When More Images Do Not Give Better Data (a Cytoplasm-to-Nucleus Translocation Study)

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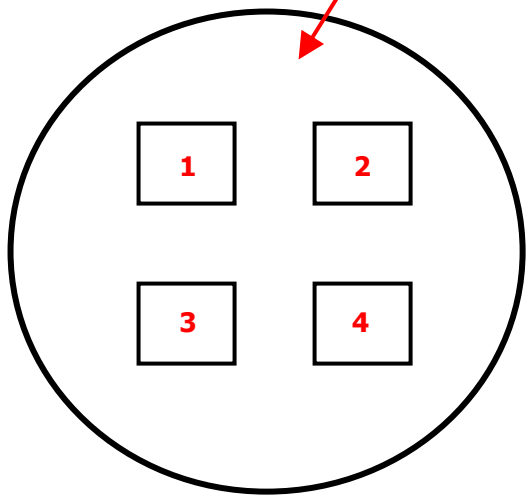
Cytoplasm to nucleus translocation was studied in A549 cells as a function of IL-1 concentration as shown in the plate map.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	0.0010	50.0000	16.6700	5.5600	1.8500	0.6170	0.2060	0.0686	0.0229	0.0076	0.0025	0.0010
C	0.0010	50.0000	16.6700	5.5600	1.8500	0.6170	0.2060	0.0686	0.0229	0.0076	0.0025	0.0010
D	0.0010	50.0000	16.6700	5.5600	1.8500	0.6170	0.2060	0.0686	0.0229	0.0076	0.0025	0.0010
E	0.0010	50.0000	16.6700	5.5600	1.8500	0.6170	0.2060	0.0686	0.0229	0.0076	0.0025	0.0010
F	0.0010	50.0000	16.6700	5.5600	1.8500	0.6170	0.2060	0.0686	0.0229	0.0076	0.0025	0.0010
G	0.0010	50.0000	16.6700	5.5600	1.8500	0.6170	0.2060	0.0686	0.0229	0.0076	0.0025	0.0010
H												

Concentration in ng/ml.



Four camera fields were acquired in each well with a microscope-based imaging system.

Images were analyzed by slope algorithm as described in:

I. Ravkin et al. "Multiplexed high-throughput image cytometry using encoded carriers", Proc. SPIE Vol. 5322, pp. 52-63, 2004.

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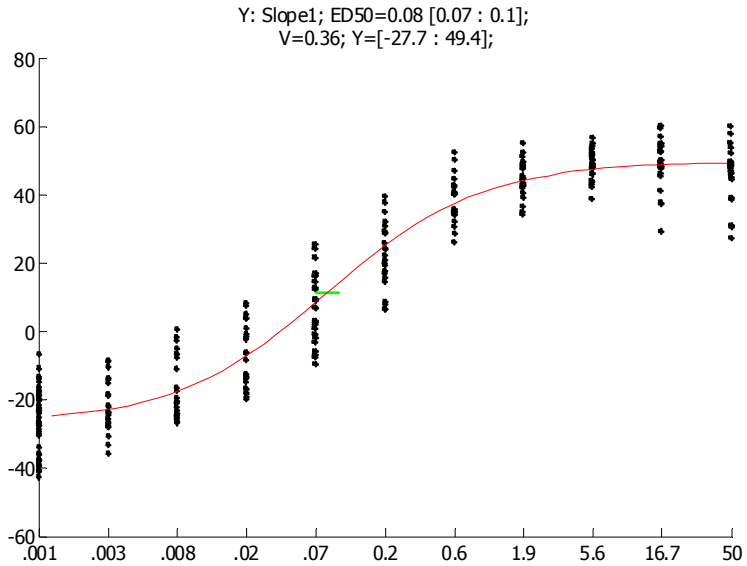
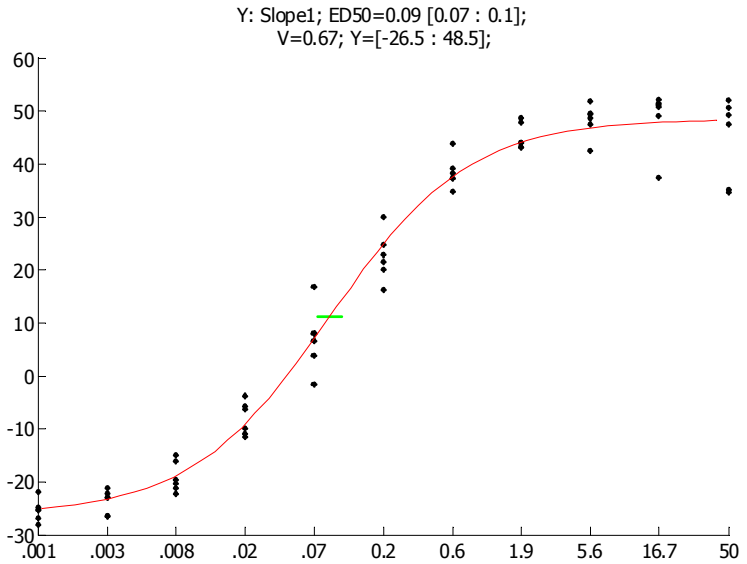
Analysis of Dose Curves with Replica Data Points. Individual and Pooled Camera Fields.



Dose curve model is fitted to data produced from individual images. This gives 24 replicas (6 rows * 4 fields) for each dose (except dose 0.001, which has 48 replicas).



Camera fields are pooled together to produce a single value. In this case this value is the median of the values of individual fields. Dose curve model is fitted to the pooled data. This gives 6 replicas (6 rows) for each dose (except dose 0.001, which has 12 replicas).

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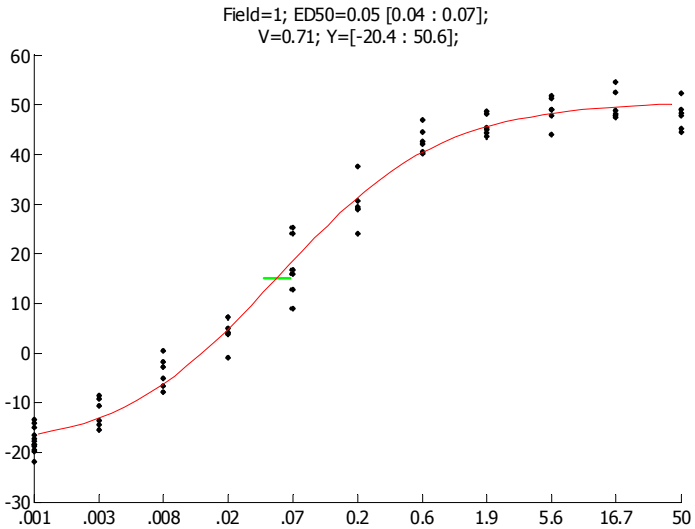
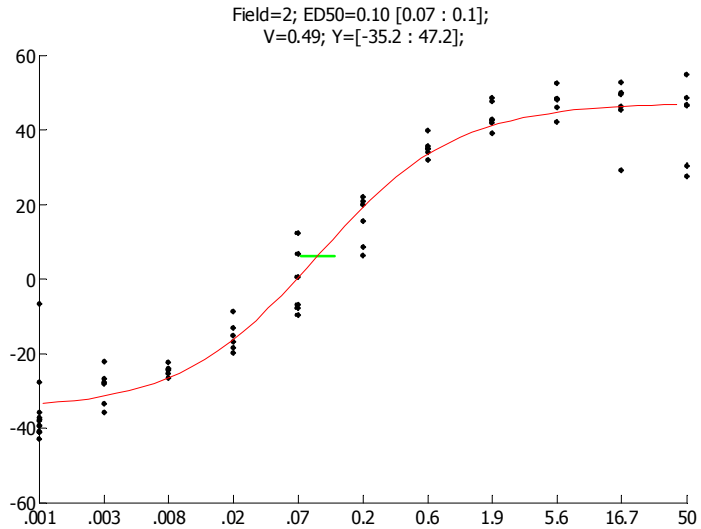
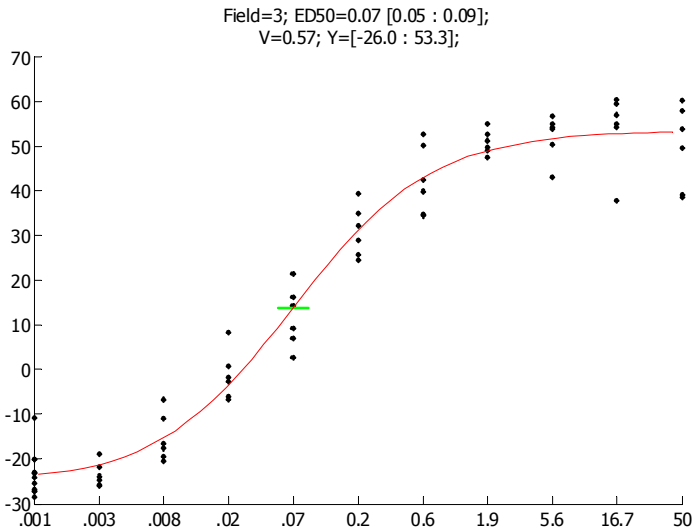
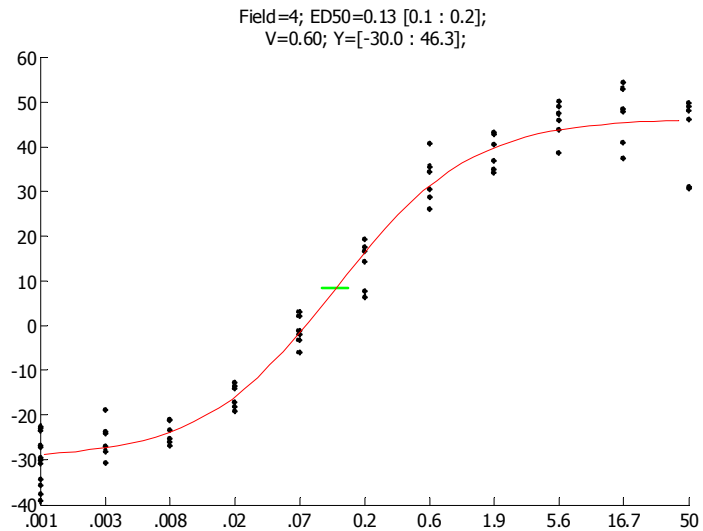
Analysis of Dose Curves with Replica Data Points. Camera Fields Analyzed Separately.









Dose curve model is fitted separately for each camera field using 6 replica rows for each dose (12 replicas for dose 0.001).

Significant difference among Ymin, Ymax, ED_{50} and V-factors suggests systematic difference among fields.

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Analysis of Variance.

Source of variation	Degrees of Freedom	Sum of squares	Mean square	Fisher's F	Pr > F
Row	5	680.2	136.0	3.4	0.019
Field	3	8526.3	2842.1	70.7	< 0.0001
Dose	10	267290.3	26729.0	665.0	< 0.0001

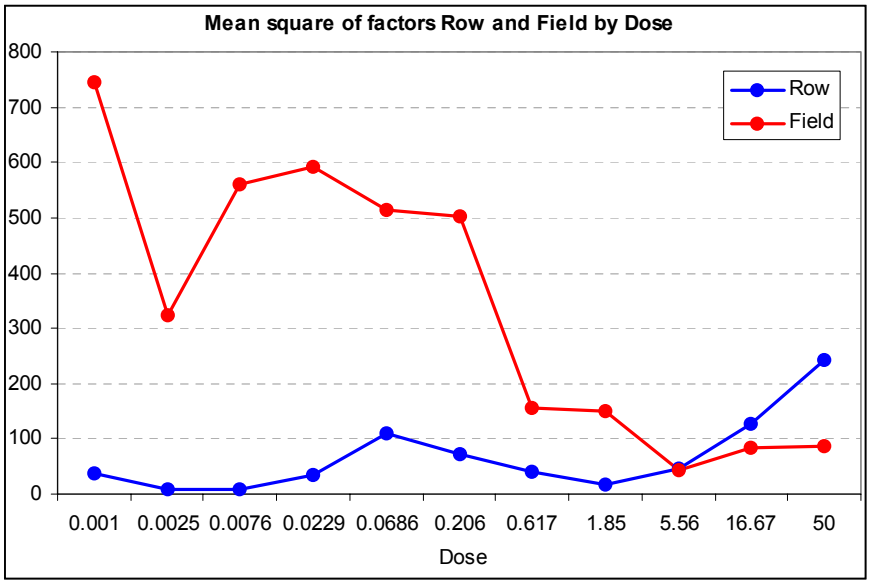
Analysis of variance of the CNT measure slope1 shows that:

factor Row is not significant – desired result;

factor Field is significant – **undesired** result;

factor Dose is significant – desired result.

Factors Row and Field are analyzed in more detail at each Dose



Variation among fields is very large at low doses of the stimulant (negative state) and decreases at higher doses (positive state).

Dose	0.001	0.003	0.008	0.023	0.069	0.206	0.617	1.85	5.56	16.67	50
Row	38	10	9	33	109	71	39	18	46	128	241
Field	746	324	559	591	515	503	156	150	43	83	87

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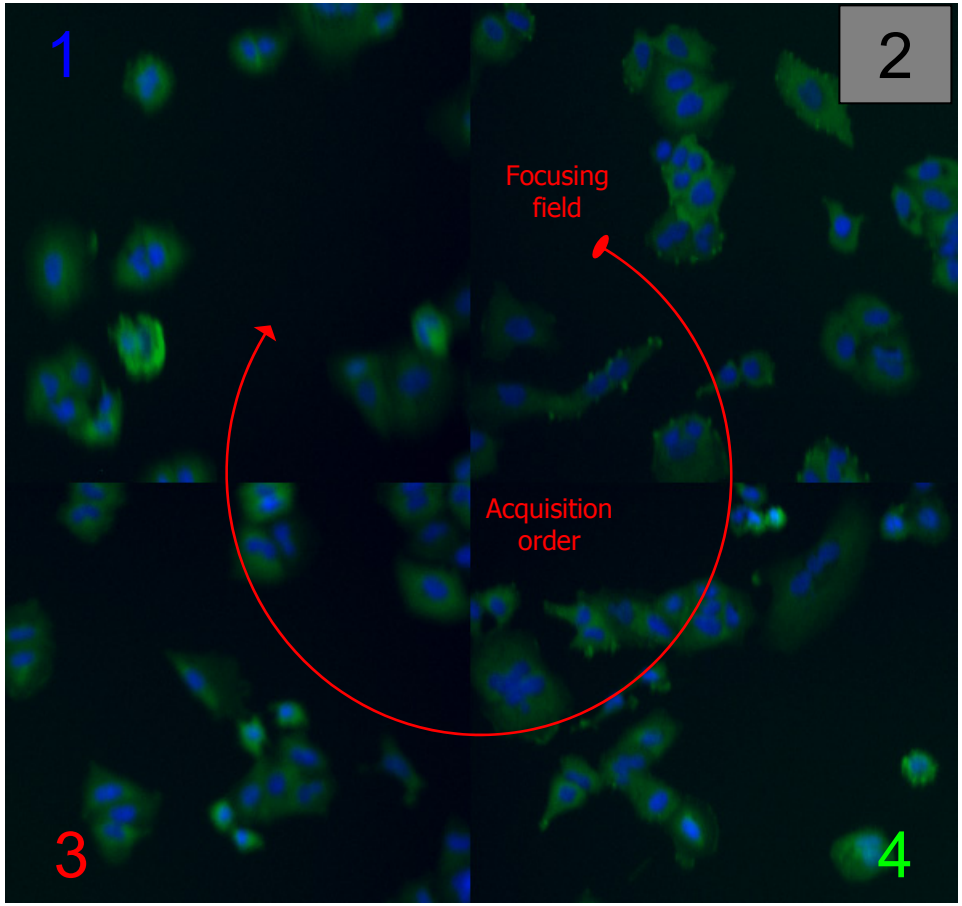
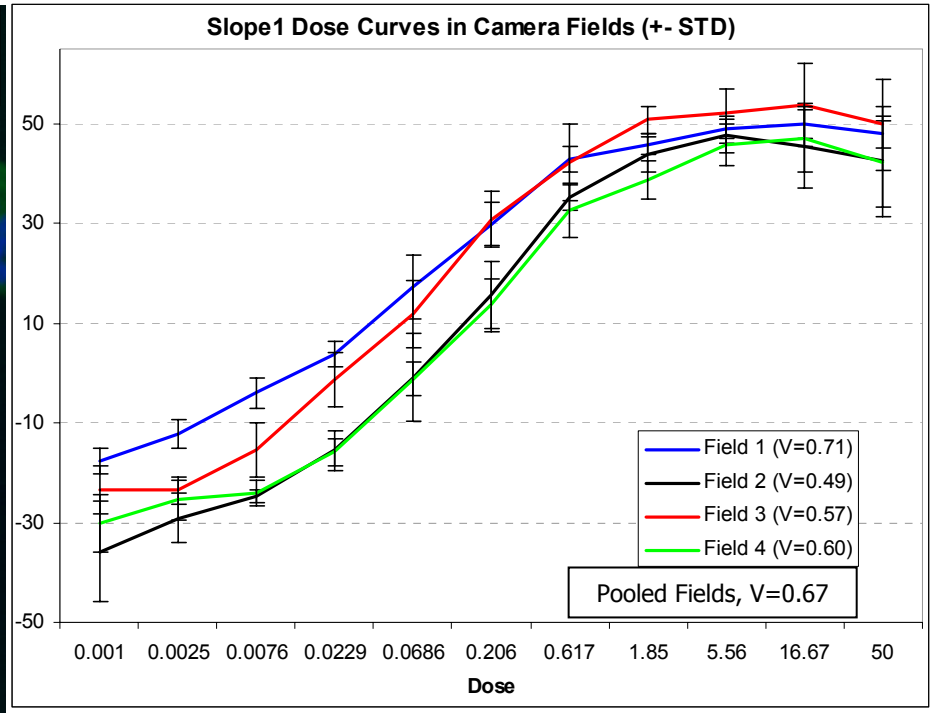
Focusing is the Cause of Difference Among Fields.





In each well focusing was done on field 2. The fields were acquired in the following sequence : field 2, field 4, field 3, field 1.

The overlaid dose curves show shift up in this order. For slope measures up-shifting is a clear sign of defocusing, especially in the negative state.

A single field (field 1) gives better V-factor than all fields pooled together. The reason is the systematic focus difference in the sequence of fields.

It is ironic that the field with the highest quality is the most defocused and the field with the lowest quality is the best focused. This is probably caused by inconsistent focusing among rows. When a field is slightly defocused, this inconsistency plays a relatively smaller role.

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Conclusion

Increasing the number of images improves data only if it is not accompanied by systematic shift among them.