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Analysis of the Spatio-Temporal Dynamics of Infection

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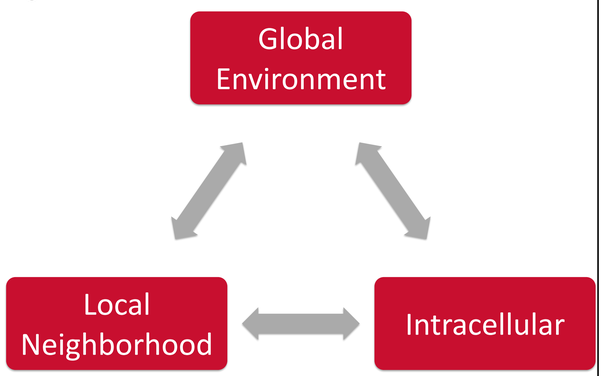
Introduction

- Spatio-temporal dynamics are vital in understanding the course of infection, particularly for infections that lead to the formation of granulomas such as Mycobacterium tuberculosis [1] which significantly impact the course of infection.
- In *in vitro* studies, the observable data is gathered at the global environment level (a single well), but this lacks the correlation and relationship between an individual cell, its local neighborhood and its global environment .



- Traditional 2D models of infection allow for easily replication and rapid sampling but, devoid of an extracellular matrix (ECM) are unable to fully replicate the spatial dynamics of an *in vivo* system.
- *In vivo* models, while providing multi-cellular response and spatial dynamics do not allow the freedom of sampling granted *in vitro*.
- We aim to develop corresponding in vitro and in silico platforms to adequately capture and analyze the multidimensional nature of immune response to infection.
- By connecting the *in vitro* and *in silico* platforms with confocal imaging, we are able to observe, quantify, and correlate cellular behaviors on all levels and determine the characterizes that lead to different outcomes of infection.

References

[1] N. Kapoor, et al. PLoS One, vol. 8, no. 1, Jan. 2013.
[2] G. Y. Lee Nat. Methods, vol. 4, no. 4, pp. 359–365, Apr. 2007.
[3] A. Colom J. Biomed Mater.Res.A, v102, pp. 2776–2784, Aug. 2014.
[4] M. E. Gough et al. Innate Immun, vol. 23, no. 6, pp. 506–523, Aug. 2017.
[5]] D. Birant Data Knowl. Eng., vol. 60, no. 1, pp. 208–221, Jan. 2007.
[6] Z. Zhang European Cells and Materials Vol. 22 pp. 344-358 2011
[7] M. Sheppard MATLAB, Fit all probability distributions to data Apr. 2012

Acknowledgements

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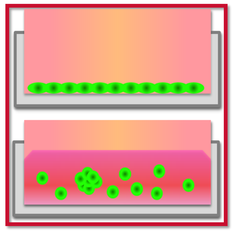
Methods



In Vitro Model

- 2D [4] and 3D [2] infection studies were preformed using GFP tagged *RAW 264.7* murine macrophages and mcherry tagged *M. smegmatis.*
- Supernatant samples were collected every 24 hours and CFUs enumerated for 72 hours.

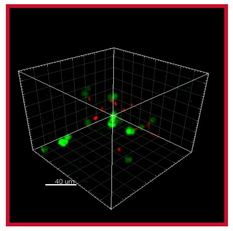


Image Acquisition

- In parallel, we utilized confocal scanning laser microscopy (Olympus/Fluoview) equipped with a stage-top incubator (Tokai Hit) to conduct multi-area time-lapse imaging of multiple experimental conditions.

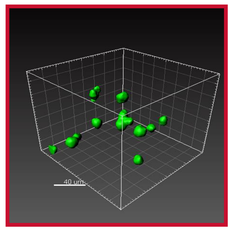
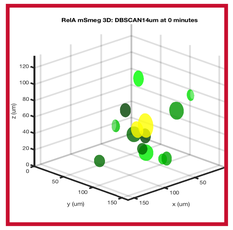


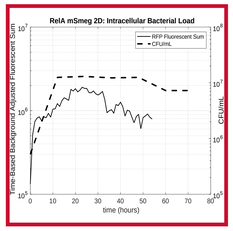
Image Analysis

- Images were rendered and analyzed using Imaris 8.1.2 (Bitplane) with surface creation and tracking.
- The same image processing parameters were used for all conditions.



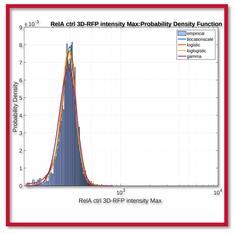
DBSCAN Clustering

- In order to determine cluster formation analysis of each time point was performed using DBSCAN algorithms [5].
- This allows us to determine when clusters form and what conditions of the cells, the system, and the local neighborhood lead to their formation.



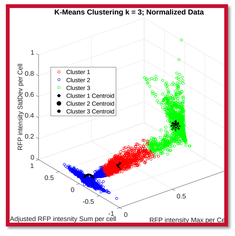
Background Subtraction

- We established a time-based noise reduction algorithm to calculate the average background noise for each time point to remove noise from all objects based on their volume.
- This can then be used to correlate in vitro CFU analysis with *in silico* fluorescent intensity.



Data Analysis

- Kruskal Wallis and rank-sum tests were run on all conditions with non normally distributed data.
- Utilizing allfitdist [7] we are able to quantify probability distribution functions for each given data-set to inform predictive infection models

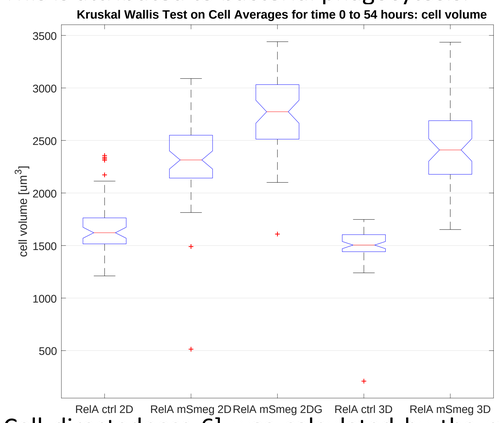


Machine Learning

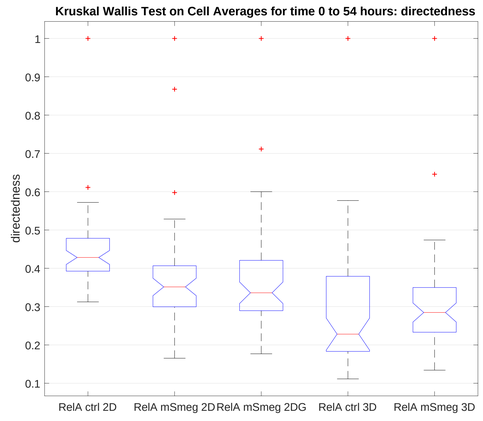
- Preliminary unsupervised learning techniques have been used to segment the heterogeneous population of infected cells into those that contain bacteria (under infected condition, actively infected), and those that do not (under infection condition, not actively infected)

Results

- Cell volume showed significant differences between most conditions, with a tendency towards higher volume under infection.
- This is attributed to bacterial phagocytosis.



- Cell directedness 6] was calculated by the ratio of the position of a cell to its original point over total distance traveled.
- The results show lower directedness (more random movement) in 3D than 2D.
- Studies using primary cells may provide results more comparable to *in vivo* response.



Conclusion

- The platform is able to quantify and characterize the dynamics of infection in 3D and correlate spatial response to bacterial load.
- *In vitro* 3D host-pathogen studies can provide novel insight regarding the role of the physiological environment and cell mobilization on response.
- The *in silico* platform analysis allows for implementation of further statistical analysis and quantification including machine learning applications that can inform predictive 3D models of bacterial infection.