

IDENTIFYN RESOLUTION STEPDOWN

# SECONDARY ANTIBODY MICROSCOPY EVIDENCE ASSESSMENT

Identifyn™ SRM Alexa Fluor™ 790 Affinity Purified Donkey Anti-Mouse, IgG (H + L)

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

4

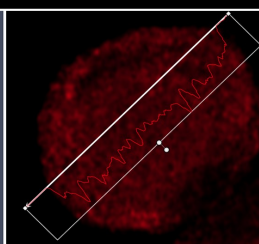
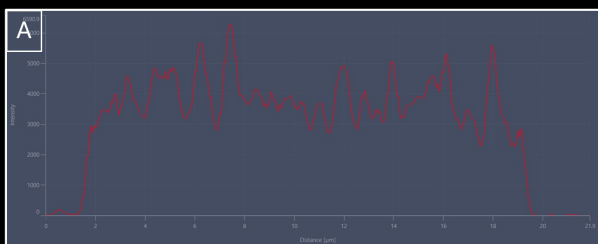
ORDERED EVIDENCE TIERS

1

SUPPORTING CONTROL

PENDING

SCIENTIST REVIEW

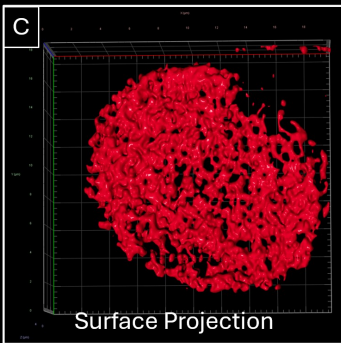
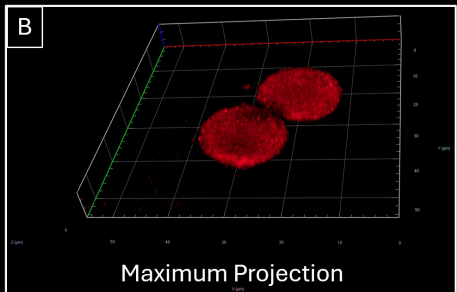


**D**

|                  |                 |
|------------------|-----------------|
| Track 1          | none            |
| Contrast Method  | Fluorescence    |
| Pinhole          | 1.00 AU / 79 µm |
| LSM MBS          | MBS 488/561     |
|                  | MBS 405/730     |
| LSM SBS          | SBS LP 740      |
| Laser Wavelength | 730 nm: 0.8 %   |
| Laser Blanking   | Enabled         |
| Scan Mode        | Frame           |
| Scan Zoom        | 2.4             |
| Rotation         | 0°              |
| Sampling         | 1.20            |
| Pixel Time       | 0.36 µs         |
| Frame Time       | 3.08 s          |
| LSM Scan Speed   | 12              |
| Scan Direction   | Bidirectional   |
| Line Step        | 1               |
| Averaging        | 8               |

Laser Wavelength

730 nm: 0.8 %



**Identifyn™ SRM Alexa Fluor™ 790 Affinity Purified Donkey  
Anti-Mouse, IgG (H + L)**  
Cat ID# - SA 000100

**Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7)**  
Cat ID# - PA-MM 000002

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / CONFOCAL

SA-000100 | PRODUCT-LEVEL EVIDENCE DOSSIER

SOURCE-GROUNDED MICROSCOPY + BIOLOGICAL REVIEW / IDENTIFYN EVIDENCE PRESERVED

## CRO CAPABILITY DEMONSTRATED

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

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

## SCIENTIFIC QUESTION

What source-bounded experimental and microscopy evidence is preserved for Identifyn™ SRM Alexa Fluor™ 790 Affinity Purified Donkey Anti-Mouse, 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-000100 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-000100 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; 49 DAPI; 672 - 748; 335 - 383; 765 - 855; 420 - 470; 752 |
| Widefield SIM — Apotome | 405/DAPI; 790                   | 2; AF750-49007; 49 DAPI; 672 - 748; 335 - 383; 765 - 855; 420 - 470; 752 |
| Confocal                | 405/DAPI; 488; 790              | 2; None; 730 nm @0.8%; 789; 794                                          |
| Control                 | 405/DAPI; 790                   | 2; None; 730 nm @0.8%; 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  $\mu\text{m}$  X 0.073  $\mu\text{m}$ ; Image size (Pixels): 1709 X 1305; Image Size (Pixels): 1709 X 1305; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Hamamatsu ORCA-Quest. Timing: Exposure Time: 350 ms; 200 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @35.00%; X-Cite Xylis Lamp Intensity @20.00%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 31.

#### 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 (8.8  $\mu\text{m}$ ); Channels: 2; Scaling (Per Pixel): 0.073  $\mu\text{m}$  X 0.073  $\mu\text{m}$  X 0.100  $\mu\text{m}$ ; Image size (Pixels): 997 X 979; Image Size (Pixels): 997 X 979; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Hamamatsu ORCA-Quest. Timing: Exposure Time: 350 ms; 200 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @35.00%; X-Cite Xylis Lamp Intensity @20.00%. 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: 38.

#### 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: 201 Slices (6 µm); Channels: 2; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 337 X 316; 930 X 930; Image Size (Pixels): 337 X 316; 930 X 930; Bit Depth: 16 Bit. Detector/camera: Imaging Device: NIR Ch2; Detector Type: GaAs-PMT. Timing: Pixel Time: 0.36 µs; Frame Time: 3.08 s. Illumination: Laser Wavelength: 730 nm @0.8%. Optical/scan: Pinhole: 1.00 AU / 79 µm; Scan Zoom: 2.4; LSM Scan Speed: 12; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 109%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); View Angle 45°, 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: 7.3 (3D, Auto). Structured source metadata values: 48.

#### 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; 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  $\mu\text{m}$  X 0.059  $\mu\text{m}$ ; Image size (Pixels): 1737 X 1737; Image Size (Pixels): 1737 X 1737; Bit Depth: 16 Bit. Detector/camera: Imaging Device: NIR Ch2; GaAsP-Pmt3; Detector Type: GaAs-PMT; GaAsP-PMT. Timing: Pixel Time: 0.22  $\mu\text{s}$ ; Frame Time: 6.49 s. Illumination: Laser Wavelength: 730 nm @0.8%; 405 nm @0.2%. Optical/scan: Pinhole: 1.00 AU / 79  $\mu\text{m}$ ; 1.00 AU / 44  $\mu\text{m}$ ; Scan Zoom: 1.3; LSM Scan Speed: 11; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: 6.6 (3D, Auto); 6.0 (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-000100 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  $\mu$ M; 2 $\mu$ M -> Etoposide = 2  $\mu$ M. The governing scientist identified every 200  $\mu$ M occurrence as a technician transcription error. The canonical historical concentration is 2  $\mu$ 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

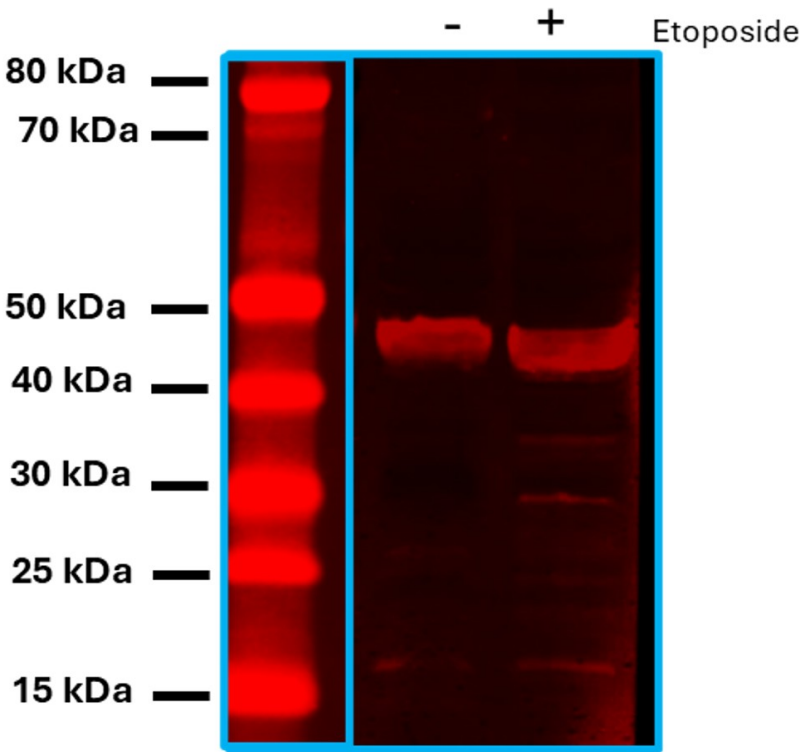
This dossier preserves the complete available evidence progression for SA-000100. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

| 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 Donkey Anti-Mouse, IgG (H + L)

Cat ID# - SA 000100

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

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

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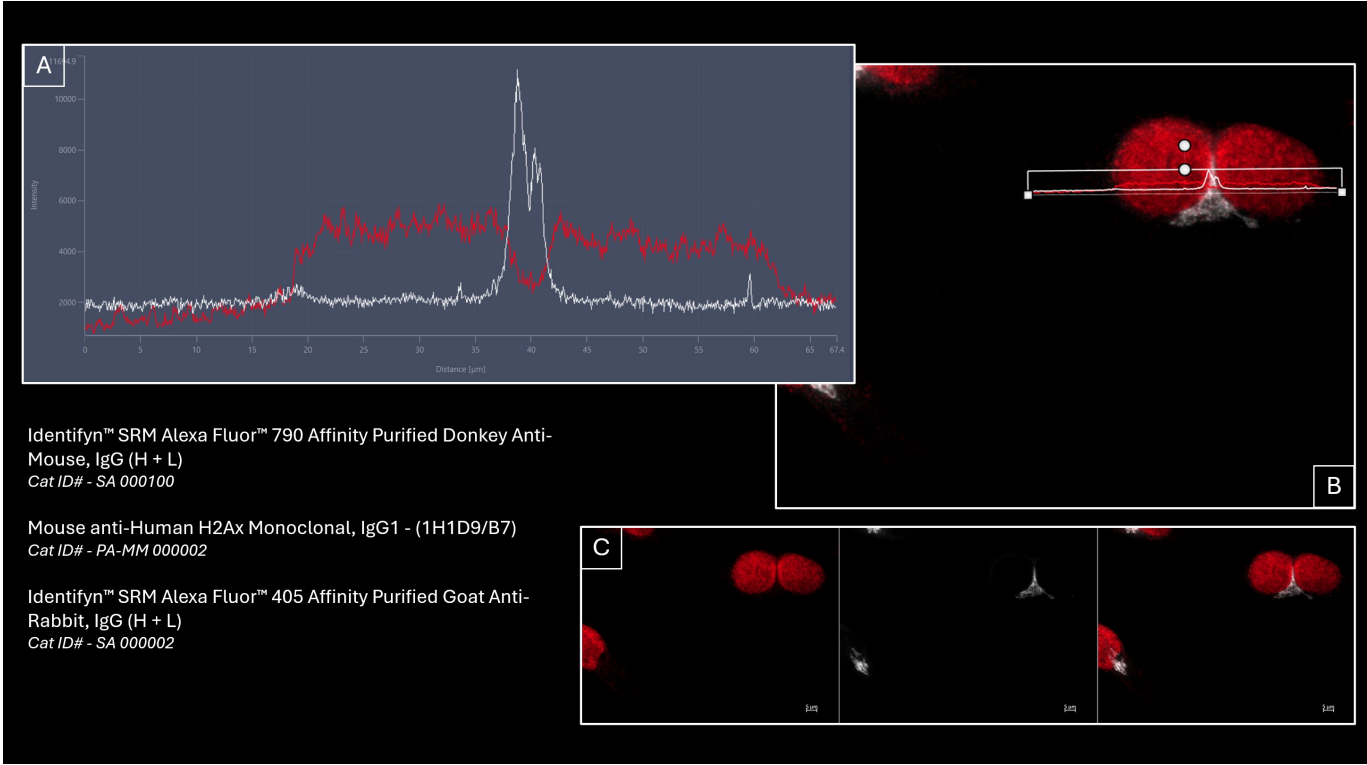
Image: K. Small

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## SOURCE PROVENANCE

|                                   |                                                                                               |
|-----------------------------------|-----------------------------------------------------------------------------------------------|
| Catalog                           | SA-000100                                                                                     |
| 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                     | 2DA42A3570191D5369AF3EF52EEB6162AA501C4BD56BE31685946DCE5D6A5352                              |
| Method PDF SHA-256                | F2E538997FE0B69AA14D51B3D233E25D661627B39C4B69A5A09EE5910BC102F7                              |

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



|                 |                                  |
|-----------------|----------------------------------|
| EVIDENCE TIER   | Widefield                        |
| INSTRUMENT      | ZEISS Axio Observer.Z1/7 Apotome |
| CELL MODEL      | HeLa                             |
| DETECTED DYES   | 405/DAPI; 790                    |
| METADATA VALUES | 31                               |

## 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 $\mu$ m X 0.073 $\mu$ m                                                                                                                                                      |
| Image size (Pixels)          | 1709 X 1305                                                                                                                                                                        |
| Image Size (Scaled)          | 124.78 $\mu$ m X 95.29 $\mu$ m                                                                                                                                                     |
| Bit Depth                    | 16 Bit                                                                                                                                                                             |
| Image Center Position        | X: 0.00 $\mu$ m, Y: 0.00 $\mu$ m                                                                                                                                                   |
| ROI Center Offset            | X: 0.00 $\mu$ m, Y: 0.00 $\mu$ 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 @35.00%   X-Cite Xylis Lamp Intensity @20.00%                                                                                                          |
| Exposure Time                | 350 ms   200 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 $\mu$ m   0.82 $\mu$ 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 67.4), Y (Min 710 and Max 11695)                                |

## 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 Donkey Anti-Mouse, IgG (H + L)

Cat ID# - SA 000100

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

Cat ID# - SA 000002

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, 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, and Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Donkey Anti-Mouse, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, Golgi GOLPH2 Polyclonal Antibody, was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 405 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:100. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant, 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) = 1709 X 1305

Image Size (Scaled) = 124.78 µm X 95.29 µm

Bit Depth = 16 Bit

Image Center Position = X: 0.00 µm, Y: 0.00 µm

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

## 790 -

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

## 405 -

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 @20.00%  
 Exposure Time = 200 ms  
 Excitation Wavelength = 401  
 Emission Wavelength = 422  
 Effective NA = 1.25  
 Imaging Device = Hamamatsu ORCA-Quest  
 Camera Adapter = 1X  
 Depth of Focus = 0.82  $\mu$ m  
 Binning Mode = 1,1

## Profile View Display - Panels A & B

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 67.4), Y (Min 710 and Max 11695)

## Split View - Panel C

The left panel features Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7) visualized with Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Donkey Anti-Mouse, IgG (H + L), the middle panel features golgi visualized with Identifyn® SRM Alexa Fluor™ 405 Affinity Purified Goat Anti-Rabbit, IgG (H + L), and the right panel is the merged image of all channels.

## Image Correction

Identifyn® SRM Alexa Fluor™ 405 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was pseudo-colored in Zen Software from blue, the default color, to white to provide visual contrast.

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Image by E.F. Simon

Image Analysis by B.T. Bennett

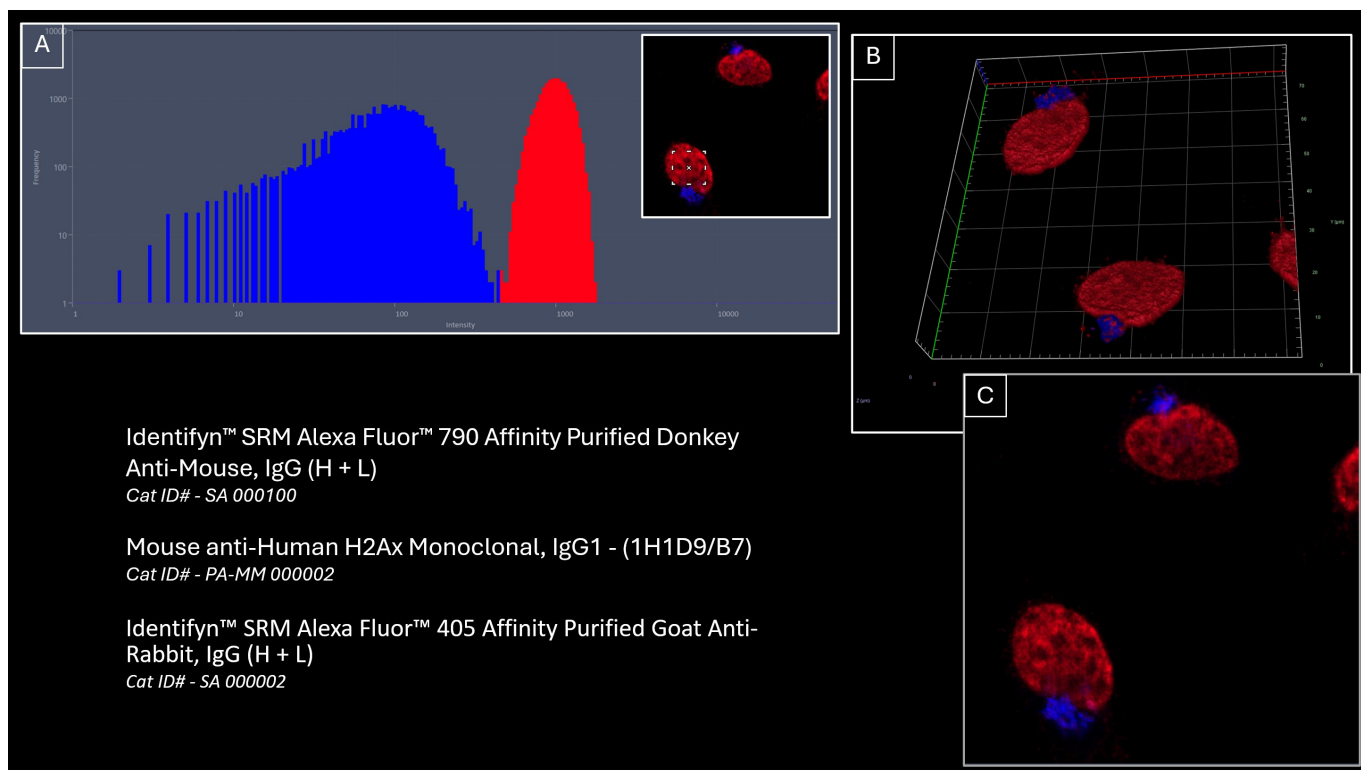
Data Presentation by P.D. Amistoso

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## SOURCE PROVENANCE

|                                   |                                                                                                                                                                                    |
|-----------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Catalog                           | SA-000100                                                                                                                                                                          |
| 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 | 31                                                                                                                                                                                 |
| Metadata source                   | PRESERVED_SOURCE_METHOD_PDF_TEXT                                                                                                                                                   |
| Cell model                        | HeLa                                                                                                                                                                               |
| Image SHA-256                     | 576D94AEDCB081973D66F238DC7E78DB7DCE2D3BA44D9C67845BB757518EBB67                                                                                                                   |
| Method PDF SHA-256                | 1C9A9F03D5EE30C8335A145DBC9A027B3E3A9C1C60BB1B6C84897BD98D3F4F9C                                                                                                                   |

## 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 | 38                               |

## 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 (8.8 µm)                                                                                                                                                                 |
| Channels                     | 2                                                                                                                                                                                  |
| Scaling (Per Pixel)          | 0.073 µm X 0.073 µm X 0.100 µm                                                                                                                                                     |
| Image Size (Pixels)          | 997 X 979                                                                                                                                                                          |
| Image Size (Scaled)          | 72.80 µm X 71.48 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   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 @35.00%   X-Cite Xylis Lamp Intensity @20.00%                                                                                                          |
| Exposure Time                | 350 ms   200 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 µm   0.82 µm                                                                                                                                                                  |
| Binning Mode                 | 1,1   2,2                                                                                                                                                                          |
| Apotome SIM Image Processing | Display Mod" "Optical Sectioning" - enabled correction selected.                                                                                                                   |
| 3D Volume Projection         | Precise                                                                                                                                                                            |
| Appearance                   | Threshold 13% all channels, Ramp 50% all channels, Maximum 100% all channels                                                                                                       |
| Background                   | Black                                                                                                                                                                              |
| Light                        | Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, 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® 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 Donkey Anti-Mouse, IgG (H + L)

Cat ID# - SA 000100

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

Cat ID# - SA 000002

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, 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, and Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Donkey Anti-Mouse, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, Golgi GOLPH2 Polyclonal Antibody, was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 405 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant, 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 45 of 89 total - Panels A & C

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

#### Z-Stack = 89 Slices (8.8 µm)

Channels = 2

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

Image Size (Pixels) = 997 X 979

Image Size (Scaled) = 72.80 µm X 71.48 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 @35.00%  
 Exposure Time = 350 ms  
 Excitation Wavelength = 752  
 Emission Wavelength = 779  
 Effective NA = 1.25  
 Imaging Device = Hamamatsu ORCA-Quest  
 Camera Adapter = 1X  
 Depth of Focus = 1.51  $\mu$ m  
 Binning Mode = 1,1

## 405 -

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 @20.00%  
 Exposure Time = 200 ms  
 Excitation Wavelength = 401  
 Emission Wavelength = 422  
 Effective NA = 1.25  
 Imaging Device = Hamamatsu ORCA-Quest  
 Camera Adapter = 1X  
 Depth of Focus = 0.82  $\mu$ 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.

## Histogram View - Panel A

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, Channel Transparency 100%, Statistic Table, Frequency Table, Internal Measurements, and Image were selected.

## 3D Image Processing - Panel B

3D Volume Projection = Precise

Appearance = Threshold 13% all channels, Ramp 50% all channels, Maximum 100% all channels

Background = Black

Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, 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)

## Summary

### Panel A

Histogram - Measures the frequency and intensity of the signal within this sample. In this analysis, we focus on a Region of Interest (ROI) located in the upper right corner of the image, specifically within the nucleus. This ROI analyzes H2AX monoclonal staining, which is counterstained with the Identifyn® SRM Alexa Fluor™790 affinity-purified donkey anti-mouse IgG (H + L). Additionally, we observe Golgi staining performed with the Identifyn SRM Alexa Fluor 405 affinity-purified goat anti-rabbit IgG (H + L). Notably, there is a strong signal for the H2AX/790 immunostaining at the approximate center z-plane of a diffuse pan-nuclear protein.

## Image Correction

### None

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Image by E.F. Simon

Image Analysis by B.T. Bennett

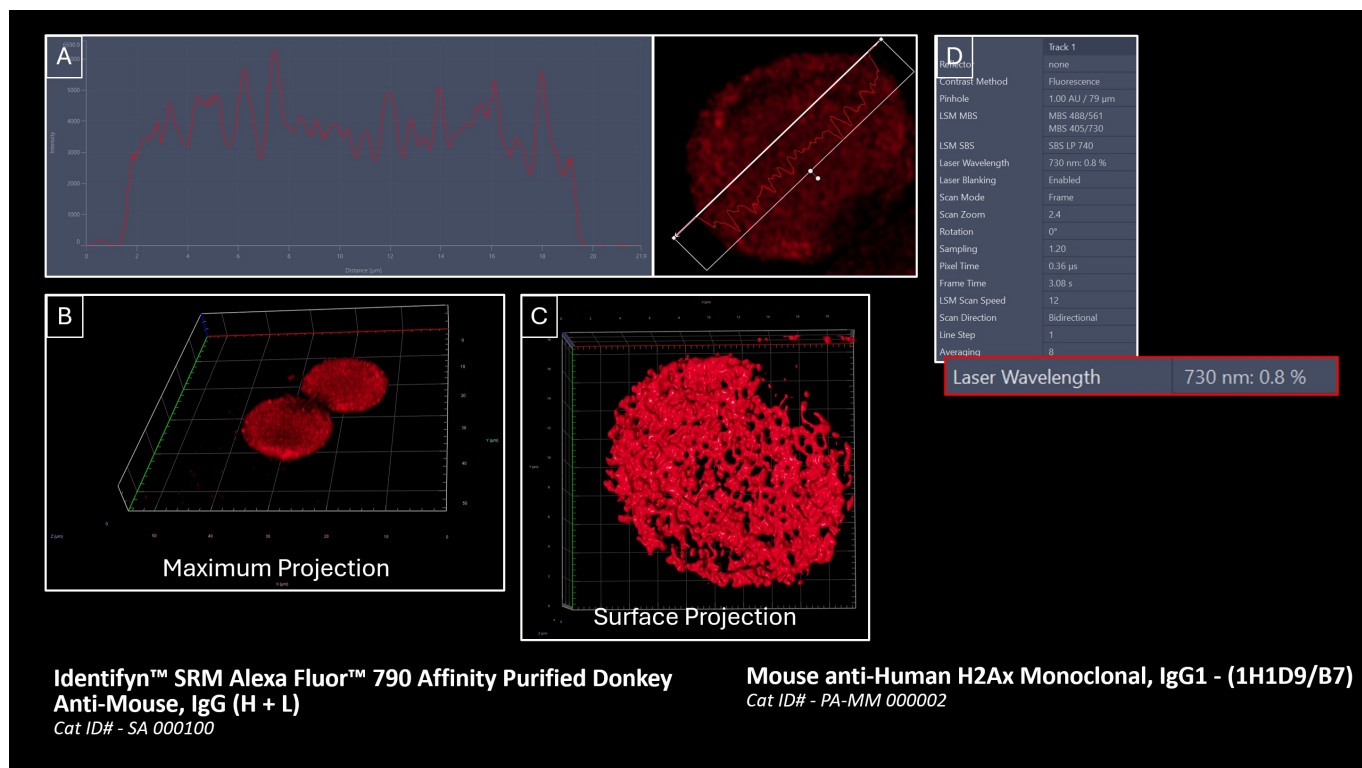
Data Presentation by P.D. Amistoso

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## SOURCE PROVENANCE

|                                   |                                                                                                                                                                                    |
|-----------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Catalog                           | SA-000100                                                                                                                                                                          |
| 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 | 38                                                                                                                                                                                 |
| Metadata source                   | PRESERVED_SOURCE_METHOD_PDF_TEXT                                                                                                                                                   |
| Cell model                        | HeLa                                                                                                                                                                               |
| Image SHA-256                     | 9E8C4E014DCA938489140176D655621EBF3441B202C070DD627A0DB4525881EE                                                                                                                   |
| Method PDF SHA-256                | 1FAF1C6EF0843B9B9ED46B7F5AF2CE8EAF48A65FBDED7421E93E6C69855F1C55                                                                                                                   |

## ORIGINAL IMAGE EVIDENCE / CONFOCAL



|                 |                    |
|-----------------|--------------------|
| EVIDENCE TIER   | Confocal           |
| INSTRUMENT      | ZEISS LSM 980      |
| CELL MODEL      | HeLa               |
| DETECTED DYES   | 405/DAPI; 488; 790 |
| METADATA VALUES | 48                 |

## 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)                                                                                                                                                 |
| Channels                 | 2                                                                                                                                                                 |
| Scaling (Per Pixel)      | 0.059 µm X 0.059 µm X 0.030 µm                                                                                                                                    |
| Image Size (Pixels)      | 337 X 316   930 X 930                                                                                                                                             |
| Image Size (Scaled)      | 19.82 µm X 18.59 µm   54.71 µm X 54.71 µm                                                                                                                         |
| Bit Depth                | 16 Bit                                                                                                                                                            |
| Image Center Position    | X: 87.60 mm, Y: 49.76 mm                                                                                                                                          |
| Stage Position           | X: 87.60 mm, Y: 49.76 mm                                                                                                                                          |
| ROI Center Offset        | X: 0.00 µm, Y: 0.00 µm                                                                                                                                            |
| Reflector                | None                                                                                                                                                              |
| Contrast Method          | Fluorescence                                                                                                                                                      |
| Pinhole                  | 1.00 AU / 79 µm                                                                                                                                                   |
| LSM MBS                  | MBS 488/561, 405/730                                                                                                                                              |
| LSM SBS                  | SBS LP 740                                                                                                                                                        |
| Laser Wavelength         | 730 nm @0.8%                                                                                                                                                      |
| Laser Blanking           | Enabled                                                                                                                                                           |
| Scan Mode                | Frame                                                                                                                                                             |
| Scan Zoom                | 2.4                                                                                                                                                               |
| Rotation                 | 0°                                                                                                                                                                |
| Sampling                 | 1.20                                                                                                                                                              |
| Pixel Time               | 0.36 µs                                                                                                                                                           |
| Frame Time               | 3.08 s                                                                                                                                                            |
| LSM Scan Speed           | 12                                                                                                                                                                |
| Scan Direction           | Bidirectional                                                                                                                                                     |
| Line Step                | 1                                                                                                                                                                 |
| Averaging                | 8                                                                                                                                                                 |
| Excitation Wavelength    | 789                                                                                                                                                               |
| Emission Wavelength      | 794                                                                                                                                                               |
| Effective NA             | 1.4                                                                                                                                                               |
| Detection Wavelength     | 755-900                                                                                                                                                           |
| Imaging Device           | NIR Ch2                                                                                                                                                           |

| FIELD                 | SOURCE-DOCUMENTED VALUE                                                                                                                                                                                                                                                                                                 |
|-----------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Detector Type         | GaAs-PMT                                                                                                                                                                                                                                                                                                                |
| Detector Gain         | 700 V                                                                                                                                                                                                                                                                                                                   |
| Detector Offset       | 0                                                                                                                                                                                                                                                                                                                       |
| Detector Digital Gain | 1.0                                                                                                                                                                                                                                                                                                                     |
| LSM Plus Mode         | 7.3 (3D, Auto)                                                                                                                                                                                                                                                                                                          |
| Profile Definition    | Stroke Thickness 2.00, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 21.9), Y (Min 0 and Max 65.91)                                                                                                                                                                    |
| 3D Maximum Projection | Precise                                                                                                                                                                                                                                                                                                                 |
| Appearance            | Threshold 4% all channels, Ramp 0% all channels, Maximum 100% all channels   Threshold 20%, Ambient Light 0%, Specular Light 100%, Shininess 26%                                                                                                                                                                        |
| Background            | Black                                                                                                                                                                                                                                                                                                                   |
| Light                 | Brightness 89%, Tone Mapping Enabled   Azimuth 80°, Elongation -130°, Enabled Tone Mapping                                                                                                                                                                                                                              |
| Projection            | View Angle 35°, Scale Z 109%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)   View Angle 45°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected) |
| 3D Surface Projection | Precise                                                                                                                                                                                                                                                                                                                 |

## SOURCE METHOD

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

### Identifyn Standards for Optical Microscopy Methods™

#### Identifyn® Primary Antibody

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

Cat ID# - PA-MM 000002

#### Identifyn® Secondary Antibody

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

Cat ID# - SA 000100

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, 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, and Identifyn® SRM Alexa Fluor™ 790 Affinity Purified Donkey Anti-Mouse, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:100. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant 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:

#### Single Plane (2D) - Z-plane 90 of 201 total - Panels A

#### Z Stack - (3D) - Panels A & C

#### Z-Stack = 201 Slices (6 µm)

Channels = 2

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

Image Size (Pixels) = 337 X 316

Image Size (Scaled) = 19.82 µm X 18.59 µm

Bit Depth = 16 Bit

Image Center Position = X: 87.60 mm, Y: 49.76 mm

Stage Position = X: 87.60 mm, Y: 49.76 mm

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

## Z Stack - (3D) - Panel B

### Z-Stack = 201 Slices (6 µm)

Channels = 2  
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm  
Image Size (Pixels) = 930 X 930  
Image Size (Scaled) = 54.71 µm X 54.71 µm  
Bit Depth = 16 Bit  
Image Center Position = X: 87.60 mm, Y: 49.76 mm  
Stage Position = X: 87.60 mm, Y: 49.76 mm  
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

### 790 -

Reflector = None  
Contrast Method = Fluorescence  
Pinhole = 1.00 AU / 79 µm  
LSM MBS = MBS 488/561, 405/730  
LSM SBS = SBS LP 740  
Laser Wavelength = 730 nm @0.8%  
Laser Blanking = Enabled  
Scan Mode = Frame  
Scan Zoom = 2.4  
Rotation = 0°  
Sampling = 1.20  
Pixel Time = 0.36 µs  
Frame Time = 3.08 s  
LSM Scan Speed = 12  
Scan Direction = Bidirectional  
Line Step = 1  
Averaging = 8  
Excitation Wavelength = 789  
Emission Wavelength = 794  
Effective NA = 1.4  
Detection Wavelength = 755-900  
Imaging Device = NIR Ch2  
Detector Type = GaAs-PMT  
Detector Gain = 700 V  
Detector Offset = 0  
Detector Digital Gain = 1.0  
LSM Plus Mode = 7.3 (3D, Auto)

## Profile View Display - Panel A

Profile Definition = Stroke Thickness 2.00, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto  
- X (Min 0.0 and Max 21.9), Y (Min 0 and Max 65.91)

## 3D Image Processing - Panel B

3D Maximum Projection = Precise

Appearance = Threshold 4% all channels, Ramp 0% all channels, Maximum 100% all channels

Background = Black

Light = Brightness 89%, Tone Mapping Enabled

Projection = View Angle 35°, Scale Z 109%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

## 3D Image Processing - Panel C

3D Surface Projection = Precise

Appearance = Threshold 20%, Ambient Light 0%, Specular Light 100%, Shininess 26%

Background = Black

Light = Azimuth 80°, Elongation -130°, Enabled Tone Mapping

Projection = View Angle 45°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

## Image Corrections

### None

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Image by J.K. Bennett

Image Analysis by B.T. Bennett

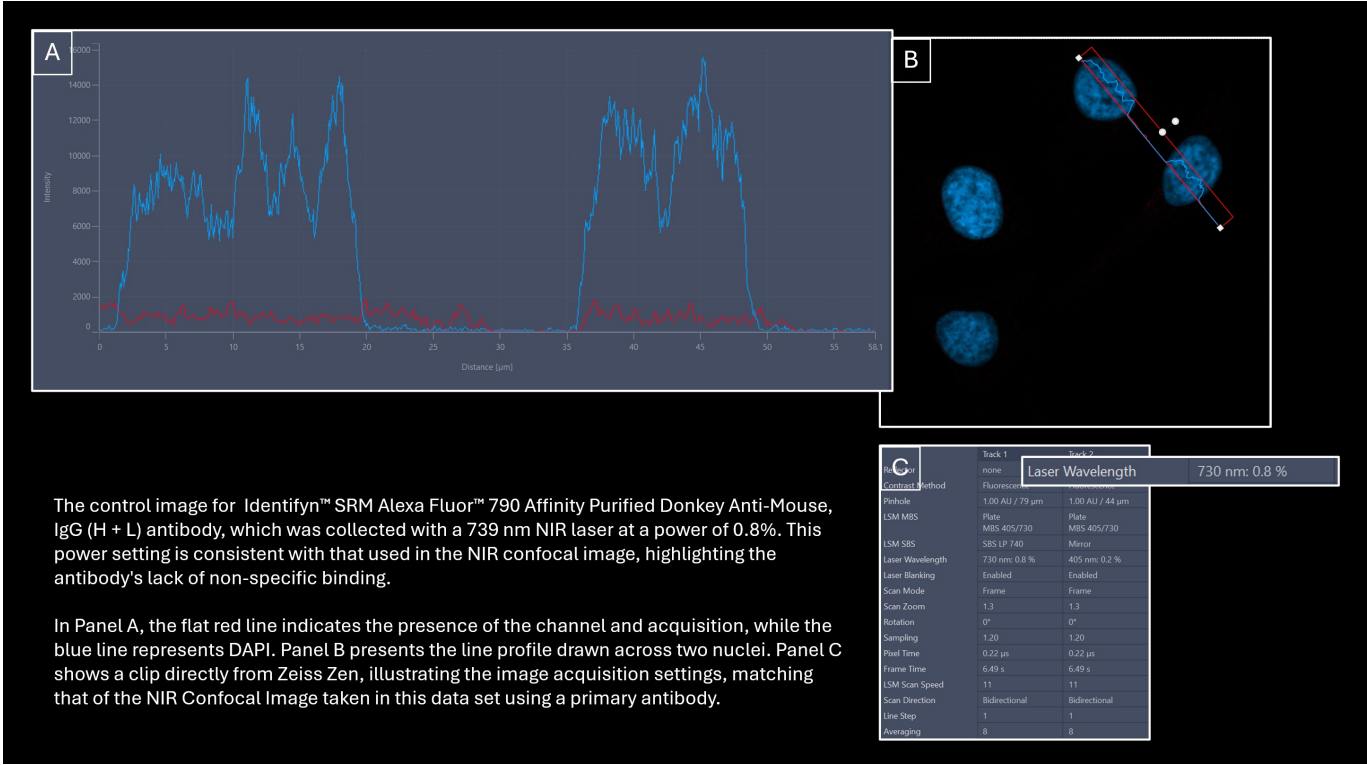
Data Presentation by P.D. Amistoso

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## SOURCE PROVENANCE

|                                   |                                                                                                                                                                   |
|-----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Catalog                           | SA-000100                                                                                                                                                         |
| 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 | 48                                                                                                                                                                |
| Metadata source                   | PRESERVED_SOURCE_METHOD_PDF_TEXT                                                                                                                                  |
| Cell model                        | HeLa                                                                                                                                                              |
| Image SHA-256                     | 1DA21471857DFC1B313DF2A31EAFD7078665A619EDEAEC0F39CC082484E590DB                                                                                                  |
| Method PDF SHA-256                | 3668C99BCAE5E2E18992E20A908CBFC642B075FFB48082E8B3B5AD040CF1F469                                                                                                  |

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 $\mu\text{m}$ X 0.059 $\mu\text{m}$                                                                                                                         |
| Image size (Pixels)      | 1737 X 1737                                                                                                                                                       |
| Image Size (Scaled)      | 102.19 $\mu\text{m}$ X 102.19 $\mu\text{m}$                                                                                                                       |
| Bit Depth                | 16 Bit                                                                                                                                                            |
| Image Center Position    | X: 92.68 $\mu\text{m}$ , Y: 51.29 $\mu\text{m}$                                                                                                                   |
| Stage Position           | X: 92.68 $\mu\text{m}$ , Y: 51.29 $\mu\text{m}$                                                                                                                   |
| ROI Center Offset        | X: 0.00 $\mu\text{m}$ , Y: 0.00 $\mu\text{m}$                                                                                                                     |
| Reflector                | None                                                                                                                                                              |
| Contrast Method          | Fluorescence                                                                                                                                                      |
| Pinhole                  | 1.00 AU / 79 $\mu\text{m}$   1.00 AU / 44 $\mu\text{m}$                                                                                                           |
| LSM MBS                  | Plate, MBS 405/730                                                                                                                                                |
| LSM SBS                  | SBS LP 740   Mirror                                                                                                                                               |
| Laser Wavelength         | 730 nm @0.8%   405 nm @0.2%                                                                                                                                       |
| Laser Blanking           | Enabled                                                                                                                                                           |
| Scan Mode                | Frame                                                                                                                                                             |
| Scan Zoom                | 1.3                                                                                                                                                               |
| Rotation                 | 0°                                                                                                                                                                |
| Sampling                 | 1.20                                                                                                                                                              |
| Pixel Time               | 0.22 $\mu\text{s}$                                                                                                                                                |
| Frame Time               | 6.49 s                                                                                                                                                            |
| LSM Scan Speed           | 11                                                                                                                                                                |
| Scan Direction           | Bidirectional                                                                                                                                                     |
| Line Step                | 1                                                                                                                                                                 |
| Averaging                | 8                                                                                                                                                                 |
| Excitation Wavelength    | 789   353                                                                                                                                                         |
| Emission Wavelength      | 794   465                                                                                                                                                         |
| Effective NA             | 1.4                                                                                                                                                               |
| Detection Wavelength     | 755-900   430-490                                                                                                                                                 |
| Imaging Device           | NIR Ch2   GaAsP-Pmt3                                                                                                                                              |
| Detector Type            | GaAs-PMT   GaAsP-PMT                                                                                                                                              |

| FIELD                 | SOURCE-DOCUMENTED VALUE                                                                                                                              |
|-----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------|
| Detector Gain         | 700 V   500 V                                                                                                                                        |
| Detector Offset       | 0                                                                                                                                                    |
| Detector Digital Gain | 1.0                                                                                                                                                  |
| LSM Plus Mode         | 6.6 (3D, Auto)   6.0 (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 58.1), Y (Min 0 and Max 16354) |

## SOURCE METHOD

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

### Identifyfyn Standards for Optical Microscopy Methods™

#### Identifyfyn® Secondary Antibody

Identifyfyn® SRM Alexa Fluor™ 790 Affinity Purified Donkey Anti-Mouse, IgG (H + L)

Cat ID# - SA 000100

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, Identifyfyn SRM Alexa Fluor 790 Affinity Purified Donkey Anti-Mouse, 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) = 1737 X 1737

Image Size (Scaled) = 102.19 µm X 102.19 µm

Bit Depth = 16 Bit

Image Center Position = X: 92.68 µm, Y: 51.29 µm

Stage Position = X: 92.68 µm, Y: 51.29 µm

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

## 790 -

Reflector = None  
 Contrast Method = Fluorescence  
 Pinhole = 1.00 AU / 79  $\mu$ m  
 LSM MBS = Plate, MBS 405/730  
 LSM SBS = SBS LP 740  
 Laser Wavelength = 730 nm @0.8%  
 Laser Blanking = Enabled  
 Scan Mode = Frame  
 Scan Zoom = 1.3  
 Rotation = 0°  
 Sampling = 1.20  
 Pixel Time = 0.22  $\mu$ s  
 Frame Time = 6.49 s  
 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 = 700 V  
 Detector Offset = 0  
 Detector Digital Gain = 1.0  
 LSM Plus Mode = 6.6 (3D, Auto)

## DAPI -

Reflector = None  
 Contrast Method = Fluorescence  
 Pinhole = 1.00 AU / 44  $\mu$ m  
 LSM MBS = Plate, MBS 405/730  
 LSM SBS = Mirror  
 Laser Wavelength = 405 nm @0.2%  
 Laser Blanking = Enabled  
 Scan Mode = Frame  
 Scan Zoom = 1.3  
 Rotation = 0°  
 Sampling = 1.20  
 Pixel Time = 0.22  $\mu$ s  
 Frame Time = 6.49 s  
 LSM Scan Speed = 11  
 Scan Direction = Bidirectional  
 Line Step = 1  
 Averaging = 8  
 Excitation Wavelength = 353  
 Emission Wavelength = 465  
 Effective NA = 1.4  
 Detection Wavelength = 430-490  
 Imaging Device = GaAsP-Pmt3  
 Detector Type = GaAsP-PMT  
 Detector Gain = 500 V  
 Detector Offset = 0  
 Detector Digital Gain = 1.0  
 LSM Plus Mode = 6.0 (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 58.1), Y (Min 0 and Max 16354)

## Image Correction

None

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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-000100 |
| Stage   | Control   |

## SOURCE PROVENANCE

|                                   |                                                                                                                                                                   |
|-----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Instrument                        | ZEISS LSM 980                                                                                                                                                     |
| Microscope configuration          | Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye: |
| Structured source metadata values | 46                                                                                                                                                                |
| Metadata source                   | PRESERVED_SOURCE_METHOD_PDF_TEXT                                                                                                                                  |
| Cell model                        | HeLa                                                                                                                                                              |
| Image SHA-256                     | 5FC0543A99FBCE5B04BEB99FB1DBD8F267F0F0C2E556D34DE36B2236BAED2F21                                                                                                  |
| Method PDF SHA-256                | 86967935490B01C2F8A41C76F945B81D593D6FFE6998FA15B6F398F4F7F0AF9F                                                                                                  |