CSU-W1 CONFOCAL SCANNER UNIT
Application Note

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Live-cell microscopy for long-term observation of mitosis by spinning disk confocal, CSU-W1

Long-term observation of mitosis by live-cell microscopy is required for uncovering the role of Cohesin on compartmentalized nuclear architecture which is linked to nuclear functions. To perform long-term observation of mitosis devices are needed that have low phototoxic effects on living cells and enable high speed imaging. Cohesin, a ring-like protein complex with its major subunits RAD21, SMC1, and SMC3 is involved in sister chromatid entrapment to ensure proper chromosome segregation during mitosis, in double-strand break repair and gene regulation. Its impact on a compartmentalized nuclear architecture, linked to nuclear functions, is less well understood.

By using the CSU-W1 confocal scanner unit for time-lapse imaging entrance into mitosis, mitotic progression and exit can be examined (Figure 1). It was found that in control cells ~80% of all recorded mitoses passed mitosis within <1 h and formed two daughter nuclei (Figure1 a). Whereas in cohesin depleted cells mitotic progress was delayed up to 14 h (median 4.5 h). The delayed mitotic passage was associated with the formation of abnormal, e.g., multipolar mitotic figures persisting over several hours with subsequent formation of one endomitotic multilobulated nucleus (MLN) (Figure1 b) .

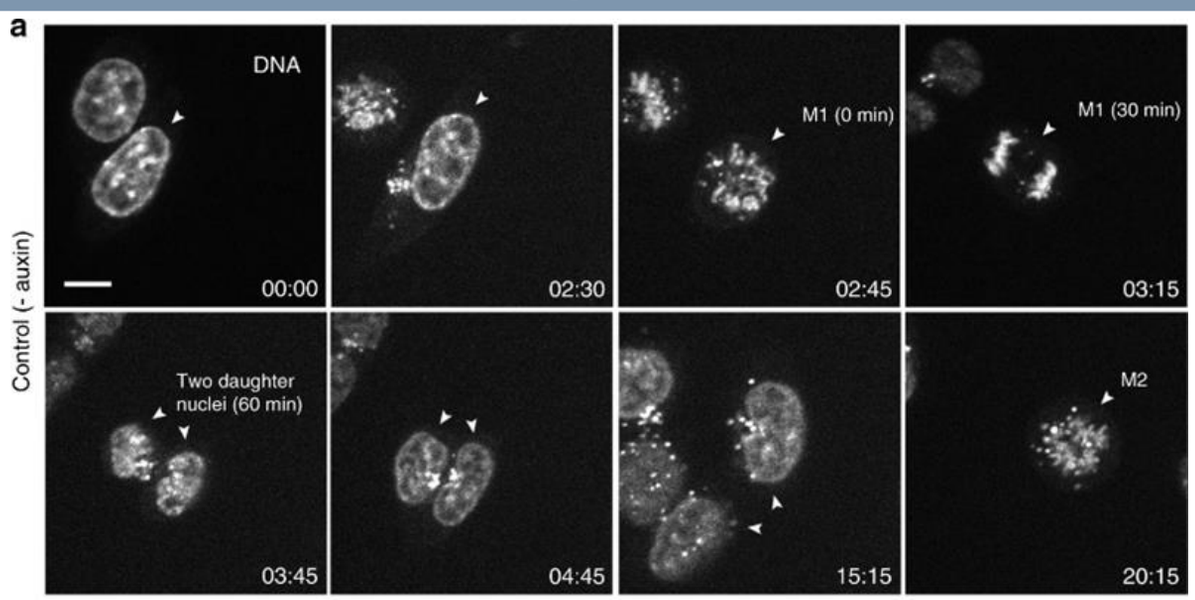
Live-cell microscopy demonstrating prolonged abnormal mitosis in cohesin depleted cells

Human colon cancer cells (HCT116-RAD21-mAID-mClover cells (kindly provided by Kanemaki lab, Shizuoka, Japan)) were plated on poly-L-Lysine-coated glass-bottom 2-well imaging slides (ibidi), allowing to image control and auxin-treated conditions in parallel. For DNA staining cells were incubated in media containing 500 nM SiR-DNA (Spirochrome) for 1 h before imaging. Cohesin was depleted by auxin-inducible degron (AID). The auxin-inducible degron (AID) system has emerged as a powerful tool to conditionally deplete proteins in a range of organisms and cell types.

Time-lapse imaging of untreated control cells (-auxin)

Figure1 a — The following figure shows selected points from time-lapse imaging (Σt = 21 h, Δt = 15 min) of untreated control cells (− auxin) with the accomplishment of mitosis (M1) with in 1h (time 02:45–03:45) and subsequent formation of two daughter nuclei. A second mitosis (M2) of one daughter nucleus is shown at time 20:15. Scale bar: 10 µm.

Figure1 a

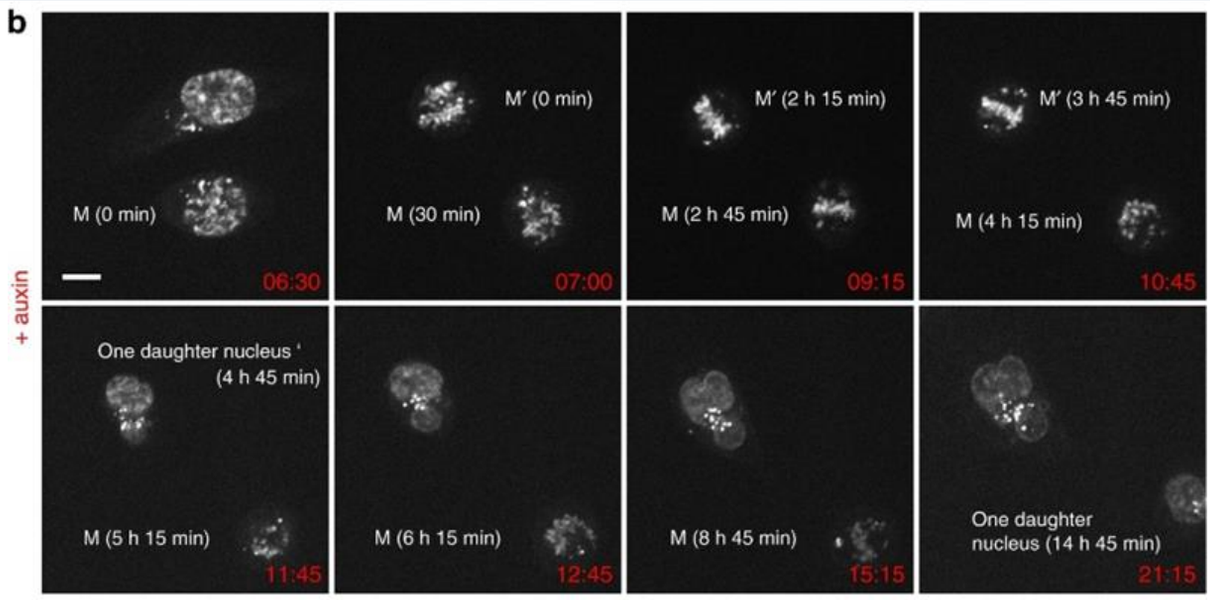


Time-lapse imaging of cohesin-degraded cells (+auxin)

Figure1 b

Selected time-lapse images of nuclei after cohesin degradation (+ auxin) conducted in parallel to control cells demonstrate a prolonged mitotic stage.
Mitosis (M) emerges at time 6:30 after auxin treatment, transition into one abnormal multilobulated daughter nucleus (MLN) is seen 14:45 h later (time 21:15). Mitosis (M') emerges 7 h after auxin treatment (time 07:00), transition into an MLN is seen 4:45 h later (time 11:45). Scale bar: 10 µm.

Figure1 b



System

- Confocal scanner unit: CSU-W1 (pinhole: 50µm)
- Laser: 488 and 640 nm
- Microscope: Nikon TiE microscope
- Camera: Andor iXon 888 Ultra EMCCD camera
- Objective lens: Nikon PlanApo 60x/1.49 NA oil immersion objective
- Control software: Nikon NIS-Elements, ver. 5.02.00
- Software: Fiji software (ImageJ 1.51j)

Imaging conditions

Images were recorded every 15 min for 21 h as z-stacks with two planes and a step size of 6 µm, unbinned and with a pixel size of 217 nm

Long-term observation of mitosis by live-cell microscopy enables the analysis of nuclear architecture of cells in mitosis. The CSU-W1 confocal scanner is a powerful tool for the analysis of the role of Cohesin on compartmentalized nuclear architecture.

Data: Cremer, M., Brandstetter, K., Maiser, A. et al. Cohesin depleted cells rebuild functional nuclear compartments after endomitosis. Nat Commun 11, 6146 (2020)

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Printed in Japan, 204 [Ed:01]

AN 80C01D02-01EN