Multiplex 3D MINFLUX and DNA-PAINT resolves nanoarchitecture of HIV-1 particles

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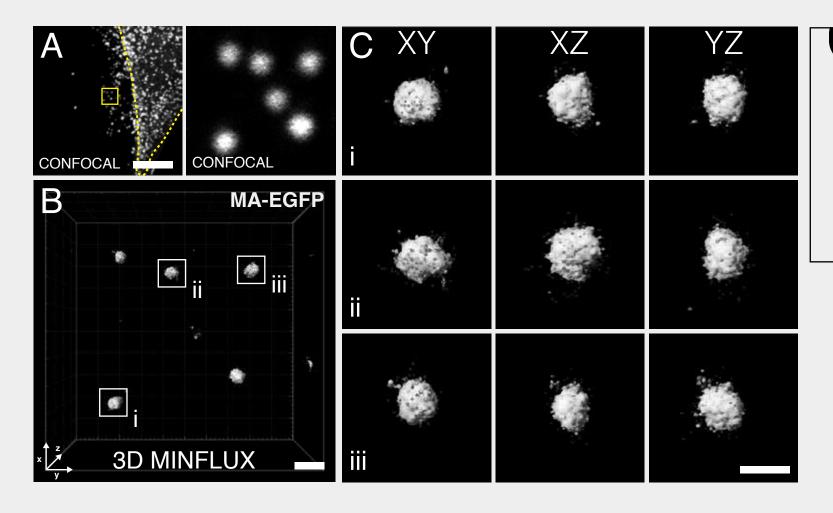
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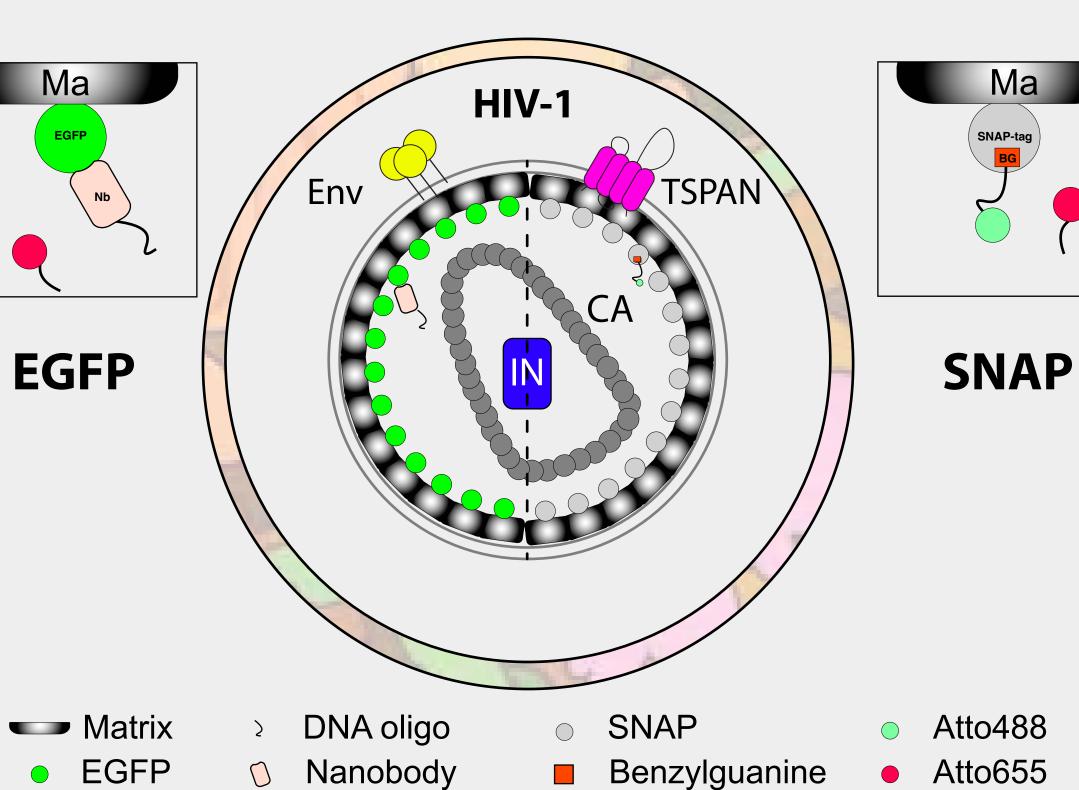
Abstract

Resolving the nanoscale organization of viral and host proteins is key to understanding HIV-1 assembly and infectivity. We present a framework for multiplexed 3D super-resolution microscopy of HIV-1 particles using MINFLUX and DNA-PAINT, achieving isotropic precision below 10 nm for up to five targets. Using the matrix protein as a reference, we quantified labeling-induced linkage errors and mapped the spatial distribution of viral and host proteins within individual particles. To support analysis, we developed matFLUX, an open-source software for quantitative visualization and alignment of multi-color MINFLUX data.

DNA-PAINT Labeling Approaches for HIV-1 Particles



GFP-labeled VLPs by confocal microscopy (**A**) and 3D MINFLUX (**B**). Three individual particles from panel B shown in XY, XZ, and YZ projection (**C**). **Scale bars: 5 μm (A), 200 nm (B), 100 nm (C).**



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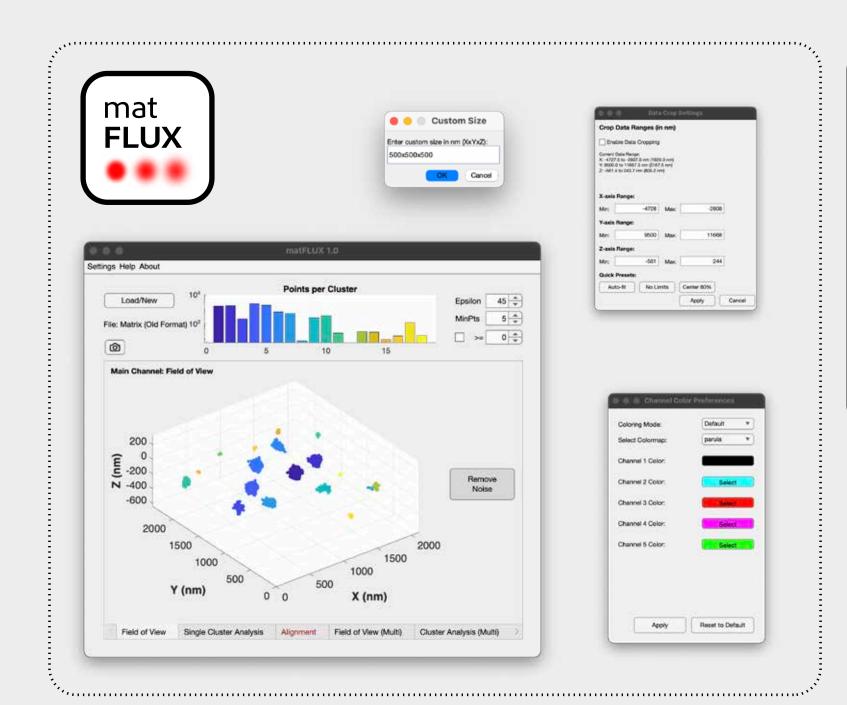
E MA-SNAP

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SNAP-labeled VLPs by confocal microscopy (**D**) and 3D MINFLUX (**E**). Three individual particles from panel D shown in XY, XZ, and YZ projection (**F**). Scale bars: 5 μ m (**D**), 200 nm (**E**), 100 nm (**F**).

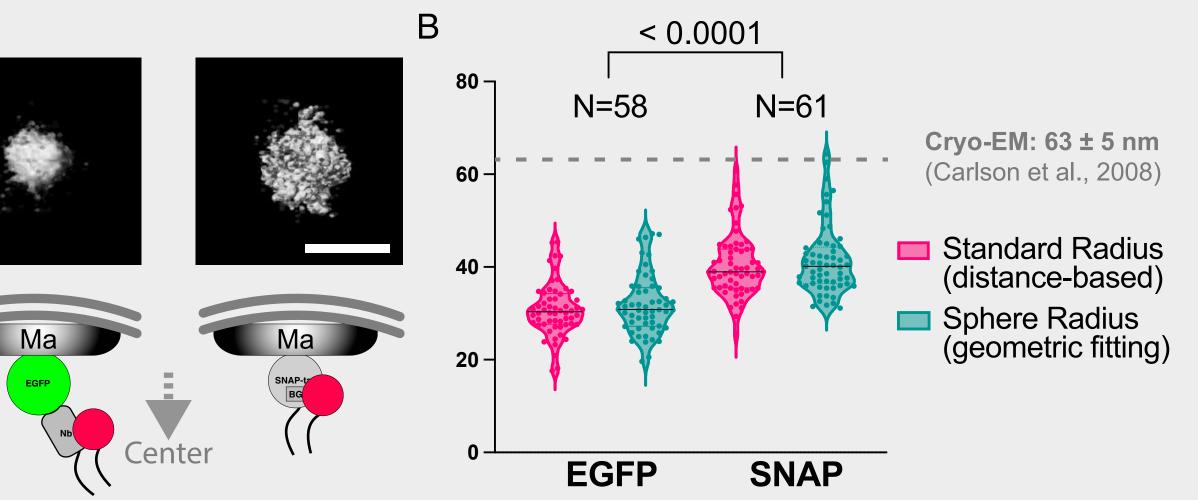
matFLUX - Particle-Centered Analysis Software



3D MINFLUX Data Processing

- Workflow-oriented & Intuitive
- Modular
- Multi-Channel Alignment
- Single and Batch Data
- Open Source
- All Platforms

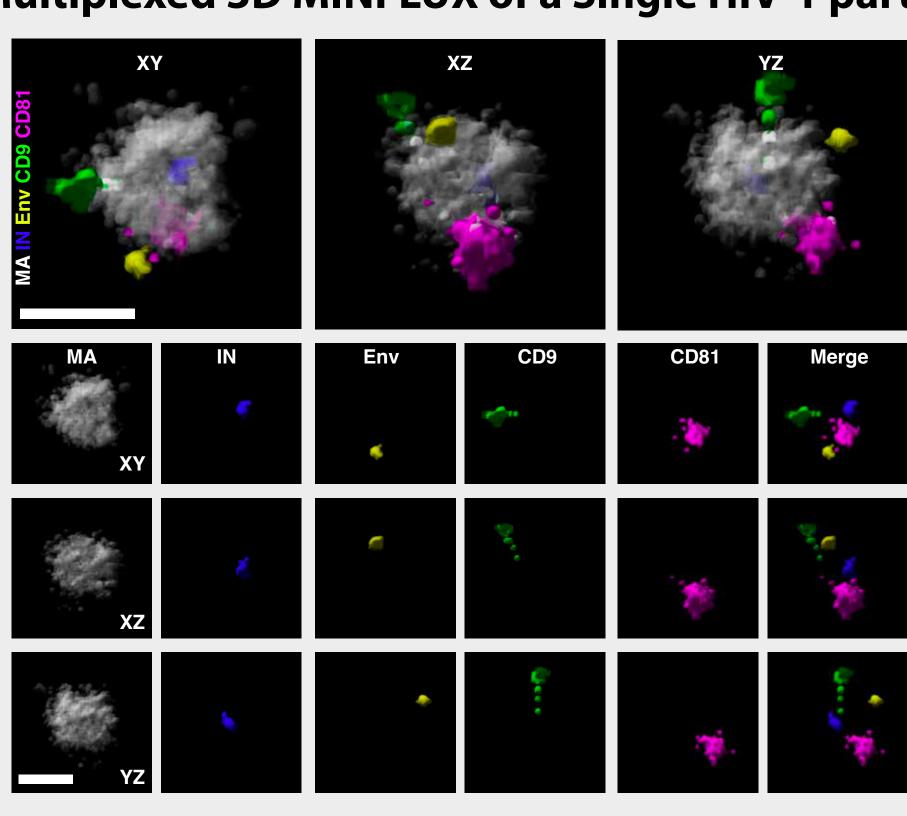
MINFLUX Reveals Linkage Error A B



Morphological comparison of EGFP- vs. SNAP-labeled HIV-1 particles. Representative particles and labeling schematic (**A**). Particle radii quantified by distance-based and geometric fitting methods, showing significant size differences (**B**).

Scale bar: 100 nm.

Multiplexed 3D MINFLUX of a Single HIV-1 particle



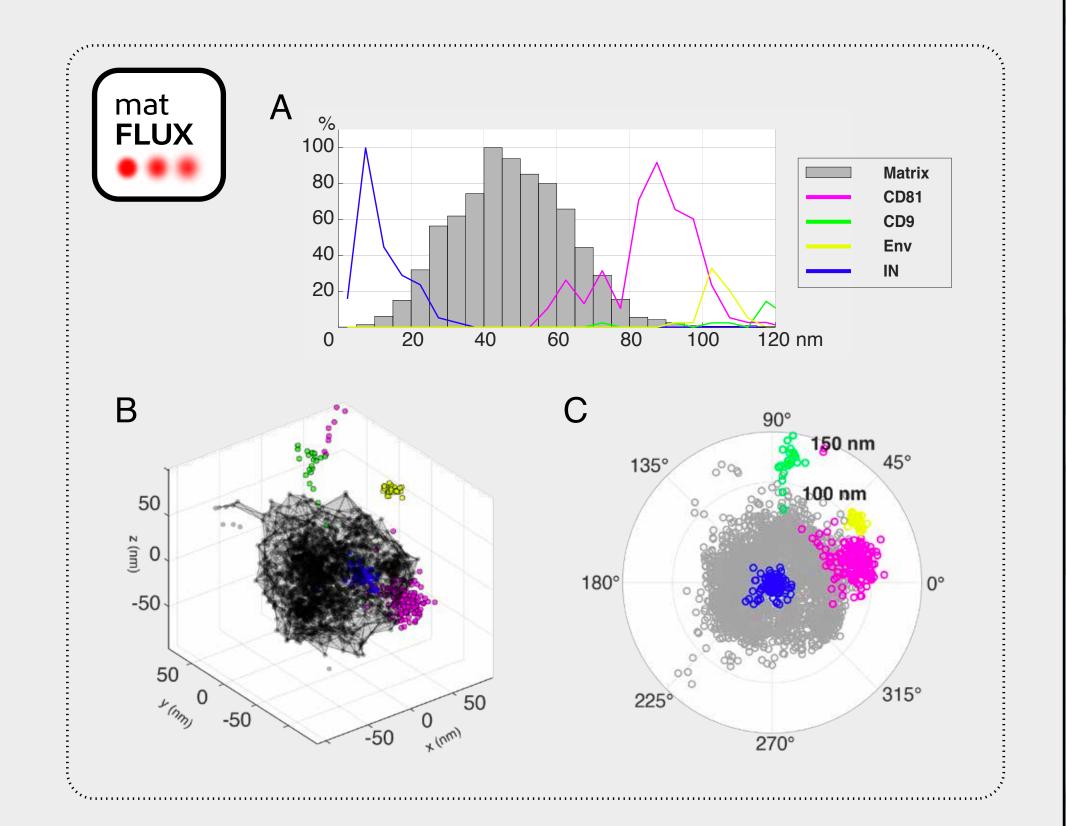
Five-target 3D MINFLUX of a single HIV-1 particle, visualizing nano- organization of three viral (HIV Matrix, Integrase and Envelope) and two host proteins (Tetraspanins CD9 and CD81).

100 nm

HIV-1

Scale bar: 100 nm.

matFLUX visualizations of HIV-1 protein organization from 5-target 3D MINFLUX: concentric analysis (**A**), 3D surface reconstruction (**B**), 2D polar plot (**C**).



Outlook

Software: Release matFLUX as open-source software with tutorials for all platforms.

Tetraspanins: Characterize tetraspanin-enriched nanodomains in viral particles and at the plasma membrane.

HIV: Visualize HIV assembly and budding processes at the plasma membrane.

Multiplexed Viral Imaging: Apply framework to other viruses to uncover shared and unique nanoscale architectures.

