Quantification of biofilm structure from confocal imaging
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What is a biofilm ?
In microbiology, knowledge has traditionally been gained from studies of suspensions of cells grown from a single cell in laboratory culture plates. These free-floating or "planctonic" cells have for instance been used in studies of how well antibiotics can kill bacteria. However, microbes can also aggregate as biofilms – i.e. organized layers of cells attached to a surface. In nature, probably 99% or more of all bacteria exist in biofilms. For instance, in an alpine stream there is typically only 10 bacteria per ml., whereas bacteria living in slimy biofilms on nearby rocks can occur in numbers like 5×10^8 per square centimetre. Biofilms, which the bacteria form naturally, provide a way of surviving e.g. UV and drying, as the slimy mass they are living within acts as a protecting layer. When a bacterium attaches to a hard surface in a moist environment gene expression is adapted to the new environment. Some genes are up-regulated whereas others are depressed or turned off.

Biofilms can be beneficial when they break down contaminants in soil and water as used in waste-water treatment but can also cause severe problems in industrial settings, corroding everything from pipes in heating systems to computer chips or causing problems on the hulls of ships

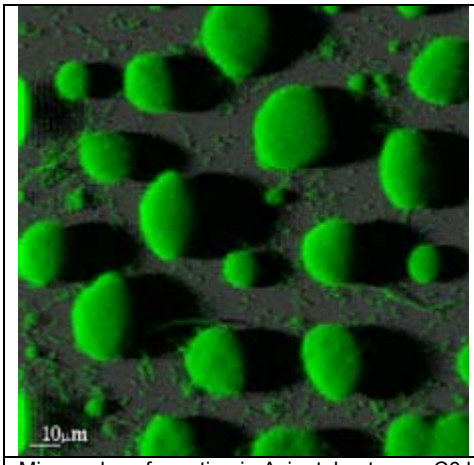
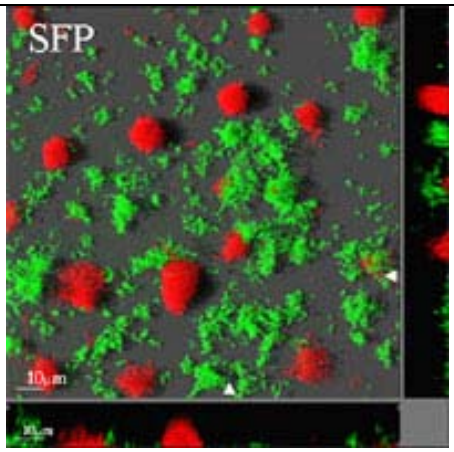
Biofilm in human diseases
Bacteria in biofilms behave differently from their planctonic counter parts. They are notoriously difficult to eradicate and the source of many chronic infections. It is believed that more than 60% of all microbial infections in humans are caused by biofilms. A well known example of a microbial biofilm in human disease is dental plaque, which is the most known and studied biofilm (secreting acids that destroy teeth and gums). Other biofilm caused diseases are, urinary tract infection, middle ear infection, and prostatisis. Less common but more threatening (causing morbidity and mortality) are infections caused by biofilms growing on implants (e.g. catheter or heart valve infections) and infections in cystic fibrosis patients.

A general observation is that bacteria which normally would be easy to kill when in suspension are highly resistant to treatment when found in biofilms. For instance, it is not unusual that the dose of an antibiotic to which the bacterium is sensitive needs to be administered at a thousand fold higher dose to have any effect on biofilm embedded cells. Then the dose of the medication may be detrimental to the patient, effectively making the antibiotic useless.

While much emphasis is on the adverse effects of biofilms and the difficulty in treating diseases, many also have a protective role, e.g. biofilms in the vagina prevent colonisation by exogenous pathogens.

Studying biofilm
The application of new molecular and microscopic techniques to analyse biofilms has revolutionised the understanding of their structure, composition, organisation, and activities. In the early 1990s, Confocal Laser Scanning Microscopy (CLSM), which magnifies live cell in real time and in 3D without destroying them, revolutionised the understanding of biofilm behaviour. Until CLSM began to be used for studying biofilm structure, there was little evidence that biofilm displayed any organised structure. In reality structure ranges from a relatively feature-less, flat type to one involving mushroom-like aggregates separated by water channels.

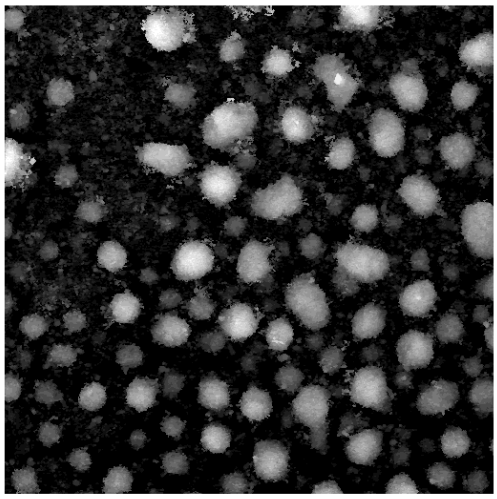
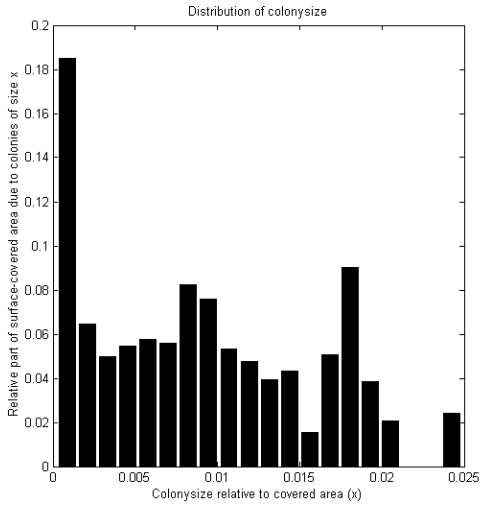
Biofilms can be cultivated *in vivo* by inoculating laboratory flow cells. Staining methods such as commercially available probes or green fluorescent protein (GFP) expression are used for fluorescence image acquisition using CSLM. The choice of staining method is system specific and done according to the individual characteristics and the purpose of the analysis.

	
Micro-colony formation in Acinetobacter sp. C6 biofilms. Image obtained using CLSM.	CLSM image of two species biofilm: Acinetobacter (DNA stain, red) and P. putina KT2440 (GFP tagged, green)

Quantifying biofilm growth and structure

From CLSM one can obtain 3D image sequences of the biofilm development within the laboratory flow cells. Using CLSM images it is possible to quantify the development of different kinds of structures and quantify the amount of biomass produced. In the case of multi-species biofilms, quantification of the interaction between different types of bacteria is also of concern. The obtained quantification is together with the molecular techniques important tools for describing, discriminating, and understanding different histories of biofilm development. A first step is to discriminate biofilm mass from the background and preferably this should be accomplished in an automated way to avoid operator bias and to speed up the process. Also alignment relative to substratum surface is often needed. When the biomass is segmented from the background a number of features (believed or empirically proved to be of value in discriminating biofilm structure) are calculated. This includes roughness of the film, porosity, thickness, number of micro-colonies etc. The working hypothesis is than in any heterogeneous biofilm, there is a finite number of parameters that can be measured and used to quantify unique features of biofilm structure.

The presentation will illustrate image sequences that can be obtained using CLSM for biofilm studies and how informative measures and descriptive features can be obtained using image processing techniques such as mathematical morphology.

	
From a 3d image stack of a biofilm an thickness image with intensity proportional to thickness is constructed	From thickness image, colonies are segmented and the areas are estimated.

References:

A. Heydorn et al, “Quantification of biofilm structures by the novel computer program COMSTAT”, Microbiology (2000), **146**, 2395–2407