

# Guidelines for Capturing Linear Images

## General guidance:

- White balance once at beginning of imaging session, then do not adjust light intensity on microscope again during that same session.
- Use camera exposure or shutter control to adjust image brightness.
- Use histogram to ensure all color channels are within the range (saturation = lost data!).
- Do not adjust contrast, saturation, hue or gamma unless such guidance is provided below.

**Software and version: Zeiss Zen (v2012, v2), Zen Lite (v2012, vBlack and Blue)**

**Camera(s): Zeiss AxioCam MRc5 / AxioCam 503 color / AxioCam 506 color**

## Recommended Acquisition Settings

Zeiss separates the image display properties within the Zen software from the image acquisition properties. Image acquisition is linear by default, however image display properties can be saved with the image file.

To visualize a linear image within Zen, refer to the Display tab in the view options section of the software:

- Select a Gamma setting of 1.0 (blue circle below)
- Ensure that the left and right arrow heads (red circles) are stretched completely to the left and right, respectively, and the middle arrow head is in the middle.

By default when saving images in the Zeiss .CZI file format, the images are linear. A non-linear image may result during export from .CZI to .TIFF or .JPEG.

If using Export from the File menu:

- Choose either JPEG or TIFF
- We suggest choosing Convert to 8-bit
- DO NOT use any compression
- DO NOT SELECT Apply Display Curve

If exporting from a previously saved .CZI file:

- Use File > Save As with Options and choose JPEG or Tagged Image File, OR
- Use File > Export/Import > Export, choose JPEG or Tagged Image File, no compression (for TIF)

## View Options

