Hand's On! Active Matter Experiments For Everyone

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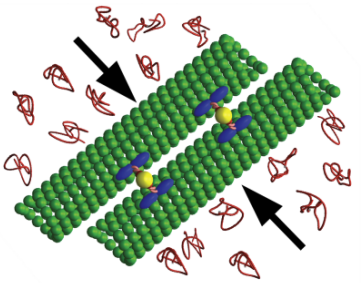
1 Microtubule Active Fluid



Today, we'll be making the 3D microtubule active fluid. Kinesin-powered microtubule bundles shear fluid at the nanoscale to generate large-scale vorticity. But there is more to the system than water, microtubules, and kinesin. Let's explore all the reagents necessary for this experiment. (Figure: Stephen DeCamp)

1.1 Premix Reagents

Ingredient	Purpose
Glucose and DTT	Antioxidants (prevent photobleaching of fluorescent tubulin)
Glucose Oxidase and Catalase	Antioxidants
ATP	Fuel Source
Pyruvate Kinase/Lactic Dehydrogenase	ATP Regeneration System
Kinesin-Streptavidin Motor Cluster	Transduces chemical energy (ATP) into mechanical work
Trolox	Antioxidant
Phosphoenolpyruvic acid (PEP)	ATP Regeneration System
Polyethylene glycol (PEP)	Bundles microtubules via entropic forces

We have prepared the premix for you ahead of time. Today, you will just combine premix and microtubules to make the active mixture.

1.2 Procedure

1. From the -80C fridge, *each person* should grab 1 premix aliquot and *each group* should grab 1 microtubule aliquot

2. Place the premix aliquot immediately in your ice bucket and the microtubule aliquot on the lab bench. **It is very important the microtubules not go on ice** or else they will depolymerize.

3. Get a third eppendorf tube for your final active solution. Keep this tube on the lab bench, not on ice. Label all three tubes with a sharpie *(e.g. 'MT' for microtubules, 'AM' for active mix, 'PM' for premix).*

4. Pipette 15 μL of premix into the empty active solution tube.

5. Let the sample sit for 30 seconds to warm up. Then pipette 3 μL of microtubules into the active solution tube.

6. Vortex and centrifuge.

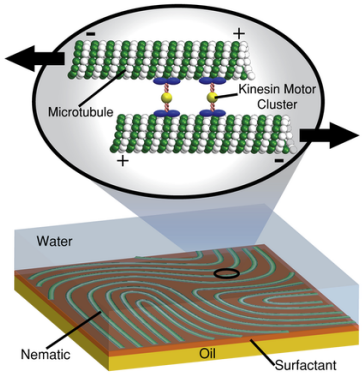
7. Pipette ~ 6 μL of active solution into the channel. To do so, place the pipette tip loaded with solution next to the channel you want to fill. Slowly depress the plunger and let capillary action draw the sample into the channel.

8. Seal the chamber with UV curable glue. More glue is better to avoid leaks.

Congratulations! You just made the microtubule active fluid.

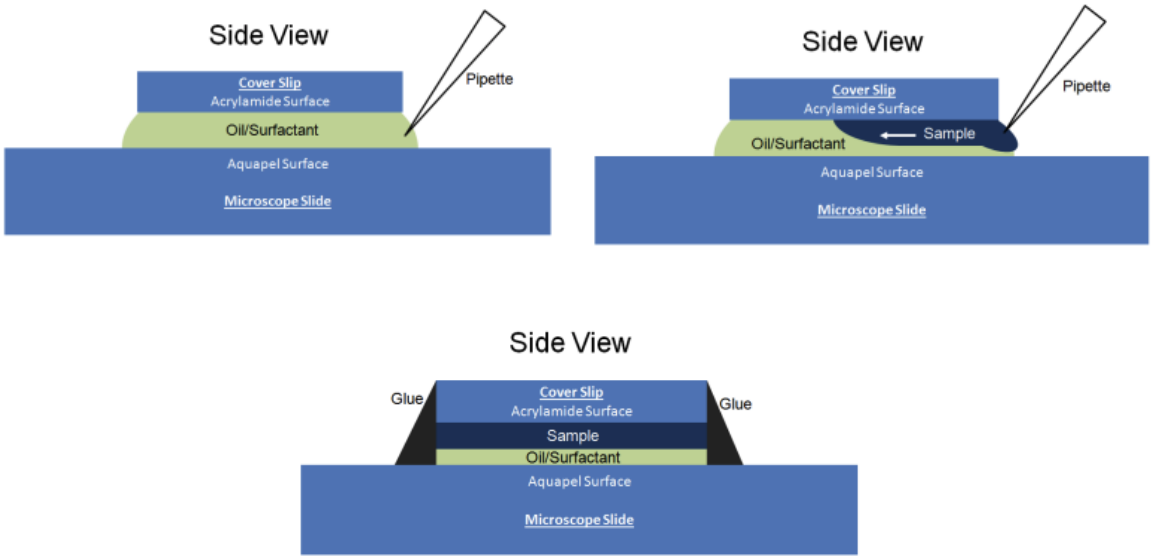
2 2D Active Nematic

2.1 What's In The Sample?



The 2D active nematic has all the same reagents as the 3D active fluid. The recipe is identical, so we can use the sample we already prepared. To collapse the microtubules into 2D, we will sediment them onto an oil-water interface.
To create the interface, we first flow oil on a hydrophobic glass slide (treated with Aquapel). With oil adhering to the slide, we then flow in our aqueous active solution which preferentially adheres to the hydrophilic cover slip. (Figure: Stephen DeCamp)

2.2 Premix Reagents



2.3 Procedure

1. Flow in the oil/surfactant mixture. If you pipette out near the entrance to the channel, capillary force will do the work for you and draw the solution into the channel.

2. Once the chamber is filled with oil/surfactant, flow in the active solution very *slowly* and displace the oil. We often use a Kimwipe at one end of the chamber to wick out the oil while pipetting in the water phase at the other end. You should see an oil/water interface moving across the channel.

3. Seal the chamber with UV curable glue. Make sure the channel is well sealed as the oil will easily seep out.