UC San Diego

Deep Learning Content-Aware Image Restoration

Anna Sim

4D CELL Biology Lab

Background

ARTICLES
https://doi.org/10.1038/s41592-018-0216-7
nature methods

Content-aware image restoration: pushing the limits of fluorescence microscopy

Martin Weigert[1,2*], Uwe Schmidt[1,2], Tobias Boothe[1,2], Andreas Müller[3,4,5], Alexandr Dibrov[1,2], Akanksha Jain[2], Benjamin Wilhelm[1,6], Deborah Schmidt[1], Coleman Broaddus[1,2], Siân Culley[7,8], Mauricio Rocha-Martins[1,2], Fabián Segovia-Miranda[2], Caren Norden[2], Ricardo Henriques[7,8], Marino Zerial[2], Michele Solimena[2,3,4,5], Jochen Rink[2], Pavel Tomancak[2], Loic Royer[1,2,9*], Florian Jug[1,2*] and Eugene W. Myers[1,2,10]

Fluorescence microscopy is a key driver of discoveries in the life sciences, with observable phenomena being limited by the optics of the microscope, the chemistry of the fluorophores, and the maximum photon exposure tolerated by the sample. These limits necessitate trade-offs between imaging speed, spatial resolution, light exposure, and imaging depth. In this work we show how content-aware image restoration based on deep learning extends the range of biological phenomena observable by microscopy. We demonstrate on eight concrete examples how microscopy images can be restored even if 60-fold fewer photons are used during acquisition, how near isotropic resolution can be achieved with up to tenfold under-sampling along the axial direction, and how tubular and granular structures smaller than the diffraction limit can be resolved at 20-times-higher frame rates compared to state-of-the-art methods. All developed image restoration methods are freely available as open source software in Python, FIJI, and KNIME.

Fluorescence microscopy is an indispensable tool in the life sciences for investigating the spatio-temporal dynamics of cells, tissues, and developing organisms. Recent advances such as light-sheet microscopy[1–3], structured illumination microscopy[4,5], and super-resolution microscopy[6–8] enable time-resolved volumetric imaging of biological processes within cells at high resolution. The quality at which these processes can be faithfully recorded, however, is determined not only by the spatial resolution of the optical device used, but also by the desired temporal resolution, the total duration of an experiment, the required imaging depth, the achievable fluorophore density, bleaching, and photo-toxicity[9,10]. These aspects cannot all be optimized at the same time—trade-offs must be made, for example, by sacrificing signal-to-noise ratio (SNR) by reducing exposure time to gain imaging speed. Such trade-offs are often depicted by a design space that has resolution, speed, light exposure, and imaging depth as its dimensions (Fig. 1a), with the volume being limited by the maximal photon budget compatible with sample health[11,12].

These trade-offs can be addressed through optimization of the microscopy hardware, yet there are physical limits that cannot easily be overcome. Therefore, computational procedures to improve the quality of acquired microscopy images are becoming increasingly important. Super-resolution microscopy[4,13–16], deconvolution[17–19], surface projection algorithms[20,21], and denoising methods[22–24] are examples of sophisticated image restoration algorithms that can push the limit of the design space, and thus allow the recovery of important biological information that would be inaccessible by imaging alone. However, most common image restoration problems have multiple possible solutions, and require additional assumptions to select one solution as the final restoration. These assumptions are typically general, for example, requiring a certain level of smoothness of the restored image, and therefore are not dependent on the specific content of the images to be restored. Intuitively, a method that leverages available knowledge about the data at hand ought to yield superior restoration results.

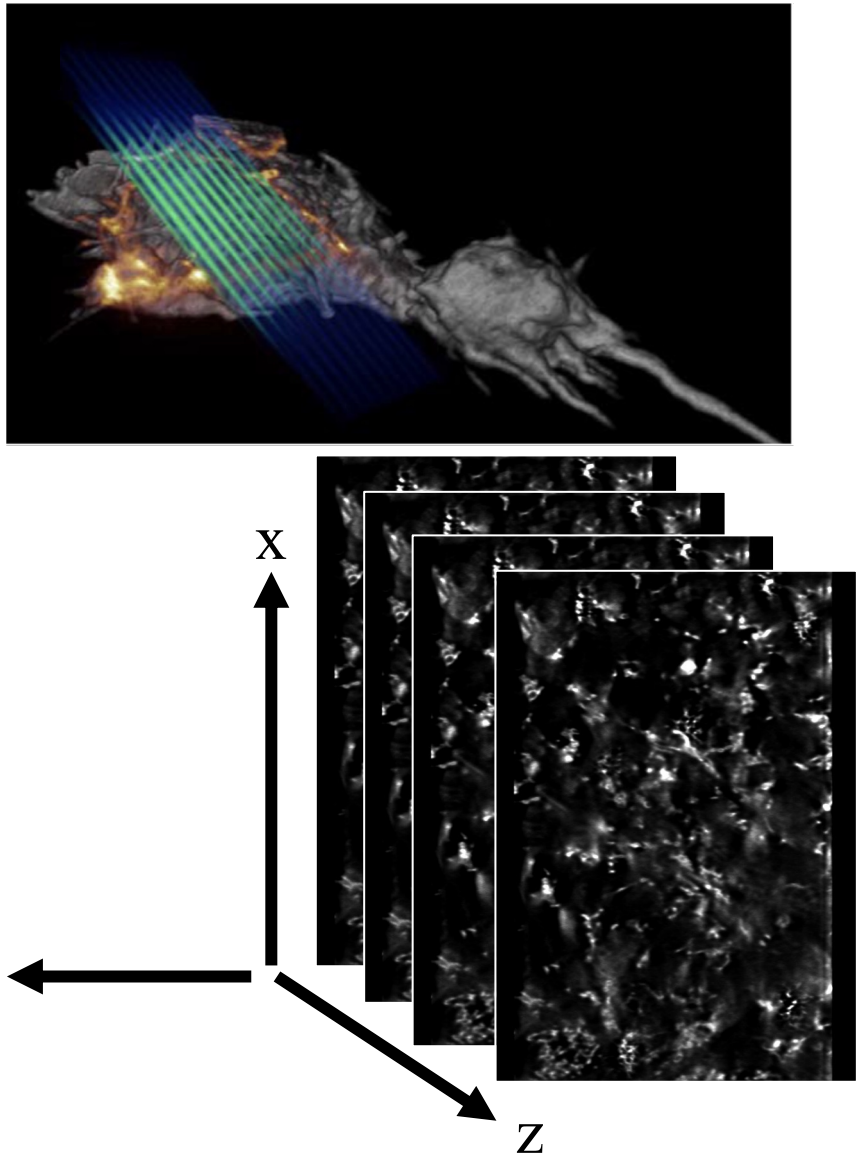
Deep learning is such a method, because it can learn to perform complex tasks on specific data by employing multilayered artificial neural networks trained on a large body of adequately annotated example data[25,26]. In biology, deep learning methods have, for instance, been applied to the automatic extraction of connectomes from large electron microscopy data[27], for classification of image-based high-content screens[28], fluorescence signal prediction from label-free images[29,30], resolution enhancement in histopathology[31], or for single-molecule localization in super-resolution microscopy[32,33]. However, the direct application of deep learning methods to image restoration tasks in fluorescence microscopy is complicated by the absence of adequate training data and the fact that it is impossible to generate them manually.

We present a solution to the problem of missing training data for deep learning in fluorescence microscopy by developing strategies to generate such data. This enables us to apply common convolutional neural network architectures (U-Nets[34]) to image restoration tasks, such as image denoising, surface projection, recovery of isotropic resolution, and the restoration of sub-diffraction structures. We show, in a variety of imaging scenarios, that trained

[1]Center for Systems Biology Dresden, Dresden, Germany. [2]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany. [3]Molecular Diabetology, University Hospital and Faculty of Medicine Carl Gustav Carus, TU Dresden, Dresden, Germany. [4]Paul Langerhans Institute Dresden of the Helmholtz Center Munich at the University Hospital Carl Gustav Carus and Faculty of Medicine of the TU Dresden, Dresden, Germany. [5]German Center for Diabetes Research (DZD e.V.), Neuherberg, Germany. [6]University of Konstanz, Konstanz, Germany. [7]MRC Laboratory for Molecular Cell Biology, University College London, London, UK. [8]The Francis Crick Institute, London, UK. [9]CZ Biohub, San Francisco, CA, USA. [10]Department of Computer Science, Technical University Dresden, Dresden, Germany. *e-mail: mweigert@mpi-cbg.de; loic.royer@czbiohub.org; jug@mpi-cbg.de

- Nature | Methods
- Martin Weigert et al.
- Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.
- Published: 26 November 2018

Background



Spatial Resolution & Temporal Resolution

Goals:

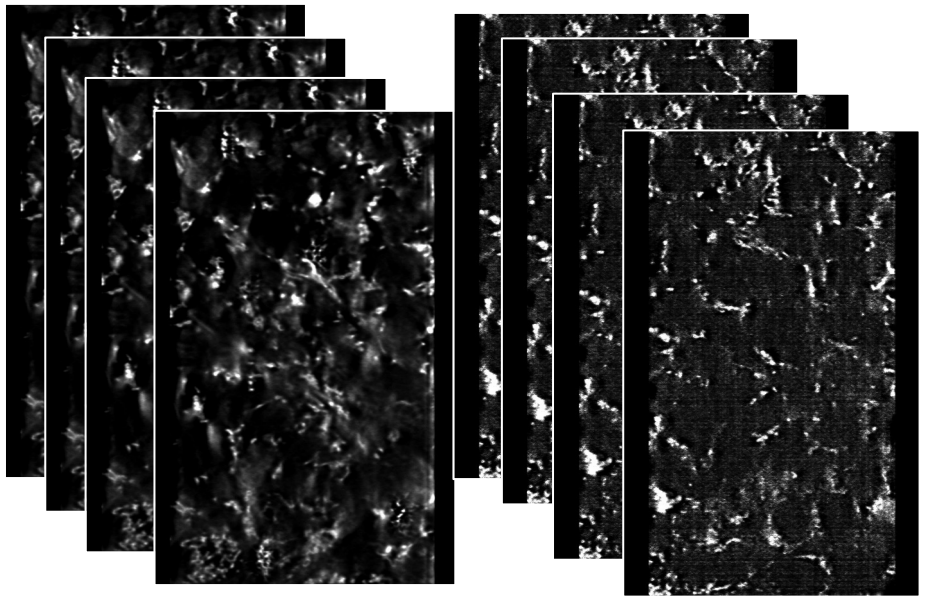
- High spatial resolution
- Prolong time of the experiment
- Required Imaging Depth
- Addressing Bleaching and Toxicity

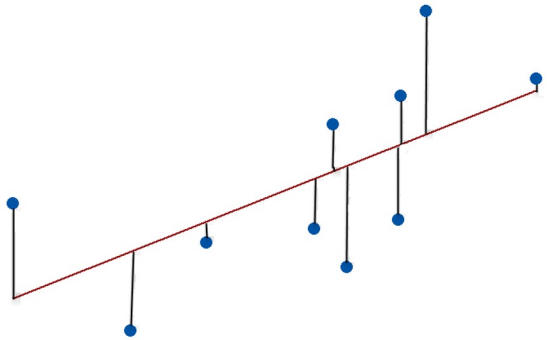
Chen, B. C., Legant, W. R., Wang, K., Shao, L., Milkie, D. E., Davidson, M. W., ... & Betzig, E. (2014). Lattice light-sheet microscopy: imaging molecules to embryos at high spatiotemporal resolution. *Science*, *346*(6208), 1257998.

Background

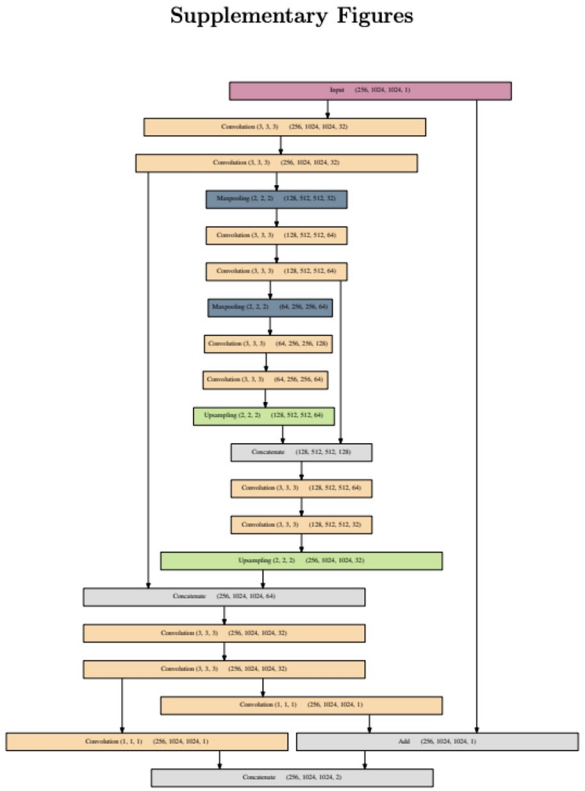
Deep Learning methods are based on:

Large number of Annotated Examples Mathematical Models Artificial Neural Network

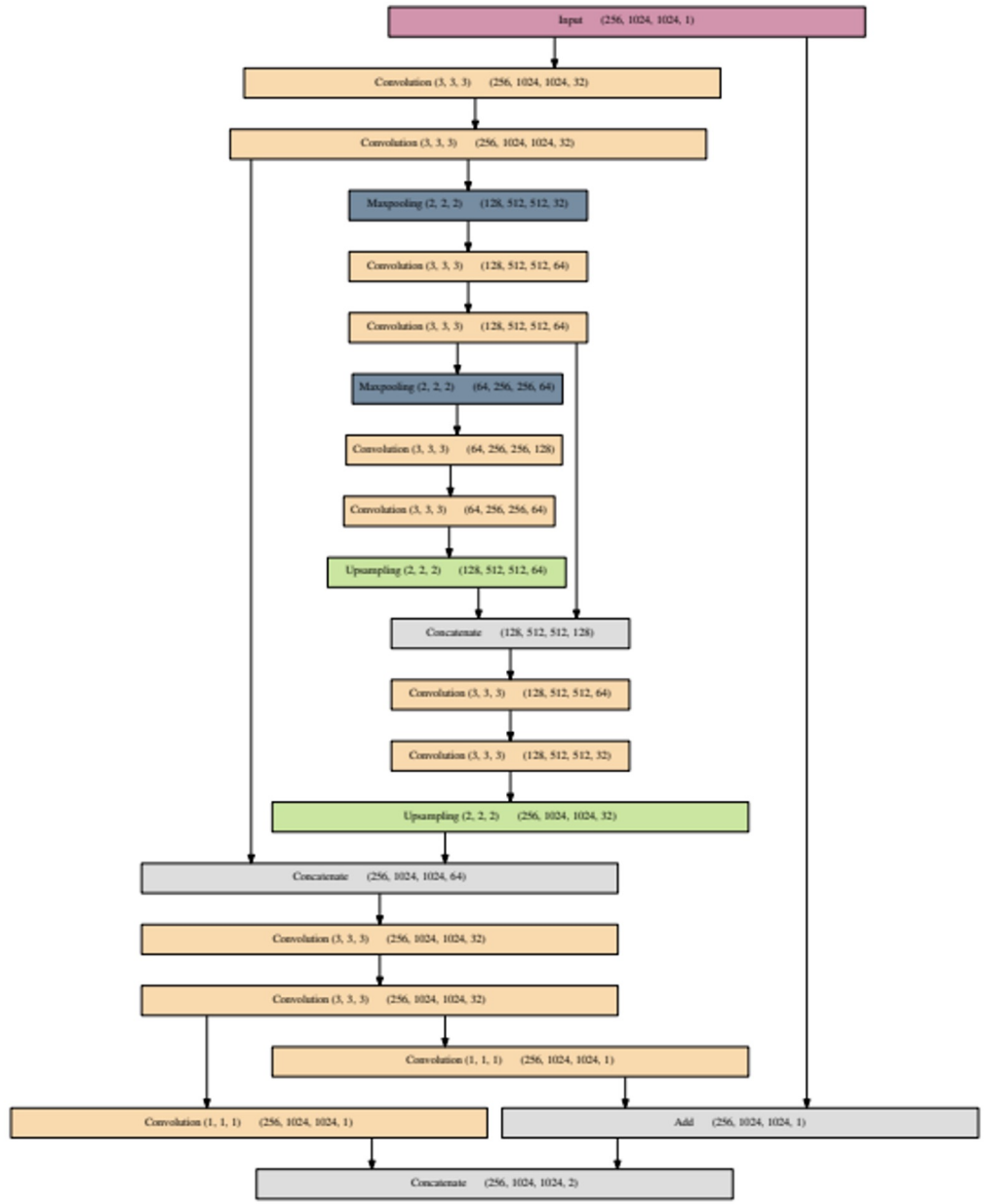




$$\mathrm{MSE}\,(u, v) = \frac{1}{N}\sum_{i=1}^{N}(u_i - v_i)^2$$



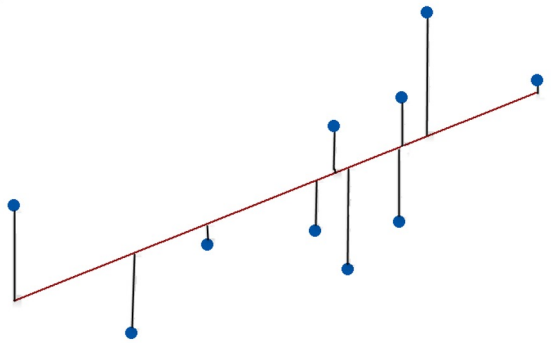
Supplementary Figures



Background

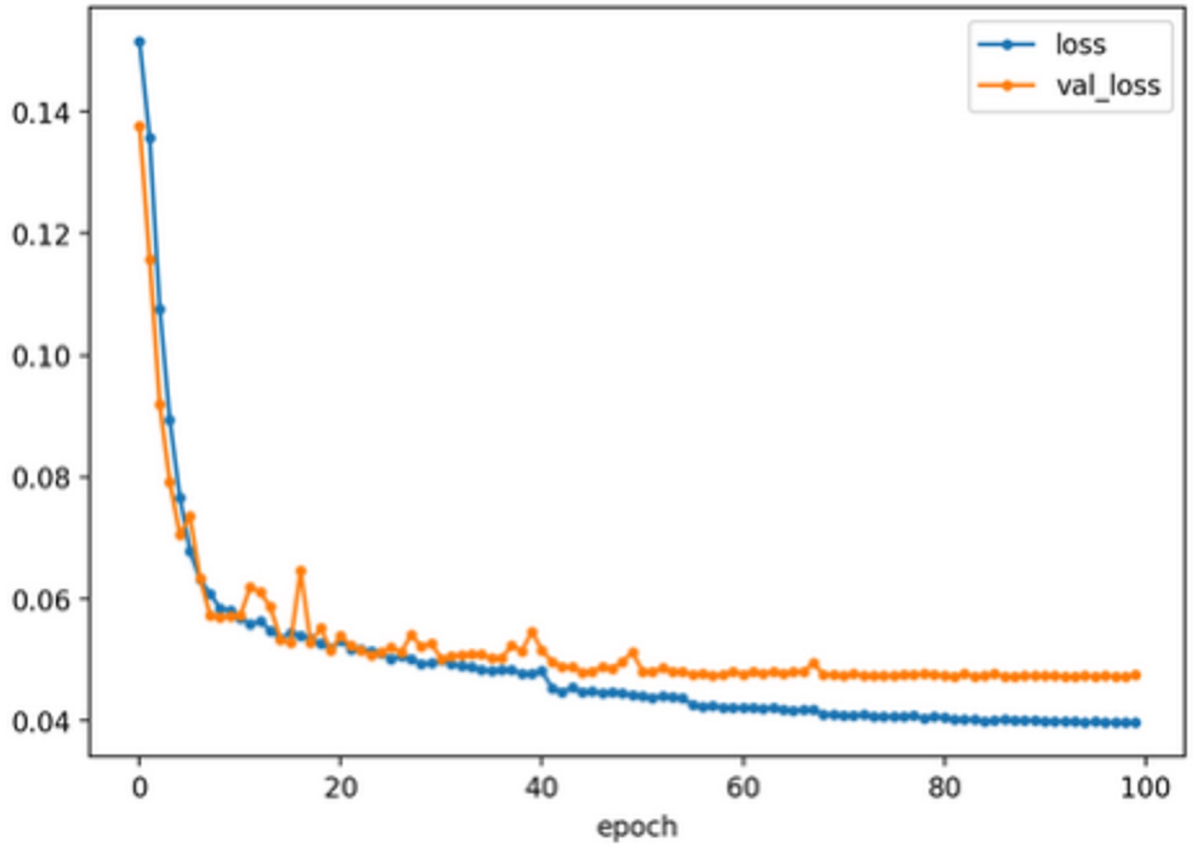
Deep Learning methods are based on:

Mathematical Models

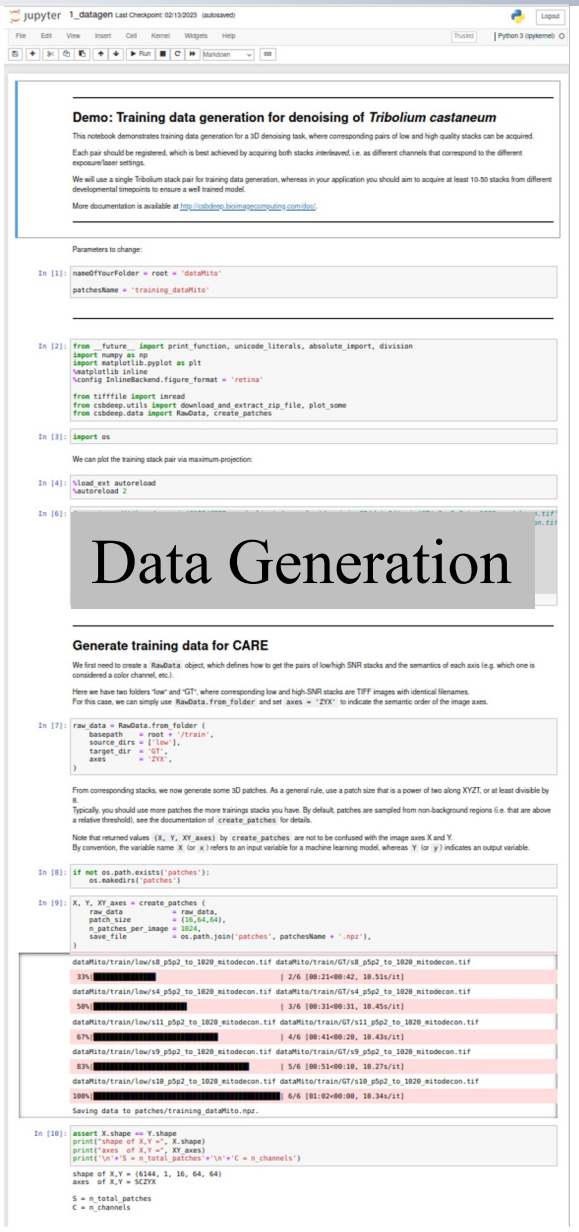


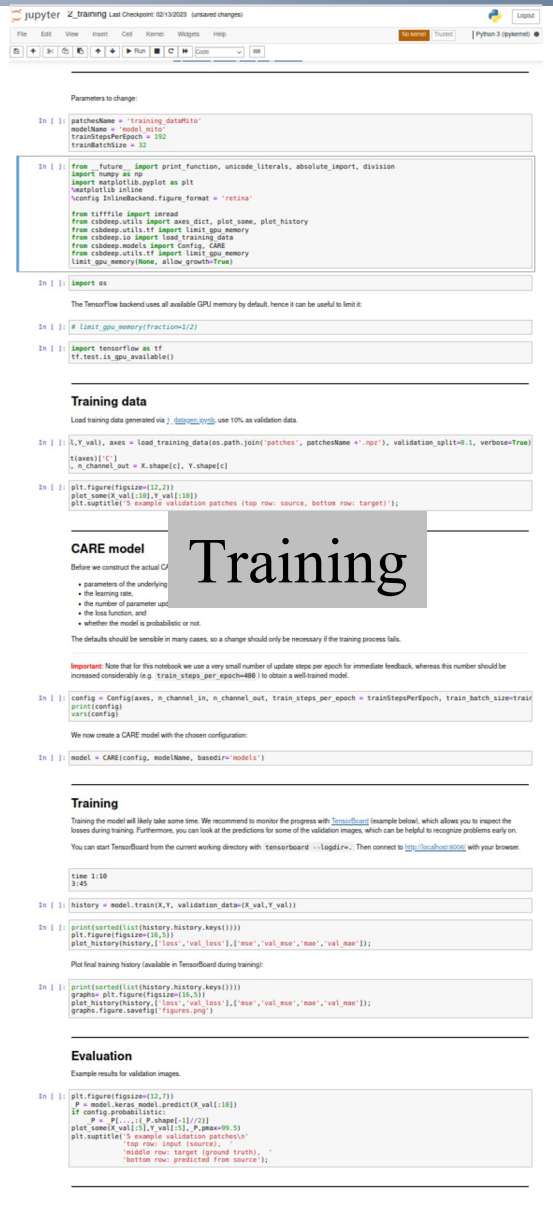
Mean Error Squared

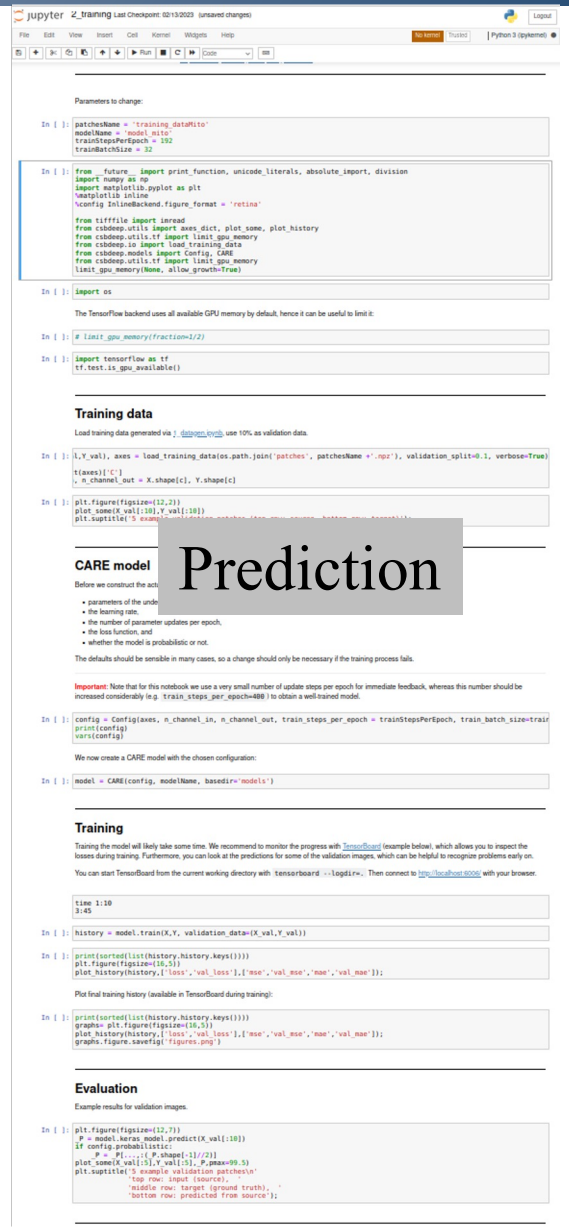
$$\text{MSE} = \frac{1}{n}\sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2$$



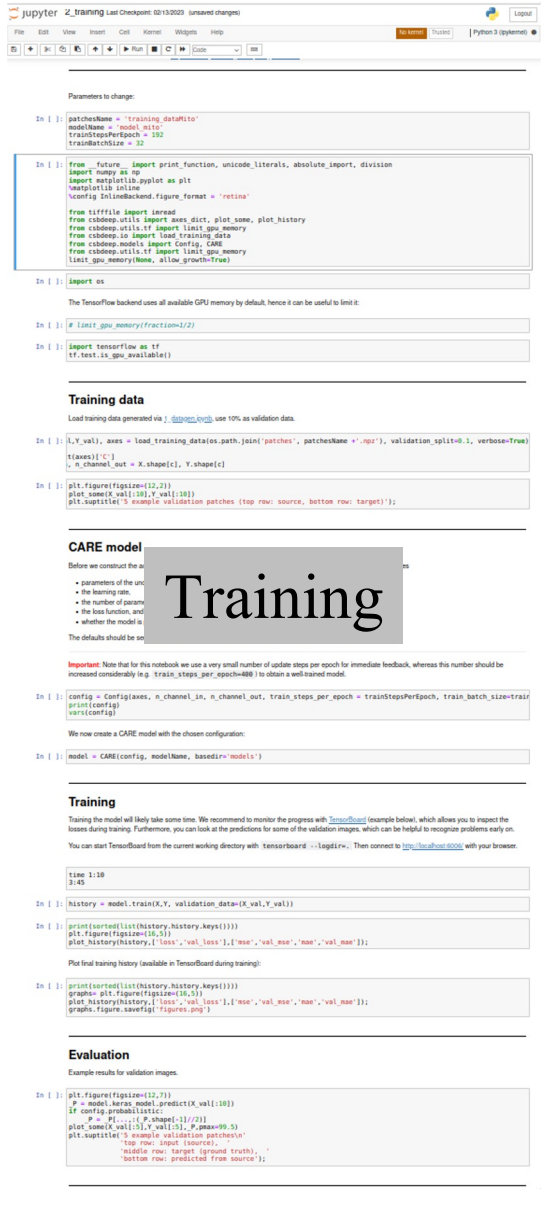
Three essential parts of any Machine Learning Model

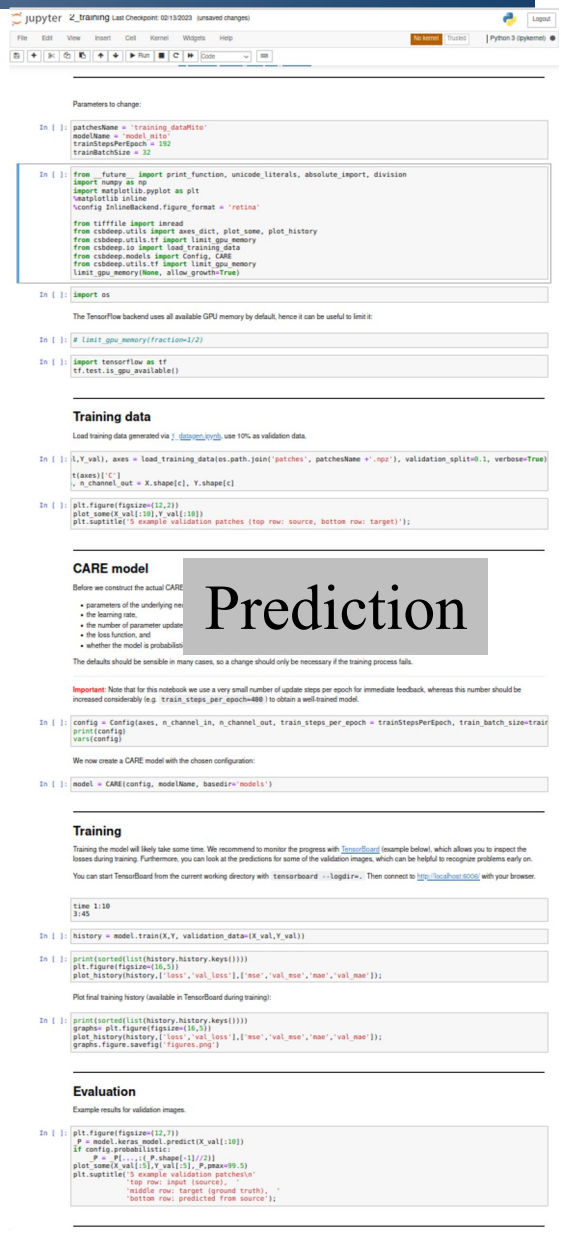






~~Three~~ Four essential parts of any Machine Learning Model





Results

Three models trained:

PreProcessed Data → Denoised Data

Raw Data → PreProcessed and Denoise Data

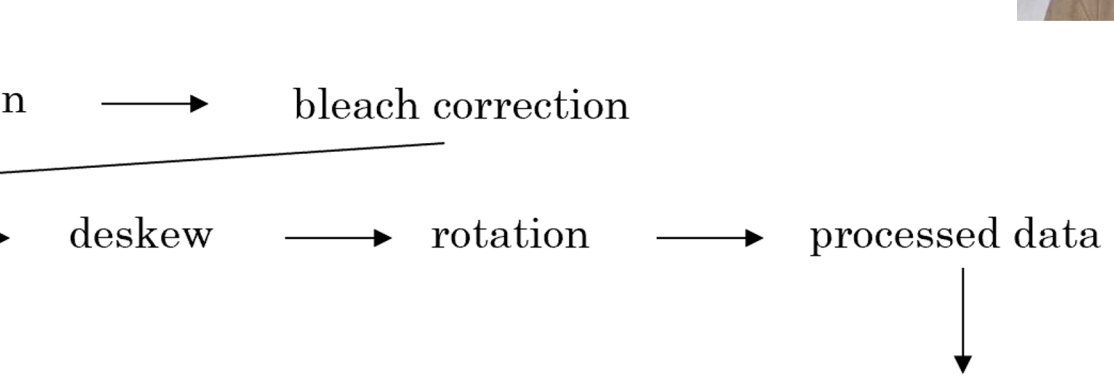
Raw Mito Data → PreProcessed and Denoise Data

Results

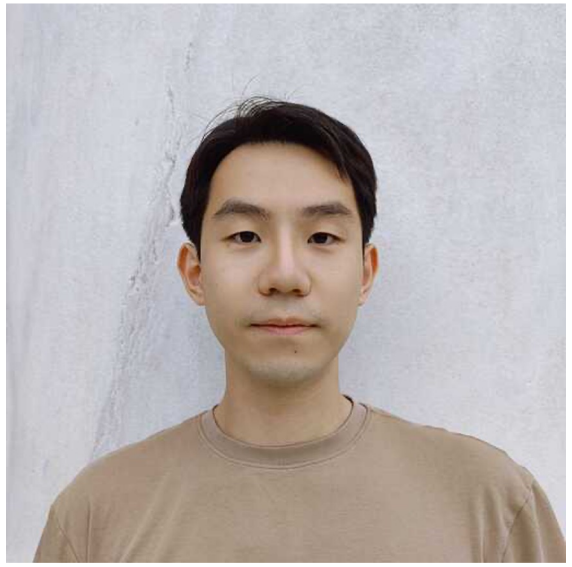
Full pipeline

Iterate:

- Date
  - Sample
    - Location
      - Channel
        - Frame



Save the data under a different folder with same file structure

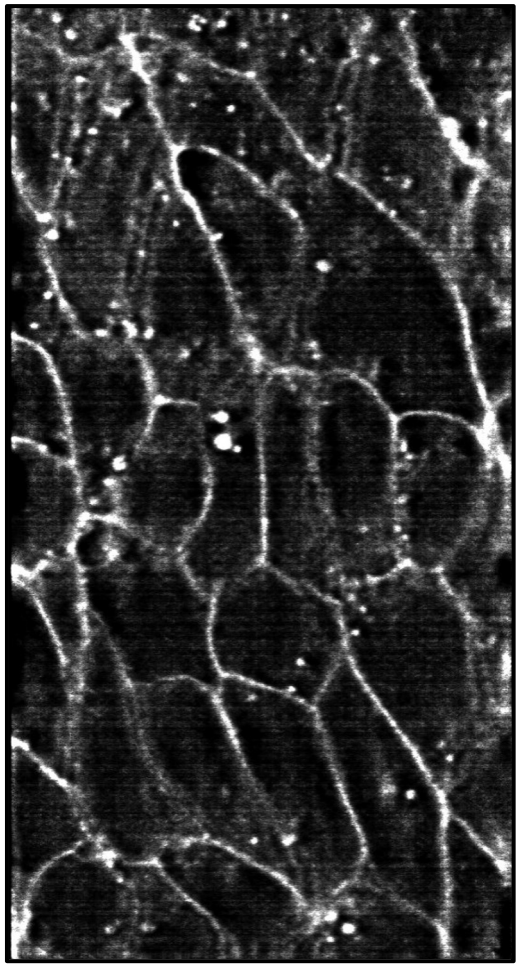


Zachary Wang

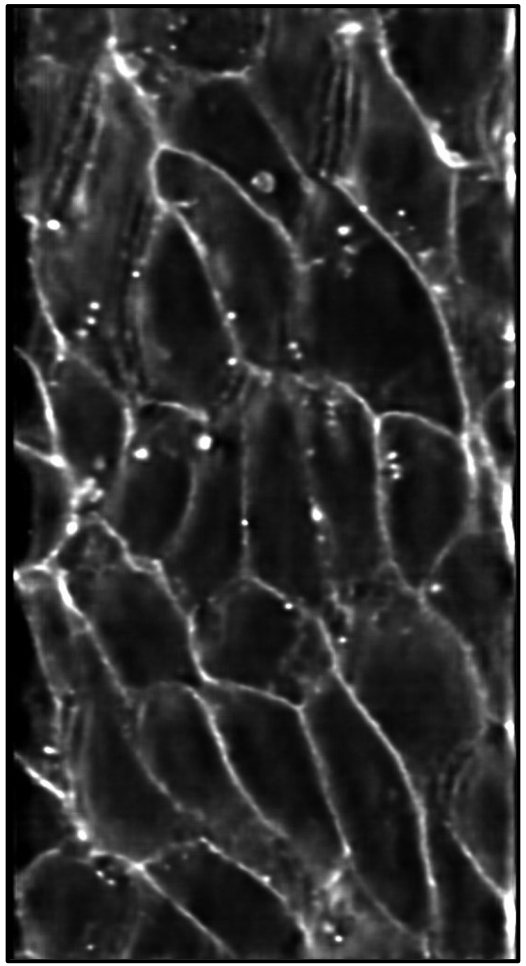
Model_1.0 PreProcessed Data – Denoised Data

sample 10

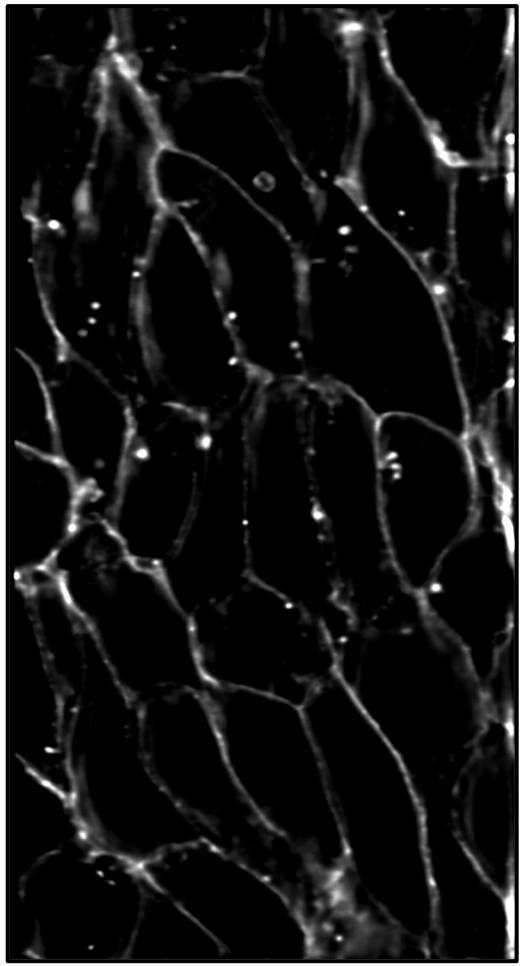
Membrane



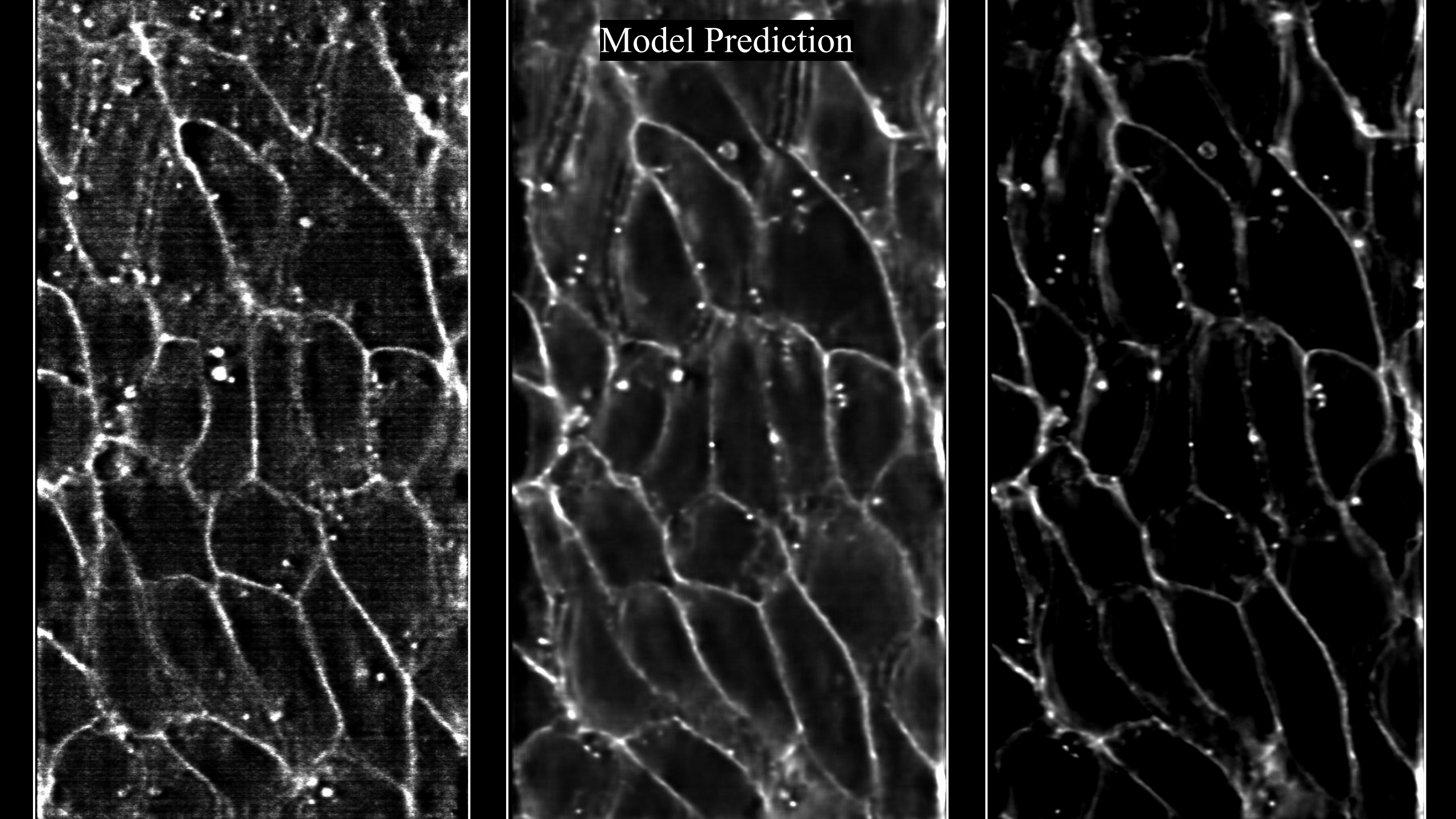
Low – Laser Power (0.5 % | 0.2%)



Prediction



GT – Laser Power (20% | 10%)

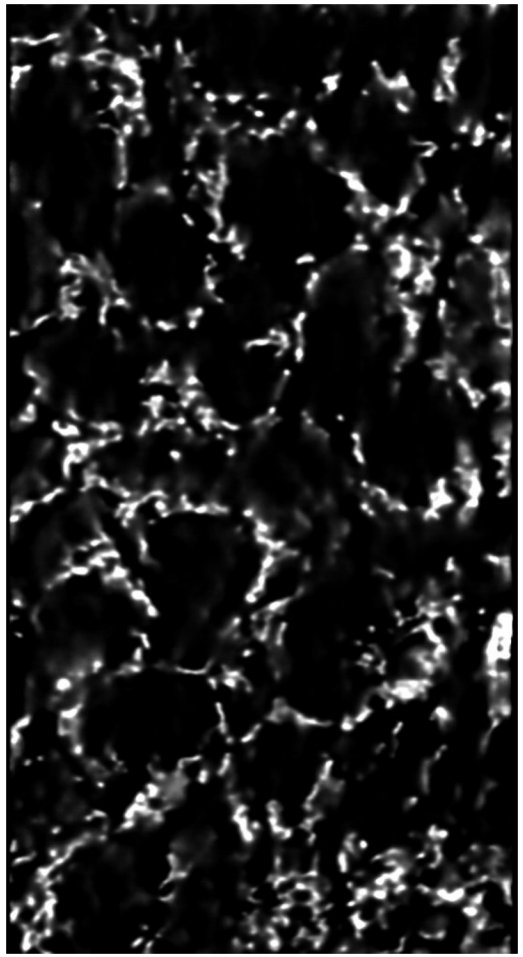

Model Prediction

Model_1.0 PreProcessed Data – Denoised Data

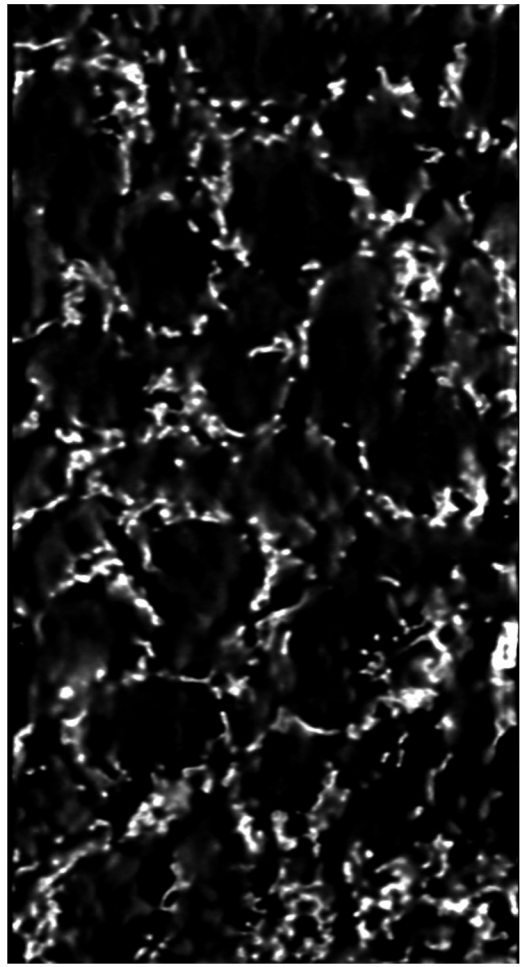
sample 6

Mitochondria

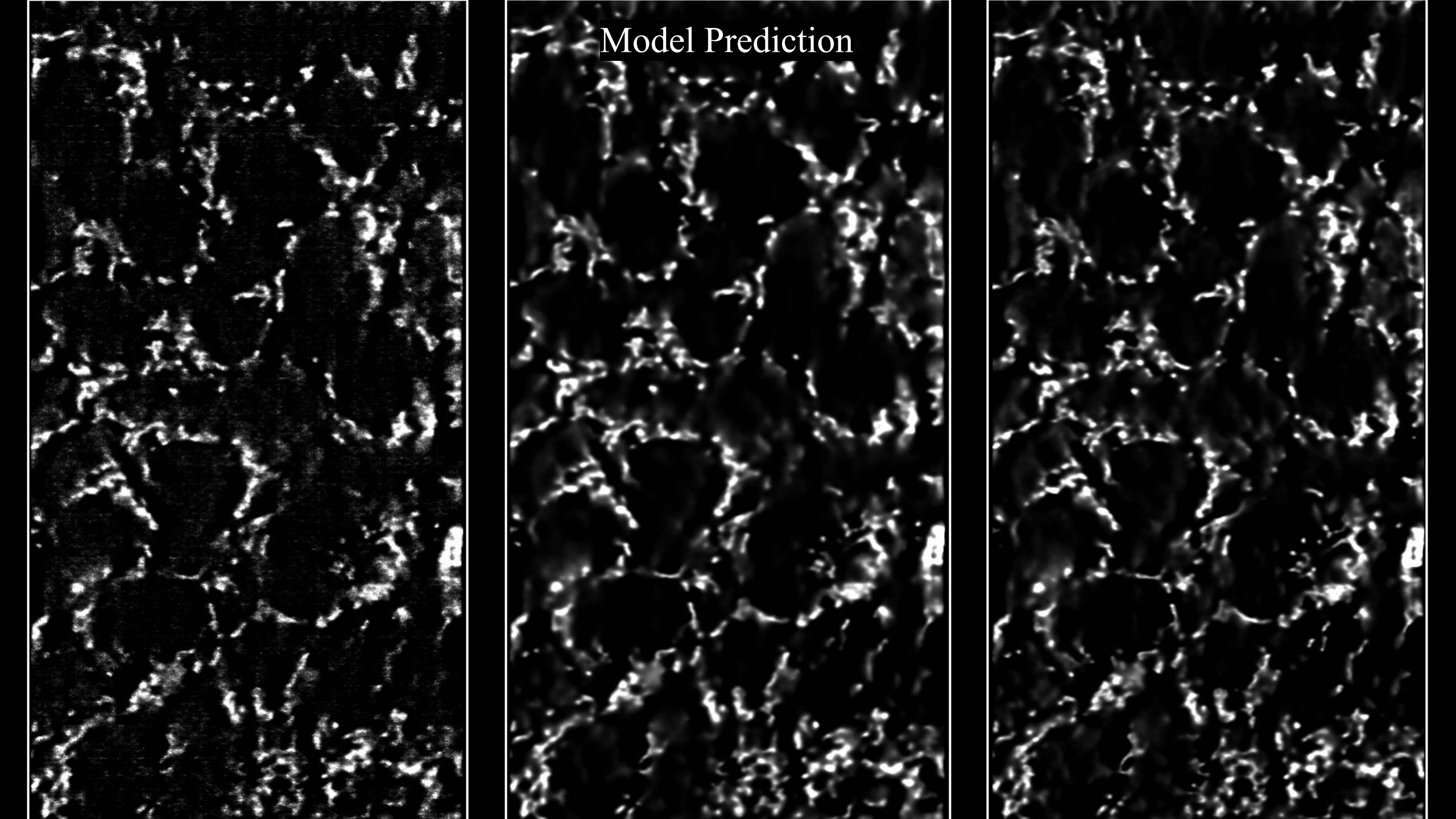
Low – Laser Power (0.5 | 0.2)



Prediction



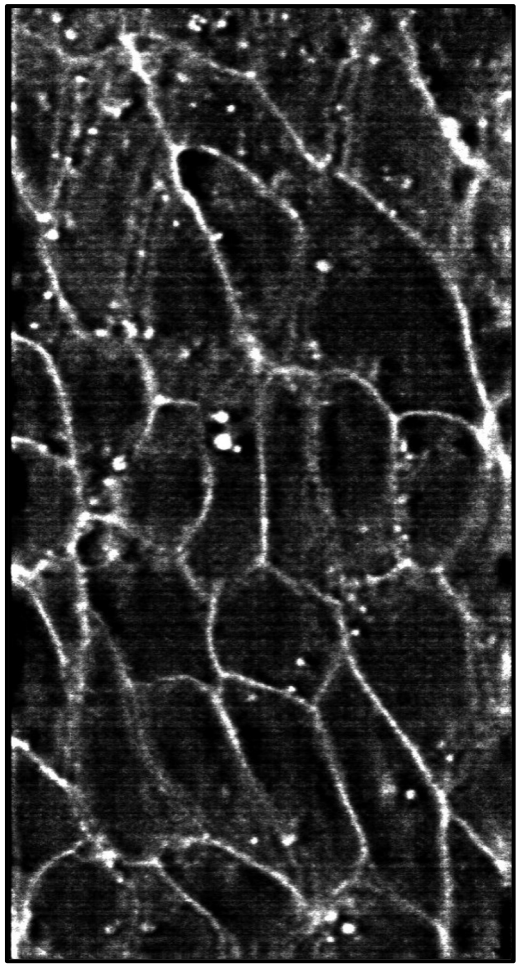
GT – Laser Power (20% | 10%)


Model Prediction

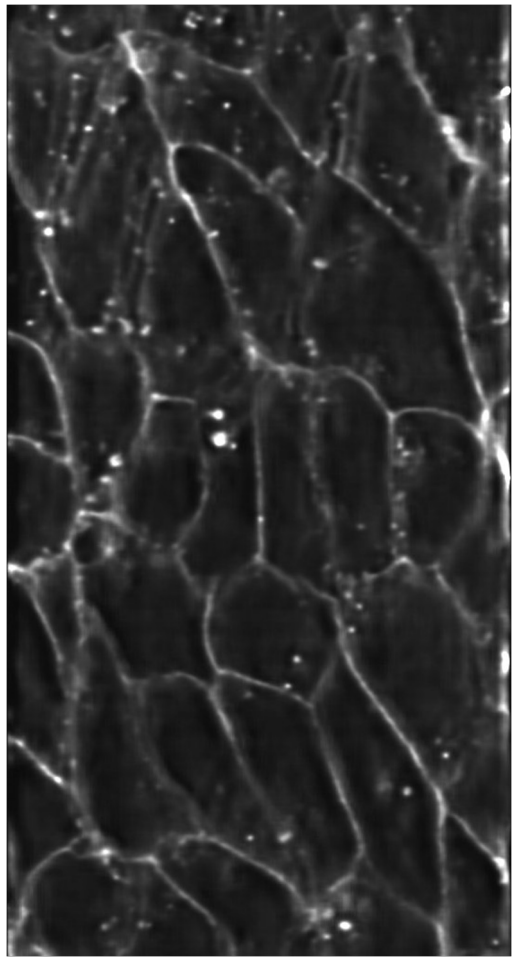
Model_1.1 Raw Data – Denoised Data

sample 10

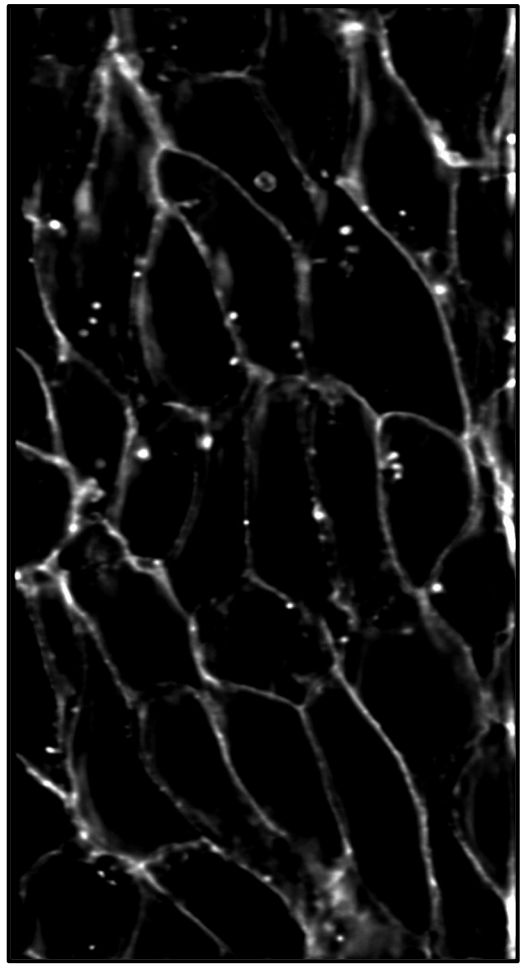
Membrane



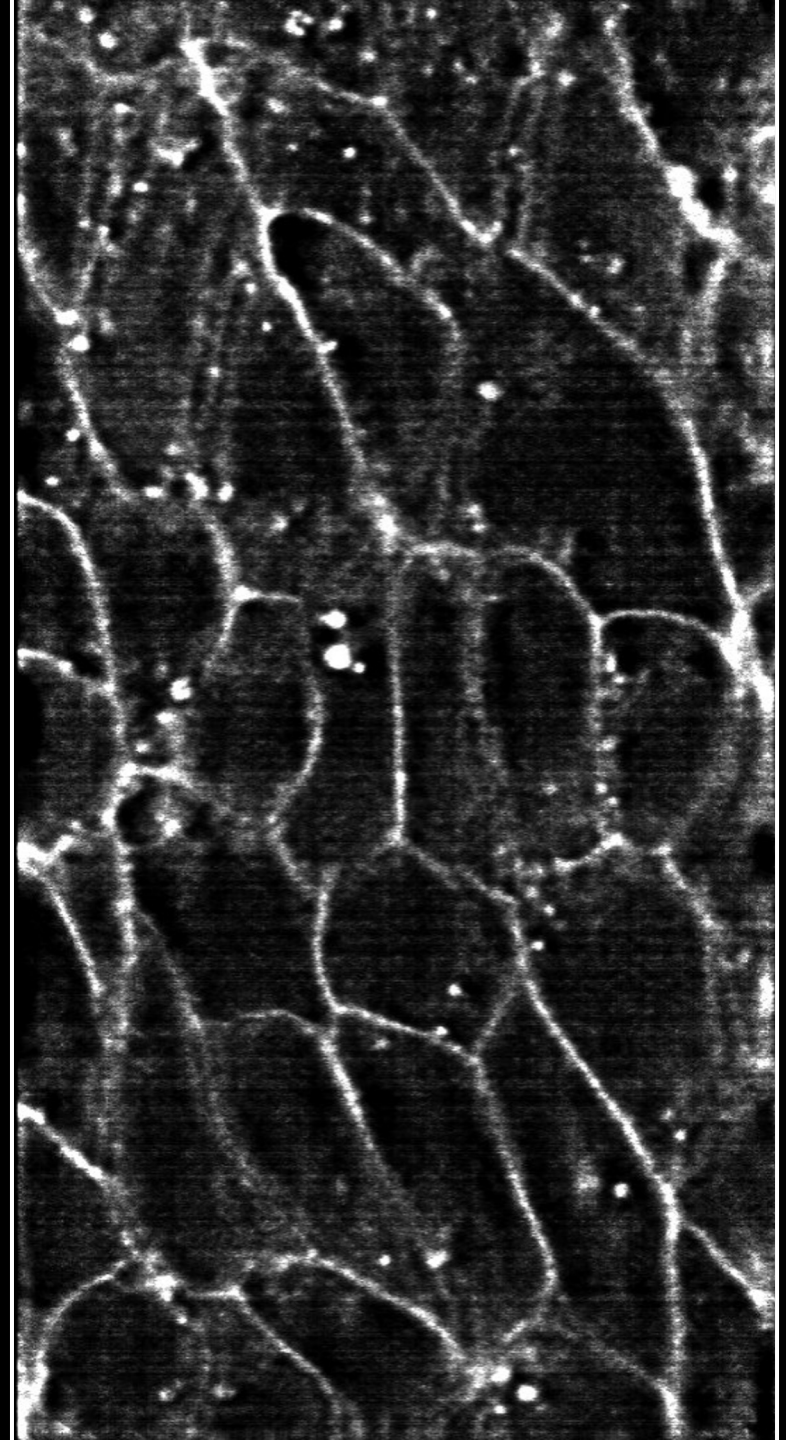
Low – Laser Power (0.5 | 0.2)

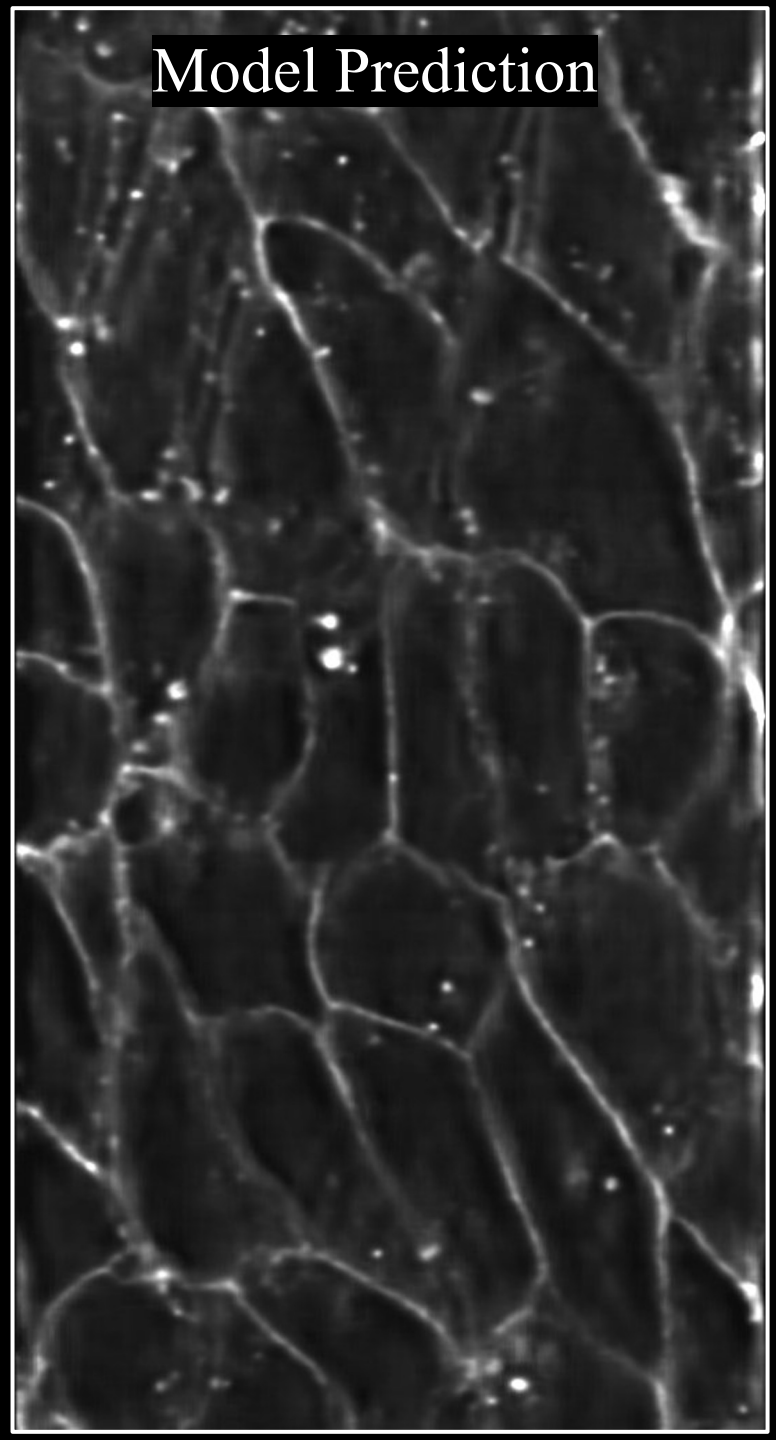


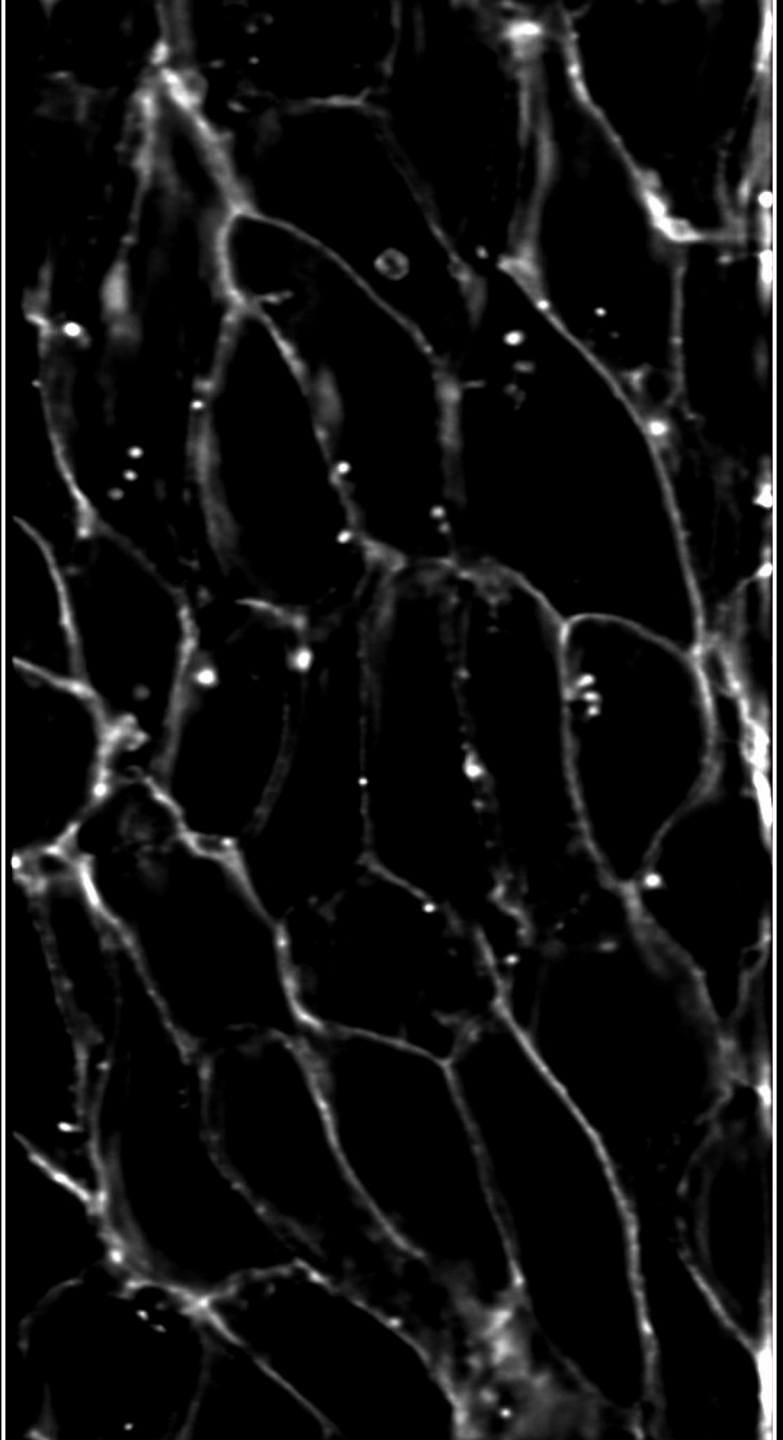
Prediction



GT – Laser Power (20% | 10%)

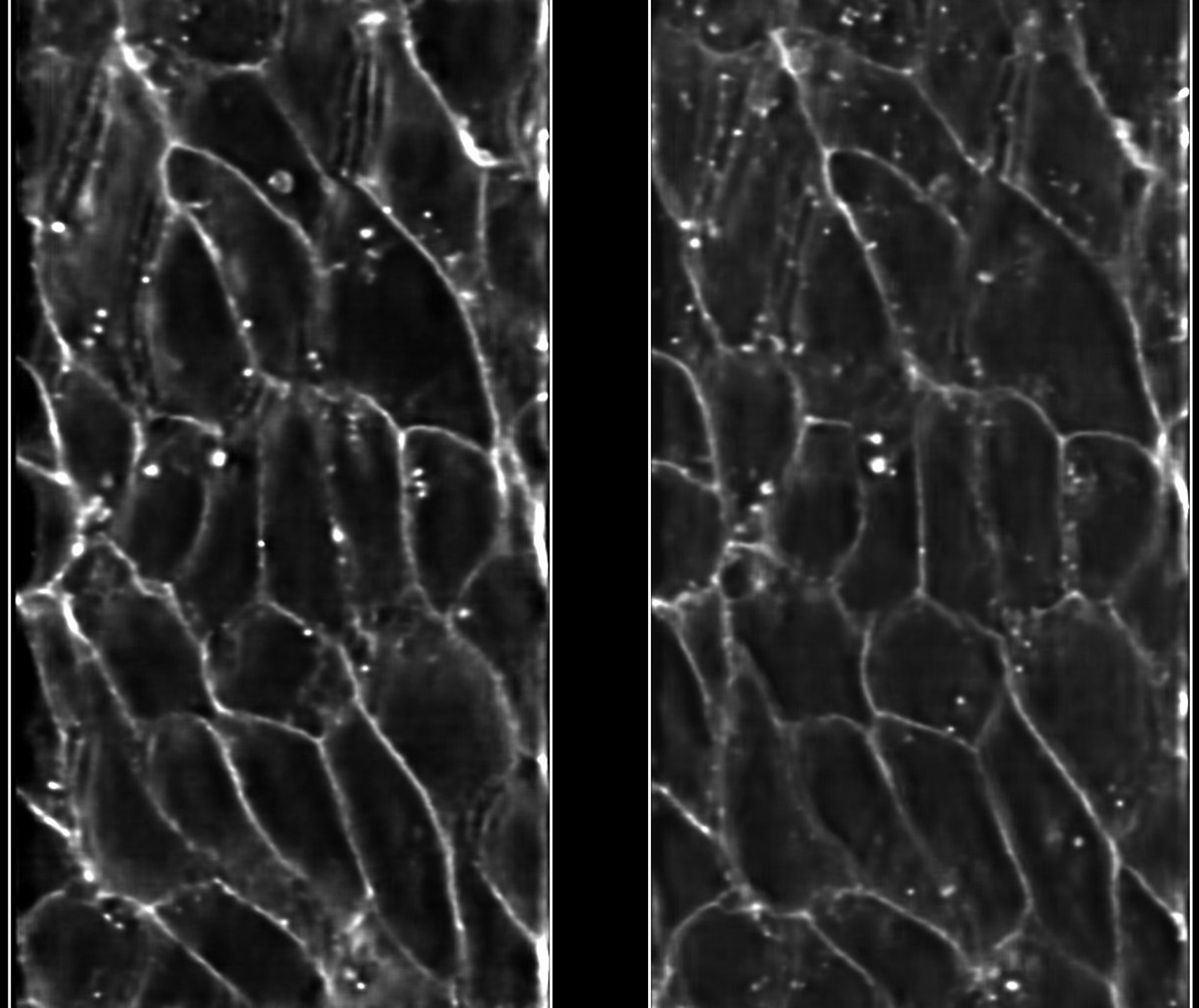



Model Prediction



Sample 10

Model 1.0

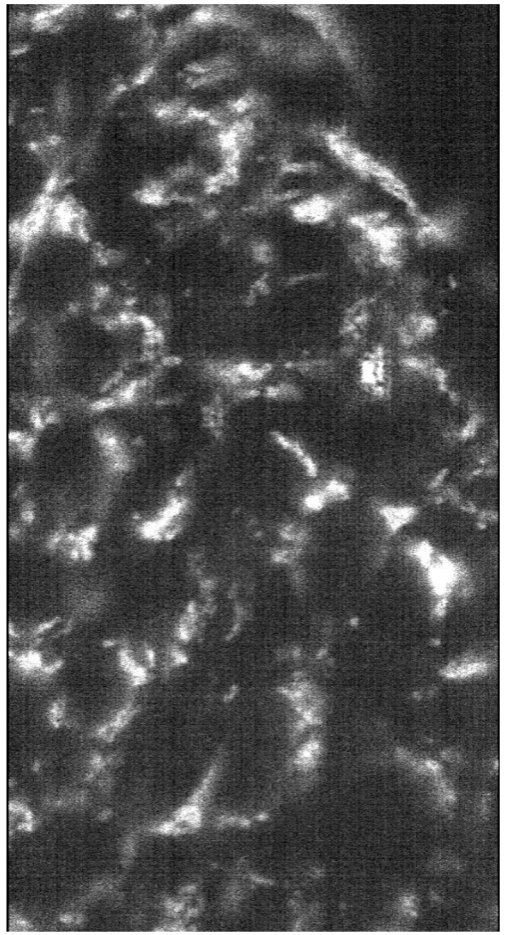


Model 1.1

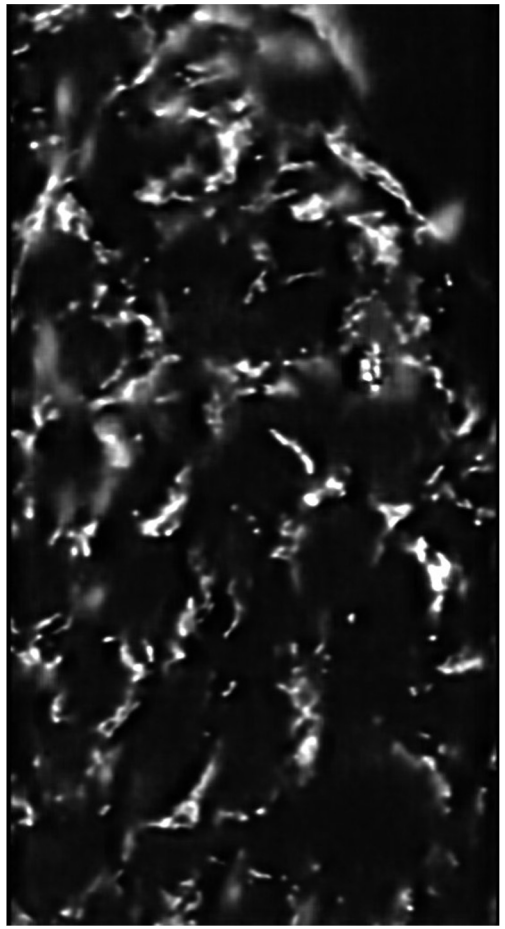
Model_1.1 Raw Data – Denoised Data

sample 8

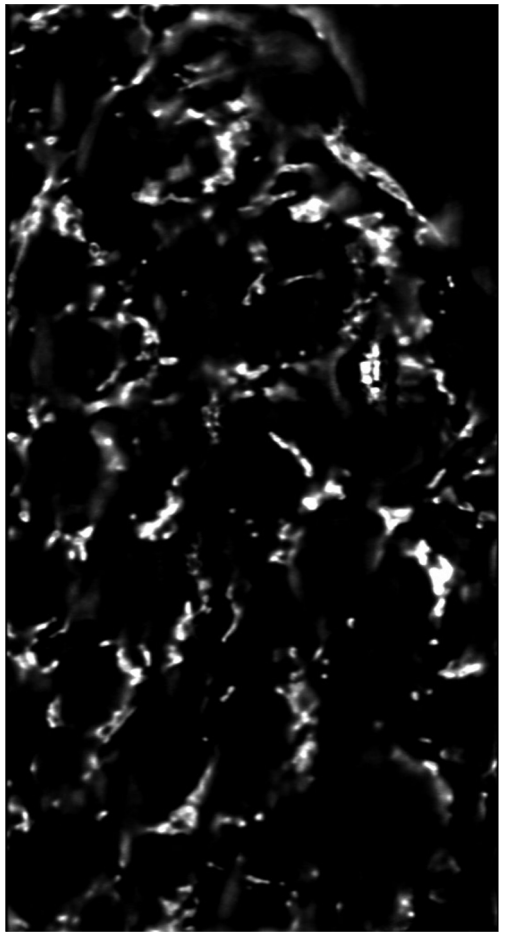
Mitochondria



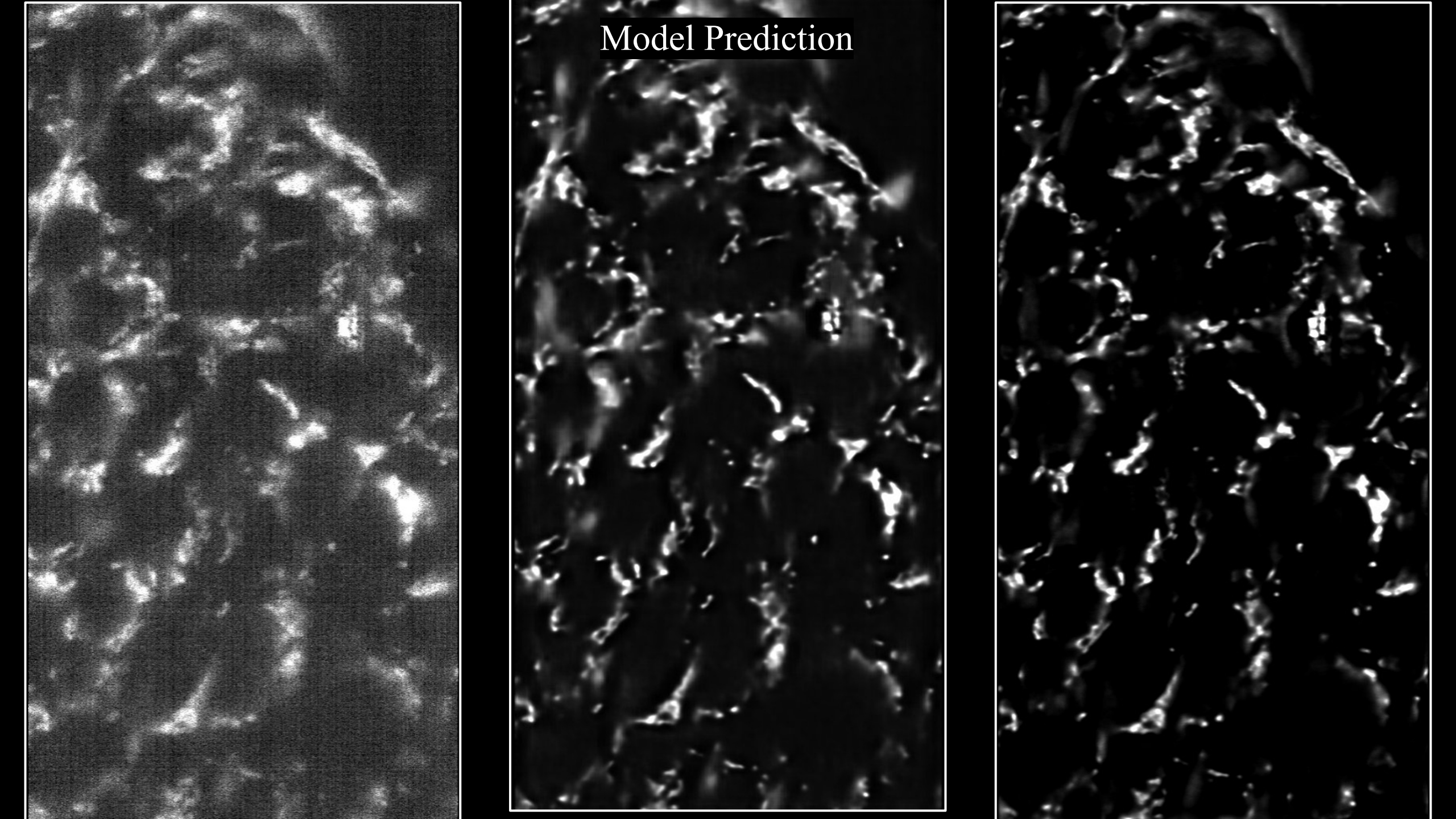
Low – Laser Power (0.5 | 0.2)



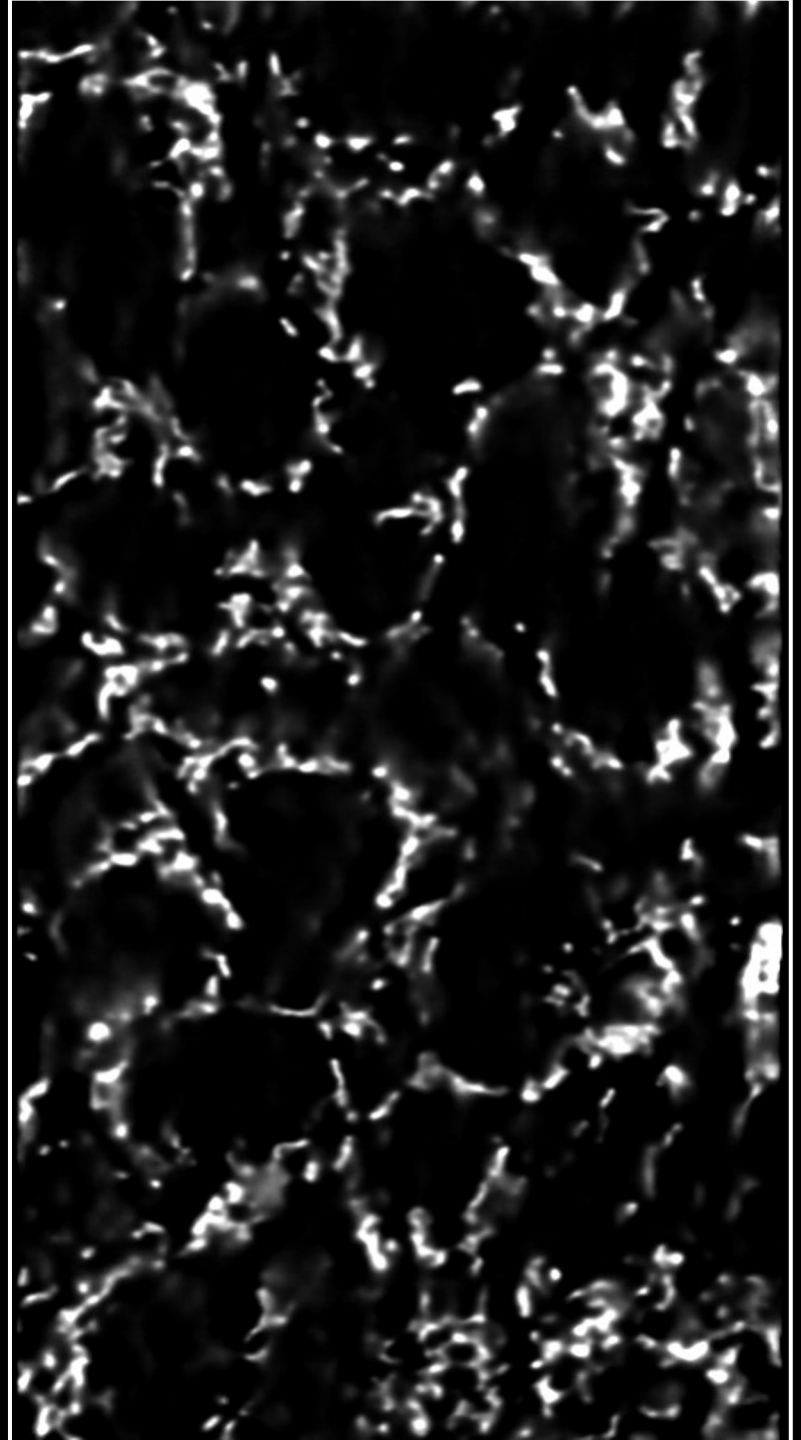
Prediction

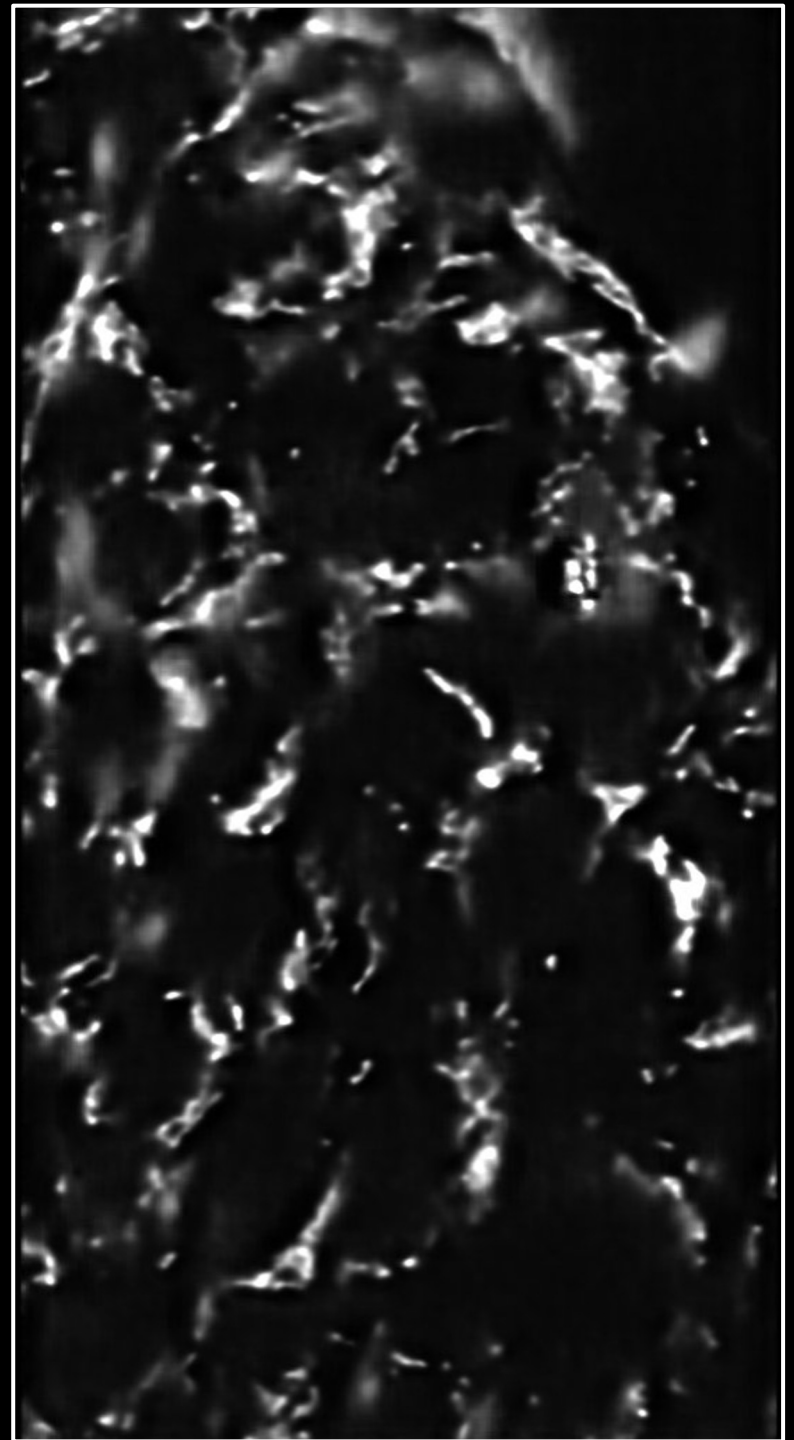


GT – Laser Power (20% | 10%)


Model Prediction

Model 1.0





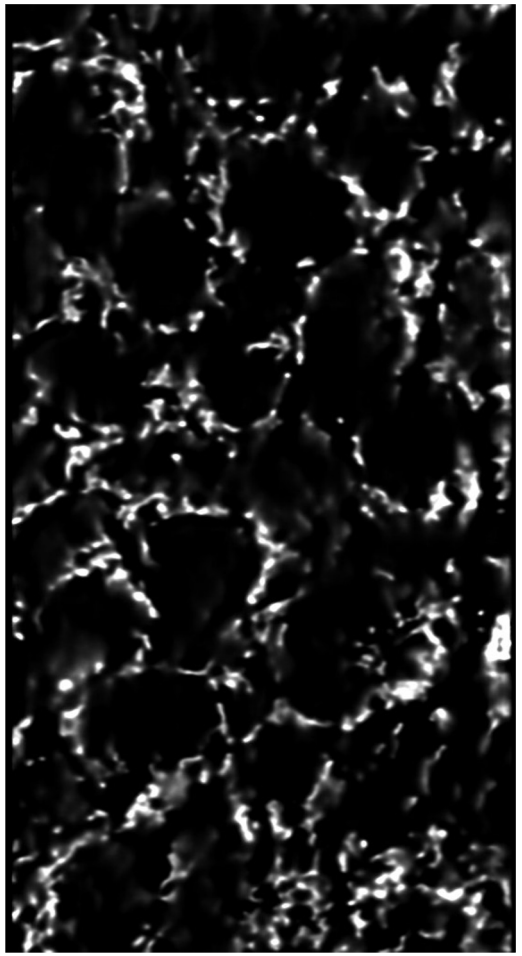
Model 1.1

Model_Mito Raw Data – Denoised Data

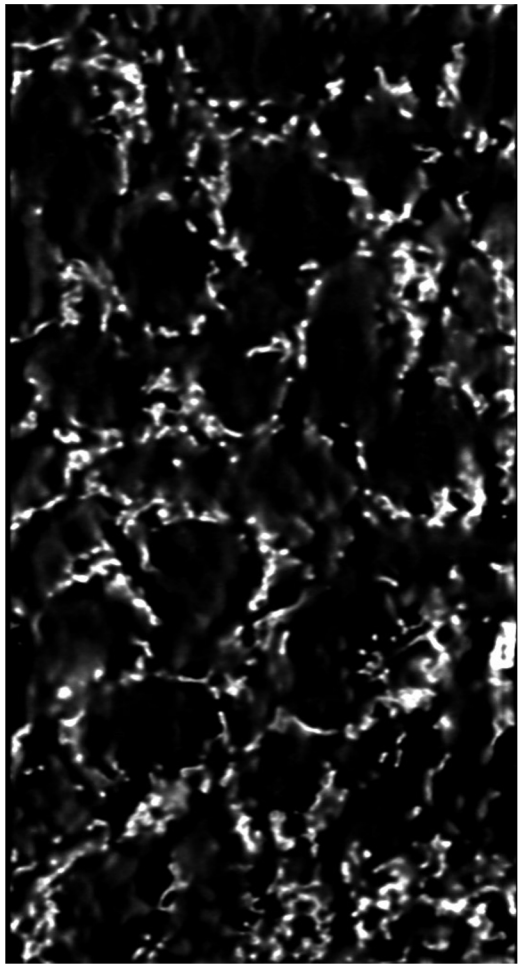
sample 6

Mitochondria

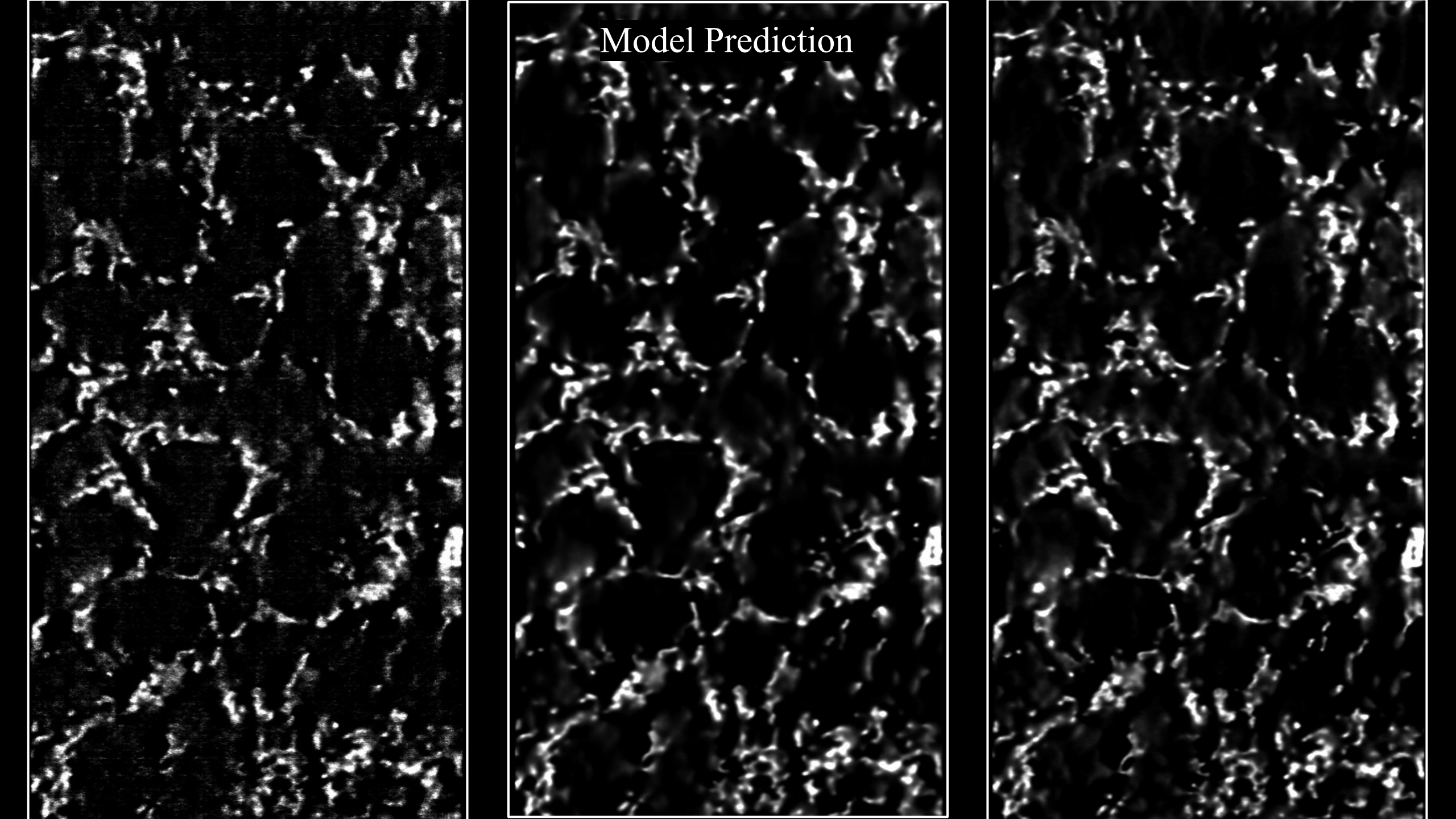
Low – Laser Power (0.5 | 0.2)

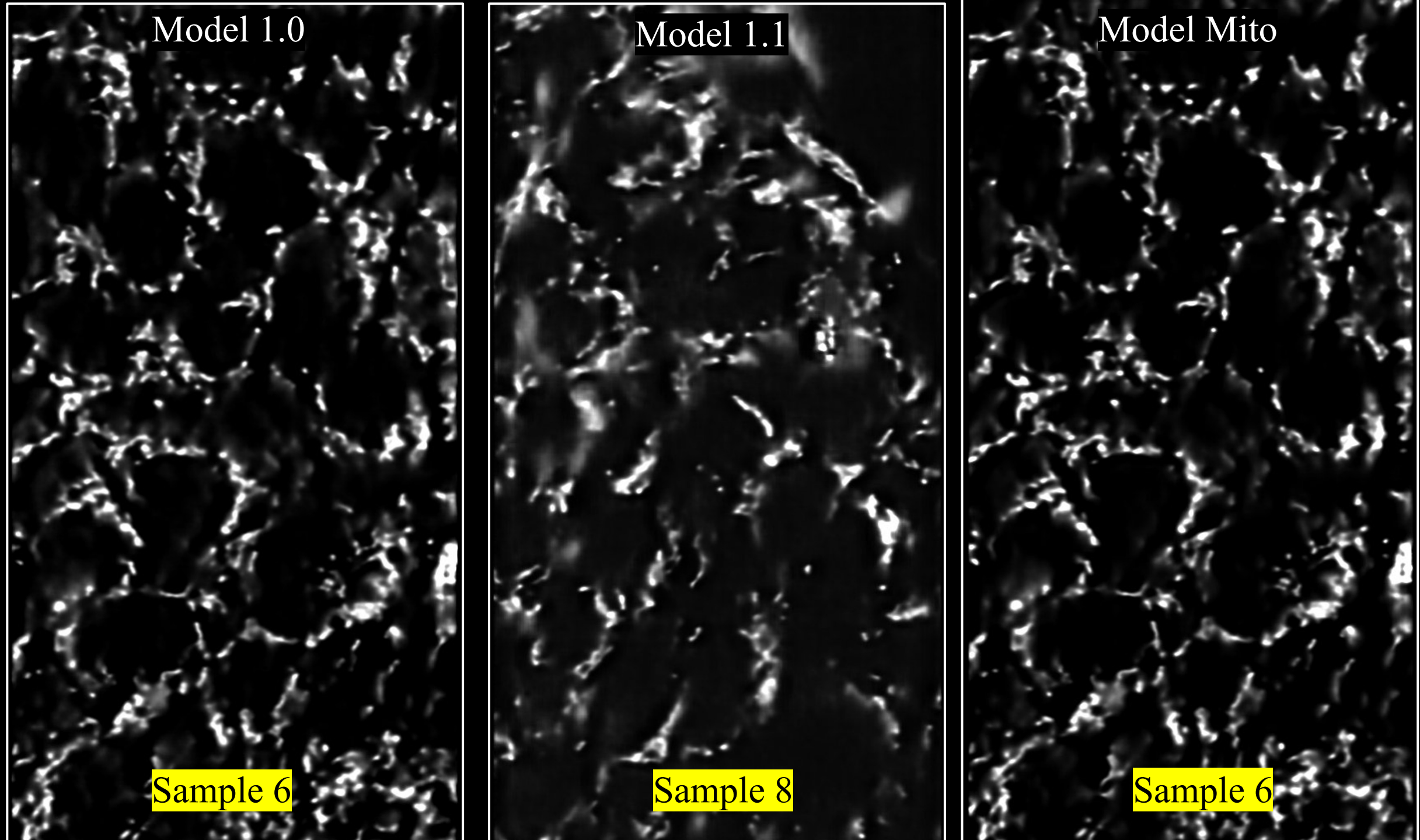


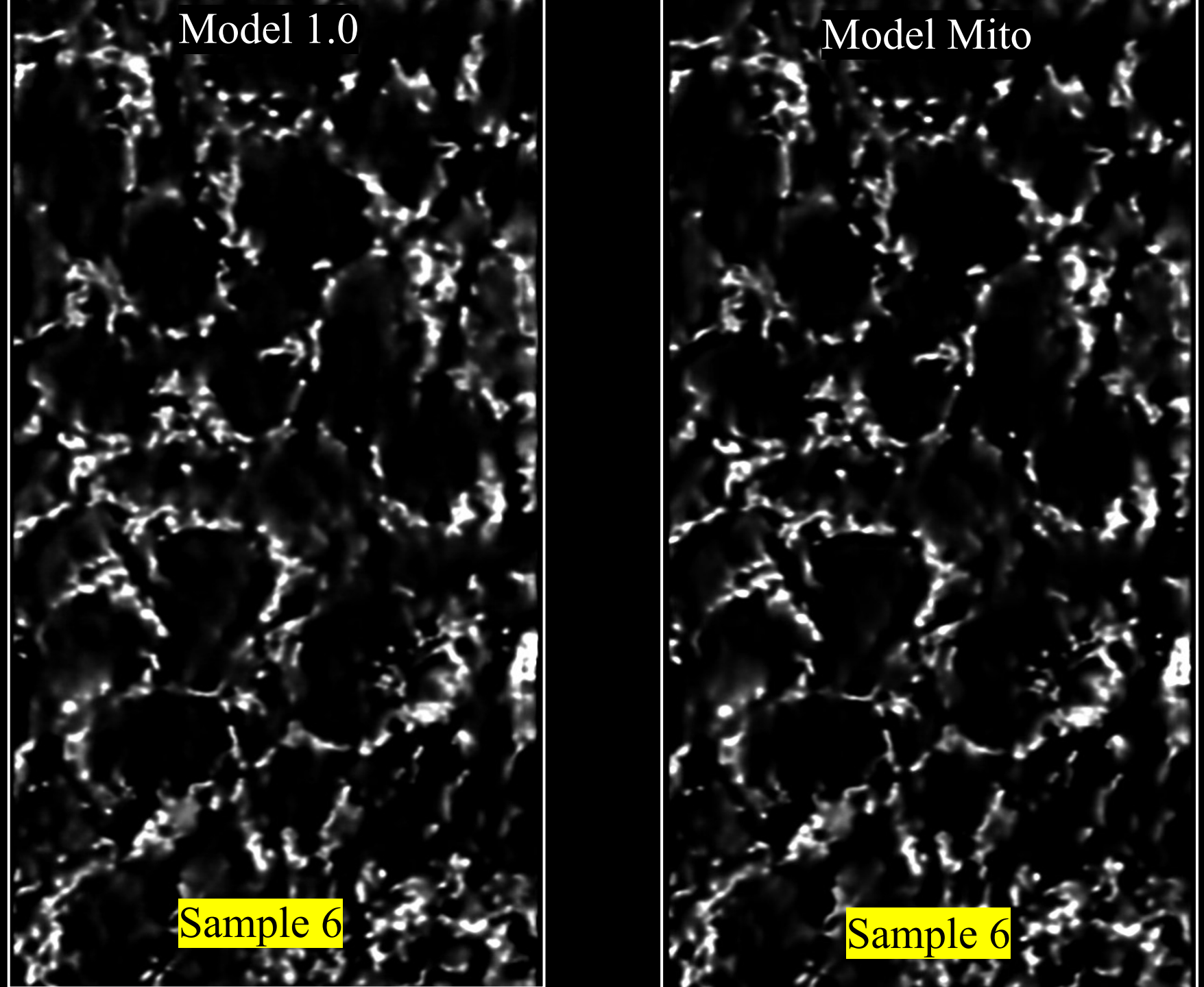
Prediction



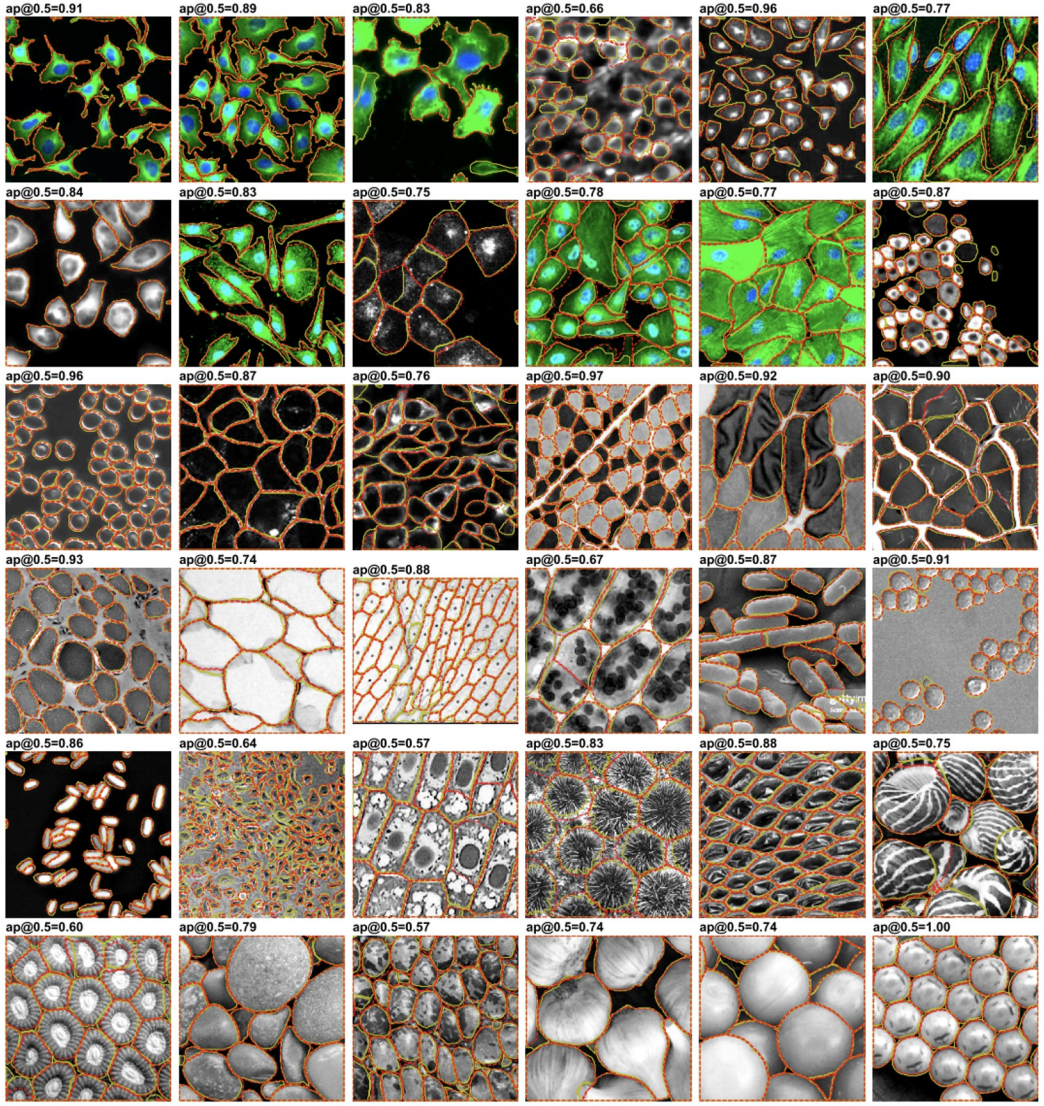
GT – Laser Power (20% | 10%)


Model Prediction


Model 1.0
Model 1.1
Model Mito
Sample 6
Sample 8
Sample 6


Model 1.0
Sample 6
Model Mito
Sample 6

CARE | CellPose



Cellpose: a generalist algorithm for cellular segmentation

Carsen Stringer[1]*, Michalis Michaelos[1], Marius Pachitariu[1]*
[1]HHMI Janelia Research Campus, Ashburn, VA, USA
* correspondence to (stringerc, pachitarium) @ janelia.hhmi.org

Many biological applications require the segmentation of cell bodies, membranes and nuclei from microscopy images. Deep learning has enabled great progress on this problem, but current methods are specialized for images that have large training datasets. Here we introduce a generalist, deep learning-based segmentation algorithm called Cellpose, which can very precisely segment a wide range of image types out-of-the-box and does not require model retraining or parameter adjustments. We trained Cellpose on a new dataset of highly-varied images of cells, containing over 70,000 segmented objects. To support community contributions to the training data, we developed software for manual labelling and for curation of the automated results, with optional direct upload to our data repository. Periodically retraining the model on the community-contributed data will ensure that Cellpose improves constantly.

Introduction

Quantitative cell biology requires simultaneous measurements of different cellular properties, such as shape, position, RNA expression and protein expression [1]. A first step towards assigning these properties to single cells is the segmentation of an imaged volume into cell bodies, usually based on a cytoplasmic or membrane marker [2–8]. This step can be straightforward when cells are sufficiently separated from each other, for example when a fluorescent marker is expressed sparsely in a subset of cells, or in cultures where cells are dissociated from tissue. However, cells are commonly imaged in tissue, where they are tightly packed and difficult to separate from one another.

Methods to achieve cell body segmentation typically trade off flexibility for automation. In order of increasing automation and decreased flexibility, these methods range from fully-manual labelling [9], to user-customized pipelines involving a sequence of image transformations with user-defined parameters [2, 8, 10, 11], to fully automated methods based on deep neural networks with parameters estimated on large training datasets [4, 5, 7, 12, 13]. Fully automated methods have many advantages, such as reduced human effort, increased reproducibility and better scalability to big datasets from large screens. However, these methods are typically trained on specialized datasets, and do not generalize well to other types of data, requiring new human-labelled images to achieve best performance on any one image type.

Recognizing this problem in the context of nuclear segmentation, a recent Data Science Bowl challenge amassed a dataset of varied images of nuclei from many different laboratories [14]. Methods trained on this dataset can generalize much more widely than those trained on data from a single lab. The winning algorithms from the challenge used established computer vision algorithms like Mask R-CNN [15, 16] and adapted the algorithms for the biological problem. Following the competition, this dataset generated further progress, with other methods like Stardist and nucleAIzer being developed specifically for this dataset [17, 18].

In this work, we followed the approach of the Data Science Bowl team to collect and segment a large dataset of cell images from a variety of microscopy modalities and fluorescent markers. We had no advance guarantee that we could replicate the success of the Data Science Bowl competition, because images of cells are much more varied and diverse than images of nuclei. Thus, it was possible that no single model would segment all possible images of cells, and indeed we found that previous approaches performed poorly on this task. We hypothesized that their failure was due to a loss of representational power for the complex cell shapes and styles in our dataset, and developed a new model with better expressive power called Cellpose. We first describe the architecture of Cellpose and the new training dataset, and then proceed to show performance benchmarks on test data. The Cellpose package with a graphical user interface (Figure S1) can be installed from `www.github.com/mouseland/cellpose` or tested online at `www.cellpose.org`.

Results

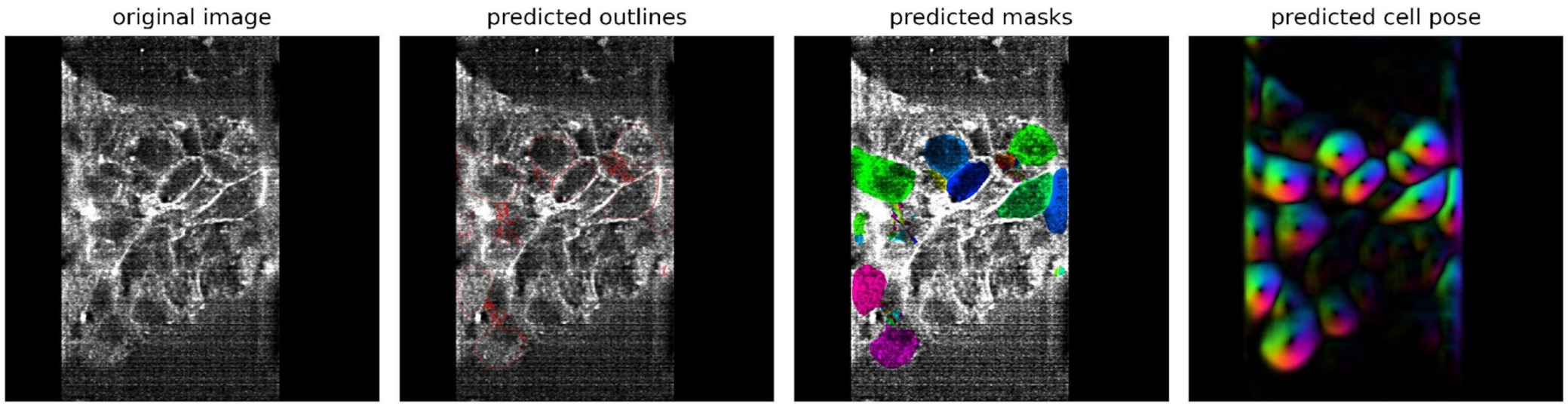
Model architecture

In developing an architecture for instance segmentation of objects, we first noted that a neural network cannot directly predict the cell masks in their standard representation as an image of pixel labels, because the labels are assigned arbitrary, inter-changeable numbers. Instead, we must first convert the masks of all cells in the training set to a representation that *can*

CARE | CellPose

Slice 98

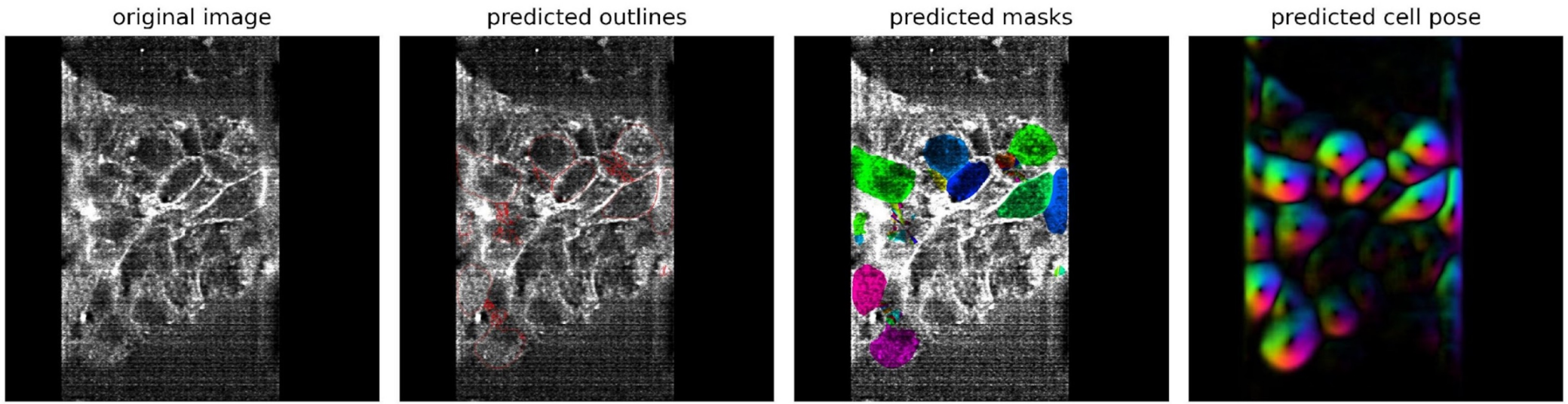
Raw



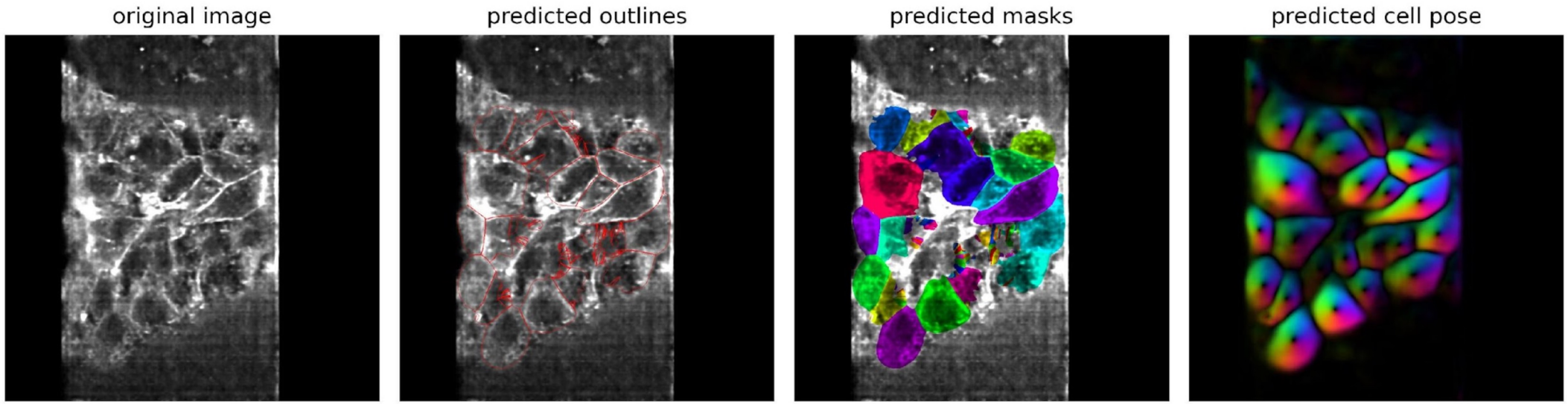
CARE | CellPose

Slice 98

Raw

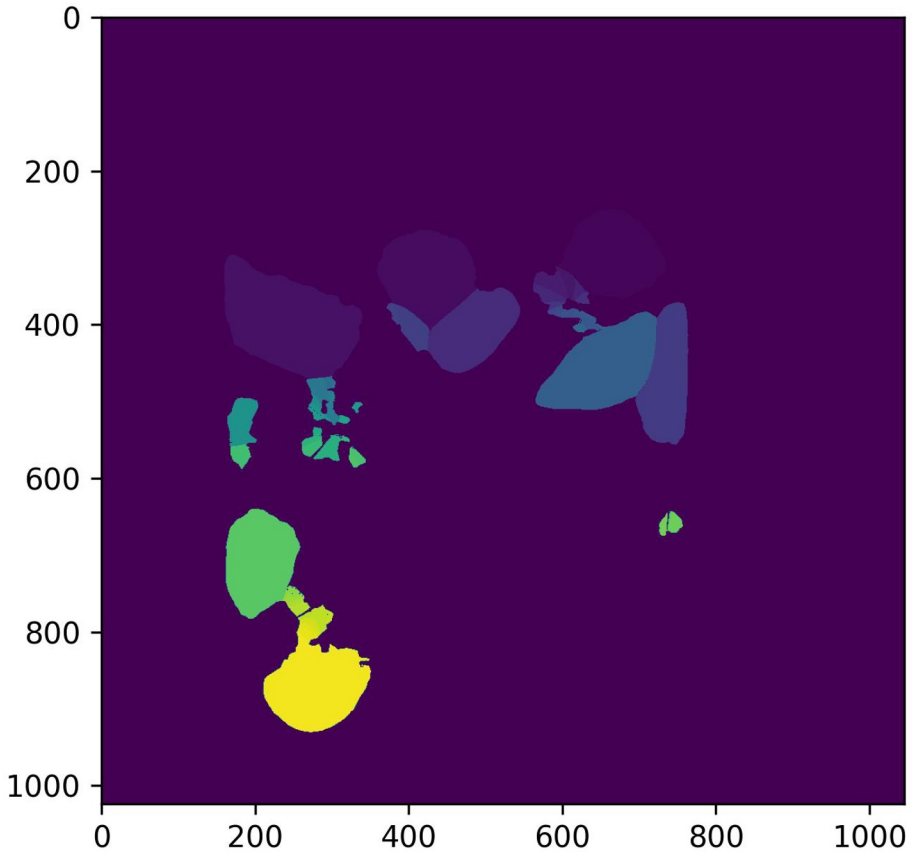


CARE

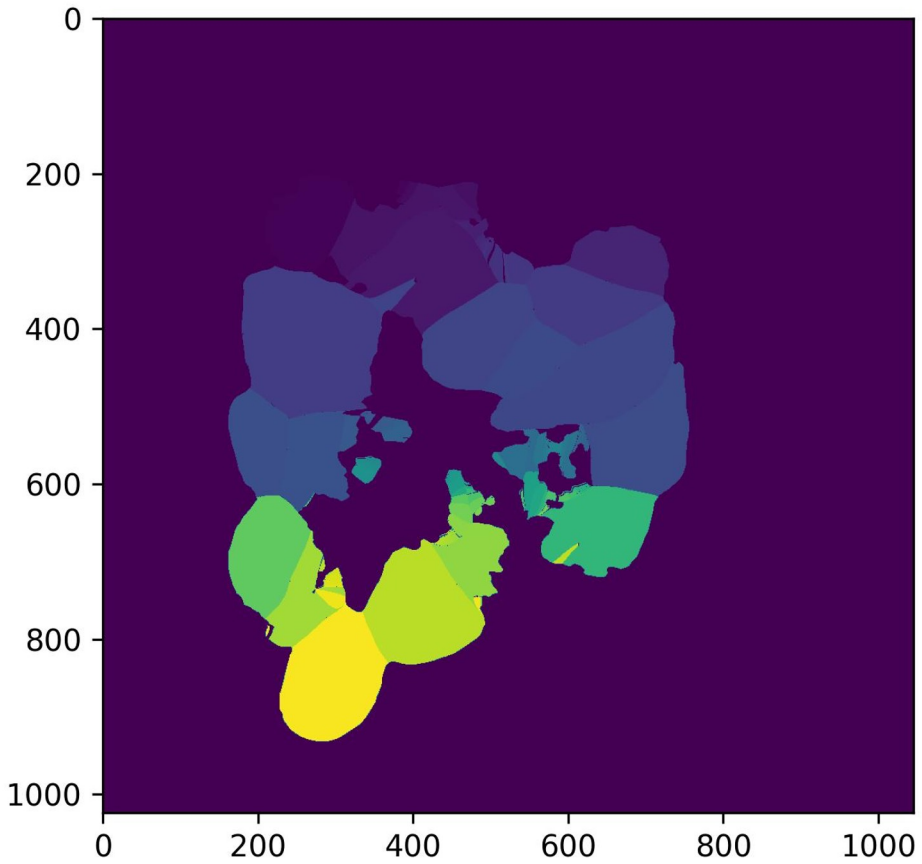


CARE | CellPose

Slice 98



Raw

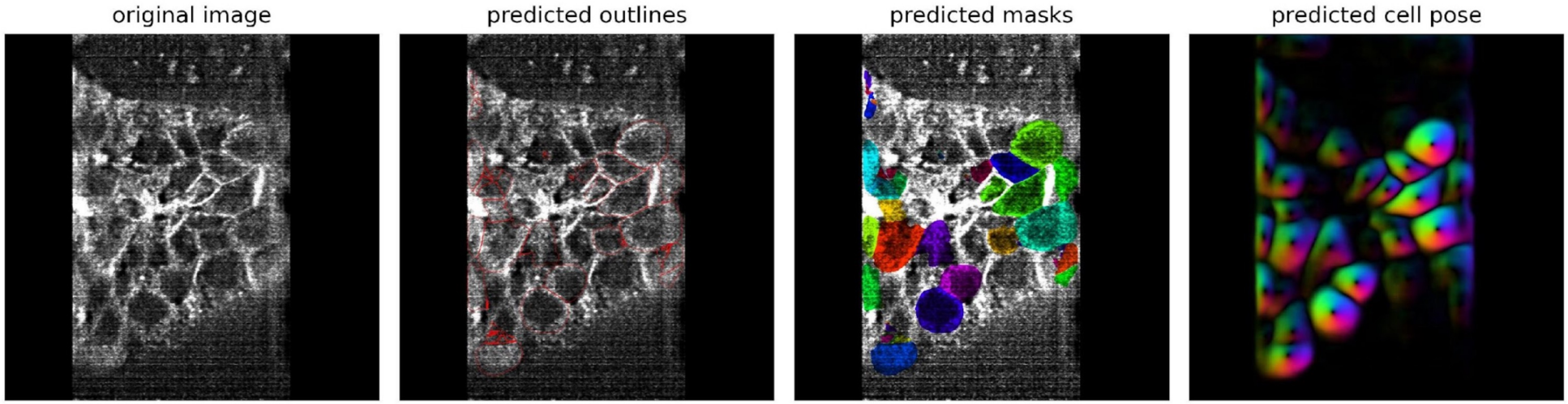


CARE

CARE | CellPose

Slice 117

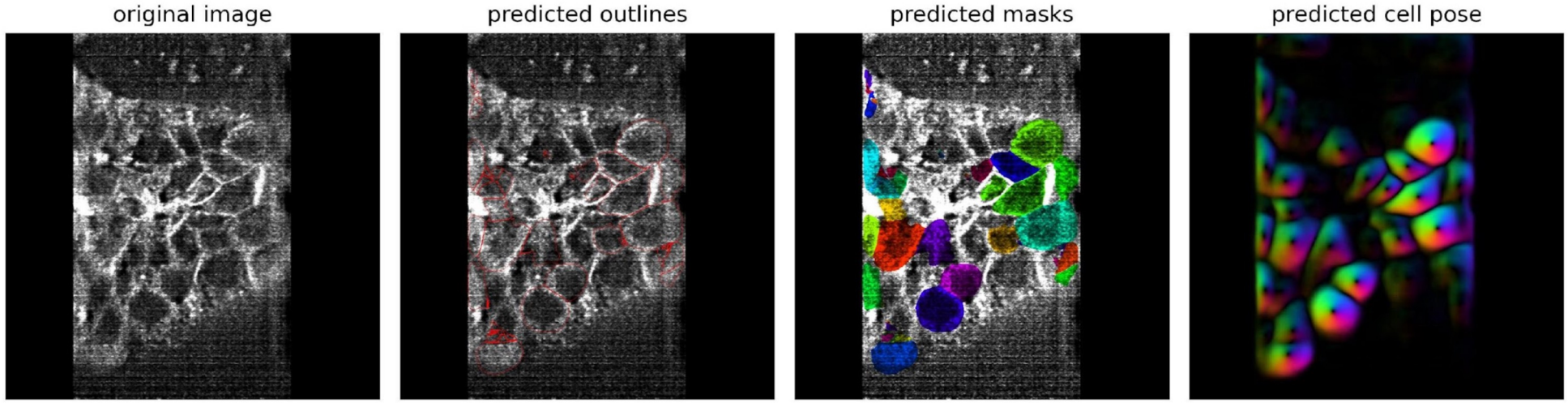
Raw



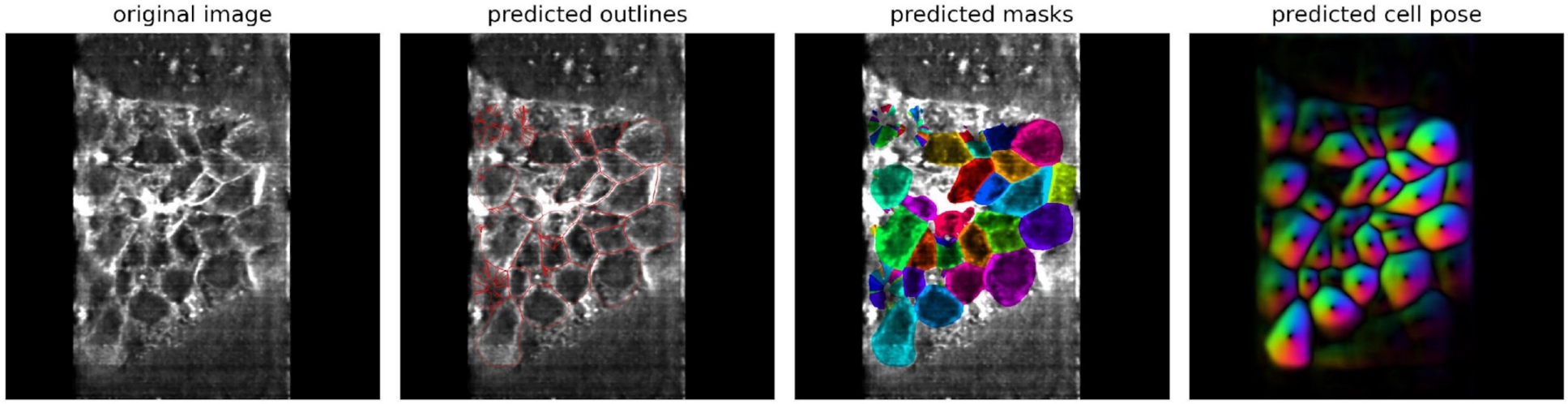
CARE | CellPose

Slice 117

Raw

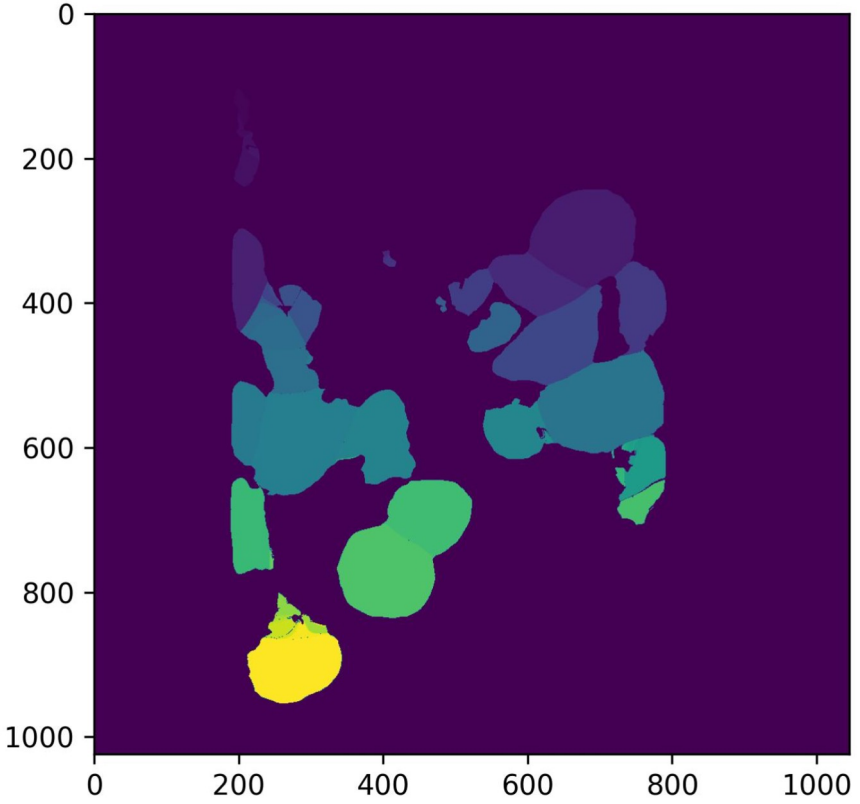


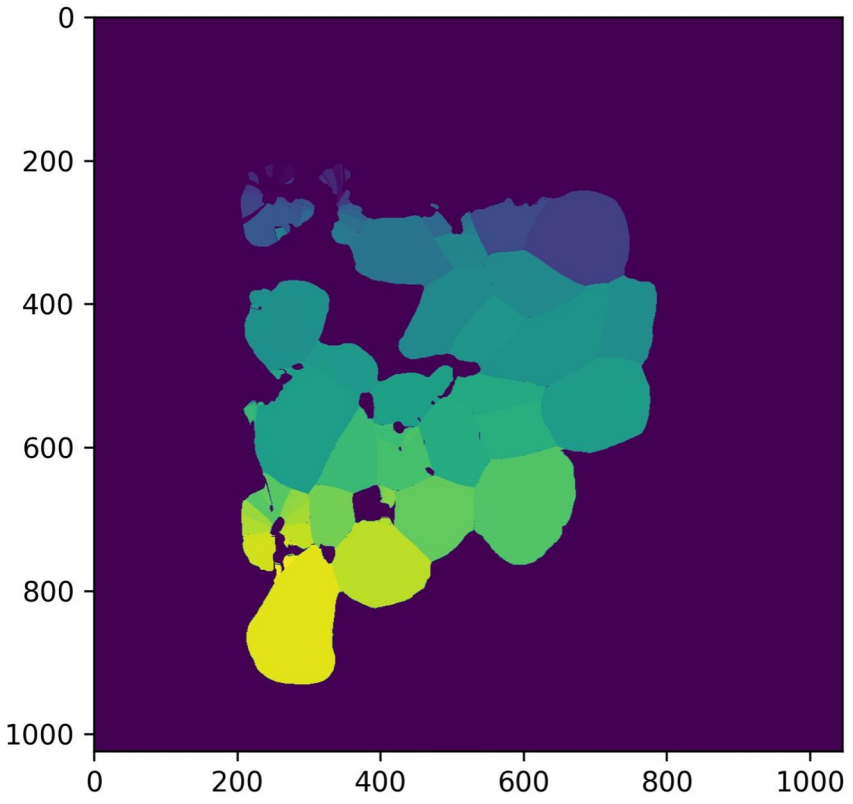
CARE



CARE | CellPose

Slice
117





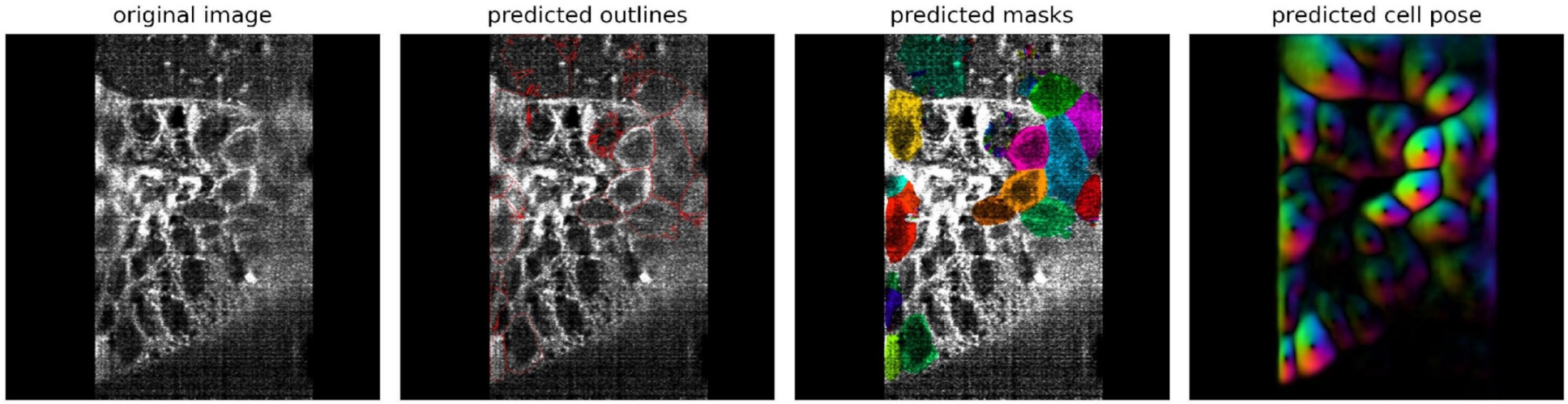
Raw

CARE

CARE | CellPose

Slice 150

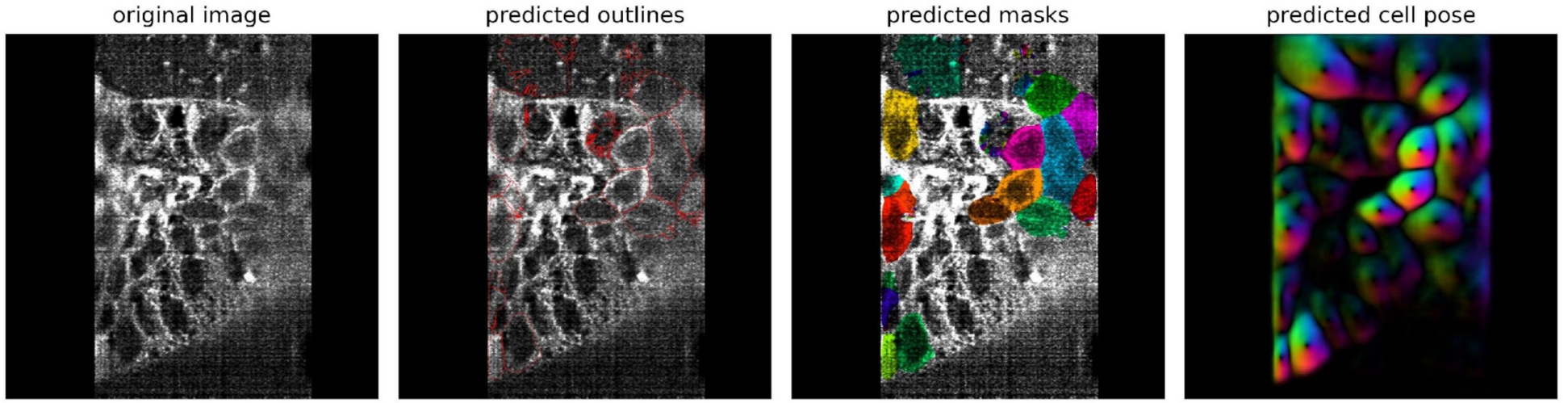
Raw



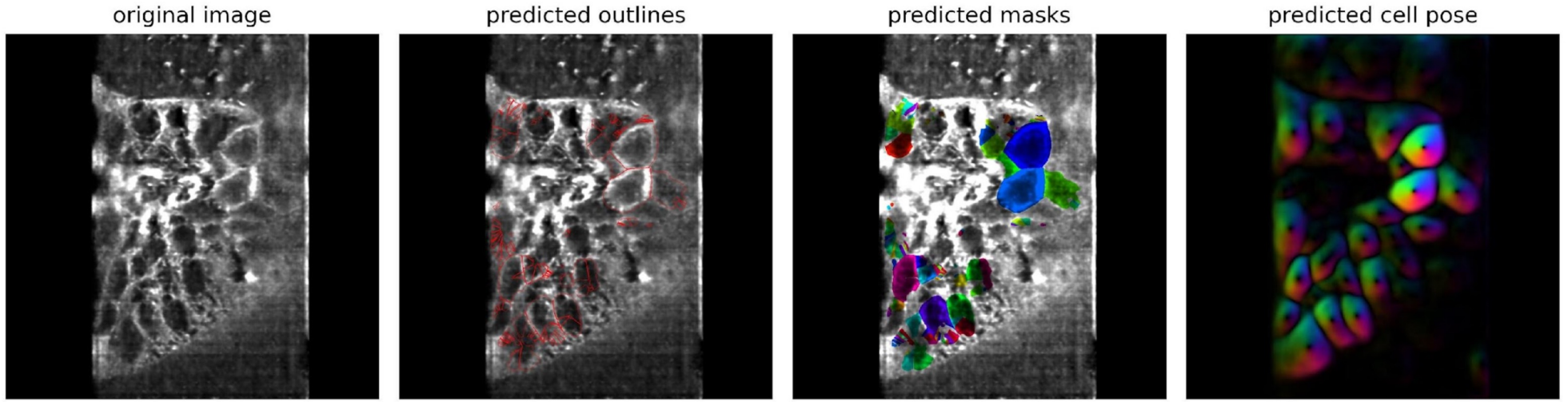
CARE | CellPose

Slice 150

Raw

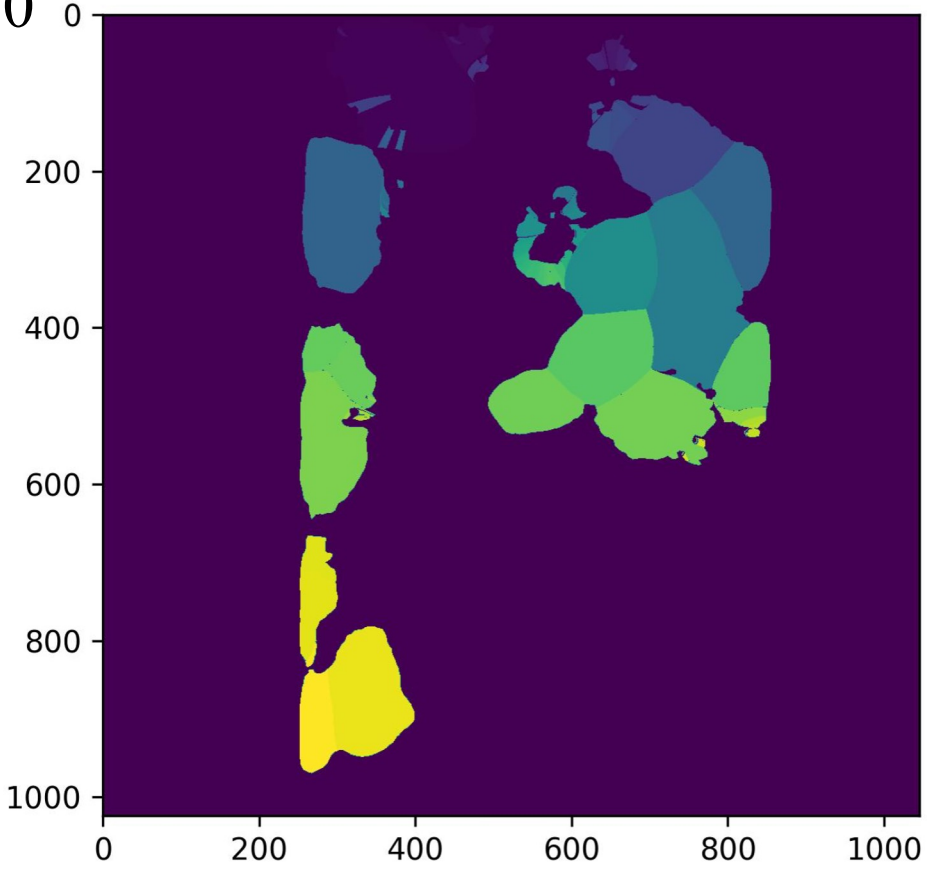


CARE

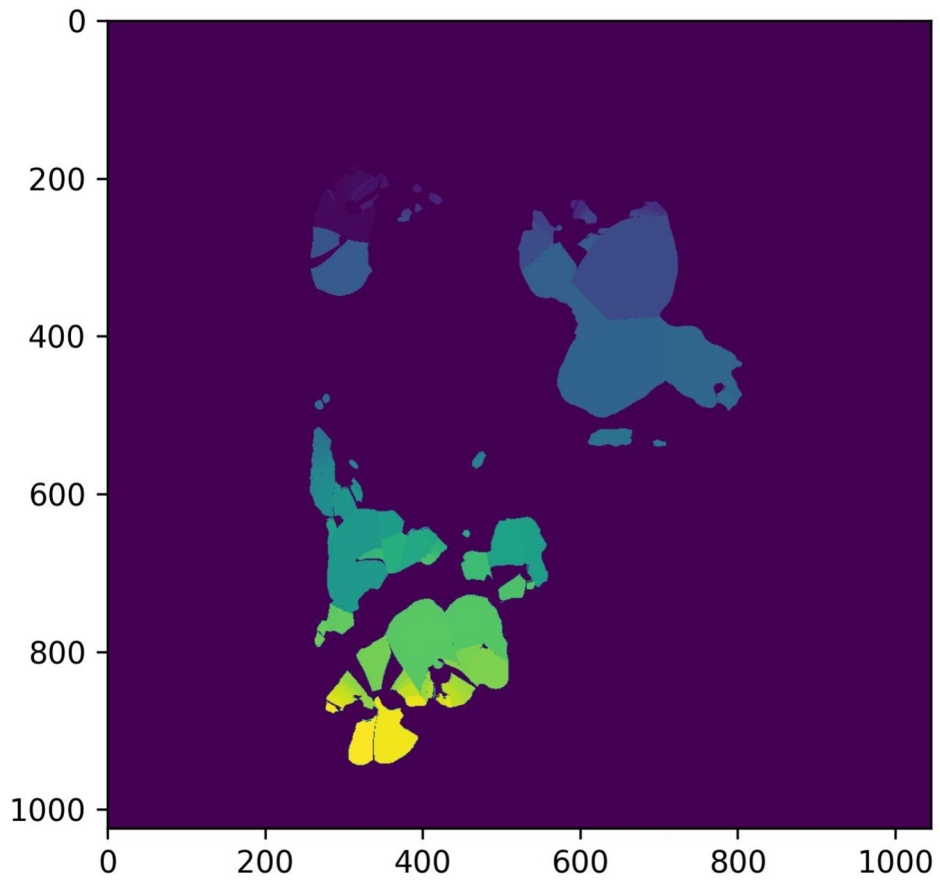


CARE | CellPose

Slice 150



Raw



CARE

Thank you! You are an amazing team!!!

Supplementary

Deep Learning methods are based on:

Mathematical Models

Mean Error Squared

$$\mathrm{MSE} = \frac{1}{n}\sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2$$

Average Error Across the Model

$$\mathrm{MAE} = \frac{\sum_{i=1}^{n}|y_i - x_i|}{n} = \frac{\sum_{i=1}^{n}|e_i|}{n}.$$

$$\mathrm{MAE} = \frac{\Sigma|\text{actual - prediction}|}{\text{number of observations}}$$

```
['loss', 'lr', 'mae', 'mse', 'val_loss', 'val_mae', 'val_mse']
```