

DISCOVERING MEDICINES USING ROBOTS AND COMPUTERS

Anne Carpenter, Ph.D., Broad Institute of Harvard and MIT

Contributors:

Mark Bray Ph.D., Kate Madden, Megan Rokop Ph.D., and Sarah Stanley Ph.D.

Hi! My name is Anne Carpenter and I'm a scientist at the Broad Institute of Harvard and MIT. We're a group of scientists in Cambridge, MA in the USA and we're researching basic biology. Our goal is to understand how does the human bodywork? How is the body affected by different diseases? And we're also trying to find medicines to help cure diseases.

The human body is made of many individual cells, actually quite a lot of individual cells. These include brain cells, skin cells, muscle cells, all kinds of cells. And in total, one adult human being has 100 trillion cells. So that's 100 thousand, million, billion, trillion. But did you also know that the human body is carrying a lot of guests around? In fact, these are bacteria. Bacteria are single celled organisms that live in our gut, on our skin, even in our mouths.

I have a question for you. How many bacteria do you think are growing in or on a single human being? Which of these answers do you think is closest? I'll pause now, give you a chance to think about it. Write down your best guess: which of these is closest to the number of bacteria growing in a single human being. We'll pause the video now and come back in a moment.

OK, welcome back. Are you ready with your answer? The actual answer is closest to D. But it's a bit of a trick question because the number of bacteria that are growing in or on an adult human being is actually closer to 100 trillion. In fact, many scientists think that there's more bacteria living in a human being than there are human cells. Most of these bacteria do not harm us. In fact we need them in order to digest our food properly, especially those living in our gut. But some bacteria do cause disease, like these. This slide shows *Mycobacterium tuberculosis*. These are the bacteria that cause the disease tuberculosis. In general to prevent the spread of bacteria and diseases, it's great to wash your hands and wash your food before eating it.

Let's run an experiment and see why. This is a petri dish. It's made out of plastic and it's also called a petri plate sometimes. This gives us a nice, consistent environment in which to conduct experiments. This one, as I said, is made of plastic. There's no food inside and so bacteria, probably, if they fall inside of this plate, they probably won't grow. There's no food, there's no source of energy for them. So if we want to try to grow bacteria, try to find bacteria in our everyday environment, we should put some food source into this plate. So this plate has agar in it. Agar is a somewhat squishy material that has some nutrients mixed into it and it looks like this. It's got a slightly yellow color. And this provides all the food and energy and nutrition that bacteria need to grow.

Now I've just touched this plate with my fingers. Do you think that if I hold onto this plate for several days that it will stay clean and smooth like it is right now? Probably not! Within a few days some bacteria and some mold will start to grow onto the plates and the entire plate will be covered. So I have an example of this that I did a few days ago. It looks like this. You can see that individual colonies of bacteria are starting to grow on the plate. Each one of those little dots is a colony of bacteria, so each one contains millions of individual bacterial cells.

Now we're going to interpret an experiment like this together in class. I touched different things to the surfaces of some plates that are shown here, and I took photos of the results after a

few days. This is what the plates looked like after a few days. What I would like you to do in your class is to predict which samples go with which plates. So that you can give an idea of what samples you think would have the most bacteria growing on them, what kind of pattern or shape would the bacteria look like growing on the different sample plates?

Now I designed my experiment very carefully to make sure that I included a positive control and a negative control. A positive control is a sample that I'm sure will produce the effect that I'm looking for. So, for example in this experiment, my positive control is a culture of bacteria that was growing in the laboratory and I streaked it on one of the plates. I'm sure that should grow. A negative control is also designed into my experiment. In this case I took a plate that did not have anything growing on it. I kept it sealed shut and never allowed anything to get inside. This plate is my negative control and it's the plate that should not have any bacterial growth after several days. Why do I include positive and negative controls in my experiment? Well, it's because - what would happen if my negative control, the one without anything streaked inside, what would happen if bacteria started growing on this plate? Well, I would know something went wrong in the experiment. Perhaps there were some bacteria invisible in the food before I started my experiment. And what would happen if the positive control did not work out well? What would happen if the plate that I had streaked a lab culture of bacteria on didn't grow after several days? Well, I would know something went wrong in the experiment. Perhaps there was no food, not the proper nutrients, in the plates.

Your teacher will give you a handout now so that you can predict the results of this experiment. First, check over the positive and negative controls to be sure that the results turned out how you expected. And then predict which of the remaining samples match by writing the name of the sample next to the plate that you think matches. We'll see you back here when you finish that and discuss the results with your teacher.

How well did you do in predicting the results of that experiment? Did you notice how the antibiotic tablets were effective at killing the bacteria that were growing nearby? The whole world is constantly in need of new antibiotics. As scientists develop new antibiotics, bacteria over the course of a few years start to develop resistance to that antibiotic and then scientists have to go back the drawing board and find a new medicine, a new antibiotic, that is effective at killing bacteria again. This keeps scientists like me very busy!

Do you know how medicines are usually discovered? Well, throughout most of human history, medicines were discovered by accident. For example, in 1928 Alexander Fleming was trying to find an antibiotic to kill nasty infections by a bacterium called *Staphylococcus aureus*. He designed and tested many chemical substances, but in the end he discovered the new antibiotic by accident. This is how it happened. He went on vacation. He had many plates of bacteria growing. When he came back from vacation most of those plates were overgrown with bacteria, but he happened to notice before he threw the plates away, that in some plates the bacteria had been killed or were just not growing. And do you think they were not growing because of some of the chemicals that he had tested and designed? Nope! It turns out that those plates that were not growing bacteria actually had had some mold settle onto the plates. The mold started to grow and the mold was apparently producing a substance that could kill the bacteria nearby. So what do you think he did? Did he start feeding patients this mold to try to cure their bacterial infections? No, that would probably just give them a mold infection! What he did is he purified that chemical substance that the mold was producing, and this became the

chemical known as penicillin. It's an antibiotic that was called a miracle medicine because it was so effective at curing patients with bacterial infections.

As scientists we don't want to wait around for accidental discoveries, so we often design experiments where we gather many chemicals, all different chemical structures, and we test them systematically to identify which chemicals might have the effect we're looking for. For example, having the effect of killing bacteria.

Today we're going to do an experiment in class where you try to identify some new antibiotics to treat tuberculosis. We're going to take a collection of chemicals and we're going to see which chemicals can kill the tuberculosis bacteria. First, I want you to write down your guess for how many chemicals structures do we typically test in an experiment like this in the laboratory? Now quickly. We're not going to pause. Just write down your best guess, what do you think it is? Turns out the answer is 400,000. That's the typical number of chemicals that we screen in an experiment like this to try to find one new medicine.

Sometimes some pharmaceutical companies even screen 1 or 2 million different chemicals. Some of these chemicals, you might be wondering where do they come from? Some of the chemicals are synthetic, which means that they've been made from scratch by chemists in the laboratory, whereas other chemicals are natural products. These are chemicals that are found naturally (in nature) and are purified and identified and then we solve their structure to try to figure out what exactly they are.

Your teacher is going to give you a chemical to test in our experiment today and I want you to take a look at the structure of the chemical that you received. Do you think it's a natural product or do you think it's synthetic? Was it created by chemists or was it found in nature? Take a minute to think about this and circle your best guess, and then come back.

It's probably hard to tell which chemicals are natural and which are synthetic just by looking at the structure isn't it? One clue is that natural products are often, but not always, more complex than synthetic ones. This is because natural products are made by organisms that have enzymes capable of some very intricate reactions. It's hard for a chemist to compete with them. But in general it is very hard to tell just by looking at the structure whether a compound is natural or synthetic—even for experts.

So we want to test some chemicals to find a new antibiotic for tuberculosis, right? This instrument is a pipette. We use it to take each chemical out of our collection and transfer it into a test tube where we can do the experiments. In this case for example, if we're looking for a chemical to kill bacteria, we might put bacteria into the test tube and then measure a few days later to see if the chemical has killed the bacteria. But how are we going to test 400,000 chemicals in an experiment like this? Let's say it takes maybe 10 seconds for me to transfer a chemical from one tube to another. If I work at that rate, I could test maybe 3,000 chemicals in a day if I worked for eight hours straight. And that would take three months total to do the entire experiment if I wanted to test 400,000 compounds.

Luckily, we have an instrument like this. This is a pipette with twelve tips on it and it allows me to transfer many chemicals at once. So I typically - instead of using test tubes - I start to use a multi-well plate. This is basically a collection of little test tubes all lined up so that I can take chemicals from one plate and transfer them over into another plate to test. These plates are much easier to handle than test tubes when we are dealing with a lot of samples.

But if we're really lucky, we can use robots to help us with this experiment. Engineers have designed robots that are fast and accurate at transferring liquids like chemicals from one plate to another, and they allow us to do these experiments very quickly. In fact, we can test 400,000 chemical compounds in a day using the robots. Here's a question. If we do this experiment and we find some chemicals that kill the bacteria, should we immediately give them to patients? Would there be any problems with that? Take a moment to discuss it with your class and come back here when you're finished.

So, if I find a chemical that kills bacteria, should I immediately begin giving it to patients? Not a good idea! You hopefully have realized by now that chemicals that kill bacterial cells might also kill human cells and thus entire human beings. Some of my fellow researchers have designed a better type of experiment to find new antibiotics. Mark Bray works together with me and he's going to tell you more about the experiment.

We're working on a new test with Sarah Stanley and Deb Hung. They had the idea of testing each potential drug to see its effects on bacterial cells and human cells to see if we can find a chemical which causes the bacteria to die, while leaving the human cells healthy. They're working on new antibiotics to treat tuberculosis, which is caused by the *M. tuberculosis* bacteria. You can see on the map that dark purple shows where most cases of tuberculosis occur, but you can also see on the map that it's basically a problem everywhere. So in each sample, we're putting human cells together with bacterial cells. In this case the bacterial cells are stained, so they appear green, whereas the human cells are stained so they appear blue. And then we use a microscope to see whether each chemical causes the green bacterial cells to disappear while leaving the blue human cells healthy. Any chemical that would do this would potentially make a very good antibiotic.

But when we designed the experiment we found that to look at all of these samples on a microscope would take about as much time as it did to actually pipette them all. But luckily we have automated microscopes, which allow the microscope to automatically move to each site, taking a picture of each one.

So the setup for our experiment is this. We place the bacteria and the human cells into each well of a multi-well plate. Then we add a different chemical to each well, and then we use an automated microscope to move to each well, taking a picture. And then we look at the pictures to see whether the green bacteria are dead while the healthy blue cells are alive.

That's great! Let's do this kind of experiment with the chemicals that your teacher has already handed out to you. You're going to prepare each sample by taking first of all the human cells and putting some into each of our test tubes. This is our multi-well plate here that we're working with. Then secondly you're going to add the tuberculosis bacteria, add a little bit of bacteria to each of the wells as well. That's going to yield an infection where the human cells are infected with tuberculosis. And then lastly each of you is going to test your chemical. So tear off the bottom with the small little structures of chemicals and prepare your sample like this. And then choose one of these test tubes in the multi-well plate. Add your chemical to that sample and then we're going to incubate it for a while.

Now your teacher is going to include some positive and negative controls in the experiment. The negative control actually is water. We don't expect water to kill tuberculosis so we should expect the bacteria to grow very heavily when we just add water to a test tube. The positive control is Rifampicin. Rifampicin is a great positive control because it's a currently used antibiotic that is effective against tuberculosis. It should kill the bacteria but it should leave the human cells looking fairly normal and healthy. You might wonder if Rifampicin is such a good positive control, if it's a good antibiotic, why are we looking for new antibiotics? Don't forget that bacteria are constantly becoming resistant to antibiotics. In fact there's some strains of tuberculosis now that are not killed by Rifampicin. So we need to find some new antibiotics.

It's important to keep good records of any experiment you do, in a lab notebook. We've got a lab notebook written here that will help us keep track of which chemical structures we put into which test tube inside the multi-well plate. I put Rifampicin into this well, so I'm going to write my name and the name of the chemical into that well. Now go and prepare all of your samples and when you finish that and they're all incubating, come back and we'll talk about how are we going to score the samples to know which chemicals make good antibiotics.

Are you finished preparing your samples? Are they incubating now? While those are incubating, let's learn how to examine images that come from the robotic microscope. This slide is showing you a real image of what the cells look like, both the human cells and the bacteria. The human cells are blue, the bacteria are green, and we want a chemical that has a low number of bacteria, so in other words very few of these green blobs, and a normal number of human cells, so that is a normal number of those blue stained objects. We also want to check to make sure that the human cells have a normal shape. So the blue objects should be fairly round, maybe oval, but they shouldn't have wiggly edges. They should not have an irregular shape.

Your teacher is going to use a pretend robotic microscope and give you the results of your experiments. So for each chemical you've tested, the robotic microscope will produce one image of that sample. And your goal is to assign a measurement and score your sample. These are the criteria. We're looking again, first of all to count up the number of bacteria. And since these are printed in black and white you'll see the bacteria look like just little blobs. Sometimes they're big blobs, sometimes they're kind of small. Just use your judgment to count up the number of bacteria. Secondly, you're looking for a normal number of human cells. The human cells on your printout look like outlines, like this. You count those up. And lastly, you want to score the shape of the human cells. This is a judgment call, but what you're looking for is to give a sample a high score if most of the human cells are fairly round or fairly oval. So samples like this would receive a ten probably. Whereas if the human cells have funny shapes like this, you should give it a lower number. So on average, what is the typical shape of the human cells in your sample? Once you finish scoring those, come back and we'll talk about the results.

OK. So you've finished scoring your samples? You've recorded the results in the laboratory notebook? Let's decide if any of the chemicals that you tested in class might make a good antibiotic. Now a good antibiotic would look like the positive control. Remember your teacher scored the Rifampicin samples, and a good positive control would have a low number of bacteria, a normal number of human cells and normal shape of the human cells. Look at your lab

notebook now for your class and discuss with the class which of those would make a good potential antibiotic. Are there several chemicals that might be worth pursuing? Are any of the chemicals that you tested better than the positive control? Whose in the class is the best chemical? Discuss it and we'll meet back here.

Now, after you've scored the samples with your classroom, how long do you think it would take to score 400,000 samples? How long would it take your classroom to accomplish that kind of experiment? You're probably thinking that you hope there's a better way to score this kind of experiment. Was the way that you did it accurate? Was it fast to look at the samples by eye? Take a break and discuss whether it's feasible to do an experiment with 400,000 samples, scoring the images by eye. What would be some of the problems with that?

It really isn't ideal to look at 400,000 samples by eye is it? It's slow, it's tedious, it's boring and it's not very accurate and it's not very objective. In fact sometimes I find it's hard to tell whether a blob should be counted as a bacterium or maybe it's just a speck of dust. We really do need to figure out a better way to do this experiment, unless I can hire thousands of students to help look at the images by eye for me! So it turns out we did come up with a better solution. My research group wrote some software that can identify and measure the individual bacteria in every image and also identify the human cells. So it counts all the bacteria, it counts all the human cells, and it measures the shape of the human cells to see if the shape is normal. The algorithms trace outlines of the human cells and the bacterial cells as shown on this slide. And the software we have made freely available to everyone in the world. You can download it on your own and do your own experiments with it. The software takes about 30 seconds for each image. So we have an entire roomful of computers that we use to process the images. With all of these computers working for us, we can process images from 400,000 samples overnight. This makes the experiment go much faster. So now, Sarah Stanley is preparing the samples for this experiment to look for new antibiotics for tuberculosis. Mark Bray and I are both helping to work the software so that the images can be analyzed automatically and quickly. And we're hoping to find a new antibiotic to treat tuberculosis.

Well, that's the end of the lesson today. I hope you enjoyed learning about how we use robots and microscopes and computers to identify new antibiotics and new medicines to help keep human beings healthy.

Hi! This is Anne Carpenter and this is the teacher's video guide to the lesson, "Discovering medicine using robots and computers." You'll find that the written guide will help you step by step through the video segments, explaining how to do the activities and giving you ideas for what the students should learn prior to doing this video lesson. During this short video guide, I will give you an overview of the lesson and its activities, and I'll point you to some further resources in case you want to do some class sessions that have related topics to this video lesson.

I love my work as a scientist because it allows me to study so many different topics. I'm always learning. Hopefully your students will appreciate that to accomplish something like discovering a new medicine requires knowledge of all different types of areas, from chemistry, biology, engineering, math, computer science. Hopefully they'll enjoy seeing the connections between these different areas of study as they go through this lesson.

In the lesson I describe a real experiment that is going on right now at the Broad Institute at Harvard and MIT. This is a university laboratory which is located in Cambridge, MA in the US. Our goal in this experiment is to identify new antibiotics to treat tuberculosis. In the lesson I will help you to guide the students through two main activities. First, they'll be learning about concepts about bacteria and antibiotics by predicting the growth of bacteria on petri plates. And second, we will conduct an experiment where each student tests their own chemical to see if it would make a good antibiotic to treat tuberculosis.

In this work, in the real experiment, we use robots to prepare the samples and to take microscopy images of each sample. And then we use computer software to analyze the images that come from these microscopes. So in your class session you'll be pretending to prepare samples. You'll take pictures with a pretend microscope of some sort. And it's fun if you can really use some creativity. The students certainly will enjoy it if you act out the session as if it's a real experiment.

The students will also be learning about CellProfiler, which is the name of some free software that my research group that helps us with this experiment and helps many scientists around the world with their own experiments.

After teaching the lesson in this classroom, you might want to explore certain topics further with the class. For example, you could teach lessons on tuberculosis and other infectious diseases. You could talk about antibiotics, how they work. You could talk about good hygiene. You could talk about how many bacteria and viruses develop resistance to the medicines that we have available, and that's why we always need to be looking for new medicines.

If you're interested in exploring how computer software actually identifies and measures cells and images, the students could download the free software that my team wrote called CellProfiler. On its website you'll find that there are many examples of how to use the software. Now keep in mind the software is designed for professional scientists, but if you choose a simple case, for example counting yeast colonies, the students might be able to process example images that are provided on the website. And in fact if you can prepare plates with colonies growing on them, the students might be able to analyze their own images. You'll just need a digital camera or a scanner to take pictures of the plates and then those pictures can be fed into the software to count each colony of bacteria that's growing on the plate.

For a simpler, more student-friendly type of software or computer lesson, you might check out the software called Scratch. It's available from MIT and it is easy enough, even for very young children to learn. Older students will certainly enjoy piecing together different components in the software to create different games or visual displays. It's a lot of fun to use

and the students, without even noticing it, will learn some of the very basic principles of computer programming.

Lastly, to explain bacterial growth and antibiotics, you might want to have the class replicate the activity that we did after the segment two of the video. This was the activity where we tested various every day samples on agar plates. You can do this in real life because the materials are not terribly expensive. You just need plastic petri dishes and some agar growth medium to put inside them. Then the students can go around in their everyday life and take swabs of different samples. So this could be something like a faucet, a drinking fountain, boiled water, tests of their desks in their school, and see what grows from these different samples. The students definitely find this entertaining! I hope you have fun teaching students how useful many different disciplines of science can be when we're tackling such a challenging project as identifying new antibiotics for disease.

END OF SESSION