Application of Likelihood methods to FRAP data

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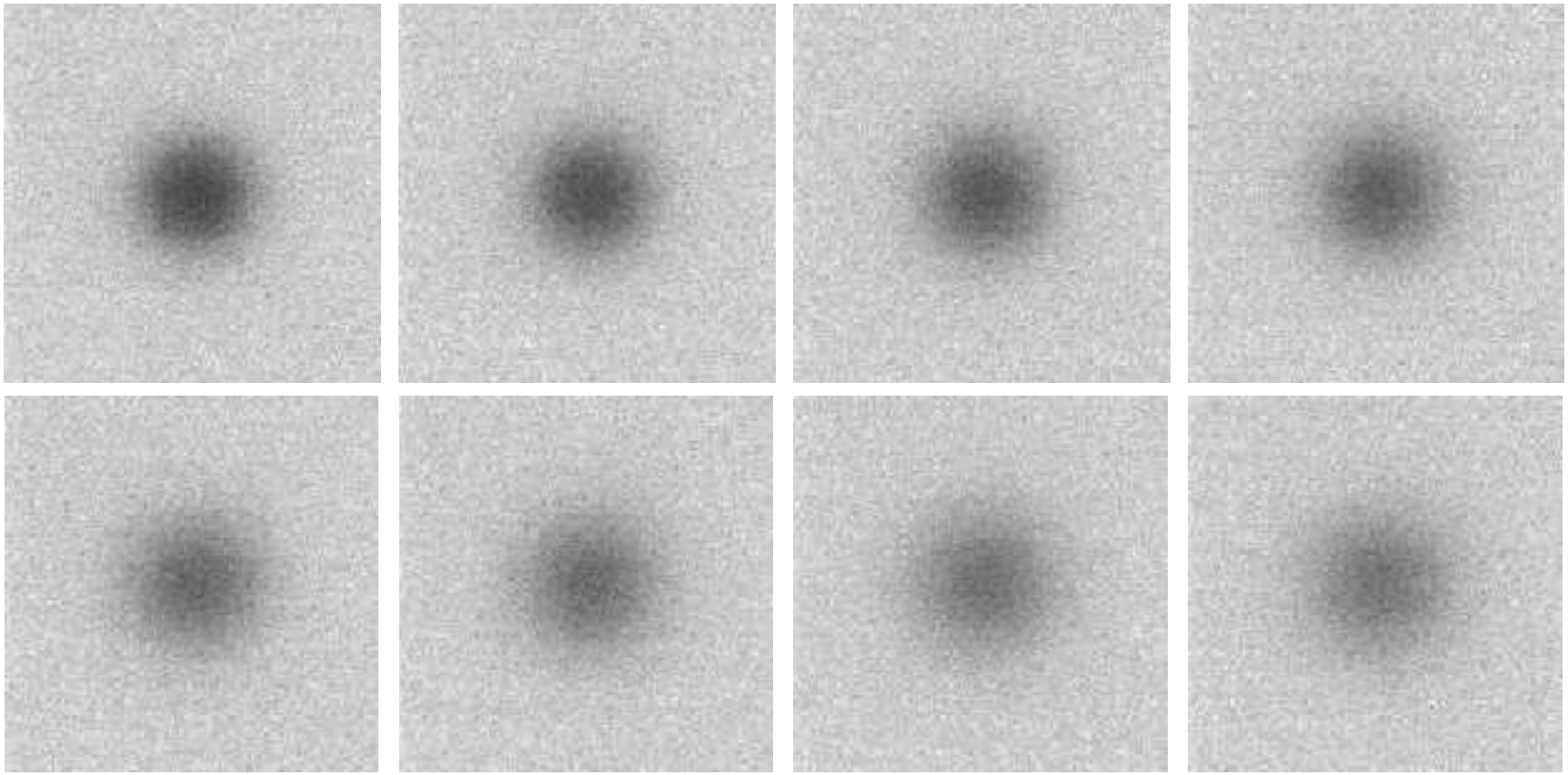
Aim

- Of interest in drug industry and food industry
- The influence of the structure of a food on the release of taste substances.
- Improve the analysis of FRAP data (slowly diffusing molecules).

Images



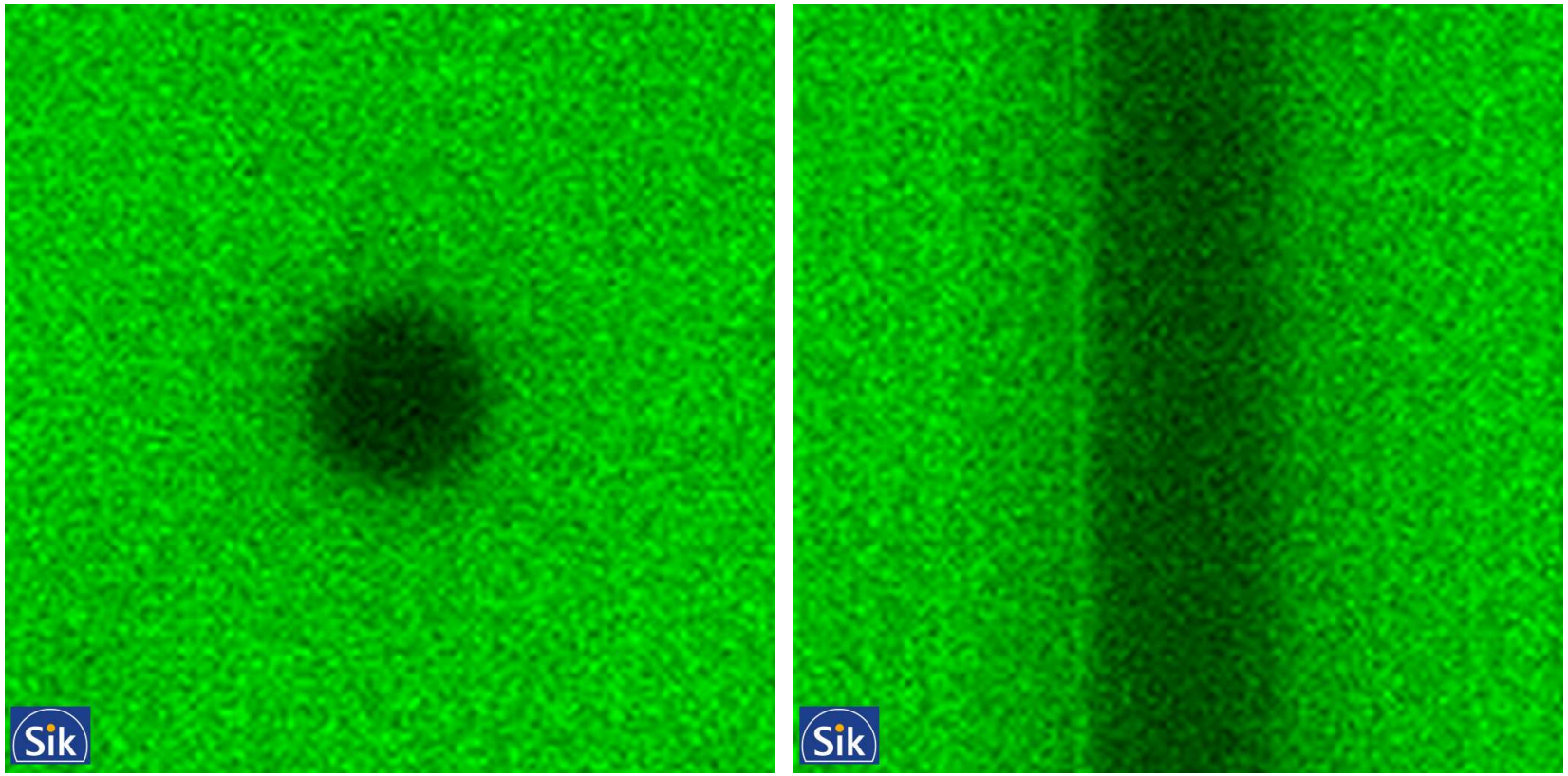
FRAP

- FRAP - Fluorescence Recovery After Photobleaching
- Technique for studying diffusion.
- Diffusing molecules should
  - be fluorescing and stable when radiated with a low intensity laser.
  - change structure and lose the fluorescing property irretrievably when radiated with a high intensity laser.
- A region of the sample is “bleached” with a high intensity laser. Fluorescing molecules are then observed as they diffuse into the bleached region.
- Can be used to study diffusion in heterogeneous media and in cells.

CSLM

- CSLM Confocal Scanning Light Microscope
- Scanning of the sample pixel by pixel.
- Almost all light from above or below the focal plane is prevented from contributing to the image.
- The intensity of the bleaching light is constant within the sample, in z-direction.
- The result is a diffusion of fluorescing molecules in 2D.
- The bleaching is repeated several times.

Depth profile



Model

Diffusion equation

$$\frac{\partial C}{\partial t} = D\left(\frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial y^2} + \frac{\partial^2 C}{\partial z^2}\right)$$

Assume rotational symmetry and as a first approximation that the initial concentration is of the form

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

Model

The solution of the differential equation is of the form

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

C concentration, D diffusion coefficient, r distance from the centre of the bleached spot, r_0 radius of the bleached spot.

Model

The images are noisy and therefore we assume that the pixel value is proportional to the concentration, but with a normally distributed noise. The pixel value at distance r from the centre of the bleached spot at time t is normally distributed with mean $C(r,t)$ and variance σ^2. The probability density function for the pixel value $p(r,t)$ is

$$\frac{1}{\sqrt{2\pi\sigma^2}}\exp\left(-\frac{(p(r,t)-C(r,t))^2}{2\sigma^2}\right).$$

Model

Assuming that the pixel values are independent, the joint likelihood function for all pixels at all times can be written,

$$\prod_{t\in T}\prod_{i=1}^{N}\frac{1}{\sqrt{2\pi\sigma^2}}\exp\left(-\frac{(p(r,t)-C(r,t))^2}{2\sigma^2}\right).$$

The likelihood is maximised with respect to the parameters $c_0, c_1, r_0, D, \sigma^2$ and the centre coordinates of the bleached spot.

Results

Sample no	D	95% confidence interval
1	20.9	(20.6, 21.2)
1	20.4	(20.1, 20.8)
1	21.3	(20.9, 21.6)
1	21.2	(20.9, 21.5)
2	26.2	(25.8, 26.6)
2	27.4	(27.0, 27.8)
2	26.1	(25.8, 26.5)
2	24.7	(24.4, 25.1)
3	22.9	(22.6, 23.3)
3	23.0	(22.6, 23.3)
3	23.9	(23.6, 24.3)
3	23.6	(23.2, 23.9)

Table 1: All values are in 10^{-12} m^2/s.

Simulation

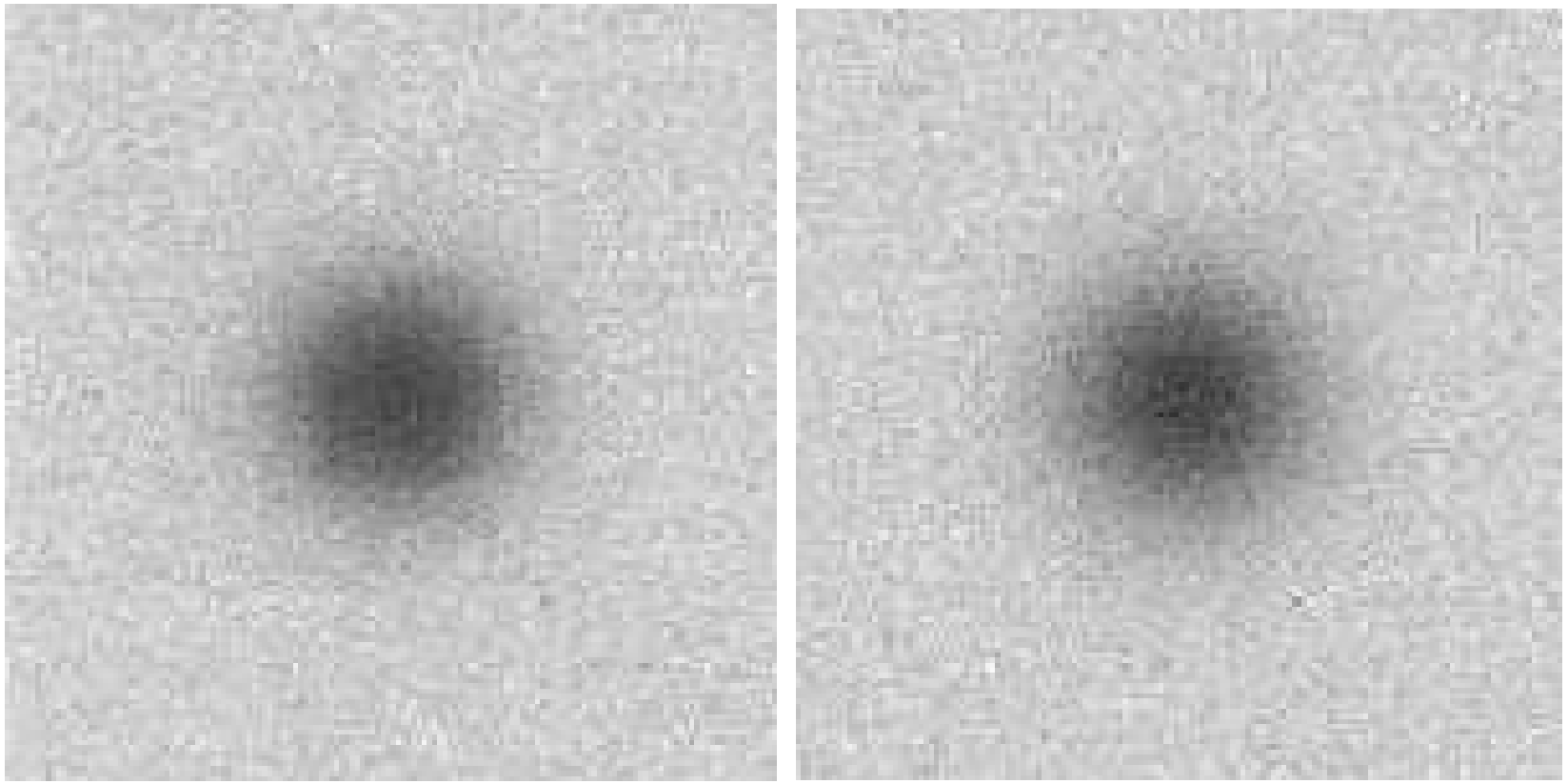


Figure 1: Image at time 0.34 s to the left and simulation of the model to the right.

Fit

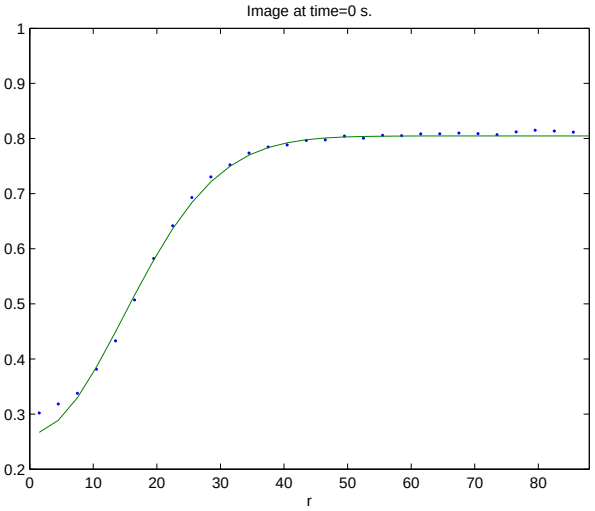


Image at time=0 s.
r

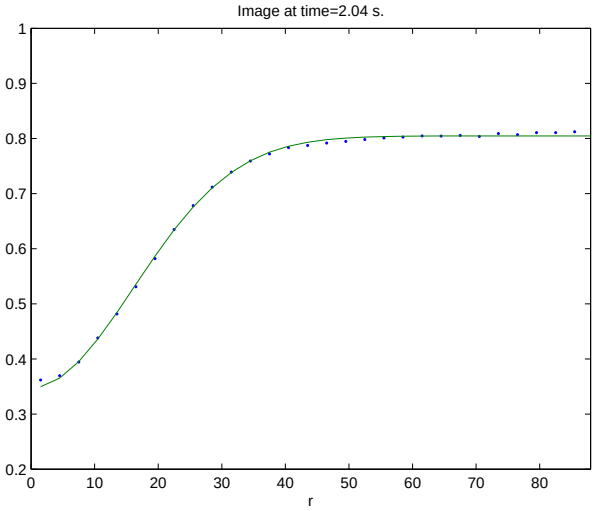


Image at time=2.04 s.
r

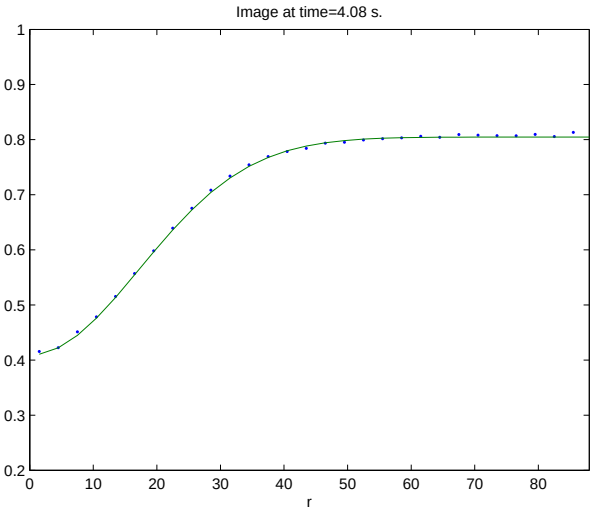


Image at time=4.08 s.
r

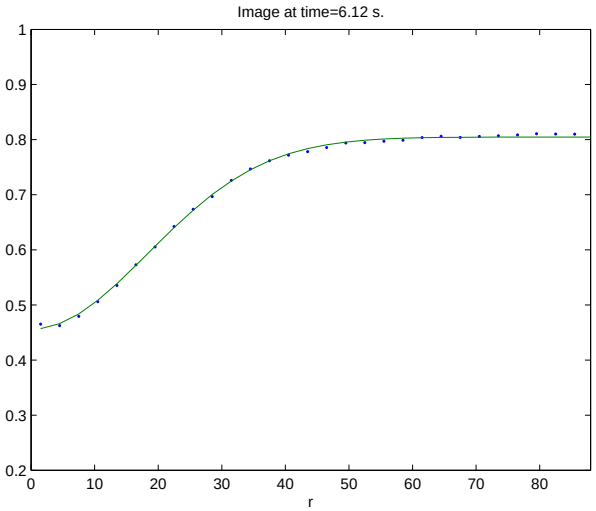


Image at time=6.12 s.
r

Fit

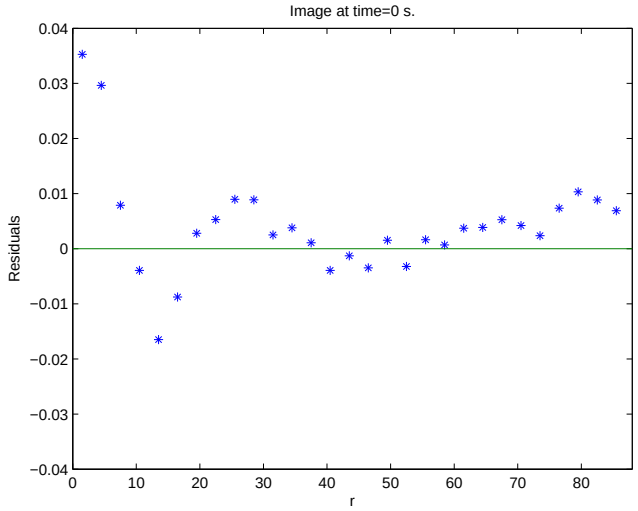
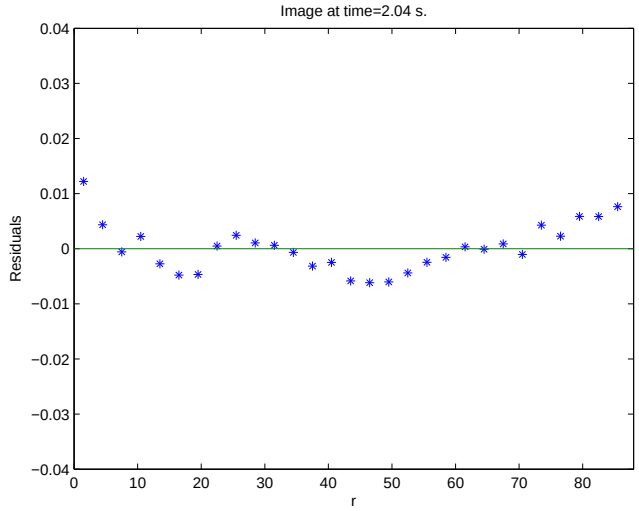


Image at time=0 s.
Residuals
r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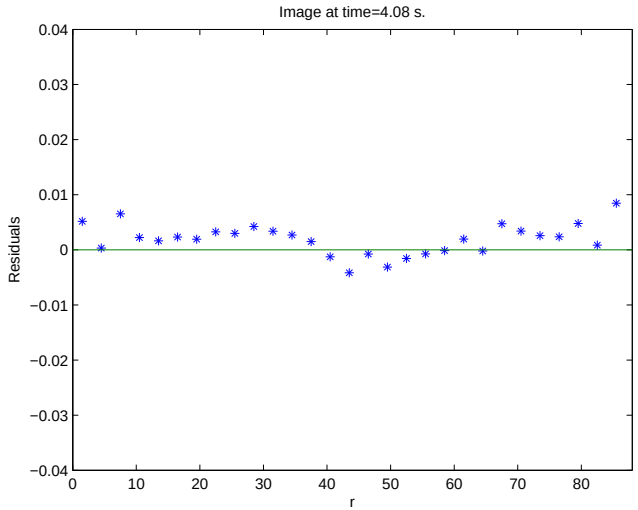
Residuals
r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Residuals
r

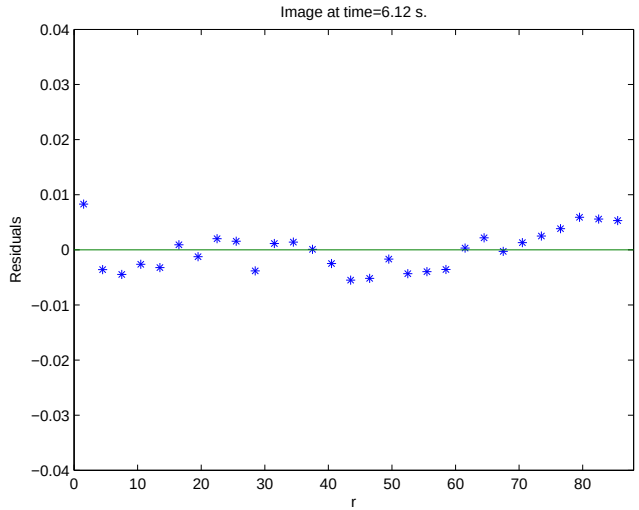
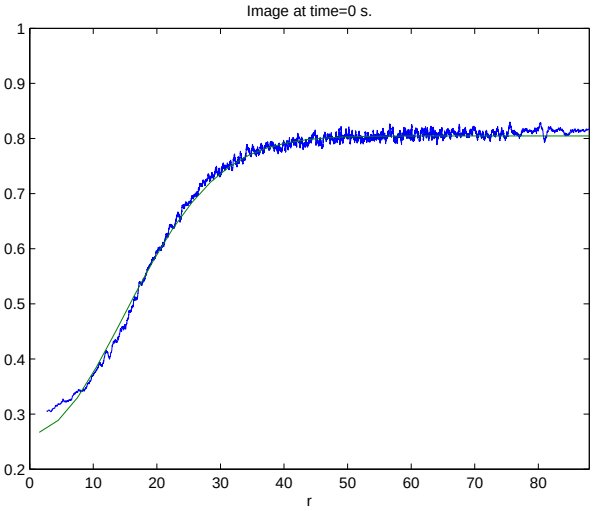


Image at time=6.12 s.
Residuals
r

Fit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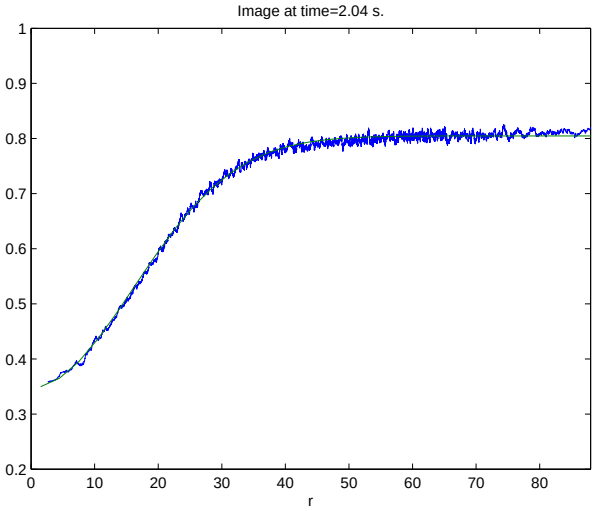
r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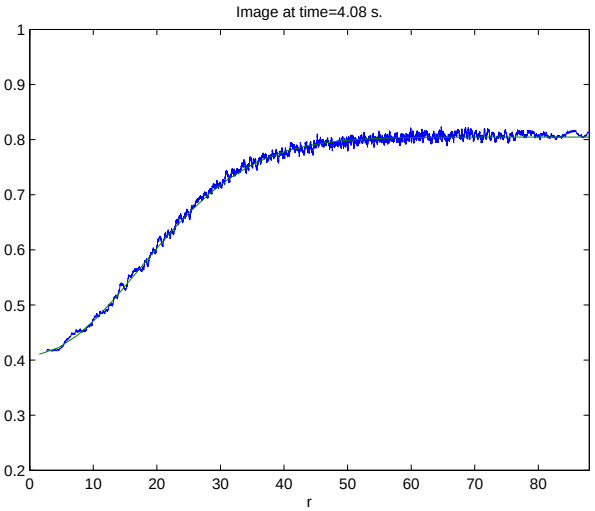
r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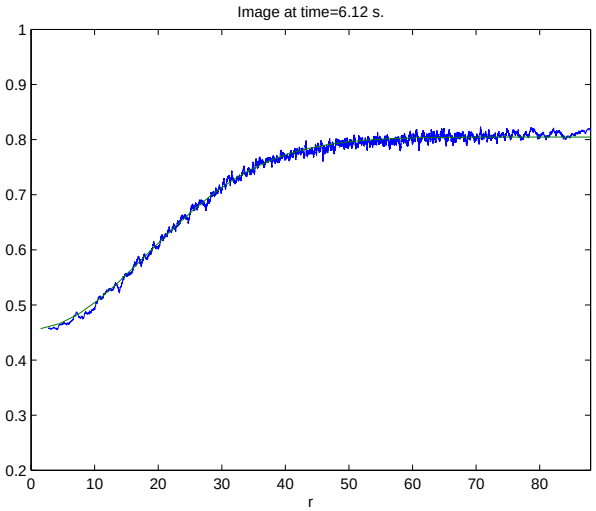
Image at time=6.12 s.
r

Fit

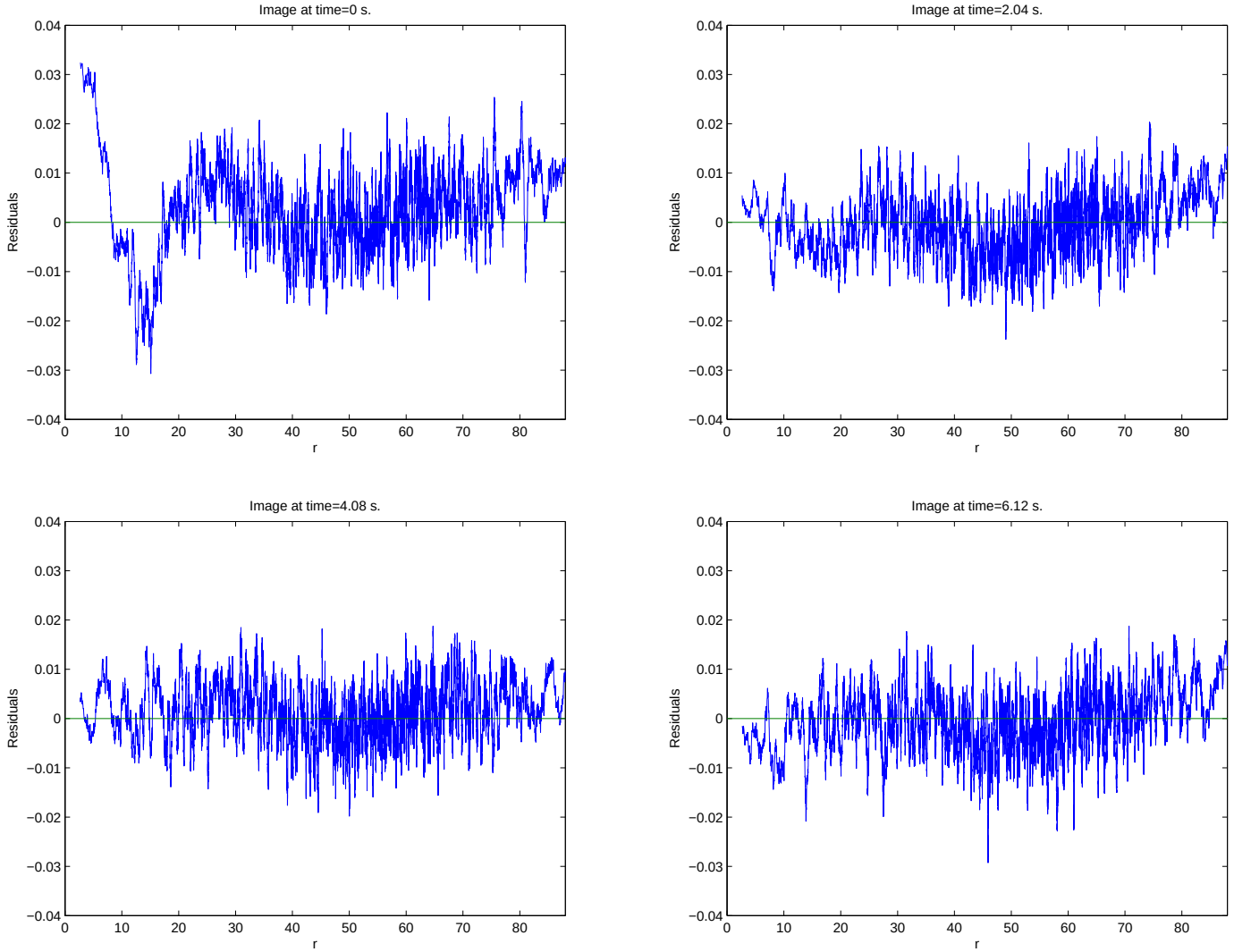
Image at time=0 s.
Image at time=2.04 s.
Image at time=4.08 s.
Image at time=6.12 s.
Residuals
r
0.04
0.03
0.02
0.01
0
−0.01
−0.02
−0.03
−0.04
0
10
20
30
40
50
60
70
80

Future work

- Try another function for the start concentration, for example of the form

$$C_o(r) = c_0 - c_1 \exp\left(-\frac{r^2}{r_0^2}\right) + \alpha_1 \exp\left(-\frac{r^2}{\alpha}\right).$$

- Solve the the differential equation analytically/numerically with arbitrary start concentration.
- Let the variation depend on concentration.
- Account for the fact that the laser scans the image.
- Check the independence assumption.
- Molecules of different sizes.

Future work

- FRAP experiments for κ-carrageenan gel.
- Validation of compution procedure by simulating from the model.
- Validation by comparing with NMR measurements.
- Calculate variance components both for measurements at different locations within the same sample and for measurements on replicated samples.
- Adjust measurement parameters
  - The amount of bleaching (intensity, times)
  - Spatial resolution versus time resolution
  - Numerical aperture (high NA - cylindrical beam)
  - Size of bleached spot.