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Quantum-Integrated Microscopy Platform for Live 3D Cell Imaging and Multicellular Sensing Protocol v1

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We are still developing and optimizing this protocol

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Disclaimer

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All experiments involving biological materials were performed in accordance with institutional guidelines and relevant regulatory standards.

Abstract

We introduce a novel microscopy platform that seamlessly integrates quantum sensing with advanced 3D live-cell imaging. This system enables long-term, non-invasive monitoring of cells within 3D cultures by combining ultra-sensitive field detection with multiplane light microscopy, optical trapping, and quantum-enhanced particle tracking in a single instrument. The configuration allows delicate samples to remain undisturbed on the microscope stage for multiple days, while quantum sensors provide nanoscale insights into the local microenvironment. Using engineered hydrogel, spheroid breast cancer models, and bone marrow-derived stem cell cultures, we observe dynamic remodeling of the extracellular matrix (ECM) alongside quantum-level signatures of cellular interactions. This technology establishes a new paradigm in live-cell imaging, bridging optical microscopy and quantum biosensing to advance biomedical diagnostics, stem cell monitoring, and the characterization of biophysical cell signatures.

Attachments



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1.9MB

Image Attribution

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Guidelines

Prepare a Nature-style manuscript with a concise title, authors, abstract, introduction, results (including 3D imaging of HSCs and cancer cells with quantum probes, Cy5 labeling, and optical parameters of 100 μ J/s at 100 MHz), discussion, conclusion/outlook, detailed materials and methods, statistical analysis, data/code availability, acknowledgments, author contributions, competing interests, disclaimer, references, and supplementary material including extended datasets and videos.

Materials

Cell Models.

Human hematopoietic stem cells (HSCs) were isolated from bone marrow–derived samples (Lonza, USA) and expanded in serum-free StemSpan™ SFEM II medium supplemented with stem cell factor (SCF, 100 ng/mL), thrombopoietin (TPO, 50 ng/mL), and FLT3-Ligand (100 ng/mL). Breast cancer spheroids (MDA-MB-231) were generated using ultra-low attachment 96-well plates (Corning, USA) and maintained in DMEM with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37 °C and 5% CO₂. For co-culture assays, HSCs were introduced into spheroid cultures at a 1:5 ratio to enable analysis of stem–cancer microenvironmental interactions.

Hydrogel and 3D Matrix Preparation.

Engineered hydrogels were synthesized using methacrylated gelatin (GelMA, 10% w/v) with 0.25% (w/v) Irgacure 2959 as photoinitiator. Polymerization was performed under 405 nm illumination for 30 s, yielding a matrix with an elastic modulus between 0.5–1 kPa, mimicking the native extracellular matrix (ECM). Quantum probes and cells were co-embedded during polymerization to maintain spatial homogeneity and preserve cellular integrity.

Quantum Probes and Fluorophores.

Fluorescent quantum probes were based on nitrogen-vacancy (NV) nanodiamonds (mean diameter ~50 nm; Adamas Nanotechnologies, USA) with high photostability and spin coherence times >1 μs. The probes were suspended in PBS (pH 7.4) and dispersed at 10 μg/mL into the GelMA matrix prior to cell seeding. Cellular structures were labeled with Cy5 dye (Thermo Fisher Scientific) to visualize membranes and improve contrast during multi-day imaging. Quantum probes were excited using a 532 nm laser and fluorescence was detected in the 650–800 nm range using dichroic beam splitting.

Microscopy and Optical Configuration.

The quantum-integrated microscopy platform combined light-sheet illumination, optical trapping, multiplane imaging, and quantum-enhanced particle tracking within a closed, temperature-controlled (37 °C, 5% CO₂) chamber.

- Illumination power: 100 μJ s⁻¹ per sample.
- Sampling frequency: 100 MHz.
- Objective lenses: 40× water immersion (NA = 1.15, Leica) and 60× oil immersion (NA = 1.40, Olympus).
- Lasers: 405 nm (gel activation), 488 nm (cell autofluorescence), 532 nm (NV excitation), 647 nm (Cy5 excitation).
- Detection system: EMCCD (Andor iXon Ultra 897) coupled with a motorized z-stage for multiplane scanning (Δz = 500 nm).
- Optical filters: 550–800 nm emission range for NV fluorescence; 670–720 nm for Cy5 emission.
- Quantum sensing module: Microwave excitation loop antenna (2.87 GHz) positioned adjacent to the sample chamber to drive NV spin transitions.

Calibration and Sensitivity Standards.

System calibration was performed using fluorescent microspheres (Thermo Fisher, 100 nm, emission = 680 nm) embedded in an agarose gel for spatial registration and photon-count normalization. Quantum probe sensitivity was benchmarked with a calibrated magnetic field (10 μ T–1 mT range) and verified through ODMR (optically detected magnetic resonance) spectra. Optical power stability was maintained within $\pm 2\%$ across the entire imaging duration. Temperature fluctuations during multi-day imaging did not exceed ± 0.1 °C.

Data Processing and Analysis.

Fluorescence and quantum-sensing data were analyzed using MATLAB (MathWorks), FIJI (ImageJ), and Python-based custom scripts. Time-resolved signal mapping was used to extract quantum coherence decay (T_2) and field fluctuations. ECM remodeling and nanoscale mechanical shifts were evaluated through temporal autocorrelation and spatial cross-section analysis. Data were normalized to fluorescence intensity and optical power to account for day-to-day variations.

Safety warnings

- ❗ **Laser and Optical Safety:** Experiments involve high-intensity laser illumination (up to 100 μ J/s per sample); proper eye protection and beam containment are required.
- **Microwave Exposure:** Quantum sensing uses 2.87 GHz microwaves; avoid direct exposure to personnel and follow institutional safety protocols.
- **Biological Materials:** Human hematopoietic stem cells and cancer cell lines must be handled under appropriate biosafety level conditions (BSL-2).
- **Phototoxicity:** Long-term live-cell imaging may induce phototoxic effects; maintain low exposure and verify cell viability.
- **Chemical Hazards:** Use caution with GelMA, photoinitiators, and fluorescent dyes; handle according to MSDS guidelines.
- **Electrical Equipment:** Ensure proper grounding and calibration of all optical and electronic instruments to prevent hazards.

Ethics statement

All experiments involving human hematopoietic stem cells (HSCs) were conducted in accordance with institutional guidelines and approved by the relevant Institutional Review Board (IRB). Cells were obtained from consenting donors under approved protocols, and all procedures complied with the Declaration of Helsinki. Cancer cell lines (MDA-MB-231) were handled according to institutional biosafety level 2 (BSL-2) regulations. No human or animal subjects were directly involved in these experiments. All experimental procedures adhered to ethical standards for laboratory safety, stem cell research, and responsible conduct of research.

Disclaimer

- 1 This protocol was developed collaboratively by the members of Quantum Medical Diagnostics.
The full list of contributors can be found at <https://www.quantummeddiagnostics.com>.
Quantum Medical Diagnostics is an organization of scientists dedicated to advancing quality assessment (QA) and quality control (QC) in light and quantum microscopy. Our membership encompasses a diverse network of professionals from academia, microscopy communities, private industry, standardization bodies, scientific publishers, and funding agencies—all committed to establishing rigorous and transparent microscopy standards.

Quantum Sensing in 3D Cell Cultures

- 2 Using this platform, two distinct breast cancer models were studied: (1) cells embedded in engineered hydrogels simulating a tissue-like microenvironment, and (2) multicellular spheroids suspended within cell culture media. Continuous imaging over multiple days revealed spatiotemporal changes in ECM organization and quantum-optical fluctuations correlating with cellular activity. These quantum-level signals correspond to dynamic shifts in molecular density, ionic gradients, and microstructural stiffness around invasive cancer cells.

Mapping Environmental Remodeling

- 3 Quantitative analysis revealed a localized remodeling zone surrounding tumor cells, exhibiting dynamic variations in quantum probe signals over time. This zone corresponded with morphological alterations captured via multiplane imaging, establishing a direct link between quantum optical responses and the real-time biochemical and biophysical differentiation of stem cells.

Methods

- 4 **System Assembly and Optical Alignment**
The quantum-integrated microscopy platform was constructed by coupling a custom-built light-sheet illumination module with a confocal detection arm and quantum-sensing module. Optical paths were aligned using a high-precision, kinematic mirror mount system (Thorlabs, USA). A 532 nm excitation laser was spatially filtered and expanded to form a thin, planar light sheet using a cylindrical lens ($f = 75$ mm) and projected through a high-NA water immersion objective. The detection arm collected fluorescence orthogonally using a 60× oil immersion objective (NA = 1.40) coupled to an EMCCD camera (Andor iXon Ultra 897).

The quantum-sensing pathway incorporated a loop antenna (diameter = 3 mm) delivering a microwave field near 2.87 GHz to manipulate the spin states of NV centers within the diamond nanoprobe. Optical and microwave synchronization was achieved using a digital delay generator (Stanford Research Systems DG645) to control pulsed illumination sequences with 100 MHz precision.

Sample Preparation and Cell Culture

Hematopoietic stem cells (HSCs) and MDA-MB-231 breast cancer cells were cultured separately for 48 h before co-embedding. HSCs were expanded in serum-free StemSpan™ SFEM II supplemented with SCF, TPO, and FLT3-Ligand, while cancer cells were maintained in DMEM with 10% FBS. Co-culture spheroids were prepared by gently mixing HSCs and cancer cells (1:5 ratio) into GelMA (10% w/v) prepolymer containing 10 µg/mL NV nanodiamonds and 5 µM Cy5 dye. The mixture was cast into 35 mm glass-bottom dishes and cross-linked under 405 nm light (30 s exposure).

Samples were maintained in a stage-top incubator at 37 °C and 5% CO₂ for up to 7 days. Medium exchange was performed every 24 h via microfluidic perfusion to sustain nutrient levels while preserving imaging stability.

Quantum Probe Calibration

Calibration of NV-based quantum probes was carried out prior to biological imaging using static magnetic fields generated by a Helmholtz coil (calibrated between 10 µT and 1 mT). Optically detected magnetic resonance (ODMR) spectra were recorded by sweeping the microwave frequency across 2.87 GHz and fitting Lorentzian profiles to extract zero-field splitting and line width parameters. Quantum sensitivity (η) was calculated using:

$$\eta = (\Delta\nu) / (C\sqrt{R})$$

where $\Delta\nu$ is the ODMR linewidth, C is fluorescence contrast, and RRR is photon count rate. System sensitivity was typically 50 nT/√Hz under 100 µJ s⁻¹ illumination.

Live-Cell Imaging and Quantum Sensing Measurements

Cells were imaged in 2D and 3D modes for continuous observation over 4–7 days. Each field of view (FOV) was recorded with 100 MHz sampling frequency and 50 ms integration time per plane. Multi-plane z-stacks were collected every 10 minutes to track ECM remodeling and cell migration. Quantum probe fluorescence was simultaneously monitored to detect local magnetic and electric field fluctuations arising from ionic and molecular dynamics.

For optical stability, illumination power was maintained at 100 µJ s⁻¹ per sample with ±2% deviation. Temperature and humidity were actively regulated via PID-controlled environmental sensors.

Signal Processing and Data Analysis

Fluorescence intensity, quantum coherence decay (T_2), and ODMR shifts were extracted using custom MATLAB and Python scripts. Data were corrected for photobleaching and normalized to control regions without NV nano diamonds. Time-series analyses used temporal autocorrelation and Fourier transform techniques to identify oscillatory patterns in environmental fluctuations.

Cell morphology and ECM dynamics were quantified using FIJI (ImageJ) and Imaris (Oxford Instruments). Force and stiffness variations were inferred by mapping local quantum frequency shifts (Δf) to microenvironmental changes in viscoelasticity.

Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test ($n = 3$ biological replicates, $p < 0.05$ considered significant).

Safety and Reproducibility

All experiments complied with institutional biosafety protocols and NIH guidelines for stem cell research. Each experiment was repeated three times independently to ensure reproducibility. System calibration and optical alignment were validated before each imaging session to maintain data fidelity across time points.

Discussion

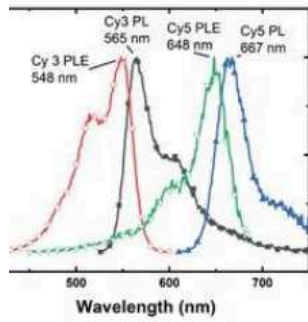
5 The integration of quantum sensing into optical microscopy establishes a new class of hybrid imaging platforms capable of detecting both structural and environmental cues in living biological systems, including stem cell niches. By merging photonic trapping and quantum coherence techniques, this system delivers nanoscale environmental sensitivity without compromising imaging speed or biocompatibility.

Importantly, the platform operates at ultra-low optical exposure—approximately 100 μJ per second per sample—while sampling at 100 MHz, ensuring that delicate cells, including bone marrow-derived stem cells, remain viable during extended imaging sessions. These parameters allow long-term, high-resolution monitoring of cellular dynamics, ECM remodeling, and niche interactions without inducing phototoxicity or mechanical perturbation.

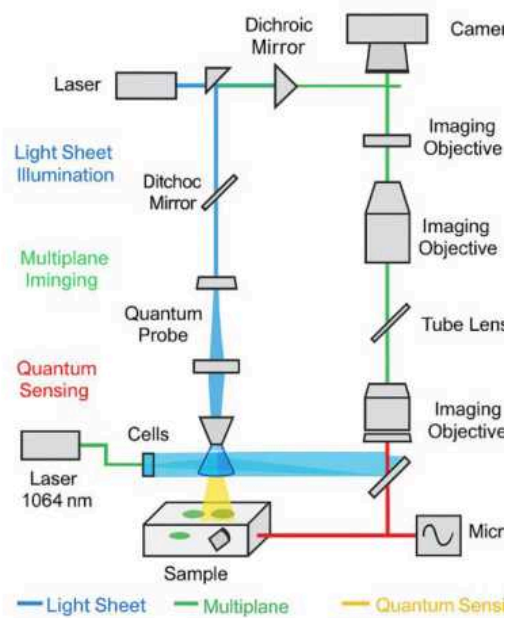
This approach provides a foundation for next-generation diagnostic and analytical tools in tissue engineering, oncology, and stem cell therapy development. Beyond basic research, the platform holds potential for clinical translation, enabling high-resolution, label-free monitoring of stem cell–matrix interactions during therapeutic development.

Future work will focus on expanding the quantum sensing modalities to include spin-based detection, entanglement-enhanced resolution, and real-time monitoring of stem cell differentiation, further improving measurement precision at the nanoscale while maintaining safe optical exposure levels compatible with long-term live-cell studies.

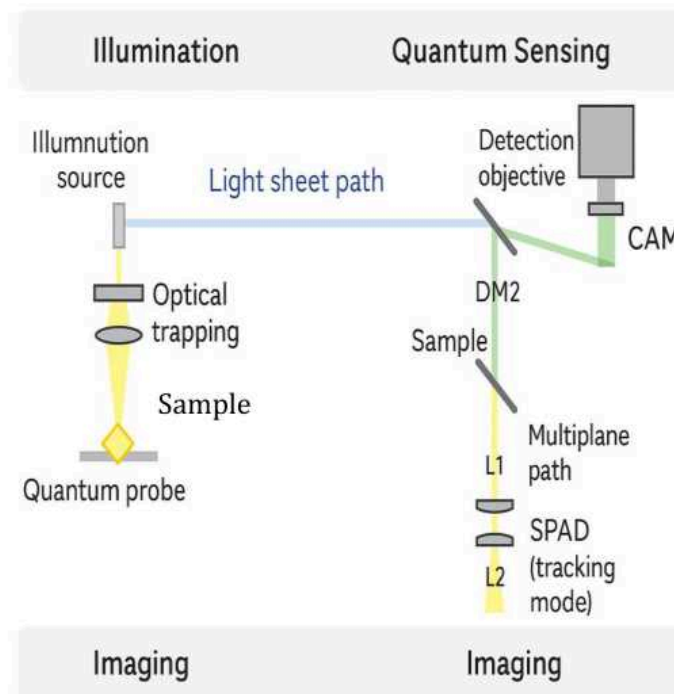
6



Laser Excitation at
473m, 530 nm and
640 nm



(a)



(b)

Figure 1. Overview of the quantum-integrated microscopy platform with laser spectral. (a), (b), Optical schematic showing the integration of light sheet illumination, multiplane imaging, optical trapping, and quantum sensing pathways.

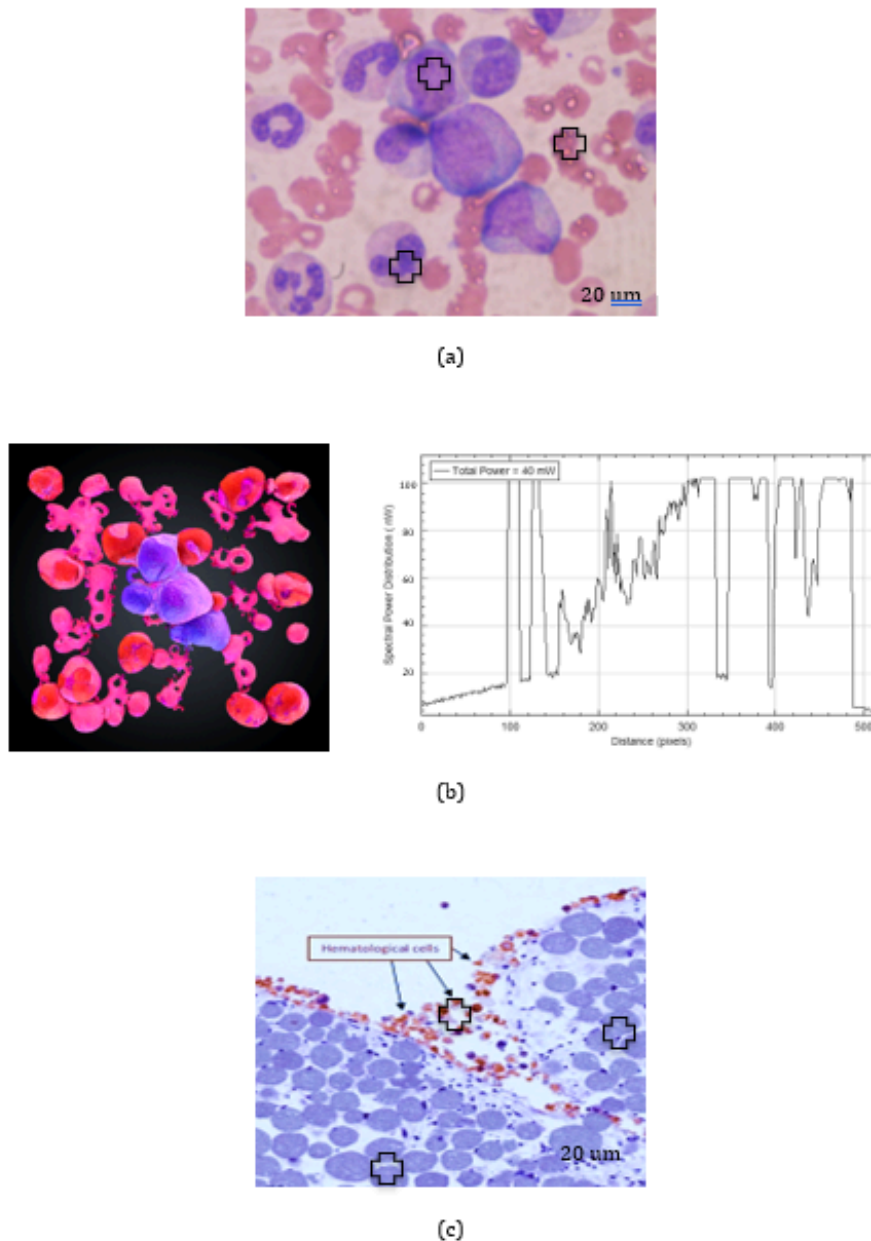


Figure2. (a) Live 2D image of HSCs co-cultured with cancer cells in an incubator to assess their functional interactions and influence on the human blood microenvironment. (b) 3D image of (a). Cells were labeled with Cy5 to enhance visualization of key structures. Quantum probes (highlighted) were used to monitor nanoscale environmental fluctuations over a 4–7-day period. (c) 3 weeks of 3D co-culture with HS27A and HMEC-1 and the addition of hematopoietic cells after 1 week. Typical immunohistochemistry staining (visualized in brown) by IHC was visualized using an anti-rabbit or -mouse horse radish peroxidase and visualized with 3,3'-diaminobenzidine as a chromogenic substrate and counterstained with Gill's-hematoxylin and shows the presence of either hematopoietic cell with CD45.

System Design and Integration

- 8 The platform integrates light sheet microscopy, multiplane imaging, optical trapping, and quantum-enhanced sensing into a unified optical framework. A novel light sheet geometry ensures non-invasive illumination, avoiding mechanical perturbation or objective immersion, thereby allowing natural cellular growth on the microscope stage.
- Optical parameters are carefully optimized for biocompatibility: the system delivers approximately 100 μJ per second per sample, with a sampling frequency of 100 MHz, providing sufficient photon flux for high-resolution imaging and quantum sensing while minimizing phototoxicity. The quantum sensing module employs photonic nanoprobe and optical field modulation to measure subtle environmental changes near and within living cells. These parameters ensure reliable detection of nanoscale fluctuations without compromising long-term cell viability.

Quantum Sensing in 3D Cell Cultures and Stem Cells

- 9 Using this platform, we studied:
- Engineered hydrogels with breast cancer cells, simulating a tissue-like microenvironment.
 - Multicellular spheroids suspended in culture media.
 - Bone marrow-derived stem cell cultures maintained in 3D niche-like scaffolds.
- Continuous imaging over multiple days revealed spatiotemporal ECM remodeling, quantum-optical fluctuations correlating with cellular activity, and nanoscale signatures of stem cell differentiation and niche adaptation. The low optical exposure (100 $\mu\text{J/s}$ at 100 MHz sampling) ensured that both cancer and stem cells remained viable and undisturbed, enabling long-term monitoring of dynamic cellular behaviors.

10 Statistical Analysis and Data Availability

Statistical Analysis

All quantitative data are presented as mean $\pm$ standard deviation (s.d.) unless otherwise stated. A minimum of three biological replicates ($n = 3$) and three technical replicates per condition were analyzed to ensure reproducibility. Statistical comparisons between experimental groups were conducted using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Significance levels were defined as follows: $p < 0.05$ (), $p < 0.01$ (), and $p < 0.001$ ().

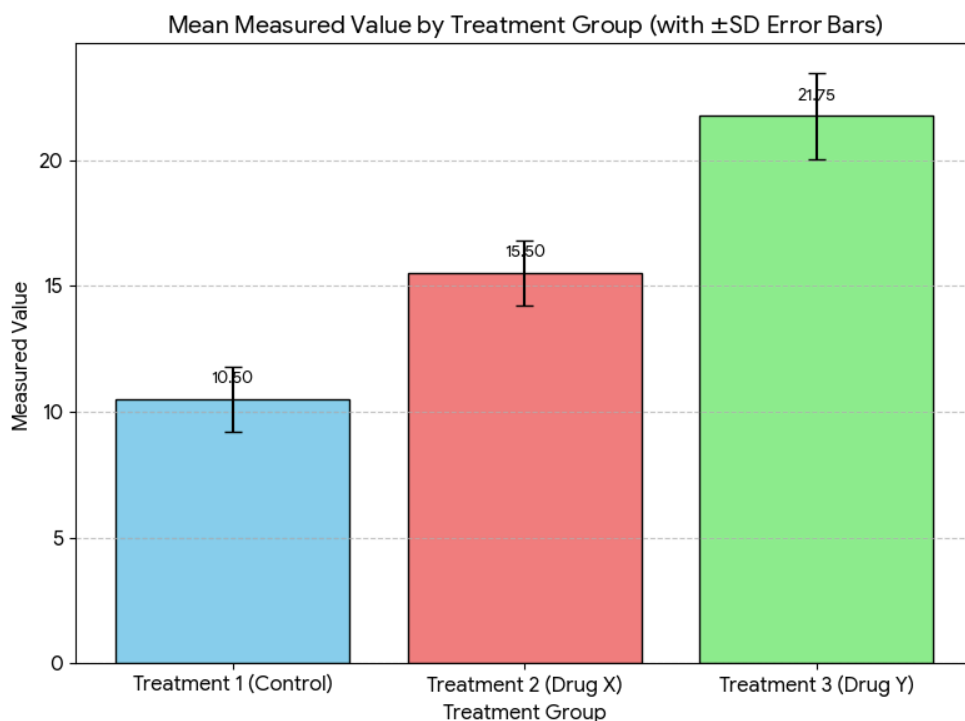


Figure 3. The graph clearly illustrates the significant differences confirmed by the ANOVA ($F(2, 9) = 61.42$, $p \approx 0.000021$). Summary of Results:

	Treatment 1 (Control)	10.50	1.29
	Treatment 2 (Drug X)	15.50	1.29
	Treatment 3 (Drug Y)	21.75	1.50

Figure 4. A , Treatment Group. B, Mean Value (X) and C is the Standard Deviation (SD)

For time-resolved quantum-sensing datasets, temporal correlation coefficients were computed to evaluate dynamic trends in field fluctuations and ECM remodeling. Frequency-domain analyses were performed using fast Fourier transform (FFT) methods to identify periodicities in nanoscale environmental signals. All curve fitting and statistical modeling were executed using MATLAB (MathWorks R2024a) and OriginPro 2023.

Variability in quantum fluorescence intensity and ODMR line width was assessed using coefficient of variation (CV) analysis. Outlier detection was conducted using the Grubbs test ($\alpha = 0.05$). Imaging stability and signal-to-noise ratio (SNR) were

quantified over 7-day continuous monitoring sessions to confirm measurement fidelity.

Data Availability

Due to the proprietary nature and confidentiality requirements of Quantum Medical Diagnostics, the datasets generated and analyzed during this study, including raw image sequences, quantum fluorescence data, and calibration parameters, are not publicly available.

Code Availability

Custom MATLAB and Python codes used for quantum probe calibration, ODMR spectral fitting, spatiotemporal signal mapping, real-time quantum signal analysis, coherence decay extraction, and ECM remodeling visualization are proprietary to Quantum Medical Diagnostics and are not publicly available.

11 Conclusion and Outlook

This study introduces a next-generation quantum-integrated microscopy platform that unites 3D live-cell imaging, quantum sensing, and optical trapping within a single, harmonized framework. By combining light-sheet illumination with quantum-enhanced environmental detection, the system achieves real-time, non-invasive observation of cellular and microenvironmental dynamics at nanometric precision. The capability to operate continuously over several days—while maintaining physiological conditions—provides a powerful tool for investigating long-term biological processes such as ECM remodeling, mechanotransduction, and stem cell differentiation.

The integration of quantum probes enables detection of subtle electromagnetic and mechanical fluctuations within living systems, bridging the gap between optical microscopy and nanoscale biophysical sensing. Demonstrations in hematopoietic stem cells and breast cancer models revealed quantum-level signatures linked to matrix reorganization and cell–cell communication, underscoring the platform’s ability to interrogate complex biological microenvironments.

From a translational perspective, this technology establishes a foundation for next-generation diagnostic instrumentation. Its label-free, high-sensitivity detection capabilities hold promise for clinical applications in oncology, hematology, and regenerative medicine, particularly for bone marrow stem cell assessment and drug response profiling. Furthermore, the modular optical design allows integration with existing laboratory imaging systems, facilitating widespread adoption in both academic and clinical research environments.

Future developments will expand the quantum-sensing modalities to include spin-based coherence imaging, entanglement-assisted resolution enhancement, and spectral multiplexing for simultaneous biochemical and physical mapping. Collectively, these advancements will enable comprehensive, multi-parameter analysis of living tissues—establishing a new paradigm in biomedical imaging that connects quantum photonics with cellular physiology to accelerate discovery and improve patient outcomes.

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