# DIC
# Differential Interference Contrast on LSM780

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**DIC – Differential Interference Contrast.**

- **Allows you to view thin transparent specimens at high resolution and high contrast.**
- **Only works with glass. It will not work with plastic.**
- **Sample preparation can affect DIC. Mounting medium. Need difference in Refractive index.**

1. **Get a fluorescent image like you normally do.**

*Note: Sometimes it is easier to set up DIC with the 10x dry objective first then go to the oil objective.*
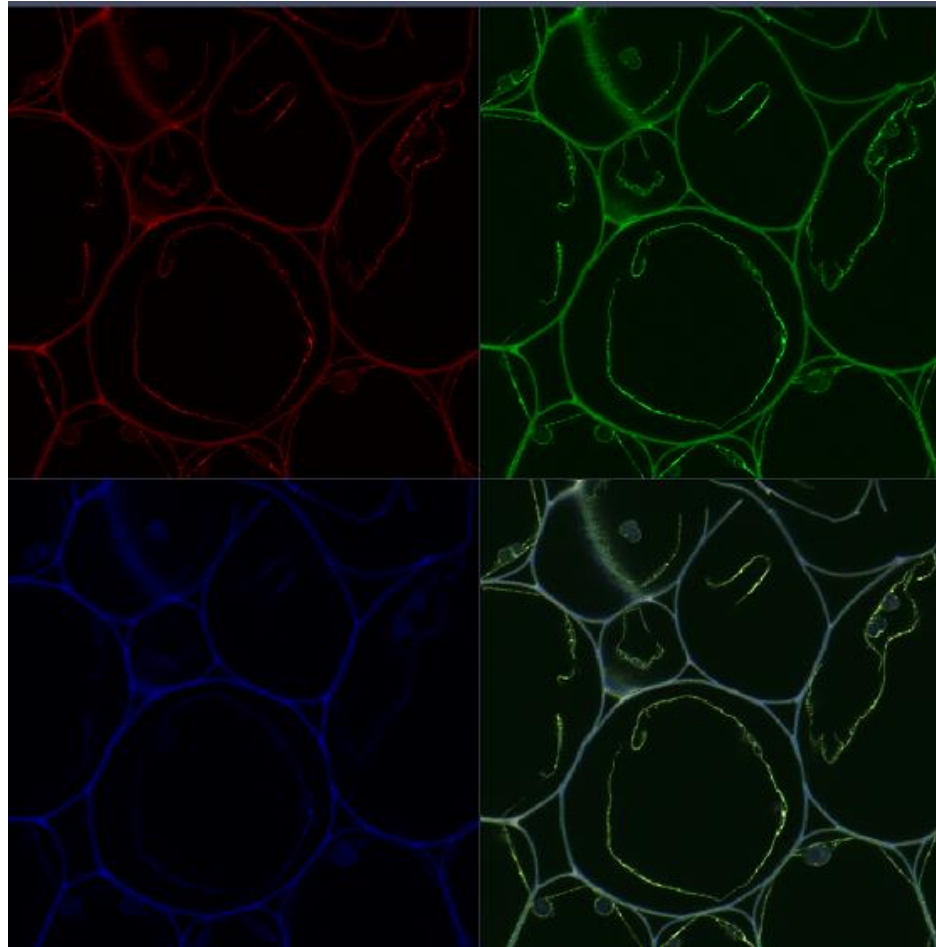
**In the Zen Software:**

2. **Click Transmitted Light On.**
3. **Click the Aperture.**
4. **Select the proper aperture for the objective. Example: DICIII for the 63x Oil Objective.** *See following slide for how to determine which filter to select.*
5. **In Configuration click Bright field.**
6. **If you need to change the intensity click on the bulb icon and adjust.**
7. **Select BS-MP-76.**
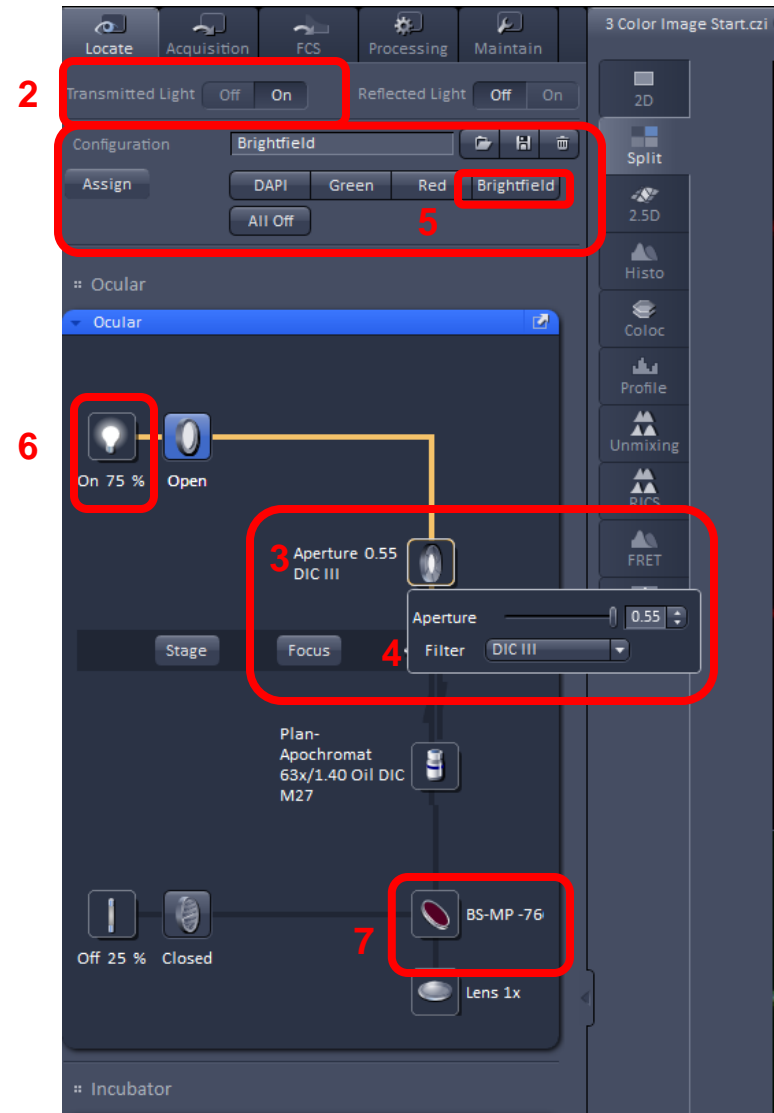
# Step 4. Select the proper aperture for the objective.

You can find the aperture setting for the objective on the touch pad in two different places :
A.   On the home screen it will be listed under Objective.
B.   In the Control tab it will be listed under Objectives.

**A**



**B**
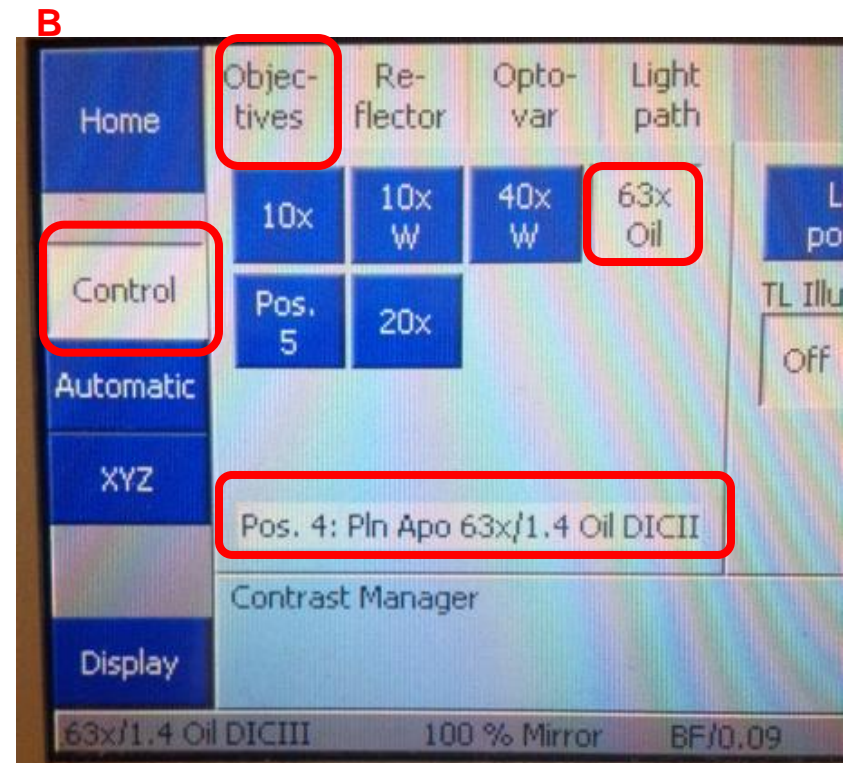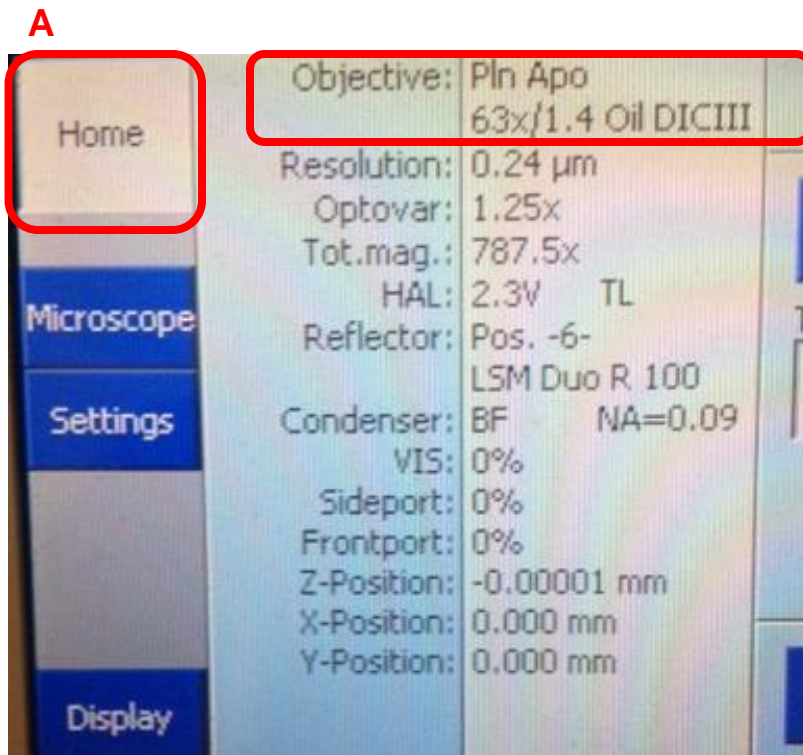
**On Microscope:**

8. **Move the silver lever of the analyzer to the left.**
9. **Put the analyzer slider into the first or far left position.**
10. **Adjust light if necessary.**
11. **Close the field diaphragm until you can see the octagon shape.** ⬡
12. **Adjust the condenser focus using the condenser focus knob until the octagon is in sharp focus.**
13. **Using the silver aperture diaphragm knobs on each side to adjust octagon to center.** *See following slide for illustration of this.*

*Steps 11-13 may have to be done in smaller increments and repeated until you can fully see the octagon shape and move it to center. It may also help to do this first with the 10x dry objective then repeat with the 63x oil objective.*
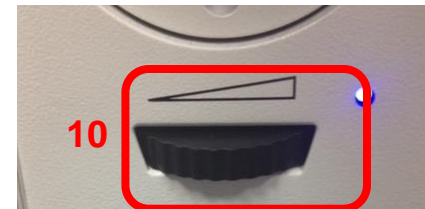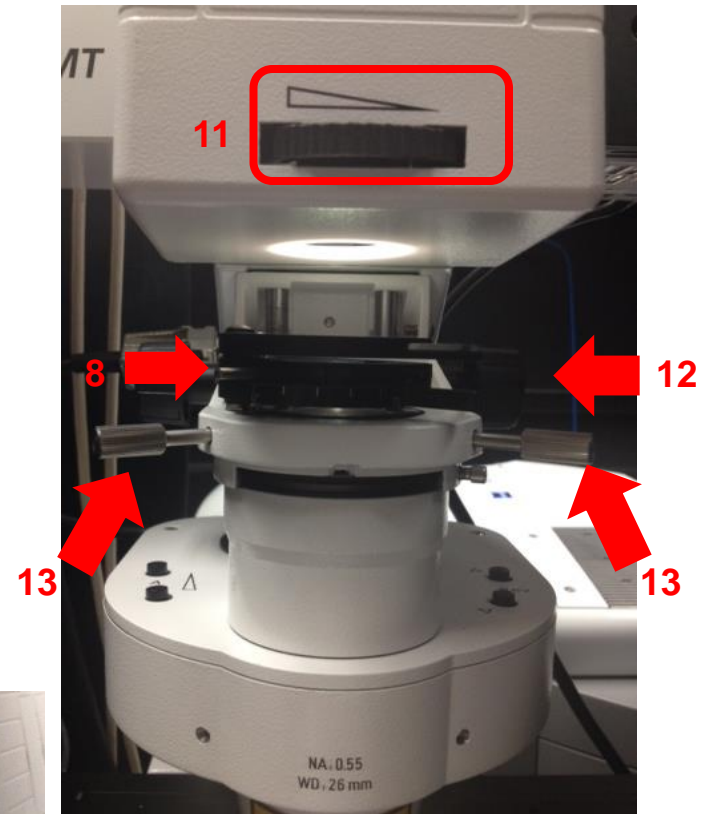
**Step 13: Using the silver aperture diaphragm knobs on each side to adjust octagon to center.**
**Here are 3 images showing the movement of the octagon to the center aligning the condenser.**
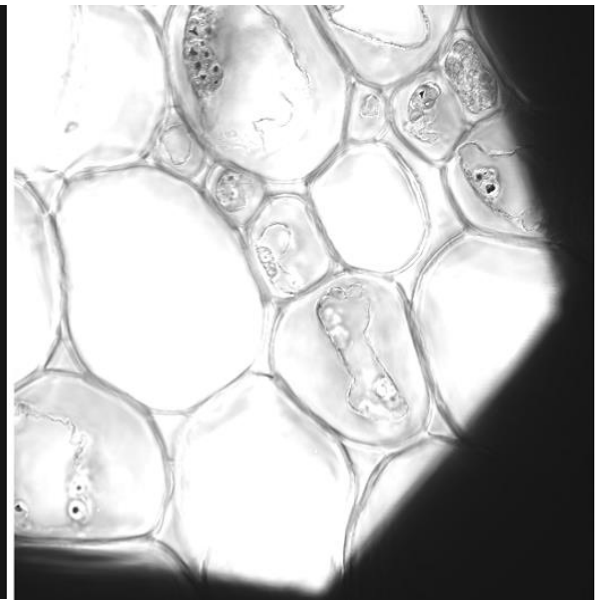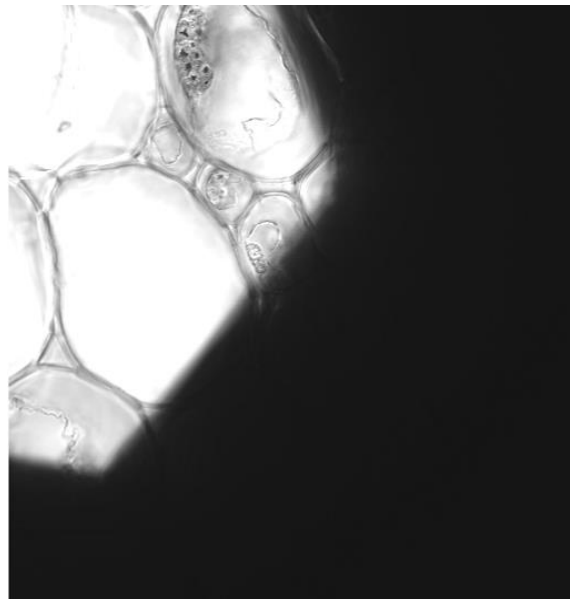
**Step 9: This is the analyzer slider showing the left hand position having the filter [left] and once again illustrating it's position on the microscope for DIC [right].**

14. **In preparation for imaging, return silver lever of the analyzer to the right.**
15. **Open up the field diaphragm.**

*Note: The right 75⁰ position is for laser scanning. The lever remains on the left for viewing DIC through the eyepiece.*
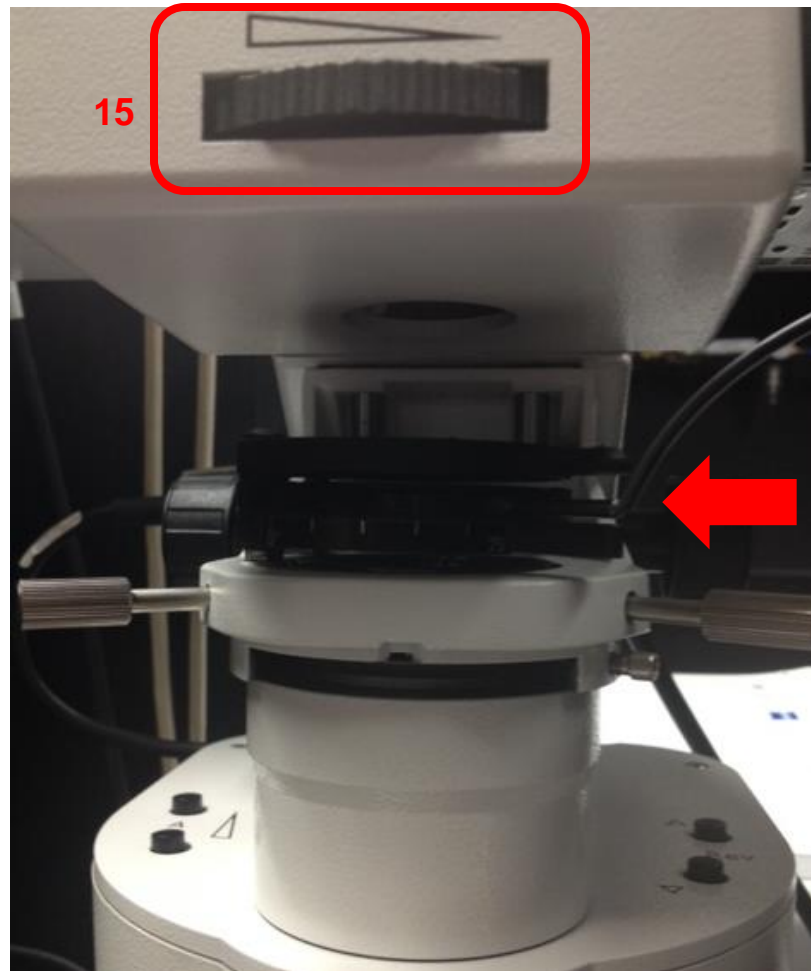
**Back on the software:**

16. **In Channels window select the track you wish to attach DIC to.**
17. **In Light Path window check the T-PMT box. This attaches DIC to that Track.**
18. **In the Channels window you will see T-PMT listed.**
19. **You also now have T-PMT controls.**

*Note: T-PMT controls off the laser power of the fluorescence track. If you change that when adjusting settings for fluorescence you will have to change your DIC settings*
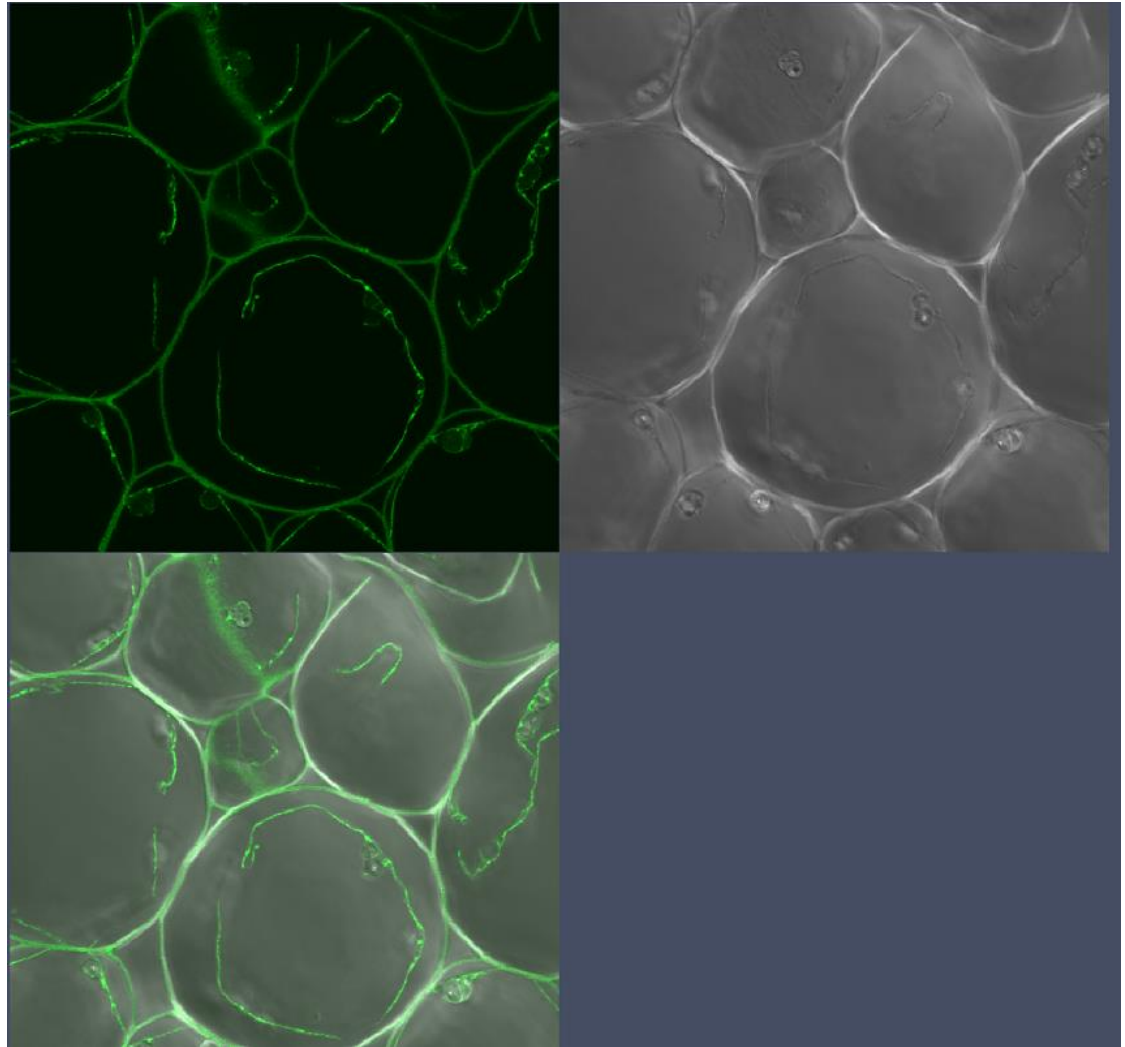
**20. While scanning in Fast XY Adjust your settings for fluorescence and DIC using controls seen in Step 19.**

**21. You will have to adjust the DIC slider to balance out the illumination.** The next slide will show you where to locate the DIC slider.
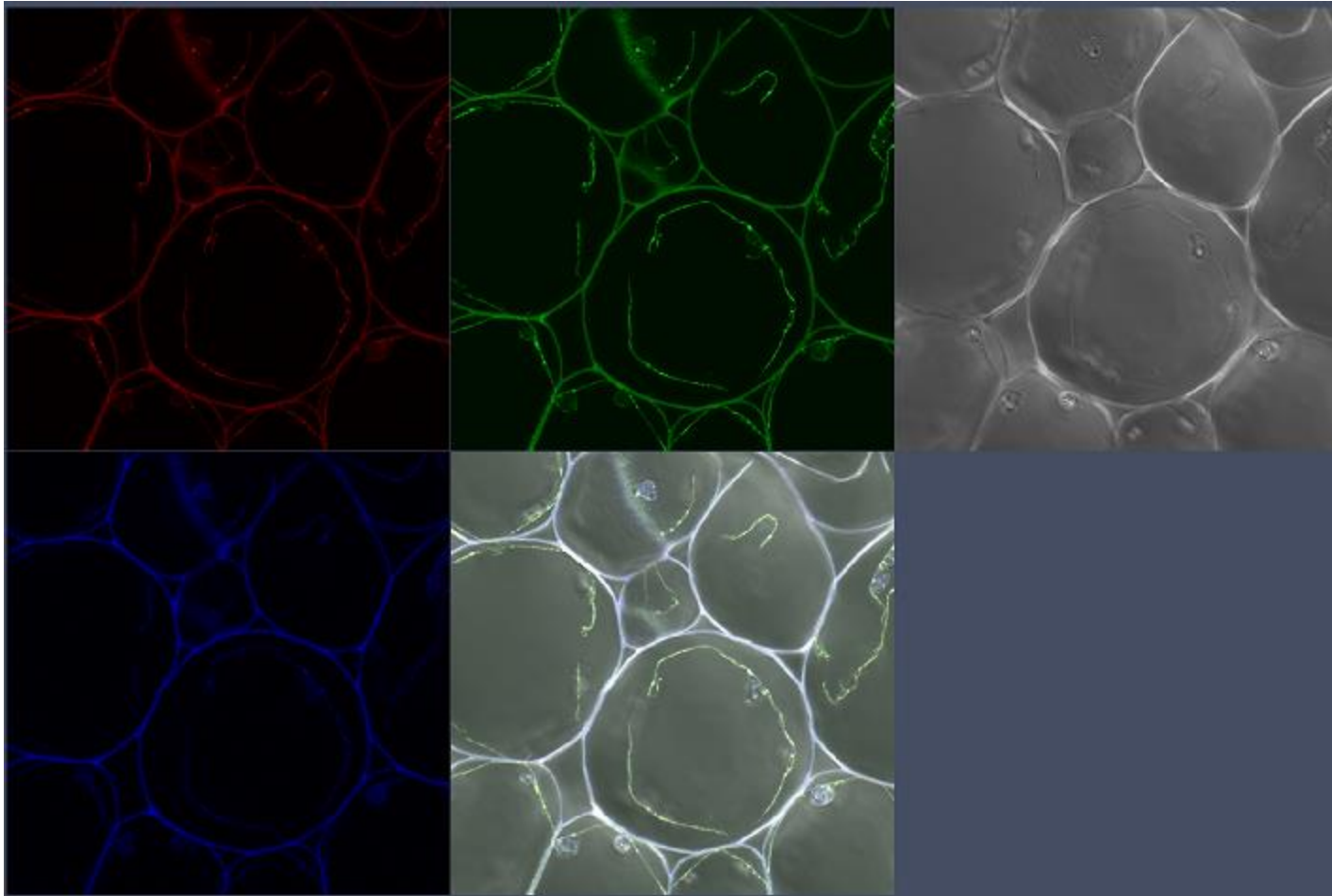
# Adjust DIC slider below the objective

**Step 21: You will have to adjust the DIC slider to balance out the illumination. The slider adjustment knob can be found on the microscope beneath the objective.** *See photo from previous slide for DIC Image.*

**22. Now take your multi-colored image plus DIC. Below is the final image in split channel view.**

**23.** In order to get the best DIC images you will need to adjust the contrast when post-processing the image. Here just T PMT-T2 was selected to adjust the contrast.