Mid-Infrared Photothermal Microscopy

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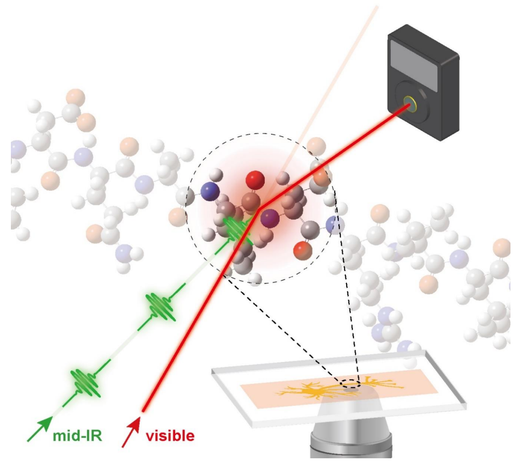
Abstract

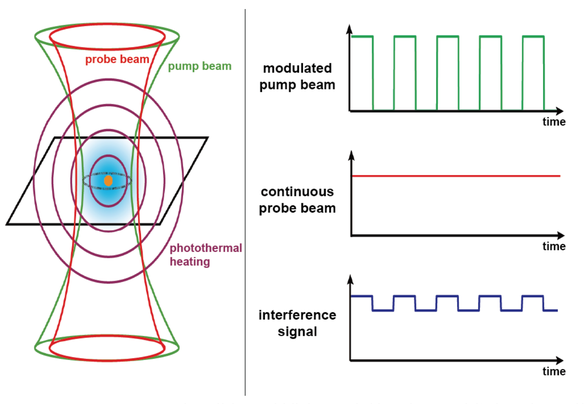
There have been various imaging techniques applying infrared (IR) source for its advantages such as chemical selectivity, non-destructiveness, and label-free character. Recently, one of promising imaging techniques utilizing IR source is an infrared photothermal microscopy. In this IR technique, a mid-IR excitation of specific vibration mode induce a temperature increase and refractive index changes in surrounding medium, which causes the deflection of a probe beam. The nonresonant 640 nm laser source was adopted as a probe to achieve sub-micrometer spatial resolution with high NA reflective objective.

We performed real-time imaging of oligodendrocytes to investigate cellular dynamics throughout the life cycle of a cell, revealing details of the cell division and apoptosis, as well as cellular migration. In the case of live neurons, we observed a photothermal contrast associated with travelling protein complexes on an axon, which correspond to transport of vesicles from the cell body to the dendritic branches of the neuron through the cytoskeleton. We anticipate that mid-infrared photothermal imaging will be of great use for gaining insights into the field of the biophysical science, especially with regard to cellular dynamics and functions.



Introduction

· Infrared photothermal microscopy



http://sites.middlebury.edu/durst/research/#photothermal/

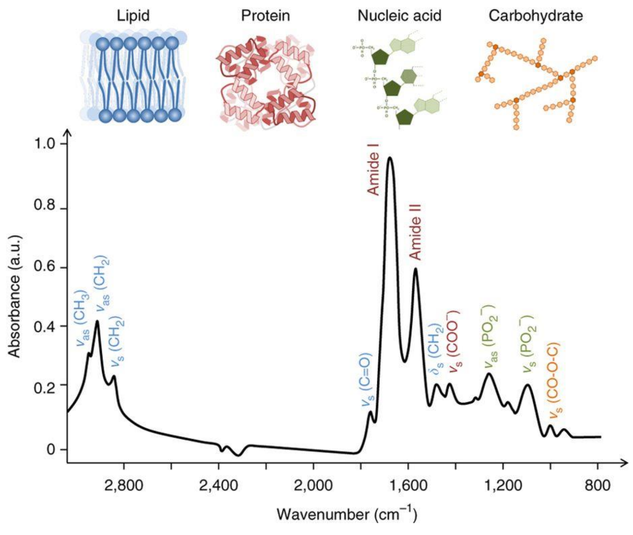
This technique is based on detection of non-fluorescent objects. It relies on infrared absorption properties of objects and can be realized using a **resonant modulated heating beam**, **non-resonant probe beam** and **lock-in detection of photothermal signals**.

The high sensitivity and selectivity of photothermal microscopy allows even the detection of single molecules by their absorption.

$$\Delta P_{probe} \propto \frac{\sigma N}{\kappa C_p}\frac{\partial n}{\partial T}P_{probe}P_{pump}$$

σ : absorption cross section
N : number density
κ : heat conductivity
C_p : heat capacity
n : refractive index
T : temperature
P_{probe} : probe power
P_{pump} : pump power

· Infrared in biology

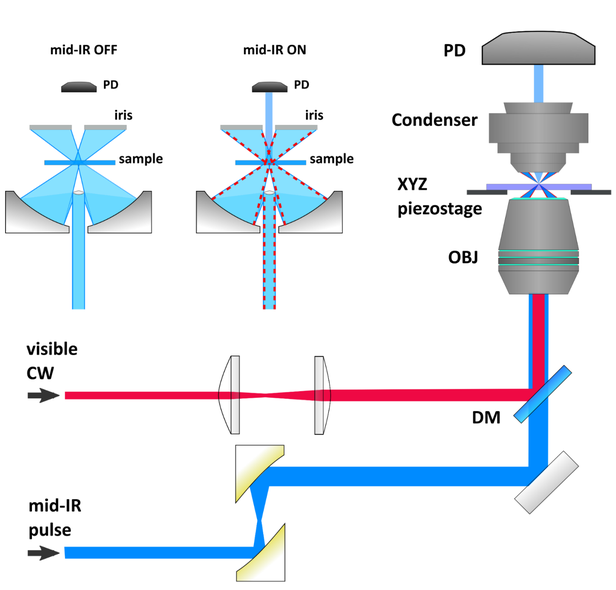


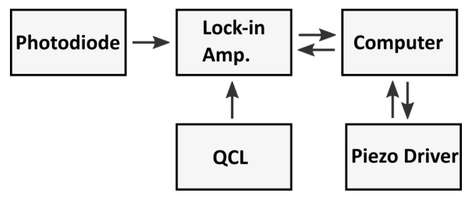
Baker, M. J. et al. *Nature protocols* **2014**, 9, 1771–1791.

A typical biological IR spectrum is shown left. These vibrational modes are quantitatively measurable by IR spectroscopy, providing a **unique, label-free** tool for studying molecular composition and dynamics **without perturbing the samples**. For interrogating biological samples, the most important spectral regions measured are typically the fingerprint region (600–1,450 cm^{-1}) and the amide I/II region (1,500–1,700 cm^{-1}). The higher-wavenumber region (2,550–3,500 cm^{-1}) is associated with stretching vibrations such as C-H, N-H and O-H.

Result & Discussion

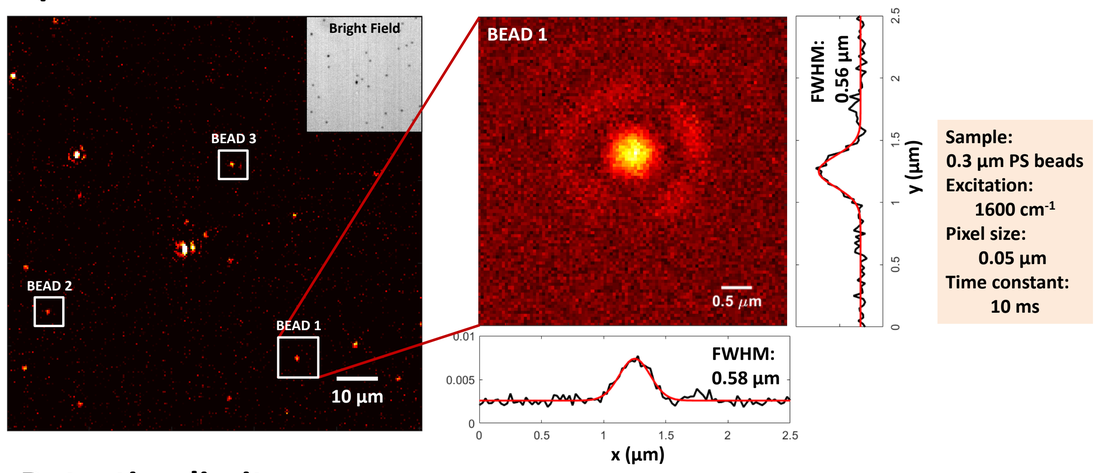
· Experimental setup & Detection scheme





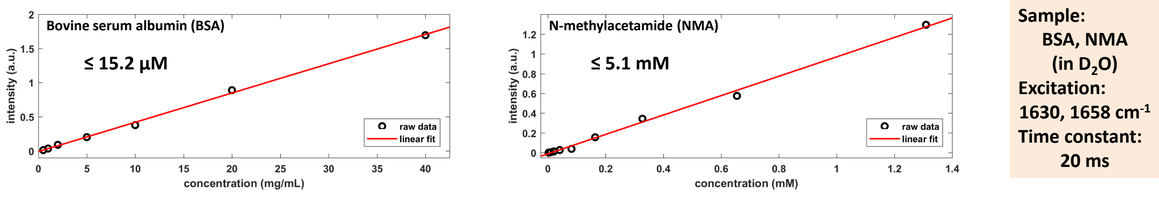
Mid-IR source (QCL):
1495-1730 cm^{-1}
Visible (DPSS laser): 640 nm
Microscope objective:
74x, NA 0.66, reflection-type
Condenser: 0.55 NA
Multi-axes scanning stage:
200 µm × 200 µm × 200 µm
HF2LI Lock-in Amplifier:
50 MHz, 210 MSa/s

· Spatial resolution



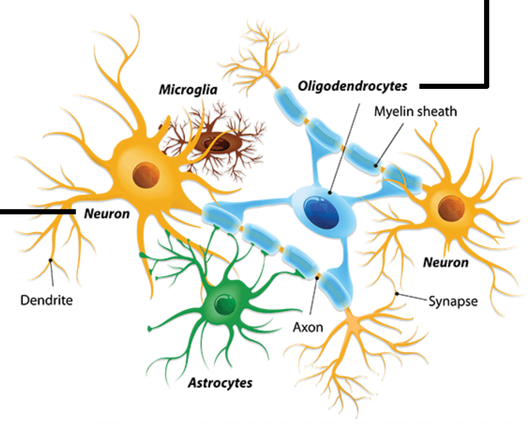
Sample:
0.3 µm PS beads
Excitation:
1600 cm^{-1}
Pixel size:
0.05 µm
Time constant:
10 ms

· Detection limit



Sample:
BSA, NMA
(in D_2O)
Excitation:
1630, 1658 cm^{-1}
Time constant:
20 ms

· Live-cell imaging



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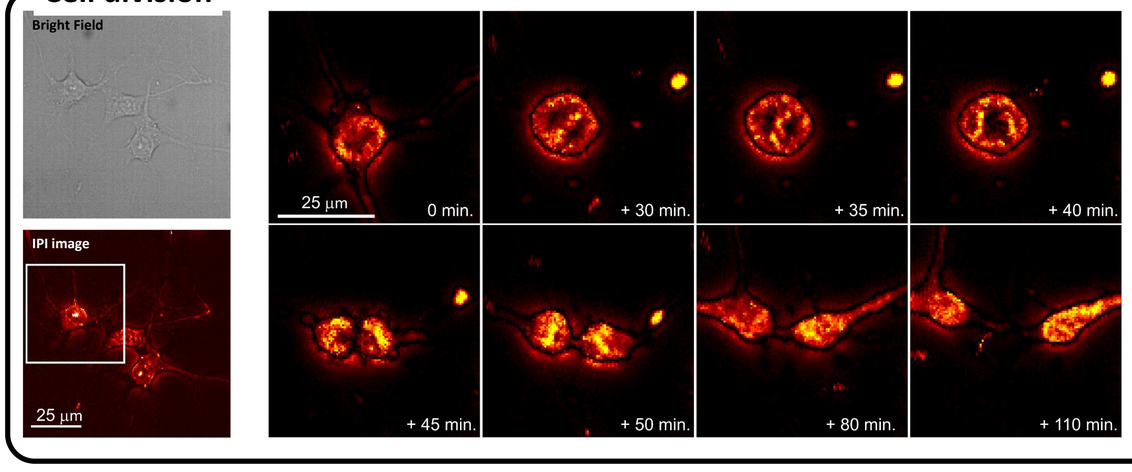
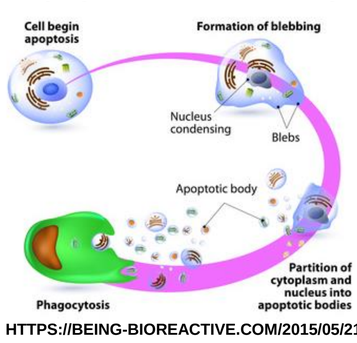
Cell division



Play video 1

Sample: Oligo-dendrocyte
Excitation:
1650 cm^{-1}
Image size:
50X50 µm²

Apoptotic disassembly



Play video 2

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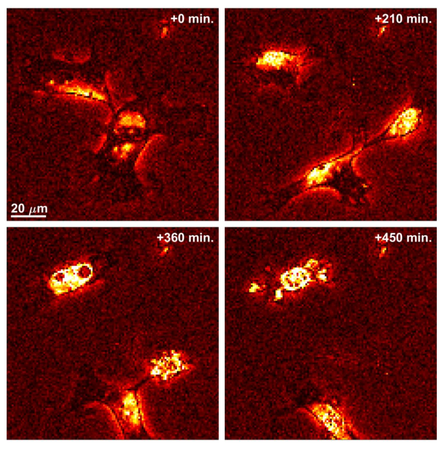
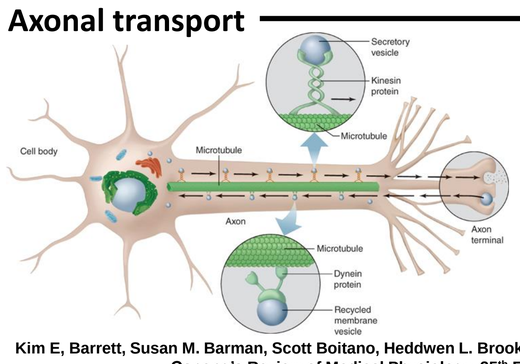
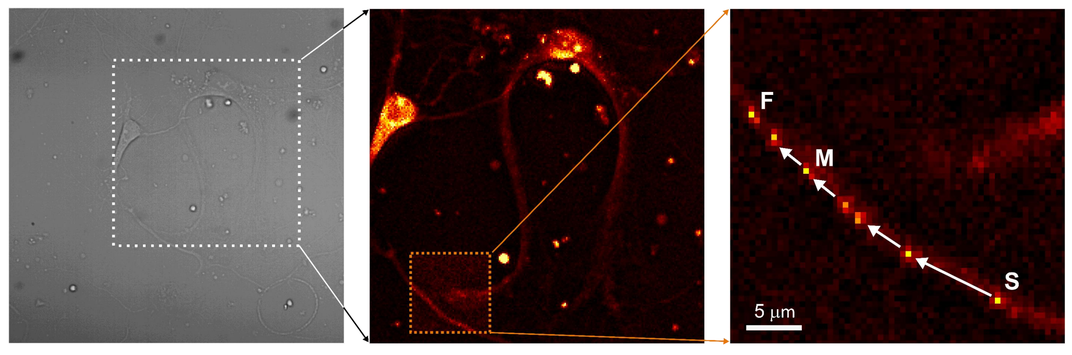
Sample: Oligo-dendrocyte
Excitation:
1650 cm^{-1}
Image size:
120X120 µm²



Axonal transport



Kim E, Barrett, Susan M. Barman, Scott Boitano, Heddwen L. Brooks: Ganong's Review of Medical Physiology, 25th Ed.



Play video 3

Sample:
Neuron
Excitation:
1650 cm^{-1}
Image size:
30X30 µm²

Publication

- Lim, J. M.; Park, C.-J.; Park, J.-S.; Kim, C.-H.; Chon, B.-H. and Cho, M.-H. *J. Phys. Chem. Lett.* **2019**, 10, 2857−2861.