FINAL SCRIPT: APPROVED FOR FILMING

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Title: A Multimodal Wide-Field Fourier-Transform Raman Microscope

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 17
Number of Shots: 27

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Martina Riva:** We developed a multimodal hyperspectral microscope to acquire the Raman or photoluminescence spectrum from each pixel of an image which reduces the measurement time.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.
AUTHOR'S NOTE: Please remove the symbol "P1" that appears at the top-left of the scene, above the drawing on the blackboard in file 0538.

What technologies are currently used to advance research in your field?

1.2. **Giulio Cerullo :** Conventional raster-scanning approach relies on dispersive spectrometers. Advanced techniques enhance the Raman signal either using light at resonance or with synchronized ultrashort laser pulses which drive molecular vibrations.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.
Videographer's Note : Use file 0540

What are the current experimental challenges?

1.3. **Gianluca Valentini:** The low Raman scattering cross-section poses two main challenges: (1) faint signal, which requires long acquisition times; (2) Raman signal may be overwhelmed by a stronger photoluminescence background.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.17*
Videographer's Note : Use file 0542

What advantage does your protocol offer compared to other techniques?

1.4. **Cristian Manzoni:** Fourier-transform spectroscopy enables parallel spectrum acquisition across all pixels, for faster measurement and a tunable sampling to access specific spectral information, such as isolating weak Raman peaks from strong luminescence background.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.1*
Videographer's Note : Use file 0543

What research questions will your laboratory focus on in the future?

1.5. **Martina Riva:**. The Fourier transform approach enables smart sampling and data analysis that leverage information in temporal traces. We aim at tailoring undersampling strategies to further shorten acquisition time.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.15*

AUTHOR'S NOTE: Please remove the symbol "P1" that appears at the top-left of the scene, above the drawing on the blackboard in file 0539.

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

2. Sample Preparation and Hyperspectral Raman Imaging
Demonstrator: Alessia Di Benedetto, Martina Riva

2.1. To begin, use a scraper to deposit 20 milligrams of each pigment powder on a precision balance, obtaining a one-to-one-to-one weight proportion **[1]**. Mix the powders with a mortar to remove clumps **[2]**.

2.1.1. Talent placing pigment powders on the precision balance.
Videographer's Note : Use file A7sIII_0545, A7sIII_0546, A7sIII_0547

2.1.2. Talent grinding powders to break up clumps.
Videographer's Note : Use file A7sIII_0549

2.2. Pour the resulting mixture onto a microscope slide **[1].** Then use the scraper tip to gently press it to obtain an almost uniform thickness of the layer **[2]**.
Videographer's Note : 2.2.1-2.3.2 merged. Use files A7sIII_0551, A7sIII_0552, A7sIII_0553

2.2.1. Talent pouring mixture onto the slide.

2.2.2. Talent using scraper tip to press mixture into uniform layer.

2.3. Apply nail polish on the edges of a microscope coverslip **[1]**. Place it on the mixture with the nail polish facing down and apply enough pressure to seal it **[2-TXT]**.

2.3.1. Talent applying nail polish to coverslip edges.

2.3.2. Talent placing coverslip with nail-polish-side down and pressing to seal. **TXT: Let it dry to harden the polish**

2.4. Next, set the excitation wavelength and focus the excitation laser at the input of the large-core multimode fiber **[1]**. Put a test sample on the microscope stage and switch on the camera to view the laser illuminated spot **[2]**.

2.4.1. Talent setting excitation wavelength and focusing laser into fiber.
Videographer's Note : Use files A7sIII_0555, A7sIII_0556, A7sIII_0557

2.4.2. Talent putting a test sample on the stage and switching the camera on, showing the illumination spot.
Videographer's Note : Use files A7sIII_0559, A7sIII_0560, 2_4_2

Added shot: SCREEN: 68515_screenshot_8.mp4 00:04-00:08, 00:14-00:20
Video editor: Please play 2.4.2 and added shot side by side in a split screen

2.5. Attach a middle section of the fiber to the vibrating membrane of a voice coil which will

remove speckle **[1]**. Mechanically scramble the fiber by tightly bending it to merge all its spatial modes **[2]**.

2.5.1. Talent attaching fiber section to voice-coil membrane.
Videographer's Note : Use files A7sIII_0565

2.5.2. Talent tightly bending fiber to scramble modes.
Videographer's Note : Use files A7sIII_0562

Added shot: SCREEN: 68515_screenshot_9.mp4 00:02-00:16
Video editor: Please play 2.5.2 and added shot side by side in a split screen

2.6. Insert a narrow bandpass filter at 532 nanometers with 2.0 nanometer bandwidth to clean the laser line and reject any unwanted pump spectral sidebands**[1]**. Use a dichroic mirror to reflect the laser light toward the sample and transmit the redshifted back-scattered radiation collected by the objective **[2]**.

2.6.1. Talent inserting the narrow bandpass filter at 532 nm in the microscope.
Videographer's Note : Use files A7sIII_0568

2.6.2. Talent inserting the dichroic mirror to direct excitation and collect backscattered signal.
Videographer's Note : Use files A7sIII_0569

2.7. Insert a long-pass filter at 532 nanometers to reject residual illumination light **[1]**.

2.7.1. Talent inserting the longpass filter at 532 nm.
Videographer's Note : Use files A7sIII_0567, A7sIII_0566

2.8. Measure the power on the sample plane with a power meter **[1]**. Adjust the pump beam to obtain an intensity at the sample which does not lead to damage **[2]**.
Videographer's Note : 2.8.1-2.8.2 merged. Use files A7sIII_0571, A7sIII_0572

2.8.1. Talent measuring sample-plane power with power meter.

Talent adjusting pump beam to safe intensity.

2.9. Now put the sample on the microscope stage and adjust the focus **[1]**.

2.9.1. Talent placing slide on stage and focusing the microscope.
Videographer's Note : use file A7sIII_0573

2.10. Switch on the driver of the motor that performs the wedge translation and press the **Acquire** option **[1]**. Then set the camera acquisition parameters like time frame and hardware binning to optimize the signal intensity **[2-TXT]**.

2.10.1. SCREEN: 68515_screenshot_1.mp4 00:00-00:16

2.10.2. SCREEN: 68515_screenshot_2.mp4 00:00-00:13
TXT: Acquisition of photoluminescence.

2.11. Now set the step length, home position and number of steps **[1-TXT].**

2.11.1. SCREEN: 68515_screenshot_3.mp4 00:00-00:16

TXT: Wait until the sample has reached a stationary condition

2.12. Add the name of the directory and the file name **[1].** Move the cross point to the target position and click on **Measure** to start the acquisition of a monochrome image for each wedge position **[2]**. Once the measurement is finished, switch off the laser before data analysis **[3].**

2.12.1. SCREEN: 68515_screenshot_4.mp4 00:00-00:14

2.12.2. SCREEN: 68515_screenshot_4.mp4 00:16-00:40

2.12.3. Talent switching off the laser.

Videographer's Note : use file A7sIII_0576

2.13. Next, generate the spectral hypercube from the acquired dataset by loading the motor positions correction file specific to the stepper motor **[1]** and the frequency calibration file corresponding to the interferometer **[2]**.

2.13.1. SCREEN: 68515_screenshot_5.mp4 00:00-00:19

2.13.2. SCREEN: 68515_screenshot_5.mp4 00:20-00:25

2.14. Set the wavelength for the spectra to be computed by Fourier transforming the interferograms of all pixels **[1]**.

2.14.1. SCREEN: 68515_screenshot_5.mp4 00:26-00:32

2.15. Apply the apodization function which offers a good trade-off between spectral broadening and artifact reduction, such as the Happ-Genzel window and select the pixel **[1]**. Then generate the spectral hypercube and save it in complex values **[2]**.

2.15.1. SCREEN: 68515_screenshot_5.mp4 00:33-00:46

2.15.2. SCREEN: 68515_screenshot_5.mp4 00:47-00:53

2.16. Launch the analysis software and open the spectral hypercube in complex values **[1].** Generate a false-colour RGB image **[2]** and obtain the average spectrum in selected areas to analyze the spectral hypercube **[3]**.

2.16.1. SCREEN: 68515_screenshot_6.mp4 00:00-00:08

2.16.2. SCREEN: 68515_screenshot_6.mp4 00:09-00:19

2.16.3. SCREEN: 68515_screenshot_6.mp4 00:20-00:32

2.17. For the Raman measurement on the same field of view, set the time frame and hardware binning **[1].** Then set the set length, home position and number of steps **[2].** Choose the file name and directory for saving and move the crosshair to the target position before pressing **Measure [3].**

2.17.1. Talent inserting the short pass filter at 600 nm.
Videographer's Note : use file A7sIII_0577
2.17.2. SCREEN: 68515_screenshot_7.mp4 00:00-00:10 .
2.17.3. SCREEN: 68515_screenshot_7.mp4 00:11-00:20
2.17.4. SCREEN: 68515_screenshot_7.mp4 00:21-00:44

Results

3. Results

3.1. Three pigments are clearly distinguished within the field of view based on their Raman spectral signatures **[1]**, with characteristic peaks for rutile at 454 and 616 inverse centimeters **[2]**, anatase at 396, 514, and 641 inverse centimeters **[3]**, and cadmium yellow at 301 and 605 inverse centimeters **[4]**.

3.1.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the color-coded regions labeled 1, 2, and 3.*

3.1.2. LAB MEDIA: Figure 4B. *Video editor: Highlight the two peaks at 454 and 616 in the top cyan-colored graph labeled "Rutile (1)".*

3.1.3. LAB MEDIA: Figure 4B. *Video editor: Highlight the three peaks at 396, 514, and 641 in the middle yellow-colored graph labeled "Anatase (2)".*

3.1.4. LAB MEDIA: Figure 4B. *Video editor: Highlight the two peaks at 301 and 605 in the bottom magenta-colored graph labeled "Cadmium Yellow (3)".*

3.2. In the photoluminescence map, instead, cadmium yellow is the only pigment visibly distributed across the field of view **[1]**, with strong emission dominating the entire image **[2].** Indeed, the faint defect emission from rutile and anatase cannot be spectrally distinguished from the yellow powder **[3]**.

3.2.1. LAB MEDIA: Figure 5A.

3.2.2. LAB MEDIA: Figure 5B. *Video editor: Highlight the dominant magenta-colored spectrum rising across the full wavelength range labeled "Cd Yellow (3)".*

3.2.3. LAB MEDIA: Figure 5B. *Video editor: Highlight the much smaller cyan and yellow peaks labeled "Rutile (1)" and "Anatase (2)" in the lower portion of the graph*.

Pronunciation List

1. Multimodal
   Pronunciation link: https://www.merriam-webster.com/dictionary/multimodal
   IPA: /ˌmʌltiˈmoʊdəl/
   Phonetic Spelling: mul-tee-moh-dəl
2. Fourier-Transform
   Pronunciation link: https://www.merriam-webster.com/dictionary/Fourier-transform
   IPA: /ˈfʊəri.eɪ-ˌtrænsˌfɔːrm/ (American: /ˈfʊri.eɪ-ˌtrænsˌfɔrm/)
   Phonetic Spelling: foor-ee-ay-trans-form
3. Raman
   Pronunciation link: https://www.merriam-webster.com/dictionary/Raman
   IPA: /ˈrɑːmɑːn/ or /ˈreɪmən/ (American commonly /ˈreɪmən/)
   Phonetic Spelling: ray-mən
4. Microscope
   Pronunciation link: https://www.merriam-webster.com/dictionary/microscope
   IPA: /ˈmaɪkrəˌskoʊp/
   Phonetic Spelling: my-kruh-skohp
5. Hyperspectral
   Pronunciation link: No confirmed link found
   IPA: /ˌhaɪpərˈspɛktrəl/
   Phonetic Spelling: hy-per-spek-trəl
6. Photoluminescence
   Pronunciation link: https://www.merriam-webster.com/dictionary/photoluminescence
   IPA: /ˌfoʊtoʊˌluːməˈnɛsəns/
   Phonetic Spelling: foh-toh-loo-muh-nes-uhns
7. Interferogram
   Pronunciation link: No confirmed link found
   IPA: /ˌɪntərˈfɪrəˌgræm/
   Phonetic Spelling: in-ter-fi-ruh-gram
8. Apodization
   Pronunciation link: No confirmed link found
   IPA: /əˌpoʊdəˈzeɪʃən/
   Phonetic Spelling: uh-poh-duh-zay-shun
9. Birefringent
   Pronunciation link: https://www.merriam-webster.com/dictionary/birefringent
   IPA: /ˌbaɪrəˈfrɪndʒənt/
   Phonetic Spelling: by-ruh-frin-juhnt

10. Dichroic
    Pronunciation link: https://www.merriam-webster.com/dictionary/dichroic
    IPA: /daɪˈkroʊɪk/
    Phonetic Spelling: dy-kroh-ik
11. Nanometer
    Pronunciation link: https://www.merriam-webster.com/dictionary/nanometer
    IPA: /ˈnænəˌmiːtər/
    Phonetic Spelling: nan-oh-mee-ter
12. Spectral
    Pronunciation link: https://www.merriam-webster.com/dictionary/spectral
    IPA: /ˈspɛktrəl/
    Phonetic Spelling: spek-trəl
13. Pixel
    Pronunciation link: https://www.merriam-webster.com/dictionary/pixel
    IPA: /ˈpɪksəl/
    Phonetic Spelling: pik-səl
14. Binning
    Pronunciation link: No confirmed link found
    IPA: /ˈbɪnɪŋ/
    Phonetic Spelling: bin-ning
15. Interferometer
    Pronunciation link: https://www.merriam-webster.com/dictionary/interferometer
    IPA: /ˌɪntərˌfɪrəˈmɛtər/
    Phonetic Spelling: in-ter-fi-ruh-meh-ter