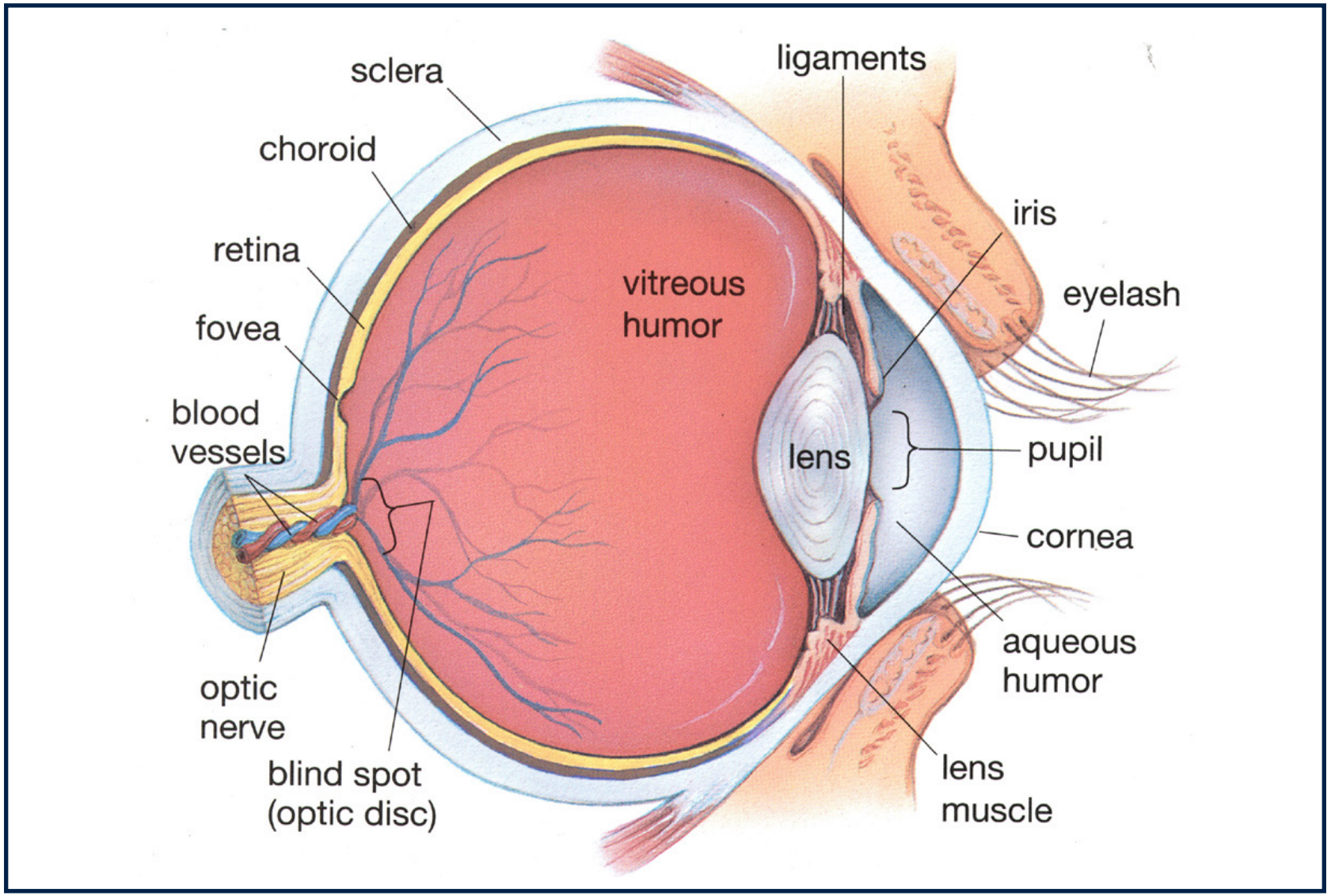
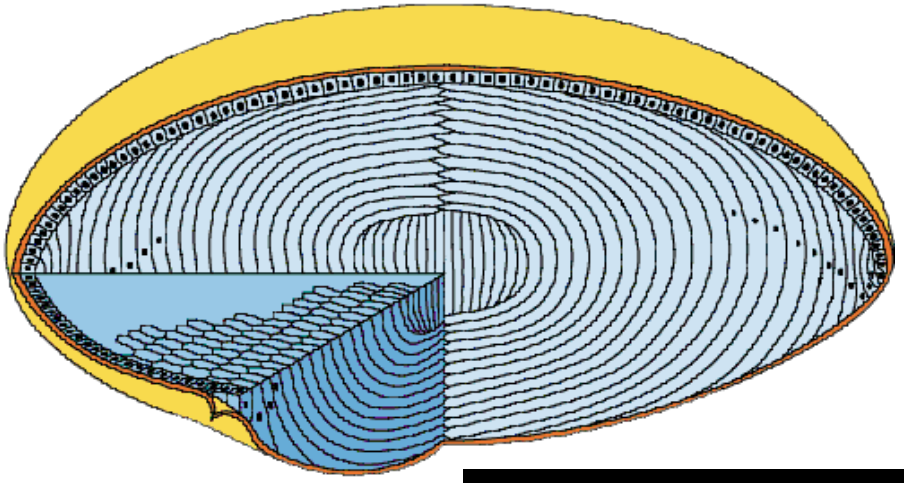
Using FRAP to determine the diffusivity of molecules in the eye

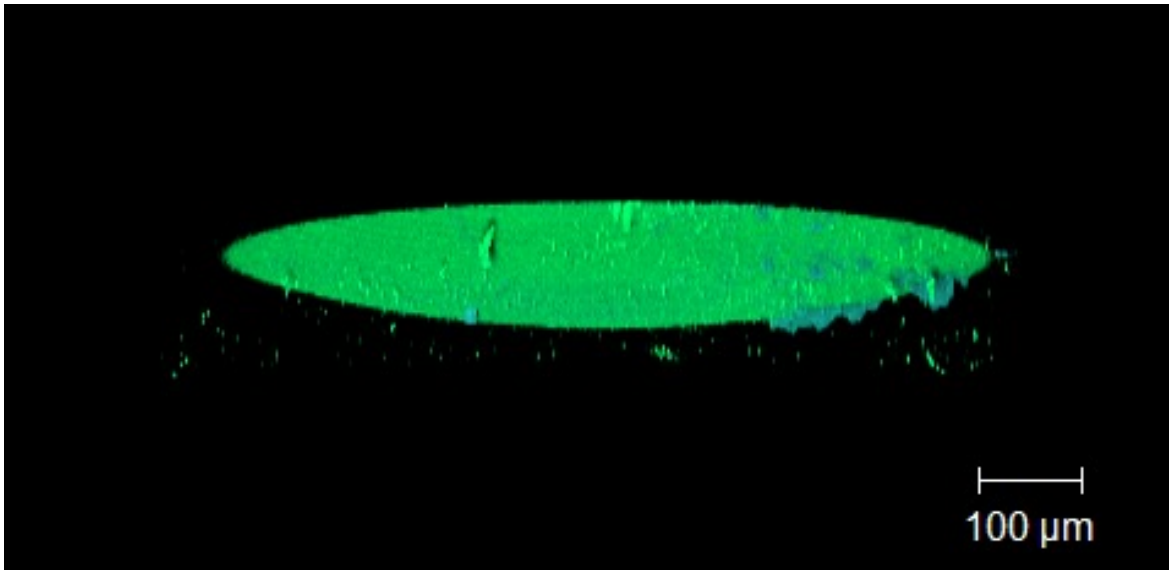
Peter Howell

The eye


sclera
choroid
retina
fovea
blood vessels
optic nerve
blind spot (optic disc)
ligaments
vitreous humor
iris
eyelash
lens
pupil
cornea
aqueous humor
lens muscle

Schematic of the lens





Capsule thickness

Mouse ~10 µm

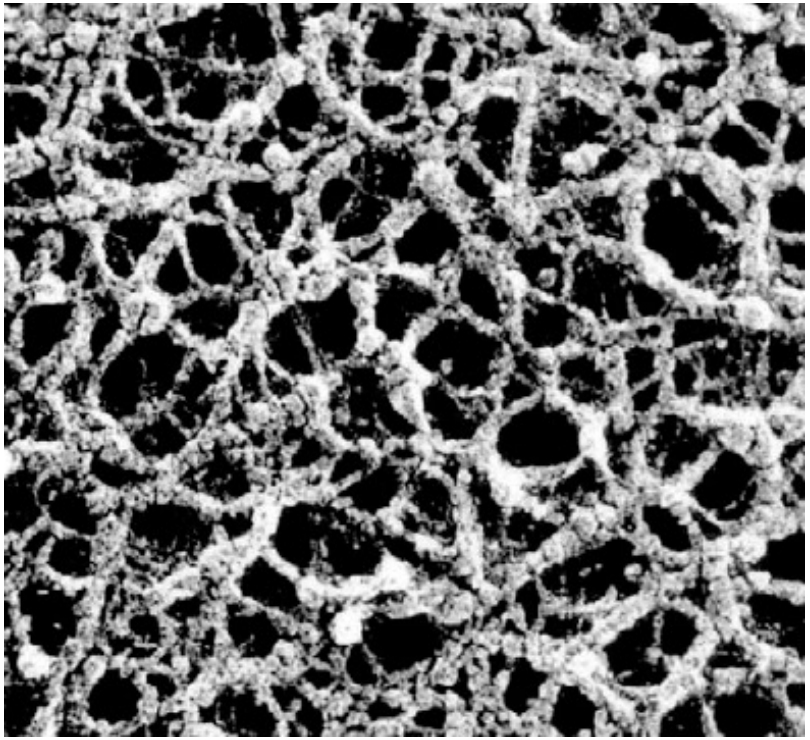
Human ~26 µm

Lens capsule composition

Structure:

Porous matrix with various pore sizes

- 99% ~4-5 nm
- 1% ~10 nm



100,000X

Function:

Allows for selective diffusion based on size, shape and charge

Diffusion in lens capsule

Important for:

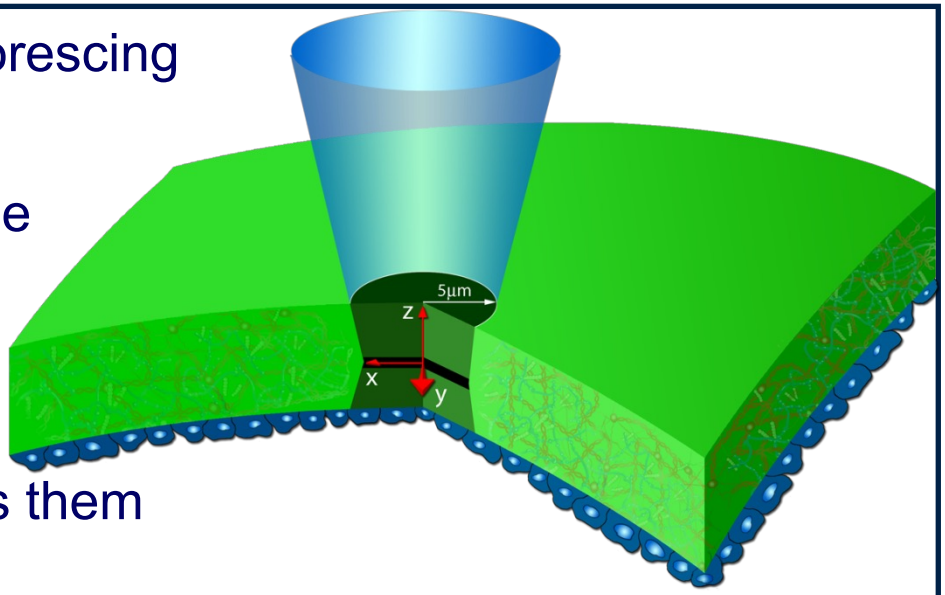
- Lens development and growth
- Nutrition and waste release
- Drug delivery
- Uveitis (Ocular inflammation)
- Cataract formation and treatment

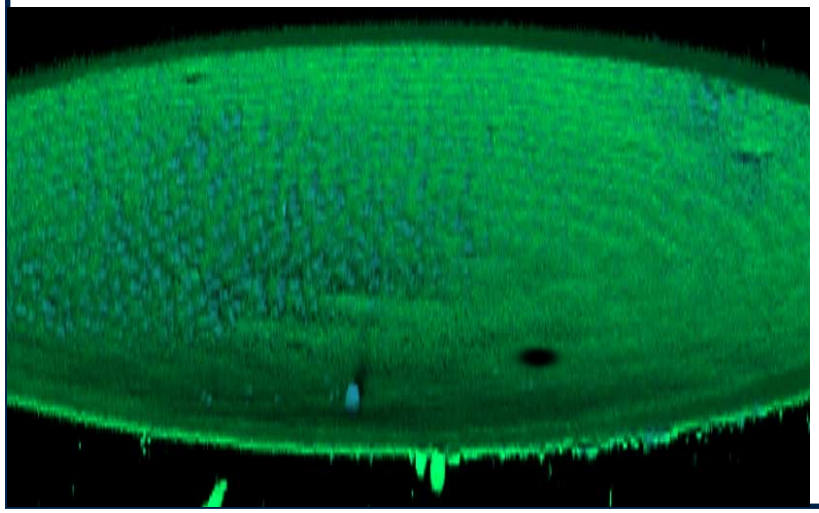
Fluorescence Recovery After Photobleaching (FRAP)

(i) Lens soaked in bath of fluorescing molecules for 1 hr

(ii) Some molecules free, some bound to scaffold

(iii) Laser blasts the molecules in an ROI (radius 5μm) for 250 msec and bleaches them

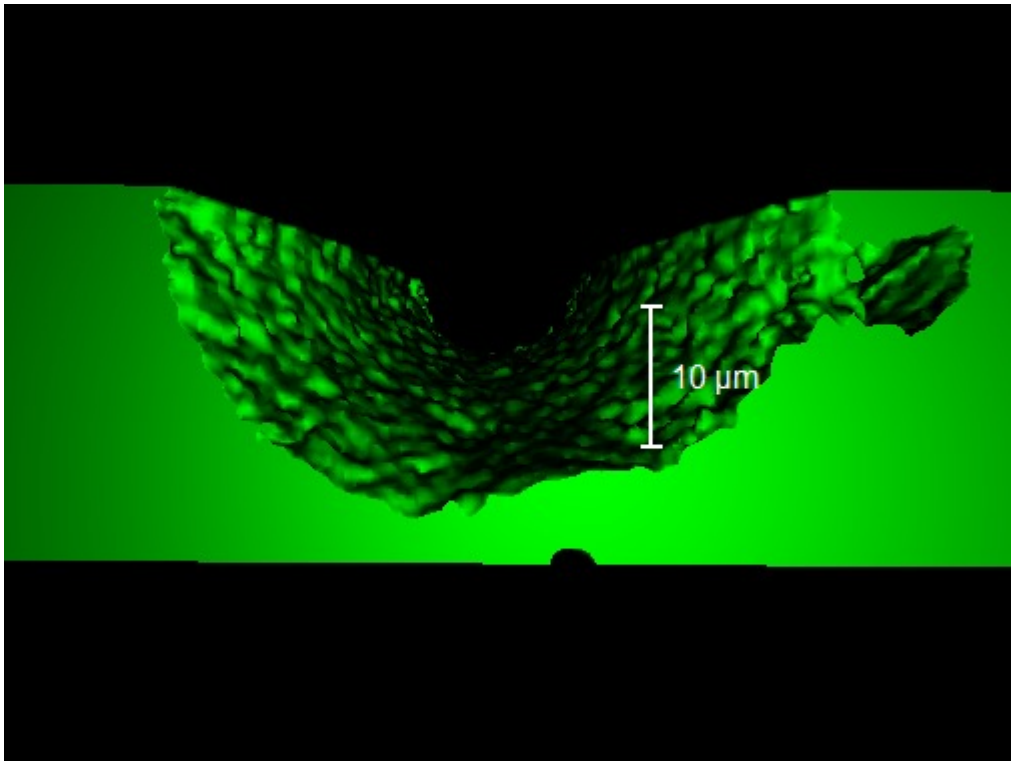




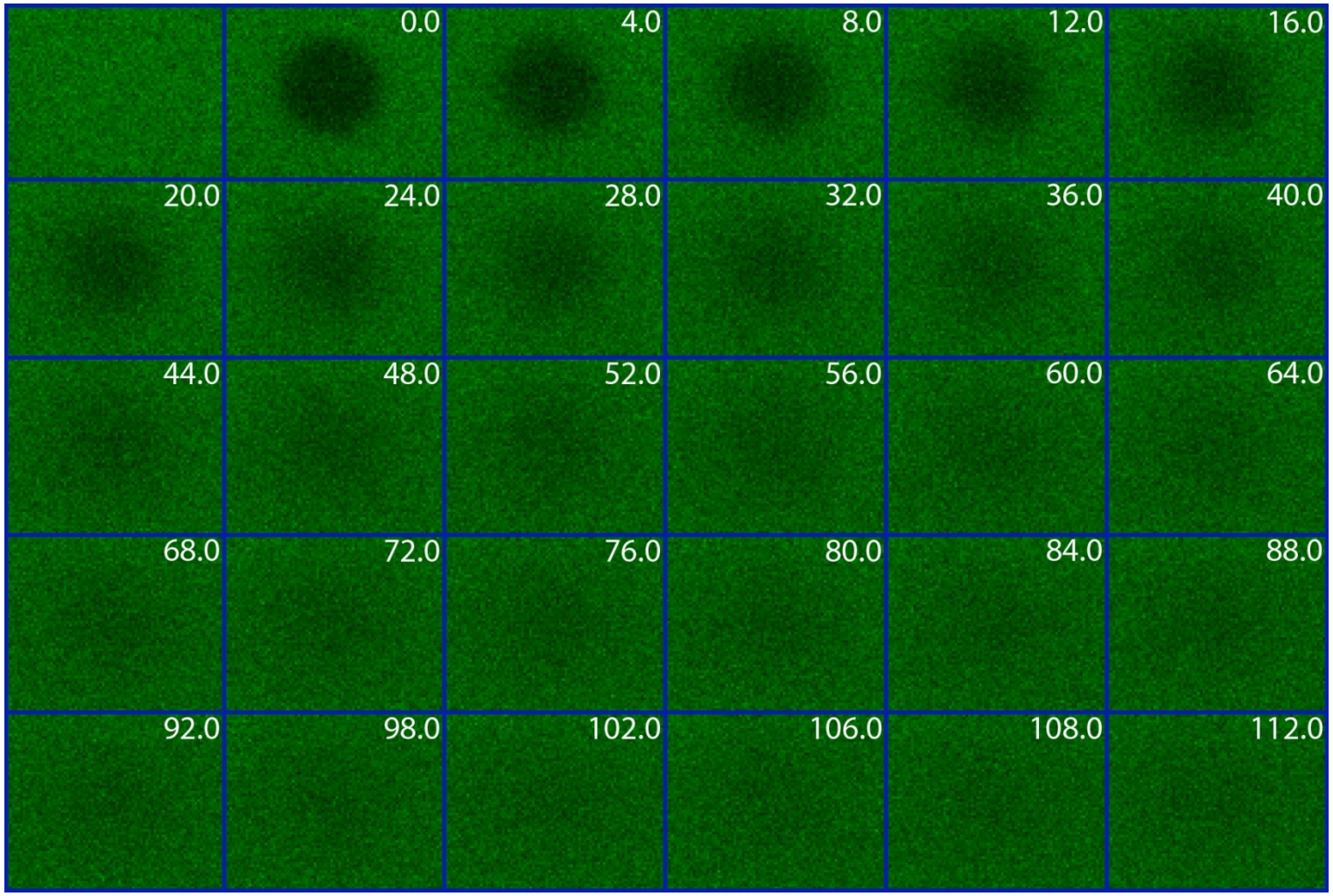
(iv) Diffusion of unbleached molecules into the ROI from outside re-establishes fluorescence

(v) Intensity is calculated by counting intensity of number of pixels in circle and dividing by area

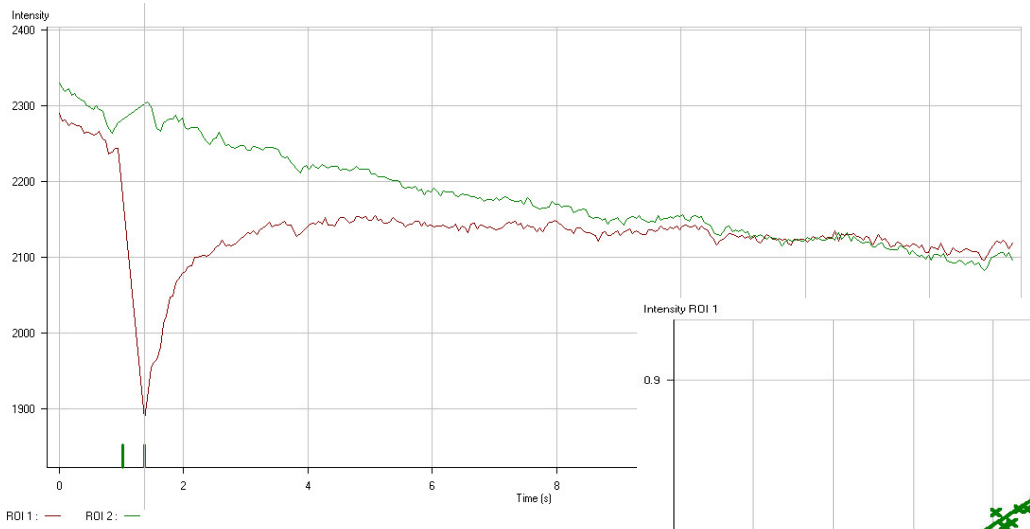
Region of interest (ROI) Bleached profile

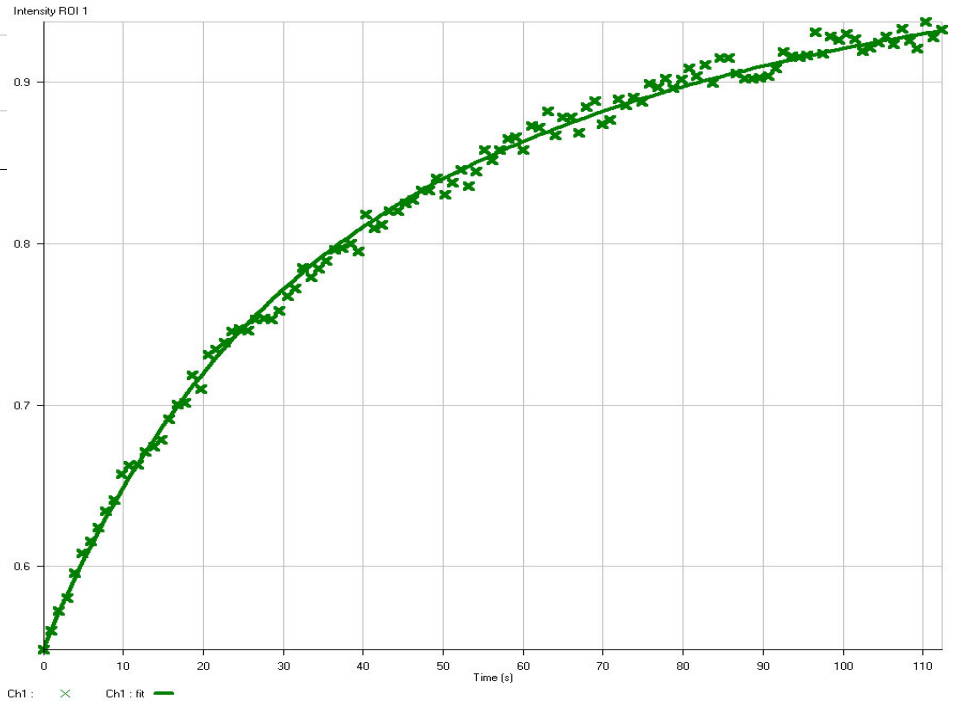


Fluorescence Recovery



Data





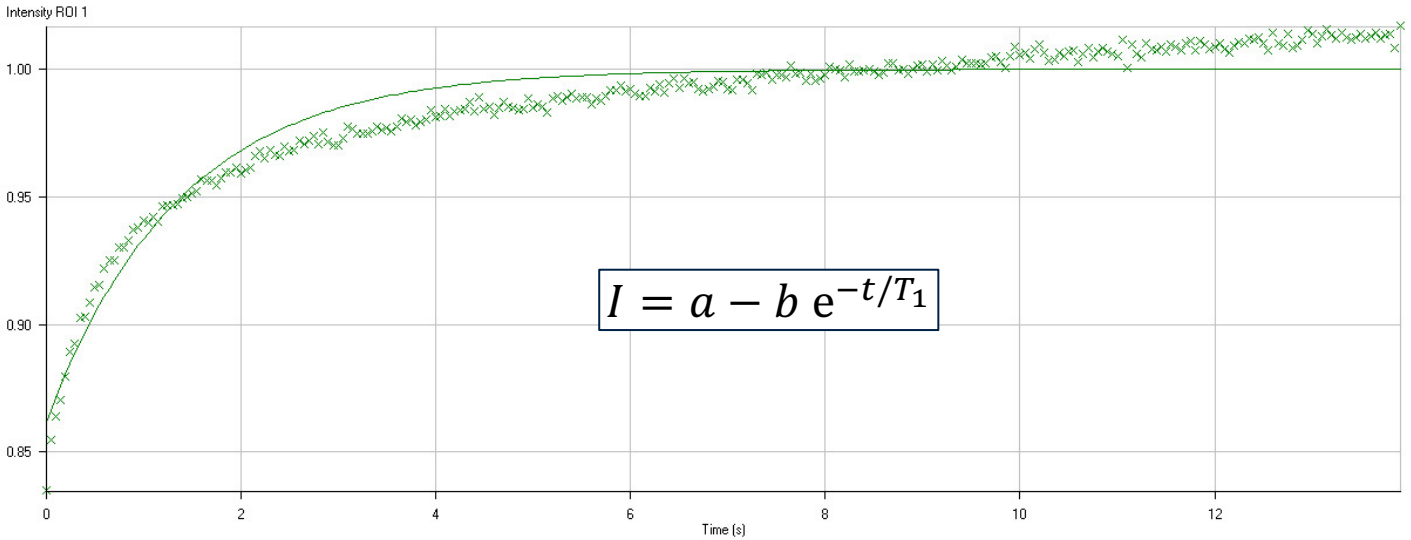
$$D = \frac{\gamma_D w^2}{4\tau_{1/2}}$$

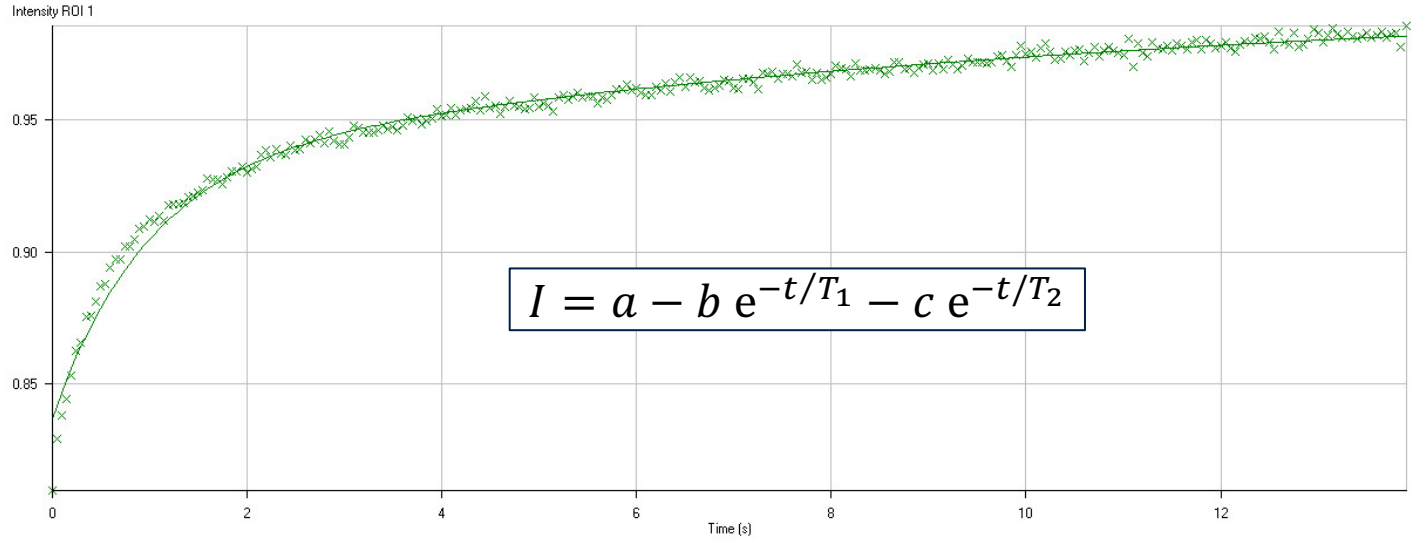
w = ROI radius

$\tau_{1/2}$ = half recovery time

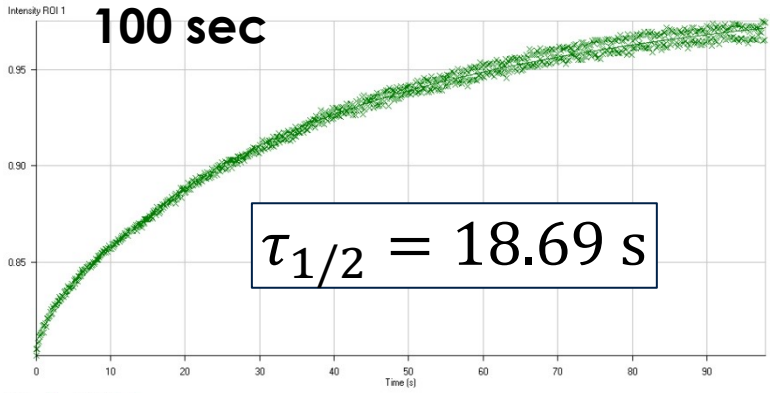
γ_D = correction factor (0.88 for circular beam)

Effectiveness of curve fit

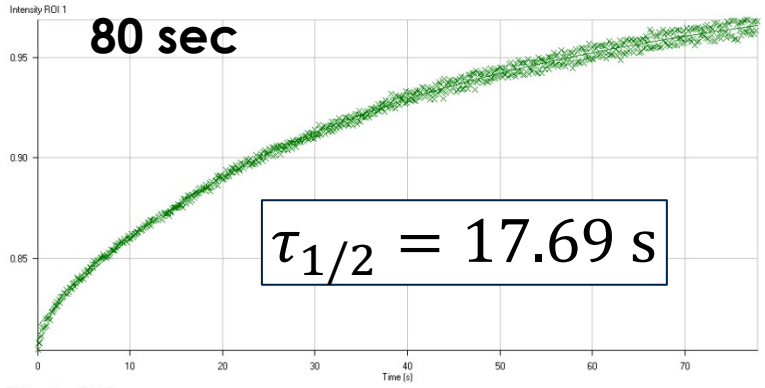




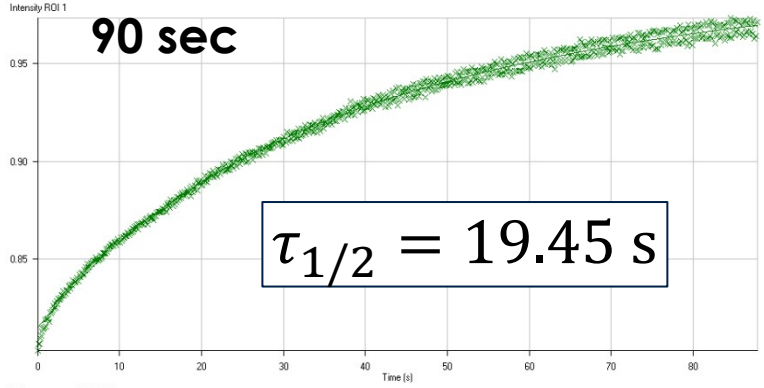
Termination of data collection



I0	I1 mobile fraction	T1 [s]	t1 half [s]	K1 [1/s]	I2 mobile fraction	T2 [s]	t2 half [s]	K2 [1/s]	I delta immobile fraction
1.000000	0.073546	18.693370	12.957257	0.053495	0.115148	69.064153	47.871623	0.014479	-0.081171



I0	I1 mobile fraction	T1 [s]	t1 half [s]	K1 [1/s]	I2 mobile fraction	T2 [s]	t2 half [s]	K2 [1/s]	I delta immobile fraction
1.000000	0.050849	17.690501	12.262121	0.056528	0.132939	56.384263	39.082593	0.017735	-0.078226



I0	I1 mobile fraction	T1 [s]	t1 half [s]	K1 [1/s]	I2 mobile fraction	T2 [s]	t2 half [s]	K2 [1/s]	I delta immobile fraction
1.000000	0.064170	19.448597	13.480740	0.051418	0.121084	62.321316	43.197844	0.016046	-0.079587

Issues

- Truncating at different times gives different values for D
- “Double exponential” (5) vs “single exponential” (3)
  - How many free parameters does the problem really have?
- Some data has structure not captured by either type of fit
- Are assumptions used to get the diffusivity from the “half-life” (e.g. cylindrical profile) justifiable?

Aim

- To quantify the results more accurately by:
  - modelling the activity within the lens cap;
  - determining which equation best fits the curve to the raw data, and why.
- To develop a tool to reliably fit a curve to the raw data and thus estimate the diffusion coefficient.