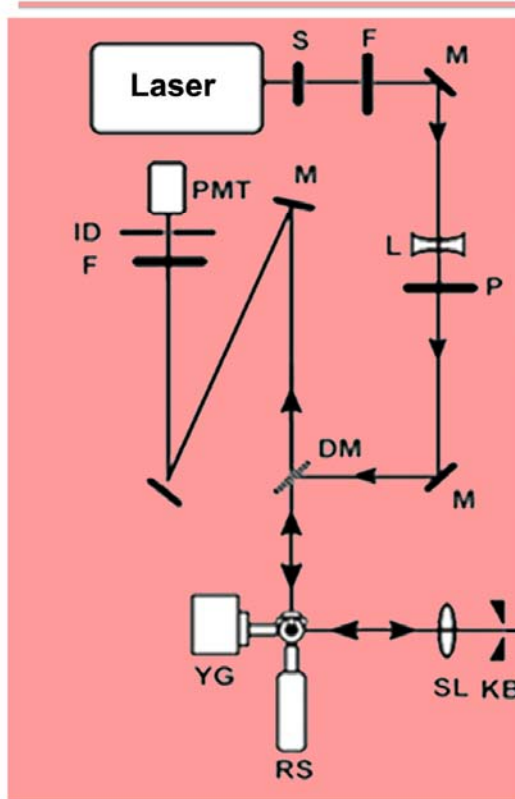


**Supplementary Figure 1. Dual confocal imaging system.**

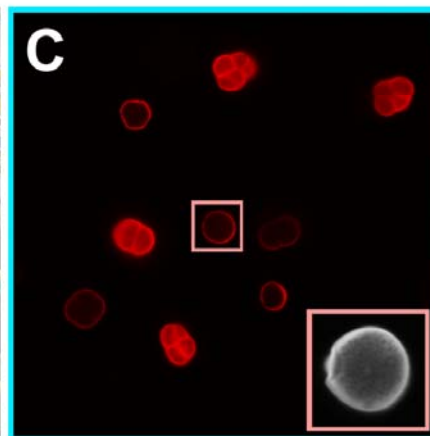
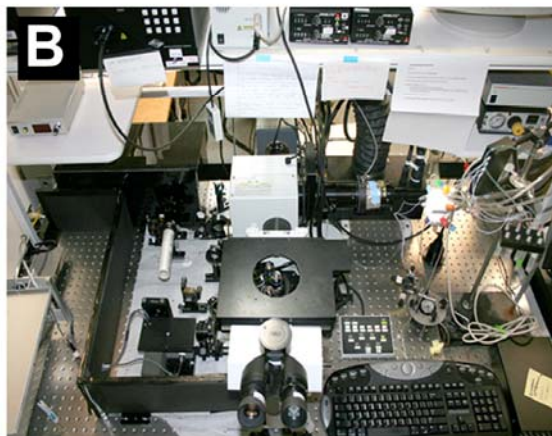
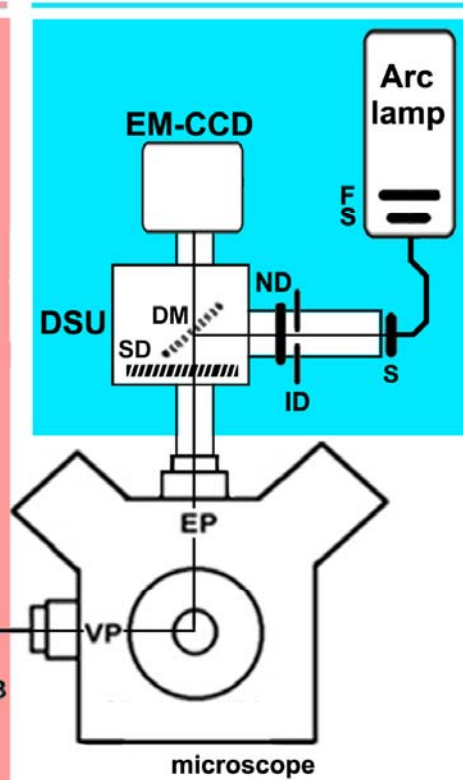
(A) Simplified optical schematic of the dual confocal system. The resonant mirror, video-rate confocal system (pink), described in detail previously (Callamaras and Parker, 1999), is built around the video-port (VP) of an Olympus automated IX81 microscope. The spinning-disc confocal assembly (blue) was interfaced to the epifluorescence port (EP) and comprises (i) an arc lamp illumination system with output ranges suitable for flash photolysis (either a rapid wavelength switching system permitting ratiometric excitation imaging (Lambda DG-4, Sutter Instruments), or more standard epifluorescence hardware (X-Cite 120, EXFO) have been used successfully); (ii) a spinning disc confocal unit (Olympus Disk Scanning Unit, DSU), and (iii) an electron multiplication CCD camera (Hamamatsu, C9100-02, EM-CCD). S = shutters (Uniblitz, VMM-T1 driver), M = mirrors, DM = dichroic mirrors, YG = y-galvanometer mirror, RS = resonant scanner mirror, L = plano-concave lens, P = polarizer, F = filter or filter wheel, SL = scan lens, KB = knife-blade, ID = iris diaphragm, ND = neutral density filters, PMT = photomultiplier tube (Hamamatsu, H7422-40). (B) Photograph of the dual confocal system. (C) Images of the same sample (mixed pollen grain slide, Carolina Biological Supply) captured to show spinning disc illumination (RFP cube, blue box, 200 $\mu$ m) centered around video-rate scan area ( $\lambda_{EX}$ =488nm, greyscale image in pink box). *Inset*, higher magnification view of indicated pollen grain. Scalebar = 10 $\mu$ m.

# A

## RESONANT-MIRROR VIDEO-RATE CONFOCAL



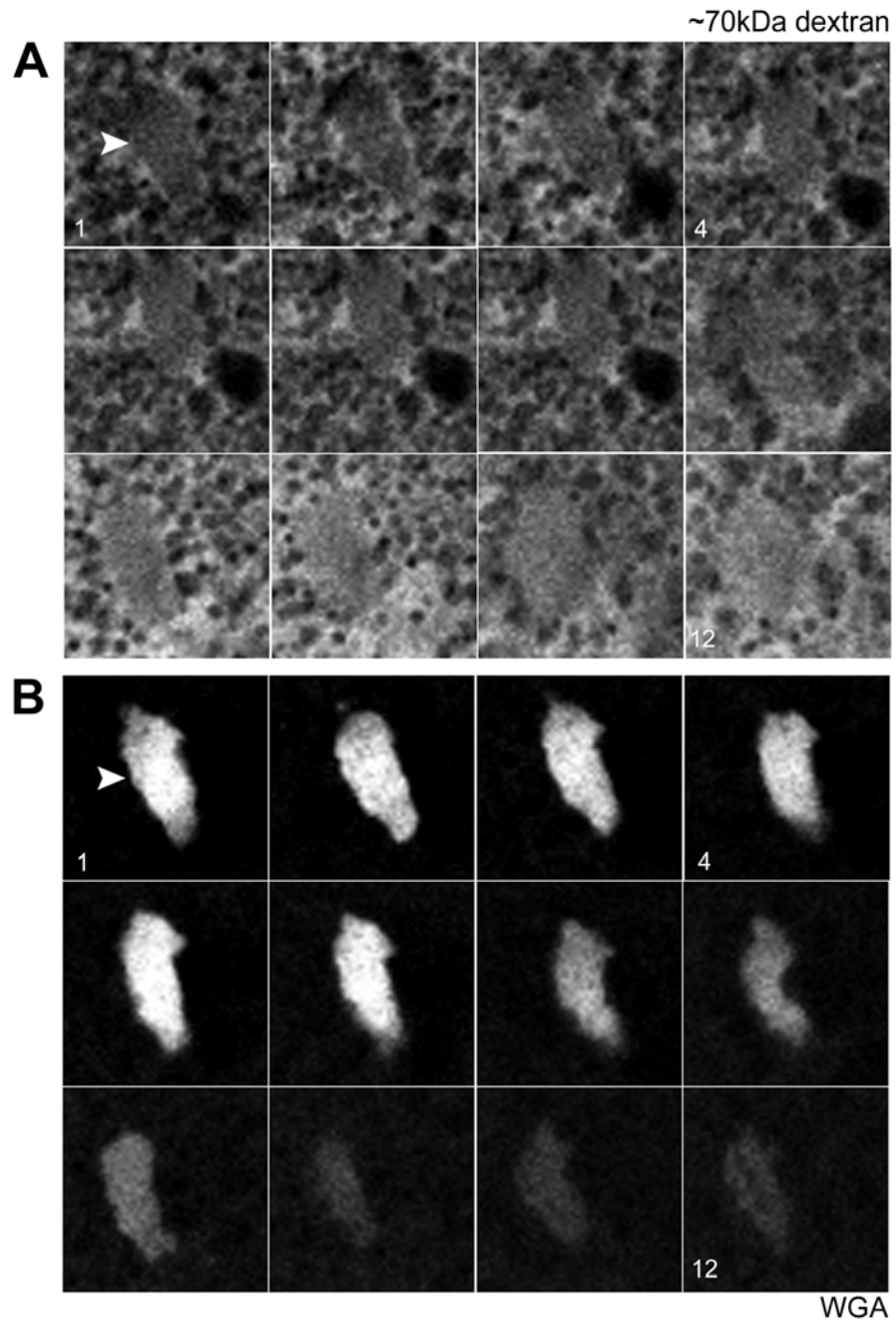
## SPINNING DISC CONFOCAL



Supplementary Figure 1

**Supplementary Figure 2. Changes in AL organization during maturation.**

Corresponding image series encompassing ~120 mins, showing (A) Distribution of ~70kDa dextran and (B) fluorescently-tagged WGA around an AL at 10 minute intervals (images 1 -12) during progesterone-evoked oocyte maturation. Scalebar =10 $\mu$ m. Images are representative of those used to generate data shown in Figure 6C.



## **Supplementary Movies**

### **Supplementary Movie 1 (SM1). *AL dynamics in the resting oocyte.***

Timelapse movie of AL dynamics (stained with rhodamine red conjugated WGA, 200µm by 200µm field of view) in the vegetal hemisphere of a stage VI oocyte recorded over ~4.5 hrs (images captured every 10 mins) .

### **Supplementary Movie 2 (SM2). *ER dynamics during maturation***

Thresholded timelapse movie (~14hrs, images captured every 10mins) of ER morphology (DsRed2-ER) during progesterone-evoked maturation. Images from this timelapse are depicted in Figure 1.

### **Supplementary Movie 3 (SM3). *NPC dynamics during oocyte maturation.***

Timelapse movie (~14hrs, images captured every 10mins) depicting NPC dynamics (stained with AlexaFluor488<sup>®</sup>-WGA) during progesterone-evoked maturation. Images from this timelapse, as well as the corresponding ER morphology in the same cell, are depicted in Figure 2A.

### **Supplementary Movie 4 (SM4). *Heterogeneous NPC dissociation from AL.***

Movie collation of lateral ('xy') confocal images (taken ~7-15 mins apart) showing several AL spanning the phase of NPC dissociation ('phase 2', Figure 1C). For ease of visualization, ER morphology (DsRed2-ER) is shown in grey and NPC (AlexaFluor488<sup>®</sup>-WGA) distribution is shown in pink. Individual image frames are shown in Figure 2B.

### **Supplementary Movie 5 (SM5). *Fluorescently-labeled vesicles during AL disassembly.***

Higher resolution images of NPC dynamics (duration, ~4hrs) during the phase of NPC disassembly frequently revealed a 'starburst' type appearance of vesicles emanating from AL during remodeling.

### **Supplementary Movie 6 (SM6). *AL dynamics during maturation with jasplakinolide.***

Thresholded timelapse movie (~6.5hrs, images captured every 10mins) of ER morphology (DsRed2-ER) during progesterone-evoked maturation in cells incubated with jasplakinolide. A lamellogram from this timelapse movie is shown in Figure 3A.

### **Supplementary Movie 7 (SM7). *Initiation of Ca<sup>2+</sup> waves from AL***

Simultaneous projection of ER morphology (greyscale, left) and fluo-4 fluorescence (pseudocolor, right) showing the initiation and propagation of a Ca<sup>2+</sup> wave from an AL in response to photorelease of IP<sub>3</sub> (the scoring phenotype for measurements reported in Figures 4A & B).

### **Supplementary Movie 8 (SM8). *Dynamics of GFP-vimentin in a Xenopus oocyte***

Dynamics of GFP-vimentin (field of view, 130µm by 130µm, 500ms per frame) within the *Xenopus* oocyte periphery. Similar data was processed in Figure 5C.