PERIODICUM BIOLOGORUM
VOL. 125, No 1–2, 133–137, 2023
DOI: 10.18054/pb.v125i1-2.25140

UDC 57:61
CODEN PDBIAD
ISSN 0031-5362

Review

A young researcher's guide to three-dimensional fluorescence microscopy of living cells

VEDRANA FILIĆ
IGOR WEBER*

Laboratory of Cell Dynamics, Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia

***Correspondence:**
Igor Weber
E-mail address: iweber@irb.hr

Keywords: laser scanning confocal microscopy; optical sectioning; photobleaching; phototoxicity; long-term imaging

Abbreviations
3D – three-dimensional
CCD – charge-coupled device
EMCCD – electron-multiplying charge-coupled device
LLSM – lattice light-sheet microscopy
PMT – photomultiplier tube
PSCM – point-scanning confocal microscopy
sCMOS – scientific complementary metal–oxide semiconductor
SDCM – spinning-disk confocal microscopy
SNR – signal-to-noise ratio

Received January 23, 2023
Revised February 3, 2023
Accepted February 20, 2023

Abstract

Three-dimensional imaging of fast intracellular processes by fluorescence microscopy should provide decent spatial and high temporal resolution while minimizing fluorophore bleaching and cytotoxicity. We give a condensed introductory overview of three contemporary methods mostly used for imaging of living cells in 3D and compare their performance in terms of temporal and spatial resolution, imaging flexibility and specimen photodamage: point-scanning confocal microscopy, spinning-disc confocal microscopy, and lattice light-sheet microscopy. While point-scanning instruments are unsurpassed in terms of confocal performance, flexibility and configurability of their optical path, spinning-disc and lattice light-sheet optical designs excel in acquisition speed and low levels of light-inflicted specimen deterioration.

INTRODUCTION

Modern fluorescence microscopy abounds with a multitude of concepts and methods not easily accessible to young scientist trained within a traditional life sciences curriculum. Although many excellent expert reviews on the subject are available *(1,2)*, we felt that simple introductory material should be provided to serve as a stepping stone for novice users of light microscopy in biological research. In this short review, we are focusing specifically on three types of microscopes best suited to explore the dynamics of, and in, living cells by optical sectioning. To perform optical sectioning simply means to image the volume of a specimen in a serial manner plane by plane. In this way, three-dimensional (3D) distribution of fluorescently labeled intracellular components can be obtained and their evolution over time can be followed. We are primarily referring to experiments that are designed to monitor fast intracellular processes and should therefore be optimized for temporal resolution at the cost of spatial resolution. Another important consideration is the reduction of phototoxicity since many important processes need to be followed continuously over several minutes or longer while preserving the sample viability.

It has been pointed out repeatedly that the key requirements for obtaining ideal imaging data are interdependent and cannot all be optimized at the same time, resulting in necessary compromises between sample protection, temporal resolution, spatial resolution, and image quality, i.e., the signal-to-noise ratio (SNR) *(3,4)*. For instance, higher temporal resolution necessitates shorter exposure times per pixel and, consequently, reduces the image quality. On the other hand, improved spatial resolution comes at the price of decreased photon counts per pixel. For example, doubling the resolution in 3D yields eight times fewer

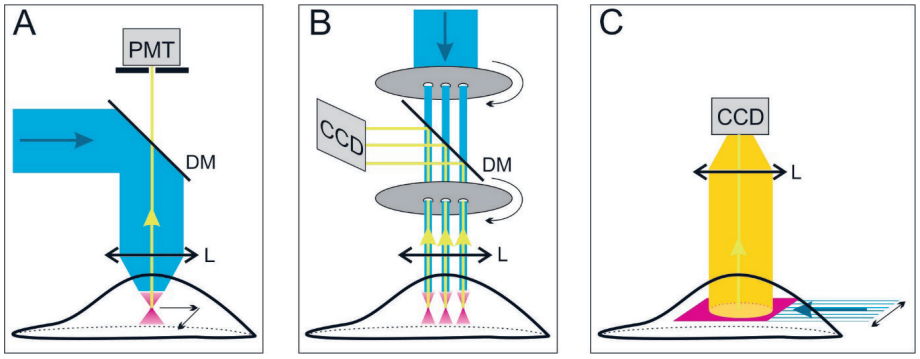

Figure. 1. *Basic optical configurations of presented microscopy methods. (A) Point-scanning confocal microscopy. Excitation laser light (cyan) is focused through the objective lens to a diffraction-limited spot in the sample focal plane. Only light emanating from the focus (yellow) passes through the pinhole and is detected by the photomultiplier (PMT), although fluorescence is also excited above and below the focal plane (magenta). The focal plane is scanned point by point in a rectangular fashion to obtain an optical section. (B) Spinning-disk confocal microscopy. Excitation light (cyan) passes through disks perforated by equivalent pinhole arrays that scan the specimen by rotating at high speed. The illumination (upper) disk is equipped with microlenses to improve the illumination efficiency. Fluorescence light from the focal plane (yellow) passes through the detection (lower) disc pinholes, and is detected by an array detector such as a CCD camera. The focal plane is scanned by a large number of pinholes in parallel, thereby speeding up the optical sectioning. (C) Lattice light-sheet microscopy. A thin sheet of excitation light is generated by dithering an array of parallel beams (cyan) and illuminates the specimen orthogonally to the axis of imaging, thus exciting only the fluorescence in the focal plane (magenta). Because all fluorescence emanates from the focal plane (yellow), a pinhole is not required. Also, since the whole focal plane is illuminated at once, no horizontal scanning is needed, and the optical section is captured by a CCD camera. DM, dichroic mirror; L, lens. The thin arrows designate the direction of orthogonal (x-y) scanning of the laser beam in (A), the disk rotation in (B), and the dithering direction of the lattice light-sheet in (C).*

photons per imaged volume, and thus eight times more light or time is needed to achieve the same signal per pixel, at the cost of sample health or temporal resolution *(3)*.

In this review, we cover the main features of the well-established and widely used point-scanning confocal microscopy (PSCM), increasingly popular spinning-disk confocal microscopy (SDCM), and still not so commonly used lattice light-sheet microscopy (LLSM). While point-scanning confocal microscopes can be configured in many ways to match diverse experimental needs, their performance in terms of fast and gentle optical sectioning is limited. Spinning-disk confocal microscopes are less configurable but surpass PSCM in terms of rapid and sample-friendly 3D imaging. Finally, lattice light-sheet microscopes are highly specialized and sophisticated devices optimized specifically for imaging full cell volumes at high frequency over long periods of time. In this article, we will not be considering several important aspects of light microscopy in general, and 3D imaging in particular, such as the anisotropic resolution, optical aberrations caused by refractive index mismatch, general effects of the light passing through thick specimens, etc., and we refer the reader to articles that discuss these issues in more detail *(1, 4)*.

POINT-SCANNING CONFOCAL MICROSCOPY

Widespread application of the concept of confocal microscopy, in which only light emitted from a diffraction-limited focal point in the specimen is collected by a detector, has revolutionized the fluorescence microscopy in the 1990s *(5,6)*. The central advantage of confocal imaging, compared to the wide-field fluorescence microscopy, is the feature of optical sectioning: sharp images of thin specimen sections can be obtained in a serial manner, thereby providing a three-dimensional reconstruction of the distribution of fluorescent molecules. The confocal microscopy is mostly implemented in the form of point-scanning instruments, where a beam of laser light scans over the specimen and emitted light is collected sequentially point by point by a photomultiplier (Figure 1A). The main advantages of this implementation include flexibility of the optical path (i.e., freedom to insert optical elements in the excitation and emission branches of the microscope optics), freely adjustable zoom factor defined by the scanning area, variable scanning speeds, possibility of simultaneous imaging in multiple spectral channels, and adjustable size of the detection pinhole that defines the degree of confocality, i.e. the spatial resolution *(7)*.

However, because of the sequential acquisition of the fluorescence signal pixel by pixel, the point-scanning confocal microscopy is intrinsically slow. As already pointed out, higher scanning rates necessitate shorter pixel-dwell times (i.e., time intervals during which the specimen area corresponding to a single pixel in the image is illuminated), which leads to unfavorable SNR in the image. This deterioration of the image quality can be somewhat compensated for by applying higher illumination densities, but at the cost of increased photobleaching, fluorophore saturation, and phototoxicity, which is particularly detrimental to living specimens. Saturation of fluorescence occurs when

the excitation photon flux is high enough to deplete the electronic ground state of the fluorophore, effectively decreasing the amount of 'excitable' fluorophore in the sample *(8)*. In addition, the laser-induced photodamage is inflicted to the specimen along the depth much thicker than the extent of the focal plane from which the fluorescence is collected. Taken together, the point-scanning confocal instruments are limited in their ability to monitor fast processes in living cells over long periods of time in 3D.

SPINNING-DISK CONFOCAL MICROSCOPY

Several attempts were made to increase the speed of confocal microscopes, including high-frequency resonant scanners and scanning-slit designs *(9,10)*. Ultimately, however, the spinning-disk approach proved to be the favorite solution for fast confocal imaging. The concept of spinning-disk confocal microscopy goes back to the 1960s and is based on the optomechanical parallelization of the point-scanning principle *(11)*. Instead of scanning a single focused light beam across the focal plane in the specimen, a doublet of synchronously rotating disks perforated by an appropriately arranged array of pinholes is placed at intermediate focal planes (Figure 1B). While disks are rotating, the array of pinholes illuminates the full field of view of an array detector, typically an EMCCD or a sCMOS camera. In this way, fast acquisition of several hundred images per second can be achieved with extended total pixel exposure times which, together with a significant increase in the overall light capture efficiency, contributes to improved SNR compared to point-scanning instruments *(8,12)*. In fact, the camera readout speed used to be the dominant limiting factor for fast acquisition of large image formats *(13)*.

In addition to improved image acquisition rates, it has been established that spinning-disk illumination is much gentler to the specimen due to a dispersed delivery of illumination energy over time, intercepted by intervals of "darkness" defined by the distance between the perforated pinholes *(8)*. In this way, the fluorophore saturation is avoided due to a lower peak excitation light density impinging on the specimen. Thus, the superior performance of the SDCM results not only from a more efficient mechanism of fluorescence collection, but also from a more efficient mechanism of fluorescence excitation *(8)*. The initial shortcomings of the early spinning-disk scanners, such as the low light-collection efficiency, non-uniform illumination, and a significant crosstalk between pinholes has been largely resolved in contemporary designs *(14)*. Implementations with two sets of spinning disks carrying pinholes of different sizes adapted to different objective magnification factors add to a growing flexibility of the method. Taken together, their improved acquisition speed and reduced photodamage make spinning-disk confocal microscopes much more adequate for fast, long-term imaging of living cells in 3D.

LATTICE LIGHT-SHEET MICROSCOPY

A common drawback of both point-scanning and spinning-disk microscopes is that the illuminated volume of the specimen is much larger than the volume from which the fluorescence is collected, leading to excessive photodamage *(15,16)*. This effect is due to the relative orientation of the excitation light beam and the imaged optical section of the specimen, which are perpendicular to each other. The recent advent of light-sheet microscopy is based on the optical arrangement in which the illuminating light sheet and the imaged optical section are coplanar and orthogonal to the imaging axis of the microscope (Figure 1C). In such an arrangement, only the imaged volume of the specimen is illuminated, thus making the use of confocal pinholes obsolete and strongly reducing the unnecessary photodamage outside of the focal plane *(3,17,18)*. Furthermore, recording the entire field of view in a single exposure brings about another steep increase in the acquisition speed. While PSCM scanners typically collect 1 pixel at a time, in SDCM approximately 10^3 pixels are detected in parallel, whereas light-sheet instruments record in parallel the entire image plane containing approximately 10^6 pixels *(12)*.

While light-sheet microscopy has been used for more than 20 years to image larger samples under low magnification, more recently it has been adapted to imaging single cells with diffraction-limited spatial resolution *(19)*. The most successful variant of high-magnification light-sheet microscopy is the lattice light-sheet microscopy, which uses sophisticated optoelectronic techniques to generate a thin and stable sheet of light to illuminate and image only a single section of the specimen at a time *(20,21)*. Lattice light-sheet microscopy has got its name because the continuous sheet of light used to illuminate the specimen is obtained by dithering a train of parallel light beams that are generated by a diffraction mask of holes arranged in the form of a lattice. While early instruments required specialized expertise to align and use, contemporary setups are much more user-friendly, can be used by non-experts, and allow usual mounting of samples on standard coverslips and cell culture dishes *(22,23)*. Since the illuminating light sheet can rapidly scan vertically through the sample, the lattice light-sheet microscopy surpasses even the spinning-disk confocal microscopy in terms of speed, and at significantly lower levels of photodamage *(20,24,25)*. As a bonus, LLSM operated in the diffraction-limited dithered mode, i.e., limited only by the optical laws of diffraction, provides superior spatial resolution, especially improving the resolution along the imaging axis: 230 nm in *xy* and 370 nm in *z (17, 20)*.

Although the light sheets used for illumination in LLSM and related methods are vulnerable to distortions brought about by the specimen, the use of adaptive optics has the potential to extend the use of the method to thicker samples *(21)*. Like many advanced methods, the use of LLSM comes at a cost - and not only of the relatively high

purchasing price of the microscopes themselves. The necessary processing of raw recorded images is computationally intensive, and the sheer volume of data obtained at the full capacity of the instrument easily reaches several terabytes per week. This data overload greatly increases the demands on computational resources of microscopy facilities for networking, storage space and image processing. In summary, lattice-light sheet microscopy is obviously a highly specialized method and is at the time best-suited for non-invasive imaging of rapid processes in living cells. This point is clearly illustrated in a recently published study that includes a comparison of the performance of PSCM, SDCM and LLSM applied to imaging highly dynamic cellular and subcellular features of migrating leukocytes *(24)*.

CONCLUSIONS

When purchasing state-of-the-art high-end light microscopes, which are nowadays best described as imaging stations, one has to carefully consider their intended purpose and use. These expensive instruments are mostly being installed in multiuser facilities and flexibly configurable optical designs are therefore usually favored. In addition to prevalence of such universal microscopes with broad range of applications, a trend towards specialization of commercial instruments in two directions can be recognized: towards superior spatial resolution, i.e., superresolution, and towards fast and sample-friendly 3D imaging *(2,22)*. Although these two demands, superresolution vs. fast and sample-preserving imaging, are difficult to reconcile in a single commercial instrument for practical reasons *(4)*, improvements of the spatial resolution in both SDCM and LLSM can be achieved *(21,26,27)*. Ideally, researchers should have at their disposal both types of specialized instruments, but research environments that are more oriented towards the live-cell work should primarily consider the options presented in this article (Table 1).

Whereas a young researcher might wish for an advice that would recommend a particular method for each specimen type, such universal recommendation is difficult to provide. It is a general rule in microscopy that each experiment should be carefully planned, and the demands placed on the imaging method considered in detail. For instance, distortion of the illumination light sheet by thick specimens, e.g., larger organoids, or the limited field of view that is uniformly illuminated in LLSM might preclude the application of this, otherwise superior, method to large living samples. On the other hand, the quality of images covering a small region with intracellular organelles might benefit from the oversampling capability provided by the adjustable zoom of PSCM. Ultimately, the best approach is to directly compare the performance of several methods applied to the same specimen *(24)*. An optimal strategy to gain experience with and evaluate new microscopy techniques is to make use of initiatives such as Euro-BioImaging that is providing access to the majority of available imaging technologies for biological community *(28)*. Finally, since the contemporary light microscopy is constantly bringing out exciting new developments, we are confident that more methods will soon become widely available for three-dimensional imaging of biological samples in their native state *(29,30)*.

Table 1. *Comparison of the performance parameters between the point-scanning confocal microscopy (PSCM), spinning-disc confocal microscopy (SDCM) and lattice light-sheet microscopy (LLSM), ordered according to the relevance for long-term 3D imaging of living cells; for more detailed comparison, see (13,17,18,20). The listed ranges of image acquisition speeds are representative of typical values used in practice for megapixel images rather than maximal instrument capabilities, and based on selected examples from the literature (20,24,25,27,31–33). The markings in the table can be interpreted as suitable (o), improved (+), and optimal (++).*

Feature	PSCM	SDCM	LLSM
Speed of acquisition (frames/s)	1-2	10-20	50-100
Phototoxicity reduction	o	+	++
Photobleaching reduction	o	+	++
Optical sectioning	+	+	+
Lateral resolution	+	+	+
Axial resolution	+	o	++
Flexible zoom factor	++	+	o
Flexible confocality	++	o	+
Flexible field of view	++	+	o
Simultaneous multicolor imaging	++	+	o
Spectral multiplexing	++	+	o

Acknowledgments: *This work has been partly supported by the Croatian Science Foundation under the project IP-2019-04-5382.*

REFERENCES

1. FISCHER RS, WU Y, KANCHANAWONG P, SHROFF H, and WATERMAN CM 2011 Microscopy in 3D: a biologist's toolbox. Trends Cell Biol 21: 682–691. https://doi.org/10.1016/j.tcb.2011.09.008
2. LEMON WC, MCDOLE K 2020 Live-cell imaging in the era of too many microscopes. Curr Opin Cell Biol 66: 34–42. https://doi.org/10.1016/j.ceb.2020.04.008
3. SCHERF N, HUISKEN J 2015 The smart and gentle microscope. Nat Biotechnol 33: 815–818. https://doi.org/10.1038/nbt.3310
4. JONKMAN J, BROWN CM, WRIGHT GD, ANDERSON KI, NORTH AJ 2020 Tutorial: guidance for quantitative confocal microscopy. Nat Protoc 15: 1585–1611. https://doi.org/10.1038/s41596-020-0313-9
5. PADDOCK SW, ELICEIRI KW 2014 Laser scanning confocal microscopy: history, applications, and related optical sectioning techniques. Methods Mol Biol 1075: 9–47. https://doi.org/10.1007/978-1-60761-847-8_2
6. PAWLEY J, Ed, 2006 Handbook of Biological Confocal Microscopy, Springer, New York, NY

7. ELLIOTT AD 2020 Confocal Microscopy: Principles and Modern Practices. Curr Protoc Cytom 92: e68 https://doi.org/10.1002/cpcy.68
8. WANG E, BABBEY CM, DUNN KW 2005 Performance comparison between the high-speed Yokogawa spinning disc confocal system and single-point scanning confocal systems. J Microsc 218(Pt 2): 148–159. https://doi.org/10.1111/j.1365-2818.2005.01473.x
9. BENEDETTI PA, EVANGELISTA V, GUIDARINI D, VESTRI S 1992 Confocal-line microscopy. J Microsc 165(Pt 1): 119–129. https://doi.org/10.1111/j.1365-2818.1992.tb04309.x
10. WINTER PW, SHROFF H 2014 Faster fluorescence microscopy: advances in high speed biological imaging. Curr Opin Chem Biol 20: 46–53. https://doi.org/10.1016/j.cbpa.2014.04.008
11. PETRÁŇ M, HADRAVSKÝ M, EGGER MD, GALAMBOS R 1968 Tandem-Scanning Reflected-Light Microscope. J Opt Soc Am, 58: 661–664. https://doi.org/10.1364/JOSA.58.000661
12. MURRAY JM, APPLETON PL, SWEDLOW JR, WATERS JC 2007 Evaluating performance in three-dimensional fluorescence microscopy. J Microsc 228(Pt 3): 390–405. https://doi.org/10.1111/j.1365-2818.2007.01861.x
13. GRÄF R, RIETDORF J, ZIMMERMANN T 2005 Live cell spinning disk microscopy. Adv Biochem Eng Biotechnol 95: 57–75. https://doi.org/10.1007/b102210
14. OREOPOULOS J, BERMAN R, BROWNE M 2014 Spinning-disk confocal microscopy: present technology and future trends. Methods Cell Biol 123: 153–175. https://doi.org/10.1016/B978-0-12-420138-5.00009-4
15. COLE R 2014 Live-cell imaging. Cell Adh Migr 8: 452–459. https://doi.org/10.4161/cam.28348
16. ICHA J, WEBER M, WATERS JC, NORDEN C 2017 Phototoxicity in live fluorescence microscopy, and how to avoid it. Bioessays 39: https://doi.org/10.1002/bies.201700003
17. WHITEHEAD LW, MCARTHUR K, GEOGHEGAN ND, ROGERS KL 2017 The reinvention of twentieth century microscopy for three-dimensional imaging. Immunol Cell Biol 95: 520–524. https://doi.org/10.1038/icb.2017.36
18. HEDDLESTON JM, CHEW T-L 2016 Light sheet microscopes: Novel imaging toolbox for visualizing life's processes. Int J Biochem Cell Biol 80: 119–123. https://doi.org/10.1016/j.biocel.2016.10.002
19. TERANIKAR T, LIM J, IJASEUN T, LEE J 2022 Development of Planar Illumination Strategies for Solving Mysteries in the Sub-Cellular Realm. Int J Mol Sci 23: 1643. https://doi.org/10.3390/ijms23031643
20. CHEN B-C, LEGANT WR, WANG K, SHAO L, MILKIE DE, DAVIDSON MW, JANETOPOULOS C, WU XS, HAMMER JA, LIU Z, ENGLISH BP, MIMORI-KIYOSUE Y, ROMERO DP, RITTER AT, LIPPINCOTT-SCHWARTZ J, FRITZ-LAYLIN L, MULLINS RD, MITCHELL DM, BEMBENEK JN, REYMANN A-C, BÖHME R, GRILL SW, WANG JT, SEYDOUX G, TULU US, KIEHART DP, BETZIG E 2014 Lattice light-sheet microscopy: Imaging molecules to embryos at high spatiotemporal resolution. Science 346: 1257998. https://doi.org/10.1126/science.1257998
21. LIU T-L, UPADHYAYULA S, MILKIE DE, SINGH V, WANG K, SWINBURNE IA, MOSALIGANTI KR, COLLINS ZM, HISCOCK TW, SHEA J, KOHRMAN AQ, MEDWIG TN, DAMBOURNET D, FORSTER R, CUNNIFF B, RUAN Y, YASHIRO H, SCHOLPP S, MEYEROWITZ EM, HOCKEMEYER D, DRUBIN DG, MARTIN BL, MATUS DQ, KOYAMA M, MEGASON SG, KIRCHHAUSEN T, BETZIG E 2018 Observing the cell in its native state: Imaging subcellular dynamics in multicellular organisms. Science 360: eaaq1392. https://doi.org/10.1126/science.aaq1392
22. HOBSON CM, AARON JS 2022 Combining multiple fluorescence imaging techniques in biology: when one microscope is not enough. Mol Biol Cell 33: tp1. https://doi.org/10.1091/mbc.E21-10-0506
23. ELGASS K, HARTMANN D, SIEBENMORGEN J, KALKBRENNER T, WEISSHART K 2021 Breakthrough instruments and products: Lattice Lightsheet 7-Non-invasive imaging of the subcellular dynamics of life. Rev Sci Instrum 92: 109501. https://doi.org/10.1063/5.0069728
24. MANLEY HR, POTTER DL, HEDDLESTON JM, CHEW T-L, KEIGHTLEY MC, LIESCHKE GJ 2020 Frontline Science: Dynamic cellular and subcellular features of migrating leukocytes revealed by in vivo lattice lightsheet microscopy. J Leukoc Biol 108: 455–468. https://doi.org/10.1002/JLB.3HI0120-589R
25. FRITZ-LAYLIN L K, RIEL-MEHAN M, CHEN B-C, LORD S J, GODDARD TD, FERRIN TE, NICHOLSON-DYKSTRA SM, HIGGS H, JOHNSON GT, BETZIG E, MULLINS RD 2017 Actin-based protrusions of migrating neutrophils are intrinsically lamellar and facilitate direction changes. eLife 6: e26990. https://doi.org/10.7554/eLife.26990
26. SCHULZ O, PIEPER C, CLEVER M, PFAFF J, RUHLANDT A, KEHLENBACH RH, WOUTERS FS, GROSSHANS J, BUNT G, ENDERLEIN J 2013 Resolution doubling in fluorescence microscopy with confocal spinning-disk image scanning microscopy. Proc Natl Acad Sci U S A 110: 21000–21005. https://doi.org/10.1073/pnas.1315858110
27. HAYASHI S, OKADA Y 2015 Ultrafast superresolution fluorescence imaging with spinning disk confocal microscope optics. Mol Biol Cell 26: 1743–1751. https://doi.org/10.1091/mbc.E14-08-1287
28. PFANDER C, BISCHOF J, CHILDRESS-POLI M, KEPPLER A, VIALE A, AIME S, ERIKSSON JE 2022 Euro-BioImaging - Interdisciplinary research infrastructure bringing together communities and imaging facilities to support excellent research. iScience 25: 103800. https://doi.org/10.1016/j.isci.2022.103800
29. WU Y, SHROFF H 2022 Multiscale fluorescence imaging of living samples. Histochem Cell Biol 158: 301–323. https://doi.org/10.1007/s00418-022-02147-4
30. KIM K 2022 Single-Shot Light-Field Microscopy: An Emerging Tool for 3D Biomedical Imaging. BioChip J 16: 397–408. https://doi.org/10.1007/s13206-022-00077-w
31. VELTMAN DM, WILLIAMS TD, BLOOMFIELD G, CHEN B-C, BETZIG E, INSALL RH, KAY RR 2016 A plasma membrane template for macropinocytic cups. Elife 5: e20085. https://doi.org/10.7554/eLife.20085
32. MARINOVIĆ M, MIJANOVIĆ L, ŠOŠTAR M, VIZOVIŠEK M, JUNEMANN A, FONOVIĆ M, TURK B, WEBER I, FAIX J, FILIĆ V 2019 IQGAP-related protein IqgC suppresses Ras signaling during large-scale endocytosis. Proc Natl Acad Sci USA 116: 1289–1298. https://doi.org/10.1073/pnas.1810268116
33. JONKMAN J, BROWN CM 2015 Any Way You Slice It-A Comparison of Confocal Microscopy Techniques. J Biomol Tech 26: 54–65. https://doi.org/10.7171/jbt.15-2602-003