controls_1st_run_metrics

June 19, 2025

0.1 Control Dataset Overview

This notebook analyzes spontaneous calcium activity in BxPC3 pancreatic cancer cells imaged under identical control conditions across six image sequences.

Experimental context: - Cell line: BxPC3 (pancreatic ductal adenocarcinoma) - Imaging: DIV2, seeded at 1.2 million cells per well (high confluence) - Ca^{2} dye: Fluo-4 AM (standard loading protocol) - Nuclei: Hoechst 33342 (10 µg/mL) - Acquisition: FITC, 1 Hz, 30 ms exposure for 30 minutes

This is the **first run** of spontaneous activity under control conditions. The goal is to assess the **reproducibility** and **biological variability** of peak-, cell-, and event-level metrics across replicates.

0.2 Setup & Utilities

Standard scientific libraries (Pandas, NumPy, Seaborn) and custom plotting utilities are loaded. These functions enable streamlined metric visualization and layout control across multiple datasets.

[1]:

```
# Libraries
import seaborn as sns
import numpy as np
from pathlib import Path
import sys

# Add /Source to sys.path if not already present
project_root = Path.cwd().parent
source_path = project_root / "src"

if source_path.exists() and str(source_path) not in sys.path:
    sys.path.insert(0, str(source_path))

# Utility functions
from calcium_activity_characterization.analysis.visualizers import␣
  ↪plot_metric_by_dataset, plot_category_distribution_by_dataset,␣
  ↪plot_raster_plots_by_dataset
from calcium_activity_characterization.analysis.loaders import␣
  ↪load_and_merge_datasets

sns.set(style="whitegrid")
```

```
# Define paths to control datasets
control_paths = {
    "20250326 - IS1": "D:/Mateo/20250326/Output/IS1",
    "20250326 - IS3": "D:/Mateo/20250326/Output/IS3",
    "20250404 - IS1": "D:/Mateo/20250404/Output/IS1",
    "20250404 - IS3": "D:/Mateo/20250404/Output/IS3",
    "20250409 - IS09": "D:/Mateo/20250409/Output/IS09",
    "20250409 - IS11": "D:/Mateo/20250409/Output/IS11",
}

# Load and merge datasets
peaks = load_and_merge_datasets(control_paths=control_paths, file_name="peaks.
 ↪csv")
cells = load_and_merge_datasets(control_paths=control_paths, file_name="cells.
 ↪csv")
events = load_and_merge_datasets(control_paths=control_paths, file_name="events.
 ↪csv")
```

0.3 Preprocessing

Before visual comparison, we normalize event labels (e.g., missing `in_event` entries set to `'individual'`) and compute additional derived fields (`is_active`, `periodicity_score`) to ensure consistent comparisons across all datasets.

These steps are crucial for **ensuring fair metric aggregation** and enabling downstream comparative analysis.

[2]:
```
peaks["in_event"] = peaks["in_event"].fillna("individual").str.lower()

if "is_active" not in cells.columns:
    cells["is_active"] = cells["num_peaks"].apply(lambda x: 1 if x > 0 else 0)

if "periodicity_score" not in cells.columns:
    cells["periodicity_score"] = np.nan
```

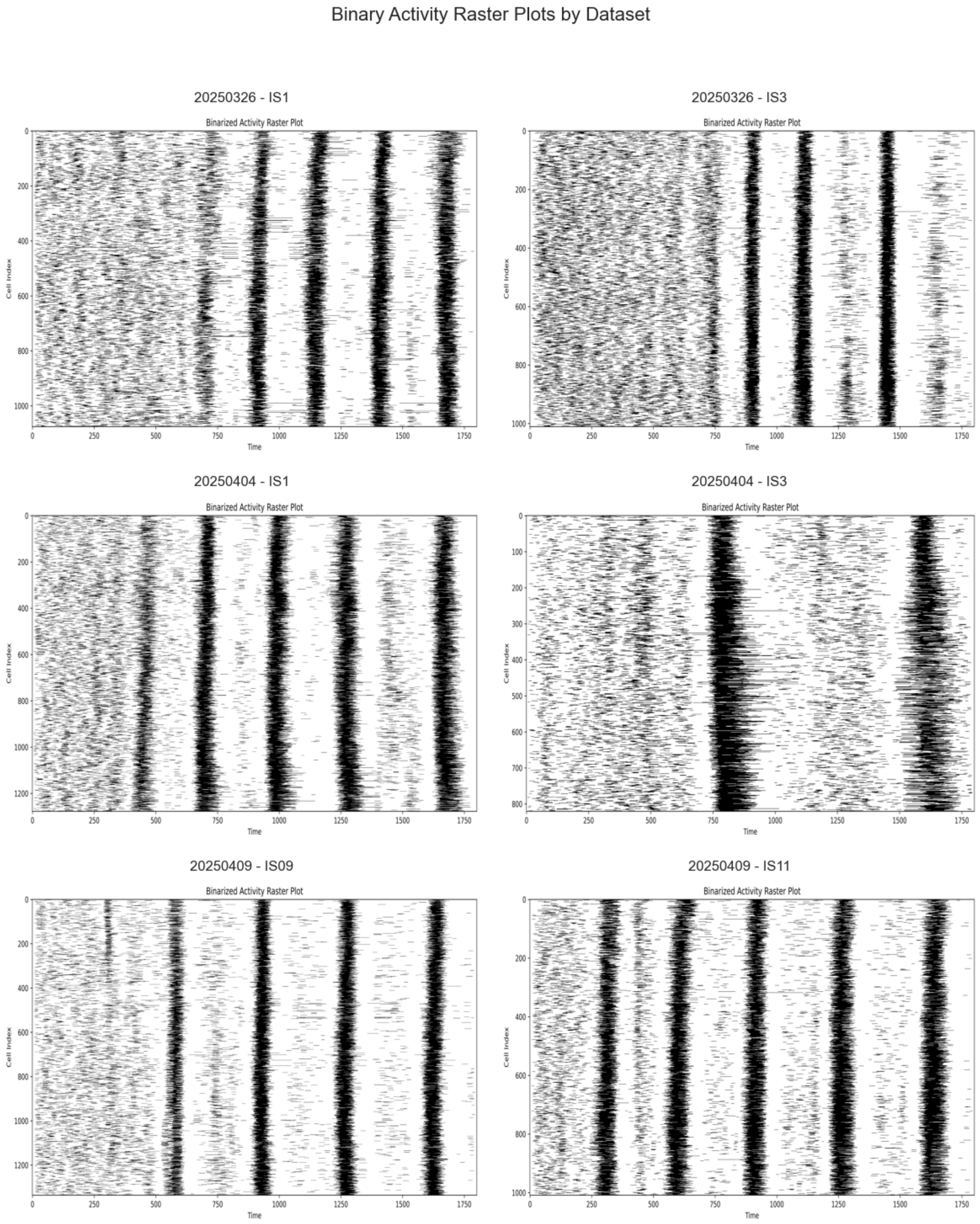
0.4 Raster Plot Inspection

Raster plots provide a binary overview of calcium activity over time per cell.

Remarks: TODO

Use these plots to **screen visually for anomalies or batch effects** before statistical analysis.

[3]:
```
plot_raster_plots_by_dataset(
    dataset_paths=control_paths,
    title="Binary Activity Raster Plots by Dataset"
)
```



0.5 Peak Type Distribution

Pie charts quantify the proportion of detected calcium peaks classified as: - `individual`: isolated peaks - `sequential`: propagating local events - `global`: large-scale synchronized events

Interpretation:

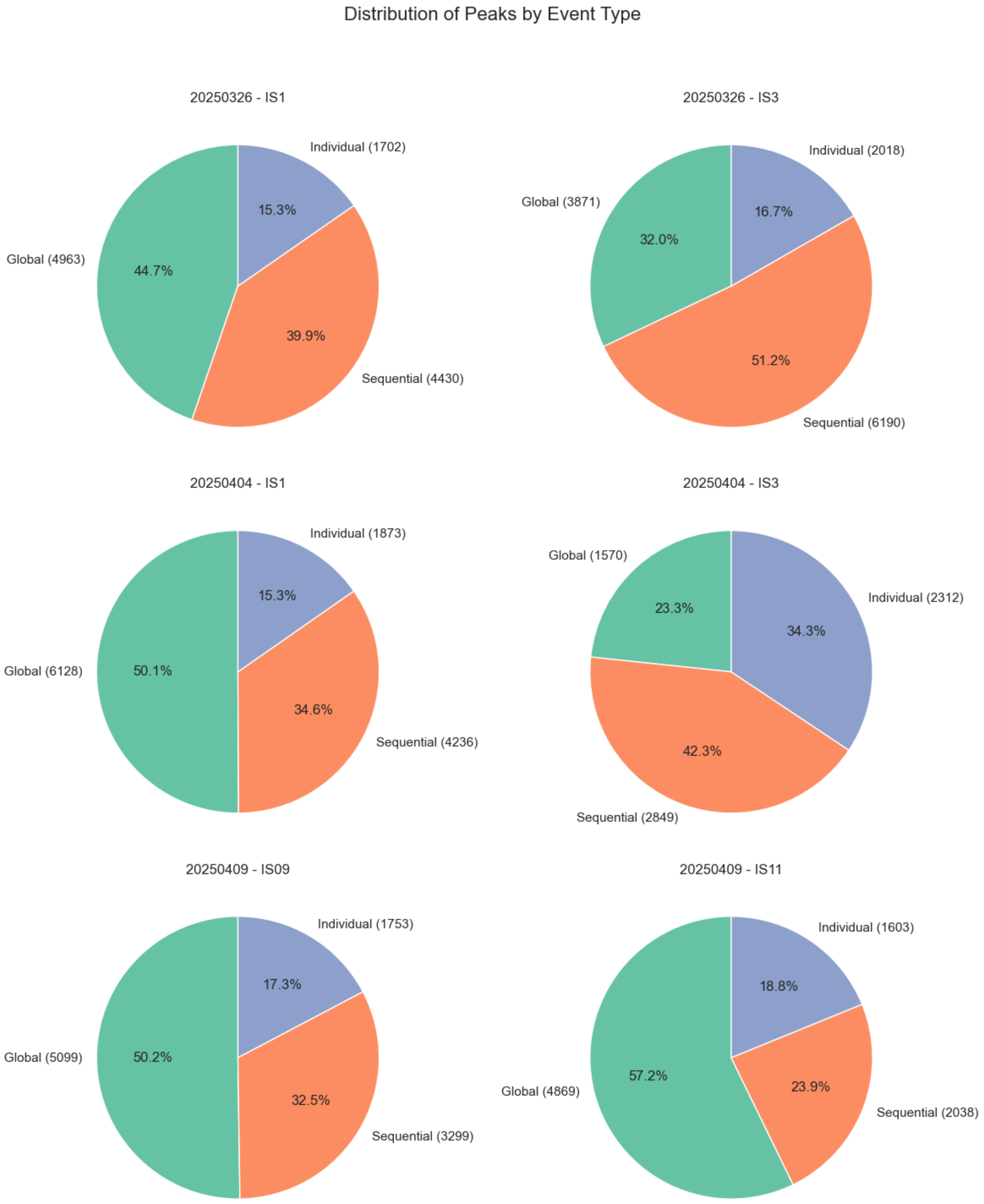
The distribution of peaks by event type (global, sequential, individual) across First Run control datasets is highly consistent.

- *Approximately* ***30-40% global events*** *are consistently observed, except in image sequence 2025-04-04 IS3, where fewer global events were detected.*
- *Sequential and individual peaks maintain stable proportions across datasets, indicating reproducible spontaneous calcium signaling activity under standard conditions.*

First run image sequences demonstrate reproducible and robust peak-type distribution, validating their reliability as control data.

[4]:

```
peak_type_colors = {
    "global": "#66c2a5",
    "sequential": "#fc8d62",
    "individual": "#8da0cb"
}

plot_category_distribution_by_dataset(
    df=peaks,
    column="in_event",
    category_order=["global", "sequential", "individual"],
    colors=peak_type_colors,
    title="Distribution of Peaks by Event Type"
)
```



0.6 Peak Metrics per Event Type

Metrics like **duration**, **prominence**, and **symmetry** are visualized per event type.

Interpretation:

- *Global peaks: Characteristically long durations (~50 frames).*
- *Sequential peaks: Duration means around **26–28 frames**; symmetry values around **0.9 ± 0.12**.*
- *Individual peaks: Duration around **26–27 frames**, also with symmetry ~0.9 ± 0.12.*

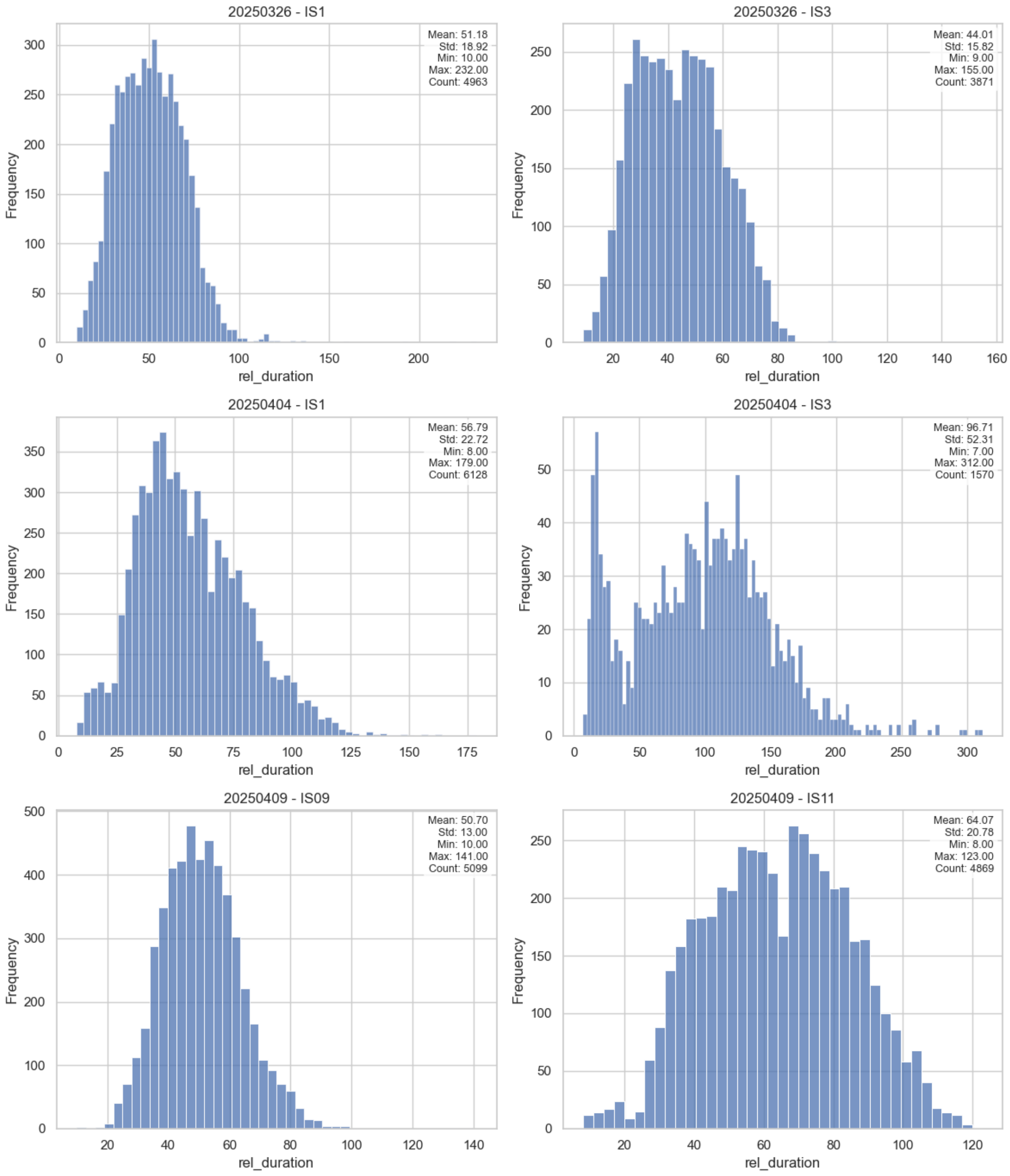
Overall, the peak metrics in the First Run are stable, biologically plausible, and reproducible.

[5]:

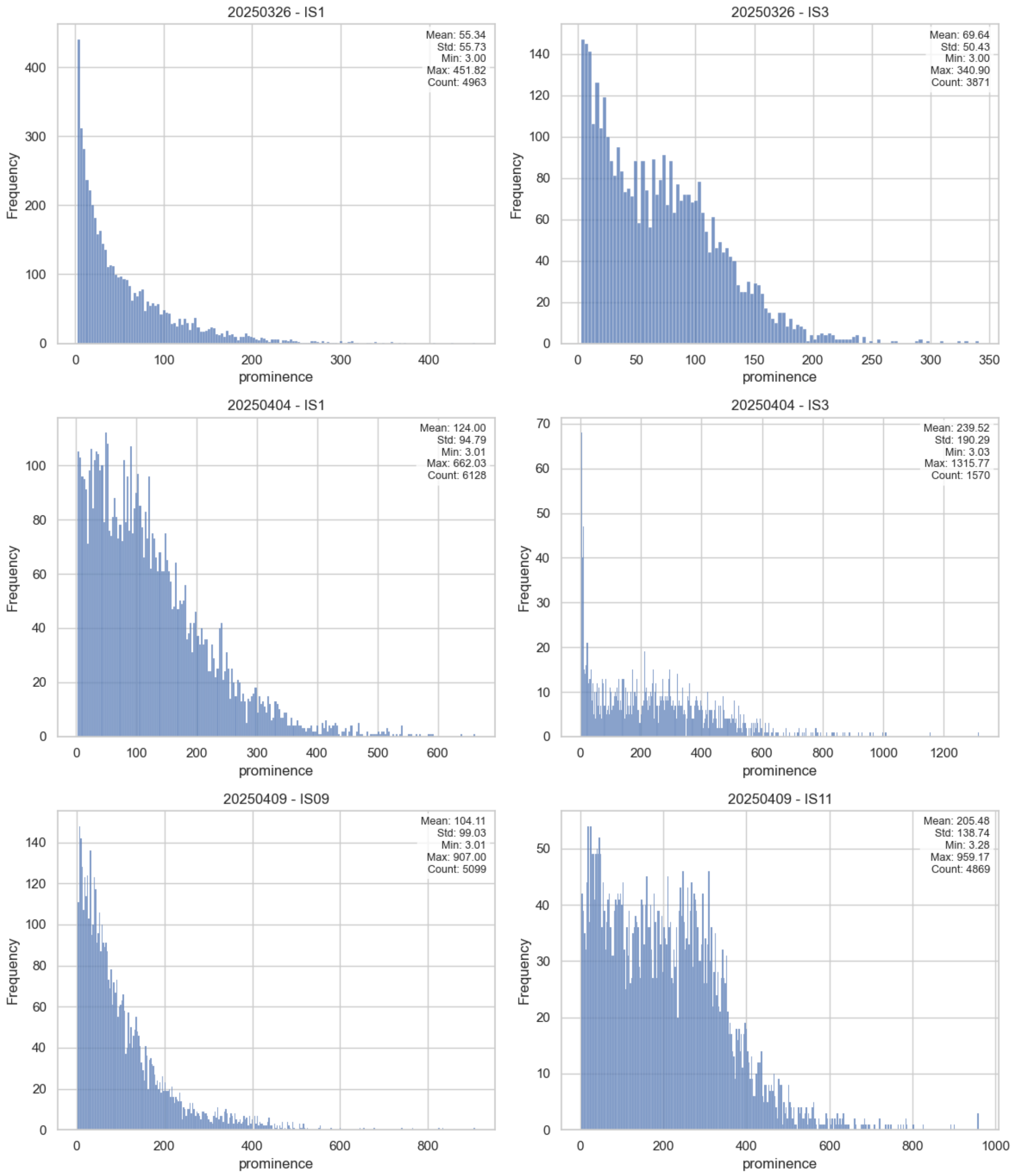
```python
for event_type in ["global", "sequential", "individual"]:
    subset = peaks[peaks["in_event"] == event_type]
    print(f"\n# Peak Type: {event_type.title()} - {len(subset)} peaks")
    plot_metric_by_dataset(subset, "rel_duration", f"{event_type.title()} Peaks: Duration", bin_width=3)
    plot_metric_by_dataset(subset, "prominence", f"{event_type.title()} Peaks: Prominence", bin_width=3)
    plot_metric_by_dataset(subset, "rel_symmetry_score", f"{event_type.title()} Peaks: Symmetry", bin_count=30)
```

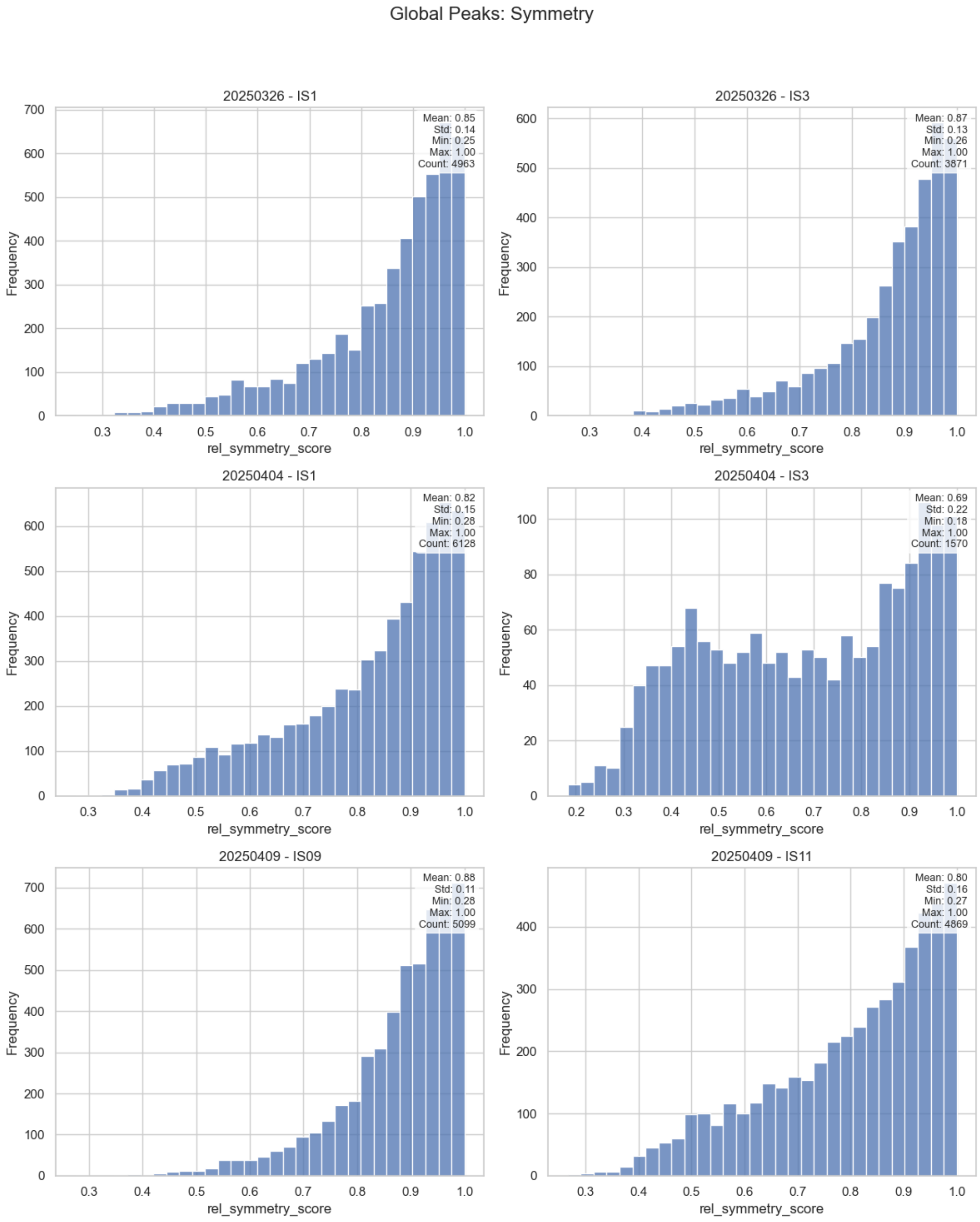
```
# Peak Type: Global - 26500 peaks
```

Global Peaks: Duration



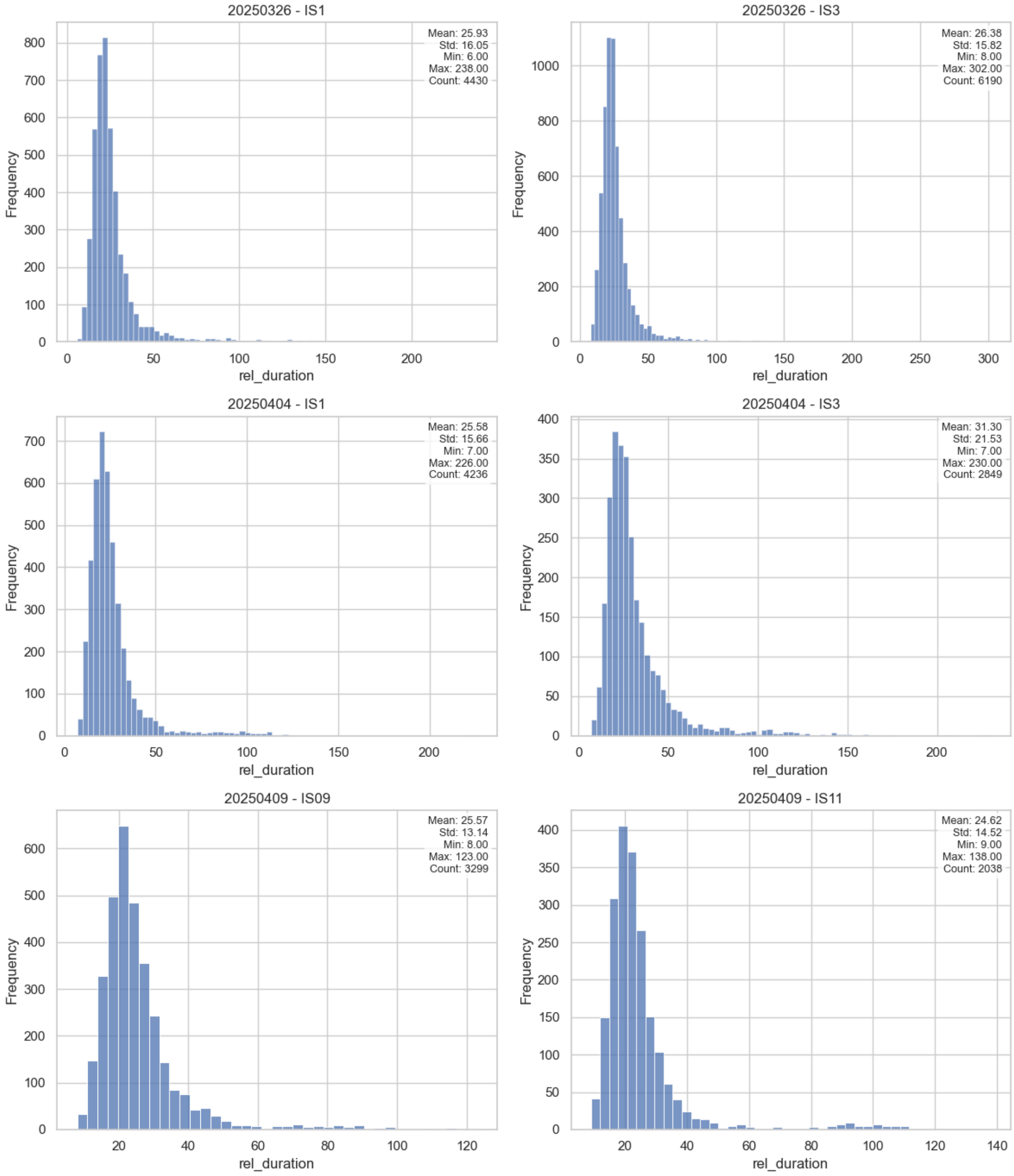
Global Peaks: Prominence



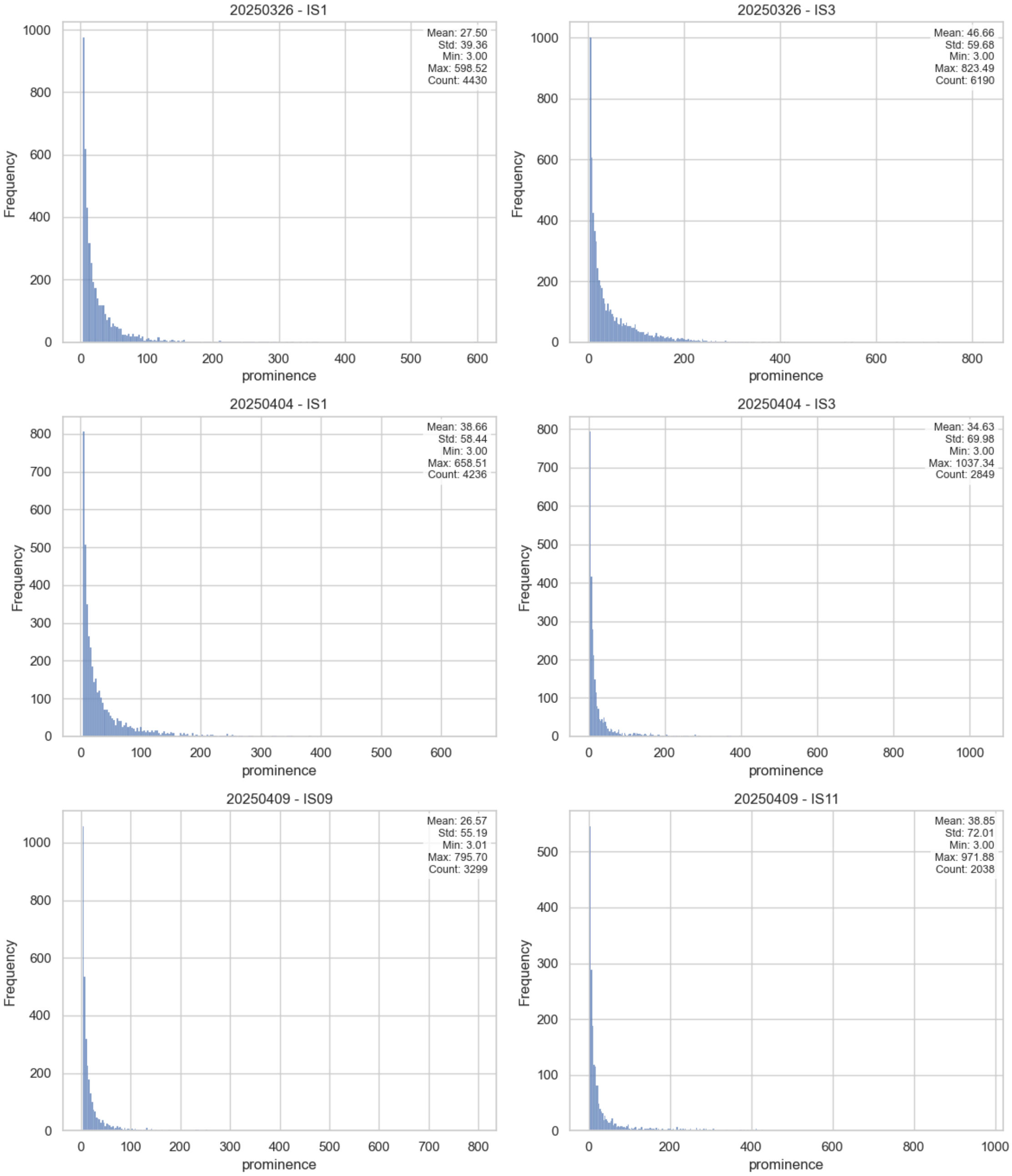


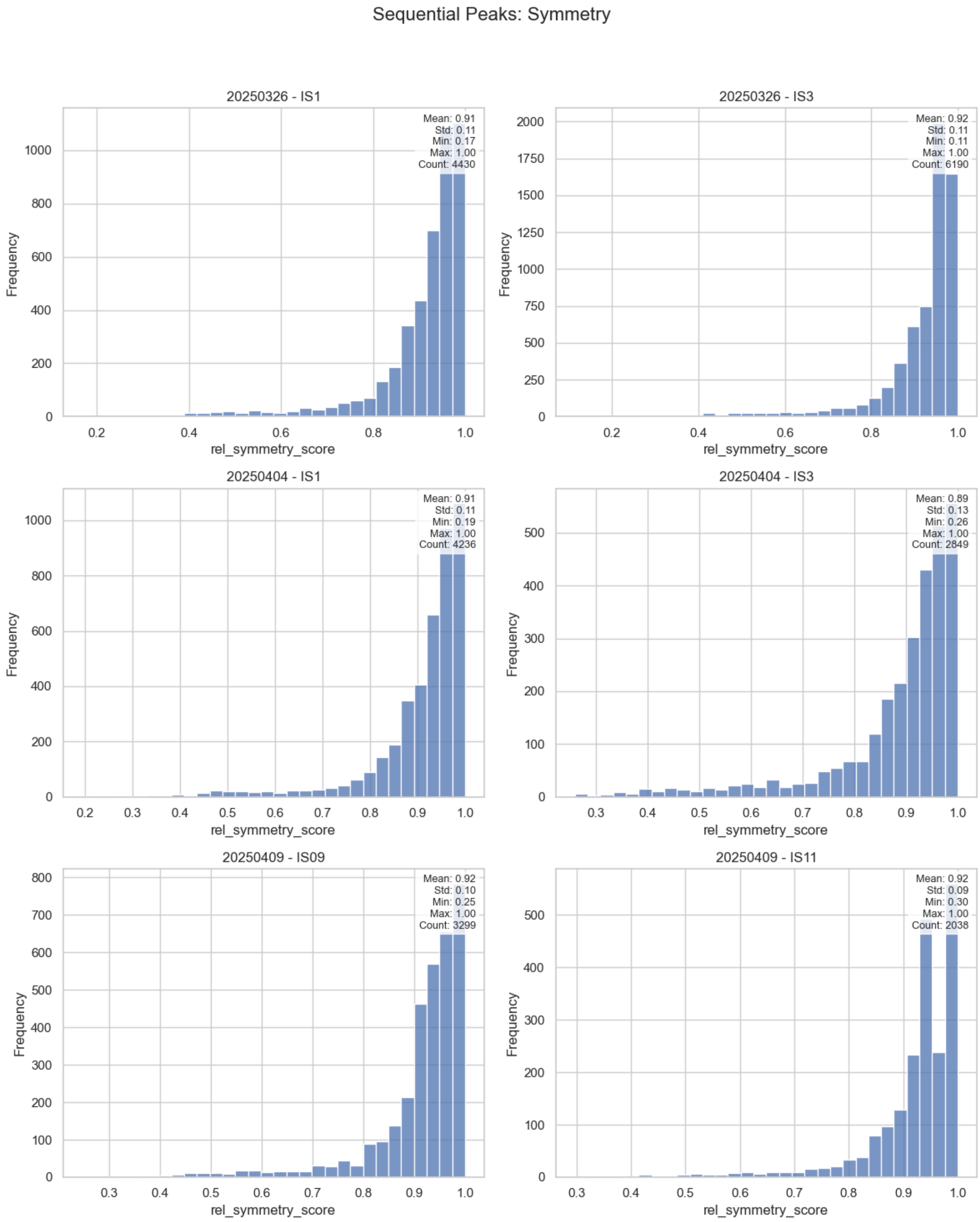
# Peak Type: Sequential - 23042 peaks

Sequential Peaks: Duration



Sequential Peaks: Prominence





Sequential Peaks: Symmetry

20250326 - IS1

20250326 - IS3

20250404 - IS1

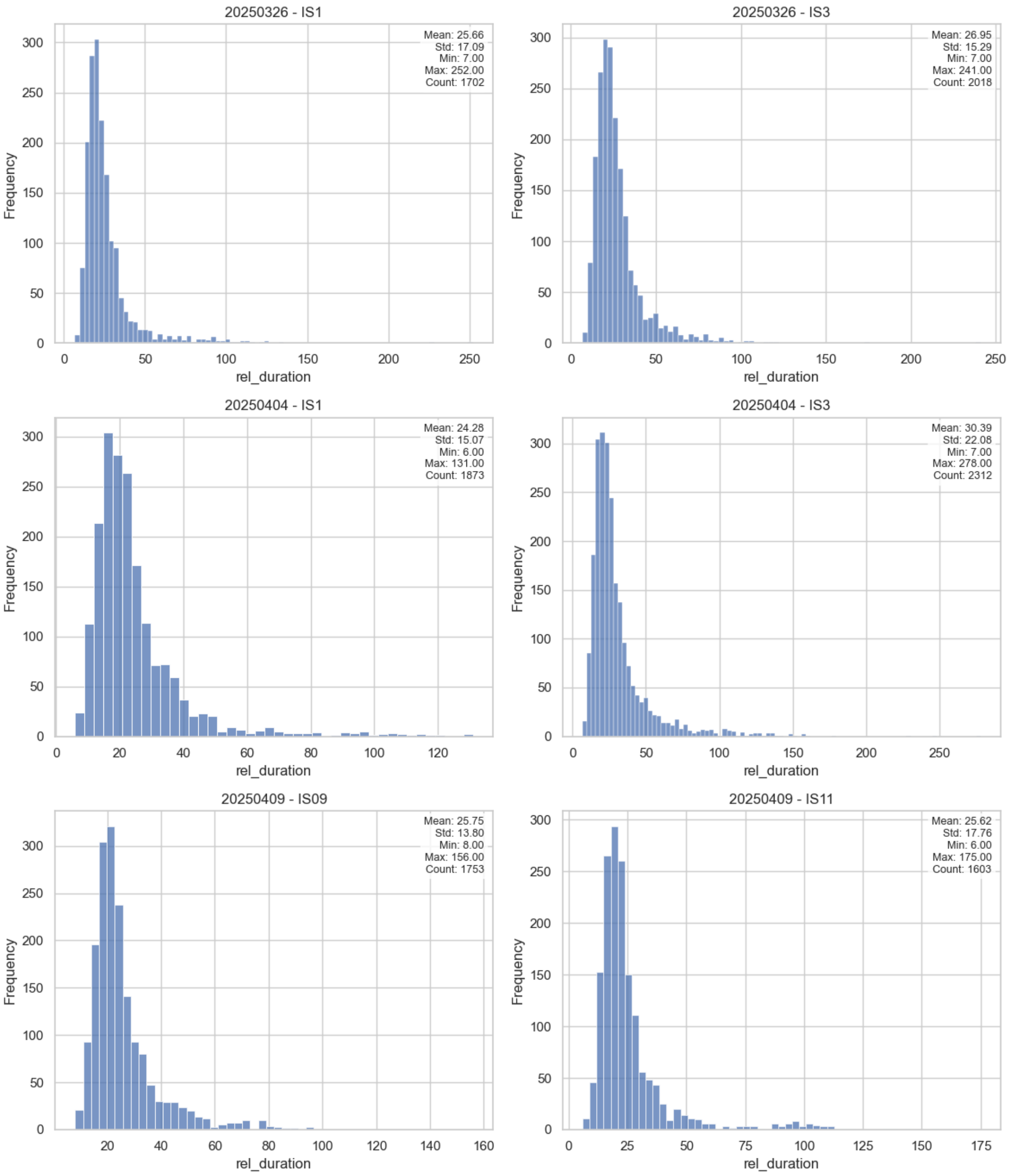
20250404 - IS3

20250409 - IS09

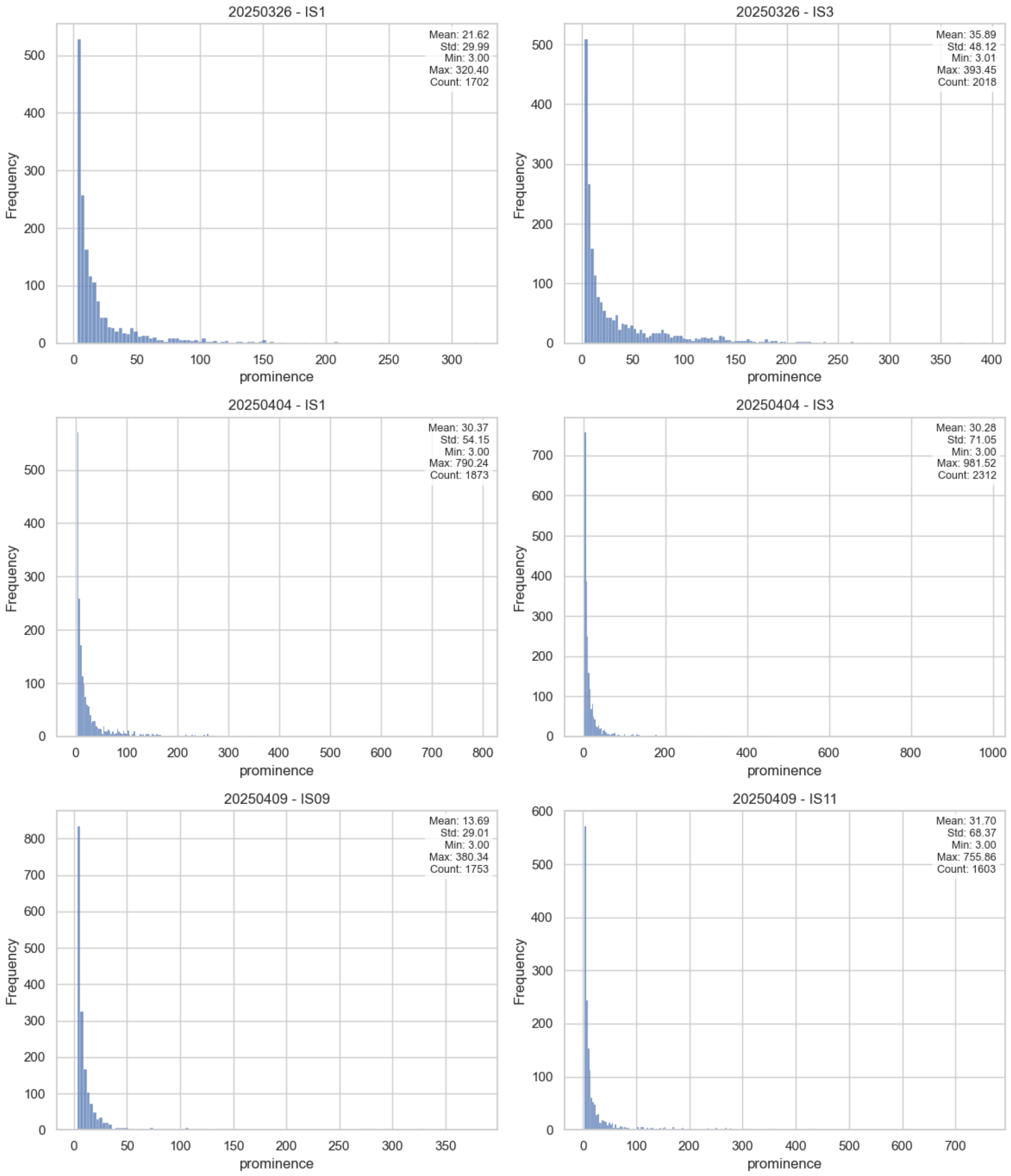
20250409 - IS11

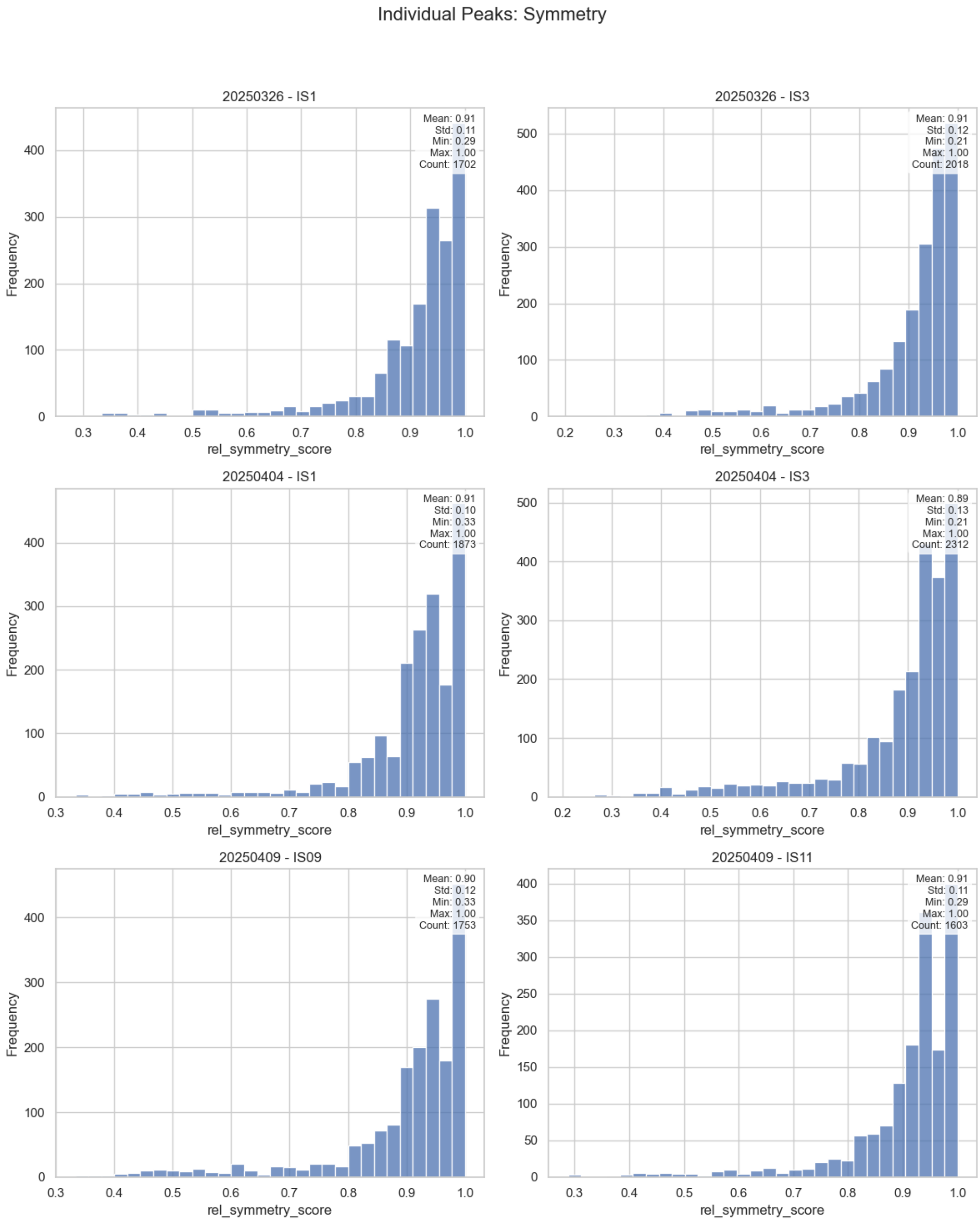
# Peak Type: Individual - 11261 peaks

Individual Peaks: Duration



Individual Peaks: Prominence





0.7 Cell Metrics

We analyze: - Number of peaks per cell (activity) - Periodicity score (oscillatory behavior) - Proportion of active vs inactive cells

Interpretation:

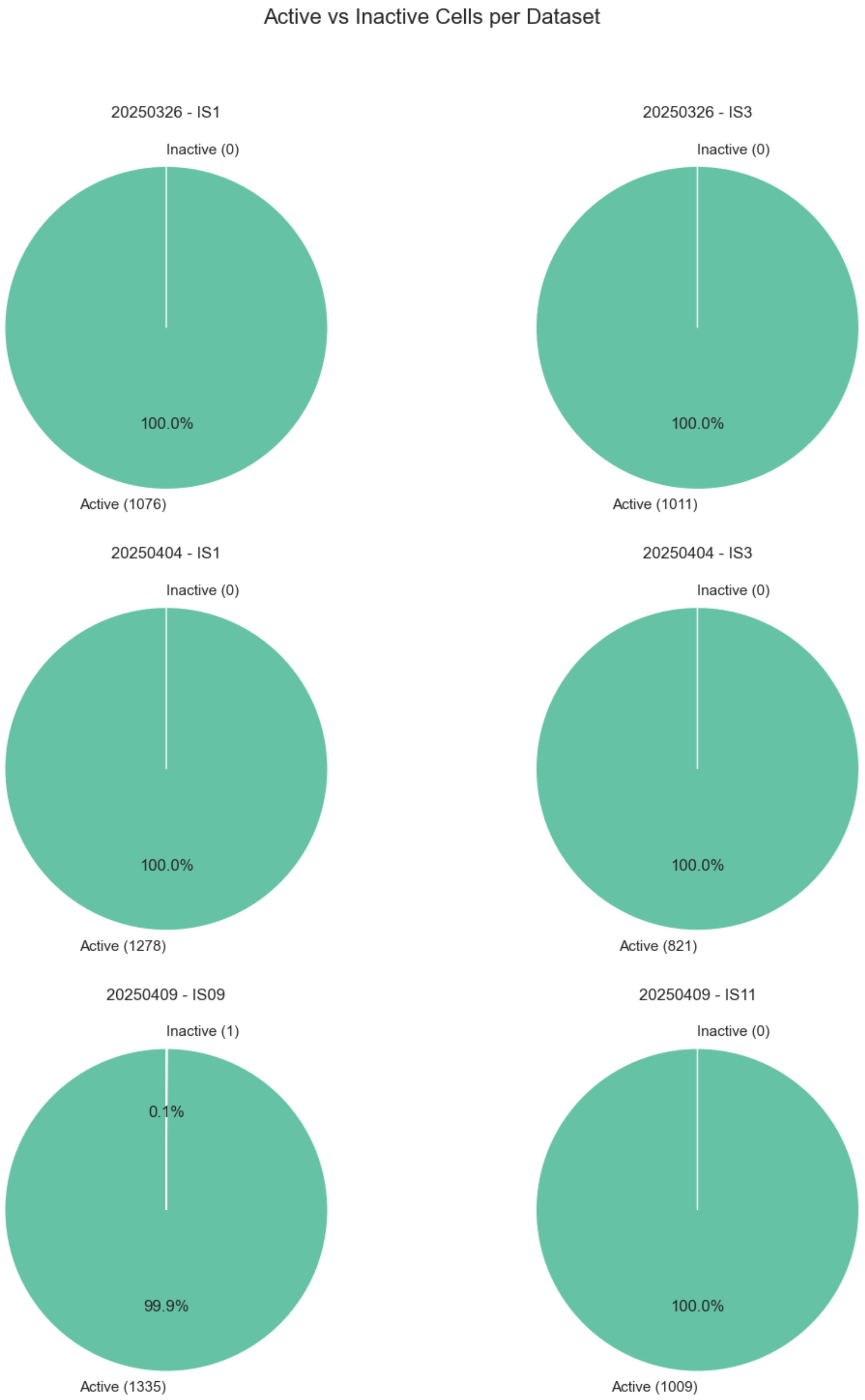
Active cells: Nearly all cells (>99.9%) are active in every dataset. This indicates excellent signal integrity and robust experimental conditions. Activity is highly homogeneous across datasets.

Number of peaks per cell: High total peak count (>10,000 peaks per dataset), with a balanced and near-normal distribution. This reflects widespread and stable activity across the population.

Periodicity: Periodicity scores (~0.7 ± 0.1) remain consistent across both imaging runs. This suggests that rhythmic calcium oscillations are preserved, even when global excitability drops.

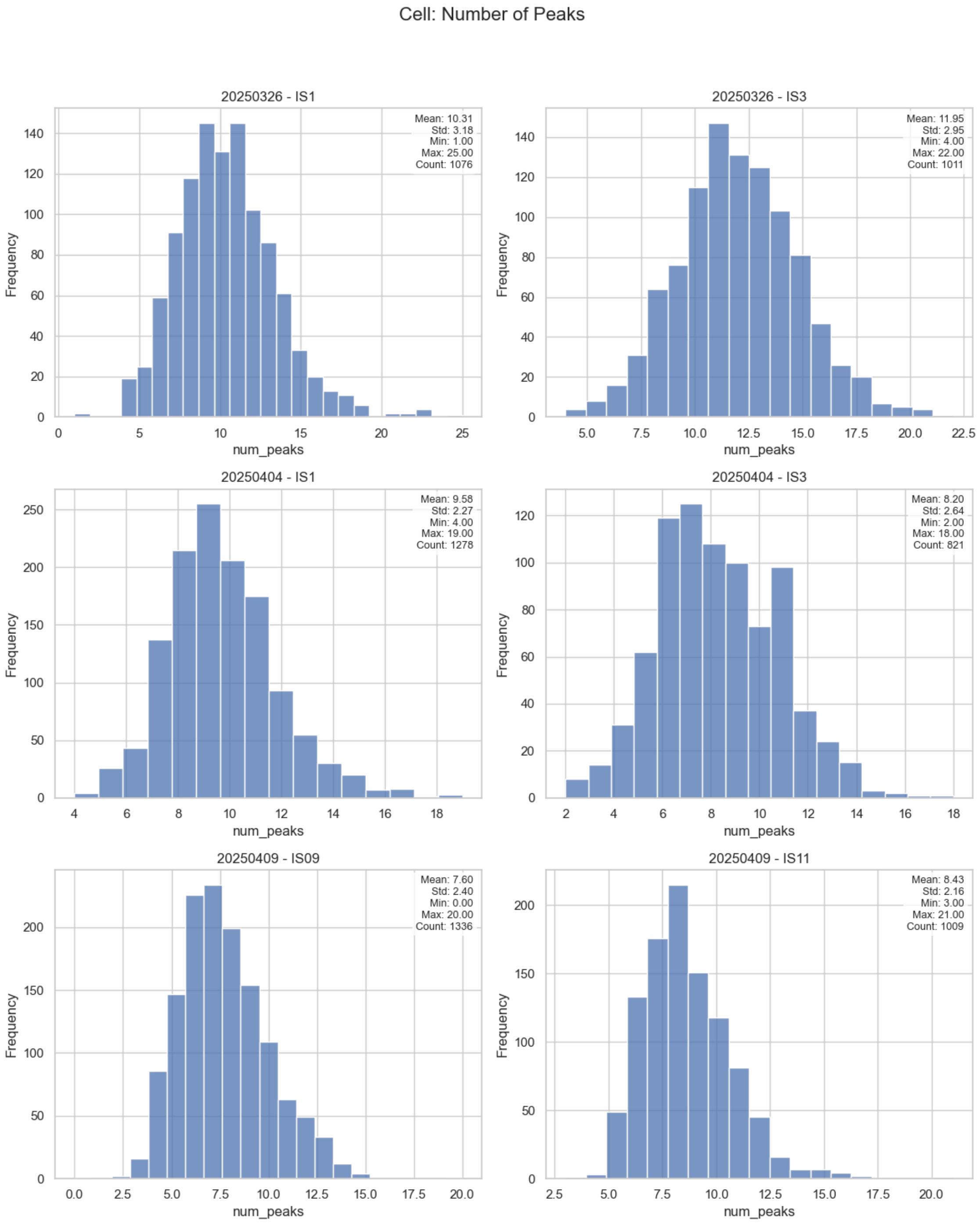
[6]:

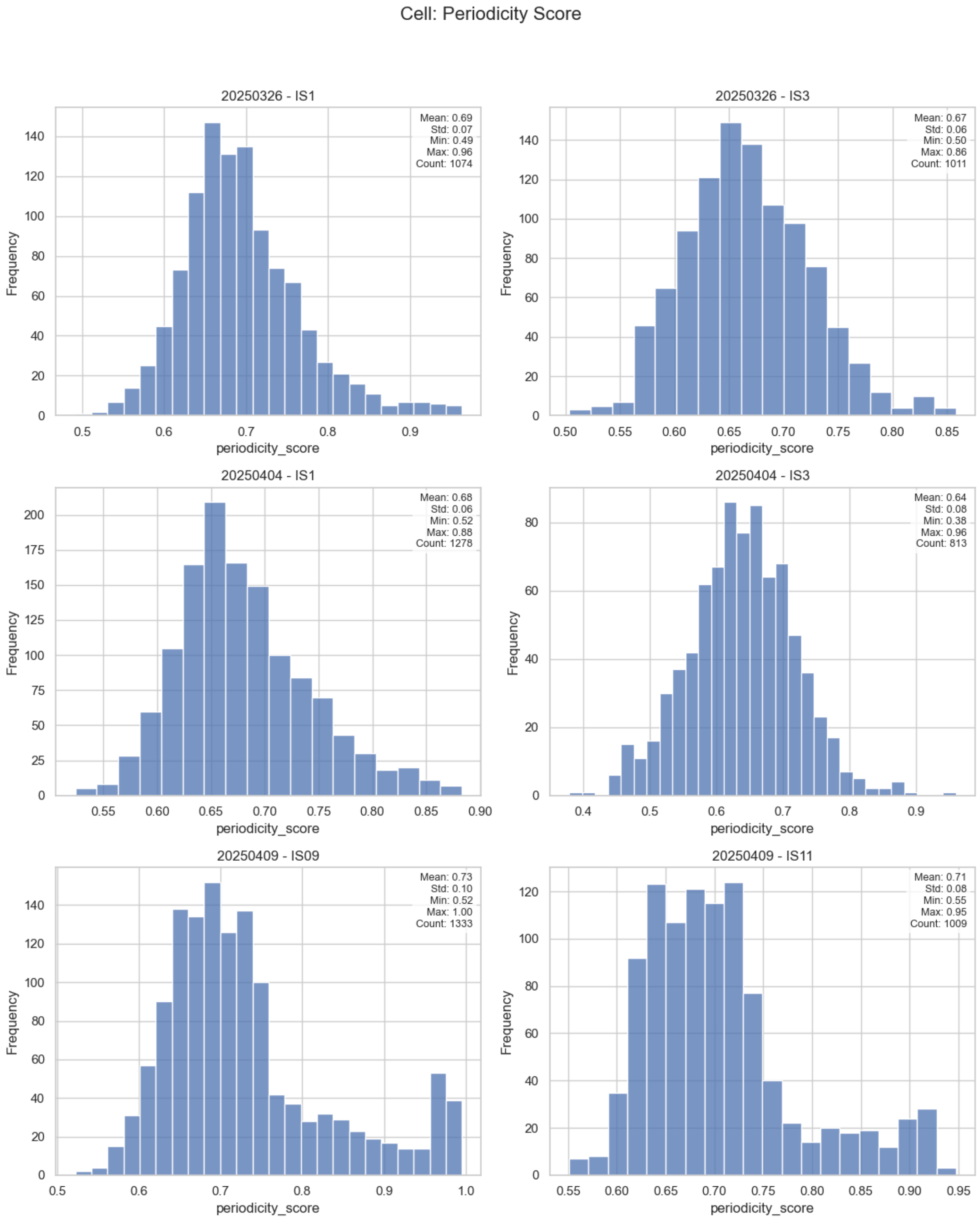
```
# Convert is_active to label for display purposes
cells["active_label"] = cells["is_active"].map({1: "Active", 0: "Inactive"})

plot_category_distribution_by_dataset(
    df=cells,
    column="active_label",
    category_order=["Active", "Inactive"],
    colors={"Active": "#66c2a5", "Inactive": "#d3d3d3"},
    title="Active vs Inactive Cells per Dataset"
)
```


Active vs Inactive Cells per Dataset
20250326 - IS1
Inactive (0)
100.0%
Active (1076)
20250326 - IS3
Inactive (0)
100.0%
Active (1011)
20250404 - IS1
Inactive (0)
100.0%
Active (1278)
20250404 - IS3
Inactive (0)
100.0%
Active (821)
20250409 - IS09
Inactive (1)
0.1%
99.9%
Active (1335)
20250409 - IS11
Inactive (0)
100.0%
Active (1009)

[7]:

```
plot_metric_by_dataset(cells, "num_peaks", "Cell: Number of Peaks", bin_width=1)
plot_metric_by_dataset(cells, "periodicity_score", "Cell: Periodicity Score",␣
 ↪bin_width=0.02)
```





0.8 Sequential Event Metrics

We focus on **spatiotemporal events**, extracting: - Mean communication time and speed - Geometric descriptors (elongation, radiality) - Graph depth, number of involved cells

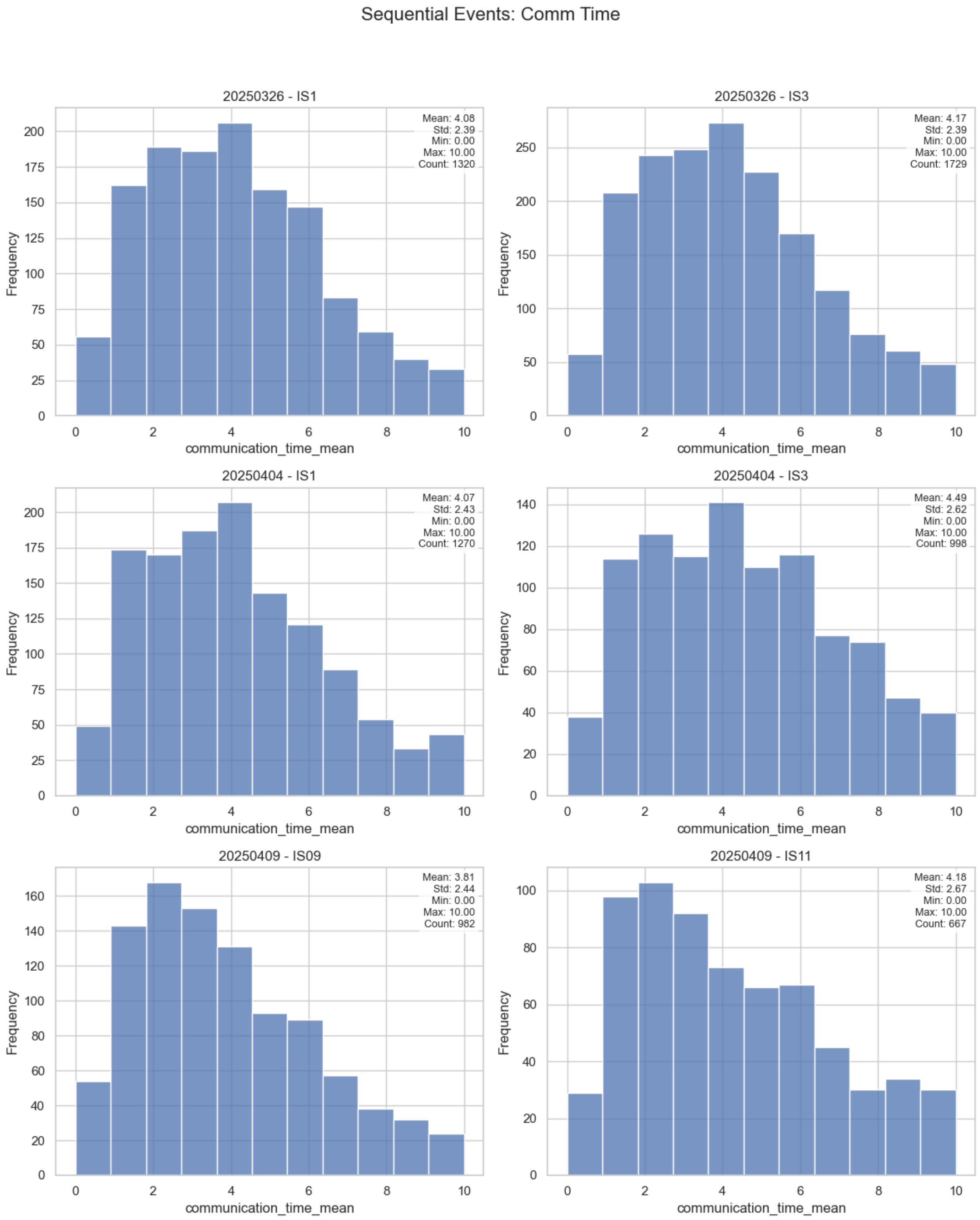
Interpretation:

- *Communication time: ~4.0 frames (STD ~2.5).*
- *Communication speed: ~6.2 units/frame.*
- *Cells involved: ~3.2 ± 2 cells per event.*

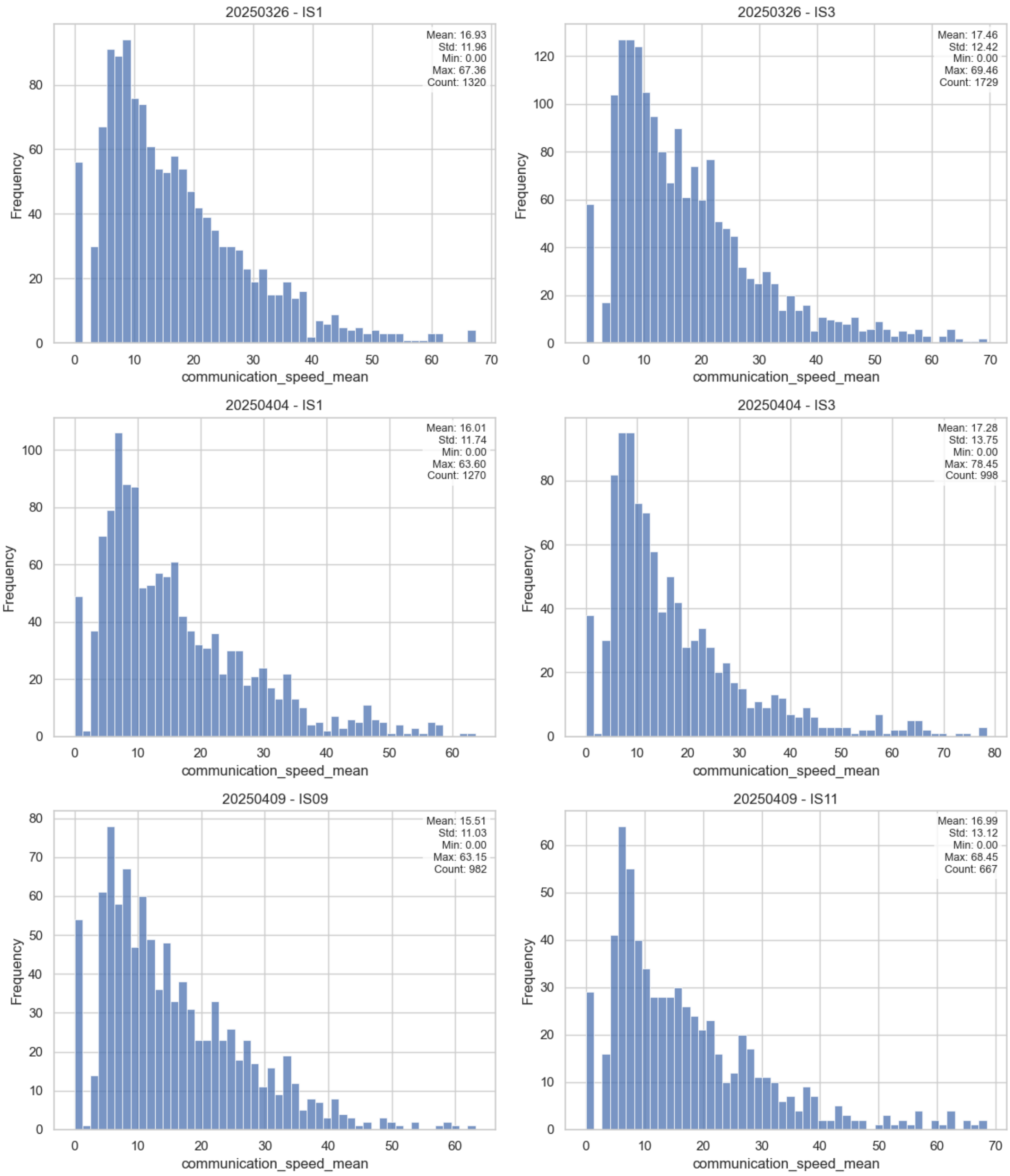
These metrics are consistent across datasets and define the propagation baseline for healthy spontaneous events.

[8]:

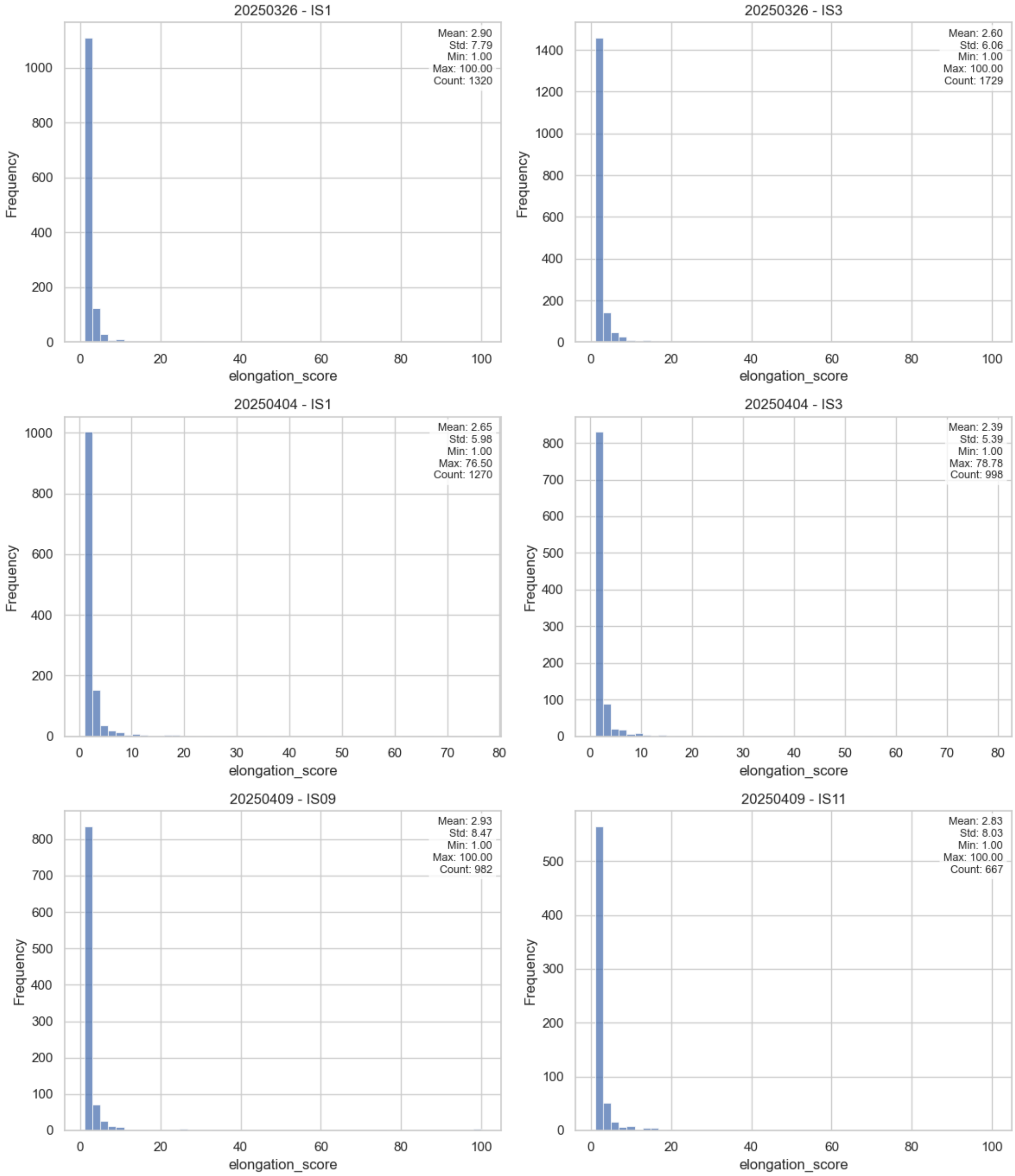
```python
seq_events = events[events["event_type"] == "SequentialEvent"]
plot_metric_by_dataset(seq_events, "communication_time_mean", "Sequential Events: Comm Time", bin_width=1)
plot_metric_by_dataset(seq_events, "communication_speed_mean", "Sequential Events: Comm Speed", bin_count=50)
plot_metric_by_dataset(seq_events, "elongation_score", "Sequential Events: Elongation", bin_count=50)
plot_metric_by_dataset(seq_events, "radiality_score", "Sequential Events: Radiality", bin_width=0.05)
plot_metric_by_dataset(seq_events, "dag_depth", "Sequential Events: DAG Depth", bin_width=1)
plot_metric_by_dataset(seq_events, "n_cells_involved", "Sequential Events: Cells Involved", bin_width=1)
```

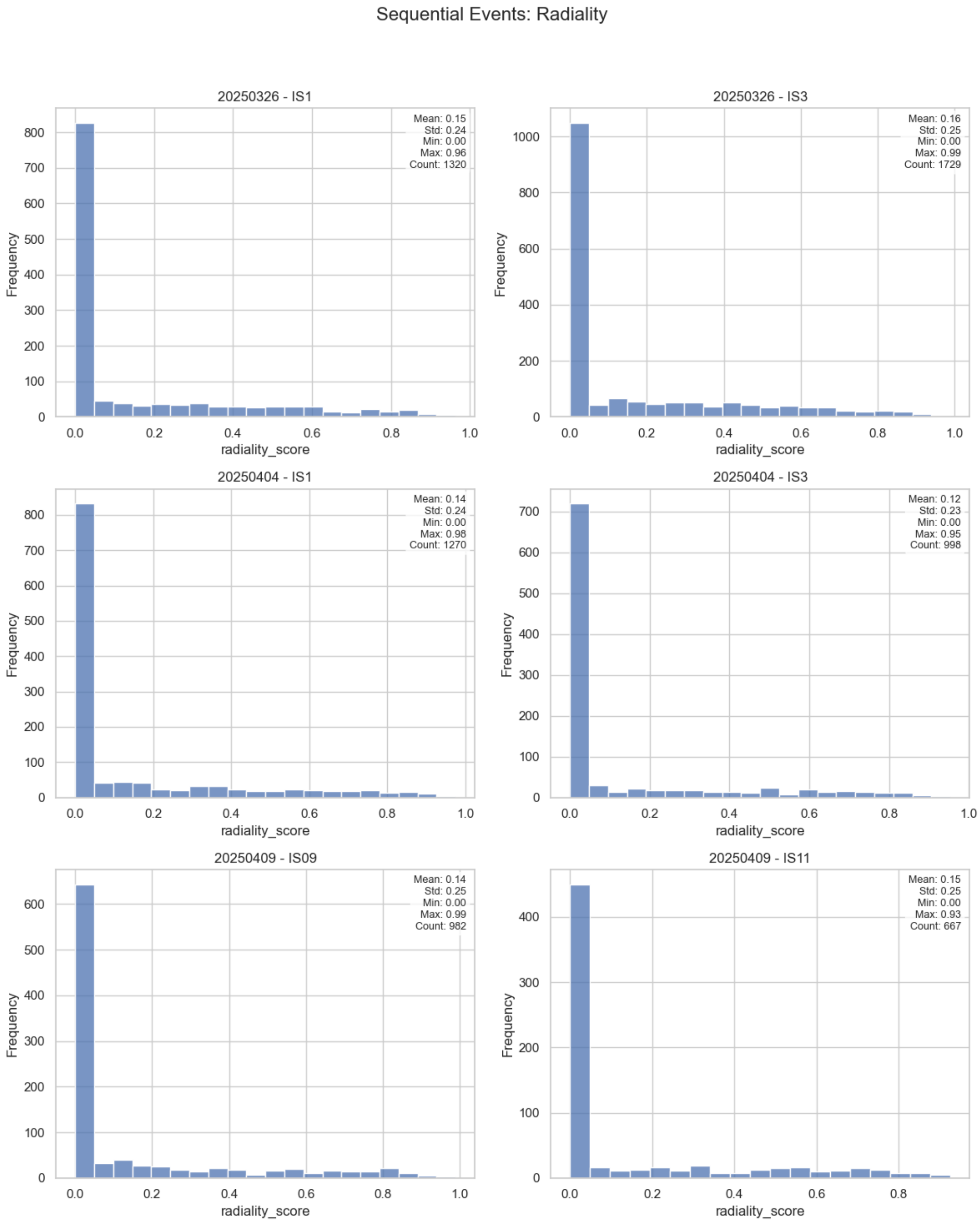

Sequential Events: Comm Time
20250326 - IS1
Mean: 4.08
Std: 2.39
Min: 0.00
Max: 10.00
Count: 1320
Frequency
communication_time_mean
20250326 - IS3
Mean: 4.17
Std: 2.39
Min: 0.00
Max: 10.00
Count: 1729
Frequency
communication_time_mean
20250404 - IS1
Mean: 4.07
Std: 2.43
Min: 0.00
Max: 10.00
Count: 1270
Frequency
communication_time_mean
20250404 - IS3
Mean: 4.49
Std: 2.62
Min: 0.00
Max: 10.00
Count: 998
Frequency
communication_time_mean
20250409 - IS09
Mean: 3.81
Std: 2.44
Min: 0.00
Max: 10.00
Count: 982
Frequency
communication_time_mean
20250409 - IS11
Mean: 4.18
Std: 2.67
Min: 0.00
Max: 10.00
Count: 667
Frequency
communication_time_mean
0
2
4
6
8
10

Sequential Events: Comm Speed



Sequential Events: Elongation




Sequential Events: Radiality
20250326 - IS1
Mean: 0.15
Std: 0.24
Min: 0.00
Max: 0.96
Count: 1320
Frequency
0
100
200
300
400
500
600
700
800
0.0
0.2
0.4
0.6
0.8
1.0
radiality_score
20250326 - IS3
Mean: 0.16
Std: 0.25
Min: 0.00
Max: 0.99
Count: 1729
Frequency
0
200
400
600
800
1000
0.0
0.2
0.4
0.6
0.8
1.0
radiality_score
20250404 - IS1
Mean: 0.14
Std: 0.24
Min: 0.00
Max: 0.98
Count: 1270
Frequency
0
100
200
300
400
500
600
700
800
0.0
0.2
0.4
0.6
0.8
1.0
radiality_score
20250404 - IS3
Mean: 0.12
Std: 0.23
Min: 0.00
Max: 0.95
Count: 998
Frequency
0
100
200
300
400
500
600
700
0.0
0.2
0.4
0.6
0.8
1.0
radiality_score
20250409 - IS09
Mean: 0.14
Std: 0.25
Min: 0.00
Max: 0.99
Count: 982
Frequency
0
100
200
300
400
500
600
0.0
0.2
0.4
0.6
0.8
1.0
radiality_score
20250409 - IS11
Mean: 0.15
Std: 0.25
Min: 0.00
Max: 0.93
Count: 667
Frequency
0
100
200
300
400
0.0
0.2
0.4
0.6
0.8
radiality_score

Sequential Events: DAG Depth

20250326 - IS1 (No Data)

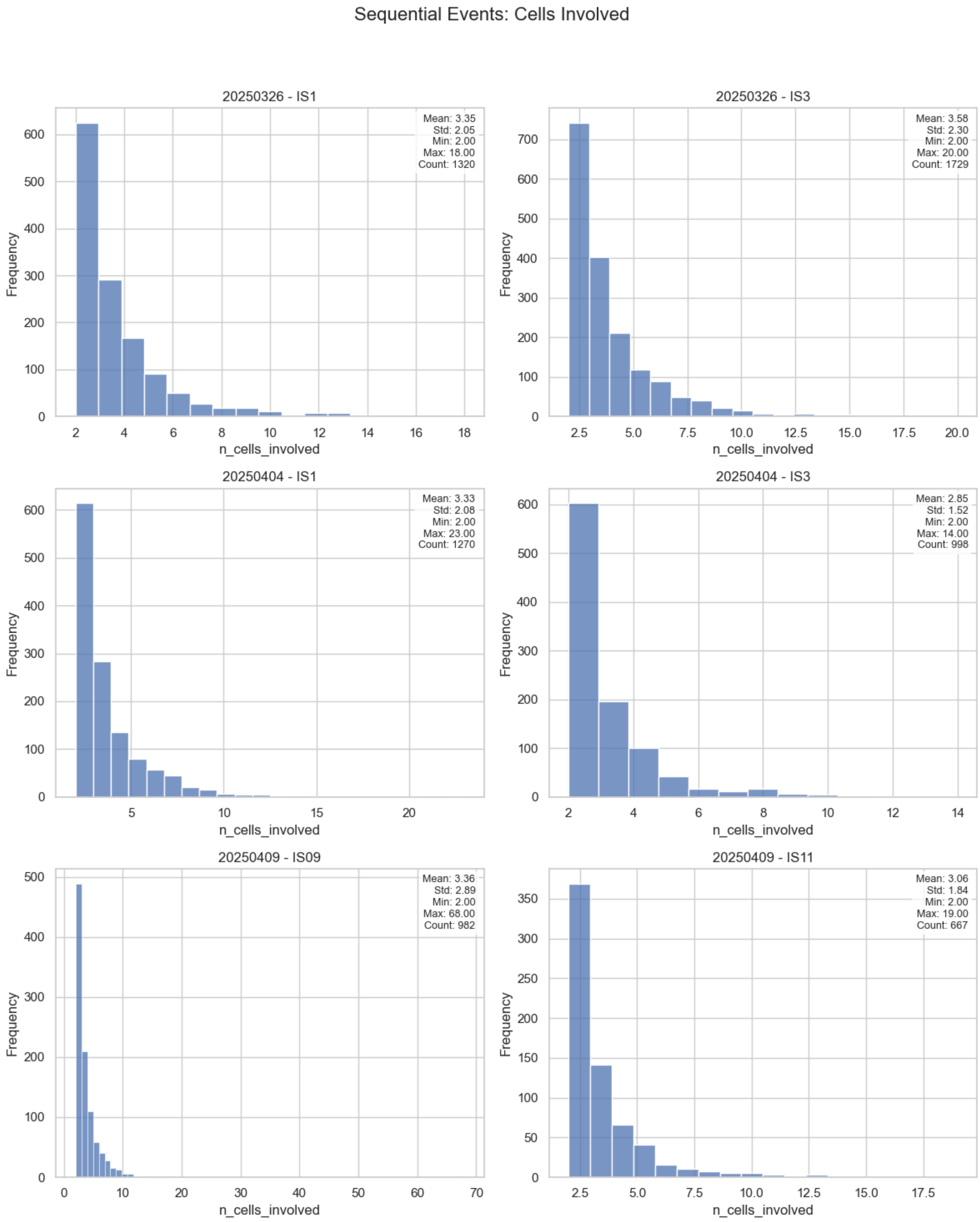
20250326 - IS3 (No Data)

20250404 - IS1 (No Data)

20250404 - IS3 (No Data)

20250409 - IS09 (No Data)

20250409 - IS11 (No Data)



Sequential Events: Cells Involved

20250326 - IS1

20250326 - IS3

20250404 - IS1

20250404 - IS3

20250409 - IS09

20250409 - IS11

0.9 Conclusion & Next Steps

0.9.1 Summary of Observations

- **Visual Patterns:** Briefly describe observed visual trends (raster plots, activity levels, etc.).
- **Statistical Results:** Mention key metrics or KS-test outcomes that stood out.

0.9.2 Dataset Reproducibility

- Which datasets appear consistent or divergent?
- Are any replicates potentially faulty or biologically distinct?

0.9.3 Actionable Decisions

☐ Exclude dataset(s) due to technical issues
☐ Flag dataset(s) for further inspection
☐ Select representative samples for downstream analysis

0.9.4 Planned Deep-Dive

- Which direction will the next notebook take? (e.g., spatial clustering, propagation analysis, signal decomposition)
