

# Compact Fluorescence Microscope

**BZ-X** Series

# No dark room required with the fully electronic fluorescence microscope

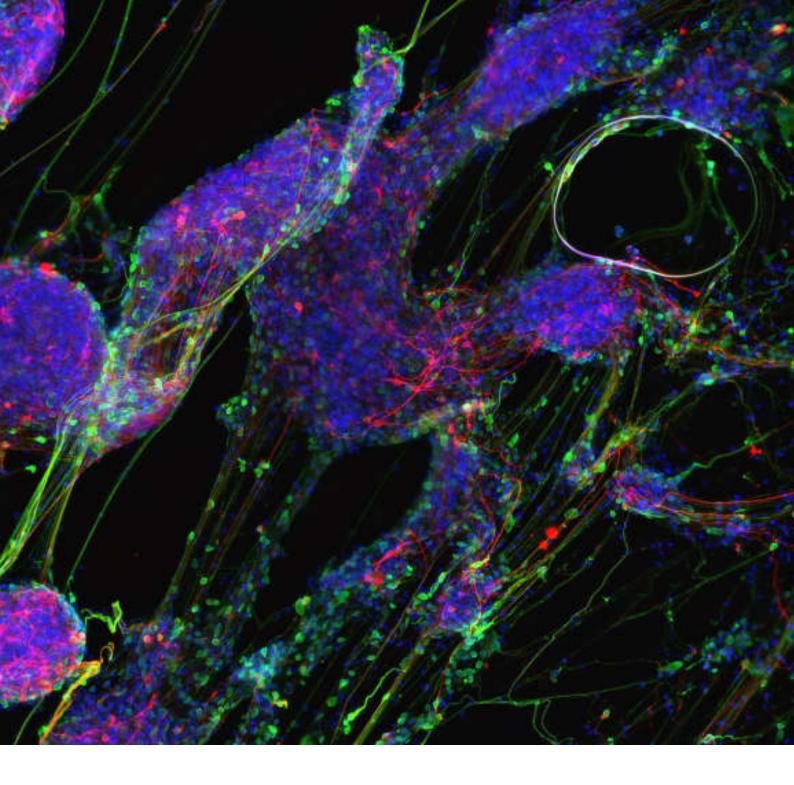
Scan the **QR code** to try the advanced functionality. Simple setup and easy operation for outstanding research results

# Built-in Darkroom and Space-saving Design

A sample container is built into the body of the microscope, allowing users to perform fluorescence imaging even in a brightly-lit room. Space-saving design means the unit can be set up in any location for optimal testing efficiency.

# Any User Can Easily Capture Images

Clear images can be easily captured. With a single click, any user can capture publication-quality images.

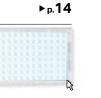


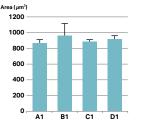
# Batch Analysis of Large Data Sets

Offers capture and analysis in short time frames. The image cytometer module reinforces testing reliability.

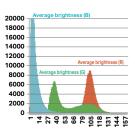
# Image cytometer module

Copying settings enables bulk observation and analysis of large data sets in one operation.





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All-in-One System

# Enhanced Core Performance

# No darkroom required

- High-contrast fluorescence imaging even in a brightly-lit room
- Enables an optimal working environment with space-saving design

#### Full electronic control

- All operations controlled within an easy-to-use software
- High-reproducibility and user independent imagingRemote controllable

# Publication-quality images

- Built-in high-sensitivity, highresolution cooled monochrome camera
- Supports clear fluorescence, brightfield and phase contrast observation over a range of samples





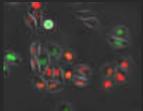
Expandable Design

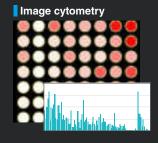
# Adaptable to Research Needs

#### Well scanning



Live cell imaging

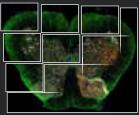




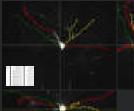
Video capturing



Image stitching



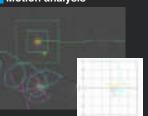
3D measurement and analysis



Optical sectioning

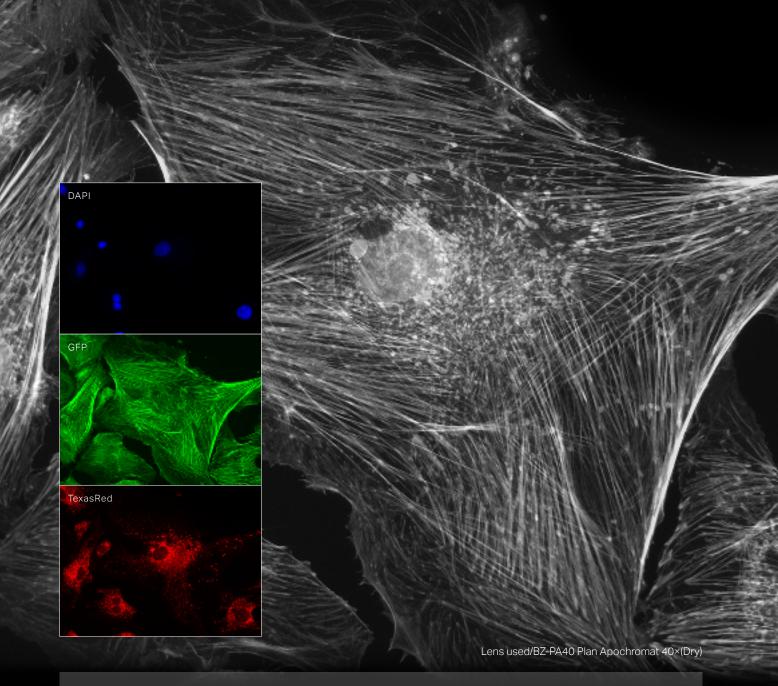


Motion analysis



Built-in high-sensitivity cooled monochrome camera and high-intensity LED light source

# Advanced Observation Delivers High-Resolution Images



#### **Cooled CCD Camera**

Even when a CCD Camera is not exposed to any light, dark current signals are generated and create unwanted noise in an image. This noise is largely temperature-dependent, increasing as a CCD gets warmer. The Peltier-cooled CCD in the BZ-X Series is cooled to 25°C below the ambient temperature to achieve high-sensitivity imaging with little noise.

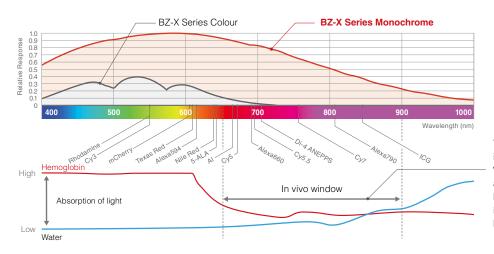
# Bright, High-definition Imaging

#### Camera with low noise and high sensitivity

The cooled monochrome camera provides clear images that combine high sensitivity and low noise. This enables clear fluorescence imaging even with low excitation light in order to minimise photobleaching and damaging of the cells, which are sensitive to phototoxicity.

#### High sensitivity across short and long wavelengths

The camera is also able to image dyes such as Cy7 in the near-infrared range, allowing for observation of cells located even in deep tissue layers. Additionally, it uses a high-intensity LED as the fluorescence light source for its broad wavelength range from UV through to IR. It supports a range of fluorescence pigments without adding a light source, simply by changing filters.



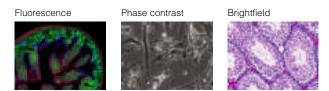
The 650 to 900 nm wavelength range is referred to as the "in vivo window." With low levels of autofluorescence and light scattering in this range, long-wavelength fluorescent dyes are ideal for visualising deep regions of living tissue.

# One-click switching between colour and monochrome camera

Switching between colour and monochrome imaging modes can be easily performed with just one click. An electronic liquid crystal filter enables high-definition imaging with superior colour reproducibility. This creates ideal conditions for brightfield applications such as H&E, DAB and similar dyes.

#### High versatility across various samples

The system supports fluorescence, brightfield and phase contrast imaging. Users can observe various specimens in different vessel types, enabling versatility across a wide range of experiments.

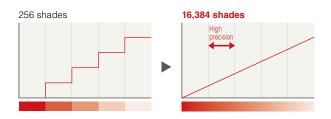


# Accurate Detection for Reliable Data

Unlike colour cameras, the CCD camera does not use colour filters. This eliminates variations in light quantities received on the CCD due to the fluorescence colour. This allows for accurate quantification of fluorescence intensity, which is important for evaluating properties such as protein weights.

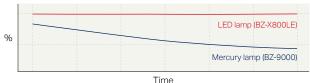
#### 14-bit high-level gradation

Data capturing with 16,384 gradations allows for accurate measurement of expression levels and precise quantification.



#### Stable light intensity over a long period

The BZ-X800LE has a fluorescence light source that incorporates a long-life LED lamp. Stable light intensity is secured both in the short term and in the long term, and quantitative comparison is possible even for data that was captured on different days.



Large motorised stage equipped to observe an entire well plate

# Easy Operation for Dramatically Improved Observation



#### Anti-vibration construction

The BZ-X Series uses a floating stage structure with anti-vibration dampers to stablise highprecision imaging. High magnification capture, image stitching and observation of cultured cells in liquid media can be performed anywhere, unaffected by vibration.

#### Variety of containers supported

Sample holders for slides, dishes, flasks, and multi-well plates are included. Sample holders for special containers are also available upon request.

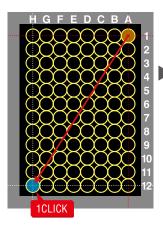




# Easy Navigation

#### Stage view

Users can simply click on each map image corresponding to the stage holder to easily access the location to view. Even with large well plates, users can find regions of interest quickly and easily.





The map and motorised stage are linked with high precision. The interlinked stage moves instantly to the clicked location.

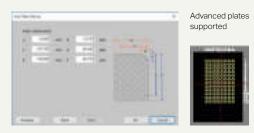


Plate customisation function

Create plate maps for use in experimental systems that use advanced plates, as well as for conventional plates in order to greatly improve the efficiency of daily observation work.

#### Point memo

Record coordinates of regions of interest. Clicking on a registered point easily moves to that point.



#### Six-mount electronic lens revolver

Both field of view and focus can be maintained even when changing magnification for easy observation.

Any combination Magnification 2x-100x Oil-immersion lenses

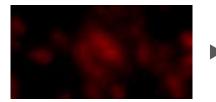
Dry lenses Phase contrast lenses

# Simple Focusing for All Users

#### High-speed auto focus

With a single click the user can instantly focus on any sample in fluorescence, brightfield or phase contrast at any magnification. Focusing is simple, regardless of magnification, not only for brightfield and phase contrast images, but also for fluorescence images observed in low light conditions. This enables the capture of clear images.

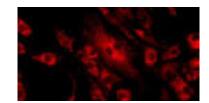
One-click auto focus



High-sensitivity partial scan for high-speed processing



Accurate focus



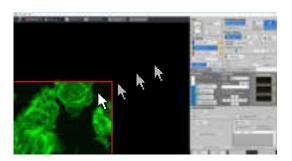
#### High-sensitivity partial scan

By combining the CCD's partial reading and binning processing, this mode enables the display of images with even higher sensitivity. Weak fluorescence signals normally require a long exposure time, but this mode makes it possible to read them at high speed for rapid focusing. The BZ-X Series uses a dedicated focus control motor for high-precision control in the Z axis, enabling accurate, user-independent auto focus.

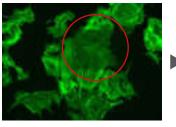
# High-Efficiency Imaging

#### Low photobleach mode

When changing the focus or field of view, the excitation light is only pulsed long enough to display an image. The excitation light is then turned off until another adjustment is made, minimising photobleaching and prolonging the life of the specimen.

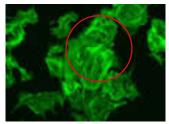


Without low photobleach mode



Photobleaching during high-magnification observation leads to sample damage with irregularities in brightness.

With low photobleach mode



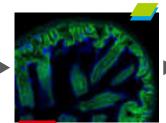
Photobleaching is minimised, resulting in uniform brightness.

#### Real-time overlay

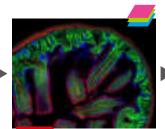
Capture settings such as focus and exposure time on an overlaid image can be viewed and adjusted prior to image capture. Usually a multi-channel overlay would need to be captured, adjusted and recaptured to obtain the desired result. The BZ-X Series saves time by providing a real-time overlay prior to image capture.



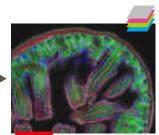
1CLICK CH1/DAPI image



1CLICK CH2/GFP image



1CLICK CH3/TexasRed image



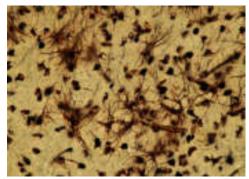
1CLICK CH4/Phase contrast image

#### Quick full focus

With a single click, the system automatically scans the height of the sample and creates a fully-focused composite image in real-time. This greatly reduces the time and effort required to interpret several partially-focused images of a thick target.

Rat brain, Golgi staining Sample courtesy of Dr. Seiji Otani, Cell Technology Laboratory





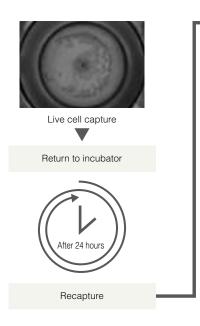
One-click, automatic scanning

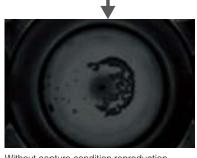
Fully-focused image

# Capture Condition Reproducibility

#### Load capture settings

Capture conditions such as the filter settings, magnification, exposure time and capture position can be read from previous images for easy reproducibility. Any user can capture images using the same conditions, eliminating variability between operators. This also allows for accurate observation of changes over time, with a higher degree of repeatability.

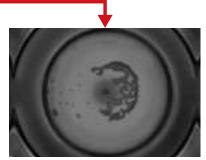




Without capture condition reproduction function

Manual location	Manual condition	Less accurate
search	reproduction	image capture

Users must keep track of what settings were used, such as the location coordinates and exposure time and then manually reproduce them in future captures. This takes time and decreases accuracy.



With capture condition reproduction function

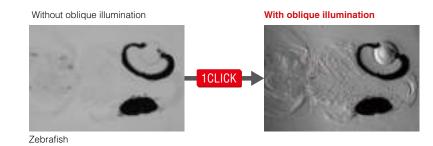


Every setting such as location coordinates, exposure time, imaging channel and magnification are automatically saved with each image. These conditions can then be loaded directly from an image, saving time and ensuring experiment accuracy.

# Enhance contrast of unstained transparent specimens

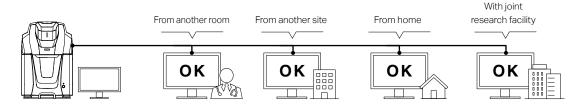
#### Oblique illumination

Observe images similar to those obtained by using differential interference contrast (DIC), but without any additional lenses, prisms or other hardware. Unlike DIC, this technique can be performed through plastic containers, making it suitable for observing ova and other clear specimens.



# Fully motorised to support remote control

All processes from imaging to analysis are performed with a single mouse, allowing remote control via a network. Observation and analysis can be performed while holding discussions with off-site joint research facilities. Among a host of possible uses, the microscope can be utilised in laboratories that cannot be accessed frequently due to a high biosafety level, and as a tool to reduce crowding in laboratories.



# Enhanced Observation and Analysis

## Expandable to support diverse applications while maintaining ease-of-use

The built-in configuration includes all of the hardware required for the optional modules. Upgrades are easy and fast for on-demand expandability. The software interface remains the same as modules are added, allowing users to easily operate the system after upgrading.

#### LED transmitted illumination

The long-lifetime LED has little to no change in colour temperature over time. This allows for accurate hue representation in brightfield, ideal for quantification.

# High-intensity LED excitation lighting

Strong lighting is provided over a wide wavelength range, from below 400 nm to above 700 nm, to support a range of fluorescent dyes simply by changing fluorescence filters. There is little fluctuation in light intensity over a long time, making this microscope perfect for quantitative evaluation using the strength of the fluorescence signal.

#### Large motorised XY stage

With a movable range of  $114 \times 80$  mm, an entire well plate can be imaged. The stage can be controlled down to 1 µm for high-precision scanning.

#### Electronic filter turret

#### Electronic projection element

The excitation light can be changed to a slit or a pinhole, enabling optical sectioning capture through a single operation. As opposed to a mechanical component, the electronic element enables high-speed and more customisable projection patterns.

#### Six-mount electronic lens revolver

Lenses are positioned to facilitate stable, continuous capture of various points and conditions. With phase contrast and oil-immersion lenses, applications ranging from cultured cells to sectioned tissue can be imaged on a single platform. The lens can be controlled down to a 0.1 µm step size in the Z axis for high-precision 3D analysis.

# Observation and Capture Modules

<b>BZ-H4XI</b> Image Cytometer Module Batch capture and analysis of large amounts of data including well plates.	▶ p.14	
<b>BZ-H4XD</b> <b>Advanced Observation Module</b> High-precision image stitching and Z-stacking for multilayer capture.	▶ p.16	
<b>BZ-H4XF Sectioning Module</b> Optical sectioning capture with structured illumination.	▶ p.20	
<b>BZ-H4XT Time-lapse Module</b> Automated capture at user-specified intervals for video and time-series measurements.	▶ p.24	No 7 8

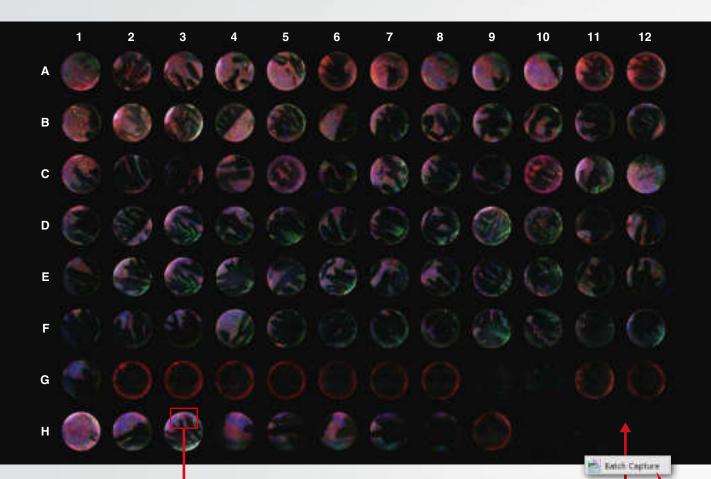
# Analysis Applications

<b>BZ-H4C/BZ-H4CM</b> <b>Hybrid and Macro Cell Count</b> KEYENCE's original algorithm enables accurate quantification of image data.	► p.26	Ratio 20.5%
BZ-H4R		NU
3D Application		
Creation of 3D images from Z-stack data. 3D measurement of localisation and configuration available.	▶ p.30	
ВZ-Н4К		
Motion Analysis Application		
Tracking of user-specified targets to measure travel range, speed and coordinate positions.	► p.32	
BZ-H4M		
Measurement Application		
Manual 2D measurements including area.	▶ p.33	and the state of the

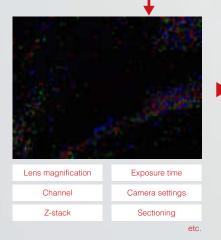
#### **Image Cytometer Module**

# High Throughput for Capture and Analysis

Capture settings in one location can instantly be applied to all fields of view on a well plate. Users can select any or all wells to be scanned with uniform conditions for high reproducibility of data. This work flow can be completed in just three simple steps. The system will then automatically execute the capture without any additional user configuration.

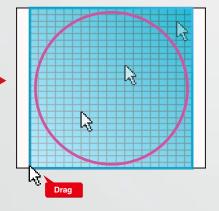


**STEP 1** Set capture conditions.

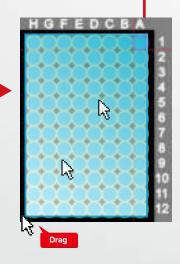


#### STEP 2

Click and drag to specify the range of capture within a well.



**STEP 3** Click and drag to specify wells to capture. 1CLICK





#### **Image Cytometer Analysis**

# Accurate, High-Content Analysis with High-Resolution Images

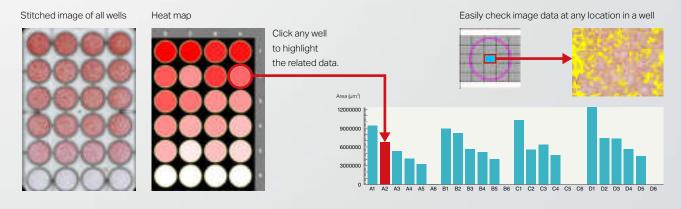
Set analysis conditions for a single image and apply to all data points automatically. This saves time and reduces variability from one image to the next. The BZ-X Series's advanced optics capture high-resolution images, resulting in highly precise data acquisition.



▲ Heatmap function Gradated display visually represents different measurement values between fields of view and wells.

#### Statistical analysis

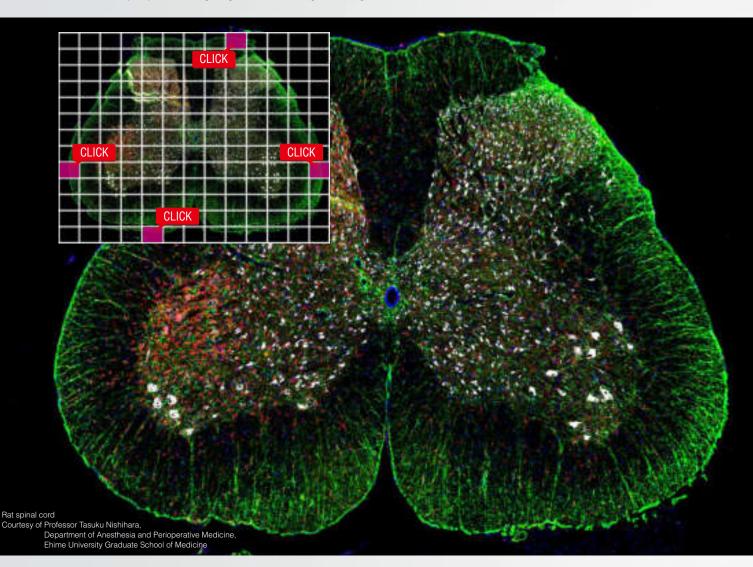
This function enables the creation of graphs for each measured item, such as sample counts, area, and light intensity. As well as graphs by well and by field of view, each field of view and measured value can be combined to create graphs covering the whole plate as a target.



#### **Image Stitching**

# High-resolution, High-speed Capture of Wide Fields

Viewing a specimen at high magnification often requires an expansion of the viewing area beyond a single field of view. Image stitching allows the user to easily capture an entire specimen at high-magnification and seamlessly create a single high-resolution image. Up to 50,000 x 50,000 pixels can be rapidly joined together without stitch lines or brightness variations. These two conflicting demands can be achieved by any user through high-resolution image stitching.



#### Automatic specimen capture

Capture an entire specimen automatically by registering the coordinates of its outermost positions.

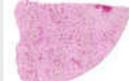
#### High precision shade correction

Uneven light intensity caused by lens aberration or non-uniform light sources appear as seams in the stitched image. This results in an unnatural appearance and affects the accuracy of quantification. The BZ-X Series eliminates uneven light intensity with its high-precision shade correction algorithm in order to create seamless, high-resolution images. Without high precision shade correction



Uneven light intensity causes stitch lines.

# With high precision shade correction



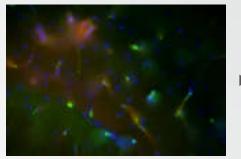
Shade correction eliminates stitch lines.

#### Full-focus Image Stitching

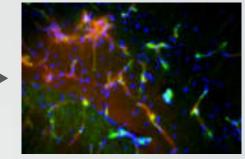
# Fully-Focused Images of Thick Samples

The automated stage captures a Z-stack for each individual field of view being stitched. This allows for a depth of field wide area image to be obtained for thick or dense samples.

Without Full-focus Image Stitching



With Full-focus Image Stitching

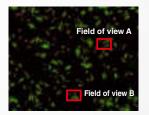


#### **Auto-focus Image Stitching**

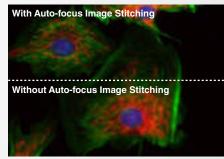
# Rapidly Focus Each Field of View

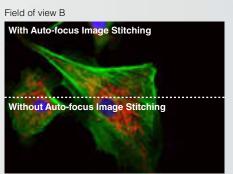
Each field of view is auto-focused prior to image capture. Optimally focused stitched images of samples with height variations, such as an unevenly sliced tissue section, can be captured without user input.





Field of view A





#### **Edge-focus Image Stitching**

# Set Z Point Positions for Fast, Focused Stitching

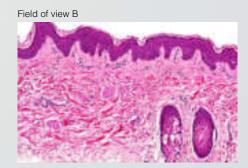
Set the focal plane for a few fields of view and then execute a rapid, single layer stitch with fewer captures. The Z axis will change gradually as the sample is scanned for rapid image stitching and minimal photobleaching.



Field of view A Field of view B

#### Field of view A







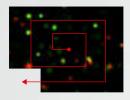
#### **Navigation**

# Easily Locate Areas of Interest

With a single click, adjacent fields of view are rapidly stitched together to create a navigation image of the entire sample. Clicking anywhere on the navigation screen will immediately move the stage to that location. The current field of view is always displayed on the navigation image, so users never lose sight of the relative viewing position, even at high magnifications.

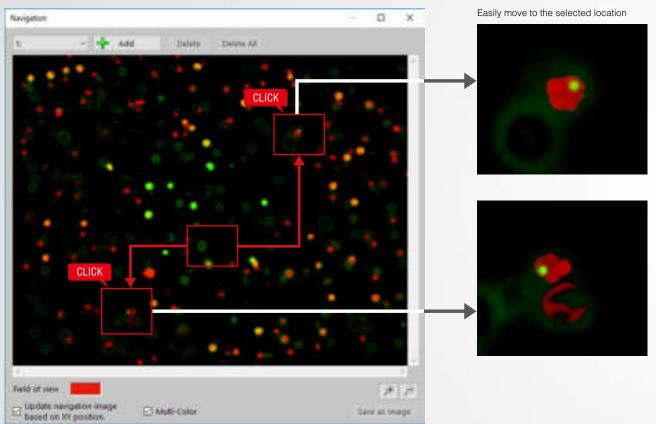






Current position

High-speed scan and real-time image stitching of surrounding area



Micronucleus genotoxicity testing

#### Image stitching made simple

#### STEP 1

While viewing the entire image of the specimen on the navigation screen, click the four points on the outside edge of the specimen to register their coordinates.



#### STEP 2

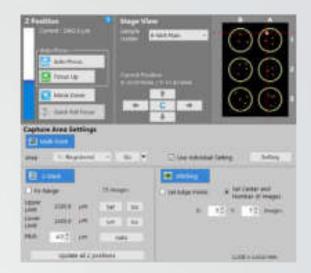
The stitched image is then captured without missing any part of the specimen. This eliminates the time and effort spent recapturing images due to some areas missing from the stitched image.



#### Multi-point & Multi-condition Capture

# Efficient Image Capture of Multiple Specimens

Up to 999 coordinate points can be recorded. A variety of capture conditions such as magnification, exposure time, Z-stack settings and image stitching can be set individually for each point. As with normal observation, simply click "Set" to register capture conditions. Multiple points of data can be obtained at the same time and this function is also useful when performing repeated evaluations of the same location on multiple specimens, such as with sequential sections and well plates.

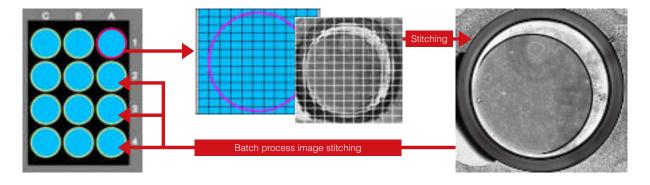


#### **Batch Process Image Stitching**

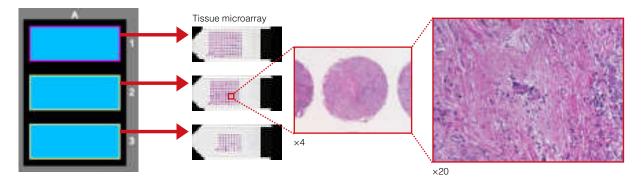
#### $\textbf{BZ-H4XD} \times \textbf{BZ-H4XI} \times \textbf{BZ-H4C}$

# Stitch Multiple Samples

Automatically carry out image stitch processing for multiple wells using macros. Acquire high-quality images without compromising on lens magnification or image resolution.



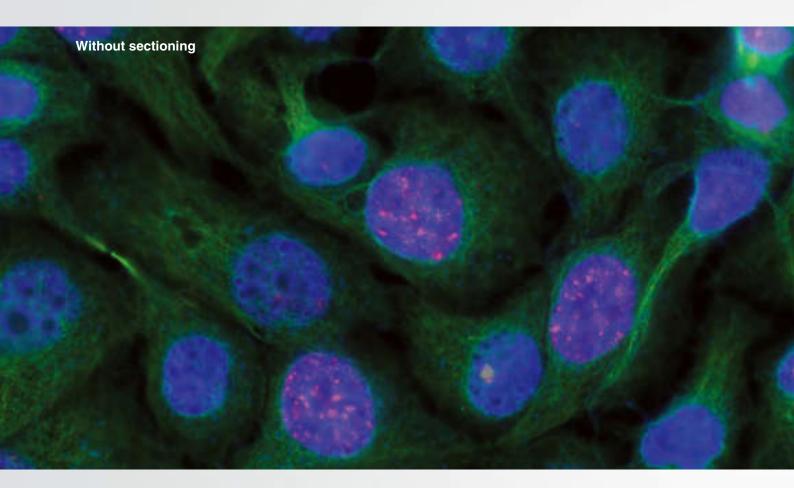
Whole slide scans The BZ-X800LE Wide Image Viewer saves uncompressed images with the highest possible resolution, allowing users to observe fine details of large samples.



## **Optical Sectioning**

# Capture Clear Images Without Fluorescence Blurring

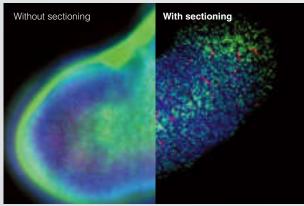
Captures high-definition images with no fluorescence blurring. This does not require special techniques or large-scale systems. The unique optical sectioning technology in the BZ-X Series uses an electronic projection element for structured illumination and provides high-definition images.

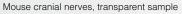


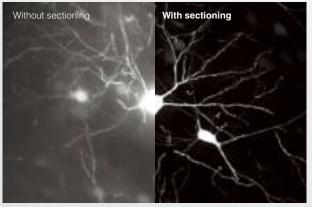
#### Clear capture of thick specimens

Optical sectioning accurately detects fluorescence signals in the desired focal plane, providing clear optical slices of thick samples. A wide range of samples, including animal cells, plant cells and cultured tissue can be easily observed.

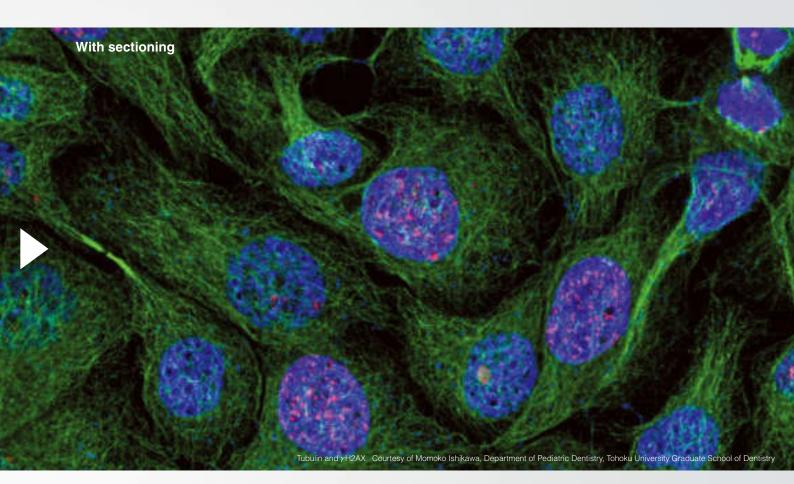








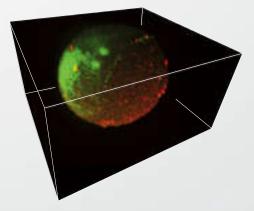




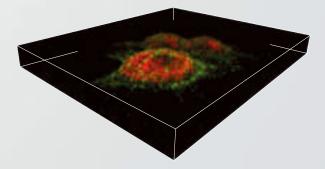
#### 3D localisation analysis

Optical sectioning provides high-accuracy, cross-sectional images without fluorescence blurring from other focal planes. Clear Z-stacks can then be transformed into realistic 3D renderings, allowing for accurate localisation analysis.

#### Ascidiacea egg



HEK293 cell



Courtesy of Assistant Professor Taku Uchida, Graduate Student Tsuyoshi Takeishi, Department of Neuroscience, Section of Integrative Physiology, Faculty of Medicine, Graduate School of Medicine, University of Miyazaki

#### **Sectioning Algorithm**

# High-Precision Optical Sectioning Using White Light

The electronic projection element enables a high-speed structured illumination scan. When compared to the effects of lasers, the white light source minimises damage to the specimen. The use of white light also provides the ability to image over a wide wavelength range, delivering high-precision optically sectioned images.

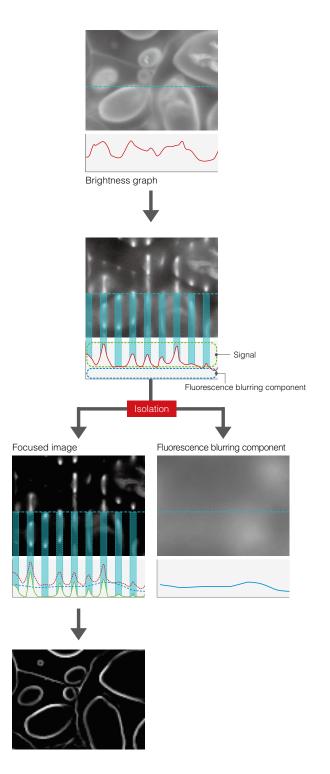
#### Normal image

Thick specimens cannot be easily captured with widefield microscopes due to scattered light in the Z plane. This fluorescence blurring obscures true signals in the focal plane of interest.

#### BZ-X sectioning

#### **STEP 1: Pattern projection**

The light passes through the electronic projection element and a structured pattern is projected onto the desired focal plane. Only signals within this focal plane are illuminated by the excitation light.



#### **STEP 2: Scan and capture**

Multiple images are captured while the illumination pattern scans across the sample. Since the brightness of scattered signals does not change significantly as the pattern moves, the fluorescence blurring can be extracted and eliminated.

**STEP 3: Sectioning image** 

The fluorescence blurring is eliminated from the multiple images captured. These images are then automatically combined to produce a clear optical section.

# Benefits of Optical Sectioning

#### Electronic projection element

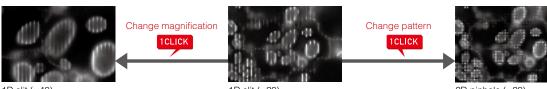
The electronic component provides a rapid, flexible excitation light configuration.

#### POINT 1

Optimal pattern automatically determined based on magnification.

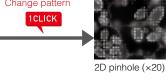
#### POINT 2

Easily complete optical sectioning image mode. No complex configuration or special skills needed.



1D slit (×40)

1D slit (×20)



POINT 3

Pattern width and structure can be easily

for higher resolution capture.

changed. A 2D pinhole pattern can be used

White light source

Easy for any user to capture high-resolution images.

#### POINT 1

Simple, compact setup.





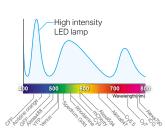
High-sensitivity detection using a monochrome cooled CCD reduces sample damage and photobleaching.





#### POINT 2

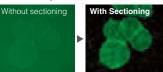
Simply change the filter to image any wavelength from UV to IR instead of dedicated laser lines.



)-o

#### **POINT 4**

Capture images in various containers, including plasticbottom multi-well plates. No complex configuration required. T-iPS cells in plastic dish

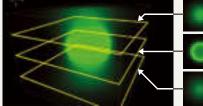


Courtesy of Assistant Professor Kyoko Masuda, Hiroshi Kawamoto Laboratory, Institute for Frontier Medical Sciences, Kyoto University

# More Accurate 3D Analysis Using Sectioning

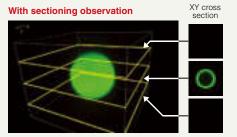
XY cross section

Fluorescent bead, 3D image Without sectioning observation





Fluorescence blurring is eliminated and signals from the desired focal plane are captured.



#### **Time-lapse**

# Temperature and CO<sub>2</sub> Regulation for Live-Cell Imaging

Perform time-series capture of brightfield, fluorescence and phase contrast images at user-specified intervals. The temperature and CO<sub>2</sub> regulation chamber can hold a variety of vessels, including well plates, to create an ideal environment for specimen during prolonged time-lapse imaging.

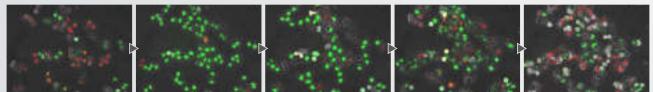


#### **Time-series Brightness Measurement Function**

# Quantify Changes Over Time

This function can provide time-series measurement of changes in RGB brightness in time-lapse images, allowing for quantitative evaluations along the time axis for experiments such as changes in gene expression. The high-intensity LED light source experiences little fluctuation in light intensity over time, enabling accurate quantitative measurement even during extended time-lapse processes.

FUCCI cell cycle checkpoints



Courtesy of Assistant Professor Atsushi Kaida, Oral Radiation Oncology Department, Tokyo Medical and Dental University

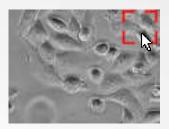




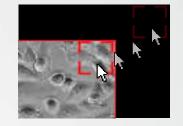
# Position Adjustment During Time Lapse

#### Adjust the field of view during time lapse capture

Adjust the capture position in the X, Y and Z directions during time lapse in response to morphology changes and temperature drift. The function is performed using previously captured images, so sensitive samples are spared from additional light exposure.



The target is about to move out of the viewing area.



Readjust the X, Y and Z capture position.



Image capture resumes using the updated position.

#### BZ-H4XT Time-Lapse Module × BZ-H4XD Advanced Observation Module

# Coordinate-specific condition settings

Different capture conditions such as focal plane, exposure time, lens magnification, filters and Z-stack width/step size can be set individually for each registered point. Multiple samples with different conditions can be imaged in the same time-lapse experiment for increased efficiency.



-	-
ens	Phase contrast 10×
Observation mode	Phase contrast image
mage stitching	7×9 images
Z-stack	N/A
Exposure time	1/70 s

#### For transfection efficiency

Lens	Phase contrast 20×
Observation mode	Phase contrast + fluorescence overlay
Z-stack	1.5 µ pitch, 8 images
Exposure time	Phase contrast 1/50 s, fluorescence 1/5 s

#### For cultured nerve cells

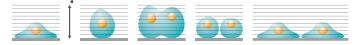
Lens	Oil immersion 60×
Observation mode	Fluorescence 2CH overlay
Z-stack	0.5 µ pitch, 10 images
Exposure time	CH1 1/6 s CH2 1/12 s

#### Focus tracking function

The optimal focal plane is automatically selected from Z-stack data. This plane is then set as the centre of Z-stack for the next capture to ensure that the sample continues to be in focus. This decreases the number of images captured at each interval, which not only reduces capture time and file size, but also reduces the risk of photobleaching.

#### Without focus tracking function

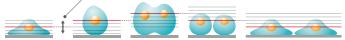
Sets a larger imaging range than necessary to allow for possible movement and morphology changes



- Larger Z-stack means more images captured
- · More exposure to excitation light increases risk of photobleaching

#### With focus tracking function

— Extracts the optimal focal plane based on previously captured images



- Less images captured for more efficient review and analysis
- Minimises sample's exposure to excitation light and reduces risk of photobleaching

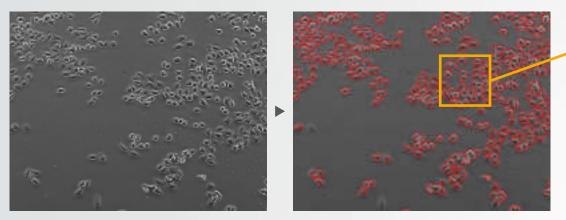
## **Hybrid Cell Count**

# High Accuracy Quantification Across Various Specimens

KEYENCE's original algorithm provides accurate quantification even for phase contrast images of cultured cells. The area of interest can be extracted and quantified quickly and accurately from phase contrast, brightfield and fluorescence images. This easy-to-use software produces repeatable, user-independent results.

#### Phase contrast

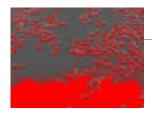
Even with low contrast between the measurement target and the background, an accurate outline for quantification can be easily extracted.





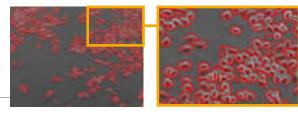
Accurate separation and extraction of adjacent cells.

#### Cell counting without phase difference mode

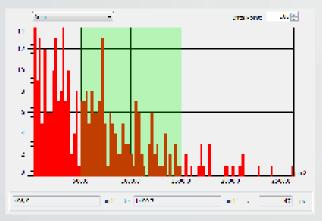


Uneven background brightness prevents cells from being extracted properly.

Low contrast makes it impossible to accurately differentiate and count the cells.



#### Data output in spreadsheet format



#### Area

Perimeter
 Major axis

Minor axis

Brightness (INT/MAX/MIN/AVE)

- RGB brightness (INT/MAX/MIN/AVE)Ferret diameter (X/Y)
- Count

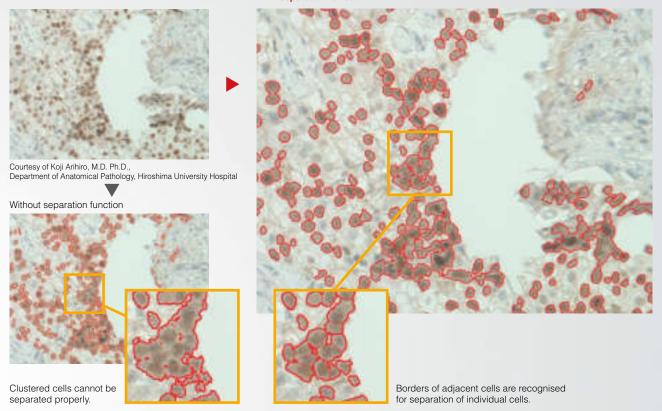
Area ratio, etc.





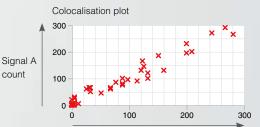
#### Colour extraction

Cells are extracted based upon hue differences and brightness information. Even clusters of cells can be separated and accurately quantified.

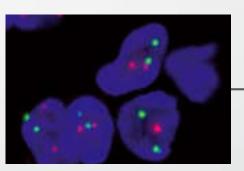


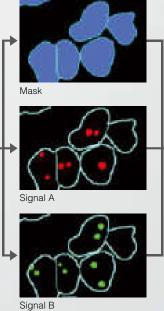
#### Masking function

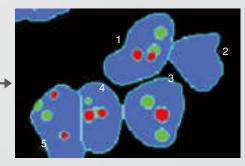
Users can specify a mask area from which to extract individual measurement areas. This allows for both individual measurement data and area ratios to be reported with ease. Up to two different extractions can be performed within the same mask area in order to quantify and compare multiple stains or conditions.



Signal B count





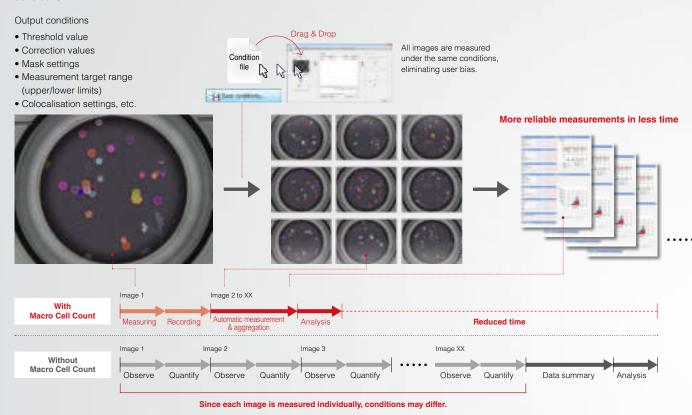


#### With seperation function

## **Macro Cell Count**

# Batch Processing for Repeatable Quantification

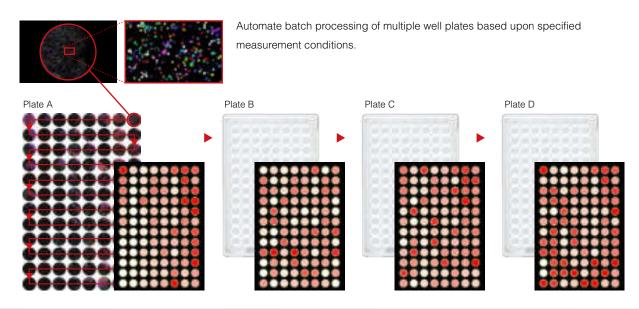
Once the appropriate measurement parameters are set for a single image, the same conditions can be applied to multiple images. This drastically reduces the amount of time needed for measurement, while improving data reliability by eliminating variations in measurement conditions.



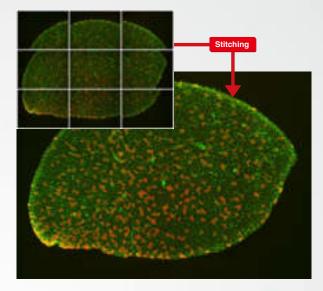
#### **Batch Analysis of Multiple Plates**

#### $\textbf{BZ-H4C} \times \textbf{BZ-H4XD} \times \textbf{BZ-H4XI}$

## Optimising Quantitative Measurements Using Well Plates

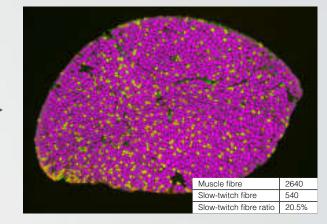


#### Hybrid & Macro Cell Count Application Examples

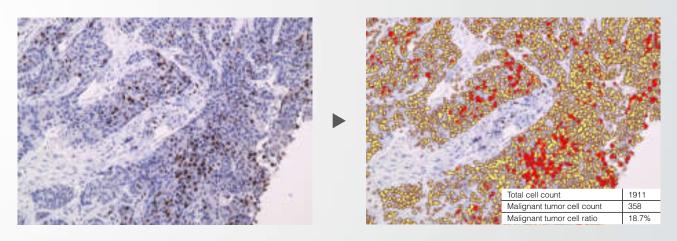


Slow-twitch skeletal muscle fibre ratio

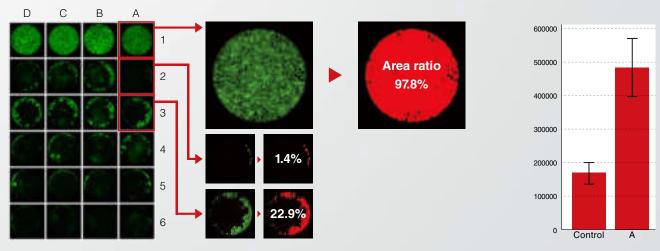
Courtesy of Lecturer Hideki Yamauchi, Division of Physical Fitness, Department of Rehabilitation Medicine, Jikei University



Malignant tumor cell (MIB-1) count



Cell migration assays using multi-well plates (24 wells)



#### **3D Analysis**

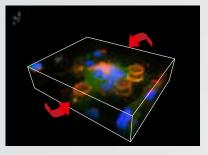
# Accurate Analysis of 3D Localisation

Transform Z-stacks into 3D images with a single click to accurately observe three-dimensional structures. Use new 3D measurement functions to quantify features such as shape and localisation. Results can then be saved in image or video format for convenient viewing.

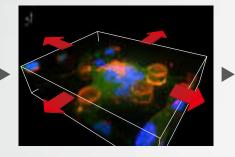


#### Intuitive operation

Rotation Click and drag to rotate.

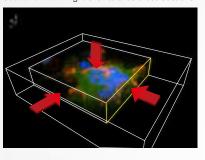


Zoom Use the mouse wheel to zoom in and out.



Sectional view Right-click to slice cross sections.

Macrophages on nanomaterials



#### Advanced 3D analysis

 YY cross section
 YZ cross section

 Image: Constraint of the section
 Image: Constraint of the section

 Image: Constraint of the section
 Image: Constraint of the section

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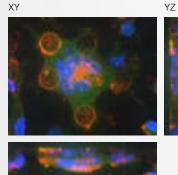
 Image: Constraint of the section
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XZ cross section

#### XYZ slicing

An image can be sliced at any XYZ position to observe the crosssectional view.



ſΖ

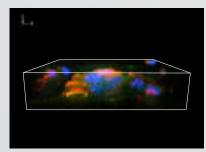
Maximum projection

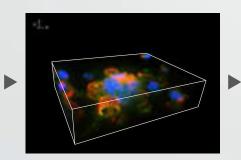
Pixels with the maximum brightness in the Z-axis are combined to display an image with a large depth of field.

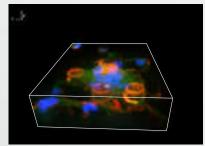
#### Video creation

3D images can be saved and played back as a video. Since videos are saved in a standard format, they can be viewed in any standard software and embedded within presentations and other documents.

X7





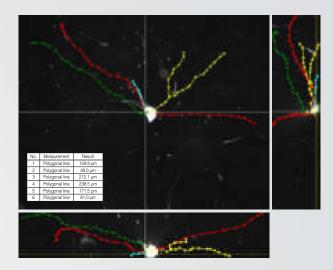


#### **3D Measurement**

Click a measurement point on a cross section and scroll through the Z-stack images to accurately measure even complex 3D shapes, such as axons of neurons. The count function enables simple and convenient counting of 3D localisation for FISH studies.

#### Intuitive measurement menu



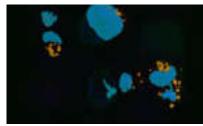


#### **3D Cell Count**

#### **BZ-H4R × BZ-H4C**

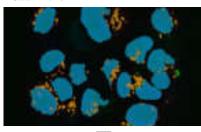
# One-Step Three-Dimensional Quantification

#### Z-Stack: Plane A

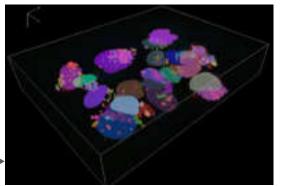


Instantly apply quantification conditions to an entire Z-stack. Quantify features such as volume, surface area and intensity of extracted areas. Specified measurement conditions are applied to the Z-stack in real-time, allowing users to quickly view and optimise settings.

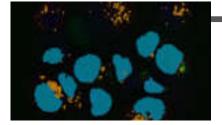
Z-Stack: Plane B



Measured areas that overlap on the Z axis are automatically integrated.



Z-Stack: Plane C



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## **Motion Analysis**

# Track Movement Over Time

Select a target and track it using brightness, hue and appearance information. Automatically record changes in coordinates over time to measure travel range, speed and movement over time.



#### Time-series data output

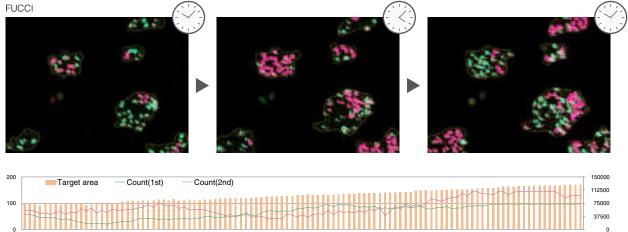


#### **Time-series Cell Count**

#### $BZ-H4K \times BZ-H4C$

# Quantify Specimen Changes Over Time

Perform batch processing of high-precision quantification for video and time-lapse recordings. Quantify cell counts, surface areas and signal intensities of extracted targets and visualise results with time-series graphs. The data can then be exported for more in-depth analysis, such as correlating surface area expansion with changes in signal intensity.

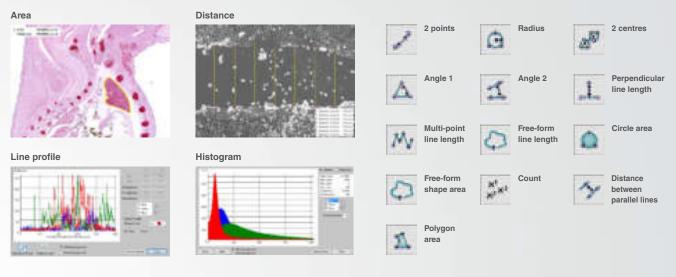


0 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61 63 65 67 69 71 73 75 77 79 81 83 85 87 89 91 93 95 97 99 101 103 105 107 105

#### Measurement

# Perform Point-and-Click 2D Measurements

A variety of 2D measurements can be made directly on the image simply by clicking the desired end points. This enables easy and accurate measurement, such as quantifying the axon length of neurons. RGB brightness values can also be quantified and visually displayed on a histogram.



#### BZ-H4A BZ-X800LE Analyzer

# Advanced Analysis Software

Perform analysis in the easy-to-use BZ-X800LE Analyzer. Capture conditions are stored in image metadata for automatic processing of Z-stacks, time-lapse, image stitching and quantification.



# Analyse data captured with conventional models

The "BZ-X800 Image Converter" analysis application is included as a standard feature. This allows data that has been captured with the previous BZ Series to be converted to the latest format. Group settings data can also be converted, allowing the various functions of the BZ-X800LE analysis application to be used for advanced analysis.



# **Objective Lenses for Fluorescence Microscope**

# Bright and Clear

Compact Fluorescence Microscope Lenses

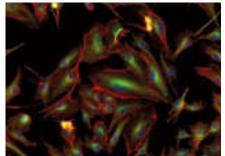


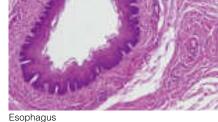


#### Bright and clear with a wide wavelength

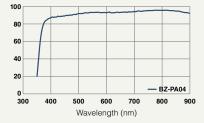
The wide wavelength range from ultraviolet to near-infrared yields a high transmission ratio. You can clearly observe both fluorescence and brightfield images.

Ideal for live cell imaging as bright fluorescence images can be obtained even with weak excitation light, minimizing damage to the cells.





The wide wavelength range from ultraviolet to near-infrared yields a high transmission ratio.



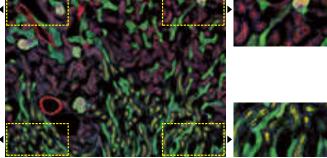
With low phototoxicity due to minimal light diffusion and absorption by organic materials, the lenses have been greatly improved to handle the wavelength range of 650–900 nm, indispensable for deep observation and live imaging.



#### High-grade optical design that minimizes distortion at the periphery of the field of view

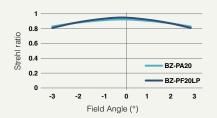
Thoroughly corrects color and screen field curvature aberrations to respond to all capture conditions, from low magnification to high magnification, and from ultraviolet to near-infrared. Maintains high level of flatness extending to the periphery of the field of view. Can easily capture natural, vivid, multi-colored stitched images seamlessly.





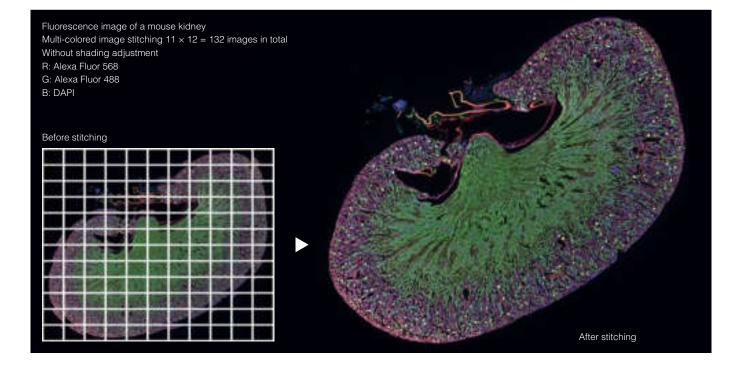
Mouse kidney

Achieves a high Strehl ratio from the center of the optical axis to the periphery

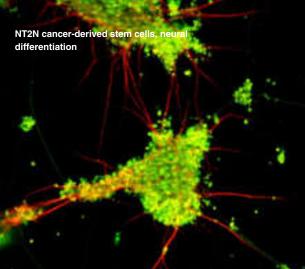


#### What is the Strehl ratio?

The Strehl ratio is the ratio of actual light intensity when compared to the maximum light intensity of the point source in an ideal optical system with absolutely no aberrations. It is generally preferable for objective lenses to have a ratio of 80% or higher.



Medical and Life Sciences

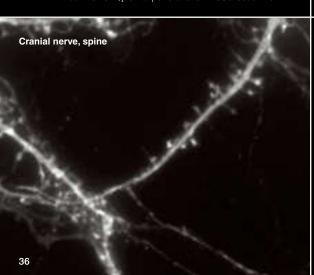


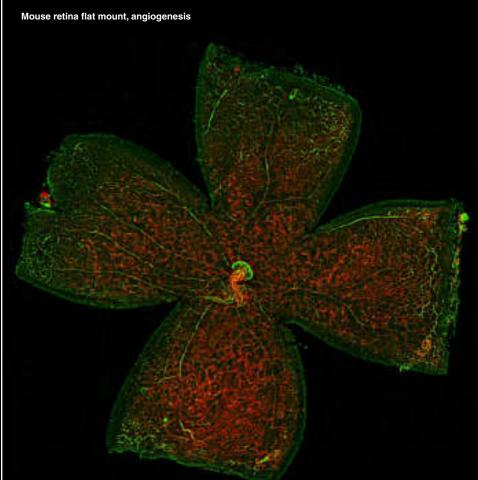
# Esophageal cancer

Heart, sectioning image



Courtesy of Dr. Koki Yokoyama, Department of Cardiovascular Medicine, Osaka University Hospital Yokoyama et al. PLoS One. 2017 Jul 28;12(7):e0182072. doi: 10.1371/journal.pone.0182072. eCollection 2017.

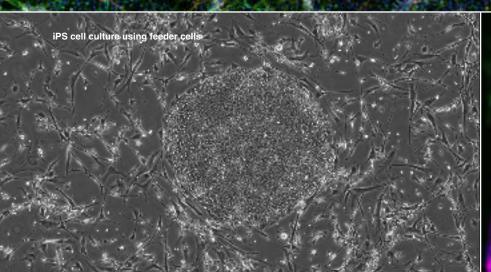




Courtesy of Professor Shigeki Higashiyama, Department of Biochemistry and Molecular Genetics, Ehime University Graduate School of Medicine

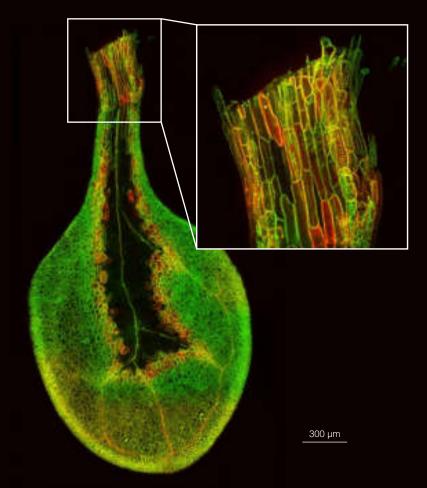


Courtesy of Assistant Professor Asuka Morizane, Department of Biological Repair, Field of Clinical Application, Center for iPS Cell Research and Application, Kyoto University



Water flea nerve and muscle, sectioning image

Arabidopsis duct, sectioning image



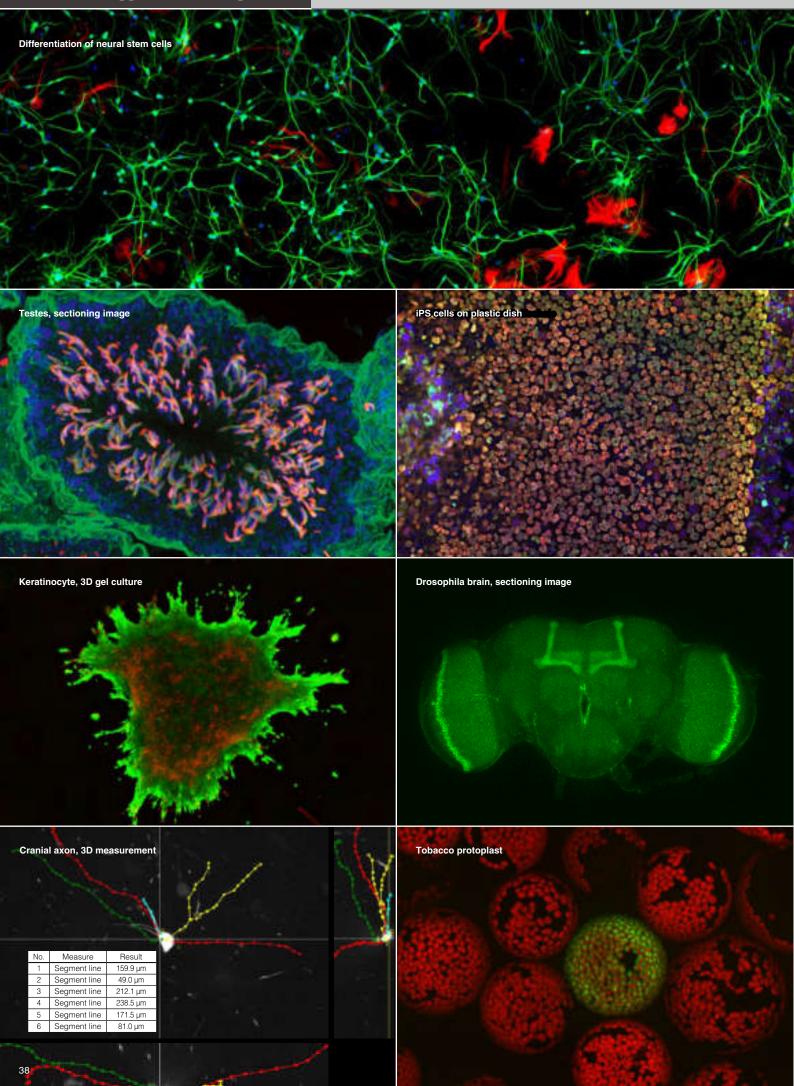
Courtesy of Assistant Professor Yasuhiro Shiga, Laboratory of Environmental Molecular Biology, School of Life Sciences, Tokyo University of Pharmacy and Life Sciences

Whole mouse embryo

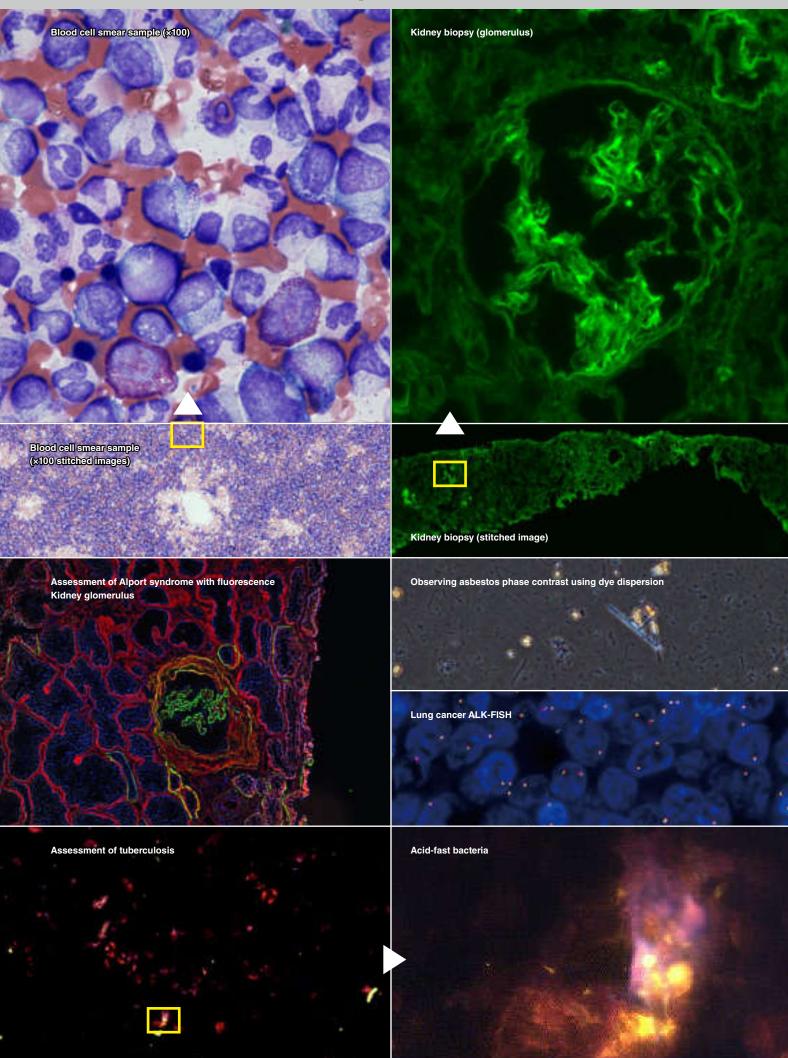
Courtesy of Lecturer Shingo Nakamura, Division of Biomedical Engineering, National Defense Medical College

# BZ Series Application Examples

## Medical and Life Sciences



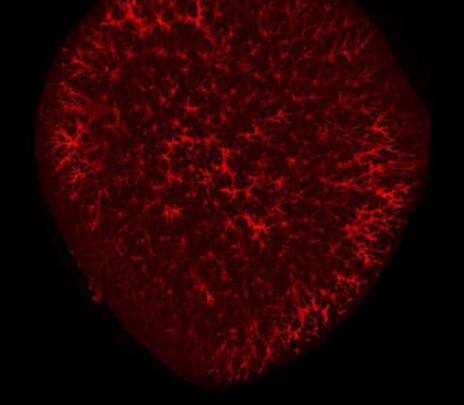
# Hospitals/Clinics

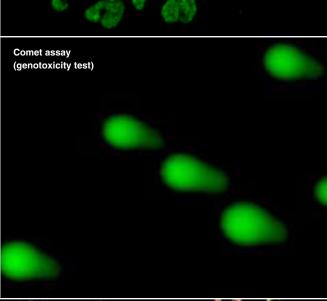


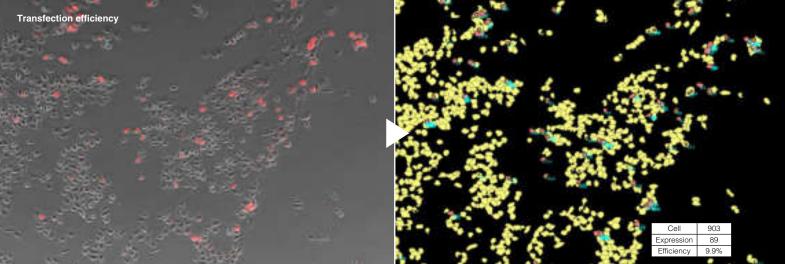
# Pharmaceuticals and Cosmetics

Cleared kidney tissue, whole mount

# 3D nephroid structure derived from tissue stem cells (renal toxicity test)

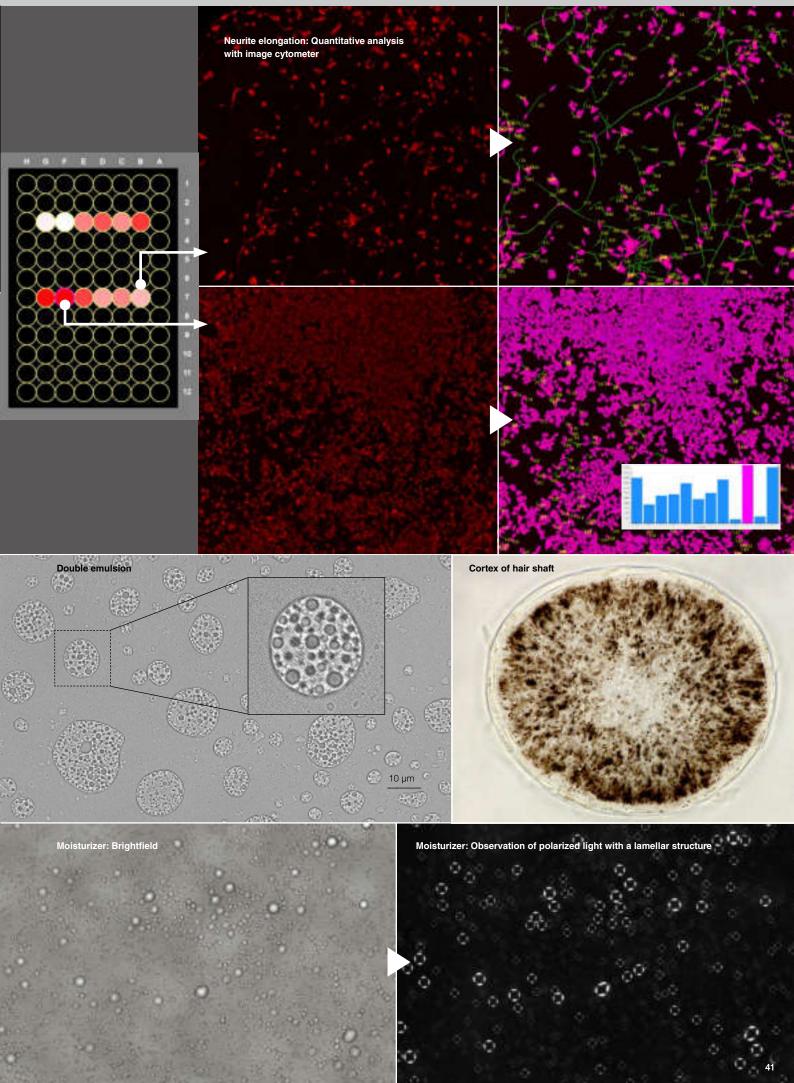






UV-damaged parts of the epidermis





#### **Specifications of BZ Lens**

(1)	Plan Apochromat 2X	BZ-PA02	NA 0.10	WD 8.5 mm	
(2)	Plan Apochromat 4X	BZ-PA04	NA 0.20	WD 20.0 mm	
(3)	Plan Apochromat 10X	BZ-PA10	NA 0.45	WD 4.0 mm	
(4)	Plan Apochromat 20X	BZ-PA20	NA 0.75	WD 0.6 mm	
(5)	Plan Apochromat 40X	BZ-PA40	NA 0.95	WD 0.25-0.17 mm	
(6)	Plan Apochromat 60X Oil	BZ-PA60	NA 1.40	WD 0.13 mm	Oil immersion
(7)	Plan Apochromat 100X Oil	BZ-PA100	NA 1.45	WD 0.13 mm	Oil immersion
(8)	Plan Fluorite 4X PH	BZ-PF04P	NA 0.13	WD 16.5 mm	Phase contrast
(9)	Plan Fluorite 10X PH	BZ-PF10P	NA 0.30	WD 14.5 mm	Phase contrast
(10)	Plan Fluorite 20X LD PH	BZ-PF20LP	NA 0.45	WD 8.8–7.5 mm	Phase contrast
(11)	Plan Fluorite 40X LD PH	BZ-PF40LP	NA 0.60	WD 3.3–2.2 mm	Phase contrast



#### Options

- BZ-X800LE desktop PC 972326
- Wide monitor **972072**
- Temperature and CO₂ regulation chamber (with mixing unit) 972082
- Temperature and CO<sub>2</sub> regulation chamber (for 5% CO<sub>2</sub> gas) 972083
- Immersion oil 971806

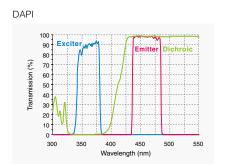


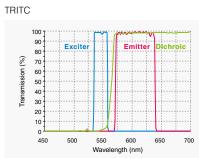
BZ-X blank filter cube **OP-87767** 

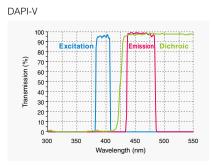
#### **Specifications of Fluorescence Filter Sets**

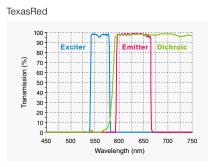
opooliiouliolio o				Units: (nm)
Set name	Model	Excitation wavelength	Emission wavelength	Dichroic mirror wavelength
BZ-X filter DAPI	OP-87762	360/40	460/50	400
BZ-X filter DAPI-V	OP-88359	395/25	460/50	425
BZ-X filter GFP	OP-87763	470/40	525/50	495
BZ-X filter TRITC	OP-87764	545/25	605/70	565
BZ-X filter TexasRed	OP-87765	560/40	630/75	585
BZ-X filter Cy5	OP-87766	620/60	700/75	660

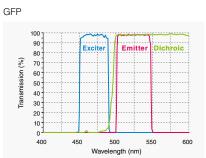
#### **Spectra of Fluorescence Filters**



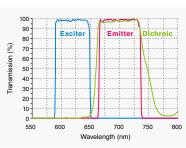








Cy5



#### Specifications

Model		BZ-X800LE/BZ-X810
	Basic optical system	Inverted fluorescence phase contrast microscope
	Objective lenses	BZ Series infinite optical system
	Observation modes	Brightfield, Fluorescence (wide-field/sectioning), Phase contrast (PhL, Ph1, Ph2), Oblique illumination
	Objective lens switching	Six-mount electronic revolver
	Image-formation optical system	Fixed image-forming lens, electronic LC filter insertion/removal mechanism
	Motorised XY stage	114 × 80 mm stroke, minimum 1 μm pitch min.
	Motorised Z stage	8 mm stroke, minimum 0.1 µm pitch min.
Microscope unit	Motorised filter turret	Up to four filters can be mounted. Automatic position recognition and automatic excitation shutdown during filter replacement
	Fluorescent incident illumination	Optical sectioning system
	Fluorescence dimming mechanism	Electronic dimming (0.3%, 5%, 10%, 20%, 40%, 100%)
	Transmitted illumination optical system	Operating distance: 45 mm, Pop-up mechanism (with automatic lamp shut off function)
	Transmitted illumination mechanism	Electronic brightfield aperture (0%, 20%, 40%, 60%, 80%, 100%)/Phase contrast slit (PhL, Ph1, Ph2)
	Transmitted light source	3.7 W LED
	Fluorescent light source	40 W LED
	Sample container	The stage is fully contained in a built-in darkroom
	Image receiving element	2/3 inch, 2.83 million pixel monochrome CCD (colourised with LC filter)
	CCD cooling mechanism	Peltier cooling: 5°C (Reduction amount: 25°C)
	Output signal, gradations	14-bit/8-bit monochrome, 8-bit R/G/B
	Frame rate	15 fps for monochrome recording (up to 95 fps with binning), 8.5 fps for colour recording
	Binning	On-chip binning (2 × 2, 3 × 3, 4 × 4, 8 × 8, 12 × 12)
	Number of pixels in recorded image	4080 × 3060 max (12.5 megapixel, high-quality interpolation)
Camera unit	Video capture	8-bit monochrome: 15 fps for 1280 $\times$ 960 With binning: 29 fps for 960 $\times$ 720, 40 fps for 640 $\times$ 480, 50 fps for 480 $\times$ 360, 75 fps for 240 $\times$ 180, 100 fps for 160 $\times$ 120 Colour: 8.5 fps for 1280 $\times$ 960
	Electronic shutter	Auto; 1/7500 to 60 sec. (77 increments)
	Gain	0 dB, +6 dB, +12 dB, +18 dB, +24 dB
	White balance	Push-set, manual
	Black balance	Push-set, manual
	Observation software	Multi-colour image capturing, Auto focus, Quick full-focusing, Scale display, Electronic revolver control, Electronic stage contro
	Applicable OS	Windows <sup>®</sup> 10 Professional 64 bit
	PC interface	USB2.0
	Ambient temperature	+15 to 35°C
	Relative humidity	35 to 80% RH (No condensation)
		Head: 517 (H) × 340 (W) × 496 mm (D) <sup>*1</sup>
Controller	Dimensions	Controller: 227.5 (H) × 125 (W) × 408 mm (D)
	Weight	Head: Approx. 33 kg, Controller: Approx. 4.8 kg
	Power supply	100 to 240 VAC ± 10%, 50/60 Hz
	Power consumption	200 VA or less
	Overvoltage category	
	Pollution degree	2
	BZ-H4XF/Sectioning Module	Optical sectioning image mode
	BZ-H4XD/Advanced Observation Module	Navigation, Image stitching, Z-stack, Coordinate-specific condition setting
Functional Modules	BZ-H4XI/Image Cytometer Module	Batch capture (user location specified/all locations specified/random location specified) *BZ-H4XD required/Image cytometer analysis, batch analysis *BZ-H4C required
	BZ-H4XT/Time-lapse Module	Time-lapse imaging, Video capturing, Time-series brightness measurement
	BZ-H4A/Advanced Analysis Software	Image stitching, Haze reduction, Full focus
	BZ-H4M/Measurement Application	Dimension measurement. Area measurement. Brightness measurement (line profile, histogram)
Analysis	BZ-H4M/Measurement Application	Dimension measurement, Area measurement, Brightness measurement (line profile, histogram) 3D display 3D measurement, XYZ slicing, Maximum projection, Video saving, (with addition of BZ-H4C) 3D cell coun
	BZ-H4R/3D Application	3D display, 3D measurement, XYZ slicing, Maximum projection, Video saving, (with addition of BZ-H4C) 3D cell coun
Analysis Applications		Dimension measurement, Area measurement, Brightness measurement (line profile, histogram) 3D display, 3D measurement, XYZ slicing, Maximum projection, Video saving, (with addition of BZ-H4C) 3D cell count Motion tracking, Motion analysis, (with addition of BZ-H4C) Time-series cell count Cell count (Phase contrast, Brightfield, Fluorescence), Mask cell count

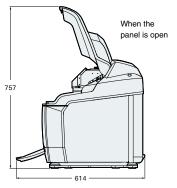
\*1 Panel closed • Windows® 10 is a registered trademark of Microsoft Corporation in the United States.

#### Dimensions

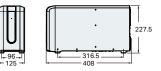
Head unit **BZ-X810** 







Controller unit BZ-X800LE



Units: mm

Imaging and analysis examples, customer feedback, and related articles

# A special site where you can access the latest information on fluorescence microscopes







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SAFETY INFORMATION Please read the instruction manual carefully in order to safely operate any KEYENCE product.

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