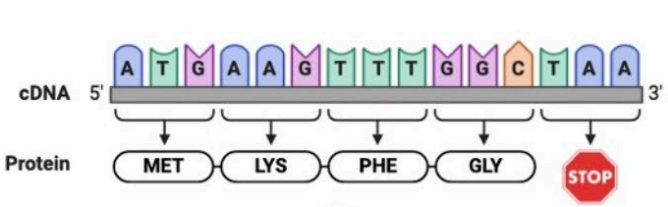
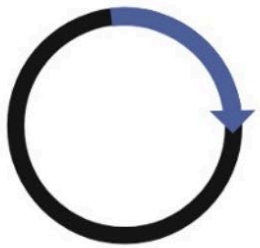
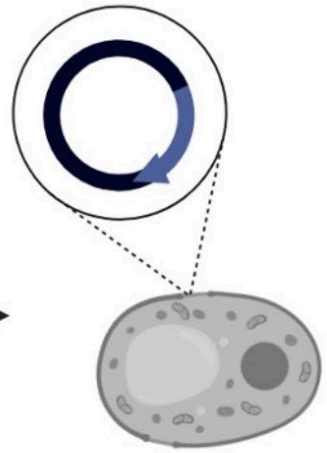
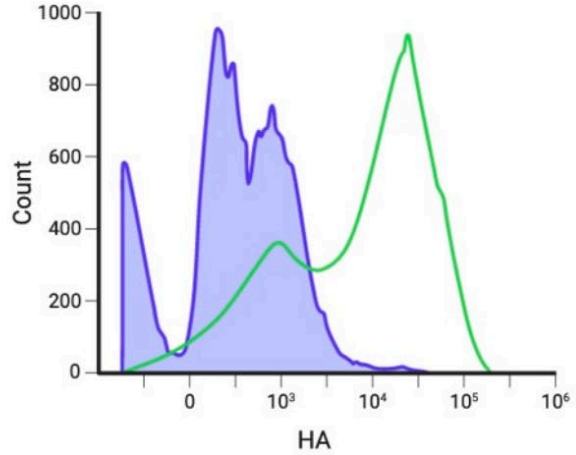
Flow Cytometry Pre-Lab

4/10/2024





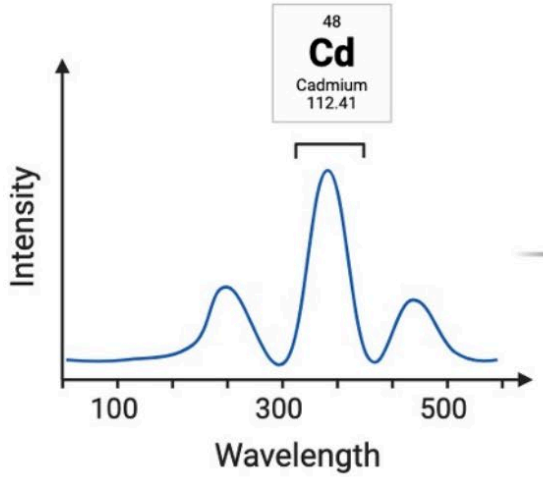


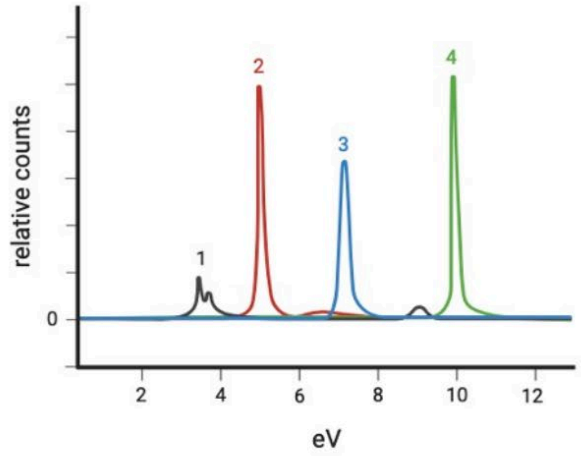


D1&D2: Insert DNA sequence for YSD peptide

D3: M17 yeast transformation with plasmid

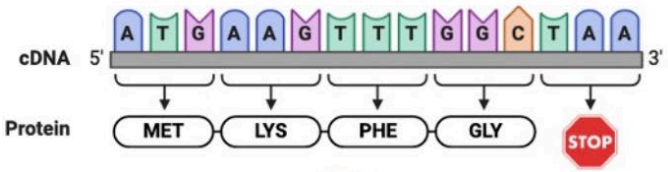
D5: Flow cytometry confirmation of cell surface expression

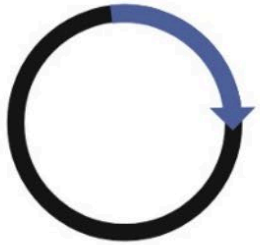




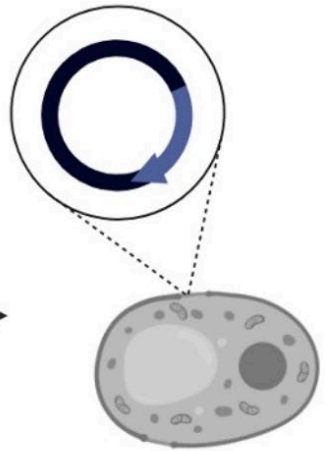
D6: ICP-OES analysis of heavy metal uptake

D7: Examine CdS sequestration pattern and fluorescence

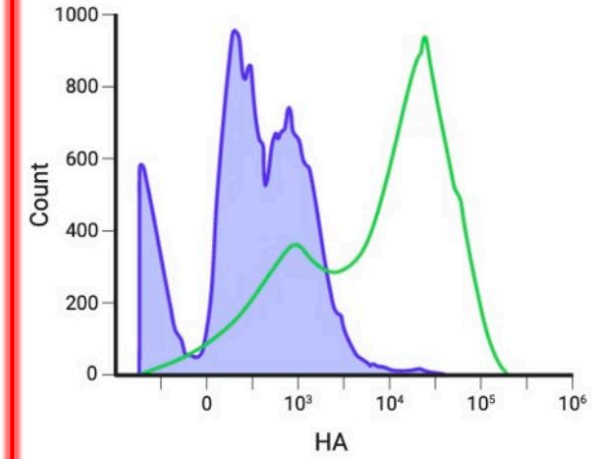




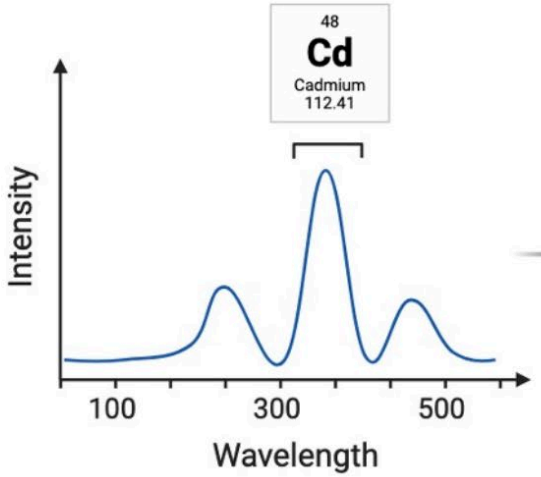
D1&D2: Insert DNA sequence for YSD peptide



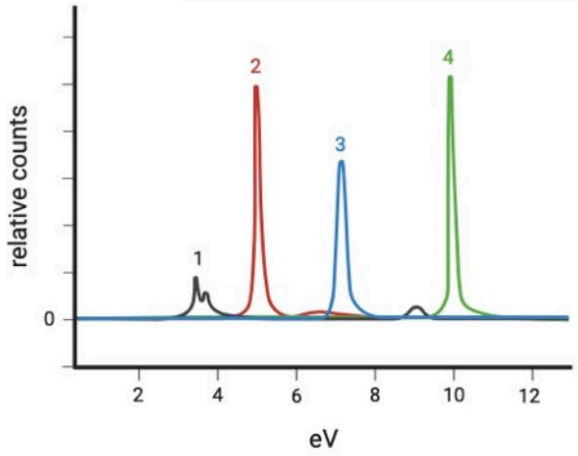
D3: M17 yeast transformation with plasmid



D5: Flow cytometry confirmation of cell surface expression

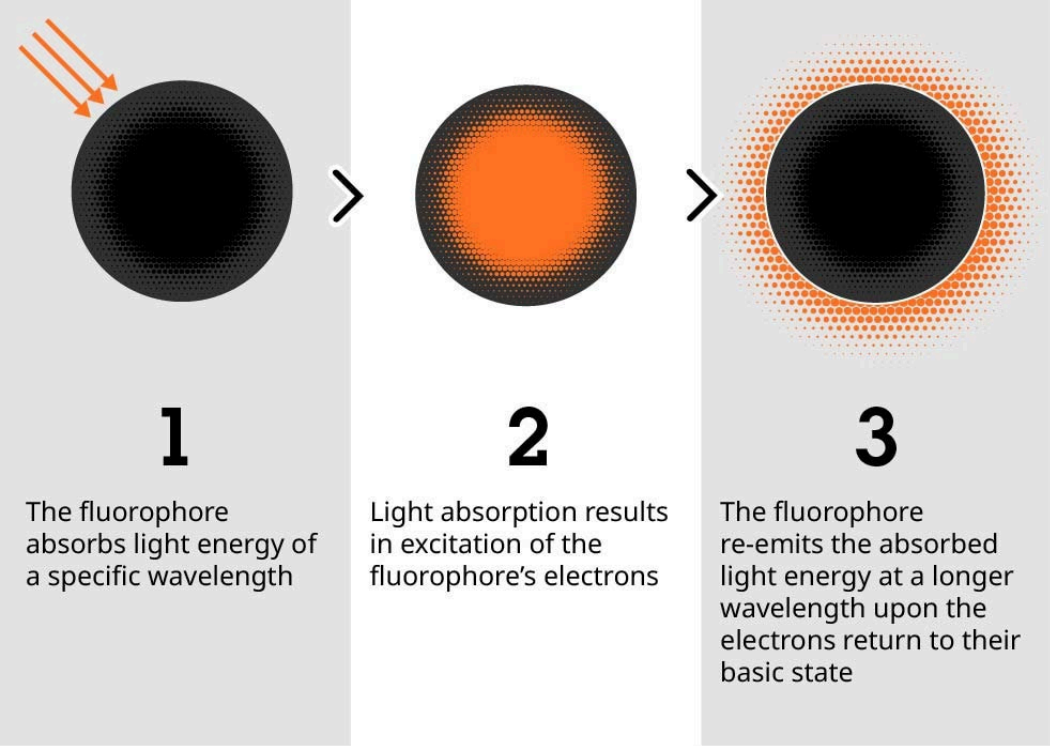


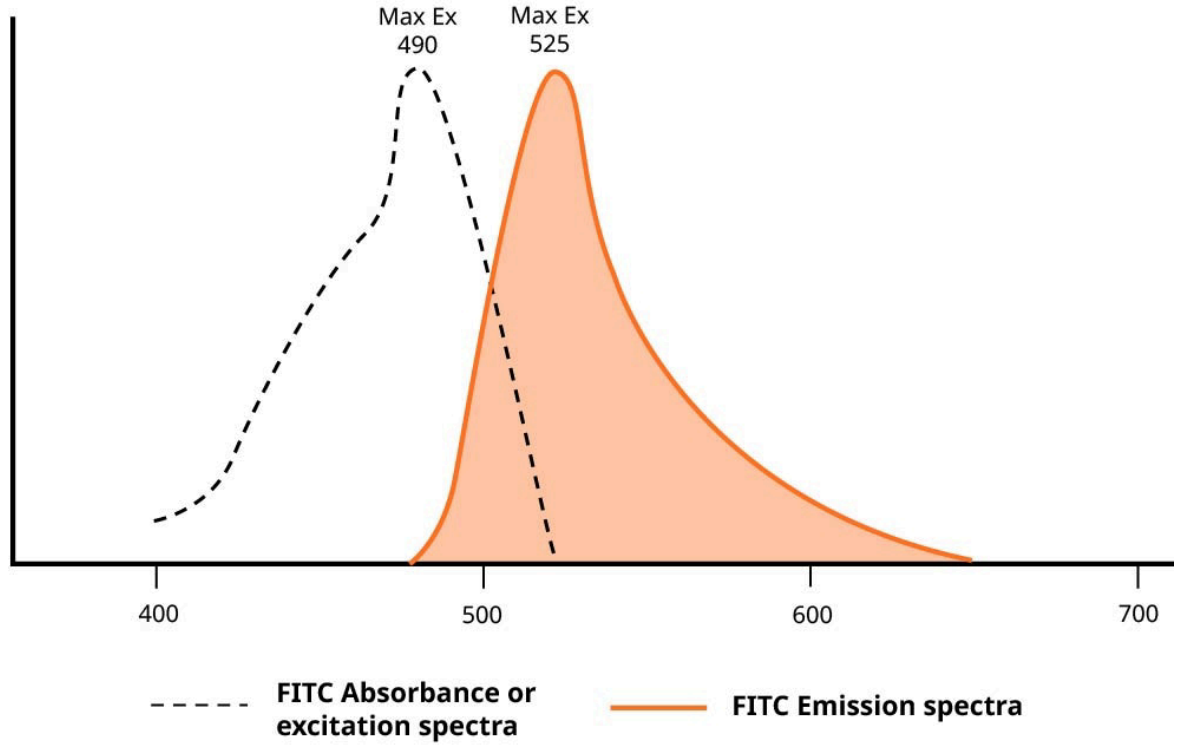
D6: ICP-OES analysis of heavy metal uptake



D7: Examine CdS sequestration pattern and fluorescence

Fluorescence



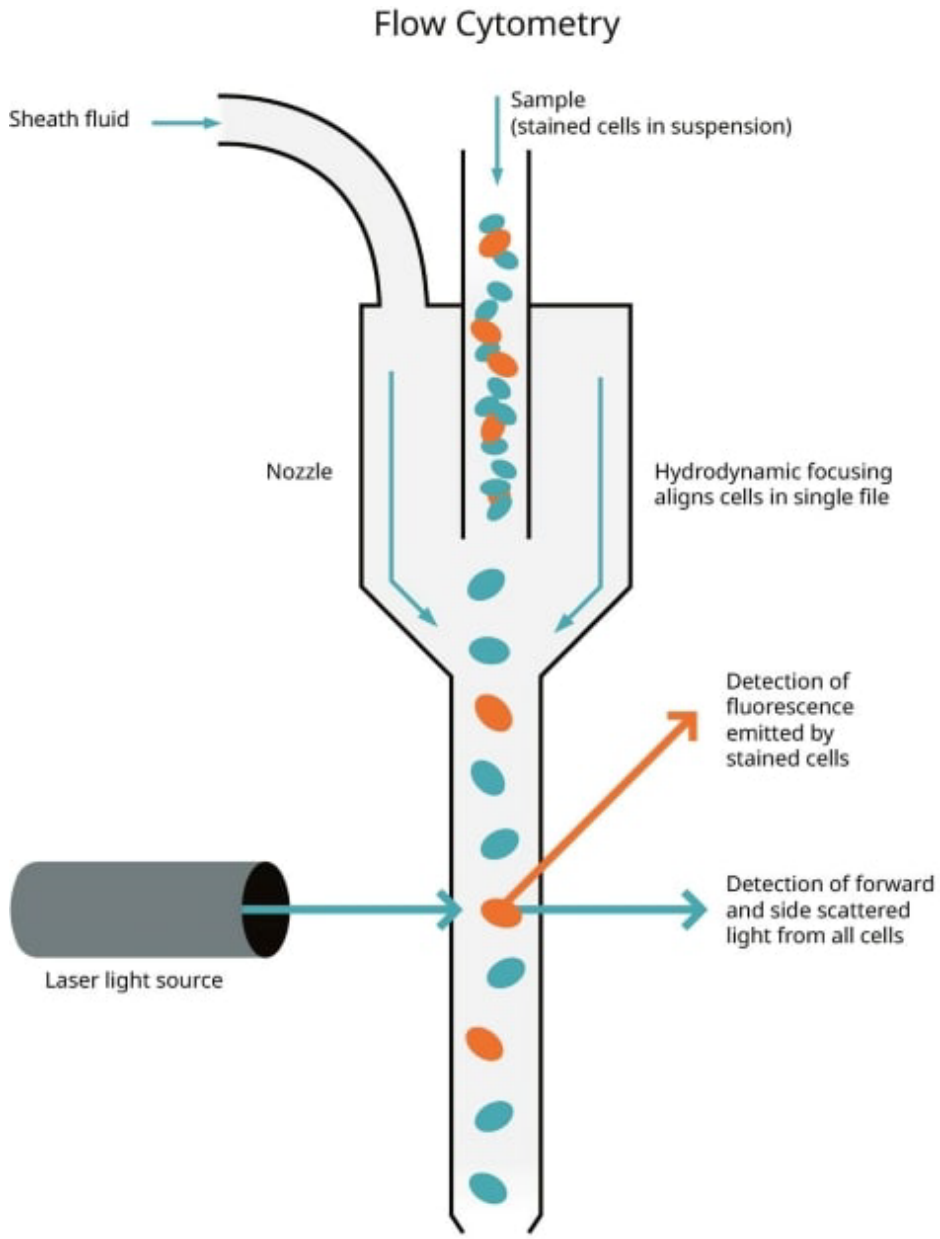


https://www.abcam.com/protocols/introduction-to-flow-cytometry

Principle

Analyze cells based on physical and fluorescent qualities

1. Sample entry and hydrodynamic focusing
2. Event recording
3. Mirrors and optical filters



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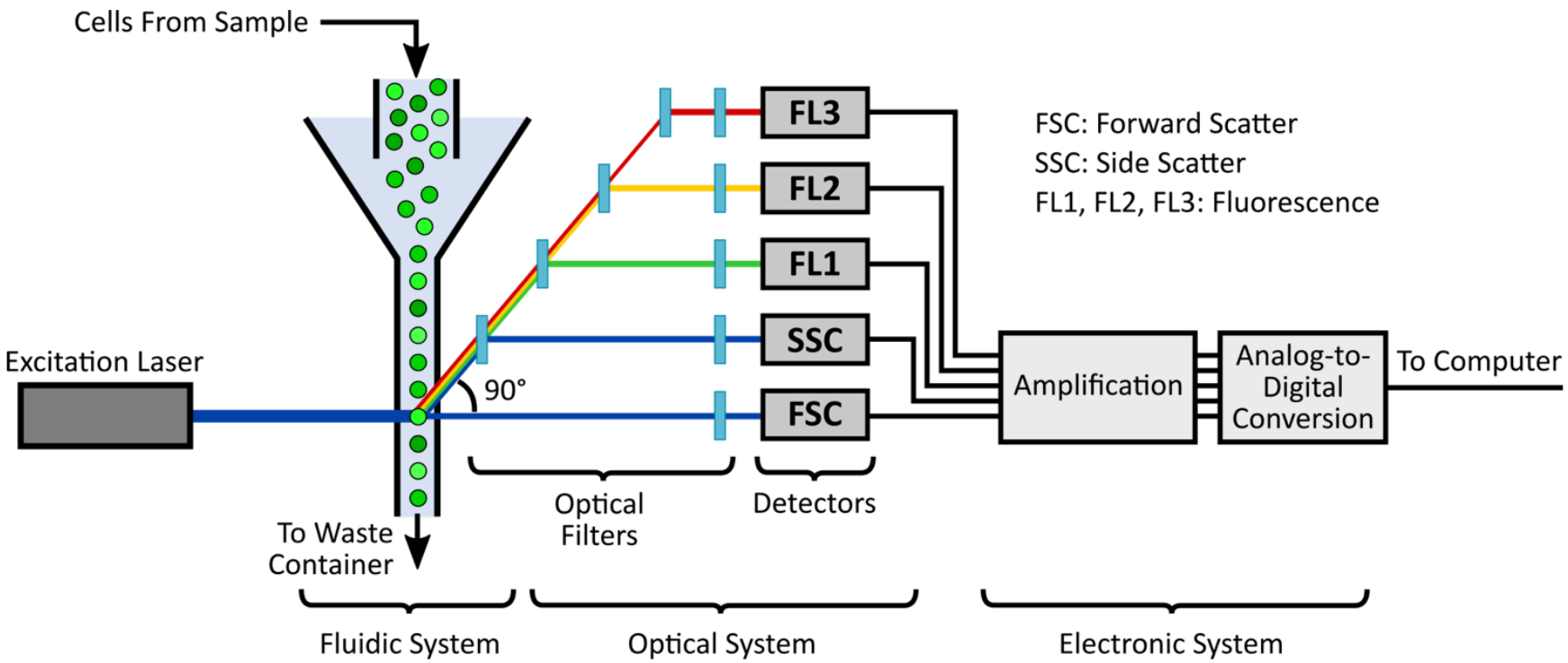
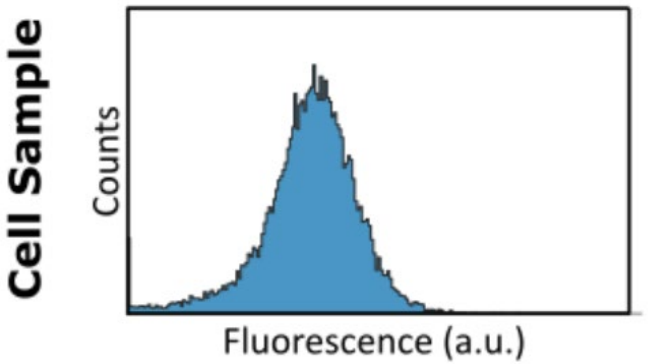
Instrumentation





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Sample Preparation

- Endogenous fluorescence vs. stained fluorescence

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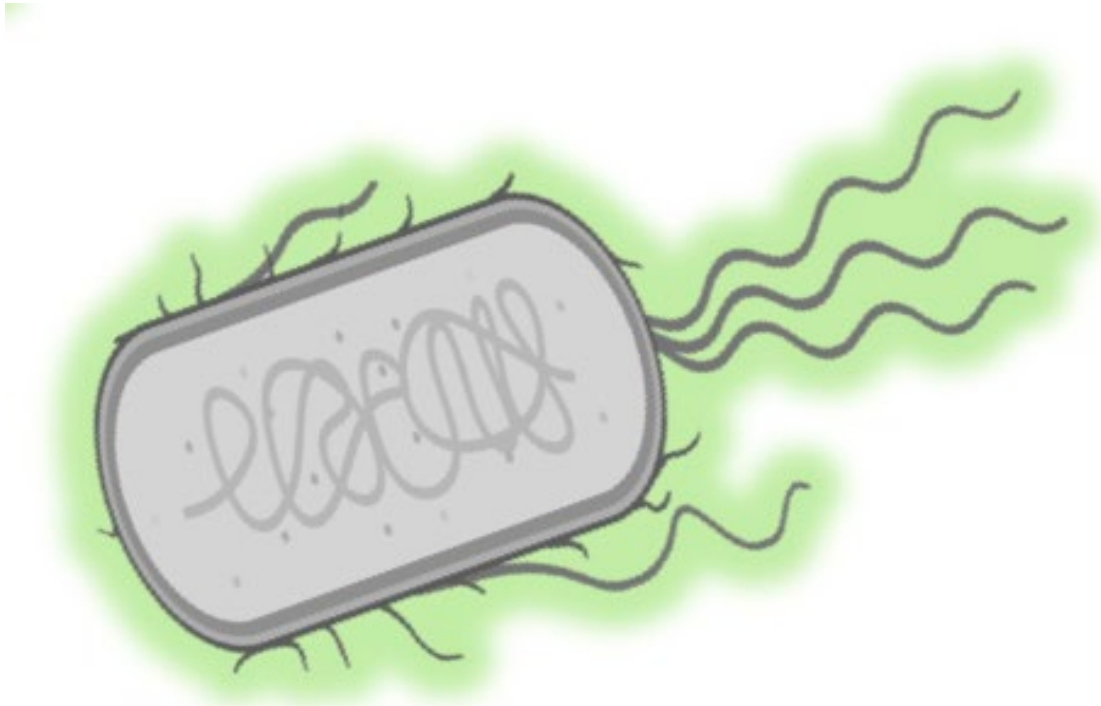
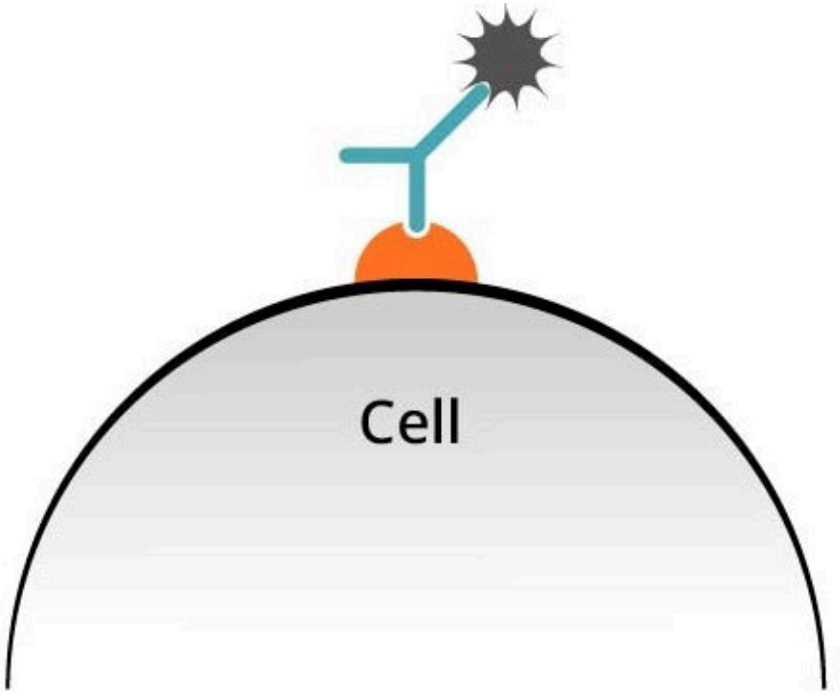
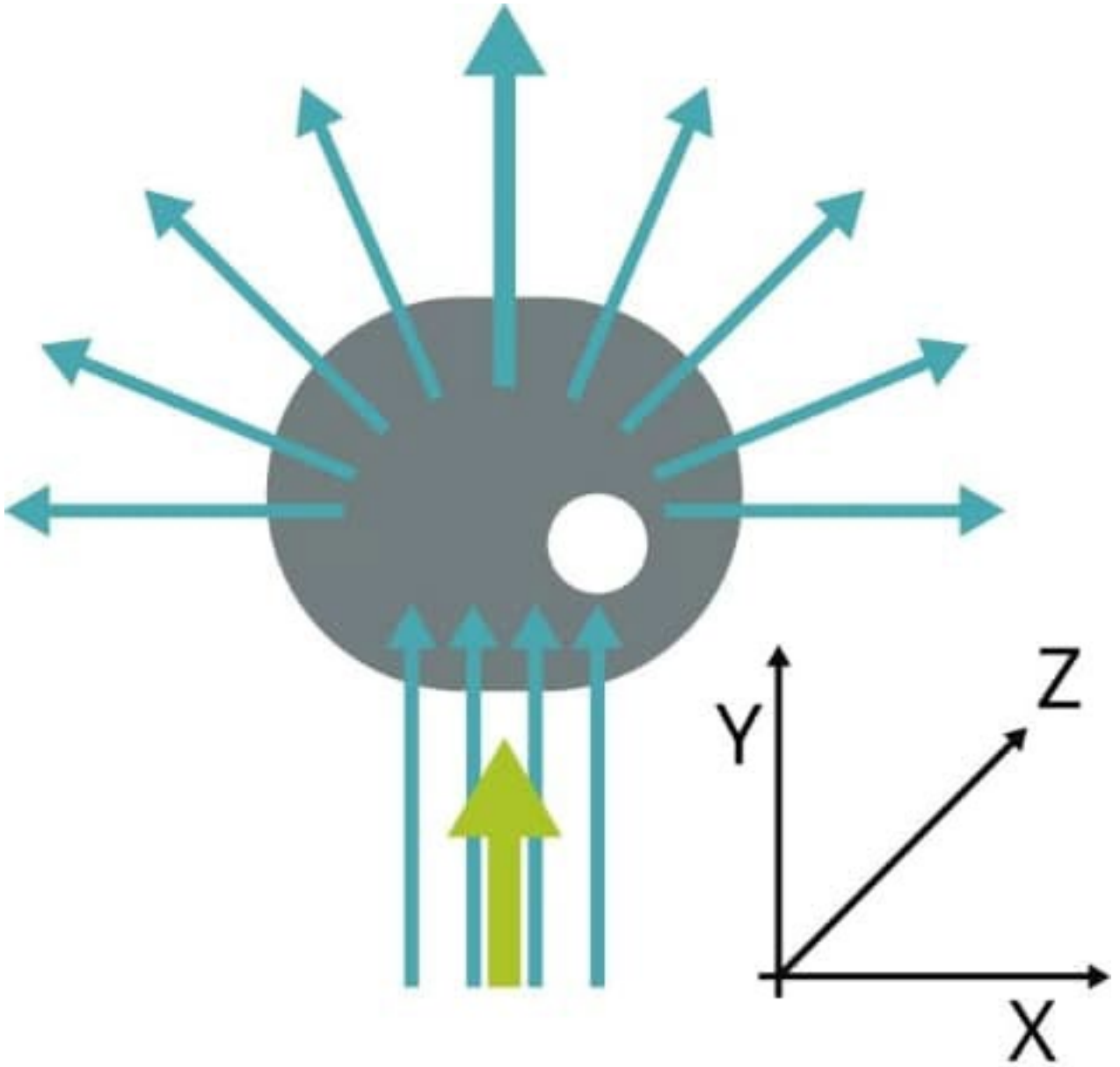




Analysis

- Light scatters as a laser interrogates the cell
- Direction of light scattered correlates to size and granularity



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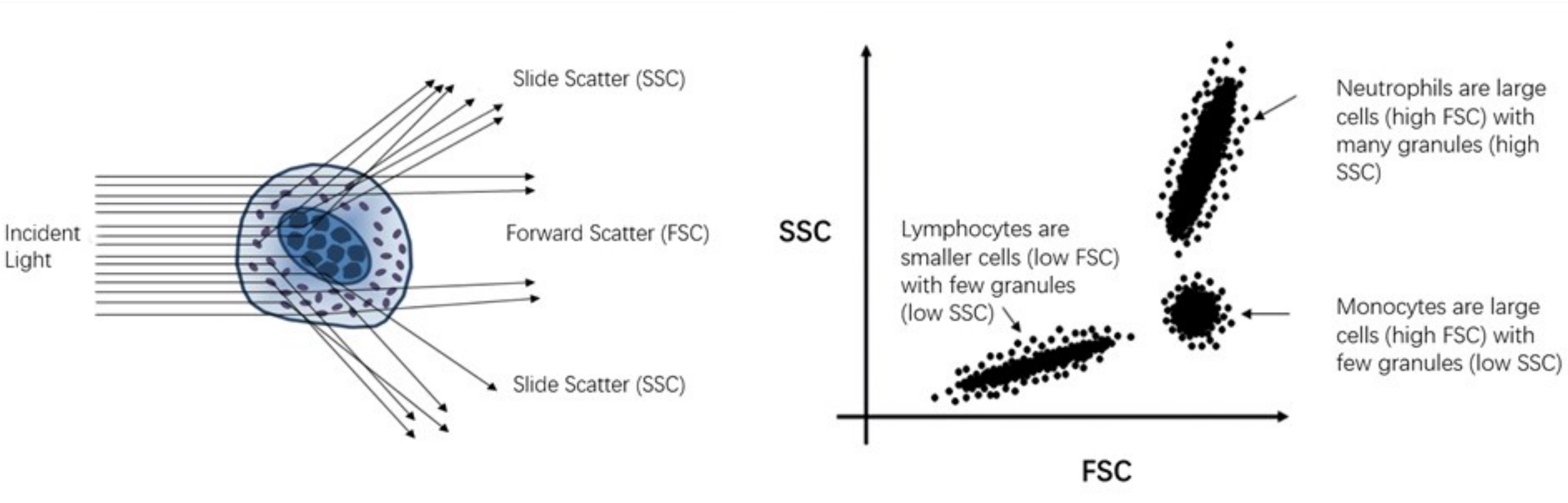
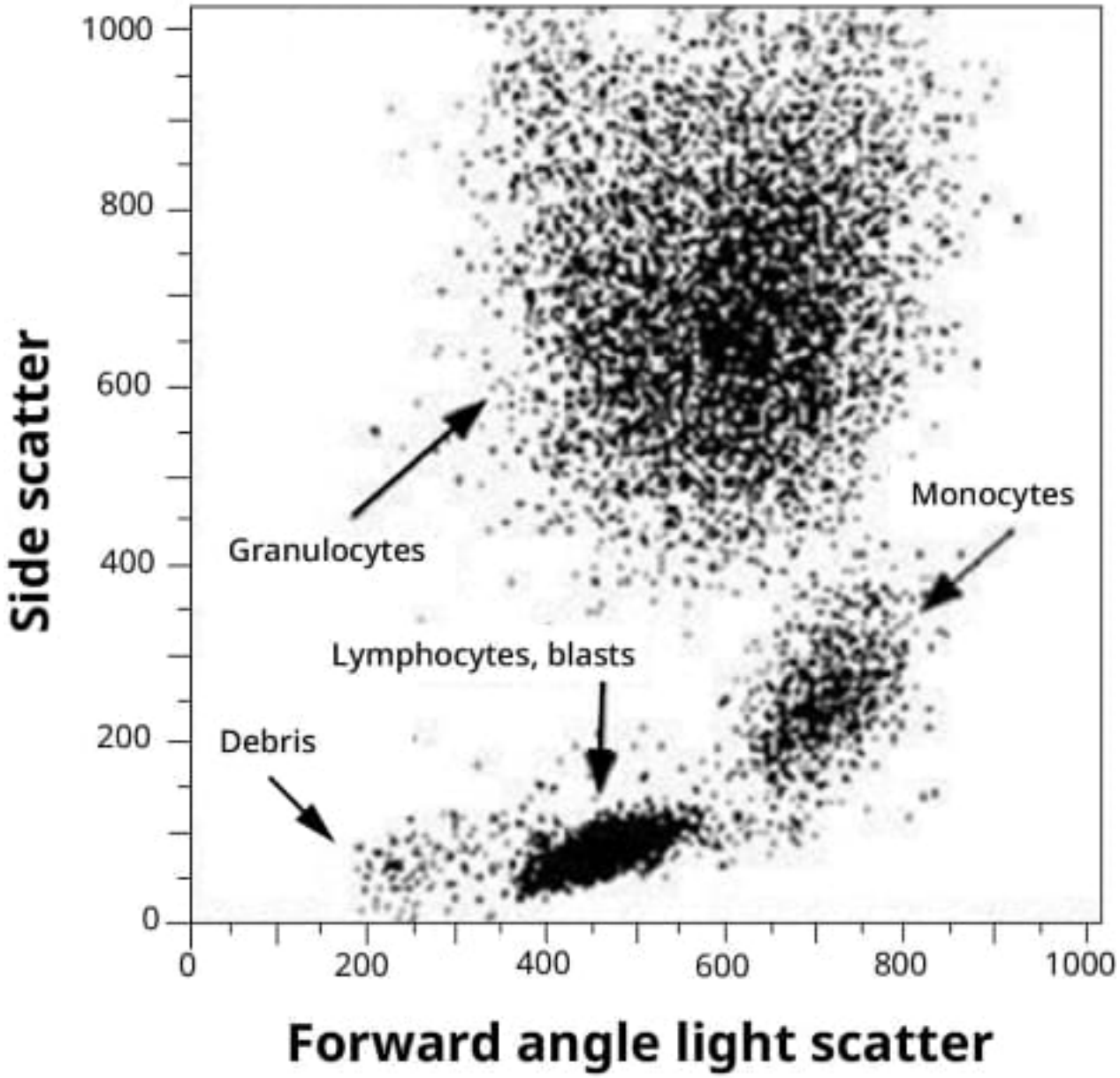
Analysis



https://www.antibody-creativebiolabs.com/flow-cytometry.htm

Analysis

- Dot plot of FS versus SS
- Each dot = single cell
- Differences in cell size and granularity determine position of cell population



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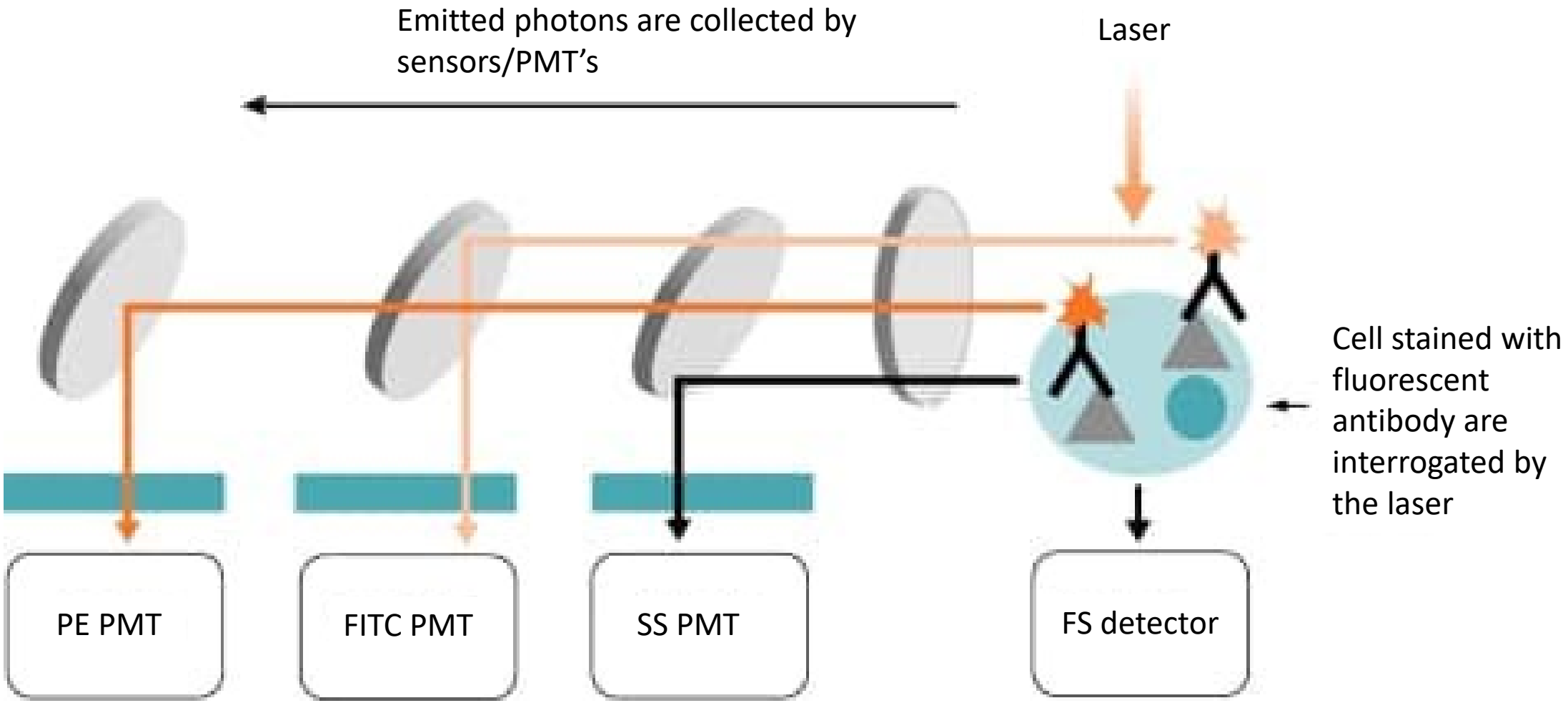
Parameters



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Data Analysis

	FITC-A	FSC-A	FSC-H	FSC-W	Omit	PE-Texas Red-A	SSC-A	SSC-H	SSC-W	Time	Zinc	cells	col	name	nu
0	950.950012	1531.139893	2774.0	36173.316406	N	-11.520000	7805.070312	8082.0	63290.410156	125.000000	0.0	EcN	1	RBS_1	
1	992.810059	1551.419922	2590.0	39256.316406	N	-136.080002	8603.140625	9079.0	62101.046875	141.100006	0.0	EcN	1	RBS_1	
2	1342.250000	3935.099854	2256.0	114313.257812	N	-84.240005	7039.760254	7688.0	60010.109375	277.600006	0.0	EcN	1	RBS_1	
3	831.740051	213.719986	2060.0	6799.201172	N	-98.640007	7786.870117	8357.0	61065.011719	228.500000	0.0	EcN	1	RBS_1	
4	753.480042	1978.859985	2178.0	59543.878906	N	59.760002	3995.810059	4634.0	56510.445312	389.100006	0.0	EcN	1	RBS_1	
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
333993	218.400009	5026.319824	4754.0	69290.054688	N	-89.280006	9083.620117	9422.0	63182.351562	313.100006	500.0	EcN	7	RBS_163	
333994	73.709999	1889.939941	3417.0	36247.910156	N	36.720001	9167.339844	8841.0	67955.070312	456.299988	500.0	EcN	7	RBS_163	
333996	135.589996	2204.280029	2383.0	60620.941406	N	69.840004	13058.500000	13474.0	63515.054688	66.199997	500.0	EcN	7	RBS_163	
333997	14.560000	6188.519531	5954.0	68117.375000	N	-9.360001	21638.890625	21682.0	65405.699219	617.299988	500.0	EcN	7	RBS_163	
333998	126.490005	4346.159668	3379.0	84294.148438	N	-15.840000	10938.200195	11447.0	62623.039062	560.200012	500.0	EcN	7	RBS_163	

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Advantages

- High throughput
- Multiparametric analysis
- Highly sensitive

Limitations

- Specialized instrumentation and training
- Time-consuming sample prep
- Complex data analysis
- Difficulty analyzing autofluorescent or overlapping signals

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For Today

- Prepare yeast for flow cytometry to evaluate peptide expression
- Perform Metal Nucleation assay for ICP-OES

For M2D6

– Methods Section for M2D2-M2D5 (Use your downtime wisely!)