Inoculate a 10 mL AFM + Amp culture (and appropriate selection) with Ashbya clean spores (10-30 μL depending on strain and clean prep density).

Incubate 15-16 hours at 30°C.

Add 1.1 mL 37% formaldehyde (3.7% final concentration) and shake at 30°C for 1 hour.

Spin down at 1000 rpm for 5 minutes in benchtop centrifuge. Dispose of supernatant in hazardous waste.

Wash twice with 10 mL 1X PBS, spinning at 1000 rpm for 5 minutes in benchtop centrifuge.

Resuspend pellet in 100 μL PBS and transfer to 1.5 mL eppendorf tube.

Add 10 μL Alexa Fluor™ Phalloidin (6.6 μM), mix gently by pipetting.

OPTIONAL DNA STAINING STEP: Add 0.2 μL Hoechst, mix gently by pipetting.

Incubate at room temperature for 1 hour in the dark.

Spin at 10,000 rpm for 2 minutes in microfuge.

Wash twice with 1 mL 1X PBS. Remove as much supernatant as possible.

Add 10 μL Prolong Gold™ mounting medium. Spot 15 μL cells in small drops over slide. Cover with 24 x 50 mm coverslip.

Compress to flatten and press out excess liquid for at least 30 minutes. Seal with nailpolish and store at -20°C.