

## Fluidlab R-300 Evaluation

### OVERVIEW



#### Evaluation Overview

- We received a Fluidlab R-300 with Acella 50 slides and Acella 100 slides for 10 and 20  $\mu$ L load volumes respectively and 3 adapters for slides and/or cuvettes.
- Cell Count experiments were performed using the Acella 50 slides. Reproducibility experiments were performed with both the Acella 50 & 100 slides.
- **Cells:**
  - **9 different cell lines**
  - **4 cell types (fibroblast, cancer, immune T cells, and iPSC's)**
  - **3 species (human, chicken, and pig)**
- The criteria we used to evaluate the instrument were 1) Ease of Setup 2) Ease of Operation 3) Accuracy compared with manual counts 4) Reproducibility



#### Conclusion

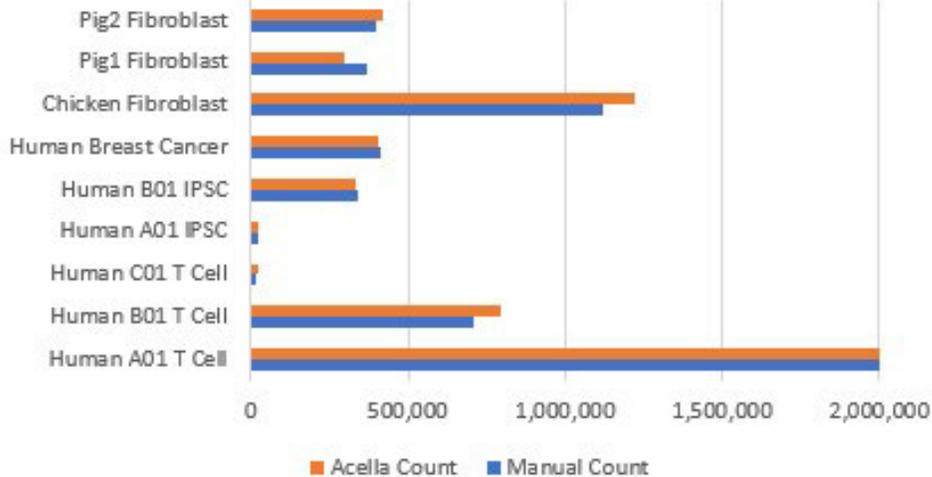
- The Fluidlab R-300 was easy to set-up (charging/data lab software upload), the cell counts were in line with the manual counts at cell/mL concentrations  $>50$ k/mL, with mixed results at lower concentrations, and intra and inter slide reproducibility was good. **Overall, the Fluidlab R-300 performed well in our hands!**

## Fluidlab R-300 Evaluation Cell Counts

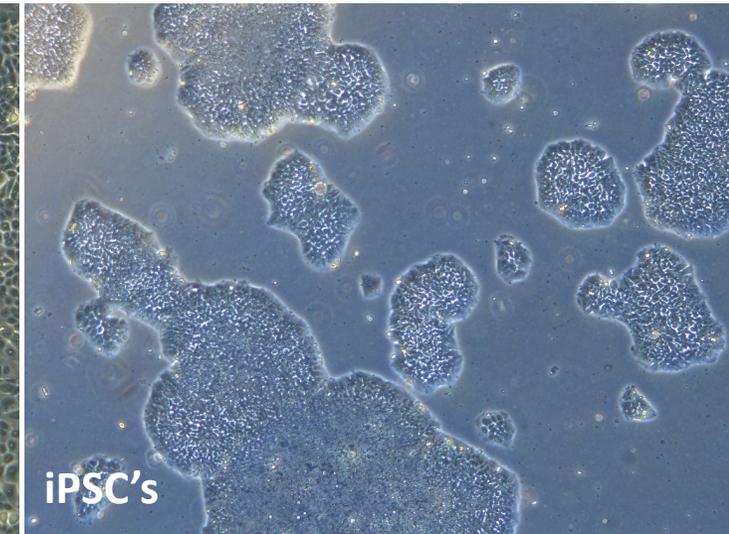
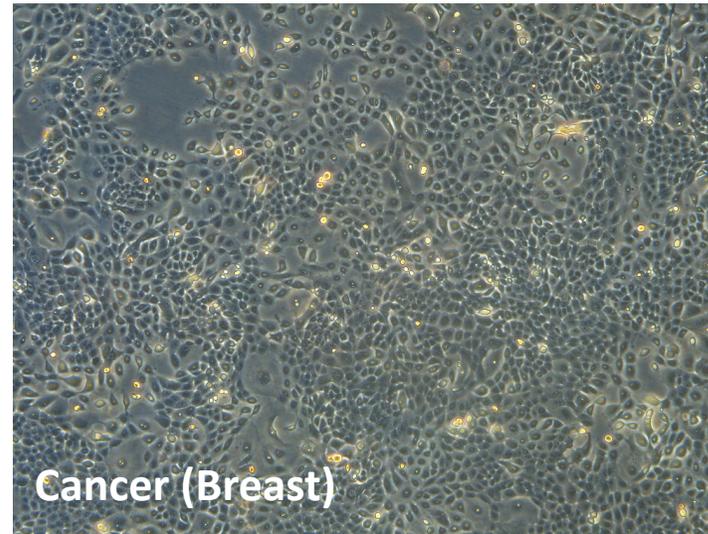
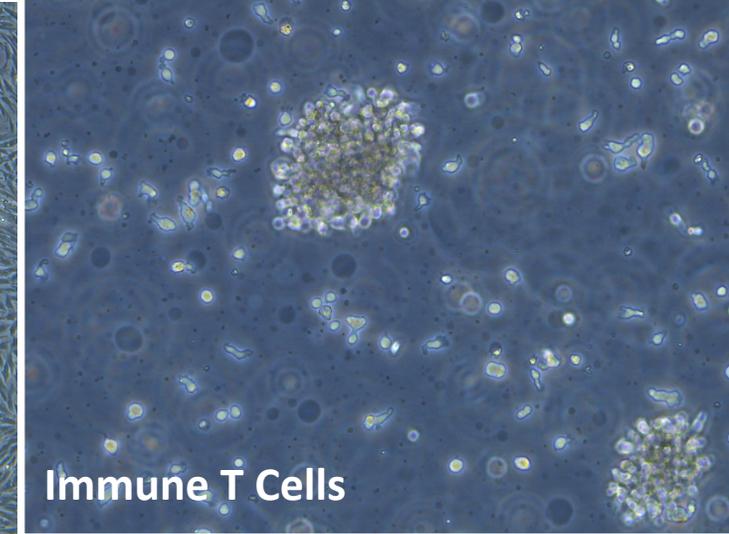
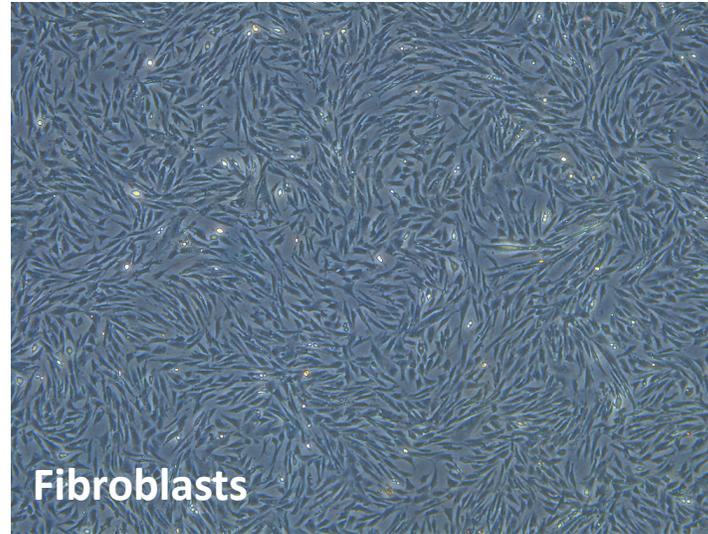
### Cell Count: Manual vs Acella 50

Sample ID	Manual Count	Acella Count	%Manual
Human A01 T Cell	2,000,000	2,000,000	100
Human B01 T Cell	710,000	794,000	112
Human C01 T Cell	18,000	20,000	111
Human A01 iPSC	23,670	22,808	96
Human B01 iPSC	340,000	333,000	98
Human Breast Cancer	410,000	408,000	100
Chicken Fibroblast	1,120,000	1,220,000	109
Pig1 Fibroblast	370,000	300,000	81
Pig2 Fibroblast	400,000	418,000	105
Average			101

### Compare Manual vs Fluidlab R-300 Cell Count



### Cell Types



## Fluidlab R-300 Evaluation

### Reproducibility

#### Intra Slide Reproducibility:

**Fluidlab R-300:** Load an Acella 50 and 100 slide with cancer cells. Read each slide 6 times with laying slide flat between readings as recommended.

**Manual:** Load a hemocytometer with cancer cells. Read one large square (WBC-square) 6 times.

#### Inter Slide Reproducibility:

**Fluidlab R-300:** Load 6 separate Acella 50 and 100 slides with cancer cells. Read each slide once.

**Manual:** Load a hemocytometer with cancer cells 6 times and read one large square (WBC-square) with each load.

**Reproducibility looks good!**

INTRA	cells/mL	1 slide read 6 times	
Read#	Manual	Acella 50	Acella 100
1	4.10E+05	4.11E+05	3.27E+05
2	4.10E+05	4.07E+05	3.29E+05
3	4.10E+05	3.80E+05	3.33E+05
4	4.10E+05	4.03E+05	3.27E+05
5	4.10E+05	4.07E+05	3.25E+05
6	4.10E+05	4.37E+05	3.40E+05
Average	<b>4.10E+05</b>	<b>4.08E+05</b>	<b>3.30E+05</b>
SD	0	18218	5529
<b>%CV</b>	<b>0.00</b>	<b>4.47</b>	<b>1.67</b>

INTER	cells/mL	6 slides each	
Chip#	Manual	Acella 50	Acella 100
1	3.70E+05	3.38E+05	3.90E+05
2	3.60E+05	3.65E+05	3.59E+05
3	4.70E+05	3.80E+05	3.17E+05
4	4.00E+05	4.18E+05	3.14E+05
5	4.40E+05	3.50E+05	3.08E+05
6	4.30E+05	3.73E+05	3.27E+05
Average	<b>4.12E+05</b>	<b>3.71E+05</b>	<b>3.36E+05</b>
SD	42622	27782	32109
<b>%CV</b>	<b>10.35</b>	<b>7.50</b>	<b>9.56</b>

## Fluidlab R-300 Evaluation

### Application: T Cells +/- Microspheres

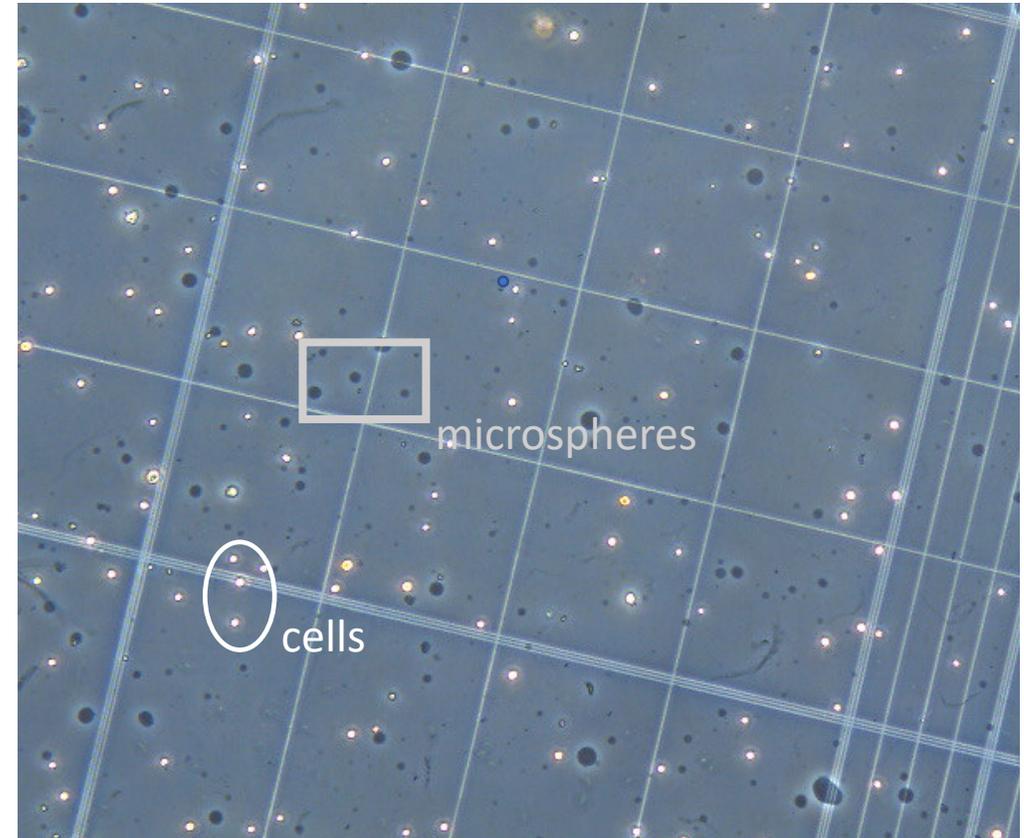
#### T Cell Activation with Microspheres

T cells do not proliferate on their own. For proliferation of T cells, a cytokine rich Renaissance T cell medium is used in combination with a hydrogel (microsphere) which is linked to antibodies for activation.

Manually counting the cells in the presence of these microspheres is easy as the cells and spheres look quite different.

**See image to the right.** But, can the Fluidlab R-300 identify the cells and microspheres as distinct populations?

To determine this, a T cell titration was prepared with and without the beads and counts were determined manually and using the Acella 50 slide.



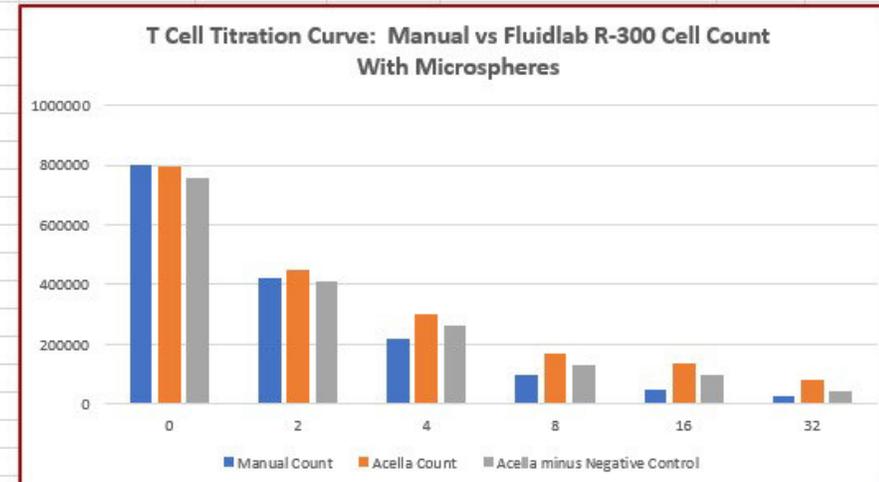
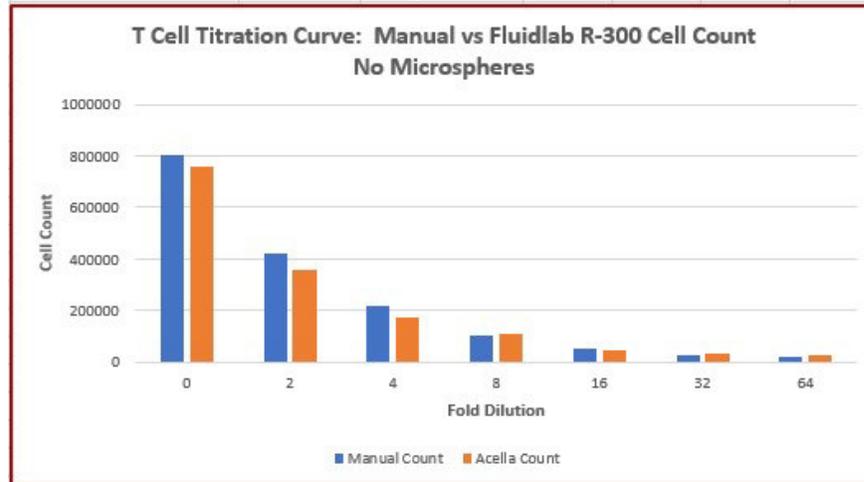
The cells are white while the microspheres are blue-gray-black of varying sizes.

## Fluidlab R-300 Evaluation

### Application: T Cells +/- Microspheres

T Cell Titration Curve (no microspheres)			
	cells/mL		
Fold Dilution	Manual Count	Acella Count	% manual
0	800000	756000	95
2	420000	354000	84
4	220000	171000	78
8	100000	106000	106
16	50000	45616	91
32	27650	34212	124
64	20000	26609	133
128	10000	164622	Warning
neg ctrl (media only)	0	0	
	Average%Manual	102	

T Cell Titration (with microspheres)				
Fold Dilution	Manual Count	Acella Count	Acella minus Negative Control	% manual
0	800000	794000	755987	94
2	420000	449000	410987	98
4	220000	300000	261987	119
8	100000	171000	132987	133
16	50000	137000	98987	198
32	27650	79828	41815	151
64	20000	297000	258987	1295
128	10000	156000	117987	1180
neg ctrl (media only)	0	38013	0	



With no microspheres, the Fluidlab R-300 does a good job in counting cells except at the 1:128 dilution where manually only 1 cell per field is observed. The instrument gives a nice warning that the count may not be correct. With the microspheres (and with correcting for microsphere only count), counts are overestimated as the cell to microsphere ratio decreases. No warning sign is give for the 1:64 and 1:128 dilutions although clearly the counts are inaccurate.

## Fluidlab R-300 Evaluation

### Summary of Results

The Fluidlab R-300 and Acella slides were evaluated for **Cell Count** accuracy, reproducibility, and challenged with a T cell application which uses hydrogel microspheres. In addition, ease of setup and software download and wireless connection were examined.

The Cell Count application was found to be accurate for cell counts routinely observed at Cellaria using 9 cell lines, 4 cell types, and cells from human, chicken, and pig.

Reproducibility of repeated counts on one slide (intra) and across slides looks good.

For T cells, at a high Cell:Microbead ratio, the instrument did well with or without beads. As this ratio decreases, the instrument overestimated the cell count. This is a challenging data set as the microbeads are not uniform in size or shape. But, it would be fantastic if we could use the instrument for this application.

**Overall, the Fluidlab R-300 was found to be easy to setup and use, accurate, and reproducible using the Cell Count feature.**