Synthetic Biology:

Quorum sensing (QS) is a prime example of paracrine signaling in which a cell affects gene expression in a neighboring cell. According to the classic QS hypothesis, bacteria communicate and count their numbers by producing, releasing, and detecting small, diffusible, signaling molecules called autoinducers (AI). Quorum-sensing has also been implicated in the regulation of processes such as bioluminescence, swarming, swimming, and virulence. But despite its appeal, the QS hypothesis may not be an accurate description of all these phenomenon.

To elucidate how cell-to-cell signaling works in bacteria, it is vital to control signal transmission between cells, yet most of the experiments used to test QS are done in a shaken culture flask, where the signal accumulates to a threshold concentration along a growth curve. It is difficult to emulate the diffusion, mixing and flow of signals found *in vivo* using a flask. In particular, bacteria naturally co-exist in sessile communities called biofilms. A biofilm is comprised of microcolonies of bacteria encapsulated in a hydrated matrix of polysaccharides, proteins and exopolymeric substances. The mass transport in a biofilm may exhibit gross deviations from Brownian diffusion—in some cases the diffusion coefficient is 50x smaller than in aqueous solutions—and so the chemistry can vary drastically over a short (100 μm) distance and have a profound effect on signal

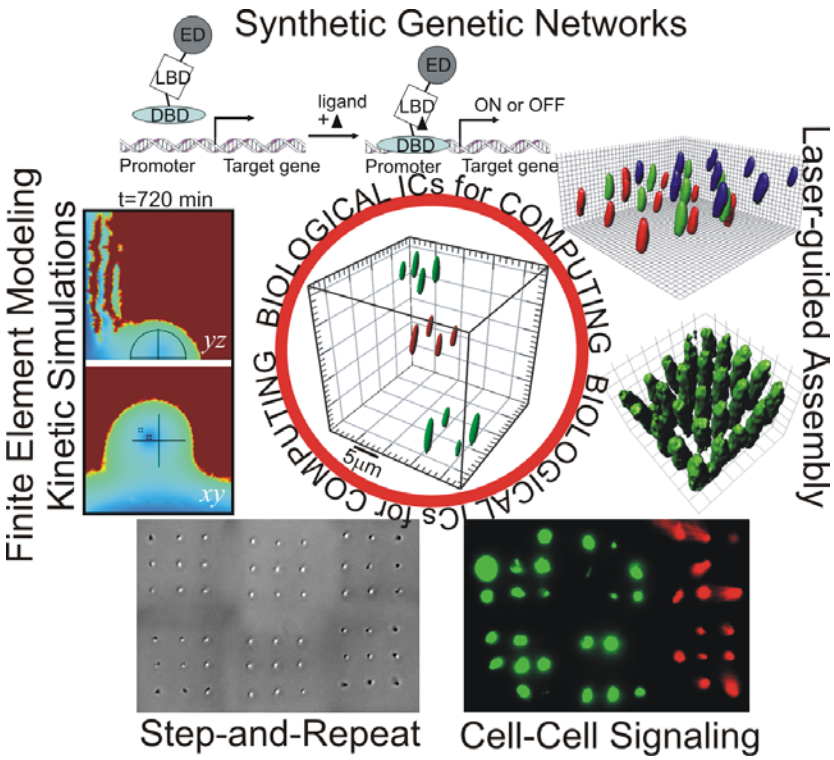

FIGURE 1. Biological Integrated Circuits for Computing. (center) At the core of this research is our demonstrated ability to assemble arrays of genetically engineered, living cells. A reconstruction from confocal microscopy an 3x2x2—3D array of transmitter and receiver bacteria is shown. **(top)** The bacteria are genetically engineered to express a logical function. A generic two component gene switch is shown consisting of a *NA* binding domain (DBD) specific for the sites in the controllable promoter, a ligand binding domain (LBD), and an effector domain (ED) (activation domain (AD) or repression domain (RD)); and a controllable promoter. **(right-top)** Laser-guided assembly is used to precisely position the genetically engineered bacteria into an array. A reconstruction from confocal microscopy of 3x3x3—3D array of bacteria with the top(blue), middle(green) and bottom (red) arrays shifted by 2μm from each other along the optical (z)-axis, and embedded in hydrogel. (right-center) A confocal image of densely packed bacteria <0.5μm apart. **(bottom-left)**The size of an array can be extended indefinitely with high precision using a step-and-repeat algorithm. A transmission image of a 2D array of *E. coli* programmed to transmit and receive a QS signal, C6-HSL. **(bottom-right)** Fluorescent image of the same array showing transmitters (red) producing C6-HSL and the receivers (green) detecting a concentration above threshold. **(left-center)** Diffusive and convective molecular transport, protein and signal production are modelled using kinetics and a finite element analysis. Shown are cross-sections through a hydrogel containing the array shown in the center of the figure under a flow condition of 0.8 μl/min.

transmission, production rate, and half-life.

We have been exploring the physical parameters governing prokayrotic cell-to-cell signaling in in a model biofilm. The model biofilm is comprised of bacteria that are genetically engineered to transmit and receive QS signals. The biofilm is formed using arrays of time-shared, holographic optical traps in conjunction with microfluidics to precisely position bacteria and then it's encapsulated within a hydrogel that mimics the extracellular matrix. Using fluorescent protein reporters functionally linked to QS genes we assay the intercellular signaling with microscopy. Contrary to the QS hypothesis, there isn't a single cell density for which QS-regulated genes are induced or repressed. On the contrary, the "information" communicated by the AI concentration depends on the environmental conditions. Cell-to-cell signaling is largely governed by diffusion, and it is acutely sensitive to mass-transfer to the surroundings and the cell location. These observations are consistent with the view advocated by Redfield and others, which posits that AI acts simply as a probe measuring mixing, flow, or diffusion in the microenvironment of the cell.

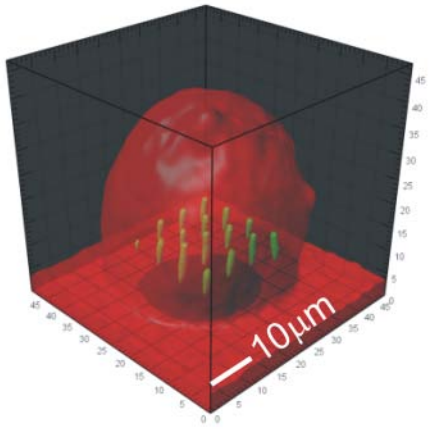

Figure 2. Trap arrays are formed using a high NA objective in a commercial optical microscope in conjunction with two AODs and an SLM to produce a time-multiplexed 3D array of optical traps. A typical hydrogel microstructure encapsulating a 4x4 array of E. coli.

This work represents one aspect of our studies in synthetic biology.

Right now, we are working to synthesize gene circuits specially designed for high sensitivity and high signal-to-noise protein production, transform bacteria with them, and then assemble with submicron precision the different bacteria into large arrays using QS signals to wire them together to express a complex computing function. The various components of the work are illustrated in the Figure 1. Because of the stochastic nature of the computation, we anticipate sorting through a succession of gene circuits using directed evolution and rational design in pursuit of sensitivity and stability with respect to noise, some of which may regulate a gene using its own products (i.e. autoregulation). To efficiently produce proteins with high signal-to-noise ratio and diminish the excessive energy cost, we also plan to leverage a protocol developed by Suzuki et al. that uses MazF, an mRNA interferase, to produce only the proteins of interest in living *E. coli* and otherwise arrest cell growth. Once the gene networks are designed and tested, we will assemble the different bacteria into large arrays to express a computing function. We plan to integrate different bacteria together with submicron precision into specific, 3D locations in a circuit using optical tweezers formed from multiple time-shared holographic optical traps. The layout of the bacteria will represent a compromise balancing diffusive transport of the signals and the sensitivity. While optical trapping can be used to create vast networks of cells laid out in an arbitrary pattern, the trapping beam has to be held on the cells to maintain the array. However, cell viability can be adversely affected by long duration exposure to the beam. To avoid photodamage, we will permanently fix the cell positions on a scaffold made from a photopolymerizable, biocompatible hydrogel embedded in the microfluidic network.

Using a step-and-repeat method, we have already shown that it is feasible to assemble heterologous arrays of bacteria and use QS signals to wire them together. Step-and-repeat is an optical lithography strategy used prevalently in semiconductor manufacturing to print small chips on large wafers. We use the same strategy to

precisely assemble small microarrays of bacteria formed using optical tweezers into a large community. Following Whitesides, we first convey genetically engineered *E. coli* to an assembly area via multiple laminar fluid flows in a microfluidic network. Then <100 bacteria are assembled into a complex 3D array using multiple, time-shared optical tweezers. The attached movie entitled "arraying" illuminates the process of organizing bacteria into arrays. Next, we fix the position of the cells by photopolymerizing hydrogel in a volume about 30μmx30μmx50μm like that shown by the confocal image of Figure 2 and then, after stepping to an adjacent location, repeat the process. As a demonstration, we formed a heterologous array of *E. coli* genetically engineered to *transmit and receive* QS signals of the type intended for use as interconnections, induced the array using ligands delivered through a microfluidic and then followed the space-time development of functionally-linked fluorescent reporters. This demonstration follows pioneering work on intercell signaling by Weiss et al.,[62] but with a difference: the location of the cells in the array are not random but instead are dictated by the optical tweezers. In turn, the 3D position of a cell in the array dictates its interconnectivity and affects function.

The different cells comprising the integrated circuit will be designed to communicate and compute using signals similar to naturally occurring QS molecules, like N-acyl homoseine lactone (C6-HSL). It is known that small molecular intermediates like these can be used to regulate development, pathogenesis, antibiotic production, symbiosis, and bioluminescence in bacteria. Using an assortment of QS-like signals as isolated communication channels, each highly specific to the cognate transcriptional activator, we plan to enable multiple biochemical inputs and parallel interconnections between the bacteria that depend on the precise layout of the cells in the array. Then we intend to biochemically induce the array and clock the computation exogenously using the microfluidic network. It is feasible to clock the array using the microfluidic network: in the preliminary data we show that paracrine signaling with C6-HSL can be utterly quenched and revived depending on the flow in the microfluidic and the position of the cell in the array. And finally, we plan to follow the space-time development of the computation throughout the array by employing fluorescent reporters functionally linked to the regulatory sequence on the output.