ONI

Super-resolution with the Nanoimager
what is the most suitable imaging technique for you?

	dSTORM Direct Stochastic Optical Reconstruction Microscopy	PALM PhotoActivated Localization Microscopy	SPT Single-Particle Tracking	smFRET Single-molecule Förster Resonance Energy Transfer
Resolution achieved	20nm	20nm	N/A	0.3–10nm
# of colors	Up to 4 (2 simultaneously)	Up to 4 (2 simultaneously)	Up to 4 (2 simultaneously)	2 FRET pairs at once
Live or fixed samples?	Mostly fixed	Both	Live (in solution or cells)	Both
Best for	• Quantification and localization of single molecules • Very high resolution	• Counting and tracking of single molecules in fixed & live samples • Lower laser powers than for dSTORM	• Tracking single particles and molecules • Quantitative data (i.e. diffusion coeff., sizing and counting)	• Real-time measurement of interor intramolecular interactions • Individual traces and population averages
Added value	TIRF/HILO for a better SNR Astigmatic 3D Microfluidics compatibility	TIRF/HILO for a better SNR Astigmatic 3D Whole body heating for live imaging	TIRF/HILO for a better SNR Astigmatic 3D Whole body heating for live imaging Microfluidics compatibility	Use of Alex (Alternating Laser EXcitation) mode for stoichiometry of molecules
Data analysis tools on Nanoimager	Automation, Real-time rendering, python	Automation, Real-time rendering, python	Automation, Dedicated tracking tool, python	Dedicated smFRET analysis tool, python
Pro tips	• Choose blinking dyes and special buffers • Very clean coverslips	• Photoactivable and photoconvertible fluorophores • Very clean coverslips	• Choose bright and long-emitting fluorophores • Very clean coverslips	• Donor emission to overlap with acceptor's absorption spectrum • Exceptionally clean coverslips
Additional notes	Live imaging not feasible due to high laser power and non-physiological buffers Longer acquisition times	Limited availability of fluorophores Acquisition can be time-consuming (depending on number of molecules)	Tricky to use in densely labeled population Fluorophores need to be sparse	Only works over short distances (up to 10nm) Compatible FRET pairs required

Legend

Automation · Real-time rendering · python Special software features · Dedicated tracking tool · Dedicated smFRET analysis tool

For the best flourophores and imaging guides visit https://oni.bio/resources/resource-library/

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