STOmics

MICROSCOPE ASSESSMENT GUIDELINE FOR ImageStudio SOFTWARE

Manual Version: B1

REVISION HISTORY

Manual Version:	A1	**Description**:
Kit Version:	V1.0 & V2.0	
Date:	May. 2023	· Initial release

Manual Version:	B1	**Description**:
Kit Version:	V1.0	
Date:	Oct. 2023	· Added microscope evaluation procedures for Stereo-seq Transcriptomics Multi-immunofluorescence (mIF) solution and Stereo-seq Transcriptomics H&E solution. · Added instructions for microscope hardware evaluation. · Eliminated the evaluation requirements for blank Stereo-seq Microscope Assessment Chip T. · - Modified compatible image formats (only compatible with TIFF/TIF formats).

Note: Please download the latest version of the manual and use it with the software specific to this manual.

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CRITICAL STEPS: Pay extra attention for these steps to avoid experimental setbacks or problematic results.

NOTE: Additional operation tips and guidance.

SOLUTION: Provides a solution to the operation procedure.

CAUTION: Proceed with extra care; improper handling or carelessness may cause experimental failure or accidents.

CHAPTER 1
INTRODUCTION

STOmics Stereo-seq Solution quantifies mRNA of tissue sections followed by mapping and visualizing the transcriptomic data on an anatomical image. Hence, the tissue image quality determines the success of downstream analysis and spatial clustering of gene expression data. This guidebook provides hardware recommendations, image acquisition and evaluation guidelines, as well as microscope compatibility test procedures. Additionally, a BGI-developed image evaluation and processing software – ImageStudio is also introduced in this guidebook.

1.1. Microscope Assessment Channels

a. Stereo-seq Transcriptomics application(ssDNA-stained nuclei image) -> FITC Channel

b. Stereo-seq Transcriptomics with mIF co-detection application (DAPI-stained nuclei image & mIF images) -> DAPI Channel

c. Stereo-seq Transcriptomics H&E application (H&E-stained image) -> Brightfield/Epi-illumination

1.2. Assessment Software and Criteria

All microscope images are evaluated for image quality via the "Image Quality Control" module in ImageStudio software, including the scoring evaluation of Trackline clarity and image clarity. All evaluations are based on the standard of Trackline QC score. For detailed scoring rules, please refer to Chapter 4.4 and Chapter 5.3. The image clarity score is for reference only and is not used as a pass/fail standard for image QC.

1.3. Assessment Duration

About half a day.

CHAPTER 2
MICROSCOPE HARDWARE EVALUATION

2.1. Imaging System Recommendations

The microscope hardware evaluation mainly evaluates whether the microscope hardware parameters meet the Stereo-seq experiment requirements. The microscope hardware evaluation contents are as follows:

It is recommended to make an appointment in advance with your manufacturer's microscope engineer to accompany the evaluation on site.

Index/ Parameter	Description
XY stage travel distance of microscope	At least 25*75 mm
Focusing approach	Pre-focus map and/or real-time autofocus
Brightfield light	Reflective light source (Epi-illumination)
Objective lens	10X (NA ≥ 0.3)
Fluorescent channel	· DAPI (Excitation 358nm, Emission 461nm) · FITC filter cube (Excitation 480/40nm, Emission 525/50nm) · TRITC filter cube (Excitation 545/25 nm, Emission 605/70 nm) · CY5 filter cube (Excitation 620/50 nm, Emission 690/50 nm)
Camera resolution	High image resolution: ≥ 1800 pixels (height) and ≥2000 pixels (width)
Image bit depth	· Grayscale image: 8bit/16bit (fluorescent image) · Color image: 3*8bit/3*16bit (H&E or other brightfield image)
Exposure time	· ssDNA/DAPI: 1 milli sec - 2 sec · H&E: 0.1 milli sec - 100 milli sec
Background balance	Adjustable background balancing function
Distortion correction	Adjustable distortion correction function
Overlap ratio	Adjustable, no less than 5%
Pixel size	Maximum pixel size ≤ 5 μm/pixel
File format	Capable of viewing or exporting stitched images or Field-of-View (FOV) original images in TIFF format, 8/16 bit
PC requirement	Windows 10 x 64 system, 16G memory or beyond