

The Metropolitan

Water Reclamation District

of Greater Chicago

**WELCOME
TO THE NOVEMBER EDITION
OF THE 2012
M&R SEMINAR SERIES**

BEFORE WE BEGIN

- **SILENCE CELL PHONES & SMART PHONES**
- **QUESTION AND ANSWER SESSION WILL FOLLOW PRESENTATION**
- **PLEASE FILL EVALUATION FORM**
- **SEMINAR SLIDES WILL BE POSTED ON MWRD WEBSITE**
(www.MWRD.org: Home Page ⇒ Reports ⇒ M&D Data and Reports ⇒ M&R Seminar Series ⇒ 2012 Seminar Series)
- **STREAM VIDEO WILL BE AVAILABLE ON MWRD WEBSITE**
(www.MWRD.org: Home Page ⇒ MWRDGC RSS Feeds)

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Editorships: Limnology and Oceanography - Fluids and Environments; Water Resources
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Published 68 papers (two book chapters); served as numerous panelists and keynote
speakers and technical committee members and chair
- Honors & Awards:** Fulbright Distinguished Chair , 2012
Huber Research Prize, American Society of Civil Engineers , 2008
Career Award, National Institutes of Health (NIAID K25), 2006
McCormick Excellence Award, Northwestern University, 2006
Career Award, National Science Foundation, 1999

Implications of Pathogen- Biofilm Interactions for Surface Water Quality

Aaron Packman

Civil and Environmental Engineering
Northwestern University

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Dr. Yang Liu (now at U. Alberta)
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Andrea Salus
Molly Baker
Lisa Marx

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Dave Chopp, Applied Math
Paul Joos

Funders:



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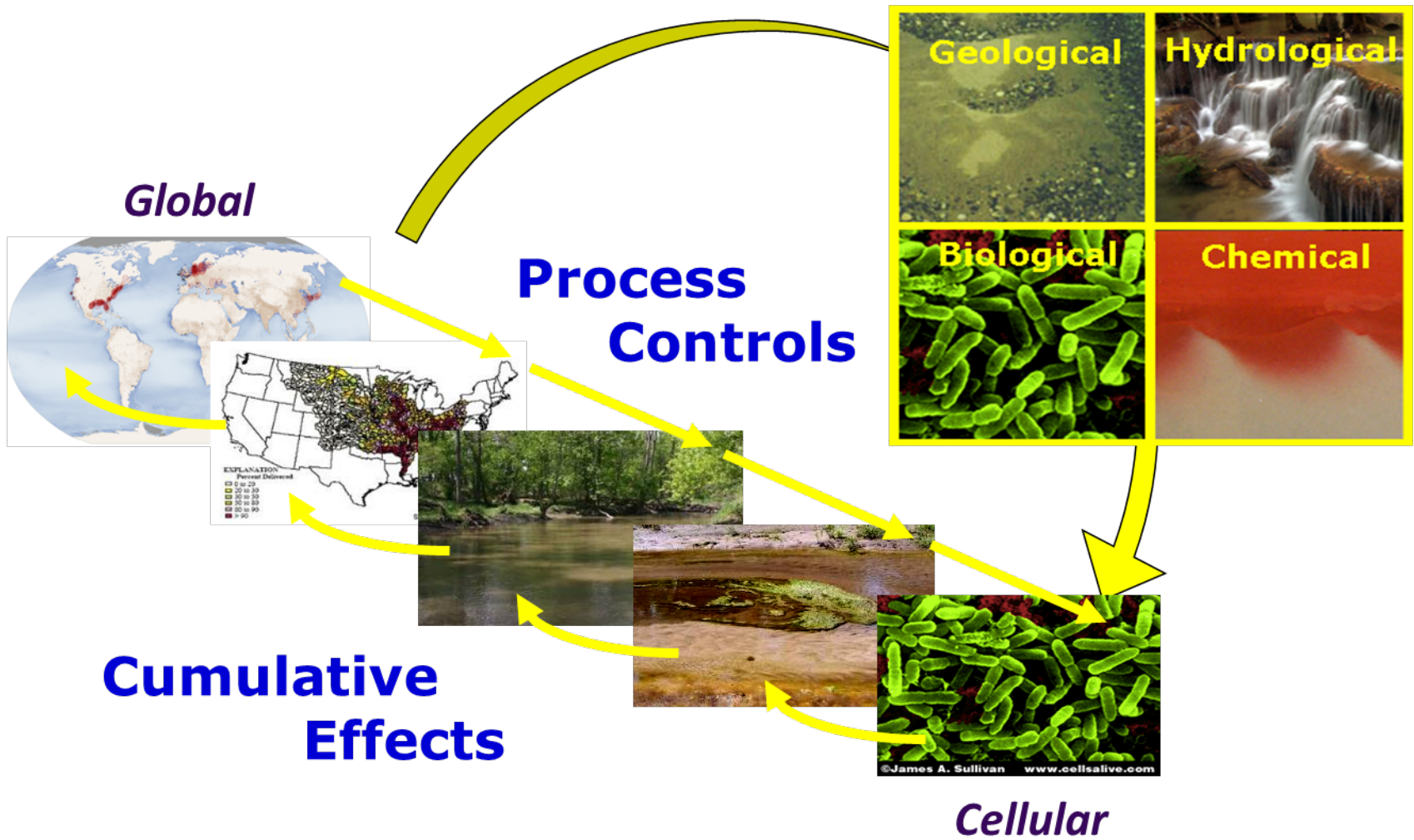
Collaborators at U. Washington:

Matt Parsek
Dr. Boo-Tsan Sheng

Additional Collaborators:

Jud Harvey, USGS
Doug Jerolmack & Raleigh Martin, UPenn

Microbial interactions across scales



Biofilm heterogeneity

Complex morphology

- Results from integrated adherence, motility, and growth
- Morphology varies with cell physiology and nutritional conditions
- Clusters and channels common (macroporous)

Complex composition

- Cells, polymer matrix (EPS), cellular products (lysed cells, eDNA, etc.)
- Complex microstructure (microporous)

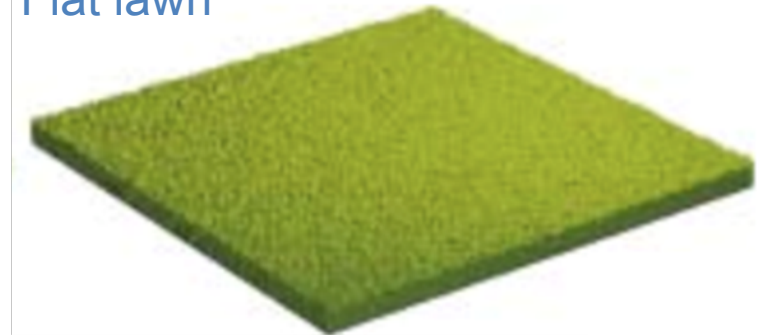
Mushroom structures



Cell clusters



Flat lawn



Biofilm heterogeneity

Complex morphology

- Results from **integrated adherence, motility, and growth**
- Morphology varies with **cell physiology and nutritional conditions**
- Clusters and channels common (**macroporous**)

Complex composition

- Cells, polymer matrix (EPS), cellular products (lysed cells, eDNA, etc.)
- Complex microstructure (**microporous**)

Multi-scale interactions

- Internal heterogeneity develops over time
- Internal heterogeneity (habitat) is coupled with external nutritional environment
- Coordinated activity builds biofilm
- Structure is an *emergent* property

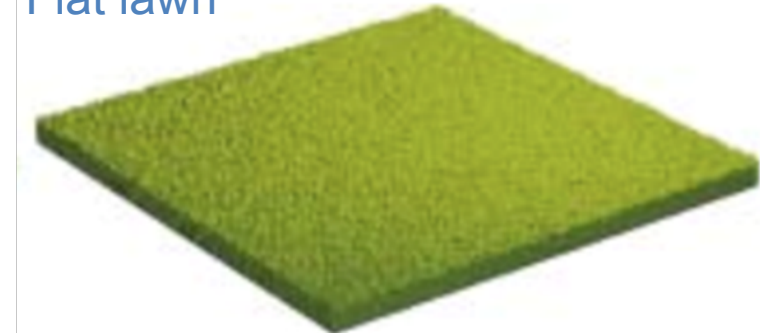
Mushroom structures



Cell clusters



Flat lawn



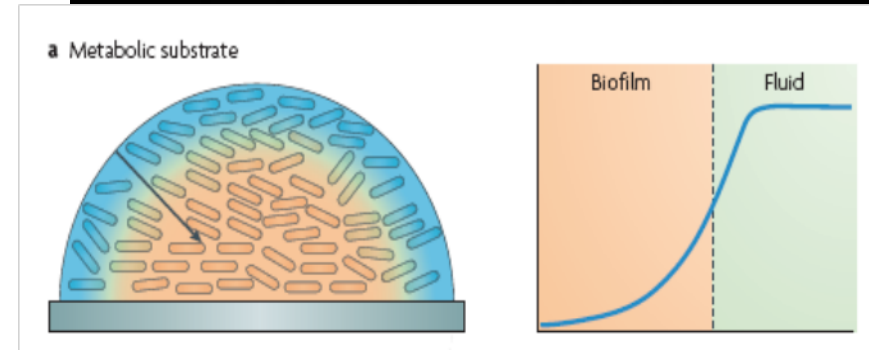
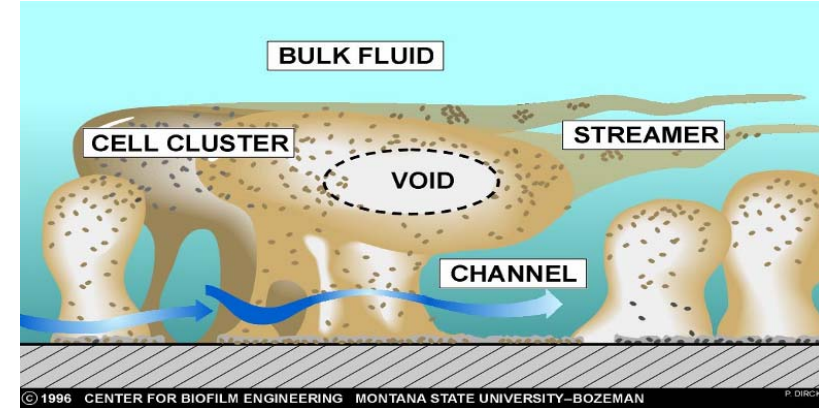
Biofilm heterogeneity

Transport-limited environment

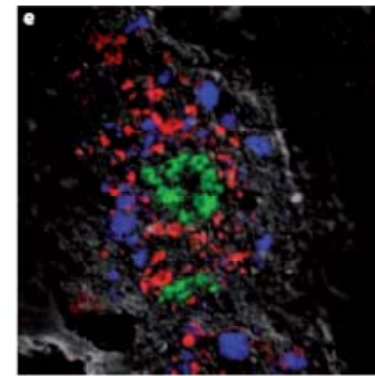
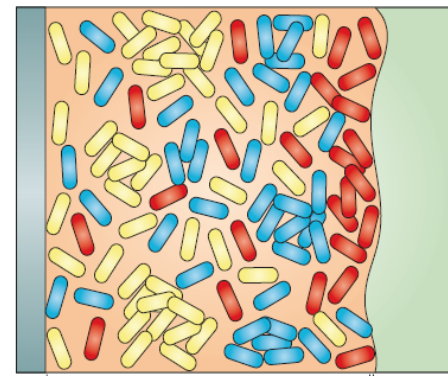
- Hindered transport environment around and within cell clusters
- Complex advective flowfield transitioning to diffusion at small (unknown) scales

Patterns of community activity

- High community metabolism plus limited transport → internal depletion
- Strong selective pressures (variable oxygen, carbon, nutrients)
- Diverse ecological micro-niches supports diverse community



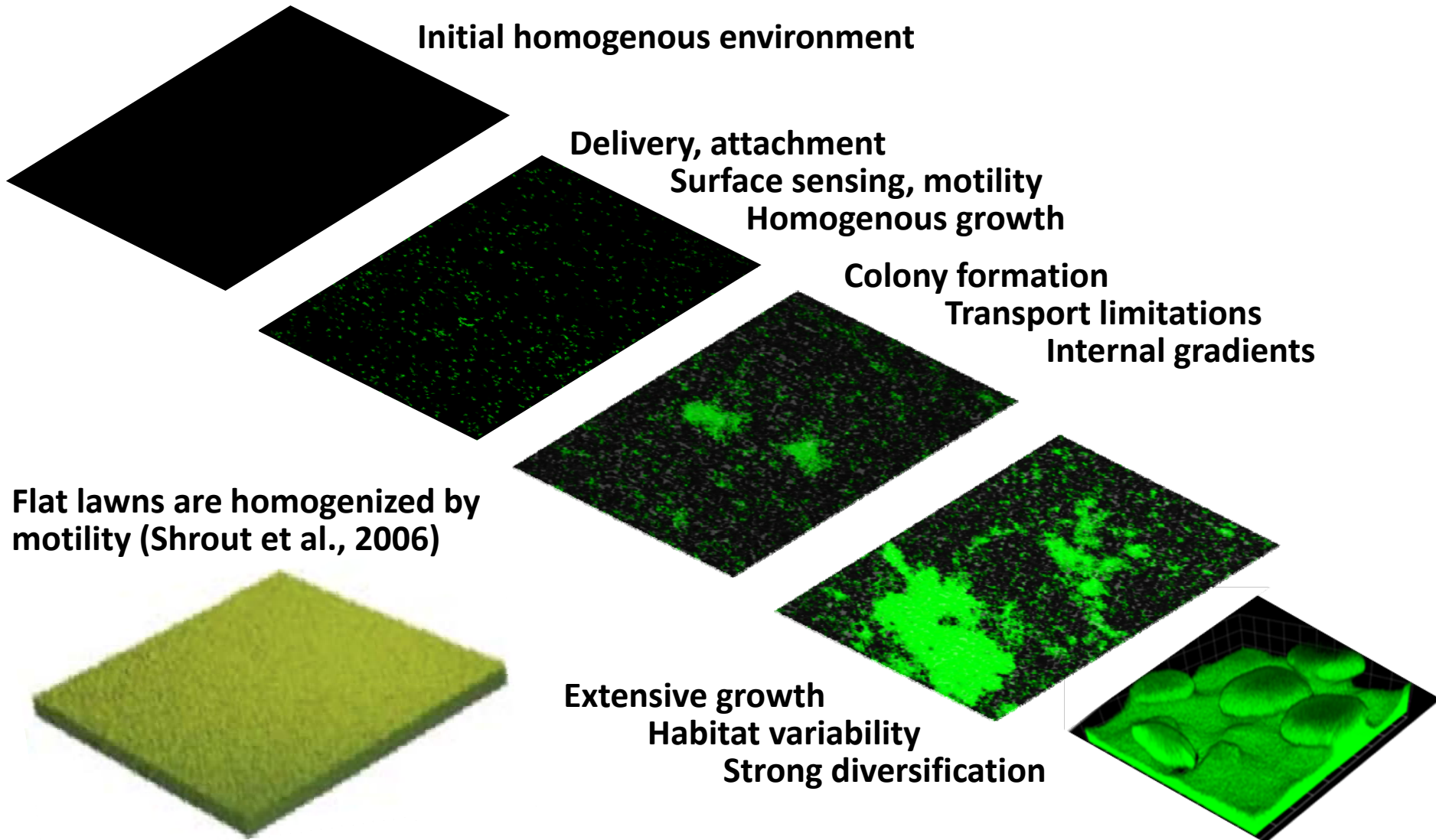
■ Sulphate-reducing bacteria
 ■ Sulphide-oxidizing bacteria
 ■ Aerobic heterotrophs



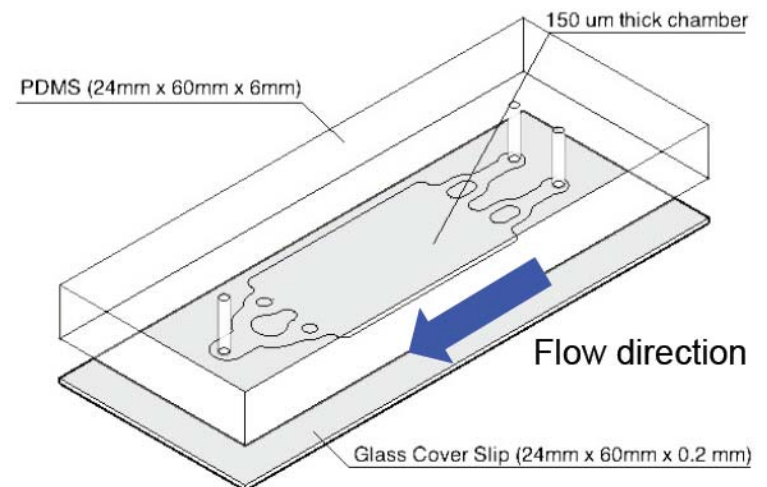
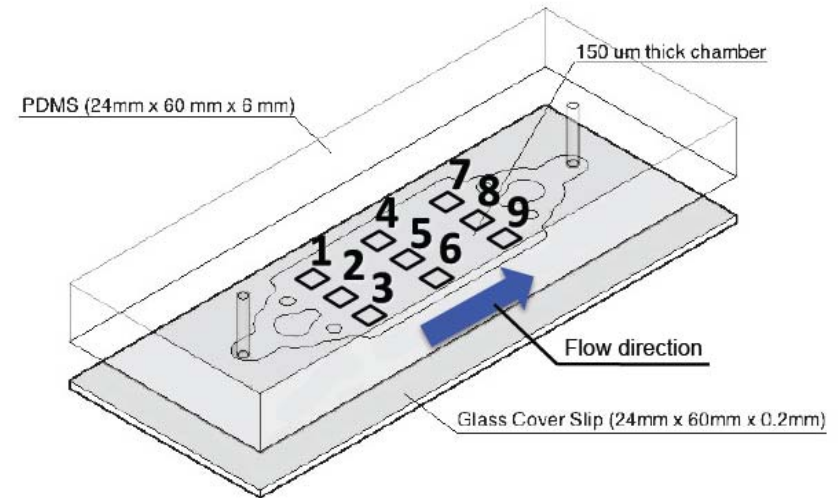
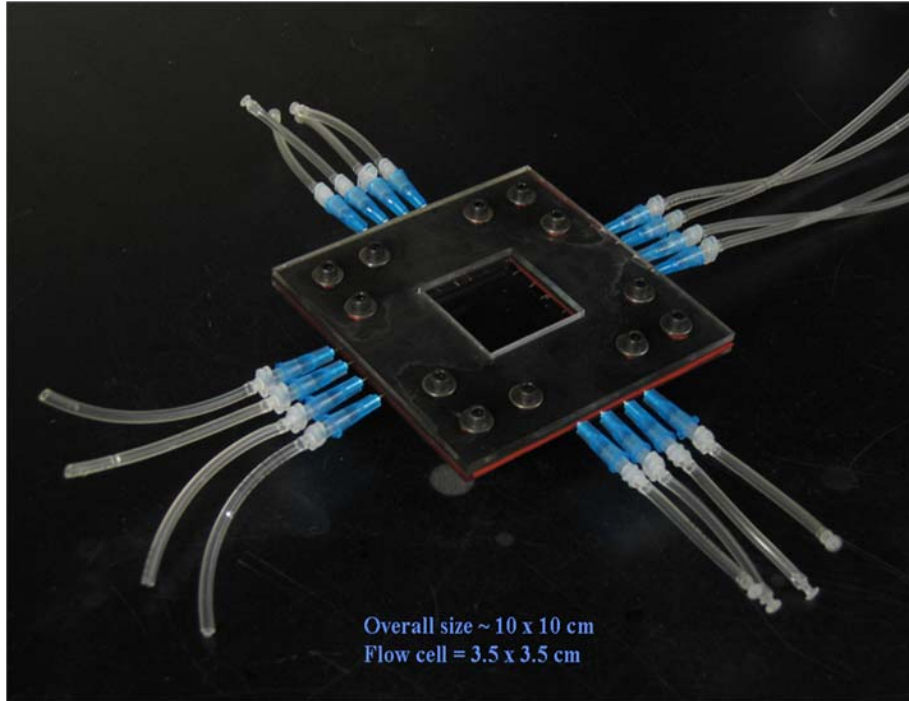
Objective is to address fundamental, multi-scale challenges in biofilms

- 1. Develop capability to observe and quantify spatial patterns and transport dynamics within biofilms**
- 2. Predict longer-term, larger-scale temporal dynamics of biofilm growth and diversification (i.e., development of heterogeneity)**
- 3. Assess important outcomes in pathogen transmission in natural and engineered water systems**

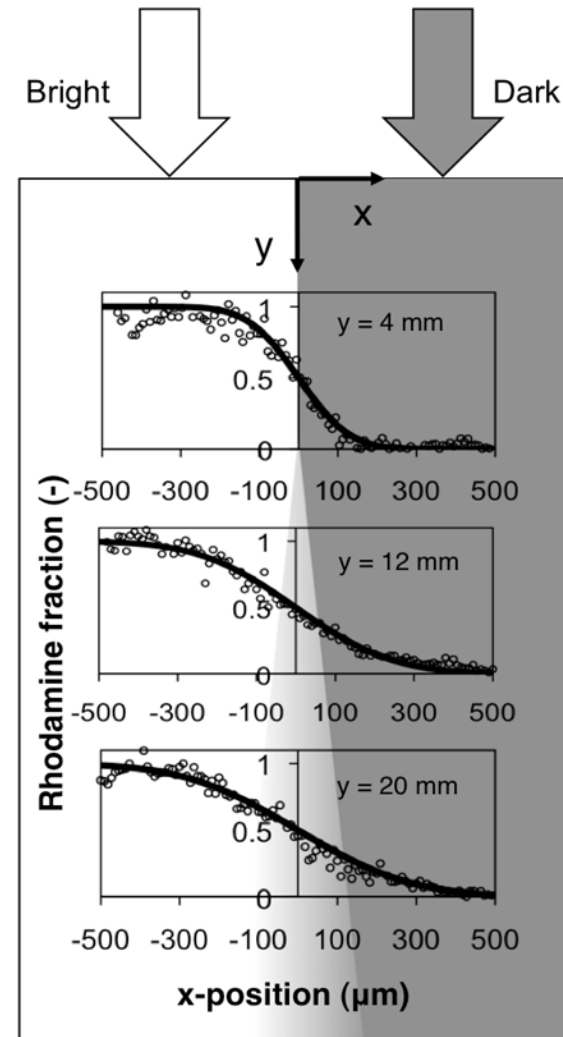
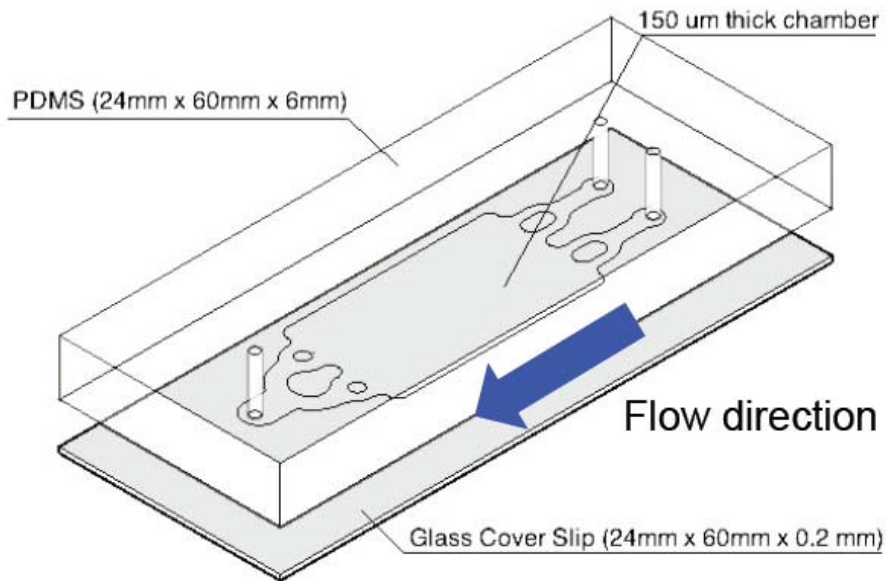
Development of biofilm heterogeneity



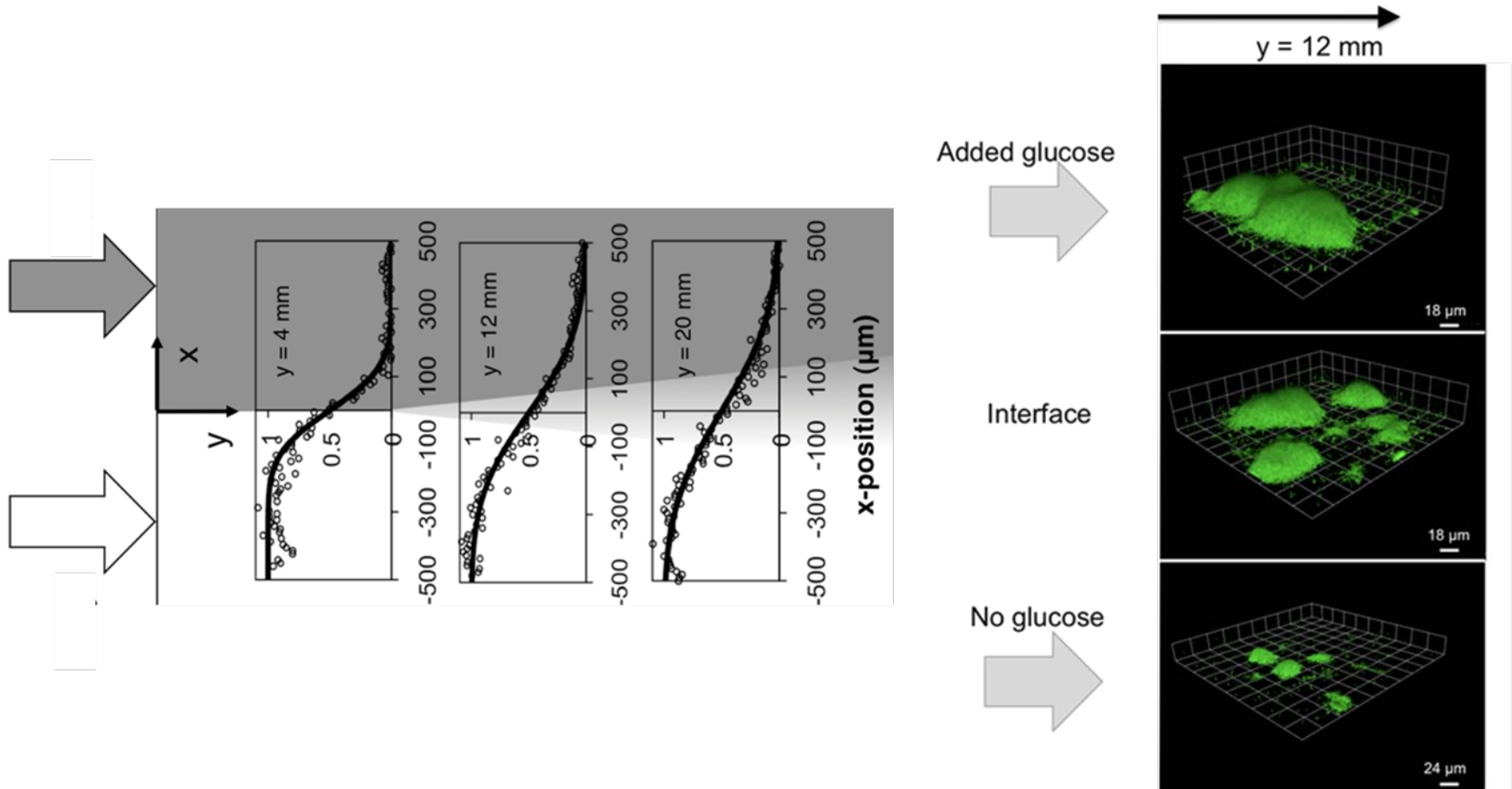
New flow cell designs for biofilm-environment interactions



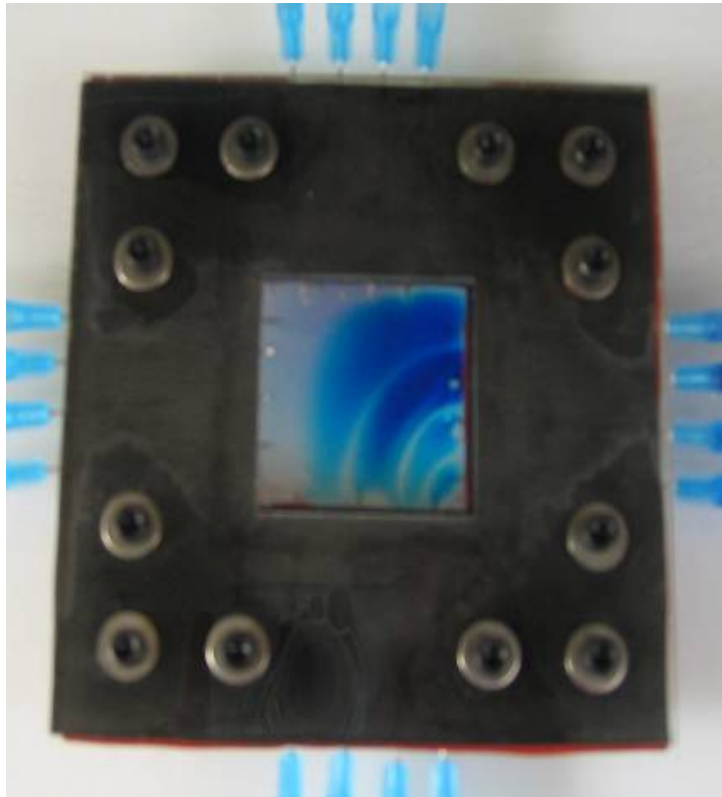
Flow cell with imposed chemical gradient



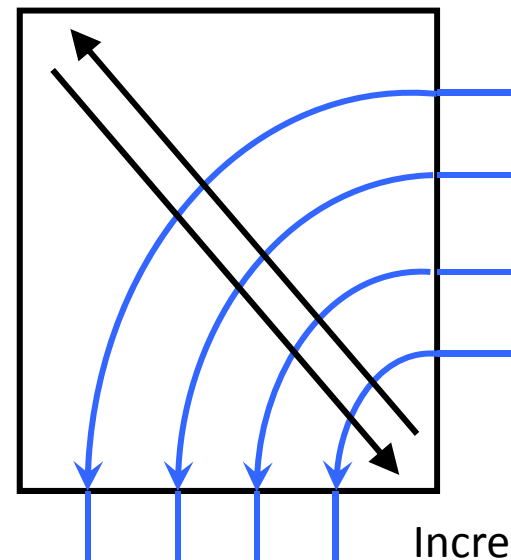
Differential growth under nutritional gradient



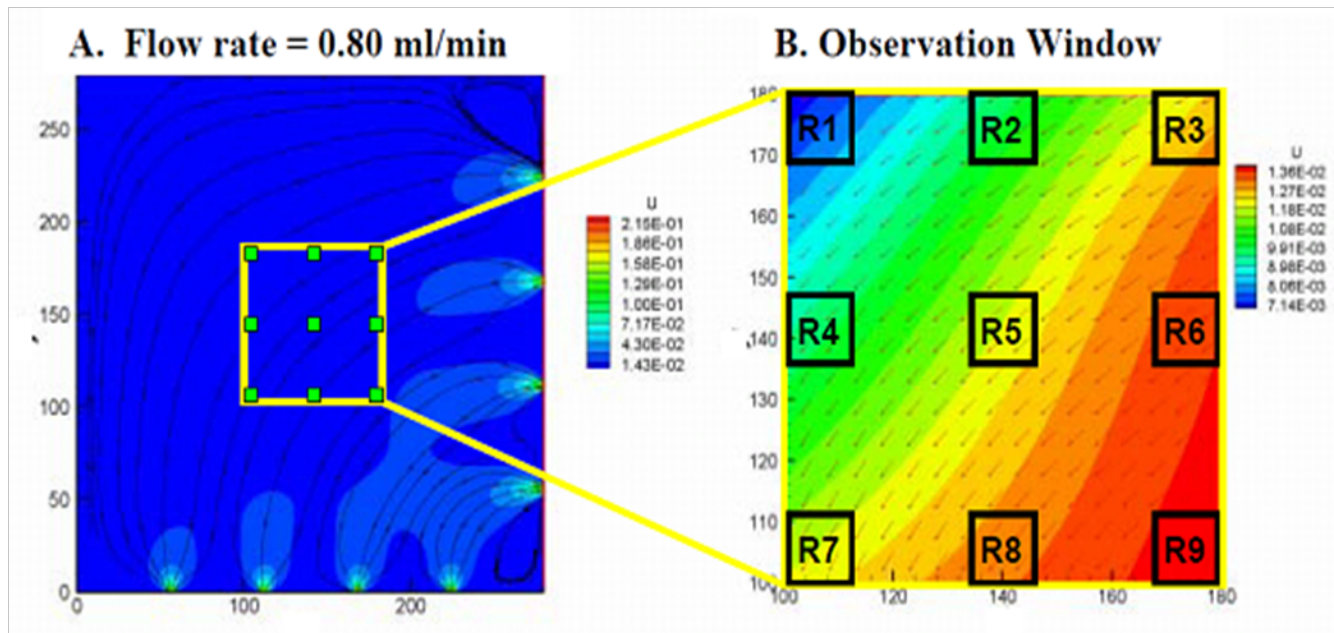
Flow cell with imposed turning flow: Simultaneous flow and influx gradient



Decreasing transport
(oxygen, nutrient influx)



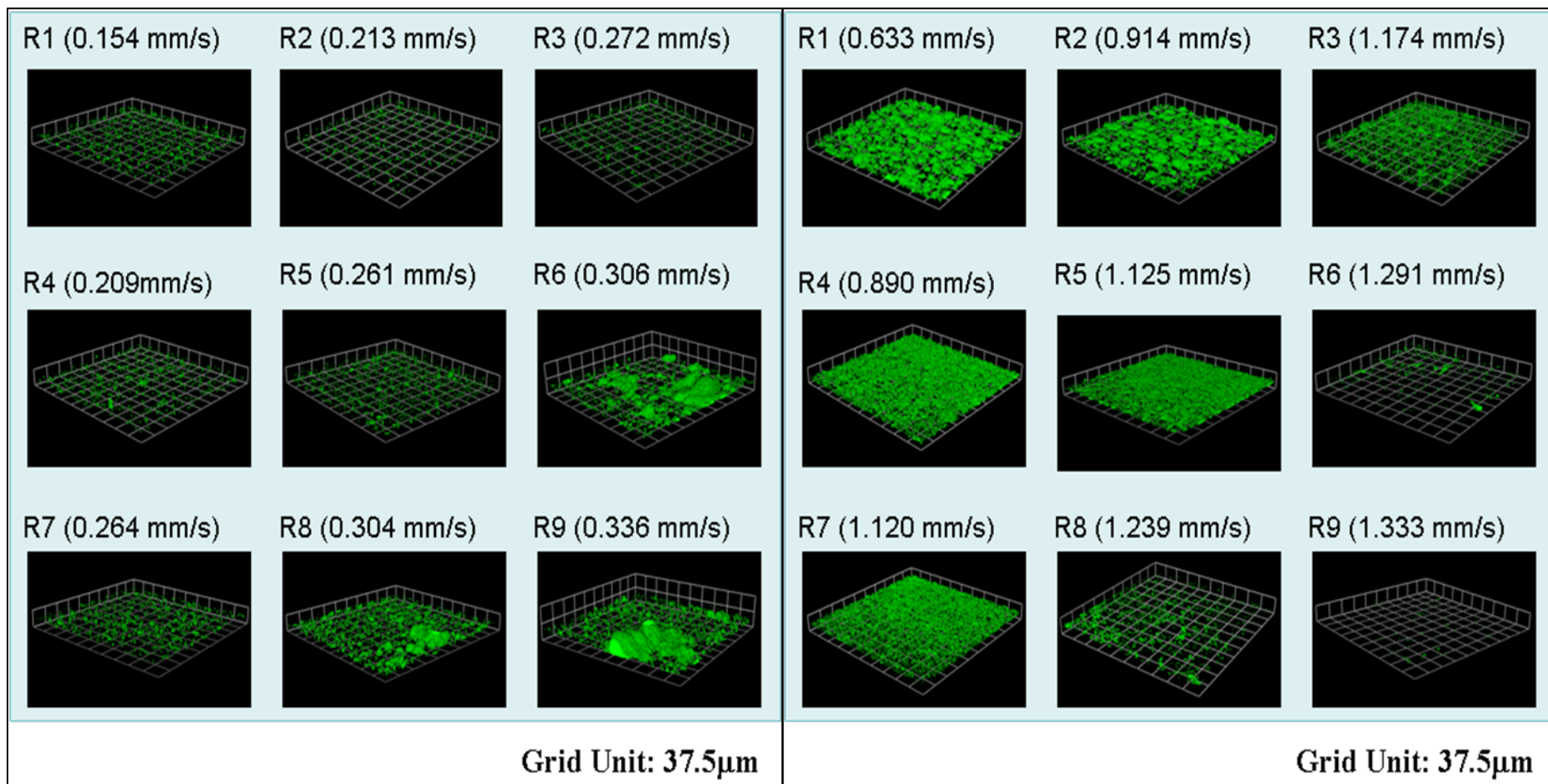
Flow cell with imposed turning flow: Simultaneous flow and influx gradient



Dramatic spatial patterns under flow gradient

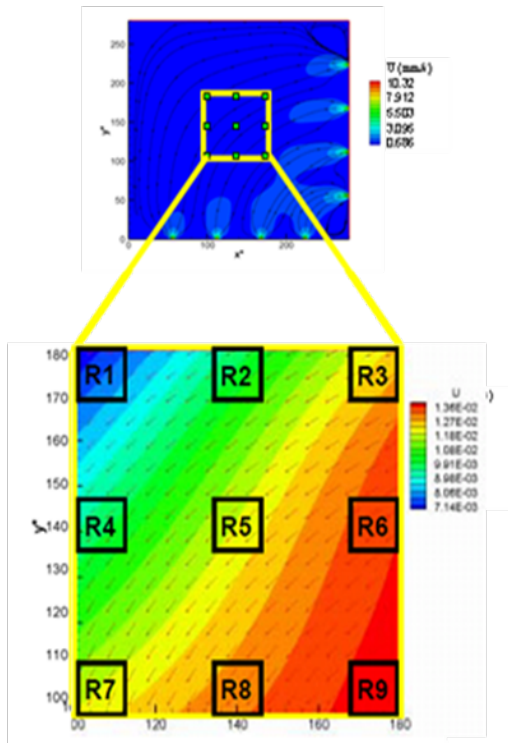
Flow rate: 0.16 ml/min

Flow rate: 0.8 ml/min

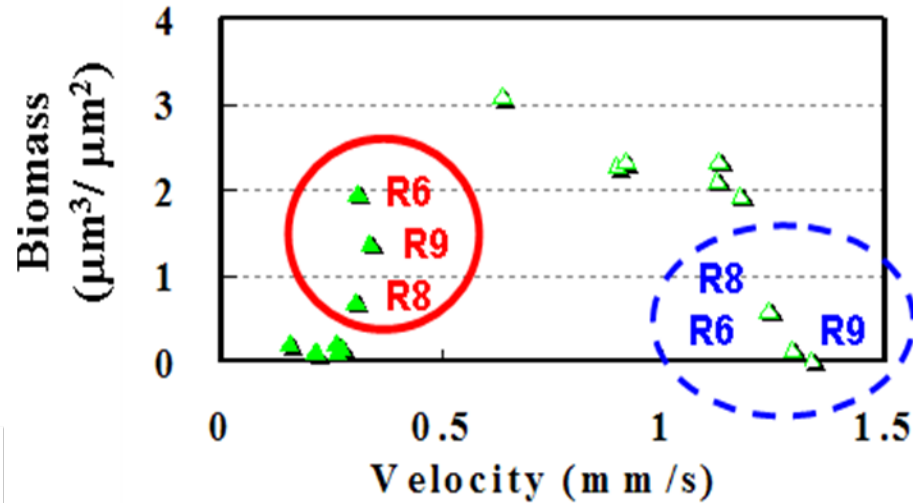


P. aeruginosa PAO1-*gfp*, Medium: 1% LB, T = 7 days

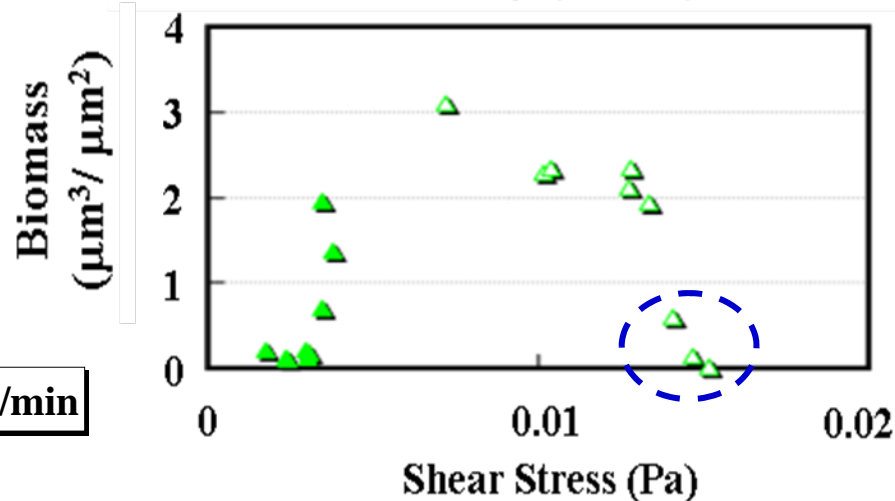
Dual transport limitation on growth: Influx and Shear



▲ 0.16 ml/min ▲ 0.80 ml/min



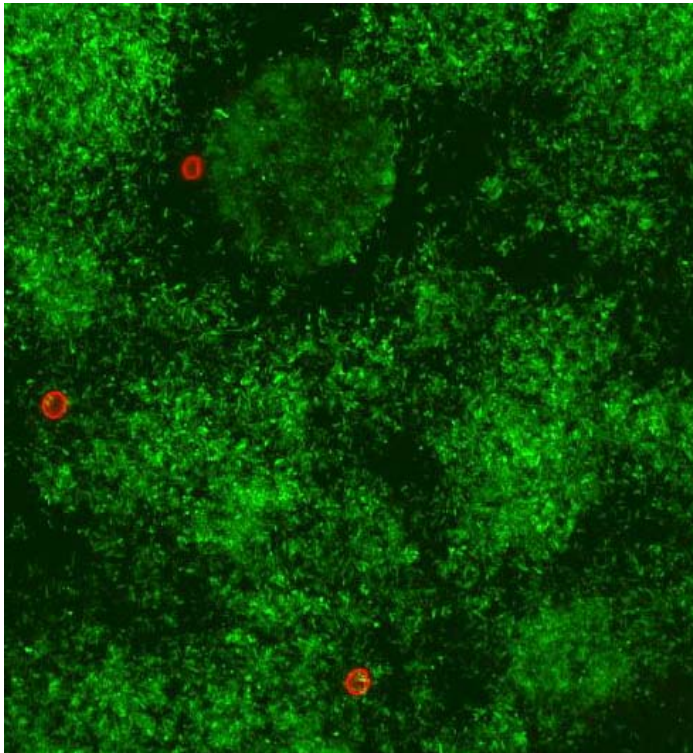
Growth limitation from substrate availability. Biofilm growth initially increases with influx.



Strong detachment starting at boundary shear of ~0.013 Pa. Almost no biofilm persists at >0.015 Pa.

P. aeruginosa PAO1-gfp, Medium: 1% LB, T = 7 days

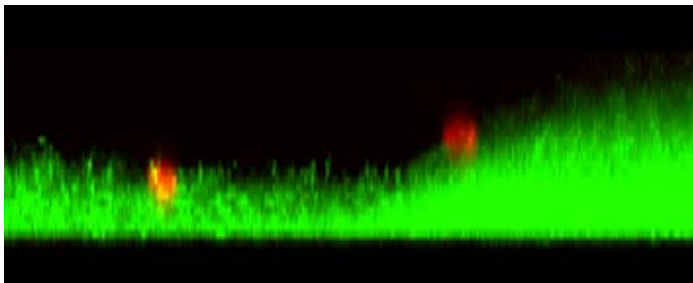
Biofilms capture pathogens



C. parvum oocysts (red)
captured by a *Pseudomonas aeruginosa* biofilm (green)

Confocal microscopy resolves
3D spatial structure

Oocyst capture increases with
biofilm roughness



Dynamic colloid interactions with biofilms

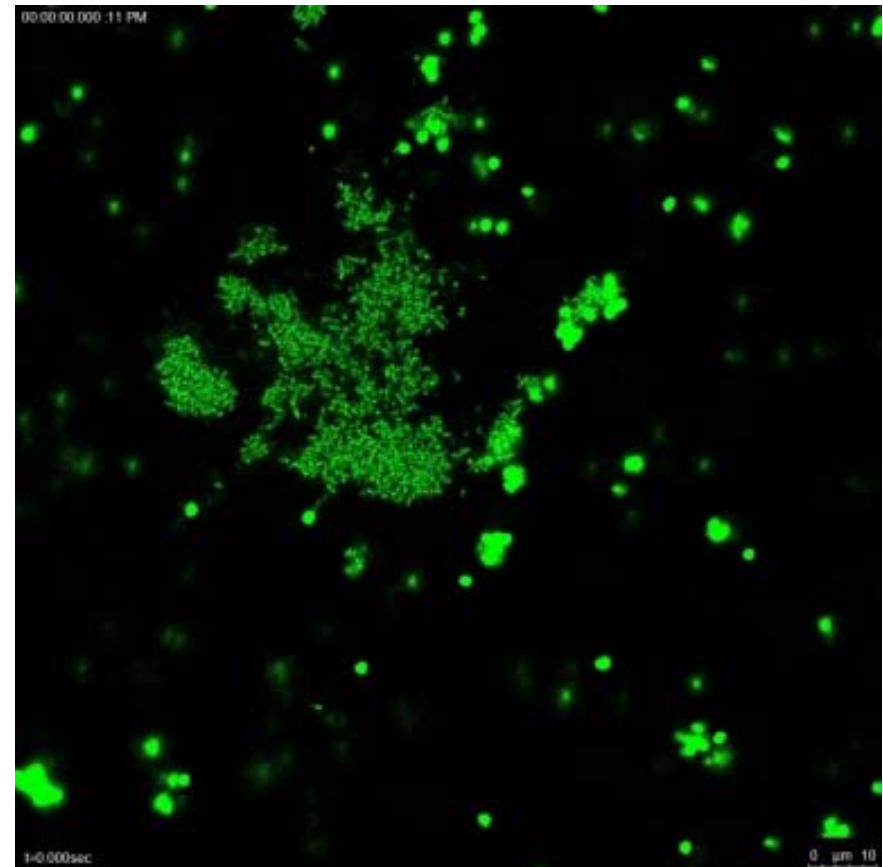
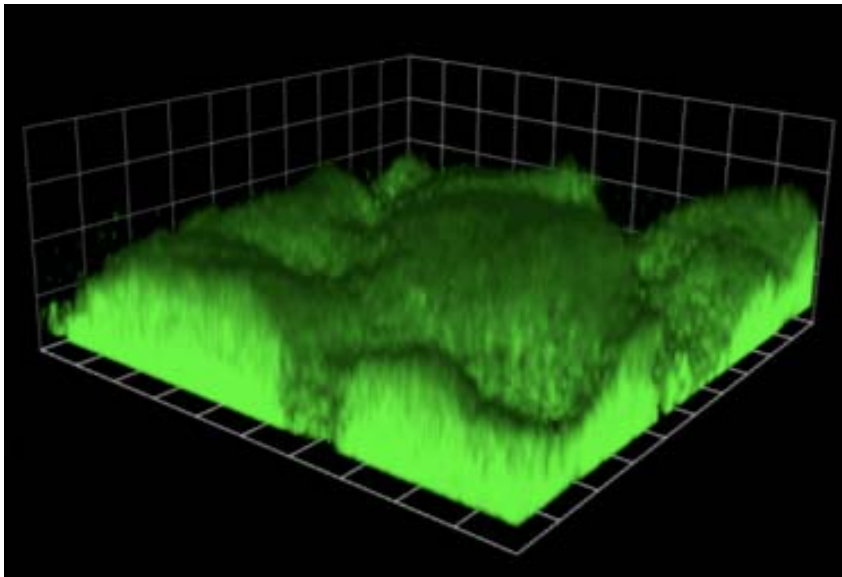
P. aeruginosa biofilm

Cultured in flow cell under 1% TSB medium

Flow rate = 0.01 ml/min, $Re = \mathcal{O}(1)$

3D image: 109 x 109 x 35 μm

Particle tracking with 1 μm fluorescent beads



Dynamic colloid interactions with biofilms

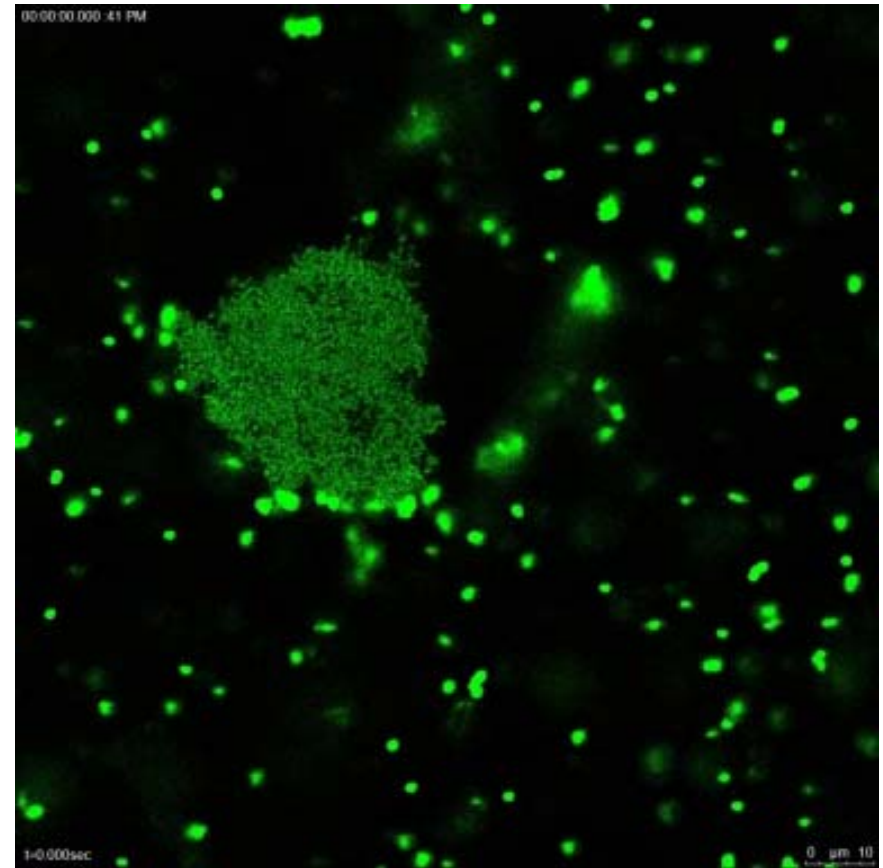
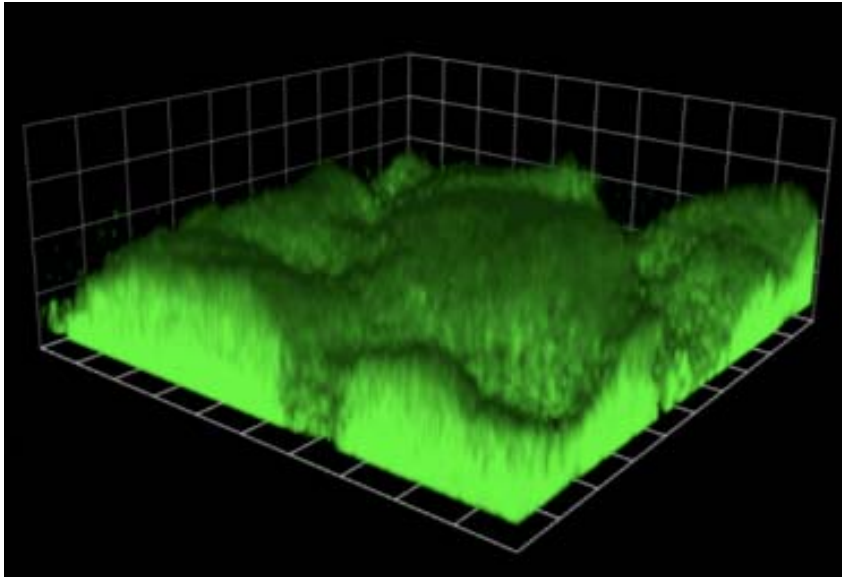
P. aeruginosa biofilm

Cultured in flow cell under 1% TSB medium

Flow rate = 0.01 ml/min, $Re = \mathcal{O}(1)$

3D image: 109 x 109 x 35 μm

Particle tracking with 1 μm fluorescent beads



Flow quantification by particle tracking velocimetry

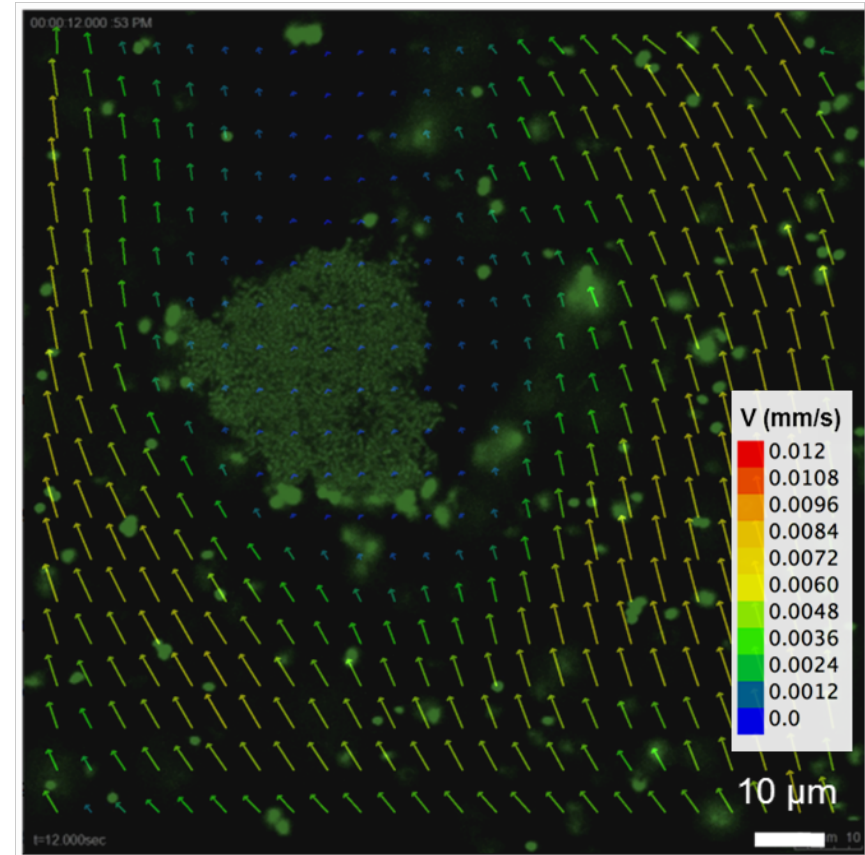
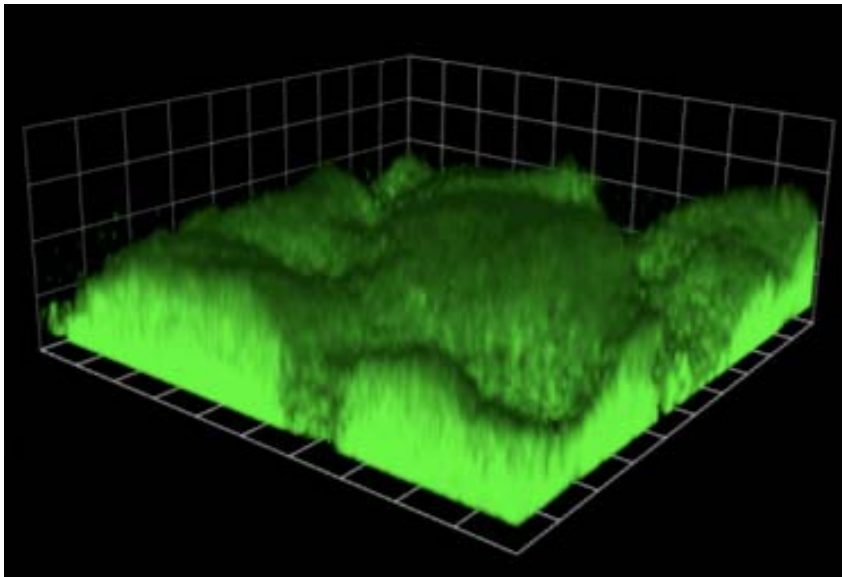
P. aeruginosa biofilm

Cultured in flow cell under 1% TSB medium

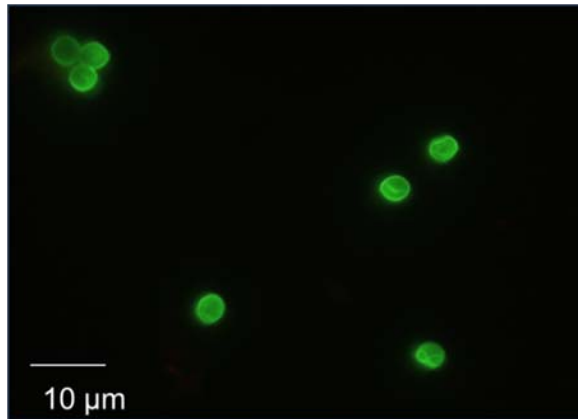
Flow rate = 0.01 ml/min, $Re = \mathcal{O}(1)$

3D image: 109 x 109 x 35 μm

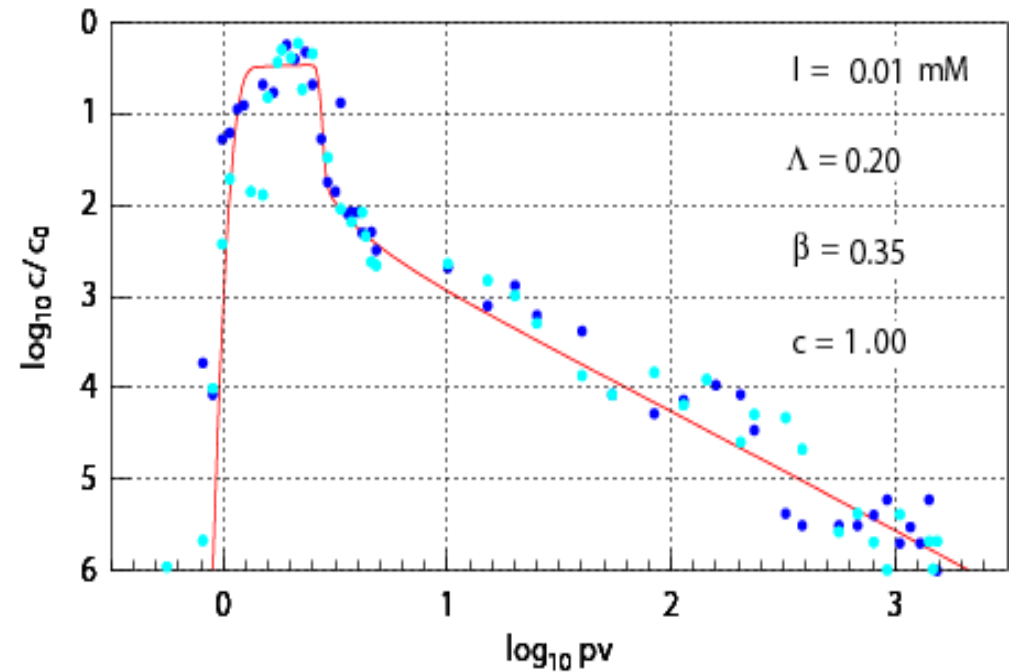
Particle tracking with 1 μm fluorescent beads



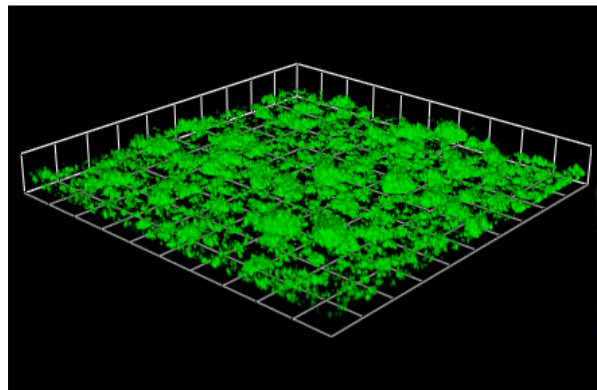
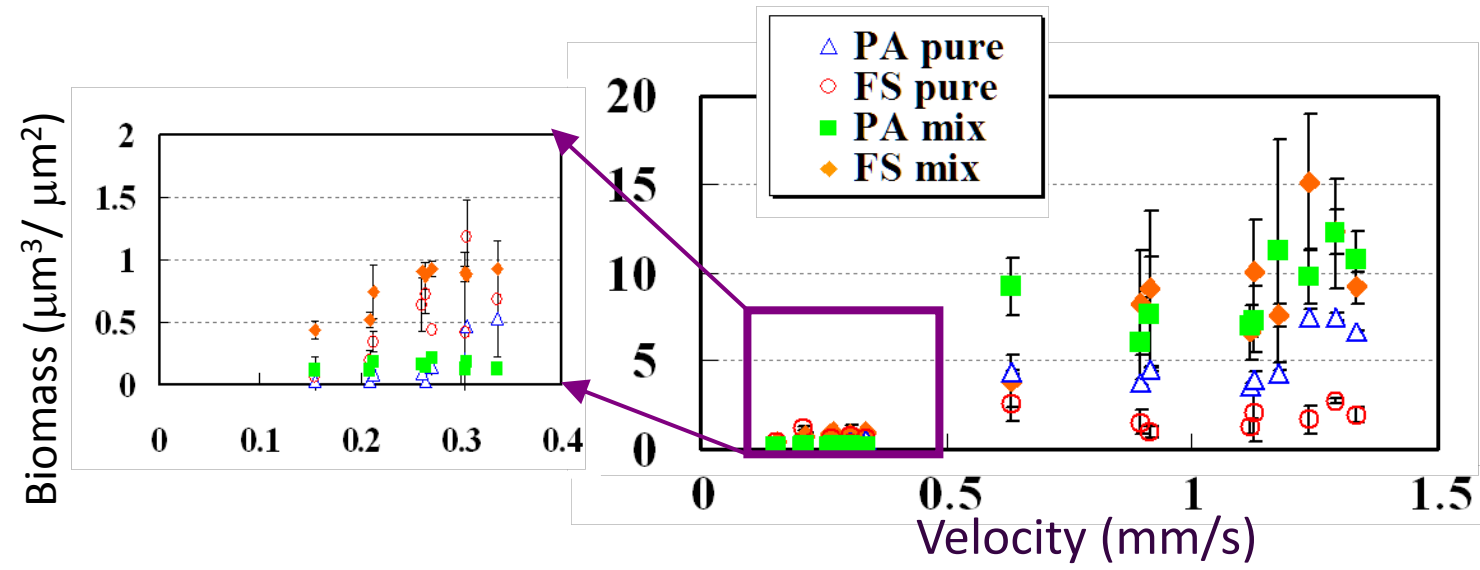
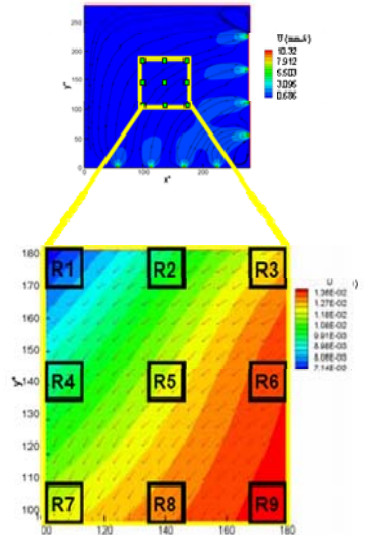
Pathogen filtration is also often reversible



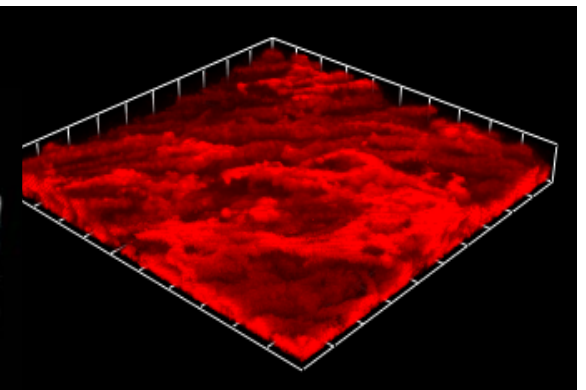
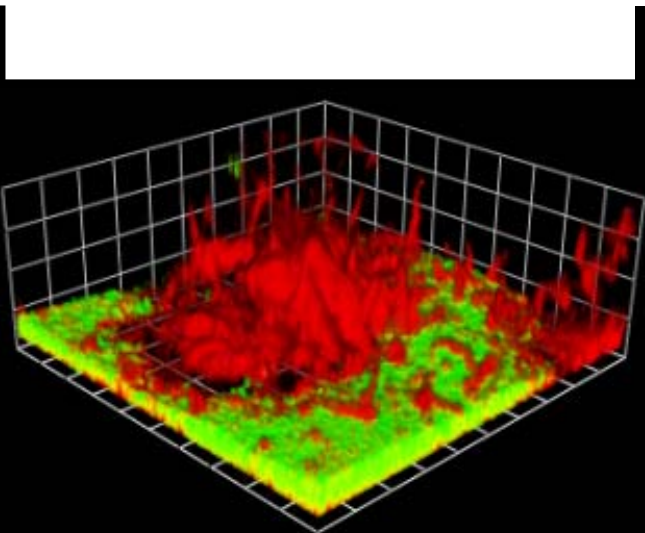
C. parvum transport in a sand column



Multi-species biofilms show complex dynamics



P. aeruginosa

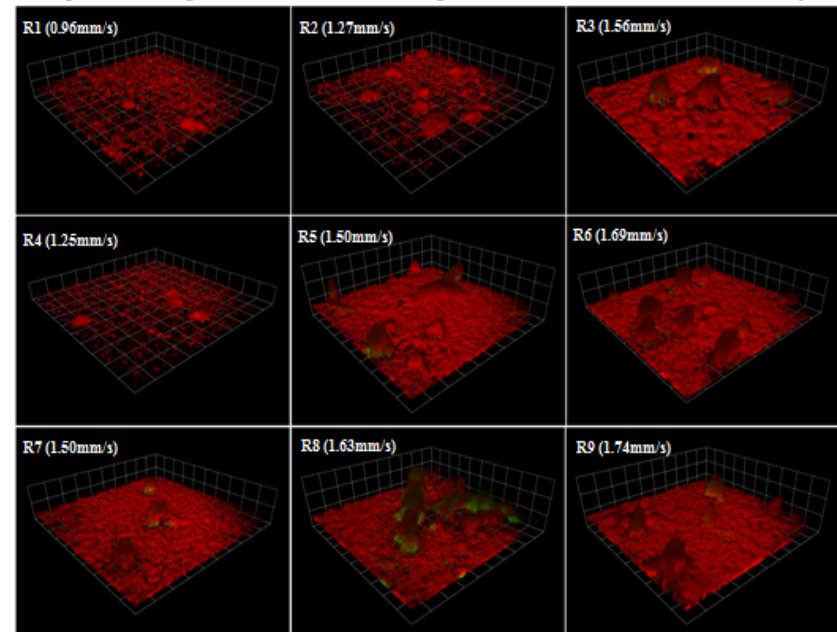
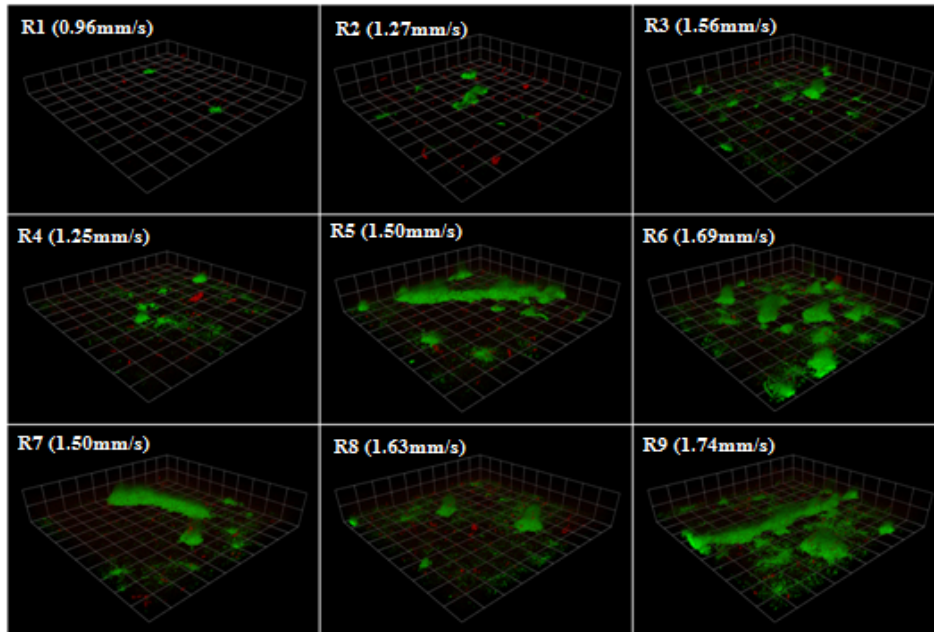


Flavobacterium CDC-65

Colonization of biofilms by planktonic cells also depends on flow environment

Colonization of *P. aeruginosa* biofilms by *E. coli* in R2A medium

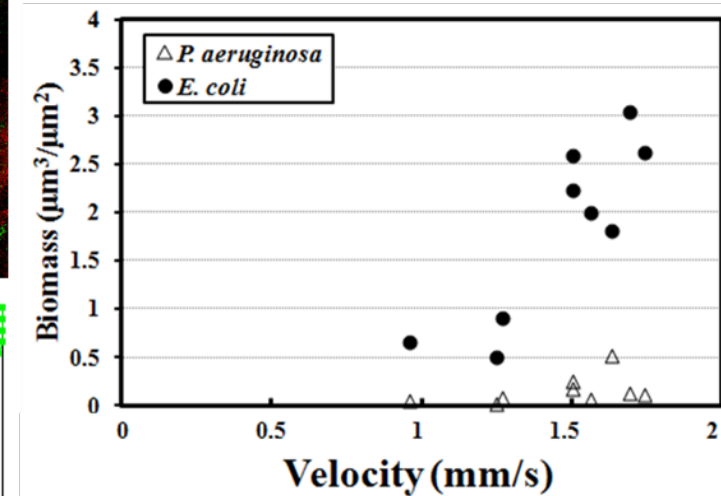
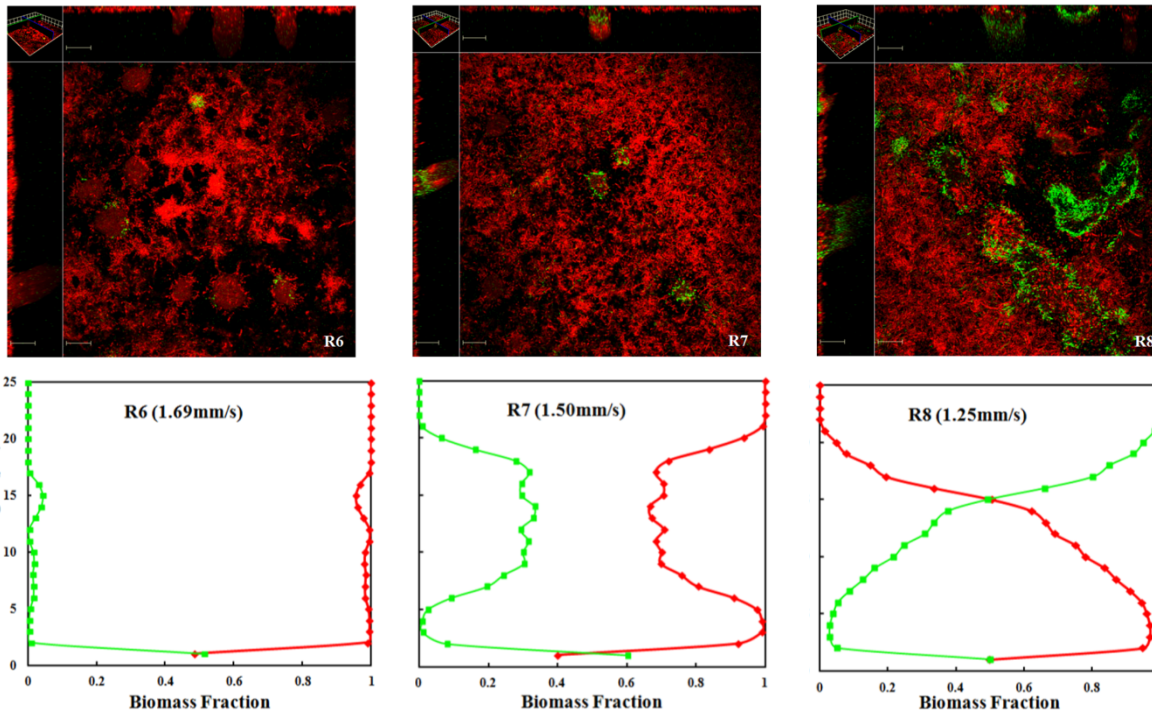
Deposition of *E. coli* on 3-day *P. aeruginosa* biofilm Overgrowth by *E. coli* after add'l 3 days



P. aeruginosa

E. coli

Colonization of biofilms by planktonic cells also depends on flow environment



P. aeruginosa

E. coli

Implications for colloid and pathogen dynamics in rivers



North Saskatchewan River



Difficult Run, VA, USA

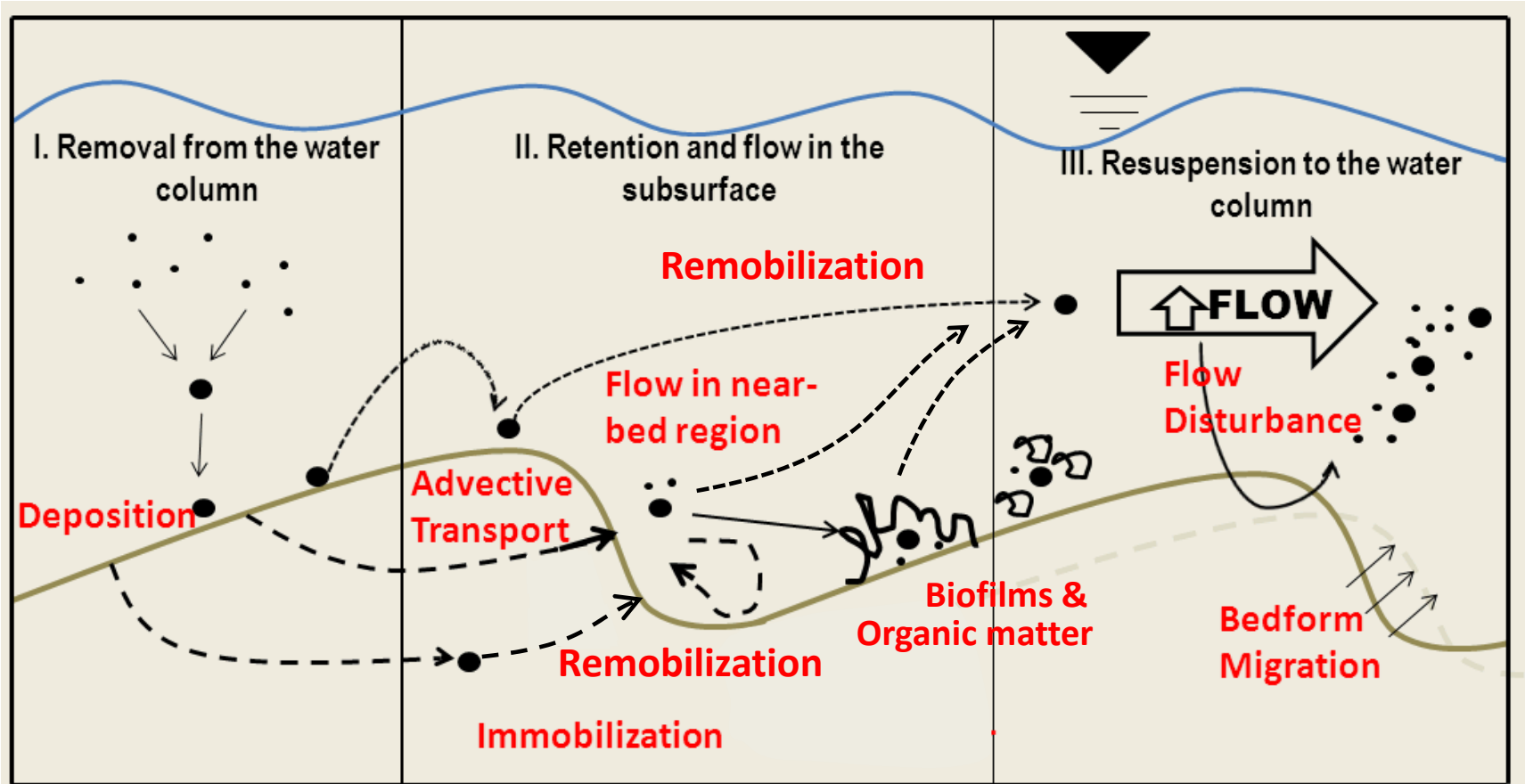


Sugar Creek, Indiana, USA



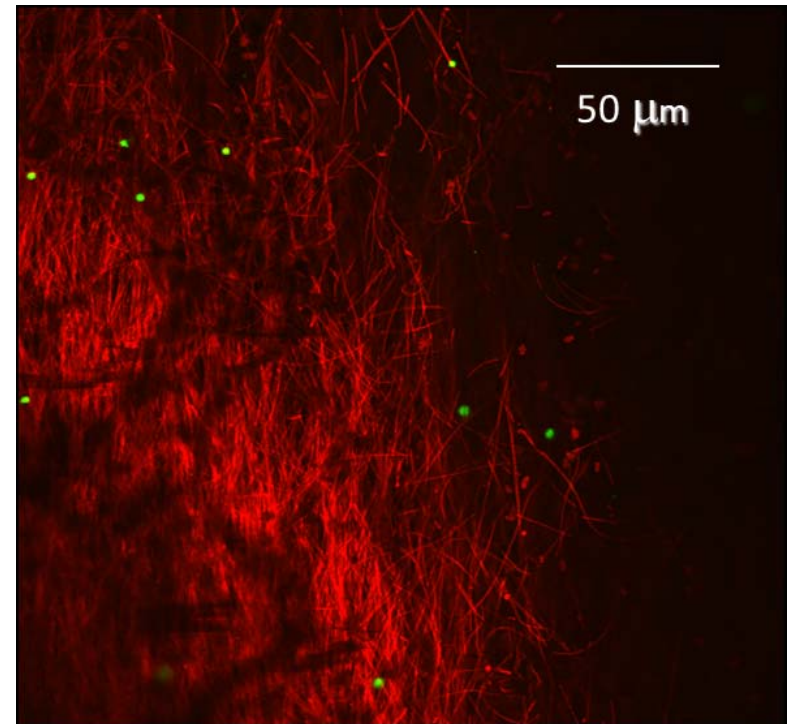
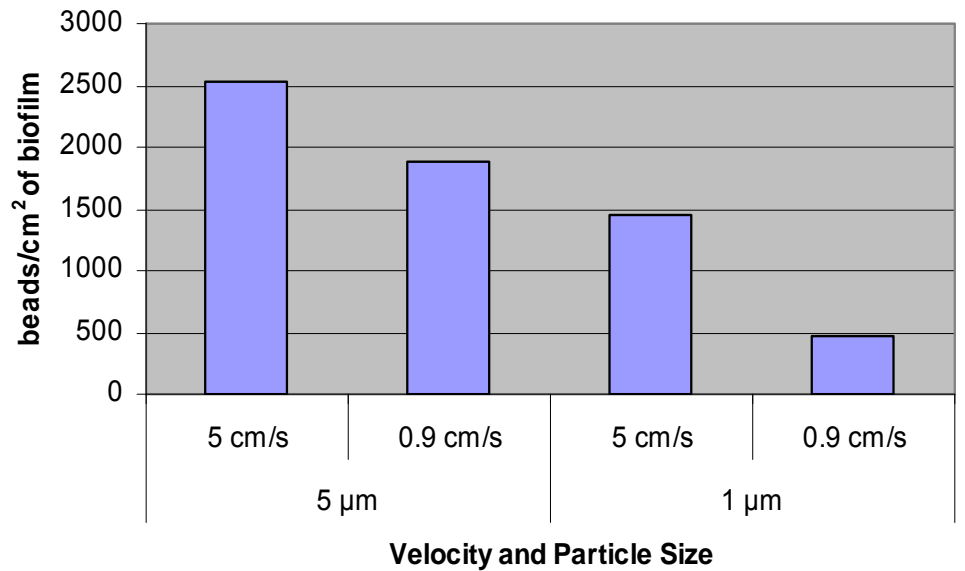
Clear Run, NC, USA

Processes hypothesized to control fine particle deposition, retention, and resuspension in streams



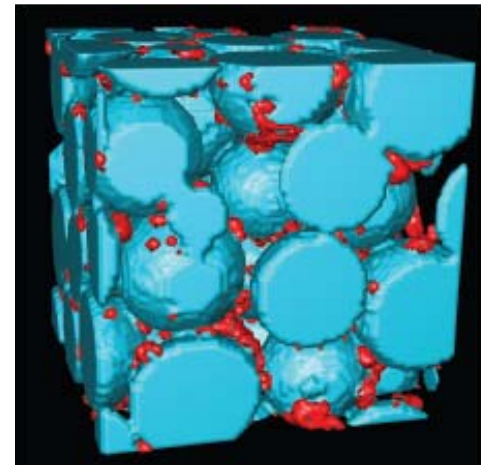
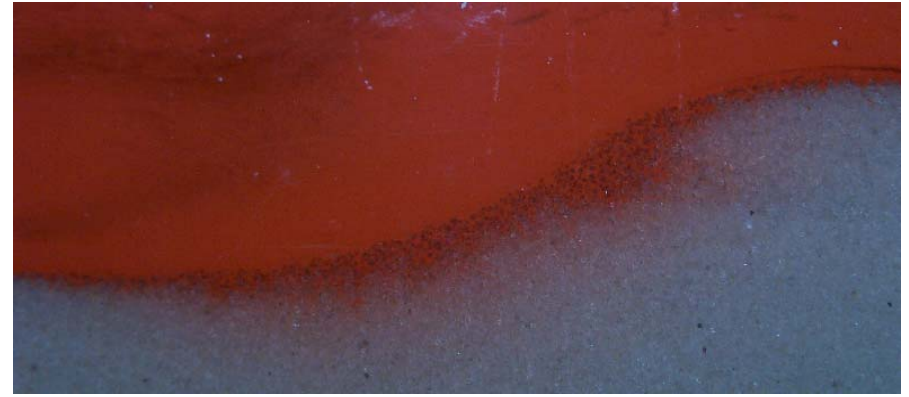
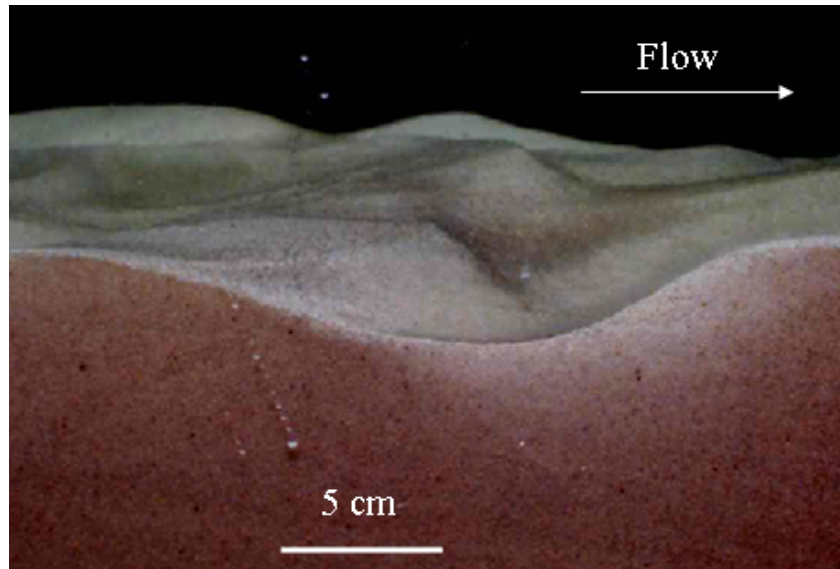
Drawing by Jennifer Drummond and Aaron Packman, Northwestern University

Streambed biofilms capture particles



Greater capture of larger colloids particles (5 μm > 1 μm microbeads).
Deposition increased with overlying stream velocity (5 cm/s > 0.9 cm/s)

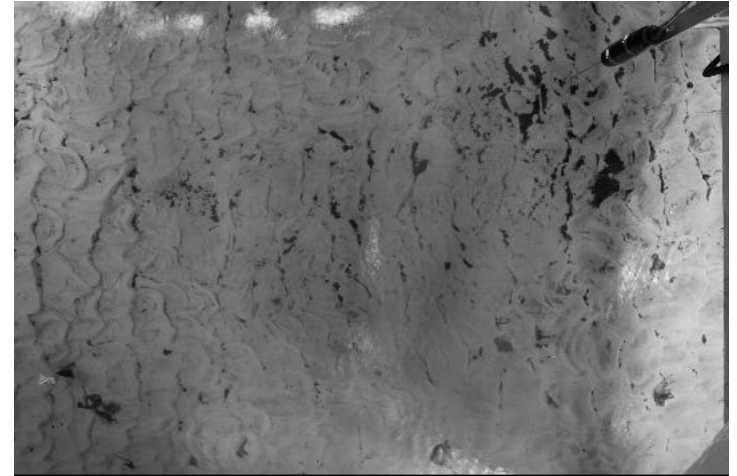
Streambed sediments capture particles too



Solute and particle injections in Clear Run, NC

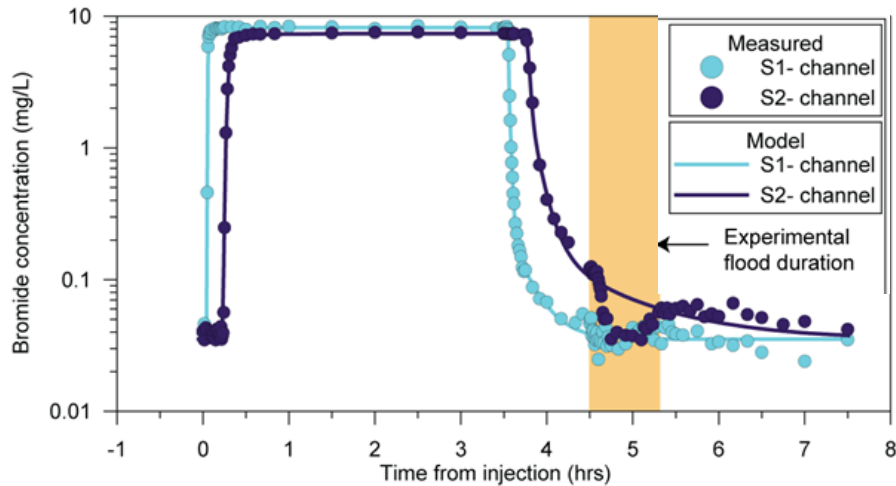


$Q \approx 60 \text{ L/s}$, Width $\approx 3 \text{ m}$, Depth $\approx 6 \text{ cm}$

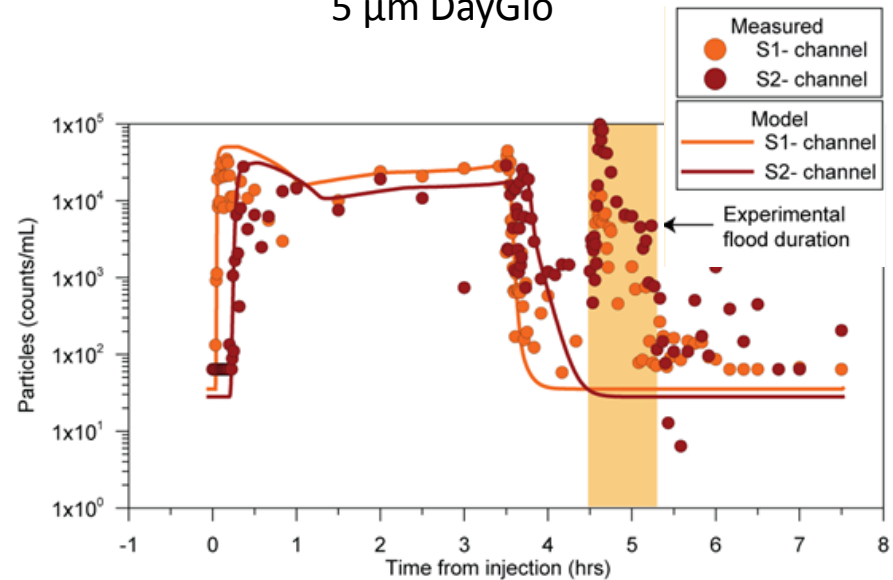


Solute and particle injections in Clear Run, NC under baseflow and artificial flood

Conservative tracer

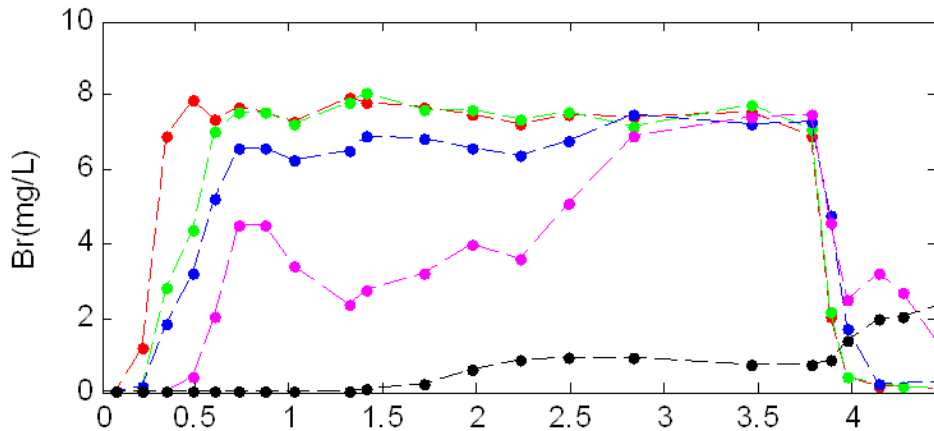


Fluorescent fine particles
5 μ m DayGlo

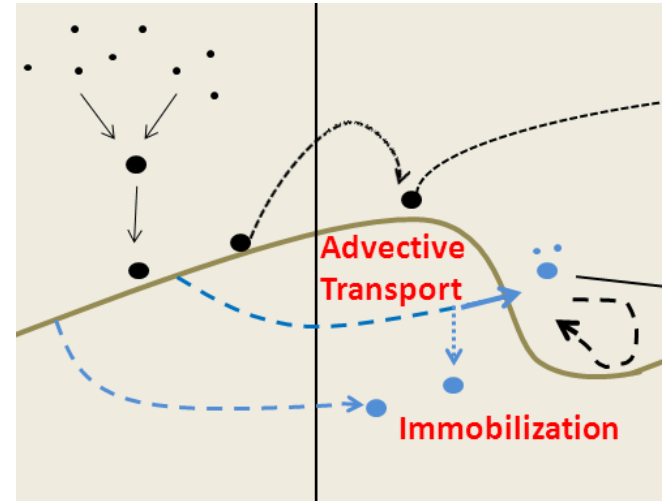
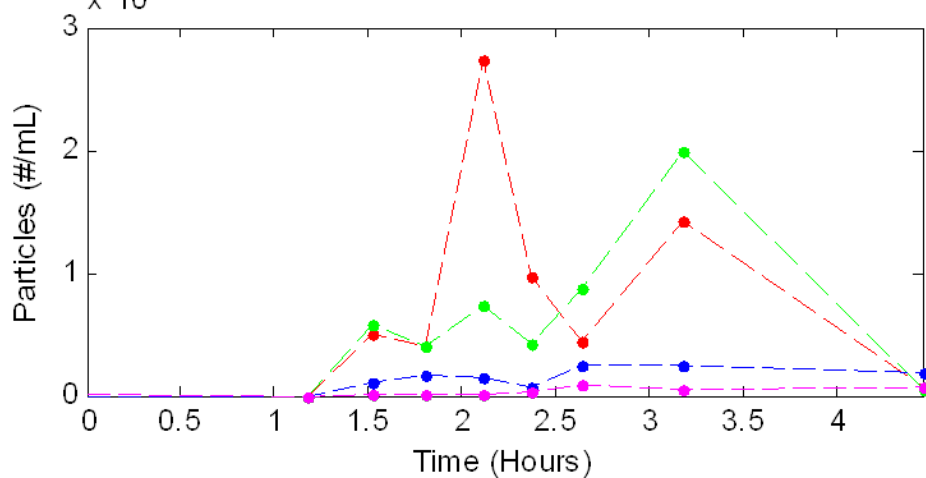


Direct evidence of subsurface transport of stream-borne colloids

Conservative Tracer: Bromide

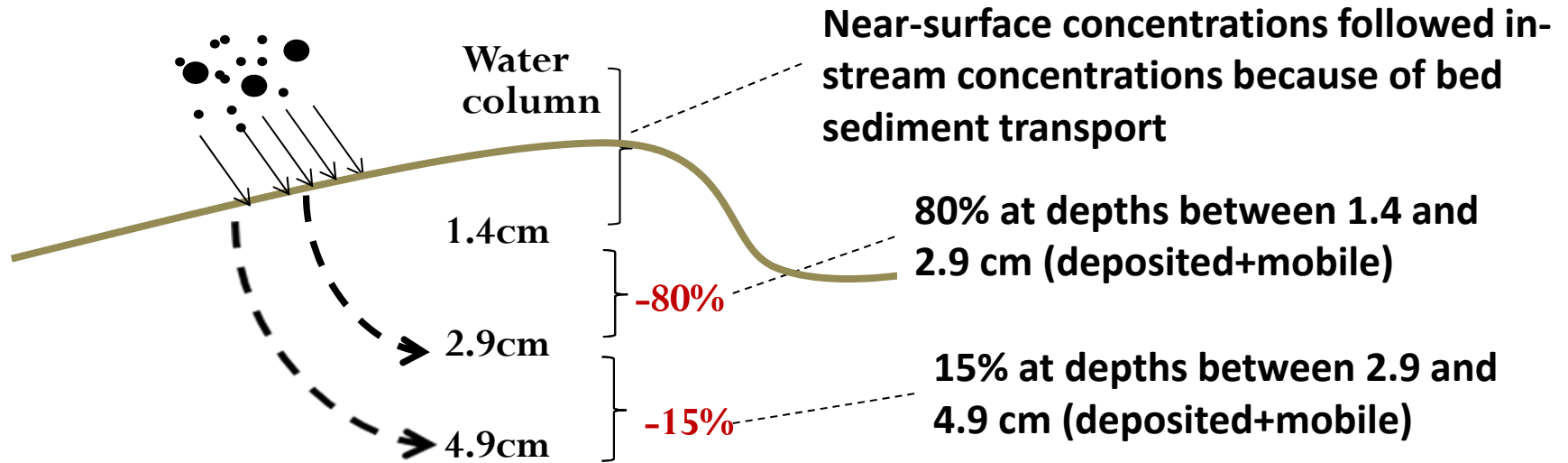
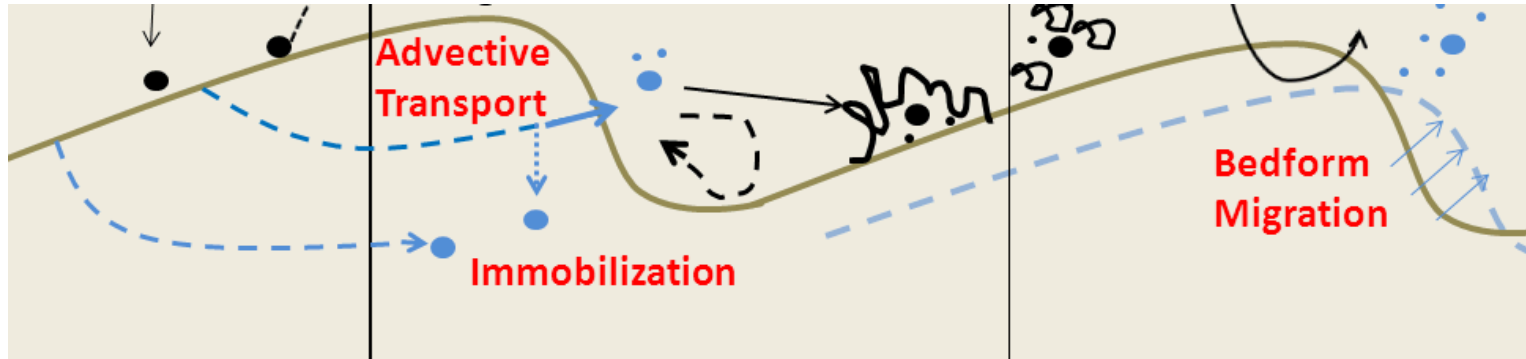


5 μm DayGlo fluorescent particles

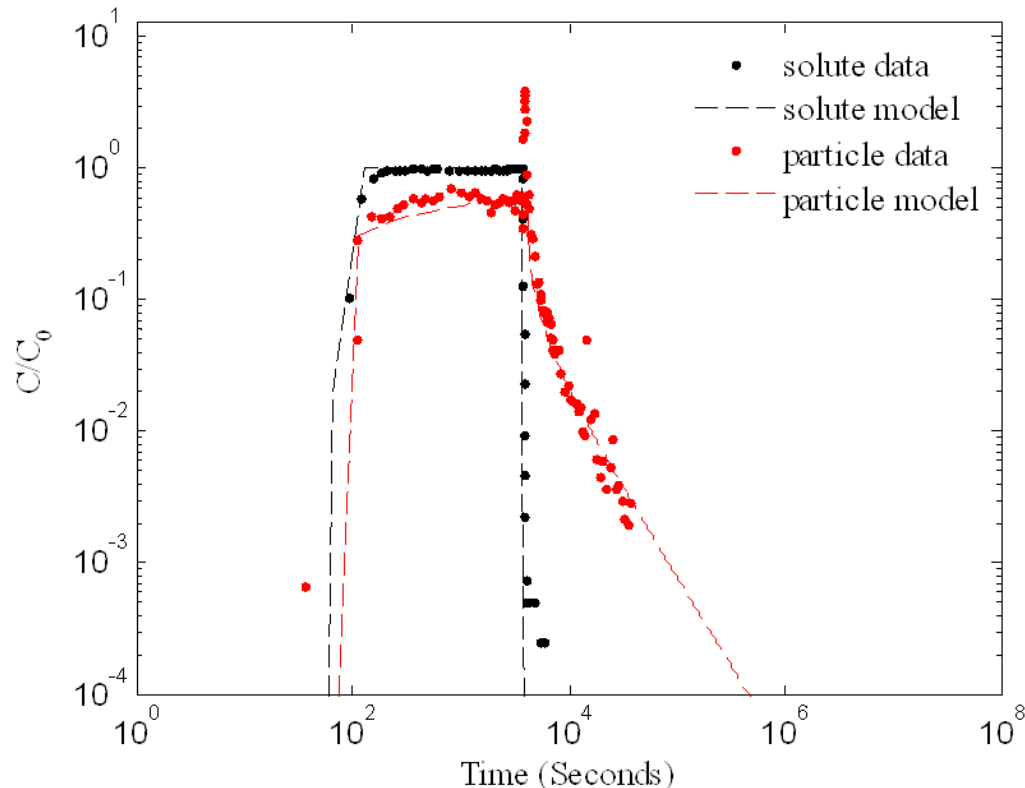


- Porewater sampling depth
- -2.1 cm
 - 1.4 cm
 - 2.9 cm
 - 4.9 cm
 - 7.9 cm

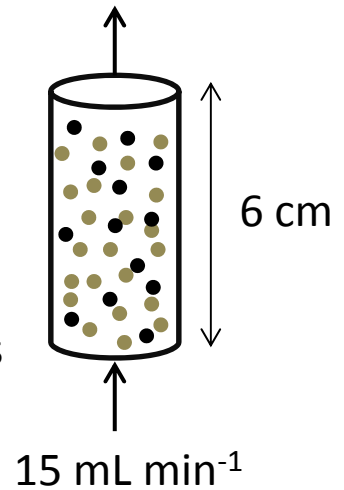
Direct evidence of colloid accumulation in sediments



Reversible filtration observed with natural streambed sediment in column experiment



Conservative Tracer:
Lithium
Fine Particle Tracer:
5 μm DayGlo
fluorescent particles



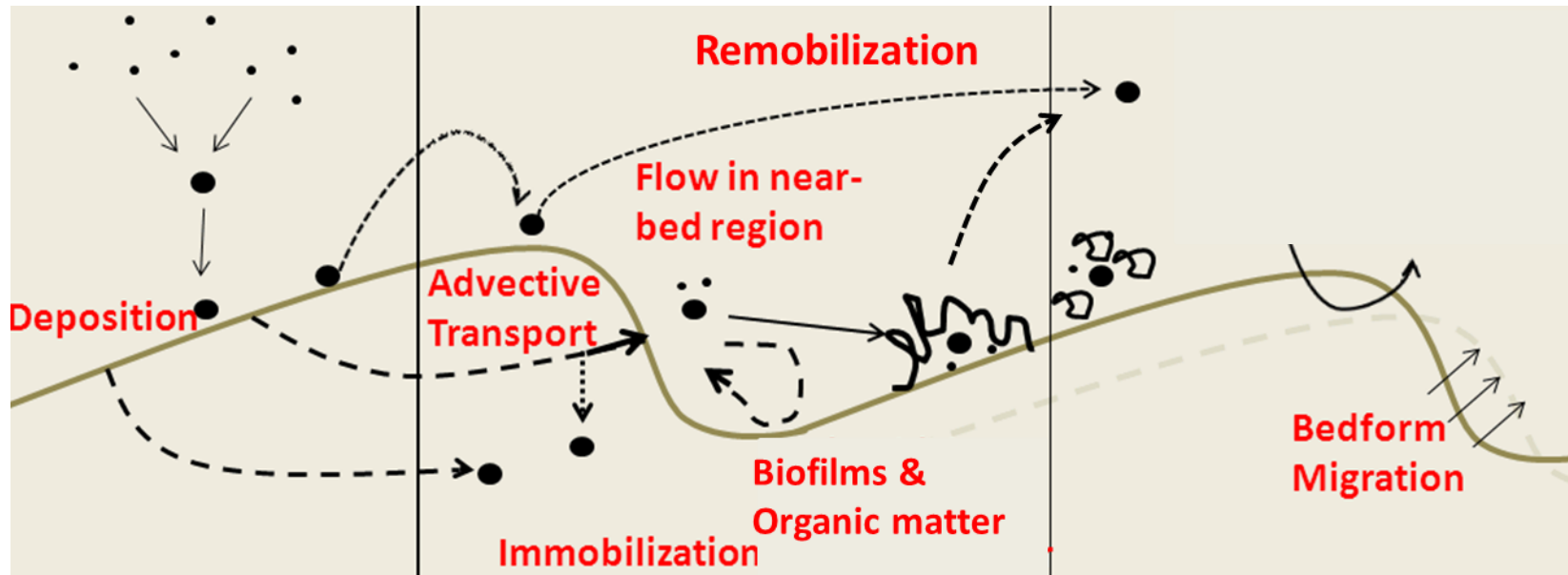
Mobile-Immobile Random Walk Model fit:

Lithium Advection-Dispersion + almost no storage

Colloid Advection-Dispersion + power law storage (heavy tail)

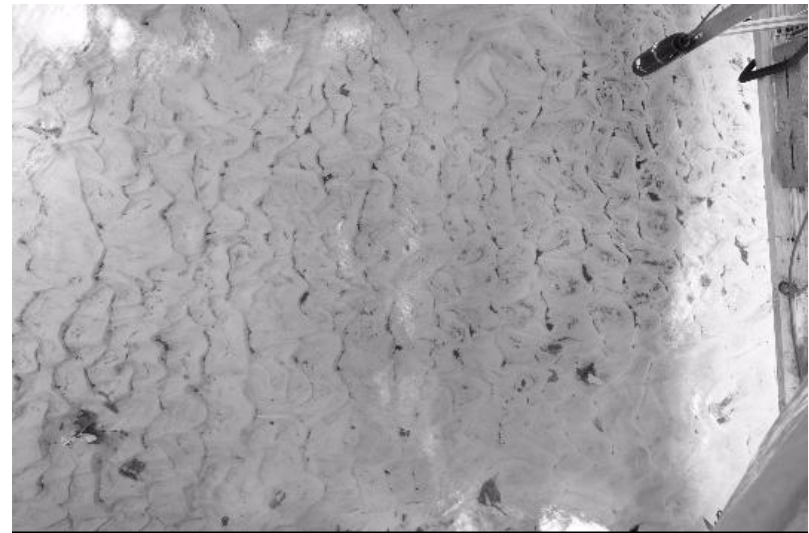
Colloid remobilization by hyporheic exchange, reversible filtration, and bed sediment transport

Direct evidence for ongoing efflux of suspended particles from bed during baseflow. Likely also ongoing turbulent resuspension.

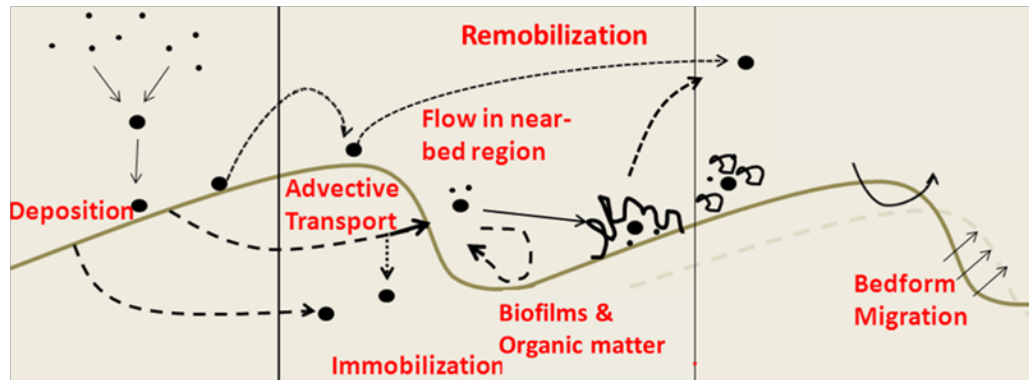


Strong colloid resuspension during floods associated with bed sediment transport

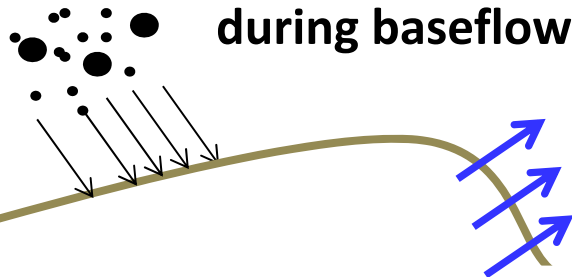
Floodflow



Baseflow

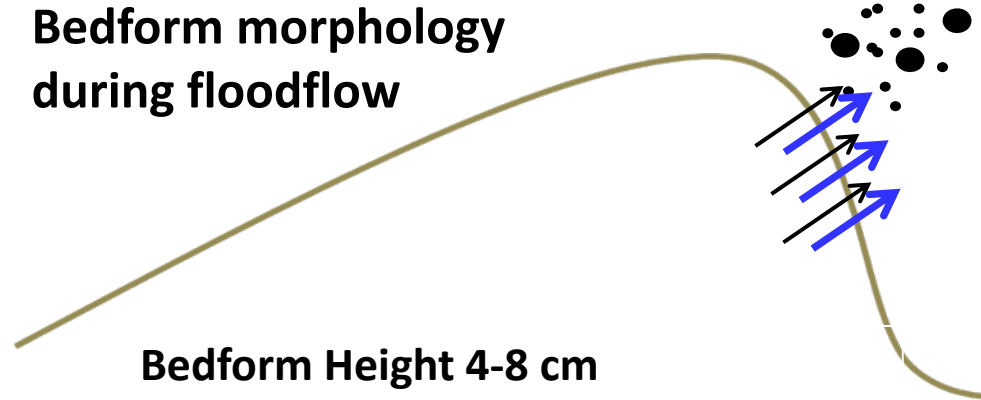


Bedform morphology during baseflow



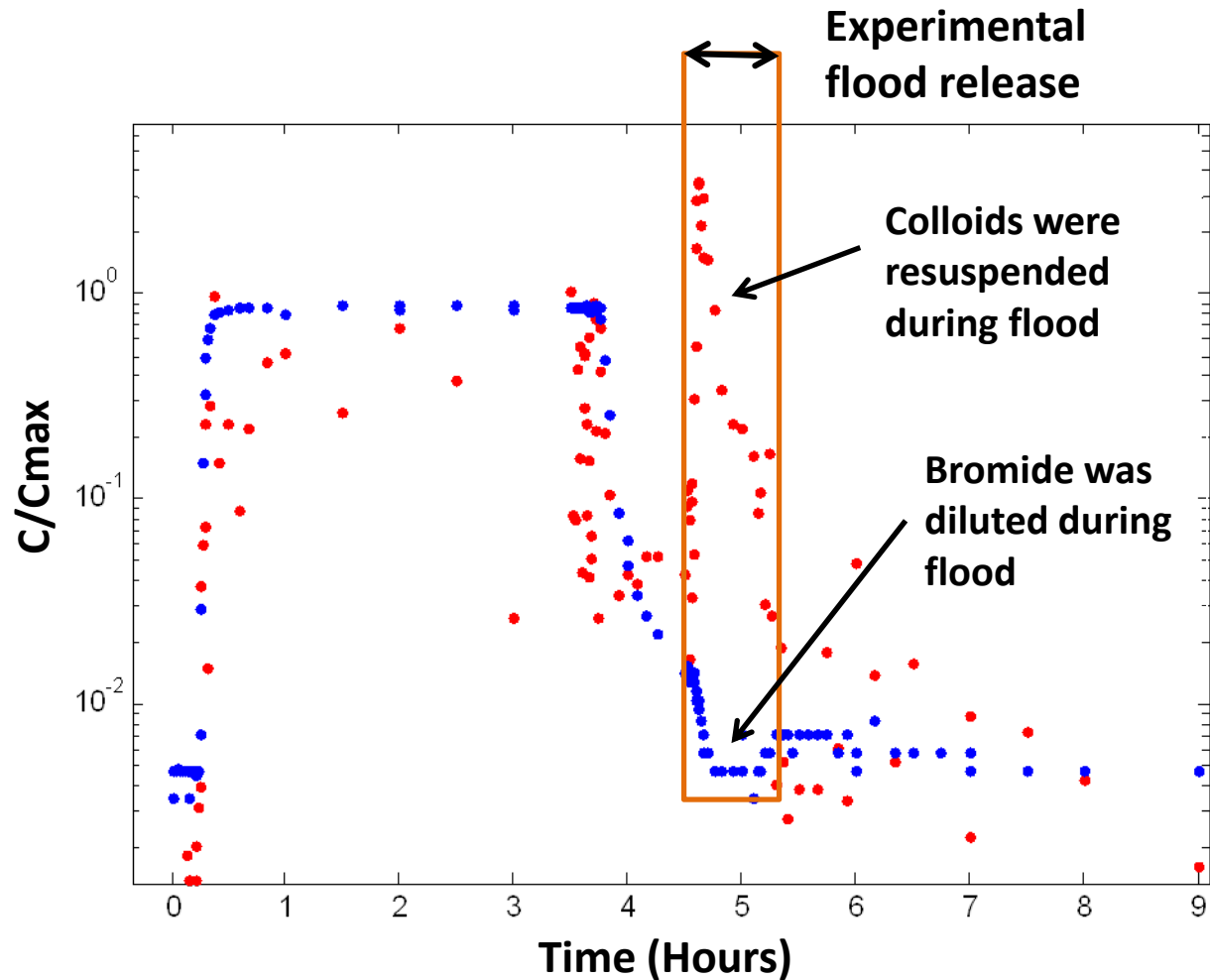
Bedform Height 1-2 cm
Crest Migration Rate $0.4 \pm 0.3 \text{ cm s}^{-1}$

Bedform morphology during floodflow

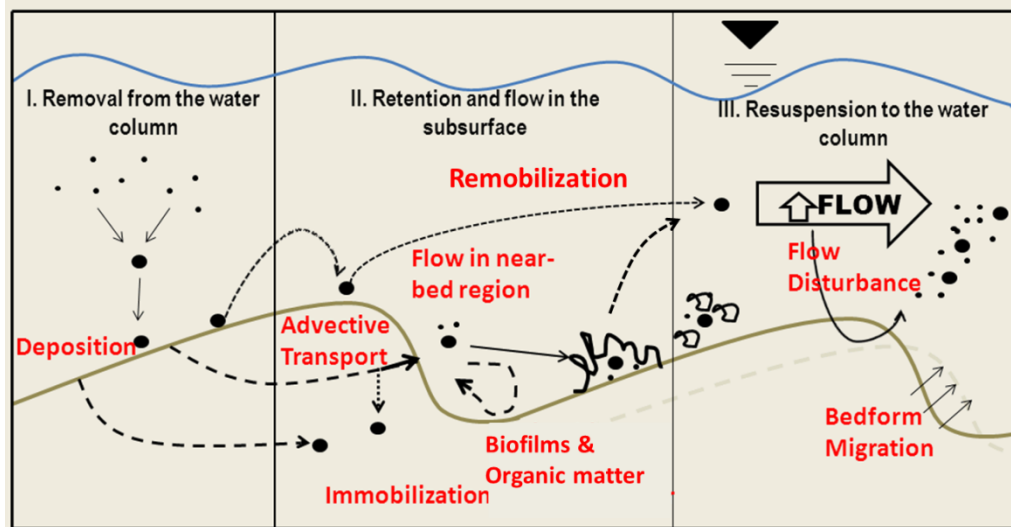


Bedform Height 4-8 cm
Crest Migration Rate $3.4 \pm 2.4 \text{ cm s}^{-1}$

Direct evidence of strong colloid resuspension during floods: Colloid export in stream



Proposed mobile/immobile model for colloid and pathogen transport in rivers with multiple convolution of different processes



Convolution of the following processes:

- **Advective exchange**
- **Settling/sedimentation**
- **Subsurface filtration**
- **Subsurface heterogeneity**
- **Turbulent deposition/resuspension**
- **Retention/release in biofilms**
- **Bedform turnover**
- **Streamflow variations**

Mobile/immobile model for downstream transport

Assume motion can be represented by an uncoupled jump PDF:

$$\Psi(x, t) = \lambda(x) \psi(t)$$

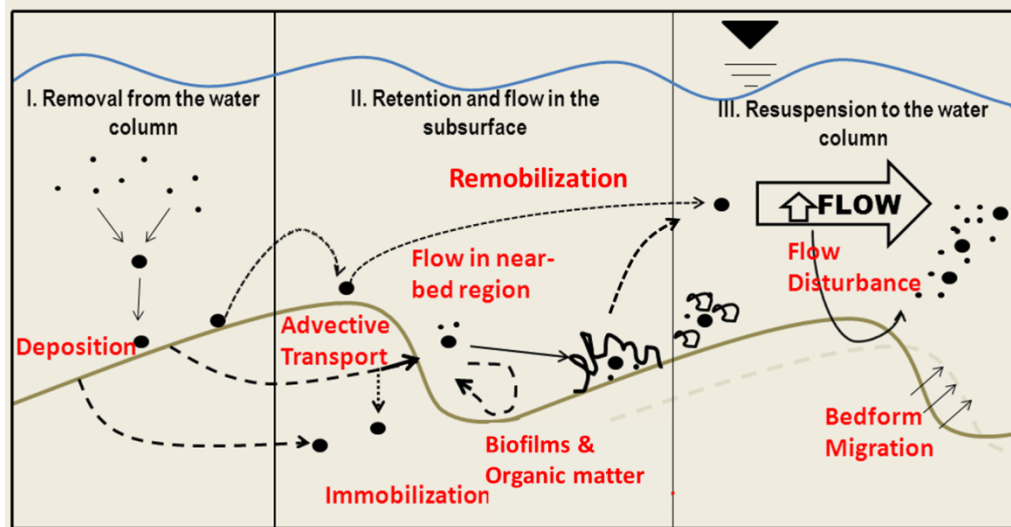
$$\frac{\partial C}{\partial t} = -U \frac{\partial C}{\partial x} + K \frac{\partial^2 C}{\partial x^2} - \int_0^t \frac{\partial C(t - \tau)}{\partial t} M(\tau) d\tau$$

Mobile, in-stream transport

Immobile, subsurface

Can represent multiple processes in memory function by convolution

Proposed model: Mobile/immobile random walk with multiple convolution of different processes

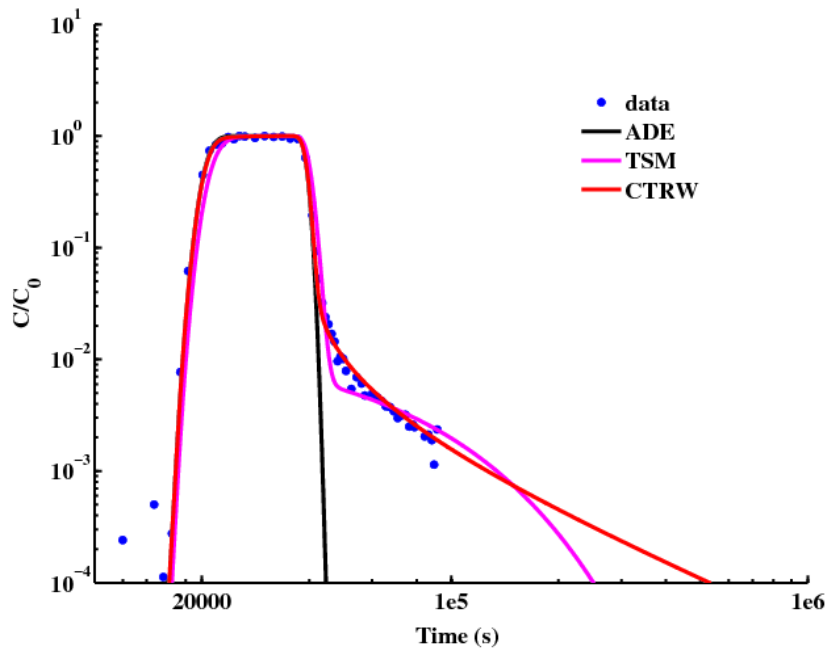


Convolution of the following processes:

- **Advection exchange**
- **Settling/sedimentation**
- **Irreversible filtration**
- **Weak subsurface heterogeneity**
- **Turbulent deposition/resuspension**
- **Retention/release in biofilms**
- **Bedform turnover**
- **Streamflow variations**

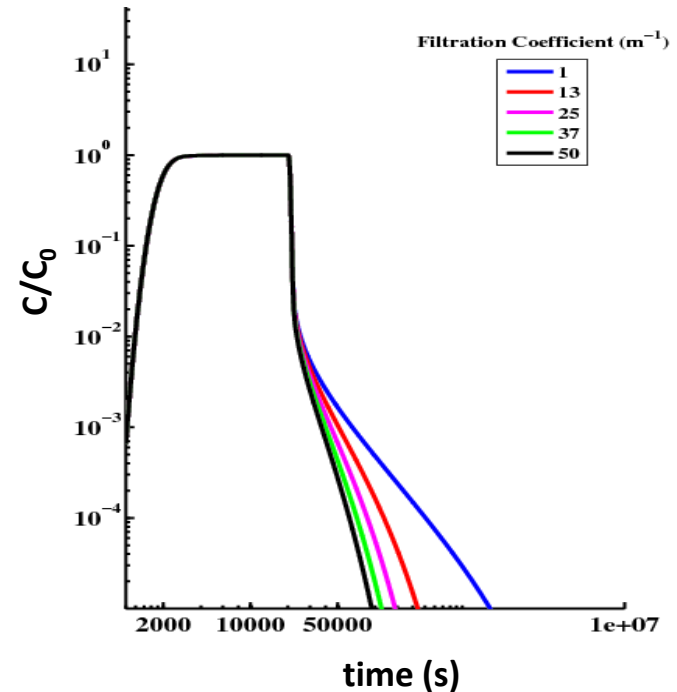
Solute and particle BTC's

Simulation of conservative solute transport
(data from Johansson et al., 2001)



$$\tilde{\psi}(u) = \tilde{\psi}_0 [u + \sum_{i=1}^n \Lambda(i) - \Lambda(i) \tilde{\varphi}_i(u)]$$

Simulation of particle transport with irreversible filtration (1st order removal)



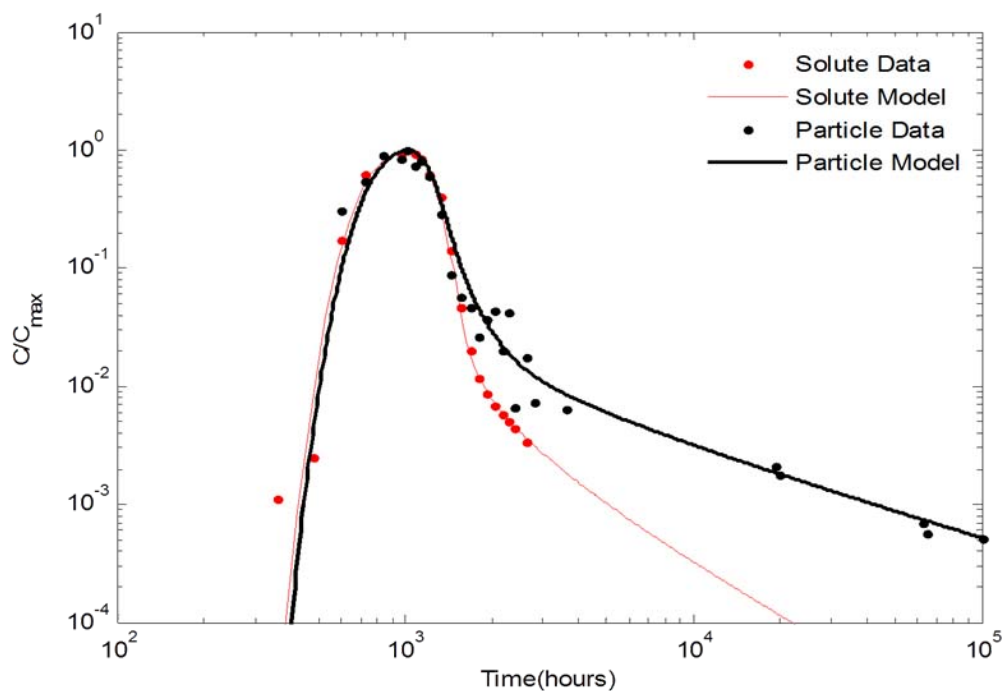
$$\tilde{\psi}(u) = \tilde{\psi}_0 [u + \sum_{i=1}^n \Lambda(i) - \Lambda(i) \tilde{\varphi}_i(u) * (1u - k_i)]$$

Inverse modeling of fine particle dynamics

Newbold et al., 2005

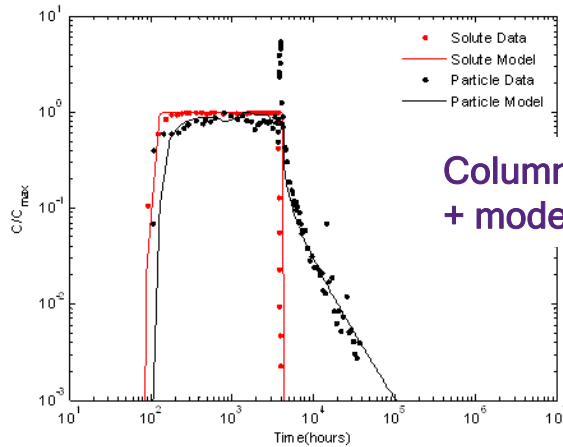
Bloomington Creek, Idaho

Tracers: Rhodamine + ^{14}C -labeled fine particulate organic matter



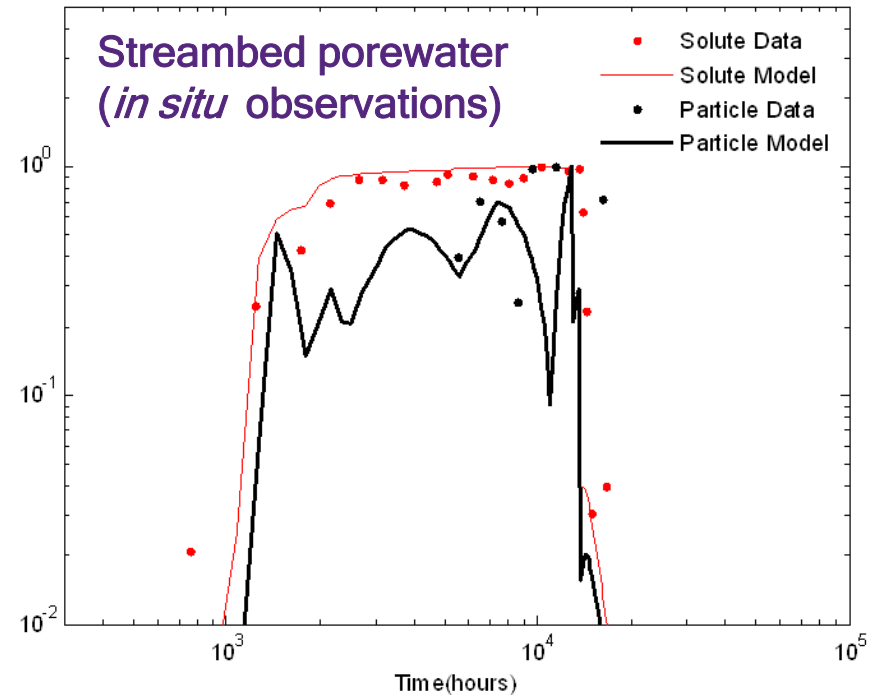
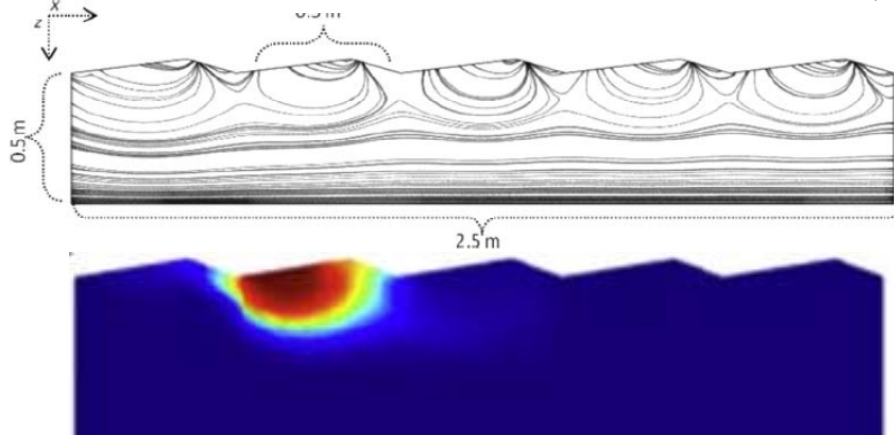
Predictive modeling

Clear Run, NC, *Harvey et al.*, 2012
 Tracers: Bromide; Dayglo fluorescent microspheres



Column experiment data + model (*ex situ*)

Porewater flow and filtration
Karwan and Sainers, 2012



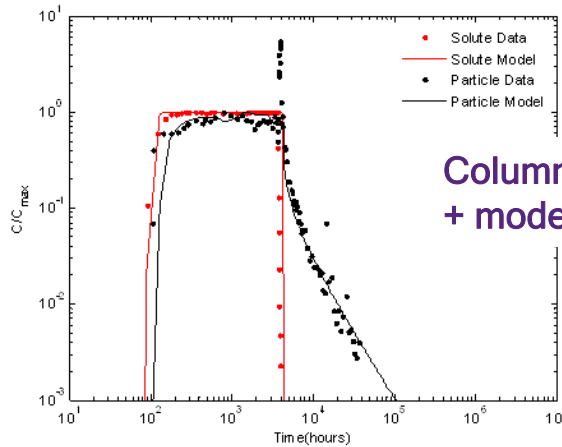
Streambed porewater (*in situ* observations)



Predictive modeling

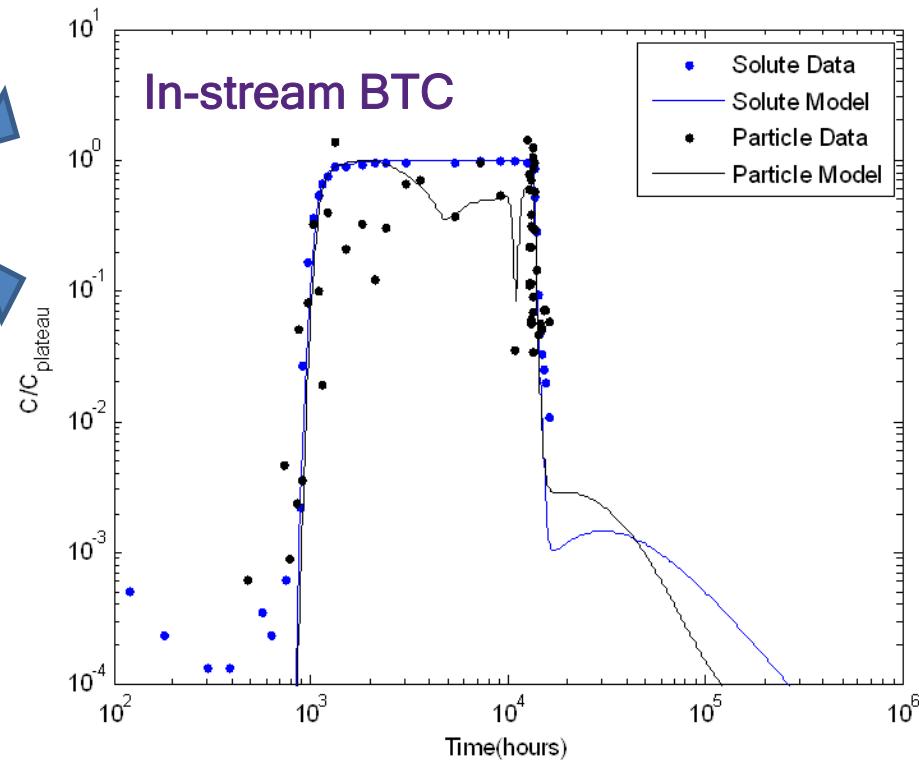
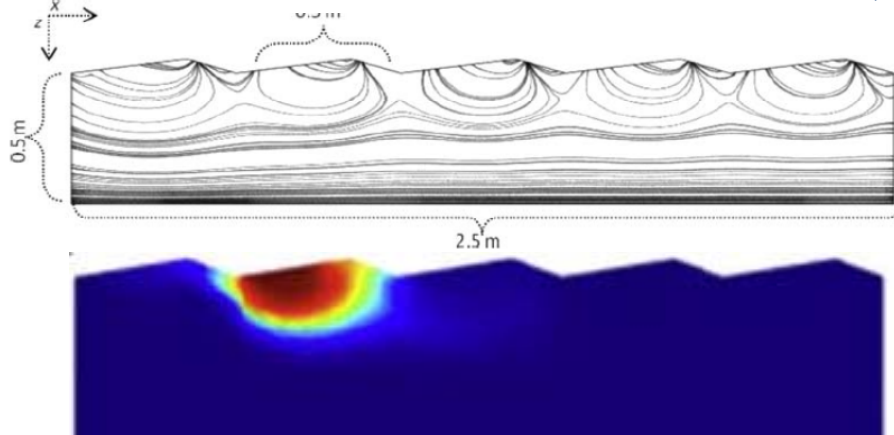
Clear Run, NC, *Harvey et al.*, 2012

Tracers: Bromide & Dayglo
fluorescent fine particles

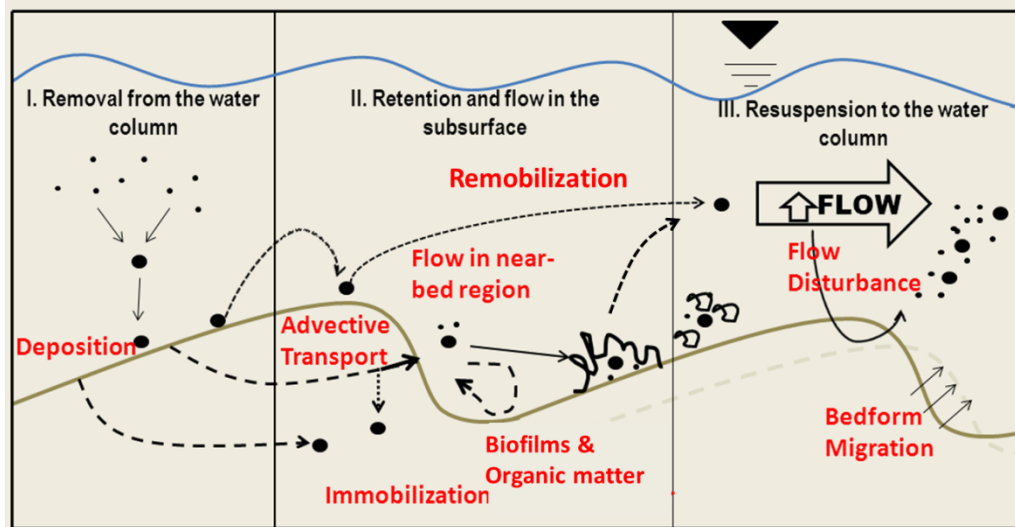


Column experiment data + model (*ex situ*)

Porewater flow and filtration
Karwan and Sairs, 2012



Proposed model: Mobile/immobile random walk with multiple convolution of different processes

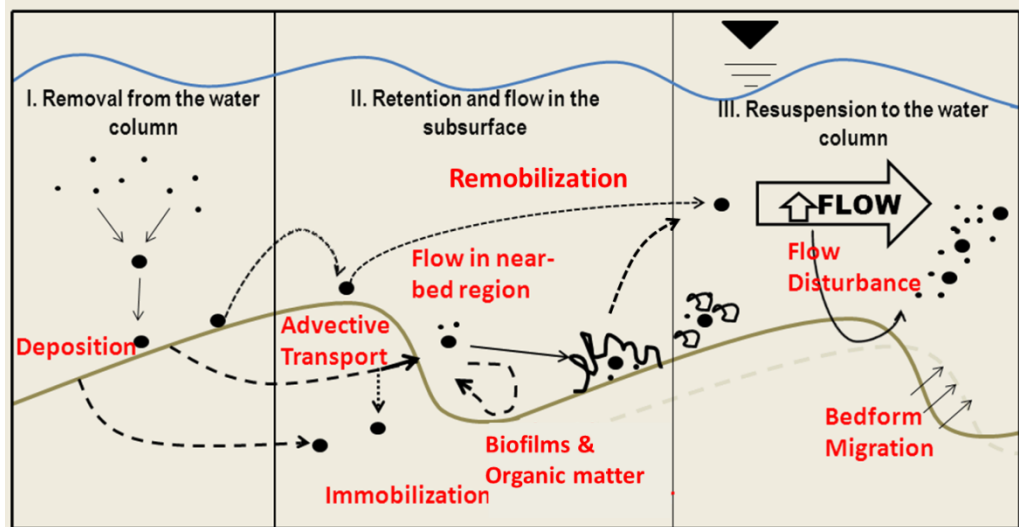


Convolution of the following processes:

- **Advection exchange**
- **Settling/sedimentation**
- **Reversible filtration**
- **Weak subsurface heterogeneity**
- **Turbulent deposition/resuspension**
- **Limited retention/release in biofilms**
- **Bedform turnover**
- **Limited streamflow variations**

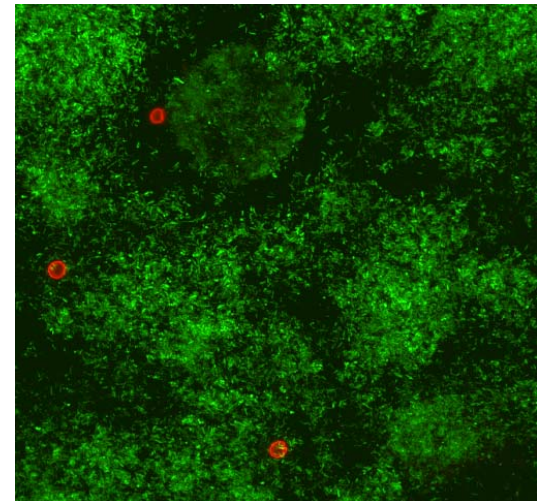
Can be extended to floodflow by considering changing morphodynamics during flood.

Proposed model: Mobile/immobile random walk with multiple convolution of different processes

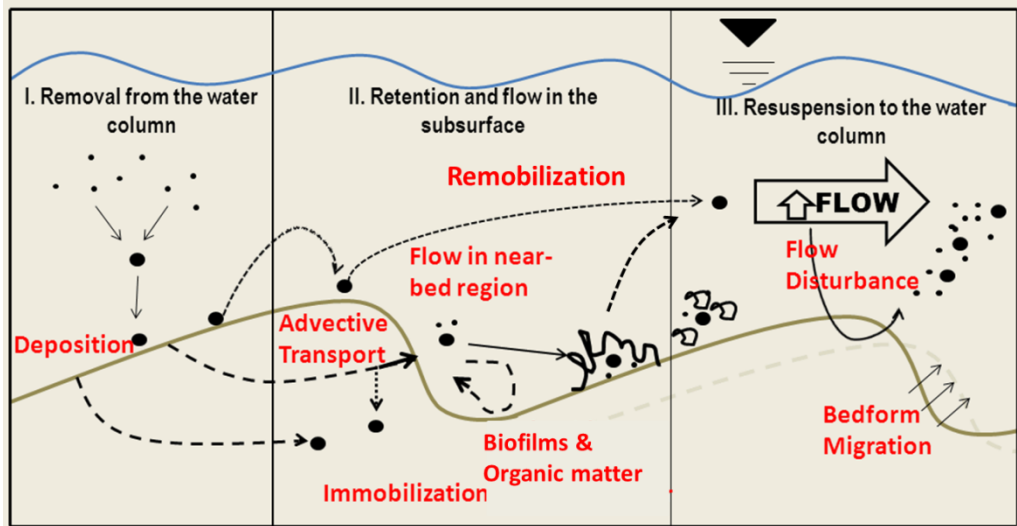


Biological processes affecting passively transmitted cysts & spores

- Deposition in biofilm EPS
- Shear-induced detachment
- Biofilm sloughing (biological)
- Predation/die-off

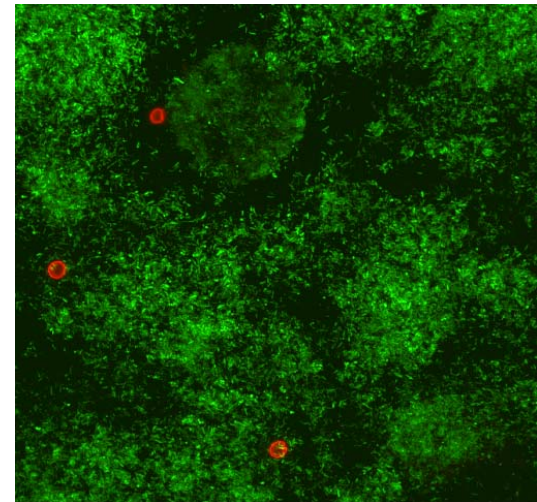


Proposed model: Mobile/immobile random walk with multiple convolution of different processes



Biological processes affecting passively transmitted cysts & spores

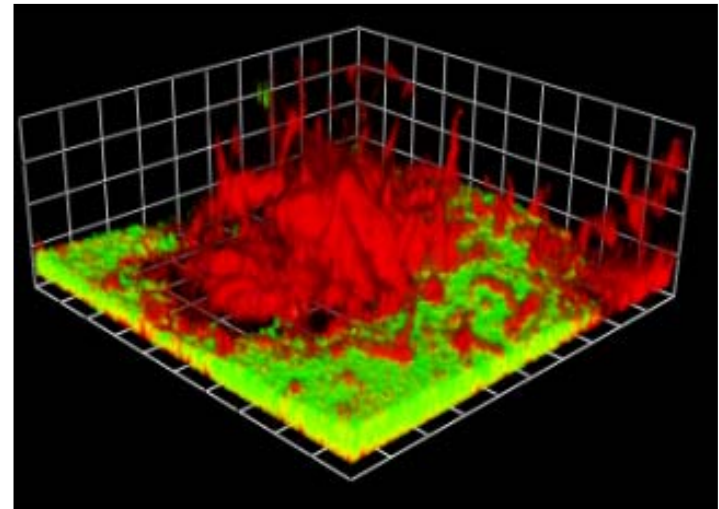
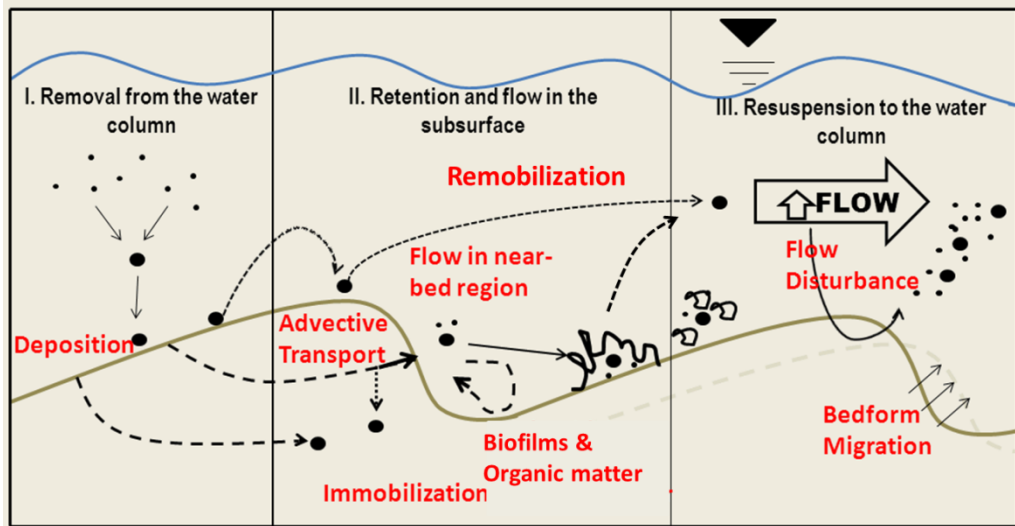
- Deposition in biofilm EPS
- Shear-induced detachment
- Biofilm sloughing (biological)
- Predation/die-off



Incorporate information from 3D flow-biofilm interactions and associated shear-induced detachment and biologically-induced sloughing.

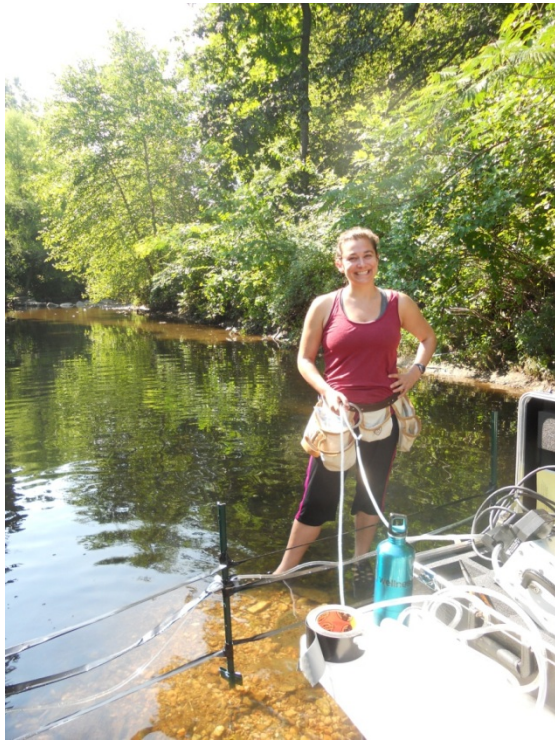
Proposed model: Mobile/immobile random walk with multiple convolution of different processes

For opportunistic bacteria, potential colonization of biofilms and sediments must also be considered.

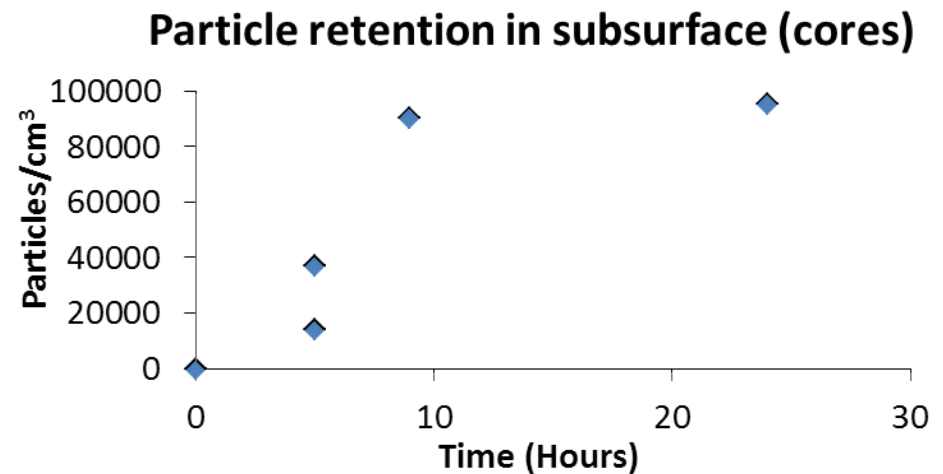
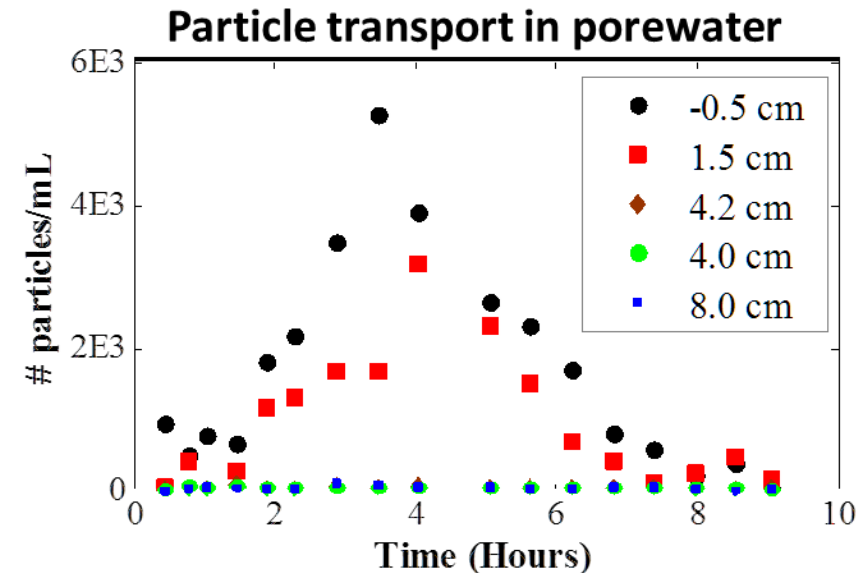
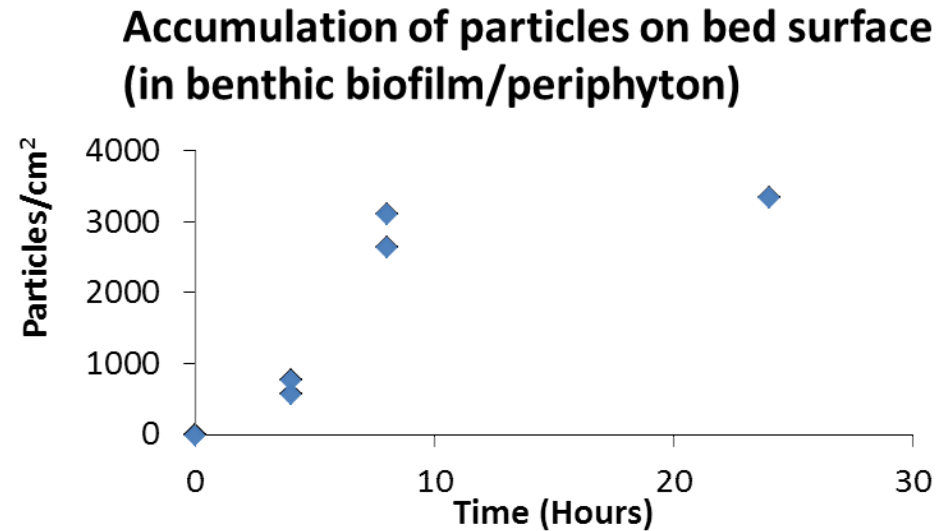
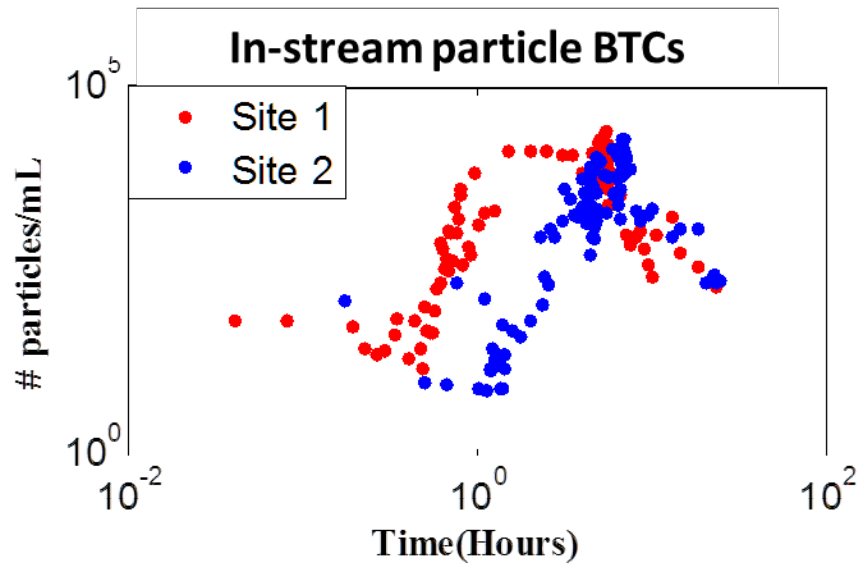


We are obtaining information on limited multispecies interactions of indicators, pathogens, and base biofilms (defined monospecies and natural base biofilms)

Solute and particle injections in Accotink Creek and Difficult Run, VA



Accotink Creek: Direct evidence of deposition/resuspension from benthic biofilms and bed sediments



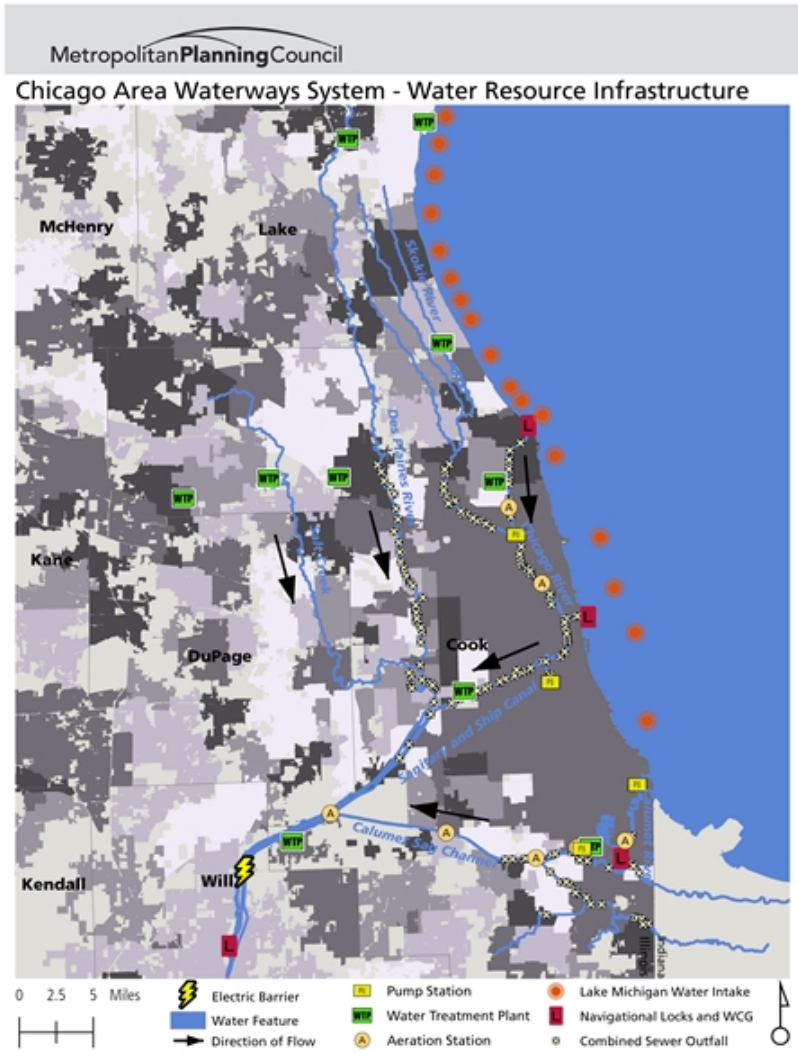
Seven Important Processes

1. **Biofilms capture and release particles & pathogens**
2. **Pathogen filtration is often reversible**
3. **Multi-species biofilms show complex dynamics**
4. **Streambed biofilms and sediments capture and release particles**
5. ***Direct evidence* of particle capture in benthic biofilms and underlying hyporheic sediments**
6. ***Direct evidence* of slow, ongoing remobilization from biofilms and bed sediments during baseflow**
7. ***Direct evidence* of rapid, extensive remobilization from the streambed during flood flow**

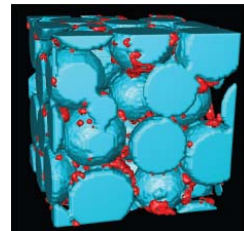
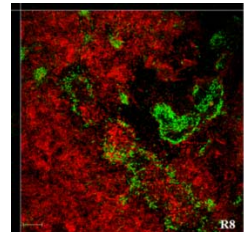
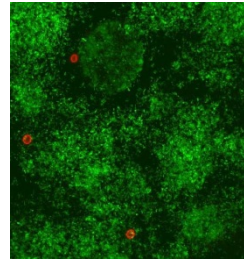
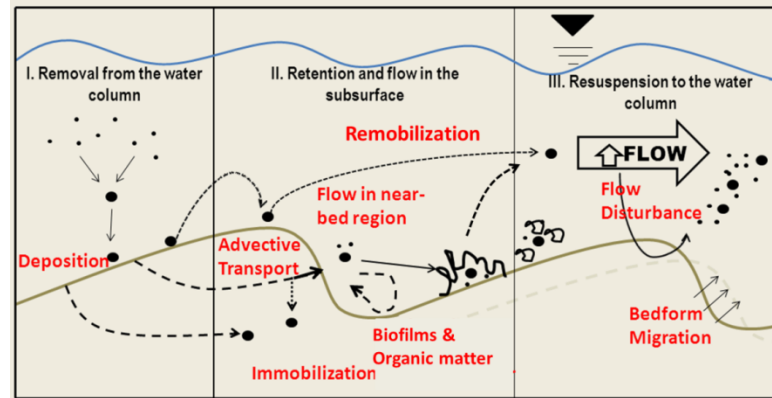
Conclusions and Implications

- 1. Rivers are strong horizontal filters, but deposited particles and pathogens become resuspended over time. Deposited particles are slowly mobilized during baseflow and rapidly & extensively mobilized by high flows that scour the bed.**
- 2. New theoretical frameworks provide the potential for quantitative assessment of transmission risks under varying loading scenarios and hydrological and climatological conditions.**
- 3. Organisms having an active life-cycle in aquatic environments show complex interactions with pre-existing biofilms.**
- 4. More information is needed on the dynamics of important pathogens in natural and engineered water systems (interactions with surfaces and biofilms, metabolism & motility, persistence & die-off in all relevant reservoirs)**

Proposed approach for Chicago-area waterways



Coupling of anomalous transport, microbial ecology, and network dynamics is a major challenge!



Proposed solution:

- Collect extensive data on microbial community in all important reservoirs
- Build hydraulic model for water flow
- Measure solute and particle transport dynamics, and biofilm processes
- Incorporate anomalous transport submodels for passive (cysts, spores) and active (bacteria) pathogens

