FRAP protocol 6/15/2004

Guidelines for MDCK monolayer prep:

- Use sub-confluent culture (< 70%) for plating – never good idea to trypsinize confluent culture
- Plate cells at least 24 hours before FRAP experiments
- Count cells and plate at different density (~$1x10^6$ cells per P35)

Laser calibration:

1. Focus on the mirror using GFP filter set and Bright field
2. Focus the laser if necessary (see the handout from Photonic Instrument)
    a. For 70/20 mirror: minimum power 25 to cut the mirror
3. At power 40-50, calibrate using FRAP alignment tab in Focus Window
    a. "Fire Next"
    b. Double-click on the laser spot on the mirror
    c. Repeat 4 more times (total 5 spots)
4. Check the center of laser coordinates by pressing "Center" – tweak by X and Y
5. Save X and Save Y
6. Test on the mirror for accuracy of the calibration

FRAP hardware setup:

- Laser dye: Coumarin 480 for GFP (Do not use Coumarin 481!)
- Laser power: 20
- Dichroic mirror: 70/20 mirror

FRAP procedure:

1. Set FRAP parameters in Capture Preferences
    a. Figure 1: Rectangular region
    b. Figure 2: Small circular region
2. Search a region to be FRAPed using Focus Window
3. Start the capture (see Figure 3)
4. To FRAP:
    a. Rectangular region
        i. Define FRAP region (see Figure 4)
        ii. Pause the Capture
        iii. Click on FRAP-ROI
    b. Small circular region
        i. Pause the Capture
        ii. Double-click on the Current Frame to FRAP
5. Acquisition should automatically continue

Figure 1: FRAP setting: Rectangular region

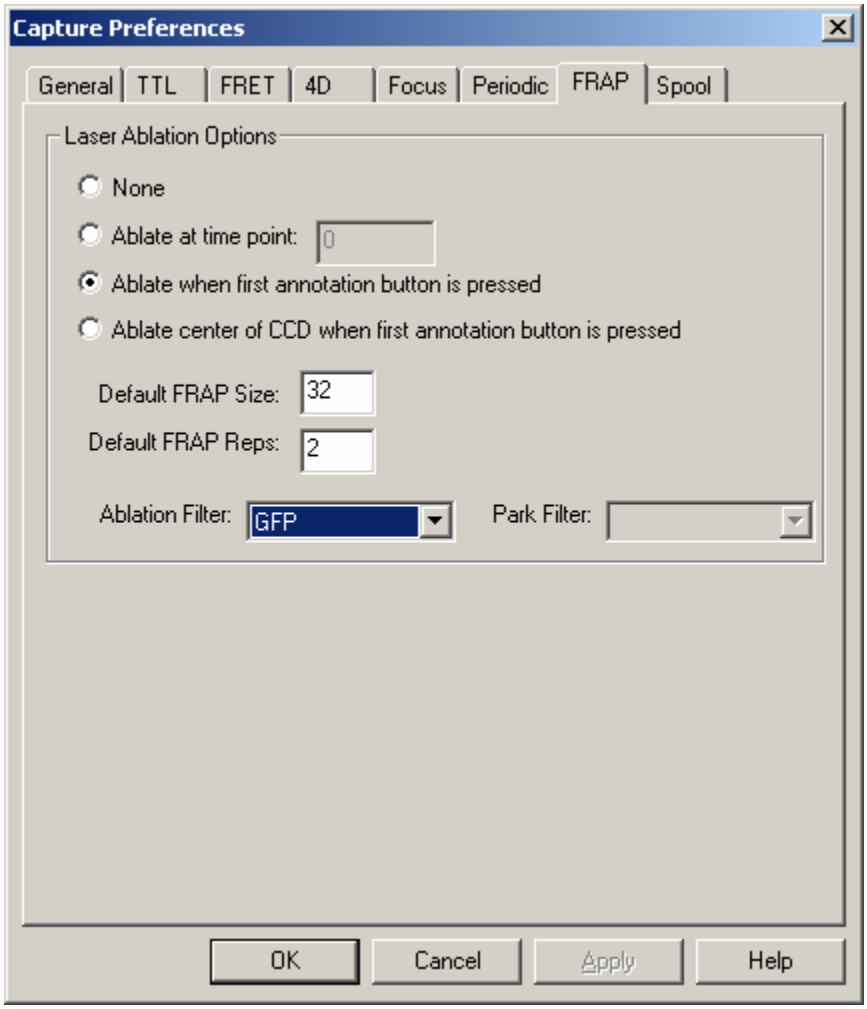

Figure 2: FRAP setting: Small circular region

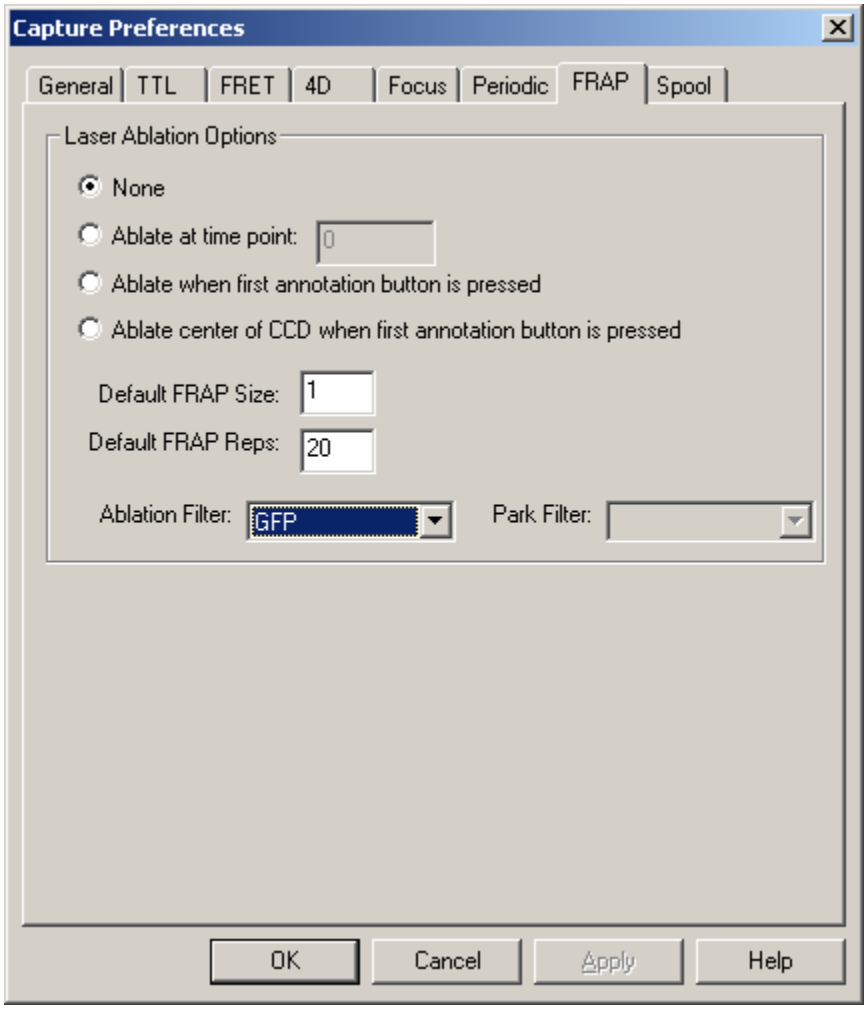

Figure 3: Capture Window

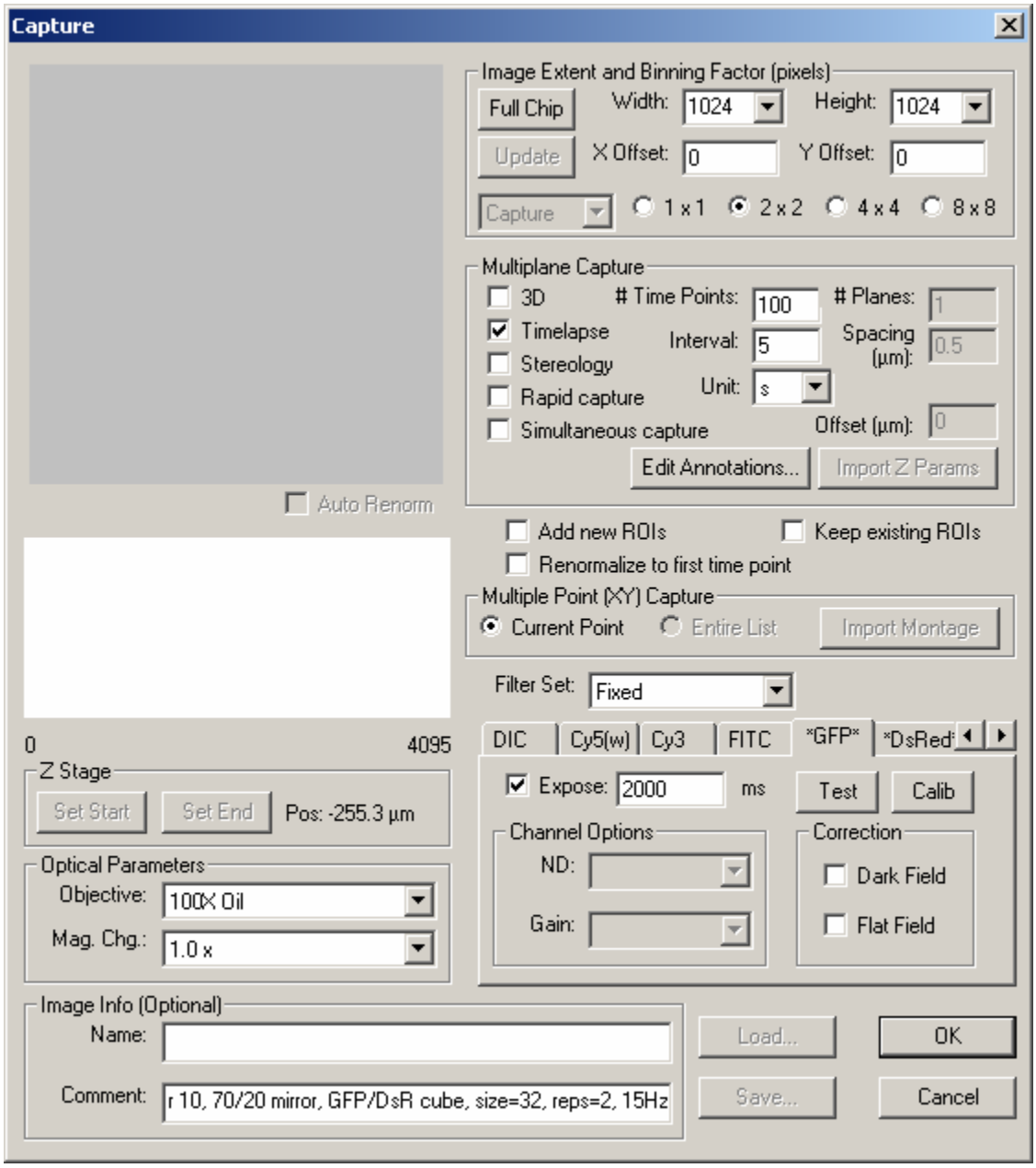

Figure 4: Defining FRAP region: Rectangular region only