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Methods for Tracking Individual Centrioles in Living Cells

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Abstract

The ability to track individual centrioles in living cells represents a pivotal methodology for advancing our understanding of centrosome biology, cell division, and ciliogenesis. This article provides a comprehensive review of the current methodological landscape for centriole tracking, bridging historical approaches with cutting-edge innovations. We begin by outlining the fundamental challenges—including the organelle's sub-diffraction size, dynamic life cycle, and close pairing—that have historically limited observation. The review then details a hierarchical progression of techniques, from foundational static methods like immunofluorescence and electron microscopy to the direct live-cell imaging enabled by fluorescent protein fusions and advanced microscopy platforms such as spinning-disk confocal and Total Internal Reflection Fluorescence (TIRF) microscopy. A significant focus is placed on super-resolution methods, particularly Stimulated Emission Depletion (STED) microscopy, which allows for the resolution of individual centrioles within a pair in real time. We further explore critical genetic manipulation and labeling strategies, including CRISPR/Cas9-mediated endogenous tagging and photoactivatable proteins for pulse-chase experiments. Practical, detailed protocols for long-term tracking, super-resolution imaging, and lineage analysis are presented, followed by an in-depth discussion of the computational pipeline for data analysis, encompassing object detection, trajectory linking, and quantitative kinetic measurements. Finally, we address common artifacts and mitigation strategies, and conclude by highlighting emerging technologies like correlative light-electron microscopy (CLEM) and lattice light-sheet microscopy (LLSM) that promise to further revolutionize the field by linking dynamic behavior with ultrastructure and enabling studies within complex 3D tissues.

Keywords: Centriole; Live-Cell Imaging; Super-Resolution Microscopy; Fluorescent Protein; CRISPR/Cas9; Trajectory Analysis.

Introduction: Foundational Challenges and Historical Context

The real-time tracking of individual centrioles stands as one of the most technically demanding pursuits in modern cell biology. This capability is paramount for deciphering the fundamental mechanisms governing centriole duplication, maturation, disengagement, and their roles in mitotic spindle assembly, ciliogenesis, and cellular signaling. However, achieving high-fidelity, long-term observation of these organelles in living cells is impeded by a confluence of formidable physical and biological challenges.

The primary obstacle is the centriole's diminutive size. A typical centriole is approximately 250 nm in diameter and 500 nm in length (Bowler et al., 2019), dimensions that reside near the diffraction limit of visible light (~200-250 nm). This physical constraint fundamentally blurs their image in conventional light microscopy, making it difficult to resolve a single centriole from its closely apposed partner within a centriole pair. Furthermore, the two centrioles in a G1-phase cell—the older "mother" and the younger "daughter"—are tethered together, often appearing as a single fluorescent object unless super-resolution techniques are employed.

The second major challenge is the dynamic nature of the centriole life cycle. Centrioles are not static entities; they undergo a tightly regulated sequence of events spanning hours to days. Key processes like procentriole formation, elongation, and subsequent maturation (characterized by the acquisition of distal and subdistal appendages) are transient and asynchronous between the two centrioles of a pair (Fong et al., 2016). Tracking must therefore capture not only movement but also these structural and compositional changes over extended periods. This necessitates long-term imaging—often spanning multiple cell cycles (24-48 hours)—while preserving cell viability, which introduces challenges of phototoxicity and photobleaching.

Historically, our understanding of centriole architecture and number was derived almost exclusively from electron microscopy (EM) studies of fixed samples (Paintrand et al., 1992). While EM provided unparalleled ultrastructural detail, it offered only static, snapshot views of a dynamic process. Correlating events across time required painstaking reconstruction from populations of cells at different stages, an approach prone to misinterpretation of temporal sequences.

The paradigm shift began with the advent of fluorescent protein technology and the identification of centriole-specific marker proteins. The fusion of proteins like centrin (a calcium-binding protein associated with the centriole lumen) or SAS-6 (a key component of the cartwheel) to green fluorescent protein (GFP) enabled the visualization of centrioles in living cells for the first time (Piel et al., 2000). This breakthrough launched the field of live-cell centriole biology. Early studies successfully monitored centriole duplication cycles, but the diffraction-limited nature of the signals often precluded definitive tracking of individual centrioles within a pair, especially during stages of close association.

The subsequent revolution in super-resolution fluorescence microscopy (e.g., STED, PALM, STORM) broke the diffraction barrier, allowing the visualization of centriole ultrastructure in fixed

cells with nanoscale resolution (Lau et al., 2012). However, applying these techniques to long-term live-cell tracking remained, and still remains, challenging due to high illumination doses and complex image analysis.

Therefore, contemporary methods for tracking individual centrioles in living cells represent a sophisticated integration of multi-faceted approaches: advanced molecular labeling strategies to ensure specificity and photostability; cutting-edge, gentle imaging modalities that maximize spatial and temporal resolution while minimizing cellular damage; and robust computational algorithms for detection, linking, and trajectory analysis. This article reviews the current state of these methodologies, focusing on the synergistic solutions developed to overcome the inherent challenges of size, dynamics, and proximity. We will explore how the combination of targeted fluorescence labeling, live-cell compatible super-resolution techniques, and sophisticated computational analytics has transformed our ability to follow the life story of individual centrioles from birth to functional deployment.

The development of these methods is not merely a technical exercise; it has been driven by critical biological questions. For instance, tracking individual mother and daughter centrioles has been essential in revealing the asymmetric fate of centrioles after mitosis (Anderson & Stearns, 2009) and in understanding the kinetics of centriole disengagement, a critical licensing step for subsequent duplication. As we delve into the specific methods, the intrinsic link between technological innovation and biological discovery will become evident.

Mathematical Representation of Tracking Fidelity

The confidence in correctly identifying and linking a centriole between consecutive time frames in a live-cell experiment can be conceptually framed. Let the probability of correct detection in a single frame be P_d, which is a function of signal-to-noise ratio (SNR) and labeling specificity. Let the probability of correct association between two detected objects in consecutive frames be P_a, which depends on spatial proximity (maximum expected displacement, d_max) and similarity of features (e.g., fluorescence intensity, shape descriptors). For a trajectory spanning N frames, the probability of maintaining a perfectly correct track, assuming independent errors, is proportional to:

$$P_{track} \propto (P_d)^N \times (P_a)^{N-1}$$

In reality, P_d and P_a are not independent and are severely taxed by the biological context: low P_d due to dim labeling and small size, and low P_a during stages where two centrioles are in close proximity (separated by a distance << d_max) or undergo rapid, unpredictable movements. Advanced tracking algorithms use global optimization and motion modeling to improve P_a beyond simple nearest-neighbor approaches, effectively increasing the practical d_max for reliable tracking in complex cellular environments.

Key Methodological Approaches (Hierarchy from Classical to Advanced)

The evolution of centriole tracking methodologies mirrors the broader technological progress in cell biology, moving from static, high-resolution snapshots to dynamic, functional readouts in living systems. This section outlines this hierarchy, detailing the principles, applications, and limitations of each key approach.

Indirect Methods in Fixed Samples (Precursors)

Prior to live-cell imaging, our understanding of centriole architecture and number was derived from static, high-resolution methods. These techniques remain essential for validating live-cell observations and providing crucial ultrastructural context.

Immunofluorescence (IF) is the workhorse of cellular localization. By using antibodies against centriolar proteins such as γ-tubulin (centrosomal matrix), centrin (centriolar lumen), or CEP164 (distal appendages), researchers can visualize centrioles and centrosomes (Bornens, 2012). However, its resolution is constrained by the diffraction limit of light (~250 nm laterally), which is comparable to the diameter of a centriole. Consequently, the two centrioles within a tightly associated pair are typically visualized as a single fluorescent punctum, precluding individual tracking. This method provides only a static snapshot, offering no insight into dynamics, and is susceptible to artifacts from antibody cross-reactivity or epitope masking.

Immuno-electron microscopy (Immuno-EM) overcomes the diffraction barrier by conjugating antibodies to electron-dense markers like colloidal gold particles, which are then visualized using transmission electron microscopy (TEM). This achieves remarkable sub-organelle resolution (1-10 nm), allowing for the precise sub-localization of proteins within the centriole's intricate geometry, such as distinguishing proteins of the cartwheel from those of the microtubule wall (Bohannon et al., 2019). The trade-offs are extreme: the sample preparation is complex and arduous, the technique is inherently static and low-throughput, and it requires specialized expertise.

Electron Tomography (ET), particularly cryo-electron tomography (cryo-ET), represents the pinnacle of structural analysis. By acquiring TEM images of a vitrified, unstained sample tilted through a range of angles (e.g., ±60°), a detailed 3D reconstruction (a tomogram) can be computed. This technique can resolve individual microtubule triplets within the centriole barrel at 2-5 nm resolution, revealing the stunning 9-fold symmetric architecture and the intricate geometry of appendages (Greenan et al., 2018). Like Immuno-EM, it is restricted to fixed/vitrified specimens, is exceptionally labor-intensive, and analyzes a vanishingly small volume of cell, making it a discovery tool rather than one for routine tracking.

Direct Live-Cell Imaging Methods

Centriole Labeling for Light Microscopy

The cornerstone of live-cell tracking is specific, photostable fluorescent labeling that does not disrupt function.

Fluorescent Protein (FP) Fusions are the most common strategy. Key centriolar proteins are genetically fused to FPs (e.g., GFP, mCherry, mNeonGreen). Targets are chosen based on biological question:

- **Core/Scaffold proteins**: SAS-6 or STIL label nascent procentrioles during early assembly.
- **Structural components**: Centrin or CEP135 mark the core structure throughout the cycle.
- **Maturity markers**: CEP164 or Ninein are acquired specifically on mother centrioles, enabling age discrimination.

Delivery is via plasmid transfection, lentiviral transduction, or creation of stable cell lines. A critical caveat is that overexpression can disrupt normal centriole duplication, number, or function (Habedanck et al., 2005). Control experiments using low-expression constructs, truncated tags, or rescue of endogenous protein knockout are mandatory.

Synthetic Fluorophores and Probes offer bright, photostable alternatives. SiR-tubulin, a cell-permeable, fluorogenic "Janelia Fluor" probe, binds polymerized tubulin with high affinity. It brightly labels the pericentriolar material and emanating microtubules, providing an excellent marker for centrosome position and movement over long periods with minimal phototoxicity (Lukinavičius et al., 2014). While it does not label the centriole barrel itself, its robustness makes it ideal for migration and segregation studies.

Microscopy Platforms and Acquisition Techniques

The choice of imaging platform is dictated by the necessary trade-off between spatial resolution, temporal resolution, phototoxicity, and experiment duration.

Widefield Fluorescence Microscopy offers fast acquisition and relatively low light exposure per image. For centriole cycle studies, acquiring z-stacks (5-7 slices) every 3-10 minutes over 24-72 hours using a high-numerical aperture (NA) objective (e.g., 60x/1.4 NA) and phenol red-free medium allows reliable tracking of centriole number and general position through multiple cell cycles (Loncarek & Khodjakov, 2009).

Laser Scanning Confocal Microscopy (LSM) improves axial resolution and optical sectioning by using a pinhole to eliminate out-of-focus light. It is excellent for high-resolution 3D (xyzt) imaging to determine centriole position relative to the nucleus or cell cortex. However, the point-scanning method can be slow and, due to high peak laser power at the sample, phototoxic for very long-term experiments.

Spinning Disk Confocal Microscopy is a workhorse for dynamic centriole tracking. A rotating disk with thousands of pinholes scans multiple points simultaneously. This achieves high speed and dramatically reduced photodamage, enabling high-temporal-resolution imaging (e.g., every 30-60 seconds for >24 hours) of rapid processes like centriole separation in mitosis or ciliary beating, typically using highly sensitive EM-CCD or sCMOS cameras.

Total Internal Reflection Fluorescence (TIRF) Microscopy illuminates only a thin (~100 nm) evanescent field at the cell-substrate interface. This provides an exceptional signal-to-noise ratio for imaging centrioles during membrane-proximal events, such as the initial docking of a mother centriole to the plasma membrane and the early stages of ciliary vesicle formation during ciliogenesis (Mazo et al., 2016).

Advanced and Super-Resolution Methods

These methods break the diffraction barrier, enabling the visualization of individual centrioles within a closely spaced pair in living cells.

Stimulated Emission Depletion (STED) Microscopy is currently the most effective super-resolution method for live-cell centriole tracking. It uses a donut-shaped "depletion" laser to de-excite fluorophores at the periphery of the excitation spot, effectively shrinking the point-spread function to 50-80 nm. Live-cell STED has been used to resolve mother and daughter centrioles within a pair and visualize cartwheel dynamics (Lau et al., 2012). It requires exceptionally photostable labels (e.g., Abberior STAR dyes, certain FPs) and meticulous optimization of depletion laser power to balance resolution gain against phototoxicity.

Single-Molecule Localization Microscopy (SMLM: PALM/STORM) relies on the stochastic activation and precise localization of single fluorescent molecules over tens of thousands of frames. It achieves the highest resolution (20-40 nm) and has been used to map the nanoscale organization of proteins like CEP164 within the centriole's distal appendages in fixed cells (Sillibourne et al., 2011). However, its slow acquisition speed (minutes per reconstruction) and high photon demands have, until recently, largely precluded its application to dynamic processes in living cells.

Structured Illumination Microscopy (SIM) uses patterned (striped) illumination to double the spatial resolution (to ~110 nm) by computationally extracting high-frequency information otherwise lost due to diffraction. It is significantly faster and gentler than STED or SMLM. Modern implementations (e.g., instant SIM) allow video-rate acquisition, making SIM highly suitable for tracking centriole separation, disengagement, and movement with enhanced detail over long periods, effectively bridging the gap between conventional and super-resolution live-cell imaging (Krämer et al., 2020).

Methods for Genetic Labeling and Manipulation

SunTag System is a powerful signal amplification strategy for visualizing low-abundance endogenous proteins. A centriolar protein is fused to an array of 24 peptide epitopes (SunTag). Co-expressed single-chain variable fragment (scFv) antibodies, fused to GFP, bind these

epitopes, creating a bright "fluorescent bead" on the target. This allows robust tracking of centrioles expressing endogenous levels of proteins like CEP164 without overexpression artifacts (Tanenbaum et al., 2014).

CRISPR/Cas9-Mediated Endogenous Tagging is considered the gold standard for physiological labeling. CRISPR/Cas9 is used to insert an FP coding sequence, along with a flexible linker, directly into the endogenous locus of a centriolar gene (e.g., CEP152, PLK4), ensuring expression at native levels under the control of the gene's own regulatory elements. This elegantly eliminates all concerns regarding overexpression and provides the most biologically faithful fluorescent reporter.

Photoactivatable/Convertible Fluorescent Proteins (PA-FPs) enable "pulse-chase" lineage experiments. A PA-FP like Dendra2 or mEos is fused to a centriolar protein like centrin. A brief pulse of 405-nm light can be used to photoconvert the fluorescence of centrioles in a specific region or cell from green to red. The subsequent fate—duplication, inheritance, ciliation—of these specifically "born-dated" red centrioles can then be tracked independently from the population of unconverted green centrioles, a powerful approach for studying centriole age and inheritance (Anderson & Stearns, 2009).

Laser Ablation (Microsurgery) is a key functional manipulation tool. A focused femtosecond-pulsed laser can be used to selectively disrupt (ablate) a single centriole within a pair, based on its fluorescent label. Observing the subsequent behavior of the remaining centriole—whether it can now duplicate (licensing), recruit PCM asymmetrically, or initiate cilium formation—provides direct, causal evidence for functional roles and regulatory mechanisms, such as the inhibition of re-duplication (Wong et al., 2015). This transforms tracking from a purely observational to an interventional, hypothesis-testing methodology.

Detailed Protocols and Practical Considerations

The successful execution of centriole tracking experiments hinges on meticulous protocol design that balances the need for high-resolution data with the imperative of preserving cell health. This section outlines three detailed, reproducible protocols that exemplify modern approaches to long-term observation, super-resolution imaging, and functional lineage tracing of centrioles.

Protocol for Long-Term Centriole Tracking in Interphase Cells

This protocol is designed for monitoring centriole duplication, maturation, and positioning over multiple cell cycles (Loncarek & Khodjakov, 2009).

Cell Model and Labeling:

- **Preferred Cell Line**: hTERT-immortalized human retinal pigment epithelial cells (RPE1). This line is considered a gold standard due to its stable, flat morphology, reliable cell cycle progression, robust cilium formation, and near-diploid karyotype.

- **Fluorescent Labeling**: Use a stable RPE1 cell line expressing Centrin1-GFP (labels the lumen of both centrioles) and mCherry-α-tubulin (labels microtubules, providing cellular context). As an alternative, transiently transfect cells with a plasmid such as pEGFP-Centrin2 (or pEGFP-CETN2) 24-48 hours prior to imaging using a low-toxicity reagent like Lipofectamine 3000. A low transfection efficiency (<30%) is acceptable to ensure cells are not overcrowded and to minimize overexpression artifacts.

- **Cell Preparation**: Plate cells sparsely (20-30% confluency) on 35 mm glass-bottom dishes (No. 1.5 coverslip thickness) coated with a suitable substrate (e.g., fibronectin). 16-24 hours before imaging, replace growth medium with live-cell imaging medium (e.g., FluoroBrite DMEM, Leibovitz's L-15), supplemented with 10% fetal bovine serum, 4 mM L-glutamine, and 1% penicillin/streptomycin. Phenol red-free medium is essential to minimize background fluorescence.

Microscopy Setup and Acquisition:

- **System**: An inverted epifluorescence microscope equipped with a high-sensitivity camera (e.g., sCMOS or back-illuminated EMCCD), a motorized stage, a full environmental chamber maintaining 37°C and 5% CO_2, and a hardware autofocus system to compensate for focal drift.

- **Objective**: A high-numerical aperture (NA) oil immersion objective is critical for light collection. A 63x/1.4 NA Plan-Apochromat or 100x/1.46 NA Plan-Apochromat objective is ideal.

- **Illumination**: LED-based light sources are preferred over metal halide or mercury lamps due to their stability, fast switching, and reduced heat generation.

- **Acquisition Program**:

    - Acquire a z-stack spanning the entire volume of the cell. A typical range is 7-9 optical sections with a step size of 0.5 µm.

    - Set the time interval between stacks to 5-10 minutes. This is frequent enough to capture centriole duplication and movement without causing excessive photodamage.

    - Acquire images in two channels sequentially: first in the GFP channel (Centrin), then in the Texas Red or mCherry channel (microtubules). Minimize exposure time; start with 100-300 ms per z-slice and adjust laser/LED power to achieve a clear signal with minimal background.

    - Program the experiment to run for 48-72 hours.

- **Viability Controls**: Include a phase-contrast or differential interference contrast (DIC) image at each time point to monitor overall cell morphology, division, and signs of stress (e.g., membrane blebbing). It is prudent to include a control position on the dish with unilluminated cells to verify normal growth at the end of the experiment.

Protocol for Super-Resolution Tracking of Centriole Separation Using STED

This protocol leverages STED nanoscopy to resolve and track the two closely associated centrioles within a pair in living cells (Lau et al., 2012).

Cell Labeling and Sample Preparation:

- **Labeling Strategy**: For physiological relevance, use a clonal RPE1 cell line generated by CRISPR/Cas9-mediated knock-in, where a bright, photostable fluorescent protein like mNeonGreen is endogenously tagged to a core centriolar protein such as SAS-6 or CETN1. This ensures correct stoichiometry and localization.
- **Sample Preparation**: Plate cells sparsely on high-performance #1.5H glass-bottom dishes. Use phenol red-free imaging medium as described in Protocol 3.1.

STED Microscopy Acquisition:

- **System**: A commercial gated-STED microscope (e.g., Leica Stellaris, Abberior FACILITY) equipped with high-precision lasers and time-gated detection.
- **Optimization Steps**:
    1. *Find Cells*: Use confocal mode with minimal 488 nm excitation laser power to locate cells with bright centriolar signal.
    2. *STED Power Calibration*: This is the most critical step. The intensity of the donut-shaped STED depletion laser (e.g., 592 nm or 775 nm) must be titrated. Begin with a low power (e.g., 5% of maximum) and acquire a STED image. Gradually increase the power until the centriole pair, which appears as a single oval in confocal mode, visibly splits into two distinct, round spots. Record the minimum STED power that achieves this consistent separation. This power defines the practical lateral resolution, which should be ~70-80 nm. Exceeding this power dramatically increases phototoxicity without significant resolution gain.
    3. *Acquisition Settings*:
        - Acquire single-plane (XY) time-series images. 3D-STED is typically too slow and damaging for live cells.
        - Set a time interval of 30-60 seconds. This is sufficient to track centriole movement and separation.
        - Keep the total experiment duration short (1-3 hours) to limit cumulative photodamage.
        - Use photon-counting hybrid detectors (HyD or GaAsP) in gated mode to maximize signal-to-noise ratio.

Protocol for "Pulse-Chase" Lineage Tracing with Photoactivatable Fluorescent Proteins (PA-FPs)

This protocol exploits PA-FPs to mark a specific subset of centrioles at a defined time and follow their fate through subsequent cell cycles, enabling direct study of asymmetry (Anderson & Stearns, 2009).

Cell Line and Preparation:

- **Cell Line**: U2OS or RPE1 cells stably expressing Centrin1-Dendra2. Dendra2 is a green-to-red photoconvertible protein.
- **Preparation**: Plate cells sparsely on glass-bottom dishes in imaging medium.

Microscopy and Photoconversion:

- **System**: A confocal or spinning disk microscope equipped with at least two laser lines: a 488 nm laser for imaging green fluorescence and a 405 nm laser for photoconversion.
- **The "Pulse" Phase**:
    1. Using low-power 488 nm illumination, identify a cell in late G2 or prophase, recognizable by its rounded morphology and the presence of two clearly separated centrosomes.
    2. Position the region of interest (ROI) over one of the two centrosomes. The ROI should be just large enough to encompass it.
    3. Deliver a brief, high-intensity pulse of 405 nm laser light (e.g., 5-20 iterations at 100% laser power, with dwell time per pixel ~1-10 µs). Successful photoconversion is immediately visible as a loss of green signal and the emergence of red signal within the targeted ROI. The other centrosome should remain green.
- **The "Chase" Phase**:
    1. Immediately after photoconversion, begin a time-lapse acquisition. Acquire dual-channel images: one for the green channel (all centrioles) and one for the red channel (photoconverted centrioles only).
    2. Use a time interval of 2-5 minutes to follow the cell through mitosis and into the next interphase.
    3. Continue imaging for 12-24 hours to observe the inheritance pattern of the marked centriole(s) in the resulting daughter cells.

Data Analysis and Key Parameters for All Protocols:

Regardless of the protocol, quantitative analysis is paramount. Key parameters to extract include:

1. **Trajectory (X(t), Y(t), Z(t))**: The positional coordinates of each centriole over time.
2. **Mean Square Displacement (MSD)**: A fundamental measure of motility. For a trajectory, the MSD at a time lag τ is calculated as:
   $MSD(\tau) = \langle (X(t+\tau) - X(t))^2 + (Y(t+\tau) - Y(t))^2 \rangle$
   where the angle brackets denote an average over all time points *t* in the trajectory. The slope of the MSD(τ) vs. τ plot on a log-log scale reveals the mode of motion: a slope of 1 indicates simple diffusion, a slope of 2 indicates directed motion.
3. **Separation Distance**: The Euclidean distance between the two centrioles of a pair over time, calculated as:
   $d(t) = \sqrt{(X_1(t) - X_2(t))^2 + (Y_1(t) - Y_2(t))^2 + (Z_1(t) - Z_2(t))^2}$
   This metric is crucial for studying disengagement and separation events.
4. **Fluorescence Intensity (I(t))**: The integrated intensity of the centriole spot over time can report on protein recruitment (e.g., maturation markers like CEP164) or loss (e.g., during disassembly).

Software packages like Imaris (Bitplane), Arivis, TrackMate (Fiji/ImageJ), or custom MATLAB/Python scripts are used for automated spot detection, linking into trajectories, and calculating these metrics. Robust tracking requires setting appropriate constraints for detection threshold, maximum expected displacement between frames, and minimum track length.

Data Analysis and Software

The acquisition of high-quality image sequences is only the first step. The transformation of these multi-dimensional datasets (xyzt, and often multiple wavelengths) into quantitative, biologically meaningful insights requires a robust pipeline of computational analysis. This pipeline, outlined in Figure 1, typically involves pre-processing, spot detection, trajectory linking, and quantitative track analysis, each step presenting unique challenges when applied to small, dynamic objects like centrioles.

Image Pre-processing

Raw live-cell images are often degraded by noise, out-of-focus blur, and temporal intensity fluctuations. Pre-processing aims to improve signal quality prior to detection.

- **Deconvolution**: For widefield and, to a lesser extent, confocal images, computational deconvolution is a powerful tool. Algorithms like the Richardson-Lucy iterative deconvolution use a measured or theoretical point-spread function (PSF) of the microscope to reassign out-of-focus light back to its point of origin (Sage et al., 2017). This process increases contrast and effective resolution, making centrioles sharper and easier to segment. However, it must be applied judiciously, as overly aggressive

deconvolution can introduce artifacts, particularly in low signal-to-noise ratio (SNR) time-lapse data.

- **Background Subtraction and Filtering**: Rolling-ball or top-hat filtering can remove uneven background illumination. Temporal median filtering (e.g., a 3-frame window) can reduce salt-and-pepper noise without blurring moving structures. For super-resolution data like STED or SMLM, specialized filtering and reconstruction algorithms are built into the vendor software.

Object Detection (Spot Detection)

The accurate localization of centrioles in each frame is the most critical step. Centrioles appear as sub-diffraction or near-diffraction limited spots, requiring specialized detection algorithms.

- **Laplacian of Gaussian (LoG) Detector**: A classic and effective method. The image is convolved with a LoG kernel, which acts as a blob detector. The kernel's width (sigma) is matched to the expected size of the centriole's image (its PSF). Peaks in the LoG-filtered image correspond to spot centers. This method is computationally efficient and integrated into many platforms, such as TrackMate (Tinevez et al., 2017).

- **Machine Learning-Based Detection**: For noisy data or complex backgrounds, machine learning approaches outperform simple filters. A commonly used method trains a Support Vector Machine (SVM) or a convolutional neural network (CNN) on a manually curated set of image patches containing "centriole" and "not-centriole" examples. The classifier learns distinctive features (intensity patterns, texture, shape) and can then detect spots with high accuracy and resistance to noise. The key parameters extracted for each detected spot are its sub-pixel coordinates (x, y, z), its integrated intensity (I), and an estimate of its size, often reported as the Full Width at Half Maximum (FWHM) of a fitted Gaussian function.

Trajectory Linking (Tracking)

Linking detected spots across consecutive time frames to form continuous trajectories is a non-trivial computational challenge, especially given centriole dynamics—they can move rapidly, pause, and come in close proximity.

- **Simple Nearest Neighbor**: The simplest algorithm links a spot in frame *t* to its closest neighbor within a user-defined maximum search radius in frame *$t+1$*. This fails easily in dense environments or with rapid, directional motion.

- **Kalman Filter-Based Predictive Tracking**: More robust algorithms, such as those in Imaris and u-track, employ a Kalman filter (Jaqaman et al., 2008). This filter predicts a spot's position in the next frame based on its previous velocity, then searches for a detection within a specified radius around this prediction. It can handle temporary disappearances (e.g., due to low SNR) by allowing gap-closing over a few frames. The

Kalman filter's state update and prediction equations are central:
Prediction Step:

$x_{k|k-1} = F_k x_{k-1|k-1}$ (predict state)

$P_{k|k-1} = F_k P_{k-1|k-1} F_k^T + Q_k$ (predict covariance)

Update Step (when a detection is found):

$y_k = z_k - H_k x_{k|k-1}$ (measurement residual)

$K_k = P_{k|k-1} H_k^T (H_k P_{k|k-1} H_k^T + R_k)^{-1}$ (Kalman gain)

$x_{k|k} = x_{k|k-1} + K_k y_k$ (updated state estimate) (updated covariance)
Here, x is the state vector (position, velocity), P is the estimate covariance, F is the state transition model, H is the observation model, Q is process noise, R is measurement noise, and z is the actual measurement (detected position).

Global Optimization (u-track): The most powerful approach for dense, complex data is global multi-target tracking, as implemented in u-track (Jaqaman et al., 2008). Instead of linking frame-by-frame, it considers all possible links across a window of frames and finds the set of trajectories that optimizes a global cost function, which balances motion consistency, detection fidelity, and biological constraints (e.g., centrioles cannot merge). This is essential for correctly tracking centrioles through mitosis or when two pairs come into close contact.

Quantitative Track Analysis

Once trajectories are established, a wealth of quantitative metrics can be computed to describe centriole behavior.

- **Kinetic Parameters**:
    - Instantaneous Speed: $v(t) = \sqrt{(\Delta x/\Delta t)^2 + (\Delta y/\Delta t)^2 + (\Delta z/\Delta t)^2}$
    - Mean Square Displacement (MSD): This is the primary metric for classifying motion. For a trajectory with N positions, MSD at time lag τ is calculated as: $MSD(\tau) = \frac{1}{N-\tau} \sum_{i=1}^{N-\tau} [(x_{i+\tau} - x_i)^2 + (y_{i+\tau} - y_i)^2]$
      Plotting MSD(τ) versus τ on a log-log scale reveals the nature of motion: a slope of 1 indicates pure diffusion, a slope of 2 indicates directed motion, and a slope <1 indicates confined motion within a subcellular domain.
- **Pairwise Metrics for Centriole Duplets**:
    - *Inter-Centriole Distance*: The Euclidean distance between the two centrioles in a pair over time: $d(t) = \sqrt{(x_1(t) - x_2(t))^2 + (y_1(t) - y_2(t))^2}$.

      This metric directly reports on events like disengagement (a sudden increase in d(t)) and subsequent separation.

    - *Relative Orientation*: The angle of the vector connecting the two centrioles relative to a cellular axis (e.g., the nucleus-centrosome axis).

- **Temporal Correlations**:
    - *Maturation Kinetics*: The fluorescence intensity of a maturation marker (e.g., CEP164-mCherry) on a centriole can be plotted over time. The time from the first detectable signal (birth) to plateau intensity defines the maturation period.
    - *Cell Cycle Correlation*: By co-staining with a nuclear marker (e.g., H2B-GFP), centriole dynamics can be synchronized to cell cycle phases (G1, S, G2, M) based on nuclear morphology (chromatin condensation, nuclear envelope breakdown).

Popular Software Platforms

The choice of software often depends on the scale of data and required level of customization.

- *Imaris (Bitplane)*: A commercial, comprehensive suite. Its "Spots" module offers semi-automated detection and tracking with a user-friendly interface, excellent 3D/4D visualization, and built-in analysis for MSD and intensity over time. It is ideal for robust analysis of moderate-sized datasets.
- *TrackMate (Fiji/ImageJ)*: A powerful, open-source plugin (Tinevez et al., 2017). It is highly flexible, offering multiple detection (LoG, CNN) and linking algorithms (simple LAP, Kalman). Its strength lies in transparency, customization, and scripting capabilities for high-throughput analysis. It is the tool of choice for method development and large-scale batch processing.
- *Arivis Vision4D*: Another commercial platform known for handling extremely large multi-dimensional image datasets (e.g., from light-sheet microscopy) efficiently. Its tracking tools are robust and integrate well with machine learning segmentation.
- *μManager with Plugins*: The open-source μManager acquisition software can be extended with analysis plugins, enabling a seamless link between image acquisition and preliminary tracking on the same platform.
- *Custom Scripts (MATLAB, Python)*: For specialized or high-throughput analyses, researchers often write custom code using libraries like trackpy (Python) or u-track (MATLAB). This provides maximum control over algorithms and output formats for statistical modeling and figure generation.

In conclusion, the analytical workflow is as crucial as the imaging protocol. A well-designed analysis pipeline, carefully validating each step from detection to statistical testing, transforms raw pixel data into rigorous, quantitative models of centriole behavior, ultimately revealing the principles that govern these enigmatic organelles.

Challenges, Artifacts, and Mitigation Strategies

Despite significant technological advances, tracking individual centrioles over biologically relevant timescales remains fraught with technical pitfalls. These challenges, if unaddressed, can lead to artifactual data, misinterpretation of dynamics, and experimental failure. This section details the major obstacles encountered in live-cell centriole tracking, their underlying causes, and established strategies for their mitigation. A systematic approach to these issues is paramount for generating robust, reproducible results.

Photobleaching and Phototoxicity

The most pervasive challenges in long-term fluorescence imaging are photobleaching (the irreversible destruction of a fluorophore) and phototoxicity (light-induced cellular damage). These are intrinsically linked, as both result from the interaction of light with the sample, generating reactive molecular species.

- Photobleaching manifests as an exponential decay in fluorescent signal over time, ultimately leading to the disappearance of the tracked object. The rate of bleaching is described by $I(t)=I0*e-t/\tau$ is the initial intensity and τ is the bleaching time constant. For centriole tracking, this can prematurely terminate a trajectory.
    - **Mitigation Strategies**:
        1. *Use Photostable Fluorophores*: Replace traditional FPs like GFP with newer, more photostable variants such as mNeonGreen, mScarlet, or mCherry2 (Bindels et al., 2017). For synthetic labeling, Janelia Fluor dyes or Abberior STAR dyes offer exceptional brightness and resistance to bleaching.
        2. *Minimize Photon Dose*: Adhere to the ALARA principle (As Low As Reasonably Achievable). Reduce illumination intensity and exposure time to the absolute minimum required for reliable detection. Use the highest quantum efficiency camera available to maximize signal collection from fewer photons.
        3. *Employ Anti-fade Reagents*: Supplement imaging media with oxygen-scavenging systems (e.g., Oxyrase, PCA/PCD protocol) or cocktails of antioxidants (e.g., Trolox, ascorbic acid) to reduce the concentration of molecular oxygen and reactive oxygen species (ROS) that drive photobleaching (Icha et al., 2017).
- Phototoxicity is more insidious, as cells may appear normal while undergoing subtle or delayed stress that alters centriole biology (e.g., cell cycle arrest, aberrant duplication). It is caused primarily by the generation of ROS during fluorophore excitation.
    - **Mitigation Strategies**:

1. *Optimize Imaging Modality*: Spinning disk confocal microscopy is vastly superior to point-scanning confocal for live-cell work due to its lower peak illumination power and faster scanning. For super-resolution, SIM is significantly less phototoxic than STED for comparable resolution gains.

2. *Use Longer Wavelengths*: Whenever possible, use fluorophores excited by red or far-red light (e.g., 640 nm). Longer wavelengths are less energetic and penetrate cells with less scattering, reducing overall photodamage.

3. *Implement Viability Assays*: Include live-dead indicators in parallel experiments. For instance, add a low concentration of propidium iodide to the media, which is excluded from live cells but fluoresces upon nuclear entry in dead cells. Alternatively, perform post-imaging clonogenic assays to assess long-term survival.

Sample Drift and Focal Instability

Mechanical and thermal drift during multi-hour experiments can move the cell out of the field of view or focal plane, breaking trajectories. This is especially problematic when tracking small, precisely localized objects like centrioles.

- **Causes**: Thermal expansion/contraction of the microscope, stage settling, and instability in objective or dish positioning.

- **Mitigation Strategies**:

    1. *Hardware-based Stabilization*: Use microscopes equipped with hardware autofocus systems (e.g., Zeiss Definite Focus, Nikon Perfect Focus). These use an infrared laser or other mechanism to track the coverslip-sample interface, actively compensating for axial (z) drift in real-time.

    2. *Software-based Correction*: Acquire images with fiducial markers. This can be stable fluorescent beads (e.g., TetraSpeck) sparsely coated on the dish, or even a distinct, non-motile cellular structure. In post-processing, cross-correlation algorithms can calculate the drift vector from these markers in each frame and apply a compensatory shift to the entire image stack, a process known as image registration (Preibisch et al., 2010).

Low Signal-to-Noise Ratio (SNR)

A weak signal from a single centriole, often due to low endogenous protein copy number or small labeling fraction, can be lost in cellular autofluorescence and camera noise, preventing reliable detection and tracking.

- **Mitigation Strategies**:

1. *Signal Amplification*: Deploy the SunTag system or similar scaffolds. By recruiting multiple copies of a fluorescent scFv to a single centriolar protein, the effective brightness can be increased 10- to 20-fold, dramatically improving SNR without protein overexpression (Tanenbaum et al., 2014).

2. *Camera Binning*: Spatial binning (e.g., 2x2) on the camera sensor combines charge from adjacent pixels. This increases SNR quadratically (by reducing read noise) at the cost of a linear reduction in spatial resolution. For tracking centroid position (rather than resolving structure), this can be an excellent trade-off.

3. *Computational Enhancement*: As detailed in Section 4, deconvolution (e.g., Richardson-Lucy) can reassign blurred light, sharpening the centriole signal. Background subtraction algorithms (rolling ball, top-hat filter) can remove uneven illumination.

Inability to Resolve Two Closely Spaced Centrioles

A fundamental physical limitation is the diffraction limit (~250 nm). When the mother and daughter centrioles are closer than this distance—as they are for much of the cell cycle—they appear as a single fused object in conventional microscopy.

- **Mitigation Strategies**:

    1. *Super-Resolution Microscopy*: The direct solution is to use techniques that break the diffraction barrier. Live-cell STED can provide 50-80 nm resolution, sufficient to visualize the separation (Lau et al., 2012). Live-cell SIM (~110 nm resolution) can also resolve centriole pairs during disengagement and separation events.

    2. *Indirect Analysis of the Point Spread Function (PSF)*: When super-resolution is not available, valuable information can be extracted by analyzing the shape of the diffraction-limited spot. A single centriole produces a symmetric, 2D Gaussian intensity profile. A pair of unresolved centrioles produces an elliptical or asymmetric PSF. By fitting the spot with an elliptical Gaussian model or analyzing its moment of inertia, one can infer the presence of two objects and even estimate their separation if it is above ~180 nm. The Full Width at Half Maximum (FWHM) along the major and minor axes can be compared; a significant difference suggests a duplet. This method, while indirect, has been successfully used to score centriole disengagement in fixed and live-cell assays (Fong et al., 2016).

Artifacts from Fluorescent Protein Overexpression

Overexpressing a centriolar protein-FP fusion can dysregulate the centriole duplication cycle, leading to the formation of excess centrioles (centriole amplification) or aberrant structures, completely confounding any tracking study aimed at normal physiology.

- **Mitigation Strategies**:

    1. *Endogenous Tagging*: The gold standard is CRISPR/Cas9-mediated knock-in of the FP at the native genetic locus, ensuring physiological expression levels and regulation.

    2. *Low-Level Transient Expression*: If stable lines or knock-ins are not feasible, use minimal amounts of transfection reagent and short expression times (24-48h). Carefully select cells with low, barely detectable fluorescence for analysis, as these are least likely to have overexpression artifacts.

    3. *Functional Rescue Assay*: Co-express the FP-tagged protein in a background where the endogenous protein is knocked down or out. Demonstrate that the fusion protein rescues the normal centriole number and function, proving it is functional and not disruptive.

Awareness of these potential pitfalls and the systematic implementation of controls and corrective measures are non-negotiable for rigorous live-cell centriole tracking. The optimal experiment is not necessarily the one with the highest resolution or longest duration, but the one that carefully balances signal, survival, and biological fidelity to yield an accurate representation of centriole dynamics in a living system.

Emerging Directions and Future Methods

The field of centriole biology is on the cusp of a new era, propelled by methodologies that promise to bridge current gaps between dynamics, ultrastructure, and molecular-scale mechanics. While established techniques provide robust frameworks, several emerging and future-oriented approaches are poised to deepen our understanding by offering unprecedented spatial and temporal context, moving beyond the cell line model into more complex systems.

Correlative Light and Electron Microscopy (CLEM)

A primary ambition is to directly link the dynamic behavior of a centriole observed in a living cell with its high-resolution, static ultrastructure. Correlative Light and Electron Microscopy (CLEM) fulfills this by imaging the same cell sequentially with fluorescence and electron microscopy. In a typical workflow, a cell expressing a centriolar FP (e.g., Centrin1-GFP) is imaged live to capture a specific event—such as disengagement, ciliary docking, or a laser ablation experiment. The cell is then rapidly fixed, often using high-pressure freezing to preserve ultrastructure, and processed for EM (Karreman et al., 2016). Using fiduciary markers (e.g., fluorescent nanodiamonds or etched grid patterns), the region imaged by light microscopy is relocated in the EM. This allows researchers to ask, for example, what specific structural changes in the distal appendages or cartwheel correlate with the dynamic separation of two centrioles observed minutes earlier. CLEM thus provides the ultimate validation and context, transforming a fluorescent spot into a detailed 3D model with known prior history.

Single-Particle Tracking Photoactivated Localization Microscopy (sptPALM)

While PALM/STORM has been largely confined to fixed samples due to slow acquisition, its application to live cells—single-particle tracking PALM (sptPALM)—holds immense potential for centriole studies. This method uses photoactivatable or photoswitchable fluorescent proteins (PA-FPs) sparsely fused to a centriolar protein. By using very low activation laser power, only a stochastic subset of molecules are fluorescent in each frame, allowing their precise nanoscale localization (20-40 nm). Tracking these single molecules over time reveals their diffusion dynamics, binding kinetics, and exchange rates within the centriole structure (Manley et al., 2008). For instance, sptPALM of SAS-6 could reveal the real-time turnover and stability of cartwheel components during procentriole assembly. The key challenge for centriole application is achieving sufficient labeling density and acquisition speed to track molecules within a small, dense organelle without blurring its overall architecture.

Artificial Intelligence and Deep Learning for Analysis

The complexity and volume of imaging data, especially from super-resolution or 3D time-lapse experiments, increasingly surpasses the limits of traditional analytical software. Deep learning, particularly convolutional neural networks (CNNs), is revolutionizing image analysis in biology. A U-Net architecture, for example, can be trained on manually annotated images to perform robust, automated segmentation of centrioles even in very noisy or crowded environments (Rivenson et al., 2017). Beyond detection, recurrent neural networks (RNNs) can be used to predict future centriole positions or classify behavioral states (e.g., "engaged," "separating," "ciliating") based on prior trajectory and morphological features. Furthermore, AI can be employed for computational super-resolution, using trained networks to predict high-resolution structures from diffraction-limited input images, potentially allowing long-term, gentle imaging with subsequent resolution enhancement.

Lattice Light-Sheet Microscopy (LLSM) and Tissue Imaging

Most centriole tracking studies are confined to monolayer cell cultures. Understanding centriole dynamics in their native physiological context—within 3D tissues, organoids, or developing embryos—is the next frontier. Lattice Light-Sheet Microscopy (LLSM) is uniquely suited for this challenge (Chen et al., 2014). It illuminates the sample with a thin, optically sectioned "sheet" of light, capturing an entire plane in a single camera exposure. This enables extremely fast, high-resolution 3D imaging with orders of magnitude less phototoxicity and photobleaching than point-scanning methods. LLSM allows for the tracking of centrioles deep within a living organoid or zebrafish embryo over hours or days, observing how their behavior is influenced by complex tissue architecture, cell-cell signaling, and mechanical forces. The ability to correlate centriole dynamics with large-scale morphogenetic events in development or disease models will open entirely new avenues of research.

Conclusion

The modern arsenal for tracking individual centrioles represents an integrated technological pipeline, a convergence of precise genetic labeling, advanced optical physics, and sophisticated computational analytics. We have progressed from inferring dynamics from static electron micrographs to directly observing, quantifying, and perturbing the life cycle of these organelles in real time within living cells.

This journey has been marked by sequential breakthroughs: the advent of FP tagging provided the first dynamic glimpse; spinning-disk and TIRF microscopy enabled long-term and high-contrast observation; super-resolution techniques like STED and SIM broke the diffraction barrier to resolve individual centrioles; and CRISPR/Cas9 endogenous tagging established a new gold standard for physiological relevance. Parallel developments in analytical software have transformed qualitative observations into rigorous, quantitative metrics of motion, separation, and maturation kinetics.

These methodological advances have been the engine for fundamental discoveries across cell biology, development, and disease. They have elucidated the mechanistic principles of centriole duplication licensing, revealed the asymmetric inheritance and age-dependent functions of mother and daughter centrioles, and detailed the early steps of ciliogenesis. In cancer biology, tracking has revealed how centrosome amplification leads to mitotic errors and genomic instability.

Looking forward, the integration of the emerging methods outlined here—CLEM, sptPALM, AI-driven analysis, and light-sheet imaging of complex systems—promises a more holistic view. We will no longer see the centriole as just a fluorescent dot to be tracked, but as a complex, molecularly dynamic machine whose behavior is intimately linked to its nanoscale architecture and its cellular and tissue microenvironment. The continued refinement and creative application of these tracking methodologies will undoubtedly remain central to unlocking the remaining secrets of these enigmatic and essential organelles.

References

Anderson, C. T., & Stearns, T. (2009). Centriole age underlies asynchronous primary cilium growth in mammalian cells. Current biology : CB, 19(17), 1498–1502. https://doi.org/10.1016/j.cub.2009.07.034

Bindels, D. S., Haarbosch, L., van Weeren, L., Postma, M., Wiese, K. E., Mastop, M., Aumonier, S., Gotthard, G., Royant, A., Hink, M. A., & Gadella, T. W. J., Jr. (2017). mScarlet: A bright monomeric red fluorescent protein for cellular imaging. Nature Methods, 14(1), 53–56. https://doi.org/10.1038/nmeth.4074

Bornens, M. (2012). The centrosome in cells and organisms. Science, 335(6067), 422–426. https://doi.org/10.1126/science.1209037

Bowler, M., Kong, D., Sun, S., Nanjundappa, R., Evans, L., Farmer, V., Holland, A., Mahjoub, M. R., Sui, H., & Loncarek, J. (2019). High-resolution characterization of centriole distal appendage morphology and function by cryo-electron tomography. Journal of Cell Biology, 218(5), 1633-1647. https://doi.org/10.1083/jcb.201809048

Chen, B. C., Legant, W. R., Wang, K., Shao, L., Milkie, D. E., Davidson, M. W., Janetopoulos, C., Wu, X. S., Hammer, J. A., 3rd, Liu, Z., English, B. P., Mimori-Kiyosue, Y., Romero, D. P., Ritter, A. T., Lippincott-Schwartz, J., Fritz-Laylin, L., Mullins, R. D., Mitchell, D. M., Bembenek, J. N., … Betzig, E. (2014). Lattice light-sheet microscopy: Imaging molecules to embryos at high spatiotemporal resolution. Science, 346(6208), 1257998. https://doi.org/10.1126/science.1257998

Fong, C. S., Mazo, G., Das, T., Goodman, J., Kim, M., O'Rourke, B. P., Izquierdo, D., & Tsou, M. F. B. (2016). 53BP1 and USP28 mediate p53-dependent cell cycle arrest in response to centrosome loss and prolonged mitosis. eLife, 5, e16270. https://doi.org/10.7554/eLife.16270

Greenan, G. A., Keszthelyi, B., Vale, R. D., & Agard, D. A. (2018). Insights into centriole geometry revealed by cryotomography of doublet and triplet centrioles. eLife, 7, e36851. https://doi.org/10.7554/eLife.36851

Habedanck, R., Stierhof, Y. D., Wilkinson, C. J., & Nigg, E. A. (2005). The Polo kinase Plk4 functions in centriole duplication. Nature Cell Biology, 7(11), 1140–1146. https://doi.org/10.1038/ncb1320

Icha, J., Weber, M., Waters, J. C., & Norden, C. (2017). Phototoxicity in live fluorescence microscopy, and how to avoid it. BioEssays, 39(8), 1700003. https://doi.org/10.1002/bies.201700003

Jaba, T. (2022). Dasatinib and quercetin: short-term simultaneous administration yields senolytic effect in humans. Issues and Developments in Medicine and Medical Research Vol. 2, 22-31.

Jaqaman, K., Loerke, D., Mettlen, M., Kuwata, H., Grinstein, S., Schmid, S. L., & Danuser, G. (2008). Robust single-particle tracking in live-cell time-lapse sequences. Nature Methods, 5(8), 695–702. https://doi.org/10.1038/nmeth.1237

Karreman, M. A., Hyenne, V., Schwab, Y., & Goetz, J. G. (2016). Intravital correlative microscopy: Imaging life at the nanoscale. Trends in Cell Biology, 26(11), 848–863. https://doi.org/10.1016/j.tcb.2016.07.003

Smeets, D., Markaki, Y., Schmid, V.J. et al. Three-dimensional super-resolution microscopy of the inactive X chromosome territory reveals a collapse of its active nuclear compartment harboring distinct Xist RNA foci. Epigenetics & Chromatin 7, 8 (2014). https://doi.org/10.1186/1756-8935-7-8

Lau, L., Lee, Y. L., Sahl, S. J., Stearns, T., & Moerner, W. E. (2012). STED microscopy with optimized labeling density reveals 9-fold arrangement of a centriole protein. Biophysical journal, 102(12), 2926–2935. https://doi.org/10.1016/j.bpj.2012.05.015

Loncarek, J., & Khodjakov, A. (2009). Ab ovo or de novo? Mechanisms of centriole duplication. Molecular Cells, 27(2), 135–142. https://doi.org/10.1007/s10059-009-0017-z

Lukinavičius, G., Reymond, L., D'Este, E., Masharina, A., Göttfert, F., Ta, H., Güther, A., Fournier, M., Rizzo, S., Waldmann, H., Blaukopf, C., Sommer, C., Gerlich, D. W., Arndt, H. D., Hell, S. W., & Johnsson, K. (2014). Fluorogenic probes for live-cell imaging of the cytoskeleton. Nature Methods, 11(7), 731–733. https://doi.org/10.1038/nmeth.2972

Manley, S., Gillette, J. M., Patterson, G. H., Shroff, H., Hess, H. F., Betzig, E., & Lippincott-Schwartz, J. (2008). High-density mapping of single-molecule trajectories with photoactivated localization microscopy. Nature Methods, 5(2), 155–157. https://doi.org/10.1038/nmeth.1176

Mazo, G., Soplop, N., Wang, W. J., Uryu, K., & Tsou, M. F. B. (2016). Spatial control of primary ciliogenesis by subdistal appendages alters sensation-associated properties of cilia. Developmental Cell, 39(4), 424–437. https://doi.org/10.1016/j.devcel.2016.10.006

Paintrand, M., Moudjou, M., Delacroix, H., & Bornens, M. (1992). Centrosome organization and centriole architecture: their sensitivity to divalent cations. Journal of Structural Biology, 108(2), 107-128. https://doi.org/10.1016/1047-8477(92)90011-X

Piel, M., Meyer, P., Khodjakov, A., Rieder, C. L., & Bornens, M. (2000). The respective contributions of the mother and daughter centrioles to centrosome activity and behavior in vertebrate cells. Journal of Cell Biology, 149(2), 317-330. https://doi.org/10.1083/jcb.149.2.317

Preibisch, S., Saalfeld, S., Schindelin, J., & Tomancak, P. (2010). Software for bead-based registration of selective plane illumination microscopy data. Nature Methods, 7(6), 418–419. https://doi.org/10.1038/nmeth0610-418

Rivenson, Y., Göröcs, Z., Günaydin, H., Zhang, Y., Wang, H., & Ozcan, A. (2017). Deep learning microscopy. Optica, 4(11), 1437–1443. https://doi.org/10.1364/OPTICA.4.001437

Sage, D., Donati, L., Soulez, F., Fortun, D., Schmit, G., Seitz, A., Guiet, R., Vonesch, C., & Unser, M. (2017). DeconvolutionLab2: An open-source software for deconvolution microscopy. Methods, 115, 28–41. https://doi.org/10.1016/j.ymeth.2016.12.015

Sillibourne, J. E., Specht, C. G., Izeddin, I., Hurbain, I., Tran, P., Triller, A., Darzacq, X., Dahan, M., & Bornens, M. (2011). Assessing the localization of centrosomal proteins by PALM/STORM nanoscopy. Cytoskeleton, 68(11), 619–627. https://doi.org/10.1002/cm.20536

Tanenbaum, M. E., Gilbert, L. A., Qi, L. S., Weissman, J. S., & Vale, R. D. (2014). A protein-tagging system for signal amplification in gene expression and fluorescence imaging. Cell, 159(3), 635–646. https://doi.org/10.1016/j.cell.2014.09.039

Tinevez, J. Y., Perry, N., Schindelin, J., Hoopes, G. M., Reynolds, G. D., Laplantine, E., Bednarek, S. Y., Shorte, S. L., & Eliceiri, K. W. (2017). TrackMate: An open and extensible platform for single-particle tracking. Methods, 115, 80–90. https://doi.org/10.1016/j.ymeth.2016.09.016

Tkemaladze, J. (2023). Reduction, proliferation, and differentiation defects of stem cells over time: a consequence of selective accumulation of old centrioles in the stem cells?. Molecular Biology Reports, 50(3), 2751-2761. DOI : https://pubmed.ncbi.nlm.nih.gov/36583780/

Tkemaladze, J. (2024). Editorial: Molecular mechanism of ageing and therapeutic advances through targeting glycative and oxidative stress. Front Pharmacol. 2024 Mar 6;14:1324446. DOI : 10.3389/fphar.2023.1324446. PMID: 38510429; PMCID: PMC10953819.

Tkemaladze, J. (2026). Old Centrioles Make Old Bodies. Annals of Rejuvenation Science, 1(1). DOI : https://doi.org/10.65649/yx9sn772

Tkemaladze, J. (2026). Visions of the Future. Longevity Horizon, 2(1). DOI : https://doi.org/10.65649/8be27s21

Zhang P. (2013). Correlative cryo-electron tomography and optical microscopy of cells. Current opinion in structural biology, 23(5), 763–770. https://doi.org/10.1016/j.sbi.2013.07.017

Wong, Y. L., Anzola, J. V., Davis, R. L., Yoon, M., Motamedi, A., Kroll, A., Seo, C. P., Hsia, J. E., Kim, S. K., Mitchell, J. W., Mitchell, B. J., Desai, A., Gahman, T. C., Shiau, A. K., & Oegema, K. (2015). Cell biology. Reversible centriole depletion with an inhibitor of Polo-like kinase 4. Science, 348(6239), 1155–1160. https://doi.org/10.1126/science.aaa5111

