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1 **Unveiling mechanical interactions between cell division and extracellular**
2 **matrix in human colonic epithelium organoids: A 4D study using DVC**

3

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20

21

22

23 **Abstract**

24 Cell division is a major event in tissue homeostasis, enabling renewal and regeneration. Stem
25 cells, in particular, play an important role in this homeostasis, thanks to their ability to perform
26 symmetric or asymmetric cell divisions. To study cell division, the human colon epithelium represents
27 a model of choice due to its rapid renewal and therefore high proliferative potential. Currently,
28 studying the live mechanical interactions between the epithelium and its matrix *in vivo* is challenging
29 due to the lack of suitable methods. 3D human colon organoids seeded in Matrigel® are good models
30 for this purpose as, from isolated stem cells, they recapitulate the tissue architecture organization and
31 properties. This culture set-up also allows to study the matrix displacements around the organoid.

32 Here, we studied the impact of cell division within the colonic epithelium on the extracellular
33 matrix. We performed and validated an original experimental and analytical process with a 3D time-
34 lapse confocal microscopy to follow cell mitosis and matrix movements on which we performed Digital
35 Volume Correlation. We showed that these two different types of cell division impact the matrix
36 differently with the asymmetric divisions causing a mainly uniaxial displacement, whereas symmetric
37 ones involved a multiaxial and more important one.

38

39 **Keywords:** 4D Digital Volume Correlation, Organoid, Extracellular matrix, Cell division, 4D
40 displacements

41 Introduction

42 Cell division is a crucial process in tissues as it is involved during development, homeostasis as
43 well as in regenerative process ¹. Once adult, the epithelia, which represent the boundaries between
44 our inner body and the outside world, display high number of cell division events to assure their
45 renewal and maintain their barrier function. Among them, the colonic epithelium acts as a selective
46 barrier between the content of the intestinal lumen (food bolus, microbiota, mucus...) and the lower
47 layers of the colonic mucosa. This epithelium consists in a monolayer of columnar cells lining two
48 distinct compartments, the crypts that form invaginations and the plateaus corresponding to flat areas.
49 This epithelium is among the most proliferative and fastest-renewing one of our body assuring the
50 renewal of the entire intestinal lining within a week ². It finds its origin at the bottom of the crypts
51 which shelter the intestinal stem cells (ISC) assuring the renewal of the epithelium. Extensive
52 proliferation occurs within the crypt compartment due to the stem cells and the transient amplifying
53 cells, corresponding to progenitors, which will differentiate when reaching the plateau to generate the
54 lineages of differentiated cells assuring the functions of the tissue (water and ions absorption,
55 protection against pathogens...).

56 The intestinal epithelium renewal is precisely controlled and depends on the spatial
57 organization of signals from the crypt environment, namely the intestinal niche. In physiological
58 conditions, the intestinal niche controls the homeostasis of the epithelium and the integrity of the
59 crypt ³. This niche includes the extracellular matrix (ECM) which participates to the cell fate ⁴. The
60 dynamics of the ECM is a key element in the evolution of tissue architecture, participating to the
61 establishment and maintenance of stem cell niche, cell differentiation but also branching or wound
62 repair ⁵. Its biochemical properties, such as its components or growth factors and cytokines gradients,
63 have been studied since a long date, however the last decade has seen considerable interest in
64 understanding the effects of its mechanical interaction with the epithelium ⁴. Indeed, cells perceive
65 physical stimuli from the matrix notably via interactions between their cell adhesion molecules linked
66 to their cytoskeleton such as integrins ^{6,7} or ion channels such as Piezo ⁸. The epithelium can also sense
67 the mechanical properties of the ECM (topography, stiffness, elasticity) and translate them into
68 intracellular messages to control a range of physiological processes regulating cellular phenotypes and
69 behaviours ⁹. Reciprocally, the epithelium itself applies mechanical loads to the matrix, notably radial
70 and/or orthoradial tensile forces, as a result of biological processes involved in homeostasis ¹⁰⁻¹².
71 Among these processes, the contribution of stem cell and progenitor divisions are major given the high
72 proliferation rate of these cells in the colon. Regarding their mode of division, both cell types can
73 proliferate by symmetric division, assuring their expansion during tissue regeneration process ¹³.
74 However, only stem cells have the capacity to self-renew indefinitely by asymmetric division, leading

75 to their own renewal while giving birth to a progenitor cell ^{14,15,16}. Progenitors, on the other hand, have
76 a very limited capacity for self-renewal, and after division cannot maintain their undifferentiated state
77 indefinitely. They can divide several times by symmetrical division, but eventually differentiate. In the
78 case of symmetric division, the mitotic spindle is mainly parallel (0-30°) to the matrix support, while in
79 asymmetric division, the mitotic spindle will be mainly perpendicular (60-90°) to the support ¹⁷. In
80 consequence, both types of division do not exert the same type of cell-matrix interrelationship, and
81 from a biophysical perspective may strain the ECM differently ¹⁸.

82 To date, while the consequence of the mitotic spindle orientation during the cell division on
83 cell fate determination, tissue organization, or development is increasingly better understood ^{19,20},
84 how it affects the extracellular matrix remains largely unexplored. A main explanation is the lack of
85 biological model allowing to combine both live cell division and ECM displacement observation in three
86 dimensions. Considering the proliferative capacity of the colonic epithelium, the human colonic three
87 dimensions (3D) organoid model embedded into Matrigel[®] represents an appropriate model to
88 address this question. Indeed, human colon organoids are established from crypts isolated from
89 patient's colon biopsy that include intestinal epithelium stem cells. Cultured in 3D in Matrigel[®], playing
90 the role of their supporting matrix, these organoids display the different tissue cell populations
91 organized in a monolayer of epithelial cells ²¹. As a consequence, they display both asymmetric and
92 symmetric divisions as illustrated in Figure 1. Moreover, their *in vitro* culture permits their live
93 observation by microscopy, allowing the follow-up of the tissue culture evolution.

94 In this study, we hypothesized that asymmetrical and symmetrical divisions involving different
95 orientations of the mitotic spindle relative to the matrix will have a different impact on the 3D
96 deformations of the surrounding ECM. Thus, the present study aims to investigate over time the impact
97 of either asymmetric or symmetric divisions on 3D displacements of the ECM. The human colon 3D
98 organoid model embedded in Matrigel[®] was used and a framework based on confocal imaging, 3D
99 image reconstruction, cell segmentation and Digital Volume Correlation (DVC) on the matrix was
100 developed to evaluate this hypothesis.

101 **Results**

102 As aforementioned, we chose the human colon 3D organoid model to study the impact of the
103 mitotic spindle orientation on the extracellular matrix displacements. To this end, it was essential to
104 be able to monitor the cell divisions and the ECM displacements over time. Regarding cell division,
105 DNA and tubulin, which forms the mitotic spindle, were visualized using Hoechst (blue channel) and a
106 BioTracker 488 Green Microtubule Cytoskeleton Dye (green channel) respectively (figure 2a). Matrix
107 displacement was investigated by adding fluorescent beads (red channel) into the Matrigel® (figure
108 2a). The organoid culture evolution was followed by image acquisition every twenty minutes over 3
109 hours using the Opera Phenix™ confocal microscope. We then performed 3D reconstruction of the
110 acquired images for each of the three channels (figure 2a) and analysed firstly the mitotic spindle
111 orientation and secondly the displacements of the nuclei as well as the matrix displacements.

112 **Quantification of asymmetric and symmetric divisions in the human colon 3D organoid model**

113 We first confirmed that both symmetric and asymmetric divisions occur over our epithelial
114 organoid culture (figure 2b).

115 We measured the orientation of mitotic spindle during the cell divisions occurring in the
116 human colon organoids over the three hours of observation. Mitotic spindle orientation was
117 determined as the angle formed between the mitotic spindle axis and the organoid-Matrigel®
118 boundary (figure 2b). In the case of asymmetrical divisions, the mitotic spindle is oriented
119 perpendicularly, or radially, to the Matrigel. Conversely, in the case of symmetrical divisions, the
120 spindle orientation is parallel, or orthoradial, to the support. Based on the literature ¹⁷, we chose to
121 classify the angles measured into 3 intervals: [0°-30°], [30°-60°] and [60°-90°]. From a total of 83
122 observed cell divisions visualized in 30 different organoids of 4 different patients (a minimum of 4
123 organoids/patient and a maximum of 9 organoids/patients were analysed), we counted 45 mitoses
124 with a mitotic spindle angle between 0° and 30°, i.e. 54% of divisions clearly classified as asymmetric.
125 Mitotic spindle angle between 60° and 90°, defining symmetric divisions, were 28, i.e. 34% of divisions.
126 It is also noticeable that a small fraction of mitoses (12%) cannot be assigned to any of the
127 asymmetrical or symmetrical categories, with a mitotic spindle angle between 30° and 60°.

128 These data also demonstrate the relevance of our biological model for the study of mitotic
129 spindle orientation.

130

131 **Impact of asymmetric and symmetric divisions on neighbouring cells**

132 We investigated whether asymmetric and symmetric divisions might affect the neighbouring
133 cells by focusing on the positioning of neighbouring nuclei in relation to the dividing cell (figure 3).

134 Metaphase being the most easily identifiable stage of the mitosis in the images, we quantified
135 the number of neighbouring cells directly surrounding the dividing cells and measured the distance
136 separating the nuclei of the neighbouring cells from the cell at this step of the mitosis. The barycentre
137 of the nuclei serves as reference point for the measurements. The measurements have been done on
138 11 divisions of both categories picked at random among the 45 asymmetric and 28 symmetric divisions
139 and observed on 8 organoids from 3 patients (a minimum of 2 organoids/patient and a maximum of 3
140 organoids/patients were analysed).

141 Asymmetric and symmetric dividing cells have a mean of respectively $7,55 \pm 0,37$ (mean \pm SEM)
142 and $7,82 \pm 0,55$ neighbouring cells. No significant difference is thus observed (Mann Whitney t-test, p-
143 value = 0,9394). In the absence of mitosis, we counted an average of $6,25 \pm 0,28$ neighbouring cells for
144 a given cell, thus slightly fewer than in the presence of division (Mann Whitney t-test, p-value = 0,0285
145 between control group and [60°-90°] group, and p-value = 0.0150 between control group and [0°-30°]
146 group).

147 Regarding the distances, our data showed that in the absence of cell division, nuclei are
148 significantly closer together (Mann Whitney t-test, p-value < 0,0001 between control group and [60°-
149 90°] group, and p-value < 0,0001 between control group and [0°-30°] group). Comparing the two types
150 of division, in the case of asymmetric division, the nuclei of surrounding neighbour cells are
151 significantly closer to the dividing cell (Mann-Whitney test, p-value = 0.0022) than in the case of
152 symmetric division (figure 3). This result could suggest that the displacement of the matrix induced by
153 asymmetric division could be less important than the one induced by symmetric division.

154 **Impact of asymmetric and symmetric divisions on the 3D matrix displacements**

155 We then studied the displacements induced by both types of division on the matrix
156 surrounding the epithelium. The signals obtained by confocal fluorescence microscopy, thanks to the
157 cell labels (nuclei in blue, tubulin in green, figure 2a) and the beads inserted into the matrix (in red,
158 figure 2a) to visualize the organoids and the ECM respectively, were processed in two distinct ways.

159 First, we established a framework that enables the tracking of the nuclei displacement over
160 the three hours of culture (figure 4a): on the raw images, we first performed a 3D segmentation of the
161 nuclei signal using Cellpose, and then used the Imaris software to track the nuclei over time. Indeed,
162 Cellpose allows to train the segmentation model according to a machine-learning principle and, if
163 necessary, to manually correct the segmentation. Segmentation using Cellpose therefore enables
164 customizable segmentation with a high degree of precision, unlike segmentation using Imaris, where
165 global segmentation parameters are selected and applied to all objects. This approach allowed us to
166 perfectly perform the nuclei segmentation in 3D for our study, facilitating the follow-up of the nuclei
167 displacement using Imaris.

168 As shown in figure 4a and based on the measurements reported in table 1, the global analysis
169 of nuclei displacements within the organoids enabled us to establish that, for a given organoid, the
170 nuclei display an overall uniform displacement as shown in a representative experiment (figure 4a).

171

Organoid from	Patient 1	Patient 2	Patient 3	Patient 4
Number of nuclei per organoid	176	291	154	220
Length of nuclei displacement (Mean (μm) \pm SEM)	14.91 ± 0.62	7.93 ± 0.29	9.34 ± 0.27	5.55 ± 0.17

172

173 **Table 1.** Nuclei displacements in organoids.

174

175 Next, the signal allowing to follow the displacement of the matrix is reported by the tracking
176 of the fluorescent beads dispersed within the Matrigel[®] between the time-points acquired every 20
177 minutes over the three hours of acquisition. It is used to perform image correlation, and more precisely
178 DVC, using the VicVolume software. DVC is an experimental technique based on the use of two
179 volumetric images (3D), one in a reference state, and the other in a deformed state. The principle of
180 DVC is to slice the reference volumetric image using a grid to obtain sub-volumes (composed of voxels).
181 Then, the correlation is performed to search individually each of the sub-volumes obtained from the
182 reference image in the deformed image. The result is the displacement field of the material under
183 study. According to our observations, mitosis is completed within one hour. In our analysis, we used
184 as initial time (T_0) the initiation of the mitosis (prophase) and performed the correlation between the
185 four images taken every 20 minutes up to $T_0+60\text{min}$ once the mitosis is completed.

186 Dense volume correlation shows that the extracellular matrix undergoes global displacements,
187 in each of the three spatial directions (figure 4b). However, unlike nuclei displacements, matrix
188 displacement is not uniform. In line with our hypothesis, this suggests that specific localized biological
189 events may affect the matrix differently, and in particular cell divisions and their orientation. Indeed,
190 the colour map used, where green corresponds to zero displacement, clearly shows that the matrix is
191 stressed as a whole, but not homogeneously. For example, from the first column onwards, we can see
192 the appearance of a displacement along the x axis (“u displacements”), which propagates over time
193 and gains in amplitude. In addition, we observed that nuclei displacements and matrix displacements
194 do not occur at the same scale. Indeed, nuclei exhibit displacements of the order of ten micrometers
195 (Table 1), while matrix displacements occur over greater distances. Indeed, as seen in figure 4b, the
196 matrix undergoes a global displacement of up to several tens or even hundreds of micrometers around
197 the organoid.

198 We postulated that local displacements of the matrix could correspond to cell division events
199 and hypothesized that monitoring the 3D ECM displacements could be used as a read out for mitoses
200 impact onto the matrix.

201

202 **Symmetric and asymmetric divisions impact the matrix differently**

203 Penetrating the volume correlation along the z axis, we found that the presence of cell division
204 generated a local displacement of the matrix, as exemplified in figure 5a. Indeed, on the right-hand
205 side of the images, the green colour indicates a matrix displacement of 0 μm (so no displacement),
206 while on the left-hand side we observed a wave of displacement whose intensity is strongest at the
207 level of mitosis, identified in the first photo, and propagates throughout the (x,y) plane.

208 Having established that cell divisions participate in local ECM movements, we set out to
209 determine the role of mitotic spindle orientation in these displacements. To solely investigate the
210 impact of independent mitosis on matrix displacements and not of combined mitosis events, we only
211 studied cases where mitosis was isolated, in both time and space. Here, 8 divisions of each type,
212 observed in 5 organoids from 2 patients, were studied using these criteria. For each mitosis, matrix
213 displacements in each of the three directions were measured at the cell-ECM frontier, and sorted
214 relative to the two others under the labels of “longest displacement”, “intermediate displacement”
215 and “smallest displacement” with values between 0 (i.e. no displacement) and 10 μm . For asymmetric
216 and symmetric divisions, the longest displacement is significantly greater than the intermediate one
217 (paired t test; asymmetric: p-value = 0.0146; symmetric: p-value = 0.0152). The intermediate
218 displacement is significantly greater than the smallest one only for symmetric divisions (paired t test;
219 asymmetric: p-value = 0.1109; symmetric: p-value = 0.0474) as shown in figure 5b.

220 Only 1 out of 8 asymmetric cell divisions causes the matrix to stain in the three directions, 2
221 cause a displacement in two directions while 5 cause a movement in the matrix in only one direction.
222 In this case, the axis of matrix displacement corresponds to the axis of mitotic spindle orientation in
223 the same plane. The longest displacement observed is an average of $4.08 \pm 1.90 \mu\text{m}$ (mean \pm SEM) for
224 this type of mitosis. We can see that displacements in the other two directions are much smaller,
225 averaging $1.11 \pm 1.39 \mu\text{m}$ (mean \pm SEM) for the intermediate displacement and $0.16 \pm 0.28 \mu\text{m}$ (mean
226 \pm SEM) for the smallest displacement (figure 5b).

227 Symmetric cell divisions, on the other hand, always impact the matrix in several directions,
228 either in two (3 out of 8 symmetric mitosis) or three directions (5 out of 8). In fact, the mean of the
229 longest displacements is $5.44 \mu\text{m} \pm 2.31 \mu\text{m}$ (mean \pm SEM), slightly greater than for asymmetric
230 divisions, but without any significant difference. On the other hand, the mean of the intermediate

231 displacement is $3.76 \pm 2.21 \mu\text{m}$ (mean \pm SEM). Similarly, the smallest displacement is $1.7 \pm 1.48 \mu\text{m}$
232 (mean \pm SEM) (figure 4b).

233 From a qualitative standpoint, we can conclude that asymmetric divisions involve a rather
234 uniaxial displacement, whereas symmetric mitoses cause a multiaxial one. In addition, from a
235 quantitative standpoint, the displacements induced in the case of symmetric division are
236 systematically greater. Considering the longest displacement, there is no significant difference
237 between the one observed in symmetric divisions and the one in asymmetric division (respectively of
238 $5.44\mu\text{m} \pm 2.31$ and $4.08 \pm 1.90 \mu\text{m}$, unpaired t test, p-value = 0.3421). Nevertheless, considering the
239 intermediate displacement, we can note that it is 3 times greater in the case of symmetric divisions
240 than the one observed in asymmetric divisions (3.76 ± 2.21 and $1.11 \pm 1.39 \mu\text{m}$, unpaired t test, p-value
241 = 0.0373). At last, we observed a factor of 10 between the smallest displacements of symmetric and
242 asymmetric cell divisions (1.7 ± 1.48 and $0.16 \pm 0.28 \mu\text{m}$, unpaired t test, p-value = 0.0453).

243

244 Discussion

245 The study of mechanical interactions between the epithelium and its matrix, particularly the
246 impact of cell division in real time and in 3D, remains complex to this day due to technical constraints
247 ¹⁰. By choosing the human colon organoid, we ensured the suitability of the biological model on some
248 major criteria allowing this type of study: physiological relevance (different types of cell division), rapid
249 growth and the accessibility of the ECM. In order to study the ECM displacements, we developed a
250 DVC approach, never used before for studying ECM displacements in a three-dimensional environment
251 *in vitro*. In this article, we have thus provided a new method for measuring and analysing the
252 mechanical interactions between the colonic epithelium and its ECM. In fine, this approach allowed us
253 to visualize and analyse over time in 3D the displacements of both the organoid and its surrounding
254 matrix. We observed a strong overall contraction of the epithelium overtime. The displacements of the
255 matrix obtained by DVC over a period of one hour correspond to a contraction following the “u”
256 direction and an expansion following the “v” direction. Analysis of the positions of neighbouring cells
257 during mitoses indicates greater proximity during the asymmetrical divisions than during the
258 symmetrical divisions. Moreover, the analyses carried out during the cell division phases show greater
259 movements of the matrix during symmetrical mitoses than during asymmetrical division.

260 Previous studies have highlighted the ability of stem cells and early progenitors to divide
261 respectively symmetrically and asymmetrically which is crucial in adapting the needs of the colon
262 during homeostasis, growth, or regeneration ^{14,16}. Each type of division is involved in distinct processes
263 of intestinal homeostasis: symmetric mitosis are responsible for epithelial renewal, while asymmetric

264 ones are involved in the differentiation process ¹⁶. However, while a number of factors that can orient
265 direct cell division are well known ²²⁻²⁵, how spindle orientation affects the matrix environment
266 remains largely unexplored. Our results show that asymmetric divisions appear to have a local and
267 uniaxial impact on the matrix, in contrast to the symmetric divisions that affect the matrix more widely
268 in different axis. From a biological perspective, this finding could be of interest to be further study in a
269 pathological context. As an example, it could provide clues to the formation of bifid crypts, one of the
270 first visible sign of tumour initiation, which rely on area of multiple hyperproliferative cells within the
271 crypt. As changes in ECM composition, rigidity and/or organization may activate pro-tumour signalling
272 pathways, it is possible that matrix remodelling due to symmetric division participate to this process
273 ²⁶. In addition, it is well known that intestinal stem cells can increase the proportion of symmetric
274 mitosis in response to environmental cues. In order to assess the mechanical interactions between the
275 epithelium and its matrix in a cancer context, it may therefore be interesting to work with tumour
276 organoids.

277

278 **Methods**

279 ***Human colon organoids***

280 The organoids used in this study are part of the registered COLIC biobank (DC-2015-2443) (for details,
281 refer to ²⁷). The culture was established from colonic samples obtained from surgical resections of
282 patients treated for colorectal cancer at the Toulouse University Hospital that gave informed consent.
283 Tissues were harvested in healthy zones at the margin of the resection and at least 10 cm away from
284 the tumour. Organoids are stored in liquid nitrogen to ensure optimum preservation and are thawed
285 when needed. After thawing, organoids undergo a passage: 3D structures are dissociated using trypsin
286 (*TrypLE*[®], Invitrogen) supplemented with 10 μ M Y27632 during 10 min at 37°C. Mechanical agitation
287 takes place every 3 minutes to promote dissociation. Digestion is stopped by adding washing medium
288 (DMEM/F12 supplemented with 5% SFV and 10 μ M of Y27632) at 4°C. The solution containing
289 dissociated organoids is then centrifuged 5 min at 400g, at 4°C. The cell pellet is recovered and re-
290 suspended in Matrigel[®]. Matrigel[®] domes are deposited in 96-well culture plates (10 μ L/well) with glass
291 bottom. Next, 100 μ L of pro-stemness culture medium ²⁸ is added to each well, and renewed every two
292 days. Cell growth occurs in an incubator at 37°C and 5% CO₂, during 10 days.

293 ***Fluorescent Beads Adding***

294 Fluorescent silica beads are added into the Matrigel[®] to obtain a speckle pattern (10% v/v Matrigel[®]
295 beads) just before the Matrigel[®] dome formation stage. The beads (Sicastar Red-F, Micromod) are
296 1 μ m-diameter silica beads covered with a red fluorophore (excitation 569 nm / emission 585 nm).

297 ***Fluorescent probes***

298 3h prior microscope image acquisition, organoid culture medium is removed and replaced by fresh
299 medium containing the fluorescent probes to stain the DNA (Hoechst, 1:3000 v/v) and the tubulin
300 (Biotracker 488 Microtubules, Merck, dilution 1:500 used with Verapamil 1:1000 to contain the probe
301 inside the cells).

302 ***Imaging Set-up***

303 At day 10 of culture, the organoids are imaged in a thermostatically-controlled chamber (37°C),
304 buffered with CO₂ (5%) with the Opera Phenix™ microscope (Perkin Elmer). The objective used is a x20
305 water objective. The z-step used is 2 μ m, covering a z-depth of around 100 μ m (i.e. around 50 z-slice).
306 Images are acquired every 20 minutes during 3 hours.

307 ***Image pre-processing***

308 The images of the beads are processed using the "Basic" plugin on ImageJ, which enables illumination
309 defects to be corrected in terms of depth. This step is required to obtain a uniform signal that can be
310 used for DVC. The nuclear signal is denoised with the "Subtract Background" option on ImageJ.

311 ***Mitotic orientation measurements***

312 The angular orientations correspond to the angle between mitotic spindle and ECM at the basal side
313 of the cell. The measurements of mitotic spindle orientations are made in the acquisition plane (x,y),
314 and normalized relative to the basal side of the organoid in ImageJ. The angles thus obtained are
315 classified into 3 categories, based on the literature ¹⁷: [0-30°], [30-60°] and [60-90°].

316 ***Nuclei segmentation***

317 Nuclei were segmented using the Cellpose 2.0 machine-learning-based approach. The system is trained
318 with nuclei images from several organoids. The training images are 2D slice, taken in all the possible
319 plans (x,y), (x,z) and (y,z) ²⁹. After the training, z-stack are segmented plan by plan and then the 2D
320 segmentations were assembled in 3D using the same software to obtain 3D labels of nuclei.

321 ***Nuclei tracking***

322 The segmentation mask obtained with CellPose is used in the commercial Imaris software to track the
323 nuclei over time. The barycentre of each nucleus is used to perform the tracking thanks to the
324 “Surface” Tool of Imaris.

325 ***Matrix displacement analysis***

326 Digital volume correlation (DVC) was performed using VicVolume software (*Correlated Solutions*®) on
327 fluorescent beads images. A subset size between of 29 voxels and a step of 3 are optimal. As illustrated
328 in figure 6, the image z-stack made up of voxels is digitally sliced using a grid of user-selected size,
329 adapted to the size and density of the speckle pattern. Each window thus created (orange square) on
330 the reference image, i.e. the first time point “t”, is then searched for in the other z-stacks,
331 corresponding to the deformed states, i.e. the subsequent time points “t +Δt”. By obtaining the
332 translational and rotational displacements for each window, a displacement field map is obtained for
333 the entire image. Matrix displacements were thus analysed in 3D, along each of the three axes
334 corresponding to the three dimensions. The reference frame used is (x,y,z), where (x,y) corresponds
335 to the microscope acquisition plane. Displacements in u correspond to matrix displacements along the
336 x axis. Similarly, v displacements correspond to displacements along the y axis, and w displacements
337 to displacements along the z axis. The size of a voxel is (0.64, 0.64, 2) μm.

338 ***Statistical analysis***

339 Data are expressed as mean ± SEM. Analyses were performed using the GraphPad Prism 9 software.
340 Statistical significance was determined by paired or unpaired t-test or Mann Whitney test, the use of
341 one or the other is mentioned in the text. Significance was accepted for p<0.05 for each test
342 performed.

343 **Figures legends**

344

345 **Figure 1.** Stem cell divisions. Human colonic organoids are used as biological model to study
346 the mechanical interactions between the epithelium and its extracellular matrix including during
347 mitosis. Stem cell (purple on the scheme) division can be symmetric (i.e. parallel to the ECM) or
348 asymmetric (i.e. perpendicular to the ECM). Differentiated cells are represented in blue (secretory
349 lineage) and red (absorbent lineage). Orange arrows represent orthoradial traction and green arrows
350 represent radial one. The width of arrows corresponds to the hypothetical importance of the traction.

351 **Figure 2.** Validation of the biological model (a) Imaging process. Organoids are imaged with
352 the Opera Phenix[®] microscope (Perkin Elmer). Nuclei are labelled with Hoechst, laser 405 nm (blue
353 lut), tubulin is labelled with Biotracker Tubulin, laser 488 nm (green lut) and beads are imaged with
354 laser 561 nm (red lut). (b) On the left, representative images of symmetric and asymmetric divisions.
355 On the right, polar representation of the mitotic spindle orientations distribution (n=83 cells divisions
356 from 30 organoids from 5 patients).

357 **Figure 3.** Measurements of the distances between the dividing nucleus and its surrounding
358 nuclei. On the left, graph presenting the distances between the dividing nucleus in metaphase and the
359 nuclei of neighbouring cells for each type of division and for controls (without mitosis), (Mann-Whitney
360 test, the measurements have been done on 11 divisions of both categories picked at random among
361 the 45 asymmetric and 28 symmetric divisions and observed on 8 organoids from 3 patients (a
362 minimum of 2 organoids/patient and a maximum of 3 organoids/patients were analysed), p-value =
363 0,0022 between the two types of division and p-value < 0,0001 between each of these groups and the
364 control one, mean \pm SEM). On the right, representative images for each type of division and a condition
365 'control' without cell division, with the distances represented. Points for measuring distances are
366 positioned at the barycentre of nuclei obtained through segmentation. A cell is considered as a
367 neighbouring cell if its membrane is adjacent to the membrane of the dividing cell, visualized with the
368 tubulin labelling.

369 **Figure 4.** Displacements of organoid nuclei over time obtained after segmentation and
370 tracking. (a) Nuclei are segmented and tracked over time, respectively with CellPose and Imaris. After
371 the labelling and acquisition, nuclei are segmented with CellPose at each time point during 3h
372 (Segmentation over time, visualisation of the segmentation masks in Imaris viewer) and tracked over
373 time with Imaris tracker (Tracking). The global displacement (Displacement vectors) illustrates the
374 uniform movement of nuclei. All scales are on the concerned images. (b) Displacement fields of the
375 matrix obtained by digital volume correlation. Displacements of the matrix after 20 min (left), 40 min

376 (middle) and 60 min (right). Voxel displacements in each axis are shown: u in the first row, v in the
377 second row, w in the third row. The colour scale is in voxels. The voxel size is 0.64 x 0.64 x 2 μ m.

378 **Figure 5.** Impact of the mitotic spindle orientation on ECM. (a) Zoom on a z-axis section
379 containing mitosis. From left to right: tubulin, ECM displacements along the u axis in the same section
380 plane, merge of tubulin channel and matrix displacements, zoom on the area where cell division takes
381 place. All scales are on the concerned images. (b) ECM displacements due to symmetric or asymmetric
382 division. On the top panel, tubulin labelling showing the mitotic spindle. The arrows indicate the
383 mitotic spindle. On the bottom panel, representation of matrix displacements on the mitosis z-slice,
384 calculated in 3D, in the three axes. Orange and magenta squares represent the areas impacted by the
385 mitosis. On the right, representation of the longest, intermediate and smallest displacements of the
386 ECM for each type of cell division. Representation of the mean \pm SEM (t test, 8 measurements in each
387 group, p-value = 0.045 between both smallest displacements, p-value = 0.037 between intermediate
388 displacements and p-value = 0.034 between longest displacements. t test between longest and
389 intermediate displacement in asymmetric division: p-value = 0.0146; and in symmetric: p-value =
390 0.0152. t test between intermediate and smallest displacement in asymmetric divisions: p-value =
391 0.1109; and in symmetric one: p-value = 0.0474).

392 **Figure 6.** Digital Volume Correlation (DVC) principle. DVC is performed on the beads stack
393 images thanks to VicVolume to obtain ECM displacements fields between a reference state and a
394 deformed state of the matrix.

395

396 **Data availability**

397 The data used and/or analyzed during this study are available from the corresponding author
398 on reasonable request.

399

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407

408 **Author contributions**

409 Audrey Ferrand, Florian Bugarin, Stephane Segonds and Gaelle Recher organized the project.
410 Léa Magne, Déborah Michel and Julien Laussu collected the data. Léa Magne and Thomas Pottier
411 performed data analysis and visualization. Léa Magne, Audrey Ferrand and Florian Bugarin drafted the
412 manuscript. Audrey Ferrand and Florian Bugarin led the project and oversaw manuscript preparation.
413 All authors have read and approve the submitted manuscript.

414

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420

421 **Competing interests**

422 The authors declare no competing interests.

423

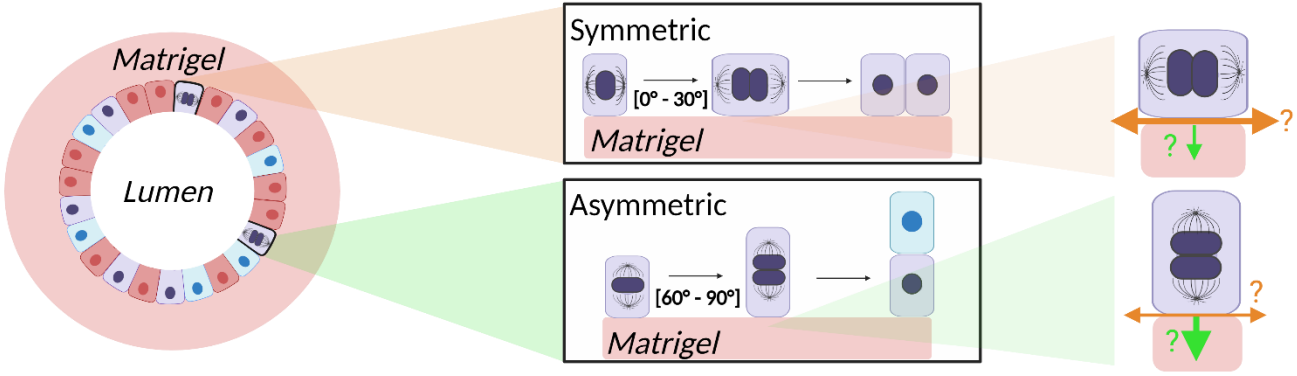
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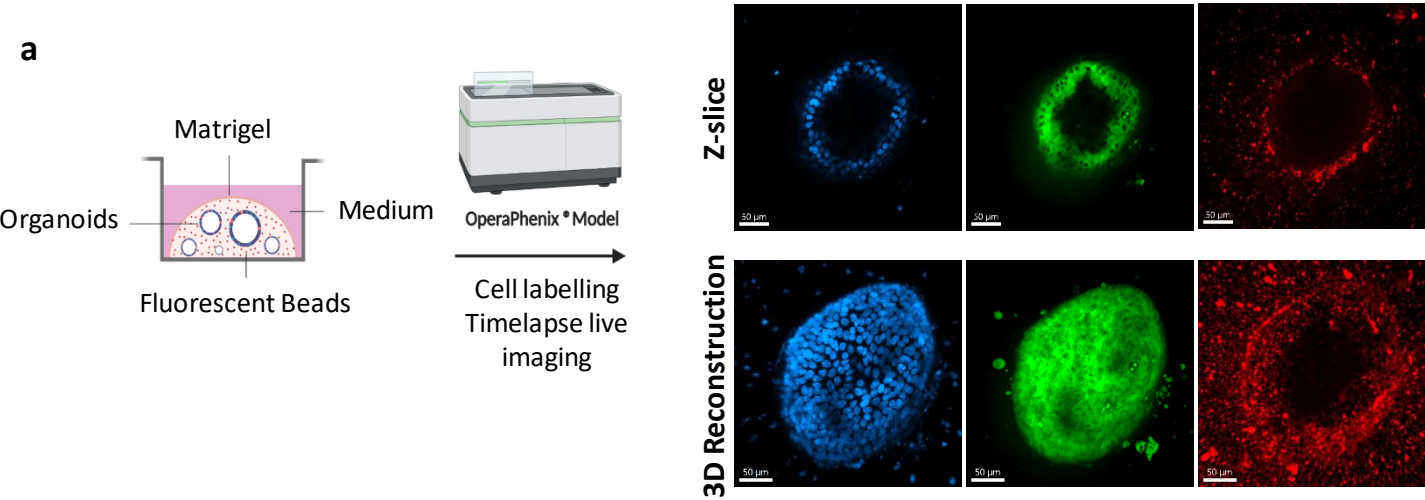
Figure 1



Human colonic organoid

Figure 2

a



b

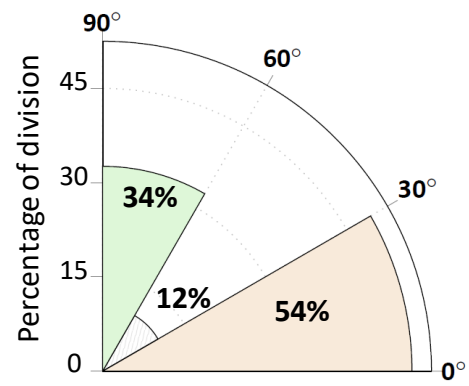
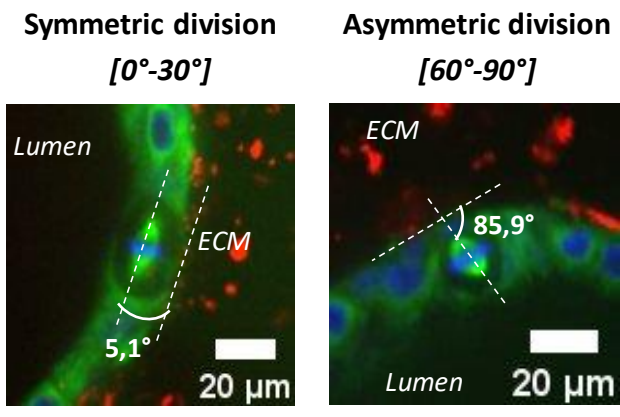


Figure 3

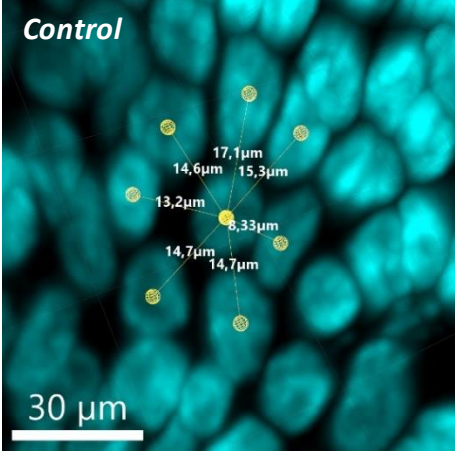
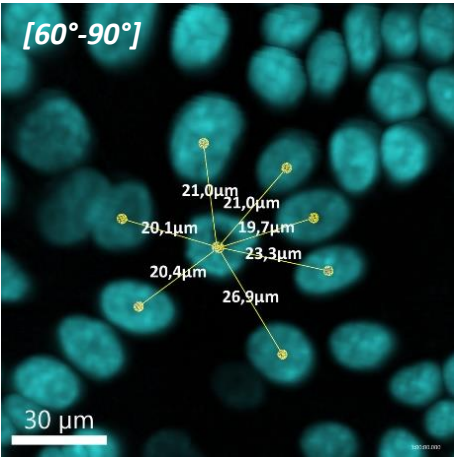
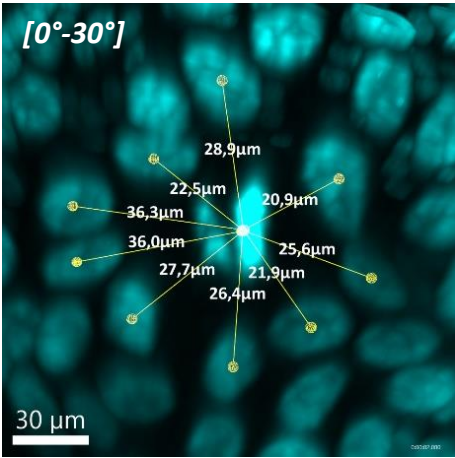
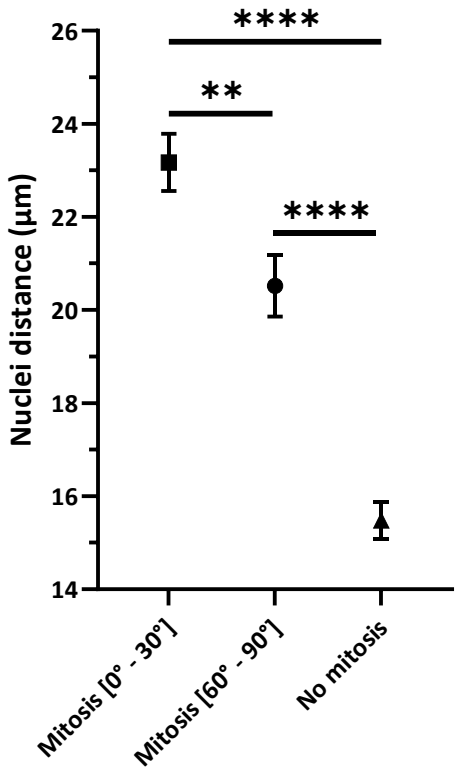
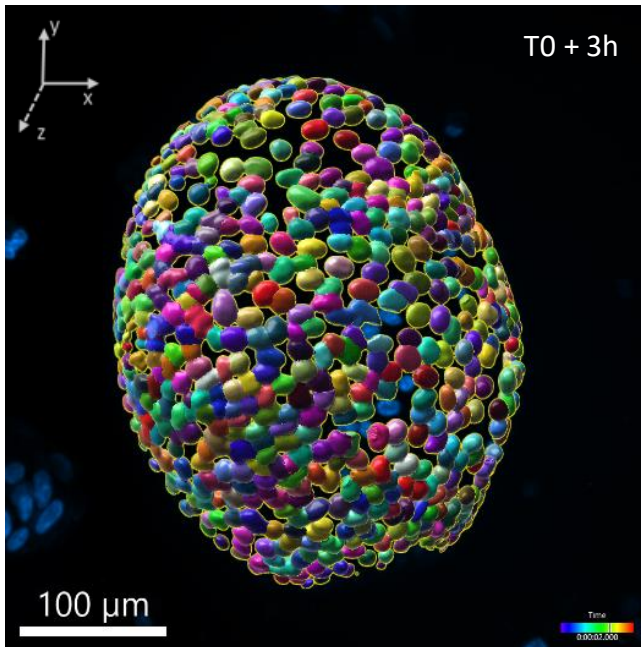
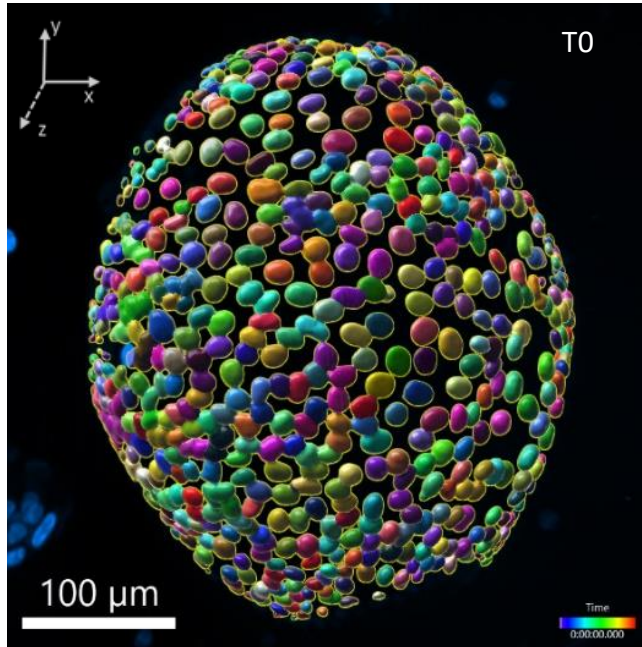


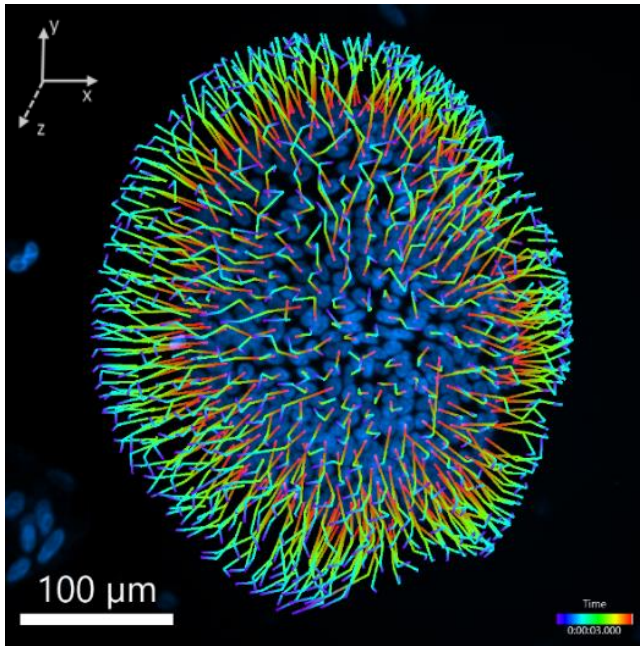
Figure 4

a

Segmentation over time



Tracking



Displacement vectors

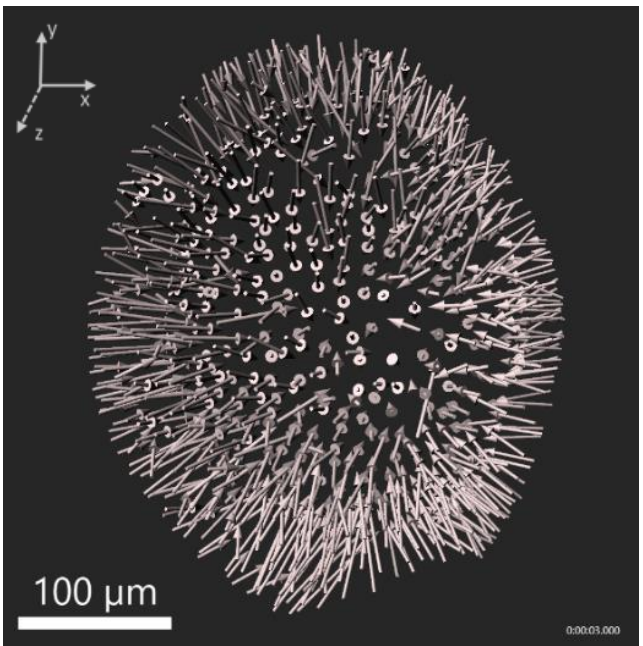


Figure 4

b

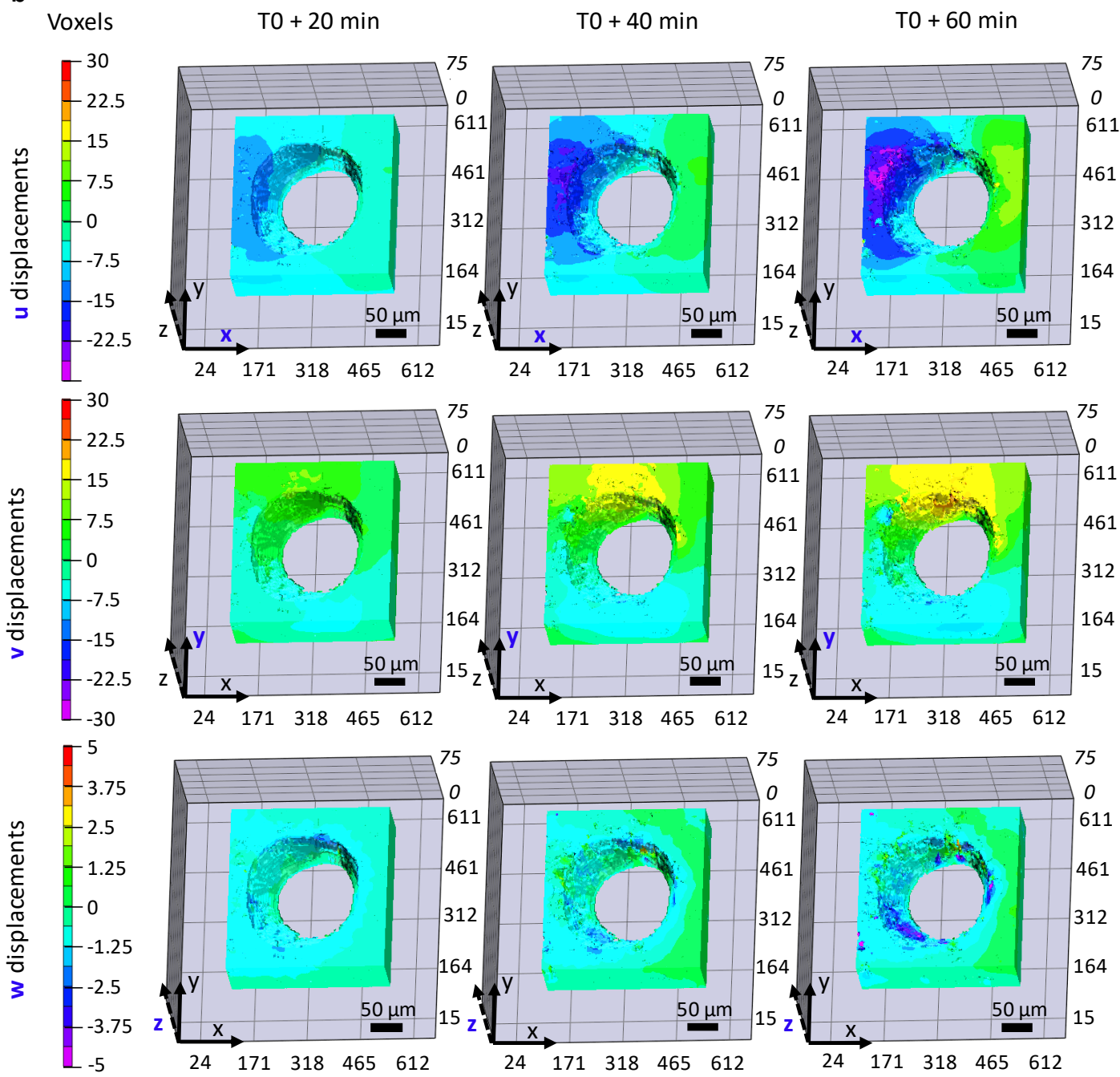
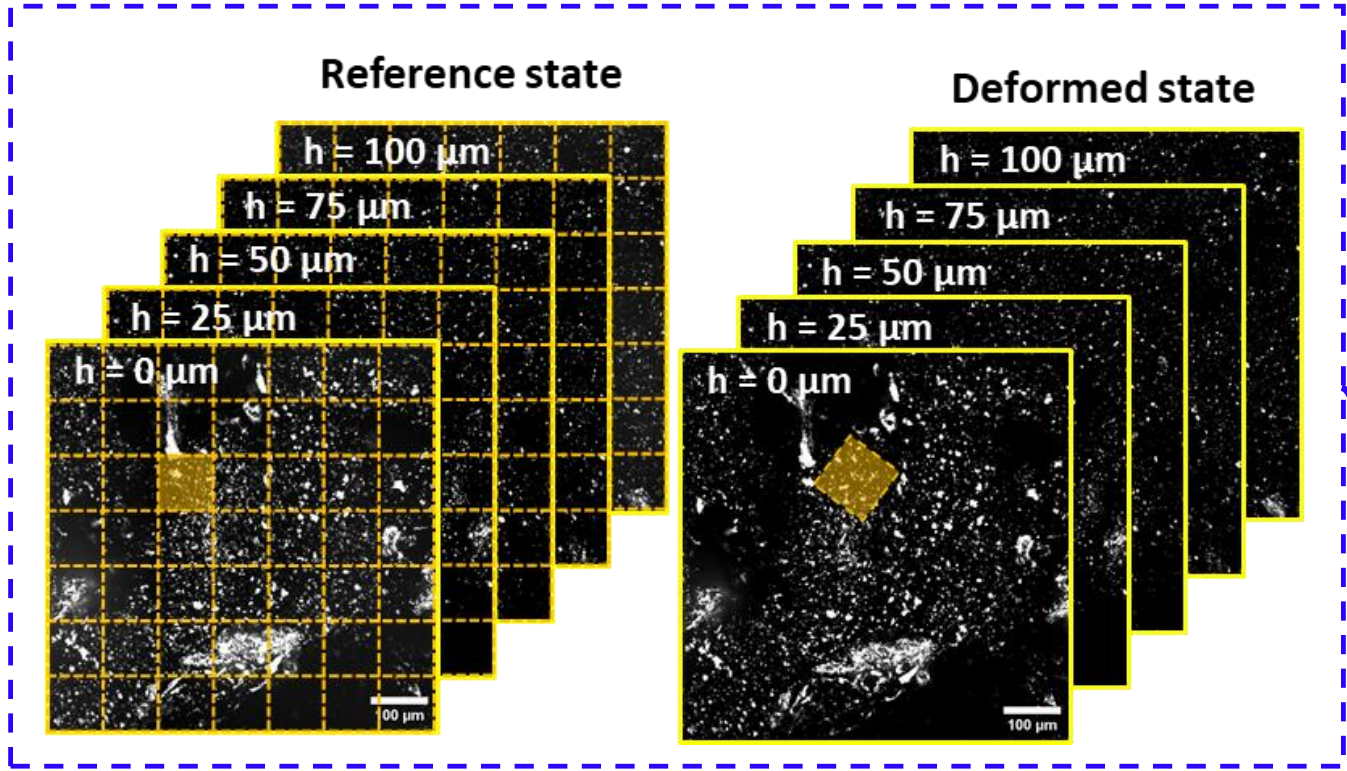


Figure 6



Digital Volume Correlation

