Detection Platform Compatibility with SmartFlare™ Probes

Technology	Imaging						Non-Imaging			
		Microscopy								
	High Content	Wide field/ Epi-fluorescence	Confocal	Two-photon Confocal	Imaging plate readers*	Imaging Flow Cytometry	Flow Cytometers		Fluorescence-Activated Cell Sorters	Plate Readers*
Examples	Biotek Cytation™ 3 PerkinElmer Opera™	Evos® Station	Nikon C2	Zeiss LSM 510-NLO	PerkinElmer EnSight™	Merck Millipore Amnis® brand FlowSight® and ImageStream®^{X} Systems	Merck Millipore Muse® Cell Analyzer	Merck Millipore guava easyCyte™ Flow Cytometer	FACSAria™	Tecan Infinite® M1000
SmartFlare™ Fit	HIGH	MED	HIGH	HIGH	MED	HIGH	HIGH	HIGH	HIGH	LOW*
Dye Compatibility Cy 3 Ex 550 Em 570	**Yes** (mono or filters)	**Yes** (530 nm excitation via lightcube)	**Yes** (561 nm excitation)	**Yes** (543 nm excitation)	**Yes** (mono)	**Yes** (561 nm excitation)	**Yes** (532 nm excitation)	Not optimal (488 nm excitation only)	**Yes** (561 nm excitation)	**Yes** (530 nm excitation)
Cy 5 Ex 625 Em 670	**Yes** (mono or filters)	**Yes** (via optional Cy5 lightcube)	**Yes** (633 nm excitation)	**Yes** (633 nm excitation)	**Yes** (mono)	**Yes** (642 nm excitation)	No	**Yes** (640 nm excitation)	**Yes** (633 nm excitation)	**Yes** (635 nm excitation)
Cy5 / Cy3 cross-compatible	**Yes** (mono)	No (Bleed over from channels can occur if no PMT)	**Yes**	**Yes**	**Yes** (+25% detection time for two-color)	**Yes** (with 561 nm and 642 nm laser options)	No	Not optimal	**Yes**	**Yes**
Applications	• HTS screens • Time-lapse experiments • Live cell assays	• Live cell applications (using heating tray)	• Morphology - Timecourse/ Live cell imaging with incubator	• Morphology in whole cells • Timecourse/ Live cell imaging with incubator	• Cell population imaging • Identification of positive cell events within population	• Single-cell imaging, morphology • Identification of positive cell events within population • Detection of rare events in populations	• Single cell analysis	• Single cell analysis	• Separation of cell populations from heterogeneous samples • Enrichment tool	• Top and bottom well readings
Typical sample size (# cells)	Up to 384-well plate, T-25 flask, up to 100 mm dishes; cell number varies	Live cell culture vessels (dishes up to 100 mm, flasks up to T-75); cell number varies	8-well or 4-well chamber slides; 35 mm dishes and multiwell plates (~10^5 cells per well)	8-well or 4-well chamber slides; 35 mm dishes and multiwell plates (~10^5 cells per well)	Up to 384-well plates	Both systems will image ~95% of cells in a 200 µL sample, regardless of the number of cells present.	Single-sample reads in tubes (200 µL) 30,000 cells per tube	96-well plate reads (~200 µL per well) 30,000 cells per tube	5 mL polypropylene tube (1 - 3 mL volume) up to ~10^7 cells per sample	Up to 1536 well plate; volume and cell numbers vary

*Usage on non-imaging plate readers as not been validated in our labs but is likely provided the platform has the correct Ex and Em wavelength capability and can perform bottom. Most Non-imaging readers only allow for whole-well fluorescence reads, resulting in population averages, not single cell resolution which SmartFlare™ probes provide.

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