Design for the Modern Prometheus:
Towards an Integrated Biodesign Workflow

by

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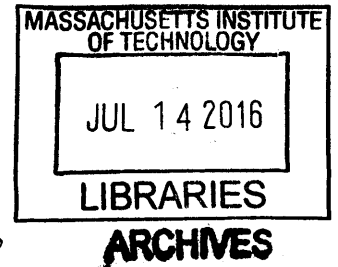in partial fulfillment of the requirements for the degree of

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Abstract

Biodesign is a growing field that harnesses the re-engineering capabilities of synthetic biology and the principles of design to create functional products on a variety of scales. It is now possible to precisely modify and program living organisms to create products useful for medicine, fabrication, and more. These capabilities are today inspiring designers to consider, and design for opportunities associated with, the incorporation of biological and otherwise living matter into the built environment. Standard Computer Aided Design (CAD) software used in design and engineering often does not have resolution required for living systems, whereas many known bioCAD software do not allow for larger scales. In addition, simulations and animations are usually limited to a short timescale, and do not allow for predictive models over days or weeks. For creating environments in which living materials or systems, from bacterial biofilms to functional swarms, can intertwine with synthetically fabricated constructs, rapid prototyping software must be developed that can allow for both design and simulation in different conditions over time. This thesis is an attempt at creating a design methodology and finding a software platform for inclusion of living material systems in manufacturing of products on multiple scales. Existing biological CAD software is surveyed and applied to two case study projects engaging multiple scales (i.e. human scale, and architectural scale) for which the digital fabrication of living materials provides additional functionality and augments the biological or ecological environment. In the process, novel work is presented in the areas of apiary management and 3D printing with biology. Additionally, several computational approaches, including rule based and agent-based techniques, are applied to both projects and evaluated for accuracy and usability. This research took place in Mediated Matters newly constructed BL2 Wet Lab, and serves as a demonstration of research that lies at the intersection

of additive manufacturing and synthetic biology.

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Acknowledgements

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Chapter 1

Introduction

Contextualization

1.1 Historically Controlling Life

In a fit of enthusiastic madness I created a rational creature and was bound towards him to assure, as far as was in my power, his happiness and well-being...

<div align="right">

Victor Frankenstein, Chapter 24

Frankenstein or *The Modern Prometheus*

Mary Shelley

</div>

In Mary Shelley's classic gothic novel, *Frankenstein* [84], the scientist Victor Frankenstein looks upon his monster in the final scene, realizing that he created a living, rational being and failed to create an environment in which it could thrive. He could not predict the morality and actions of his monster, and the novel ends with Victor, the modern Prometheus, dying after attempting to kill his own creation.

Frankenstein, while clearly fantastical and dramatic, illustrates the difficulties in dealing with and exercising control over living organisms. While designers and engineers have largely learned how to modify and fabricate with materials like metals, challenges are still apparent when working with natural materials and biomaterials that have less predictable, more time-dependent properties. For example, cellulose and chitin are two of the most abundant biopolymers existent in nature [85], yet are still being continually researched because complete predictive control has yet to be established. This challenge is further exacerbated in the case of living organisms in standard built environments and fabricated products.

As the world of design and manufacturing progressively intersects with the world of biology and the natural sciences, it is becoming increasingly important that essential elements of each are exchanged. In the past five years in particular, significant progress has been made in exploring digital fabrication as a means to computationally control manufacturing on multiple scales. In addition, the democratization of rapid prototyping, user-friendly software, and low-cost manufacturing tools such as desktop 3D printers [48] are allowing many more people to experiment with design and creation, and thereby partake in the dialogue. Online communities, marketplaces, and makerspaces such as Shapeways [21] and Sculpteo [20], allow anyone to design prints and even sell or share with others. Additive manufacturing is being hailed as the third revolution, both for industry and individual makers [37]. Whether this is true or not, the improvements in computer aided design and modeling software have correlated with an expansion of purposes for products and the utilizable material catalog, and are now being pushed further as living organisms are considered.

Living organisms themselves are now being engineered, too. Born out of the momentum of genetic engineering and systems biology, the field of synthetic biology aims to rigorously create, control, and program cellular behavior [33] and [57]. Though both the story of synthetic biology and its definition are still being discussed, it is

now fact that several techniques have been established that allow organisms to be forward-engineered to allow for designed form and function. These methods are continually being improved and reinvented [40], and applications are acknowledged to be widespread - from medicine to materials science - and the field is now capturing the imagination of designers as well.

1.2 Mediated Matter and the Media Lab

This work is a reflection of the environment in which it was done; both the research group in which I work and the department on the whole are described as "antidisciplinary" [50]. This means that traditional disciplines are not accurate metrics to contain the research - many projects deliberately exceed these bounds.

Mediated Matter began atop the newly constructed tenets of Material Ecology, a field pioneered and coined by Professor Neri Oxman [72]. This approach highlights how context and environment are intrinsically linked to material design and fabrication. In the past five years, the research group has expanded to include students trained in materials science, product design, architecture, mechanical engineering, computer science, structural engineering, computational design, and biology.

Throughout this time, several key concepts have arisen, one of which is "design across scales" [73]. Similar to the idea of ultrascale engineering of Markus Buehler [32] and Eames' Powers of Ten [71], this idea encapsulates how principles of design can apply on every scale - from the atom to the universe. Complexity is gained by assessing projects with variable focus and resolution, though the approach - top-down or bottom-up - may vary. The concept is an underlying current in this work, which seeks to combine fields and tools across scales with an overarching workflow that is applicable to products and platforms alike.

1.3   Lens and Perspective

While this work is firmly in the realm of interdisciplinary exploration, my own formal background is in biology - including molecular and micro - and my research experiences and mentors have allowed me to learn about mammalian disease models, clinical science, imaging, neuroscience, biotechnology, biophysics, cancer biology, and more. Two formative experiences were in the labs of Professor Ed Boyden at MIT and Dr. Jean Livet at the Institut de la Vision. From each PI, I learned cutting edge techniques such as optogenetics [29] and Brainbow [62], but also how crossing from one field to another can yield new perspectives for invention and problem solving. My lens is that of a science student, which applies to the experiments discussed in this work and the perspective through which I have learned and explored the world of design. In context, this thesis is one of many current pushes to apply biology and design, both in the Media Lab and in the world, and serves as a foundation for further study.

1.4   Core Focus

Several gaps remain between precise computational tools for biology (which tend to focus on the micron scale and smaller) and those for product and architectural design (which rarely move below the millimeter scale). Furthermore, sophisticated and aesthetic design tools often lack the simulation duration and parameter control needed for accurate representation of living systems, thereby curtailing full realization of biological components in designed pieces.

The broad question at hand is:

How can we design hybrid products with living and non living materials?

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As discussed in the next pages, this question is broken down further - namely:

What are the steps in current workflows for traditional product design and biological experimentation?

Is it possible to apply these workflows to biodesign?

How can we bridge the gap between in silico and physical tools for both design and biology to allow for efficient and deliberate creation?

The overarching goals of this thesis are:

- To contribute novel and significant knowledge or products to the Media Lab and biodesign community

- To address the problem of product realization in biodesign

- To further collaborations between designers and biologists

- To increase my own depth and breadth of knowledge as a student

Chapter 2

Background

Foundations

2.1   Biodesign

Biodesign is, most simply, designing with living systems [18]. This definition is very flexible; it may also encompass bio-inspired design [43], biomimicry [13], medical device design [60], global problem solving, environmentally sustainable product design, and other areas. The field has gained attention in recent years and is linked to other burgeoning spheres, including synthetic biology, bioprinting [22], biocomputation [47], bio-art [16], DIY Bio [19], citizen science [14], and biohacking [54]. In every case, however, it appeals to both designers and biologists and often has an application or education driven focus. In the spectrum of laboratory work to consumer markets, biodesign attempts to find a happy medium - in which rigorous biology embeds functionality, and design offers application and physical embodiment on a larger scale.

Biodesign is seen as a step deeper into both fields (Figure 2-1), as it moves past theory or pure aesthetic into function, integration, and use [69]. In bio-inspired design and biomimicry, phenomena seen in nature are often replicated with standard materials, to be both aesthetically and metaphorically representative, as well as to harness the nature-perfected solution to a problem. For example, mimicking skin denticles - a particular pattern used by Galapagos Sharks to repel bacteria - can create antimicrobial surfaces [28]. In this case, the discovery of the skin could be done by biologists, but the physical product and application is done by designers. In contrast, a biodesign approach can harness an organism itself - in natural or engineered state - in a functional product. In this case, both designer and biologist must become familiar with the other field, as they work with novel environments and materials. Cutting edge techniques in both design and biology are garnered to explore this interface, which has both breadth and depth. This allows for complex exploration that sheds light on new questions - how can we "design life" [93]? Can we make biology a "new material for design" cite? How can synthetic biology function as a new enabling technology for makers across fields? Pioneers in biology such as George Church, J. Craig Venter, and Drew Endy, as well as designers such as Daisy Ginsberg, Kathryn Fleming, and students at the Royal College of Art have asserted that this area is a very shared domain.

In the manifestations of biodesign that include organisms, there are parts that are living and therefore highly dynamic, though they can be synthetically engineered to yield more predictable behavior; and there are parts that are non living and can be more readily controlled. The latter can be deliberately created to interact precisely with the living parts, through processes like templating. For example, irrigation systems that deposit water to specific areas on a plant bed will more or less ensure that plants will thrive in hydrated areas and be absent elsewhere. Ideally, however, the living components can respond and influence the nonliving components, creating a feedback loop. As in biology, this loop could serve as a type of symbiosis to a desirable
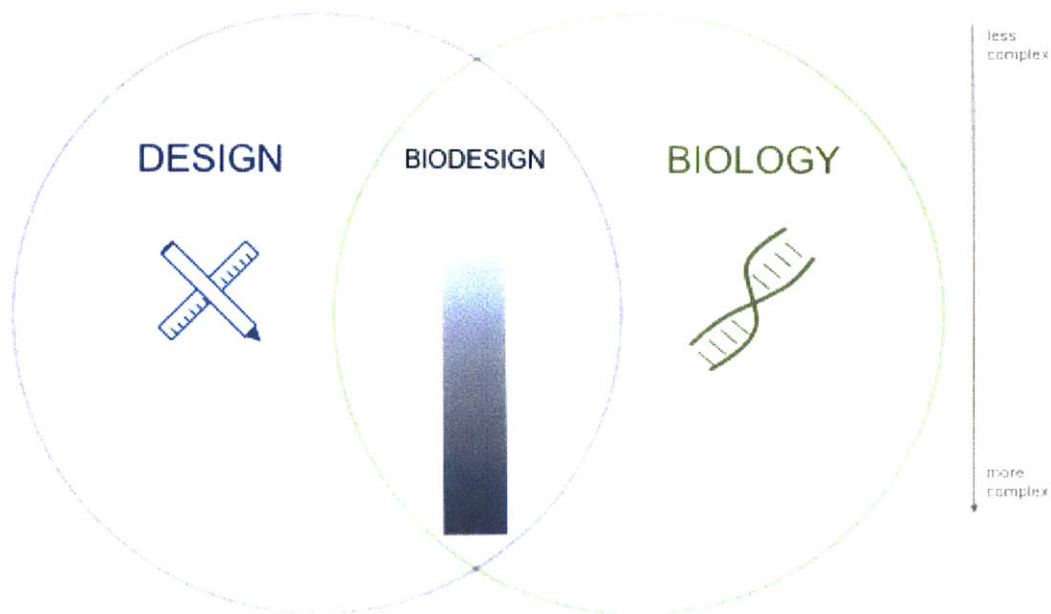
Figure 2-1: **Venn Diagram of Biodesign.** Design and biology both are deep fields; in their intersection lies the spectrum of biodesign, which can grow in complexity based on contribution to and from the parent fields.

end state or theoretically ad infinitum, or could amplify or dampen outputs.

In addition to symbiosis, concepts such as biosensing can be very relevant and have historically been used in many real world applications. A classic example of a biosensor is a canary in a mine: coal miners used to bring caged canaries into tunnels, as the birds would die first if dangerous but undetectable gases were present, alerting the miners. In far less gruesome adaptations, biotic components can change in response to external stimuli, such as chemicals, and yield a response or set off a cascading pathway. For example, glucose-oxidase based blood monitors act by using an enzyme to bind to blood glucose with a readable output [6]. In another example, Tangible Media Group's piece BioLogic [101] includes embedded bacterial spores that sense humidity and change, thereby opening pores on a dancer's wearable suit. The connection between sensing and actuation is also very closely studied in synthetic biology, and is integral in the programming of cellular life [58].

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The language used to describe these biological concepts and even life itself often overlap with the language of design. Terms such as *hierarchy, form, function, space, light, heavy,* and *structure* - and even architectural expressions such as *metabolism* - comprise a shared lexicon between the two fields, though connotations vary. Furthermore, certain methods that have gained foothold in the world of design, such as Finite Element Analysis, have now been applied in biology - to discretize complex systems into more tractable parts [49].

2.2 Realizations of Biodesign

There are today several examples of biodesign - which in this context means *a physical object which includes fabricated and living components.* These realizations can be found in product design, biomedical research, art, and architecture (Figure 2-2), as well as several other fields. While some examples of biodesign are decades old commercial products, such as ChiaPets [2], others are recent projects closer to the realm of speculative design [77].

2.3 System Components

Most of the projects and products can be divided into three components: living, non-living, and environment. In addition, all of them are inherently multiscale and dynamic systems, which contribute to both complexity and application potential.

2.3.1 Environment

The environment, as considered here, refers to the *local and global surroundings which interact with the built or designed components.* Given the highly dynamic nature of

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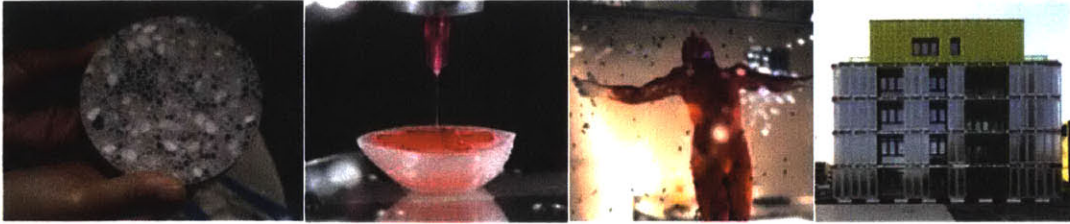
Figure 2-2: **Examples of Biodesign Across Fields.** Four examples of objects that include living components. From left to right: self healing concrete [8], bioprinted cartilage tissue [56], a bee-fabricated sculpture [61], and a building powered by algae [82].

living and natural elements, even small changes may cause a great effect. The environment is intrinsically linked to the scale and application; for example, the environment for a building will encompass the landscape (e.g. urban, rural), terrain (e.g. mountainous, ocean), natural phenomena (e.g. earthquakes, tornadoes), dynamic features (e.g. influence from humans and other organisms), weather (e.g. temperature, humidity, seasons), local ecosystems (e.g. tundra, rainforest), and climate (e.g. temperate, tropical). In contrast, a consumer product has a more localized environment that may affect performance - such as heat from sunlight, motion by a wearer, or submersion in water. Importantly, the environment can provide energy to generate a sustainable and renewable product.

2.3.2 Material

The material in this context refers to the non-living components, which may be scaffolding, enclosures, or other structures that contact the living components. The material makes up the more traditionally designed or fabricated component of a piece, and can therefore be created using existing computational and fabrication tools. Materials can be characterized based on their properties, especially those that are intrinsic or intensive - such as surface energy, specific strength, or stiffness [96]. These can be quantitatively measured and used in computational modeling and prediction. In ad-

dition, the measured properties can allow for functionalities, for example, the surface charge allowing for antimicrobial utility. The material catalog contains composites, metals, ceramics, and more, as shown in Figure 2-3 [5].
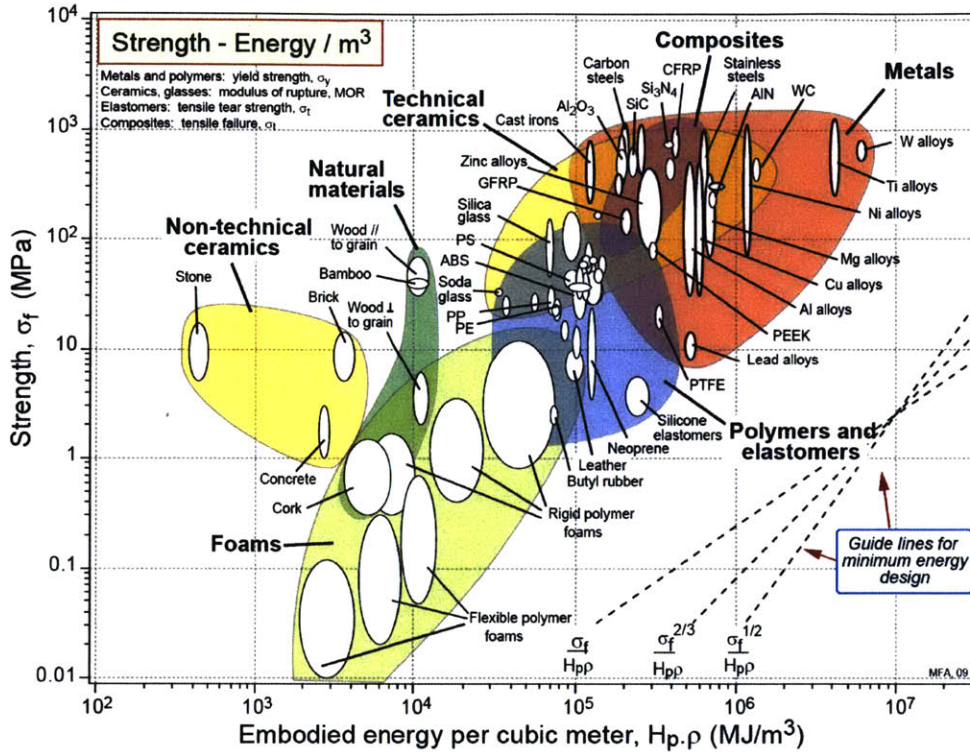
Figure 2-3: **A Typical Ashby Plot.** An Ashby plot or chart allows for material comparison and selection by along different parameters - for example embodied energy and strength. Adapted from Manufacturing Toolbox Blog (Autodesk).

While traditional building materials and even fabricated materials are considered "static", they do have time-dependent behaviors such as degradation that affect their properties. However, most representations such as Ashby plots exist in 2D. This may be tested in the future as natural and biomaterials, which may be more dynamic, are added to the catalog (Figure 2-4) [97]. One important note is that biomaterials include those that are naturally biocompatible, and thus may be good candidates for interfacing with living organisms.
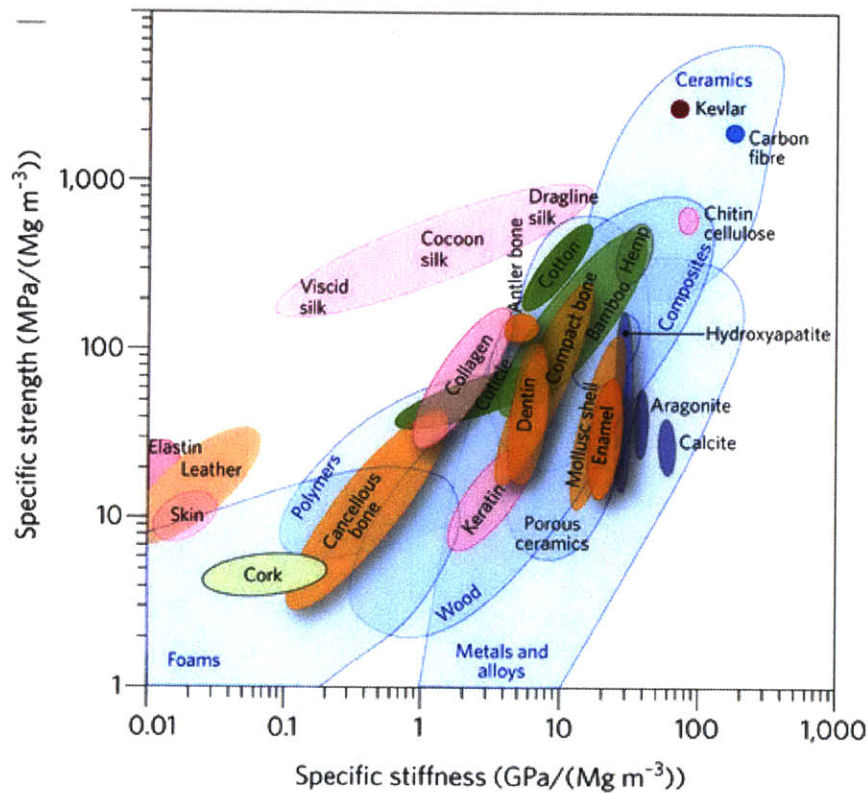
Figure 2-4: **Ashby Plot Focusing on Bio and Natural Materials.** This chart, adapted from Wegst et al. 2015, focuses on biomaterials and materials found in nature. These can actually surpass traditional materials in different qualities, and occupy new spaces on the chart.

2.3.3 Living

The living catalog, by contrast, theoretically includes all of organisms currently in existence (Figure 2-5) - as well as those that are synthetically engineered and altered [10]. Organisms, like materials, also have measurable properties, which can be considered in the context of the characteristics of life [64]. These can generally be considered:

1. Growth

2. Homeostasis

3. Reproduction

4. Responsiveness

5. Organization

6. Adaptation

7. Metabolism

Certain model organisms found in the phylogenetic tree, such as types of bacteria, yeast, and now even some mammals, have been genetically altered to create new outputs [55] [35]. Synthetic biology techniques can allow biologists and designers to program in behaviors, which can be considered as functionalities that can be useful in products or for humans.

2.4 Prior Work

In 2014 and 2015, two projects were developed in Mediated Matter that explored the biodesign space that was initiated with the Silk Pavilion the year before [66].
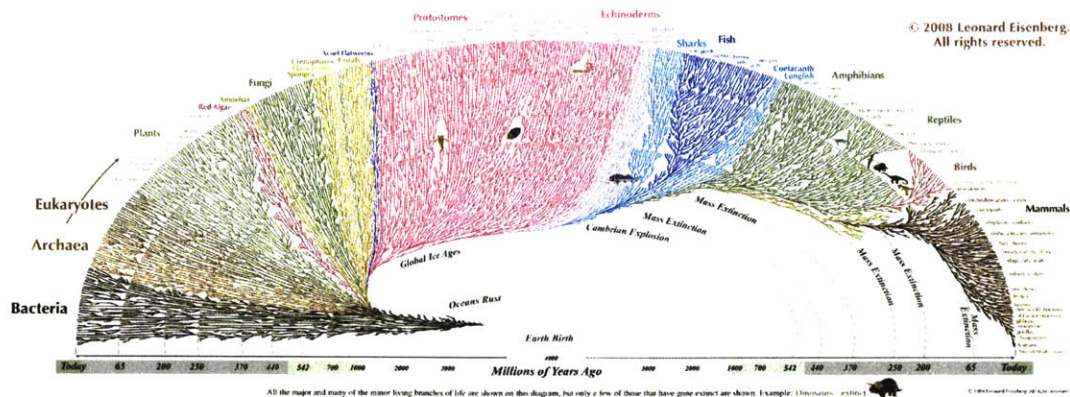
Figure 2-5: **Phylogenetic Tree of Life.** Shown is a full phylogenetic tree that includes mainly organisms currently in existence. Adapted from Evogeneao.

The first of these was the Ocean Pavilion, which was a foray into water-based fabrication. In this project, a KUKA robotic arm was fitted with a custom deposition head which could extrude different concentrations of chitosan. Chitosan is a water-soluble derivative of chitin, which is an abundant biopolymer that forms exoskeletons of crustaceans, insects, and other organisms; it is also a huge waste product of the food industry [52]. By tuning the amount of water in the liquid chitosan mixture, properties such as viscosity, optical transparency, and stiffness could be controlled. Over the course of the project, large scale (3m) pieces were 3D printed and responded to environmental conditions to fold into unique configurations, partially informed by the hierarchical deposition pattern. These pieces could be returned to aqueous environments and fully biodegrade, illustrating the use of biopolymers as greener materials. In addition, chitosan was explored as a scaffold for certain types of bacteria, which could theoretically coat or be otherwise embedded in a piece and provide some sort of fuel or chemical output. The initial experimentation was done with a photosynthetic algae called cyanobacteria, which survived on certain chitosan concentrations and has previously been engineered to produce fuels such as ethanol [100]. Ideally, an architectural scale chitosan scaffold containing this microorganism could be used as an alternative building facade, converting sunlight into usable energy, similar to

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Figure 2-6: **Prior work by Mediated Matter.** Two projects that provided a foundation for this further exploration are *Mushtari* (left) and Water Based Fabrication (related to the Ocean Pavilion) (right). In both of these projects, the same strains of bacteria were used to augment functionality (center). Images by Mediated Matter Group, Paula Aguilera, Jonathan Williams, Stephanie Hays, Tom Ferrante.

work shown in Figure 2-2.

The second project, which was on the wearable scale, was the creation of an "artificial gut" called *Mushtari* [65] [74]. This was a 3D printed piece, worn on the hips, which featured a 59 meter long continuous channel that could house microorganisms in solution. The 3D print was the first of its kind, and used soluble support in order to clear the full channel. The microorganism was the same strain used in the Ocean Pavilion - cyanobacteria (*Synechococcus elongatus PCC7942*) engineered by the Silver Lab at Harvard WYSS Institute to export large amounts of sucrose. This capability could make it a good candidate for co-culture, as many other strains metabolize sucrose and can produce other useful compounds. Given that it was a prototype piece, only small patches were used for experimentation; however, the idea was novel and paved the way for future work in this area.

2.5 Current Workflows

Overall, it is difficult to find any examples of deliberately structured biodesign pieces existent today, partially because the process of design and that of science are very different. It appears that design projects can begin and develop in many different directions: inspiration from nature or other sources, desire to explore a single material and its capabilities, illustration of a particular tool and its utility, and many more. Scientific inspiration can also come from many areas, but a more standardized workflow is followed - the scientific method. This consists of creating an initial hypothesis, developing testable and specific predictions, gathering data through observation and experimentation, and comparing against the hypothesis. The cycle can continue until significant conclusions are drawn.

In both the previous projects done in Mediated Matter, the approach to biodesign was experimental. The traditional materials could be designed using computational aids, but the interaction with the living components was more surmised than tested. For instance, the fact that chitosan is naturally bacteriostatic or antimicrobial was not acknowledged until late in the project, and presented a significant obstacle. Similarly in the wearable, it was unknown how long the cells could survive, how to supply them with nutrients and remove waste, if there would be plaque or film formation in the walls, or even if the pumping of liquid could cause shearing.

The challenge of a biodesign workflow is to allow for creativity while maintaining rigor to highlight the best of both fields. The often used "guess and check" method does not often lead to sustainable or replicable results, and lessens the impact of biodesign projects. When the project is in the realm of art, application is not always a high priority, so the project process is not constrained and many different approaches work. The only area in which there seems to be a standardized workflow is in medical device design, in which the application offers many conditions that drive the purpose and

the creation. In addition, simulations of common medical disorders are often used before *in vitro* or *in vivo* testing, and can inform design at an early stage. Thus, an application driven model could be useful in biodesign in general, and adding a computational component could be highly useful to maximize efficiency and precision.

Chapter 3

Proposal

Motivations and Specific Goals

3.1   Motivations

The motivation of this thesis is to propose a new biodesign workflow that takes inspiration from the fields of biology and design. It can be used to create multiscale projects that incorporate living organisms as well as non living components that interact with the environment for a variety of applications. This will ideally allow for biologists and designers to create greater impact with their projects more efficiently and allow for reproducibility. Instead of using a "guess and check" methodology which makes iteration costly, *in silico* (computational) methods borrowed from design and engineering can be implemented prior to physical work. It can be validated using scientific approaches *in vitro* (which in this case, means in a physical realization).

While there are many potential workflows that can be taken from biology or design, the proposed workflow has these steps: formulation, simulation, experimentation, and

deployment (Figure 3-1). The observations from each step go back into the design. Similar to the steps of the scientific method, these steps can be done repeatedly to change the design before deployment.

SYSTEM DESIGN

FORMULATION ⟷ SIMULATION ⟷ EXPERIMENTATION ⟷ DEPLOYMENT

in silico   *in vitro*

Figure 3-1: **Proposed Workflow.**

Two underlying concepts here are *scale* and *feedback*. Scale is naturally at play with the inclusion of biological systems, which follow levels of hierarchy (from cell to organism); this mirrors the hierarchy of material systems (from atom to compound material). Furthermore, existing tools from different fields allow designers to modify components on many scales - from the gene to the colony, or molecule to block - and thus the complexity leads to opportunity. Feedback on the other hand is a common theme in biology, and describes how components interact to influence one another (Figure 3-2). One of the benefits of a biodesign system that has modifiable living and non living parts is that feedback loops may be established - and can lead to dynamic, responsive products that offer flexibility and adaptation over time.

3.2 Steps and Evaluation

The process of biodesign will be explored through the creation of two products, or "case studies" - one on the architectural scale and one on the wearable scale. In each

Figure 3-2: **Feedback Loops in Biodesign Systems.** In the ideal system, the living and non living components should exist in a dynamic feedback loop that exchanges with the environment.

product, a living organism community will be contained within a built environment, and control will be explored through templating. Because the process has several parts, the focus of this thesis will be on the computational *in silico* piece - which in itself is a significant area. Therefore, the existing field of computational biodesign-related tools will be surveyed, and a single tool will be selected to be used in both case studies.

These goals can be summarized in the following steps:

1. **Survey of Computational Tools for Biodesign**. A survey of *in silico* tools will be conducted, and selection criteria developed. Evaluation will consist of testing the criteria in the process, and the selection of a single tool.

2. **Case Study Architectural Scale Biodesign**. A large scale project will be created that includes a community of living organisms in a built space. The design of the space should be informed by the environment, materials, and living components. When the set up is complete, a simple assay will be developed to

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compare the simulation of project performance to actual performance. This will serve as the evaluation.

3. **Case Study Wearable Scale Biodesign**. A smaller scale project will be created that includes a community of microorganisms in a fabricated piece. The design of the piece should be informed by the environment, materials, and living components. When the set up is complete, a simple assay will be developed to compare the simulation of project performance to actual performance. This will serve as the evaluation.

In order to create a feasible scope, two key choices have been made. The first is an assumption that a single computational tool exists that can be used for both projects though they are on different scales, though they both focus on a living community within a built space. Software exists that can be used with many different characterized materials for a myriad of applications; possibly the same approach could be done with characterized organisms replacing materials. However, it is possible that existing tools are organism or species specific. Either way, the survey process will still provide a current census of what exists in the field, and inform the creation of new tools in the future that build on these foundations.

The second choice made is to consider organisms in the biological context - not to adapt existing computer-aided design software by creating a fluid or material that approximates the living mass. While this approach could be valid, it may not work for organisms that do not form physical communities (e.g. biofilms). Nevertheless, this is an interesting and highly relevant area to explore for the future.

Chapter 4

Survey

Existing Computational Design Tools

4.1 BioCAD and Related Terms

In the area of design and engineering, there are many options for software to assist in the creation and evaluation of physical pieces before and during actual fabrication. One subset of computational tools includes Computer Aided Design (CAD), Computer Aided Manufacturing (CAM), and Computer Aided Engineering (CAE) [23]. While many software platforms and programs integrate various aspects of these three and definitions are not entirely set, generally CAD refers to those programs that can be used to create 2D and 3D graphical representations. By contrast, CAM software is often used to exert control over manufacturing tools in a production environment; and CAE software simulates performance to verify engineering and design [87]. A commonly associated term is digital prototyping, which refers to the entire process of exploring ideas and designs before physical execution of products [36].

In more recent years, the terms CAD, CAM, CAE, and digital prototyping have all begun to enter the vernacular of biologists and scientists. The relatively new field of "bioCAD" is widely applied to almost any software tool used in biology - including those that allow for plasmid design, graphical pathway configuration, bioinformatics, 3D imaging reconstructions, data representation, molecular design, experimental design, and community behaviors. The unifying factor is that all of these tools are *in silico* as opposed to *in vitro* or *in vivo*, which simply means computational. These tools have been applied to medical engineering, clinical science, systems biology, and synthetic biology, and have great potential to improve efficiency and standardization in both experimental and applied biology. Concepts such as FEM have even been considered for computer-assisted biology, with the argument that compounds can be analyzed for coordination and compatibility just as discretized engineering materials can. Objectively, a significant amount is known about common compounds and model organisms used in life sciences research, so computer-assisted tools should be relevant.

BioCAD tools can take in a variety of inputs and create outputs for many purposes and scales. Certain programs can be useful in visualizing large data sets, for example in computational biology. Others feature graphical user interfaces with simplified diagrams for plasmid engineering or pathway design (e.g. VectorNTI [92]). In the scope of this thesis, a tool for predictive simulation based on verified models would be most useful. Additionally, an interface similar to those found in SolidWorks [89] or Rhinoceros [79] would allow for creation of detailed renders. Ideally, the tool would be applicable on multiple scales - by incorporating different modeling approaches on each scale and integrating in a bottom-up approach. Thus, the same tool could be used to model bees and microorganisms as living components in their built environments.

4.2 Experimental Framework

The purpose of this survey was to identify if there is an existing *in silico* simulation tool that could be useful in informing the design of two biodesign case studies, one on the architectural scale with insects in communities, one on the wearable scale with unicellular organisms in communities. The first step was to survey the existing tools, which span a variety of fields - molecular, synthetic, systems, micro, and computational biology; bio engineering and bio physics; mathematical biology; and computational design and manufacturing. One tool would be selected to generate *in silico* simulations and compared against *in vitro* and experimental results for each case study. This process would serve as both a useful review, as such a comprehensive overview of the current field does not currently exist in literature; identify the gaps in the domain; and most importantly, establish a component of the proposed workflow.

4.3 Selection Criteria

Given the breadth of the bioCAD field, there are literally hundreds of existing computational tools for modeling and simulation. In order to be as inclusive as possible, 130 software tools, packages, platforms, plug-ins, and scripts were surveyed, found in the non-exhaustive list in Figure 4-1. These were found through a literature search using Web of Science, PubMed, and Google Scholar; these were linked to the keywords: "computational", "design", "biology", "modeling", "simulation", "systems biology", "synthetic biology", "whole cell", "organism", "cellular interaction", "biofilm", "mathematical", "cellular potts", "cellular automata", "game of life", "bacterial", "agent based", "lattice based", "in silico", "in vitro", "experimental", "parameters", "growth", "behavior", "bioCAD", "bioCAM", "bioCAE", "biodesign".

In order to select the appropriate computational tool for the two proposed projects, certain criteria were established, namely:

1. **Active community, updates, and support.** It is critical to select a tool that is being used by the community, as this is an indicator that it is useful and valid. In addition, those that have support and updates - like any other commonly used software - will be the most rigorously tested and refined. In this case, "activity" is defined as any documented update, publication, or community usage in the last year (2015) (Figure 4-1).

2. **Focuses on the cell (micron) level and above.** Because biology by nature spans many scales, the corresponding software must focus on different scales as well. In this case, the two projects deal with full organisms as units - so the software must be at least cell level or greater (Figure 4-2).

3. **Has an interactive rendering GUI.** One aspect that makes traditional CAD software useful is the graphical representation and renders that can be generated and modified by the user. The visual component allows for possibly more specification and simulation capability, versus numerical data and plots, and focuses on the object. In this case, any graphical or visual representation that the user can interact with - rather than modify only via command line - would be desirable (Figure 4-3, 4-4).

4. **Simulation is based on a verified modeling approach and is modifiable based on user input.** Simulation differs from animation because it can be based on a verified model of behavior. For example, how a protein folds is based on its hierarchical structure, and can be mathematically predicted. In addition, different conditions - such as the amino acids in the primary sequence - can be changed to yield different global results that can be seen in the folding simulation. For biochemical kinetics, certain organism behaviors, growth pat-

terns, and other processes, a palette of models have been created and tested over time. These can all serve as potential bases for simulation and would be useful as predictive tools.

BIOCAD TOOLS

CellGEO	Autodock	Foldit	MoE	Benchware 3DEx	Gene Designer	IBM Rational Rhapsody	BacSim	BioSPICE	RiboMaker
Asmparts	HEX	PROTdes	MolMol	CADnano	Breve	Kasim Kasa	mcell	BioNexGen	Bionetsolver
GEC	Biodesigner	TeselaGen	NAMD	Molecular Flipbook	CellSim	Pathway Logic	cellblender	xCellerator	JCASim
AutoBioCAD	BetaWB	Genome Compiler	GROMACS	QuteMol	Tumor Simulator	CellSys	Vcell	BioCHAM	Deshatiky
RoVeRGene	BioDIVINE	Genabler	AMBER	Pymol	Ascape	CellModeller	ERATO	BioSketchPad	Mobi
GeneDesign	PRISM	GeneCoder	CHARMM	JSMol	SBaddon	cellPACK	BioBlender	Cellware	PkSim
uNAfold/DINAMelt	BioNetGen	GEPASI	LAMMPS	Jolecule	BMA	Vaa3D	GRO	COPASI	BioShape
mFOLD	SPiM	CellDesigner	DESMOND	Jmol	Tissue Simulation TK	nToxer	E-CELL	Geneious	StarLogo
ZBit	OpenCell	JigCell	PLUMED	Chimera/MDS	CompuCell3D	Bio Computational Evolution	ePMV	VectorNTI	Mason
Vienna RNA	PySMol	PySCES	MARTINI	nanoCAD	Breach	mMaya	OpenCMISS	GenoCAD	RePast
Rosetta	Towercell	Pathway Studio	Folding@Home	ChemDraw	FLAME	nDynamics	BioSpi	Benchling	BSim
Bioinformatics Toolkit	SynBioSS	CCP4	SYBYL xSuite	Clotho	GinSim	nCloth	NetLogo	Aquarium	Swarm
cellML*	Foldit	COOT	Muse Invent	BioJADE	GreatSPN	nParticles	StochSS	Snoopy	SPNP

Figure 4-1: **Table of Surveyed Software Tools.** A complete list of the software and computational tools surveyed for this thesis. Each was evaluated on the basis of four criteria; shown here is selection based on the first - activity - with the qualifying programs in black and those that did not pass in gray. *cellML refers to those simulations directly based on cellML, including PyCML and CHASE.

ORGAN	Vaa3D	Mobi	PkSim			
TISSUE	BioShape	Benchware 3D Explorer				
	cellML*	TumorSimulator				
CELL	mcell	BMA				
	CompuCell3D	CellModeller				
	SYBYL xSuite	cellblender	VCell	mMaya		
	NAMD	cellPACK	Rosetta	BetaWorkBench	OpenCell	
MOLECULE	Folding@Home	COOT	Vienna RNA	CCP4	PySCES	Jolecule
	Pymol	Muse Invent	Molecular Flipbook	StochSS	COPASI	Chimera/MDS
	MoE	QuteMol	ChemDraw	MARTINI	Bioinformatics Toolkit	JSMol
	CADnano	Autodock	AMBER	CHARMM	GROMACS	nanoCAD
	ePMV	Jmol	Foldit	TeselaGen	LAMMPS	BioBlender
	GeneCoder	PLUMED	DESMOND	BioCHAM	Breach	mFOLD
GENE	GeneDesigner	uNAfold/DINAMelt	RoVeRGene	BioJADE	AutoBioCAD	GenoCAD
	Pathway Logic	Geneious	Pathway Studio	Clotho		
	Genome Compiler	VectorNTI	GEC	GinSim		

Red box: Aquarium, Snoopy, GreatSPN, Swarm, MASON, Benchling, Kasim Kasa, Maya with plugins**, FLAME, NetLogo, REPAST

Figure 4-2: **Selection of Tools based on Scale.** The qualifying tools from Figure 4-1 were arranged based on scale of operation (criterion 2). Highlighted in blue are those that operate in the scale of interest for these studies; highlighted in red are those that are not scale-based, so cannot be evaluated under this criterion. **Maya with plugins refers to nDynamics, nCloth, and nParticles.

Criterion 2 was notably broken, since several tools were not scale-based. These tools followed different modeling approaches, and offered another alternative: a multiscale tool that applies the same model to an integrated system - more of a "top-down" approach. In order to accommodate for this new option, the tools that fell into this subcategory were exempted from criterion 2. The six tools that could be evaluated under the original criteria (i.e., were based on scale) were next tested against criterion 3, which focuses on user interface (Figure 4-3).

| CompuCell3D | TumorSimulator | mCell |
| cellML Applications | CellModeller | BMA |
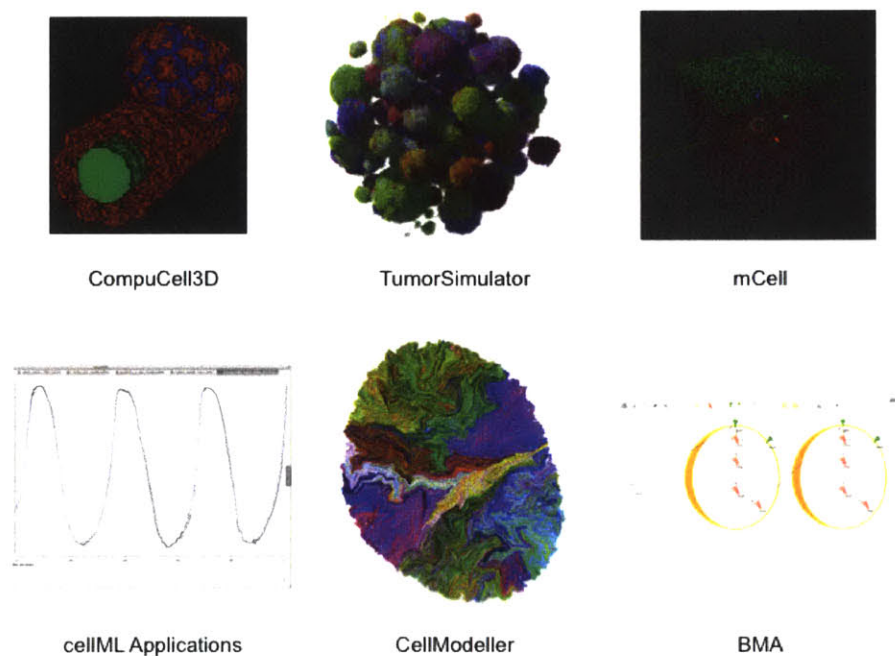
Figure 4-3: **Selection of Tools based on User Interface and Graphical Output.** The six tools that passed the first two criteria were evaluated for user interface and graphical output. Publications and documentations of completed projects using these tools were compared.

Five tools passed the first three criteria, and were further investigated. Thirteen tools passed criterion 1 but not 2, and were also under consideration. At this point, it was apparent that two key aspects that were not described by the criteria were
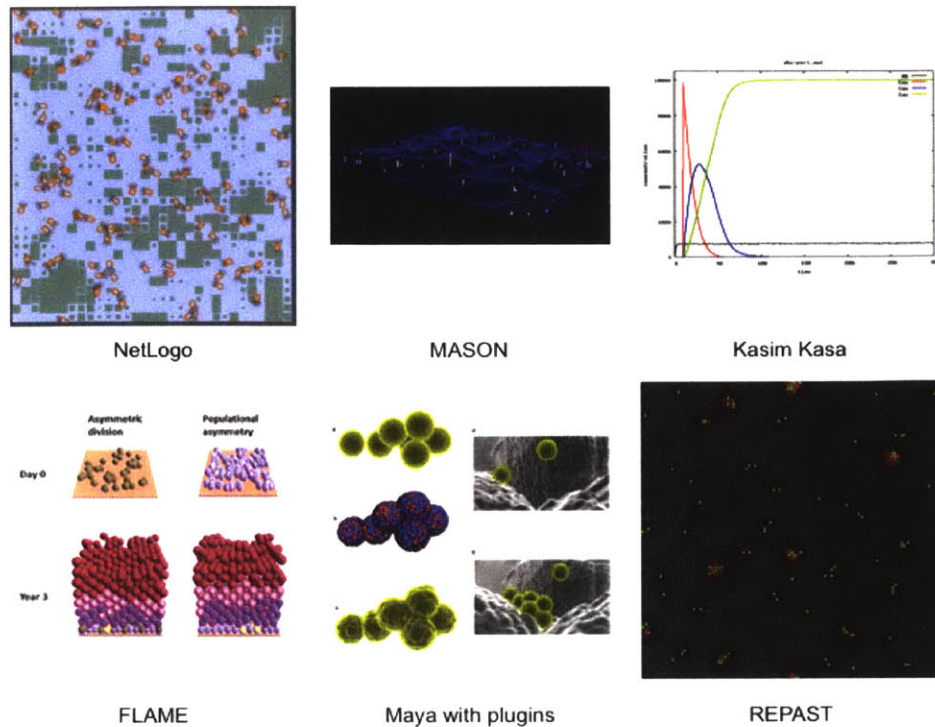
Figure 4-4: **Selection of Tools based on User Interface and Graphical Output.** Tools that were exempted from criterion 2 were evaluated for user interface and graphical output. Publications and documentations of completed projects using these tools were compared.

purpose and feasibility of implementation, which are interlinked. Certain tools, such as TumorSimulator, GreatSPN, and Snoopy, were created for specific purposes and thus were difficult to generally apply to a variety of conditions; Benchling (digital lab notebook and assistant) and Aquarium (for molecular biology workflow) were also excluded for these reasons. Others, such as BioModel Analyzer (BMA), were most applicable as teaching tools, as they had simplified models that would not scale or bend to fit differing contexts. After these exclusions, the tools that failed criterion 2 were evaluated for criterion 3 - user interface and graphical output (Fig 4-4).

At the end of the first three evaluations, the remaining tools were: CompuCell3D, CellModeller, mCell, NetLogo, MASON, FLAME, Maya with plugins, and REPAST.

4.4 Modeling Approaches

Criterion 4 initially was designed to separate simulation software from animation software, to ensure that user input would not be the sole input for behavior, which would be unrealistic. However, given the issue that arose with criterion 2, it became important to first gain an understanding of different modeling approaches that are not purely bottom-up mathematics.

Shown in Figure 4-5 are nine types of modeling approaches used in systems biology, which are applicable to this study of living and non-living hybrid systems. Given that each case study is focused on a living community, systems biology would possibly offer an elegant perspective. The software tools surveyed fall into four of these approaches: rule-based, hybrid, agent-based, and lattice-based [24].

4.4.1 Rule Based

Rule based systems are those that follow defined models with specific rules, such as enzyme-substrate binding. The terms themselves can be easily exchanged and modified, unlike in equations, and established rules can be implemented to define particular interactions. Certain add-ons for mCell, such as BioNetGen, employ this technique.

4.4.2 Hybrid

Hybrid systems apply the logic of state based systems but add in dynamics, and often feature ordinary differential or partial differential equations (ODEs and PDEs). This allows for representation of continuous dynamics. CellModeller incorporates

both of these equation types in a cellular biophysics-based bottom-up approach that can model thousands of cells. This model is useful for illustrating qualitative and quantitative data, and is fairly user-friendly and modifiable.

4.4.3   Agent Based

Agent based systems feature many individual autonomous entities that have defined rules of interaction with each other and the environment. They are flexible and dynamic, and take a top-down approach to systems by modeling overall behavior without consideration of cellular or sub-cellular equations. Tools such as FLAME, REPAST, and NetLogo - many of which are inspired by swarm communities - are well suited to model behaviors such as growth and chemotaxis. However, most agent based systems do not offer high resolution of individual agents or enhanced visual representations with which the user can graphically interact.

4.4.4   Lattice-Based

Lattice-based models assign discrete dynamic systems (which are defined as single units) to a spatial area on micro, meso, or macro scale. This is best suited for inter-connected networks and systems, or conceivably, communities made up of individual parts. The Cellular Potts model, also known as the Glazier-Graner-Hogeweg model, is a special case of this. CompuCell3D uses the Cellular Potts model; mCell uses a similar spatial framework that also employs Monte Carlo dynamics to account for stochasticity [95].
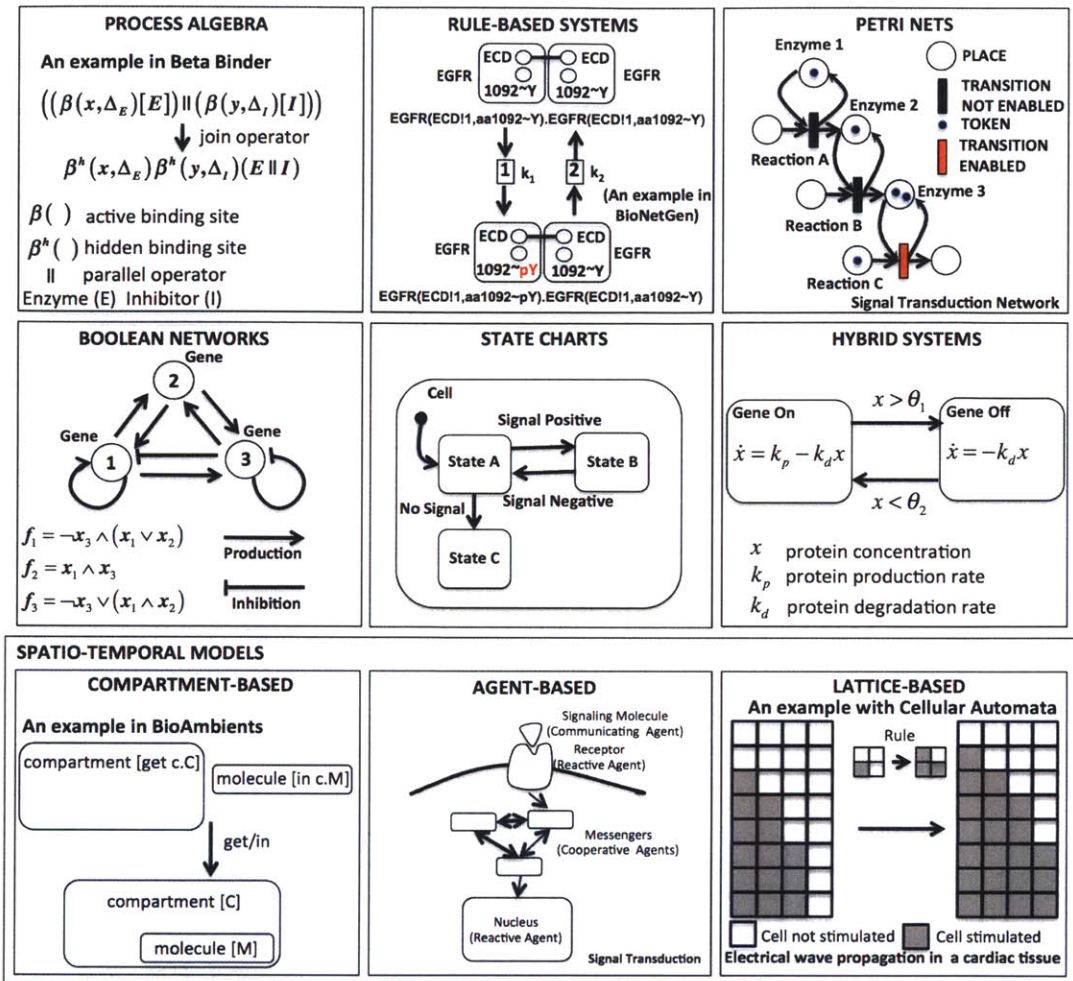
Figure 4-5: **Comparison of Modeling Approaches for Systems Biology.** Nine common approaches for systems modeling and simulation. Adapted from Bartocci and Lio 2016.

4.4.5 Selection

Some of the tools could not be verified as following any standardized modeling approach that was based on a biological system, and were therefore excluded. At the end of this round of evaluation, six tools remained: mCell, CellModeller, NetLogo, MASON, FLAME, and REPAST.

4.5 Final Selection and Considerations

At this point, the qualifying tools were explored in detail and their capabilities tested. Some of these tools were incompatible with the available working system, had bugs, and support was unavailable to rectify issues prior to implementation. The final selection was NetLogo. NetLogo was created in 1999 as a agent based language and modeling environment [98]. While mCell and CellModeller may have been better suited to the wearable scale case study which featured microorganisms, they could not exceed that scale, which suggests that software that has a bottom up approach to modeling are not easily made to be "multiscale". This assumption - that one tool may be used for both biodesign projects - is made to ideally simplify a modular workflow. However, this could be an infeasible assumption. NetLogo had the most examples and tutorials available, as well as precedent in creating simulations of both bacteria and bees. In addition, it is a long running project with an active user community, and a fairly user friendly interface.

While these tools did pass all the given criteria, almost all of them were object or system focused, meaning there was no way to create an advanced environment or substrate. In addition, any unique or programmed outputs of cells - such as fibril formation for biofilms - would be difficult to implement in some of them, though others (such as CompuCell3D) have been used explicitly for this purpose. Furthermore, the

graphics of some of the programs were very primitive and not very interactive; the Maya plugins, while better suited for animation than simulation, did allow users to precisely define details and examine a render in 3D space.

It is important to note that certain published models and simulations are not available as source code or packages, so were not considered, such as LINDSAY Composer, which is used in the LINDSAY Virtual Human Project [11]. Conversely, some software packages have been highly modified for new applications relevant in this area. Notable examples include Maya plugins nTinker and Biocomputational Evolution created by Deepak Chandran and David Benjamin respectively [34] [27]. However, neither of these were available to use in an updated form, and were instead used as inspiration for multiscale Maya-style rendering platforms. This method, however, could provide new insight into moving to larger system modeling and simulation, and can implement an accepted material catalog as well as more "real world" constraints. However, in the scope of this thesis, the existing software packages were first surveyed and evaluated to clearly define the possibilities and needs within this field.

The applied test of the selected tool would be comparing *in silico* parameters and results to *in vitro* to evaluate the accuracy and efficacy of the simulation. In the next two chapters, the *in silico* simulation is evaluated against the two *in vitro* case studies: architectural swarm fabrication and wearable microorganism 3D printing.

Chapter 5

Case Study 1

Swarm Fabrication

5.1 Background

For hundreds of years, humans have observed natural living systems, and have remained fascinated with sociobiological dynamics. A subset of animals within the insect class exhibit eusocial behavior; that is, they live in groups as adults, cooperatively take care of juveniles, have a reproductive division of labor, and overlap generations [76] [99]. Social or swarm insects fall in five orders: Isoptera, Hemiptera, Thysanoptera, Coleoptera, and Hymenoptera. Ants, bees, and wasps all fit within this final order, and are commonly seen examples of highly organized societies.

In the last few decades, a large amount of research has been done observing behaviors and capabilities of all three groups, and how swarm communities may have advantages over other social systems such as by creating widespread but connected sensor networks, responding dynamically and predictively to environmental changes, and co-

ordinating for population success. Many surprising revelations have been unearthed, such as bees' ability to detect explosives [81] [78] and air quality [30], and how ants leave pheromone trails in order to communicate [88] have influenced how many human systems are designed [39].

Additionally, ants, bees, and wasps conduct natural forms of fabrication, by tunneling (subtractive) or hive building (additive). For this reason, their behaviors are intrinsically applicable to building and manufacturing practices. Honeybees in particular are known for creating beautiful and complex wax hives, which can weigh hundreds of pounds [67] and house up to 20,000 (feral) 80,000 (managed) bees [3]. The honeycomb hexagonal packing has been mimicked in architecture [51] and product design [4], while the naturally created products wax, propolis, and honey have been harnessed for human health [12] and products [31].

Controlled production of these materials has been explored by artists and designers, and a number of parameters characterized that affect the amount, rate, and quality, including gravity [80], and foundation structure [61]. In most cases, bees were used as co-fabricators they conducted natural wax building on a novel structure or base, but their intrinsic behavior was purposely unaltered.

Apis mellifera typically live in temperate and tropical zones, and are active outside the hive when temperatures are above about 5C; below this temperature, such as during the winter, honeybees survive within a colony. A colony can have between 5,000 and 80,000 bees, a majority of which are female worker bees. Male drones are used mainly for breeding and excluded from the hive during the winter, while a single fertile queen, that is larger and lives for 3-5 years, lays eggs [25]. This hierarchical society is further divided into workers of various types dependent on role nurses, foragers, and possibly others [53].

Honeybees, *Apis sp.*, are an ideal model organism due to their well-characterized and easily observable natural behaviors, reproduction rate, population size, complex

Figure 5-1: *Apis mellifera.*

communication and organization, and visible social behaviors. There are many sub-species that have been domesticated for hundreds of years, including *Apis mellifera*, the European honeybee. This subspecies is relatively gentle, produces large amounts of harvestable honey, create hives above ground, and hold an integral place in many cultures around the world [46].

Honeybees have complex forms of communication that include physical (such as the waggle dance [7] and chemical signaling [68], and thus are a highly studied example of swarm collective motion by large numbers of a single species [15]. The combination of swarm behavior and additive manufacturing makes bees particularly interesting for designers and scientists alike, and suggests possible applications such as new robotic systems, computational methods, and even urban-scale interaction platforms.

Bees have been scientifically studied for years, both on the population and individual scale. Generally, assays are conducted in the field wooden hives are placed in open

areas that have unlimited access to food source, and researchers observe their behavior with regard to particular areas over time [94] [83]. Since the 1970s, different methods have been used to tag bees to more easily gather data in the field, and range from paint marking [1] to attaching small RFID tags [91]. However, while a natural environment is highly useful for examining intrinsic behaviors, it is difficult to identify which factors influence hive activities precisely. Individual bees are studied in lab environments for short periods of time, and can even be caged or harnessed instead of free flying in small areas.

Generally, bees used in experimentation and art are allowed to live naturally or within traditional hive environments. Beekeeping is done in an outdoor environment using wooden hives often with removable frames in Langstroth, top bar, or Warre styles. In recent years, the urban beekeeping movement has gained foothold in several countries, and bees are being moved from remote farms into metropolis roofs and buildings. In regions above the frost line, such as in New England, beekeeping concludes when the temperature drops below 5C, for approximately 4-5 months in the winter season, during which the hives are wintered either indoors (at low temperature) or outdoors. Alternatively, bees may be housed in observation hives with plastic or glass windows, but also must forage outdoors and thus cease activity in cold weather. During this time, both outdoor and indoor bees remain inside the hive for warmth, precluding any material creation or brood formation. While bees generally store honey during the spring and summer months in preparation for fall, beekeepers often give them extra sugar water or sugar syrup, often mixed with health supplements, as well as pollen substitute that contains essential building blocks.

In recent years, several diseases, pests, and widespread phenomena that affect honeybees have come to national attention. Small pests, such as *Varroa* mites, and diseases such as European foulbrood and wing rot, can impact population health and bee productivity. Colony collapse disorder, in which bees take their queen and leave the

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hive entirely, was observed between the mid-2000s and early 2010s [41]. Since then, patterns of absconding, dwindling, and swarming have been seen as well. In addition, about 40% of hives in the United States have died during the winters; in colder areas, such as New England, over 60% of hives have died [in discussion with professional beekeeper Noah Wilson-Rich, 2015].

In the most mysterious cases of absconding and other hive abandonment events, bee-keepers do not know where bees go; as the swarm leaves the hives, there is no easy way to track or trap them, and it is often difficult to understand why this occurs. Attempts have been made previously at enclosing honeybees within structures, most commonly greenhouses, which would theoretically limit absconding events. Insertion of honeybee hives in greenhouses for plant pollination have been unsuccessful due to hive disorientation, pesticide use, as well as nutrition and lighting issues. Further-more, in greenhouses as well as other built structures such as observation hives, a passage is always provided to the outside to allow for foraging, which reduces the amount of control and delivers a means of escape. However, there has yet to be found an enclosure in which solely honeybees are kept enclosed indoors during the winter season without the purpose of pollination and with free access within a space.

Such an enclosure could serve as a new laboratory for behavioral studies of hon-eybees and other organisms that traverse great distances and are highly dependent upon natural environmental conditions. Other model organisms from zebrafish to monkeys have standard protocols and environments for controlled observation and behavioral assays, and the creation of a honeybee-focused enclosure could allow for the establishment of more extensive and replicable protocols. In the wake of widespread phenomena such as hive abandonment, it is increasingly important to understand where outdoor colonies fit within nature to contextualize their behavior and survival. Additionally, in order to understand behaviors such as fabrication, material produc-tion, foraging, and hierarchical dynamics, further research must be done to rigorously

identify and modulate environmental and social parameters and thus, new laboratory environments must be established.

5.2   Focus

In this project, the aim is to create a space in which honeybees can thrive - with access to resources and minimal pests and disease - and create a model that can be used across similar species. The ideal natural environment could be maintained without regard to season, and research activities such as data collection and behavior observation can be done in a controlled and rigorous manner. Such an environment can be adapted in the future, especially in urban contexts, to create safe spaces for honeybees while complementing the built human landscape. This attempt at such an environment, termed the Synthetic Apiary, is an interdisciplinary foray into a complex natural problem, and yielded several novel proofs-of-concept that may be improved and further demonstrated in the future.

5.3   Materials and Methods

An entirely sealed tensile structure within the 200m^2 experimental space was designed and built using a framework of wooden crossbeams stretched between white elastic fabric (Dazian Trapeze Plus white 122, 90% polyester 10% Spandex), above which full spectrum low heat lights (144 Spectra Brite full spectrum 48 fluorescent tubes, 32 watts, 93 cri) were fixed (Figure 5-4). This allowed for a large space for habitation and foraging with minimal crevices and corners, as well as easy viewing of hives as well as individual bees. Additionally, it provided a bright space with dispersed light to mitigate one commonly seen issue in greenhouses, in which honeybees are attracted to light and injure themselves through repeated contact.

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The architecture of the space consisted of a central rectangular sealed enclosure with two entrance rooms. Each entrance room had netted magnetically self-sealing doors into the main enclosure as well as to the outside parameter of the entire enclosure, thus preventing dislocation of bees. The concrete floor of the enclosure was cleaned and painted white, and was swept and mopped every two days with low concentration bleach in hot water.

Figure 5-2: **Top down rendering of designed enclosure.** Illustration by Markus Kayser and Jorge Duro Royo.

8 wooden Langstroth hives (approx. 160,000 insects) of European honeybees, *Apis mellifera*, were introduced (Best Bees Company) (Figure 5-5, Figure 5-6). Each hive had 1-3 boxes, each of which had 10 frames with foundation wax. Climate conditions were maintained at between 10-21 Celsius, at least 50% relative humidity, 15 hours simulated and incremental daylight, and appropriate air circulation and ventilation; this mimicked a spring-like natural environment, during which bees are highly active. This may also be tuned to represent other seasonal climates during
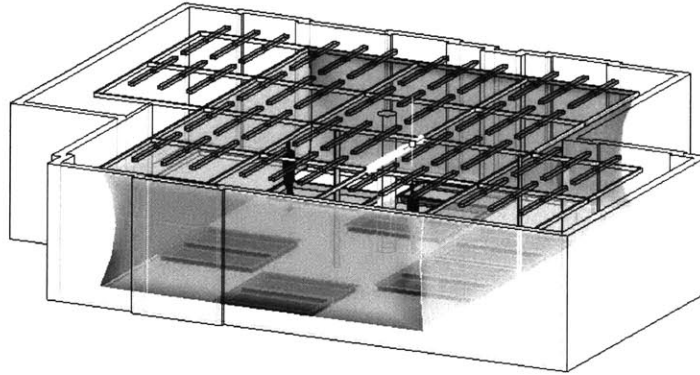
Figure 5-3: **Side view rendering of designed enclosure.** Illustration by Markus Kayser and Jorge Duro Royo.

which particular behaviors and activities may be observed. The system features standard residential heating, a heating ventilator unit, bladeless fan (Dyson AM03 Air Multiplier Bladeless Adjustable Pedestrial Fan), and space humidifiers (AIRCARE HD1409 Digital Whole-House Console-Style Evaporative Humidifier) and additional electric heaters as needed. Air quality is additionally improved by exhaust vent fans (Panasonic FV-15VQ5 WhisperCeiling 150 CFM Ceiling Mounted Fan) as well as by a fresh air ventilation unit (Panasonic FV-04VE1 WhisperComfortTM Spot ERV Ceiling Insert Ventilator). The air quality may also be tested and monitored to ensure a healthy environment.

Nutrients and water were provided in abundance in a central foraging area. Pollen substitute (BeePro) and 1:1 pure sugar water (supplemented with HoneyBee Healthy to prevent fermentation) are provided in trays equipped with wooden posts for cleaning, or floating colored foams to prevent drowning, respectively. All nutrient sources were disease and pesticide-free, and replaced regularly to ensure quality. Hives were

Figure 5-4: **Actual built enclosure, before insertion of hives.**

also regularly checked for presence of pests such as mites (*Varroa sp.*) and hive bee-
tles (*Aethina sp.*), as well as signs of viral diseases such as deformed wing virus. Used
wax frames may also be tested for pathogens, and products could be evaluated for
material composition. Researchers were all trained and approved by Massachusetts
Institute of Technology Environmental Health and Safety, in partnership with Best
Bees Company, and wore protective mesh and fabric suits, along with gloves and
boots (Mann Lake Ltd.), while inside the space (Figure 5-5, Figure 5-6). Out of the 8
hives, two were established as stable controls, and basic experiments were initiated in
the others, such as frame removal, wire foundation insertion, and removal of natural
stored honey to encourage wax and honey formation.

After about 7-14 days, hives were assessed for overall acclimatization and health. At
this point, most hives were generally thriving and thus suggested that a sustainable

ecosystem was created, due to the presence of fertile queens and developing eggs (Figure 5-7, Figure 5-8). Activity was visible in and around the hive and in the foraging area.

New wax structures were found, indicating that the bees used nectar substitute (sugar water) provided, and also stored pollen substitute, which serves as food for young brood (Figure 5-8). Clusters of several hundred to thousands of bees outside the hives were observed, laying wax on the enclosure itself, apparently without a queen, indicating absconding but highlighting the use of an enclosure to observe hive abandonment.

5.4   Experimental Framework

Once the space was created and verified to be a sustainable environment for honeybees, the experimental framework was developed. The assay in this case was actually just observed behavior over time.

The specific question to answer regarding the workflow was:

Does a prepackaged model in NetLogo (the selected computational tool) accurately represent activity of real bees in the space?

The evaluation of the success of this model was done by comparison of parameters present *in silico* versus *in vitro* (meaning in reality), and the quantities of bees surviving over time in each hive. Qualitatively, the amount of activity seen around the foraging area and hive could also be compared to the generated data from the simulation.

5.4.1　In laboratory

The experiment began after the hives had been installed for about two weeks, to account for acclimatization. The hives were introduced in a staggered manner, meaning two hives were initially considered, one of which died soon thereafter (Figure 5-9). At this point, about 2000-4000 bees were in the hive used in this experimentation, and were examined over a month for overall activity and population. As is customary with beekeeping, it was assumed that some pests existed in the hive, though they were untracked.

5.4.2　In silico

An existing NetLogo model called BEEHAVE was used as the basis for experimentation. This model simulates the development of a honey bee colony and its foraging of nectar and pollen in a realistic landscape [26] (Figure 5-10). It is an attempt at an all-inclusive tool that includes several common stressors - *Varroa* mites, deformed wing virus (DVW), acute paralysis virus (APV), pesticides, poor food sources - to provide a realistic representation of colony growth and behavior over a day, month, and year. It is being used by several research groups as well as the European Food Safety Authority as an accurate simulator of honeybee behavior that can help combat recent losses.

The default set up variables were modified to allow for constant light, no honey harvesting, no *Varroa* treatment, optimal levels of nectar and pollen available, minimal colony size of 1000, distance traveled to 1km, laziness probability of bees due to winter and age to 25%.

Given that the NetLogo BEEHAVE software can be tuned for a variety of situations, the same simulation was run several times with different conditions, including different

time of the year, and higher levels of disease and pests. The conditions, labeled C1-C5, were as follows:

1. (C1) Start with 2000 bees over days 0-45, no mites or disease. This is the simplest scenario.

2. (C2) Start with 2000 bees over days 30-60, no mites or disease. This is the scenario accounting for different ages of a hive and after a two week acclimatization.

3. (C3) Start with 2000 bees over days 0-60, 10 healthy 10 diseased mites carrying other disorders, such as DFW. This is to incorporate the realistic presence of parasitic bugs.

4. (C4) Start with 40000 bees over a full year, noting the pattern starting in November, no mites or disease.

5. (C5) Start with 50000 bees over a full year, noting the pattern starting in November, 1000 healthy mites and 1000 infected mites.

5.5   Evaluation

As shown in Figure 5-10 and Figure 5-11, the real conditions in the Synthetic Apiary were partially represented in the BEEHAVE environment. While BEEHAVE accounted for colony size and disease, it only partially considered light, humidity, and temperature, which were instead lumped together under "weather", and completely ignored air quality, pollutants, and inter hive interactions.

The populations over time in each simulation were generated and compared against hand-counted population over time in the physical hive, with the null hypothesis that

there was no difference between the control (reality) and the experiments (*in silico*) (Figure 5-12). A two tailed, equal variance student's t-test was performed, with significance at p less than .1 and p less than .05. C1 and C2 were deemed significant at p less than .1 but not p less than .05 (with null hypothesis rejected) with values of .056 and .057 respectively; C3 was deemed highly significant at p less than .1 and p less than .05 with value .038; and C4 and C5 did not reject the null hypothesis and were not significant at values .841 and .905 respectively.

5.6   Results and Discussion

The Synthetic Apiary, while successful in some regards with the formation of eggs and fresh wax, overall saw few cases of sustained survival. Unfortunately, many hives decreased in population and two died completely; notably however, this is a higher survival rate than that of New England hives in typical wintering conditions.

While the software BEEHAVE did not include several vital components of the real situation, certain simulations did appear to give similar results. While C1, C2, and C3 assumed that this experiment took place at the beginning of the year, with or without mites and disease, C4 and C5 were evaluated in the late fall and winter. This seasonal change, regardless of the number of starting worker bees, made a notable impact. It suggested that the bees in the Synthetic Apiary - though in a simulated "spring" environment - were still acting in accordance to a yearly seasonal cycle. However, given the low n (a single hive was tested against the simulations), any insight should be seen as a correlation at best. Still, the *in silico* simulation was able to somewhat accurately represent the situation in a top-down fashion and could likely be modified to fit better as more research is done. Thus, the specific experimental question was answered. That being said, several features of BEEHAVE were limiting, such as the inability to define a starting time at any point during the year or lessen the quality

of the food source. These hindered an accurate and easy representation, but could likely be compensated for in a restructuring of the code.

The rapid decrease in population in the Synthetic Apiary could have its roots in a variety of uncontrolled variables. Some key issues that arose were: disease and contamination, air quality, lack of nutrition, and shock. First, it was found several weeks into the experiment that nearly all hives had some form of pest, such as beetle, mite, or disease. Following a natural beekeeping practice, however, prevented the application of medicine or treatment, as bees can actually overcome these stressors in the best conditions. However, a shared food and water source meant that disease could theoretically have been passed from hive to hive, leading to more failure. Second, air quality was not measured and tested prior to installation of hives. Generally, bees survive in both urban and rural environments, which have greatly differing air qualities, so this was not considered. However, after noticing many bees clinging to fabric near the unsealed windows in the larger room, an air circulation system was set up with an auxiliary fan. This seemed to improve the air quality, but this was not quantitatively measured. Third, the food given to the bees - while stated to be a complete nutritional source - may have actually been lacking essential nutrients. In nature, bees can gain different benefits from different flowering species, which may strengthen their immunity and bolster health; however, this was not possible in the synthetic set up. Finally, there may have been an element of shock and disorientation in the initial bees and maybe even those born in the Apiary, as the environment is significant non natural. For instance, there are fewer sign posts towards food, the flying area is less, and density of hives significantly more than in nature. These conditions could have led to unnatural behavior of the bees.
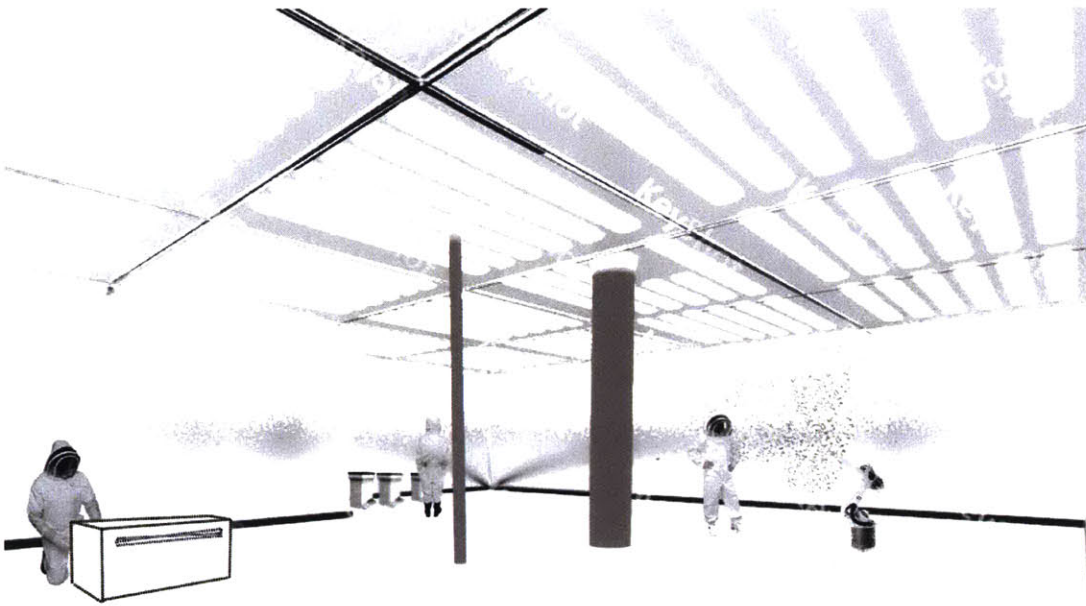
Figure 5-5: **Top down rendering of designed enclosure.** Illustration by Markus Kayser and Jorge Duro Royo.

Figure 5-6: **Researcher pictured with two initial hives.**

Figure 5-7: **Small eggs within freshly created wax.**
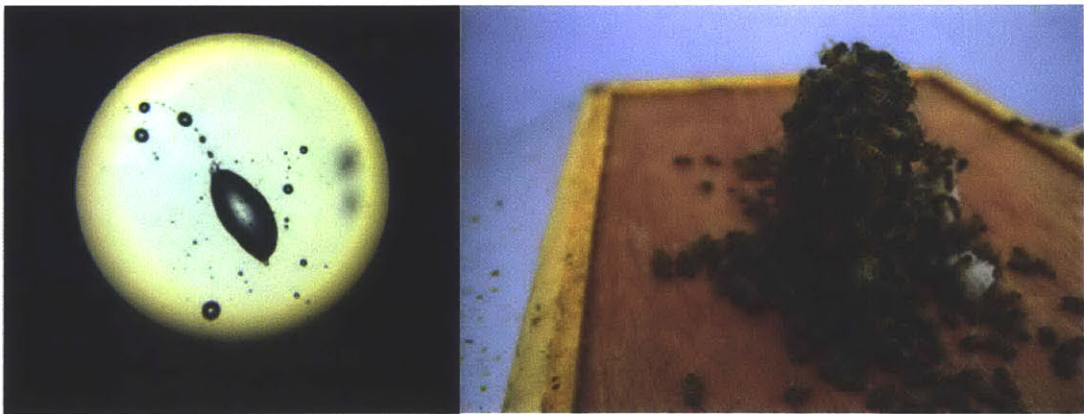
Figure 5-8: **Material Products of the Synthetic Apiary.** Shown here are a light microscope image of honey (40x) (left) and a natural wax structure (right), both of which indicate that the bees were actively building and generating food stores.
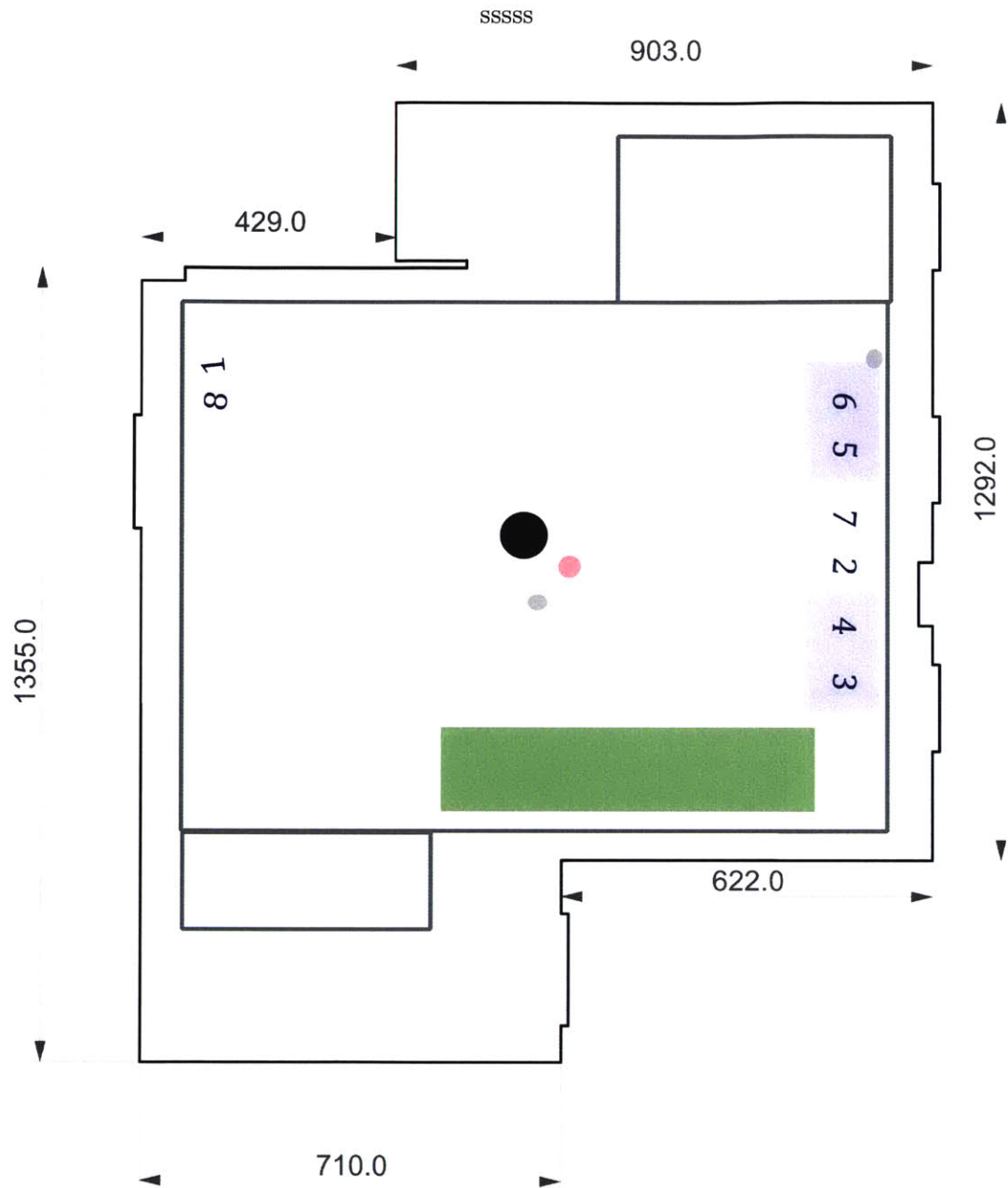
Figure 5-9: **Synthetic Apiary Experimental Set up.** The Apiary experimental space featured hives (numbered 1-8) some of which were on tables (purple), support poles (black and pink circles), and a foraging area for water, nectar, and pollen (green).
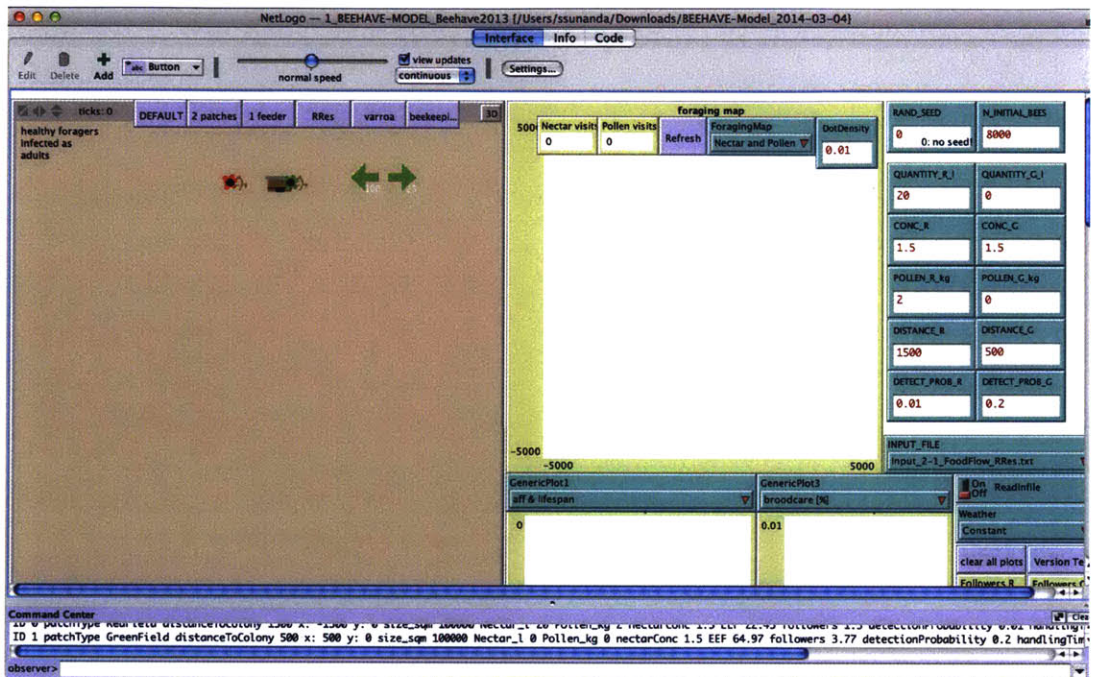
Figure 5-10: **NetLogo BEEHAVE Experimental Set up.** The environment of BEEHAVE featured a graphical representation of factors and data over time, as well as plots of measured and predicted values.

Parameter	In vitro	In silico
Light	◆	❖
Colony size	◆	◆
Disease	◆	◆
Air Quality	◆	
Pollutants	◆	
Other Hives	◆	
Humidity	◆	
Temperature	◆	❖

Figure 5-11: **Comparison of Parameters.** The NetLogo BEEHAVE set up was compared to the real conditions to evaluate how many parameters were accounted for.
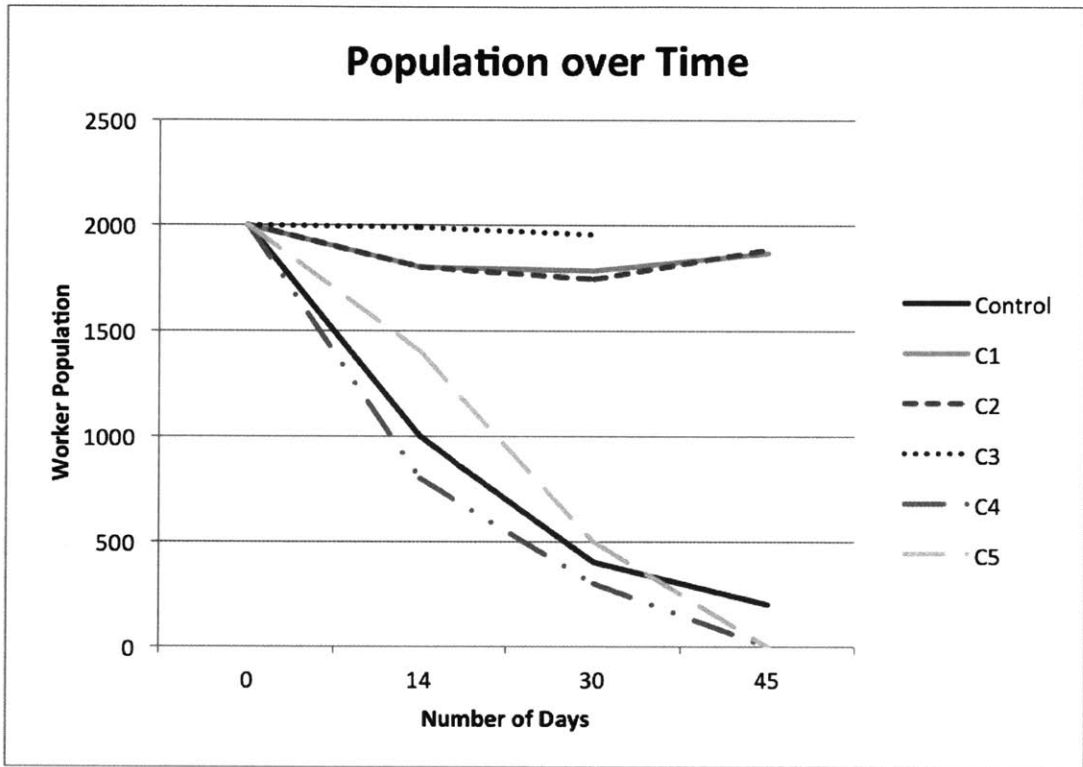
Figure 5-12: **Population over Time.** The population of worker bees were counted by hand over different days.

Chapter 6

Case Study 2

Living Wearables

6.1 Background

As noted previously, *Mushtari* was the first wearable prototype featuring living microorganisms created by the Mediated Matter group [74]. While this experiment was highly useful and original, it had several obstacles which hindered full realization of the biodesign system. Most notably, the size of the wearable and the cleaning process of the channels made the experimentation with living communities very difficult, as smaller and quicker tests could not be done, cleaning was tedious, and this could have distorted growth of cells. In addition, a more expansive set of tests moving past cytotoxicity into biocompatibility to strengthen the case for a sustainable wearable. These sorts of test could be usefully simulated *in silico*, as well. For these reasons, a smaller wearable without internal channels could be tested as a new environment that is easier to handle, clean, and experiment with.

One of the smaller wearables that bridges the gap between accessories and apparel is the mask. Inspiration for this project was drawn from the concept of death masks [38], which have historically been used to preserve the likeness of a person at the time of his death, provide protection and gifts for the afterlife, or for studies such as phrenology. This tradition is tied to many ancient cultures, from Romans to Egyptians, and has been practiced for hundreds of years. Several concepts, such as spirit, last breath, final words, and emotion have all be profoundly expressed in the face, making it an interesting starting point for an artistic wearable. Some current artists, such as Nick Reynolds, have resurrected the "lost art" of mask making, and have brought the idea back. Often, famous historical figures such as Napoleon have death masks that are observed as a window into the person himself.

Masks are also commonly found in the realm of the living. From preventing the spread of disease to filtering air, they are frequently tied to a function useful for the wearer and often provide an interface to the environment. They are also explored as a way of extending emotion, for example, through exaggerating expressions [17], of selecting sensory inputs [75], and more. With the advent of 3D printing, masks can be designed that fit a particular persons face or cover certain areas, lending flexibility and specification at once. With 3D bioprinting, medical research is even exploring facial reconstruction and transplantation [59], suggesting that scaffolding and embedding living cells may be possible.

Many masks are created to keep microorganisms away from humans, and are not often seen as a desirable scaffold for microbial communities. However, many microorganisms regularly exist on human faces, and may actually aid in the health of skin and prevention of disease [45], suggesting a commensal or mutualistic relationship. Microbiota of the body - most notably explored in the gut - provide a living "fingerprint" for non-human parts of the human ecosystem. The Bellybutton Diversity project illustrated how a skin community existent on almost every human differs and can be

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used nearly as a form of identification [42]. It is conceivable that facial microbiota could also act in a similar way, though there are many more factors that influence growth. This opens a range of new questions: does your facial microbiome change based on your habits, mood, relationships, diet, and more? Can new communities of microorganisms be transplanted to create mental or physical change (such as in the gut with Fecal Matter Transplants)? Can the facial microbiome act as a footprint and a log for traveling and activity?

A mask can act as a piece for exploration of these questions and more. It can serve as a second skin where a new microbial community can thrive and provide a useful function to the living wearer - such as air or water purification, production of anti-fungals and antibiotics for skin infection, burn and injury treatment - or for a dead wearer by theoretically accelerating the process of decomposition, creating a form of cremation, or harvesting decaying matter into new fuel or growth. Wild type and natural microorganisms can be harnessed to execute these functions, demonstrating an applied use of synthetic engineering on the human scale. Additionally, 3D printing is approaching the resolution of cell communities, and certain printers can also create multimaterial pieces. Using such technology, it is possible to create microstructures or patterns that can influence cell growth and behavior.

In this case, there are many options for print design as well as experimental procedure that can impact the growth and survival of cells on different materials. There are a few examples of simulations of bacteria-material growth [44] which are precedents to this work, which is a newly developing field. Ideally, materials can be selected from a catalog (similar to what is done in some engineering simulations) and a few characterizations and properties are inputted by the user; then the simulation can take place and both growth and pattern formation can be observed.

6.2 Focus

The focus of this work was to create a wearable mask that can be worn by the living or the dead, and has a 3D printed design that templates the behavior of embedded microorganisms. The mask(s) may serve as a prototype for further study and ideally will allow for novel findings regarding the application of 3D printing to microbiology, as well as generate a tangible product that can be exhibited. This particular case study focuses on early development of the mask process, which is mainly about applying living cells to these materials for periods of time.

6.3 Materials and Methods

The materials used as substrates for bacterial growth were polystyrene as control (Sigma), and two covalently cross-linked thermoset 3D printed resins called VeroGrey and TangoBlackPlus (Stratasys) [63]. The printed materials were created as chips (3mm depth x 30mm diameter). Chips were cleaned of support material by power washing and soaking in 2% sodium hydroxide for 20-30 minutes, alternating the cycle three times. All materials were then sterilized with 10% bleach soak for 20 minutes and wiping down with 70% ethanol (Figure 6-1).

Wild type *Escherichia coli* K12 bacteria (ATCC) were grown in liquid Luria Bertrani (LB) media until in log phase. Cells were then resuspended in 2mL of fresh LB in 6-well plates.
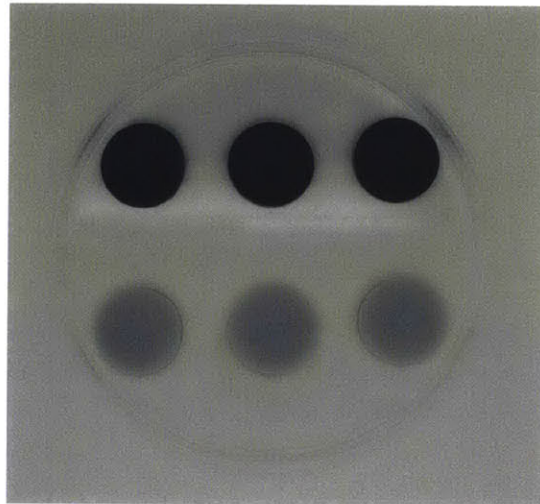
Figure 6-1: **Printed chips of VeroGrey and TangoBlackPlus after cleaning.**

6.4 Experimental Framework

The assay selected for comparison between *in silico* and *in vitro* behavior was cytotoxicity, which is one component of biocompatibility. It is critical that a printed piece is not toxic to the microorganisms; certain materials are already verified to be compatible with mammalian cells [63].

The specific question to answer regarding the workflow was:

Does a prepackaged model in NetLogo (the selected computational tool) accurately represent growth in a cytotoxicity assay using bacteria?

The evaluation of the success of this model was done by comparison of parameters present *in silico* versus *in vitro*, and the quantity of surviving bacteria over time.

6.4.1 In laboratory

Into the 6 well plates were placed VeroGrey and TangoBlackPlus chips, with control wells being empty (only polystyrene). Cells were then grown for 12 hours at 37C in a shaking incubator (150 rpm). At the end of this time, cells were prepared using the LIVE/DEAD Baclight Protocol to assess viability; another subset of cells was diluted and plated onto LB agar and grown for 24 hours at 37C (non shaking). The cells were sampled in two ways: directly from the well, or after a wash step. In the wash step, chips were placed in fresh 2mL of ddH2O and placed in a shaker for 30 minutes. The remaining liquid was then used for analysis. The data shown here, however, only represents cells that were directly sampled.

6.4.2 In silico

There are several models that have been appplied in NetLogo to bacterial growth, including those focused on growth in the presence of antibiotics and those focused on inter-species "rock paper scissors" style competition. For this case study, the basic package bacteria.nlogo was selected [86], with different amounts of antibiotic (Figure 6-2):

1. (Sim1) Start with 1000 bacteria, all resistant, no antibiotic, no external flora.

2. (Sim2) Start with 1000 bacteria, 75% resistant, 25% sensitive, antibiotic level 1, no external flora.

3. (Sim3) Start with 1000 bacteria, 50% resistant, 50% sensitive, antibiotic level 1, no external flora.

4. (Sim4) Start with 1000 bacteria, 25% resistant, 75% sensitive, antibiotic level 1, no external flora.
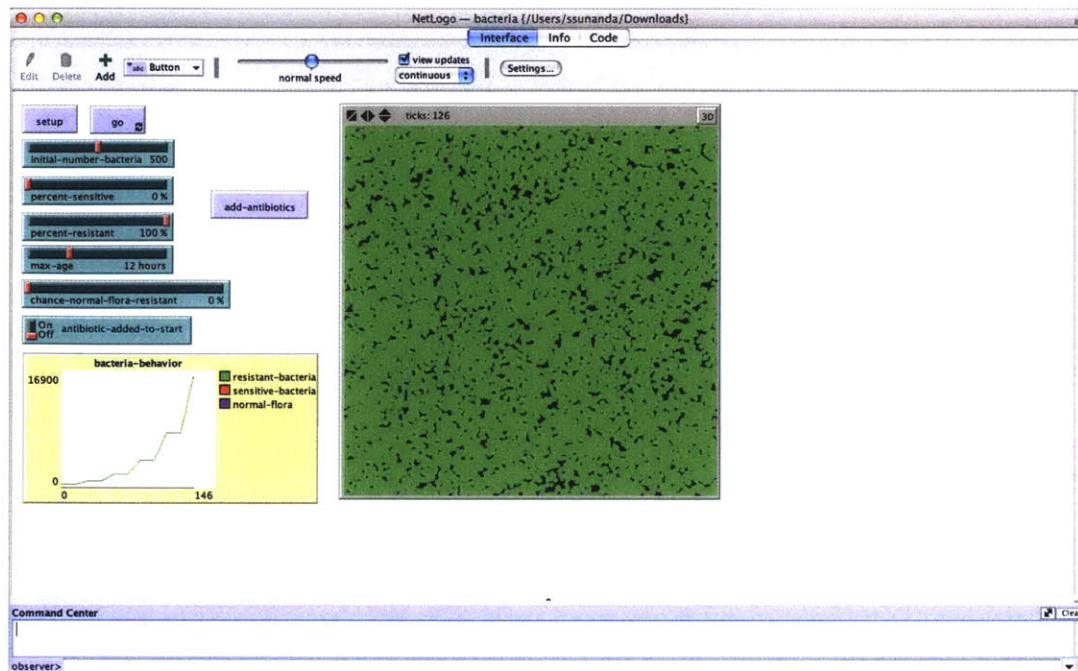
Figure 6-2: **NetLogo Bacteria Experimental Set up.** The environment featured a graphical representation of the bacterial population and plots over survival over time, as well as sliders to change parameters.

5. (Sim5) Start with 1000 bacteria, 0% resistant, 100% sensitive, antibiotic level 1, no external flora.

6.5   Evaluation

The computational package bacteria.nlogo did not consider many aspects of the actual physical conditions, notably, the temperature, media, material, or growth substrate (i.e. liquid or plate). It is possible that the creators assumed the conditions would be standard 37C on an LB agar plate, but this leaves little room for environmental modification. Additionally, the material substrate is unaccounted for, which is key to this case study. Since this was not possible, antibiotic concentrations and bacterial sensitivity were used to show how population would change as a result of local inputs.

Parameter	In vitro	In silico
Temperature	◆	
Media	◆	
Antibiotic	◆	◆
Material	◆	
Growth substrate	◆	
Coculture	◆	❖

Figure 6-3: **Comparison of *In vitro* and *in silico* Parameters.**

6.6   Results and Discussion

This project is an ongoing one; the data shown here are preliminary results, as, given the depth of this area,the project will continue for the foreseeable future. The results from the *in vitro* comparisons of materials are highly interesting and will be verified especially with regards to how surface area of material impacts the growth of bacteria, and if mixes of different materials (in multimaterial prints) can impact bacteria survival or morphology.

The top-down approach for *in silico* modeling in this case was a gross oversimplification of the scenario. Even if edits were made to the program, it is likely that it would not capture the biochemical kinetics or surface material-bacterial interactions and thus would not be an accurate predictor. A bottom-up modeling system, such as CellModeller or CompuCell3D, may be better in this case.

In terms of the experimental setup, several changes could be implemented to yield more robust results in the future. For example, a strain that has antibiotic resistance could allow for better prevention of contamination; data should be taken at multiple

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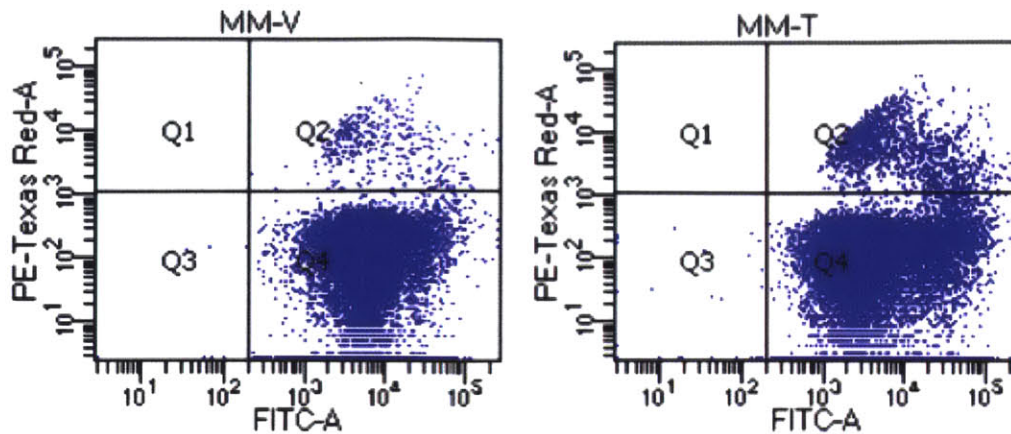
Figure 6-4: **LIVE/DEAD Assay Results.** Cell samples exposed to control polystyrene, VeroGrey, and TangoBlackPlus were stained and analyzed. Shown here are results from VeroGrey (left) and TangoBlackPlus (right). Points in Q3 indicate dead cells, points in Q4 illustrate living.

time points to show growth and not simply output at the end; and overall the number of trials should increase.

Regardless of these changes, the results here offer an alternative to the prevailing mindset in the community that prints with Stratasys resins, which is that Tango materials are highly toxic and Vero are entirely biocompatible. In this study, colony counts could only be done after a 10^6 dilution, as cells grew after being exposed to all materials (Figure 6-5, Figure 6-6). This was verified by the results of the LIVE/DEAD assay, which only showed a slight difference between the materials (Figure 6-4). Importantly, several of the unpublished studies that claim knowledge of toxicity are done in microfluidic pieces, which feature a high surface area and low volume; this study had the reverse, so it is possible there is a "dose curve" based on material exposure to cells.

The difference in growth, if it is verified, could be used as a templating strategy in a mask. Alternatively, a lack of change based on material increases the types of
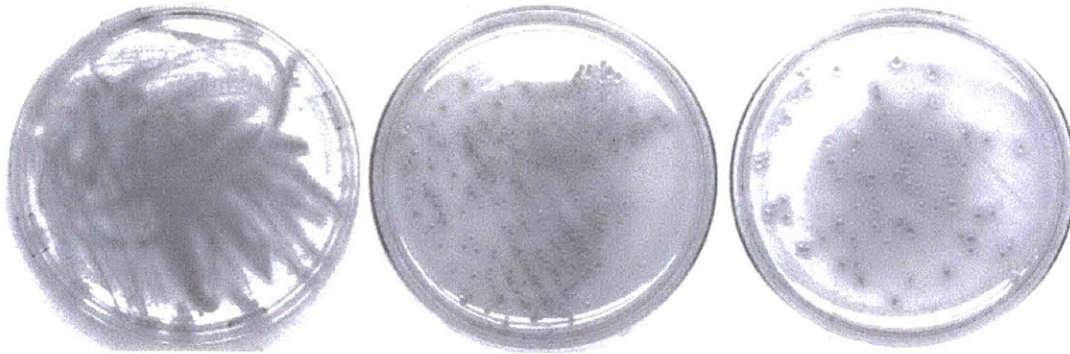
Figure 6-5: **Colony growth post-cytotoxicity assay.** After cells were grown overnight in 6 well culture plates with control (glass), VeroGrey, and TangoBlack, cells were sampled from each to assess viability. Shown from left to right is colony growth with Control (lawn), VeroGrey (562 colonies), and TangoBlack (99 colonies).
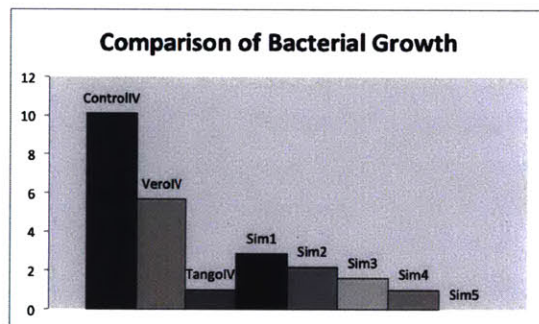
Figure 6-6: **Cell counting *In vitro* and *in silico*.** Counted colonies in both conditions are represented as ratios to one another.

material that can be used in this project to interface with the living human and microorganisms both. However, there are other differences in the materials - such as swelling in solvents - that can yield future templating strategies.

Chapter 7

Conclusions and Future Work

Discussions

7.1 Overall Evaluation

The overall results indicate that NetLogo as a platform works fairly well on the large, architectural scale, and a lot of work has been done to adapt its structure to honeybee behavior in particular. While the simulations were not perfect, they did achieve a similar result to the actual physical study, and can certainly be adapted in the future to account for a variety of environmental conditions. However, there is no way for the user to interact with the visualization itself and examine in 3D space, which is limiting and hinders the transition from CAD tools to bioCAD. In this case, the main purpose is simulation and not just rendering, though, so it remains useful. If the model organism changed though - to those not so well studied - the entire process may have to be changed.

On the cellular scale, in comparison, NetLogo does not have any established models

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that can be useful in analyzing even simple behavior for hybrid built objects. Great effort could be put into modifying this top-down approach to account for new materials and environmental conditions, but it is possible that more specified tools, such as CompuCell3D and CellModeller, would be better suited to the job. These would perhaps illustrate spatial patterning better, instead of having visual "placeholders" and having the bulk of simulation information being in the form of graphs.

There was also something missing in regards to software that can truly account for the self-assembly and autonomous nature of both biomaterials and living organisms as part of a designed structure - that acts irrespective of the designer. The criteria used in Chapter 4 could be re-assessed to priortize this true modeling component, and thus graphical representation or DNA editing systems, like VectorNTI, would be excluded.This may fall into the discussion of purpose, which is difficult to define while there is still a focus on modularity.

With regard to the proposed workflow, it is clear that implementation is not feasible until robust computational tools are developed either using existing software or from scratch. In addition, it is clear that the criteria for useful bioCAD and simulation software must be refined - though careful consideration was done in this case, the criteria still had to be modified to include all possible approaches. For experimentation, there may also have to be adjustment made because it is likely that real life scenarios will always have a greater range of possibilities than simulations can include.

In terms of the biodesign projects in general, they may not be determined as successes or failures. While the Synthetic Apiary did lead to the creation of a novel space, maintenance was difficult and costly, and the solution was not fully sustainable yet. The project did offer the opportunity to learn about swarm and dynamic systems on a scale that has not yet been done, and is informing new work on other swarm systems, such as ants. The living wearables project is currently ongoing, with a projected deployment date in the fall. There is still a great deal of exploration going on, but

the first prototype series will be another opportunity to try *in silico* modeling before moving into the physical world.

7.2   Future Work

The next steps for improving upon the *in silico* to *in vitro* transition in hopes of establishing a useful biodesign workflow can be broken down into three categories: software improvement, standardization of simple assays, and expansion of comparisons. These will be implemented in the existing case study - living wearables.

The software selected here was one option out of over a hundred. Even during the course of this thesis, new evidence was generated that suggest other more powerful agent based systems, such as Shell for Simulated Multi Agent Systems (SeSAM) [9] and Recursive Porous Agent Simulation Toolkit (RePAST). These, while they have no existing examples for both case studies discussed, could be used as a basis for developing more accurate models. In addition, a new approach - using different biodesign tools for different scales - could be tested. In this case, a lattice-based or hybrid model for microorganism growth could be considered.

More simple assays, with standardized conditions and sampling, must also be established over the course of this project. For instance, the difference in cell sampling (with wash step or without) could significantly alter results regarding cytotoxicity, and thus results should always be presented in context. After cytotoxicity, behaviors such as chemotaxis of cells towards materials soaked in different chemicals can be tested. For all assays done, more effort must be made to compare *in silico* simulations to *in vitro* results, so that a tangible product as well as a verified workflow may be presented at the end.

7.3 Issues and Perspectives

Two of main issues that came to light in this process were regarding the structure of software. Often, computational tools for biology seemed overly application specific and with user dictated outcomes, both of which lessen the applicability and simulation power. There seems to be less support for modularity in general. Ideally, a tool could be created that would take in measurements such as known growth rate, colony size and morphology, as well as mathematical models about interactions and behavior for each of the three components (living, non living, and environment). While this would be a bottom up approach, the detail may allow for more accurate simulation and incorporation of previous work. Furthermore, a range of possible simulations - instead of a single outcome - could be shown, because rarely in nature does the same thing happen twice. Natural errors that often lead to aberrations from a simulation could then be included and allow researchers to work backwards from an experimental result to determine what went wrong. However, it is no trivial task to create an accurate rendering and simulation engine that is both modular and artfully "broken" so that it may more accurately represent a wide range of realistic scenarios. Only one company known, Autodesk, is currently working on creating such a system that combines features from molecular Maya with other tools to allow users to build hybrid renders.

At the same time, there is a large push to bring agent based modeling and other computational approaches to biology. A huge amount of work is being done to standardize languages and formats in biology and computation, bringing with it terms such as Synthetic Biology Markus Language (SBML), Synthetic Biology Open Language (SBOL), and Cell Markup Language (CellML).The field is now being stretched and changed to dig into new problem contexts, and offers a top down approach to complex systems. In the future, however, the right perspective may not be bottom

up or top down - but, as Nobel Laureate Sydney Brenner once stated, a "middle out" approach may be the correct one [70].

Chapter 8

Reflections

Impacts

Man, one harmonious soul of many a soul

Whose nature is its own divine control

The Earth, Act IV

Prometheus Unbound

Percy Bysshe Shelley

8.1 Contributions

8.1.1 E14-470 BL2 Lab

Throughout the process of this thesis, several novel contributions have been made in both tangible and intangible forms. The most significant physical contribution was

the creation of the first Biosafety Level 2 (BL2) Biology "wet" Lab in the E14 Media Lab. The lab, found in E14-470, is a new space for interdisciplinary exploration (Figure 8-1). The process of creating the first "designer's wet lab" began in fall 2014, with the initial renderings and concept done by myself, Chikara Inamura, and Neri Oxman. I was spearheading the scientific aspects of design, such as how to include space for benches, storage, and BL1 or BL2 material. Given the ever-changing nature of Mediated Matter's work, it was a significant challenge to include features for experimentation with virtually any chemical, living system, material, or tool.

Furthermore, the space had to align with the E14 Media Lab's commitment to transparency, embodied in the architecture. Many traditional labs have limited access and physical visibility, so are naturally away from public areas. The E14-470 lab is clearly visible from the 3rd floor atrium, 5th floor seating area, and accessible to E15 as well; it has nearly two full walls made of transparent sheet glass; it is deliberately designed as an active display of investigative science. To achieve a monolithic look, great care was taken to select the material and profile of the main bench, cabinets, and casework; for example, chemical testing was done in-house to assess various surface materials. Each decision was examined from both a design perspective as well as a scientific one, to marry aesthetic with functionality.

In January 2016, E14-470 was certified and approved by MIT Facilities, MIT Environmental Health and Safety, and the Cambridge City as a usable space for research. I then took on the roles of manager, technician, and researcher, and was fortunate to form cross-campus connections to quite literally bring the lab to life. At present, the E14-470 lab houses 12 strains of bacteria and fungi, has a mix of traditional and state-of-the-art additive manufacturing tools (such as the KUKA Robotic Arm), and is the site of collaboration between the Mediated Matter group, the Voigt and Lu Labs at the MIT Synthetic Biology Center, and the Silver Lab at the Harvard WYSS Institute for Biologically Inspired Engineering. We also are fortunate to share re-
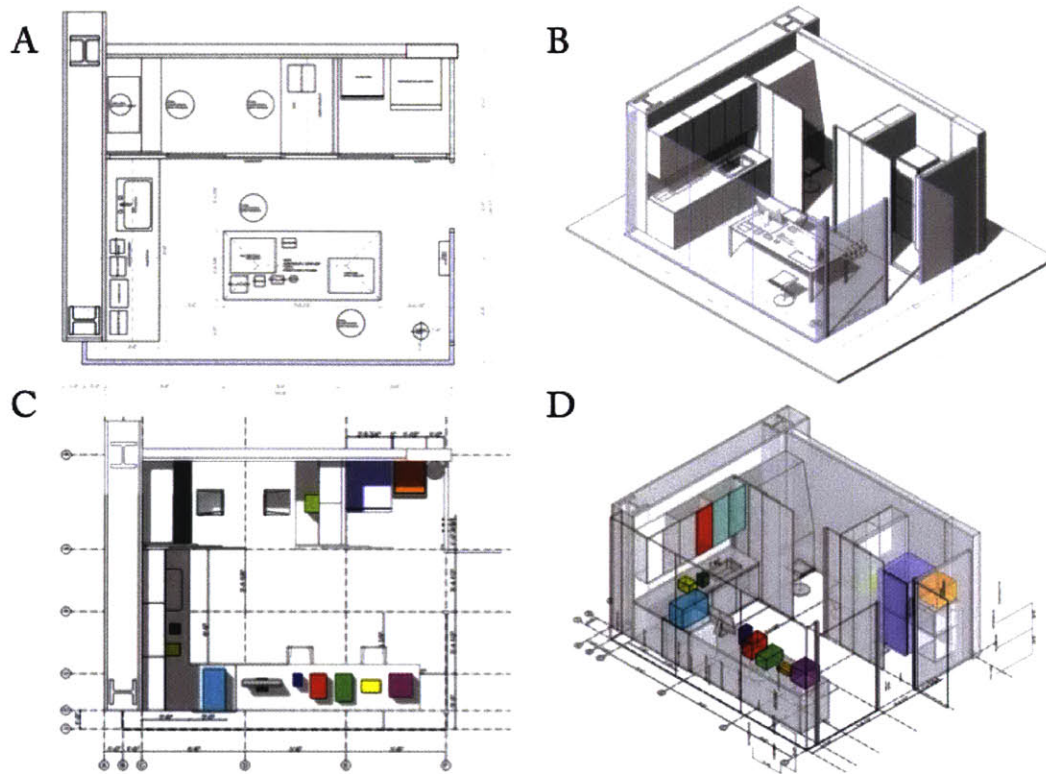
Figure 8-1: **E14-470 Design Progression.** Initial renders of the space (top (A) and side (B)). The design went through many iterations to accommodate regulations of the Media Lab, MIT, and the city of Cambridge. A later version is shown in C (top) and D (side) including equipment. Illustrations by Chikara Inamura.

sources with Professor Ed Boyden and the Synthetic Neurobiology Group, Professor Neil Gershenfeld and the Center for Bits and Atoms, Professor Joe Jacobson and the Molecular Machines Group, Professor Mriganka Sur and his lab at the Department of Brain and Cognitive Sciences, Dr. Vanessa Cheung and Anthony Fuccione in the Department of Biology, and the Synthetic Biology Center at MIT. In the future, we hope to facilitate an exchange of knowledge and tools with these labs and others, to contribute novel research and execute significant projects.

8.1.2 Synthetic Apiary

Another physical contribution made during the course of this thesis is the Synthetic Apiary itself, which is the first of its kind large scale indoor controlled laboratory environment. In our case, it was used to examine honeybees; however, it may be adapted for a range of other organisms. The full patent description, which was filed on April 11, 2016, can be found in Appendix B.
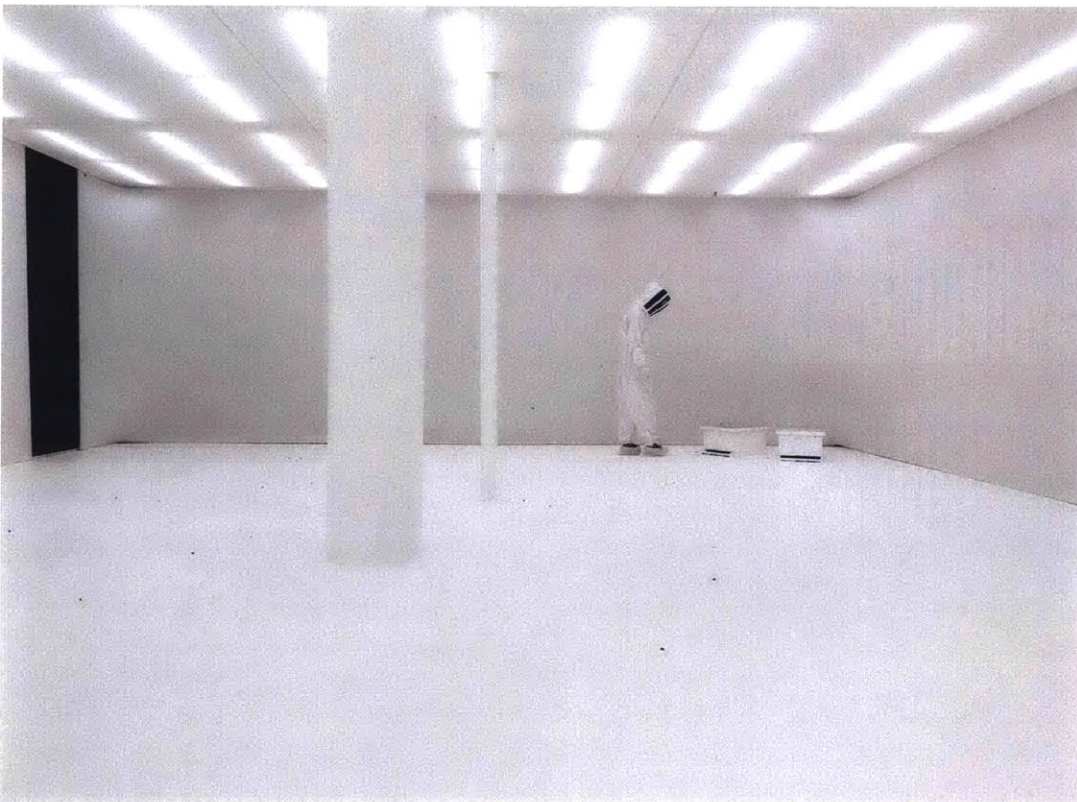
Figure 8-2: **Inside the Synthetic Apiary.** A view of the two initial hives inside the Synthetic Apiary, installed November 2015.

The process of creating the Synthetic Apiary began in the summer of 2015, when two hives of honeybees (Best Bees Company) were installed on the roof of E15 (Figure 8-3). These were used to observe and establish typical, healthy living conditions

for honeybees, as summer is a particularly active time for those in New England. Automated monitoring of these hives was done using TetraScience systems, which provided real-time data of temperature, humidity, and traffic in and out of each hive.

Figure 8-3: **Honeybee Hives on E15 Roof.** Two *Apis mellifera* hives were installed on the roof of building E15 in summer 2015 for initial observations in a semi-natural environment.

As the temperature dropped below 40F, the hives were moved indoors into the newly designed Synthetic Apiary. The novelty of the Apiary lies in the amount of control provided; air, temperature, light, food, water, and other factors were highly controlled and could be modulated to decouple hive behaviors, without risk of complete loss. Such a setup can be useful for farmers looking to pollinate crops within greenhouses, for hive-scale behavioral experimentation (e.g., swarm), and for inter-hive community observation. Given the known interdependency between bees and the environment,

being able to tune specific factors for any period of time to examine responses can elucidate bees' biosensing capabilities and how the changing surroundings may aid or impair their survival. The laboratory environment itself allows for easy isolation and sampling of products for testing (Figure 8-2). For example, pollen from frames or natural structures can be sampled and sequenced as a correlate to the type of synthetic nutrition provided, which may help decode how bees process foraged material (Figure 8-4). Furthermore, such an environment can serve as a negative control for bee microbiome studies, which focus on how the microbial community in the bee gut changes with respect to the surroundings (e.g., urban versus rural hives).
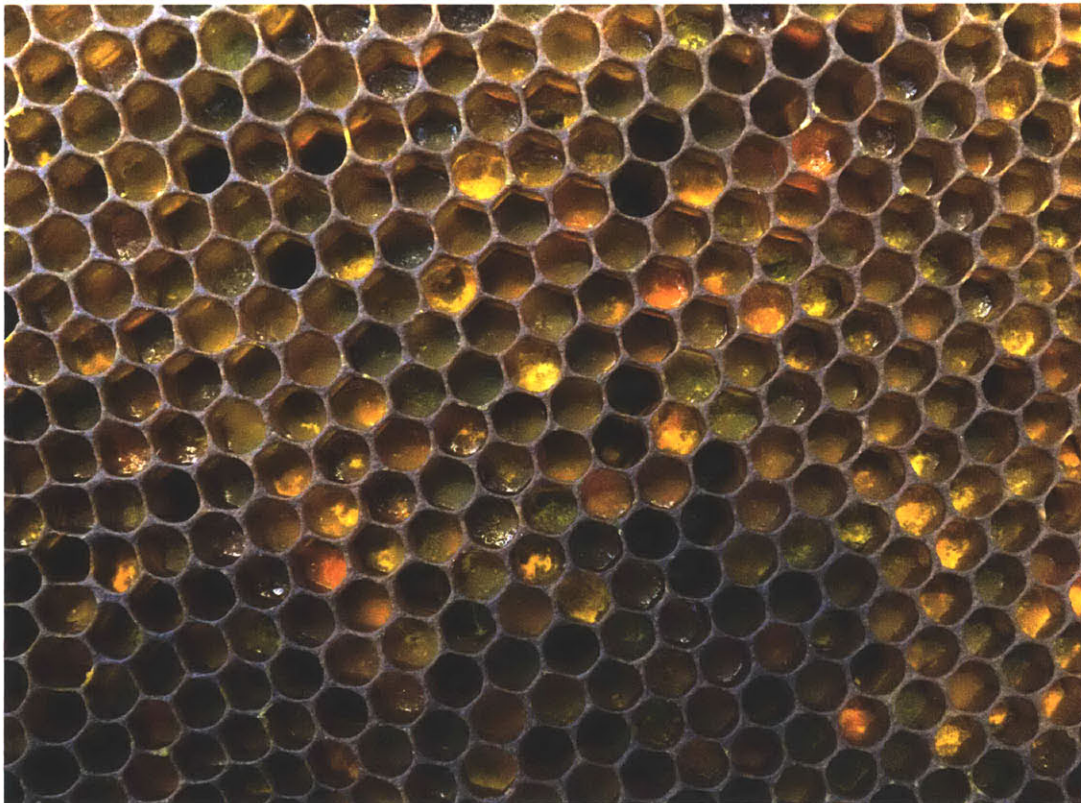
Figure 8-4: **Pollen within Langstroth Frame.** Pollen stored in cells of a natural Langstroth hive. The variety of colors indicates the variety of flowering plants that bees visited.

8.1.3 3D Printed Materials for Experimental Biology

Further novel information found in this thesis is currently under preparation for papers in 2016. As a group, we aim to release papers regarding the methods and new techniques used in *Mushtari*; the capabilities of high resolution 3D printing; and the functions of multimaterial prints in biomimetic and actual biological systems, which has its roots in the second case study presented here. All of this work is done using the Object Connex500, which can now print several materials with digital on-the-fly mixing, with down to 60 micron resolution [90]. The catalog includes flexible, rigid, opaque, and transparent materials, which we hope to use in conjunction with living, possibly synthetically engineered, systems on multiple scales. *Mushtari* was the first prototype in this line of work, and expanded lab-on-a-chip style 3D printed microfluidics into meso and macrofluidics for larger systems. The goal in that case was to open a new interface for experimentation; the small patch tests examining flow of liquid media in fluidics set the stage for the more rigorous biocompatibility studies reported in this thesis. The preliminary data shown here is relevant for any 3D printing and synthetic scaffold work applicable to biology - and is actually contrary to current unpublished work from other groups - and is a step towards standardizing a very varied approach. New insights have already been found, such as the impact of surface area on cytotoxicity, and new methods for low-cost sterilization of prints.

8.1.4 Computational Tools for Biological Systems

There is currently a large push for development of new computational tools for biological systems. One of the major arms of this movement is for agent based modeling as a top-down approach to representing intracellular and intercellular interactions. This work, while it uses a fairly low-power agent based engine, still gives an example of the utility of the approach - and is one of the only projects that offers the simulation

and a physical test in reality.

8.2 Broader Impacts

There is a notable amount of inspiring work happening at the intersection of biology and many other fields, making this an exciting time in science. The broader impacts of this particular work are mainly based in the approach - which is an attempt to introduce a new method for generating replicable results into the field, and expand the catalog of projects in biodesign. While the inspiration and techniques do incorporate some rigor from both biology and design, there is also deliberate naivete. A biologist entering biodesign should not have to be a trained designer, and a designer should not need to be a practicing biologist for a profound impact to be made; in fact, the lack of experience can allow for creative and unrestrained thinking, which will add novelty to each respective field. Moreover, interdisciplinary collaborations are then founded that foster original and infectious thought. Mediated Matter, for instance, is in its essence rooted firmly in design - but has worked with organisms from three different kingdoms (and ten species) of life in the past year; and now collaborates with several biology labs and companies to execute its projects. E14-470 is a physical space where this exploration can be carried out, and each project is a demonstration of this naive (yet, hopefully powerful) thinking. The work of this thesis adds another interesting push to the new and burgeoning field of biodesign, both in its philosophy and its tangible outputs.

Appendix A

Appendix

Publications, Patents, and Awards

2016

Methods and Apparatus for Synthetic Apiary (patent pending). M. Kayser, S. Sharma, J. Duro Royo, N. Wilson-Rich, N. Oxman

3D Printed Multimaterial Microfluidic Valves (submitted paper). S. Keating, I. Gariboldi, W. Patrick, S. Sharma, D. Kong, N. Oxman.

Director's Focus Group for Future of Art Design Science and Engineering

2015

Emerging Voices Award (Architectural League NY) as part of Mediated Matter

Innovation by Design Award: Fashion (FastCompany) for *Mushtari* as part of a team - W. Patrick, D. Kolb, C. Bader, S. Sharma, S. Hays, P. Silver, N. Oxman.

Designing the Ocean Pavilion: Biomaterial Templating of Structural, Manufacturing, and Environmental Performance. L. Mogas Soldevila, J. Duro Royo, M. Kayser, D. Lizardo, W. Patrick, S. Sharma, S. Keating, J. Klein, C. Inamura, and N. Oxman, *Proceedings of the International Association for Shell and Spatial Structures (IASS) Symposium, Amsterdam.*

Appendix B

Appendix

Synthetic Apiary for Controlled Year-round Observation of Apis mellifera

Electronic Acknowledgement Receipt

EFS ID:	25458863
Application Number:	62321068
International Application Number:	
Confirmation Number:	8405
Title of Invention:	Methods and Apparatus for Synthetic Apiary
First Named Inventor/Applicant Name:	Markus Kayser
Customer Number:	88723
Filer:	Stephen Riley Otis
Filer Authorized By:	
Attorney Docket Number:	MIT_20160411_A
Receipt Date:	11-APR-2016
Filing Date:	
Time Stamp:	18:32:29
Application Type:	Provisional

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$ 260
RAM confirmation Number	5532
Deposit Account	506454
Authorized User	OTIS, STEPHEN
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	
Charge any Additional Fees required under 37 CFR 1.16 (National application filing, search, and examination fees)	
Charge any Additional Fees required under 37 CFR 1.17 (Patent application and reexamination processing fees)	

Charge any Additional Fees required under 37 CFR 1.19 (Document supply fees)

Charge any Additional Fees required under 37 CFR 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Specification	apiary_specification_E.pdf	789364 3789a94d029f44cae4b8bc21ea311ae65cf2 f5a9	no	27
Warnings:					
Information:					
2	Provisional Cover Sheet (SB16)	apiary_coversheet.pdf	1477120 33afad51108eb2320b1509ef9c79d74b0b4 ecbb4	no	4
Warnings:					
Information:					
3	Application Data Sheet	apiary_ads.pdf	1793280 57f8e2636c1a910aadf174a5a2a6dc1e8063 56c5	no	9
Warnings:					
Information:					
4	Fee Worksheet (SB06)	fee-info.pdf	29255 435a659321957d27432983423fd4dd545bc 4accf	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	4089019		

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National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Provisional Application for Patent Cover Sheet
This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c)

Inventor(s)

Inventor 1
[Remove]

Given Name	Middle Name	Family Name	City	State	Country ¡
Markus		Kayser	Cambridge	MA	US

Inventor 2
[Remove]

Given Name	Middle Name	Family Name	City	State	Country ¡
Sunanda		Sharma	Cambridge	MA	US

Inventor 3
[Remove]

Given Name	Middle Name	Family Name	City	State	Country ¡
Jorge		Duro Royo	Cambridge	MA	US

Inventor 4
[Remove]

Given Name	Middle Name	Family Name	City	State	Country ¡
Noah		Wilson-Rich	Boston	MA	US

Inventor 5
[Remove]

Given Name	Middle Name	Family Name	City	State	Country ¡
Neri		Oxman	Cambridge	MA	US

All Inventors Must Be Listed – Additional Inventor Information blocks may be generated within this form by selecting the **Add** button.   [Add]

Title of Invention	Methods and Apparatus for Synthetic Apiary
Attorney Docket Number (if applicable)	MIT_20160411_A

Correspondence Address

Direct all correspondence to (select one):

● The address corresponding to Customer Number	○ Firm or Individual Name
Customer Number	88723

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.
⦿ No.
○ Yes, the invention was made by an agency of the United States Government. The U.S. Government agency name is:
○ Yes, the invention was under a contract with an agency of the United States Government. The name of the U.S. Government agency and Government contract number are:

Entity Status
Applicant asserts small entity status under 37 CFR 1.27 or applicant certifies micro entity status under 37 CFR 1.29

○ Applicant asserts small entity status under 37 CFR 1.27

○ Applicant certifies micro entity status under 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.

◉ No

Warning

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

Signature

Please see 37 CFR 1.4(d) for the form of the signature.

Signature	/Stephen Otis/			Date (YYYY-MM-DD)	2016-04-11
First Name	Stephen	Last Name	Otis	Registration Number (If appropriate)	64226

PTO/AIA/14 (11-15)
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| Application Data Sheet 37 CFR 1.76 | Attorney Docket Number | MIT_20160411_A |
| | Application Number | |

Title of Invention	Methods and Apparatus for Synthetic Apiary

| Customer Number | 88723 |
| Email Address | stephen.otis@gmail.com | Add Email | Remove Email |

Application Information:

Title of the Invention	Methods and Apparatus for Synthetic Apiary		
Attorney Docket Number	MIT_20160411_A	Small Entity Status Claimed	☐
Application Type	Provisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)		Suggested Figure for Publication (if any)	

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For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	◯ US Patent Practitioner	◯ Limited Recognition (37 CFR 11.9)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	MIT_20160411_A
	Application Number	

Title of Invention	Methods and Apparatus for Synthetic Apiary

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)

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Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

			Remove
Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

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Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

| **Application Data Sheet 37 CFR 1.76** | Attorney Docket Number | MIT_20160411_A |
| | Application Number | |

| Title of Invention | Methods and Apparatus for Synthetic Apiary |

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	MIT_20160411_A
	Application Number	
Title of Invention	Methods and Apparatus for Synthetic Apiary	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

[Clear]

◯ Assignee	◯ Legal Representative under 35 U.S.C. 117	◯ Joint Inventor
◉ Person to whom the inventor is obligated to assign.		◯ Person who shows sufficient proprietary interest

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here.	☒
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Additional Applicant Data may be generated within this form by selecting the Add button.

Assignee Information including Non-Applicant Assignee Information:

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	MIT_20160411_A
	Application Number	

Title of Invention	Methods and Apparatus for Synthetic Apiary

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

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Prefix	Given Name	Middle Name	Family Name	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1	
Address 2	

City		State/Province	
Country i		Postal Code	
Phone Number		Fax Number	
Email Address			

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Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Stephen Otis/			Date (YYYY-MM-DD)	2016-04-11
First Name	Stephen	Last Name	Otis	Registration Number	64226

Additional Signature may be generated within this form by selecting the Add button.

| **Application Data Sheet 37 CFR 1.76** | Attorney Docket Number | MIT_20160411_A |
| | Application Number | |

| Title of Invention | Methods and Apparatus for Synthetic Apiary |

SYNTHETIC APIARY

April 11, 2016

Mediated Matter Group
MIT Media Lab

Sunanda Sharma
Markus Kayser
Jorge Duro Royo
Noah Wilson-Rich
Neri Oxman

TABLE OF CONTENTS

I. Introduction

Laboratory environments for behavioral studies are often created and finely tuned for particular species. Model organisms – from zebrafish to monkeys – have standard protocols and environments for observation and behavioral assays. However, insects such as honeybees, which traverse great distances and are highly dependent upon natural environmental conditions, present a significant challenge to this control-focused paradigm. In the wake of widespread phenomena such as hive abandonment, it is increasingly important to understand where outdoor colonies fit within nature to contextualize their behavior and survival. Additionally, in order to understand behaviors such as fabrication, material production, foraging, and hierarchical dynamics, further research must be done to rigorously identify and modulate environmental and social parameters – and thus, new laboratory environments must be established. In this text, we introduce a novel controlled setup for experimentation with swarm insects that operate in large areas, and highlight features that mimic seasonal natural atmospheres.

II. Background

A. Eusocial Insects

For hundreds of years, humans have observed natural living systems, and have remained fascinated with sociobiological dynamics. A subset of animals within insect class exhibit eusocial behavior; that is, they live in groups as adults, cooperatively take care of juveniles, have a reproductive division of labor, and overlap of generations[1,2]. Social or "swarm" insects fall in five orders: *Isoptera*, *Hemiptera*, *Thysanoptera*,

Coleoptera, and *Hymenoptera*. Ants, bees, and wasps all fit within this final order, and are commonly seen examples of highly organized societies.

In the last few decades, a large amount of research has been done observing behaviors and capabilities of all three groups, and how swarm communities may have advantages over other social systems – such as by creating widespread but connected sensor networks, responding dynamically and predictively to environmental changes, and coordinating for population success. Many surprising revelations have been unearthed, such as bees' ability to detect explosives[3,4] and air quality[5], and how ants leave pheromone trails in order to communicate[6] have influenced how many human systems are designed[7].

B. Fabrication

Additionally, ants, bees, and wasps conduct natural forms of fabrication, by tunneling (subtractive) or hive building (additive). For this reason, their behaviors are intrinsically applicable to building and manufacturing practices. Honeybees in particular are known for creating beautiful and complex wax hives, which can weigh hundreds of pounds[8] and house up to 20,000 (feral) – 80,000 (managed) bees[9]. The honeycomb hexagonal packing has been mimicked in architecture[10] and product design[11], while the naturally created products – wax, propolis, and honey – have been harnessed for human health[12] and products[13].

Controlled production of these materials has been explored by artists and designers, and a number of parameters characterized that affect the amount, rate, and quality, including gravity[14], and foundation structure[15]. In most cases, bees were used as "co-fabricators" – they conducted natural wax building on a novel structure or base, but their intrinsic behavior was purposely unaltered.

C. Honeybees – (Apis)

Honeybees, *Apis sp.*, are an ideal model organism due to their well-characterized and easily observable natural behaviors, reproduction rate, population size, complex communication and organization, and visible social behaviors. There are many subspecies that have been domesticated for hundreds of years, including *Apis mellifera*, the European honeybee. This subspecies is relatively gentle, produces large amounts of harvestable honey, create hives above ground, and hold an integral place in many cultures around the world[16].

Apis mellifera typically live in temperate and tropical zones, and are active outside the hive when temperatures are above about 5C; below this temperature, such as during the winter, honeybees survive within a colony. A colony can have between 5,000 and 80,000 bees, a majority of which are female worker bees. Male drones are used mainly for breeding and excluded from the hive during the winter, while a single fertile queen, that is larger and lives for 3-5 years, lays eggs[17]. This hierarchical society is further

divided into workers of various types dependent on role – nurses, foragers, and possibly others[18].

Honeybees have complex forms of communication that include physical (such as the "waggle dance"[19] and chemical signaling[20], and thus are a highly studied example of "swarm" – collective motion by large numbers of a single species[21]. The combination of swarm behavior and additive manufacturing makes bees particularly interesting for designers and scientists alike, and suggests possible applications such as new robotic systems, computational methods, and even urban-scale interaction platforms.

Bees have been scientifically studied for years, both on the population and individual scale. Generally, assays are conducted in the field – wooden hives are placed in open areas that have unlimited access to food source, and researchers observe their behavior with regard to particular areas over time[22-25]. Since the 1970s, different methods have been used to tag bees to more easily gather data in the field, and range from paint marking[26] to attaching small RFID tags[27]. However, while a natural environment is highly useful for examining intrinsic behaviors, it is difficult to identify which factors influence hive activities precisely. Individual bees are studied in lab environments for short periods of time, and can even be caged or harnessed[28,29] instead of free flying in small areas[30].

D. Traditional and Urban Beekeeping

Generally, bees used in experimentation and art are allowed to live naturally or within traditional hive environments. Beekeeping is done in an outdoor environment using wooden hives often with removable frames in Langstroth, top bar, or Warre styles. In recent years, the urban beekeeping movement has gained foothold in several countries, and bees are being moved from remote farms into metropolis roofs and buildings[31,32]. In regions above the frost line, such as in New England, beekeeping concludes when the temperature drops below ~40F, for approximately 4-5 months in the winter season, during which the hives are "wintered" either indoors (at low temperature) or outdoors[33]. Alternatively, bees may be housed in observation hives with plastic or glass windows, but also must forage outdoors and thus cease activity in cold weather. During this time, both outdoor and indoor bees remain inside the hive for warmth, precluding any material creation or brood formation. While bees generally store honey during the spring and summer months in preparation for fall, beekeepers often give them extra sugar water or sugar syrup, often mixed with health supplements, as well as pollen substitute that contains essential building blocks.

In recent years, several diseases, pests, and widespread phenomena that affect honeybees have come to national attention. Small pests, such as Varroa mites, and diseases such as European foulbrood and wing rot, can impact population health and bee productivity. "Colony collapse disorder", in which bees take their queen and leave the hive entirely, was observed between the mid-2000s and early 2010s[34]. Since then,

patterns of absconding, dwindling, and swarming have been seen as well. In addition, about 40% of hives in the United States have died during the winters; in colder areas, such as New England, over 60% of hives have died[35].

III. Motivation and Prior Art

In the most mysterious cases of absconding and other hive abandonment events, beekeepers do not know where bees go; as the swarm leaves the hives, there is no easy way to track or trap them, and it is often difficult to understand why this occurs. Attempts have been made previously at enclosing honeybees within structures, most commonly greenhouses, which would theoretically limit absconding events[36-39]. Insertion of honeybee hives in greenhouses for plant pollination have been unsuccessful due to hive disorientation, pesticide use, as well as nutrition and lighting issues. Furthermore, in greenhouses as well as other built structures such as observation hives, a passage is always provided to the outside to allow for foraging, which reduces the amount of control and delivers a means of escape. To date, the authors have not found any existing project in which solely honeybees are kept enclosed indoors during the winter season without the purpose of pollination and with free access within a space.

The most recent prior art was a patented indoor bee raising apparatus consisting of a single small beehive, netted flying area, and light source[40]. However, the emphasis here was to create an environment to temporarily raise a single hive of bees for observation

while indoors, and not a sustainable long term setting for multiple honeybee populations.

In this project, the aim is to create a space in which honeybees can thrive - with access to resources and minimal pests and disease - and create a model that can be used across similar species. The ideal natural environment could be maintained without regard to season, and research activities such as data collection and behavior observation can be done in a controlled and rigorous manner. Such an environment can be adapted in the future, especially in urban contexts, to create safe spaces for honeybees while complementing the built human landscape. Our attempt at this environment – termed the Synthetic Apiary – is an interdisciplinary foray into a complex natural problem, and yielded several novel proofs-of-concept that may be improved and further demonstrated in the future.

IV. Materials and Methods

An entirely sealed tensile structure within the 200m^2 experimental space was designed and built using a framework of wooden crossbeams stretched between white elastic fabric (Dazian Trapeze Plus white 122", 90% polyester 10% Spandex), above which full spectrum low heat lights (144 'Spectra Brite' full spectrum 48" fluorescent tubes, 32 watts, 93 cri) were fixed (Figure 1-4). This allowed for a large space for habitation and foraging with minimal crevices and corners, as well as easy viewing of hives as well as individual bees. Additionally, it provided a bright space with dispersed light to mitigate

one commonly seen issue in greenhouses, in which honeybees are attracted to light and injure themselves through repeated contact. The architecture of the space consisted of a central rectangular sealed enclosure with two entrance rooms. Each entrance room had netted magnetically self-sealing doors into the main enclosure as well as to the outside parameter of the entire enclosure, thus preventing dislocation of bees. The concrete floor of the enclosure was cleaned and painted white, and was swept and mopped every two days with low concentration bleach in hot water.
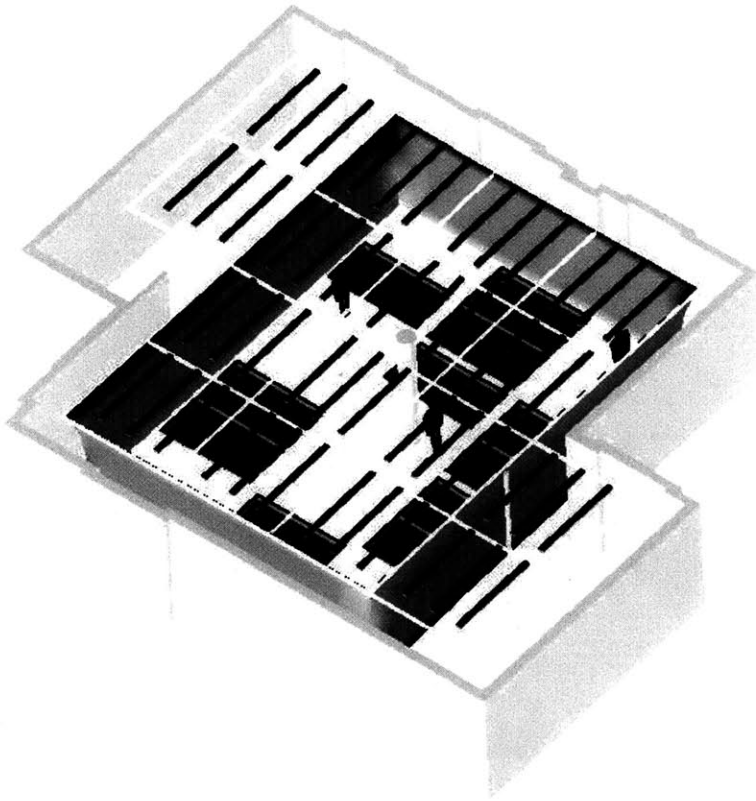

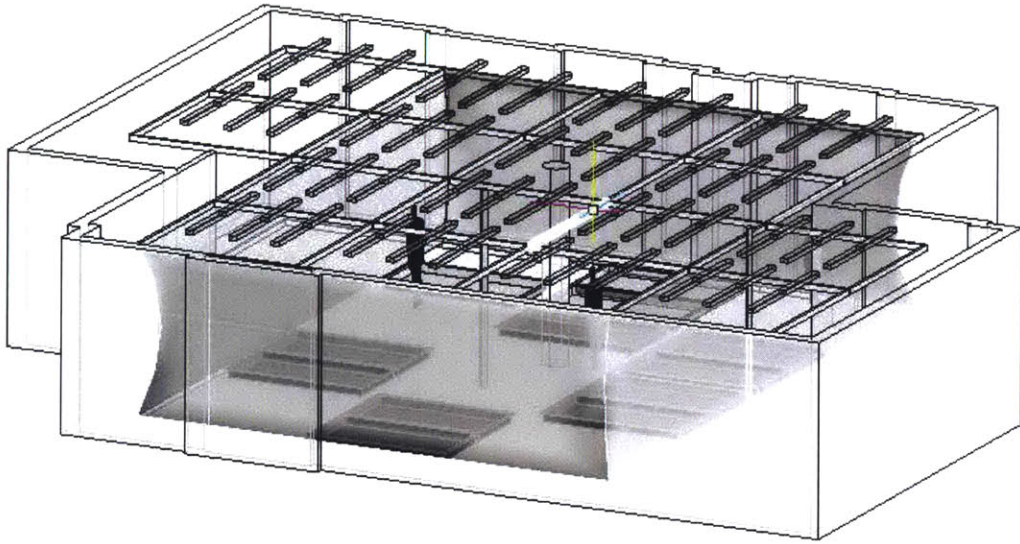*Figure 1. Top down rendering of designed enclosure.*

Figure 2. Side rendering of designed enclosure.

Figure 3. Actual built enclosure, before insertion of hives.

8 wooden Langstroth hives (approx. 160,000 total) of European honeybees, *Apis mellifera,* were introduced (Best Bees Company) (Figure 4, Figure 5). Each hive had 1-3 boxes, each of which had 10 frames with foundation wax. Climate conditions were maintained at between 50-70F, at least 50% RH, 15 hours simulated and incremental daylight, and appropriate air circulation and ventilation; this mimicked a spring-like natural environment, during which bees are highly active. This may also be tuned to represent other seasonal climates during which particular behaviors and activities may be observed. The system features standard residential heating, a heating ventilator unit, bladeless fan (Dyson AM03 Air Multiplier Bladeless Adjustable Pedestrial Fan), and space humidifiers (AIRCARE HD1409 Digital Whole-House Console-Style Evaporative Humidifier) and additional electric heaters as needed. Air quality is additionally improved by exhaust vent fans (Panasonic FV-15VQ5 WhisperCeiling 150 CFM Ceiling Mounted Fan) as well as by a fresh air ventilation unit (Panasonic FV-04VE1 WhisperComfortTM Spot ERV Ceiling Insert Ventilator). The air quality may also be tested and monitored to ensure a healthy environment.

Nutrients and water were provided in abundance in a central foraging area. Pollen substitute (BeePro) and 1:1 pure sugar water (supplemented with HoneyBee Healthy to prevent fermentation) are provided in trays equipped with wooden posts for cleaning, or floating colored foams to prevent drowning, respectively. All nutrient sources were disease and pesticide-free, and replaced regularly to ensure quality. Hives were also regularly checked for presence of pests such as mites (*Varroa sp.*) and hive beetles (*Aethina sp.*), as well as signs of viral diseases such as deformed wing virus. Used wax

frames may also be tested for pathogens, and products could be evaluated for material composition. Researchers were all trained and approved by Massachusetts Institute of Technology Environmental Health and Safety, in partnership with Best Bees Company, and wore protective mesh and fabric suits, along with gloves and boots (Mann Lake Ltd.), while inside the space (Figure 4-5). Out of the 8 hives, two were established as stable controls, and basic experiments were initiated in the others, such as frame removal, wire foundation insertion, and removal of natural stored honey to encourage wax and honey formation.
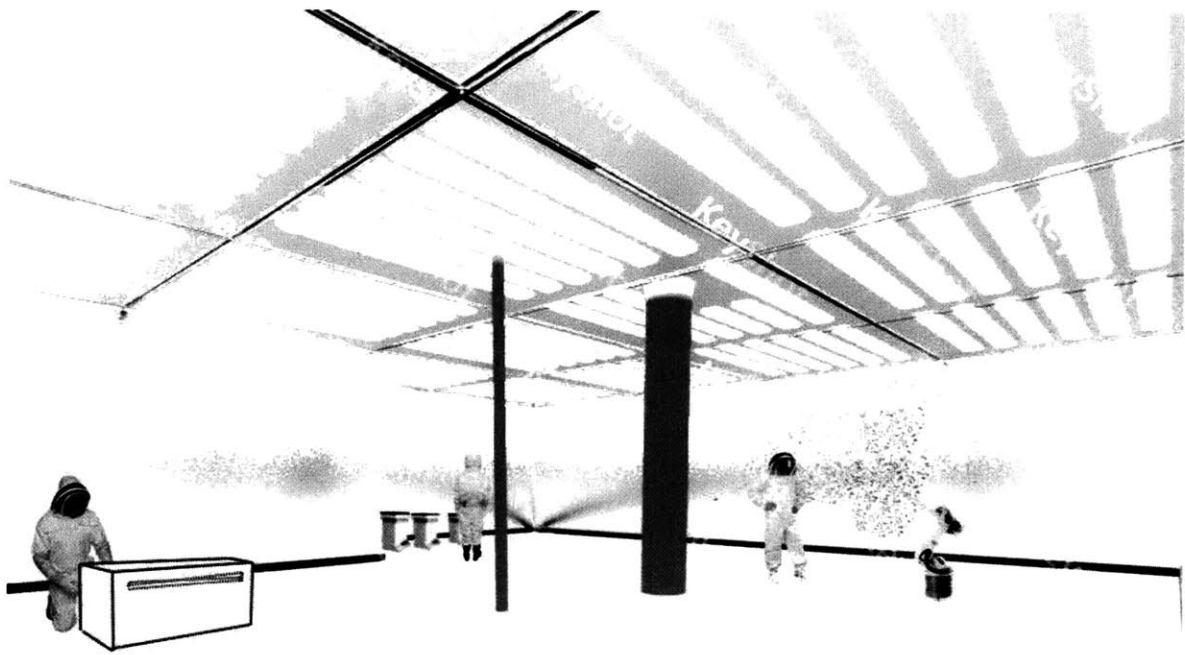
Figure 4. Rendering of proposed enclosure from inside.
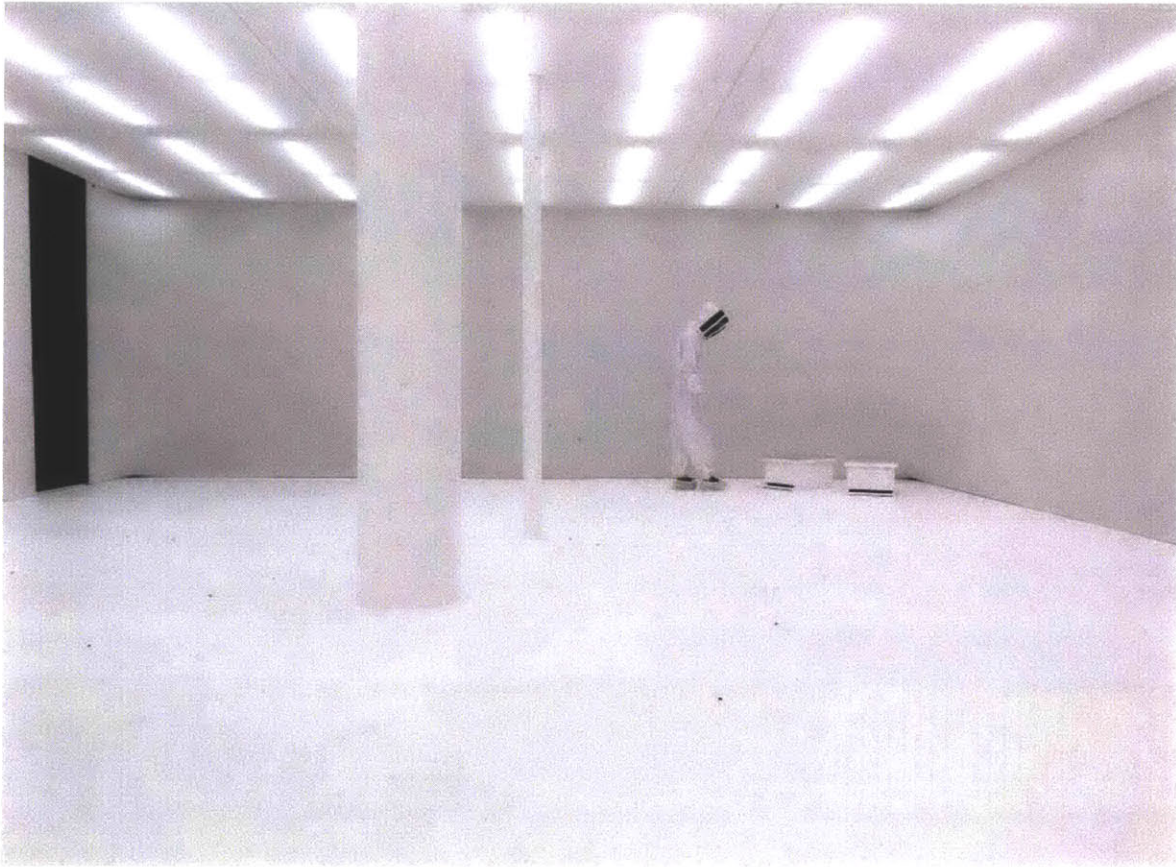
Figure 5. Researcher pictured with two initial hives.

V. Results and Discussion

After about 7-14 days, hives were assessed for overall acclimatization and health. At this point, most hives were generally thriving and thus suggested that a sustainable ecosystem was created, due to the presence of fertile queens and developing eggs (Figure 6). Activity was visible in and around the hive and in the foraging area.

Figure 6. Small eggs (marked with red circle) within freshly created wax.

New wax structures were found, indicating that the bees used nectar substitute (sugar water) provided, and also stored pollen substitute, which serves as food for young brood (Figure 7-8). Clusters of several hundred to thousands of bees outside the hives were observed, laying wax on the enclosure itself, apparently without a queen, indicating absconding but highlighting the use of an enclosure to observe hive abandonment.

Figure 7. Light microscope images of honey, wax, and a wing (40x).

Figure 8. Wooden frame with wax (top) and natural wax structures (bottom).

VI. Experimentation and Proposed Work

One experimental focus is on individual and emergent behaviors that may be templated by attracting and repelling factors including light, pheromones, and temperature. Behavioral assays are first conducted in a 20-gallon glass tank, in which researchers can observe in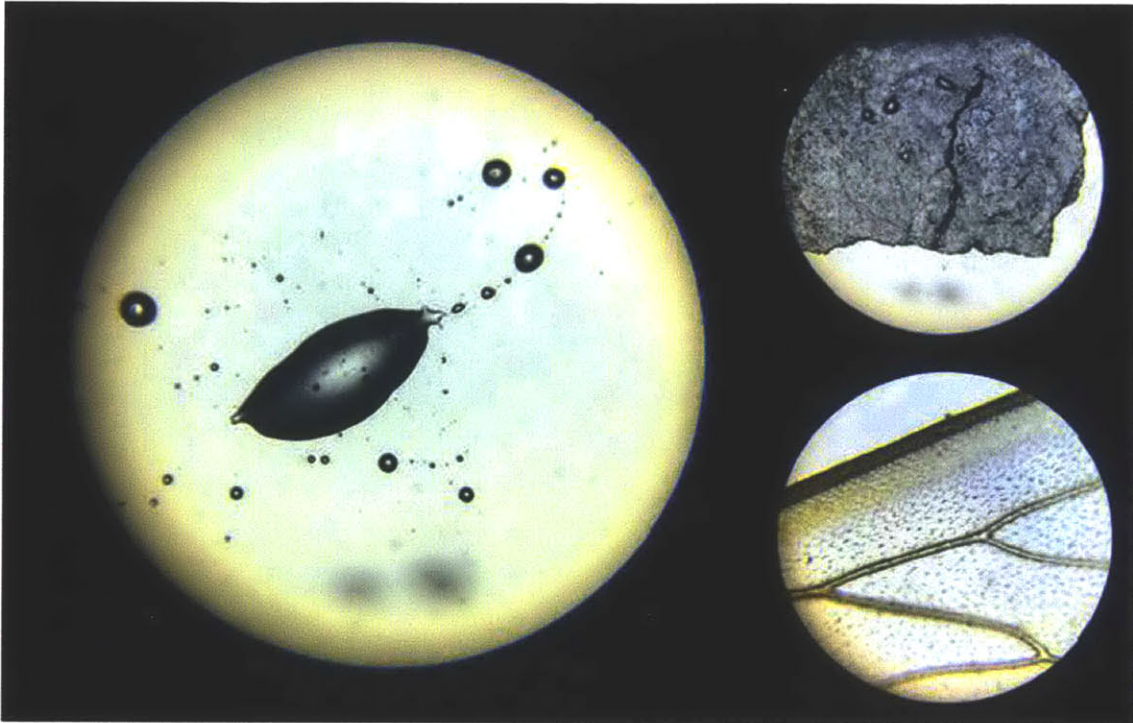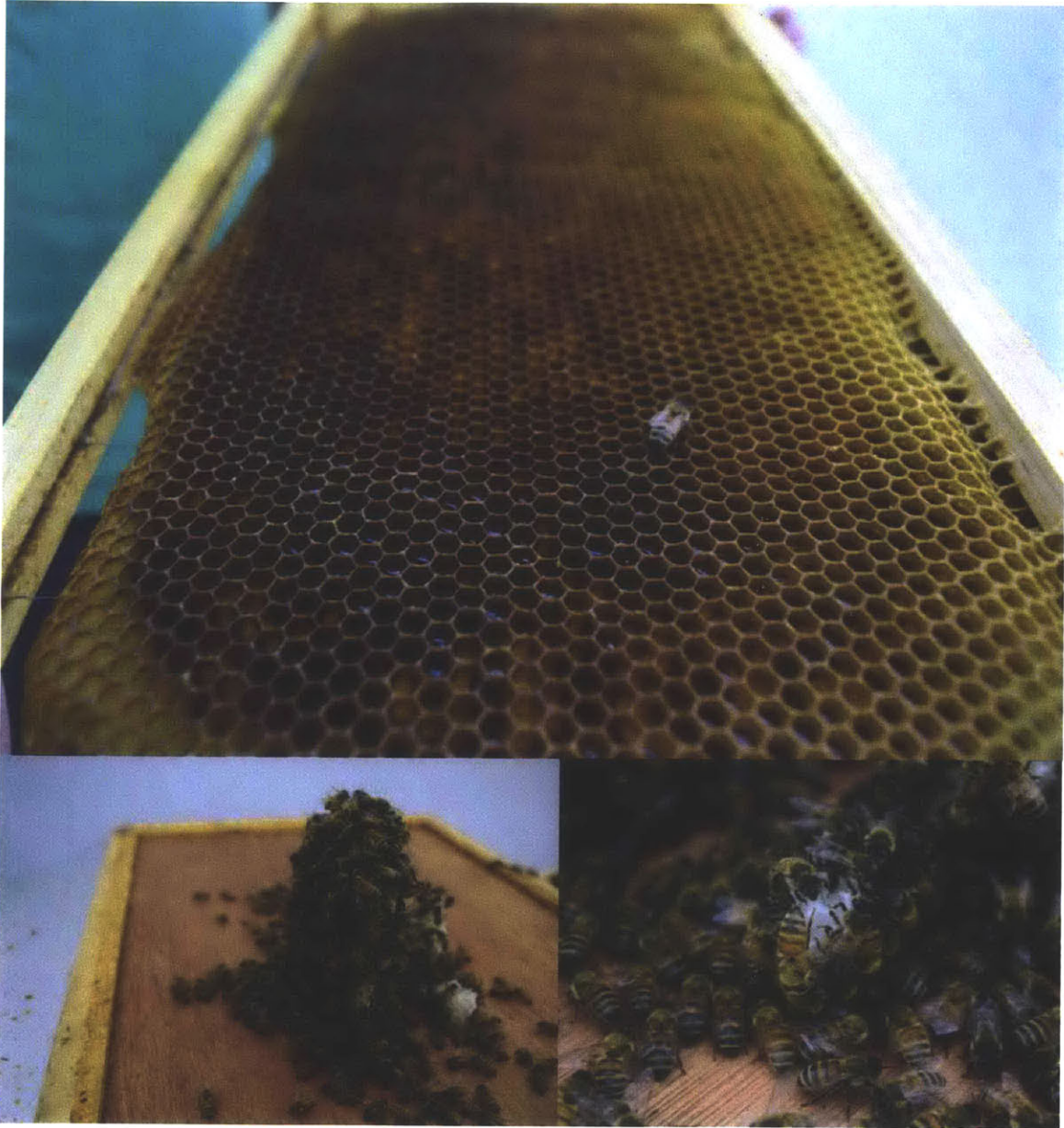dividual worker or queen bees as they navigate along prescribed paths or in relation to selected regions. Within this microenvironment, foraging-type behaviors can easily be both observed and recorded (by eye and by camera), and can then be replicated in the larger enclosure. Camera footage can be analyzed using software for computerized motion tracking and assignment of different behaviors.

Light experimentation can be done with different levels and types of light: full spectrum (144 'Spectra Brite' full spectrum 48" fluorescent tubes, 32 watts, 93 cri), UV light (American Black Light Lamp, 115 volts), red light, and darkness. The red light and darkness can be provided simply by covering the experimental areas with red transparent paper and black opaque cloth. Light intensity can be measured using a digital light meter.

In order to outline paths and make regions of interest, chemical mixtures are available; in experiments in the Synthetic Apiary, a honeybee swarm lure was used (Swarm Commander) as a positive control, and a natural repellent was used (Honey-B-Gone) as a negative control. While initially the pheromones were sprayed or dabbed with cotton, they may also be deposited using an automated system, such as a modified deposition

head designed for a KUKA robotic arm that has several degrees of freedom for maneuvering in small and large spaces[41]. The deposition head, previously patented[42], can be used release pheromones in liquid form, which then diffuse in a local area. Alternatively, small mobile robots exhibiting signals such as Queen Mandibular Pheromone[43] can act as "robotic queens" in an area, exerting influence over honeybees in a manner similar to natural queens. In this case, even small robots with wheels or small drones (commercially available) may be used and coated with queen pheromones.

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As used herein, "Synthetic Apiary" means an embodiment of the present invention.

In illustrative implementations of this invention, a controlled enclosure is created for the year-round observation of honeybees (Apis mellifera), and possibly other organisms with significant space requirements. It is designed to house both traditional hives (e.g. Langstroth bee hive) as well as free-roaming honeybees and provide essential nutrients for their survival and propagation. Full-spectrum artificial daylighting is provided, along with temperature and humidity control in order to accurately mimic a range of outdoor conditions. The enclosure prescribes a large yet limited space to observe intra-hive and inter-hive behaviors, foraging, and events such as swarming and absconding, which may then be useful in forming basic research studies, as well as applied research about swarm activities, for example.

In the Synthetic Apiary, climate conditions mimic spring conditions to allow for foraging and other natural behaviors year-round; the enclosed environment allows researchers to observe swarming and absconding behaviors; all nutrients and other inputs (e.g. light) are provided consistently and hygienically to minimize pests and disease.

The Synthetic Apiary has several features that allow for continued activity and productivity of the honeybees.

First, the enclosure was designed to ensure safety and promote observation of honeybees. It features full spectrum low-heat lights (144 Spectra Brite full spectrum 48" fluorescent tubes, 32 watts, 93 cri) placed on the outside of a light transmitting and partially dispersive fabric. This helps mitigate one commonly seen issue in greenhouses, in which honeybees are attracted to light and injure themselves through repeated contact. In addition, the entire tensile structure is made of fabric (Dazian Trapeze Plus white 122", 90% polyester 10% Spandex) stretched between wooden crossbeams, which reduces the number of crevices in which propolis or wax may be laid and unobservable hives formed. The space is white, which allows for easy viewing of hives as well as individual bees, enhancing researchers' ability to note behaviors such as swarming, foraging, and inter-hive activity. The architecture of the space consists of a central rectangular sealed enclosure with two entrance rooms. Each entrance room has netted magnetically self-sealing doors into the main enclosure as well as to the outside parameter of the entire enclosure, thus preventing dislocation of bees.

Second, climate conditions are maintained at between 50-70F, at least 50% RH, 15 hours simulated daylight, and appropriate air circulation and ventilation. This may also be tuned to represent other seasonal climates during which particular behaviors and activities may be observed. The system features standard residential heating, a heating ventilator unit, bladeless fan (Dyson AM03 Air Multiplier Bladeless Adjustable Pedestrial Fan), and space humidifiers (AIRCARE HD1409 Digital Whole-House Console-Style Evaporative Humidifier) and additional electric heaters as needed. Air quality is additionally improved by exhaust vent fans (Panasonic FV-15VQ5 WhisperCeiling 150 CFM Ceiling Mounted Fan) as well as by a fresh air ventilation unit (Panasonic FV-04VE1 WhisperComfortTM Spot ERV Ceiling Insert Ventilator). The air quality may also be tested and monitored to ensure a healthy environment.

Third, nutrients and water are provided in abundance in a central foraging area. Pollen substitute (BeePro) and 1:1 pure sugar water (supplemented with HoneyBee Healthy to prevent fermentation) are provided in trays equipped with wooden posts for cleaning, or floating colored foams to prevent drowning, respectively. All nutrient sources are disease and

pesticide-free, and replaced regularly to ensure quality. Hives are also regularly checked for presence of pests such as mites (Varroa sp.) and hive beetles (Aethina sp.), as well as signs of viral diseases such as deformed wing virus. Used wax frames may also be tested for pathogens, and products can be evaluated for material composition.

Fourth, the Synthetic Apiary space allows for humans, bees, and robotics to all interact to influence the environment. While many experiments were done directly involving humans and bees, automated systems - specifically a robotic arm - may be used to deposit physical substances (such as wax, honey, propolis, pollen, sugar water), and also chemical substances (specifically pheromones). We have previously used large scale robotic arms (KUKA) to deposit liquids of various viscosities, and similar deposition techniques can be used for depositing liquid pheromones (e.g. Swarm Commander as a positive attractant, and Honey-B-Gone as a negative repellent).

In illustrative implementations of this invention, one or more electronic computers (e.g., integrated circuits, microcontroller, controllers, field-programmable-gate arrays, personal computers, or other onboard or remote computers) are programmed and specially adapted: (1) to control the operation of, or interface with, hardware components of a synthetic apiary, including a HVAC (heating, ventilation and air conditioning) system, a thermostat, a humidity sensor, a humidifier, illumination sources, and air flow controls for air intake and air exhaust, (2) to perform any other computation, calculation, program or algorithm described or implied above; (3) to receive signals indicative of human input; (4) to output signals for controlling transducers for outputting information in human perceivable format; and (5) to process data, to perform computations, to execute any algorithm or software, and to control the read or write of data to and from memory devices. The one or more computers are in any position or positions. For example, in some cases (a) at least one computer is housed in or together with other components of the system, or (b) at least one computer is remote from other components of the system. The one or more computers are connected to each other or to other components in the system either: (a) wirelessly, (b) by wired connection, or (c) by a combination of wired and wireless connections.

In illustrative implementations of this invention, one or more computers are programmed to perform any and all computations, calculations, programs and algorithms described or implied above, and any and all functions described in the immediately preceding paragraph. Likewise, in illustrative implementations of this invention, one or more non-transitory, machine-accessible media have instructions encoded thereon for one or more computers to perform any and all computations, calculations, programs and algorithms described or implied above, and any and all functions described in the immediately preceding paragraph.

For example, in some cases: (a) a machine-accessible medium has instructions encoded thereon that specify steps in a software program; and (b) the computer accesses the instructions encoded on the machine-accessible medium, in order to determine steps to execute in the software program. In exemplary implementations, the machine-accessible medium comprises a tangible non-transitory medium. In some cases, the machine-accessible medium comprises (a) a memory unit or (b) an auxiliary memory storage device. For example, in some cases, while a program is executing, a control unit in a computer fetches the next coded instruction from memory.

As used herein, the term "computer" includes any computational device that performs logical and arithmetic operations. For example, in some cases, a "computer" comprises an electronic computational device, such as an integrated circuit, a microprocessor, a mobile computing device, a laptop computer, a tablet computer, a personal computer, or a mainframe computer. For example, in some cases, a "computer" comprises: (a) a central processing unit, (b) an ALU (arithmetic/logic unit), (c) a memory unit, and (d) a control unit that controls actions of other components of the computer so that encoded steps of a program are executed in a sequence. For example, in some cases, the term "computer" also includes peripheral units, including an auxiliary memory storage device (e.g., a disk drive or flash memory). However, a human is not a "computer", as that term is used herein.

In illustrative implementations of this invention, multiple electronic devices (e.g., computers onboard, adjacent to, or remote from the system) are configured for wireless or wired communication with other electronic devices in a network.

For example, in some cases, multiple electronic devices each include or are operatively connected to a wireless communication module for wireless communication with other electronic devices in a network. Each wireless communication module includes one or more (a) antennas, (b) one or more wireless transceivers, transmitters or receivers, and (c) signal processing circuitry. The wireless communication module receives and transmits data in accordance with one or more wireless standards.

As used herein, "including" means including without limitation. As used herein, the terms "a" and "an", when modifying a noun, do not imply that only one of the noun exists. As used herein, the term "or" is inclusive, not exclusive. For example A or B is true if A is true, or B is true, or both A or B are true. As used herein, "for example", "e.g." and "such as" refer to non-limiting examples that are not exclusive examples.

Conclusion

The above description (including any attached drawings and figures) describes exemplary implementations of the invention. However, the invention may be implemented in other ways. The methods and apparatus which are described above are merely illustrative applications of the principles of the invention. Numerous modifications may be made by those skilled in the art without departing from the scope of the invention. Also, this invention includes without limitation each combination, subcombination, and permutation of one or more of the abovementioned implementations, embodiments and features.

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