Quantifying Image Quality and its Influence on HCS Data

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Factors That Affect the Quality of HCS Data

1. **sample biology**
2. **sample prep**
3. **plate type**
4. **image acquisition system** ← **Factor we study**
5. **image analysis**

Quantifying Quality

We use assay Z' as quality metric of HCS data

(IN Cell Analyzer 1000, GFP expressing cells, nuclear translocation assay)

We manipulate image quality and investigate its effect on Z':

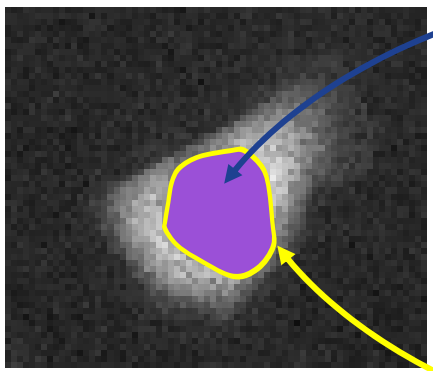
- **Image uniformity** → **By comparing images -/+ FFC**
- **Image signal/noise** → **By adding noise to images**
- **Image contrast** → **By enhancing contrast**
- **Image blur** → **By acquiring out of focus images**

Assay Data

Increasing dose of IL-1

Replicate wells

[IL-1] ng/mL	0.000	0.001	0.005	0.010	0.050	0.100	0.500	1.000	5.000	10.00	50.00	100.0
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.99	0.99	0.99	1.00	1.09	1.09	1.11	1.13	1.13	1.15	1.12	1.09
B	0.99	0.99	1.00	0.99	1.06	1.08	1.06	1.08	1.10	1.06	1.10	1.12
C	0.98	0.99	1.02	1.00	1.06	1.11	1.11	1.11	1.13	1.12	1.12	1.13
D	0.99	0.99	1.02	1.02	1.08	1.12	1.12	1.14	1.13	1.13	1.14	1.10
E	0.99	0.97	1.02	1.02	1.08	1.09	1.13	1.13	1.15	1.11	1.09	1.13
F	0.98	1.00	0.99	1.01	1.05	1.13	1.13	1.11	1.09	1.14	1.13	1.14
G	0.98	0.98	1.00	1.02	1.06	1.09	1.13	1.14	1.12	1.13	1.15	1.14
H	0.95	0.98	0.99	1.00	1.04	1.09	1.13	1.11	1.13	1.12	1.11	1.12
mean	0.98	0.99	1.00	1.01	1.07	1.10	1.11	1.12	1.12	1.12	1.12	1.12
SD	0.012	0.008	0.012	0.011	0.018	0.017	0.023	0.019	0.018	0.025	0.019	0.016



$$1.13 = \frac{\text{(GFP intensity from nuclear region)}}{\text{(GFP intensity from cytoplasmic region)}}$$

Assay Data

Increasing dose of IL-1

Replicate wells

[IL-1] ng/mL	0.000	0.001	0.005	0.010	0.050	0.100	0.500	1.000	5.000	10.00	50.00	100.0
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.99	0.99	0.99	1.00	1.09	1.09	1.11	1.13	1.13	1.15	1.12	1.09
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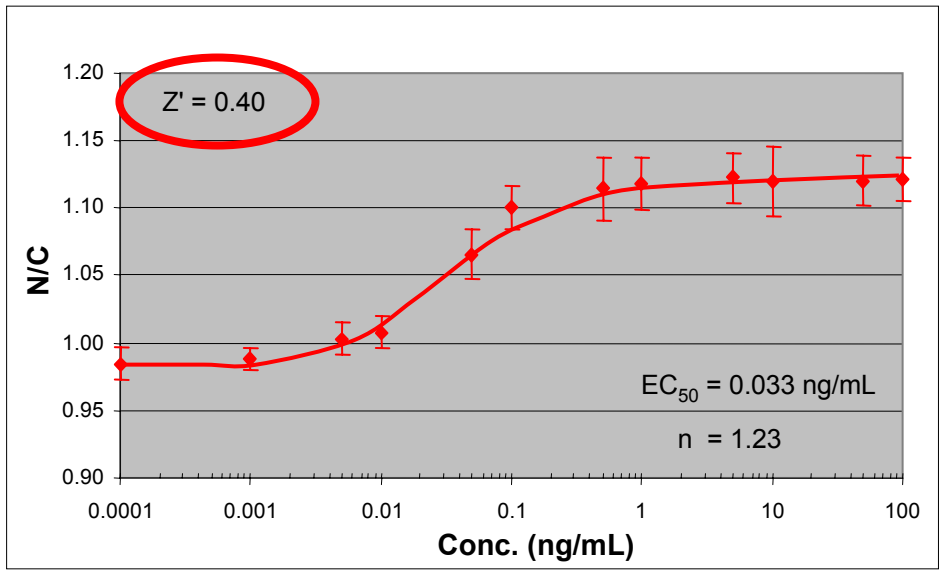

Precision of Assay Data on IN Cell Analyzer 1000

Increasing dose of IL-1

Replicate wells

[IL-1] ng/mL	0.000	0.001	0.005	0.010	0.050	0.100	0.500	1.000	5.000	10.00	50.00	100.0
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.99	0.99	0.99	1.00	1.09	1.09	1.11	1.13	1.13	1.15	1.12	1.09
B	0.99	0.99	1.00	0.99	1.06	1.08	1.06	1.08	1.10	1.06	1.10	1.12
C	0.98	0.99	1.02	1.00	1.06	1.11	1.11	1.11	1.13	1.12	1.12	1.13
D	0.99	0.99	1.02	1.02	1.08	1.12	1.12	1.14	1.13	1.13	1.14	1.10
E	0.99	0.97	1.02	1.02	1.08	1.09	1.13	1.13	1.15	1.11	1.09	1.13
F	0.98	1.00	0.99	1.01	1.05	1.13	1.13	1.11	1.09	1.14	1.13	1.14
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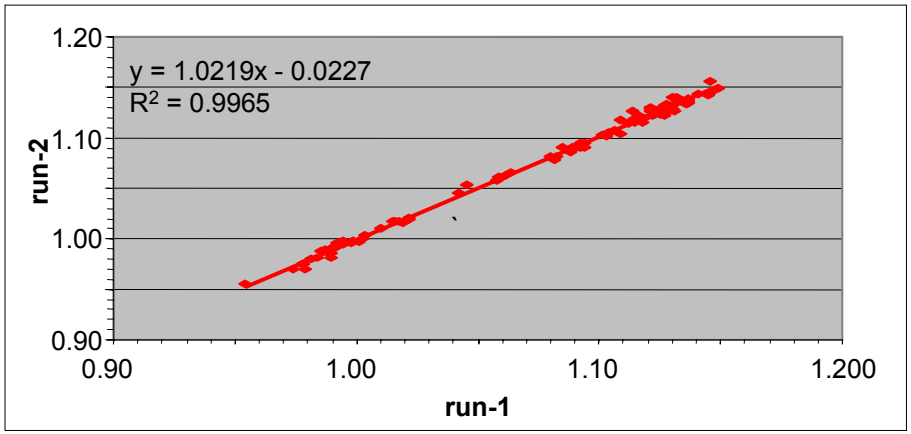
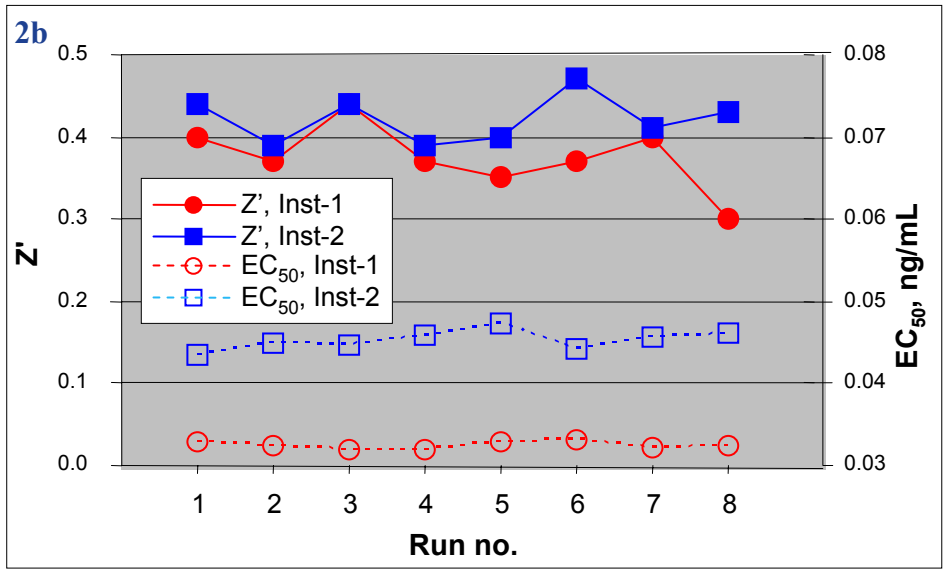
SD	0.012	0.008	0.012	0.011	0.018	0.017	0.023	0.019	0.018	0.025	0.019	0.016



N/C values are repeatable within ± 0.3% (instrument)

N/C values between wells repeat within ± 1.5% (biology)

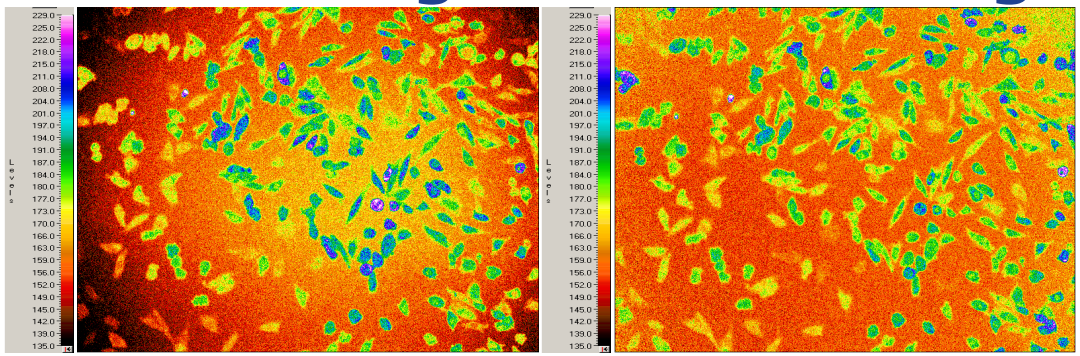
Repeatability of Z’ and EC_{50} (8 runs)



Instrument	Z’	EC_{50}, ng/mL
# 1	0.38 ± 0.04	0.032 ± 0.001
# 2	0.42 ± 0.03	0.045 ± 0.001

Influence of Image Uniformity on Z’

Raw image **FFC’ed image**



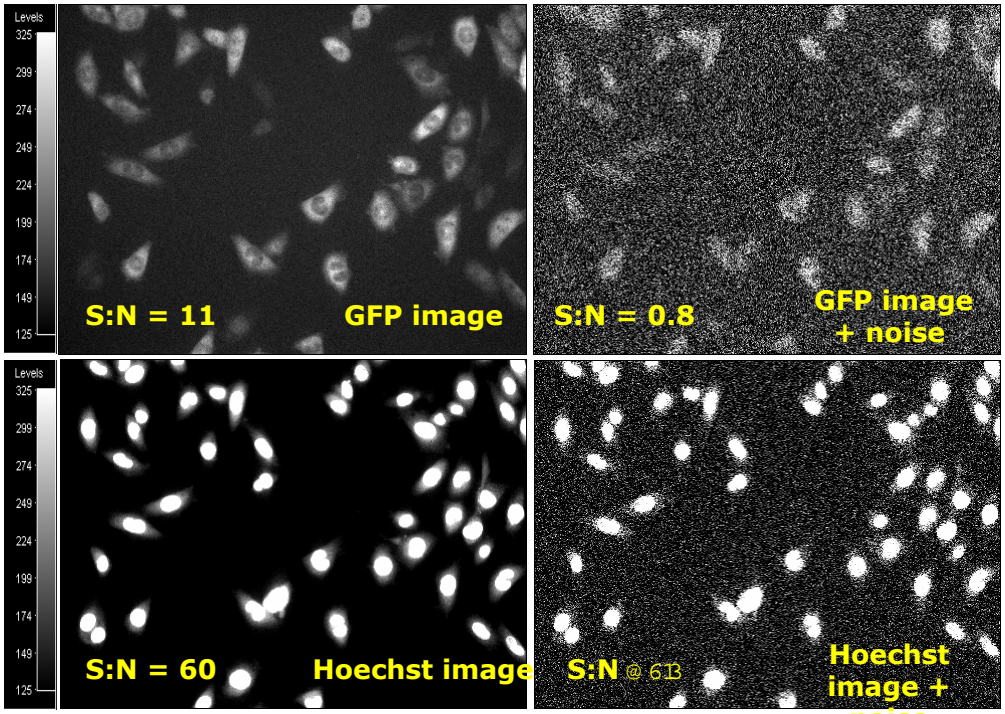
Poster # P02010

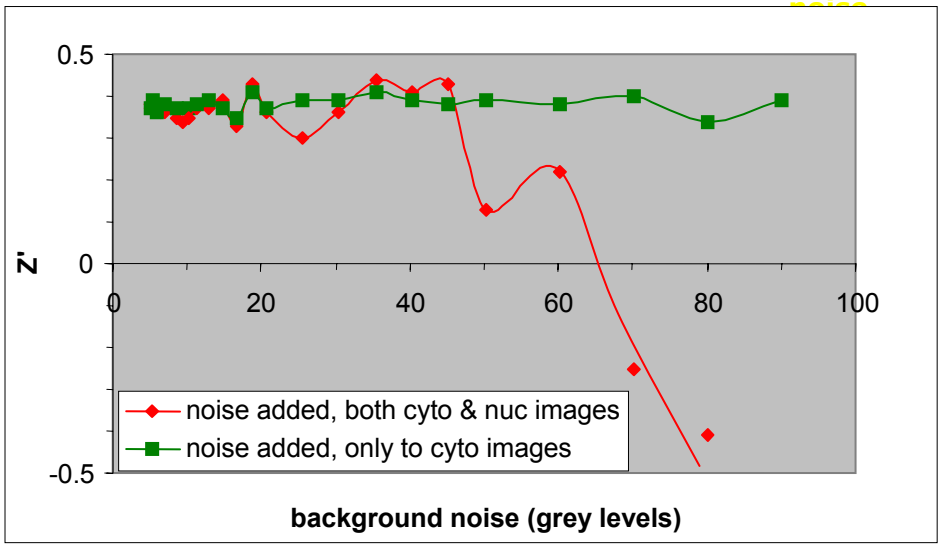
Result: No significant effect

Before FFC
Z’ = 0.38 ± 0.04

After FFC
Z’ = 0.36 ± 0.03

Influence of Image Noise on Z’





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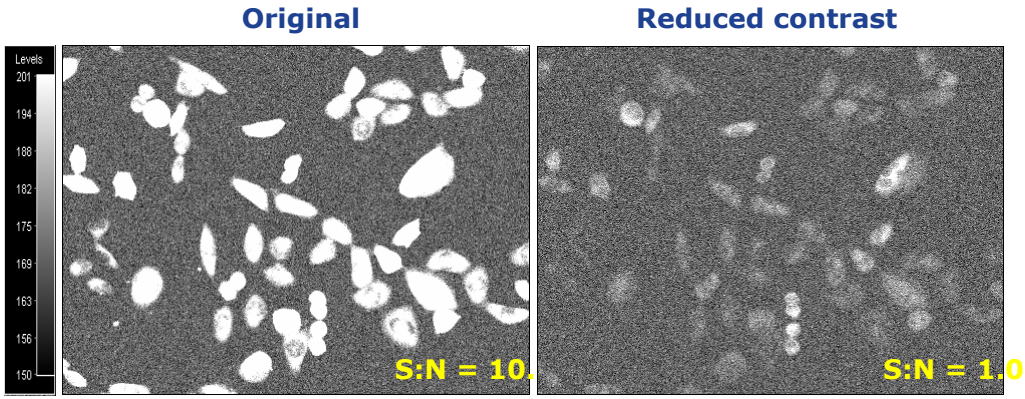
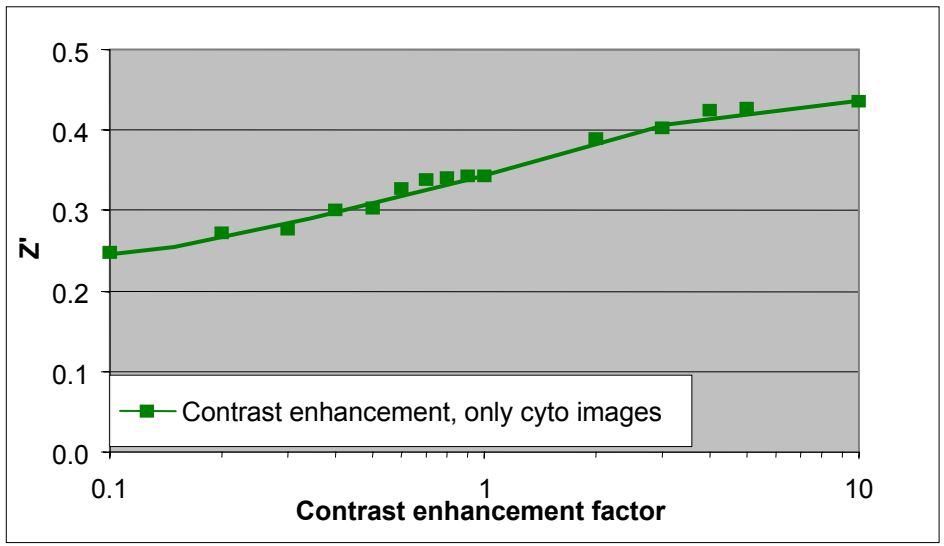
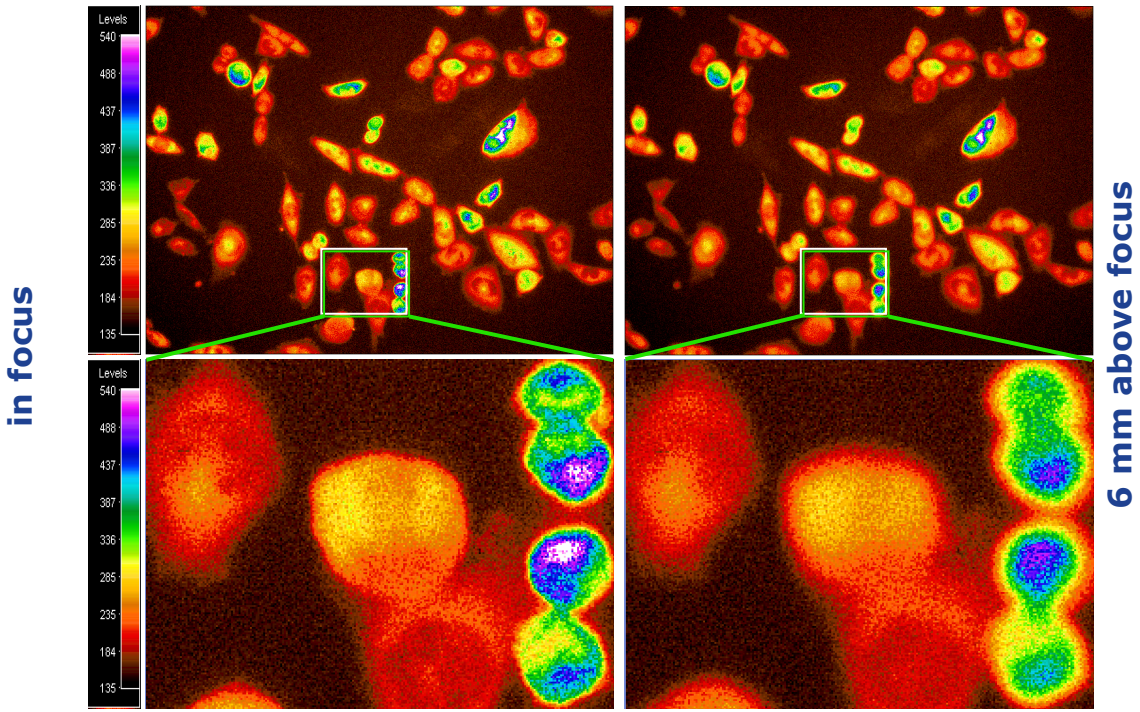
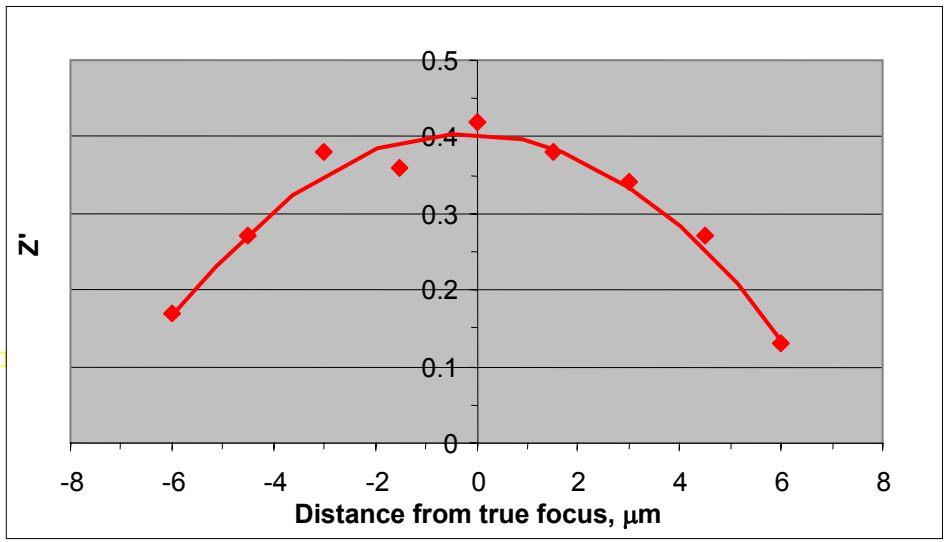
Influence of Image Contrast on Z’





Influence of Image Blur on Z’





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Summary

We have used GE Healthcare's IN Cell Analyzer 1000 to study how image quality influences assay performance as measured by Z' and EC_{50} of a nuclear translocation assay. We find:

The instrument's repeatability is high at 0.3% for the output N/C data, and ± 0.04 for Z'.

Flat field correction (for this assay) has no significant influence on Z' because the output data are based on intensity ratios.

Image noise (or contrast) in the GFP channel has very little effect on Z', up to signal/noise ≈ 1.

Image noise in the nuclear channel deteriorates Z' when signal/noise < 5.

Images blurred by defocusing more than ± 2 mm deteriorate Z'.

Legal considerations

'The IN Cell Developer Toolbox is the subject of US patent application number 11/019326 in the name of Amersham Biosciences Niagara, Inc.';

'The IN Cell 1000 is the subject of US patents 6,563,653 & 6345115 and US patent application number 10/514925, together with other granted and pending family members, in the name of Amersham Biosciences Niagara, Inc.'; and

'The IN Cell Analyzer 3000 is the subject of US patents 6,400,487 and 6,388,788 and US patent application number 10/227552, together with other granted and pending family members, in the name of Amersham Biosciences Corporation'