ADVANCED HEALTHCARE MATERIALS

Supporting Information

for *Adv. Healthcare Mater.*, DOI: 10.1002/adhm.201601356

Biocompatible Multifunctional Black-Silicon for Implantable Intraocular Sensor

*Jeong Oen Lee, Vinayak Narasimhan, Juan Du, Blaise Ndjamen, David Sretavan, and Hyuck Choo**

Submitted to ADVANCED MATERIALS

Supporting Information for

Biocompatible Multifunctional Black-Silicon for Implantable Intraocular Sensor

Jeong Oen Lee[+], Vinayak Narasimhan[+], Juan Du, Blaise Ndjamen, David Sretavan and Hyuck Choo[*]

[*] Prof. H. Choo, Corresponding-Author

hchoo@caltech.edu

[+] These authors contributed equally to this work

Dr. J. O. Lee, V. Narasimhan, Dr. B. Ndjamen, Prof. H. Choo

Department of Medical Engineering,

California Institute of Technology, Pasadena, CA, 91106, (USA)

Dr. J. O. Lee, Prof. H. Choo

Department of Electrical Engineering,

California Institute of Technology, Pasadena, CA, 91106, (USA)

Dr. J. Du, Prof. D. Sretavan

Department of Ophthalmology,

University of California, San Francisco, San Francisco, CA,

Device Working Principle

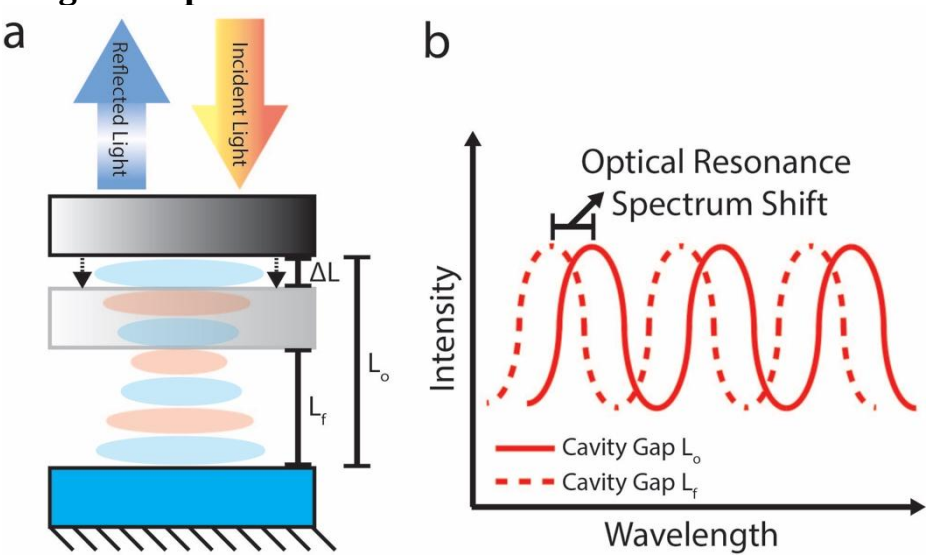

Figure S1. **(a)** The sensor primarily consists of an optomechanical cavity with a deformable transparent top layer and a fixed reflective bottom layer. The initial air gap (or cavity gap) between the two layers, L_o, is 4 µm. When the ambient pressure changes the top layer deforms, thereby altering the air gap to L_f. (b) Upon optically exciting the cavity with a NIR tungsten light source, the reflected resonance spectrum is captured by a spectrometer. A change in gap size results in a shift in the reflected resonance spectrum which can subsequently be correlated to IOP values.

B-Si Surface Profile

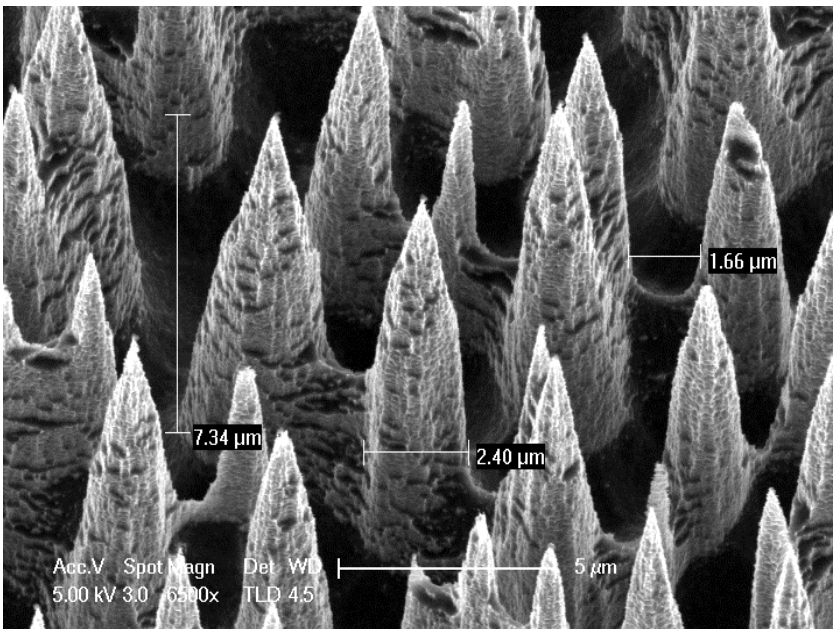

Figure S2. SEM image at 6500x magnification of b-Si texturing with annotations indicating structure height, width and spacing. Through SEM image processing, the RMS surface roughness was estimated to be 4.63 μm. Scale: 5 μm.

Absolute Reflectivity Measurements

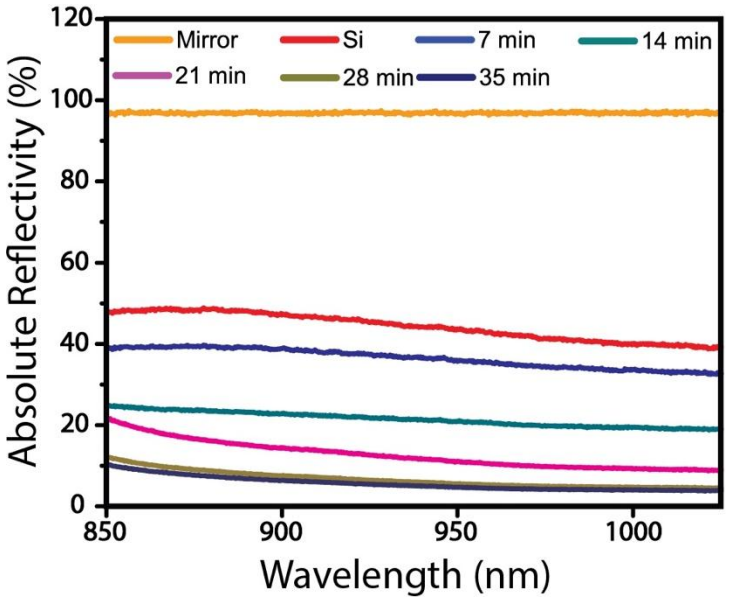

Figure S3. The absolute reflectivity of Si and five b-Si samples (categorized by process time) were characterized. b-Si samples were optimized to an absolute reflectivity as low as 3.5% in the wavelength range of interest. A NIR high-reflectivity mirror (R_{avg} > 97%, Edmund Industrial Optics) was used as a reference.

Fabrication Process Flow

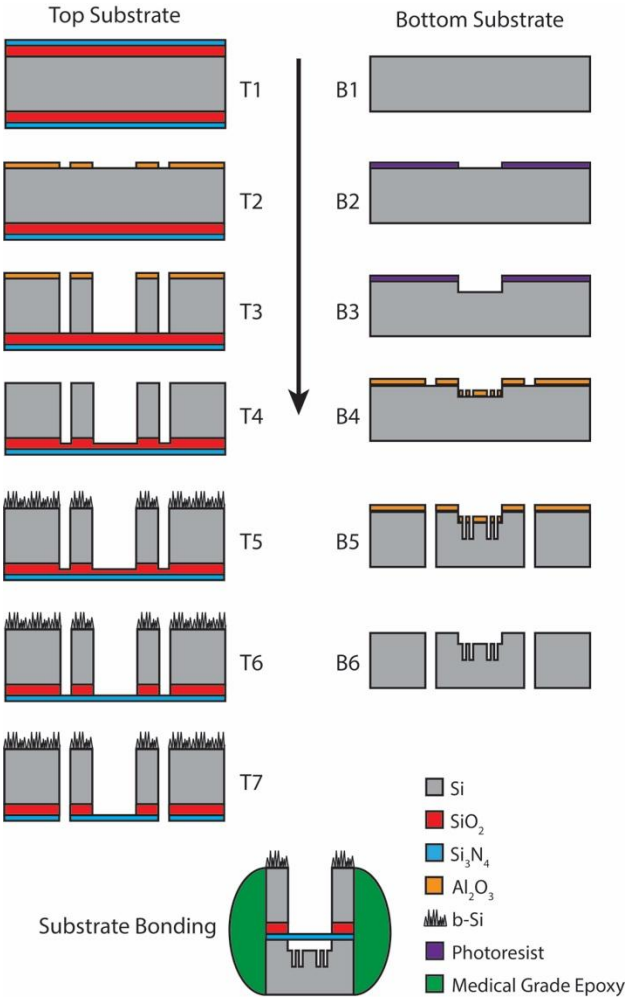

Figure S4. Illustration of the b-Si sensor fabrication process flow. The sensor consists of two parts (top and bottom) individually batch-fabricated and bonded together using medical grade epoxy to produce a hermetically sealed active core cavity. The diagram also illustrates the seamless integration of the room-temperature b-Si process into the sensor fabrication.

The top and bottom substrates that form the b-Si IOP sensor were fabricated individually. Initially the top structure consisting of a flexible Si_3N_4 membrane and a hollow circular opening in the center was processed. 2 µm thick silicon dioxide (SiO_2) and 300 nm thick silicon nitride (Si_3N_4) layers were deposited on the top and bottom surfaces of a double side polished Si wafer (thickness: 300 µm) using thermal oxidation and low pressure chemical vapor deposition (LPCVD), respectively (**Fig. S4T1**). Then the Si_3N_4 and SiO_2 layers on the top surface of the wafer were completely removed using reactive ion etching (RIE) (Plasmalab System 100 RIE/ICP, Oxford Instruments, Inc.) and buffered hydrofluoric (BHF) acid. Next, a 300 nm thick aluminum oxide (Al_2O_3) layer was deposited using an e-beam evaporator (FC-1800 E-Beam Evaporator, Temescal) and patterned the surface using photolithography and plasma etching (**Fig. S4T2**). Using the patterned Al_2O_3 layer as a hard mask, the wafer was etched down via a Bosch process (Plasmalab System 100 RIE/ICP, Oxford Instruments, Inc.) to the SiO_2 etch stop at the bottom surface of the wafer (**Fig. S4T3)**.

After removing the Al_2O_3 hard mask by soaking in BHF (**Fig. S4T4**), the b-Si process (SF_6/O_2 mixed-mode plasma process for 21 min) described in the previous section was conducted to create an anti-reflective surface on the inactive region of the hollow disk (**Fig. S4T5**). During the b-Si process, the Si_3N_4 membrane located at the bottom of the wafer was protected by the still-present SiO_2 etch-stop layer. After soaking in BHF in order to remove this SiO_2, the Si_3N_4 membrane was released from the wafer (**Fig. S4T6**). Individual parts were then diced from the processed wafer using a through-wafer deep RIE (DRIE) process (**Fig. S4T7)**.

Next, the bottom part of the sensor was fabricated using another Si wafer (**Fig. S4B1**). The mirror in the center was obtained using a precisely controlled etch process that creates a 4 μm-deep recessed surface (**Fig. S4B2, S4B3**), and the concentric circular trenches were patterned using another timed DRIE run (**Fig. S4B4**). The through-wafer DRIE was used again to dice the processed wafer into individual parts (**Fig. S4B5, S4B6**). Finally, a pair of top and bottom parts were aligned under a microscope and assembled using a water-proof medical-grade epoxy (**Fig. 2g, Fig. S4**).

Slit-Lamp Measurement Setup

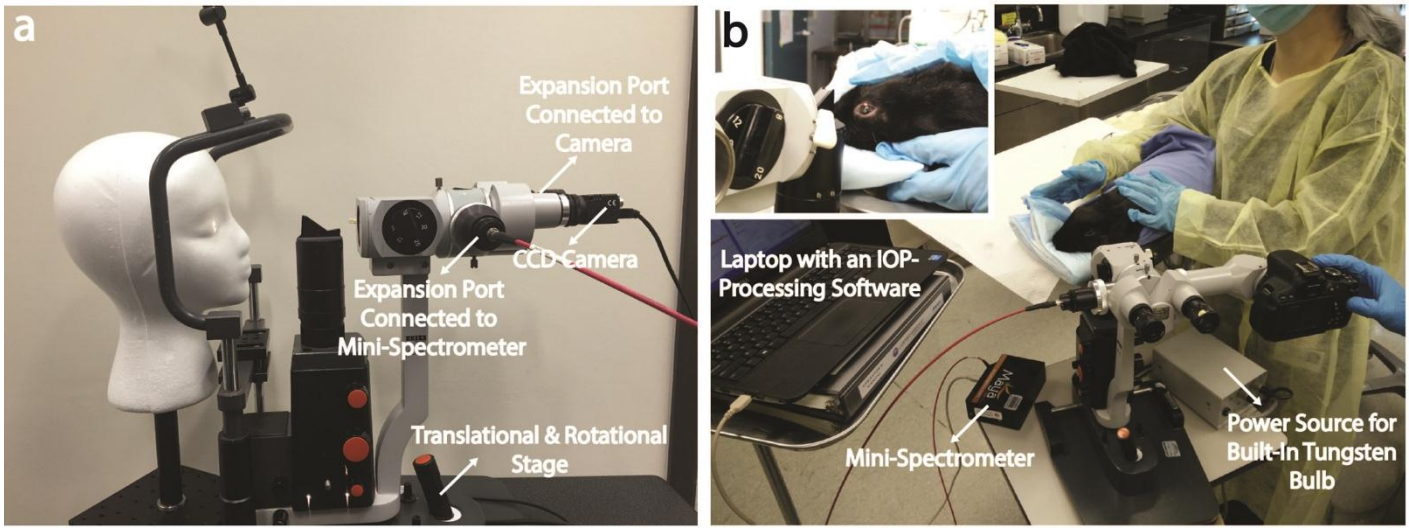

Figure S5. a) Photograph of a slit-lamp readout setup with a spectrometer and a CCD camera connected to the slit-lamp expansion ports. b) Photograph of the slit-lamp being used for *in-vivo* IOP measurements on an awake rabbit. The inset shows slit-lamp illumination in the rabbit's eye.

Variation in Peak Detection

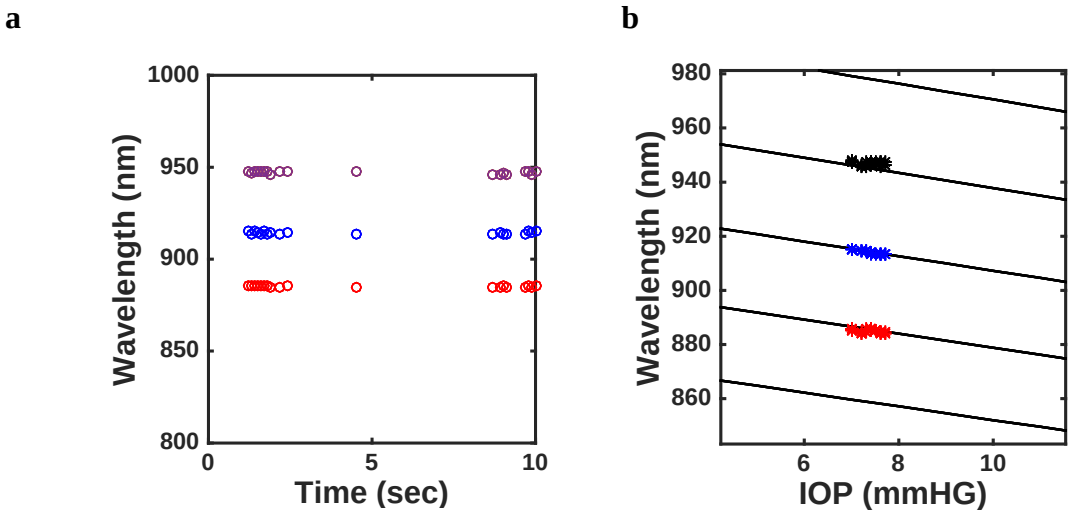

Figure S6. **(a)** Fluctuations of the major peaks during the 10-sec *in-vivo* measurement. Only the top 30 highest SNR spectra were selected and plotted. The spectral fluctuation in the peak locations is within ±1 nm. **(b)** The corresponding IOP for the spectrum was 7.3 mmHg and the peak-to-peak fluctuation was less than 0.4 mmHg.

Immunofluorescence Analyses

Sample Preparation Protocol

1) Paraformaldehyde (PFA) Fixation:
   - Upon explanting, rinse the sensor in 1xPBS/0.02% NaN_3.
   - Incubate in PFA for at least 30 min at room temperature (RT) overnight (O/N) or longer at 4°C.
2) Quenching:
   - Rinse sample in sterile 1x PBS (gently shaking for 5 min) **x3**.
   - Incubate sample in freshly prepared 1xPBS/0.02% NaN_3/50 mM NH_4Cl (quenching buffer) for 10 min at RT.
   - Rinse sample in sterile 1xPBS/0.02% NaN_3 (gently shaking for 5 min) **x3**.
3) Blocking:
   - Incubate sample in 1xPBS/0.02% NaN_3/2% BSA/0.25% TritonX100 (blocking buffer) at 4°C with gentle shaking O/N.
4) Staining:
   - Incubate sample in 1mL blocking buffer/DAPI 405 1:2000/CD62L 488 1:500/Phalloidin 555 1:1000 (staining reagent) at RT with shaking for 1 hr.
   - Incubate in staining reagent at 4°C O/N.
5) Preparation for imaging:
   - Transfer sample into blocking buffer.
   - Wash in blocking buffer **x3**.
   - Incubate in blocking buffer at 4°C O/N.
   - Rinse sample in sterile 1xPBS/0.02% NaN_3 (gently shaking for 5 min) **x3**.
   - Soak sample in sterile 1xPBS/0.02% NaN_3 for imaging.

Imaging Methodology and Biofouling

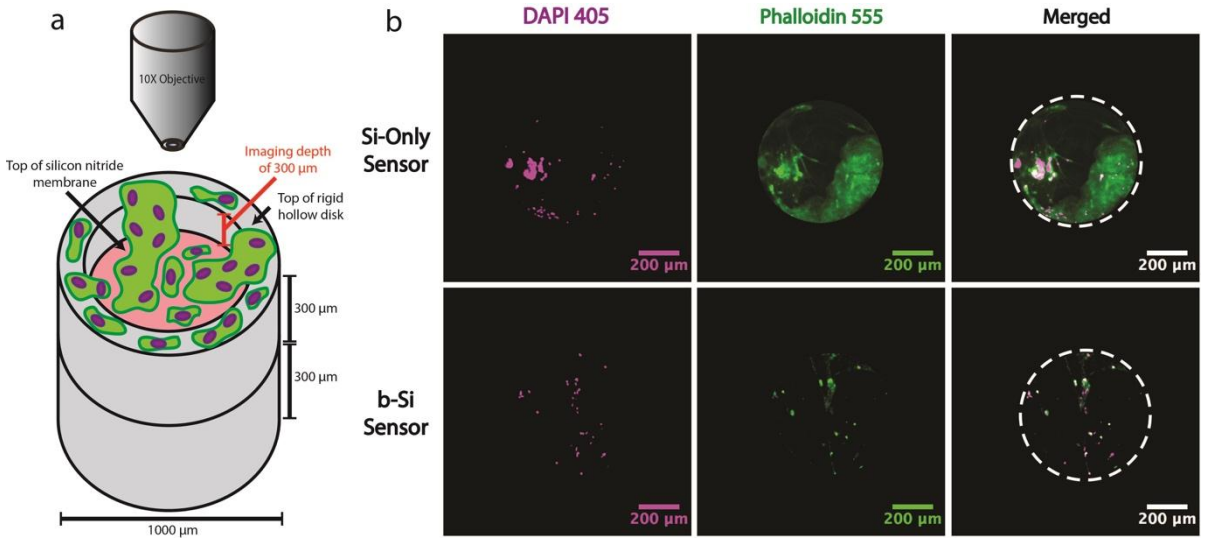

Figure S7. **(a)** Illustration showing imaging depth (shown in red) of 300 µm starting from the top of the rigid hollow disk to the top of the Si_3N_4 membrane. **(b)** Extent of sensor biofouling was evaluated through assessing the percentage of the sensor active surface area (highlighted by the white dashed circle) in merged images (DAPI 405 and Phalloidin 555) covered by tissue for the Si-only and b-Si sensors.

Immune Response Comparison

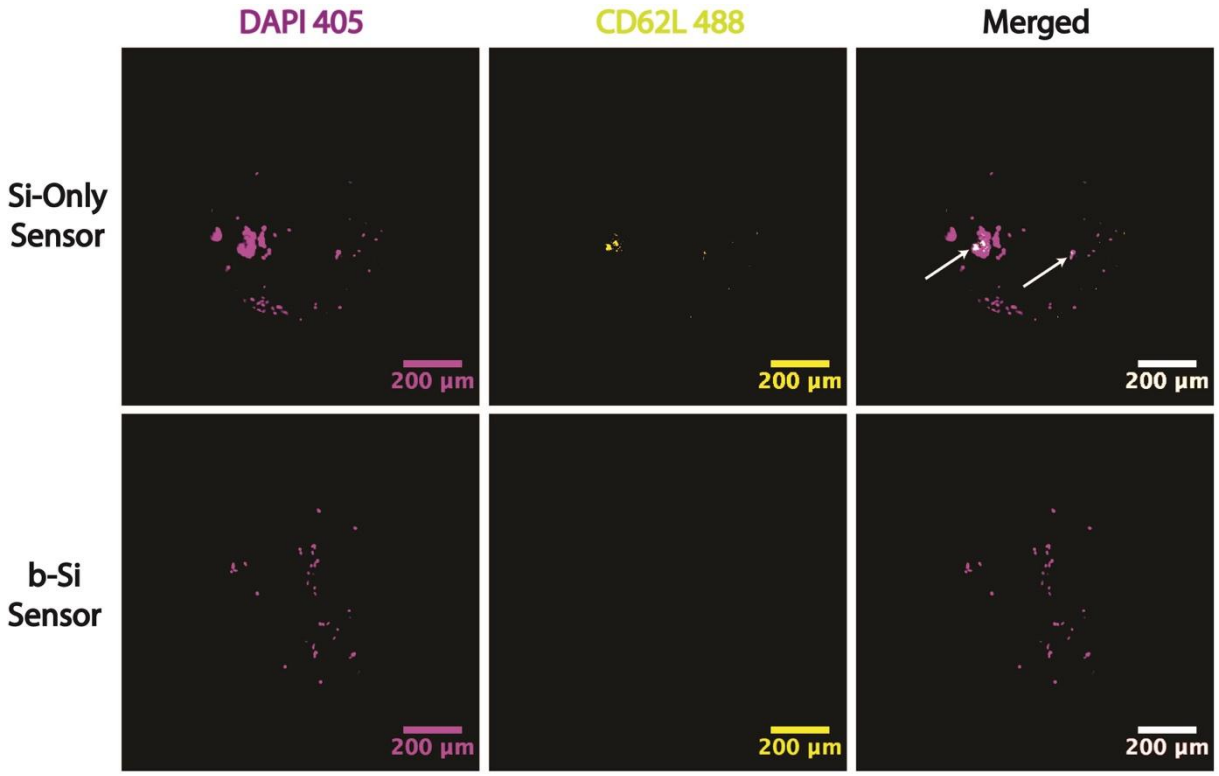

Figure S8. Immune response comparison that indicates the presence of potential inflammation in the Si-only sensor. The CD62L 488 marker is overlapped with DAPI 405 to ascertain the location of immune cells. Multiple instances of inflammation were observed in the case of the Si-only sensor (indicated with white arrows). No inflammation was detected in the b-Si sensor.