Bacteria can rapidly evolve resistance to virtually all known antibiotics, sometimes in a matter of hours. This visible example of Darwinian evolution is driven by mutations – spontaneous alterations of the genetic information carried by the bacterium in its DNA. Mutations are caused by errors during DNA replication; despite very efficient correction mechanisms, about one in five billion of newly synthesized nucleotides (building blocks of the DNA) is inserted incorrectly, leading to a mutation. When the cell reads off the DNA to synthesize proteins, the mutation results in a slightly altered protein, information about which was stored in that particular region of the DNA. Most of these variants will not work correctly, and may even kill the cell. However, very rarely, a mutation may actually improve the protein, or make it resistant to an antibiotic that targets this particular protein. This happens in about 1 in a billion cell divisions, but since bacterial populations can be very large (a billion cells fit into one cubic millimetre), such mutations occur almost all the time both in the laboratory and during bacterial infections. This has important implications for antimicrobial treatment, because if resistant mutants develop shortly before or during treatment, resistant cells will continue to grow and eventually re-establish the infection.

This simplistic view of the emergence of resistance does not take into account that the occurrence of a resistance-conferring mutation does not automatically imply that a cell carrying this mutation instantaneously becomes resistant. In fact, the new, mutated protein variant must accumulate to a sufficient level for a cell to become resistant to an antibiotic targeting that protein. The details of this transition from a mutation to a “phenotype” – the cell becoming resistant – are not fully understood. A better understanding how bacteria become resistant to antibiotics could lead to novel, “evolution-resistant” antimicrobial therapies.

The purpose of this project is to investigate the population dynamics of antibiotic-resistant mutants of the bacterium *E. coli* immediately after their emergence due to spontaneous mutations. This bacterium is an important opportunistic pathogen causing urinary and gastrointestinal infections. In the project, we will develop a new platform for microscopic imaging of very large populations of bacteria. We will scan a billion bacterial cells in order to find a few spontaneous mutants, and watch how they grow and respond to different antibiotics. To do this, we will use motorized, computer-controlled microscopes, powerful computers and machine learning algorithms to process millions of images generated in a single experiment.

The experimental data obtained in this way will help us to determine how long it takes from a mutation to resistance, how different mutations compete with each other in the bacterial population, and whether they come from all cells or only a small subset of special “mutator” cells. Besides answering fundamental biological questions that proved elusive so far, the project may suggest new ways of dealing with bacterial infections that develop resistance to antibiotics. The platform for scanning bacteria may also find applications beyond our project.