Collective Motion of Mammalian Cell Cohorts in 3D

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Supplementary Materials and Captions

Supplementary Video 1: The left panel shows the Z-projection of the images in **Gel I View i**. The right panel shows nuclei rendering of the same data as obtained after tracking, de-drifting and clustering. Each nucleus is labeled as a spot, and colors represent cluster identifiers. Black nuclei are single cells. Data represents a 560 X 560 X 100 μ m³ volume.

Supplementary Video 2: The left panel shows the Z-projection of the images in **Gel I View ii**. The right panel shows nuclei rendering of the same data as obtained after tracking, de-drifting and clustering. Each nucleus is labeled as a spot, and colors represent cluster identifiers. Black nuclei are single cells. Data represents a 560 X 560 X 100 μ m³ volume.

Supplementary Video 3: The left panel shows the Z-projection of the images in **Gel I View iii**. The right panel shows nuclei rendering of the same data as obtained after tracking, de-drifting and clustering. Each nucleus is labeled as a spot, and colors represent cluster identifiers. Black nuclei are single cells. Data represents a 560 X 560 X 100 μ m³ volume.

Supplementary Video 4: The left panel shows the Z-projection of the images in **Gel II View i**. The right panel shows nuclei rendering of the same data as obtained after tracking, de-drifting and clustering. Each nucleus is labeled as a spot, and colors represent cluster identifiers. Black nuclei are single cells. Data represents a 560 X 560 X 100 μ m³ volume.

Supplementary Video 5: The left panel shows the Z-projection of the images in **Gel II View ii**. The right panel shows nuclei rendering of the same data as obtained after tracking, de-drifting and clustering. Each nucleus is labeled as a spot, and colors represent cluster identifiers. Black nuclei are single cells. Data represents a 560 X 560 X 100 μ m³ volume.

Supplementary Video 6: Z-projections of the dataset with GFP-NLS cell cohorts (green) and 1 μ m beads (red) embedded in the collagen gels. A small region of the matrix (beads) around moving nuclei is perturbed due to pulling of the gel. Data represents a 560 X 560 X 200 μ m³ volume.

Supplementary Video 7: Z-projections of the dataset with GFP-Ecad cells. Data represents a 270 X 270 X 40 μ m³ volume.















Supplementary Figure 1: Panel A shows Z-projections of a third field of view of the same gel as in Figure 1 with 3D collagen gel with MDCK GFP-NLS cell cohorts at 0 h (red), 24 h (green) and 48 h (blue). Panels C and E are similar Z-projections for

two fields of view from a different gel. The numbers in white indicate cohort number as determined by a clustering algorithm. Panels B, D and F are 3D renderings of nuclear tracking corresponding to Panel A, C and E, respectively.



Supplementary Figure 2: Each plot has temporal analysis for the labelled Gel, View and Cohort. The left y-axis corresponds to the 1h squared displacement distribution: black line is the median, grey shaded regions are upper and lower quartiles. Vertical shaded regions represent motility events. The right y-axis and the red line correspond to 1h order parameter of the cohort.

Supplementary Tables: Parameters of 61 motility events. C is Cohort ID, Ti is the intial time, Tf is the final time, |d| is the displacement of the cohort, $\langle \phi \rangle$ is the average order, and Nc is the average number of cells in the cohort.

Gel	View	С	T _i (h)	T _f (h)	d (μm)	<φ>	N _c
I	i	1	1:20	4:20	19.20	0.79	19
I.	i	1	20:20	22:10	10.81	0.79	24
Т	i	1	26:50	29:40	14.41	0.86	24
I.	i	1	33:10	35:10	12.53	0.86	26
1	i	1	37:50	40:10	14.27	0.87	29
I	i	1	40:50	43:20	17.29	0.84	31
I	i	2	0:30	4:10	33.80	0.85	13
I	i	2	5:00	6:30	6.57	0.75	14
I	i	2	17:40	19:30	9.69	0.90	14
I	i	2	21:10	22:10	4.61	0.66	15
I	i	2	28:30	29:40	10.31	0.85	17
I	i	2	36:40	37:50	9.10	0.85	22
1	i	3	0:50	1:50	5.39	0.87	3
I.	i	3	1:50	6:50	49.07	0.93	4
Т	i	3	7:10	9:0	5.46	0.73	5
I	i	3	9:50	11:20	11.14	0.83	4
Т	i	3	11:40	13:0	7.27	0.76	5
Т	i	3	13:00	14:50	10.82	0.84	5
Т	i	3	17:10	18:50	3.35	0.39	7
T	i	3	23:00	24:40	12.36	0.78	6
Т	i	3	27:00	28:10	4.71	0.69	8
Т	i	3	28:50	30:30	10.43	0.87	8
Т	i	3	34:50	36:40	11.08	0.77	8
Т	i	3	37:30	40:10	15.35	0.69	6
1	i	3	42:30	44:40	3.84	0.78	8
Т	i	3	44:40	45:40	3.84	0.56	8
1	ii	1	0:50	2:10	12.19	0.82	14
Т	ii	1	3:20	9:30	89.06	0.92	10
I	ii	1	9:50	11:10	8.92	0.79	13
I	ii	1	16:00	18:20	19.22	0.80	9
I	ii	1	22:30	24:40	19.20	0.81	9

Gel	View	C	T _i (h)	T _f (h)	d (μm)	<φ>	N _c
T	ii	1	27:50	29:40	5.96	0.86	13
Т	ii	1	30:00	31:20	11.09	0.80	14
Т	ii	1	34:40	35:40	5.88	0.82	13
I	ii	2	0:40	4:0	28.30	0.91	6
Т	ii	2	15:40	18:40	17.97	0.84	8
Т	ii	2	23:30	25:50	9.58	0.75	10
Т	ii	2	36:30	39:0	14.60	0.50	26
Т	ii	3	0:30	4:20	26.33	0.81	14
Т	ii	3	21:30	24:30	20.62	0.77	17
Т	ii	3	32:30	33:50	6.33	0.72	23
Т	iii	1	0:30	3:50	12.61	0.72	13
Т	iii	1	26:00	27:30	9.76	0.60	18
Т	iii	2	1:10	7:0	34.39	0.87	7
Т	iii	2	26:30	29:20	19.29	0.75	15
Т	iii	2	29:20	32:10	21.11	0.87	18
Т	iii	2	35:30	38:0	16.21	0.85	21
Т	iii	2	40:20	43:40	23.88	0.67	22
Т	iii	2	44:40	46:20	10.64	0.74	22
Ш	i	1	1:30	5:50	53.09	0.84	19
Ш	i	1	18:40	22:40	31.33	0.87	24
Ш	i	1	23:40	25:40	30.28	0.93	5
Ш	i	1	27:10	28:10	11.03	0.95	3
Ш	i	2	2:00	5:30	25.08	0.77	14
Ш	i	2	28:10	29:40	6.69	0.80	15
Ш	ii	1	1:30	3:50	19.64	0.82	10
Ш	ii	2	1:00	3:0	18.82	0.78	11
Ш	ii	2	3:00	5:40	13.54	0.84	8
Ш	ii	2	25:10	27:20	11.43	0.84	15
Ш	ii	2	41:20	43:0	11.12	0.77	19
Ш	ii	2	44:30	46:40	12.14	0.76	15