

# Real-time detection of calcium ion channel events of single cells for drug efficacy and cardiac toxicity evaluations

Paul C.H. Li and XiuJun Li

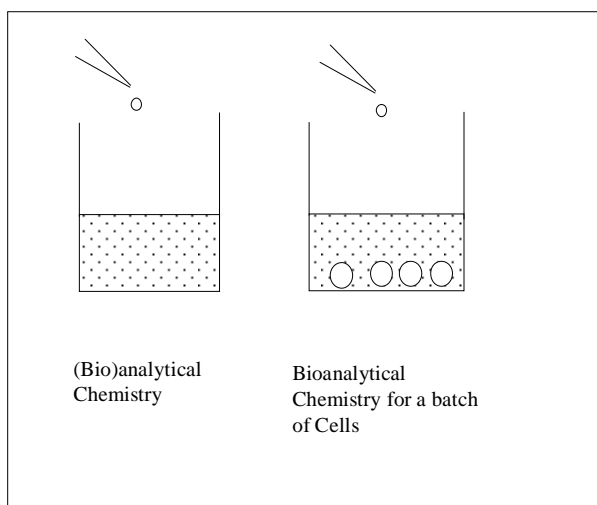
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## (Bio)Chemical analysis

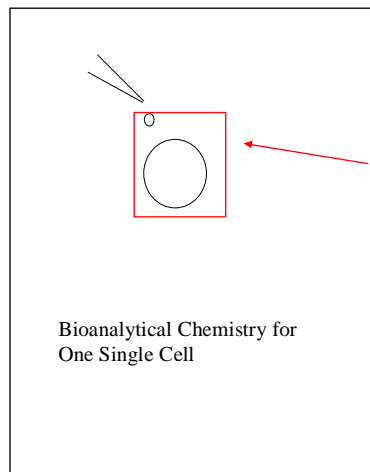


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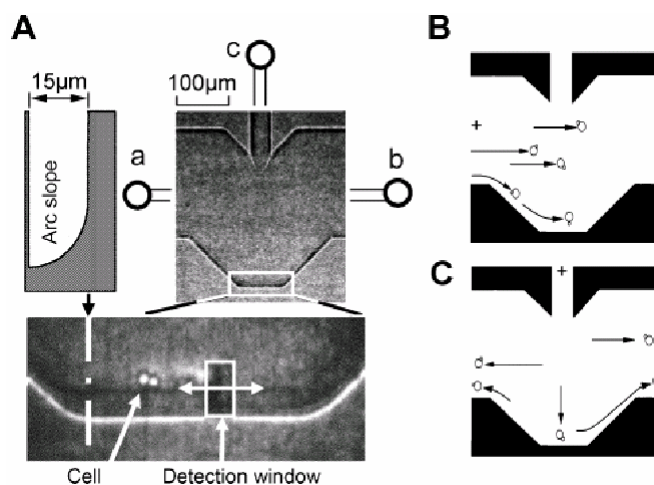
# Single-cell measurement



Microfluidic chamber

Bioanalytical Chemistry for One Single Cell

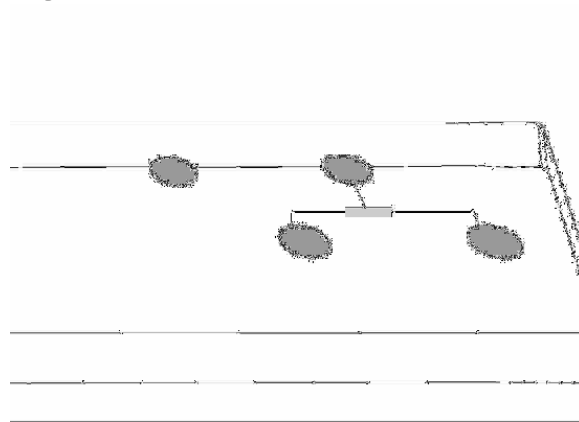
# The glass chip for single-cell retention



Larry Peng & Paul Li, *Anal. Chem.*, 2004, 76, 5273

# The single-cell biochip

- Cells placed in microfluidic channels
- Background removed
- Clean responses
- Long-term cell monitoring



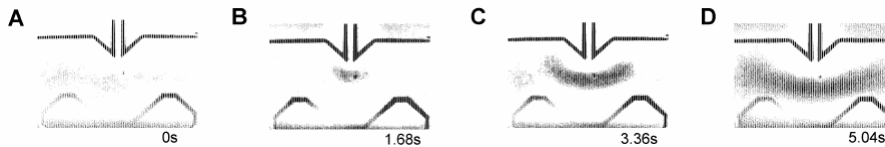
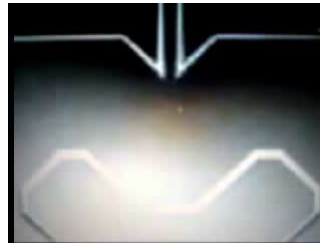
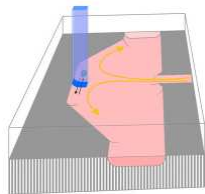
Peng and Li, LabChip, 2005, 5, 1298



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## Liquid delivery

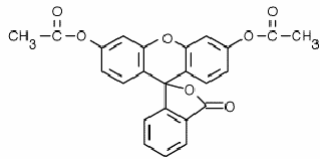
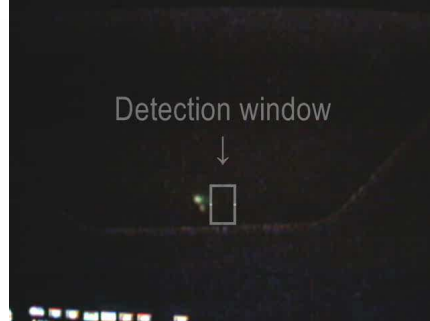
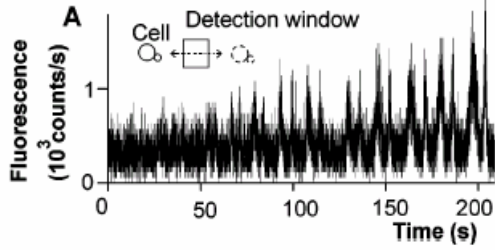


Peng & Li, Anal. Chem, 2004, 76, 5273

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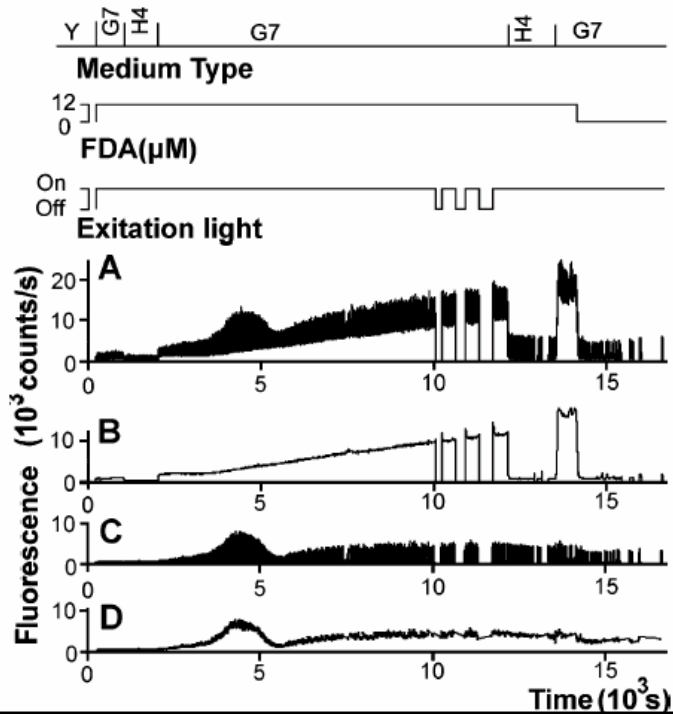
Measurement of cellular fluorescence and background signal on a single yeast cell



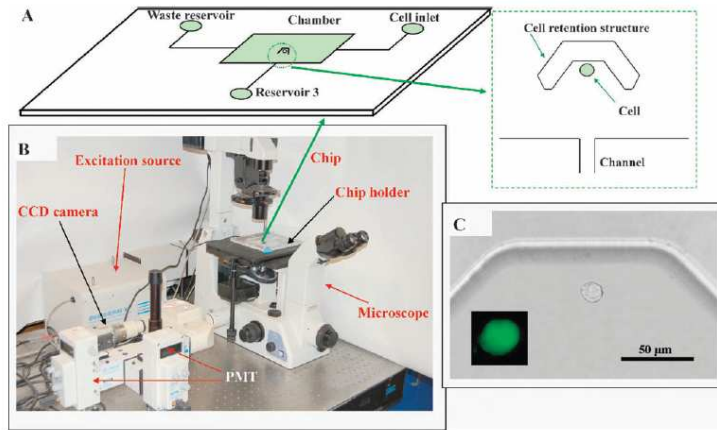
Fluorescein diacetate (FDA)

Peng and Li, Anal. Chem, 2004, 76, 5273

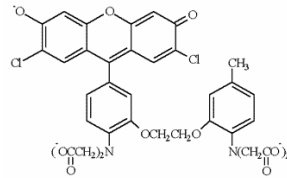
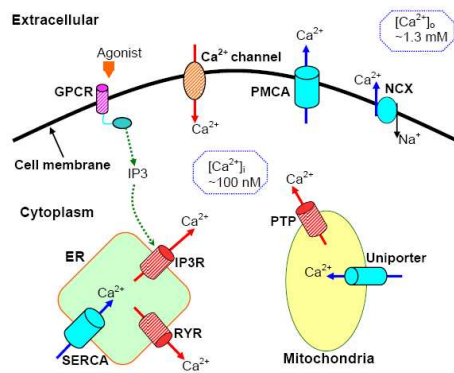
Background correction



# Working with mammalian cells

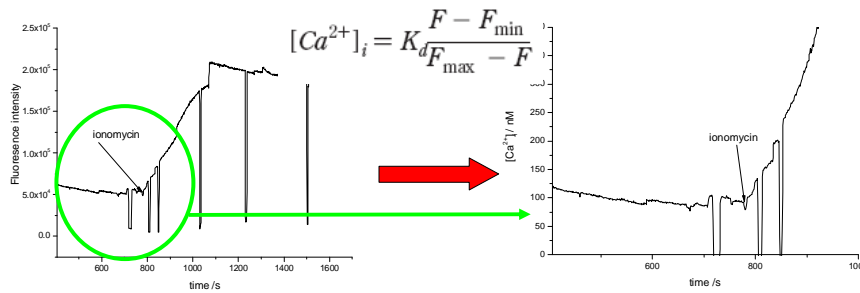


# Fluorescent measurement of $Ca^{2+}$ in single cancer cells



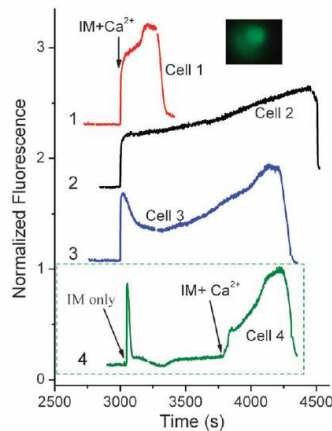
Fluo 4- binds to calcium ion to form a metal ion complex with enhanced fluorescence

## Quantitation of $[Ca^{2+}]_i$ of a single cell in the microchip



Consistent with literature value.  $[Ca^{2+}]_{i,r}$  about 100 nM.

## Calcium ion influx in response to ionomycin (IM) stimulus



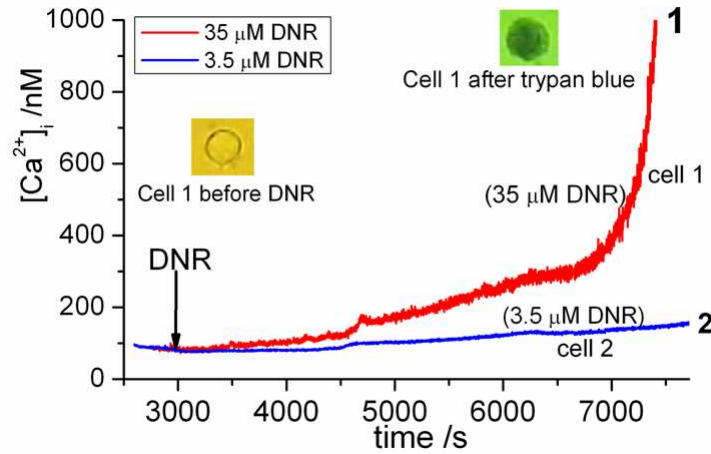
Curves 1–3, IM containing 50 mM  $CaCl_2$ .

Curve 4, first IM only, and then IM containing 50 mM  $CaCl_2$ .

Different patterns of  $[Ca^{2+}]_i$  flux from 4 different RAW 264.7 cells stimulated by  $10 \mu g ml^{-1}$  IM.

*Integrative Biology, 2009, 1, 90–98*

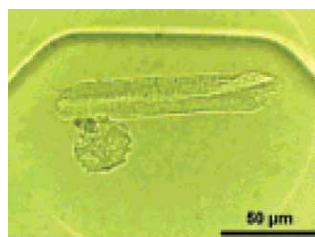
## Drug Effect (efficacy) studied on single cancer cells



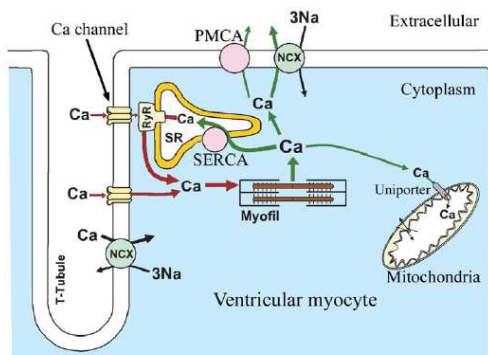
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## contraction of cardiomyocyte



Primary rabbit ventricular myocyte



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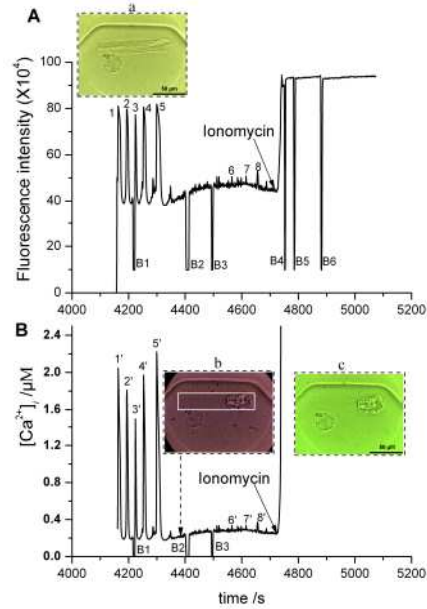
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## Fluorescent measurement of cytosol $\text{Ca}^{2+}$ in a single cardiomyocyte

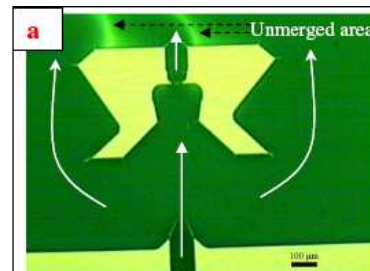
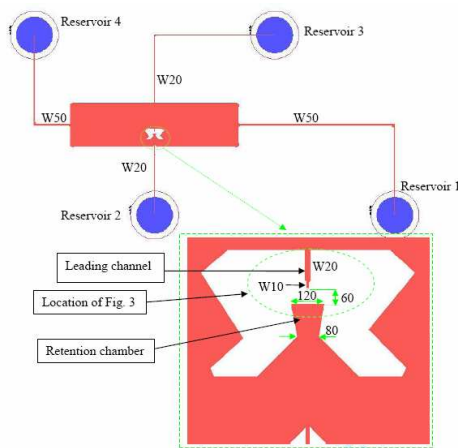
- Variation in fluorescence:12%
- Actual variation in  $[\text{Ca}^{2+}]_i$  :36%

Li and Li, Anal. Chem. 2005, 77, 4315



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## Improved cell retention by on-chip weir construction



Li et al. Electrophoresis, 2007, 28, 4723

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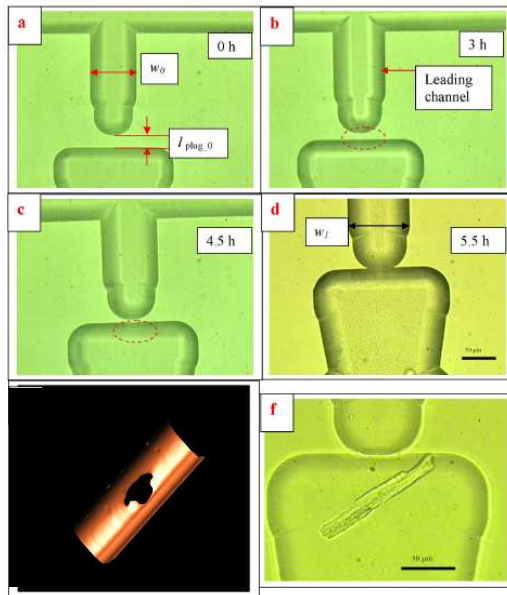
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## Improved cell retention by on-chip weir construction

On-chip fabrication of a weir structure inside a bonded chip.  
 (a) before etching.  
 (b) after etching for ~3 h, (c) after etching for ~4.5 h, (d) after etching for ~5.5 h.

(e) A three-dimensional profile of the cell retention structure from Z-sliced reconstruction obtained by confocal microscopy.

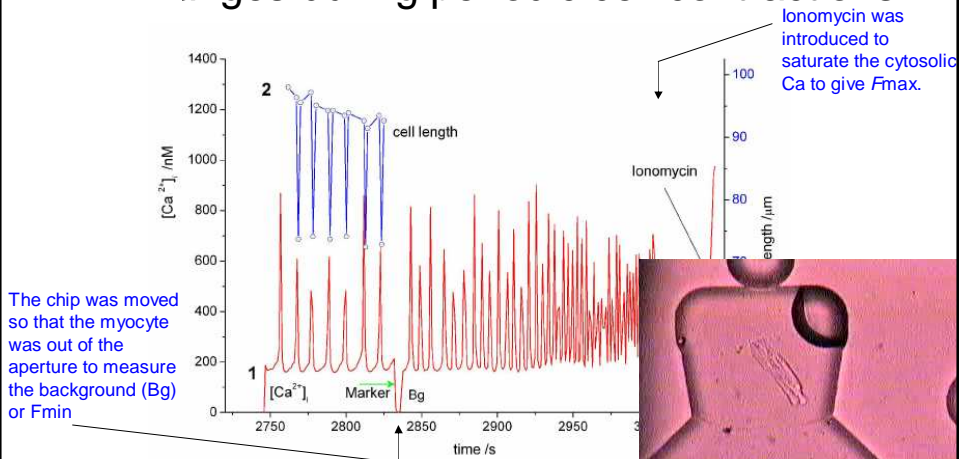
(f) A single cardiomyocyte was retained in the cell retention chamber.



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## Ca changes during periodic cell contractions

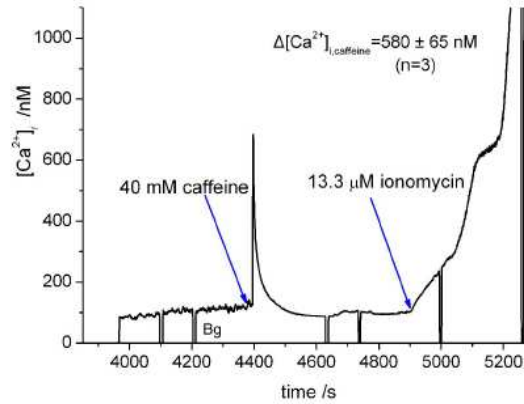


Curve (1) represents the  $[Ca^{2+}]_i$  versus time curve.  
 Curve (2), cell length versus time curve.  
 Since the cell length kept decreasing, only the cell length changes of the first six cycles of contractions were measured.

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## Ca change during caffeine stimulation

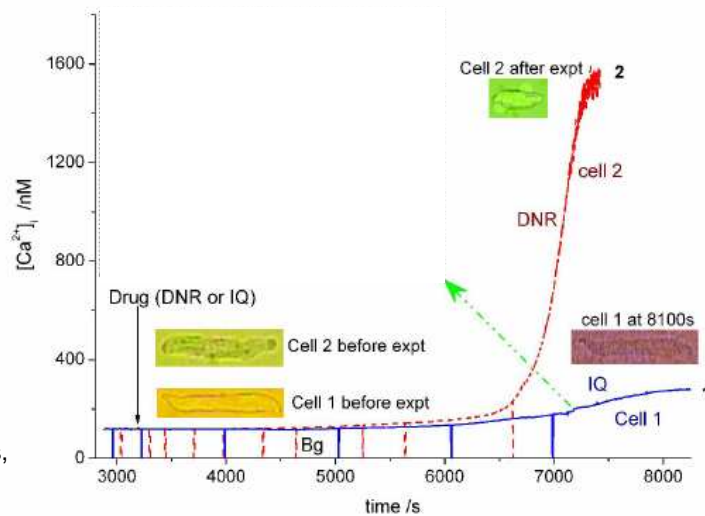


Caffeine-induced response of a single cardiomyocyte in microchip. Caffeine was added to trigger muscle contraction. Ionomycin was added to saturate cellular fluorescence for calibration.

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## Drug Effect (safety) studied on single cardiomyocyte



Li et al.  
Electrophoresis,  
2007, 28, 4723

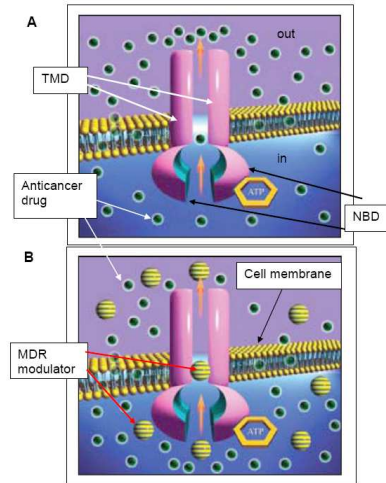
Curve (1), treatment of IQ on cell 1. Curve (2), Treatment of DNR on cell 2. Cell images before and after the drug treatments are shown.

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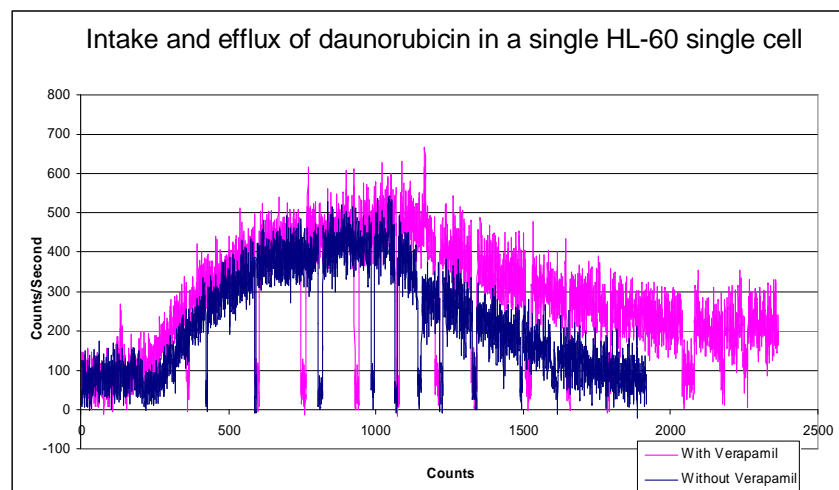
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## Drug efflux via ABC transporters

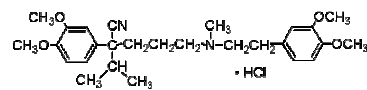
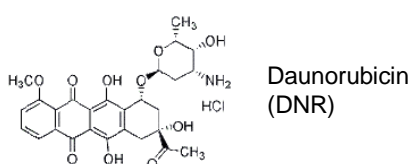
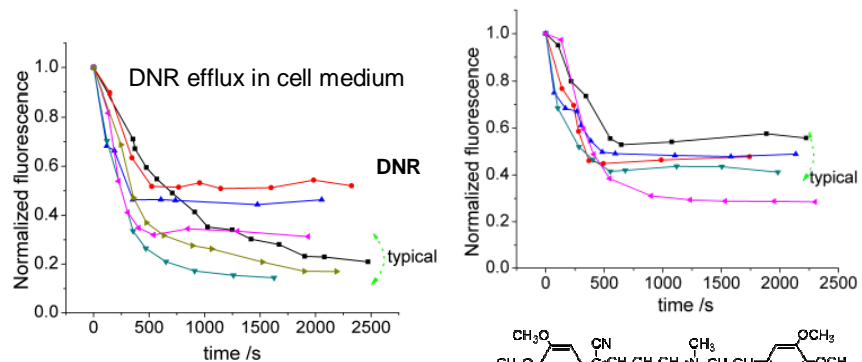


H. Lee from Moffitt Cancer Center & Research Institute

## Single-cell drug efflux measurement



## Drug-resistant cancer cells



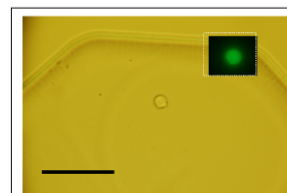
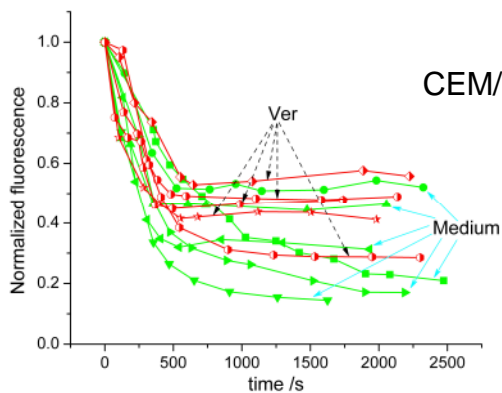
Drug efflux reversed in verapamil

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## Hard to compare cancer cells with different MDR abilities



The test cell (inhibited by Ver) may have a stronger MDR ability than the control cell

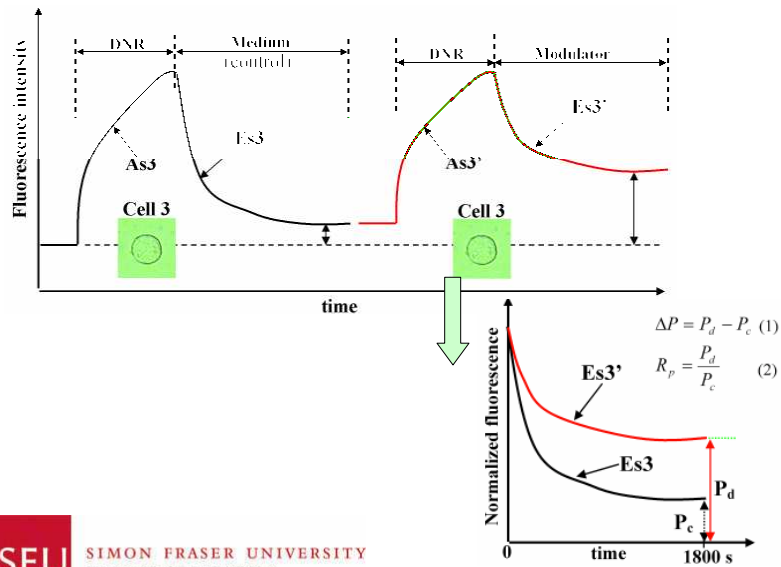
Li et al, Anal. Chem, 2008, 80, 4095.

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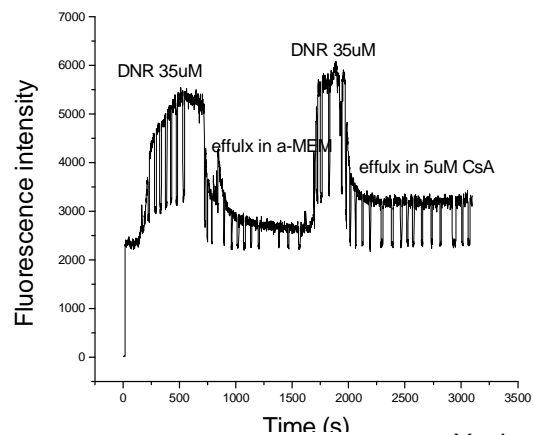
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## Use the same cell as the reference: Same-single-cell analysis (SASCA)



## Cyclosporine A (CsA) effect on DNR retention inside the MDR cells

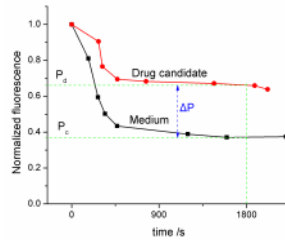


Yuchun Chen

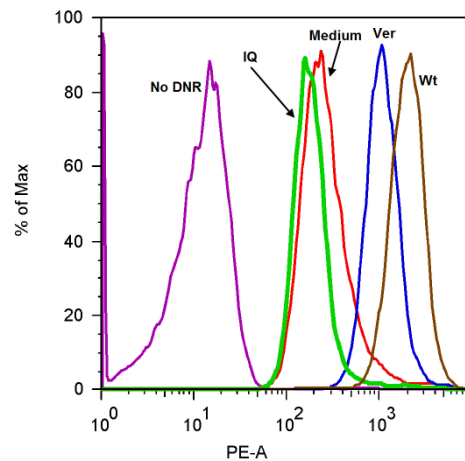
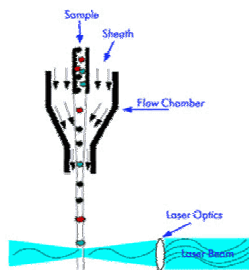


## DNR efflux in CEM/VLB cells as modulated by verapamil and IQ

	IQ			Verapamil		
	Cell 1	Cell 2	Cell 3	Cell 1	Cell 2	Cell 3
$P_d$ (%)	29.6	45.3	8.8	42.2	28.8	55.7
$P_c$ (%)	31.7	40.8	14.4	31.7	17.2	14.4
$\Delta P$ (%)	-2.1	4.5	-5.6	10.5	11.6	41.3
Average $\Delta P$ (%)	$-1.1 \pm 5.1$			$21 \pm 17$		
$R_p (P_d/P_c)$	0.93	1.11	0.61	1.33	1.67	3.87
Average $R_p$	$0.88 \pm 0.25$			$2.3 \pm 1.4$		
Geom. mean ratio	$0.72 \pm 0.21$			$3.83 \pm 0.86$		

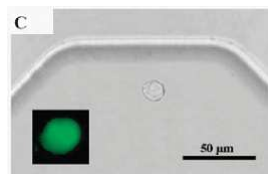
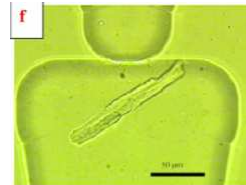
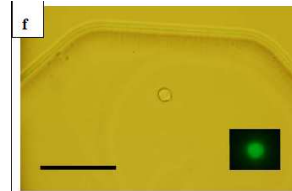


## Drug retention Studied by flow cytometry



## Applications of single cell-based studies

- Study small numbers of cells
- drugs efficacy on cancer cells
- Drug toxicity to heart muscle cells
- Not experimenting drugs on the patients, but on patients' cells



## Acknowledgements

Grad. students: Weijie Wang, Laurent de Camprieux, Jia Cai, James Li, Yuchun Chen  
Postdoc: Dr. Hong Chen, Dr. T.C. Wu, Dr. Jane Zhang,  
Undergrad students: Derek Chew, Michael Sung, Yan Xue, David Wicks, Monika Sangar

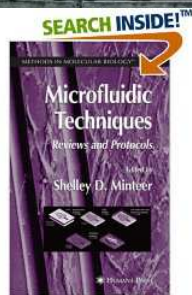
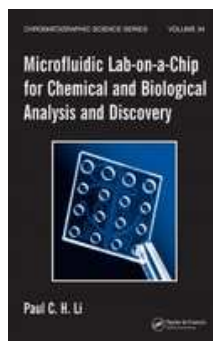
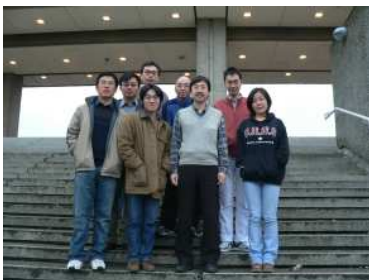
### Collaborators:

Dr Larry Peng (Xiamen University)  
Dr. Glen Tibbits (SFU Kinesiology)  
Dr. Victor Ling (BC Cancer Research Centre)

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NSERC (Discovery, Idea-to-Innovation)  
CFI, BCKDF, CIHR, CMC Protolyne technology

Graduate Student Positions Available, see  
[www.sfu.ca/chemistry/faculty/Li](http://www.sfu.ca/chemistry/faculty/Li)



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