Supplementary Document 1: Image Analysis Protocol

- 1. Import image into ImageJ (FIJI)
- 2. Split the channels of the image
- 3. On cell stained channel image (see green stain): Image > Stacks > Z- Project > Projection type: Max Intensity
- 4. On **bead channel** image (grayscale of beads): *Image* > *Stacks* > *Z*-*Project* > *Projection type: Sum Slices*



- 5. Open ROI manager: *Analyze -> tools -> ROI manager*
- 6. Trace cells of interest (cells that are completely in frame and do not overlap with other cells) using free hand selection tool. Add each cell to ROI manager by clicking 'add' or by pressing "T"

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Add [t]

Update

Delete

Rename...

Measure

Deselect

Properties...

Flatten [F]

More »



7. In **sum projected beads image**, click on an ROI. This should select the same cell in the grayscale sum projection.



- 8. To collect fluorescent signal value: *Analyze -> set measurements -> check area, mean gray value, and integrated density.* 9. To collect data: Analyze -> measure or press Ctrl+M

🗊 Set Measurements		>	<
 Area Standard deviation Min & max gray value Center of mass Bounding rectangle Shape descriptors Integrated density Skewness 	 ✓ Mean gray value ✓ Modal gray value ✓ Centroid ✓ Perimeter ✓ Fit ellipse ✓ Feret's diameter ✓ Median ✓ Kurtosis 		
Area fraction	Stack position		
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3	318252	201	205							
4	389313	100	159							
5	60124	217	229							
6	80784	492	863							
7	38251	476	773							
8	256012	263	586							
9	136264	286	806							
10	158427	512	731							
11	22632	112	/36							
12	45215	413	110							
13	244104	207	007							
15	153931	411	487							
16	23736	18	348							
17	19877	79	117							
18	116858	260	909							
19	229077	324	379							
20	198811	174	639							
21	81969	24	556							
22	70938	338	544							
23	27052	31	.002							
24	36563	328	405							
25	80310	54	599							
26	186202	46	583							
27	220574	115	215							
28	136692	127	463							
29	35908	15	676							
30	16474	518	503							
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10. Copy and paste results into Excel spreadsheet. We use the RawIntDen value, as it does not normalize to mean fluorescence of the selected cell. Thus, it captures the *sum* of the pixels generated across the z-stack.

11. To upload data to our KS UI, make a single column for each treatment/condition containing the RawIntDen values. Save this file as tab delimited .txt

Supplementary Table 1

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A	•	

	vars	n	mean	sd	median	trimmed	mad	min	max	range	skew	kurtosis	se
dist1	1	100	50	50.2518907629606	50	50	74.13	0	100	100	0	-2.0199	5.02518907629606
dist2	1	100	50.6	24.7337335587973	50	49.5	0	10	100	90	0.288785796296112	-0.176186756032821	2.47337335587973
B.													

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	vars	n	mean	sd	median	trimmed	mad	min	max	range	skew	kurtosis	se
Control	1	184	108860.467391304	95322.0949728333	73540	94902.0135135135	74820.1503	6397	430396	423999	1.18851149405518	0.718687855257543	7027.23807378872
GCM	1	143	184355.629370629	150297.774662416	140567	165178.295652174	145948.6266	9683	598962	589279	0.872976096310908	-0.177242276540173	12568.5313183291

Supp table 1. Descriptive statistics from A. hypothetical data sets *dist1* and *dist2*, and B. Data collected from BMDM in experiment 1.