# Light-based patterning of bacterial cells through induction of biofilm formation processes

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## Abstract

Microbes live in communities known as biofilm on many surfaces. Thus, understanding the spatially-resolved intercellular communication and signalling link would be important to elucidate the fundamental mechanisms that govern the division of labour within biofilm as well as the differentiated roles of different species within the community. To this end, different cell patterning approaches ranging from streak plate inoculation to more spatially-defined methods utilizing microfluidics have been shown to be useful for patterning different types of cells on the same surface. However, these approaches suffer from one major limitation: the inability to control the cellular state of the cells patterned on the surface. For example, it was not possible to control the cellular differentiation pathways activated in cells patterned on a surface by the streak plate inoculation approach. A recent article in *PNAS* described the approach of biofilm lithography that utilized light illumination to control biofilm formation and thus patterning of cells on a surface. Specifically, a light-sensitive promoter, pDawn, was coupled to a biofilm formation gene, Ag43 that enabled the induction of biofilm formation and deposition of cells on a surface upon activation of a specific wavelength of light. The approach is amenable to the use of photomask common in photolithography and enables the formation of patterns with high spatial resolution of 25 μm. However, the method suffers from unexplained degradation of the patterned biofilm after a few days and this limits its utility in long duration experiments seeking to understand cellular behaviour due to intercellular signalling. In addition, the maximal spatial resolution achieved is still multiple cell lengths away from that necessary to understand intercellular communications of cells in close contact in a biofilm. However, by coupling the light sensitive promoter to other genes important to biofilm formation processes in other microbial species, the approach could be extended in future work to the formation of different patterns of multiple microbial species to understand how different localization of different microbial species impact on the ecology and functioning of biofilms. Collectively, the approach of biofilm lithography represents an important advance in the biologist's toolkit for patterning spatially-resolved patterns of cells for understanding how spatial location influences cell-cell communications within the same community of cells.

***Keywords:*** biofilm, lithography, light-sensitive promoter, cell-cell communication, signalling, division of labour, differentiated functions, bacteria, microbe, spatial resolution,

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**Commentary**

Microbes do not live alone, rather, they assemble into multi-species and multicellular assemblage known as biofilms on various surfaces. Specifically, microbial cells often assemble into biofilm matrix with differentiated functions and roles in the consortium resulting from complex intercellular communications and signalling that mediates a division of labour amongst cells of the biofilm. Architecturally complex with multiple gradients of nutrients and oxygen where the innermost cells are generally deprived of nutrients and oxygen, studies have revealed that cells on the periphery of the biofilm behaved differently from those in the biofilm interior.[1]

As biofilms are multicellular assemblage, different species and cells are distributed across different parts of the biofilm. Thus, it would be interesting to understand how spatial segregation coupled with intercellular signalling mediated by diffusive molecules enable cross-communication between different species of microbes and cells for coordinating biofilm response to fluctuations in environmental and nutritional conditions. Such a requirement would naturally call for the development of techniques and methods for the patterning of cells and species in defined locations on a surface for understanding the ecology and mechanisms underlying cell-cell communications and signalling between species of a biofilm community.

To this end, the simple streak plate technique on agar plate is the most commonly available technique for patterning different microbial species on a surface. However, the method suffers from the lack of spatial resolution where the accuracy of cell patterning critically depends on the experimenter's skill and control over the inoculation loop. Other techniques for cell patterning uses more exotic methods such as cell deposition from controlled flow of cell-fluid suspension in narrow channels via the approach of microfluidics.[2] [3] [4] [5] One major deficiency of the above approaches is the lack of control over the cellular state of the cells patterned on the surface. For example, the cells could be in a variety of cellular differentiation programmes and might not have activated the biofilm cellular state during surface growth.

Using light-based patterning of cells on a surface that induces the biofilm formation processes, a recent study in *Proceedings of the National Academies of Sciences of USA* (*PNAS*, Link)[6] describes the approach of biofilm lithography that managed to achieve high spatial resolution (i.e., 25 µm) patterning of bacterial cells on a surface. Specifically, the authors used a light sensitive promoter pDawn to exert control over the gene expression patterns of the cells via light. In particular, the pDawn promoter was coupled to a gene important to the induction of biofilm formation processes, Ag43, which translated light illumination into biofilm formation. Experiments revealed that the approach was responsive to light illumination where light facilitated the formation of biofilm on surfaces. More importantly, the method could be coupled to photomasks of different designs, which enabled the patterning of cells into different patterns on the surface through biofilm formation.



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One key limitation of the approach is that the biofilms patterned were only stable for a few days whereupon the pattern would disintegrate. Understanding the mechanisms underlying this degradation of the biofilm pattern may help enhance the technique's ability to pattern more long-lasting biofilm pattern. Another problem stems from the relative lack of spatial resolution for patterning cells in close proximity to each other, which is important for understanding how cell-cell signalling influences biological processes of cells in a biofilm. Considering the typical length of a bacterium as 1 µm, the achieved spatial resolution of 25 µm remains too coarse for the high spatial resolution patterning of bacterial cells useful for understanding the signalling processes within the close confines of a biofilm matrix.

Conceptually, the approach could be used in patterning different species of bacteria through coupling pDawn to different biofilm formation genes pertinent to individual species. If the approach could be extended to induce biofilm formation in other bacterial species upon light illumination, it could find ready use in patterning exquisite patterns of different cells and species on the same surface; thereby, enabling the formation of spatially-defined patterns comprising different microbial species useful for understanding cell-cell interactions and signalling.

Collectively, a light-based patterning approach was shown to be effective in high spatial resolution patterning of bacterial cells on a surface. By coupling a biofilm formation gene with a light sensitive promoter, biofilm formation came under the control of specific wavelength of light that enabled the process to be utilized for patterning. However, the approach suffers from the degradation of the biofilm pattern after a few days by unknown mechanisms that precluded the use of the technique to the study of long duration processes. Future work could attempt to couple the light sensitive promoter to other biofilm formation genes from other microbial species that could enable the spatially precise patterning of different species on the same surface. Such an approach would be useful for understanding how spatial patterning and location influences cell-cell interactions and communications that could, in turn, augment our understanding of ecological functions of different species in spatially-resolved cellular clusters.

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**Conflicts of interests**

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