Electronic Supplementary Material (ESI) for Photochemical & Photobiological Sciences.

Supporting Information

Spatial distribution and temporal evolution of DRONPA-fused SNAP25 clusters in adrenal chromaffin cells.

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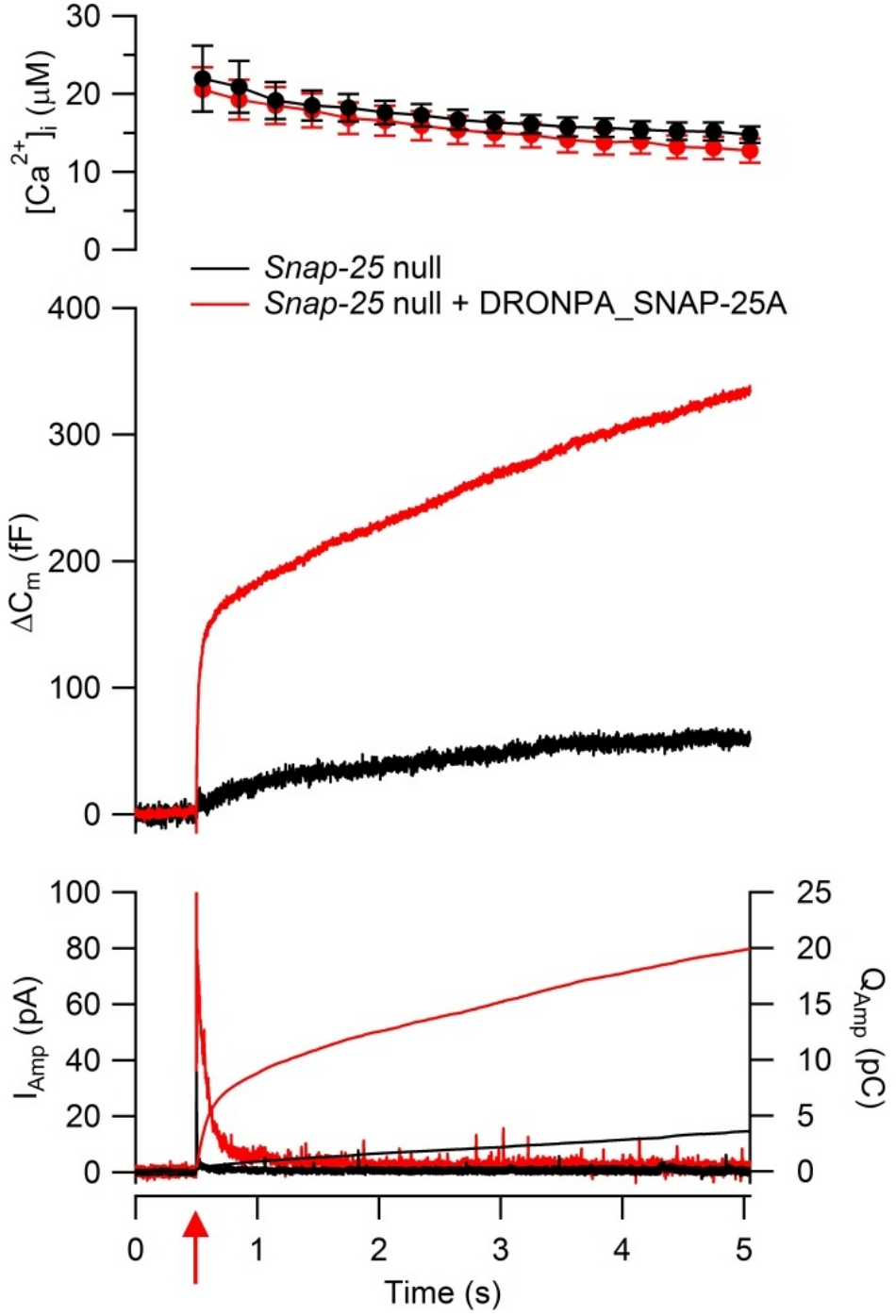


Figure SI1: Rescue experiment demonstrating full functionality of the DRONPA_SNAP-25 construct using calcium uncaging, capacitance measurements and amperometry. Top panel shows calcium concentrations measured by microfluorimetry; uncaging flash given at the time indicated by the red arrow. Middle panel shows capacitance change. Bottom panel shows amperometric current (left ordinate axis) or amperometric charge (right ordinate axis). Shown are mean traces. Black traces and symbols*: Snap-25* null cells (n= 8 cells); red traces and symbols *Snap-25* null cells expressing DRONPA_SNAP-25A (n= 8 cells). In the absence of SNAP-25, secretion is almost absent. DRONPA_SNAP-25A expression rescues secretion to normal levels.

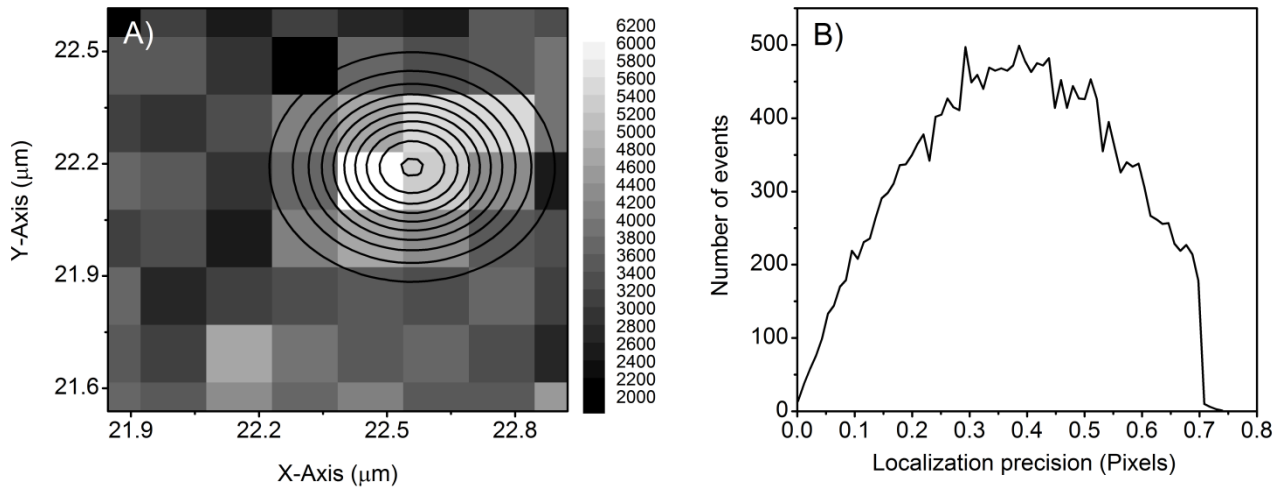


Figure SI2: A) Example of a bright spot, detected with the Localizer software and the corresponding 2D Gaussian fit, used to determine the central location of the bright spot. B) Histogram of the localization precision from an analyzed movie expressed in pixels. The size of 1 pixel is approximately 150 nm, so this leads to an average localization precision of 60 nm for the bright spots. As mentioned in the main section, this does not necessarily correspond to the physical location of a single DRONPA_SNAP25 molecule.

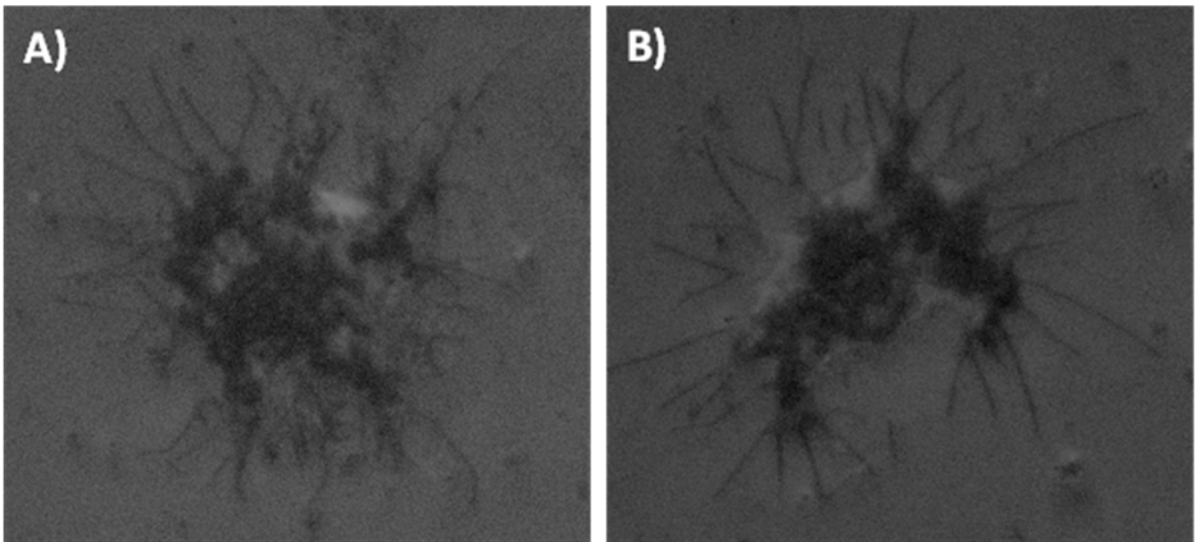


Figure SI3: A and B) Two examples of a SRIC image of living chromaffin cells, immobilized on a glass cover slip in the same way as the cells used in the paper.

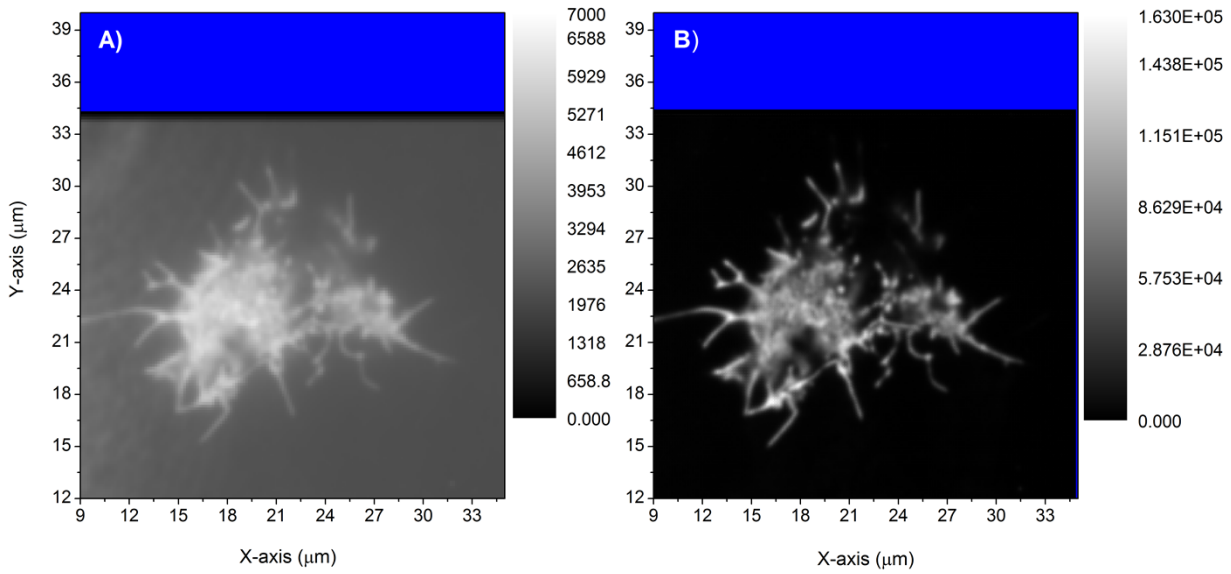


Figure SI4: A) Average intensity image (A.U.) and B) second order SOFI image (A.U.)[1] of the first 20000 frames of the movie that was also used to create the results represented in Figure 2 in the main article. The blue color represents areas with no data.

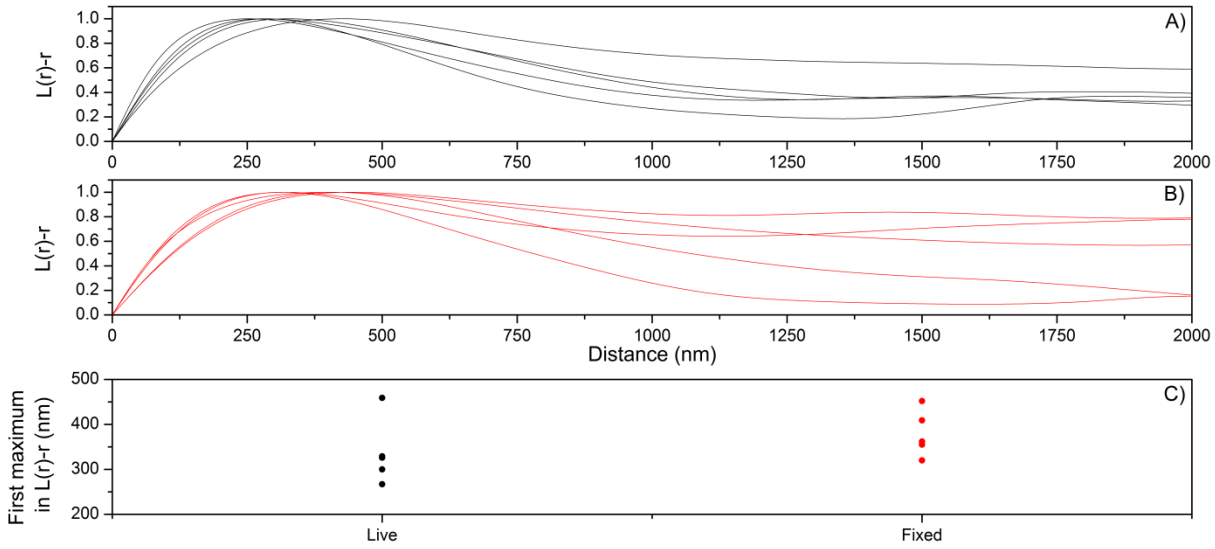


Figure SI5: L(r)-r function of (A) 5 live chromaffin cells and (B) 5 fixed chromaffin cells. C) Values of the first maximum in the L(r)-r function of 5 live chromaffin cells and 5 fixed chromaffin cells. Average of the maxima for the 5 live cells is 336 nm and for the 5 fixed cells is 380 nm.

Influence of 405 nm excitation on the number of detected bright spots in a living chromaffin cell.

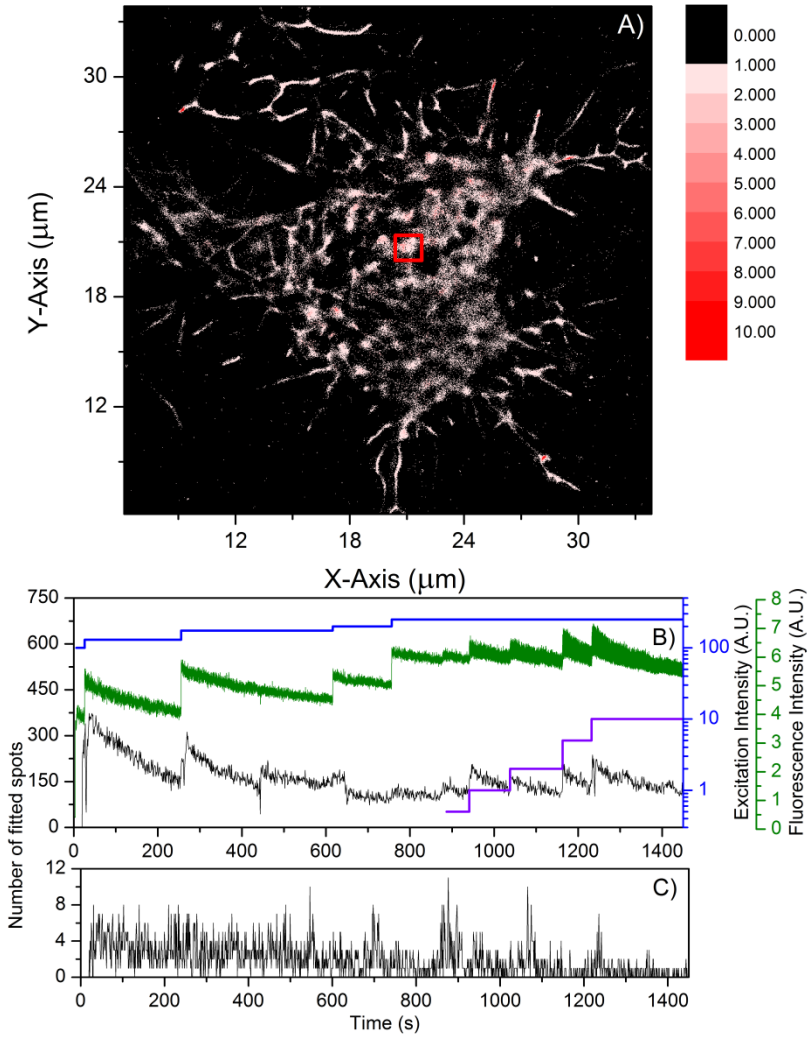
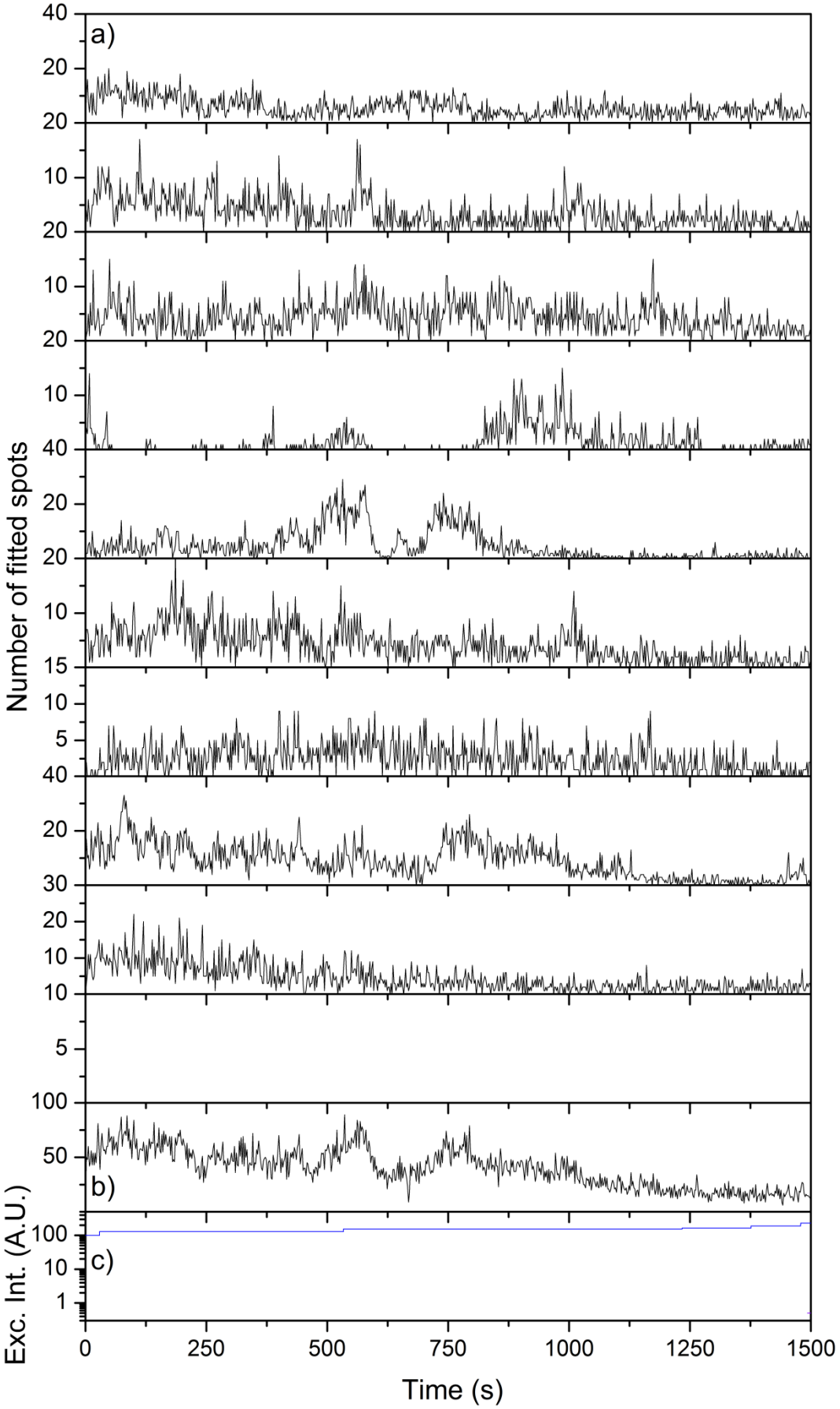
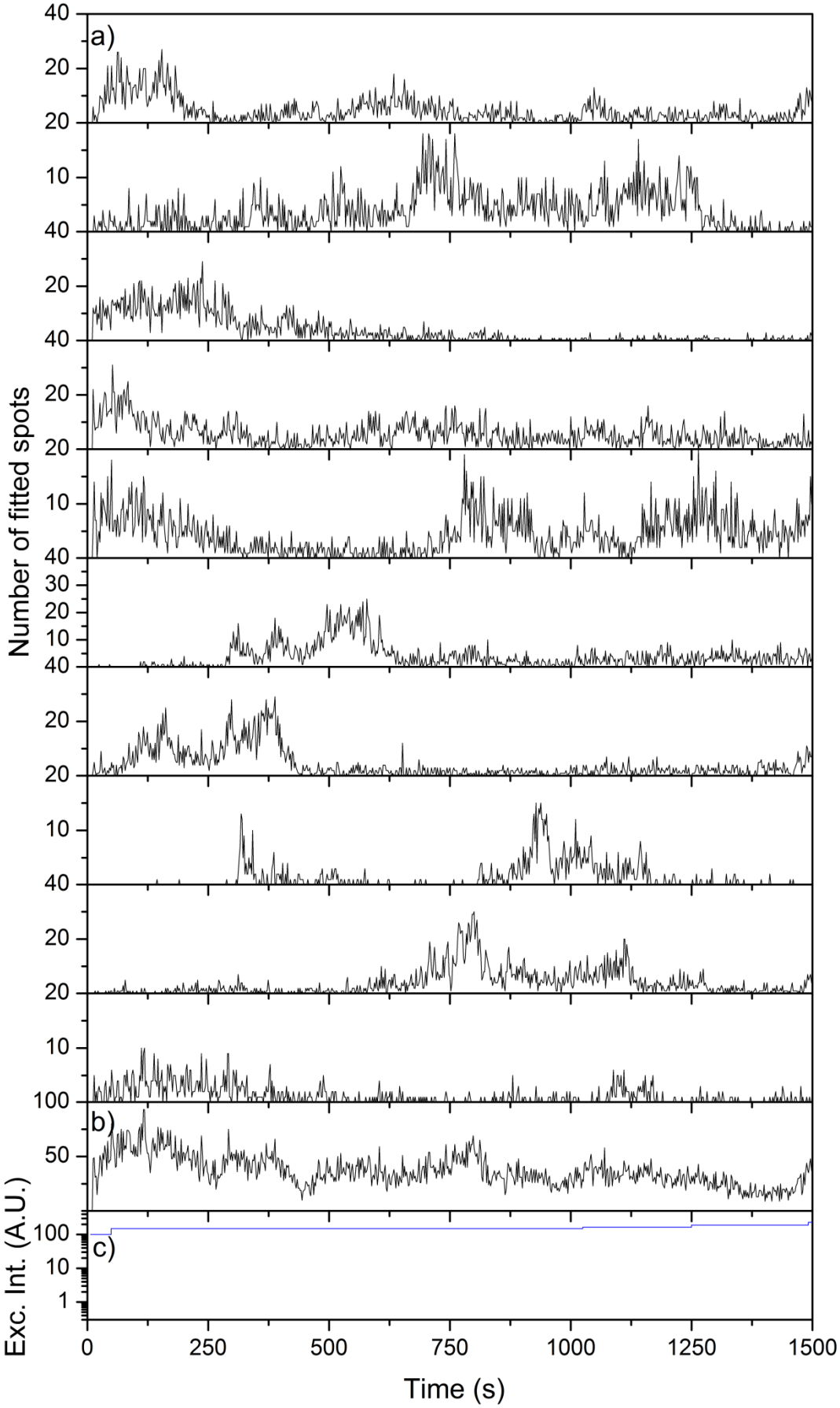


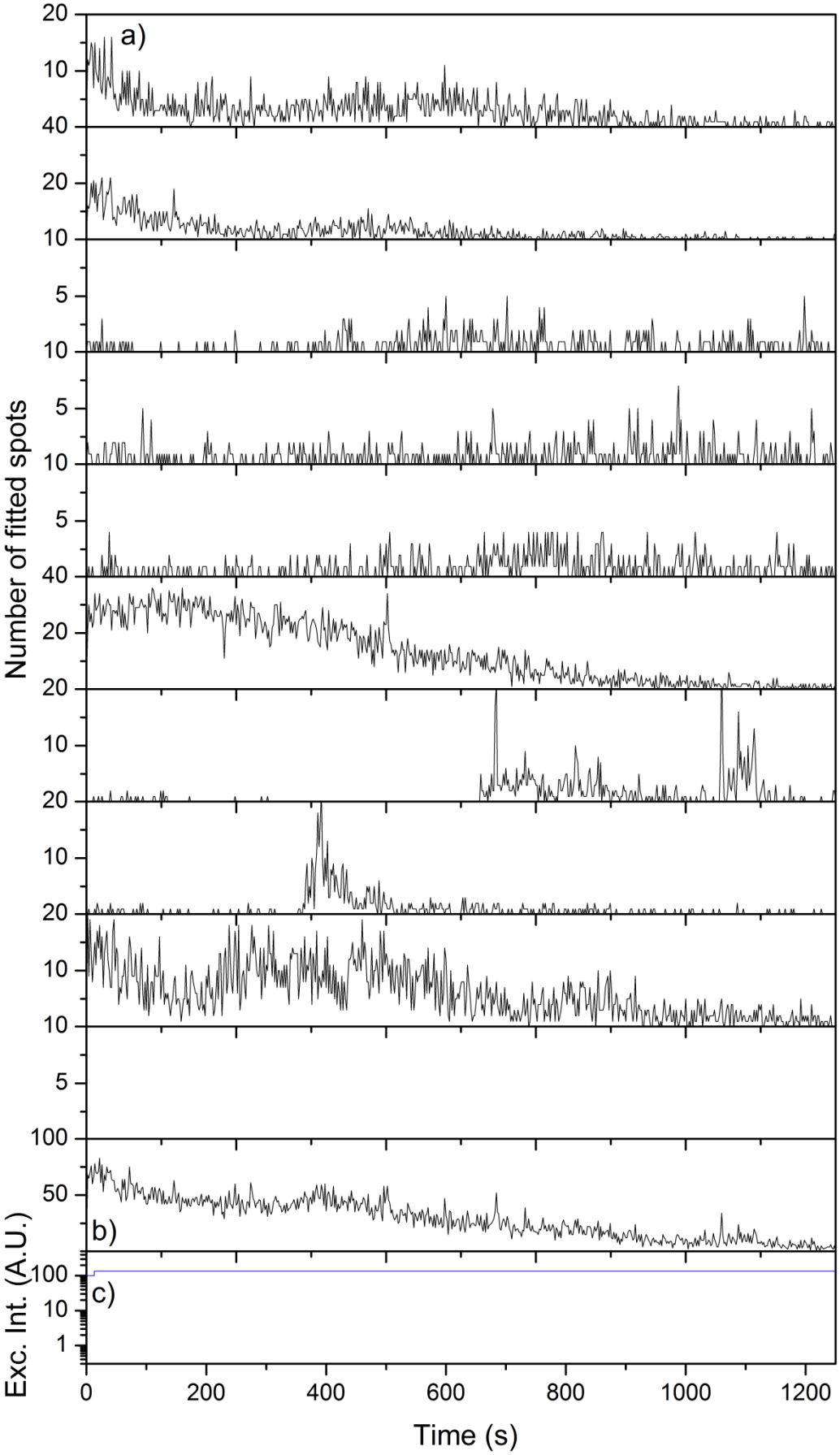
Figure SI6: A) Reconstructed image displaying the DRONPA_SNAP25 related bright spots from the first 15000 frames of a movie with 30000 frames (approximately 1450 seconds) from a live chromaffin cell for which the center was determined by the Localizer software. B) Temporal evolution of the number of detected bright spots (black curve and black Y-axis), the 488 nm excitation power (blue curve and blue Y-axis, the excitation power was indirectly determined from laser bleed through in areas of the image where no cells were present), 405 nm excitation power (purple curve and blue Y-axis). The A.U. for the 488 nm and the 405 nm are not the same. C) Temporal evolution of the number of detected and fitted bright spots in a single cluster region for which the area is depicted in SI Figure 3A by the red box.

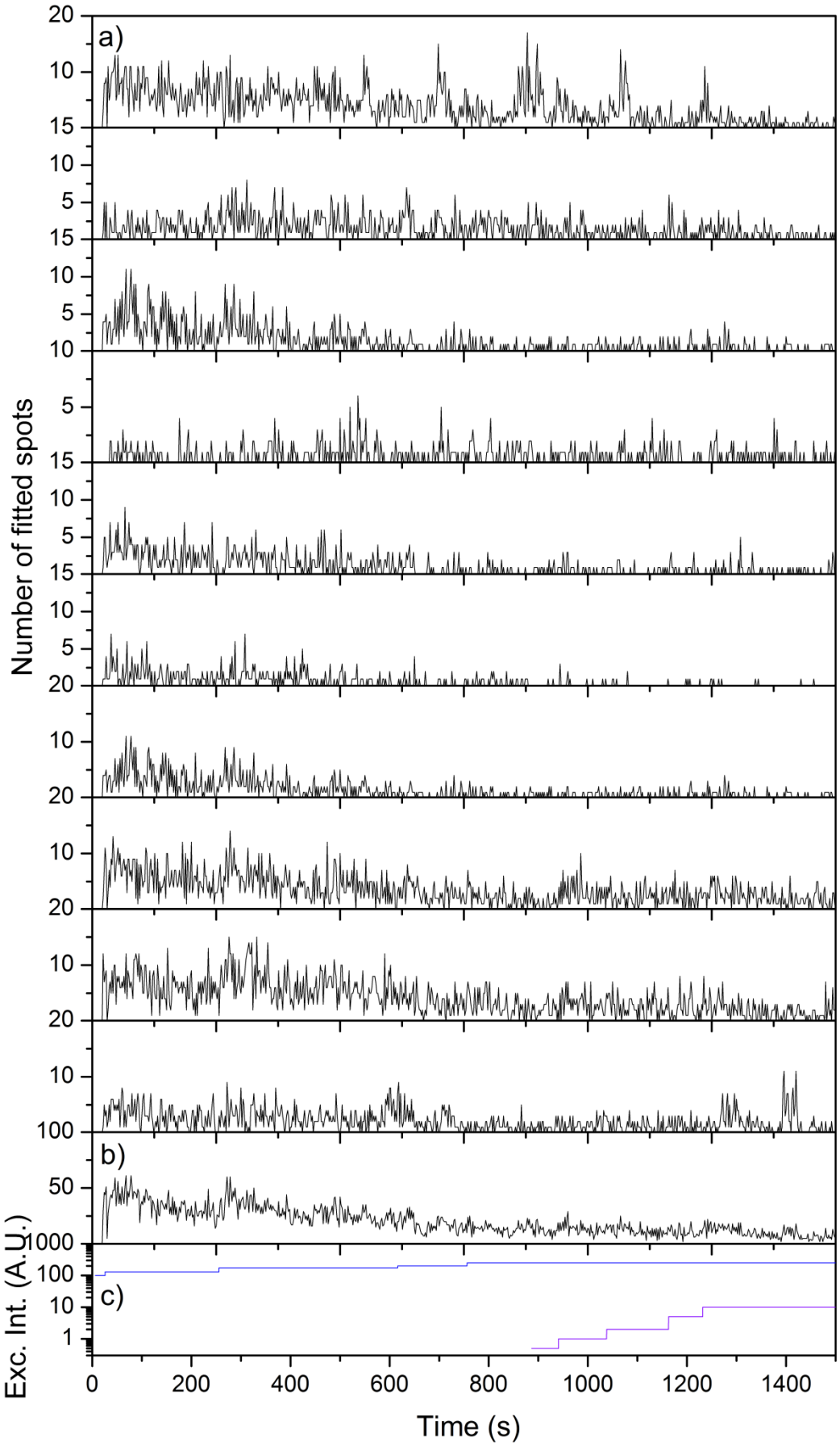
Additional data of clusters dynamics of live and fixed Chromaffin cells.

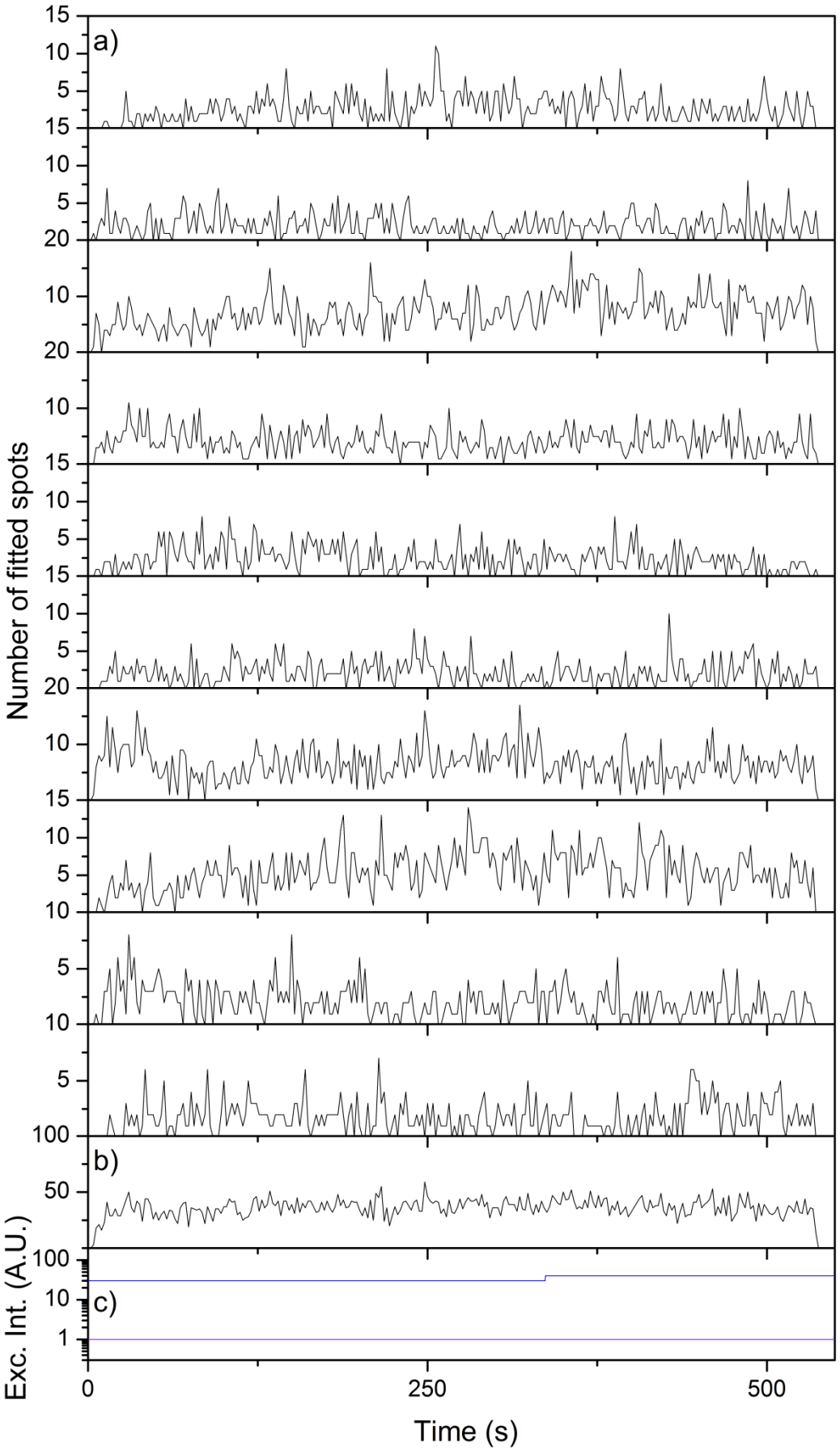
A) Live cells. The next 5 figures are from live Chromaffin cells. In part ***a*** of the figures, the temporal evolution of 9 or 10 DRONPA_SNAP25 cluster domains are represented. Part ***b*** is the sum of all the traces represented in part ***a***). Part ***c*** shows which excitation energy was used for 488 nm (blue curve) and 405 nm (violet curve; when no 405 nm was used, no curve is present).


a)
b)
c)
Number of fitted spots
Exc. Int. (A.U.)
Time (s)
0
250
500
750
1000
1250
1500

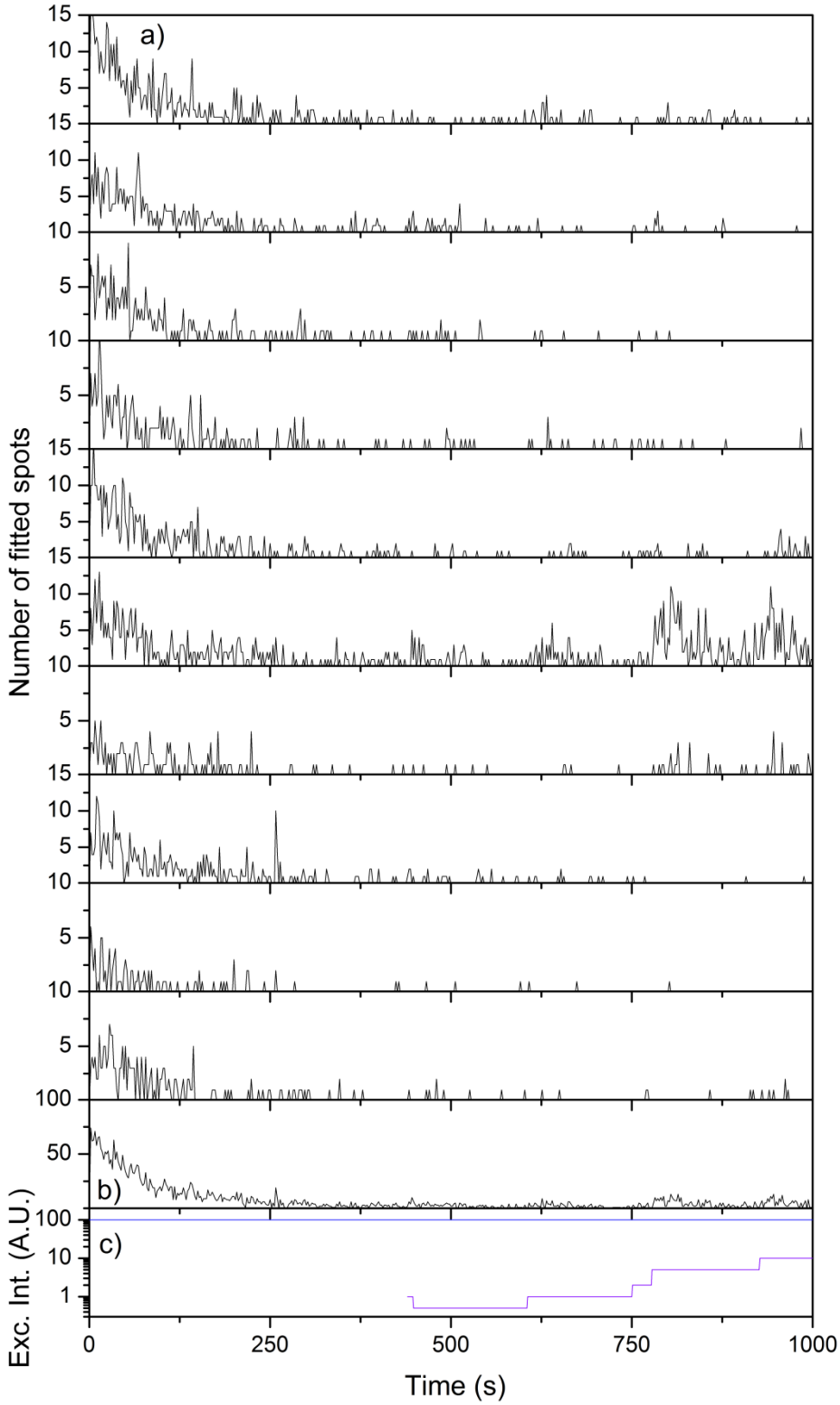

a)
b)
c)
Number of fitted spots
Exc. Int. (A.U.)
Time (s)
0
250
500
750
1000
1250
1500

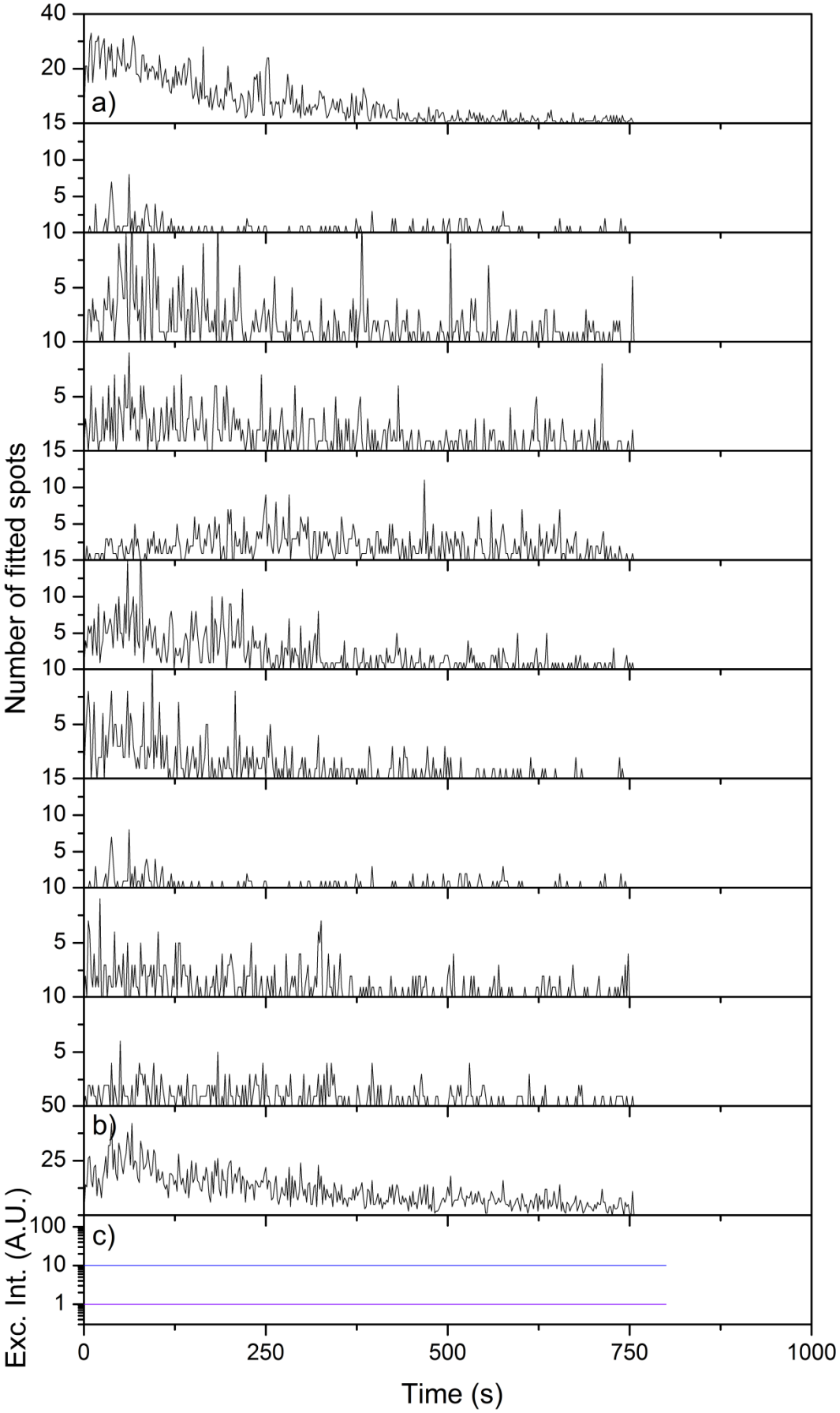

a)
b)
c)
Number of fitted spots
Exc. Int. (A.U.)
Time (s)
0
200
400
600
800
1000
1200

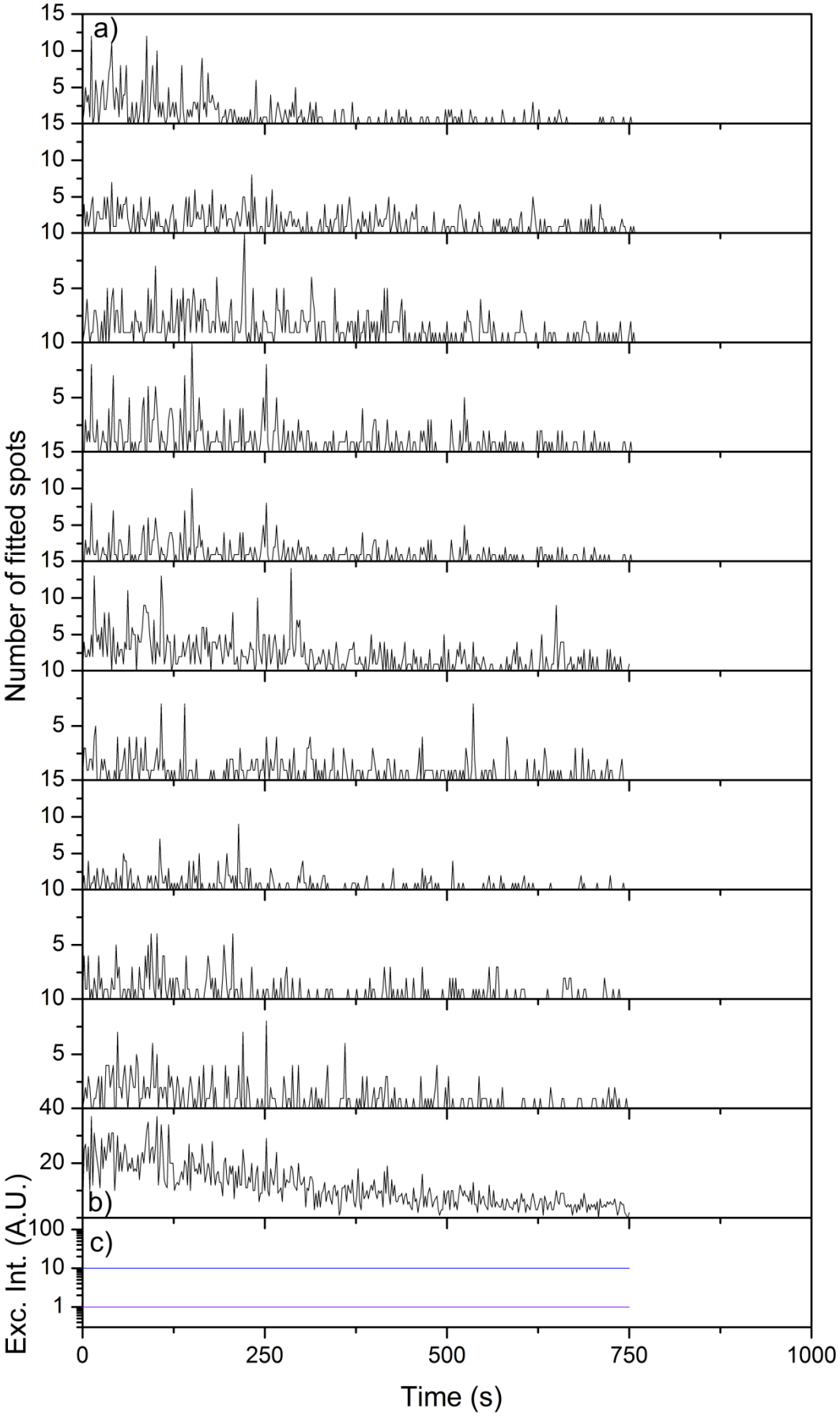

a)
b)
c)
Number of fitted spots
Exc. Int. (A.U.)
Time (s)
0
200
400
600
800
1000
1200
1400

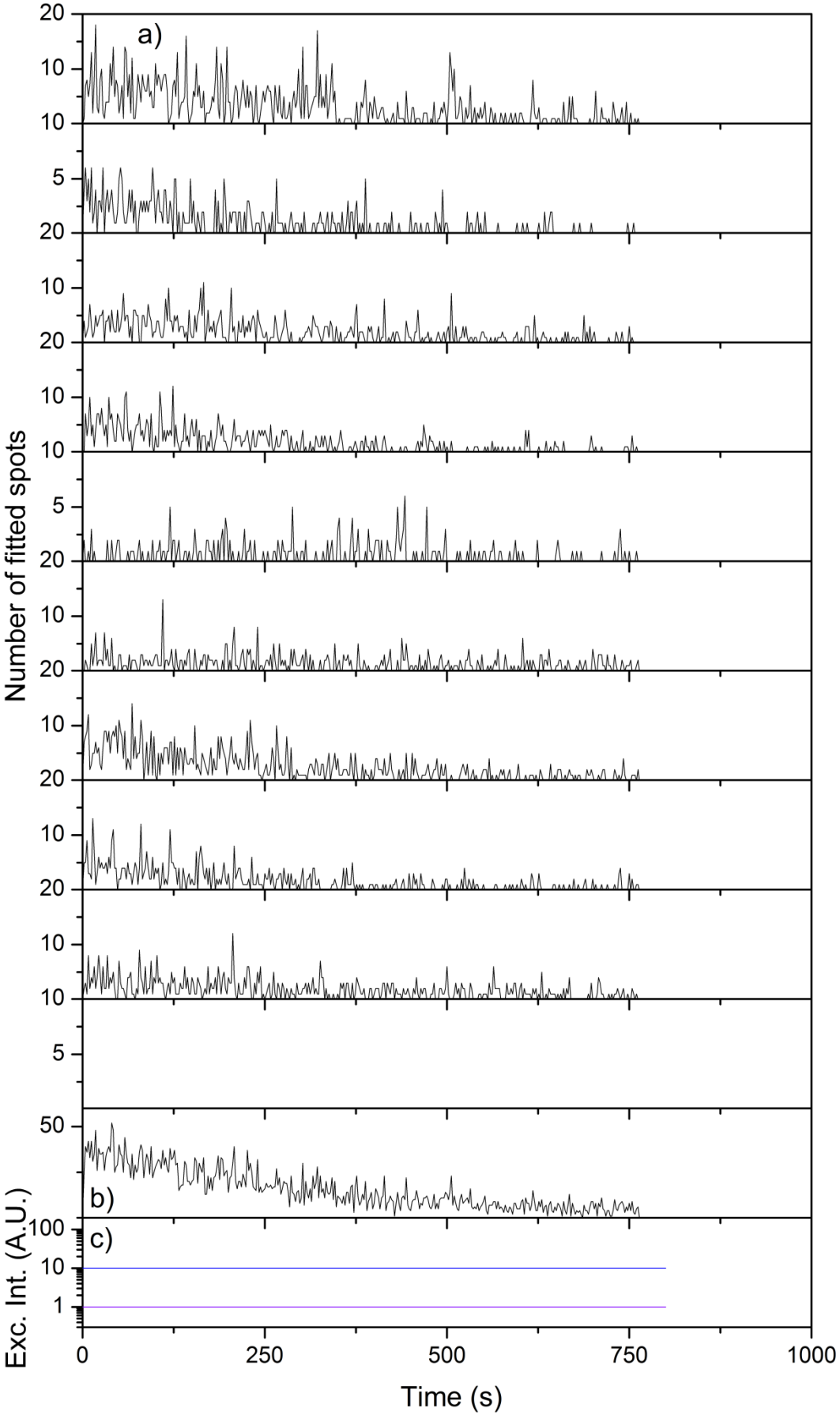

a)
b)
c)
Number of fitted spots
Exc. Int. (A.U.)
Time (s)
0
250
500

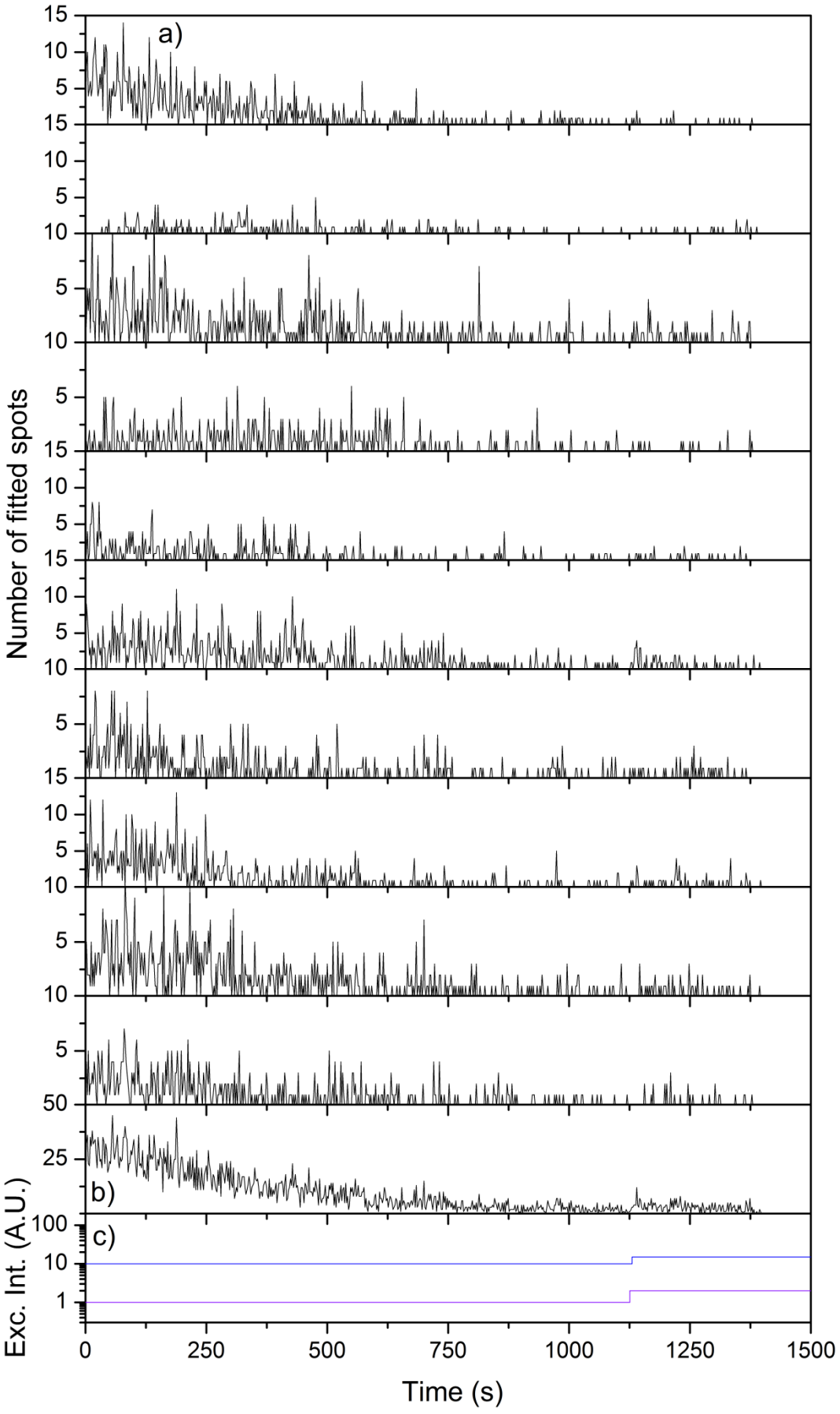
B) Fixed cells. The next 5 figures are from fixed Chromaffin cells. In part a of the figures, the temporal evolution of 9 or 10 DRONPA_SNAP25 cluster domains are represented. Part b is the sum of all the traces represented in part a. Part c shows which excitation energy was used for 488 nm (blue curve) and 405 nm (violet curve, when no 405 nm was used, no curve is present).




a)
b)
c)
Number of fitted spots
Exc. Int. (A.U.)
Time (s)
0
250
500
750
1000


a)
b)
c)
Number of fitted spots
Exc. Int. (A.U.)
Time (s)
0
250
500
750
1000


a)
b)
c)
Number of fitted spots
Exc. Int. (A.U.)
Time (s)
0
250
500
750
1000


a)
b)
c)
Number of fitted spots
Exc. Int. (A.U.)
Time (s)
0
250
500
750
1000
1250
1500

1. P. Dedecker, G. C. H. Mo, T. Dertinger and J. Zhang, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 10909-10914.