Distinguishing Bacterial Motion Quantitatively: A Diagnostic Method for Intestinal Disease

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Section I: Personal Introduction

In seventh grade, my science teacher pressed his broken piece of chalk to the board and within moments, a litany of dates appeared out of the haze of white dust. Our first science fair would take place in mere months and we were tasked with planning our own life sciences-related project from start to finish. Most of what we could feasibly experiment with ourselves, while interesting, was predictable. Growing *Brassica rapa* in a number of nutrient-deficient, light-starved conditions, as I had done in sixth grade, would not illuminate its secret environmental resilience. However, there was one realm of study teeming with mystery: microbiology. In particular, the idea of experimenting with bacteria captivated me; the phrase "there is more than meets the eye" most succinctly captures my curiosity.

Another luring facet of bacteriology was its susceptibility to reductionism. It is very easy to believe that the longevity of these organisms has made it irrelevant to our study of modern life (namely humans), but more research is cropping up about their sophistication. They can communicate in chemical pathways between and within species, acting as a previous parallel to the nuances of human language. And while many other organisms also have this capacity of communication, the sheer scale upon which bacteria can coordinate behavior speaks to a much higher degree of complexity that requires further exploration. I was interested in one manifestation of this communication — how bacteria coordinate movement in groups.

Given my limited access to higher power imaging equipment at that time, I simply quantified the extent of bacterial movement by measuring the distance from the edge of a colony to the center of the petri dish as a function of the concentration of nutritional medium I supplied. This first step into using mathematics to uncover properties of bacterial motion prompted me to inquire further. I was curious about motility patterns on a microscopic level, but I was unsure of how I could harness fluid, boundless motion in an equation or discrete variable. An even larger question soon emerged: what implications would this

research have beyond deepening our understanding of bacterial behavior? To answer these questions, I returned to the literature-scoping phase of my science fair project, entering a year-long period of stasis as I dissected numerous reviews and articles.

In my Research Seminar class during my freshman year of high school, the time came for another independent science fair investigation of a higher caliber than our previous projects. Having already spent weeks immersed in the relevant literature, I was beginning to form a clearer vision for how I would go about quantifying differences in bacterial motion types. I even took my proposal one step further: after attending a World Science Festival panel on the human gut microbiome and scouring research on gut pathophysiology, I found the end goal this project desperately needed in the beginnings of, ironically, a connection I had made in seventh grade. The parallel I made between bacterial and human communication as discrete entities can now be extended further to the communication between the two species — a relationship that has recently begun to occupy the limelight of microbial research. Particularly, the two motion types I wanted to quantify were correlated with separate physiological phenomena; bacterial swarming (dynamic, collective motion) is associated with inflammation and pathogenesis whereas swimming (individualized, chaotic motion) is associated with homeostasis. My freshman year research project began to tackle the question of not only mathematically characterizing each motion type but also significantly distinguishing them. Employing graphite particles as my tracking device under a microscope-camera system, I calculated torque and power for both swarming and swimming. Empowered by the stark differences in these mathematical values, I realized the diagnostic potential of this line of inquiry. If I could nuance my model with more mathematical parameters and an improved, controlled engineering design, this system could have applications as a diagnostic tool for gut disease (particularly Inflammatory Bowel Disease).

By this point in my research journey, I had exhausted the materials provided by my high school and in order to increase the depth of my research, I needed access to an active matter physics laboratory; additionally, I wanted a mentor to look through my self-directed proposal and offer guidance when I would encounter inevitable hurdles. This proposal wholeheartedly embraced multiple disciplines: engineering (to

design a confined system for bacterial motion), computer science (to analyze motion patterns), physics (to quantify motility), and microbiology (the backbone of the study).

Perhaps it was the amalgamation of so many scientific disciplines that hindered my success initially. As I began sending my proposal to numerous laboratories around New York City and neighboring states, my inbox filled with emails echoing the same sentiment of a lack of interest or an inability to accommodate another lab member. The Tang laboratory at Brown University took a leap of faith and graciously agreed to let me carry out my research with guidance from Weijie Chen, a Ph.D. student at the time.

The weeks that followed were filled with thinking and re-thinking experimental design. The microgears I had designed on paper needed to be synthesized in gel in order to plot the vectors of bacterial motion. I just could not get any of my designs to work. With star-shaped gears, the bacteria would get stuck on the edges, rendering the gear static. How would I quantify bacterial motion if I could not even *visualize* it using a gear?

The solution to my issue came to me when I observed the grand mechanics of how bacteria were moving the gear. I was getting stuck in the minutia of clumps of cells. Upon reviewing the recordings of my previous trials, I noticed that bacteria have "handedness." If I made my gears asymmetrical, I could use this property to my advantage.

A J-shaped gear fell perfectly into place. The bacteria rotated the gear as if it were a native part of their colony. Hours faded as I jotted down notes and downloaded video files, excitedly showing my mentor my newest findings of the day. We rejoiced for a moment, and then we went back to scribbling micropipette amounts in black ink. The subsequent weeks were filled with new ideas hastily penned onto more pages of my lab notebook, ready to transition from thought to implementation.

The time came for me to analyze the motion I had now harnessed. I learned Matlab software (particularly Particle Image Velocimetry) to optimize the accuracy and visibility of the vectors I superimposed on each bacterial sample. In order to apply formulae I had researched for active matter, I needed to learn some new concepts in calculus and physics (e.g. using integrals and dot products in the

formula for the Vortex Order Parameter). After calculating three types of biophysical parameters, I refined my Python skills in order to code a Random Forest Classification Algorithm that distinguished between the mathematical profiles of swarming and swimming.

It was thrilling to see my ideas materialize. From what seemed like a far-fetched whim of ascribing mathematical values to motility, my research project finally came together by synthesizing various scientific fields. Even more exciting was the prospect of applying this novel tool in detecting Inflammatory Bowel Disease, an ailment with an unrefined, tumultuous diagnostic process. The mystery that allured me to this field in seventh grade is still present, perhaps even more so now that I have answered some questions and have many more lingering in the aftermath of my project.

For high school students potentially interested in conducting research that straddles science and mathematics, my first piece of advice would be to read as much literature about your field(s) as you can. Constructing your experimental methodology with a strong understanding of the protocols, formulae, and algorithms at your disposal will improve your chances of obtaining the data you want. Of course, science is still unpredictable and you may not get the results you want even after exhausting all possibilities. Patience and disappointment are fixtures in the research process, so setting small goals for yourself will make the long-term project feel more achievable. Most importantly, curiosity is the greatest motivator in any line of research. The enigma of bacterial behavior gripped me from the start of my research career and this fascination has sustained my faith in my project. After encountering and overcoming the numerous obstacles in my research, I've come to truly enjoy the trial-and-error process so characteristic of scientific inquiry.

Section II: Research

Abstract

Gastrointestinal illnesses afflict more than 100 million people in the U.S. alone and are often indicated by gut microbiota motility. Typically, swarming bacteria are indicators of infection while swimming bacteria are more innocuous. Current diagnostic methods for intestinal diseases are lengthy, expensive, non-specific,

or lead to serious complications. This study proposes a novel way to diagnose Inflammatory Bowel Disease (IBD) through quantitatively distinguishing bacterial motion. Current methods of discerning bacterial motility involve only qualitative description without consideration of potential medical applications; no quantitative models to differentiate bacterial motility exist. In this study, a novel interdisciplinary diagnostic tool was developed that distinguishes swarming and swimming SM3 bacteria quantitatively for the first time. Photolithography was used to create PDMS sheets and microgears for studying both motilities. Software captured images for Particle Image Velocimetry (PIV) analysis for the calculation of Vortex, Nematic, and Polar Order Parameters, which were fed into a developed machine learning algorithm; accuracy was analyzed to ascertain the importance of each variable in motility distinction. Vortex Order Parameters (VOPs) were used to generate a Vicsek model for differentiating swimming and swarming which demonstrated the importance of cell-cell alignment force in motility distinction — the model yielded high and low VOP values for swarming and swimming respectively. Studies of motility on intestinal tissue supported modeling trends from prior PIV analysis on agar. This novel tool can be tested in a variety of intestinal diseases to provide a preliminary diagnosis, operating more economically, efficiently, specifically, and safely than conventional procedure.

Introduction

Gastrointestinal Disease

Intestinal diseases result in 14 million hospital admissions, more than 230,000 deaths, and more than $140 billion in healthcare costs annually in the U.S. (Almario et al. 2019). One facet of intestinal disease is Inflammatory Bowel Disease (IBD), which comprises Crohn's Disease (CD) and ulcerative colitis (UC) and is defined as the inflammation of the gastrointestinal tract (Seyedian et al. 2019). IBD can cause painful symptoms such as diarrhea, abdominal pain, and bleeding of the anus (Wehkamp et al. 2016). In 2017, 6.8 million people worldwide were diagnosed with IBD, with the prevalence rate increasing by 4.8 per 100,000 people from 1990 to 2017 (Alatab et al. 2019). In the US alone, $1.7 billion of healthcare costs are attributed to IBD care (Hansberry et al. 2017). Current diagnostic methods either carry risks, excess expenses, or

require long wait times to receive results. While their clear imaging makes them an important part of the diagnosis process, endoscopies can cause complications and cannot access the full intestine (Spiceland and Lodhia et al. 2018) — they are also expensive procedures and still require other supplementary tests to piece together a diagnosis (Nemati and Teimourian 2017, Papagrigoriadis et al. 2004). Scientists have been searching for noninvasive biomarkers of intestinal disease for diagnosis via blood or fecal tests (Hansberry, David et al. 2017), yet those that have been discovered are non-specific, measured at arbitrary "cut-offs," or unable to predict a relapse for patients in remission (Dragoni et al. 2020 and Ikhtaire et al. 2016). Blood tests (such as CBC panels, I-FABP, DAO, Zonulin, and D-lactate) either lack sufficient data for threshold calibration or specificity (Linsalata et al. 2020). Thus, serological tests, while helpful in assessing a grander level of physiological function, are not ideal when simple, specific tests can aid in an initial diagnosis of intestinal disease (Sellin and Shah 2012). Positivity rates in blood tests for current biomarkers (even those specific to intestinal disease) are also highly variable and can be as low as 1.4% to a maximum of 71% (Ince et al. 2019 and Lewis 2011). In addition to non-specificity, blood tests take one to three days for reporting in a non-emergent setting. Fecal tests, such as calprotectin, lactoferrin, and hemoccult tests, usually provide information for a wide range of conditions, from cystic fibrosis to cirrhosis to food allergies; they also operate on thresholds with a great degree of variability for active disease and lactoferrin, in particular, is not stable in stool for long at room temperature (Ikhtaire et al. 2016). Urine tests, which are not usually used for intestinal diagnosis, are still greatly time-consuming and non-specific (Linsalata et al. 2020). In addition to the aforementioned limitations, fecal tests take one to two weeks for reporting (Hejl et al. 2017). Colonoscopies, while a gold standard in diagnosis because of clear imaging capacity, carry a 0.25% risk of bowel perforation, burns, and bleeding; although these complications seem improbable, they are life-threatening (Zauber 2014). Increased time spent waiting for results to confirm the diagnosis of IBD (e.g. with fecal and blood tests) can have adverse health risks, such as increased strictures and necessitating invasive intestinal surgery (Lee et al. 2017, Taylor et al. 2016). Given the gravity of both the toll on individual patients and the American healthcare system, there ought to be a method for streamlining

diagnoses and prognoses as well as decreasing in-patient procedures and hospital admits — one that will be economic, efficient, safe, and accurate.

Role of Bacteria in Disease

Evidence links bacterial motility to intestinal disease, particularly Inflammatory Bowel Disease (IBD) (Rooks et al. 2014, Stantonr and Savage et al. 1984). As opposed to swimming bacteria in the intestines, swarming bacteria are also associated with virulence (Allison et al. 1994, Mazzantini et al. 2016, Overhage et al. 2008). Bacterial swarming is qualitatively characterized by a collective, dynamic flagellar movement on a partially solidified surface that bacteria employ in various environments to optimize their acquisition of resources as shown in Figure 1 (Partridge and Harshey 2013). In this type of movement, bacteria utilize their flagella to navigate, two-dimensionally, through a medium and acquire necessary materials for overall survival (Darnton et al. 2010). By contrast, bacterial swimming is qualitatively defined as an individualized, three-dimensional flagellar movement in liquid medium as shown in Figure 1 (Kearns 2010). Thus, bacterial movement on soft surfaces, and under confinement, exert distinct properties on the host that have been underexplored. Swarming manifests most starkly in intestinal diseases. *Proteus sp.*, *Salmonella enterica*, and *Campylobacter jejuni* are examples of swarming bacteria that cause a variety of symptoms of gastrointestinal distress (Golden and Acheson 2001, Hamilton et al. 2018, Kim et al. 2003). SM3 (a strain of *Enterobacter sp.*) served as the focus of the study to examine motility in the context of IBD. For this disease, it is necessary to develop a specific, accurate test with more efficiency and economy — a simple, microbiological test distinguishing bacterial motility to demonstrate infection or a presence of intestinal stress (Elhenawy et al. 2019) at the beginning of the diagnostic process to avoid unnecessary procedures as well as to prognosticate for patients in remission.

Proposal of Study

This study proposes taking advantage of differences in bacterial motility to design a potential diagnostic tool that can differentiate between swarming and swimming in IBD patients. This tool, upon further refinement, would reduce diagnostic cost by $1450, time by several days, and risk to none as well as increase specificity by at least 20% (an extension of the agar work conducted in this study). Currently, there

are no quantitative models that distinguish between swarming and swimming bacteria and current definitions for motility are only descriptive with limited studies (Hall et al. 2018). Prior research (without applications in medical diagnosis) has investigated swimming bacteria rotating microgears, in open media and confinement, as shown in Figure 2 (Lushi et al. 2014, Sokolov et al. 2010, Wioland et al. 2013). Microgears and subsequent calculations of torque and power were used to track and assess swimming movement (Di Leonardo et al. 2010, Sokolov et al. 2010, Wong et al. 2013). Studies of bacterial swimming under confinement, via polydimethylsiloxane (PDMS) sheets with circular wells, were used to amplify swimming motion and observe motion patterns (Beppu et al. 2017, Zhai et al. 2017, Wioland et al. 2013). Models have been made for swimming and swarming bacteria respectively, but they have not provided sufficient detail, not fully characterized the motility types, and not analyzed both motilities in tandem (Be'er et al. 2019). Using prior biophysical microbial research as a guide, the provision of clear mathematical distinctions between swarming and swimming will be crucial for efficiently, economically, safely, and specifically diagnosing intestinal disease in the future.

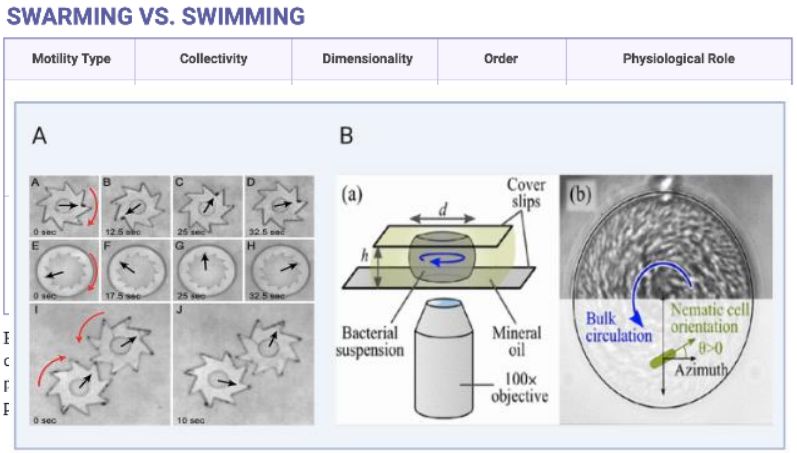

Figure 2. Microgears and confinement images in studying bacterial motion. (A) An image of swimming *B. subtilis* rotating microgears over time for the calculation of torque from Sokolov et al. 2010. (B) A schematic and image of swimming *B. subtilis* in confinement from Wioland et al. 2013.

Key Figures and Equations

PARAMETER DEFINITIONS

Parameter	Definition	
Vortex Order Parameters (VOP)	measures the particle vectoral motion as a function of noise in the system (thus providing infomation on how ordered/disordered the movement is)	$\phi_v = \frac{\left(\frac{\sum_i \lvert v_i \cdot t_i \rvert}{\sum_i \lVert v_i \rVert} - \frac{2}{\pi}\right)}{1 - \frac{2}{\pi}}$
Polar Order Parameters (POP)	measure the degree of alignment between particles and treats particles as single-arrow vectors	$\Phi_p = \frac{1}{N}\left\lvert \sum_{j=1}^{N} e^{i\varphi j} \right\rvert$
Nematic Order Parameters (NOP)	measure the degree of alignment between particles and treats particles as double-arrow vectors	$\phi_n = \frac{1}{N}\left\lvert \sum_{j=1}^{N} e^{2i\varphi j} \right\rvert$

Figure 4. Parameter definitions used in Vicsek model (VOP) and algorithm (VOP, NOP, POP). The formula for Vortex Order Parameters was used from Wioland, Hugo et al. 2013. The formulas for Polar and Nematic Order Parameters were taken from Großmann, Robert et al. 2015. These parameters quantified motility distinctions.

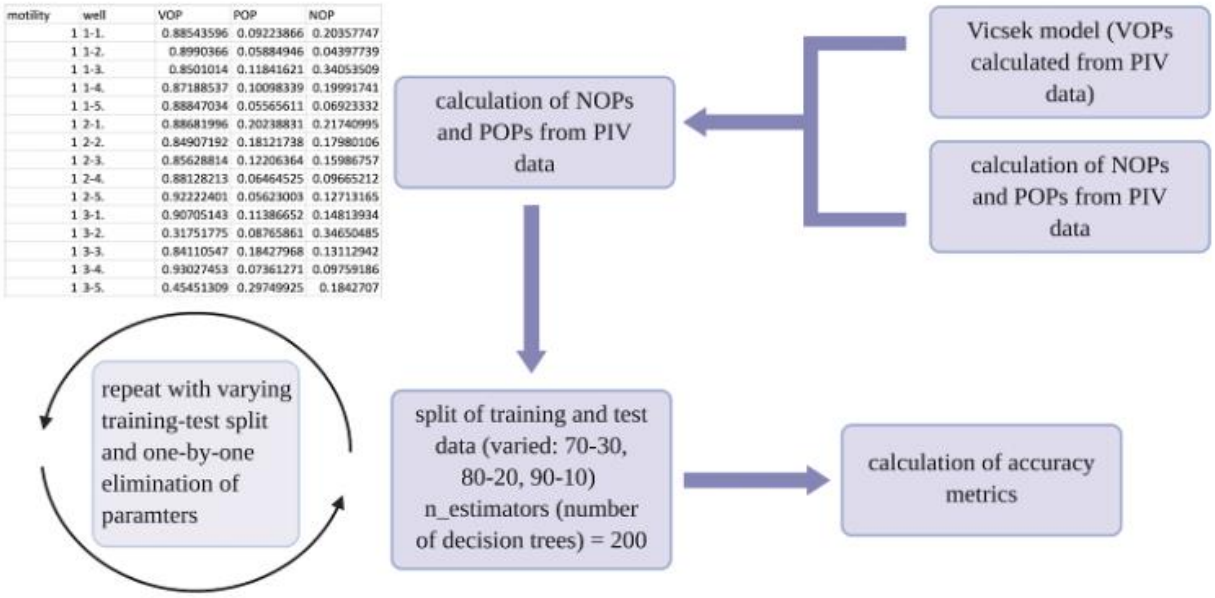

Figure 5. Flow of Random Forest Classification algorithm in Python. Parameters were computationally calculated and collated into a dataset file. The dataset was split into training and test facets (with the splits varying for analytic purposes). After running the algorithm 100 times, the mean accuracy was calculated and reported. This procedure was continued with the one-by-one elimination of parameters to compare to the algorithm with all parameters informing the classification.

Machine Learning Algorithm (with NOP and POP parameters)

Equipped with data from the Vicsek model, a Random Forest algorithm was developed to distinguish between swarming and swimming motilities in SM3 using VOPs as well as two other parameters: Nematic Order Parameters (NOP) and Polar Order Parameters (POP), both of which have been used in characterizing the motion of active matter (Großmann, Robert et al. 2015). Polar order parameters and nematic order parameters both measure the degree of alignment between particles (Figure 4). POP treats particles as single-arrow vectors and NOP treats particles as double-arrow vectors (Figure 4). Accuracy metrics were extracted and analyzed by varying test split and parameter usage (Figure 5). One-way ANOVA and Tukey's post hoc tests were performed on machine learning parameter usage data. A Kruskal-Wallis test was performed on dataset test split data. A P-value benchmark of less than 0.05 was set for consideration of statistical significance. Values are presented as means with ± standard deviation (SD).

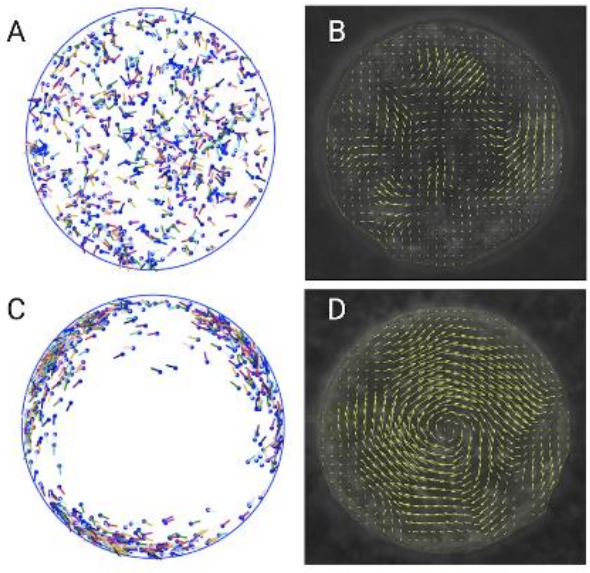

Figure 7. Vicsek Models mirror PIV analyses of SM3 swarming and swimming. (A) Vicsek Model demonstrates characteristic chaotic SM3 swimming motion visually as well as quantitatively (r=2, η=0.8, N=600, ϕ=0.004 ± 0.079). (B) PIV analysis of swimming SM3 in a 74μm diameter PDMS well, which clearly demonstrates disordered movement through vector plotting. (C) Vicsek Model demonstrates characteristic uniform SM3 swarming motion visually and quantitatively (r=6, η=0.4, N=600, ϕ=0.895 ± 0.031). (D) PIV analysis of swarming SM3 in a 74μm diameter PDMS well, whch clearly demonstratees ordered movement through vector plotting.

Vicsek Modeling Reveals Mathematical Differences between SM3 Swimming and Swarming

To uncover the mathematical variable(s) responsible for the pattern differences between swarming and swimming SM3, a Vicsek model was coded to reflect the vectorial motion shown through Particle Image Velocimetry (PIV). When generating the Vicsek model, the code was run thirty times per motility and the average Vortex Order Parameter (VOP) was generated. Two variables were manipulated to produce this result: the radius (r) of the circle encompassing the particle and other particles deemed its "neighbors" and the noise variable (η). The number of particles (N) was kept constant to reflect equalized cell density (thus inherent motility properties being the only factors influencing distinct motion patterns). The average VOP was calculated according to the aforementioned formula, which characterizes the degree of ordered, centric movement from zero to one (Wioland et al. 2013). For swimming SM3, the model reflected the disorganized behavior of swimming bacteria in reality (Figures 7A and 7B). The motion quantitatively manifests in a low VOP value of 0.004 ± 0.079. For swarming SM3, the model reflected the uniform behavior of swarming bacteria in reality (Figures 7C and 7D). The motion quantitatively manifests in a high VOP value of 0.895 ± 0.031.

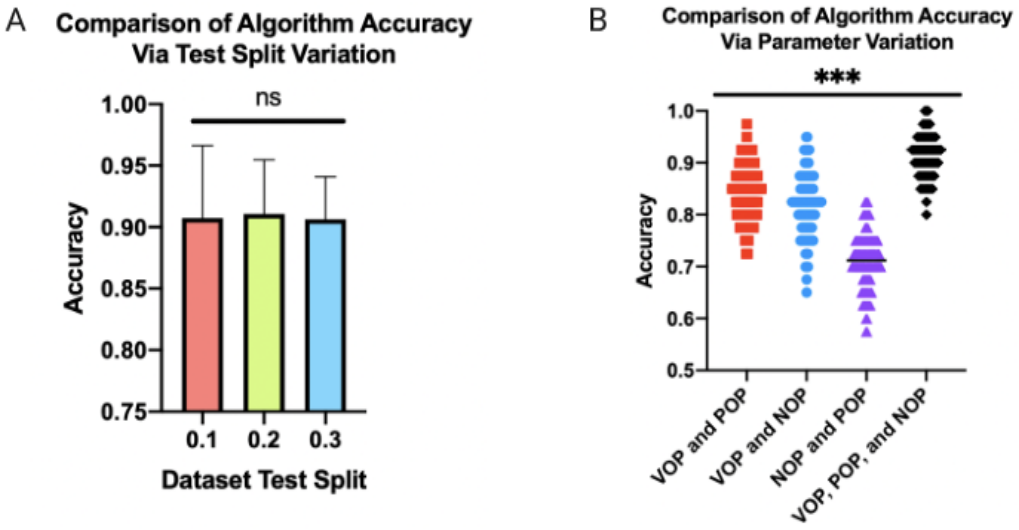

Figure 12. VOP is the most important parameter in motility classification. (A) Plot of dataset test split (10%, 20%, and 30%), with SD error bars, versus accuracy of classification (swarmring or swimming) based on three parameters (VOP, NOP, POP) for n=100. The Kruskal-Wallis test revealed no statistical significance between dataset splits, thus affirming the consistency of the model. (B) Plot of parameters used in the algorithm versus the classification accuracy. VOP proved to be the most crucial parameter in evaluating motility patterns, and NOP proved to be the least crucial parameter. The use of all three parameters still provides the highest mean accuracy percentage (0.911). One-way ANOVA revealed a p-value < 0.001, thus affirming the significance of tri-parameter usage in classifying bacterial motility.

Analysis of Machine Learning Algorithm Classification Accuracy

The machine learning model developed had classified motilities based on PIV files of 74 μm wells with swarming and swimming SM3. Analyses of the accuracy of the model were performed through manipulating dataset splits (Figure 12A) and parameter usage (Figure 12B). Algorithm accuracy was measured across dataset test splits (10% testing, 20% testing, and 30% testing) and it was observed that there was no significant difference in the model's accuracy through this manipulation (Figure 12A). Thus, the algorithm proves to be consistent. Using a test split of 20%, the impact of Vortex, Nematic, and Polar Order Parameters was ascertained in measuring classification accuracy by removing each parameter from the algorithm. It was determined that VOPs were the most influential on motility and NOPs were the least influential. Nevertheless, all three parameters were valuable in classifying motility since the algorithm's mean accuracy with only two parameters (VOP and POP) was 6.7% lower than the mean accuracy using all three (Figure 12B). Further refinement of the model must be conducted with more training data, varying well diameters, expanding to other strains and species of bacteria. In comparing positivity rates of current biomarkers in serological tests (Ince, M. Nedim et al. 2019 and Lewis, James 2011), this algorithm classifies with at least 20% more accuracy with correlation to disease because of the use of SM3.

STANDARD VS. EXPERIMENTAL DIAGNOSTIC PROTOCOL

Protocol	Cost (without insurance)	Time	Risk	Accuracy/ Positivity Rate
Standard (blood tests, fecal tests, colonoscopy)	CBC/metabolic panel blood tests: ~ $500-$1100 fecal tests: ~ $200 per test colonoscopy without polypectomy: ~ $800	blood tests: 1-3 days fecal tests: 7-14 days colonoscopy results: immediate (if outright negative) to 10 days	blood and fecal tests: no risk colonscopy: 0.25% risk of bowel perforation, burns, and bleeding (Zauber, 2014).	biomarker positivity rate in blood tests: 1.4% to 71% fecal tests: 48% to 89%
Experimental (use of stool sample and microchip)	Cost of PDMS chip + petri dish setup + analysis methods: ~ $50 per test	6 - 12 hours	use of stool sample: no risk	algorithm with VOP, POP, and NOP yields 91% accuracy

Figure 14. Cost-risk-time-accuracy analysis of intestinal diagnostic methods. Standard protocol can total a minimum of $1500 without insurance. Even for patients with insurance, many special fecal tests are considered "investigative" and subsequently not covered. The proposed method would cost approximately $50 per test given the cost of the components and acknowledgement of lower costs in mass production. The turnaround time for standard tests in a clinical, non-emergent setting ranges from days to weeks. The proposed method would take 6-12 hours. Colonoscopies in standard protocol carry risk and patient discomfort, whereas the experimental method poses no risk as it would operate on a stool sample. Biomarkers in serological and fecal tests are detected in a wide range, from barely 2% to 89%. The algorithm distinguishes bacterial motility with 91% accuracy which, upon further training with more data, could increase. The benefits of the experimental method are promising for diagnosis.

Cost Analysis of Proposed Diagnostic Tool and Standard IBD Diagnostic Protocol

Given the preliminary demonstration of bacterial motion on natural, bumpy intestinal tissue, this methodology can be extended for diagnostic use, particularly operating on a stool sample since motility distinctions maintain on uneven surfaces (i.e. not simply manifestations on agar). For a positive experimental test (i.e. presence of swarming), blood and fecal standard tests could be bypassed and a colonoscopy would not be necessary for preliminary diagnosis (only to be conducted for further information on intestinal condition). Complications listed do not include the additional complications caused by long diagnostic times (intestinal strictures and a higher risk of surgery) as mentioned before (Lee, Dong-won et al. 2017, Taylor, Sarah et al. 2016). In conjunction with the machine learning algorithm's accuracy of 91.1%, these benefits illuminate a potential avenue for intestinal disease diagnosis: one that is economical (only $50), efficient (takes only a few hours), accurate (differentiating with 91.1% accuracy), and safe (relies on a stool sample only) (Figure 14).

Brief Conclusion

Despite progress made in the field of microbiology to understand bacterial motility, prior research has not made use of idiosyncratic, microbial biophysical properties to inform the diagnosis process of intestinal diseases. In this study, a diagnostic tool based on mathematical and visual patterns of swarming and swimming SM3 was developed that is the first to quantify distinctions in bacterial motility, which has potential economic, safety, specificity, and time-saving benefits.

In developing a machine-learning algorithm and a Vicsek model, three parameters (VOP, NOP, and POP) demonstrated the uniformity of swarming motion in contrast to the fragmentation of swimming motion; VOP proved to be the most crucial variable in categorizing motility. The methodology and algorithm serve as a basis for a diagnostic tool for intestinal disease upon further testing and refinement. The Vicsek model revealed the intrinsic noise variable to be responsible for the disparate motion patterns. Biologically, intrinsic noise partially manifests in cell length differences between swarmers and swimmers.

Further genomic analyses can be conducted to elaborate on the role cell length plays in mathematical differences between swarmers and swimmers.

The most important consideration for the diagnostic tool stems from what could be perceived as a limitation. The human gut is polymicrobial and thus far, only one strain of bacteria has been analyzed at a time. A perceived limitation would be attributing one set of quantitative parameters (especially VOP) to a polymicrobial culture. Further experimentation must be done with polymicrobial cultures; however, it is hypothesized that the quantitative parameters will categorize the presence of swarming in the culture (even if the rest of the bacteria are swimming), thus allowing scientists to be aware of intestinal inflammation and categorize the strain if more studies are conducted. The presence of swarming in the culture will affirm the need for more diagnostic testing. Once the model is further streamlined with a larger volume of SM3 data and varied data (different bacterial strains), it can be used as a first assessment of intestinal disease in a safe, cost-efficient, accurate, and time-saving way.

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