Objectives

- Fourier Transform Infrared Imaging: a powerful tool for chemical analysis of micron size biological samples
- Synchrotron Imaging of a Dielectric bead demonstrates the importance of electromagnetic shape resonances, and scattering
- Accurate feature extraction and data interpretation can be done after signal correction

Infrared Spectroscopy and Scattering

- Determination of functional groups in molecules, by analyzing the interaction of infrared light and material
- Electric field interaction with object is the same as interaction of sound wave with object, for example instruments with different shape sound differently
- Geometrical Shape or size of the object could result in different spectra

Hyperspectral Imaging

- Fourier Transform Infrared (FTIR) spectroscopy
- Determine characteristic absorbance of molecular vibrations from spectra
- Collecting large number of spatially resolved spectra by array of 128x128 detectors
- Synchrotron source: brightness, fast data acquisition, higher spectral and spatial resolution
- Accurate extraction of chemical and structural features

Imaging Methods

Experiment

- A 20µl drop of a 32-80µm bead solution
- A 50µm bead is casted after evaporation
- 360 infrared images at 1 to 360 angles are taken

3D Image Reconstruction

- Images are reconstructed by inverse radon transform using filtered back projection algorithm, and linear interpolation

\[ f(x,y) = \frac{1}{2\pi} \int_{0}^{\pi} \Delta \theta g_{\theta}(x \cos \theta + y \sin \theta) \]

\[ g_{\theta}(\theta) = p_{\theta}(\theta) \cdot k(\theta) \]

Where \( \Delta \theta \) is the angular spacing between the projections and \( k(\theta) \) is the radon kernel or the filter

Wobble Correction

- Wobble Correction algorithm based on minimizing 2D entropy function of images as follows:
   - The 2-D entropy function of the image is defined as:
     \[ E = - \sum_{m=1}^{M-1} \sum_{n=1}^{N-1} f_{m,n} \ln(f_{m,n}) \]
   - Where \( f_{m,n} \) is the intensity of pixel \((m,n)\)

4D Visualization \((x, y, z, \lambda)\)

- The first 3 dimensions are \(x, y, z\), and the last one is wavelength
- A 4D infrared visualization software is designed and implemented to visualize and analyze the data

Results

- Infrared spectra of a single homogenous bead is varying spatially, due to electromagnetic resonances

Next Steps

Ideal Imaging

- Electric fields inside of the a 25µm radius sphere are simulated using CST package
- The free space green function and the trick shown in the figure are used to calculate the ideal image of the sample.
- Wavelength dependence Point Spread Function (PSF) of the system is measured (Basically and Schwarzchild objective)
- The PSF is going to be convolved with the ideal image to construct a realistic distorted image
- An algorithm Based on EMSC is going to be implemented to correct the spectra

Conclusion

- Infrared spectra of micron size objects (10 to 50 µm), could be distorted depending on the shape and size of the object. Signal analysis and corrections should be done before any interpretation.

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References

- Bassan, P. (2011), "Light scattering during infrared spectroscopic measurements of biomedical samples (Doctoral Dissertation, University of Manchester)