Research Plan

Xiaoyang Zheng

2025-10-01

Abstract

This research develops an Intelligent Adaptive Optics (iAO) workflow for hyperspectral Stimulated Raman Scattering (SRS) microscopy to enable deep-tissue, label-free chemical imaging. The project tightly integrates adaptive optics hardware (spatial light modulator based wavefront control) with machine learning across three coupled roles:

1. real-time, single-shot wavefront estimation and control using a deep predictive controller (sub-100 ms latency target),
2. AI-powered image restoration and hyperspectral unmixing to increase effective SNR and chemical contrast, and
3. downstream semi-supervised and uncertainty-aware segmentation/analysis to extract biologically meaningful metrics from large, partially labeled volumes.

The combination of these components aims to restore diffraction-limited performance in highly scattering samples, unlock deeper imaging with Ozeki Lab’s hyperspectral SRS systems, and provide robust computational tools for analyzing the resulting large datasets.

**Intelligent Adaptive Optics for Deep-Tissue Hyperspectral Stimulated Raman Scattering Microscopy**

# Research Background

Stimulated Raman Scattering (SRS) microscopy provides label-free chemical contrast by probing intrinsic molecular vibrations and achieves imaging speeds orders of magnitude faster than spontaneous Raman techniques . Its advantages—high chemical selectivity, linear concentration dependence, and three-dimensional sectioning—make it suited for live biological imaging .

The Ozeki Laboratory focuses on

(1) rapidly tunable laser sources for hyperspectral SRS ,

(2) improved detection sensitivity including quantum-enhanced approaches, and

(3) integration with microfluidics and machine learning for high-throughput analysis .

When imaging deep inside tissue, sample-induced optical aberrations degrade focus and SRS signal strength, creating a practical limit on imaging depth . Adaptive optics (AO) mitigates such aberrations by actively correcting the wavefront; spatial light modulators (SLMs) are commonly used to implement phase corrections and holographic excitation .

While classical, iterative sensorless AO algorithms (e.g., Zernike-mode hill-climbing) can improve signal quality, they are typically too slow for dynamic biological samples and cannot exploit correlations present in hyperspectral data. Modern machine learning (ML) enables new strategies: predictive single-shot correction, learned priors for denoising and unmixing, and semi-/weakly-supervised segmentation that reduce annotation burdens while providing calibrated uncertainty estimates for downstream analysis. Therefore, combining the Ozeki Lab’s hardware strengths with ML-driven control and analysis represents a natural and high-impact next step. Integrating these AI capabilities directly into the AO-SRS workflow is the central novelty of this proposal.

# Problem Statement

AO combined with SRS has been demonstrated using modal correction techniques and hill-climbing search strategies to improve signal and resolution . However, these classical iterative control algorithms test sequential correction patterns and are too slow for dynamic aberrations in living tissue .

Furthermore, hyperspectral SRS datasets are large and noisy when acquired at depth; traditional image processing pipelines struggle to extract reliable chemical maps and cellular features without laborious manual annotation. There is an opportunity to exploit deep learning both for optical control and for the analysis of the richer, corrected data produced by an iAO system.

# Proposed Work and Objectives

I propose to develop a predictive, single-shot AO controller based on deep learning, integrated with a semi-supervised hyperspectral analysis pipeline. Objectives:

1. Integrate a polychromatic AO module into an SRS microscope and implement a classical iterative control baseline.
2. Develop and validate a CNN-based predictive AO controller for single-shot wavefront estimation and correction.
3. Apply the intelligent AO-SRS system to biological imaging and create a semi-supervised segmentation workflow for hyperspectral data.

To emphasize the AI elements explicitly, I decompose the project into three coupled technical tracks that will run in parallel with frequent integration milestones:

* Track A (Hardware & Optics): SLM integration, polychromatic relay design, calibration, and classical AO baseline (Zernike modal control).
* Track B (AI for Real-time Control): dataset generation, CNN/regression models to predict corrective Zernike coefficients or SLM phase maps from single-shot images, latency optimization and hardware-in-the-loop testing.
* Track C (AI for Data Analysis): hyperspectral denoising/unmixing, semi-supervised segmentation (Mean Teacher / uncertainty-aware variants), and interpretability / visualization tools for end-users.

# Methods

Phase 1: System integration and iterative baseline

* Integrate an LCoS-SLM into the SRS optical path conjugate to the back focal plane of the objective. I will design the relay to minimize chromatic dispersion between pump and Stokes beams and will validate alignment with bead phantoms and scattering tissue-mimics.
* Implement a classical sensorless AO baseline (Zernike-mode hill-climbing and modal optimization) in Python. I will use this baseline to collect ground-truth data for Phase 2 training and to provide a performance reference for the iAO controller .

Phase 2: Predictive deep learning controller and image restoration

* Data collection: Use the Phase 1 setup to apply thousands of randomized aberration patterns (Zernike coefficient sets or measured SLM phase maps) and record the corresponding degraded SRS images across relevant spectral channels. I will acquire pairs: (aberrated image, known corrective phase) and (aberrated image, un-aberrated reference) when possible.
* Model design: Train a convolutional neural network (CNN) to perform image-to-vector regression (predict Zernike coefficients) and an alternative image-to-phase U-Net for direct SLM phase prediction. Candidate architectures: ResNet-derived encoders for robustness to noise, lightweight MobileNet-style backbones for low-latency deployment, and temporal/3D extensions if volumetric stacks are used.
* Training strategies: Employ augmented data (photon noise, motion blur, spectral variability), domain randomization to improve generalization from phantoms to biological tissue, and curriculum learning (coarse-to-fine Zernike prediction). I will evaluate both accuracy (residual wavefront error) and runtime (inference latency on target hardware).
* Image restoration: Develop learned denoisers and hyperspectral unmixers (e.g., 2D/3D U-Nets, spectral CNNs) that leverage corrected PSFs to increase effective SNR and to extract concentration maps for major chemical components (lipids, proteins, nucleic acids).

Phase 3: Biological application and semi-supervised analysis

* I will apply the iAO-SRS system to biologically relevant samples: 3D tumor spheroids, organoids, and thick brain slices. I will benchmark depth-dependent improvements to PSF, contrast, and chemical map fidelity.
* Semi-supervised segmentation: I will start from a small set of manually annotated superficial regions to train an initial U-Net; I will extend to deep regions using pseudo-labeling and Mean Teacher-style consistency regularization. I will incorporate uncertainty estimation (e.g., Monte Carlo dropout or deep ensembles) so that downstream analyses can weight predictions by confidence.
* Active learning loop: I will prioritize regions for manual annotation based on model uncertainty and expected information gain, minimizing annotation effort while maximizing segmentation performance in challenging, deep regions.

# Evaluation and Success Criteria

Success metrics include:

* Optical gains: SRS intensity increase and PSF restoration quantified by measured Strehl ratio improvement and PSF widths across depth.
* Control performance: residual wavefront error (RMS Zernike), correction accuracy relative to baseline, and controller latency (target: <100 ms single-shot inference including I/O).
* Data-analysis performance: denoising PSNR/SSIM improvements, segmentation Dice and IoU on held-out test regions, and calibration of uncertainty estimates (expected calibration error).
* End-to-end impact: demonstrable imaging depth increase (e.g., X-fold deeper diffraction-limited imaging vs. no-AO) and biological case study results (quantitative metrics of metabolic maps / feature counts enabled only with iAO).

# Risks and Mitigation

I identify the primary technical and project risks and will take the following steps to mitigate them:

* Hardware integration risk: chromatic alignment between pump and Stokes beams may require multiple optical iterations. Mitigation: design modular relay optics, procure spare alignment optics, and perform early phantom tests to validate alignment before biological samples.
* Model generalization risk: models trained on phantoms may not generalize to real tissue. Mitigation: use domain randomization, collect mixed phantom/ ex vivo tissue datasets, and adopt transfer learning with small annotated real-data subsets.
* Latency and deployment risk: achieving <100 ms closed-loop latency may require hardware acceleration. Mitigation: prototype on GPU, then optimize using pruning, quantization, or deploy on edge accelerators (e.g., NVIDIA Jetson, Coral) as needed.
* Annotation bottleneck: limited labeled deep-tissue data may slow segmentation development. Mitigation: use semi-supervised, active learning loops, and uncertainty-based prioritization to minimize manual labeling effort.

# References