Ultrasound Microrobots with Reinforcement Learning
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1. Introduction

Precise, accurate, and controlled motion of microrobots can create exciting new opportunities in 3D manipulation,[1] micro-assembly,[2] sensory applications,[3] and lithography,[4] as well as numerous biological and surgical applications. A variety of physical and chemical strategies exist for propulsion at the microscale, but their use in bioapplications is limited due to lack of biocompatibility, low propulsion speed and forces, and poor navigation capabilities; for example, micro/nanorobots powered by chemical fuels,[5,6] and electric fields[7,8] are not entirely biocompatible. However, there have been recent advances in biocompatible enzymatic microrobots[9,10] with controlled manipulation.[6] Light-induced propulsion is exciting,[11–13] but could face distinct challenges when implementing in vivo applications as light is absorbed or scattered by surrounding tissue.[14]

Magnetically-powered micro/nanorobots offer precise navigation capabilities,[15–25] but many of these robots feature complex 3D structures that require involved fabrication procedures. Recently, ultrasound-based propulsion has elicited considerable interest as an alternate means to generate propulsion and manoeuvre micro- and nanosized objects.[26–27] A brief overview of research in this area follows.

Wang et al. demonstrated propulsion of bimetallic nanorods due to asymmetrical scattering of the sound field.[17] Ahmed et al. designed microswimmers that challenged the commonly-held design paradigm that microswimmers must use nonreciprocal motion to achieve propulsion; instead, the swimmer is propelled by oscillatory motion of an air bubble trapped within its polymer body.[28] Bertin et al. developed 3D-printed extremely fast propelling microrobots through acoustic interaction of microbubbles.[29] Ahmed et al. developed a nanorobot propelled by travelling acoustic waves through the oscillation of a flexible flagellum.[30] Inspired by starfish larvae, Dillinger et al. developed a novel type of soft acoustic microrobots propelled by reciprocal motion of ciliary bands.[31] Wei et al. demonstrated bidirectional motion of microparticles on the surface of micropillars through modulation of the acoustic signal combined with visual feedback control.[32] Kaynak et al. demonstrated acoustically activated 3D-printed micromachines.[33] Janiak et al. developed a new mechanism for the self-assembly, microparticle trapping, and acoustic propulsion of discoidal microparticles in a viscous gel.[34] Ahmed et al. developed rolling microrobots inspired by the rolling of neutrophils on endovascular walls, which can execute upstream propulsion in a combination of acoustic and magnetic fields.[35] Zhang et al. further demonstrated a fundamentally new mechanism of rolling motion, i.e., rolling without dependence on real physical boundaries.[36] Dayton et al. utilized the acoustic radiation forces to concentrate microbubble contrast agents near a vessel wall in vivo.[37] Finally, an acoustic vortex beam and standing wavefield have been applied to manipulate micrometer- and millimeter-sized particles in vivo using phased-array and acoustic standing wavefield approaches.[38,39,40] For example, gas-filled microbubbles have been manipulated within the...
vasculature of zebrafish embryos by modulating the pressure antinode of a standing acoustic wavefield. However, these microbubbles can only be navigated over a small distance due to the piezo transducer's narrow frequency bandwidth. Ultimately, ultrasound-actuated micro/nanorobots can generate large propulsive forces, require low power, are safe, and have potential for in vivo use; however, they yet suffer from poor navigation capabilities, which limits their practical applications. To achieve controlled steering and manipulation in 3D, acoustically-activated swimmers have been designed with magnetic nanostructures and microcontainers; however, no reinforcement learning capabilities have been implemented for navigation purposes to date.

In the last decade, the growing availability of large annotated datasets and computation has prompted development of numerous machine learning-based applications in 3D microscopy, fluid mechanics, diagnostics, and medicine. Artificial intelligence has made significant progress in visual object recognition and detection, and deep learning architecture in reducing the computational complexity of physical processes. Accordingly, machine learning and computer vision strategies are widely utilized in large-scale robotic and control systems. However, very few works exist regarding artificial intelligence in microrobotics platforms. In general, the lack of AI in microscale can be attributed to the absence of onboard microcontrollers and sensors, which precludes direct acquisition of necessary information. Nonetheless, artificial intelligence-based techniques in microrobotics platform are an exciting field of study with a great room for development in acoustics. Adaptive behavior in complex flow has been demonstrated by masses of unevenly distributed gyrotactic microparticles using reinforcement learning. Additionally, reinforcement learning has been used to realize acoustic-mediated navigation of 600 μm particles on a flat metal surface toward a target location, and to provide optimal strategies for automating self-thermophoretic particles. Deep learning has been used to navigate and track magnetic microrobots in simple 2D fluid environments, and a deep Q-learning approach has been proposed for navigating colloidal robots in unknown environment. To the best of our knowledge, artificial intelligence with ultrasound-based microrobots does not exist.

In this work, we implement reinforcement learning control strategy to navigate ultrasound-activated microrobots, exhibiting accurate target identification and tolerance definition. This system can transport a single swarm of microrobots to a user-defined target point. We empirically explored and discussed critical factors that affect transport performance and developed different positioning settings for chosen tolerance values. After training the robot to follow a circle, we attempted to improve its path in every episode. The robot was then programmed to follow the policy that maximized the cumulative reward. As a demonstration of the method's capability in arbitrary navigation, we had it manipulate the microrobots to spell “ETH.” Further development of ultrasound robots with reinforcement learning capability will lay the foundation for the development of next-generation intelligent micro/nanorobots for application in medicine.

2. Results

In our approach to ensuring experiments are performed successfully and with consistency, the scale and simplicity of the experimental setup are central considerations. The schematic in Figure 1A depicts our experimental setup, which consists of a microfluidic channel and four piezo transducers (PZTs) that act as actuators. The channel, made from polydimethylsiloxane (PDMS). It is designed as a square aligned with the PZTs, which are glued directly to the sides of the channel (see the Experimental Section). When conducting experiments, a solution containing microbubbles is introduced into the channel. Without any acoustic actuation, they remain dispersed evenly through the solution, but when subjected to an incident acoustic field, the acoustic force brings the microbubbles together in swarms. We exploited this mechanism to cluster the microbubbles at arbitrary positions by means of pseudorandomly actuating each of the PZTs one at a time using a Mersenne Twister random number generator.

An in-house Python pipeline has been developed to facilitate communication between instruments. Iteratively, a model-free control algorithm navigates a microswarm with arbitrary size and location through a microfluidic channel from position \( \mathbf{p}_i \) toward the next target point \( \mathbf{p}_i \in \mathcal{P}_i \) along an arbitrary path \( \mathcal{P} \subseteq \mathcal{P} \in \mathbb{N}^{300\times300} \).

The goal condition is satisfied when the swarm has consecutively passed all target points that make up the target path. In this notation, \( \delta \) denotes the minimum distance that a swarm has to be from one target point in \( \mathcal{P}_i \) in order to be allowed to move to the next.

The control algorithm predicts optimal values for the output variables, which consist of peak-to-peak voltage \( V_{pp} \), frequency \( f \), and PZT index \( k \). That is, for every step \( n \), the algorithm has to choose an optimal \( V_{pp} \) and \( f \) for each \( k \). Each step is finalized by sending an AC voltage at \( V_{pp} \in [0, 20] \) V and frequency \( f \in [0, 5] \) MHz to each of the four PZTs \( k \in [1, 2, 3, 4] \). The three output variables thus form a 3D action space, the boundaries of which are set by the limits of the Tektronix AFG301C function generator and the number of PZTs. Given that this action space is in fact very large, we performed action space reduction in order to speed up the computation time and reduce complexity. An overview of the pipeline is illustrated in Figure 1c.

2.1. Dynamics of an Ultrasound Microswarm

The ultrasound waves travel through the PDMS and reach the liquid environment that contains the microbubbles. As the microbubbles are exposed to the sound field, they oscillate and scatter, giving rise to the secondary Bjerknes force. Accordingly, adjacent microbubbles cluster into a swarm, as depicted in Figure S1C (Supporting Information). The propulsion strategy relies on the combined action of the primary radiation force and the secondary Bjerknes force. Microswarms are formed by...
Figure 1. A) Schematic of the experimental setup comprises of a $4000 \times 4000 \times 50 \, \mu m$ square channel and four piezo transducers. A piezo transducer is attached to each of the walls, which allows the robot to move in all directions. B) Microbubbles form a swarm microrobots due to the effect of the secondary radiation force $F_B$, and then are manipulated the primary radiation force $F_P$. C) Sequential flow diagram of the navigation algorithm (on the right in dark red) and instruments (on the left in blue). A swarm detection algorithm, explained in the “Object detection and tracking” section, initializes a CSRT tracker, which tracks the swarm at location $p_s[n]$ for any $n > 0$. A list of past locations $[p_s[n], p_s[n-1], \ldots, p_s[n-m]]$ is continuously held in memory to update the learning dynamics matrix $Q_{local}[n]$. This is combined with the static dynamics matrix $Q_{global}$ (loaded from memory at the start of the experiment together with target path $P_t$) to form the combined dynamics matrix $Q[n]$. Policy $\pi$ uses $Q[n]$ to choose the optimal set of outputs based on current position $p_s[n]$ and target position $p_s[n]$. These outputs (voltage $V_{pp}[n,k]$ and resonant frequency $f_0[n,k]$) are then transferred as inputs to the PZTs, which produce ultrasound. This produces a response from the microswarms, which is observed by an observation module through a microscope, after which the process repeats for step $n+1$. 
secondary radiation forces,\textsuperscript{[65]} while primary radiation forces from the piezo transducers guide the microrobots along the desired trajectory as depicted in Figure 1B (see also Note S2, Supporting Information).

2.2. Action Space

We prune the action space from three dimensions to one dimension to simplify the navigation problem and increase the algorithm’s precision and convergence speed. There are three reasons that this pruning step is necessary to achieve our results. First, during initial experiments, we found the action space that results from the natural limits of our equipment, specifically the Tektronix AFG301C function generator, to be too large for the learning control algorithm to converge in an acceptable finite timespan without any other explicit constraints. That is, algorithms that search the 3D action space for an optimal action had difficulty converging into consistent navigational behavior. Second, not only are the natural boundaries too expansive, but a large part of the range of \( f \) is completely irrelevant in practice. For one, the acoustic field strength that we require can only be created by PZTs when using frequencies at or around certain resonance frequencies,\textsuperscript{[44]} one of which is the optimal theoretical resonance frequency \( f_0 \) of \( \approx 2 \text{ MHz} \) for the PZTs used in our study. Thirdly, we experimentally found that small changes in \( f \) can produce large differences in the swarm-PZT dynamics of the system; hence, by constraining \( f \) we increase reproducibility.

We thus devised a novel approach to achieve consistent navigation, which is largely enabled by reducing the action space size through pruning. We approached this pruning with the assumption that the microswarm should move at maximum speed at all times, because our algorithm aims to satisfy the goal condition as fast as possible. Thus, we can apply limitations to \( V_{pp} \) and \( f \) that promote convergence toward the goal condition. In particular, we can experimentally determine the optimal \( V_{pp} \) and \( f \) for each PZT \( k \). This reduces the action space to a 1D \( k \) and its corresponding pre-determined \( V_{pp} \) and \( f \).

Accordingly, we conducted a grid-search of the action space, collected a series of images, and measured the movement of swarms at arbitrary positions in the channel. To create the search set, we first discretized \( V_{pp}[n,k] \in [10, 20] \) to form one axis consisting of six values with a step size of 2: \( V_{pp}[n,k] \in \{10, 12, 14, 16, 18, 20\} \). We then discretized \( f[n, k] \in [1.5, 2.5] \), creating a set of 41 values with steps of size 0.025. These were combined with \( k[n] \in \{1, 2, 3, 4\} \) to form a search set having \( 6 \times 4 \times 4 \times 4 = 984 \) samples. The swarm dynamics at each point in the search set was recorded for three seconds at 33 frames s\(^{-1}\), producing a dataset of 984 \( \times 3 \times 33 = 97416 \) images. We collected the recordings over the course of 41 experiments at distinct frequencies, as swarms became less responsive during the course of a single experiment. This produced high-quality and reproducible data from which we could make assumptions about swarm-PZT dynamics. Sample footage of these recordings is provided as Video S1 in the Supporting Information.

Our approach provides a means of rigorously measuring the dynamics of an acoustic microswarm within an acoustic microfluidic system without any prior assumptions. Importantly, the lack of prior assumptions about swarm dynamics means it could be scalable to in vivo applications. Moreover, our results clearly indicate the resonance frequency \( f_0[k] \) for each of the four PZTs in the system (Figure 2B–E) as well as an approximately linear relationship between voltage and swarm velocity (Figure 3). Using that data, we applied the following constraints to the action space based on the assumption that maximizing the velocity of a swarm allows it to satisfy the goal condition as fast as possible.

\[
V_{pp} [n, k] \in \{0, 20\}, \forall n, k \sum_{i=1}^{\infty} V_{pp} [n, k] =
\]

\[
20 \forall n, k \left[ f[n, k] = f_0[k] \right] \forall n, k
\]

In other words, policy \( \pi \) only has to find a single PZT with index \( k = k_0 \) to activate at its resonance frequency \( f[n, k = k_0] = f_0[k_0] \) and maximum voltage \( V_{pp} [n, k = k_0] = 20 \), while providing the other three PZTs with \( f[n, k \neq k_0] = 0 \) and \( V_{pp} [n, k \neq k_0] = 0 \). By making the input variables dependent or constant, we simplify the control problem to a choice between only four possible actions. This allows us to achieve reproducible navigation of microswarms.

2.3. Reinforcement Learning Implementation

Our control algorithm is based on a subcategory of reinforcement learning termed “Q-learning,” originally devised by Watkins,\textsuperscript{[66]} which aims to learn the value of an action in any arbitrary state. Specifically, it is designed to find the optimal policy \( \pi^* \) that maximizes the expected value of the total future reward \( E(R) \) for any finite Markov decision process.\textsuperscript{[68]} In short, the agent attempts an action under a given state, for which it receives a reward or penalty. It then assesses that consequence and makes a new attempt. Through repeatedly trying all actions under all conditions, the agent ultimately learns which perform best according to the overall reward.

To understand the reason for our choice of learning method, it must first be understood that the behavior of the microswarm-PZT system is extremely spatially nonlinear.\textsuperscript{[69]} Even in our simple experimental setup, the environment is not static. For example, auxiliary microbubbles and swarms are present in the channel with spatial and temporal variability, as are contaminants such as dust particles and an increasing number of burst microbubbles as the experiment proceeds. These environmental factors result in a changing nonlinear bulk acoustic wavefront combined with secondary and higher order waves that are time-dependent and highly sensitive to the initial state. This spatially and temporally nonlinear behavior makes it difficult to navigate based on a continuously learning algorithm, because convergence is not guaranteed. Such dynamism might also be one of the reasons why theoretical models and simulated models often are not validated by actual acoustic manipulation experiments.

2.3.1. Global Dynamics

As outlined above, control and automation of acoustic robotic systems has remained a fundamental challenge.\textsuperscript{[70]}
mainly one of reliability and scalability as in vivo applications require moving toward more nonlinear environments. To start to overcome this fundamental limitation, we created a global dynamics matrix $Q_{global}$ for each of the four PZTs; this matrix represents the expected movement $E[p_{n+1}]$ in $\mu m s^{-1}$ of swarms at all locations in the channel, visualized in Figure 4A–D. The dynamics of the swarms can be extracted from the dataset described earlier, using linear regression to...
extrapolate a uniform grid of $E[p_{n+1} | \forall p \in P]$ from the non-uniform data.

We specifically extrapolated the dataset to a 4D array, in which two dimensions represent the position $p_k$ in the channel, the third dimension stores $E[p_{n+1} | \forall p]$ and the last dimension distinguishes the four PZTs. $Q_{\text{global}}$ was then used to perform navigation of microswarms along an arbitrary path. For demonstration purposes, we had the swarms write “ARSL” as depicted in Figure 6A–D. Video footage of these experiments is available in Video S2 in the Supporting Information.

2.3.2. Local Dynamics

We found experimentally that when using only global dynamics, swarms often become stuck. This is likely a result of the overgeneralization that the global dynamics represent; an algorithm that relies upon generalized behavior in a spatially and temporally dynamic environment is likely to encounter a local minimum. Thus, while global dynamics are necessary for navigation, they need to be supplemented by a local dynamic matrix $Q_{\text{local}}$ to account for changes in the environment. We constructed a uniformly initialized matrix of the same shape as $Q_{\text{global}}$ for $n=0$ and applied the following formula to update $Q_{\text{local}}$ after initialization

$$Q_{\text{local}}[n+1] = (1 - \alpha) * Q_{\text{local}}[n] + \mathbb{E}[\alpha * Q_{\text{local}}[n+1]]$$  (3)

where $\alpha \in [0, 1]$, the learning rate, can be used to make the function more or less sensitive to changes in dynamics. $\mathbb{E}[\alpha * Q_{\text{local}}[n+1]]$ is calculated from the average of a number of past steps collected in memory. As environments become more complex, it is necessary to adjust the learning rate in order to give greater account to local behavior. Since our reinforcement learning method is not contingent on the propulsion mechanism, it could be adjusted for any ultrasound-based microrobots.

To demonstrate the capability of the learning algorithm, we used $Q_{\text{local}}$ to direct swarms along an arbitrary path (three laps of a circle) and compared its prediction error to the use of $Q_{\text{global}}$ alone (Figure 5A,B). Video S3 in the Supporting Information shows a live visualization of the 4D $Q_{\text{local}}$ on the left, and a microswarm navigating in a circular path on the right. In this experiment, $\alpha = 0.05$.

2.3.3. Policy

We then combined $Q_{\text{global}}$ and $Q_{\text{local}}$ into one matrix encapsulating overall dynamics using the following formula

$$Q[n] = \beta * Q_{\text{global}} + (1 - \beta) * Q_{\text{local}}[n]$$  (4)

in which $\beta \in [0, 1]$ represents the bias toward global behavior. A large $\beta$ makes navigation easier, but increases the chance of the swarm getting stuck in a local minimum. The obtained matrix $Q[n]$ contains the final expected velocity $E[p_{n+1} | \forall p]$ for any position and any $k$. When navigating a swarm, the policy finds a $k_{\text{optimal}}$ such that

$$k_{\text{optimal}}[n=m] = \argmax_{k \in 1, \ldots ,4} \mathbb{E}[p_k | x=a, y=b, n=m] - p_k | x=c, y=d, n=m+1]$$  (5)

In other words, the policy chooses to activate the PZT with index $k$ at arbitrary step $n = m$ to direct the microswarm at position $x = c$, $y = d$, if and only if doing so minimizes the expected distance between the swarm and the target position at $x = a$, $y = b$. To demonstrate the method capable of precise navigation along an arbitrary path, we used swarms to spell out “ETH” (Figure 5C; and Video S4 in the Supporting Information). We found that microswarms manipulated by this policy are less prone to becoming stuck in local minima while still able to navigate long distances efficiently.

3. Discussion

To overcome difficulties in steering microrobots by ultrasound, we implemented a reinforcement learning strategy and developed a Python platform that interfaces with the piezo transducers, electronic circuits, microcontroller, and a camera for feedback. In microrobot manipulation with this platform, images are grabbed and analyzed and the microrobots are detected and tracked within those images. The algorithm was able to navigate the microrobots efficiently and to perform tasks autonomously due to the use of a large amount of training data based on experimental results; as a demonstration, we used a microswarm to spell ARSL and...
ETH. Since our system is based on a model-free control strategy, it could be adapted for the study and guidance of other acoustic microrobot propulsion systems. Previous studies have shown that the size of the microbubble clusters depends on the concentration of microbubbles, the applied acoustic power, and the duration of the applied acoustic field.\textsuperscript{[71,72]} Future work will also include programmable regulation of the cluster size. Additionally, the reinforcement learning strategy can be applied to the manipulation of living cells with a standing acoustic wavefield. That is, the simultaneous activation of at least a pair of piezo elements will result in a standing acoustic wavefield, and due to the positive contrast factor of cells in a surrounding liquid medium, they can be trapped at the pressure nodes of that wavefield. The trapped cells can then be manipulated by adjusting the excitation frequency. This is made feasible by having complete control over the frequency and number of piezo transducers activated by our platform.

To navigate microrobots in complex environments, we combined our main generalized trained matrices with local matrices that take into account the local behaviors of microrobots. By increasing the learning rate, we plan to navigate our microrobots in more sophisticated environments. As part of our future research, we intend to extend our reinforcement learning strategy to a transducer array combined with ultrasound imaging,\textsuperscript{[73]} photoacoustic,\textsuperscript{[74]} or two-photon microscopy for the imaging, training, and manipulation of microrobots in 3D in vivo. This may provide an avenue for the tracking and manipulation of microrobots in blood vessels in vivo, which has to date proven challenging.

4. Experimental Section

The experimental setup is shown in Figure S1 (Supporting Information), which includes a function generator, camera, inverted

![Figure 4. A–D) The respective global dynamics of PZTs 1–4, averaged over 100 000 images. Rows show the predicted movement of an arbitrary microswarm during 1 s as a function of the swarm's position in the channel. As most arrows point away from the PZTs, it is very evident that the primary bulk acoustic wave is dominant over secondary and higher-order waves. Irregularities in the field can mostly be attributed to the fact that the experimental data are not perfectly uniform, especially at channel edges. In addition, the primary wave is also not expected to be completely uniform because of environmental irregularities, such as dust particles and auxiliary microbubbles and swarms. In both E) and F), we show the average distribution of microbubbles injected in the microfluidic channel over all data in the x and y directions, respectively. G) Swarm manipulation using only the global dynamics matrix. The algorithm is given a path consisting of a series of points in a 300-by-300 discretized coordinate system within the channel. It iteratively tries to navigate the centroid of the swarm to consecutive points on the path, moving on to the next point once the location of the swarm has satisfied the condition $|p_t[n] - p_s[n]|^2 \leq 5\sigma_{p_s}$.](image-url)
microscope, microcontroller, electronic circuit, microfluidic channel, and piezo transducers (PZTs) that serve as actuators, which were glued to the PDMS wall with two-component epoxy glue. The ultrasound piezo transducer used has a resonance frequency of 2 MHz. A function generator (Tektronix AFG31000), a microcontroller (Arduino-Uno), and a relay module, and a camera (Hamamatsu C11440) connected to an inverted microscope (Leica DMI6000B) were connected to In-house Python code. Images are captured at 33 frames s$^{-1}$ by the camera and are processed by Python for autonomous manipulation. Additionally, the Python code controls the frequency, amplitude, and state of the piezo transducers.

Microfluidic channels were fabricated with polydimethylsiloxane (PDMS) using a standard soft lithography and mold replica technique. A master mold was used to manufacture each device, which was then lithographically patterned with SU-8 negative photoresist on a 4 in. silicon wafer and then placed inside a Petri dish. PDMS prepolymer was prepared by mixing the silicon elastomer base and curing agent at a 10:1 weight ratio, following which the PDMS prepolymers were degassed under vacuum and cast into the mold. PDMS was cured by heat treatment at 85 °C for 2 h. Bonding was conducted after plasma pretreatment for 1 min.

**Microbubble Contrast Agents:** The microbubbles used for manipulation were imaging contrast agents purchased from Bracco Sonovue. The Sonovue contrast agent is provided in a “fabrication kit” that includes a glass vial containing lyophilized sulfur hexafluoride lipid-type A powder (25 mg) and a syringe prefilled with saline solution. Following injection of the saline solution into the glass vial and gentle shaking, a microsphere dispersion is produced. The sulfur hexafluoride gas and the phospholipid monolayer shell provide stability and prevent microbubble coalescence. The produced microbubbles are tiny, having a mean diameter of 2.5 µm; most range between 2 and 9 µm, with 90% under 6 µm and 99% under 11 µm. In experiments, this MB solution was injected with a pipette into the microfluidic channel and also into the silicon tube connected to the syringe pump. The self-assembly of the microswarms under acoustic forces was recorded by a camera connected to an inverted microscope.

**Figure 5.** For the purpose of demonstrating the capabilities of the learning algorithm, we used the following algorithm $Q_{\text{local}}$ to direct swarms along an arbitrary path. A) A representation of the swarm moving in a circle in response to each piezo transducer being activated in turn. B) Images showing real-time manipulation of the swarm through a circle, the green color indicates the activation of the piezo element. C) Swarm manipulation using both global and local dynamics to spell ETH, demonstrating the ability of this combined approach to achieve precise manipulation. These experiments had a much higher success rate than those using only global dynamics.
A) Local prediction error of a swarm following an arbitrary circular path. The error is defined as the average error of the 2D \( \mathbb{E}[p[n+1]] \) and thus contains information about both angular prediction error and velocity prediction error. The graph starts similar to an exponentially decaying harmonic wave. B) Global prediction error of a swarm following the same arbitrary circular path. The error magnitude does not decrease over the course of the experiment.

Images analysis of the experiments was processed using autonomous object detection and tracking system. The experiments require a robust approach to subtract the background from images and analyze them in real-time. Automatic detection and tracking systems can process much more information than a manual operator. An operator may be unable to perform acoustic microfluidic experiments with rapid changes in behavior. Therefore, a feedback system that detects and tracks the location of any swarm in-frame of at least 10 \( \mu \text{m} \) in diameter at a speed of ≈33 frames per seconds (fps) was developed. The minimum swarm diameter is only limited by the microscope magnification and resolution, which is in turn limited by the required processing speed. The visual sensory system consists of a microscope and an observation module and that collects a 2048-by-2048 16-bit PNG image, \( I_{\text{PNG}} \), through a 5x magnifying lens to a processing pipeline in the Python programming language in PyCharm 2020.3.3. The pipeline compresses the image to 300-by-300 8-bit grayscale. BMP image, \( I_{\text{BMP}} \) shown in (Figure S3A, Supporting Information). This compression decreases timestep \( t[n] \rightarrow t[n+1] \) considerably compared to using a noncompressed image. Not only can the swarm location \( p_s \) be extracted quicker, but the size of state space \( P_s \) is considerably decreased, which increases convergence speed of the algorithm. For \( n = 0 \), a threshold condition is applied to \( I_{\text{BMP}} \) to form a binary image, \( I_{\text{thresh}} \) as shown in (Figure S3B, Supporting Information). The threshold boundaries are determined from the distribution (Figure S3E, Supporting Information) of the pixel intensity of a sample set of images. The intensity threshold filters out any pixels of higher intensity than a specific value and provides maximum contrast between microbubbles, black, and the bulk surroundings, white, but does not yet distinguish between swarms and image contaminants. The binary image is then convoluted with a 2 × 2 Gaussian blur kernel as shown in (Figure S3C, Supporting Information) in order to improve accuracy in the next step. Standard Canny edge detection\(^{[75]}\) from the OpenCV image processing library was used to produce \( I_{\text{canny}} \), an image containing the edges of \( I_{\text{thresh}} \). \( I_{\text{canny}} \) retains only edge features as shown in (Figure S3D, Supporting Information), which are used to find the contours of an image using the OpenCV implementation of topological structural analysis\(^{[76]}\). It was experimentally found that this implementation inherently filters out almost all nonmicrobubble contaminants such as dust particles due to their small scale (≈10 – 50 \( \mu \text{m} \)) compared to the microswarms were used (50 – 200 \( \mu \text{m} \)). On top of this, the OpenCV implementation of topological structural analysis only detects shapes with relatively soft curves, a property that is often not associated with dust particles. If no manual specification is presented to the algorithm, it automatically chooses the one with the largest moment to find \( p_s[0] \). For any sequential steps \( n > 0 \), tracking is used instead of detection to improve processing speed and ensure a continuity of the one that was experimentally found to have the best combination of accuracy and speed was the OpenCV implementation of a discriminative correlation filter with channel and spatial reliability\(^{[64]}\).

**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

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**Conflict of Interest**

The authors declare no conflict of interest.
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