Study: Excess breast tissue samples were obtained from UMass Medical School after surgery. pHLIP was conjugated with Alexa532 for fluorescence imaging. Fresh samples were stained with 5 μM pHLIP-Alexa532 at ambient temperature, and then imaged with wide-field and confocal systems. Reflectance and fluorescence images of pHLIP were acquired and compared to histology.
Results: pHLIP-Alexa532 wide-field imaging successfully identifies breast cancer. Imaging results correlate well with histology. High-resolution images show that pHLIP-Alexa532 stains the cytoplasm of cancer cells.
Conclusion: pHLIP fluorescence imaging has potential for intraoperative delineation of breast cancer.

#21

OPTICAL COHERENCE TOMOGRAPHY IMAGE-GUIDED SMART LASER KNIFE

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Background: In review, current state-of-the-art techniques in tumor resection (like NICO Myriad, Medtronic StealthStation, Zeiss OPMI) employ techniques like iMRI, iCT, fluoroscopy and preoperative CT/MRI to provide information on the location of the tumor and a navigation path to the location. Other current state-of-the-art techniques, the surgeries are being done under intra-operative ultrasound with prior knowledge from MRI and CT. There is a limitation of the resolution achievable using these techniques and also, preoperative imaging only provides information of the location and the pathway to the tumor. For cases like iMRI, the whole surgical space needs to be changed (plastic surgical tools) in fully utilize the accuracy of MRI technique which is still limited in millimeter resolution. Although fluorescence imaging offers much higher micron/sub-micron resolution the imaging is mostly on the surface and information about the sub-surface delicate structures remains inaccessible. Pathologist recommendation on resected tissues remains to be the gold standard in these techniques even today resulting in long surgery durations, involving the patient to be under anesthesia during that period. Considering these limitations, Optical Coherence Tomography (OCT) occupies an effective surgical spot in terms of resolution and imaging depth. Plaque classification using OCT offers an example of how effective the procedure has been in providing less than 10 micrometer resolution in comparison to IVUS (intravascular *Ultra* Sound) of 100s of micrometers. Thus, employing a surgical tool guided by the imaging technique of OCT offers effective resection of tissue or tumors in neurological surgeries. Neurological cancer surgeries require such specialized tools that enhance imaging for precise cutting and removal of tissue without damage to adjacent neurologic structures.
Study: The novel combination of high-resolution fast Optical Coherence Tomography (OCT) alongside short pulsed nanosecond thulium (Tm) lasers offers stark advantages over conventional surgical lasers utilizing the superior beam quality, high volumetric tissue removal rates of thulium lasers with minimal residual thermal footprint in the tissue, and avoiding damage to delicate sub-surface structures (e.g., nerves and microvessels); which has not been showcased before. A bench-top system is constructed, using a 15 W 1940 nm nanosecond pulsed Tm fiber laser (500 μJ pulse energy, 100 ns pulse duration, 30 kHz repetition rate) for removing tissue and a swept source laser (1310 ± 70 nm, 100 kHz sweep rate) for OCT imaging, forming a combined Tm/OCT system − a smart laser knife. The OCT image-guidance informs the Tm laser for cutting/removal of targeted tissue structures.
Results: Tissue phantoms were constructed to demonstrate surgical incision with blood vessel avoidance on the surface where 2 mm wide 600 μm deep cuts are executed around the vessel using OCT to guide the cutting procedure. Cutting up to delicate subsurface blood vessels (2 mm deep) is demonstrated while avoiding damage to vessel walls. A tissue removal rate of 5 mm3/sec is obtained from the bench-top system. We constructed a blow-off model to characterize Tm cut depths taking into account the absorption coefficients and beam delivery systems to compute Arrhenius damage integrals. The model is used to compare predicted tissue removal rate and residual thermal injury with experimental values in response to Tm laser-tissue modification.
Conclusion: In this work we describe a system that combines optical coherence tomography (OCT) and laser tissue modification with thulium (Tm) laser. A modeling of the process is carried out using COMSOL and Zemax simulation tools. The simulation results of the cutting depth show good relation to the experimental Tm etching for tissues. The OCT image guided smart laser knife demonstrates the use of tomographic imaging to differentiate between types of tissues can help prevent bleeding (by avoiding vessels/isolating them), and still offer high speed micro-precision cutting of up to 5mm3/sec

#22

MULTIMODALITY OPTICAL CHARACTERIZATION OF IMPAIRED WOUND HEALING IN A PRINCIPAL MODEL OF DIABETES MELLITUS

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Background: Diabetes Mellitus (DM) is becoming an epidemic throughout the world, so far affecting almost 194 million people. This number is expected to increase to 344 million by the year 2030. Major complications result from the accumulation of Advanced Glycation End Products (AGEs), leading to delayed wound healing and persisting skin ulcers. The high mortality rate and lack of ability to treat these wounds and ulcers raise an urgent necessity for early detection of non-healing wounds. This allows physicians the ability to provide treatment for these wounds at an early stage. In this study, using Streptozotocin (STZ) injected rats; we evaluate and find non-invasive methods for the detection of biomarkers for delayed wound healing.
Study: To this end, we compared the wound healing process of STZ induced diabetic rats with a control population using Spatial Frequency Domain Imaging (SFDI), Laser Spackle Imaging (LSI), and Multiphoton Microscopy (MPM). Using SFDI, oxyhemoglobin, deoxyhemoglobin, and water content was evaluated in the area next to the wound and within the wound itself. The changes in blood flow were assessed using LSI, and the accumulation of AGEs was measured using MPM.

Results: Our results indicate that only 2 weeks after confirmation of diabetes, accumulation of AGEs is clearly visible in collagen fiber. There is also an indication that changes in water content and deoxyhemoglobin levels during this process can be an indicator for delayed wound healing.
Conclusion: Accumulation of AGEs in the skin and especially in the collagen of diabetic animals can be detected and visualized using MPM. The accumulation of AGEs is correlated to the delay in wound healing of diabetic rats compared to non-diabetic controls. The high level of deoxyhemoglobin measured using SFDI is also a potential indicator of impaired wound healing. More investigation is needed to calculate the ratio between oxy- and deoxyhemoglobin concentration, the role of water content and inflammation during diabetic wound healing, and thus determining the potential of this assay for predicting the duration of wound healing in diabetic patients.

#23

OCT IMAGING OF SMALL BLOOD VESSEL REMODELING AFTER FRACTIONAL PHOTOTHERMOLYSIS

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Background: Fractional photothermolysis is an established procedure in modern dermatology providing convincing results of skin resurfacing and rejuvenation. However, the immediate effect on the dermal network of micro-vasculature and its impact on wound healing is not fully unveiled yet. Optical coherence tomography (OCT) allows *in vivo*, sectional imaging of dermal tissue non-invasively and is even capable to display vessel perfusion with a μm-resolution.
Study: A commercial OCT-device is used to perform imaging before and multiple times after treatment of fractional photothermolysis on rat skin for up to 3 weeks. The treatments are varied in power per pulse and density of lesions. A custom made software was implemented to generate 3D maps of the vessel network within the dermis.
Results: An instantaneous effect of fractional photothermolysis on the capillary network can be observed using OCT imaging. Perfusion is corrupted in the areas of treatment immediately after irradiation and even interrupted completely when a high density of lesions is chosen.
Conclusion: The results presented in this study lead to reconsider the parameter of fractional photothermolysis in clinical studies by also respecting the induced damage to the capillary network and its effect on the wound healing process and outcome.

#24

DERMATOSCOPE INCORPORATING LASER SPECKLE IMAGING TO ENABLE SIMUTANOUS BLOOD FLOW QUANTIFICATION AND VISUAL INSPECTION OF SKIN

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Background: Dermatoscopes are commonly utilized for the qualitative visual inspection of skin lesions. While automated image processing techniques and varied illumination strategies have been used to aid in structural analysis of lesions, robust quantification of functional information is largely unknown. We have therefore developed a compact, handheld dermatoscope which enables real time blood flow measurements of skin during conventional visual inspection.
Study: Blood flow measurements were achieved by integrating a compact laser speckle imaging (LSI) system into a dermatoscope. LSI measurements using illumination from a 785 nm laser diode was performed simultaneously with visual inspections under white LED illumination *via* spectral filtering of co-registered images. Flow measurements using the LSI-dermatoscope were validated by acquiring LSI data from a tissue-simulating phantom with syringe pump-controlled flow of optically scattering fluid across the physiologically relevant range. Measurements were also performed during post-occlusive reactive hyperemia tests ($n = 10$) on the forearm of healthy volunteers to assess the correlation of the LSI-dermatoscope measurements to a validated benchtop LSI system and to perform repeatability and signal to noise analysis.
Results: The LSI-dermatoscope was able to measure known flow rates in a tissue-simulating phantom with a correlation coefficient of 0.98. Data acquired from volunteers during post-occlusive reactive hyperemia showed the expected physiological blood flow response of decreased blood flow during occlusion and the return of blood flow above baseline (hyperemia) following occlusion release. LSI-dermatoscope data was significantly correlated ($p < 0.05$) to data acquired simultaneously using a traditional benchtop LSI system. The coefficient of variation between measurements was relatively low (0.0023) and exhibited a signal to noise ratio of 17.
Conclusion: We have developed and validated a dermatoscope that enables simultaneous blood flow measurements and visual inspection of skin. The simplicity and accuracy of LSI dermatoscope facilitate straightforward integration into the dermatology workflow and future investigation of blood flow dynamics within various cutaneous pathologies.

#25

PHOTOOXIDATION OF TRYPTOPHAN DURING PHOTOCROSSLINKING TREATMENT FOR KERATOCONUS

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Background: A recent FDA-approved clinical treatment option for keratoconus involves the use of UV-initiated photo-crosslinking with riboflavin to increase corneal stiffness. Fluorescence excitation-emission spectroscopy can be used to probe the creation and destruction of fluorescent species in cornea; we hypothesized that loss of tryptophan fluorescence would occur in the presence of riboflavin, due to the production of reactive oxygen species from tryptophan oxidation.
Study: 81 *ex vivo* rabbit eyes were treated with either riboflavin-dextran solution plus UV light, dextran solution plus UV light, or riboflavin-dextran solution only for 20, 40, and 120 minutes. Uniaxial tensiometry was performed on all samples after treatment to determine the degree to which mechanical photo-crosslinking had occurred. UV fluorescence spectroscopy to assess tryptophan fluorescence was performed after crosslinking treatment.