

Recoverable temporal and spectral information in public human macrophage imaging

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Research Article

Keywords: human macrophages, microscopy metadata, image reuse, stimulated Raman scattering, temporal redundancy, cell correspondence, secondary analysis

Posted Date: October 27th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11469316/v1>

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Additional Declarations: The authors declare no competing interests.

Abstract

Reusing microscopy images to relate an earlier cellular state to later function requires interpretable measurement axes and individual correspondence. We performed a purposive, exploratory audit of four public human macrophage videos and one fixed-cell hyperspectral stimulated Raman scattering (SRS) field from three studies. Source metadata and file checksums supported reproducible analyses of decoded frames, rendered channels and spectral blocks. One video contained 45 byte-distinct complete frames, yet a fixed interior crop contained six distinct images: 39 of 44 adjacent crop pairs were pixel-identical. Five large full-frame transitions were separated from the remaining changes by a 208-fold gap. The six interior images were consistent with the source description, without certifying acquisition timestamps. Three video renderings replicated greyscale values across RGB channels. The SRS field contained 153 planes in three spectral blocks; at the shared nominal setting of 2950 cm^{-1} , raw field means differed by 15.5% between adjacent blocks. These results change which stored elements can be counted as distinct content and which intensities can be compared without additional assumptions. A task-specific evidence matrix distinguishes established, conditional, unestablished and structurally absent information. The audit supports reuse of the examined representations while leaving individual correspondence and the added value of spectra for tracking unevaluated. Its contribution is a reproducible set of resource-specific findings, without population generalisation or claims of a new tracking method.

Introduction

Macrophages can change appearance and composition while remaining the same individual. A study relating an earlier cellular state to a later response therefore needs both interpretable measurements and a defensible link between observations. Cell type, subcellular location, measured state and individual continuity are different questions. A signal that describes state need not be unique to a cell or remain constant when that cell responds.

Published human macrophage resources include live efferocytosis imaging [1], differential interference contrast and confocal movies [2], live Raman and stimulated Raman scattering (SRS) imaging [3], and fixed-cell SRS fields [4,5]. These sources establish relevant imaging precedents. Their public representations may preserve different information from that required for a new question: a movie can contain overlays and repeated content, while a stack can sample spectral position rather than time.

This distinction is grounded in established imaging practice. The Open Microscopy Environment (OME) specification explicitly maps image planes to dimensions [6]. Recommended Metadata for Biological Images (REMBI) addresses the context needed for reuse [7], and QUAREP-LiMi checklists address transparent image presentation and analysis [8]. The Cell Tracking Challenge separates object detection, segmentation and temporal links, assessed against reference annotations [9]. These frameworks do not make a particular resource adequate for every purpose; they motivate examining what information is retained and what remains to be established.

We asked what temporal, spectral and correspondence information could be recovered from selected public human macrophage image files, and how that recovery changes their permissible reuse. We examined five files from three studies, with detailed contrasts of interior video repetition and a nominal spectral setting repeated across acquisition blocks. Related preprints considered selection and weighting in optical interpretation [10] and what a description must preserve for response comparisons [11]. The present contribution consists of distinct human source files and directly computed consequences of their representations. It neither introduces a tracking algorithm nor claims that the importance of metadata or correspondence is new.

Methods

Resource selection and analytical units

The audit began on 21 October 2026 in Colombia. A focused search for human macrophage time-lapse, confocal, Raman and SRS resources was combined with previously identified references. It was not systematic, exhaustive or representative of the literature. Eligibility required a retrievable image or video, a traceable primary source supporting the human macrophage context, and interpretable modality information. We selected Canton movies 2, 4 and 6 [2], Santulli-Marotto S1 Movie [1], and the fixed human macrophage field associated with panel 3D in S-BIAD1584 [4,5]. They supplied complementary representations rather than biological replicates. We assessed a secondary use, not the adequacy of the original studies for their own purposes.

Files and fields were the descriptive units. Frames, pixels and tiles were nested within them; no donor-level inference or population hypothesis test was performed. Cell-type attribution came from the source characterisation. We generated no new specimens or acquisitions and did not combine measurements across studies as paired observations. Stiebing et al. [3] provided a reported live-human Raman/SRS precedent, based on its publisher abstract and metadata; its raw data and complete methods were not independently audited. Supplementary File S1 preserves resource-selection decisions and distinguishes contemporaneous records from retrospective clarification. The analyses of interior repetition and the repeated spectral setting were added on 22 October after inspection of the initial results and reviewer feedback. They are exploratory, not preregistered.

File provenance and decoding

Original URLs, retrieval records, sizes and SHA-256 checksums identify the five inputs. The two video source articles distribute their materials under CC BY 4.0; the SRS archive declares CC0. Source attribution and transformations accompany all reused images. Python, OpenCV, NumPy, tiff file and Matplotlib supported decoding, numerical summaries and figures; exact versions and executable scripts are supplied in Supplementary File S2.

For each movie, we recorded frame count, dimensions, playback rate, exact decoded-frame duplication and equality of rendered RGB channels. A command-line FFmpeg route checked counts. Both routes use FFmpeg components and are not independent decoder implementations. The revised audit rejects decoder errors, missing completion information or count discrepancies before accepting its results. Pixel differences describe the representation rather than cellular motion.

We distinguished playback time, source-reported acquisition cadence, acquisition-to-frame mapping, acquisition origin and dependence between observations. These properties need not be established together. Reported cadence can support conditional relative timing if each rendered frame represents one consecutive acquisition; matching counts alone do not certify that assumption. No physical velocity or biological event rate was calculated.

Interior repetition in the PLOS movie

For all 44 consecutive frame pairs, we calculated mean absolute difference across pixels and rendered channels in stored 8-bit units. We sorted the full-frame differences and found the largest ratio between consecutive positive values, without fixing the number of large transitions. This is an exploratory separation rule, not a universal threshold or classifier.

A fixed central crop, $x=64:576$ and $y=48:432$ in zero-based, upper-exclusive coordinates, retained 512×384 pixels from each 640×480 frame. The crop was chosen during exploratory inspection to exclude the displayed clock and scales. We compared crop arrays exactly, counted unique arrays and identified runs of adjacent identical content. Complete frames and their crop bounds are retained in Supplementary Figure S1 and the numerical tables. Agreement between crop changes and large full-frame changes is a within-file check using overlapping information, not independent biological validation. Neither analysis labels cells or reconstructs acquisition timestamps.

Spectral reconstruction and repeated-setting contrast

The file `Macrophages_5hCholOA_SRS.tiff` contains a fixed preparation [4]. The source README assigns its 153 planes to three 51-plane blocks: 1625–1775, 2800–2950 and 2950–3100 cm^{-1} , each in increments of 3 cm^{-1} . The directory label 3D identifies a figure panel, and the condition name 5h does not define a time axis. Both entries at 2950 cm^{-1} were retained. We used the accompanying metadata; citing OME does not assert that this particular file implements OME-TIFF.

We calculated field means, spatial standard deviations, quantiles and extrema across all 1,048,576 pixels per plane, including background. The primary contrast compared planes 101 and 102, the final plane of block 2 and first plane of block 3 at nominal 2950 cm^{-1} . Relative difference was $100 \times (\text{mean in block 3} - \text{mean in block 2}) / \text{mean in block 2}$. A supplementary calculation subtracted each plane's documented coverglass background, retaining signs. Acquisition settings differed between blocks; this comparison

was not a controlled estimate of a single instrumental or biological cause. No concentration calibration or source molecular decomposition was reproduced.

The earlier intensity-selected region analysis was retained as a supplementary illustration. Region A maximised and region B approximated the median of 32×32 -pixel tile means over three display planes. Such selection makes an amplitude contrast expected. Neither region was treated as an identified cell or organelle. Spatial dispersion is not an uncertainty interval for a cell population.

Evidence states and computational verification

For each reuse question we distinguished **established** information directly supported by the inspected file or source; **conditional** information derivable under an explicit, unverified assumption; **not established** information not annotated or sufficiently verified here; and information **absent in the representation**, such as a live time sequence in a fixed spectral field. These are scoped audit judgments, not a validated quality score. Their evidence and adjudication provenance are recorded in Supplementary File S2. Lack of an established reference is not evidence that no useful correspondence could be annotated.

Verification included input hashes, complete decoding, per-page TIFF agreement, alternative arithmetic and rerunning the packaged analyses from an isolated copy. Earlier checks and the current revision are identified separately in the verification records; a prior receipt is not used to certify changed code. Computational agreement concerns these inputs and implementations, not replication between preparations or independent biological validation.

Results

Complete-frame uniqueness concealed repeated interior content

The four movies contained 39, 40, 20 and 45 decoded frames, respectively (Table 1). Every complete frame within each movie was byte-distinct. The three Canton exports had equal rendered R, G and B values, so their RGB arrays contained replicated greyscale information. The coloured PLOS overlay did not provide independently recoverable Raman/SRS measurements.

Table 1. Decoded videos and temporal interpretation. Counts concern exported frames, not independent biological observations. Cadence and nominal windows come from the source captions [1,2].

Resource	Frames	Source cadence and window	Acquisition-to-frame interpretation
Canton movie 2	39	30 s; nominal 15 min	Mapping unresolved; count needs additional explanation
Canton movie 4	40	15 s; nominal 10 min	Consecutive-acquisition mapping conditional; 39 intervals would span 585 s
Canton movie 6	20	15 s; nominal 5 min	Consecutive-acquisition mapping conditional; 19 intervals would span 285 s
Santulli-Marotto S1	45	Six images; 5 min; nominal 30 min	Six interior patterns recovered; exact source events and timestamps unestablished

The PLOS crop contained exactly six unique arrays. Of 44 adjacent crop pairs, 39 were pixel-identical and five changed, at boundaries 4–5, 13–14, 21–22, 30–31 and 39–40 (Figure 1). Runs occupied indices 0–4, 5–13, 14–21, 22–30, 31–39 and 40–44. Thus, complete-frame byte uniqueness did not establish new interior image content at every frame.

The largest ratio between sorted positive full-frame differences was 208.3863, separating a maximum small difference of 0.0500249566 from a minimum large difference of 10.4245138889 stored 8-bit units. The next-largest ratio was 1.3402. The five large transitions matched the crop boundaries. The output table supplies full-precision values; thresholds strictly between the exact separation endpoints select the same five boundaries.

The six interior patterns are consistent with the source's six-image description [1]. They do not certify six original acquisitions or independent observations. Under a one-to-one mapping, six images at five-minute intervals span five intervals, or 25 minutes; the nominal 30-minute window therefore does not establish exact timestamps. Likewise, the Canton 4/6 counts are compatible with their nominal windows under endpoint conventions without proving a consecutive-acquisition mapping. Relative frame order remains usable for inspection even when physical timing is unresolved.

Stored amplitudes differed between blocks at the same nominal spectral setting

All 153 TIFF pages matched the corresponding planes of the complete array. The $153 \times 1024 \times 1024$ uint16 field retained three spectral blocks and two observations at 2950 cm^{-1} . Its 320,881,706-byte size and checksum matched the input manifest. The planes describe a fixed field, not longitudinal observations of individuals.

At 2950 cm^{-1} , raw means were 211.38975 stored-signal units in block 2 and 244.16172 in block 3, a 15.5031% increase relative to block 2 (Figure 2). Subtraction of documented backgrounds gave signed means of 56.45999 and 76.41152, respectively, a 35.3375% difference. The latter comparison is supplied

in Supplementary File S1 and the output tables; background subtraction alone does not establish cross-block calibration.

Sharing a nominal coordinate alone did not establish quantitative interchangeability between these two stored planes. Retaining both planes and their block identities preserves this discrepancy; silently averaging them would conceal it. The comparison does not identify detector gain, molecular concentration or biological change, because other acquisition conditions were not held identical. No inferential error bar or population test is assigned to this single-field contrast. Supplementary Figures S3–S5 retain spatial maps, selected-region profiles and complete field summaries.

Reuse depended on the question and the evidence state

The audit established representation properties while identifying conditional and unevaluated uses (Table 2). It recovered interior redundancy and spectral block structure without establishing cellular trajectories. Some visible links might be annotated in a future evaluation. Repeated per-cell Raman/SRS spectra paired with those links are not supplied by the selected renderings or fixed field. Their combination across studies does not create that pairing.

Table 2. Evidence states for specific reuse questions. States apply to the inspected representations and records, not all information held by the source investigators. They are not a ranked quality scale.

Reuse question	State	Evidence and consequence
Count distinct interior content in PLOS S1	Established	Six exact crop arrays across 45 frames; peripheral content and acquisitions remain distinct questions
Assign relative time in Canton 4/6	Conditional	Use reported 15 s cadence only if rendered frames map to consecutive acquisitions
Interpret SRS plane coordinates	Established	Metadata identify three blocks and repeated 2950 cm ⁻¹ ; retain block-specific amplitudes
Evaluate cell links in the videos	Not established	No correspondence reference was annotated or evaluated here; possible links were not ruled out
Recover a live trajectory from the fixed SRS field	Absent in this representation	Spectral planes of fixed material do not form a live time sequence
Evaluate the added value of spectra for tracking	Not established	The admitted set supplies no paired longitudinal per-cell spectra and independent correspondence reference

Discussion

What the recovered information changes

These cases show two different consequences of reading stored elements as biological observations. In the video, different complete arrays repeated exactly the same interior content. In the SRS stack, the same nominal coordinate occurred in different blocks with different stored amplitudes. The first finding changes the count of distinct displayed interior images; the second changes the conditions under which intensities may be combined. Neither requires a claim about a new macrophage mechanism.

Metadata standards already establish the importance of dimensions, provenance and transparent transformations [6–8]. Our contribution is to make their practical consequences inspectable in particular public files, with code and quantitative outputs. The full-frame duplicate test was technically correct but insufficient for the question of interior repetition. The repeated-setting comparison was possible because block identity and both observations were preserved. These findings support task-specific reuse rather than a binary judgment that a source is adequate or inadequate for all purposes.

Why correspondence matters when state changes

Consider a constructed example with two baseline cells, one with a higher optical signal and one with a lower signal, followed by one uptake event and one non-event. With complete observation and a fixed denominator of two, either cell could be assigned the event if individual links are lost. The total remains one while the direction of the baseline signal–response association reverses. This illustration is not an observation in the audited videos or a new theorem. It shows why measurements of states and responses do not identify their individual relationship without correspondence. Aggregate outcomes need not remain identifiable when losses or eligibility also change.

Tracking evaluation separates masks and temporal links [9]. Ordered-frame references can support correspondence measures without an absolute acquisition clock; estimating physical velocities additionally requires appropriate time and spatial calibration. A useful future evaluation would compare spatial continuity and morphology with the same observations plus spectral features, using separately supported reference links and reporting ambiguity, losses and coverage. The present audit did not perform that comparison.

The biological motivation also requires keeping state and identity distinct. Live-human Raman/SRS observations are already reported [3]. A descriptor sensitive to compositional change may be useful for state measurement while changing within the same cell. Conversely, similar spectra can occur in different cells. Preserving correspondence should allow biological change rather than defining identity as an invariant spectrum. In a prospective study of repeated uptake demands, linking baseline individuals to later confirmed episodes would further require eligibility, observation windows and event adjudication. Better linkage would improve measurement of history; it would not randomise that history or establish its causal effect.

Scope and limitations

The resource set is small and purposively selected. It cannot estimate the prevalence of representation problems, variability between donors, or general tracking performance. Published videos are derived

renderings, and the spectral analysis concerns one fixed field. The crop was chosen after inspection; its exact redundancy result applies to its stated bounds. The separation rule was exploratory and has not been validated across videos. Block-specific differences are descriptive and do not isolate their cause. Intensity-selected tiles illustrate extraction procedures rather than independent biological classes.

The absence of a joint spectral tracking benchmark is largely expected from the selected modalities and sample states; it is a scope limit, not the principal empirical discovery. This audit does not establish that suitable longitudinal datasets are unavailable elsewhere. It also does not replicate the source studies' complete analyses or validate a proposed imaging configuration. The preserved source records, numerical checks and transparent distinctions make the current findings assessable while leaving those broader questions open.

Conclusion

In the selected human macrophage files, 45 distinct complete video frames contained six distinct interior images, and two spectral planes at nominal 2950 cm^{-1} differed in raw field mean by 15.5% across acquisition blocks. Recovering these properties changes how displayed observations are counted and how stored intensities can be compared. The accompanying evidence matrix identifies established, conditional and unevaluated uses without treating uncertainty as impossibility. This reproducible case audit supplies concrete information for reuse and cell-history study planning; individual correspondence and the benefit of spectral features for tracking remain to be evaluated.

Declarations

Data and code availability

The original inputs are available through the source journal supplements and BioImage Archive S-BIAD1584 [1,2,5]. Supplementary File S1 describes selection, variables, transformations and reproduction. Supplementary File S2 supplies executable analysis code, pinned requirements, source metadata, input URLs and checksums, numerical outputs, figures, verification records and four small source videos with attribution. The 320,881,706-byte SRS TIFF is retrieved from its repository by the included downloader and accepted only when its size and SHA-256 match the manifest. Remote availability is not guaranteed; changed files are rejected rather than substituted. Source licences are retained.

Funding

The author received no specific funding for this work.

Competing interests

The author declares no competing interests.

Ethics and consent

This secondary analysis used published, publicly accessible image files and metadata. No new recruitment, participant contact or collection of human specimens was undertaken, and no donor-identifying records were introduced. Source collection and consent procedures are those reported by the original studies [1,2,4]. No new institutional ethics approval or waiver is claimed.

Author contributions

Elkin Navarro Quiroz is the sole human author and corresponding author. He conceived and directed the study and its biological questions. Computational implementation, visualisation, drafting and critical review were developed with AI assistance as described below. The author is responsible for the scientific interpretation and submitted content. The cited creators retain the original experimental contributions.

AI assistance

OpenAI Codex assisted literature discovery, code generation and execution, descriptive analysis, figure production, drafting and critical review. Scientific figures derive from documented source files and numerical outputs; the retained identity schematic is conceptual. No generative image was used as empirical microscopy. AI-assisted checks are not external peer review or independent biological validation, and AI is not an author.

Acknowledgements

The author thanks the investigators who made the source images, videos and metadata available. Their inclusion does not imply endorsement of this audit.

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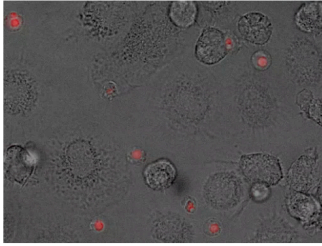
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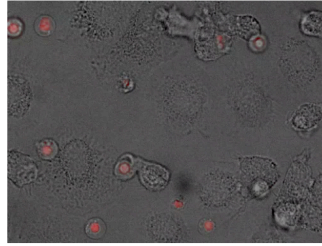
Figures

A Six distinct interior images

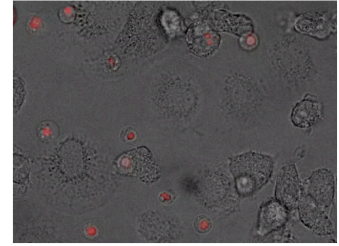
Pattern 1 | frames 0-4



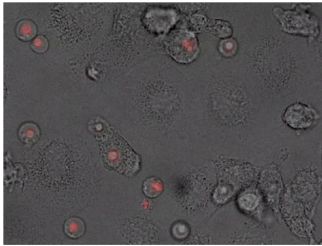
Pattern 2 | frames 5-13



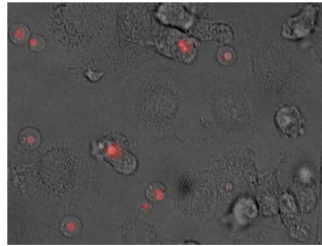
Pattern 3 | frames 14-21



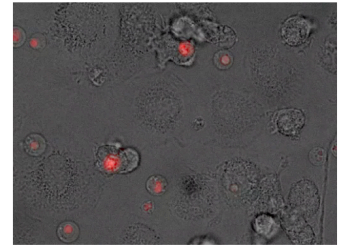
Pattern 4 | frames 22-30



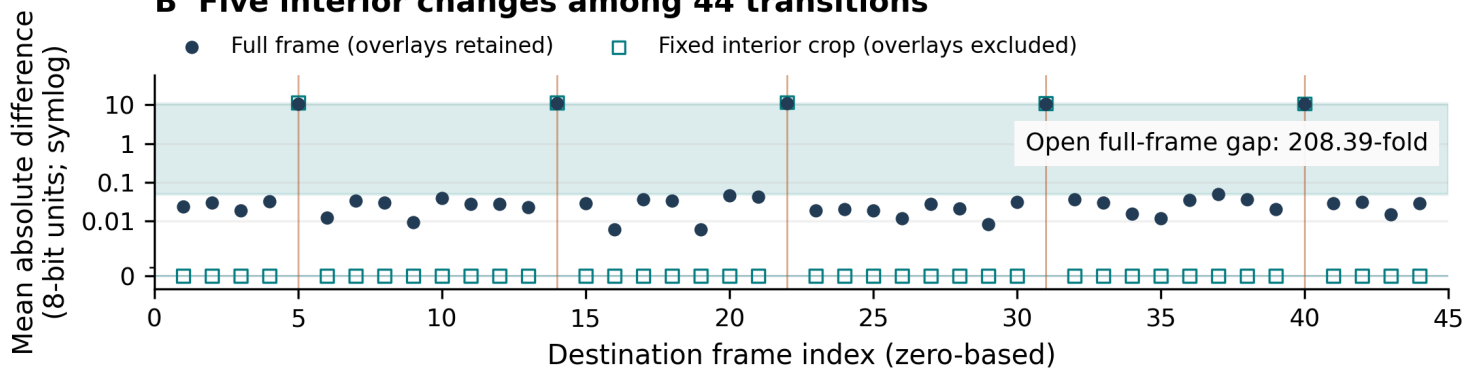
Pattern 5 | frames 31-39



Pattern 6 | frames 40-44



B Five interior changes among 44 transitions



C Frames per interior pattern



Fixed crop: $x = 64:576$, $y = 48:432$ (upper bounds excluded); 39 of 44 interior transitions equal zero.
 Panel B: symlog, linear below 0.005. Image equality does not establish timing or cell identity.
 Source: Santulli-Marotto et al., PLOS ONE (2015), S1 Movie, CC BY 4.0. Cropped and assembled.

Figure 1

Interior repetition in the published PLOS movie. Consecutive-frame differences and six representative interior images from Santulli-Marotto et al. [1], S1 Movie, CC BY 4.0. The fixed crop $x=64:576$, $y=48:432$ excludes displayed clock and scales; all indices are zero-based and upper crop bounds are excluded. Differences use stored 8-bit values across rendered channels. Thirty-nine adjacent crops are exactly equal; the five changes coincide with the largest full-frame changes. Representatives display the crop without a new physical scale or cell labels. The crop and separation rule were exploratory. Groups

identify repeated image content, not cells, acquisitions or independent biological states. Complete frames and crop bounds appear in Supplementary Figure S1.

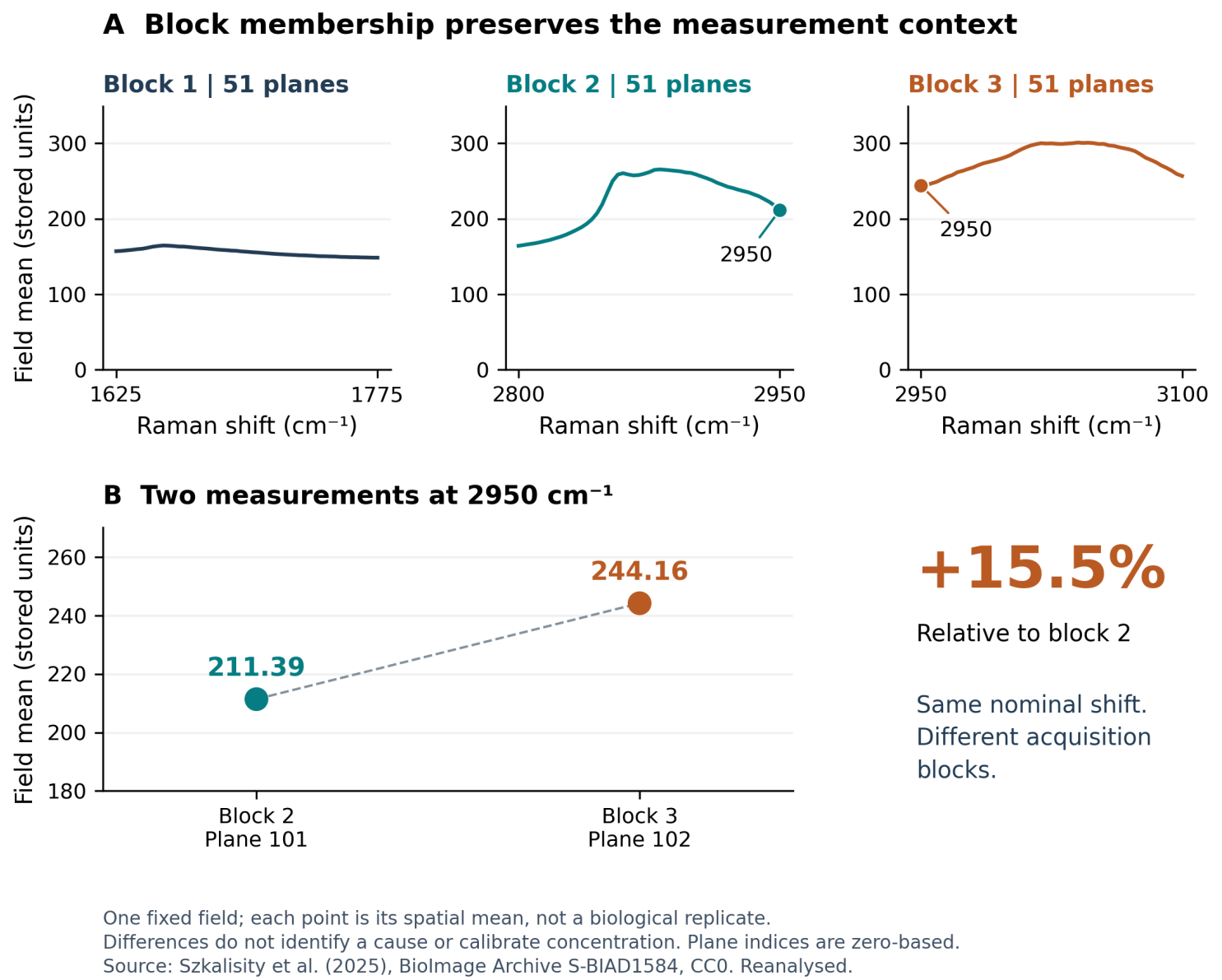


Figure 2

Spectral blocks and the repeated nominal setting.Field means from the fixed human macrophage TIFF associated with panel 3D in Szkalitsity et al. [4], S-BIAD1584 [5], CC0. The three 51-plane blocks are retained separately. Planes 101 and 102 both have nominal Raman shift 2950 cm⁻¹ but raw means of 211.39 and 244.16 arbitrary stored-signal units. The 15.50% difference uses block 2 as denominator. These are descriptive means over all pixels, including background, in one field; no biological-replicate uncertainty is represented. Distinct block settings preclude attribution of the difference to a single cause or interpretation as a concentration change.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryFileS1.pdf](#)
- [SupplementaryFileS2.zip](#)