



INTRODUCTION

Adriamycin (doxorubicin) and cisplatin are frequently used cancer therapeutics which interfere with DNA replication by different mechanisms. The A2780 series of ovarian cancer cell lines are unique to The European Collection of Authenticated Cell Cultures (ECACC) and its distributors. They have been effective *in vitro* tools for cancer research for over 20 years^(1,2).

A2780cis (ECACC 93112517) and A2780ADR (ECACC 93112520) (derivatives of A2780 (ECACC 93112519)) are resistant to cisplatin and Adriamycin respectively. They are useful in the study of cancer biology and resistance to chemotherapies, enabling the search for new treatments.

Drug resistance has been quantified in these cell lines using IC₅₀ values; the dose of a drug which causes a 50% inhibition in normal cell growth. The methods employed to determine IC₅₀ values often have different durations (4-21 days), they are labour intensive and can yield inconsistent results⁽¹⁻⁴⁾.

To independently characterise the cell lines and investigate whether they have retained their drug resistant properties, we developed a novel automated approach using the IncuCyte ZOOM and a label-free assessment of IC₅₀ values. The study demonstrated a proof of principle for the analysis of *in vitro* drug resistance using cell lines.

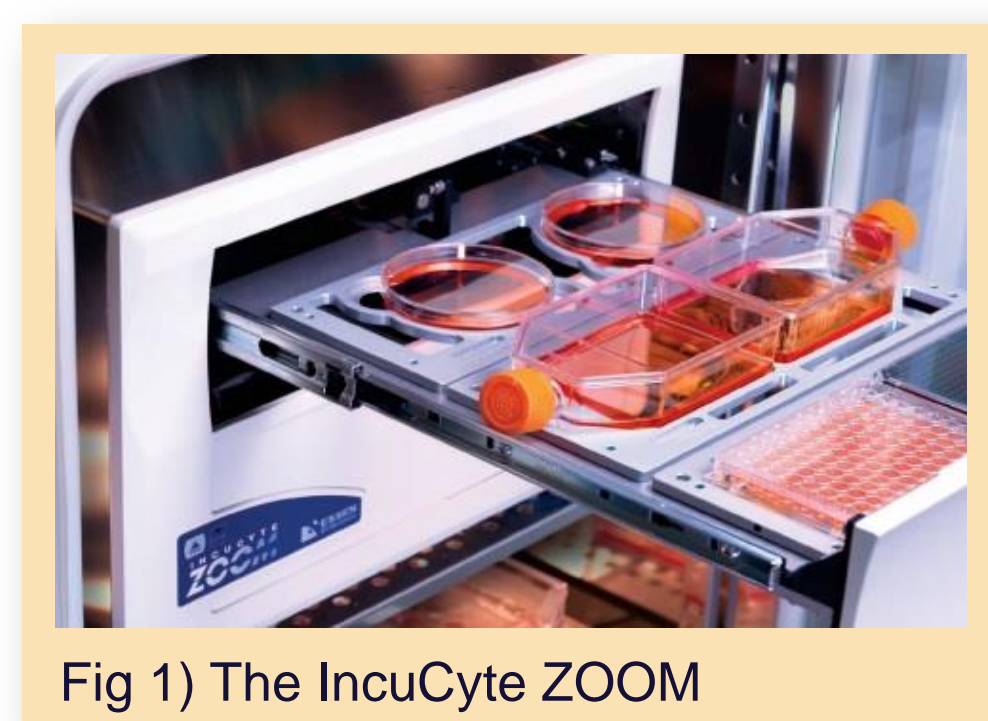


Fig 1) The IncuCyte ZOOM

METHODS

Authenticated cell lines were provided from ECACC and grown according to recommended instructions. Cells were seeded in 96-well plates at densities of 3x10⁴ cell/cm² (A2780) and 1x10⁴ cell/cm² (A2780cis, A2780ADR) followed by addition of a drug dilution series.

- Cisplatin (P4394, Sigma) dissolved in PBS was diluted in media (final well concentration: 500µM-0.001µM).
- Adriamycin (44583, Sigma) was dissolved in DMSO and diluted in media, (final well concentration: 150µM-0.001µM). DMSO was serially diluted in media to act as vehicle control.

Plates were incubated in the IncuCyte ZOOM (Essen BioScience). Phase contrast images were obtained every two hours to provide data for confluence determination. For cell number count, nuclear stain Syto16 (S7578, Life Technologies) was added after 120 hours and fluorescently imaged.

We used the two distinct parameters of confluence and cell count and subsequent analysis using GraphPad Prism7 to determine IC₅₀ values. Adriamycin treatment was normalised using DMSO controls and no difference was seen between IC₅₀ values calculated using normalised or raw values.

RESULTS

- The three cell types displayed a similar morphology and the IncuCyte accurately quantified confluence and cell number (fig 2).
- Cells reached mid-log phase after ~72 hours as determined by growth curve.
- IC₅₀ values calculated using confluence (72 hours) or cell number (120 hours) produced very similar IC₅₀ values (fig 3, fig 4).
- A2780cis showed a ten-fold resistance to cisplatin.
- A2780ADR showed over ten-fold resistance to Adriamycin.
- These results are consistent with those published over 20 years ago generated using different experimental techniques^(1,3-6) (table 1).

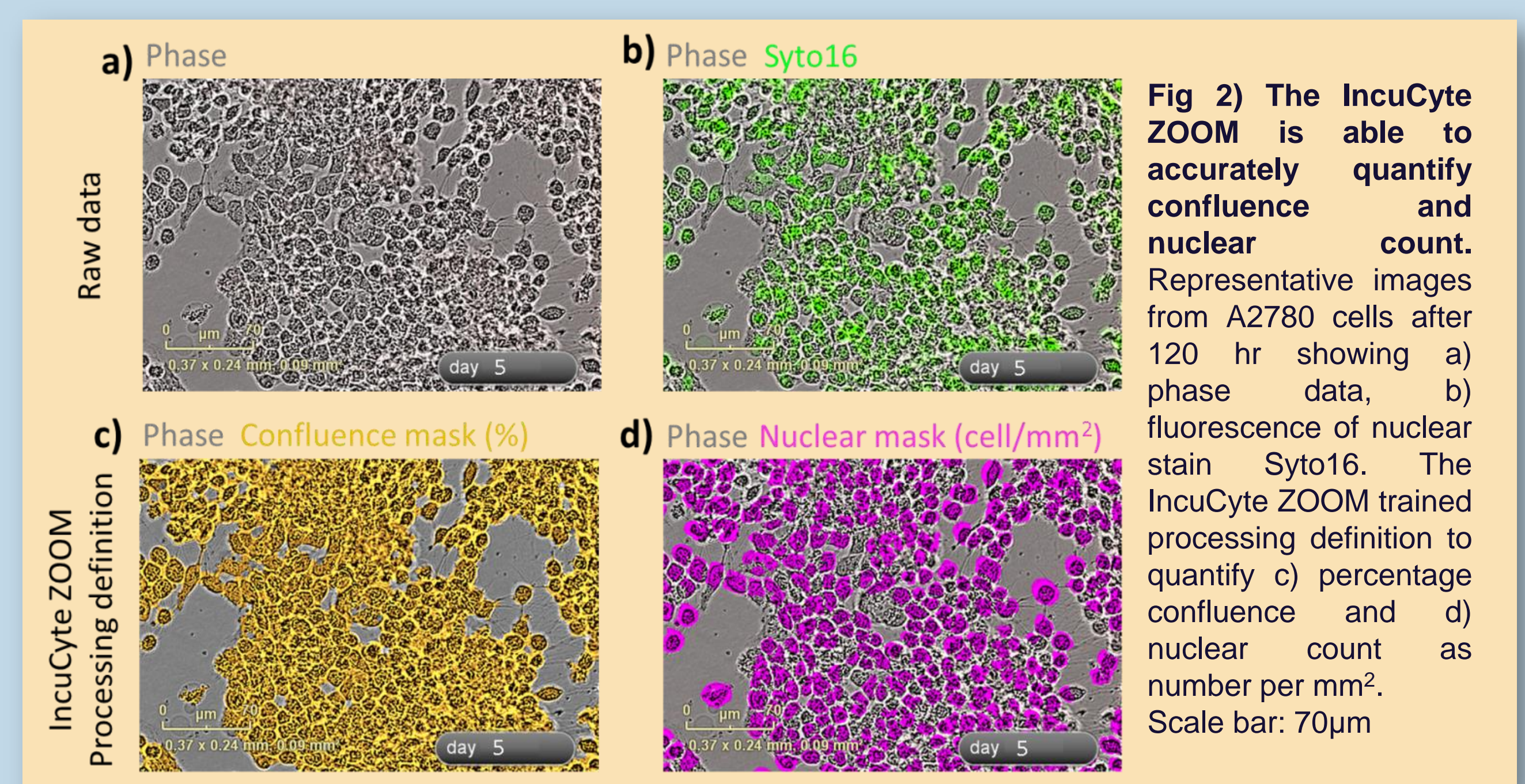


Fig 2) The IncuCyte ZOOM is able to accurately quantify confluence and nuclear count. Representative images from A2780 cells after 120 hr showing a) phase data, b) fluorescence of nuclear stain Syto16. The IncuCyte ZOOM trained processing definition to quantify c) percentage confluence and d) nuclear count as number per mm². Scale bar: 70µm

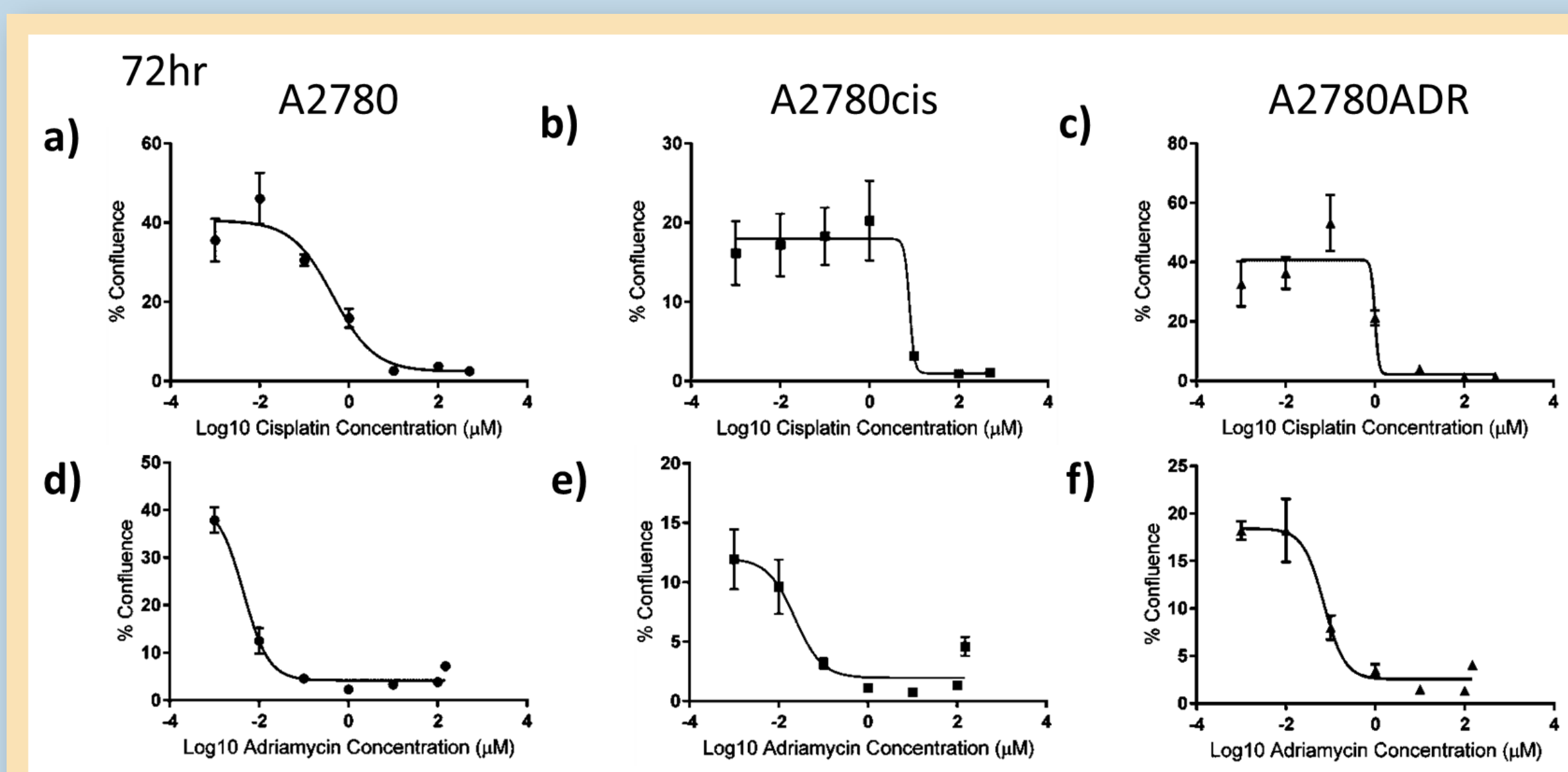


Fig 3) Confluence-based IC₅₀ values show A2780cis and A2780ADR have higher drug resistance than parental A2780 cells. Error bars are SEM generated from 6 (Adriamycin) or 8 (cisplatin) technical replicates at each drug concentration.

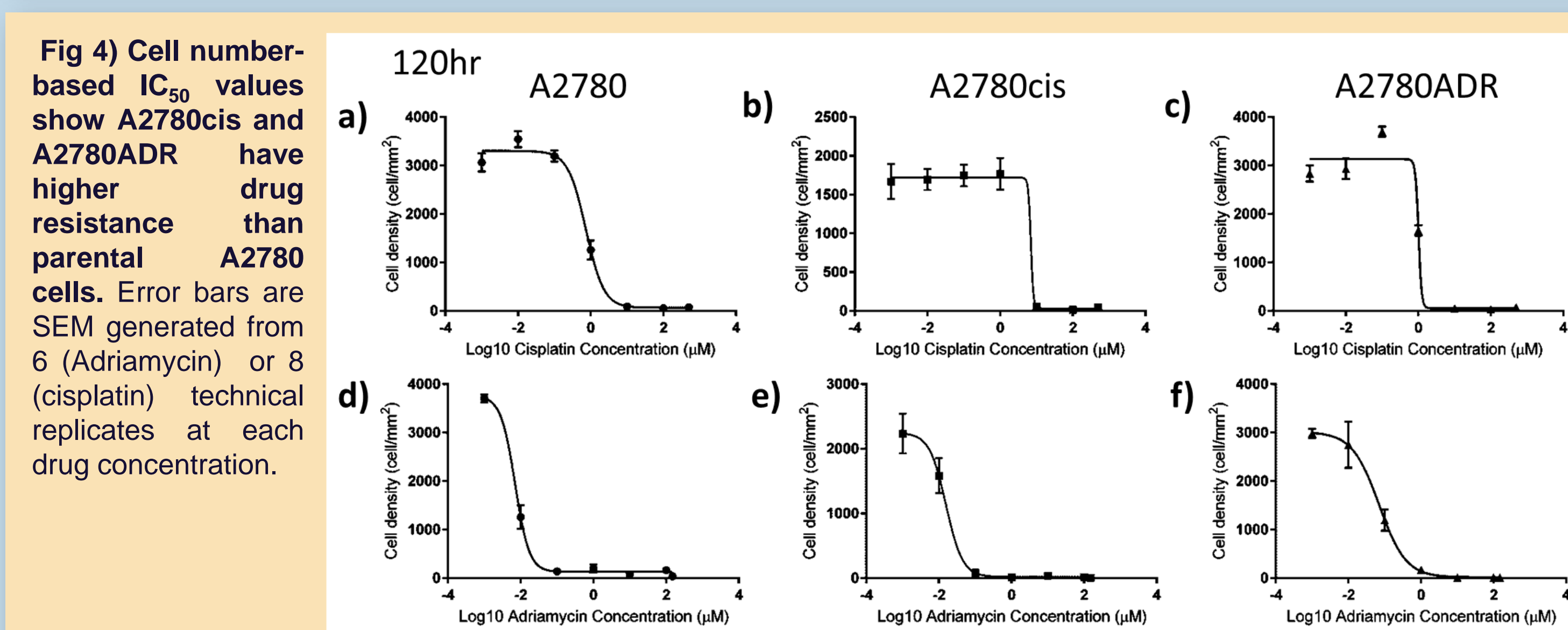


Fig 4) Cell number-based IC₅₀ values show A2780cis and A2780ADR have higher drug resistance than parental A2780 cells. Error bars are SEM generated from 6 (Adriamycin) or 8 (cisplatin) technical replicates at each drug concentration.

Table 1. IC₅₀ values for A2780, A2780cis and A2780ADR (experimental and published data)

Drug	Method	Time after seeding (hr)	IC ₅₀ values		
			A2780 (µM)	A2780cis (µM)	A2780ADR (µM)
Experimental IC₅₀					
Cisplatin	Cell number	120	0.736	7.044	1.009
	Confluence	72	0.438	7.812	0.997
	Range:		0.4 - 0.7	7 - 8	1
Published IC₅₀ (Refs: 1, 3, 5)					
Cisplatin	Range:		0.3 - 1.1	8-39	1.4

Drug	Method	Time after seeding (hr)	IC ₅₀ values		
			A2780 (µM)	A2780cis (µM)	A2780ADR (µM)
Experimental IC₅₀					
Adriamycin	Cell number	120	0.007	0.016	0.070
	Cell number (DMSO normalised)	120	0.005	0.012	0.060
	Confluence	72	0.004	0.023	0.071
	Confluence (DMSO normalised)	72	0.004	0.010	0.086
	Range:		0.004 - 0.007	0.01 - 0.02	0.06 - 0.9
Published IC₅₀ (Refs: 4, 5, 6)					
Adriamycin	Range:		0.01 - 0.1	-	1.6-710

DISCUSSION & CONCLUSION

- A2780cis and A2780ADR are an order of magnitude resistant to their respective therapeutic agents. These results are consistent with the originally published data describing these cell lines⁽¹⁺²⁾.
- The IncuCyte ZOOM enabled the automation of imaging and quantification of confluence and nuclear count data.
- By comparing IC₅₀ determination using cell number we have shown that cell confluence is a valid parameter to determine IC₅₀ in these cell lines.
- This study has verified that A2780 and its drug resistant counterparts, A2780cis and A2780ADR, have retained resistance as reported over 20 years ago. The selective culture conditions used in the production of these cell lines in ECACC has ensured the cells continue to be fit for purpose.
- This study has demonstrated the proof of principle of a label-free, fast and convenient way study *in vitro* drug resistance.

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