

**Supplementary Material:**

**Logistic proliferation of cells in scratch assays is delayed**

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## 1 Location of the subregions

A key assumption in this work is that the two subregions we choose to quantify temporal changes in cell density are located sufficiently far away from the scratch that the cell density is approximately spatially uniform in these locations. To examine this assumption, following Jin et al. (Jin et al., 2016), we use the Fisher–Kolmogorov equation, which is given by

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} + \lambda C \left[ 1 - \left( \frac{C}{K} \right) \right], \quad (1)$$

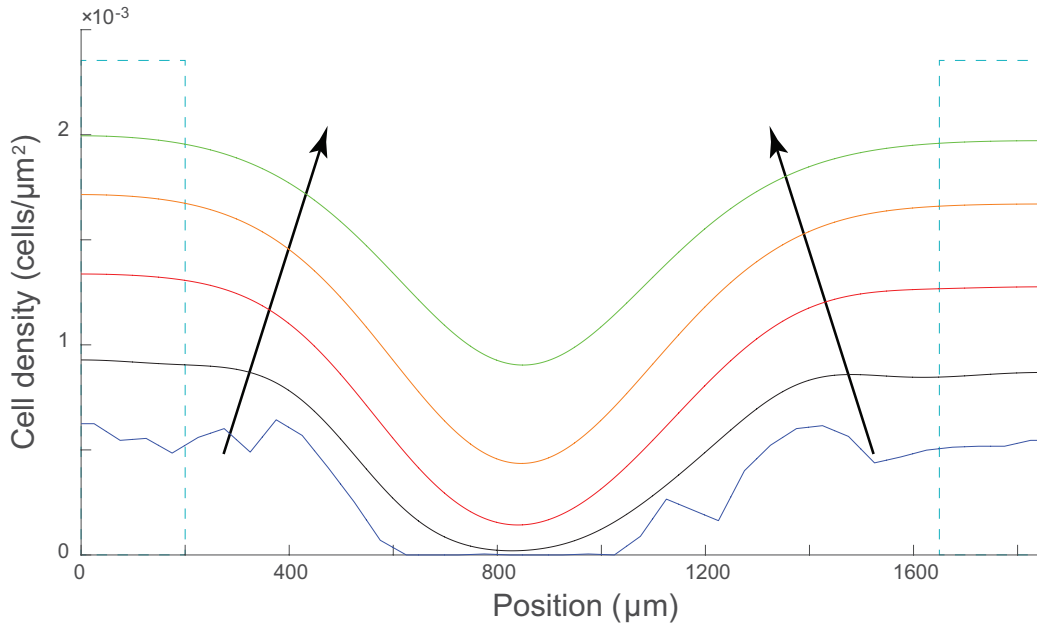
where  $C(x, t)$  is the cell density as a function of position,  $x$ , and time,  $t$ . Here,  $D$  is the cell diffusivity,  $\lambda$  is the proliferation rate and  $K$  is the carrying capacity density. To match our experiments we have  $0 \leq x \leq 1850 \mu\text{m}$  (Jin et al., 2016), and to mimic the geometry of our experiments we impose symmetry boundary conditions ( $\partial C / \partial x = 0$ ) at both  $x = 0 \mu\text{m}$  and  $x = 1850 \mu\text{m}$  (Jin et al., 2016). We specify the initial cell density,  $C(x, 0)$ , by using manual counting in a series of columns, of width  $50 \mu\text{m}$ , across the image at  $t = 0$  hours. Dividing the initial number of cells per column by the area of the column gives us an estimate of  $C(x, 0)$ . Repeating this procedure across a number of identically prepared experiments allows us to average our estimates of  $C(x, 0)$  to reduce the fluctuations in the initial density profile.

To solve Equation (1) we uniformly discretise the spatial domain with grid spacing  $\delta x$  (Morton and Mayers, 2005). The spatial derivatives are approximated using a central–difference approximation, which leads to a system of coupled nonlinear ordinary differential equations that are integrated through time using a backward–Euler approximation with constant time steps of duration  $\delta t$  (Morton and Mayers, 2005). The resulting systems of coupled nonlinear algebraic equations are linearised using Picard iteration, with absolute convergence tolerance  $\epsilon$ , and solved using the Thomas algorithm (Morton and Mayers, 2005). We always choose  $\delta x$ ,  $\delta t$  and  $\epsilon$  so that our numerical algorithm produces grid–independent results.

We present results here for a scratch assay with one particular choice of initial seeding condition, 16,000 cells per well. Note that the results shown here for this initial seeding condition are representative of the results for the other two initial seeding conditions that we consider in the main document. Our estimate for the initial cell density profile from the experimental data at  $t = 0$  hours is linearly interpolated to match the numerical discretization. Using this information, we solve Equation (1) with  $D = 570 \mu\text{m}^2/\text{h}$ ,

$\lambda = 0.059$  /h and  $K = 2.4 \times 10^{-3}$  cells/ $\mu\text{m}^2$ , which are representative estimates of  $\lambda$  and  $K$  from the main manuscript, and an estimate of  $D$  taken from our previous work (Jin et al., 2016).

Numerical solutions of Equation (1) are shown in Figure S1. Here we see that the initial fluctuations in  $C(x, 0)$  smooth out with time relatively quickly. Then, at later times, we see that there are negligible spatial gradients,  $\partial C/\partial x \approx 0$ , at the locations of the two subregions used to count the cell density profile. This indicates that the net flux of cells into these subregions is approximately zero, and hence Equation (1) for  $C(x, t)$  simplifies to the logistic growth model for  $C(t)$  locally in these subregions that are located sufficiently far from the scratch.



**Fig. S1. The numerical solution of the Fisher-Kolmogorov model for the scratch assay.** Numerical solutions of Equation (1) are shown at  $t = 0, 12, 24, 36, 48$  hours, with the arrow showing the direction of increasing  $t$ . The location of the two subregions are shown using the two cyan rectangles, as indicated. The numerical solution of Equation (1) is obtained with  $D = 570 \mu\text{m}^2/\text{h}$ ,  $\lambda = 0.059 /\text{h}$ ,  $K = 2.4 \times 10^{-3} \text{ cells}/\mu\text{m}^2$ ,  $\delta x = 0.5 \mu\text{m}$ ,  $\delta t = 0.2 \text{ h}$  and  $\epsilon = 10^{-5}$ .

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**Experimental data**

All raw experimental data used to construct the growth curves in Figure 3 (Main document) are summarised as follows: (i) data for the cell proliferation assay are shown in Tables 1-3, corresponding to initial seeding conditions 1-3, respectively; and, (ii) data for the scratch assay are shown in Tables 4-6, corresponding to initial seeding condition 1-3, respectively. In Tables 1-6 we show data from three individual experimental replicates as well as averaged density data by averaging the three individual replicates from each experimental condition.

Table 1: Proliferation assay; Initial seeding condition 1

		Time (h)															
		0	2	4	6	8	10	12	14	16	18	24	30	36	42	48	
Cell density ( $\times 10^{-3}$ cells/ $\mu\text{m}^2$ )	Replicate 1	Subregion 1	0.30	0.32	0.37	0.40	0.47	0.47	0.54	0.60	0.60	0.61	0.74	0.94	1.15	1.26	1.38
		Subregion 2	0.21	0.24	0.26	0.28	0.31	0.38	0.37	0.40	0.41	0.44	0.66	0.76	1.01	1.15	1.27
		Average	0.25	0.28	0.31	0.34	0.39	0.42	0.45	0.50	0.50	0.52	0.70	0.85	1.08	1.20	1.33
	Replicate 2	Subregion 1	0.35	0.38	0.37	0.38	0.44	0.53	0.59	0.60	0.63	0.66	0.85	1.00	1.17	1.28	1.36
		Subregion 2	0.32	0.31	0.32	0.35	0.39	0.41	0.48	0.50	0.54	0.54	0.78	0.96	1.08	1.17	1.32
		Average	0.33	0.34	0.35	0.37	0.41	0.47	0.54	0.55	0.58	0.60	0.81	0.98	1.13	1.23	1.34
	Replicate 3	Subregion 1	0.34	0.41	0.41	0.46	0.49	0.54	0.57	0.62	0.67	0.68	0.89	1.00	1.05	1.24	1.36
		Subregion 2	0.33	0.38	0.37	0.41	0.44	0.49	0.54	0.52	0.61	0.61	0.74	0.95	1.15	1.21	1.38
		Average	0.34	0.39	0.39	0.43	0.47	0.52	0.55	0.57	0.64	0.64	0.82	0.98	1.10	1.22	1.37
	Average		0.31	0.34	0.35	0.38	0.42	0.47	0.52	0.54	0.58	0.59	0.78	0.94	1.10	1.22	1.35

Table 2: Proliferation assay; Initial seeding condition 2

Time (h)		0	2	4	6	8	10	12	14	16	18	24	30	36	42	48		
Cell density ( $\times 10^{-3}$ cells/ $\mu\text{m}^2$ )	Replicate 1	Subregion 1	0.55	0.66	0.71	0.69	0.79	0.87	0.87	0.95	1.01	1.06	1.26	1.41	1.49	1.57	1.59	
		Subregion 2	0.56	0.64	0.66	0.69	0.77	0.81	0.81	0.90	0.94	0.94	1.01	1.15	1.35	1.54	1.52	1.61
		Average	0.55	0.65	0.69	0.69	0.78	0.84	0.89	0.89	0.95	0.97	1.03	1.20	1.38	1.51	1.55	1.60
	Replicate 2	Subregion 1	0.57	0.62	0.67	0.72	0.82	0.87	0.88	0.92	0.97	0.98	1.18	1.34	1.37	1.47	1.47	1.62
		Subregion 2	0.54	0.58	0.61	0.68	0.66	0.73	0.82	0.82	0.79	0.89	1.10	1.28	1.47	1.47	1.40	1.57
		Average	0.56	0.60	0.64	0.70	0.74	0.80	0.85	0.87	0.88	0.88	0.93	1.14	1.31	1.42	1.44	1.59
	Replicate 3	Subregion 1	0.51	0.54	0.61	0.66	0.74	0.84	0.89	0.94	0.95	0.99	1.21	1.32	1.56	1.57	1.66	1.66
		Subregion 2	0.57	0.58	0.62	0.67	0.73	0.84	0.83	0.85	0.95	0.98	1.12	1.31	1.40	1.51	1.60	1.60
		Average	0.54	0.56	0.61	0.66	0.73	0.84	0.86	0.90	0.95	0.98	1.16	1.32	1.48	1.54	1.63	1.63
	Average		0.55	0.60	0.65	0.68	0.75	0.83	0.87	0.90	0.93	0.98	1.17	1.33	1.47	1.51	1.61	1.61

Table 3: Proliferation assay; Initial seeding condition 3

		Time (h)															
		0	2	4	6	8	10	12	14	16	18	24	30	36	42	48	
Cell density ( $\times 10^{-3}$ cells/ $\mu\text{m}^2$ )	Replicate 1	Subregion 1	0.68	0.75	0.78	0.89	0.87	0.98	1.01	1.04	1.08	1.12	1.32	1.41	1.49	1.47	1.51
		Subregion 2	0.84	0.82	0.93	0.93	1.00	0.97	1.01	1.16	1.14	1.17	1.27	1.36	1.41	1.43	1.49
		Average	0.76	0.78	0.86	0.91	0.94	0.97	1.01	1.10	1.11	1.15	1.29	1.38	1.45	1.45	1.50
	Replicate 2	Subregion 1	0.80	0.92	0.88	0.92	0.96	1.03	1.00	1.08	1.10	1.19	1.34	1.50	1.55	1.53	1.51
		Subregion 2	0.75	0.85	0.90	0.91	1.05	1.09	1.08	1.13	1.17	1.18	1.31	1.45	1.44	1.48	1.51
		Average	0.77	0.89	0.89	0.91	1.00	1.06	1.04	1.10	1.14	1.18	1.33	1.48	1.49	1.51	1.51
	Replicate 3	Subregion 1	0.75	0.83	0.91	0.99	0.98	1.10	1.16	1.22	1.17	1.26	1.51	1.56	1.59	1.53	1.72
		Subregion 2	0.83	0.86	0.95	0.99	1.06	1.17	1.23	1.23	1.24	1.20	1.42	1.43	1.51	1.56	1.55
		Average	0.79	0.85	0.93	0.99	1.02	1.14	1.20	1.23	1.21	1.23	1.46	1.50	1.55	1.55	1.64
	Average		0.77	0.84	0.89	0.94	0.99	1.06	1.08	1.14	1.15	1.19	1.36	1.45	1.50	1.50	1.55



Table 4: Scratch assay; Initial seeding condition 1

		Time (h)															
		0	2	4	6	8	10	12	14	16	18	24	30	36	42	48	
Cell density ( $\times 10^{-3}$ cells/ $\mu\text{m}^2$ )	Replicate 1	Subregion 1	0.25	0.26	0.28	0.26	0.29	0.31	0.31	0.35	0.32	0.35	0.43	0.58	0.82	1.00	1.33
		Subregion 2	0.30	0.31	0.30	0.30	0.33	0.35	0.38	0.41	0.41	0.43	0.53	0.58	0.79	0.91	0.99
		Average	0.27	0.29	0.29	0.28	0.31	0.33	0.35	0.38	0.36	0.39	0.48	0.58	0.80	0.96	1.16
	Replicate 2	Subregion 1	0.35	0.34	0.37	0.40	0.43	0.44	0.47	0.47	0.48	0.53	0.65	0.76	0.93	1.09	1.18
		Subregion 2	0.25	0.24	0.23	0.25	0.26	0.26	0.26	0.30	0.28	0.33	0.40	0.51	0.71	0.80	0.99
		Average	0.30	0.29	0.30	0.32	0.35	0.35	0.37	0.39	0.38	0.43	0.52	0.63	0.82	0.95	1.08
	Replicate 3	Subregion 1	0.40	0.44	0.44	0.47	0.46	0.50	0.54	0.53	0.56	0.56	0.78	0.84	0.96	1.08	1.36
		Subregion 2	0.23	0.25	0.25	0.30	0.31	0.30	0.32	0.35	0.32	0.33	0.49	0.65	0.72	0.90	1.02
		Average	0.32	0.34	0.34	0.39	0.39	0.40	0.43	0.44	0.44	0.45	0.64	0.75	0.84	0.99	1.19
	Average		0.30	0.31	0.31	0.33	0.35	0.36	0.38	0.40	0.40	0.42	0.55	0.65	0.82	0.96	1.13

Table 5: Scratch assay; Initial seeding condition 2

Time (h)		0	2	4	6	8	10	12	14	16	18	24	30	36	42	48		
Cell density ( $\times 10^{-3}$ cells/ $\mu\text{m}^2$ )	Replicate 1	Subregion 1	0.53	0.57	0.58	0.65	0.64	0.66	0.69	0.74	0.76	0.73	0.92	1.13	1.31	1.53	1.64	
		Subregion 2	0.57	0.56	0.63	0.62	0.67	0.75	0.79	0.77	0.79	0.86	0.88	1.13	1.33	1.62	1.87	
		Average	0.55	0.57	0.60	0.64	0.66	0.71	0.74	0.75	0.78	0.80	0.80	0.90	1.13	1.32	1.58	1.75
	Replicate 2	Subregion 1	0.56	0.61	0.67	0.66	0.66	0.77	0.82	0.85	0.82	0.82	0.84	1.24	1.38	1.62	1.78	1.98
		Subregion 2	0.51	0.53	0.55	0.59	0.63	0.71	0.73	0.72	0.72	0.74	1.12	1.16	1.36	1.52	1.80	
		Average	0.54	0.57	0.61	0.62	0.65	0.74	0.77	0.78	0.78	0.77	0.79	1.18	1.27	1.49	1.65	1.89
	Replicate 3	Subregion 1	0.57	0.59	0.65	0.64	0.65	0.70	0.74	0.78	0.84	0.96	1.11	1.28	1.51	1.65	1.84	
		Subregion 2	0.49	0.52	0.58	0.60	0.63	0.62	0.66	0.68	0.72	0.77	1.01	1.22	1.54	1.60	1.80	
		Average	0.53	0.55	0.61	0.62	0.64	0.66	0.70	0.73	0.78	0.86	1.06	1.25	1.52	1.63	1.82	
	Average		0.54	0.56	0.61	0.63	0.65	0.70	0.74	0.76	0.78	0.82	1.05	1.22	1.44	1.62	1.82	

Table 6: Scratch assay; Initial seeding condition 3

Time (h)		0	2	4	6	8	10	12	14	16	18	24	30	36	42	48	
Cell density ( $\times 10^{-3}$ cells/ $\mu\text{m}^2$ )	Replicate 1	Subregion 1	0.85	0.84	0.87	0.91	0.98	0.94	1.03	1.07	1.09	1.14	1.30	1.52	1.60	1.66	1.93
		Subregion 2	0.73	0.75	0.72	0.77	0.82	0.82	0.85	0.89	0.90	1.01	1.14	1.35	1.56	1.68	1.88
		Average	0.79	0.79	0.80	0.84	0.90	0.88	0.94	0.98	1.00	1.08	1.22	1.44	1.58	1.67	1.90
	Replicate 2	Subregion 1	0.72	0.71	0.75	0.75	0.81	0.83	0.85	0.85	0.93	0.98	1.07	1.37	1.50	1.63	1.82
		Subregion 2	0.79	0.75	0.85	0.82	0.79	0.79	0.82	0.87	0.93	0.96	1.13	1.40	1.57	1.68	1.95
		Average	0.76	0.73	0.80	0.78	0.80	0.81	0.83	0.86	0.93	0.97	1.10	1.39	1.54	1.66	1.88
	Replicate 3	Subregion 1	0.75	0.75	0.80	0.79	0.84	0.81	0.87	0.85	0.90	1.00	1.18	1.55	1.64	1.81	1.97
		Subregion 2	0.86	0.82	0.83	0.85	0.87	0.88	0.89	0.98	1.05	1.09	1.34	1.42	1.53	1.83	1.94
		Average	0.80	0.78	0.82	0.82	0.86	0.84	0.88	0.92	0.97	1.05	1.26	1.48	1.59	1.82	1.95
	Average		0.78	0.77	0.80	0.81	0.85	0.85	0.89	0.92	0.97	1.03	1.20	1.44	1.57	1.72	1.91

## References

1. Jin W, Shah ET, Penington CJ, McCue SW, Chopin LK, Simpson MJ. 2016. Reproducibility of scratch assays is affected by the initial degree of confluence: Experiments, modelling and model selection. *J Theor Biol.* 390, 136–145.
2. Morton KW, Mayers DF. 2005. Numerical solution of partial differential equations. 2nd ed. Cambridge University Press, Cambridge.