

Introduction

- Fibrinogen is a soluble 340 kDa glycoprotein found in the blood that is essential to wound healing.
- Typical physiological concentration is 2-4 mg/ml.
- Fibrinogen is enzymatically cleaved by thrombin to generate an insoluble fibrin clot during wound healing.
- Fibrin hydrogels are being investigated in the literature for injectable scaffolding applications to improve the wound healing process.
- An effective scaffold has physical properties comparable to target tissue.

Research Questions

- What does the microstructure of fibrin hydrogels look like?
- What effect(s) does drying method have on fibrin's microstructure?

Methods

- Aliquots were prepared to achieve final mixed concentrations of fibrinogen at 6 mg/ml, thrombin at 1 U/mL, and CaCl₂ at 5 mM in 1x Tris-buffered saline.
- Fibrinogen, thrombin, and CaCl₂ aliquots were rapidly mixed with a micropipette and allowed to gel in a 12-well plate or a petri dish for 30 minutes.
- Samples were then fixed with 2.5% electron microscopy-grade glutaraldehyde for 0, 30, 60, or 120 minutes.
- One sample, Fig. 1, was treated with a 35%, 50%, 75%, 95%, 100% ethanol series and critical point dried (CPD) at MTSU.
- Remaining samples were washed with water to remove salts, rapidly frozen with liquid N₂ and freeze dried overnight, Figs. 2-5.
- Dried samples were sputter coated with Au/Pd for 2 minutes and imaged using a Hitachi SU7000 scanning electron microscope (SEM) at TN Tech.
- Fibers were randomly selected using MATLAB and diameter was measured using ImageJ.

Results

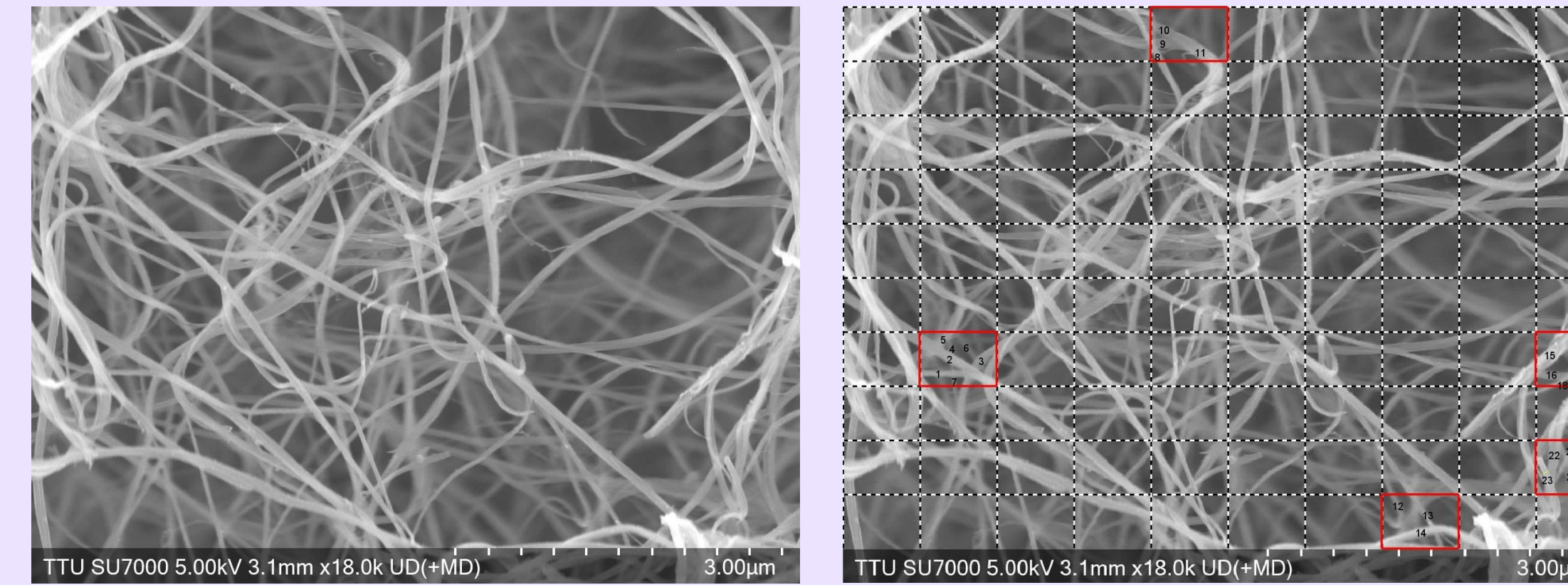


Fig 1. CPD with 120 minutes fixation. Average measured fiber diameter is 77 nm.

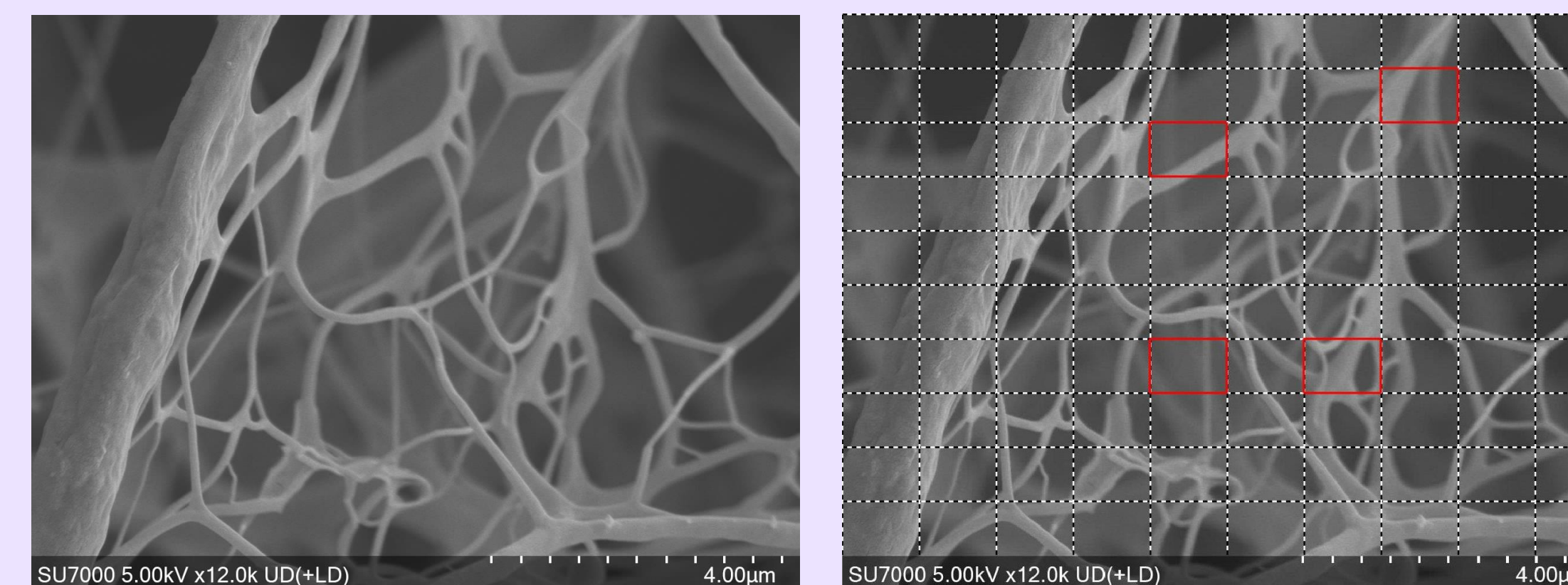


Fig 2. Freeze drying with no fixation. Average measured fiber diameter is 191 nm.

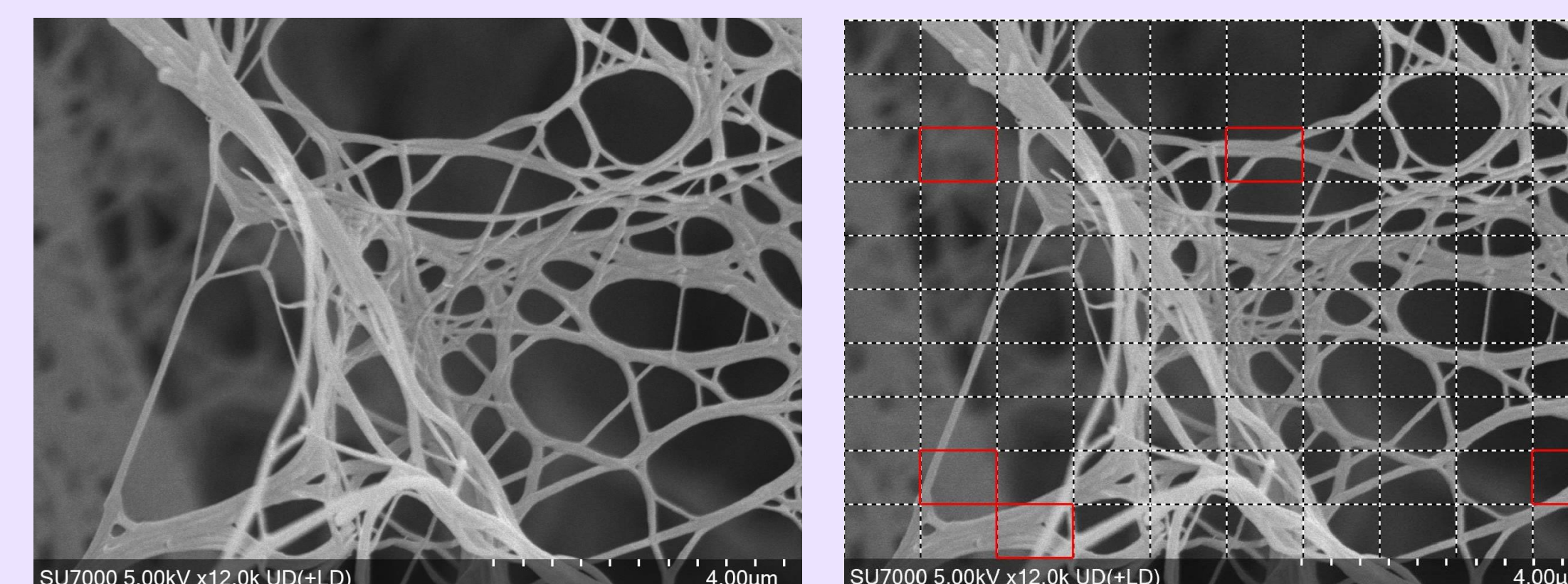


Fig 3. Freeze drying with 30 minutes fixation. Average measured fiber diameter is 159 nm.

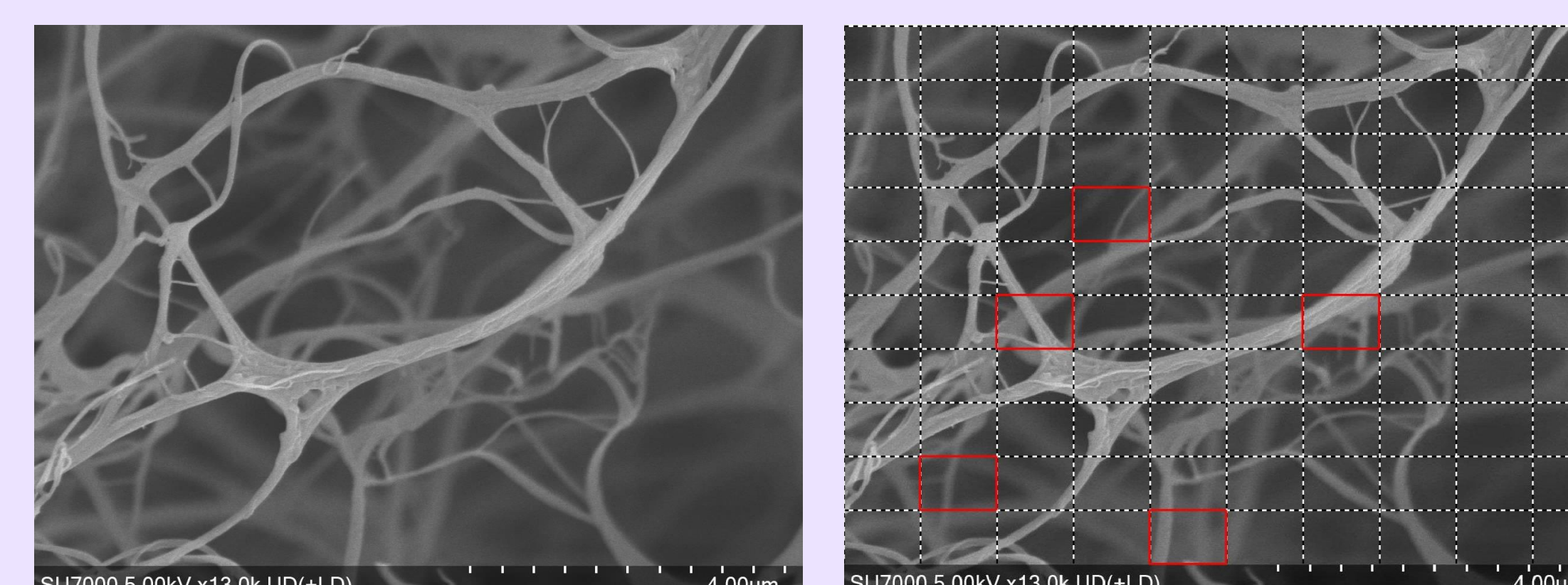


Fig 4. Freeze drying with 60 minutes fixation. Averaged measured fiber diameter is 247 nm.

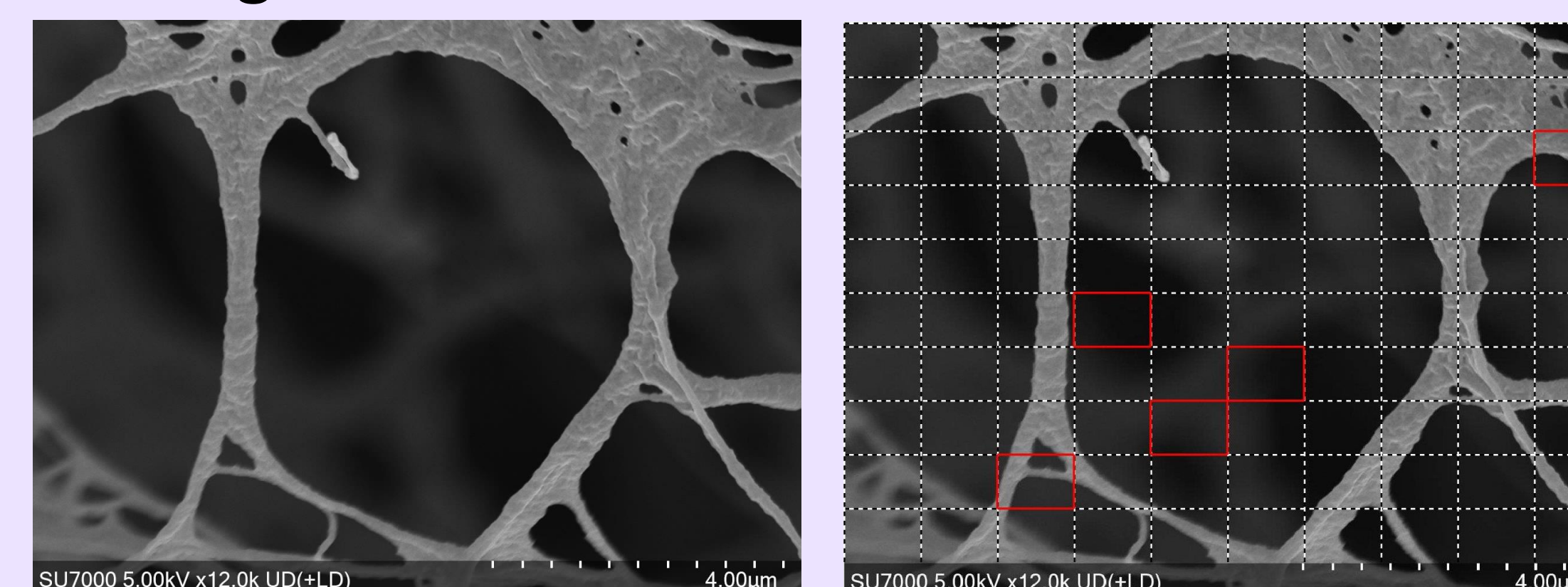


Fig 5. Freeze drying with 120 minutes fixation. Average measured fiber diameter is 197 nm.

Discussion

- Qualitatively, scanning electron micrographs seem to indicate that CPD, Fig. 1, is less destructive than freeze drying for fibrin hydrogels, Figs. 2-5.
- To identify fibers to be measured, a 10-by-10 grid was overlaid on each micrograph and 5 regions were randomly selected and highlighted in red.
- Diameters of all fibers in these selected regions were measured using ImageJ.
- Fibrin gels dried using freeze drying appear to have fibers that are more clumped together overall and more splayed out at branching points than CPD dried gels.
- Fiber diameter in freeze dried samples was 2-3 times larger than in the CPD sample.
- "Clumping" appears to be more prevalent at longer fixation times in freeze dried samples.

Future Work

- Obtain more images of critical point dried samples to serve as a comparison between the effects of drying technique on resulting microstructure.
- Definitely determine whether or not critical point drying is superior to freeze drying hydrogels as prevalence in literature would indicate.
- Compare fiber diameter as measured by SEM with results from the Carr-Hermans approach to approximating average fiber thickness from turbidity measurements.

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