# Second Harmonic Generation Microscopy of Rat Scleral Remodeling by Collagenase and Reparative Collagen Mimetic Peptides

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[1]University of Rochester David and Ilene Flaum Eye Institute & [2]Center for Visual Science, Rochester, NY, United States, [3]Stuart Therapeutics, Inc., FL, United States
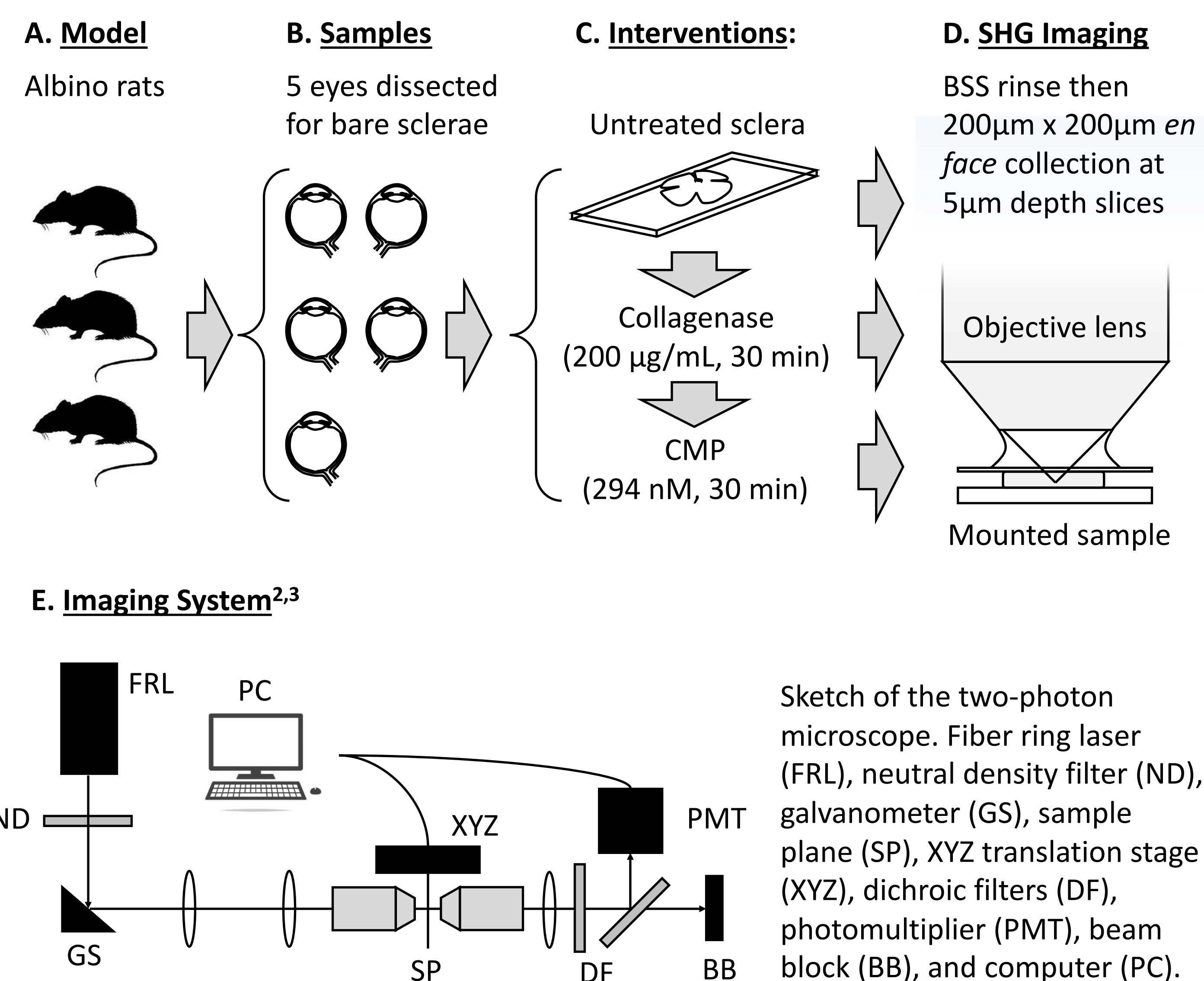*Financial Disclosures*: *Owner, financial interest in Stuart Therapeutics, Inc., **Financial support from Stuart Therapeutics, Inc.
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## 1. Purpose

- Myopia is associated with increased scleral protease activity.[1]
- Protease activity results in structural changes that can be visualized using Second Harmonic Generation (SHG) microscopy.
- Collagen mimetic peptides (CMPs) (Stuart Therapeutics) may act as reparative agents to restore collagen organization and structure after collagenase digestion.[2]
- **Aim**: test the ability of 2 distinct CMP compositions to reorganize digested collagen in rat sclera, and possibly provide a basis for new therapeutics targeting myopia progression.

## 2. Methods

**A. Model**
Albino rats

**B. Samples**
5 eyes dissected for bare sclerae

**C. Interventions:**
Untreated sclera
↓
Collagenase (200 µg/mL, 30 min)
↓
CMP (294 nM, 30 min)

**D. SHG Imaging**
BSS rinse then 200µm x 200µm *en face* collection at 5µm depth slices

Objective lens
Mounted sample

**E. Imaging System[2,3]**



Sketch of the two-photon microscope. Fiber ring laser (FRL), neutral density filter (ND), galvanometer (GS), sample plane (SP), XYZ translation stage (XYZ), dichroic filters (DF), photomultiplier (PMT), beam block (BB), and computer (PC).

## 3. Results

- SHG microscopy of the untreated scleral tissue showed a high degree of organization
- **CMP 1** treatment showed *subjective* reordering of digested collagen fibers, but the order coefficient[4] was not significantly changed from digested tissue (p < 0.05)
- **CMP 2** treatment of digested sclera resulted in a collagen organization that was not significantly different from untreated tissue at depths up to 60 µm (p < 0.05)
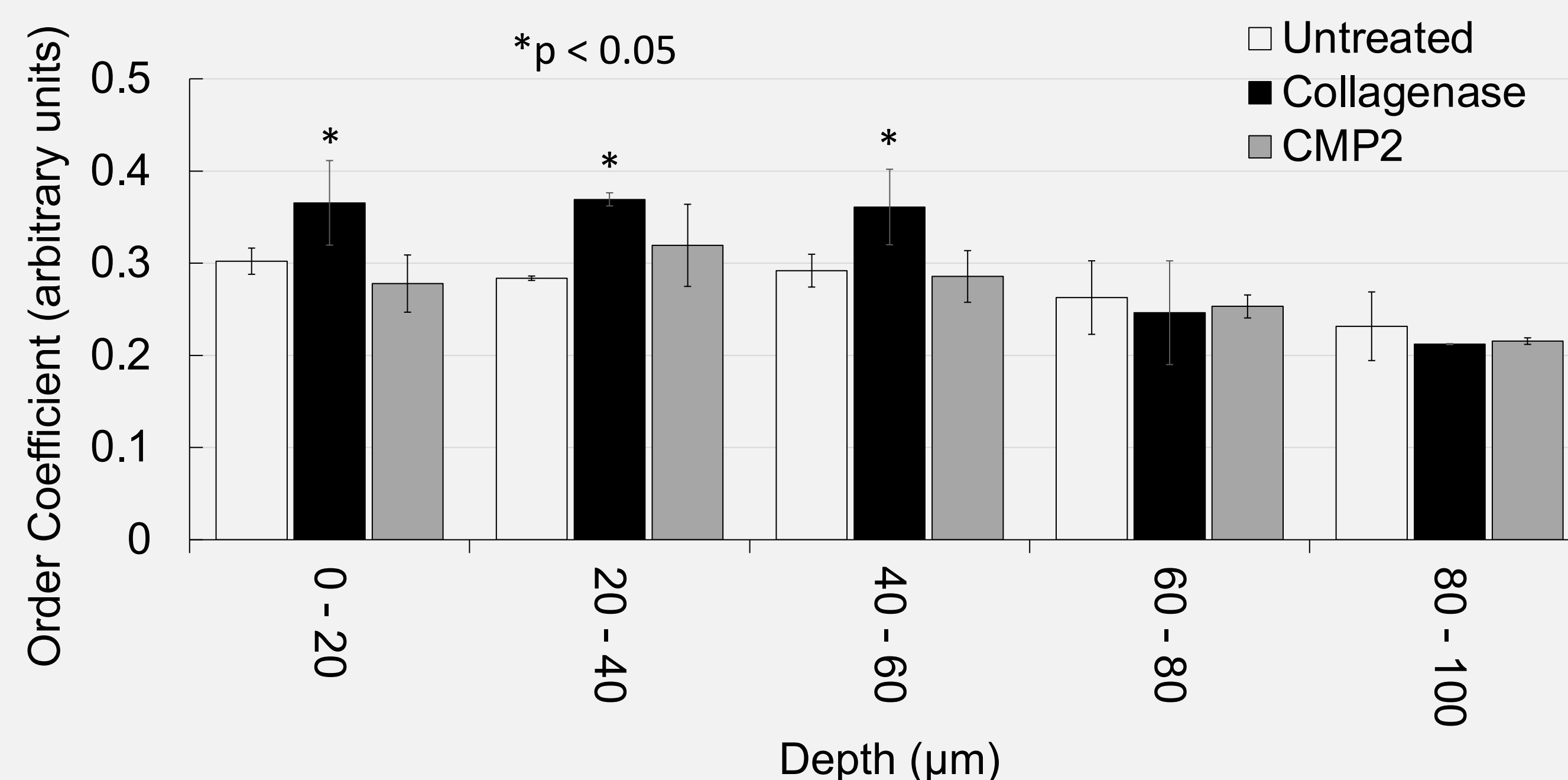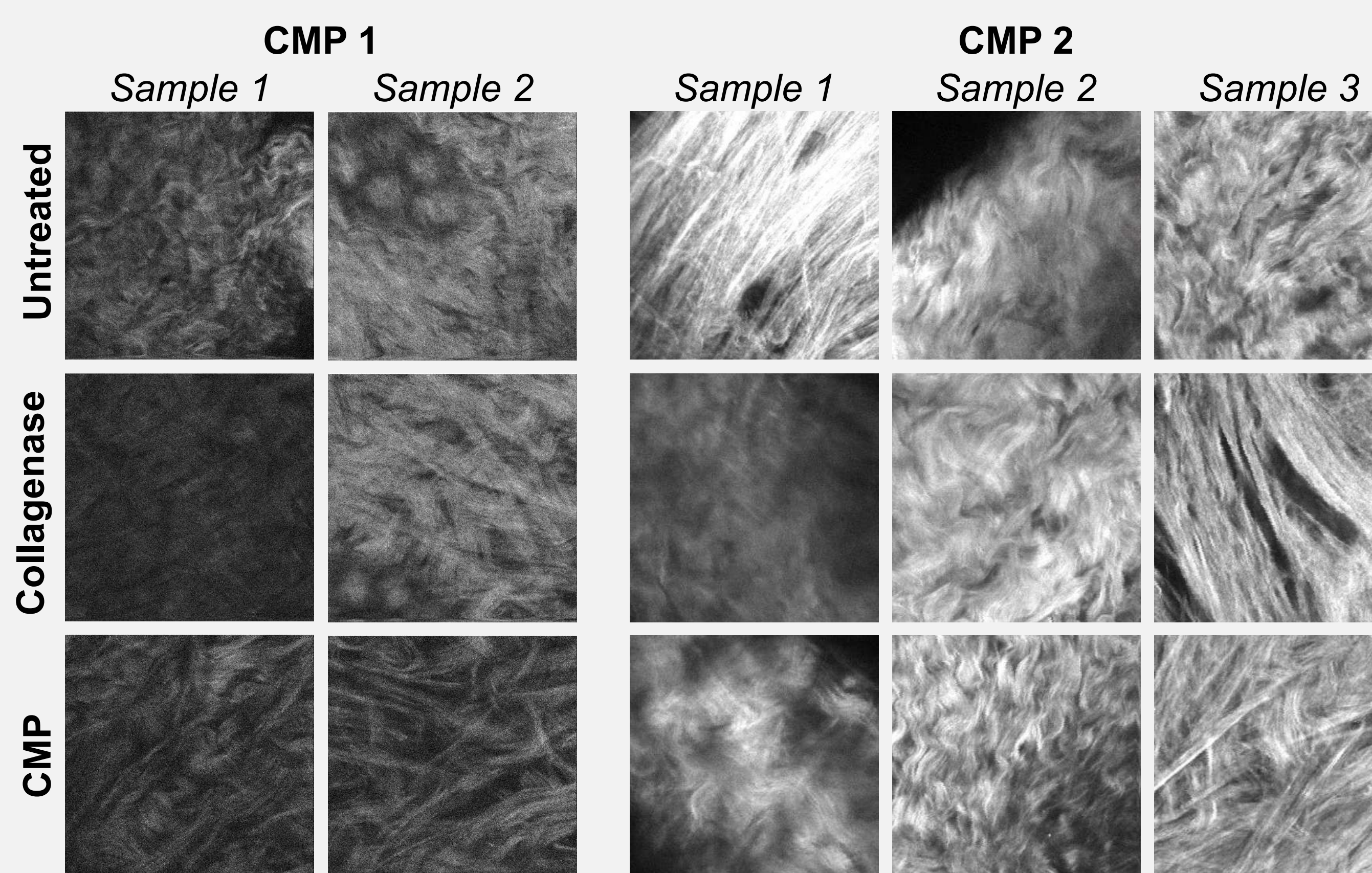




## 4. Conclusions

- SHG microscopy showed *subjective* CMP 1-induced and *objective* CMP 2-induced reorganization of rat scleral collagen after collagenase digestion
- CMP 2 treatment restored digested collagen to a similar organization as untreated tissue, (*i.e.*, the order coefficient between CMP 2-treated collagen after enzymatic digestion and untreated collagen was not statistically different)
- These changes may represent a reparative effect on enzymatically digested scleral collagen that could be useful in the treatment of myopia progression

## 5. Future Work

- Investigate mechanical properties of CMP-treated collagen using atomic force microscopy and optical coherence tomography elastography
- Better quantify CMP-induced changes in collagen organization using wavelet transform texture analysis
- Study effects of CMP treatment on collagen in an *in vivo* model

## 6. References & Acknowledgements

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