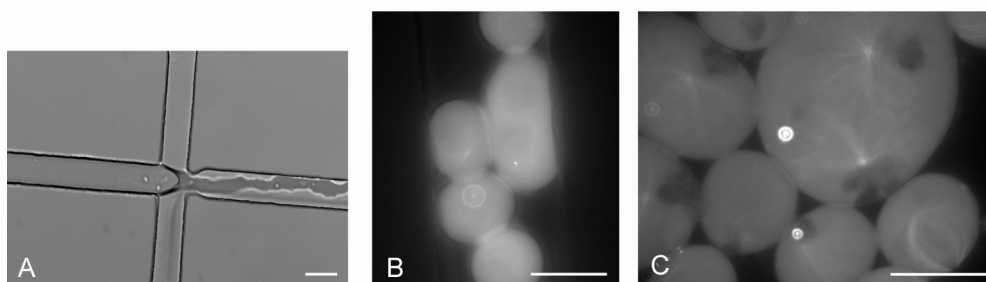
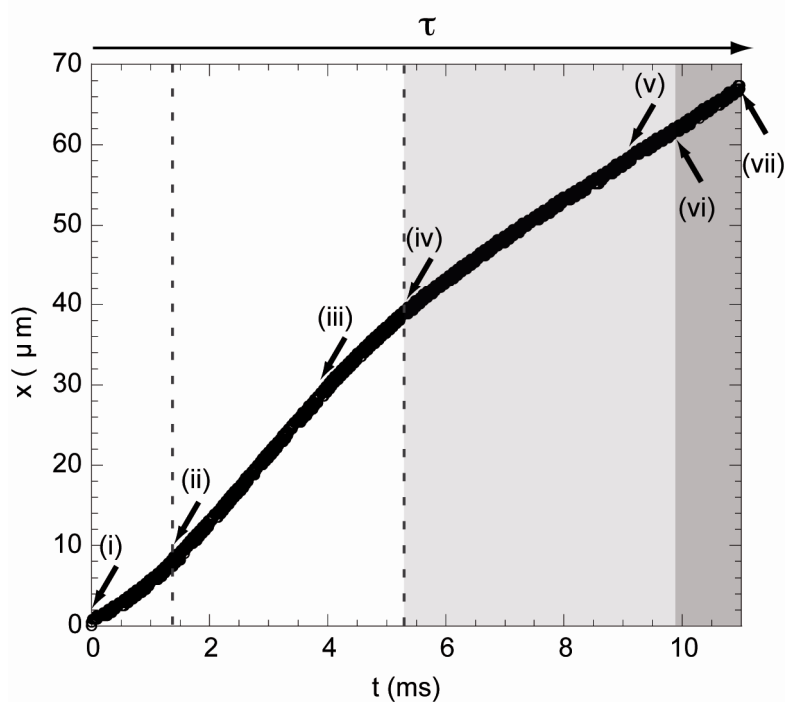


Supplementary Figure 1



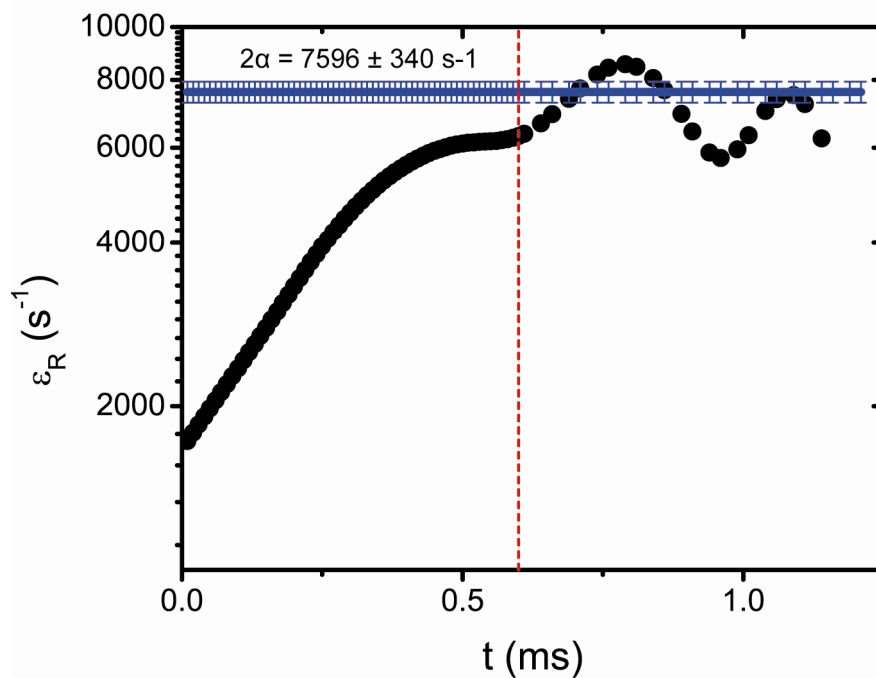
Supplemental Figure 1: A. Injection of oil (side channel) and cell extract (central channel) in a FFD sealed with a hydrophilic slide (clean glass surface): due to wetting of the cell extract with the glass cover slide, the pinching process does not occur leading to an absence of droplet formation (scale bar = 40 μm). B. C. Droplet coalescence: Contrary to the use of the block copolymer, droplets that were stabilized with E-PC coalesce with time: fluorescent observations inside the micro-channel (B) and in the reservoir (C). Scale bars = 30 μm .

Supplementary Figure 2



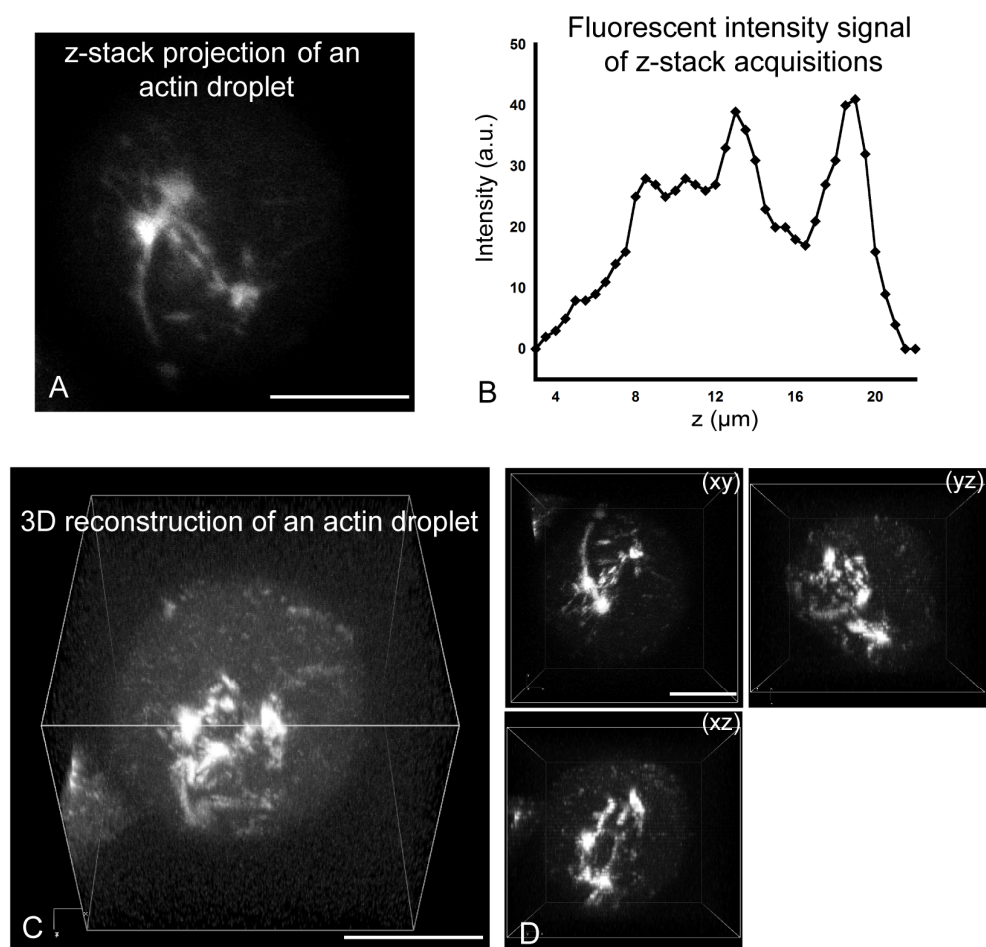
Supplemental Figure 2: Evolution of the position of the front edge of the growing droplet versus time: the three different slopes correspond to the different stages of the formation: blocking, pinching, and neck thinning. The origin of time corresponds to the event where the previous produced droplet has just detached from the cell-extract reservoir; the droplets are emitted at a constant frequency $1/\tau$.

Supplementary Figure 3



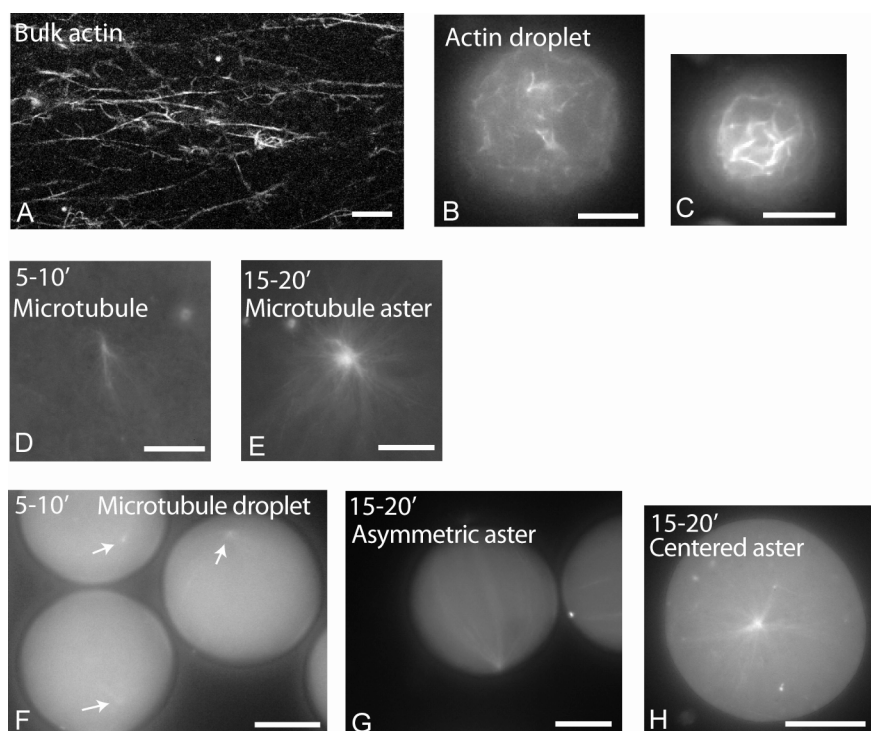
Supplemental Figure 3: Evolution of the extensional rate ϵ_R as a function of time (black filled disks). The dashed line marks the beginning of the slowdown of the thinning dynamics. After this regime, ϵ_R is roughly constant and is comparable with twice the value of the exponential decay rate α (blue line, see Fig. 1C).

Supplementary Figure 4



Supplemental Figure 4: Confocal images of the confined actin filaments.

A. z-stack projection of a 20μm-droplet. B. Plot of the fluorescent intensity signal as a function of the z-position showing that actin filaments are mainly distributed in the bulk phase of the droplet. C. 3D reconstitution of the actin droplet. D. Example of acquisition plane (xy) (yz) (xz) showing an absence of co-localization of actin filaments with the droplet boundary. Scale bars, 10 μm.



Supplemental Figure 5: A.B.C. Observation by fluorescent microscopy of actin filaments stabilized with Alexa-488-labeled phalloidin in (A.) unconfined (bulk) geometry and (B-C.) inside mono-disperse droplets (diameter: 35 μ m in B and 20 μ m in C). The structures are similar in all three cases. D. E. Examples of time-point acquisitions of aster assembly in unconfined extracts: after 5 min (D, initiation of the assembly) and 20 min (E, steady-state). F.G.H. Examples of time-point acquisitions of aster assembly in confined extracts: F. Initiation of the assembly occurs after 5 to 10 min: bright dots and small microtubule bundles can be distinguished in the droplet at a random spatial position. G. Asymmetric microtubule structures observed after 20 min of incubation at 18 °C. These asymmetric asters were observed for droplet size smaller than 30 μ m. H. symmetrical asters, morphologically identical to unconfined patterns, were observed for droplets larger than 30 μ m. The time necessary to reach fully developed asters was found to be ~ 15-20 min, equivalent for both unconfined and confined asters. Scale bars, 10 μ m.

Legends for Supplementary movies

S.Mov1. Dynamics of an asymmetric-aster pole. Droplet size = 22 μm . Time between two frames = 10 s.

S.Mov2. Dynamics of an aster center. Droplet size = 32 μm . Time between two frames = 10 s.