# Automated, handsfree water immersion

### Autoimmersion Module for ZEISS Axio Observer 7

Reliable Data Acquisition from Start to Finish



Seeing beyond

zeiss.com/autoimmersion

## **Automated Application of Immersion Water**

Minimize Your Risk & Improve Your Efficiency

Immersion media between the sample and the objective is required for high-resolution imaging. This can be a challenge for some experiments using water as immersion media. With automated multi-position data acquisition, one application of immersion media might not be sufficient as the sample moves to different locations. With live sample experiments, immersion water can evaporate over long periods. Manual addition of immersion media risks loss of data points or even microscope damage from user error; it is also tedious and inefficient. The Autoimmersion Module for ZEISS Axio Observer 7 widefield and confocal systems is your automated, easy-to-use solution for maintaining immersion media for water immersion objectives.



### How Does It Work

The Autoimmersion Module pumps water between the sample and objective via a feed pipette attached to a universal holder.

### **Minimize Your Risk**

Fully controlled by ZEISS ZEN software and the ZEISS Axio Observer 7 touch screen, application of water for immersion media is completely hands-free, minimizing potential risks to your experiment, such as disturbing your specimen or losing your plane of focus, and protecting your microscope from any mishaps.

#### **Increase Your Efficiency & Throughput**

For longer, complex experiments that require multiple applications of immersion media over many hours of experiment time, you can now increase both your efficiency and throughput. During daytime hours, you no longer need to hover near the microscope to reapply immersion media. You can dedicate your time and focus to other projects while the Autoimmersion Module maintains the immersion media. Increase your throughput even further by setting up data collection for overnight.

### **Supported Sample Carriers:**

- Slides
- Petri dishes
- Multi-chamber slides
- Multi-well plates

#### **Suggested Applications:**

- Highly automated, multi-position data acquisition of samples in slides, dishes or multi-well plates
- Extended time series of live cells, organoids and developing organisms
- Accurate three-dimensional imaging of aqueous samples
- Hands-free, error-free objective changes from large field-of-view, air objectives to high resolution, water immersion objectives

### **Easy Installation & Intuitive Operation**

Hands-Free Control for Minimized Risk



The feed pipette is located at the objective front lens for water immersion without the need to change focus or position.

### **Functionality without Compromises**

The ZEISS Autoimmersion Module is easy to install and compatible with many ZEISS water immersion objectives. No tools are needed to move components between objectives. The pipette can be switched from one objective to another within seconds. Installation of the module is a straightforward task even with difficult set-ups such as a microscope with a nontransparent XL incubator.

The ZEISS Autoimmersion Module will not compromise the capabilities of your ZEISS water objectives. It does not decrease the objective's working distance. Additionally, the transparent ring grants full visibility on the objective, allowing correction collar adjustments as needed.



The Autoimmersion Module is seamlessly integrated in ZEN imaging software.

#### **Reduce Risk to Your Experiment and Your Microscope**

The ZEISS Autoimmersion Module automates the application of immersion media for water immersion objectives. Water is pumped directly between the sample and the objective. A pipette attached to a universal holder guides the water directly to its destination. Only a few microliters are used per application and a built-in water protection system guarantees safe operation.

The immersion media is applied while maintaining objective focus and position, leaving your experiments undisturbed. This removes common user errors that occur during manual immersion media applications, such as selection of the incorrect immersion media and media spillage as well as collisions that might happen when trying to refocus the objective.



The Autoimmersion Module does not decrease working distance, nor does it block visibility or access to the correction collar.

### Full Software Integration for Simple & Intuitive Operation

The seamless integration of the Autoimmersion Module into ZEISS ZEN imaging software as well as the control touch screen for ZEISS Axio Observer 7 means simple and intuitive operation with minimal – even remote – user interactions. Apply immersion media on demand during manual or remote imaging experiments or at pre-programmed intervals during automated imaging experiments, all without touching the microscope.

If the immersion media from the water reservoir runs low, the integrated sensor will trigger a software alert to refill the tank. This alert is timed so that there will always be a sufficient supply to support lengthy experiments.

### Automated Immersion for Complex Experiments Improve Your Efficiency & Throughput

### Make Better Use of Your Time & the Off-working Hours

With the ZEISS Autoimmersion Module, you can design complex experiments for unsupervised data collection where previously you were committed to stay near the microscope to ensure there was always enough immersion media. This includes extended live cell imaging experiments and/or multiposition data acquisition. Dedicate your time to other projects while your microscope collects data autonomously. Set up imaging acquisition during non-working hours, knowing that the ZEISS Autoimmersion Module will enable reliable data collection through the end of your experiment.



Improve your throughput by up to 2.5-fold by designing experiments that collect data during non-working hours, such as overnight or over the weekend.



#### Live Cell Experiments over Extended Periods with Automated Immersion

HEK KO PEX5 cells expressing eGFP with a photocaged peroxisomal targeting signal type 1 were reconstituted with the peroxisomal import receptor PEX5. A light induced conformational change of the photocage leads to exposure of the peroxisomal targeting signal. If the WT PEX5 is expressed, accumulation of the eGFP signal in the dotted peroxisomes can be monitored (top row). In case of the mutated PEX5 (bottom row), even after 18 hours, no peroxisomal import could be detected. Sample courtesy of K. Reglinski, Institute for Applied Optics and Biophysics, Friedrich-Schiller-Universität Jena, Germany.

### Multi-position, Extended Time Lapse Experiment with Automated Immersion



When working with living samples, you might not know where your event of interest may occur. To capture the uptake of nanoparticles by macrophages, many locations from a multi-well plate are acquired as well as multiple z-planes over several hours at 37°C using re-immersion. The region shown above is a subset of the much larger dataset that was captured using automated imaging and shows the uptake of nanoparticles inside the cells (top row). The surface of the cells were also imaged to verify that the nanoparticles are inside the cells and not simple sitting on the cell surface (bottom row). Sample courtesy: F. Páez Larios and C. Eggeling, Institute for Applied Optics and Biophysics, Friedrich-Schiller-Universität Jena, Germany.

### **Fast and Exact Application of Water Immersion** For Precise Measurements in Aqueous Samples

### The Importance of Water Immersion for Precision and Image Quality

Spherical aberrations occur when the refractive indices of the sample, surrounding medium and immersion medium differ. Most organisms have aqueous compartments with refractive indices close to water. Consequently, imaging with oil or air objectives can lead to spherical aberrations. A correct three-dimensional representation of the sample is of utmost importance when quantifying distances or volumes. While oil may provide some benefits to the user for long-term live cell experiments (little to no evaporation) or multi-position experiments (less need for reapplication of media), only imaging with water objectives and water immersion media will provide the most accurate measurements of your aqueous samples.



XZ projection of a giant unilamellar vesicle prepared from the lipid DOPC doped with an Atto647N-labeled lipid, imaged with a 40×/0.95 air objective (left), a 40×/1.2 water immersion objective (middle) and a 40×/1.4 oil immersion objective (riaht).

Sample courtesy of C. Haupt and K. Bacia, University of Halle, Germany

### Accurate Data Acquisition in Aqueous Samples

For researchers testing different sample conditions for a specimen, such as drug responses, or measuring a full binding isotherm by fluorescence correlation spectroscopy (FCS), using multi-well specimen holders and automated data collection can dramatically improve throughput and increase efficiency. However, acquiring accurate measurements is critical for these types of experiments.

The ZEISS Autoimmersion Module is both fast and exact, ensuring your data collection is accurate even when moving to multiple positions of a multi-well specimen. As shown on the right, researchers prepared wells with different concentrations of a fluorescently labeled protein and were able to accurately measure the binding curve to red fluorescent liposomes using fluorescence cross-correlation spectroscopy (FCCS).



Red fluorescent small liposomes and different concentrations of Sar1p protein (partially labeled with Alexa Fluor 488) were mixed in a 96 multi-well plate and measured automatically over 15 hours. Krüger et al., Biophys. J. 2017. Sample courtesy of C. Haupt and K. Bacia, University of Halle, Germany

### **ZEISS** Autoimmersion Module

Expand Your Possibilities



### **Microscope Systems**

- ZEISS Axio Observer 7 inverted widefield research microscopes
- ZEISS LSM 900/980 confocal laser scanning microscopes mounted on ZEISS Axio Observer 7 platforms
- Simple upgrade of existing ZEISS Axio Observer 7 widefield research microscopes and ZEISS Axio Observer 7 based confocal systems is possible on site.

### **Supported Water Immersion Objectives**

- C-Achroplan 32×/0.85 W Corr M27
- LD LCI Plan-Apochromat 25×/0.8 Imm Corr DIC M27
- LD LCI Plan-Apochromat 40×/1.2 Imm Corr DIC M27
- C-Apochromat 40×/1.2 W Corr M27
- C-Apochromat 40×/1.2 W Corr FCS M27
- C-Apochromat 100×/1.25 W Corr M27
- LD C-Apochromat 40×/1.1 W Corr M27
- LD C-Apochromat 63×/1.15 W Corr M27

Carl Zeiss Microscopy GmbH 07745 Jena, Germany microscopy@zeiss.com www.zeiss.com/autoimmersion