Exploring Mid-IR Photothermal Microscopy with Bead Samples

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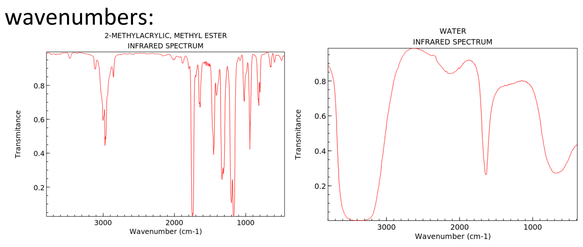
Introduction

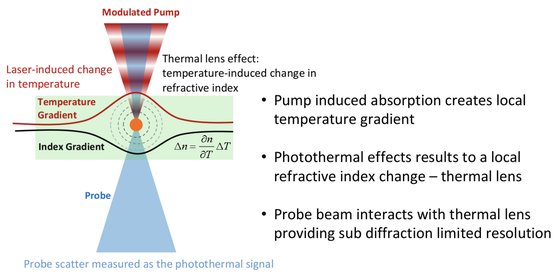
Mid-IR photothermal microscopy utilizes a mid-infrared laser at a specific wavenumber to excite certain areas within a sample. Every material has its own unique absorption spectrum, absorbing more and less at different wavenumbers:



The absorption spectra of 2-Methylacrylic Methyl Ester, or PMMA[3], and Water[4]

Included in the system are two lasers:

- The pump laser:
  - Is tuned to a specific wavenumber according to peaks in the absorption spectra of the materials
  - Is pulsed at a specific frequency in kHz
  - Has an adjustable pulse duration in ns
- The probe laser:
  - Scatters differently off of areas at different temperatures



- Pump induced absorption creates local temperature gradient
- Photothermal effects results to a local refractive index change – thermal lens
- Probe beam interacts with thermal lens providing sub diffraction limited resolution

Because the probe scatters differently off of heated areas, it can be used construct the image indicating where the material is present

Other factors:

- The position of the objective, also known as the z-offset
- The phase, changing depending on the distance between the pump and probe

The signal contrast and resolution of the images changes between samples and settings.
How might each of these factors affect the quality of the image? What could the optimal settings be?

Methods

Images were taken at many different combinations of settings. A sample of melamine beads in oil and a sample of PMMA beads in water were imaged.

Pulse Frequency:
10kHz, 50kHz, 100kHz, 200kHz, 300kHz

Pulse Duration:
150ns, 250ns, 500ns, 1000ns

Objectives Offsets:
243um, 238um, 233um

Signal Phases:
Positive and negative

Peak absorption wavenumbers:

Sample 1:
Melamine: 1580 cm^{-1}, Oil: 1731 cm^{-1}

Sample 2:
PMMA: 1728 cm^{-1}, Water: 1660 cm^{-1}

Investigations:

Frequency Sweep: Change pulse frequency

Phase: Remain in the same position and move the sample height to alter the objective-sample offset, causing phase flip

Knob used to move sample up and down

Objective Height: Change objective height

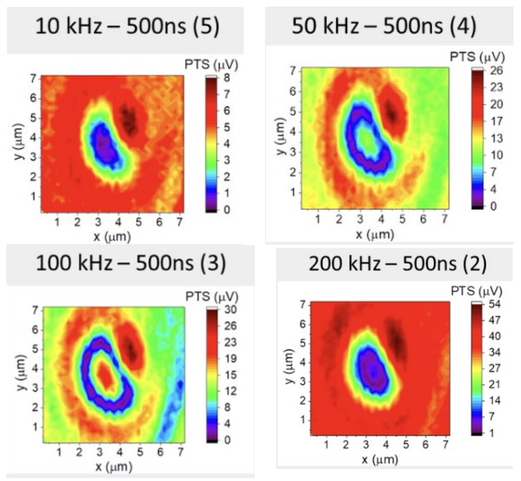
Control used to move objective up and down

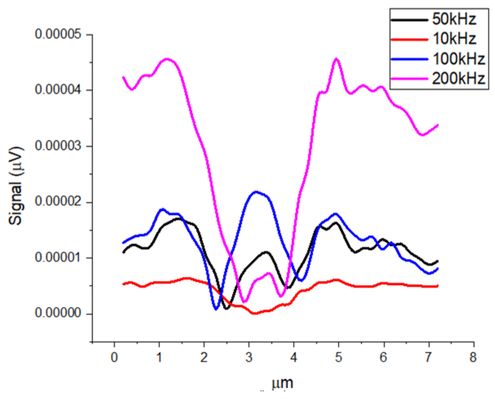
Wavenumber: Images are taken at both bead and background absorption

The data was processed using Matlab and Origin and was analyzed using **line scans, frames across time, and qualitative observations of the heat maps.**

Results

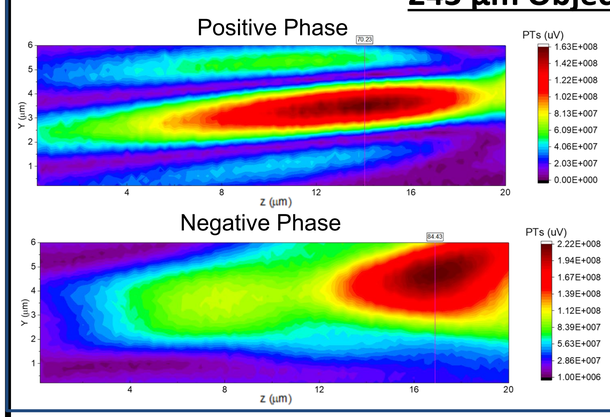
XY Graphs of Melamine Beads in Oil with 1731 cm^{-1} Wavenumber

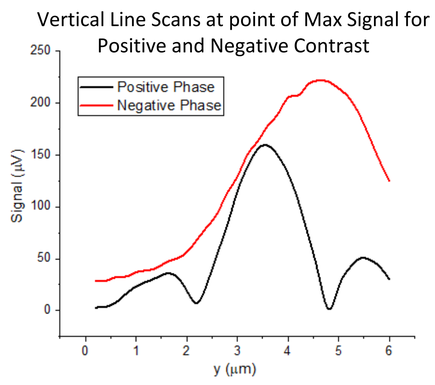




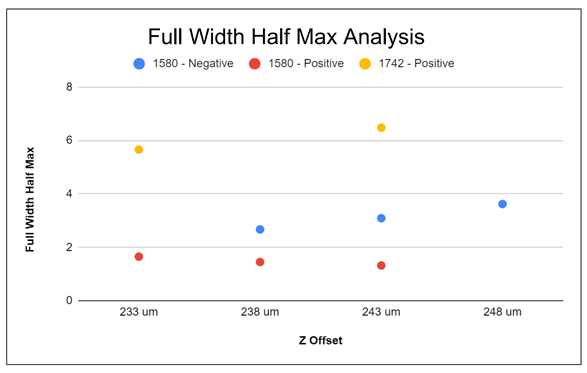
Vertical Line Scans of Signal at x=3.2 µm in the XY graphs for Different Frequencies

YZ Graphs of Melamine Bead in Oil with 1580 cm^{-1} Wavenumber at 243 µm Objective

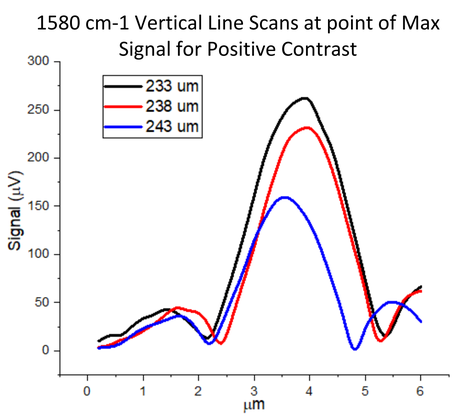




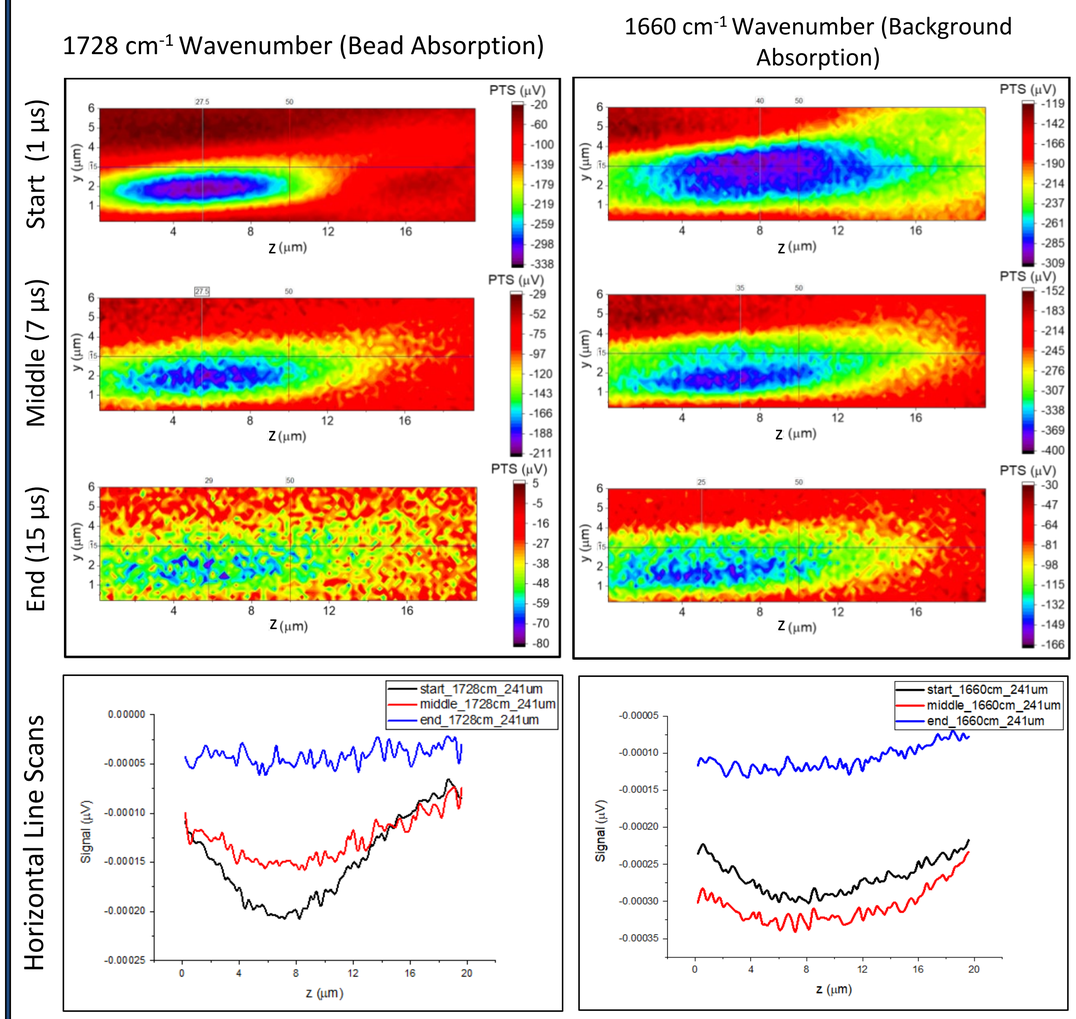
Melamine Bead in Oil Resolution and Objective Heights



Lower Full Width of half maximum indicates greater resolution for positive phase for melamine absorption



PMMA Beads in Water at 241 µm Objective Height



Discussion/Conclusions

XY Graphs of Melamine Beads in Oil

- Line scans have the highest signal at 100 kHz from the point of the bead (3 um)
- 100kHz also displays the most defined transition, having a clear boundary between bead and background
- Heatmaps show the boundaries and features of the bead are most visible at 100kHz

100 kHz displays the best results.

YZ Graphs of Melamine Beads in Oil

- Bead is better defined in the positive phase
- Bead has phase flip in the positive phase:
  - On the graph there is a dip and then rise again in the signal
- Bead does not demonstrate phase flip in the negative phase

Positive phase displays the best results, this is *not* where the signal is the highest.

FW Half Max and Objective Height

- 1580 cm-1, positive contrast, and 243 um Z offset has lowest full width of half max → the highest resolution
- Graph of overlaid line scans shows the best defined boundary occurs at 243 um

243 um Z offset displays the best results, this is *not* where the signal is the highest.

PWA Frames in Times for Bead and Background Absorption

- Projected image of the bead appears to grow with time when targeting bead absorption
- The projected image of the bead appears to shrink when focusing on the background absorption
- Curve of the signal of the bead appears to flatten out over time

Final Conclusions:

1. **The resolution and contrast are independent of the signal level**
2. **Positive contrast gives the best definition and phase flip for Melamine beads**
3. **The projected image of the bead changes according to the area absorbing the heat in time**

References

P. Samolis and M. Sander, "Increasing contrast in water-embedded particles via time-gated mid-infrared photothermal microscopy," Opt. Lett. 49, 1457-1460 (2024). https://opg.optica.org/ol/fulltext.cfm?uri=ol-49-6-1457&id=547628.

[1] Adhikari, Subhasis, et al. *Photothermal Microscopy: Imaging the Optical Absorption of Single Nanoparticles and Single Molecules*. Vol. 14, no. 12, 20 Nov. 2020, pp. 16414–16445, https://doi.org/10.1021/acsnano.0c07638.

[2] Spring, Kenneth, and Michael Davidson. "Introduction to Fluorescence Microscopy." *Nikon's MicroscopyU*, 2019, www.microscopyu.com/techniques/fluorescence/introduction-to-fluorescence-microscopy.

[3] Coblentz Society, Inc. "Methyl Methacrylate IR Absorption Spectrum," *National Institute of Standards and Technology*, 2018, webbook.nist.gov/cgi/cbook.cgi?Spec=C80626&Index=1&Type=IR&Large=on. Accessed 1 Aug. 2024.

[4] Coblentz Society, Inc. "Water IR Absorption Spectrum," *National Institute of Standards and Technology*, 2018, https://webbook.nist.gov/cgi/cbook.cgi?Spec=C7732185&Index=1&Type=IR&Large=on. Accessed 1 Aug. 2024.

Acknowledgements

Many thanks Boston University and the RISE program for giving me the opportunity to conduct this research. I would also like to thank the members of the Sander lab for all of their help and support throughout this project!