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Spindle Assembly and Architecture: From Laser Ablation to Microtubule Nucleation DANIEL NEEDLEMAN, JAN BRUGUES, VALERIA NUZZO, ERIC MAZUR, Harvard University — Spindles are arrays of microtubules that segregate chromosomes during cell division. It has been difficult to validate models of spindle assembly due to a lack of information on the organization of microtubules in these structures. Here we present a novel method, based on femtosecond laser ablation, capable of measuring the detailed architecture of spindles. We used this method to study the metaphase spindle and find that microtubules are shortest near poles and become progressively longer towards the center of the spindle. These data, in combination with mathematical modeling, high resolution imaging, and biochemical perturbations, are sufficient to reject previously proposed mechanisms of spindle assembly. Our results support a new model of spindle assembly in which microtubule polymerization dynamics are not spatially regulated, microtubule transport locally sorts microtubules – determining their proper organization in the spindle without moving them appreciable distances –, and the profile of microtubule nucleation controls the length of the spindle.

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