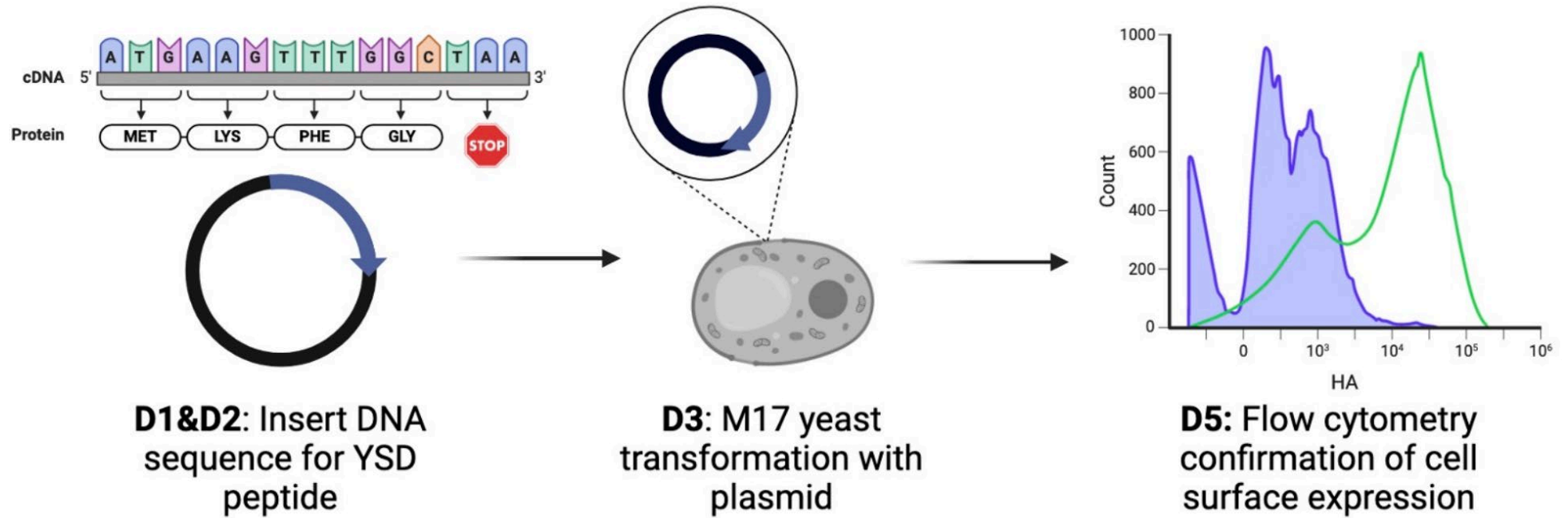# Flow Cytometry Pre-Lab

4/9/2024
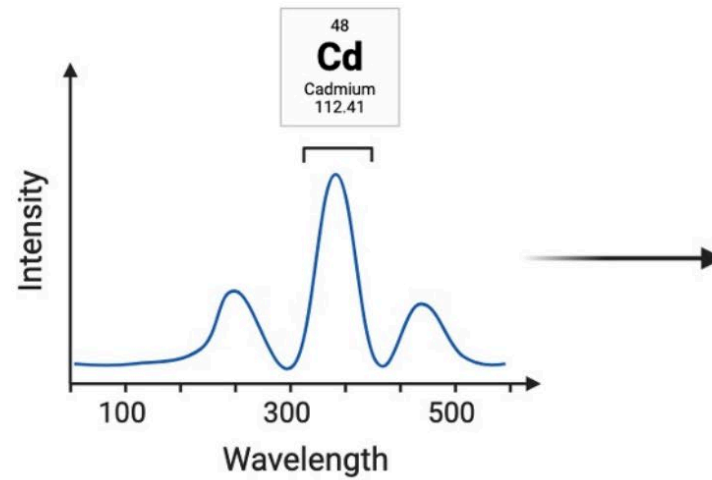
D1&D2: Insert DNA sequence for YSD peptide

D3: M17 yeast transformation with plasmid

D5: Flow cytometry confirmation of cell surface expression

D6: ICP-OES analysis of heavy metal uptake
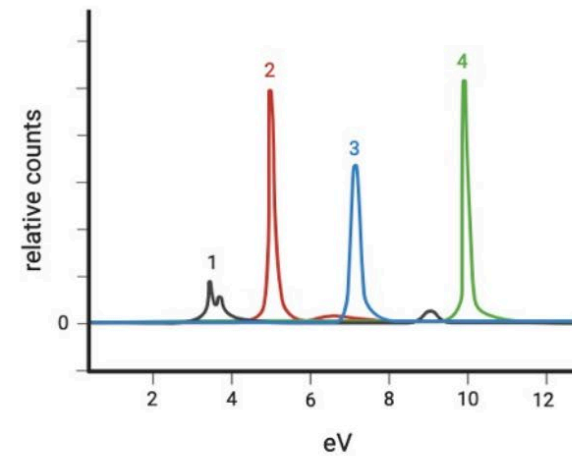
D7: Examine CdS sequestration pattern and fluorescence

D1&D2: Insert DNA sequence for YSD peptide

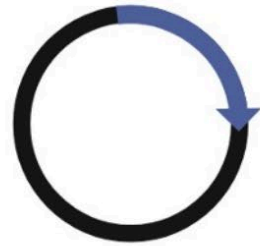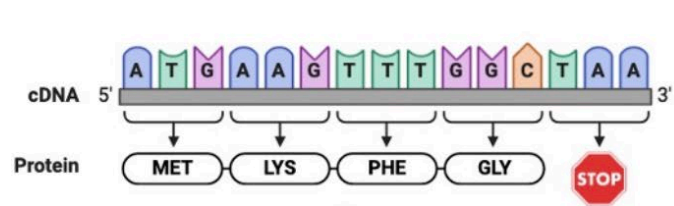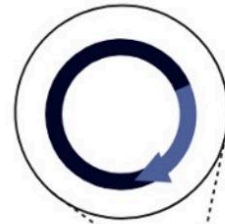**D3**: M17 yeast transformation with plasmid

D5: Flow cytometry confirmation of cell surface expression

D6: ICP-OES analysis of heavy metal uptake

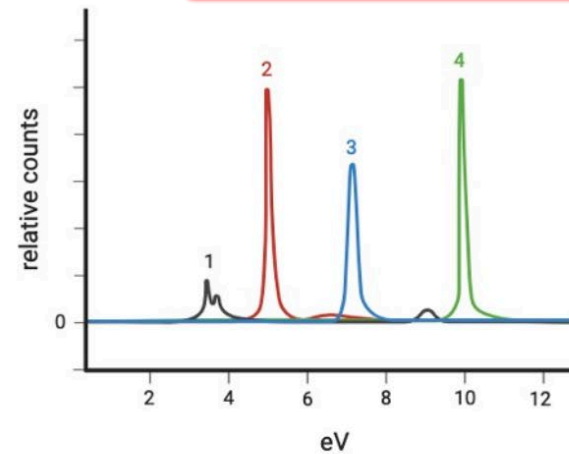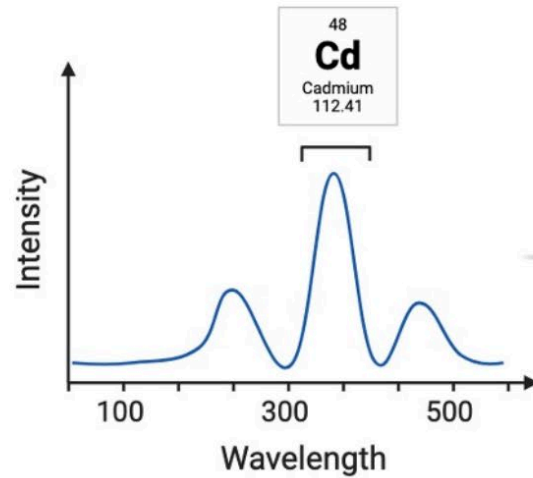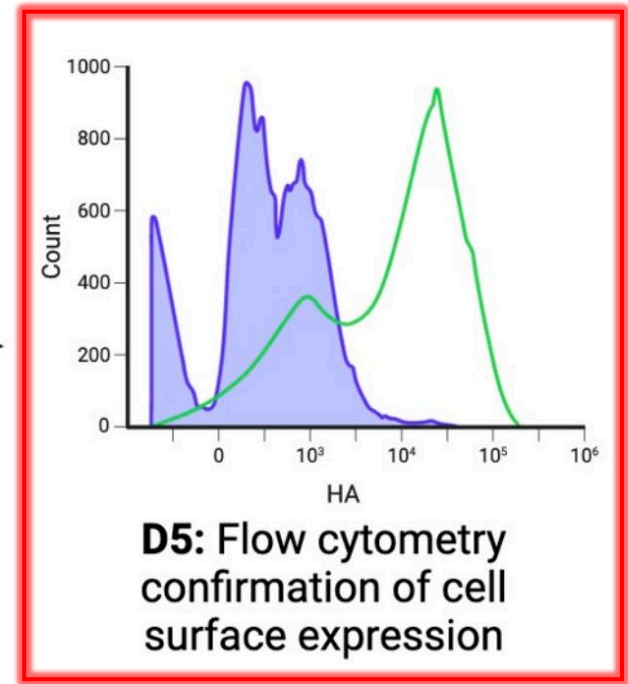**D7**: Examine CdS sequestration pattern and fluorescence
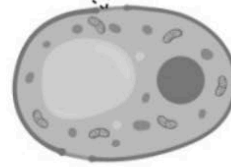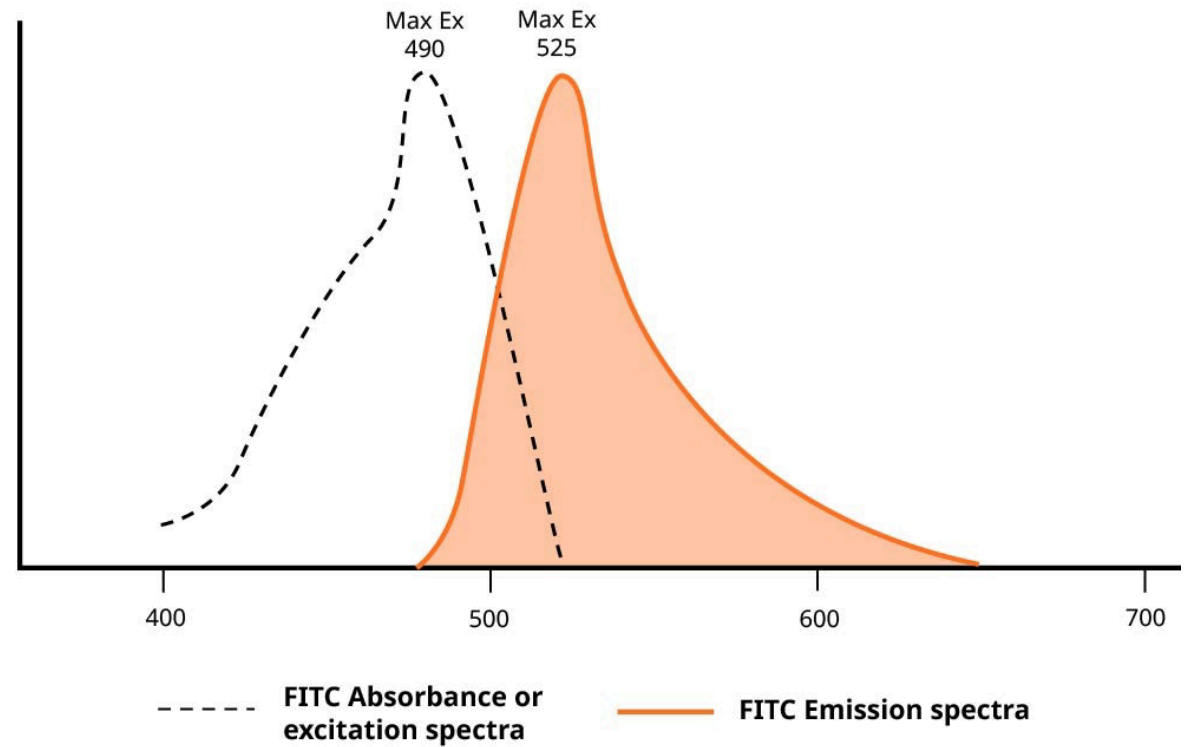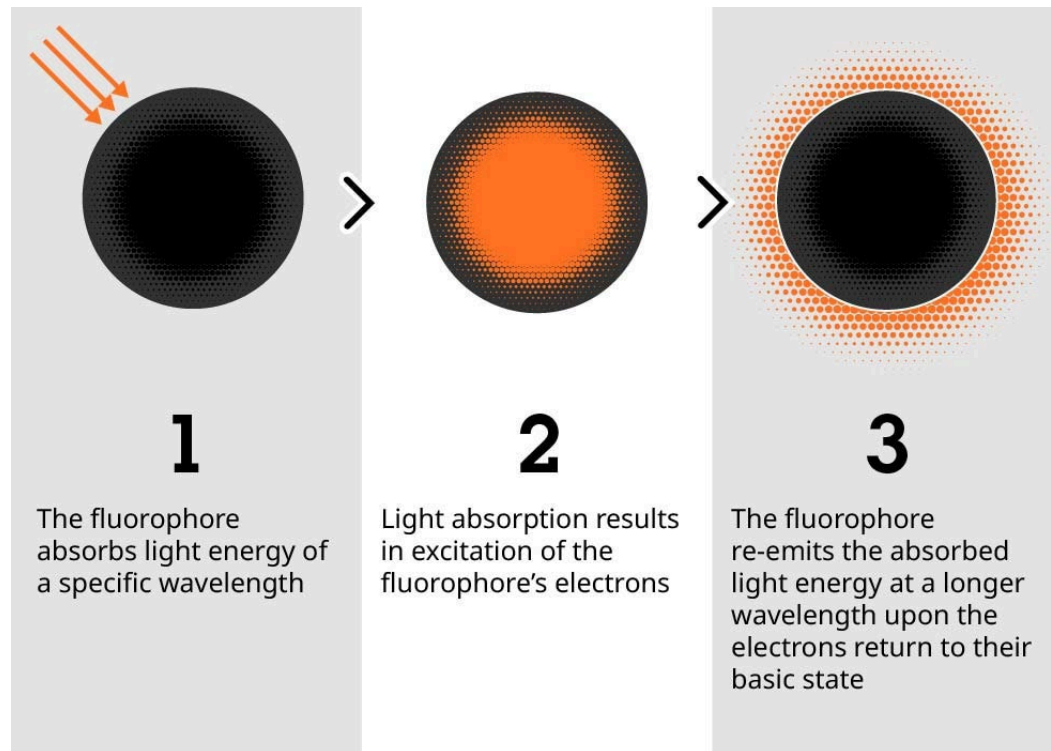
Fluorescence

Principle

Analyze cells based on physical and fluorescent qualities

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

Flow Cytometry

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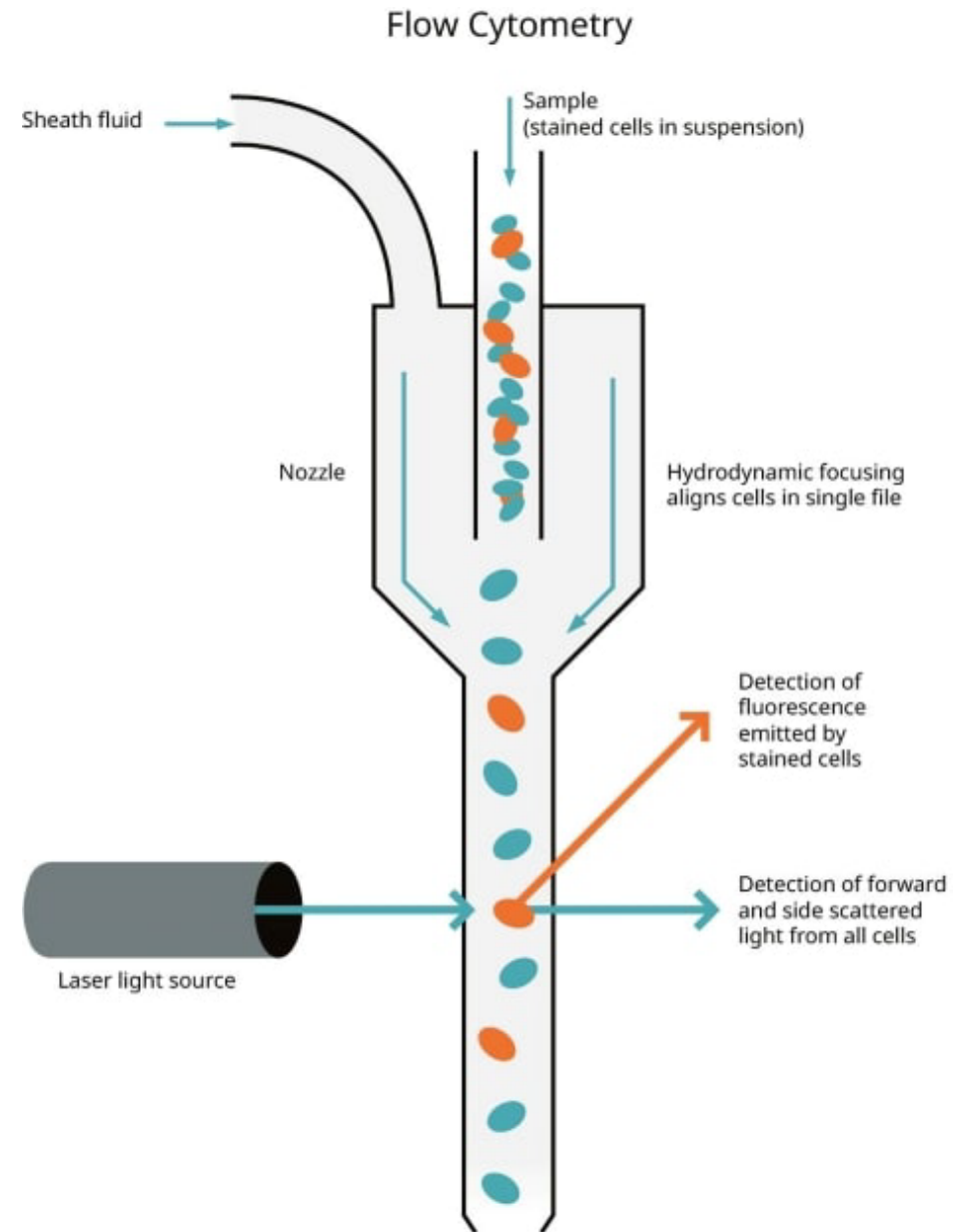
Instrumentation
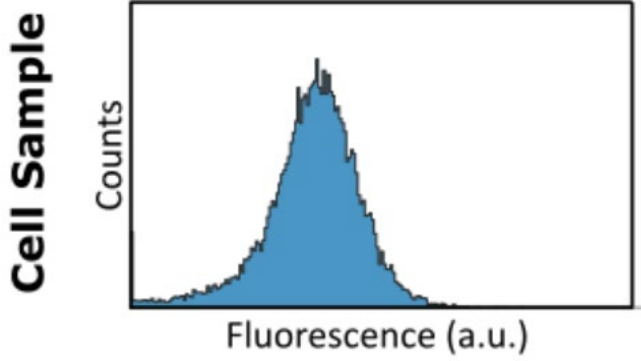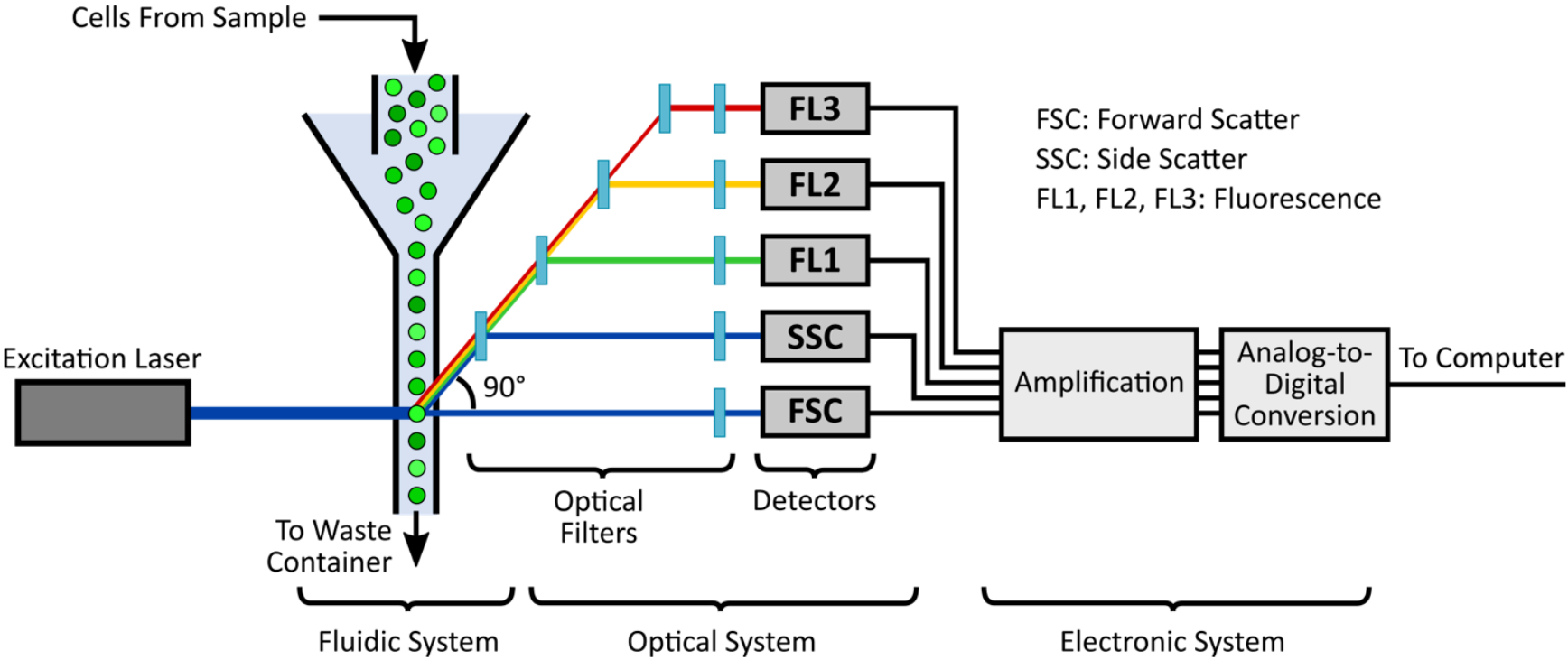
Sample Preparation

- Endogenous fluorescence vs. stained fluorescence

Sample Preparation

- Endogenous fluorescence vs. stained fluorescence
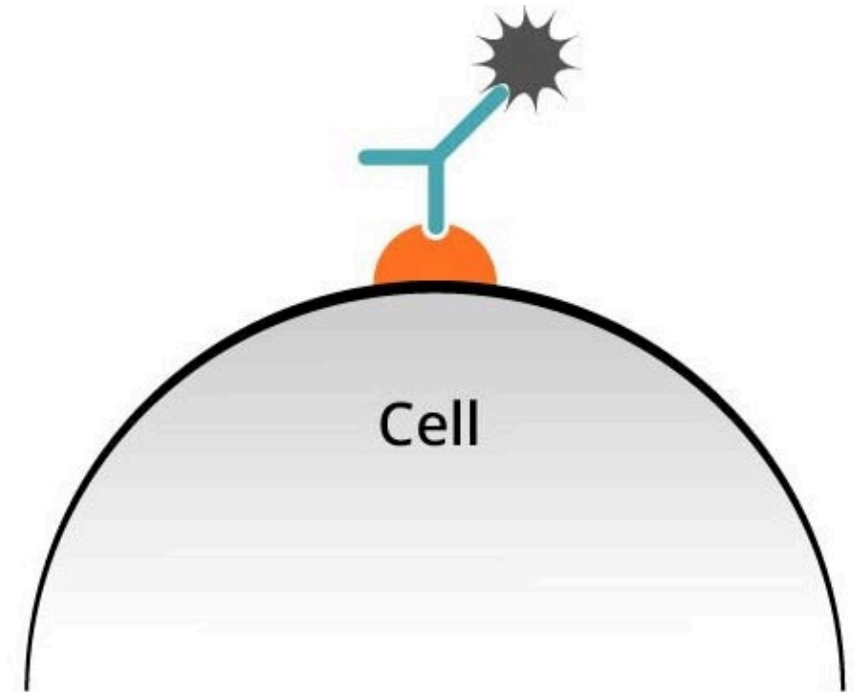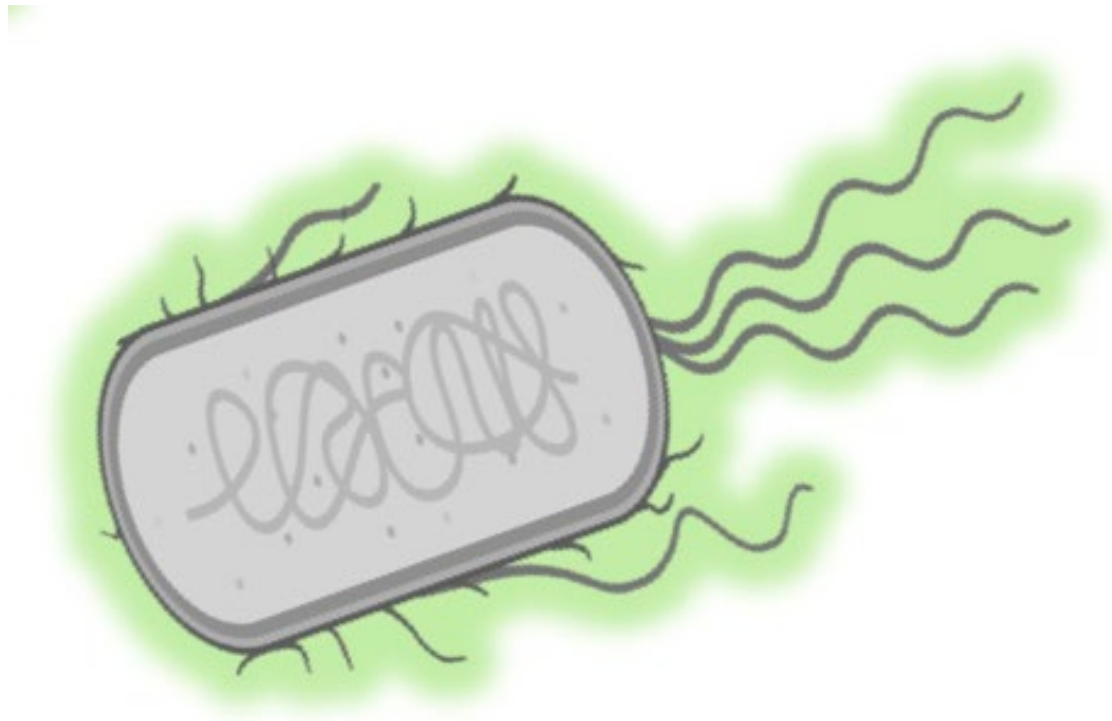
Analysis

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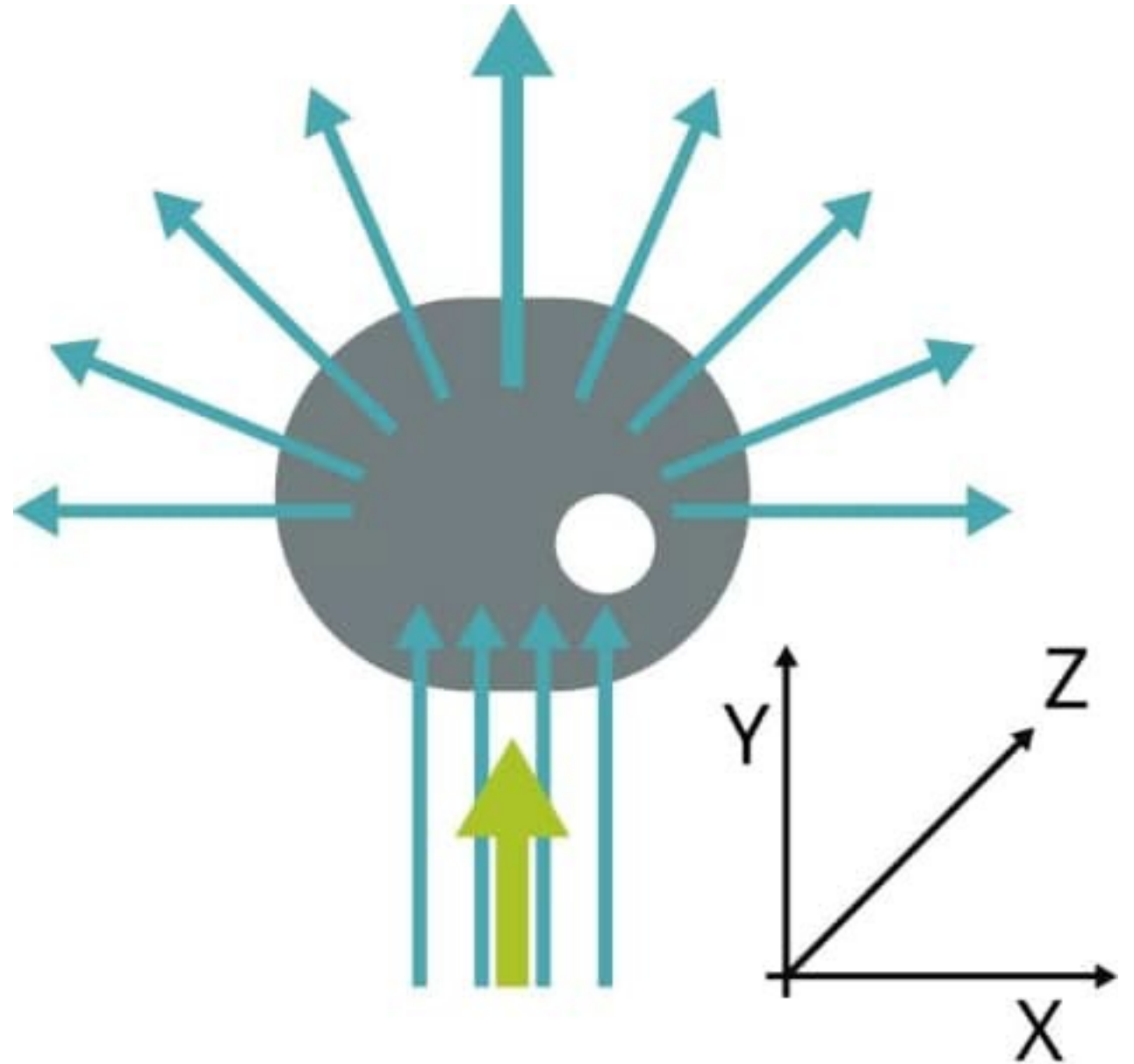
Analysis
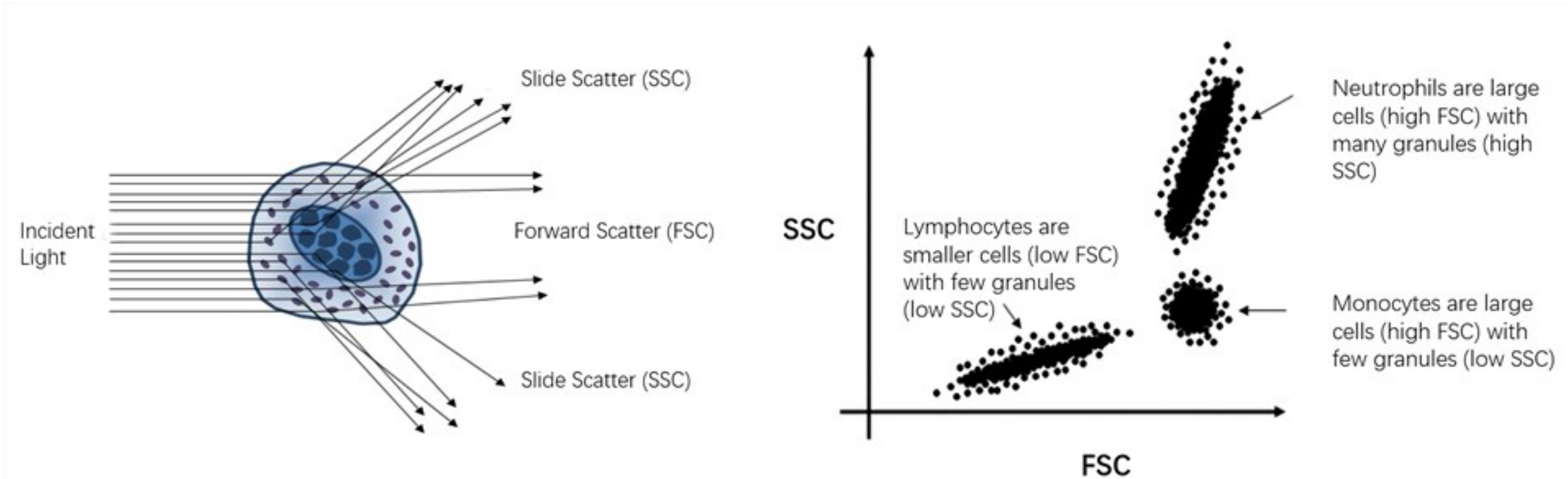
Analysis

- Dot plot of FS versus SS

- Each dot = single cell

- Differences in cell size and granularity determine position of cell population
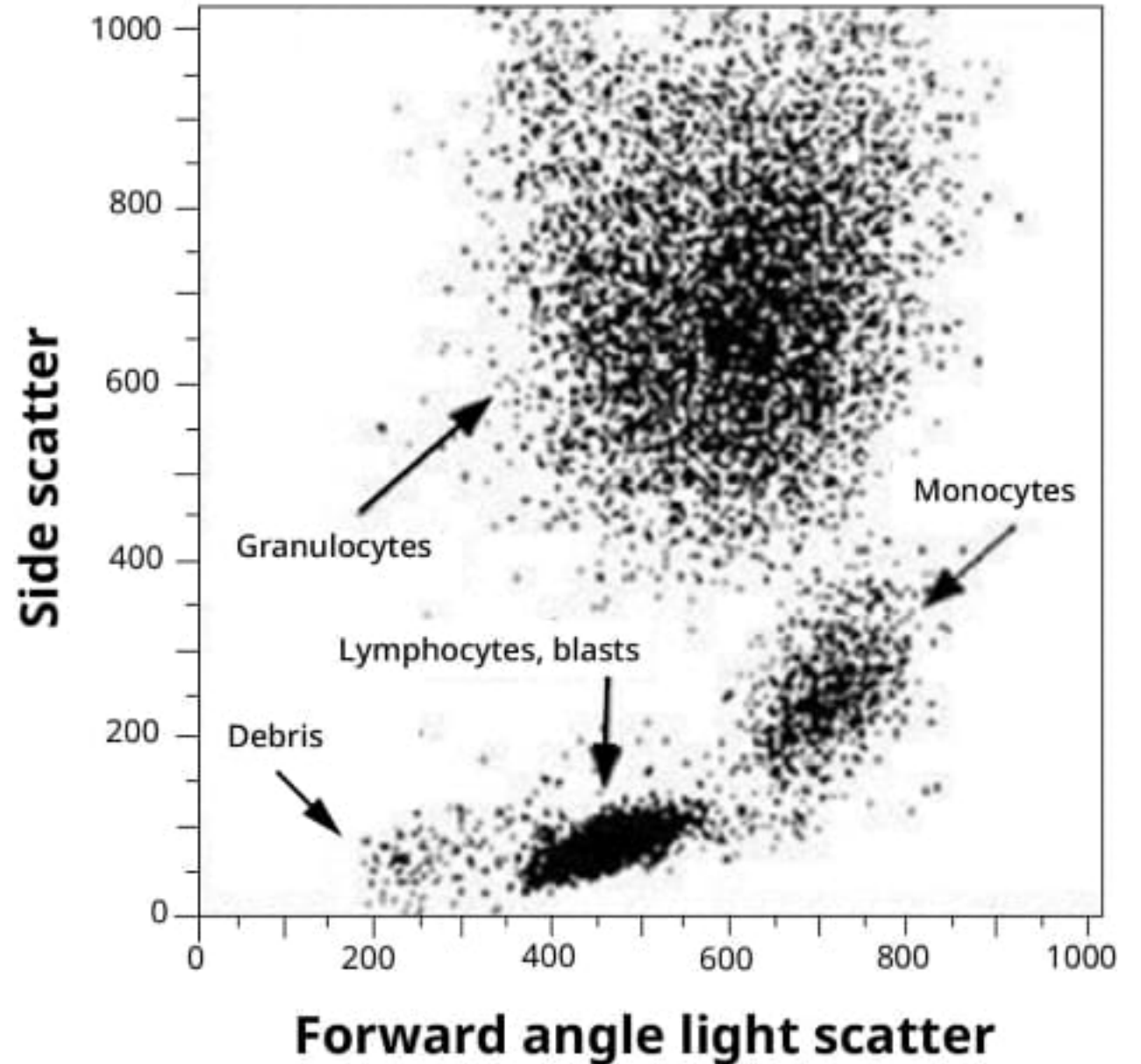
Parameters
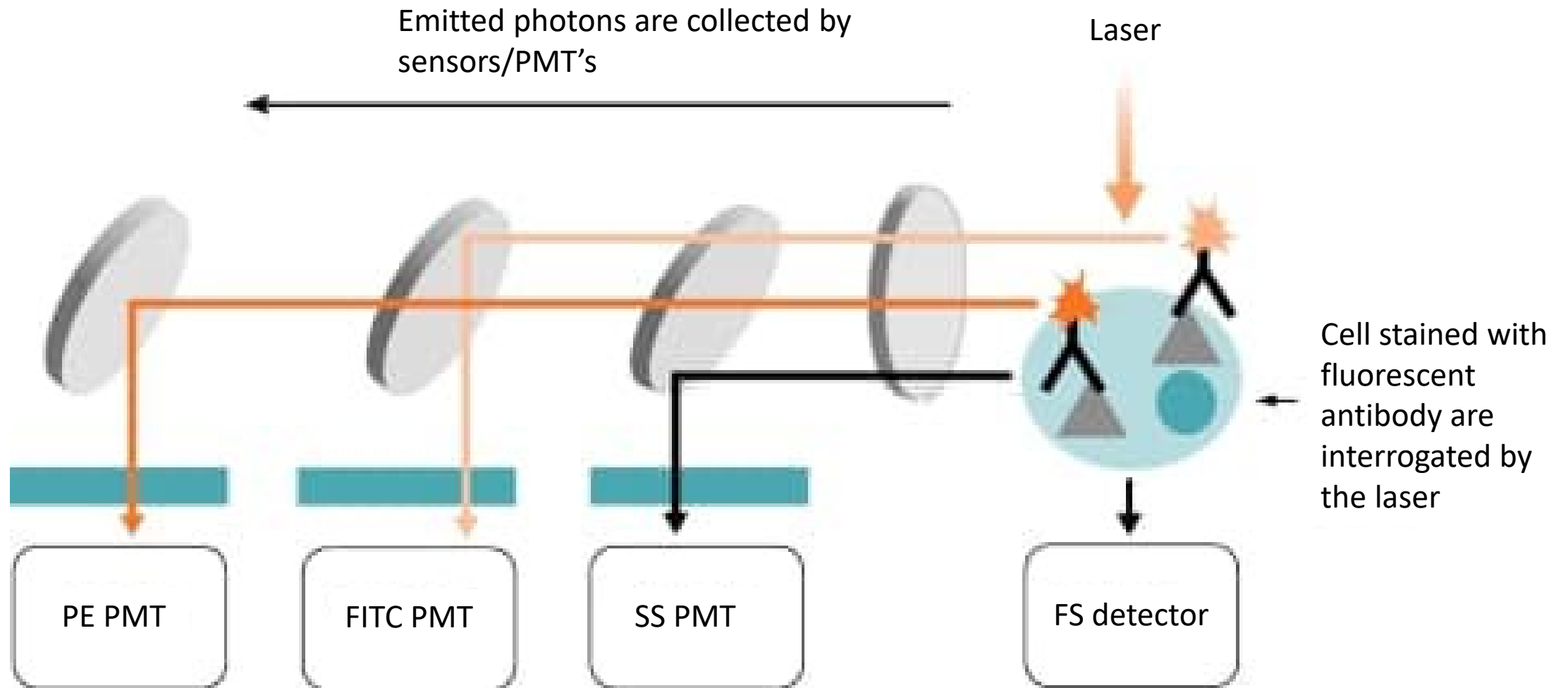
Emitted photons are collected by sensors/PMT's

Laser

Cell stained with fluorescent antibody are interrogated by the laser

PE PMT

FITC PMT

SS PMT

FS detector

Data Analysis

```python
# Imports
import matplotlib.pyplot as plt
import numpy as np
import sys
import cytoflow as flow
import string
import pandas as pd
import seaborn as sns
from scipy import stats
import re
import matplotlib.colors as colors
import matplotlib
import matplotlib.pyplot as plt

sys.path.append('../modules')
import cf_imports
import cf_helpers
```

Data Analysis

```python
# Provide full path to flow cytometry .fcs file directory and pass to fixer
exp_dirs = [
    r'/Users/danielpascal/Desktop/MIT/Voigt Lab/Flow Data/cinI_RBS_screen/minus_zinc_1',
    r'/Users/danielpascal/Desktop/MIT/Voigt Lab/Flow Data/cinI_RBS_screen/plus_zinc_1',
    r'/Users/danielpascal/Desktop/MIT/Voigt Lab/Flow Data/cinI_RBS_screen/minus_zinc_2',
    r'/Users/danielpascal/Desktop/MIT/Voigt Lab/Flow Data/cinI_RBS_screen/plus_zinc_2'
]
conds_dir = None

# Loop through all and add to one dataframe
i = 0
for exp_dir in exp_dirs:
    fcs_dir = cf_imports.dir_fix(exp_dir)
    exp = cf_imports.exp_from_dirs(fcs_dir, conds_dir, event_num = 5000) #subset 5000 events

        # Apply 2D gaussian fit on FSC/SSC to make a gate
    g = flow.GaussianMixtureOp(name = "fsc_ssc_gate",
                               channels = ["FSC-A", "SSC-A"],
                               scale = {'FSC-A' : 'log',
                                        'SSC-A' : 'log'},
                               num_components = 1,
                               sigma = 2)
    g.estimate(exp)
    exp2 = g.apply(exp)

    gg = flow.GaussianMixtureOp(name = "fsc_ssc_gate2",
                                channels = ["FSC-H", "SSC-H"],
                                scale = {'FSC-H' : 'log',
                                         'SSC-H' : 'log'},
                                num_components = 1,
                                sigma = 2)
    gg.estimate(exp)
    exp3 = gg.apply(exp)
```
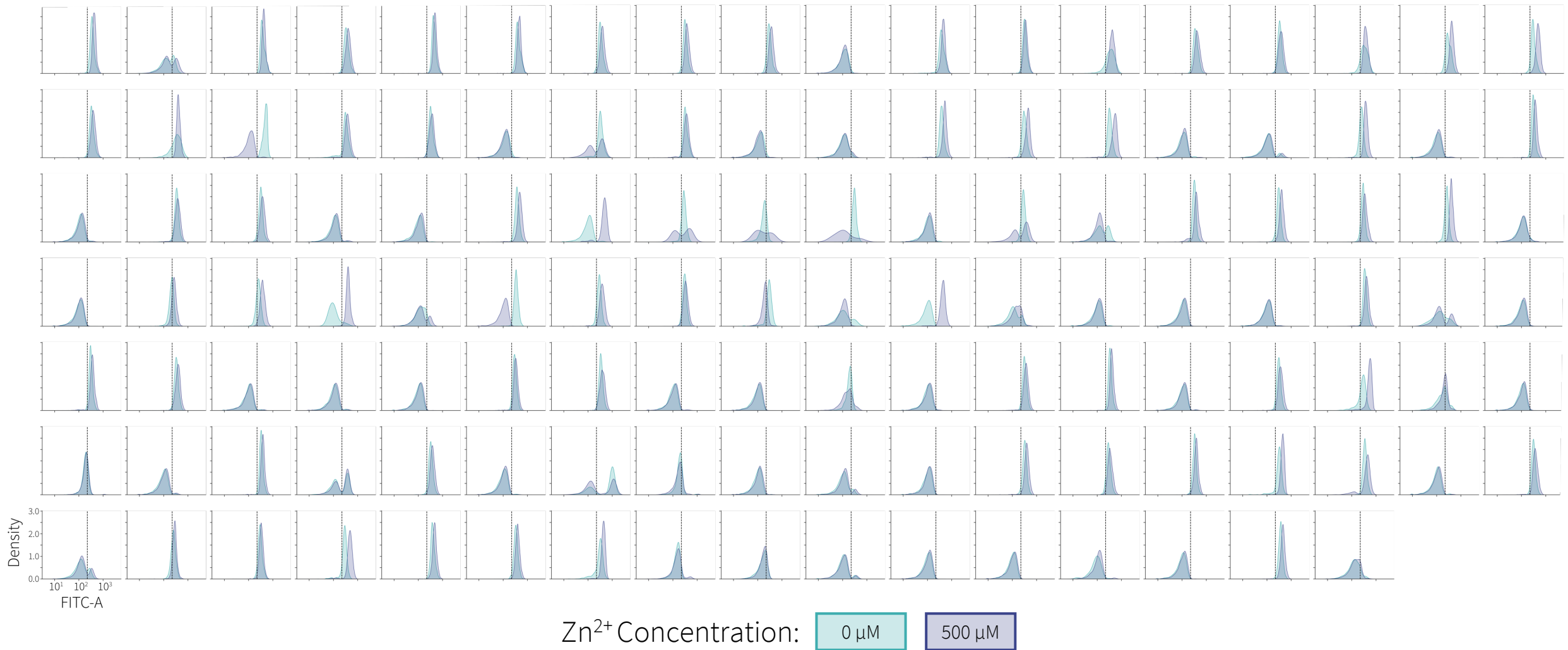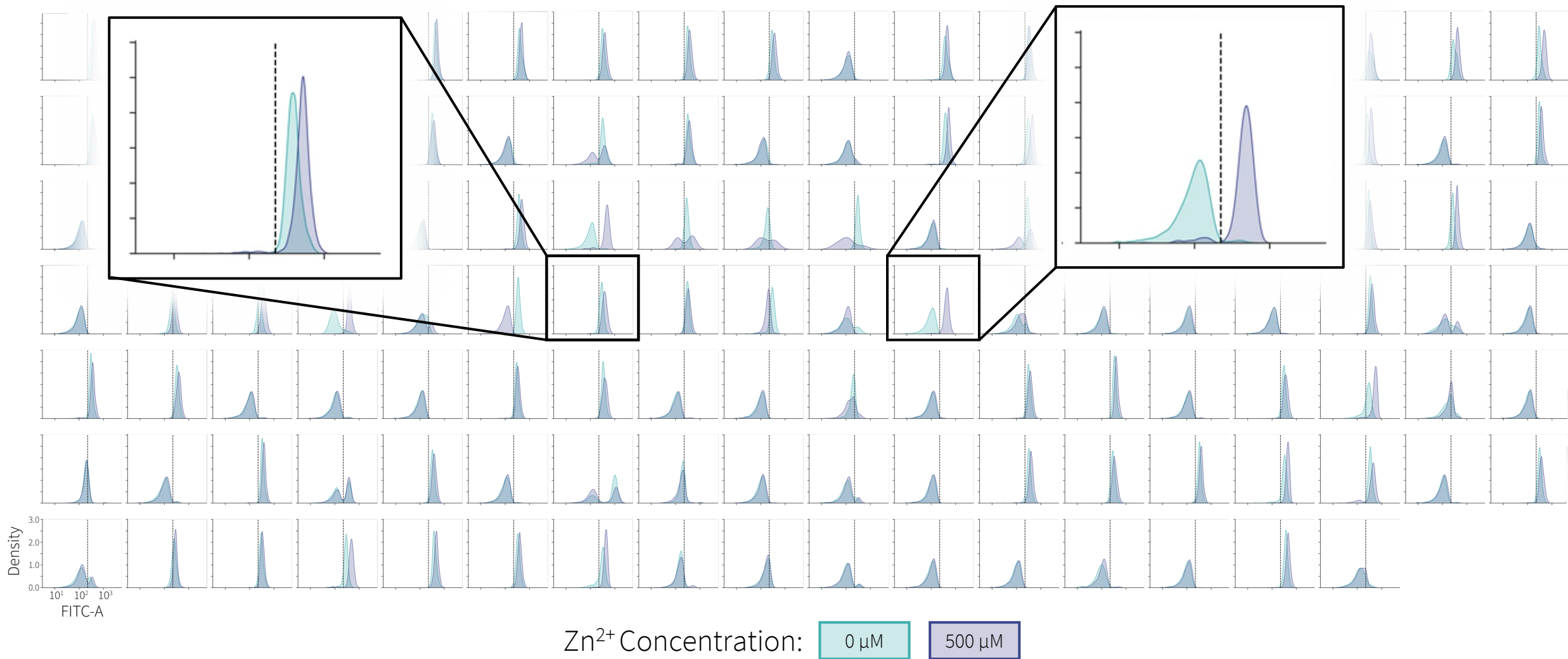
Data Analysis

	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	

1074138 rows × 18 columns

Data Analysis

Density

FITC-A

Zn²⁺ Concentration: 0 μM   500 μM

Data Analysis

Density

FITC-A

10^1 10^2 10^3

3.0
1.0
0.0

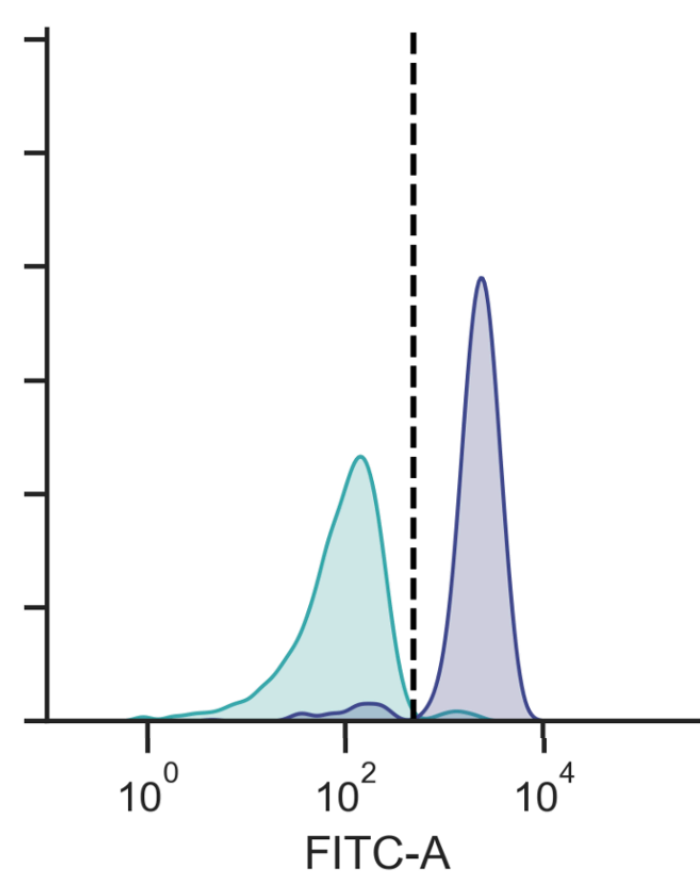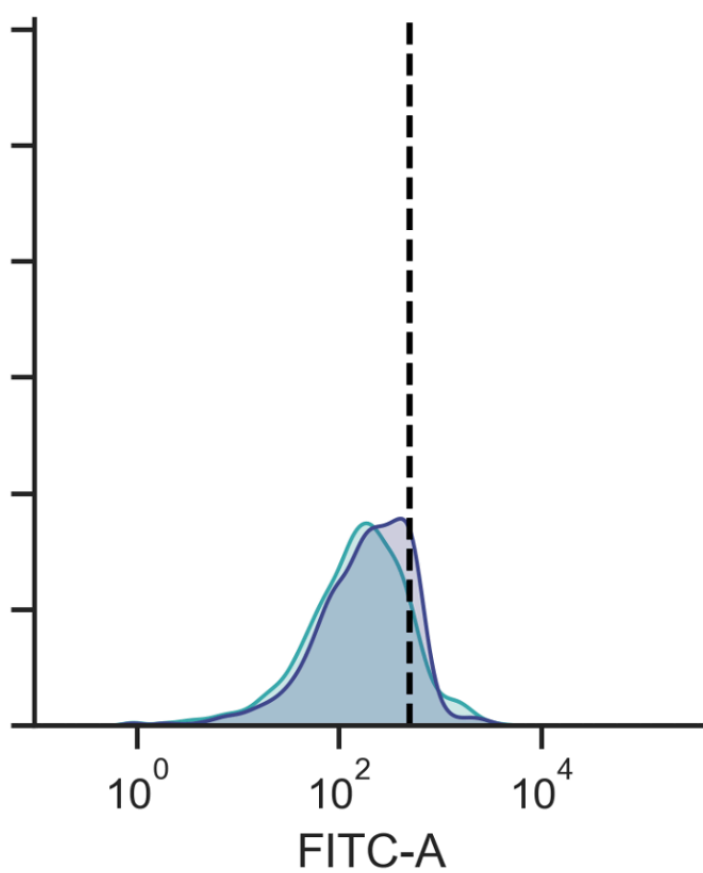Zn²⁺ Concentration: 0 μM 500 μM
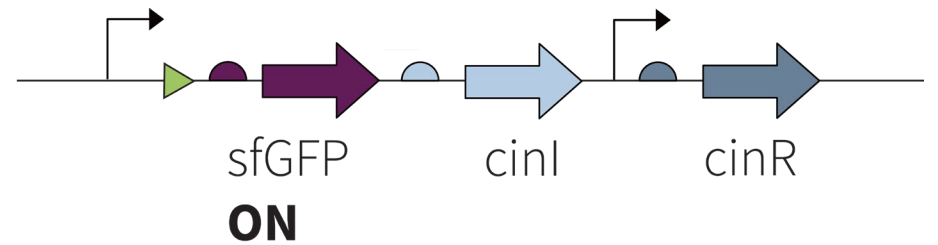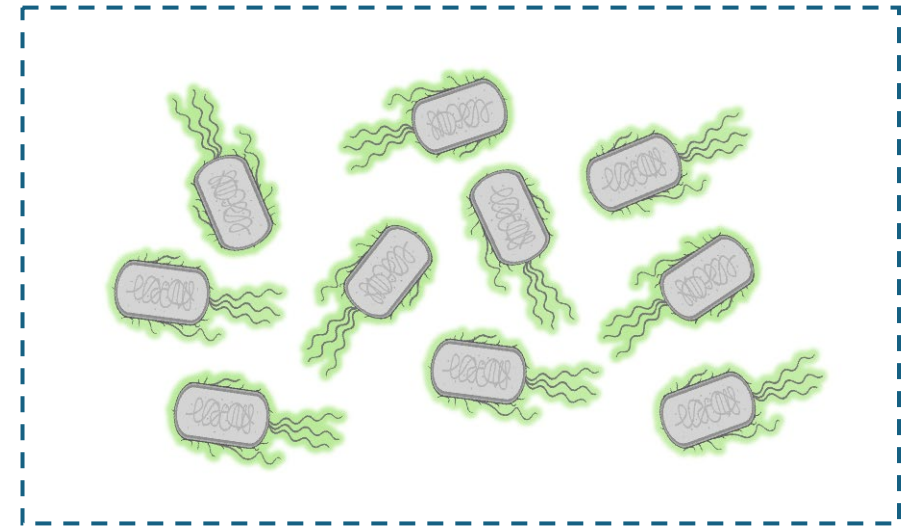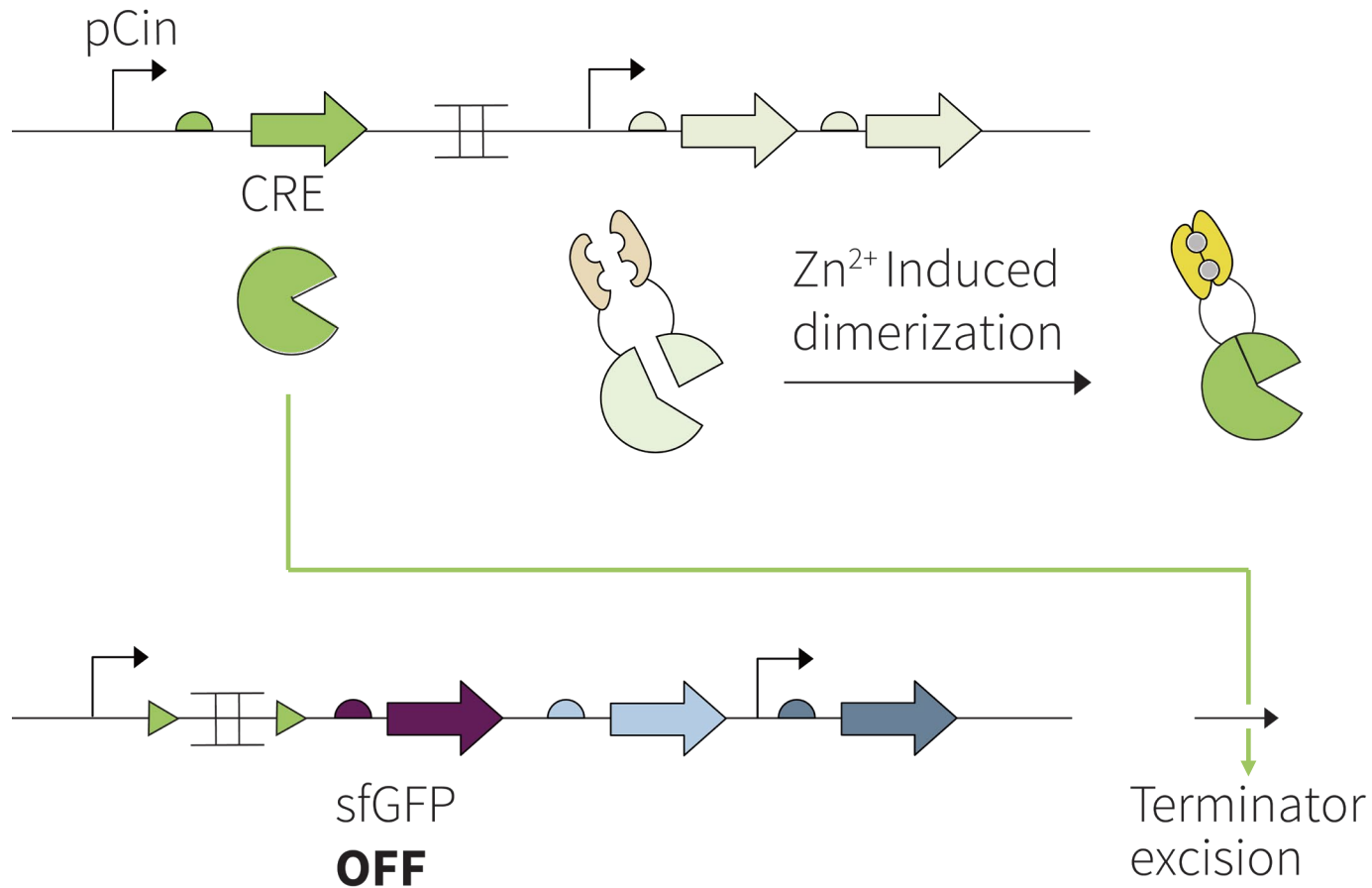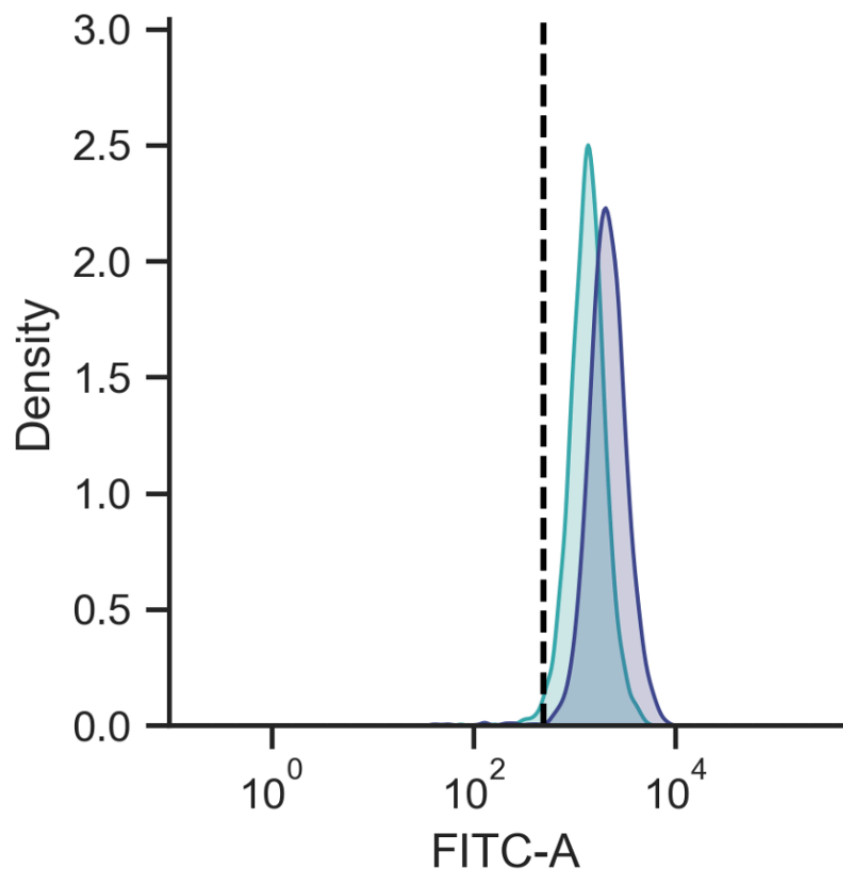
Applications

Applications

Zn²⁺ Concentration: 0 µM 500 µM

Advantages

- High throughput
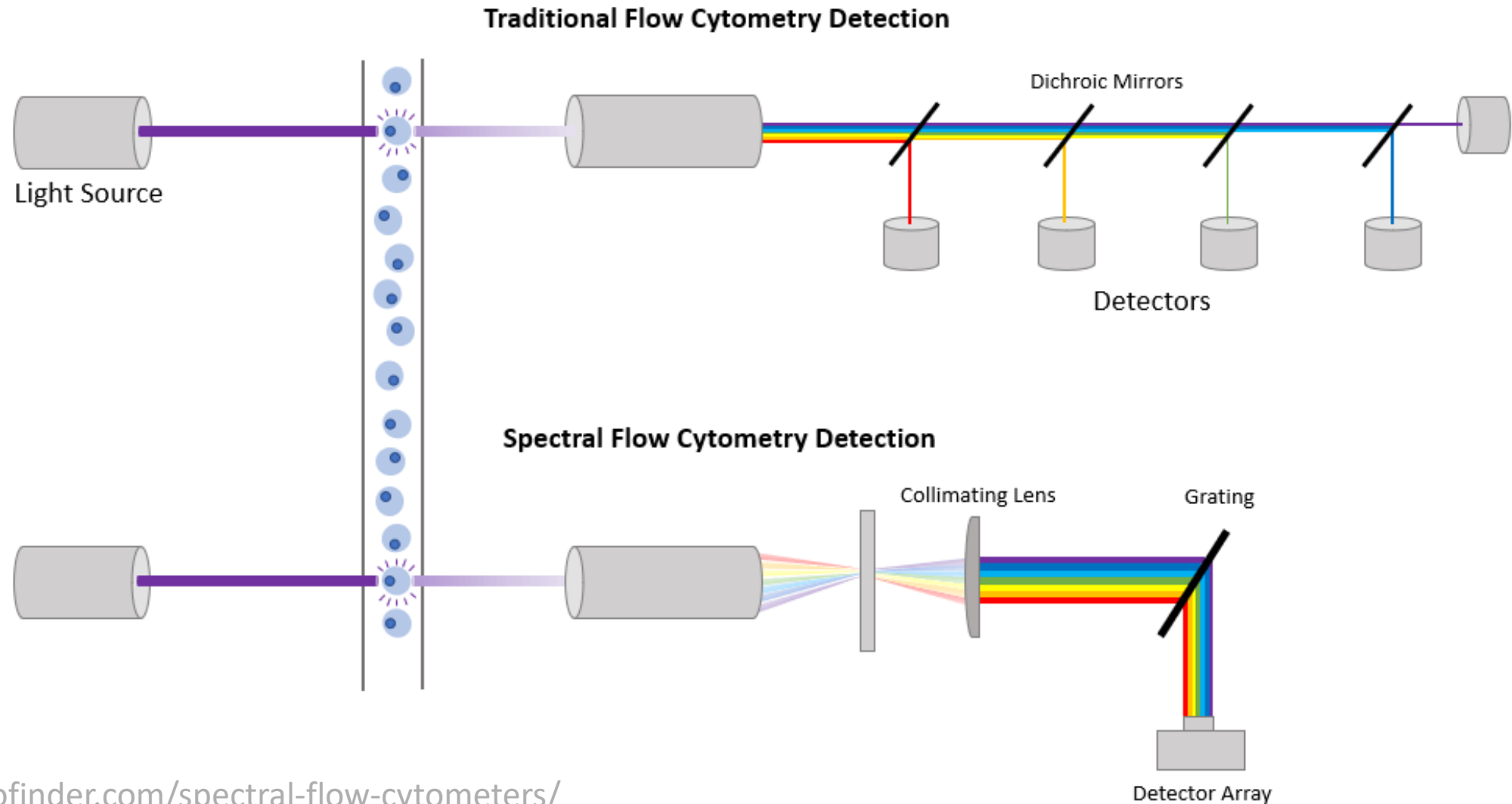- Multiparametric analysis
- Highly sensitive

Limitations

- Specialized instrumentation and training

- Time-consuming sample prep

- Complex data analysis

- Difficulty analyzing autofluorescent or overlapping signals

Future directions

- New fluorophores
- Microfluidic devices
- Combining with special information of microscopy

Spectral flow cytometry

Imaging flow cytometry