

The Limiting Effect of Cytoplasmic Volume and Microtubule Dynamics

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Molecular Biology

Honors Program

The Basics

- Cellular division!

Video Enhanced DIC Microscopy
of Mitosis in Newt Lung Cells
(*Taricha granulosa*)

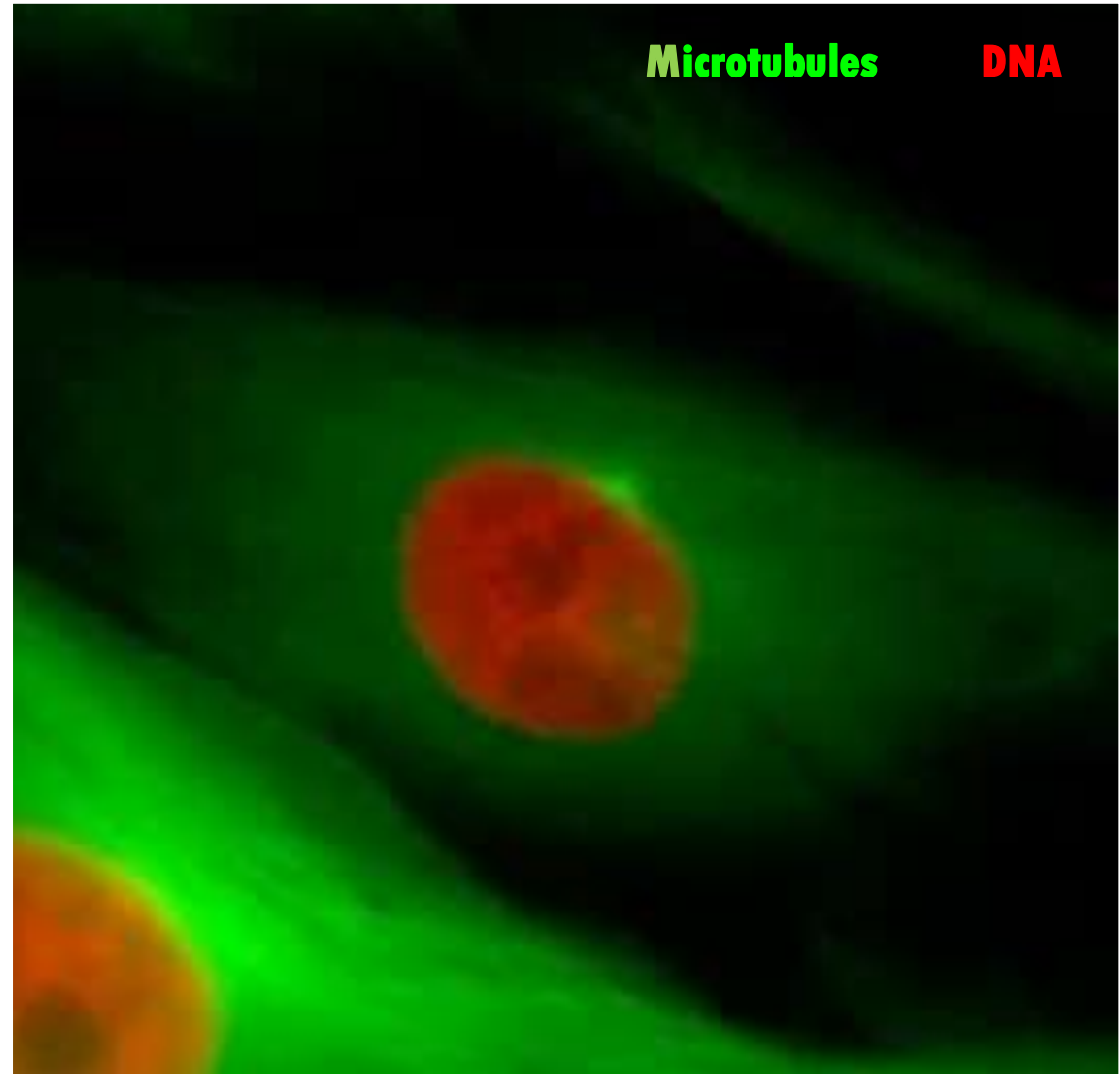
Victoria Skeen,
Robert Skibbens, and
E. D. Salmon

University of North Carolina at Chapel Hill
{see Skibbens et al., 1993, J. Cell Biol.
122:859-875}

Frame Time = HR:MIN:SEC

The Basics

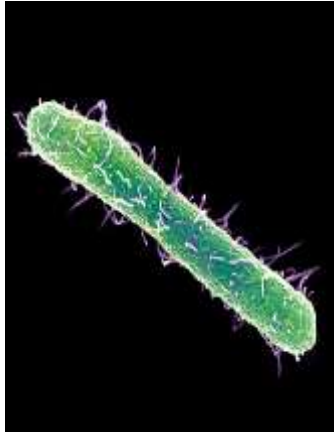
- Mitotic spindles play a key role in cellular division
- Spindles are composed of microtubules
- Microtubules are dynamic filaments



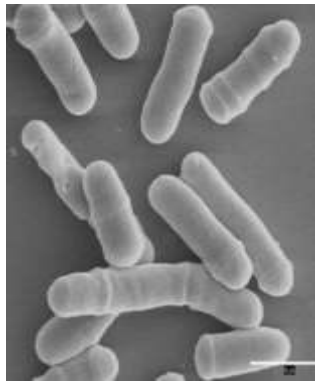
The Significance

- Shape and size are important!
- The “Scaling Challenge” essential for normal development

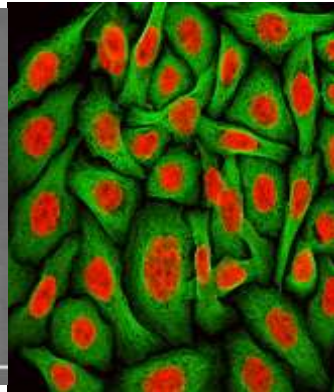
Differences in cell scale



Bacteria
1 μm



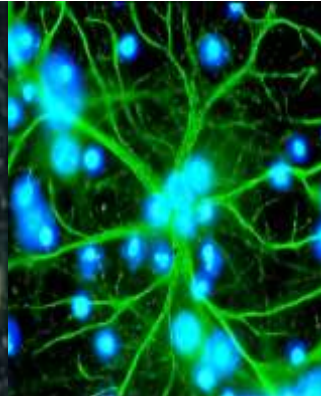
Yeast
10 μm



HeLa Cells
20 μm

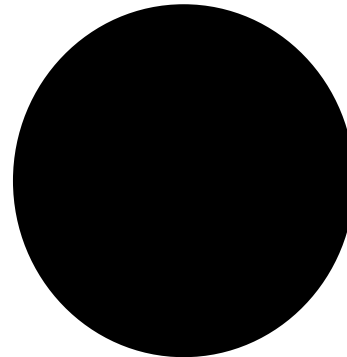


***Xenopus* eggs**
1.2 mm



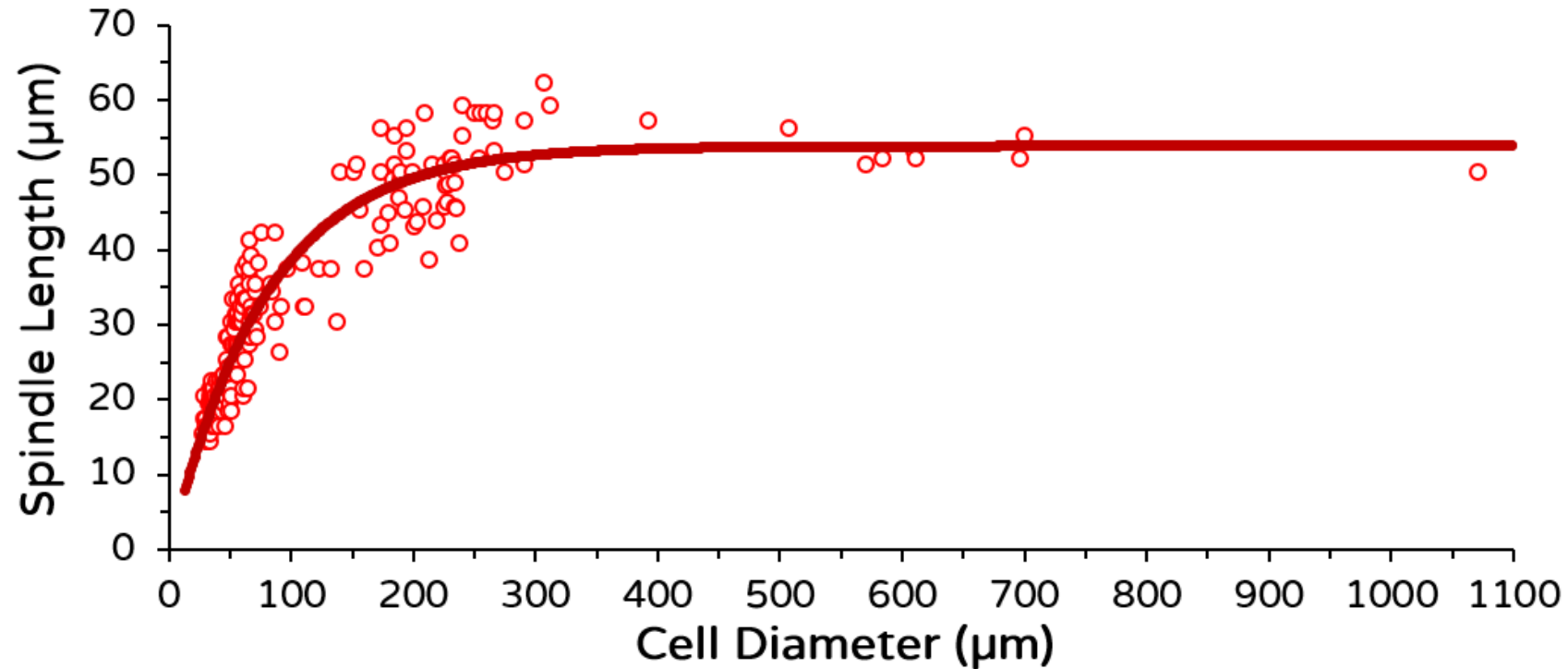
Neurons
>1 m

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Spindle size scaling

Adapted by J. Gatlin
from Wuhr et al., 2008



- Spindle size scales with cell size during development

The Significance

- Shape and size are important!
- The “Scaling Challenge” essential for normal development
- Errors in this process can lead to aneuploidy
- Enzymes regulate this process
 - Some processes are still unknown
 - Small cells vs big cells?

The Big Question

- How does cell size determine spindle size?

The Hypothesis

- Microtubule size and “speed” of growth in comparison to cell size

The Approach

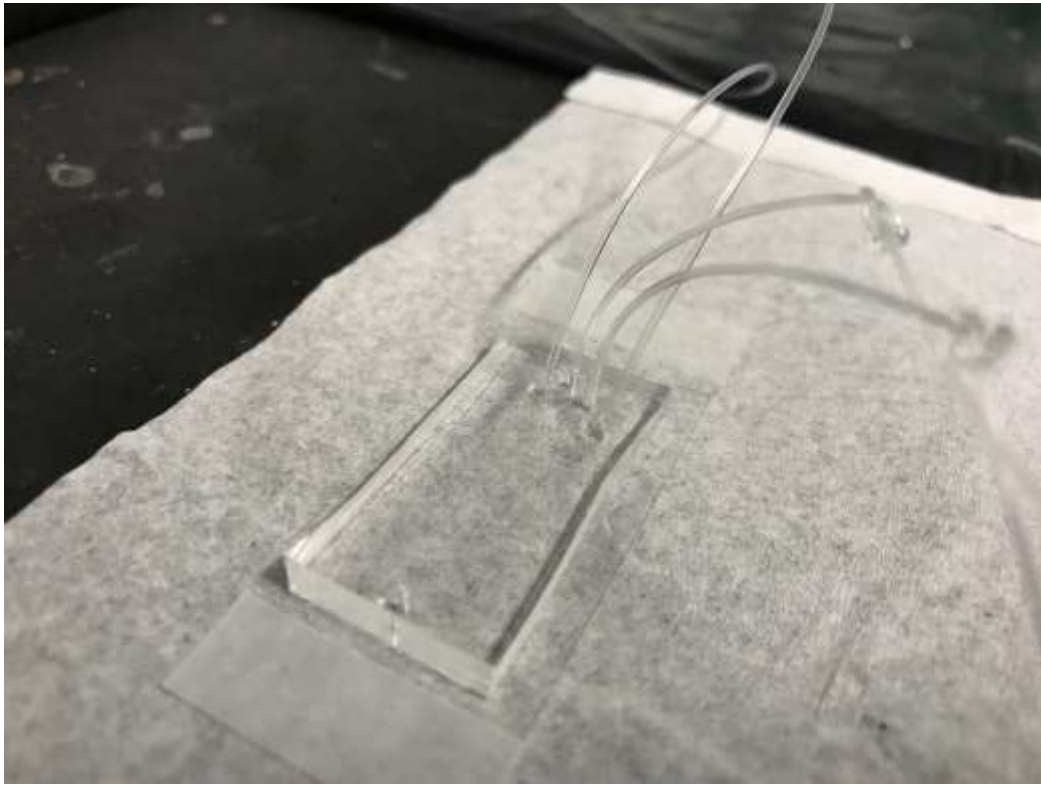
- Cell-free cytoplasmic extracts and purified proteins
 - African clawed frog (*Xenopus laevis*)
 - Tubulin
 - End-binding (EB) protein
 - Acentriolar Mitotic Tubulin Originating Centers (aMTOC)
 - Calcium
 - Cycloheximide



Source:
https://upload.wikimedia.org/wikipedia/commons/thumb/b/b4/Xenopus_laevis_02.jpg/1200px-Xenopus_laevis_02.jpg

The Approach

- Droplet Microfluidics



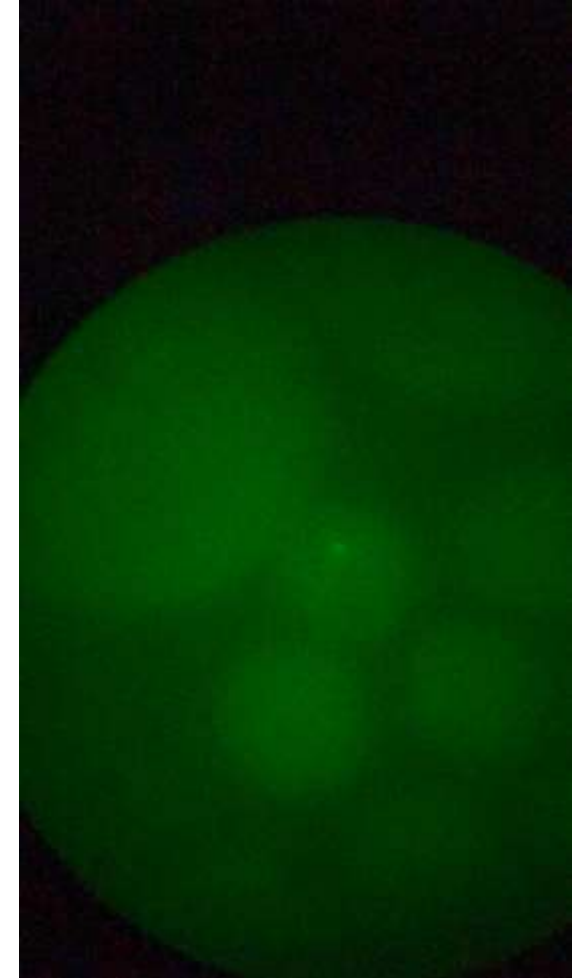
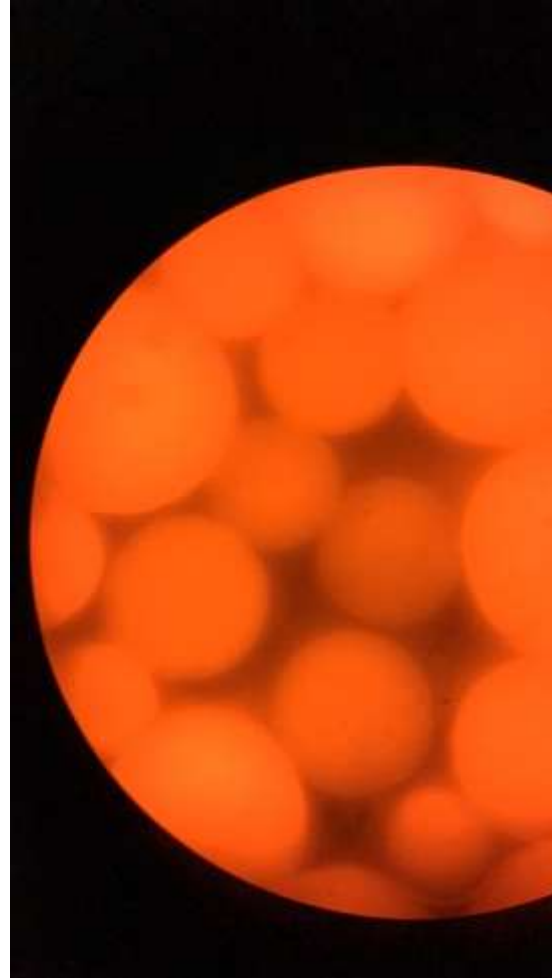
The Approach

- Droplet Microfluidics



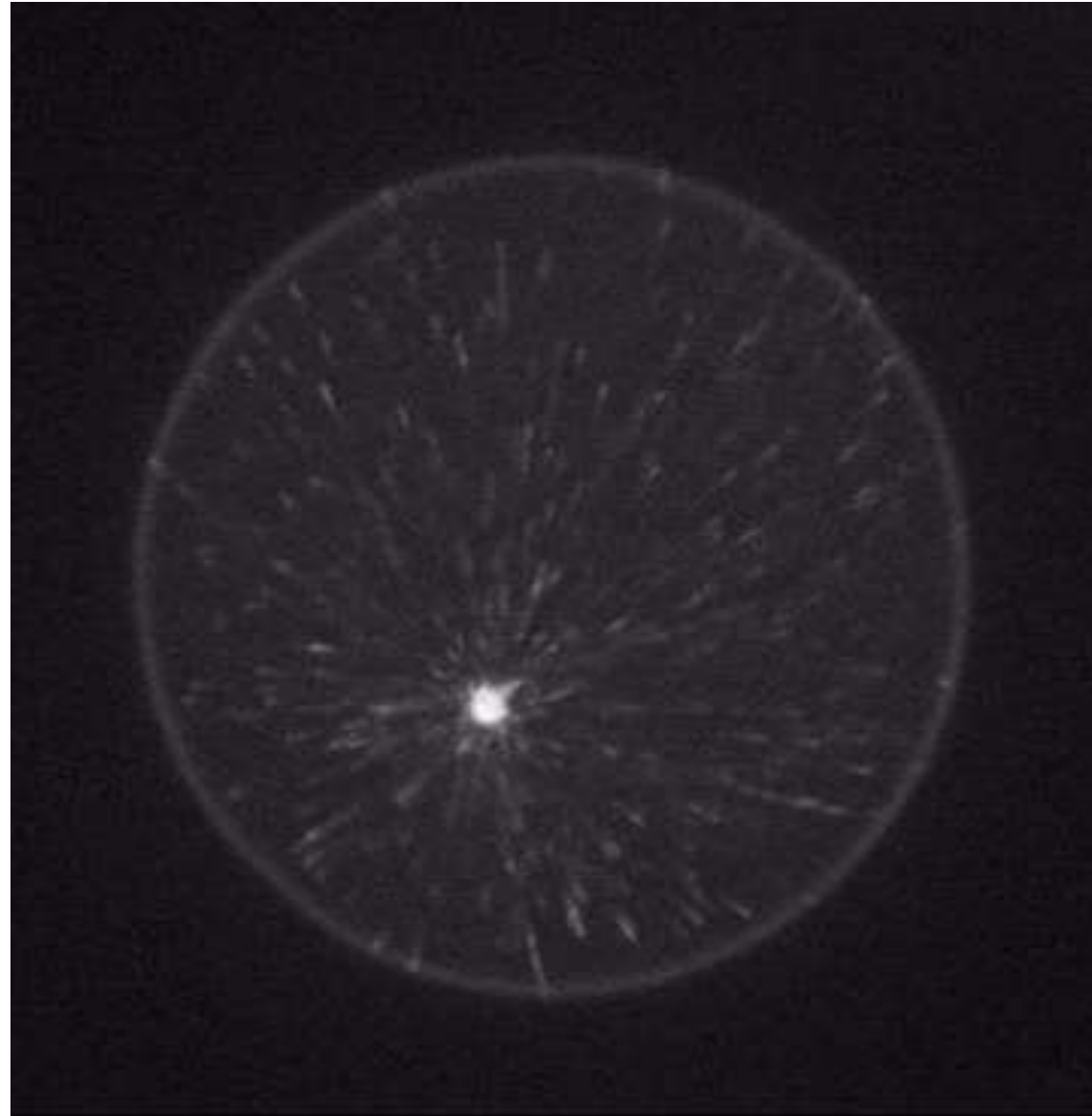
The Approach

- Confocal Microscopy



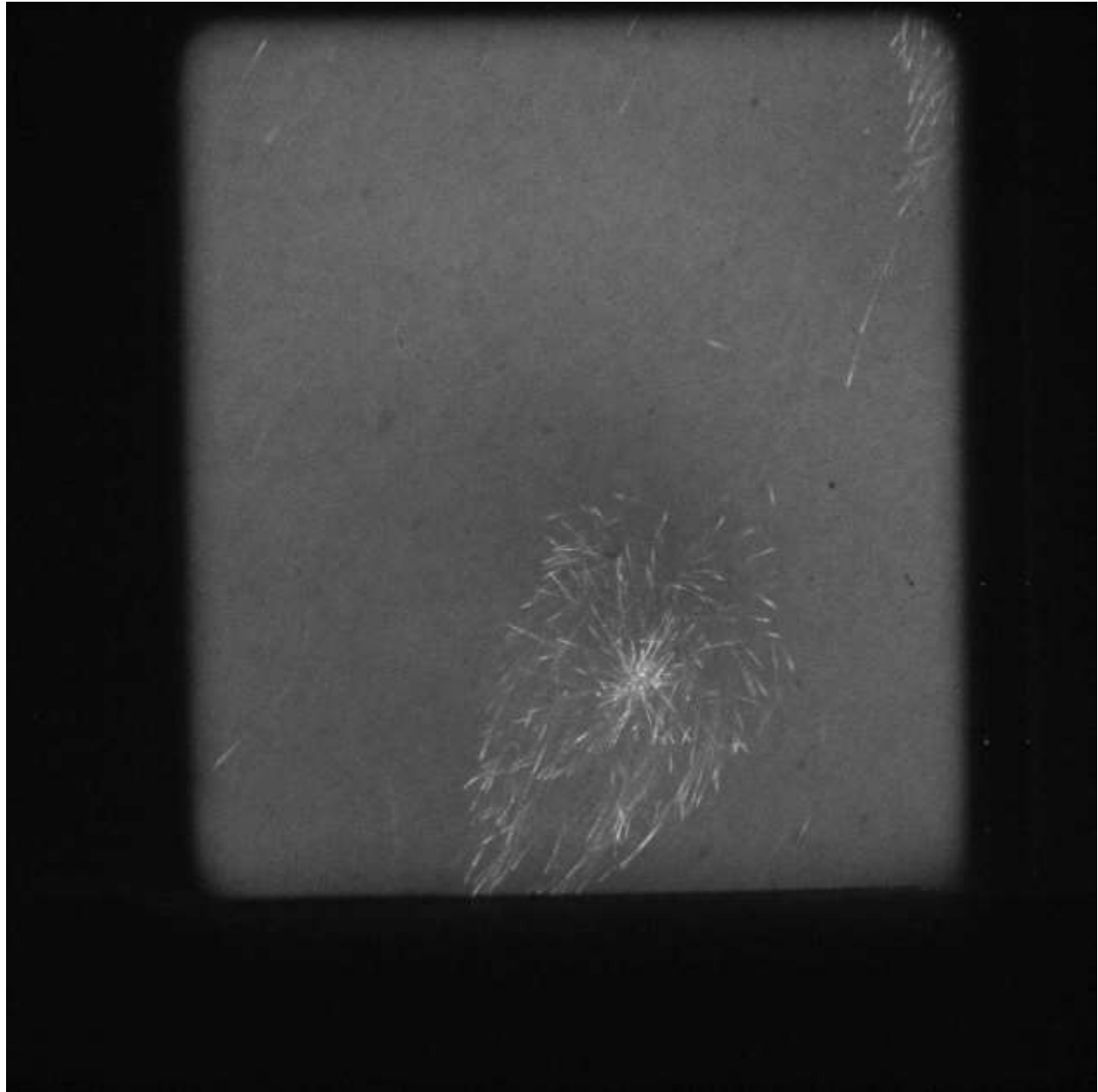
Results?

- Measure microtubule growth rates within these droplets

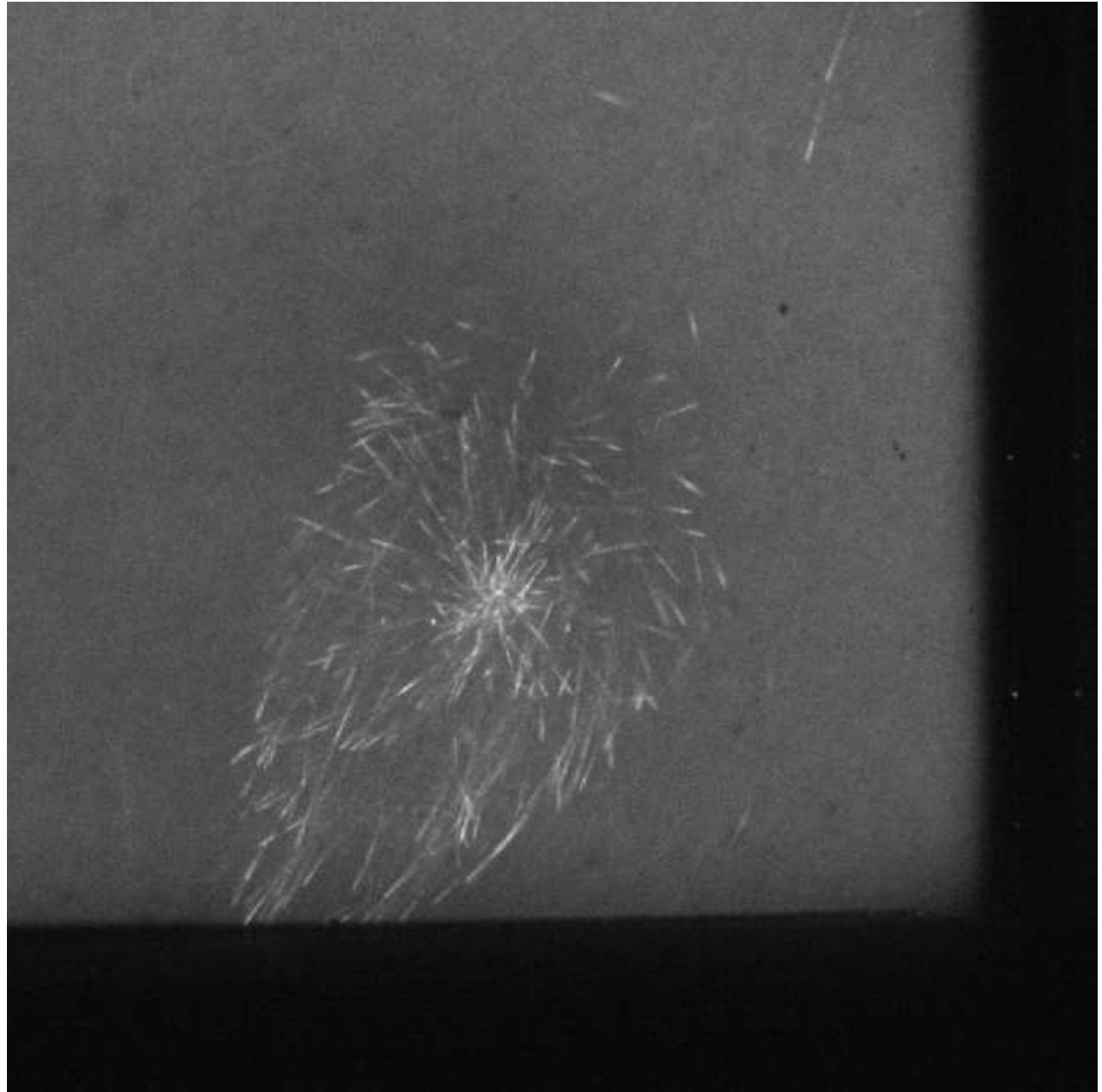


J. Gatlin

Analysis



Analysis



To be continued...

- Questions?

Acknowledgements

- Gatlin Lab Personnel and Collaborators