REPORT DOCUMENTATION PAGE

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
03/01/2011	Final Report	2/1/2008 – 10/31/2009

4. TITLE AND SUBTITLE		5a. CONTRACT NUMBER
Computation by Bacteria		
		5b. GRANT NUMBER
		W911NF-08-1-0044
		5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)	5d. PROJECT NUMBER
Robert H. Austin	
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8. PERFORMING ORGANIZATION REPORT NUMBER
The Trustees of Princeton University ORPA 4 New South Building PO Box 36 Princeton, NJ 08544-0036	

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
US Army RDECOM ACQ CTR W911NF 4300 S. Miami Blvd Durham, NC 27703	W911NF
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This work started with our original work of the invention of Micro-Habitat Patches (MHP) which allowed us to create on a silicon wafer a complex world of interacting communities with differing conditions. One can view this collection of microhabitats as a giant optimization problem where local and global fitness compete with one another. Living organisms, at least at the lower levels such as bacteria, are not viewed as ``computers'' or as capable of solving problems based on algorithms. Yet, they do form a collective problem solving entity as viewed from an evolutionary perspective. The problems solved by bacteria differ qualitatively from the problems normally solved by computers, and in some respects are actually much harder.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE	SAR	9	Robert H. Austin
					19b. TELEPHONE NUMBER (include area code) 609-258-4353

Forward

This work started with our original work of the invention of Micro-Habitat Patches (MHP) [1] which allowed us to create on a silicon wafer a complex world of interacting communities with differing conditions. One can view this collection of microhabitats as a giant optimization problem where local and global fitness compete with one another.

Bacterial Computation I: Optimizing Collective Fitness

Living organisms, at least at the lower levels such as bacteria, are not viewed as "computers" or as capable of solving problems based on algorithms. Yet, they do form a collective problem solving entity as viewed from an evolutionary perspective. The problems solved by bacteria differ qualitatively from the problems normally solved by computers, and in some respects are actually much harder.

In our first experiment we attempted to see how two competing organisms solved the problem of coexistence. We mixed two closely related but not interbreeding strains of *E. coli* together and introduced them into a simple "black-white" MHP world. The two phenotypes would seem to be antagonistic: there was a wild-type "cooperative strain" which ceases to divide upon reaching a certain population density, and a "cheater strain (actually a stress adapted mutant) which can continue growth under high stress conditions by utilizing the complex metabolic byproducts of lysed wild-type bacteria. It is known that under well-sturred but stressed conditions the "cheater" strain will replace the wild-type strain. Does the same extinction process occur in a complex environment?

The answer, surprisingly, was NO [2]. There is a fundamental difference between the population dynamics in a well-stired and an unstirred and complex environment. In fact, BOTH strains of the bacteria co-exist in the MHP world and their fitness (ultimate population levels) is increased by cohabitation, extinction does NOT occur! This can be seen most dramatically in Fig. 1, which shows how the "cooperators" and "cheaters" (a language I do not like) segregate locally within each MHP.

The segregation occurs at many different length scales: a more global segregation was also revealed by a Pearson-correlation analysis of the different populations in different MHP, as shown in Fig. 2. At the start of this seeding we poised some questions. We now have some answers. (1) what is the influence of habitat on competition? A: The open region shows significant fitness enhancement upon mixture for both strains, but the closed region shows minimal changes. (2) what is the optimum fraction of wild-type and GASP mutant cells which maximizes fitness for the combination of wild-type and GASP mutants? A: We tested three inoculation population fractions: (a) all wild-type, (b) 50/50 wild-type and GASP, and (c) all GASP. Of the three populations tested, the 50/50 mixture optimized the fitness of both strains. (3) what is the time-course approach to this solution? A: As described earlier, the pelagic stage lasts for maybe 20 hours, perhaps seeding initial fluctuations into the spatial distribution of cells. A sessile stage begins. Sessile growth freezes in spatial patterns in the open region of the chip. GASP mutants survive in the closed half of the chip. We find it interesting that these separate phases are taken since previous experiments forced cells to exhibit a uniform degree of spatial mixture throughout the duration of those experiments. Using unstirred aqueous growth media, we allowed the cells to use, at different times, different degrees of
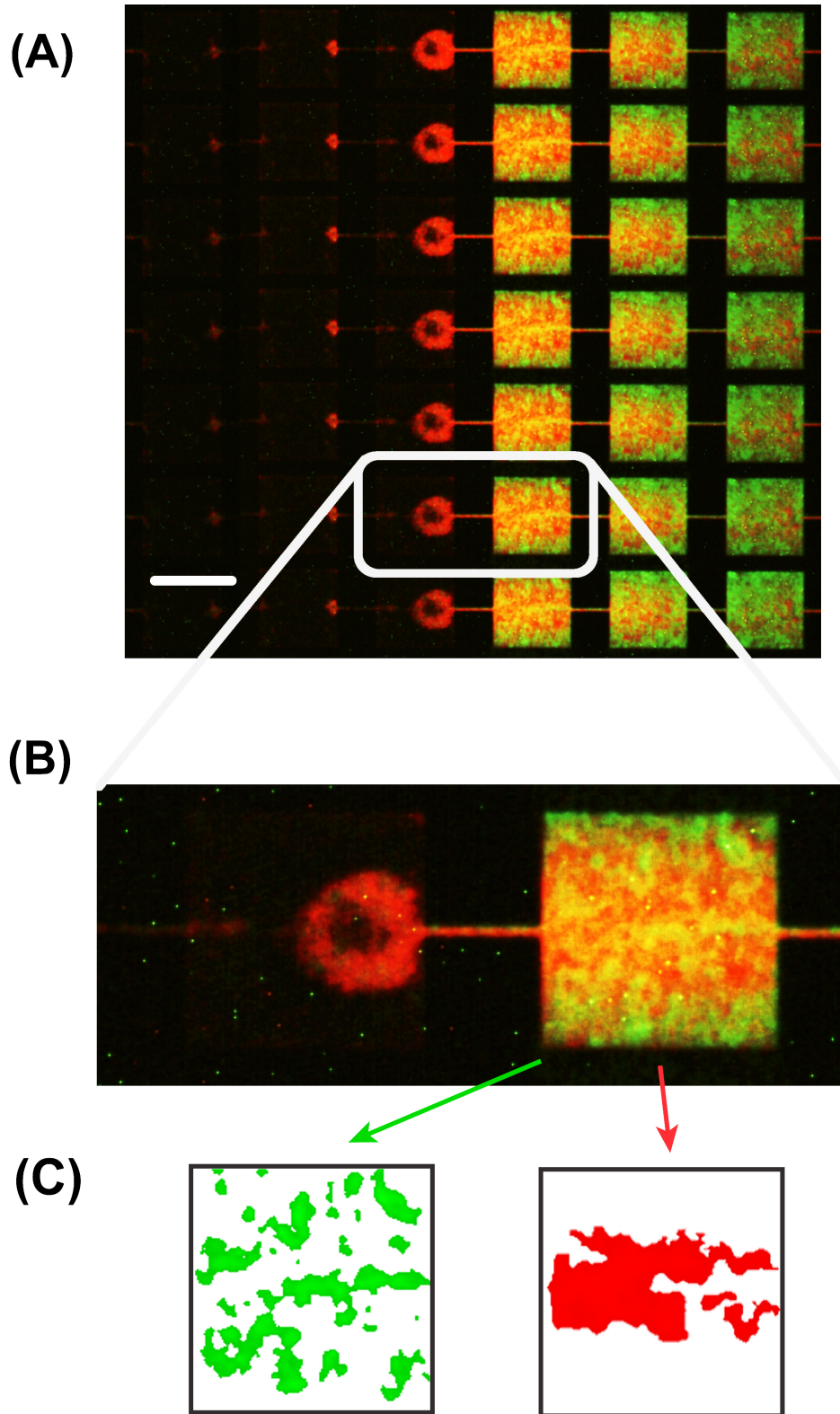
Figure 1: (A). True color image of the fluorescence of the chain of MHPs stacked vertically for JEK1033 (GASP bacteria, red RFP) and JEK1036 (wildtype bacteria, green GFP) competitive cohabitation. This image corresponds to an enlargement of the interface region between the "closed" and "open" regions about 50 hours into the run. (B) Two neighboring patches across the interface between zero external nutrient diffusion (left) and full external nutrient diffusion (right). (C) Result of the cluster size analysis performed on the rightmost patch of Fig 1B.

motility.

Our experiments should map into attempts to simulate the bacterial computations by including (a) mutually beneficial interactions at longer length scales and (b) less beneficial interactions at intermediate scales. It would be interesting to learn which of these interactions might be explained by chemical signaling at a distance or more local mechanical contact made possible by curli amyloid adhesion, for example. The identification of a mechanism for the wild-type-GASP interaction constitutes a microbiological approach, one we have not carried out in these experiments. The evolutionary approach asks a complimentary question to this work: how do the mechanisms increase a biological system's fitness in natural environments? We do not know if our present microhabitat length scale of approximately 200 microns is optimized for *E. coli* fitness enhancement, nor do we know how the length scales for fitness enhancement scale to other organisms.

Bacterial Computation II: Setting the Scale of Cooperation and Conflict

As we mentioned above, the original paper that analyzed competition in MHP used a very coarse Black-White (44 black-44 white) pattern which really did not probe the scale of competition very well. We have now conducted experiments with the highest possible spatial frequency of habitats, namely alternating black/white MHPs (a "binary" pattern). As we pointed out above, we were very surprised to find out in our first experiments that the "selfish strain" did NOT drive the cooperators but rather the bacteria on several different length scales segregated and not only coexisted but actually seemed to *increase* their individual strain fitness. Another great surprise has now struck us: as is shown in Fig. 3 in the binary MHP array, for some reason our present modeling cannot compute the "selfish" strain concentrate in the white, or open region while the "cooperator" strain concentrates in the black, or closed region. This of course is exactly the OPPOSITE of what we would expect, perhaps: when GASP competes against wild-type cells, it pushes them away from the high nutrient regions in spite of GASP adaptation to more stringent conditions.

Another surprise is the growth curves which perhaps helps explain things. The growth curves in both the high and low resource regions are plotted in Fig 4 as a function of time. Surprisingly, one sees that after approximately 30 hours, the population of wild-type bacteria in the high nutrient regions collapses to reach a steady state value much lower than that of wild-type bacteria in the low nutrient regions. This result is powerful: we find the competing strains not only affect each other, but also they do it in a way that is completely different to what one might expect: in control experiments (not shown). In the absence of competition, most cells are growing more efficiently in the high nutrient regions. This striking result, in itself, represents a *test* for the GASP phenotype. Indeed, by monitoring the way a given cell culture behaves in presence of either Wild-type or GASP individuals can tell us whether they express the GASP *phenotype*.

Bacterial Computation III: Solving the Problems of Topological Forcing

Fly traps and bee escapes have been used historically for clearing buildings of unwanted insects. In the last couple of years, we used microfabrication to adapt similar structures for bacteria.[5]. These structures could pump cells from input to output ports or distinguish between motile and immotile organism strains according to spatial distribution of cells. Numerical physicists have designed chiral micromotors that swimming bacteria could drive.[3]

We have previously reported packets of cells migrating across various microfabricated environments
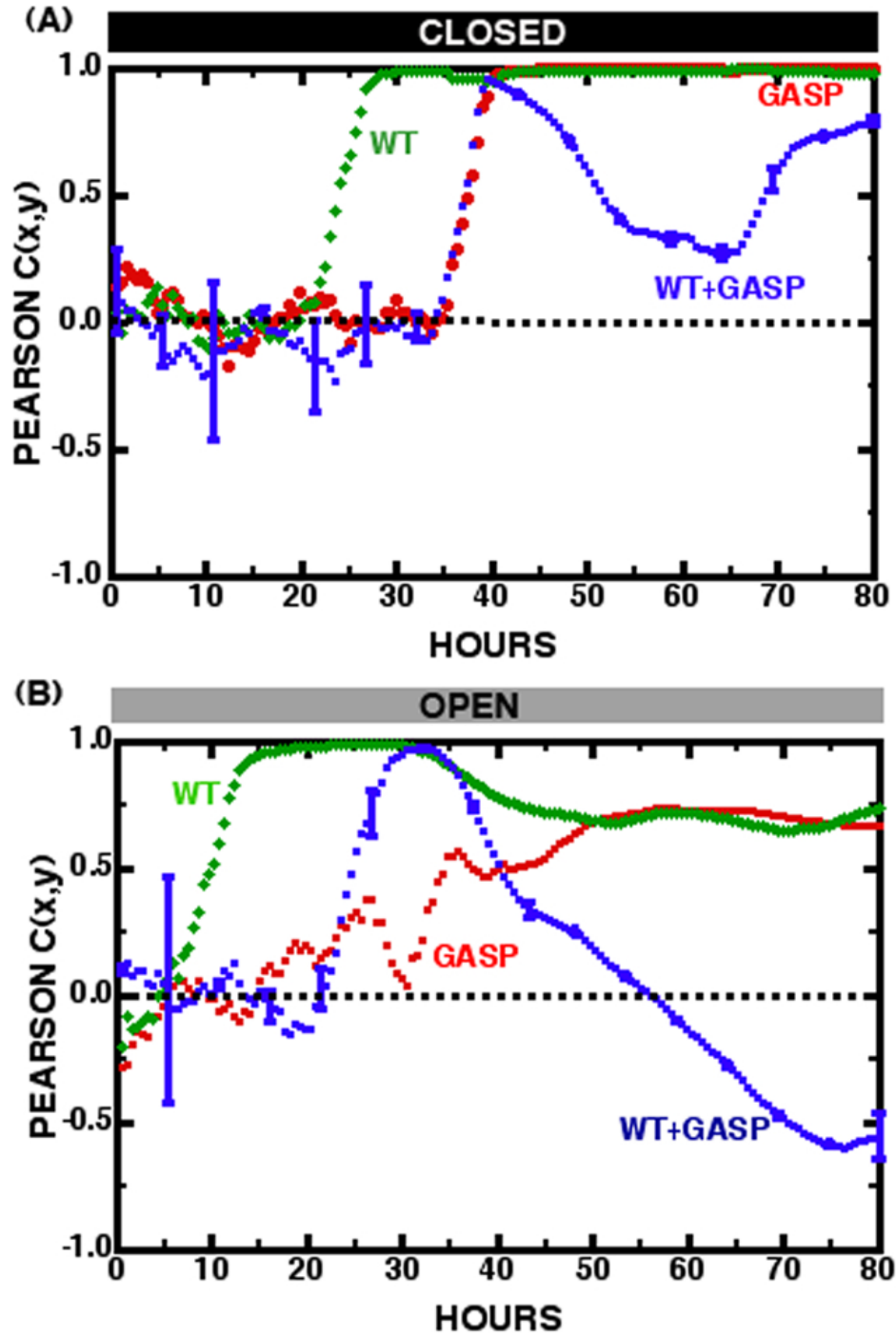
Figure 2: $C_{x,y}$ vs. time in hours after inoculation for the Closed and Open regions of the MHP array. Error bars are shown for only the WT+GASP competition experiments. (A) Closed region. WT+WT control cross-correlation between red and green strains are green diamonds, GASP+GASP control cross-correlation between red and green strains are red circles, WT+GASP competition cross-correlation between red and green strains are blue squares. (B) Open region. WT+WT control cross-correlation between red and green strains are green diamonds, GASP+GASP control cross-correlation between red and green strains are red circles, WT+GASP competition cross-correlation between red and green strains are blue squares.
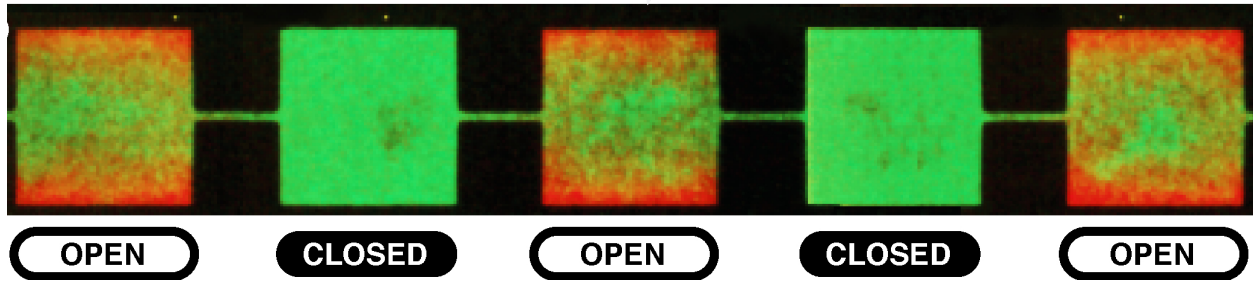
Figure 3: Pattern of cooperator (green) and GASP mutant (red) bacteria in a binary landscape of alternating full open nanoslits and full closed.

which allowed chemical heterogeneity to develop [4]. Considering the potential applications of micro-ratchets [5, 6], we performed experiments and wrote a model to study whether migratory packets could defeat micro ratchet structures. In both experiment and simulation, we saw a packet of cells migrate across the device, solving the problem of topological forcing to get at resources [7]. The application of such microratchets to a broad range of organisms and conditions requires compatibility with a range of biological relevant environments. However, reported experiments often suppressed chemotaxis by using "motility broth" or by providing nutritionally homogeneous broth throughout a microfabricated device. Numerical simulations have ignored chemotaxis. Chemical gradients could arise in implants in a biologically realistic environment, such as tissue, or in funneling devices trapping cells for extended durations.

All of these devices take advantage of the conversion of chemical energy into propulsion that occurs within bacteria. These devices break spatial inversion symmetry *and* time reversal symmetry by dissipating energy, and breaking both these symmetries allows ratcheting. The ability of ratcheting microstructures to separate microorganisms on the basis of motility, move microorganisms within a device, and for allow microorganisms to drive micromachines relying on energy that microorganisms would dissipate "anyway" even in the absence of ratchet structures suggests that researchers could harness bacteria to build diagnostic instruments, manipulate cells, and even provide alternative bioenergy. Thinking industrially, we might use bacterial motion to improve the way that the iRobot Roomba vacuum finds its battery charger. We've heard of complaints on the internet about these robots getting stuck in the worst places when they need to be recharged. More academically, we might think about bacterial motion through villus, bird feathers, and other biological objects with natural microstructures.

Future Directions: Cancer as a Fitness Computation

This DARPA sponsored work has grown considerably in its impact. Most predominantly, it played a major role in the creation of the Physical Sciences Oncology Centers (PS-OC) program http://physics.cancer.gov/ that the National Cancer Institute launched. We have shown that landscapes in ecology have a profound influence on the adaption and evolution of competing populations for resources [8]. The NCI became interested in how altruistic populations survive in the presence of selfish individuals in a non-stirred, closed and complex nutrient landscape and connected it directly to the dynamics of tumors and the transition to metastasis. Remarkably we know have experienced the creation of 12 PS-OCs across the country, co-lead by Physicists and Oncologists, which are dedicated to exploring the insights of physics
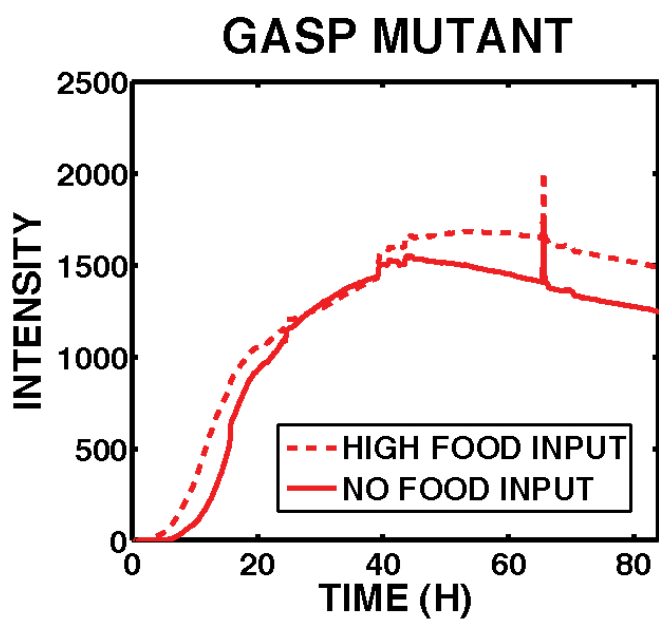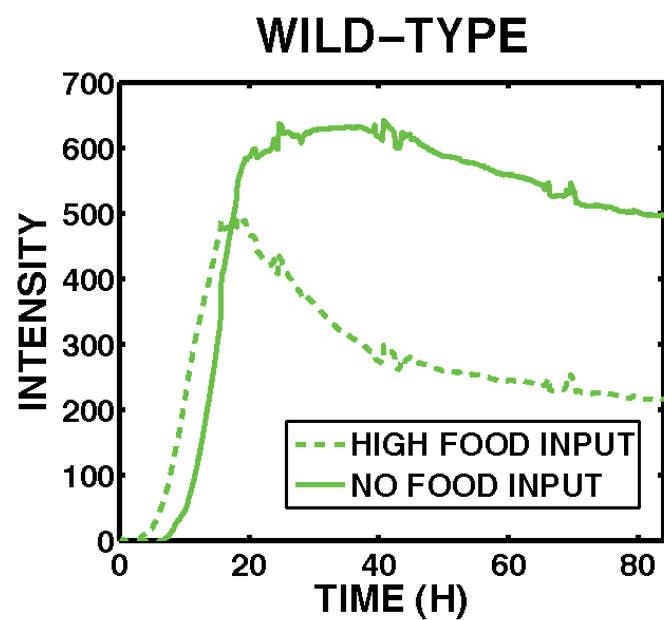
Figure 4: Growth curves of the wild type and GASP mutant as a function of time and position.

into the dynamics of cancer growth and evolution. This DARPA grant played a significant part in the creation of this program.
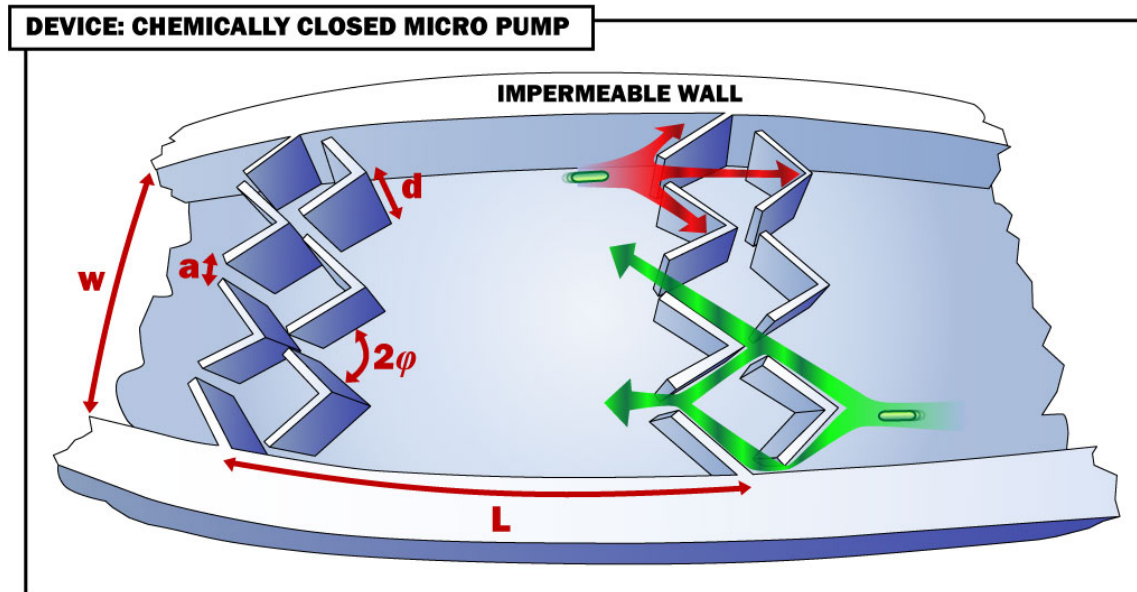
Figure 5: Ratcheting funnel arrays for removing pathogens, diagnosing microorganism conditions, and extracting alternative bioenergy. Experimental apparatus to test for migratory packets in a ratchet device with walls impermeable to chemical attractants.

References

[1] Juan E. Keymer, Peter Galajda, Cecilia Muldoon, Sungsu Park, and Robert H. Austin. Bacterial metapopulations in nanofabricated landscapes. PNAS 103:17290-17295 (2006)

[2] Juan E. Keymer, Peter Galajda, Guillaume Lambert, David Liao, and Robert H. Austin, Computation of mutual fitness by competing bacteria, PNAS 51: 20268-20273 (2008)

[3] Angelani, L. and Di Leonardo, R. and Ruocco, G., Self-starting micromotors in a bacterial bath, Phys. Rev. Lett. 102, 048104, (2009)

[4] S. Park, P. Wolanin, E. Yuzbashyan, L. Lin, N. Darnton. J. Stock, P. Silberzan and R.H. Austin, Movement to a Quorum, PNAS 100: 13910-13915 (2003

[5] Galajda, P., J. Keymer, P. Chaikin, and R. Austin. A wall of funnels concentrates swimming bacteria. J Bacteriol 189:8704-8707 (2007) .

[6] Galajda, P., J. Keymer, J. Dalland, S. Park, S. Kou, and R. Austin. Funnel ratchets in biology at low Reynolds number: choanotaxis. Journal of Modern Optics 55:3413-3422 (2008)

[7] Lambert, G., Liao, D. and Austin, R. H. Collective Escape of Chemotactic Swimmers through Microscopic Ratchets. Physical Review Letters 104, -, doi:Artn 168102 Doi 10.1103/Physrevlett.104.168102 (2010).

[8] Austin, R. H., Tung, C. K., Lambert, G., Liao, D. and Gong, X. Q. An introduction to micro-ecology patches. Chemical Society Reviews 39, 1049-1059, doi:Doi 10.1039/B911230h (2010).