

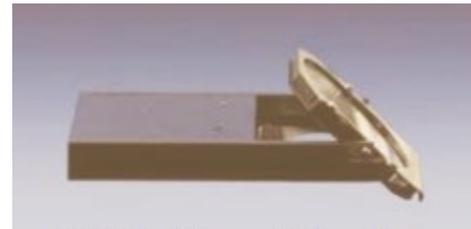
## Using Definite Focus (i880)

*The infrared light of an LED probes the distance between the objective and the base of the sample vessel. Any change in that distance, e.g. from a change in room temperature, is corrected by Definite Focus.*

### Setup

1. Raise microscope objectives all the way up with focus knob. At the objective nosepiece carrier, you will find the “No DF” slider. Loosen the screw (with SW 3.0 screwdriver on optical table) and take the slider out.
2. Replace the “No DF” slider with the “DF” slider. Push in slowly and tighten screw.

- The DF slider is in the gray Zeiss box (9031-DF) in the top right drawer of the working table.



*Definite Focus (DF) slider*

3. Start Zen software. Place your specimen on the stage of the microscope and focus it.
4. On touchscreen monitor, open the Definite Focus window in the menu Microscope > XYZ > Definite Focus.

Activate Definite Focus by clicking the Stabilization ON button. The activity of Definite Focus is displayed in the Status area of the Definite Focus window by a periodic indication of “Waiting...” and “Setting focus...”, as well as by an animated icon.

- The status is also displayed in the window of the fc12 power supply box: initialization and setting of focus.
5. On Zen, open the “Focus Devices and Strategy” tab. Under Autofocus Mode: choose Definite Focus, then choose how often Definite focus will correct focus position between time points.
  6. Start the image acquisition experiment. This can be a single or multi-channel time-lapse experiment. Definite focus automatically turns on during the time series experiment.

### When you are done. . .

1. Use focus knobs to bring objectives back to the highest focus position and take out the “DF” slider.
2. Put the “No DF slider” in its place.
3. Return the “DF” slider in its box.

**Note:** *Definite Focus is only applicable when samples are in aqueous solution and when using an immersion objective.*