# Optical Imaging and Manipulation Applied to Microbiological Systems

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## Abstract

In this thesis I have demonstrated how different optical systems can be designed to combine optical manipulation and fluorescence imaging in a microscope for single cell studies. Full control of the environment has also been achieved using micro-fabricated structures. These systems have then been applied in microbiological research.

I have shown how the viability of *E. coli* bacteria can be investigated using the membrane sensitive fluorescent markers SYTO9 and propidium iodide in combination with optical tweezers. The technique was found to give similar response as the traditional agar based technique. Further, metabolically active but non-culturable cells were found, which demonstrate that the reproductive ability might be lost prior to the collapse of the membrane potential. It was also shown that the intensity of the trapping laser light did not influence the doubling time of the trapped bacteria.

I have also shown how propidium iodide can be inserted using laser scalpels in a single bacterium and the molecular diffusion studied. This technique was used to investigate the possible exchange of cytoplasmic material in chain forming *ftsK1::cat* mutants of *E. coli* bacteria. It was found that the dye did not diffuse into the neighboring cells, which indicates that the cells in the chain do not share or exchange cytoplasmic materials. Experiments performed on filament forming ftsI and ftsK mutants showed that propidium iodide molecules could diffuse freely. In ftsK mutants we also found nucleic acid free compartments which we believe is a result of the separation of the cytoplasm into multiple cytoplasmic packages during cell division by the inner membrane of the bacterium.

Finally, I have shown how focused IR lasers can be used as a localized heat source for gene activation on a cellular level in *C. elegans*. Optically targeted cells experienced an increase in temperature due to the absorption of the light which triggered a stress responsive promoter and induced heat shock proteins. The frequency of the gene expression was found to increase with an increase in both laser power and exposure time. The data also indicates that the promoter does not react as quickly to an increase in temperature as it does to photochemical stimuli.

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**Keywords:** optical tweezers, laser scissors, laser induced heating, microfluidic system, lab-on-a-chip, microscope, epi-fluorescence, confocal, multiphoton, *Escherichia coli*, *Saccharomyces cerevisie*, *Caenorhabditis elegans*