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Zeiss Z2m Microscope Manual Supplement, Volume 1

by Donovan Harris

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14. ABSTRACT
The Zeiss Z2m microscope system is capable of subsurface analysis of translucent materials using 3-D image generation and/or employing monochromatic illuminants. This technical note provides additional instructions that address issues arising from a lack of Internet support capability or those not found in the Zeiss Z2m system manuals and software help sections. The supplement also touches briefly on issues with image annotation and measurements.

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Zeiss Z2m, AV, optical microscope, supplement, subsurface analysis, translucent, transparent

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Foreword

The issues and workarounds discussed here need to be considered valid only for one specific instrument system (Zeiss Z2m) and its software support constriction of not having Internet access. There is no way to determine the applicability to another Z2 system.

This technical note addresses operations and issues experienced by this Z2m system but not correctly covered by available help documentation. If supplemental information is not included for a specific function or application, the application was not tried or the performance did not address the current needs and attempting a similar function proved more time effective. Additional supplements will be published as more functions are tried.

1. Introduction

1.1 Overview

The Zeiss Z2m system is an upright optical microscope capable of 3-D imaging, working in the near infrared (NIR) and subsurface characterization of transparent to translucent materials. The Zeiss manuals and the AxioVision (AV) help functions rely upon having direct Internet support that is precluded by security compatibility issues. This supplement provides users with solutions to the issues experienced that are not addressed by the US Army Combat Capabilities Development Command Army Research Laboratory's Ceramics and Transparent Materials Branch's (CTMB's) existing Zeiss support materials. CTMB possesses a limited number of materials-, ceramics-, and/or metals-related software modules. Reflected and transmitted illumination controls also have issues.

The Zeiss Z2m system comprises the following:

- Hardware. High-resolution monochrome (HRm) and a medium-resolution color (MRc) cameras:
    - HRm: 1388 × 1040 pixels at 6.45 × 6.54 µm using a 1.00× projection lens
    - MRc5: 2584 × 1936 pixels at 3.4 × 3.4 µm using a 0.63× projection lens.
- Illumination
    - White LEDs, 5500K, dedicated one transmission and one reflected
    - Monochromatic LEDs, nine lamps, 385–940 nm; one UV 385; five visible, 400–710 nm; three NIR 780–940nm
- Contrasting modes
    - Reflected light: brightfield (BF); darkfield; reflected polarized light; circular–differential interference contrast (C-DIC)
    - Transmitted light: BF; cross polarization; darkfield
- Software
    - AV, 4.9.1 sec 64-bit: Z-stack
    - Topography
    - Extended Focus

- MosaiX
- Image J, 64-bit

The Zeiss “materials” applications were not purchased. Both ImageJ and MatLab plugins support the 14-bit Zeiss ZVI file format. AV will export 8- and 16-bit TIFF and JPEG images and is able to produce .avi files from Z-stacks.

The Z2m used to develop this technical note was preconfigured to perform 3-D subsurface studies of transparent ceramics and correct several issues using a 32-bit AV software version. The 16-GB workstation random-access memory (RAM) improves the 3-D and extended focus modules operability but does not provide full support for the system’s capture capabilities. The system’s illumination capability is on the back of the Z2m stand (Fig. 1). The top unit is for reflected light with a left hand lamp for the white LED source and the right hand lamp for the monochrome source. The bottom unit is for is for transmitted light, and with the white LED source on the left and the monochrome source on the right. The monochrome LED illumination sources were not acquired as part of the original Z2m system, but rather separately from Thorlabs.

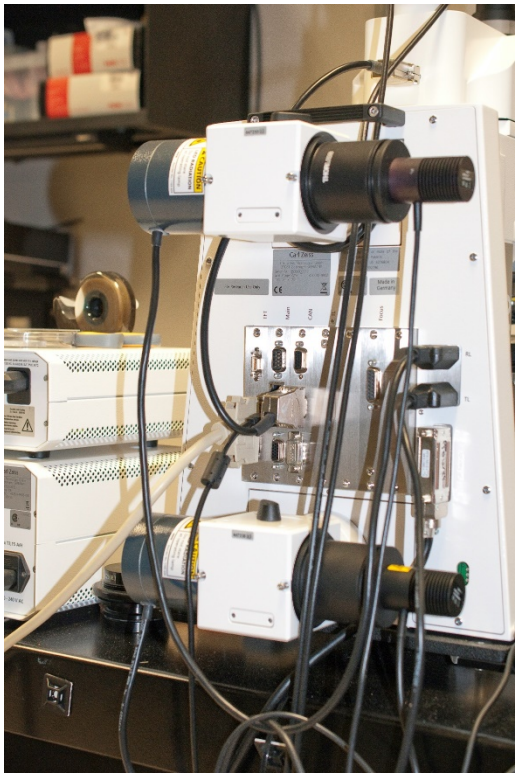

Fig. 1 Back of Z2m stand showing reflected light module (top), transmitted light module (bottom) with white LEDs (left side of each module), and monochrome LEDs (right side of each module)

The unit proper uses two computer modules, a control board housed in the stand, and a module housed in the touch pad. Both modules interact with the AV software through Zeiss's Micro Tool Box, a separate application.

IMPORTANT: Do not attempt any microscope configuration modification, as the software presumes software-readable part ID chips on all configurable hardware components and only half of the parts on this Z2m have those chips.

Required Readings

- Safety Notes AxioVision.pdf
- AxioVision Read Me.txt
- AV49_KnownBugs.pdf
    - NOTE: As of the date of publication, the AV software on the system computer is still listed as the latest version (AV, 4.9.1 sec 64-bit: Z-stack), but is in the process of being replaced. The compatibility of the two systems is yet unknown.

NOTE: AV is not Windows 10 compatible.

1.2 File Formats and Associated Issues

ZVI is the 14-bit default Zeiss format and is limited to processing approximately 2-GB monochrome Z-stack or MosaiX capture.

The Z-stack processing depends on available RAM. During subsurface studies, we were able to generate, capture, store (but not process) Z-stacks greater than 6 mm without the AV freezing. Ideally, the system should use 64-GB of RAM. The work-around is to create several smaller substacks to be processed, but this may need a separate third-party stitching application. The substacks products may be able to use MosaiX for stitching providing their aggregate is less than 2 GB.

The MosaiX, while listed as an AV 4.9 constraint, has tested as fully functional by Zeiss, but still has a 2-GB limit.

2. Z2m Startup and Shutdown

Power-on order is as follows:

- Line isolator-1
    - Stage Power Supply
    - Z2m Power Supply

    - ONLY after the touch pad is fully operational power
    - Z2m PC (This will also power the cameras)
- Line isolator-2
    - Monochromatic LED's power supply
    - Buffalo external hard drive (archival storage)

The power-on order is to ensure that both the Z2m and the AV have registered and properly identified all the hardware components.

The TFT touch pad does not control the cameras, which are discussed in Section 6.

3. AV 4.9 Application

There is a desktop icon and a task bar microscope icon for opening the AV application.

The AV application has the equivalent of a taskbar using tabs and not icons, below which sit three ribbons with functional icons. The third ribbon microscope icon opens a hardware control panel that has most of the standard controls required and is an ideal initial operational point. The bottom two ribbons were set in place by Zeiss at time of installation and are not illustrated in the AV software manual.

The second ribbon icons activate individual light controls that permit monitoring how the light manager function is actually working.

Many of the Zeiss manual descriptions for productivity improvements rely on the missing Commander module.

Shut down is the reverse of power on. **NOTE: Always turn off the computer. As long as it is on, the cameras and Peltier coolers are on and being aged.**

4. Light Managers

The hard copy 2012 manual,[1] pages 92–96, presents an overview on how the light manager functions. AV does not control the light managers but interacts with the TFT that controls the light manager. Figure 4-32 of the manual,[1] page 104, shows a TFT screen that is not available on the in-house Z2m system.

The light managers are configured to be off at present, including the BF for transmitted light (that button will not activate).

The light manager functions in Figure 4-19 of the manual[1] have not been fully explored. Only the BF tab found on the Objectives display page has been tried. It was largely ineffective for the Zeiss LED, although it was designed for transmitted light BF use.

The light managers appear to control the Zeiss LEDs and the mechanical controls for each light path. Using the monochrome LEDs, the light managers respond as if they also controlled those units. For that reason, the actual readouts from the apertures need be shown and monitored if the light managers have been activated. There are several specimens for light manager experimentation. The glass frits work with both reflected and transmitted light in the visible-NIR region.

5. Monochromatic LEDs

All the Z2m monochromatic LEDs are from Thorlabs as of February 2018, with only the UV-Vis sources addressed here; Section 11 addresses the NIR sources. The full list of available LEDs is shown in Table 1 with the Z2m LEDs highlighted.

Table 1 Available monochromatic LEDs (used with permission of Thorlabs, LLC)

Item #	Color[a]	Housing	Total Beam Power[b]
M365L2-C4	UV		80 mW
M365LP1-C4[c]	UV		400 mW
M385L2-C4	UV		110 mW
M385LP1-C4[c]	UV		630 mW
M405L3-C4	UV		600 mW
M405LP1-C4[c]	UV		570 mW
M455L3-C4	Royal Blue		430 mW
M470L3-C4	Blue		310 mW
M505L3-C4	Cyan		180 mW
M530L3-C4	Green		150 mW
Item #	**Color[a]**	**Housing**	**Total Beam Power[b]**
M590L3-C4	Amber		70 mW
M617L3-C4	Orange		280 mW
M625L3-C4	Red		330 mW
M660L4-C4	Deep Red		570 mW
M730L4-C4	Far Red		195 mW
M780L3-C4	IR		180 mW
M810L3-C4	IR		230 mW
M850L3-C4	IR		400 mW
M940L3-C4	IR		380 mW
MCWHL5-C4	Cold White		380 mW

[a] Thorlabs designation for wavelength
[b] Beampower when collimated for visible
[c] For LEDs in the visible spectrum, the nominal wavelength indicates the wavelength at which the LED appears brightest to the human eye. For UV and IR LEDs, the nominal wavelength corresponds to the peak wavelength. The nominal wavelength for visible LEDs may not correspond to the peak wavelength as measured by a spectrograph.

SAFETY NOTE: Use only the cameras with the UV sources. Limit eyepiece use in the 455–480 nm range to avoid possible retinal effects.

The green M530L3-C4 LED generally resides on the reflected light path as the preferred alternative to the white light LED being the most responsive for human vision and both cameras. The HRm curve is shown in Fig. 2.

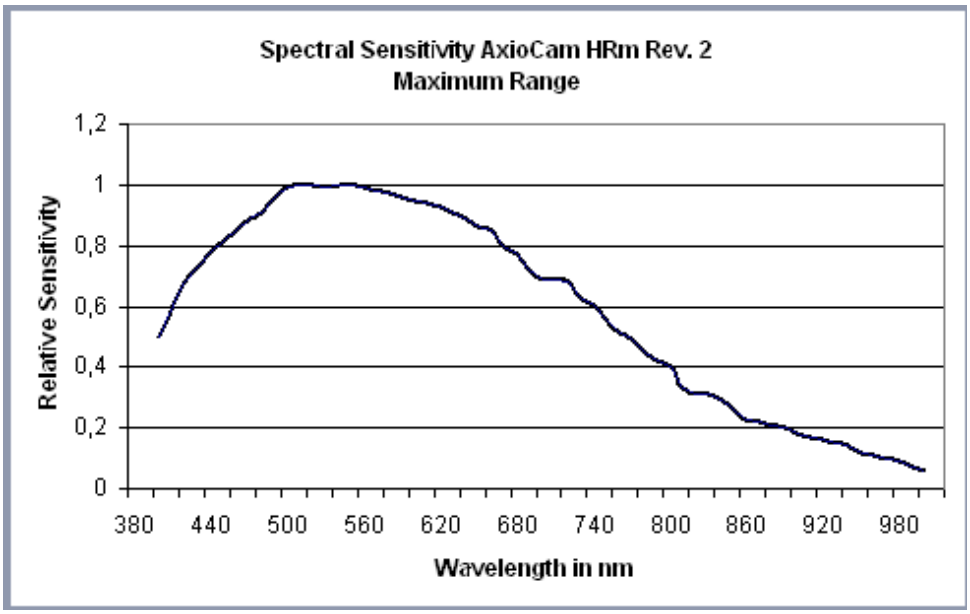

Fig. 2 HRm curve (used with permission of Carl Zeiss Microscopy, LLC)

The M660 and M730 LEDs have not yet been tried.

5.1 Known Issues

The M385, M405, and M530 LEDs have generally proven troublesome to use with the MR5c color camera using reflected light, changing observed colors to pastels, and shifting the wavelength band with increasing power. With the HRm camera, their performance generally varies with objective changes; the 10× and 20× objectives are more consistent. For reflected light microscopy, carbide surfaces prove difficult to analyze except when using reflected polarized light and darkfield illumination for generally flat surfaces.

Larger wavelengths reliably reduce resolution and thus feature detectability and measurability. Rule of thumb for estimating the feature detection limit of a monochromic illuminant is $\lambda/2$ or ideally, the wavelength should be less than half the desired resolution. Smaller surface features maybe detectable at larger wavelengths but not necessarily resolvable.

Due to machining imperfections, mounting the Thorlabs LEDs requires checking that the unit is actually centered in the adapter. Rotating the lamp unit for centering before engaging the setscrew may mean rotating the display monitor for observation. This alignment is also possible by observing the setscrew, which will draw deeper when improperly rotated.

5.2 Power Controller

The Thorlabs power controller (Fig. 3) is an analog unit operating independently of the Z2m system and AV. Precise and repeatable settings are not possible without ancillary instrumentation, which is not currently on hand. A separate line isolator is needed to avoid noisy images.

Fig. 3 Thorlabs power controller

At this time only one power supply is available, so only one light path at a time is available. Changing light paths requires changing the power to the desired lamp.

6. Cameras: HRm and MRc

The high- and medium-resolution cameras are configured as follows:

- HRm: 1388 × 1040 pixels at 6.45 × 6.54 µm using a 1.00× projection lens
- MRc5: 2584 × 1936 pixels at 3.4 × 3.4 µm using a 0.63× projection lens

NOTE: Both cameras become noisy in a warm environment (> 73 °F).

6.1 HRm

The 14-bit monochrome camera has proven to work well over 385- to 850-nm wavelengths. At 940 nm, the response is poor and grainy; the NIR boost increases that graininess.

The ZVI file format is the 14-bit AV default that can be opened by both ImageJ and a MatLab Image Tool Box add-on. Generation of 8-bit JPEG and TIFF files are possible in place of the ZVI files with a loss of the metadata. AV can export 8- and 16-bit JPEG and TIFF files from a ZVI file.

The HRm installation manual has the more complete usage description, while the AV manual[2] and software help provide limit support for either camera.

The AV HRm controls are best accessed using the properties tab of a live image, as the HRm and MR5c top ribbon tabs have reduced options and seem to conflict with the live function properties software if both are active.

The top ribbon tabs are best used to check settings prior to activating the live mode, thus ensuring reasonable initial conditions as the software stores and opens with its last settings.

NOTE: Do not use the buttons, best fit, auto snap, or auto live on any control panel, as the results are rarely usable and recovery tedious for both cameras.

Read the HRm installation manual, pdf pages 67–70, on shading/vignetting correction. The 2.5× objective has shading issues on a regular basis with both transmitted and reflected light. The higher magnification objectives experience shading issues primarily when using transmitted light with translucent materials. Sometimes the light manager will close down an aperture creating the appearance of a shading issue. Check your aperture and f-stop displays first.

NOTE: There are two readout speeds for the HRm that are located on the frame tab of the camera panel.

Use 12.5 MHz for analysis, publications, and tiling/MosaiX, and use 25 MHz for setting up Z-stacks.

The remainder of the available HRm controls has not been explored beyond a quick survey of their functions.

6.2 MRc5

The MRc5 has an issue with its white balance. The 3300 and 5000 K options do not work correctly. Autocorrect while off provides the best adjustment. A white Lambertian reference is required to generate a reliable standard working reference.

The resolution of the camera has not yet proven sufficient for materials research. Work using known references is required for the camera to become a research instrument. The MRc5 and controls are geared toward fluorescence studies of tagged biologicals.

NOTE: The MRc5 has only one available readout speed.

7. Stages

The scope has two automated stages: a Z-stage that is integral to the stand and an X-Y stage that attaches to the Z-stage and is separately powered.

The Z-stage uses control knobs on the stand and the TFT control display: a TFT control panel and an AV panel. The last option is accessed by opening the top AV/Microscope-icon tab/the stage tab and then selecting the Z option. AV control of the Z-stage provides a finer and more reliable level of control than the knobs when using the 50× and 100× objectives. Note that Zeiss never defines what TFT actually means.

The joystick controller for the X-Y stage can be difficult to master. To achieve fine displacements, use the AV X-Y control panel. The control panel is relatively simple and very accurate, which aids in performing linear scans. While a manual does exist, clarity is not its strong point.

NOTE: TFT control of the X-Y stage does not exist.

8. 3-D and Extended Focus

All the 3-D, Multidimensional Acquisition, and Extended Focus modules perform similar functions using different capture methods and producing slightly different products. Both modules make use of the same core Z-stack processing algorithms. The 3-D module yields two products and the Extended Focus yields one product.

Extended Focus is able to use individually focused images and manual inputs to produce a Z-stack and then an interpolated product image, which is more 3-D looking than the individual slices.

The 3-D module control panel allows you to define and generate a Z-stack with known slice spacing. That Z-stack can be processed using either the EF module or the 3-D Topography option that generates two ZVI files, one an interpolated image and the other the topographic/height data. The height data allow wire mesh and surface modeling generation. Similar ImageJ plug-ins have performed much better on the samples tested. The only down side to using 3D wire-mesh plug-ins in ImageJ is the resultant output print image size is small and fixed.

9. Annotations

All image annotations exist in a layer separate from the actual image layer(s) and thus do not interact with any of image processing functions. For that reason, the image exporting function must be instructed to burn the annotations into the exported file. Only annotation issues with captured ZVI files can be addressed, as no images have been captured in TIFF or JPEG formats.

The AV4 annotation process is neither intuitive nor straightforward. The micron marker module is extremely simple when using the default settings: just activate

the tab on the fourth ribbon and place the marker where desired. NOTE: Sometimes the software will allow you to reposition the marker and not force a deletion but that has not been the norm.

Modifying the appearance for any annotation must be done after the fact in the image properties window under the annotation tab. The annotation appearances cannot be modified before the fact, but those changes can be made the new default (i.e., change the default colors, text size, and line thickness). Deletion of a particular annotation is possible in a single delete command.

The first ribbon contains two active annotating tabs, Annotations and Measure. The annotating tabs are mutually exclusive, thus requiring switching between the two tabs for full documentation of some images.

Annotations cover everything that does not require a value be generated. Measure is for quantitative documentation. The appearances of both types of notations are modifiable in the image properties tab. The ZVI images can be reannotated but non-ZVI files cannot. Before exporting a ZVI to another format, the ZVI must be annotated.

10. Exporting ZVI Files to Other Formats

Most of the metadata in a ZVI file is lost in the transformation to other formats. The exception is TIFF files where XML files containing the metadata are concurrently generated. No attempt has been to use non-ZVI file formats directly instead of the ZVI. Exporting to Zeiss's LSM format has not been attempted.

All metadata is lost for existing JPEG, BMP, and the similar formats. There has been no experience with capturing to non-ZVI formats.

11. Transmitted NIR LEDs

Figure 4 shows the nominal spectral response for the Zeiss HRm monochrome camera, and Table 2 lists the Zeiss Z2m compatible NIR LED sources available through mid-2018. CTMB has all but the M810L3-C4 shown in Table 2.

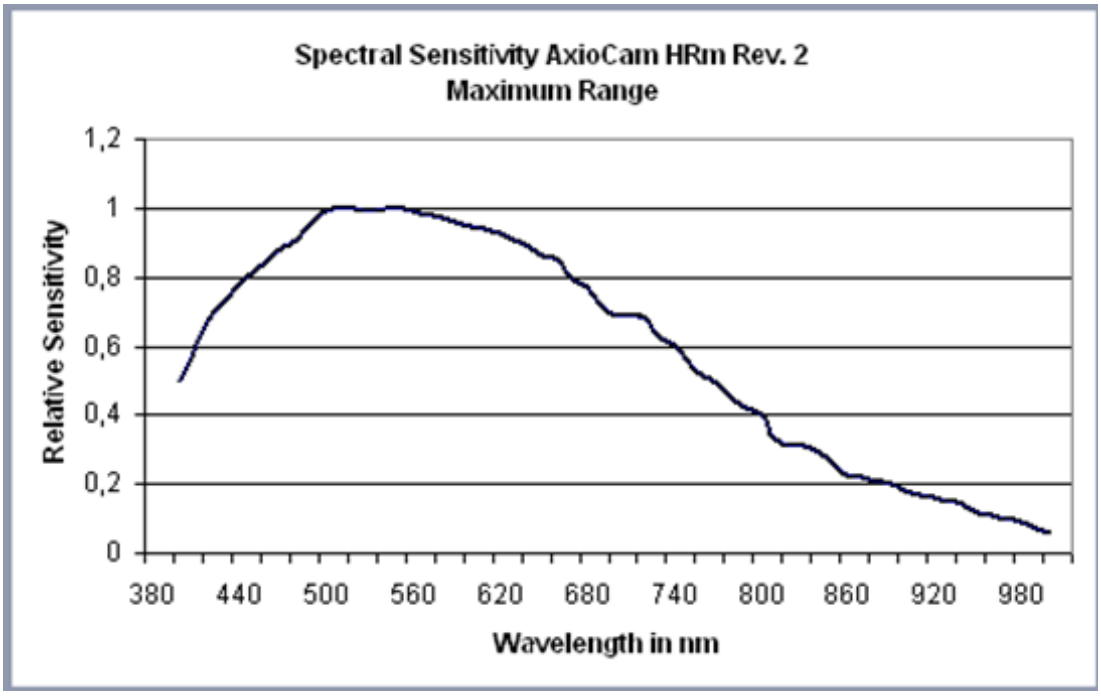

Fig. 4 **Zeiss HRm relative spectral sensitivity (used with permision of Carl Zeiss Microscopy, LLC)**

Table 2 **Z2m-compatible Thorlabs NIR LEDs (used with permission of Thorlabs, LLC)**

Item no.	Color[a]	Housing	Total Beam Power[b]
M780L3-C4	IR	[redacted]	180 mW
M810L3-C4	IR	[redacted]	230 mW
M850L3-C4	IR	[redacted]	400 mW
M940L3-C4	IR	[redacted]	380 mW

[a] At peak wavelength
[b] After colimation

The M940 LED proved too weak with the Zeiss HRm camera, producing images (not shown) with salt and pepper noise at all settings. The M780 produces an orange glow visible to the HMc5 camera but only opaque debris is discernable.

Based on the nominal relative spectral intensities shown in Fig. 4, the HRm1 response at 850 nm should be slightly higher than the 940-nm response. With this information, future users should assess whether a new camera is justified to achieve 940-nm lumination for their program. No measurements of the NIR LEDs' actual intensities are possible as the available sensors cannot detect NIR. The half

bandwidth for the 850-nm LED (Fig. 5) caused at least one chromophore to emit in the visible spectrum, supporting MR5c use and obscuring the 850-nm contribution.

The 810-nm LED, because of its narrow half-band (Fig. 5, top), would be ideal for subsurface grain studies based upon past experiences with spinel and AlON samples.

SAFETY NOTE: Use only with the cameras. Do not use the eyepieces with these sources. Use for transmitted light only.

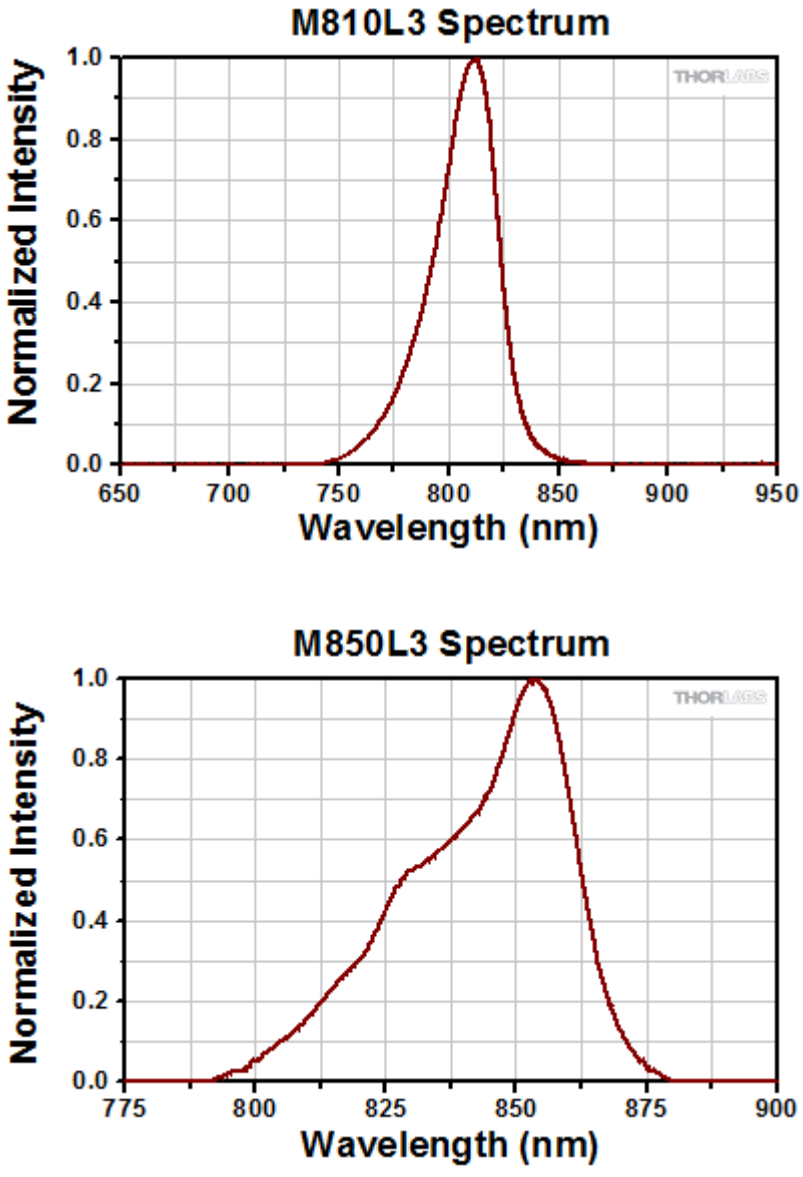

Fig. 5 Normalized spectral intensities (used with permission of Thorlabs, LLC)

The 780-nm LED generates a visible orange glow (Fig. 6), which is captured by the MR5c but is not sufficient to support imaging. The MR5c nominal relative response curve (Fig. 7) is consistent with the red image in Fig. 6. Without the Bayer array filter, the camera chip spectral response curve is very similar to that for the HRm. We only know the nominal responses but not the actual Bayer array filter or camera chip's actual spectral responses for the completed system.

NOTE: Check power cable is connected to correct source path.

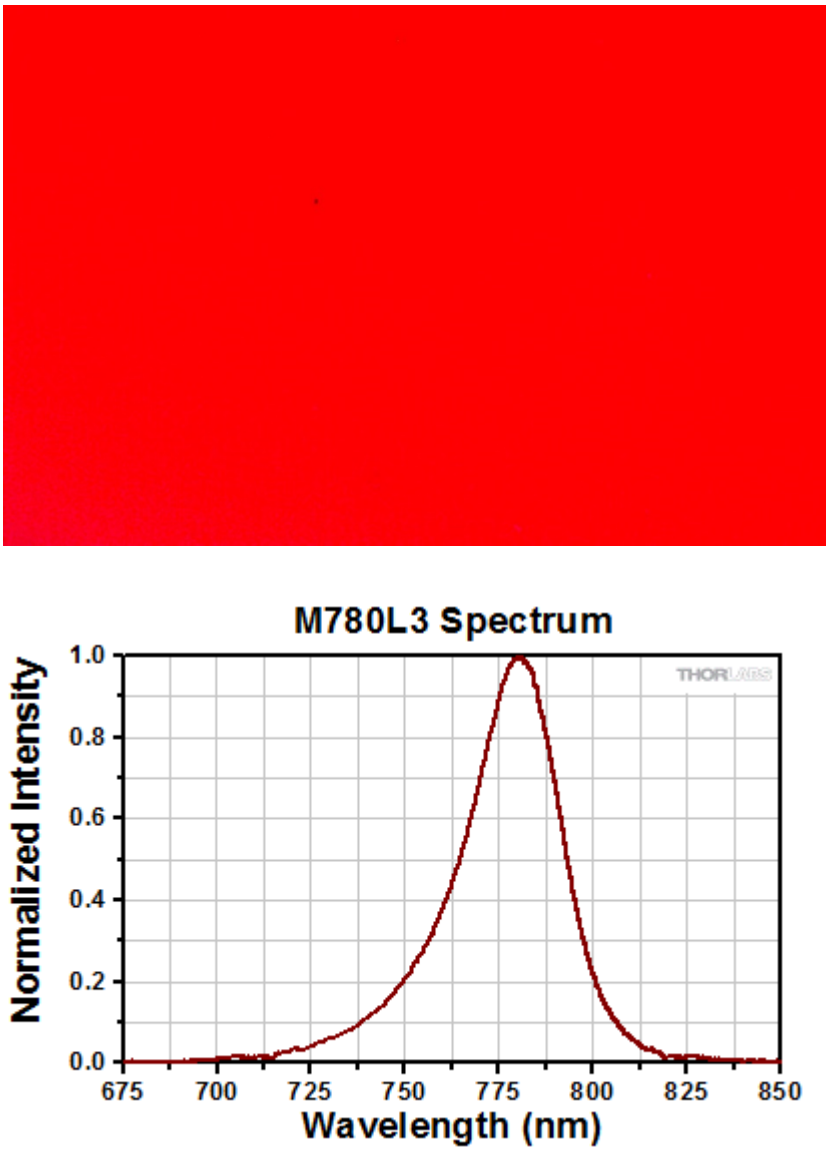

Fig. 6 Orange glow that originates at the lower end of nominal 780-nm LED spectrum (top) and M780L3 nominal intensity (bottom). Orange frame made using a nanocomposite. (Spectrum used with permission of Thorlabs, LLC.)

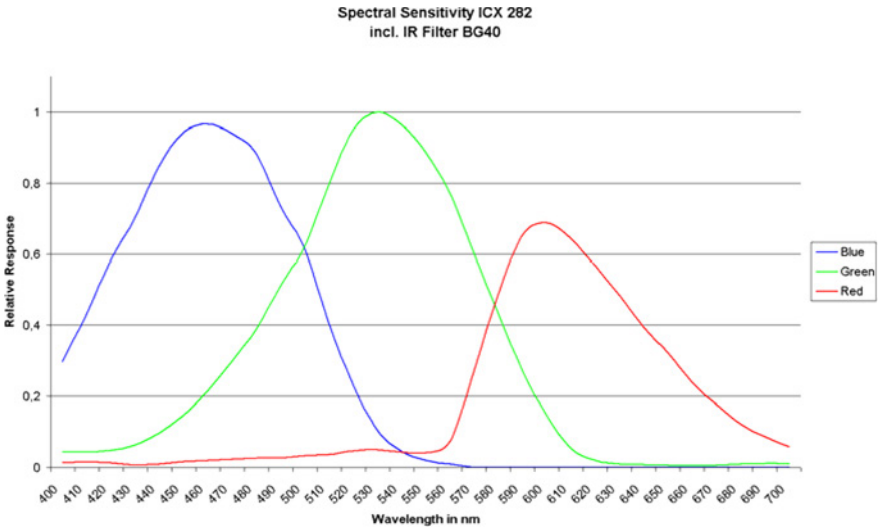

Fig. 7 MR5c nominal spectral response (used with permission of Carl Zeiss Microscopy, LLC)

12. References

1. Carl Zeiss MicroImaging GmbH. Axio Imager upright microscope operating manual. Jena (Germany): Carl Zeiss MicroImaging GmbH; 2012.
2. Carl Zeiss Microscopy GmbH. AxioVision user's guide, release 4.9 SE64. Göttingen (Germany): Carl Zeiss Microscopy GmbH; 2012 Nov.

List of Symbols, Abbreviations, and Acronyms

3-D	three-dimensional
AV	AxioVision
BF	brightfield
C-DIC	circular–differential interference contrast
CTMB	Ceramics and Transparent Materials Branch
HRm	high-resolution monochrome
LED	light-emitting diode
MRc	medium-resolution color
NIR	near-infrared
PC	personal computer
RAM	random-access memory
TFT	undefined by Zeiss

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