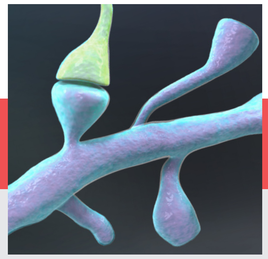
BEYOND THE DIFFRACTION BARRIER

An overview of super-resolution microscopy strategies



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ABSTRACT

Super-resolution microscopy (SRM) is a form of light microscopy that enables investigation of sub-cellular processes in living cells, beyond the diffraction barrier in conventional light microscopy. To facilitate understanding, support, and adoption of the technology, a 3D animation was created to visually explain high-level principles of several SRM techniques, using visualization of neuronal dendritic spines and subcellular processes to deliver complex and difficulty concepts to specific groups of audience.

INTRODUCTION

Light microscopy is essential to study **living neurons** in neurobiology, but is limited by the **diffraction** properties of light, which significantly lower the imaging resolution and focus of sub-cellular structures past 200nm.

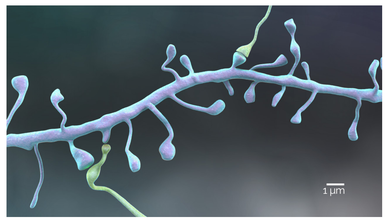

Figure 1. 3D visualization of a dendrite with growing and shrinking dendritic spines demonstrates how SRM allows observation of dynamic activities in living neurons.

Observation of sub-cellular processes in live **dendritic spines** allows greater understanding of their dynamic morphology and plasticity. Super-resolution microscopy can go beyond the diffraction barrier and allow study of living mechanisms.

Communication Goal

The main goal is to successfully explain the basic principles of various SRM techniques

- **Objective**: To create a 3D animation showing an overview of what can be seen with different SRM techniques to capture initial interests
- **Objective**: To show that SRM techniques can work together to reveal a complete picture of the dendritic spine, and thereby stimulate further research interests in SRM

The intended audience for the animation includes funding bodies, graduate students, neurobiologists, and general biologists.

Super-resolution Microscopy

The animation introduces four SRM techniques that can be grouped into two distinct sets of imaging strategies:

Laser scanning microscopy

- **ST**imulated **E**mission **D**epletion (STED)
- **S**witching **LA**ser **M**ode (SLAM)

Wide-field microscopy

- **S**ingle **P**article **T**racking (SPT)
- **P**hoto-**A**ctivated **L**ocalization **M**icroscopy (PALM)

MATERIALS & METHODS

All 3D modeling and animation were created in Autodesk Maya 2014, and composited in Adobe After Effects CS6, with audio recorded and edited in Adobe Audition CS6. References for the main neuron was obtained from NeuroMorpho, dendrite with spines from Nägerl *et al*. (2008), individual molecules from RCSB Protein Data Bank (PDB).

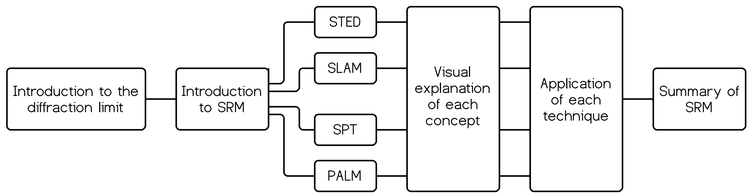

Figure 2. Summary of the narrative flow. Difficult concepts can be delivered with straightforward storytelling.

RESULTS

Figure 3. Snapshots from the opening scene where 3D models of pyramidal neurons, whose neurites are covered with dendritic spines–created with **PaintEffects** in Maya, are shown to establish the setting of optical microscopy and to demonstrate its diffraction limit.

Optical shaping of light excitation in STED and SLAM

STED and SLAM sharpen the blurry focal spot of light by either using a depletion light beam on excited fluorophores (STED), or a dark laser beam of the same wavelength (SLAM). These two techniques use similar concepts of optical shaping but are significantly different in their approaches.

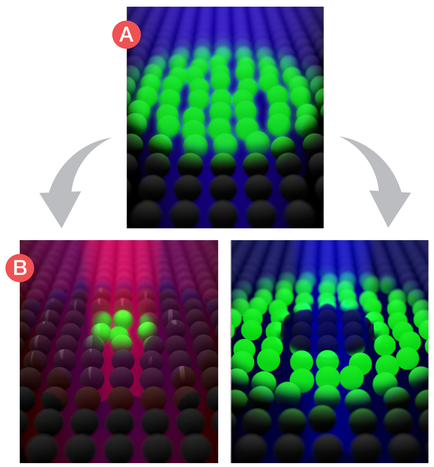

Figure 4. **(A)** The basic concept of fluorophores emitting fluorescence after laser excitation is explained by using simple **nParticles** reacting to a confined **turbulence field**. **(B)** The bottom panels show the fundamental difference between the two techniques (STED on left, SLAM on right) in shaping the original laser beam.

Single particle imaging in SPT and PALM

SPT and PALM localize single particles by calculating for the center of regular Gaussian-shaped signals. These techniques rely on wide-field beam.

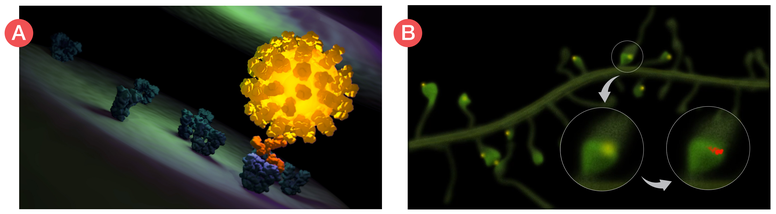

Figure 5. **(A)** An animation still shows a close-up of a fluorescent nanoparticle (quantum dot) binding to a neurotransmitter receptor at a synapse as an application of SPT. **Molecular Maya Toolkit (mMaya v1.3)** was used to import the molecules from PDB (#3KG2 for glutamate receptor; #1IGT for immunoglobin). **(B)** A simulated footage of quantum dots (in yellow) moving inside synapses (in bright green). A simulation, instead of real footage, not only gives higher image quality, but also allows flexibility and creativity in visually explaining concepts. The trajectory of the receptor (in red) was simulated using the **Wiggler** and **CC Time Blend** effects in After Effects.

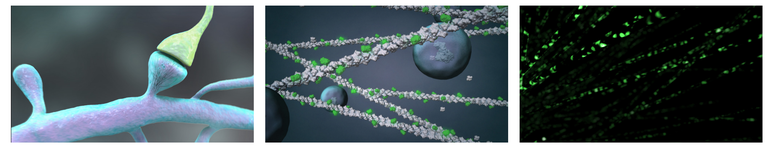

Figure 6. PALM can be applied to investigate molecules such as f-actin microfilaments inside the dendritic spine (left). Sparse GFP molecules can be can be turned on and off to obtain signals (middle), which can be stacked together over time to show the complete location of the microfilaments (right). Actin filaments were modeled with information from *In Silico* (2008) (PDB #1J6Z for actin molecules; #1EMA for GFP).

DISCUSSION

The animation will be posted on the Neurophotonics Centre website for the general public and especially scientists and students interested in super resolution microscopy. An additional interactive module with more in-depth explanation of SLAM is currently in development, and will accompany the animation when completed.

References & Acknowledgment

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Project Development

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