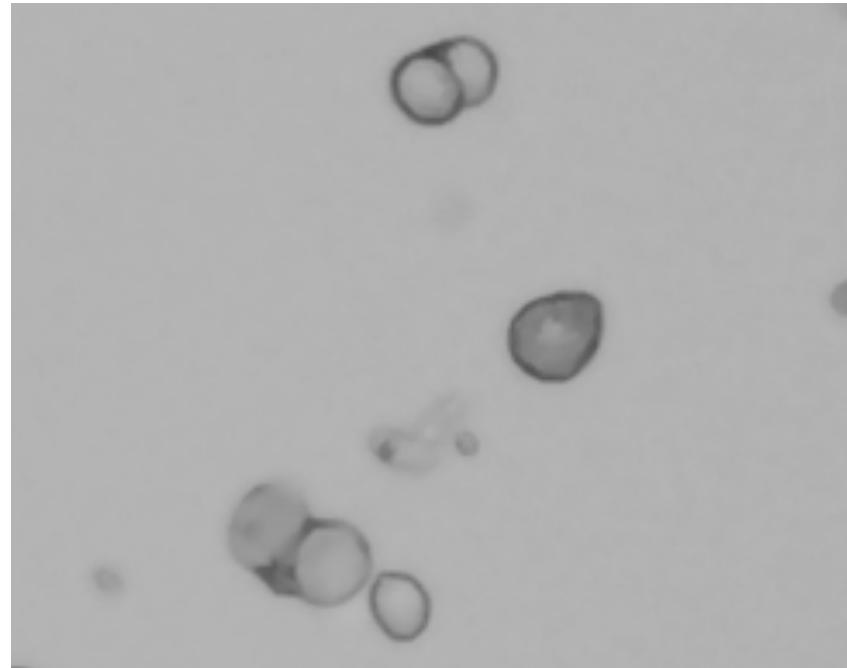


Cell preparation for 10X



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Overview



- Single cell isolation.
- Removing dead cells.
- Cell counting.
- Sample delivery / loading.

Cell isolation



- Cell isolation guides available at:

<https://www.support.10xgenomics.com/single-cell-gene-expression/sample-prep/>.

—Preparation depends on cell type.

▼ Demonstrated Protocol

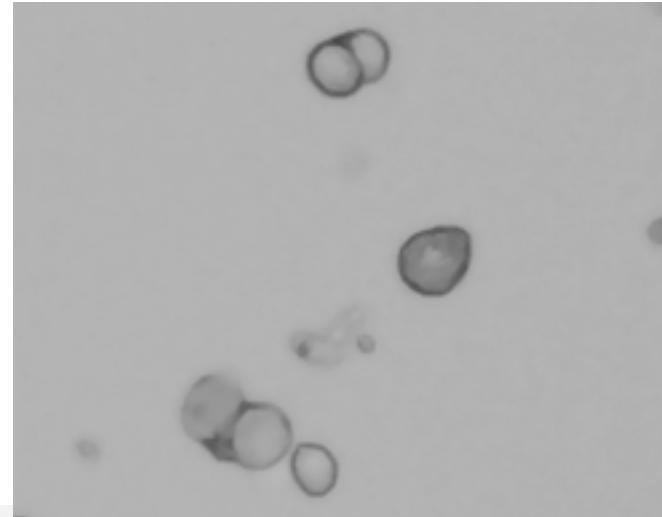
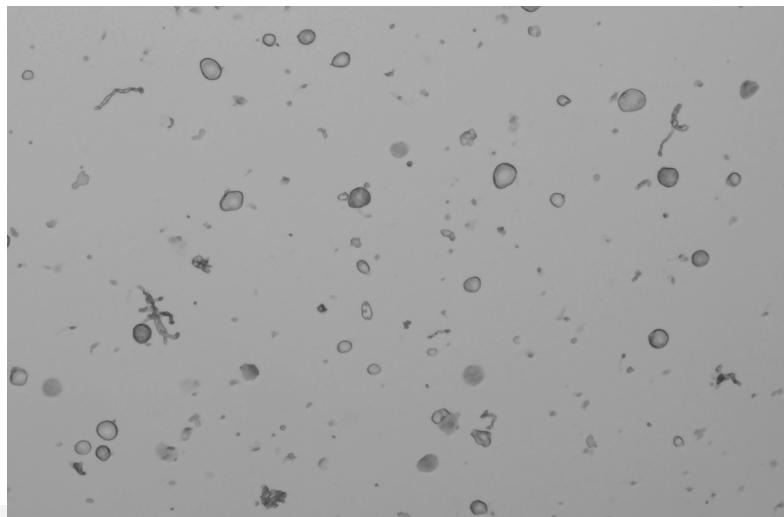
- Isolation of Nuclei for Single Cell RNA Sequencing
- Single Cell Protocols - Cell Preparation Guide
- Enrichment of CD3+ T Cells from Dissociated Tissues for Single Cell RNA Sequencing and Immune Repertoire Profiling
- Single Cell Suspensions from Cultured Cell Lines for Single Cell RNA Sequencing
- Removal of Dead Cells from Single Cell Suspensions for Single Cell RNA Sequencing
- Moss Protoplast Suspension for Single Cell RNA Sequencing
- Fresh Frozen Human-Mouse Cell Line Mixtures for Single Cell RNA Sequencing
- Fresh Frozen Human Peripheral Blood Mononuclear Cells for Single Cell RNA Sequencing
- Dissociation of Mouse Embryonic Neural Tissue for Single Cell RNA Sequencing

Factors influencing SC success

- Cell viability and counting.
 - High viability.
 - Cell concentration impacts.
 - Time from isolation to library preparation.

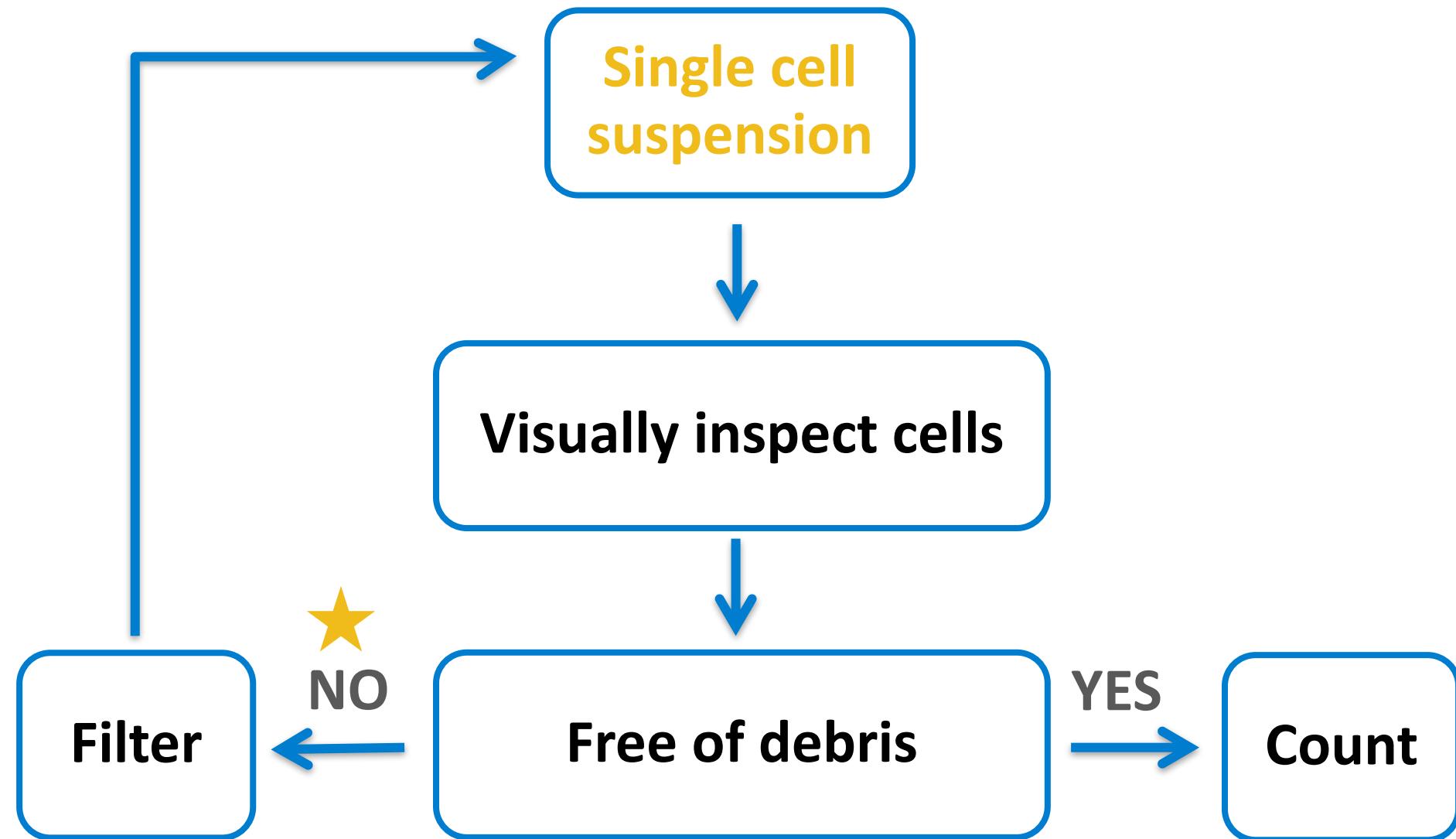


- Cell debris and aggregates.



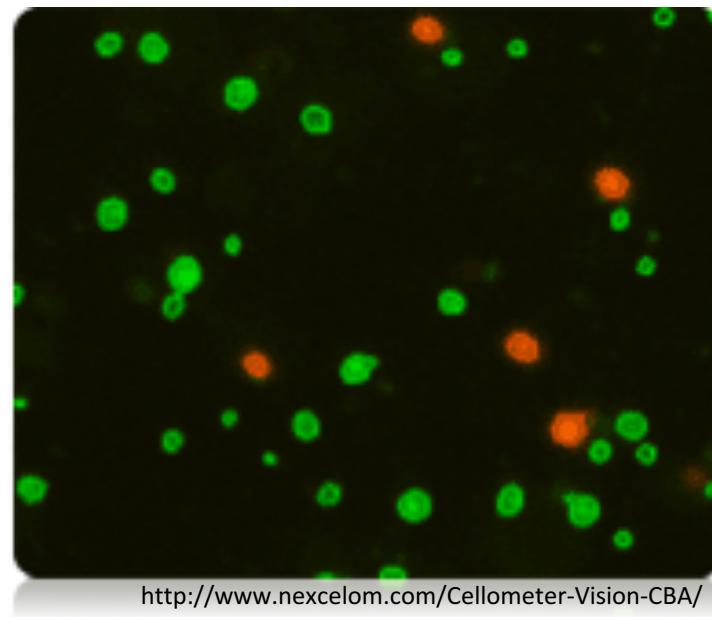
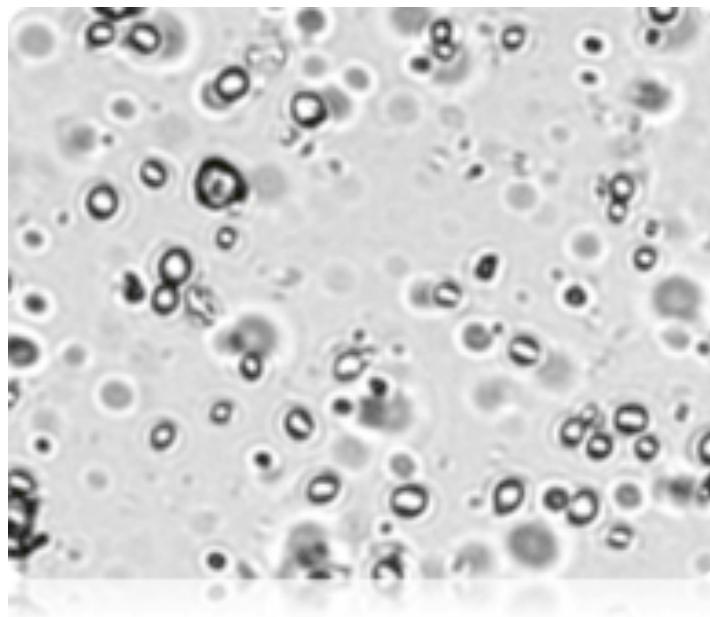
Bad for any
single cell
experiment.

Workflow



Cell debris

- Organic matter left over from dead cells.
- Impacts targeted cell recovery.
 - Free RNA → noise.
 - Hard to obtain cell counts.



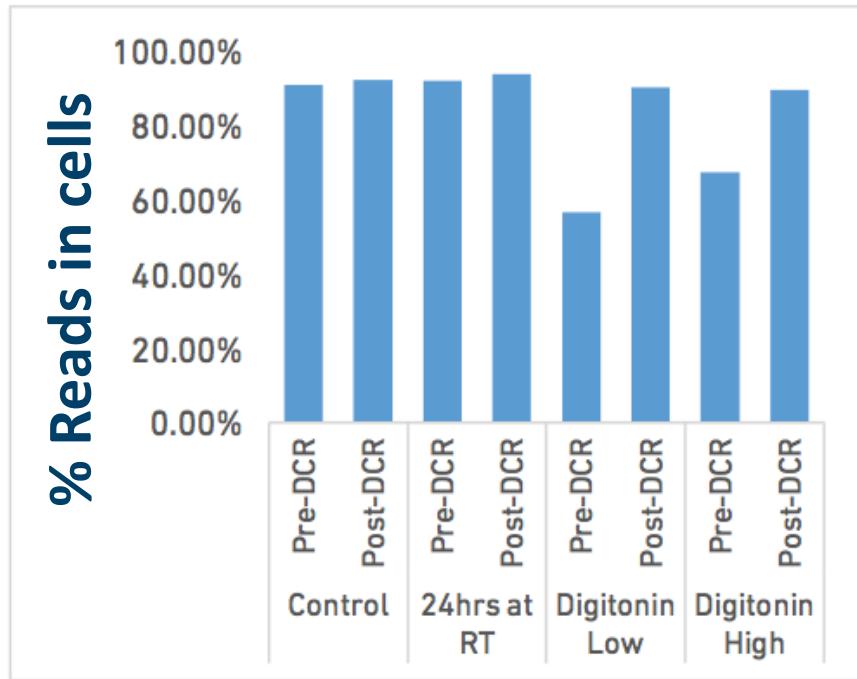
<http://www.nexcelom.com/Celldometer-Vision-CBA/>

★ 10X recommends Flowmi™ Cell Strainer or - MACS® SmartStrainer. Also washes / detergents.

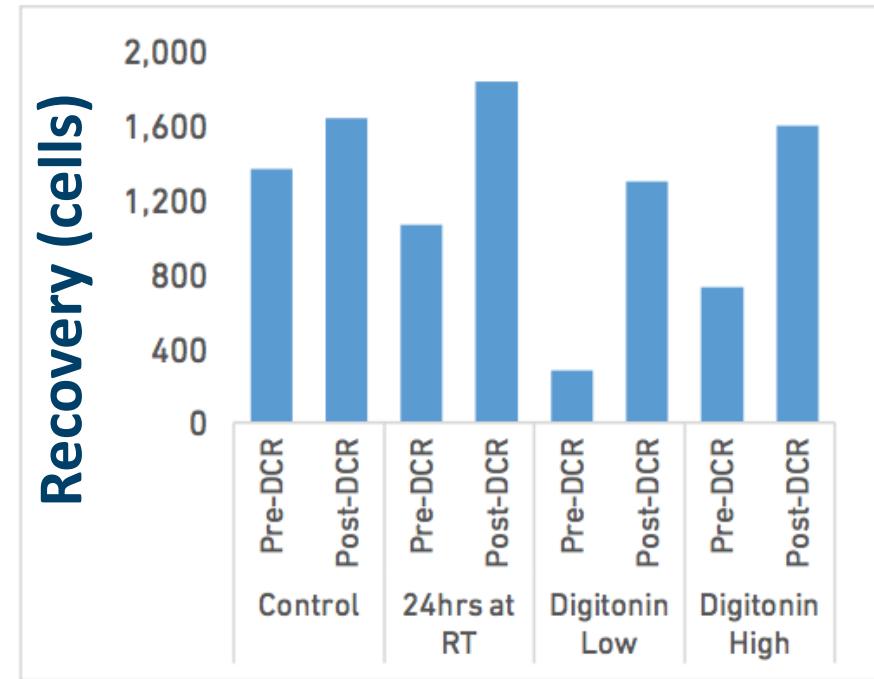
Is a clean suspension necessary?

10X
GENOMICS™

- Digitonin treated cell suspension.



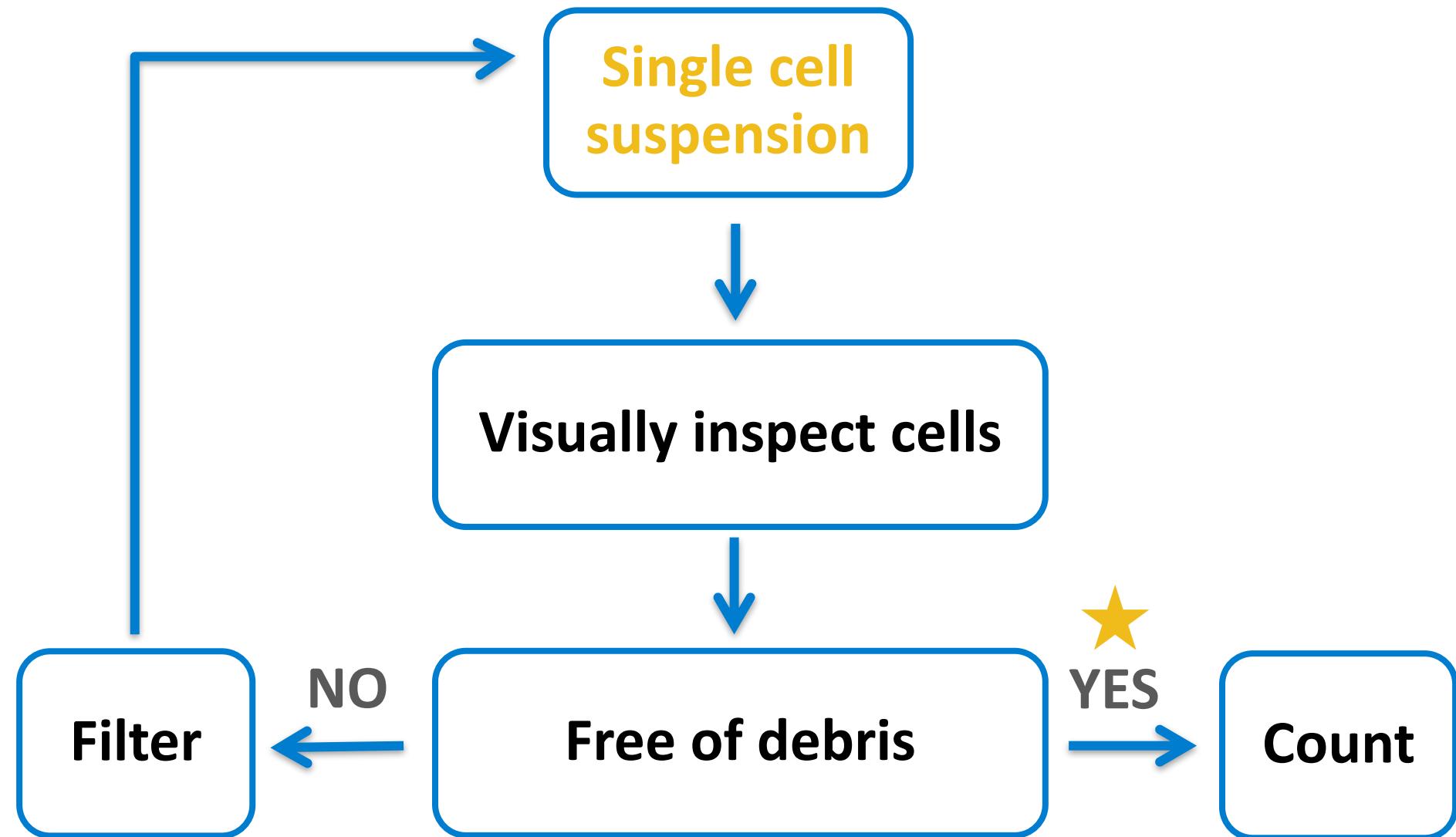
↑ Reads in cells.



↑ Targeted recovery.

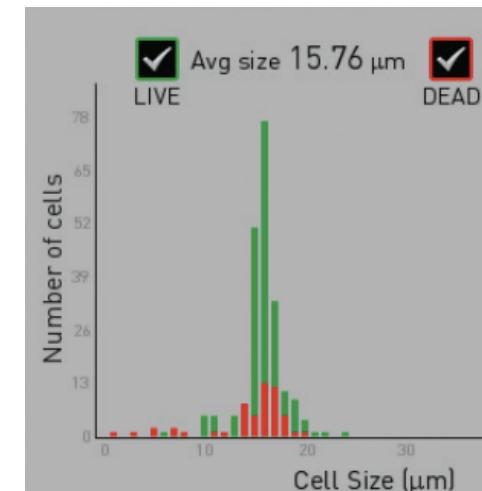


Workflow



Cell counting – automated

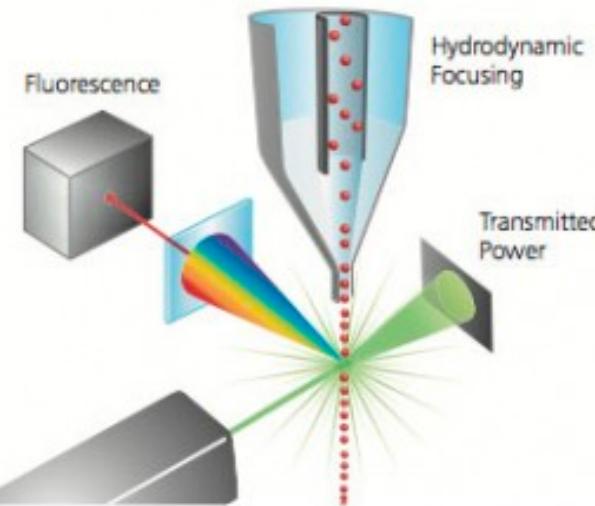
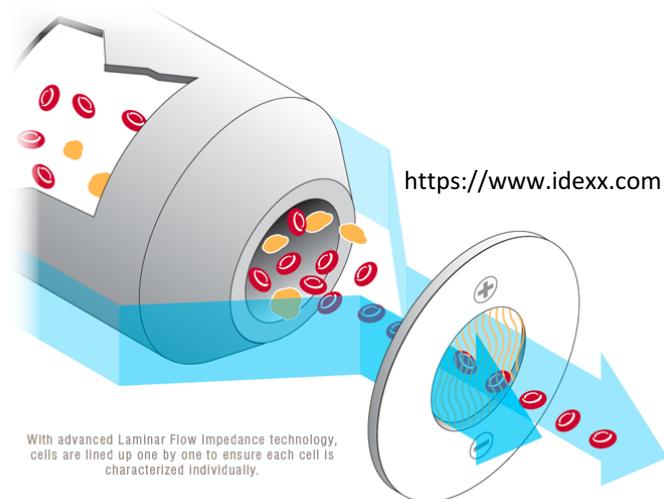
- Countess II (done by DNA Tech).



- Pros (+):
 - Fast.
 - Live/dead cell counts.
 - Cell size estimates.
- Cons (-):
 - Cell size limits (4-60um).
 - Performance poor for odd shapes.

Cell counting – automated

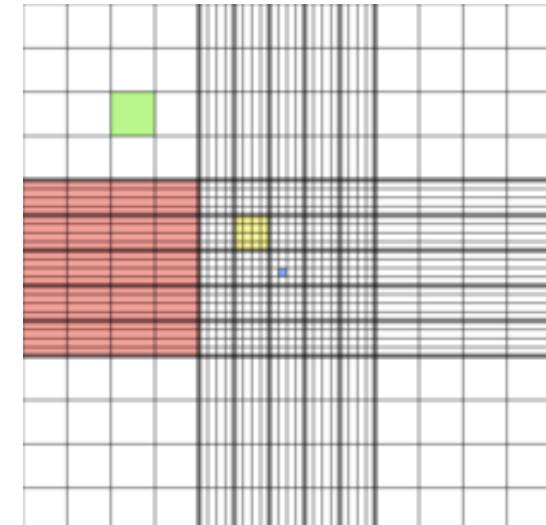
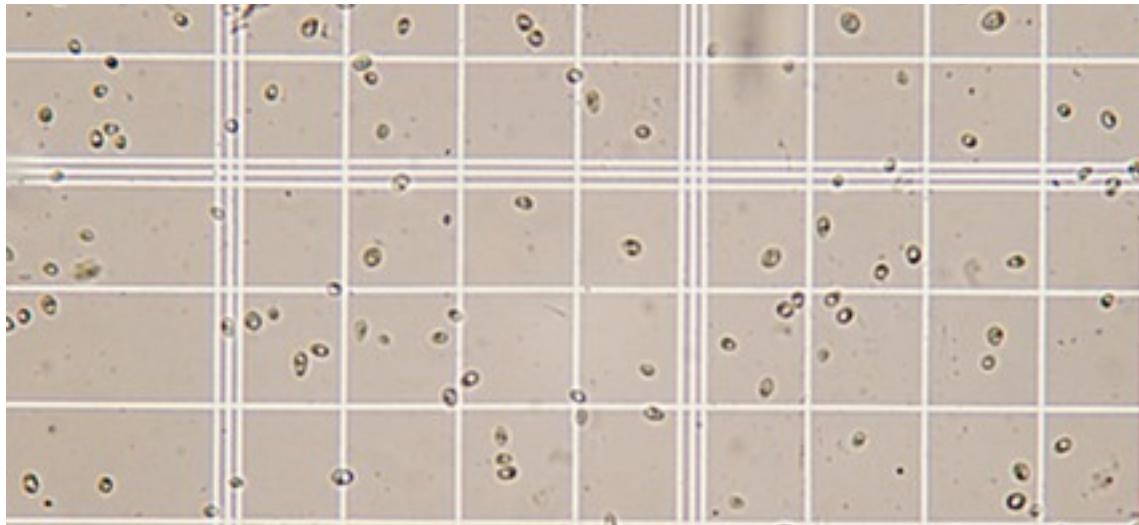
- Flow cytometry (Flow Core)



- Pros (+):
 - Fast.
 - Sort based on characteristics (fluorescence, etc).
 - Characterize cells.
- Cons (-):
 - Cannot provide absolute counts.
 - Lose a lot of input cells.

Cell counting – manual

- Hemocytometer (customer-supplied counts).



<http://www.wetnewf.org/pdfs/hemocytometer.html>

- Pros (+):
 - Reliable cell counts.
- Cons (-):
 - Slow...
 - In this time cells can die or aggregate in your suspension.

Important metrics – 10X

- Concentration: 100-2,000 cells per μl .
 - **700-1,200 cells per μl .**
 - Count in replicates!
 - We require cell counts prior to delivery.
- Viability: **70% minimum.**
 - But we'll load less.
- Sample buffer.
- Cell size and shape.
 - Chip channel is <100um.
- Treat cells gently.
 - Wide bore pipette tips, keep cells on ice.



Sample delivery – 10X

- What:
 - Counted cells at the appropriate concentration.
 - Fresh cell suspension best.
- When:
 - Before 2:00 PM Monday – Thursday.
 - Please contact dnatech@ucdavis.edu 1-2 weeks before delivery.
- Things to consider:
 - Only work with one project per day.
 - Receive up to 8 samples.

Chip loading

- Very flexible → cell concentration and recovery.

Cell Stock Concentration (Cells/µl)	Targeted Cell Recovery										
	500 cells	1000 cells	2000 cells	3000 cells	4000 cells	5000 cells	6000 cells	7000 cells	8000 cells	9000 cells	10000 cells
100	8.7 25.1	17.4 16.4	n/a								
200	4.4 29.5	8.7 25.1	17.4 16.4	26.1 7.7	n/a						
300	2.9 30.9	5.8 28.0	11.6 22.2	17.4 16.4	23.2 10.6	29.0 4.8	n/a	n/a	n/a	n/a	n/a
400	2.2 31.6	4.4 29.5	8.7 25.1	13.1 20.8	17.4 16.4	21.8 12.1	26.1 7.7	30.5 3.4	n/a	n/a	n/a
500	1.7 32.1	3.5 30.3	7.0 26.8	10.4 23.4	13.9 19.9	17.4 16.4	20.9 12.9	24.4 9.4	27.8 6.0	31.3 2.5	n/a
600	1.5 32.4	2.9 30.9	5.8 28.0	8.7 25.1	11.6 22.2	14.5 19.3	17.4 16.4	20.3 13.5	23.2 10.6	26.1 7.7	29.0 4.8
700	1.2 32.6	2.5 31.3	5.0 28.8	7.5 26.3	9.9 23.9	12.4 21.4	14.9 18.9	17.4 16.4	19.9 13.9	22.4 11.4	24.9 8.9
800	1.1 32.7	2.2 31.6	4.4 29.5	6.5 27.3	8.7 25.1	10.9 22.9	13.1 20.8	15.2 18.6	17.4 16.4	19.6 14.2	21.8 12.1
900	1.0 32.8	1.9 31.9	3.9 29.9	5.8 28.0	7.7 26.1	9.7 24.1	11.6 22.2	13.5 20.3	15.5 18.3	17.4 16.4	19.3 14.5
1000	0.9 32.9	1.7 32.1	3.5 30.3	5.2 28.6	7.0 26.8	8.7 25.1	10.4 23.4	12.2 21.6	13.9 19.9	15.7 18.1	17.4 16.4
1100	0.8 33.0	1.6 32.2	3.2 30.6	4.7 29.1	6.3 27.5	7.9 25.9	9.5 24.3	11.1 22.7	12.7 21.1	14.2 19.6	15.8 18.0
1200	0.7 33.1	1.5 32.4	2.9 30.9	4.4 29.5	5.8 28.0	7.3 26.6	8.7 25.1	10.2 23.7	11.6 22.2	13.1 20.8	14.5 19.3
	0.7	1.2	2.7	4.0	5.4	6.7	8.0	9.4	10.7	12.0	12.4

Cost – 2017 10X Single Cell

Library prep	Total Cost
1 sample	\$2,072
2 samples	\$3,909
3 samples	\$5,746



Per prep day	Cost
Labor, cell QC, chip	\$235
Labor, reagents, instrument use	\$1,837

Important resources

- 10X Genomics
 - <https://support.10xgenomics.com/single-cell-gene-expression>
- UC Davis Flow Cytometry
 - http://www.ucdmc.ucdavis.edu/pathology/research/research_labs/flow_cytometry/index.html
 - Bridget McLaughlin (Technical Director)
- UC Davis DNA Technology Core
 - <http://dnatech.genomecenter.ucdavis.edu/single-cell-analyses/>

Acknowledgements

- UC Davis DNA Technology Core.
- UC Davis Bioinformatics Core.
- 10X Genomics.
 - Nicole Rapicavoli (Lead Field Applications Scientist).

