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Quantitative Phase Imaging for detection of *Legionella* in Amoeba Vesicle of amoeba

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Aims

Legionella are parasites of amoeba. It infects their vesicles where it multiplies. Upon inhalation the bacteria can infect alveolar macrophages. The cell becomes invaded by the parasite. It is eventually expelled by the cell by means of cell explosion or exocytose.

To follow the evolution of the infection by the parasites, it is important to see and count them within the vesicles. We propose to observe parasites in infected vesicles by means of Quantitative Phase Imaging.

Methods

The quantitative phase images are generated using a patented-technique called Quadri-Wave Lateral Shearing Interferometry. It is implemented as a conventional camera at one of a microscope exit port. It offers a very great contrast enhancement for cells observation without labeling. Therefore a fast and direct observation of biological structures is made possible. The image contrast is obtained from the phase difference within a sample. The phase carries information on the cell local refractive index, which is proportional to the dry mass concentration. Therefore the measurement provides quantitative information that can be statistically analyzed for the study of different parasites.

We used objectives with high magnification (100x) with other external zoom (400x) to observe the *Legionella* inside amoeba vesicles.

Results

In our first observation we used free *Legionella* in water. We can measure and obtain values, which characterize specifically *Legionella* in this medium. We then observed amoeba vesicles and detected those infected by *Legionella*. We have seen that applying a high-pass filter on quantitative phase images greatly improved the detection of *Legionella* inside the vesicles.

Conclusions

These first studies are very encouraging for the detection of *Legionella* in amoeba vesicle. The next step is to characterize the parasite in the vesicles to have the possibility to detect and count them in environmental samples.

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