# Label-Free Nonlinear Optical Imaging Uncovers Key Biomarkers of Cellular Radiation Response

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## Introduction

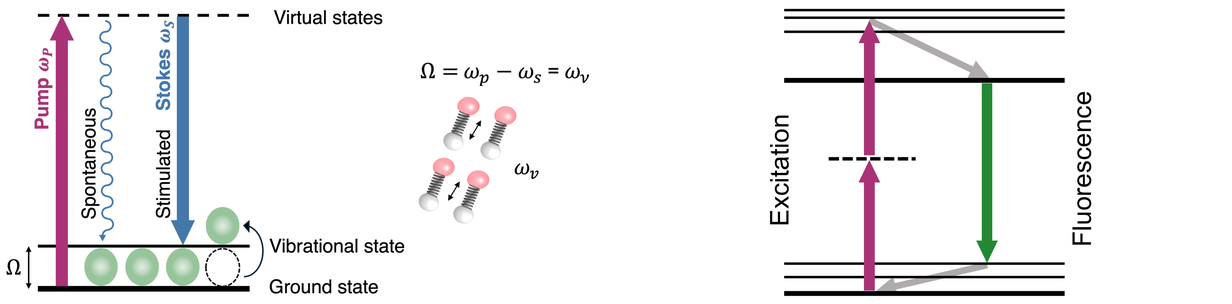
Ionizing radiation induces reactive oxygen species, causing mitochondrial dysfunction and damage to lipids, proteins, and DNA. The resulting biomolecular and metabolic responses remain poorly understood, especially at the cellular and subcellular levels. Understanding these changes is key to advancing radiotherapy and radiation safety [1].

We apply a label-free multimodal nonlinear optical imaging approach that uses stimulated Raman scattering (SRS) [2] and two-photon excitation fluorescence (TPEF) microscopy [3] to evaluate radiation-induced changes in cellular metabolism involving lipids, proteins and mitochondria in live A549 human lung cancer cells as a model system.

## SRS and TPEF Microscopy

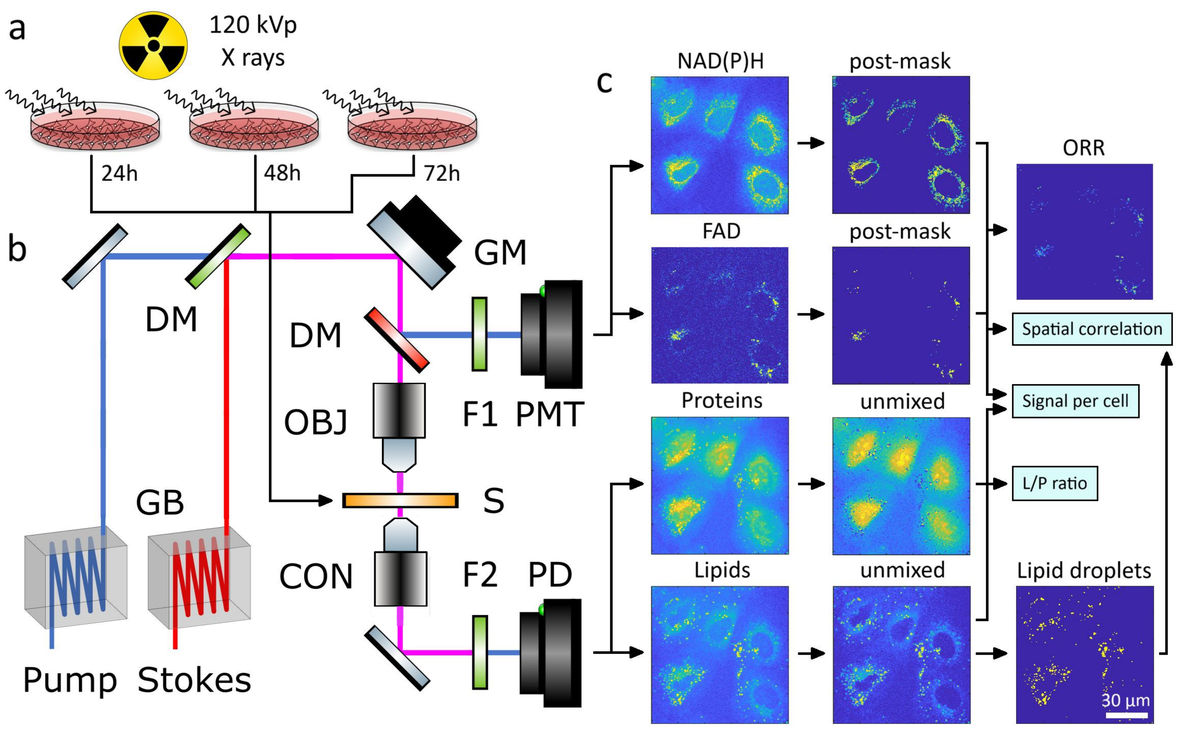
**SRS microscopy** involves the coherent excitation of vibration modes of chemical bonds of molecules. The difference frequency between two ultrafast lasers was set to match the vibrational frequency of $CH_2$ and $CH_3$ bonds to generate SRS images at 2850 $cm^{-1}$( lipids) and 2926 $cm^{-1}$ (proteins).

**TPEF microscopy** involves the simultaneous absorption of two near-infrared photons of the same frequency by a fluorescent molecule. TPEF images of endogenous fluorophores NAD(P)H and FAD were generated using 120 fs pulsed laser at 720 nm and 880 nm respectively.




## Method

- Human lung cancer A549 cells were exposed to X-ray dose of 0, 2, 4, 6, 8, or 10 Gy at a dose rate of 1 Gy/min
- Live A549 cells were imaged at 24-, 48-, and 72-hours post-irradiation using SRS and TPEF microscopy

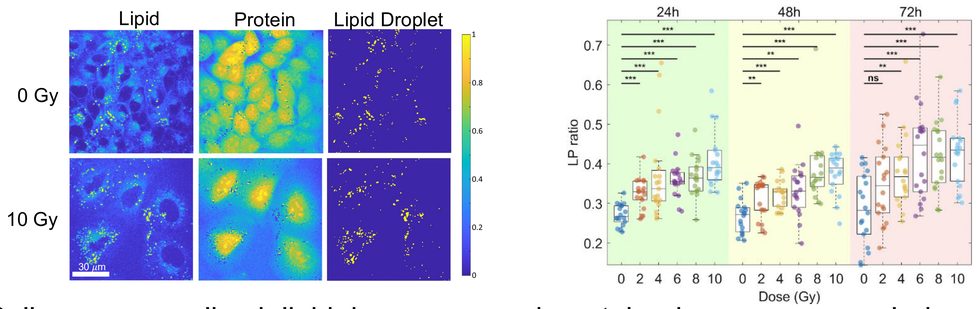



## Image Analysis

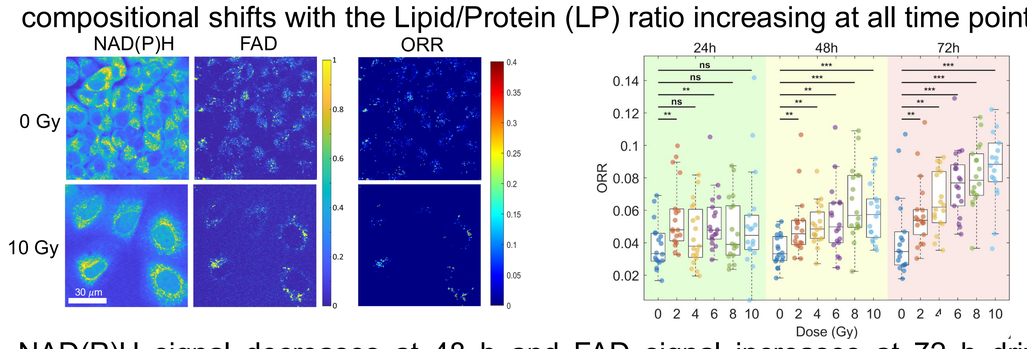
- Lipid and protein images were obtained from the raw SRS images by unmixing the images using the known intensities of pure lipids and proteins.
- A threshold mask was applied to determine the lipid droplet (LD) area/cell.
- A threshold mask was applied to the TPEF images to isolate mitochondrial signal, and and the spatial correlation between the two TPEF channels was evaluated.
- Optical Redox Ratio (ORR) was evaluated from the TPEF intensity as follows:

$$ORR = \frac{FAD}{FAD + NAD(P)H}$$

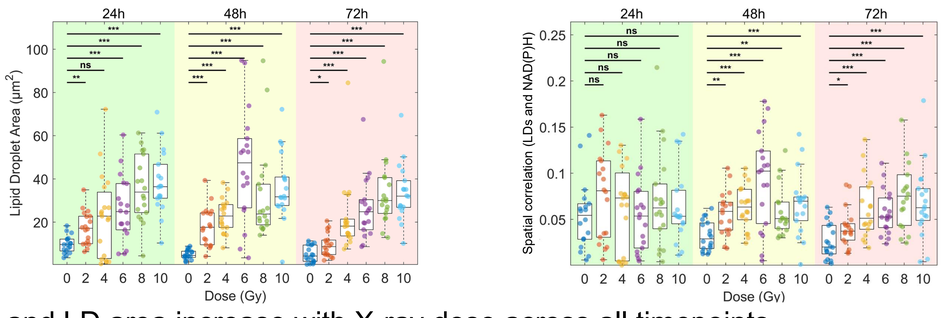
## Results




- Cell area-normalized lipid increases and protein decreases reveal dose-dependent compositional shifts with the Lipid/Protein (LP) ratio increasing at all time points




- NAD(P)H signal decreases at 48 h and FAD signal increases at 72 h drive a time-dependent increase in ORR




- Cell and LD area increase with X-ray dose across all timepoints
- Increased spatial correlation between LDs and TPEF signals indicates enhanced organelle association at 72 hours
- Spatial correlation between NAD(P)H and FAD signals increases at 72 hours

## Conclusion

Ionizing radiation induces dose- and time-dependent changes in cell structure, composition, and metabolism. Label-free multimodal nonlinear optical microscopy (SRS + TPEF) enables simultaneous quantification of lipid, protein, and redox metabolism changes, providing a sensitive approach for assessing radiation dose responses at clinically relevant doses.

## References

[1] Baskar, R. et al. *Front. Mol. Biosci* (2014) doi: 10.3389/fmolb.2014.00024
[2] Gagnon, JR. et al, *Sci. Rep.* (2025) doi: 10.1038/s41598-025-23780-8
[3] Allen, CH. et al. *Sci. Rep.* (2021) doi: 0.1038/s41598-021-93043-9