BSBS Core Equipment – Lake Nona

Microscope Systems

Gel Docs, in vivo

Plate Readers

UCF College of Medicine

20260213

BSBS Core Microscope Systems – Lake Nona

Confocal

Zeiss 710 Confocal
BBS 107C – Jeremiah Oyer

Leica TCS SP5II Confocal
Annex 116A- Moving to:
BBS 107A

Yokogawa CQ3000 Cell Voyager
BBS 107E

Near Confocal

Zeiss B/W, Apotome 3
BBS 340 to LNCC 3704

Zeiss B/W, Apotome 2
BBS 317 Color and B/W cam

Micro Confocal
BBS 557 to LNCC A3952

Inverted Fluorescence

MACSima
BBS 557 To LNCC A3986

Pico ImageXpress
BBS 557 to LNCC A3952

IncuCyte S3 and SX5- Automation
BBS 557: S3 to BBS 462 or 362
SX5 to LNCC A3952

Leica DMI3000
BMS 300

Leica DMI600
BBS 356

Keyence BZ-X800
BBS 340

Zeiss B/W Cam
BBS 456

Leica DMI6000B
BBS 456

Upright

Leica DMI2000 - color
BBS 125

Keyence VHX 7000- color
BBS 256
Specialty - 3D

Zeiss A1 B/W FL
BBS 340

Nikon Eclipse E400 B/W FL
BBS 417

Stereo/Dissection

Leica M125
BBS 153 and 156

All systems here are overseen by Alicia Willenberg except for the Zeiss 710 Confocal: BSBS 107c – Jeremiah Oyer

BSBS Core Confocal – Lake Nona



Zeiss 710 Confocal Microscope
BSBS 107c

Aging system (2010) that has some issues connecting to the computer

10, 20, 40 and 63×

BSBS Core Confocal – Annex



Leica TCS SP5II Confocal (2011)
Annex 116A

To be moved to BBS 107A

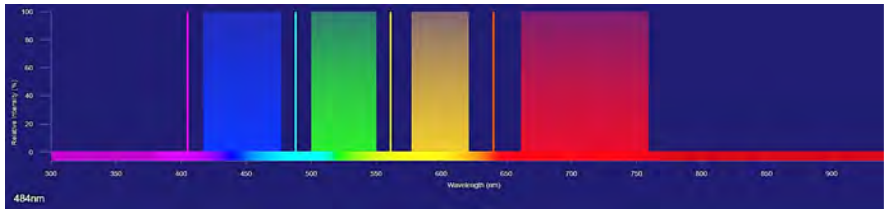
Leica AF Software

Laser Scanning Confocal
Lasers:
405 (near UV)
Argon 65mW: 458, 476, 488, 514
DPSS 20mW: 561
HeNe 10mW: 633

Objectives:
20x (Leica 20x/0.7 HC PL APO)
63x (Leica 63x/1.4-0.6 HCX PL APO, oil)
100x (Leica100x/1.44 Oil HCX PL APO

BSBS Core Confocal – Lake Nona





Yokogawa CQ300 Cell Voyager (2026)
BSBS 107E

High Content, Dual Spinning Disk Confocal

6 objectives: 4, 10, 20, 20 water, 40 and 60× water

Lasers:
Camera 1: 405, 488,
Camera 2: 561, 640

Emission filters:
Camera 1: 447/60; 525/50
Camera 2: 599/44, 710/98

Well plates, slides, 35mm dishes
CellPathFinder Analysis Software
Can image 2 simultaneous images, one on each camera (not blue/green Ca^{2+} imaging)
Can use lasers with either Em filter

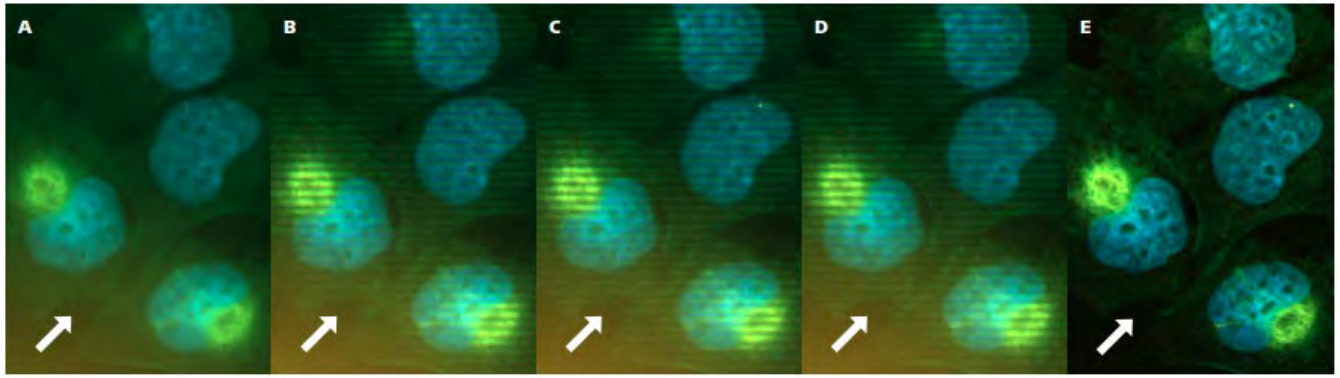
Near Confocal: Zeiss Apotome 2

BBS 317

Fluorescence: DAPI, GFP, Cy3, TR, Cy5
10, 20, 40 and 100× objectives
Cell Culture Plates, Slides
Zen Software
Z-Stack, Sectioning and Deconvolution



Video 1 , Video 2



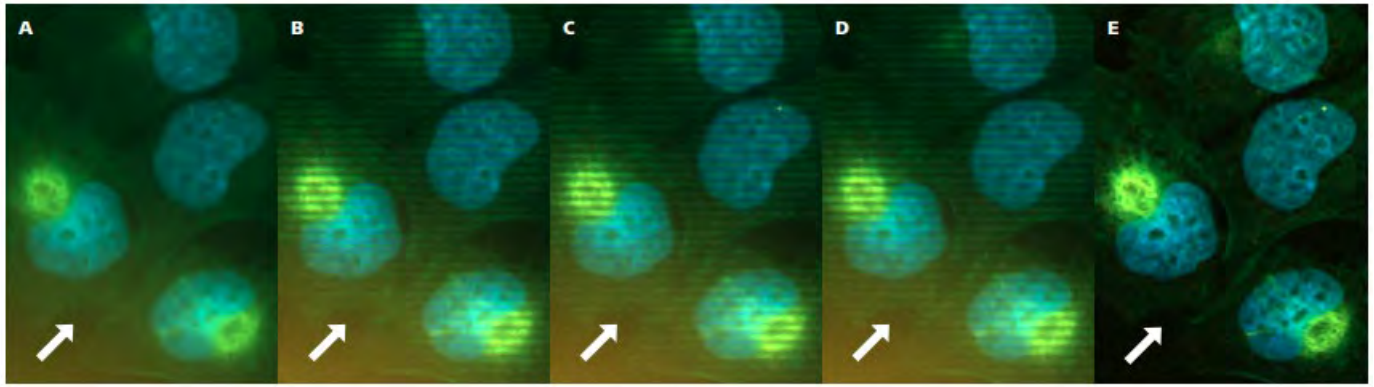
Schematic illustration of the grid projection. A: Widefield image. B – D: raw images with different positions of the grid. E: resulting optical section through the sample. Out of focus light is efficiently removed by the structured illumination (arrow).

Near Confocal: Zeiss Apotome 3

LNCC A3704

Fluorescence: DAPI, GFP, Cy3, TR, Cy5
5, 10, 20 plate, 20 slides, 40 and 60× objectives
Cell Culture Plates, Slides
Zen Software
Z-Stack, Sectioning and Deconvolution

Video 1 , Video 2



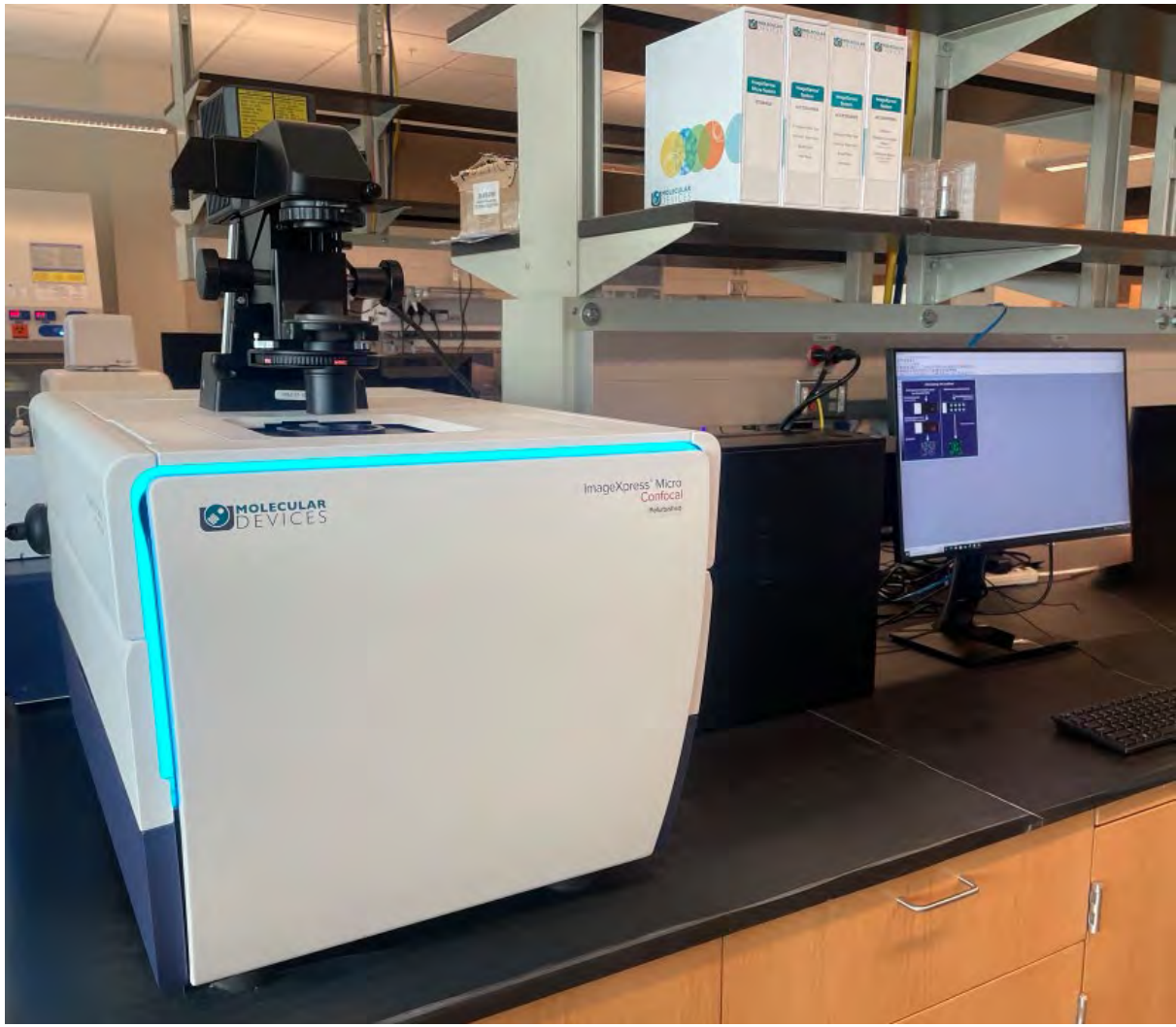
Schematic illustration of the grid projection. A: Widefield image. B – D: raw images with different positions of the grid. E: resulting optical section through the sample. Out of focus light is efficiently removed by the structured illumination (arrow).



Near Confocal: Micro Confocal

LNCC A3952

Software is less user friendly, but we have worked out several issues- updated guides; exchange of 40x in progress, current 40x is error prone
Automation: single plate, single slide (not ideal for slides)
Fluorescence:
DAPI, GFP, Cy3, TR, Cy5
4, 10, 20 phase, and 40× objectives
- (20/40 = correction collars)
60µm spinning disk
Cell Culture Plates
Slides (single slide holder)
Z-Stack and Sectioning
Stitching
Analysis



Inverted Fluorescent - Basic

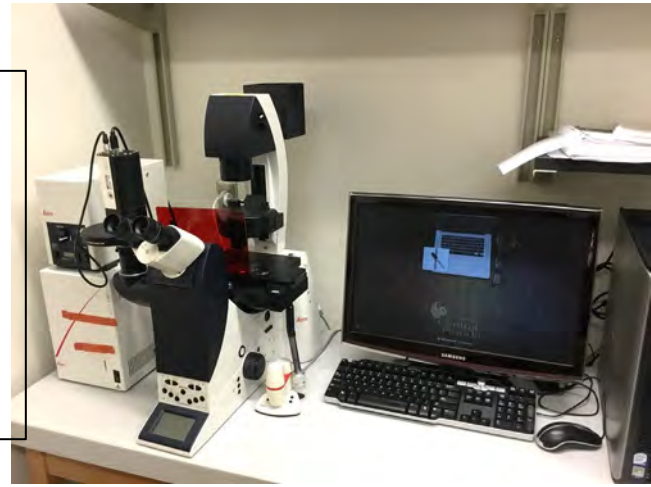


Leica BSBS 356

DMI600
Halogen Light Source (FL)
B/W Leica camera
Automated Stage
5, 10, 20, 40, 60, 100 ×
DAPI, GFP, red x
Leica LAS software

Leica BSBS 456

Halogen Light Source (FL)
20min warmup, cool down
B/W Qcapture camera
4, 10, 20, 40×
DAPI, GFP, TRIC
MicroManager software



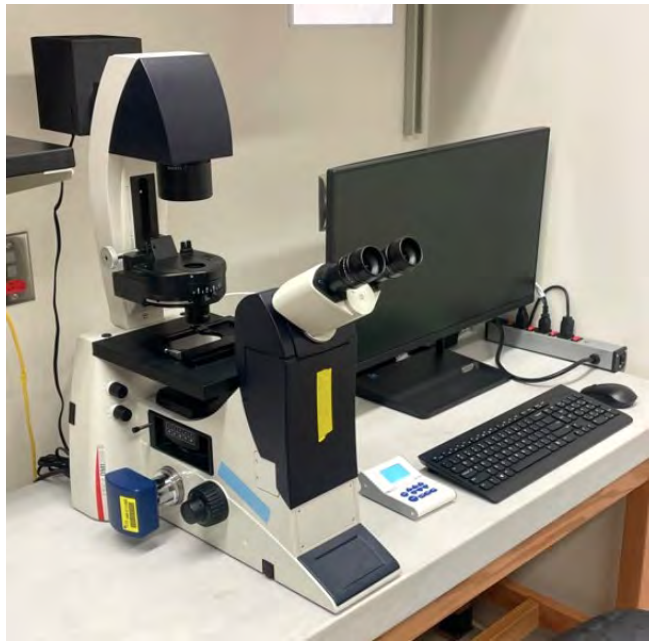
Zeiss B/W Cam BSBS 456

Halogen Light Source (FL)
20min warmup, cool down
B/W Zeiss camera
4, 10, 20, 40, 63, 100×
DAPI, GFP, TRIC
Axiovision software
Lacks overlay



Inverted Fluorescent – Basic

Leica BMS

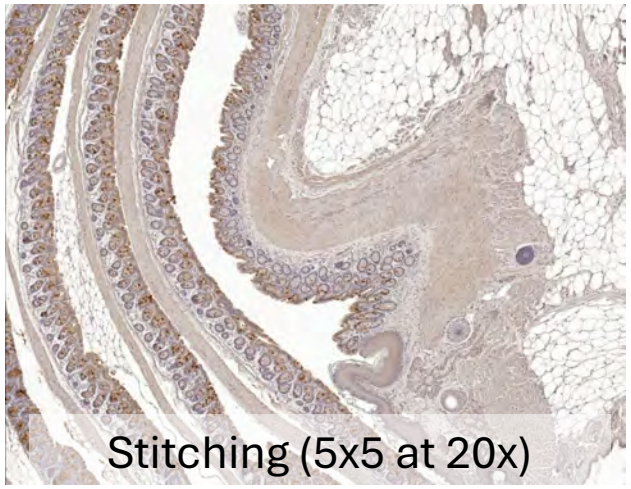


DMI3000B
LED Light Source (FL)
B/W Jenoptiks camera
4, 10, 20, 40, 60, and 100×
DAPI, GFP, TRIC
Manual Stage
Graphax software

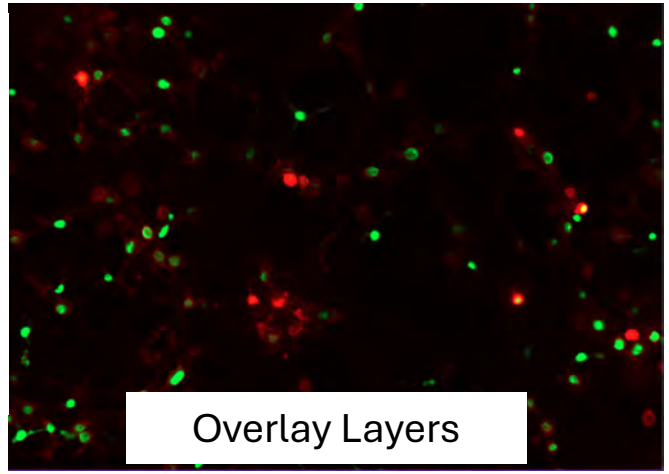
Keyence BZ-X800

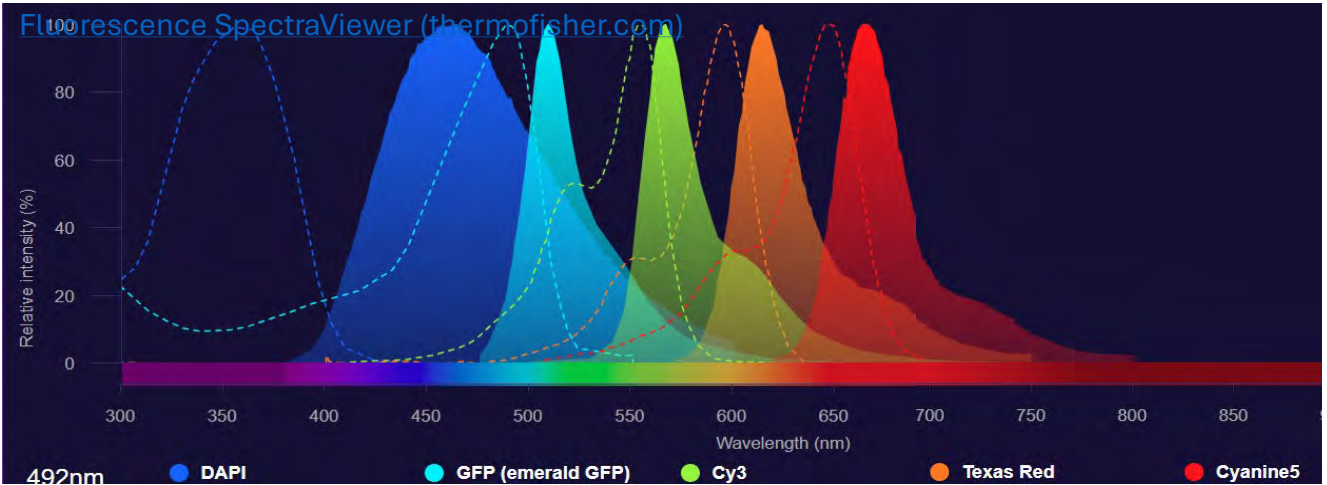
BBS 340 – Most User-Friendly & Versatile

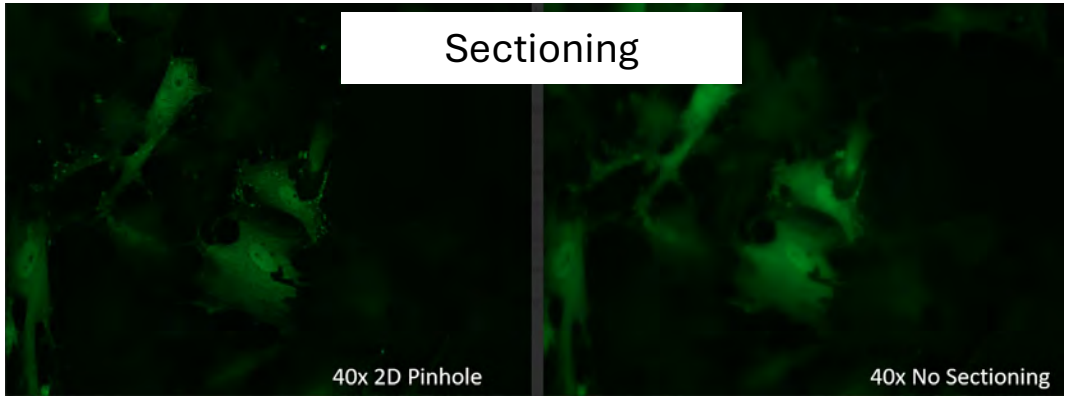
Inverted: Brightfield and Fluorescence:
DAPI, GFP, Cy3, TR, Cy5
2, 10, 20, 40 and 60× objectives; 2nd 20× objective for coverslips
Cell Culture Plates, Slides
Z-Stack and Sectioning; Pinhole light options
Stitching
Live cell imaging/videos* - not ideal
Analysis (decent: cell counts, amount/intensity of colors)


Stitching (5x5 at 20x)




Overlay Layers



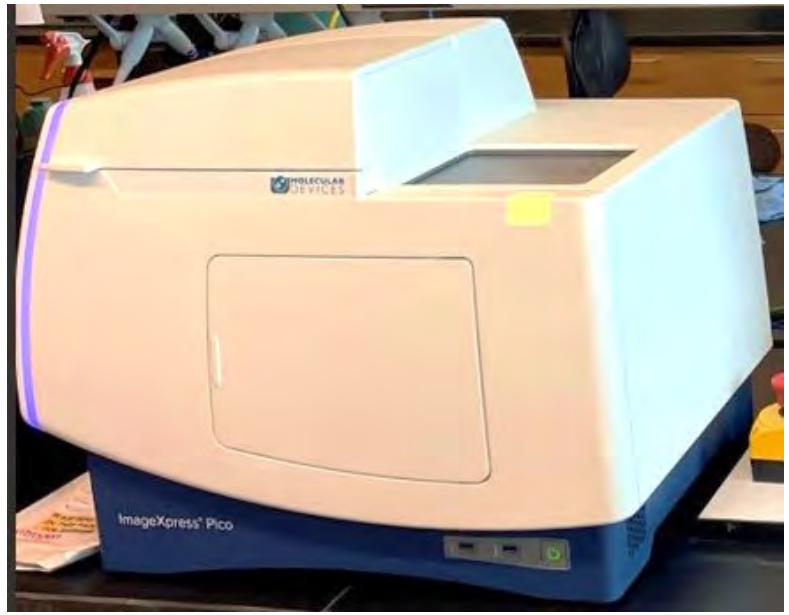


ImageXpress Pico

LNCC A3952

Automation: single plate, up to 4 slides
Brightfield (true color) and Fluorescence
DAPI, GFP, TR, Cy5
4, 10, 20, and 40× objectives
    40x: dry with correction collar – check each time!
Cell Culture Plates, Slides
Z-Stacking (no projections- flattens)
Stitching
Time Courses
Temperature controlled; CO_2 and humidity if using chamber cassette
Analysis: not perfect, but better than by eye (mostly): cell counts, amount/intensity of colors

Exporting of data = extra time

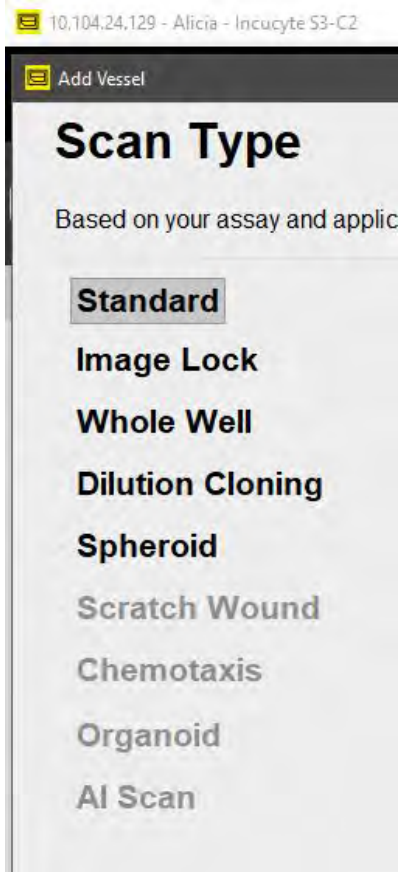


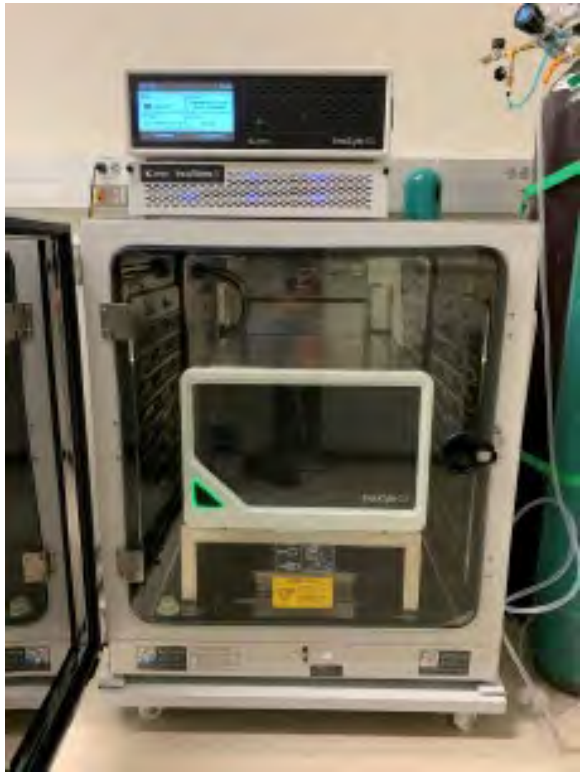
IncuCyte S3

BBS 557

Automation: 6 plates (6-384-well)
Fluorescence / Phase
1 optical module: Red/Green
4, 10, and 20× objectives
Time Courses
Analysis

[Sartorius - IncuCyte S3]





IncuCyte SX5

LNCC A3952

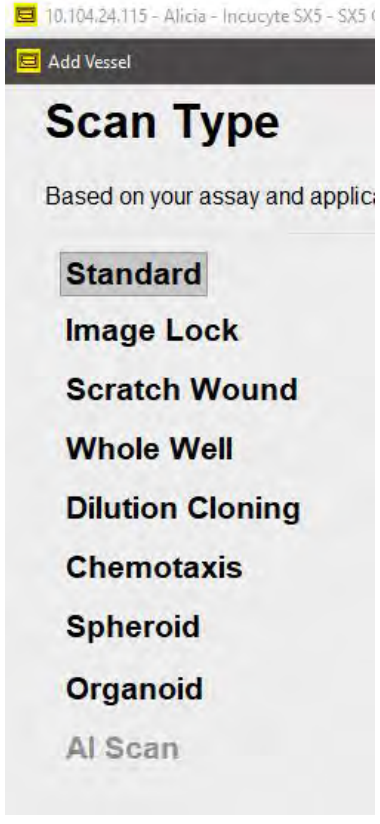
Automation: 6 plates (6-384-well)
Fluorescence / Phase
3 separate optical modules:
1. Red/Green
2. G/O/NIR
3. Metabolism (NIR)

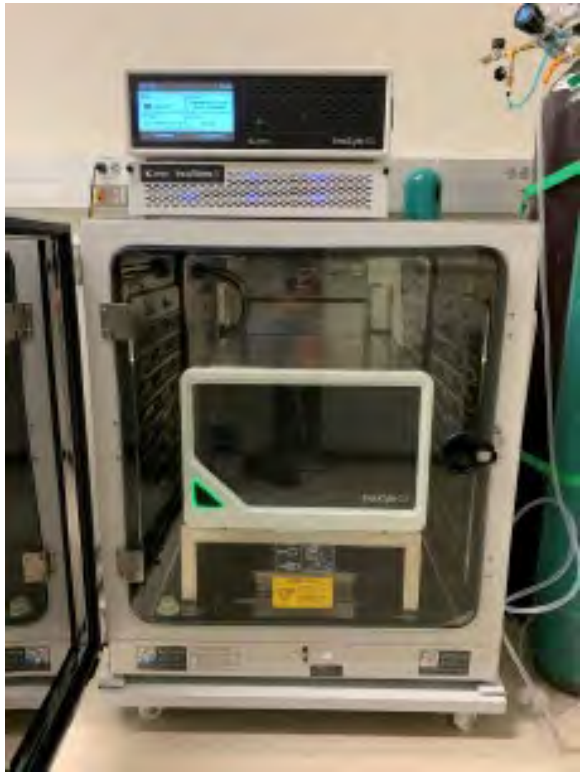
4, 10, and 20× objectives
Time Courses
Analysis

[Sartorius - IncuCyte SX5]

Optical Modules

	Channel	Excitation	Emission
G/O/NIR (standard)	Green Orange NIR	453-485 nm 546-568 nm 648-674 nm	494-533 nm 576-639 nm 685-756 nm
G/R (optional: Cat. No. 4832)	Green Red	441-481 nm 567-607 nm	503-544 nm 622-704 nm
Metabolism (optional: Cat. No. 4820)	ATP	473-498 nm 524-550 nm	565-591 nm
	Orange NIR	524-550 nm 648-674 nm	565-591 nm 685-756 nm





MACSima

BBS 557

Ultrahigh-content:
Cyclic stainer and imager
Deep phenotyping, biomarker research; 5-100+ antibodies on a single sample, plus RNASky

Powerful analysis software:
MACSIQ

Slides + Chambers
Specialty Dishes

Validated antibodies for MICS

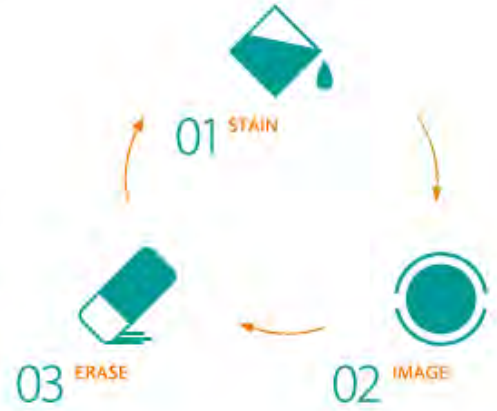
Specific staining – conclusive results

If you want to achieve high reproducibility and error-free analysis, rely on recombinant antibodies.

- World's largest portfolio of antibodies for ultrahigh-content imaging
- Recombinant antibodies specifically validated for MICS technology
- Tested for compatibility with FFPE-, PFA-, or acetone-fixed samples of human or mouse origin
- Lot-consistent and reproducible results due to sophisticated recombinant antibody technologies

The two mechanisms for signal erasure

After staining with fluorochrome-conjugated antibodies (01) and image acquisition of the stained sample (02), the fluorescent signal can be erased by either of the two mechanisms shown below.



03 ERASE BY PHOTOBLEACHING

The fluorescence signal of samples that were stained with fluorochrome-conjugated antibodies, such as our recombinant REAfinity™ Antibodies coupled to non-photostable fluorochromes, can be erased via photobleaching.

03 ERASE BY FLUOROCHROME RELEASE

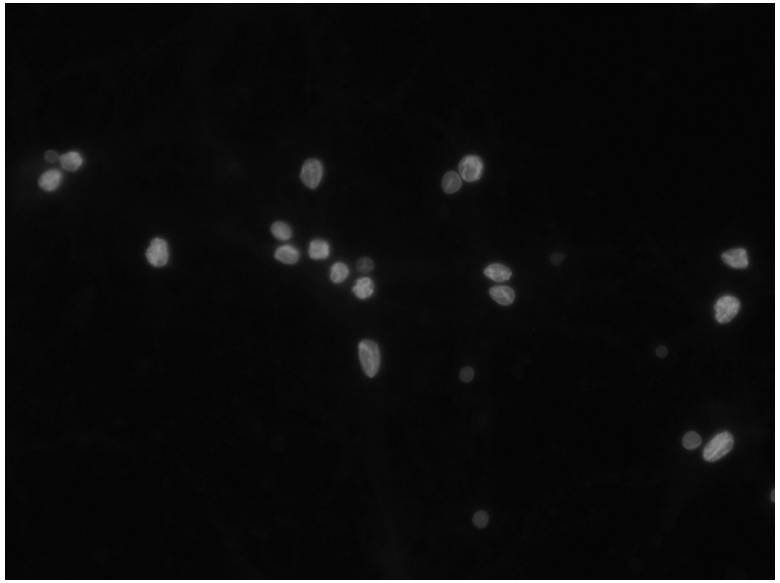
Staining of samples with REAdye_lease™ and REAlease® Fluorochrome-Conjugated Antibody complexes allows for fast and gentle signal erasure via a controlled release of fluorochromes.



REAfinity Recombinant Antibodies

REAdye lease Releasable Fluorochromes

REAlease Releasable Antibodies



Zeiss B/W Cam BSBS 456

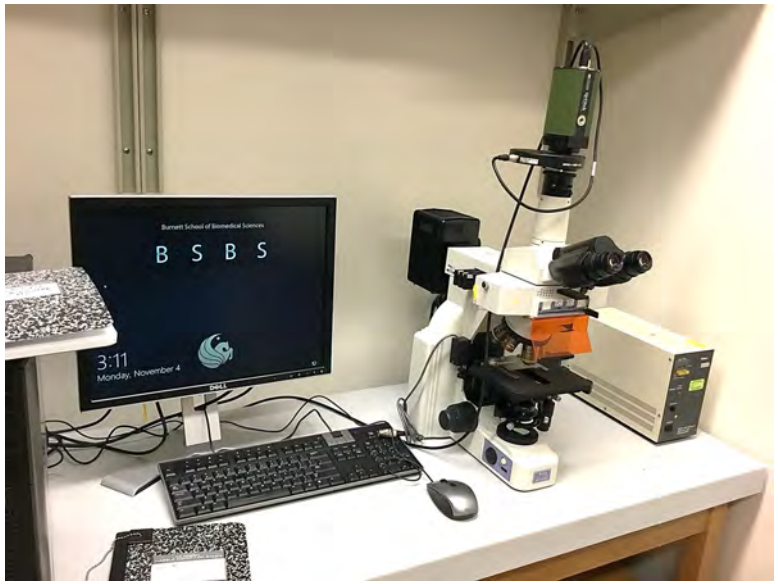
Upright – Basic



Leica DM2000
Brightfield; Upright
BSBS 125
Jenoptiks Color Camera
5, 10, 20 and 40×
Graphax Software



Zeiss Axio Scope A1
BSBS 340
Fluorescent; Upright
B/W Camera (FL)
10, 20, 40 and 100×
Zen Software



Nikon Eclipse E400
BSBS 356 – to 417?
Fluorescent; Upright
B/W Camera (FL)
2.5, 4, 10, 40, 100×
Nikon Elements Software



Zeiss – Valencia- Axioscope 5
LED Light Source (FL)
B/W Jenoptiks camera
More Objectives available:
10, 40, 100x2
4, 20 and 63x Zeiss in storage
DAPI, GFP, mCherry/TR
Needs Computer Graphax software

Keyence VHX 7000

BBS 256

Upright Brightfield – 3D Topography
Image objects
Stitching
Measurements
Particle counting
Videos

Functional Head with 3 objectives with optical zoom: 20 (20-100×); 100 (100-500×); 500 (500 to 2500×)

CAN have Fluorescent attachments (NightSea) Not purchased

Not Networked; bring drive



Gel Doc Systems – BSBS Lake Nona

(2) Licor Odyssey units for 700/800 NIR also present

B-R Gel Doc EZ

Bio-Rad Chemidoc MP



Licor Odyssey M



Thermo iBright



Azure 600





Feature	ChemiDoc MP	Odyssey M	iBright	Azure 600	Film
Fluorescence*	**	**	**	***	
Chemiluminescence	***	*	**	***	***
User-Friendly	***	**	**	***	*
Speed	***	*	**	**	*
Camera	B/W	B/W	B/W	Color + B/W	NA
Maintenance/ Service	*	*	**	***	on us
Cost	$37,000	$80,000		$34,578	$$$

*Odyssey M and Azure 600 have laser diode scanning for fluorescent imaging

Gel Doc Systems – BSBS Lake Nona

Bio-Rad Chemidoc MP and EZ Doc

EZ: BBS 207
MP: BBS 470:
Chemiluminence, FL, NIR, UV







MP: Works great when it works. Fails frequently: too many sensors, mirrors
EZ: smaller format

Azure 600

BBS 557 (BBS and LNCC)
Camera: 9.1mp CCD
In Vivo Imaging







Excellent instrument, no preview with door open; detects Cy3 better than the other instruments here, more versatile.

Licor Odyssey M

Light Sources
- RGB LED (trans-illumination)
- RGB LED (reflective illumination)
- Solid-state diode laser at 488 nm
- Solid-state diode laser at 520 nm
- Solid-state diode laser at 685 nm
- Solid-state diode laser at 785 nm



BBS 557 to LNCC

Licor Odyssey

Camera: ?mp CCD
Laser Diode: 685 and 785nm



BBS 270

Slow, analysis software does not work on networked devices (only 1 license key). For FL = good, but not as good as the Azure; worst at chemiluminescence

Gel Doc Systems – BSBS Lake Nona



Cytiva Typhoon IP
Storage Phosphor Screen

BBS 371

BAS-IP retains energy produced by ionizing radiation from isotopes (^{32}P); ~1/10 exposure compared to film

• Versatility: use one system to image multifluorescent-, radioisotope-labeled, and colorimetric samples on gels, membranes, multi-well plates, culture dishes, glass slides, and tissue sections. The IP model is for phosphor imaging only but can be upgraded

• Accurate quantitation: detect signals from as low as **3 pg of protein** and differences across a dynamic range with greater than five orders of magnitude

• High resolution: resolve fine details in your sample with a pixel resolution of as low as 10 µm

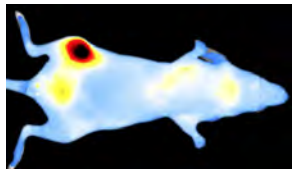
• High sample throughput: large scanning area of 40 × 46 cm enables you to simultaneously image up to 20 gels or blots, measuring 10 × 8 cm in size. It is also possible to scan up to 9 multiwell plates in a single scan. This throughput facilitates comparisons among blots and plates, reduces workload, and decreases waiting time. The IP model has a scanning area of 35 x 43 cm, which fits Cytiva's largest imaging plate

Imaging Systems – in vivo

IVIS



IVIS: in vivo large format CCD camera; imaging chamber housing, a heated, moveable Platform, filter wheel, LEDs.



Vevo 3100



Ultrasound:
Probes/Transducers:
Small tumors/items: MX250
Pregnancy checks: MX550D



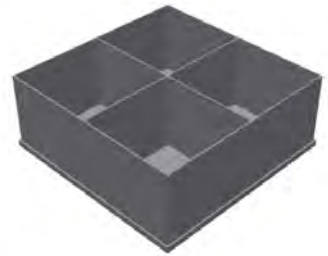
invivoXtreme



X-Ray; needs work to get it back working. Not straightforward to use

AnyMaze

ANY-maze
Behavioral Tracking Software



Software and camera system used for many behavioral set ups, including open field monitoring

Plate Readers



PerkinElmer Envision
BBS 270 and 470
filter based: most accurate for specific protocols (narrow bandwidths), no heat, does shake, special assays DELFIA, Alphascreen; least user-friendly – see quick start guide. Luminescence not recommended due to lens on aperture sits on top of the plate and gets splashed frequently



Biotek Synergy H1M in BBS270; LNCC A3770
230-999nm monochromator; heat, shaking
Full-spectrum read out down to 1nm steps



Biotek Synergy NEO2 BBS 557: fast for kinetic assays (6 vs 30s 96-well reads): monochromator 230-1000nm; Full-spectrum read out down to 1nm steps;
heat, shaking, injection;
Take3 microvolume analysis (Nanodrop) 48-position



SpectraMax iD5
BBS 557: monochromator 230-1000nm; Full-spectrum read out down to 1nm steps;
heat, shaking, injection;
24-place nanodrop plate, some filters for specialty scans

All these plate readers can read Absorbance, Fluorescence and Luminescence
All have shaking – please note sample volume and shaking level compatibility