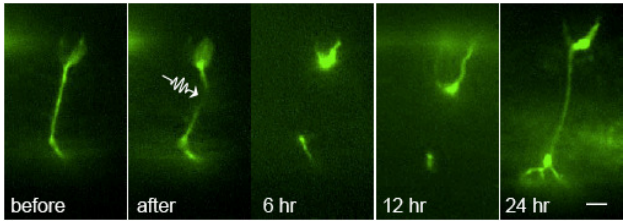
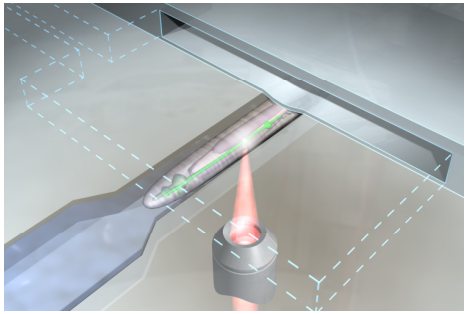
Nerve Regeneration Studies in *C. elegans* using Femtosecond Laser Axotomy

In the United state, nearly 5 million people suffer from neurodegenerative diseases and another half-million have suffered from some type of nerve injury. To this day, the mechanisms underlying nerve regeneration and degeneration remain largely unknown. Searching for the genes involved in these mechanisms will help us to better understand the causes of these debilitating diseases and thus pave the way for successful treatments and preventions. The best conditions for studying nerve regeneration would be met if one could sever axons or dendrites in a controlled manner and study what genes or molecules affect their regrowth in vivo in a simple organism. The soil nematode *Caenorhabditis elegans* (*C. elegans*) is an ideal model organism for such study. They are transparent to visible light; their nervous system comprises only 302 neurons and is entirely mapped; and their genetic versatility allows for gene manipulations. In our animal model, *C. elegans*, we are investigating mutant worms and using RNA interference, along with femtosecond laser surgery, to search for candidate genes that may be involved in this complicated regeneration process.

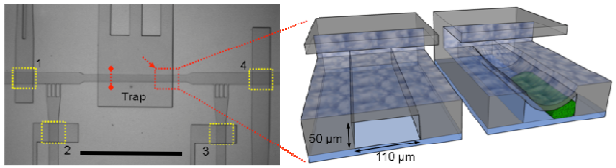


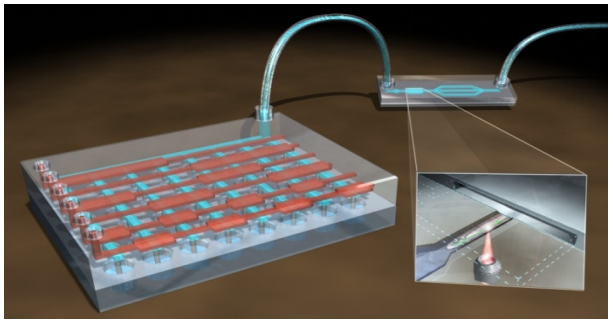


Top: Nerve regeneration after femtosecond laser axotomy in live C. elegans. Fluorescence images of GFP labeled axons before, after, and following axotomy. Bottom: Rendition of a trapped worm undergoing axotomy in a microfluidic chip.

High-throughout Microfluidics Platforms for Genome-Wide Nerve Regeneration Studies

Nerve regeneration studies should utilize an environment for both surgery and observation that has minimal impacts on biological pathways and behavior. In contrast to the conventional immobilization method using anesthetics, microfluidic devices offer several advantages, including chemical-free immobilization, ease of manipulation, and high throughput data acquisition. These devices provide a well-controlled microenvironment for severing axons and monitoring the animals' neural functions. Specifically for nerve regeneration studies, we developed a new microfluidic device to immobilize the worms for surgery without using any chemicals. This microfluidic device allows us to precisely manipulate worms inside small channels and eliminates the need for anesthetics, which we have shown to interfere with the axonal regrowth process. With the recent advancements in microfluidic interfaces, now it is also feasible to automatically load large sample populations from multiwell plates. The prospective integration and full automation of such microfluidic platforms finally offers the possibility to perform high-throughput, genome-wide screening of phenotypes in living animals.

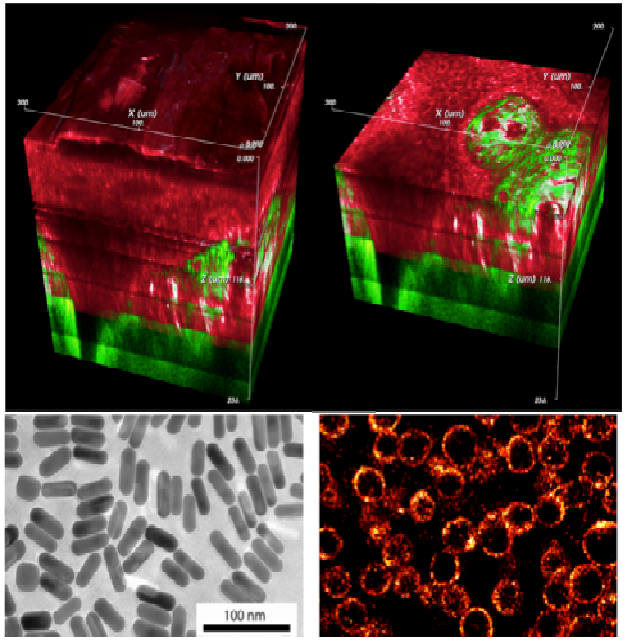




Integrated microfluidics platform will be used for worm loading, axotomy, and recovery for high throughput nerve regeneration studies. Top left: Worm immobilization chip. Scale bar is 1 mm. Top right: Schematic of the two layer trap channels without and with an immobilized worm. Bottom: Integrated microfluidics platform.

Nonlinear Optical Imaging for Cancer Diagnosis

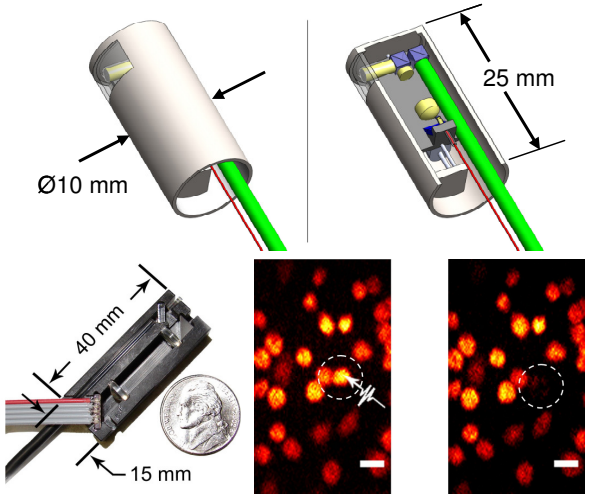
Nonlinear microscopy is a potential technology for the diagnosis, screening, and monitoring of disease that allows real time, non-invasive imaging to be performed with subcellular resolution hundreds of micrometers deep in scattering tissues. Our lab, in collaboration with surgeons at the M.D. Anderson Cancer Center, has been investigating this technology as a means to classify oral tissue biopsies as normal, precancerous, or cancerous *ex-vivo*. Using two-photon and second-harmonic generation microscopy, we have generated 3D maps of endogenous fluorescence from the samples, which provide morphological and functional information for biopsy case-finding. To improve the sensitivity of this technique, we are introducing novel contrast agents, such as gold nanospheres and nanorods, which are specifically targeted to proteins which are overexpressed in cancerous tissues. Furthermore, the increased brightness from these exogenous contrast agents allow for endoscopic imaging of tissues in our less-sensitive, but more clinically friendly miniaturized nonlinear microscope.



Top: 3D rendering of autofluorescence (red) and second harmonic generation (green) signal from human tongue tissue. Bottom left: SEM image of gold nanorods. Bottom right: Two-photon image of cancer cells labeled with gold nanorods.

Nonlinear Image-Guided Femtosecond Laser Microsurgery Endoscope

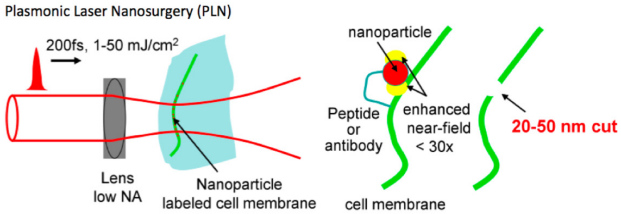
Two-photon microscopy is emerging as a useful imaging technique for diagnostic imaging of biological tissues. Additionally, femtosecond laser pulses have proven to be much more efficient in ablating tissue for microsurgery, thus greatly reducing collateral damage. Thus far, integration of these techniques into clinically-useful tools has been extremely limited. The aim of this project is to develop small flexible probes capable of delivering ultrashort laser pulses into the body for combined imaging and microsurgery. Currently, we are focused on two key clinical applications: treatment of small cancerous lesions and treatment of vocal fold scarring. This project has strong optical design and experimental components. The nature of the work is very multidisciplinary and includes fields such as MEMS devices, photonic crystal structures, manufacturing, signal processing, and cell biology. There are currently several proposals being reviewed to provide funding for this project.



Left: Photograph of the first probe prototype, measuring just 10 × 15 × 40 mm^3. Center: A two-photon fluorescence image of cancer cells taken with the prototype, indicating a cell targeted for ablation. Right: The same region, after delivering one high-energy surgery pulse to the targeted cell. The targeted cell was instantly destroyed while neighboring cells were left intact. Below: A preliminary model of a clinically packaged endoscope for treatment of vocal fold scars.

Plasmonic Laser Nanoablation (PLN)

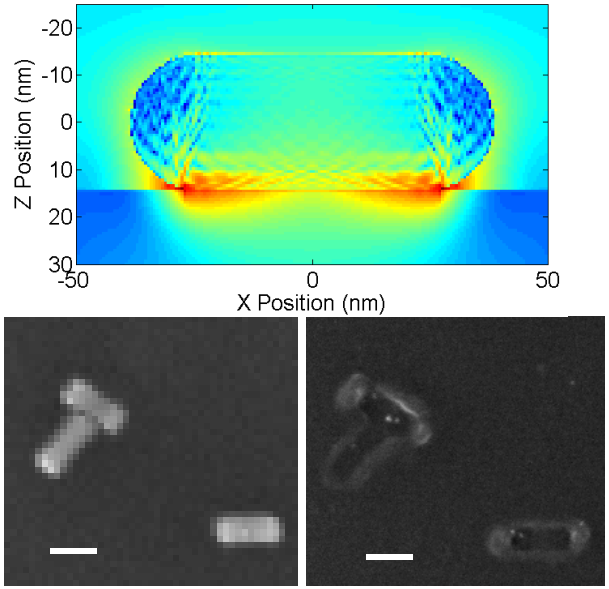
Plasmonic Laser Nanoablation or Nanosurgery (PLN) is a novel photodisruption technique that exploits the large enhancement of femtosecond (fs) laser pulses in the near-field of metal nanoparticles for the selective and non-thermal nanoscale manipulation of biological structures. The electric field, which is amplified when the laser frequency is tuned to the plasmon frequency of a metal nanostructure, can be utilized to initiate photodamage with nanoscale precision to biological targets onto which nanoparticles are attached. The use of fs-laser pulses ensures non-thermal tissue photodisruption, while functionalized nanoparticles greatly improve the localization of the photodisruption process. Moreover, the enhanced electric field around the particle reduces the fluence necessary for material photodisruption, limiting the extent of damage to the particle near-field. Since the particles themselves act as "nano-lenses", large volumes of tissue can be irradiated at a given time, decreasing the time of treatment. In addition, gold nanostructures exhibit minimal cellular toxicity, making the technique ideal for *in vivo* clinical use.



In our own research, we have demonstrated the feasibility of plasmonic laser optoporation for both cellular death by necrosis for cancer treatment and transient pore formation for cellular transfection. In clinical medicine, PLN has the potential to treat cancers localized in sensitive regions, i.e. brain tumor, or can be utilized for the removal of small epithelial cancer lesions, i.e. breast carcinomas in situ. In biological sciences, PLN could bring significant advance in the transfection of cellular systems; this includes the transfection of cells that typically do not respond well to traditional transfection methods or that of in vivo mass transfections. On a broader spectrum, the high specificity of PLN provides the possibility for membrane- and molecular-specific phototherapy; direct therapeutic prospects extend into the fields of cancer, genetics, proteomics, virology, bacteriology, and cardiology.

Optical Lithography using PLN

Moore's Law, first stated in 1965, was an observation that computer chip density doubled every two years and that it should continue to do so for the foreseeable future. This prediction has proven remarkably prescient over the last 40 years. However, conventional lithography is limited to half of the wavelength used in fabricating features for micro- and nano-electronic devices and current fabrication technology is approaching the size limits given available light sources. Increasingly sophisticated methods are now being used to develop smaller features, but increasing cost and complexity and the existence of a fundamental limit render these methods unsustainable. In light of this problem, fabrication methods that can create features smaller than the diffraction limit are of great interest. One possible nanofabrication method is the use of plasmonic laser nanoablation. Our research focuses on the use of plasmonic laser nanoablation for fabricating nanostructures. We use computational techniques to investigate the interaction between laser light and gold nanoparticles while experimentally creating nanoablation features on silicon and glass substrates using plasmon-enhanced femtosecond laser pulses.



Top: Computational electric field enhancement for infrared laser light incident on a gold nanorod on a silicon surface. Bottom: SEM images of nanorod ablation. Scale bar: 50 nm.

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