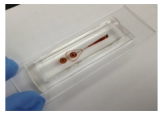
Application of Microfluidics to the Study of Single-Cell Circadian Rhythms



Sam Arsenault[1], Zhaojie Deng[2], Jonathan Arnold[3] and Leidong Mao[2]

[1]Nanotechnology and Biomedicine REU, [2]College of Engineering, [3]Department of Genetics, University of Georgia, Athens, Georgia 30602, USA

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Background & Motivation

- Circadian rhythms set the "clock" that cells live by and influence processes such as carbon metabolism and the cell cycle.
- Understanding of the clock could impact our understanding of sleeping and eating disorders, drug delivery systems, and cancer therapy.
- Traditional methods for studying circadian rhythms involved the use of fluorescence or race tubes and were only capable of seeing the rhythms in large populations of cells
- Microfluidics gives us the ability to isolate individual cells and observe their circadian rhythms without the influence of other cells.

Experimental

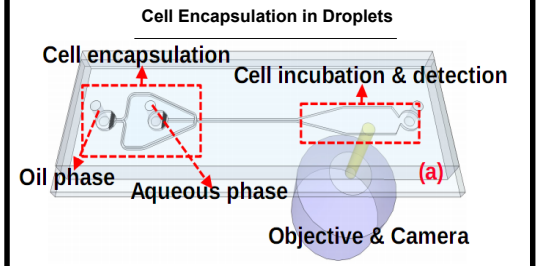
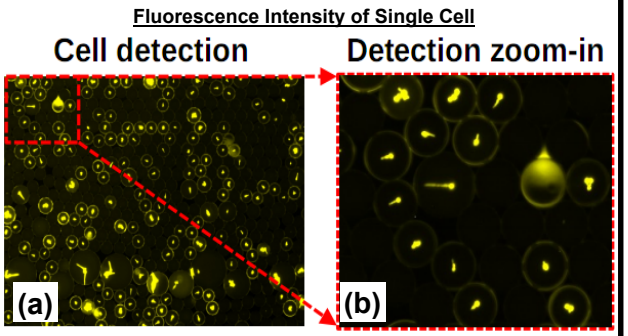


Figure (a) shows a model of the microfluidics device which we employ in order to synthesize uniformly sized droplets. Figures (b), (c), and (d) illustrate the step by step process of forming the droplets. In figure (b) the droplets are formed and in figures (c) and (d) the droplets are stored in the incubation chamber.

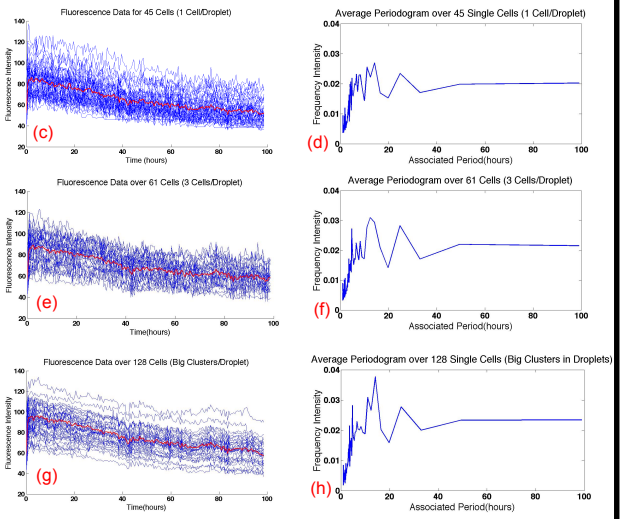
Procedure:

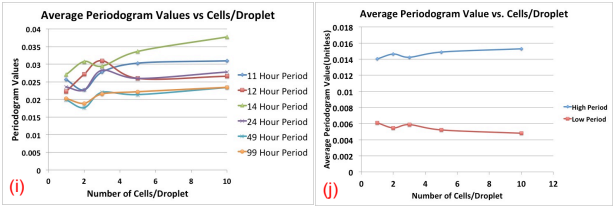
1. The device template is created using soft lithography.
2. The template is then used to create a PDMS device with the pattern embedded in the surface.
3. Oil is then injected into the oil phase port using a syringe and begins to flow through the device at a controlled rate.
4. After oil has filled the device, the cells in suspension are injected into the aqueous phase port and the flow rate is controlled as well.
5. At the junction where the oil and aqueous phases meet, droplet begin to form as seen in figure(b).
6. These droplets then travel into the incubation chamber of the device where they can stay or be removed as desired.
7. In our case, we removed the encapsulated cells and placed them into a capillary tube, sealed the tube, and then allowed the cells to stabilize.
8. The fluorescence was then captured at set time intervals using a microscope with an automatic shutter.
9. The images were then analyzed and each cell in the image was defined by a fluorescence intensity value at each time point.
10. Using the individual time series, it was possible to calculate the periodogram of each individual cell.

Results (Single Cell)



Figures (a) and (b) show examples of the fluorescence images we gather after each time interval. The fluorescent gene is linked to a clock control gene and thus can be used to track circadian rhythms.





Figures (c), (e), and (g) show the the fluorescence data for each individual cell (blue) and the average over all of the cells (red). Figures (d), (f), and (h) show the average of the periodograms of each individual cell. Figures (c) and (d) are from (1 Cell/Droplet) cells, figures (e) and (f) are from (3 Cells/ Droplet) cells, and figures (g) and (h) are from (Big Clusters/Droplet). Figure (i) shows the change in average periodogram values for various periods as there are more Cells/Droplet while figure (j) shows a more general case with the average high period periodogram values compared to the average low period values.

Results (Model)

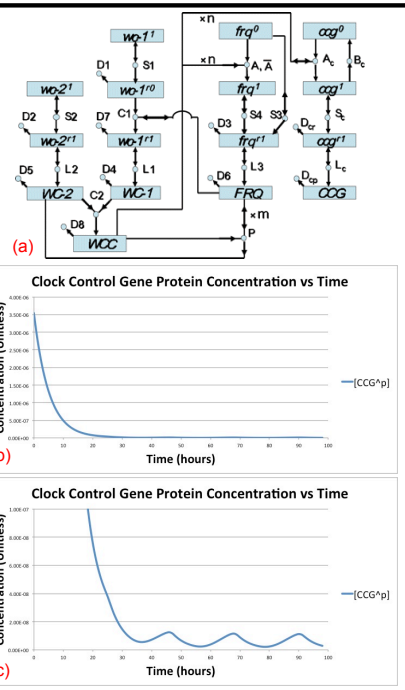


Figure (a) shows to genetic network which defines the model (Arnold 2007). For the species (blocks) in the network, superscripts of 1 or 2 indicate DNA concentrations, superscripts of r0 and r1 indicate RNA concentrations and capitalized names indicate protein concentrations. Each circle indicates a reaction and the arrows define how each reactant, product, or catalyst factors into the reaction. Figures (b) and (c) show the Concentration vs. Time graphs for the clock control gene ([CCG]). Figure (c) is zoomed in to clearly show the oscillations characteristic of the clock.

Conclusions & Future Work

- We developed a simple droplet-based microfluidic device to encapsulate and culture single cells.
- The droplets are stable in the device for tracking the fluorescence intensity of the cell over 96 hours.
- It is fast, highly efficient, low-cost and convenient for system integration.
- Using this method, we were able to observe single circadian rhythms and compare it to the circadian rhythms of cells in groups.
- The data indicates that single cells do indeed have an intrinsic rhythm but communication between cells helps to nullify some of the stochastic behavior and damping in single cell circadian rhythms.
- Investigation into this communication mechanism could yield crucial insight into how cells are able to work together synchronously.

Acknowledgements

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