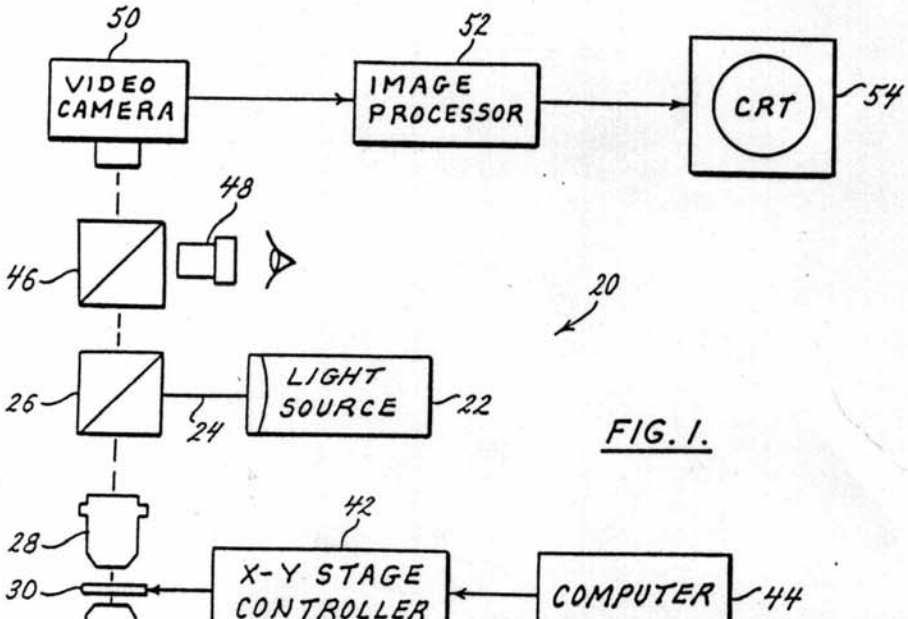


FIG. 1.

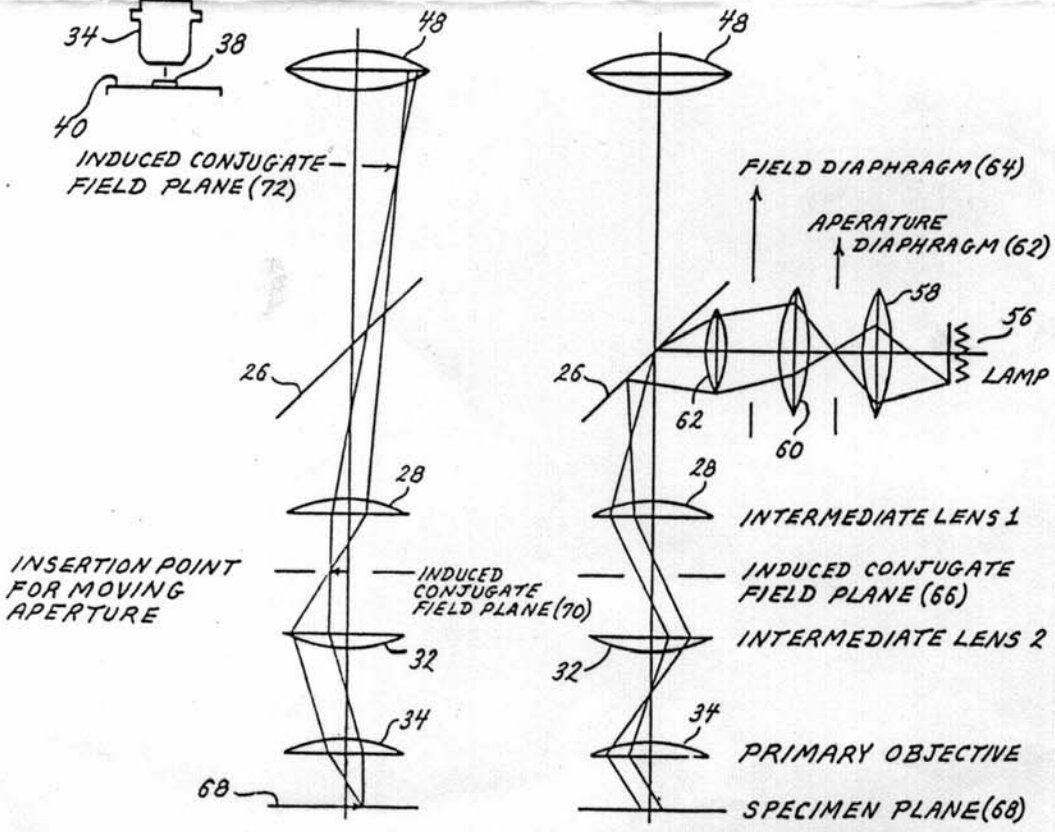


FIG. 3.

FIG. 2.

STAN.

So sorry for delay

November 1, 1988

This is for the confocal microscope. See what you think.

J. W.

Enclosed please find two drawings of our scanning epi-illumination microscope. ~~As I described over the phone~~, this device makes use of single apertures to simultaneously illuminate and view the specimen. Its principle advantages are:

1) It is inexpensive and simple to build.

2) Speed of scan, aperture size and shape (holes, slits) and method of scanning (linear, rectilinear, or rotating) can easily be changed.

3) Real time scanning or slow scanning for highly resolved images are both straightforward.

4) The scanned image can be viewed by eye (real time), by video camera and image processor (e.g., SIT and digitizer) or be obtained on film using a camera attachment at the trinocular port.

5) Magnification can be varied by rotating different objectives into the position of the first intermediate lens (see figure). In this way a high power, high NA lens as primary objective can be used to obtain both low and high magnification images.

Details about design shown in figure: We use a Leitz Laborlux microscope with 75 Watt Xenon bulb with cubes I_2 and N for fluorescence excitation. The scope has been modified in two ways: 1) the stage (which moves in the Z axis for focussing) has been lowered several inches to accommodate the extra lenses, and 2) a second "turret" has been added to allow the primary objectives to be structurally attached to the microscope stand. The first intermediate lens is either a 2.5X Leitz PL or a 10X Leitz EF. The aperture is either a slit (a "negative reticle" or two carbon-coated razor blades facing each other work well) or a series of slits (back to back rhonchi gratings (300 1/inch) slightly offset to make

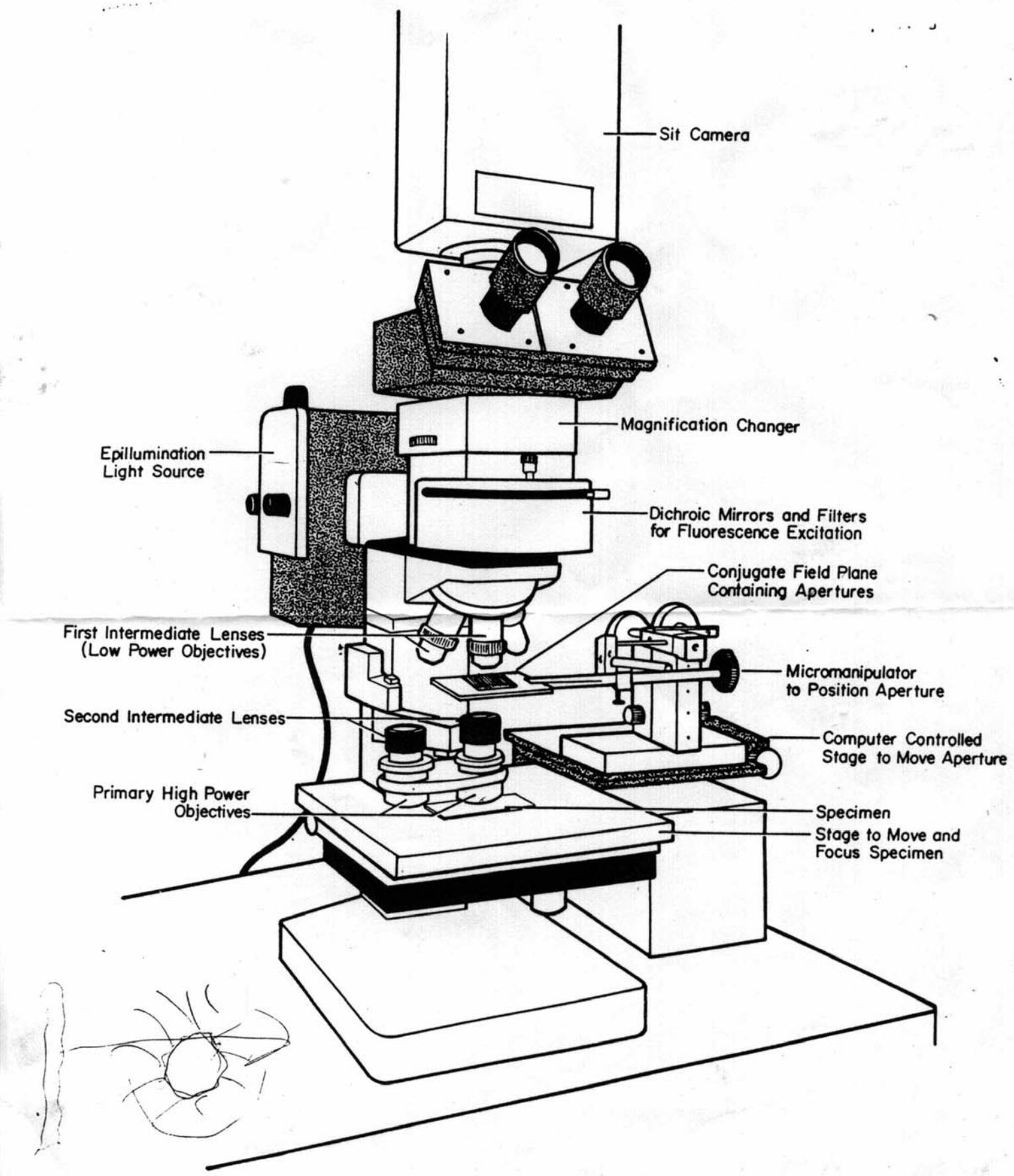
slits that illuminate and view .2-.3 μm stripes on the specimen. The slits are moved laterally with a mechanical stage. We scan passing a large series of thin slits across the specimen giving rise to a final image that is relatively uniform because the imperfections in each slit average out. The second intermediate lens is a simple planoconvex lens from the top of a Leitz 10X or Zeiss 2X eyepiece. It is positioned so as to focus the high power objective (either a 50 or 100X NPL Fluotar) onto the conjugate image plane where apertures reside.

I hope this information is useful to you. Please call (314-362-2504) if I can provide anything else.

Sincerely,

Jeff W. Lichtman, M.D., Ph.D.
Associate Professor of Neurobiology

JWL:sje


Sit Camera
Magnification Changer
Epillumination Light Source
Dichroic Mirrors and Filters for Fluorescence Excitation
Conjugate Field Plane Containing Apertures
First Intermediate Lenses (Low Power Objectives)
Micromanipulator to Position Aperture
Second Intermediate Lenses
Computer Controlled Stage to Move Aperture
Primary High Power Objectives
Specimen
Stage to Move and Focus Specimen