# School of Physics and Astronomy


## Proposed Project Title
## First Year Report and Literature Review

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**Abstract**

The abstract is a short concise outline of your project area, **of no more than 100 words**.

Signature: Date:

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# Contents

# 1 Background

Since their discovery in the early 1900s [1], antibiotics have shaped modern medicine, and indeed modern society as a whole. Yet despite their prevalence, with over 260,000,000 courses of antibiotics prescribed in the USA alone each year [2], very little is still known about the actual underlying pharmacodynamics. I.e., how these chemicals actually regulate the growth and death of bacteria. This lack of understanding is becoming increasingly significant with the rising emergence of antibiotic resistance. There were over 58,000 deaths in newborns under the age of a year in India in 2013 due to drug-resistant strains of bacteria [3], with experts predicting that the number of deaths from antibiotic-resistant bacteria will number in the millions by 2050 [4].

This project aims to shed further light on how the application of antibiotics causes bacterial populations to evolve and proliferate over time. In particular, in the cases of when the applied antibiotic concentration has the form of a gradient. A considerable portion of antibiotic research has been performed under ideal conditions with constant, uniform antibiotic concentration [5], however it has only recently been proposed that the effects of spatial heterogeneity may be a major factor in the emergence of resistance and the efficacy of drugs [6]. While these concentration gradients can simply arise due to scenarios such as diffusion throughout body tissue [7], the scenario which is most pertinent to this project is that of antibiotic concentration throughout biofilms.

Biofilms arise when microbial organisms adhere irreversibly to a surface and then begin to secrete various polymers which further aid in surface and inter-microbial attachment [8] and creates a "slimey" surface. These structures are particularly problematic as it is difficult to achieve sufficient drug penetration to adequately curtail microbial growth, which leads to an increase in the persistence of infections [9].

The ability to inhibit and even prevent biofilm growth and formation has not only a multitude of medical applications, but industrial as well. In the shipping industry it's estimated that around 10%, up to even 45%, of all fuel consumption of large shipping vessels arises from overcoming the hydrodynamic drag caused by biofilm formation on ship hulls below the water level [10]. This not only has economic influences, but also major environmental implications. When compared to other nations, the shipping industry is the 7th largest producer of $CO_2$ on the planet [11]. Therefore, research into how these marine biofilms form and develop is incredibly important.

Recently, several physical methods of reducing marine biofouling have been developed, which range from physical coatings that inhibit microbial attachment due to their topography [12] to usage of ultraviolet radiation [13]. However, the most widespread technique is that of anti-microbial paints which are applied to the boat hulls and then leach various antimicrobial compounds over time which inhibit biofouling [14]. It is this latter method which is of relevant interest to this project due to its analogous nature of applying antibiotics to bacterial biofilms.

This project will involve creating simulations of a range of scenarios where bacterial populations experience antibiotic gradients, and will investigate how these populations develop over time. Including but not limited to; the effects of bacterial evolution, differing types of growth-rate dependent antibiotics and populations with heterogeneous species and resistance distributions.

# 2 Review of Background Bibliography

- overview of antibiotics - how they work etc
- Causes of antibiotic crisis
- growth rate dependent antibiotics, $\beta$-lactams etc
- how biofilms form - the bacteria change when in a biofilm, why are they difficult to get rid of
- overview of biofouling, current ship hull anti-microbes
- difference of films and fouls
- brief mention of macrofouling
- surface colonisation
- opposite gradient directions for films and fouls
- how biocidal paint works, diffusion coefficients etc
- mention the differnt algorithms possible for the sims, gillespie etc - and give overview of what we used
- as in, mention the rates, algorithm and description of why used

## 2.1 Antibiotic gradients

The issue of antibiotic resistance is one of the key issues plaguing modern science as of today, and as such, the field commands a large volume of research dedicated to it with a wide range of methods involved, ranging from experimental to theoretical methods including both modelling and more in depth simulation [15, 16, 17]. While current research is investigating a wide variety of factors which contribute to the development of resistances, from mutational path lengths [18] to the synergistic effects of various antibiotics [19], the majority of these studies, including all studies referenced so far in this section, are performed with constant and uniform concentrations of antibiotics.

While this situation tends to be more convenient for idealised in vivo experiments, many real-world in vivo scenarios do not have these conditions. Many naturally occurring structures, typically the ones which the drugs are intended to target, do not allow said drugs to fully permeate throughout the region, creating drug gradients where the drug concentration can vary noticeable over space. These gradients can arise in a variety of situations, from tissue [20] to bacterial biofilms [21].

It is only in recent years that the effects of these spatial heterogeneities have been considered as a serious influencer on the evolution of resistance. In fact, models have been constructed which predict that drug gradients actually tend to accelerate the evolution of resistance [22]. To test this hypothesis, Zhang et al. [6] constructed an apparatus involving an array of several interconnected microhabitats with an antibiotic gradient of ciprofloxacin. This gradient ranged from no discernible antibiotic concentration at the

top of the array to a concentration of 10$\mu$g/ml at the bottom. This concentration is around 200 times the minimum inhibitory concentration (MIC) of ciprofloxacin [23]

The array was then inoculated in the central microhabitats with around $10^6$ wild type *E. coli*. Chemotaxis due to nutrient consumption then drove the bacteria towards the perimeter microhabitats. Once resistant mutants had fixed, they then spread and propagated throughout the array, as shown in Figure 1.

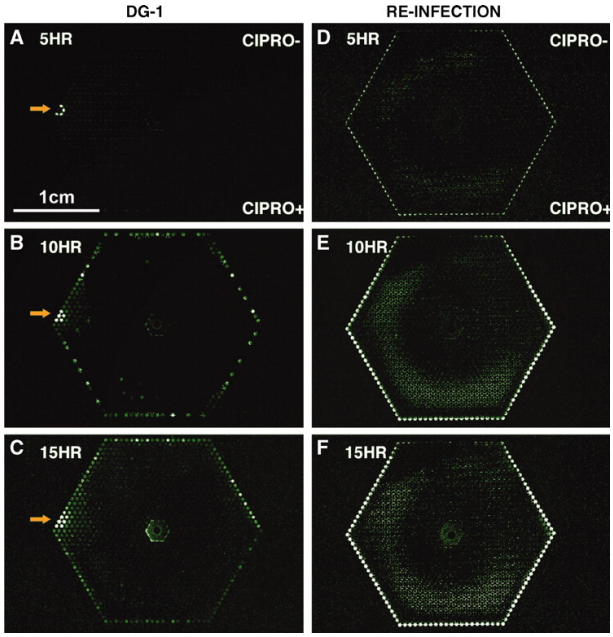


Figure 1: The proliferation of the bacterial population when exposed to an antibiotic gradient. The LHS shows the development of the population after the initial inoculation, and the RHS shows the development of an identical slide which was inoculated with the resistant mutants. Zhang et al., 2011

To confirm that it was indeed the gradient which allowed for this enhanced development of resistance, Zhang et al. conducted a range of further experiments. Firstly they eliminated the gradient by including ciprofloxacin at both ends of the array. This uniform antibiotic concentration resulted in no growth from the inoculated wild-type *E. coli*, as can be seen in Figure 2.

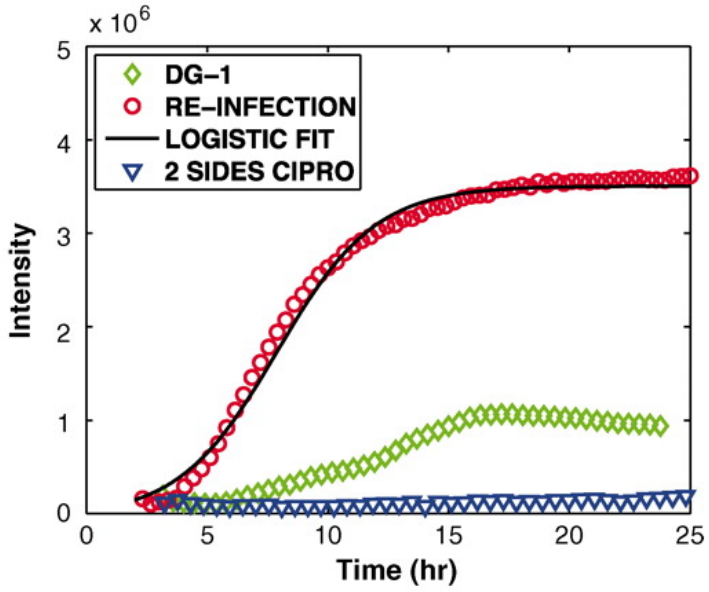


Figure 2: The summed growth over the entire array for various scenarios. The green diamonds are the initial experiment where the wild-type *E. coli* were placed in the antibiotic gradient. The red circles are the resistant mutants which were then used to re-inoculate an identically set up array. The blue triangles are the wild-type exposed to a uniform antibiotic concentration. Zhang et al., 2011

Zhang et al. then performed the experiment in a 96 well plate with a gradient present, but with the microhabits now disconnected from one another, with discrete antibiotic concentrations in each well, ranging from low to high concentrations as in the previous array. This also resulted in no resistance being developed, as the growth of the bacterial colonies simply decreased as the concentration of ciprofloxacin increased, thereby implying that bacterial motility across the gradients is what is key to the emergence of resistance.

To confirm the effects of motility on resistance development

## 2.2 Biofilms

The main articles used at this point in the project is [24] and [25]

# 3 Progress to Date

- make several sections giving brief overview of them - PRL replication, growth dependent, multispecies
- mention paper

Progress made so far in this project can be organised into several sections. The initial few weeks of the project were spent replicating the results and techniques found in [24]. Discussion was had on the subject of which algorithm would be optimal for updating the system over time. Algorithms such as Gillespie[26] and $\tau$-leaping [27] were proposed, but

# 4 Proposal

- Writing paper on growth dependent stuffs
- begin work on more intricate continuum model - surface roughness and flow
- utilise data from AkzoNobel to accurately model growth
- visit AkzoNobel to undertake lab work and metagenomic analysis

In this section, detail, as far as you are currently able, your research plan for the second and third years of your PhD, drawing from the key references [**?**] that you have highlighted in your review section. Here, try and illustrate your proposal, as in Figure 3 which is taken from the same paper as the illustration references.

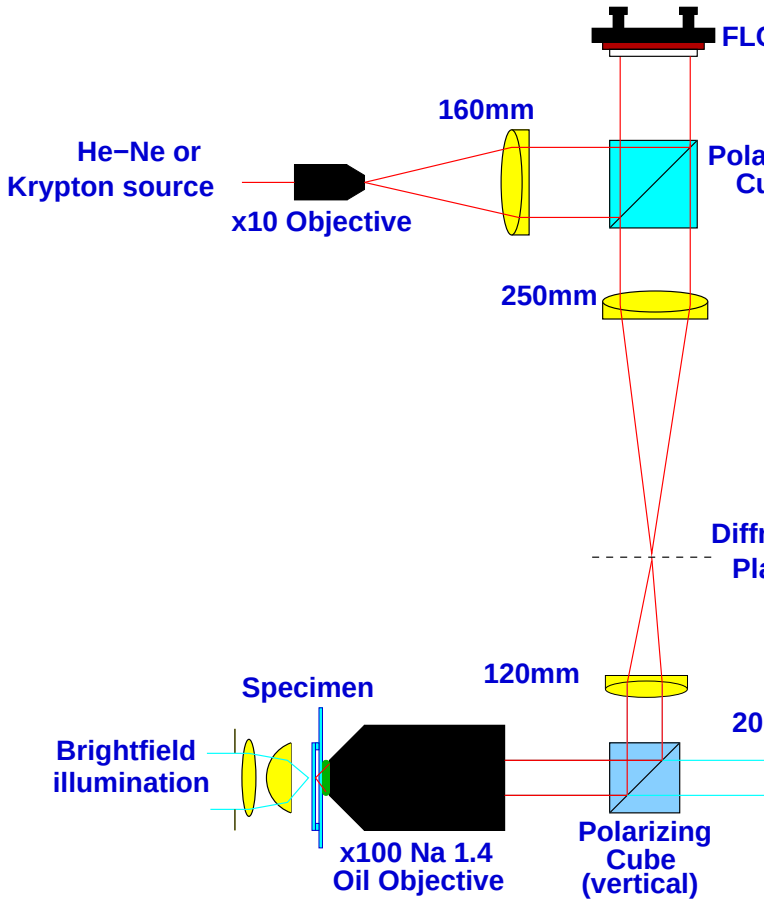


Figure 3: Here is the optical system from the same paper as the reference drawn in `xfig` and include to shown how such a figure in included.

At this stage it is *not* expected that this will be a fully-developed research proposal, but is your chance to show what you have extracted from the literature and how you see your own work will fit in. This section is not expected to exceed 2 pages.

# 5 Summary

The aim is to finish writing the paper regarding the work on the growth-dependent antibiotics, then to continue with the multispecies model and hopefully incorporate some realistic parameters contributed by AkzoNobel. Following on from this, work will begin on the proposed continuum model which should incorporate surface texture and flow into the system. This will be accompanied by some wet work at the AkzoNobel laboratory at their Newcastle compound. This is mainly for interest and not intended to form a sizable component of the project. There has also been some discussion of my involvement in AkzoNobel's metagenomic analysis of organisms gathered from their laboratory.

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