# An Open-Source Design and Implementation of Polarization and Scanning Control Modules in a Second Harmonic Generation Microscope

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## INTRODUCTION

- The dynamics of the tumor microenvironment (TME) are critical in driving cancer progression. Collagen is the dominant protein in the TME.
- Microscopes are critical in advancing quantitative biological research.
- Second Harmonic Generation (SHG) is an advanced microscopy technique that is label-free and collagen specific.
- Polarization-resolved SHG is sensitive to supramolecular collagen structures and differentiates normal and cancerous tissues.

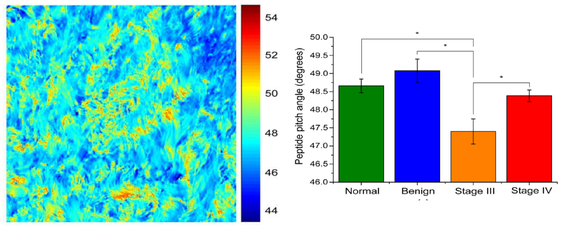


**Figure 1:** Collagen peptide pitch angle in stage III ovarian cancer. [1]

## BIG PROBLEM

- Commercially available multiphoton microscope systems with SHG capabilities can **range in price from four hundred thousand to one million dollars** and are non-modifiable to new microscopy techniques.
- There is **no widely used, open-source, and accessible platform** for optics researchers to utilize collaboratively.

## COMMUNITY RESPONSE

- Researchers have begun developing open-source solutions utilizing free-form bases and **3D printing** that incorporate readily accessible components and open-source software to **increase accessibility**. These approaches can enable configurability and functionality beyond industry standard microscopy systems.

## OUR RESPONSE

- Add polarization-resolved measurements to our SHG imaging capabilities and motorize control of the x, y, and z axis for precise scan slices using accessible, retrofitted, and **open-source components and software.**
- Develop a **parts library of common optical components** for community use along with open-sourcing all Onshape native designs developed throuhgout our microscope modifications.

## APPROACH

**Open-source Onshape designs meet open-source software and off the shelf components.**

## POLARIZATION CONTROL

- Integrate a liquid crystal device and a Glan Linear Polarizer to control both excitation and emission polarization states.
- Custom designed and 3D printed components to repurpose the infinity space of an Olympus BX-51 microscope to house the polarization control module.

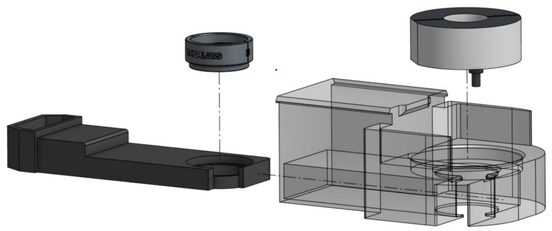

**Figure 2:** Polarization control module including liquid crystal rotator (light grey) and removeable ¼ waveplate (dark gray).

CLICK IMAGE FOR LINK TO ANIMATED MODEL.

## SCANNING CONTROL

- Computerized x, y, and z axis control allows for precise vertical slicing and tiling for 2D and 3D reconstructions.

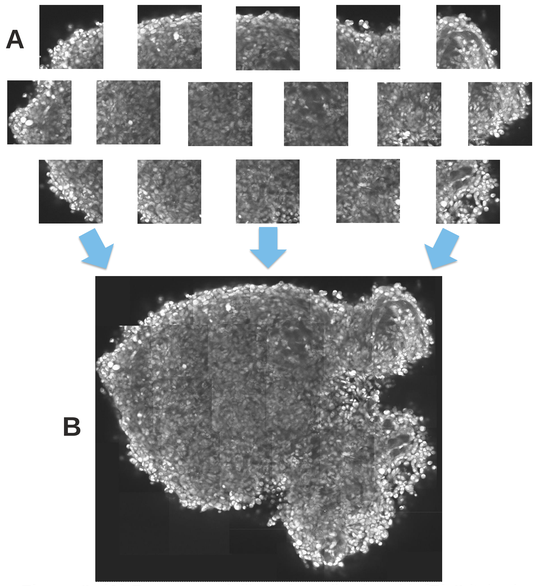


**Figure 3: (A)** Raw manual tiling of a cell spheroid. **(B)** Stitched slice of spheroid.

- Retrofit an Olympus BX-51 microscope with axis control stages for alternate Olympus microscope with **custom components and adapters**.

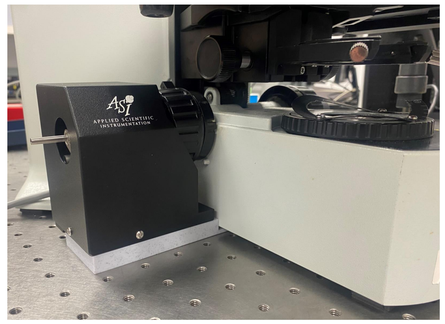

**Figure 4:** Z axis control module on Olympus BX-51.

## INTEGRATION

- The polarization and scanning modules, along with other power-controlled aspects of the optical system, will be controlled using ScanImage.
- ScanImage is an open-source laser control software for custom built and off-the-shelf microscopes.

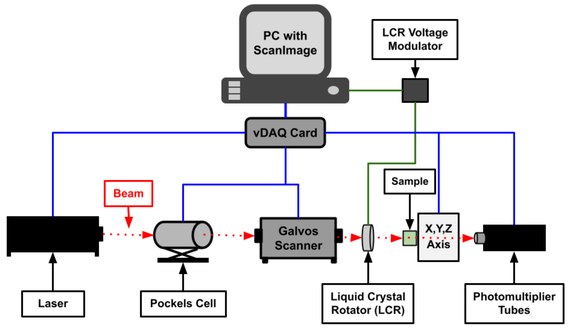


**Figure 5:** Basic control diagram of voltage-controlled components in beam path.

- All custom scripts developed for testing and microscope operation will be made **public** within the corresponding **Onshape documents**.

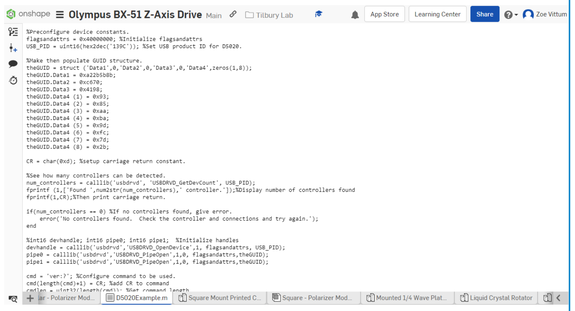


**Figure 6:** Polarizer voltage control MATLAB script in OnShape workspace

## OPTICS LIBRARY DEVELOPMENT

- Along with all custom components developed, we are working to develop an **easy-to-use library of off-the-shelf optical components.**
  - Inspired by MKCad library for robotics.
- Allow researchers to **streamline design processes** by removing the need for the download, import, and property configuration steps.

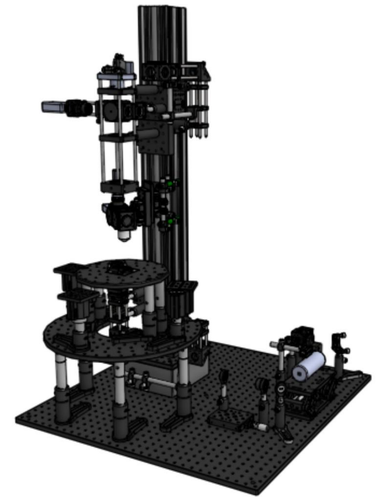

**Figure 7:** Free-form custom microscopy system previously published [2] composed of off-the-shelf components.

CLICK IMAGE FOR LINK TO ANIMATED MODEL.

## CONCLUSIONS & FUTURE WORK

- We successfully retrofitted the Olympus microscope for axis control and polarization control.
  - Current tuning and testing will prove the degree of polarization achieved.
- We will continue developing the **Onshape optics library** and begin publicizing library and custom component workspaces.
  - Track impact based on copy and insertion metrics.
- We will leverage polarization resolved SHG imaging techniques to increase our sensitivity and **understand the impact of integrin-mediated collagen remodeling in the TME.**

## REFERENCES

[1] Campbell KR, Chaudhary R, Handel JM, Patankar MS, Campagnola PJ. Polarization-resolved second harmonic generation imaging of human ovarian cancer. J Biomed Opt. 2018 Jun;23(6):1-8. doi: 10.1117/1.JBO.23.6.066501. PMID: 29900704; PMCID: PMC5998835.

[2] Rosenegger DG, Tran CH, LeDue J, Zhou N, Gordon GR. A high performance, cost-effective, open-source microscope for scanning two-photon microscopy that is modular and readily adaptable. PLoS One. 2014 Oct 21;9(10):e110475. doi: 10.1371/journal.pone.0110475. PMID: 25333934; PMCID: PMC4204885.