Centrifuge Microscopy to Analyze the Sedimentary Movements of Amyloplasts

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[Abstract] A centrifuge microscope (CMS) functionally consists of a centrifuge producing a centrifugal force (hypergravity condition) and a microscope making an enlarged image of an object. This combination of equipment allows live-cell imaging during centrifugation. We have developed a new CMS (NSK Ltd.) to observe movements of the plant organelles such as amyloplasts, under hypergravity conditions (Toyota *et al.,* 2013). This CMS is distinct from previously designed CMSs in terms of spatio-temporal resolution, ease of use and compactness. Here, we show a quick protocol to prepare a specimen of *Arabidopsis* inflorescence stem, use the CMS, obtain imaging data and analyze them using a single tracking method.

Materials and Reagents

1. *Arabidopsis thaliana* inflorescence stems
2. MS salt mixture (Wako Pure Chemical Industries, catalog number: 392-00591)
3. 1% (w/v) sucrose
4. 0.05% (w/v) MES
5. 0.1% (w/v) agar
6. Growth media (see Recipes)

Equipment

1. Fine tweezers
2. Scissors
3. Razor blade (Electron Microscopy Sciences, catalog number: 72000)
4. Kimwipes
5. Aluminum chamber (custom built) (NSK Ltd.)
6. Silicone rubber (thickness: 0.5 mm) (AS ONE Corporation, catalog number: 6-611-01)
7. Round cover glass (diameter: 12 mm) (Matsunami Glass, catalog number: CO12001)

8. CMS system (Figure 1, not commercially available) (NSK Ltd., http://www.nsk.com/)
   CMS is a newly designed compact centrifuge microscope, 30 cm in height and 20 cm in diameter. CMS consists of a direct-drive motor (NSK Ltd., MEGATORQUE MOTOR™, model: M-PS1006KN002) and optics including a 50x objective lens with a working distance of 18 mm (SLMPLN 50x, 0.35 NA, OLYMPUS), LED light source (SCHOTT MORITEX Corporation, model: MEBL-CW25) and a CCD camera (SENSOR TECHNOLOGY, model: STC172C).

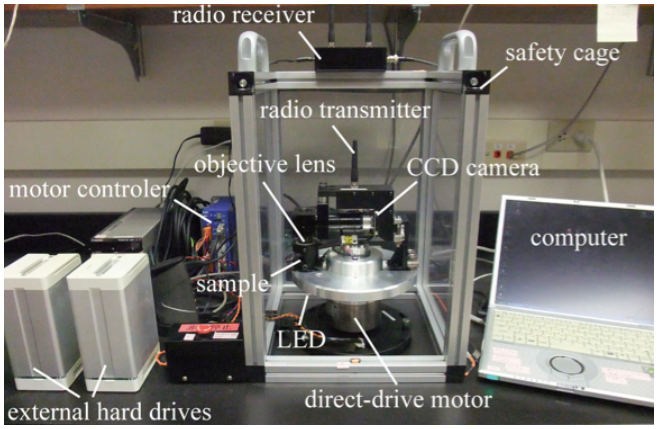


Figure 1. Overview of the CMS system

9. Windows computer [minimum computer requirements: Windows® XP or later, Pentium M 778 (1.6 GHz), RAM 1024 MB or higher]
   Note: To install the software below, Windows computers are highly recommended.

Software

1. MEGATORQUE MOTOR™ controller (EDC megaterm software) (NSK Ltd., http://www.nsk.com/)
   Note: This is free software to control the motor and is available only for Windows.
2. Video capture software (COREL, http://www.corel.com/)
   Note: You can use any video capture soft/hardware that converts analog video signal into digital signal and stores the data in a computer.
3. G-Track spot-tracking software (G-Angstrom, http://www.gangstrom.com/eng/products/index.php)
   Note: This is a piece of commercial software to trace fluorescence/bright spots and available only for Windows.

Procedure

1. Excise an approximately 1-cm-long segment of an inflorescence stem at 1-2 cm from the apex of the primary stem.
2. Place the stem segment onto a hand-made stem holder and slide a razor blade to split the stem longitudinally (Figure 2) (Saito *et al.,* 2005; Nakamura *et al.,* 2011).

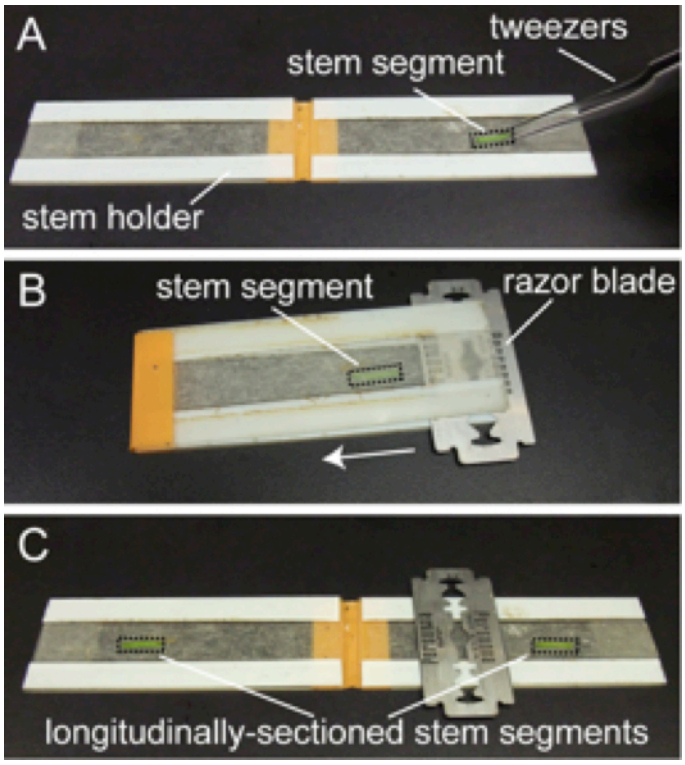
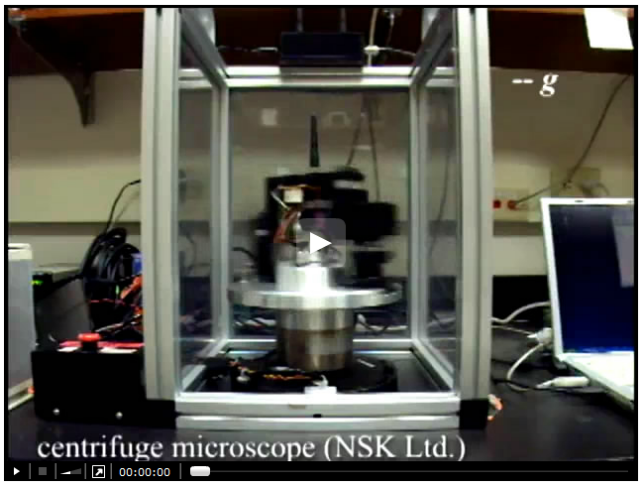


Figure 2. Making longitudinal sections of *Arabidopsis* inflorescence stems. A. Place the stem segment onto a hand-made stem holder. B. Close the holder and slide a razor blade in the direction of the arrow. C. Open the holder and retrieve the longitudinally-sectioned stem segments.

3. Drop a small amount of growth medium onto the sectioned side of the stem to prevent the tissue from drying out.
4. Keep the sectioned side up and put this segment into a slit in a round silicone rubber sheet (0.5 mm deep) on the bottom glass of an aluminum chamber (Toyota *et al.,* 2013).
5. Pour growth medium into this slit, put a cover glass onto the silicone rubber and remove spilt growth media with Kimwipes.
6. Mount the aluminum chamber in a holder under an objective lens of the CMS.
7. Turn on the LED light to illuminate the specimen and the CCD camera to acquire images.

8. Acquired bright-field images are transmitted through a radio system and shown on the video capture software in the computer.
9. Adjust focus and field of view in the CMS while monitoring the computer.
10. Set a centrifugal acceleration between 0 to 33 *x g* in the MEGATORQUE MOTOR™ controller of the computer. In case of wild-type *Arabidopsis* stems, maximum gravitropic responses are seen at 10 *x g*.
11. Start video capture and run MEGATORQUE MOTOR™ (Videos 1 and 2).

Video 1. Side view of the CMS during operation



Video 2. Top view of the CMS during operation

12. Monitor real-time images (30 frames per sec) on the computer during centrifugation.
13. Stop the motor and video capture and save the images as an avi file in the computer.
14. Open this file in G-Track spot-tracking software.

15. This software automatically recognizes many white or black spots (amyloplasts) and traces them (Video 3; Figure 2). If necessary, you can modify image parameters such as gain or contrast, and invert brightness.

Video 3. Single-particle tracking analysis of amyloplast movement during centrifugation at 10 × *g* (Toyota *et al.*, 2013). Most amyloplasts are automatically recognized by the G-Track spot-tracking software and traced while they are recognized as white or black spots. Please note that this software does not precisely recognize an amyloplast (spot) with weak contrast nor aggregated amyloplasts. Video duration = 152 s (8 x speed).

16. Run the tracking program. You can automatically get data [*i.e.*, mean square displacement (MSD) of an amyloplast, Table 1] and then calculate velocity and displacement.
17. Export the data to Excel/CSV file (Table 1).

Representative data

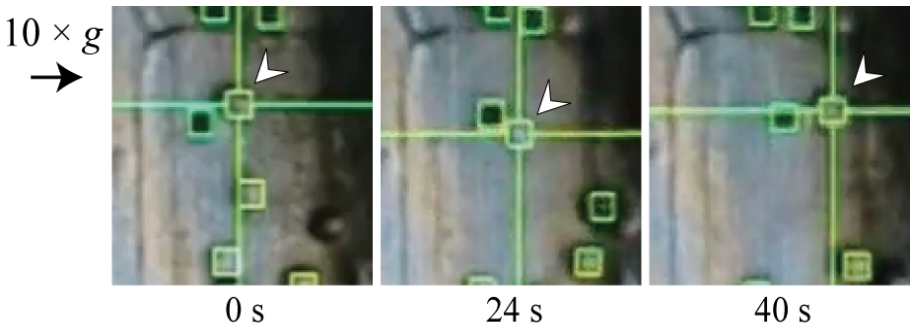


Figure 3. Representative tracking image (Toyota *et al.*, 2013). Movement of an amyloplast

(arrow head) is successfully traced by the G-Track spot-tracking software during centrifugation.

Table 1. Mean square desplacement (MSD) of the amylplast for 1 sec of centrifugation. MSD of the amyloplast traced in Figure 3 is automatically calculated by the tracking program. X, Y and 2D denote movement in the horizontal (10 x *g*) and vertical directions and in a two-dimensional (2D) plane, respectively. For downloading data, please click the image below.

	A	B	C	D
1	Time (s)	X MSD (nm^2)	Y MSD (nm^2)	2D MSD (nm^2)
2	0	0	0	0
3	0.03	6870.8873	10532.905	17403.792
4	0.06	9749.6869	14827.617	24577.304
5	0.09	11313.81	17081.064	28394.874
6	0.12	12568.488	19455.514	32024.003
7	0.15	13963.539	21543.437	35506.977
8	0.18	15569.045	23321.761	38890.807
9	0.21	17541.118	26335.084	43876.202
10	0.24	19432.915	27944.374	47377.289
11	0.27	21403.929	30141.126	51545.055
12	0.3	22938.853	32296.456	55235.308
13	0.33	24300.065	33497.584	57797.648
14	0.36	26517.51	36392.242	62909.751
15	0.39	28825.892	38763.712	67589.604
16	0.42	30869.982	40700.067	71570.049
17	0.45	32780.855	43296.53	76077.385
18	0.48	34262.313	45199.287	79461.6
19	0.51	36403.245	47060.662	83463.907
20	0.54	38598.231	50664.354	89262.585
21	0.57	40776.465	52142.989	92919.454
22	0.6	42924.119	54301.86	97225.978
23	0.63	45106.729	56725.471	101832.2
24	0.66	46994.137	58596.968	105591.1
25	0.69	49352.392	61639.01	110991.4
26	0.72	51931.826	64626.378	116558.2
27	0.75	54316.798	66399.085	120715.88
28	0.78	56531.071	69203.688	125734.76
29	0.81	58549.965	70777.191	129327.16
30	0.84	61294.082	72188.885	133482.97
31	0.87	64151.522	75361.508	139513.03
32	0.9	66568.19	76730.799	143298.99
33	0.93	69008.021	78713.241	147721.26
34	0.96	71401.731	81138.789	152540.52
35	0.99	73710.912	82665.399	156376.31
36	1.02	76116.427	84816.022	160932.45

Recipes

1. Growth media (pH 5.1)
   1x MS salts
   1% (w/v) sucrose
   0.05% (w/v) MES
   0.1% (w/v) agar

Acknowledgments

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References

1. Nakamura, M., Toyota, M., Tasaka, M. and Morita, M. T. (2011). An *Arabidopsis* E3 ligase, SHOOT GRAVITROPISM9, modulates the interaction between statoliths and F-actin in gravity sensing. *Plant Cell* 23(5): 1830-1848.
2. Saito, C., Morita, M. T., Kato, T. and Tasaka, M. (2005). Amyloplasts and vacuolar membrane dynamics in the living graviperceptive cell of the *Arabidopsis* inflorescence stem. *Plant Cell* 17(2): 548-558.
3. Toyota, M., Ikeda, N., Sawai-Toyota, S., Kato, T., Gilroy, S., Tasaka, M. and Morita, M. T. (2013). Amyloplast displacement is necessary for gravisensing in *Arabidopsis* shoots as revealed by a centrifuge microscope. *Plant J* 76(4): 648-660.

