

Very Fast Acquisition of Tilt series in Environmental TEM Tomography: Tips and Tricks

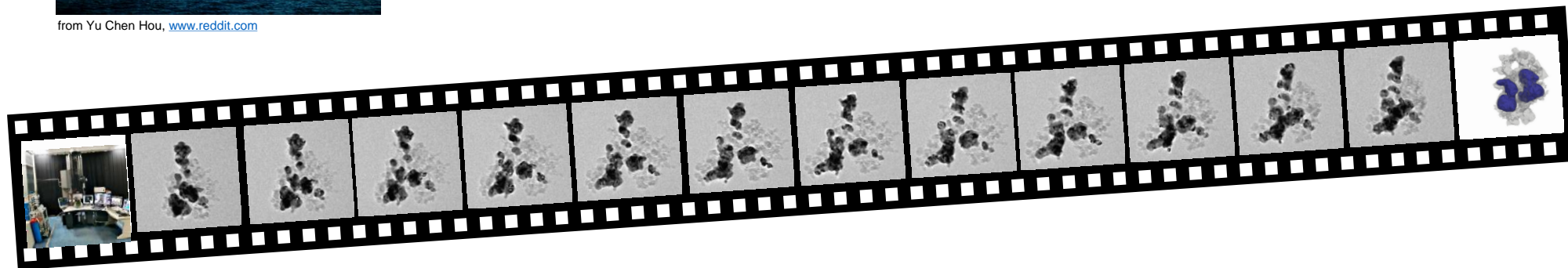
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Voichita MAXIM², Siddardha KONETI¹, Lucian ROIBAN¹



from Yu Chen Hou, www.reddit.com

¹ Univ. Lyon, INSA-Lyon, Université C. Bernard Lyon 1,
MATEIS, umr 5510 CNRS, Villeurbanne, France.

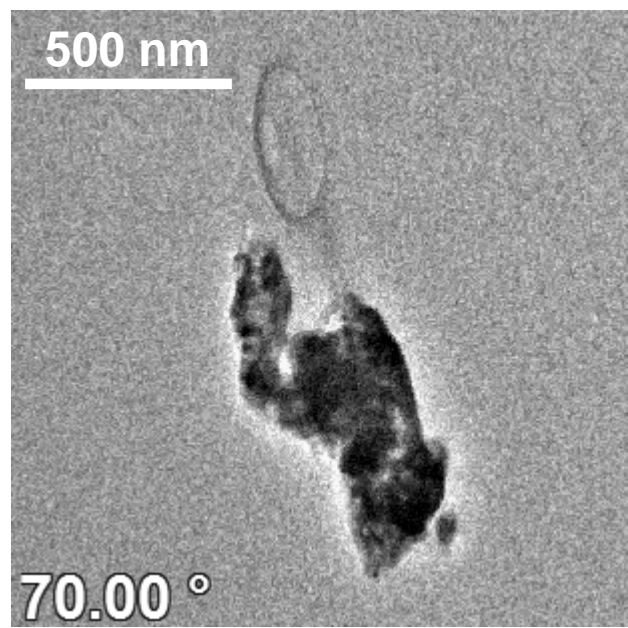
² Univ. Lyon, INSA-Lyon, Université C. Bernard Lyon 1,
CREATIS, umr 5220 CNRS, INSERM U1206, Villeurbanne, F.



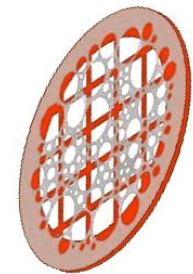
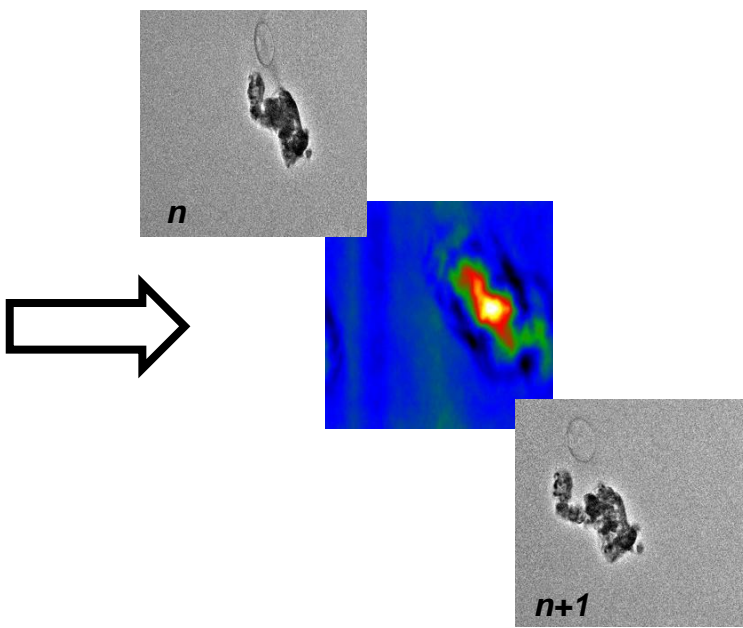
Acquiring 2D projections series for 'tilt' Electron Tomography (ET) in TEM



'step-by-step' series of 2D *BF* images
 $\approx \pm 70^\circ$ angular range



Tilt n to $n+1$:
 re-alignment (re-focussing)



classical *optimistic* step-by-step 'tilt' Bright Field* tomography

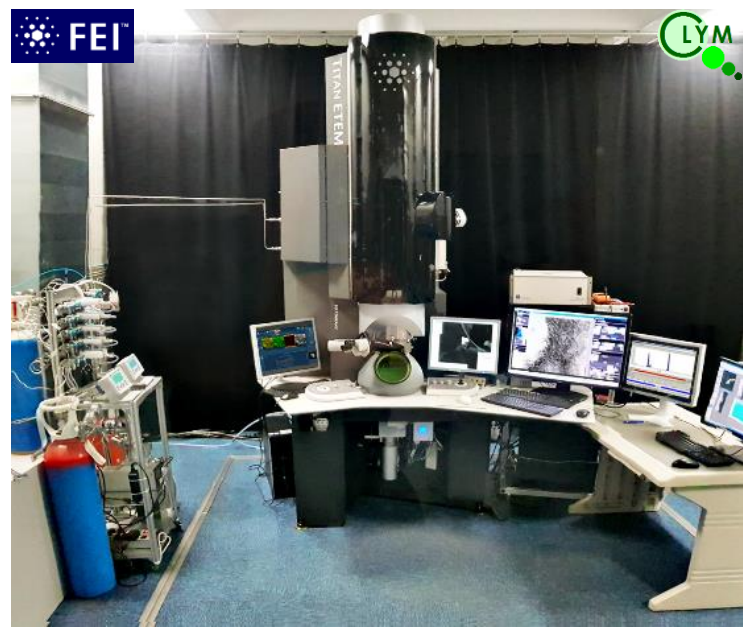
Rotation angular amplitude	140°
Angular step increment	2°
Re-alignment at each tilt	15 sec
Exposure time of each image*	0.1 sec
Time to proceed to the next tilt	0.1 sec
Total acquisition time	≈ 18 min

Adapted from S. KONETI et al., *Mater. Characterisation*, 151 (2019), 495

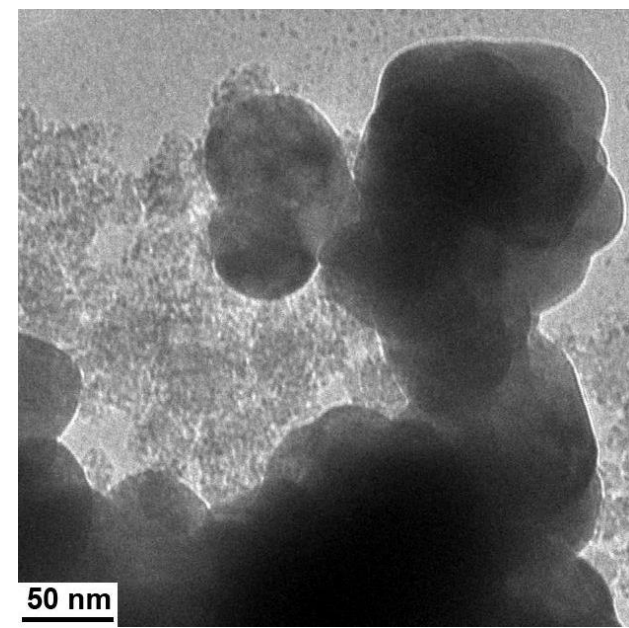
*STEM:
 typically > 5 sec at least for 2K² images



- Under gas and temperature in ETEM, morphologies change rapidly
- The longer the experiment, the higher the electron dose (irradiation damages)

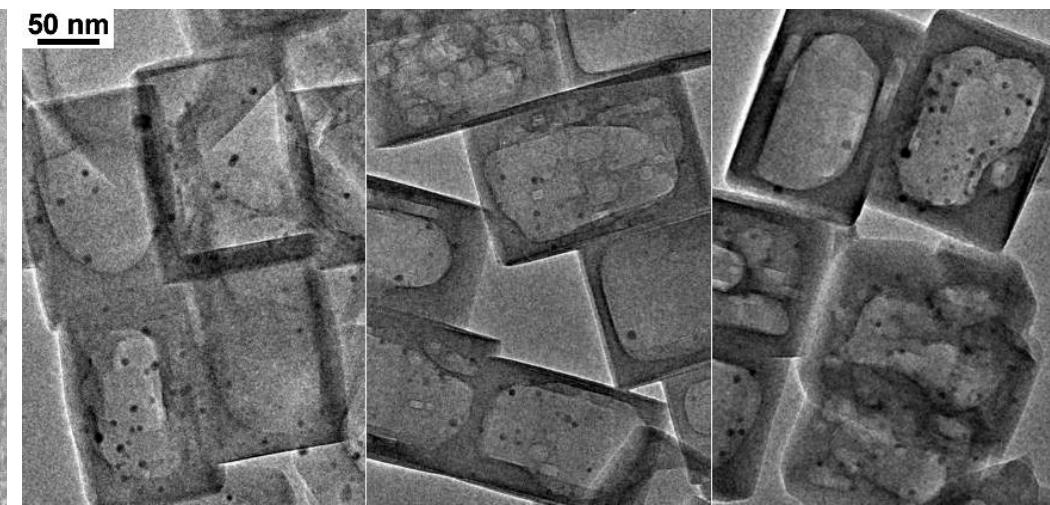


Gaseous environmental TEM
Cs-corrected Titan ETEM 80-300 kV



Soot combustion on YSZ-based catalysts
ETEM 80 kV, 500°C 3mbar O₂
Total time 2 min (speed x8)

A. SERVE et al., *Applied Catal. A* **504** (2015), 74



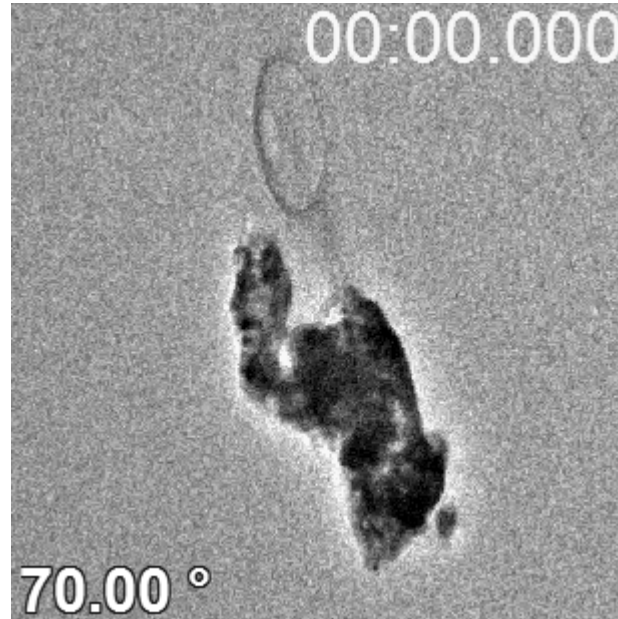
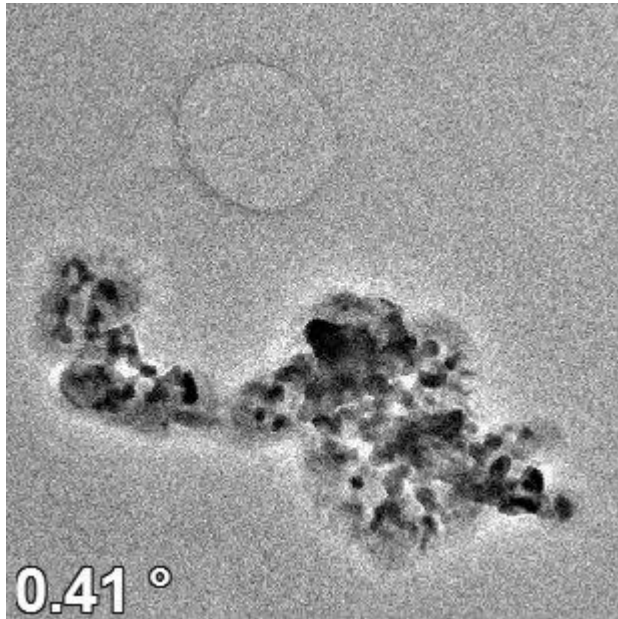
Silicalite with Ag NPs: 20°C, irradiation test 4 min
Decrease of the electron dose

8.0 10³ e⁻.Å⁻²s⁻¹ 3.2 10³ e⁻.Å⁻²s⁻¹ 2.4 10³ e⁻.Å⁻²s⁻¹ →

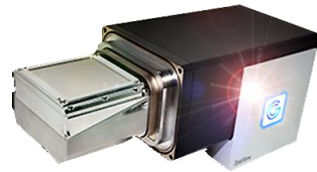
L. ROIBAN et al., *J. of Microscopy*, **269** 2 (2018) 117

'step-by-step' series of
2D *BF* images
 $\approx \pm 70^\circ$ angular range

Continuous tilt: $\pm 70^\circ$ angular
range in **5 sec**
(140° at the fastest speed
of the FEI-TITAN goniometer)



≈ 18 min



OneView camera
100 fps in 2kx2k

'tilted' electron tomography by **continuous rotation**

- diffraction** M. GEMMI et al., *J. Appl. Cryst.* **48** (2015) 718
B.L. NANNENGA et al., *Nature Methods* **11** (2014) 927
- BF image** V. MIGUNOV et al., *Sci. Rep.*, **5** 14516 (2015)

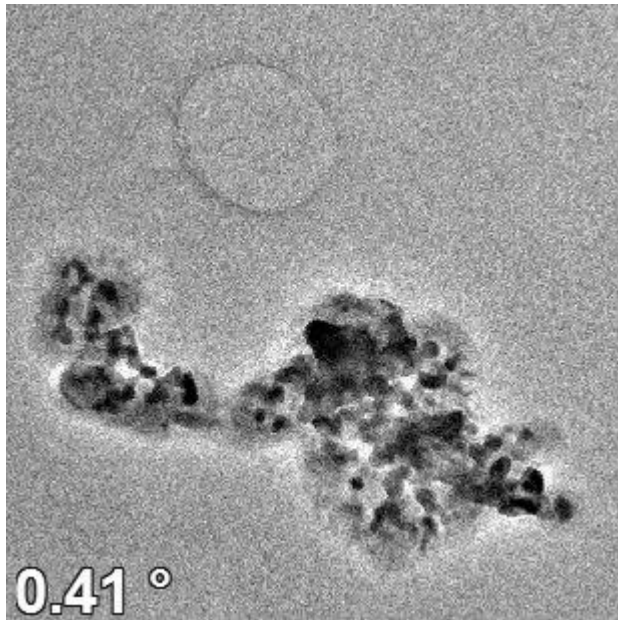
www.esrf.eu (European SRF, Eu)

X-Ray CT



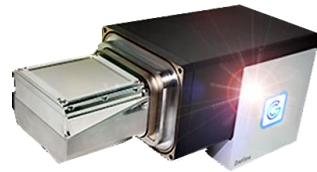
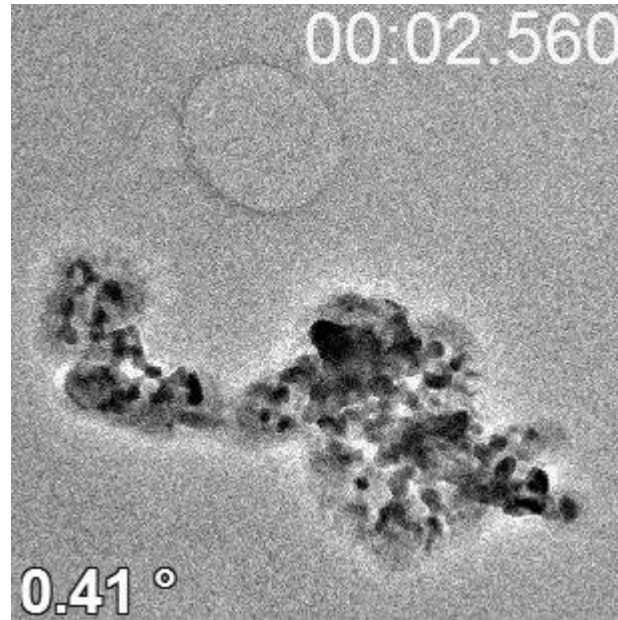
J. VILLANOVA et al., *Mat. Today*
20 7 (2017), 354

'step-by-step' series of
2D *BF* images
 $\approx \pm 70^\circ$ angular range

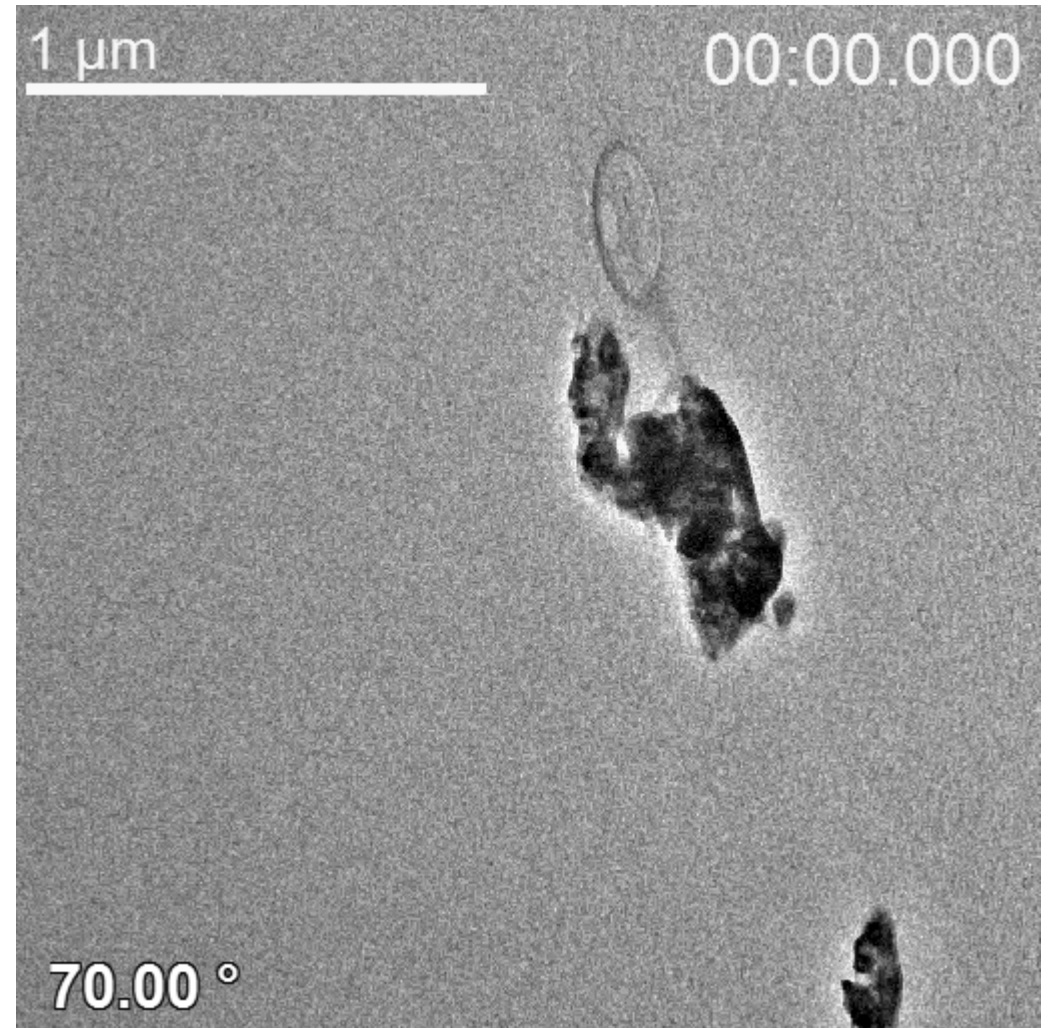


≈ 18 min

Continuous tilt: $\pm 70^\circ$ angular
range in **5 sec**
*(140° at the fastest speed
of the FEI-TITAN goniometer)*



OneView camera
100 fps in 2kx2k
 GATAN



Is it a good idea to take time to adjust properly the eucentric?..

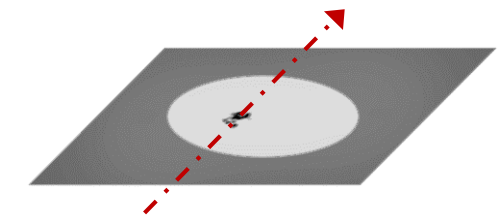
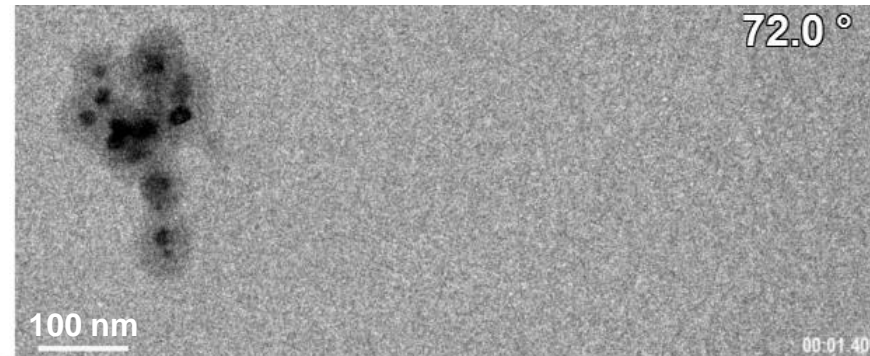
Typical ongoing in situ
(environmental) experiment



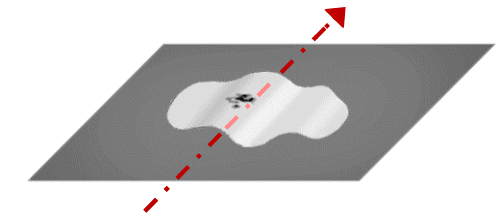
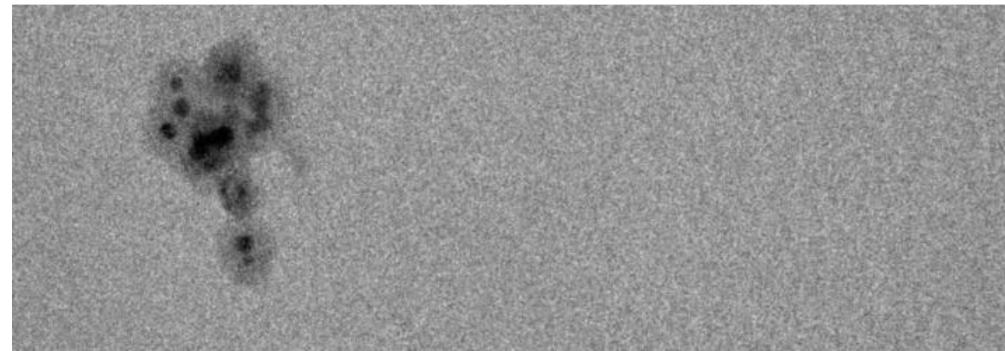
- **Eucentric re-adjustment *frequently* needed during heating experiments**

Pd@SiO₂ on SiN_x membrane

After 35' at 400°C, H₂ 4 mbar



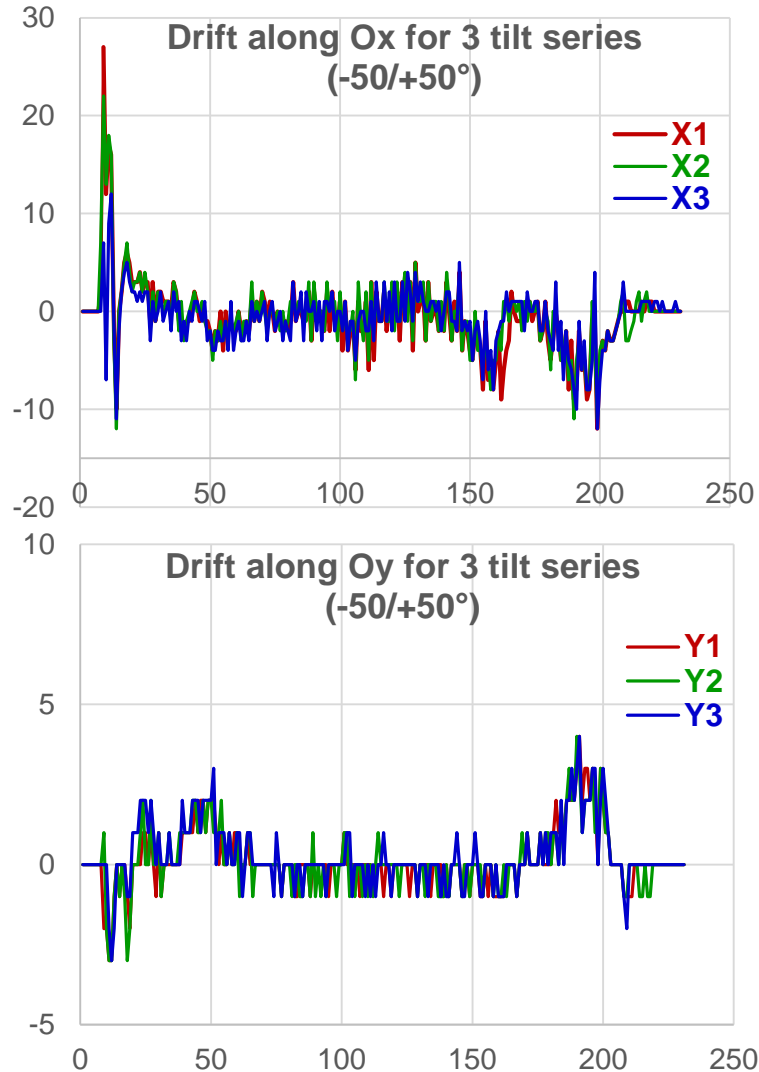
After 60' at 500°C, H₂ 4 mbar



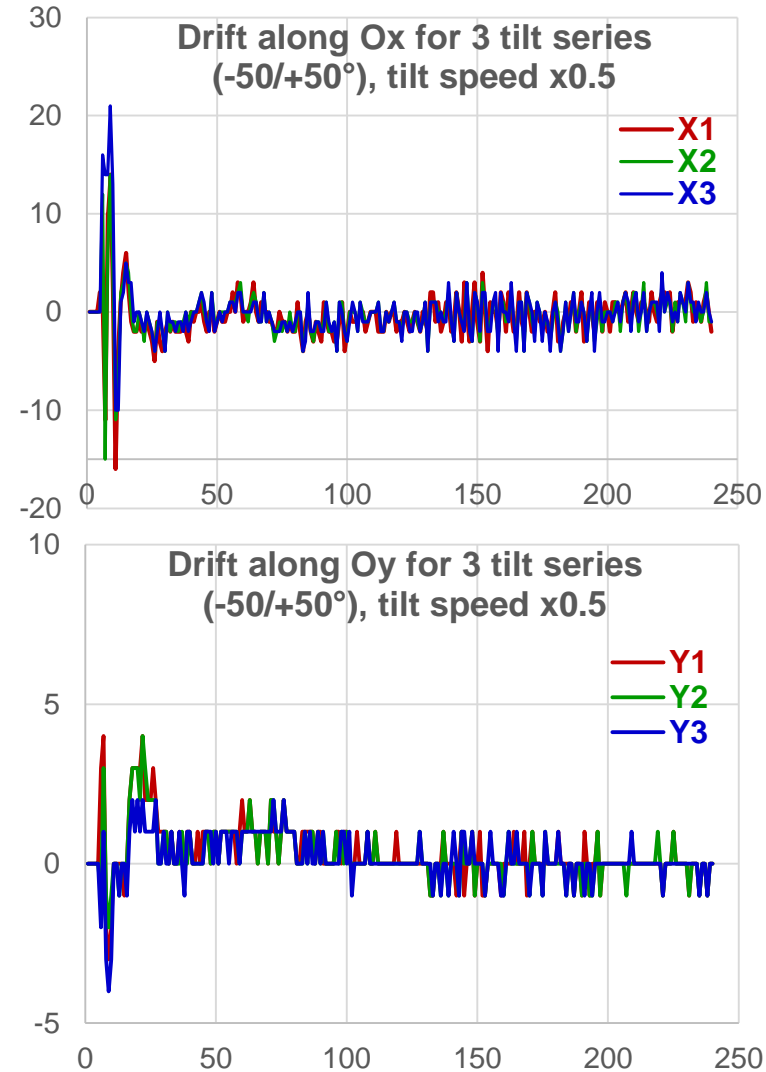
**Deformation of
the membrane**

• Reproducibility of (X,Y) DRIFTS as a function of tilting parameters

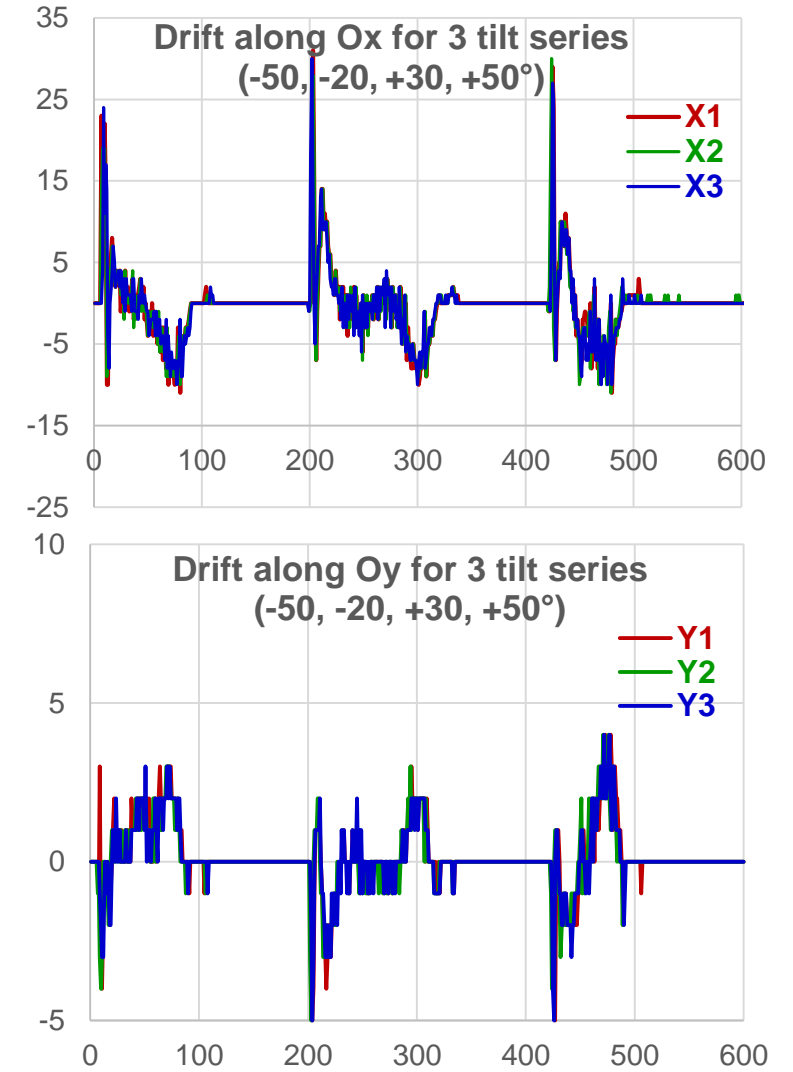
Fastest tilt speed, continuous tilt




50% of Fastest tilt speed, continuous tilt

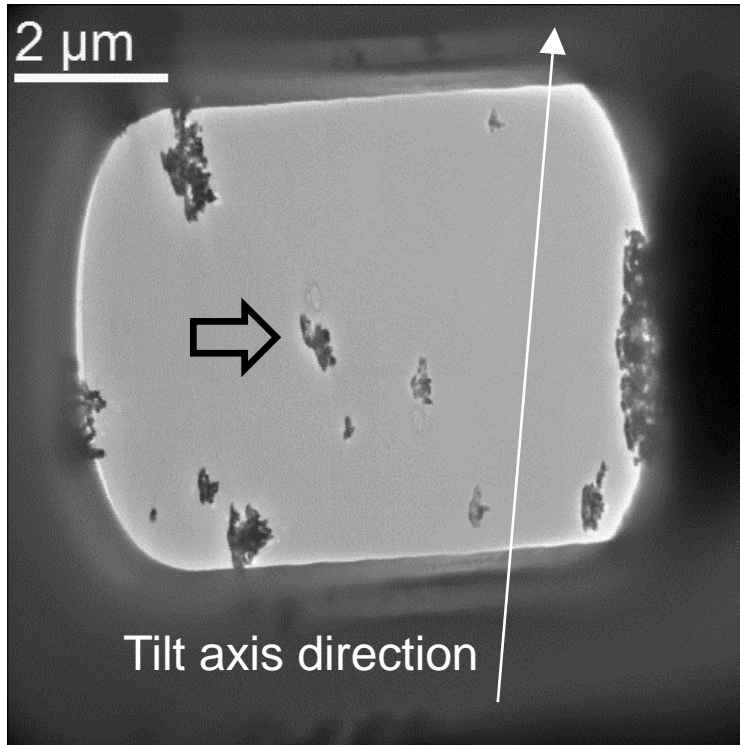


Fastest tilt speed, discontinuous tilt

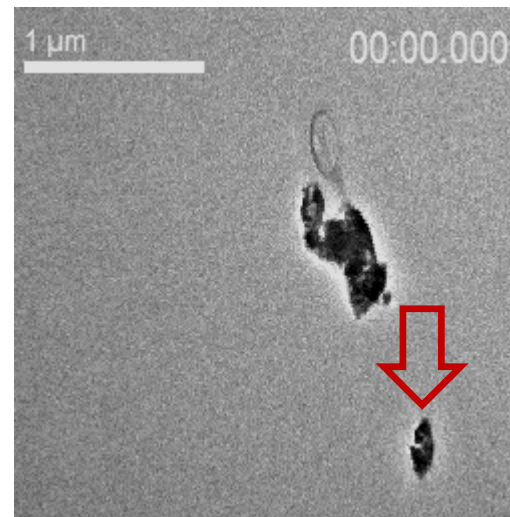


- **Fast measurement of appropriate (δX , δY) corrections**


Nanocatalyst powder, Wildfire single tilt heating holder  DENS solutions, +72 / -72°

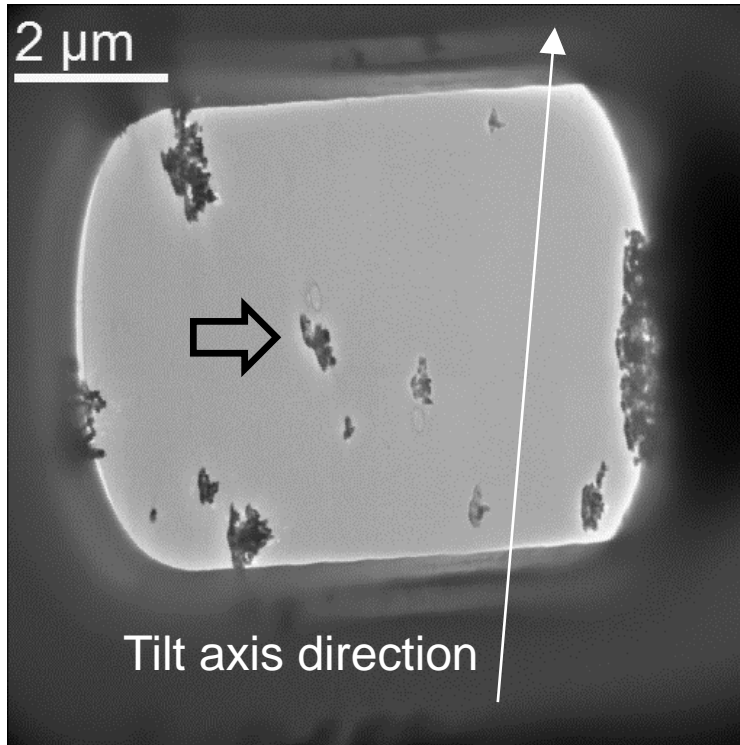


Tilt +70 to -70°
Mag 16K
Image 1Kx1K

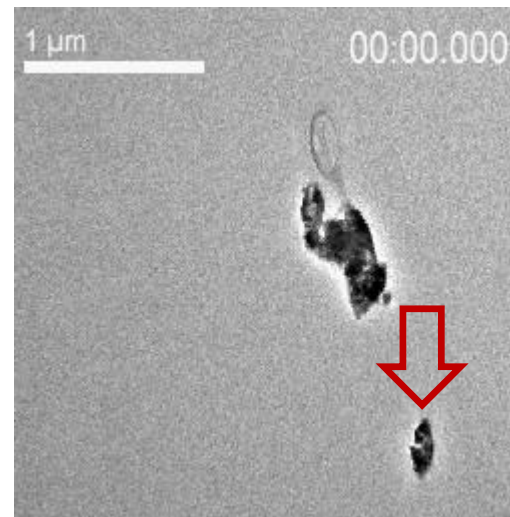


- **Fast measurement of appropriate (δX , δY) corrections**

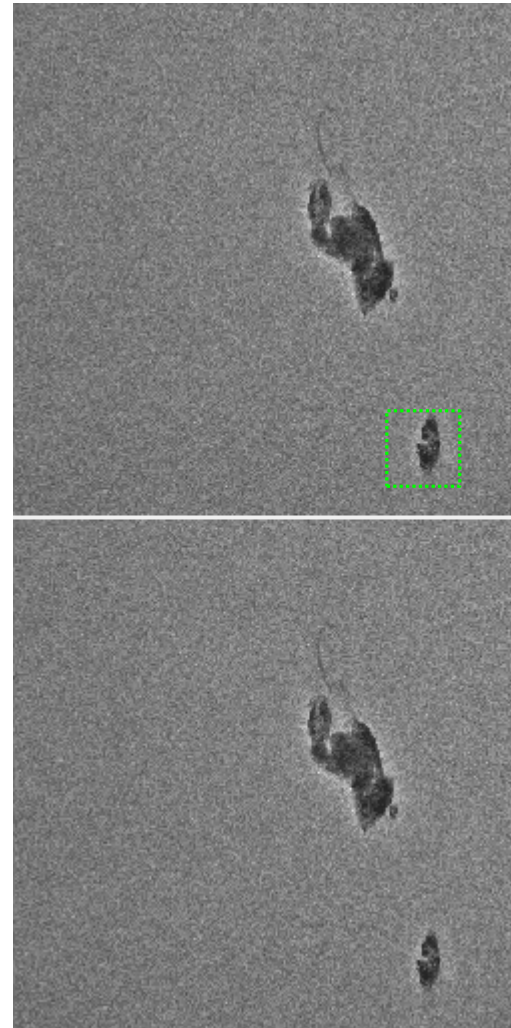
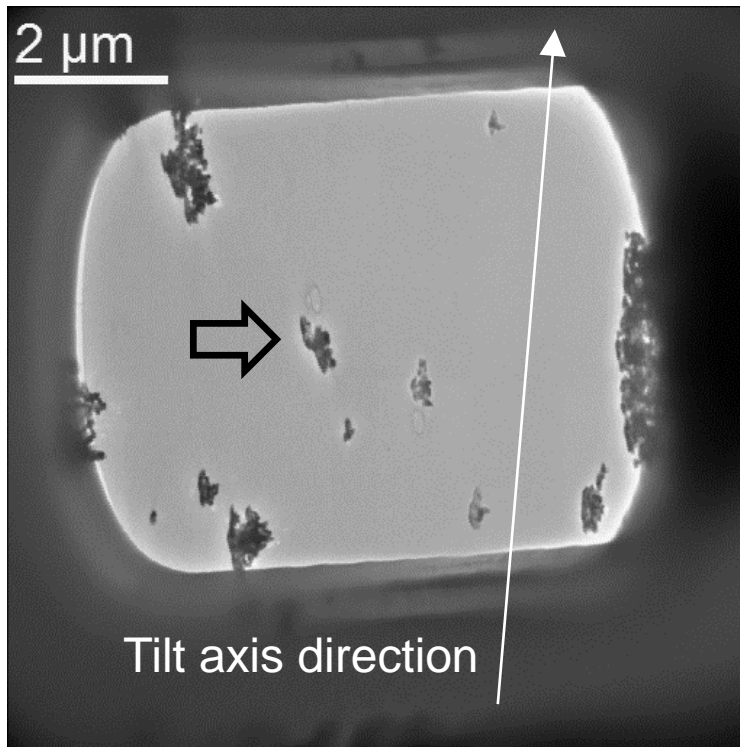
Nanocatalyst powder, Wildfire single tilt heating holder  **DENS solutions**, +72 / -72°



Tilt +70 to -70°
Mag 16K
Image 1Kx1K
100 fps
actual time 5 sec



- Fast measurement of appropriate (δX , δY) corrections



display speed x0.5

Post-mortem
FAST re-centering (at the ETEM)

tracking procedure based on the
Normed Cross-Correlation in Fourier Space
(NCCFS)

J.P. LEWIS, *Vision Interface*, (1995) 120

$$\underset{B}{\operatorname{argmax}} \frac{\sum_{m,n=1}^{M,N} \operatorname{Im}1(m,n) \cdot \operatorname{Im}2(m,n)}{\sqrt{\sum_{m,n=1}^{M,N} \operatorname{Im}2(m,n)^2}}$$

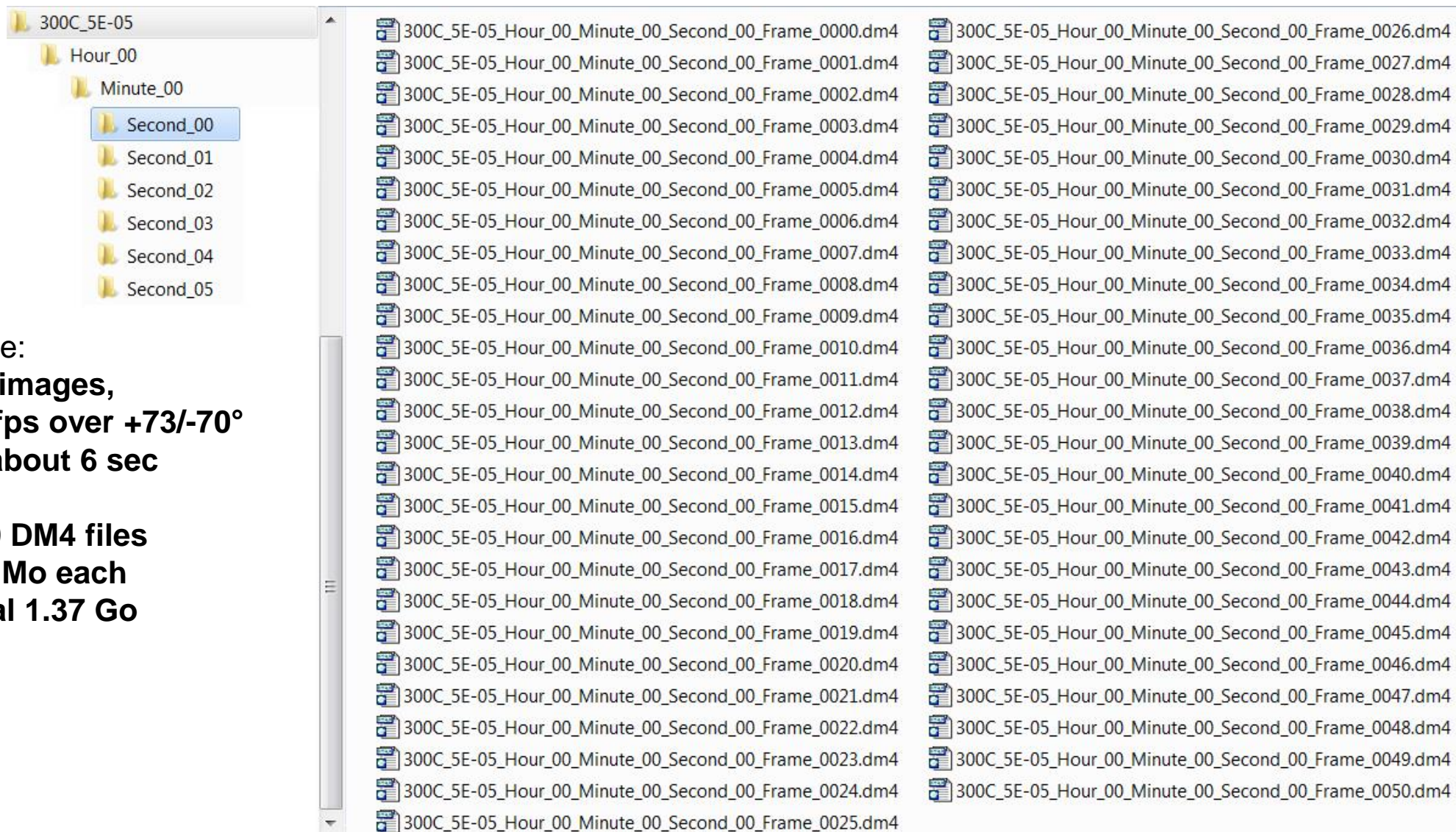


Normed Cross-Correlation in Fourier Space (NCCFS)

J.P. LEWIS, *Vision Interface*, (1995) 120

"The algorithm described in this paper was developed for the movie *Forest Gump* (1994)... Special effects sequences in that movie included ...the addition of a contemporary actor into historical film and video sequences"

- Reading "*GatanDoll*" DM4 'in situ' recorded sequences



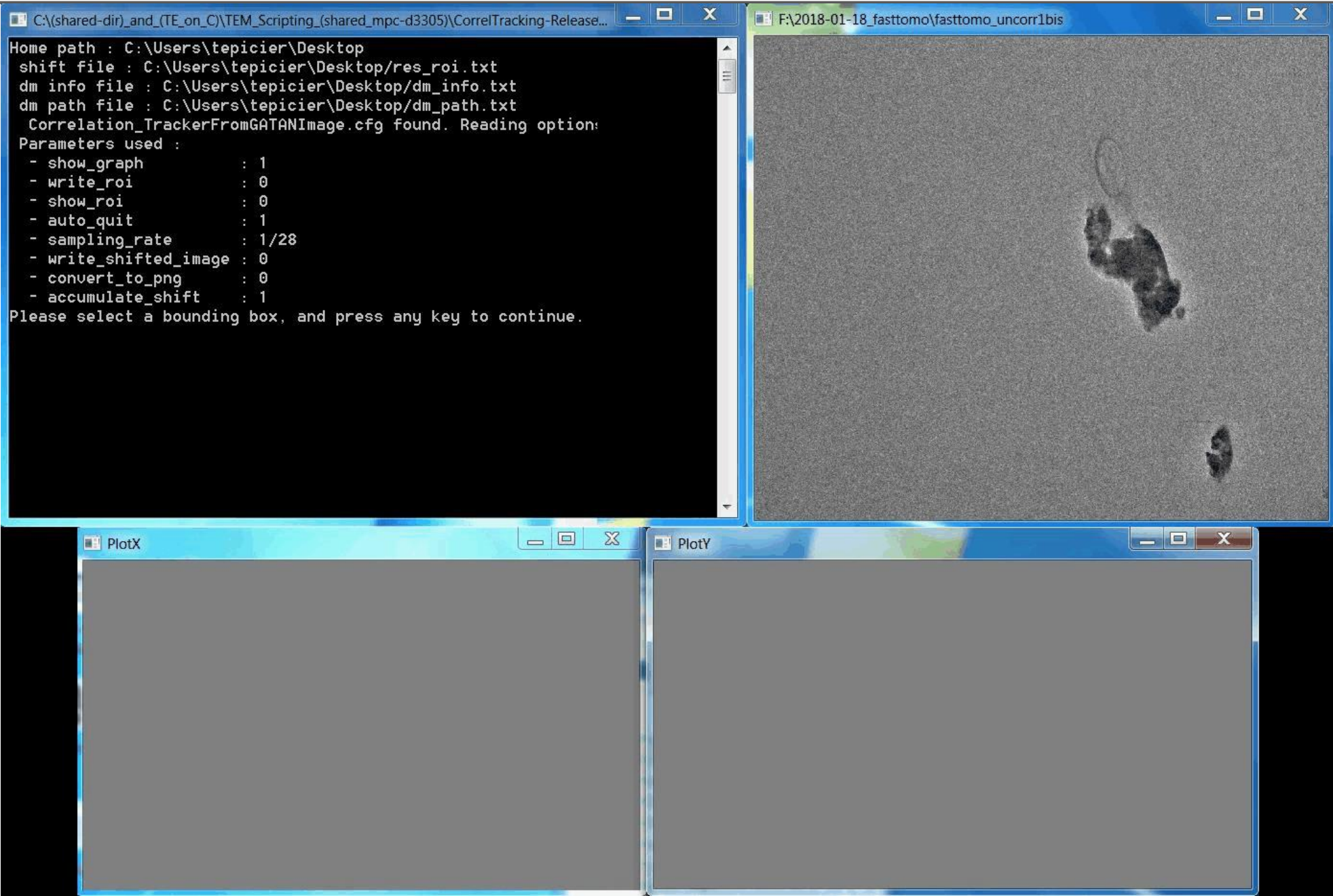
Here:
1K images,
50 fps over +73/-70°
in about 6 sec

300 DM4 files
4.6 Mo each
total 1.37 Go



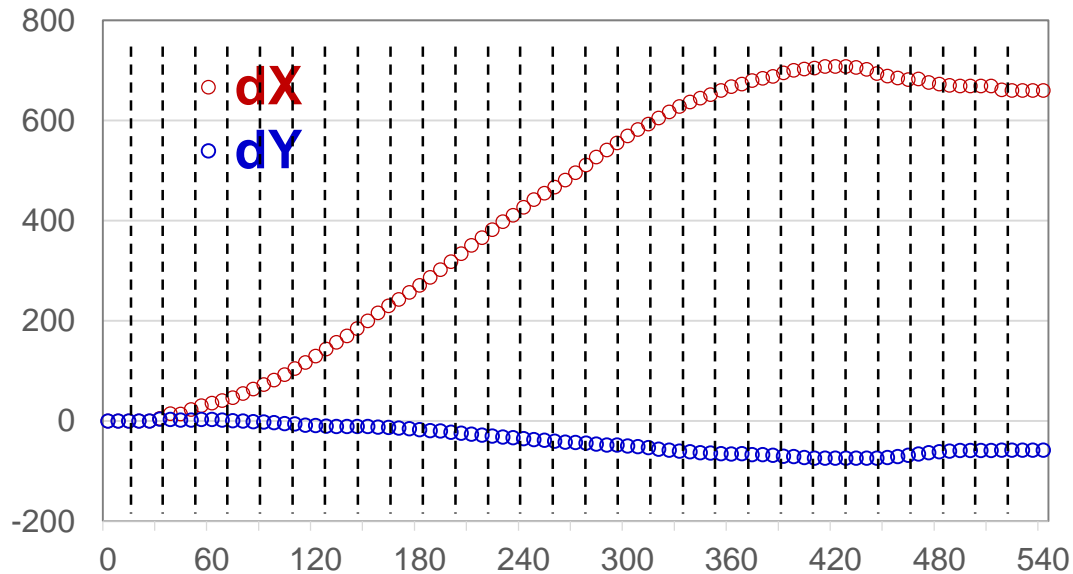
NCCFS-based tracking Procedure

display speed
x 0.3,
real full time
4 s (1Kx1K series)



- Application of an adequate set of correction drifts ($\delta X, \delta Y$)

dX and dY drifts in pixels (frame by frame)

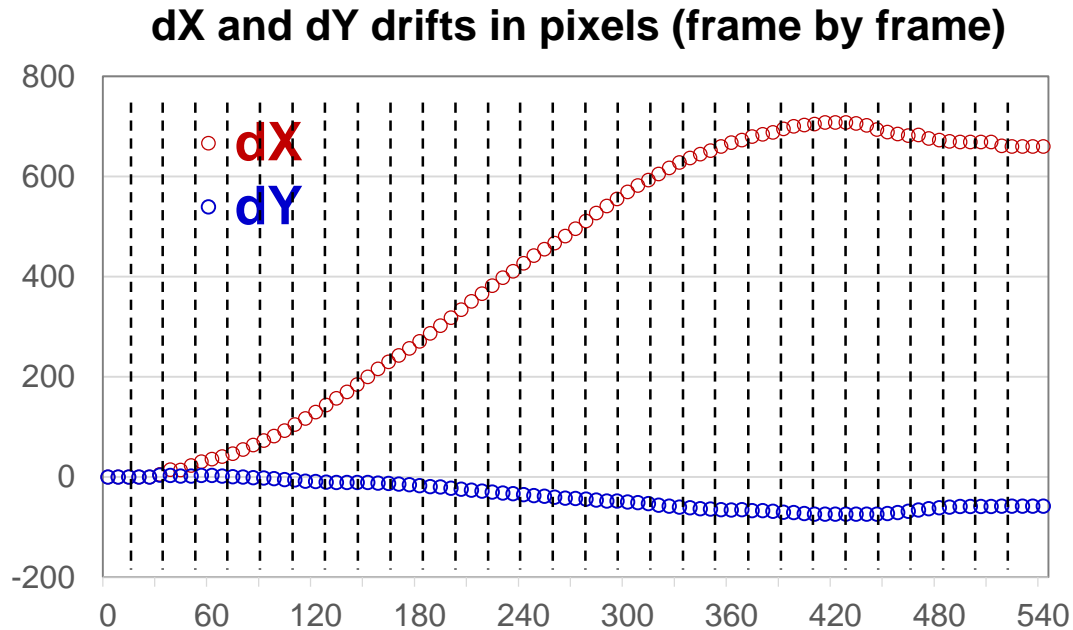


(user's) choice of 28 corrections sent during the tomographic acquisition

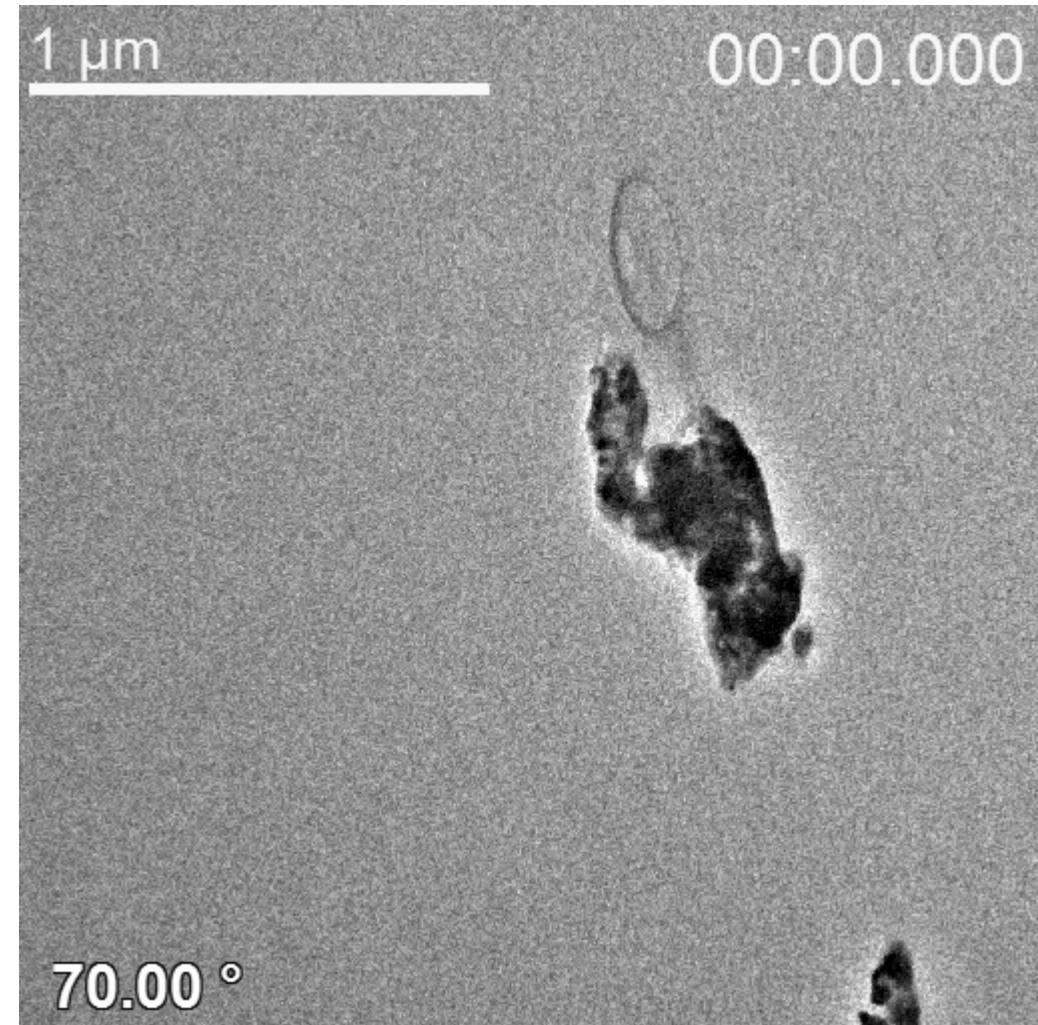
Home-made software suites
(using TEM Scripting © FEI)



- Application of an adequate set of correction drifts ($\delta X, \delta Y$)

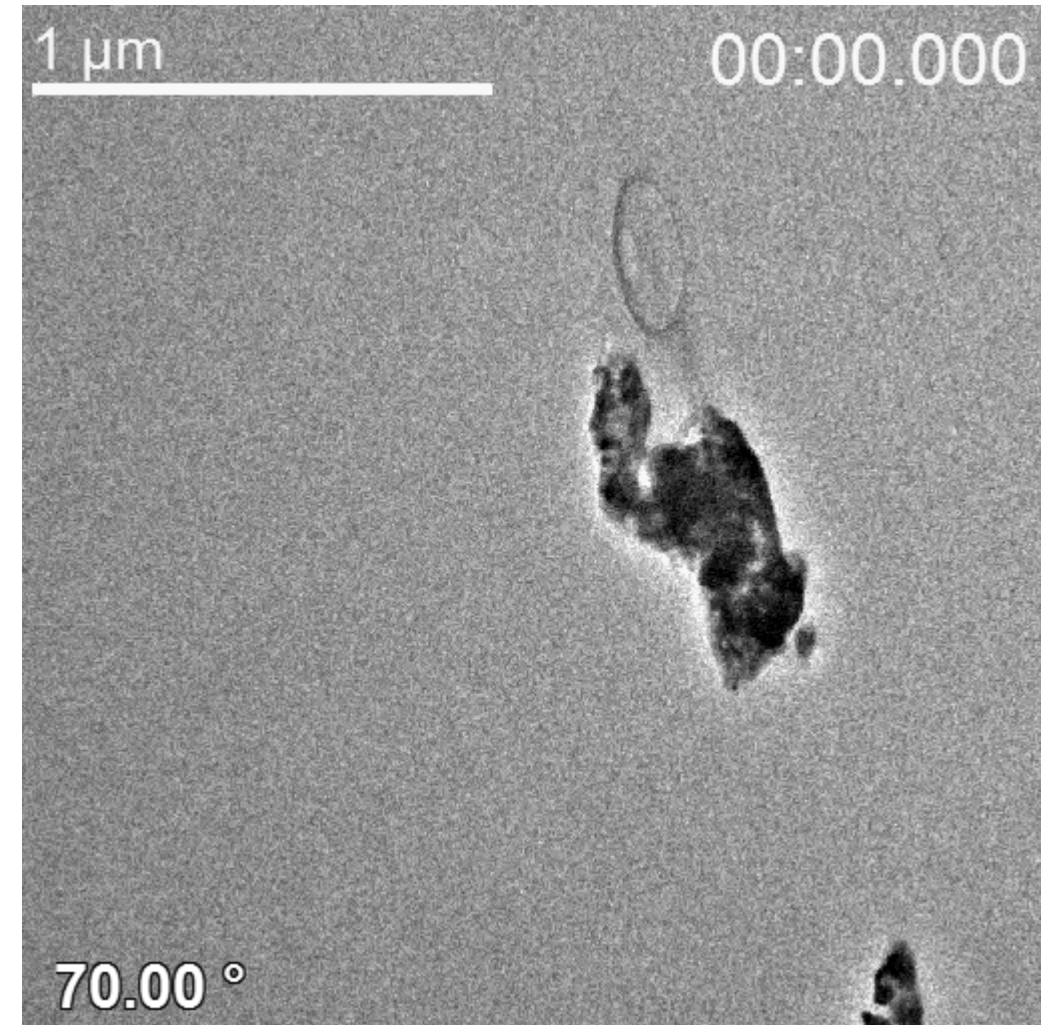
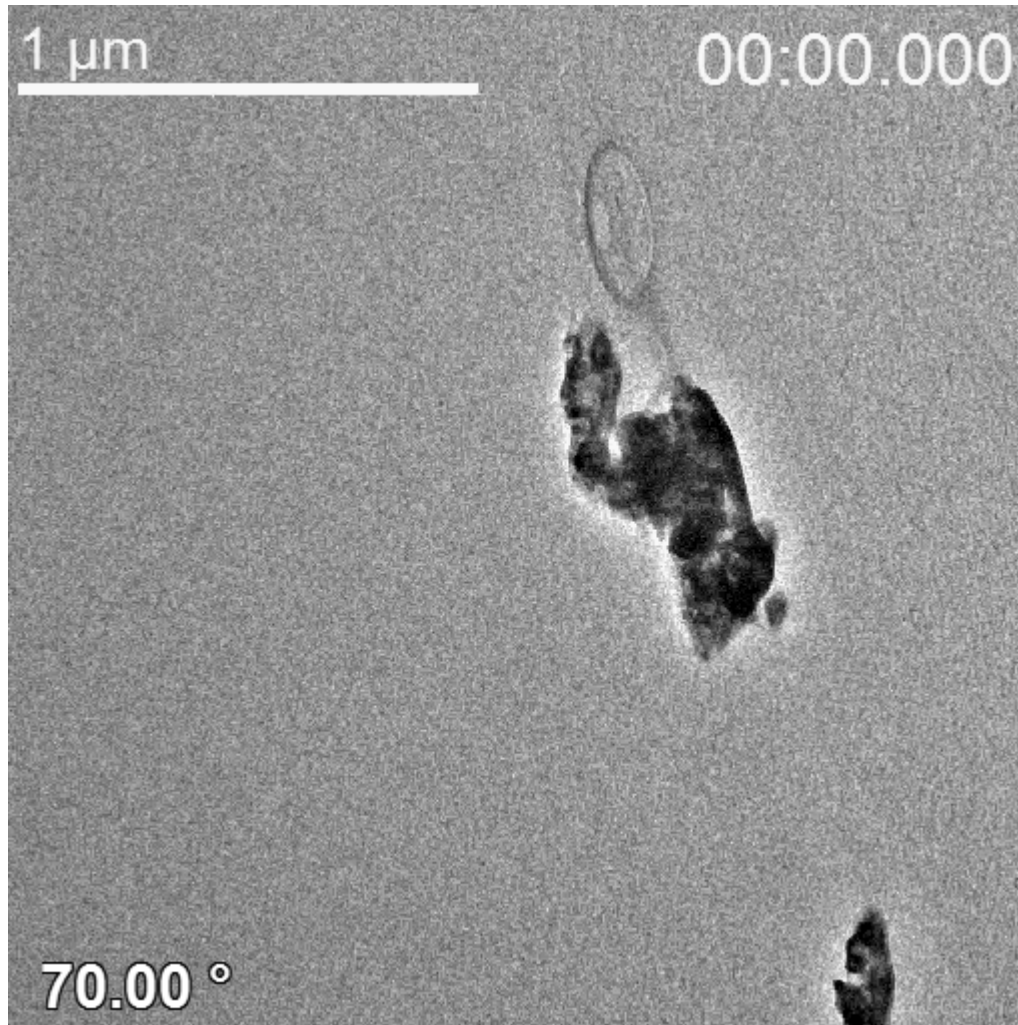


(user's) choice of 28 corrections sent during the tomographic acquisition



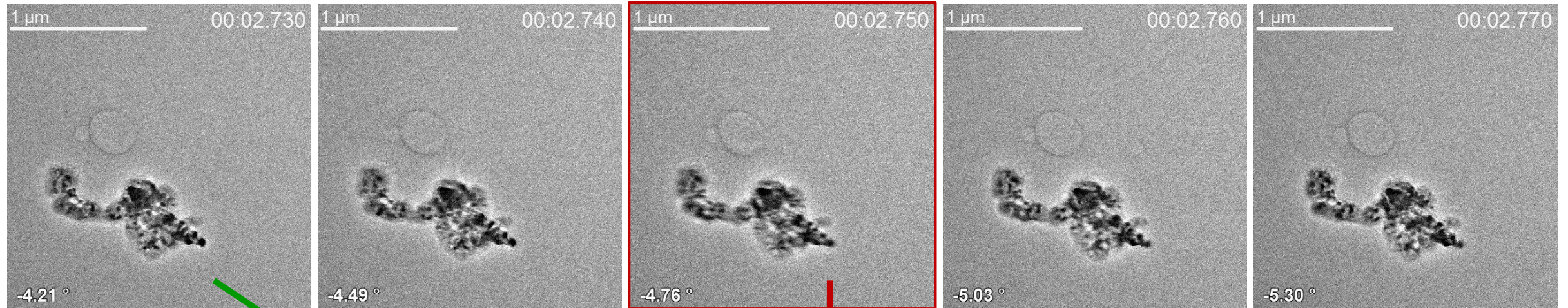


- Application of an adequate set of correction drifts ($\delta X, \delta Y$)

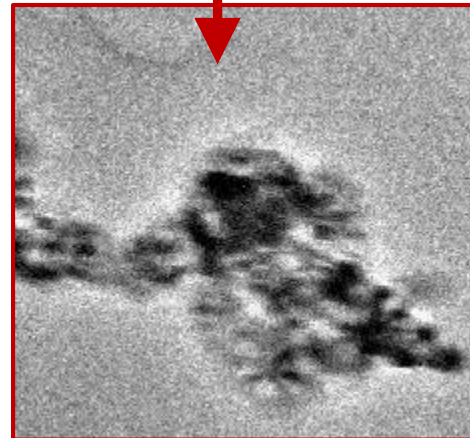
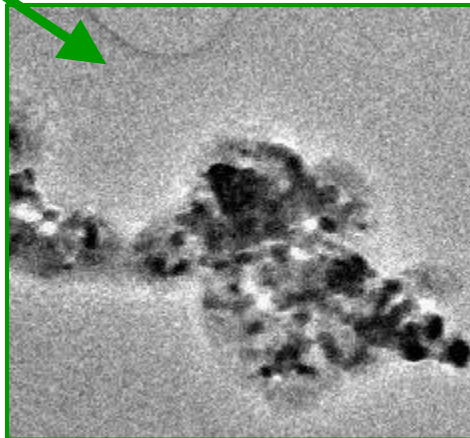


- **Example of an elementary drift correction (over 28)**

Performed with the image shifts microscope controls

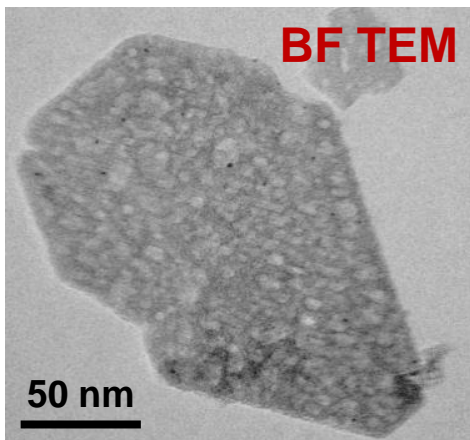


≈ only one frame significantly blurred, less than 1.5° angular amplitude affected



• Is **Bright Field** tomography adequate for crystalline objects?..

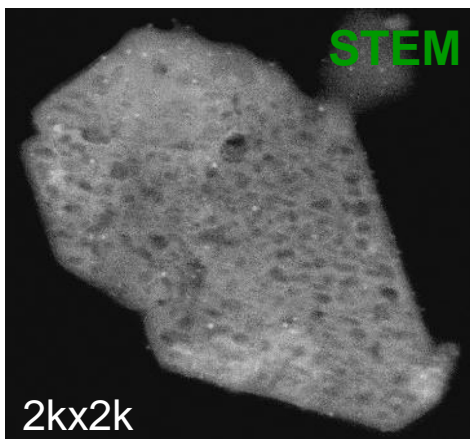
Pd nanoparticles on $\delta\text{-Al}_2\text{O}_3$
In situ ETEM: 350°C
 after 60', 2.6 mbar O_2



"Fast"
 camera:
 Acquisition
**0.01 s or
 less**

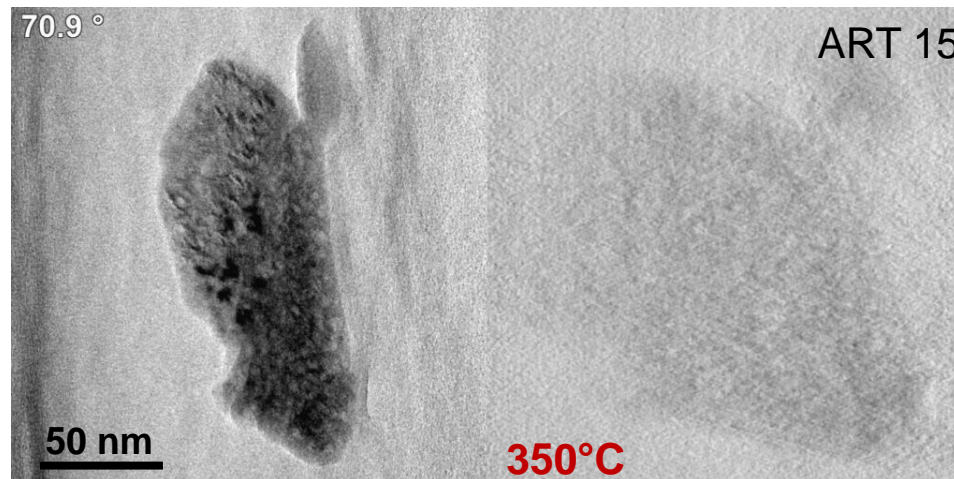


Back to 20°C

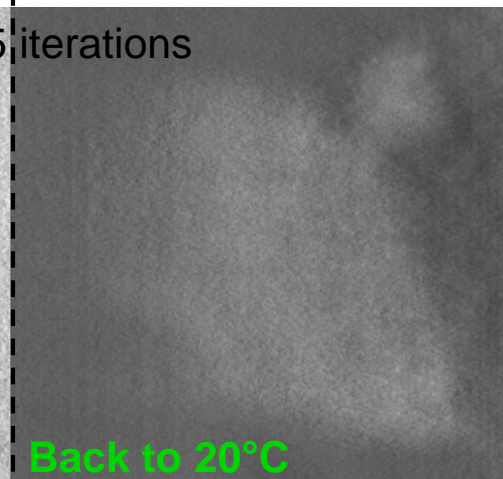


Dwell Time
 2.5 μs :
 Acquisition
10 s

BF step-by-step (2°),
 +73.4° / -66.6°,
2 min 42 sec

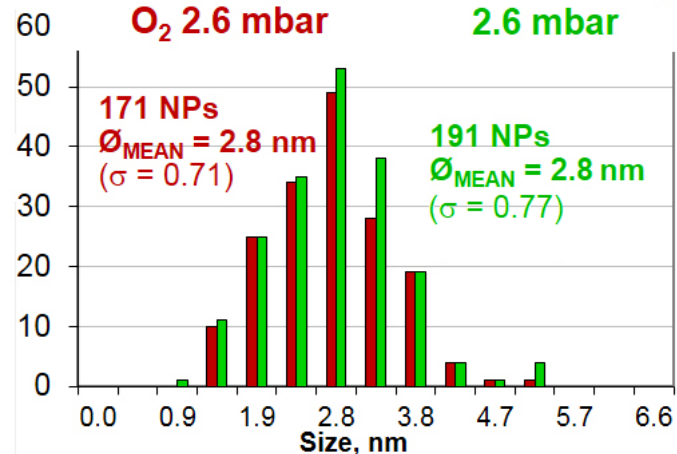


ADF step-by-step (2°),
 +75° / -67.8°,
≈ 90 min



FAST ET, 350°C,
 O_2 2.6 mbar

STEM 20°C, O_2
2.6 mbar



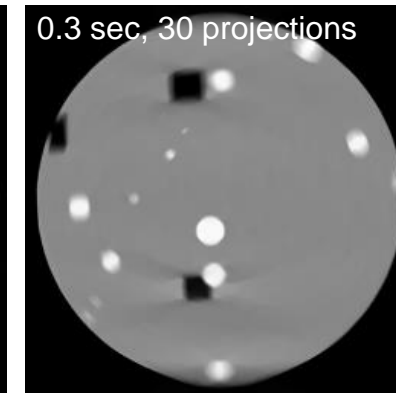
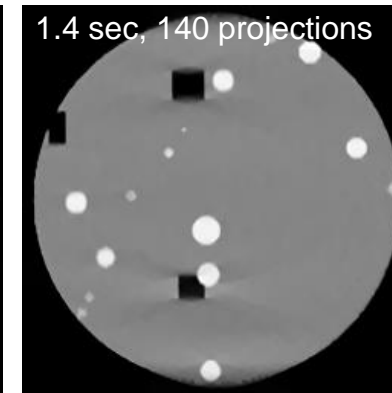
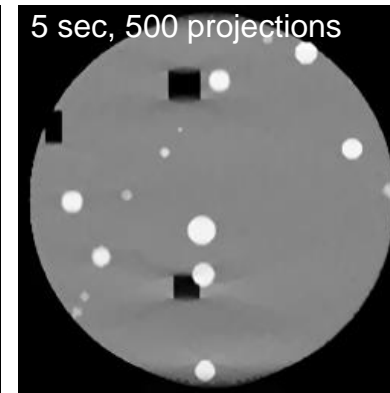
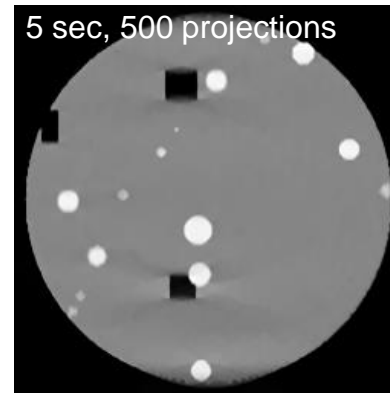
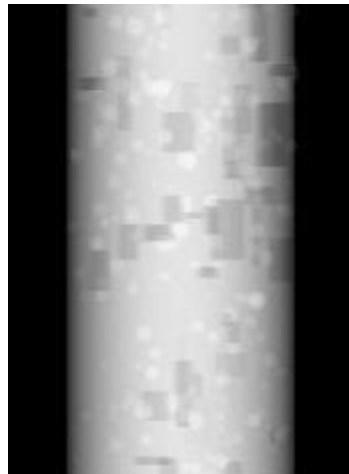
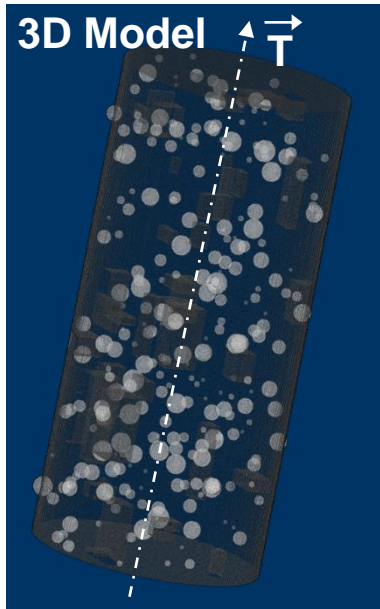
T. EPICIER et al., *Catal. Today*, **334** 15 (2019), 68



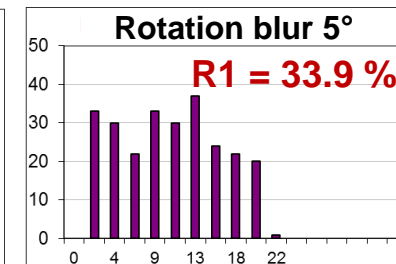
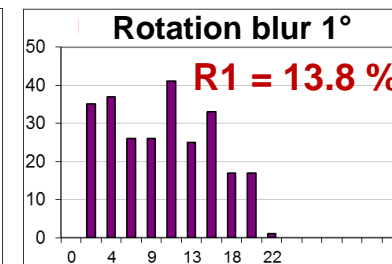
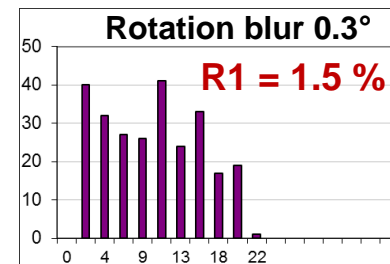
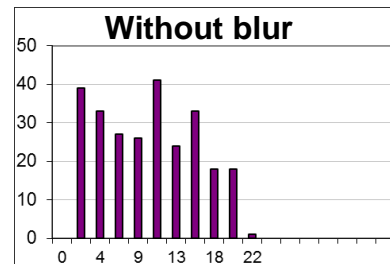
- Images are acquired during the rotation of the object: aren't they all BLURRED?

Rotation angular amplitude: 2α	140°	140°	140°	140°
Total acquisition time: t_{total}	10 sec	5 sec	1 sec	0.3 sec
Angular rotation speed $\omega = 2\alpha/t_{total}$	14°/sec	28°/sec	140°/sec	467°/sec
Number of frames per second: Fps	100	100	100	100
Rotation blur / frame: $\Delta\alpha = \frac{2\alpha}{t_{total} \cdot Fps}$	0.14°	0.28°	1.4°	4.7°

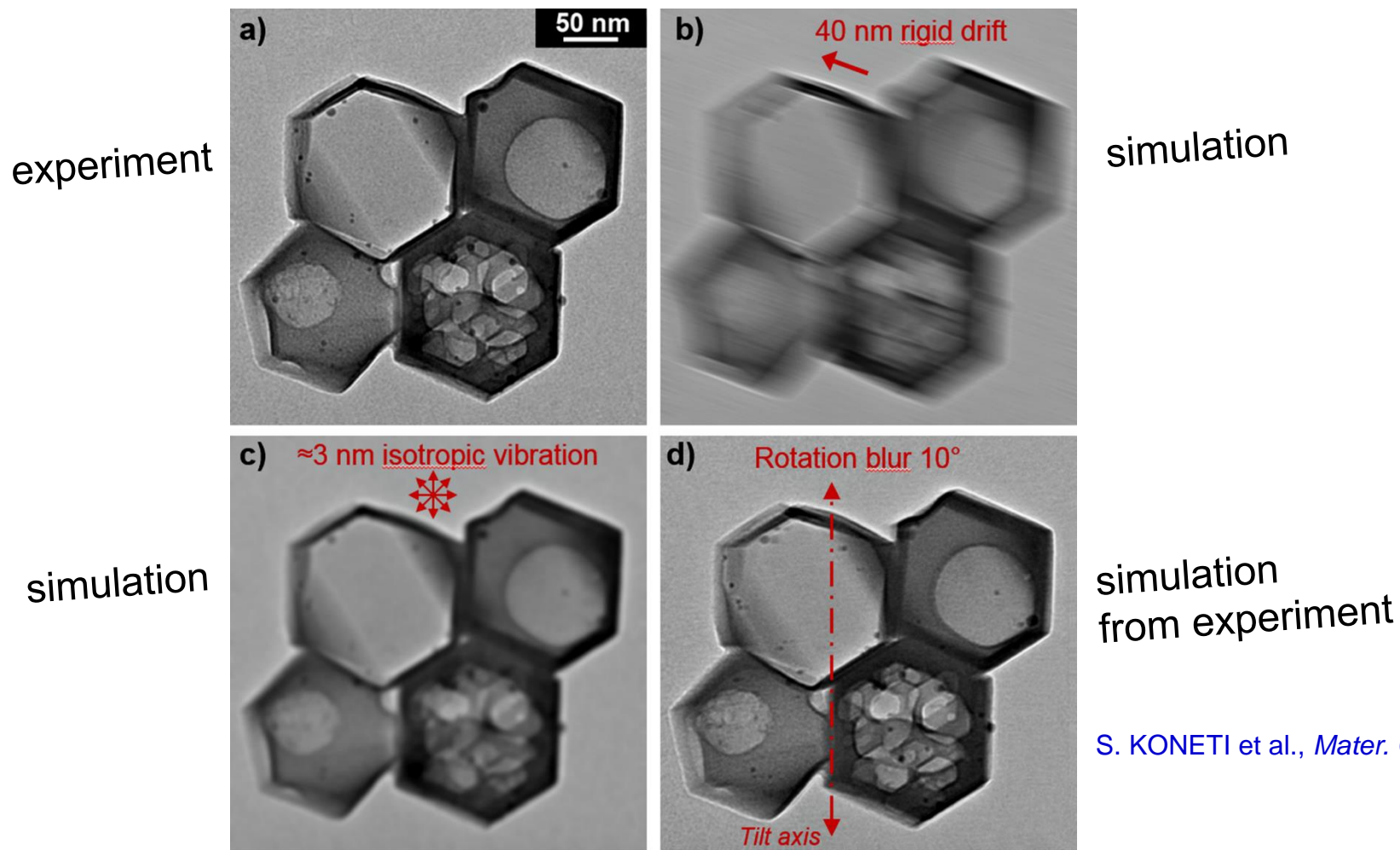
SIRT-based (SIRT-FISTA-TV) reconstructions [H. BANJAK et al., Ultramicroscopy, 189, \(2018\), 109](#)



"NP" size histograms



- Images are acquired during the rotation of the object: aren't they all **BLURRED**?



S. KONETI et al., *Mater. Characterisation*, **151** (2019), 495

The 'rotation blur' is NOT the most significant blur source

CONCLUSION *(summary of the approach)*

1
CALIBRATION
SEQUENCE

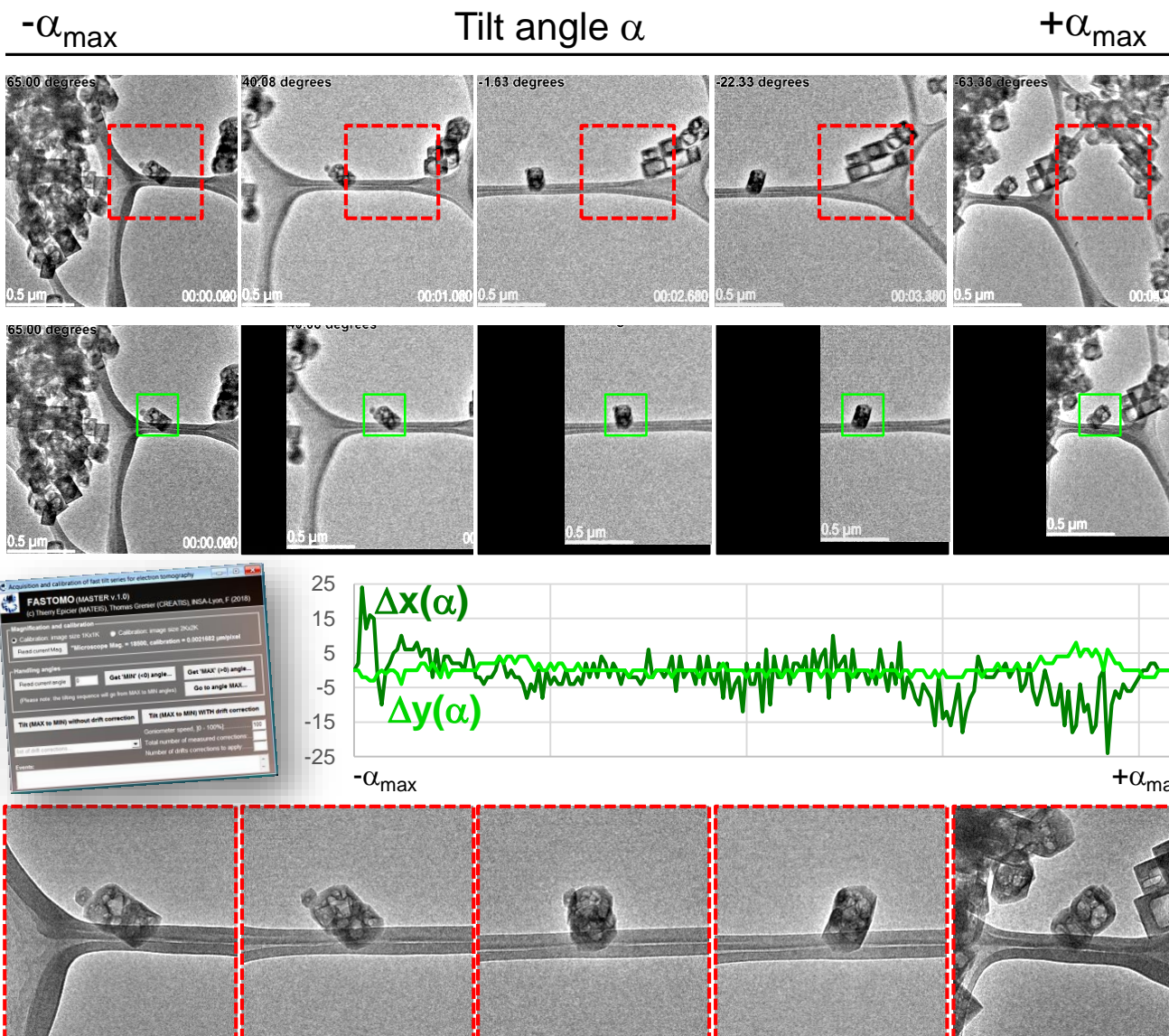
≈ 05 seconds

2
(X,Y) DRIFT
ANALYSIS

≈ 120 seconds

3
FINAL TILT
SEQUENCE
APPLICATION of
DRIFT
CORRECTIONS
TemScripting[©]FEI
Application

≈ 05 seconds



total time less than
3 minutes

Fast acquisition at a lower mag than the **final mag**

50-100 fps
typically 1k x 1k
tilt - α_{max} / $+\alpha_{max}$ in 5-10 s
 α_{max} up to 72°, Widfire heating holder

Fast acquisition at the **final mag**

100 fps
2k x 2k
tilt - α_{max} / $+\alpha_{max}$ in down to 5 s



- Mimoun AOUINE, *University Lyon I, F*



- Anne-Sophie GAY, Amandine CABIAC,
IFPen, Solaize, F



- Consortium Lyon – St-Etienne de Microscopie



- French ANR project '3DCLEAN' n°15-CE09-0009-01,
LabeX 'IMUST' University of Lyon



- DENS solutions GATAN

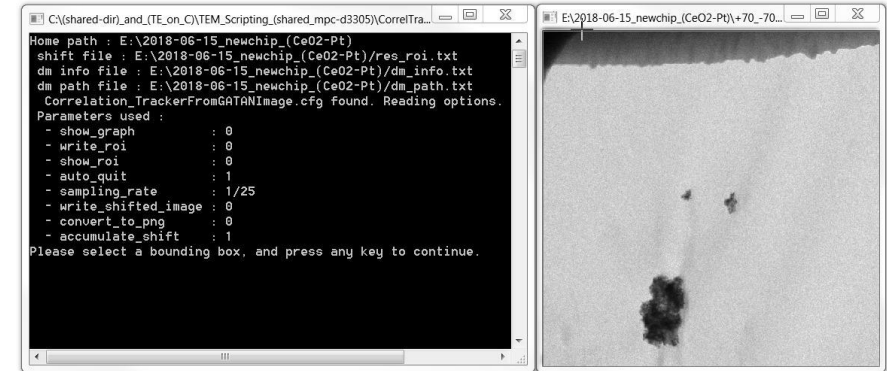


- www.metsa.fr METSA



Ceria (CeO₂) nano powder

Wildfire heating single-tilt holder DENS solutions, +70 / -70°



1K series, 100 fps, mag 16 500

Tracking every 25 frames, time of analysis ≈ 8 s