

INSTRUCTIONS FOR 40x/NA1.2 C-APO WATER IMMERSION OBJECTIVE

8.19.97

I. General

- A. You need a special training session to use this lens.
- B. This objective cost more than your car--treat it with care:
 1. Put it back in its container when you are finished
 2. Don't let water dry on the lens
 3. Use clean distilled microfiltered water (the stuff in the brown bottle)
 4. Never let immersion oil contact this lens
 5. Use extreme caution to avoid leaks, spills and other sources of water that can get into the optics and destroy the microscope.
- C. This objective is optimized for use with #1½ (thickness= 170µm) coverglass.
- D. Use the red indicator on the correction collar when working at 37°, the black one for room temperature.

E.

II. Parameters

- A. Optimal pinhole sizes (one Airy unit):
 1. 12 for rhodamine or double labeled specimens
 2. 11 for FITC alone
- B. Optimal zoom (diffraction-limited imaging): 5 (0.124µm/pixel)

III. Procedure

- A. Set the objective correction collar to 0.17 (for #1½ coverglass, 0.16 for #1 or unknown).
- B. Select 40x/1.2 objective (#4) under **Lens**.
- C. Use the provided clean filtered water, put a *small* drop on the objective front lens.
- D. Scan in a fluorescence or DIC image of the sample
- E. Center the pinhole(s), using a pinhole size of 6. Leave the objective focused on the water-glass interface, and leave the barrier filter out when you are finished. Reset the pinhole size to 12.
- F. Select **Z/Z-line**, and set the following parameters
 1. Number of sections=500
 2. Z-interval=0.4
 3. Current Pos. Number=460
 4. Refractive correction=1.14
- G. Click **Start**
 1. The resulting trace should have two peaks extending towards the right of the screen (upper figure). Adjust the gain (**Contrast**) until the peaks extend 1/2-3/4 of the way to the scale line.
 2. Click the **Measure** box, then **Posit** and move the crosses to the center of each peak (lower figure).
 3. Set the objective correction collar to the measured value indicated on the image screen.
- H. Repeat steps F and G until the measurement becomes constant.
- I. Replace the barrier filter, and reset the zoom value.

