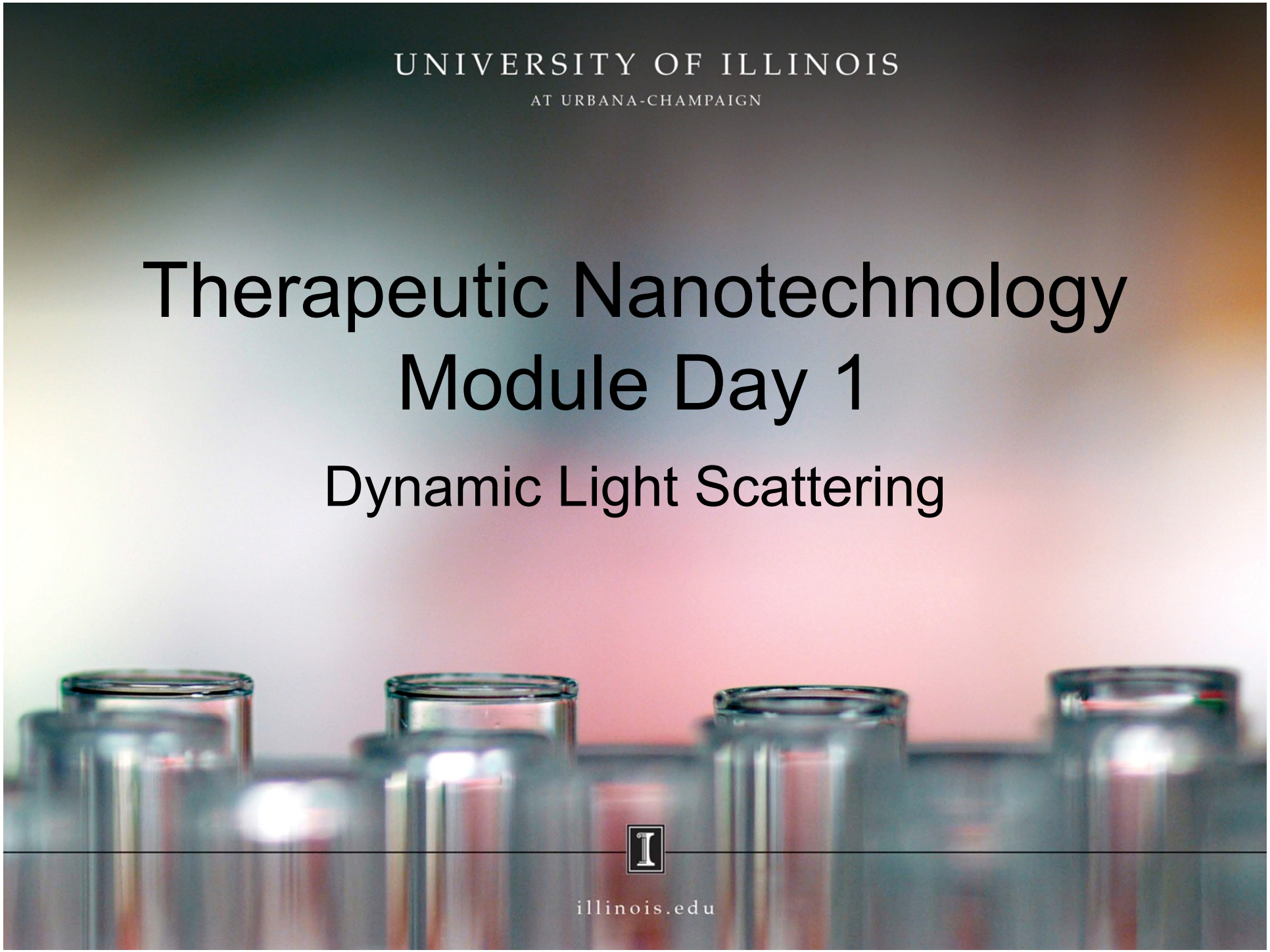
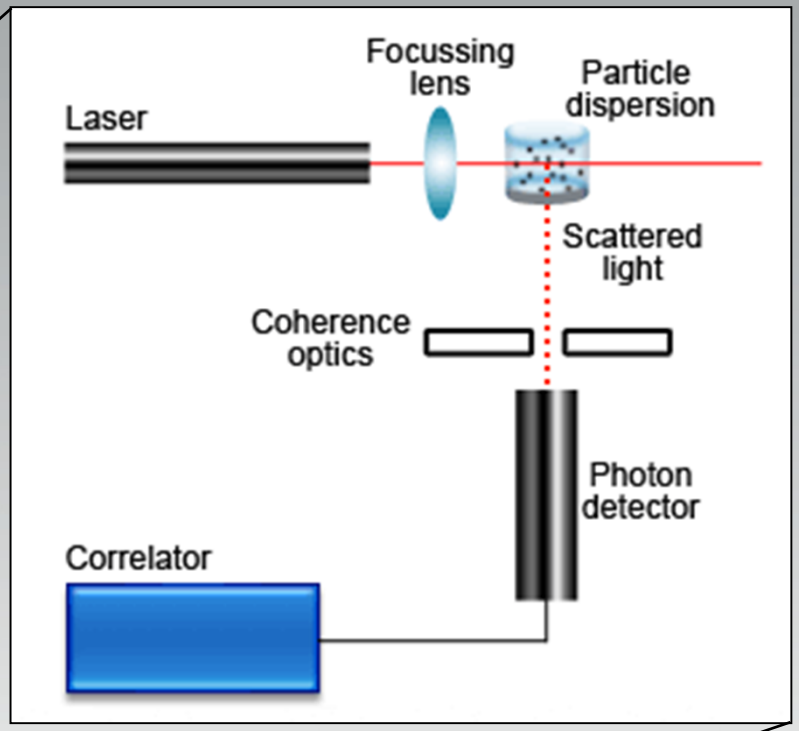


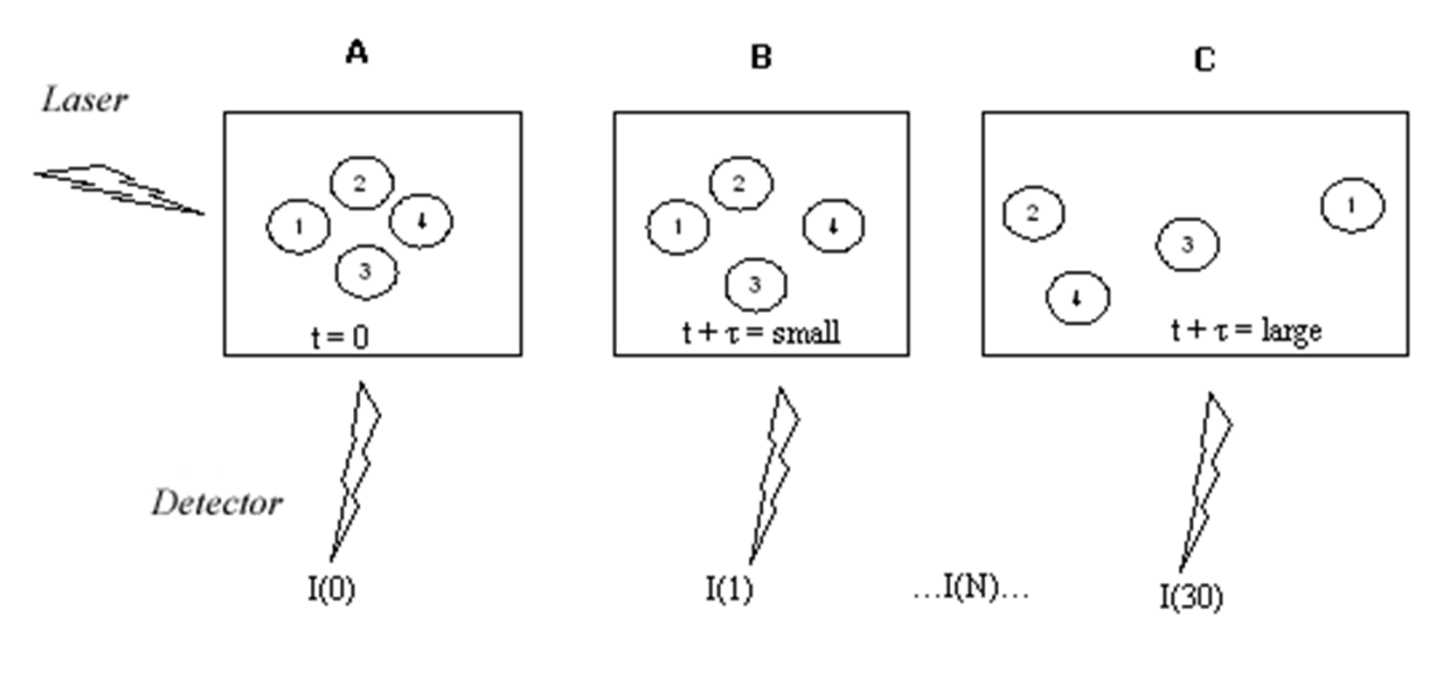
Therapeutic Nanotechnology Module Day 1

Dynamic Light Scattering

DLS Instrumentation

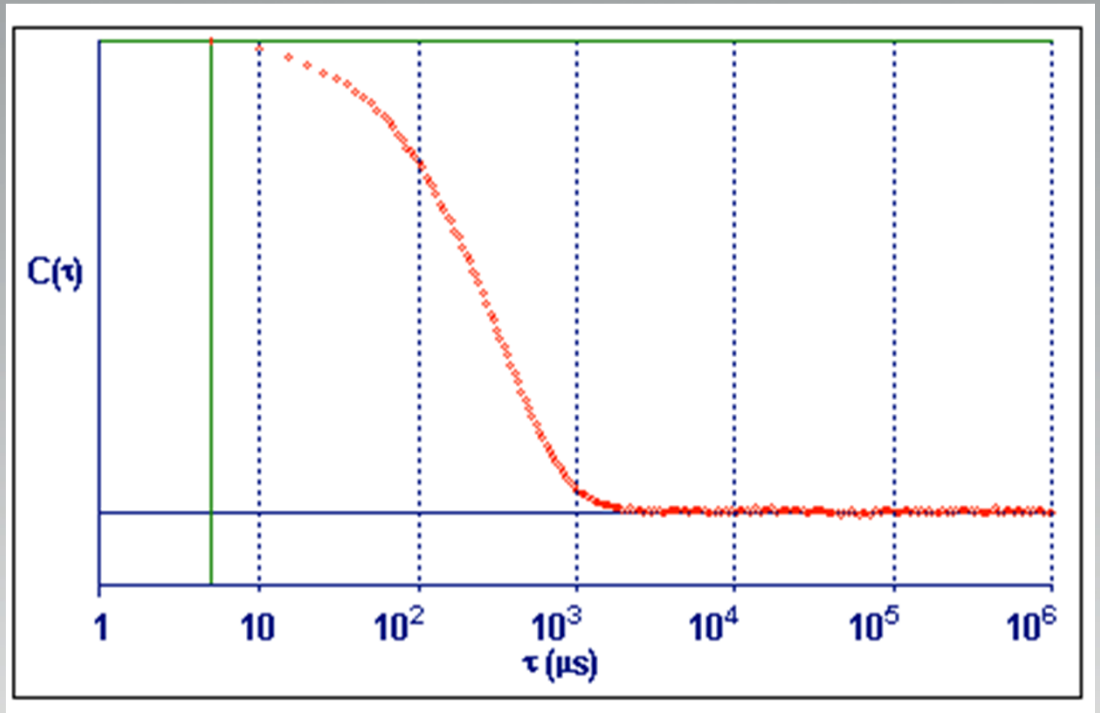


Background



- Light intensity, *I(0)*, is recorded at $t = 0$
- At a future time, $t + \tau$, the particles have moved and result in a different intensity, $I(t + \tau)$
- As time progresses, the intensity values no longer correlate with the initial intensity reading

Background



The autocorrelation function, $C(\tau)$, is used to described the correlation of intensity at time τ to the initial intensity. As the function approaches zero, there is little or no correlation

Background

- Average particle size is determined by performing curve fitting to the autocorrelation function:

$$C(\tau) \propto e^{-2Dq^2\tau}$$

Where *D* is the diffusion coefficient, and *q* is the scattering vector. *q* is calculated as follows:

$$q = \frac{4\pi n}{\lambda}\sin\left(\frac{\theta}{2}\right)$$

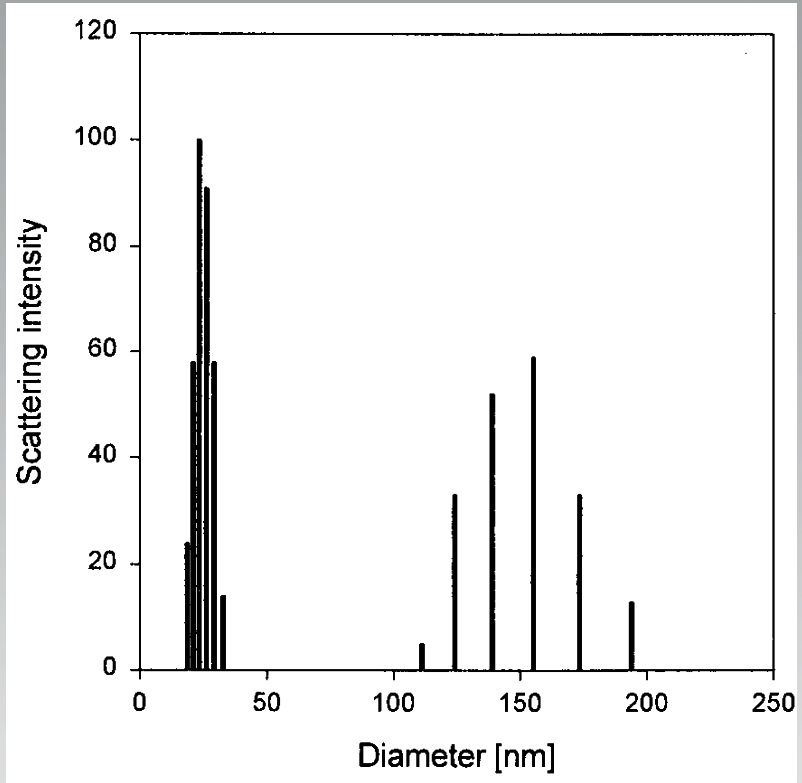
Where *n* is the refractive index of the solution, *λ* is the laser wavelength, and θ is the scattering angle

- With *q* calculated from known values, curve fitting to *C(τ)* yields *D*. Finally, particle size is calculated from the Stokes-Einstein equation:

$$D = \frac{k_B T}{3\pi\eta d}$$

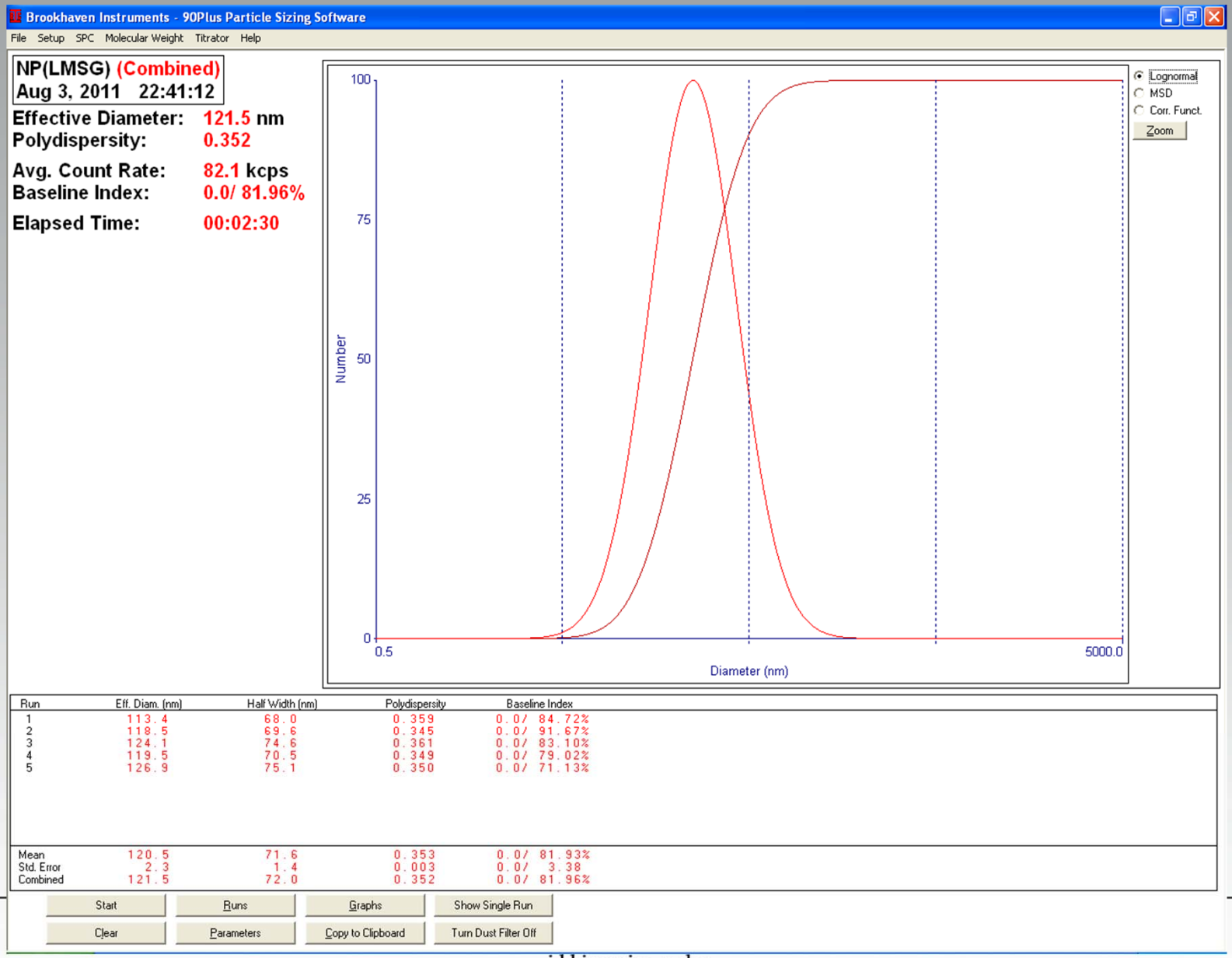
Where k_B is Boltzmann's constant, *T* is temperature, *η* is the liquid viscosity, and *d* is the particle diameter

DLS Output

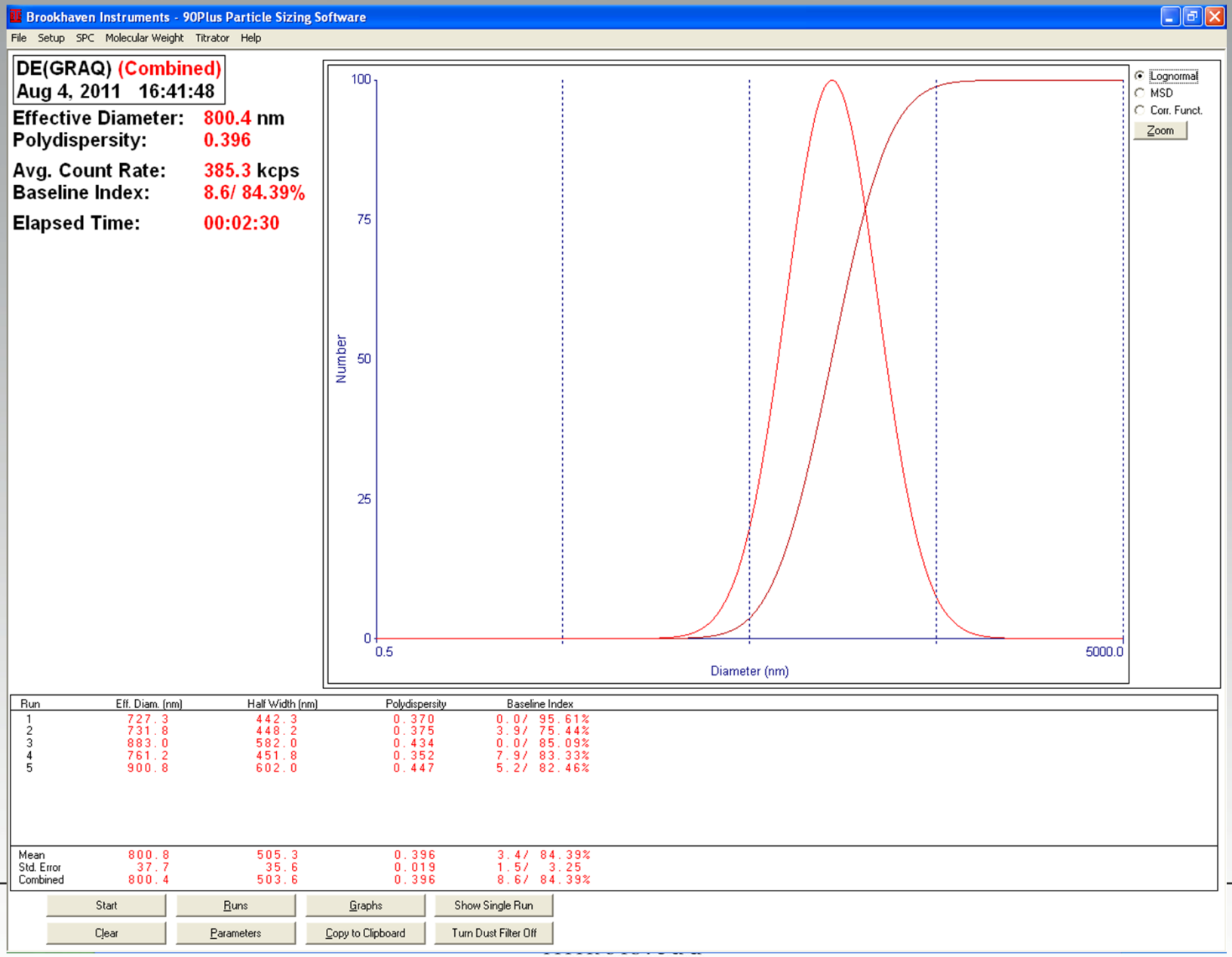


DLS experimental data is often displayed in the form of a histogram indicating the size distribution

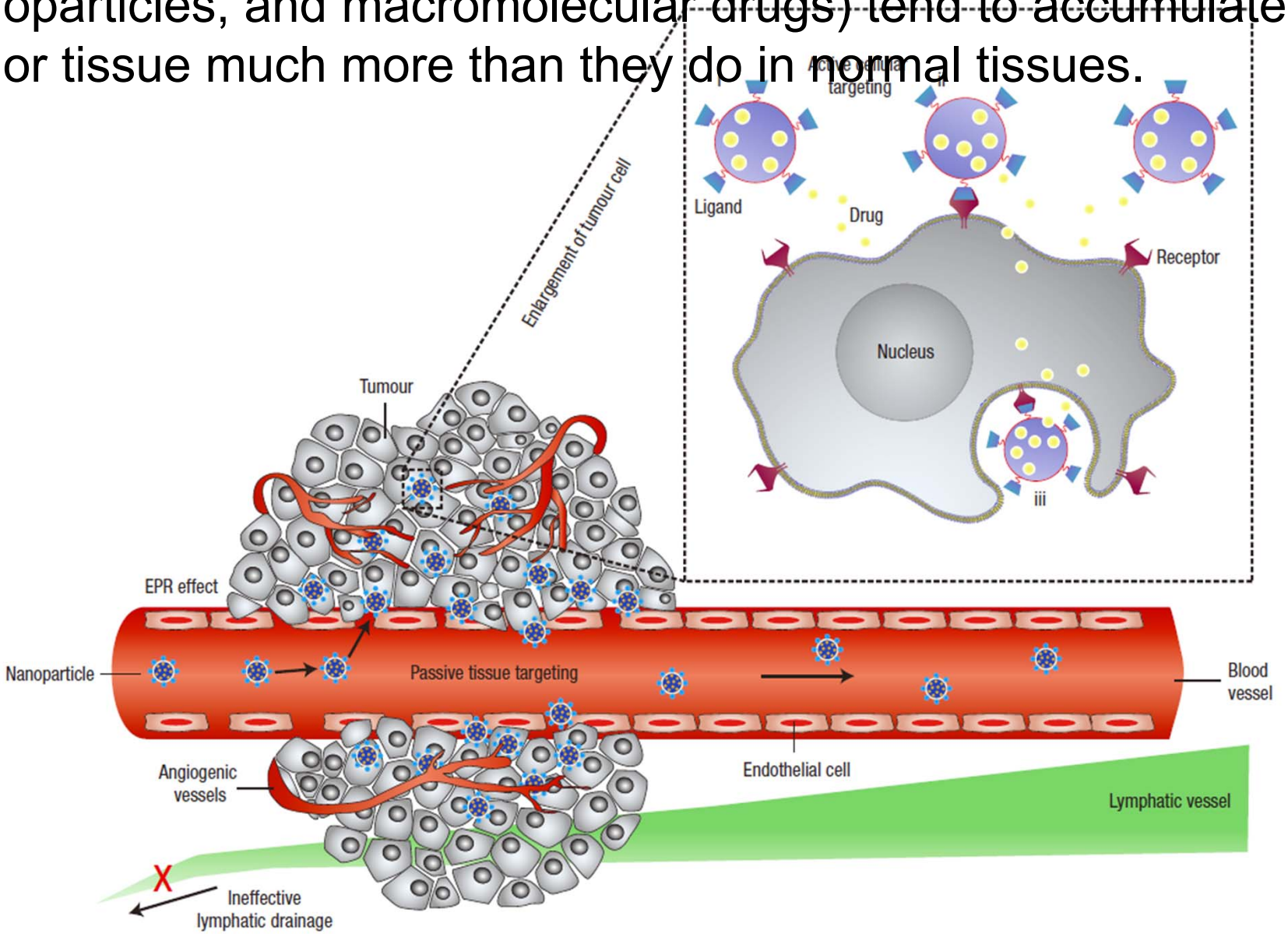
Nanoprecipitation:



Double emulsion:



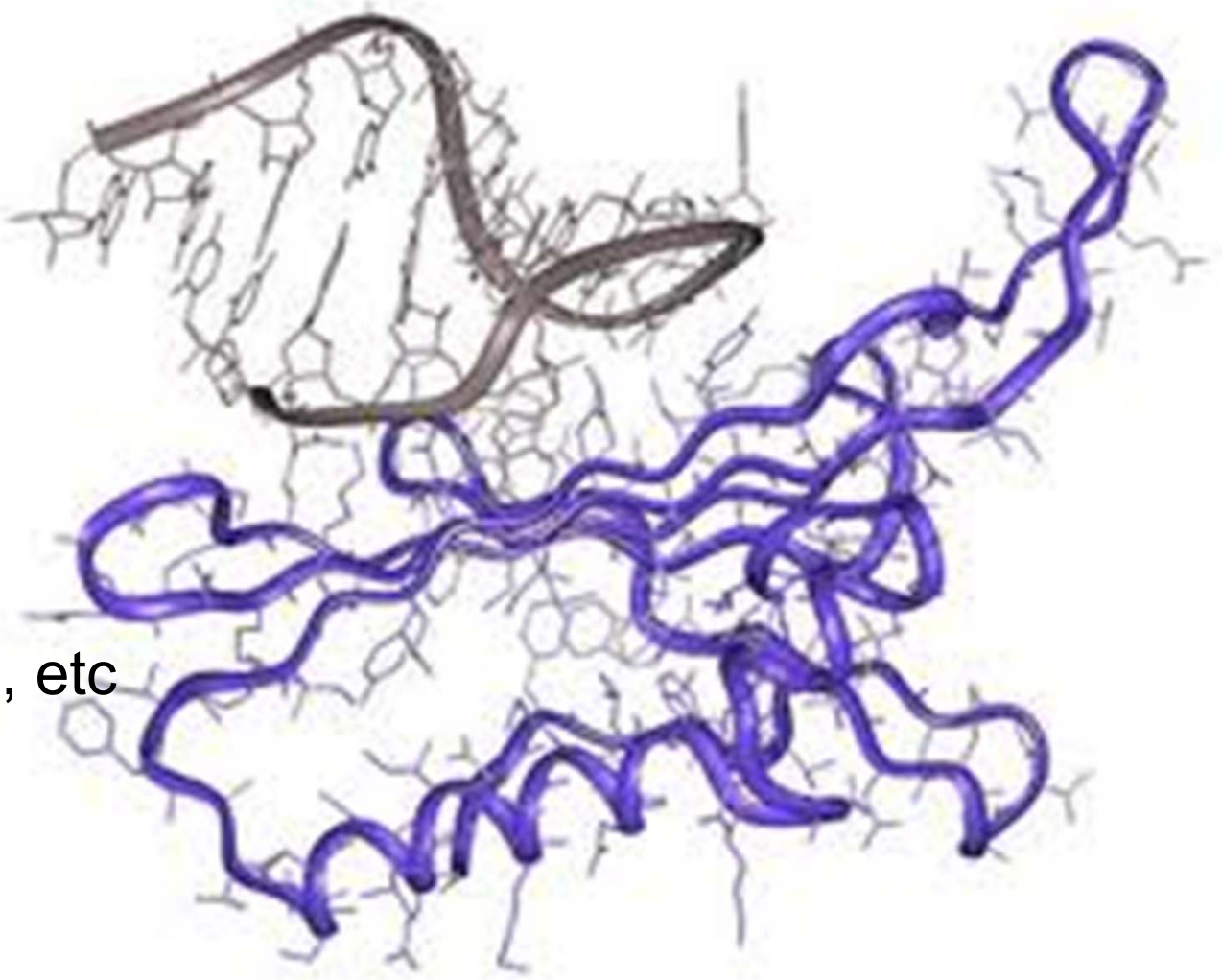
The **Enhanced Permeability and Retention** (**EPR**) **effect** is the property by which certain sizes of molecules (typically liposomes, nanoparticles, and macromolecular drugs) tend to accumulate in tumor tissue much more than they do in normal tissues.

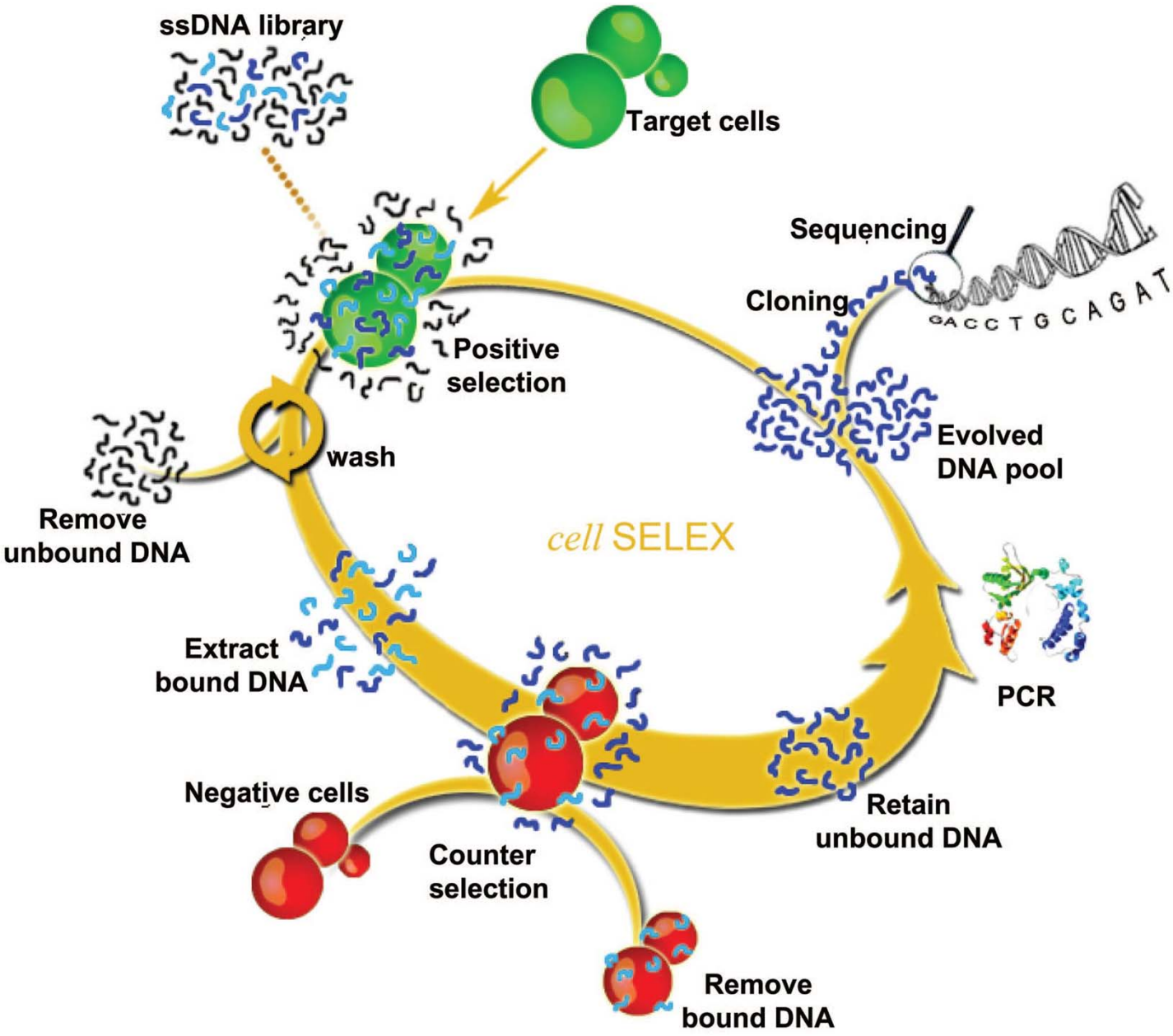


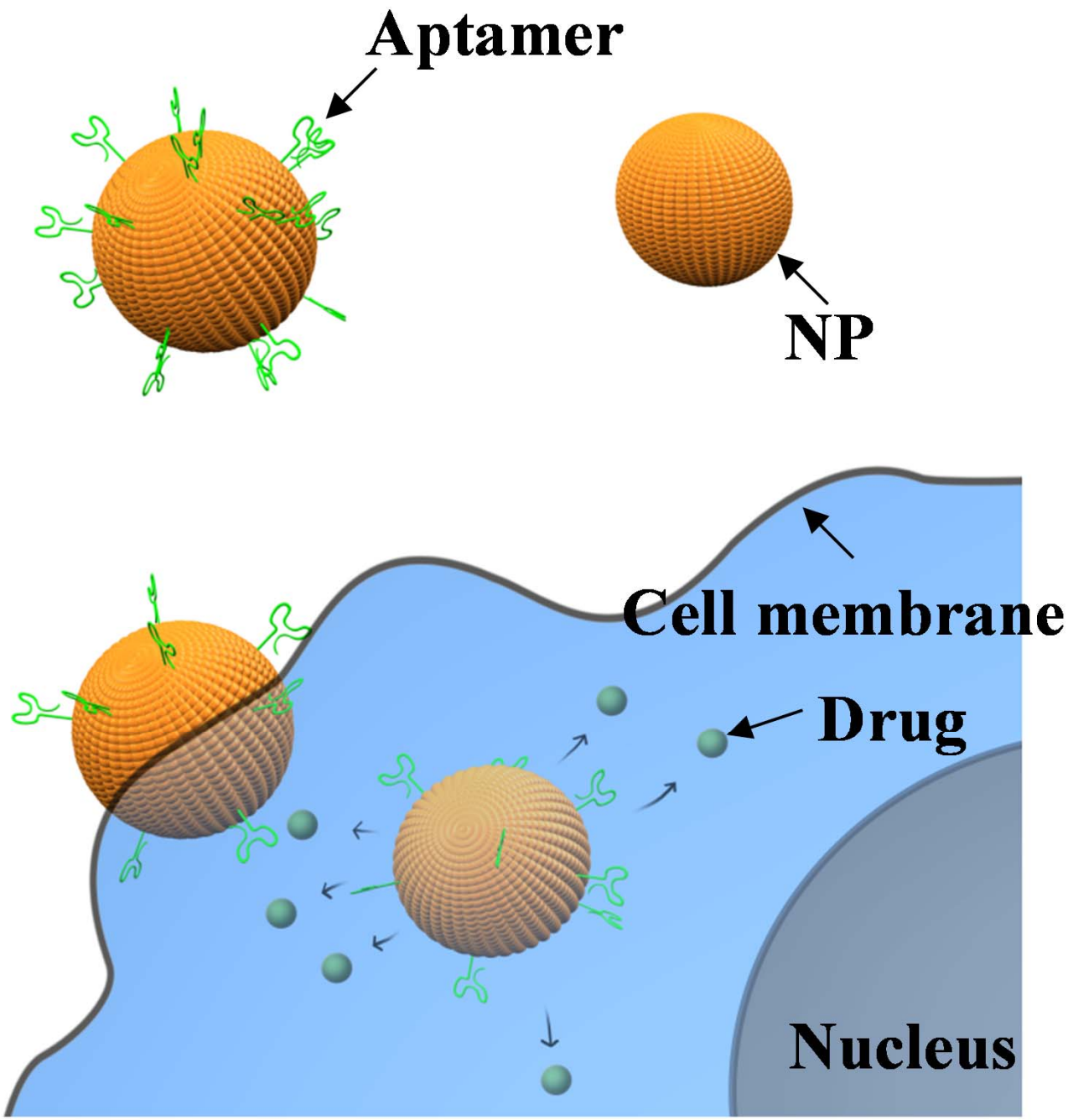
Aptamers are oligonucleic acid or peptide molecules that bind to a specific target molecule.

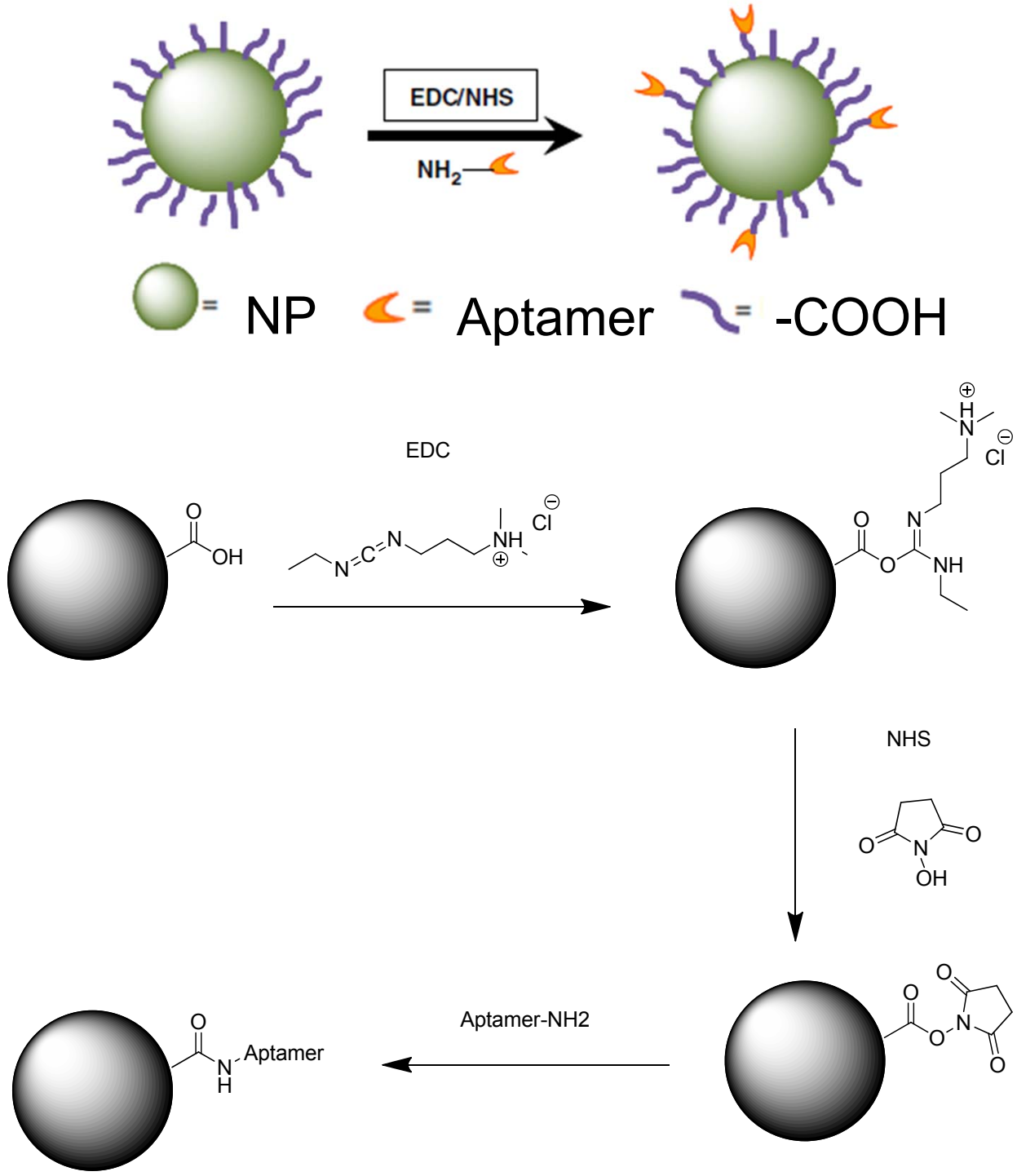
Aptamer

Target: protein, etc




ssDNA library
Target cells
Sequencing
Cloning
GACCTGCAGAT
Positive selection
wash
Remove unbound DNA
Evolved DNA pool
cell SELEX
Extract bound DNA
PCR
Negative cells
Counter selection
Retain unbound DNA
Remove bound DNA


Aptamer
NP
Cell membrane
Drug
Nucleus

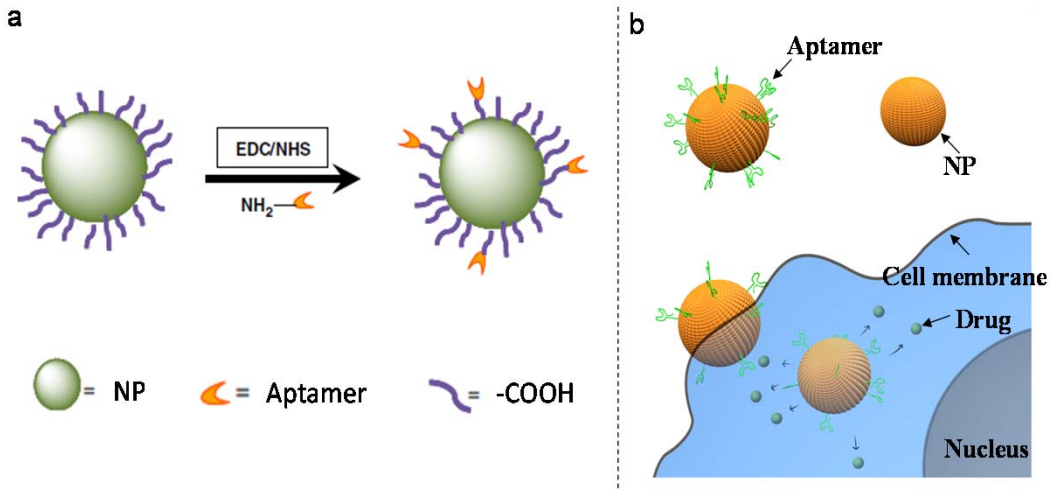

EDC/NHS
NH2
NP
Aptamer
-COOH
EDC
OH
Cl
NH
NHS
OH
Aptamer-NH2
Aptamer

Introduction for Part III

Outline of Experiment

Aptamer modified & unmodified NP

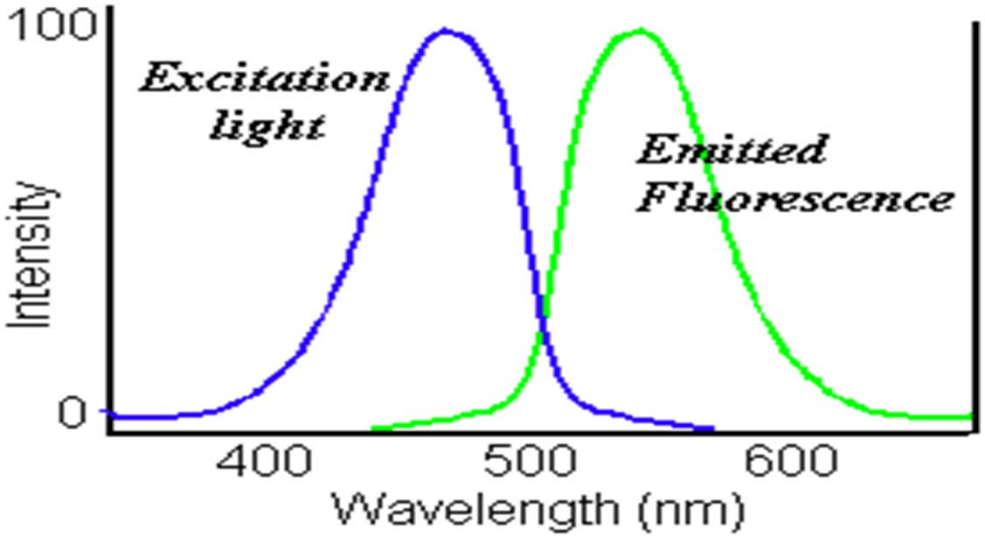
- **Cell lysis fluorescent measurement**
- **Fluorescent microscope observation**



You will learn

How to use fluorescent microscope
How to use micro-plate reader

How to use biosafety cabinet



Biosafety Cabinet and Micro-plate Reader

Wash with 200µl PBS twice

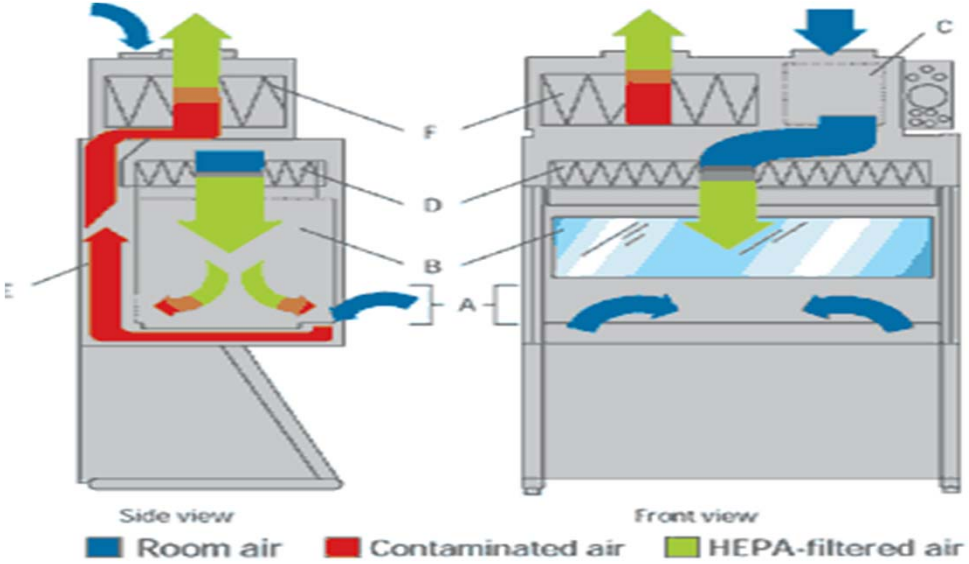
Add 90µl Opti-DMEM and then 10µl particles

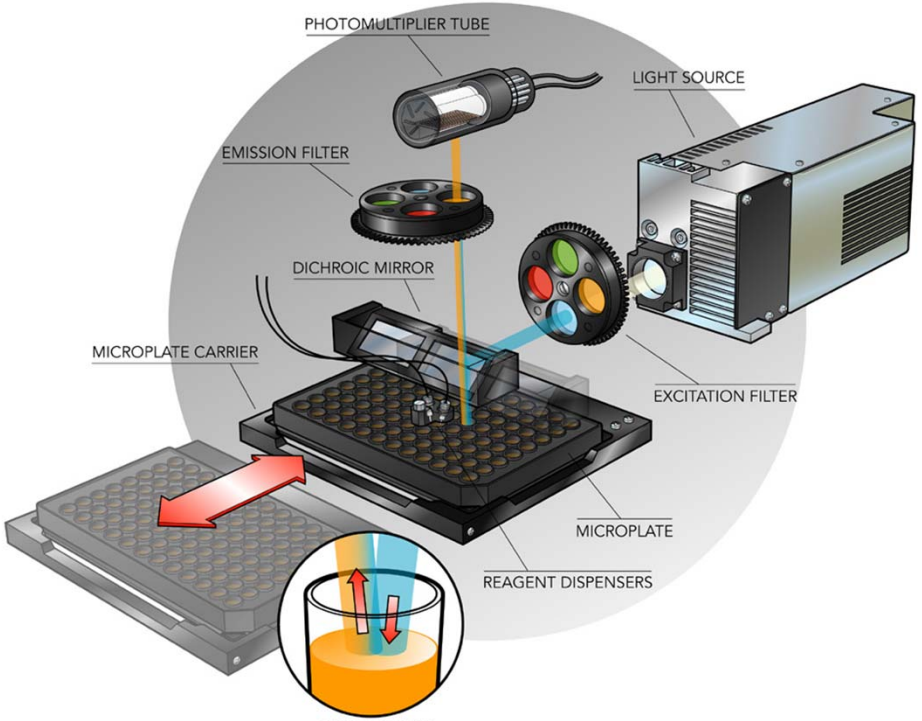
Incubate 2h

Wash with 200µl PBS 3 times

Add 200µl lysis buffer

Shake 10min and observe with micro plate reader





James P. Rydock, “Performance anxiety”, Mo

Fluorescent Microscope

Wash with 1ml PBS twice

Add 900μl Opti-DMEM and then 100μl particles

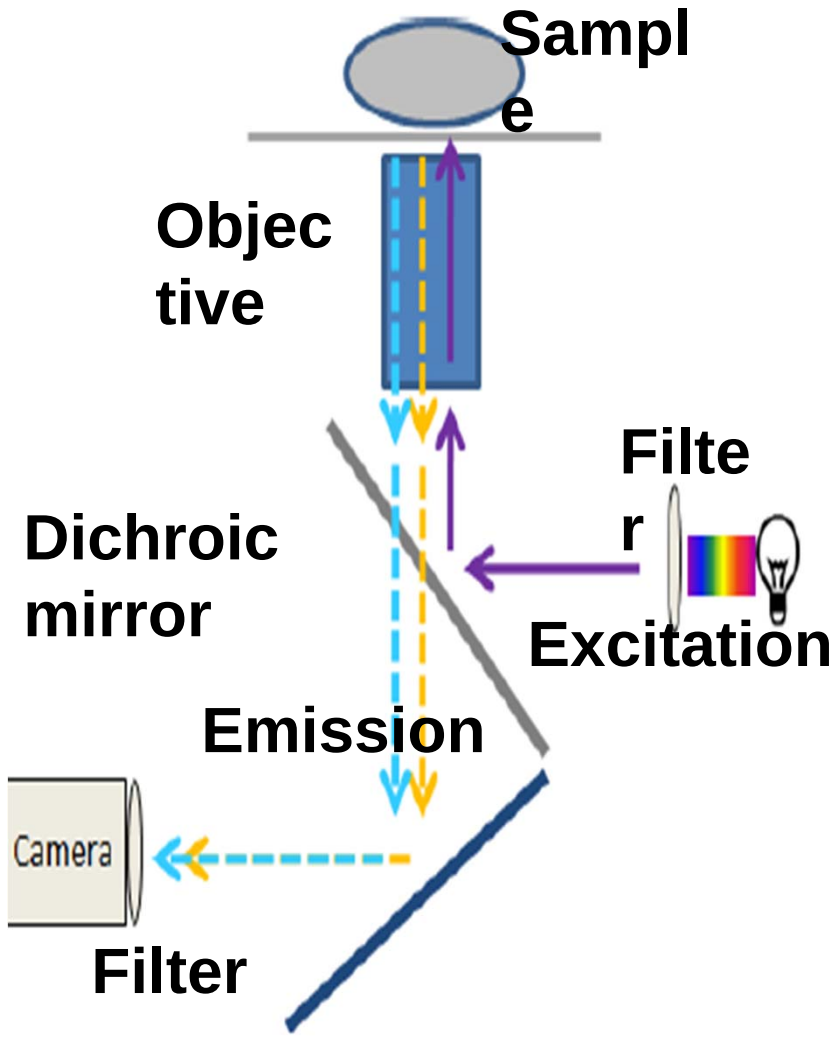
Incubate 2h

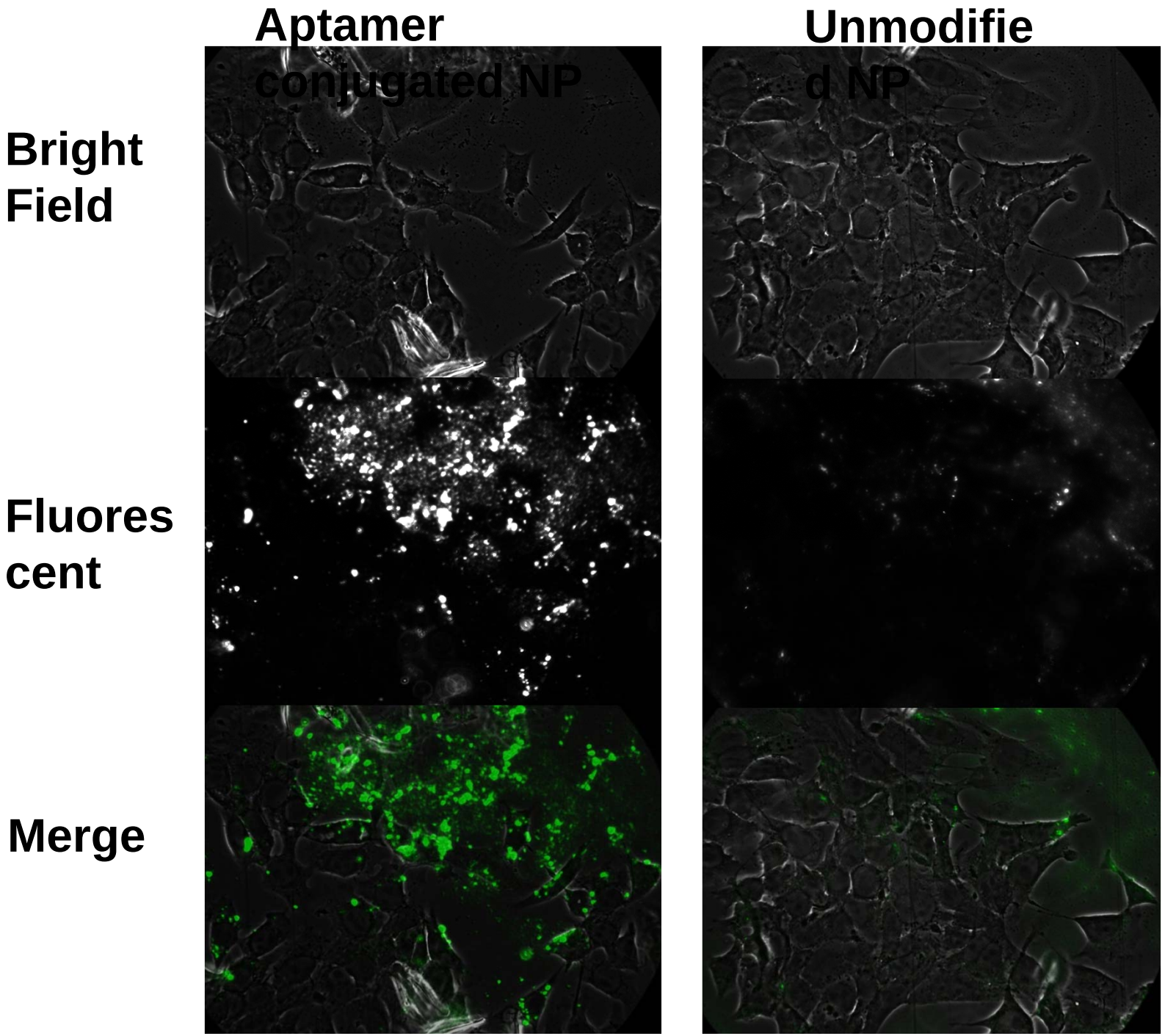
Wash with 1ml PBS 3 times

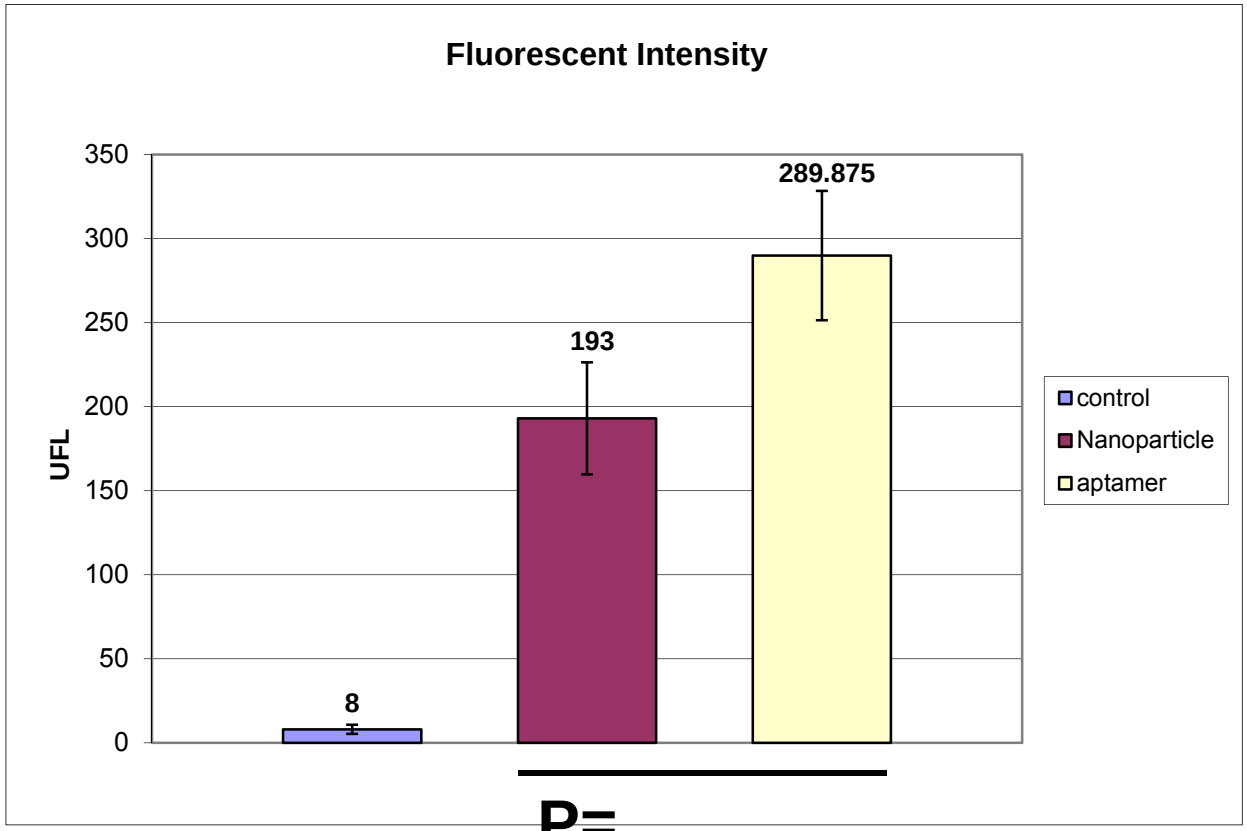
Add 200μl paraformldehyde to fix 10 min

Wash with 1ml PBS 3 times

Use mounting solution to cover cell




Aptamer conjugated NP
Unmodified NP
Bright Field
Fluorescent
Merge


Fluorescent Intensity
UFL
350
300
250
200
150
100
50
0
289.875
193
8
control
Nanoparticle
aptamer

P= 0.00012, n=8