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## CARS microscopy of cancer cells in vitro and tumors in vivo.

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Identification of cancer cells in tumors is still a challenge in the biomedical field. Coherent Anti-Stokes Raman Scattering (CARS) microscopy may be useful for fast retrieval of 3D imaging and to distinguish cancer cells from the non-cancerous environmental cells as it is a label-free imaging technique that is capable of real-time, non-pertubative examination of living cells and organisms based on molecular vibrational spectroscopy with a sub-micron spatial resolution.

In this project, we have taken CARS and Second Harmonic Generation (SHG) images of normal and cancer cells using a optical setup developed in collaboration with Genia Photonics. Epithelial cancer cells (HT29 and MCF-7) and normal epithelial cells (MCF-10A) were embedded in  $300\mu$ m-thick collagen gel laid on glass coverslips and then observed directly on a microscopic platform equipped with a 20X objective. Control observations were done with fixed (formaldehyde) and stained (dyes and fluorescent probes) specimens.

Tumor xenografts were induced after injection of cancer cells (HT29) in mouse spleen (lymphoid tissue) to reproduce lymph node metastases. Cryosections (20-30mm thick) of retrieval and unfixed tissues were performed to allow direct CARS and SHG microscopy through a glass coverslip. Routine histology sections were used to compare imaging.

Resulting 3D cell morphology was close to that seen by optical microscopy. Cancer cells were also seen in tissue sections of lymphoid tissue-grafted tumors for which imaging was clearly distinguishable from the inflammatory reaction and fibrotic reaction (observed with SHG) as well as the normal lymphoid tissue.

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