

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 interior 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
Level of skill	Nanoimager	   	   	   	   
	Data analysis	   	   	   	   
Data analysis tools on Nanoimager		  	  	  	 
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
  Special software features
  Dedicated tracking tool
  Dedicated smFRET analysis tool

For the best flourophores and imaging guides visit: www.oni.bio/resources/university