Likelihood estimation of diffusion coefficients from sequences of confocal microscope images

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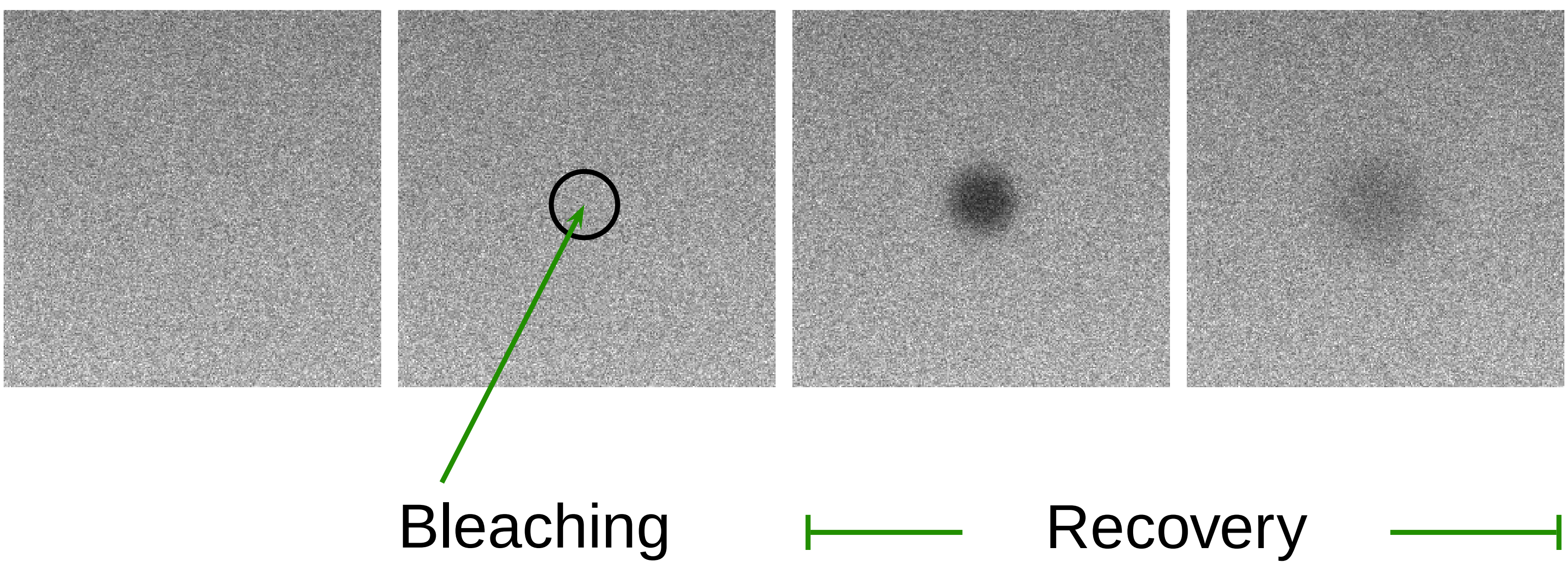
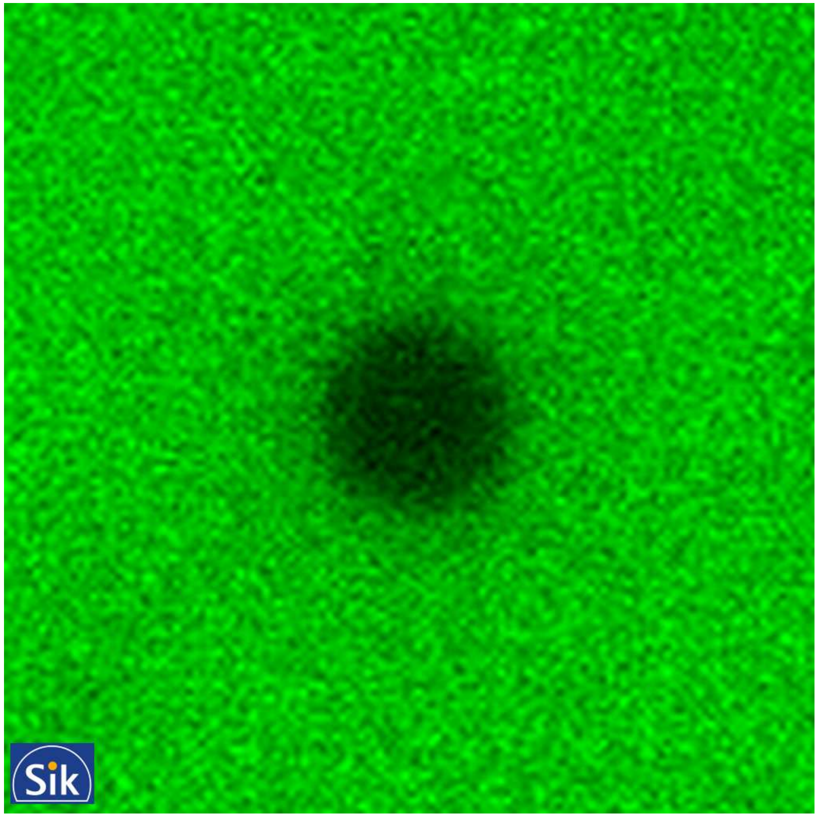
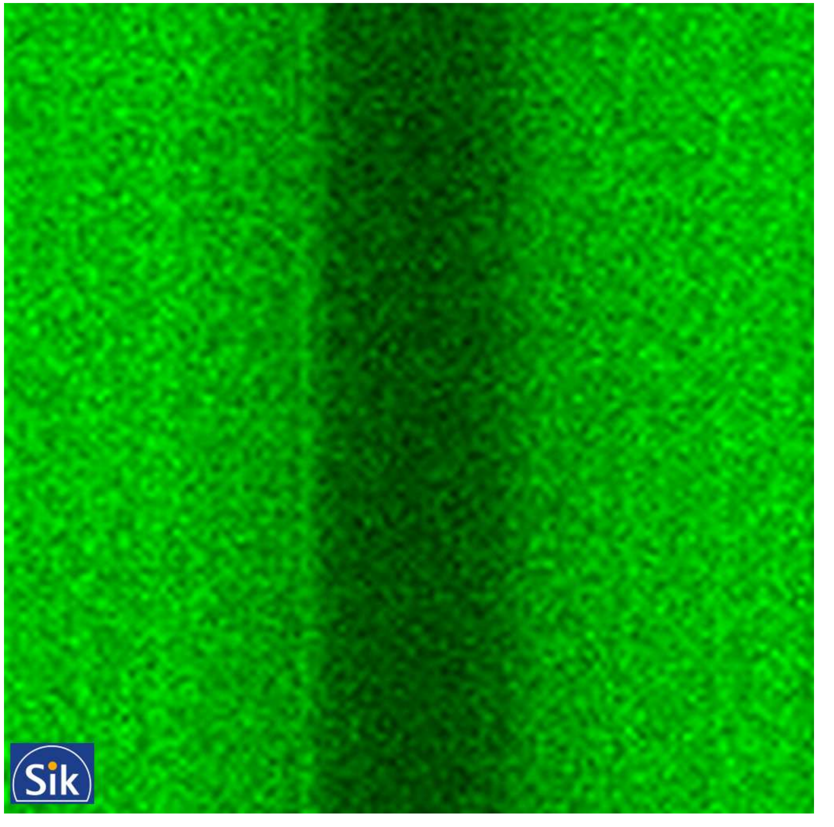
FRAP

- Fluorescence Recovery After Photobleaching



Depth profile



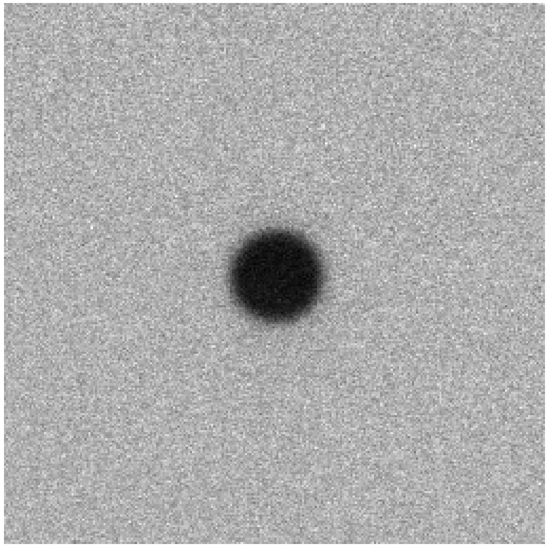


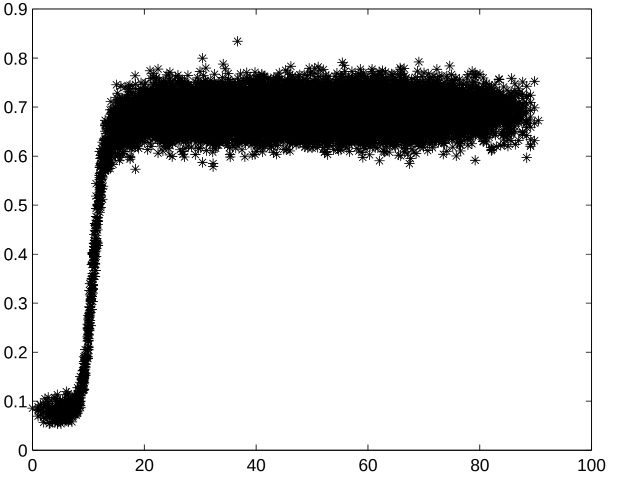
Diffusion equation

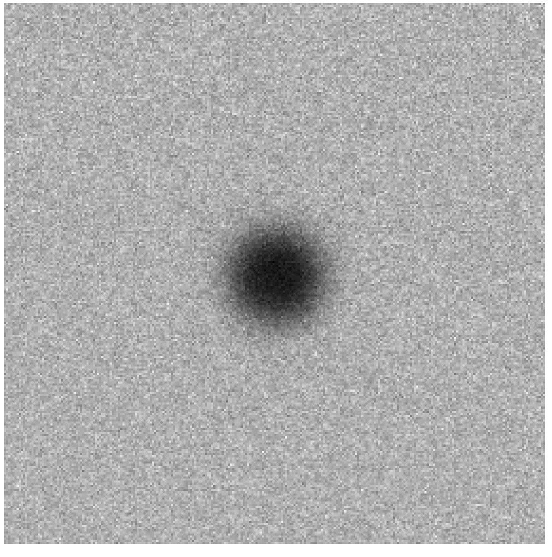
- Rotational symmetry
- No net diffusion in z-direction
- Diffusion equation

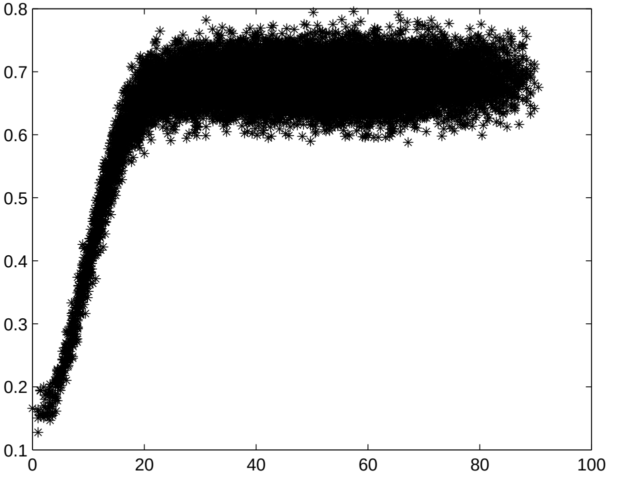
$$\frac{\partial C}{\partial t} = D\left\{\frac{1}{r}\frac{\partial C}{\partial r} + \frac{\partial^2 C}{\partial r^2}\right\}.$$

Initial concentration





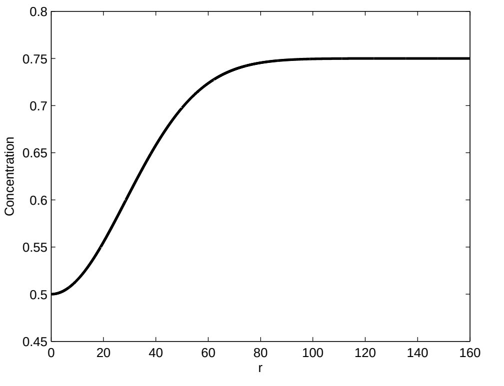




Model 1

- Parametric initial concentration profile

$$C_o(r) = c_0 - c_1 \exp\left(\frac{-r^2}{r_0^2}\right)$$



- $\Rightarrow$ Analytic solution to the diffusion equation

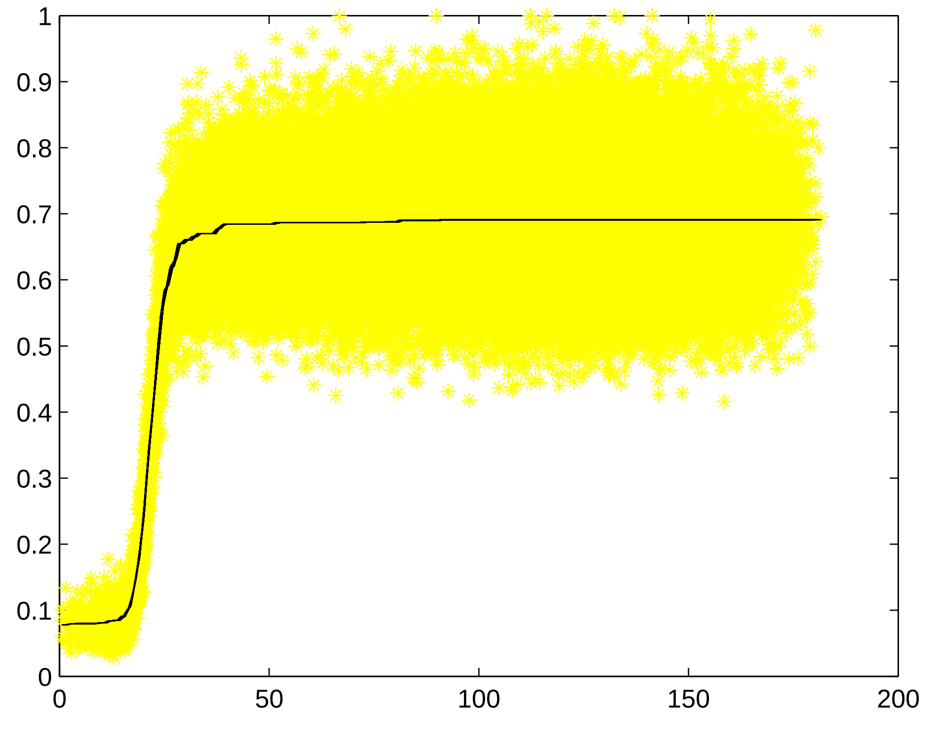
$$C(r,t) = c_0 - \frac{c_1}{4Dt + r_0^2} \exp\left(-\frac{r^2}{4Dt + r_0^2}\right).$$

Model 1

- Normally distributed noise
- Noise is independent between pixels
- Maximum likelihood estimation of the parameters $D, c_0, c_1, r_0, \sigma^2$.
- Fast
- Limited to image sequences where the first image has a profile already smoothed by diffusion

Model 2

- Initial concentration profile estimated from the first image using isotonic regression followed by kernel smoothing.



Model 2

- Let $N(x,t)$ be the photon count at x at time t and let the pixel value be $p(x,t) = kN(x,t)$.
- $N(x,t)$ is Poisson distributed with expectation $\lambda(x,t)$, which we write as

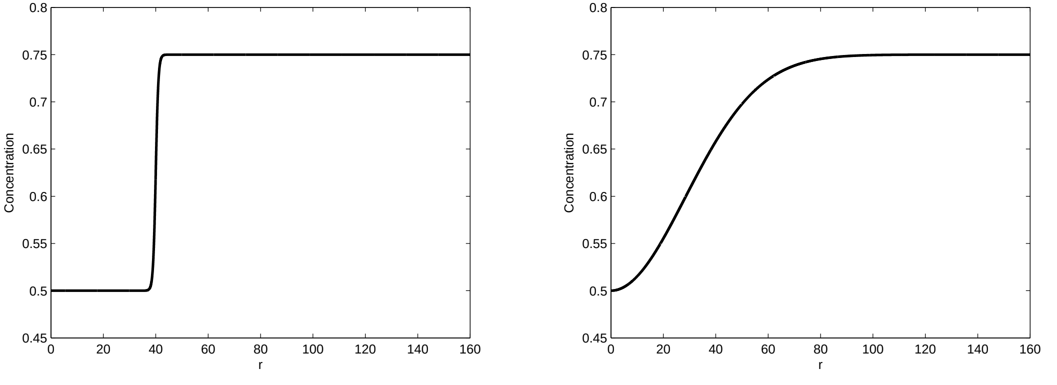
$$\lambda(x,t) = \sum_y g_0(|y|) f(|x-y|,t),$$

- $g_0(|y|)$ is the initial concentration
- “Diffusion propagator”

$$f(d,t) = \frac{1}{4\pi Dt} \exp\left(-\frac{d^2}{4Dt}\right).$$

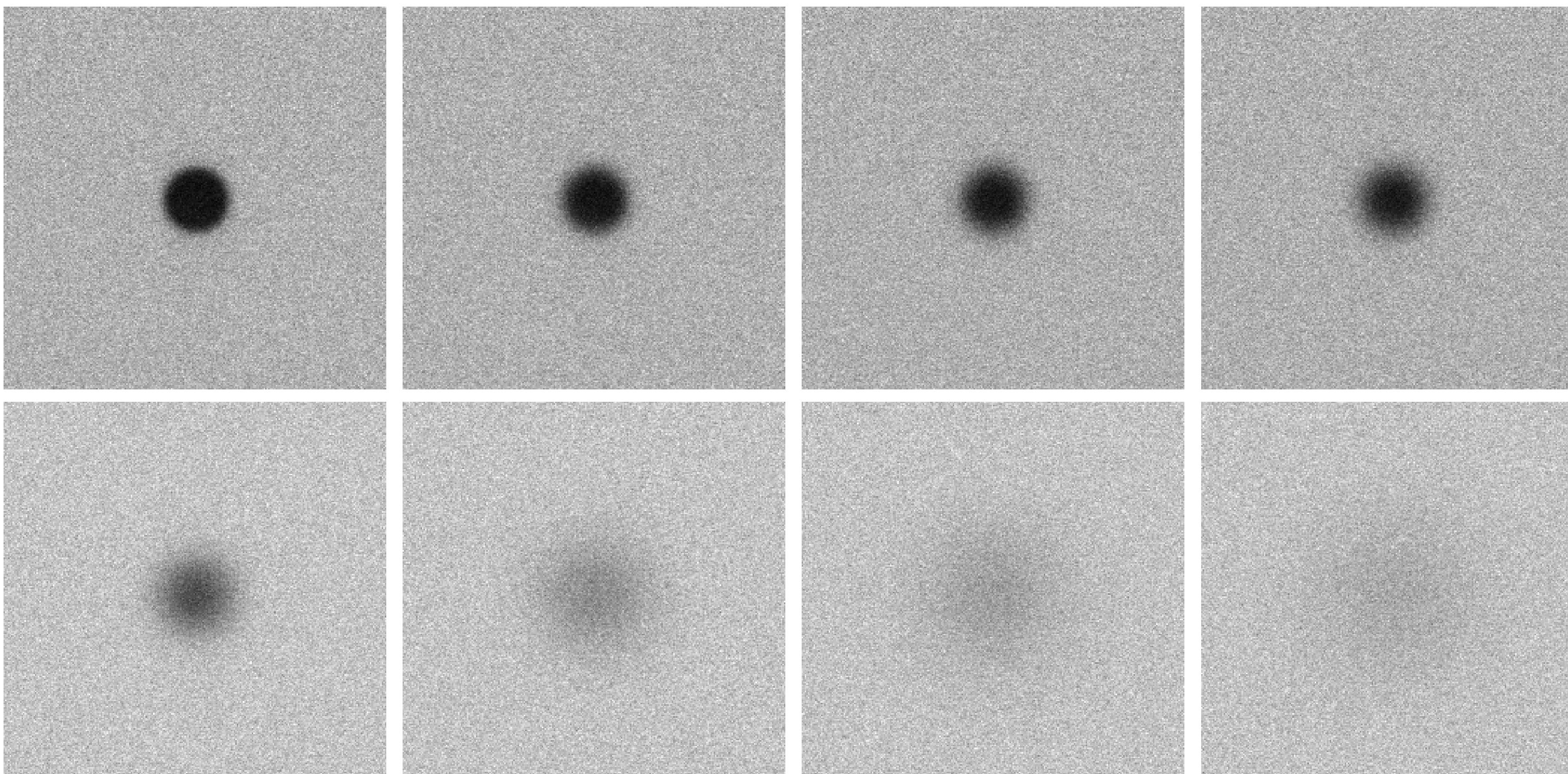
Model 2

- Maximum likelihood estimation of D. (k estimated from pre-bleach image).
- Slow
- Flexible in terms of initial concentration profile



SAP

- SAP, 0.1 mole% crosslinker, swollen 1 and 2 times it's weight.
- 50 ppm Na Fluorescein



SAP 2x - model fit

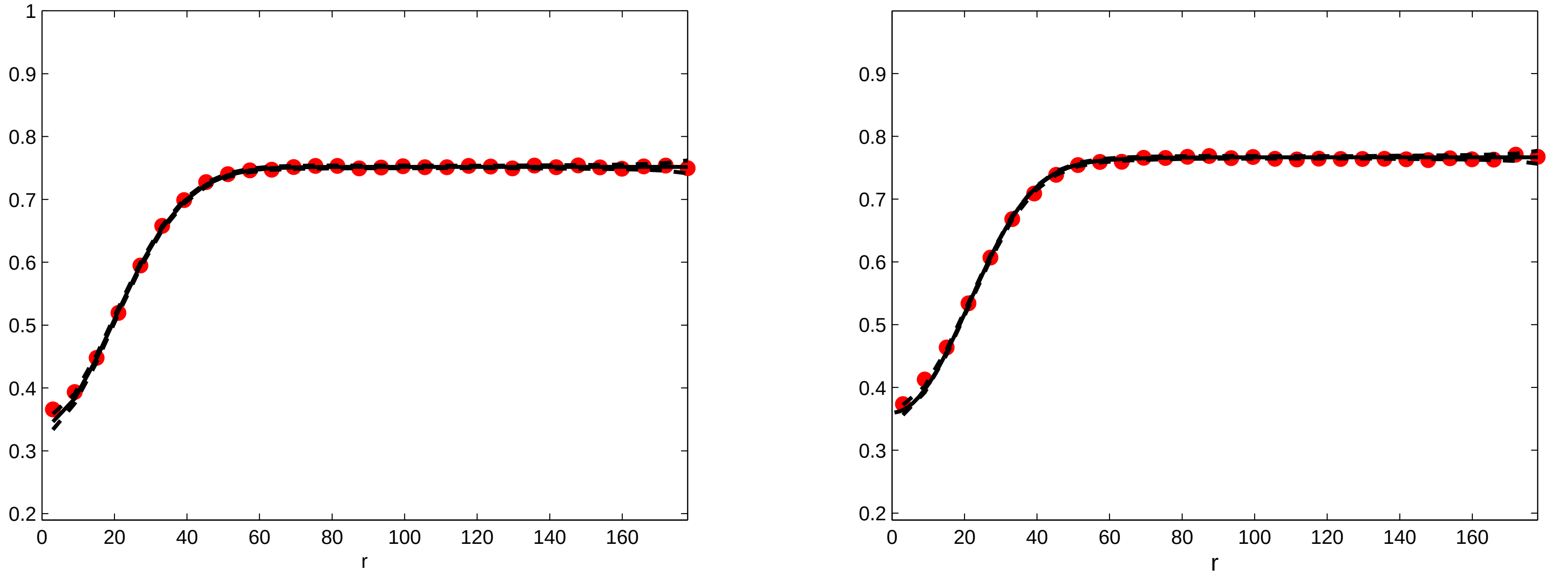


Figure 1: Model 1 to the left and model 2 to the right. Post-bleach image no 2.

SAP 2x - model fit

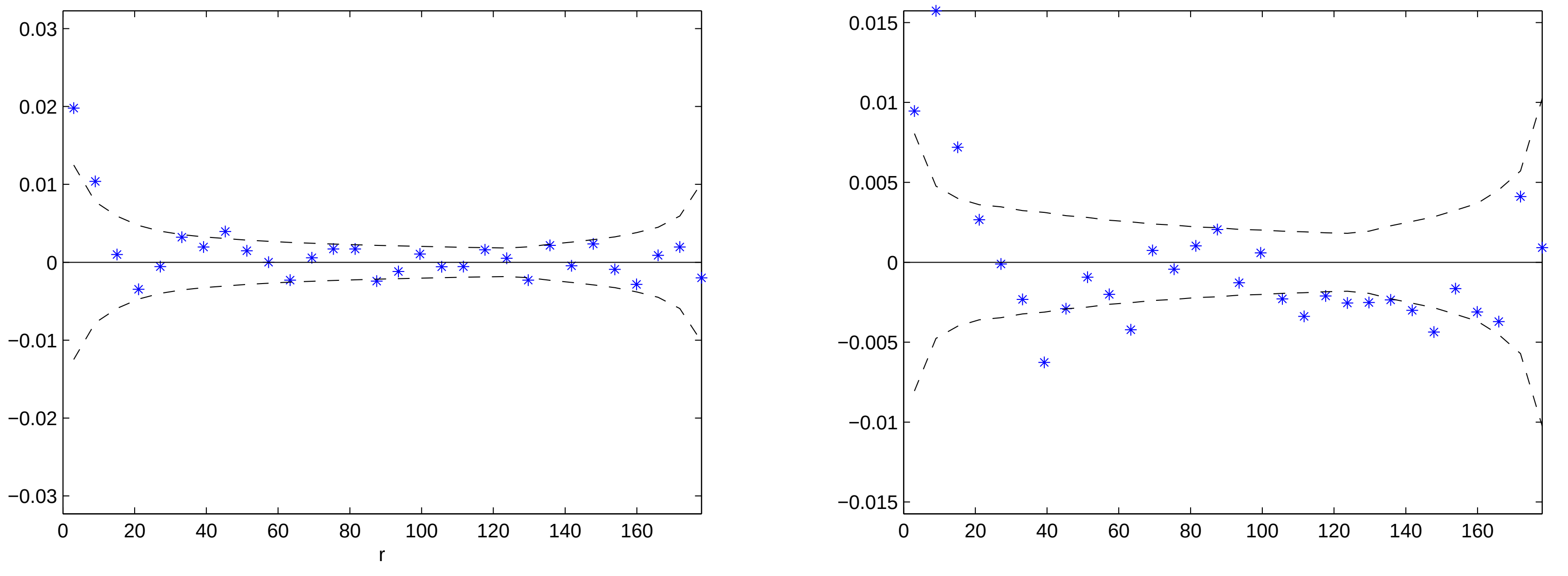


Figure 2: Model 1 to the left and model 2 to the right. Post-bleach image no 2.

SAP 2x - Diffusion coefficient

Units: $(\mu m)^2/s$

ROI 30 μm

Model 1	Model 2
29.9	30.4
30.3	30.2
29.0	29.1
$\bar{D}$=29.7	$\bar{D}$=29.9
s=0.68	s=0.67

ROI 50 μm

Model 1	Model 2
28.2	28.7
27.3	27.8
28.0	28.0
27.4	27.1
27.4	27.6
$\bar{D}$=27.7	$\bar{D}$=27.9
s=0.40	s=0.56

SAP 1x - model fit

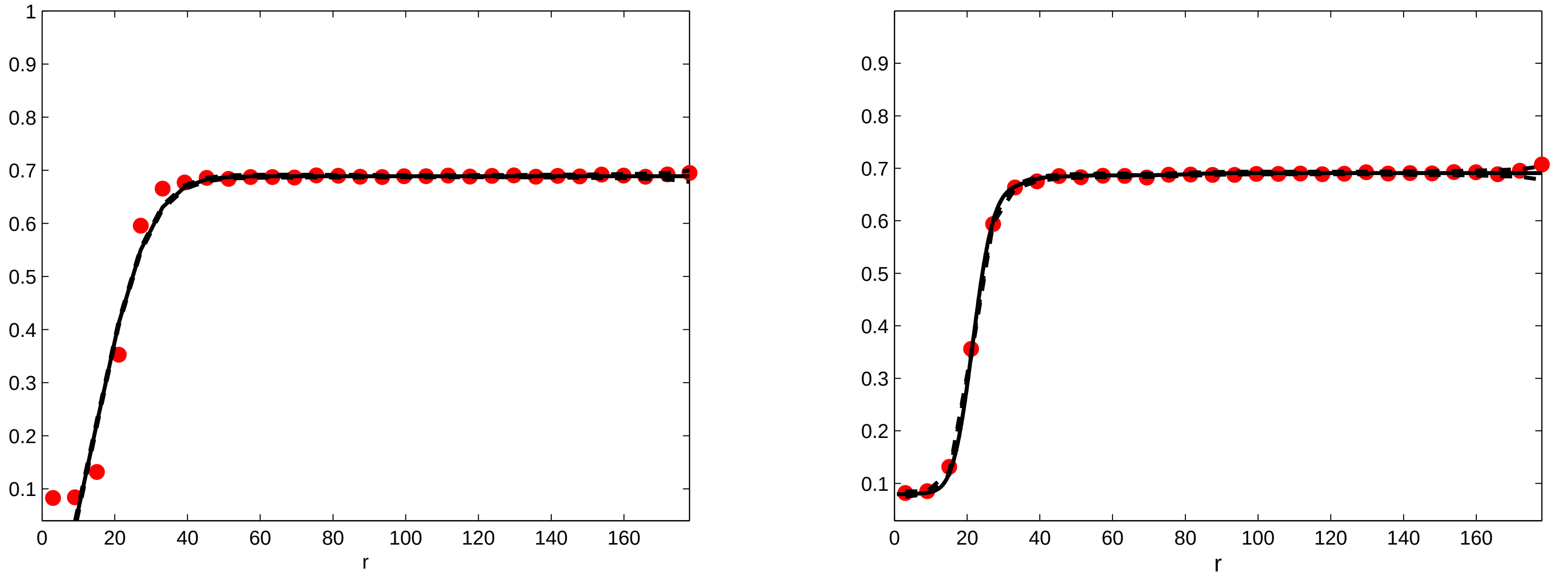


Figure 3: Model 1 to the left and model 2 to the right. Post-bleach image no 2.

SAP 1x - model fit

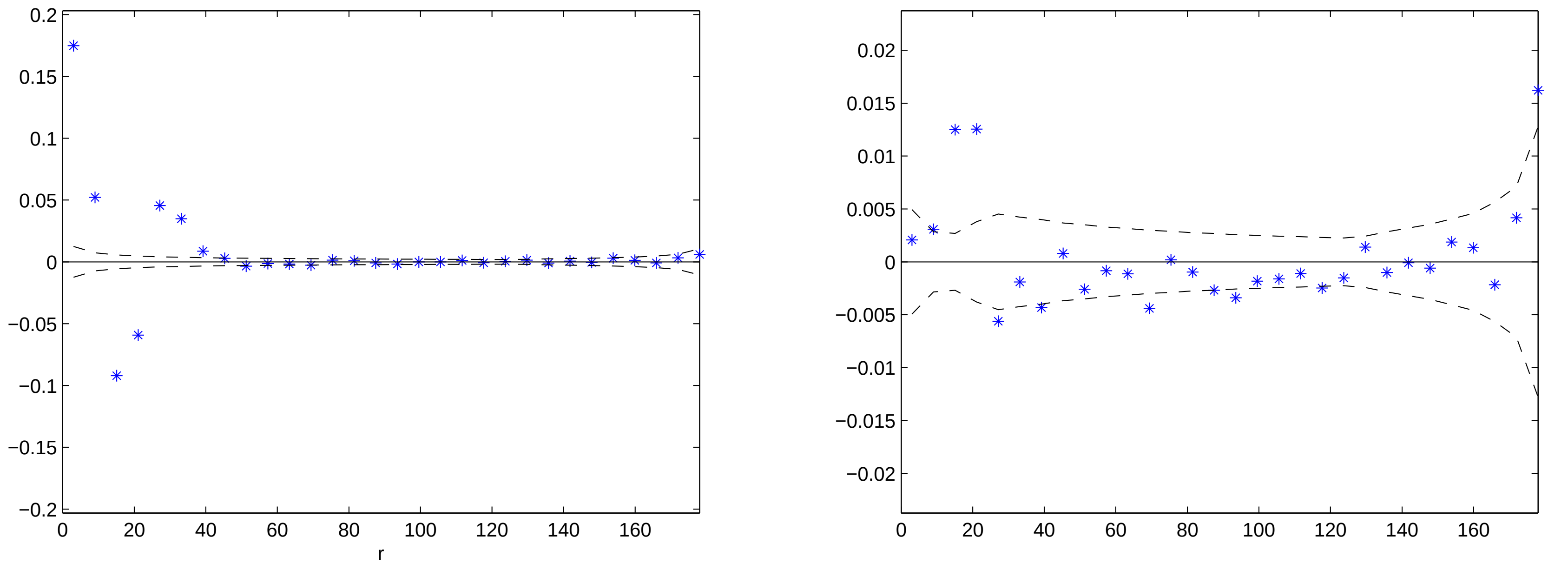


Figure 4: Model 1 to the left and model 2 to the right. Post-bleach image no 2.

SAP 1x - Diffusion coefficient

Units: $(\mu m)^2/s$

	ROI 30 μm		ROI 50 μm	
	Model 1	Model 2	Model 1	Model 2
	1.03	1.25	1.71	2.03
	0.85	1.06	0.57	0.65
	0.68	0.88	0.12	0.14
	0.89	1.13	0.09	0.09
	$\bar{D}$=0.86	$\bar{D}$=1.08	$\bar{D}$=0.62	$\bar{D}$=0.73
	s=0.14	s=0.16	s=0.75	s=0.90

Future work

- Irregularly bleached regions
- Inhomogeneous media - additional structure information is needed
- Diffusing particles of different size
- Account for the scanning
- Calculate variance components to plan experiments
- Investigate instrumental settings - spatial resolution, relative size of bleached region, the number of images

Background subtraction

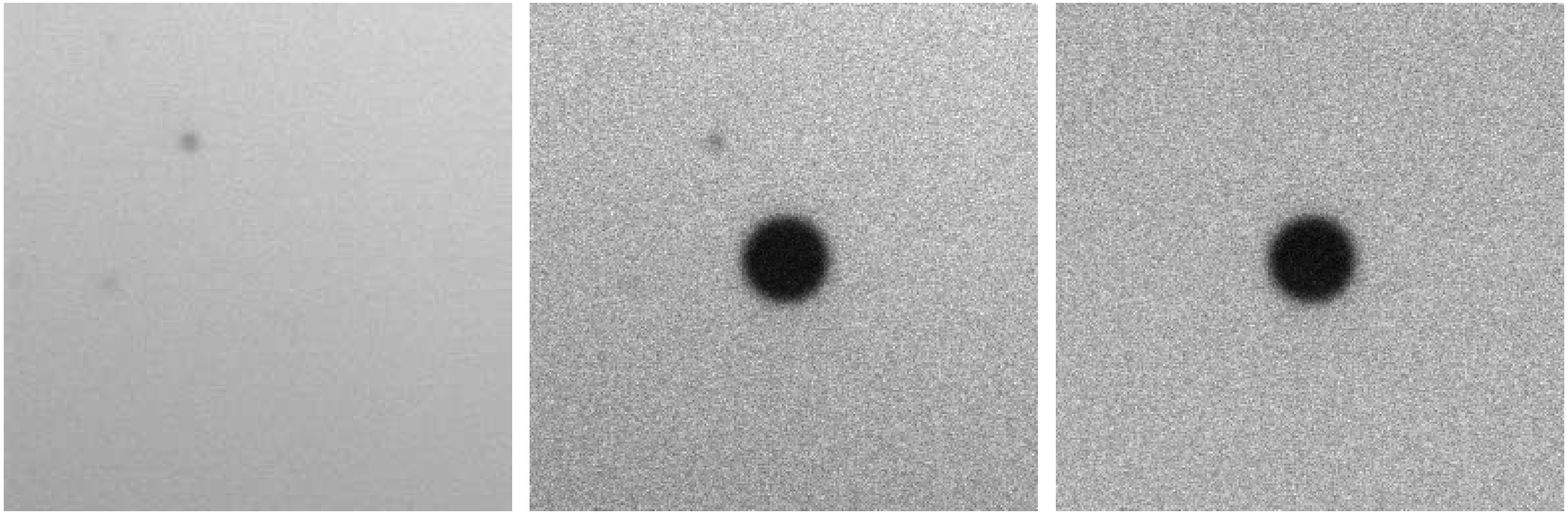


Figure 5: Average pre-bleach image, first post bleach image without and with subtraction of background.