Project Summary / Abstract

Sensing and smart systems can help us improve the quality of life within cities if properly deployed within different built environment scales. Sensing in cities could help us better understand citizen's needs, patterns and their interaction with the environment. This would allow us to create cities that fulfill citizen needs while maintaining a better equilibrium with the environment. Biosensors deployed at the scale of cities or public buildings could help us to tap into new types of information or into new ways of collecting information that can already be collected through physical sensors. This proposal presents (1) the development of a bio sensor meant to act as a bio microphone based on synthetically engineered living cell systems and (2) the design and validation of a low cost, open source, portable and modular incubator for bio sensor testing and deployment.

Relevance

Exploring and understanding ways in which we can integrate bio-sensing into current sensing and city Internet of Things (IoT) infrastructure. The first part of this project consists of the design and evaluation of modified sound sensitive E. coli cells. The cells are used to test gene expression induced by exposure of cells to specific audio wave frequencies and amplitudes. Secondly, we design and test a novel low-cost and portable bio reactor for allowing deployment of live cell experiments and bio sensors outside of the wet lab.

Specific Aims

Development of a specific sound intensity and frequency sensing system based on synthetically engineered E. coli cells. The system has the long term goal of enabling the monitoring of sound pollution in a privacy preserving way at different scales within cities. The cells will report exposure to specific sound wave frequencies and amplitudes through fluorescence by means of synthetically engineered putative genes. A low cost, modular, bio reactor is developed for being able to test and deploy the system outside of the wet lab.

Aim 1: Implementation of Bio Microphone Design and testing of an environmental sound pollution bio-sensor.

1.1. Sound responsive bacterial growth. Testing and characterizing sound stimulated bacterial growth as proven in [1, 2, 3].

1.2. Sound responsive gene expression. Using putative promoters to construct gene circuits that are capable of expressing genes according to exposure to sound. Circuits will use optic promoters to allow us to evaluate results and determine linearity of the system.

1.3. Bio - Digital Interface. Design of low cost optical or capacitive equipment and sensing instruments that can transfer and translate information obtained from the bio sensor to a digital device.

Aim 2: Taking sensors and experiments outside of the lab Design and testing of low cost, open source modular experiment and deployment bio reactor.

2.1. Agar plate E.coli culture. Growing E. coli colonies with agar plate.

2.2. Agar plate E. coli culture and sound experiments. Testing Aim 1.2. using the bio-reactor as deployment and experimental tool.

2.3. Live culture with microfluidics. Building device module that enables it to be used for live cell cultures, expanding its capabilities from agar based cultures.

Aim 3: Wireless city scale sensor networks. Researching possibilities and challenges for wireless bio-sensor nodes for better urban development.

3.1. Vision outline. Outline of how large scale deployments could look like, what type of new information could they bring and how to connect them to current IoT infrastructure.

3.2. Obstacle description. Narration of technical hurdles that need to be overcome for the systems to be a reality and exposure of possible research avenues that could solve them.

3.3. Societal and environmental impact. Description of societal and environmental impacts of large scale distribution of bio-sensing systems for cities. Outline of benefits, possible negative impacts and safe deployment strategies.

Significance

Advances in bio-sensing at the scale of the city have shown promising alternatives to understanding new ways in which we can instrument our cities [4]. These novel approaches have the potential to help us better understanding citizen's needs, behavioral patterns and their interaction with the environment [5]. Novel research has shown that it is possible to understand complex social dynamics within cities through the use bio-sensing systems [6, 7, 8]. Biology based systems could offer interesting tools that our current sensing infrastructure lacks, helping us to detect previously undetected phenomena. Furthermore, bio-sensing could improve our detection capabilities for electronically instrumented systems in ways that make them more effective and privacy preserving.

The internet of bio-nano things is an emerging field that aims to develop new applications for sensing enabled by biology [9]. Current publications suggest that the application of biology based sensors can complement our current electro mechanical systems in order to further develop the capabilities that we currently have to understand the complexity of cities and the ways that the urban environment impacts the natural environment [10, 11, 9, 12, 13].

The goal of the proposed research is to prove the feasibility of bio based sensing systems that can be deployed outside of a wet lab setting. This research represents an intersection of bio-sensing, behavioral recognition, living labs and the internet of things. From bio-sensing we take the tecniques and tools to engineer living systems that can . Behavioral recognition then allows us to contextualize sensor readings as they interact with human activities. The concept of living labs pushes us to develop systems that can be tested in real life scenarios, meaning that the sensors must be able to function outside of the wet lab. Finally, the internet of things serves as inspiration to think of the broader developments that need to emerge around communication and bio-digital interfaces so that bio sensing can be fully integrated with our current IoT infrastructure.

Innovation

Understanding sound pollution within the urban context has been of great intrest for researchers over the last decades. Approaches that use different internet of things architectures have been developed, investigators have developed custom electronics and have also appropriated smartphones [14, 15, 16]. The problem with these systems is that they rely on the use of microphones for collecting audio data. The use of microphones introduces great privacy concerns if this systems are to be adopted at large scales.

Furthermore, researchers have also used cameras and microphones to analyze and predict human behavior within smaller scales like office buildings and homes [17, 18, 19]. These techniques can introduce great benefit by actively understanding and changing the environment in which citizens spend time in as to make it more healthy or pleasant. Privacy and surveillance concerns are even more relevant at this scale.

The bio microphone serves as an alternative for understanding the soundscape of an environment without the need of recording, saving and processing granular acoustic information. The bio microphone uses sound stimulated gene expression (upward and downward regulation), these expression can be reported through specific proteins and captured through optics. The information would allow us to understand exposure of bacteria to specific frequencies and amplitudes of interest but it would not allow anyone to access the granular information from the device. This bio sensing device would be a means to encrypt information through the mechanism used to sense as opposed to protecting it after it has been extracted, therefore enhancing our current physical sensor capabilities. In the future, bio microphones could be connected to bio materials and the creation of more complex genetic circuits could allow for seamless sensing and actuating at the built environment scale.

Live cell sensors are not easy to deploy or test outside of the lab. These systems require specialized equipment that can give appropriate conditions of growth to the cells. For city bio sensing to become a reality, we must develop devices that are able to be deployed outside of wet labs at a low cost in a reliable manner. In order to do that we propose a low cost, modular incubator design that can allow for deployment and testing of live cell sensors outside of a wet lab. The system would complement capabilities being developed through the use of cell free systems. Ideally, freeze dried cell free systems could be used to monitor the correct performance and safe operation of live cell systems deployed outside of labs.

Approach

0.1 Implementation of Bio Microphone

Research has shown that sound plays an important function in regulating biological pathways and gene expression in multiple organisms [20, 21, 22, 23, 3]. Natural gene expression changes induced by sound have been studied in order to understand the impact of sound waves on the tomato ripening cycle [24], and on razor clams digging behavior [25]. In addition, chimeric genes have been synthesize to control gene activation through sound on rice plant cells [26], hamster ovary cells [27] and multiple bacterial cells [28, 29].

Using the above knowledge, our aim is to create a system that can be deployed within public spaces around a city, the system, based on modified and live E. coli cells, will interact with various sounds at specific amplitudes and frequencies, as the cells interact with sound, genes will be up regulated and down regulated. Gene regulation will result in lost or gain of fluorescence which will be analyzed using optical instrumentation. The final result will be knowledge of the soundscape that surrounds the system in a privacy persevering manner. Figure 1 outlines the basic operation of the system.

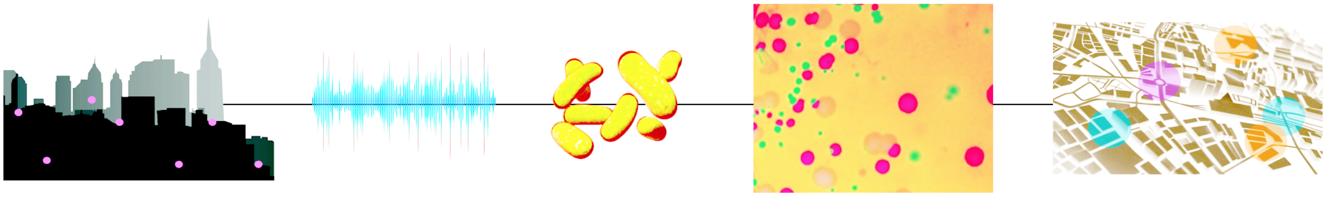

Figure 1: Translating the sounds of the city into privacy preserving acoustic information through the use of genetically engineered E. coli cells.

0.1.1 Sound responsive bacterial growth

Several bacterial strains have been proven to modify their growth patterns when exposed to different sound amplitudes and frequencies [2, 21, 1]. Indicators of increased bio mass of cultures could be essential to correctly characterize and calibrate our sensor system and would serve as a solid proof to the proper functioning of the proposed system.

Based on [2], we will carry out growth experimentation and proof by exposing a solid culture to sound at 8kHz and 80dB for 24 hours. We expect to see increased colony count and bio mass on the culture that is exposed to sound. Experiment schematic and expected results are shown on figure 2.

0.1.2 Sound responsive gene expression

In order to add more control to our system we will make use of putative promoters that are capable of upregulating and downregulating gene expression when exposed to specific frequencies and amplitudes. Up regulation and dowregulation linked to different color proteins will allow our system to be capable of detecting various frequencies with the same device.

Upregulation and dowregulation of gene expression through the use of sound responding putative promoters has been proven on [29]. For our design, we will be making use of the putative promoters BBa_K112401 and BBa_K112402 used on the same study. In order to test behavior of these promoters when exposed to different sound frequencies, four different genes were designed based on the two promoters. Two of the genes express a red fluorescent protein (RFP), one of them expresses green fluroscent protein (GFP) and the last one expresses the well known AmilCP gene in its visible purple variant (AmilCp expression could be used by non

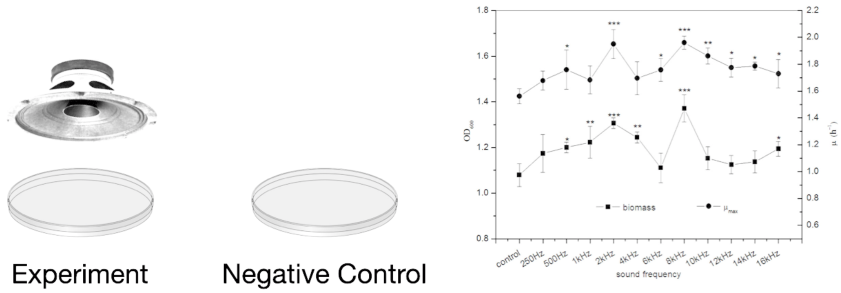

Figure 2: Experiment plate will be exposed to sound at 8kHz and 80 dB. Negative control will be grown from same cell culture but will not be exposed to the sound. We expect to see higher biomass on the culture exposed to the sound.

specialized equipment to determine growth and gene expression in future work). The vectors are synthesised within commercially available Amp resistant vectors from TWIST bio sciences and optimized for expression on E. Coli.

Figure 3: Plasmid design for sound sensitive gene regulation. Promoters upregulate and downregulate expression of color reporting proteins.

Experiments will be carried out in liquid cultures as ilustrated on figure 4. Experimentation will be setup through the use of an Opentrons 2 robot. The cells will be exposed to the same sounds as the cells on the first growth experiment. We expect to see upregulation of gene expression through the use of fluorescence spectometry as opposed to the fluorescence shown by non stimulated cells.

It is relevant to note that approaches that use gene activation instead of regulation have been explored and could lead to better sensor specificity but have not been developed enough for iterative testing [27, 29]. These

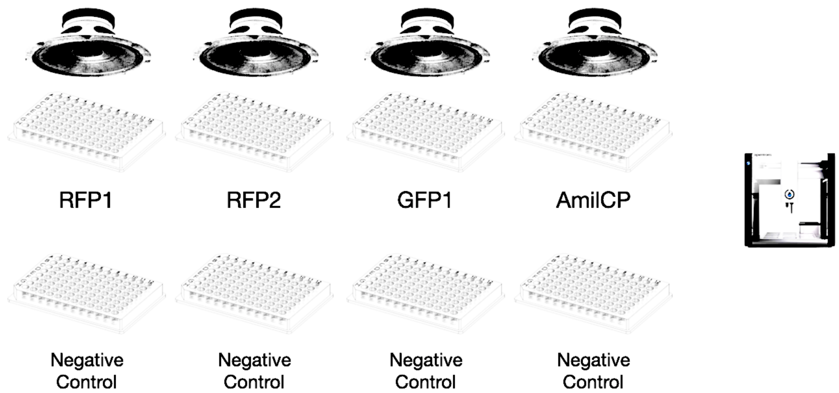

Figure 4: Experimental setup for testing putative promoter gene expression regulation cinduced by sound at 8kHz and 80db. Experimental setup will be prepared using an Opentrons robot.

approaches are based on the well know mechanosensitive transduction effects that have been observed on various cell membranes [30, 31, 32] Future developments could improve upon our proposed system through the addition of activation switches and oscillators.

0.1.3 Bio - Digital Interface

For the first experiments we will be using fluorescence spectometry analysis in order to determine if system does react as expected to sound. Nevertheless, in order to have this system scale to large deployments outside of the lab, we will need to design optical systems that use low cost, light sensors in order to determine gene expression. Optical sensors could be complimented by capacitive sensors that allow the system to quantify protein production and biomass in a highly accurate manner.

The optical system could be connected directly to the existing IoT infrastructure through wired or wireless means in order to send data to a centralized server. Further developments of new ways to make use of the biological information generated by bio sensors would be a crucial area of research for making this systems a reality.

0.2 Taking sensors and experiments outside of the lab

Low cost incubators and synthetic biology equipment has been of great interest to the scientific community for the past years [33, 34, 35]. Research has been carried out in order to develop low cost but highly specialized machines. Some examples include human incubators [36], incubators for carrying out field studies in remote or under served communities [37], laser cut incubators for optogenetic bacterial culture [38] and hypoxia chambers for cell culture [39]. Analysis reveals that none of the published devices has all of the following characteristics; low-cost, portable and multi purpose (modular).

In order to scale bio sensing for city scale implementations, we must build the necessary tools for deployment and testing of the systems. With the goal of allowing easy deployment of bio sensors outside of laboratory settings, we propose and test the design of a low cost, open source, portable and modular sensor incubator.

The design would allow for professional synthetic biologists and the DIY biology communities to build and test different types of sensors outside of professional equipment. This device would speed up development times for the bio sensing infrastructure of the future of our cities.

The first design of the system is illustrated on figure 5. The device uses a 2500mAH battery that operates at 3.7V and an open source PCB module for controlling a peltier element for heating and cooling actions. The pieces can be easily changed and stacked on top of each other depending on the different experiments that are to be carried out with it. The device also allows for the use of different types of culturing platforms such as personalized or commercial petri dishes. Lastly, the upper most part is the experiment module, this module can be used to mount different systems that can then be used to carry out different experiments. An example would be mounting a speaker to the module to carry out experiments 1 and 2 from this proposal.

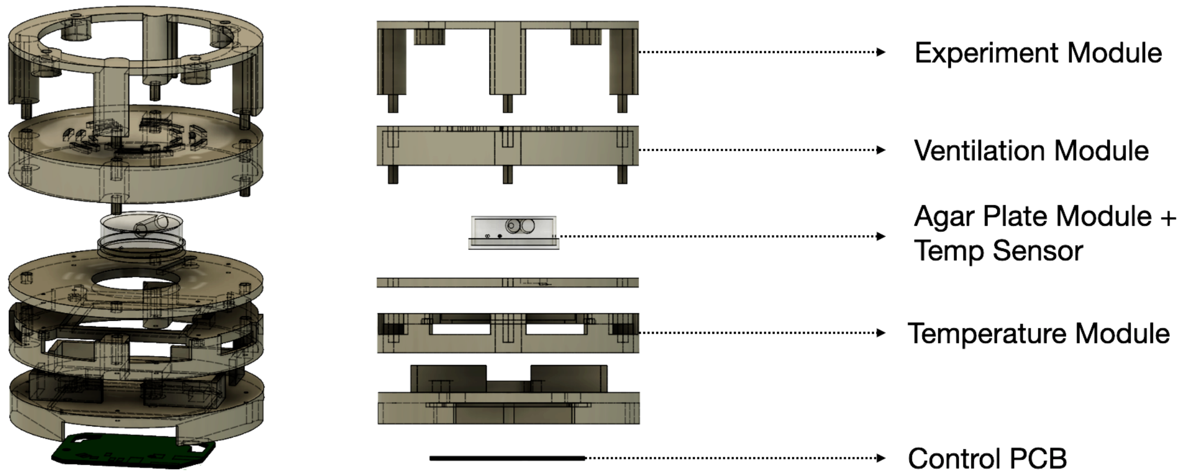

Figure 5: Low cost, modular design for portable bio sensor reactor.

0.2.1 Agar plate E.coli culture.

In order to test the proposed design we started by experimenting with growing a solid agar based culture of modifies E. Coli, the strain of E. Coli used was transformed with the Puc19 plasmid and was designed to express the AmilCp gene in its purple mutation. Using this cells, we would be able to verify the capability of temperature regulation from the device.

Two batches of experiments were set up in order to test multiple variable combinations and controls. The variables on each experiment were the petri dish ((1) our design and (2) a commercial design) used and the incubator used ((1) our design, (2) a professional design and (3) no incubator). Figure 6 outlines the different combinations used for testing.

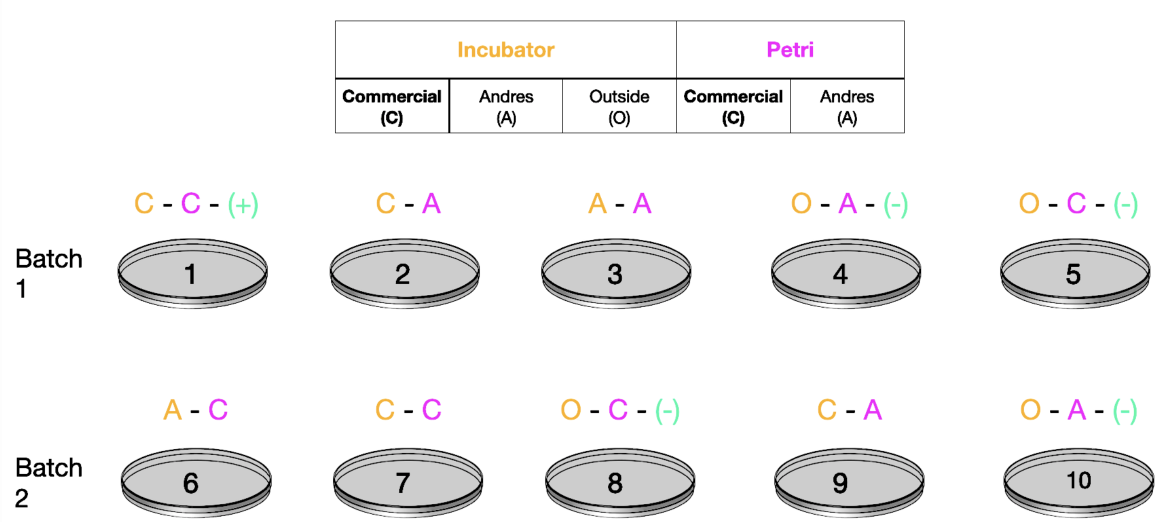

Figure 6: Experiment 3 layout for two batch experimentation. Experiments were combinations of type of incubator and type of petri dish used. Negative and positive controls are marked with (+) and (-) respectively.

Result from experiment are shown below on figure 7

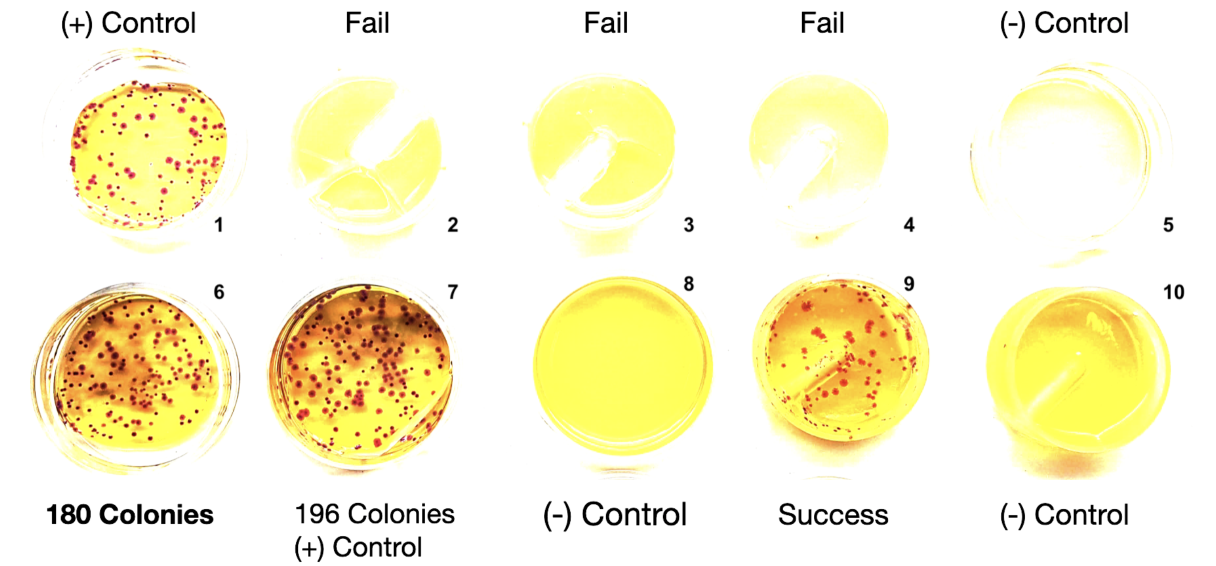

Figure 7: Experiment results from two batches of testing illustrated on figure 6 1 is the positive control for the first batch. 2 - 4 failed pointing to a problem with the curing of the resins used to manufacture the A dish design. 6 is the succesful culture carried out within our proposed incubator design. 9 is a succesful culture carried out inside of our own petri dish design and cultures with a commercial incubator. With the shown results we are able to prove proper functionality of both our incubator and petri dish designs for solid culture of E. coli.

0.2.2 Agar plate E. coli culture and sound experiments

Once we have proven that cultures can successfully grow within the low cost, portable and modular device. We will test the devices capabilities to carry out sensor experimentation and deployment by using the devices experimental module to mount a speaker and stimulate cultures described on Aim 1.

In order to properly test multi frequency response, we will build multiple incubators that can be used in parallel to test different sound frequencies and amplitudes on the same cultures. Experimentation and incubator setup is illustrated on figures 8.

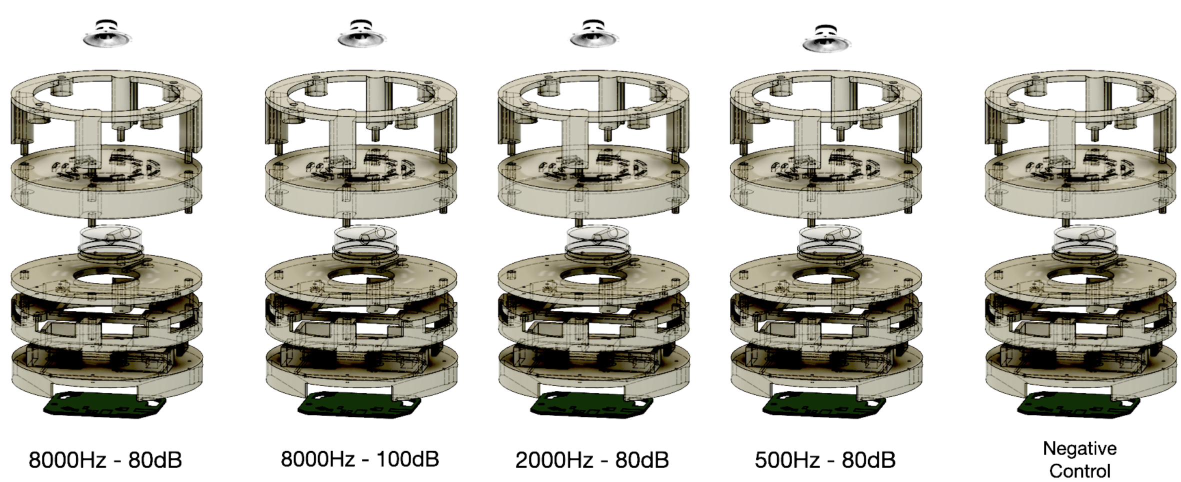

Figure 8: Parallel sound stimulation of cultures at multiple frequencies and amplitudes.

0.2.3 Live culture with microfluidics

Future modules of the device would allow for the placement of microfluidic chambers that can be controlled through the use of pump enabled modules. These modules would extend the device's capabilities as to being able to keep live systems within its chambers. This advances would be meaningful for more sustainable and long lasting deployments of bio sensors. Modularity of the device allows for the creation of endless combinations that can nurture systems with completely contrasting designs and aims.

0.3 Wireless city scale sensor networks

Sensor networks around cities can help us to better understand the complexities innate to the urban environment [40]. Advances in sensing will enable us to create cities that are better designed for citizens and that built in harmony with the natural environment [41]. Bio sensing will play a key role in helping us understand aspects of the urban environment by improving ways in which we currently sense, such as using the bio microphone as a privacy preserving sound sensing system, and adding new unseen capabilities such as being able to sense particles that are impossible to sense with physical sensors.

0.3.1 Vision outline

Looking into the future, it would be desirable that our engineered systems start to use biological properties for computation and embodiment. This would allow sensor networks to be better en grained into the natural environment. Furthermore, questions regarding data privacy and how data is currently used by centralized stakeholders could be addressed through understanding the ways that nature processes and acts upon information. Information in nature is usually local and decentralized.

Furthermore, bio sensing could enable us to see cities through lenses that could not be possible before. Analyzing movement of molecules, pollutants, microbes and pathogens could allow us to untangle the complex social interactions that cities create. Bio inspired and bio based systems could help to create a more balanced interaction between the built and natural environment.

A key question that can arise when thinking of the future of bioIoT is if we would seek to completely substitute electronic sensing with biological or if we would use them as complements. With the rise of low power electronics, RFID systems and extended memory capabilities, I believe that systems will make use of both types of components. As a first step it might not make much sense to completely get rid of our current sensing systems due to economies of scale but as synthetic biology advances we might reach a point where it makes sense to do so. I would still argue that the best systems would be hybrid ones. As hybrid systems take the spotlight it is crucial to start thinking about ways in which we can interface electronics with biological components in a low cost and reliable manner. It is important to understand the capabilities and shortcomings of both technologies.

[12] Gives a great comparison between characteristics that electronics and biological parts have. Some of the most relevant differences are about the types of changes they can sense, the speed at which they can work and the nature of their sensing (deltas). Further understanding of how physical and biological systems could complement each other is needed in order for engineered systems to make use of the best aspects from each one of the technologies.

0.3.2 Obstacle description

Some of the major obstacles will be to find ways in which systems can be properly deployed as to last a long time with reliable performance. Aim 2 of this proposal outlines a device that seeks to mark the way in which bio sensors can be deployed outside of the lab. Further research on how to use this types of devices for living cultures would be required. Research on freeze dried cell free sensing systems will also be crucial for deploying systems that are low cost, reliable and safe for the environment such as the one shown in [42].

Bio sensors usually rely on the interaction of molecules coming from an external environment into a genetic circuit. The design of membranes that are capable of capturing the desired molecules and at the same time stopping any of the device's components from filtering out into the environment is a crucial and non trivial advancement that needs to be developed.

Aim 1.3 talks about ways in which we can connect bio sensors to our existing digital infrastructure. It will be crucial to develop devices that will allow for this connection to be seamless and reliable. This would allow bio sensing to benefit from advancements in digital sensing and IoT.

The internet of things infrastructure does not only include devices for sensing, it also relies on devices for actuation. Actuation within the context of cities, can enable a dynamic reformation of our infrastructure as to create spaces that are more pleasant, healthier and efficient. A simple actuation device would be motorized window that can shut down when sound levels outside are high as to allow the person within the room to concentrate on the task at hand. Bio sensing, could be well complimented with bio actuation in order to reach a broader impact and set of applications as outlined on [11]. Following the window example, we could imagine a system that uses E. Coli to sense sound (as the one proposed above), and instead of synthesizing a reporter protein, they would produce a molecule that could activate a secondary bacterial system that would open/close micro pores in a flexible membrane window by mechanical forces coming from bacterial motility, such as the induced movements shown in [43]. Engineering this types of systems would more closely resemble how nature senses, communicates and actuates to maintain ecosystems at equilibrium.

0.3.3 Societal and environmental impact

As any technological development, city scale bio sensing could bring negative consequences to society. It is relevant to address important questions around devices being re purposed, ecosystem - bio sensor interactions, surveillance practices, sensor tampering and waste management. Addressing this key issues will allow systems with this nature to be kept benign. Figure 9 outlines the key questions on each of the relevant areas stated above along with some possible developments that need to happen in parallel to the development of the sensing technologies described above.

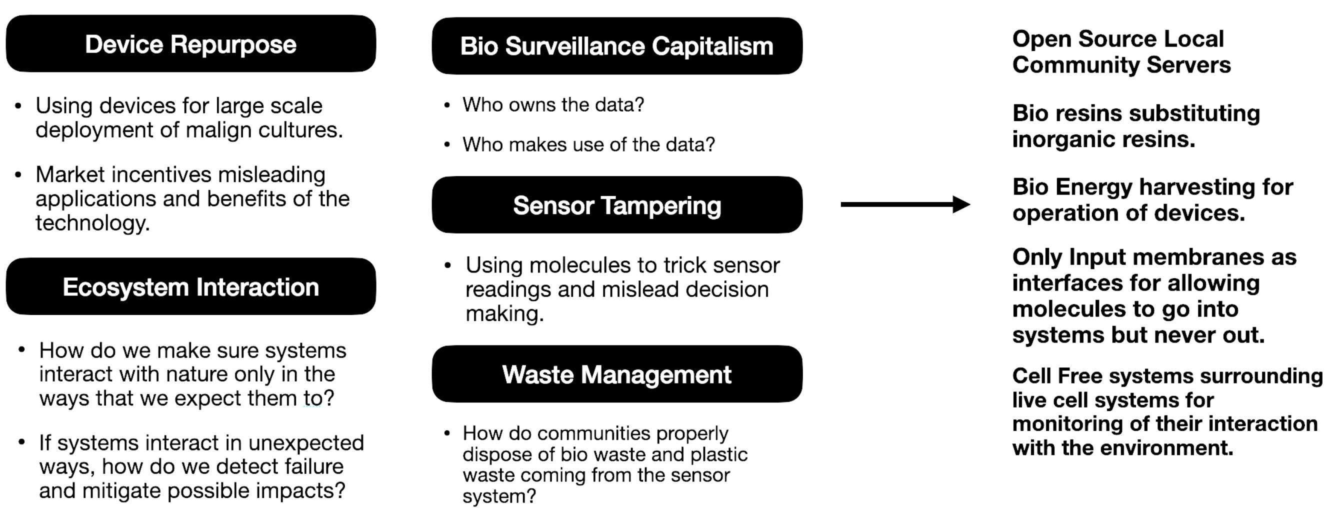

Figure 9: Potential negative impacts and questions that need further answers in order for city bio sensing to be kept as a beneficial and safe technology. To the right of the arrow are some of the key concepts and mechanisms that could be put in place as a starting point for this types of systems.

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Budget

Item	Estimated Cost
Plasmid Design & Manufacture	$1,500.00
PCB Components	$500.00
3D Printing Resins	$700.00
Speakers	$300.00
Opentrons Robot	$5,000.00
Fluorescence Spectometer	$4,000.00
Consumables	$10,000.00
Total	$22000.00

Timeline

Aim	Completion Date
Aim 1.1	June 2021
Aim 1.2	July 2021
Aim 1.3	November 2021
Aim 2.1	May 2021
Aim 2.2	July 2021
Aim 2.3	December 2021
Aim 3.1	June 2022
Aim 3.2	June 2022
Aim 3.3	June 2022