



Article

Specular Microscopy of Human Corneas Stored in an Active Storage Machine

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SUPPLEMENTARY DATA

Number of cells counted per image (Table S1)

For the 1-month storage study, the overall mean number of counted cells per SM image was 1364 ± 510 (range 224–3022) at each time point. The mean number of counted cells per SM image increased over time (ANOVA, $P < 0.001$): at D2 1008 ± 426 cells (range 224–3022), at D26 1437 ± 415 cells (range 813–2630), at D28 1646 ± 470 cells (range 949–2866). The final endothelial assessment with HoeschtECD allowed extra-large wide field of analysis with a mean of 4768 ± 1769 nuclei cells (range 2751–9122) analyzed per image.

For the 3-month storage study, the overall mean number of counted cells per SM image was 1353 ± 194 (range 877–1729) at each time point. The mean number of counted cells per SM image increased similarly over time (ANOVA, $P < 0.001$): at D2 1033 ± 105 cells (range 877–1286), at D23 1241 ± 95 cells (range 1085–1456), at D44 1360 ± 81 cells (range 1282–1597), at D65 1433 ± 88 cells (range 1284–1583), at D86 1493 ± 68 cells (range 1355–1657), and at D88 1558 ± 67 cells (range 1475–1729). The final endothelial assessment HoeschtECD allowed extra-large wide field of analysis with a mean of 3235 ± 266 nuclei cells (range 2954–4035) analyzed per image.

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Table S1. Number of cells counted per image in the 1 and 3-month studies.

1-month Study [8]	D2	D26	D28	Final Hoechst			
Mean N of cells±SD	1008±426	1437±415	1646±470	4768±1769			
3-month Study [9]	D2	D23	D44	D65	D86	D88	Final Hoechst
Mean N of cells±SD	1033±105	1241±95	1360±81	1433±88	1493±68	1558±67	3235±266

N = number analyzed ; D = day ; SD = standard deviation

Thanks to our custom SM, a precise follow-up of the same area all along the storage was possible. **Figure S1** and **Video S1** highlight EC redistribution over three months.

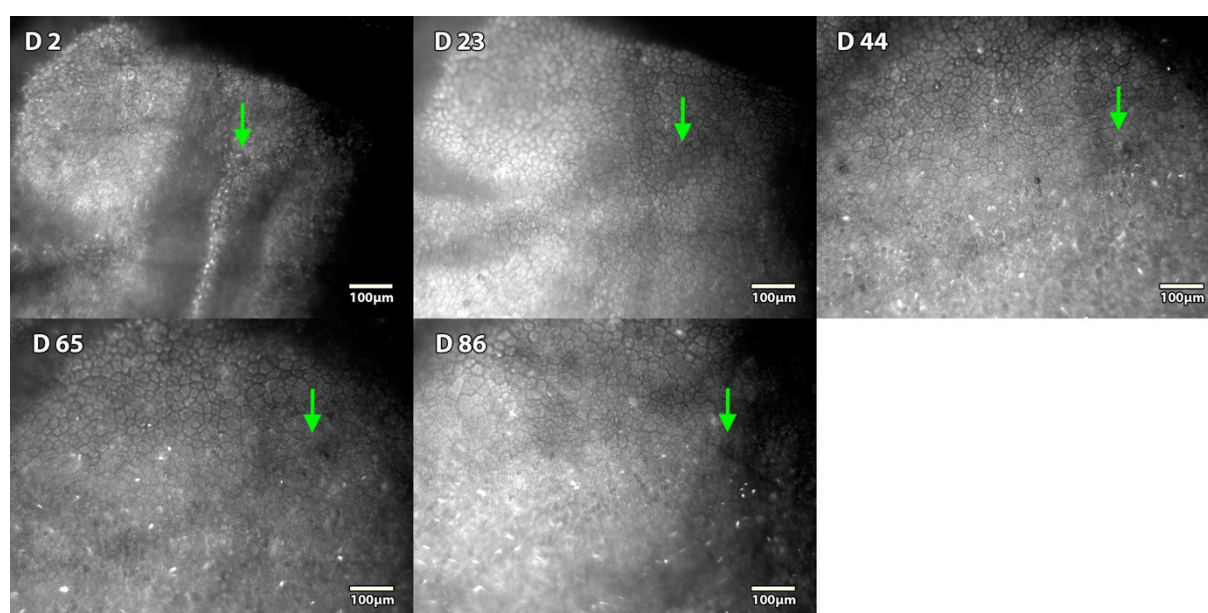
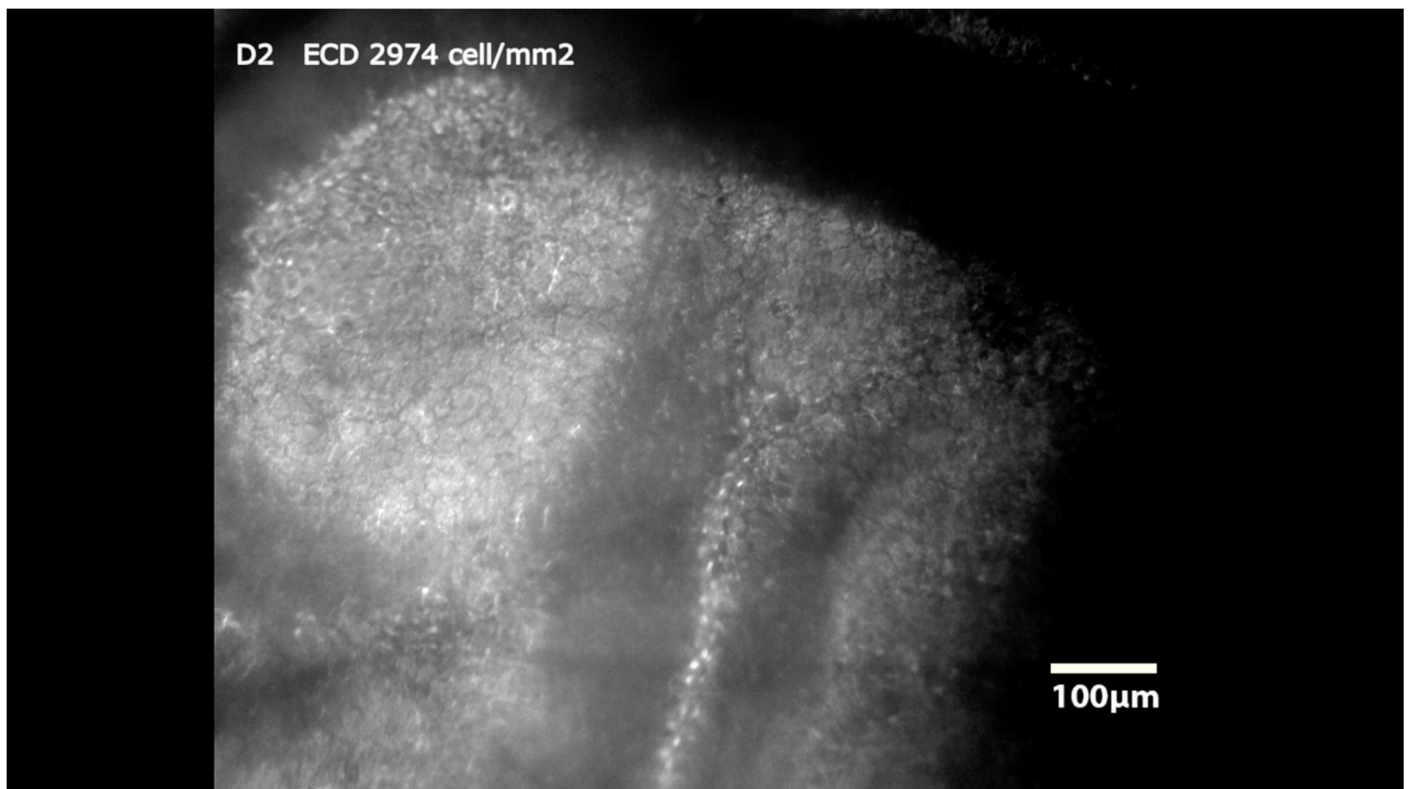
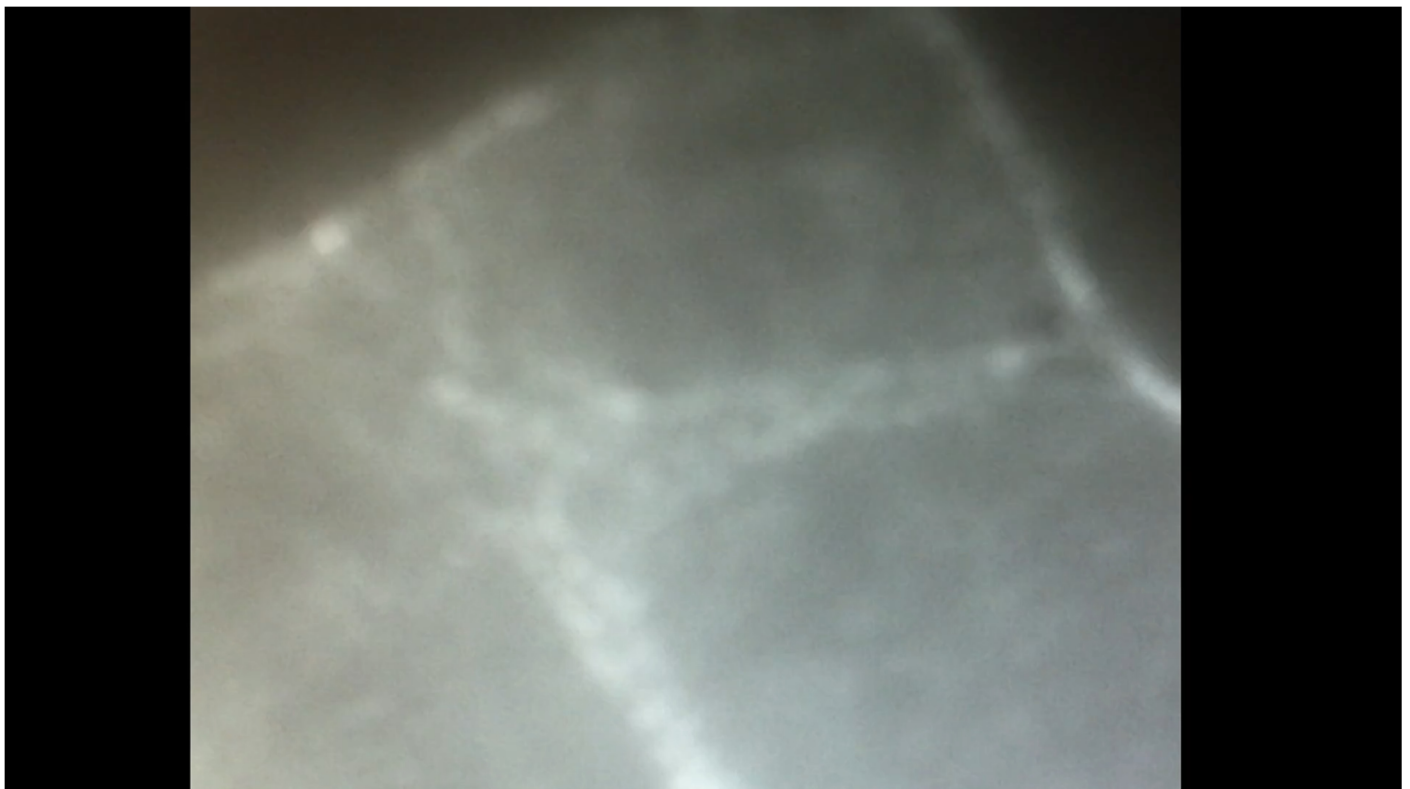


Figure S1. Time lapse of the same endothelial area followed during three months in the active storage machine. The same area for each field was precisely acquired repeatedly from Day 2 to Day 86 thanks to micrometric stage of our specular microscope. Green arrow indicated the redistribution of endothelial cells at the same point of the field. Endothelial cell density decreased over time, with larger cells, a progressive slight increased polymorphism and pleiomorphism.



Video S1. Time lapse showing EC redistribution over three months.

Our custom allowed movie recording in the same area, by modulating depth on Z axis (**Video S2**).



Video S2. Movie recording example on a defined area.