FIF Technique (transcribed from H. R. Horvitz notes)

[This is more for historical interest, than as a protocol to use - it is as exact a transcription of the material as possible. Done while I was a postdoc in the Kenyon lab (1988-93), from a photocopy of Bob's handwritten notes, presumably from Cynthia's files, brought with her from the MRC(?). I'm not sure what the date of those notes were. Additional note: the MS Word doc 'date created' metadata for the transcription indicates 'Feb 2, 1990, 1:50 PM' -- CL]

1. Ice in ice bucket! flatten
2. Black disc (actually from Wild Stage) put into large petri dish
   Sit flat on ice, but not for too long (> condensation problems)
3. Cover slip 22x64 mm. No. 1 1/2 (sturdier; good for liq. N_2)
   Clean w/ Kimwipe
   Use "Staedtler Lumocolor 317 waterproof ("permanent") Pen"
   > Note: new (PCB-free; brown) Zeiss oil dissolves this ink.
   Drawn line around edge > visible after putting into liq. N_2
   If many samples, mark off areas w/ "Chinagraph" (white) pen (waxy pencil)
   (keeps specimens apart (blocks H_2O flow); [?unclear?] mark on side for specimens,
   whereas if marking w/black pen, do non-specimen side)
   Write labels >> on non-specimen side
4. Place wrong side up on disk
5. Buy ovalbumin (lyophilized)
   Make 10% soln. in dH_20
   Store frozen
   Cool ovalbumin soln in ice bucket
6. Pull out Pasteur pipette (e.g. break on dish; don't make super thin point).
   Use small Pastuer pipette bulb (easier to control)
7. Prepare dessicator.
   Make sure its airtight!!
   Empty and rinse petri dish in bottom (if old P_2O_5 still white and solid,
   simply top up; o'e, refill. if rinsed, be sure to dry thoroughly!)
   Put P_2O_5 (phosphoric oxide) into Petri dish. Use a lot. [=phosphorus pentoxide]
   (Transfer with large spatula) Spread P_2O_5 around petri dish.
   Above Petri dish w/ P_2O_5 is metal screen.
   above screen is upside-down petri dish, which will
   support aluminum block
   Clean (i.e., run hand around) rim of dessicator
   Also clean tubing. Occ'ly, grease w/ silicone high vacuum grease
   Clean top, [?unclear?] valve (no air bubbles) etc.
   Note: pump must be good (test on vacuum gauge).
   Leave dessicator covered but not closed.
   Put aluminum blocks in ice bucket
8. Get liq. N_2
9. Worms. Bit of food, but plenty of worms of all ages.
   (just about to clear). (Too many bacteria > higher background)
10. Put empty ice bucket, slightly tilted, next to ice bucket w/ ice and ovalbumin
    Put petri plate w/ black disc, w/ coverslip on top of ice in ice bucket
    Put small drop of ovalbumin on to spot for worms
    (Note: too much ovalbumin > high background; can use 0.05M NaCl [?unclear?] M9
...when not singling worms)
11. Transfer worms
    Scrape edge of paper strip across plate.

put into drop. Push around to transfer. Spread a bit
[Put strip into drop by edge; don't spread drop yet]
Try to avoid bacteria.
If starting to dry, breathe on coverslip.
CRUCIAL; do fast; don't let dry.

12. Pour liq. N_2 into empty ice bucket
13. Spread worms in each area evenly out w/ paper strips. (If not spread enough > increased background; too much spread > drying)
14. Put coverslip into liq. N_2, (using fine forceps)
    Put edgewise down.
15. Put liq. N_2 into ice bucket w/aluminum block.
    covering one block (bottom) and 1/2 way up the other
16. Put on pump (but with TAP SHUT)
17. Wait until liq. N_2 down well below level of top of bottom block
    and until "N_2" drops stopped dancing
    Then, put coverslips, specimen side up, on to bottom block
    Put top block over.
    Transfer (wearing glove) to dessicator. Close. Turn tap.
    TRANSER MUST BE RAPID!
18. Pump for 1 hr. Close tap. Pull off pipe. Turn off pump. Tape (cellophane) piping on!
19. Wait ~ 24 hrs. (or less)
20. If less heat with heat lamp (~ 1' from dessicator).
    Normal protocol: Heat 20', cool 15', heat 20', cool 15', open
    Prob. best to do some heating no matter what > dry
21. Paraformaldehyde, kept for at least 1 week over 70% humidity (= H_2SO_4 + H_20) in petri dish, in dessicator [?unclear?] (see next page)

{from next page - Note: 70% relative humidity = 33% H_2SO_4 (weight by weight?) = 33 ml conc. H_2SO_4 into (slowly) 134 ml d H_2O. Let it cool}
NOT kept under vacuum, but in closed dessicator > 70% r.h.
Put ~1/2 teaspoon of 70% r.h. paraformaldehyde into "fruit jar"
w/ copper([?unclear?]) screening for support
Seal tightly

22. Open dessicator by SLOWLY releasing vacuum.
    Then rapidly remove upper block (which should NOT be cold)
    and, w/fine forceps, transfer coverslip(s) to jar, standing up.
    TRANSFER RAPIDLY
23. Place jar at back (for people-protection!) of 67° C (lower temp prob okay, since prob lower in original protocol) oven for 1 hour.
24. Remove jar from oven.
    Put 3-4 drops of old (white; w/PCB) Zeiss immersion oil onto slide. Note: new oil dissolves ink. Also, more hydrophilic > could be worse.
    Slowly and carefully, put coverslip (worm-side down) onto slide; don't move unnec'ly, or else worms will disintegrate
    If nec'y, add more oil to edges.
25. Sit at least 1 hr. (ON prob OK) IN DARK.
26. Empty paraformaldehyde into sink in fumehood; wash down w/H_2O. Do not wash jar, but reclose.

Here is the FIF method as it appears in Sulston et al., 1975 (pp 215-216):

Formaldehyde induced fluorescence (FIF)

The general method has been reviewed recently (Fuxe and Jonsson, '73).

C. elegans

Two aluminum blocks (9 X 7 X 4 cm) were milled so as to fit together leaving a 1 mm gap between them. The blocks were cooled in liquid nitrogen. The nematodes were placed in the minimum volume of 0.1 M NaCl on a cover slip, quenched in liquid nitrogen, and transferred to the space between the blocks. The assembly was placed on a plastic petri dish over $P_2 0_5$ in a desiccator, which was immediately evacuated to about 0.01 torr. After 30 minutes, the desiccator was sealed and left at room temperature. If the drying time was less than 24 hours, the blocks were warmed with a heat lamp before the desiccator was opened. The cover slip was transferred, with no special precautions, to a 1-lb preserving jar containing 1-2 g of paraformaldehyde equilibrated at 70% humidity. The jar was sealed, and placed in an oven at 67° for one hour. The nematodes, still supported on the cover slip,

were mounted in Zeiss immersion oil and allowed to clear for several hours before examination.
For multiple assays, each 22 X 64 mm cover slip carried ten samples separated from one another by Chinagraph lines and two cover slips were placed between a pair of blocks. Usually the nematodes were transferred from plate cultures and spread out by means of a paper strip, which also served to absorb excess saline. During transfers, the cover slip was kept on ice to prevent evaporation; if it became too wet, a plastic petri dish was interposed between it and the ice. When individual nematodes were examined, as in genetic mapping, they were transferred with a pointed stick to 10% ovalbumin.