

LOUIS STOKES CLEVELAND VA MEDICAL CENTER  
Medical Research Service  
Subcommittee on Research Safety Policy

Effective Date: February 1, 2018

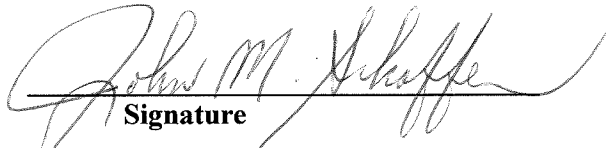
SOP Title: USE OF THE RAMAN MICROSCOPE

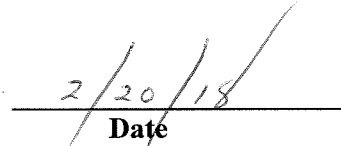
SOP Number: SRS--022

SOP Version: .04

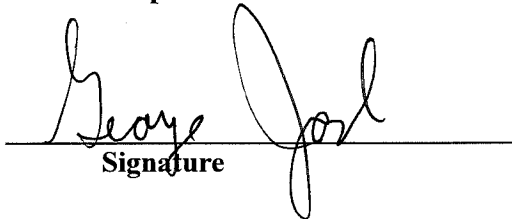
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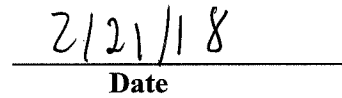
Name: John M. Schaffer, B.A.  
Title: Research Safety Coordinator  
Department: Medical Research Service

  
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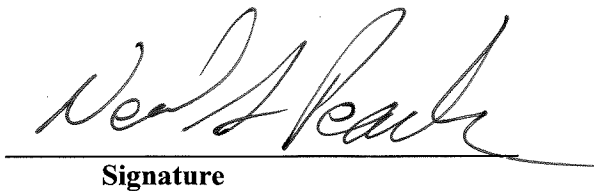
**Research & Development Committee Chair:**

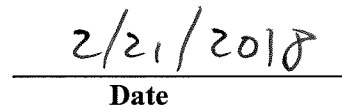
  
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**Associate Chief of Staff/Research**

  
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**LOUIS STOKES CLEVELAND VA MEDICAL CENTER**  
**Medical Research Service**  
**Standard Operating Policy**

Effective Date: February 1, 2018  
SOP Title: Use of the Raman Microscope  
SOP Number: SRS--022  
SOP Version: .04

1. **PURPOSE:** To establish a general protocol for the operation of the Raman microscope system located in Research, Room K109.

2. **POLICY:** The Raman microscope is a complicated experimental instrument that should be operated by experienced users in a safe manner.

3. **DEFINITIONS:**

a. Raman-last name of the physicist who discovered the optical effect that bears his name; a form of vibrational spectroscopy wherein light is scattered from a sample leaving the sample in a vibrationally excited state. The vibrational states are measured to yield structural information about samples.

b. Kr-Krypton, an inert gas that is found in the Kr ion laser.

c. Class IV-OSHA designation of laser power.

d. CCD-charge coupled device; type of camera that is used to detect the Raman signal with the microscope.

e. PPE-personal protective equipment

4. **RESPONSIBILITIES:**

a. **INVESTIGATOR/SUPERVISOR**

i. Informs the Research Safety Coordinator whenever a new employee starts work in the Raman laboratory.

ii. Provides new employees with training on safe working practices, policies and procedures appropriate for the Raman laboratory. Maintains documentation of this training.

iii. Performs all other duties of Investigator/Supervisor as outlined in SOP Number SRS-001 v. 04 "Medical Research Safety Program", June 11, 2008.

b. **EMPLOYEE**

i. Performs all other duties of EMPLOYEE as outlined in SOP Number SRS-001 v. 04 "Medical Research Safety Program", June 11, 2008.

ii. Adheres to the Raman microscope usage protocol as outlined below:

In the event of an emergency, contact Dr. Skalweit at 1-440-562-0170 (pager), x 4682 or 216 502 5955 (mobile, home).

5. PROCEDURES: The Raman microscope and associated Kr ion Class IV laser (hereafter referred to as the “Raman system”) are used to obtain Raman vibrational spectra of small molecules, proteins and potentially live cells or tissue samples. Only trained personnel familiar with the operation of the Raman system should have access to and use the instrument. Other users can be present and participate in experiments using the Raman system only following general safety training and only under the direct supervision of a trained user. Following general safety training, Raman system users will be trained by Dr. Skalweit or a trained user designated by Dr. Skalweit. In addition, prior to use of the system, operators must undergo an eye examination (baseline health maintenance) and obtain a retinal photograph to document the appearance of the retina in the event of ocular exposure to the laser.

Note: Prior to use, certification and maintenance (per the manufacturer) must be followed. If the unit has been dormant, refer to the manufacturer’s instructions before operating unit.

The Raman system, located in the inner room of K109, is comprised of the following equipment:

- 1) Coherent 70C Class IV Kr ion laser
- 2) Laser power supply
- 3) Heat exchanger
- 4) Raman microscope
- 5) Raman monochromator and CCD camera
- 6) Personal computer
- 7) Assorted optics
- 8) Laser interlock system
- 9) Personal protective equipment (PPE) consisting of goggles, lab coat, disposable gloves for preparation and handling of samples

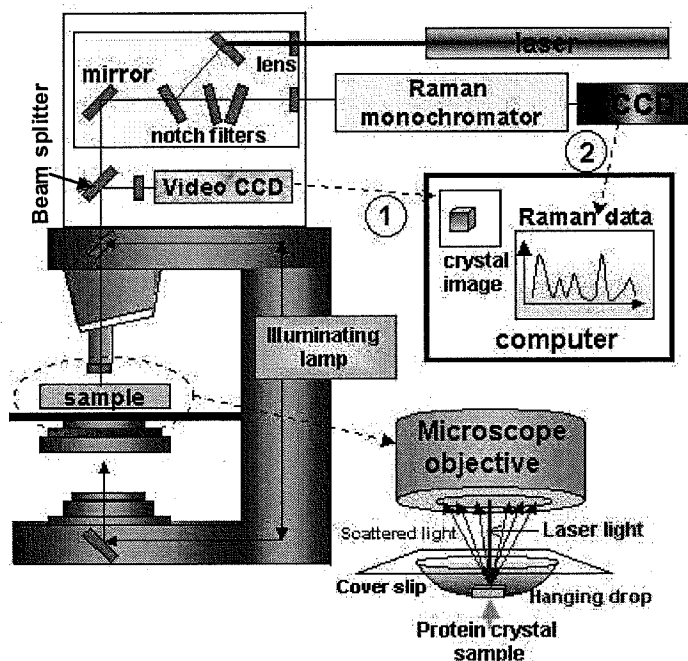


Figure 1: Raman microscope

General protocol for use of the Raman system-entering and exiting the room

- 1) Trained users don PPE prior to entering inner room of K109.

- 2) If the Green “laser safe” LED is illuminated solid, the system is safe and the interlock is ready to arm. Goggles are required once the system is armed.
- 3) If the yellow “Laser On” is flashing, the room interlock is set, the laser is running and goggles are required on entering the room.
- 4) If the Green “laser safe” LED is flashing, this indicates that the system has been crashed deliberately by a user and should be reset prior to use
- 5) If the Green “laser safe” LED is off, this indicates that the system has lost power and this must be reestablished prior to use.
- 6) If the yellow “Laser On” light is flashing, the user must enter the primary entry code to momentarily defeat the interlock, allowing entry of authorized users into the room without disrupting data acquisition or turning off the laser.
- 7) If the system is not armed prior to entry, the user should close the inner room door, don goggles and push the “Push to Arm” button to activate the interlock system
- 8) To disarm the laser interlock under normal circumstances, the user pushes the “Push to Disarm” button.
- 9) To disarm the laser interlock emergently (in the case of a fire or other emergency in the laser room), the red “emergency crash module” can be activated. This button must be turned 1/8 turn clockwise to reset the system once the emergency is resolved.

#### General protocol for use of the Raman system—turning on the Kr ion laser

- 1) Open valves to the house water supply to the heat exchanger
- 2) Throw the circuit breaker to the on position
- 3) Turn laser power supply key to the on position
- 4) Press the start button on the laser controller module—there will be a 60 second delay before laser emission is noted
- 5) Check flow and temperature of the house supply n.b.; this drains to a utility sink in the basement of the research wing in engineering space via a green garden hose. Also, check that the circulating bath pump is running to supply water to the laser head.
- 6) Set the desired laser power using the laser controller module, measuring power at the microscope objective.

#### General protocol for use of the Raman system—turning on the Raman microscope

- 1) Switch microscope light to on position
- 2) Open the Holograms application on the PC
- 3) Open the video camera application on the PC
- 4) Open the GRAMS/AI data analysis application on the PC
- 5) Turn on video camera
- 6) Turn off room lights
- 7) Calibrate wavelength and intensity of the system using the Ne lamp

#### General protocol for use of the Raman system—data acquisition

- 1) Measure the laser power at the sample
- 2) Place appropriate sample on microscope stage
- 3) Visualize sample using the video camera
- 4) Focus on the area of sample from where spectra are to be obtained
- 5) Illuminate sample with laser radiation
- 6) Turn off room lights
- 7) Initiate data collection using Holograms

#### General protocol for use of the Raman system—system shut down

- 1) Turn off laser power at controller module
- 2) Turn off power supply with key
- 3) Allow circulating bath/heat exchanger to run for 10 minutes

- 4) Remove all samples from the laser table
- 5) Discard trash
- 6) Wipe surfaces with 70% EtOH solution
- 7) Once circulating bath has shut itself off (when laser is cooled), throw circuit breaker to off position and turn off the house cooling water
- 8) Push "Push to Disarm" button to shut off the laser interlock (can also do this following step 2).

General protocol for use of the Raman system-emergency shut down

- 1) Press red emergency Crash Module button
- 2) In the event of fire, there is a fire extinguisher located just outside the door of the inner room where the laser is housed. Apply to base of fire as appropriate.
- 3) Assess other emergencies and respond accordingly.
- 4) To reset system later if appropriate, turn red button 1/8 turn clockwise.

6. REFERENCES: VHA Handbook 1200.08; Manufacturer Instructions of Use

7. RECISSION: Medical Research Service SOP SRS--22 dated December 10, 2008. The rescission date of this SOP is February 1, 2021.

8. FOLLOW-UP RSC/CHO