The importance of using multi-scale and multi-organ approaches to understand host-pathogen dynamics in TB

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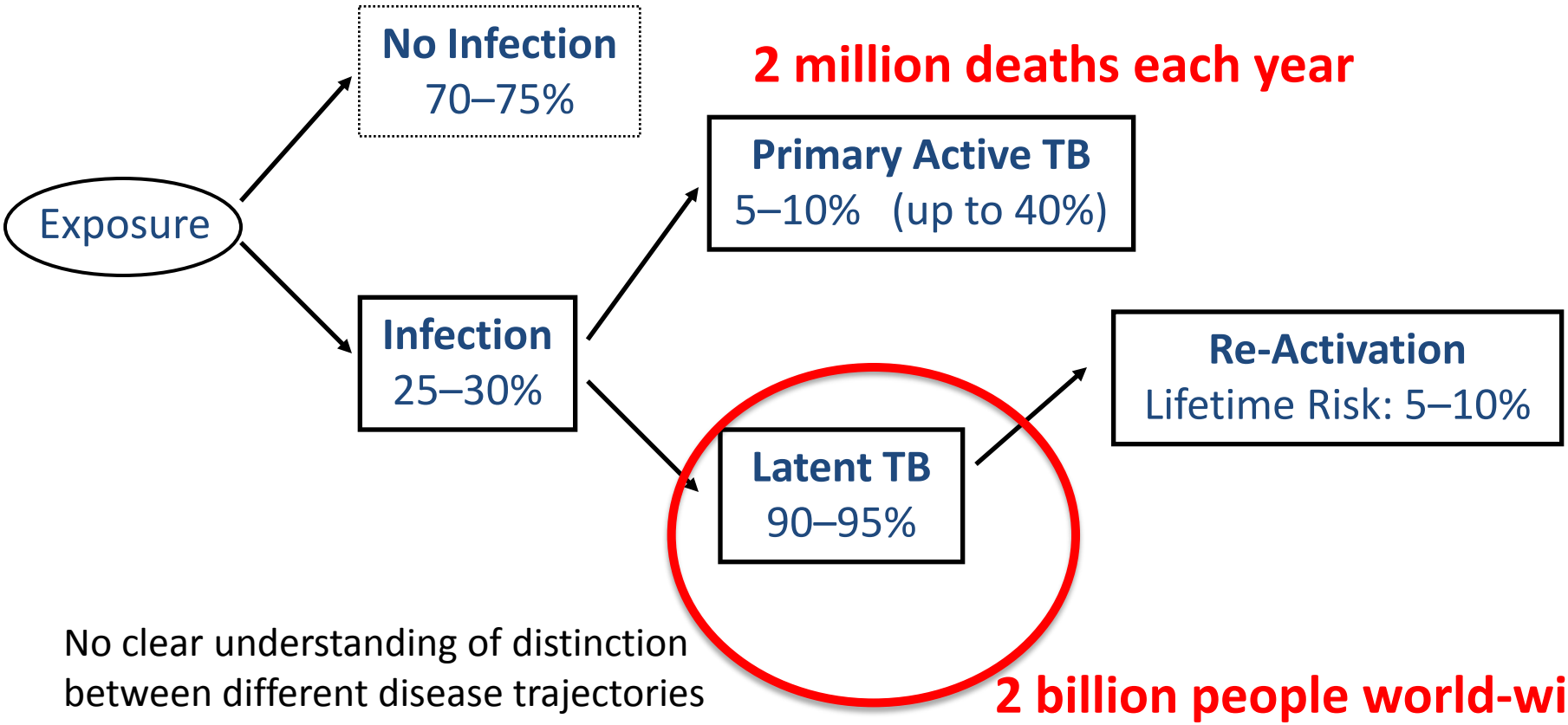
NIH support funding this work

- **Systems Biology** (PIs: Kirschner, Linderman, Flynn, Kunkel)
- R33HL092853, “A multi-scale and multi-system approach to understand granuloma formation in *Mycobacterium tuberculosis*”, 7/15/2008 – 5/30/2012
- RENEWAL submitted (A1 status)

- **MSM**-(PIs Kirschner, Linderman, Flynn)
- **2**R01EB012579-04A1 “Multi-scale methods to predict outcomes of immuno-modulation and drug therapy during TB” 8/2011-8/2016**.**
- **MSM** (completed): R01 LM 10020661: “A multi-scale approach to understanding antigen presentation in human immunity” 9/05-8/09

Infection Outcomes Associated with Exposure to the bacteria *M. tuberculosis*



Flynn Lab: Non-human primate model of tuberculosis

Cynomolgus macaque

+ M. tb Erdman
25 cfu
via bronchoscope

100%

2-6 weeks

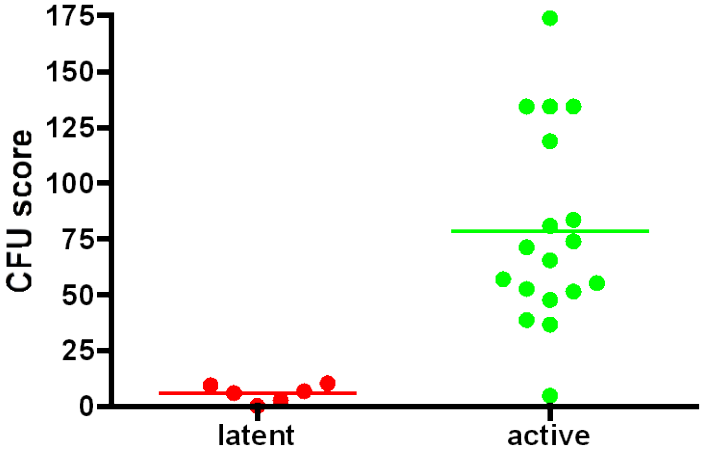
Tuberculin Skin Test +
Other immunologic tests +

6-8 months

ACTIVE TB (43%)

Positive Chest x-ray
Mycobacterial culture
- + GA or BAL culture

Clinical signs
- weight loss,
- appetite loss
- cough

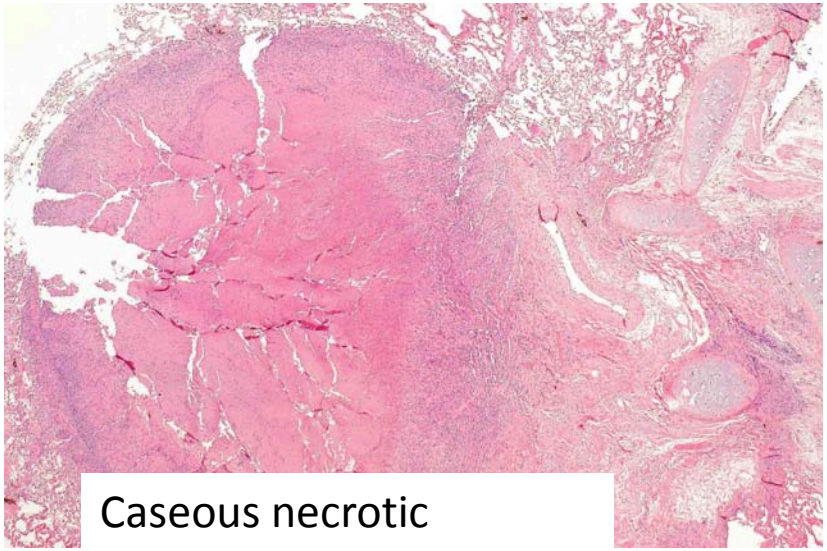


LATENT TB (52%)

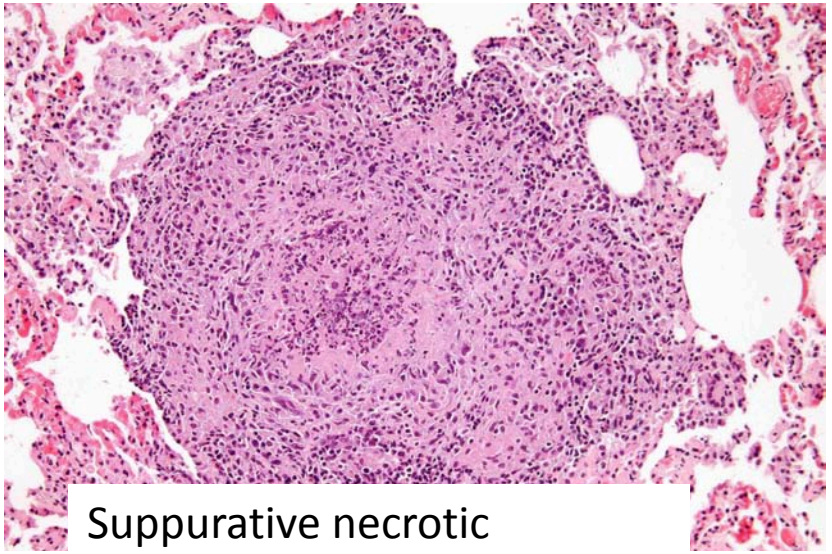
No signs of disease
CXR negative by 2 months
Mycobacterial culture
negative after 2 months
Clinical signs--none

Lin, et al IAI 2009

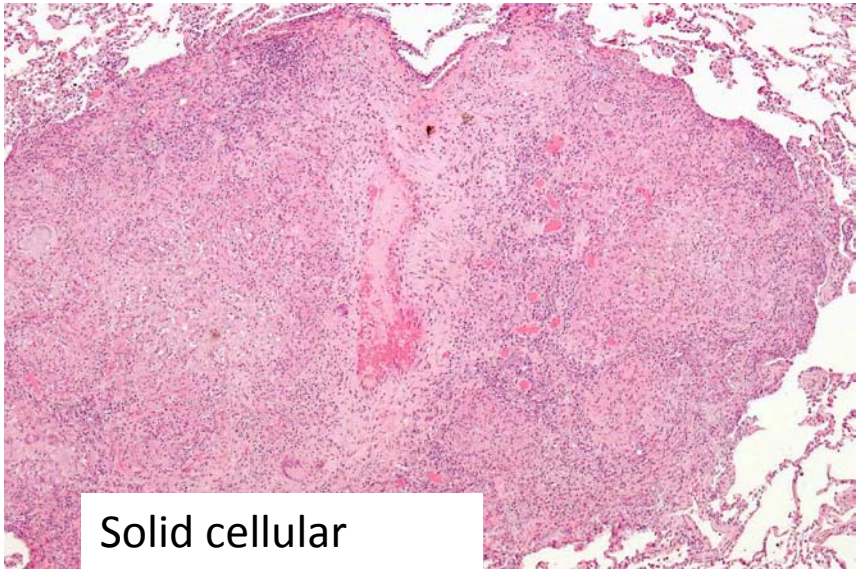
Spectrum of granulomas in macaques: Immune and bacterial microenvironments



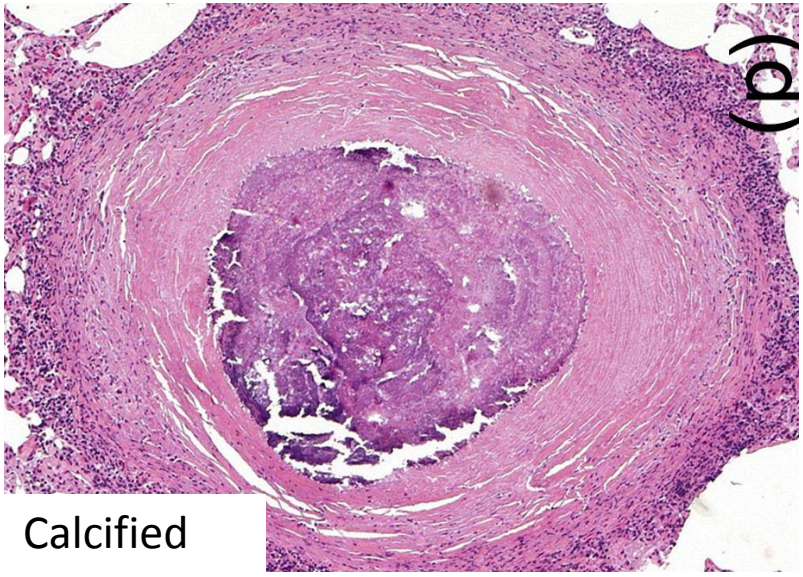
Caseous necrotic



Suppurative necrotic

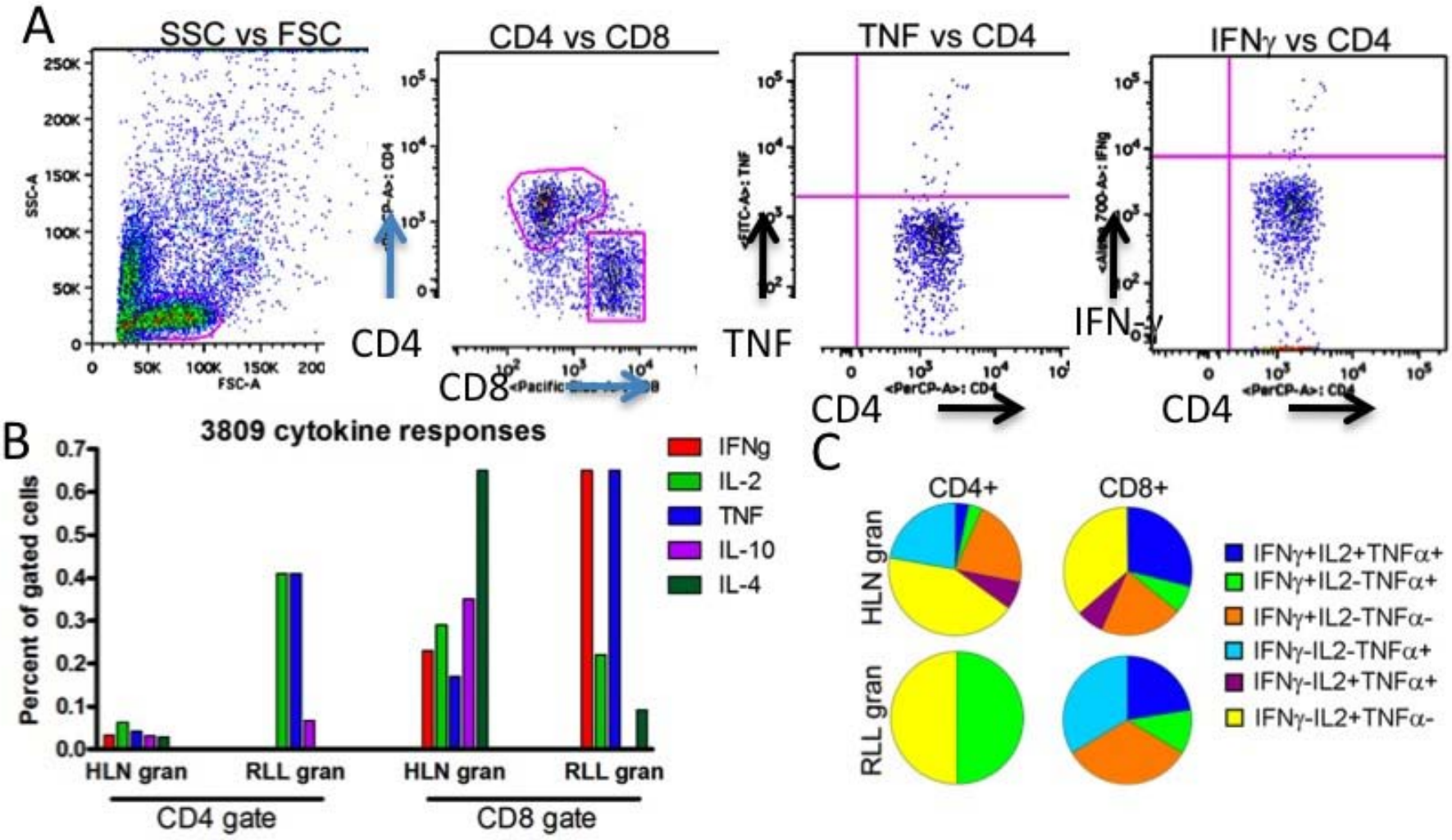


Solid cellular



Calcified

From granulomas at necropsy:
obtain data on cell types, cytokines, chemokines
in different granulomas at a specific time point

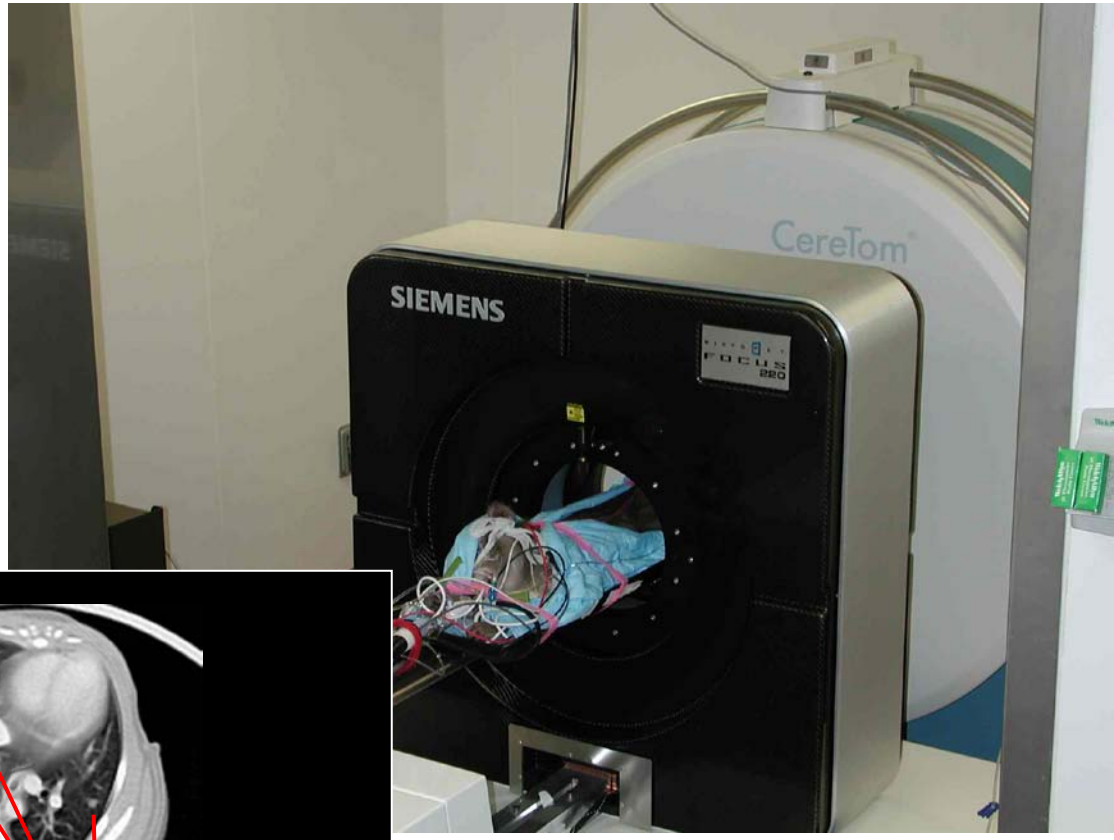


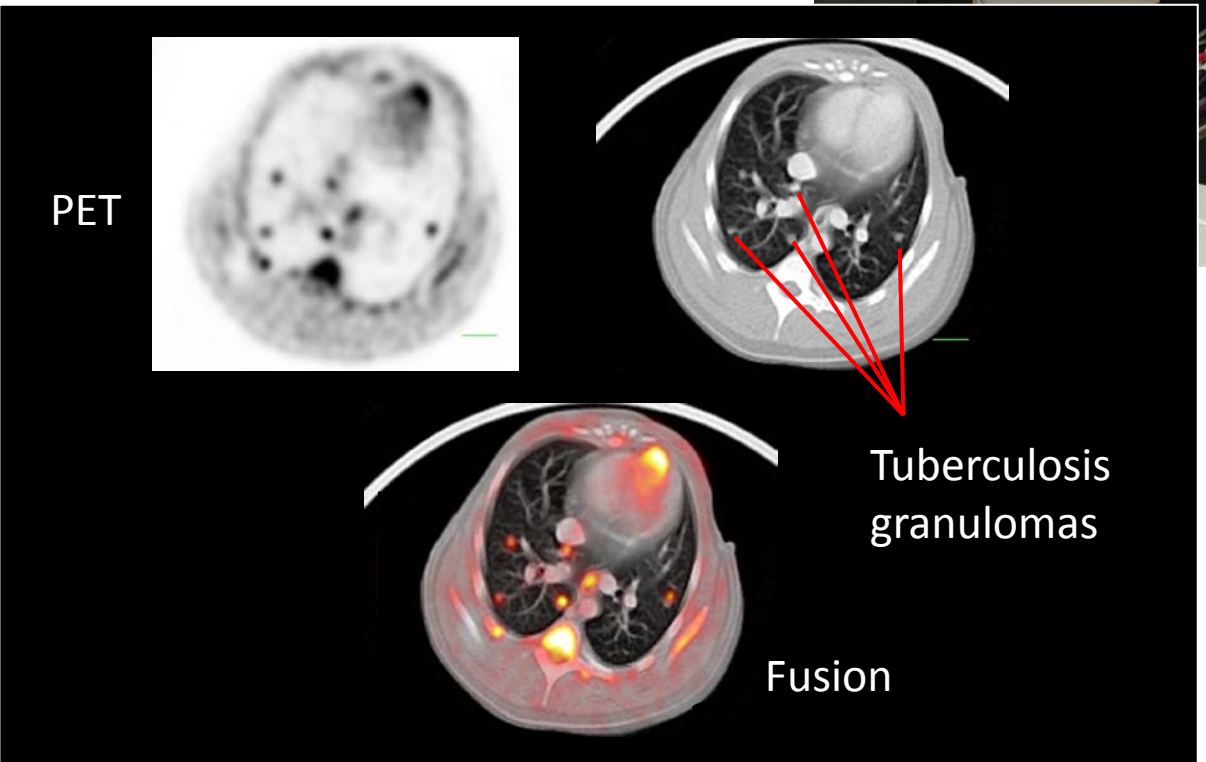
Flow cytometry, intracellular cytokine staining, boolean gating, SPICE analysis

PET/CT: Imaging modality for serial tracking of lesions and disease

CT: structural map of lesions in organs

PET: functional map of lesions in organs

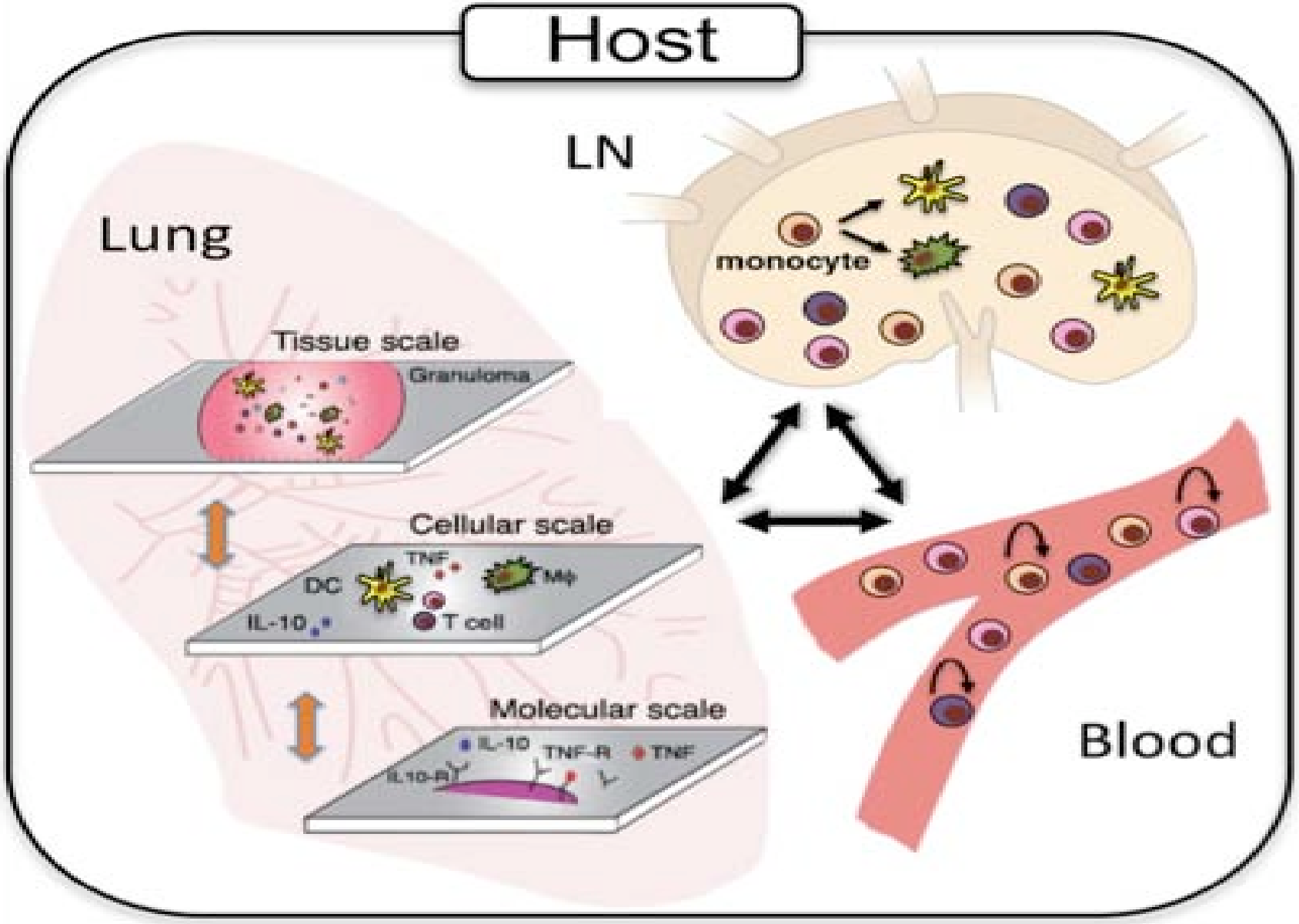




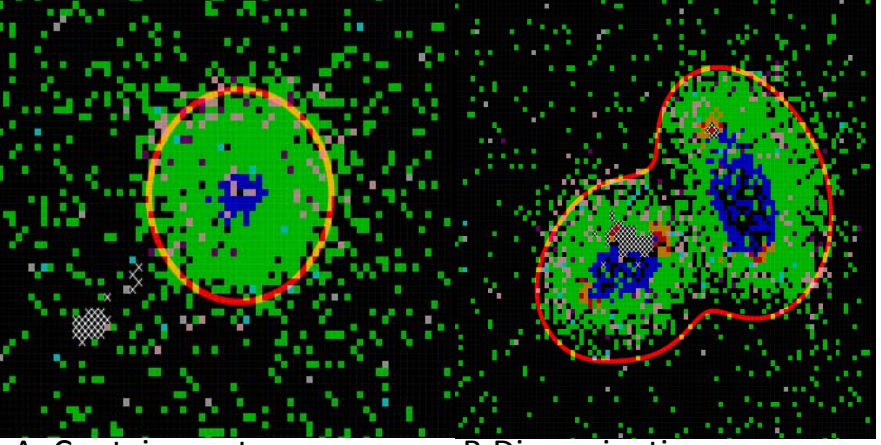
BSL3 imaging suite
Regional Biocontainment Lab (RBL), Flynn Lab
University of Pittsburgh

Understanding dynamics at the level of a granuloma using systems biology

- To this end, necessary to build/refine a model of granuloma forming in lungs of infected primates –integrate data
- Add relevant organs/compartments that feed into and out of the lung-integrate data
- Add relevant scales (genetic, molecular, cellular, tissue)
- Build and validate model on NHP and other data
- Test model against different data
- Perform analysis, make predictions

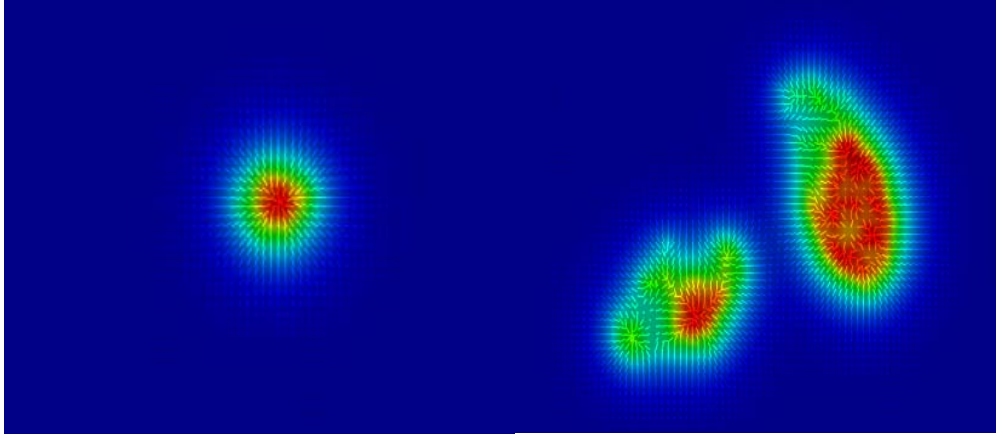


We apply tuneable resolution to toggle between detail and none as appropriate



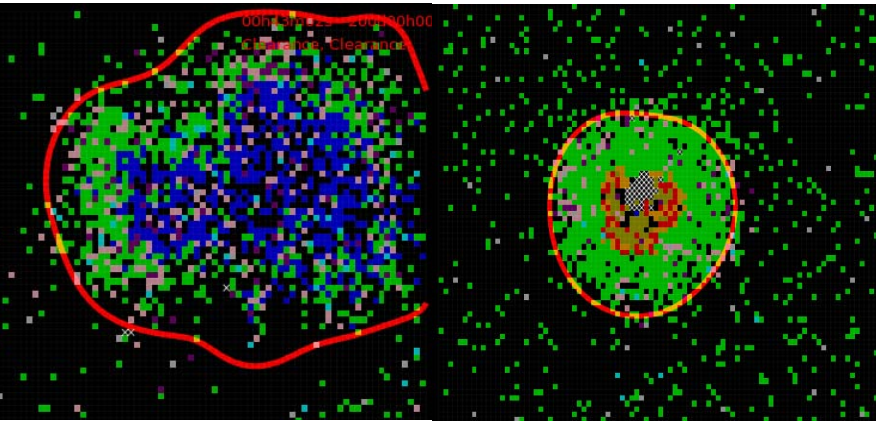
A: Containment

B.Disseminating



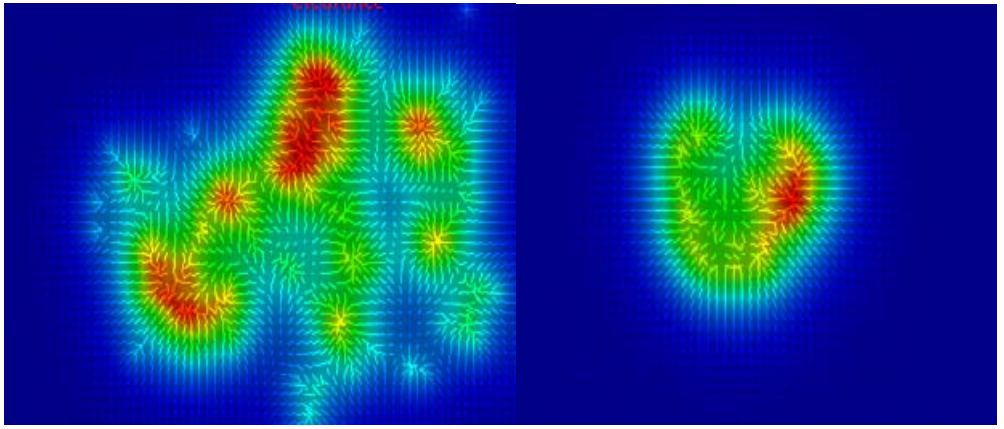
A

B



C: Clearance with inflammation

D: uncontrolled growth

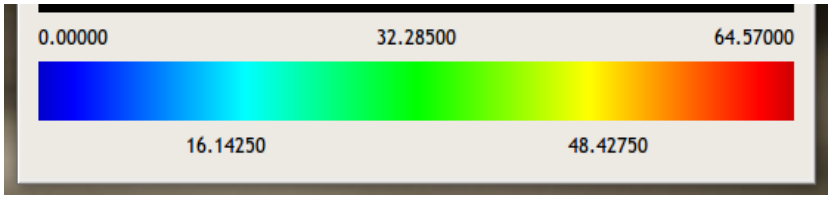


C

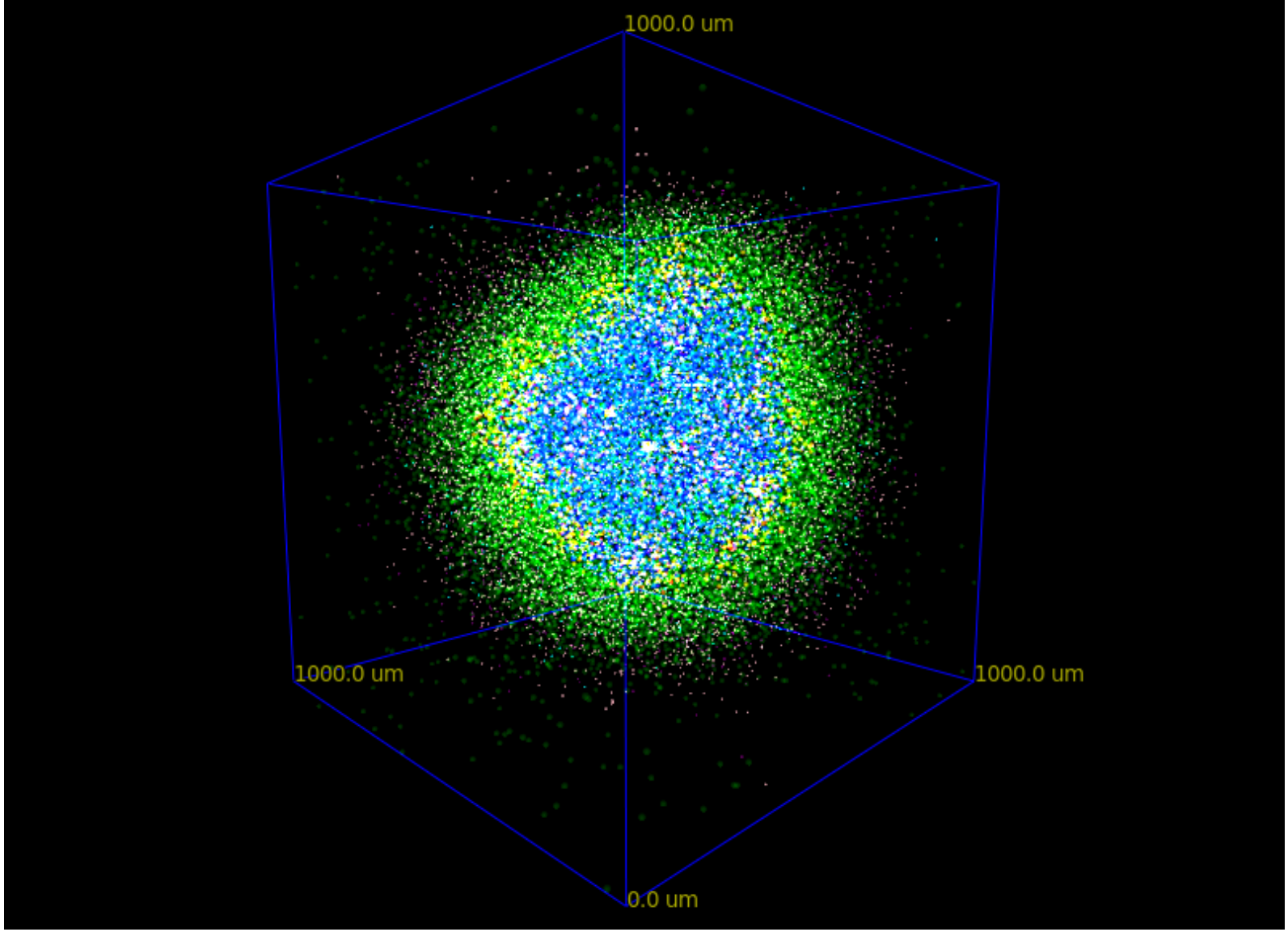
D

	Be	Bi
A:	0	10
B:	232	437
C:	0	0
D:	1930	950

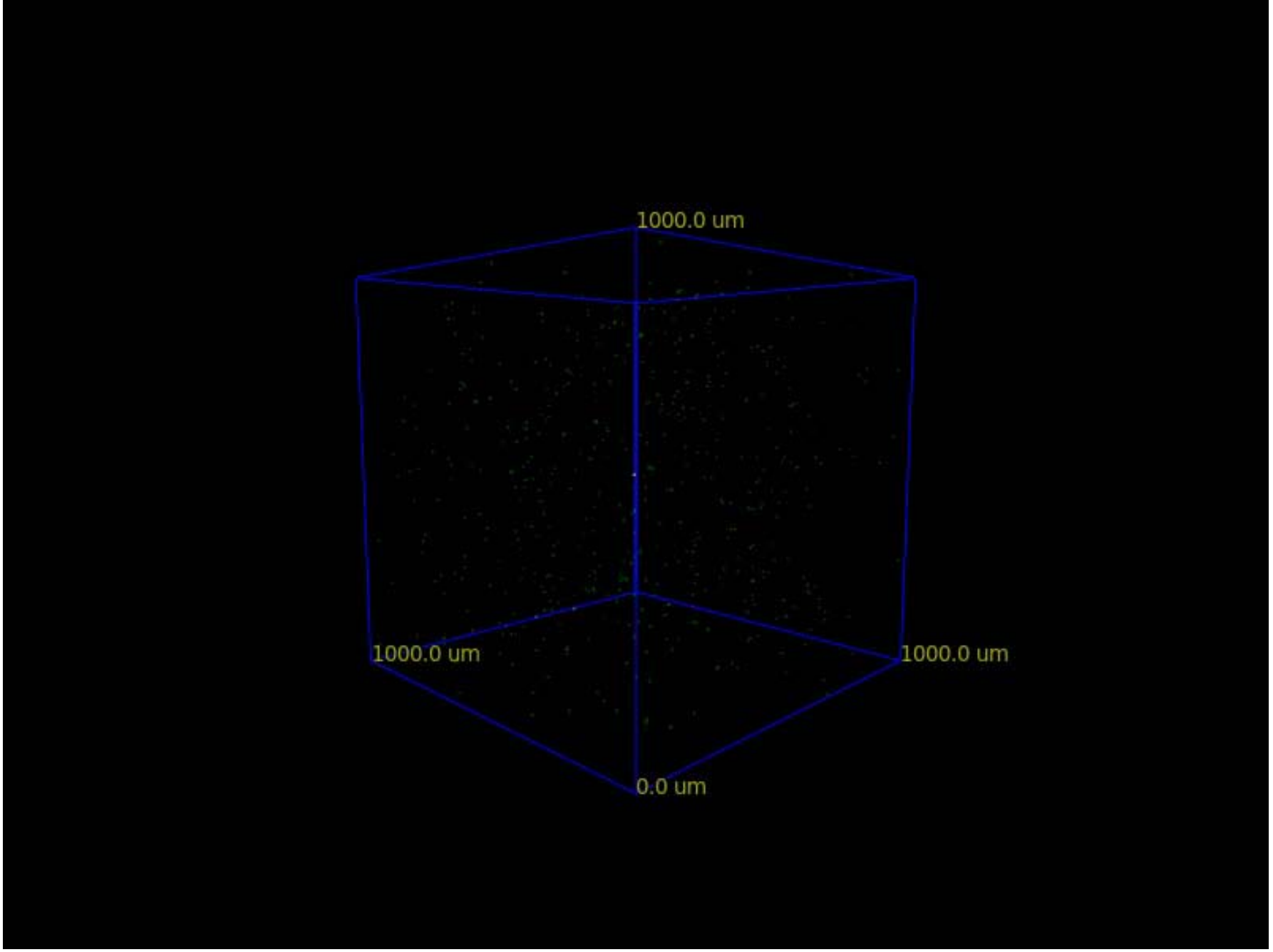
Concentration of TNF scale



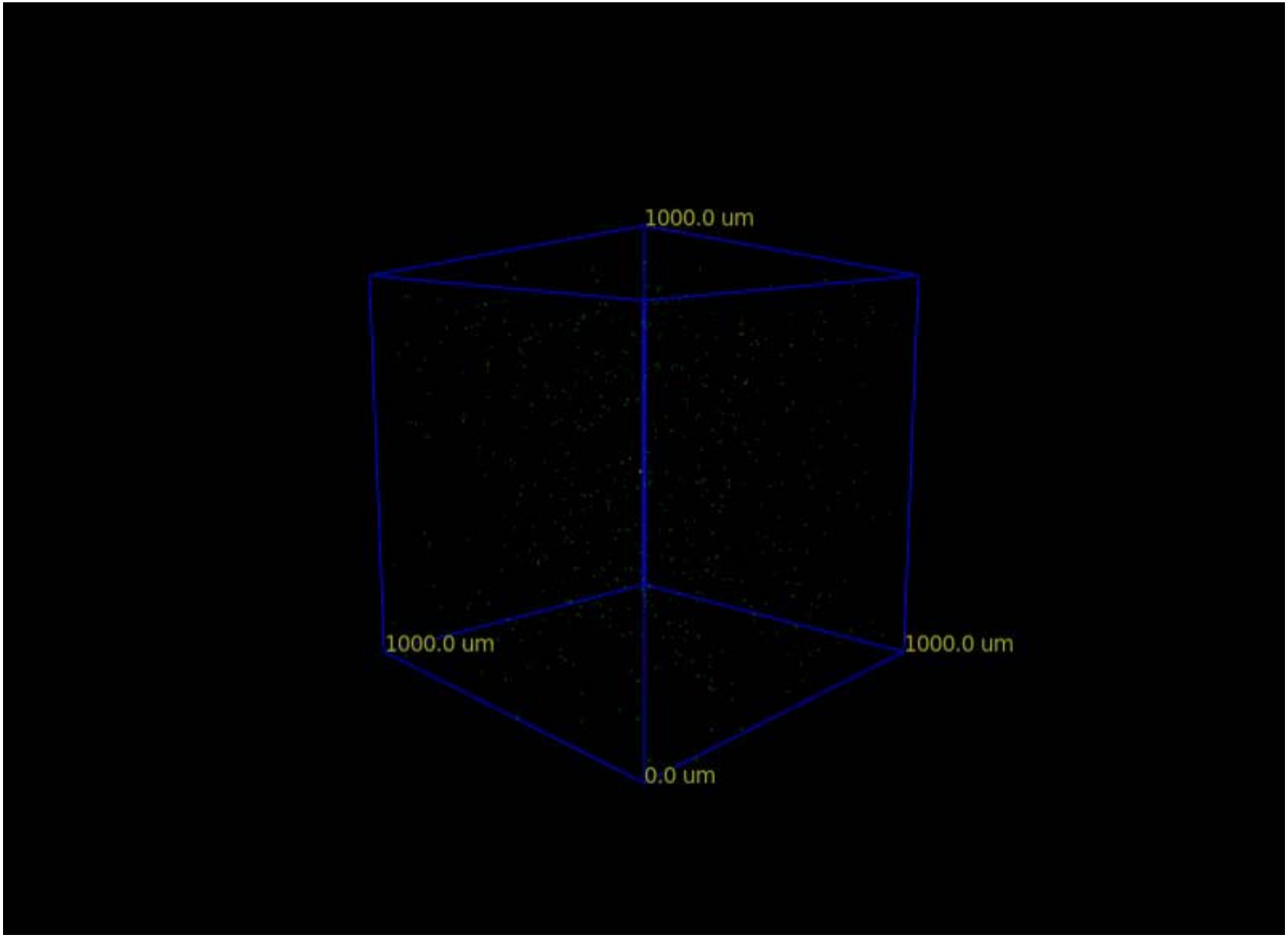
*Gray hashed areas are caseous



Snapshot of a 3D ABM simulation of a granuloma at 200 post-infection. Shown is a 'transparent front-end' granuloma. We can see inside the granuloma because we can control the transparency level of different cell types from a transparency level of 0 (opaque) to 100 (invisible). The colors correspond to cells as follows: Resting MΦ (green), activated MΦ (blue), infected MΦ (orange), chronically infected MΦ (red), Treg (teal), Tgamma (pink), Tcyt(purple). Necrotic regions



It takes 60 hours to generate one 200 day simulation in the right dimension of tissue (2 mm x 2mm x 2mm)

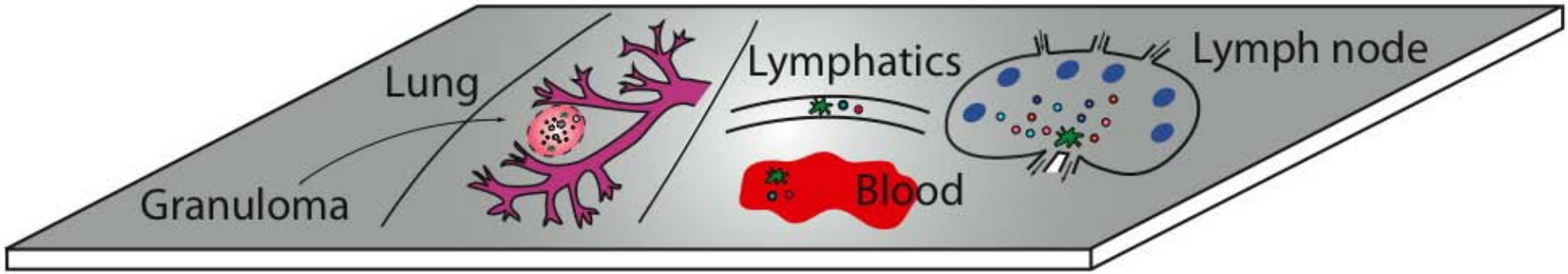

1000.0 um
1000.0 um
1000.0 um
0.0 um

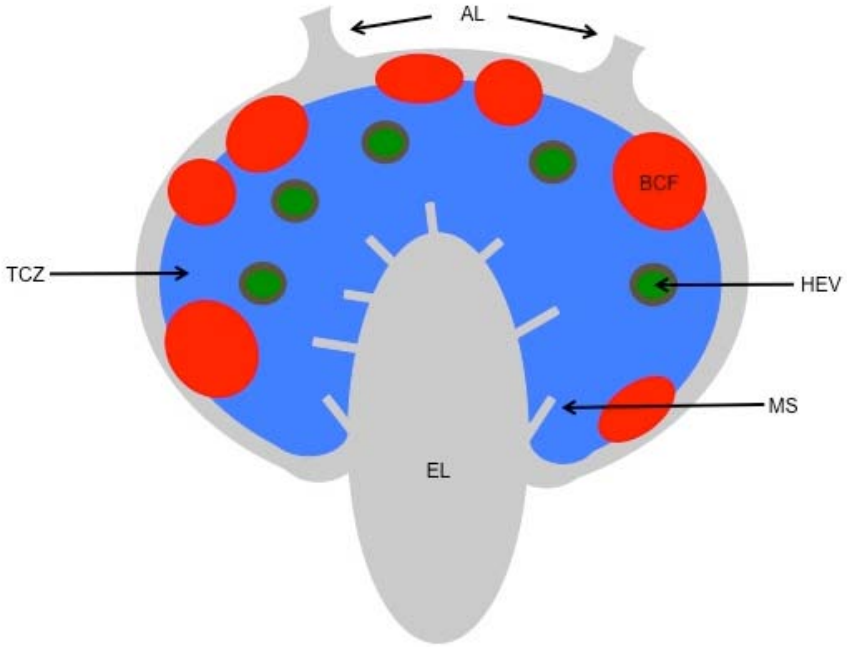
Parameter estimates and analysis: how do we begin to distinguish mechanisms driving observed differences?

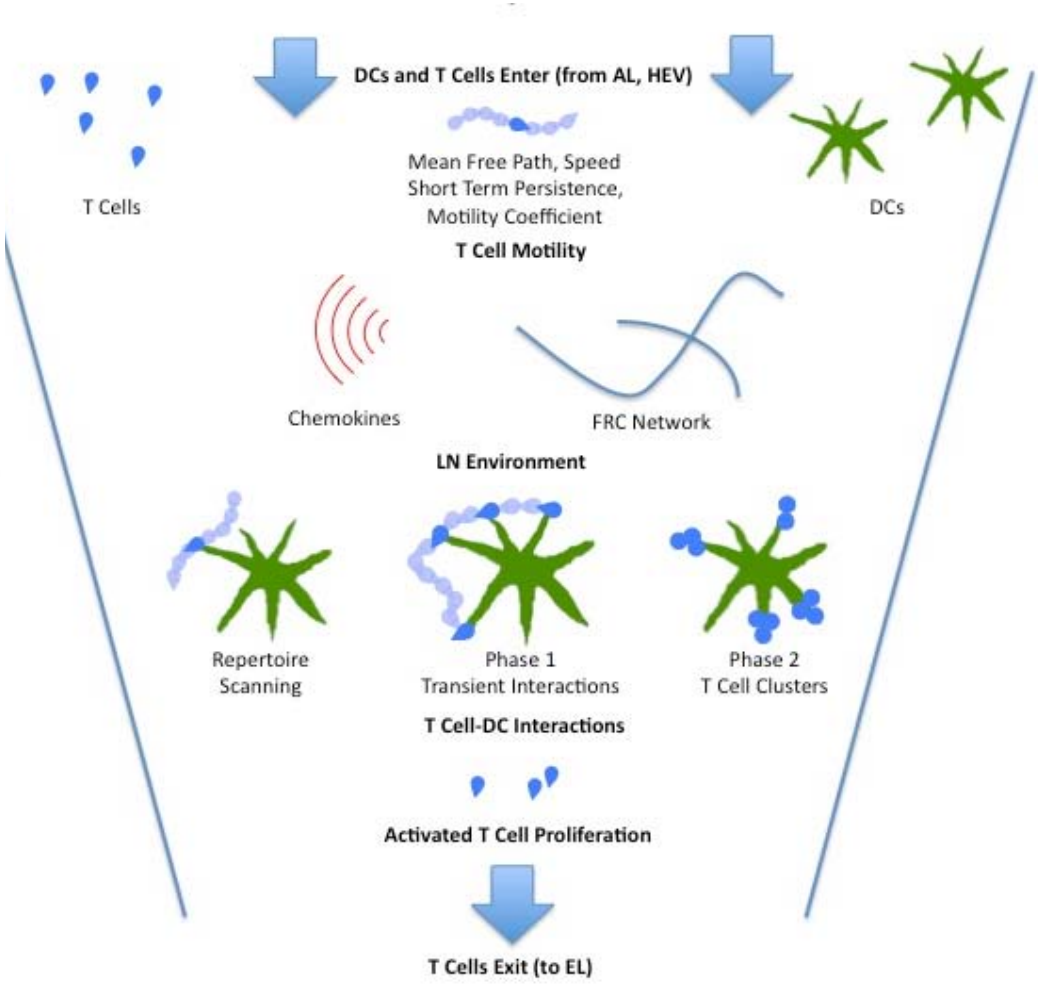
- **Uncertainty Analysis - determines how much variability in outcome is induced by variability in parameter values**
  Latin Hypercube Sampling (LHS)
- **Sensitivity Analysis– measures which parameters induce this variability and ranks the correlations** ***Partial Rank Correlation (PRC)***
- ***KEY FEATURE - can quantify relationships over time***
- ***Simeone Marino, Ian B. Hogue, Christian J. Ray, Denise E. Kirschner. A Methodology For Performing Global Uncertainty and Sensitivity Analysis in Systems Biology Journal of Theoretical Biology, Vol 254, pp 178-196, 2008, NIHMS ID #68202.***

(programs available on our website)

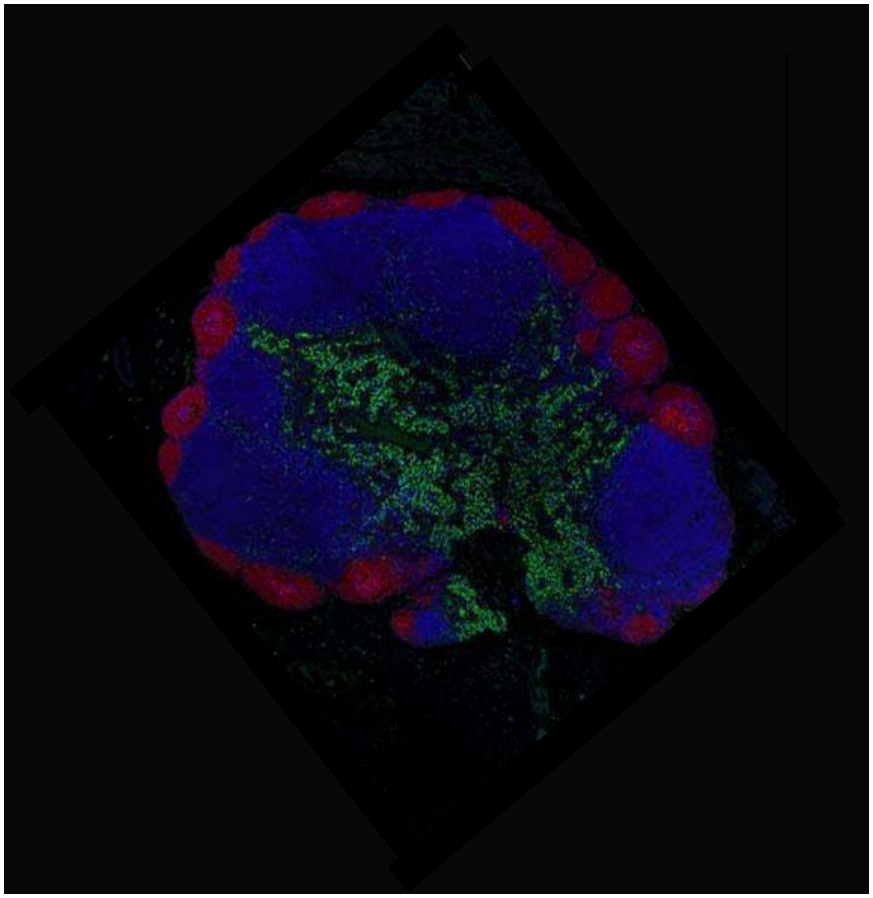
Understanding granulomas is a Multi-scale problem in both space and time: multi-organ scale



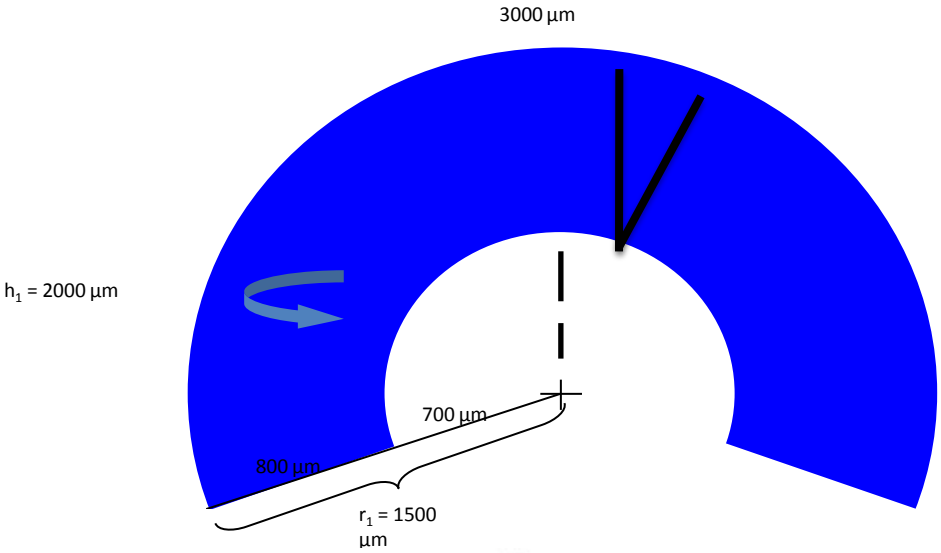


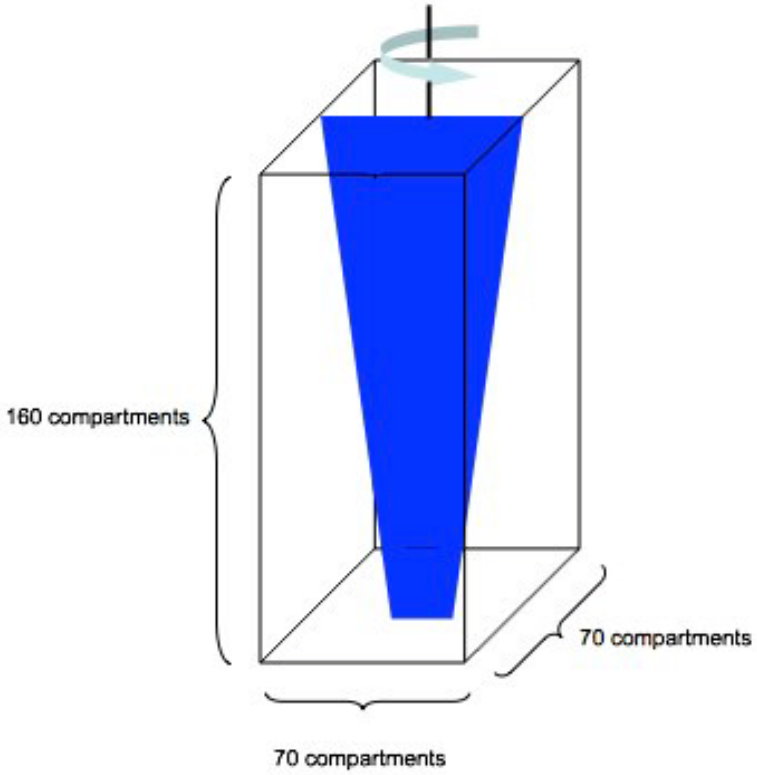


Mark Miller, Wash U Collaborator

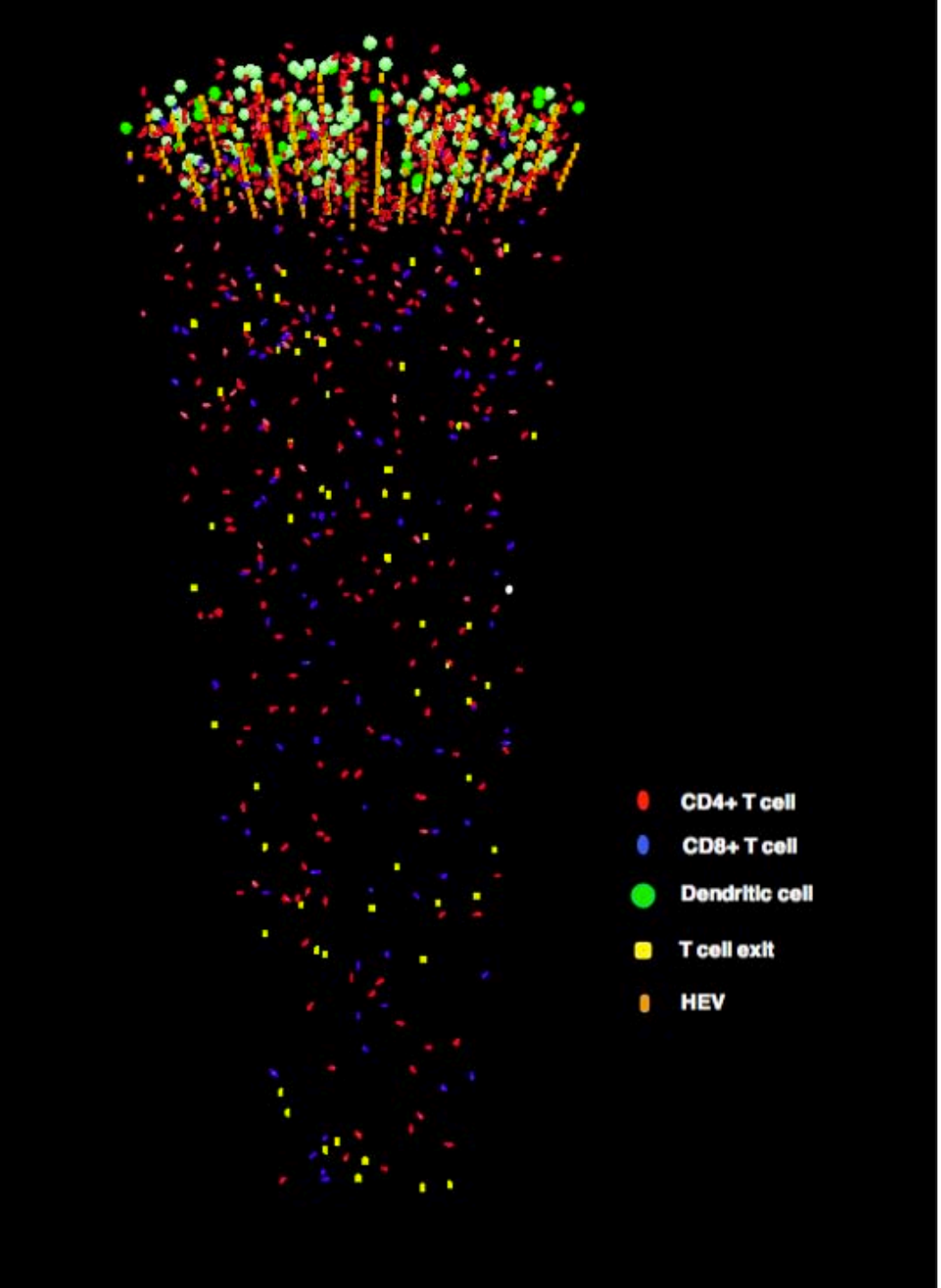


LN image of a NHP by Flynn Lab





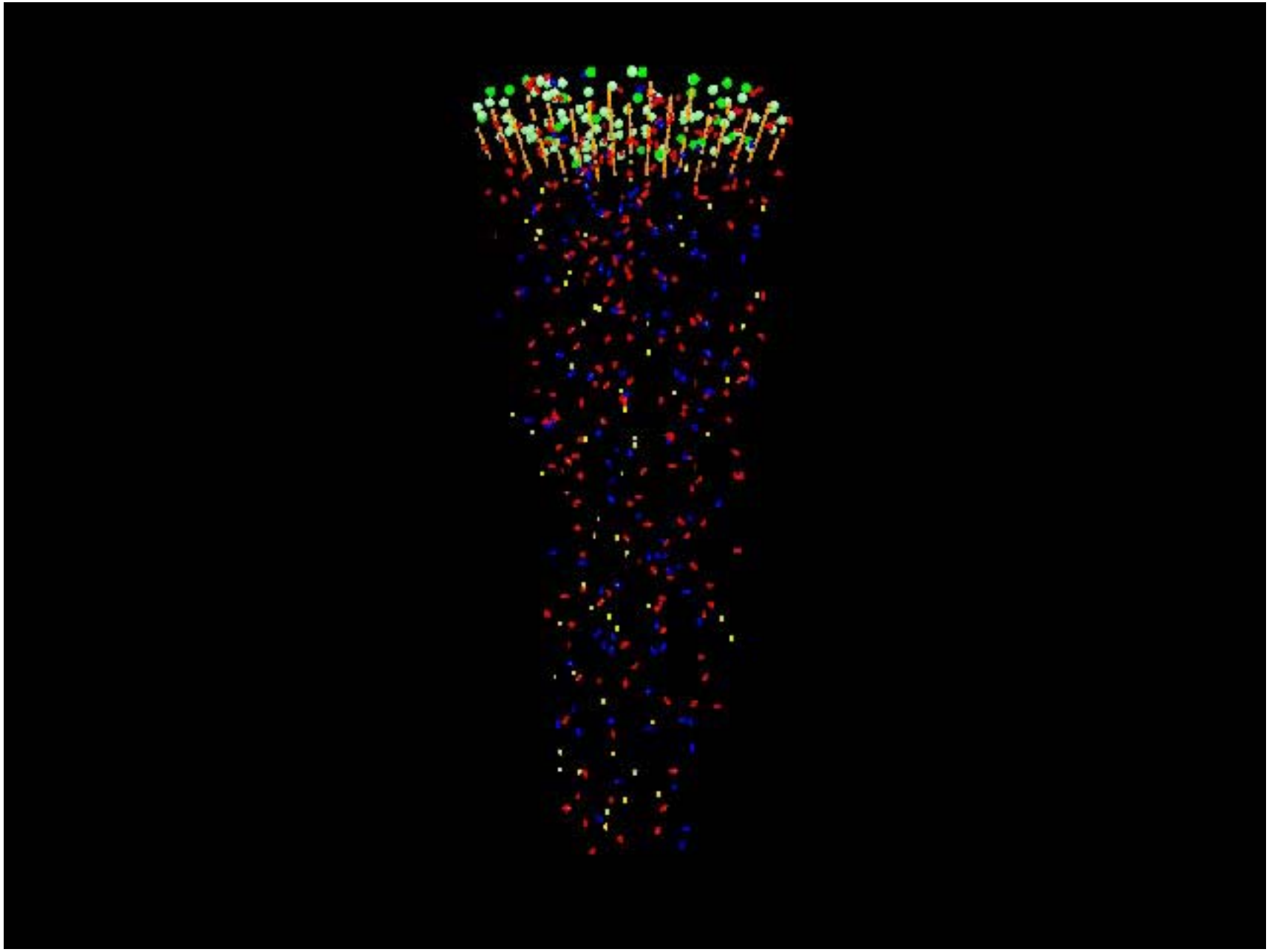
Conical simulation area

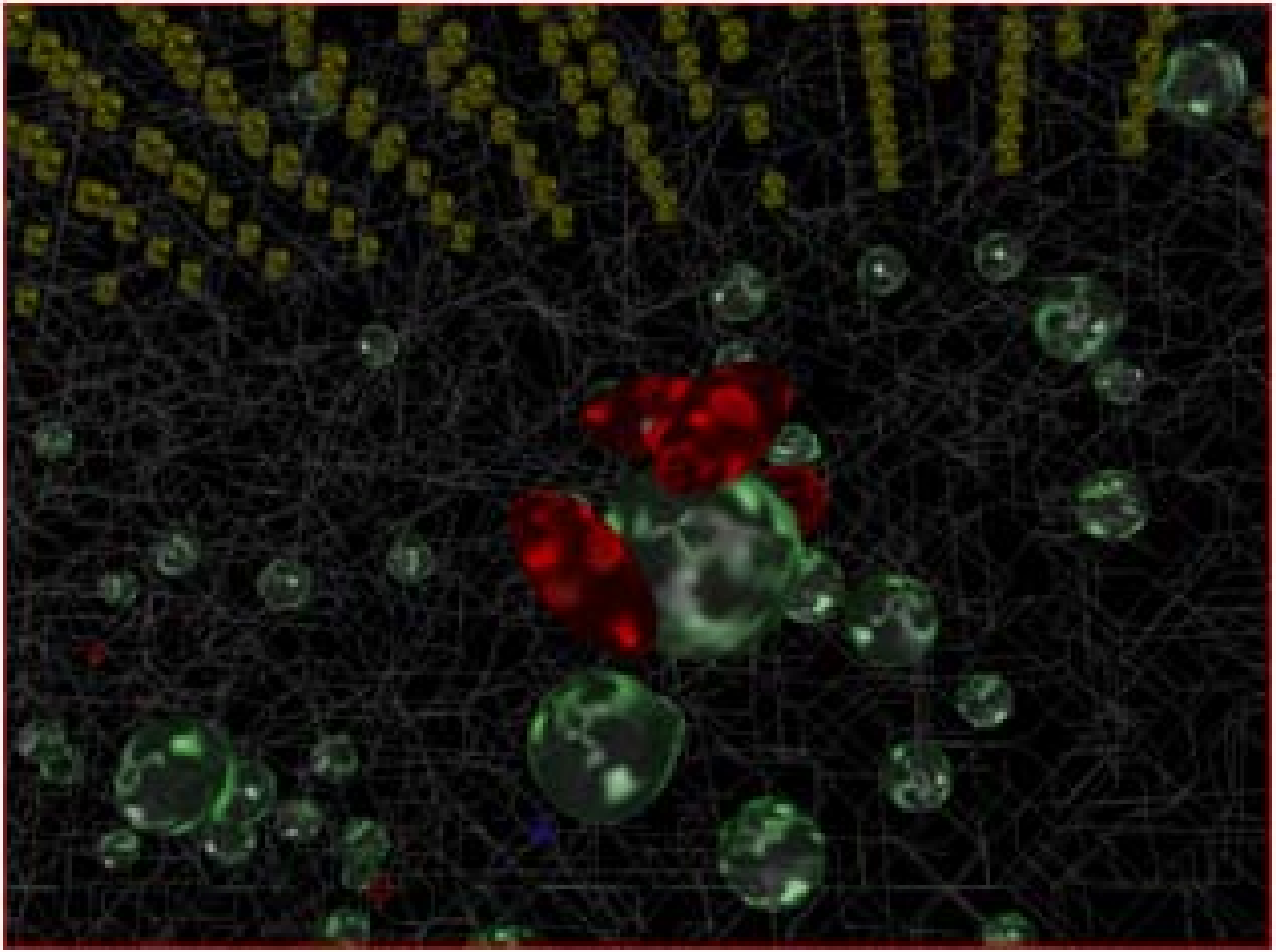


3D ABM Design for LN

CD4+T cells (active, effector)
CD8+ (active, effector)
DCs (naïve, antigen-bearing and licensed)
HEV, AL, EL—simulate acute infection
500 DCs
Simulated over 7 days

Need to optimize size of cone to be sure We are capturing all relevant dynamics Without having to simulate the entire T zone, which is computationally prohibitive



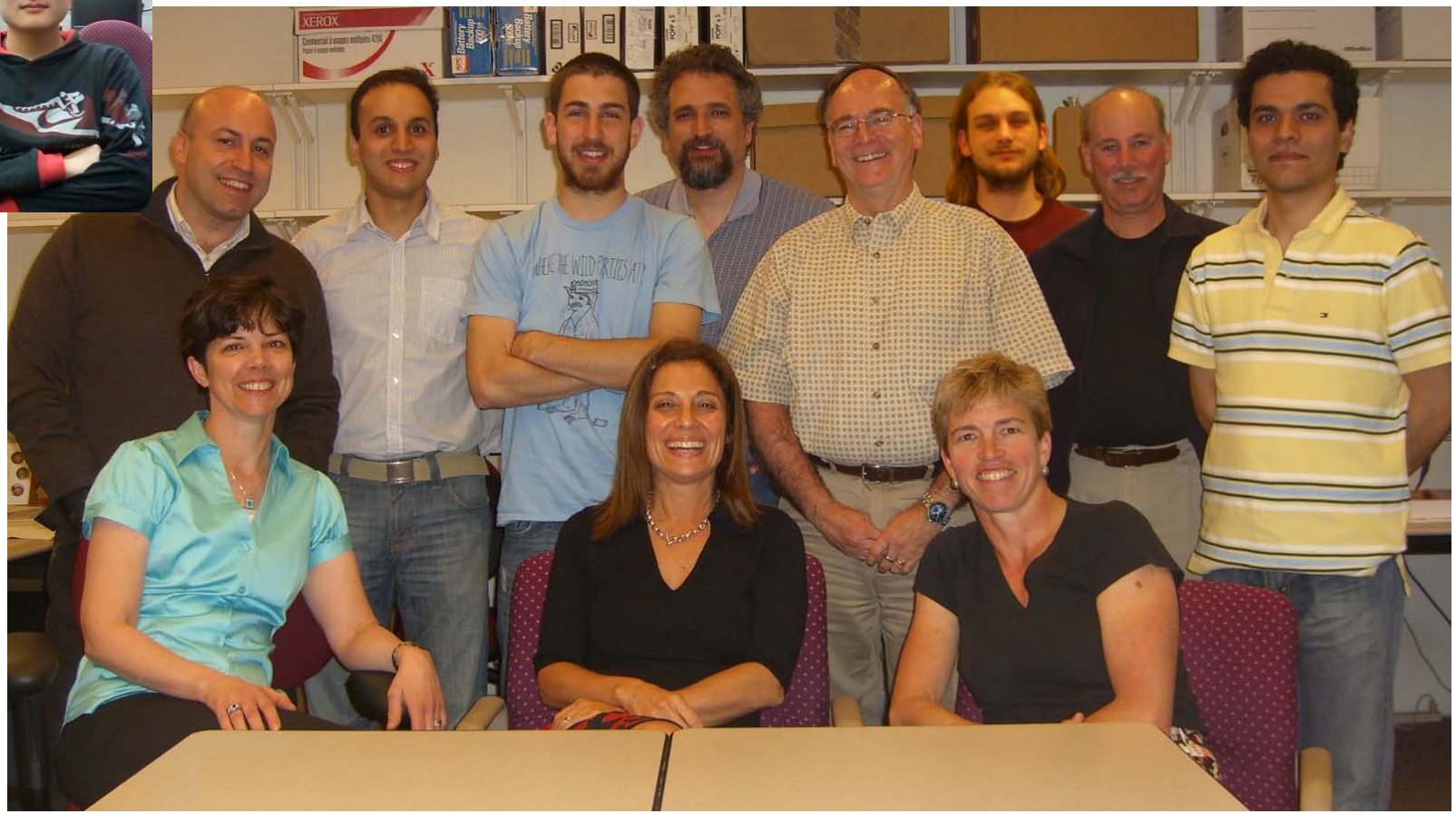


Fibroblastic Reticuluar Cell Network, red football T cell, DC is green sphere

Summary of points for Theme 3

- We build single scale models of a variety of types and link them in different ways
  - Link multi-scale, Link multi-organ
- We use tuneable resolution to turn on and off at will the different scales and organs
- We developed and use a detailed uncertainty and sensitivity analysis for determining parameters and indentifying model features
- We are currently focusing on 2-D versus 3-D representations of the ABMs

Acknowledgments



- **Collaborators: Jennifer Linderman, (Univ of Mich)**
- **JoAnne Flynn (Pitt)**
- **Steve Kunkel, (Univ of Mich)**

Website: http://malthus.micro.med.umich.edu/