



# Classification of Biofilm Formation using Artificial Intelligence

Jaliyah Herbert; Dipak Singh, PhD  
Department of Computer Science, Stephen F. Austin State University



## Abstract



Fig. 1A

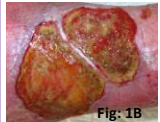


Fig. 1B



Fig. 1C

Artificial intelligence (AI), particularly deep learning, has lately allowed extraordinary advancements within the biotech industry. Classifying objects, such as cancerous tumor, in images is one of the fundamental challenges that deep neural networks can handle. This project explore the classification of biofilm formation using deep learning object classification algorithms. The presence of biofilm facilitates the development of infections by compromising a patient's immune system and contributing to the failure of the antibiotic therapy, which results in recurrent infections and the emergence of multiresistant pathogens.

The traditional methods of biofilm detection, for example, Tissue Culture Plate, Tube method, bioluminescent assay, are tedious, costly and time-consuming. To overcome these limitations, we deployed an automated AI-based deep learning approaches, such as Convolutional Neural Networks (CNN), to identify biofilm formation. Our results show that the VGG-CNN model with transfer learning performs the best with an accuracy of 92.47%.

## What is Biofilm?

A biofilm is a bacterial cluster encased in a self-produced exopolysaccharide matrix that attaches to a biotic or abiotic surface. Biofilm forms anywhere there is a combination of moisture, nutrients, and a surface by producing a long, stringy, slimy, glue-like substance. Considering biofilm plays a role in pathogenesis, there is greater importance in identifying biofilm and its growth.

## Data

To train and test our CNN-based models, we used a raw dataset consisting of Brightfield images (biofilm and single-cell) provided by Mississippi State University. To avoid incorrect results, we cleaned our dataset before training our CNN-based models.

Table 1. Biofilm dataset

	Biofilm	Single-cell
Raw dataset	276	190
Training/Validation	221	152
Test	55	38

## References

1. J. Yosinski, J. Clune, Y. Bengio, and H. Lipson, "How transferable are features in deep neural networks?," Advances in Neural Information Processing Systems, 08-Dec-2014. [Online]. [Accessed: 01-Aug-2022].
2. K. Simonyan and A. Zisserman, "Very deep convolutional networks for largescale image recognition," arXiv, 10-Apr-2015. [Online]. [Accessed: 01-Aug-2022].
3. R. R. Selvaraju, M. Cogswell, A. Das, R. Vedantam, D. Parkhi, and D. Batra, "Grad-cam: Visual explanations from deep networks via gradient-based localization," Papers With Code, 03-Dec-2019. [Online]. [Accessed: 01-Aug-2022].
4. Sengupta B, Adhikari P, Mallet E, Havner R, Pradhan P. "Spectroscopic study on pseudomonas aeruginosa biofilm in the presence of the aptamer-DNA scaffolded silver nanoclusters," Molecules (Basel, Switzerland), 10-Aug-2020. [Online].

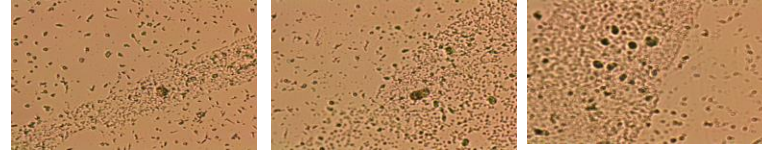


Fig 2A: Biofilm sample images ( the long chain clusters indicate biofilm formation)



Fig 2B: Single-cell sample images ( the transparent spots indicate single cell bacteria presence)

## Model Design and Performance

We used VGGNet16 model which is a CNN-based model. The model consists of 16 weighted layers, which includes thirteen convolutional layers, five Max Pooling layers, and three Dense layers.

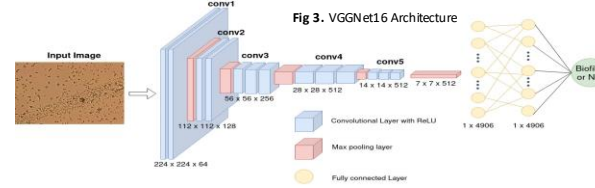


Fig 3. VGGNet16 Architecture

After preprocessing our dataset, we began training our model using regularization layers, K-fold cross-validation, and simplified our model to avoid overfitting during the training process. To ensure our model was not overfitting, we documented the loss and accuracy throughout the training phase.

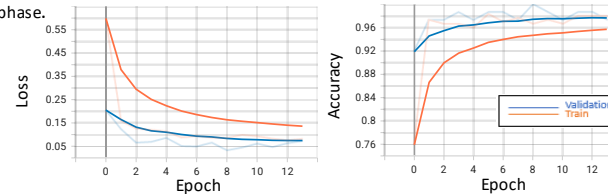


Table 2. Model Performance on Test Dataset

	Accuracy	Precision	F1 Score
With TL	92.47%	88.71%	96.49%
Without TL	81.72%	76.39%	74.35%

## Model Interpretation: Why the model think biofilm is located in a picture?

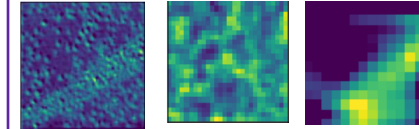


Fig 4A: Visualization of the feature maps at layer 1, 3, 5 (left to right) for a biofilm image

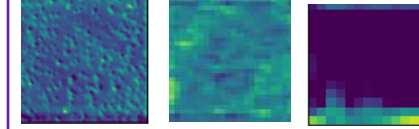


Fig 4B: Visualization of the feature maps at conv layer 1, 3, 5 (left to right) for a single cell image

As an input image passes through the deeper conv layers of the VGG model, it learns higher level abstraction information about a biofilm and a single cell image which empowers it with classifying ability.

Observations:

1. The output at layer 1, is retaining most of the information of input image and mostly acting as edge detector.
2. At the layer 5, we can see the model is activated at the long chain cluster region of a biofilm image (light green) and inactive (dark purple) for a single cell. This shows that the model is learning the correct abstraction features.

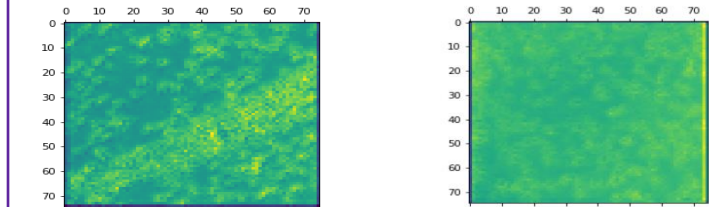


Fig 5. Superimposed class activation heatmap on biofilm (left) and single cell (right) image

Moreover, to ensure that our model is not classifying images by mistake (with wrong set of features), we used GRAD-CAM algorithm, to visualize the region in the image, that filters of the model are concentrating on when making classification decision between a biofilm and single cell image. As we can see in Fig 5, the model is concentrating on the long chain cluster region of the biofilm image (light yellow) to label the image as a biofilm. Such region is missing in single cell image.

## Conclusion and Future Work



In our project, we showed that the CNN based model are efficient and cost-effective method to identify biofilm images. The VGGNet 16 model with transfer learning performed better than our VGGNet16 model without transfer learning. In future work, we want to use image segmentation to outline where biofilm is present in images. Image segmentation is more efficient than image classification because it extracts pixel-level features within the images which helps in localizing an object and also increases the accuracy of the model.

## Acknowledgments

This research was supported by the College of Sciences and Mathematics as a part of the Summer Undergraduate Research Experience. Further, I would like to thank Angel Robledo-Torres, Rajesh Shrestha, Dr. Bidisha Sengupta (Stephen F. Austin State University), Dr. Prabhakar Pradhan (Mississippi State University) for providing the dataset, as well as my advisor Dr. Dipak Singh and the SFA Department of Computer Science.

## Contact

Jaliyah Herbert  
Stephen F. Austin State University  
Herbertjm@jacks.sfasu.edu  
To view source code:  
<https://github.com/jaliyahherbertSFA/VGGNet>