**Standard Operating Procedure, SOP**

**Title:** **Imaging,**

**Data Processing,**

**Data Storage**

**Valid: ERC Project**

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*(Surname, Name)*

**Staff Signature:**

*(signing below indicates that you have read this SOP and understand the material contained in it)*

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**Revision History**

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| **Version** | **Reason for changes** | **Changed at** | **Changed from** |
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**Table of contents**

1 Purpose 5

2 Description 5

3 Materials & Reagents 5

4 Procedures 6

4.1 Preparations 6

4.2 InCell Software preparation 6

4.3 Overlord Software preparation 6

4.4 Imaging 6

4.5 Raw Data Analysis 7

4.6 Data Storage 8

4.5 Clean up 8

5 Safety information 9

6 Other applicable documents 9

7 Attachments 10

7.1 Start automatic image analysis 10

7.2 Imaging Modes 10

# 1 Purpose

This standard operation procedure explains describes the procedures and workflows needed for imaging, automatic image processing and storage of several 384 well flat bottom transparent plates. It starts with a stack of plates and ends with archived raw data and analyzed data.

# 2 Description

# 3 Materials & Reagents

* GE IncellAnalyzer 2200 GE
* HP Z220 Workstation HP
* HP Custom ProLiant Server Cluster (96 CPU, 70 TB storage) HP
* Inatek FD2102 Double HDD Docking Station Inatek
* Seagate 8 TB Archive HDD ST8000AS0002 Seagate
* Seagate 2 TB HDD ST2000DM001 Seagate

# 4 Procedures

**Note:** For more information on the instruments, please ask the *responsible persons (see black board Lab jobs or WIKI)*

## 4.1 Preparations

## 

a) Start the InCell 2200 and PC if not already running

b) Wait until only a green light is visible at the InCell 2200

c) Let plates equilibrate at room temperature for 30 min

d) Clean plates with dust free towel to remove dust and hair

## 4.2 InCell Software preparation

a) Open InCell Analyzer Software (v 6.4)

b) Go to File -> Open -> “ERC\_4x\_4t.xaqp”

c) Go to Application -> Performance Manager -> remove check marks -> ok

d) Go to Application -> Robotics Mode -> enable

## 4.3 Overlord Software preparation

a) On Desktop Pull the “RUN ERC.ovp” icon onto the Overlord3 icon

## 4.4 Imaging

a) Clean each plate with a lint free towel

b) Put plates into rack starting from stack 1 (max. 30 Plates per stack)

c) Overlord3 Software -> Click Run

d) Answer questions: ok -> ok -> yes -> no

e) As protocol choose: “ERC\_20x\_4t.xaqp”

f) As base path choose: select the smaller of the two choices

i) goto: Computer -> share (X://) or share2 (Y://) -> raw\_data -> Dmel\_HP\_20x\_4tiles\_ERC\_SynGene

choice 1) X:/raw\_data/Dmel\_HP\_20x\_4tiles\_ERC\_SynGene

choice 2) Y:/raw\_data/Dmel\_HP\_20x\_4tiles\_ERC\_SynGene

## 4.5 Raw Data Analysis

a) The analysis will start automatically and data will appear at

i) X:/results/Dmel\_HP\_20x\_4tiles\_ERC\_SynGene

ii) Y:/results/Dmel\_HP\_20x\_4tiles\_ERC\_SynGene

b) The progress can be monitored depending on the input folder via the web

i) open Firefox

ii) open new tab

iii) type the address belonging to the input folder:

choice1: [b110-sc2hn01/controls\_plates.pl?FOLDERTOWATCH=data/results/Dmel\_HP\_20x\_4tiles\_ERC\_SynGene/](http://b110-sc2hn01/controls_plates.pl?FOLDERTOWATCH=data/results/Dmel_HP_20x_4tiles_ERC_SynGene/)

choice2: [b110-sc2hn01/controls\_plates.pl?FOLDERTOWATCH=data2/results/Dmel\_HP\_20x\_4tiles\_ERC\_SynGene/](http://b110-sc2hn01/controls_plates.pl?FOLDERTOWATCH=data2/results/Dmel_HP_20x_4tiles_ERC_SynGene/)

iv) both paths are pinned in the favorites bar of each browser

v) there you will find a self-refreshing page giving live insight into the imaging and analysis progress

c) the script *“all\_in\_one\_v1.9.cluster.watcher.data.pl”* and *“all\_in\_one\_v1.9.cluster.watcher.data2.pl”* will watch both input folder and analyze always all images of 4 wells in a block until the entire plate is imaged

notes:

- a plate will never be analyzed twice, unless the services are restarted

- a plate, who’s barcode has not been read properly is not analyzed

d) the script in b) will automatically source the R analysis *“image\_analysis\_ERC.v6.R”* for the set of 48 images comprising 4 wells

e) Once all plates are imaged and analyzed check if all barcode were read

## 4.6 Data Storage

a) connect barcoded and “exFat” formatted Hard drive via Inatek FD2102 Double HDD Docking Station to the USB-3 slot of the workstation (Blue slot on the PC)

b) open a windows shell by pressing the “shell” symbol in the task bar

c) type:

robocopy /e “source path” “destination path”

e.g.:

robocopy /e X:/raw\_data/Dmel\_HP\_20x\_4tiles\_ERC\_SynGene L:/

d) when this is done -> store Hard drive in Office 3.301, Schrank2, enter Hard drive list in tracer sheet and hard drive inventory list

## 4.5 Clean up

1. Remove all drives from computer
2. Switch off Microscope (InCell Software -> Application -> Hardware -> Switch off instrument)
3. Switch off computer
4. Store Imaged plates at 4 °C in cold-room until needed

# 5 Safety information

**None Applicable**

# 6 Other applicable documents

* **Tracer sheet** (PowerFolder -> ERC -> SOPs)
* PowerFolder -> ERC -> Analysis-methods:
  + *all\_in\_one\_v1.9.cluster.watcher.data.pl*
  + *all\_in\_one\_v1.9.cluster.watcher.data2.pl*
  + *image\_analysis\_ERC.v6*
  + *waiter.quick.pl*
  + *start\_the\_watchers.pl*
  + *RUN ERC.ovp*
  + *ERC\_20x\_4t.xaqp*

# 7 Attachments

## 7.1 Start automatic image analysis

a) log into b110eng@b110-sc2hn01

i) username: b110eng and password: incell2000

b) type: killall perl

c) type: ./start\_the\_watchers.pl

## 7.2 Imaging Modes

* 4 non-overlapping tiles perl well
* 3 channels per tiles
* FITC-channel:
  + a-Tubulin-FITC (ex:475±28, em:511±23) = 0,3 sec.
* TRITC-channel:
  + Phalloidin-TRITC (ex: 542±27, em:597±45) = 0,2 sec
* Hoechst-channel:
  + Hoechst (ex:390±18, em:435±48) = 0,1 sec

## 7.3 Interrupting imaging

1. Open overlord2 software
2. Wait until the robot is about to out the just finished plate into the stack
3. Press stop and immediately accept with yes to stop the robot
4. Gently remove the plate from the robot
5. Close all programs
6. Refill the stacker
7. Start at the beginning of this SOP