Electronic supplementary information (ESI): Programming fibril alignment and mechanical response in reconstituted collagen fibers using reagent-free biomimetic energetic electron crosslinking

Anastassiya Bublikova[a,b,‡], Friedrich Schütte[a,b,‡,*] and Stefan G. Mayr[a,b,*]

a) Leibniz Institute of Surface Engineering (IOM), Permoserstr. 15, 04318 Leipzig, Germany

b) University of Leipzig, Division of Surface Physics, Department of Physics and Earth System Sciences, University of Leipzig, Linnéstr. 5, 04103 Leipzig

‡: These authors contributed equally to this work.

* Corresponding authors: Friedrich Schütte, Email: friedrich.schuette@uni-leipzig.de and Stefan G. Mayr, Email: stefan.mayr@iom-leipzig.de

14 Pages, 14 Figures, 1 Table

Collagen sample preparation

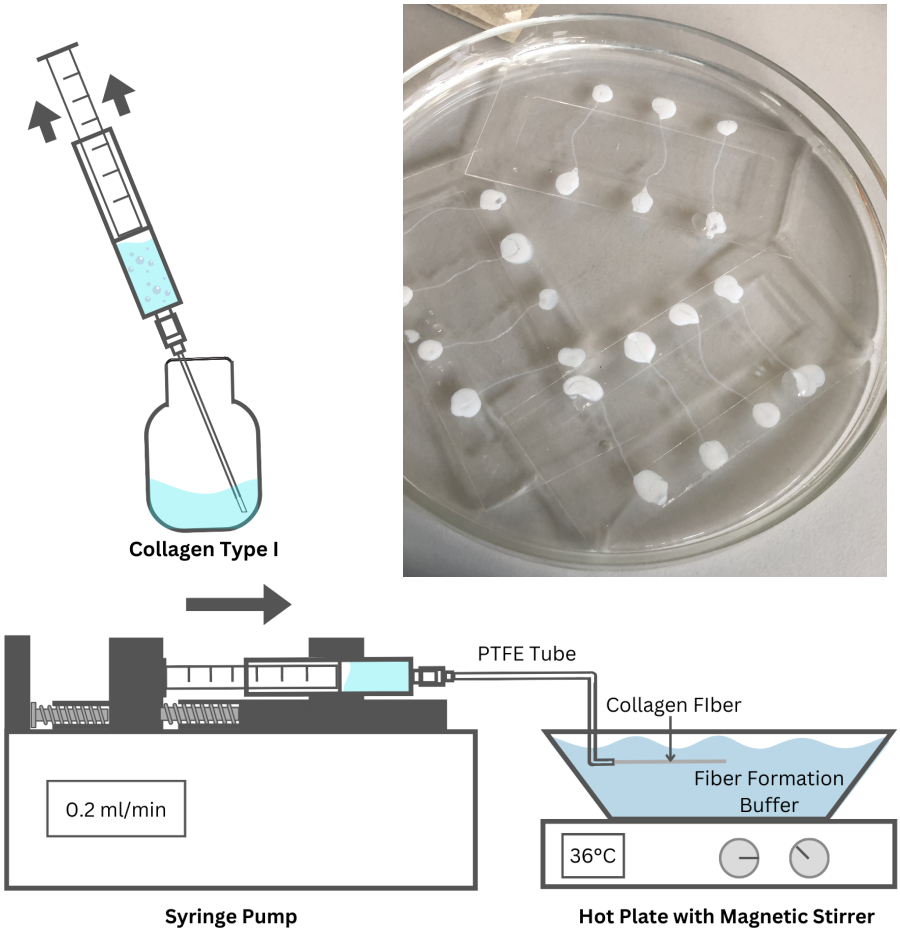

Figure S1: Sample preparation using a syringe pump to extrude collagen type I at a constant rate into a fiber formation buffer placed on a hot plate with magnetic stirrer.

Tensile testing setup

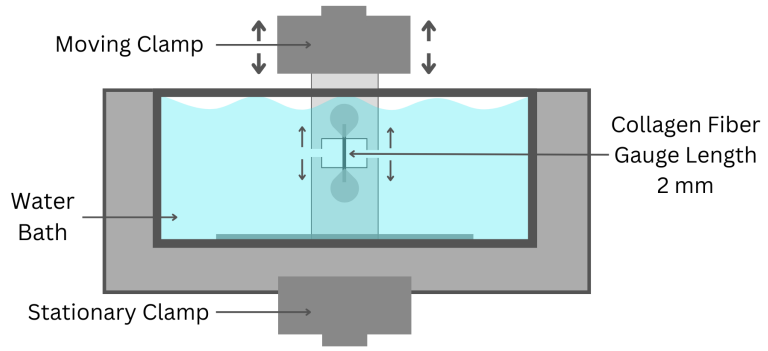

Figure S2: Schematic diagram of a collagen type I fiber glued with cyanoacrylate on test slides with a fixed gauge length of 2 mm and placed in water bath with transparent glass walls made of microscope carrier platelets. The sample holder is clamped inside the tensile testing machine (see **??**) and cut to allow the collagen fiber to be stretched vertically.

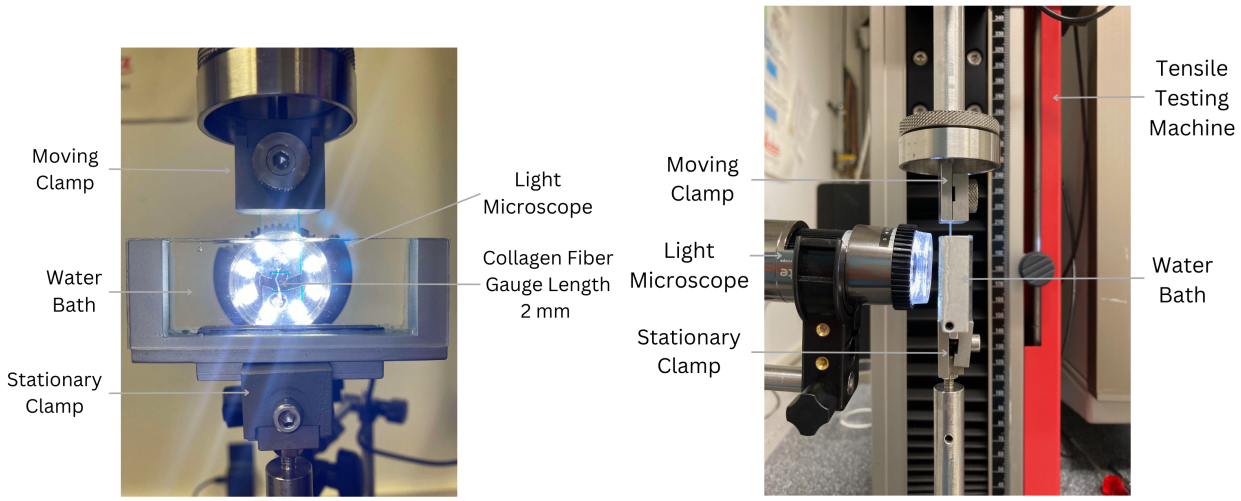

Figure S3: Setup for measuring collagen fibers in a water bath with zwickiLine tensile testing machine (type Z0.5 TS, art. no. 058992, ZwickRoell GmbH & Co. KG, Germany) and DinoLite AM7915MZT microscope (AnMo Electronics Corporation, Taipei, Taiwan) allowing real-time observation and imaging in parallel. The upper clamp moves in y-direction during the measurement (see **??**).

Light microscopy

In **??** a fully hydrated as-extruded collagen fiber is imaged in a water bath (see **??**) with a light microscope DinoLite AM7915MZT (AnMo Electronics Corporation, Taipei, Taiwan).

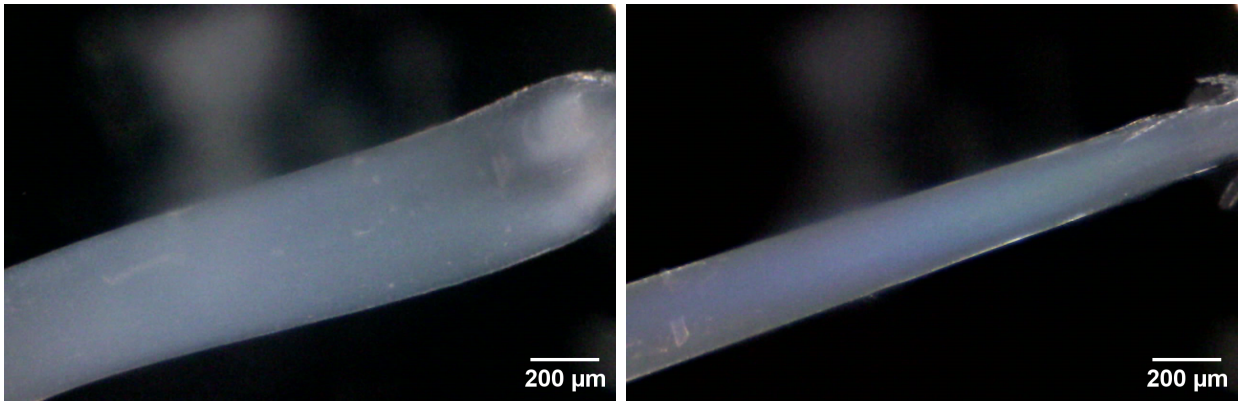

Figure S4: A fully hydrated collagen fiber exemplary imaged with DinoLite light microscope before tensile test (left) and after tensile test (right).

Uniaxial mechanical characterization

Basic mechanical properties

Table S1: Basic mechanical properties from uniaxial mechanical characterization depending on fiber diameter. The strain ϵ_{max} and rupture stress σ_{max} correspond to the mean rupture strain and stress, respectively.

Dose (kGy)	Prestrain (%)	Diameter (μm)	Strain ϵ_{max}	Stress σ_{max} (MPa)
50	60	258.45 ± 19.02	0.1630 ± 0.0046	3.499 ± 0.213
50	25	294.38 ± 19.25	0.5701 ± 0.0110	1.466 ± 0.186
50	0	301.93 ± 17.85	1.5495 ± 0.0025	1.388 ± 0.144
25	60	320.87 ± 21.72	0.1572 ± 0.0011	1.958 ± 0.120
25	25	365.48 ± 23.02	0.8507 ± 0.0001	1.433 ± 0.072
25	0	374.85 ± 21.34	1.5501 ± 0.0001	1.010 ± 0.110
10	60	372.20 ± 29.41	0.8141 ± 0.0033	1.765 ± 0.094
10	25	423.94 ± 27.45	1.1478 ± 0.0001	1.011 ± 0.008
10	0	434.81 ± 30.37	1.8358 ± 0.0001	0.771 ± 0.077
5	60	418.45 ± 26.35	1.5088 ± 0.0078	0.899 ± 0.103
5	25	476.63 ± 27.59	1.5109 ± 0.0017	0.681 ± 0.056
5	0	488.85 ± 20.64	1.5501 ± 0.0001	0.567 ± 0.070
0	0	642.44 ± 24.67	2.6279 ± 0.0024	0.165 ± 0.002

Differential moduli

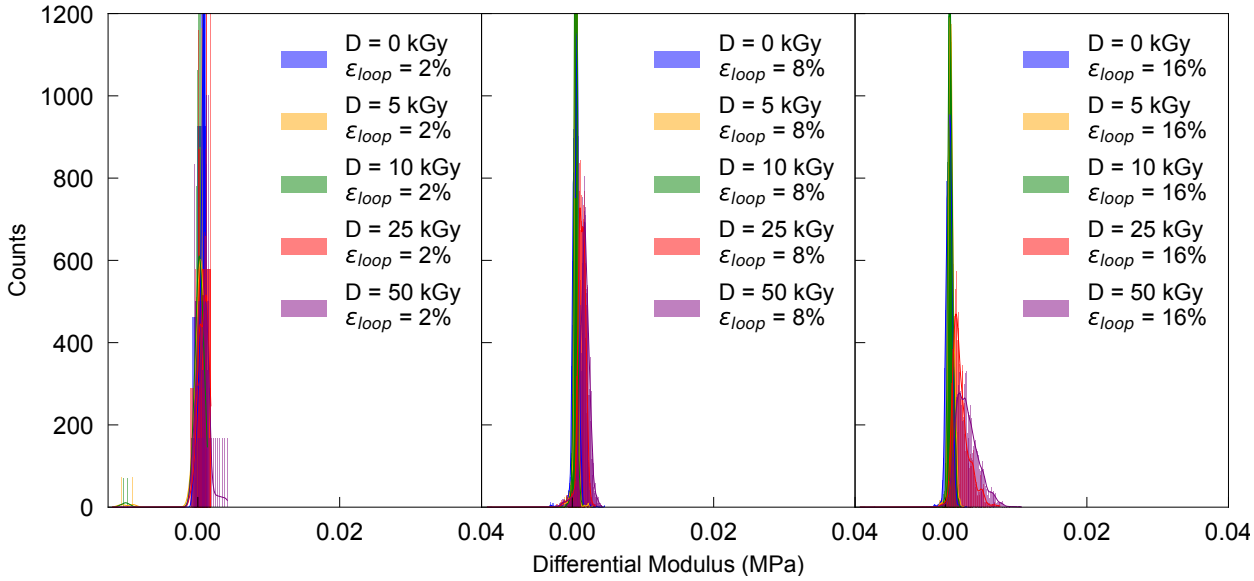

Figure S5: Differential moduli for a prestrain of $\epsilon_{pre} = 0\%$ for increasing maximal strains in the corresponding loop ϵ_{loop} with ten cycles each. The dose D was achieved in 5 kGy steps.

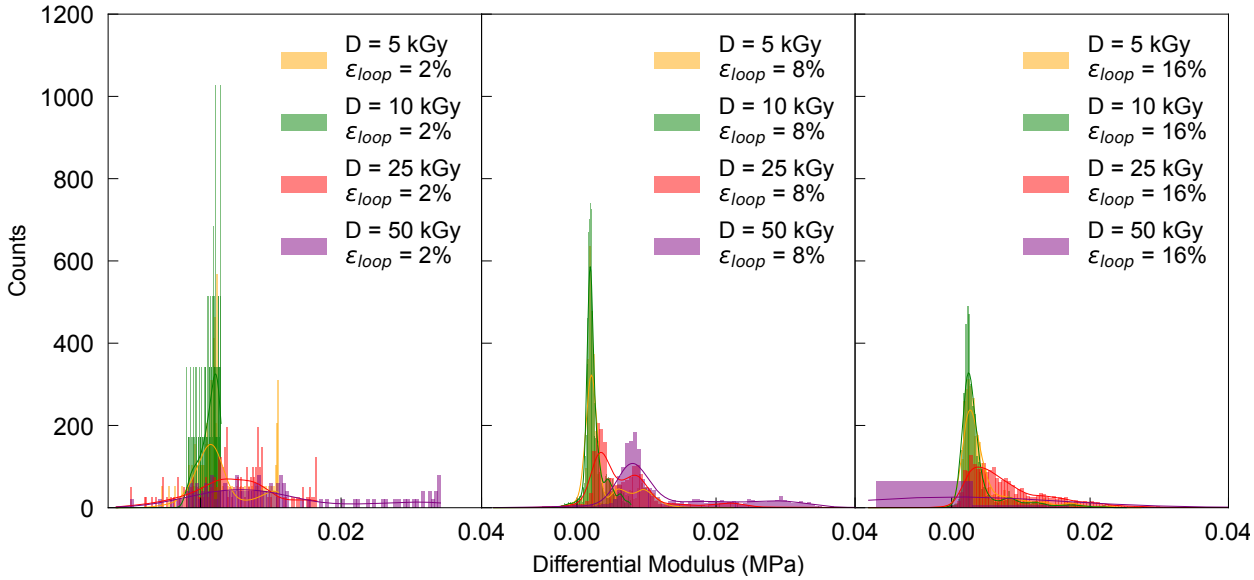

Figure S6: Differential moduli for a prestrain of $\epsilon_{pre} = 25\%$ for increasing maximal strains in the corresponding loop ϵ_{loop} with ten cycles each. The dose D was achieved in 5 kGy steps.

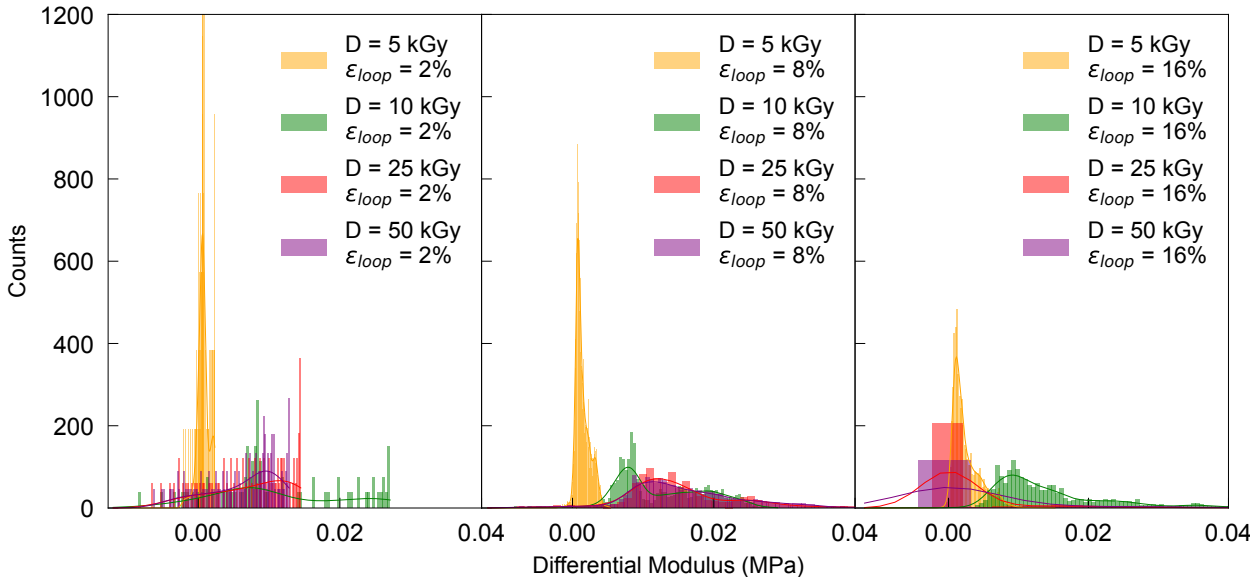

Figure S7: Differential moduli for a prestrain of $\epsilon_{pre} = 60\%$ for increasing maximal strains in the corresponding loop ϵ_{loop} with ten cycles each. The dose D was achieved in 5 kGy steps.

Energy dissipation

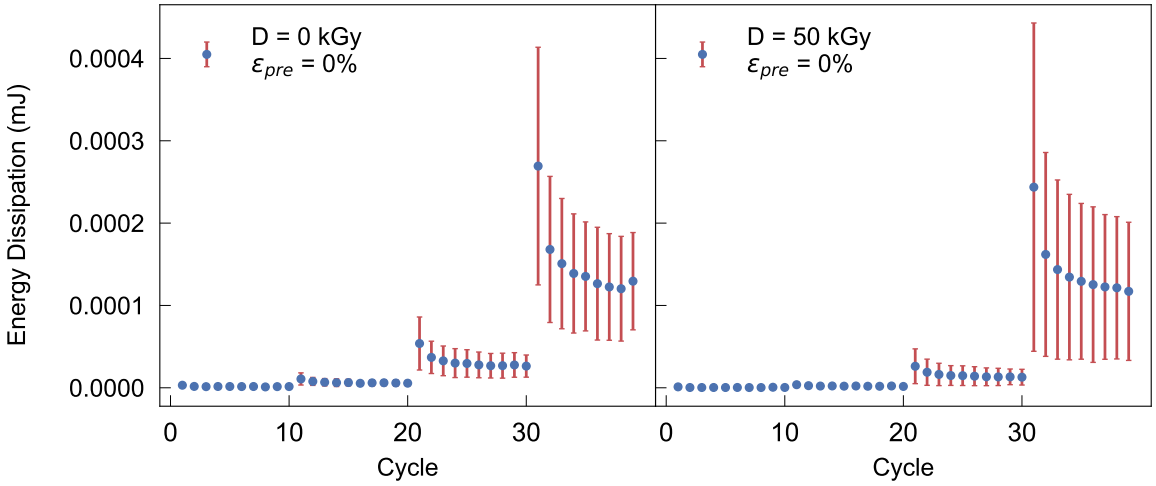

Figure S8: Energy dissipation $\Delta E \approx W$ within a single measurement cycle, i.e. stretching and retracting the fiber sample once within a defined strain range. Shown are the mean values for unirradiated and irradiated collagen fibers without prestraining ϵ_{pre}. A higher irradiation dose D does not initially appear to have a particularly large effect on energy dissipation.

Polarization microscopy

The microscope used for polarization microscopy is the Leica DM2700M (Leica Microsystems GmbH, Wetzlar, Germany).

Imaging control samples

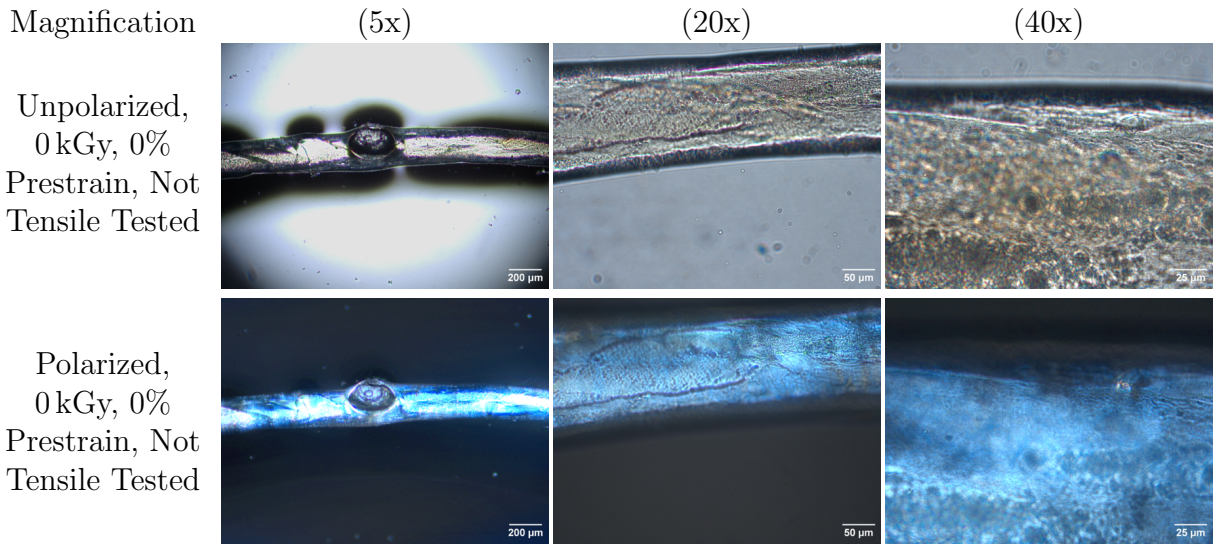

Figure S9: In the first and second row unpolarized and cross-polarized images, respectively, of a non-modified (unirradiated, non-prestrained) and not tensile tested collagen fiber are shown. Imperfections are visible on the surface of the sample.

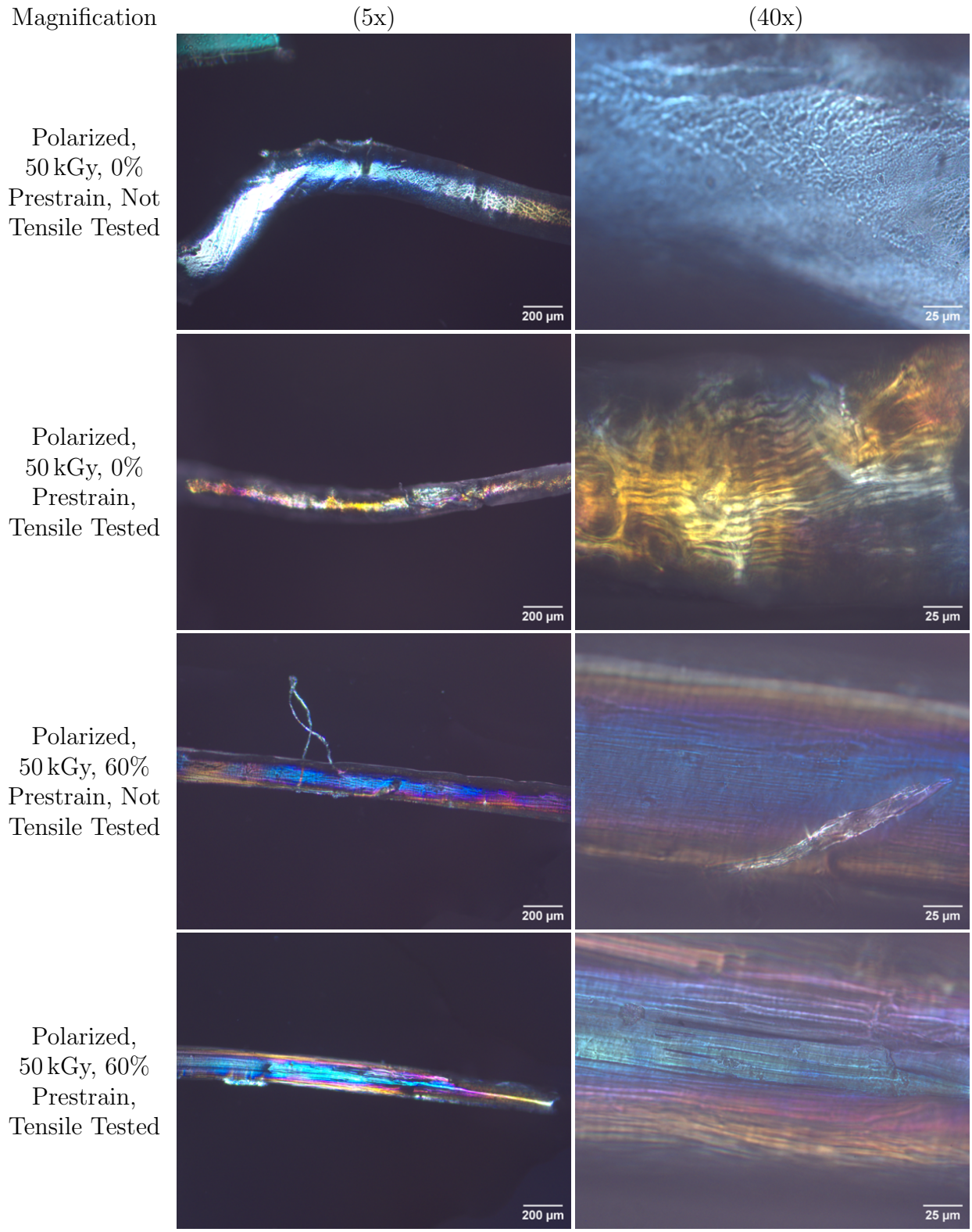

Figure S10: Polarisation microscopy images of collagen fibres crosslinked with a dose of 50 kGy at varying prestrains, before and after the tensile test.

Sample rotation

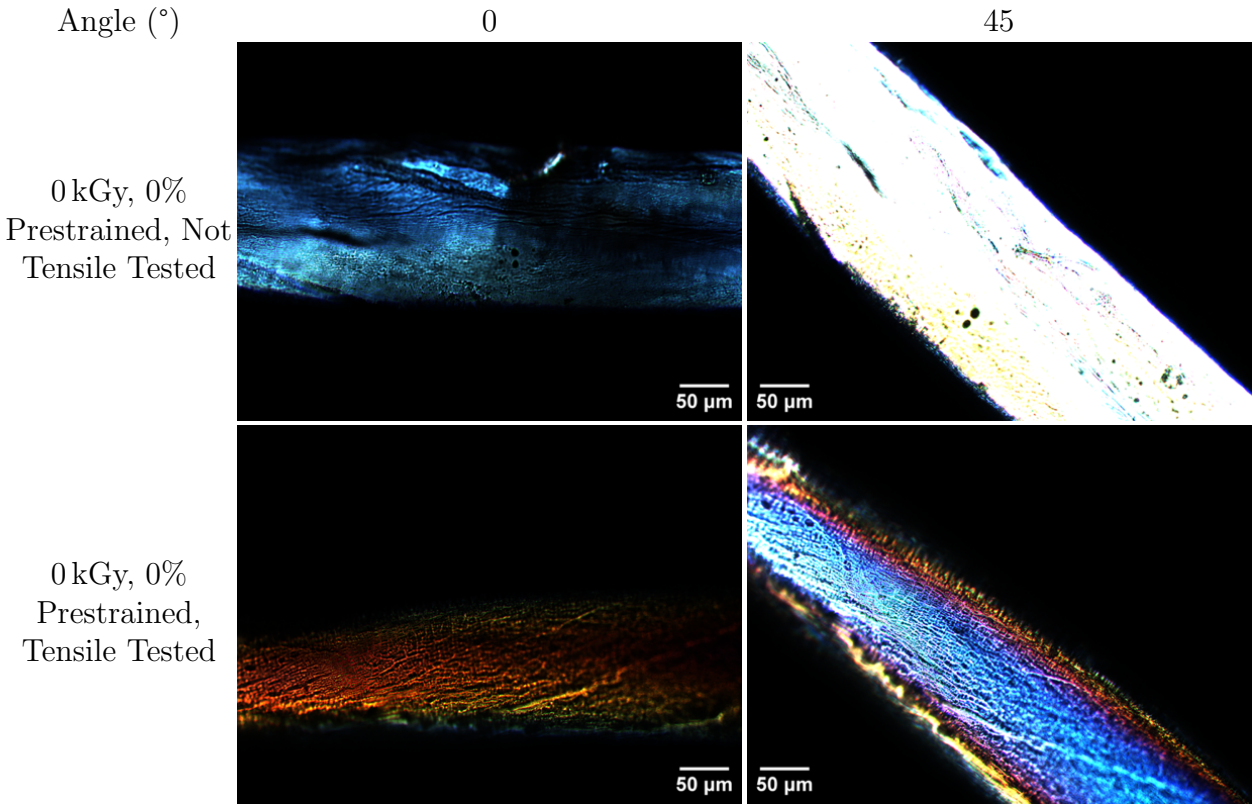

Figure S11: Rotated polarisation microscopy images of non-prestrained and uncrosslinked collagen fibres, before and after the tensile test.

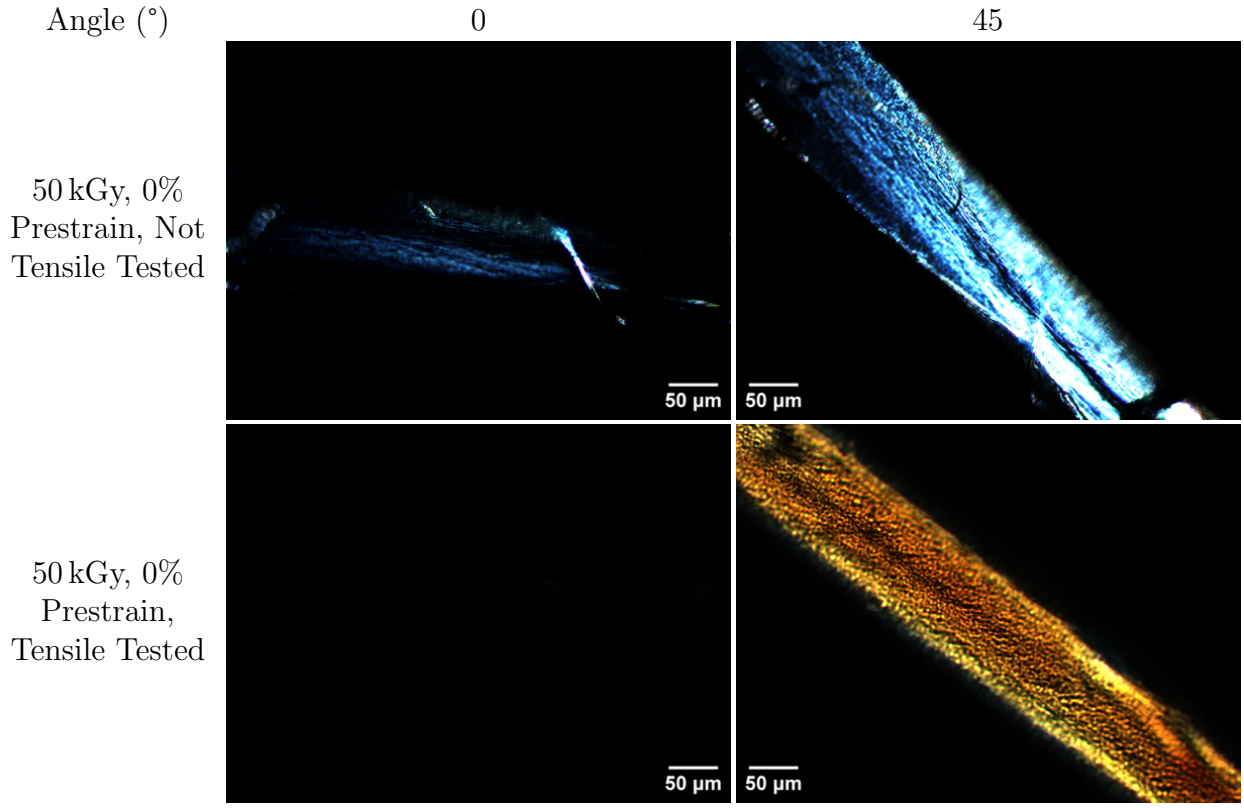

Figure S12: Rotated polarisation microscopy images of non-prestrained collagen fibers crosslinked with 50 kGy, before and after the tensile test.

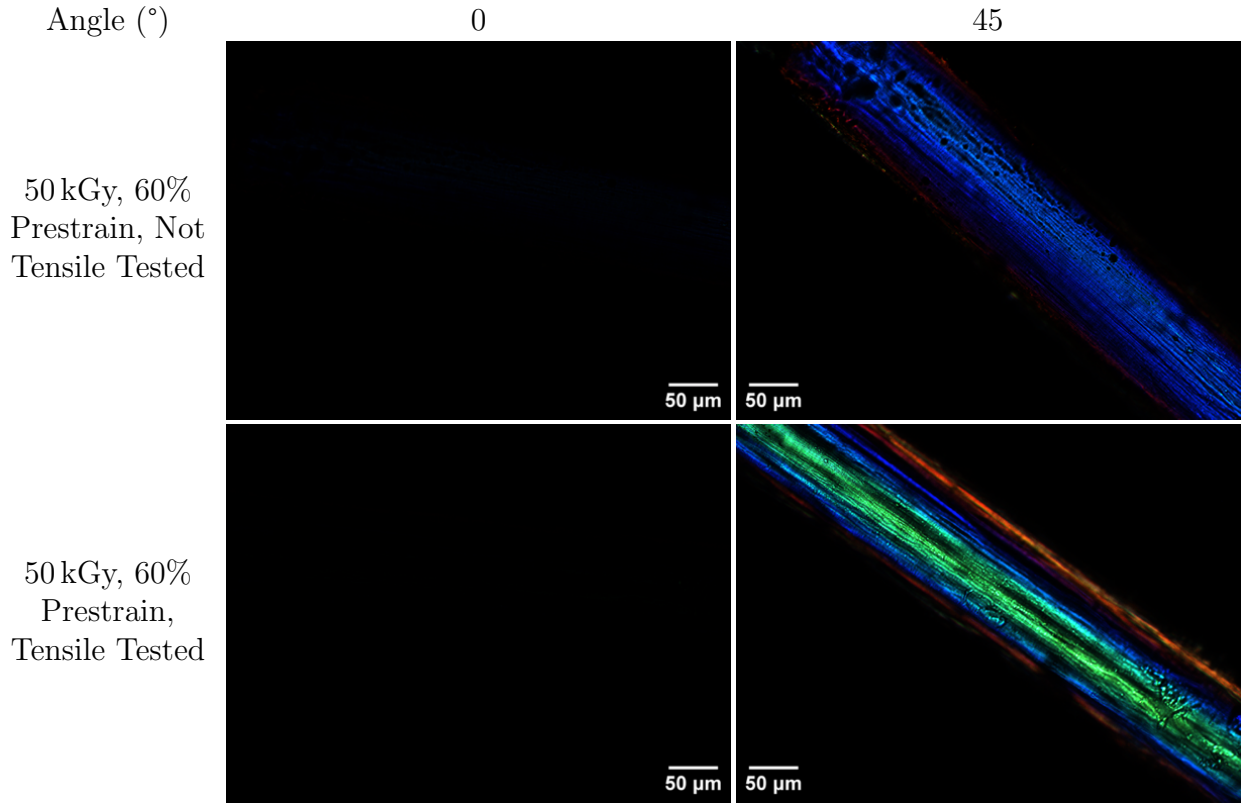

Figure S13: Rotated polarisation microscopy images of prestrained and crosslinked collagen fibres, before and after the tensile test.

Polar plots

For the purpose of generating polar plots, the corresponding intensity profile was generated for each channel. The maximum circle area in the polarized light microscopy images was considered, with the center of the circle in the middle of each image and the edge of the circle at the minimum and maximum y-positions, respectively (see **??**). Weighted mean intensities of the individual red (R), green (G), blue (B) channels were examined.

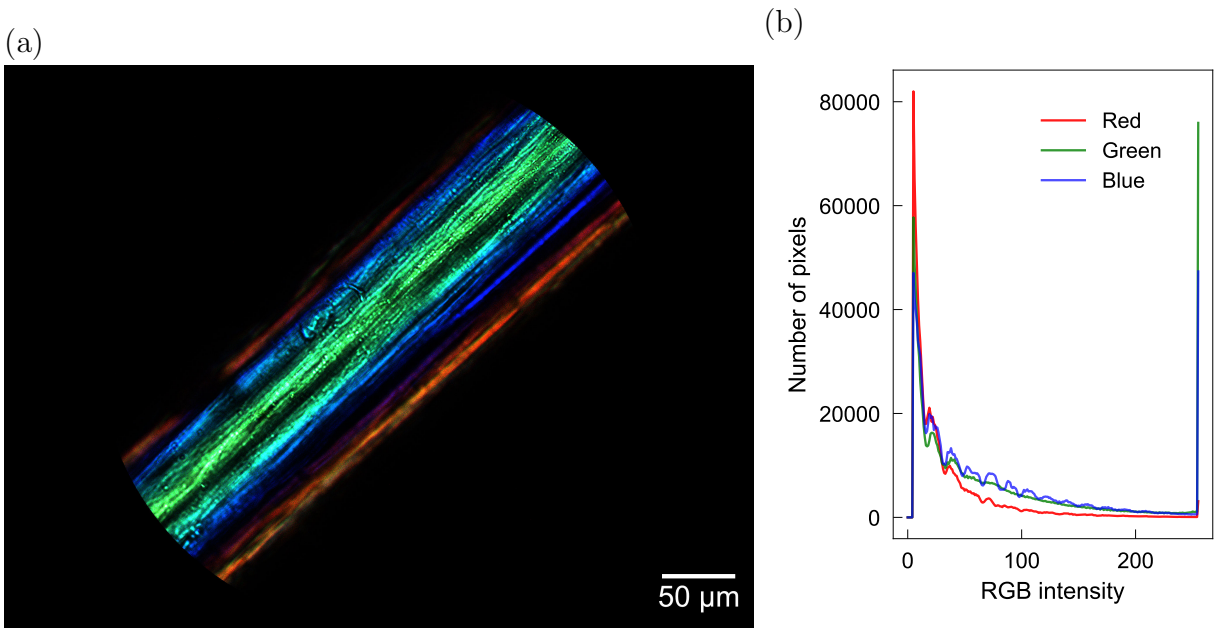

Figure S14: (a) Collagen fiber measured with polarization microscopy at rotation angle $\alpha = 140°$ masked with circular area, whereby outlined areas were set zero (R=0, G=0, B=0). (b) Histogram of the previous image **??** (a).