Second Harmonic Generation Microscope
Product Requirements Document
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B	11-13-2017
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Table of Contents:

Rev	Description	Date	Authorization
A	Initial PRD	10-30-2017	AH
B	PRD 2	11-13-2017	AH
C	PRD 3	11-27-2017	AH
D	PRD Final	12-15-2017	AH

The SHG microscope design is based on inputs from our customer, Dr Robert Hill, faculty advisor Dr Wayne Knox, and customer advisor Dr Ed Brown.

Vision:

The product is a prototype system for scanning and predicting metastasis in breast cancer cells.

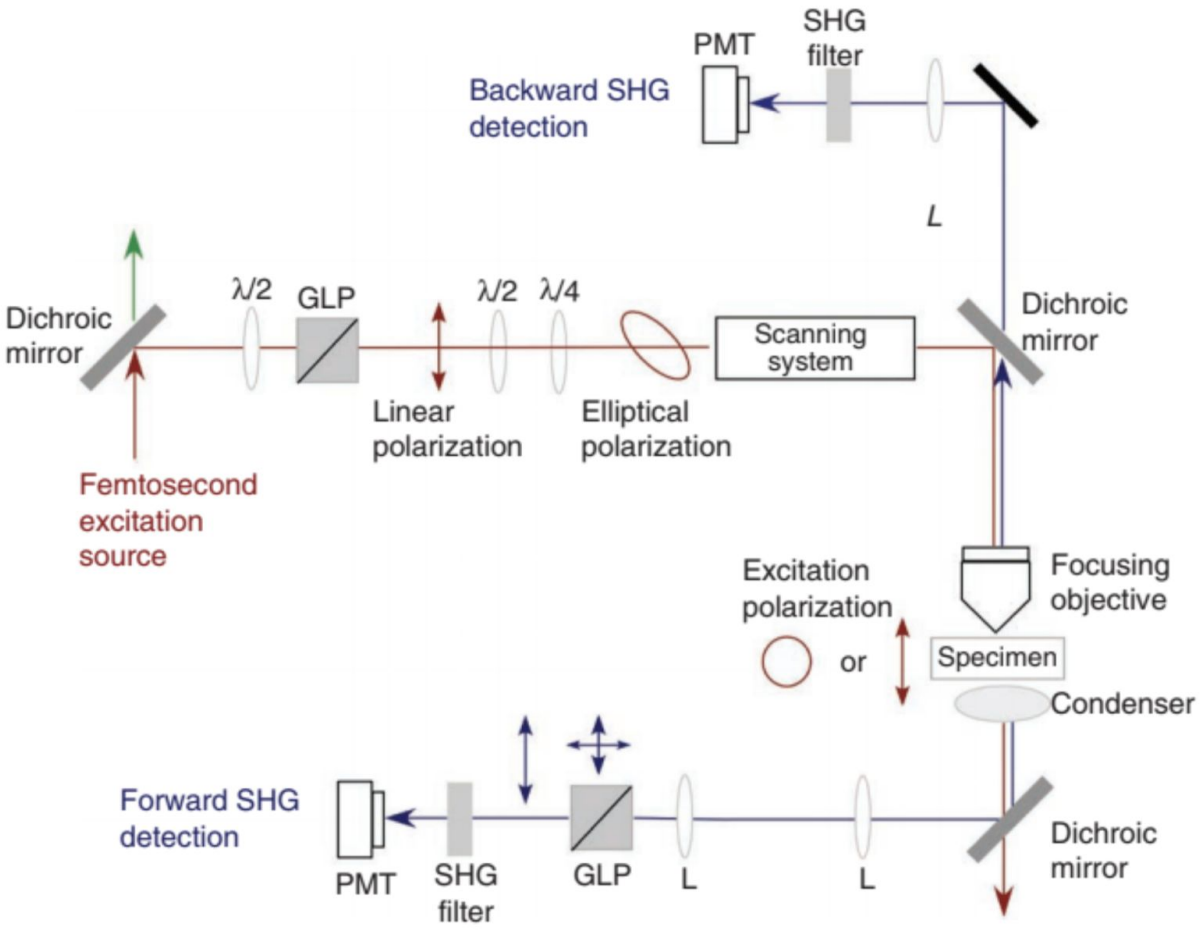


Figure 1. An example SHG microscopy setup with illumination light (red) and SHG signal (blue).[1]

Environment:

This device is intended to work in a central laboratory setting, not in smaller laboratories in hospitals.

Temperature

65-75 °F – Operation range (room temperature)

Relative Humidity

Non-condensing

Power

Outlet (120 V)

Regulatory Issues:

The microscope must conform with ANSI Z136 laser safety standards.

The microscope must receive FDA approval as a diagnostic device or operate under the CLIA exemption to the FDA rules for laboratory developed tests (LDT).

Fitness for use:

The scanner system will:

Improve signal-to-noise threefold

Use a pulsed titanium-sapphire laser to illuminate the sample

Be optimized for signal strength with respect to the following parameters:

- Laser wavelength within the tuning range of the Ti-Saph Laser (~700 - 1000 nm)
- Laser average pulse power
- Laser repetition/pulse rate
- Dwell time of the optical scanner
- Choice of numerical aperture for focusing and collection optics
- Polarization of incident light and analyzer orientation

Not damage the tissue sample in any way:

- The SHG signal cannot change the collagen structure (detectable as a decrease in SHG signal intensity)
- Paraffin (used for sample fixing) cannot melt
- Avoid creating autofluorescent or two-photon fluorescent signals, or at least understand and know how to remove this signal.

Be simple enough to be operated consistently by a technician

Make accurate, repeatable measurements of F/B to objectively predict tumor metastasis

Use two microscope objectives (forward, backward) one to focus the beam into the sample and both to be used as collection optics

Use a standard oncology lab prepared slide with cover slip as the object

Have a standardized and repeatable calibration method

Use a detector optimized for the SHG signal wavelength

If time and resources allow, the system may:

Be accompanied by new image processing software to efficiently analyze results with larger data sets

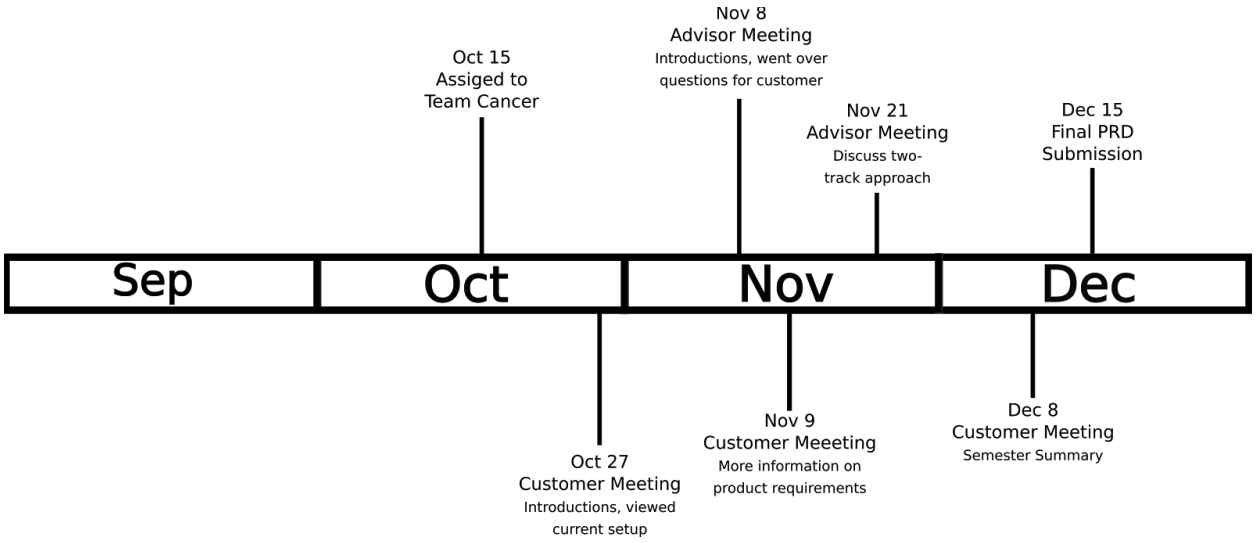
Use a different detector to the PMTs already in use, such as an APD. Choice will be based on noise floor and quantum efficiency

What we are not responsible for:

Getting FDA approval for the microscope as a diagnostic device.

Getting biopsy samples and rat tail samples for the experiment

Timeline for the fall semester:



Planned Spring Testing Timeline:

Approx Time:	**January**	**February-March**		**April**
Track I (EB Lab):	Dosimetry Plans	Experiments		Results/Redesign
Track II (WHK Lab):	Plan New System	Parametric Study	Experiments	Results/Redesign

SOURCE	**TRACK I (BROWN LAB)**	**TRACK II (KNOX LAB)**
Laser	**Ti:Sapphire**	**Yb Fiber**
Pulse Duration	**110 fs**	**250 fs**
Rep Rate	**80 MHz**	**1-15, 60 MHz**
Wavelength	**810nm**	**1035nm**
Power	**0-2 W**	**0-30 W**

Two Track Overview:

TRACK I

We will utilize the existing optical setup in Professor Ed Brown's lab to test the effect of altering laser power on signal to noise. As laser power is increased, we expect to see a proportional increase in SHG signal. As power is increased, eventually the collagen structure will be damaged and SHG will decrease. We aim to experimentally determine this damage threshold.

TRACK II

In Dr. Knox's lab, we will utilize a similar laser (see above chart) to parametrically determine the effects of other variables on signal to noise. We plan to optimize:

-Repetition rate of the laser
-Polarization
-Detector Choice
-Collection and Focusing optics (NA, spot size, etc.)
-General Optical setup (Filters, beam path etc.)

While monitoring SNR and avoiding the damage threshold determined in track I.

Track I:

In the first research track, based in Dr Brown's lab, we will use the current system to explore the limits of exposure that collagen samples can take to input light before becoming damaged. We will consider the input power of the current setup, and measure how the SHG signal changes as we increase the input power. Other signals, such as autofluorescence and two-photon fluorescence must also be detected as they are a sign of fibril bonds breaking. If time allows, the scan speed may also be considered.

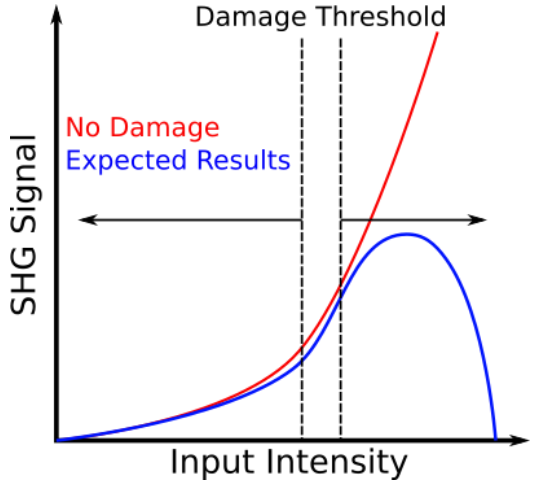


Figure 2. The expected change in SHG signal strength as a function of input laser power assuming no sample damage (red), and expected results on real samples (blue).

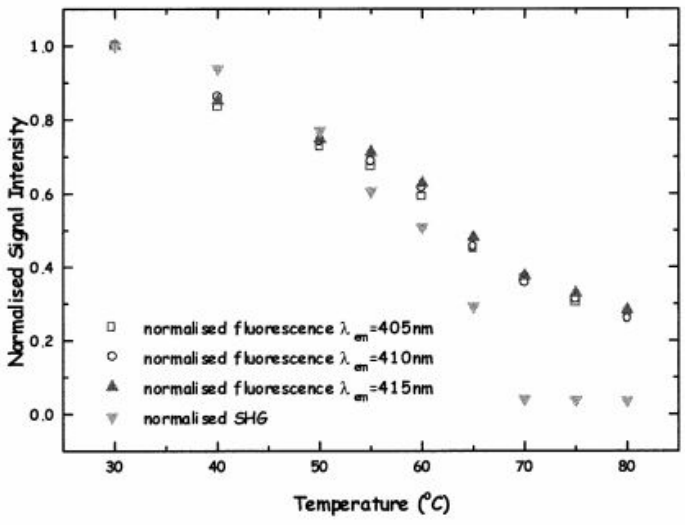


Figure 3. Various collagen signal intensities as a function of temperature. SHG signal (▼) is shown to decrease significantly past room temperature (23°C). (Reference 3)

Track II:

In the second research track, based in Dr. Knox's lab, we will consider what can be changed for the first prototype system and what factors affect the conversion efficiency from input energy to SHG signal (rep rate, peak power, etc.).

We will consider the results of the first track experiments to perform a top to bottom optical redesign of the instrument. This design will attempt to

-Maximize SHG signal from collagen

-Minimize system noise

-Minimize build cost

Our end goal is a threefold increase in signal to noise ratio.

References

1. K.Burke, M. Smid, R. P. Dawes, M. A. Timmermans, P. Salzman, C. H. M. can Deurzen, David G. Beer, J. A. Foekens, E. Brown. Using Second Harmonic Generation to Predict Patient Outcome in Solid Tumors. 2015, *BMC Cancer 15:929*
2. Juan M. Bueno, Francisco J. Avila, Pablo Artal, Comparison of Second HArmonic Microscopy images of Collaged-based Ocular Tissues with 800 and 1045 nm. 2017, *Biomedical Optics Vol. 8 No. 11*
3. Theodossiou T., G. S. Rapti, V. Hovhannisyan, E. Georgiou, K. Politopoulos, D. Yova, Thermally Induced Irreversible Conformational Changes in Collagen Probeed by Optical SEcond Harmonic Generation and Laser-induced Fluorescence. 2002, *Lasers Med Sci 17:34-41*
4. Williams, Rebecca M., Warren R. Zipfel, Watt W. Webb, 2005, Interpreting Second-Harmonic Generation Images of Collagen I Fibrils. *Biophysical Journal Vol. 88* 1377-1386.
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