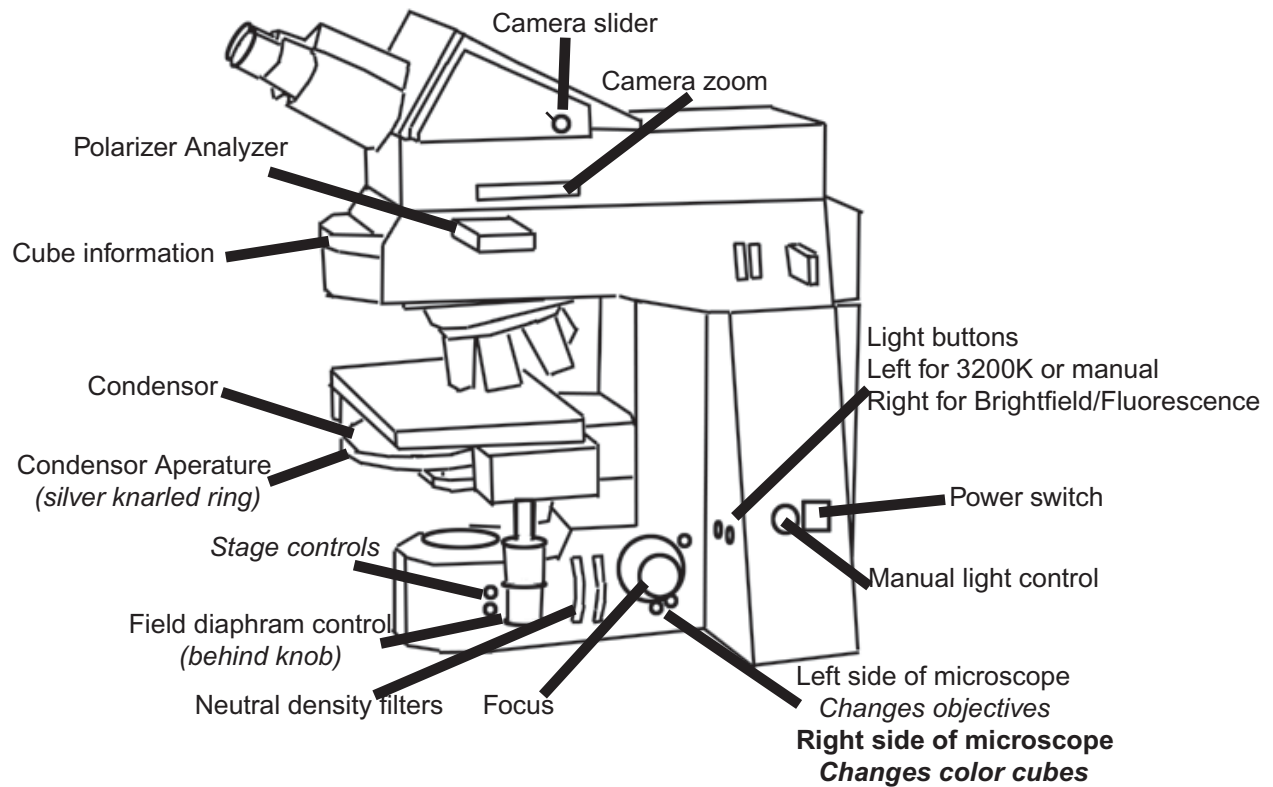


Zeiss Brightfield

1. Turn on equipment

- a. Turn on the SPOT
- b. Turn on the microscope



2. Set up the microscope

- a. Press Brightfield button and adjust brightness
Sometimes the field diaphragm is closed. Open it by rotating the field diaphragm dial.
- b. Make sure neutral density filters dials on the microscope are at **100%**
- c. Check that the condensor is set to **H/DIC2**
- d. Select the empty cube position
No label appears at that cube position
- e. Check that the polarizer analyzer is out of the light path
- f. Make sure the zoom for the camera is at **1**
- g. Keep the metal camera slider at halfway out
Half of the light will go to the eyepieces and the other half will go to the camera

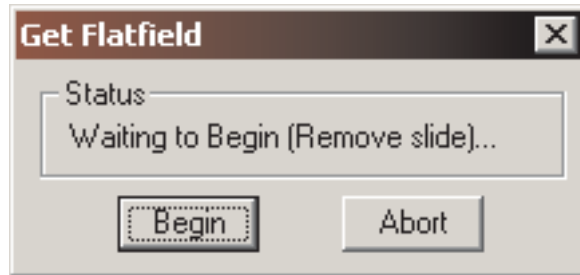
Zeiss Brightfield *continued*

3. Koehler Illumination (see pictorial view on following page)

- a. Put a slide on the stage. Use the 10x objective.
- b. Open the condenser aperture and the field diaphragm aperture all the way.
- c. Move the substage condenser up close to the slide, using the condenser focus.
Make sure the top lens of the condenser is flipped up into place.
- d. Focus the sample. Use the neutral density filters (disks by the microscope) if it's too bright.
- e. Close the field diaphragm as far as it goes. A small circle of light should be seen in the eyepieces.
- f. Center the small circle using the condenser centering rods.
- g. Focus the condenser until the blade edges can be seen using the condenser focus. It will probably have to be re-centered again after this.
- h. Open the field diaphragm all the way.
- i. Take off one of the eyepieces and close the condenser so that 10% of the opening is blocked

4. Correct for Uneven Illumination

- a. Choose Brightfield Auto from drop down list at bottom right
- b. Choose any slide and focus on a specimen
- c. Get the Flatfield Image: Camera > Get Flatfield Image



- d. REMOVE the slide and click begin
- e. Enter the date as the name for the flatfield image and click OK
- f. Place the slide back on the stage

The microscope is now corrected for uneven illumination

Zeiss Brightfield *continued*

5. Typical Settings for Brightfield (*example on following page*)

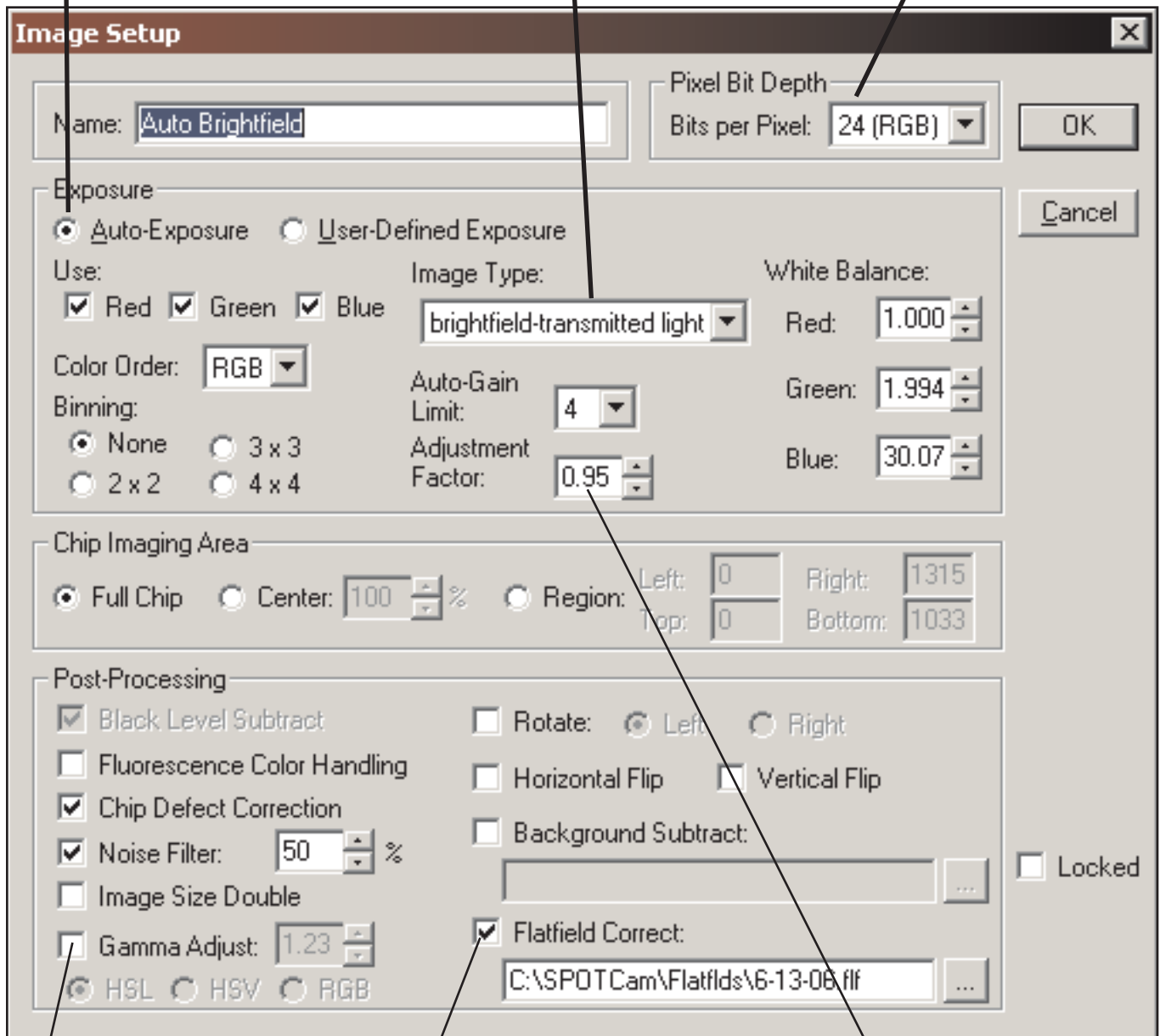
- a. Setup > Image Setups
- b. Find desired setup from list and click Modify
- c. Select **24 (RGB)** for Bits per Pixel
- d. Choose **Auto-Exposure**
*Select **User-Defined Exposure** only if measuring densities later or if you are attempting to show differences in densities*
- e. Image Type is **brightfield-transmitted light**
- f. **Auto-Gain Limit** set to **4**
Higher gain equals more noise and less exposure time
- g. Set **Adjustment Factor** to 0.95
Change if the image is too light overall. Change in increments of -0.5.
- h. Adjust **Gamma** only if dark to white areas are too contrasty
Higher Gamma lightens dark features
- i. Check **Flatfield Correct** to apply correction for uneven illumination if not checked already
Do not check if you are not correcting for uneven illumination

Zeiss Brightfield *continued*

Select Auto-Exposure

Choose brightfield-transmitted light

Select 24 (RGB)



Adjust gamma here if necessary

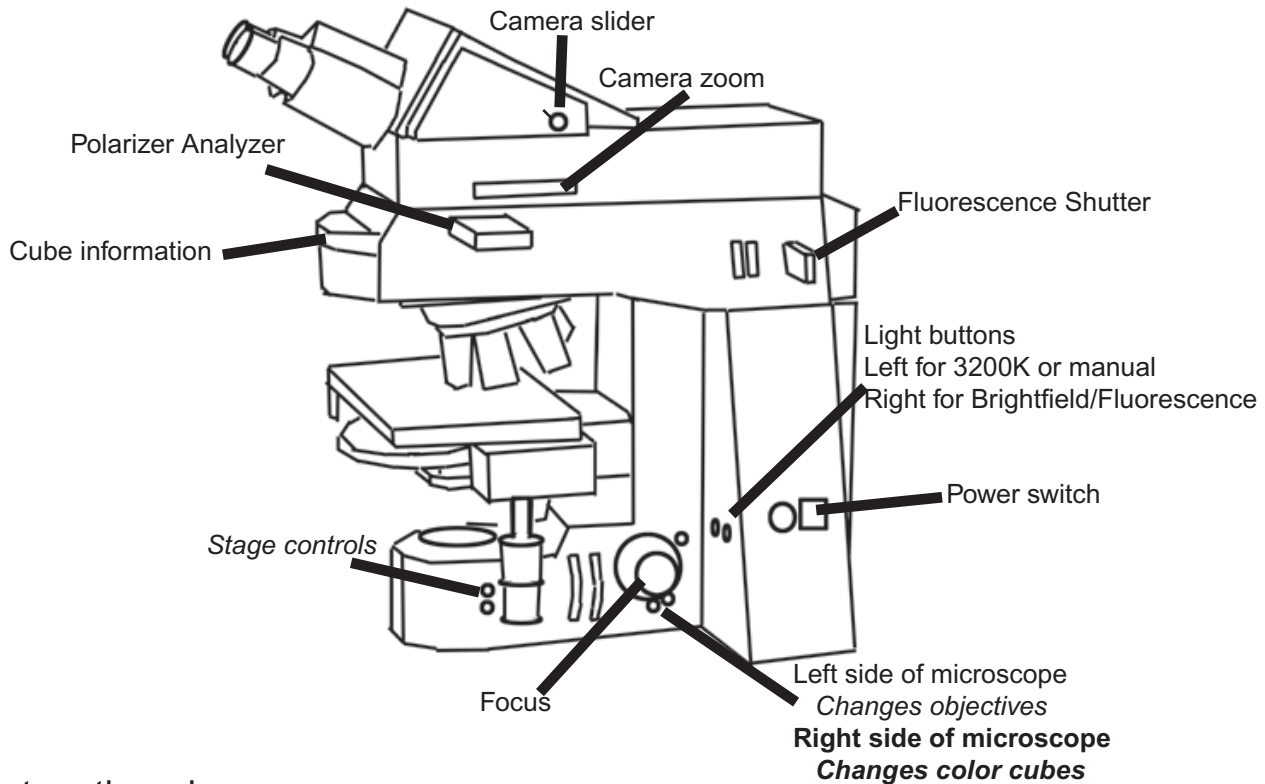
Check to apply Flatfield Correct
or uncheck if not correcting for
uneven illumination

Change Adjustment if too light

Zeiss Fluorescence

1. Turn on equipment

- a. Turn on the HBO
It is fully on when Atto Arc turns on and is at 100%
- b. Turn on the SPOT
- c. Turn on the microscope



2. Set up the microscope

- a. Set light button to Fluorescence
Press once firmly
- b. Select desired color cube
Use buttons adjacent to focus knob on the right side of the microscope
- c. Check that the polarizer analyzer is out of the light path
- d. Make sure the zoom for the camera is at 1
- e. Keep the metal camera slider at halfway out
Half of the light goes to the eyepieces and the other half goes to the camera
- f. Open the fluorescence shutter (slide out two "clicks")

3. Correct for Uneven Illumination

You **MUST** do this if subsequently measuring fluorescence intensities

- a. Choose red, green, or blue fluorescence auto or your own settings at the bottom right of the screen.
- b. Use a fluorescence reference slide (attached to wire cable)
- c. Go to clear area of the slide and focus into center of the slide so that no spots or dust appears
- d. Get the Flatfield Image: Camera > Get Flatfield Image
- e. DO NOT remove the slide and click begin
- f. Enter the date as the name for the flatfield image and click OK

Zeiss Fluorescence *continued*

4. Typical Settings for Fluorescence (*example on next page*)

- a. Setup > Image Setups
- b. Find blue, green, or red auto fluorescence setup from list and click Modify
- c. Select **24 (RGB)** for Bits per Pixel
- d. Choose **Auto-Exposure**
*Select **User-Defined Exposure** only if measuring intensity or if showing differences in intensities among several images*
- e. Select color of Fluorophore
- f. Image Type is **fluorescence**
- g. **Auto-Gain Limit** set to **4**
Higher gain equals more noise and less exposure time
- h. Set **Adjustment Factor** to 1.00
Change if the image is too dark overall. Change in increments of +0.5.
- i. Adjust **Gamma** only if dark areas are too dark
Higher Gamma lightens dark features
- j. Check **Flatfield Correct** to apply correction for uneven illumination
Do not check if you are NOT correcting for uneven illumination

Zeiss Fluorescence *continued*

Select Auto-Exposure

Choose color of Fluorophore

Choose fluorescence

Select 24 (RGB)

The screenshot shows the 'Image Setup' dialog box with the following settings and annotations:

- Name:** Auto Blue Fluorescence
- Pixel Bit Depth:** 24 (RGB) (Annotated: Select 24 (RGB))
- Exposure:** Auto-Exposure (Annotated: Select Auto-Exposure), User-Defined Exposure
- Use:** Red, Green, Blue (Annotated: Choose color of Fluorophore)
- Image Type:** fluorescence (Annotated: Choose fluorescence)
- White Balance:** Red: 1.000, Green: 1.003, Blue: 4.002
- Color Order:** B
- Binning:** None, 3 x 3, 2 x 2, 4 x 4
- Auto-Gain Limit:** 16 (Annotated: Auto-Gain at 8 Unless image is too dim)
- Adjustment Factor:** 1.00 (Annotated: Increase adjustment factor if too dark overall)
- Chip Imaging Area:** Full Chip, Center: 100%, Region: Left: 0, Right: 1315, Top: 0, Bottom: 1033
- Post-Processing:**
 - Black Level Subtract
 - Fluorescence Color Handling
 - Chip Defect Correction
 - Noise Filter: 50%
 - Image Size Double
 - Gamma Adjust: 1.60 (Annotated: Adjust gamma typically to 1.5 if dark features are too dark)
 - Rotate: Left, Right
 - Horizontal Flip, Vertical Flip
 - Background Subtract
 - Flatfield Correct: (Annotated: Check to apply Flatfield Correct or uncheck if not correcting for uneven illumination)
 - Locked

Adjust gamma typically to 1.5 if dark features are too dark

Auto-Gain at 8 Unless image is too dim

Check to apply Flatfield Correct or uncheck if not correcting for uneven illumination

Increase adjustment factor if too dark overall