Quantitative Comparison of Microscope Setups for Lipid Droplet Identification and Characterisation

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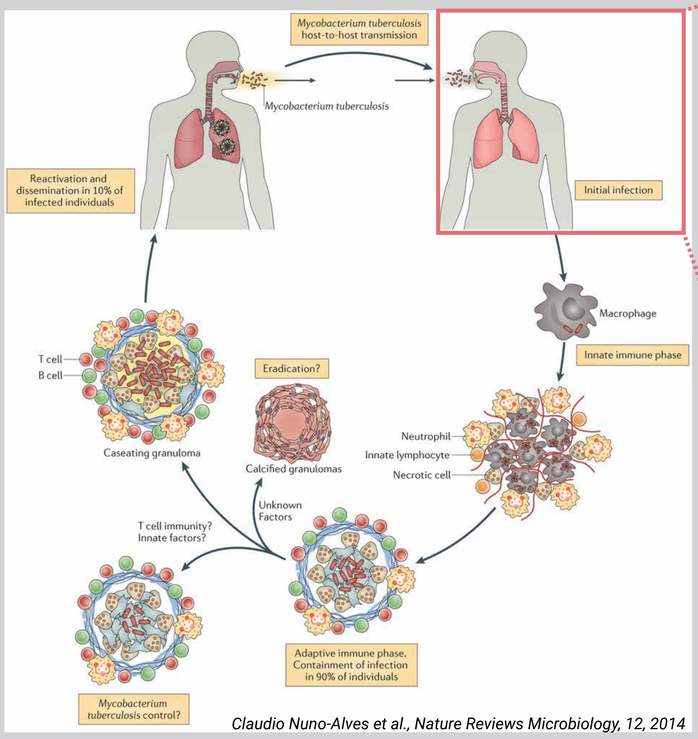
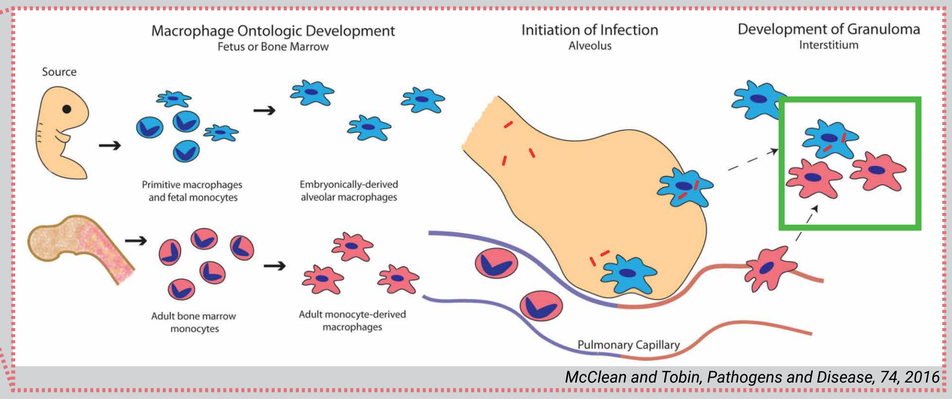
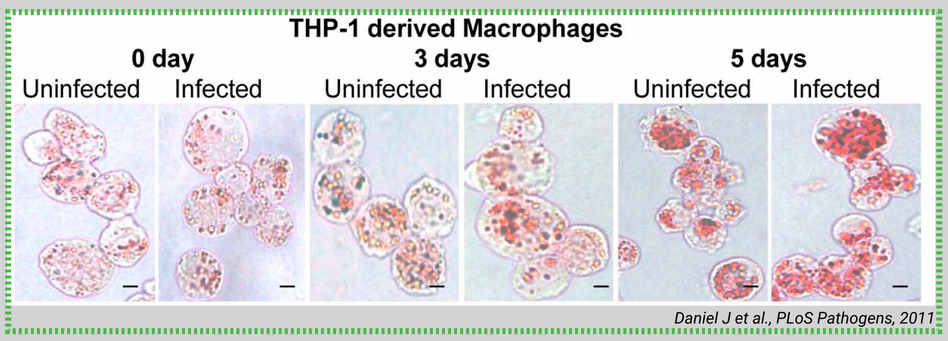
Lipid droplets are highly dynamic cellular organelles known to be involved in the regulation of intracellular lipid metabolism and lipid storage essential for membrane formation and maintenance (Bozza, PT and Viola, JP; 2010). Pathogens such as *Mycobacterium tuberculosis* are known to exploit lipid droplets present in macrophages in order to propagate their infectious cycle (McClean, CM and Tobin, DM; 2016). In order to further understand the dynamic behaviour of lipid droplets and how it is affected by pathogenic agents, the need for an automated imaging assay for lipid droplet identification and characterisation has arisen.



Claudio Nuno-Alves et al., Nature Reviews Microbiology, 12, 2014

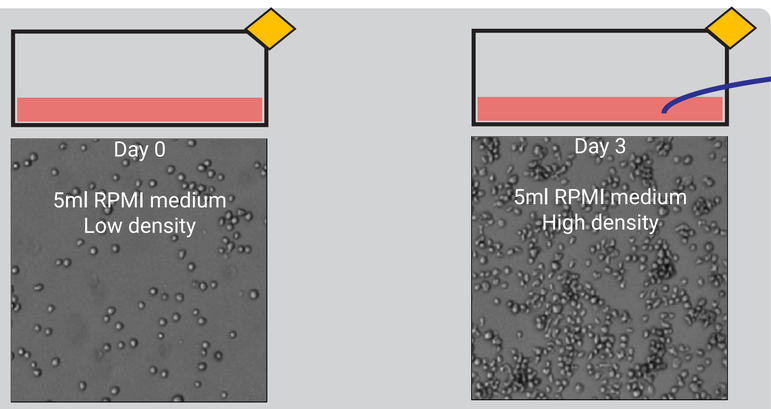


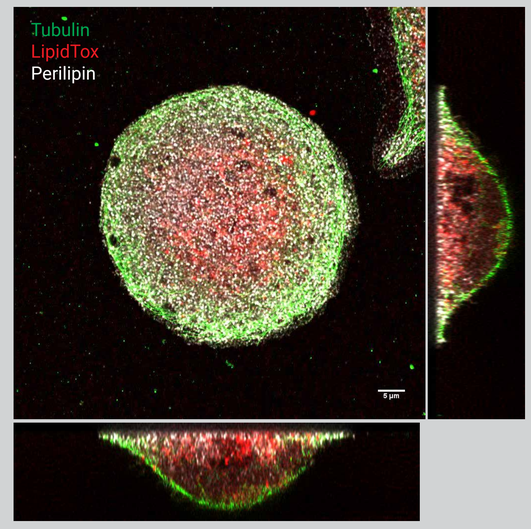
McClean and Tobin, Pathogens and Disease, 74, 2016



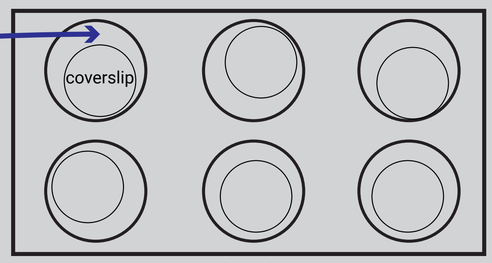
Daniel J et al., PLoS Pathogens, 2011

Tuberculosis Pathogenesis (left) Infection is initiated by the inhalation of aerosol droplets that contain bacteria. The initial stages of infection are characterized by innate immune responses that involve the recruitment of inflammatory cells to the lung (pink box). Following bacterial dissemination to the draining lymph node, dendritic cell presentation of bacterial antigens leads to T cell priming and triggers an expansion of antigen-specific T cells, which are recruited to the lung (pink closeup). Upon infection with *Mycobacterium tuberculosis*, macrophages accumulate lipid droplets as shown with a staining against Oil Red-O (green closeup). The recruitment of T cells, B cells, activated macrophages and other leukocytes leads to the establishment of granulomas, which can contain *Mycobacterium tuberculosis*. Most infected individuals will remain in a 'latent' state of infection, in which no clinical symptoms are present. A small percentage of these people will eventually progress and develop active disease, which can lead to the release of *M. tuberculosis* from granulomas that have eroded into the airways. When individuals with active tuberculosis (TB) cough, they can generate infectious droplets that transmit the infection.



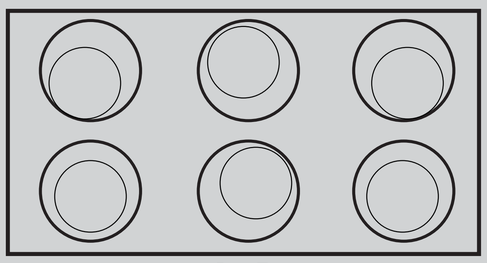


Undifferentiated THP1 cells



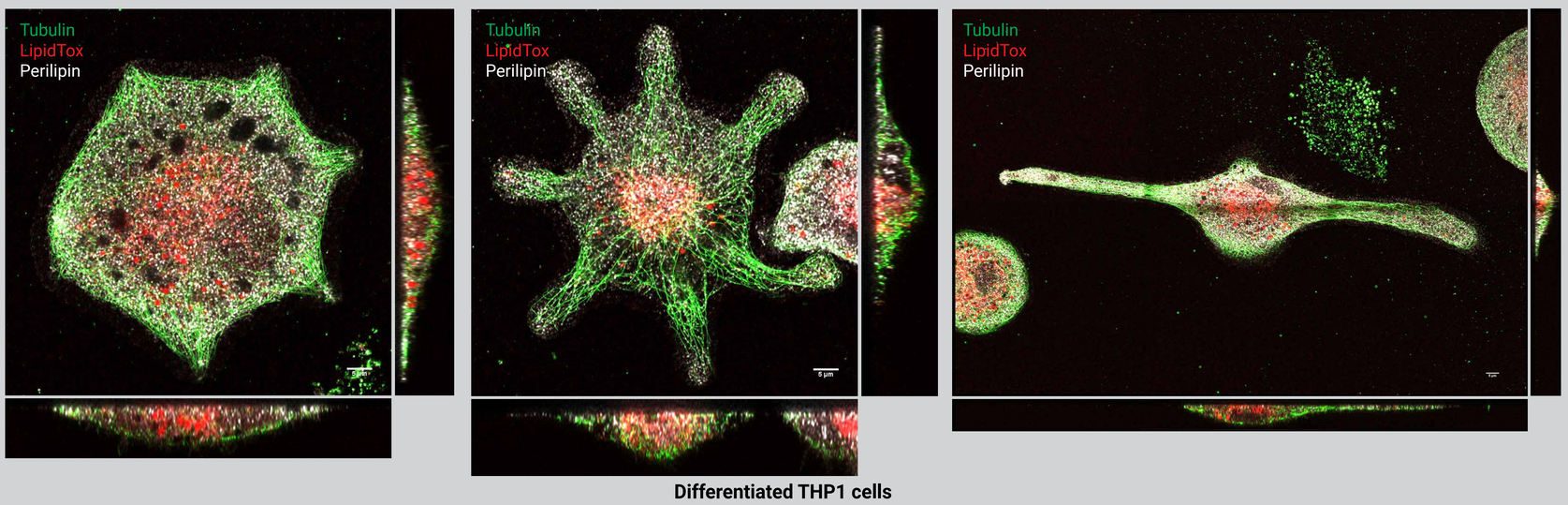
Day 4

2 ml RPMI medium no phenol red + 100nM PMA



Day 7

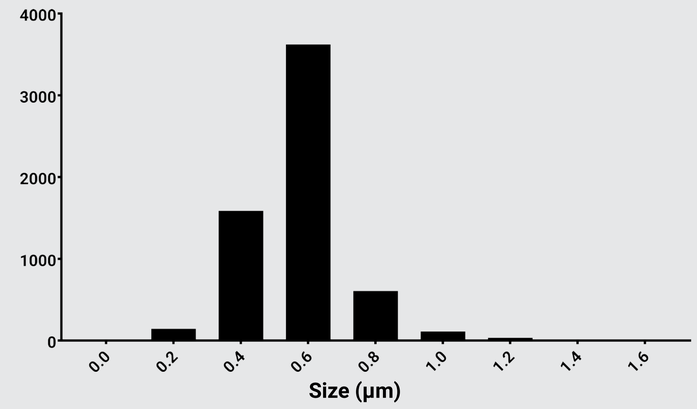
2ml RPMI medium no phenol red



Differentiated THP1 cells

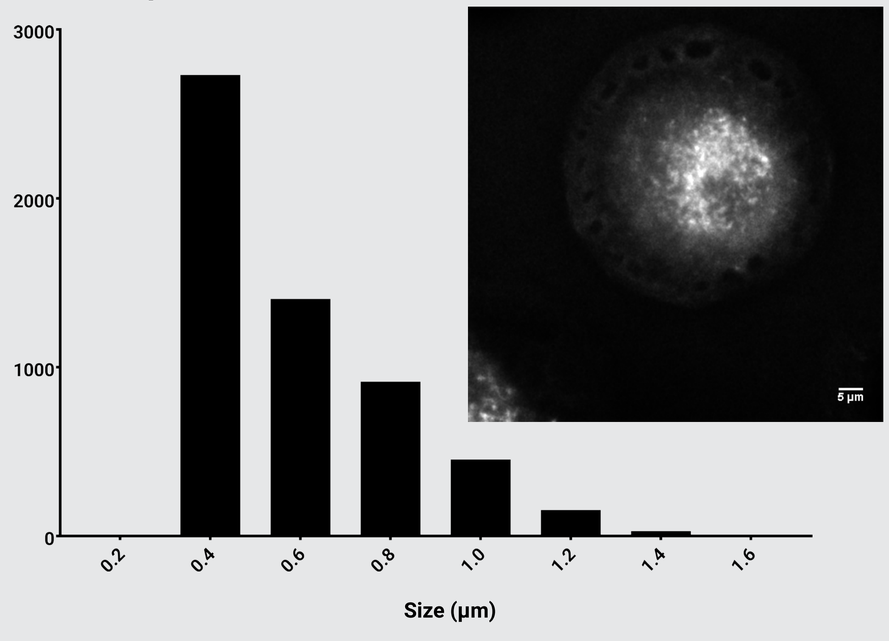
We started comparing different microscope systems and techniques to assess the achievable spatial and temporal resolution and its overall usability to image lipid droplets as well as other cellular compartments in macrophages differentiated from THP1 cells. Furthermore, we used a lipid droplet associated protein marker (perilipin) to assess the specificity of our live imaging marker staining (LipidTox) and its size distribution.

Confocal Perilipin Size Distribution (Imaris Spot Detector)



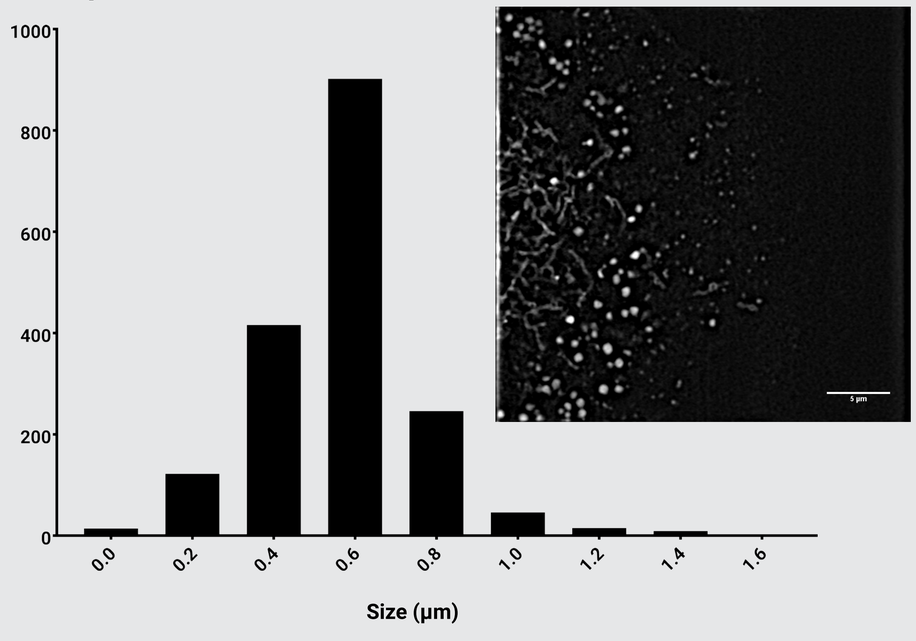
Average Particle Size: 570nm
Percentage of Particles smaller than 200nm ≈ 2%

Widefield LipidTox Size Distribution



Average Particle Size: 640nm

SIM LipidTox Size Distribution



Average Particle Size: 550nm
Percentage of Particles smaller than 200nm ≈ 5%

Take Home Message

Most lipid droplets within a cell are well above 200nm.

Notwithstanding, the smaller vesicles are the ones that show the highest dynamics in live imaging movies of THP1 cells. Therefore it is important to identify them and resolve them best.

From the different setups we have tested so far, SIM has shown the most promising results regarding LipidTox.

It is the first time that THP1 macrophages have been imaged using super resolution techniques.

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