MicrosCopilot: An Agentic, Physics-Aware AI Framework for Confocal Microscopy

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Abstract

Quantitative confocal microscopy is central to soft-matter and biological physics, enabling measurements of particle dynamics, structure, and transport in complex environments. However, transforming raw 3D or 4D confocal image stacks into reliable physical insight remains labour-intensive, parameter-sensitive, and highly expertise-dependent. In this work, we present **MicrosCopilot**, a lightweight, physics-aware analysis framework that integrates a configurable digital twin, a modular multi-agent analysis pipeline, and an interactive user interface. The system bridges raw confocal data and interpretable physical metrics such as mean-squared displacement (MSD), anomalous diffusion exponents, and imaging diagnostics while explicitly exposing analysis assumptions and potential biases. By combining established particle-tracking tools with physics-informed simulation and an extensible explanation layer, the Copilot lowers the barrier to robust quantitative analysis and supports rapid diagnosis and optimization of confocal experiments. We demonstrate the framework on diverse datasets (Brownian particles, soft-matter gels, cell nuclei, colloidal monolayers) and show that the agentic planning layer can recommend follow-up analyses based on detected anomalies (e.g., bleaching, noise, crowding). This framework is designed as a practical, transparent alternative to black-box microscopy pipelines and is well-suited for prototyping, education, and reproducible research.

1. Introduction and Motivation

Three-dimensional confocal microscopy underpins a wide range of studies, from colloidal glasses and microrheology to intracellular transport and active matter. Despite major advances in microscope hardware, the downstream analysis pipeline particle detection, tracking, and physical interpretation remains a dominant bottleneck. Researchers must manually tune parameters on noisy datasets, iterate through trial-and-error workflows, and rely on expert intuition to diagnose artifacts such as photobleaching, depth-dependent signal loss, and particle crowding. These challenges limit scalability, hinder reproducibility, and complicate collaboration across distributed teams where experimental and analysis expertise are decoupled. While recent machine-learning approaches and "microscopy copilot" concepts have shown promise, most existing tools focus on 2D data, require heavy deep-learning infrastructure, or operate as opaque black boxes. MicrosCopilot addresses this gap by combining physics-informed simulation, modular analysis agents, and an interactive interface designed to encapsulate expert knowledge while remaining transparent and interpretable.

2. Methodology

Physics-Informed Digital Twin

At the core of the framework is a configurable digital twin that generates realistic synthetic confocal datasets with known ground truth. The twin simulates 3D Brownian particle trajectories with tunable diffusion coefficients, particle density, voxel size, and frame interval. These trajectories are rendered into 4D confocal-like image stacks using an anisotropic 3D Gaussian point-spread function (PSF) that approximates lateral and axial resolution. To reproduce key experimental failure modes without incurring the cost of full wave-optics simulations, the digital twin incorporates three dominant imaging artifacts: **Depth-dependent intensity attenuation**, modeling signal loss due to scattering and optical aberrations, **global photobleaching**, implemented as an exponential decay in fluorescence intensity over time, and **additive Gaussian noise**, representing detector noise and background fluctuations.

Modular Multi-Agent Analysis Pipeline

Rather than relying on a monolithic algorithm, the Copilot decomposes confocal analysis into a transparent, four-agent pipeline with clearly defined roles.

Agent 1: Planner (Orchestrator) - The planner ingests the user's natural-language query, dataset metadata (voxel size, frame interval, estimated PSF), and analysis preferences to produce an analysis plan specifying the analysis mode (e.g., diffusion-focused), whether to invoke the digital twin for comparison, and initial parameters for detection and tracking. This step formalizes heuristic reasoning typically performed implicitly by experts.
Agent 2: Detection and Tracking - This agent converts 3D or 4D image stacks into particle trajectories using established tools. For the current implementation, frames are optionally max-projected along the axial direction and processed using Trackpy's feature localization and linking algorithms. Outputs include trajectory tables and basic quality metrics such as detection counts and track-length distributions.
Agent 3: Physics Analysis - Trajectories are mapped to interpretable physical descriptors. The agent computes ensemble-averaged MSD curves and fits an anomalous diffusion model to extract the exponent $\alpha$and effective diffusion coefficient D. In parallel, it evaluates imaging diagnostics including depth-dependent intensity profiles, bleaching curves, and a crowding metric based on nearest-neighbour distances.
Agent 4: Explainer - The explainer translates numerical outputs into concise, physics-aware interpretations at an experimentalist level. It highlights limitations (e.g., unreliable long-lag MSDs due to short trajectories) and suggests actionable next steps (e.g., adjusting laser power or frame rate). While currently implemented as a placeholder, the design supports integration with large language models for richer explanations.

User Interface and Workflow

The full pipeline is exposed through a lightweight Gradio-based web interface. Users can upload confocal datasets or select bundled examples, enter metadata, pose natural-language questions, and run the analysis with a single action. Outputs including MSD plots, depth profiles, and explanatory text—are displayed interactively, and follow-up questions reuse cached intermediate results to enable conversational analysis.

3. Discussion and Outlook

Confocal Microscopy Copilot demonstrates how physics-informed simulation, modular analysis, and interactive explanation can be combined into a practical, transparent alternative to black-box microscopy pipelines. By explicitly encoding expert heuristics and exposing diagnostic metrics alongside physical results, the framework supports both reliable analysis and informed experimental decision-making.

Future extensions include richer optical models, viscoelastic motion in the digital twin, deeper integration with learning-based detectors, and deployment of real LLM backends for adaptive explanation and experiment planning. More broadly, the Copilot illustrates a general design pattern for scientific "assistants" that prioritize interpretability and domain knowledge over purely data-driven automation.

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