

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

Effective Date: APRIL 5, 2018

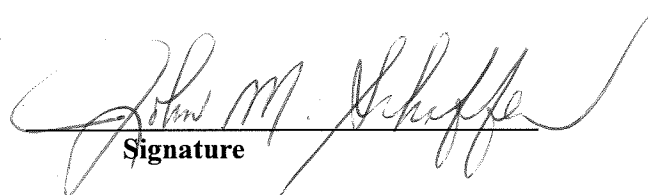
SOP Title: USE OF THE CONFOCAL MICROSCOPE (ZEISS PASCAL)

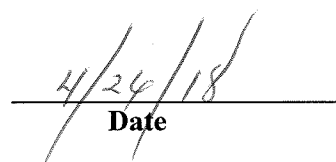
SOP Number: SRS--023

SOP Version: .04

Author:

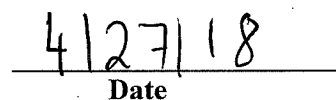
Name: John M. Schaffer, B.A.
Title: Research Safety Coordinator
Department: Medical Research Service


Signature


Date

Research & Development Committee Chair:

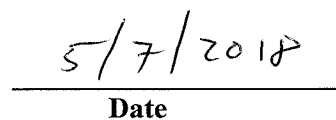

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Approved By:

Associate Chief of Staff/Research


Signature


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1. **PURPOSE:** To establish protocol for the safe use and operation of the Confocal Scanning Microscope located in Research, Room K205A.

2. **POLICY:** The Confocal Scanning Microscope operates with the use of class 3 B laser. This instrument must be operated only by trained users.

3. **DEFINITIONS:**

a. **Confocal Microscopy** – Confocal microscopy offers several advantages over conventional optical microscopy, including controllable depth of field, the elimination of image degrading out-of-focus information, and the ability to collect serial optical sections from thick specimens. The key to the confocal approach is the use of spatial filtering to eliminate out-of-focus light or flare in specimens that are thicker than the plane of focus.

b. **HeNe1 and HeNe2 – Helium-neon lasers**, usually called HeNe lasers, are a type of small gas laser.

c. **Argon Laser** – A laser with ionized argon as the active medium.

4. **RESPONSIBILITIES:**

a. **INVESTIGATOR/SUPERVISOR**

i. Provides new employees with training on safe working practices, policies and procedures appropriate for the safe use and operation of the Confocal Scanning microscope and maintains documentation of this training.

ii. Performs all other duties of Investigator/Supervisor as outlined in SOP Number SRS-001 Medical Research Safety Program.

b. **EMPLOYEE**

i. Performs all other duties of EMPLOYEE as outlined in SOP Number SRS-001 Medical Research Safety Program.

ii. Adheres to the Confocal Scanning Microscope usage protocol as outlined below in Section 5, Procedures.

5. PROCEDURES: The Confocal Scanning Microscope is operated with a class 3 B laser. This moderate-risk class includes medium-power lasers. You must take care not to expose yourself to the radiation of such lasers. *Never look into the beam of a laser!*

If used properly, the Confocal Scanning Microscope will not pose any laser radiation risks for operating staff. The dangerous laser radiation area is limited to the beam path, which is approximately 10 cm from the specimen. Warning and information labels must be observed. Operators must observe the following:

- a. Do not place any reflecting objects into the beam path.
- b. Do not open any covers or paneling.
- c. Do not look at the laser beam to view specimens. Damage to the eye may occur.
- d. Do not leave any screw positions of the nosepiece uncovered.

Suitable protective measures must be taken if gases, dust or vapors hazardous to health, secondary radiation or explosive objects should arise on the specimen resulting from laser radiation.

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.

General protocol for use of the Confocal Scanning Microscope:

- a. Turn on room fan to cool off instruments.
- b. Turn on Arc Lamp (Mercury lamp for fluorescent microscopy):
-Green switch under microscope.
- c. Turn on power strip located on table leg.
- d. Turn on HeNe lasers by turning (2) keys from vertical to horizontal position.
- e. Turn on Argon laser power source (black box next to HeNe lasers):
-Press button 1st, then turn key.
-Light inside laser box will turn from orange to purple when is has warmed up. After the light turns purple, flip the silver toggle switch UP*.

*When the confocal microscope is not in use for a prolonged period, turn off (down) toggle switch

- f. Turn on computer (when asked for password, leave line blank and press "OK")
- g. Use of Confocal Software:
 - i. Double-click PASCAL for confocal.
 - ii. Click ONLINE mode.
 - iii. Click START.
 - iv. Make sure the ACQUIRE button is pressed on top row of toolbar.
 - v. Open 3 windows: 1. MICRO 2. CONFIG 3. SCAN

MICRO box lets you control things on the microscope such as the objective lens, the reflectors you need to use for fluorescence and the shutter---In order to see the specimen through the microscope, the VIS button must be selected on the upper toolbar---

CONFIG lets you configure the settings (e.g. laser power, dichroic mirrors, emission filters, etc.) for confocal image acquisition.

SCAN is used to control the laser scanning microscope. Here is where you choose pixel depth, scan time, z stacks, etc. The LSM button must be selected in the upper toolbar.

- h. To turn microscope OFF:
 - i. Clean objective lens and move to open position.
 - ii. Turn toggle switch off (down).
 - iii. Turn off key next to toggle switch.
 - iv. AFTER THE FAN GOES OFF, turn off power on black box.
 - v. Turn off 2 keys on HeNe laser box.
 - vi. Turn off/shut down computer
 - vii. Turn off power strip
 - viii. Turn off Arc lamp under microscope

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

7. RECISSION: Medical Research Service Policy SRS--23 dated May 7, 2009. The rescission date of this Policy is April 1, 2021.

8. FOLLOW-UP RSC/CHO