

# Inflammatory response of macrophages and trophoblasts investigated using structured illumination microscopy and quantitative phase microscopy

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**Introduction:** The super-resolution capability of structured illumination microscopy (SIM) (100 nm) enables 3D imaging of mitochondria, and label-free quantitative phase microscopy (QPM) provides nanoscale quantitative phase values relating to cellular thickness and the refractive index of cellular content.

**Methods:** We examined morphological changes in response to LPS and TNF- $\alpha$  (agents which are known to cause inflammation) challenge on live RAW264.7 (macrophage) and HTR-8/SVneo (trophoblast) cell lines using SIM and QPM, and quantitatively measured nitric oxide (NO) production.

**Results:** SIM imaging showed changes in the morphology of mitochondria (Fig.1) and plasma membrane (Fig.2) in approximately 50-60% of macrophages following LPS challenge (1 $\mu$ g/ml for 24 h), but no detectable changes in mitochondria or plasma membrane were observed after TNF- $\alpha$  challenge (1ng/ml for 24 h). Mitochondrial and plasma membrane morphology appeared unaffected in trophoblasts following either LPS or TNF- $\alpha$  challenge under similar conditions. LPS-challenged macrophages produced approximately 22fold more NO as compared to controls, whereas no significant increase was seen after TNF- $\alpha$  challenge. QPM revealed that the phase value decreased by approximately 18% in LPS-challenged macrophages as compared to controls. In contrast, no notable changes in the phase value or NO production were observed in trophoblasts with either LPS or TNF- $\alpha$  challenge.

**Conclusion:** Our results suggest that the different cells which are responsible for the mother-fetus cross-talk, especially macrophages and trophoblasts, respond differently to various inflammatory agents. High-resolution optical microscopy is shown to be a live-cell friendly, useful tool to evaluate sub-cellular mechanisms associated with inflammation complications during pregnancy.

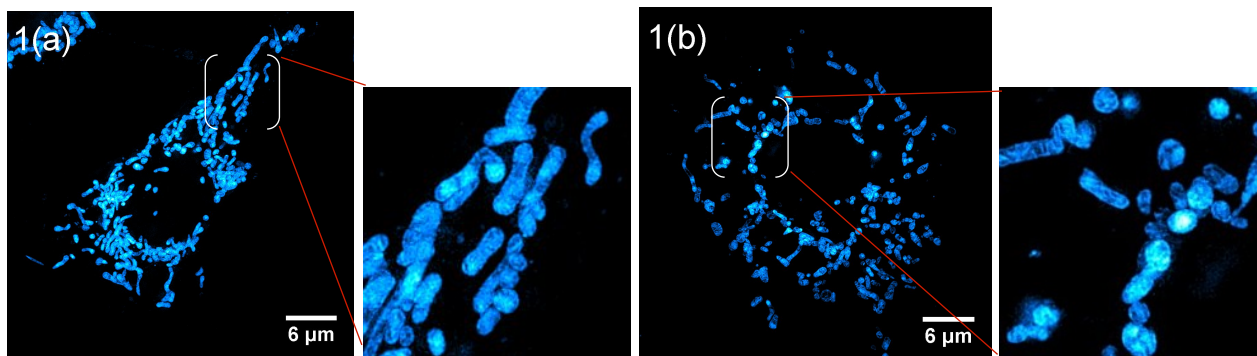


Fig. 1. In controls (a), mitochondria appeared longer and more uniform while in LPS-challenged macrophages (b) they were smaller and rounded in shape.

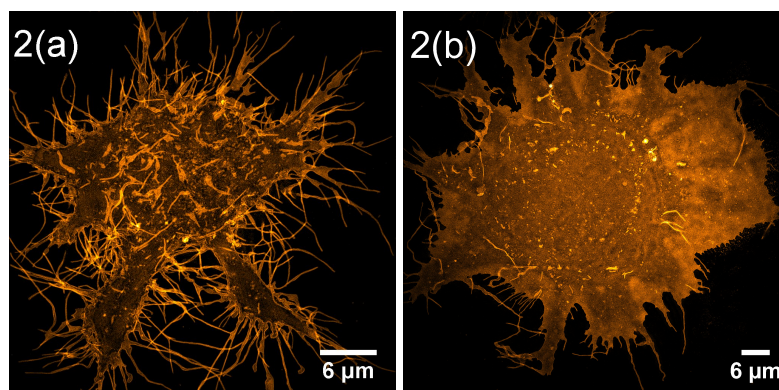


Fig. 2. Compared to controls (a), LPS-challenged macrophages (b) have larger footprints and fewer protruding extensions.