

# Dimensionality Matters: Exploiting UV-Photopatterned 2D and Two-Photon-Printed 2.5D Contact Guidance Cues to Control Corneal Fibroblast Behavior and Collagen Deposition

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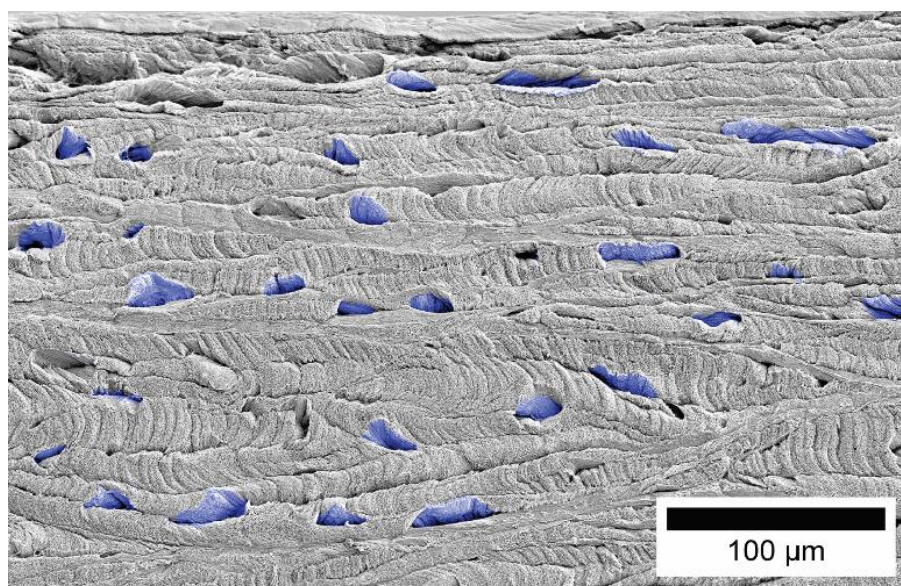
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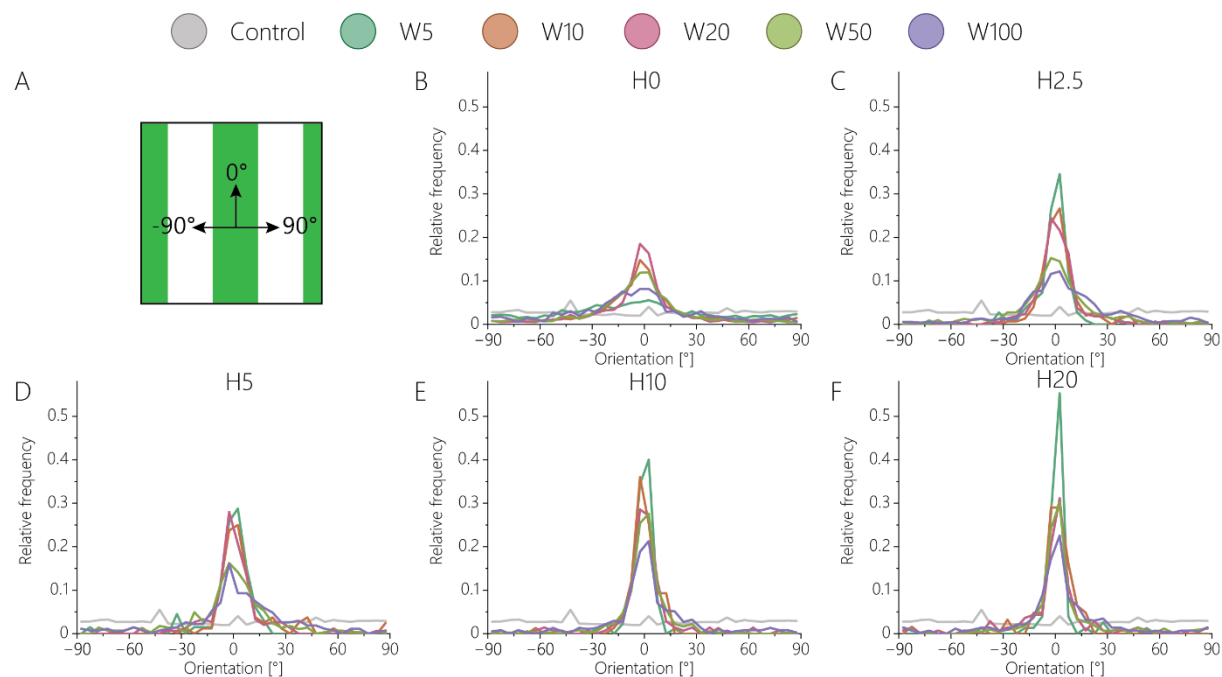
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## Supplementary Information

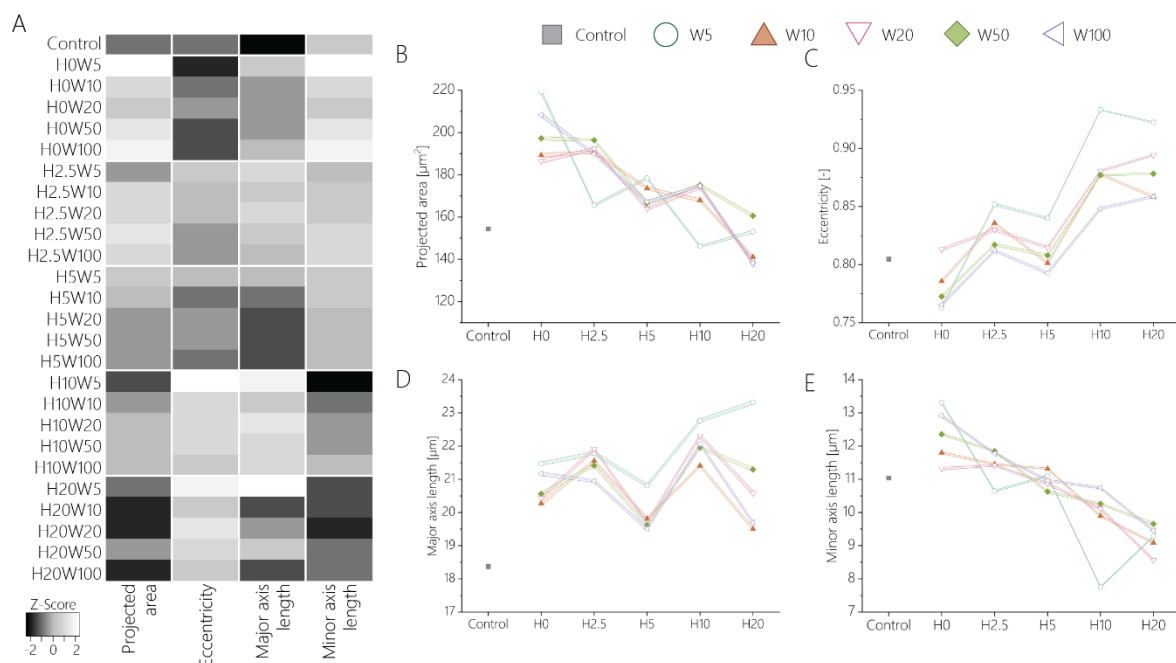


**Figure S1.** False colored SEM image of the corneal stroma cross-section shows voids in blue, which are possible hosts where keratocytes reside in their native environment.

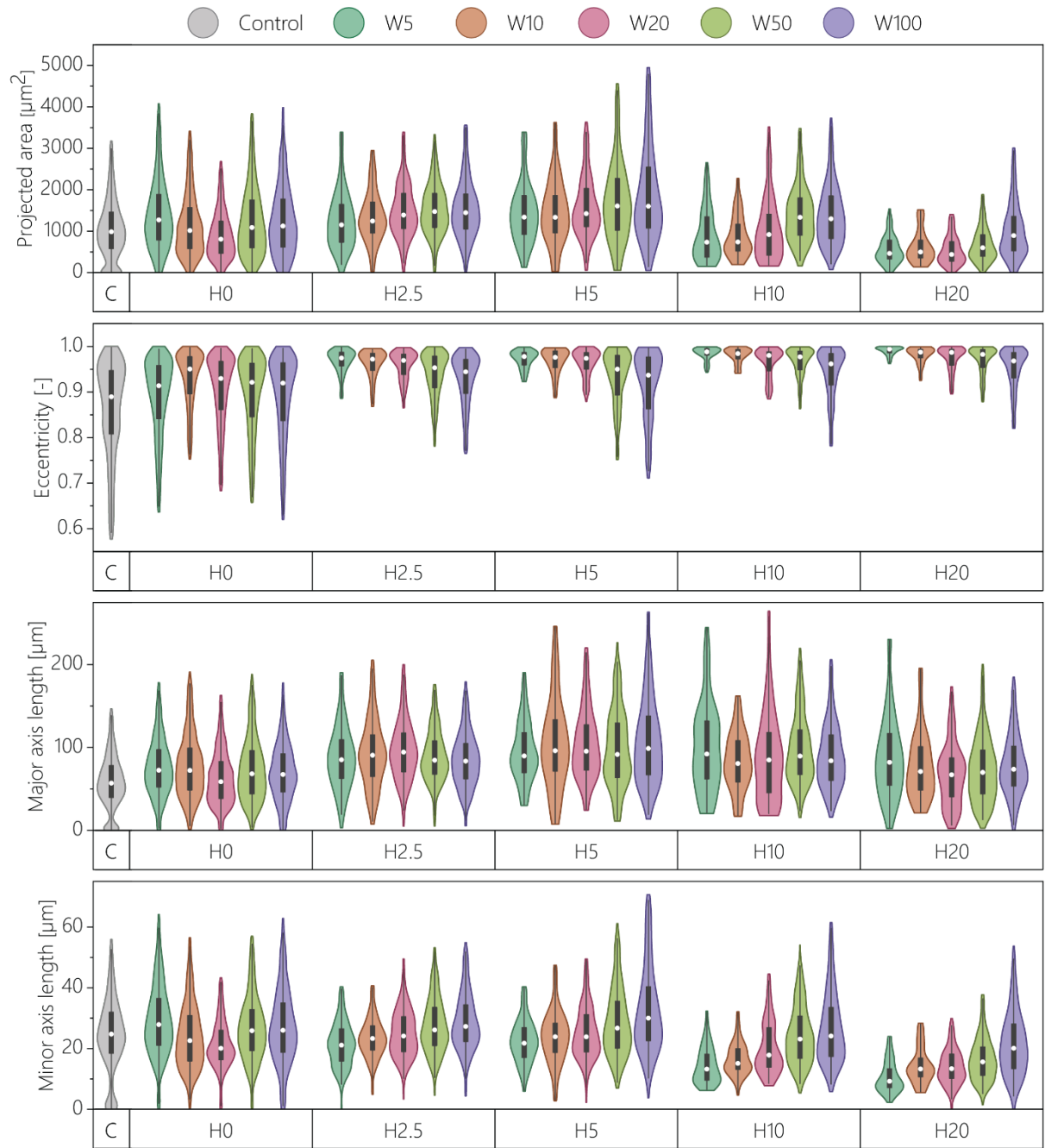


**Figure S2.** The orientation distributions of activated human primary keratocytes on contact guidance cue presenting substrates. A) Quantification of orientation with respect to the cue direction, which means 0° represents cell alignment towards pattern line direction. Orientation distributions of B) 2D cues (H0), 2.5D cues of C) 2.5 μm, D) 5 μm, E) 10 μm, and F) 20 μm

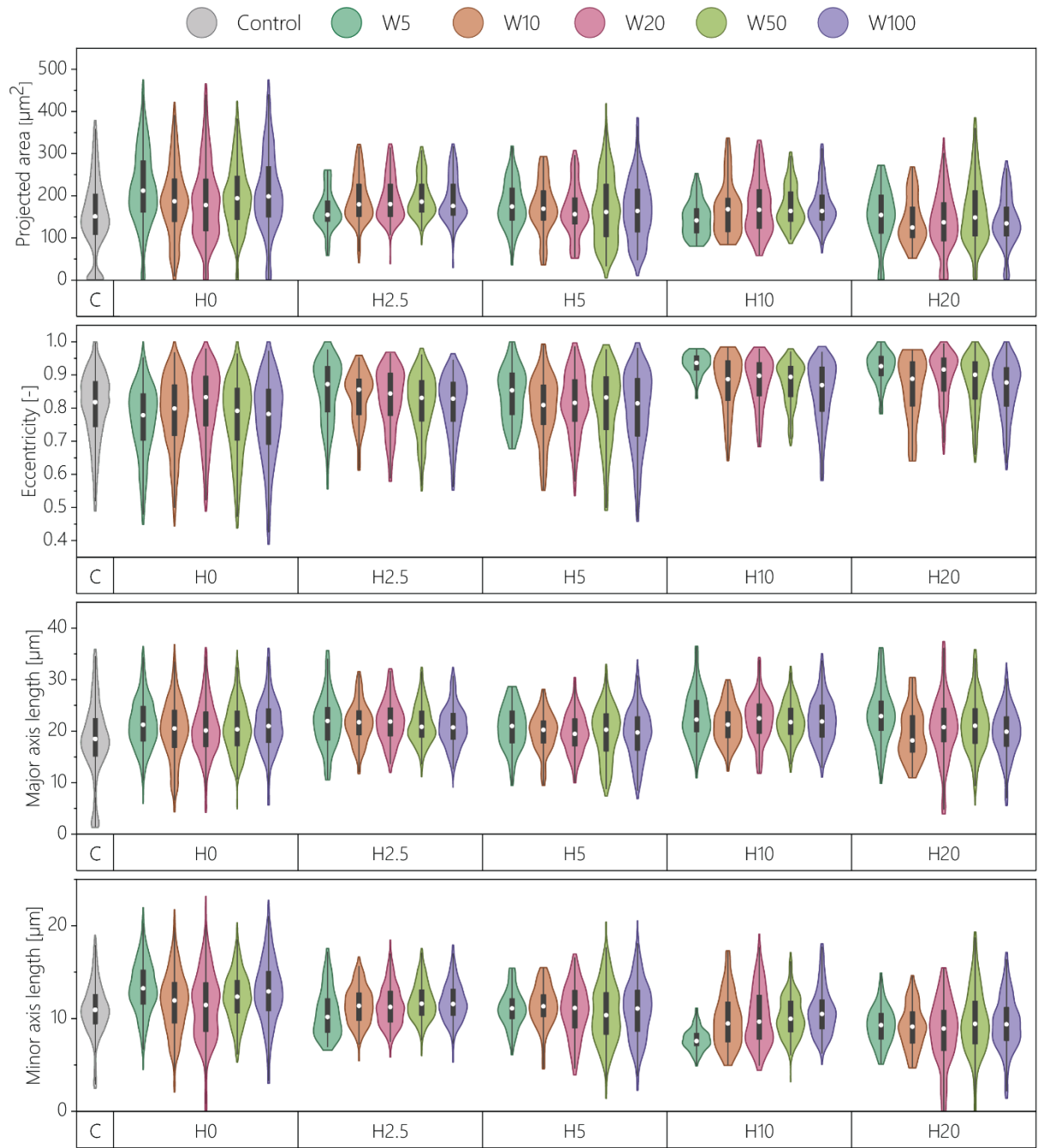
tall;  $-90^\circ$  represents a counter-clockwise rotation, and  $90^\circ$  represents a clockwise rotation. Statistical differences between distributions are described in Table S1.



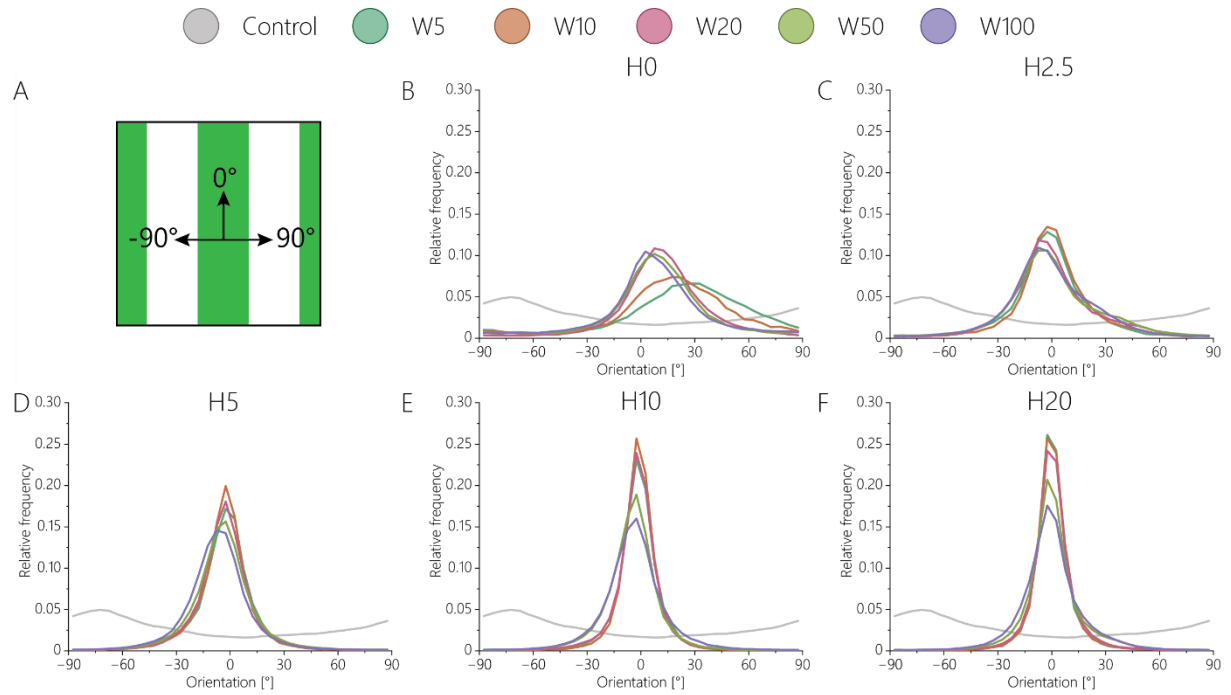
**Figure S3.** A) Heatmap of morphological parameters of the nuclei of fibroblastic keratocytes when cultured on substrates presenting 2.5D and 2D contact guidance cues. Gray scales are normalized for each readout. B) The projected area, C) eccentricity, D) major axis length, and E) minor axis length of activated keratocytes on substrates with and without (control, grey) cues. All data are presented as mean  $\pm$  standard error of the mean. Statistical differences between conditions are described in Tables S2-S5.



**Figure S4.** Violin plots for the projected area, eccentricity, major and minor axis length of fibroblastic keratocytes on contact guidance cue presenting substrates. The white points represent the median of each violin dataset. Statistical differences between conditions are described in Tables S2-S5.



**Figure S5.** Violin plots for the projected area, eccentricity, major and minor axis length of nuclei on contact guidance cue presenting substrates. The white points represent the median of each violin dataset. Statistical differences between conditions are described in Tables S2-S5.



**Figure S6.** The orientation distributions of fibroblastic keratocytes with respect to the contact guidance cues, meaning that 0° represents cells aligning according to the protein pattern (H0) or 2.5D cues (H2.5-H20) (A). Orientation distributions of B) H0, C) H2.5, D) H5, E) H10, and F) H20; -90° represents a counter-clockwise rotation, and 90° represents a clockwise rotation.











**Table S6.** Primer sequences used for RTqPCR analysis of gene expression.

<b>Peptidylprolyl isomerase A (PPIA)</b>	For	GGCAAATGCTGGACCCAACACA
	Rev	TGCTGGTCTTGCCATTCCTGGA
<b>Aldehyde dehydrogenase 3 Family member A1 (ALDH3A1)</b>	For	CTCGTCATTGGCACCTGGAACT
	Rev	CTCGCCATGTTCTCACTCAGCT
<b>Lumican (LUM)</b>	For	AACATACCAACTGTCAATGAAAACC
	Rev	TGCCATCCAAACGCAAATGCTTG
<b>Keratocan (KER)</b>	For	TGCTCATCTGCAGCACCTTCAC
	Rev	ATCCAGACGGAGGTAGCGAAGA
<b>Alpha smooth muscle actin (<math>\alpha</math>-SMA)</b>	For	CTATGCCTCTGGACGCACAACCT
	Rev	CAGATCCAGACGCATGATGGCA
<b>Cluster of differentiation 90 molecule (CD90)</b>	For	GAAGGTCCTCTACTTATCCGCC
	Rev	TGATGCCCTCACACTTGACCAG
<b>Vimentin (VIM)</b>	For	AGGCAAAGCAGGAGTCCACTGA
	Rev	ATCTGGCGTTCCAGGGACTCAT
<b>Cluster of differentiation 34 molecule (CD34)</b>	For	CCTCAGTGTCTACTGCTGGTCT
	Rev	GGAATAGCTCTGGTGGCTTGCA

**Video S1.** Live cell imaging of fibroblastic keratocytes on 2D protein patterns (H0W20). Imaging started 24 hours after seeding and continued for 6 days. Over time, cells adhere to both the protein pattern and the non-adhesive area in between the patterned areas.