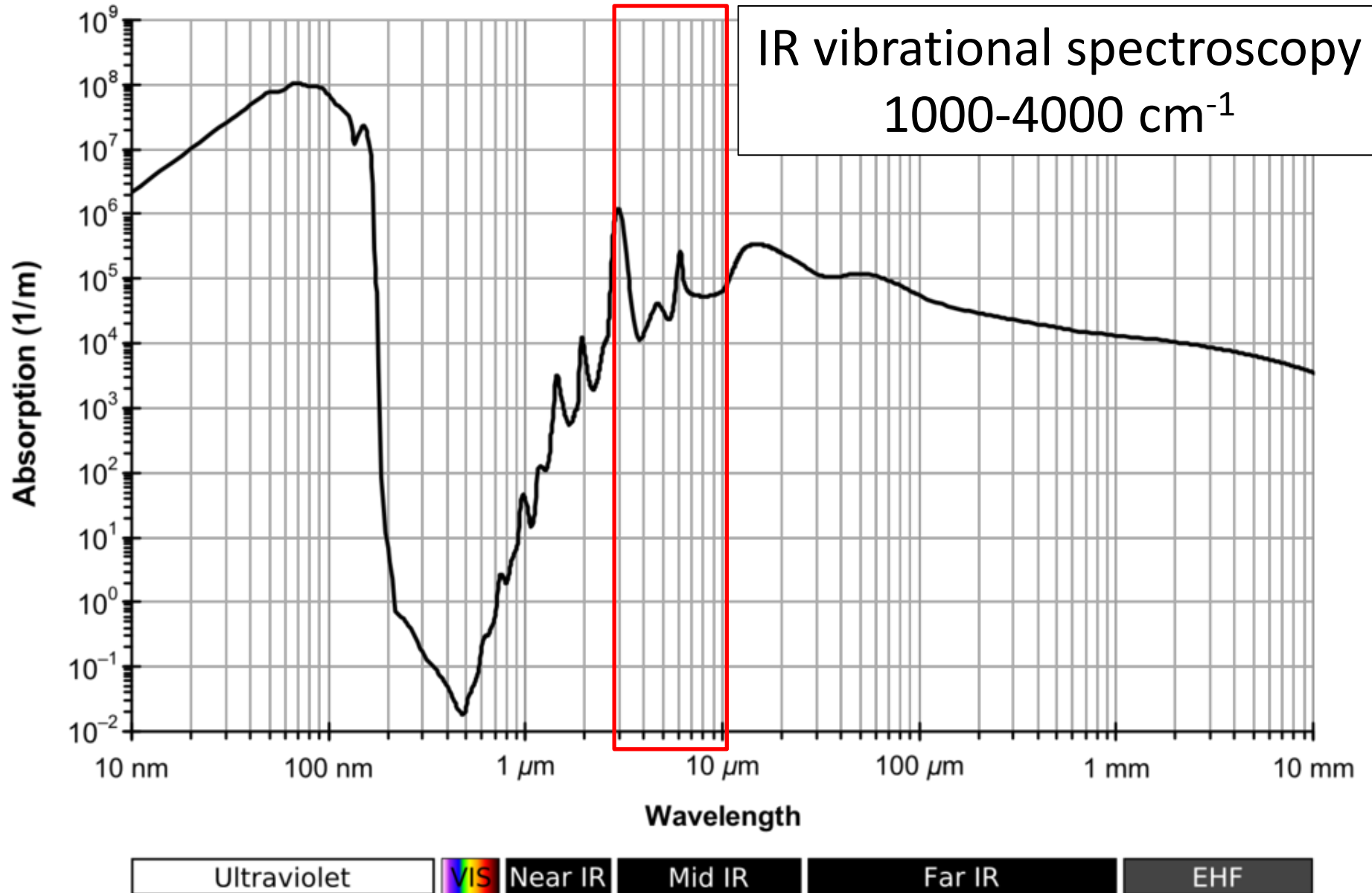
Energy levels of the molecule



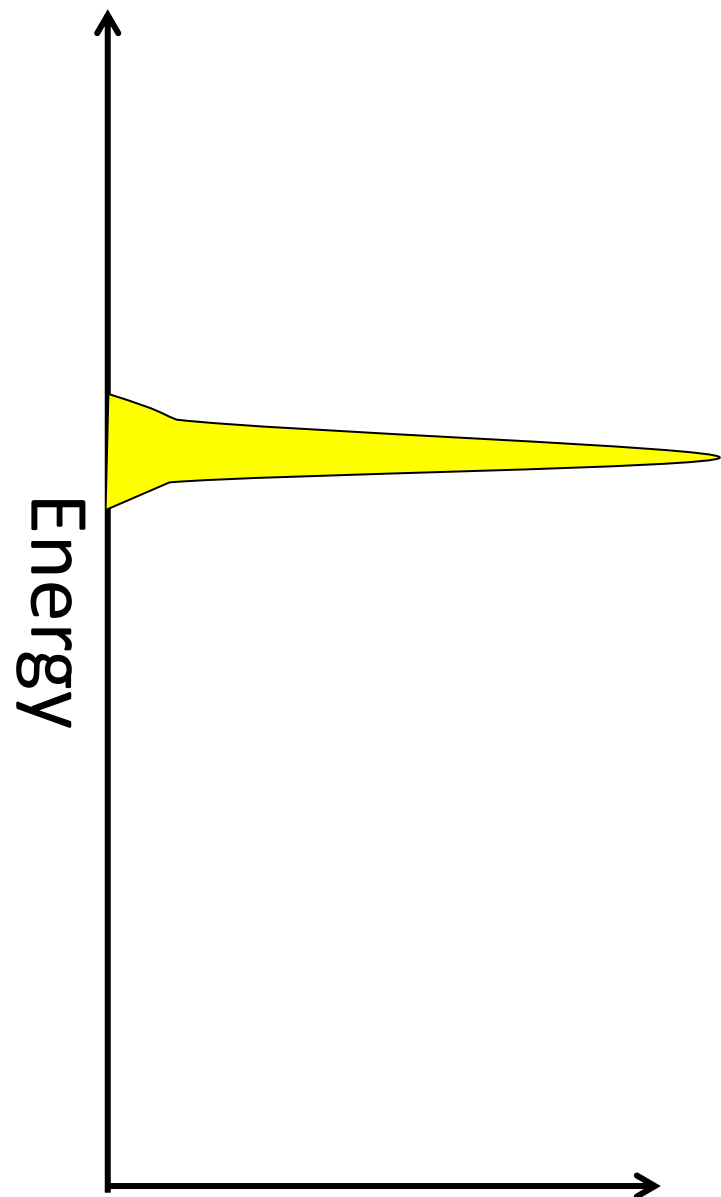
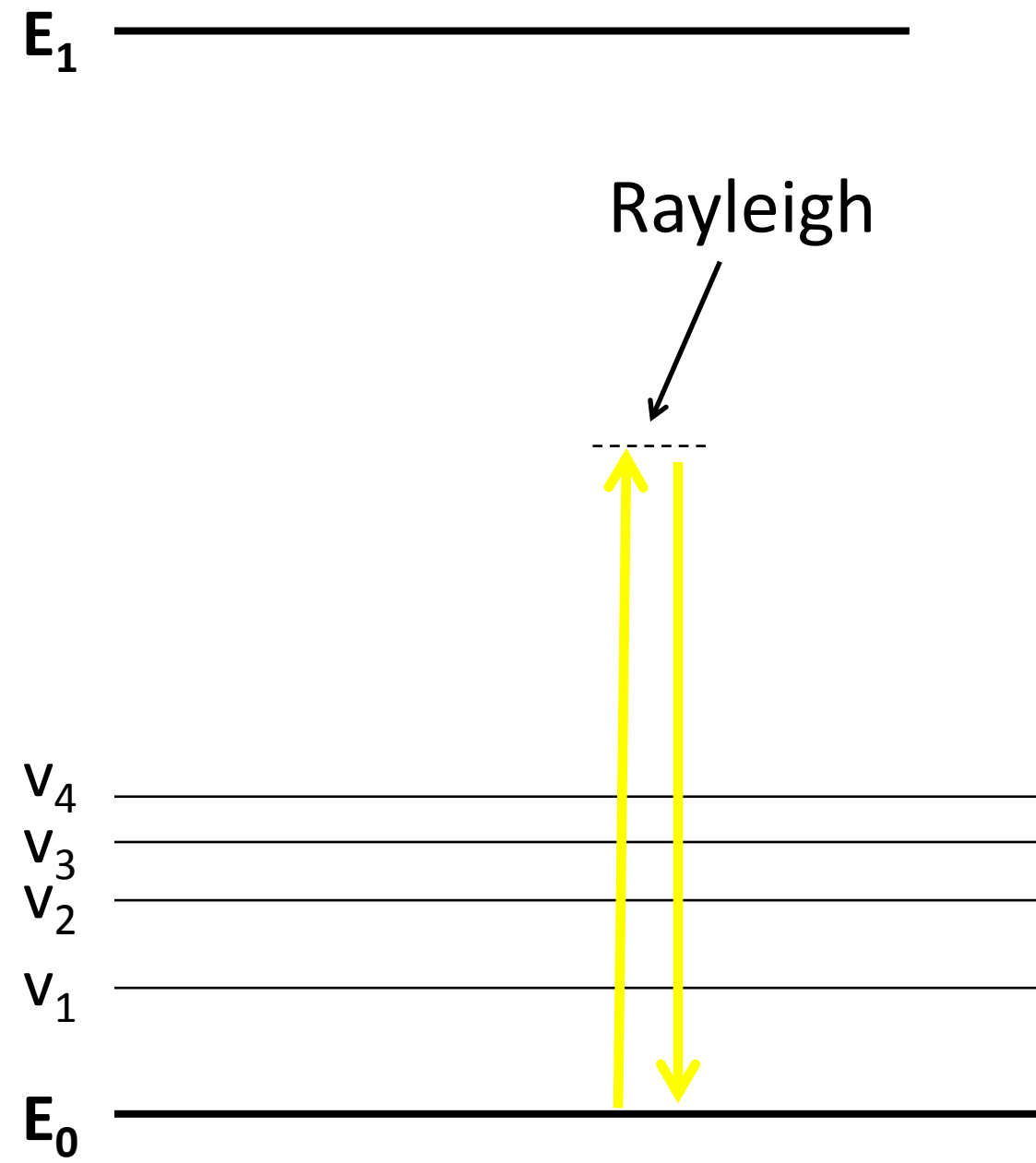
Absorption spectrum of liquid water



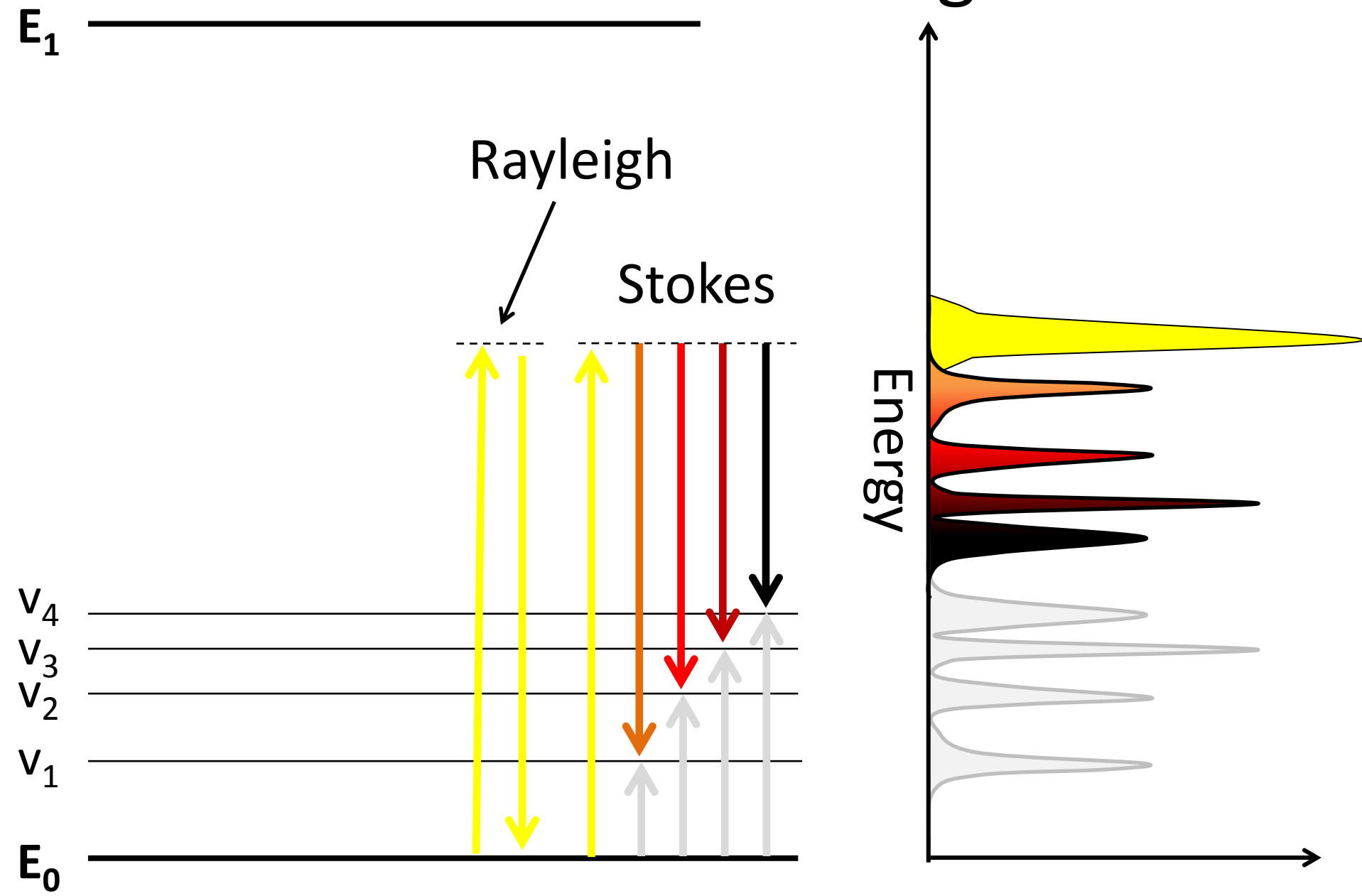
Absorption spectrum of liquid water



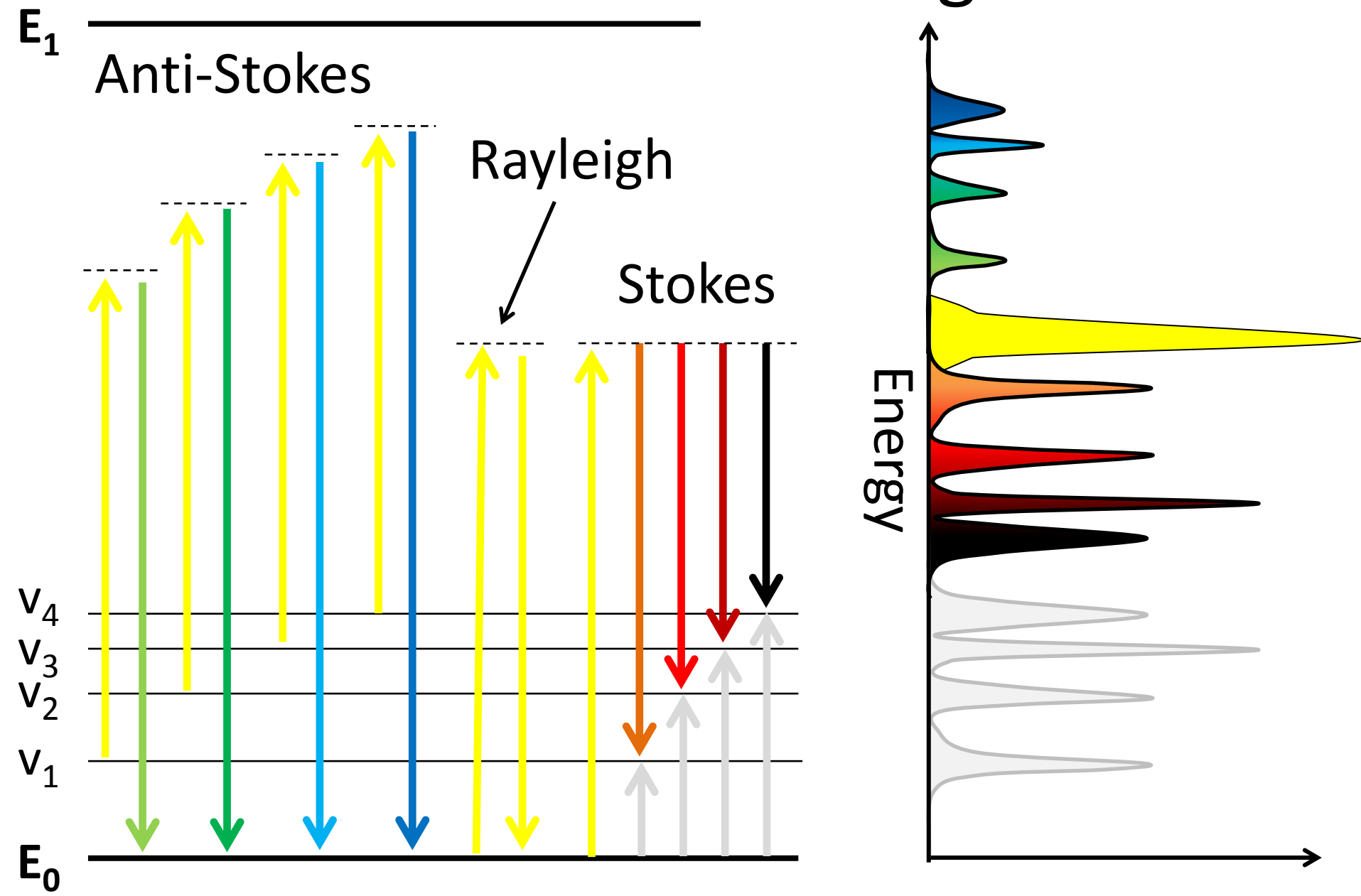
Raman scattering



Raman scattering



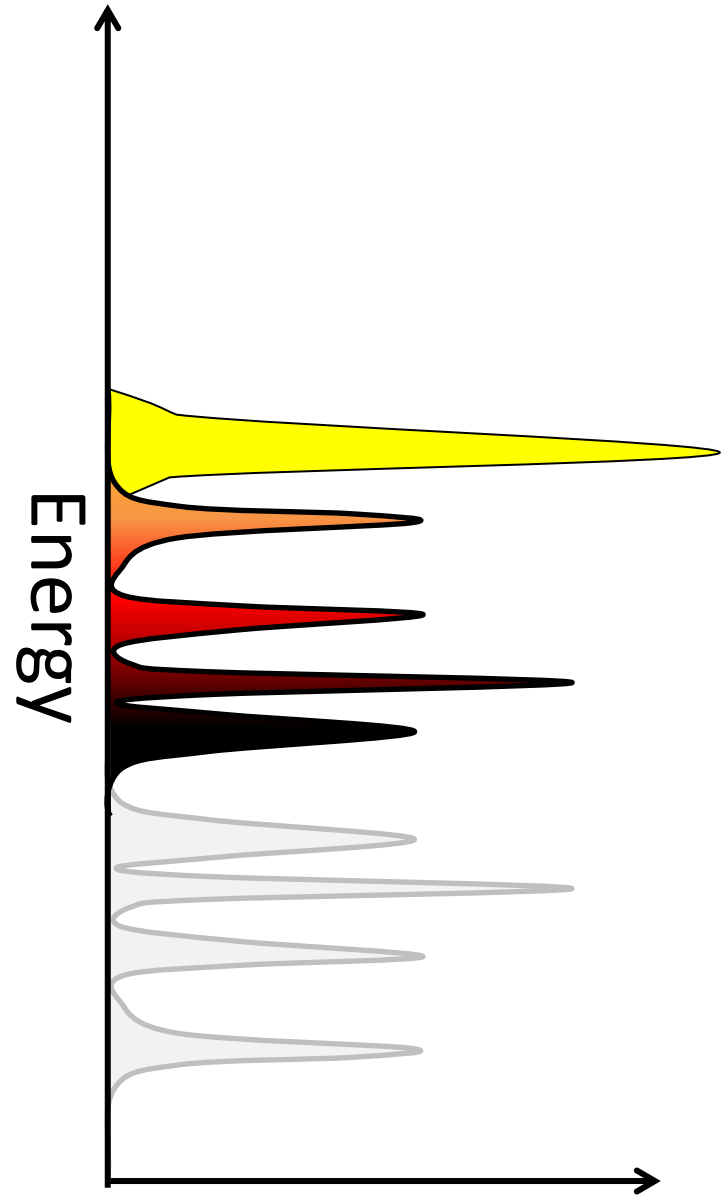
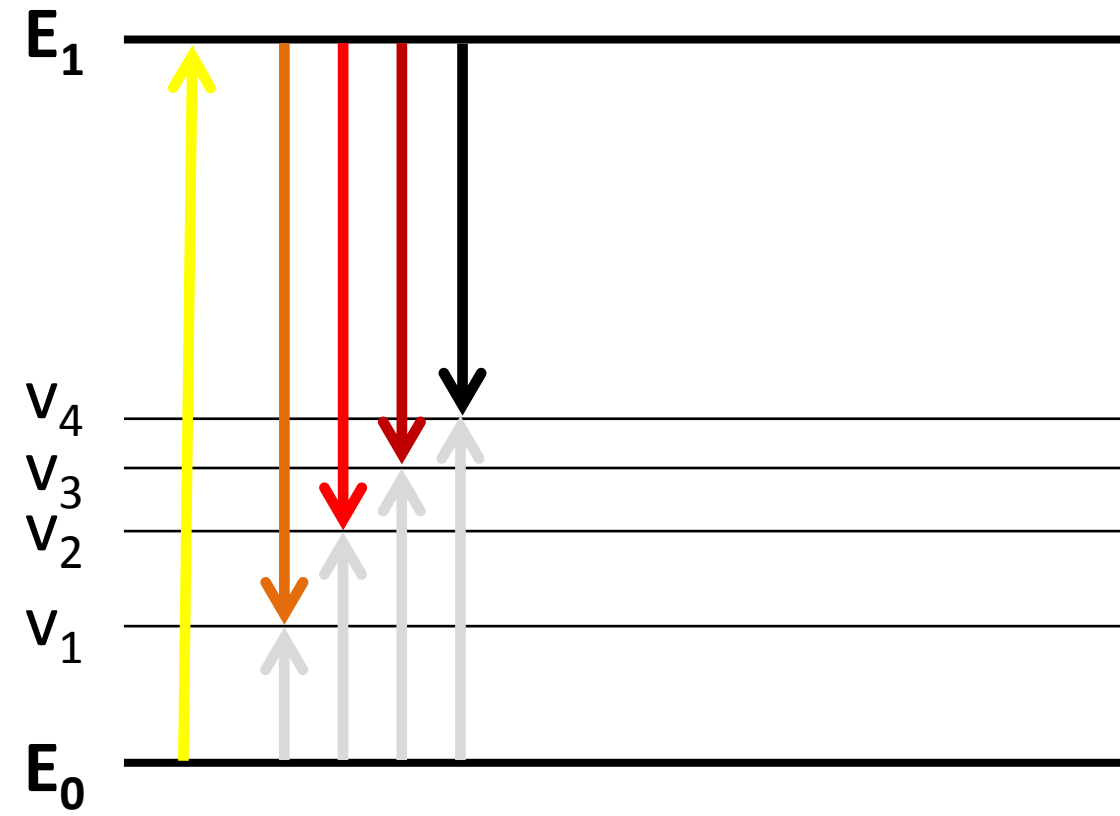
Raman scattering



Fluorescence background in Raman

Stokes

Raman scattering

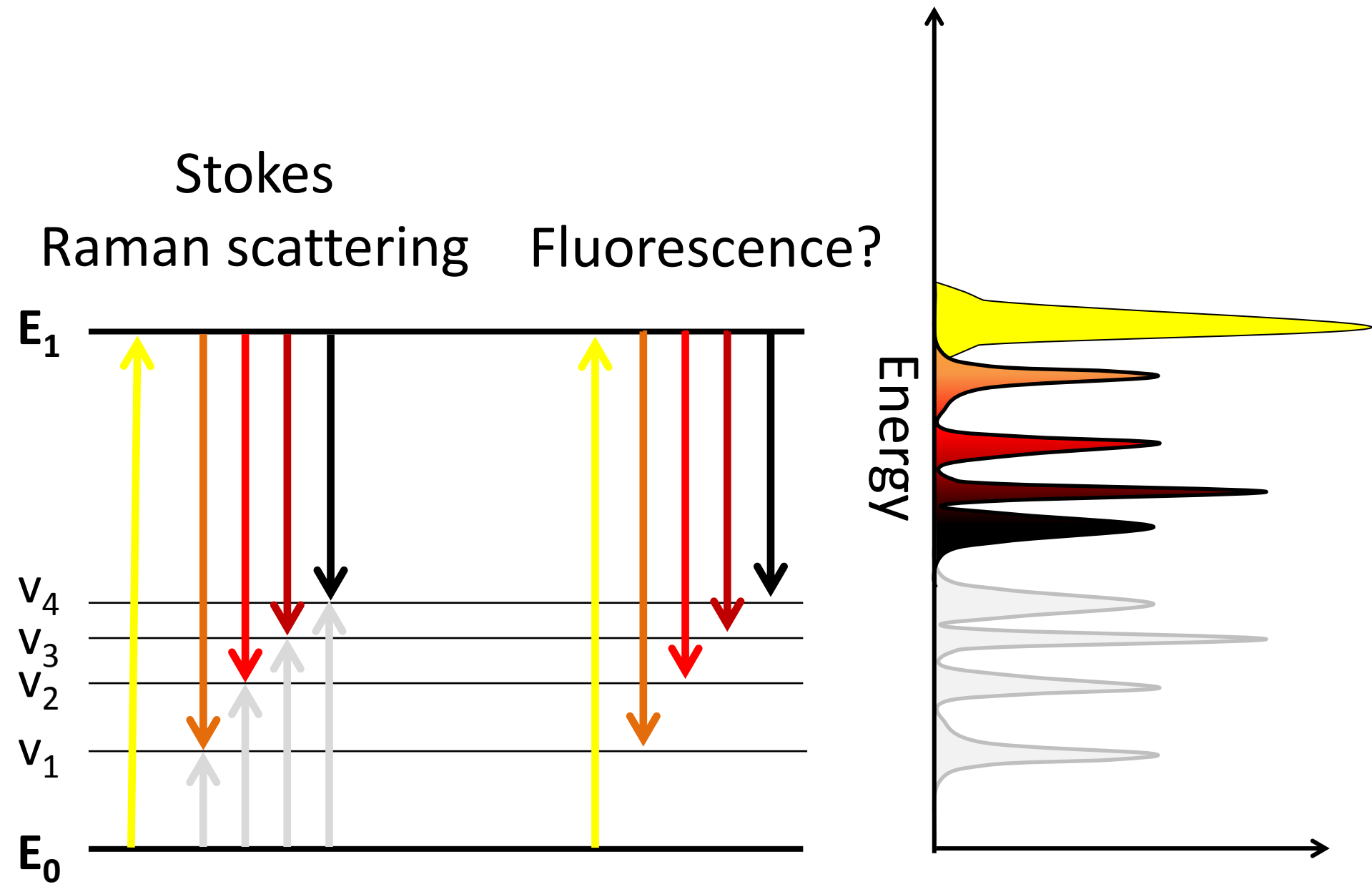


Fluorescence background in Raman

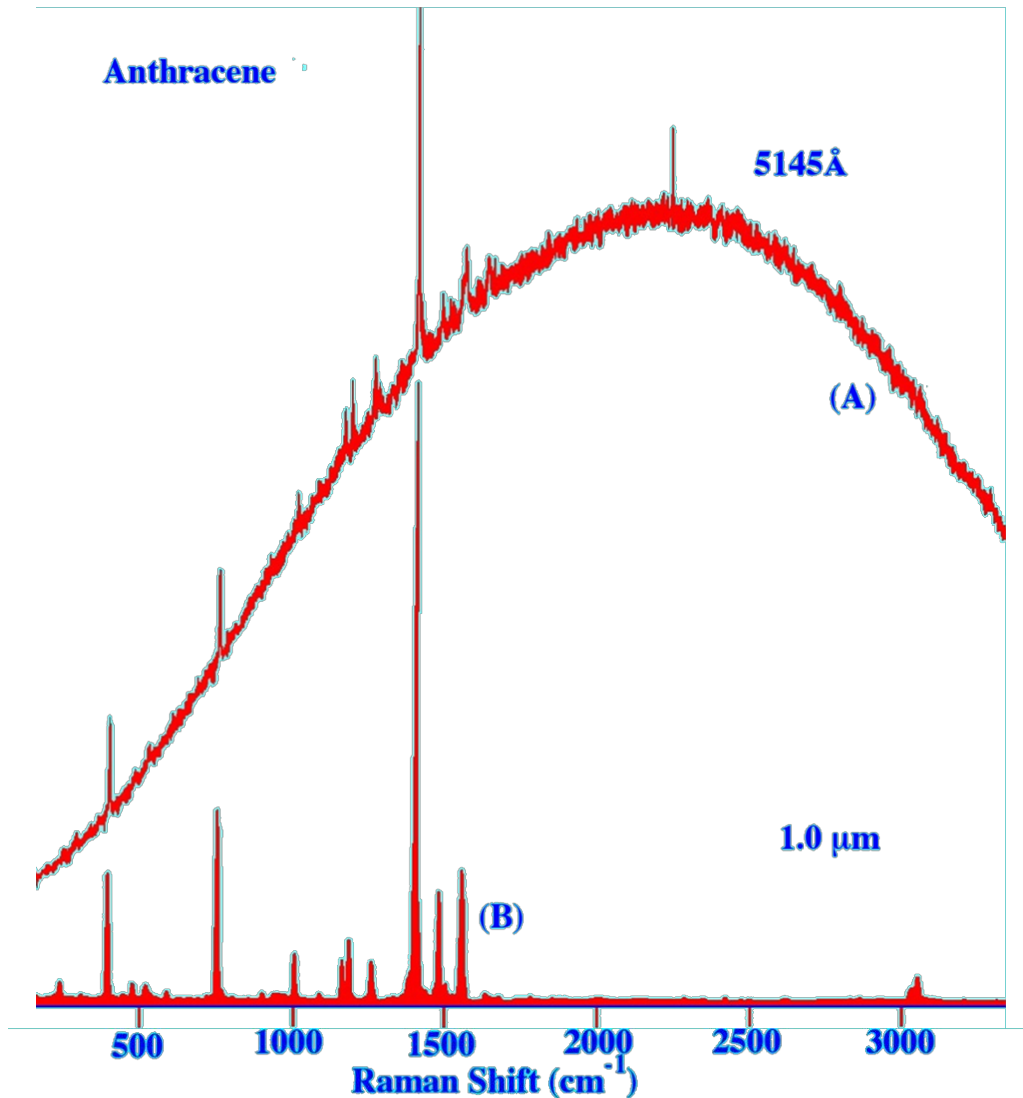
Stokes

Raman scattering

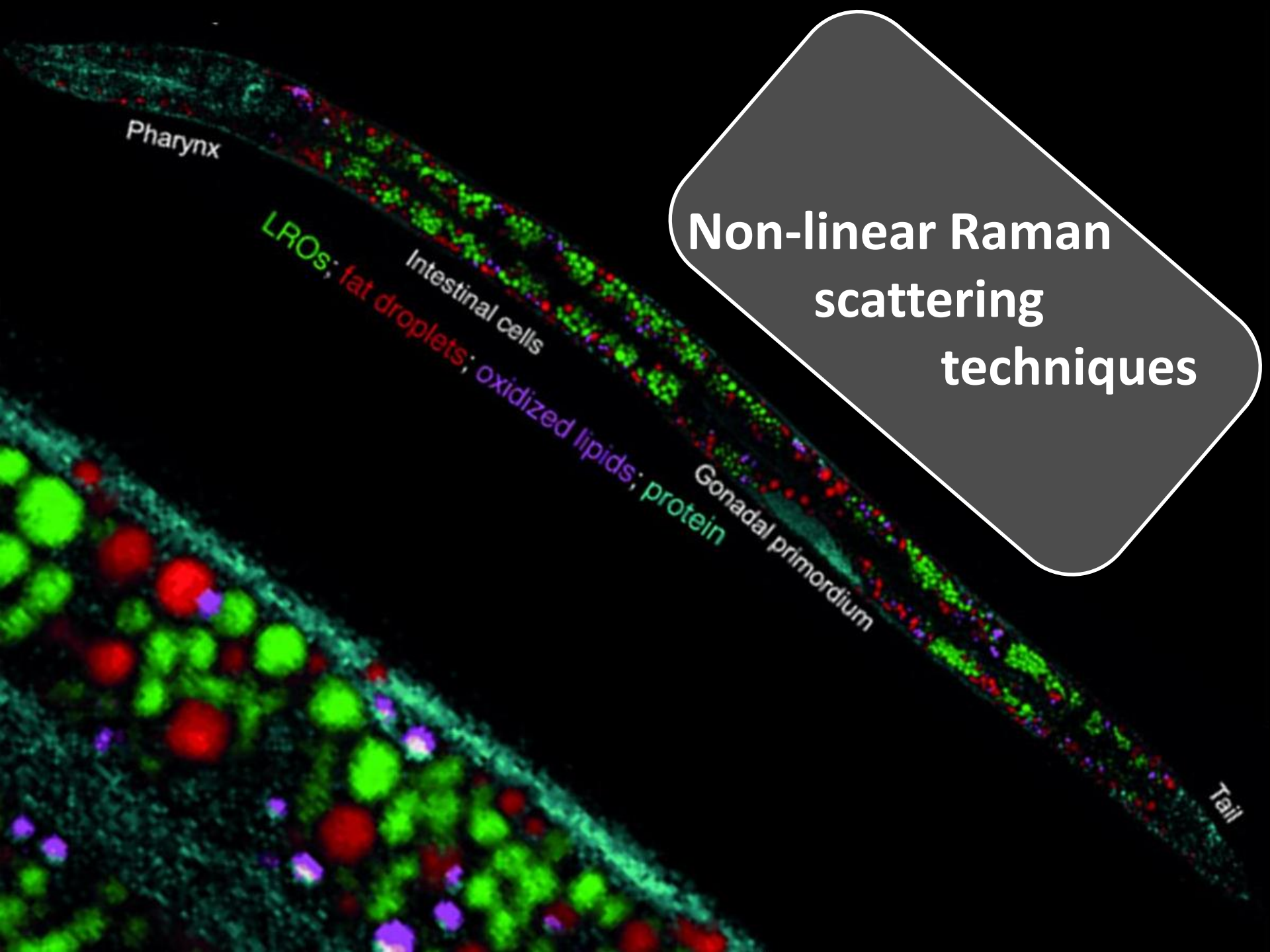
Fluorescence?



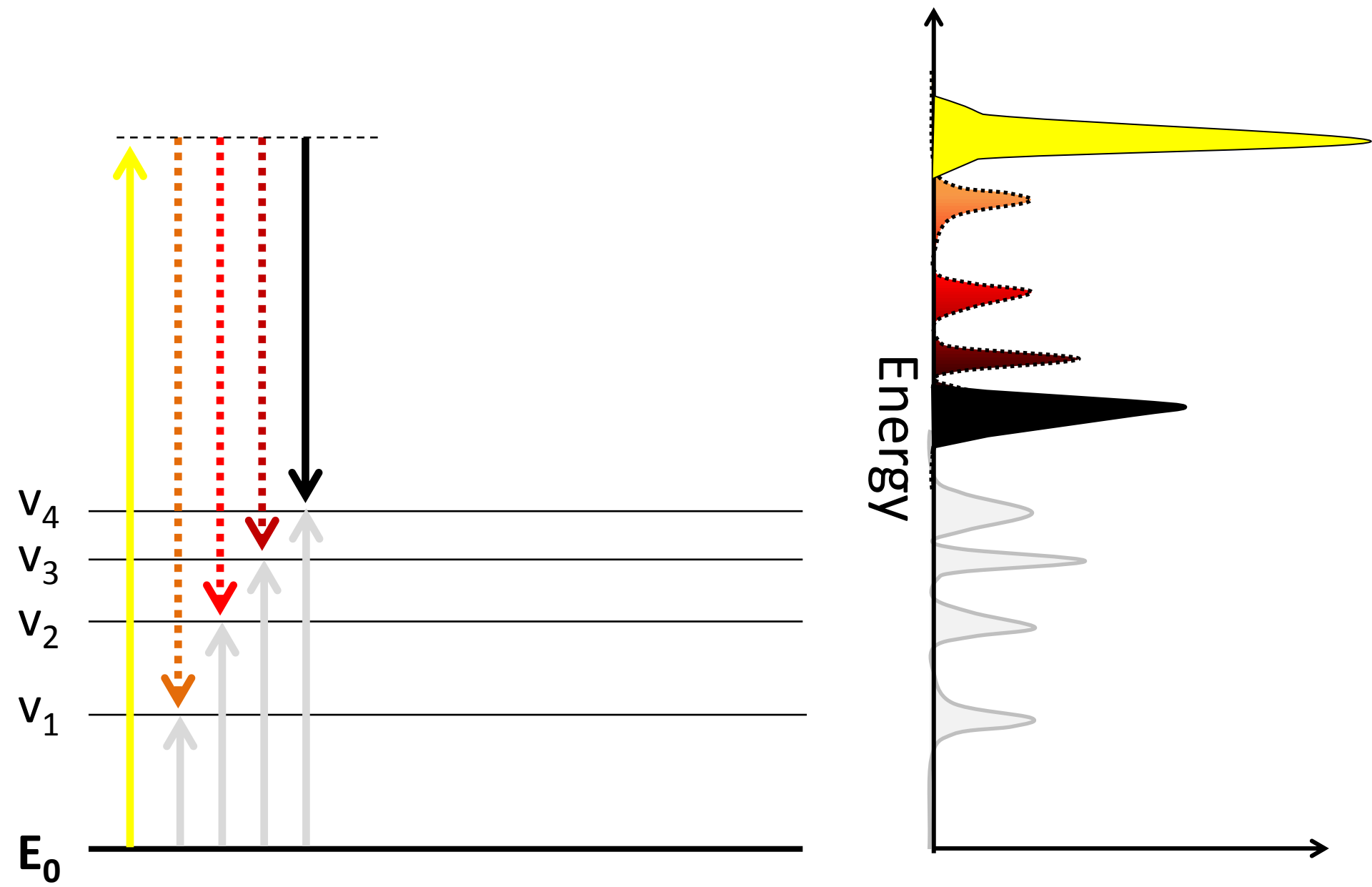
Fluorescence background in Raman



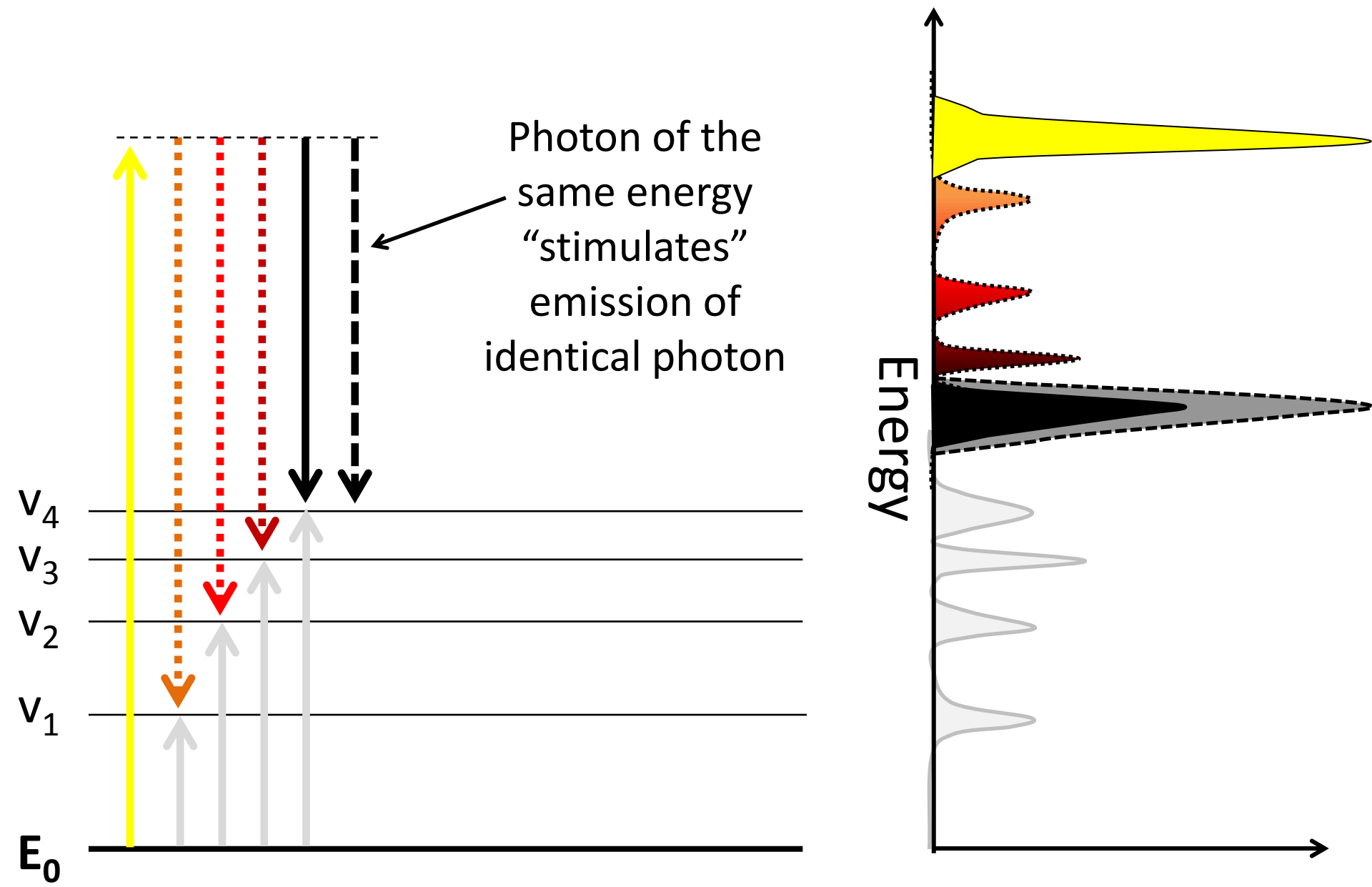
Non-linear Raman scattering techniques



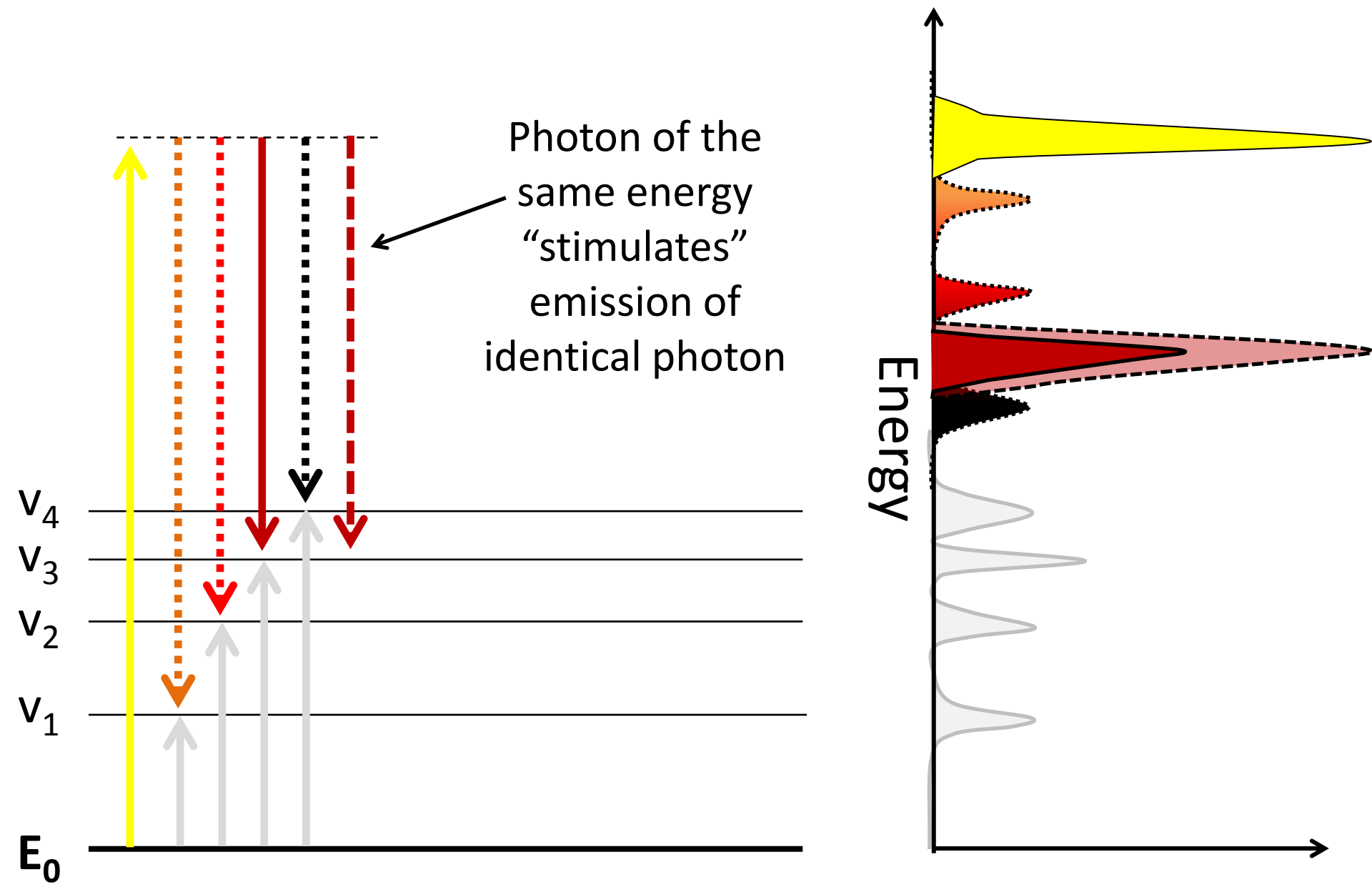
Stimulated Raman scattering (SRS)



Stimulated Raman scattering (SRS)

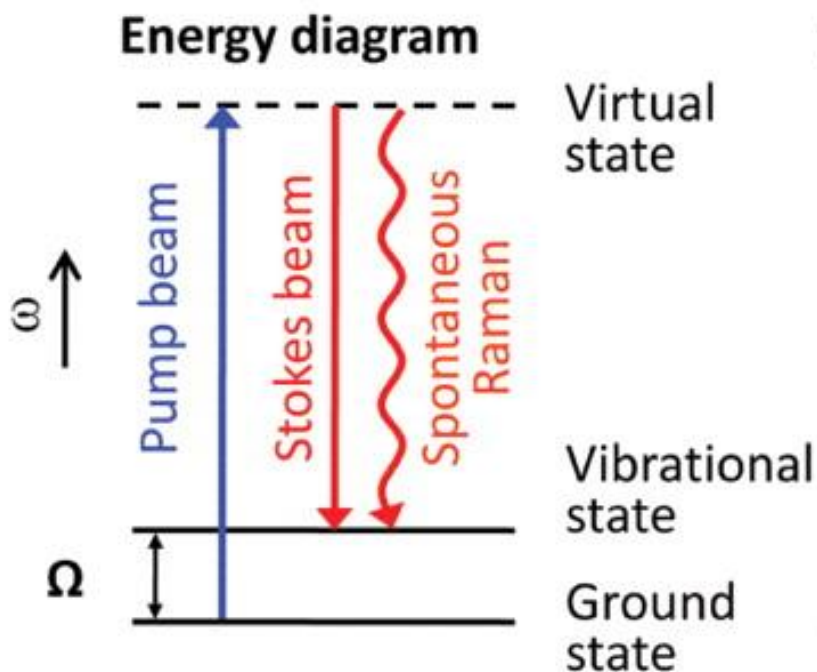


Stimulated Raman scattering (SRS)

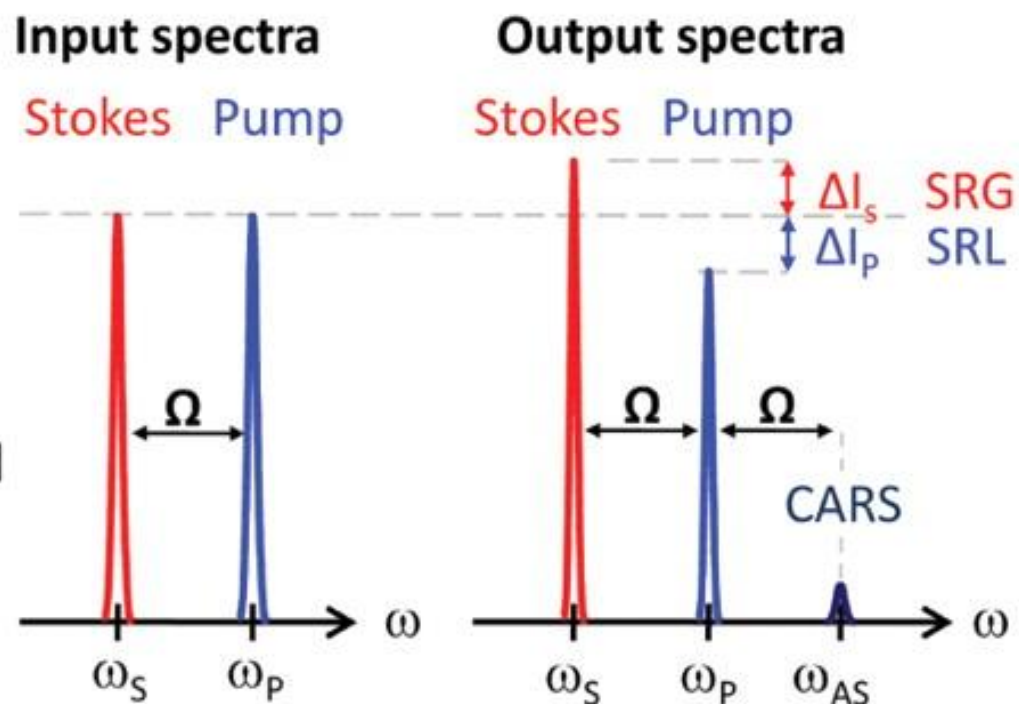


Stimulated Raman scattering (SRS)

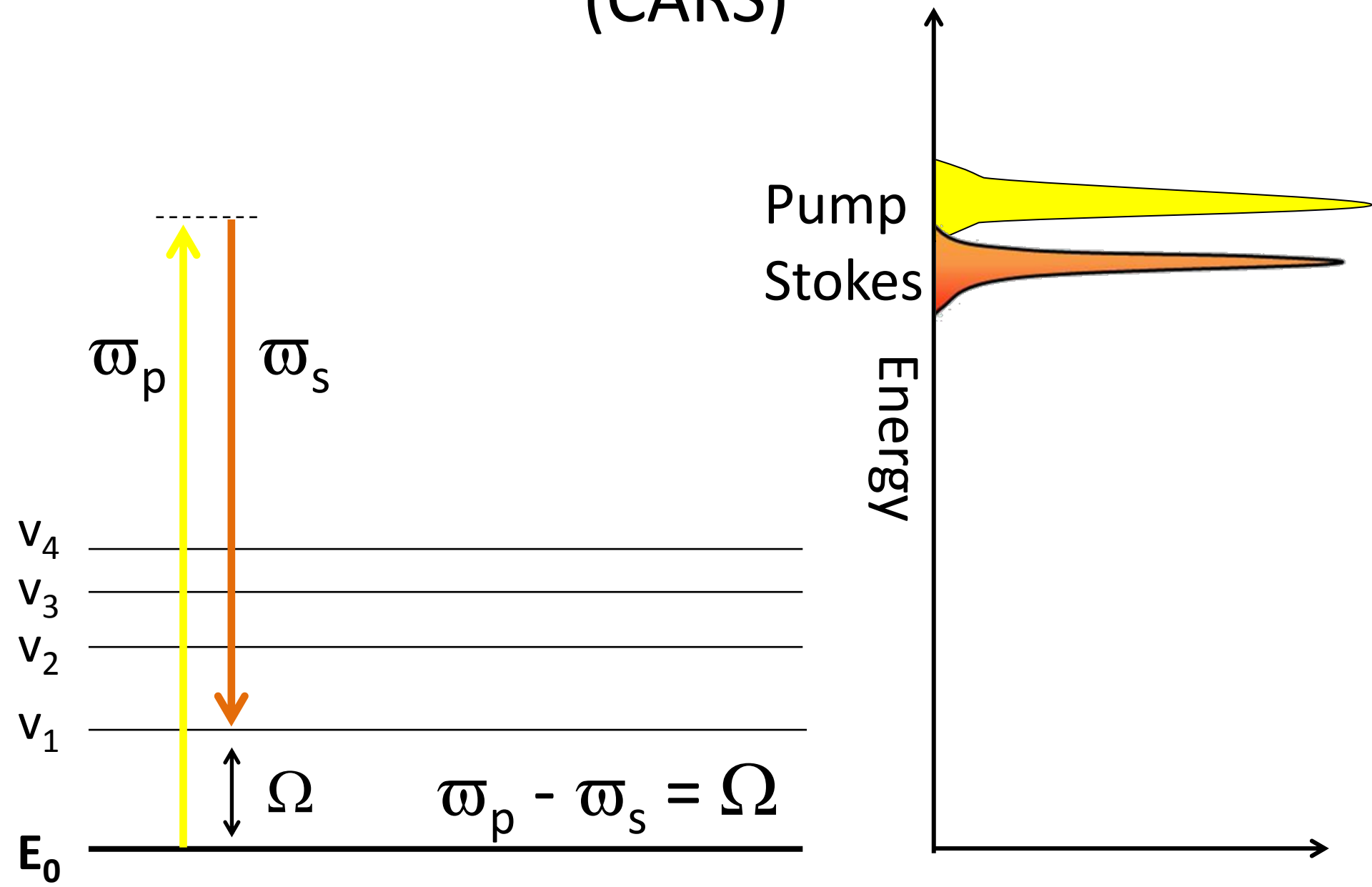
A



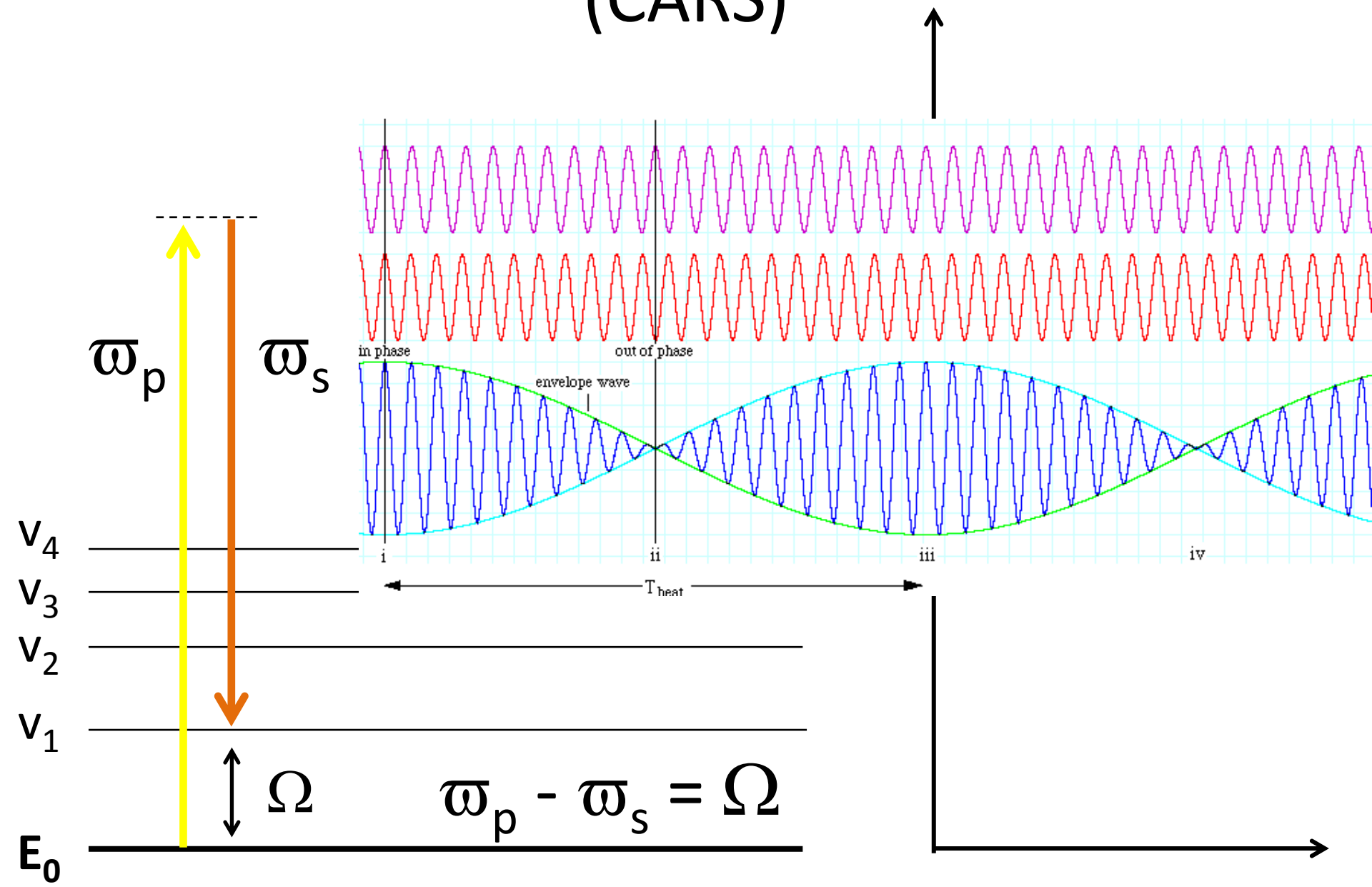
B



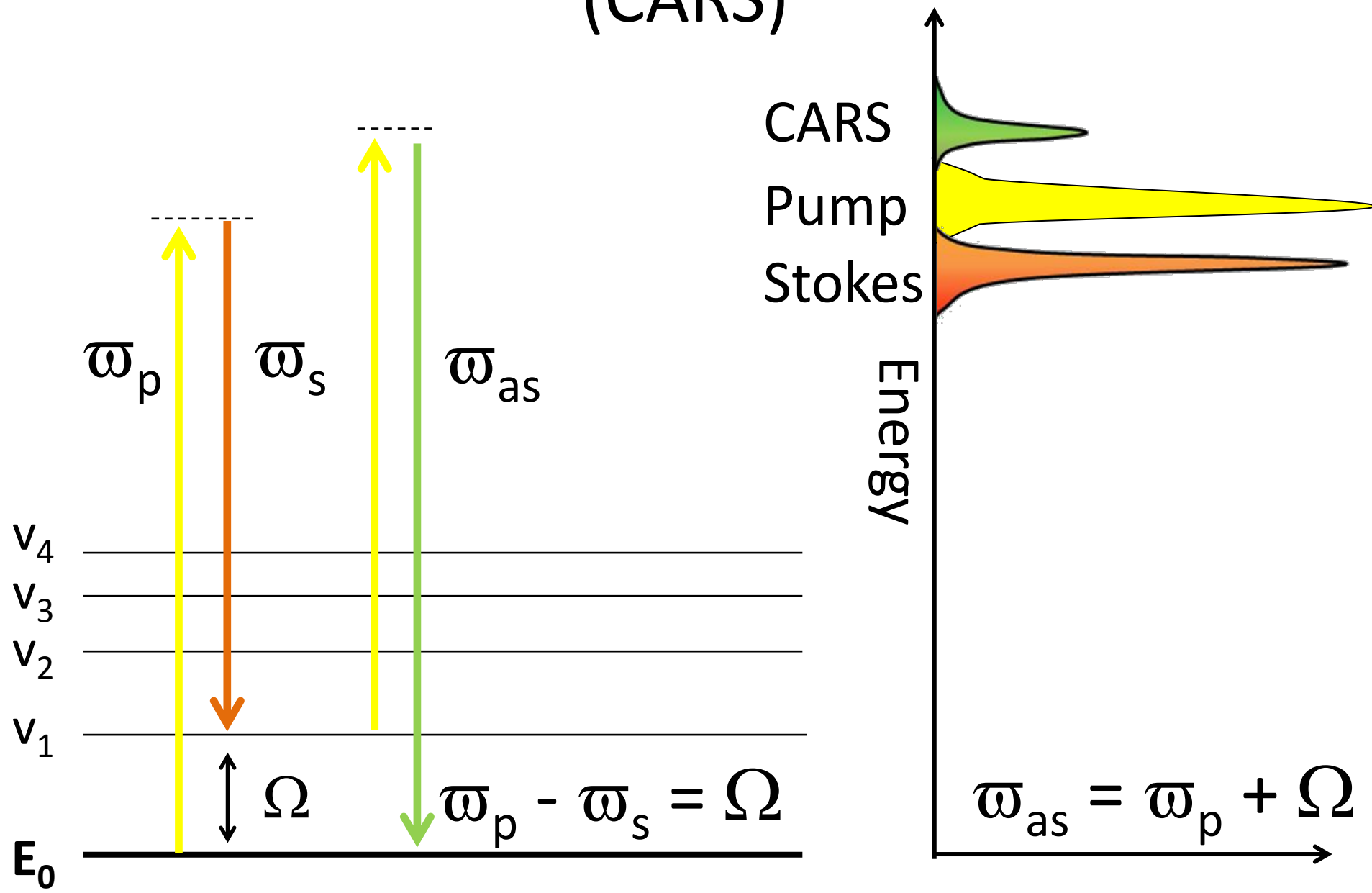
Coherent anti-Stokes Raman scattering (CARS)



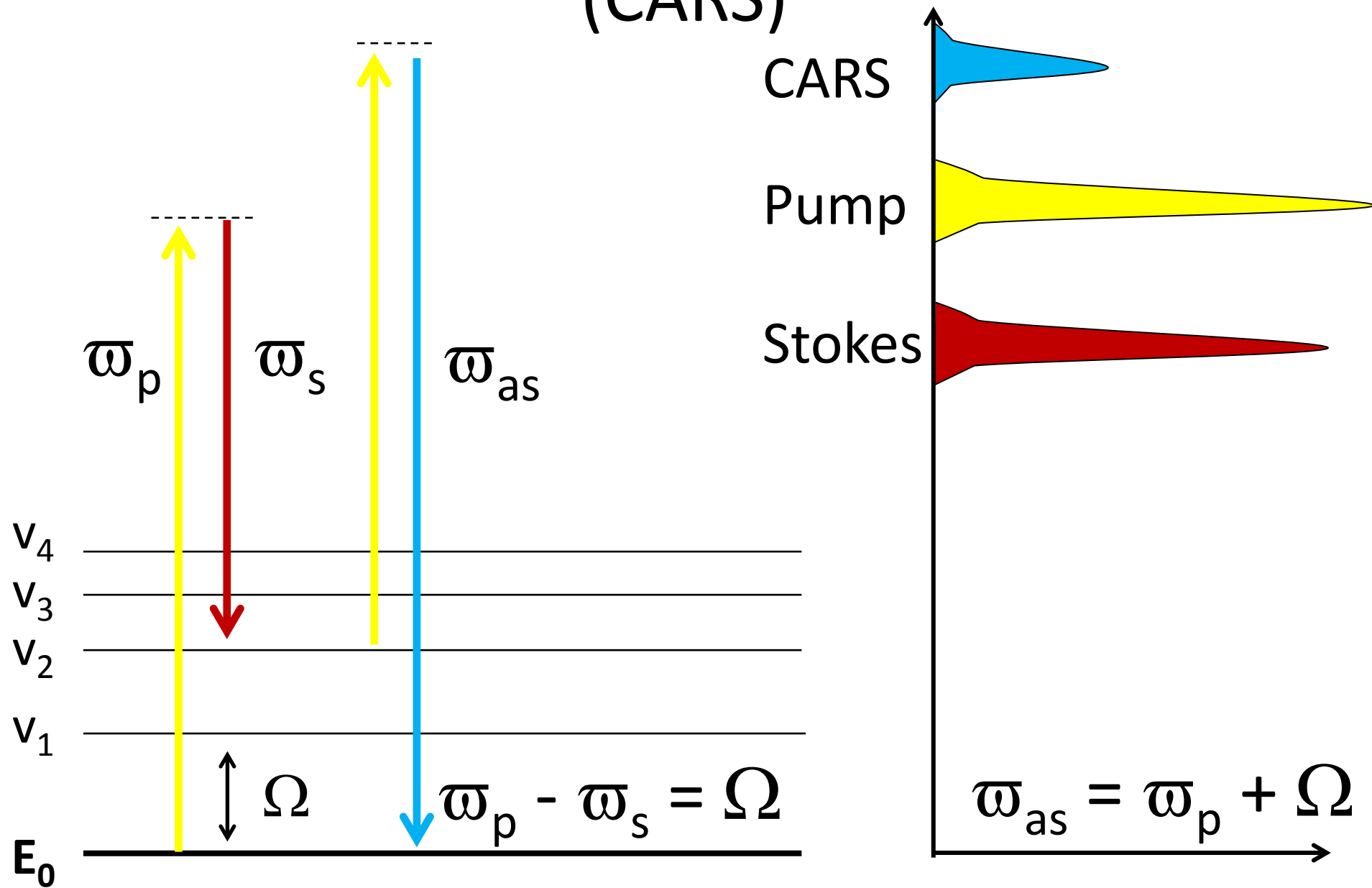
Coherent anti-Stokes Raman scattering (CARS)



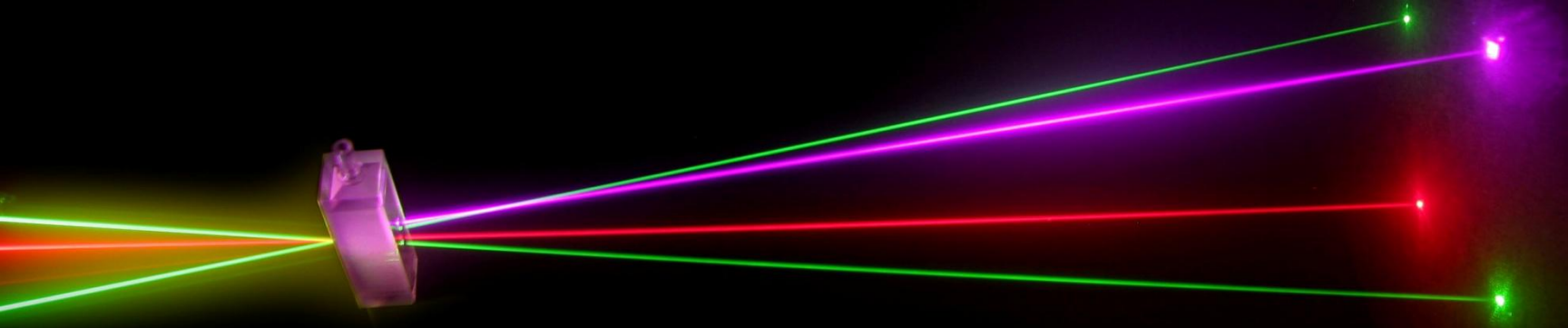
Coherent anti-Stokes Raman scattering (CARS)



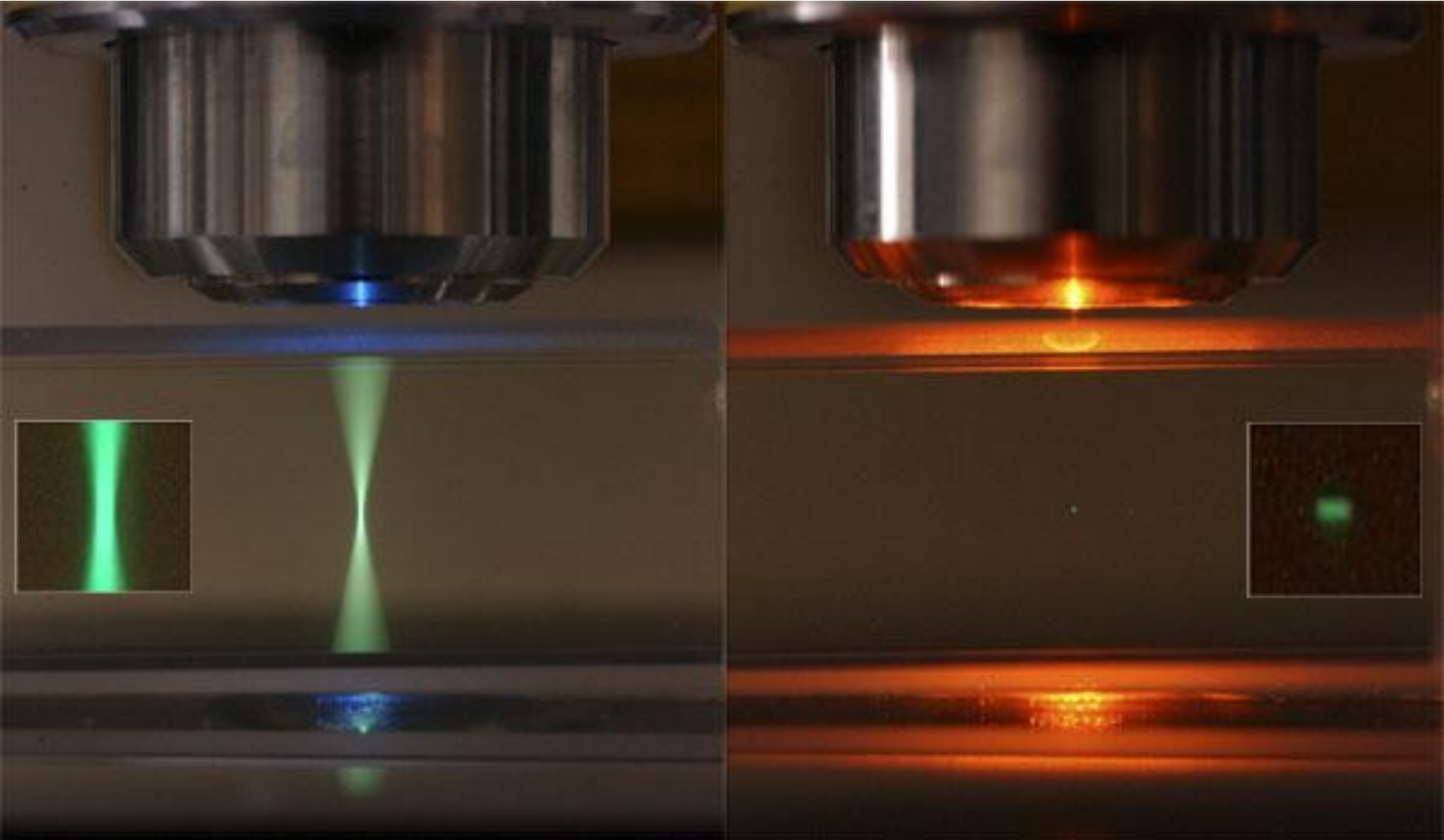
Coherent anti-Stokes Raman scattering (CARS)



Nonlinear optics

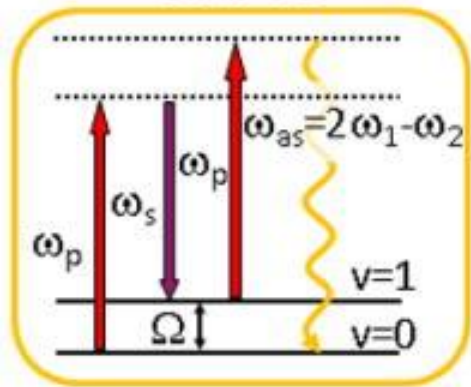


Single photon vs two photon excitation fluorescence

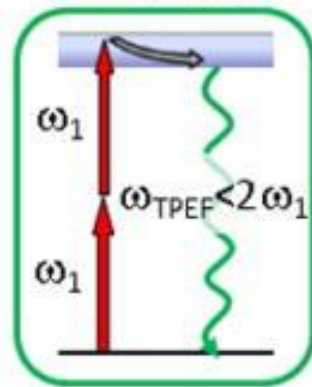


Non-linear processes

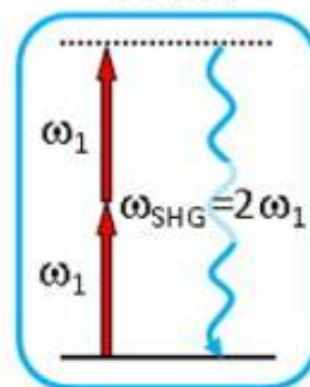
CARS



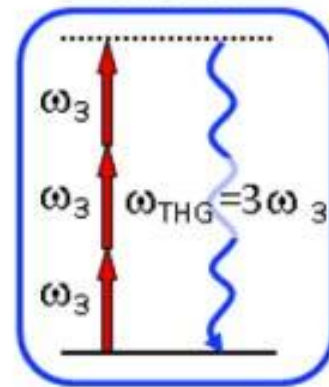
TPEF/TPL



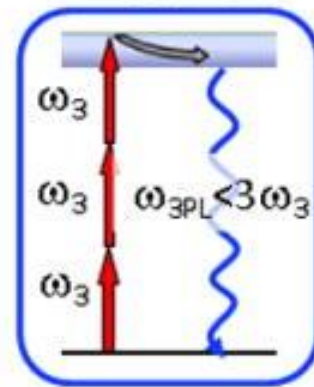
SHG



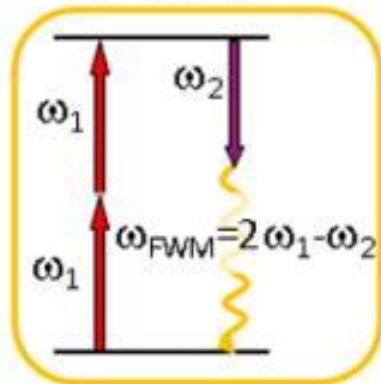
THG



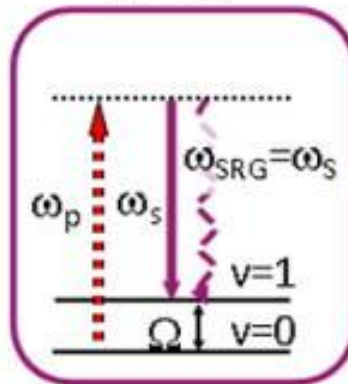
3PL



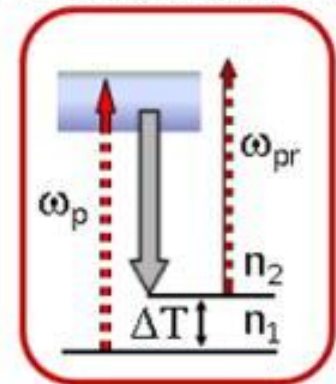
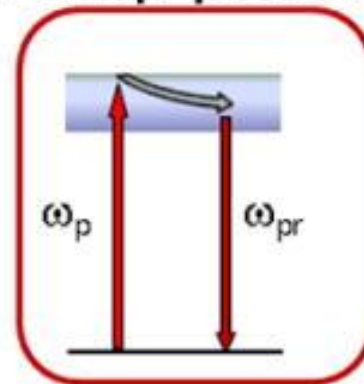
FWM



SRS

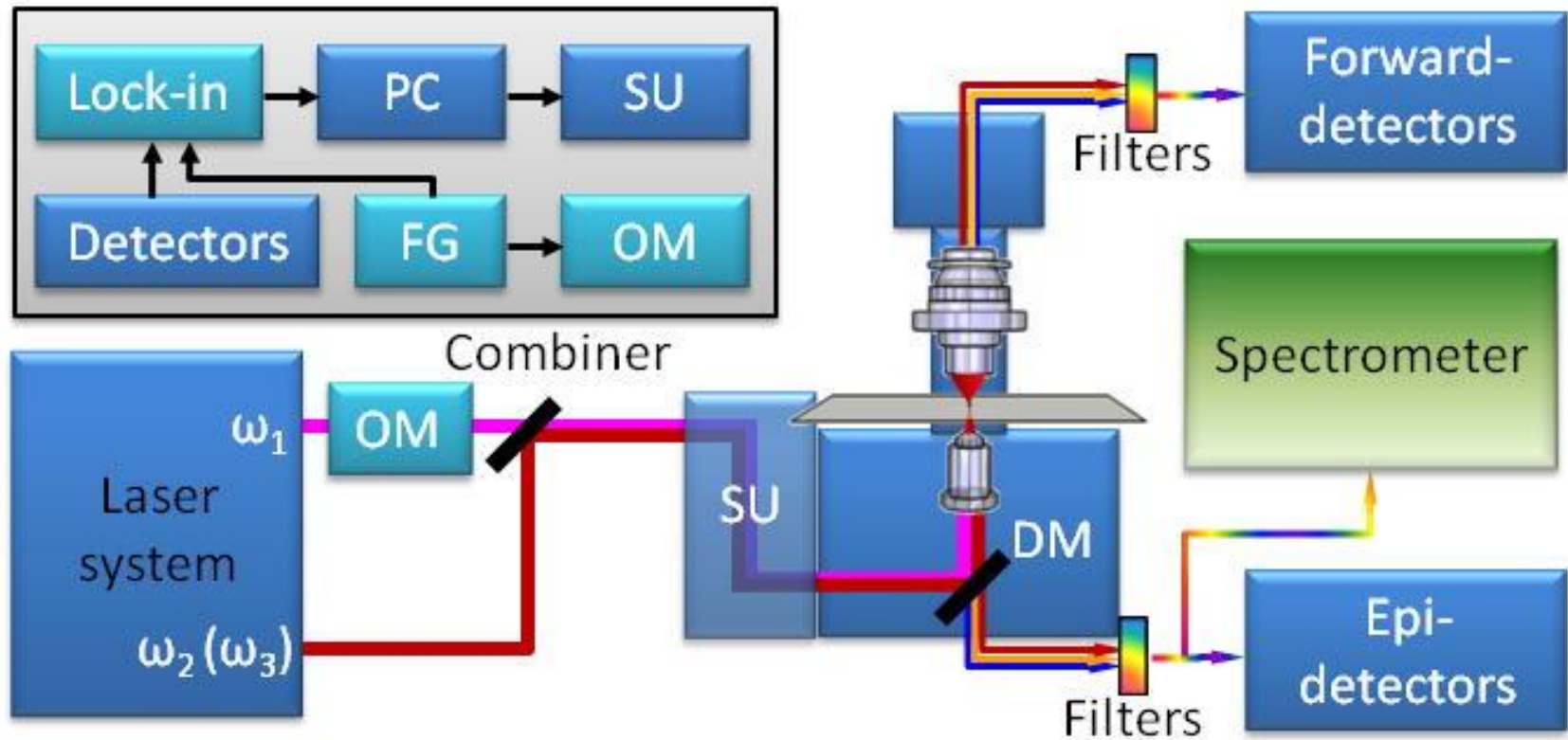


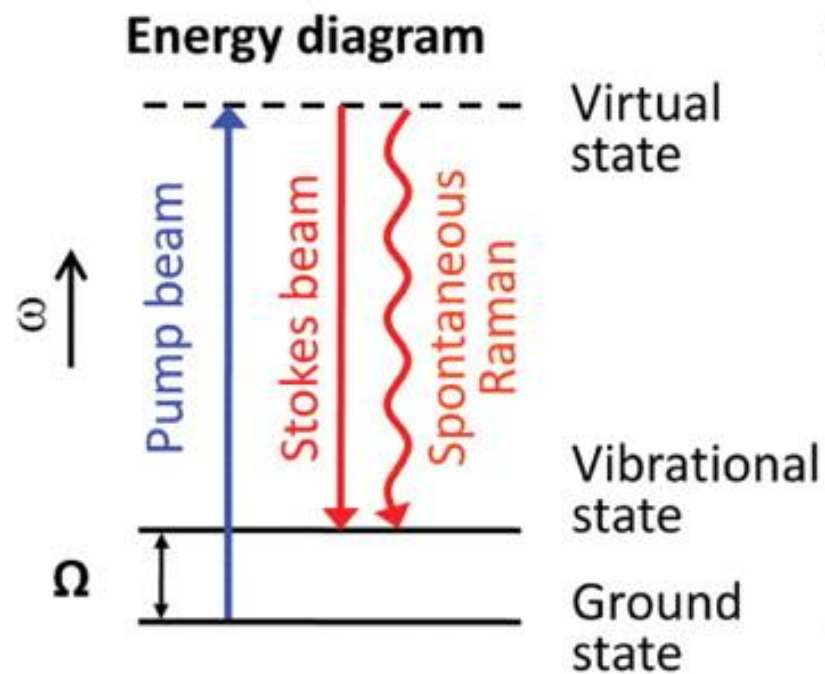
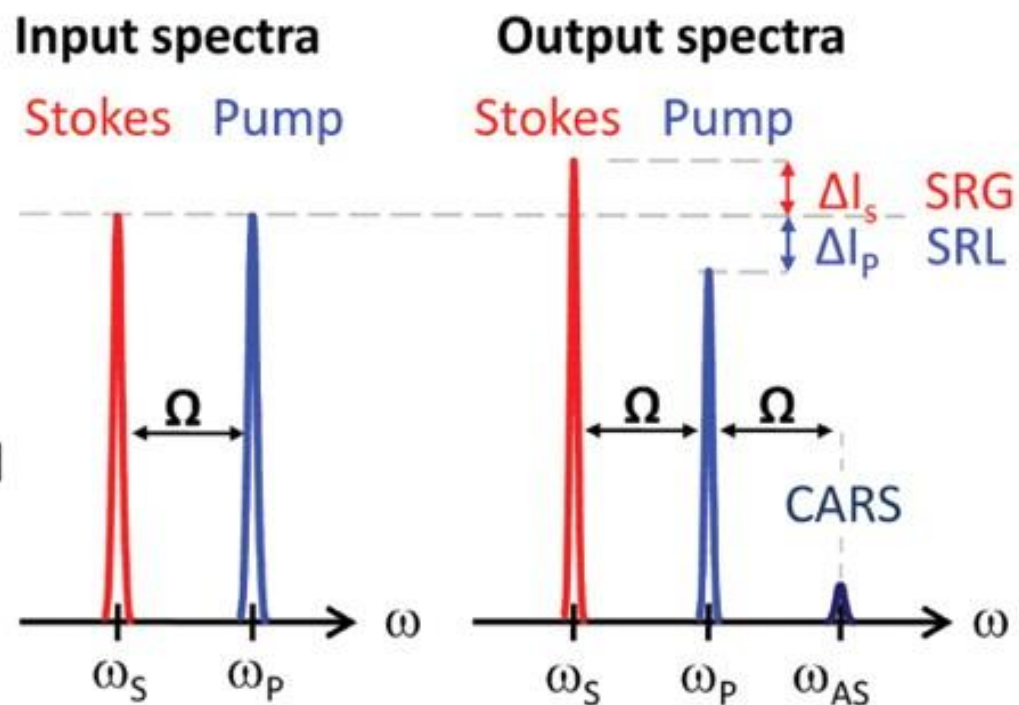
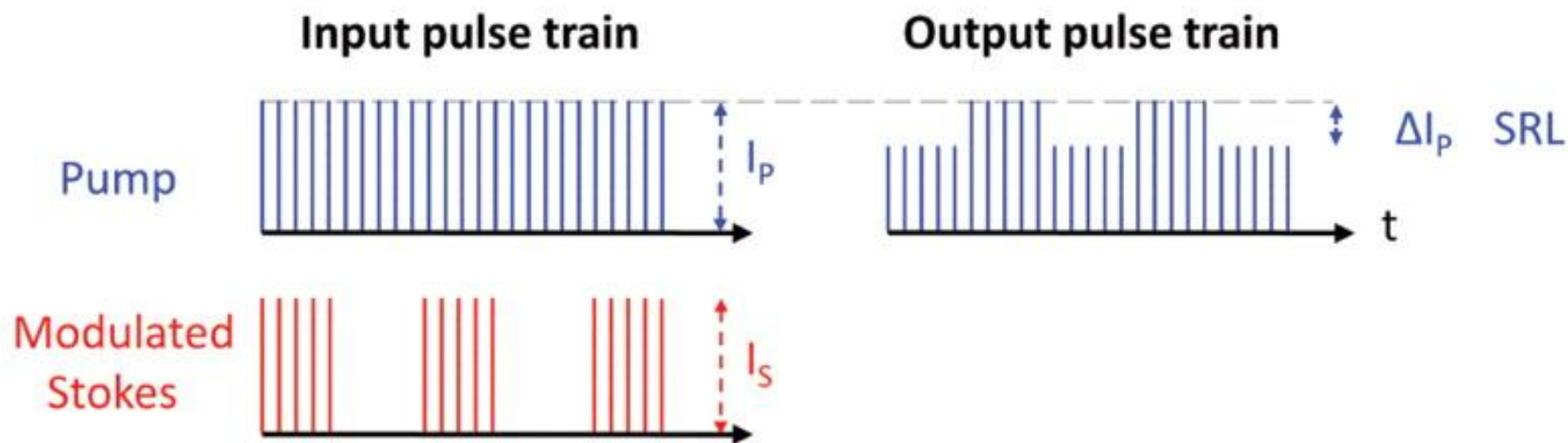
Pump-probe Photothermal



Multimodal non-linear microscope

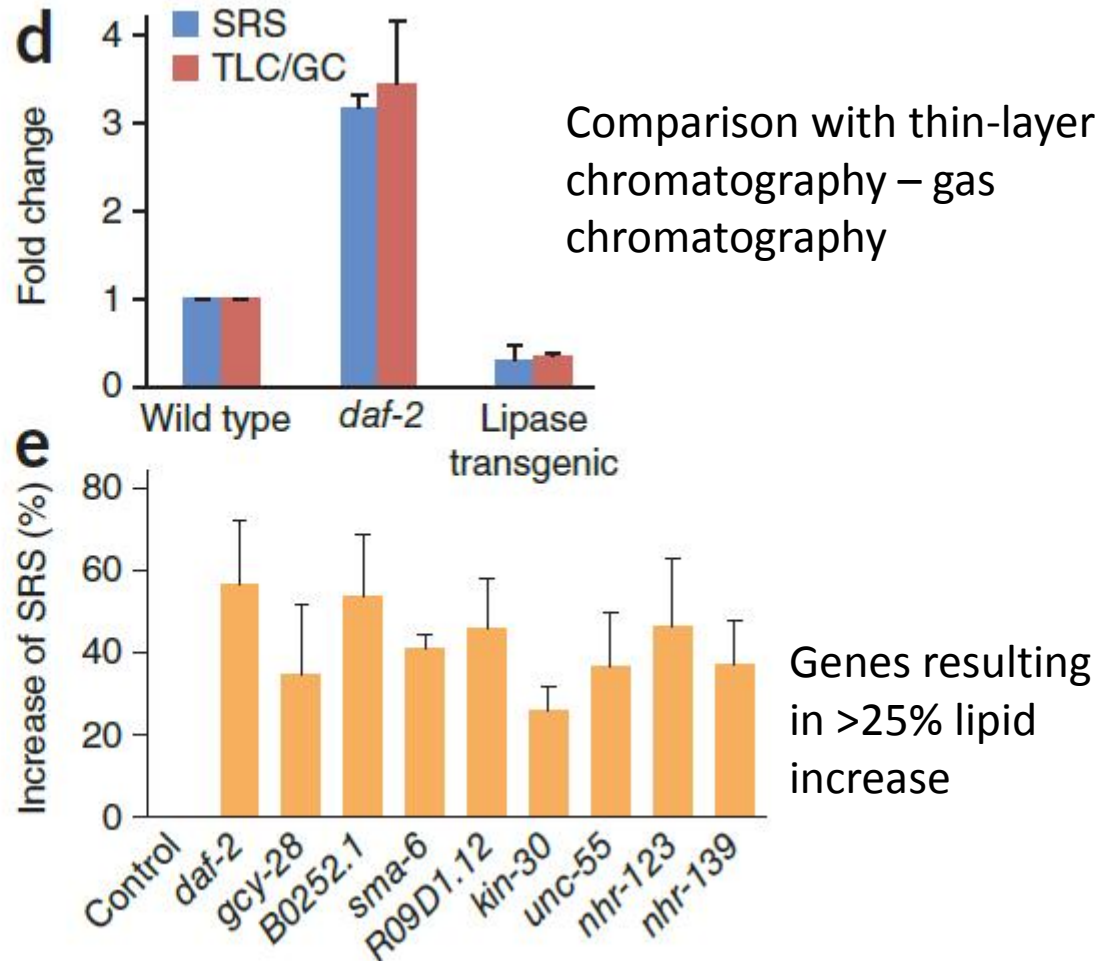
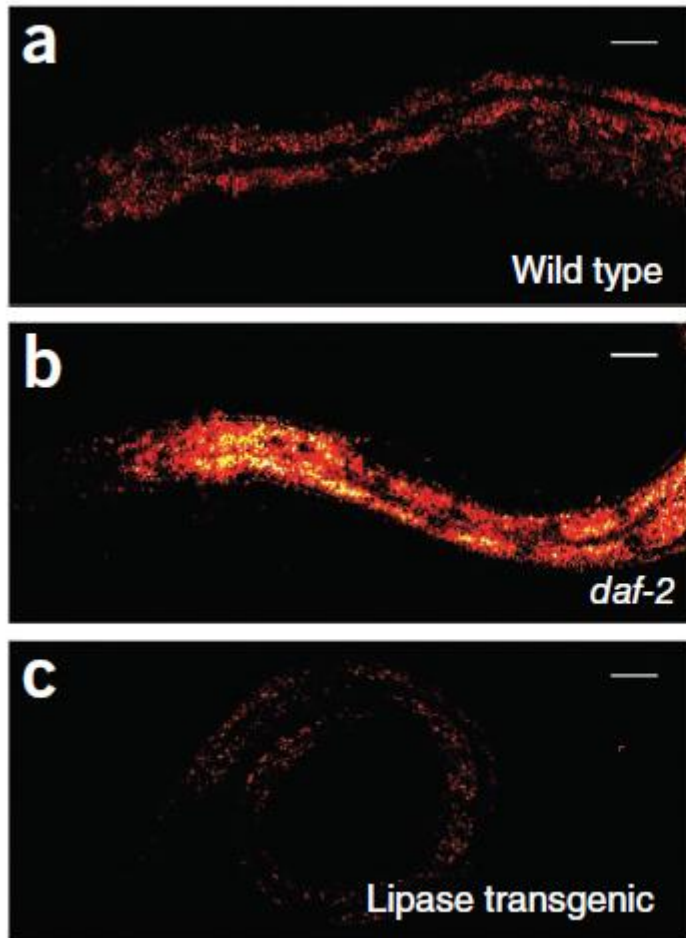
SU - scanning mirror
FG - function generator
OM - optical modulator



A**B****C**

Applications

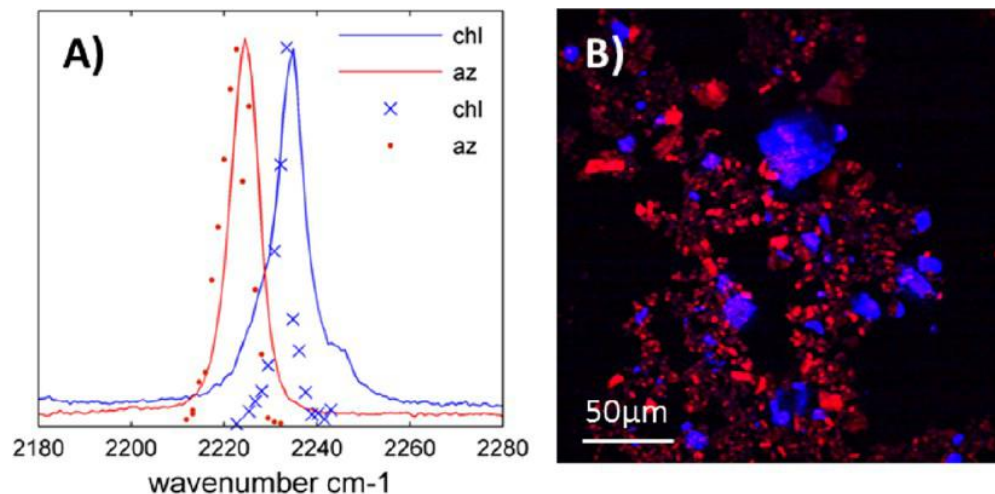
RNAi screening for fat regulatory genes with SRS microscopy



Wang, Meng C., et al. "RNAi screening for fat regulatory genes with SRS microscopy." *Nature methods* 8.2 (2011): 135-138.

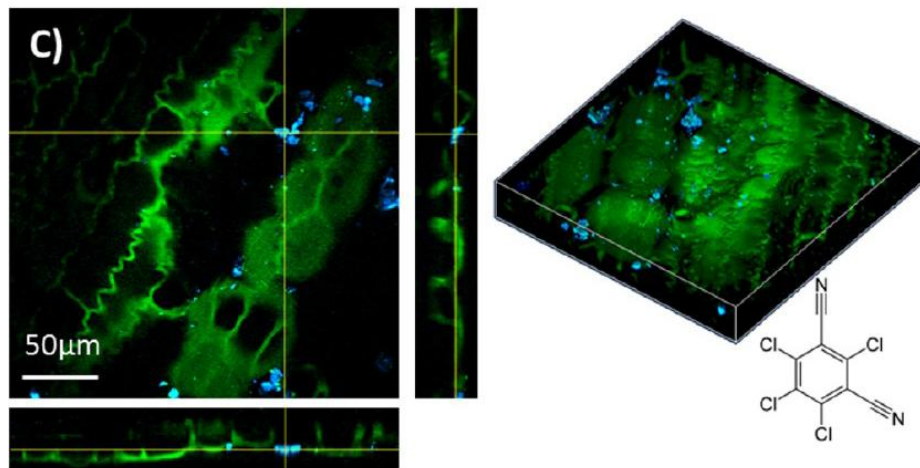
Label-free Chemically Specific Imaging in Planta with Stimulated Raman Scattering Microscopy

Commercially available fungicides azoxystrobin and chlorothalonil

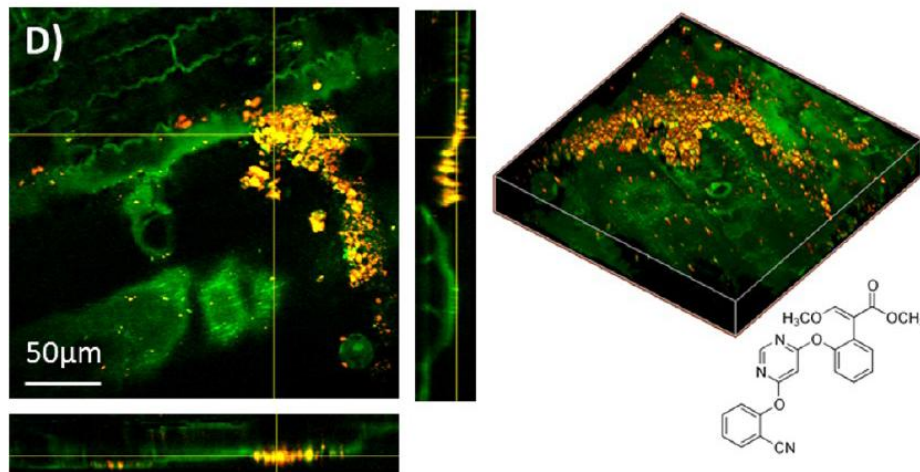


Mansfield, Jessica C., et al. "Label-free chemically specific imaging in planta with stimulated Raman scattering microscopy." *Analytical chemistry* 85.10 (2013): 5055-5063.

Label-free Chemically Specific Imaging in Planta with Stimulated Raman Scattering Microscopy



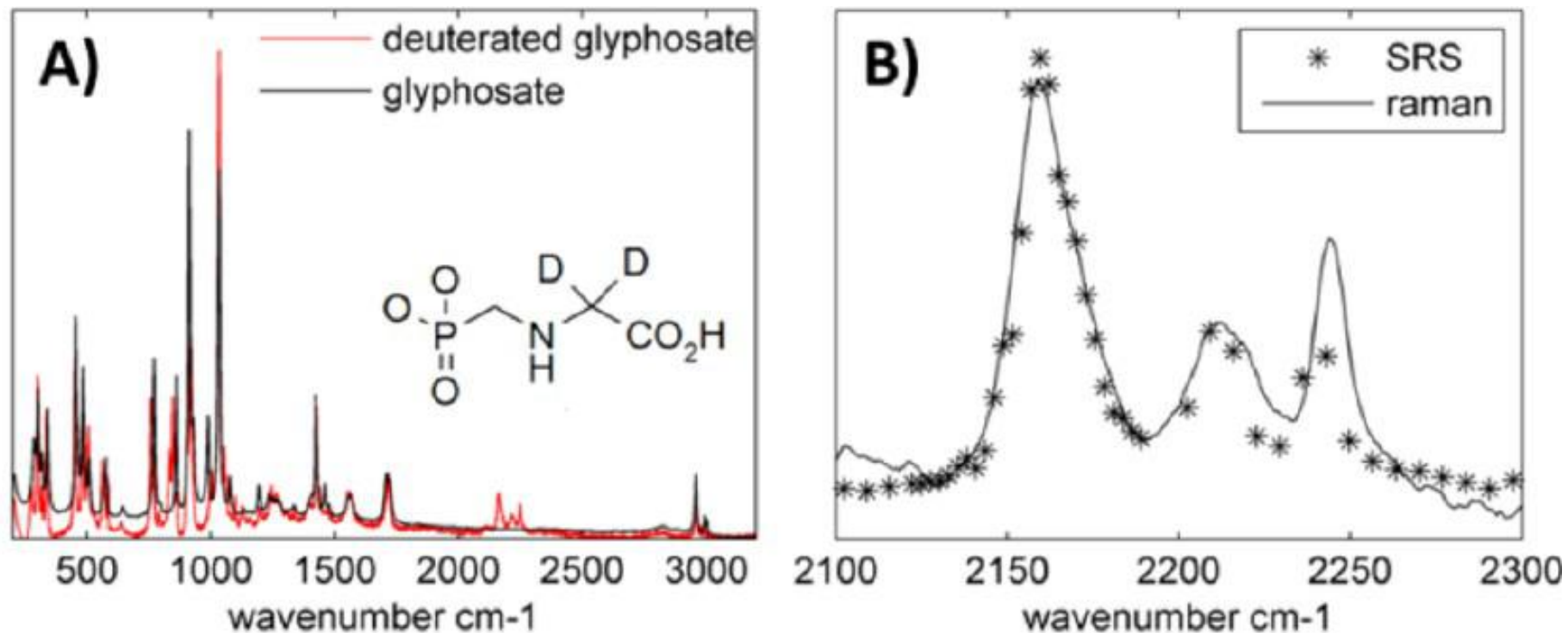
(C) Chlorothalonil applied to a maize leaf
blue = SRL at 2234 cm^{-1} from the CN bond,
green = SRL at 2930 cm^{-1} from the CH₃
vibrations).



(D) Azoxystrobin applied to a maize leaf
red = SRL at 2225 cm^{-1} from the CN bond,
green = SRL at 2930 cm^{-1} from the CH₃
vibrations)

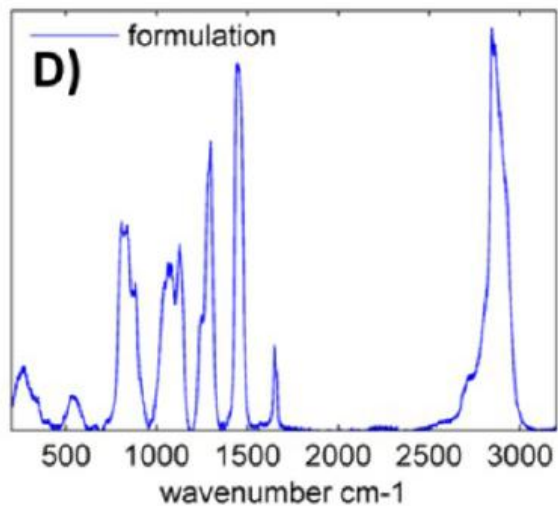
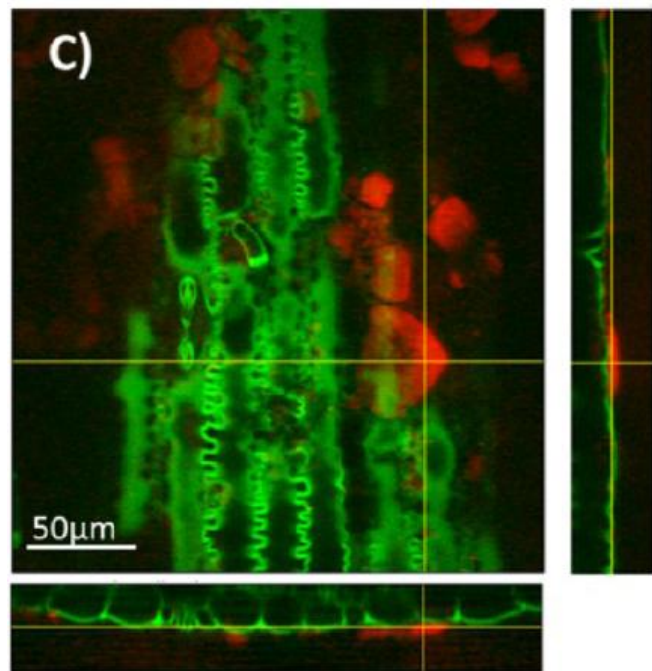
Label-free Chemically Specific Imaging in Planta with Stimulated Raman Scattering Microscopy

Many agrochemicals do not contain Raman vibrations within the **silent region**. To aid chemically specific imaging of these compounds **deuterium labeling** was investigated.

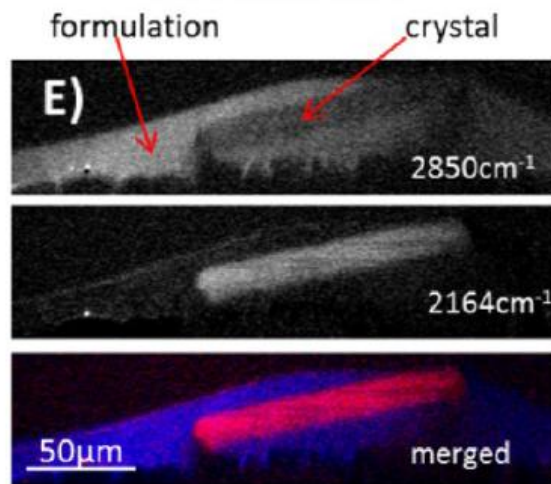
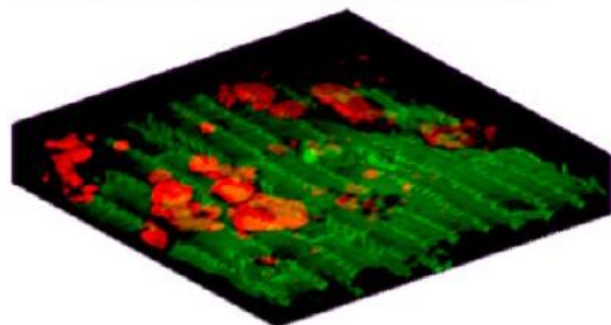


Mansfield, Jessica C., et al. "Label-free chemically specific imaging in planta with stimulated Raman scattering microscopy." *Analytical chemistry* 85.10 (2013): 5055-5063.

Label-free Chemically Specific Imaging in Planta with Stimulated Raman Scattering Microscopy

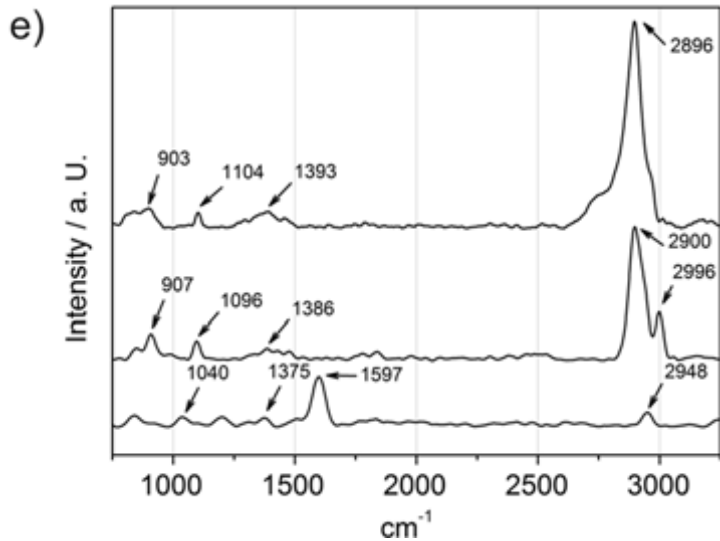


Deuterated Glyphosate on maize leaf



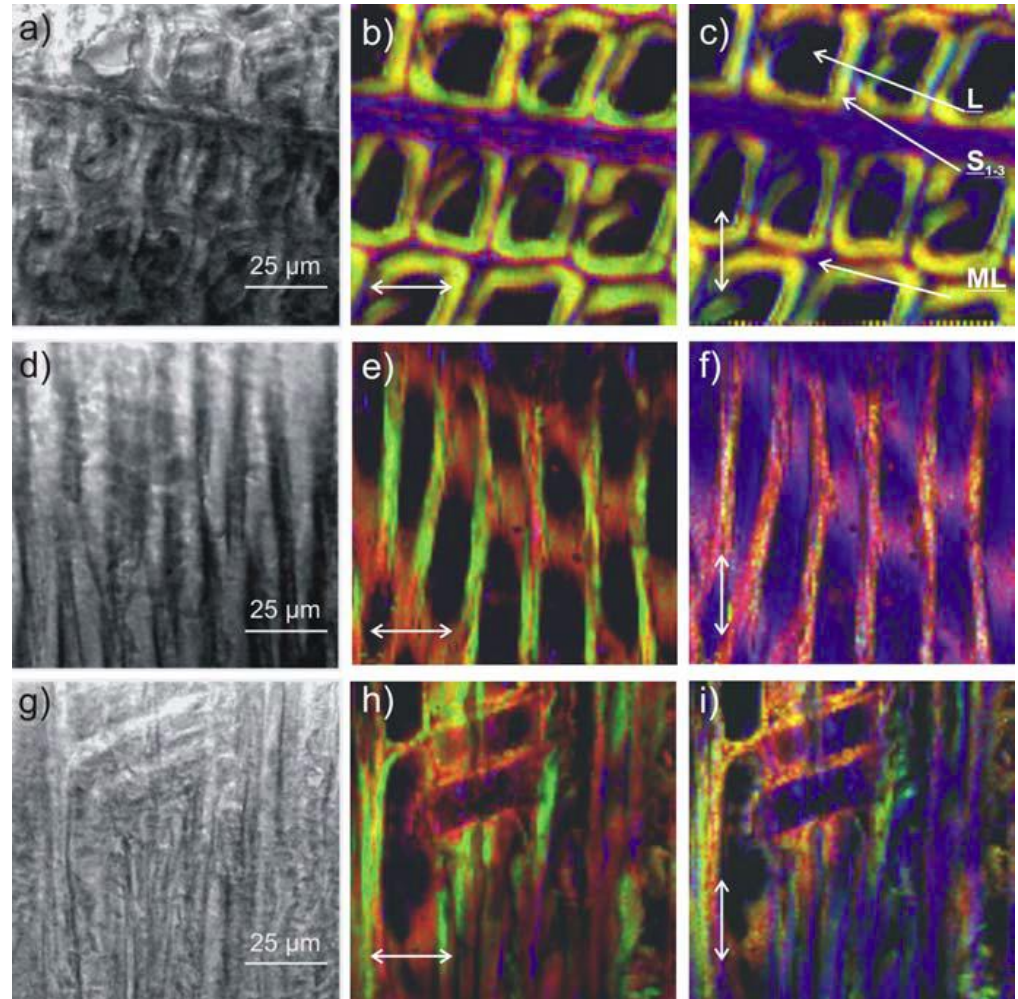
Deuterated Glyphosate crystal in formulation.

Chemical imaging of lignocellulosic biomass by CARS microscopy



Green - cellulose
 Red - xylan
 Blue - lignin

Cellulose alignment



spruce

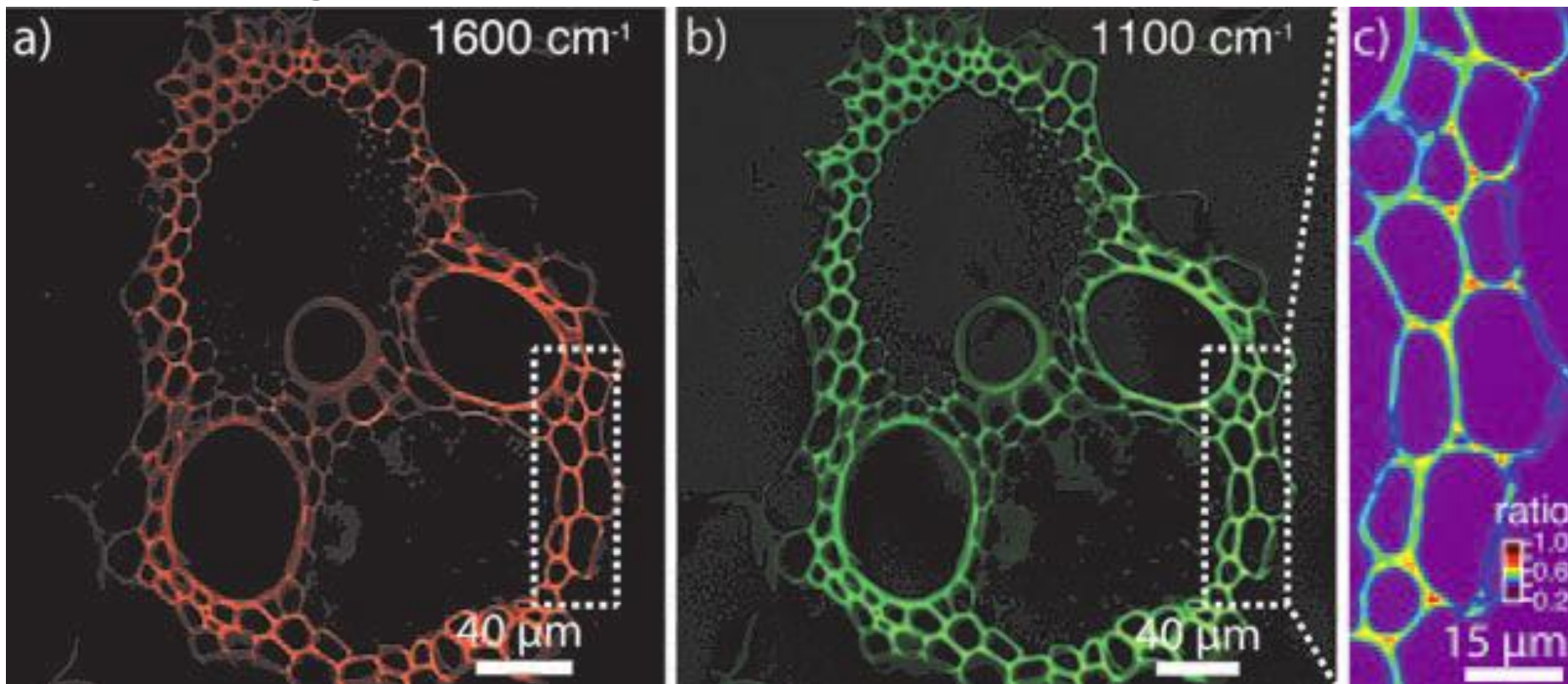
birch

oak

Label-Free, Real-Time Monitoring of Biomass Processing with Stimulated Raman Scattering Microscopy

Lignin

Cellulose



Saar, Brian G., et al. "Label-Free, Real-Time Monitoring of Biomass Processing with Stimulated Raman Scattering Microscopy." *Angewandte Chemie International Edition* 49.32 (2010): 5476-5479.

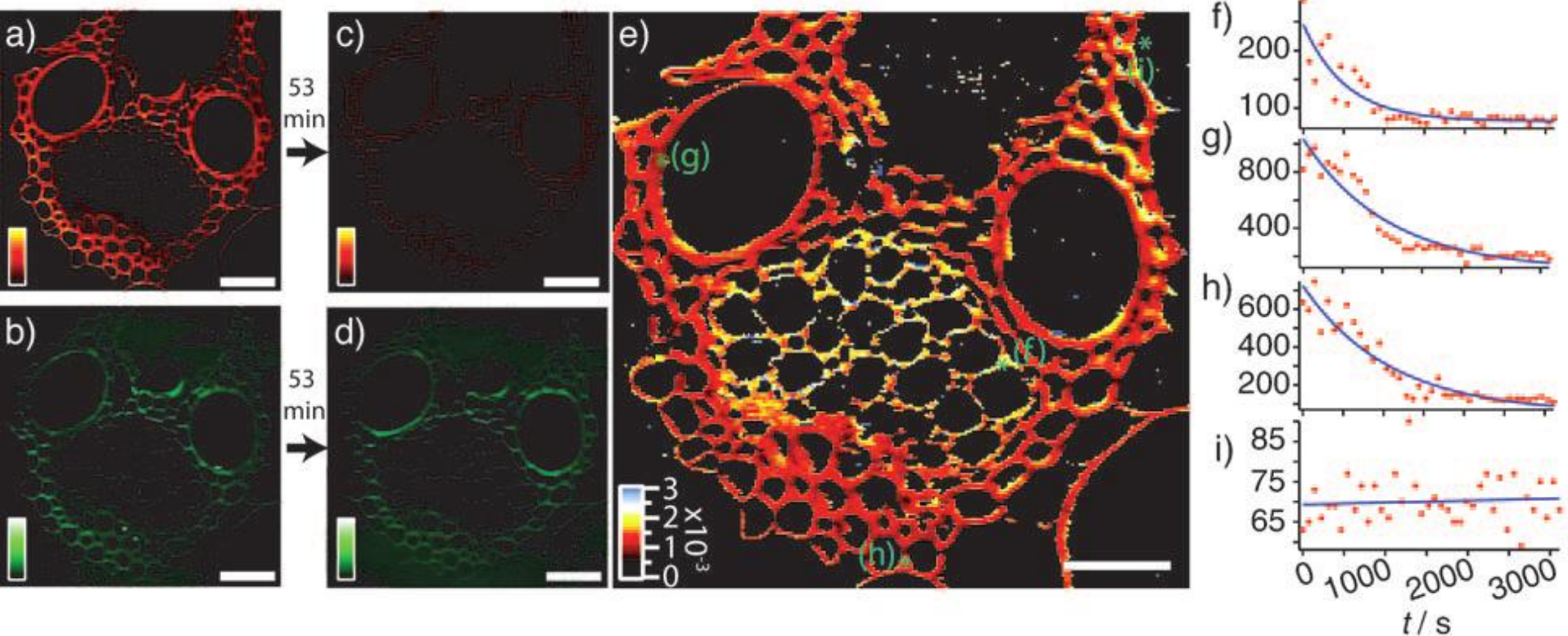
Label-Free, Real-Time Monitoring of Biomass Processing with Stimulated Raman Scattering Microscopy

delignification reaction in corn stover

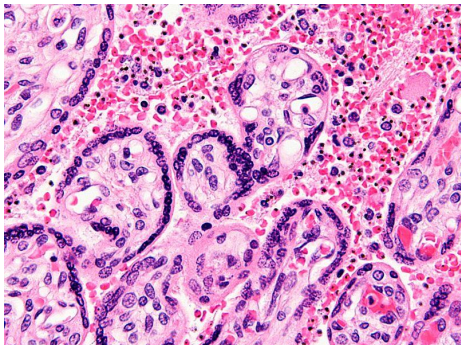
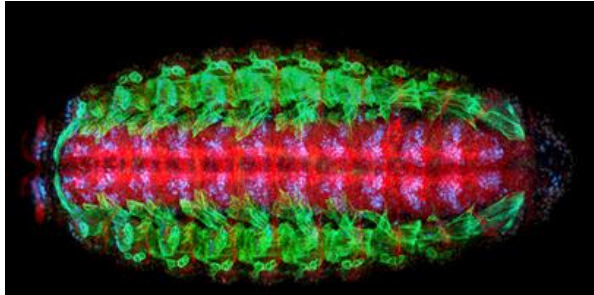
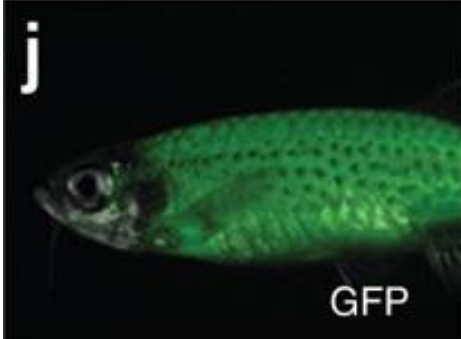
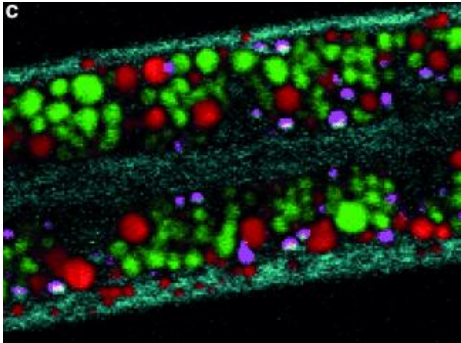
$t = 0$

$t = 53 \text{ min}$

False-color heat map of the reaction rate constant



Saar, Brian G., et al. "Label-Free, Real-Time Monitoring of Biomass Processing with Stimulated Raman Scattering Microscopy." *Angewandte Chemie International Edition* 49.32 (2010): 5476-5479.

Method	Selectivity	Sample image
Basophilic and acidophilic staining	Acidic/Basic components	
Immuno-histochemistry	What antibodies bind	
Fluorescent protein labeling	Expressed genetically modified proteins	
Raman scattering	Molecular vibrations	

Objectives of the lecture

After the lecture I hope that you are able to:

- Using energy diagram of the molecule explain how different light-molecule interactions can be used in microscopy and what information about molecule can be extracted.
- Make a sketch of spontaneous, stimulated and coherent anti-Stokes Raman scattering using energy diagram of the molecule and explain how Raman spectra are measured.
- Compare fluorescence labeling microscopy with coherent Raman scattering microscopy in terms of chemical specificity, resolution and invasiveness of the method.

Nonlinear Raman – staining/labeling

Raman

- Specific to what?

Think in terms of your research

- Invasive? How?
- Resolution. What are limiting factors?
- What else might be of interest to compare?

Staining/labeling

Nonlinear Raman – staining/labeling

Raman

- Probes vibrations. Specific only if substances have different spectral components
- High powers, in case of intrinsic pigments can lead to photodamage
- NIR light + nonlinearity: resolution comparable to VIS. Advantage – intrinsic confocality.

Staining/labeling

- Specificity depends on the label. In case of antibodies – as specific as antibodies can be.
- Staining/labeling modifies the object of study. Care should be taken about the level of interference.
- Can be used with multi-photon excitation techniques

Do you have questions?

Do you have questions?

- What is Raman scattering?
- Molecular vibrations – what is it?
- Why nonlinear microscopy is intrinsically “confocal”?
- There are many nonlinear optical processes?
Why should I care? 😊

Thanks!

If you still have questions,
feel free to contact me at
juris@chalmers.se