Setting up Koehler Illumination on the ImageXpress System.

To ensure high-quality illumination with brightfield or phase contrast imaging, set up the Transmitted Light option for Koehler illumination. Do this alignment anytime there is concern about the quality of the transmitted light images, or when changing plate type or slide type. Since the objectives are parfocal, repeating this procedure after switching objectives is unnecessary.

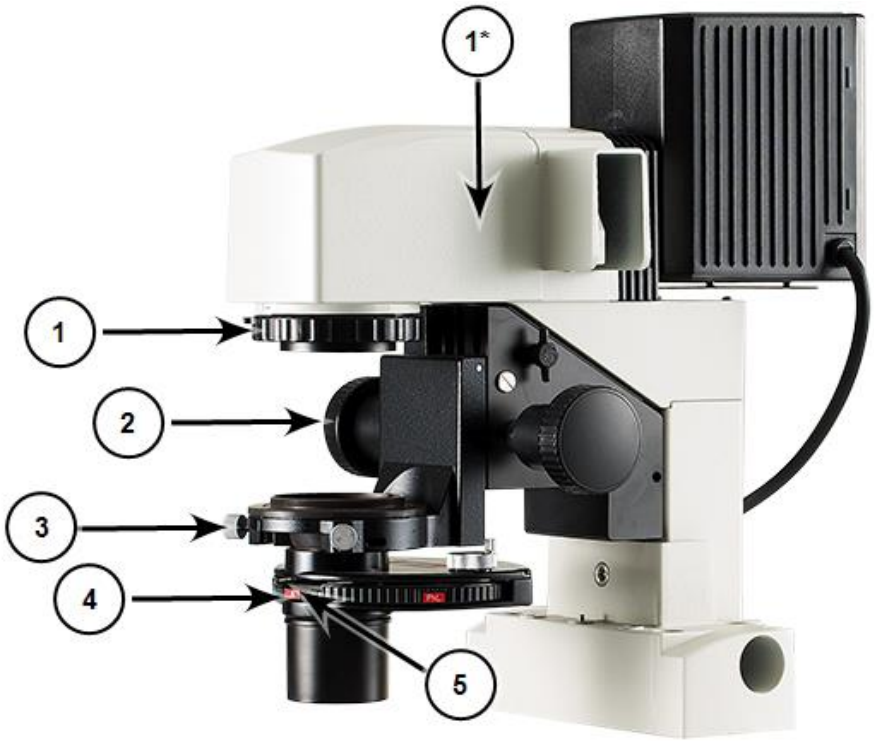

Table 8-3: ImageXpress Transmitted Light option overview

Item	Description
1	Field Aperture Diaphragm Control (* this control can be located here on older Transmitted Light models)
2	Condenser Focus Control
3	Condenser Adjustment Screws (Aperture Lateral Adjustment Control)
4	Condenser Turret
5	Field Stop Slider (Condenser Aperture Diaphragm Control)

To set up the ImageXpress Transmitted Light option for Koehler Illumination:

1. Load the sample (multi-well plate or slide).
2. Select a low magnification objective such as the 10x Plan Fluor or a 4x objective.
3. Rotate the condenser turret (4) so that the **A** position is selected (at the front). Open the condenser aperture diaphragm by moving the field stop slider (5) above the **A** to the right.
4. In the MetaXpress Software, click **Screening > Plate Acquisition Setup**. In the simplified menu, click **Screening > Acquisition Setup**.

5. In the **Plate Acquisition Setup** dialog, move the stage to a well or slide area where there is visible sample.
6. Select one of the available Transmitted Light illumination settings.
7. Use the laser-based autofocus, image-based autofocus, or live mode to focus on the sample.
8. Click **Start Live**.
9. Close the field aperture diaphragm (1) most of the way by moving the lever left, or down if you have the older model (1*).
10. Adjust the condenser height with the condenser focus control (2) until a bright polygon surrounded by sharp dark edges is visible.

Figure 8-57: Condenser height focus adjustment comparison

11. If you cannot detect sharp edges, open the field aperture diaphragm (1) further, and try adjusting the condenser height again.
12. Center the polygon in the field of view using the condenser adjustment screws (3).
13. Open the field aperture diaphragm (1) by moving the lever right, or up if you have the older model (1*), until the whole field is illuminated and the dark edges are just outside of the field of view. Opening this too far can increase glare in the images.
14. Close the condenser aperture diaphragm slightly by moving the field stop slider (5) above the **A** to the left, to optimize the sharpness and contrast of the transmitted light image.
15. Click **Stop Live**.
16. Click **Focus** to confirm proper illumination. If you see dark edges in your field of view, repeat step 13.
17. The adjustment is now complete.

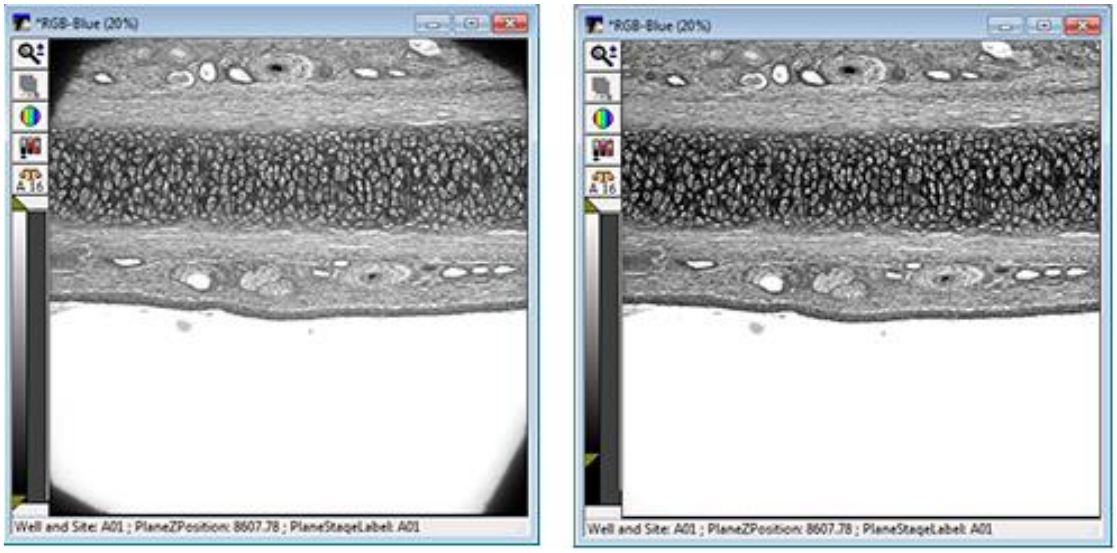

Figure 8-58: Adjustment of the Field Aperture Diaphragm Comparison