Fluorescence imaging as a tool to study the 3D structure of *in vitro* and *ex vivo* mycobacterial biofilms

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Biofilm forming bacteria contribute to many chronic and recurring diseases. The respiratory illness tuberculosis is caused by *Mycobacterium tuberculosis* that forms biofilm *in vitro* and *in vivo*. The high antibiotic tolerance of biofilms reduces the efficacy of tuberculosis treatment. Therefore, novel treatment methods, such as antibiofilm compounds, are needed.

The zebrafish pathogen *Mycobacterium marinum* is a close relative to the human pathogen. Fluorescent *M. marinum* strains were created by introducing plasmids for fluorescent protein expression into the bacterium. We also created fluorescent deletion mutant strains and strains that overexpress certain *M. marinum* proteins. The selected proteins were hypothesized to affect biofilm formation. Mature biofilms were imaged with confocal microscopy to reveal their 3D-structures. The fluorescent bacteria were also used to infect adult zebrafish. Bacterial granulomas were collected from the fish 2–13 weeks after infection and used either for whole-mount staining or to prepare paraffin sections for staining. A variety of dyes that stain ECM components were used to visualize the structure and organization of bacteria in granulomas.

The 3D-structures of submerged-type biofilms revealed that *M. marinum* forms cord-like structures *in vitro*. Mutants forming thicker cords and overall thicker biofilm had increased tolerance. This suggests a strong link between biofilm structure and antibiotic tolerance.

Granulomas are composed of bacteria encapsulated by host immune cells. The imaged granulomas contained known ECM components indicating that *M. marinum* forms biofilm *in vivo*. Understanding the structure and organization of mycobacterial biofilm *in vivo* could help the development of more rapid and effective tuberculosis treatment.