Investigating the Assembly Pathway of SBA Protein Microtubules Using Single-Molecule Super-Resolution Imaging

Chen Xu

Introduction

Induced by specific ligands, soybean agglutinin (SBA) spontaneously assembles into highly uniform protein microtubes. However, current Cryo-EM techniques can only capture the static, “dead” structures of the final product. How these micro-tubes actually nucleate and grow in an aqueous environment remains a mystery. To crack this, we developed a closed **Flow Cell system** coupled with **TIRF-STORM** super-resolution imaging, successfully capturing the in-situ dynamic assembly of microtubes at the single-molecule level.

Method

Traditional open systems are highly susceptible to oxygen in the air, which can easily disrupt the normal formation of tubulin.

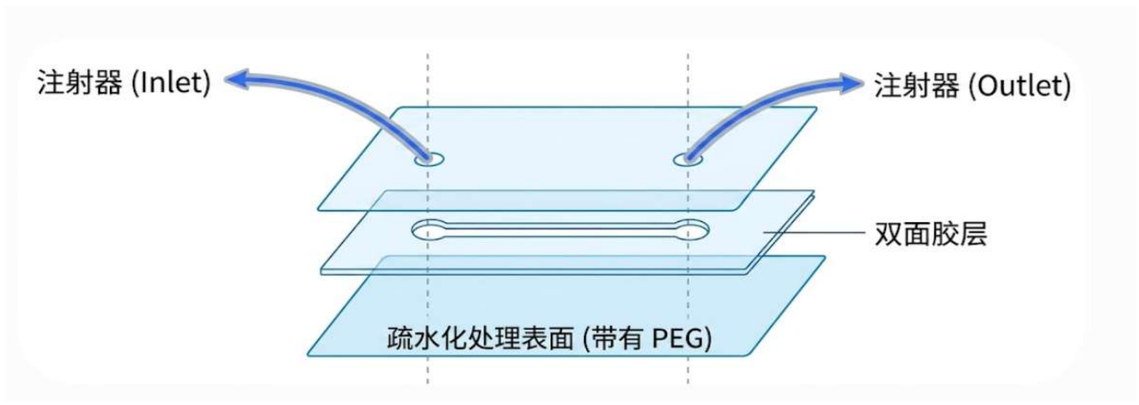

Figure 1:The structure of the flow cell.

To tackle this, we use a closed Flow Cell system, as shown in the figure. The core advantage of this system lies in its flexible fluid control. During the process, we can use a micro-syringe to temporarily draw out the reagents for in situ washing and observation, and then seamlessly inject them back to resume the self-assembly. This allows us to successfully capture the intermediate states in action.

TIRF&STORM

The excitation light in traditional microscopes penetrates the whole sample, which inevitably causes significant back-ground noise. To solve this pain point, Total Internal Reflection Fluorescence (TIRF) microscopy cleverly utilizes an ‘evanescent wave’ to excite just a very thin layer right next to the coverslip.

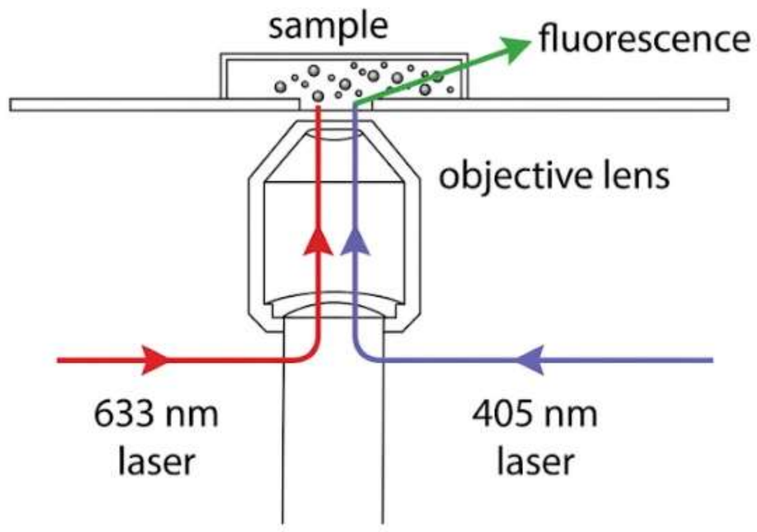

Figure 2:The optical path diagram of TIRF.

The first step is to capture the random 'blinking' of fluorescent molecules using high-speed imaging, generating a raw sequence of tens of thousands of frames. Next, we use the ThunderSTORM plugin in ImageJ to analyze this sequence. By applying a 2D Gaussian fit, we can precisely pinpoint the sub-pixel coordinates of each molecule while filtering out the cluttered background noise.

Finally, by accurately overlaying and rendering all these extracted single-molecule coordinates together, we can reconstruct an ultra-clear image with incredibly high spatial resolution.

Results

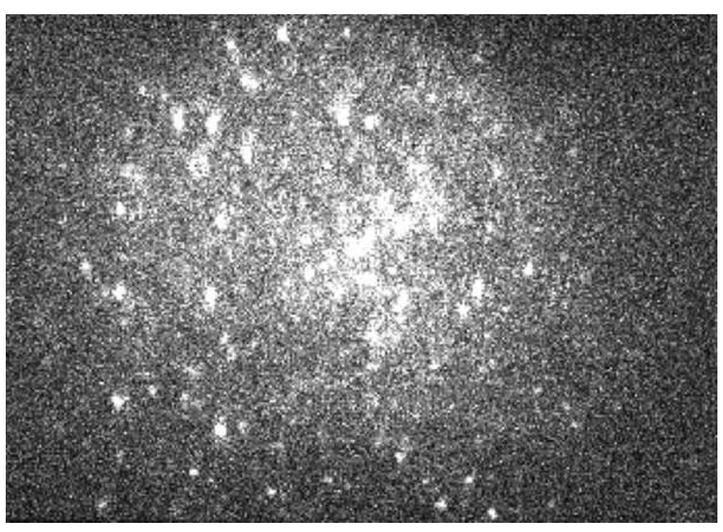

Figure 3:The fluorescence spots observed through TIRF.

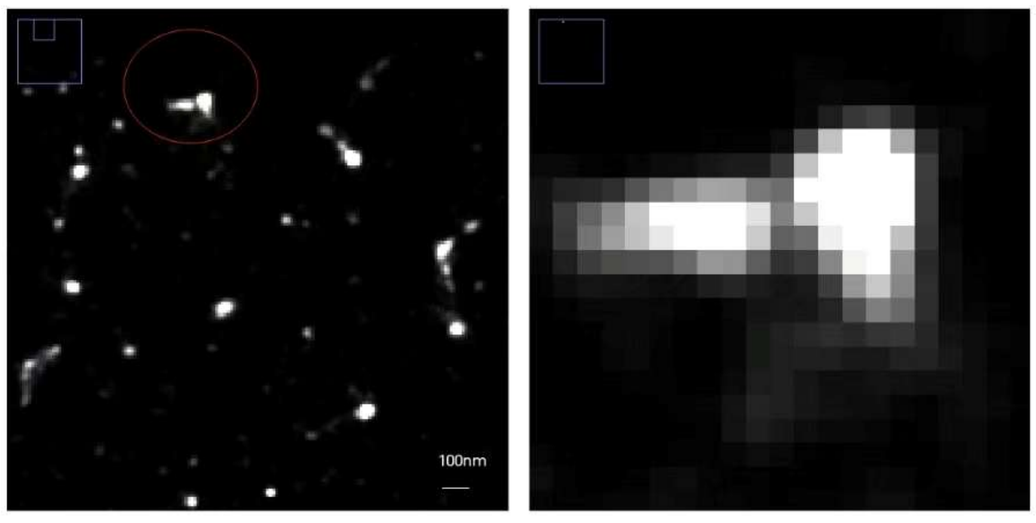

Figure 4: STORM imaging results. The rod-like structures likely represent successfully assembled microtubules.