

SONIFICATION OF CHEMOTACTIC WAVES OF BACTERIA

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ABSTRACT

Chemotaxis is the ability of certain microscopic organisms to sense and swim towards beneficial or away from detrimental chemicals in their surroundings. Identifying this behavior is important for understanding the relationships between species and their environments in the natural world. Predicting migration of an entire population from known characteristics of individual microorganisms is a key contribution, but can be a laborious process and requires watching and waiting for visual evidence of the process on a large population scale. Sonification offers a novel solution to this problem by allowing the observer to tap into our auditory sensory system to process information. In this project, we developed and assessed a proof-of-concept sonification tool as a high throughput, real-time screening tool for chemotaxis in populations of swimming bacteria. The tool operates by reporting the y-axis position of bacteria that appear in the microscope image as microsecond duration pitched notes, giving the user a sense of the average location of the population. In this paper, we present how it has been used as a chemotaxis assay and as a tool to locate traveling waves of bacteria as they pass through the field of view in order to capture data at specific timepoints, which is used to analyze individual swimming patterns of microorganisms within the wave.

1. INTRODUCTION

In recent decades, microorganisms such as bacteria have come into the spotlight for their various roles in human life. They restore polluted environments by cleaning up dangerous toxins, they form difficult-to-remove sticky layers of bacteria called biofilms that can spread disease, and they are critical to brain and body health as part of the human microbiome. Chemotaxis is the ability of many bacteria, as well as many other microorganisms, to migrate in a favorable direction based on nearby chemical cues. These chemicals can include food, chemical signals from other cells, and toxins [1]. In bioremediation, which is the ability for microbes to clean up oil and chemical spills, chemotaxis plays an important role as it can increase the ability of bacteria to find and consume oil droplets in the ocean [2] and in soils and sediments [3], [4]. It can also be critical to biofilm formation - for example, chemotaxis towards oxygen is critical for the formation of biofilms at air-water interfaces for certain bacteria [5], [6]. Finally, it is an essential part of ecological diversity, as many microbes use chemotaxis in order to infect hosts and become established communities [7]. As such, studying chemotaxis has the potential to impact

many aspects of life, and therefore detecting chemotactic behaviors in species of interest through assays to understand the underlying dynamics of their motion is of significant interest.

In most bacterial suspensions, chemotaxis arises in the form of a bias in their natural swimming patterns. Bacteria such as *Escherichia coli* swim in a random-walk pattern. When they sense a chemical of interest (called a chemoattractant), they bias their swimming so that they are moving towards the source of the chemoattractant [8], [9] (Figure 1). When observing millions of bacteria at once, they accumulate into moving waves that travel towards the source of the attractant [10], which we call traveling chemotactic waves. The presence of accumulation (Figure 1) or traveling waves (Figure 9A) acts as a litmus test to assess whether chemotaxis is occurring, and identifying these phenomena is the first essential step for conducting research to understand how the behavior of individual bacteria leads to the observed collective patterns.

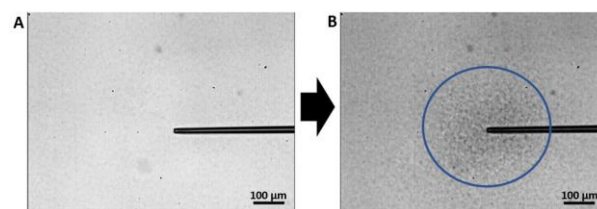


Figure 1: (A) A capillary (black rod) filled with chemoattractant was placed into a bacterial suspension to begin the assay. The chemoattractant diffuses into the surrounding medium. (B) Bacteria near the tip of the capillary sense the chemoattractant and swim toward it, creating a visible increase in bacterial density (darker gray in blue circle). Image adapted from Abe et al. [11].

Many effective assays and setups for conducting chemotaxis research have been developed using these visual phenomena, but they have certain shortcomings that can limit their usefulness. Traditional assays generally use both the observation of thousands of bacteria accumulating towards a source [12][13], and/or mathematical models of chemotaxis in order to determine relevant parameters [14][15]. Since these assays rely on long-term visible evidence of bacterial accumulation, they take between hours and days to complete, as hundreds of thousands of bacteria must accumulate to produce stark visual evidence of chemotaxis, or otherwise the assays require sophisticated mathematical analyses. Setup for these experiments is also time consuming even for those that are fast, making it difficult for scientists to quickly and



efficiently survey many chemicals when studying a bacterium or locate traveling waves in response to them. Finally, these are in all cases purely visual setups, making them only accessible to sighted users. As such, there is no current experimental platform for chemotaxis that gives quick, simple, and accessible feedback in a high-throughput way, thus limiting the utility of existing experimental systems.

In order to address this problem, we aim to expand the toolbox available to researchers by using sonification to report chemotaxis and traveling waves in real time. We aimed to design a sonification tool that augments a visual chemotaxis setup to improve experimental design and research efficiency, as well as create a more accessible form of data for those who are visually impaired. Similar work has been conducted in the past by Ngo et al. [16], who created a cellular stethoscope that sonified the x and y locations of fluorescent heart cells on special scaffolds to report their movement. This auditory feedback would allow for the user to make more efficient conclusions about the response of the cells, allowing the methodology to be more high-throughput. Work by Edwards et al. [17] also highlights how sonification can be used to augment a researcher's understanding of information portrayed by microscopy images of cells, specifically exploring features of cervical cells that are associated with cancer. By applying sonification to bacterial chemotaxis, we can create a platform that similarly takes advantage of sound as a data processing avenue to augment the research experience.

In this paper, we introduce a sonification platform that translates visual microscopy feeds of bacterial populations swimming in liquid media into audible sound for the user. The output sound captures critical information about the movement dynamics of the bacterial population, which can be used to determine whether chemotaxis is occurring in the system. This tool can be used to assay chemotaxis by giving instantaneous auditory feedback on the direction the population is moving and can also help identify traveling chemotactic waves of bacteria as they move across the visual field. This knowledge can then be used to make quick determinations about chemotactic response, or prompt data collection for deeper research. We first describe the design of the sonification algorithm. Then, we examine two use-case scenarios. In the first, the platform was used to determine if a chemical caused an attractive or a repulsive chemotactic response. In the second, the platform was used to identify when a chemotactic wave entered the field of view. This was then used to trigger high-quality visual data collection that was then analyzed to determine individual bacterial paths. These scenarios are intended to demonstrate how the sonification platform can supplement the toolbox of the chemotaxis researcher by offering informative audible feedback.

2. THE SONIFICATION PROCESS

The sonification is designed to give a broad sense of the location of bacteria as they move through a region that may or may not contain a source of a chemical of interest. In essence, the audio sonification process (Figure 2) uses the microscope video feed as input and interprets it as an audio sonogram. The input video, which can be fed in as a video file or captured live through the computer screen, is interpreted frame-by-frame as grayscale values. Bacteria

appear in the image as bright dots against a black background. The sonification scans across the columns of pixels in the x direction for each individual frame of the input video. Within a column of pixels being sonified, each bright pixel in the image (corresponding usually to a bacterium) contributes a very brief frequency based on the y position. Frames are scanned in succession at the same speed as the video's frame rate, so the sound evolves over time as the bacteria move from frame to frame.

What the user hears as a result is all of the net frequencies that are contributed to the sound over time, and their relative y-positions. So, if there are more bacteria overall, more frequencies are contributed, and if there are fewer, then fewer frequencies are contributed. If there are more bacteria near the top of the image (greater y values), then more high frequencies will be contributed, and similarly, if the bacteria gather at the bottom of the image, more low frequencies are contributed. Thus, the overall loudness (number of total frequencies) as well as the proportion of high frequencies to low frequencies (spectral centroid) informs how many bacteria are in the field of view, and where they have gathered. The sonification is designed to run with minimal user input once the sonification starts, allowing for audio feedback over long times that can capture changing bacterial population dynamics. The following subsections delve into the specifics of how the sonification is implemented.

2.1. Mapping Video to Sound

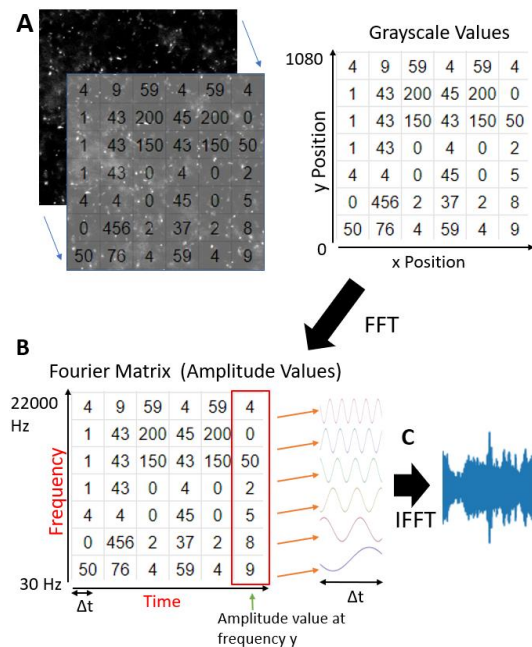


Figure 2: The sonification process of mapping video to audio. (A) Videos are interpreted as grayscale values. (B) These values are then placed into the FFT matrix, where their value represents an amplitude, their y-index represents a frequency, and their x-index represents an instantaneous timepoint. (C) The resulting amplitude-adjusted frequencies at each timepoint are played back together as one sound waveform.

To start the process a pre-recorded video or real-time video was run with a frame rate of 26 fps in grayscale using the Max/MSP environment. The video was turned into a matrix of values using a Fast Fourier Transform and analyzed for magnitude (Figure 2A). Every pixel with a black grayscale

value in the image was assigned a magnitude of zero, and every non-black pixel (for example, a bacterium in focus) was assigned a magnitude based on how bright it was. This information filled the Fourier Transform bins with audio amplitude and phase information (Figure 2B). This is a similar process to analyzing audio to view in a sonogram, however, instead of using audio the algorithm uses video. Once the FFT filled the bins with data, an IFFT or Inverse Fourier Transform was used to read the data at frame rate (Figure 2C). Each bin that had data produced a frequency between 20 Hz and 20 kHz (or between 20Hz and 10kHz for an “easy resolution” mode). This corresponded to the location on the sonogram where the bacteria were located. For example, if the FFT frame size was 4096 and the audio sample rate was 44.1 kHz then each bin represented 10.76 Hz. If a bacterium on the Y axis was in bin number 50, then a frequency of 538 Hz was produced. If a bacterium was between bins, then both bins were filled with the appropriate information and on playback played both bins at the same time.

In order to make the process as accurate as possible, a threshold was applied to the image to filter out any part of the grayscale spectrum that was not the brightest white or a set white value controlled by the user. This in turn showed only the desired values and filters out any out of focus bacteria or glare in the microscope field of view. The frame rate of the video corresponds to how fast the computer is scanning the video as well – generally, 26 frames are used so every time a new frame of video is loaded, it is scanned instantly. Scanning at this rate allows for differences between successive frames to be heard as a slow change in frequency and/or density while any bacteria on the slide not moving are heard as constantly repeating frequencies.

2.2. Sound Analysis Features

Apart from turning the video into sound the software can analyze the sound for various spectral properties such as centroid, peak frequencies, and root-mean-square loudness (RMS). These descriptors serve as another way of interpreting the sound spectrum produced from the video images. For example, Spectral Centroid represents the center of the spectrum which corresponds to, on average, where bacteria are gathered together in the field of view, which is particularly relevant for identifying traveling waves. The sound analysis is supplemental in the sonification process as a way of checking what was heard with trackable data.

2.3. Experimental Features

The software used for this sonification also has an experimental feature linking the sonification to the generation of sheet music. While the software is sonifying the data there is a notation function that analyzes the peak frequency of the sound and onset in time and notates it on a music staff spatially. The software does this by looking at the FFT bin that has the largest magnitude and rounding the note to a note in a microtonal musical scale. The software then quantizes the spatialized score into a music score in 4/4 time. This feature is provided for artistic purposes. Figure 3 shows how the notes are spatially notated and how the computer software quantizes them into a music staff in 4/4 time with the shortest note being a 16th note.



Figure 3: Notes spatially notated on a music staff and quantized into sheet music.

3. EXPERIMENTAL TESTS

3.1. Experimental Setup

In order to observe how bacteria respond to a chemical of interest, we first needed to design a platform that put them near a source of the chemical. In order to effectively see the bacteria, we used a strain of *Escherichia coli* bacteria (strain W3110) tagged with green fluorescent protein that would glow under a fluorescent microscope. We then exposed them to chemicals of interest in an agar bridge platform [18], which allowed the chemical to spread out from a semi-solid agar plug into a pool of bacteria on a microscope slide (Figure 4). For this test an observation chamber was constructed using glass coverslips and sealed with vacuum grease. Melted agar containing the chemical of interest was injected on one side to create a plug. A bacterial suspension was then injected around the plug, and a region near the agar plug was imaged under a microscope at a total magnification of 20x. The edge of the agar is aligned with the bottom of the imaging area so that accumulation of bacteria would vary along the y axis. Video was output using a camera exposure time of 200 ms (5fps) and sonified for 200 s. For these experiments, both video and sonification output sound were recorded (see Supplementary Files, section 5).

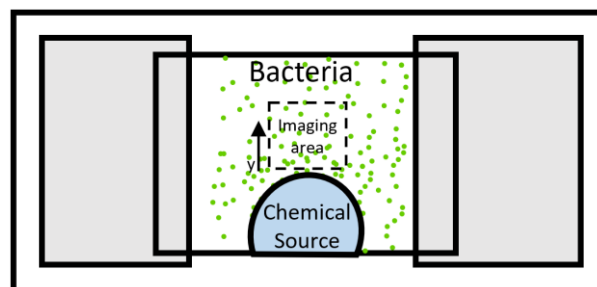


Figure 4: Top-down depiction of the imaging chamber constructed on a microscope slide. Gray squares show the placement of coverslips, and blue semi-circle represents the agar droplet that may contain chemicals of interest. The



green dots represent bacteria (not drawn to scale). The area imaged by the microscope is represented by the dotted box.

3.2. Assaying Chemotaxis with Sonification

In this set of experiments, we conducted an initial evaluation of whether the sonification algorithm could effectively test for chemotaxis based on our design. We exposed a suspension of *E. coli* bacteria to a known chemoattractant (attractive chemical) or chemorepellent (repulsive chemical) [1]. If bacteria were attracted to the chemical, the population concentration should slowly increase at the interface, which we hypothesized would lead to an audible and measurable increase in sound density or perceived noisiness. If bacteria were repelled by the chemical, we expected the population to move away from the interface, leading to a decreased population concentration, which we hypothesized would lead to an audible and measurable decrease. In the control without a chemical, no net movement was expected other than some minimal accumulation near the agar, which we hypothesized would occur because bacterial are unable to penetrate the agar surface. We sonified the results in “easy resolution mode” so the sound frequency was scaled from 20 Hz to 10000 Hz.

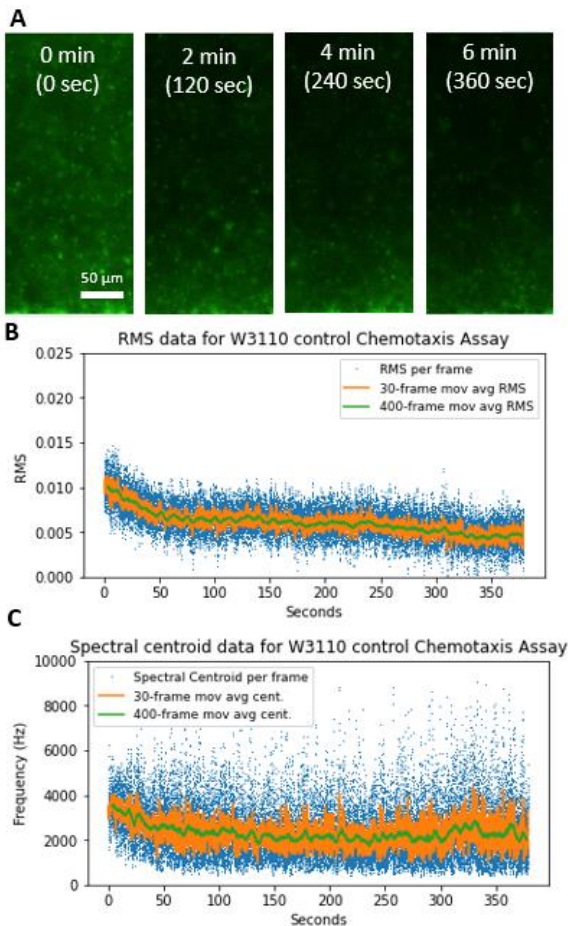


Figure 5: (A) Visual images at several times, (B) RMS values and (C) spectral centroid values of the sonification output for the chemotaxis assay control case without chemical stimuli.

The assay was conducted for a chemoattractant α -methylaspartate or a chemorepellent nickel, as well as a control experiment without a chemical stimulus.

Representative frames of the video feed (Fig 5A, 6A, 7A), as well as plots showing spectral analyses of the sonification output (Fig 5B-C, Fig 6B-C, and Fig 7B-C) are shown. To measure noisiness, root-mean-square loudness (RMS) was used, as an increase in the number of frequencies correlated with the magnitude of the RMS value. Spectral centroid was also reported as it relates to the physical insight of location along the y axis where bacteria have accumulated. Sound-frame-rolling-averaged lines are plotted in order to reduce the noisiness of the data. Averaging over 30 sound frames provides insight into how the sound appears audibly to the ear moment-to-moment and averaging over 400 frames provides insight into the overall trend our brain senses as the sound changes over periods of time.

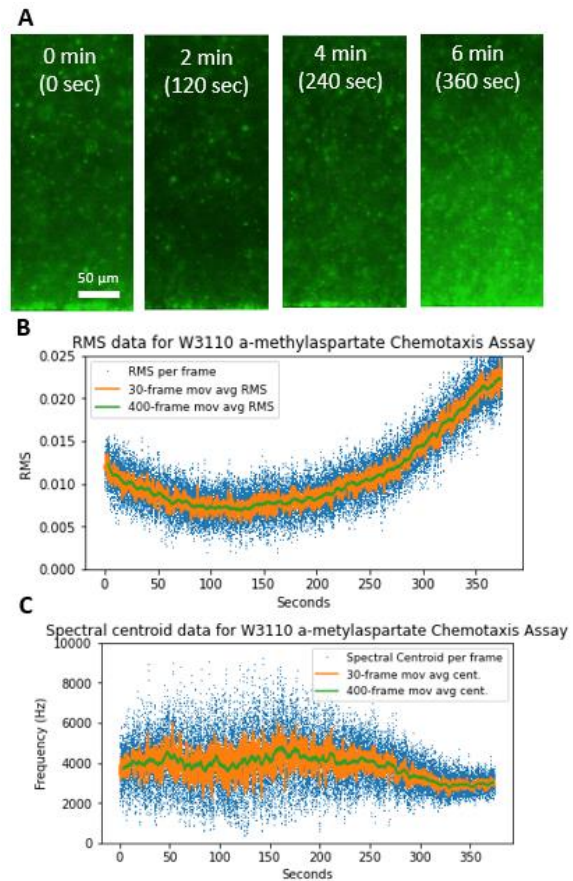


Figure 6: (A) Visual images at several times. Time evolution of (B) RMS and (C) spectral centroid values of the sonification output for bacterial chemotaxis toward a chemoattractant α -methylaspartate.

The first experiment conducted was the control. The control is conducted to establish a baseline for the behavior expected from the bacteria when no chemicals are present, but they are still under exposure to lasers under the microscope and interacting with the agar. It provides baseline sound to expect when there is no response from the bacteria, such that any deviation from this baseline sound indicates a response to the chemical. Figure 5 shows the results for the control. Figure 5A shows the visual output of the microscope (the sonification input) at different timepoints. After 6 minutes of exposure to the fluorescent laser, the brightness of the bacteria has decreased. This is because the fluorescent proteins in the bacteria have been exposed to the laser for so long that they lose their ability to glow, in a process called

photobleaching. This leads to a slight decrease in RMS over time (Figure 5B), and the spectral centroid remains mostly constant but low, reflecting accumulation close to the agar edge (Figure 5C). Bacteria lose fluorescence to the same extent regardless of position, but accumulate towards the bottom as they collide with the impenetrable agar, so this sound provides a baseline response to which the user can calibrate their expectations. The resulting sound and trends can be heard in the attached file for this figure.

The second test conducted looked at a chemical that was a known attractant for the bacteria. Figure 6 shows the results for this compound, α -methylaspartate at a concentration of 1 mM. Figure 6A shows the visual output of the microscope image (the sonification input) at different timepoints. After 6 minutes of exposure to the chemical, a visible accumulation near the bottom of the field of view (near the agar edge) is visible. As expected, at around 300 seconds, the audio shows a clear increase in overall noisiness measured by the RMS of the sound signal (Figure 6B), as well as a decrease in spectral centroid (Figure 6C), as bacteria that are near the bottom of the field of view contribute more frequencies. The resulting sound and trends can be heard in the attached file for this figure.

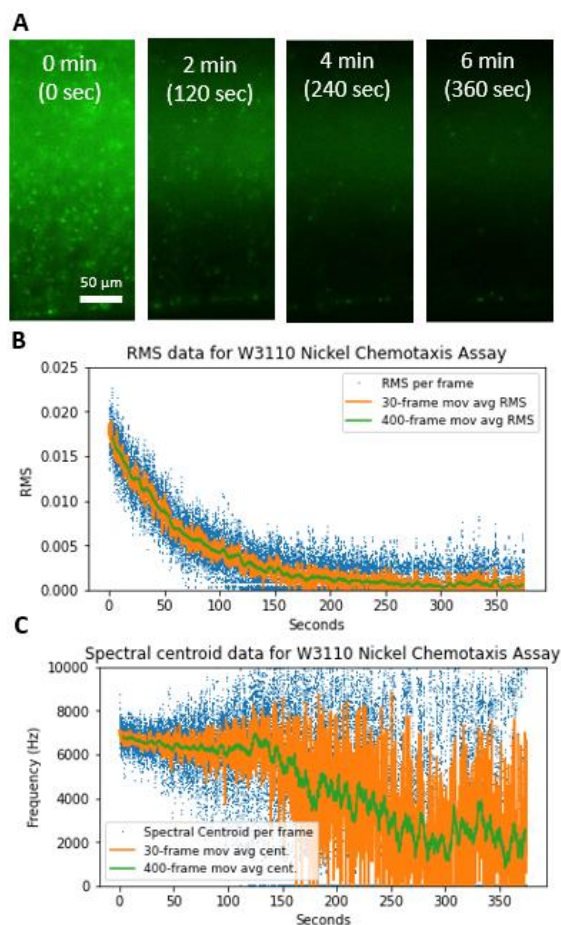


Figure 7: (A) Visual images at several times. Time evolution of (B) RMS and (C) spectral centroid values of the sonification output for bacterial chemotaxis away from a chemorepellent nickel.

The final test conducted looked at a chemical that was a known repellent for the bacteria. Figure 7 shows the results

for this chemical, nickel at a concentration of 0.1mM. Figure 7A shows that upon exposure to nickel, there is a rapid decrease in the bacterial population near the interface. Figure 7B shows the rapid decrease and leveling out of RMS, while the overall average of the spectral centroid starts out high, as the bacteria are moving away from the interface at the bottom, but then decreases and becomes broader as there are fewer bacteria to contribute frequencies (Figure 7C). The resulting sound and trends can be heard in the attached file for this figure.

All three results – the control, attractant, and repellent - showed that sonification, consistent with visual output, provided relevant information about the response of bacteria to the chemical and overall changes in on-screen dynamics. Based on the numerical evaluation, the sound yielded distinct audible markers of chemotactic response within a reasonable time period. Positive and negative chemotaxis were clearly distinguished from one another and from the control in both RMS and spectral centroid. The control without chemicals will be especially important to conduct at the beginning of the experiment, as it provides a baseline for the user to calibrate their ears to changes associated with photobleaching.

3.3. Detection of Chemotactic Waves with Sonification

The second test is another use-case scenario of the sonification software that takes advantage of some benefits as outlined above. In this test, sonification was used to give real-time auditory background feedback on the population distribution from the microscope feed. The user passively listens to and then evaluates, based on auditory and/or visual input, the correct time to start collecting video data for a subsequent analysis. Specifically, we listened for a traveling chemotactic wave of bacteria so that individual cell trajectory data could be recorded as the peak of the wave passed through the center of the image. Based on mathematical models of chemotaxis [26], the peak of the traveling wave corresponds to the chemical concentration at which the bacteria are most sensitive to the chemical signal. The experimental setup was similar to the chemotaxis assay described earlier, but instead of imaging at the edge of the agar, imaging was conducted about one millimeter away from the edge, where traveling waves of bacteria commonly pass through in response to repellents.

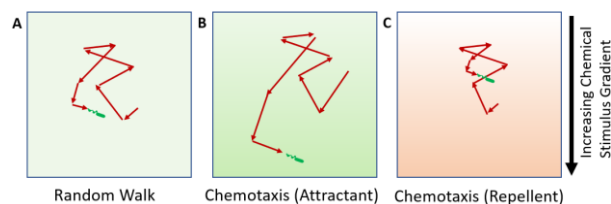


Figure 8: (A) Run and tumble motility of *E. coli*. Bacteria alternate runs with reorienting tumbles, resulting in a random-walk behavior. (B) By extending run lengths in the direction of a chemoattractant, they can bias their overall movement towards the source. (C) By shortening run lengths in the direction of a repellent, they can bias their movement away from the source.

By locating the traveling wave, we analyzed how individual bacteria swimming patterns were altered when their chemotactic response was strongest. *E. coli* bacteria swim in a random-walk pattern called run-and-tumble, where the



bacteria swim forward in a relatively straight line, which is called a “run”, or the bacteria temporarily stops moving and changes direction, which is called a “tumble” [19]. To perform chemotaxis, the bacteria change the length of their runs to bias the random walk so that the mean position of the bacteria moves towards or away from a chemical of interest [8], [9] (Figure 8). Using the collected data, we analyzed the bacterial tracks using TrackMate in ImageJ [20] and evaluated the overall directional bias of the tracks. Since theory suggests that the peak of the wave is where the bacteria are most sensitive, we expected to see a strong bias in the bacterial run lengths in the center of the wave [10]. In our case, we expected to see a decrease in the lengths of the runs in the direction of increasing chemorepellent concentration. While this is a rudimentary analysis as presented here, this platform can be extended to efficiently capture and quantify data in this region and, combined with mathematical diffusion models, be used to predict signaling parameters related to the sensitivity of the bacteria [8].

For this test, bacteria were exposed to nickel as it diffused from the agar plug into solution. For the sonification, we hypothesized that we would hear a distinct increase in the noisiness of the sound as the traveling wave passed through the field of view. We also expected a significant change in the spectral centroid as the wave traveled from the bottom of the field of view to the top. Figure 9 confirms these hypotheses, with Figure 9B showing an increase and decrease in RMS as the wave passed through, and Figure 9C showing a steady increase in spectral centroid. Both of these audible qualities of the sound are recognizable to the user, who can be trained to determine when the sound is sufficiently loud and the pitch is around the center of the spectrum (implying the center of the wave is in frame).

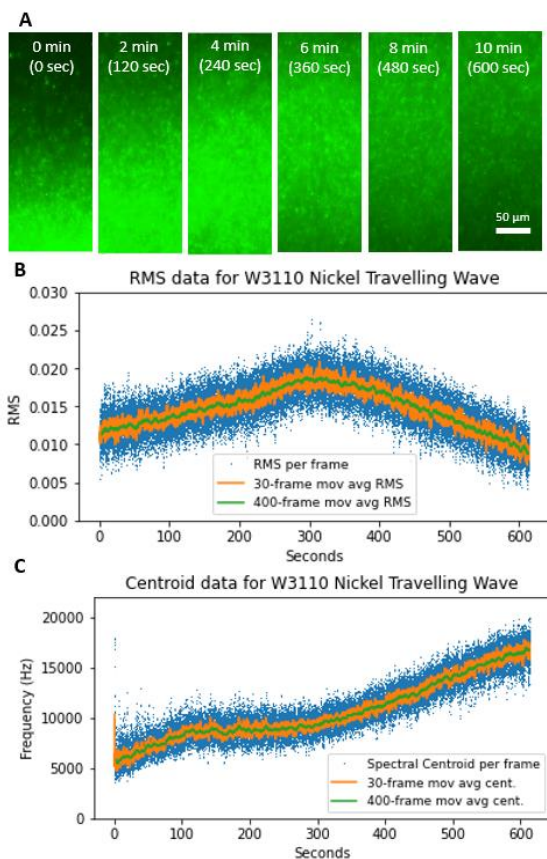


Figure 9: (A) Visual images of bacterial traveling wave over time, with corresponding (B) RMS and (C) spectral centroid values of the sonification output in response to a nickel chemorepellent.

3.4. Analysis of Swimming Behavior within the Wave

Using data collected from the center of the wave, we analyzed bacterial tracks. We hypothesized that the bacterial tracks in the direction of the nickel source would be shorter in length compared to a control, as bacteria tumble and change direction more frequently when they travel up a chemorepellent gradient (the opposite response to an attractant). The tracks in the negative y direction towards the nickel source are far shorter than the tracks in the positive y direction (Figure 10B) and the control tracks (Figure 10A) – overall, we observed no long tracks in the direction of the repellent, which was consistent with our hypothesis. When combined with the population-scale assay to detect traveling waves these results show how the sonification algorithm can be used to relate bacterial population dynamics to changes in swimming trajectories of individual bacteria.

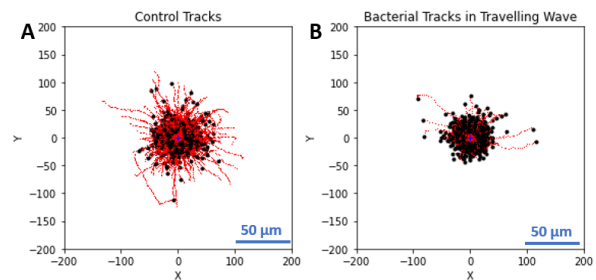


Figure 10: Bacterial tracks plotted with their starting point at the origin (0,0) – (A) control tracks without chemorepellent on the left, (B) nickel-repelled traveling wave tracks on the right. Endpoints of certain tracks are denoted by a black dot. X and Y positions shown in pixels.

4. DISCUSSION & FUTURE WORK

In this work, we examined two use-case scenarios – (1) a basic chemotaxis assay, and (2) the identification of a chemotactic wave entering the field of view. In both cases, our sonification platform was able to give distinguishable responses, although this conclusion is mainly supported by numerical analysis of the sound and basic confirmation by the designers. While both use-case scenarios could be conducted solely visually to a high accuracy, we believe the benefits of the sonification approach are threefold. First, it allows for a new stream of audible information in a way that would allow BVI scientists to get as accurate an interpretation of the bacterial population dynamics as the visual input would provide. However, on this front, there are other accessibility improvements that would need to be made to the process as a whole to make the microscopy assay usable as well; for example, locating the interface in the first place or assembling the device. The second benefit is that by being auditory, the information can be processed in the background. Thus, the user does not have to watch the screen while waiting for a visual signal to begin recording. The user can listen to the dynamic population change while preparing the next sample, cutting a significant portion of time from the process if multiple samples are being assayed. Finally, using the “live” video output function of microscopes and

recording the sonification data saves on expensive memory and storage that would be required to save several gigabytes of video data. Since the population behavior is symmetric, the original shape of the population along the y-axis is the most relevant component of the data, and this is preserved in the sonogram of the sonification output at a high fidelity. This allows for more efficient use of data storage. The second use scenario takes advantage specifically of the second and third benefits of sonification – instead of waiting for a visible traveling wave at a lower magnification, the user can stay at a high magnification, which provides superior data quality for single-cell tracking and listen to the population behavior in the background. Then, once the peak has been audibly identified, the user collects only as much data is needed. The transient population dynamics are still effectively preserved in the sound signal.

In this paper, the analysis of the efficacy is limited to providing sound analysis and sound files. However, for effective use of this sonification platform, especially in the absence of visual data, training will be required. In future work, an evaluation of the platform using a perceptual study group would be necessary to improve the tool and gauge its limitations. We also aim to expand our use-case scenarios, using the tool to expedite experiments related to individual and population-scale swimming in order to gain further knowledge of the bacterial chemosensory system.

5. SUPPLEMENTARY AUDIOVISUAL FILES

Sonified microscope videos for the three assays and the traveling wave can be found at [21]:

<https://zenodo.org/record/7937652>

DOI: [10.5281/zenodo.7937651](https://doi.org/10.5281/zenodo.7937651)

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