Dynamic Mode Structure of Active Turbulence Modeling Results from 2019-2022 (VIC project)

Website: https://www.bco-dmo.org/dataset/885923
Data Type: model results, experimental
Version: 1
Version Date: 2022-12-28

Project
» Collaborative Research: Viral induced chemotaxis mediating cross-trophic microbial interactions and carbon flux (VIC)

Contributors	Affiliation	Role
Guasto, Jeffrey	Tufts University	Principal Investigator
Henshaw, Richard J.	Tufts University	Scientist, Contact
Newman, Sawyer	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Dense suspensions of swimming bacteria exhibit chaotic flow patterns that promote the mixing and transport of resources and signalling chemicals within cell colonies. While the importance of active turbulence is widely recognized, the structure and dynamics of the resulting collective flows are the subject of intense investigation. Here, we combine microfluidic experiments with proper orthogonal decomposition (POD) analysis to quantify the dynamical flow structure of this model active matter system under a variety of conditions. In isotropic bulk turbulence, the modal representation shows that the most energetic flow structures dictate the spatio-temporal dynamics across a range of suspension activity levels. In confined geometries, POD analysis illustrates the role of boundary interactions for the transition to bacterial turbulence, and it quantifies the evolution of coherent active structures in externally applied flows. Beyond establishing the physical flow structures underpinning the complex dynamics of bacterial turbulence, the low-dimensional representation afforded by this modal analysis offers a potential path toward data-driven modelling of active turbulence

---

Table of Contents

- Coverage
- Dataset Description
  - Methods & Sampling
  - Data Processing Description
  - BCO-DMO Processing Description
- Data Files
- Supplemental Files
- Related Publications
- Parameters
- Instruments
- Project Information
- Funding

Coverage

Temporal Extent: 2019-09-30 - 2022-05-01

Methods & Sampling

Culturing:

Wild-type Bacillus subtilis bacteria (strain OI1084) were taken from −80°C frozen stock and streaked onto 1.5% agar plates prepared with Terrific Broth (TB, Sigma). Plates were incubated at 25°C for 24 hours, after which time a single colony from the plate was used to inoculate an overnight liquid TB culture at 30°C with

shaking (200 rpm). The bacterial suspension was then subcultured (1.5 ml of cell culture into 60 ml of pre-warmed TB) and grown at 35°C and 200 rpm for 6 hours to mid-log phase (OD600 ≈ 0.2). Immediately prior to experiments, dense cell suspensions (~ 1010 cells.ml−1) were prepared by centrifugation at 5000 g for 5 minutes, and the pellet was resuspended with 2 µl of fresh TB media.

Microfluidics and image analysis

Polydimethlysiloxane (PDMS) microfluidic channels were fabricated through soft lithography and plasma bonded to No. 1 thickness glass coverslips. The PDMS channels were thinly cast to ensure ample diffusion of oxygen to the bacterial suspension and prolonged cell activity. Dense cell suspensions were gently loaded into the microfluidic devices via pipette, and the channel inlet and outlet were sealed with wax to prevent residual flows. For all experiments, bacterial suspensions were imaged with brightfield illumination on an inverted microscope (Nikon Ti-E) using a sCMOS camera (Zyla 5.5, Andor Technology). Time-resolved velocity fields, u(x, t), of the bacterial suspensions were measured by performing Particle Image Velocimetry (PIV) using PIVLab implemented in MATLAB. The subsequent velocity fields were then lightly smoothed using a Guassian kernel with a standard deviation of one PIV pixel (1.73 µm) in space and one frame (9.5 ms) in time. Vorticity fields, ω(x, t), were computed from measured velocity fields using central differencing (MATLAB).

Bulk suspensions with decaying activity (quasi-2D)

To capture the bulk dynamics of dense bacterial suspensions in the absence of lateral wall effects, large microfluidic chambers (2 mm ×2 mm ×26 µm) were prepared, where the side length corresponds to ≈80 correlation lengths of the turbulent bacterial suspension. Imaging was performed with a 20 × objective (0.45 NA) at 105.5 fps. Bacterial activity was varied using a previously established approach. Briefly, the cell suspensions were imaged periodically over the course of 30 minutes, during which time the cell swimming speed naturally decayed due to oxygen depletion, where the decay rate of cell activity was controlled via the thickness of the PDMS device. Without the need to manipulate the cell suspension, this approach ensured that the bacterial concentration was constant across varying activity levels. Seven data sets were captured in total, which consisted of 6, 300 frames each (≈ 1 min per video) and a 2 − 3 min delay between acquisitions. Data analysis was restricted to the central portion of the chamber, ≈ 30 correlation lengths from the lateral boundaries.

Cell suspensions under varying confinement

To quantify POD modes for bacterial suspensions under varying degrees of confinement experiments from Wioland et al (2016). were replicated with minor modifications. Microfluidic devices (19 µm deep) were fabricated, which comprised a series of racetrack geometries connected by narrow inlets (30 µm wide). Nine racetrack widths were chosen between 30 µm ≤ W ≤ 171 µm in fractional increments of our measured characteristic vortex size (38 µm) taken as the minimum point in the spatial ACF in quasi-2D conditions (Fig. 1C, dotted red line). Cell suspensions were imaged at 40× (0.6 NA) magnification and 100 fps for 10 s per channel width. All confined microfluidic geometries were simultaneously loaded with the same cell suspension and imaged within the first 10 min of loading to ensure consistent bacterial activity. Experiments were 8 repeated across multiple days with freshly cultured bacteria to verify repeatability. The net flow ψ was calculated following the definition in [18]: ψ = |(Σu · eˆx) / (Σ||u||)|, where eˆx is the unit vector along the principal channel direction and the sum is over all PIV sub-windows over 5 s (500 frames) of video. ψ = 1 indicates uni-directional circulation flow around the racetrack, and ψ = 0 corresponds to a globally stationary suspension. The stream-wise flow profiles, F(y), were generated from the velocity field by averaging over time and space as F(y) = hu(x, y, t) · eˆxi(x,t) , where eˆx was chosen as defined in [18], such that F(y) is on average positive.

Data Processing Description

Flow fields were calculated using PIV (PIVLab, MATLAB), publicly available here. https://www.mathworks.com/matlabcentral/fileexchange/27659-pivlab-particle-image-velocimetry-piv-tool-with-gui . Box sizes of 32 pixel then 16 pixel (50%) overlap were used.

The subsequent velocity fields were then lightly smoothed using a Guassian kernel with a standard deviation of one PIV pixel (1.73 µm) in space and one frame (9.5 ms) in time.

An example script of the POD analysis is included in the submitted data files - this makes uses of the in-built "svd" function in MATLAB, and should be compatible with all versions.

BCO-DMO Processing Description

Primary data file set to a csv representation of POD Flow Field Averages from each of the seven experiments.

Spaces removed from parameter names in primary data file (csv).

[table of contents | back to top]

Data Files

File	
8855923_v1_PODFlowFieldAverages.csv File processed with laminar pipeline "8855923_v1_PODFlowFieldAverages" at path 8855923/1/data/8855923_v1_PODFlowFieldAverages.csv	(Comma Separated Values (.csv), 313 bytes) MD5:232d3912229b3bd8dc6dcc50a39ccac5

[table of contents | back to top]

Supplemental Files

File	
Example script for POD analysis filename: Example_POD_Analysis.m Sample MATLAB script to conduct POD analysis on an input flow field. Uses in-built "svd" function for the decomposition	(MATLAB Programming Script (.m), 1.21 KB) MD5:ea781c05d5bf2d34a263875d4e09f0c7
Exp01_PODFiles filename: Exp01_PODFiles.zip POD files for decomposition of both the velocity and vorticity fields of Exp01 (corresponds to Exp01 of the FlowFields data)	(ZIP Archive (ZIP), 27.04 GB) MD5:b7a2468408159922473fe74e693067e8
Exp02_PODFiles filename: Exp02_PODFiles.zip POD files from the decomposition of both velocity and vorticity fields. Corresponds to Exp02 of the flow fields data.	(ZIP Archive (ZIP), 32.91 GB) MD5:ce32fa8c9e511724bacf511e1083eb66
Exp03_PODFiles filename: Exp03_PODFiles.zip POD files from the decomposition of both velocity and vorticity fields. Corresponds to Exp03 of the flow fields data.	(ZIP Archive (ZIP), 32.91 GB) MD5:6c936fb2a0f5958353b9f00b25e83abe
Exp04_PODFiles filename: Exp04_PODFiles.zip POD files from the decomposition of both velocity and vorticity fields. Corresponds to Exp04 of the flow fields data.	(ZIP Archive (ZIP), 32.91 GB) MD5:6bb9adaedd59ef8f041077faf07b87cb

File
Exp05_PODFiles filename: Exp05_PODFiles.zip (ZIP Archive (ZIP), 32.90 GB) MD5:a7effa92c515644bacf754571da484e8 POD files from the decomposition of both velocity and vorticity fields. Corresponds to Exp05 of the flow fields data.
Exp06_PODFiles filename: Exp06_PODFiles.zip (ZIP Archive (ZIP), 32.90 GB) MD5:2345816b9c63d0e20f87f1dd58fc7020 POD files from the decomposition of both velocity and vorticity fields. Corresponds to Exp06 of the flow field data.
Exp07_PODFiles filename: Exp07_PODFiles.zip (ZIP Archive (ZIP), 32.91 GB) MD5:563b94bd2137996a9e2617639e658228 POD files from the decomposition of both velocity and vorticity fields. Corresponds to Exp07 of the flow field data.
Figure data and plotting scripts filename: PaperFigures_PlottingScripts_RefinedData.zip (ZIP Archive (ZIP), 420.48 MB) MD5:bba55aefef255d8713f2dd1e7842eafd Associated plotting scripts and refined panel data to reproduce all plots in results publication: Henshaw et al - Dynamic mode structure of active turbulence.
Flow fields for dense suspensions of bulk bacterial turbulence. filename: POD_FlowFields.zip (ZIP Archive (ZIP), 41.21 GB) MD5:12a8c54f8ce5ade614d354c9b0dd5d6c
POD Data: Racetracks filename: PODData_Racetracks.zip (ZIP Archive (ZIP), 3.41 GB) MD5:8250825ff35a398916572ef3e4924737 POD files from the decomposition of the flow fields measured from a turbulent suspension under confinement (of various widths). Corresponds to the flow fields in FlowFields_Racetracks.zip
PODFlowFields_AverageSpeeds.xls (Microsoft Excel, 30.00 KB) MD5:cb607d43989458e8191e86c764175831 Average flow speeds (um/s) of each of the flow fields in POD_FlowFields.zip
Racetrack PIV flow fields filename: FlowFields_Racetracks.zip (ZIP Archive (ZIP), 860.27 MB) MD5:14edbd8616c22c52cc4f23cd1508726f PIV fields of the flow fields measurement in racetracks of widths: 30, 38, 45, 57, 66, 76, 95, 114, 171um. All flow fields were measured from the same suspension and within the first <10 mins (see methods for full details)

[table of contents | back to top]

Related Publications

Henshaw, R. J., Martin, O. G., & Guasto, J. S. (2022). Dynamic mode structure of active turbulence. https://doi.org/10.1101/2022.04.15.488501
Results

Wioland, H., Lushi, E., & Goldstein, R. E. (2016). Directed collective motion of bacteria under channel confinement. New Journal of Physics, 18(7), 075002. https://doi.org/10.1088/1367-2630/18/7/075002
Methods

[table of contents | back to top]

Parameters

Parameter	Description	Units
Experiment_Number	ID representing a unique experiment. These IDs correspond with the POD data files available for download in the Supplemental Files section of the BCO-DMO metadata page for this representative dataset (https://www.bco-dmo.org/dataset/885923).	unitless
Average_speed	Average flow speeds in um/s of each of the flow fields in each of the experimental PODFiles zip files.	um/s
Dataset	Name of corresponding experimental POD zip file	unites

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Nikon Ti-E Inverted Microscope
Generic Instrument Name	Inverted Microscope
Dataset-specific Description	For all experiments, bacterial suspensions were imaged with brightfield illumination on an inverted microscope (Nikon Ti-E) using a sCMOS camera (Zyla 5.5, Andor Technology).
Generic Instrument Description	An inverted microscope is a microscope with its light source and condenser on the top, above the stage pointing down, while the objectives and turret are below the stage pointing up. It was invented in 1850 by J. Lawrence Smith, a faculty member of Tulane University (then named the Medical College of Louisiana). Inverted microscopes are useful for observing living cells or organisms at the bottom of a large container (e.g. a tissue culture flask) under more natural conditions than on a glass slide, as is the case with a conventional microscope. Inverted microscopes are also used in micromanipulation applications where space above the specimen is required for manipulator mechanisms and the microtools they hold, and in metallurgical applications where polished samples can be placed on top of the stage and viewed from underneath using reflecting objectives. The stage on an inverted microscope is usually fixed, and focus is adjusted by moving the objective lens along a vertical axis to bring it closer to or further from the specimen. The focus mechanism typically has a dual concentric knob for coarse and fine adjustment. Depending on the size of the microscope, four to six objective lenses of different magnifications may be fitted to a rotating turret known as a nosepiece. These microscopes may also be fitted with accessories for fitting still and video cameras, fluorescence illumination, confocal scanning and many other applications.

[table of contents | back to top]

Project Information

Collaborative Research: Viral induced chemotaxis mediating cross-trophic microbial interactions and carbon flux (VIC)

Coverage: Culture-based work

NSF Award Abstract:
Drifting photosynthetic microbes in surface ocean waters carry out nearly half of global carbon (C) fixation, both supporting the marine food web and reducing atmospheric carbon dioxide (CO_2) levels. The fate of C in ocean ecosystems is controlled by myriad individual interactions within a highly interconnected planktonic food

web, the sheer complexity of which has hindered predictive understanding of global C cycling. Chemical cues govern microbial interactions, and during infection, marine viruses manipulate the metabolism of phytoplankton and bacteria, facilitating the release of dissolved organic matter from infected cells. This research aims to determine how viral metabolic reprogramming of and organic matter release from intact, infected phytoplankton influences microbial interactions and C cycling. The interdisciplinary, collaborative nature of the project will enable direct training of two postdoctoral researchers, one graduate student, and undergraduate students in viral ecology, microfluidics, and metabolomics. An educational outreach program that engages middle school students in hands-on, high speed imaging of microbes will be expanded, and the project will culminate in a three-day workshop to advance the application of microfluidic devices and mass spectrometry analyses in microbial ecology.

The overarching hypothesis behind this research is that viral infection alters the chemical landscape of intact, infected picophytoplankton cells, attracting neighboring chemotactic bacteria and protistan zooplankton, and altering C flux pathways. To test this idea, a series of linked multi-scale laboratory-based experiments will be run to 1) Characterize the response of diverse model marine microbes to dissolved organic matter (DOM) released from intact, virus-infected picophytoplankton using microfluidics-based chemotaxis assays, 2) Identify key viral-derived DOM compounds eliciting chemotactic responses using stable isotope labeling, metabolomics analyses, and chemotaxis assays, and 3) Quantify micron-scale cross-trophic encounter dynamics and evaluate their impact on bulk-scale C cycling using liter-scale measurements of C dynamics linked to high spatiotemporal resolution live imaging of microbial food webs. The ultimate goal of the project is to develop a mechanistic understanding of the role of intact, virus-infected cells in oceanic C cycling.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1829827

[table of contents | back to top]