LCAM info, laser safety & lab rules

Van Leeuwenhoek Centre for Advanced Microscopy
Swammerdam Institute for Life Sciences
University of Amsterdam

Van Leeuwenhoek Centre for Advanced Microscopy (LCAM)

LCAM: Formal collaboration since 2011 between three microscopy centres in the Amsterdam region

Section of Molecular Cytology, SILS, University of Amsterdam
Department of Cell Biology and Histology, Academic Medical Center
Division of Cell Biology, Netherlands Cancer Institute

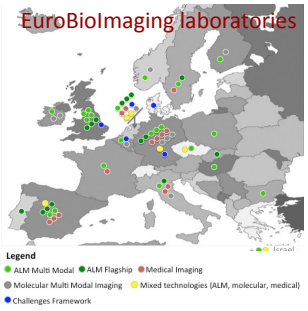
LCAM: Eurobioimaging laboratory

NL-BioImaging AM and Euro-BioImaging (EuBI) is an extensive research infrastructure that provides open access, services and training to a broad range of state-of-the-art biological and medical imaging technologies.

NL-BIOIMAGING AM EURO-BIOIMAGING

Currently 29 EuBI Node laboratories offer high quality microscopy services to life scientists in Europe and beyond, in a coordinated and harmonized environment, hosted by top research institutes across Europe.

EuroBioImaging laboratories

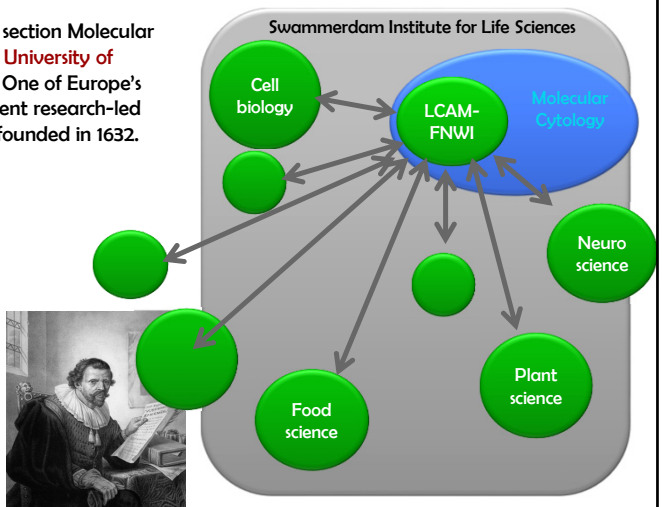


29 EuBI Nodes

www.eurobioimaging.eu

van Leeuwenhoek Centre for Microscopy (LCAM)

Housed in the section Molecular Cytology, University of Amsterdam: One of Europe's most prominent research-led universities, founded in 1632.



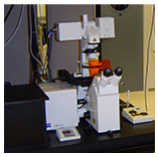
The Swammerdam Institute for Life Sciences (SILS) is the largest institute of the Faculty of Science.

The institute comprises biological disciplines including molecular and cell biology, microbiology, plant science, physiology and neurobiology, supported by modern enabling technologies for the life sciences.

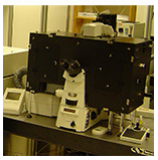
LCAM facilities

Over 15 microscope setups for fluorescence imaging & spectroscopy

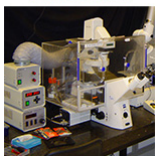
Zeiss LSM510 confocal microscope



Nikon A1-R confocal microscope

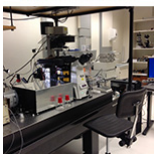


Zeiss Cell Observer widefield mic.



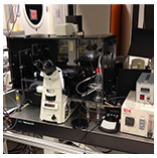
Leica stereo macroscope

Nikon SIM/RCM super-resolution

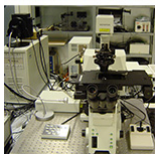


FD-FLIM widefield mic.

Lambert/Nikon FLIM screener



Olympus/Picoquant confocal microscope



more info (microscopes, manuals, contact persons, etc.):
www.lcam-fnwi.nl

LCAM-FNWI staff

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LCAM-FNWI director
room 2.266
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LCAM director

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LCAM-FNWI manager,
New users & ICT

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Laser-safety

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Data analysis

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Technician
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Cell culture

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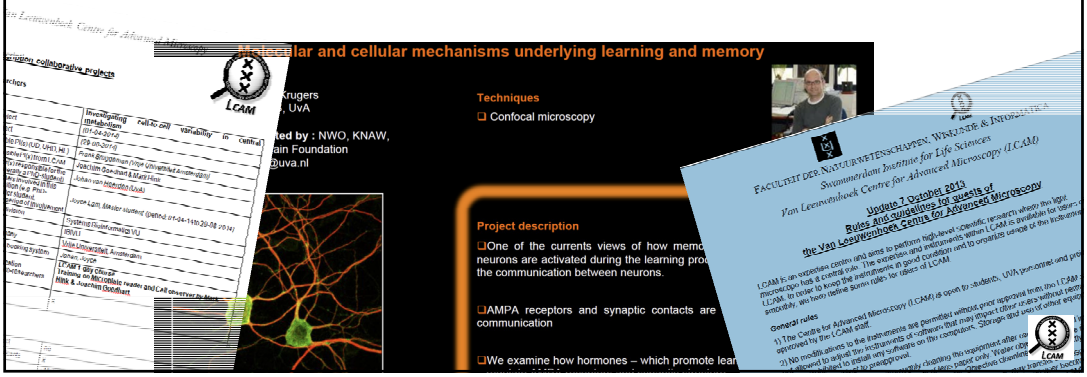
GMO's

New LCAM users

LCAM is not a facility; it is an expertise centre.
This implies that the LCAM-staff works together with the guests of LCAM based on a scientific collaboration.

Researchers interested should contact Mark Hink for more information



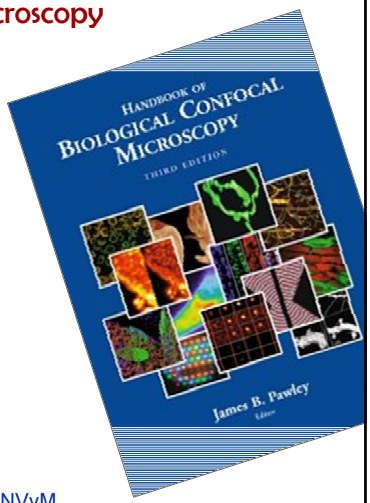
Education: Learn more about microscopy

Microscopy websites

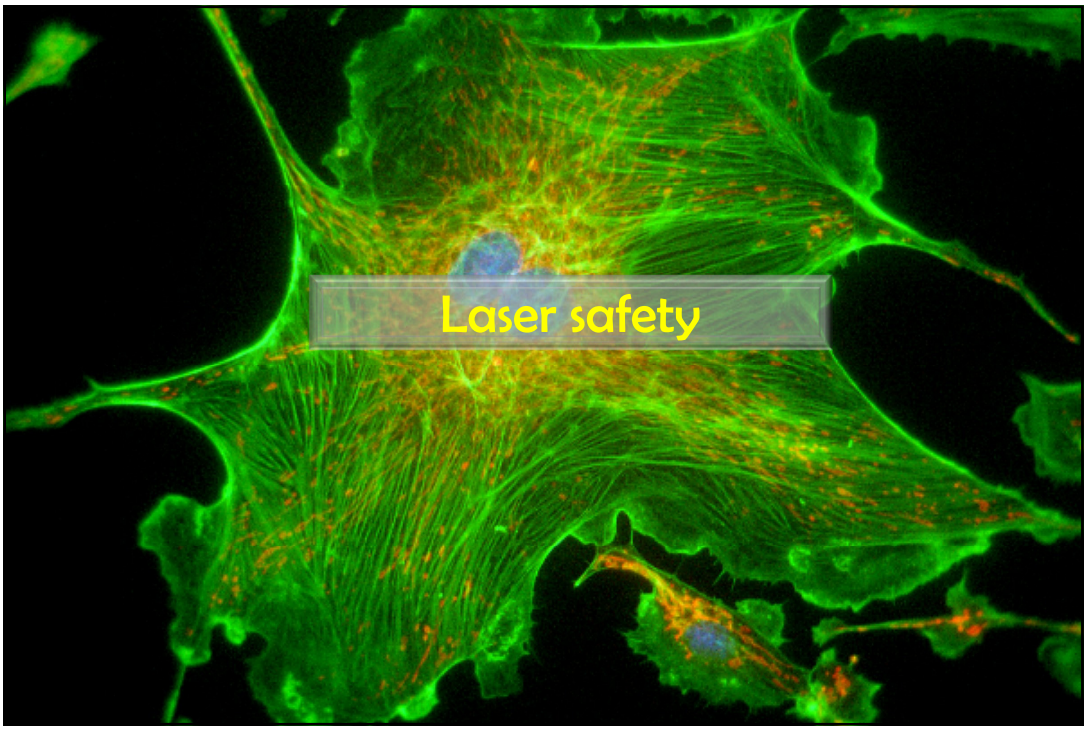
- MyScope online microscopy course
- Nikon microscopy education website
- Zeiss microscopy education website
- Olympus microscopy education website
- Invitrogen spectra viewer
- SIP chart analysis
- ImageJ

Organisations (incl. course overviews)

- NVvM (Dutch Microscopy Society)
- EuroBioImaging
- ELMI (European Light Microscopy Initiative)
- EMS (European Microscopy Society)



NVvM
elmi
EURO-BIOIMAGING
European Microscopy Society



Laser Safety Instructions

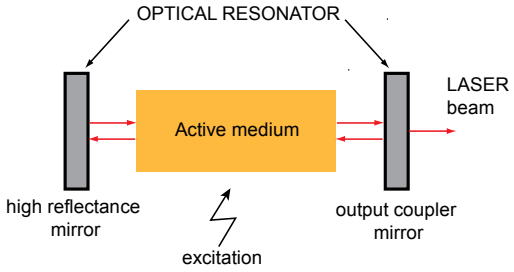
updated: September 2016

EURO-BIOIMAGING
Node Candidate

van Leeuwenhoek Centre for Advanced Microscopy
LCAM

Laser Components

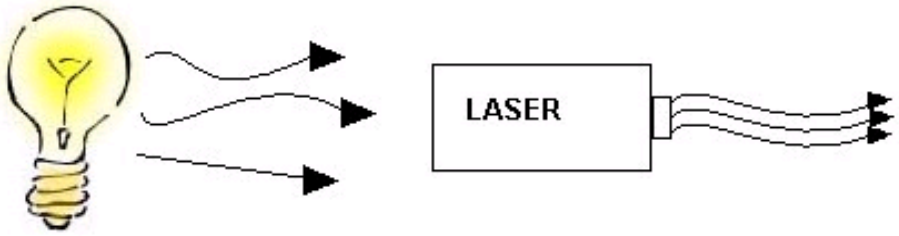
- Light Amplification by Stimulated Emission of Radiation



Associated hazards:

1. Laser Beam: eye injury, burns, skin cancer (UV), fire hazard
2. Excitation source: high voltage, water cooling

Ordinary Light vs. Laser Light



1. Many wavelengths
2. Multidirectional
3. Incoherent

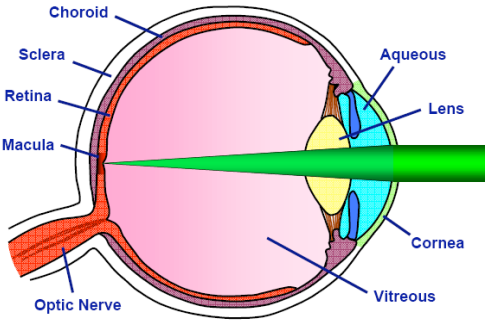
1. Monochromatic
2. Directional
3. Coherent

These three properties of laser light are what can make it more hazardous than ordinary light.

Laser light can deposit a lot of energy within a small area.

Human Eye

- laser beam can be focused by cornea and the lens to a very tight spot on the retina



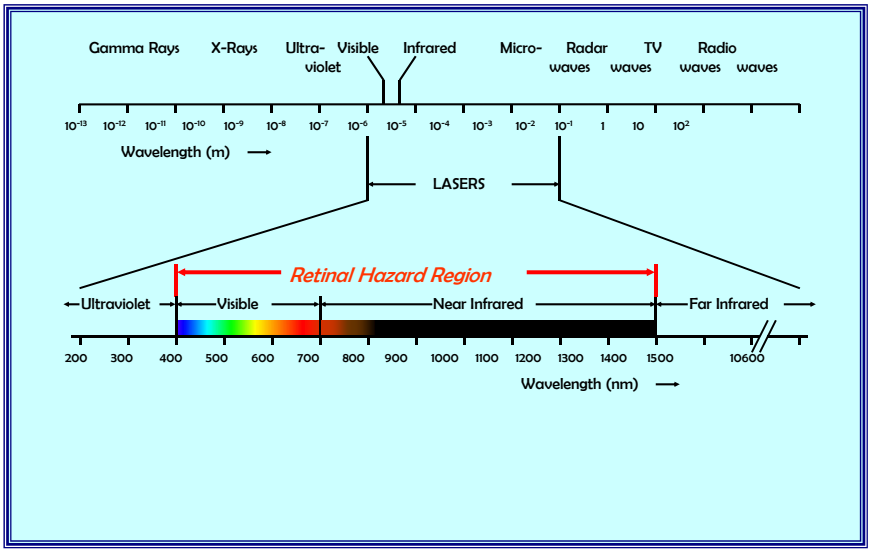
400-1400 nm

Retinal damage

300-400 nm

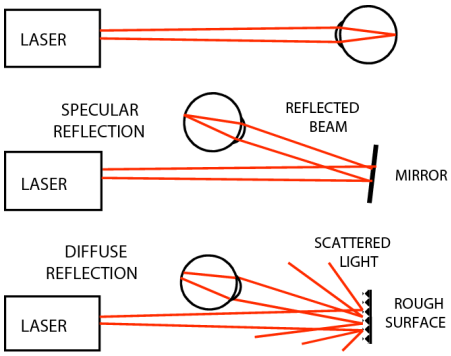
- Most hazardous: visible light – NIR. Eye is designed to focus visible light; focal spot can be 10E6 times greater than intensity impinging on the iris
- Hazardous: Near UV. Presents internal damage hazard, but not to retina
- Least hazardous: mid-infrared & middle UV:
  - do not penetrate. do not present retinal hazard
  - UV light can do photochemical damage to cornea, skin

LASER SPECTRUM



Laser-Professionals.com

Types of laser eye exposure



- Diffuse reflections (matter surface) from a high powered laser (Class 3B) can result in an eye injury.
- An observation distance of more than 13 cm for a max. time of 10s is considered as safe. Specular reflections (mirror) can be just as dangerous as direct exposures.

Laser hazard classes

- Classification by wavelength and output power, according to their ability to produce damage

Class	Power	Remarks	Typical examples
I	Very low or beam completely enclosed	•Inherently safe, •No possibility of exposure	CD, DVD drives, laser printers…
II	1 mW Visible only	•Staring into the beam is hazardous •Eye protected by aversion response	Supermarket laser scanners, some pointers
IIIa	1-5 mW	•Aversion may not be adequate	Laser pointers
IIIb	5-500 mW	•Direct exposure is a hazard	**Diode laser** **HeNe laser** **Ar lasers**
IV	>500 mW	•Exposure to direct beam and scattered light is eye and skin hazard •Fire hazard	**Pump laser 2photon** **High power Diode /DPSS* lasers**

*DPSS: Diode pumped solid state laser

Laser classes in the LCAm lab

Class 3B: Visible and near-IR lasers are very dangerous to the eye.

- This laser classification will cause injury upon direct viewing of the beam and specular reflections, but is usually not a fire hazard, or diffuse viewing hazard unless done under conditions of intentional staring within the diffuse hazard distance.
- Eye-wear is required for all Class 3B unenclosed laser use.

Class 4: Hazardous to eye and skin from direct viewing and diffuse reflection

- This laser or laser system is a hazard to the eye and skin under any viewing conditions, if viewing directly, specularly or within the diffuse reflection safety distance.
- Eye-wear is required for all Class 4 unenclosed laser use.

Best practices

Reliable protection depends on both protective equipment, and safe lab practices:

- rigorously avoid procedures that might result in direct exposure
- always think before doing, when aligning laser/optical systems
- keep the room lights on (smaller eye iris lets-through less light, focus is larger)
- avoid situations where the beam is, or might be deflected upwards
- avoid "eye level" beams
- exercise caution when leaning down to beam-level
- always look away from table area when bending-down
- think twice before leaning to table level to get a better look at your experiment
- use always the lowest possible laser class for the whole experiment
- do not use or bring flammable material near the possible beam paths!

Responsibilities

Your responsibilities

- Read laser safety signs at microscopes and watch the *Laser on* signs near the microscopy rooms.
- Wear protective equipment as appropriate but always follow safe lab practices. Note: Using a standard microscope means working with laser class 1 equipment. Eye wear is then not necessary.
- Never undertake any actions with lasers without asking permission first
- Follow the operating procedures as laid down by the LCAM lab personnel (manuals at microscope pages of www.lcam-fnwi.nl)
- Do not eat, drink, or use tobacco products in the laboratory
- Keep laboratory doors closed

Laser Safety Officer

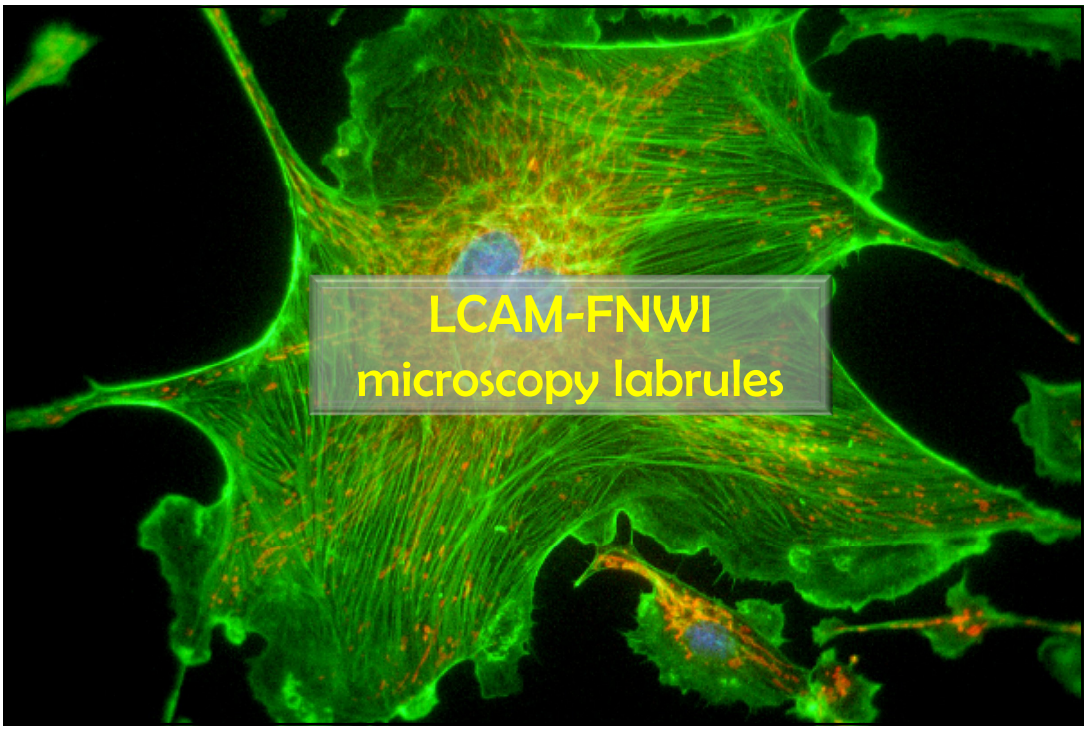
In case of questions ask:

Ronald Breedijk
LCAM laser safety officer
+7860
r.m.p.breedijk@uva.nl

other LCAM personnel
cam.microscopy@gmail.com

EURO-BIOIMAGING
Node Candidate

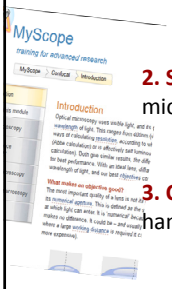
LCAM



New users

1. Intake discussion. Collaborative project will be discussed with LCAM staff.
Contact: Mark Hink

Dr. ir. Mark Hink
assistant professor
room 2.264
tel: +31 (0)20-525 6211



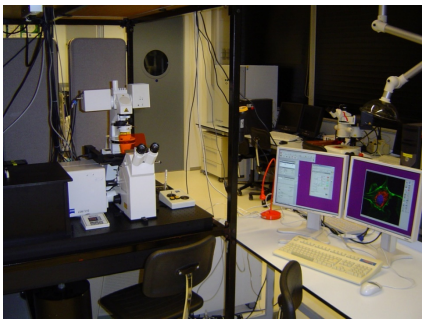
2. Self-study course microscopy. For non-experienced users that will carry out the microscopy experiments.

3. Confocal training day. Lectures and practical to understand basic principles and handling of a confocal microscope. Including laser safety training.

4. Microscopy exam. Multiple choice test to make sure that the user does have enough knowledge to handle the 300-1000 kEuro microscope equipment.

New users

5. Microscope training session. Together with the microscope contact person the user will get a personal training at the specific microscope that will be used during the project. After this session the user is able to work at the microscope itself.
Note: New users should not be trained by LCAM users themselves



Microscope contact persons
(Check website to find appropriate person for each mic)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MSc. Anna Chertkova
Technician
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Dr. ir. Mark Hink
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Dr. ir. Joachim Goedhart
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Prof. dr. Dorus Gadella
LCAM-FNWI director
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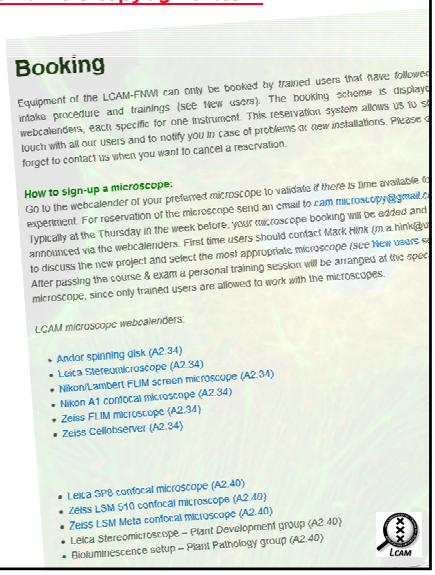
Dr. Marten Postma
assistant professor
room 2.267
tel: +31 (0)20-525 5189

Booking

- The equipment has to be reserved in advance via email: cam.microscopy@gmail.com
- No reservation more than 1.5 weeks in advance
- The schedule is made at Thursday afternoon, the week before and can be viewed at: www.lcam-fnwi.nl/booking/ For last-minute adjustments contact Ronald Breedijk personally.


Ing. Ronald Breedijk
technician
room 2.267
tel: +31 (0)20-525 7860

- The staff can break into the schedule. (i.e. maintenance, emergency adjustments)
- Microscope use after working hours must be booked. Not allowed for master- or bachelor-students.


Booking

Equipment of the LCAM-FNWI can only be booked by trained users that have followed
intake procedure and trainings (see New users). The booking scheme is displayed
webcalenders, each specific for one instrument. This reservation system allows us to st
touch with all our users and to notify you in case of problems or new installations. Please
forget to contact us when you want to cancel a reservation.

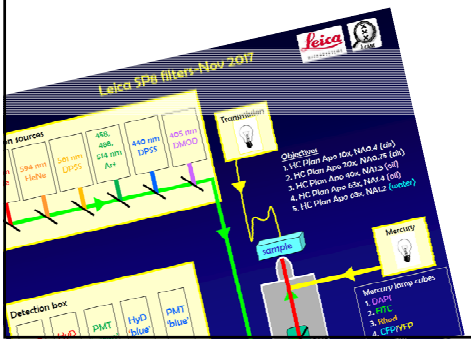
How to sign-up a microscope:
Go to the webcalender of your preferred microscope to validate if there is time available fo
experiment. For reservation of the microscope send an email to cam.microscopy@gmail.c
Typically at the Thursday in the week before, your microscope booking will be added and
announced via the webcalenders. First time users should contact Mark Hink (m.a.hink@u
to discuss the new project and select the most appropriate microscope (see New users s
After passing the course & exam a personal training session will be arranged at the spec
microscope, since only trained users are allowed to work with the microscopes.

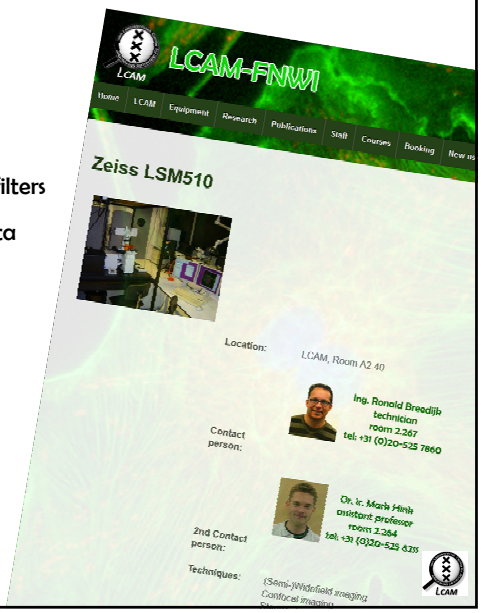
LCAM microscope webcalenders:

- Andor spinning disk (A2.34)
- Leica Stereomicroscope (A2.34)
- Nikon/Lambert FLIM screen microscope (A2.34)
- Nikon A1 confocal microscope (A2.34)
- Zeiss FLIM microscope (A2.34)
- Zeiss Cellobserver (A2.34)
- Leica SP8 confocal microscope (A2.40)
- Zeiss LSM 510 confocal microscope (A2.40)
- Zeiss LSM Meta confocal microscope (A2.40)
- Leica Stereomicroscope – Plant Development group (A2.40)
- Bioluminescence setup – Plant Pathology group (A2.40)

Microscope info

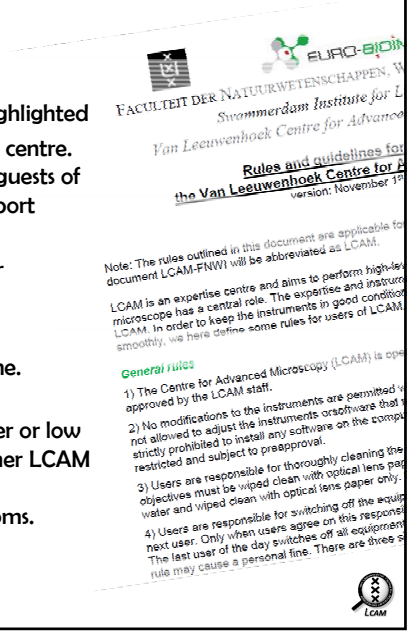
- Information about the microscope can be found at www.lcam-fnwi.nl/facilities
- You'll find here:
  1. The two contact persons for each microscope
  2. Description of available objectives and lasers
  3. Sheet with all dichroic mirrors and detection filters
  4. Startup and turn-off manual
  5. RDM file for correct management of your data





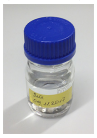
General rules

- All users accept and sign the LCAM user rules
  downloadable at www.lcam-fnwi.nl
  In this ppt only a part of these regulations will be highlighted
- Please remind that LCAM is not a facility but an expertise centre. This implies that the LCAM-staff works together with the guests of LCAM based on a scientific collaboration: Not to only support users.
- Don't make any modifications to the instrument (hard- or software)
- Clean the equipment after each session.
- Switch off the equipment after use. In case one forgets: Fine.
- Immediately report instrument damages or apparent misfunctioning (i.e. blinking mercury lamp, low laser power or low detection signals) to the microscope contact persons or other LCAM staff.
- No food and beverages are allowed in the microscope rooms.



How to handle objectives

- At the start: If you find the microscope not cleaned notify the contact persons (or staff)
- Immersion: Use correct type (air, water, glycerol, silicon or oil)
  water = MilliQ (not tap or distilled water)



Use correct brand (Leica, Nikon, Olympus or Zeiss) NEVER EXCHANGE!!!


Sometimes: Select immersion for correct temperature

- When moving the stage check first if the objective is not too high
  (otherwise objective damage -> 6.000 – 13.000 Euro)
- When changing sample -> First press *Escape* to park objective to lowest position

Clean objectives

- Quality of images highly depends of cleanness of the objective lens
- Remove immersion with lens paper (not tissues or paperwraps!!)
- If required clean with ethanol
- If still dirty ask the microscope contact persons to use more stringent cleaning solutions
- Have a close look at the objective if it's really clean


GMO's and cell culture options

- All LCAM microscopy labs have the ML1 status. In principle labcoats should be worn in the labs.
- For ML1 work fill out the GMO-nr in the intake form. GMO issues can be handled by the biological safety officer Joachim Goedhart.

- Live cells, media, sample containers and gloves must be discarded in the yellow biohazard containers.
- Clean the equipment and lab/computer tables at the end of the experiment.
- Living cells (ML1 level) can be maintained in 37°C-5% CO_2 incubators present in the LCAM labs. For more info contact Anna Chertkova. Remove cell containers at the end of your measurement session.

MSc. Anna Chertkova
Technician
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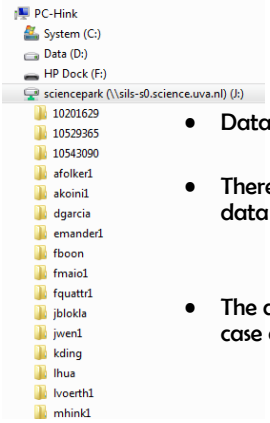
tel: +31 (0)20-525 8366



Data Storage & RDM

- Data CAN NOT be stored at the microscope PC's for longer than a day.
- Therefore each LCAM user will be allocated 200 GB storage space at the data server *SILS-S0* that can be accessed using UvANETID
- The dataserver can be accessed at the microscopes and office computers. In case of malfunction directly contact Mark Hink

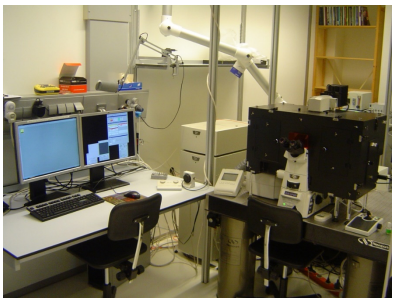
Dr. ir. Mark Hink
assistant professor
room 2.264
tel: +31 (0)20-525 6211

- There is no active backing up of the SILS-S0 server. We strongly recommend not to rely exclusively on the server for data safekeeping! Remember that users are responsible for their own data.
- For optimal Research Data Management conditions LCAM can provide users a recommended digital RDM logbook for each microscope. For more info contact Marten Postma

Dr. Marten Postma
assistant professor
room 2.267
tel: +31 (0)20-525 5189

After using the microscope

- Remove the sample safely (ESC mode of objective to lower it)
- Clean objective with lens tissue (and ethanol if needed)
- Clean microscope surrounding (oil stains, lens paper, etc.)
- Discard GMO's in a proper way and remove cells from the 37°C incubator
- Turn off the microscope as mentioned in the LCAM microscope manual. Check if other users will use the system afterwards -> don't turn off lasers and mercury lamp if the other user will start within 30 min.



Data analysis and publications

- As discussed during intake: LCAM might be able to help you with data analysis. For more info contact Marten Postma.

Dr. Marten Postma
assistant professor
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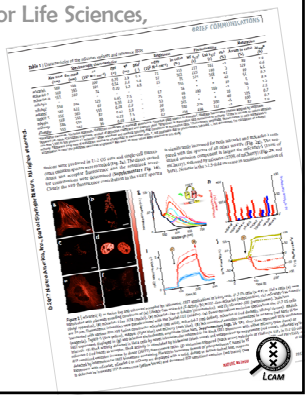
- When data from LCAM microscopes is used in publications LCAM should appropriately be acknowledged and should be cited as: "Van Leeuwenhoek Centre for Advanced Microscopy, Section Molecular Cytology, Swammerdam Institute for Life Sciences, University of Amsterdam".



- The LCAM staff wants to receive a pdf-file of these publications.
- We ask all LCAM users to supply a powerpoint slide to advertise your microscopy project and results at the promo-screen in the corridor of Sciencepark.