

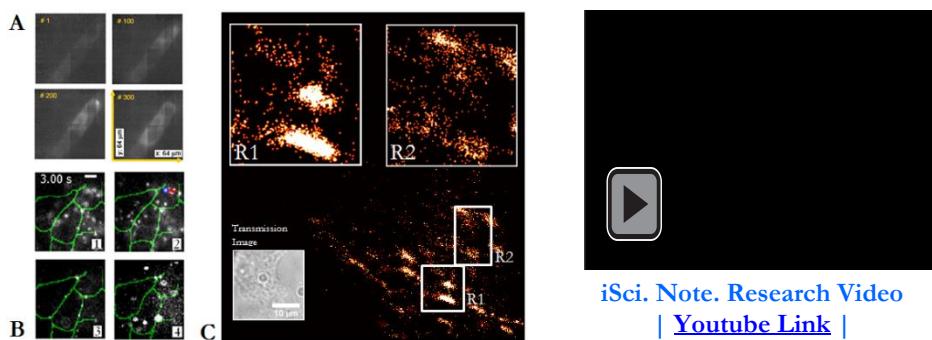
SPATIO-TEMPORAL SUPER-RESOLUTION OPTICAL FLUORESCENCE MICROSCOPY

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Figure 1. |A| Multifocal multiphoton microscopy A system showing contraction of cardiac myocytes (labeled with Fluo3 dye) [7,8,11]. |B| Multifocal plane microscopy showing the transfer of Tf molecules in Z310 at simultaneous 4 different focal planes within the cell monolayer [8,11]. |C| One of the variant of fPALM super-resolution localization microscopy (Clean microscopy) shows the distribution of Actin in NIH-3T3 mouse fibroblast cells (scale bar=1μm) [12].



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Fields ranging from the physical sciences to biology mostly rely on indirect techniques to understand the underlying biological processes. Only now are they being interrogated and visualized with the advent of super-resolution optical microscope techniques (PLAM, fPALM, STORM, GSDIM, STED and SIM) [1]. More recently the field of super-resolution has expanded with its integration with light-sheet microscopy[2]. These techniques can resolve features better than the diffraction limit [3]. Several interesting biophysical studies have been reported in the literature. For example, the Samuel Hess group have shown how super-resolution microscopy can address key problems in medicine by directly imaging clustering of membrane protein during viral infection [4] ; The Stefan Hell group have shown video-rate observation of individual vesicle movement (continuously transiting through the axons) using STED microscopy[5] ; The X. Zhuang group showed that, poliovirus exhibit microtubule-independent fast intracellular movement with a speed of approximately few microns/sec to quickly explore the entire cell during infection [6]. These are some of the few selected studies and this is just the beginning.

Most of these techniques are capable of spatial super-resolution, often at the expense of poor temporal resolution. Specifically, this is true for localization techniques. Recent techniques that are capable of high temporal resolution include, multiphoton multifocal microscopy (MMM) [7,8], multifocal plane microscopy [9], multiple excitation spot optical (MESO) microscopy and multiple light-sheet microscopy (MLSM)[10]. Fig.1 shows images obtained using state-of-the-art

temporal and spatial super-resolution techniques. Table.1 compares key features. To enable spatio-temporal super-resolution, one needs to explore techniques that can simultaneously capture spatial information from multiple specimen planes for instant volume reconstruction, a step closer to 4D imaging. A simple way to realize such a spatio-temporal super-resolution microscope is through the integration of spatial super-resolution with high temporal resolution techniques. The next generation microscope is expected to achieve this feat.

Much has been suggested to further improve spatial resolution whereas temporal resolution is somewhat neglected. This becomes essential for imaging rapidly occurring biophysical processes. Temporal resolution of an imaging system depends on many factors including, detector efficiency (Quantum yield) and complex molecular properties. However, the fundamental property that puts a lower-bound on temporal resolution is limited by the recycle time ($S_0 \rightarrow S_1$ and $S_1 \rightarrow S_0$) of the ensemble of excited molecules or equivalently their excitation-emission cycle. This ensures that the molecule is available for re-excitation and is only limited by the recycle time between ground and excited state (assuming negligible photobleaching) [11]. In a simplistic three state system (S_0 , S_1 , T_1), the minimum recycle time for the ensemble of excited molecule is found to be approximately, $3\tau_p = 3(\log 10)/(k_f + k_{nr})$, where k_f and k_{nr} are the radiative and non-radiative emission rates respectively [11]. For the idealistic assumption of zero photobleaching, $t=3\tau_p$ is the lower limit on the temporal resolution of the fluorescence microscope.

Table.1 | Advantages and disadvantages of different super-resolution techniques [13].

Microscopy Technique	Probe Type	Spatial Reso.	Temp. Reso.
Typical fluorescence Microscope	Conventional	250 nm (diffrac. limit)	typical, 100 – 500 ms
PALM fPALM	Photo-activatable	10nm – 55nm	typical, 5 – 10 min
STORM GSDIM	Photo-switchable	10nm – 55nm	typical, 5 – 10 min
STED	Conventional	30nm – 70nm	few seconds
SIM	Conventional	≈ 100 nm	few seconds

In near future, it is hoped that the availability of spatio-temporal super-resolution system may enable us to: 1| Visualize molecular events (such as, neuronal firing, Ca^{2+} ion influx) in ultraslow motion, 2| Reconstruct single-shot volume images and, 3| Acquire spatio-temporally super-resolved 4D video in real-time.

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Review Editor's Comments : Partha Mondal wrote an interesting and timely article about the need for improved temporal resolution in microscopy.