

IDENTIFYN RESOLUTION STEPDOWN

SECONDARY ANTIBODY MICROSCOPY EVIDENCE ASSESSMENT

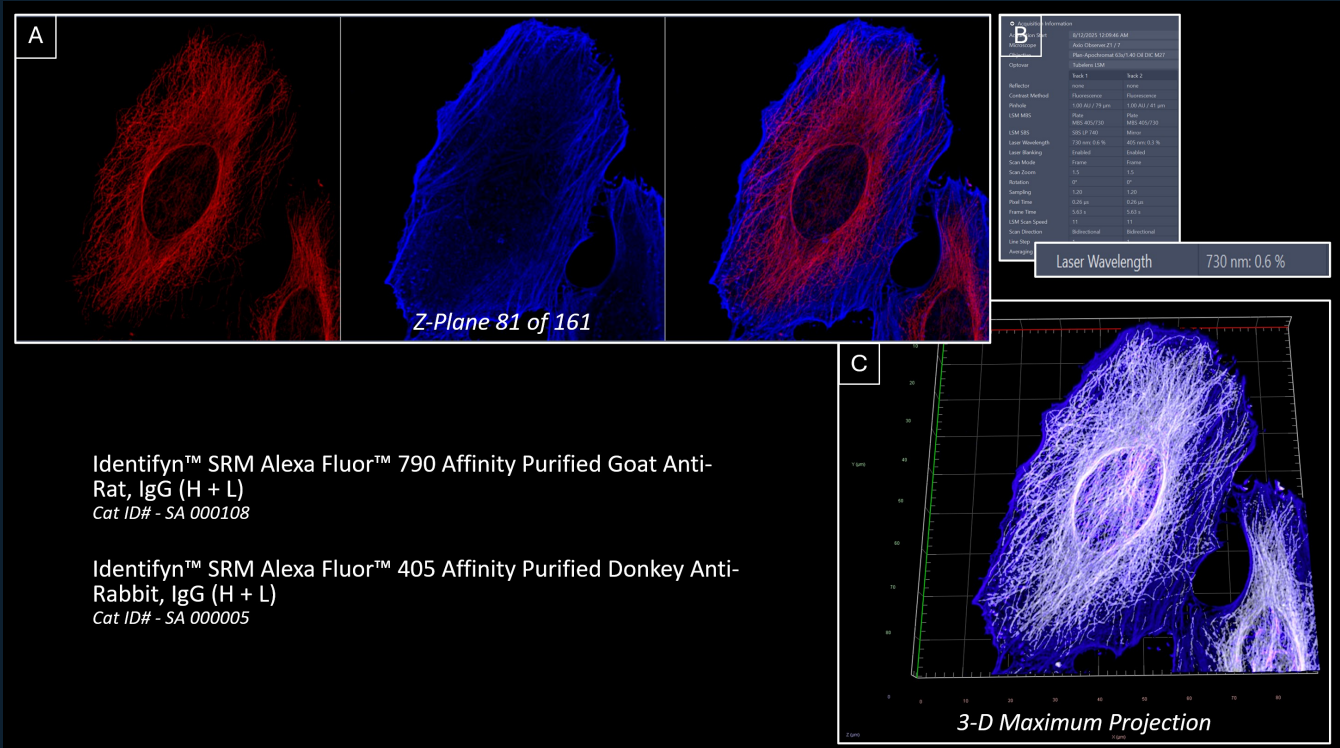
Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Goat Anti-Rat, IgG (H + L)

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

4
ORDERED EVIDENCE TIERS

1
SUPPORTING CONTROL

PENDING
SCIENTIST REVIEW



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 Goat Anti-Rat, IgG (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-000108 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-000108 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; 790	2; AF750-49007; AF405-49027; 672 - 748; 372 - 408; 765 - 855; 417 - 445; 752
Widefield SIM — Apotome	405/DAPI; 790	2; AF750-49007; AF405-49027; 672 - 748; 372 - 408; 765 - 855; 417 - 445; 752
Confocal	405/DAPI; 790	2; None; 730 nm @0.6%; 405 nm @0.3%; 789; 401; 794; 422
Control	405/DAPI; 790	2; None; 730 nm @0.6%; 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: 2; Scaling (Per Pixel): 0.073 μm X 0.073 μm ; Image size (Pixels): 2011 X 1441; Image Size (Pixels): 2011 X 1441; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Hamamatsu ORCA-Quest. Timing: Exposure Time: 250 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @25.00%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 29.

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; 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: 2; Scaling (Per Pixel): 0.073 μm X 0.073 μm X 0.100 μm ; Image size (Pixels): 1871 X 1564; Image Size (Pixels): 1871 X 1564; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Hamamatsu ORCA-Quest. Timing: Exposure Time: 250ms; 250 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @25.00%. 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: 37.

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; 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: 161 Slices (4.8 μm); Channels: 2; Scaling (Per Pixel): 0.059 μm X 0.059 μm X 0.030 μm ; Image size (Pixels): 1502 X 1502; Image Size (Pixels): 1502 X 1502; Bit Depth: 16 Bit. Detector/camera: Imaging Device: NIR Ch2; GaAsP-Pmt3; Detector Type: GaAs-PMT; GaAsP-PMT. Timing: Pixel Time: 0.26 μs ; Frame Time: 5.63s; 5.63 s. Illumination: Laser Wavelength: 730 nm @0.6%; 405 nm @0.3%. Optical/scan: Pinhole: 1.00 AU / 79 μm ; 1.00 AU / 41 μm ; Scan Zoom: 1.5; LSM Scan Speed: 11; 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: 7.6 (3D, Auto); 6.7 (3D, Auto). Structured source metadata values: 52.

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; 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): 1248 X 1248; Image Size (Pixels): 1248 X 1248; Bit Depth: 16 Bit. Detector/camera: Imaging Device: NIR Ch2; GaAsP-Pmt3; Detector Type: GaAs-PMT; GaAsP-PMT. Timing: Pixel Time: 0.27 μs ; Frame Time: 4.10 s. Illumination: Laser Wavelength: 730 nm @0.6%; 405 nm @0.2%. Optical/scan: Pinhole: 1.00 AU / 79 μm ; 1.00 AU / 43 μm ; Scan Zoom: 1.8; LSM Scan Speed: 12; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: 6.4 (3D, Auto); 5.6 (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-000108 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; 2 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.

UNRESOLVED - PRESERVED AS CONFLICT

Cross-tier / treatment duration: {"Western blot": ["20 hours"], "Widefield": ["24 hours"], "Widefield SIM — Apotome": ["24 hours"], "Confocal": ["24 hours"]} -> Preserved as modality-specific historical duration values. Genuinely different treatment durations are present across evidence stages, and no source or scientist correction resolves them. Supporting evidence: Only duration values from explicit etoposide-treatment sentences were classified; concentration, wavelength, resolution and pixel-size values were excluded.

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

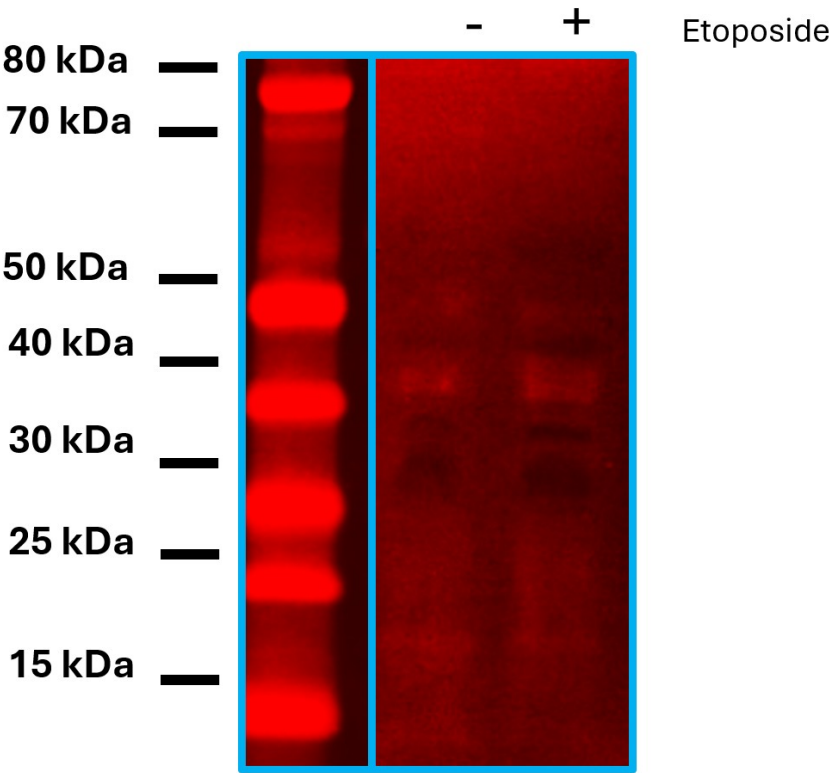
This dossier preserves the complete available evidence progression for SA-000108. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

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 Goat Anti-Rat, IgG (H + L)

Cat ID# - SA 000108

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 Goat Anti-Rat, IgG (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

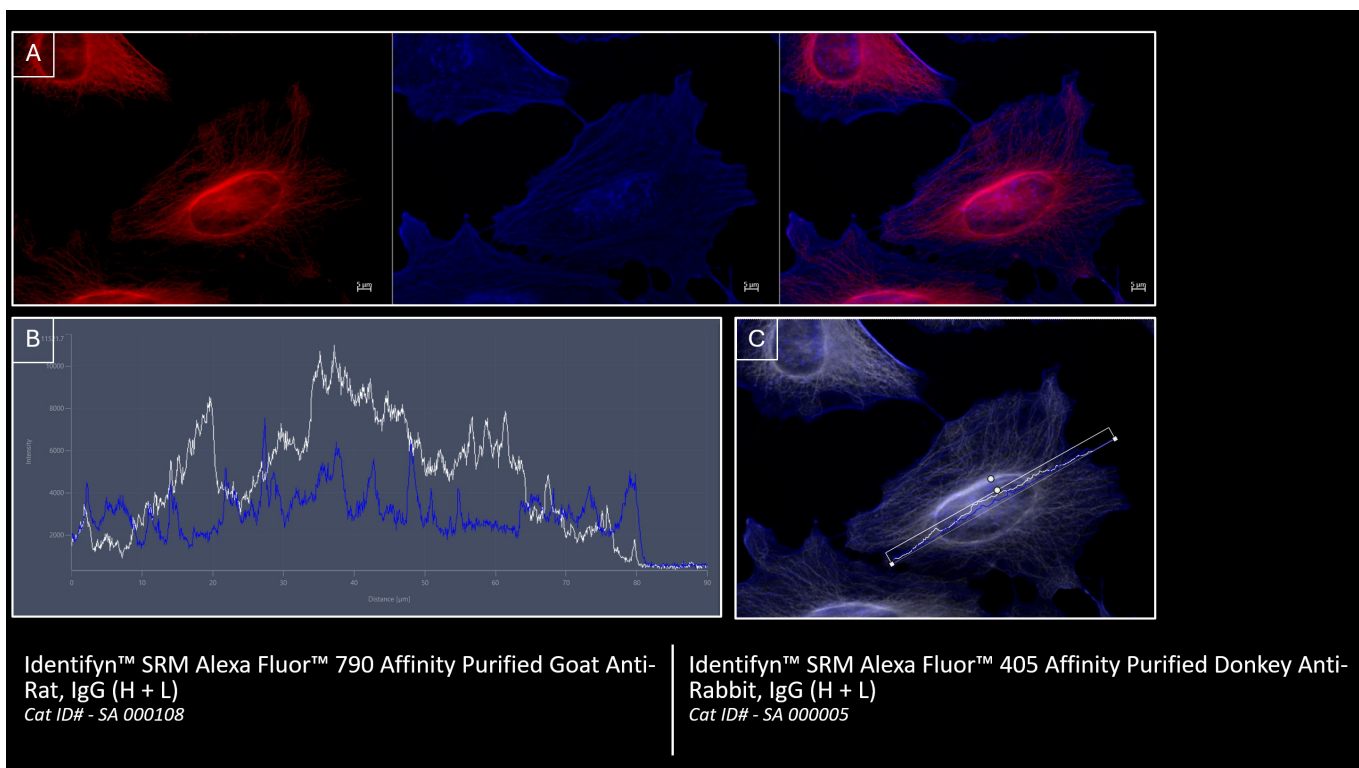
Image: K. Small

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SOURCE PROVENANCE

Catalog	SA-000108
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	84A778053895CE65C84C1151E8E0F018175CB89DFD9A982A63799F135106A542
Method PDF SHA-256	A3119F5CA61E18C8F312B04ADF969D87723A5CD4FC1B843841972B11293F4F04

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 790
METADATA VALUES	29

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	2
Scaling (Per Pixel)	0.073 μ m X 0.073 μ m
Image size (Pixels)	2011 X 1441
Image Size (Scaled)	146.83 μ m X 105.22 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 AF405-49027
Beam Splitter	760 412
Filter Excitation Wavelength	672 - 748 372 - 408
Filter Emission Wavelength	765 - 855 417 - 445
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @25.00%
Exposure Time	250 ms
Excitation Wavelength	752 401
Emission Wavelength	779 422
Effective NA	1.25
Imaging Device	Hamamatsu ORCA-Quest
Camera Adapter	1X
Depth of Focus	1.51 μ m 0.82 μ m
Binning Mode	1,1
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 90.0), Y (Min 323 and Max 11522)

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 Goat Anti-Rat, IgG (H + L)

Cat ID# - SA 000108

Identifyn® SRM Alexa Fluor™ 405 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)

Cat ID# - SA 000005

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Goat Anti-Rat, IgG (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™ 405 Affinity Purified Donkey 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, cat#P36961.

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 = 2

Scaling (Per Pixel) = 0.073 µm X 0.073 µm

Image size (Pixels) = 2011 X 1441

Image Size (Scaled) = 146.83 µm X 105.22 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 @25.00%
 Exposure Time = 250 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

405 -

Reflector = AF405-49027
 Beam Splitter = 412
 Filter Excitation Wavelength = 372 - 408
 Filter Emission Wavelength = 417 - 445
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @25.00%
 Exposure Time = 250 ms
 Excitation Wavelength = 401
 Emission Wavelength = 422
 Effective NA = 1.25
 Imaging Device = Hamamatsu ORCA-Quest
 Camera Adapter = 1X
 Depth of Focus = 0.82 μ m
 Binning Mode = 1,1

Split View - Panel A

The left panel features Alpha Tubulin counterstained with Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Goat Anti-Rat, IgG (H + L), the middle panel features Actin counterstained with Identifyn® SRM Alexa Fluor™ 405 Affinity Purified Donkey Anti-Rabbit, IgG (H + L), and the right panel is the merged image of all channels.

Profile View Display - Panels B & C

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 90.0), Y (Min 323 and Max 11522)

Image Correction - Panel C

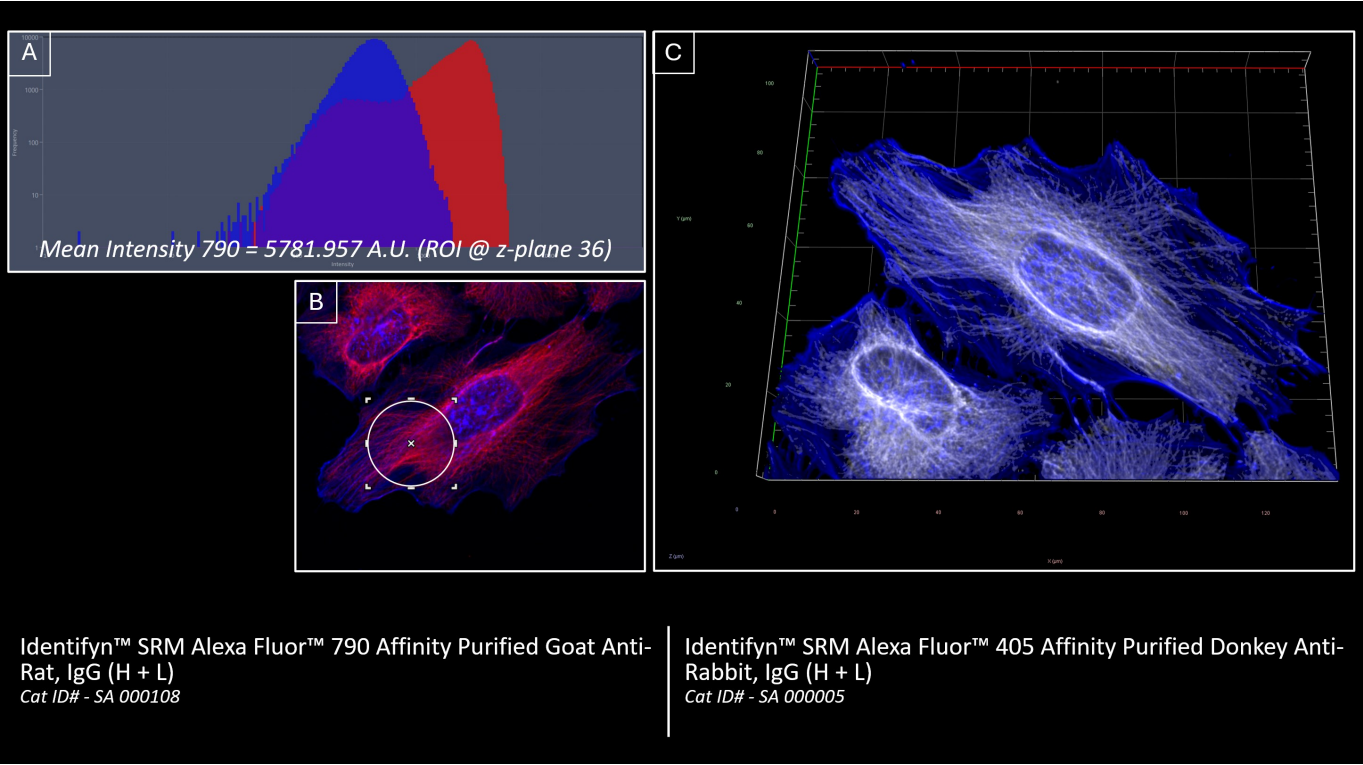
Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Goat Anti-Rat, IgG (H + L) was pseudo-colored in Zen Software from red, the default color, to white to provide visual contrast.

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

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SOURCE PROVENANCE	
Catalog	SA-000108
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	29
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	91361945C205701471251EF6DB4B6FF7CA740BABB6B2552238CC536A71D52A35
Method PDF SHA-256	2DE5BD7C9542D98422ED9C3E9A1EF74D515450B3B387B43FB12C5FA914C93234

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; 790
METADATA VALUES	37

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 μ m)
Channels	2
Scaling (Per Pixel)	0.073 μ m X 0.073 μ m X 0.100 μ m
Image Size (Pixels)	1871 X 1564
Image Size (Scaled)	136.61 μ m X 114.20 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 AF405-49027
Beam Splitter	760 412
Filter Excitation Wavelength	672 - 748 372 - 408
Filter Emission Wavelength	765 - 855 417 - 445
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @25.00%
Exposure Time	250ms 250 ms
Excitation Wavelength	752 401
Emission Wavelength	779 422
Effective NA	1.25
Imaging Device	Hamamatsu ORCA-Quest
Camera Adapter	1X
Depth of Focus	1.51 μ m 0.82 μ m
Binning Mode	1,1
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
Histogram View - X and Y axis auto, X	Min 1 and Max 65535, Y = Min 1 and Max 100000, Channel Transparency 61%, Statistic Table, Frequency Table, Internal Measurements, and Image were selected.
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)

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 Goat Anti-Rat, IgG (H + L)

Cat ID# - SA 000108

Identifyn® SRM Alexa Fluor™ 405 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)

Cat ID# - SA 000005

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Goat Anti-Rat, IgG (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™ 405 Affinity Purified Donkey 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, cat#P36961.

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 36 of 81 total - Panel B

Z Stack - (3D) - Panel C

Z-Stack = 89 Slices (9.6 µm)

Channels = 2

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

Image Size (Pixels) = 1871 X 1564

Image Size (Scaled) = 136.61 µm X 114.20 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 @25.00%
 Exposure Time = 250ms
 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

405 -

Reflector = AF405-49027
 Beam Splitter = 412
 Filter Excitation Wavelength = 372 - 408
 Filter Emission Wavelength = 417 - 445
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @25.00%
 Exposure Time = 250 ms
 Excitation Wavelength = 401
 Emission Wavelength = 422
 Effective NA = 1.25
 Imaging Device = Hamamatsu ORCA-Quest
 Camera Adapter = 1X
 Depth of Focus = 0.82 μm
 Binning Mode = 1,1

Post Processing - SIM Processing

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

Histogram View - Panel A and Image Panel B with Region of Interest (ROI)

Histogram Definition - Normal, Histo Table was Frequency, Bin Count 256, Bin Size 256, Lower Threshold 0, Upper Threshold 65535, Showing Threshold, and deploying Logarithmic Binning

Histogram View - X and Y axis auto, X = Min 1 and Max 65535, Y = Min 1 and Max 100000, Channel Transparency 61%, Statistic Table, Frequency Table, Internal Measurements, and Image were selected.

3D Image Processing - Panel C

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)

Image Correction

None

Image by E.F. Simon

Image Analysis and Data Presentation B.T. Bennett

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SOURCE PROVENANCE

Catalog	SA-000108
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	37
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	0277A052CACE2FE4B6FBBE6E2B8DF43A76800F7BB4D960D0AFB10BBA968E99A6
Method PDF SHA-256	E9D32056F487C86A3AC76C0A0F831FFFB8C997CBEBD5AFC727256BCF17B7E135

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	161 Slices (4.8 μm)
Channels	2
Scaling (Per Pixel)	0.059 μm X 0.059 μm X 0.030 μm
Image Size (Pixels)	1502 X 1502
Image Size (Scaled)	88.38 μm X 88.38 μm
Bit Depth	16 Bit
Image Center Position	X: 79.10 mm, Y: 50.42 mm
Stage Position	X: 79.10 mm, Y: 50.42 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 / 41 μm
LSM MBS	Plate MBS 405/730
LSM SBS	SBS LP 740 Mirror
Laser Wavelength	730 nm @0.6% 405 nm @0.3%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.5
Rotation	0°
Sampling	1.20
Pixel Time	0.26 μs
Frame Time	5.63s 5.63 s
LSM Scan Speed	11
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	789 401
Emission Wavelength	794 422
Effective NA	1.4
Detection Wavelength	755-900 405-450
Imaging Device	NIR Ch2 GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAs-PMT GaAsP-PMT
Detector Gain	600 V 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	7.6 (3D, Auto) 6.7 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 1% 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® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Goat Anti-Rat, IgG (H + L)

Cat ID# - SA 000108

Identifyn® SRM Alexa Fluor™ 405 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)

Cat ID# - SA 000005

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Goat Anti-Rat, IgG (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™ 405 Affinity Purified Donkey 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, cat#P36961.

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:

Z Stack - (3D) - Panel B, Z-Plane 81 of 161 Panel A, Split-View

Z-Stack = 161 Slices (4.8 µm)

Channels = 2

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

Image Size (Pixels) = 1502 X 1502

Image Size (Scaled) = 88.38 µm X 88.38 µm

Bit Depth = 16 Bit

Image Center Position = X: 79.10 mm, Y: 50.42 mm

Stage Position = X: 79.10 mm, Y: 50.42 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 = Plate MBS 405/730
 LSM SBS = SBS LP 740
 Laser Wavelength = 730 nm @0.6%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.5
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.26 µs
 Frame Time = 5.63s
 LSM Scan Speed = 11
 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 = 600 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = 7.6 (3D, Auto)

405 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00 AU / 41 µm
 LSM MBS = Plate MBS 405/730
 LSM SBS = Mirror
 Laser Wavelength = 405 nm @0.3%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.5
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.26 µs
 Frame Time = 5.63 s
 LSM Scan Speed = 11
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 401
 Emission Wavelength = 422
 Effective NA = 1.4
 Detection Wavelength = 405-450
 Imaging Device = GaAsP-Pmt3
 Detector Type = GaAsP-PMT
 Detector Gain = 500 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = 6.7 (3D, Auto)

Split View - Panel A

The left panel features Alpha Tubulin counterstained with Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Goat Anti-Rat, IgG (H + L), the middle panel features Actin counterstained with Identifyn® SRM Alexa Fluor™ 405 Affinity Purified Donkey Anti-Rabbit, IgG (H + L), and the right panel is the merged image of all channels. Z-Plane 81 of 161 shown.

Power Input - Panel B

The 730nm laser required 0.6% input to deliver high brightness, Scan Zoom 1.5, Sampling 1.20, Pixel Time 0.26s, Frame Time 5.63s, Scan Speed 11, Bidirectional, Line Step 1, and Averaging 8.

3D Image Processing - Panel C

3D Maximum Projection = Precise
 Appearance = Threshold 1% 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 Donkey Anti-Rabbit, 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

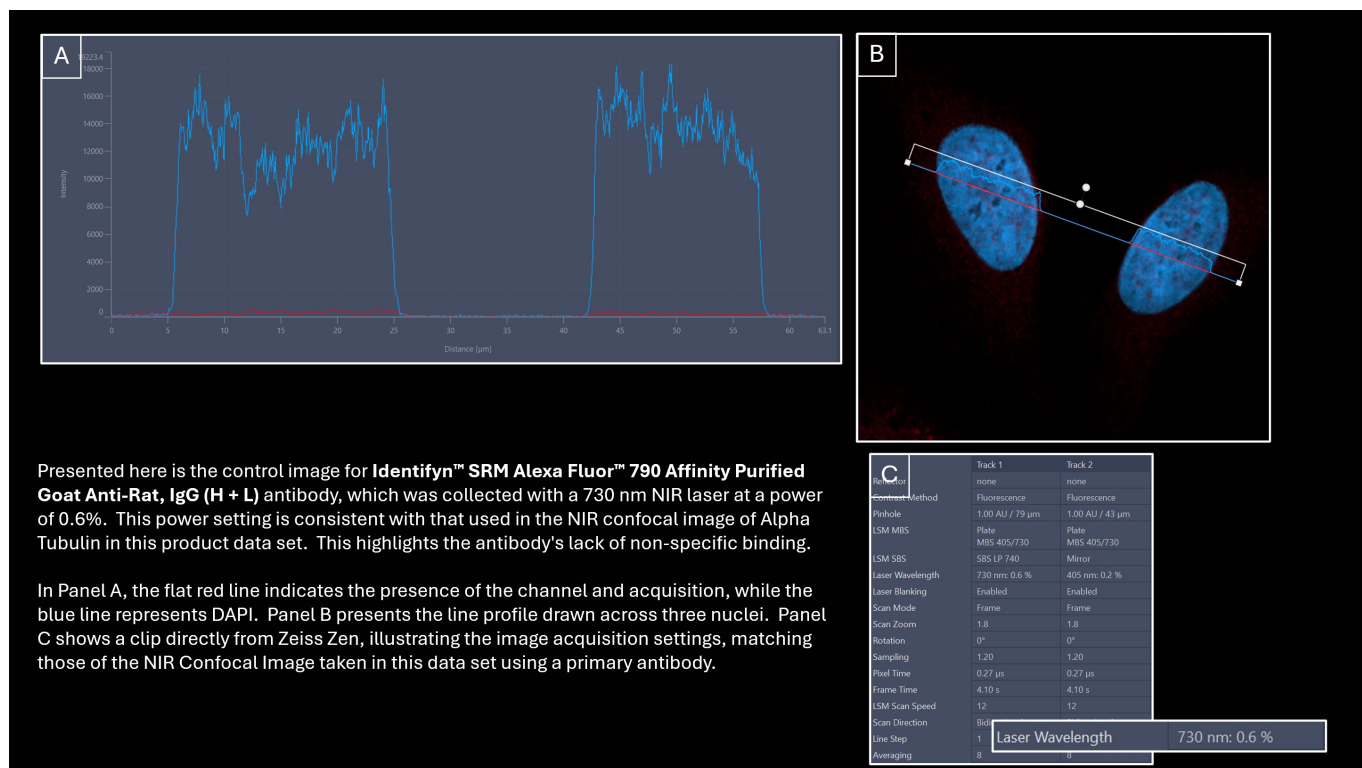
Image Analysis and Data Presentation B.T. Bennett

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SOURCE PROVENANCE

Catalog	SA-000108
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	52
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	0D01A58CD12A482CAA93254987737078D39885FC49C881EB8FB6C178C2AEE717
Method PDF SHA-256	00407D1CDC235BF3FA827DE276F676DA2579EEAD04DE9A29A7CE9E4838FD0072

ORIGINAL IMAGE EVIDENCE / CONTROL



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)	1248 X 1248
Image Size (Scaled)	73.41 μm X 73.41 μm
Bit Depth	16 Bit
Image Center Position	X: 76.27 μm , Y: 49.69 μm
Stage Position	X: 76.27 μm , Y: 49.69 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 / 43 μm
LSM MBS	Plate, MBS 405/730
LSM SBS	SBS LP 740 Mirror
Laser Wavelength	730 nm @0.6% 405 nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.8
Rotation	0°
Sampling	1.20
Pixel Time	0.27 μs
Frame Time	4.10 s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	789 353
Emission Wavelength	794 465
Effective NA	1.4
Detection Wavelength	755-900 430-470
Imaging Device	NIR Ch2 GaAsP-Pmt3
Detector Type	GaAs-PMT GaAsP-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	600 V 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	6.4 (3D, Auto) 5.6 (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 63.1), Y (Min 0 and Max 19223, DAPI and 538 for 790)

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 Goat Anti-Rat, IgG (H + L)
Cat ID# - SA 000108

HeLa Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocking with Thermo Fisher Fish Serum Blocker (cat#37527X3). In the absence of a primary antibody, Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Goat Anti-Rat, 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)

Channels = 2
Scaling (Per Pixel) = 0.059 µm X 0.059 µm
Image size (Pixels) = 1248 X 1248
Image Size (Scaled) = 73.41 µm X 73.41 µm
Bit Depth = 16 Bit
Image Center Position = X: 76.27 µm, Y: 49.69 µm
Stage Position = X: 76.27 µm, Y: 49.69 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

790 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00 AU / 79 μ m
 LSM MBS = Plate, MBS 405/730
 LSM SBS = SBS LP 740
 Laser Wavelength = 730 nm @0.6%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.8
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.27 μ s
 Frame Time = 4.10 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 = 600 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = 6.4 (3D, Auto)

DAPI -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00 AU / 43 µm
 LSM MBS = Plate, MBS 405/730
 LSM SBS = Mirror
 Laser Wavelength = 405 nm @0.2%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.8
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.27 µs
 Frame Time = 4.10 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 = 430-470
 Imaging Device = GaAsP-Pmt3
 Detector Type = GaAsP-PMT
 Detector Gain = 500 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = 5.6 (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 63.1), Y (Min 0 and Max 19223, DAPI and 538 for 790)

Summary -

Presented here is the control image for Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Goat Anti-Rat, IgG (H + L) antibody, collected with a 730 nm NIR laser at 0.6% power. This power setting is consistent with that used in the NIR confocal image of Alpha Tubulin in this product data set. This highlights 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, which match those of the NIR Confocal Image taken in this data set using a primary antibody.

Image Correction

None

Image by J.K. Bennett

Image Analysis and Data Presentation B.T. Bennett

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SOURCE PROVENANCE

Catalog	SA-000108
Stage	Control
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	13F9BA12F5E8933078F320F710D2AFEF882B926CE29CE10EEF22520246CA5A77
Method PDF SHA-256	B4D5B2B9DA2E4864E62E7784A91A57C54D52A6DE68D93BD98CF3A065D53EC4C1