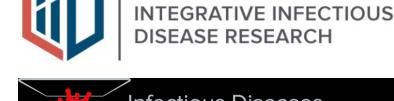
Multiplex 3D MINFLUX and DNA-PAINT resolves nanoarchitecture of HIV-1 particles

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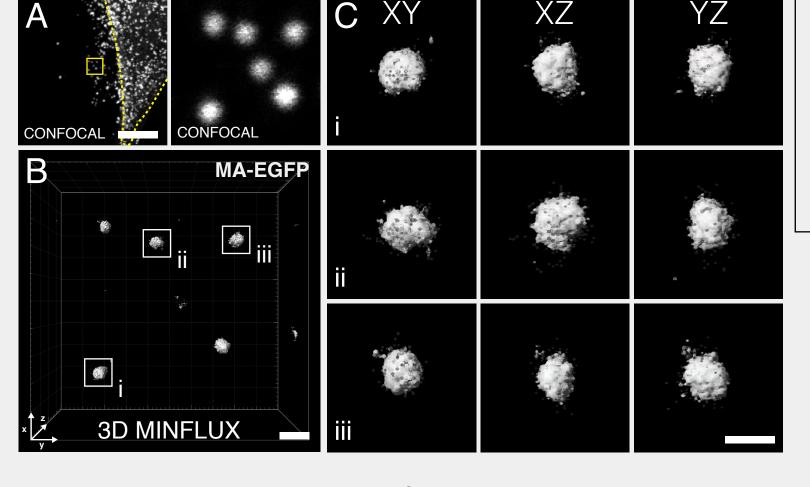




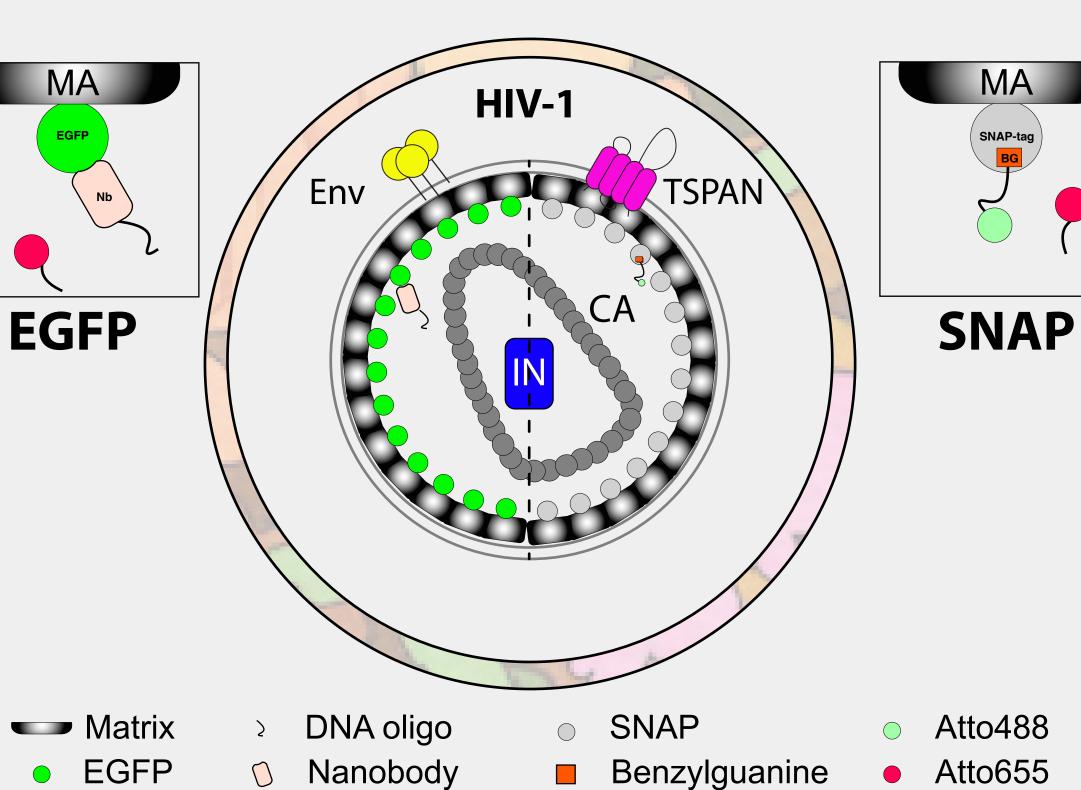
Abstract

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. This framework revealed distinct nanodomains of viral and host proteins at the HIV-1 surface, highlighting their nanoarchitecture.

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).



MA-SNAP 3D MINFLUX

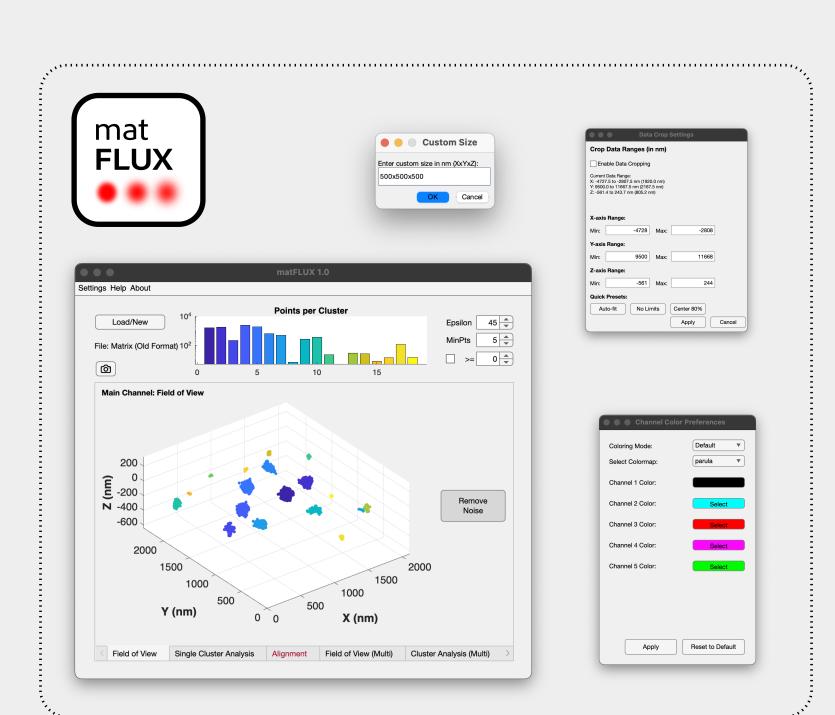
SNAP-labeled VLPs by confocal microscopy (**D**) and 3D MINFLUX (**E**). Three individual particles from panel D shown in XY, XZ, and YZ projection (F). Scale bars: 5 μm (D), 200 nm (E), 100 nm (F).

SNAP

(distance-based)

Sphere Radius (geometric fitting)

matFLUX - Particle-Centered Analysis Software



3D MINFLUX Data Processing

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

< 0.0001 N=61 N=58 Cryo-EM: 63 ± 5 nm (Carlson et al., 2008) Standard Radius

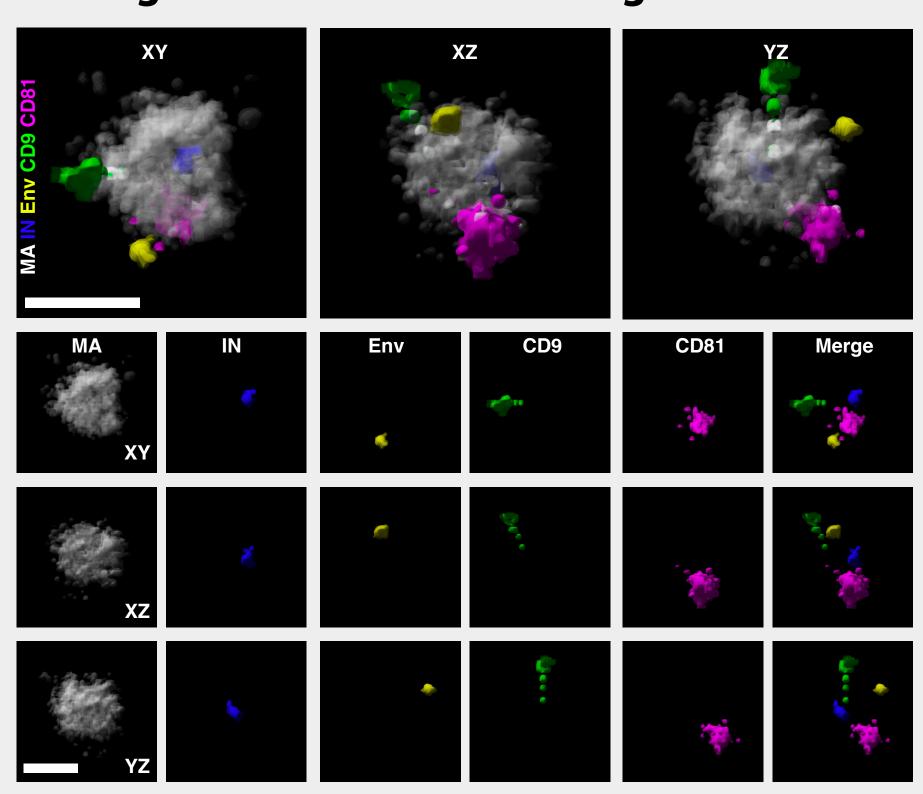
EGFP

MINFLUX Reveals Linkage Error

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.

Five-target 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

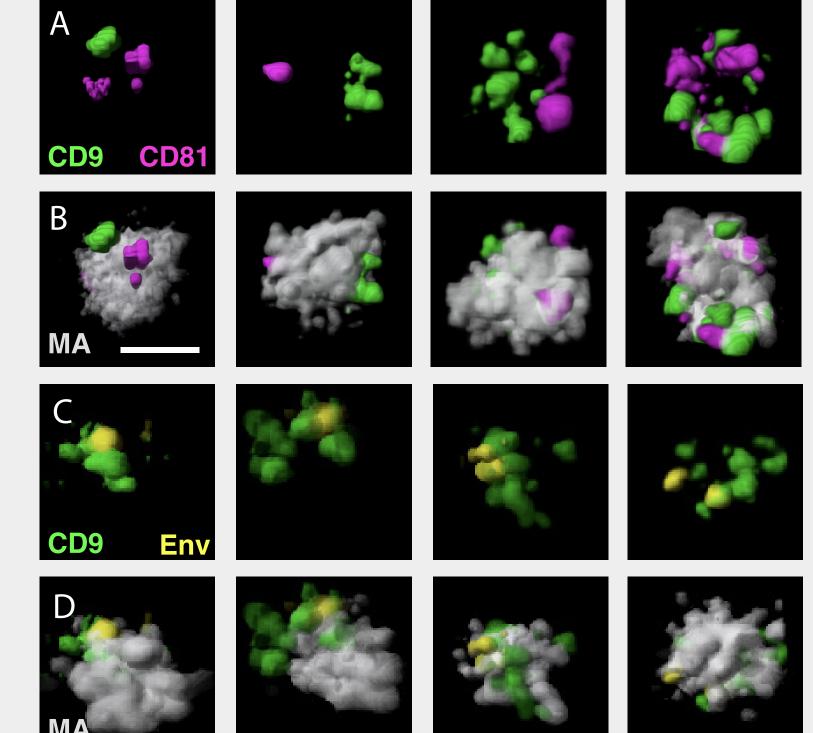
HIV-1

Scale bar: 100 nm.

3D MINFLUX imaging of viral nanodomains. CD9 (green) with CD81 (magenta) or Env (yellow), visualized alone (A,C) and together with MA (gray) (B,D). Multiple representations from single virus particles.

Scale bar: 100 nm.

Tetraspanin Nanodomains on HIV-1 Particles



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.

