Point-by-Point Response to Editorial Comments – Manuscript JoVE60826R3

1. The highlighted protocol steps are over the 2.75 page limit (including headings and spacing). Please highlight fewer steps for filming.

Please see at the following page for a cut version of the highlighted protocol steps (trying to respect the request to include all subsections of a mentioned point).

2. Please ensure that all figures are numbered in the order of their appearance in the manuscript. For example, Figure 6 (line 126) was cited after Figure 2. So, it should be numbered as Figure 3.

The figure numbering has been adjusted accordingly. Previous figure 6 is now figure 3.

3. There is one footnote on page 13. Please remove.

The footnote has been removed and implemented into the text.

1. Configuration of the hyperspectral microscope

1.2 Set the appropriate **optical cube configuration** (Figure 2b and c).

1.2.1 Starting from the microscope stage (1 in Figure 2a) and following the emission beam pathway towards the detectors (3 in Figure 2a), leave the first position for an optical cube (4 in Figure 2b) vacant and place the *confocal microscope optical cube* (DFM1-P01) in the position indicated as 5 in Figure 2b, so that the emission from the sample is directed through the visible light path.

1.2.2. Looking along the optical path towards the detector, place the *visible optical cube* (CM1-P01), which contains the dichroic mirror and the filters to direct the visible emission to the detection paths, in the position indicated as 6 in Figure 2b.

1.2.3. Continuing the path towards the detector, place the *confocal pinhole optical cube* (DFM1-P01) in the position indicated as 7 in the Figure 2b to direct the light through the visible light detection path. Then, following the path, place the *confocal spectrometer optical cube* (DFM1-P01) in position 8 in Figure 2c so that the emitted light reaches the detector.

1.2.4. For the HSI mapping, manually control the *detector slit opening* (9 in Figure 2c) in order to match with the size of the *pinholes* that are used (around 50 μm is optimal).

1.2.5. In the **PHySpec** software, choose the aperture of the *pinhole* (5 in Figure 3).

2. Hyperspectral imaging of a [TbEu(bpm)(tfaa)$_6$] single crystal

2.5. Manually position the 20X objective (indicated by 5 in Figure 5) under the sample and press the white button (6 in Figure 5) on the left side of the microscope to turn on the white light.

2.5.1. Adjust the brightness by turning the knob underneath the white light power button (7 in Figure 5).

2.6. In the **PHySpec** software, press the play (video) button on the color camera window, which will cause the acquisition of a live scan.

2.6.1. If the color camera window shows a black image, increase the *Exposure Time* (2 in Figure 3) and/or the *Gain Value* (3 in Figure 3) found in the instrument control panel, under the *Color Camera* tab. If the image viewed is too bright, decrease the exposure time and/or gain value.

2.6.2. Ensure the forward knob on the right side of the microscope (2 in Figure 5) is set to R in order to send 20% of the signal to the camera/binoculars and 80% of the signal to the detector.

2.7. Focus on the sample by adjusting the distance between the objective and the stage (Figure 4b). This is done by turning the knobs shown in Figure 4d on the right side of the microscope.

2.10. Open the **broadband lamp** shutter (12 in Figure 2) to allow the UV excitation of the sample to take place. Turn the intensity knob (11 in Figure 2d) to the desired position (*e.g.* 8 – intermediate intensity) to control the intensity of the broadband lamp (UV) excitation.

2.10.1. To choose between wide field illumination (open aperture) or a smaller spot illumination (more closed aperture), control the size of the UV lamp field aperture using the stick and knobs shown in 4 in Figure 5.

2.11. Under the *SpectraPro SP-2300* tab, select a wavelength to observe the sample emission.

2.13. Adjust the *Exposure Time* (*e.g.* 0.5 s – 2 in Figure 3) and *Gain* (3 in Figure 3) of the *Color Camera* accordingly, to obtain a good quality image. If needed, add the scale bar to the image by clicking at the button *Show/hide scale bar* at the second row of the menu in the top of the **PHySpec** software window.

2.14. Recommendation: Prior to acquiring the hyperspectral cube, record a bright field optical microscopy image of the crystal under white light (Figure 6a) and/or UV full (Figure 6b) or confined (Figure 6c) illumination (UV illumination controlled by the shutter aperture, shown as 4 in Figure 5). To do so, with the sample in focus, click on the play button of the color camera.

2.17 To obtain the hyperspectral cube, write a new sequence. Therefore, in the sequencer, click on the + sign to add a new node.

2.17.1. Click on *Confocal Imager*.

2.17.1.1. Click on *Multi-Spectrum Acquisition*. Here, the desired field of view is defined by the number of points to acquire in the *x* and *y* directions and the step size. For example, use 100 points in *x* and 100 points in *y* with a 5 µm step size to obtain an image of 500 by 500 µm.

2.17.1.1.1. Input the desired *X Position* (*e.g.* 100) and *Y Position* (*e.g.* 100) counts as well as the desired *Step Size* (*e.g.* 5 µm).

2.17.1.1.2. Select the *Hardware* option for the camera sync, for visible emission mapping (and *Software* option in case of NIR detection). Click OK.

2.18. In the sequencer, click on the newly added *Multi-Spectrum Acquisition* line to highlight the node.

2.19. Click the play button to run the selected node.

3. Hyperspectral data analysis

3.3.1. In case of the spectral distribution from an image, use the *Crop and Bin* function to increase the signal-to-noise ratio in the image. In order to do that, click in the top menu *Processing* then choose *Data* and then the option *Crop and Bin*.

3.3.2. For an emission intensity profile, on the cube image, right click and select the *Create Target* or *Create X profile* or *Create Y profile* depending if only one point (Target - 5 and 6 in Figure 7) or a line (Profile - 7 and 8 in Figure 7) needs to be analyzed. Select the area of analysis by dragging the target, the horizontal or the vertical line profile with the cursor and move it across the cube.

3.3.2.1. Once the profile has been properly selected, right click on the region and select the *Add Target to Graph*. Chose the option to create a new graph to display the emission intensity (*y* axis) as a function of the physical position of the target (*x* axis). The spectrum will appear on the new graph which was inserted (6 and 7 in Figure 7).

3.3.3. Alternatively, obtain an emission spectrum of a specific area of the sample (9 in Figure 7). To begin with, hover the cursor over the cube image and right click. Click on the *Rectangle Selection* or *Ellipse Selection* options on the tab that pops-up.

3.3.3.1. Then draw the selection shape (*e.g.* a rectangle) over the desired region by clicking and dragging the cursor across the cube. Once the area has been properly selected, right click on the region and select the *Add Selection to Graph*.

3.3.3.2. At the appearing window *Add to Graph*, select *Create a New Graph* to display the emission spectra of the target and click OK.

3.3.4. Once the spectrum is obtained, save it before selecting a new region because only one region can be selected at a time. To do so, select the window in which you have your graph. Then, in the *File* menu select *Save as* and you can choose to save the graph in the folder of choice, using the name of choice, either in .h5 format, which can be opened in the **PHySpec** software, or in .csv format, which can be imported in Excel.