

## **Title:** AI-Enabled Construction of Aligned Collagen Using Two-Photon Techniques

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**Introduction:** Laser-based collagen biofabrication process is a novel approach for generating customizable 3D collagen structures as an in-vitro tissue scaffold and has the potential for in-vivo tissue engineering. The technology stems from the ability of a femtosecond (fs) laser pulse to optically generate a controlled and localized pH gradient via a two photon (2P) effect that facilitates the generation of collagen protein assembly, called fibrillogenesis, into fibers and bundles. Unlike all other collagen biofabrication techniques, this process utilizes the spatial and temporal precision attributed to light, which allows for accurate 3D modeling of native tissue structure. *Tseng et. al, 2020* proposed a framework for a functional biopolymer that could alternate between the two  $\beta$ -sheet structures in response to pH changes. *Chiba et. al, 2003* have extensively studied amyloidogenicity showing a significant correlation with the stability of the amyloid fibrils with PH, and little correlation with that of the native state. It has been proposed that the stability of the native state and the unfolding rate to the amyloidogenic precursor as well as the conformational preference of the denatured state is influenced by PH. The most immediate impact of collagen fabrication would be in the creation of custom, patterned cell culture scaffolds. Here, we propose an AI based innovative laser collagen alignment technique to be developed into an in-situ scaffold formation technology for producing next generation of tissue engineering-based implantable biomedical devices.

**Study Objectives:** This research investigates if the use of trained convolutional neural net architecture (CNN) can effectively segment aligned collagen fibers deep inside tissue, this study set out to train a U-Net CNN architecture to accurately classify aligned collagen-positive pixels within a SHG image volume. This AI-enabled collagen image processing opens a new way to create real-time scaffold formation technique that mimic various in vivo tissue structures.

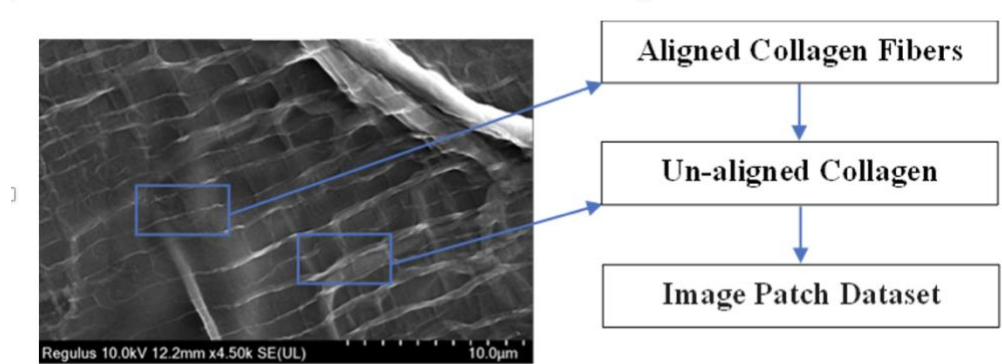
**Methods:** To achieve this, we have used PyTorch, a python library to initialize the conventional U-Net CNN with four encoding and decoding units on 2000 images (Figure 1). The synthetic images were generated utilizing a Stable Diffusion 3.5 Large technique. A non-linear rectified non-linear unit (ReLU) layer comes after two 2D convolution layers in each encoding and decoding block. The network's weights and biases are modified by an adaptive moment (ADAM) optimizer. These findings will assess whether a trained CNN can accurately and precisely segment aligned collagen-positive pixels at a variety of imaging depths assisting our understanding of fibrillogenesis and subsequently collagen biofabrication.

**Results:** Primary training found the model to have a training loss of 0.009 with a precision and recall of  $> 0.90$ . However, an overfitting has been observed with this run. To fix this, we intend to increase data (via augmentation), simplify the model by having fewer layers, try regularization (L1/L2), implement early stopping, or will apply K-Fold cross-validation to improve generalization. As an alternative, transfer learning can be used to modify the trained CNN for use

in drastically distinct fiber networks or to retrain the network for images at significantly different magnifications.

**Discussion:** Here, we propose an AI based innovative laser collagen alignment technique to be developed into an in-situ scaffold formation technology for producing next generation of tissue engineering-based implantable biomedical devices. This AI-enabled collagen image processing opens a new way to create real-time scaffold formation technique that mimic various in vivo tissue structure. These findings will assess whether a trained CNN can more accurately and precisely segment aligned collagen-positive pixels at a variety of imaging depths.

**Figure 1:** Sample image for aligned and un-aligned collagen fibrils used in CNN training.



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