

IDENTIFYN RESOLUTION STEPDOWN

SECONDARY ANTIBODY MICROSCOPY EVIDENCE ASSESSMENT

Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Rabbit Anti-Mouse, F(ab')₂ (H + L)

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

4

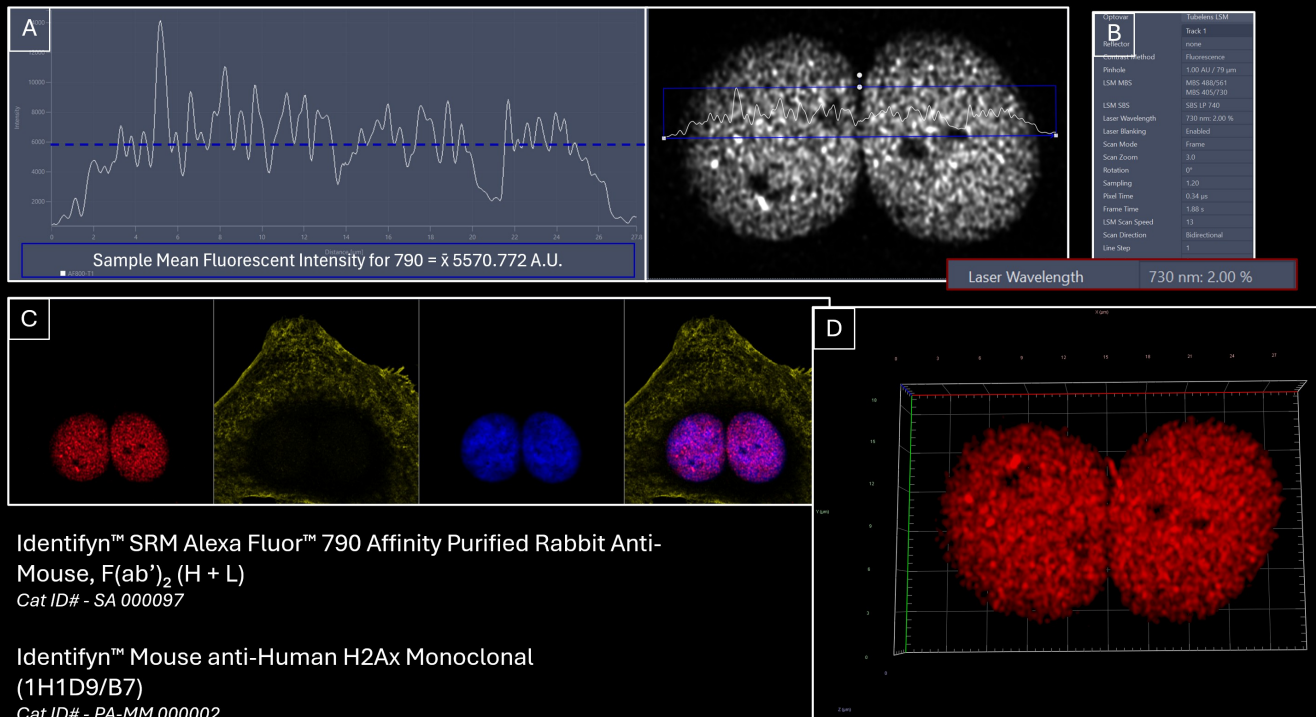
ORDERED EVIDENCE TIERS

1

SUPPORTING CONTROL

PENDING

SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / CONFOCAL

SA-000097 | 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 Rabbit Anti-Mouse, F(ab')₂ (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-000097 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-000097 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; 2; AF750-49007; AF532-49014; 49 DAPI; 672 - 748; 515 - 545; 335 - 383
Confocal	405/DAPI; 488; 532; 555; 790	3; None; 730 nm @2.00%; 488 nm @0.08%; 405 nm @0.3%; 789; 528; 353
Control	405/DAPI; 790	2; AF750-49007; 49 DAPI; 672 - 748; 335 - 383; 765 - 855; 420 - 470; 752

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): 2002 X 1548; Image Size (Pixels): 2002 X 1548; 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. 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: 97 Slices (9.6 μm); Channels: 3; 2; Scaling (Per Pixel): 0.073 μm X 0.073 μm X 0.100 μm ; Image size (Pixels): 1986 X 1429; 1088 X 783; Image Size (Pixels): 1986 X 1429; 1088 X 783; 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: 66.

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: 201 Slices (6 μm); 102 Slices (3.03 μm); Channels: 3; Scaling (Per Pixel): 0.059 μm X 0.059 μm X 0.030 μm ; Image size (Pixels): 739 X 739; 504 X 332; Image Size (Pixels): 739 X 739; 504 X 332; Bit Depth: 16 Bit. Detector/camera: Imaging Device: NIR Ch2; GaAsP-Pmt3; Detector Type: GaAs-PMT; GaAsP-PMT. Timing: Pixel Time: 0.34 μs ; Frame Time: 1.88 s. Illumination: Laser Wavelength: 730 nm @2.00%; 488 nm @0.08%. Optical/scan: Pinhole: 1.00 AU / 79 μm ; 1.00 AU / 53 μm ; Scan Zoom: 3.0; LSM Scan Speed: 13; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. 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; LSM Plus Mode: 7.0 (3D, Auto); 6.5 (3D, Auto). Structured source metadata values: 62.

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; 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

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 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: 75 Slices (8.5 μm); Channels: 2; Scaling (Per Pixel): 0.073 μm X 0.073 μm X 0.100 μm ; Image size (Pixels): 1597 X 1593; Image Size (Pixels): 1597 X 1593; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Hamamatsu ORCA-Quest. Timing: Exposure Time: 300 ms; 25 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @30.00%; X-Cite Xylis Lamp Intensity @10.00%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 33.

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 Axio Observer.Z1/7 Apotome, 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-000097 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 μ M -> Etoposide = 2 μ M. The governing scientist identified every 200 μ M occurrence as a technician transcription error. The canonical historical concentration is 2 μ M. 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 EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

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



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

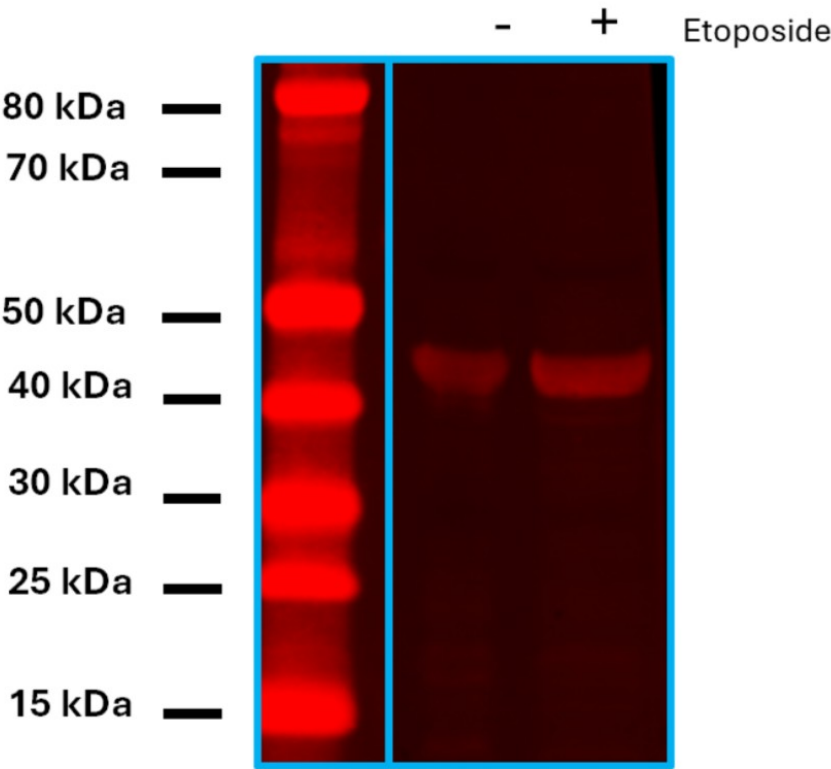
This dossier preserves the complete available evidence progression for SA-000097. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

CANONICAL STEPDOWN	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 Rabbit Anti-Mouse, F(ab')₂ (H + L)

Cat ID - SA 000097

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 Rabbit Anti-Mouse, F(ab')₂ (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

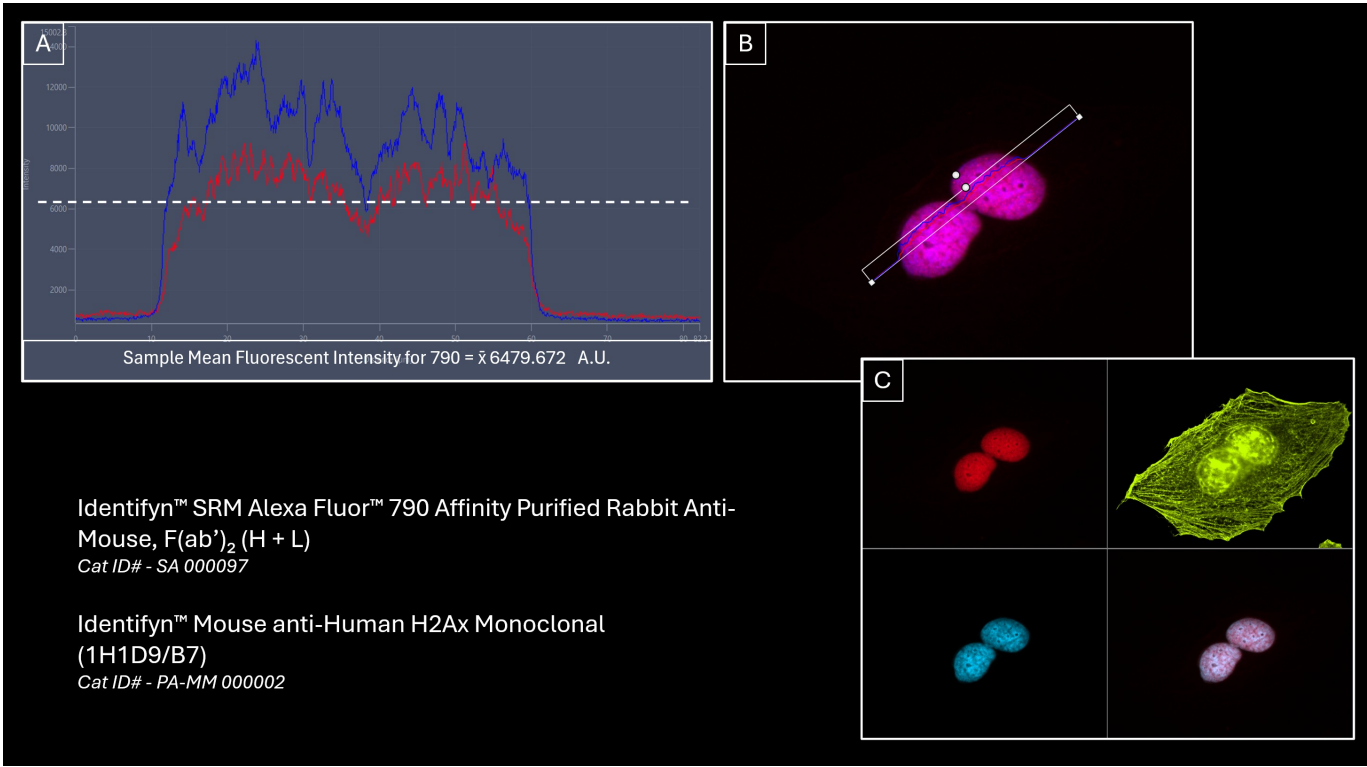
Image: K. Small

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SOURCE PROVENANCE

Catalog	SA-000097
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	84D81B3069E5229339B976DA6F65EE794622281033F0837A041EBABF14BB16D8
Method PDF SHA-256	D5FB2BBE3015F5F6A70A155DB02CF280C9CC292D8FE83F2F4AF3298691243C5C

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



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 μ m X 0.073 μ m
Image size (Pixels)	2002 X 1548
Image Size (Scaled)	146.18 μ m X 113.03 μ m
Bit Depth	16 Bit
Image Center Position	X: 0.00 μ m, Y: 0.00 μ m
ROI Center Offset	X: 0.00 μ m, Y: 0.00 μ m
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 μ m 1.07 μ m 0.90 μ m
Binning Mode	1,1 2,2
Profile Definition	Stroke Thickness 2.5, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 82.2), Y (Min 331 and Max 15003)

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® Primary Antibody

Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7)

Cat ID# - PA-MM 000002

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Rabbit Anti-Mouse, F(ab')₂ (H + L)

Cat ID# - SA 000097

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)

Cat ID# - SA 000027

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 primary antibody, Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Rabbit Anti-Mouse, F(ab')₂ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, Actin Recombinant Rabbit Monoclonal Antibody (JF47-01), 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 Donkey Anti-Rabbit, IgG (H + L) was incubated for 1 hour at room temperature 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) = 2002 X 1548

Image Size (Scaled) = 146.18 µm X 113.03 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 @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 μ m
 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 μ m
 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 μ m
 Binning Mode = 2,2

Profile View Display - Panels A & B

Profile Definition = Stroke Thickness 2.5, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 82.2), Y (Min 331 and Max 15003)

Split View - Panel C

The top-left features Rabbit anti-Human γ H2Ax Recombinant Monoclonal, Mouse Fc, IgG (BLR014M) visualized with Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Rabbit Anti-Mouse, F(ab')₂ (H + L), the top-right panel features actin visualized with Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Donkey Anti-Rabbit, IgG (H + L), the bottom-left panel shows DAPI nuclear staining, and the bottom-right panel shows the merged image of all channels.

Image Correction

None

Image by E.F. Simon

Image Analysis by B.T. Bennett

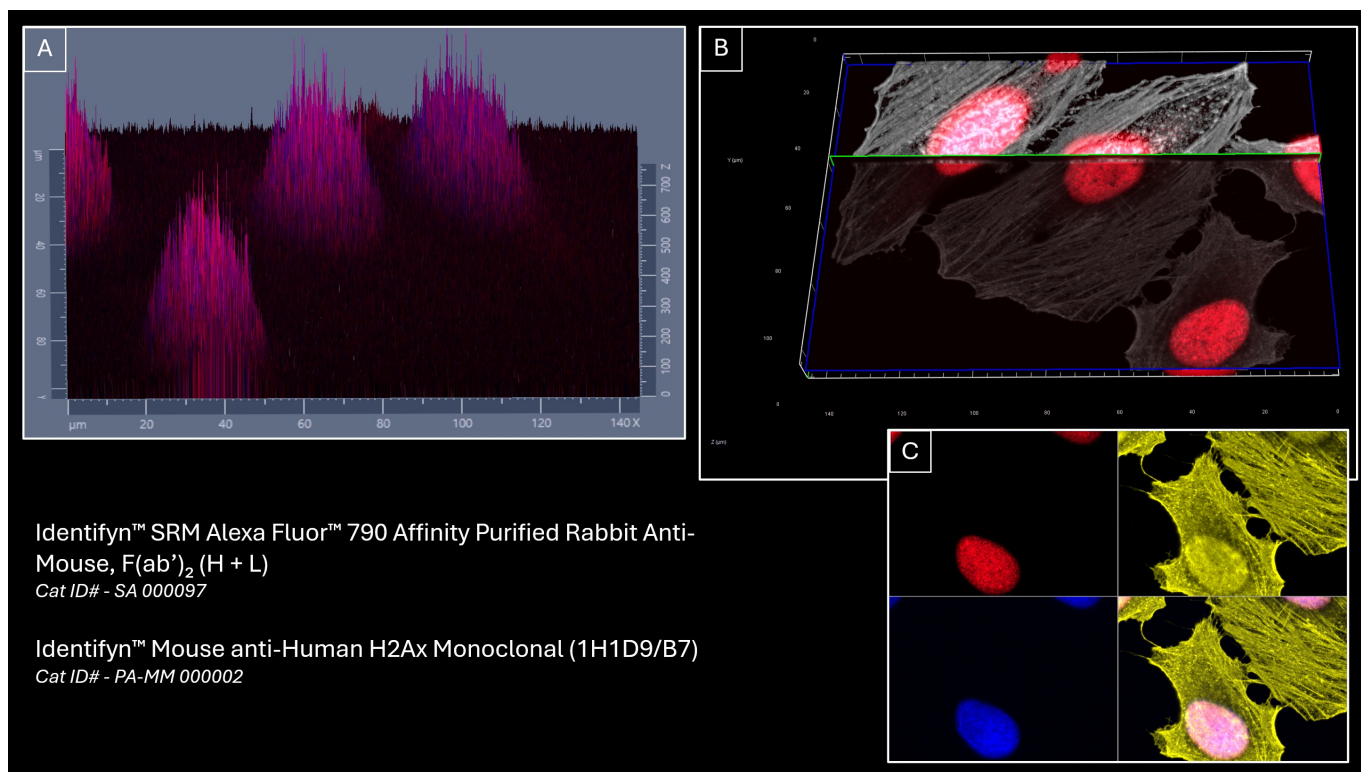
Data Presentation by P.D. Amistoso

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SOURCE PROVENANCE

Catalog	SA-000097
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	D6298C1C6A24CEBF7C45FE183BC6E46B8E21FE44A8901BC22CB94C18B8A365D7
Method PDF SHA-256	3867FAA819B311266711E4437959245DD839A149B8F12E4F335FA83319A5998F

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	66

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	97 Slices (9.6 μm)
Channels	3 2
Scaling (Per Pixel)	0.073 μm X 0.073 μm X 0.100 μm
Image Size (Pixels)	1986 X 1429 1088 X 783
Image Size (Scaled)	145.01 μm X 104.34 μm 79.44 μm X 57.17 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	3
Angle X	53
Angle Y	0
Z Scaling	1.0
Ambient	50
Specular	20
Shininess	50

FIELD	SOURCE-DOCUMENTED VALUE
Light Height	2.0
3D Maximum 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	-4% -21%
Clip X	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® Primary Antibody

Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7)

Cat ID# - PA-MM 000002

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Rabbit Anti-Mouse, F(ab')₂ (H + L)

Cat ID# - SA 000097

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)

Cat ID# - SA 000027

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 primary antibody, Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Rabbit Anti-Mouse, F(ab')₂ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, Actin Recombinant Rabbit Monoclonal Antibody (JF47-01), 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 Donkey Anti-Rabbit, IgG (H + L) was incubated for 1 hour at room temperature 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:

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

Z-Stack = 97 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

Single Plane (2D) - Z-plane 49 of 97 total - Panel C

Z Stack - (3D)

Z-Stack = 97 Slices (9.6 µm)

Channels = 2

Scaling (Per Pixel) = 0.073 µm X 0.073 µm X 0.100 µm

Image Size (Pixels) = 1088 X 783

Image Size (Scaled) = 79.44 µm X 57.17 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 μ m
 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 it shares similar intensities.

2.5D Display

Render Mode = Surface
 Grid distance = 3
 Palette and Show Faces at Side are both Selected

2.5D Display Options

Angle X = 53
 Angle Y = 0
 Z Scaling = 1.0
 Ambient = 50
 Specular = 20
 Shininess = 50
 Light Height = 2.0

3D Image Processing - Panel A

3D Maximum 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 = -4%

Clip X = 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.

Position of Clip = -21%

Clip X = 0°

Clip Y = 0°

Image Correction

None

Image by E.F. Simon

Image Analysis by B.T. Bennett

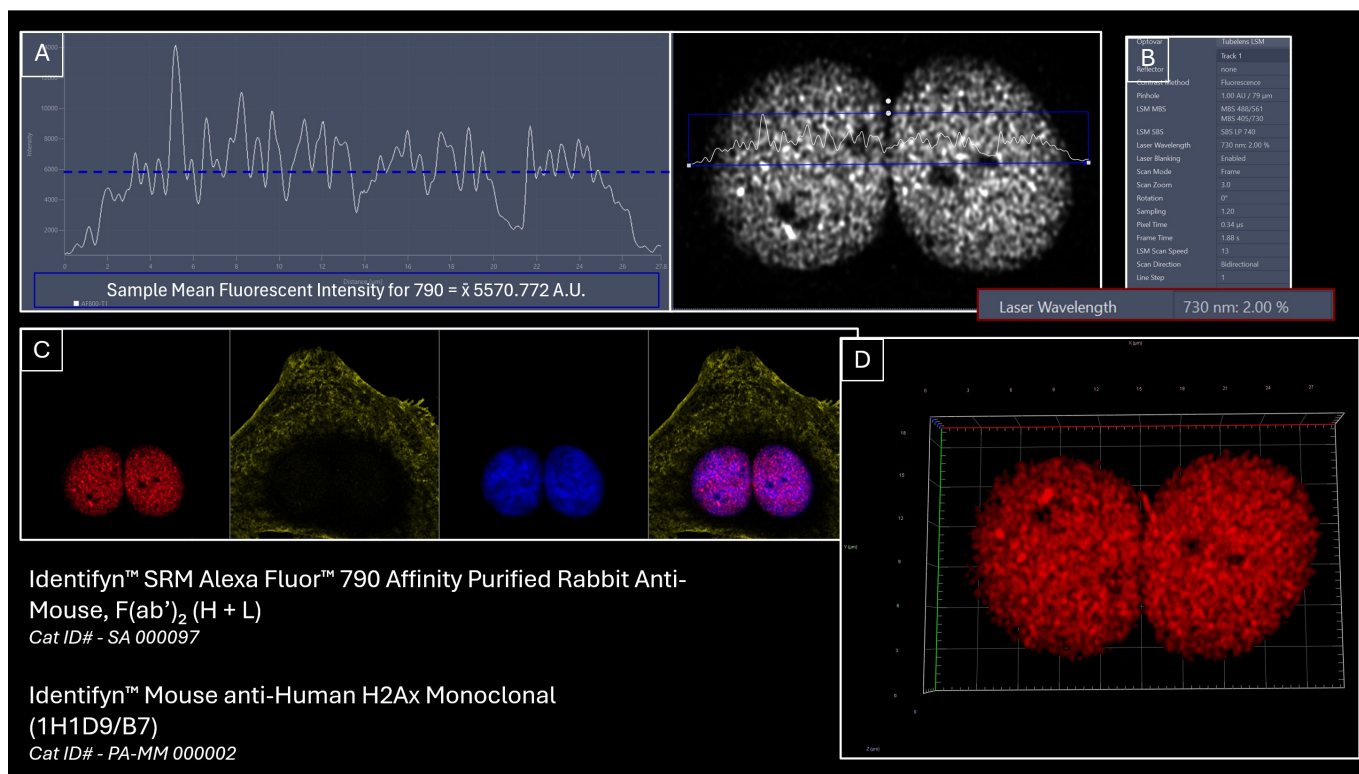
Data Presentation by P.D. Amistoso

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SOURCE PROVENANCE

Catalog	SA-000097
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	66
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	39E05A91463BEE438AD6B3EC0FD67E5693BFB88E6414AA09C360024A65E30CEF
Method PDF SHA-256	DF519E52B05E4828E4B3080147AE1A86C3EE06A24C35F5DA83FC80922311DEBB

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532; 555; 790
METADATA VALUES	62

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	201 Slices (6 μm) 102 Slices (3.03 μm)
Channels	3
Scaling (Per Pixel)	0.059 μm X 0.059 μm X 0.030 μm
Image Size (Pixels)	739 X 739 504 X 332
Image Size (Scaled)	43.48 μm X 43.48 μm 29.66 μm X 19.54 μm
Bit Depth	16 Bit
Image Center Position	X: 75.79 mm, Y: 49.69 mm
Stage Position	X: 75.79 mm, Y: 49.69 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 @2.00% 488 nm @0.08% 405 nm @0.3%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	3.0
Rotation	0°
Sampling	1.20
Pixel Time	0.34 μs
Frame Time	1.88 s
LSM Scan Speed	13
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	700 V 600 V 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	7.0 (3D, Auto) 6.5 (3D, Auto) 5.8 (3D, Auto)
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 27.8), Y (Min 360 and Max 14826)
3D Maximum Projection	Precise
Appearance	Threshold 2% all channels, Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Tone Mapping Enabled
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

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® Primary Antibody

Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7)

Cat ID# - PA-MM 000002

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Rabbit Anti-Mouse, F(ab')₂ (H + L)

Cat ID# - SA 000097

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)

Cat ID# - SA 000027

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 primary antibody, Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, Actin Recombinant Rabbit Monoclonal Antibody (JF47-01), 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 Donkey Anti-Rabbit, IgG (H + L) was incubated for 1 hour at room temperature 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 52 of 102 total - Panel C

Z Stack - (3D)

Z-Stack = 201 Slices (6 µm)

Channels = 3

Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm

Image Size (Pixels) = 739 X 739

Image Size (Scaled) = 43.48 µm X 43.48 µm

Bit Depth = 16 Bit

Image Center Position = X: 75.79 mm, Y: 49.69 mm

Stage Position = X: 75.79 mm, Y: 49.69 mm

ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

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

Z-Stack = 102 Slices (3.03 µm)

Channels = 3

Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm

Image Size (Pixels) = 504 X 332

Image Size (Scaled) = 29.66 µm X 19.54 µm

Bit Depth = 16 Bit

Image Center Position = X: 75.79 mm, Y: 49.69 mm

Stage Position = X: 75.79 mm, Y: 49.69 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 @2.00%

Laser Blanking = Enabled

Scan Mode = Frame

Scan Zoom = 3.0

Rotation = 0°

Sampling = 1.20

Pixel Time = 0.34 µs

Frame Time = 1.88 s

LSM Scan Speed = 13

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 = 700 V

Detector Offset = 0

Detector Digital Gain = 1.0

LSM Plus Mode = 7.0 (3D, Auto)

532 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00 AU / 53 μ m
 LSM MBS = MBS 488/561, 405/730
 LSM SBS = Mirror
 Laser Wavelength = 488 nm @0.08%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 3.0
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.34 μ s
 Frame Time = 1.88 s
 LSM Scan Speed = 13
 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 = 600 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = 6.5 (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 @0.3%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 3.0
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.34 µs
 Frame Time = 1.88 s
 LSM Scan Speed = 13
 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 = 5.8 (3D, Auto)

Split View

The first panel on the left features Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7) visualized with Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Rabbit Anti-Mouse, F(ab')₂ (H + L), the second panel shows Actin visualized with Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Donkey Anti-Rabbit, IgG (H + L), the third panel features DAPI nuclear staining, the last panel on the right features the merged image of all channels. This split view was taken at Z-Position 51 of 102.

Profile View Display

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 27.8), Y (Min 360 and Max 14826)

3D Image Processing

3D Maximum Projection = Precise
 Appearance = Threshold 2% all channels, Ramp 0% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness 100%, Tone Mapping Enabled
 Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Rabbit Anti-Mouse, F(ab')₂ (H + L) was pseudo-colored in Zen Software from dark red, the default color, to white to enhance visual contrast.

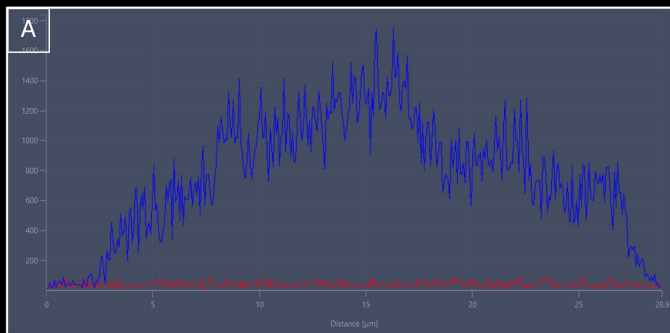
Image by J.K. Bennett
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

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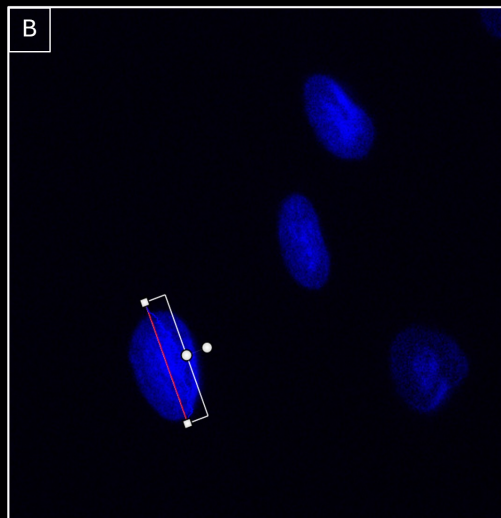
SOURCE PROVENANCE

Catalog	SA-000097
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	62
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	C044E2E103729026B0EE5633E1665273ED4D357788FB2C48F540C5526127BD31
Method PDF SHA-256	F9C4D34F30AE8D11FFAB85FF222E5737EC0ED0C8B672B58376046A96CACAD3AE

ORIGINAL IMAGE EVIDENCE / CONTROL



Panel A - The Line Profile shows that the 790 channel was collected because there is a baseline for this channel. If it had not been collected, the flat red background line could not have been recorded.



Identifyn™ SRM Alexa Fluor™ 790 Affinity Purified Rabbit Anti-Mouse, F(ab')₂ (H + L)
Cat ID# - SA 000097

EVIDENCE TIER	Control
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 790
METADATA VALUES	33

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 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	75 Slices (8.5 μ m)
Channels	2
Scaling (Per Pixel)	0.073 μ m X 0.073 μ m X 0.100 μ m
Image Size (Pixels)	1597 X 1593
Image Size (Scaled)	116.61 μ m X 116.31 μ m
Bit Depth	16 Bit
Image Center Position	X: 0.00 mm, Y: 0.00 mm
ROI Center Offset	X: 0.00 μ m, Y: 0.00 μ m
Reflector	AF750-49007 49 DAPI
Beam Splitter	760 395
Filter Excitation Wavelength	672 - 748 335 - 383
Filter Emission Wavelength	765 - 855 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @30.00% X-Cite Xylis Lamp Intensity @10.00%
Exposure Time	300 ms 25 ms
Excitation Wavelength	752 353
Emission Wavelength	779 465
Effective NA	1.25
Imaging Device	Hamamatsu ORCA-Quest
Camera Adapter	1X
Depth of Focus	1.51 μ m 0.90 μ m
Binning Mode	1,1 2,2
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 28.9), Y (Min 1 and Max 1840)

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 Rabbit Anti-Mouse, F(ab')₂ (H + L)

Cat ID# - SA 000097

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 Rabbit Anti-Mouse, F(ab')₂ (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) - Z-plane 38 of 75 total - Panel A

Z Stack - (3D)

Z-Stack = 75 Slices (8.5 µm)

Channels = 2

Scaling (Per Pixel) = 0.073 µm X 0.073 µm X 0.100 µm

Image Size (Pixels) = 1597 X 1593

Image Size (Scaled) = 116.61 µm X 116.31 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

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 μ m
 Binning Mode = 2,2

Profile View Display - Panel A & B

The Line Profile indicates that the 790 channel was collected due to the presence of a baseline for this channel. If it had not been collected, the flat red background line could not have been recorded.

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 28.9), Y (Min 1 and Max 1840)

Image Correction

None

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

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SOURCE PROVENANCE

Catalog	SA-000097
Stage	Control
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	33
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	1B14287C96418B47379F4C37DEFEB4EBB08C54CF824DEF4EF396CC38C896DA2
Method PDF SHA-256	217A3C7494F733FAD14F8B29F6CD20911AE4202E82FE7F7FC284E220AACFEF28