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Optical Methods to Quantify Oxygen Tension in Microfluidic Devices

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Rapidly dividing tumor cells have high metabolic needs, which results in high oxygen consumption rates and an oxygen gradient in the tumor microenvironment. This change in oxygen tension also affects the neighboring vascular network which often results in tumor angiogenesis, the sprouting of vessels. To study the oxygen gradients present in the tumor microenvironment, a microfluidic device made of polydimethylsiloxane (PDMS) was used to recapitulate this phenomenon. Subsequently, to ensure physiological oxygen tensions, it was of interest to quantify this concentration in the devices in order to visualize the oxygen gradients.

Phosphorescent lifetime imaging microscopy (PLIM) utilizes a phosphorescent oxygen probe called Oxyphor G4 which was added directly to the media 24 hours before measurements were made. Oxyphor G4 does not permeate into cell membranes so the dye does not interfere with the cell behavior in the experiment. Measurements were made using a laser pulsed confocal microscope that measured the dye's lifetime decay. Oxyphor G4 relies on phosphorescent quenching such that high oxygen tensions result in lower lifetimes and low oxygen tensions yield higher lifetimes. At least 100 counts were taken at each pixel and the data was exported as an array of counts and an array of lifetimes.

To translate the array of lifetimes to an array of oxygen concentrations, a MATLAB script was first developed to filter out the PDMS in the oxygen map. The array of counts was used to threshold the image and the lifetimes of the remaining pixels were then mapped to the final image. By using PLIM to measure oxygen tension, heterogeneous distributions of oxygen were able to be quantified and visualized on an oxygen map.