

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  $1364 \pm 510$  (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  $1008 \pm 426$  cells (range 224-3022), at D26  $1437 \pm 415$  cells (range 813-2630), at D28  $1646 \pm 470$  cells (range 949-2866). The final endothelial assessment with HoeschtECD allowed extra-large wide field of analysis with a mean of  $4768 \pm 1769$  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  $1353 \pm 194$  (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  $1033 \pm 105$  cells (range 877-1286), at D23  $1241 \pm 95$  cells (range 1085-1456), at D44  $1360 \pm 81$  cells (range 1282-1597), at D65  $1433 \pm 88$  cells (range 1284-1583), at D86  $1493 \pm 68$  cells (range 1355-1657), and at D88  $1558 \pm 67$  cells (range 1475-1729). The final endothelial assessment HoeschtECD allowed extra-large wide field of analysis with a mean of  $3235 \pm 266$  nuclei cells (range 2954-4035) analyzed per image.

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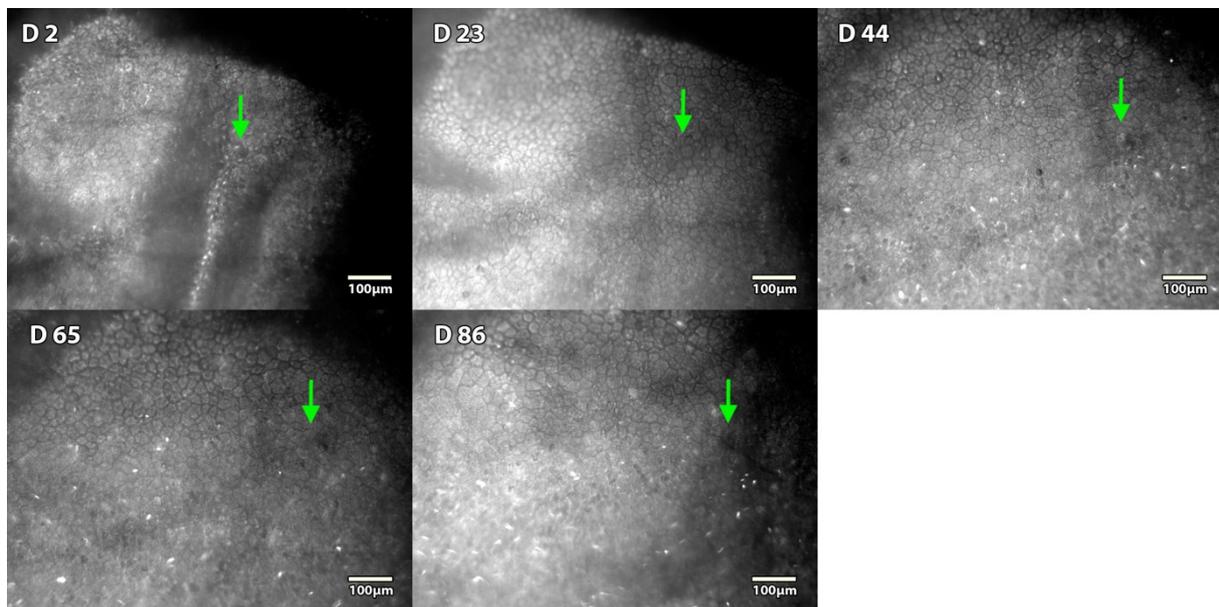
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**Table S1.** Number of cells counted per image in the 1 and 3-month studies.

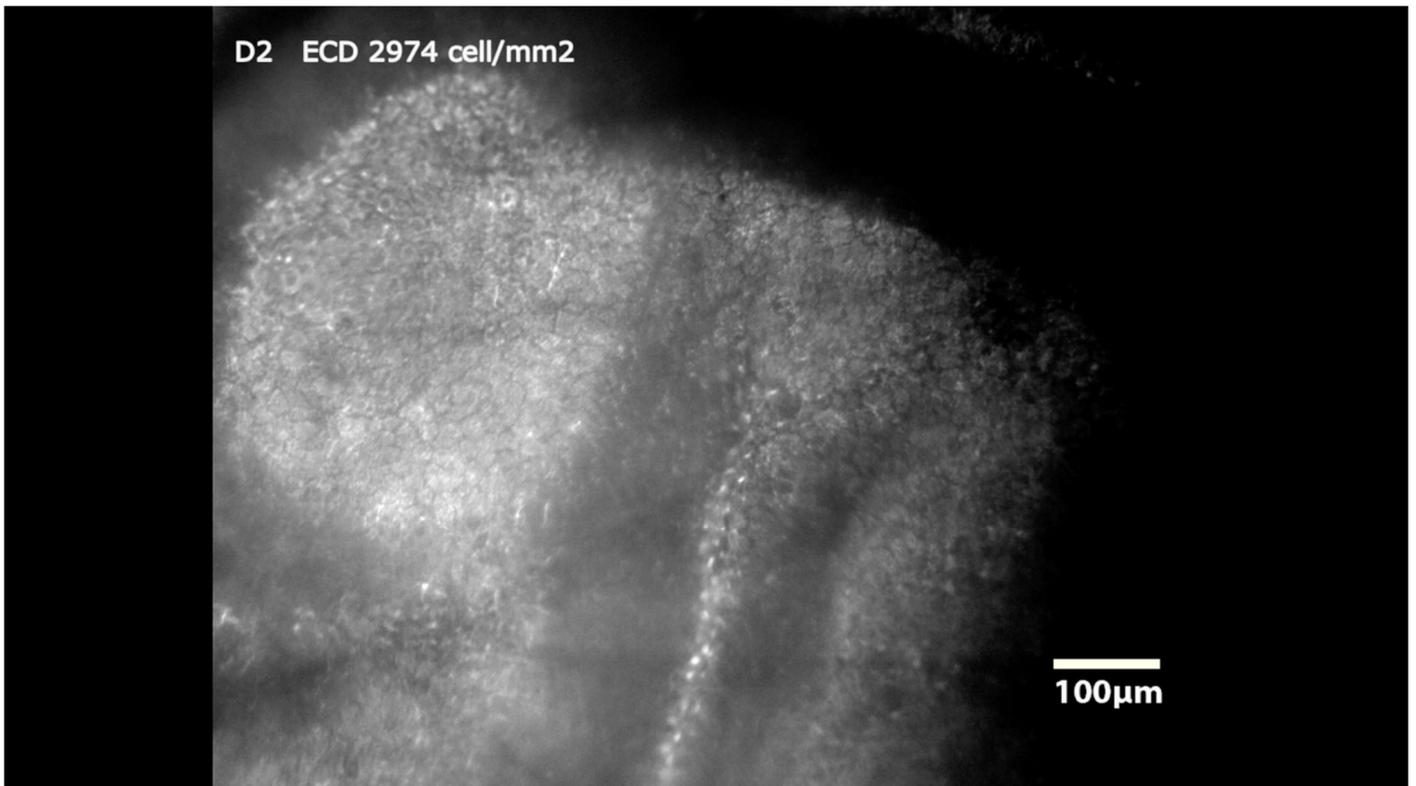
<b>1-month Study [8]</b>	<b>D2</b>	<b>D26</b>	<b>D28</b>	<b>Final Hoechst</b>			
Mean N of cells±SD	1008±426	1437±415	1646±470	4768±1769			
<b>3-month Study [9]</b>	<b>D2</b>	<b>D23</b>	<b>D44</b>	<b>D65</b>	<b>D86</b>	<b>D88</b>	<b>Final Hoechst</b>
Mean N of cells±SD	1033±105	1241±95	1360±81	1433±88	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.

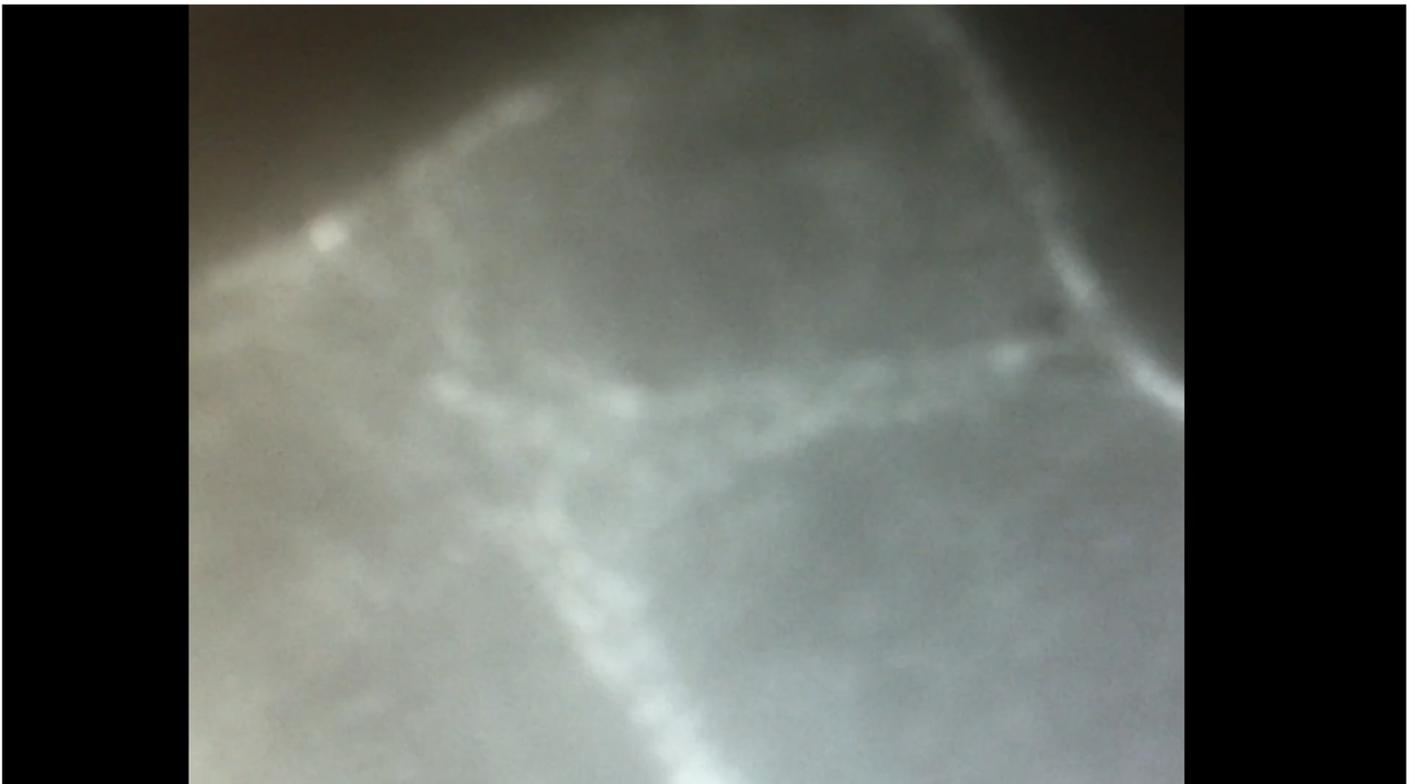


**Figure S1.** Time lapse of the same endothelial area followed during three months in the active storage machine. The same area for each field was precisely acquired repeatedly from Day 2 to Day 86 thanks to micrometric stage of our specular microscope. Green arrow indicated the redistribution of endothelial 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.