Lagrangian Structure and Stretching in Bacterial Turbulence Modeling Results from February 2020 (VIC project)

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

Contributors	Affiliation	Role
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Abstract
Dense suspensions of active, self-propelled agents spontaneously exhibt large-scale, chaotic flow structures. Descriptions of their dynamics have predominately focused on characterization of spatiotemporal correlation of the velocity field, but their transport and mixing properties remain largely unknown. In this work, we use Lagrangian analysis techniques to study the chaotic flow field generated by "bacterial turbulence" in dense suspensions of Bacillus subtilis. High-resolution velocity fields are simultaneously measured along with individual tracer and cell trajectories across a range of bacterial swimming speeds. The flow kinematics are quantified through the Lagrangian stretching field and used to characterize the mixing induced by the stretching and folding of the active bacterial colony. The distribution of the finite-time Lyapunov exponent (FTLE) field reveals swimming-speed dependent transitions reminiscent of intermittent dynamics in classical chaotic dynamical systems. Finally, measured trajectories of both passive beads and individual swimming cells directly demonstrate how the striking active Lagrangian flow structures regulate transport in bacterial turbulence.

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Coverage

Temporal Extent: 2020-02-18

Dataset Description

Velocity Field Variables:

U: u-component of the velocity fields

V: v-component of the velocity fields

X,Y: XY coordinates produced from the PIV analysis (meshgrid layout)

Stretching Field Variables:

TINT: Integration time for tracer particles (s)

choice: parameter choices for numerical experiment, see code for details

stretch: a Nx1 cell of the stretching field for this particular integration time, where each cell is a frame

tintegrate: time vector of the tracer particle integration

x_field, y_field: Initial xy coordinates of tracer particle grids. Formed using meshgrid

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) at 40x magnification. 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 in space and one frame in time.

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 in space and one frame in time.

Stretching fields were calculated using custom-MATLAB script, based from the analysis of Parsa et al, and a condensed summary can be found in the Appendix of Dehkarghani et al. Briefly, the analysis:

1) Loads and smooths the input velocity field

2) Calculates a starting grid of particle locations

3) Creates "clusters" of 4 particles centered on these initial locations

4) Advects the particles through the velocity field, rescaling the clusters periodically to preserve the order

orientation and shape of the cluster

5) Calculates the stretching fields using the Cauchy-Green deformation tensors

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Data Files

File
Velocity field data (PIV) filename: LCS_VelocityFieldData.zip (ZIP Archive (ZIP), 52.96 GB) MD5:caa8a1a54e22d7ea98985fbadce0cc2c

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Supplemental Files

File
Example MATLAB scripts for calculation of stretching fields filename: ExampleCode.zip (ZIP Archive (ZIP), 6.39 KB) MD5:5639797ce8c40550c436eff982a1797e Example of analysis used to calculate the stretching fields of "LCS_Stretching_Files" using the flow fields of "LCS_VelocityFieldData".

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Related Publications

Parsa, S., Guasto, J. S., Kishore, M., Ouellette, N. T., Gollub, J. P., & Voth, G. A. (2011). Rotation and alignment of rods in two-dimensional chaotic flow. Physics of Fluids, 23(4). https://doi.org/10.1063/1.3570526
Methods

R.J. Henshaw and J.S. Guasto. Lagrangian structure and stretching of bacterial turbulence. In preparation
Results

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Parameters

Parameters for this dataset have not yet been identified

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Instruments

Dataset-specific Instrument Name	sCOMOS camera (Zyla 5.5, Andor Technology)
Generic Instrument Name	Camera
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) at 40x magnification.
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

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) at 40x magnification.
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.

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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.

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Funding

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

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