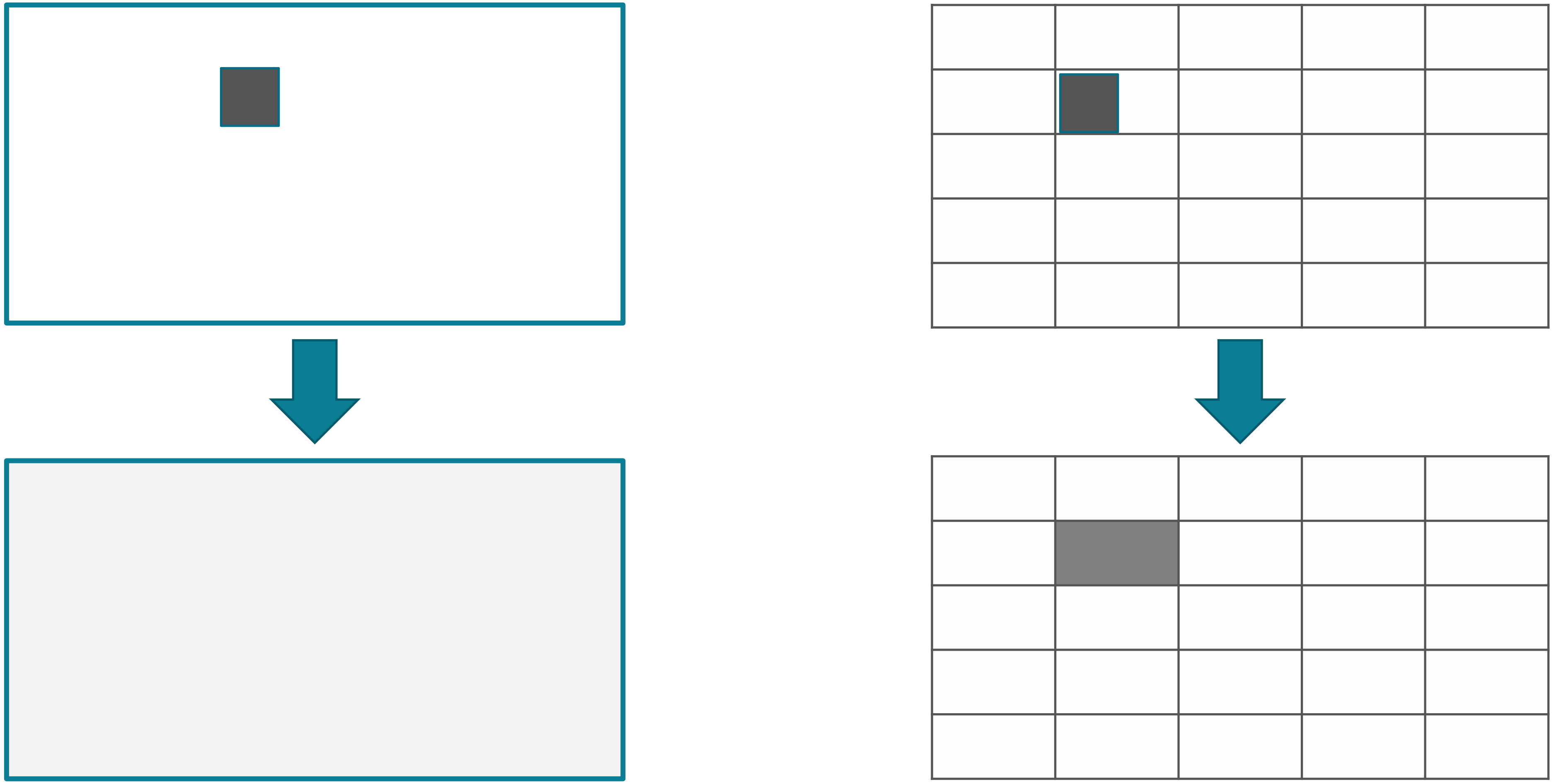
SETE SUMMER SCHOOL

Arnaud REITZ

SUMMARY

- Why droplets microfluidics ?
- How to do encapsulation
- Encapsulation statistics
- Application : DropSeq protocol

BULK VS COMPARTMENTALIZED



Compartmentalization allows for higher sensitivity as it dilutes less the sample

BULK VS COMPARTMENTALIZATION

	Robot	Microfluidic drops
Total reactions	5×10^7	5×10^7
Reaction volume	100 µL	6 pL
Total volume	5,000 L	150 µL
Reactions/day	73,000	1×10^8
Total time	~2 years	~7 h
Number of plates/devices	260,000	2
Cost of plates/devices	$520,000	$1.00
Cost of tips	$10 million	$0.30
Amortized cost of instruments	$280,000	$1.70
Substrate	$4.75 million	$0.25
Total cost	$15.81 million	$2.50

J. Agresti et al., 2009, doi: 10.1073/pnas.0910781107

ALLOWS FOR SINGLE CELL MANIPULATIONS

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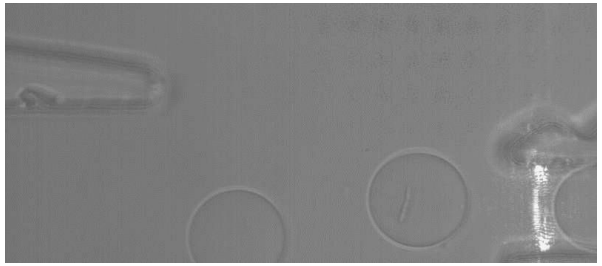

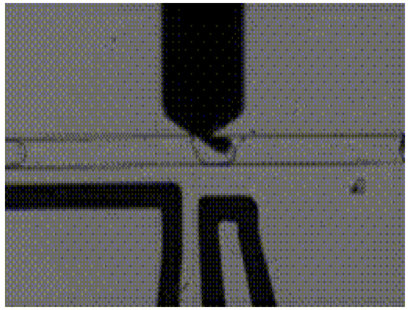

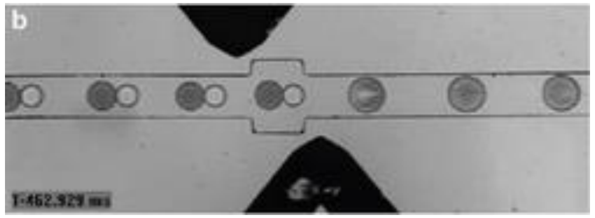

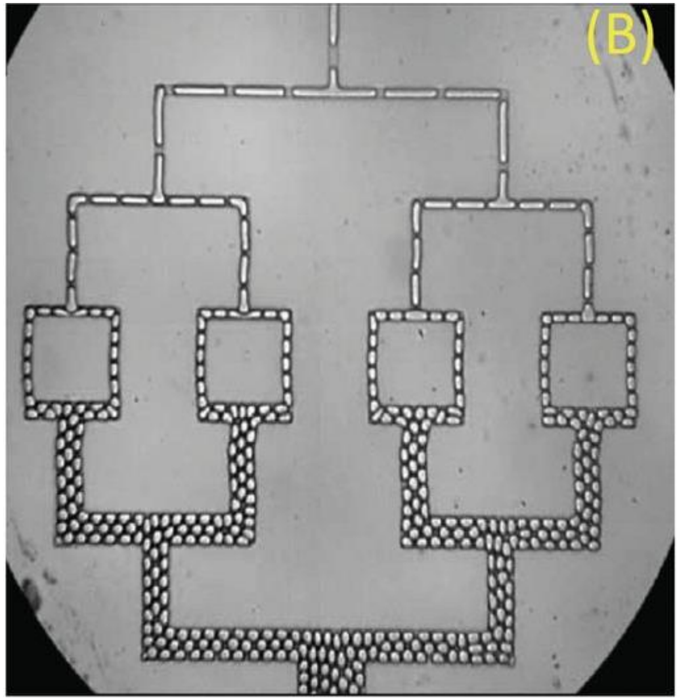

And many more :
The sky is the limit !

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- **How to do encapsulation ?**
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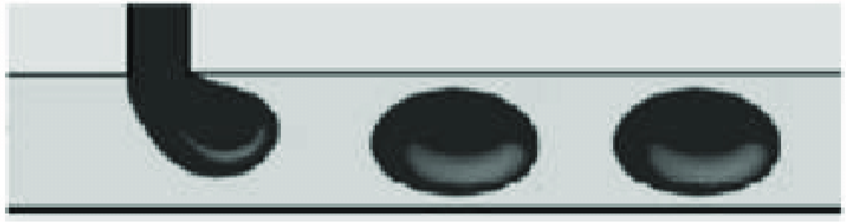
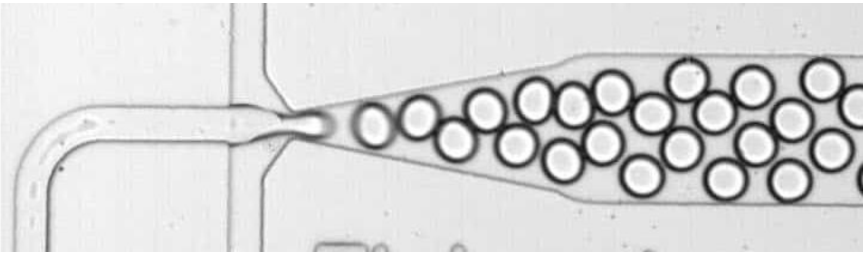
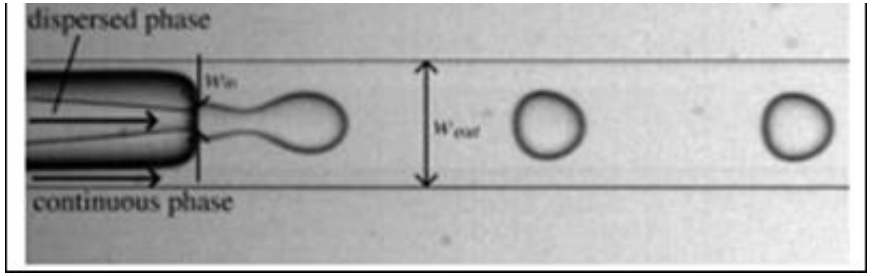
HOW TO DO ENCAPSULATION



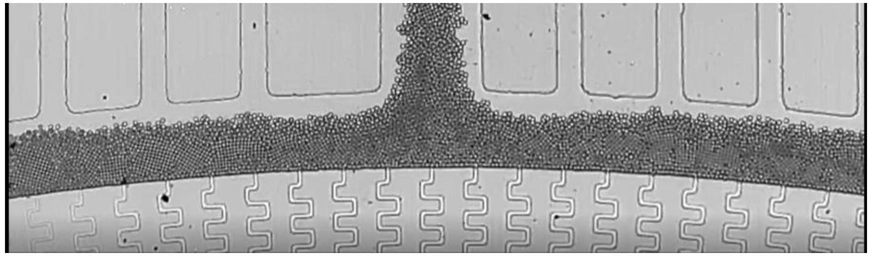
T-junction



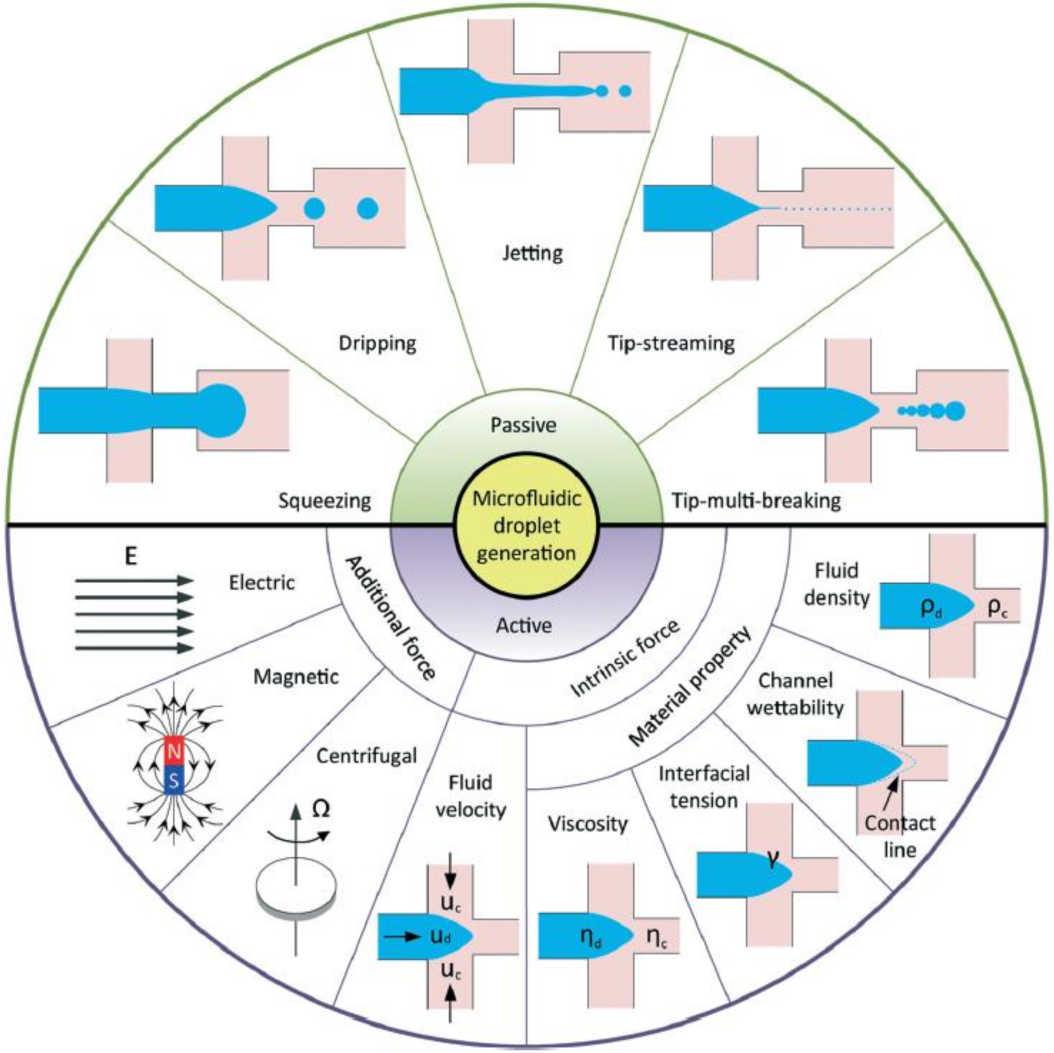
Flow-focusing



Co-flowing



Step emulsification



PHASE DIAGRAMS

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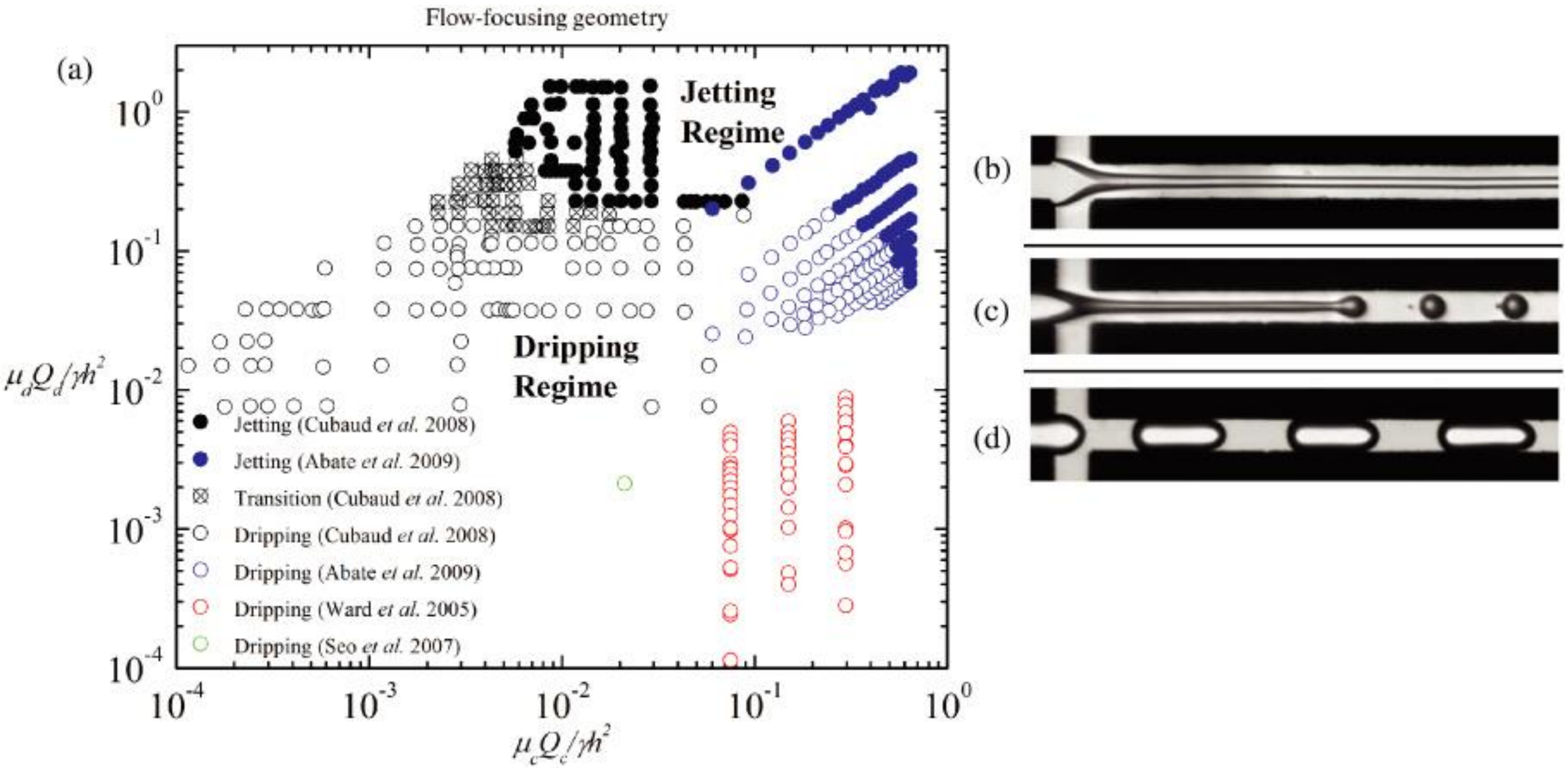

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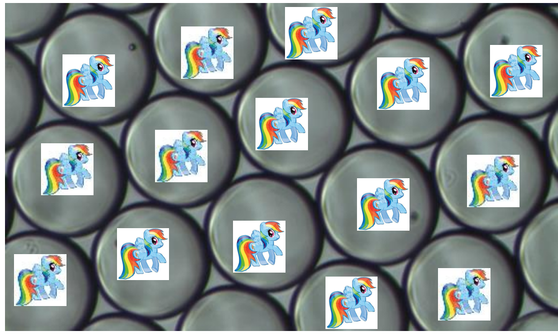
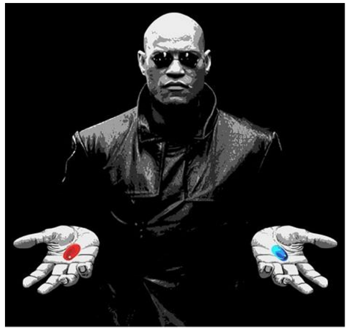
ENCAPSULATION STATISTICS

Try to encapsulate 10 000 cells in 10 000 drops

Ideal world

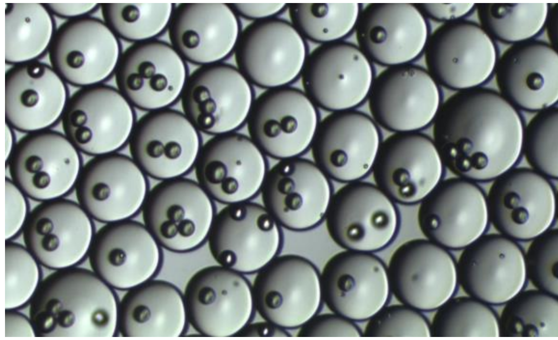
10 000 drops containing 1 cell each



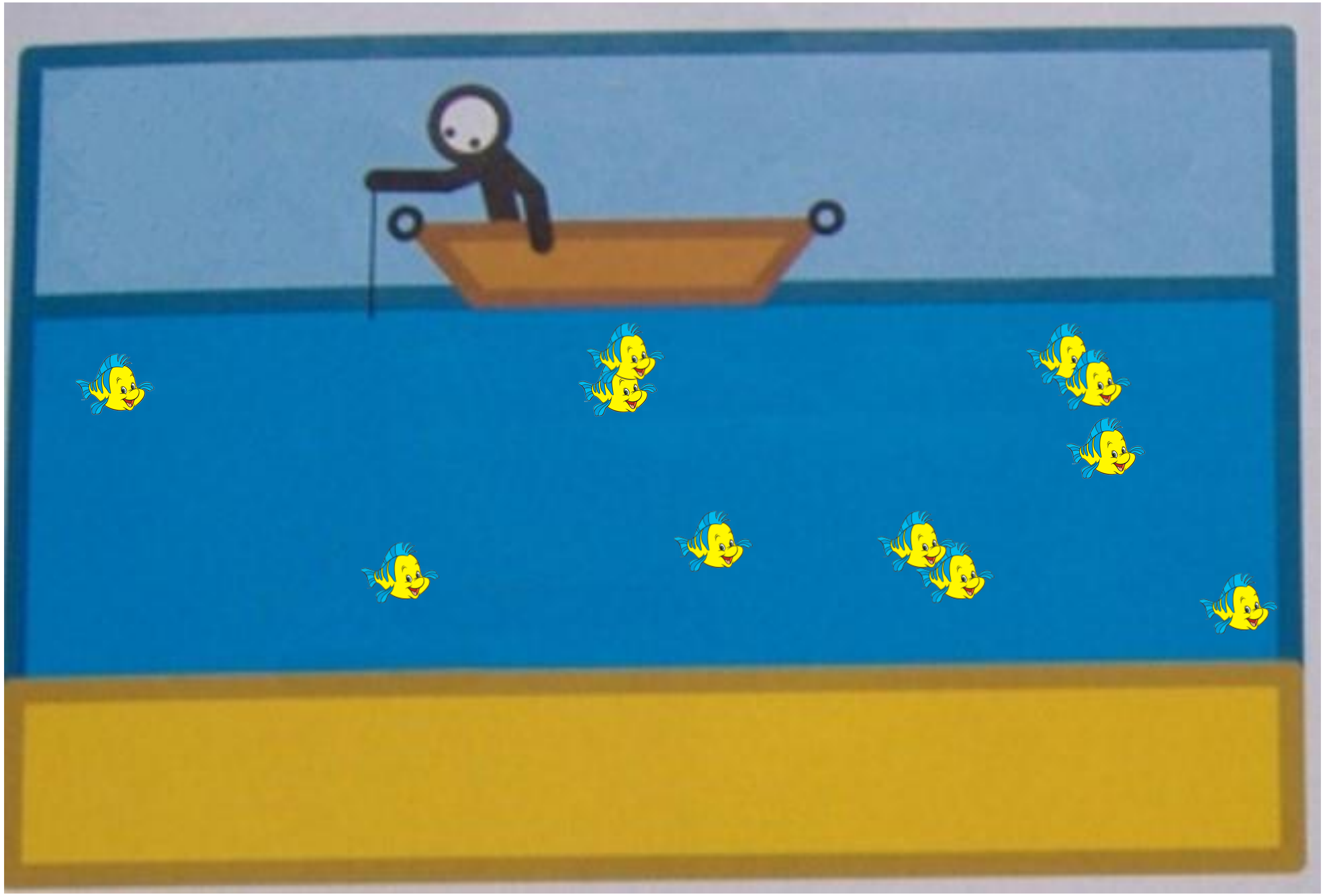


Real world

3679 drops containing 1 cell
--AND--
3679 drops containing 0 cells
1839 drops containing 2 cells
613 drops containing 3 cells
153 drops containing 4 cells
31 drops containing 5 cells
5 drops containing 6 cells
1 drop containing 7 cells

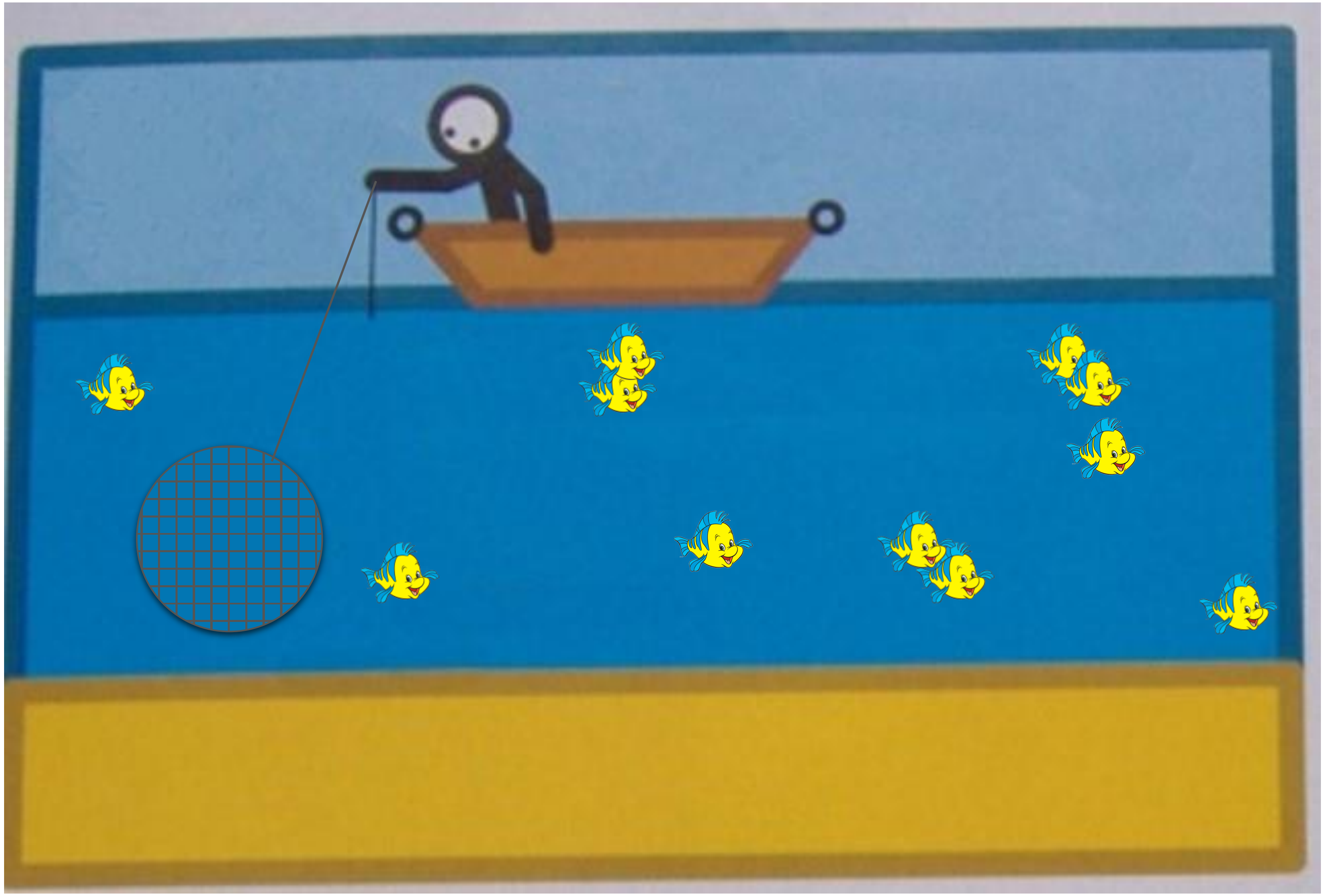


EXPERIMENT OF THE FISHERMAN



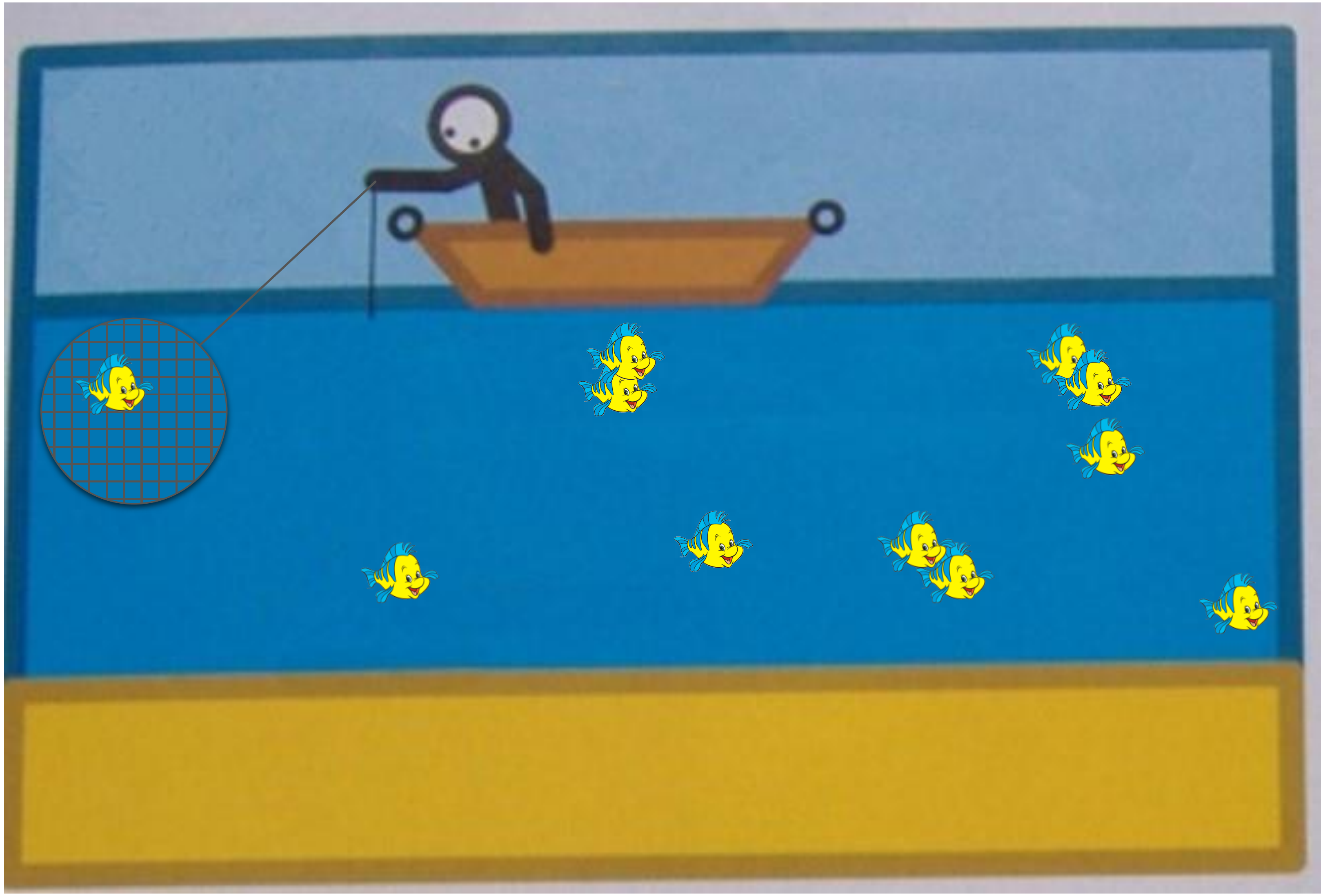
How many fish will he capture in his net ?

EXPERIMENT OF THE FISHERMAN



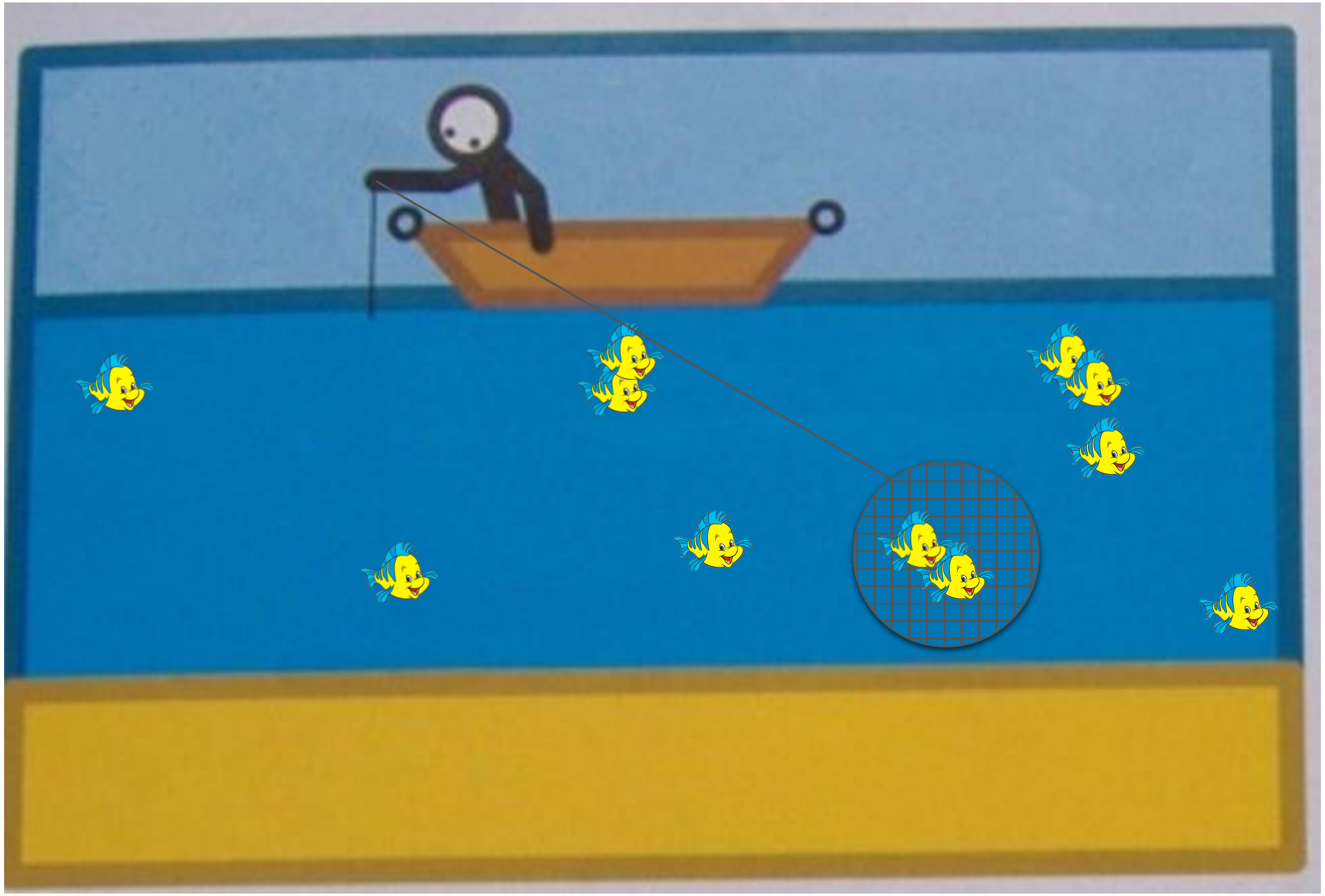
It can be o

EXPERIMENT OF THE FISHERMAN



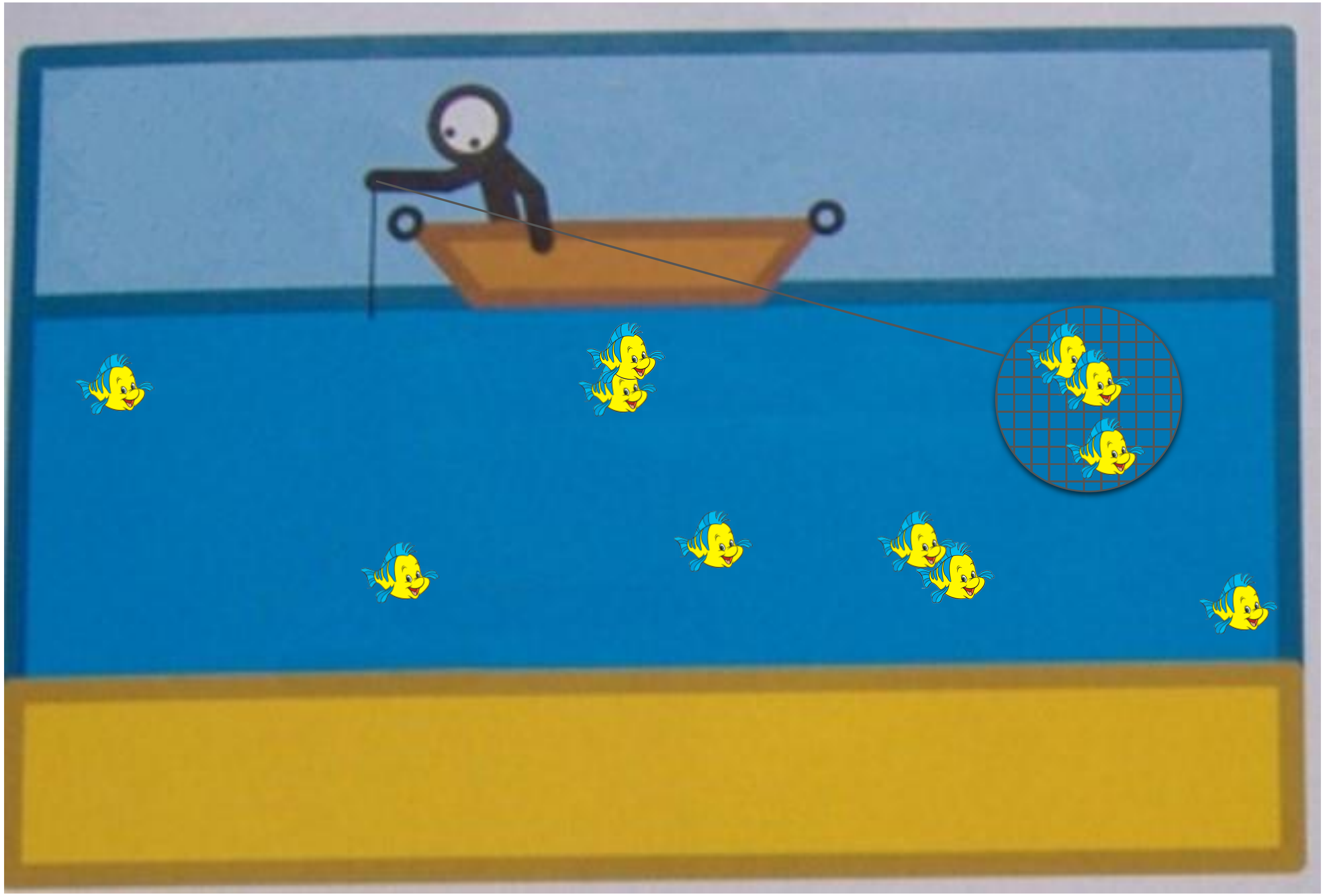
It can be 0
It can be 1

EXPERIMENT OF THE FISHERMAN



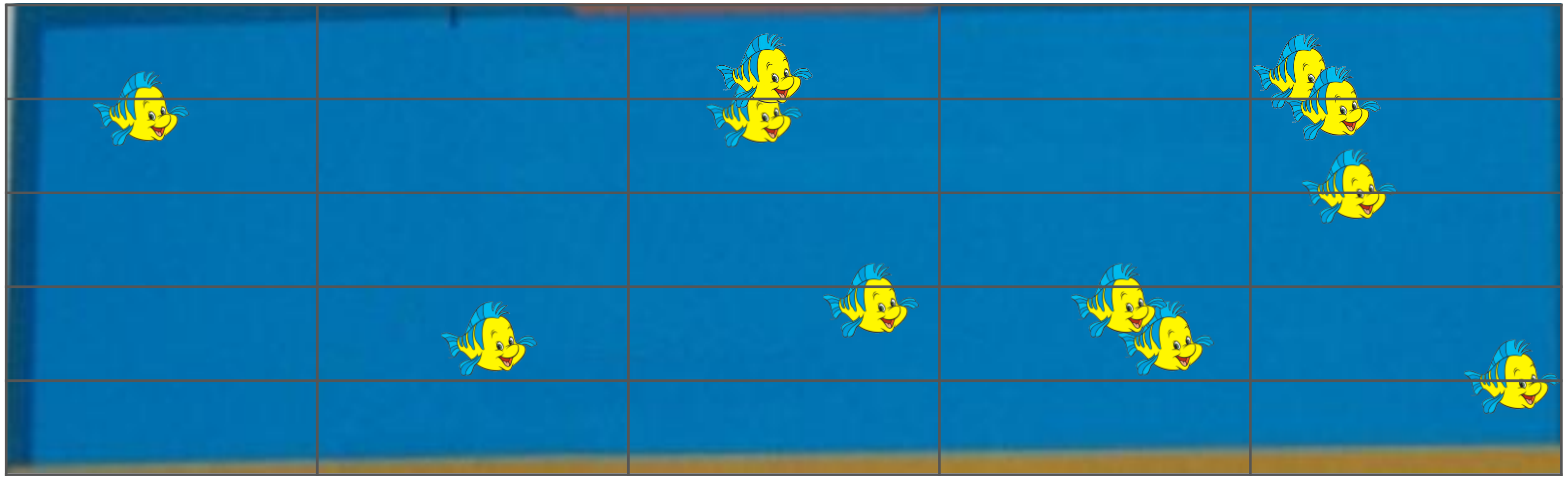
It can be 0
It can be 1
It can be 2

EXPERIMENT OF THE FISHERMAN

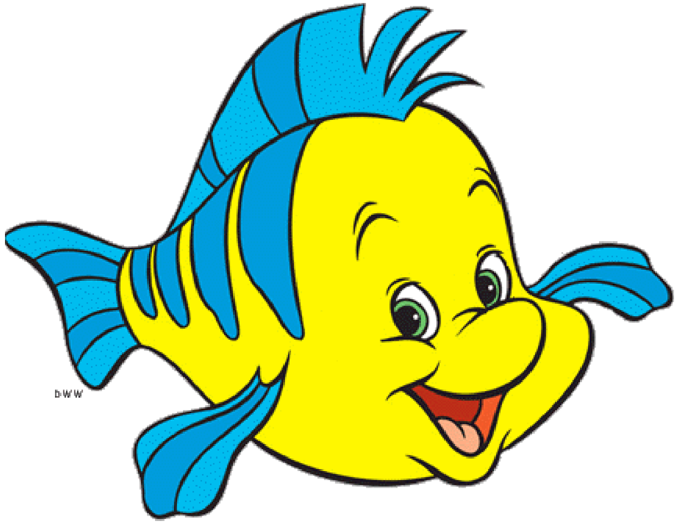


It can be 0
It can be 1
It can be 2
It can be 3

PHENOMENON BASED ON RANDOMIZED DISTRIBUTION



Loi de Poisson



PHENOMENON BASED ON RANDOMIZED DISTRIBUTION

$$P[X = k] \approx \frac{\lambda^k}{k!} e^{-\lambda}$$

X = the probability you are calculating (eg : how many drops contain X cells, or what is the probability to have X cells in a drop)
Lambda = mathematical expectation (e.g : 10 000 cells in 10 000 drops will lead on average to 1 cell per drop ➔ Lambda = 1 in this case

Loi de (Siméon Denis) Poisson

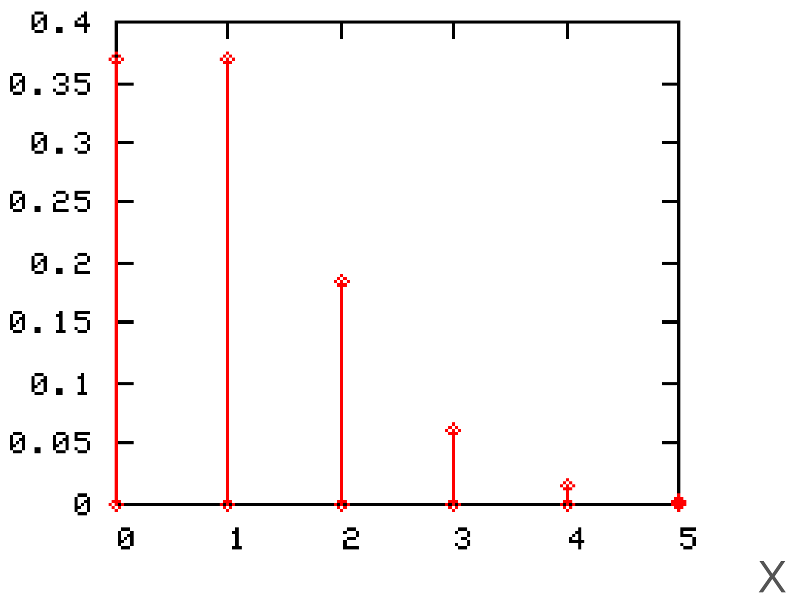
$$P[X = k] \approx \frac{\lambda^k}{k!} e^{-\lambda}$$

Try to encapsulate 10 000 cells in 10 000 drops

Lambda = 1 ➔ $P[X=k] = \frac{0,3679}{k!}$

P(X=0) = 36,79% ➔ 3679 drops with 0 cells,
P(X=1) = 36,79% ➔ 3679 drops with 1 cell,
P(X=2) = 18,39% ➔ 1839 drops with 2 cells,
P(X=3) = 6,13% ➔ 613 drops with 3 cells,
P(X=4) = 1,53% ➔ 153 drops with 4 cells,
….

P(X) with lambda = 1



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APPLICATION : THE DROPSEQ PROCESS

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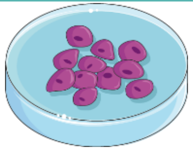


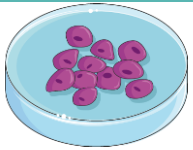
Cells preparation
The sample

DROPSEQ

Next gen sequencing

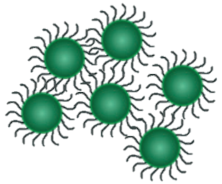
Data processing

APPLICATION : THE DROPSEQ PROCESS



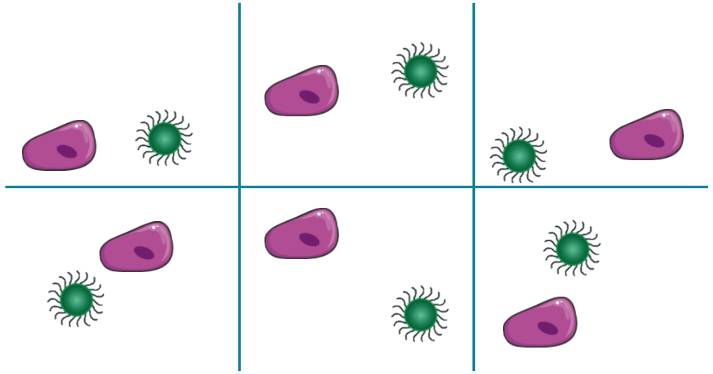
Cells preparation

The sample



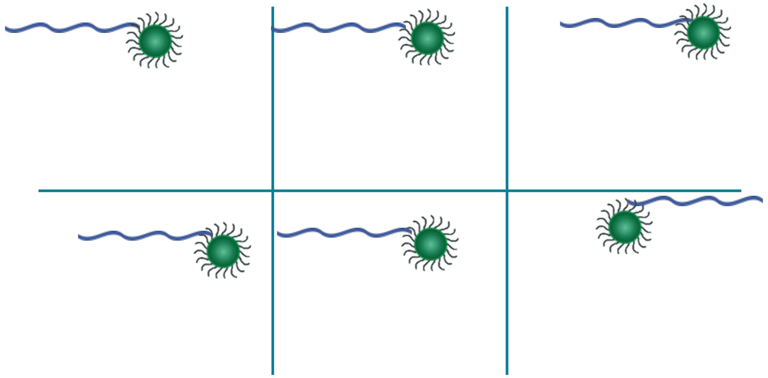
DropSeq beads

Will allow for retrieval of RNA and identification during the sequencing run

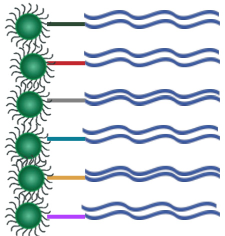


Sample compartmentalization

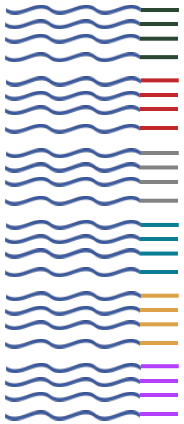
Cells are lysed individually to allow correct tagging of the RNA



RNA retrieval and tagging



RNA processing into barcoded cDNA

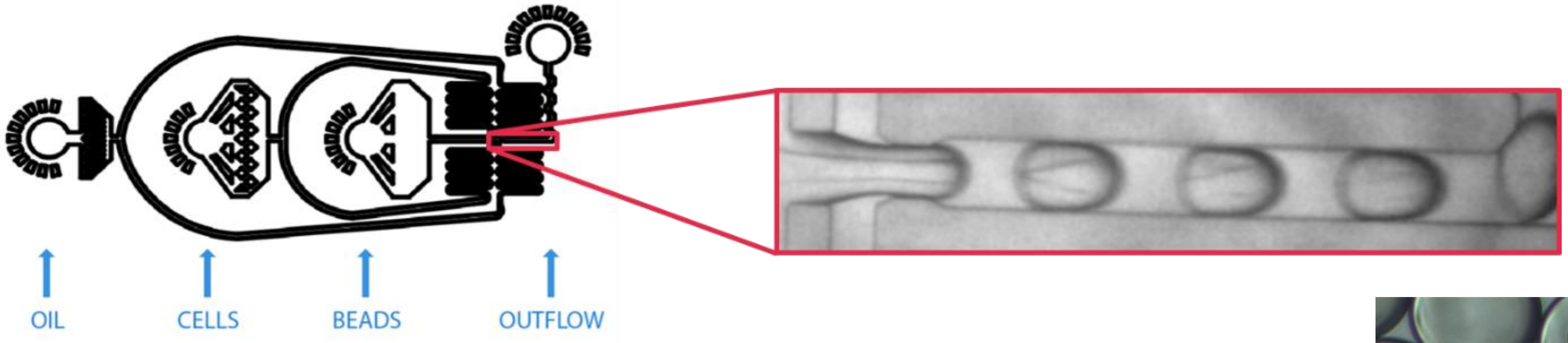


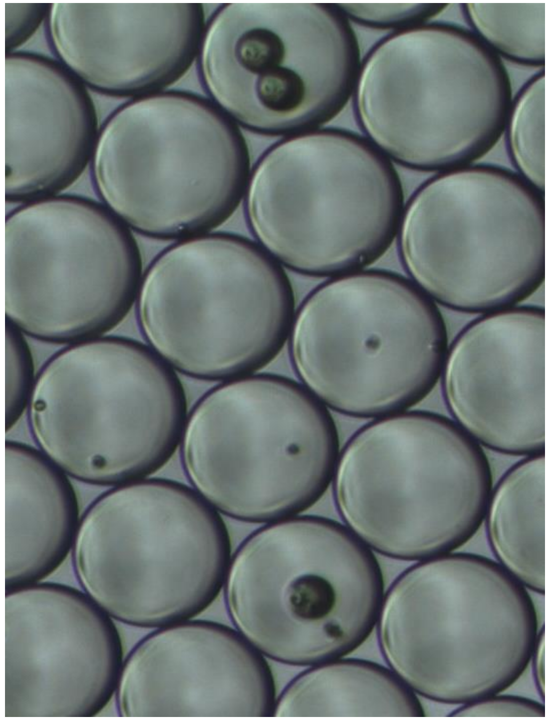
Amplification by PCR

Next gen sequencing

Data processing

DROPSEQ ENCAPSULATION PROCESS





A DOUBLE POISSON DISTRIBUTION ?

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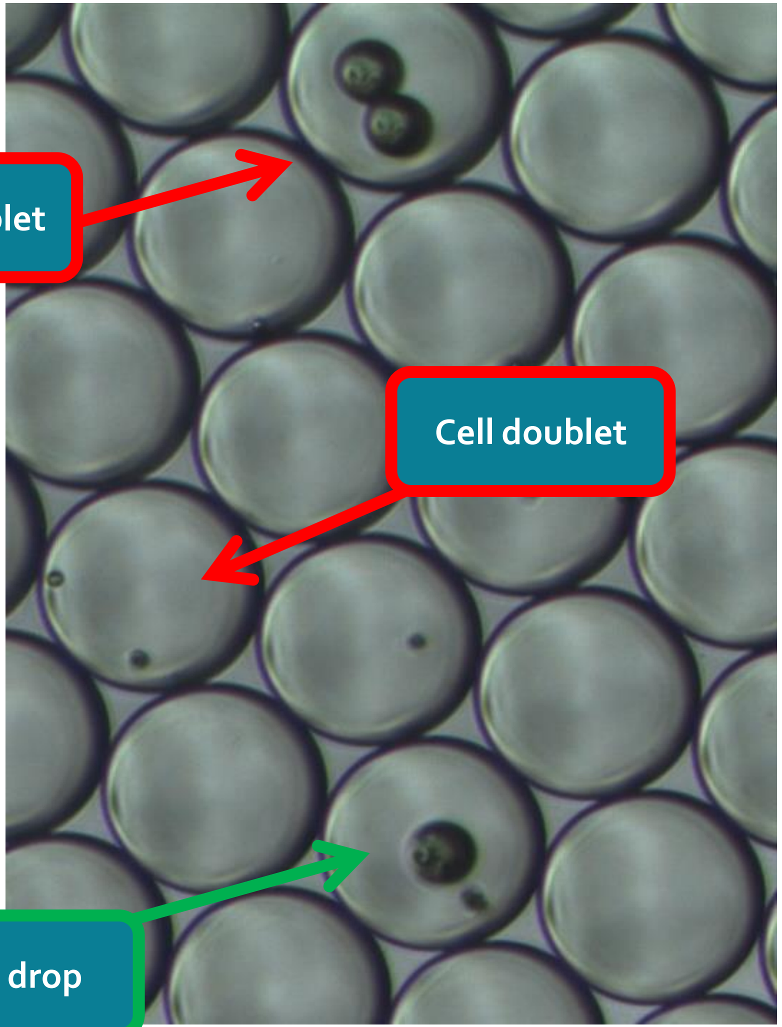
Beads Poisson distribution → Lambda Beads

Cells Poisson distribution → Lambda Cells

What will the final distribution be ?



HOW TO HAVE A LOW DOUBLET RATE

$$P[X = k] \approx \frac{\lambda^k}{k!} e^{-\lambda}$$

$$\frac{P[X=2]}{P[X=1]}(\lambda) = \frac{\lambda}{2}$$

To have a negligible doublet rate (and in general multiplets), you need to have Lambda << 1

HOW TO CAPTURE MORE SEQUENCES

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$$P[X = k] \approx \frac{\lambda^k}{k!} e^{-\lambda}$$

$$\frac{P[X=0]}{P[X=1]}(\lambda) = \frac{1}{\lambda}$$

To have a good encapsulation rate (low number of empty drops), you need to go at high Lambdas

WHAT TO CHOOSE ????

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To have a negligible doublet rate (and in general multiplets), you need to have Lambda << 1

To have a good encapsulation rate (low number of empty drops), you need to go at high Lambdas

WHAT TO CHOOSE ????

To have a negligible doublet rate (and in general multiplets), you need to have Lambda << 1

To have a good encapsulation rate (low number of empty drops), you need to go at high Lambdas

FINAL CHOICE

Why Lambda = 0,1 ?

- Doublets will generate noise ➔ unusable data
- Uncaptured cells will generate noise
- Uncaptured cells will not be sequenced properly
- Beads can capture environmental mRNA ➔ noise again

DropSeq protocol performs best when you have a decent redundancy in your sample

DROPS DISTRIBUTION

The two distributions are independant

↓

Any subpopulation has the same statistics than the global drops sample with regards to the other distribution

λ = 0,1

K = 0 : 90.5%
K = 1 : 9%
K = 2+ : 0.5%

No bead and no cell : 0.905 x 0.905 = 82% empty drops
1 Bead and no cell : 0.09 x 0.905 = 8%
No bead and 1 cell : 0.905 x 0.09 = 8%
1 bead and 1 cell : 0.09 x 0.09 = 0.8%
2+ beads and/or 2+ cells = 1,2%

YOUR TURN TO WORK !

- Theoretical questions :
  - Calculate precisely how many cells you will theoretically capture if you start with 100 000 cells
  - Why do people use Ficoll solutions and a stirring system ? Try to calculate the ideal concentration of ficoll solution for beads mix.
  - Calculate how many beads you need for making 1mL of bead solution for making 1nL droplets
  - Bonus 1 : try to design the system to produce drops
  - Bonus 2 : try to calculate the pressure required to get 40µL/min water and 80µL/min HFE flowrates through the system

YOUR TURN TO WORK !

- Practical questions :
  - How to calculate the size of the drops while flowing
  - How to then determine the frequency ? Is it matching the real frequency ?
  - How to calculate the size of the drops post experiment ? Is it matching the previous calculation ?

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CELLS CAPTURE : DROPS CONTAINING 1 CELL

The two distributions are independant

Any subpopulation has the same statistics than the global drops sample with regards to the other distribution

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K = 0 : 90.5%
K = 1 : 9%
K = 2+ : 0.5%

Drops containing 1 cell and K beads :

K = 0 : 90.5%
K = 1 : 9%
K = 2+ : 0.5%

Drops containing 1 cell and K beads :

90.5% uncaptured cells
9% correctly captured cells
K = 2+ : 0.5% generating noise

OVERALL CELL CAPTURE

9% of drops contain 1 cell
0,5% contain 2 or more cells

Let's say we have 100 000 cells

➔ How many cells will be alone in a drop ?

$$\frac{9 * 1}{9 * 1 + 0.5 * 2} = 90\%$$

➔ How many cells will be 2 in a drop ?

$$\frac{0.5 * 2}{9 * 1 + 0.5 * 2} = 10\%$$

HOW MANY CELLS WOULD BE CAPTURED CORRECTLY THEORETICALLY

90 % of cells are in a 1 cell droplet

9 % of cells are with 1 bead

100 000 x 90% x 9% = 8100 cells

Faster calculation : 0.81% of drops contain 1 bead and 1 cell
There are 10 times more drops than cells (lambda = 0,1)
➔ 8,1% of cells are with 1 bead only ➔ 100 000 * 8,1% = 8 100

FICOLL MATCHING BEADS DENSITY

- Beads density is roughly 1.05
- Water density is roughly 1
- Ficoll is roughly 1,2 at 50% w/v concentration and 1,05 at 12% w/v
- Roughly 25% of Ficoll 50% in the mix

HOW MANY BEADS NEEDED

- 1mL of bead solution : total aqueous solutions is 2 mL
- 1nL drops from 2mL solution : 2 million drops
- Lambda = 0,1 ➔ 200 K beads needed

SYSTEM DESIGN