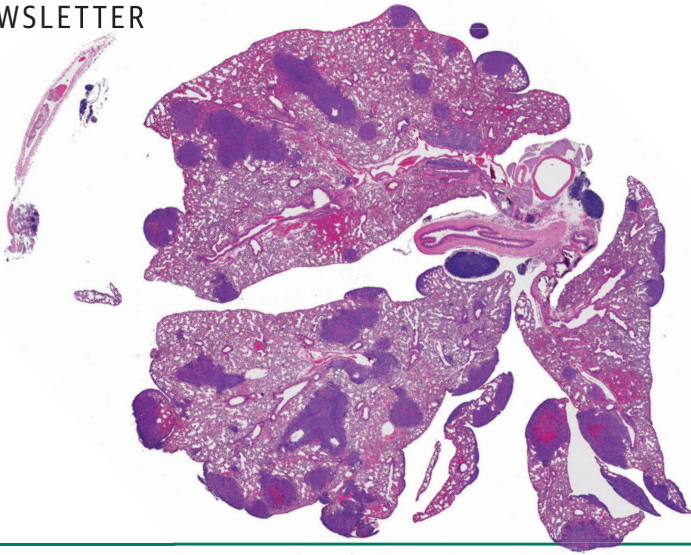


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# BioCoder

BIO NEWSLETTER

APRIL 2018



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## **Cancer Detection, One Slice at a Time**

Courtney Webster

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## **How Synthetic Biology Startups Are Building the Future at RebelBio**

Elsa Sotiriadis

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## **The Community Lab: A Student's Door to Opportunity**

Shreya Thiagarajan

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## **Where Beauty and Biotechnology Intersect**

Meghan Tahbaz

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## **DARPA and the Future of Synthetic Biology**

Benjamin Wolfson

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# BioCoder #13

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## BioCoder #13

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# Cancer Detection, One Slice at a Time

*Courtney Webster, MM DD, 2017*

Ke Cheng's story begins in a way that's achingly familiar. She recalled late nights and long hours as a grad student, repeating a tedious task in the name of research. Like many of us, Ke dreamed of starting a company that would make that job easier and faster. She ended up building the world's largest online, pre-clinical pathology database, which catalyzes and could ultimately lead to the automated detection of cancer. But let's take a step back—how did Ke go from an over-worked grad student to building a fountain of digital data?

For her, the tedious grad-school task was cutting tissue and making slides for histopathology (the analysis of tissue to identify or determine changes due to a disease). The process starts by preserving a tissue sample and embedding it within a block of paraffin wax. A microtome is used to section the tissue into slices thin enough for microscopy (typically, about five microns thick). Each slice is placed on a microscope slide, where it can be stained to color different structures. The most common stain, H&E, is a mixture of an acidic dye (hematoxylin) and a basic one (eosin), which will stain cytoplasm pink and nuclei purple. This alone can be used to detect disease, just by allowing researchers to observe the organization (or disorganization) of the tissue structure. But there are hundreds of “special stains” to detect tissue elements (e.g., muscle fibers, glycoproteins, and mucins), microorganisms (e.g., fungi and bacilli), or specific ions like ferric iron ( $\text{Fe}^{3+}$ ). Immunohistochemistry (IHC) can also be performed on tissue slides. IHC uses antibody-based detection to confirm cancer subtypes. While standard H&E staining is automated, using special stains or IHC can take significant time to optimize. Most researchers prep the tissue themselves and then send it off for sectioning and staining. At the time of Ke's graduate work, it took two or more weeks to get the slides back.

The key step that transformed Ke's histology-service company into a pathology data powerhouse was simple: digitization. When Ke founded HistoWiz four years ago, there were already instruments available that would automate histopathology (though they were uncommon to see in everyday research labs). But automating slide histology wasn't the final vision; it was just the first step. Ke was inspired by the open source genomic research model, in which searchable databases exist that everyone can access and contribute to. She saw firsthand that a trove of histopathology data existed but was unable to be easily shared because it was siloed in slide boxes (and tucked away in dusty cabinets or drawers). Even in the literature, only representative images (tiny sections of the entire slide) were used; and of course, publishable results are a small fraction of the all data collected.

Ke started HistoWz on her own and drew an initial customer base by promising a three-day turnaround (compared to the two-week industry standard at the time). She spent her days going door to door in her old research buildings, asking if people would be interested in doing a free trial. Then she spent her nights processing the samples using core facility equipment. Four years later, HistoWiz supports a number of major research institutions (including Harvard, MD Anderson, and HHMI), as well as big pharma (Pfizer, Regeneron, and Novo Nordisk).

The difference between HistoWiz and the other histology competitors was that Ke was the first to digitize the slides. After placing an order, the customer receives a **high-res, microscope-quality image** of the entire slide that can be viewed (with up to 40x magnification) on a laptop, smartphone, or tablet (**Figure 1-1**). If desired, the customer can receive the raw image or have the slide analyzed by one of HistoWiz's in-house pathologists. While providing faster, better histology as a service, HistoWiz had an open pipeline to data. Each customer receives a discount if they choose to contribute their data, so each slide HistoWiz processed could be incorporated into a database.

HistoWiz's **PathologyMap** now has over 30,000 slides and is growing at a rate of over 200 percent each year. While most of the data is from mouse tissue, PathologyMap has data from **human and other preclinical/experimental models** (zebrafish, rats, rabbits, etc.) as well. Ke's mission is to "fight cancer cooperatively." No more slide boxes or static representative images—this database allows researchers to do tissue-driven data mining by using whole slides in a massive dataset. It's important to Ke that this data is not restricted to just pathologists or academic researchers. For her, this opens a door to allow anyone to step inside the histology field and access the latest discoveries in cancer research.

## H&E lung metastasis

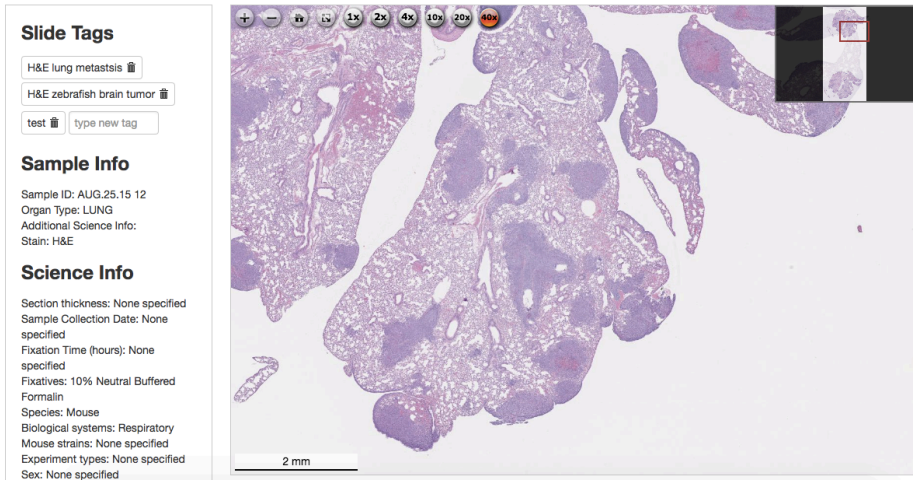


Figure 1-1. An H&E stained slide of metastatic murine lung cancer, available in HistoWiz' demo slide gallery. Source: Ke Cheng.

The possibilities of what can be done with this database are pretty fascinating. This is a new era of “digital pathology.” The FDA **recently approved digital pathology for primary diagnosis**, but acquiring enough data is still a very limiting factor. Ke’s database provides the raw data needed to accomplish that goal.

Secondly, Ke sees a potential for researchers to use the HistoWiz database as a tissue bank. She’s collecting extra unstained slides from her contributors, with a vision that someday researchers could request an unstained slide to analyze with their experimental tools instead of having to develop a mouse model themselves. While clinical tissue banks exist, there are few analogous resources for the pre-clinical research community.

And last but not least, the moonshot. Ke’s team is in the process of developing a machine learning algorithm to automate the diagnosis and prognosis of cancer. The goal is, from histology alone, to be able to predict whether a patient will respond to a certain type of therapy (or be a “nonresponder”). This approach would be cheaper and faster than current methods (genetic sequencing, for example). And this future may be closer than we think! A team at Stanford working on genetics and biomedical informatics recently published an algorithm that can predict non-small cell lung cancer prognosis just from H&E slides (**Snyder and Rubin, 2016**).

HistoWiz has a number of pathologists on staff to contribute “tagging” (identifying tumor types, tumor margins, etc.) for the database. An online annotation

tool is under development so that the data and the analysis can be crowdsourced. Different users will have different levels to help distinguish tags from the in-house pathologists, versus the slide owner, versus the general public. But all in all, the more data, the better to feed the machine learning algorithm. Ke doesn't expect machine learning to take the place of pathologists for diagnosis any time soon but can see the value of starting with prognosis of preclinical models.

While the potential to automate cancer detection would get anyone up in the morning, Ke finds inspiration from the end goal and the artistic beauty of the data itself. The company [posts on Twitter](#) "images of the day," sometimes with holiday themes ([Figure 1-2](#)). If you'd like to get to know Ke or learn more about HistoWiz, sign up to join one of the monthly dinners.

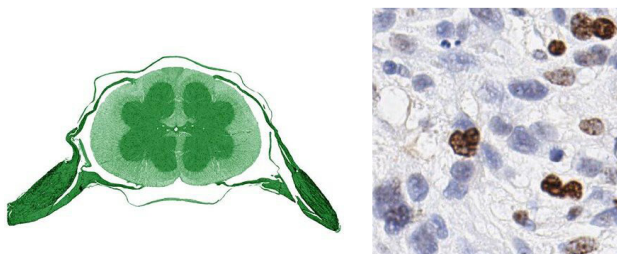


Figure 1-2. Left: Clover pattern for St. Patrick's Day; Right: A heart for Valentine's Day. Source: Ke Cheng.

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**Courtney Webster** ([@automorphyc](#)) is a freelance writer with professional experience in laboratory automation, automated data analysis, and the application of mobile technology to clinical research.

# How Synthetic Biology Startups Are Building the Future at RebelBio

*Elsa Sotiriadis*

Biology is becoming the **new digital**. As the engineering of biology starts delivering solutions at industrial scale while becoming more data-driven and automated, investors are getting excited to fund breakthrough biotechnologies that unlock this potential for a whole range of industries.

Europe has been steadily rising as a global hub for innovation and is attracting big bets from **veteran VC investors**. It has even been hailed as the “**New Silicon Valley**.” Boasting an active DIY biology scene, world-class institutions and productive innovation ecosystems, the continent is a fertile base to grow global startups from the early stage to the next level.

Accelerators are indispensable elements to harness this early-stage innovation, which often remains locked up in institutions or falls into the “Valley of Death,” the often deadly funding gap between those first discoveries and a working prototype.

This is the stage the RebelBio accelerator comes in to help startups solve global grand challenges—with life itself.

## An Innovation Engine

It’s as exciting in biology today as it was in the computer industry in the late ’70s, when the Apple II came out. Computers became personal.

As Steve Jobs said, “I think the biggest innovations of the 21<sup>st</sup> century will be at the intersection of biology and technology,” the ethos of RebelBio is to bend

and break the rules of the status quo at the intersection between these two disciplines.

A total of 15 multidisciplinary teams from across the world have begun the latest program at RebelBio, garnering an investment of over \$100,000 for each company. In addition to gaining access to fully equipped labs and office spaces, they also draw from a network of hundreds of mentors, including RebelBio-founder Bill Liao, who also cofounded Xing, Davnet, and CoderDojo.

The program helps the founders to make their longer-term moonshot visions (the “innovation” part) into feasible projects which generate revenue early on (the “engine” part). It is transforming scientists into entrepreneurs across diverse areas of life sciences and is currently in its fourth batch.

*From novel biomaterials to new ways to brew the foods we love, from speeding up cancer lab tests turning days into hours, to a microbe-miner discovering life-saving antibiotics. We even have a machine learning startup for drug discovery, and another working on microbial fuel cell modules to treat wastewater while generating electricity!*

**Bill Liao, founder of RebelBio and general partner at SOSV**

So what kind of startups are brewing at RebelBio?



Figure 2-1. The lab and some of the cohort IV at University College Cork (Image: RebelBio)

## Diagnostics 2.0: Rapid, Portable, and Personal

One RebelBio startup is **Sex Positive**, founded by Nico Bouchard and Mary Ward, cofounders of **Counter Culture Labs** and both biohackers from California. Developed as a smart diagnostic device for sexually transmitted infections, Sex Positive



is enabling rapid self-testing of sexual health in the privacy of one's own home, without the need for a hospital lab. The first test will be for chlamydia. The device combines fluid dynamics, immunology, genetics and electronics designed by a Tesla engineer remotely in Palo Alto.

Meanwhile, **KaitekLabs** is founded by Emilia Diaz from Chile, who is turning bacteria into living computers. They function as a ready-to-use, portable biosensor kit to detect shellfish toxin directly from shellfish—a major food source for many people living in coastal regions around the world. It makes the invisible poison orange! The startup has already sold out a batch of the first prototype.



*Figure 2-2. KaitekLabs' founder Emilia with the "MOSES" (Microbial Optic Shellfish Evaluation Sensor) toxin-detection kit (Image: KaitekLabs)*

Then, OaCP (Oncology and Cytogenetic Products) is a university spin-out from Bologna, Italy, using its patented reagents to speed up diagnostics. Since many in vitro diagnostic tests for cancer can take over three days of agonized waiting, OaCP uses a novel reagent which enhances hybridization of nucleic acid probes. This reduces the diagnosis time to as little as two hours. As the applications are versatile, the reagent can also be used to accelerate bottlenecks in genome sequencing, genome editing, or liquid biopsies.



Figure 2-3. Sex Positive's personal in vitro diagnostic test to detect sexually transmitted infections (Image: Sex Positive)

## DNA As the New Silicon

As biology is becoming an information technology, DNA emerges as the new silicon.

For instance, **Helixworks Technologies** is the first to offer storage of digital data in DNA. The startup is now developing a new product: a portable molecular machine (dubbed OpenMOSS) that converts digital data into DNA—in your home.

By storing data completely offline in a medium as durable and dense as DNA, Helixworks could provide a biological solution to the rapidly expanding need for cost-effective, long-term data storage while addressing the rising threat of cybersecurity. Storing large or sensitive sets of data completely offline in a physical medium offers safety from cyberthreats. The startup recently won the “**Most Innovative**” award at pitch competition SXSW.

Tools that make it easier to program life are a running theme across the cohorts, with another example, **Briefcase Biotec**: it's building a DNA synthesizer called Kilobaser, “the Nespresso Machine of DNA synthesis”: simply add a reagent-cartridge and enter your sequence! The machine will make oligos and primers quickly and cheaply directly on the lab bench.

Moirai Biodesign is also adopting this modular approach, in their case to build programmable cancer therapeutics with “Plug-and-Play RNA”. The molecule consists of two domains, whereas the sensor part reacts to the presence of cancer-specific biomarkers, while the trigger part encodes a certain protein. Allowing for switch-like activation of the biodevice, specifically in cancer cells, this targeted therapeutic holds great promise to alleviate side-effects of cancer therapy in the future.



Figure 2-4. Left, Conor Crosbie, Eshna Gogia, Sachin Chalapati, Nimesh Chandra, and feline buddy of the Helixworks team (Image: Sachin Chalapati); right, Kilobaser’s new quality-control platform (Image: Briefcase Biotec)

## Personal Maker Kits to Build with Biology

Tools like these could be used in conjunction with a bio-maker kit, like BentoLabs and AminoLabs—or an entirely new kind. Hence, **Cell-Free** is breaking a billion-year-old processor out of the cell to enable anyone, anywhere to manufacture biomolecules with a cell-free machinery: a “Raspberry Pi” model for biology.

In coming years, point-of-care synthesis of products like insulin and vaccines could drastically improve the availability of medicines. For now, the startup is envisioning consumer biotech applications to enable anyone to make custom colors, smells, logic circuits—even glow-in-the-dark ink! The founders aim to bring technology and biology closer together.

*Biological sensors, detectors and processors will be core to this. We are building the tools that will allow innovators from all backgrounds to engineer the materials of the future.*

**Dr. Thomas Meany, cofounder and CEO of Cell-Free Technologies**



Figure 2-5. Right, Rapid prototyping kit to take biology from imagination to creation (Image: Cell-Free); left, a design-test-build cycle with *Bio-Pixel visualization* on the existing open-source maker platform (BioDesign Challenge), such that synthesis can be followed live via an app (Image: Helene Steiner, Biodesign Challenge, Royal College of Art)

## From Machine Learning to a “MicrobeMiner” to Unlock New Medicines

Continuing on the health theme, clinical trials and approval for new medicines are devastatingly time-consuming, costly, and risky; while the rise of antibiotic resistance poses a rising challenge to global health.

*Galactica Biotech* is using machine learning algorithms with multiple highly trained modules to identify potential new uses for existing medicines, from small molecules to complex plant alkaloids. The process even works backward to find new targets for these drugs, as well as identifying off-target effects for toxicology studies. This means they will offer their multiapproach A.I. as a service to pharmaceutical companies to help them unlock the full potential of their drug discovery pipeline toward a future of precision medicine. They’ve just used their algorithm successfully to identify a new anticancer lead molecule in the lab, which is already approved for another indication. The team hails from Russia, Spain, Mex-

ico, and the UK. It brings together expertise from PhD research in computational medicinal chemistry, artificial intelligence, systems, and synthetic biology.

**CyCa OncoSolutions** is founded by Dr. Nusrat Jahan and curious things can happen when a chemist is doing biology. She discovered a biomolecular machinery to permeate the cell membrane. This allows for more effective and targeted delivery, for instance, of cancer drugs. The young innovator and principal investigator worked relentlessly all around the world, from the universities of Oxford, Leiden, and Kyoto to ETH Zurich, driven to find a solution to her father's life-shattering cancer diagnosis.

**Valanx Biotech**, on the other hand, makes programmable designer proteins, for instance, to conjugate them to a targeting antibody. It uses versatile click-chemistry to easily dock new molecules to the protein with its patented technology "SnapIt."

In software, we mine for bitcoins, whilst in biology, we mine for antibiotics! **Prospective Research, Inc.** has developed a platform that mimics stimuli in the soil to mine for novel, life-saving antibiotics from *Streptomyces* bacteria.

How? With what it calls the MicrobeMiner platform. Why? Because the next billion-dollar drug could be buried in your backyard. Therefore, the team also sends out the MicrobeMiner kits for sample collection to crowd-source that discovery.

About 90% of natural products remain hidden in the silent operons of the microbe's DNA, unless the pathway is induced. The biosynthetic machinery of antibiotics can be initiated by certain stimuli in the soil matrix though. Hence, the microbes are first screened with the GeneMiner technology, which identifies the gene clusters that are silenced in the absence of these inducers.

For the second step, StimKeys comes into play: this proprietary platform launches a variety of chemical inducers with the aim to unlock these potent chemical pathways. They've indeed identified molecules which turn a seemingly uninteresting "dirt microbe" into a powerful factory for novel, potentially life-saving antibiotics.



Figure 2-6. The Prospective Research team has built the MicrobeMiner platform to mine novel antibiotics from soil microbes. Pictures show *Streptomyces* cultures used for the applications MicrobeMiner, GeneMiner, and StimKeys. (Image: Prospective Research)

## Growing the Circular Economy: Bio-inspired Design, High-tech Ecosystems and new Biomaterials

Urbanization is a global trend that will drastically change how we live. By 2050, up to 66% of the world's population will live in cities, according to the [United Nations](#). That means we need better solutions to power, feed and clean up our future megacities.

Therefore, developing a productive circular economy is imperative to make human activity more sustainable and improve the health of our planet. Biomimicry can help us unlock nature's most resource-efficient blueprints to future-proof humanity. Hence, building smarter, zero-carbon cities with biology has already started.

For example, [NuLeaf Tech](#) is combining the technologies of engineered ecosystems with microbial plant fuel cells as part of a biologically inspired hardware module that treats wastewater to create clean water and generate energy. These were the ideas that gave rise to NuLeaf Tech in the NASA Ames Advanced Studies Lab in 2015.

The team is testing a first prototype in collaboration with local farmers, with the vision to create high-tech ecosystems and artificial, modular wetlands—even in vertical arrangement for use in the home.

*Our bio-inspired technology will create purified water and clean energy solutions for industry and residential use.*

**Rachel Major, cofounder and CEO of NuLeaf Tech**

So, bio-inspired design helps us uncover powerful engineering solutions—and even novel materials!

Examples of sustainably manufactured materials include the ability to 3D-print degradable bioplastics and make useful items from the plastic waste, in which the planet is drowning. This is an area **Saphium** and **BioCellection** are working on.

On the other hand, **Pili** is growing beautiful, living color pigments for print and design from bacteria at an industrial scale, and **Chinova Bioworks** is turning to mushrooms for new biomaterials.



Figure 2-7. Left, NuLeaf Tech; right, microbial fuel cell prototype (Images: NuLeaf Tech)

### The Foods of the Future? They're Brewed, Too!

It's never been a more exciting time for animal lovers, because we're entering the post-animal economy. A rapidly increasing number of animal-free products are in development around the world. Examples include allergy-free peanuts (**Ara-nex Biotech**), genome-edited plants to grow the designer foods of the future (**PlantEdit**), and *in vitro* meat at our sister program IndieBio SF (**Memphis Meats**).

But what about sustainable beverages?

**Perfect Day** (formerly Muufri) is a vegan alternative to milk which has been hitting the headlines as long-awaited animal-free dairy. At the same time, Spira is looking to lock on to the health market with a tasty, nutritious drink produced by *Spirulina* algae.

**Afineur**, is cofounded by CEO Dr. Camille Delebecque. Their Cultured Coffee, produced by microbial fermentation, is taking New York by storm. And what better alternative to sweeten your caffeine fix, but with **MilisBio's** sweetener proteins?

Seeing as these plug-and-play vegan plant proteins are up to 700 times sweeter than sugar, the MilisBio team is addressing the demand for non-carbohydrate-based artificial sweeteners in a world hooked on sugar.



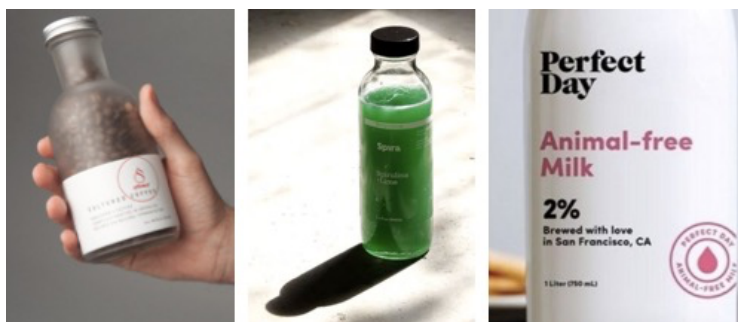


Figure 2-8. Afineur Cultured Coffee (Image: Afineur); Spira Spirulina-based drink (Image: Spira); and Perfect Day cow-free milk (Image: Perfect Day)

## Hacking the Plant Biofactory

Another major area of biotech innovation is programming microorganisms and plants to produce useful compounds and novel biomolecules.

For example, Hemoalgea is cofounded by a team of bioengineers from Costa Rica. The startup is using an optimized microalgal factory to make Hirudin, a major anticoagulant originally produced in leeches. The algae has been found to produce complex glycosylation patterns unlike other biomanufacturing platforms. Meanwhile, SwaLife Biotech is extracting novel alkaloids from plants, which have already shown promising results against DNA breakage. They could find applications in cosmetics, and later on, medicine.

Similarly, **Alternative Plants** is cofounded by CEO Anna Ramata-Stunda in Latvia. It's unlocking the hidden treasures of active compounds found in plants by using plant tissue stem cells. This allows to access nature's reservoir of compounds, while producing them sustainably at large scale, without harm to the often rare and endangered species. The team has already optimized the powerful platform for high yield and is now scaling up the production of cosmetic ingredients with industrial partners.

Canuevo is an Uruguayan-Canadian startup cofounded by Dr. Nils Rehman to launch a paradigm shift for cannabinoid medicine. Their nanoparticle-encapsulation technology can be applied to creams, pills, supplements, and later on, new medicines. Finally, **Hyasynth Bio** in Canada produces THC and other cannabinoids in highly efficient yeast factories as featured [here](#) and in [here](#).



As we see, very exciting things are happening at the intersection of biology and technology. Synthetic biology startups are at the forefront of tackling diverse areas of life sciences to build a better world by programming life.

Hopes are high for astonishing consumer biotech products, delicious foods and beverages, biomaterials and sustainable living, as well as novel medicines and therapeutics—in short, to share the benefits of scientific innovation with people around the world.

## Accelerating the Biorevolution

Find out more [on the website](#) or [this youtube video](#) and stay up to date by connecting on [Twitter](#) and [Facebook](#). You can apply for next year's cohort [on the application portal](#).

**RebelBio** (previously IndieBio EU) is the world's first and leading early-stage startup accelerator and part of **SOSV**, the accelerator VC. The fund has \$300 million in assets under management and is the world's **most active investor in synthetic biology**. SOSV is also running the leading seed-stage accelerator **IndieBio SF** in San Francisco.



*Figure 2-9. The 2017 cohort of 15 startups from around to world with the team and the RebelBio and SOSV teams*



Figure 2-10. Some of the RebelBio team (from left to right): John Carrigan (chief scientist), Elsa Sotiriadis (program director), Steven O’Connell (program manager) and Bill Liao (founder and SOSV general partner)

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*Elsa Sotiriadis is a bioengineer, technologist, and program director of the “moonshot factory” RebelBio @SOSV. Her background is a PhD in synthetic biology and she has experience in startups, emerging technologies (including digital biology, machine learning, and high-tech), and future thinking. She’s also a poet and cyborg—her hand speaks IoT.*

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- Twitter: [@thebiofuturist](https://twitter.com/thebiofuturist)
- Facebook: [biofuturist](https://www.facebook.com/biofuturist)

# The Community Lab: A Student's Door to Opportunity

*Shreya Thiagarajan*

In what seems like a revolutionary rise in citizen science, hackerspaces (fondly called community labs by their users) have emerged at the forefront as a potential solution to the lack of scientific resources available to ordinary citizens. They provide everyone with access to ample resources and materials needed to kickstart a project that may otherwise require years of funding to begin. Hackerspaces are on the rise nationwide, popping up in several big cities all over the United States such as New York, Los Angeles, and San Francisco.

Hackerspaces vary in nearly every aspect, from size to accessibility to funding. Many hackerspaces specialize in a particular field of science, such as nanotechnology or computational biology, while many others are hosts to projects in a variety of different scientific subfields. The consensus seems to be that the typical hackerspace is a location for a meeting of the minds, that is, an open resource center for a scientific community to meet and exchange ideas about potential laboratory-common projects. For example, Counter Culture labs, a hackerspace in Berkeley, California, plays host to the Open Insulin project, one that the most lab members work on as a laboratory-common project. In most cases, hackerspaces specialize in one or two projects the entire community works together on like the Open Insulin project, but they usually also serve as laboratories for their users to conduct individual projects in.

Such individual projects have bloomed into successful products, some of which can even be found on the market today. What most citizen scientists have not considered, however, is the remarkable impact these hackerspaces have on college students and high-school students like me.

One such hackerspace is [BioCurious](#), located in Sunnyvale, California, deep in the heart of the Silicon Valley. Not only does BioCurious hold regular classes to teach students basic laboratory procedures and techniques, but it also encourages

them to take part in its various community projects, many of which are submitted to the annual [International Genetically Engineered Machine \(iGEM\) jamboree](#), a contest that attracts teams from universities and hackerspaces worldwide to present their innovative ideas at a conference-style event in Boston. The iGEM jamboree is unique because it fosters collaboration among participating teams by allowing them to submit their engineered biological components to their database from which future teams can request certain “bio-blocks” to use in their projects. In 2014, when iGEM had just begun accepting community labs as participants, BioCurious won a gold medal in its category for its Real Vegan Cheese project. The following year, it was able to secure a bronze medal in its category for its Bio Sunblock project.

Having joined BioCurious in the December of 2014 I was unfortunately unable to participate in the Real Vegan Cheese project. I was, however, graciously invited to participate in the 2015 iGEM project, first helping to compile a list of possible topics to do further research on. It was here that my involvement in BioCurious’s community projects and my interest in citizen science began.

My first dosage of scholarly research came from perusing various publications and journal articles to gain information about the topic I had chosen to vouch for from an ideas list that included everything from inducing bacteria to grow into three-dimensional shapes to using natural compounds as biological ink. Along with my high-school and college peers, I was able to design a poster-style slide presentation detailing specific information about each project topic, including the required materials, feasibility, and societal impact. When the voting was completed, only one project remained.

Our idea of producing biological sunblock-like compounds stemmed from the issue of chemical pollution in freshwater rivers and lakes, as well as one of the most important ecosystems on our planet, the ocean. Information from a [study](#) published by the journal *Ecotoxicology* and reported by the National Ocean Service indicated that certain compounds in sunscreen that washed off of swimmers in the ocean were contributing to coral bleaching and reef death. Our goal was to find a method to keep ourselves protected from dangerous ultraviolet rays while simultaneously combating the effects of chemical pollution in the ocean.

Our team noted that several marine organisms such as algae and some coral species already produced UV-protectant substances to shield growing polyps from harmful solar radiation. Mycosporine-like amino acids (MAAs), as they were called, could be induced upon prolonged exposure to ultraviolet light and were full of anti-oxidative compounds that fought radiation-caused skin damage.

Shinorine, a certain MAA produced by the cyanobacteria *Anabaena variabilis*, had already been studied extensively and had shown promising results as a poten-

tial biological sunblock. The gene for the compound in *A. variabilis* was first discovered in 2010 by researchers [Emily Balskus and Christopher Walsh](#) at Harvard University; and in 2012, the [University of Minnesota iGEM team](#) succeeded in creating the gene cluster containing the shinorine producing gene ([Figure 3-1](#) and [Figure 3-2](#)). Synthetic shinorine production in *Escherichia coli* containing the transformed gene cluster was yet to be observed, so our team took to the task.

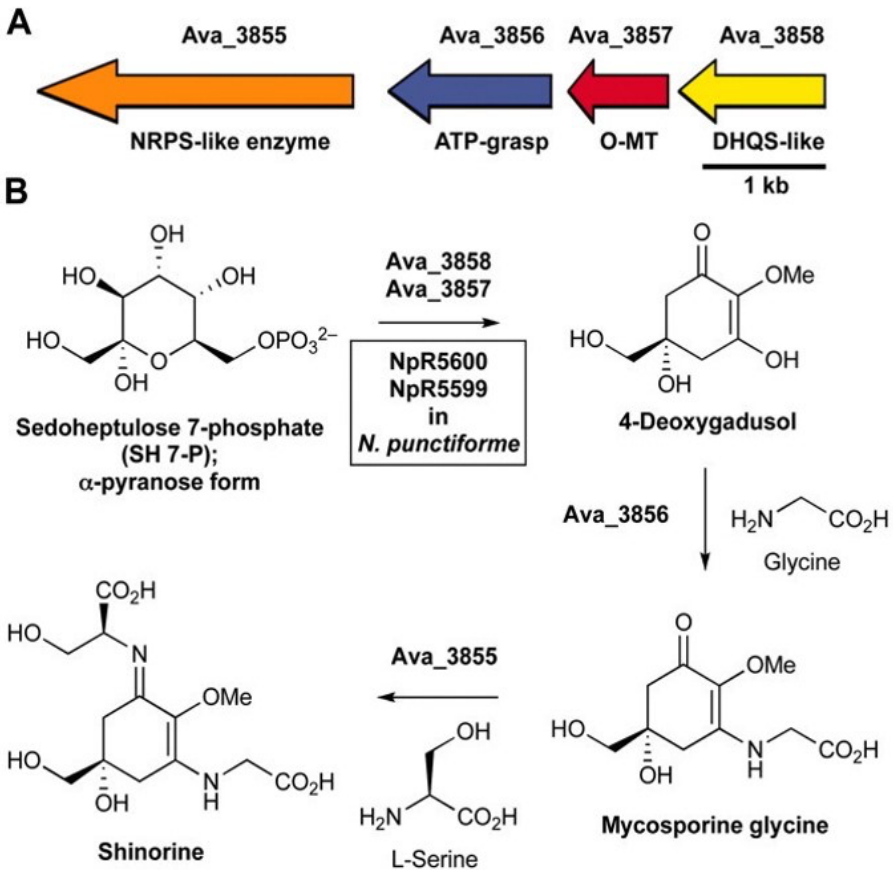


Figure 3-1. Gene cluster from *A. variabilis* with chemical diagrams of MAAs shown

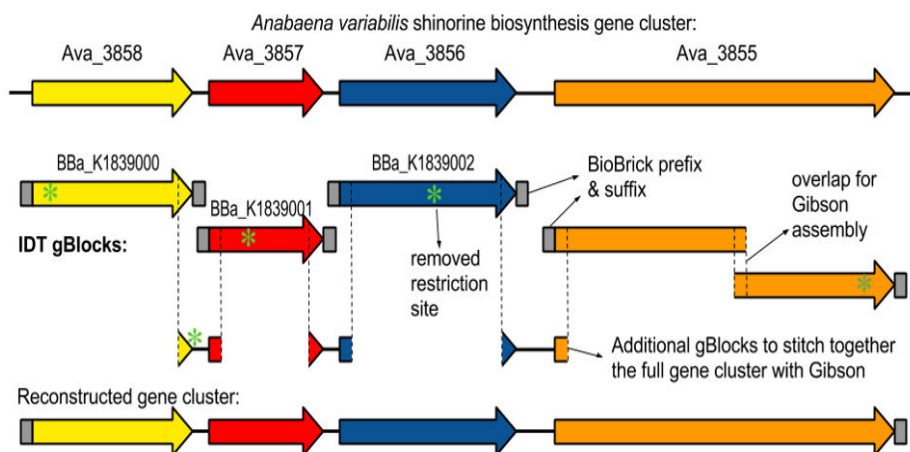


Figure 3-2. Shinorine pathway engineered by BioCurious from template from the University of Minnesota

Up to this point, the most cutting-edge scientific procedures I had ever done were preparing agar plates and culturing bacteria. I was understandably surprised then when I (along with some responsible adults, of course) was assigned, as a learning opportunity, the task of transforming the completed gene cluster into *E. coli*, using the “bio-blocks,” or editing tools, provided by IDT, an iGEM sponsor. Gene editing had only been briefly mentioned in my freshman biology class, so all this time I had thought of the process as an expensive and extremely delicate procedure only performed in the most secure and highly funded of laboratories. Yet I was handed a series of different-sized micropipettes and small vials of liquid presumably holding the gene cluster that needed to be transformed into the bacteria.

Not many high-school students can boast about having had the opportunity to edit genes, and I owed my privilege to BioCurious for placing such resources into my hands. Although accuracy in results was what we aimed for, the team emphasized education as a critical component of the experimental process. It was under their guidance that one Saturday morning, I was able to transform the shinorine-producing gene into a culture of *E. coli* with little difficulty.

Over the following six months before the jamboree, I could be found at BioCurious every Saturday morning helping perform spot assays (a process that uses serial dilution of a bacterial culture sample to measure bacterial growth) in the fume hood at the corner of the lab or fiddling with the DeNovix spectrophotometer.

Two years after the project won BioCurious a bronze medal at the 2015 iGEM jamboree, the project was discontinued, yet it continues to have an impact on my life and my opportunities as a student researcher. My work at BioCurious has opened several doors for me, and not only has my experience helped me obtain research positions in university laboratories, but with BioCurious's help, I was able to carry out two projects of my own, both of which I submitted to the local Synopsis science fair.

My first project focused on the neurodegenerative disease amyotrophic lateral sclerosis (ALS), whose growing prevalence in the elderly population, combined with the popularity of the ALS ice bucket challenge at the time, piqued my interest around the same time the deadline for submissions to the local science fair was approaching. The rapid progression of the disease is closely correlated to an increase in the production of the protein TDP-43. However, it was the clumping of this protein in motor neurons that is believed to lead to rapid cell death and paralysis (Mackenzie, Ian R.A.; Rademakers, Rosa).

From reading journal articles, I discovered that a particular natural compound known as curcumin had been effective in inhibiting the clumping of proteins in motor neurons that lead to similar diseases such as Parkinson's disease and Huntington's disease. In fact, curcumin was proven by a research team in UCLA and confirmed by two other scientists to have disbanded beta-amyloid plaque buildup in cell models with Alzheimer's disease (Yang, Fusheng, et al., 2004) With this information in mind, I decided to test the effects of curcumin on the clumping of TDP-43 in ALS yeast models.

This particular project was especially unforgettable since it was the first time I would complete a science project that I myself designed and carried out on my own. Of course, I could not have accomplished a single part of the project without the resources and materials provided by BioCurious which were so generously shared by their respective owners for the whole lab to use. Additionally since it was my first time performing some other laboratory procedures (using the fluorescent microscope, gel electrophoresis, etc.) I had significant help from several other lab members (mentioned in the acknowledgments) to carry out these procedures. In time, these resources allowed me to finish my project before the regional science fair, and with the help I received from my fellow lab members, I was able to present my data seamlessly to all who chose to listen.

The following year, another pressing issue took root in my mind, prompting me to research its implications and impact on society today: antibiotic resistance, particularly the presence of antibiotics in livestock feed. It was a "solution" that had been devised to combat the growing prevalence of disease in livestock that resulted from crowded and dilapidated pens and uncleaned feed. Through the

repeated administration of antibiotics to livestock through crops and feed, mutant bacteria with innate resistance have survived with each increased concentration of antibiotics and found their way into the animals we interact with and the meat we consume.

Since traditional antibiotics were the problem itself, other chemicals proven to be antimicrobial would not serve as an adequate solution since bacteria could evolve a resistance to those chemicals as well. An ideal solution would be one that evolved along with the resistance in bacteria. That ideal solution was the *bacteriocin*, a category of compounds naturally produced by some bacteria species to kill other bacterial species that compete for the same resources. Since these bacteria coexist, a developing resistance in one bacteria species could trigger evolution of the other bacteria species, favoring individuals that produce an “evolved” bacteriocin that could now kill the resistant bacteria.

One species of bacteria *Lactococcus lactis*, commonly found in the cow’s digestive tract, produces a specific bacteriocin called *nisin*. Past studies proved nisin as a bacteria effective in killing gram-positive bacteria, including two particular species that are commonly found in animal feed.

Though nisin itself would have been an optimal choice as a deterrent to use in my experiment, past studies had proven its effectiveness in reducing the growth of bacteria. Thus, I decided I would primarily research the effects of nisin combined with either ascorbic acid or acetic acid on bacterial growth. Acetic acid, or vinegar, was itself antimicrobial, so I hoped a combination of the two would enhance nisin’s performance.

The results from my research were enough to win me a special award at the regional science fair and a first-place cash award from the Northern California Institute of Food Technology. I give credit for both to BioCurious for providing me with the materials and help I needed to succeed in my scientific endeavors.

Overall, my past two years at Biocurious have opened numerous doors. I was able to present at the annual Bay Area Makerfaire on behalf of the laboratory. Maker Faire is one of the area’s most popular annual attractions and plays host to hundreds of booths and organizations that flock to the event to display their creativity in various ways. I still remember my excitement from being given a free ticket to the event, a perk that all participating organizations received for sharing their innovations with the public. What stuck in my mind as one of the most rewarding moments of the experience was watching two children, still in elementary school, waddle up to our booth with excitement. Their eyes widened in fascination as I spoke to them about how we had stripped a sheep heart of cells, leaving a protein scaffold behind that could be used as a template to create a functional human heart out of stem cells.



Their mother approached me and began inquiring about the lab as her children worked on extracting their own DNA from their cheek cells, an activity we had designed for interested children and adults alike to try. I recall being congratulated and commended for my passion for science and for my will to take initiative and accomplish so much at a rather young age.

The truth is most high-school students are interested in cutting-edge research, and those who are currently uninterested are soon to change when exposed to the kind of technology that is changing the way we innovate. That is where community labs make their biggest impact, inviting anyone, regardless of his or her experience and interests, to make dreams come to life. I think I can speak for all my high-school peers at Biocurious that community labs have been a key part of our development as future scientists.

I would like to thank Maria Chavez for letting me join BioCurious as a high-school student and participate in the 2015 iGEM project, Patrick D'haeseleer and Jay Hanson for guiding me through each step of the experimental process in the Bio Sunblock project, and Johan Sosa for assisting me in both my personal projects and allowing me to help him on the Real Vegan Cheese project. Special thanks to Eric Harness for teaching me how to use the autoclave and the fluorescent microscope.

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**Shreya Thiagarajan**, a Bay Area native, is a rising senior at Westwood High School in Austin, Texas. While living in the Bay Area, she was a member of the community lab BioCurious, carrying out two of her own projects on the progression of ALS and antibiotic resistance as well as participating in the lab's project on biological sunblock synthesis, which was awarded the bronze medal in the 2015 iGEM jamboree. In Austin, she interns at two laboratories at the University of Texas at Austin working on a gene editing therapeutics project aimed at curing cystic fibrosis and a psychopathology project studying the basic behavior of slime molds. She can be contacted via email at [shreya-thiagarajanoo@gmail.com](mailto:shreya-thiagarajanoo@gmail.com).



# Where Beauty and Biotechnology Intersect

**THERE'S BEAUTY IN BIOLOGY—AND THE BIOTECH INDUSTRY IS READY TO MAKE A MOVE**

*Meghan Tahbaz*

With the ever-expanding and innovative uses of biotechnology in medicine, agriculture, and other equally essential fields, there comes an intriguing question: where can biotechnology *not* be applied? As with all scientific pursuits, arguably the sky's the limit. That seems to be the ethos of two entrepreneurial forces which are each working to bring biotech into a completely new domain: beauty. For biotech-turned-beauty company Amyris, this means introducing Biossance, a plant-based skincare product with engineered ingredients that mimic molecules naturally produced by the human body. Then there's AOBiome, parent company to Mother Dirt, that is working to incorporate beneficial bacteria into facial sprays with the thought that our skin biomes actually need such microbes to thrive.

## Biossance is Bringing Biology to the Beauty Counter

What initially began as a Berkeley lab project to manufacture a readily available cure for malaria has led to a skincare revolution founded on the principles of sustainability and health-conscious living. Biossance is redefining beauty, bringing the industry closer to newer environmental standards. And the best part of the company? It is utilizing biotechnology to accomplish this.

When it comes to skincare products, Biossance believes that the best source of inspiration is something we're all inherently familiar with—the human body. Therefore, the basis of its unique products is squalane (C<sub>30</sub>H<sub>62</sub>), a completely saturated form of the lipid squalene that is naturally produced by the body's seba-

ceous glands. The production of squalene in the human body decreases beginning around age 20, leading to less moisture retention in the skin. Biossance's goal is to supplement this decrease in squalene with its manufactured molecule squalane (Figure 4-1).

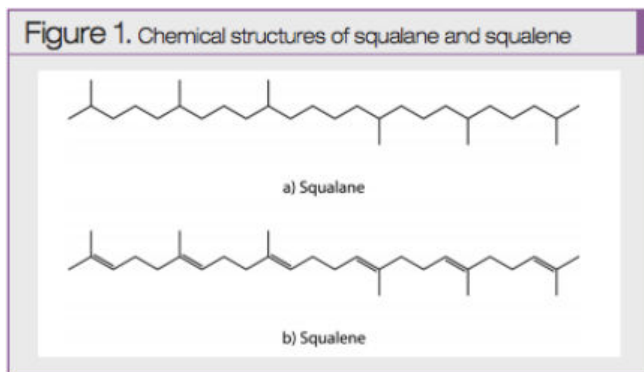


Figure 4-1. The chemical structures of squalane and squalene. (Source: "Deriving Renewable Squalane from Sugarcane")

The difficulty with squalane is deriving it from an eco-friendly source. Its precursor, squalene, can be isolated from shark liver oil, but this animal source poses two major problems: one, it is highly unethical to target such marine life in order to extract the desired compound; and two, it is entirely unsustainable.

That's where bioengineering comes in. Parent company Amyris employs a manufacturing process that generates squalane from sugarcane-fed yeast, ensuring that the product is not only completely pure but also continually renewable. This production method is commercially feasible for manufacturing squalane on a much larger scale and is a step up from experiments of the 1990s and before. It hinges on the isoprenoid pathway and on the use of  $\beta$ -Farnesene, the molecule that precedes the synthesis of squalene.

Amyris has engineered a microbe, a yeast called *Saccharomyces cerevisiae*, which is essentially a microcosm for factory production of  $\beta$ -Farnesene. The metabolic pathway for the yeast has been altered to produce, in response to fermentation with raw sugarcane as feedstock, a mixture of squalene and other natural by-products; the yeast itself is removed after fermentation. The form of squalene produced through this pathway is highly unstable, which would make isolation a difficult task. However, a natural chemical coupling, using the pure hydrocarbon  $\beta$ -farnesene as feedstock, occurs after removal of the yeast, which makes isolation unnecessary (Figure 4-2).

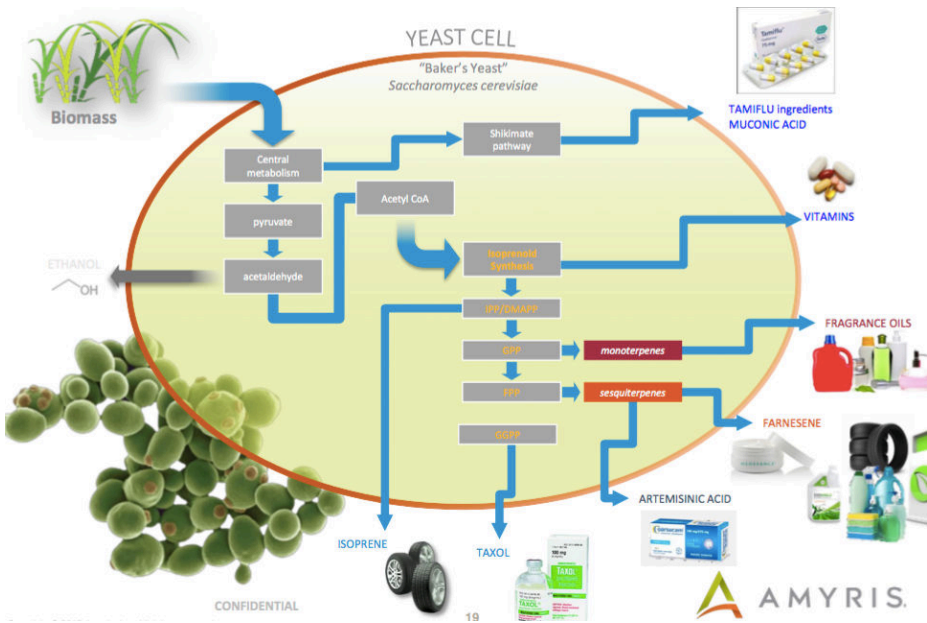


Figure 4-2. The production of  $\beta$ -Farnesene and other molecules through interaction of yeast and feedstock/biomass. (Source: Amyris)

Squalane is subsequently produced through catalytic hydrogenation of squalene. Squalene's unsaturated double bonds are broken upon introduction of a catalyst, which is later recovered, thus leaving behind completely saturated single bonds in the hydrocarbon which is now known as squalane. Fractional distillation allows for further purification by separating substances according to their differing boiling points (Figure 4-3).

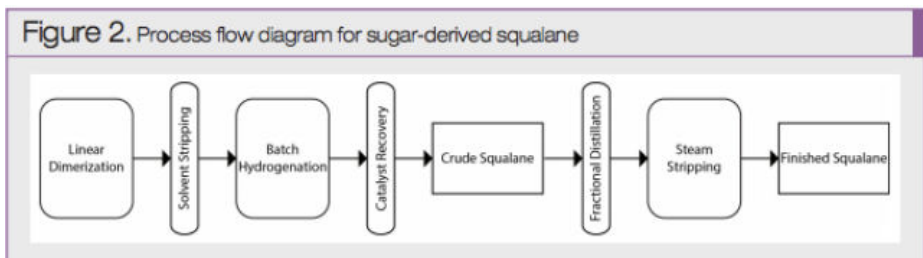


Figure 4-3. Linear dimerization and solvent stripping of yeast microbes followed by the hydrogenation of squalene to produce, and later purify, squalane. (Source: "Deriving Renewable Squalane from Sugarcane")

This process offers several advantages in the production of commercial squalane, being that it makes such wholesale manufacturing possible in the first place. Firstly, development can be easily regulated, which ensures a higher degree of quality control and homogeneity in the resulting compound. Secondly, each batch of product can be reproduced thanks to the abundance of feedstock needed for creation. And perhaps most importantly, this biological-based method upholds the environmental consciousness that Biossance espouses.

Each of the products manufactured is a combination of squalane and other natural oil/gel products such as vitamin C rose oil, ensuring that only the safest ingredients are included. The products are bottled and then packaged in sugar-cane fiber boxes, achieving both an eco-friendly interior and exterior (Figure 4-4).



Figure 4-4. Squalane + Peptide Eye Gel product (Source: Biossance.com)

While Biossance maintains its own website, it has also partnered with Sephora to expand its sales and outreach. The brand became available at the cosmetics retailer in February 2017, with the Squalane + Peptide Eye Gel product quickly becoming the best-selling eye product.

As customers scour the aisles for more bioengineered beauty products, Biossance will surely continue to deliver on its promise to make safe skincare: “because the products you use make a difference for your health and the health of the planet.”

## AOBiome Doesn’t Mind Getting Its Hands (or Face) a Bit Dirty

With the large focus on antibacterial cleanliness in today’s market, it’s a bit of a shock to find a company that is advocating for the introduction of bacteria *into* skincare regimens. But that’s exactly what Mother Dirt and parent company AOBiome are doing. AOBiome believes that certain ammonia-oxidizing bacteria are essential to preserving the health of human skin biomes, and their products have the goal of introducing and maintaining such healthy bacteria. Mother Dirt is starting a trend, and it revolves around probiotics—this time for the skin.

The skin biome is an amazingly diverse habitat, home to a large population of microorganisms which survive through symbiosis. Our skin provides these microorganisms with the energy they need to thrive; and simultaneously they protect our skin from harmful pathogens in the external environment.

There are several factors that influence the type of flora found on the skin: lifestyle, the external environment, contact with nature, etc., all contribute to the diversity of our bacterial cohabitants, or our lack thereof. In today’s modern times, urban lifestyles and the focus on cleanliness to the point of sterility have combined to eliminate a portion of the positive bacteria in the skin biome. AOBiome speculates that our lack of helpful bacteria could potentially cause or allow for noticeable skin sensitivity, acne, eczema, and other skin conditions. It hypothesizes that reintroducing certain beneficial bacteria through specially designed skincare products will begin to solve some of these healthcare concerns (Figure 4-5).



Figure 4-5. Mother Dirt AO+Mist product (Source: motherdirt.com)

AOBiome's Mother Dirt products focus on *Nitrosomonas*, which are a type of ammonia-oxidizing bacteria (henceforth referred to as AOB). AOB have a large role in sustaining the nitrogen cycle and therefore can be found anywhere that ammonia is present. The only exception is human skin; ammonia is present on skin, and yet there are no corresponding AOB. This type of bacteria is highly sensitive to soaps and therefore may have been completely eliminated by modern cleaning agents. A lack of AOB is problematic due to the helpful functions that these bacteria perform.

*Nitrosomonas* can be part of bioremediation by eliminating pollutants from the environment, a process which is appealing to the health-boosting intentions of Mother Dirt. *Nitrosomonas*, in general, oxidize the ammonia and urea found in bodily sweat, producing nitrite and nitric oxide critical to antibacterial and anti-inflammatory care, respectively (Figure 4-6).





Figure 4-6. *Nitrosomonas europaea* (Source: Stan Watson, Woods Hole Oceanographic Institute)

Nitric oxide is an entirely desirable product because of the many functions it has in conjunction with the human body. Due to its small size, Nitric oxide can permeate cell membranes and aqueous solutions, acting as a signaling molecule and performing chemical modifications on certain proteins. It affects the body in several regions: the brain and its ion channels, smooth muscle cells in blood vessel walls, and in targeting inflammation. If *Nitrosomonas* can establish themselves on the skin biome, it is thought that inflammation will be held at bay. Therefore, AOBiome's product development has the goal of sustaining these healthy bacteria colonies.

The point of integrating AOBiome's research and biotechnology base into Mother Dirt products is to establish a "biome-friendly" product formula that correlates to these research findings. The survivability and success of ammonia-oxidizing bacteria in the presence of the Mother Dirt products is used as a test marker to ensure that other equally sensitive microorganisms will be able to thrive as well. The company has developed a patent-pending assay to determine that both the raw ingredients put into its products and the final formula are safe for AOBs. This intent to sustain bacteria also means that no preservatives are used in the product formula due to the detrimental effect they have on beneficial bacteria.

Much like Biossance products, Mother Dirt's merchandise is manufactured to uphold certain standards of skin safety. The products are hypoallergenic, non-irritating, and sensitive-skin friendly, as they lack any fragrance, sulfates, parabens, phthalates, or preservatives. In accordance with its research foundation, AOBiome has conducted clinical tests to gauge the overall effectiveness of the Mother Dirt formula.

The data drawn from the individual conducted studies, each set up on a timeline of four weeks, illustrated improvement in three separate categories: "skin

clarity,” “look and feel of rough and bumpy skin,” and “reduction in shine.” Improvement in skin clarity occurred in 35% of the 28 “problem” skin type study participants. Improvement in the look/feel of rough and bumpy skin occurred in 35% of the 24 “dry” skin type study participants. Lastly, there was a 22% “reduction in shine” for those with an “oily” skin type.

As was mentioned, AOBiome is parent company to Mother Dirt. Therefore, research preceded, or better yet facilitated, the formation of the product. While the skincare products are cosmetic in nature, AOBiome conducts clinical research in treating inflammatory conditions such as acne vulgaris. Such in-depth research enables product development to have a better idea of the function of bacteria on the skin biome and therefore can potentially lead to more effective treatment results, even at the cosmetic level.

## What Do Biossance and Mother Dirt Have to Do with the Average Consumer?

Companies like Biossance and Mother Dirt are pioneering the introduction of biotechnology into the mainstream cosmetics market. The two companies sell products similar in function (moisturizers, facial sprays, the like) but with very different niches. Biossance operates under the pretense that what the body produces is inherently best while Mother Dirt believes in the benefits of external microorganisms on the health of our skin biomes. Despite these marked differences, both cosmetics designers are highlighting nature as a key source of inspiration. Biomimicry has officially entered the beauty world.

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# DARPA and the Future of Synthetic Biology

*Benjamin Wolfson*

Synthetic biology is one of the fastest growing fields in terms of both information and capital generation. In 2012, The World Economic Forum ranked it as the second most important emerging technology of the 21st century; and while it does not explicitly appear on the 2016 list, half of the emerging technologies are enabled by or involve synthetic biology. The number of synthetic biology articles published per year in peer-reviewed journals has doubled since 2010, and the synthetic biology market is experiencing a compound annual growth rate of 24% and is expected to reach \$11.4 billion dollars by 2021.

This rapid growth belies the true state of synthetic biology research. While the majority of US science funding comes from federal organizations such as the National Institutes of Health (NIH) and the National Science Foundation (NSF), the interdisciplinary nature and strong engineering focus of synthetic biology have excluded it from many biomedical funding initiatives, including most NIH funding. The NSF supports research in all nonmedical fields, and is the US's largest funder of basic research. While the NSF invested \$140 million into synthetic biology through the Synthetic Biology Engineering Research Center (SynBERC) over the past 10 years, the program ended in 2016 and has been replaced with the NSF-funded Engineering Biology Research Consortium (EBRC). This group aims to guide the advancement of engineering biology but is not a funding agency. The EBRC will foster the growth of a synthetic biology community and seeks to provide training and define best practices. While these are laudable goals, the EBRC will not fill the hole left by the ending of SynBERC.

In the absence of funding from traditional basic science focused sources, where is the money driving the synthetic biology boom coming from? There are two answers: private capital and the Department of Defense (DOD). There are more than 160 private synthetic biology companies, and since 2009 they have

drawn more than **\$5.4 billion dollars** in private investment venture capital. Private funding is an increasingly important source across most scientific disciplines, but it comes with its own cadre of problems. Private ownership of data means limited sharing, restricting the growth of science as whole. Profit prioritization limits science with no explicit application, and reliance on the markets leads to funding instability. Today, the majority of high-quality, open, basic research is still supported by federal funding. However, while organizations like the NSF primarily fund basic science, not all public funds are without issue. Between 2008 and 2014, the United States invested approximately **\$820 million** in synthetic biology research at both academic and industry institutions, with 67% of that coming from the DOD. Within the DOD, the primary driver of synthetic biology research is the Defense Advanced Research Projects Agency, or DARPA.

One of the primary reasons for the creation of the Department of Defense in 1947 was funding scientific research; as **President Truman** stated when forming the DOD, “No aspect of military preparedness is more important than scientific research.” Since its foundation, the DOD has taken this mission seriously, and DOD research has contributed to the development of the internet, radar, integrated circuits, GPS, wireless mobility connections, and many more of the technologies necessary for modern life.

President Eisenhower founded DARPA in 1957 as the Advanced Research Projects Agency (ARPA), following the Soviet Union’s launch of Sputnik in 1957. He charged ARPA with “preventing technological surprises like Sputnik, and developing innovative, high-risk research ideas that hold the potential for significant technological payoffs” (Beyond Sputnik: U.S. Science Policy in the Twenty-first Century, Neal, Smith and McCormick, 2008). ARPA was designed to undertake high-risk, high-reward basic research and push for transformational changes in lieu of the incremental advances that drive most scientific progress.

While DARPA was providing almost no funding to synthetic biology in 2010, the organization had increased its investment to \$100 million per year by 2014. Funding increases were followed by the creation of the DARPA program Living Foundries: Advanced Tools and Capabilities for Generalizable Platforms, which sought to increase the speed and decrease the cost of generating new production strains of organisms. This program started in 2012, and in 2014, Living Foundries transitioned to a new program, **Living Foundries: 1000 Molecules**, which will invest \$110 million through 2019 to enable facilities to generate organisms capable of producing 1,000 molecules of industrial and defense interests. These funds are going to a diverse group of projects. Some are incredibly broad, such as funding provided to **MIT’s Broad Institute** to advance our ability to assemble large genetic systems. Other projects have a specific, discrete purpose, such as the

DARPA-funded biotech startup *Ginkgo Bioworks*, which is developing probiotics to help prevent common infections for soldiers.

Recognizing the importance of biotechnology, in 2014 the DOD created the Biology Technology Office (BTO), which houses the Living Foundries program as well as numerous others, such as *Battlefield Medicine*, which is developing platforms for creating medicine on demand in the field, and *Safe Genes*, a program to create tools for safe genome editing. While the purview of the BTO is greater than synthetic biology alone, further development of synthetic biology tools will result in the application of synthetic biology tools and techniques in greater numbers of projects. Synthetic biology is one of the most important research fields in the present day, and the DARPA approach to synthetic biology research has proven successful so far: the first iteration of Living Foundries succeeded in increasing the speed of creating production strains of new organisms by 7.5-fold, while decreasing the cost 4-fold.

Despite these successes, DARPA is not an ideal organization to serve as the primary federal funder of synthetic biology research. While in its early days DARPA funded large amounts of diverse basic research, the passage of the *Mansfield Amendment in 1970* prevented the Defense Department from funding any research that “did not have a direct and apparent relationship to a specific military function or operation.” This fundamentally reshaped the US research funding landscape, as projects amounting to approximately 60% of the DOD research budget were either dropped or transferred to other federal agencies.

DARPA sometimes offers competitive grants, but they are directed towards specific projects that DARPA has prioritized and are often issued exclusively to a subset of qualified investigators rather than being open for submission. These competitive grants are offered in conjunction with contracts to private companies for specific research services or investigations toward a specific project. Due to the restrictions of the Mansfield Amendment, researchers often shift their focus or identify potential military applications of their research in order to fit with DARPA’s requirements.

Moreover, it is unlikely that DARPA support of synthetic biology is sustainable. While the fiscal year 2017 appropriations bill increases the budget for DOD research, test, and evaluation activities by 3.6%, this includes a cut to basic research by 1.4%, followed by a proposed cut of 2.1% in President Trump’s 2018 budget. This ignores the fundamental fact that for applied sciences to flourish, basic bench research must also be funded. Without an additional agency providing funds for basic synthetic biology research, applied sciences will likely slow.

These problems are largely the same as those facing the entirety of US science. Decreases in federal support send researchers looking for alternative fund-

ing sources, and while industry support for science research continues to increase, differences in motivation mean it can't replace open, federally funded science. One potential funding avenue is private donation. On the small scale [crowdfunding](#) platforms for research have been created, while on the large scale biomedical fields are waiting to see whether the donations and nonprofit research institutions started by Silicon Valley's latest crop of [billionaire philanthropists](#) will help fill the gaps left by decreased federal support.

At present, the Defense Department obviously sees the value of synthetic biology for the US military. A [recent report](#) from the DOD's Office of Technical Intelligence suggested that grant funding include a requirement for researchers to spend two to four weeks at military laboratories, providing training and guiding research. The OTI also proposed that military academy students spend at least one summer doing research at civilian institutions, and participate in the [International Genetically Engineered Machine competition](#) (iGEM) in order to gain synthetic biology expertise. The OTI recognizes the significant human capital available to them and sees the value in divorcing military science from civilian institutions through creating their own scientific workforce.

While DARPA will continue to be a primary source of federal funding for synthetic biology in the near future, synthetic biology must be able to survive outside the purview of military and private funding. Military research funding is often justified through the principle of dual use, recognizing that any knowledge or product may have both military and civilian purposes. This is frequently realized, but the original intent of a research project shapes its future growth and applications. Researchers must remember the trap that many Manhattan Project scientists fell into. After the creation of the atom bomb, the very scientists who had built it urged President Truman to not use it. They recognized the horror of what they had created, and formed the Federation of American Scientists and the *Bulletin of the Atomic Scientists*, both organizations focused on arms control and [disarmament](#). While researchers often have civilian uses at the forefront of their minds, their discoveries can be repurposed to devastating effect without their consent.

Synthetic biology is growing into an amazing tool that offers great promise in improving lives throughout the world, but it can't fulfill this destiny without funding from a source that can help shape it for the better. The best hope of the field is to follow the path forged by research into computers. Significant gains in computing technology were made in WWII for code breaking and other military applications. However, civilian computer research was progressing quickly prior to the war, and continued during and after with the help of diverse funding sources. While much research into computing technology is now done by private companies, funded by their own revenue stream, they remain accessible to academic and



amateur researchers as well. The current focus of synthetic biology is basic research that is fueling the development of an engineering toolset, which will then be applied in for future in-depth investigations as well as product development. At the present, the best hope is that DARPA funding will create the tools, after which revenue-driven business models will be sufficient to propel synthetic biology investigations, similar to the current state of other engineering disciplines.

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# Bioprinting: From a DIY Revolution to Patients

*Nieves Cubo Mateo*

*Luis Rodríguez-Lorenzo*

Advances in health concern us all. It is important to keep in mind that at the end of research and development, there are patients who need these advances to recover and survive. That's why when a new technology that promises to shorten transplant waiting lists is released, it should reach the entire world. To reach this aim, researchers will have to work together to innovate low-cost and higher throughput technologies.

[Openbioprinting.org](https://openbioprinting.org) does just that. The initiative allows bioprinting developers to discuss, collaborate on, and share pieces, protocols, and research results from anywhere in the world.

Most of the technologies used in bioprinting come from the free movement that emerged after the expiration of the first 3D printers patents ([Figure 6-1](#)). These developments have Creative Commons licenses, in many cases SA (Share-Alike). That's why when we look for information about how to make bioprinters, what we find are several initiatives belonging to the field of DIYbio, where researchers and citizen scientists together develop new methods and materials to be used in bioprinting (not only for medical applications, but also for arts, basic research, and ecology printing with algae and bacteria).

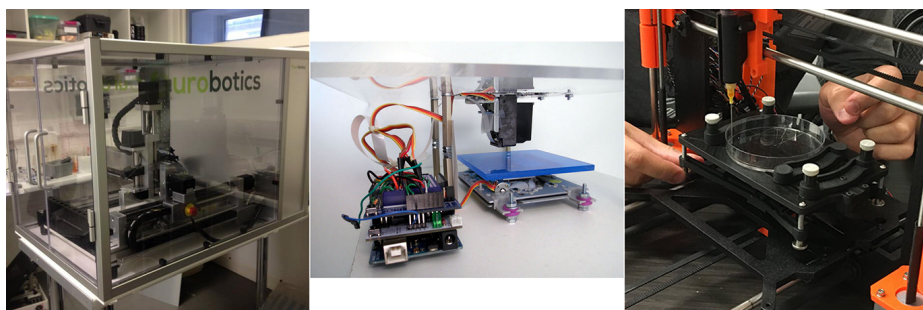


Figure 6-1. Open source bioprinters, from left to right: Ourobotics, bioCURIOUS/Hackteria, and EvanRoche

It's trendy to use terms like DIY (Do It Yourself), or the analog one when referring to a group of people (DIWO, Do It With Others). However, these practices have been done for many years in scientific environments where the necessary machines did not exist or existed but were beyond the reach of researchers for economic reasons.

This is how our project to create human tissue began. Starting with our own 3D designs and taking parts from open source projects, such as the [Printrobot](#), from pages where some people had voluntarily uploaded their designs, like [Thingiverse](#). The first 3D bioprinter we developed was able to print tissues, as human skin, without causing any damage to the cells or to the other biological components used. For more information about this work, please look at the published article on [Biofabrication](#).<sup>1</sup>

These kinds of projects show that it is possible to do an investigation, despite having few resources, when people collaborate and share their knowledge for a common, greater good. When the purpose of an investigation is to improve the life of a patient, everybody has to push in the same direction.

The most important fact about bioprinting is that it allows researchers to build personalized tissues with the patient's own cells, reducing the probability of rejection and enabling a better recovery. With this technology, we are not just replacing a part of the body but regenerating it, so at the end of the process there is no artificial prosthesis inside the patient, but instead human tissues.

Another important issue is that if we are able to emulate these tissues in vitro (at the lab), we could use them as alternative methods for animal testing.

1 Cubo, N., Garcia, M., del Cañizo, J. F., Velasco, D., and Jorcano, J. L. (2016). "3D bioprinting of functional human skin: production and in vivo analysis." *Biofabrication*, 9(1), 015006.

## How to Build a Bioprinter<sup>2</sup>

First of all, we will be working with cells either from human or animal origins, so we will need to adapt normal printers in order to avoid any damage or contamination. The normal 3D printing techniques use high temperatures (from 60 to 300° C) to change the state of the plastic and to build a layer by layer structure in a very controlled pattern. However, we will not be able to handle such temperatures with cells, so new techniques and technologies are required.

Knowing this, these are the basic elements that anyone will need to build a bioprinter (Figure 6-2):

- *A robotic platform* that moves in three dimensions within a certain space. It can be a Cartesian configuration like in 3D printers, or robotic arms like those used in automation processes (SCARA, spherical, polar, etc).
- *A system of pumping/cell deposition*, to infuse cells. It can also be used to deposit other liquid or gel materials. The commonly used systems are peristaltic pumps, piezoelectric systems, and syringe-pumps.
- *A support* that would replace the conventional *head extruder* in the printer, as we do not want to fry cells at temperatures over their survival one, nor contaminate them with the mechanism.
- An extra *conventional head* (optional) to work with biocompatible thermoplastics (PCL, PLA, PGA, etc.) that can be used to reinforce the cell matrices, without damaging the cells with these high temperatures.
- *A disinfection or decontamination system* for items to be reused.
- Single-use (*disposable*) *materials* that will be in direct contact with the cells of each patient.

<sup>2</sup> A complete tutorial will be published on Openbioprinting.org at the OpenLab section.

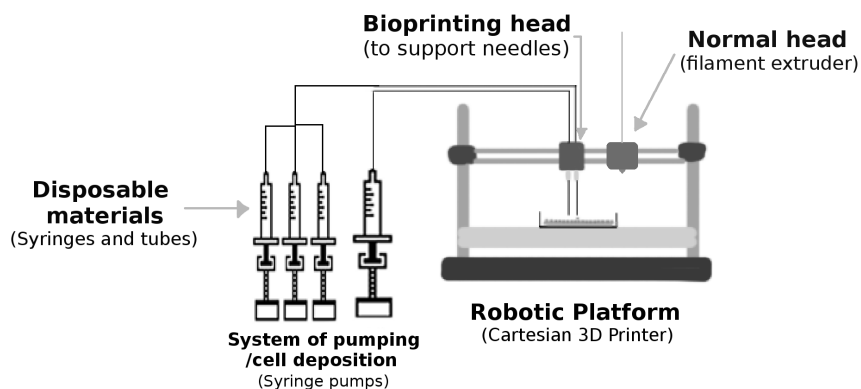


Figure 6-2. Basic elements of a bioprinter. Image reused with modifications from Cubo, 2016.

Again, when working with human cells, we must offer them an environment in which they can live, nourish themselves, and breathe. In addition, when depositing them, there are some factors that can be critical in their viability (survival), such as shear stress, pressure, and other mechanical factors that could damage the cell membrane. In addition, we must work in a sterile environment, so we must avoid elements that can hold bacteria or can generate cross-contamination (from one element to another).

## Synthesis of the Bioprinting Process

In the printing process we normally find three phases: *pre-printing*, *tissue printing*, and *post-printing*. It is very important to understand each of them:

### *Pre-printing*

In this phase the main objective is to multiply the number of available cells obtained from a little biopsy. This is usually the bottleneck of the process, because despite presenting an exponential growth, we start with a very limited number of cells from the patient.

### *Tissue printing*

The deposition of the cells and the biological materials that will form the extracellular matrix as a suitable space to grow and develop. More materials

can be added at this step to improve, for example, the mechanical properties of the scaffold.

### *Post-printing*

Or maturation of the printed tissues to prepare them for their normal function when implanted into the patient. This process has to also be carried out when used as alternative method, as some of the materials used have to degrade to be substituted by a new tissue.

During tissue printing, machines for cell culture (incubator, laminar flow cabinets, etc.) are required in order to culture and grow the cells. At post-printing, we will need also bioreactors, machines that simulate the physiological environment for each kind of cells and that allow the maturation of the tissues for their later introduction into the body. It's important for muscle and bone tissues to be mature; otherwise they could collapse.

## Conclusions

We are in a moment of history full of changes and revolutions at the social level, where citizen participation is increasing, even to fund and to carry out scientific projects. This is the perfect moment to create a more collaborative and scientific environment, to impulse potential technologies that can be used to advance in the field of medicine, as bioprinting, that is one of the technologies that may transform medicine as it is known now.

Bioprinting will become an indispensable tool in the field of personalized medicine, to patients with damaged organs or tissues, as it will allow to regenerate tissues from cells from the own patient. Despite bioprinting is still a developing technology, new materials and methods are being published every day. We are sure that in a few years, the technology will be a routine technique at hospitals.

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# Environmental Sensing with Recycled Materials

*Trevor M. Tomesh*

*Daryl H. Hepting*

Environmental sensing—the process of gathering information from ecological systems—is an essential part of ecology and sustainable agriculture. However, sensors can be expensive and difficult for citizen scientists to obtain, even though their parts are all around us, in the form of technological waste. When a gadget breaks, it is often easier and cheaper to throw it away and purchase a new one than to attempt to repair it. Citizen scientists can take advantage of this unfortunate by-product of “throw away culture” by harvesting the sensor technology that is often found in e-waste. In this article, we discuss an approach to the development of such sensors.

When assessing and addressing environmental issues, especially at a local level, it is more advantageous to involve community members—those who are directly affected by such issues—than scientists and academics. Such an approach has been found to be both faster and more efficient, as dedication amongst local volunteers has been found to be much higher than those with little attachment or stake in the success of the project (Danielsen et al. 2010, 1166–1168). However, oftentimes there is very little funding and resources available to citizen scientists and thus necessitates support from non-local institutes.

There are a number of projects that purport to address exactly this issue. However as we traverse this technological landscape of citizen sensing, although the economic climate is shifting toward affordability, mass distribution of these devices (in detector arrays, for example) is still quite out of the scope of many budgets.

According to Sui and Elwood in “Crowdsourcing Geographic Knowledge”, there exists four levels of participation in citizen science activities. The majority of the projects outlined here fall within the first two levels of engagement; however, this should not be interpreted as an inability for citizen scientists to participate in environmental sensing projects at higher levels. While there are many ecological sensing projects worthy of examination, to list them all would be well outside the scope of this paper; therefore only a select few are outlined (Figure 7-1).

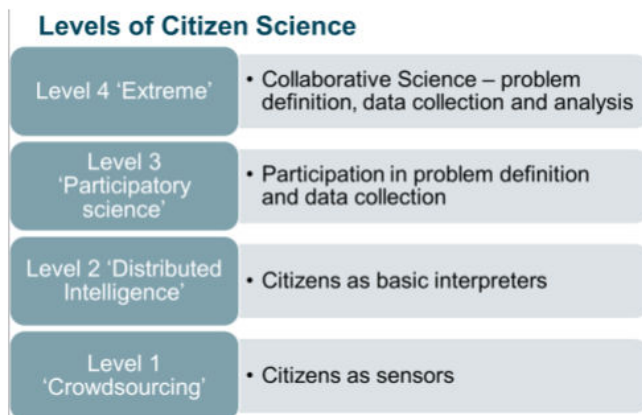


Figure 7-1. Sui and Elwood propose 4 levels of involvement in citizen scientists ranging from “passive sensors” to “active collaborators” (Sui and Elwood, 2013)

## Smart Citizen

One of the most polished options for citizen-scientist environmental sensing is the **Smart Citizen** project. The Smart Citizen Kit is billed as “an Open-Source Environmental Monitoring Platform consisting of arduino-compatible hardware, data visualization web API, and mobile app” (Smart Citizen 2014). It is the result of a crowdfunding effort on Kickstarter by Fab Lab Barcelona at the Institute for Advanced Architecture of Catalonia. The sensor board can measure air composition (CO and NO<sub>2</sub>), temperature, light intensity, sound levels, and humidity (Figure 7-2). It is capable of communicating data wirelessly to iOS devices via the Smart Citizen App. The kit itself consists of three boards: the ambient board, which houses the sensors; a data-processing board based on an ATmega32u4; and a Baseboard with USB socket, SD card reader, EEPROM, battery holder, and clock (The Smart Citizen Kit: Crowdsourced Environmental Monitoring 2014). The

Smart Citizen Kit places a particular emphasis on large-scale collaboration. Users can register their sensor board on the Smart Citizen website and communicate their local conditions over the web. The result is an international “sensornet” that is openly available for anyone to make use of (Smart Citizen 2014). There are a number of shortcomings with the Smart Citizen, however, especially the price tag — the Smart Citizen kickstarter edition sells for 105 USD unassembled. This sensor would be considered a one or two on Sui and Elwood’s engagement scale because, currently, it only affords citizens the role of data collector and reporter.

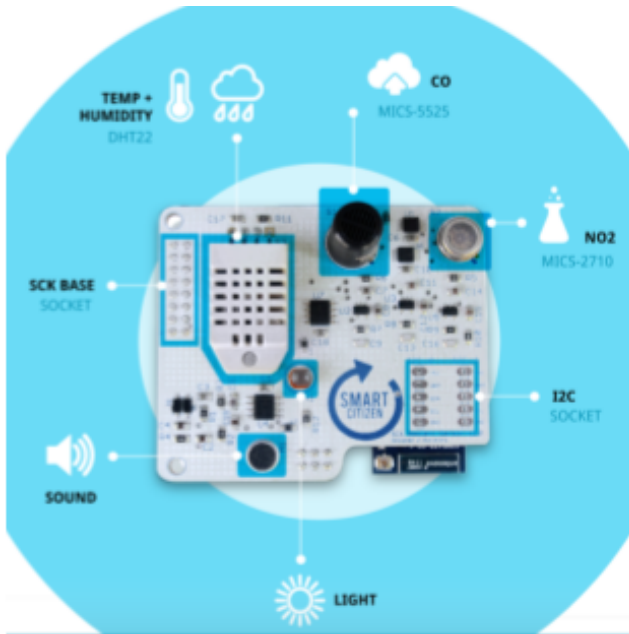


Figure 7-2. Smart Citizen sensor board (*The Smart Citizen Kit: Crowdsourced Environmental Monitoring*, 2014)

## GardenBot

According to the [gardenbot.org website](http://gardenbot.org), GardenBot “is an open source garden monitoring system” (Frueh 2014). However, GardenBot is more than just a “garden monitoring system”; it is a comprehensive how-to on small-scale environmental monitoring. The bot itself is composed of an Arduino Uno as the “brain,” an LM335 temperature sensor, a small photocell (for monitoring light), and a

moisture sensor made of galvanized wire. Furthermore, the system incorporates a water valve as the control for an automated watering system that responds to soil moisture levels. The system, despite being quite simple, has been featured on [wired.com](http://wired.com), [treehugger.com](http://treehugger.com), [OpenSourceEcology](http://OpenSourceEcology), and [SparkFun.com](http://SparkFun.com) (Figure 7-3).



*Figure 7-3. Close-up of a part of the gardenbot (Ganapati, 2010)*

There are, however, a number of notable drawbacks to this system. Most notably is the fact that it must be connected to a PC. The system works on PC-Arduino serial communication and therefore requires a dedicated PC to run and thus does not allow for the sort of portability that one might hope from an environmental monitoring system. Moreover, because the system incorporates an Arduino board and a number of other components that must be purchased, the setup is not particularly scalable on a tight budget (an Arduino board alone is upwards of 40 USD). Given the rather involved nature of constructing the device—there is no option to order it pre-assembled—any citizen scientist working with it must have a fair bit of aptitude. Therefore, the GardenBot rises above the first and possibly the second level of engagement to level three “participatory science,” at least for the principal stages.

## Growerbot (formerly garduino)

The **Growerbot**, another Arduino-based garden monitor, is billed as a “gamified gardening assistant”. The Growerbot is equipped with a soil probe, a temperature/humidity sensor, and a TSL2561 luminosity sensor. It is also WiFi-enabled with an Electric Imp connectivity platform for connecting to the Internet of Things, thus giving the Growerbot the capacity to share data both locally and over the web. The standard kit also contains an LCD interface and a light/pump controller. The final system is a handsome and well-polished device which is ready to be crowd-sourced (**Figure 7-4**).



Figure 7-4. **Growerbot**—polished, yet expensive

## The Problem of Electronic Waste

While purchasing commercially available tools for environmental sensing is an attractive option, introducing new technological mass into circulation would be a self-defeating exercise.

In 2012 alone, the average Canadian generated 24.72 kg of electronic waste. This is a staggering fact, given that the average Canadian consumed 28.59 kg of electronic products that same year (StEP 2014). In a year, we dispose of 86% of the electronics we purchase. Many of these items, if not disposed of properly, pose environmental and health hazards. Cathode ray tubes, for example, are known to leach heavy metals such as lead into groundwater. Gold-plated components such as IC's discharge hydrocarbons and bromide into bodies of water, turning them acidic and killing fish. Plastics from electronics housings emit dioxins and hydrocarbons (Wath et al. 2011). In industrialized countries, these problems are well-managed with recycling and reclamation programs.

However, a good deal of electronic waste is exported to developing countries where there is little infrastructure to deal with it, and so those people are often forced to live alongside hazardous waste (Grossman 2006).

The irony of producing e-waste to develop environmental sensors is not lost on the researcher and it is, therefore, the philosophy of this project—so much as possible—that no new materials should be produced to develop it.

## Environmental Sensor Development

The intended final application of this project is an agricultural sensor grid spaced throughout a local crop field. To begin with, we consider three important growing variables for most crops: the amount of light received, the local temperature, and the local soil moisture (i.e., conductivity). Therefore, three separate sensors must be developed: a light-meter, a thermometer, and a conductivity sensor.

### Light Meter

A new light meter is around \$6 (Adafruit Industries 2014). While this is perfectly sensible for a single meter, scaling into the hundreds gets expensive. However, electronic waste is rife with photoresistors, which can be used as rudimentary luminosity sensors (Figure 7-5). However, a reading from a photoresistor will not be in the standard measurement of light (lumens), and we therefore must calibrate any photoresistor that we use for this purpose. Calibration can be accom-

plished by measuring the voltage across a photoresistor, as a light source provides different levels of light, and then comparing that value to a calibrated light meter (Figure 7-6). Because lab-grade light meters are not readily available, a simple solution is to use the light meter that most smartphone devices have. Using a three-level desk lamp as a light source and [Sensor Logger by i-RealitySoft](#) on a Samsung Galaxy Note 2 smartphone, we calibrated a simple photoresistor circuit to respond with acceptable accuracy. To do so, we plotted the average luminosity against the reading from our photoresistor, and we found a nearly linear relationship of  $\text{lux} = 5.3(\text{lx}/(\text{unit}/3\text{v})) * (\text{resistor}) - 2401.5 \text{ lx}$  with an  $R^2$  of 0.97 (Figure 7-7).

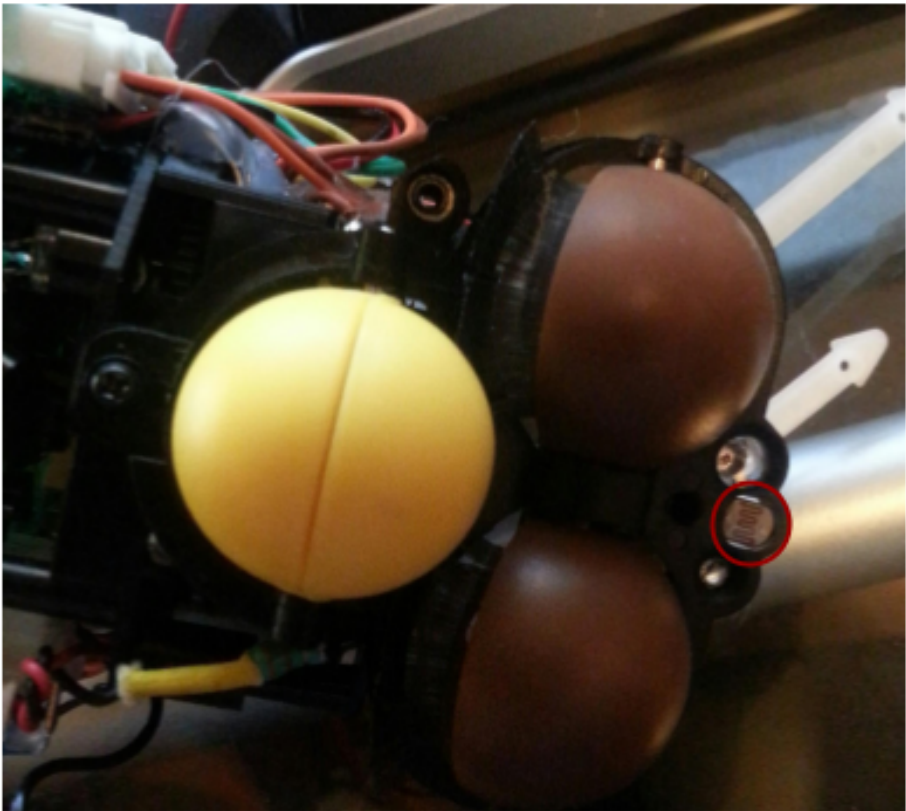


Figure 7-5. A deceased Furby with a photoresistor exposed (circled)



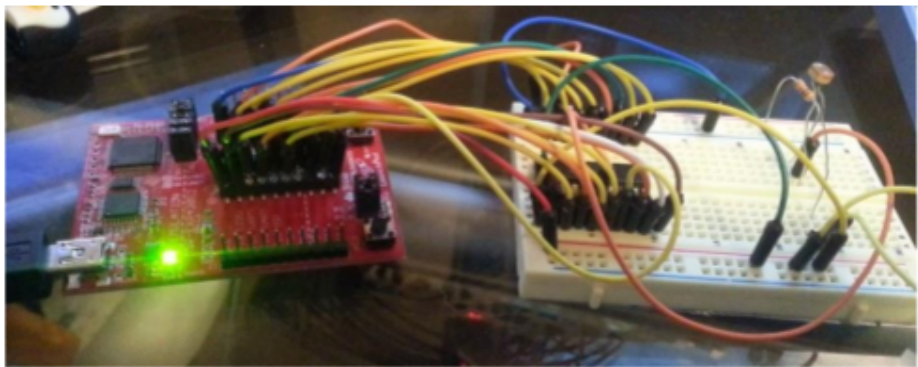


Figure 7-6. Set-up for calibrating the photoresistor. A 10k pull-down resistor provides a steady ground and an MSP430 reads the voltage values.

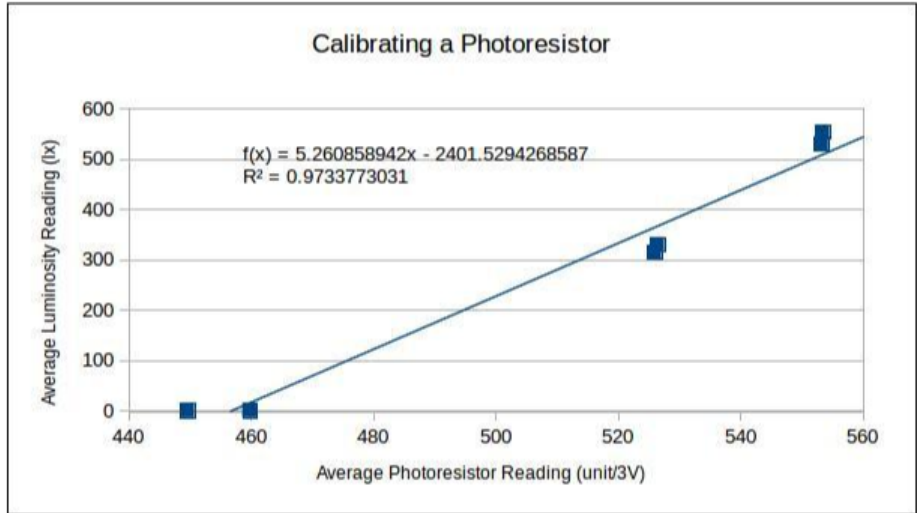


Figure 7-7. Plotting the average LUX reading against the average photoresistor reading gives us a near linear relationship ( $R^2 = 0.97$ ) for the few light conditions tested. We find that:  $\text{lux} = 5.3 (\text{lx}/(\text{unit}/3\text{v})) * (\text{resistor}) - 2401.5 \text{ lx}$

## Temperature Sensor

The MSP430G2553 microcontroller unit which serves as the “brain” of this project contains an internal temperature sensor that can be easily read via either a serial connection or an attached display. These chips are very common both in industrial applications and in commercial ones such as children’s toys. (Four-Three-Oh!



2012) Before attempting to utilize the internal temperature sensor, an exact idea of how accurate it is must be determined.

The procedure for cross-checking the temperature was simple: a digital house thermometer was placed next to the MSP430 Launchpad Development board, which was in turn connected via serial over USB to a computer where the temperature was read out. Randomly over a week the temperatures read by both devices were recorded and compared (Figure 7-8).

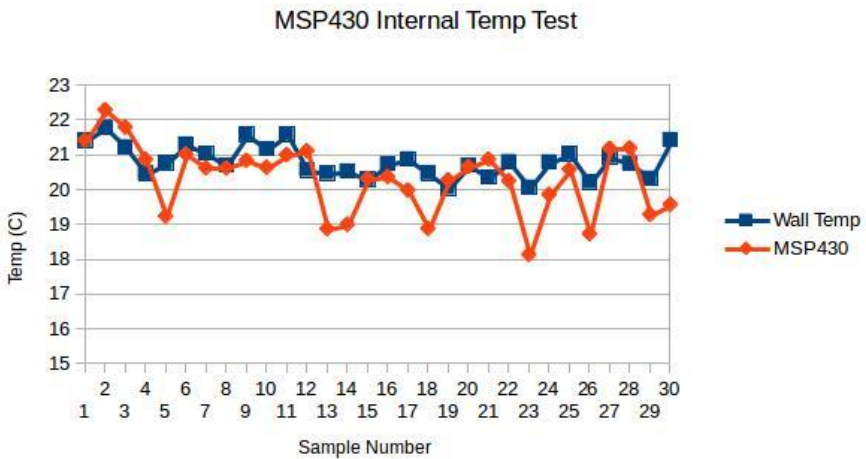


Figure 7-8. Determining the accuracy of the internal temperature sensor of the MSP430G2553

Over 30 samples recorded during the week, the average percent error was approximately 2.4%. Although this is certainly not lab-quality scientific accuracy, as a makeshift environmental sensor, this small bit of seemingly systematic error is quite acceptable.

## Soil Moisture Sensor

To determine the moisture of soil, a pair of galvanized nails are soldered to a pair of wires, with one lead connected to +3.3 V and the other connected via a 10K pull-down resistor and to an analog input on the MSP430. The simple idea behind this setup is that as moisture is added to the soil, it becomes more conductive and thus there is less and less resistance between the sensor nail and the voltage source nail. To test this, three quarters of a cup of potting soil was placed in a cup with the nails spaced a few centimeters apart, and water was added in one-eighth

cup increments until the soil was saturated (Figure 7-9). The values were recorded over the serial port and plotted to examine what constitutes “underwatering” and “overwatering”. It was found that in completely dry soil, the reading is nearly zero. Once the first 1/8 cup of water is added, that value changes quickly to be around 600. Another 1/8 cup brings the value up to a little over 650, and it appears to max out at around 700, whereby the soil is saturated with water (Figure 7-10).



Figure 7-9. Testing the soil moisture sensor

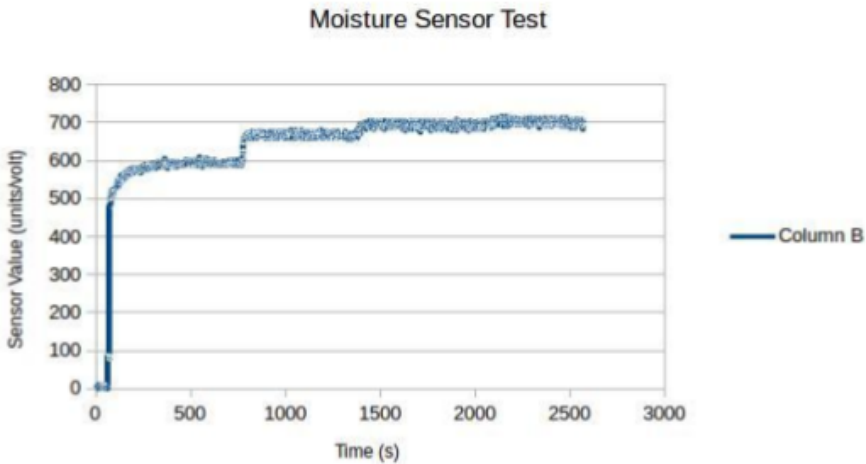


Figure 7-10. A soil moisture reading is taken to determine what sorts of values correspond with dry, under-watered, properly watered, and over-watered plants

## Demonstrator Build

With the sensors (mostly) working, a final build to enclose the sensors was constructed. The components were removed from the breadboard and soldered directly to a small prototyping PCB. A recycled pill bottle was chosen as a housing, and a 3 V coin-cell battery installed. Holes for the moisture sensor probes and the light sensor were drilled on the bottom and top, respectively. Hot glue served to water-tighten the ports for the sensors ([Figure 7-11](#)).



*Figure 7-11. The final build is ultra portable and extremely inexpensive (the MCU was the only nonrecycled part, but was free)*

While it is yet to be tested, the final build is meant to be watertight and weather-resistant, with the ability to survive an entire season in a field or garden. Afterward, the capsule can be extracted and the chip removed from the device. The data periodically stored to the nonvolatile flash memory within the MSP430 can then be collected and interpreted. It is intended that the final product will fall within the first level of citizen science involvement, allowing volunteers to passively collect data (literally burying the equipment in the ground and forgetting about it) without needing to concern themselves with the broader work ([Figure 7-12](#)).



Figure 7-12. The final build displaying the internal circuitry

## Conclusion

Here we described building a sensor system constructed entirely of electronic waste as a way for citizen scientists to participate in mass sensing without spending much money.

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**Daryl Hepting** is an associate professor in the Department of Computer Science and an associate member of the Department of Film at the University of Regina. His research is focused on the development of tools to help individuals deal with and navigate complex information spaces, in application areas as diverse as environmental decision support, eyewitness identification, and multimedia composition. He is a senior member of the ACM and member of IFIP Working Groups 2.13 (Open Source Software) and 5.11 (Computers and Environment).

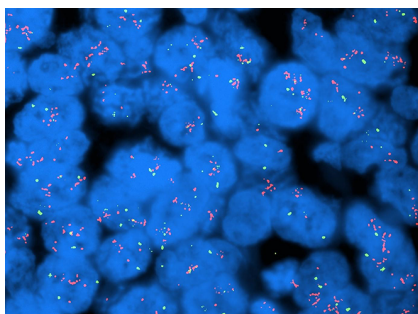
# Time Machine for Cancer Diagnosis!

*Enrico Di Oto, Ph.D*

One of the most important improvements in oncology was the introduction of target therapy. It allowed clinicians to prescribe a pharmacological treatment specifically programmed to fight and kill only cancer cells, unlike wide-spectrum chemotherapy (Slamon D.J. et al. 2001). To define a patient as eligible to receive target therapy, it is necessary to define the genetic profile of the cancer cells. Among the techniques that have been developed in the past 20–30 years, one of the most used and considered the gold standard is in situ hybridization. This technique is based on the principle of the specificity of the DNA sequences and uses genetic probes to recognize a specific gene, chromosome, or part of them. This enables labs to see if there are numerical alterations, such as multiple copies of the genes; a reduction in the genes' copies; or structural alterations such as deletions, inversions, or rearrangements; and then, to emit a report. For example, in breast cancer it is important to define the number of the HER2 gene to assess the eligibility for the dedicated therapy; or in brain cancer where the loss of some parts of chromosome 1 and chromosome 19 is related to a specific cancer type (Slamon D.J. et al 2001; Barbashina V. et al 2005). Today it is common to label these DNA probes with fluorescent dyes. **Figure 8-1** shows one example of fluorescent in situ hybridization (FISH): the red and green dots are respectively two different genes. The blue big bodies are the cell nuclei.

Following the last WHO guidelines for cancer diagnosis, the molecular characterization of an individual's cancer cells is becoming mandatory, and it is fundamental to do a complete and accurate diagnosis. The main problem is that these tests require at least two or three working days and are costly. The wait reduces the lab's capability to quickly emit a genetic report and also reduces the number of tests that could be performed daily. The delay in diagnosis is not only a problem for the hospital administration in planning patients' followup but is also related to

an increasing amount of anxiety disorders among cancer patients (Baqutayan SMS 2012). This limit of the FISH and the general ISH techniques started to become relevant once the new therapeutic targeted drugs for specific genetic assets were enlisted among the first-line treatments being prescribed to patients after their first biopsy. Some examples of cancer treatment based on the ISH techniques results include the use of an antibody called Trastuzumab for the breast cancers with the amplification of the HER2 gene, or another antibody called Crizotinib, specific for a lung cancer type characterized by ALK gene genetic alterations (Voegel et al. 2002; Solomon B.J et al. 2014)



*Figure 8-1. FISH test: red indicates the test gene; green, the control gene; blue bodies, the cell nuclei*

## What Can Technology Do to Fix This Problem?

In the last 10 years, different approaches were used to reduce the time it takes to get a diagnosis and its cost. One of them is a combination of engineering and biology: automate the diagnostic instead of performing it manually. Automation will allow for tests to be run rapidly and with high accuracy. It will also reduce the cost of each test. However, there are still some limitations:

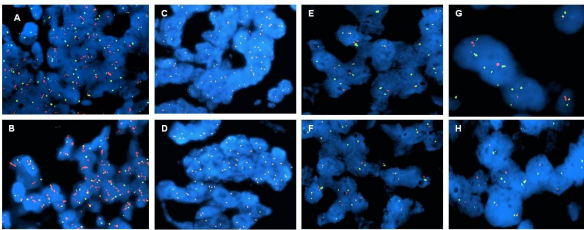
1. The time required for a test procedure is about six to eight hours, even if automated, so it will still take two days to receive the report.
2. The equipment is costly.
3. Lab protocols will have to change to accommodate automation

4. Labs will have to find a way to archive data properly such that the data are compatible with the readouts from the new equipment.

The other solutions proposed in the past four years are chemical-based (Dako-IQ FISH, and Abbott Intelli FISH, for example). Most of them reduce the test time to at least four hours (or more) but have the same problems (e.g., high costs and incompatibility with lab reagents and procedures).

Is it possible to have a fast test without disrupting lab procedures?

Some second generations of these chemical solutions are available. One of the solutions we developed, Rapid ISH integra, is on the market and allows labs to maintain their reagents while reducing the test time to only 2 hours and the cost by up to 50%. The ISH techniques consist in a labeled DNA fragment that match its complementary fragment in the DNA of the sample. We can empower these techniques because our solution act by increasing the efficiency of the DNA interactions. To give you an idea let me make a parallelism among a DNA “meeting” and a meeting between friends: The standard DNA-DNA hybridization act as the same as meeting a friend in a pub: you’re at the same table, but because of background noise, it’s harder to pay attention to each other. With our products, we bring the meeting to a dedicated and isolated room in a library: no noise, no distraction, great efficiency. Our reagents increase the efficiency of the DNA-DNA interactions while reducing the “noise” in the background and therefore the competition for the specific DNA regions in the sample making the test faster and more cost effective. **Figure 8-2** compares the standard FISH test and fast tests with Rapid ISH integra A-B HER2 gene (red dots) and chromosome 17 centromeric region (green dots) in breast cancer; C-D Alk gene (red dots) and eml4 gene (green dots) in lung cancer; E-F chromosome region 1p (red dots) and 1q (green dots) in brain cancer; G-H chromosome region 19q (red dots) and 19p (green dots) in brain cancer. These markers are important both for the diagnosis and the therapy assignment.



*Figure 8-2. FISH test with standard technique (A,C,E, and G) and with Rapid ISH Integra (B,D,F, andH)*

In cancer diagnosis and research, the ISH techniques are not the only ones used but are part of an arsenal of techniques both DNA-based ( generically “genomics” ) and protein- based. Thanks to the universal applicability on DNA of our chemical mixtures, we are working to improve the “genomics” so that labs can perform more tests more quickly. We have also developed, thanks to participation in the RebelBio accelerator program, a new chemical-based technology that will improve the efficiency of the protein tests.

Currently the technology we developed is used both in human and in veterinary pathology to improve the efficiency of the ISH tests. In the coming months we will start with global distribution due to the worldwide need for faster diagnostics and solutions. Most of the technology, both in genomics and in informatics, is working to provide us quick and easy data and solutions. We are working to make the technology better by combining our knowledge and passion and by cooperating with universities and companies. We strongly believe in this cooperative approach to bring the science to the next level. Just as the ‘70s informatic revolution that gave us the personal computer, we want to make this technology accessible to all the cancer patients around the world. Making the test more cost-effective allows labs in developing economies to adopt our technology and improve both the quality of care and opportunities to grow their scientific knowledge. By making the test faster, we enable labs to increase their productivity (more tests run in parallel ) and their patients’ treatments and quality of life.

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# Google, Venture Capital, and BioPharma

*Neal Ungerleider*

Biotech is a different world from Silicon Valley's tech scene, and one with its own ways and traditions. Where Silicon Valley has a reputation for being brash and anarchistic, biotech developments are perceived—even if it's inaccurate—as being bound by regulation and a cat's cradle of connections with academia, big pharma, and government. But they have one thing in common: an overlapping venture capital community ready to fund the next big thing, be it cloud computing or CRISPR.

And, for many biology startups, there's something unexpected: Google is there.

GV, formerly and popularly known as Google Ventures, has been making a major push into biopharma and life sciences. The venture capital arm of Google holding company Alphabet has investments in many of biopharma's best known names, including Editas, 23andMe, Grail, and Denali Therapeutics.

The fund's life science and health division includes dozens of other firms, including Foundation Medicine, Cambridge Epigenetix, Metabiota, and Oscar.

For Alphabet and Google, pharmaceuticals and biology occupy the convergence of two intersecting trends—a rise in massive data sets used to develop drugs and treatments, and the ability to turn a profit by backing potentially world-changing technologies in their early stages. Venture capital for biopharma has traditionally been dominated by pharma company funds such as Pfizer Venture Investments, Amgen Ventures, and GSK's.

GV is part of a wave of tech-centric VC firms like Andreessen Horowitz, Kleiner Perkins Caufield & Byers, and Venrock Associates that are joining the party.

Speaking by phone, GV general partner Krishna Yeshwant credits his firm's involvement in biopharma and life sciences to a rise in potentially transformative products both on the market and in development.

"At a high level, GV is set up as a fund where Alphabet is given a mandate to invest profitably," Yeshwant explains. "When GV was founded in 2009, some people were surprised to see us involved in therapeutics—externally, it can be a little confusing. We always look at Alphabet as less search engine, and more about new tactical insights and tech that comes to the top and is transformative."

This could take the form inside the firm's life science and health division of Editas, the publicly traded gene editing firm; Impossible, which makes vegan hamburgers that "bleed" like real beef; or Flatiron, which develops data platforms for oncology.

One example of GV's involvement comes from the experiences of Denali Therapeutics. The Bay Area firm is working on treatments for neurodegenerative conditions such as Alzheimer's, Parkinson's, ALS, and frontotemporal dementia. Denali focuses on drug discovery and development, and shares DNA with Genentech. The company's executives are predominantly Genentech veterans, and Denali's office is just down the street from Genentech's corporate headquarters in South San Francisco.

Denali launched in 2015 with a massive \$217 million first-round venture financing. The company had a subsequent \$130 million funding round; GV is an investor in the company, alongside the Alaska Permanent Fund, ARCH Venture Partners, Baillie Gifford, Flagship Pioneering, and F-Prime Capital Partners.

Steve Krognos, Denali's CFO, characterizes GV's investment as a "passive investment." In Denali's case, GV does not have a board seat or act in formal advice-giving capacity. However, Krognos says, "As part of the Google Ventures umbrella, a number of services are expended to us. We can access resources Google Ventures has—for instance, the benefit of working with companies under the Google Ventures umbrella to do things like providing advice on websites, and more technical IP-related subjects."

Yeshwant confirms this: one big advantage biopharma and life sciences companies in the GV portfolio can leverage is expertise with design, recruiting, marketing, and data work.

The bulk of GV's investment in the bio/life sciences space consists of minority investments in a wide range of firms rather than concentrating on a few high-risk, marquee investments. As Yeshwant puts it, "We try to be helpful in the relationship, and to be careful about keeping a distance from places that are confusing to Google or Alphabet." It's a more hands-off approach to life sciences invest-

ing where portfolio firms have access to Alphabet's resources but largely avoid more interventionist forms of investor oversight.

*Speaking with Business Insider* (Ramsey, 2017), Yeshwant identified seven key areas which GV is focusing on: new tools for physician burnout, adherence, neurology, learning healthcare systems, inflammatory conditions, infectious diseases, and medical devices

Biopharma is also an area of increasing monetary and strategic interest for GV—as well as Silicon Valley in general. According to a *June 2017 report* by CB Insights (2017), GV has more than doubled its pharmaceuticals investments in each of the last two years. In 2017 alone, Google Ventures has invested first-time deals in Fulcrum Therapeutics (gene therapy), Arsanis (anti-bacterials), Spero Therapeutics (anti-bacterials), and BlackThorn Therapeutics (neurobehavioral disorders).

Before departing GV to start his own fund, Google Ventures founder and first CEO Bill Maris wrote in a blog post that *life science and health* are GV's biggest area of investment (Maris, 2015), contributing to nearly 31% of investment. In late 2016, *Maris reportedly walked away* from starting a new \$230 million healthcare fund.

Maris previously outlined his—and GV's—*eight key areas of focus in the life sciences*: Machine learning and artificial intelligence, understanding the brain, reinventing antibiotics, battling cancer with immunotherapy and pharmacogenomics, genetic repair with tools like CRISPR, the microbiome, organ generation, and stem cell technology.

GV's interest is paralleled by that of other firms. *Writing for Andreessen Horowitz's blog* (Conde, 2017), the venture capital firm's bio fund general partner Jorge Conde noted the existence of a sort of Moore's law of genomics:

*To me what's most fascinating for entrepreneurs and startups is that we've gone from a 'single lens' view of biology where the focus was on genomics (the A, C, T, G code of DNA) to where we can now look at biology via multiple lenses... That is, various biological signals—DNA, RNA expression levels, proteomics—in a more multi-dimensional and high-throughput way. We can integrate all these different lenses together to get a much clearer picture of what's happening from a disease biology standpoint.*

Of course, Alphabet also has a longstanding interest in the life sciences, healthcare, and some of biopharma's more far-out frontiers. Alphabet's life sciences division, Verily, says its mission is to “make the world's health data useful so

that people enjoy healthier lives.” By Alphabet’s estimation, this includes developing tools for making sense of health data, and building platforms and interventions based on those insights.

While the bulk of Verily’s on-record projects are in the healthtech space rather than biopharma, the company is experimenting with both biological initiatives and pharmaceutical projects. For instance, Verily is partnering with Biogen and Brigham and Women’s Hospital on a multiple sclerosis observational initiative, and is working with the National Institutes of Health on a precision medicine initiative. Another company under the Alphabet umbrella, Calico, is working on life-extension tools, and includes molecular biology, genetics, and computational biology experts.

This bounces back to a recurring theme in GV’s investments: the fact that massive data sets, machine learning, and pattern recognition are becoming an integral part of modern biotech and drug development. While precision medicine is arguably the best known expression of that, the rise of massive data sets and cloud computing has created opportunities for Google, even as cloud computing has expanded to all ends of pharma.

According to Yeshwant, when looking for companies to invest in, GV looks for what he calls “transformative” factors ranging from new biological toolsets like CRISPR to innovative approaches to cancer, neurology, and pain. The fact that all of these intervention approaches require mass quantities of data is just icing on the cake.

Denali’s Krognès notes that GV is especially interested in the intersection of healthcare and data. “For Denali,” he adds, “we select our targets—the genes we go after—to modify disease based on a proven link to the diseases in question like Alzheimer’s and Parkinson’s. The identification of this link is something done through technology, and data analysis.” For instance, Denali tracks biomarkers in population subsegments into responders and uses that as a therapeutics guide, which takes up a mass amount of computing power.

Interventions such as innovative neurological and brain treatments create an overlap for tech companies such as Alphabet, venture capital firms with tech experience such as GV, and life sciences firms like Denali. Whether the drug development world likes it or not, their fate is tied to the tech sector.

This also ties into a larger trend in the biotech and pharma VC worlds: the ongoing symbiotic relationship between Silicon Valley and Boston’s venture capital communities. Thanks to the local academic community and research institutions like Brigham and Women’s Hospital (where Yeshwant is also a practicing clinician), Boston has traditionally held an edge in life sciences. However, the Bay Area has largely been where the money is.

Much like other venture capital firms from the tech community developing bio funds of their own, GV focuses on making sense of what can be a very different landscape both in terms of business and regulation. A scrappy tech startup can bootstrap its way toward Series A funding with just an Amazon Web Services subscription, a business plan, a rich relative to offer a loan, and a handful of early-stage employees willing to work 70-hour weeks. However, launching pharma and bio startups require both years of education and access to existing institutional knowledge that can't be easily replicated.

Understanding the regulatory landscape is another challenge for venture capital firms entering the life sciences. One of the great advantages of health-oriented VC funds is an understanding of the arcane details of the drug approval pipeline that can often puzzle outsiders. Despite the fact that GV is backed by one of the world's largest companies and has amazingly talented experts working on its health investments, the dynamics are often a long way off from conventional tech investing.

However, companies exiting GV investment also offer opportunities for the firm to turn a tidy profit going forward. For instance, Yeshwant has a board seat on Foundation Medicine, a molecular information company specializing in cancer care, which went public in 2013. Pharmaceutical firm Roche has majority control of Foundation, and GV previously had a 4% stake.

Yeshwant himself took a more circular path to pharma and life sciences. He comes from a computer science background, and before joining GV helped found two companies that were acquired by Hewlett-Packard and Symantec. He decided to go to medical school after his father became ill; while working on his MD, he came aboard at Google Ventures through connections from the tech world. Yeshwant then found himself in what he describes as an “unusual environment where I was basically an intern and investing millions as an intern.”

When asked how he views life sciences and biopharma investment as a whole, Yeshwant takes the long view: he notes that investment in life sciences is cyclical and that GV is one participant in a much larger ecosystem of venture funds that fulfill different roles.

“Lots of tech investors have naivete about healthcare,” Yeshwant adds. “There’s a huge regulatory burden and lots of negatives, but for us it’s so critical to someone’s life and the bar is so high.” Cycling back to GV’s twin pillars of looking for new biological innovations and looking for market invitations that make those innovations fit into patient care, he also notes that development and building up new companies takes time.

Unless unforeseen factors change things, 2017 and 2018 are likely to continue the same pattern of aggressive growth in the biopharma venture capital

world. GV's role, essentially, is to leverage Alphabet/Google's expertise and use it to make money by supporting smaller life sciences firms that have a decent shot at making it big. Other venture funds will keep on fulfilling other roles in the ecosystem, ranging from accelerating the R&D pipeline for big pharmaceutical firms to pushing products especially reliant on machine learning or massive data sets. One thing's for sure: we haven't seen the last mega-funding round for a biopharma company.

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# DUO: Connecting the Home to the Hospital

*Meghan Tahbaz*

Detection of heart sounds in the early 1800s and before was limited to direct contact between the physician's ear and the patient's chest. Auscultation changed markedly in 1816 with René Laënnec's invention of the stethoscope, though the wooden tube prototype was far from the bi-aural apparatus of modern medical practice. Throughout the years, the stethoscope has witnessed minor adjustments to improve material quality and ease of use; however, the fundamental design has remained largely the same. Biotech startup Eko addresses this stagnancy with a digital take on a tool utilized by over 30 million clinicians around the world.

Like many pioneers, Berkeley-based company Eko began with a question: if there is a technical gap in cardiology, how can it be resolved? Its answer came in the form of a digital stethoscope known as CORE, which transmits heart sound data straight to a clinician's compatible device. While CORE represented an unprecedented improvement in auscultation, according to cofounder and COO Jason Bellet, the company's newest device provides an equally, if not more, powerful tool.

Its latest product, DUO, aids those suffering from heart disease by combining the function of a digital stethoscope and an electrocardiogram into a minimalistic, handheld device operable by patients and doctors alike. DUO enables physicians to gain insight into advanced patient cardiovascular data while helping those prescribed the device to monitor personal well-being, directly from their own homes.

Eko was founded in 2013 by three UC Berkeley affiliates, Connor Landgraf, Jason Bellet, and Tyler Crouch. Landgraf, then a master's student in Berkeley's bioengineering program, was inspired by a UC San Francisco presentation highlighting the inefficiency of the stethoscope, arguably one of the most used tools in the medical industry. "The fundamental premise for the company was to bring

the stethoscope, this icon of medicine ... used every day for the frontlines of cardiovascular screening, to bring that tool into the digital age,” says Bellet.

According to the Centers for Disease Control and Prevention, heart disease is the leading cause of death in America, killing 610,000 and adding up to \$26 billion in healthcare expenditures.

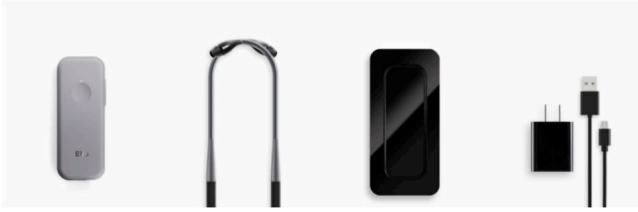
“When you look at some of the statistics, 50% of [cardiovascular] patients will get readmitted to the hospital within six months, 25% of them will get readmitted within 30 days,” Bellet says. “If we can help cut down some of those readmissions, that has both a tremendous impact on patient care, as well as on healthcare economics.”

Aside from death due to cancer, specifically concerning malignant neoplasms, heart disease causes a staggering number of deaths, surpassing most other medical concerns, such as chronic lower respiratory diseases and stroke. Eko believes that empowering patients with DUO will combat this cardiovascular epidemic by providing doctors with live patient data that comes in the form of easily accessible digital updates.

DUO demonstrates an intriguing concept in innovation—combining two entirely distinct, but equally utilized medical tools into one simple apparatus. It is the first FDA-cleared device to incorporate both a digital stethoscope and electrocardiogram, transmitting data on both heart sounds and electrical cardiac output. The device is equipped with two stainless steel electrodes that comprise the ECG portion and a digital diaphragm to detect heart sounds. The design is minimalistic in look, with only a power button on the front surface and an LED light ring to indicate when it is in use. The simple structure is meant to simplify patient and clinician use.

“It doesn’t look like a traditional stethoscope,” says Bellet. “It looks like something that patients can use pretty intuitively for their own monitoring at home under the prescription of a physician.”

Aside from the unique apparatus, DUO’s appeal is in the facile connection that it provides between the home and the hospital. Patients suffering from heart disease can be prescribed DUO as a part of their healthcare regimen. This allows them to track their cardiac function and wirelessly provide medical information to their physicians. The tool “fits in the palm of either the physician’s hand or the patient’s hand... [and] streams that data back to Eko’s HIPAA compliant software,” Bellet explains. This type of technology alters the traditional outpatient experience and has the intention of giving cardiac patients the consistent attention they require.



*Figure 10-1. From left to right: DUO device, included earpiece, wireless charging pad, charging cable, and USB adapter (Source: Eko)*

Bellet describes the product as “affecting healthcare primarily by being prescribed to patients with congestive heart failure or a chronic cardiac condition as a means for them to be able to monitor and capture recordings of their heart at home and stream it back to their physician.” DUO “bridges the gap in care between what they receive in the hospital and the lack of care they traditionally receive at home.”

Eko’s pioneering pathway goes beyond producing unique physical technology and extends further to software programs. The company has constructed the necessary software to digitize and synch real-time cardiac waveforms and acoustics to several platforms (Windows, iOS, and Android). Any data collected by DUO can be added to patient records, giving doctors the ability to not only instantly annotate but also replay any transmitted recordings. Eko provides a system of data encryption that is HIPPA-compliant, ensuring patient confidentiality while allowing for second opinions from physicians who can be manually connected via the app. An additional feature of the product is the ability for it to be included in telemedicine. The beauty of telemedicine is, again, the connectivity it furnishes between the home and the hospital—and connectivity is Eko’s goal.

“Medical technology is becoming more connected in terms of not only capturing a vital sign recording but taking that data and pushing it into the health record and making it possible for the physicians to analyze more effectively,” Bellet says. “If we can take devices and make them connected devices such that the data they are capturing is sent directly to the physician, is pushed into the health record, and is analyzed potentially by tools like the one that Eko is building, it’ll make them much more impactful, both in the clinic and at home.”

The latter analysis that Bellet is referring to is Eko’s upcoming project, the development of algorithms to analyze cardiac data. Ultimately, it would like to increase the scope of available analytics by releasing machine-learning algorithms as “physician support tools” that alert doctors of potential declines in cardiac function. These tools “will be focused on actual analytics of the [heart] sounds themselves.”



Figure 10-2. The application of the DUO in bedside care (Source: Eko)

Until the complete development and release of Eko's software, the most innovative use of the company's product lies in the collection and dissemination of patient data. To better clarify the process of syncing cardiovascular waveforms to a smart device, the following is a basic summary of the steps taken during a live-stream of data:

1. Download the Eko app for smartphone or tablet, and log in using secure credentials.
2. Power on the Eko device (DUO or CORE), and navigate through the Bluetooth menu in the Eko app to search for the device connection.
3. Connect the device to the app, and automatically sync real-time cardiovascular waveforms.
4. To begin a stream of cardiovascular data, enter the live stream menu, and select the streaming option.
5. Every account is given a unique streaming link, which can be sent to through the app to any desired recipient.
6. Streaming can occur at the same time as a HIPAA-compliant video conference session for use in telemedicine.

DUO is a device for cardiovascular treatment. However, the idea of taking highly utilized medical tools, combining them, and bringing them forward to meet the demands of the 21st century can be applied in any health specialization.

"Looking holistically at the market, I think the one thing that's worth noticing is how inefficient the status quo is," comments Bellet. The team at Eko was able to

see the stethoscope as “ripe for innovation,” a perfect candidate for a technological upgrade. Other medical tools may have the same potential for advancement, specifically regarding connectivity, if only there are innovators who can envision such progress.

While the doctor’s office will most likely remain the focal point of patient care, with products such as DUO, the home is quickly becoming a practical space for patient data sharing and advanced medical monitoring.

Eko will begin shipping DUO to clinicians across the country in fall 2017. For more information, visit <https://ekodevices.com>.

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# Clinical Trial? There's an App for That

*Neal Ungerleider*

When the Cleveland Clinic was looking for participants in its more than 130 cancer trials, its outreach teams didn't just cold-call doctors and hospitals. They tried something new: they launched an app. The [Cancer Trial App](#) is designed for two distinct populations: patients looking for clinical trials and doctors who are treating those patients. Users who download the app either on iOS or Android receive information on trials by disease, phase, physician, and hospital location. In addition, the app details each trial's objective, eligibility rules, and progress.

The Cleveland Clinic's app is a simple solution to a complex problem: how to make clinical trials easier for individuals to participate in. Because of a lack of vendors, complex regulatory tools, and institutional inertia, many of the digital workflow and recordkeeping tools that are commonplace in other parts of biology never made it to clinical trials.

According to [Premier Research](#), a life sciences consulting firm, only several hundred of the more than 150,000 mobile health applications published as of December 2016 focus on clinical trials. And of those, most are directory apps like the Cleveland Clinic's, rather than more complicated apps that enhance the patient experience.

However, innovation is happening in the clinical trial mobile app space—even if it's taking longer than expected. In the United States, the Food & Drug Administration is working with stakeholders in clinical trials to examine ways smartphone apps can enable speedier, more cost-effective clinical trials that speed up the new drug approval pipeline. The FDA has a public docket out on [“Using Technologies and Innovative Methods to Conduct FDA-Regulated Clinical Investigations of Investigational Drugs”](#) that seeks to create consensus.

## The Challenges

Designing apps for cancer studies and automating patient data is far more complicated developmentally and legally than creating a new smartphone game, which is why development has been slow.

Stakeholders need to manage the following factors:

1. The cost of app development, which is a challenge when many pharmaceutical companies don't have robust, in-house Android and iOS teams and may not know what questions to ask outside contractors to keep costs down
2. Privacy and quality expectations in randomized clinical trial research
3. Regulatory issues
4. Inside the clinical trial world, a culture that prioritizes written documentation and written paperwork over digital recordkeeping, at least when interacting directly with patients

There are several ways of dealing with these challenges. Additionally, new innovations such as Apple's [ResearchKit](#) and [CareKit](#) open source frameworks break down the barriers that prevent researchers, pharma companies, and others from building mobile apps that enhance the patient experience in clinical trials.

In [promotional materials aimed at researchers](#), Apple emphasizes how easy these apps are to use: "Perform(ing) activities using the advanced sensors in iPhone to generate incredibly precise data wherever you are, providing a source of information that's more objective than ever before."

## Helping Researchers with Trials

The potential of smartphone apps for easing patient experience during the research and trial process has fascinated clinicians.

[No 483 For Me!](#) ([Figure 11-1](#)) is an iOS app developed by William Tobia, a lead clinical research instructor at GlaxoSmithKline (GSK). It's designed for clinical research site staff and aims to sharply reduce FDA inspection findings by training them on what to look for, thus enhancing the patient experience.



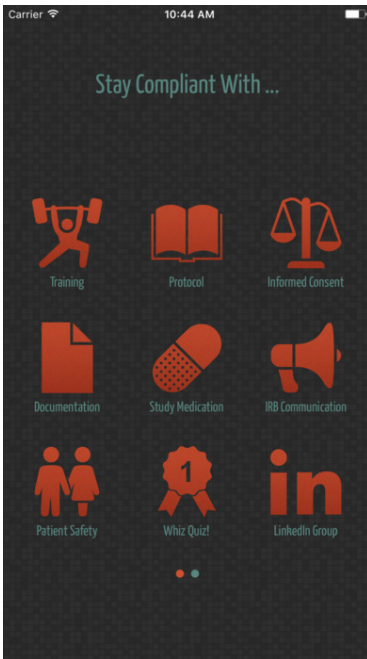


Figure 11-1. No 483 For Me! (Screenshot by Neal Ungerleider.)

In an email interview, Tobia said that “compliance with the protocol and FDA regulations is a priority in clinical research but, for some staff, it is difficult to locate the applicable regulations. The purpose of this app is to make it faster and easier to find the information they need on the FDA’s website.”

Once users open the app, they click on an icon corresponding to their area of interest and see the regulations—never having to search on the FDA’s sprawling website for them.

No 483 For Me! also contains information about and quizzes on noncompliance.

## Styluses and Tablets

**Clinical Ink** (Figure 11-2) is a Massachusetts-based company whose product, Sure-Source, is a tablet-centric software suite designed for entering data via stylus during clinical trials—something the company says provides cleaner data faster and can eliminate paper documents.

“The stylus works with tablet, and is meant to emulate –it’s really for the ability to collect data at site,” says Ron Quinn, Clinical Ink’s vice president of marketing. “It emulates the paper and pen process. You can enable styluses to be touch enabled, to type or use the stylus to make a mark, and it records. Once you ink something, it’s permanent.”

According to one case study published on Clinical Ink’s website, the app **also has capabilities** for remote monitoring, video uploads, image capture, and push-button randomization.

EST-123123123 | Switch User | Logged in: Ed Segura Coordinator

Visit List | Med Hx | AE | ConMed | Visit 1 Screen

Screening Number: 0049  
Visit 1 Screening and Enrollment  
Visit 1 General Procedures Form

Visit Information ☐ Transcribed

Visit Date: 30 / Nov / 2016 30

Informed Consent ☐ Transcribed

☒ Yes ☐ No Has the subject signed the Informed Consent?

Signed date: 30 / Nov / 2016 30 Signed time: 09 : 57

Version date: 30 / Nov / 2016 30

☒ Yes ☐ No Was Informed Consent/HEPPA signed prior to any study procedures being performed?

☒ Yes ☐ No Was a signed copy of the Informed Consent/HEPPA given to the subject for his/her records?

☒ Yes ☐ No Did the subject have all of their ICF/HEPPA questions answered to their satisfaction?

Notes: subject read ICF at home which was mailed to her prior to the screening visit.

Steel 01 / Full Study 1 - 5 V 1.1.1604 (Date: 2016-05-08)

Figure 11-2. Clinical Ink (Credit: Clinical Ink, used with permission.)

Clinical Ink's product strategy ties into a fact of clinical research: clinicians tend to prefer pen and paper and time-tested workflows for data entry rather than new methods, even when they could enhance patient experience. In sales materials, Clinical Ink emphasizes its product sharply cuts down on transcription and data entry time and costs.

Due to factors including regulatory considerations, lack of time to train on new methods, a preference for pen and paper, and cost considerations, tablets and smartphone apps aren't used on a regular basis in clinical trials. Clinical Ink hopes to change this, and hopes that their product has enough selling points to convince researchers and pharma companies of its worth.

This means overcoming existing worries about using tablets in clinical research. According to a recent [white paper](#) published by Clinical Ink, a survey of 517 research coordinators and investigators using the company's product found that key difficulty points for sponsors and on-site personnel dealing with electronic data capturing (EDC) include transcription time, having to verify transcribed data is correct and completing paper sources, and updating sources and responding to queries. Systems like Clinical Ink are designed to reduce the time and expense involved.

## Smartphones and Parkinson's

One of the most audacious attempts to leverage smartphones for patient-oriented clinical trial innovations comes from mPower. A massive smartphone-based patient study involving more than 9,000 participants, [mPower](#) ([Figure 11-3](#)) uses an app to monitor and understand the causes of variations in symptoms of Parkinson disease.

What makes the study especially fascinating is how it leverages the sensors inside iPhones and optional wearable device data to collect 24-7 information.

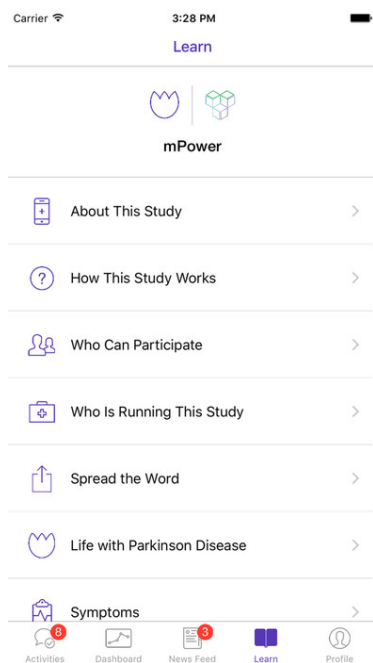


Figure 11-3. mPower (Screenshot by Neal Ungerleider.)

According to a 2016 [Science Data report on mPower](#) (Bot et al. 2016), the study takes a highly untraditional approach to recruitment and onboarding. The app itself is the primary recruitment mechanism and uses what lead author Bot calls a “novel remote approach to enrollment” where participants—both those with Parkinsonian conditions and a non-Parkinson’s control group anyone can join—self-guide themselves through the consent process before opting in to join the study. The consent process also includes an explicit decision point requesting if data participants donate to the study can also be used for secondary research purposes.

Sage Bionetworks, the nonprofit biomedical research organization that developed mPower, says that the study lets users track symptoms through fitness tracker and game-like activities, including finger tapping, a memory game, speaking, and walking. In early results, Sage found distinct correlations between intake of medication and participants’ Parkinson symptoms which potentially could help researchers personalize treatment plans and care. New windows of intervention could be developed as well.

The study is one of the first of its kind and includes massive open source data repositories such as a [developer portal](#) and a large [presence on GitHub](#).

## Science 37 and Smartphone-based Studies

Another approach comes from **Science 37** (Figure 11-4), a Los Angeles-based firm whose platform NORA (Network Oriented Research Assistant) is designed for telemedicine participation in clinical trials through smartphones or computers. By using NORA for clinical studies, sponsors are able to reach trial participants who live in rural areas, work during daytime hours, or otherwise could not take part in a conventional trial.

As part of Science 37's business model, sponsors pay for iPhones, which are then provided to trial participants in order to input data. Kelly Chu, Science 37's chief technology officer, notes that participants can input info through form entries, text messaging, video chats, or whatever other communication method suits both them and the trial stakeholders.

"People gravitate to video chat, and seeing who is on the other end," Chu says. "They don't see us in a brick-and-mortar setting." He calls messaging as opposed to video chat "lower friction" and adds that it benefits patients when no visual data needs to be given and they may feel uncomfortable on camera. His company sees telemedicine as a way to reduce the traditional withdrawal rate of 30% over a three-year-long clinical study.

Science 37 is well funded. The company raised an initial \$31 million in Series A and Series B funding, and raised another \$29 million in early 2017. Glynn Capital Management led the latest funding round, and other investors include Amgen Ventures and Sanofi-Genzyme BioVentures. Sanofi has an agreement with the company to look at improving recruitment and reducing trial times.

Noah Craft, Science 37's cofounder and CEO, describes his company as "one-third tech and two-thirds clinical." This means creating a platform which steers information to researchers and sponsors as quickly as possible while using behavioral tools to make sure patients stay engaged in trials. As he puts it, "Even though you can ping a patient twice a day to ask if they have taken their medications, people don't like being pinged twice a day by anybody."

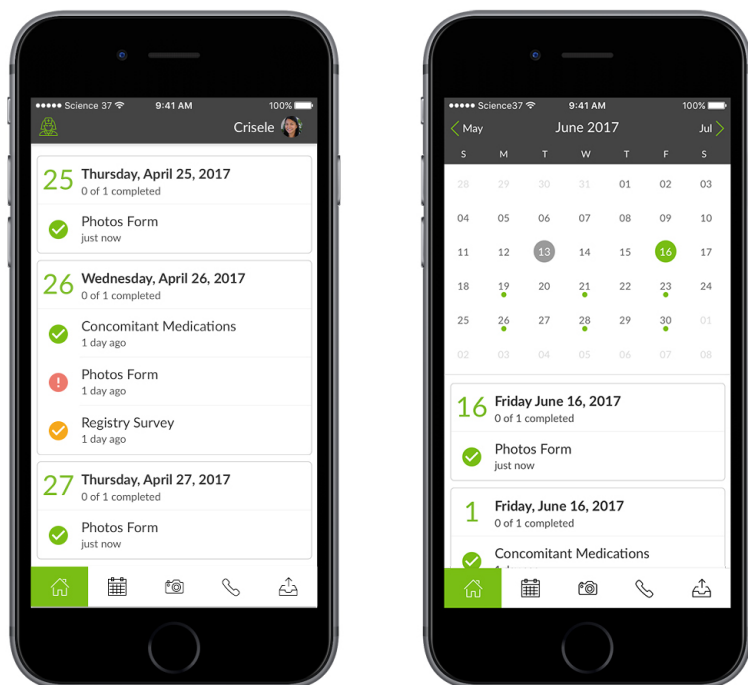


Figure 11-4. Science 37 (Credit: Science 37, used with permission.)

## Into the Future

Smartphone- and tablet-based, patient-facing technologies hold great potential for improving clinical trials and creating a better patient experience. But despite big steps forward like ResearchKit and Patient Kit, changes won't happen overnight.

Tobia is optimistic—he says mobile health technologies can better engage patients and help them understand clinical research options with healthcare providers and have an easier time participating.

However, Craft notes that there are big regulatory and development factors at play. As he puts it, “Regulations around authenticating and identifying who a patient is and their signature is when someone is signing something” are just the beginning. From a tech perspective, Craft says, companies like Science 37 use processes similar to banking that include multifactor authentication, and multilevel

email address sign-in that are primarily designed for patient safety and that privately and securely identify who patients are.

While the interfaces for these apps are left up to the designer, they—and rightfully so—have to live up to electronic signature guidance dictated by the FDA and other stakeholders.

It also fits into a larger and ongoing problem in the world of biology and pharma: The shift from paper-based recordkeeping to electronic medical records, electronic data sets and massive, multi-use databases. Stakeholders have to make sure they're collecting the exact same data digitally as they would normally be collecting with paper, which is not the easiest thing to do.

Large-scale studies like mPower are ongoing as well; and as Apple improves their platforms, it's inevitable that other influential industry players will try smartphone app, mass population studies. The challenge will be reconciling the expectations of patients who play high-tech smartphone games, and use communication apps like Facebook, with the design and functionality required by regulations.

Phone and tablet apps also hold the potential to work in tandem with other workflow and patient-experience improvement tools in the clinical trial world. For instance, **Cancer Commons** is a massive nonprofit data crowdsourcing project designed for any patient to send in a question about their cancer type, stage, or treatment and quickly receive an answer from a physician. Science 37's efforts are also paralleled by other companies like **Global Care Clinical Trials**, which maintains a network of clinicians who can conduct at-home visits with patients in clinical trials, which opens opportunities for trial participation.

In the end, it's all about the patients and how technology can improve the trial experience for them. Because mobile apps can reduce the timeframe for new products to make it to market, make the clinical trial process easier, and even help patients better understand their own conditions, they hold enormous potential. As the life sciences world becomes more comfortable with mobile apps, they're going to become increasingly commonplace. And while they might be a novelty today, smartphones and tablets are going to become a much more prominent part of clinical trials.

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**Neal Ungerleider** (@nealunger) is a Los Angeles-based writer and journalist. His work appears in *Fast Company*, the *Los Angeles Times* op-ed section, *Wired*, *Slate*, and many other venues. Neal's honest, dorky hope is that mobile apps and better patient-facing technology will decrease the time for lifesaving innovations to make it to the general public.





# HVMN's Better-Body Biohacking

*Meghan Tahbaz*

Technology is unique in the fact that its improvement provides an intuitive next step. Products are refined, updated, and necessarily upgraded at any given time. Optimization is never viewed as a bonus in the tech industry; it's the name of the game.

But what happens when we attempt to expand optimization goals to include the very facilitators of progress: our minds? Crossing the boundary between hard science and pseudoscience, biohacking companies are exploring the principle of “upgrading” the human body in the hopes that our inherited genetics are more malleable than we think. One such company is HVMN (pronounced “human”). The company’s main product is NOOTROBOX, a line of nootropics or colloquially-dubbed “smart drugs” meant to enhance neural performance in areas such as memory, learning, and focus.

HVMN was founded by two Stanford alumni, Geoffrey Woo and Michael Brandt, both of whom have a background in computer science. While computer science may not seem directly tied to the biology behind nootropics, Woo takes an unconventional approach to formulating his products in which he sees a parallel between self-hacking and computer engineering.

“I look at biohacking, this sort of niche community if you will, as the home brew club of computer hacking. The original home brew computer hackers really changed how people live today. I see the same thing happening with biohacking” Woo says. “You have all these interesting hobbyists and hackers tinkering with biology, tinkering with human performance, measuring biometrics, optimizing for them with interesting input and our goal with HVMN is to be an anchor company within an ecosystem.”

Nootropics are by no means a new concept, given that the term was coined in 1972 by Corneliu E. Giurgea, a Romanian chemist/psychologist. The word itself

has Greek origins and literally translates to “mind turning” (“noos tropē”). These dietary supplements fall under the broader category of stimulants, drugs as widely available as caffeine or as abused as Adderall. HVMN’s products form their own niche in the market as they lack the casual consumption associated with coffee but also don’t require the prescriptions necessary to obtain over-the-counter drugs like Adderall and Ritalin. With advertising that pushes for hyperactivity (“Go write a book. Go run a marathon. Go ace that test...”), HVMN wants to expand human capabilities or at least push the idea that with the help of science, people can pursue all of their passions.

Woo’s idea for HVMN originated from his personal interest in self-optimization. He ordered specific bulk powders online from different research laboratories in the hopes that he could begin creating his own nootropic concoctions. But after a point he had a well-founded concern. “You kind of realize that maybe this is a little bit crazy in the sense of safety” Woo explains.

Taking the leap between personal experimentation and safe product formulation, HVMN was formed based on the desire to be “as quantitative as possible,” a concept that inevitably originated from the co-founders’ engineering backgrounds. Every product stack undergoes a safety analysis to determine product purity, microbial presence, and ingredient content.

These analyses are available at the following link: <https://github.com/Nootrobox/COAs>.

The proposed science behind HVMN’s products (denoted as “stacks” due to the presence of several nootropics in each formulation) is grounded in three main principles:

1. Supplying neurons with the precursors to selected neurotransmitters and building block molecules that promote neuron membrane health.
2. Accelerating neuron growth and protecting the brain from oxidative stress.
3. Promoting acute performance in specific categories such as sleep, focus, and recovery.

HVMN supports their products (Figure 12-1) with scientific publications that intend to validate the success of their marketed formulas. The effects of major ingredients such as caffeine and L-theanine have been investigated and are shown below in (Figure 12-2). One stack, RISE, is noted to have increased verbal learning rates by 12.28% (measured by “how quickly participants were able to memorize a list of words”) and decreased mental fatigue by 15.38% (measured by a “custom

fatigue index”). Each stack is associated with certain cognitive goals and the company encourages long-term use of their products to maximize results.

In terms of product formulation, HVMN strives to be transparent with providing information on active ingredients. RISE, for example, is primarily a combination of  $\alpha$ -GPC, Bacopa Monnieri, and Rhodiola Rosea. The latter ingredients are derived from perennial plants used in traditional South and East Asian cultures while the former is a precursor to acetylcholine, a neurotransmitter required for memory, cognition, and motor control.



Figure 12-1. NOOTROBOX selection of four products (Source: HVMN)

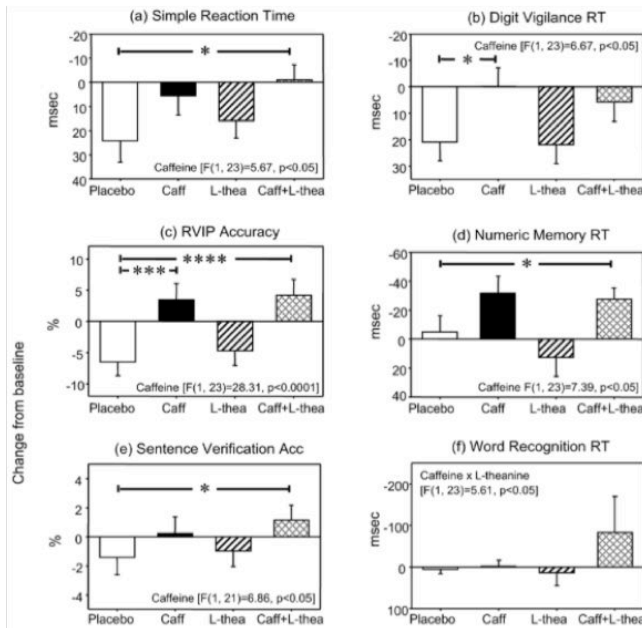


Figure 12-2. Psychological effects of placebo, caffeine, L-theanine, and a combination of caffeine and L-theanine (Source: HVMN)

HVMN's second product line is equally concerned with “hacking” the human body, this time with the help of the world's most consumed drug: caffeine (Figure 12-3). GO CUBES are a fusion of 50 mg pure caffeine and select nootropics, a potent mixture that is advertised as “4 hours of focus and energy” per cube consumed. The combination of one part caffeine to two parts L-Theanine reportedly produces a “synergistic effect on cognition” that optimizes performance gains. L-Theanine is a non-dietary amino acid associated with reducing stress perception and improving attention. It is primarily a relaxant and therefore is thought to reduce the jitteriness typically associated with caffeine.



Figure 12-3. GO CUBES product design (Source: HVMN)

Woo describes the process behind HVMN's product formulation as “looking at a few different sources of inspiration. What are hobbyists talking about that is interesting? And then the second inspiration point is what does the clinical research say?...Then of course lastly we make sure everything that's in our products is FDA regarded safe. We're not putting anything into our products that the FDA is going to have any problems with.”

With its products, HVMN is contributing its own unique take on health-consciousness to an already booming wellness industry. The company presents a new perspective on where health-based innovations should be targeted, or rather in what ways health itself can be addressed. It has established itself as a biohacking hub meant to produce both a series of products for optimization of the human body and to inspire consumers to develop self-hacking knowledge and skills.

“We started tapping into the broader cultural shift around how people have thought about human performance and their own health” Woo says. “Biohacking to me is empowering the individual to really take control of their own health information and be active about it. I think in classic health care it's very much sick care

where people don't feel empowered, they don't know what to do with their health, whereas biohacking is very active. You are part of manipulating yourself."

While the effectiveness, and even ethics, of cognitive enhancers is controversial, HVMN's general vision for human improvement as well as the resources it provides to further this agenda marks an interesting point in industry where people are beginning to be looked at as candidates for scientific development not out of necessity but out of luxury.

"The way I look at it is that humanity has always been manipulating our environment or creating tools to better manipulate our environment. I see this as the next step. The next level of tools that people are making are about making ourselves more efficient."

For more information on clinical studies and the effectiveness of the supplements, please visit <https://hvmn.com/biohacker-guide/nootropics/summary-of-nootropic-compound-effects>.

## References

<https://hvmn.com>

<https://www.trubrain.com/pages/science>

<https://nootroo.com/about>

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