Lec 4. Droplet microfluidics

Zida Li
Associate Professor

深圳大学
SHENZHEN UNIVERSITY

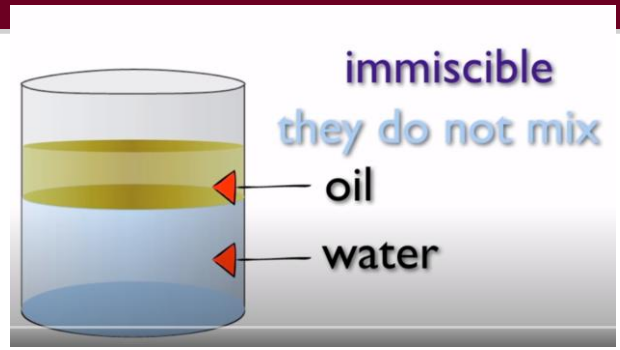
Content

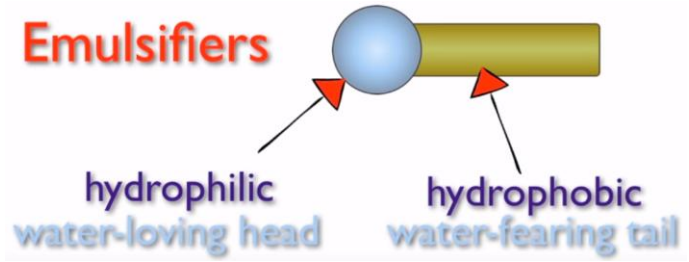
- Importance of droplet microfluidics
- Flow setup
- Surfactant
- Device design and fabrication

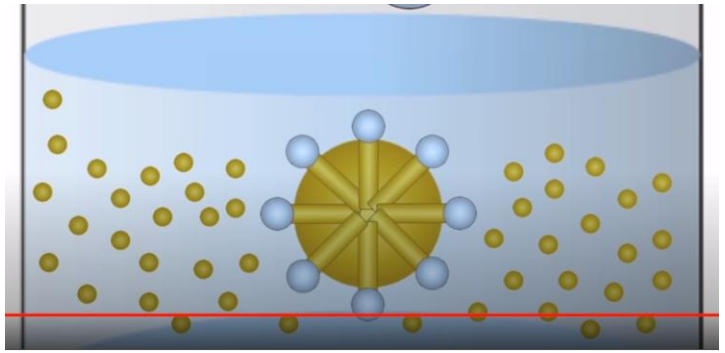
Why droplets?

Emulsion (乳液)

- A mixture of two immiscible liquids where one is dispersed as droplets in the other.
- Key distinction: Emulsions are stabilized by surfactants/emulsifiers → prevent coalescence.
- Everyday examples: milk (O/W), butter (W/O), lotions, mayonnaise.
- Relevance to microfluidics: Droplets = “tiny test tubes,” allowing isolated, controlled reactions.





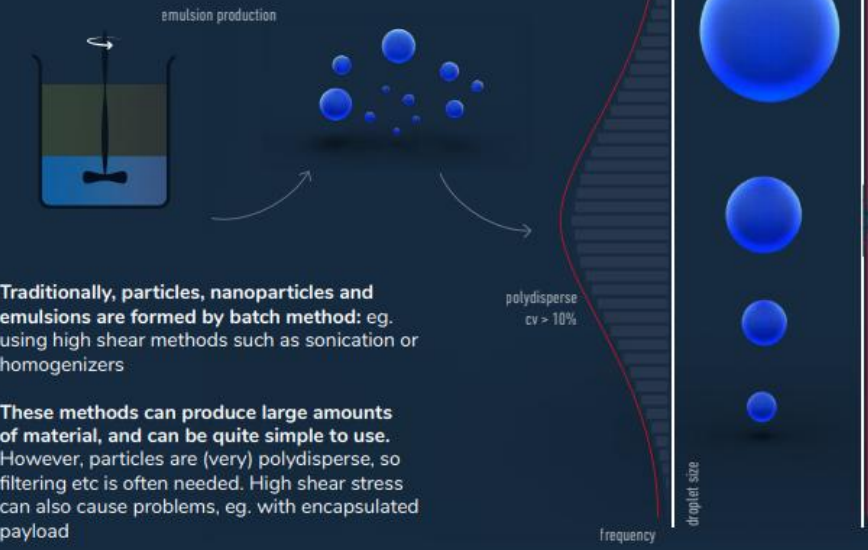


- Oil in water emulsion

MICROFLUIDICS AND DROPLETS

Why use microfluidics?

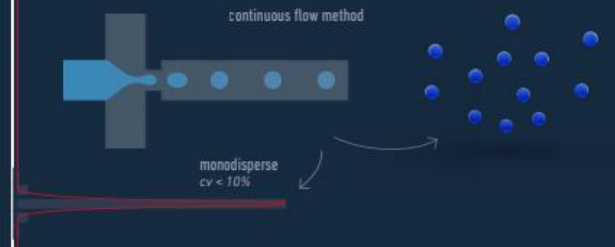
BATCH METHOD



Traditionally, particles, nanoparticles and emulsions are formed by batch method: eg. using high shear methods such as sonication or homogenizers

These methods can produce large amounts of material, and can be quite simple to use. However, particles are (very) polydisperse, so filtering etc is often needed. High shear stress can also cause problems, eg. with encapsulated payload

MICROFLUIDICS

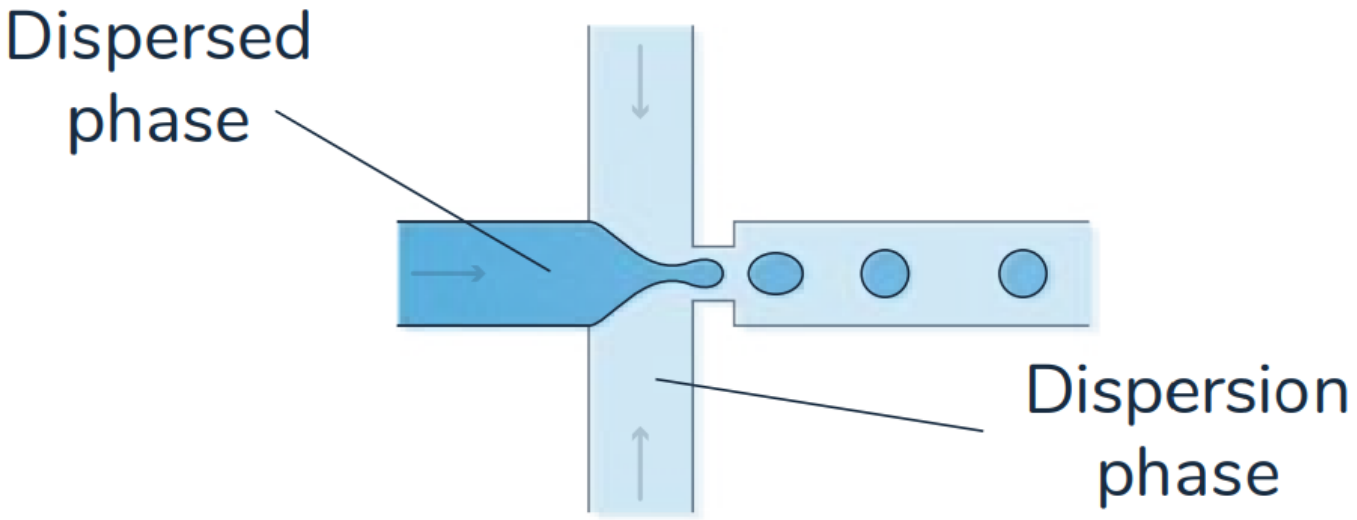


Microfluidic methods offer a number of significant advantages:

- 1MHz or 10kg/day of droplets/ particles is feasible by a very compact system
- Highly monodisperse (typically 1 to 5%)
- Tunable to any particle size below 1mm
- Complex particles can also be produced
- Amenable to real time Quality Control
- Wide range of particle formation methods
- Consistent and reproducible

Terminology

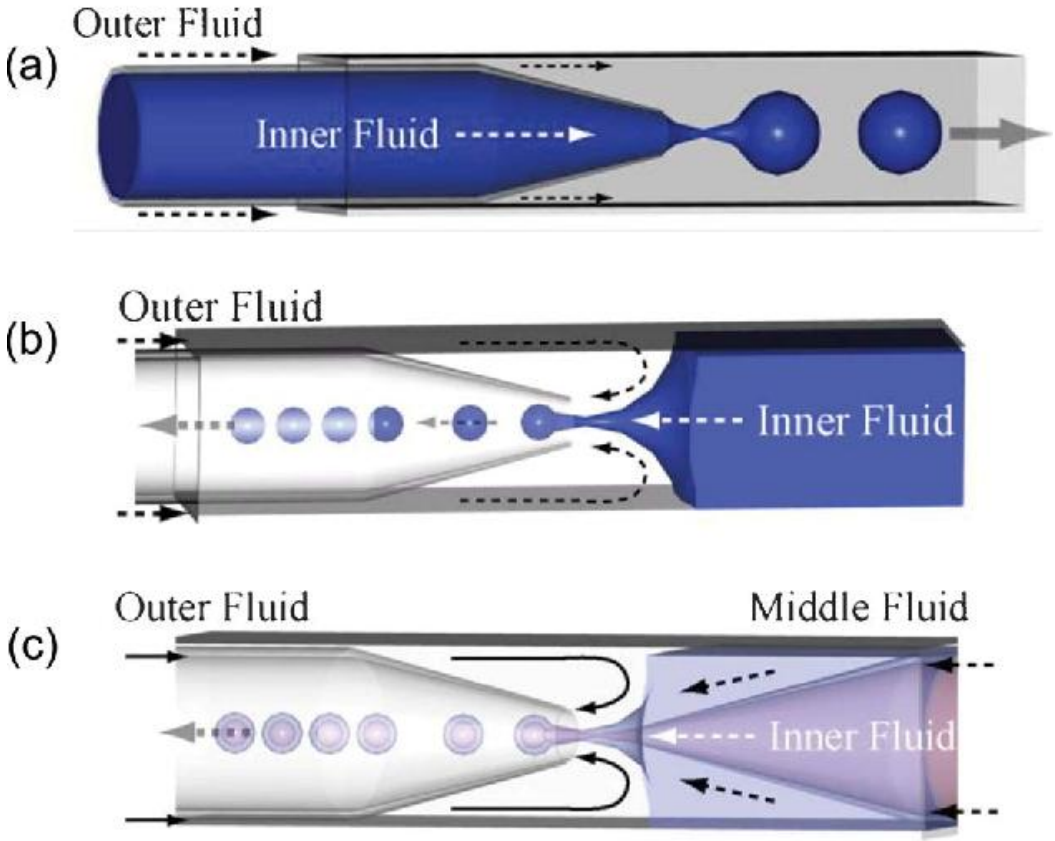
- Dispersed phase：分散相
- Continuous phase：连续相



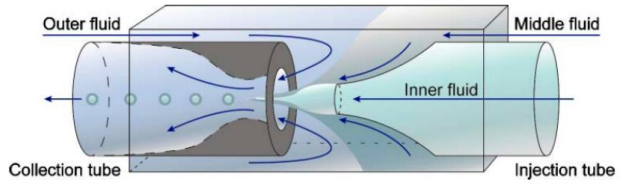
Different setups

- Co-flow
- Flow focusing
- T junction

Different setups: co-flow

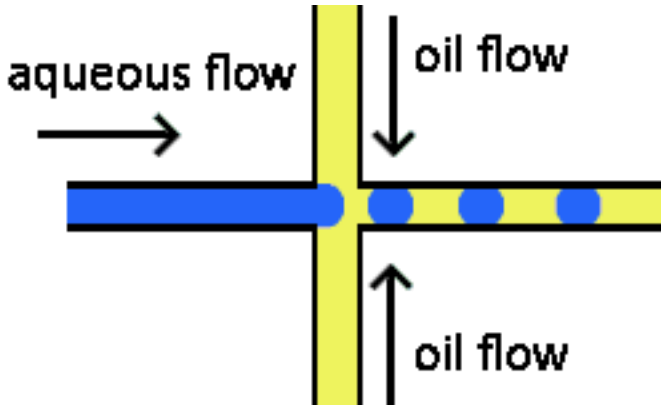


Generating droplets with capillaries



Utada AS, Lorenceau E, Link DR, Kaplan PD, Stone HA,Weitz DA. 2005. Monodisperse double emulsions generated from a microcapillary device. *Science 308:537*–41

Different setups: flow focusing



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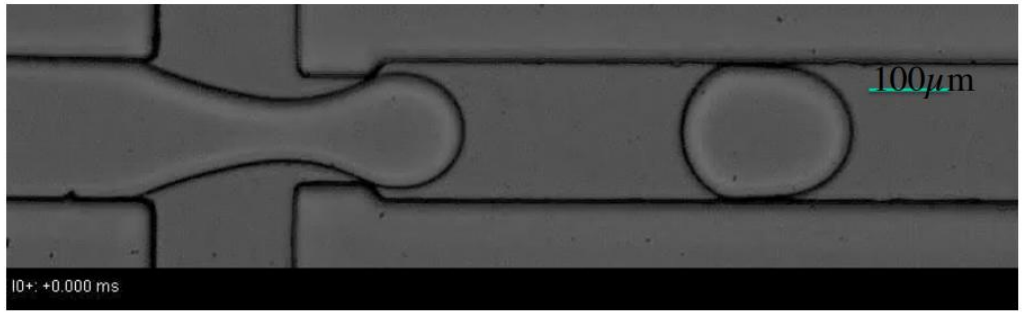
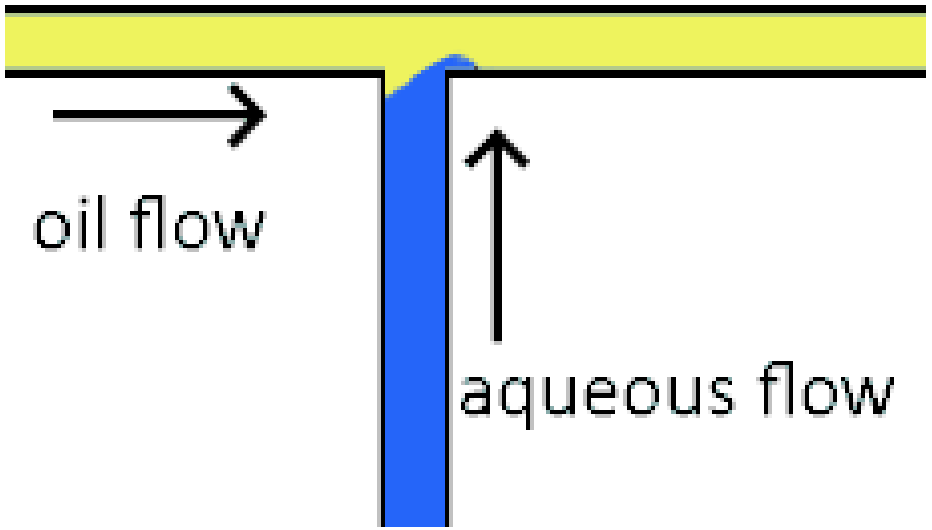
Flow focusing geometry

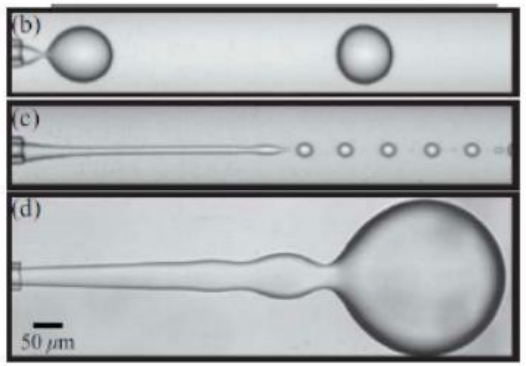


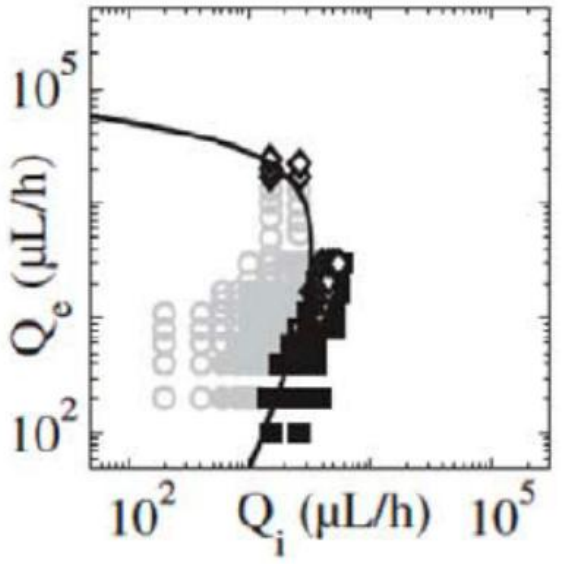
Different setups: T-junction



Flow regimes: dripping vs jetting

Theory of droplet formation with capillaries





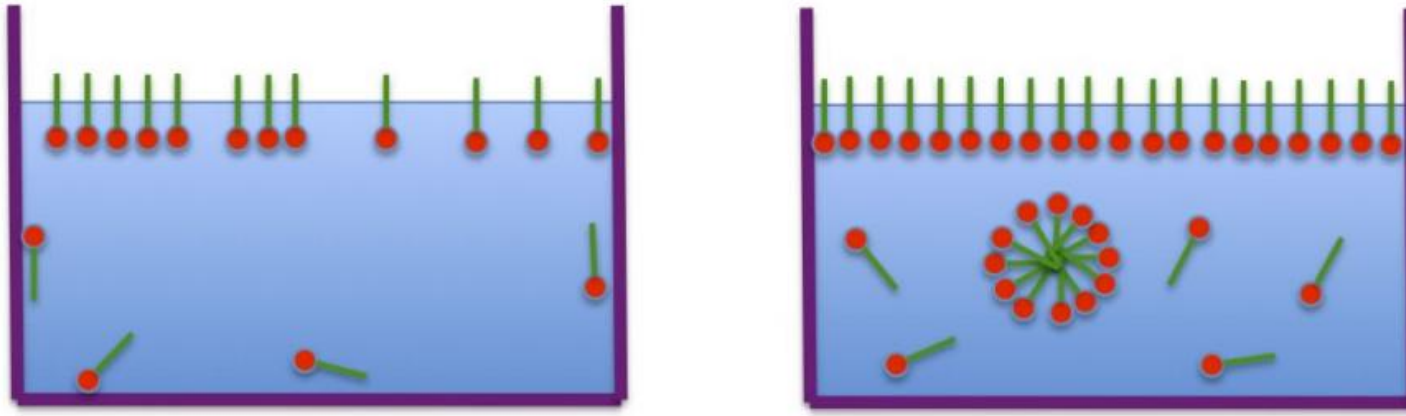
P. Guillot et al, *Phys. Rev. Lett.* (2007)

Surfactant

Surfactant Basics

- Amphiphilic molecule: hydrophilic head, hydrophobic tail
- Adsorb at oil–water interface
- Reduce interfacial tension
- Prevent droplet coalescence

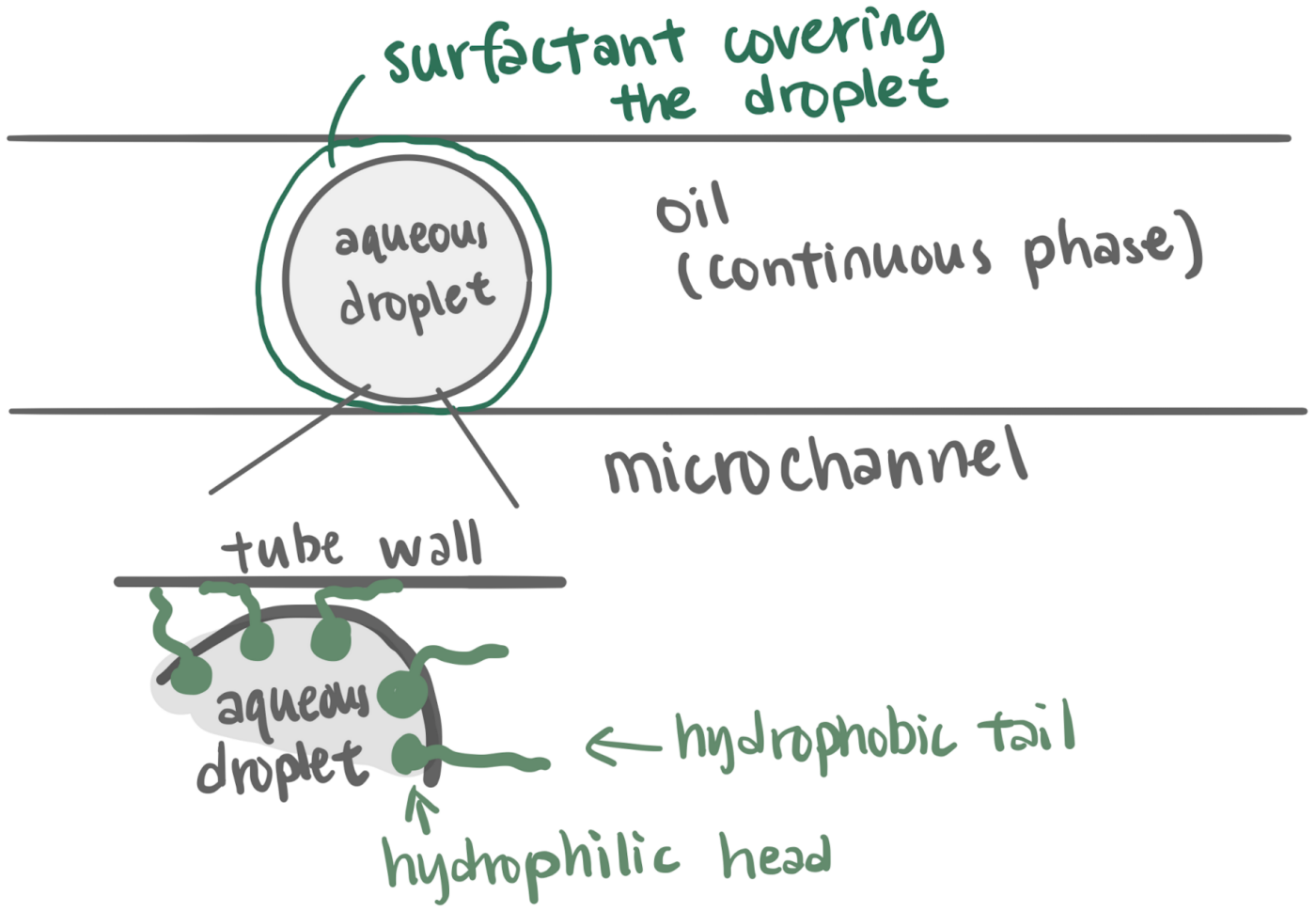
→ stable emulsions



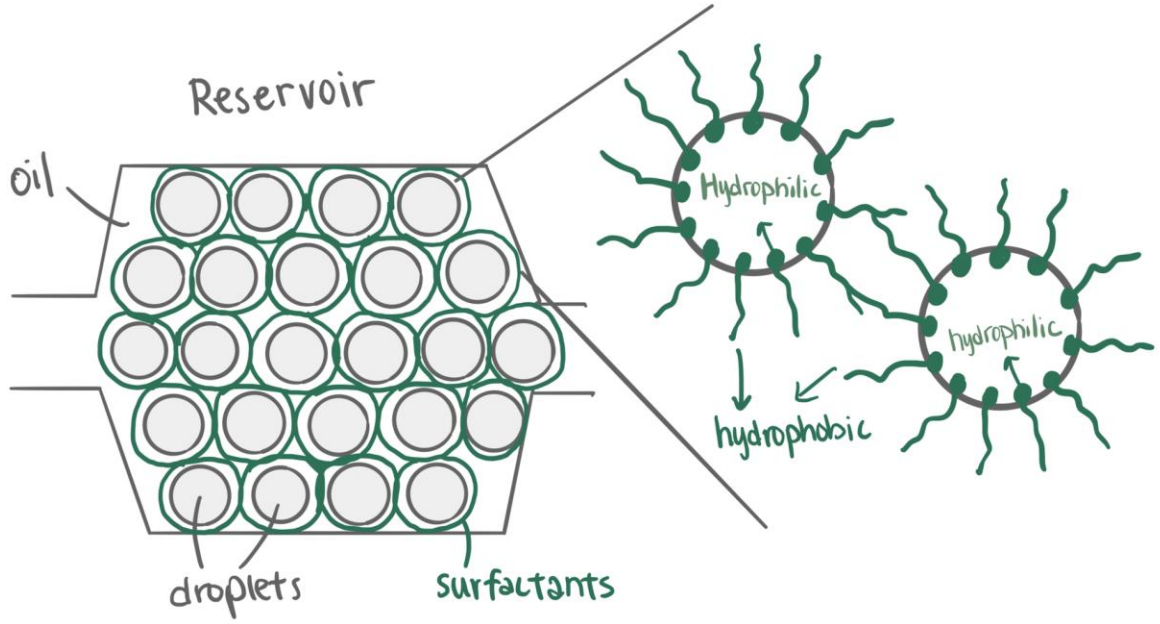
Type	Function	Example
Detergents	Remove dirt/oil by forming micelles	Laundry detergent
Foaming agents	Stabilize gas-liquid interfaces	Shaving foam, beer head
Wetting agents	Lower the contact angle so liquids spread better on solids	Paints, inks
Dispersants	Prevent solid particles from aggregating	Pigment dispersants in paint
Emulsifiers	Stabilize emulsions (= droplet mixtures of oil-in-water or water-in-oil)	Mayonnaise, lotions
Solubilizers	Help dissolve poorly soluble substances	Perfume in water-based sprays
Conditioners	Deposit active ingredients on hair/skin	Hair conditioners (often cationic surfactants)

Surfactant in Microchannels

- Coat droplets in microchannels
- Prevent sticking to channel walls
- Create protective barrier around droplets
- Controlled coalescence possible via surfactant coverage

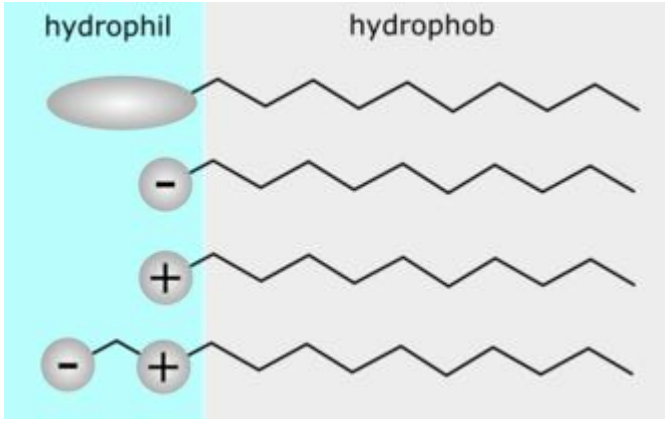


- Maintain droplet stability during storage
- Hydrocarbon oils not biocompatible
- Fluorosurfactants (e.g., PFPE–PEG block copolymers) enable stability + biocompatibility
- Essential for live cell work & biochemical assays



Types of surfactants

- non-ionic,
- anionic,
- cationic,
- amphoteric.



Common Surfactants in Microfluidics

1. **Fluorosurfactants (most widely used)**

- **PFPE–PEG block copolymers**
  - Structure: perfluoropolyether (PFPE) tails + PEG head
  - Properties: high droplet stability, biocompatible
  - Applications: single-cell assays, biochemical reactions
- **Fluorinated polyglycerols**
  - Customizable functional groups
  - Tunable surface chemistry

2. **Conventional Surfactants**

- **Span 80 (sorbitan monooleate)**
  - Oil-soluble surfactant (often with hydrocarbon oils)
  - Less biocompatible, mainly for material synthesis
- **Tween series (Tween 20, Tween 80)**
  - Water-soluble surfactants
  - Sometimes used for O/W emulsions

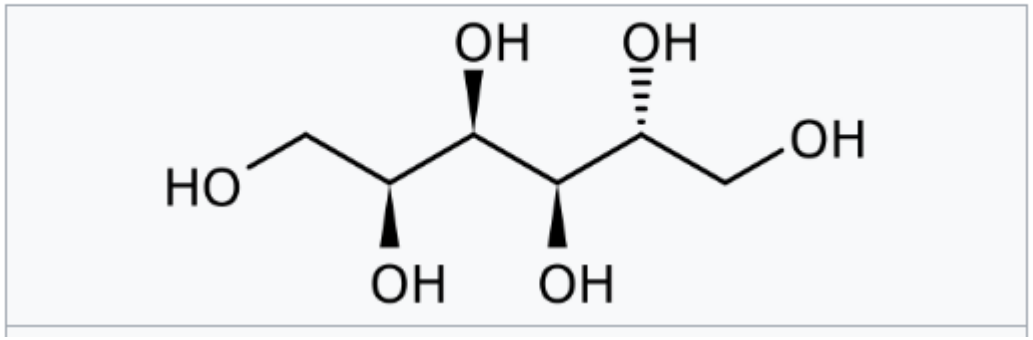
3. **Specialized Additives**

- Block copolymers and triblock surfactants for tailored stability
- Fluorinated surfactants from commercial suppliers:
  - RainDance / BioRad formulations
  - Miller–Stephenson fluorosurfactants

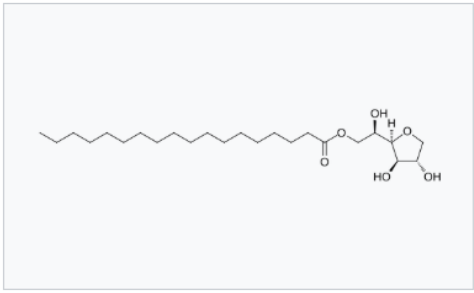
Span

- Sorbitol
  - Sugar alcohol
  - Sweetener

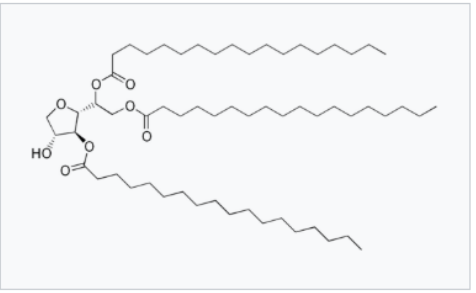
Sorbitol



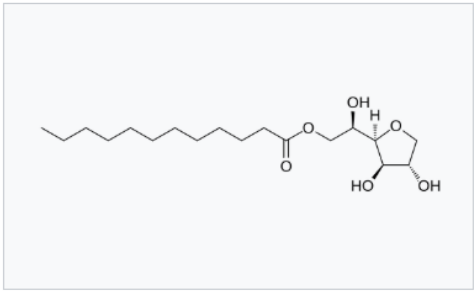
Sorbitan esters (Spans)



Sorbitan monostearate
(Span 60, E number: E491)

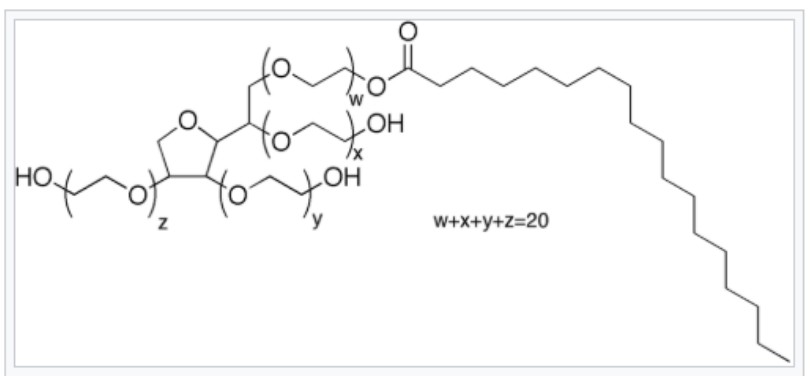


Sorbitan tristearate
(Span 65, E number: E492)



Sorbitan monolaurate
(Span 20, E number: E493)

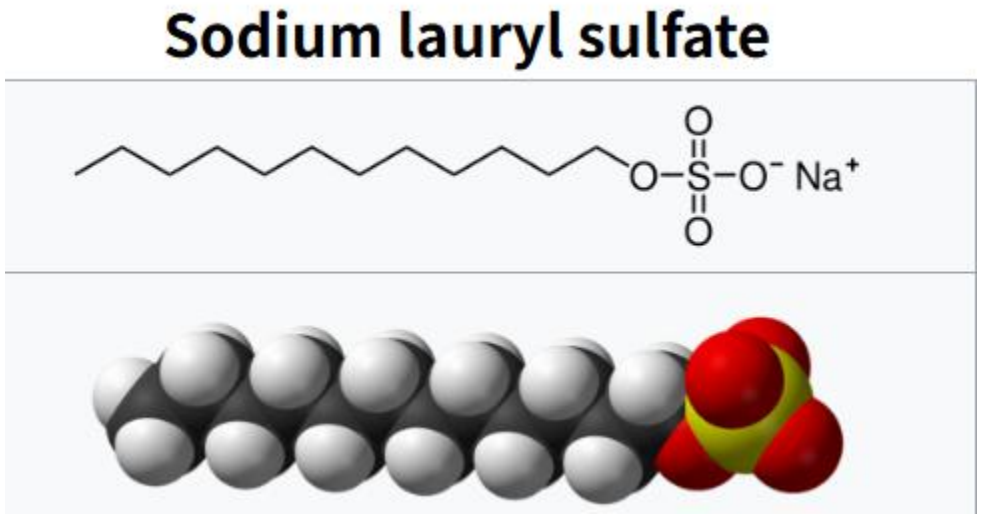
Tween



- **Tween** is the trade name for **Polysorbates.**
  They are **nonionic surfactants** made by **polyoxyethylene (PEG) derivatives of sorbitan esters**.
- Chemically:
- Start with **sorbitol** → **sorbitan** (dehydrated form).
- Esterify with fatty acids (as in Span).
- Then add **polyethylene oxide (ethoxylation)** to the free hydroxyl groups.
- So, **Tween = Polyoxyethylene sorbitan fatty acid ester**.

SDS

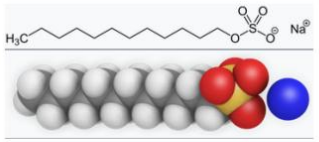
- **Full name**: Sodium dodecyl sulfate (also called sodium lauryl sulfate, SLS).
- **Type**: **Anionic surfactant**.
- **Structure**:
  - Long hydrophobic tail = **C_{12} hydrocarbon chain** (dodecyl group).
  - Hydrophilic head = **sulfate group (–$OSO_3^-Na^+$)**.
- So it's amphiphilic: hydrophobic tail + charged hydrophilic head.



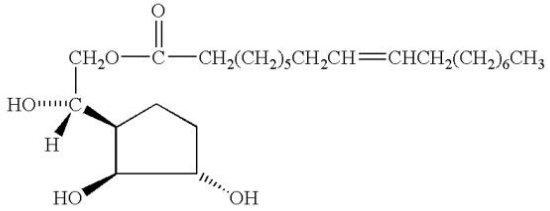
Surfactant

- Tween 20
- SDS
- SPAN

SDS



SPAN80



- The choice of surfactant depends on the oil phase.

Fluorinated oil as continuous phase

- **1. The Challenge with Fluorinated Oils**
- In droplet microfluidics, emulsions are usually **water-in-oil (W/O)**.
- Oils like **HFE-7500** (hydrofluoroether) and **Novec 7500** are *fluorinated*, meaning they are **very chemically inert, extremely hydrophobic, and lipophobic**.
- This makes them difficult to stabilize with conventional surfactants (like SDS, Span, or Tween), because those surfactants don’t dissolve well in fluorinated oils.

- **2. The Surfactant Solution: Fluorophilic Surfactants**
- To stabilize droplets in fluorinated oils, researchers use **amphiphiles with fluorinated tails** (not just hydrocarbon tails).
- **Fluorophilic tails**: Dissolve in the fluorinated oil.
- **Hydrophilic heads**: Interact with the aqueous droplet.
- These surfactants sit at the oil–water interface and prevent droplets from coalescing.

- **3. Common Surfactants for HFE and Novec Oils**

(a) PFPE–PEG block copolymers

- **PFPE** = Perfluoropolyether (fluorophilic block, soluble in fluorinated oils).
- **PEG** = Polyethylene glycol (hydrophilic block, stabilizes water phase).
- Example: **EA Surfactant (Ran Biotechnologies)**, **Krytox-PEG surfactants**.
- Widely used in droplet microfluidics for biological assays.

(b) Ionic fluorosurfactants

- Variants where PFPE tails are linked to charged groups.
- Can tune droplet stability depending on application.

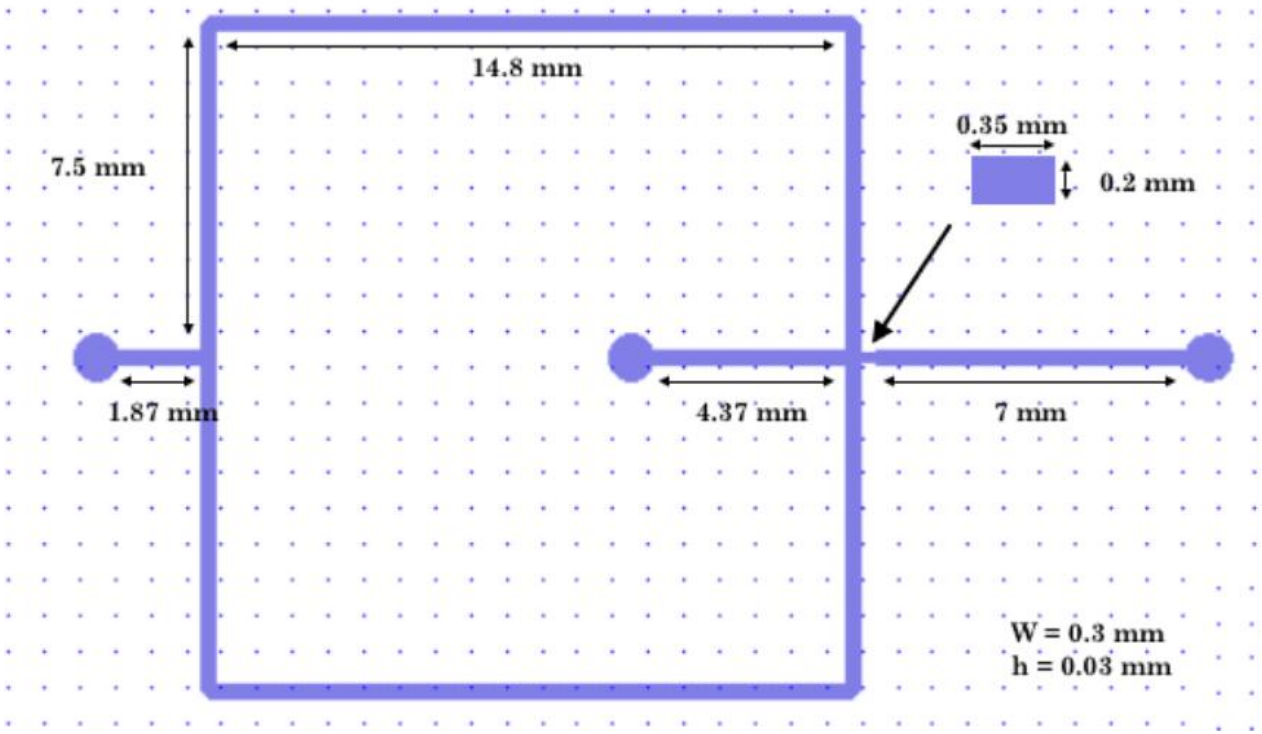
(c) Krytox derivatives

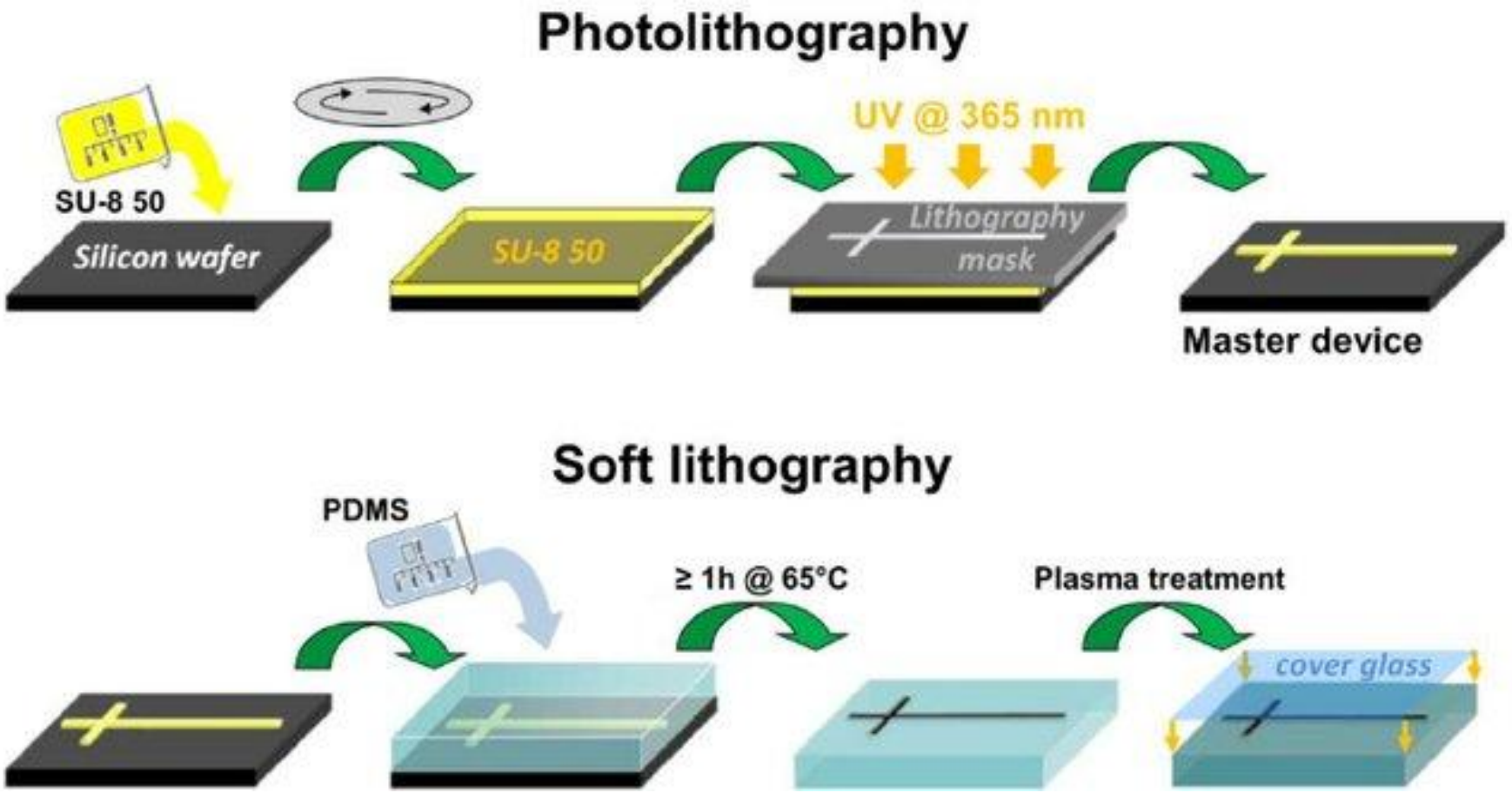
- Krytox (a commercial PFPE oil) can be modified with **carboxylic acid or ammonium heads** to act as a surfactant in HFE oils.
- Example: **Krytox 157 FSL** (carboxylic acid terminated PFPE).

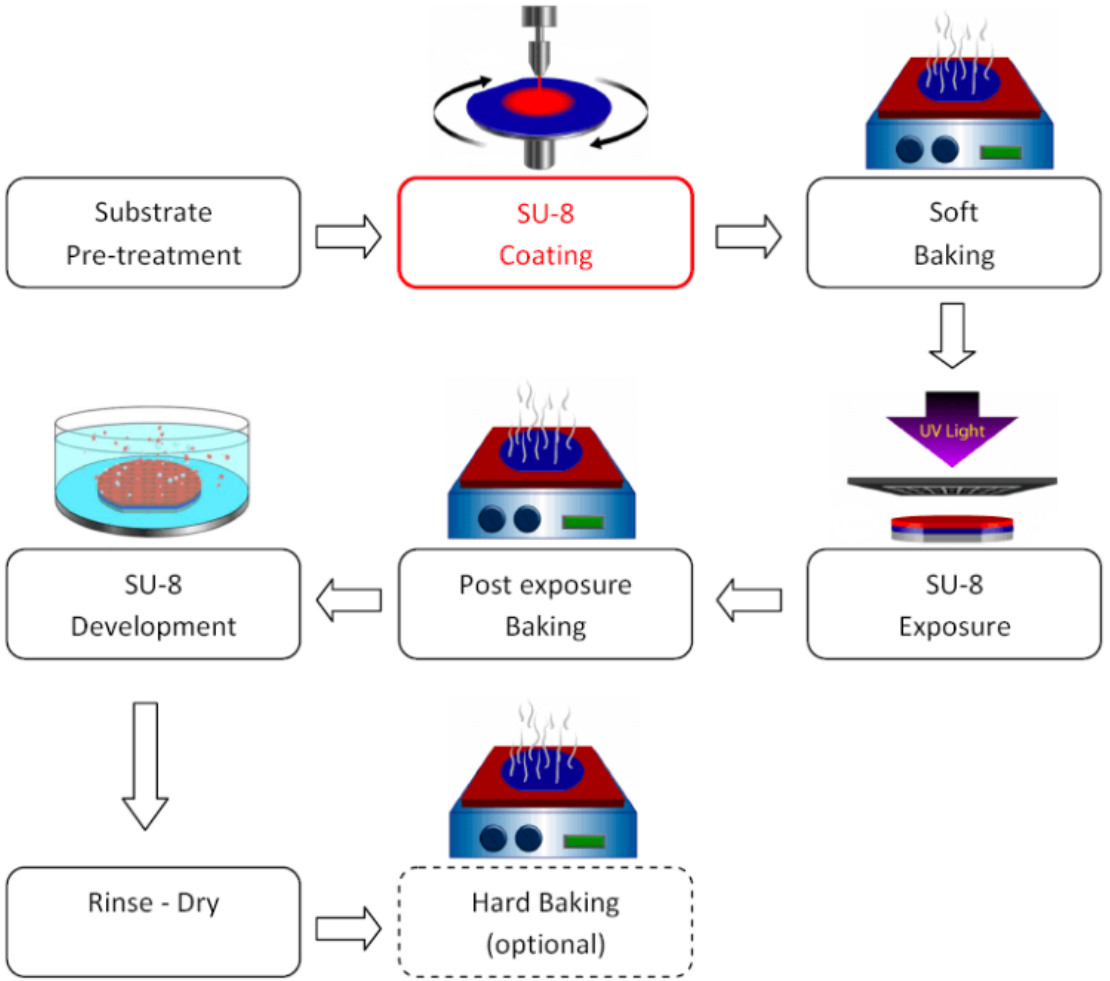
- **4. Why They Work**
- Fluorinated oils "like" fluorinated tails (fluorophilic interactions).
- A PEG (or ionic) headgroup provides droplet stabilization.
- This unique design prevents droplet merging and allows for **stable, monodisperse droplets** in microfluidic chips.

Device fabrication

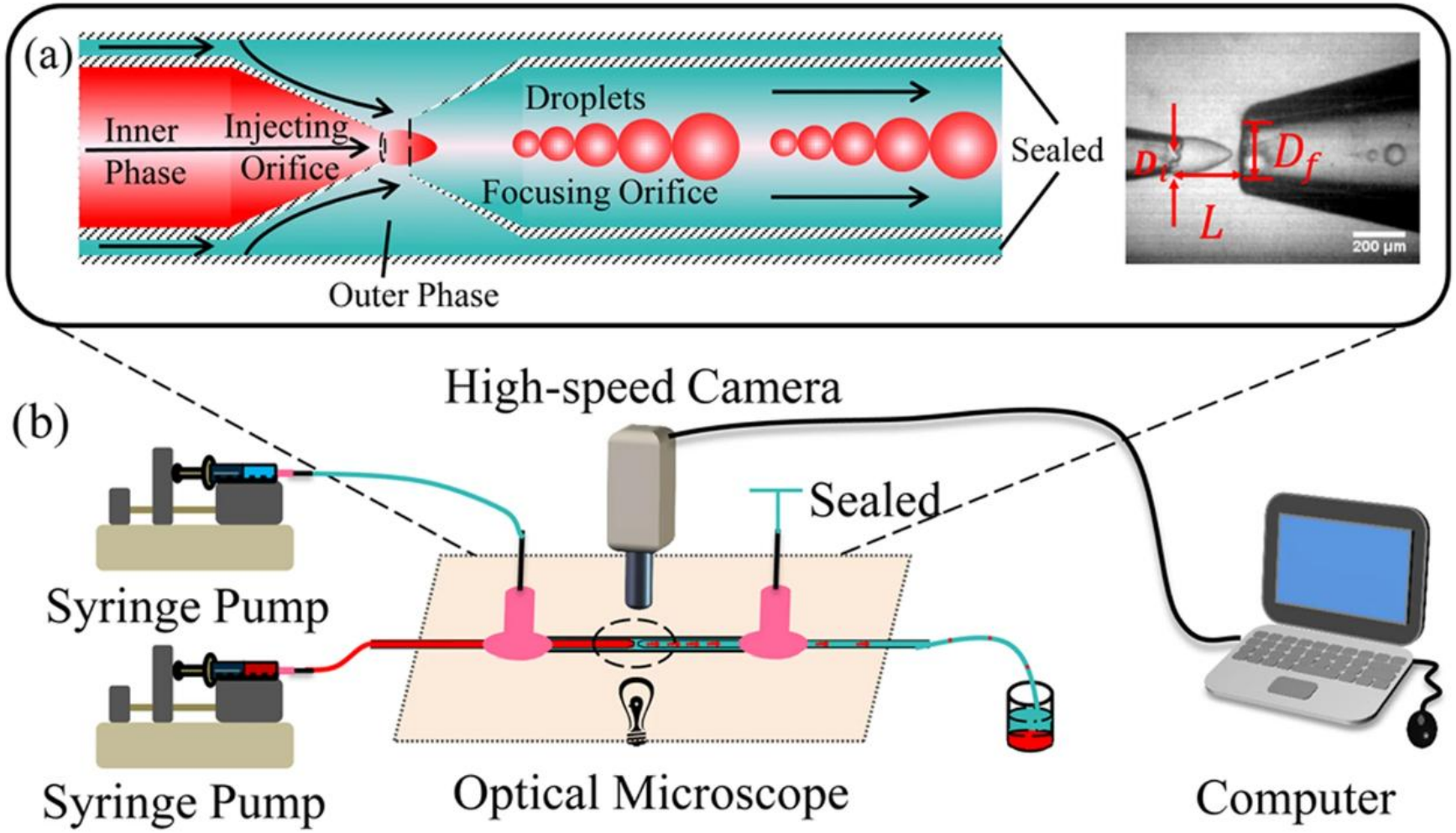
- Flow focusing
- Design mask in AutoCAD
- Generate SU-8 mold
- Replica molding PDMS
- Bonding
- Done

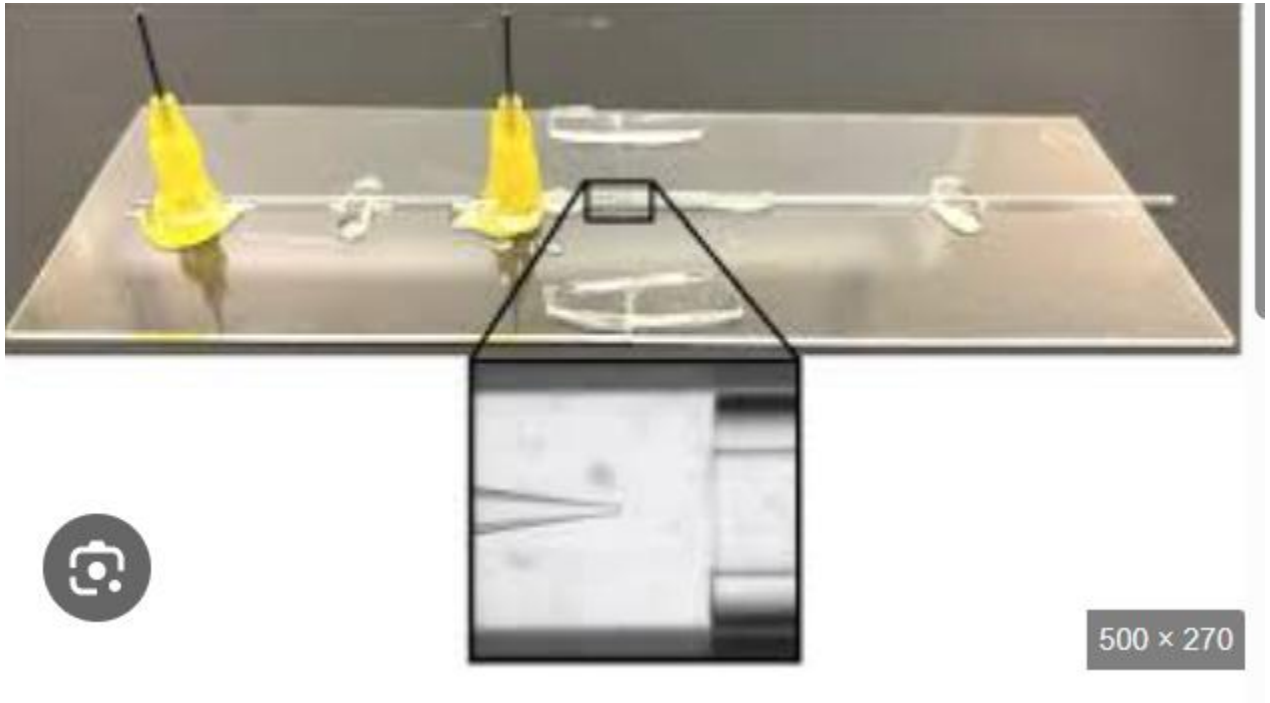



Photolithography
SU-8 50
Silicon wafer
SU-8 50
UV @ 365 nm
Lithography mask
Master device
Soft lithography
PDMS
≥ 1h @ 65°C
Plasma treatment
cover glass


Substrate
Pre-treatment
SU-8
Coating
Soft
Baking
UV Light
SU-8
Exposure
Post exposure
Baking
SU-8
Development
Rinse - Dry
Hard Baking
(optional)

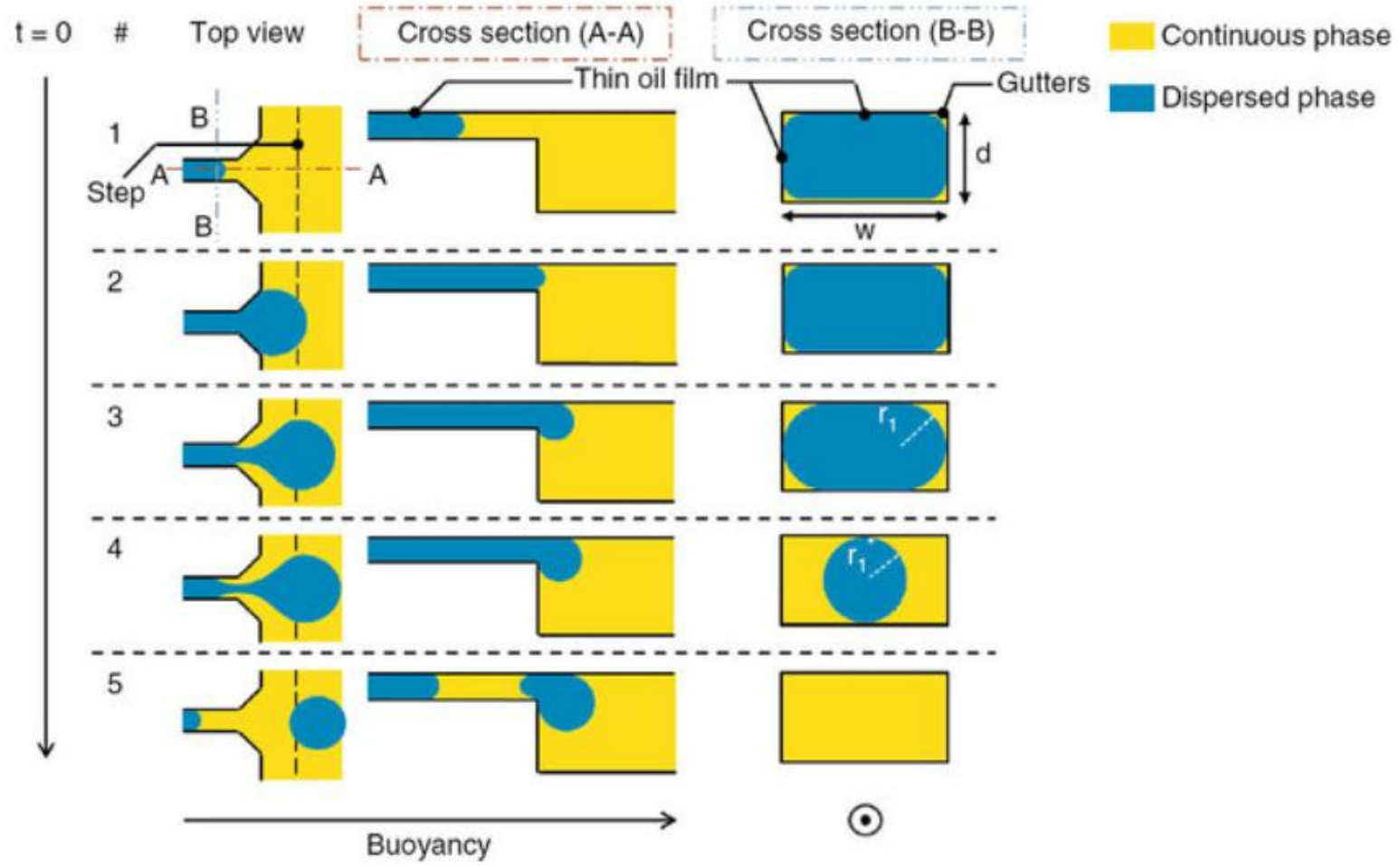
Glass capillary device



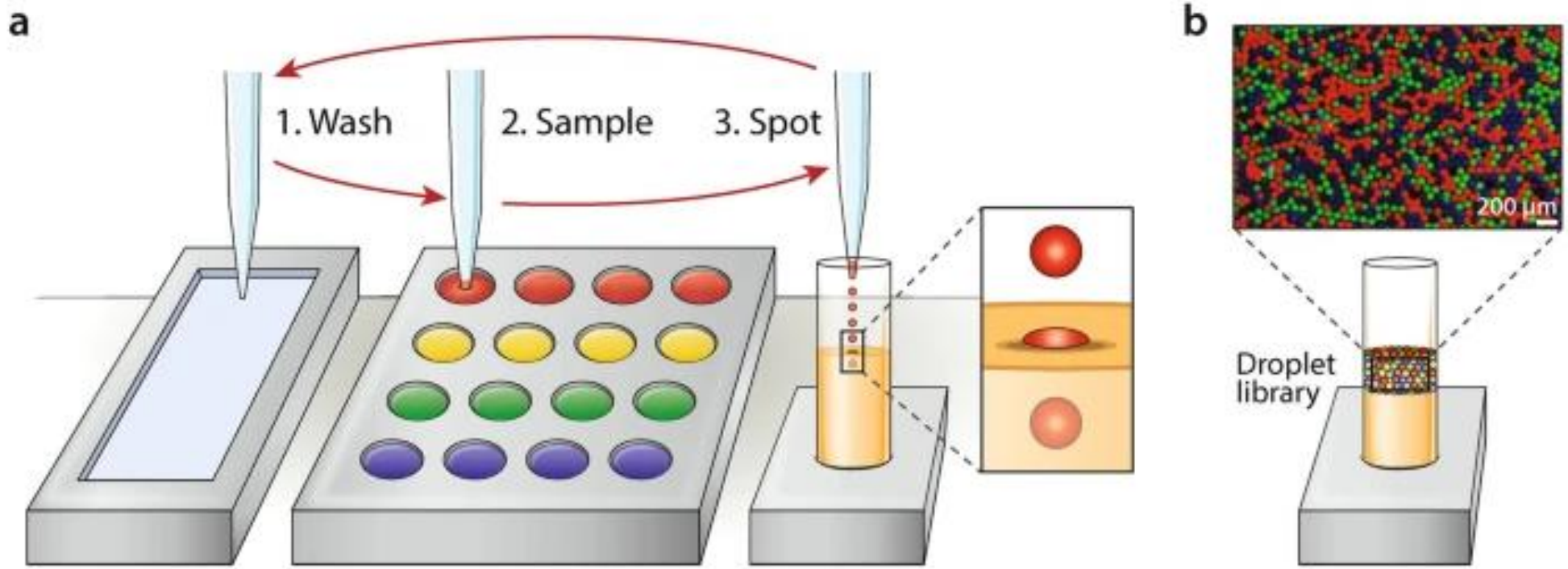

500 × 270

Non-conventional methods

Step emulsification



- Printing



Droplet manipulation techniques

- https://www.abatelab.org/microdroplets

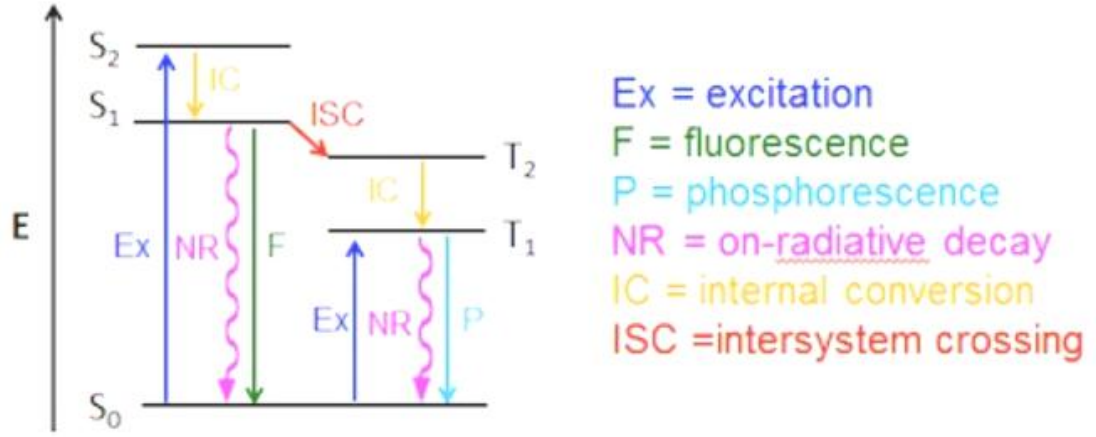
Droplet detection

- Fluorescence
- Absorption
- Impedance
- Microscopic brightfield imaging

Fluorescence

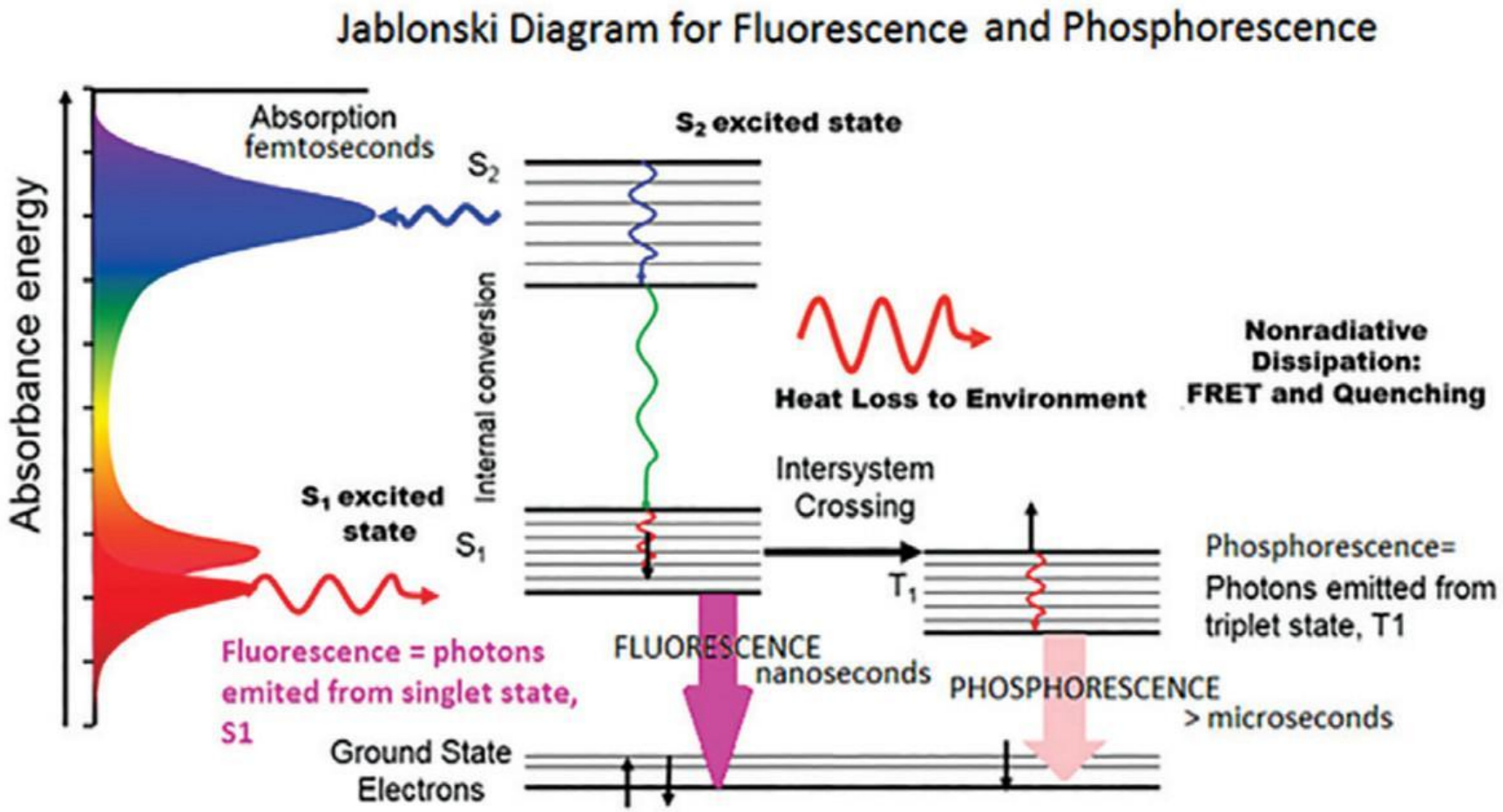
What is Fluorescence?

- **Definition**: Fluorescence is the emission of light by a substance that has absorbed light (or other electromagnetic radiation).
- **Key feature**: Emission occurs at a **longer wavelength** (lower energy) than excitation.
- **Timescale**: Very fast (nanoseconds).



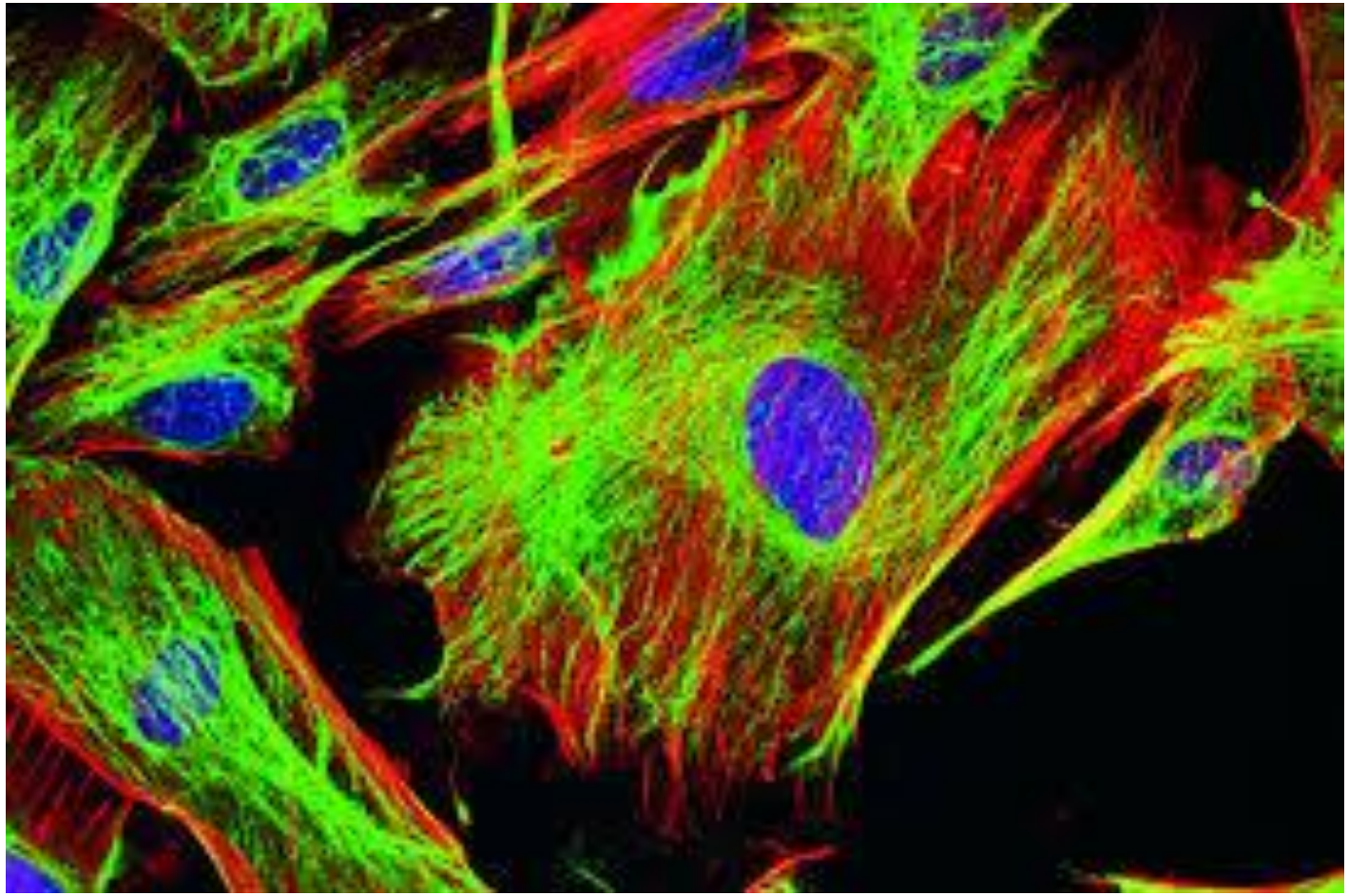
Principles of Fluorescence

- **Excitation**: A fluorophore absorbs a photon → electron promoted to higher energy state.
- **Relaxation**: Electron loses some energy (non-radiative).
- **Emission**: Electron falls back → emits photon of lower energy.
- **Stokes shift**: Difference between excitation and emission wavelengths.


Jablonski Diagram for Fluorescence and Phosphorescence
Absorbance energy
Absorption
femtoseconds
S2 excited state
S2
Internal conversion
Heat Loss to Environment
Nonradiative
Dissipation:
FRET and Quenching
Intersystem
Crossing
S1 excited
state
S1
T1
Phosphorescence=
Photons emitted from
triplet state, T1
Fluorescence = photons
emited from singlet state,
S1
FLUORESCENCE
nanoseconds
PHOSPHORESCENCE
> microseconds
Ground State
Electrons

Fluorophores

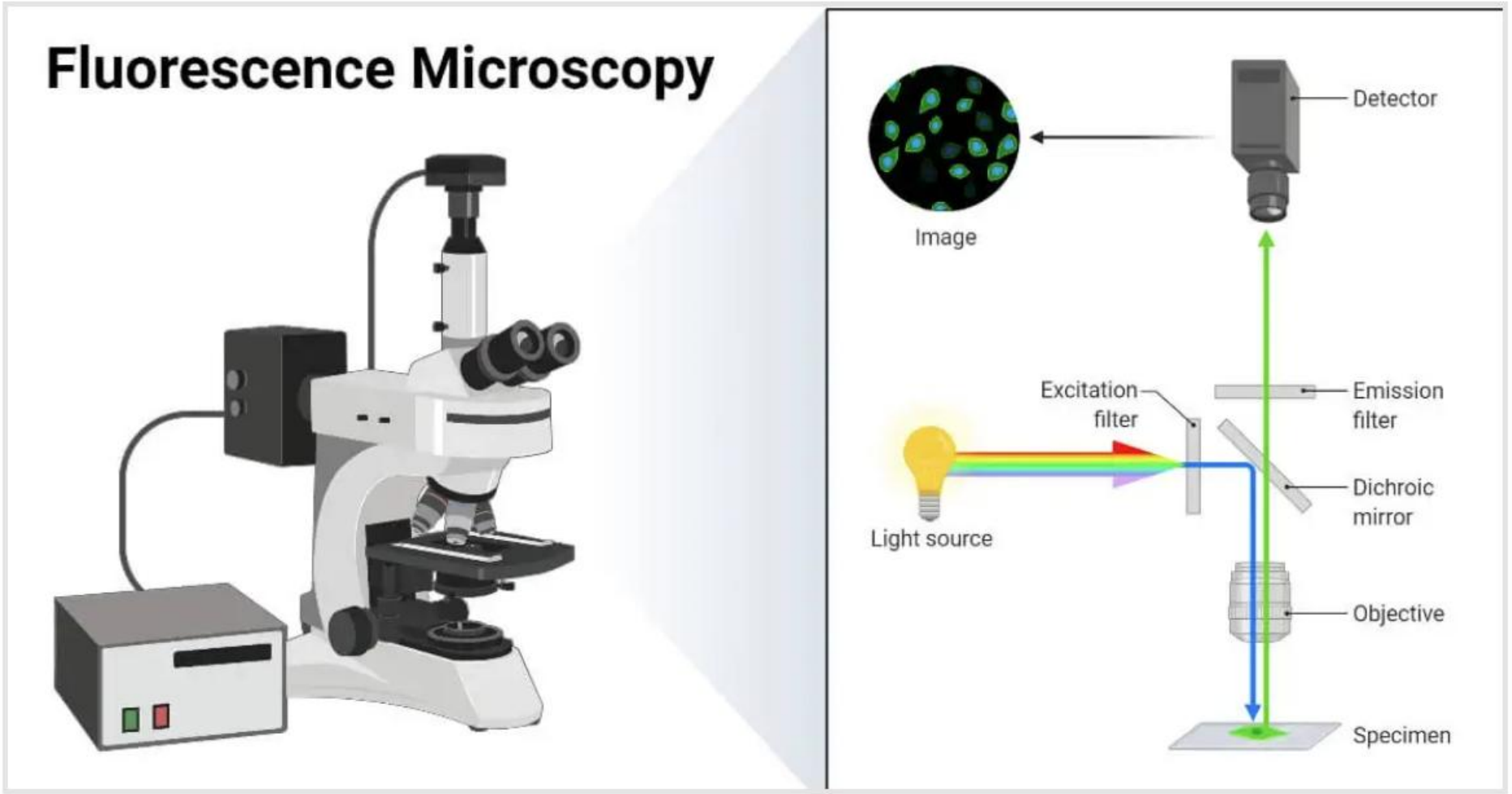
- **Intrinsic fluorophores** (e.g., NADH, tryptophan).
- **Synthetic dyes** (e.g., fluorescein, rhodamine).
- **Fluorescent proteins** (e.g., GFP, mCherry).
- Chosen based on **spectral properties, brightness, and photostability**.



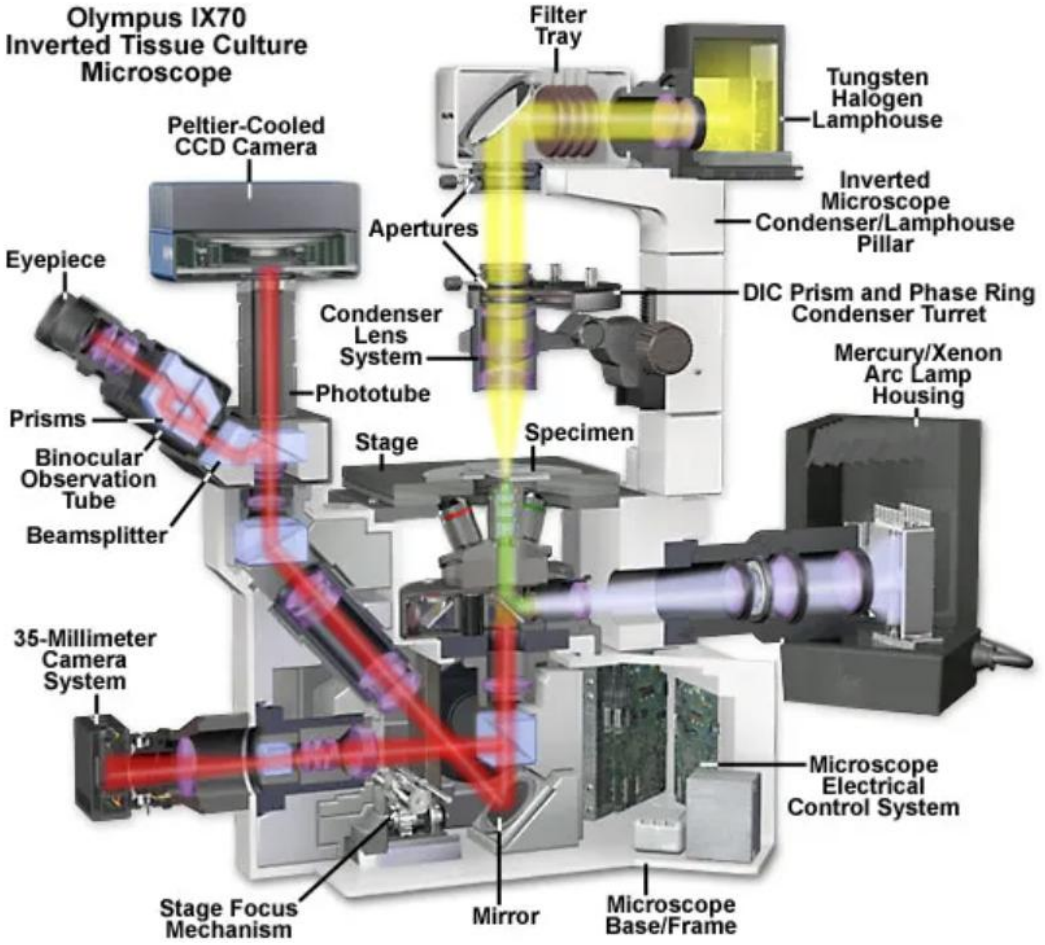
What is Fluorescence Microscopy?

- A technique to **visualize specific molecules** inside cells or tissues using fluorophores.
- Allows detection of **localization, abundance, and dynamics**.
- Widely used in cell biology, neuroscience, medicine.

Upright


Fluorescence Microscopy
Image
Detector
Excitation filter
Emission filter
Dichroic mirror
Light source
Objective
Specimen

Inverted microscope



Common Fluorescence Channels

1. DAPI / Hoechst (Blue channel)

- **Excitation**: ~350–405 nm (UV/violet)
- **Emission**: ~450 nm (blue)
- **Used for**: Nuclear/DNA stains (DAPI, Hoechst).

2. FITC / GFP (Green channel)

- **Excitation**: ~470–490 nm (blue light)
- **Emission**: ~510–530 nm (green)
- **Used for**: GFP, FITC, Alexa Fluor 488, SYBR Green.

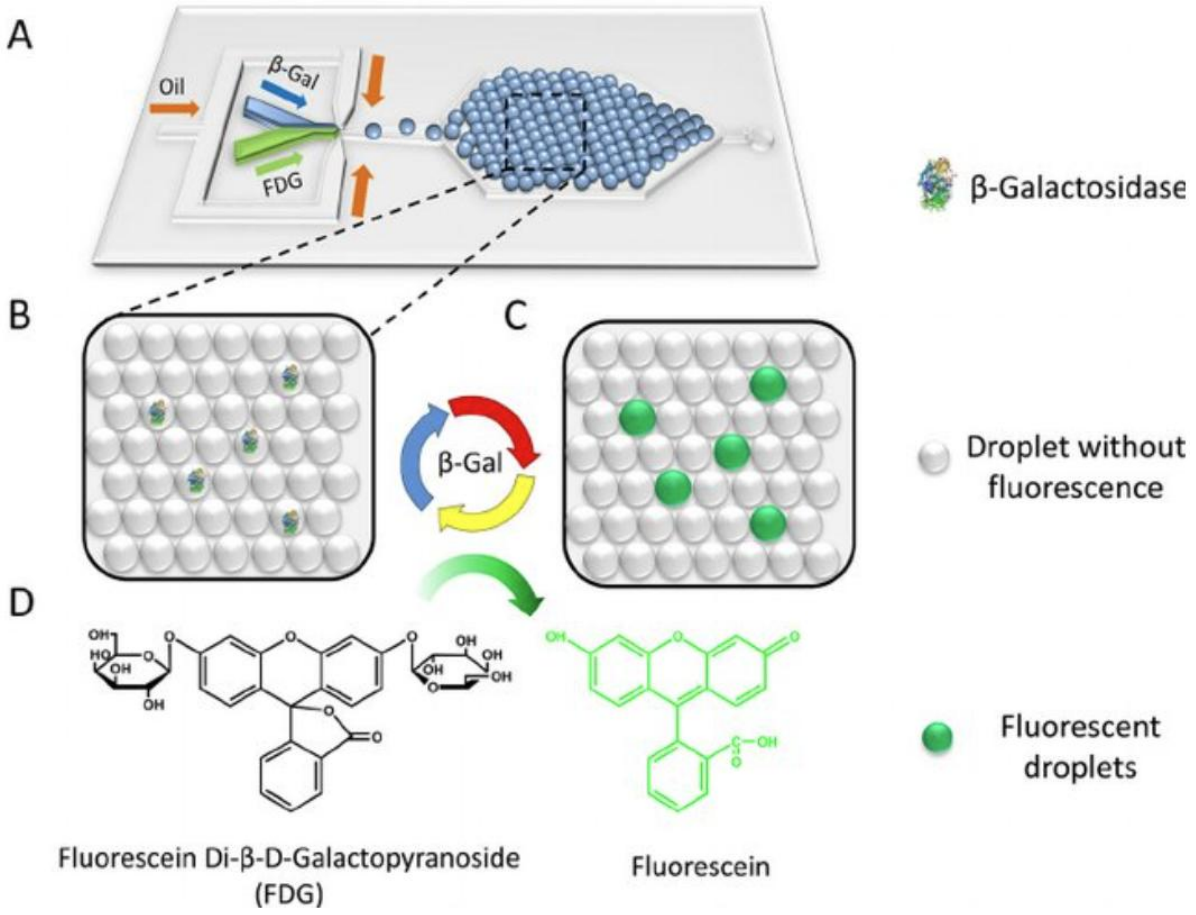
3. TRITC / RFP / mCherry (Red–orange channel)

- **Excitation**: ~540–560 nm (green light)
- **Emission**: ~570–620 nm (orange–red)
- **Used for**: TRITC, DsRed, mCherry, Alexa Fluor 555/568.

4. Cy5 / Far-red channel

- **Excitation**: ~630–650 nm (red light)
- **Emission**: ~660–700+ nm (far red)
- **Used for**: Cy5, Alexa Fluor 647, APC.

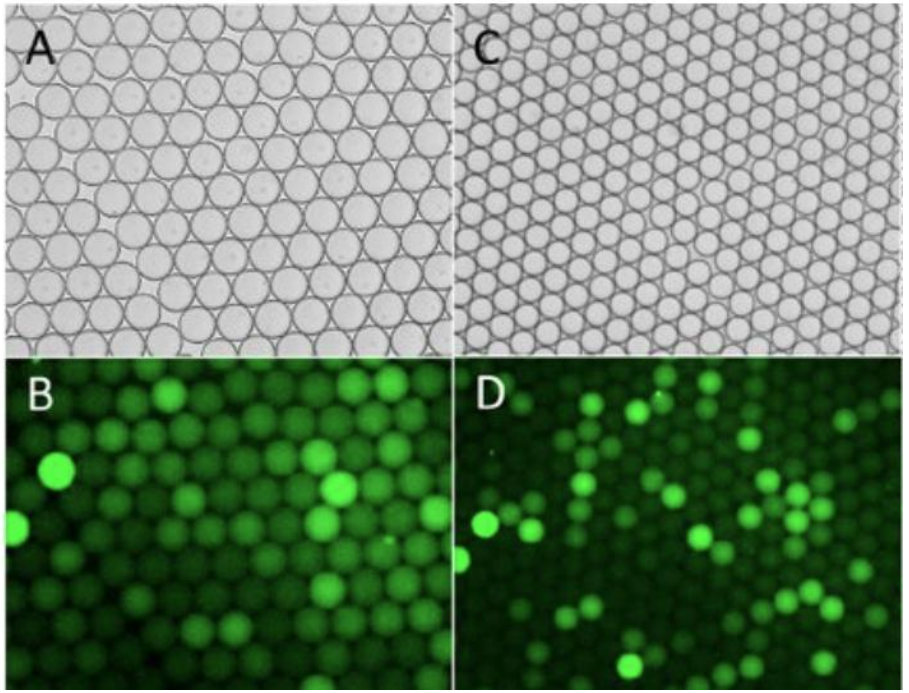
Fluorescence of droplets



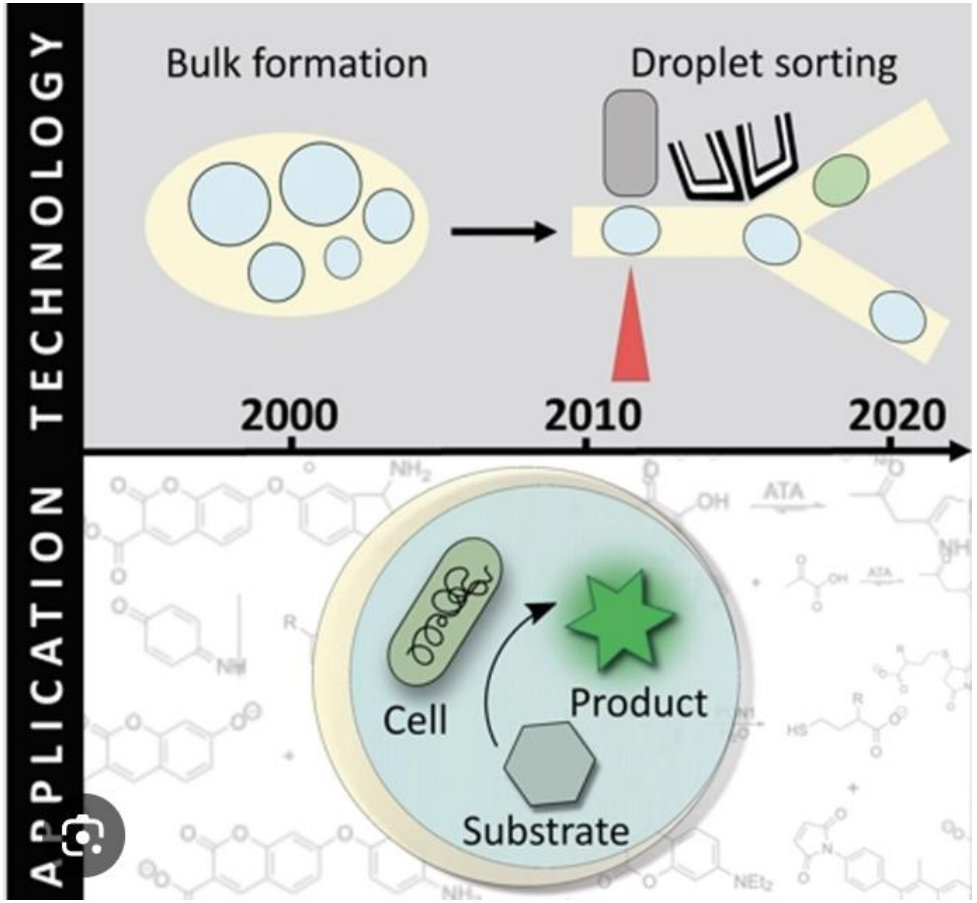
β-Galactosidase

Droplet without fluorescence

Fluorescent droplets

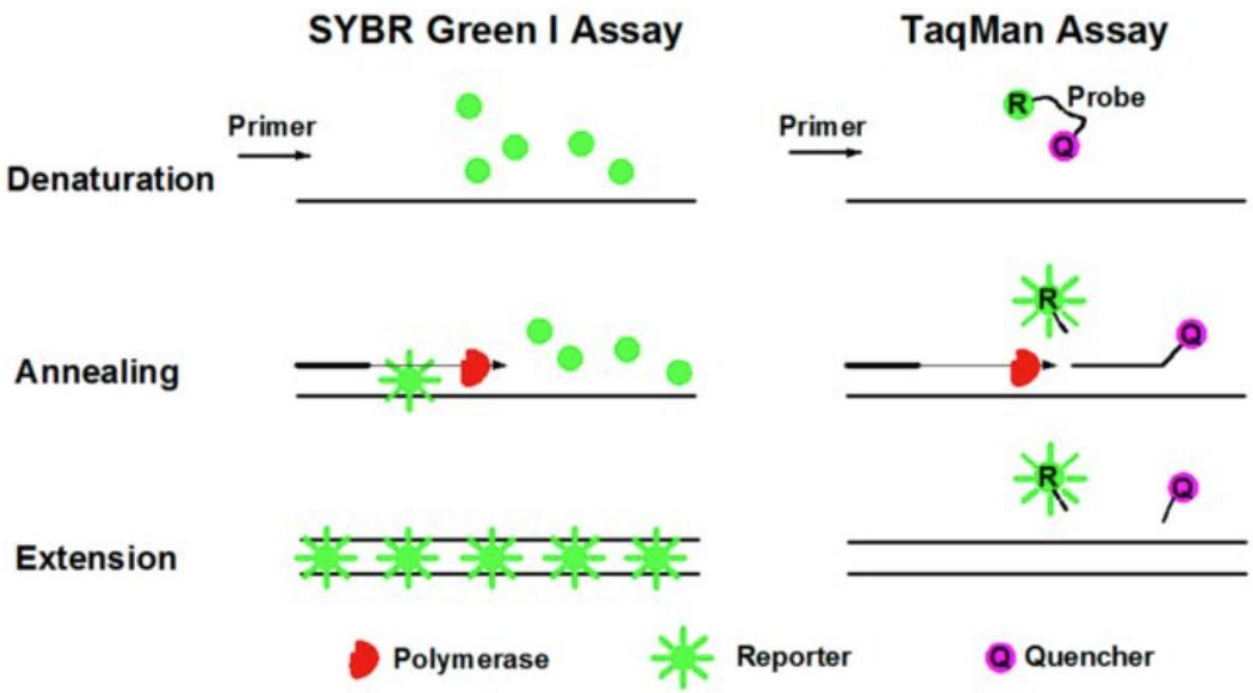


Fluorescence-activated droplet sorting



Example 1: droplet digital PCR

- SYBR Green I
- TaqMan Probe



What is SYBR Green I?

- **SYBR Green I** is a **fluorescent dye** commonly used in molecular biology.
- It is an **asymmetric cyanine dye** that specifically binds to **double-stranded DNA (dsDNA).**
- Excitation maximum: ~495 nm (blue light)
- Emission maximum: ~520 nm (green light)

Mechanism of DNA Staining

- **Free dye in solution**: SYBR Green I has **very little fluorescence** when unbound.
- **Binding to dsDNA**: When SYBR Green I intercalates (or sits in the minor groove of) double-stranded DNA, its **fluorescence increases >1000-fold**.
- **Why?** DNA binding restricts the dye's rotation, which prevents non-radiative energy loss → resulting in strong green emission.

Use in PCR (specifically Real-Time PCR / qPCR)

- During PCR, as DNA is amplified, **more dsDNA is produced**.
- SYBR Green I binds to this dsDNA.
- The fluorescence intensity increases proportionally with the amount of DNA.
- A real-time PCR machine measures this fluorescence **cycle by cycle** → allowing quantification of DNA.

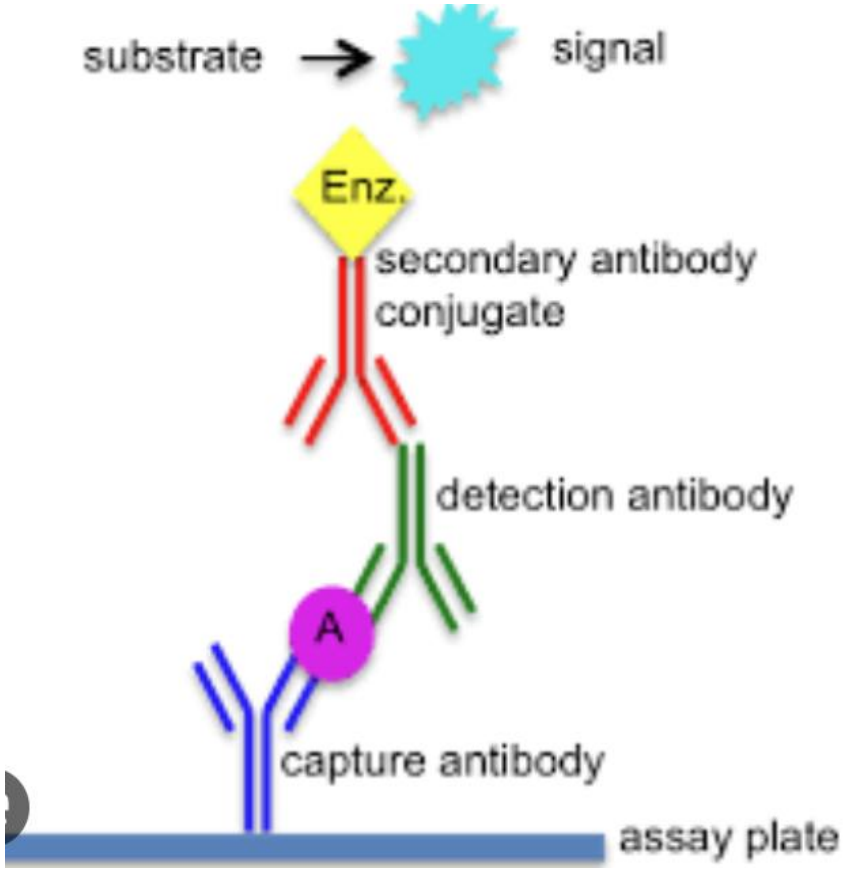
Advantages

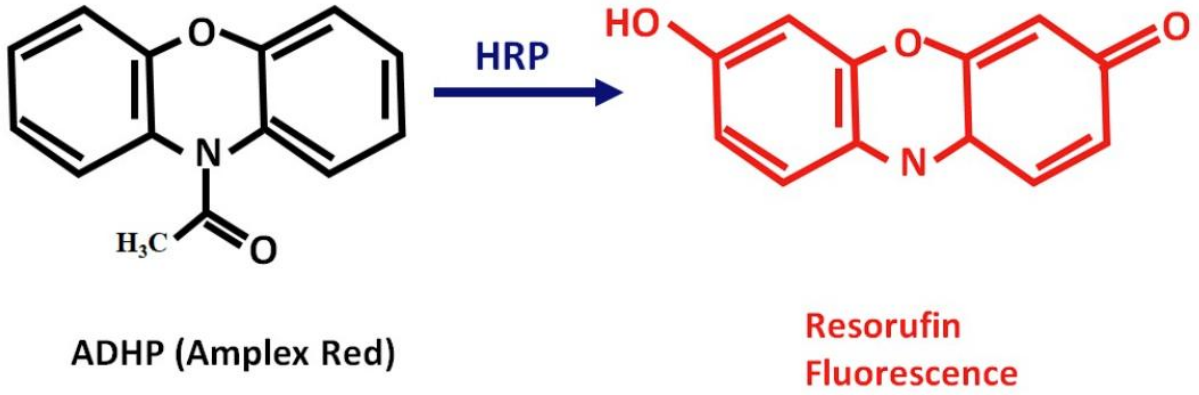
- Simple, inexpensive, and highly sensitive.
- Works with any dsDNA sequence (no need for specific probe design).

Limitations

- **Non-specific**: SYBR Green I binds to *all* dsDNA, including:
  - Specific PCR products
  - Primer dimers
  - Nonspecific amplification products
- Because of this, **melt-curve analysis** is often performed after qPCR to distinguish true product from artifacts.

Example 2: droplet digital ELISA





ADHP (Amplex Red)

Resorufin
Fluorescence

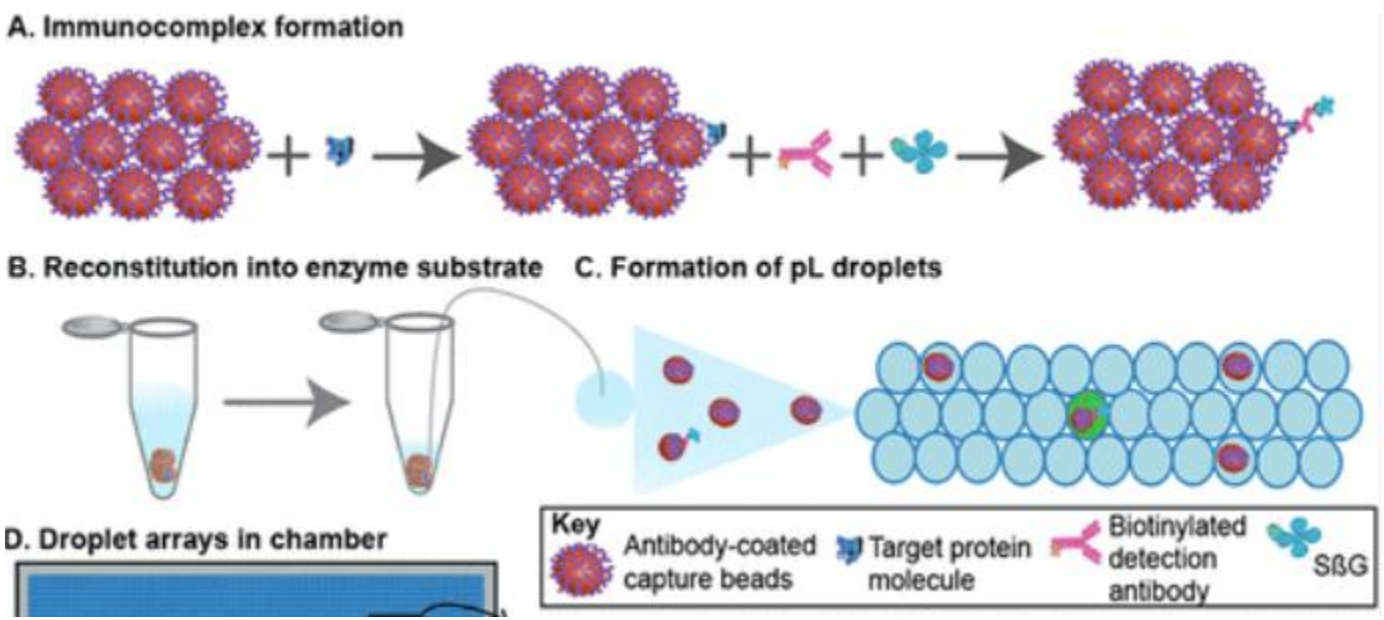

A. Immunocomplex formation
B. Reconstitution into enzyme substrate
C. Formation of pL droplets
D. Droplet arrays in chamber
Key
Antibody-coated capture beads
Target protein molecule
Biotinylated detection antibody
SßG

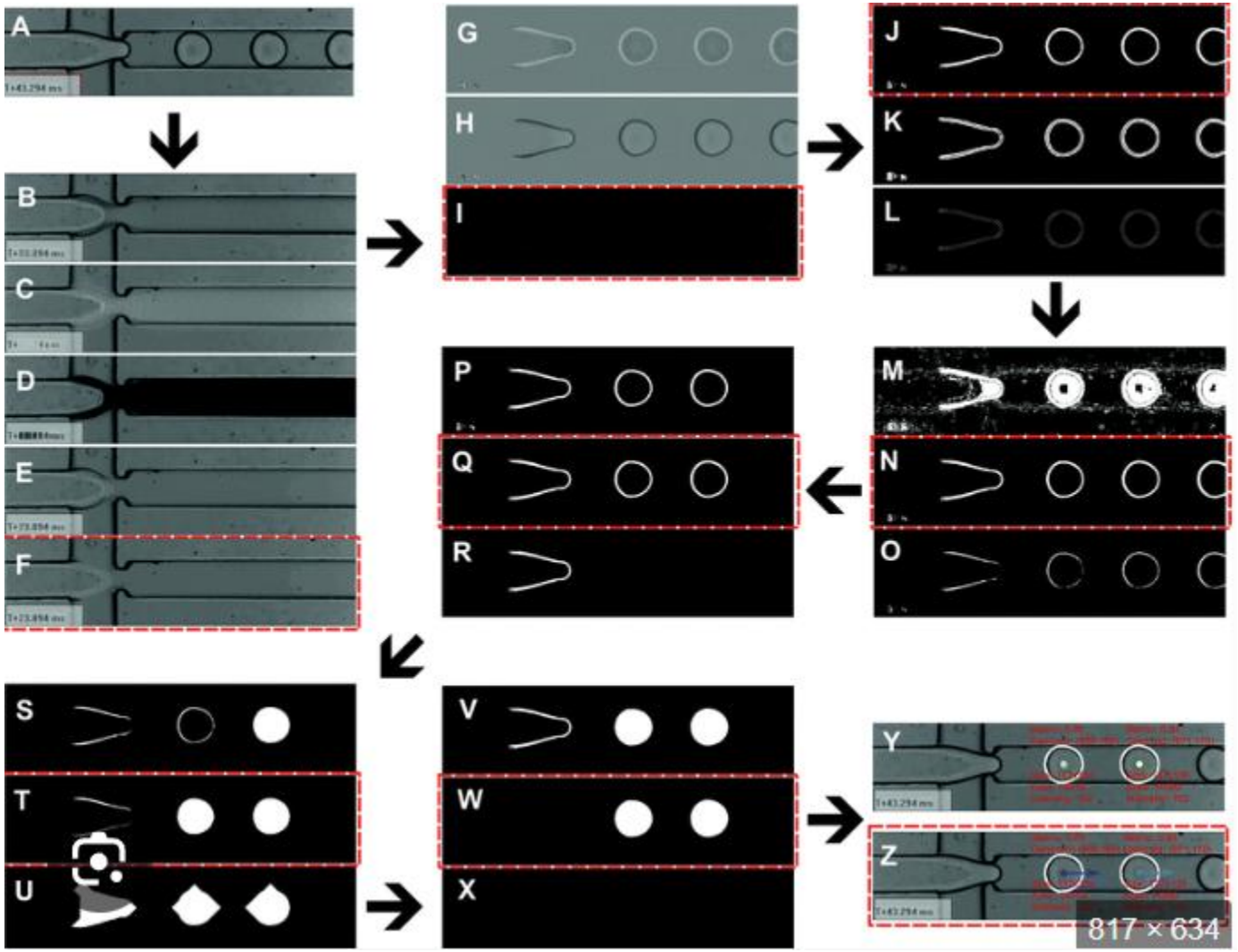
Image analysis

Why Image Analysis Matters

- Droplet microfluidics produces **large datasets** (millions of droplets).
- Accurate analysis is essential for:
  - Droplet size distribution
  - Encapsulation efficiency
  - Fluorescence intensity measurements
  - Sorting and classification
- Key challenge: **Speed vs. accuracy vs. scalability**.

Conventional Image Analysis Methods

- **Thresholding / segmentation** (e.g., Otsu's method)
- **Edge detection** (Canny, Sobel filters)
- **Circular Hough Transform** (for droplet detection by circular features)
- **Morphological operations** (erosion, dilation, watershed for separating touching droplets)
- **Advantages:**
  - Simple, interpretable, low computational cost
- **Limitations:**
  - Sensitive to noise, lighting variations
  - Difficult with overlapping or irregular droplets
  - Limited adaptability to new datasets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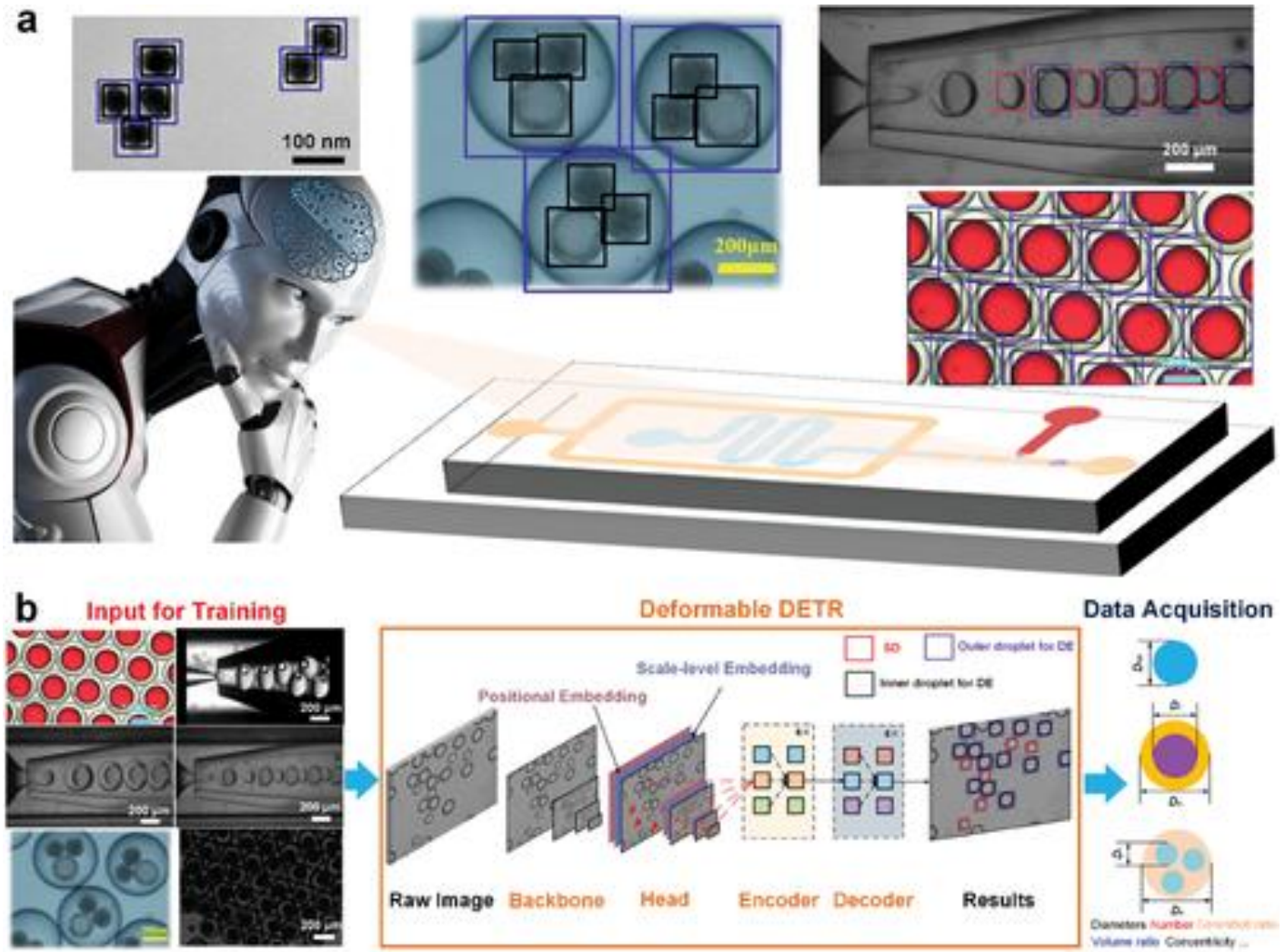
817 × 634

Machine Learning / AI-Based Methods

- **Classical ML**:
  - Feature extraction (size, shape, intensity) + classification (SVM, random forest).
- **Deep Learning (CNNs, U-Nets):**
  - Pixel-wise segmentation (semantic/instance segmentation).
  - Detect droplets even in noisy, overlapping, or irregular conditions.
- **Advantages:**
  - High accuracy, robust to noise
  - Can automate feature extraction
  - Scales to large datasets
- **Limitations:**
  - Requires large, labeled training datasets
  - Computationally expensive (GPU resources)
  - Less interpretable (“black box”)


a
100 nm
200μm
200 μm
b
Input for Training
Deformable DETR
Data Acquisition
Positional Embedding
Scale-level Embedding
Raw Image
Backbone
Head
Encoder
Decoder
Results

Aspect	**Conventional**	**Machine Learning**
Setup	Simple, rule-based	Needs training data
Speed	Fast for small sets	Fast at inference, slower to train
Robustness	Sensitive to noise/contrast	More robust, generalizable
Accuracy	Good if droplets uniform	Handles irregular/overlapping
Interpretability	Transparent	Black box, harder to interpret

- **When to Use Which?**
- **Conventional methods**
  - Small datasets
  - Uniform droplets, good image quality
  - Quick prototyping
- **ML-based methods**
  - Large datasets, need automation
  - Complex droplet populations (polydisperse, irregular)
  - Noisy or high-throughput experiments