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Viewpoint

Vibrational Probes in Bioimaging and Chemical Biology

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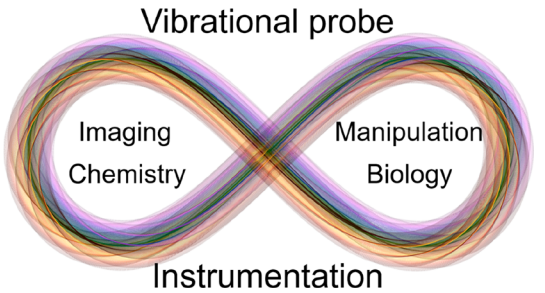
ABSTRACT: The integration of vibrational probes with instrumental technologies heralds a broad and transformative future for biomedical imaging and chemical biology.

Imaging techniques are indispensable in modern biological research, providing insight into dynamic molecular processes with high spatial and temporal resolution. While fluorescence microscopy has dominated the field, its inherent limitations, such as broad spectral overlap, photobleaching, and the relatively large size of fluorescent probes, have driven the exploration of alternatives. Vibrational microscopy has gained increasing attention due to its unique capability for label-free imaging, utilizing intrinsic molecular vibrations to visualize the chemical composition of biological systems.



By detecting bond-specific vibrational signals, techniques such as Raman scattering and infrared absorption are particular suitable for molecular imaging with rich chemical information. Especially, the development of nonlinear vibrational microscopy including coherent Raman scattering and vibrational photothermal microscopy has greatly enhanced the sensitivity and imaging speed to facilitate its application in life sciences and biomedical research, opening new avenues for studying cellular dynamics, molecular interactions, and biochemical processes.

In addition to the label-free modality, the integration of vibrational probes has unlocked a new dimension in bioimaging. These synthetic labels exhibit sharp spectral signatures in biologically "silent" regions, enabling noninvasive, multiplexed, and quantitative imaging of living systems. The fusion of probe chemistry with advanced vibrational techniques, such as stimulated Raman scattering (SRS) microscopy, has laid the foundation for an emerging field: vibrational chemical biology.

Recently, Nature Methods published a Focus issue on bond-selective imaging. Several leading research groups in vibrational microscopy have written insightful comments and reviews on the historical perspectives[1,2] and the recent progress and future directions[3−6] in this vibrant field. A variety of technical advances and instrumental innovations have been highlighted in the past two decades, including signal amplification strategies by coupling to electronic or plasmonic resonance, fluorescence emission, and photothermal detection. With enhanced sensitivity down to single-molecule level and machine learning assistance, spatial resolution of vibrational imaging has also broken the optical diffraction limit to reach tens of nanometers.

In this viewpoint, I will focus on the role of vibrational probes in these advanced imaging techniques, discussing their design, applications, and potential challenges. For more comprehensive reviews on both technological and chemical developments, readers are encouraged to refer to the articles in the Nature Methods Focus issue (https://www.nature.com/collections/hbbgiihcge).[7]

As fluorescent probes are integral to fluorescence microscopy, vibrational probes play crucial roles in vibrational microscopy. Fluorescent probes often face limitations such as spectral overlap and probe bulkiness, which hinder multiplexing and can interfere with biological function of labeled molecules. To overcome these challenges, vibrational probes have emerged as a powerful alternative, capable of visualizing multiple species in living systems with high chemical specificity and minimal perturbation.

Compared with fluorescent probes, one significant advantage of vibrational probes lies in their much smaller physical size, often comprising a single chemical bond with 2−3 atoms. These probes produce distinct signals within the "cell-silent" region of the vibrational spectrum (1800−2700 cm^{-1}), a window largely free from endogenous cellular signals, offering a clean spectral background for high-contrast detection. Additionally, vibrational probes exhibit much sharper spectral line widths, enabling supermultiplexed frequency detection. Moreover, vibrational probes are typically resistant to photobleaching and quenching, and can be applied in aggregated forms or polymeric structures for intense signal and long-term imaging.

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Common vibrational probes include alkyne (C≡C), nitrile (C≡N), azide (N_3), carbonyl (C═O), and carbon-deuterium (C−D) bonds. Among these, Raman imaging probes are better developed. Based on the strength and symmetry of chemical bond vibration, alkyne (2100−2300 cm^{-1}) and C−D bonds (2100−2300 cm^{-1}) are frequently used in Raman imaging due to large hyperpolarizability changes. And azide (2100−2200 cm^{-1}) and carbonyl (1600−1800 cm^{-1}) bonds are often applied in infrared imaging with large dipole moment changes. Nitrile bonds (2200−2300 cm^{-1}) can be applied in both Raman and infrared-based imaging, and are found in naturally occurring molecules and pharmaceutical compounds.

Due to their small size and bioorthogonality, vibrational probes with a single chemical bond have been directly applied to visualize molecular localization, uptake, transport, interactions, and metabolism in living systems. Many species including small metabolites, DNA, RNA, proteins, various lipids, carbohydrates, and drug molecules have been successfully imaged by both Raman and infrared photothermal microscopy in living cells and organisms such as C. elegans, zebrafish, Drosophila, mice and plants. Besides labeling specific species, deuterium-labeled water (D_2O) is shown to serve as a universal tracer for imaging nucleic acids, proteins and lipids via metabolic incorporation of deuterium into biomass synthesis.

Thanks to the narrow vibrational line width (~10 cm^{-1} or ~1 nm), these chemical bonds can be further engineered for multiplexed imaging and profiling with MARS (Manhattan Raman scattering) and Carbow (Carbon rainbow) probes. Based on conjugated alkyne and nitrile bonds, more than 20 vibrational "colors" have been developed through π-system tuning, isotope editing and chemical substitution. Live-cell ten-color organelle imaging and one-shot 11-color immunoimaging of epitopes in thick brain tissues have been achieved.

In addition, through up-converting vibrational excitation to fluorescence emission, fluorescent probes can be applied in stimulated Raman excited fluorescence (SREF) and fluorescence encoded IR imaging (FEIR and BonFIRE), demonstrating single-molecule sensitivity with bond-selective specificity. Furthermore, Raman dots, polymer nanoparticles, and liposome drug carriers have been enriched with vibrational probes for multiplexed cell barcoding, profiling, and phenotyping with single-particle sensitivity.

Beyond static structural labels, vibrational probes are sensitive to environmental changes. By coupling vibrational signal to physical change or chemical reactivity, turn-on and ratiometric sensors have been developed for functional vibrational imaging of electric fields, pH, reactive species and enzyme activities in live cells. Photoactivatable and photoswitchable vibrational probes have also been reported for dynamic and super-resolution imaging with high spatiotemporal precision.

To summarize, the integration of chemical probes into vibrational microscopy offers exciting opportunities for high-content imaging of biological dynamics with enhanced sensitivity, specificity and functionality. Through innovations in organic chemistry, isotope incorporation, polymer-based amplification, and live-cell labeling strategy, the Nature Methods Focus issue have showcased the great potential of vibrational probes in metabolic imaging, multiplexed profiling, drug development, and disease diagnostics.

With a large toolbox in optics and engineering, sensitivity will be the key to achieve superior resolution and speed in vibrational imaging for broader biomedical applications. Increasing the detection limits by 100 folds from current μM-mM levels to sub-μM or low nM range will be crucial. Innovative signal-enhancing mechanisms for molecular design are greatly needed to push the limit. One strategy could be self-stacked small molecules with large Raman enhancements.[8]

Another promising direction is the integration of both label-free spectra and vibrational probes with multiple omics platforms for comprehensive phenotypic analysis. Chemical proteomics for site-specific target identification in proteins with ATRaS (alkyne-tag Raman screening)[9] and single-cell mid-infrared spectral profiling with VIBRANT (vibrational painting)[10] are useful in drug discovery. Advances in data processing techniques, such as spectral analysis and machine learning,[11] will help to address these challenges.

Looking even further, vibrational control could be another direction to manipulate biochemical activities in living systems, through either direct vibrational excitation[12] or indirect effects such as bond-selective photothermal stimulation and inhibition.[13] This will complete the picture of vibrational chemical biology to both explore and regulate biological processes.[14]

While challenges remain in sensitivity and throughput, the modularity and multiplexing capability of vibrational probes open new directions toward vibration-based live-cell study. Continued development of more sensitive and functional probes and advanced data analysis techniques will undoubtedly expand the scope and capabilities of vibrational imaging. As these technologies become more accessible and widely used, they are poised to make a profound impact on our understanding of cellular processes and disease mechanisms.

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Notes

The author declares no competing financial interest.

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