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INVESTIGATION OF CELL TRAJECTORIES IN CHAOTIC FLOW FIELDS GENERATED BY A HELICAL STATIC MICROMIXER FOR RAPID CROSSLINKING KINETICS

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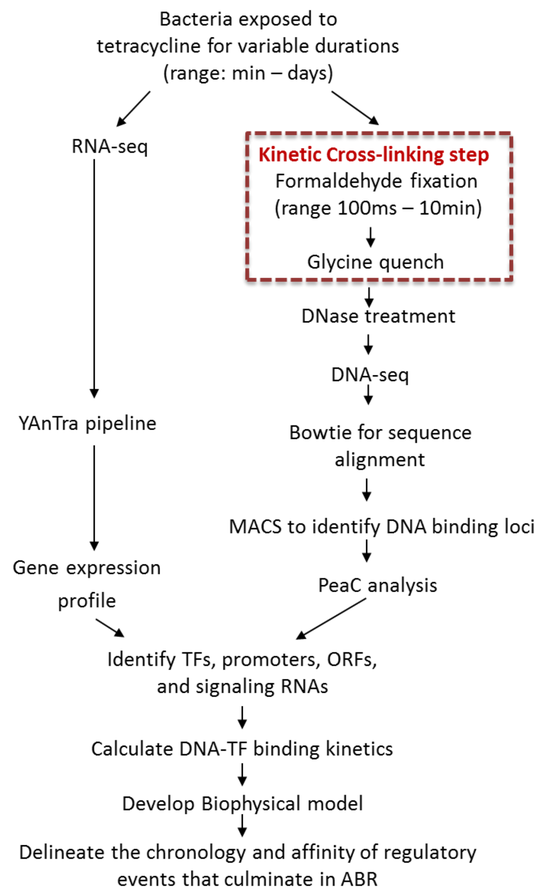
Background and significance

- The use of antibiotics has caused the evolution of antimicrobial resistance (AMR) in bacteria through the expression of antibiotic resistance which is reliant on transcription factors (TFs) associated with the resistance gene of interest.
- Therefore there is a need to develop specialized drugs which identify and target sites of gene regulation by TFs for antibiotic resistance in such multi drug resistant (MDR) bacteria.
- One of the most robust techniques to study the interaction of chromatin and associated binding protein involves isolation of chromatin fragments from cells via antibodies specific to the chromatin binding factor of interest. This method called chromatin immunoprecipitation (ChIP) involves crosslinking chromatin fragments with formaldehyde so that the associated DNA can be isolated and assayed via PCR or sequencing.
- Thus there is a need for an in-vivo ChIP analysis system capable of resolving chromatin interactions in the sub-second to minute resolution range



Immunoprecipitation-seq

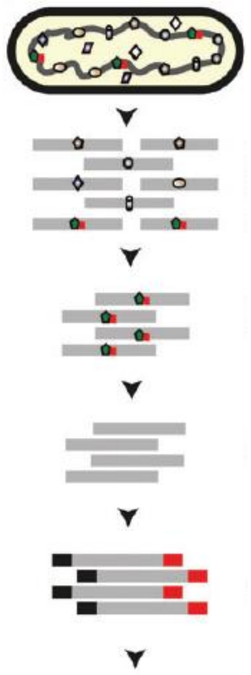
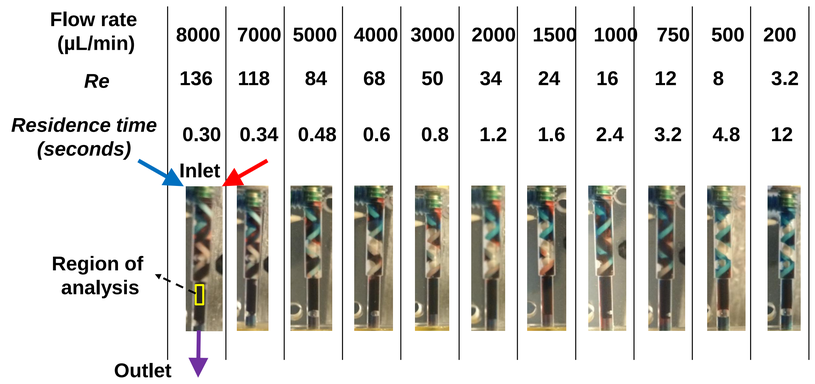
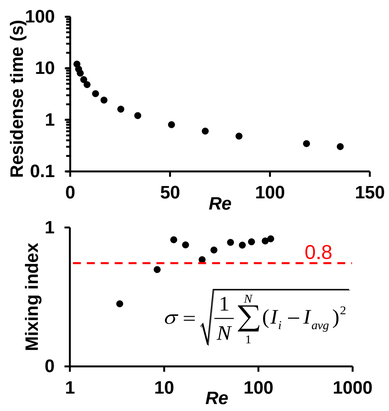


Figure adapted from Chumsakul et.al. DNA research **20**, 325-377 (2013)

Characterizing mixing index



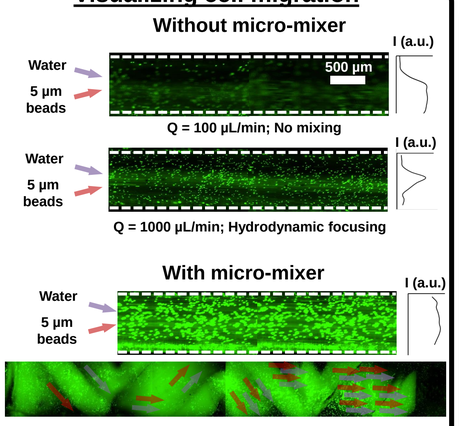


$$\text{Mixing index} = \frac{\sigma_{max} - \sigma}{\sigma_{max}}$$

- Mixing efficiency was quantified by calculating the standard deviation of pixel colors downstream from the mixing chamber.
- Where I_i is the intensity of the red channel of the i'th pixel, I_{avg} is the average red channel intensity and N is the total number of pixels.

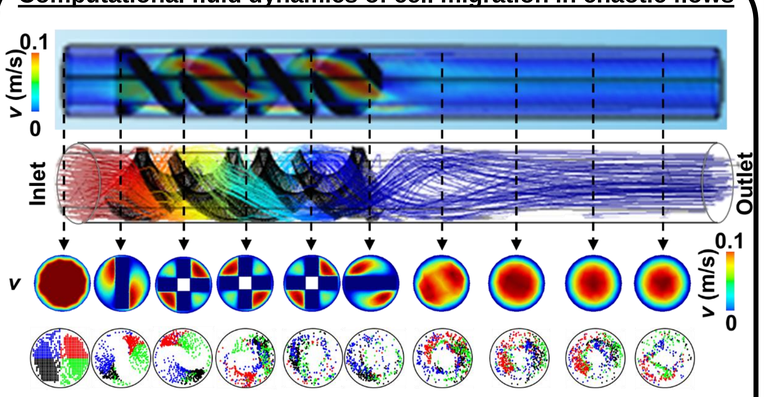
Cell migration in micro-mixer driven flow

Visualizing cell migration



- Without the mixer water and bead solution remains separated at low (laminar separation) and high flow rates (hydrodynamic forces focus the beads along the center).
- With the micro-mixer, the chaotic flow field downstream disrupts enables efficient cross flow for rapid mixing..

Computational fluid dynamics of cell migration in chaotic flows



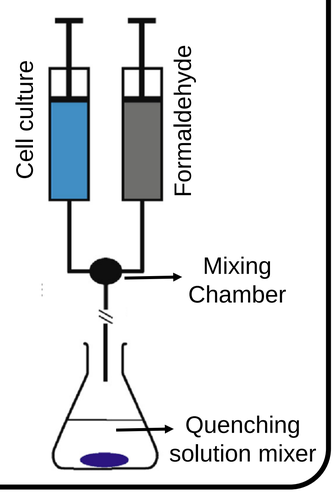
Poincaré maps of cell migration with the helical mixer

- Cross sectional velocity profiles reveals areas of high and low local velocities near the mixer.
- Time resolved trajectories of 5 micron cells originating at the inlet.
- Poincaré maps of cells originating at the inlet in four distinct islands evolve rapidly at the onset of chaotic advection.

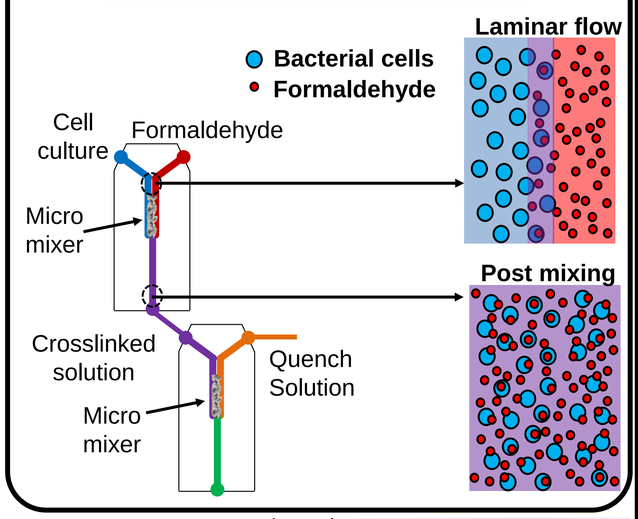
A microfluidic quench flow system

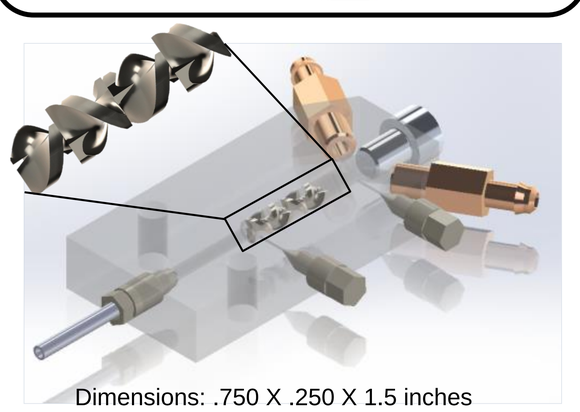
Conventional Stop flow for rapid mixing

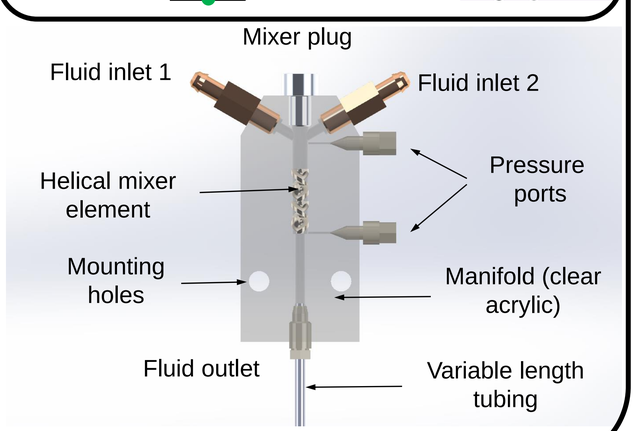
- High pressure damages cell
- Multiple time points require multiple
- Run to run variation between different time points



Micro mixers for automated ChIP

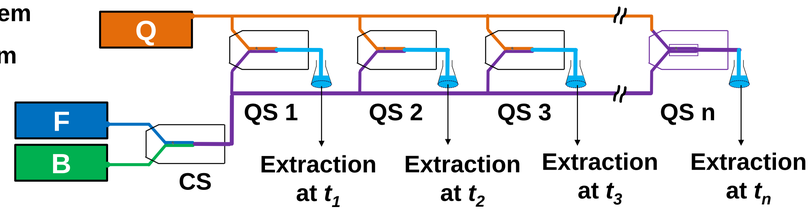




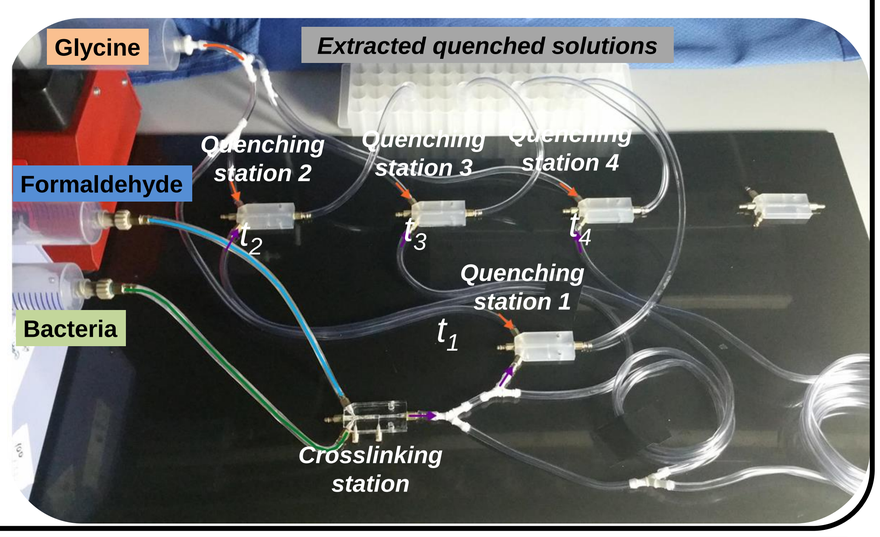


Series of micro-mixers for automated ChIP

Q : Glycine quench Pump System
F : Formaldehyde Pump System
B : Bacteria Pump System
CS : Crosslinking Station
QS : Quenching Station



- The assembled dosing apparatus consists of 1 crosslinking station for rapid crosslinking (sub second time scale) and 4 quenching stations for quenching the crosslinked mixture at different time points.
- Ability to obtain multiple time data sets from a single experiment
- Different quenching time points can be obtained by simply changing the tubing length.



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