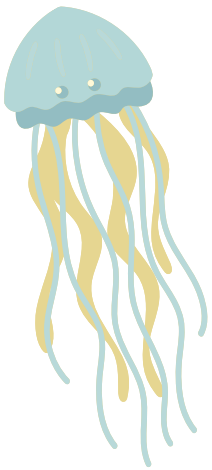
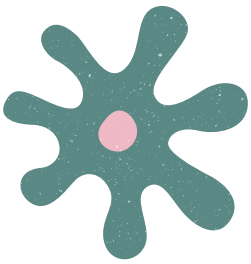
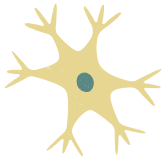
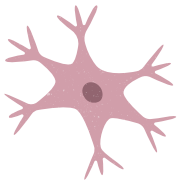
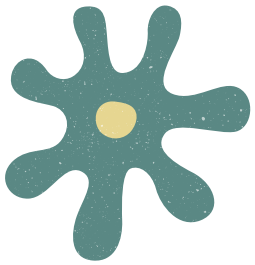




# CCMAR
# BIO-IMAGING






T Correia v2023.03.20

# About us

CCMAR BIO-IMAGING

**Training, assistance and services in Bio-Imaging**

## TEAM

**Teresa Matias Correia**
Coordinator & Group Leader

**Carina Mónico**
Microscopy Service Specialist

**Rute Castelo Félix**
Team Member | Sample Preparation

**Sandra Silva**
Team Member | Clearing

## CONTACT

**ccmarimaging@ualg.pt**

# How can we help?

## CONSULTATION

Meet us to discuss your needs and optimal bio-imaging approach.


## TEST

(if necessary)
book a test with us on a specific microscope.

## LARGE PROJECT

## TRAINING

Mandatory training session to learn how to operate the microscope.

## SMALL PROJECT


## WE DO IT

Only need a few images? Book the microscope and we do the acquisition for you. No training required.

## ASSISTED USE

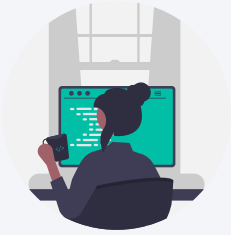
Get hands-on experience with our assistance. The training is finished when the researcher can operate the microscope safely and correctly.

## INDEPENDENT USER

Book and use the microscope with regular user privileges. We are available for additional training or assistance.

## PROCESSING & ANALYSIS

We can show you how to open and convert raw data to generic file formats, basic process & analysis procedures. We can also help you prepare a few images for a publication.



## COLLABORATION

In you need, for example, custom-building hardware or software, novel (or uncommon/untested) protocols for sample preparation, image acquisition, processing and/or analysis, we can discuss a scientific collaboration.

# Equipment

## LSFM FACILITY

### ZEISS LIGHTSHEET Z.1

3D Fluorescence Microscope

### WORKSTATION

Image Processing and Analysis

## BIOTERIUM FACILITY

### ZEISS AXIO ZOOM.V16

Large-field Stereo Fluorescence Microscope

### ZEISS AXIOSCOPE 5

Upright Fluorescence Microscope

## CELL CULTURE FACILITY

### ZEISS PRIMOVERT

Inverted Microscope with a Colour Camera

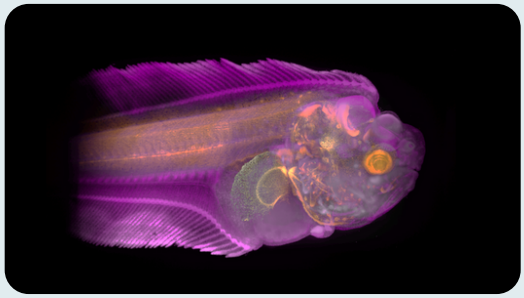
### ZEISS AXIO VERT.A1

Inverted Fluorescence Microscope

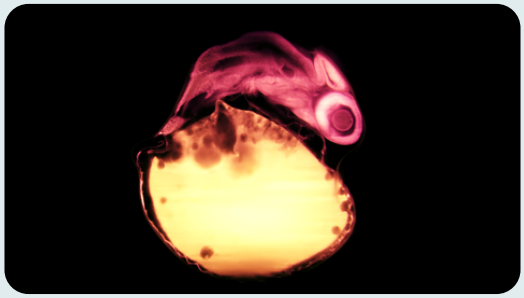
# Applications

## ZEISS LIGHTSHEET Z.1

3D imaging of whole living or cleared samples.



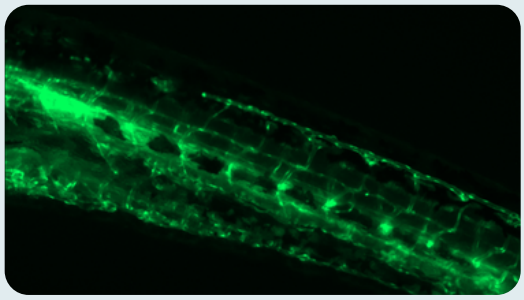
Senegalese sole (flatfish)



Trout embryo

## ZEISS AXIO ZOOM.V16

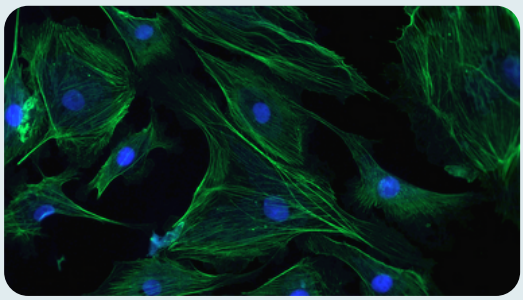
Observing, preparing, sorting and microsampling (dissecting, stimulating, manipulating) samples.



Zebrafish larvae blood vessels expressing fli1a:GFP

## ZEISS AXIOSCOPE 5

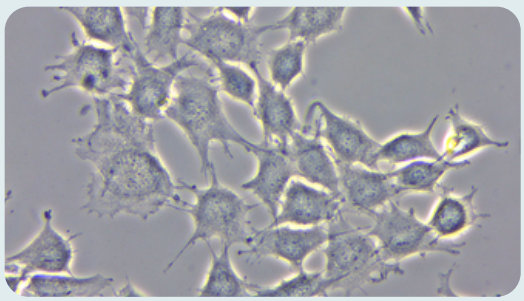
Viewing samples with multiple fluorescent labels at high magnification (up to 100x).



BPAE cells stained to show nuclei (blue) and actin (green)

## ZEISS PRIMOVERT

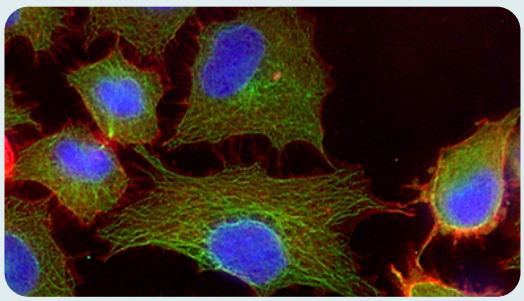
Cell culture routine work. Examine cell morphology and viability assays in brightfield and phase contrast.



Micrasterias radiata (algae), brightfield contrast

## ZEISS AXIO VERT.A1

Investigate cell cultures using brightfield, phase contrast and multicolour fluorescence.



A549 cancer cells stained to show nuclei (blue), alpha-tubulin (green) and actin (red)

# Zeiss Lightsheet Z.1

## Building 8 | Lab 1.16

The Zeiss Lightsheet Z.1 is a lightsheet fluorescence microscope that enables fast volumetric imaging of large cleared samples (up to 1cm x 1cm x 2cm) or live transparent samples with low photobleaching and low phototoxicity.

### LASER LINES

**488** nm 50mW
**561** nm 50mW
**638** nm 75mW

### OBJECTIVES | ILLUMINATION

**5x**/0.1
**10x**/0.2

### OBJECTIVES | DETECTION

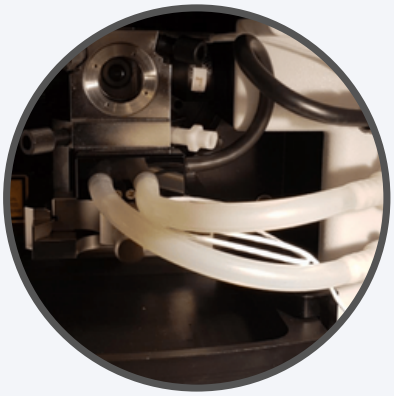
**5x**/0.16 EC Plan-NEOFLUAR
**10x**/0.5 W Plan-APO
**20x**/1.0 W Plan-APO DIC Corr UV-VIS-IR nd=1.33
**20x**/1.0 Clr Plan-APO Corr VIS-IR nd=1.38

### FILTERS | CAMERA 1 & CAMERA 2

**GFP** | 505-545 nm & **Cy3** | 575-615nm
**GFP** | 505-545 nm & **mCherry** | 585nm
**YFP** | 525-565 nm & **Cy3** | 575-615nm
**Cy3** | 575-615 nm & **DRAQ5** | LP 660 nm

### CAMERAS

2 PCO.edge sCMOS Cameras | 30 fps | pixel 6.5 µm



### CHAMBERS AND INCUBATION

Aqueous media (n=1.33)
Clearing media (n=1.38/20x, n=1.45/5x)
Incubation and temperature control options

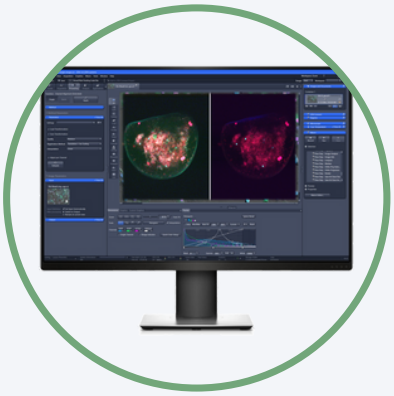
### ACQUISITION DESKTOP ZEN 2014

**CPU** 2x Intel XEON CPU E5-2623 @3.0GHz
**RAM** 64 GB
**Storage** 4x 2TB | 4TB RAID 10
**GPU** NIVIDIA Quadro K2000 4 GB
**OS** Windows 7

# Zeiss Lightsheet Z.1 Workstation

## Building 8 | Lab 1.16

Handling, processing and visualisation of lighsheet microscopy data is extremely computer intensive and requires the use of a high performance computer. The Zeiss Lightsheet Z.1 Win 7 workstation is specifically configured for storing and handling large experimental dataset obtained with the Zeiss Lightsheet Z.1 microscope.



## WORKSTATION

**CPU** Intel XEON CPU E5-2620 @2.4GHz
**RAM** 192GB
**Storage** 6x HDD 8TB | RAID 5 config 36TB
**GPU** NIVIDIA Quadro K2000 4 GB
**OS** Windows 7

## SOFTWARE

Zeiss Zen 2014
Arivis vision4D
Fiji/ImageJ

## WARNING

Save your data in D:\SWAP*your name* and not in the C:\ drive.

# Zeiss Axio Zoom.V16

## Building 9 | Lab 0.08

The Zeiss Axio Zoom.V16 is a motorised fluorescence stereo zoom microscope that combines a large field of view, zoom and working distance. It is suitable for applications in which observation and careful handling of specimens exceeds the need for greater magnification. Applications include microinjections, dissections, movement or separation of small organisms, and more.


## FEATURES

**16x motorised zoom** with high NA
Eyepieces 10x
Magnifications from 7x to 253x
**Imaging modalities** | brightfield, darkfield, oblique light, Epi-fluorescence
Acquisition of multi-channel images and **Z-stack**

## ILLUMINATION

LED transmitted light for brightfield
Metal halide fluorescence lamp with 3 **fluorescence** excitation lines: UV (385) | Blue (475 ) | Green (555)


## OBJECTIVES

**1x**/0.25 Plan-NEOFLUAR (WD 56mm)
**2.3x**/0.57 Plan-NEOFLUAR (WD 10.6mm)

## FILTERS BLUE, GREEN, RED

**49 DAPI** | Ex 365 | BS 365 | EM BP 445/50
**38HE GFP** | Ex BP 470/40 | BS 495 | EM BP 525/50
**63 mRFP** | Ex BP 572/25 | BS 590 | EM BP 629/62


## CAMERA

**Axiocam 305** mono | 2.8 MP CMOS | 36 fps | pixel 4.54 µm

## ACQUISITION DESKTOP ZEN 3.1 PRO

**CPU** Intel i7
**RAM** 8 GB
**OS** Windows 10

# Zeiss Axioscope 5

## Building 9 | Lab 0.08

The Zeiss Axioscope 5 is a manual upright microscope with transmitted white light LED and fluorescence LED illumination Colibri 7. The Zeiss Axioscope 5 has a wide selection of objectives, including a 100x oil lens for images with increased level of detail and resolution. It is a standalone microscope, which can acquire images without the need of a computer.

## FEATURES

**Imaging modalities** | brightfield and epi-fluorescence
Eyepieces 10x


## ILLUMINATION

Transmitted light LED 10W
Colibri 7 with 5 **fluorescence** excitation lines: UV (385) | Blue (475 ) | Green (555) | Yellow (590) | Red (630)

## OBJECTIVES

**10x**/0.25 A-Plan
**20x**/0.45 A-Plan
**40x**/0.65 A-Plan
**100x**/1.25 A-Plan oil immersion


## FILTERS BLUE, GREEN, RED

**96 HE DAPI** | Ex 390/40 | BS 420 | EM BP 450/40
**38 HE GFP** | Ex BP 470/40 | BS 495 | EM BP 525/50
**45 HQ mCherry** | Ex BP 560/40 | BS 585 | EM BP 630/75


## CAMERA

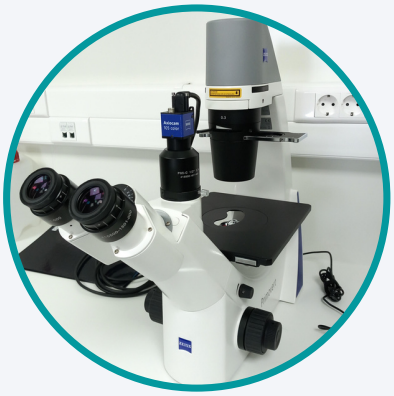
**Axiocam 202 mono** | 2 MP CMOS | pixel 5.86 µm

## ACQUISITION DESKTOP ZEN 3.1 PRO

**CPU** Intel i7
**RAM** 8 GB
**OS** Windows 10

# Zeiss Primovert

## Building 7 | Lab 3.38



The Zeiss Primovert microscope is a manual inverted microscope equipped with the Axiocam 208 colour camera, designed for cell culture routine work.


## FEATURES

**Imaging modalities** | brightfield and phase contrast
Eyepieces 10x

## ILLUMINATION

Transmitted light HAL 30W

## OBJECTIVES

**4x**/0.10 Plan-Achromat Ph0
**10x**/0.25 Plan-Achromat Ph1
**20x**/0.30 Plan-Achromat Ph1 LD
**40x**/0.50 Plan-Achromat Ph1 LD


## PHASE RINGS

Ph0 and Ph1

## CAMERA

**Axiocam 105 colour**| 5 MP CMOS | pixel 2.2 µm

## BRING YOUR LAPTOP OR TABLET

Download Zen Lite available for free from the Zeiss Portal

# Zeiss Axio Vert.A1

## Building 7 | Lab 3.38

The Zeiss Axio Vert.A1 is a manual inverted microscope suitable for brightfield, phase contrast and epi-fluorescence to investigate cell cultures.

## FEATURES

**Imaging modalities** | brightfield, phase contrast, and epi-fluorescence
Eyepieces 10x
**Sample holders**: suitable for multiwell plates, flasks, Petri dishes of 95 mm, microscope slides 76x26 mm, Ibidi chambers µ-SlideTM and Lab-TekTM II

## ILLUMINATION

Transmitted light LED
Colibri 7 with 5 **fluorescence** excitation lines: UV (385) | Blue (475 ) | Green (555) | Yellow (590) | Red (630)

## OBJECTIVES

**5x**/0.15 LD A-Plan
**10x**/0.25 LD A-Plan
**20x**/0.35 LD A-Plan
**40x**/0.55 LD A-Plan


## FILTERS BLUE, GREEN, RED

**96 HE DAPI** | Ex 390/40 | BS 420 | EM BP 450/40
**38 HE GFP** | Ex BP 470/40 | BS 495 | EM BP 525/50
**45 HQ mCherry** | Ex BP 560/40 | BS 585 | EM BP 630/75


## CAMERA

**Axiocam 202 mono** | 2 MP CMOS | pixel 5.86 µm

## ACQUISITION DESKTOP ZEN 3.1 PRO

**CPU** Intel i7
**RAM** 8 GB
**OS** Windows 10

# How to access our equipment?

1

**Email** us at **ccmarimaging@ualg.pt** to schedule a consultation meeting to discuss your needs, experimental strategies and select the most adequate microscope.

Depending on your needs, we may acquire (a few) images for you or train you to become an independent user

2

Once you become an **independent user**, you will have access to the **online calendar** and will be able to **book** CCMAR's Bio-Imaging microscopes.

**Note**: "Assisted use" sessions need to take place within 1 week of the training session. If an independent user does not use a microscope for more than 6 months or misuses the microscope, we will contact the user to assess if a refresher session is required.

# How to store and transfer data?

Save your data in E:\Users\your name (or D:\SWAP*your name*).
**Never save data in the C:\ drive**.
Use **cloud** services to transfer data (e.g. Dropbox , Google drive, OneDrive).
**Do not use external hard drives** to transfer your data.
Note: Files older than 1 month will be removed from the local storage.

# Usage Fees

**SPECIAL OFFER**

**TRY FOR FREE**

*until 31/05/2023*

| Instrument charge rates per hour from 1st of June 2023 | | | |
|---|---|---|---|
| **Imaging System** | **CCMAR Internal Users** | **Academic visitors & non-profit organisations** | **Industry** |
| **Zeiss Lightsheet Z.1** | 10.00€ | 20.00€ | As per quote |
| **Zeiss Axio Zoom.V16** | 1.30€ | 2.60€ | 10.00€ |
| **Zeiss Axioscope 5** | 0.90€ | 1.80€ | 6.00€ |
| **Zeiss Axio Vert.A1** | 0.90€ | 1.80€ | 6.00€ |
| **Zeiss Primovert** | 0.70€ | 1.40€ | 4.00€ |

# Usage Fees

| CCMAR Bio-Imaging Services | | | |
|---|---|---|---|
| **Services** | **CCMAR Internal Users** | **Academic visitors & non-profit organisations** | **Industry** |
| **Project Consultation and Advice** | Free | | As per quote |
| **Training** | Free | Free but instrument time is charged | |
| **Testing Samples** | Free | Free but instrument time is charged | |
| **Sample Preparation** | As per quote collaboration can be considered | | |
| **Assisted Imaging** | As per quote collaboration can be considered | | |
| **Custom Image Analysis** | As per quote collaboration can be considered | | |
| **Writing Support \| Imaging Methods (publications)** | Free | | |
| **Writing Support \| Grant Applications (imaging sections)** | Free | | |
| **Quotations for grant applications (imaging projects)** | Free | | |
| **Advice concerning the purchase of microscopes and accessories** | Free | | |
| **Microscope Maintenance and Quality Checks** | As per quote | | |

# Resources

## STEP-BY-STEP INSTRUCTIONS

*Microscope manuals coming soon!*

## ZEISS MANUALS

*Microscope, sample preparation and image processing manuals*

Zeiss Lightsheet Z.1 sample preparation
Zeiss Lightsheet Z.1 advances in tissues clearing
Zeiss Axio Zoom.V16 info and manual
Zeiss Axioscope 5 info and manual

## ONLINE RESOURCES

*Educational websites and videos*

Zeiss webinars and how-to videos | Zeiss Microscopy Insights Hub
Zeiss Microscopy videos | Zeiss Microscopy YouTube Channel
Zeiss Lightsheet Z.1 Sample Preparation | Zeiss How-to Video
Zeiss Axioscope 5 Fluorescence Imaging | Zeiss How-to Video
Zeiss Axioscope 5 Transmitted Light Imaging | Zeiss How-to Video
Zeiss Education in Microscopy | Carl Zeiss Microscopy Online Campus
iBiology Microscopy Videos | iBiology Microscopy Series
Royal Microscopy Society Microscopy Videos | RMS Technical Tea Breaks

## BIO-IMAGING SOFTWARE

*Image visualization, processing and analysis software*

Zeiss software downloads | Zeiss Portal
Zeiss Image Viewer | Zeiss Zen Lite
Open Source Image Processing Tool | Fiji
Fluorescence Spectra Viewer | ThermoFisher
Fluorescent Protein Database | FPbase

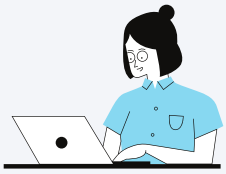
# Grants, Publications & Acknowledgments

## HELP WITH GRANT PROPOSALS

We can work with you on bio-imaging aspects of your grant application, help you write supporting statements and estimate microscopy access costs. We encourage you to discuss your planned work with us at the earliest opportunity.

## MATERIALS & METHODS IN PUBLICATIONS

We can help you writing the Materials and Methods sections regarding microscopy and image processing.



Example: "Microscopy experiments were performed at the Bio-Imaging Facility at CCMAR."
"Images were acquired on a Zeiss [Axio Zoom.V16, Lightsheet Z.1 , etc] microscope equipped with a Zeiss [Axiocam 202 mono CMOS, etc] camera and controlled with the Zeiss Zen [3.1 Pro Blue Edition, etc] software, using the [1x and 0.25NA, etc] objective and the fluorescence filter sets [GFP and mCherry, etc].
"The [image analysis software] was used to generate [maximum intensity projections, 3D renderings, etc] of the acquired dataset.

## ACKNOWLEDGMENTS IN PUBLICATIONS

When you use our equipment, please remember to acknowledge us and our funding sources in publications or other formal presentations. This is very important to guarantee future funding!

**Acknowledgments**: This study received Portuguese national funds from FCT - Foundation for Science and Technology through project UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020, and from the operational programmes CRESC Algarve 2020 and COMPETE 2020 through project EMBRC.PT ALG-01-0145-FEDER-022121. The authors would like to thank CCMAR Bio-Imaging for support & assistance in this work.

**Collaborations:** acknowledgments will be determined on a case by case basis.

CCMARIMAGING@UALG.PT