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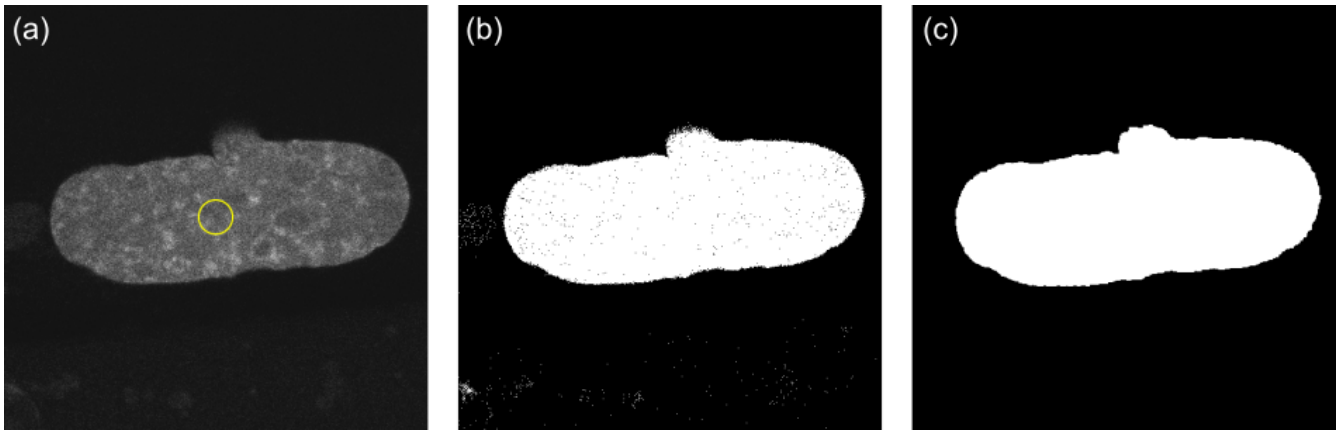
Supplemental Information

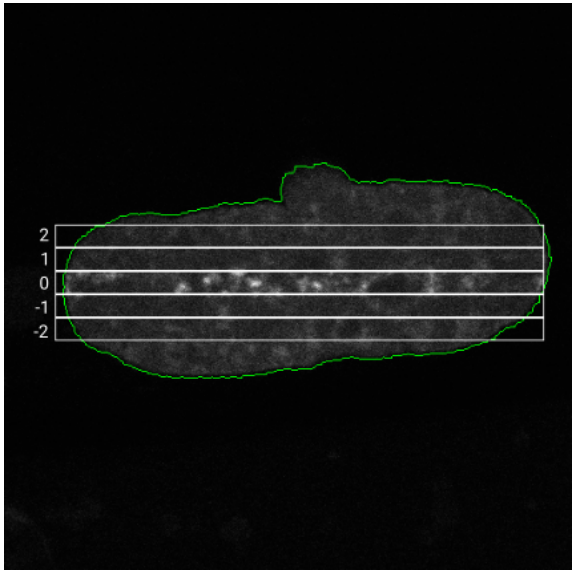
Q-FADD: A Mechanistic Approach for Modeling the Accumulation of Proteins at Sites of DNA Damage

Jyothi Mahadevan, Johannes Rudolph, Asmita Jha, Jian Wei Tay, Joseph Dragavon, Erik M. Grumstrup, and Karolin Luger



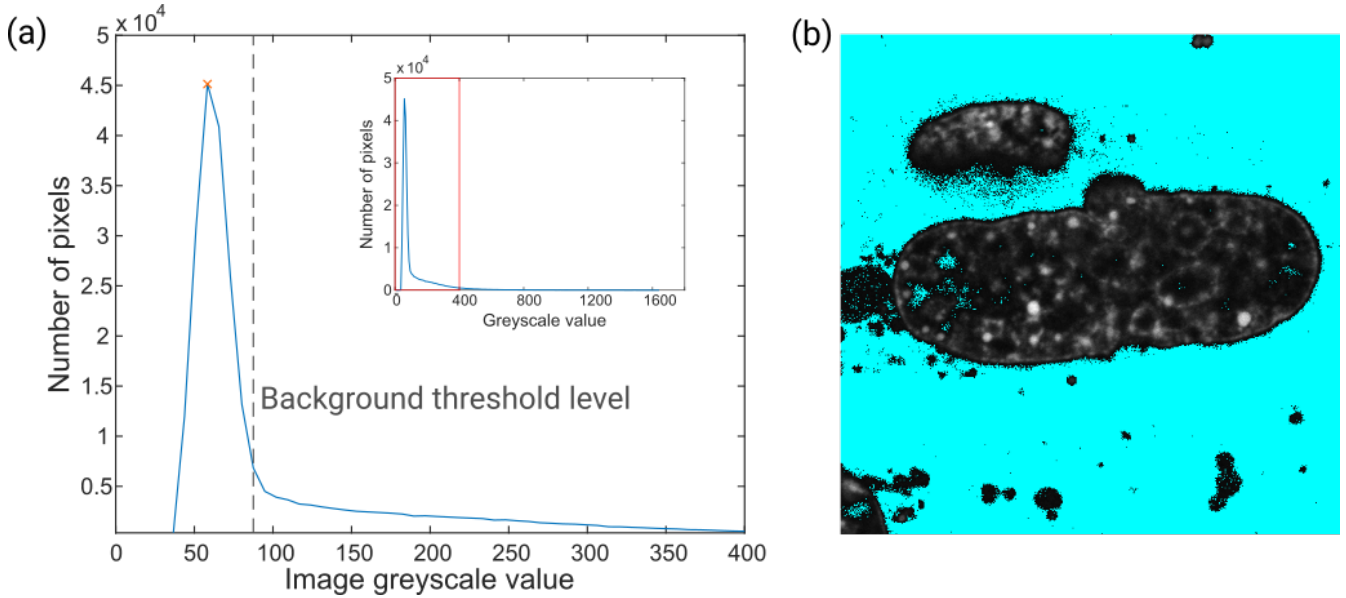
Supplementary Figure 1:

(a) Greyscale image of the EGFP channel. Yellow circle indicates region used to calculate the intensity threshold. (b) The initial nuclear mask after calculating the intensity threshold. (c) The final nuclear mask after removing small objects, filling in gaps, and morphological dilation.



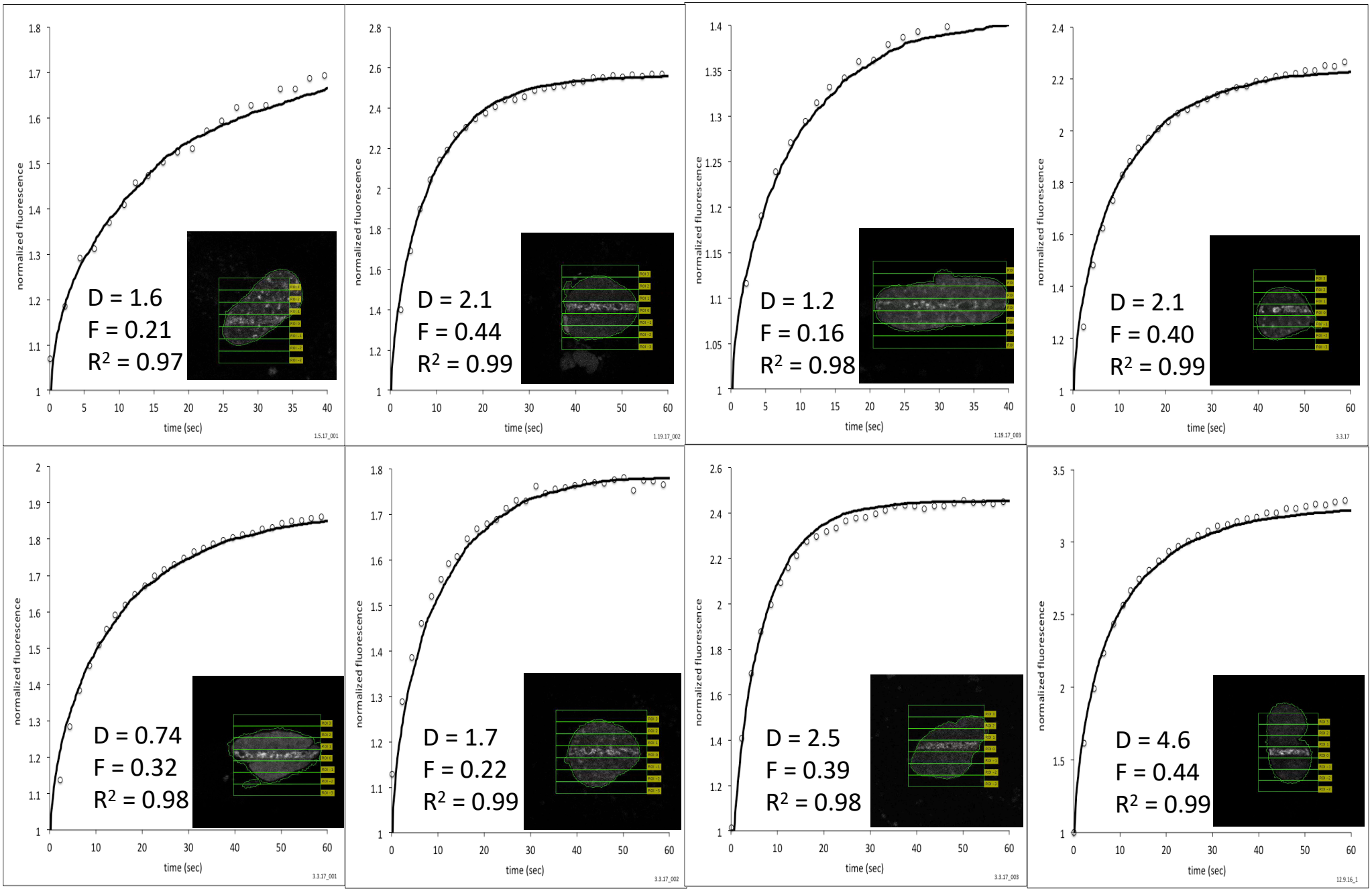
Supplementary Figure 2:

The white boxes correspond to the ROIs, while the outline of the nucleus is shown in green.



Supplementary Figure 3:

(a) The intensity histogram of the image was used to determine the background threshold level. The inset shows the full histogram, and the red box indicates the enlarged region. (b) The background (cyan) of each image was segmented and the mean intensity value was used for background subtraction for each ROI.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time (sec)
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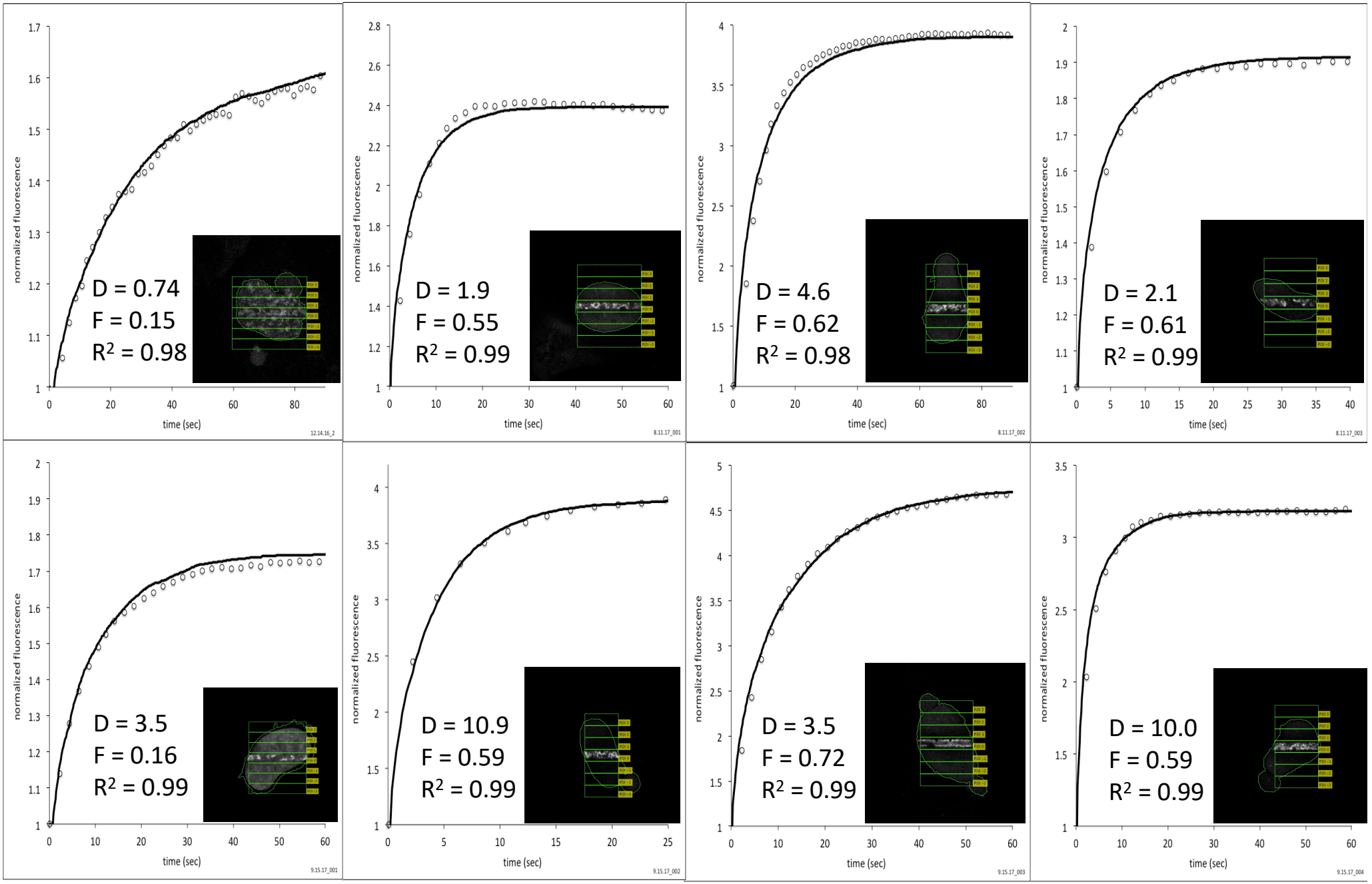
F = 0.21
R² = 0.97
1.5.17_001
D = 2.1
F = 0.44
R² = 0.99
1.19.17_002
D = 1.2
F = 0.16
R² = 0.98
1.19.17_003
D = 2.1
F = 0.40
R² = 0.99
3.3.17
D = 0.74
F = 0.32
R² = 0.98
3.3.17_001
D = 1.7
F = 0.22
R² = 0.99
3.3.17_002
D = 2.5
F = 0.39
R² = 0.98
3.3.17_003
D = 4.6
F = 0.44
R² = 0.99
12.9.16_1


normalized fluorescence
time (sec)
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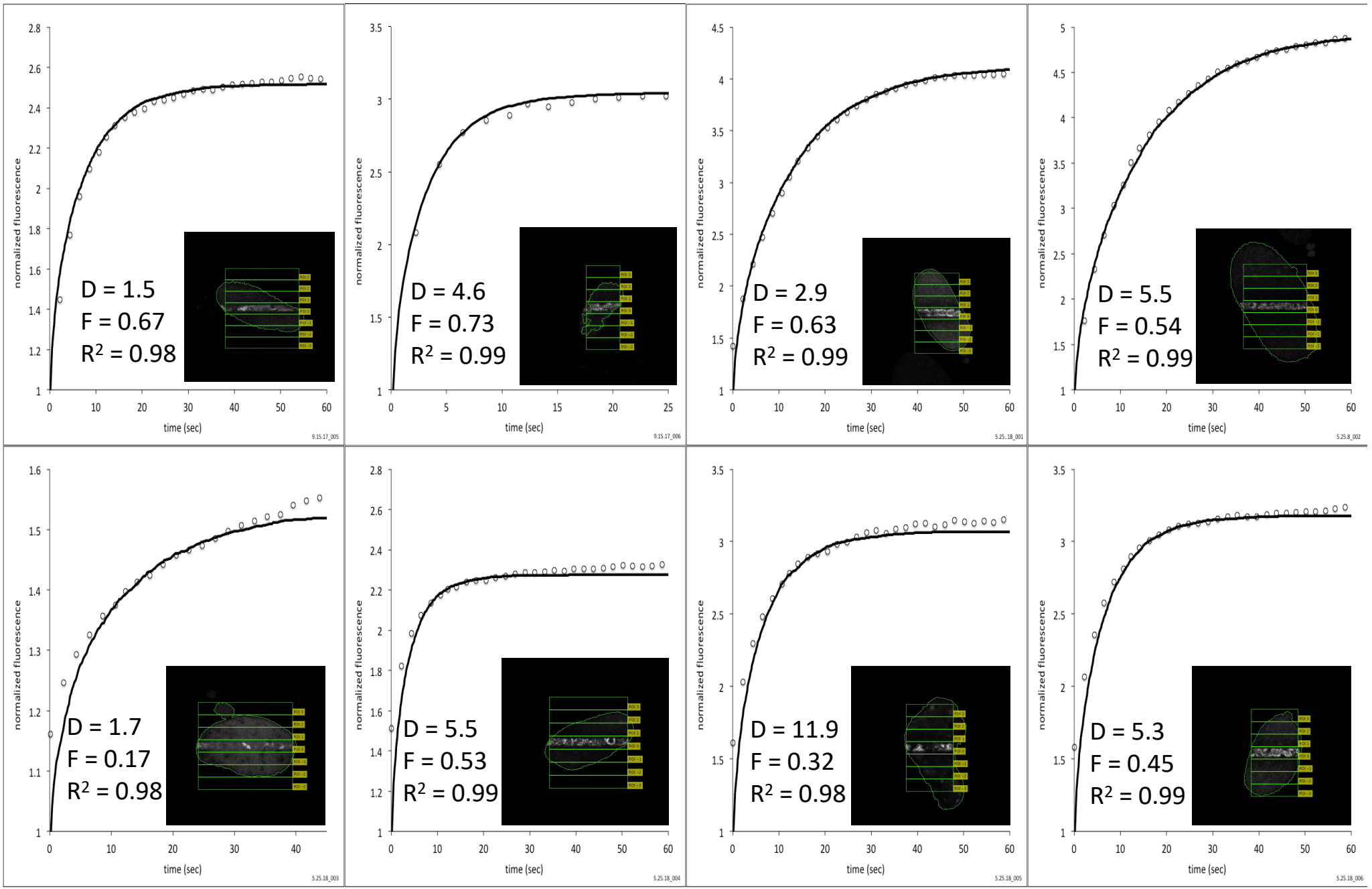
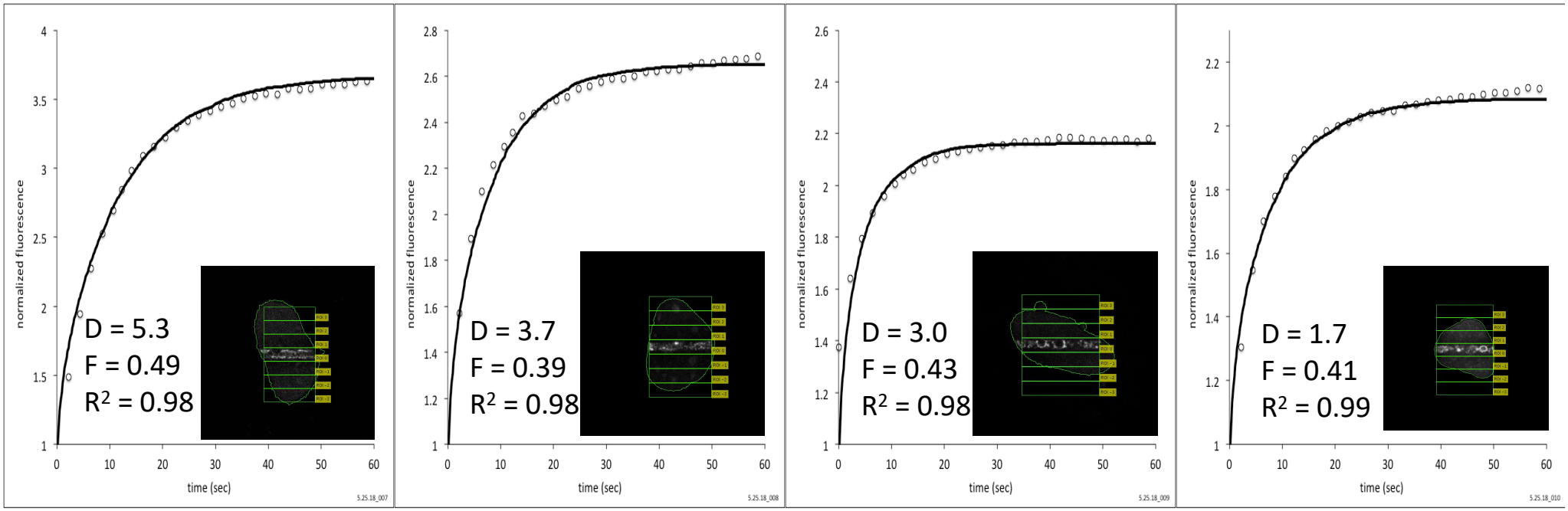
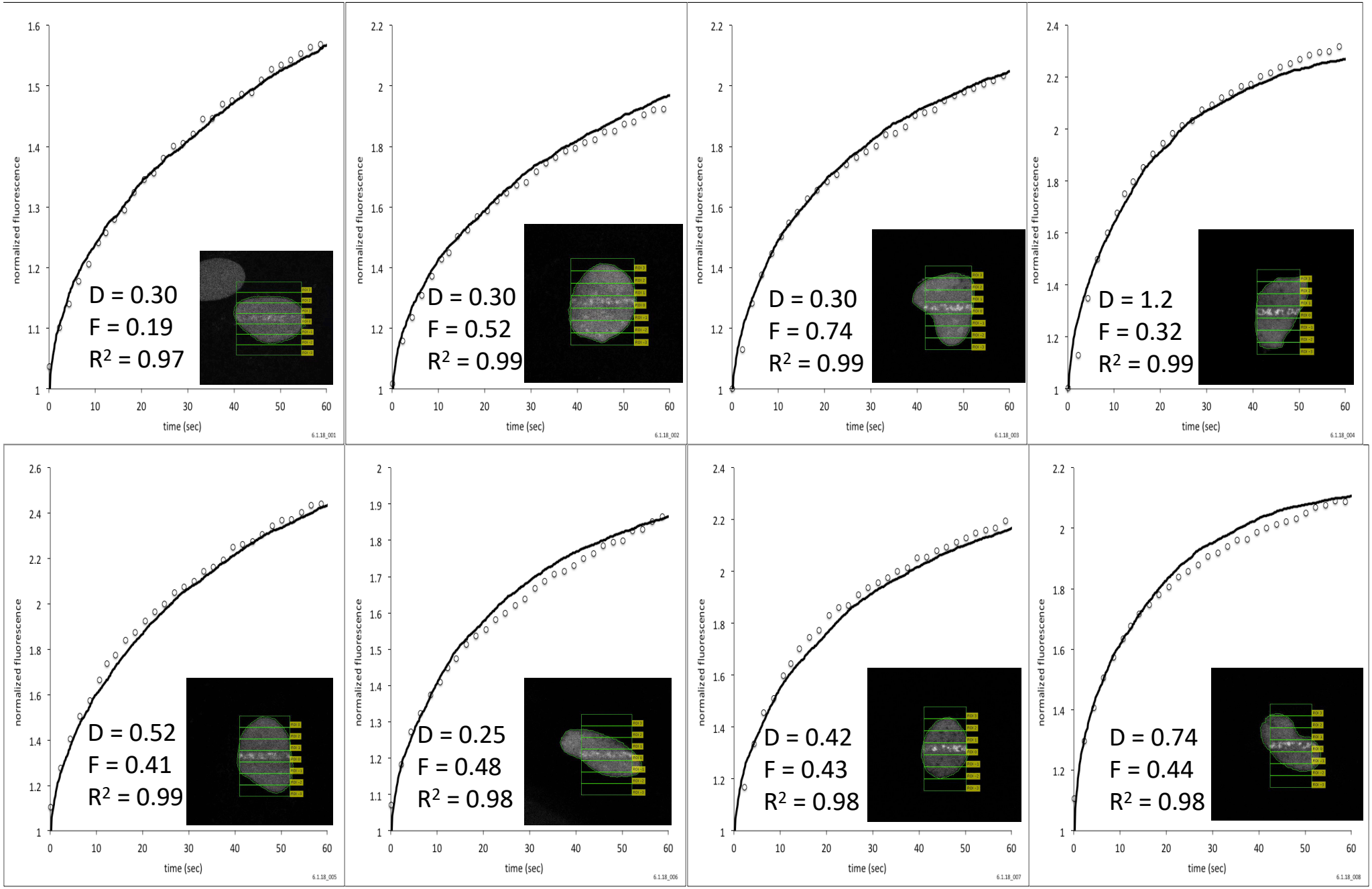
F = 0.15
R2 = 0.98
12.14.16_2
D = 1.9
F = 0.55
R2 = 0.99
8.11.17_001
D = 4.6
F = 0.62
R2 = 0.98
8.11.17_002
D = 2.1
F = 0.61
R2 = 0.99
8.11.17_003
D = 3.5
F = 0.16
R2 = 0.99
9.15.17_001
D = 10.9
F = 0.59
R2 = 0.99
9.15.17_002
D = 3.5
F = 0.72
R2 = 0.99
9.15.17_003
D = 10.0
F = 0.59
R2 = 0.99
9.15.17_004


normalized fluorescence
time (sec)
D = 1.5
F = 0.67
R² = 0.98
D = 4.6
F = 0.73
R² = 0.99
D = 2.9
F = 0.63
R² = 0.99
D = 5.5
F = 0.54
R² = 0.99
D = 1.7
F = 0.17
R² = 0.98
D = 5.5
F = 0.53
R² = 0.99
D = 11.9
F = 0.32
R² = 0.98
D = 5.3
F = 0.45
R² = 0.99



Overlay plots showing experimental data from FADD with the simulation of free diffusion used to determine D_{eff} and F for GFP-PARP1 in MEF cells. For each of the 28 different nuclei, the values of D (in $\mu m^2/s$), F, and R (r-squared coefficient) for each plot are shown along with a snapshot from each nucleus taken in the first frame after laser irradiation. The frame size of each cell image is 512 pixels x 512 pixels = 44 µ x 44 µ.


normalized fluorescence
time (sec)
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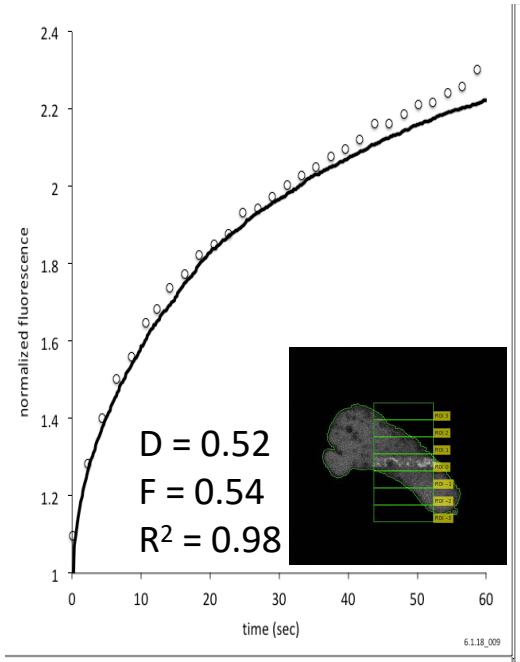
R² = 0.97
6.1.18_001
D = 0.30
F = 0.52
R² = 0.99
6.1.18_002
D = 0.30
F = 0.74
R² = 0.99
6.1.18_003
D = 1.2
F = 0.32
R² = 0.99
6.1.18_004
D = 0.52
F = 0.41
R² = 0.99
6.1.18_005
D = 0.25
F = 0.48
R² = 0.98
6.1.18_006
D = 0.42
F = 0.43
R² = 0.98
6.1.18_007
D = 0.74
F = 0.44
R² = 0.98
6.1.18_008


normalized fluorescence
time (sec)
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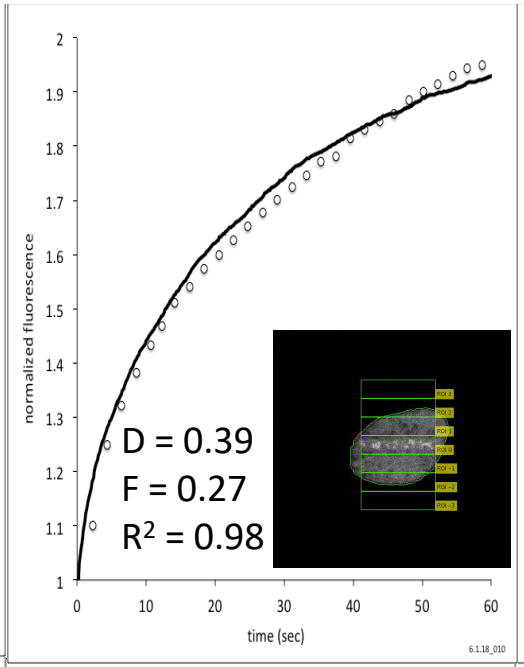
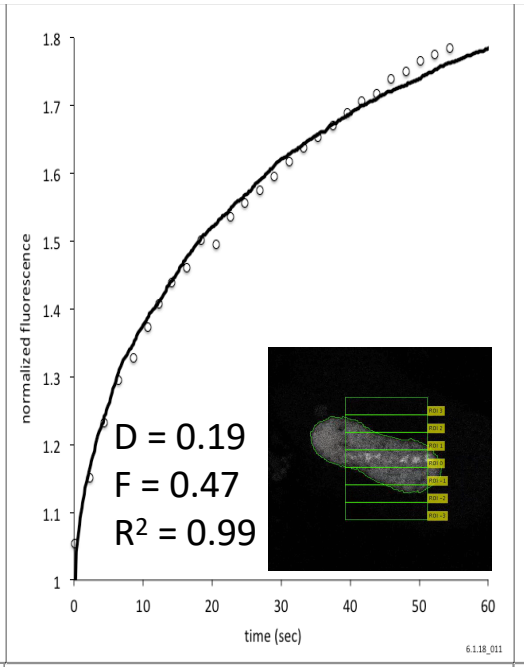
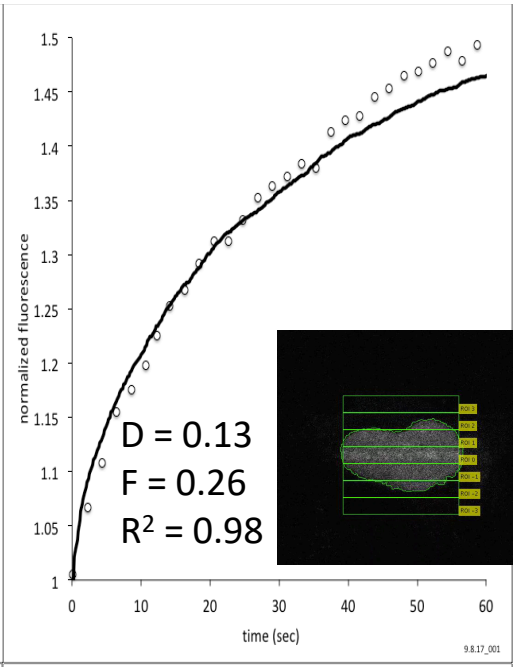
F = 0.54
R² = 0.98
6.1.18_009


normalized fluorescence
time (sec)
D = 0.39
F = 0.27
R² = 0.98
6.1.18_010


normalized fluorescence
time (sec)
D = 0.19
F = 0.47
R² = 0.99
6.1.18_011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F = 0.26
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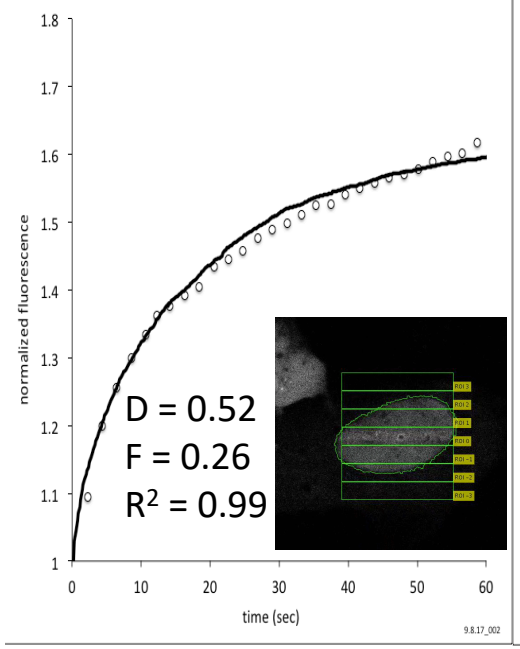
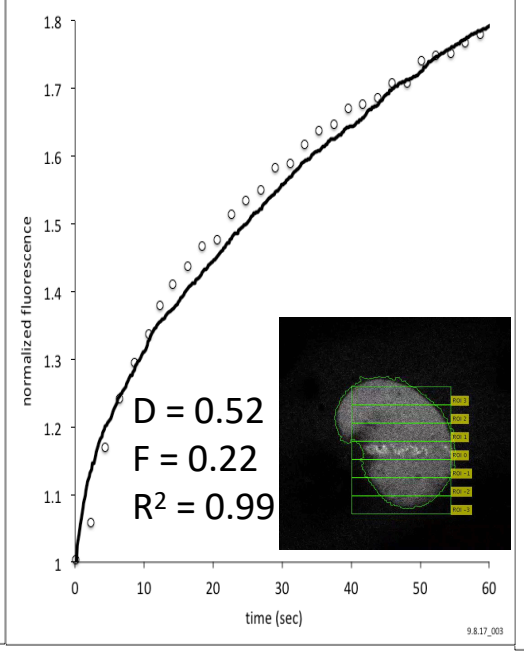
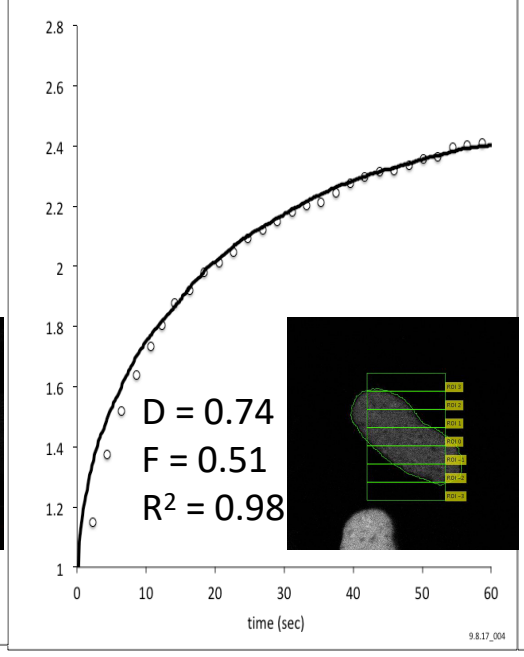
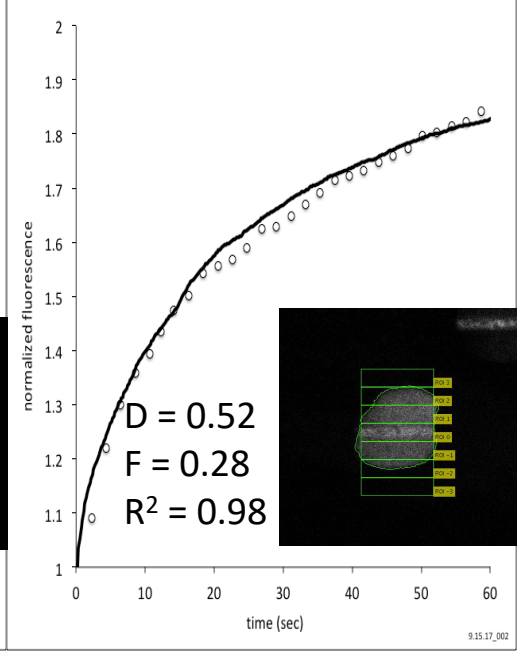
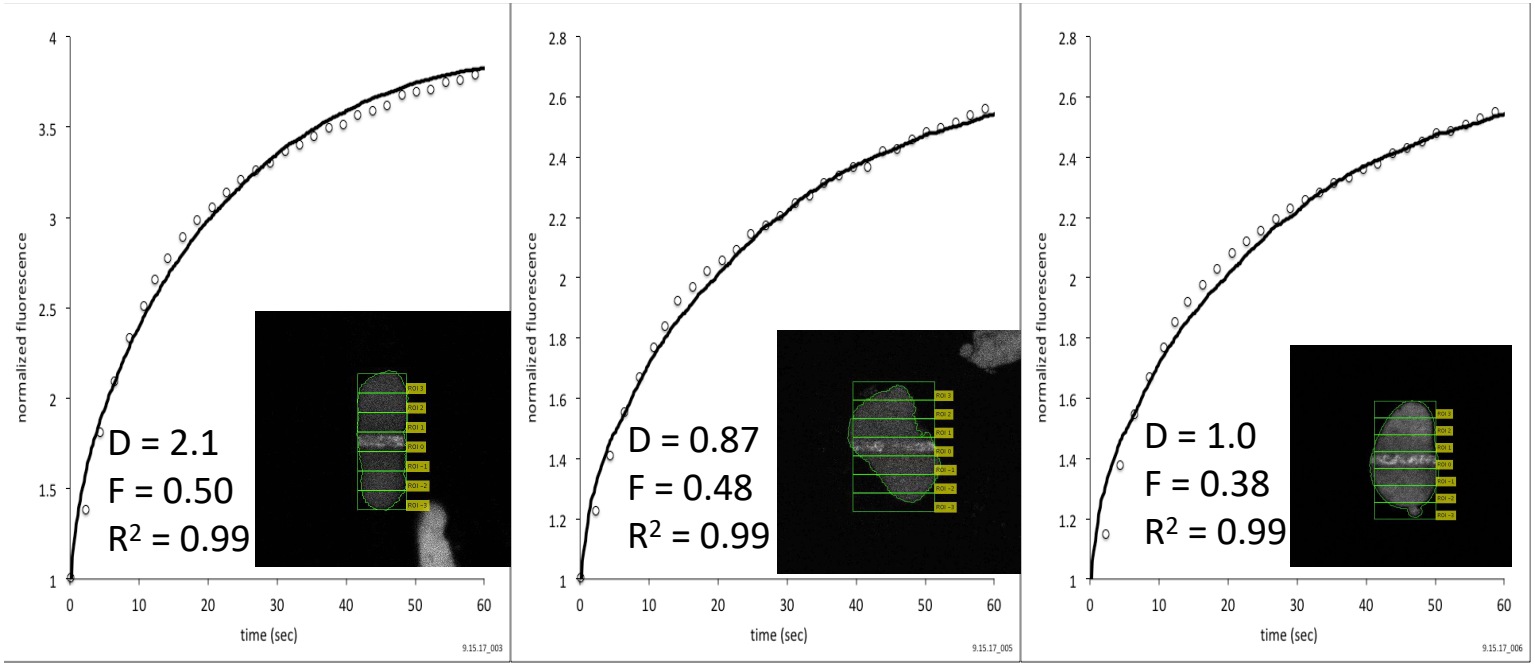
9.8.17_001


normalized fluorescence
time (sec)
D = 0.52
F = 0.26
R² = 0.99
9.8.17_002


normalized fluorescence
time (sec)
D = 0.52
F = 0.22
R² = 0.99
9.8.17_003

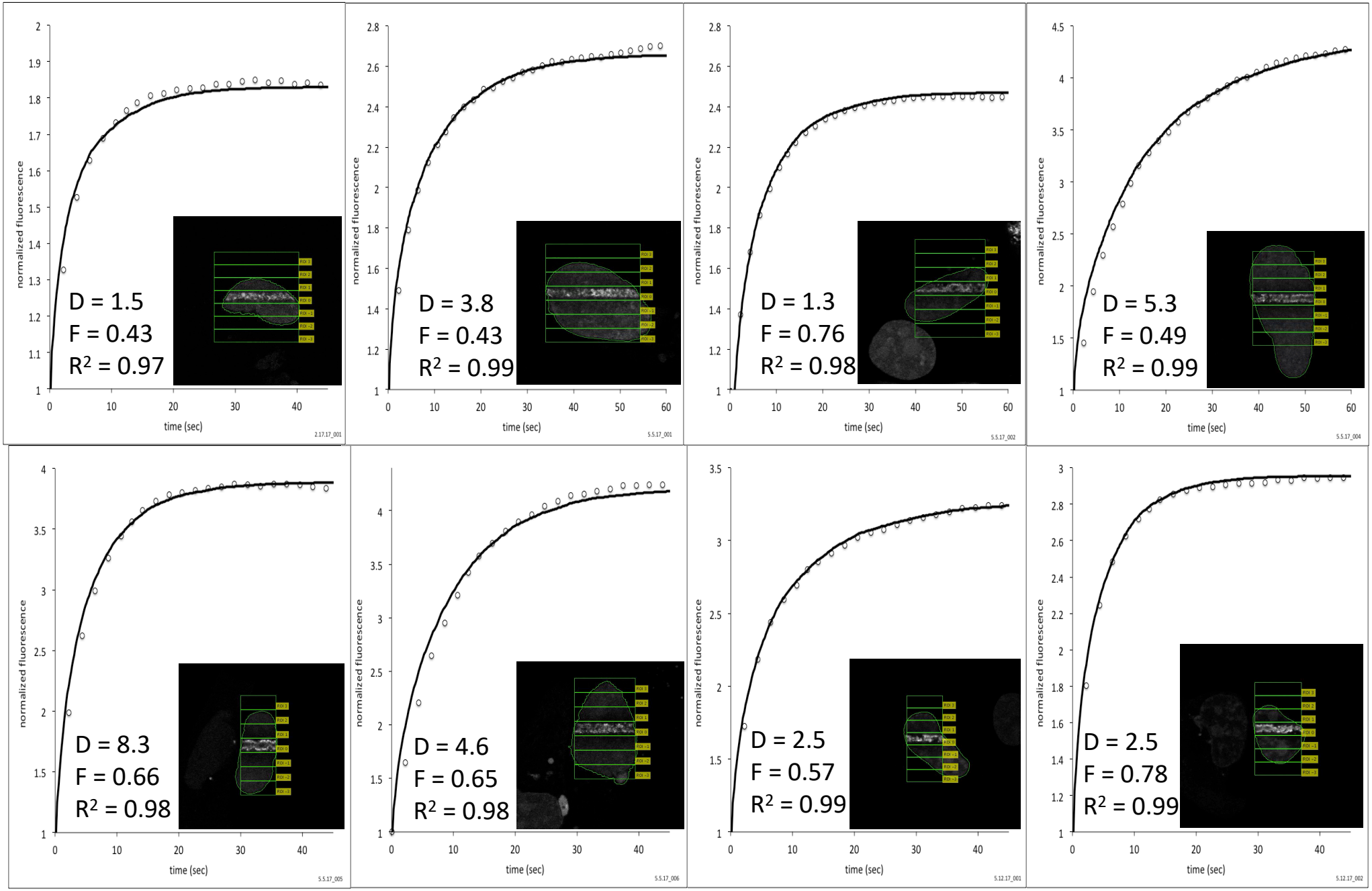

normalized fluorescence
time (sec)
D = 0.74
F = 0.51
R² = 0.98
9.8.17_004

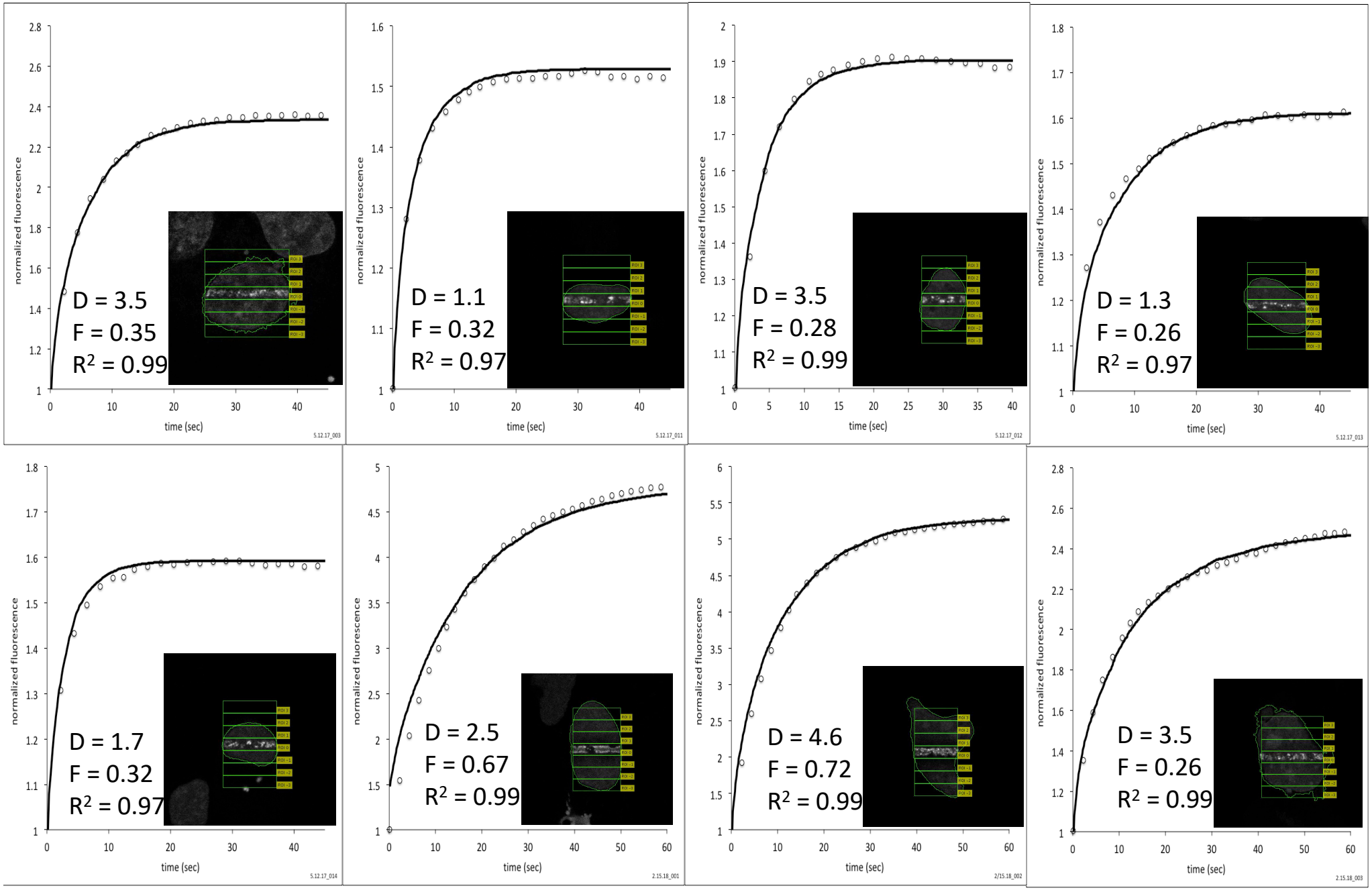

normalized fluorescence
time (sec)
D = 0.52
F = 0.28
R² = 0.98
9.15.17_002

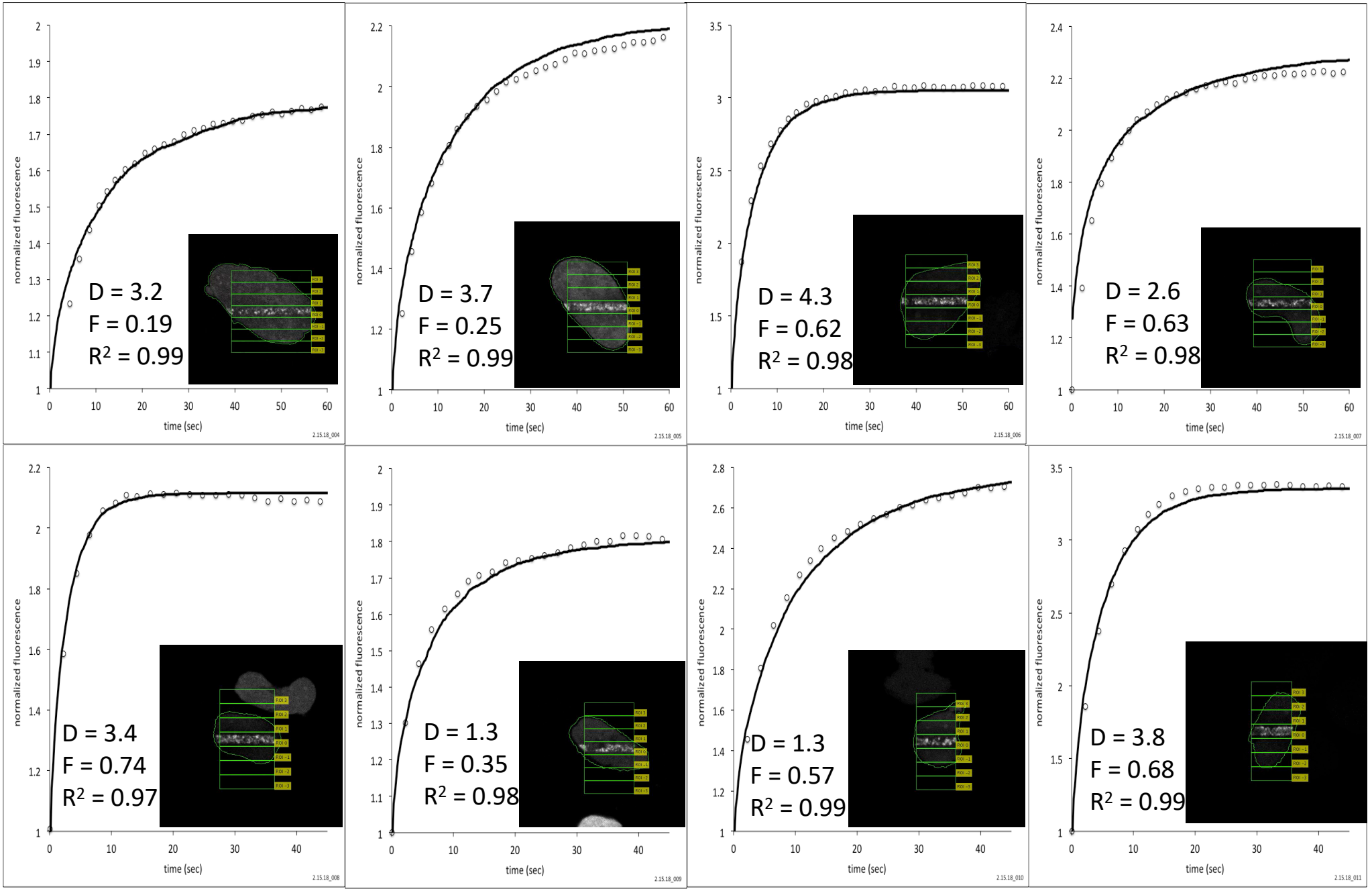


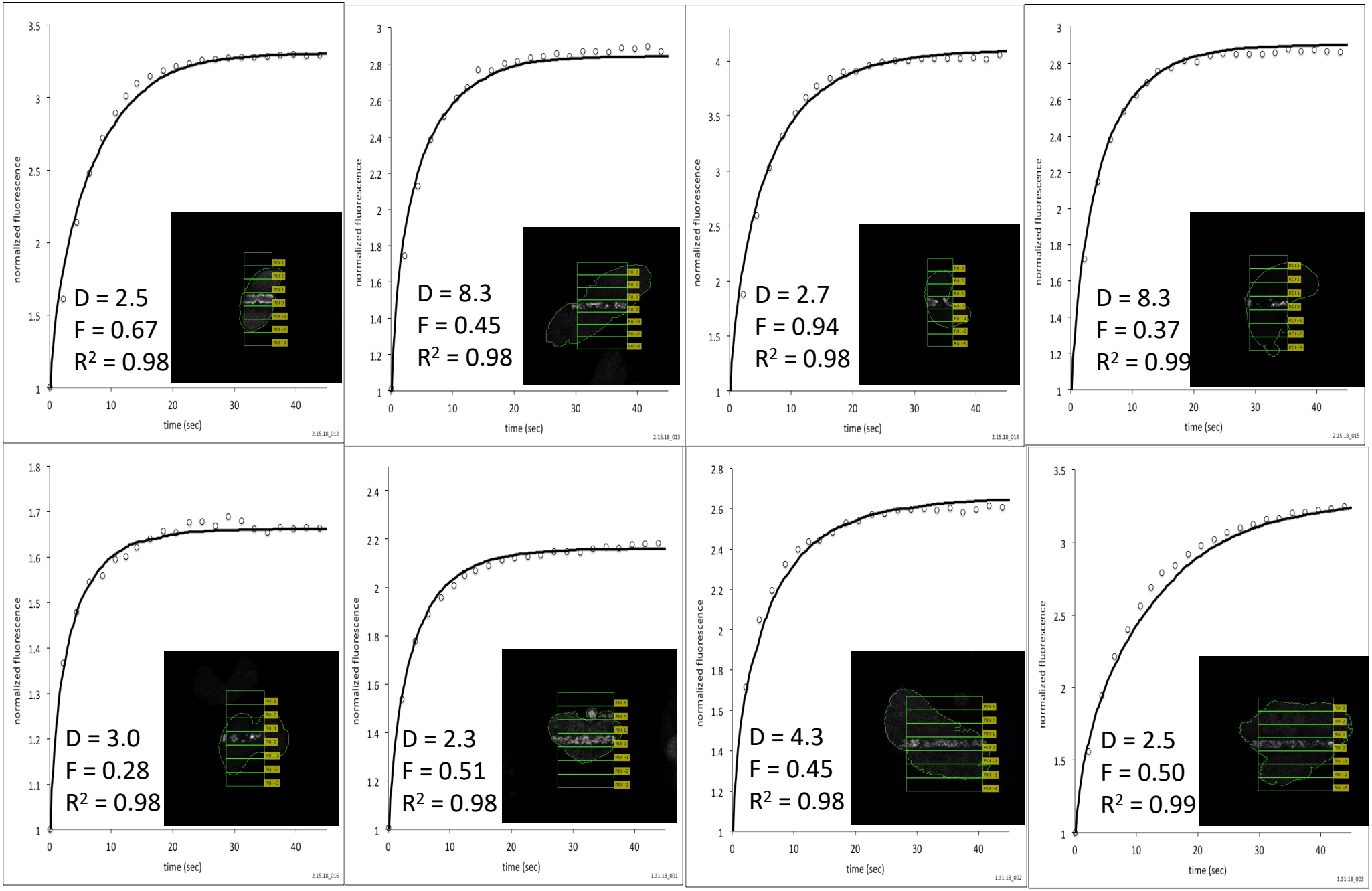
Supp. Fig. 5, page 3 of 3

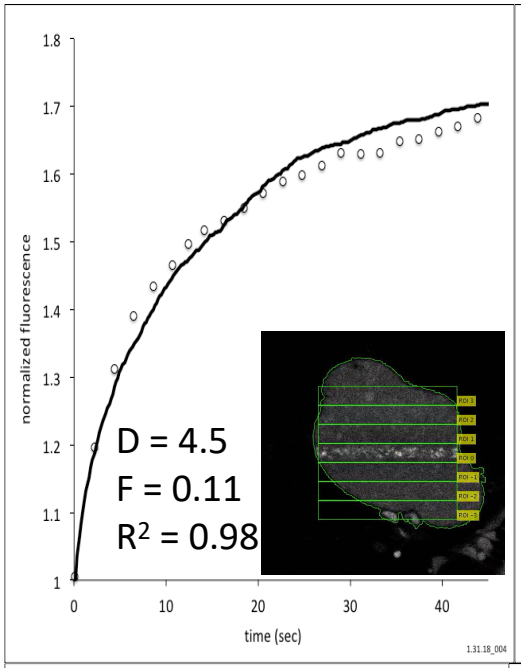
Overlay plots showing experimental data from FADD with the simulation of free diffusion used to determine D_{eff} and F for GFP-PARP2 in MEF cells. For each of the 19 different nuclei, the values of D (in $\mu m^2/s$), F, and R^2 (r-squared coefficient) for each plot are shown along with a snapshot from each nucleus taken in the first frame after laser irradiation. The frame size of each cell image is 512 pixels x 512 pixels = 44 µ x 44 µ.

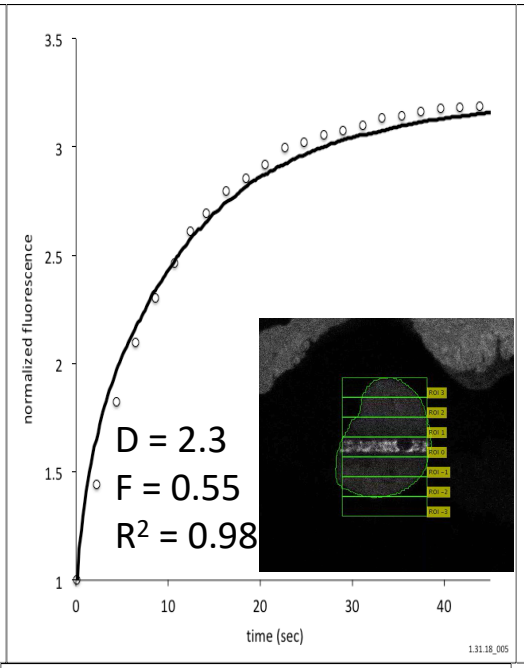

normalized fluorescence
time (sec)
D = 1.5
F = 0.43
R² = 0.97
D = 3.8
F = 0.43
R² = 0.99
D = 1.3
F = 0.76
R² = 0.98
D = 5.3
F = 0.49
R² = 0.99
D = 8.3
F = 0.66
R² = 0.98
D = 4.6
F = 0.65
R² = 0.98
D = 2.5
F = 0.57
R² = 0.99
D = 2.5
F = 0.78
R² = 0.99

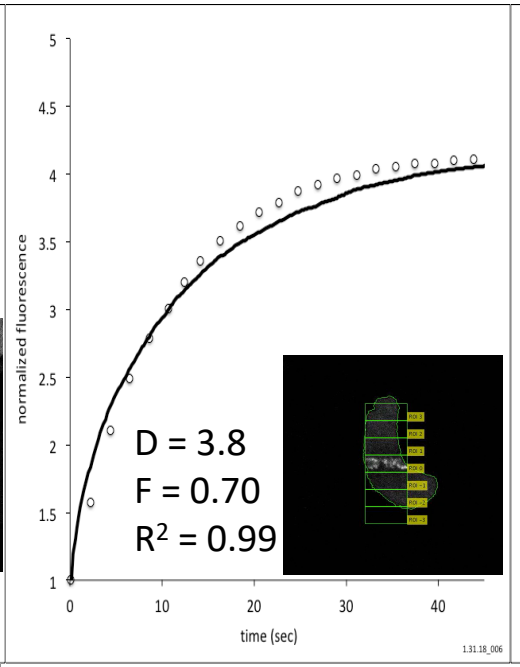

normalized fluorescence
time (sec)
D = 3.5
F = 0.35
R² = 0.99
D = 1.1
F = 0.32
R² = 0.97
D = 3.5
F = 0.28
R² = 0.99
D = 1.3
F = 0.26
R² = 0.97
D = 1.7
F = 0.32
R² = 0.97
D = 2.5
F = 0.67
R² = 0.99
D = 4.6
F = 0.72
R² = 0.99
D = 3.5
F = 0.26
R² = 0.99

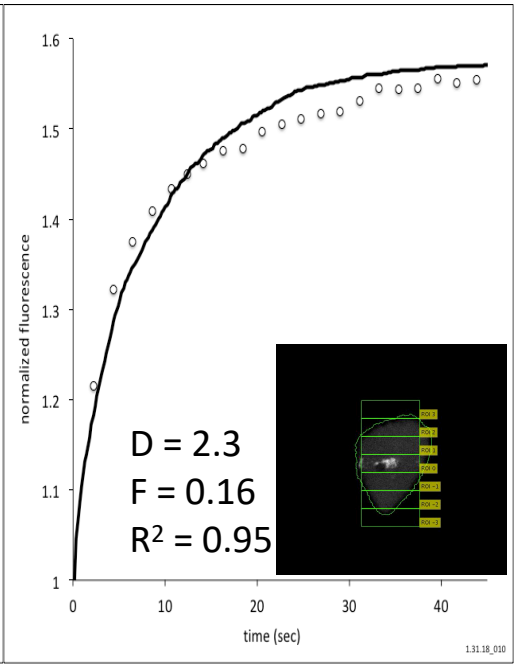

normalized fluorescence
time (sec)
D = 3.2
F = 0.19
R² = 0.99
D = 3.7
F = 0.25
R² = 0.99
D = 4.3
F = 0.62
R² = 0.98
D = 2.6
F = 0.63
R² = 0.98
D = 3.4
F = 0.74
R² = 0.97
D = 1.3
F = 0.35
R² = 0.98
D = 1.3
F = 0.57
R² = 0.99
D = 3.8
F = 0.68
R² = 0.99

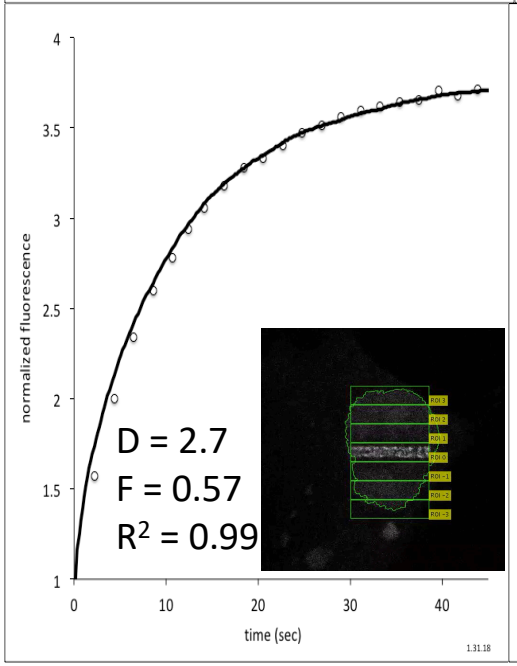

normalized fluorescence
time (sec)
D = 2.5
F = 0.67
R² = 0.98
2.15.18_012
D = 8.3
F = 0.45
R² = 0.98
2.15.18_013
D = 2.7
F = 0.94
R² = 0.98
2.15.18_014
D = 8.3
F = 0.37
R² = 0.99
2.15.18_015
D = 3.0
F = 0.28
R² = 0.98
2.15.18_016
D = 2.3
F = 0.51
R² = 0.98
1.31.18_001
D = 4.3
F = 0.45
R² = 0.98
1.31.18_002
D = 2.5
F = 0.50
R² = 0.99
1.31.18_003

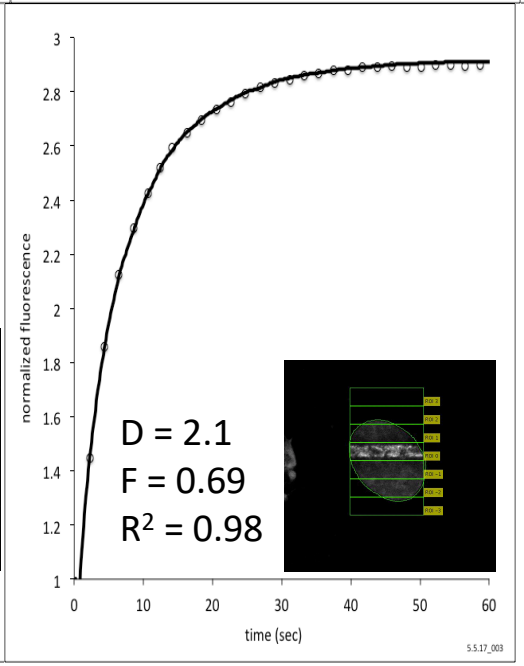






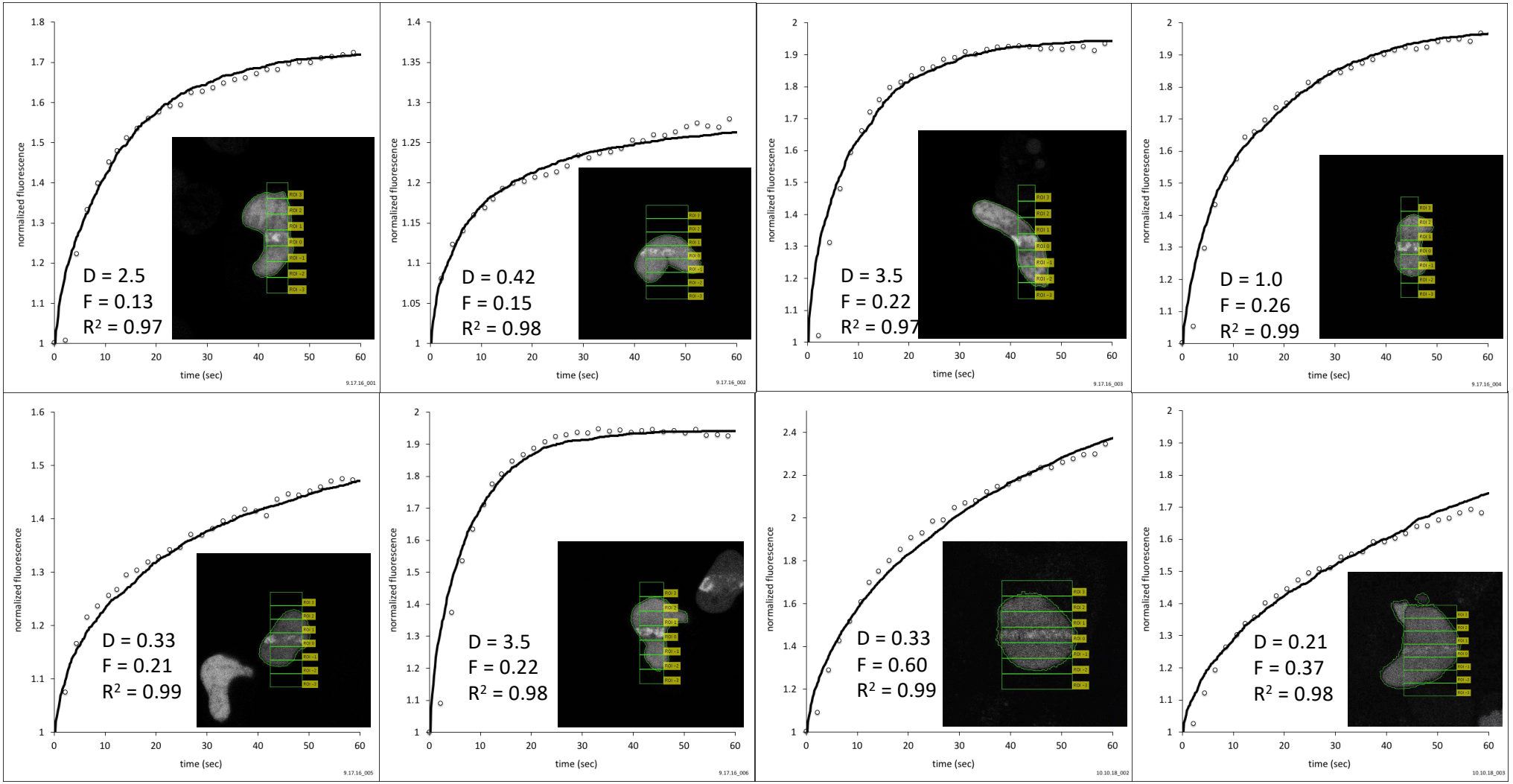




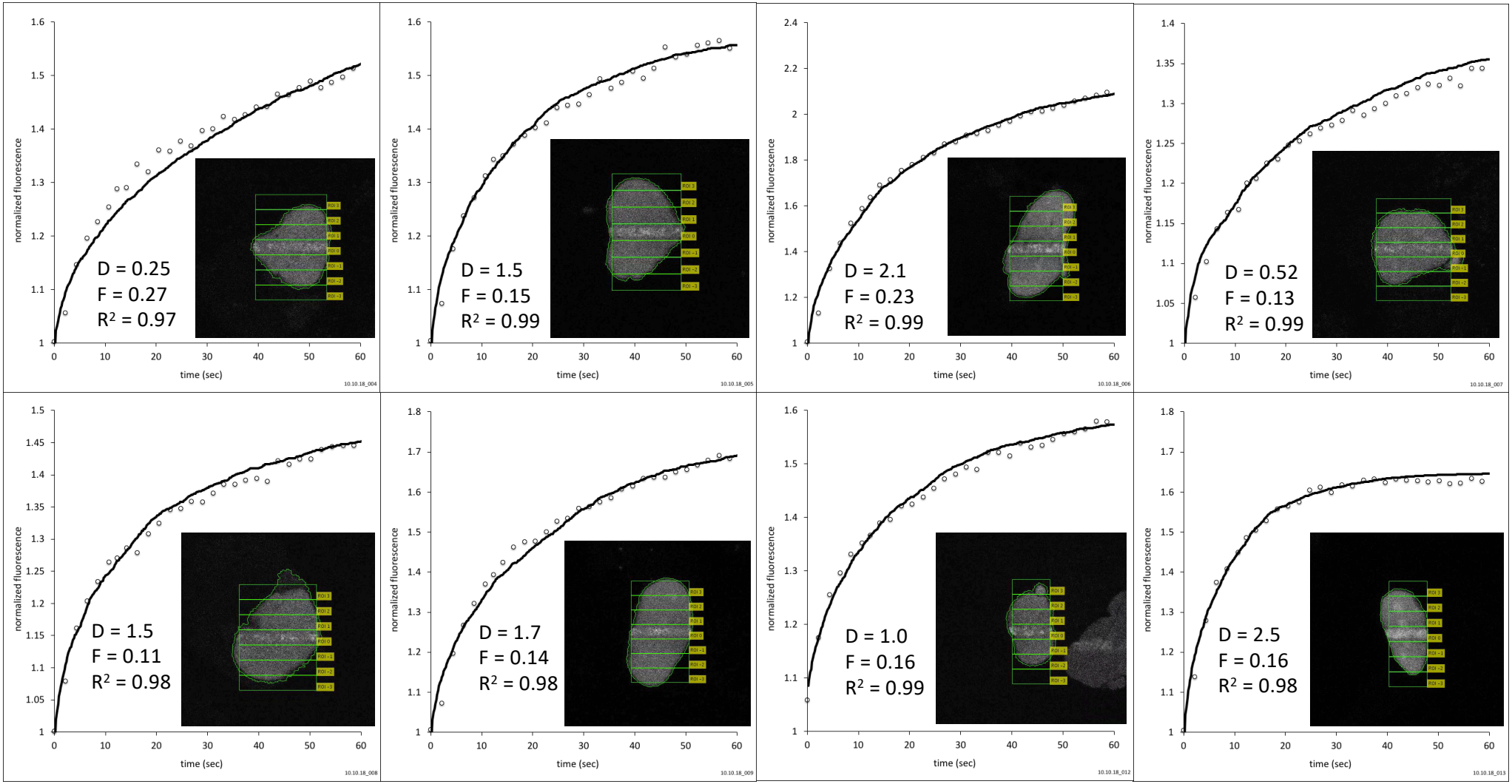


Supp. Fig 6, page 5 of 5

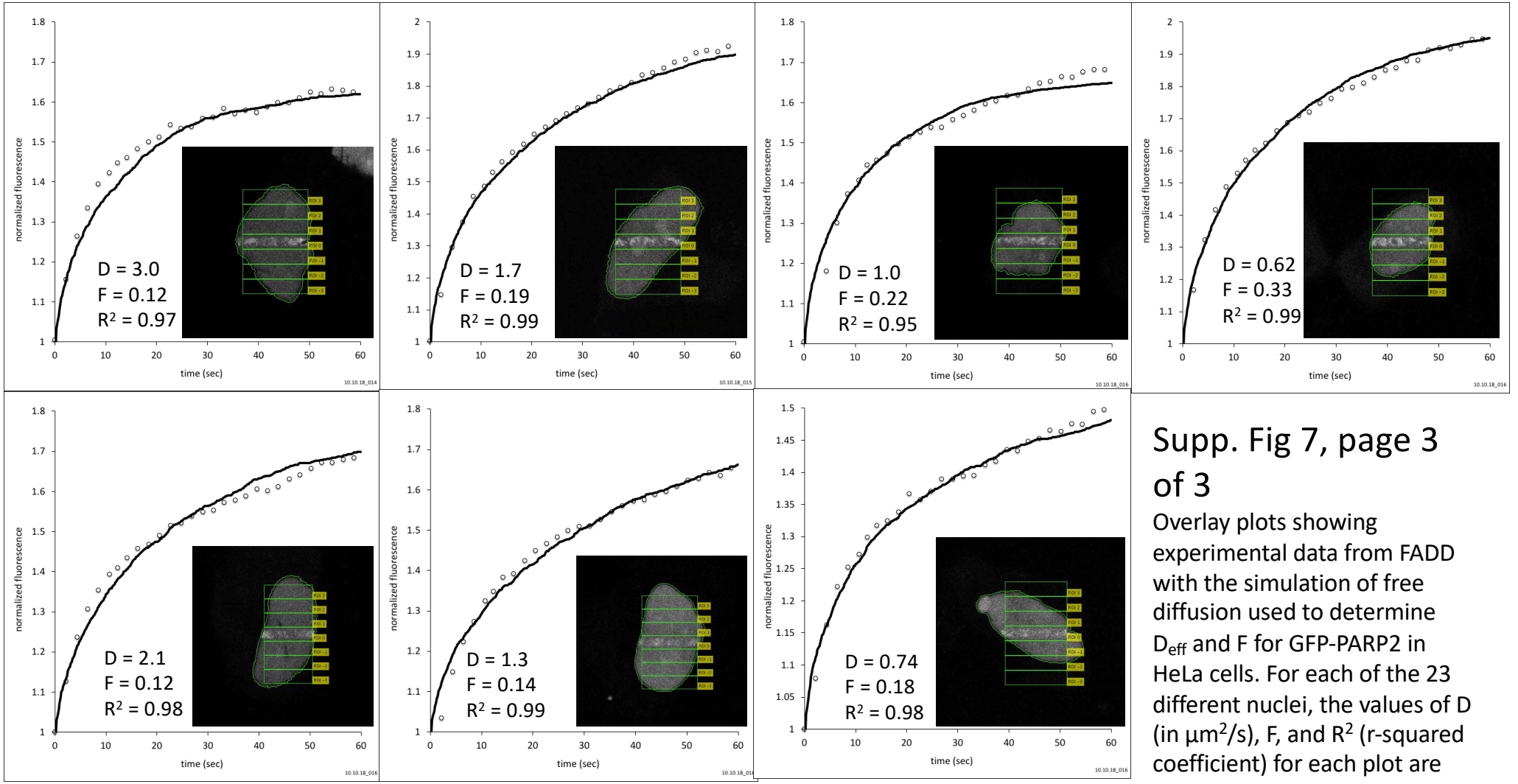
Overlay plots showing experimental data from FADD with the simulation of free diffusion used to determine D_{eff} and F for GFP-PARP1 in HeLa cells. For each of the 38 different nuclei, the values of D (in $\mu m^2/s$), F, and R^2 (r-squared coefficient) for each plot are shown along with a snapshot from each nucleus taken in the first frame after laser irradiation. The frame size of each cell image is 512 pixels x 512 pixels = 44 µ x 44 µ.



Supp. Fig. 7, page 1



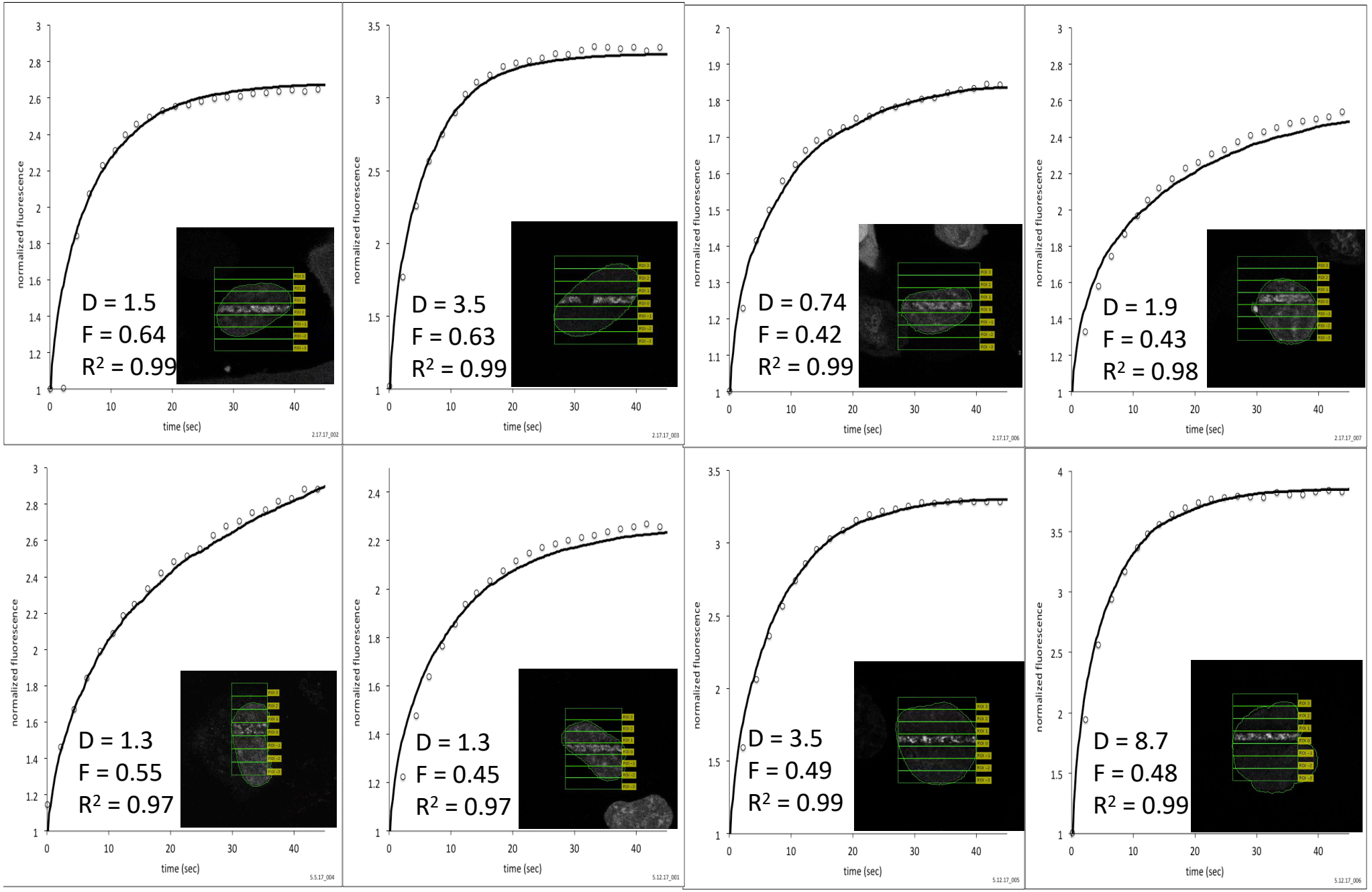
Supp. Fig. 7, page 2

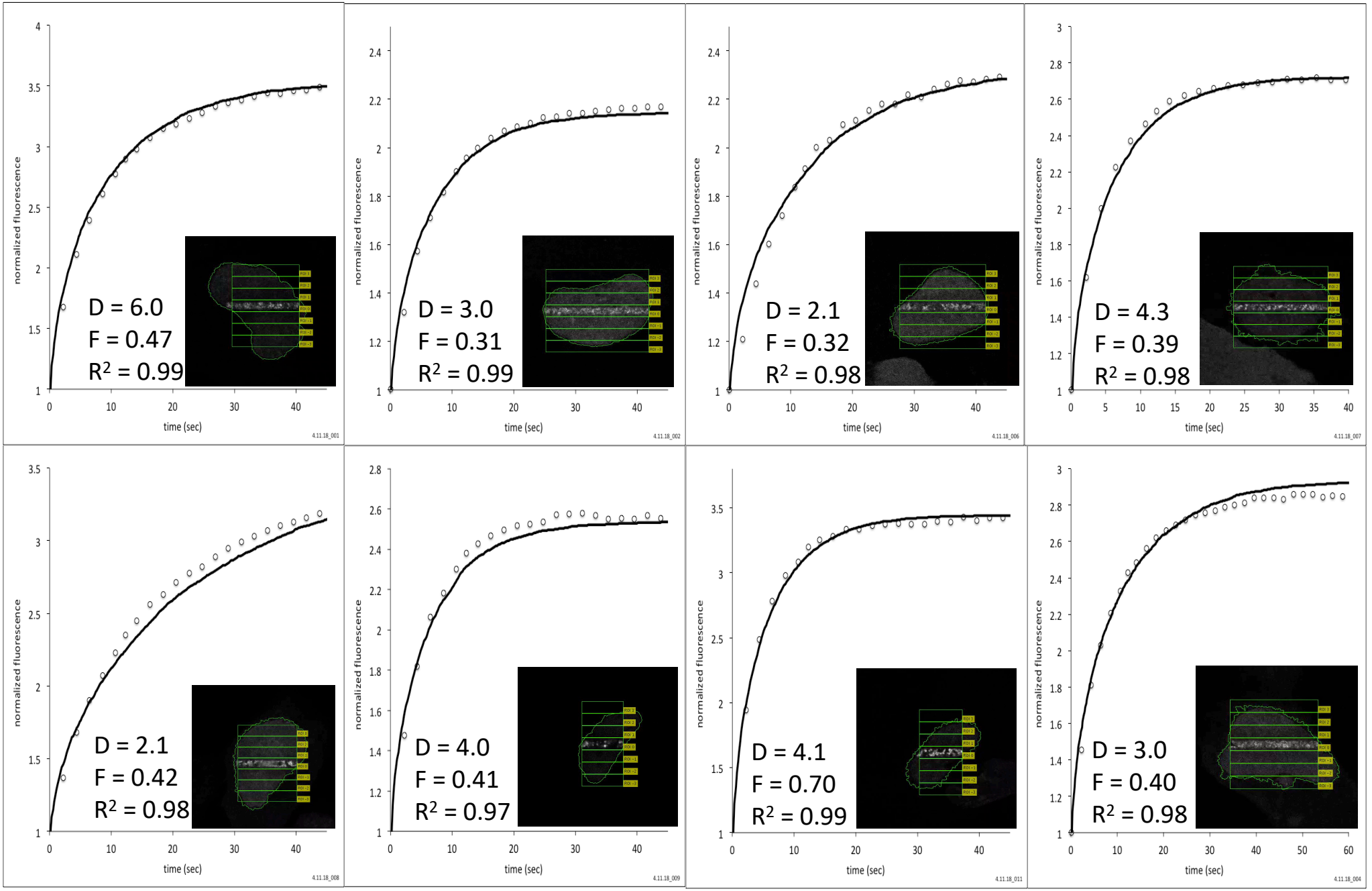


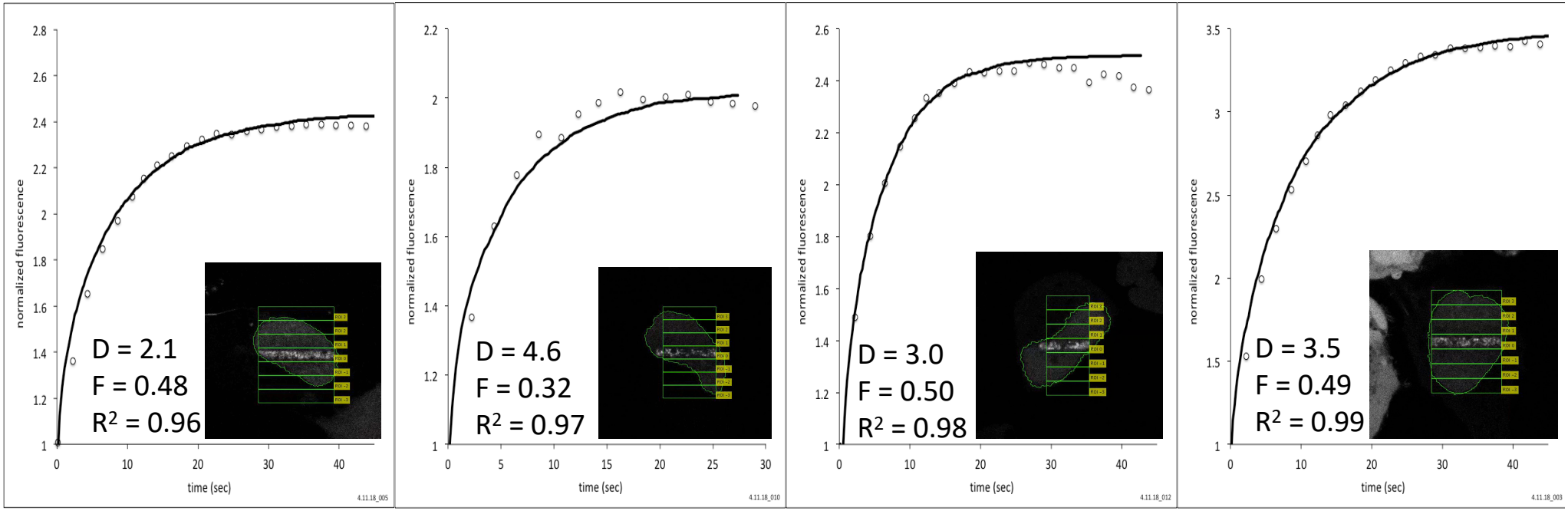
Supp. Fig. 7, page 3

Supp. Fig 7, page 3 of 3

Overlay plots showing experimental data from FADD with the simulation of free diffusion used to determine D_{eff} and F for GFP-PARP2 in HeLa cells. For each of the 23 different nuclei, the values of D (in $\mu m^2/s$), F, and R^2 (r-squared coefficient) for each plot are shown along with a snapshot from each nucleus taken in the first frame after laser irradiation. The frame size of each cell image is 512 pixels x 512 pixels = 44 µ x 44 µ.

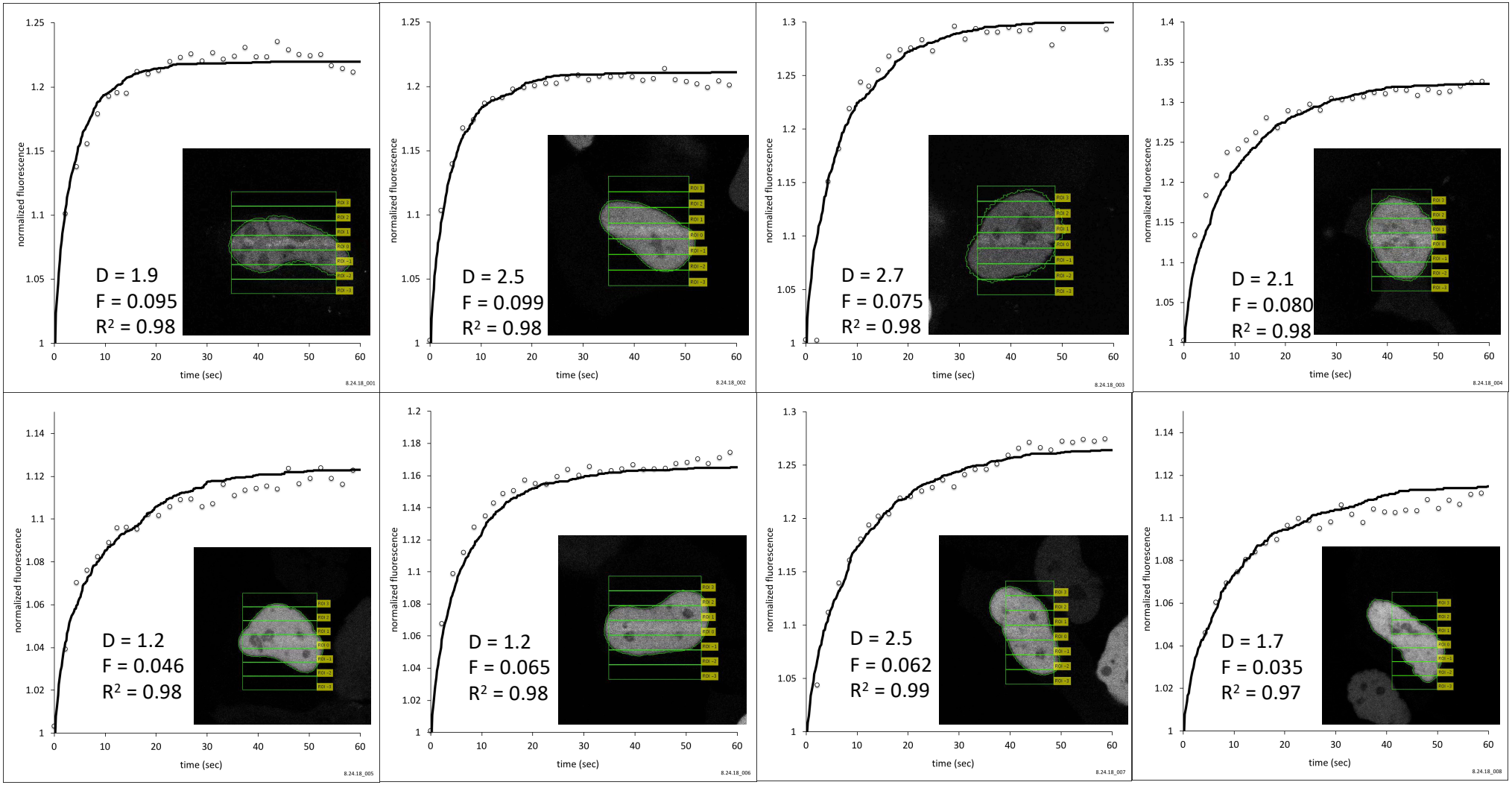

normalized fluorescence
time (sec)
D = 1.5
F = 0.64
R2 = 0.99
D = 3.5
F = 0.63
R2 = 0.99
D = 0.74
F = 0.42
R2 = 0.99
D = 1.9
F = 0.43
R2 = 0.98
D = 1.3
F = 0.55
R2 = 0.97
D = 1.3
F = 0.45
R2 = 0.97
D = 3.5
F = 0.49
R2 = 0.99
D = 8.7
F = 0.48
R2 = 0.99


normalized fluorescence
time (sec)
D = 6.0
F = 0.47
R² = 0.99
D = 3.0
F = 0.31
R² = 0.99
D = 2.1
F = 0.32
R² = 0.98
D = 4.3
F = 0.39
R² = 0.98
D = 2.1
F = 0.42
R² = 0.98
D = 4.0
F = 0.41
R² = 0.97
D = 4.1
F = 0.70
R² = 0.99
D = 3.0
F = 0.40
R² = 0.98

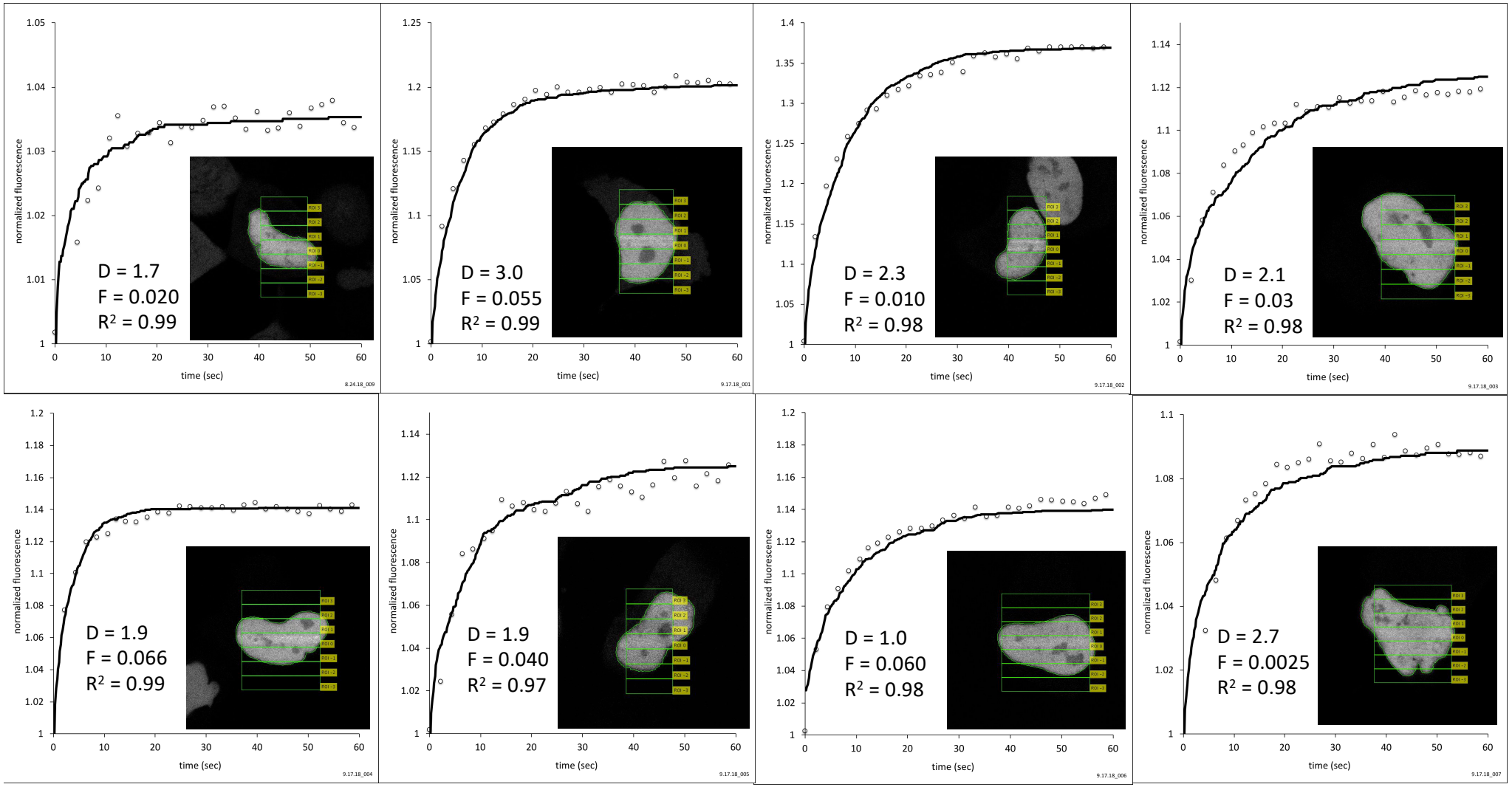


Supp. Fig. 8, page 3 of 3

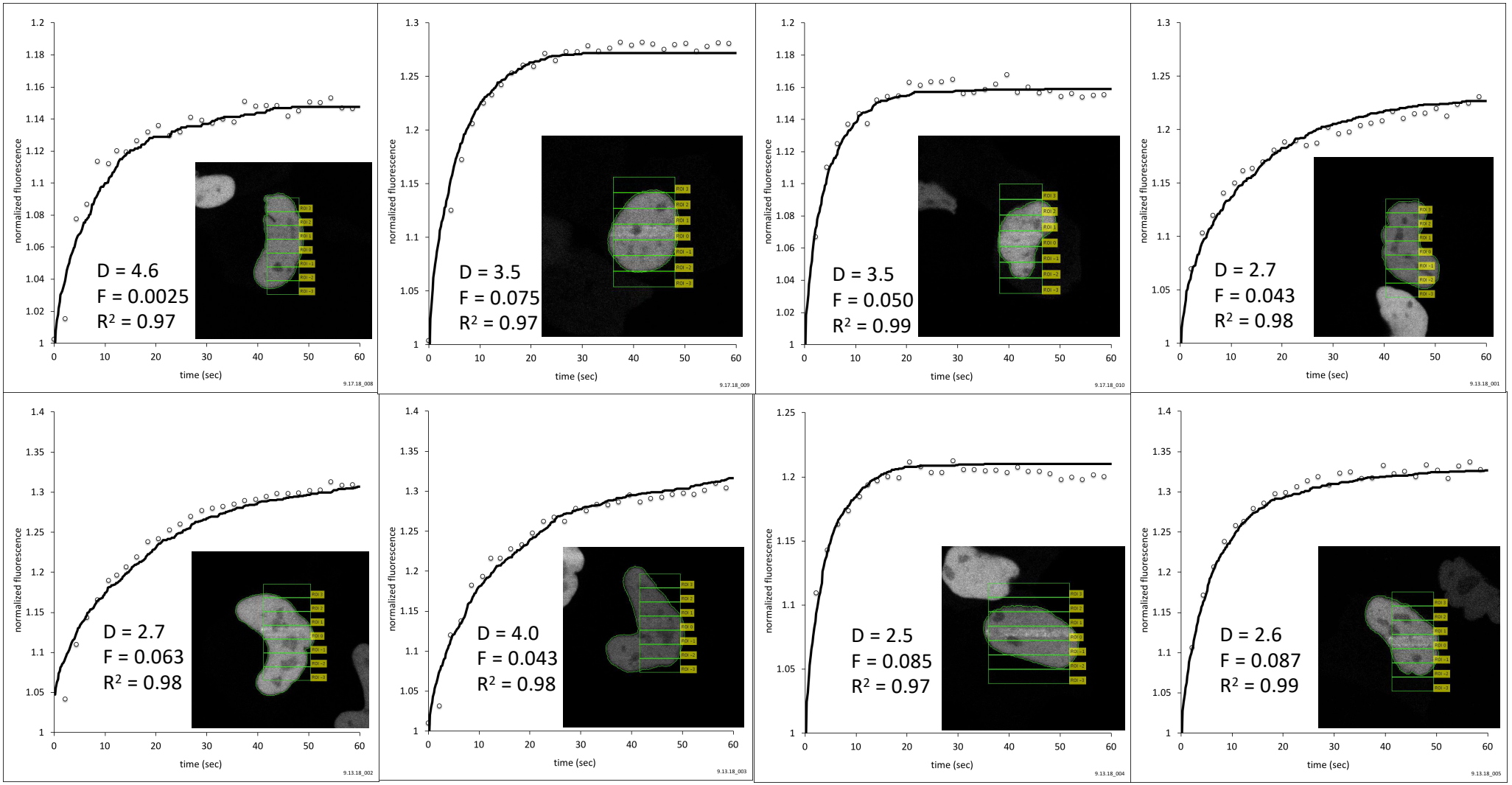
Overlay plots showing experimental data from FADD with the simulation of free diffusion used to determine D_{eff} and F for chromobody experiments detecting accumulation of endogenous PARP1 in HeLa cells. For each of the 20 different nuclei, the values of D (in $\mu m^2/s$), F, and R^2 (r-squared coefficient) for each plot are shown along with a snapshot from each nucleus taken in the first frame after laser irradiation. The frame size of each cell image is 512 pixels x 512 pixels = 44 µ x 44 µ.

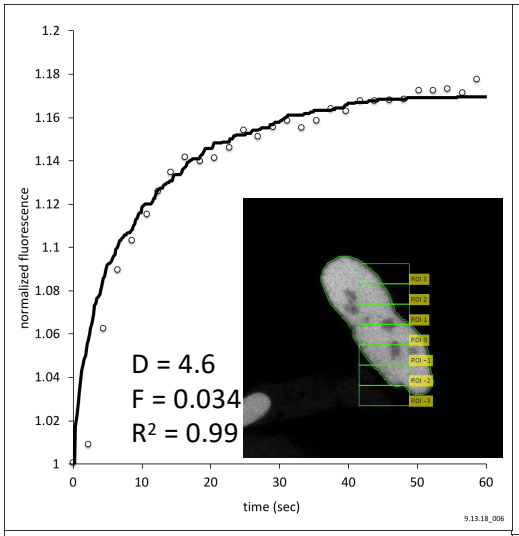


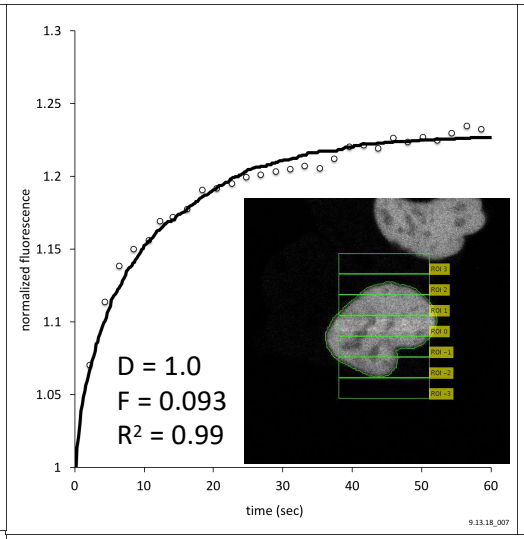
Supp. Fig. 9, page 1

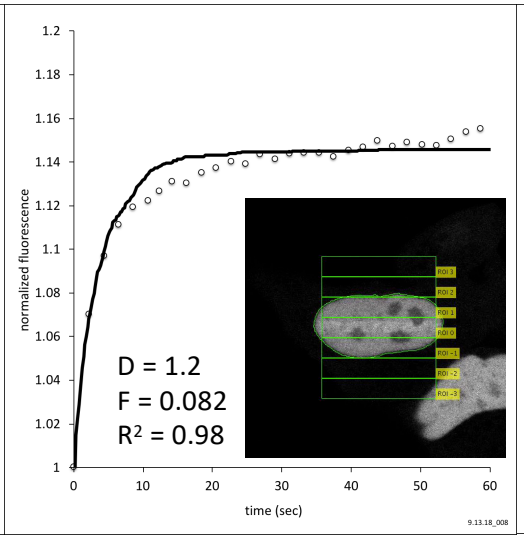


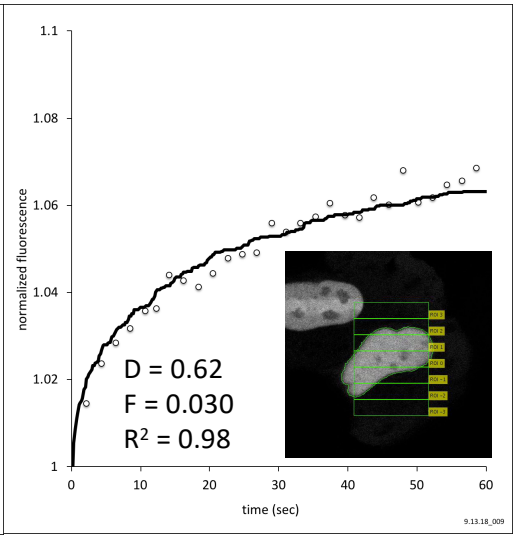
Supp. Fig. 9, page 2

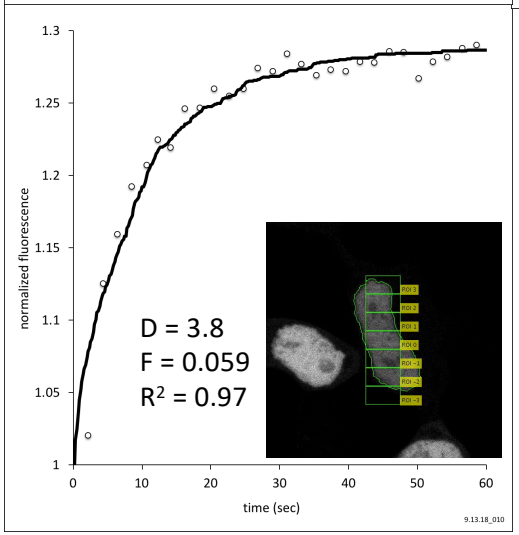

normalized fluorescence
time (sec)
D = 4.6
F = 0.0025
R² = 0.97
9.17.18_008
D = 3.5
F = 0.075
R² = 0.97
9.17.18_009
D = 3.5
F = 0.050
R² = 0.99
9.17.18_010
D = 2.7
F = 0.043
R² = 0.98
9.13.18_001
D = 2.7
F = 0.063
R² = 0.98
9.13.18_002
D = 4.0
F = 0.043
R² = 0.98
9.13.18_003
D = 2.5
F = 0.085
R² = 0.97
9.13.18_004
D = 2.6
F = 0.087
R² = 0.99
9.13.18_005





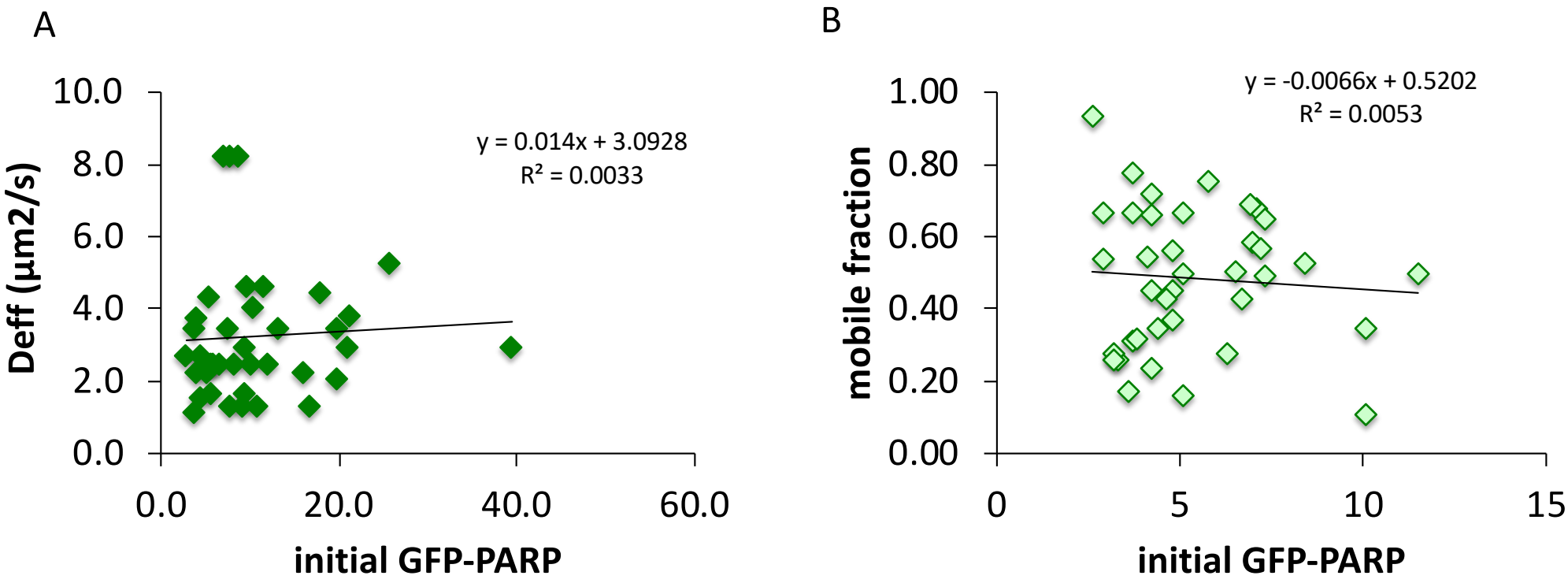






Supp. Fig 9

Overlay plots showing experimental data from FADD with the simulation of free diffusion used to determine D_{eff} and F for GFP-HPF1 in HeLa cells. For each of the 29 different nuclei, the values of D (in $\mu m^2/s$), F, and R^2 (r-squared coefficient) for each plot are shown along with a snapshot from each nucleus taken in the first frame after laser irradiation. The frame size of each cell image is 512 pixels x 512 pixels = 44 µ x 44 µ.



Supp. Fig 10

There is no correlation between initial levels of GFP-PARP1 with either Deff or F for 38 HeLa nuclei. A) Initial fluorescent signal of GFP-PARP1 vs. D_{eff}. B) Initial fluorescent signal of GFP-PARP1 vs. F. All fluorescent signals were divided by 10^6. A similar lack of correlation was seen for GFP-PARP2 (data not shown).