

# STANDARD OPERATING PROCEDURE

## JuliBr Live Cell Movie Analyser

### PURPOSE

This document describes the operational setup and procedures for the JuliBr live cell movie analyser (JuliBr). This document offers users, particularly beginners, a brief step-by-step instruction on how to operate the JuliBr.

JuliBr is a live-cell imaging system offering bright field (BF) imaging of live samples to monitor cell changes/growth over time of live cells, tissue or organoids while culturing them. Acquisition is from within a tissue culture incubator and compatible with your culture dish. With stable CO<sub>2</sub> supply and temperature control, cell culturing and imaging can be performed in a stable environment over long periods. The system comes with dual microscopes and a controller enabling single field of view simultaneous comparisons between experimental samples (i.e. treatment vs control or two different treatments).

### SCOPE

The procedure applies to basic image acquisition including focusing, getting confluence value and generating time lapse videos/cell monitoring. In addition, the document covers additional information about specifications of the JuliBr.

For more detailed operational instructions such as data saving, viewing and access, users are to refer to the full version manual provided by the vendor, which is available via the Westmead Imaging internet site and in the instrument lab.

### SAFETY

Acquisition of samples on the JuliBr must only occur after the approval of an associated project in PPMS. Any projects to be run on the JuliBr involving hazardous chemicals must have appropriate approval. OGTR requirements for safe work in a PC2 laboratory apply.

For general Biosafety information, refer to the latest Safe Work Procedure WIMR-SWP-WHS-GEN, "Using a fluorescence or laser scanning microscope":

<https://sydneyuni.atlassian.net/wiki/spaces/WIF/pages/768016621/Manuals+Protocols>

### TRAINING / COMPETENCIES

All personnel require training prior to independent operation of the instrument. Training is conducted by imaging staff and competency demonstration is necessary before authorisation.

Competency is assessed via demonstration of independent instrument operation, in conjunction with verbal explanation of all aspects of operation and troubleshooting common faults. After training is carried out, a training competency quiz is to be completed and passed before instrument access is authorised by imaging staff. All instrument operation is to be conducted by trained operators.

## EQUIPMENT & SUPPLIES

The JuliBr is located in the shared prep. lab J206, Level 2, WIMR with the microscopes in the top incubator and the control unit outside the incubator.

## PROCEDURE

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### 1. Starting JuliBr

Turn on the **Power Button**.  
Place the sample on the stage.



### 2. Focusing

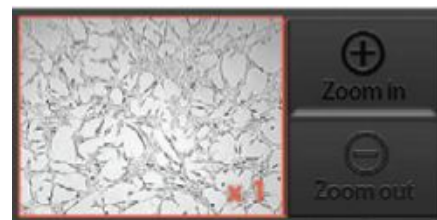
Adjust the focus for each scope unit if second unit is also connected to station unit. Press **Channel** tab to select each scope unit.



Adjust the illumination intensity using the **Exposure** and **Brightness** bar of Focusing menu.



Set the viewing region of image by pressing the **Zoom** in/out button and dragging preview.



Adjust focus of the image using the **Focus knob** on the scopes or **Focus interface** in the software.



Press **Confluence** and **Save** button to acquire the image if it is necessary.



### 3. Getting confluence value

Press **Confluence** button within the **Focusing** menu. If the value of confluence should be more accurate, try again after adjusting parameters of



Press the **Capture** and **Save** button to acquire the image with confluence circles if it is necessary.



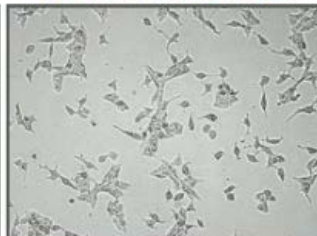
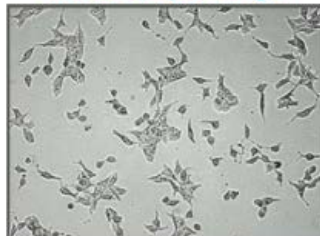
### The reference images for correct focus and confluence

Du-145 (Parameter A)

Good sample image

Normal sample image

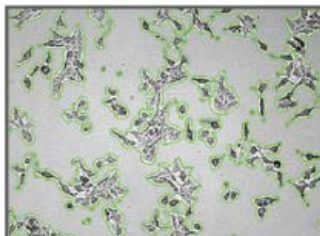
Poor sample image



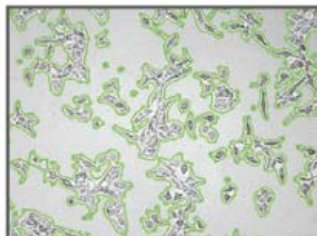
Reference: Exposure 4 / Brightness 1

Reference: Exposure 6 / Brightness 3

Reference: Exposure 6 / Brightness 5



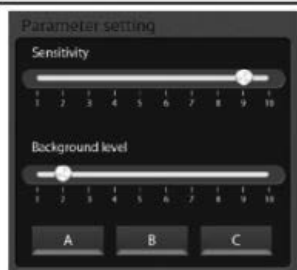
Confluence 35.11 %



Confluence 36.22%



Confluence 43.03%

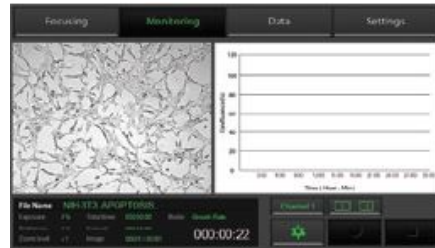
Parameter	Cell line	Recommendation
	A HepG2, GH3, Hep3B, A549, MCF7, SH-SY5Y, SCN2.2, NIH-3T3, F9, HeLa, Du-145	Sensitivity level: 9 Background level: 2
	B LNCaP	Sensitivity level: 10 Background level: 2
	C U-2 OS, ADSC	Sensitivity level: 10 Background level: 8

#### 4. Making time lapse imaging

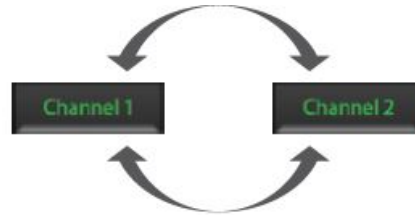
**Biosafety notes**

Double pack live sample when transporting to the Cell Imaging Facility.

Biohazardous samples must be sealed in the sample container i.e. capped flask while imaging.



Each scope **Channel** can be viewed by pressing channel tab if second scope unit is connected to station unit.



Press **Setting** button of Monitoring menu.



Press **Name** to type file name and **Save** button to save.



Choose the monitoring type among **Growth rate**, **Wound healing** and **Movie only**.



Set up **Total time** and **Time interval**.  
**Note:** time Interval should be set up more than 1 minute for the best quality result in case of monitoring growth rate and wound healing. However, in the case of dual monitoring, more than 5 minutes is recommended.



Press the **Apply** button to save all options.



Press the **Rec.** button to begin recording.

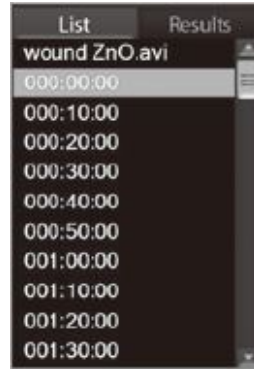


## 5. Data save and delete

Press the **Data** tab to access saved images/movies options and select data.



Data can be opened or shown by double tapping folder name in **List** category or single tapping folder in Result category.



Movie file can be viewed by pressing **Play** button



Insert USB drive into USB port to export data.



## 6. After your experiment is done

Clean the surfaces of the scopes and incubator door handles with 70% ethanol and paper towel.

## 7. Specifications

Cat no	Device JULI-BR04 (Single set, 1 Scope & 1 Station) JULI-BRD04 (Dual set, 2 Scopes & 1 Station) JULI-BRSC (2 <sup>nd</sup> Scope) Accessory JULI-BRCM (Counting starter kit) JULI-BRTB (XY Stage)
Magnification	Objective 4 X and digital zoom (~ 400 X)
Image resolution	2560 x 1920 pixels (5M)
Exported formats	JPEG (image), AVI (movie), CSV (raw data)
Display	10.1" LCD touch screen
Light source	White LED
Dimensions & weight	Scope: 300 x 190 x 188 mm, 4 kg Station: 282 x 285 x 160 mm, 3.2 kg
Storage	320 GB Hard drive 4 GB USB drive

## DATA & RECORDS MANAGEMENT

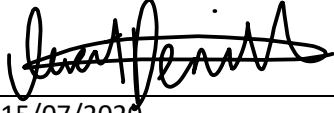
This system does not come with a computer and therefore our Auto Data Workflow does not apply. All monitoring/movie data must be saved onto the Control Unit in “Data” menu (kept for 7 days). Any other data except monitoring movies are to be saved in a default folder called “captured Images”. Users need to manually transfer data with the USB drive provided by Cell Imaging and return it within 24h.

## REFERENCES

Hardcopies of both quick and full manuals provided by the instrument vendor are available in the instrument lab. They are also published on the Imaging Website:

<https://sydneyuni.atlassian.net/wiki/spaces/WIF/pages/768016621/Manuals+Protocols>.

## DOCUMENT CONTROL

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## REVISION HISTORY

Revision Date	Version No.	Amended by:	Change