Lab on a Chip Supporting Information

Silicon-on-glass pore network micromodels with oxygen-sensing fluorophore films for chemical imaging with retained spatial structure information

Jay W. Grate, Ryan T. Kelly, Jonathan Suter, and Norman C. Anheier, Jr

Microfabrication and Assembly

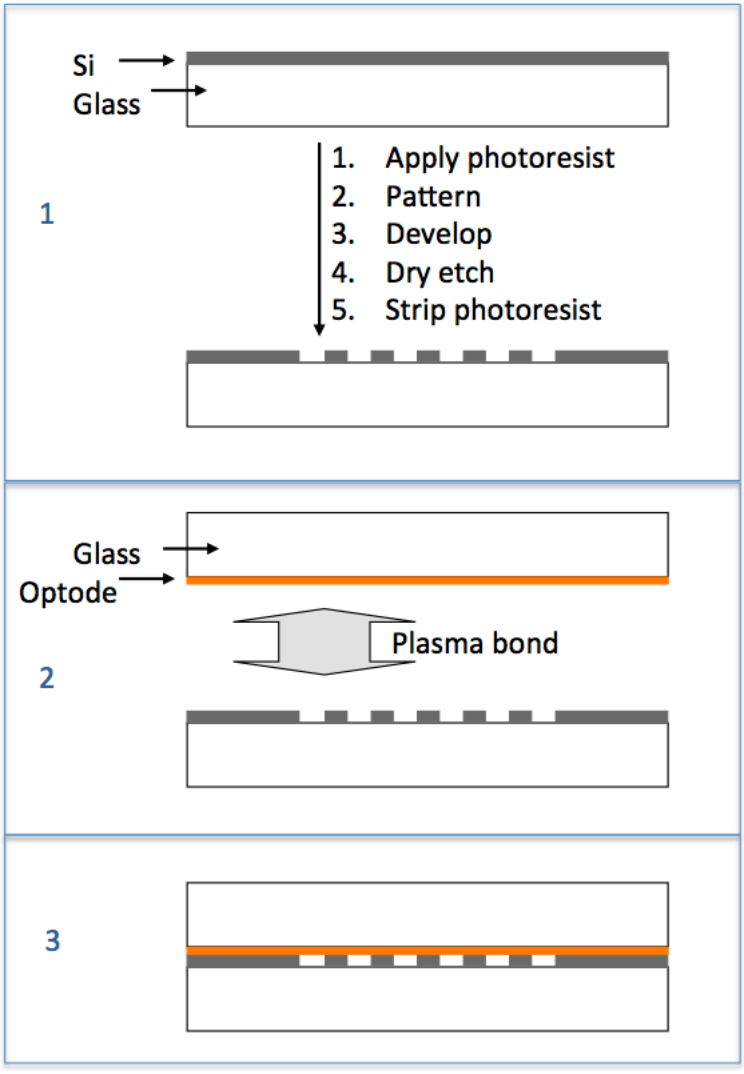

Figure ESI_1. Simplified schematic process sequence showing key steps of the microfabrication and assembly process. 1) Creation of the silicon-on-glass pore network. 2) Assembly of the micromodel by plasma bonding a glass cover plate with the optode previously applied by spin-coating. 3) The assembled micromodel with the optode incorporated that enables imaging through transparent glass from either side.

Fluorescence Lifetime Imaging System

The custom imaging system is shown in **Figure ESI_2**. Prior to scanning the sample, a zero phase reference was established using a red fluorescent reference slide (Model 2273-R, Ted Pella Inc., Redding, CA). The fluorescence lifetime of the emitting compound in this slide is so small compared to the lifetime of PtTFPP that it can be treated as zero. The measured phase shift, ϕ_ω, was then used to directly calculate the single exponential lifetime, τ ,of the fluorophore using the relationship,

$$\tan\phi_\omega = \omega\tau ,$$

where ω is the modulation frequency (5 kHz).

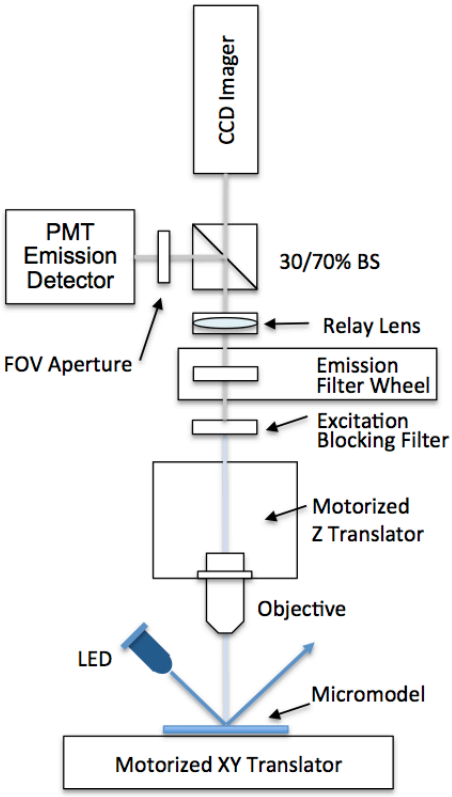

Figure ESI_2. Simplified schematic diagram of the fluorescence lifetime imaging microscope with a motorized stage, off axis LED illumination, fluorescence detection with a PMT, and a CCD camera for wide field fluorescence intensity imaging. The iris assembly for the LED is not shown.

Silicon Micromodel with Fluorescence Lifetime Imaging

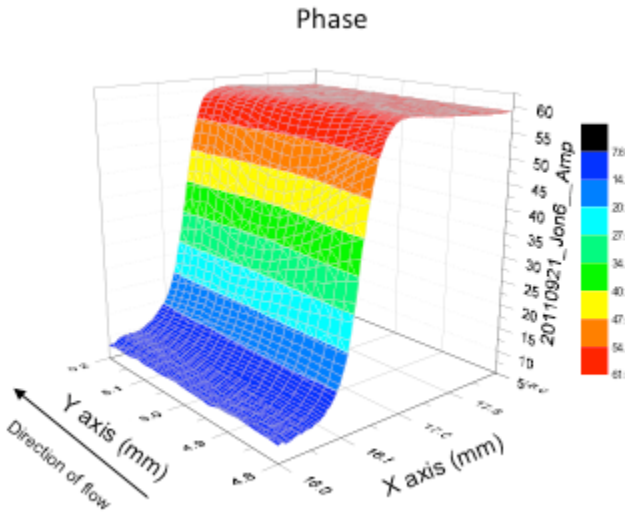

Figure ESI_3. Scanning fluorescence lifetime imaging result for a dissolved oxygen gradient in a silicon micromodel, imaged through the glass coverplate to the optode film under the glass coverplate. Although silicon pillars are within the image field, they cannot be seen in the fluorescence lifetime image.

Calibrations Models

Fluorescence imaging of oxygen concentrations by the collisional quenching mechanism typically fits the Stern-Volmer-equation below (1), which can be expressed as either a ratio of intensities or a ratio of fluorescence lifetimes.

$$I_o/I = \tau_o/\tau = 1 + K_{SV}\,[O2] \qquad (1)$$

In this equation, [O2] is the oxygen concentration, I is the observed intensity of the luminescence, I_o is the intensity at [O2] = 0, τ is the lifetime of the excited dye triplet state, τ_o is the lifetime at [O2]=0, and K_{SV} is the Stern-Volmer constant.

Using the observed fluorescence lifetimes at the two endpoints of our gradient imaging, i.e. where the dissolved [O2] = 0 and the dissolved [O2] is equilibrated with 100% oxygen gas (i.e. ca. 42 mg/L), we modeled the calibration curves assuming a linear Stern-Volmer relationship. Two point calibrations are common in the usage of fluorescence oxygen sensors. The modeled calibration curves are shown below, illustrating different dynamic ranges and sensititivies for the PDMS and polystryrene matrix optodes (see text of paper).

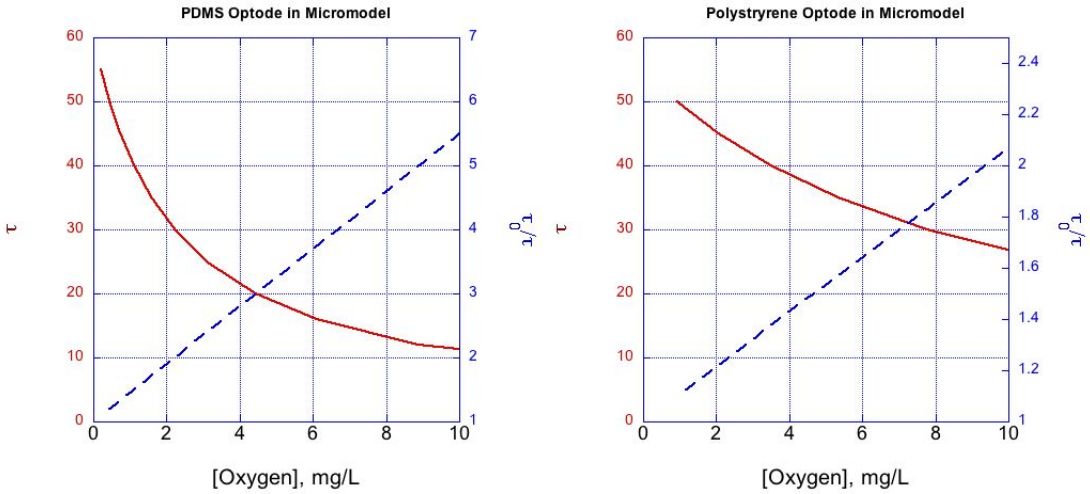

Figure ESI_4. Modeled calibration curves for the PDMS and polystyrene optodes showing the fluorescence lifetimes, τ(solid red line) and the fluorescence lifetime ratio, τ_o/τ, of the Stern-Volmer relationship (dashed blue line). The portion of the calibration curves corresponding to dissolved oxygen from zero to air saturated water (ca. 8 mg/L) is shown.

PDMS Membrane Structure for Covering Silicon-on-glass Micromodels

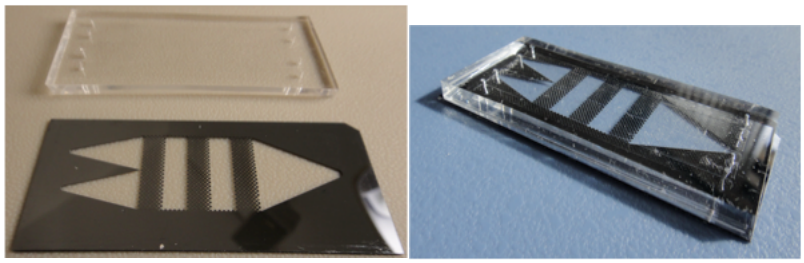

Figure ESI_5. Images showing the silicon-on-glass pore network and a PDMS slab containing gas channels, separately and together. With the PDMS structure on the pore network micromodel, the glass base provides a quality transparent interface through which processes in the pore network can be imaged.