BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

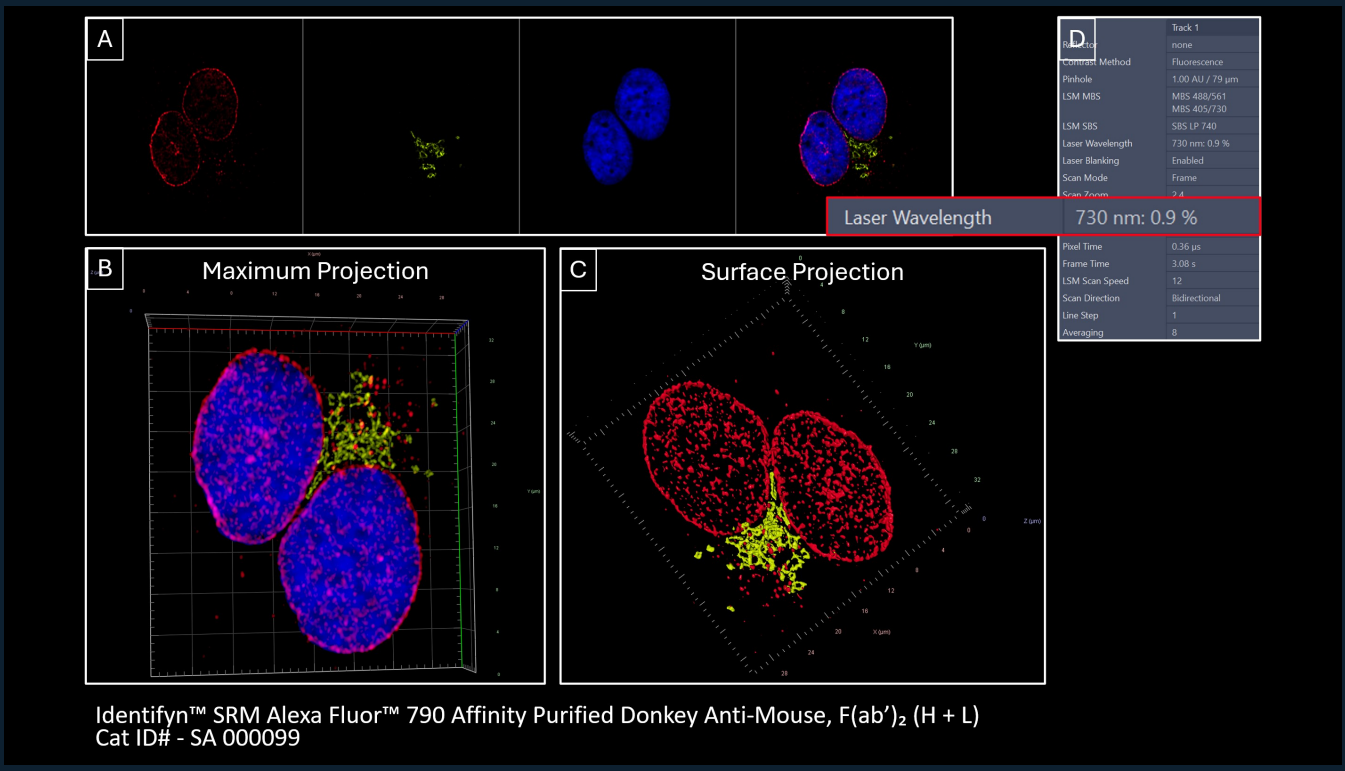
IDENTIFYN RESOLUTION STEPDOWN

SECONDARY ANTIBODY MICROSCOPY EVIDENCE ASSESSMENT

Identifyn™ SRM Alexa Fluor™ 790 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)

WESTERN BLOT -> WIDEFIELD -> WIDEFIELD SIM / APOTOME -> CONFOCAL

4	1	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / CONFOCAL

SA-000099 | PRODUCT-LEVEL EVIDENCE DOSSIER

SOURCE-GROUNDED MICROSCOPY + BIOLOGICAL REVIEW / IDENTIFYN EVIDENCE PRESERVED

CRO CAPABILITY DEMONSTRATED

Identifyn performed the experimental and imaging work documented below. BENNETT converts that operating history into a governed, source-bounded scientific record.

- Source-documented sample preparation and antibody/reagent workflow
- Western blot imaging
- Widefield fluorescence microscopy
- Widefield structured illumination / Apotome optical sectioning
- Confocal microscopy
- Preserved control experiment
- Treatment-response experimental workflow
- Z-stack / 3D acquisition
- Multi-channel imaging

SCIENTIFIC QUESTION

What source-bounded experimental and microscopy evidence is preserved for Identifyn™ SRM Alexa Fluor™ 790 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L), and what is the strongest interpretation allowed by the available modalities and controls?

EVIDENCE AVAILABLE

Western blot -> Widefield -> Widefield SIM — Apotome -> Confocal -> Control

BENNETT ASSESSMENT

The preserved SA-000099 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal. The historical boundary reflects the validated application available for the specific conjugate, deployed platform and workflow, not a universal scientific impossibility.

BENNETT / MICROSCOPY AND BIOLOGICAL EVIDENCE REVIEW

SOURCE-GROUNDED RETROSPECTIVE REVIEW / PENDING SCIENTIST REVIEW

SOURCE STATE	SOURCE HASH VERIFIED / EVENT RECORDED
BENNETT EVIDENCE REVIEW	COMPLETED / SOURCE-BOUNDED
NATIVE IMAGE REPROCESSING	NOT PERFORMED
LITERATURE SOURCES	INDEPENDENTLY VERIFIED
SCIENTIST REVIEW	PENDING
PUBLICATION	NOT AUTHORIZED

OVERALL CALL / NIR-BOUNDED PARTIAL STEPDOWN

The preserved SA-000099 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal. The historical boundary reflects the validated application available for the specific conjugate, deployed platform and workflow, not a universal scientific impossibility.

SETTINGS ASSESSMENT

The record preserves 4 ordered evidence tier(s). Structured source metadata values are reported by stage and remain tied to the original method objects.

BIOLOGICAL SUPPORT

The record supports retrospective, source-bounded microscopy review. Target identity, specificity, treatment effect, binding, interaction and mechanism are not inferred from presentation images alone.

CONTROL ASSESSMENT

A true preserved control record is present and appears last.

CLAIM CEILING

This draft supports historical capability documentation, source registration, modality ordering, settings review and bounded interpretation. It does not independently validate antibody specificity, quantify a population response, establish binding, interaction, causation or mechanism, or authorize publication.

PROCESSING DISCLOSURE

Retrospective BENNETT architectural evaluation of preserved Identifyn source evidence. BENNETT did not perform native-file reprocessing, new deterministic measurement or the historical image post-processing represented in this record.

REAGENTS AND ACQUIRED CHANNELS

Preparation reagents are reported separately from channels actually documented in the acquisition settings. Fluorophores mentioned in staining are not automatically treated as acquired channels.

EVIDENCE OBJECT	REAGENTS / FLUOROPHORES PRESENT	CHANNELS ACTUALLY ACQUIRED
Western blot	790	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 532; 555; 790	3; AF750-49007; AF532-49014; 49 DAPI; 672 - 748; 515 - 545; 335 - 383; 765 - 855
Widefield SIM — Apotome	405/DAPI; 532; 555; 790	3; AF750-49007; AF532-49014; 49 DAPI; 672 - 748; 515 - 545; 335 - 383; 765 - 855
Confocal	405/DAPI; 488; 532; 790	3; None; 730 nm @0.9%; 488 nm @0.1%; 405 nm @0.2%; 789; 528; 353
Control	405/DAPI; 790	2; None; 730 nm @0.9%; 405 nm @0.2%; 789; 353; 794; 465

MODALITY ASSESSMENT / PART 1 OF 5

Western blot / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Azure Sapphire FL. Objective: not explicitly stated. Platform: Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s). Structured source metadata values: 11.

VISIBLE OBSERVATION

A preserved presentation image is available for Western blot. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Western blot, documents platform context as Azure Sapphire FL, and records detected dye/channel descriptors as 790.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy and antibody-validation guidance that presentation evidence must remain bounded by modality, controls and source documentation.

INTERPRETIVE LIMIT

Band position and displayed intensity do not independently establish analytical specificity, target identity, linearity, or treatment effect.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Western blot evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 2 OF 5

Widefield / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63X/1.25 oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Hamamatsu ORCA-Quest, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Channels: 3; Scaling (Per Pixel): 0.073 µm X 0.073 µm; Image size (Pixels): 1920 X 1515; Image Size (Pixels): 1920 X 1515; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Hamamatsu ORCA-Quest. Timing: Exposure Time: 350 ms; 50 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @35.00%; X-Cite Xylis Lamp Intensity @5.00%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 40.

VISIBLE OBSERVATION

A preserved presentation image is available for Widefield. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Widefield, documents platform context as ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 532; 555; 790.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy and antibody-validation guidance that presentation evidence must remain bounded by modality, controls and source documentation.

INTERPRETIVE LIMIT

A presentation image supports gross localization and source-bounded co-occurrence, not direct binding, molecular colocalization, or native quantitative measurement.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Widefield evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 3 OF 5

Widefield SIM — Apotome / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63X/1.25 oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Hamamatsu ORCA-Quest, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Z-Stack: 89 Slices (9.6 µm); Channels: 3; Scaling (Per Pixel): 0.073 µm X 0.073 µm X 0.100 µm; Image size (Pixels): 1986 X 1429; Image Size (Pixels): 1986 X 1429; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Hamamatsu ORCA-Quest. Timing: Exposure Time: 300 ms; 100 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @30.00%; X-Cite Xylis Lamp Intensity @10.00%. Optical/scan: Effective NA: 1.25. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 64.

VISIBLE OBSERVATION

A preserved presentation image is available for Widefield SIM — Apotome. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Widefield SIM — Apotome, documents platform context as ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 532; 555; 790.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy and antibody-validation guidance that presentation evidence must remain bounded by modality, controls and source documentation.

INTERPRETIVE LIMIT

Improved optical sectioning does not by itself establish reconstructed super-resolution or molecular interaction.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Widefield SIM — Apotome evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 4 OF 5

Confocal / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 181 Slices (5.4 µm); Channels: 3; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 528 X 583; 930 X 930; Image Size (Pixels): 528 X 583; 930 X 930; Bit Depth: 16 Bit. Detector/camera: Imaging Device: NIR Ch2; GaAsP-Pmt3; Detector Type: GaAs-PMT; GaAsP-PMT. Timing: Pixel Time: 0.36 µs; Frame Time: 3.08 s. Illumination: Laser Wavelength: 730 nm @0.9%; 488 nm @0.1%. Optical/scan: Pinhole: 1.00 AU / 79 µm; 1.00 AU / 53 µm; Scan Zoom: 2.4; LSM Scan Speed: 12; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 109%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; LSM Plus Mode: 7.4 (3D, Auto); 8.0 (3D, Auto). Structured source metadata values: 63.

VISIBLE OBSERVATION

A preserved presentation image is available for Confocal. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Confocal, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 532; 790.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy and antibody-validation guidance that presentation evidence must remain bounded by modality, controls and source documentation.

INTERPRETIVE LIMIT

Optical sectioning and source-bounded 3D presentation do not establish binding, mechanism, or population-level effect.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Confocal evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 5 OF 5

Control / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:. Acquisition: Channels: 2; Scaling (Per Pixel): 0.059 µm X 0.059 µm; Image size (Pixels): 1884 X 1884; Image Size (Pixels): 1884 X 1884; Bit Depth: 16 Bit. Detector/camera: Imaging Device: NIR Ch2; GaAsP-Pmt3; Detector Type: GaAs-PMT; GaAsP-PMT. Timing: Pixel Time: 0.23 µs; Frame Time: 7.77 s. Illumination: Laser Wavelength: 730 nm @0.9%; 405 nm @0.2%. Optical/scan: Pinhole: 1.00 AU / 79 µm; 1.00 AU / 44 µm; Scan Zoom: 1.2; LSM Scan Speed: 10; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: 6.8 (3D, Auto); 6.1 (3D, Auto). Structured source metadata values: 46.

VISIBLE OBSERVATION

A preserved presentation image is available for Control. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Control, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 790.

LITERATURE CORRELATION

Consistent with peer-reviewed antibody-control guidance that a no-primary or related control bounds background but is not a complete specificity system.

INTERPRETIVE LIMIT

A control bounds the recorded comparison only; it does not establish universal specificity, zero background, zero cross-reactivity, or absence of free dye.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Control evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

CROSS-MODALITY SYNTHESIS

The preserved SA-000099 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal. The historical boundary reflects the validated application available for the specific conjugate, deployed platform and workflow, not a universal scientific impossibility.

Different acquisitions are not treated as a quantitative intensity ladder. Any continuity statement remains qualitative and source bounded.

BENNETT LITERATURE CORRELATION — SOURCES INDEPENDENTLY VERIFIED

BENNETT uses independently verified primary scientific sources to test whether the interpretation is scientifically consistent and properly bounded. Literature correlation does not retroactively validate the Identifyn dataset or replace scientist review.

- Antibody and microscopy evidence require orthogonal controls and modality-appropriate interpretation; presentation images alone do not establish analytical specificity.

- Advanced-resolution presentation depends on acquisition, reconstruction or localization quality control before numerical resolution, molecular dimensions or cluster identity can be claimed.

REFERENCES

1. Uhlen M, et al. A proposal for validation of antibodies. Nat Methods. 2016;13:823-827. doi:10.1038/nmeth.3995.
2. Gustafsson MGL. Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy. J Microsc. 2000;198:82-87. doi:10.1046/j.1365-2818.2000.00710.x.
3. Sage D, et al. Quantitative evaluation of software packages for single-molecule localization microscopy. Nat Methods. 2015;12:717-724. doi:10.1038/nmeth.3442.

RELEASE BOUNDARY

This assessment remains a draft pending scientist review. No publication is authorized. Any future native-file analysis must enter BENNETT as a new evidence snapshot with routed engine authority, native-data QA, methods, limitations, and an independently reviewed claim ceiling.

SOURCE RECONCILIATION / QA / PROVENANCE

SOURCE STATUS - PRESERVED / AUTO-RECONCILED

Historical Identifyn source material was subjected to BENNETT retrospective QA. Original source state and correction history remain preserved in provenance.

HIGH CONFIDENCE - SCIENTIST-AUTHORIZED CORRECTION

Cross-tier / treatment concentration: 200 µM -> Etoposide = 2 µM. The governing scientist identified every 200 µM occurrence as a technician transcription error. The canonical historical concentration is 2 µM. Supporting evidence: Scientist-authorized correction supplied for the Identifyn historical archive; original technician text remains preserved unchanged. Authority: Dr. Brian T. Bennett. Preservation: Original source text and hashes remain unchanged; only the derivative canonical field is corrected.

BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

BENNETT has completed the bounded retrospective assessment.
The following pages switch ownership to the preserved Identifyn record.

IDENTiFYN®

IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

Images, source methods, acquisition settings and provenance follow in the locked Stepdown order. Scientific wording is preserved; missing modalities are not invented.
A preserved control follows all available Stepdown tiers as supporting evidence.

PRODUCT-LEVEL STEPDOWN MAP

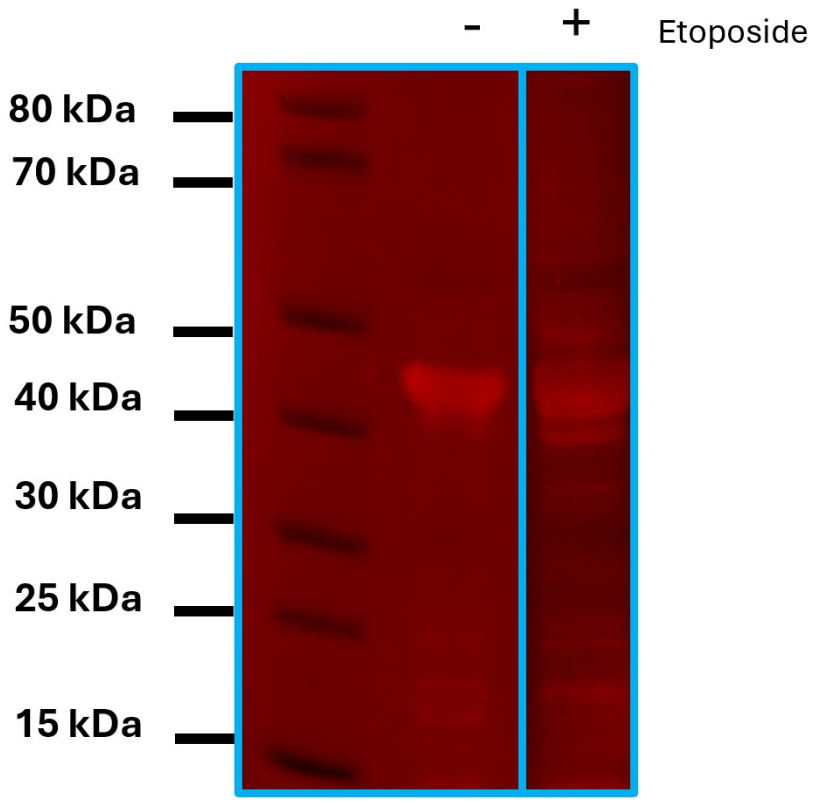
This dossier preserves the complete available evidence progression for SA-000099. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

CANONICAL STEPDOWN TIER	EVIDENCE STAGE	INSTRUMENT	STATUS
1	Western blot	Azure Sapphire FL	PRESERVED
2	Widefield	ZEISS Axio Observer.Z1/7 Apotome	PRESERVED
3	Widefield SIM - Apotome	ZEISS Axio Observer.Z1/7 Apotome	PRESERVED
4	Confocal	ZEISS LSM 980	PRESERVED

INTERPRETIVE BOUNDARY

Ordering evidence by resolution tier does not make the modalities interchangeable. Each stage retains its acquisition environment and scientific limitations. This pilot organizes the record; it does not synthesize new biological claims.

ORIGINAL IMAGE EVIDENCE / WESTERN BLOT



EVIDENCE TIER	Western blot
INSTRUMENT	Azure Sapphire FL
CELL MODEL	HeLa
DETECTED DYES	790
METADATA VALUES	11

STRUCTURED ACQUISITION METADATA / WESTERN BLOT

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Scanner	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Detection mode	Fluorescence
Laser	730nm
Emission Filter	829±62nm
Detector	PMT
Intensity	3
Pixel size	100 µm
Scan Speed	High
Quality	High
Focus	Membrane (0 µm in Z-axis)
Image	K. Small

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000099

HeLa cells were treated with 200 µM etoposide for 20 hours to induce DNA damage. Cells were then homogenized using an ultrasonication probe and RIPA lysis buffer, and then centrifuged to pellet the cell debris The lysate supernatant (100 µg) was mixed with NuPAGE™ LDS Sample Buffer (4X), heated at 80 °C for 10 min, and subjected to electrophoresis using NuPAGE™ 4 to 12% Bis-Tris, 1.0-1.5 mm, Mini Protein Gels in a Mini Gel Tank. The Thermo Fisher's PageRuler™ Prestained Protein Ladder, 10 to 180 kDa, was used as a molecular weight marker.

Post electrophoresis, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane using the iBlot™ 3 Starter Kit PVDF. The transfer membrane was blocked with Blocker™ Casein for 1 hour and then washed five times with PBS. The blocked membrane was incubated with Actin Monoclonal Antibody (MA1-744) for 2 hours, followed by five washes in 1X PBS. Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L) was incubated for 1 hour and washed 5X with PBS. The membrane was dried for 10 minutes at room temperature before imaging.

Scanner = Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)

Scan Settings

Detection mode = Fluorescence
Laser = 730nm
Emission Filter = 829±62nm
Detector = PMT
Intensity = 3
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

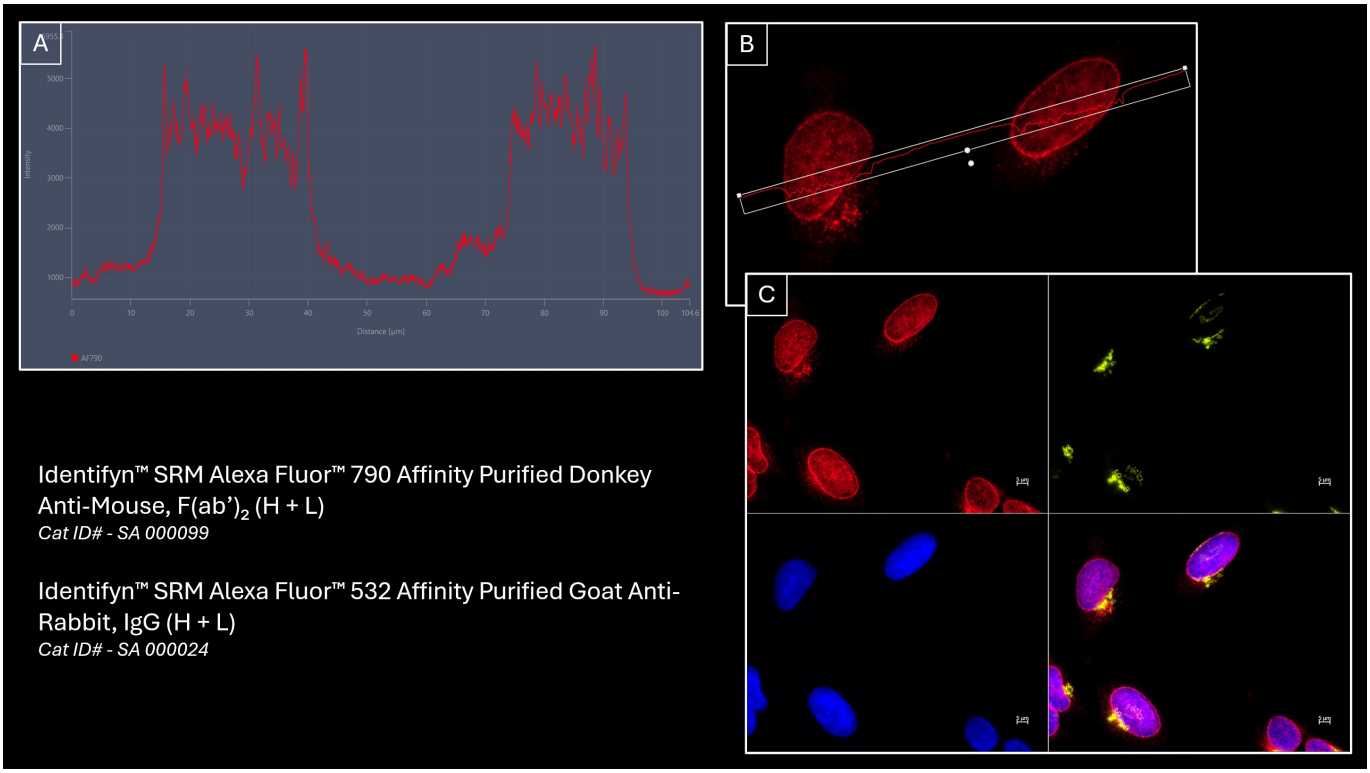
None

Image: K. Small

SOURCE PROVENANCE

Catalog	SA-000099
Stage	Western blot
Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	E248BFD49B5527F66FCB43BEA186E4E4B9A7C0D727B3723685E47B3AEBF46F34
Method PDF SHA-256	C5EC6DA4F8539D71F95BCAA9B0FEF1FF5C121C898F09290B7ECB1F856A8ADD8A

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



Identifyn™ SRM Alexa Fluor™ 790 Affinity Purified Donkey Anti-Mouse, F(ab')$_2$ (H + L)
Cat ID# - SA 000099

Identifyn™ SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000024

EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 532; 555; 790
METADATA VALUES	40

STRUCTURED ACQUISITION METADATA / WIDEFIELD

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Hamamatsu ORCA-Quest, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63X/1.25 oil M27
Channels	3
Scaling (Per Pixel)	0.073 µm X 0.073 µm
Image size (Pixels)	1920 X 1515
Image Size (Scaled)	140.19 µm X 110.62 µm
Bit Depth	16 Bit
Image Center Position	X: 0.00 µm, Y: 0.00 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	AF750-49007 | AF532-49014 | 49 DAPI
Beam Splitter	760 | 550 | 395
Filter Excitation Wavelength	672 - 748 | 515 - 545 | 335 - 383
Filter Emission Wavelength	765 - 855 | 555 - 595 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @35.00% | X-Cite Xylis Lamp Intensity @5.00% | X-Cite Xylis Lamp Intensity @10.00%
Exposure Time	350 ms | 50 ms | 25ms
Excitation Wavelength	752 | 528 | 353
Emission Wavelength	779 | 553 | 465
Effective NA	1.25
Imaging Device	Hamamatsu ORCA-Quest
Camera Adapter	1X
Depth of Focus	1.51 µm | 1.07 µm | 0.90 µm
Binning Mode	1,1
Profile Definition	Stroke Thickness 2.25, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 104.6), Y (Min 564 and Max 5955)

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000099

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000024

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The first primary antibody, Nuclear Pore Complex Monoclonal Antibody (39C7), was incubated overnight at 4°C at a dilution of 1:100 Cells were washed 3X in PBS, and Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Donkey Anti-Mouse, F('b')□ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, Golgi GOLPH2 Polyclonal Antibody, was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Hamamatsu ORCA-Quest, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Single Plane (2D)

Channels = 3
Scaling (Per Pixel) = 0.073 µm X 0.073 µm
Image size (Pixels) = 1920 X 1515
Image Size (Scaled) = 140.19 µm X 110.62 µm
Bit Depth = 16 Bit
Image Center Position = X: 0.00 µm, Y: 0.00 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

790 -

Reflector = AF750-49007
Beam Splitter = 760
Filter Excitation Wavelength = 672 - 748
Filter Emission Wavelength = 765 - 855
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @35.00%
Exposure Time = 350 ms
Excitation Wavelength = 752
Emission Wavelength = 779
Effective NA = 1.25
Imaging Device = Hamamatsu ORCA-Quest
Camera Adapter = 1X
Depth of Focus = 1.51 µm
Binning Mode = 1,1

532 -

Reflector = AF532-49014
Beam Splitter = 550
Filter Excitation Wavelength = 515 - 545
Filter Emission Wavelength = 555 - 595
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @5.00%
Exposure Time = 50 ms
Excitation Wavelength = 528
Emission Wavelength = 553
Effective NA = 1.25
Imaging Device = Hamamatsu ORCA-Quest
Camera Adapter = 1X
Depth of Focus = 1.07 µm
Binning Mode = 1,1

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @10.00%
Exposure Time = 25ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = Hamamatsu ORCA-Quest
Camera Adapter = 1X
Depth of Focus = 0.90 µm
Binning Mode = 1,1

Profile View Display - Panels A & B

Profile Definition = Stroke Thickness 2.25, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 104.6), Y (Min 564 and Max 5955)

Split View - Panel C

The top-left panel features nuclear pore complexes visualized with Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L), the top-right panel features Golgi visualized with Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Rabbit, IgG (H + L), the bottom-left panel shows DAPI nuclear staining, and the bottom-right panel is the merged image of all channels.

Image Correction

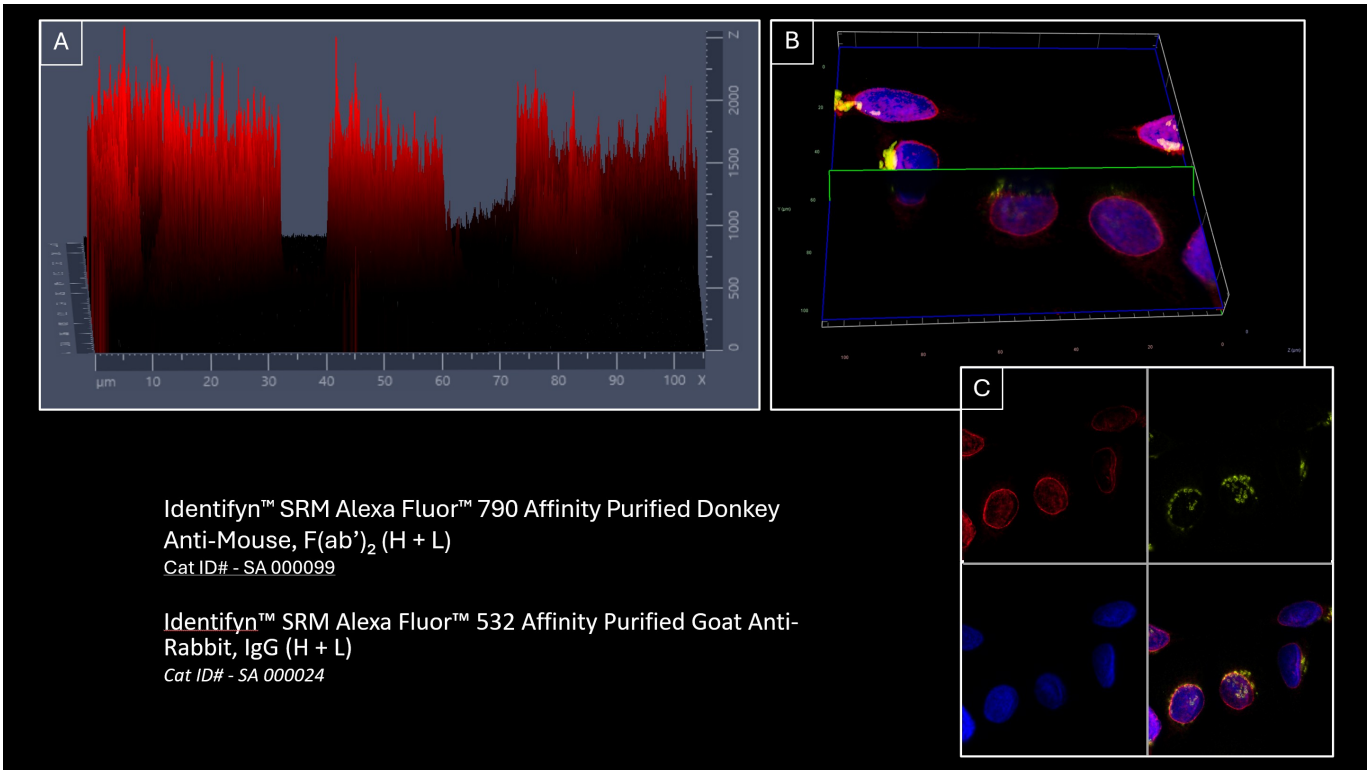
None

Image by E.F. Simon
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

SOURCE PROVENANCE

Catalog	SA-000099
Stage	Widefield
Instrument	ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Hamamatsu ORCA-Quest, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	40
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	302A0AFE0B37B1227DF60E9CB5FD828FF34F3B1F1559AA8E3915A2F1B32B2B01
Method PDF SHA-256	0C6C98621DF218F1F1A04E787B29FEB262A3CD545DA85BBCF52A28CE9ACC282E

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 532; 555; 790
METADATA VALUES	64

STRUCTURED ACQUISITION METADATA / WIDEFIELD SIM - APOTOME

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Hamamatsu ORCA-Quest, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63X/1.25 oil M27
Z-Stack	89 Slices (9.6 µm)
Channels	3
Scaling (Per Pixel)	0.073 µm X 0.073 µm X 0.100 µm
Image Size (Pixels)	1986 X 1429
Image Size (Scaled)	145.01 µm X 104.34 µm
Bit Depth	16 Bit
Image Center Position	X: 0.00 mm, Y: 0.00 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	AF750-49007 | AF532-49014 | 49 DAPI
Beam Splitter	760 | 550 | 395
Filter Excitation Wavelength	672 - 748 | 515 - 545 | 335 - 383
Filter Emission Wavelength	765 - 855 | 555 - 595 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @30.00% | X-Cite Xylis Lamp Intensity @10.00%
Exposure Time	300 ms | 100 ms | 25 ms
Excitation Wavelength	752 | 528 | 353
Emission Wavelength	779 | 553 | 465
Effective NA	1.25
Imaging Device	Hamamatsu ORCA-Quest
Camera Adapter	1X
Depth of Focus	1.51 µm | 1.07 µm | 0.90 µm
Binning Mode	1,1 | 2,2
Apotome SIM Image Processing	Display Mod" "Optical Sectioning" - enabled correction selected.
Render Mode	Surface
Grid distance	1
Angle X	580
Angle Y	0
Z Scaling	1.0
Ambient	50
Specular	20
Shininess	50

FIELD	SOURCE-DOCUMENTED VALUE
Light Height	2.5
3D Volume Projection	Precise
Appearance	Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
Clipping Image Process	Clipping planes are introduced to pronounce regions of special interest.
X-Z	Activate was selected, textured opaque was chosen, and a blue outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip	-20% | -38%
Clip X	45° | 0°
Clip Z	0°
X-Y	Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Clip Y	0°

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000099

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000024

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The first primary antibody, Nuclear Pore Complex Monoclonal Antibody (39C7), was incubated overnight at 4°C at a dilution of 1:100 Cells were washed 3X in PBS, and Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Donkey Anti-Mouse, F('b')□ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, Golgi GOLPH2 Polyclonal Antibody, was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:100 . Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Hamamatsu ORCA-Quest, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Single Plane (2D) - Z-plane 45 of 89 total - Panel C

Z Stack - (3D) - Panels A & B

Z-Stack = 89 Slices (9.6 µm)

Channels = 3
Scaling (Per Pixel) = 0.073 µm X 0.073 µm X 0.100 µm
Image Size (Pixels) = 1986 X 1429
Image Size (Scaled) = 145.01 µm X 104.34 µm
Bit Depth = 16 Bit
Image Center Position = X: 0.00 mm, Y: 0.00 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

790 -

Reflector = AF750-49007
Beam Splitter = 760
Filter Excitation Wavelength = 672 - 748
Filter Emission Wavelength = 765 - 855
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @30.00%
Exposure Time = 300 ms
Excitation Wavelength = 752
Emission Wavelength = 779
Effective NA = 1.25
Imaging Device = Hamamatsu ORCA-Quest
Camera Adapter = 1X
Depth of Focus = 1.51 µm
Binning Mode = 1,1

532 -

Reflector = AF532-49014
Beam Splitter = 550
Filter Excitation Wavelength = 515 - 545
Filter Emission Wavelength = 555 - 595
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @10.00%
Exposure Time = 100 ms
Excitation Wavelength = 528
Emission Wavelength = 553
Effective NA = 1.25
Imaging Device = Hamamatsu ORCA-Quest
Camera Adapter = 1X
Depth of Focus = 1.07 µm
Binning Mode = 1,1

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @10.00%
Exposure Time = 25 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = Hamamatsu ORCA-Quest
Camera Adapter = 1X
Depth of Focus = 0.90 µm
Binning Mode = 2,2

Post Processing - SIM Processing

Apotome SIM Image Processing = Display Mod" "Optical Sectioning" - enabled correction selected. Normalization, Phase Corrector, Fourier Filter, and Deconvolution were NOT used.

2.5D Image Processing - Panel A

2.5D - Zeiss Zen Software converts a two-dimensional image into a height map, known as a 2.5D Image. The most significant extension in the Z-direction represents the intensity values, creating a pseudo-three-dimensional image that displays the fluorescent localization or colocalization of the collected signal, along with its intensity in arbitrary units Here we show the fluorescent intensity of the Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Rabbit Anti-Mouse, F('b')□ (H + L) along with DAPI a to show that is shares similar intensities.

2.5D Display

Render Mode = Surface
Grid distance = 1
Palette and Show Faces at Side are both Selected

2.5D Display Options

Angle X = 580
Angle Y = 0
Z Scaling = 1.0
Ambient = 50
Specular = 20
Shininess = 50
Light Height = 2.5

3D Image Processing - Panel B

3D Volume Projection = Precise
Appearance = Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process = Clipping planes are introduced to pronounce regions of special interest.

X-Z = Activate was selected, textured opaque was chosen, and a blue outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = -20%
Clip X = 45°
Clip Z = 0°

X-Y = Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = -38%
Clip X = 0°
Clip Y = 0°

Image Correction

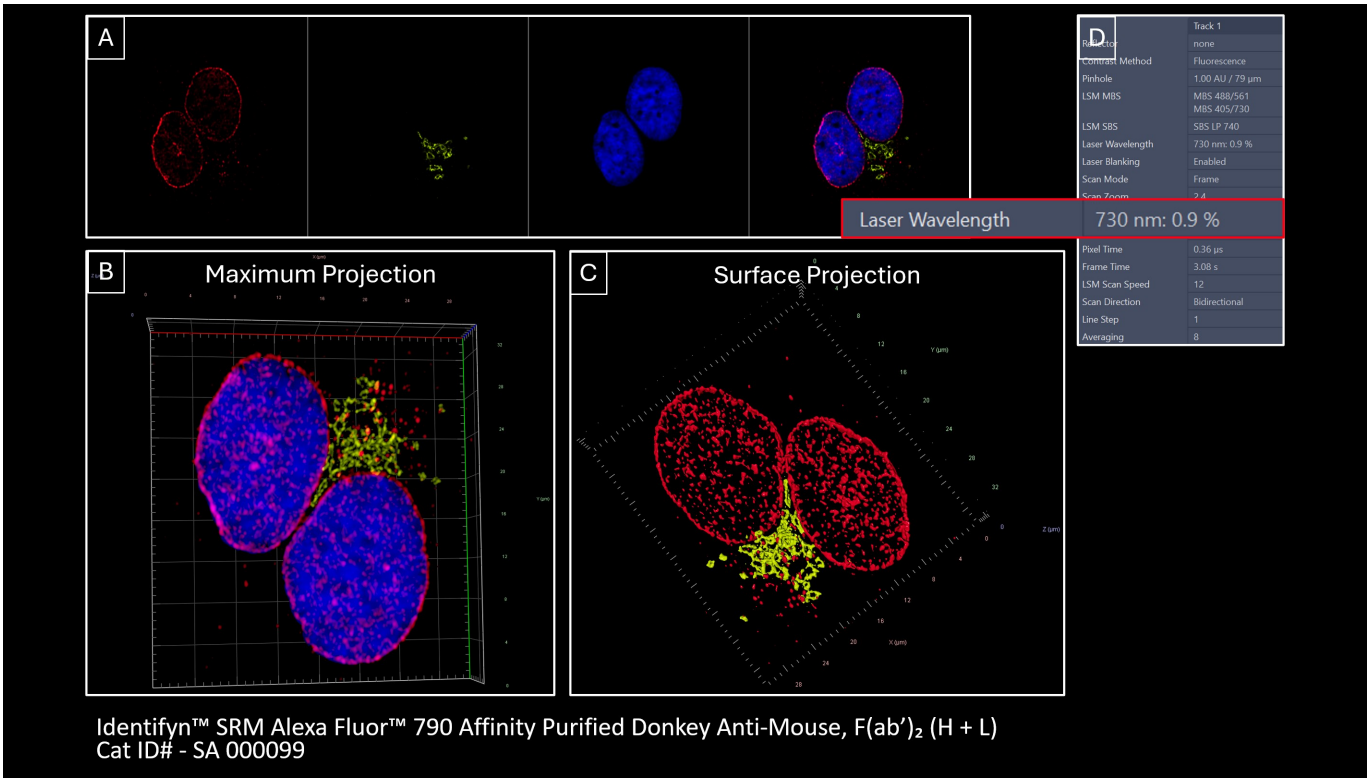
None

Image by E.F. Simon
Image Analysis by B.T. Bennett
Data Presentation by B.T. Bennett

SOURCE PROVENANCE

Catalog	SA-000099
Stage	Widefield SIM - Apotome
Instrument	ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Hamamatsu ORCA-Quest, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	64
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	1136471EA43B3C644AEFE10CB356C8191C4B7DCE210D25A17523062324598007
Method PDF SHA-256	3EDD333E75DA5B01FB08A18D57FB2F78AC3DE6690D39869FAC21EA8A35416A9D

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532; 790
METADATA VALUES	63

STRUCTURED ACQUISITION METADATA / CONFOCAL

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	181 Slices (5.4 µm)
Channels	3
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels)	528 X 583 | 930 X 930
Image Size (Scaled)	31.06 µm X 34.30 µm | 54.71 µm X 54.71 µm
Bit Depth	16 Bit
Image Center Position	X: 88.80 mm, Y: 52.25 mm
Stage Position	X: 88.80 mm, Y: 52.25 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 79 µm | 1.00 AU / 53 µm | 1.00 AU / 44 µm
LSM MBS	MBS 488/561, 405/730
LSM SBS	SBS LP 740 | Mirror
Laser Wavelength	730 nm @0.9% | 488 nm @0.1% | 405 nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.4
Rotation	0°
Sampling	1.20
Pixel Time	0.36 µs
Frame Time	3.08 s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	789 | 528 | 353
Emission Wavelength	794 | 553 | 465
Effective NA	1.4
Detection Wavelength	755-900 | 530-580 | 430-499
Imaging Device	NIR Ch2 | GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAs-PMT | GaAsP-PMT
Detector Gain	650 V | 550 V | 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	7.4 (3D, Auto) | 8.0 (3D, Auto) | 6.3 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels | Threshold 20%, Ambient Light 0%, Specular Light 100%, Shininess 30%
Background	Black
Light	Brightness 100%, Enabled Tone Mapping | Azimuth 80°, Elongation -130°, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 109%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Surface Projection	Precise

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Donkey Anti-Rabbit, F(ab')□ (H + L)
Cat ID# - SA 000101

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000024

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The first primary antibody, Nuclear Pore Complex Monoclonal Antibody (39C7), was incubated overnight at 4°C at a dilution of 1:100 Cells were washed 3X in PBS, and Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Donkey Anti-Rabbit, F(ab')□ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, Golgi GOLPH2 Polyclonal Antibody, was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Microscope

Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:

Single Plane (2D) - Z-plane 91 of 181 total - Panel A

Z Stack - (3D) - Panels A & B

Z-Stack = 181 Slices (5.4 µm)

Channels = 3
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 528 X 583
Image Size (Scaled) = 31.06 µm X 34.30 µm
Bit Depth = 16 Bit
Image Center Position = X: 88.80 mm, Y: 52.25 mm
Stage Position = X: 88.80 mm, Y: 52.25 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel C

Z-Stack = 181 Slices (5.4 µm)

Channels = 3
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 930 X 930
Image Size (Scaled) = 54.71 µm X 54.71 µm
Bit Depth = 16 Bit
Image Center Position = X: 88.80 mm, Y: 52.25 mm
Stage Position = X: 88.80 mm, Y: 52.25 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

790 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 79 µm
LSM MBS = MBS 488/561, 405/730
LSM SBS = SBS LP 740
Laser Wavelength = 730 nm @0.9%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.36 µs
Frame Time = 3.08 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 789
Emission Wavelength = 794
Effective NA = 1.4
Detection Wavelength = 755-900
Imaging Device = NIR Ch2
Detector Type = GaAs-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 7.4 (3D, Auto)

532 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 53 µm
LSM MBS = MBS 488/561, 405/730
LSM SBS = Mirror
Laser Wavelength = 488 nm @0.1%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.36 µs
Frame Time = 3.08 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 528
Emission Wavelength = 553
Effective NA = 1.4
Detection Wavelength = 530-580
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 8.0 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 44 µm
LSM MBS = MBS 488/561, 405/730
LSM SBS = Mirror
Laser Wavelength = 405 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.36 µs
Frame Time = 3.08 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 430-499
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 6.3 (3D, Auto)

Split View - Panel A

The first panel on the left features nuclear pore complexes visualized with Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Donkey Anti-Rabbit, F(ab')□ (H + L), the second panel features Golgi visualized with Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Rabbit, IgG (H + L), the third panel shows DAPI nuclear staining, and the last panel on the right shows the merged image of all channels. This image was taken at Z-Position 91 of 181.

3D Image Processing - Panel B

3D Maximum Projection = Precise
Appearance = Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 109%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel C

3D Surface Projection = Precise
Appearance = Threshold 20%, Ambient Light 0%, Specular Light 100%, Shininess 30%
Background = Black
Light = Azimuth 80°, Elongation -130°, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 109%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

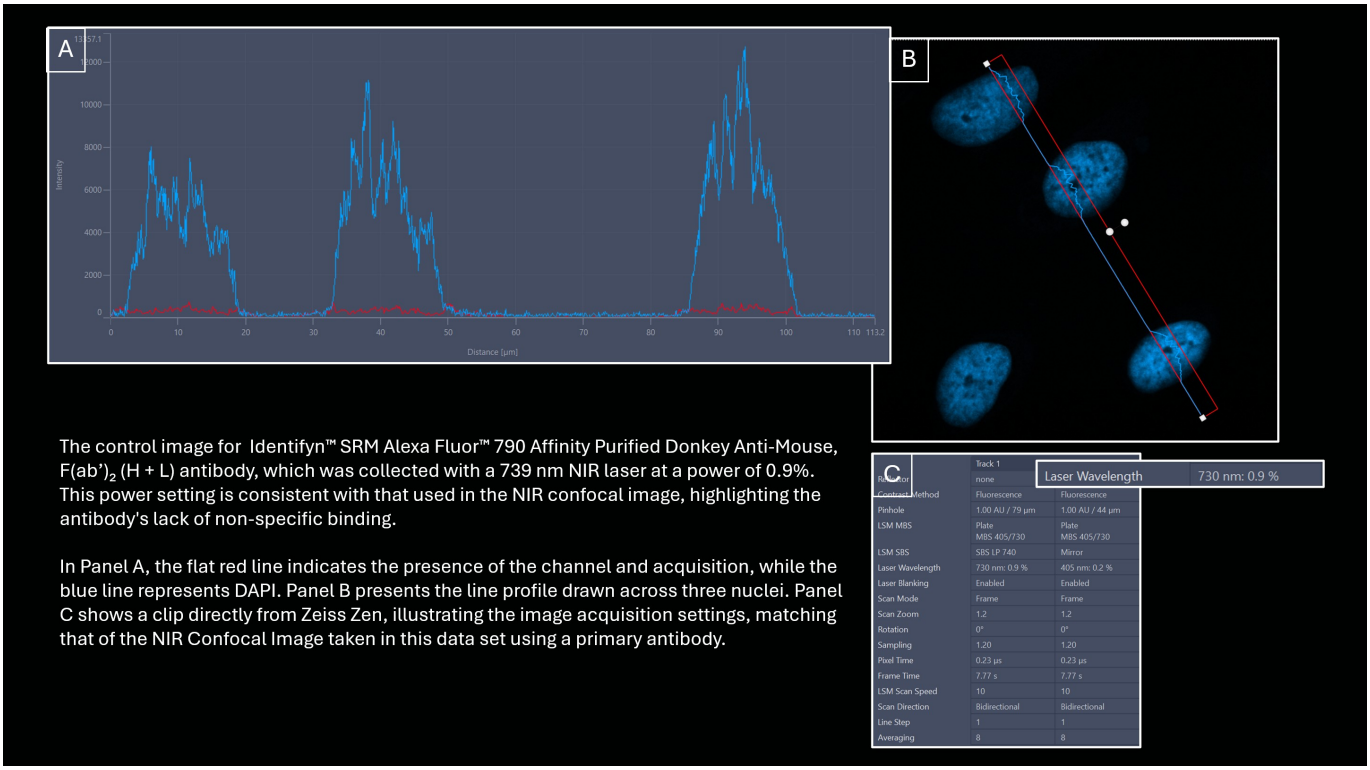
None

Image by J.K. Bennett
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

SOURCE PROVENANCE

Catalog	SA-000099
Stage	Confocal
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	63
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	9626A4B12FF3EB339D5193A34E7F96DFA8811C2F02E633FEED4EBCF4E28CA5B1
Method PDF SHA-256	734BD476865BC540E0884F600908401C49D844375AD52D5439B5F7D017B543AF

ORIGINAL IMAGE EVIDENCE / CONTROL



The control image for Identifyn™ SRM Alexa Fluor™ 790 Affinity Purified Donkey Anti-Mouse, $F(ab')_2$ (H + L) antibody, which was collected with a 739 nm NIR laser at a power of 0.9%. This power setting is consistent with that used in the NIR confocal image, highlighting the antibody's lack of non-specific binding.

In Panel A, the flat red line indicates the presence of the channel and acquisition, while the blue line represents DAPI. Panel B presents the line profile drawn across three nuclei. Panel C shows a clip directly from Zeiss Zen, illustrating the image acquisition settings, matching that of the NIR Confocal Image taken in this data set using a primary antibody.

EVIDENCE TIER	Control
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 790
METADATA VALUES	46

STRUCTURED ACQUISITION METADATA / CONTROL

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Channels	2
Scaling (Per Pixel)	0.059 µm X 0.059 µm
Image size (Pixels)	1884 X 1884
Image Size (Scaled)	110.83 µm X 110.83 µm
Bit Depth	16 Bit
Image Center Position	X: 89.72 µm, Y: 51.47 µm
Stage Position	X: 89.72 µm, Y: 51.47 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 79 µm | 1.00 AU / 44 µm
LSM MBS	Plate, MBS 405/730
LSM SBS	SBS LP 740 | Mirror
Laser Wavelength	730 nm @0.9% | 405 nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.2
Rotation	0°
Sampling	1.20
Pixel Time	0.23 µs
Frame Time	7.77 s
LSM Scan Speed	10
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	789 | 353
Emission Wavelength	794 | 465
Effective NA	1.4
Detection Wavelength	755-900 | 430-490
Imaging Device	NIR Ch2 | GaAsP-Pmt3
Detector Type	GaAs-PMT | GaAsP-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	650 V | 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	6.8 (3D, Auto) | 6.1 (3D, Auto)
Profile Definition	Stroke Thickness 1.00, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 113.2), Y (Min 0 and Max 13357)

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000099

HeLa Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher, Fish Serum Blocker cat#37527X3. In the absence of a primary antibody, Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:

Single Plane (2D)

Channels = 2
Scaling (Per Pixel) = 0.059 µm X 0.059 µm
Image size (Pixels) = 1884 X 1884
Image Size (Scaled) = 110.83 µm X 110.83 µm
Bit Depth = 16 Bit
Image Center Position = X: 89.72 µm, Y: 51.47 µm
Stage Position = X: 89.72 µm, Y: 51.47 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

790 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 79 µm
LSM MBS = Plate, MBS 405/730
LSM SBS = SBS LP 740
Laser Wavelength = 730 nm @0.9%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.2
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.23 µs
Frame Time = 7.77 s
LSM Scan Speed = 10
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 789
Emission Wavelength = 794
Effective NA = 1.4
Detection Wavelength = 755-900
Imaging Device = NIR Ch2
Detector Type = GaAs-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 6.8 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 44 µm
LSM MBS = Plate, MBS 405/730
LSM SBS = Mirror
Laser Wavelength = 405 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.2
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.23 µs
Frame Time = 7.77 s
LSM Scan Speed = 10
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 430-490
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 6.1 (3D, Auto)

Profile View Display - Panels A & B

Profile Definition = Stroke Thickness 1.00, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 113.2), Y (Min 0 and Max 13357)

Image Correction

None

Image by E.F. Simon
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

SOURCE PROVENANCE

Catalog	SA-000099
Stage	Control

SOURCE PROVENANCE

Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	46
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	16FCB476BC6C7A8AF02ED7D2C076AA17126AD23B511007510D1E29D4A91EB5EB
Method PDF SHA-256	81FCFBAD656E1817405D86FD970B7FD1ED9F1C0904AC594D483FA697B12D538C