MICROFLUIDIC CHAMBER DEVICE TO TEST QUORUM SENSING THEORY

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ABSTRACT

We report a microfluidic device that mimics an artificial tissue to test the theory of quorum sensing as a method for synchronization of a model fungal system, *Neurospora crassa* (*N. crassa*). High synchronicity between cells were observed by calculating the Kuramoto order parameter (K) between different fields of view.The dimensions of the microfluidic chamber allows us to also calculate an upper limit of the radius of a hypothesized quorum sensing signal by using the diffusion approximation for signal travelling within the device.

KEYWORDS: Quorum Sensing, Cell Analysis, Communication

INTRODUCTION

Collective behavior is seen in a variety of biological organizations, varying from the collective attack of viruses on bacterial cells to troops of baboons. This may lead to the synchronized oscillations that includes either fire flies or cells being able to synchronize their time clocks. In order to understand how cells synchronize, we hypothesize that *N.crassa* cells are able to communicate through the mechanism of quorum sensing[1]. This is also tested with a quorum sensing model[2].When the density of cells reach a certain threshold, the single *N. crassa* cells will then be able to secrete a signaling molecule to produce a coordinated response. Here, we fabricated a microfluidic device to mimic a tissue in order to obtain a macroscopic limit for phase synchronization to occur in *N. crassa*.

EXPERIMENTAL

The microfluidic device consists of one inlet and one outlet for sample loading, it had dimensions with a width,(W) of 1150µm and a length, (L) of 1800µm with a height (H) of 10µm as seen in Figure 1(A).The strain *ccg-2P:mCherry* with a *clock-controlled gene-2 Promoter* region (*ccg-2P*) fused in frame with an mCherry recorder was used for fluorescence imaging. To ensure that cells were not growing, the media chosen contained sorbose to ensure that the cells would not grow without control. Cells were loaded into the microfluidic device from one end at a concentration of 6.88×10^7 cells/mL as seen in Figure 1(B) before placing under a LED light for two hours. Imaging with a CCD camera through a microscope was then done for a time period of ten days. After obtaining the fluorescence intensities, Cell Profiler was used to track the fluorescence intensity of single cells. mCherry beads (Takara Bio) were placed in a separate microfluidic chamber on the same device as a control. Results were obtained by normalizing the fluorescence intensity obtained from the cells with beads, following a twenty four hour detrending method to obtain Figure 1(C).

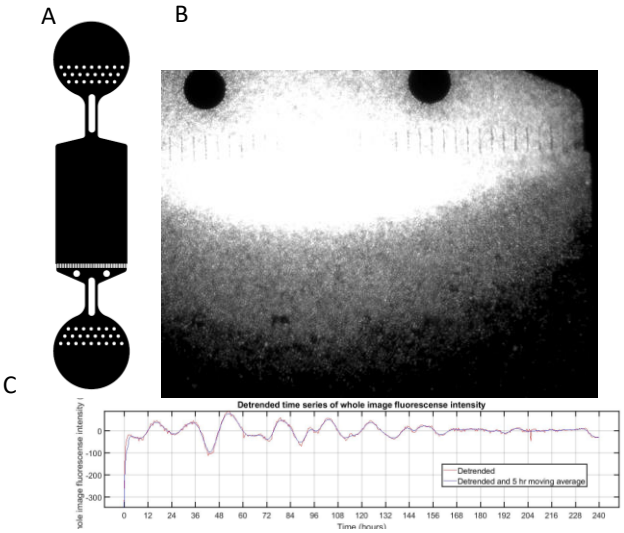

Figure 1 : (A) Schematic of the microfluidic device to trap cells. (B) Image captured of cells densely packed in the device prepared for fluorescence imaging. (C) Detrended average fluorescence intensity of single cells trapped with the microfluidic device.

RESULTS AND DISCUSSION

From Figure 1(C) , we were able to observe synchronicity between cells when they were imaged over a period of 10 days. The synchronicity was further confirmed by obtaining five different field of views and comparing the trajectories of it as seen in Figure 2(A). We can observe from Figure 2(B) that the trajectories of each field of view aligns with other. From these results, we were able to proceed with obtaining an estimate on the upper limit of the physical size of the possible communication signal. This was achieved by using the Stokes-Einstein Equation, and

we were able to obtain an estimation of 13.05nm. The synchronization measure known as the Kuramoto order parameter (K) :

$$K = \langle \left| n^{-1} \sum_{j=1}^{n} exp(iM_j) - \langle n^{-1} \sum_{j=1}^{n} exp(iM_j) \rangle \right| \rangle \quad (1)$$

where the brackets denote an expectation over time and M_j is the phase of the jth giant cell. The quantity n is the number of oscillators being compared (e.g., n=2 for two fields of view). When the fields of view were perfectly synchronized, the K value would be 1.00 while unsynchronized areas would give us 0.00. The synchronization measure (K) observed between any two fields of view was over 0.97 across this artificial tissue.

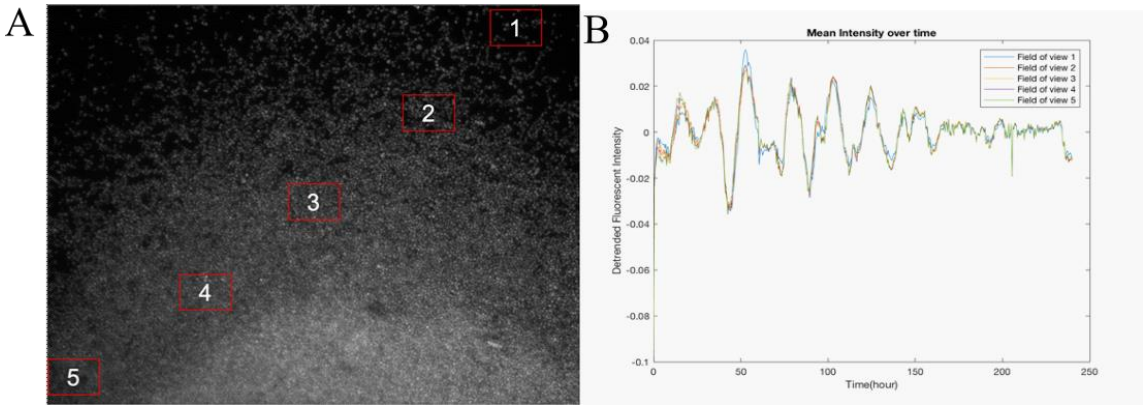

Figure 2: (A) Five different field of view's were selected to observe their respective mean fluorescence intensity as well as calculating the Kuramoto value. (B) Detrended fluorescence intensity of each field of view.

Table 1. Kuramoto value between field of views

K	FOV 1	FOV2	FOV3	FOV4
FOV1	0.9894	-	-	-
FOV2	0.9895	0.9954	-	-
FOV3	0.9820	0.9926	0.9821	-
FOV4	0.9778	0.9892	0.9893	0.9939
FOV5	0.9894	-	-	-

CONCLUSION

We fabricated a microfluidic chamber device to examine the full range of phase synchronization to test the quorum sensing theory. This device was able to fit around 150,000 cells to examine the limits on synchronization. We were also able to observe a high synchronicity between cells in the microfluidic device.

ACKNOWLEDGEMENTS

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REFERENCES

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