E. Coli as a Model System for Cancer Evolution

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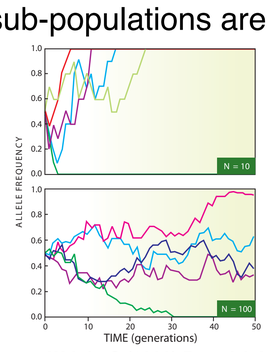
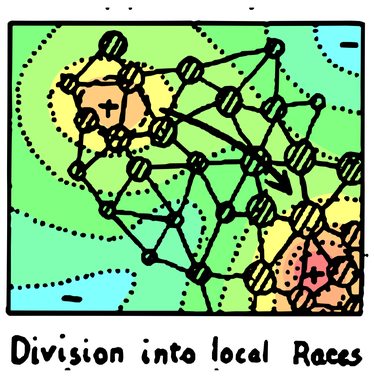
Introduction

Evolution drives cancer. Whether it is during the development of drug resistance or through the adaptation to highly stressful environments, cancer cell populations generally benefit from having a wildly mutating genome.

Indeed, studies have shown that small, related sub-populations are able to adapt more easily to a changing environment than large, clonal ones.



We build upon those ideas to study the adaptation of biological systems under stress, and use *E. coli* bacteria to study the evolution and adaptation of cancer cells.



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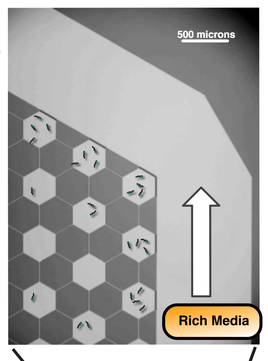
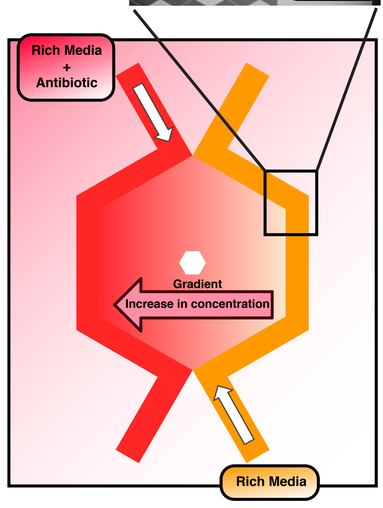
The Death Galaxy

Many cancers eventually develop resistance to chemotherapies. Questions that arise are 1) how does a cell population adapts to an influx of drug and 2) how does resistance spread *at the population level*.

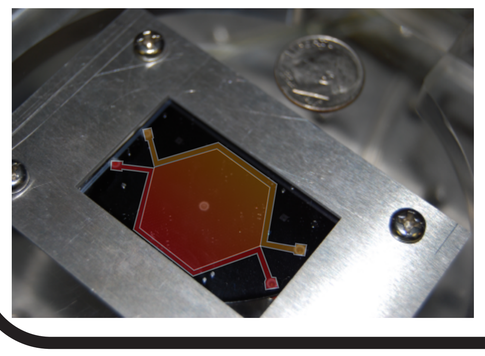
Since a cancer micro-environment is highly complex and heterogeneous, the final drug concentration reaching a given cancer cell may be much lower than the physician's intended dose. To study the effect of such chemical gradients on the emergence of drug resistance, we create a microfluidics device which physically impose small, related subpopulations on the cell population.



The device consists of an interconnected mesh of micro chambers, lying on a hexagonal lattice. We flow nutrient in channels on each sides of the device; adding antibiotic in only one channel will create a stable gradient as the flow is maintained.

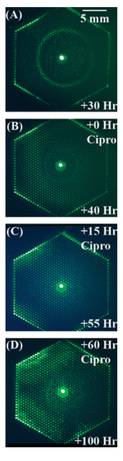


We inoculate the device with *E. coli* bacteria, and study the emergence of antibiotic resistance. We are not necessarily interested in the genetic details of how resistance arises in E. coli. Rather, we wish to study how a given cell population, bacterial or cancerous, adapts to a heteregenous environment and evolves drug resistance.



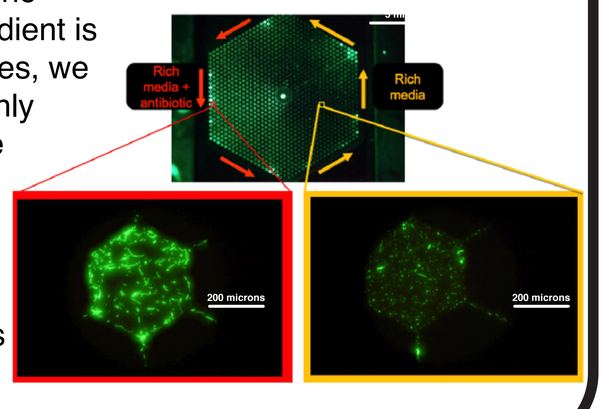
Cipro in Outer Space

We start by inoculating GFP-producing wild-type *E. coli* in the center of the device. The side channels initially contain LB broth. After 40 hours, Ciprofloxacin, a powerful antibiotic which targets DNA gyrase is added to one channel only. Cipro inhibits cell division but does not stop transcription: cells are thus still able to synthesize proteins and digest nutrients.



Since Cipro is added to one channel only, a stable gradient is created. As time progresses, we observe that bacteria mainly populate the region where the cipro concentration is low. At later times, however, cell density on the Cipro side is increasing.

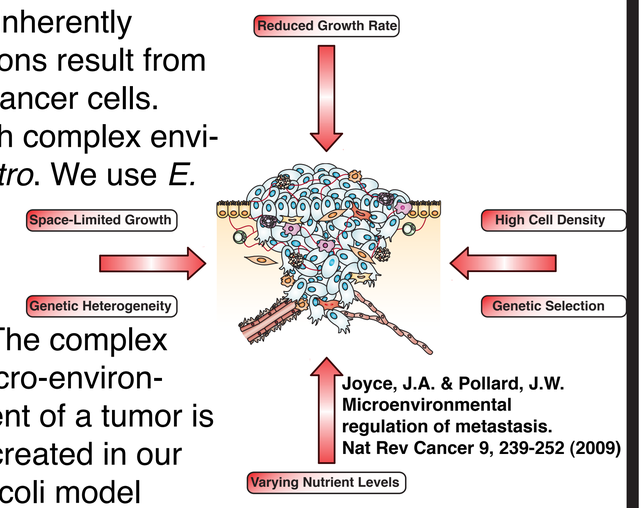
Indeed, we still observe that cells are physiologically different than those on the nutrient rich side.



Micro-Habitat Patch System

A cancer micro-environment is inherently stressful. Several of these conditions result from the seemingly chaotic growth of cancer cells.

It is challenging to recreate such complex environments for cancer cultures *in vitro*. We use *E. coli* bacteria cultured inside a microfluidics device to study the population dynamics of cancer growth within a tumor.



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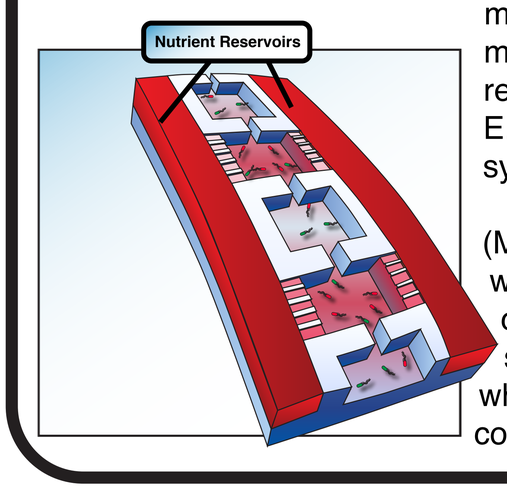
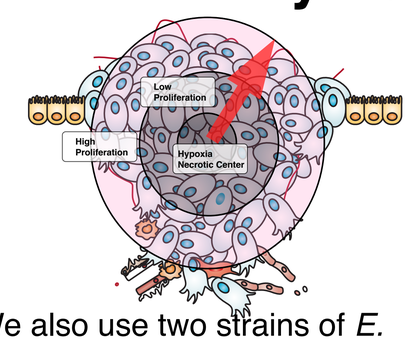
The complex micro-environment of a tumor is recreated in our E. coli model system by using a microfluidics device.



A linear array of micro-habitat patches (MHPs) 100x100x8 microns in size are linked weakly linked through small channels. We create regions of high or low metabolic stress by tuning the number of nanoslits, which are small (200nm thick) openings that couple the MHPs to the nutrient reservoirs

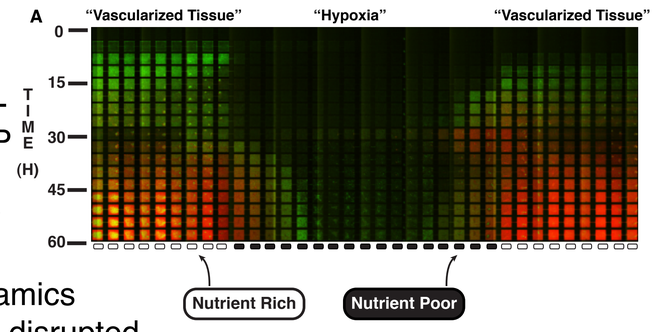
Physiological Tumor

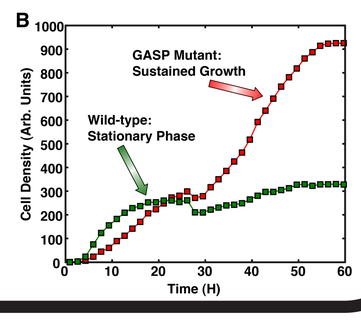


We use the MHP system to recreate the stressful conditions inside a tumor. Since the oxygen and nutrient levels dramatically decrease near the center of the tumor, a phenomenon that results from insufficient access to blood supplies, we create a "hypoxic" region surrounded by a "well-vascularized" environment.

We also use two strains of *E. coli* to model the growth of a tumor within healthy tissues.

One of the strains of *E. coli* inoculated in the device is a GASP mutant, a strain which has a **G**rowth **A**dvantage in **S**tationary **P**hase over the wild-type strain. The GASP mutant's growth dynamics reflect that of cancer cells with a disrupted *p53* gene: they keep on proliferating under stress because their cell cycle regulator (*rpoS* gene) is disrupted (46 bp insertion).





Therefore, using a combination of physical stresses and population pressures, we believe that we can use this system to study the physiological dynamics of cancer development.

Conclusion

We have shown how we use *E. coli* as a biological model for the evolution of cancer. By using a combination space-limited habitats and chemical gradients, we study how a population of bacteria adapt to varying levels of antibiotics.

Furthermore, by using a suitable co-culture of wild-type and GASP mutant *E. coli* strains, we aim to study large-scale population dynamics of cancer cells inside a tumor as they progress within healthy tissues.