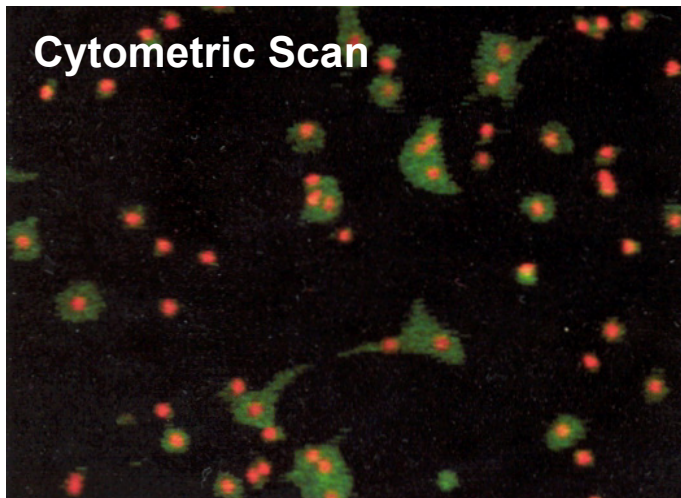
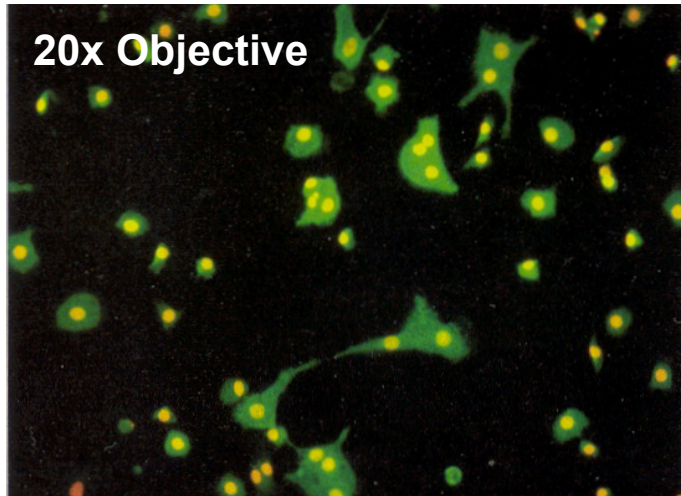


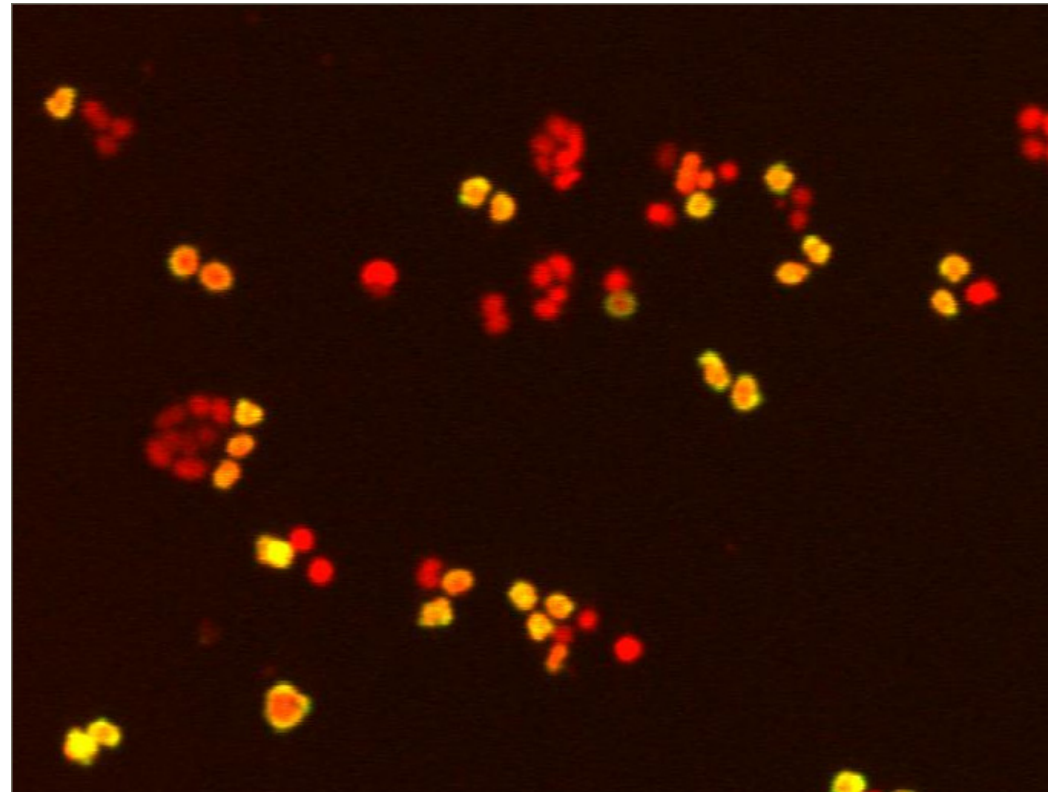
Whole Well Image Capture using a Laser Scanning Fluorescence Microplate Cytometer

Wayne Bowen
TTP LabTech Limited
Melbourn, UK





TIFF Image – Mitotic Index



- 1 μ m x 1 μ m scan
- Anti-phospho-histone H3 (FITC)
- Nuclear counterstain (PI)

Kriauciunas et al., Eli Lilly & Company
Eur.J.Pharmacol. 3, 42-51 (2006)

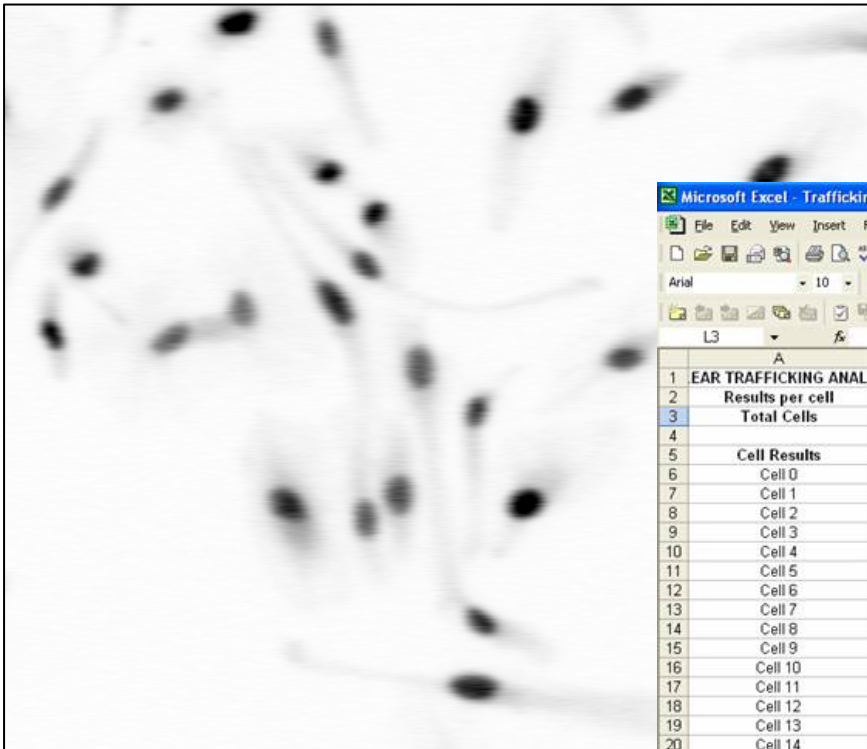


Nuclear Translocation

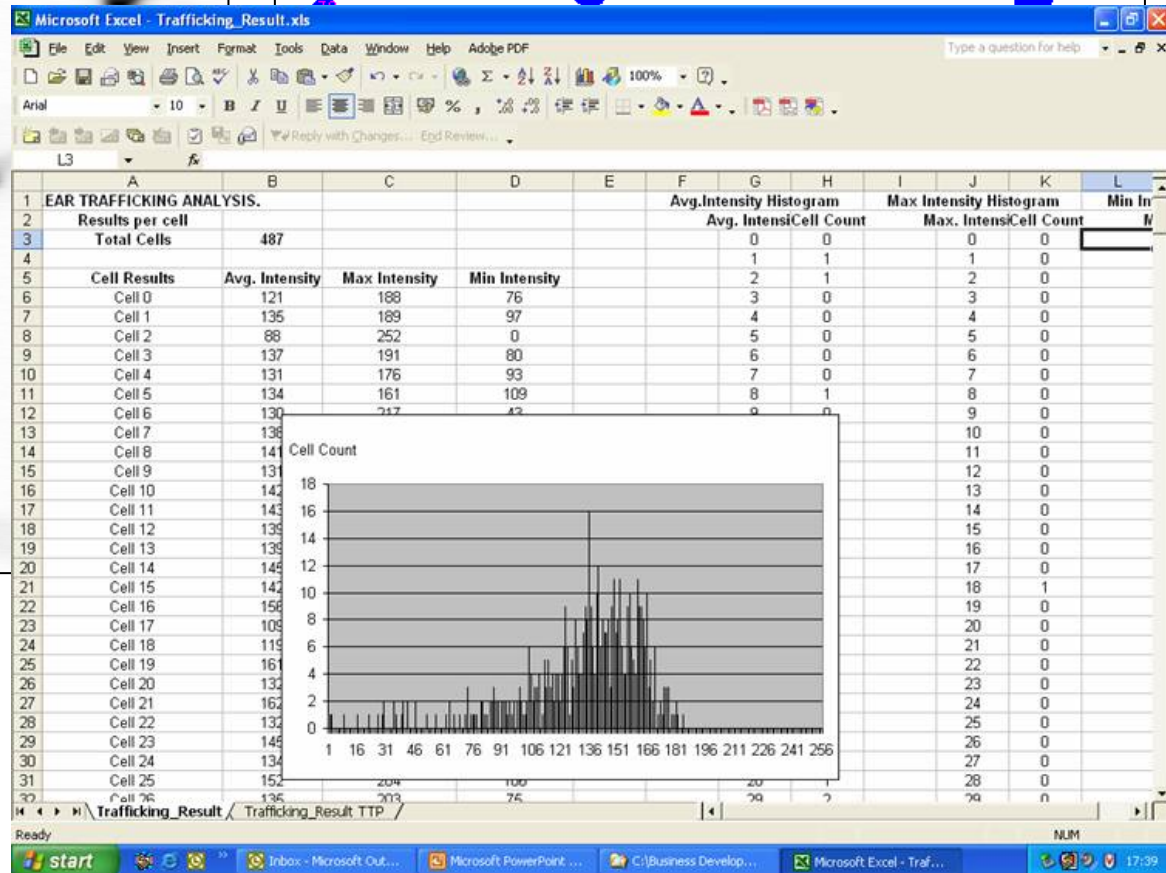
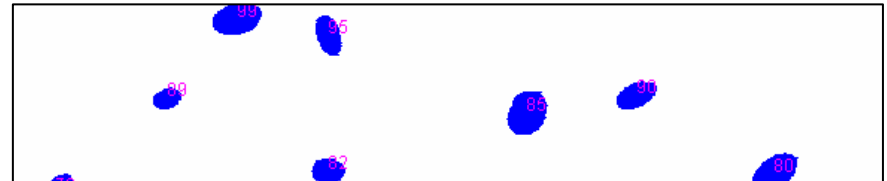
Data & Image Analysis



TIFF Image



Nuclear Mask



- 1 μ m x 1 μ m scan
- Anti-tubulin (FITC)
- Nuclear counterstain (PI)

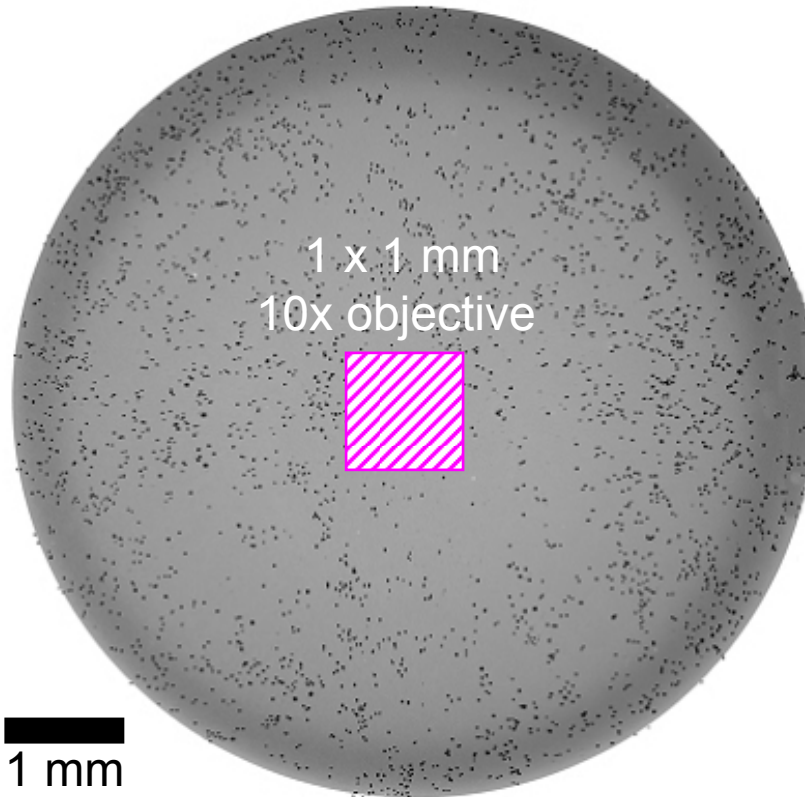


What Scan Area is Required?

Data & Image
Analysis



Whole Well TIFF Image



- HeLa
- DyeCycle Orange
- $1\mu\text{m} \times 1\mu\text{m}$

	Area	1 mm ² Image
Well	38 mm ²	2.6%
Scan	49 mm ²	2.0%

Benefits

- Data for all cells (> sample size)
- Ideal for rare event detection
- Permits normalisation of responses to total cell number

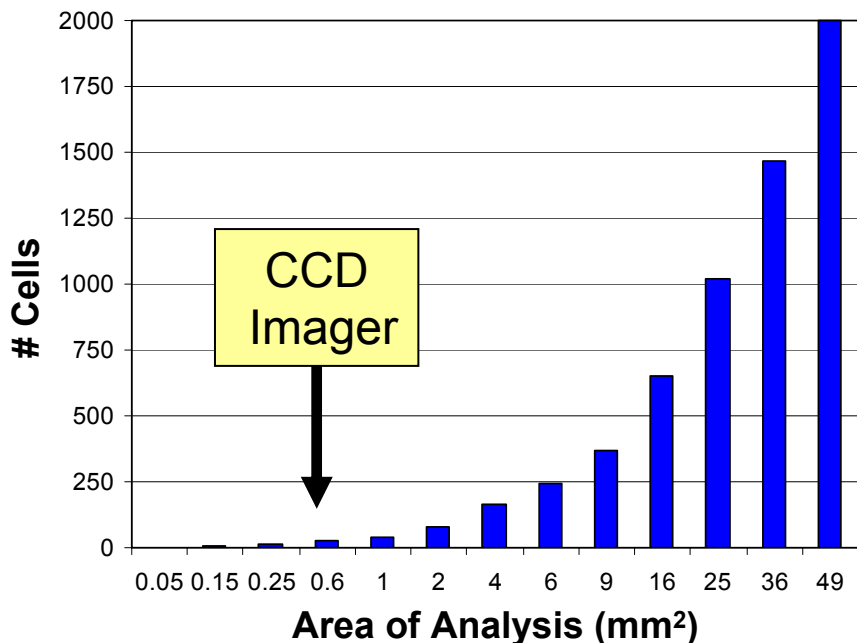


Effect of Scan Area on Assay Performance

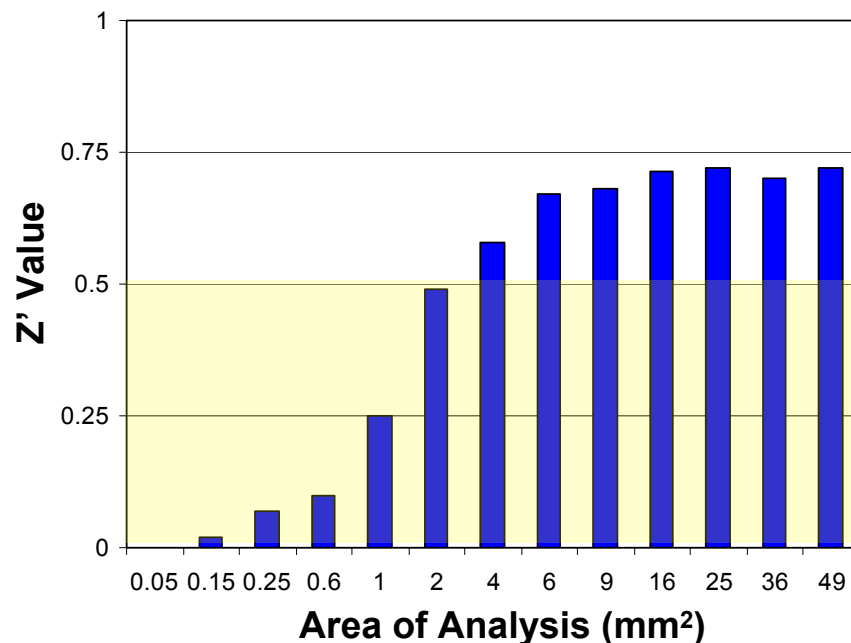
Data & Image Analysis



Effect on Cell Number (96 well)



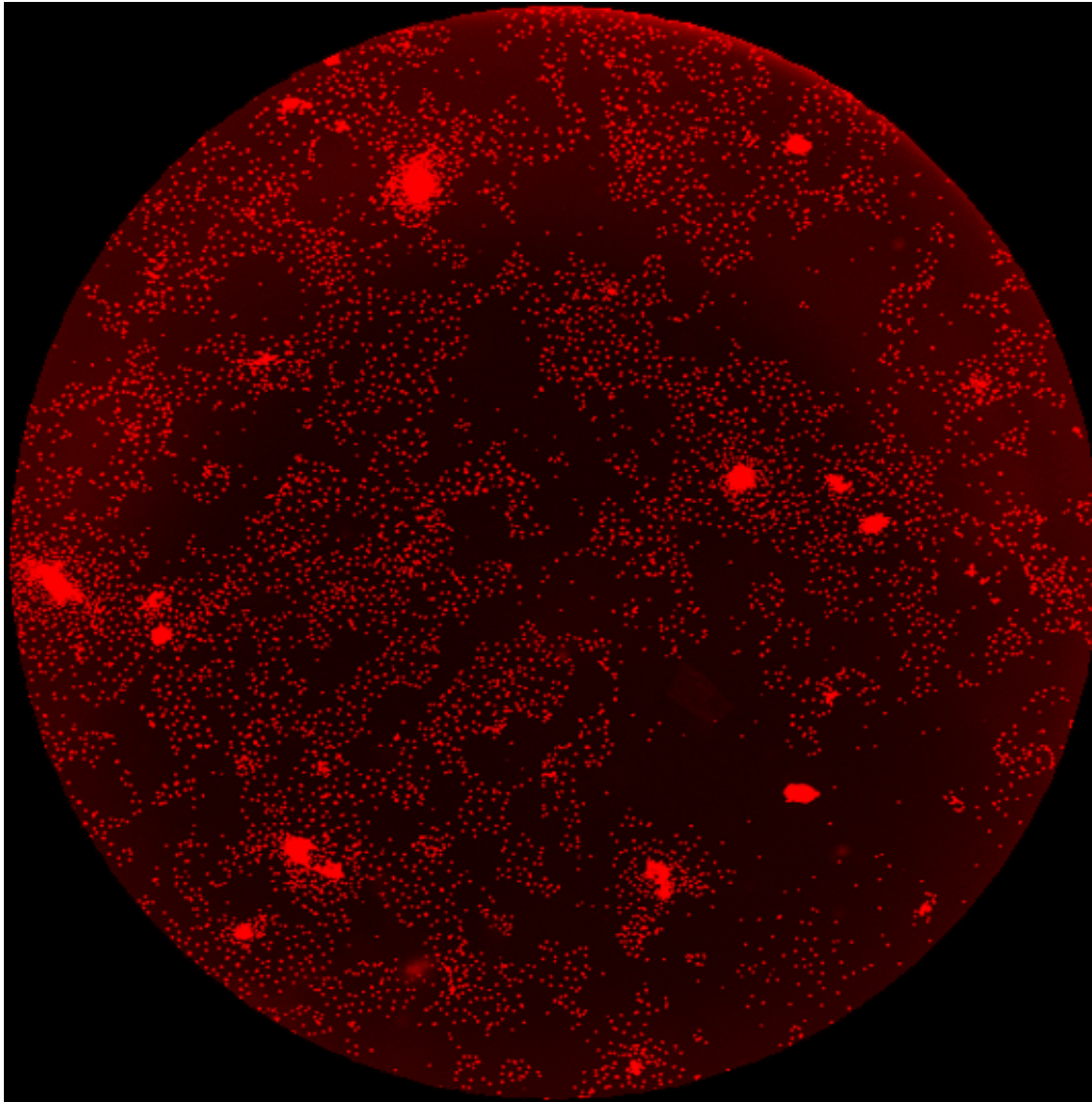
Effect on Z' (96 well)



	Lens	FOV
InCell 3000	40x	0.56 mm ²
Opera	20x	0.15 mm ²
Cellworx	20x	0.55 mm ²

- Cell morphology assay
- Requires 20x objective on CCD imager
- Total cell stain (calcein-AM)





Cell Proliferation

- Fixed cells
- Whole well scan
- $1\mu\text{m} \times 1\mu\text{m}$
- BrdU–Alexa 488
- + Nuclear stain

Rare event detection
(250/6,000 – 4%)

