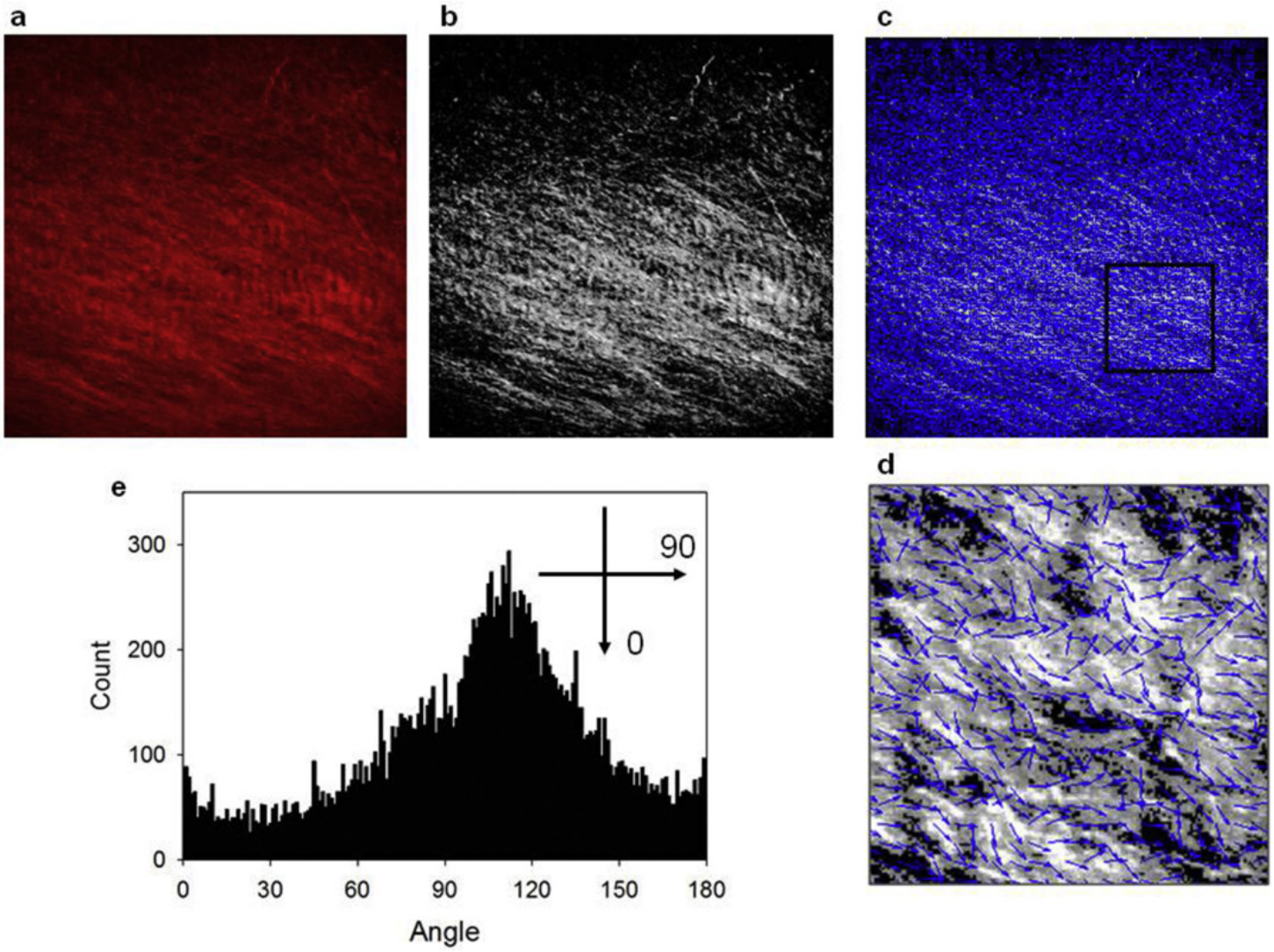
Appendix A. Supplementary data

The following are the supplementary data related to this article:



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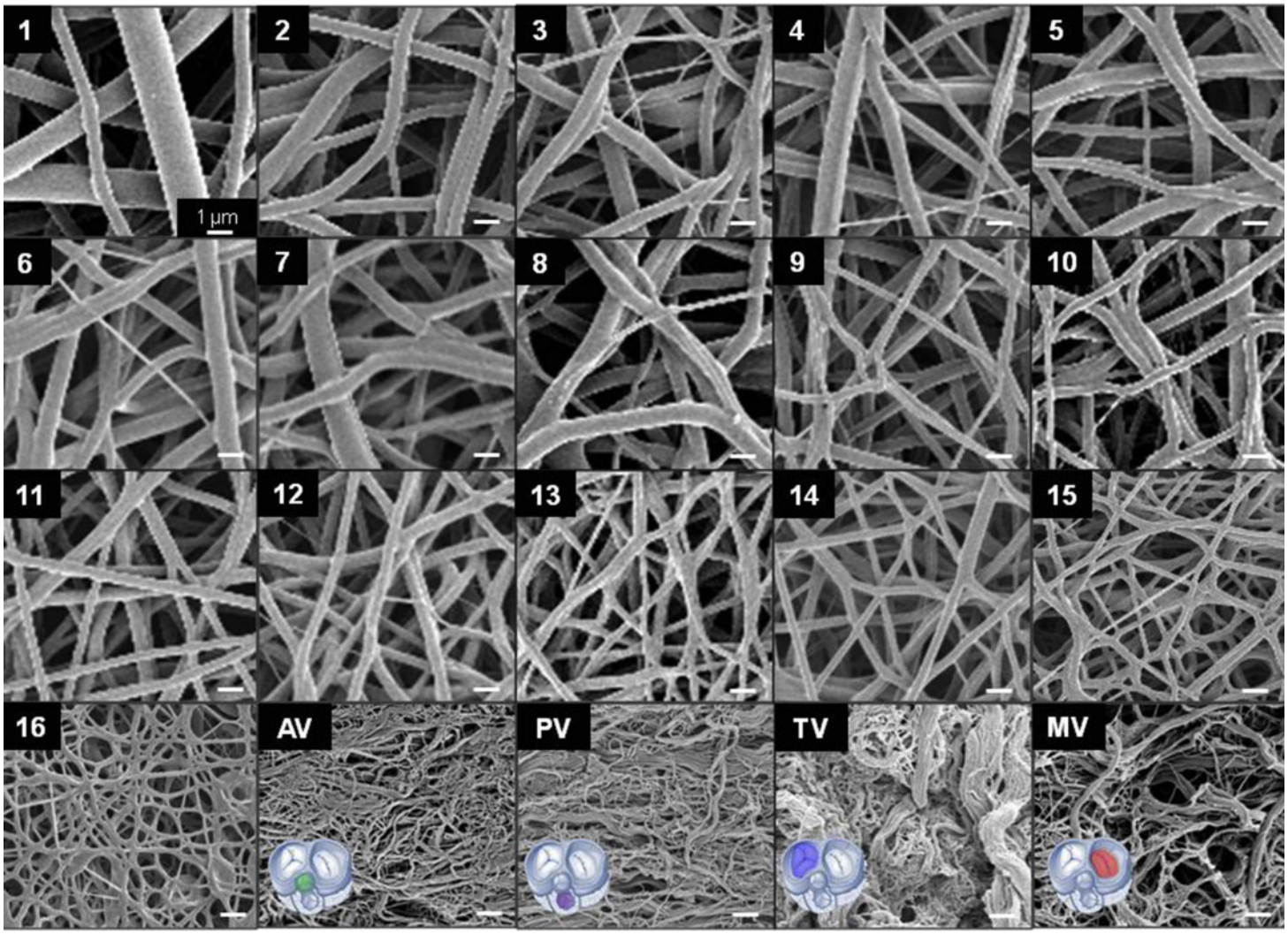
Supplemental Fig. 1. **Multi-photon microscopy and digital image analysis**. **a**) Image stacks of 500 x 500 x 100 μm for a native leaflet. **b)**Threshold collagen second harmonic generation. **c)** Processed image [47] showing identified fiber direction. **d)** Detailed inset. **e)**Related fiber orientation histogram.



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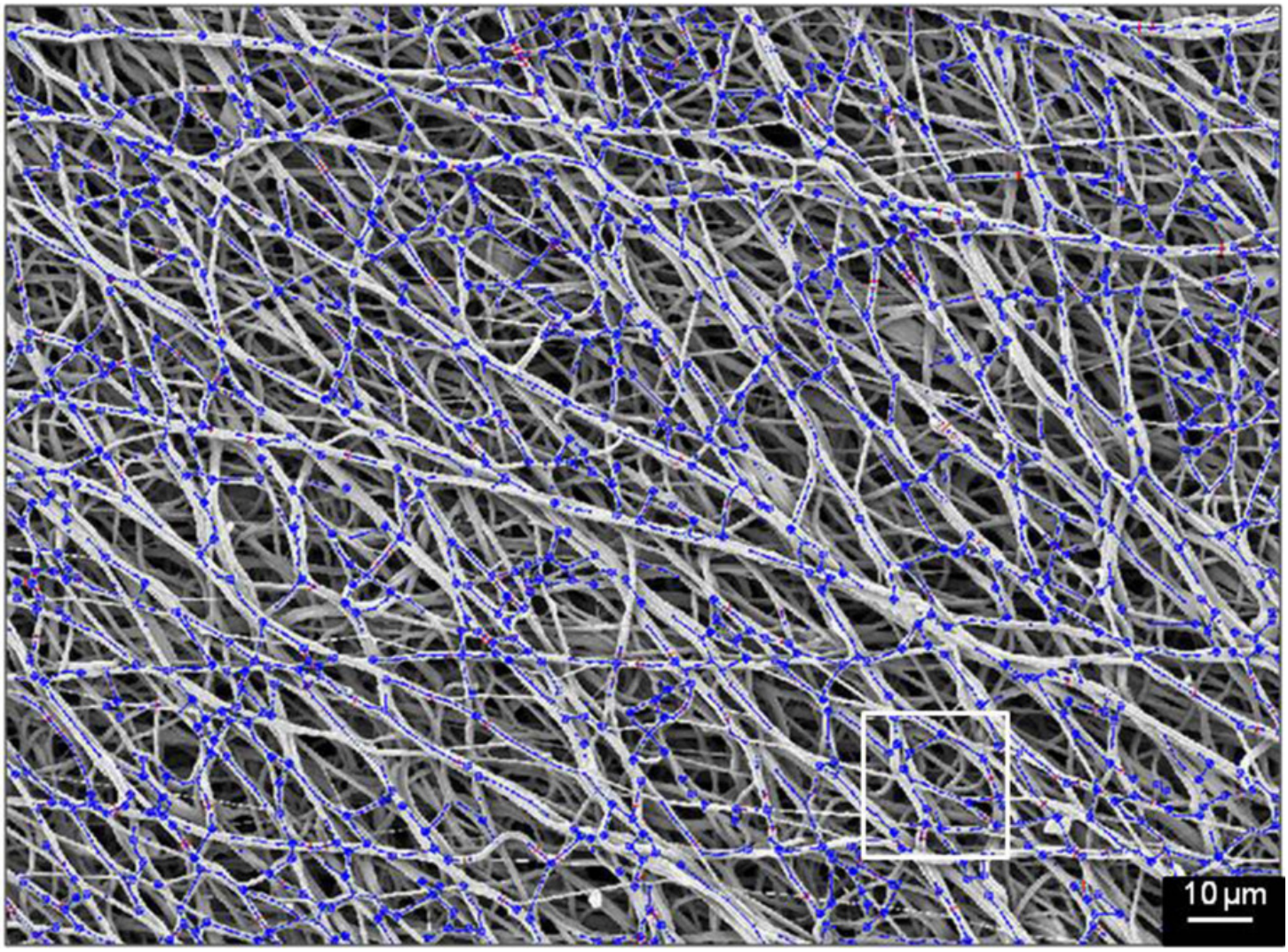
Supplemental Fig. 2. **Scanning electron microscopy of DCD processed and native leaflets.** Fabrication conditions changed as described in Fig. 8d with polymer - mandrel voltage difference: 4 - 32 kV, gap: 5.5-7.5 cm and polymer/solvent concentration: 4-12%. Representative SEM images showed the impact of fabrication conditions (1-16) on leaflet micro-structure. Comparison of 15 and 16 with SEM micrographs obtained at random locations of AV, PV, TV, MV showed comparable fiber bundle and pore size (Fig. 8g,i).



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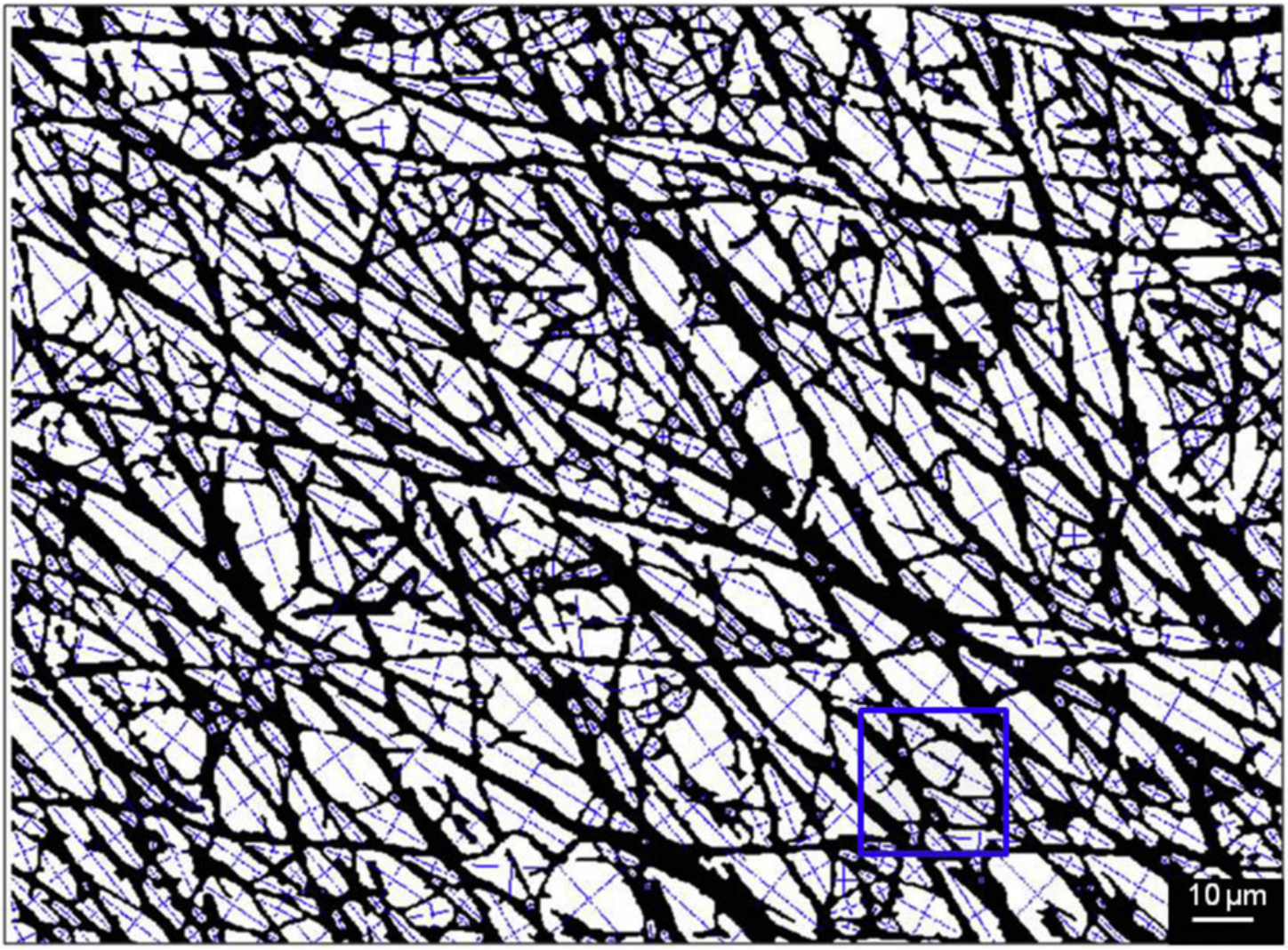
Supplemental Fig. 3. **Scanning electron microscopy and digital image analysis**. Example of fiber network detection performed [48] to quantify leaflet micro-architectural features. Blue solid line indicated the identified fiber, circles represented fiber intersections, and red segment highlighted the measured fiber diameter. White box: area showed in the inset Fig. 8f.



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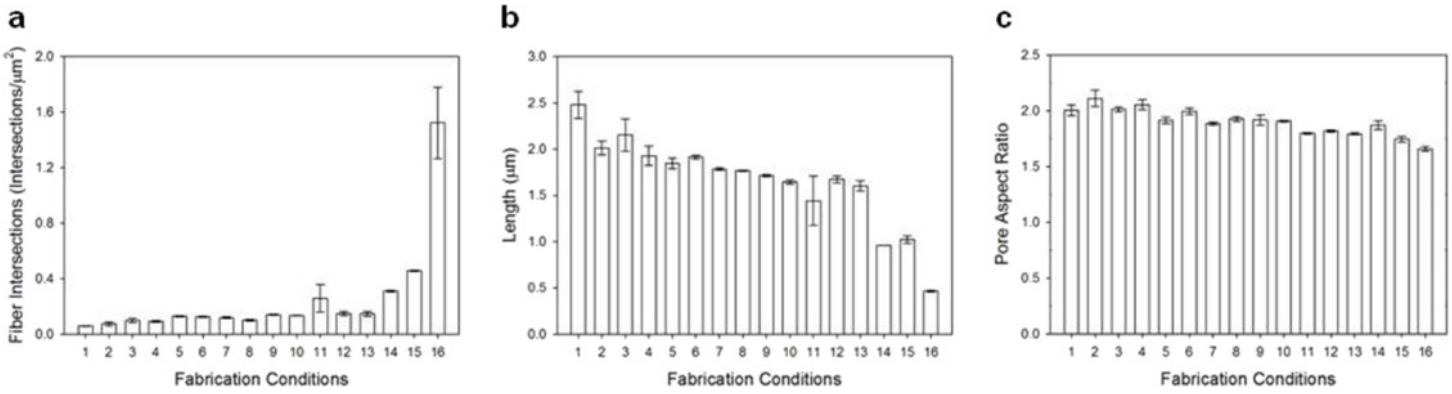
Supplemental Fig. 4. **Scanning electron microscopy and digital image analysis**. Example of pore geometry detection performed [48] to quantify leaflet micro-architectural features. White objects indicated the identified scaffold pores, blue segment represent the pore major and minor axes. Blue box: area showed in the inset Fig. 8h.



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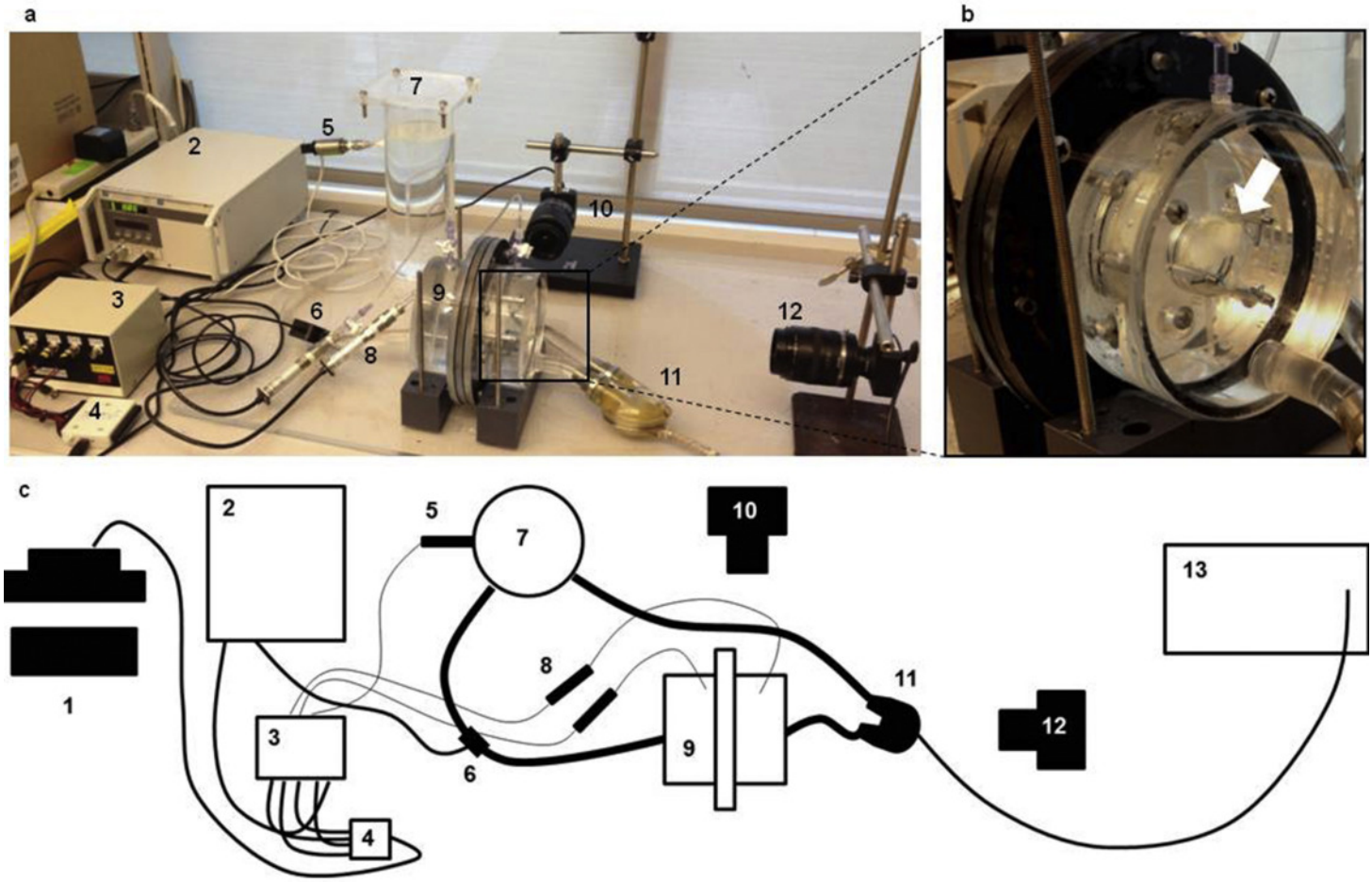
Supplemental Fig. 5. **DCD process control on engineered valve microstructure, additional micro-scale parameters. a**) Fiber intersection spatial density for the fabrication conditions in Fig. 8d. **b)** Average fiber intersection distance. **c)** Pore aspect ratio. All measurements were performed using the algorithm described in [48].



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Supplemental Fig. 6. **Pulse duplicator apparatus. a**) Flow duplicator for valve functional assessment under physiological flow conditions (40% glycerol solution, VAD system set at 80 BPM and 30% ejection time, flow 4–5 L/min), the system included: 1) desktop computer, 2–4) pressure and flow signal acquisition system, 5) pre-load pressure sensor, 6) flow meter, 7) capacitor, 8) pressure sensors, 9) valve holder with flanges, 10) side camera, 11) Thoratec Percutaneous VAD System (Thoratec Corporation, Pleasanton, CA), 12) frontal camera, and 13) VAD controller. A 40% glycerol solution was utilized to mimic blood viscosity, and the VAD system was set to 80 BPM and 30% ejection time. **b)** Valve holding apparatus details, the white arrow points at the engineered valve. **c)** Flow duplicator schematic.

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