# Label-free Multimodal Multiphoton Microscopy Reveals Spatial Lipid Alterations Associated with Inflammation in Triple-negative Breast Cancer Following NOS2 Suppression and COX-2 Inhibition

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## Introduction

Triple-negative breast cancer (TNBC): 20% of breast cancers
- Metastatic, few effective treatments

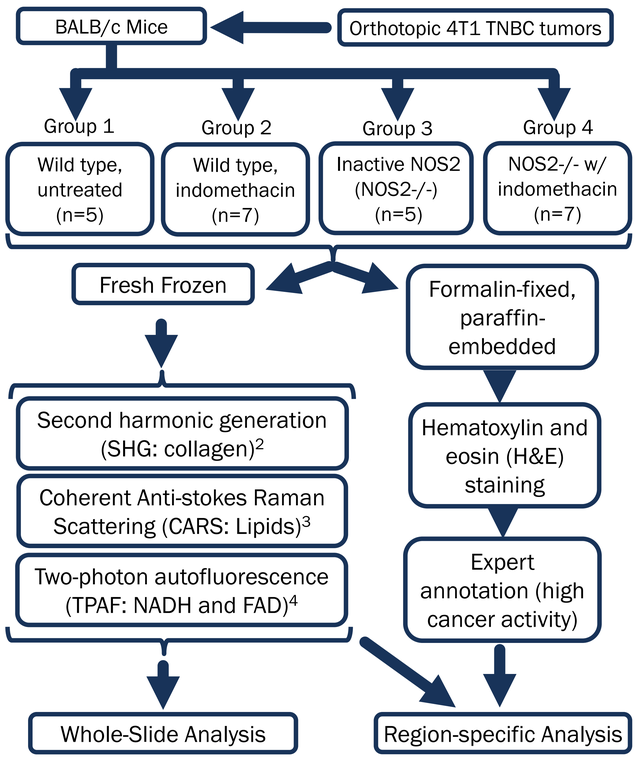
Pro-inflammatory factors in the tumor microenvironment (TME) facilitate cancer growth, including:
- Nitric oxide synthase-2 (NOS2): ↑Nitric oxide (NO) → ↑angiogenesis, ↑cell proliferation. ↑NOS2 → poor survival, via EGFR and NK-κB (immunosuppression, pro-metastasis)[1]
- Cyclooxygenase-2 (COX-2): ↑prostaglandins → ↑NOS2, immunosuppress, and remodel ECM proteins[1]

Spatially heterogeneous TME in TNBC with groupings of functionally different cancer cell niches
- Microscopy enables quantitative spatial analysis.
- Label-free imaging enables specific study of structural proteins (collagen) and metabolism (NADH, FAD, lipids) without exogenous dyes (bulk/live tissue, preserves sample)

## Purpose

To examine NOS2-mediated NO signaling and COX-2 impacts on TNBC extracellular collagen, lipid storage, and oxidative phosphorylation in TNBC via multimodal label-free microscopy.

## Methods




## Result

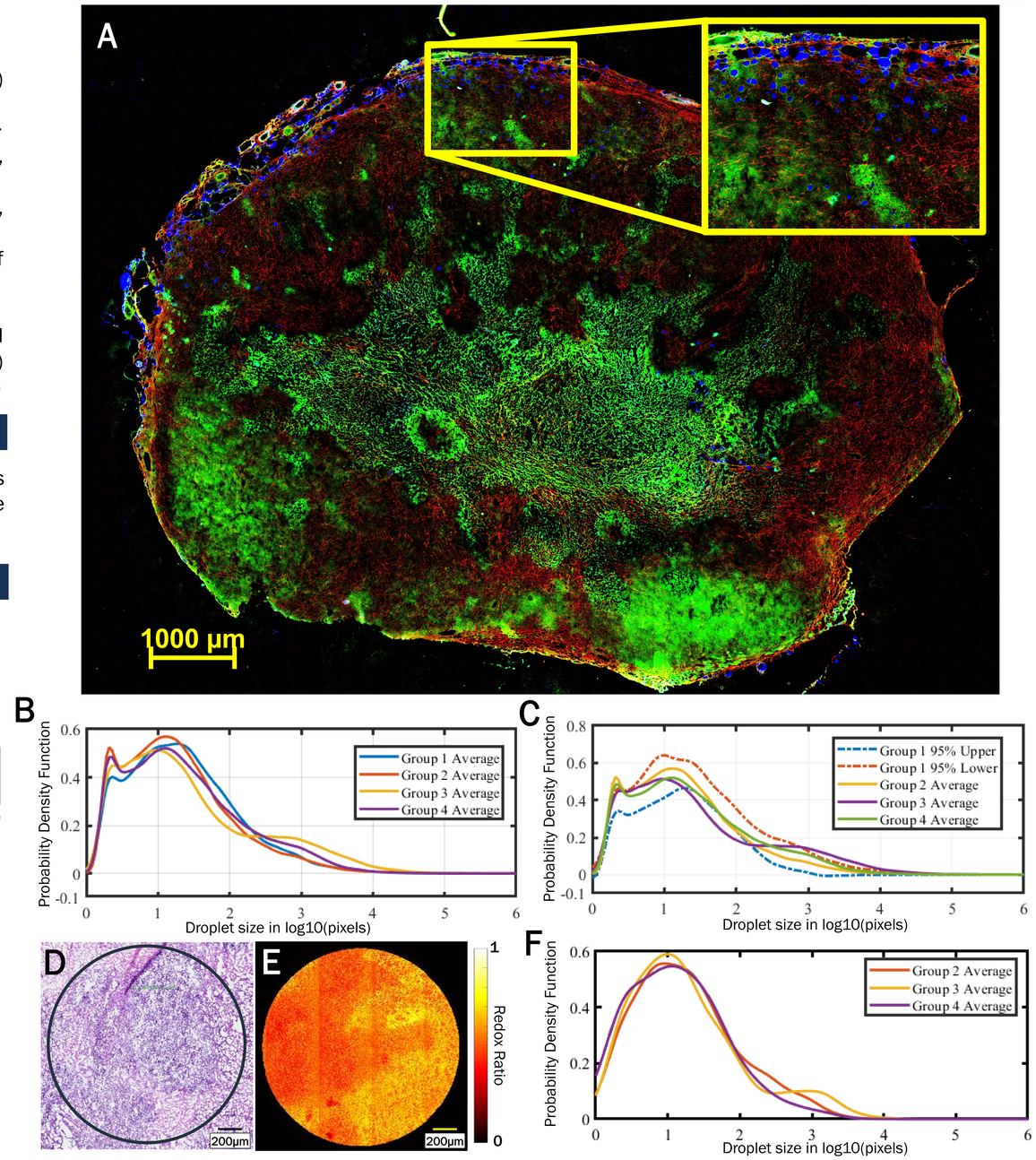



**Fig 1. A**: Merged CARS (blue), SHG (red), and TPAF (NADH, green) image of one sample. **B**: Average probability density functions (PDFs) for each group. Each PDF is a curve representing the relative lipid droplet sizes (logarithmic area in pixels) for each, normalized to an area under the curve of 1. **C**: The average PDFs for groups 2, 3 and 4 (solid lines) compared to the 95% confidence interval band of Group 1 (dotted lines). **D**: Representative annotated cancer region on a corresponding H&E slide. **E**: Representative corresponding region in redox ratio map to Fig 1E. **F**: Average PDFs for groups 2 – 4 for lipid droplet size distribution in annotated regions.

## Results

NOS2-/- samples have increased proportion of large lipid droplets (1,000 – 10,000 pixels) versus wild type.
- ↑ lipid accumulation, altered β-oxidation or storage without NO signaling

Indomethacin-treated groups showed increased proportion of small lipid droplets (1 – 10 pixels).
- ↑ turnover or lipolysis
- NOS2-/- and indomethacin together had increased the proportion of small lipid droplets.

Annotated regions showed similar droplet size distributions, but low counts in small regions make statistics challenging.

High spatial heterogeneity in redox ratio
- Low redox ratio areas next to high redox ratio in multiple annotated regions
- Possible mismatch between H&E annotations and masking out areas in multiphoton images
- Expect low redox ratio for rapidly dividing cancer cells (Warburg effect), but extensive infiltration of leukocytes may contribute highly varying redox ratios

Multimodal label-free imaging utilizing CARS and TPAF reveals spatial heterogeneity and enables correlation between traditional H&E analysis with label-free molecular and metabolic analysis.

## Future Actions

Further imaging of TNBC samples are ongoing to verify differences in visualized lipid droplet size distribution. Additional multimodal quantifications are being developed to investigate the relationships between the differences in metabolic markers measured here by label-free imaging and extracellular structural changes in SHG imaging of collagen.

## Acknowledgments

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## References

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