## Introduction

The Axio Observer 7 Microscope from Zeiss is an extremely versatile and multi-functional instrument. With a myriad of available fast-switching excitation wavelengths, multiple objectives and many other features, this microscope can accommodate a wide variety of both live and fixed samples.

Due to the broad range of modalities and adaptive equipment, the Axio Observer 7 provides a flexible platform to optimize the perfect environment for high-resolution imaging.

### **Features**

### Seven 'Autocorr' objectives

- Objectives available at the SBC facility include 5, 10, 20, 40, 63 long working distance, 20X/0.8 Ph2 and 63X oil
- Correction ring adjustment to compensate for spherical aberrations, giving efficient and high contrast fluorescence detection.

### Adaptive compensatory stabilization

• *'Definite Focus.2'* technology compensates for changing conditions and drifts in focus

### Wide Range of excitation wavelengths

- Seven fast-switching 'Colibri 7' LED lamps (red, yellow, green, cyan, blue, violet, UV) for flexible dye analysis
- Fast-switching excitation filter wheel including DAPI, TRITC, CFP, GFP, mCherry, DsRED, Texas Red, Fura-2 calcium imaging, Cy3/Cy5, CFP/YFP FRET and other wavelengths

### Axiocam 702 mono CMOS Camera

 Low light sensitivity with low read noise and high speed burst-mode function (up to 128 fps at full resolution)

#### Peltier-controlled stage

• 4°C to 95°C for isothermal and temperature gradient experiments

#### Hypoxia Chamber

• Humidity, temperature, CO<sub>2</sub> and O<sub>2</sub> controls



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SickKids The hospital for SICK CHILDREN

Produced by James Magnus Jorgensen

## The Hospital for Sick Children's

## Structural and Biophysical Core Facility



## ZEISS Axio Observer 7 Microscope

Bright Field and Fluorescence Inverted Microscope with Peltier Controlled Thermal Stage

- Multimodal imaging of living and fixed specimens and other samples types
- Quantification and qualification of immunofluorescence labelled samples
- Evaluate morphology and growth rate of cells or tissue samples
- Evaluate transfection rate and stability of live cell cultures
- Particle tracking analysis and optical sectioning deconvolution software

# Axio Observer 7 Microscope

## **Theoretical Background**

An inverted microscope has the objective pointing upwards from below the stage, with the sample being illuminated from above.

The Axio Observer 7 can be used for bright field (BF), differential interference contrast (DIC), and fluorescence microscopy.

### Bright Field Microscopy

- Considered the traditional method, the specimen can be stained and the entire field of view is illuminated.
- Used when low contrast is sufficient and for specimens where the observed colour does not need to be the actual colour.



### Differential Interference Contrast Microscopy

• DIC is used in observing live and/or transparent specimens. Some regions of the specimen will give differing refractive indices and therefore differing gradients in the optical path length, which become translated into varying levels of contrast. The resulting image appears shadow cast but is pseudo-three dimensional (not the actual topological structure)

### Fluorescence Microscopy

 The specimen must be fluorescent itself, or else is fluorescently labelled or dyed. A light source emits excitation wavelength(s) which are absorbed by fluorophores in the specimen. A dichroic mirror isolates the re-emitted wavelength for detection.

## Peltier Plate and Definite Focus.2

A noteworthy feature of the Axio Observer 7 is the Peltier plate, used for thermoelectric heating and cooling. This allows for the cultivation of temperaturedependent cells or tissues, and control over temperature-dependent processes.

The '*Definite Focus2*' stabilization technology compensates for many changes in conditions, including temperature changes induced by the Peltier system. The expansion or contraction induced by alterations in temperature can cause focus drift, and the '*Definite Focus.2*' adapts and accounts for this, ensuring maximum reliability.

### Fast-Switching Emission Wavelength Technology

The Axio Observer 7 has both the flexible *Colibri* 7 fluorescent LED illumination source, and the fast switching excitation filter wheel. The *Colibri* 7 provides seven excitation wavelengths from red light to UV, the intensity of which can be individually adjusted quickly and precisely to observe fast processes in live specimens.



The Colibri 7 utilizes narrow-band LED excitation to reduce any cross-stimulation, and has very little UV leakage to prevent bleaching & maintain specimen viability.

### Software

The intuitive software interface makes it easy to make customized systems. Particle tracking analysis and optical sectioning deconvolution software is also available to access. Integrate Python scripts and external image analysis applications easily into experiments. The Fast-switching double filter wheel gives a selection of emission wavelengths and dichroic mirrors, allowing for flexible combinations of wavelengths.



## 'Autocorr' Objectives

The new generation of lenses are designed to provide the high numerical aperture needed to image subcellular structures, while also avoiding spherical aberrations. This allows for crisp contrast and more efficient fluorescence detection.



## Axiocam 702 monos CMOS Camera

A 2.3 Megapixel camera, ideal for live cell imaging and low light applications. Up to 128 frames per second at full resolution, for fast and sensitive fluorescent imaging and time-lapse video for longterm observation.