

# NPC SMLM data for image alignment and single particle averaging

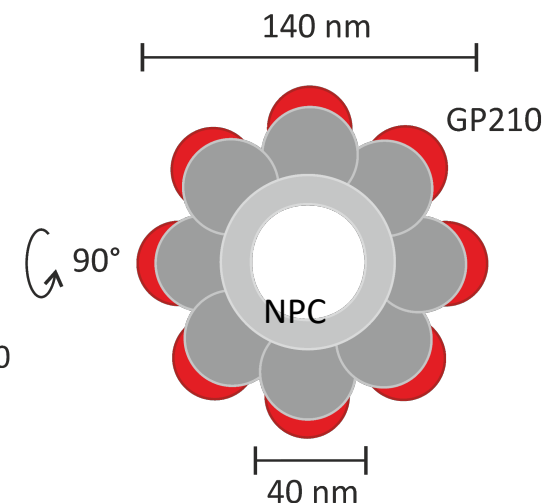
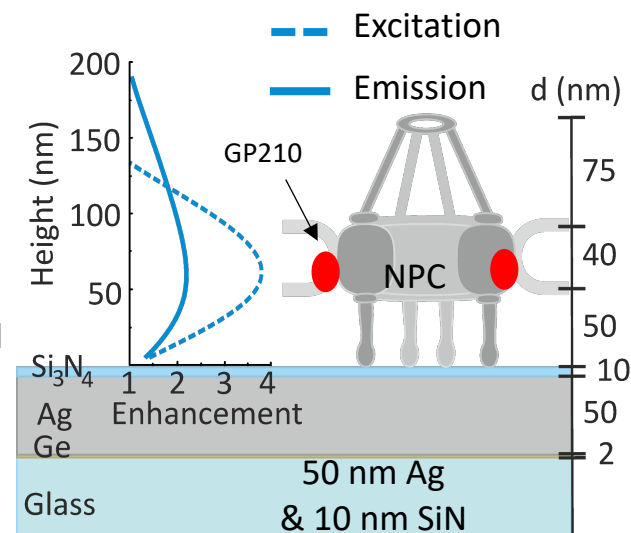
Hannah S. Heil, AG Heinze, RVZ, University of Würzburg (2018-04-09)

This is a depository for two single molecule localization microscopy datasets of nuclear pore complex (NPC) structures for single particle averaging.

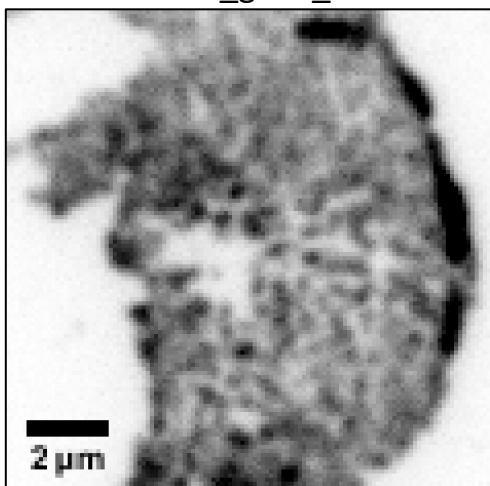
The data was published in:

Heil, H.S., Schreiber, B., Götz, R. *et al.* Sharpening emitter localization in front of a tuned mirror. *Light Sci Appl* **7**, 99 (2018). <https://doi.org/10.1038/s41377-018-0104-z>

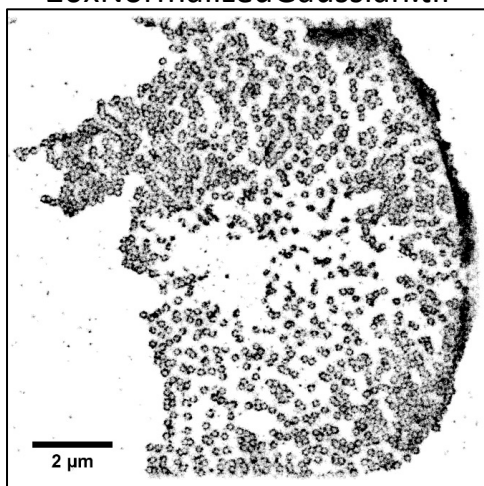
Both datasets have two different levels of localisation precision as one is a conventional STORM experiment and the second a mirror-enhanced STORM experiment. A detailed description of the sample preparation and imaging conditions can be found in the related publication. In short the NPC structures are placed on the surface of a glass coverslip or nano-mirror coated coverslip by manual isolation and spreading of nuclear envelopes from xenopus laevis oocytes, fixed and stained by indirect immunolabeling. The primary antibody targets GP210, the secondary F(ab')<sub>2</sub> fragment is conjugated with Alexa Fluor 647.



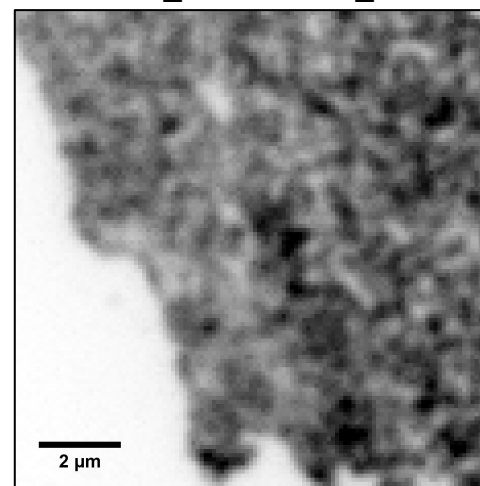
NPCData\_glass\_EPI.tif



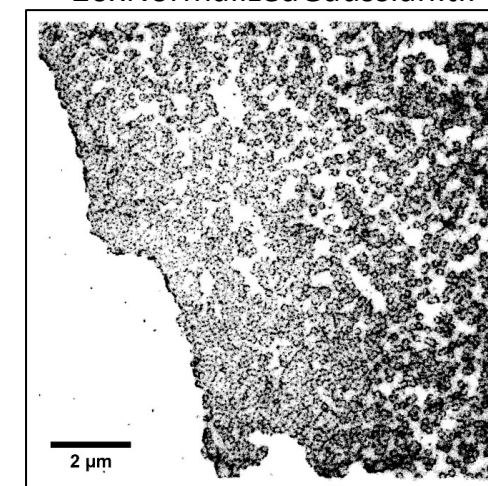
NPCData\_glass\_STORM\_20xNormalizedGaussian.tif



NPCData\_nanomirror\_EPI.tif



NPCData\_nanomirror\_STORM\_20xNormalizedGaussian.tif



In this depository I'm providing the raw images data, localisation data and super-resolved reconstruction for the two experiments, as well as the localisation data and super-resolved reconstruction of single NPC rings.

I'm also providing a MatLab script that allows to select single NPC positions in the super-resolved image and export the localization data of the single NPC ROI: **P01\_ImageAlignment\_PickElements.m**

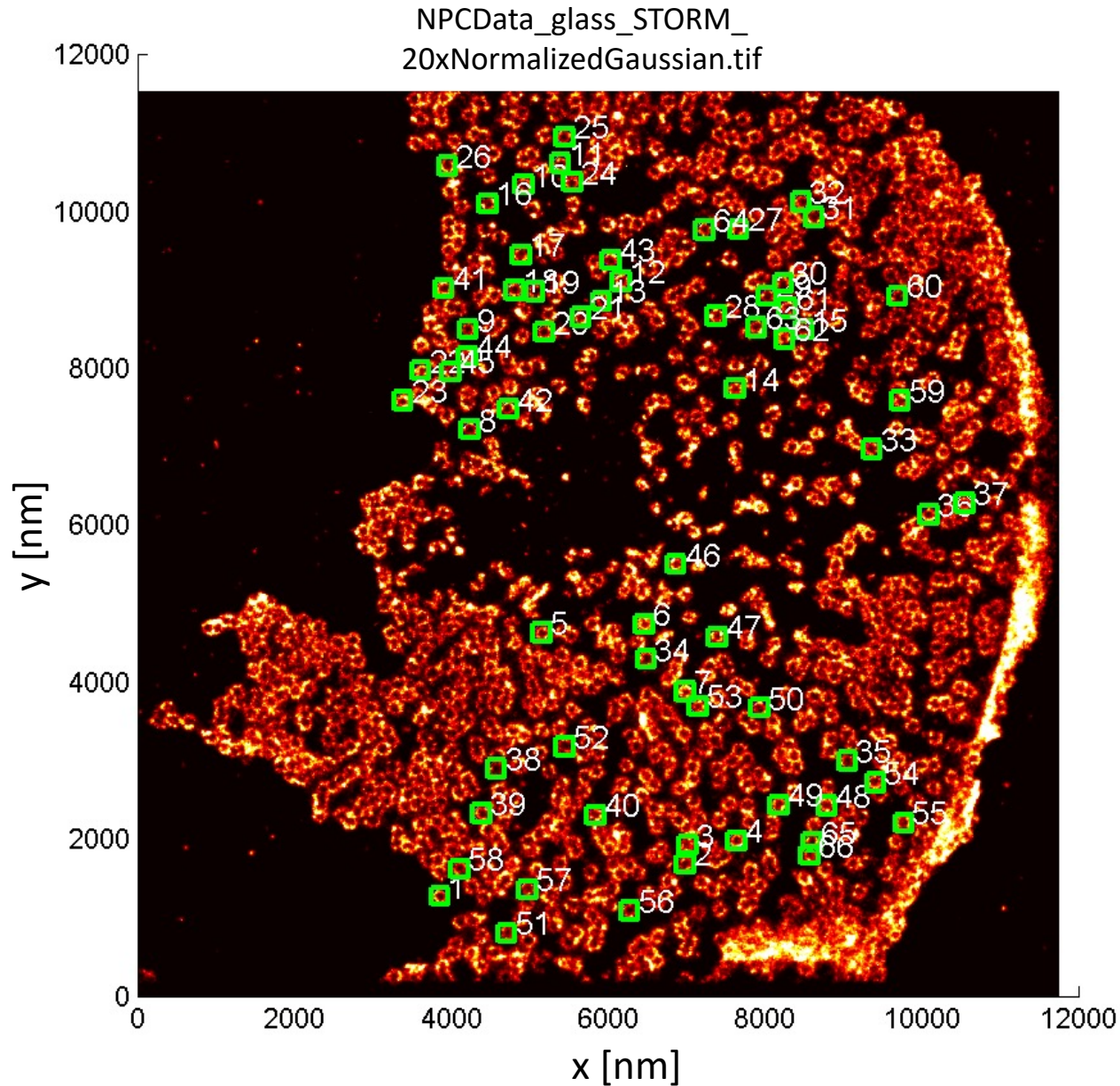
Image parameters: 102 nm pixel size, EM Gain 100, Photoelectrons per A/D count

The localization analysis was performed with ThunderSTORM (M. Ovesný et al., (2014) 10.1093/bioinformatics/btu202) and the protocols with the analysis and data filtering parameters are provided.

Column structure of the localisation text files:

1	2	3	4	5	6	7	8	9	10	11
Id	Frame	x [nm]	y [nm]	sigma [nm]	intensity [photon]	offset [photon]	bkgstd [photon]	chi2	Uncertainty [nm]	detections

# Selection of single NPC ROIs (ROI size: 240x240 nm<sup>2</sup>)



Selection 2

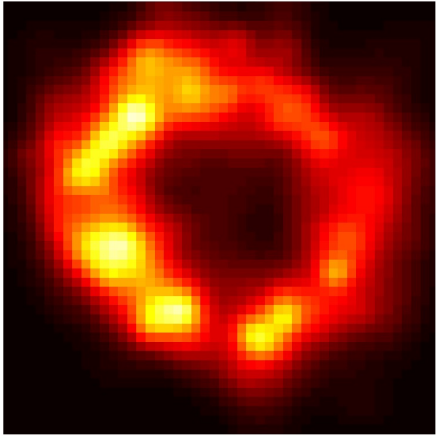
1	11	21	31	41	51	61
2	12	22	32	42	52	62
3	13	23	33	43	53	63
4	14	24	34	44	54	64
5	15	25	35	45	55	65
6	16	26	36	46	56	66
7	17	27	37	47	57	
8	18	28	38	48	58	
9	19	29	39	49	59	
10	20	30	40	50	60	



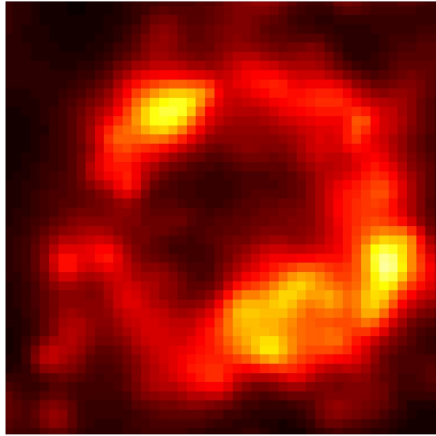
# Single NPCs, STORM on glass, 240x240 nm<sup>2</sup> ROIs 1/7

20x normalized Gaussian reconstruction -> 5.1 nm pixelsize

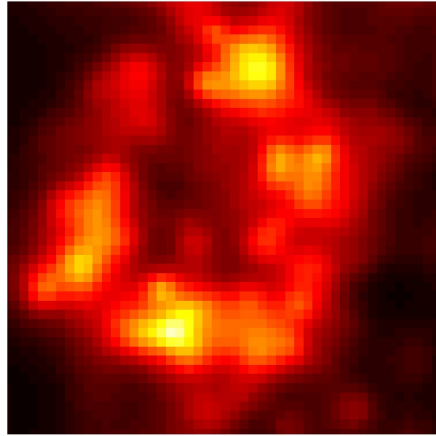
201707149\_glass\_4: Element1



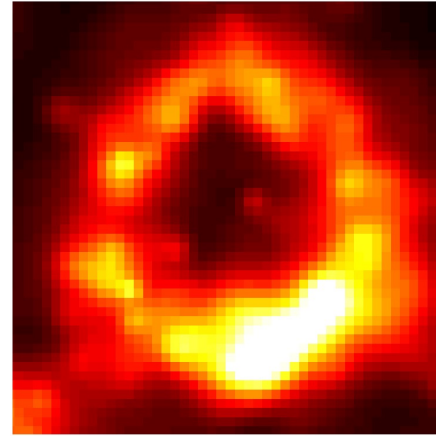
201707149\_glass\_4: Element2



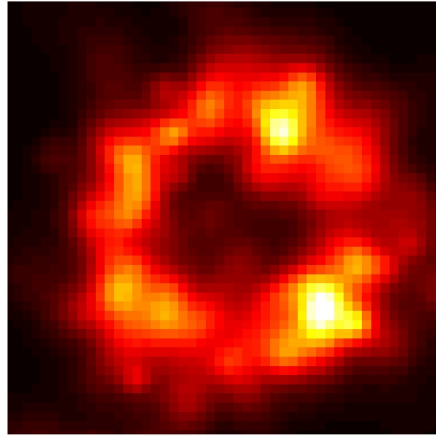
201707149\_glass\_4: Element3



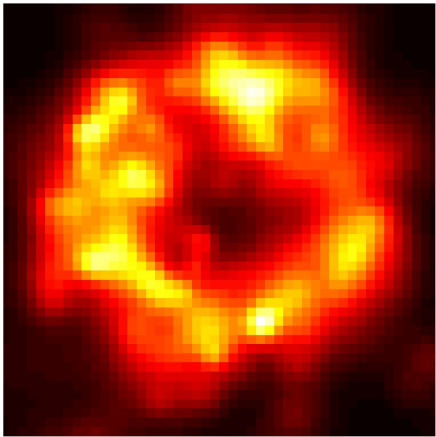
201707149\_glass\_4: Element4



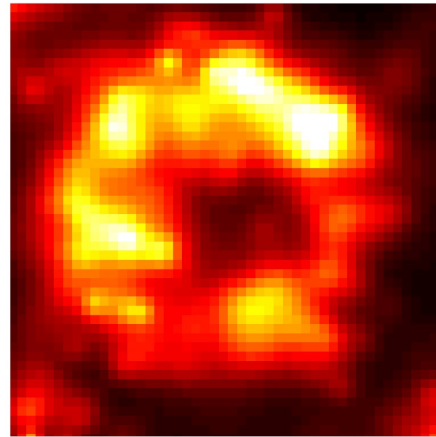
201707149\_glass\_4: Element5



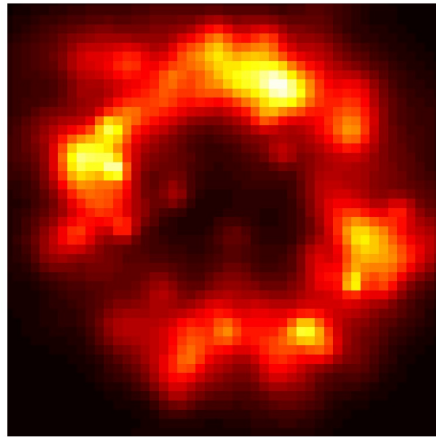
201707149\_glass\_4: Element6



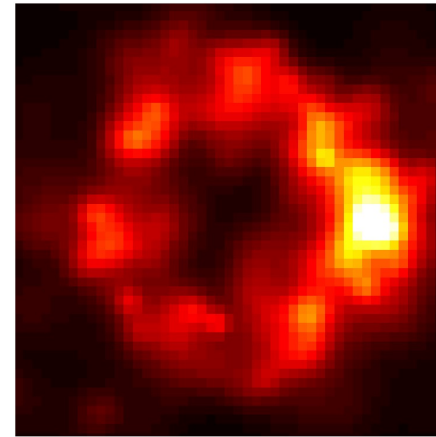
201707149\_glass\_4: Element7



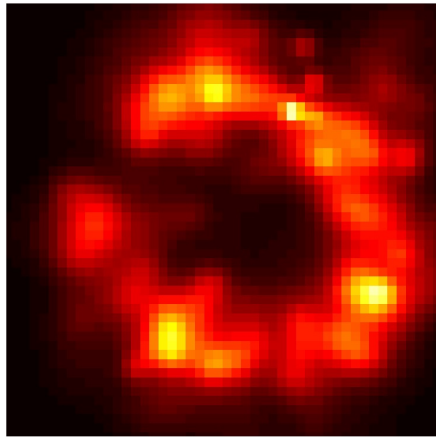
201707149\_glass\_4: Element8



201707149\_glass\_4: Element9



201707149\_glass\_4: Element10

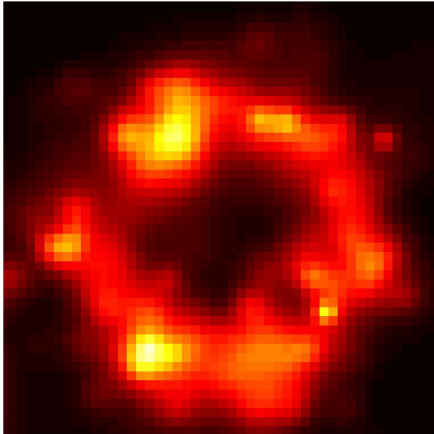




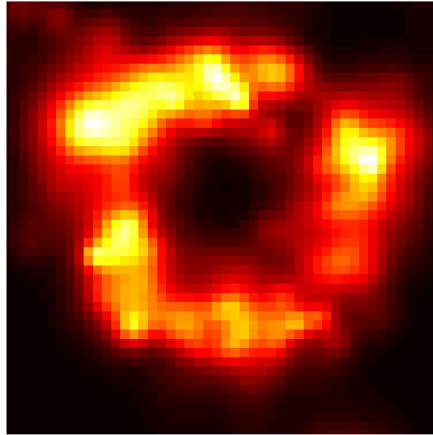
# Single NPCs, STORM on glass, 240x240 nm<sup>2</sup> ROIs 2/7

20x normalized Gaussian reconstruction -> 5.1 nm pixelsize

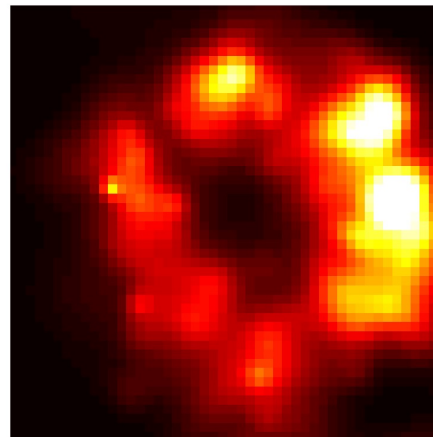
201707149\_glass\_4: Element11



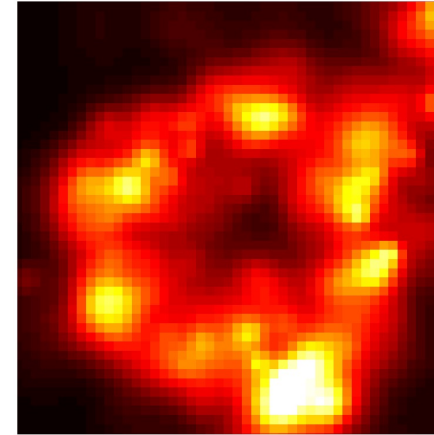
201707149\_glass\_4: Element12



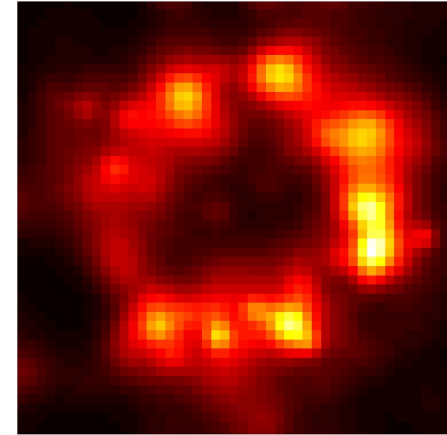
201707149\_glass\_4: Element13



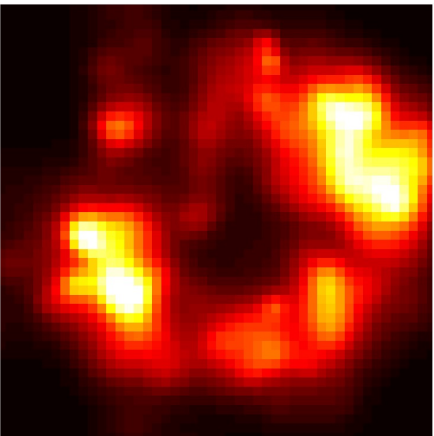
201707149\_glass\_4: Element14



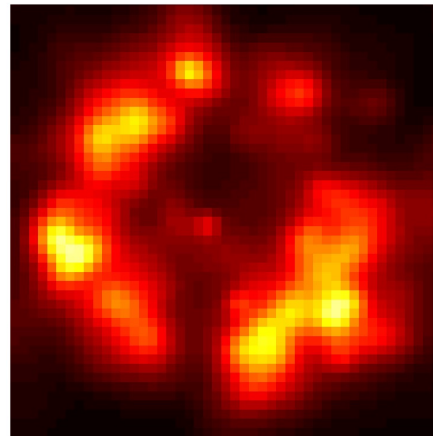
201707149\_glass\_4: Element15



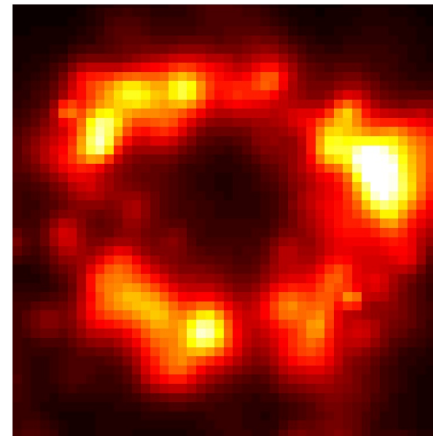
201707149\_glass\_4: Element16



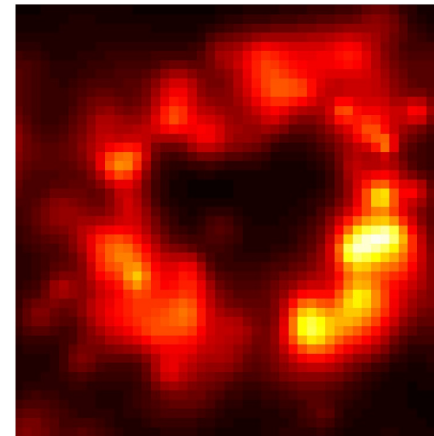
201707149\_glass\_4: Element17



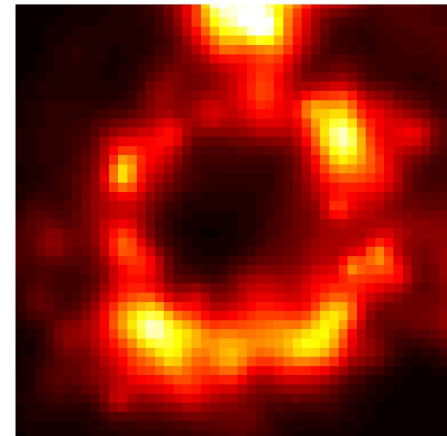
201707149\_glass\_4: Element18



201707149\_glass\_4: Element19



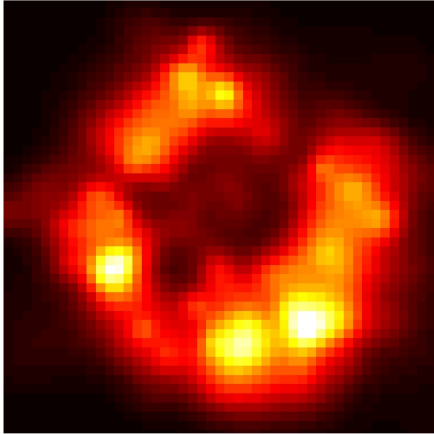
201707149\_glass\_4: Element20



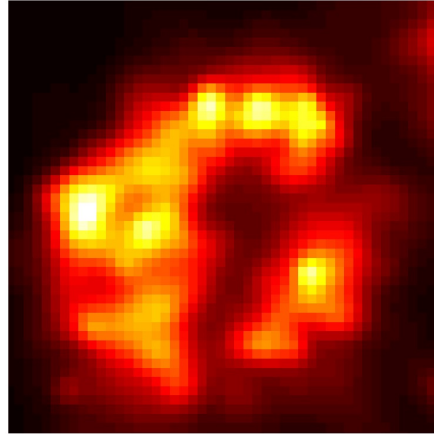
# Single NPCs, STORM on glass, 240x240 nm<sup>2</sup> ROIs 3/7

20x normalized Gaussian reconstruction -> 5.1 nm pixelsize

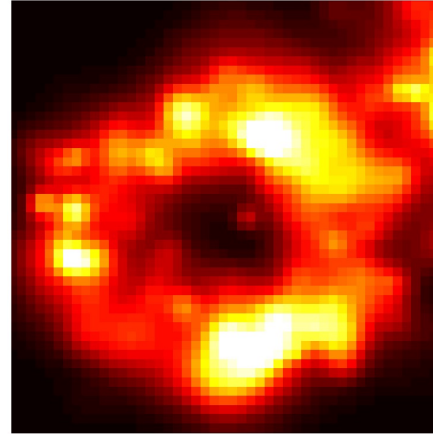
201707149\_glass,4: Element21



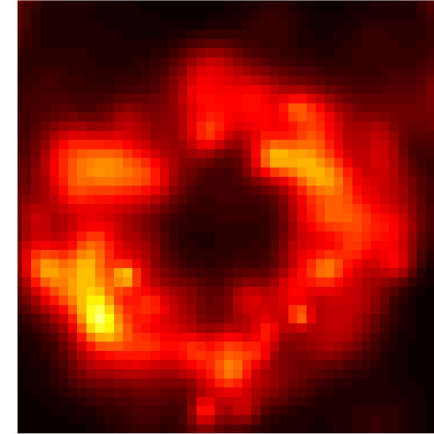
201707149\_glass,4: Element22



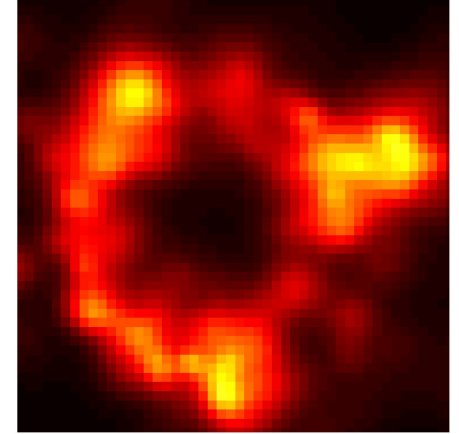
201707149\_glass,4: Element23



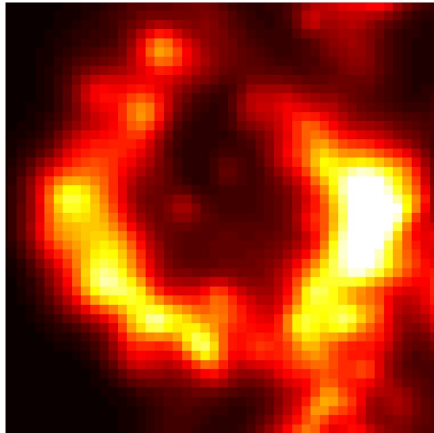
201707149\_glass,4: Element24



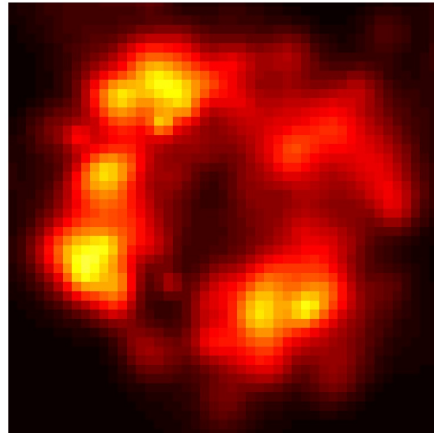
201707149\_glass,4: Element25



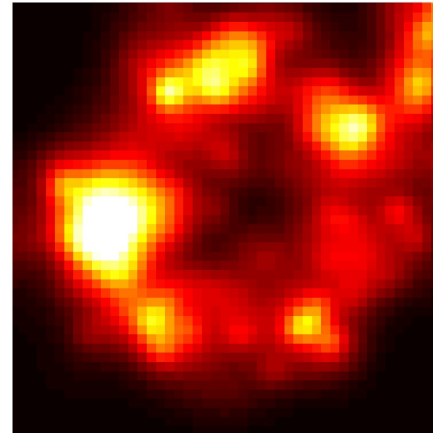
201707149\_glass,4: Element26



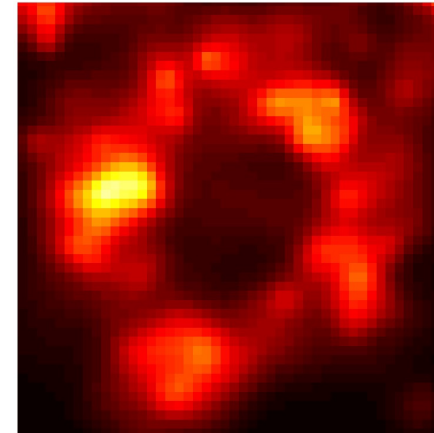
201707149\_glass,4: Element27



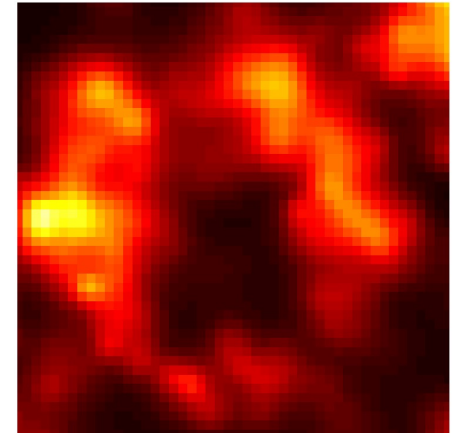
201707149\_glass,4: Element28



201707149\_glass,4: Element29



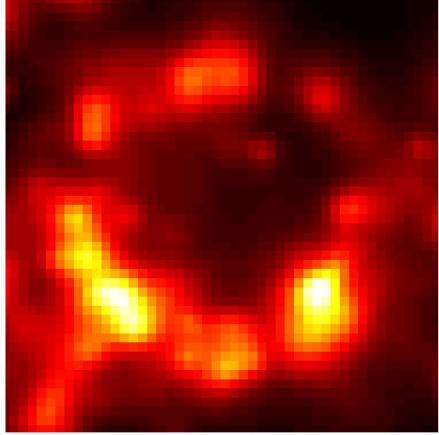
201707149\_glass,4: Element30



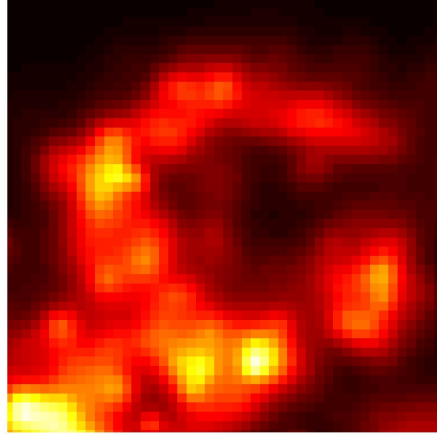
# Single NPCs, STORM on glass, 240x240 nm<sup>2</sup> ROIs 4/7

20x normalized Gaussian reconstruction -> 5.1 nm pixelsize

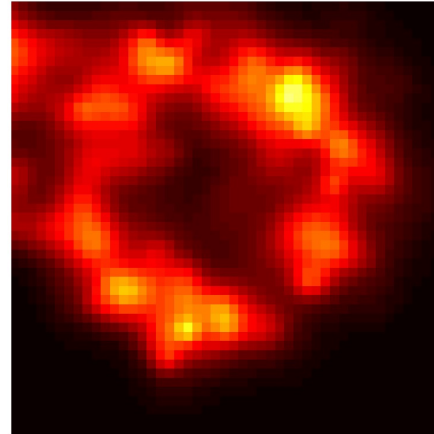
201707149\_glass\_4: Element31



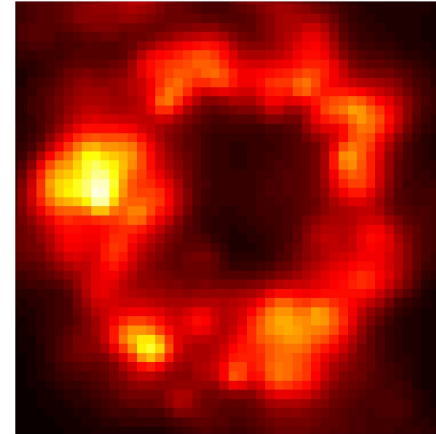
201707149\_glass\_4: Element32



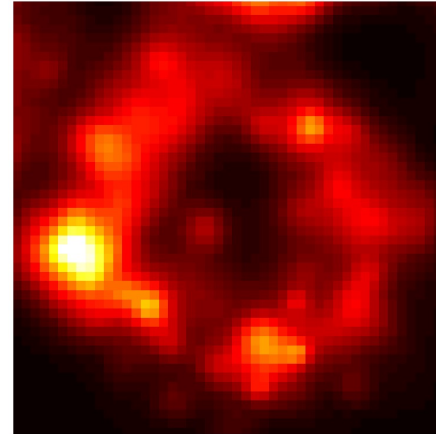
201707149\_glass\_4: Element33



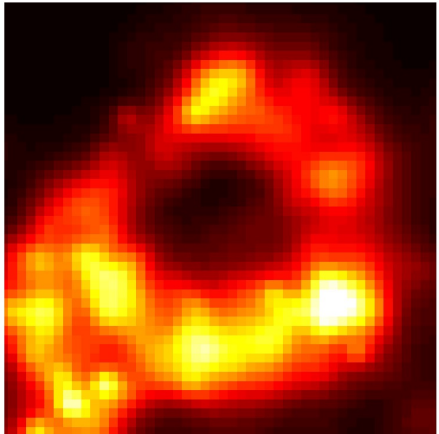
201707149\_glass\_4: Element34



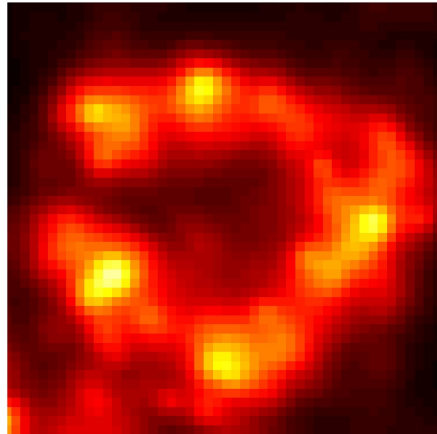
201707149\_glass\_4: Element35



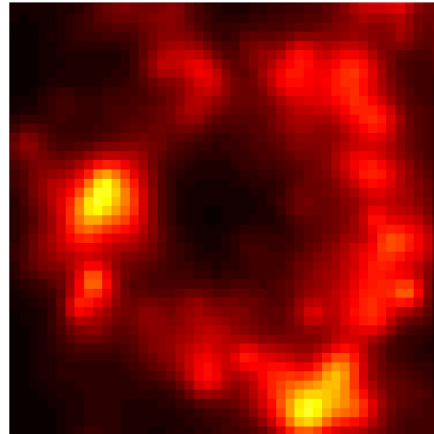
201707149\_glass\_4: Element36



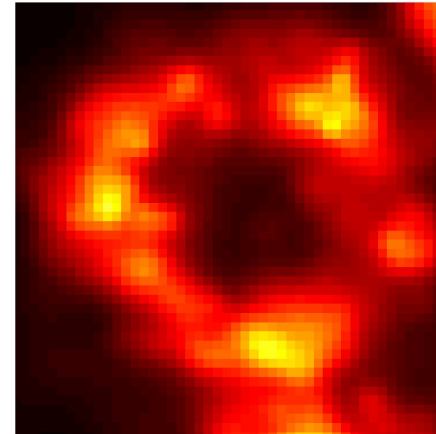
201707149\_glass\_4: Element37



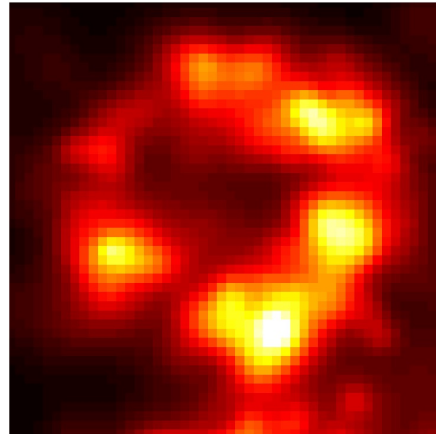
201707149\_glass\_4: Element38



201707149\_glass\_4: Element39



201707149\_glass\_4: Element40

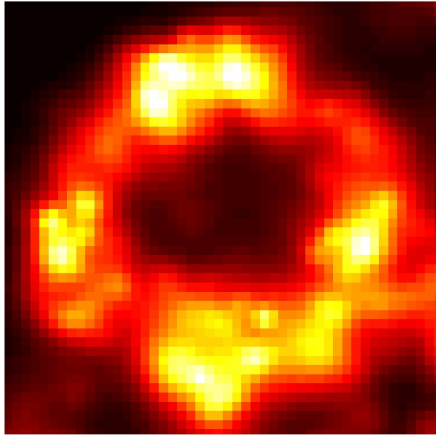




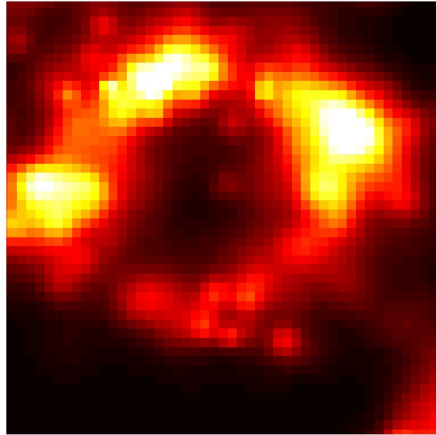
# Single NPCs, STORM on glass, 240x240 nm<sup>2</sup> ROIs 5/7

20x normalized Gaussian reconstruction -> 5.1 nm pixelsize

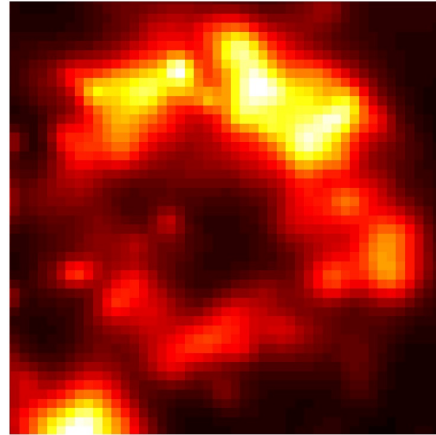
201707149\_jass,4: Element41



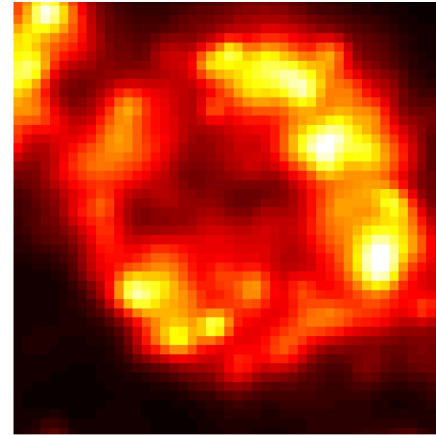
201707149\_jass,4: Element42



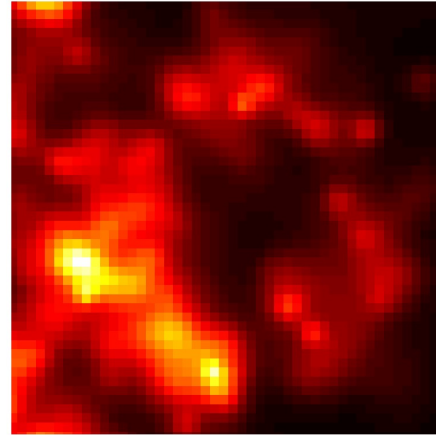
201707149\_jass,4: Element43



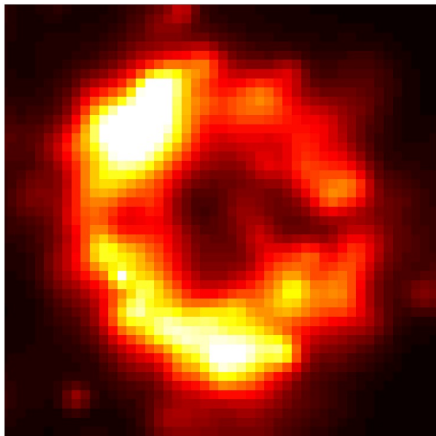
201707149\_jass,4: Element44



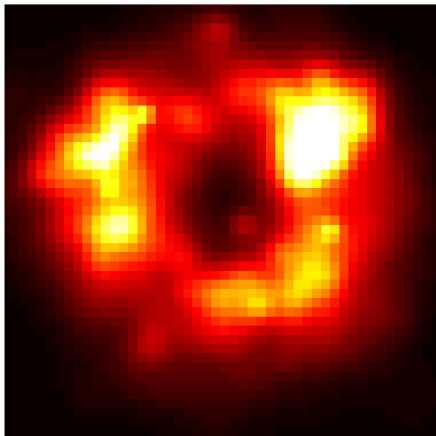
201707149\_jass,4: Element45



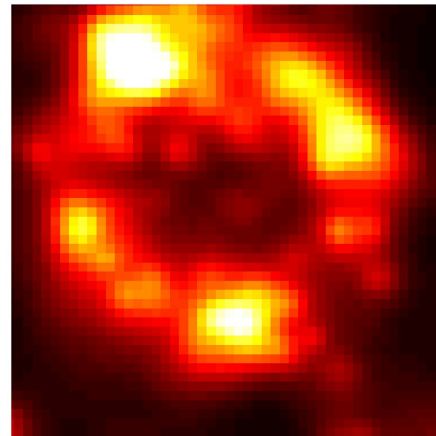
201707149\_jass,4: Element46



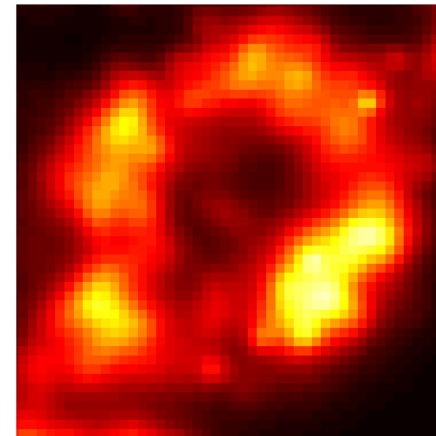
201707149\_jass,4: Element47



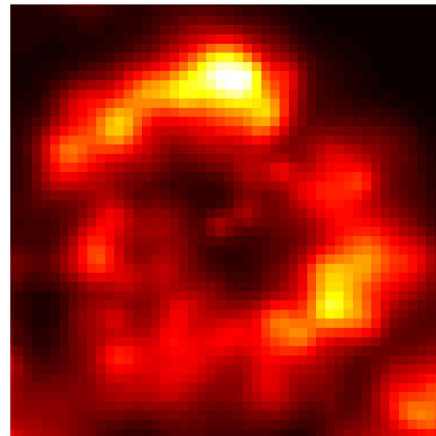
201707149\_jass,4: Element48



201707149\_jass,4: Element49



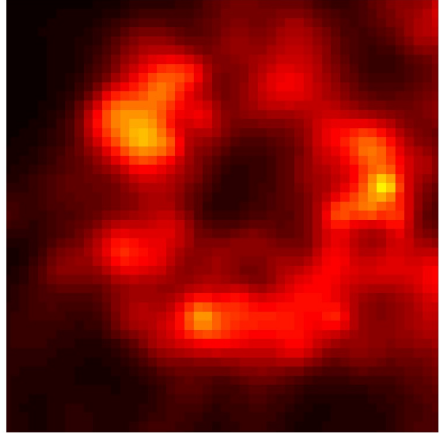
201707149\_jass,4: Element50



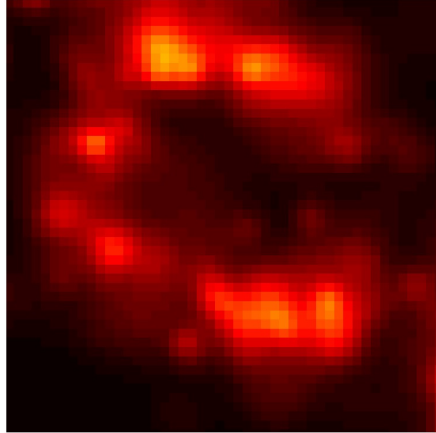
# Single NPCs, STORM on glass, 240x240 nm<sup>2</sup> ROIs 6/7

20x normalized Gaussian reconstruction -> 5.1 nm pixelsize

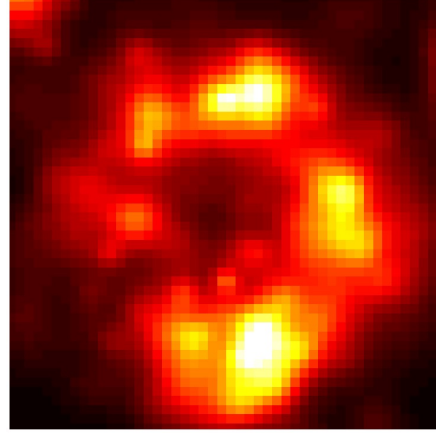
201707149\_glass\_4: Element51



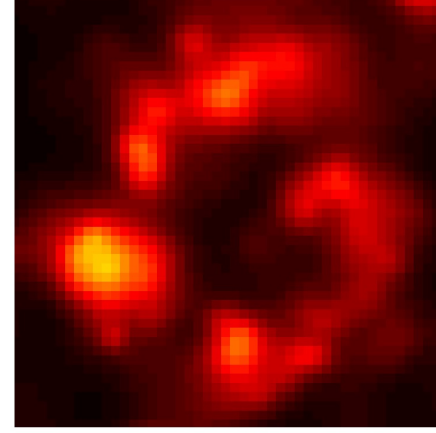
201707149\_glass\_4: Element52



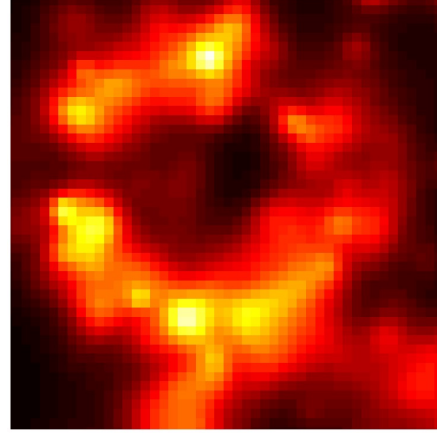
201707149\_glass\_4: Element53



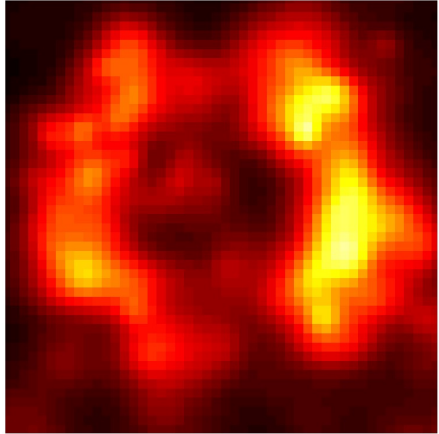
201707149\_glass\_4: Element60



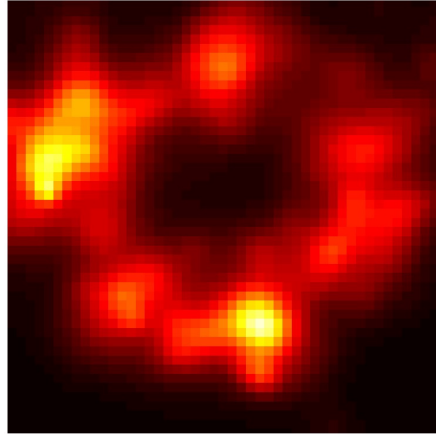
201707149\_glass\_4: Element54



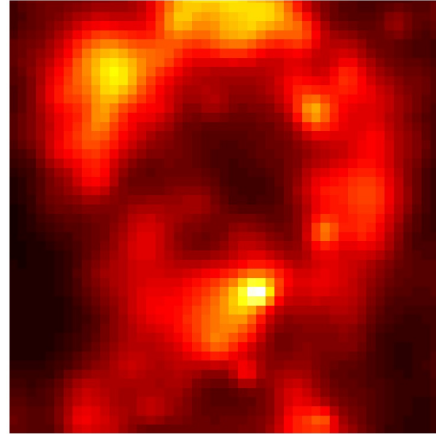
201707149\_glass\_4: Element55



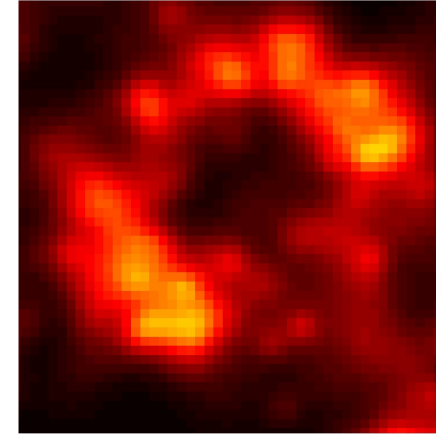
201707149\_glass\_4: Element56



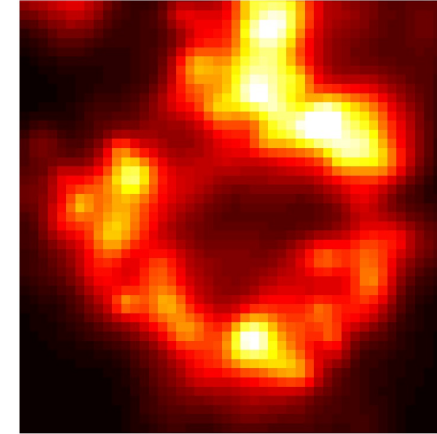
201707149\_glass\_4: Element57



201707149\_glass\_4: Element58



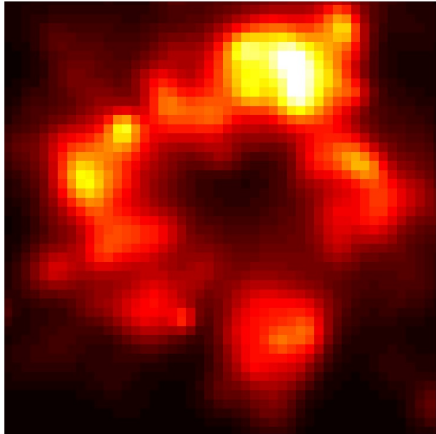
201707149\_glass\_4: Element59



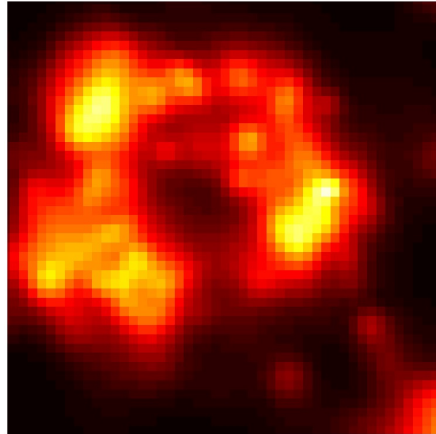
# Single NPCs, STORM on glass, 240x240 nm<sup>2</sup> ROIs 7/7

20x normalized Gaussian reconstruction -> 5.1 nm pixelsize

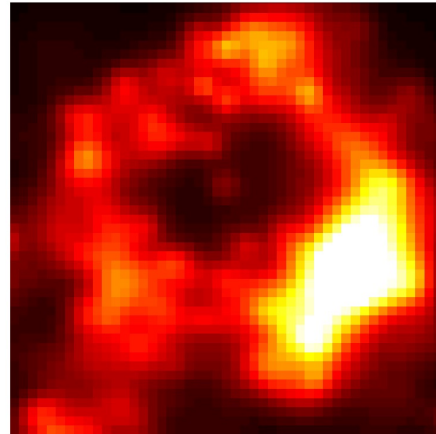
201707149\_glass,4: Element61



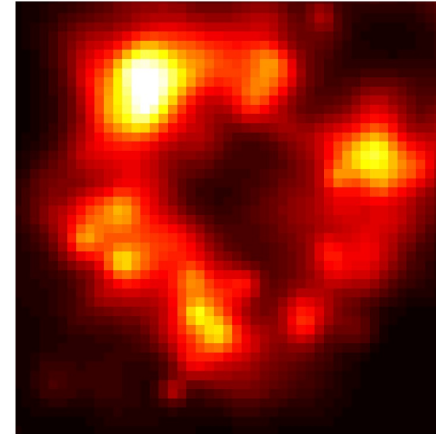
201707149\_glass,4: Element62



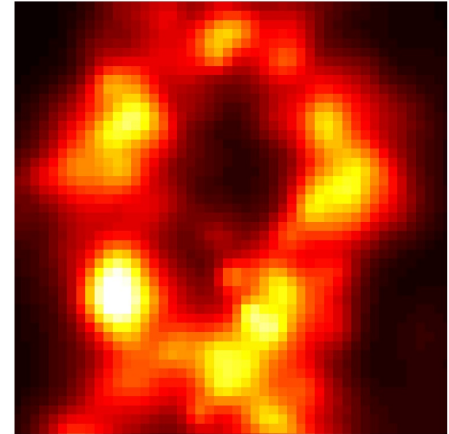
201707149\_glass,4: Element63



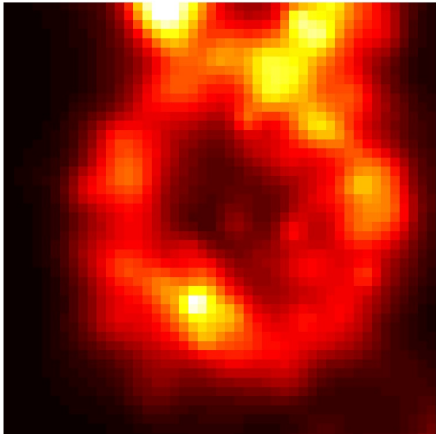
201707149\_glass,4: Element64



201707149\_glass,4: Element65

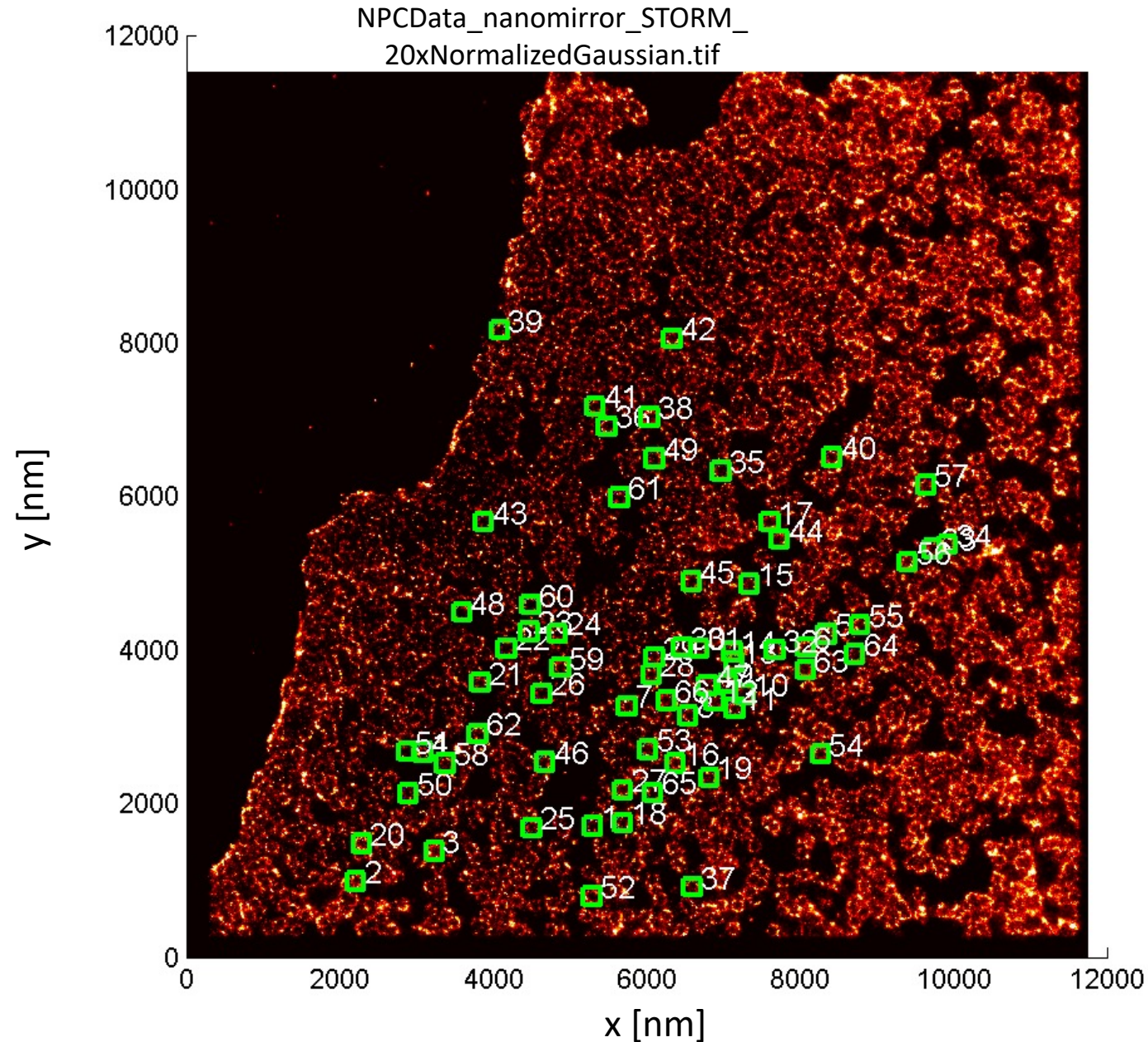


201707149\_glass,4: Element66





# Selection of single NPC ROIs (ROI size: 240x240 nm<sup>2</sup>)



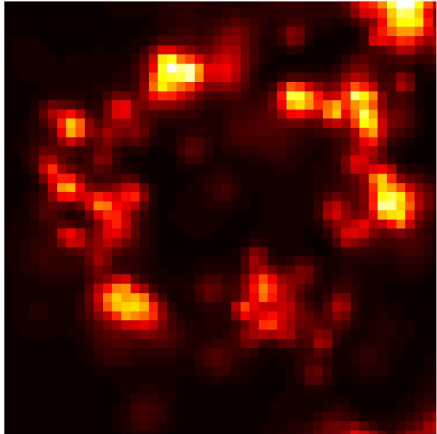
Selection 2

1	11	21	31	41	51	61
2	12	22	32	42	52	62
3	13	23	33	43	53	63
4	14	24	34	44	54	64
5	15	25	35	45	55	65
6	16	26	36	46	56	66
7	17	27	37	47	57	
8	18	28	38	48	58	
9	19	29	39	49	59	
10	20	30	40	50	60	

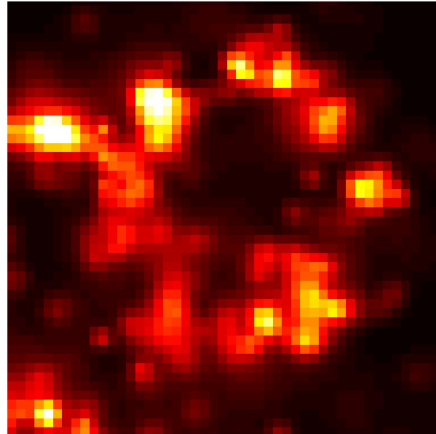
# Single NPCs, STORM on nanomirror, 240x240 nm<sup>2</sup> ROIs 1/7

20x normalized Gaussian reconstruction -> 5.1 nm pixelsize

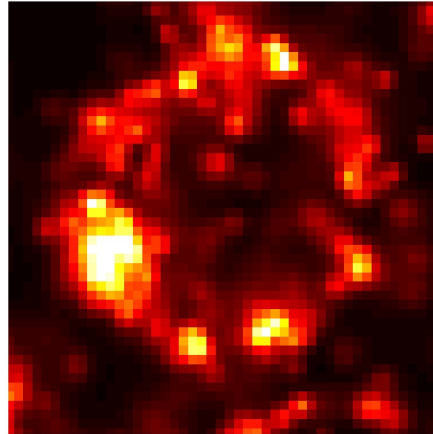
20170718\_sub1\_3: Element1



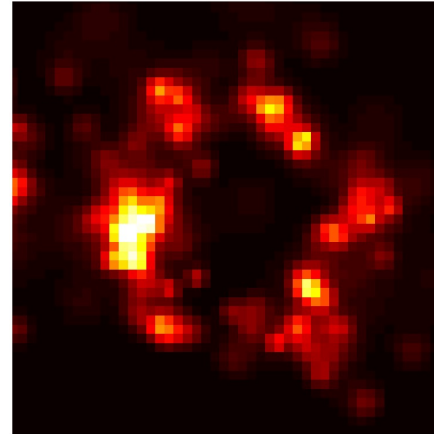
20170718\_sub1\_3: Element2



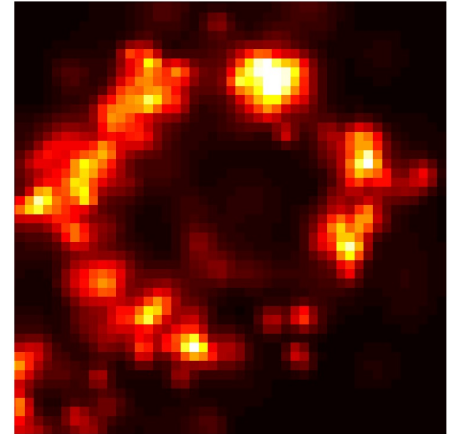
20170718\_sub1\_3: Element3



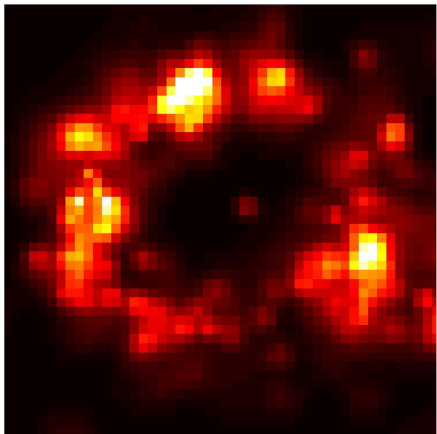
20170718\_sub1\_3: Element4



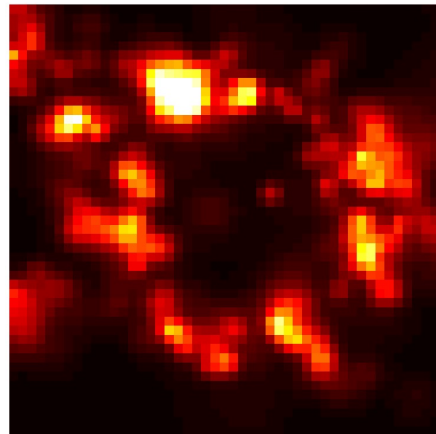
20170718\_sub1\_3: Element5



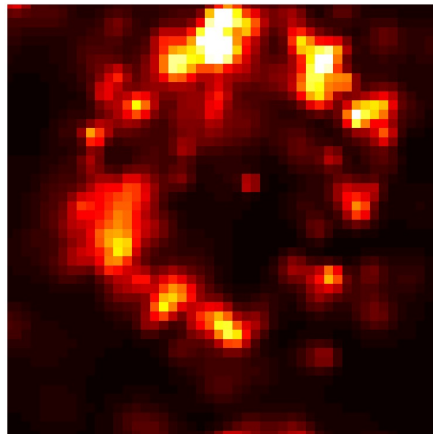
20170718\_sub1\_3: Element6



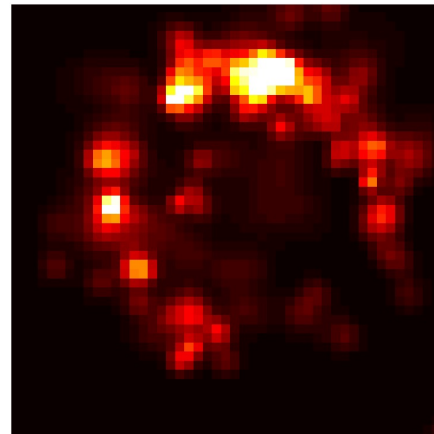
20170718\_sub1\_3: Element7



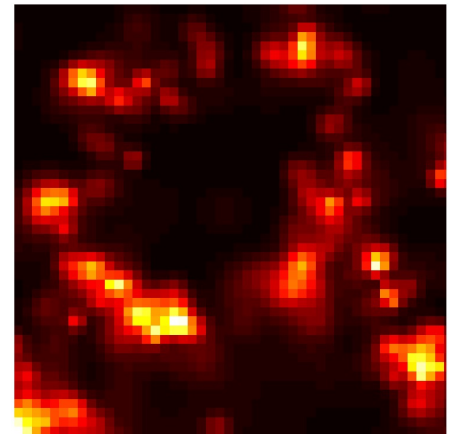
20170718\_sub1\_3: Element8



20170718\_sub1\_3: Element9



20170718\_sub1\_3: Element10

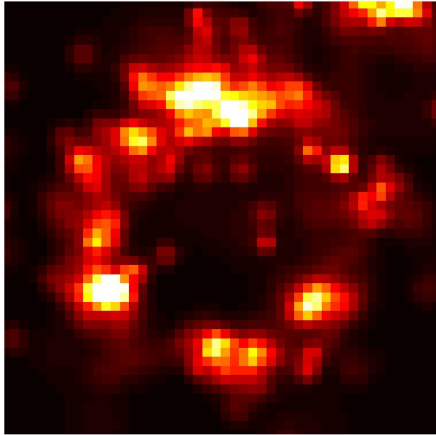




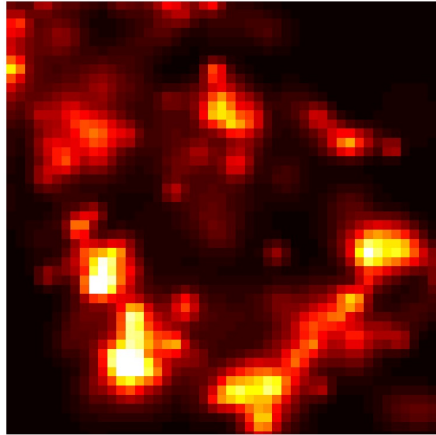
# Single NPCs, STORM on nanomirror, 240x240 nm<sup>2</sup> ROIs 2/7

20x normalized Gaussian reconstruction -> 5.1 nm pixelsize

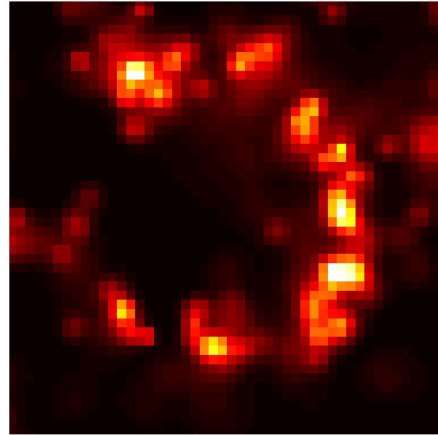
20170718<sub>g</sub>ub1<sub>3</sub>: Element11



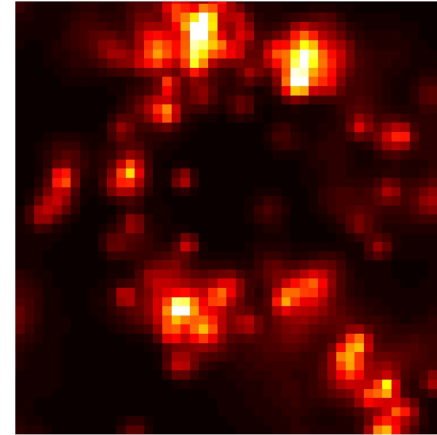
20170718<sub>g</sub>ub1<sub>3</sub>: Element12



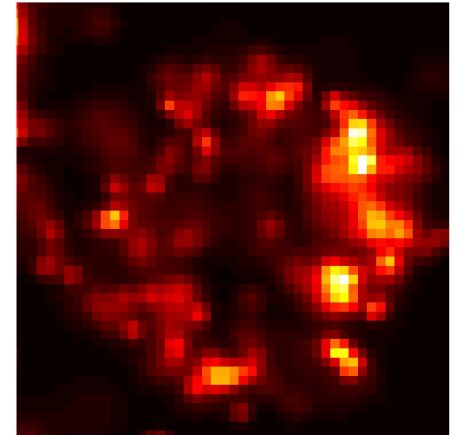
20170718<sub>g</sub>ub1<sub>3</sub>: Element13



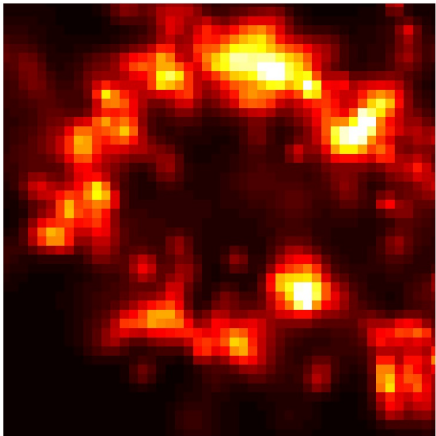
20170718<sub>g</sub>ub1<sub>3</sub>: Element14



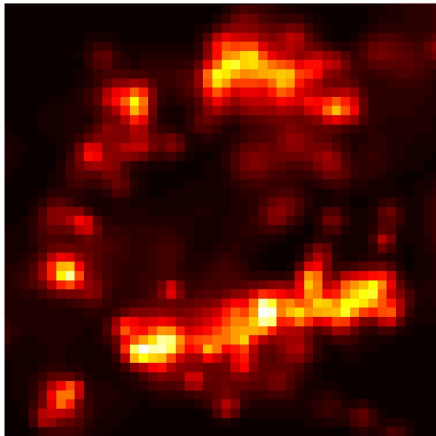
20170718<sub>g</sub>ub1<sub>3</sub>: Element15



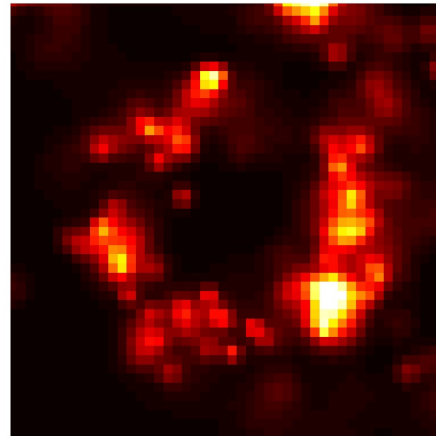
20170718<sub>g</sub>ub1<sub>3</sub>: Element16



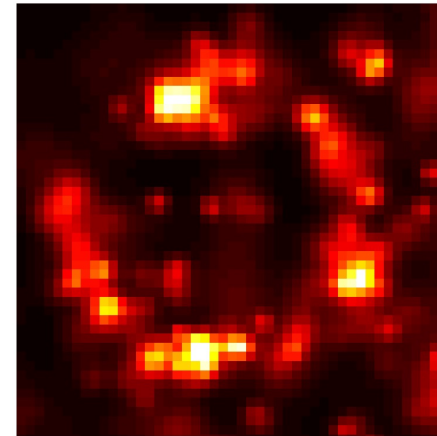
20170718<sub>g</sub>ub1<sub>3</sub>: Element17



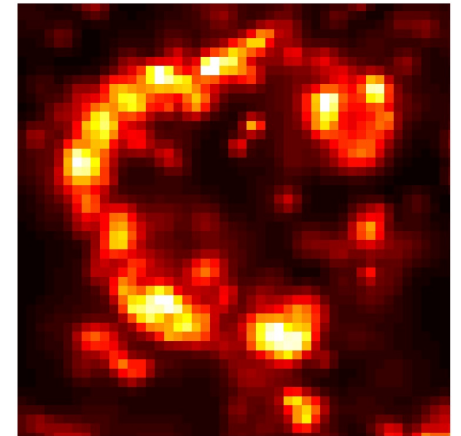
20170718<sub>g</sub>ub1<sub>3</sub>: Element18



20170718<sub>g</sub>ub1<sub>3</sub>: Element19



20170718<sub>g</sub>ub1<sub>3</sub>: Element20

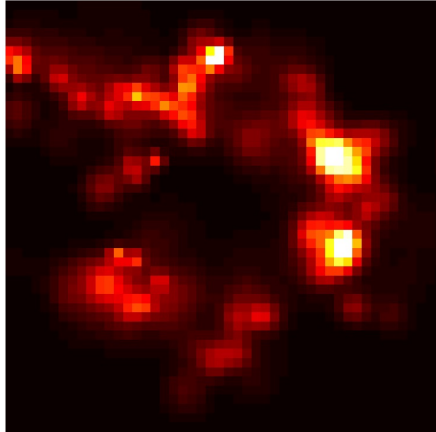




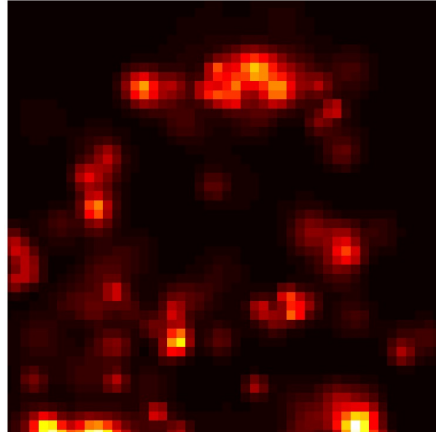
# Single NPCs, STORM on nanomirror, 240x240 nm<sup>2</sup> ROIs 3/7

20x normalized Gaussian reconstruction -> 5.1 nm pixelsize

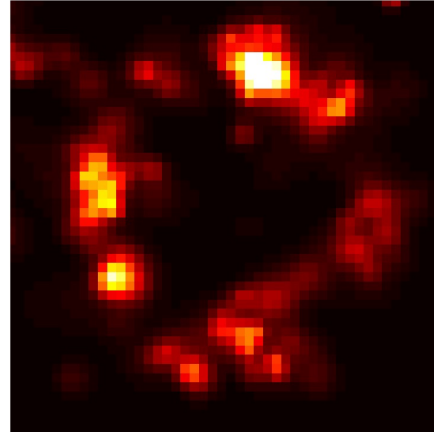
20170718<sub>g</sub>ub1<sub>3</sub>: Element21



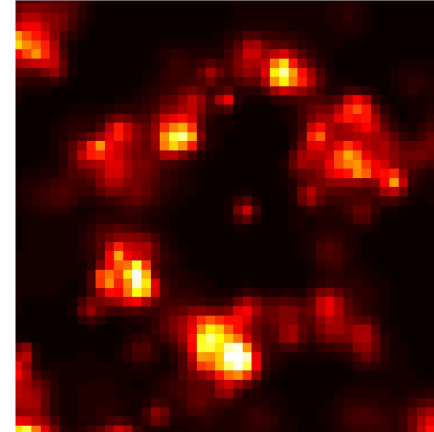
20170718<sub>g</sub>ub1<sub>3</sub>: Element22



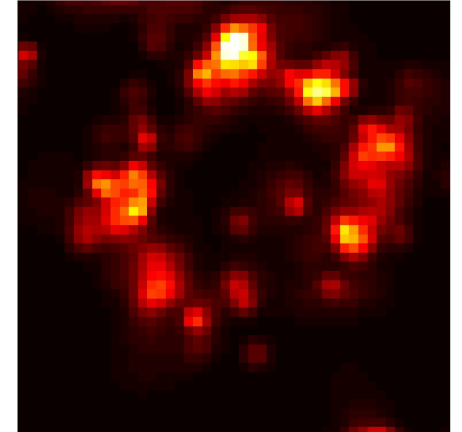
20170718<sub>g</sub>ub1<sub>3</sub>: Element23



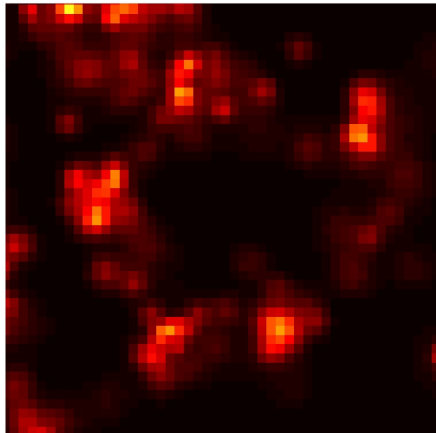
20170718<sub>g</sub>ub1<sub>3</sub>: Element24



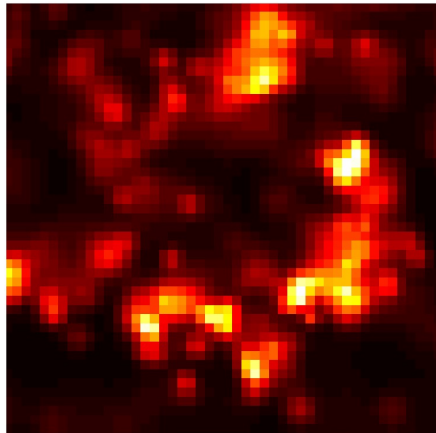
20170718<sub>g</sub>ub1<sub>3</sub>: Element25



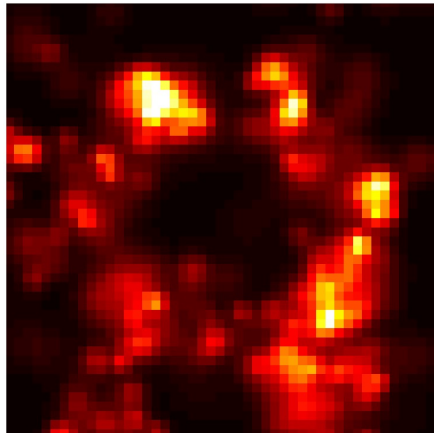
20170718<sub>g</sub>ub1<sub>3</sub>: Element26



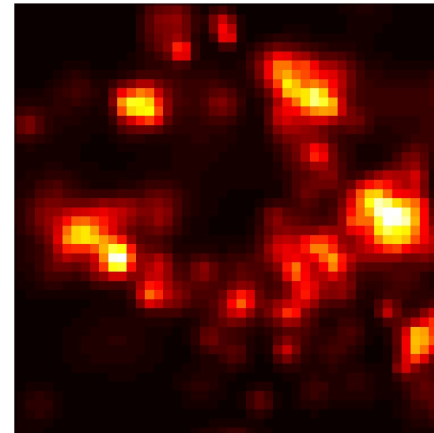
20170718<sub>g</sub>ub1<sub>3</sub>: Element27



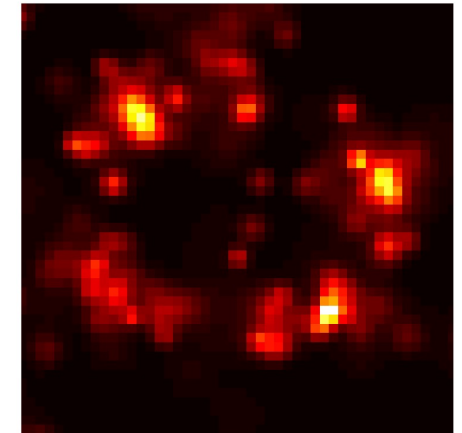
20170718<sub>g</sub>ub1<sub>3</sub>: Element28



20170718<sub>g</sub>ub1<sub>3</sub>: Element29



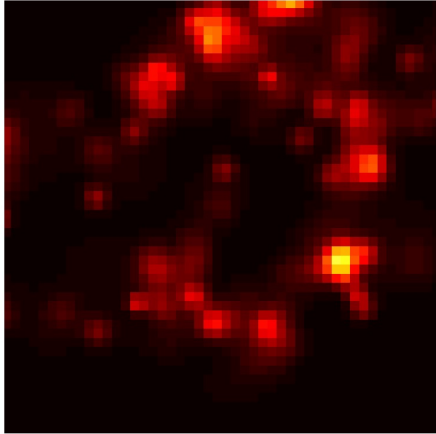
20170718<sub>g</sub>ub1<sub>3</sub>: Element30



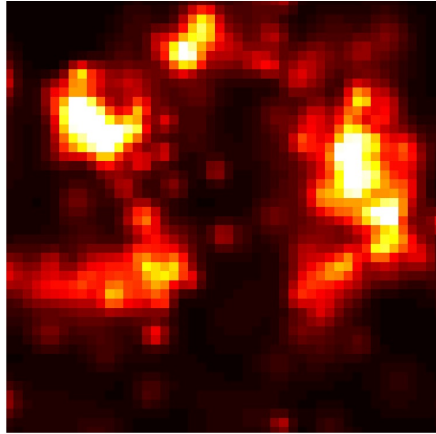
# Single NPCs, STORM on nanomirror, 240x240 nm<sup>2</sup> ROIs 4/7

20x normalized Gaussian reconstruction -> 5.1 nm pixelsize

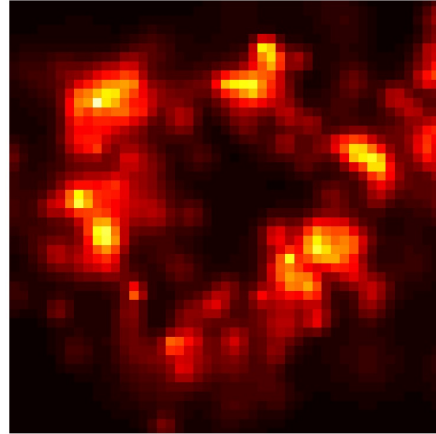
20170718<sub>sub</sub>1<sub>3</sub>: Element31



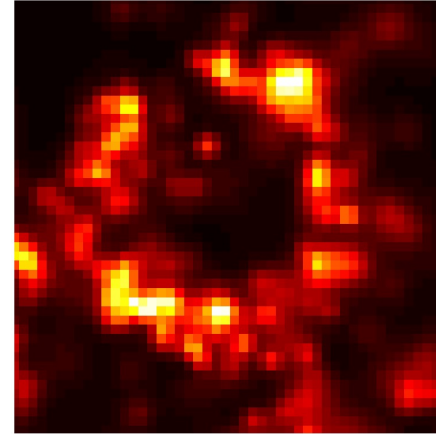
20170718<sub>sub</sub>1<sub>3</sub>: Element32



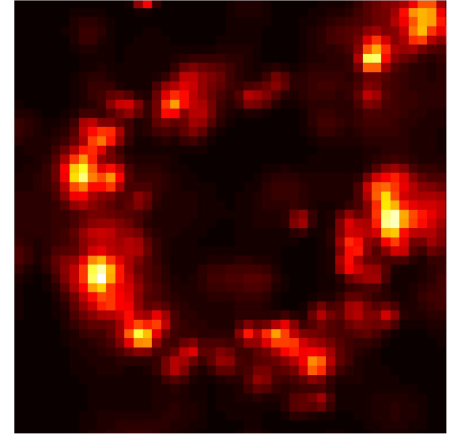
20170718<sub>sub</sub>1<sub>3</sub>: Element33



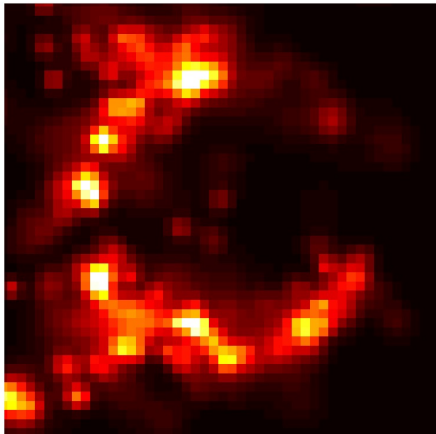
20170718<sub>sub</sub>1<sub>3</sub>: Element34



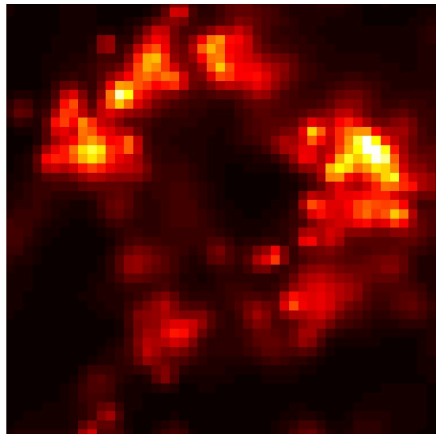
20170718<sub>sub</sub>1<sub>3</sub>: Element35



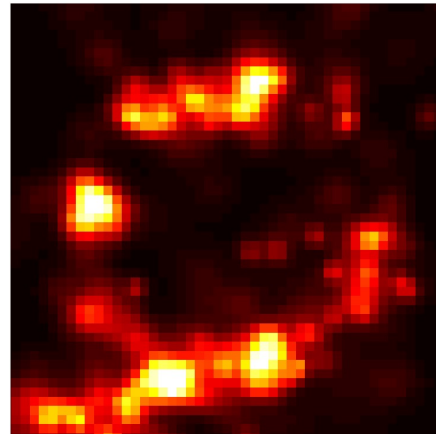
20170718<sub>sub</sub>1<sub>3</sub>: Element36



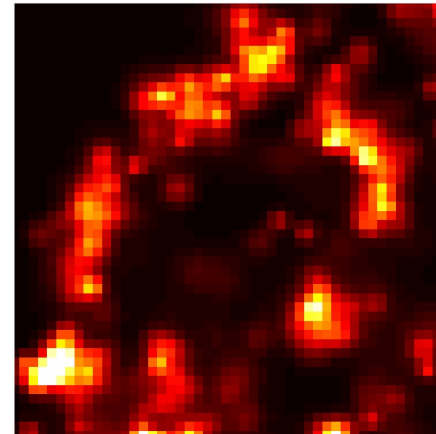
20170718<sub>sub</sub>1<sub>3</sub>: Element37



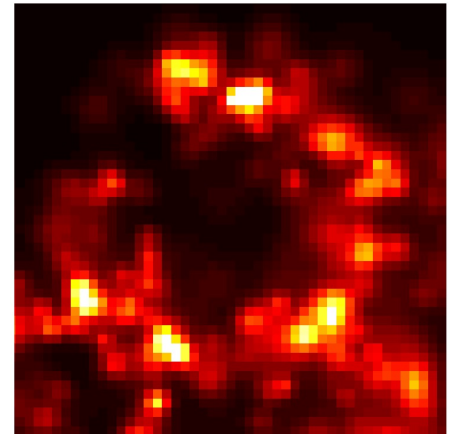
20170718<sub>sub</sub>1<sub>3</sub>: Element38



20170718<sub>sub</sub>1<sub>3</sub>: Element39



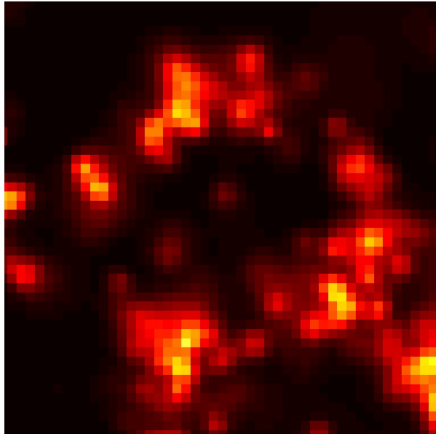
20170718<sub>sub</sub>1<sub>3</sub>: Element40



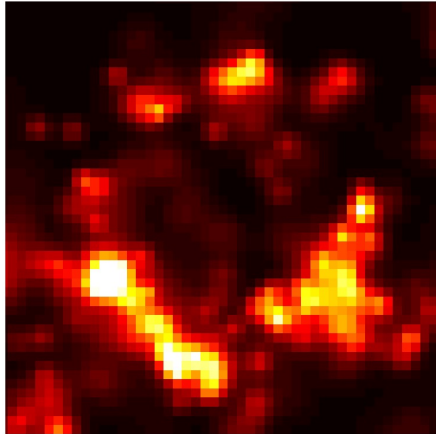
# Single NPCs, STORM on nanomirror, 240x240 nm<sup>2</sup> ROIs 5/7

20x normalized Gaussian reconstruction -> 5.1 nm pixelsize

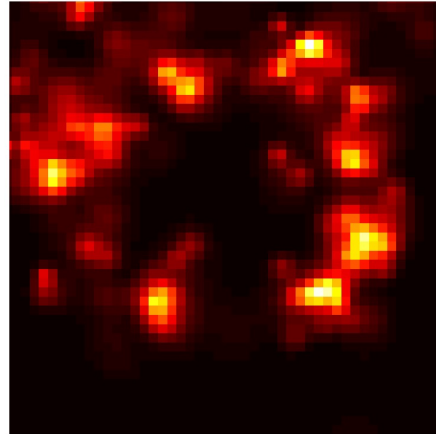
20170718<sub>g</sub>ub1<sub>3</sub>: Element41



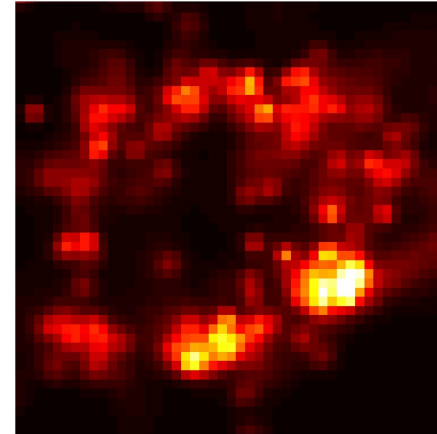
20170718<sub>g</sub>ub1<sub>3</sub>: Element42



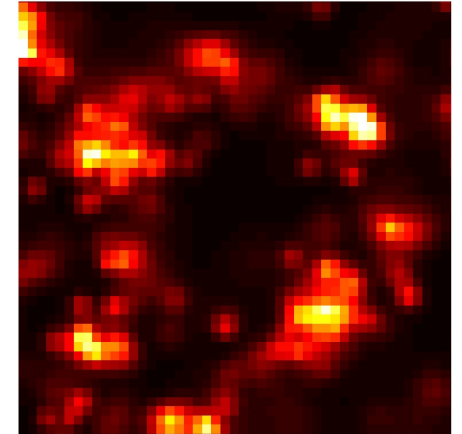
20170718<sub>g</sub>ub1<sub>3</sub>: Element43



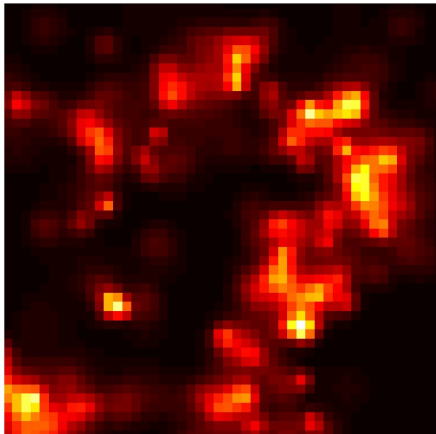
20170718<sub>g</sub>ub1<sub>3</sub>: Element44



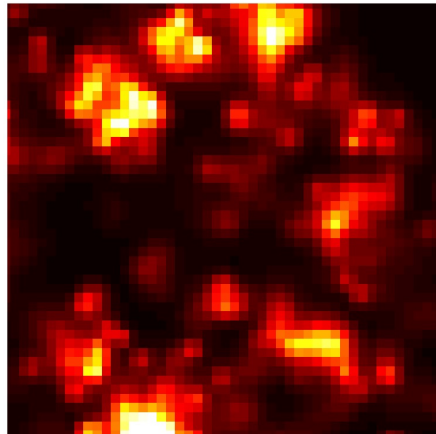
20170718<sub>g</sub>ub1<sub>3</sub>: Element45



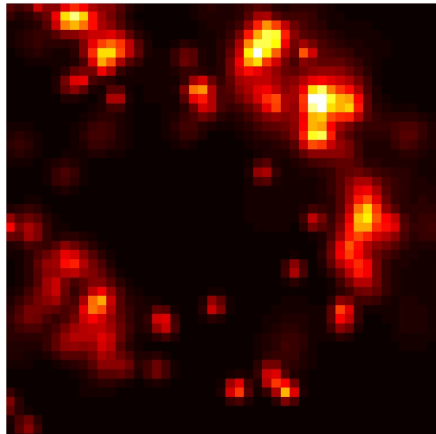
20170718<sub>g</sub>ub1<sub>3</sub>: Element46



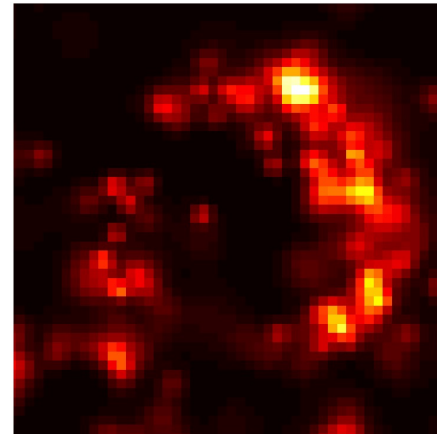
20170718<sub>g</sub>ub1<sub>3</sub>: Element47



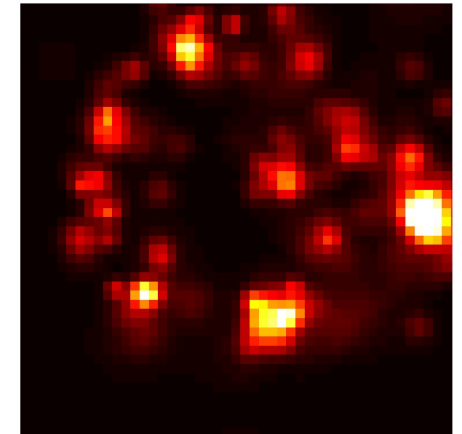
20170718<sub>g</sub>ub1<sub>3</sub>: Element48



20170718<sub>g</sub>ub1<sub>3</sub>: Element49



20170718<sub>g</sub>ub1<sub>3</sub>: Element50

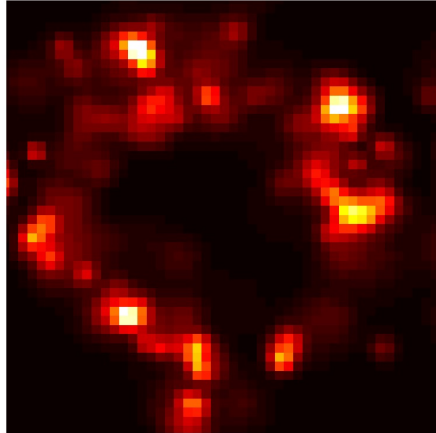




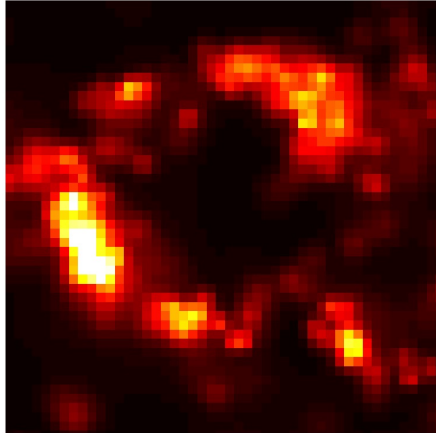
# Single NPCs, STORM on nanomirror, 240x240 nm<sup>2</sup> ROIs 6/7

20x normalized Gaussian reconstruction -> 5.1 nm pixelsize

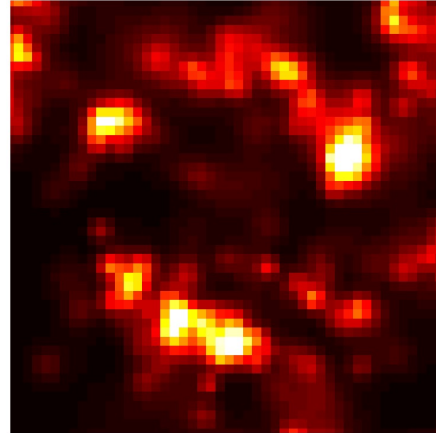
20170718<sub>g</sub>ub1<sub>3</sub>: Element51



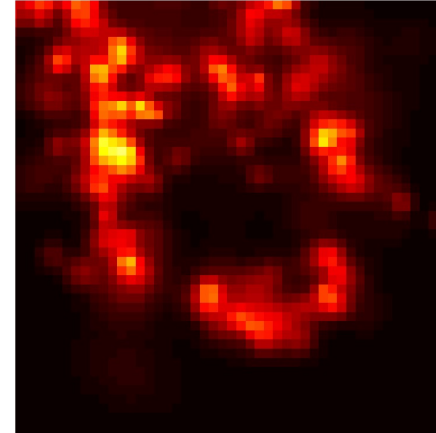
20170718<sub>g</sub>ub1<sub>3</sub>: Element52



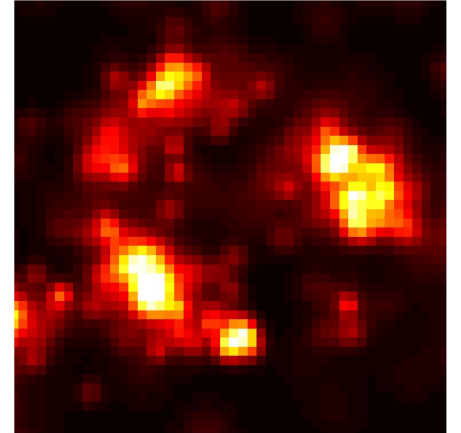
20170718<sub>g</sub>ub1<sub>3</sub>: Element53



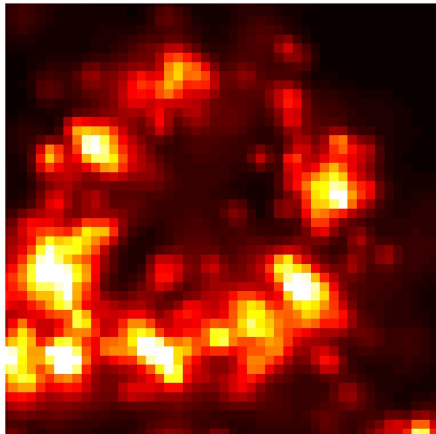
20170718<sub>g</sub>ub1<sub>3</sub>: Element54



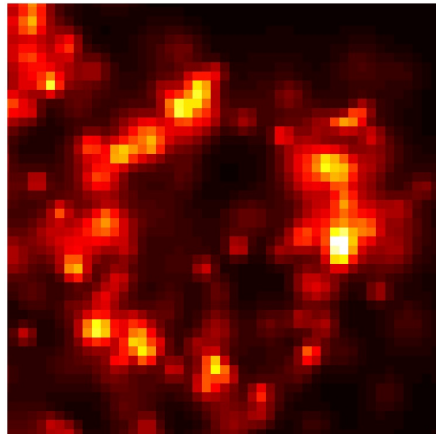
20170718<sub>g</sub>ub1<sub>3</sub>: Element55



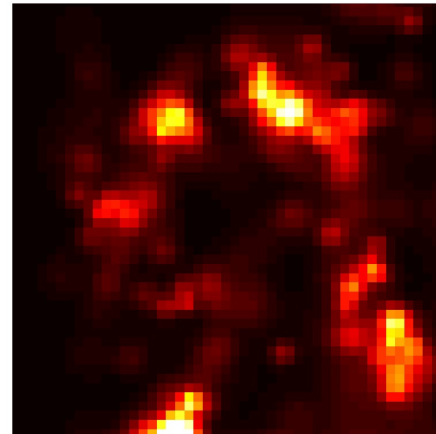
20170718<sub>g</sub>ub1<sub>3</sub>: Element56



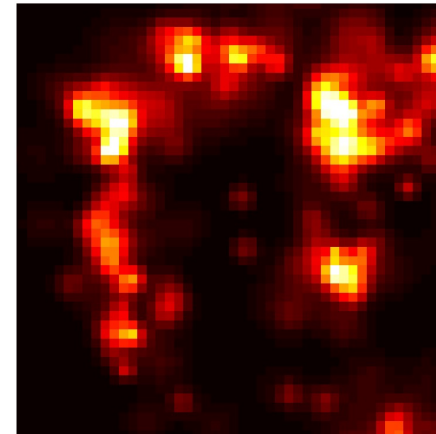
20170718<sub>g</sub>ub1<sub>3</sub>: Element57



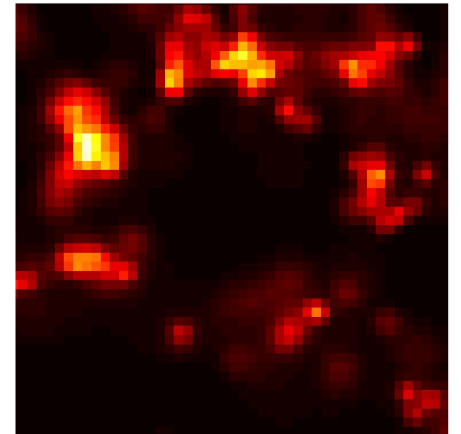
20170718<sub>g</sub>ub1<sub>3</sub>: Element58



20170718<sub>g</sub>ub1<sub>3</sub>: Element59



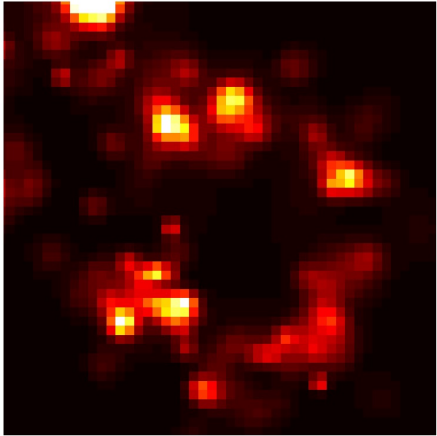
20170718<sub>g</sub>ub1<sub>3</sub>: Element60



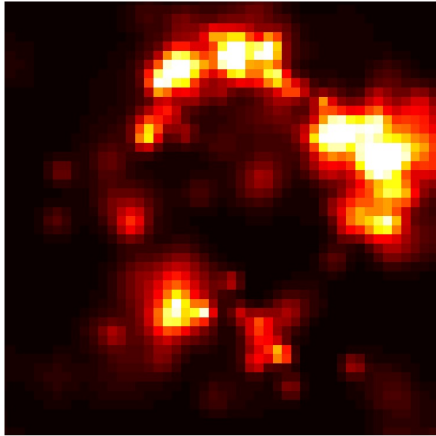
# Single NPCs, STORM on nanomirror, 240x240 nm<sup>2</sup> ROIs 7/7

20x normalized Gaussian reconstruction -> 5.1 nm pixelsize

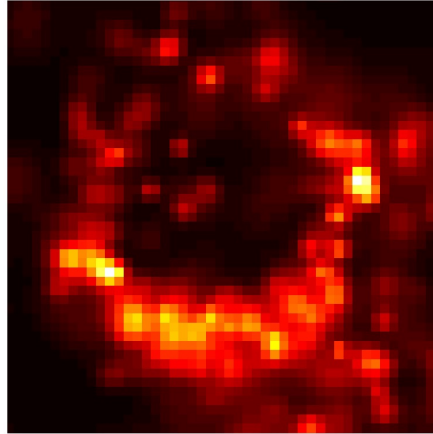
20170718\_gub1\_3: Element61



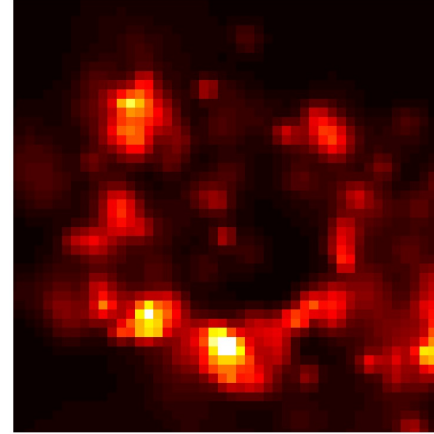
20170718\_gub1\_3: Element62



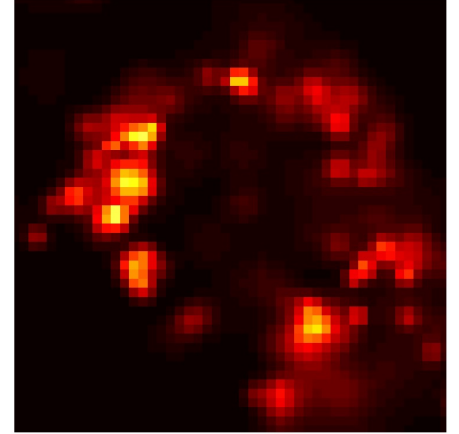
20170718\_gub1\_3: Element63



20170718\_gub1\_3: Element64



20170718\_gub1\_3: Element65



20170718\_gub1\_3: Element66

