

# **Spatial Metabolic Imaging via Functional Bond-Selective Microscopy**

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California Institute of Technology

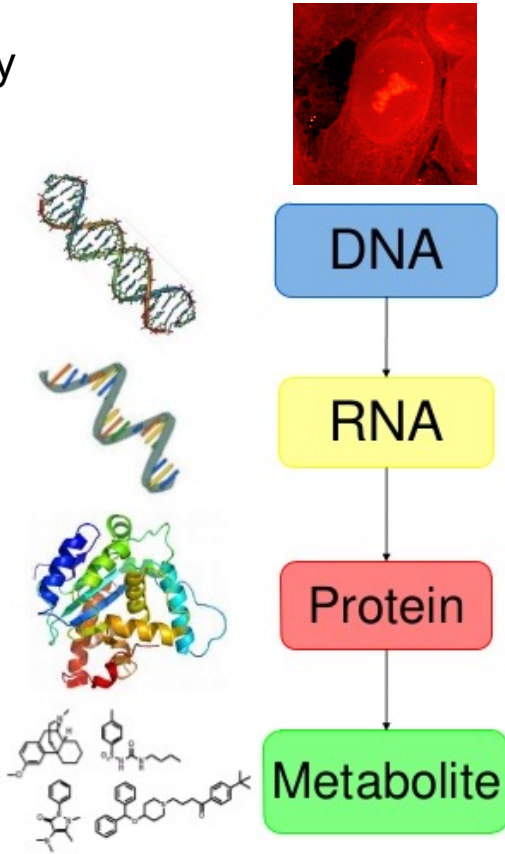
Spatial Multi-Omics for Cancer Systems Biology Workshop, 2024

2/12/24

# The Central Dogma of Human Biology

Complexity

Function/phenotype  
relevance



DNA

*What can happen*

RNA

*What appears to  
be happening*

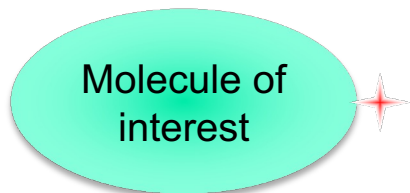
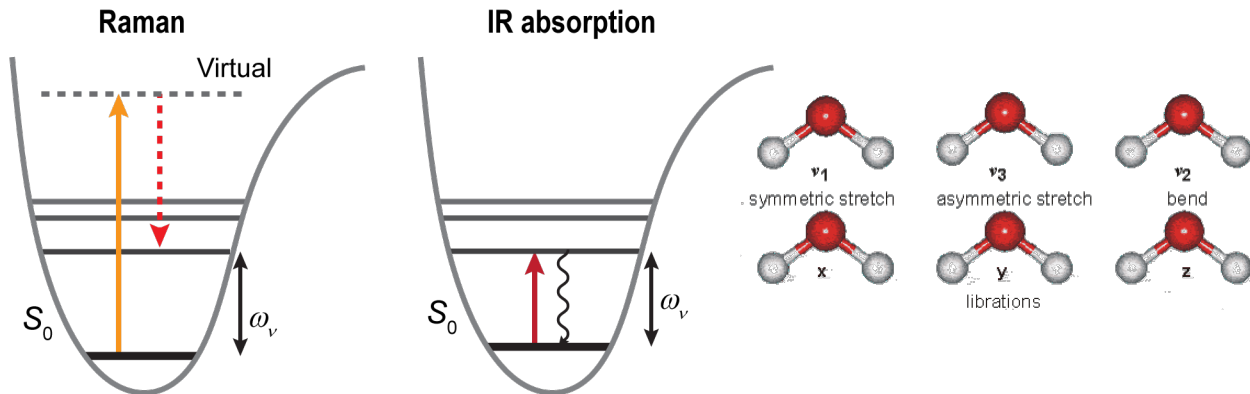
Protein

*What makes it  
happen*

Metabolite

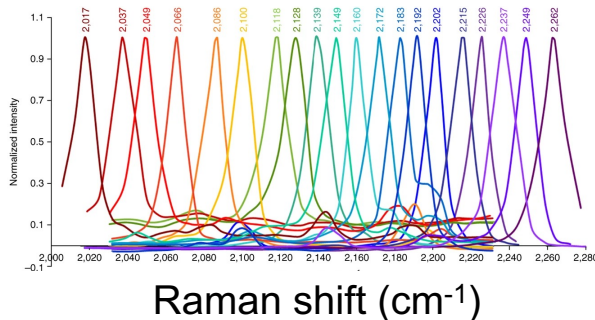
*What has happened  
and is happening*

# Bond-Selective Imaging Harnessing Molecular Vibrations



C-D; C $\equiv$ N; C $\equiv$ C

J. Du & L. Wei, *ACS Chem. Biol.*, (2022).



L. Wei, *et al. Nature* (2017).  
F. Hu, *et al. Nat. Methods* (2018).

# Next-generation Optical Microscopy: Functional Bond-selective Imaging

- Weak signal
- Low sensitivity ( $\sim$ mM)
- High cellular fingerprint background
- Low spatial resolution (IR)



- Going Nonlinear
- ✓ Stimulated Raman Scattering Microscopy
- ✓ **Bond-Selective Fluorescence-Detected IR-Excited (BonFIRE) Spectro-Microscopy**
- Using Chemical Tags
- ✓ C-D; C $\equiv$ N; C $\equiv$ C vibrational tags
- ✓ **C $\equiv$ N & C $\equiv$ C bearing fluorescent dyes**

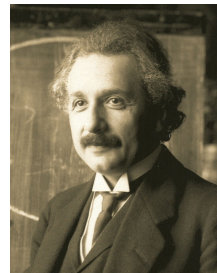
# From Spontaneous Raman Scattering to Stimulated Raman Scattering

Raman scattering

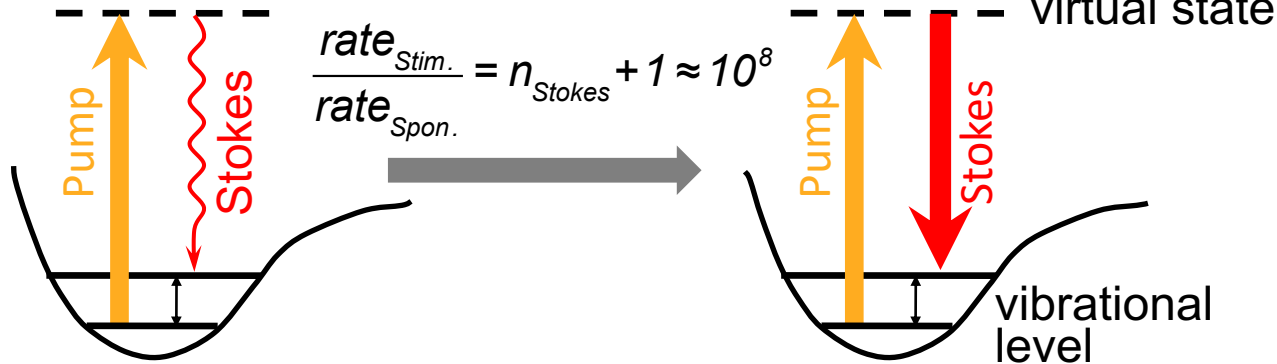


C. V. Raman

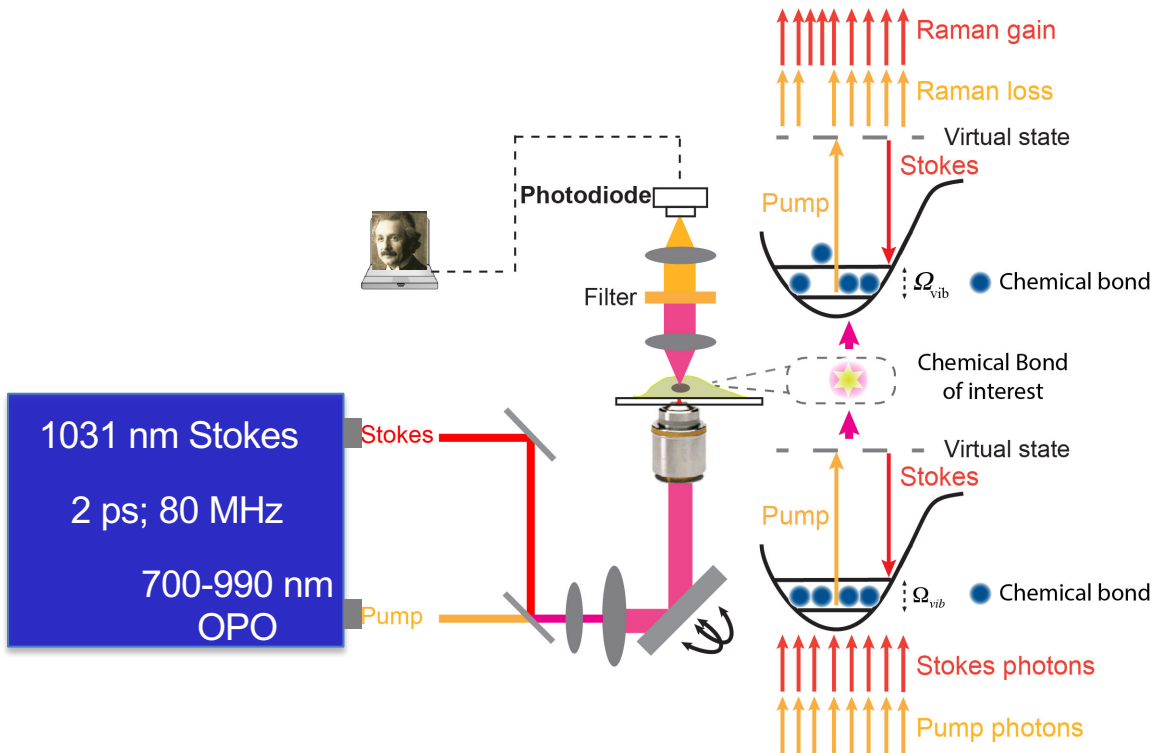
Stimulated emission



A. Einstein

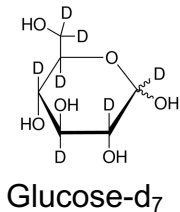
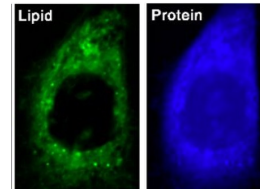
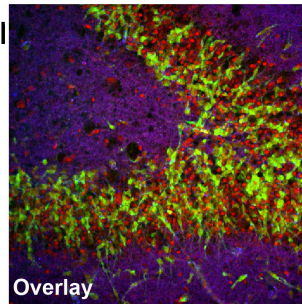
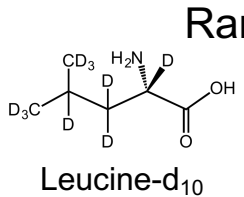
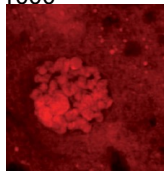
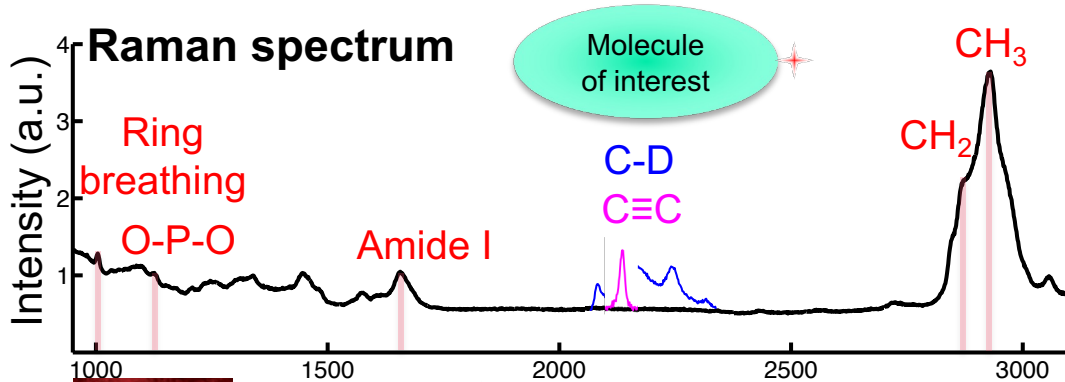


# Stimulated Raman Scattering (SRS) microscopy

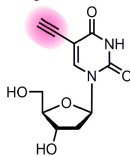


C. Freudiger\*, W. Min\* *et al.* *Science*, 322, 1857 (2008).  
W. Min *et al.* *Annu. Rev. Phys. Chem.*, 62, 507 (2011).

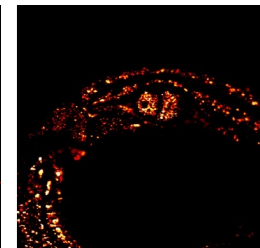
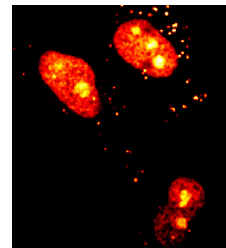
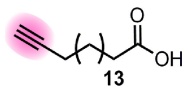
# Stimulated Raman Scattering (SRS) microscopy



**5-Ethynyl-2'-deoxyuridine  
(Deoxyribonucleoside)**



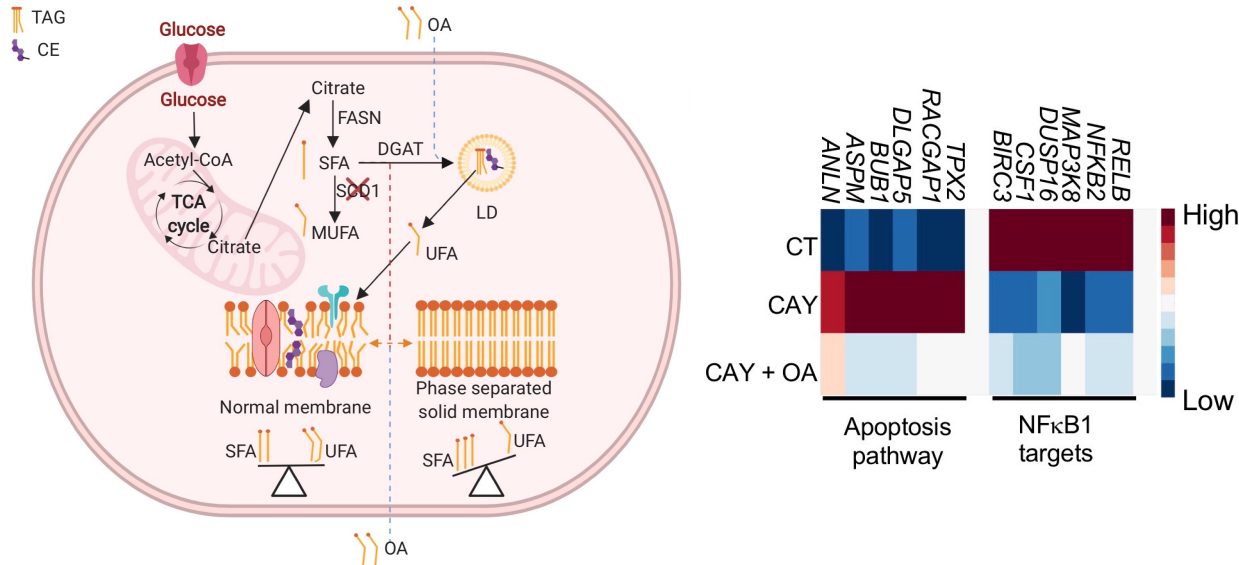
**17-Octadecynoic acid  
(Fatty acid)**



# Interrogating Cellular Metabolism in Live Cells and Tissues

Chemical composition; synthesis, conversion, transportation, degradation

## Raman-guided subcellular pharmaco-metabolomics for metastatic melanoma cells



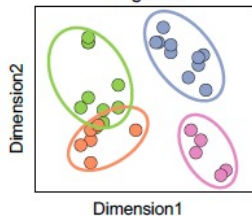


# Raman-guided Subcellular Pharmaco-Metabolomics for Metastatic Melanoma Cells

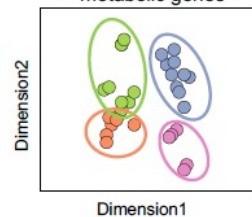
a

● Melanocytic ● Neural crest  
● Transitory ● Undifferentiated

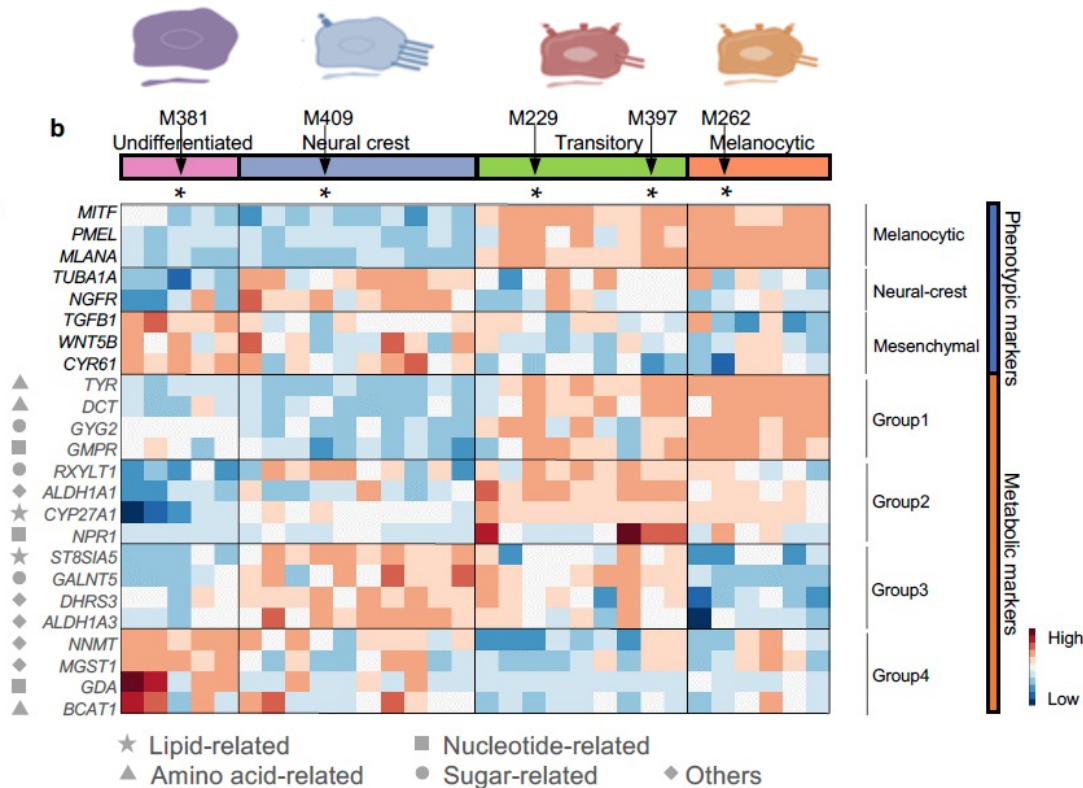
Clustering based on all genes



Clustering based on metabolic genes



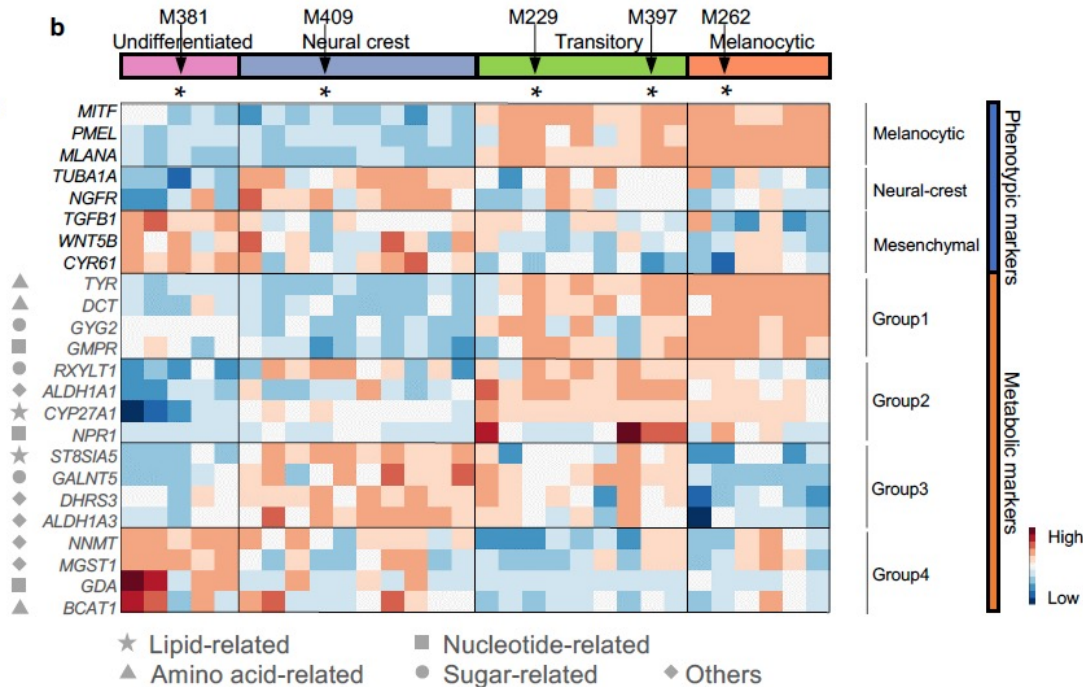
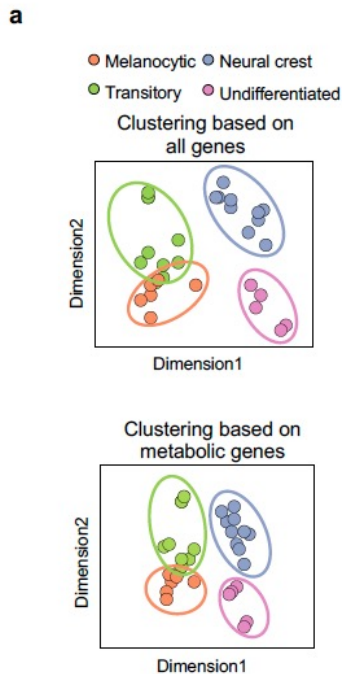
b



*J. Tsoi, et al. Cancer Cell 33, 890 (2018)*

*J. Du, Y. Su et al... J. Heath\* & L. Wei\*, Nat. Commun. 4830 (2020)*

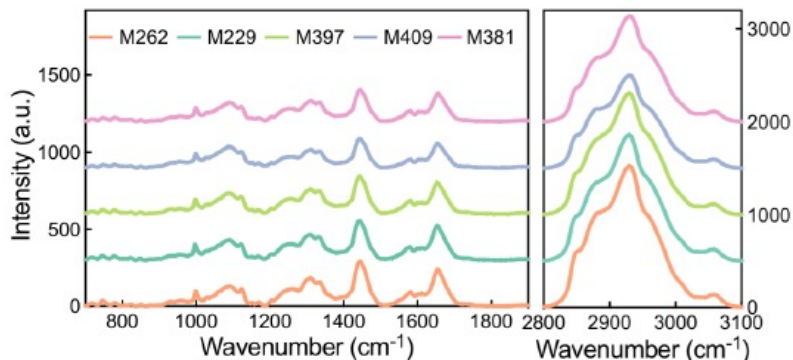
# Raman-guided Subcellular Pharmaco-Metabolomics for Metastatic Melanoma Cells



*J. Tsoi, et al. Cancer Cell 33, 890 (2018)*

*J. Du, Y. Su et al... J. Heath\* & L. Wei\*, Nat. Commun. 4830 (2020)*

# Raman Analysis For Metastatic Melanoma Cells

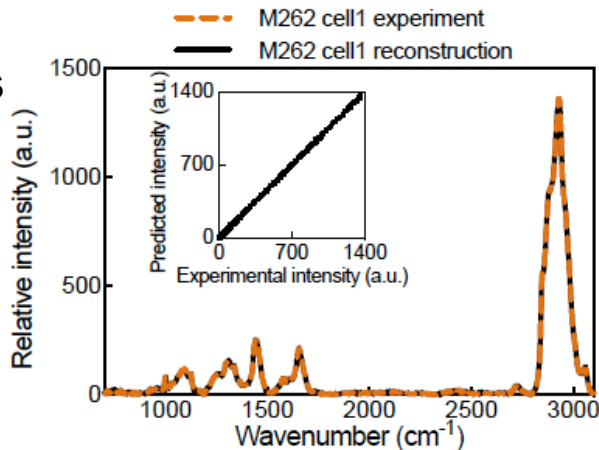


5 spots/cell  
10 cells/sample  
50 spectra/sample

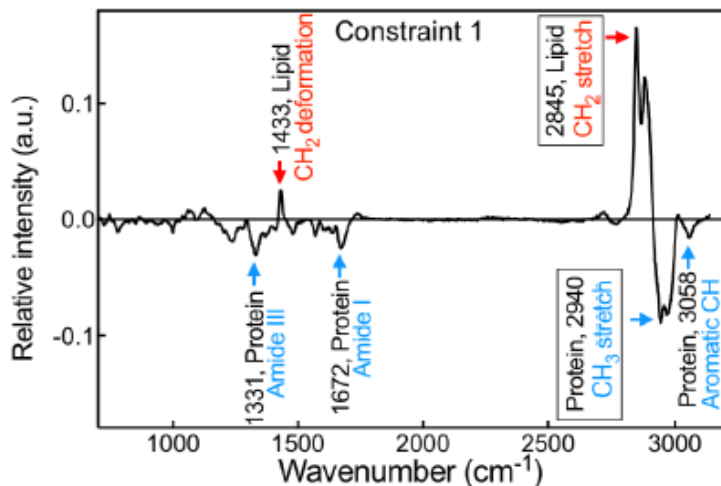
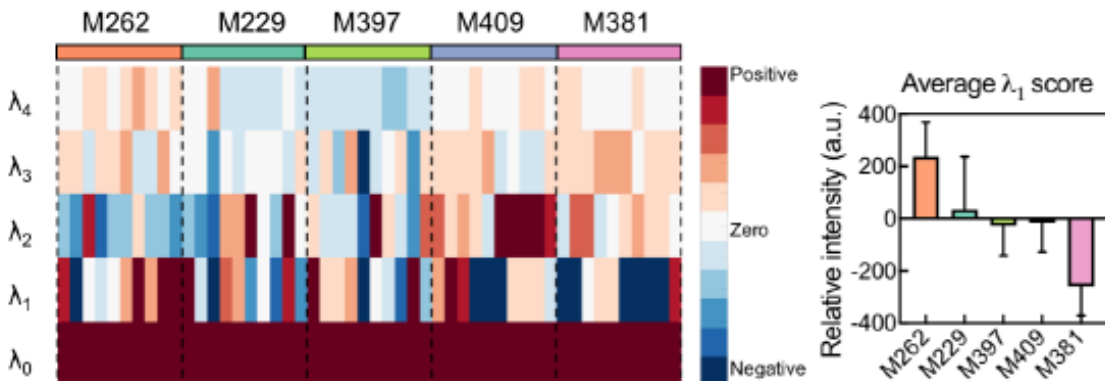
## Unsupervised Surprisal Analysis for dimension reduction

S. Zadran et al. PNAS, 111, 13235 (2014)

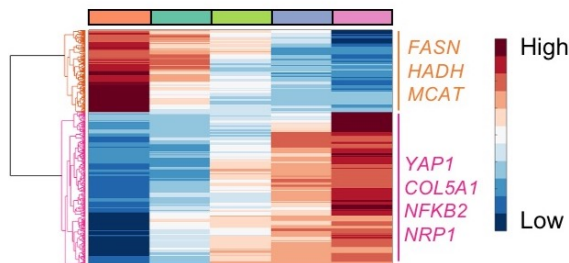
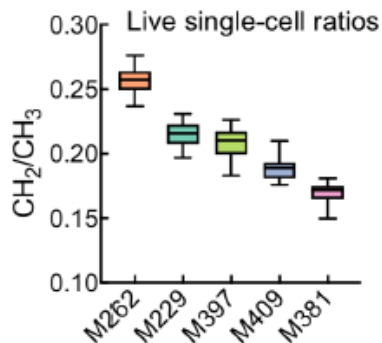
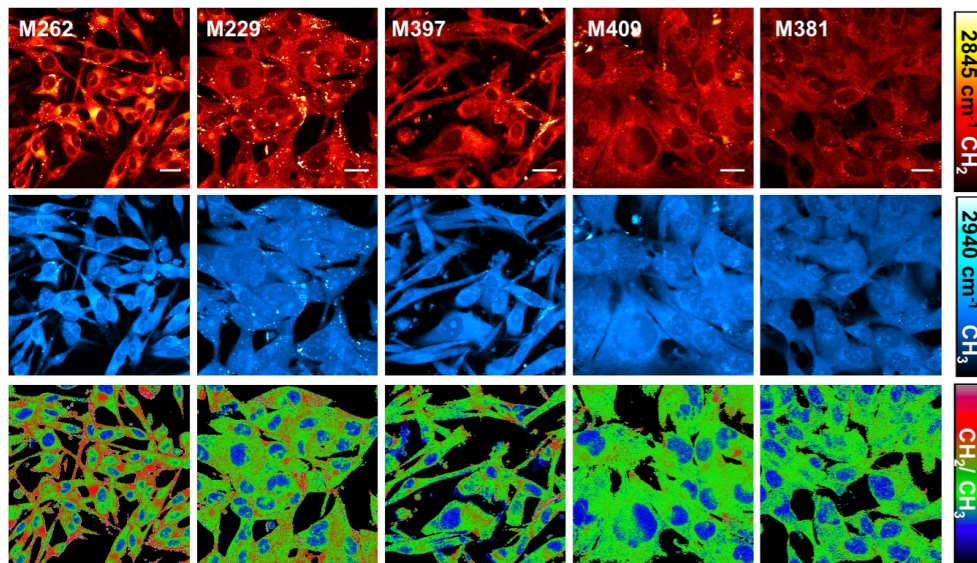
<https://github.com/mesako/Melanoma-Publication>



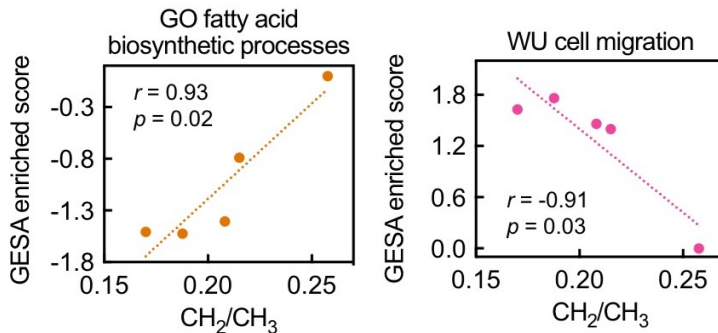
# Raman Analysis Reveals Relative Higher Lipid Signals in the Melanocytic Phenotype



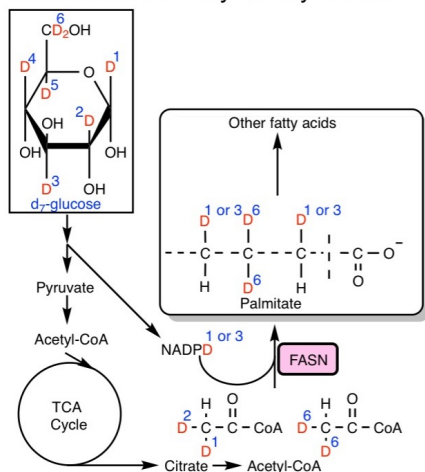
# SRS Imaging on Live Metastatic Melanoma Cells



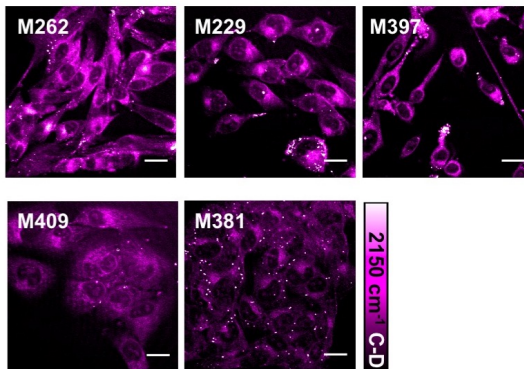
# Correlation Between Raman and Gene Expression Analysis



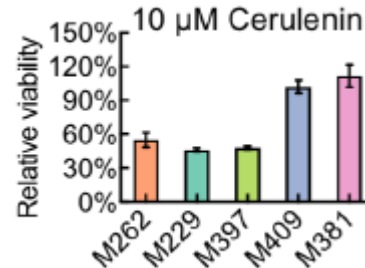
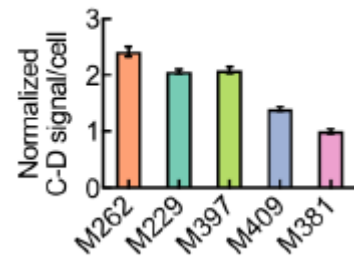
Glucose derived fatty acid synthesis



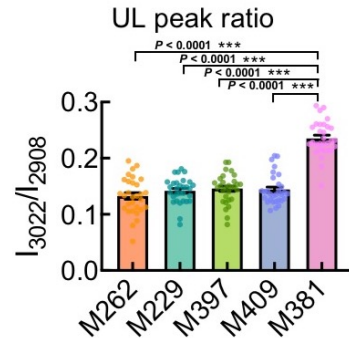
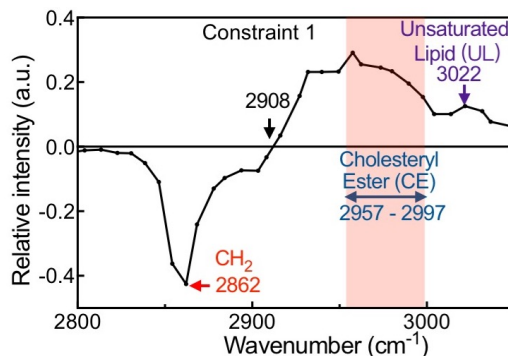
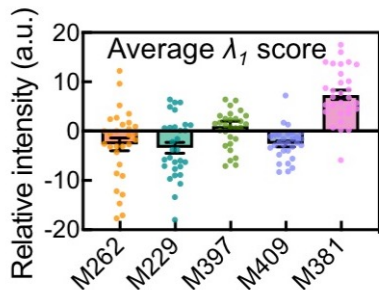
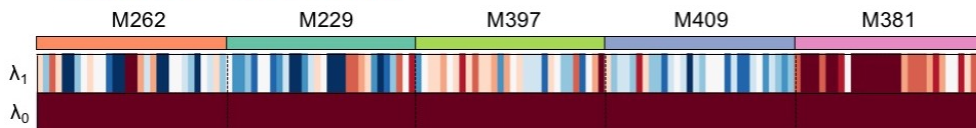
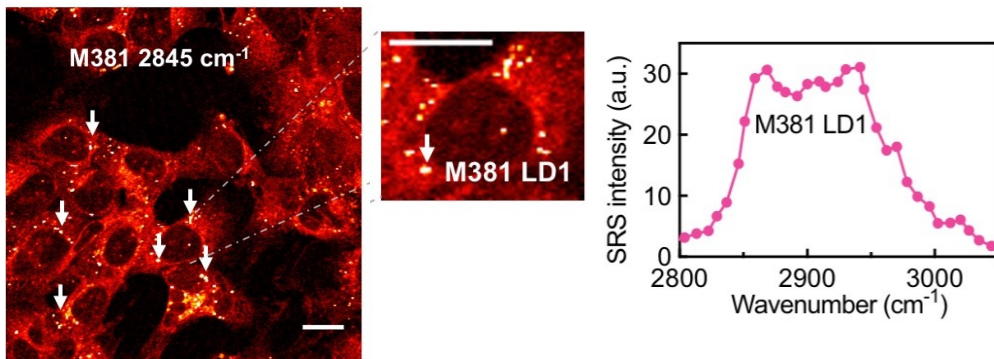
72h d<sub>7</sub>-glucose labeling → Imaging



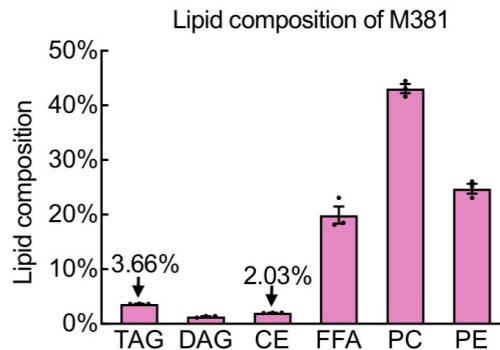
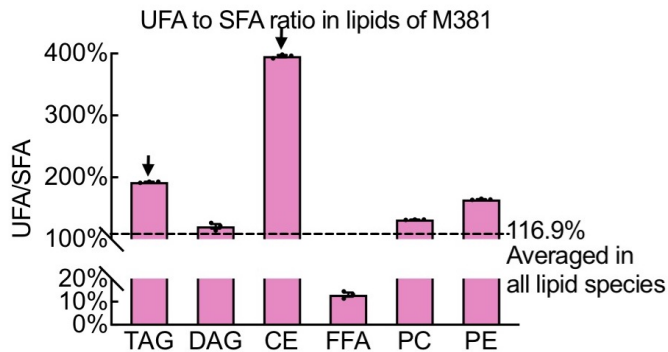
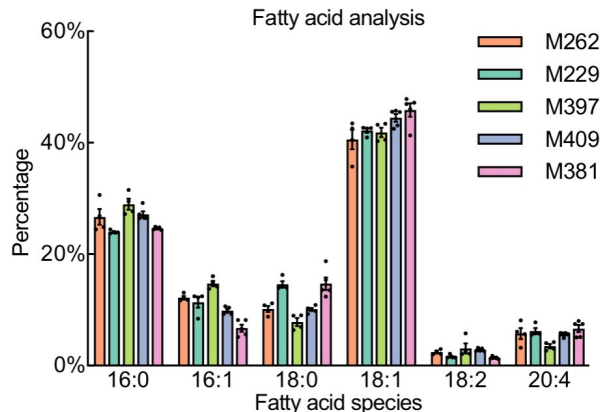
d<sub>7</sub>-glucose labeling



# Raman-guided Subcellular Pharmaco-Metabolomics from Lipid Droplets



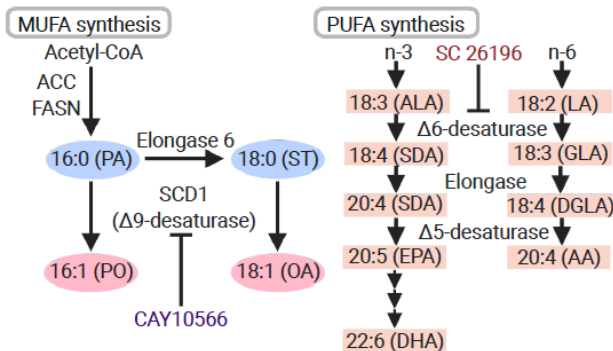
# Raman-guided Subcellular Pharmaco-Metabolomics for Metastatic Melanoma



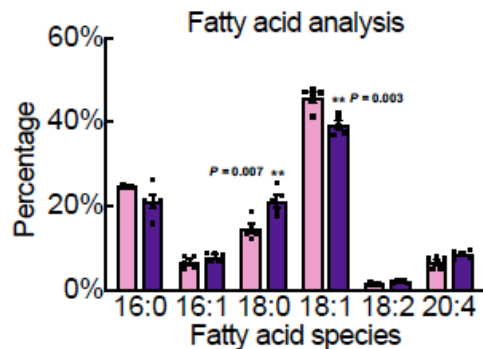
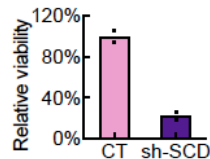
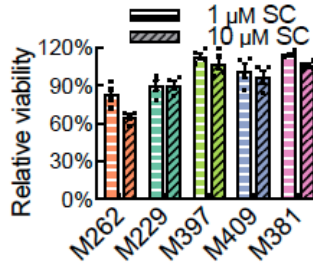
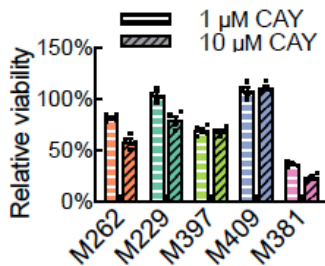
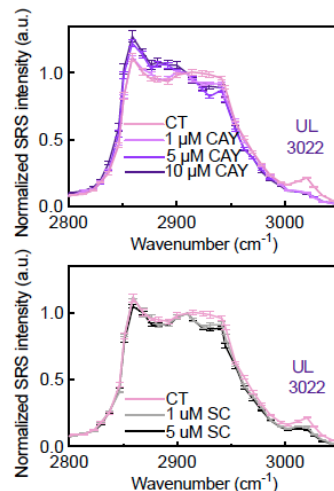


# Raman-guided Subcellular Pharmaco-Metabolomics for Metastatic Melanoma

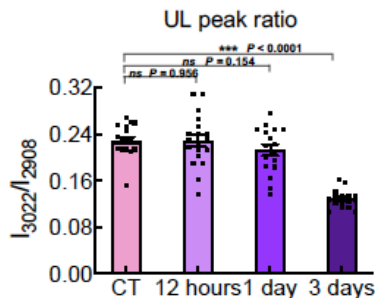
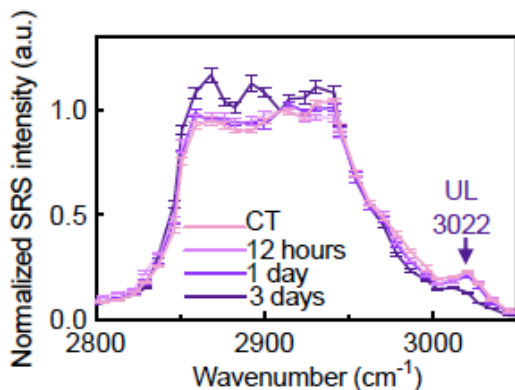
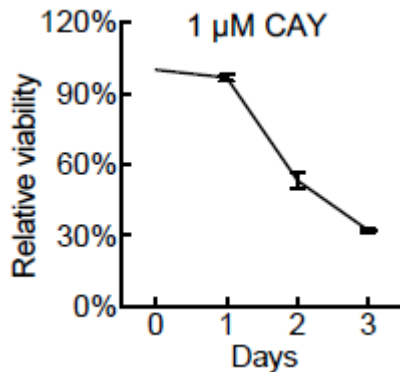
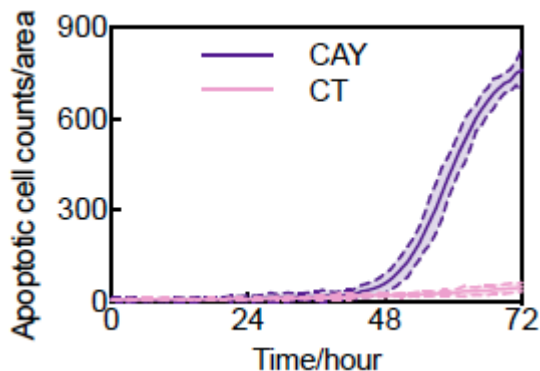
a



b

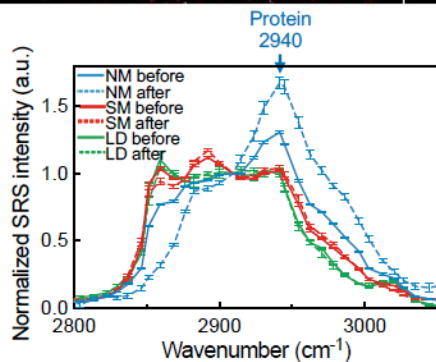
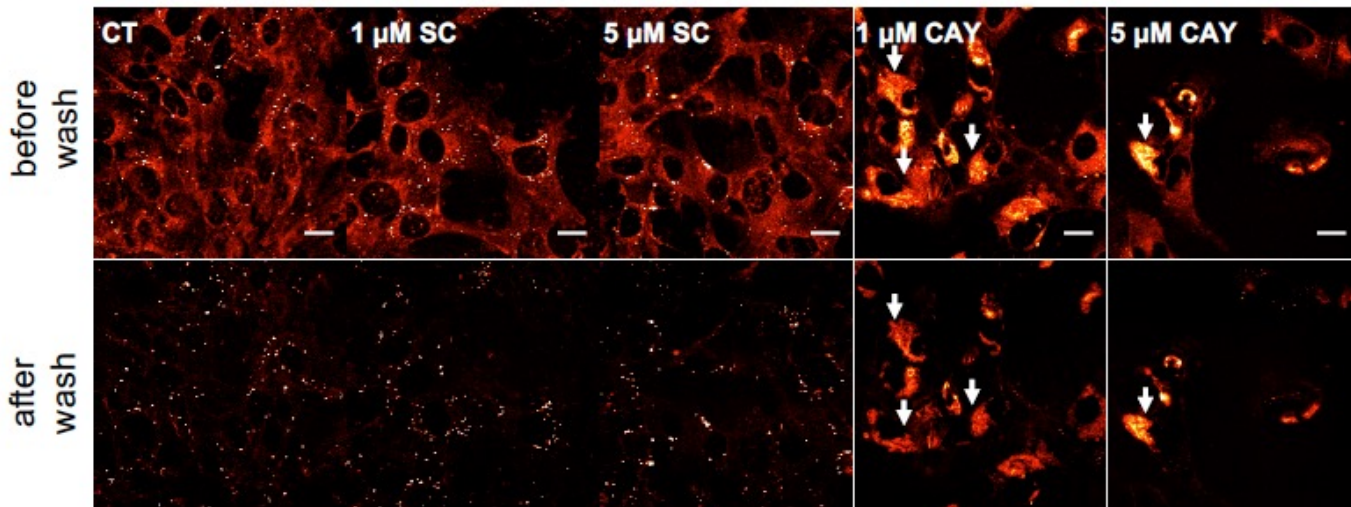


# Raman-guided Subcellular Pharmaco-Metabolomics for Metastatic Melanoma

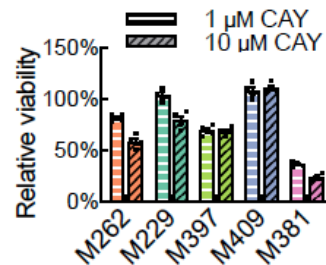
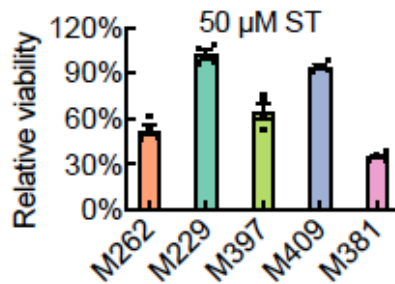
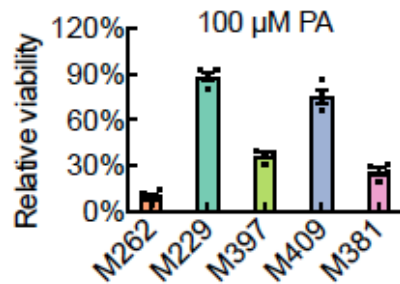
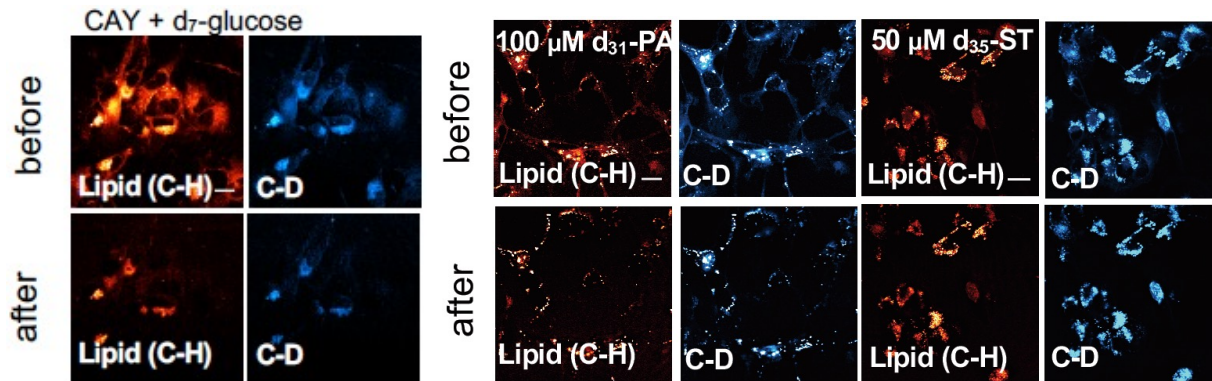


# Raman-guided Subcellular Pharmaco-Metabolomics for Metastatic Melanoma

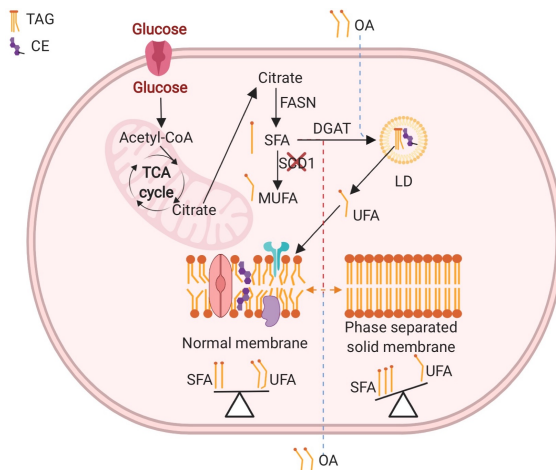
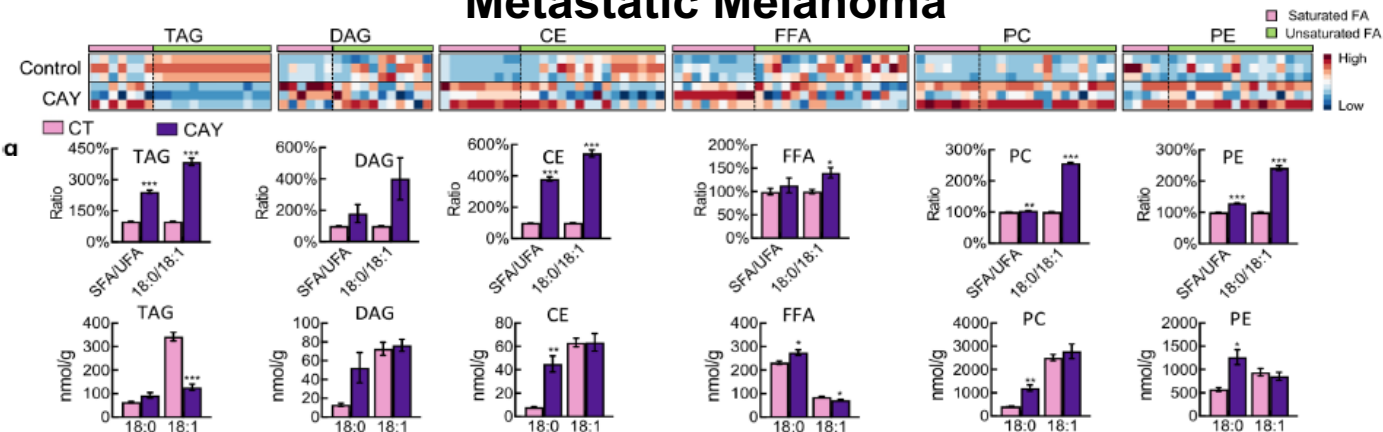
Lipid channel:



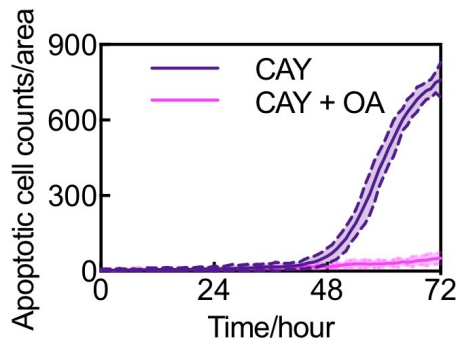
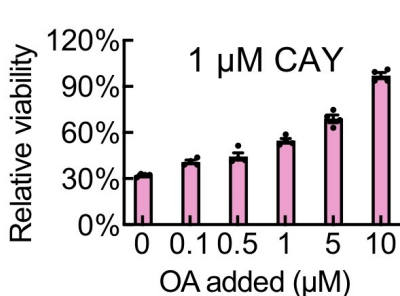
# Raman-guided Subcellular Pharmaco-Metabolomics for Metastatic Melanoma



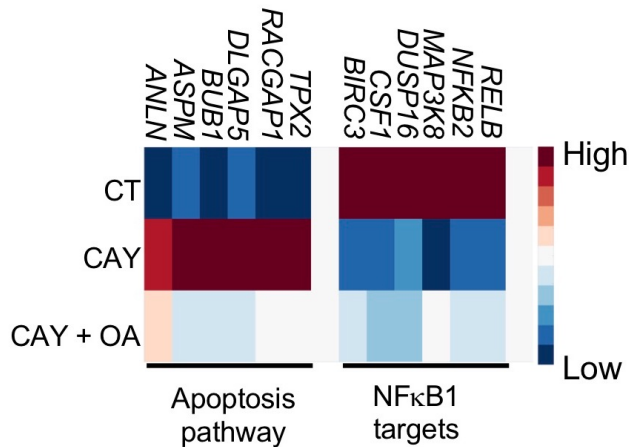
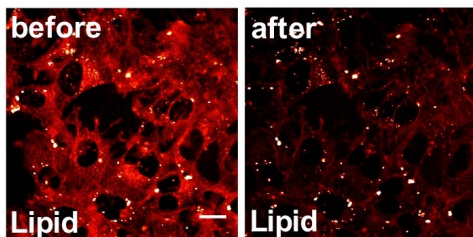
# Raman-guided Subcellular Pharmaco-Metabolomics for Metastatic Melanoma



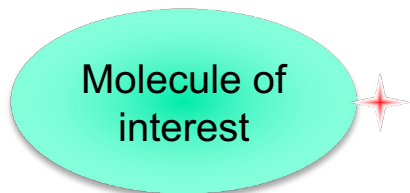
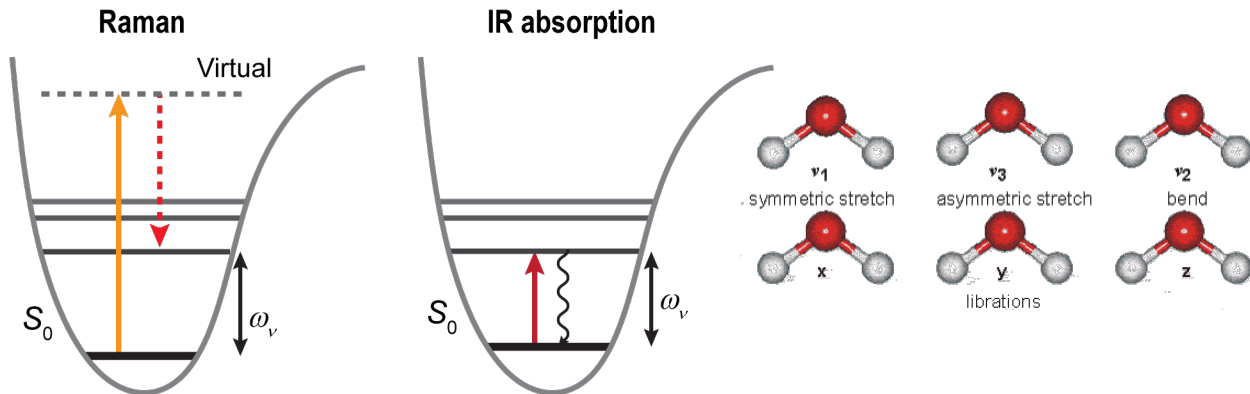
# Raman-guided Subcellular Pharmaco-Metabolomics for Metastatic Melanoma



5  $\mu$ M CAY + 10  $\mu$ M OA

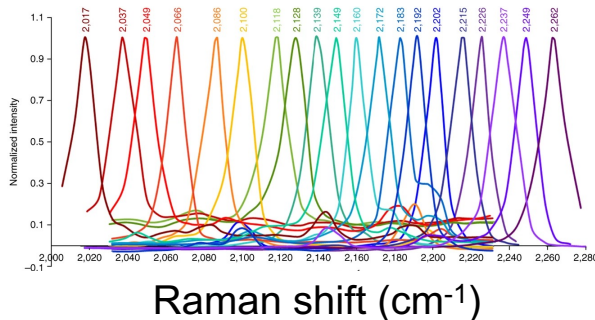


# Bond-Selective Imaging Harnessing Molecular Vibrations



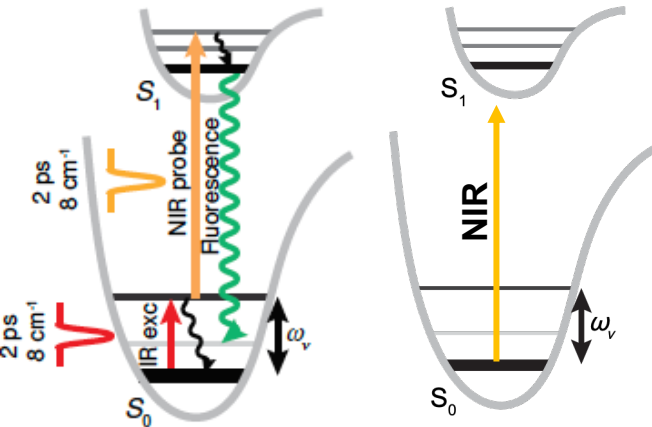
C-D; C $\equiv$ N; C $\equiv$ C

J. Du & L. Wei, *ACS Chem. Biol.*, (2022).



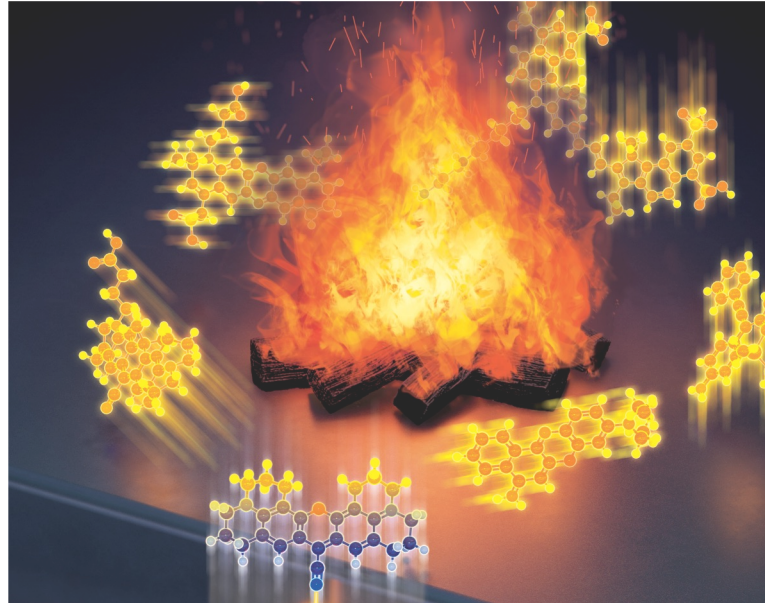
L. Wei, *et al. Nature* (2017).  
F. Hu, *et al. Nat. Methods* (2018).

# From Raman to IR to fluorescence detection: Bond-Selective Fluorescence Imaging with Single-Molecule Sensitivity



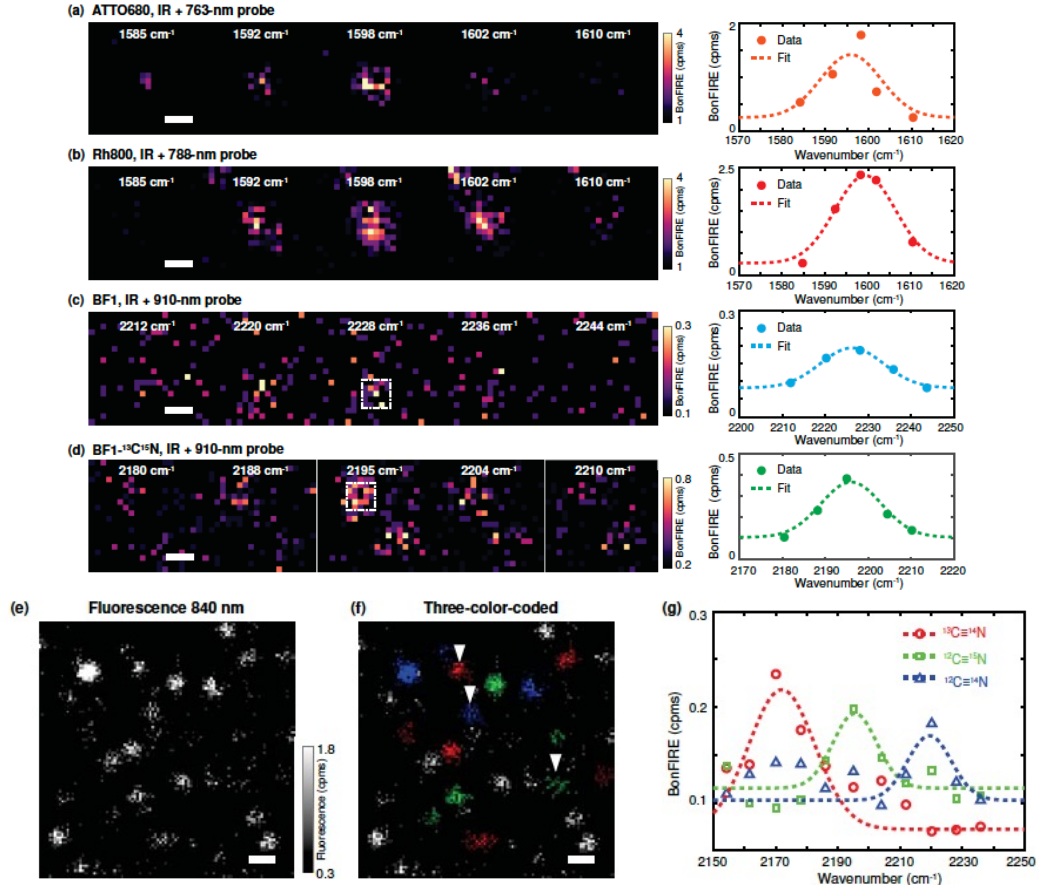
## Bond-Selective Fluorescence-Detected IR-Excited (BonFIRE) Spectro-Microscopy

- Chemical bond selectivity
- Single-molecule sensitivity

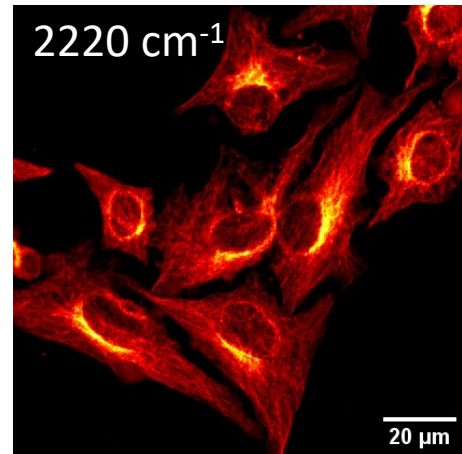
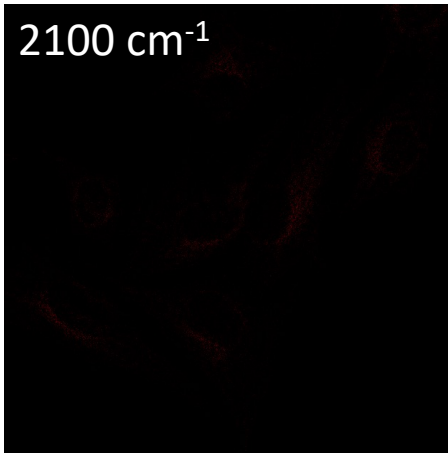
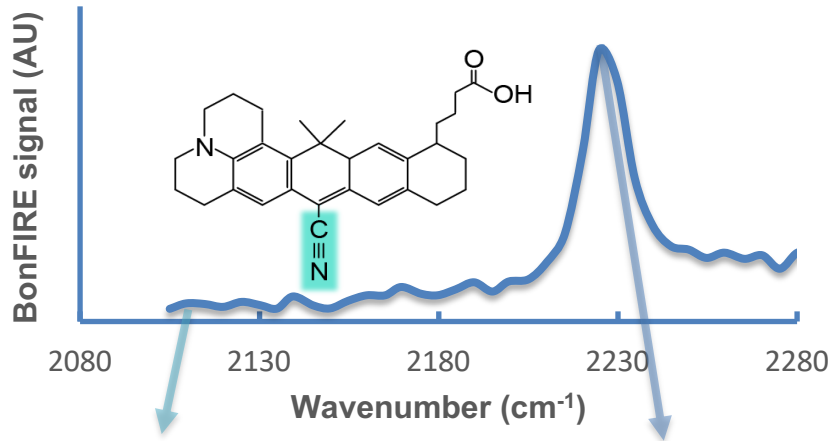




# Single-molecule BonFIRE Spectroscopy and Imaging

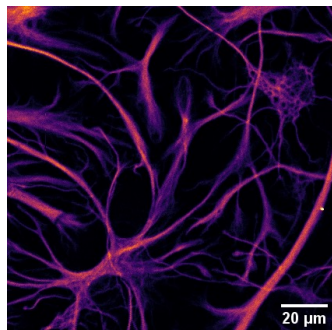


# BonFIRE Bio-Imaging

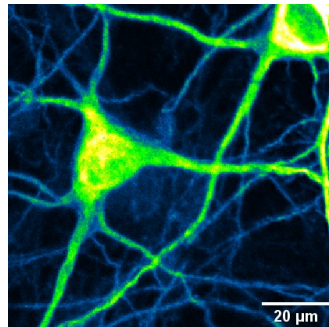


# BonFIRE Bio-Imaging

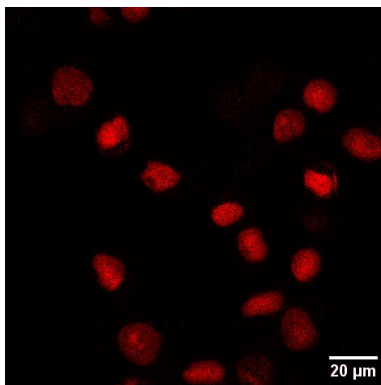
Neuron cells, GFAP,  
MARS2228, 2170  $\text{cm}^{-1}$



Neuron cells, MAP2,  
MARS2228, 2220  $\text{cm}^{-1}$



4-color imaging on nuclei in HeLa cells



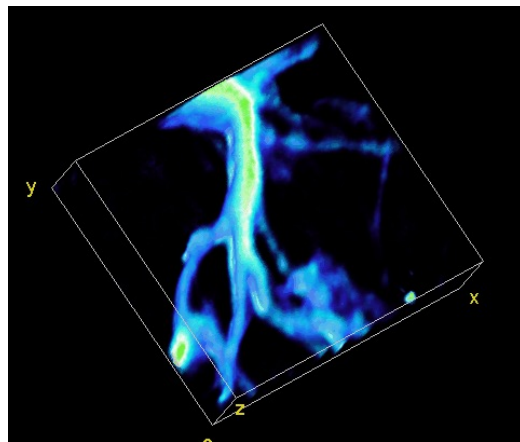
$^{13}\text{C}^{15}\text{N}$ -EdU

$^{13}\text{C}^{14}\text{N}$

$^{12}\text{C}^{15}\text{N}$

$^{12}\text{C}^{14}\text{N}$

3-D brain tissue imaging



# Acknowledgement



Thank you!