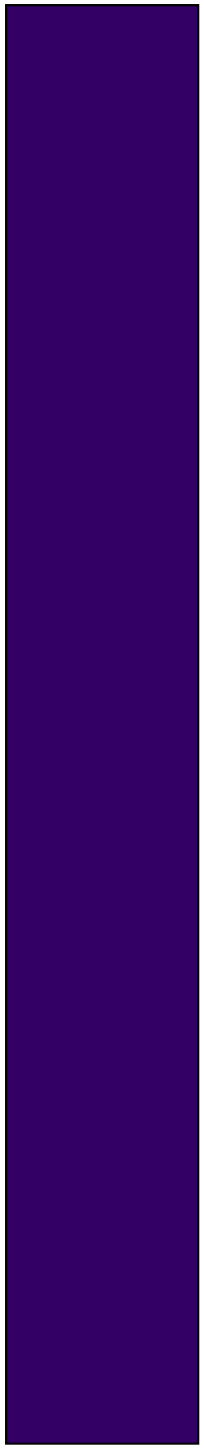
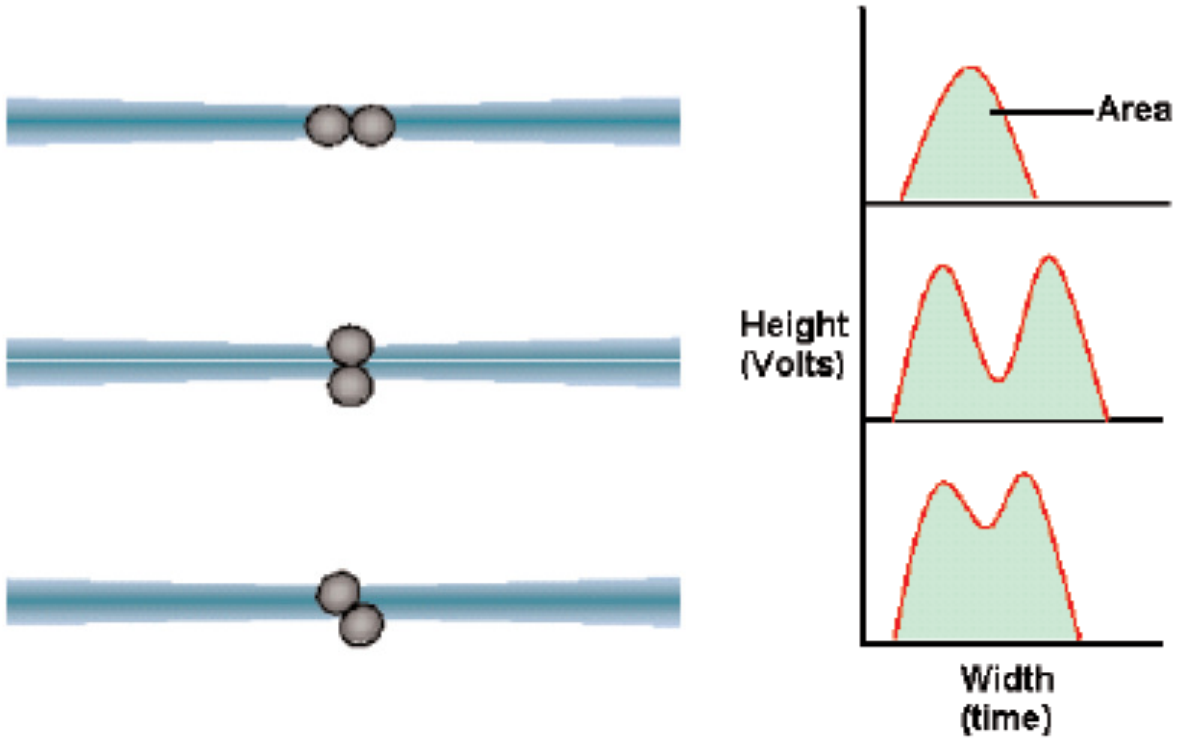


Sorting Theory

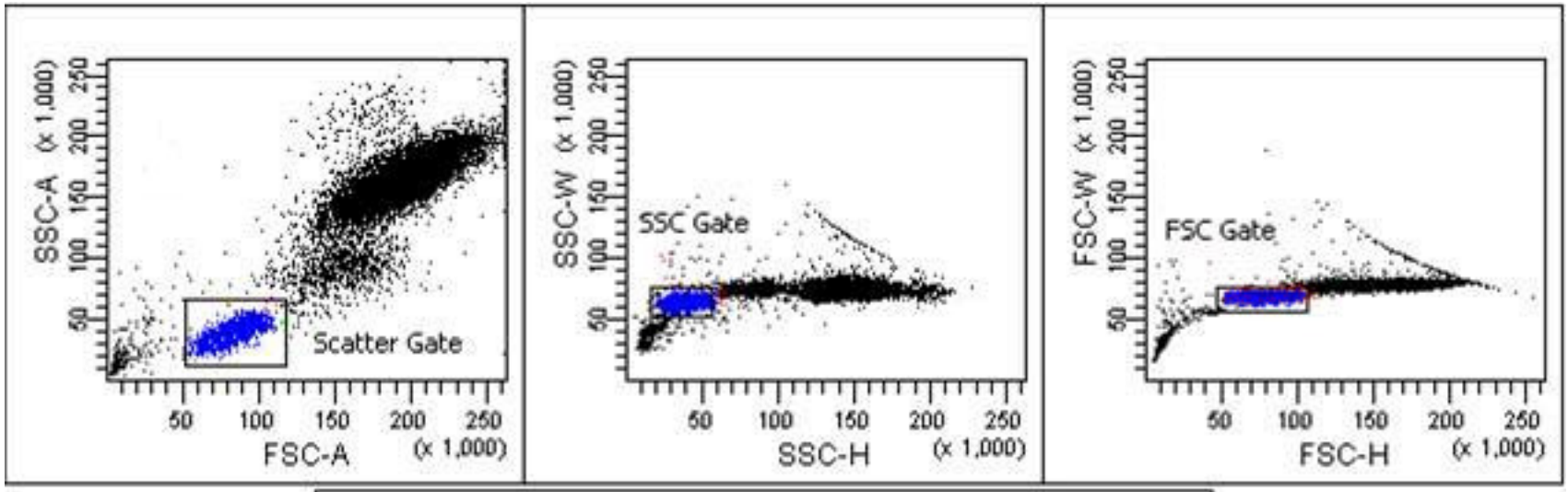
Doublet Discrimination

Doublets can be distinguished from singlets based on differences in voltage pulse shape.



Doublet Discrimination

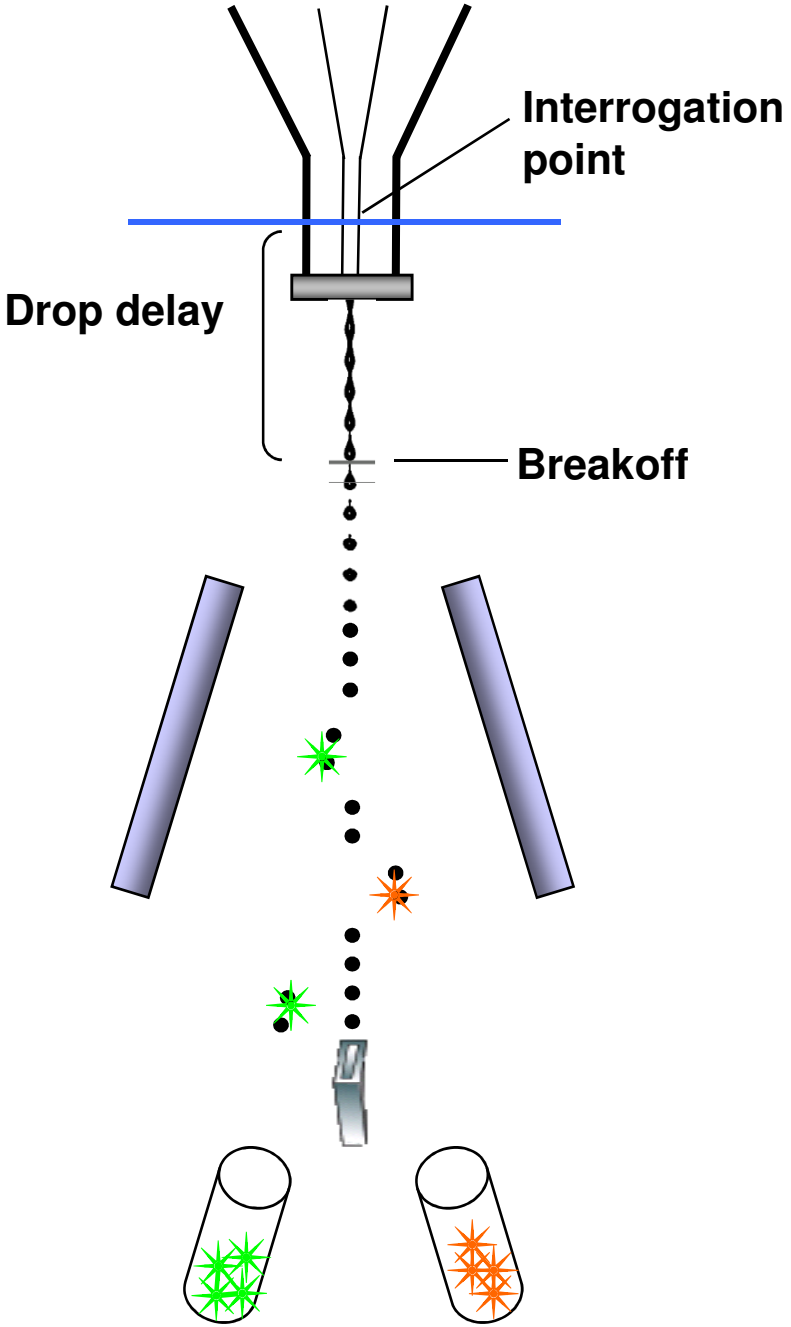
The doublet discrimination gating template includes the following plots.

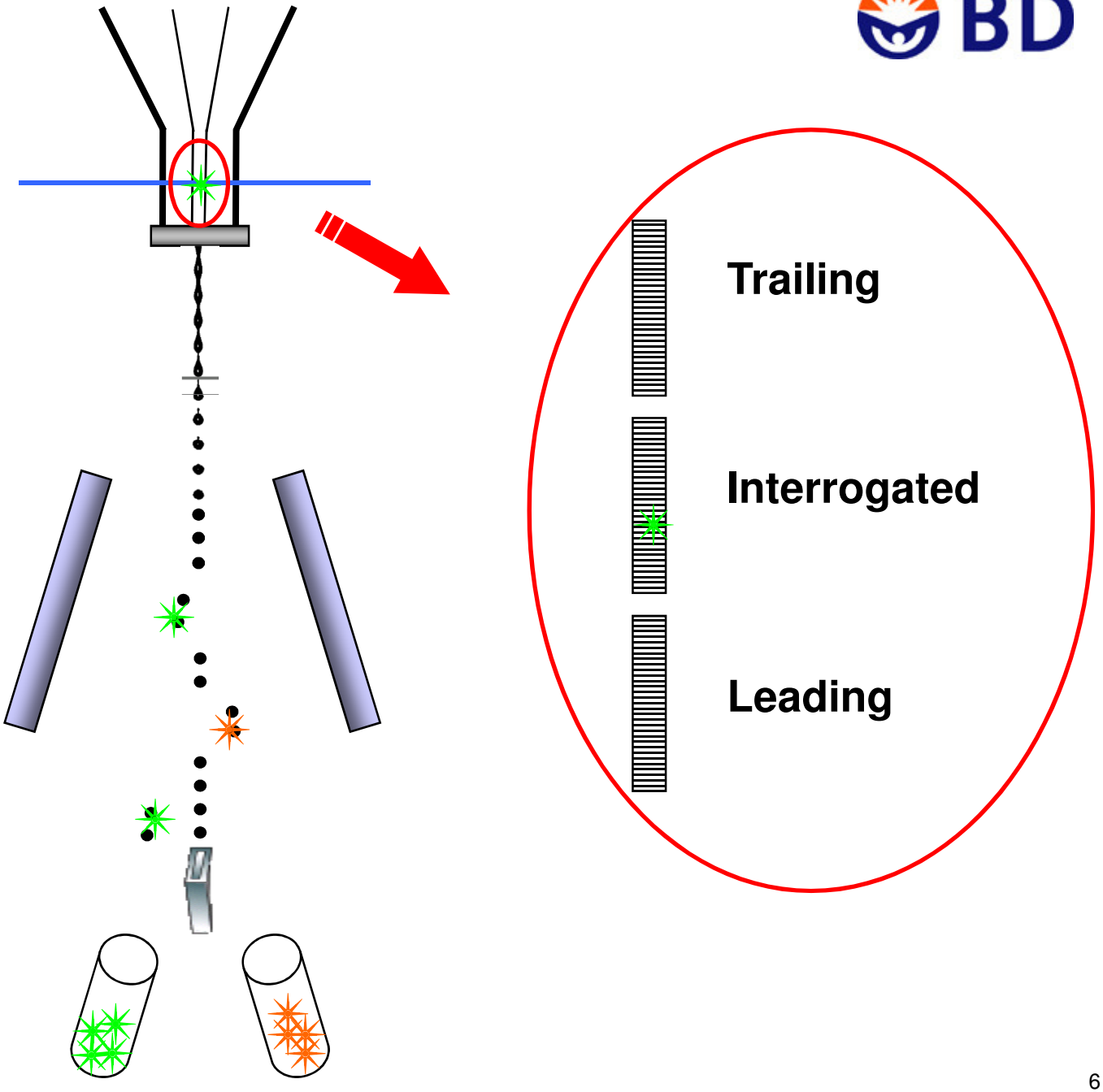


Tube: pre sort

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
Scatter Gate	1,022	10.2	10.2
SSC Gate	996	97.5	10.0
FSC Gate	989	99.3	9.9

Conflict Resolution


Interrogation point
Drop delay
Breakoff


Trailing
Interrogated
Leading

Mask

- A region of the stream monitored for the presence of cells.
- Determines how drops will be deflected if a sorting conflict occurs.
- Measured in 1/32 drop increments.

Conflict Resolution

- Precision modes include three types of masks:
  - Yield
  - Purity
  - Phase

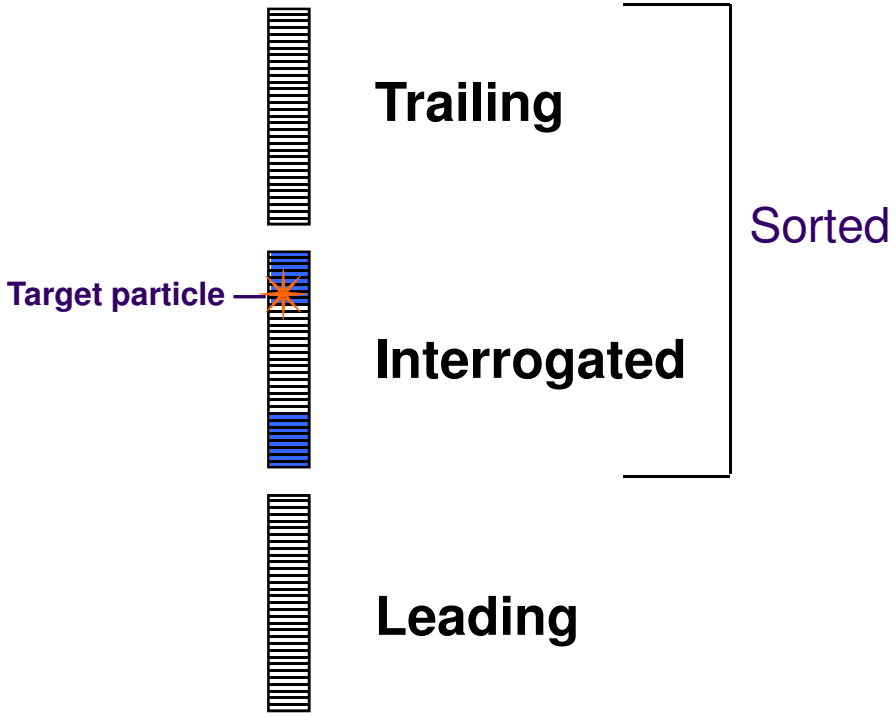
	Precision Mode					
	Purity	4-Way Purity	Yield	Single Cell	Initial	Fine Tune
Yield Mask	32	0	32	0	32	0
Purity Mask	32	32	0	32	0	0
Phase Mask	0	0	0	16	0	0
Single Cell	☐	☐	☐	☒	☐	☐

Yield Mask

- If a target particle is within the yield mask, the interrogated drop and the adjacent drop are sorted.

- For this example,
  the yield mask = 16
  (8/32 on each side)

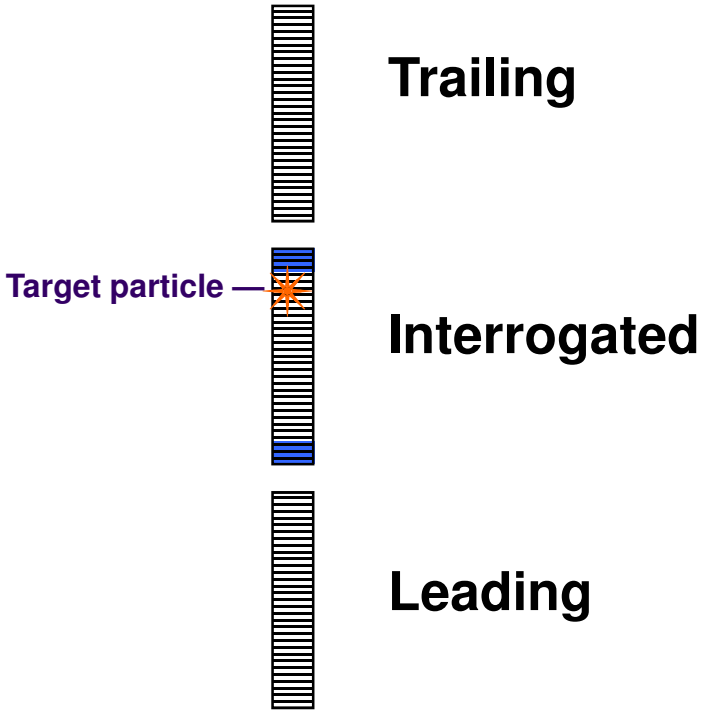
- 2 drops will be sorted



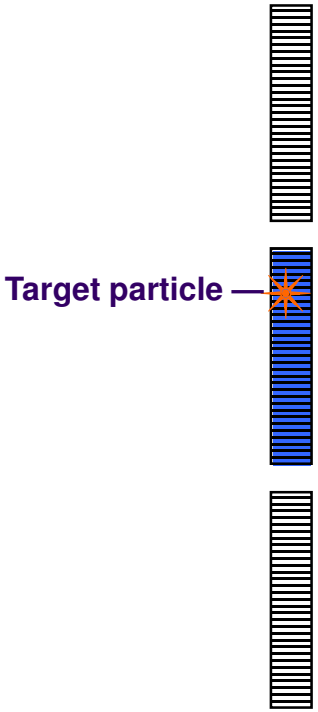
Yield Mask (continued)

- If the yield mask = 8

  How many drops will be sorted?

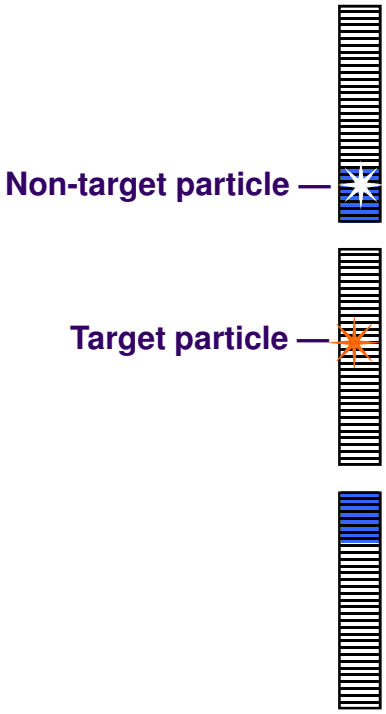


Yield Mask (continued)



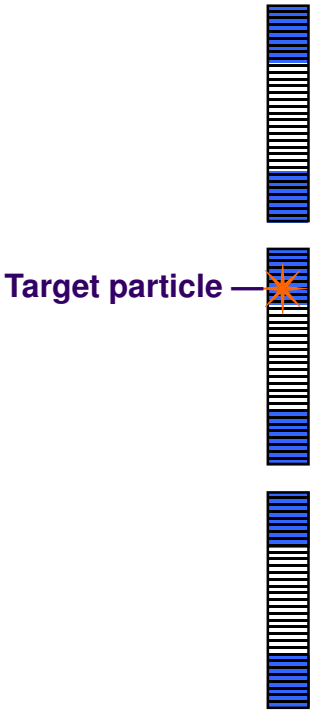
- Yield mask = 32
  __ drop(s) will always be sorted
- Yield mask = 0
  __ drop(s) will always be sorted

Purity Mask



- If a target particle is in the interrogated drop and a non-target particle is within the purity mask, the interrogated drop will not be sorted.
- If the purity mask = 16, the target particle in this example will not be sorted.
- If the purity mask = 8, will the target particle be sorted?

Phase Mask



- If a target particle is within the phase mask, the interrogated drop will not be sorted.
- Phase mask = 16
  In this example, the particle is not sorted.
- Phase mask = 8
  Will this particle be sorted?

Precision Modes

	Precision Mode					
	Purity	4-Way Purity	Yield	Single Cell	Initial	Fine Tune
Yield Mask	32	0	32	0	32	0
Purity Mask	32	32	0	32	0	0
Phase Mask	0	0	0	16	0	0
Single Cell	☐	☐	☐	☒	☐	☐

2-Way sorting

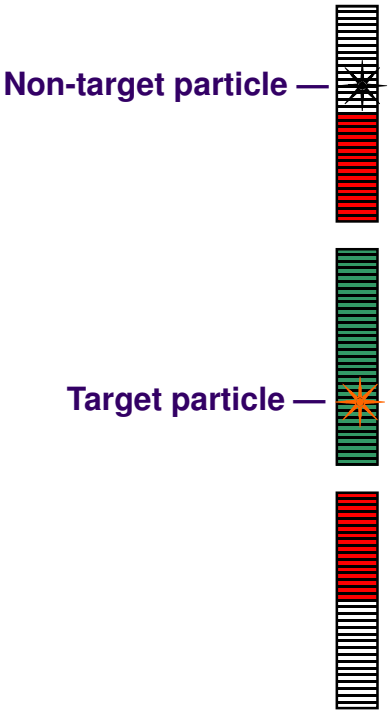
4-Way sorting

Enrichment

Plate sorting

Accudrop

Purity Precision Mode



Trailing

Interrogated

Leading

Yield = 32

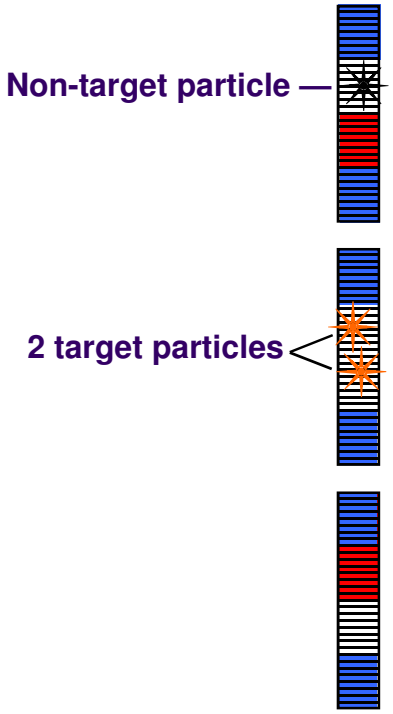
Purity = 32

Phase = 0

Single cell =

Is the target particle in this example sorted?

Single Cell Precision Mode



Yield = 0

Purity = 32

Phase = 16

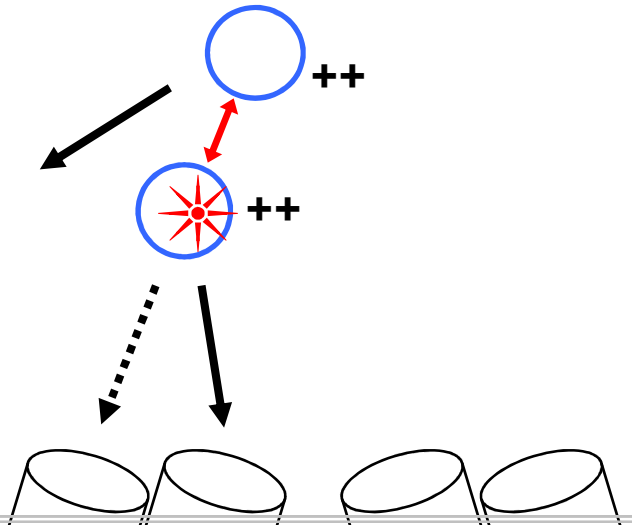
Single cell = ☒

Are the target particles in this example sorted?

Precision Mode Tips

- A yield mask and a phase mask cannot be used together.
- When performing a 4-way sort or sorting onto plates, you can improve the trajectory of the sorted droplet by:

Using a 1-drop envelope
(Yield mask = 0)



Using a phase mask