

Spatial calibration of fluorescence microscope

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Summary: This procedure describes how spatial calibration of the CCD camera with a particular fluorescence optical setting is achieved.

1. Identify a calibrated microscope scale that is in a form that can be placed on a microscope stage. Ideally, this slide is transparent. A ruled microscope slide or the USAF 1951 resolution target can be used.
2. Place a piece of fluorescent glass (i.e. Schott) that provides a fluorescence signal under the desired fluorescent filter conditions over the top of the ruled microscope slide.
3. Set the microscope for fluorescence imaging and identify the measurement features of the slide in the CCD camera. The ruled features should selectively prevent or permit fluorescence from the glass to reach the CCD camera.
4. Align the ruler so it is approximately square with the CCD field.
5. Collect an image of the ruler.
6. If required, rotate the ruler 90 and collect a second image.
7. By using these images and image analysis software (i.e. Image J) measuring functions, the x and y-length per pixel can be calculated. Record these values for image metadata.
8. To ensure proper calibration calculations, it is worthwhile to compare the calibrated pixel length to that expected from an ideal objective and the actual binned pixel sizes of the CCD camera. If these values are not similar, it may suggest a non-par-focal CCD camera configuration exists or the presence of an unidentified accessory lens in the optical path.

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