controls_2nd_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 **second run** of spontaneous activity under control conditions.

Goal: Determine if the second imaging run can serve as reliable control data or if phototoxicity, photobleaching, or altered culture conditions (room temperature exposure without optimal CO and humidity) have significantly impacted cellular behavior.

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 - IS2": "D:/Mateo/20250326/Output/IS2",
    "20250326 - IS4": "D:/Mateo/20250326/Output/IS4",
    "20250404 - IS2": "D:/Mateo/20250404/Output/IS2",
    "20250409 - IS10": "D:/Mateo/20250409/Output/IS10",
    "20250409 - IS12": "D:/Mateo/20250409/Output/IS12",
}

# 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
```

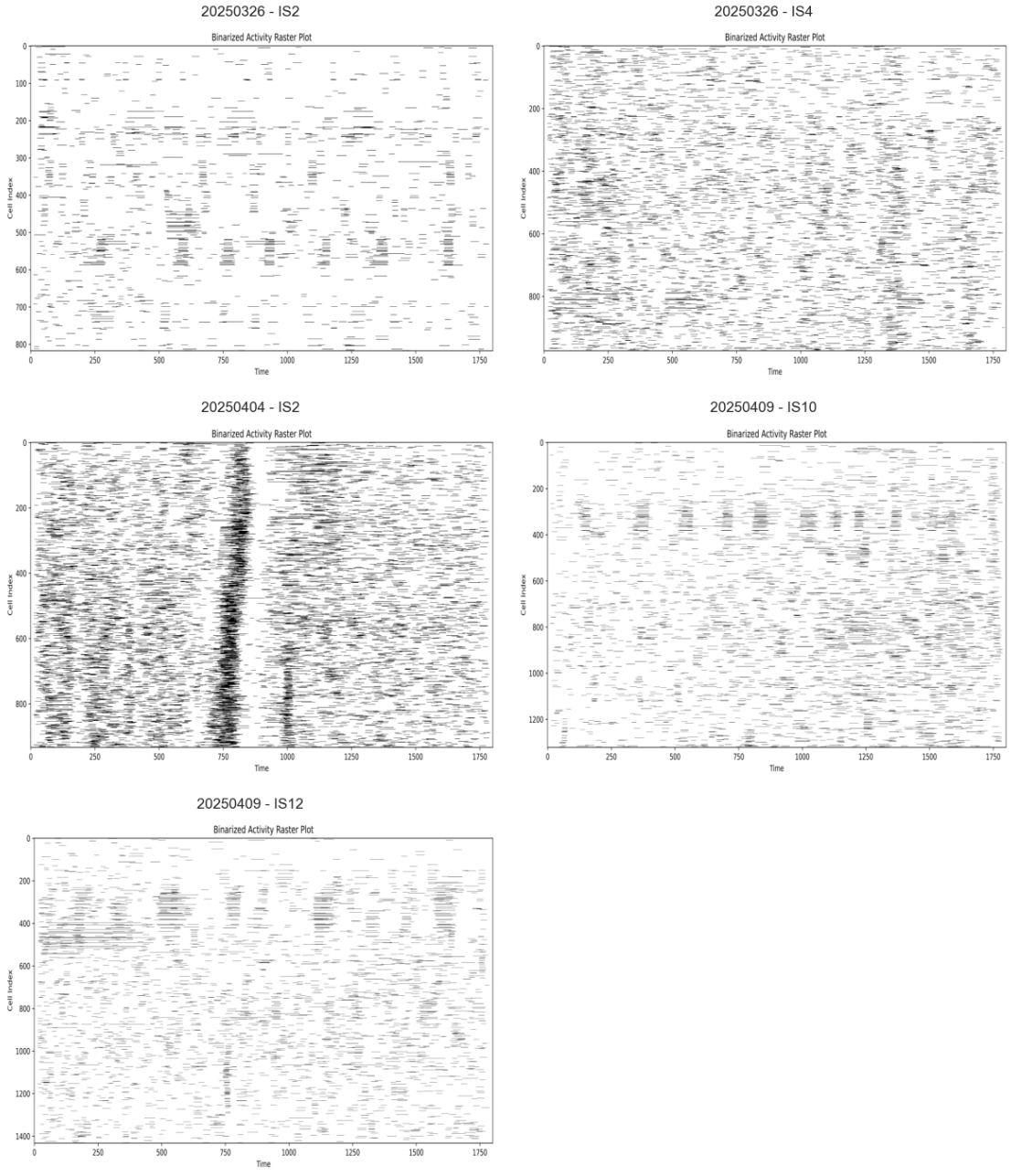
0.4 Raster Plot Inspection

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 second imaging run displays striking differences compared to the first.

- *Global events are almost entirely absent, with 4 out of 5 datasets showing* ***no global events****. Only one dataset (`2025-04-04 IS3`) exhibited minimal global activity.*
- *Sequential and individual peaks maintain a similar ratio compared to the first run, but the absence of global events indicates an altered system-wide behavior.*

This suggests a breakdown of coordinated signaling mechanisms, likely caused by phototoxicity or environmental stress.

[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:

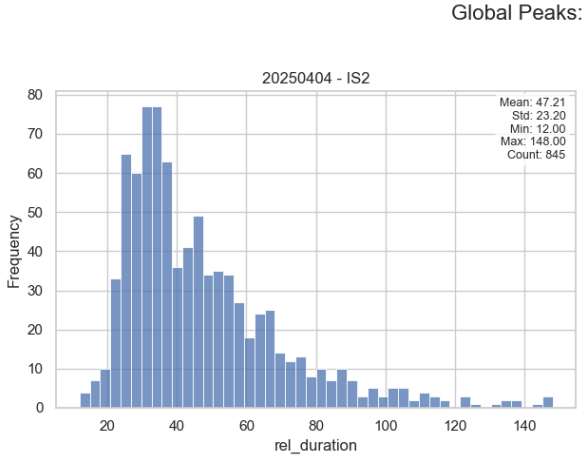
- *Global peaks: Only present in one dataset; insufficient for analysis.*

- *Sequential peaks: Slightly longer durations (~33 frames) than in First Run; symmetry remains consistent (~0.9 ± 0.12).*
- *Individual peaks: Also slightly longer (~30 frames), with similar symmetry.*

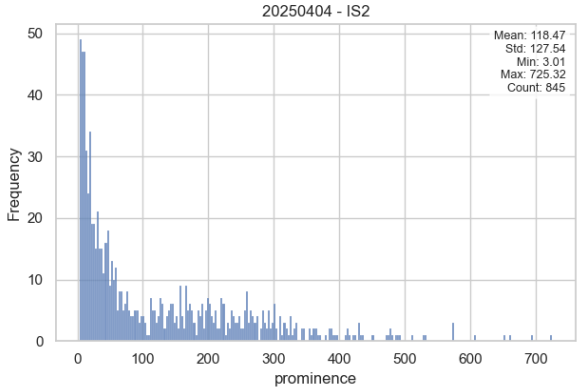
These longer durations might result from subtle shifts in intracellular calcium handling, possibly caused by phototoxic stress or under-detection of broad events misclassified as individual.

[5]:
```
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)
```

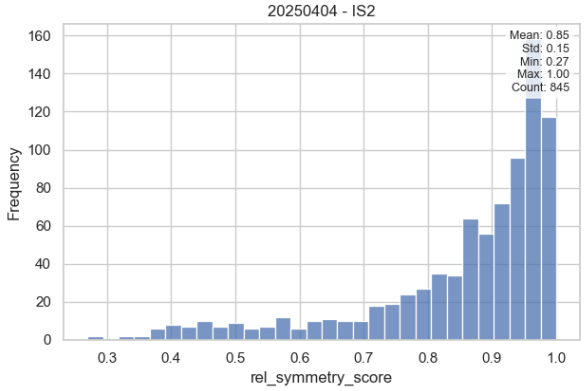
```
#  Peak Type: Global - 845 peaks
```



Global Peaks: Prominence

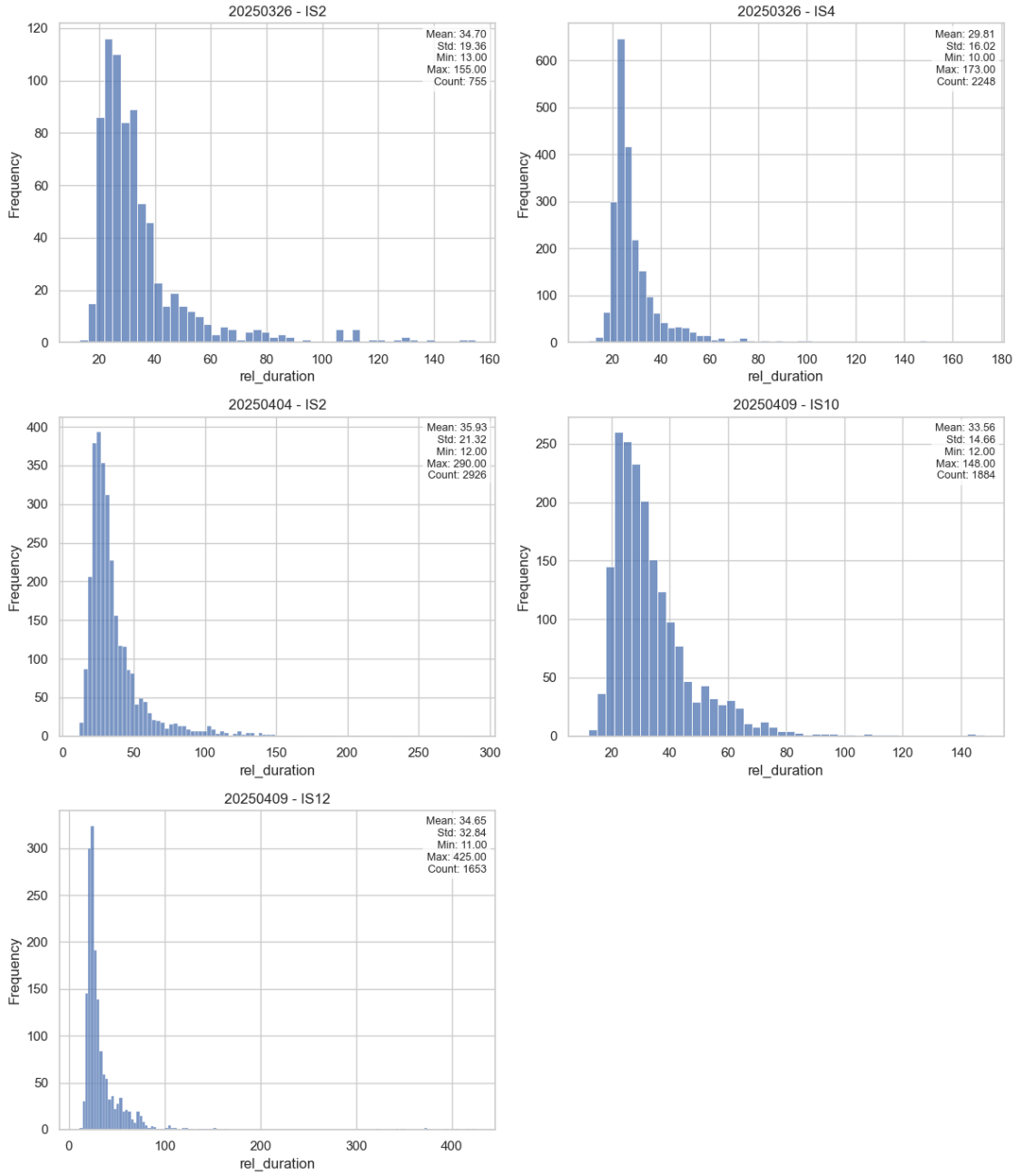


Global Peaks: Symmetry

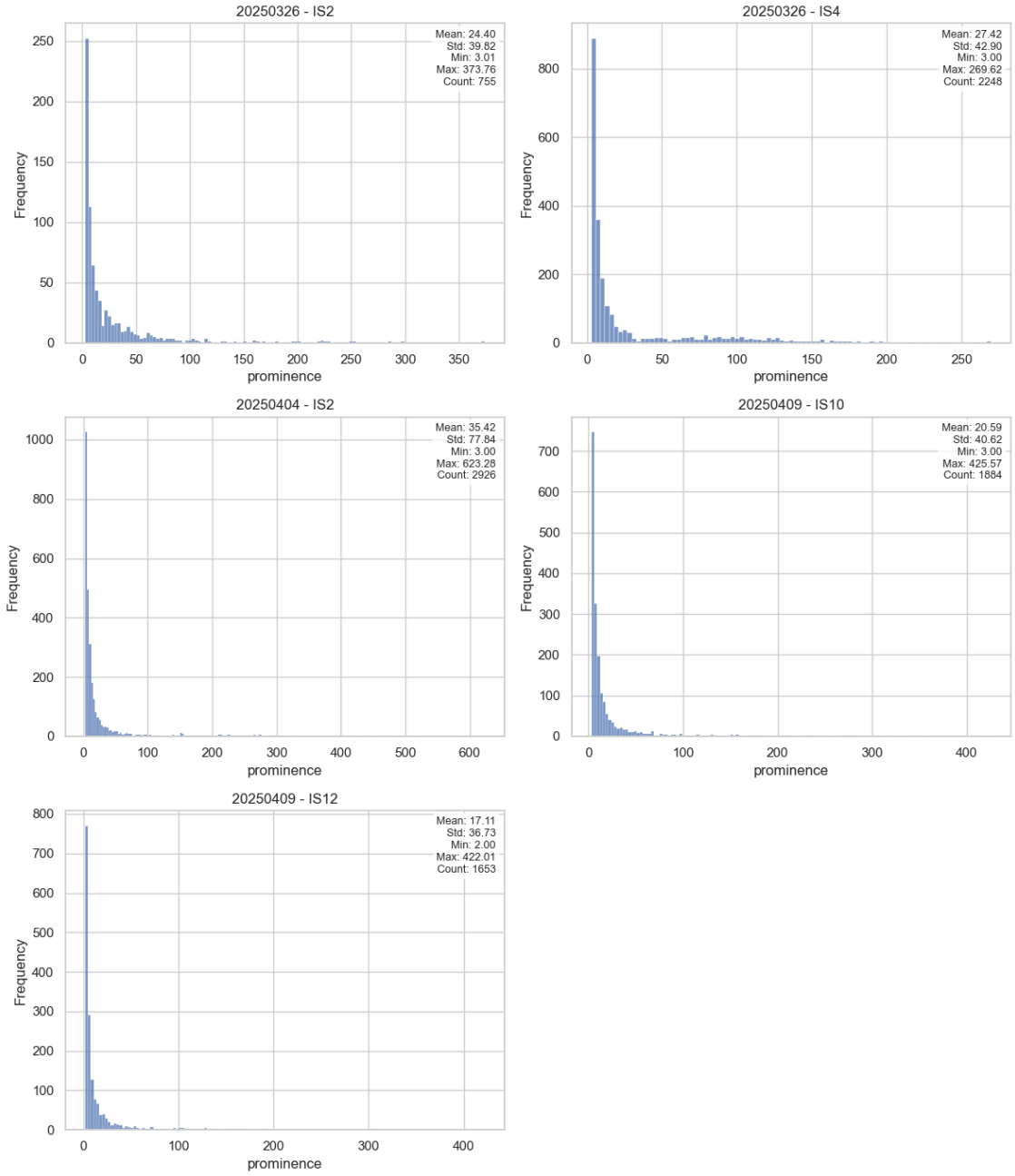


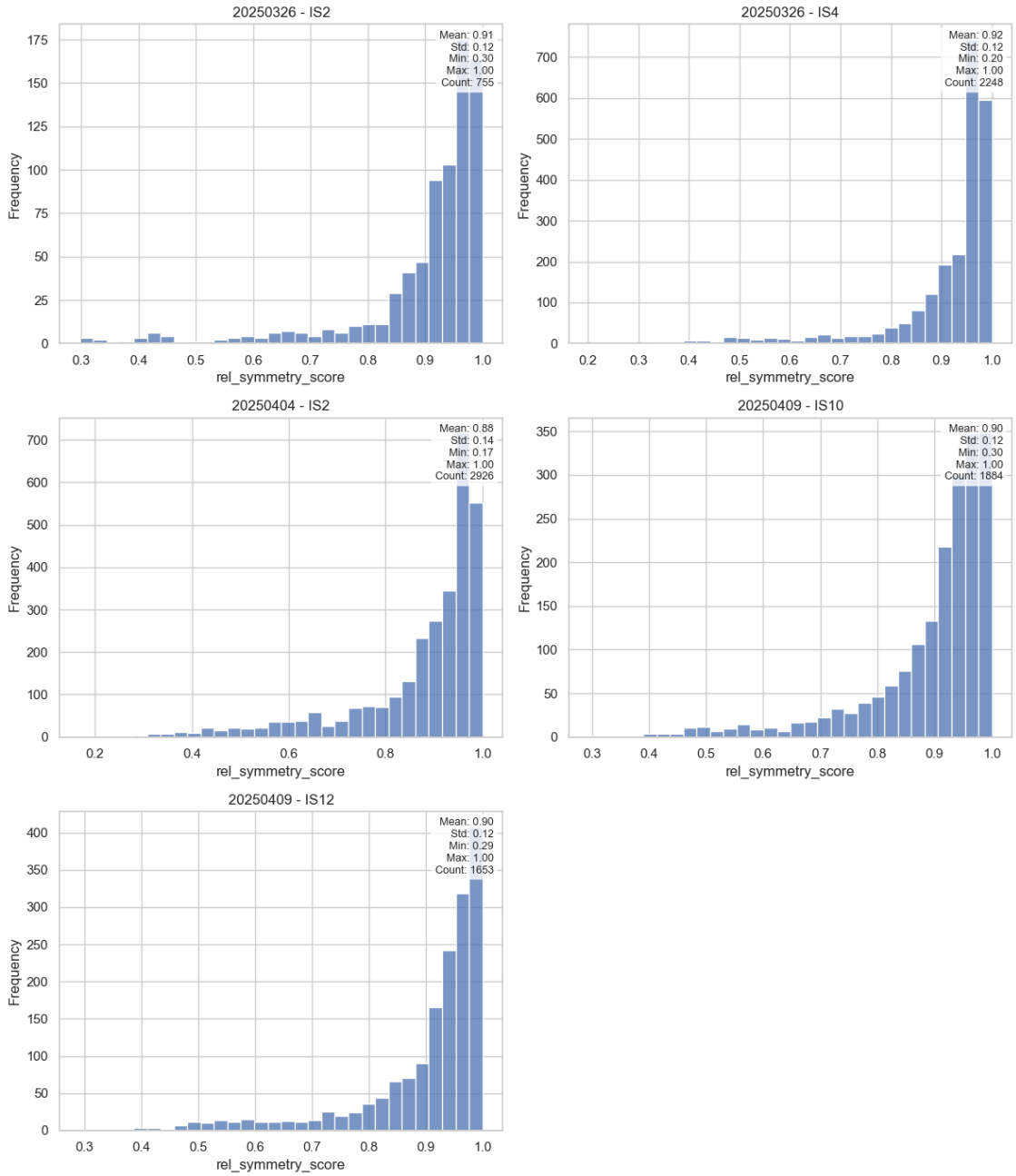

```
#  Peak Type: Sequential - 9466 peaks
```

Sequential Peaks: Duration



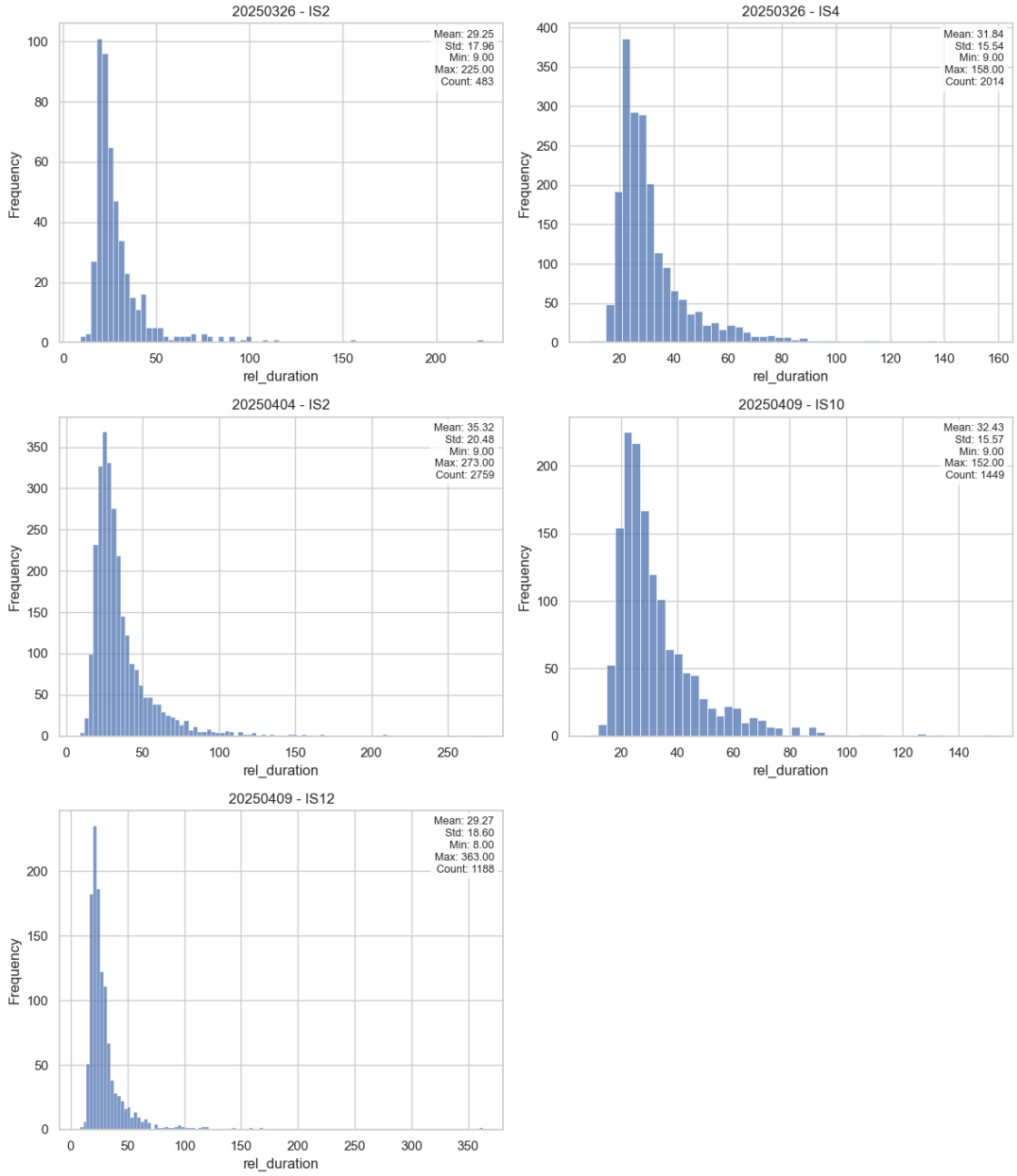
Sequential Peaks: Prominence



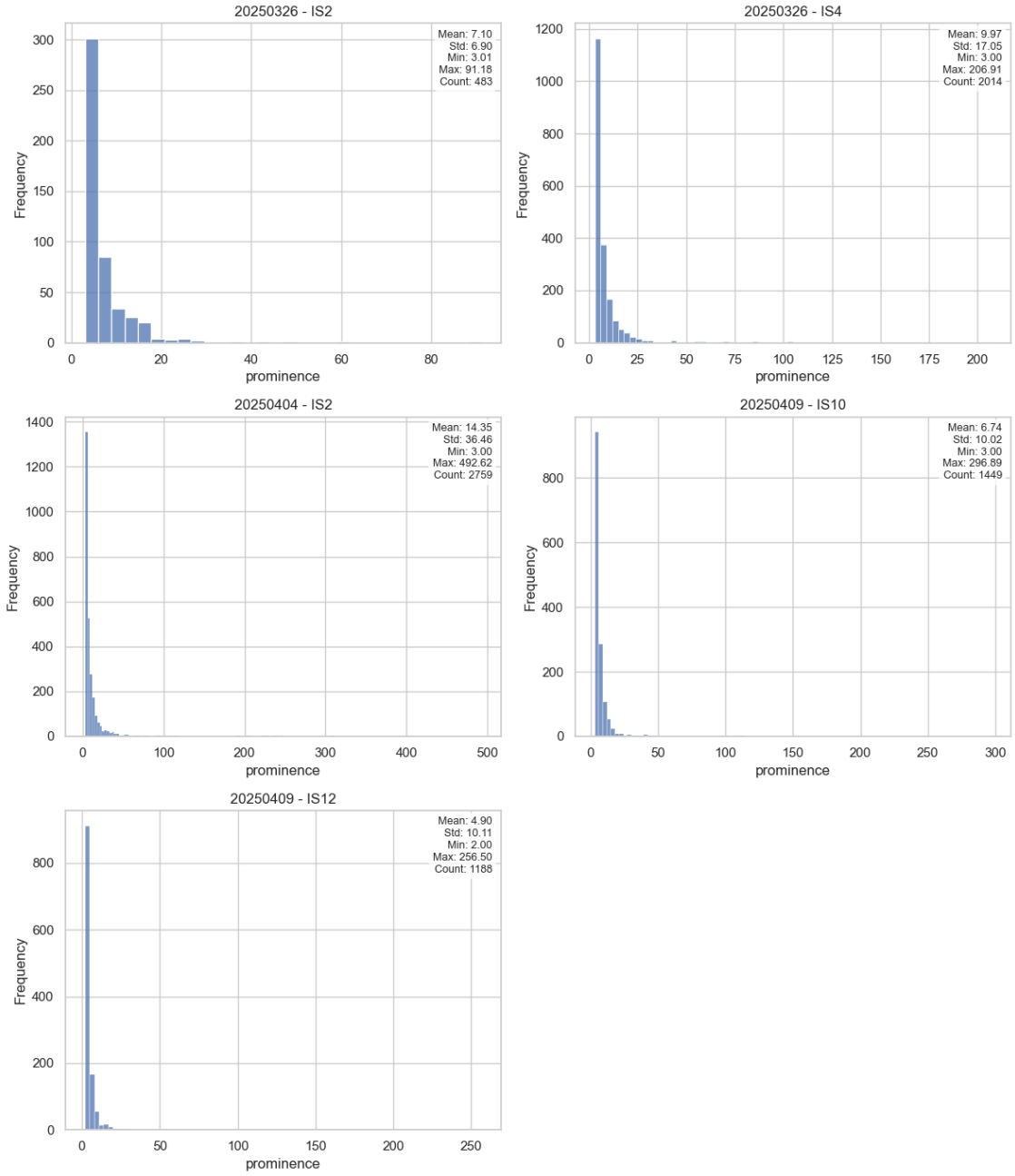


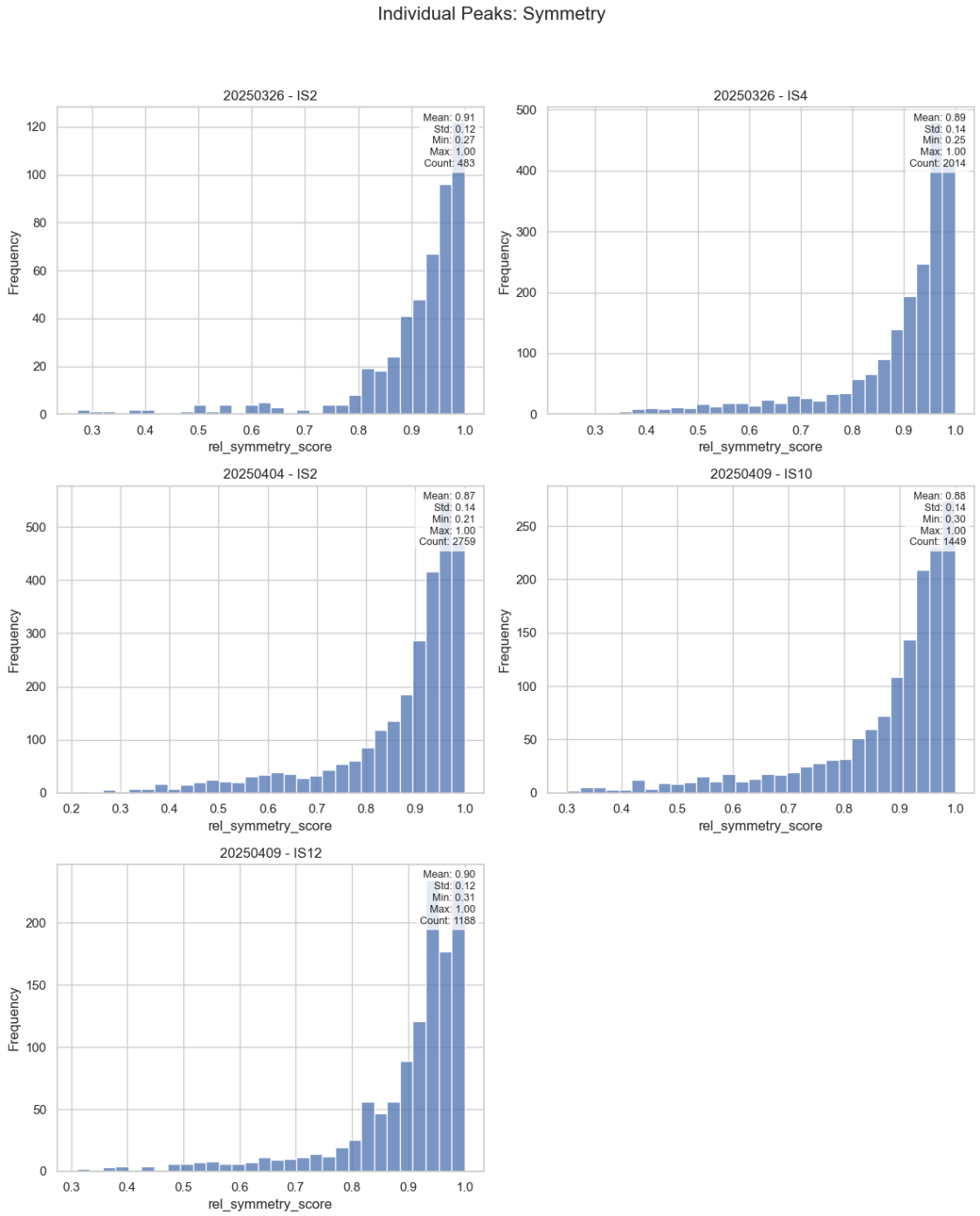
Peak Type: Individual - 7893 peaks

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

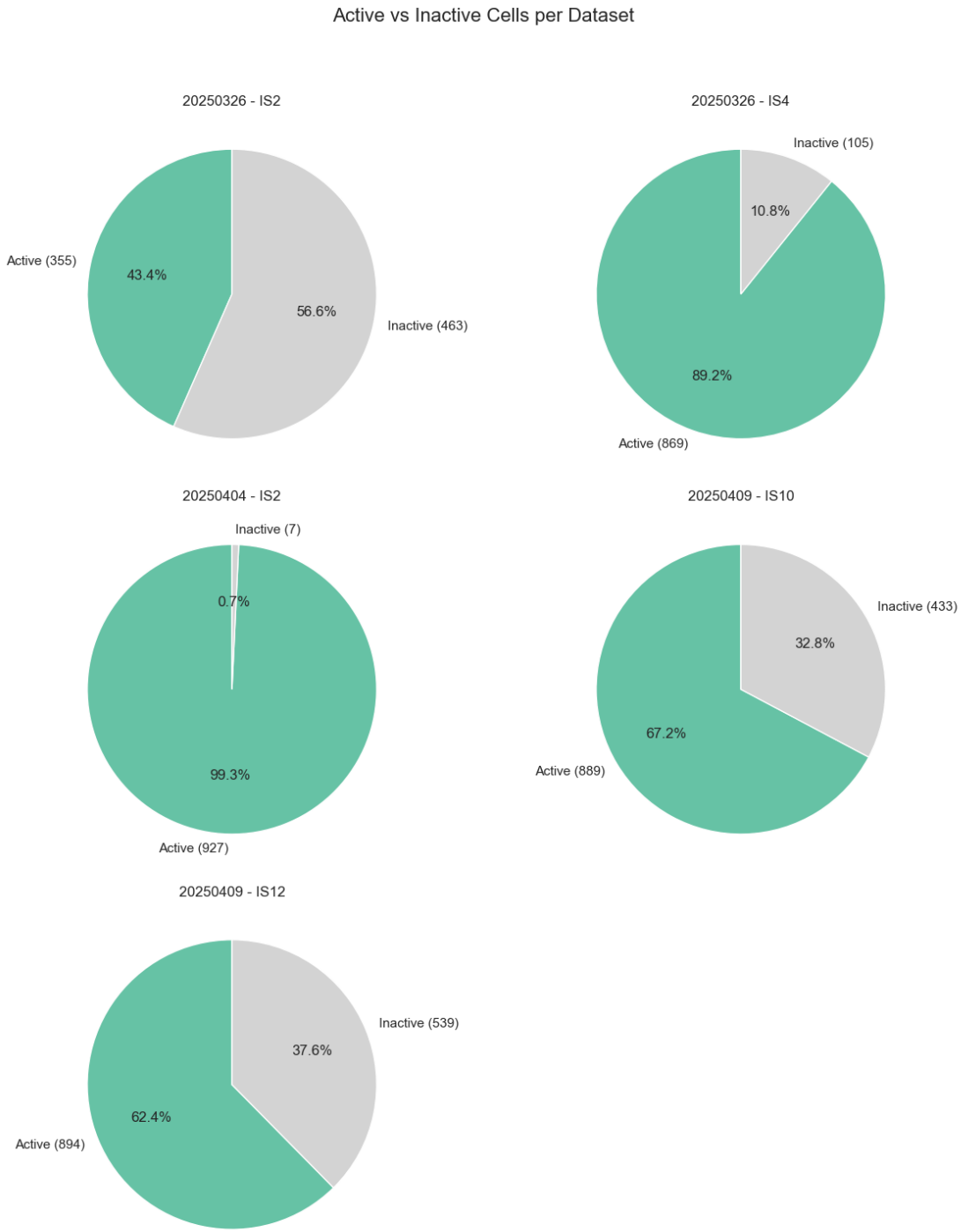
Interpretation:

Active cells: Active cell fraction is significantly lower and more variable (10%–50% inactive). Only the dataset containing a global event exhibits >99% active cells. This suggests that only a subset of cells remains responsive, potentially those participating in larger coordinated events. This change may be due to phototoxicity, room temperature exposure, or photobleaching leading to reduced responsiveness.

Number of peaks per cell: Significant reduction in total peak count (1,000–4,000 per dataset). - Distribution shifts to an exponential decay pattern, rather than normal. Only the one dataset with global activity shows similar activity levels to the first run. The altered distribution strongly suggests reduced overall excitability, possibly from Fluo-4 photobleaching or environmental stress.

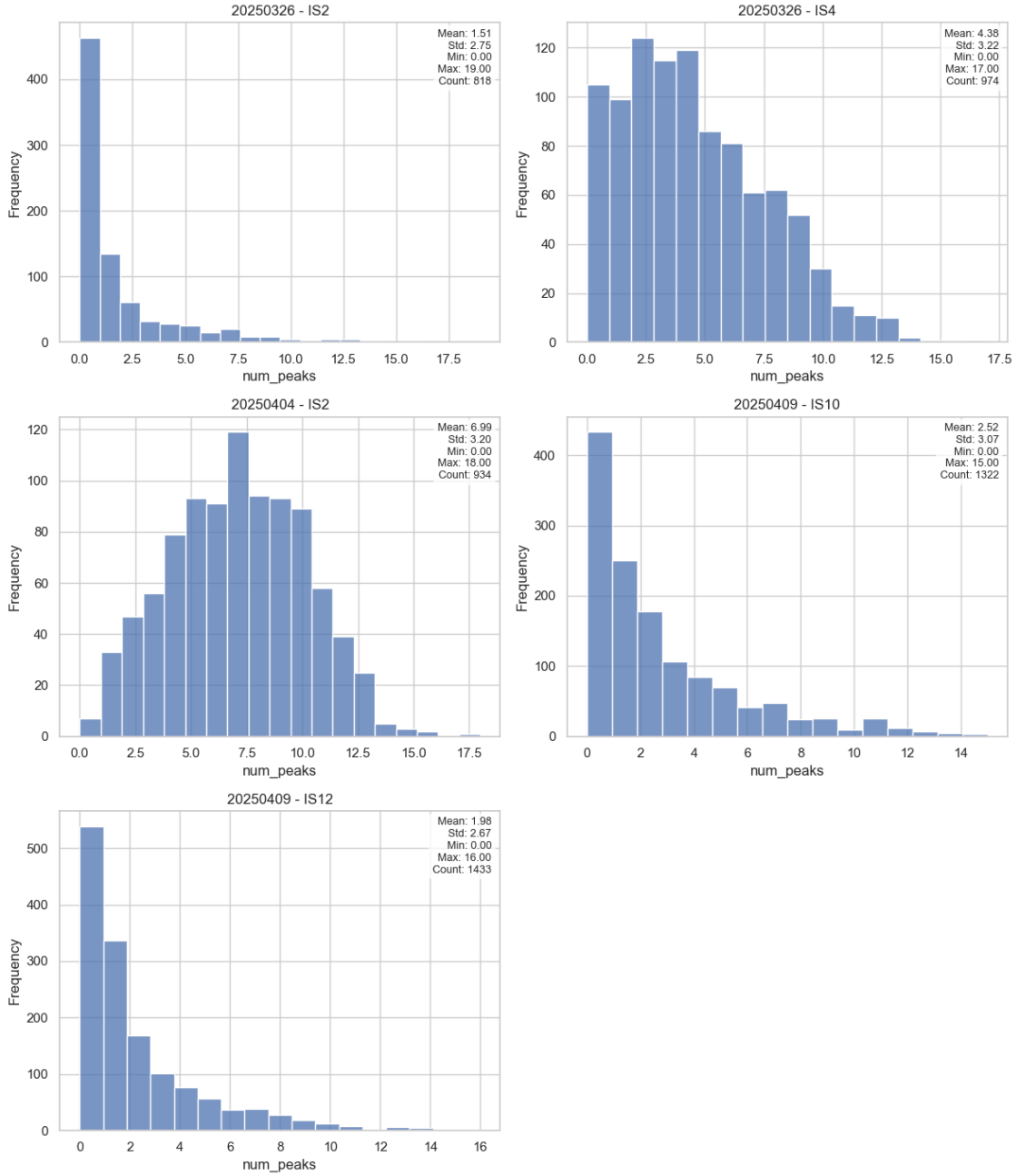
Periodicity: Periodicity scores (~0.7 ± 0.1) remain consistent across both imaging runs. This suggests that rhythmic calcium oscillations are preserved, even when global excitability drops.

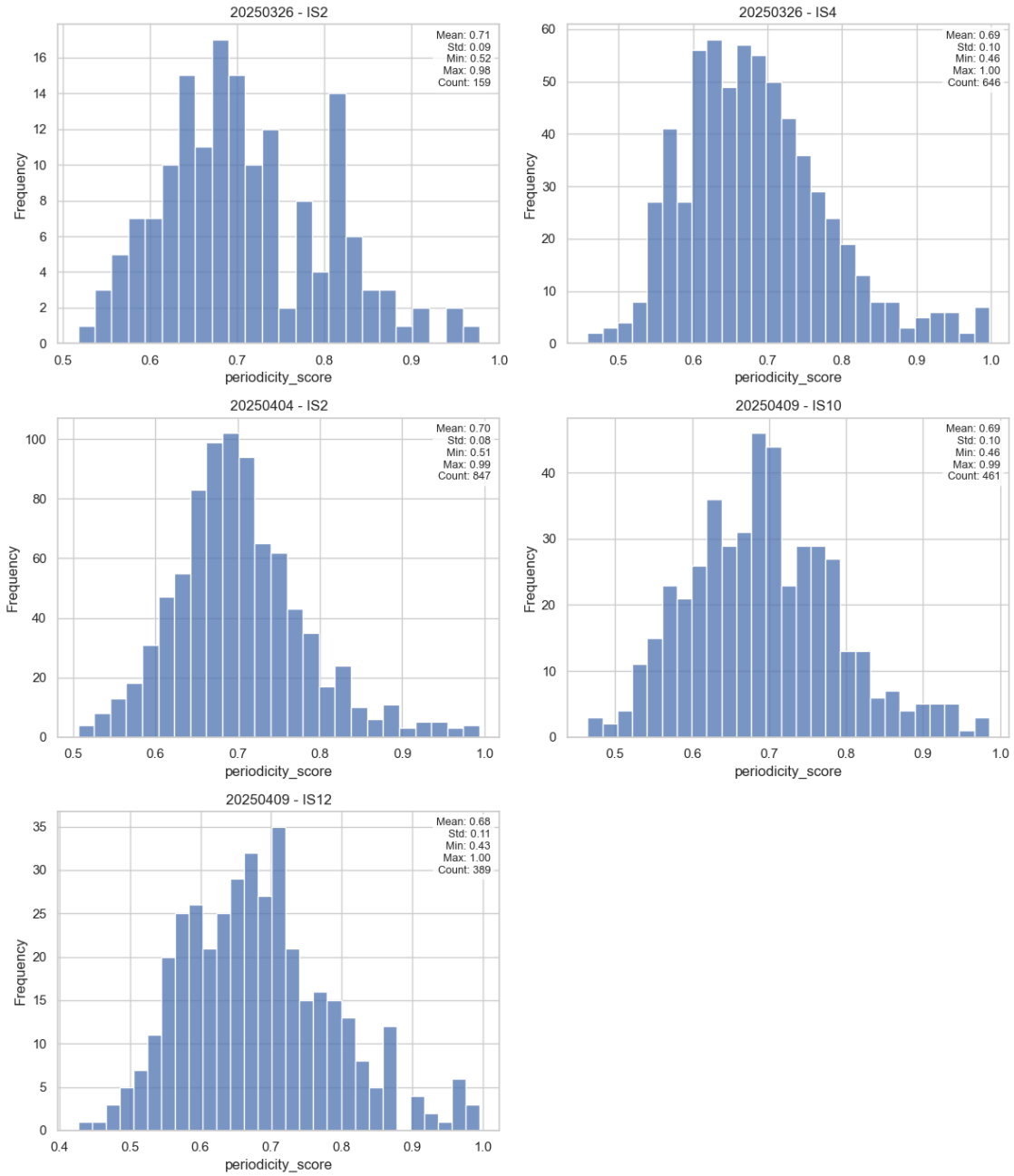
[6]:
```
# Convert is_active to label for display purposes
cells["active_label"] = cells["is_active"].map({1: "Active", 0: "Inactive"})

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"
)
```



[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)
```

Cell: Number of Peaks





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

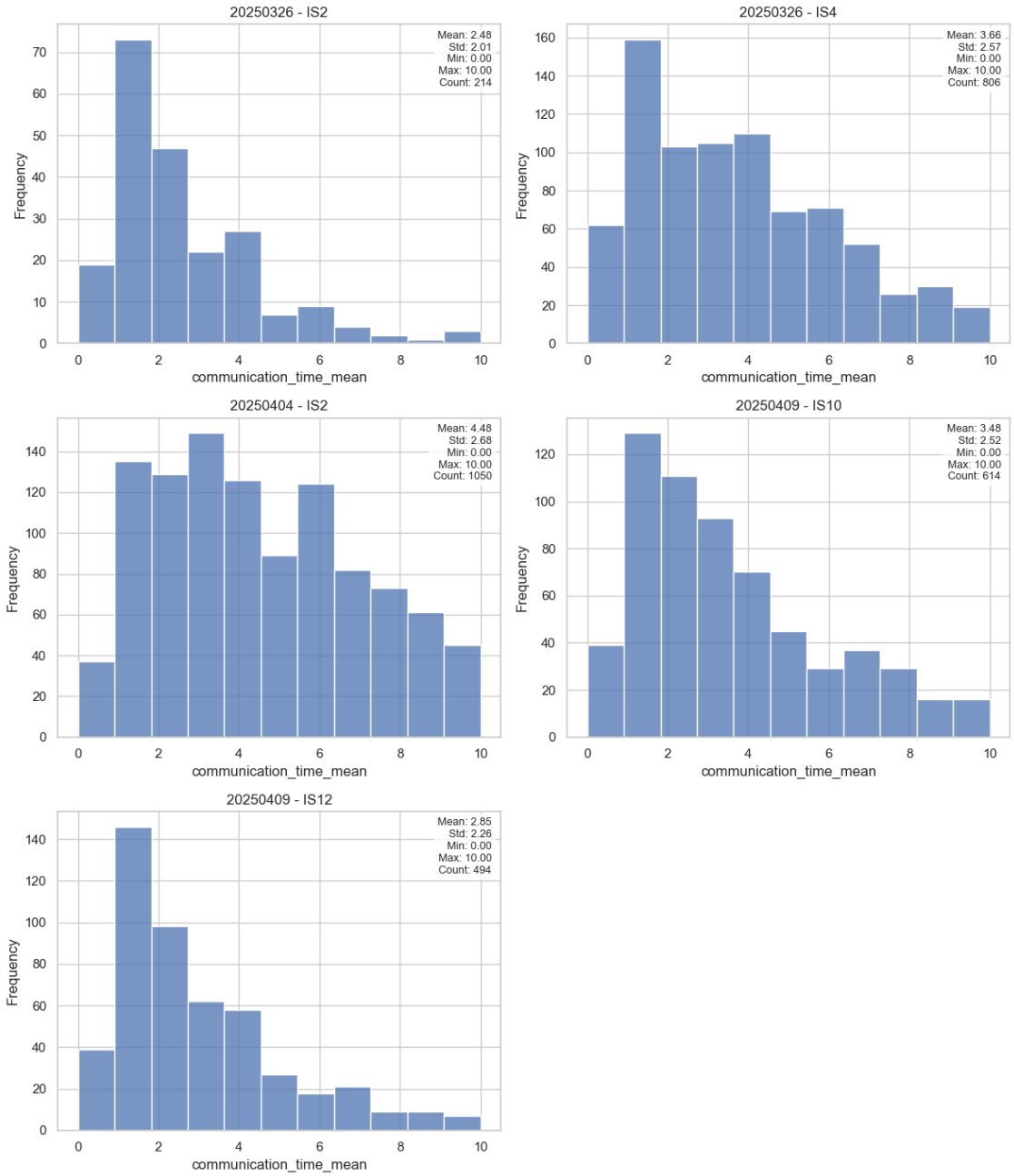
Interpretation:

- *Communication time: Slightly shorter (means between 2.5 and 4.5), with more variation.*
- *Speed: Slightly higher (~16–18), but similar spread.*
- *Cells involved: Unchanged (~3.2 ± 2).*

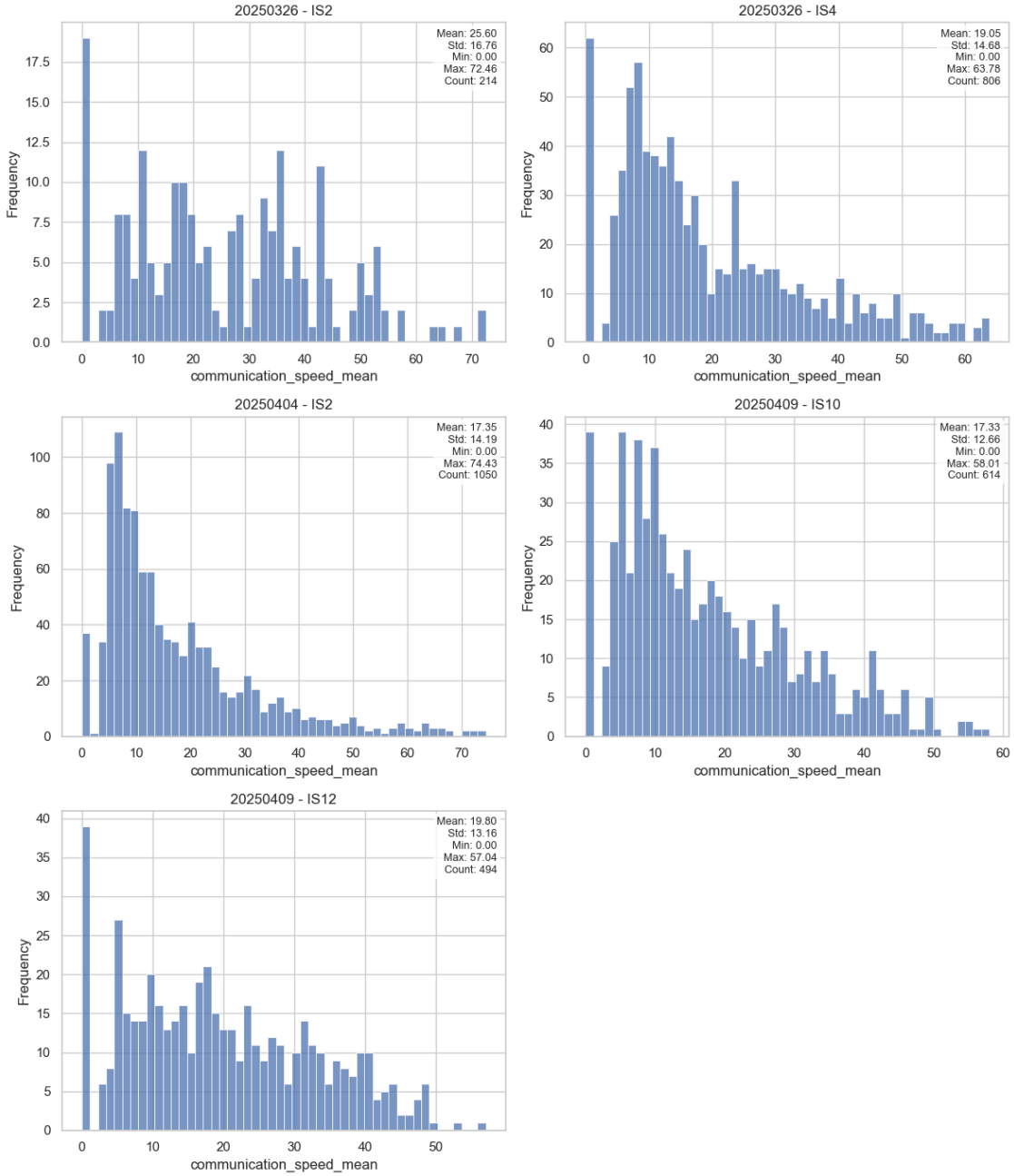
These findings indicate that sequential event mechanisms persist but may occur under altered physical or signaling constraints.

[8]:
```
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)
```

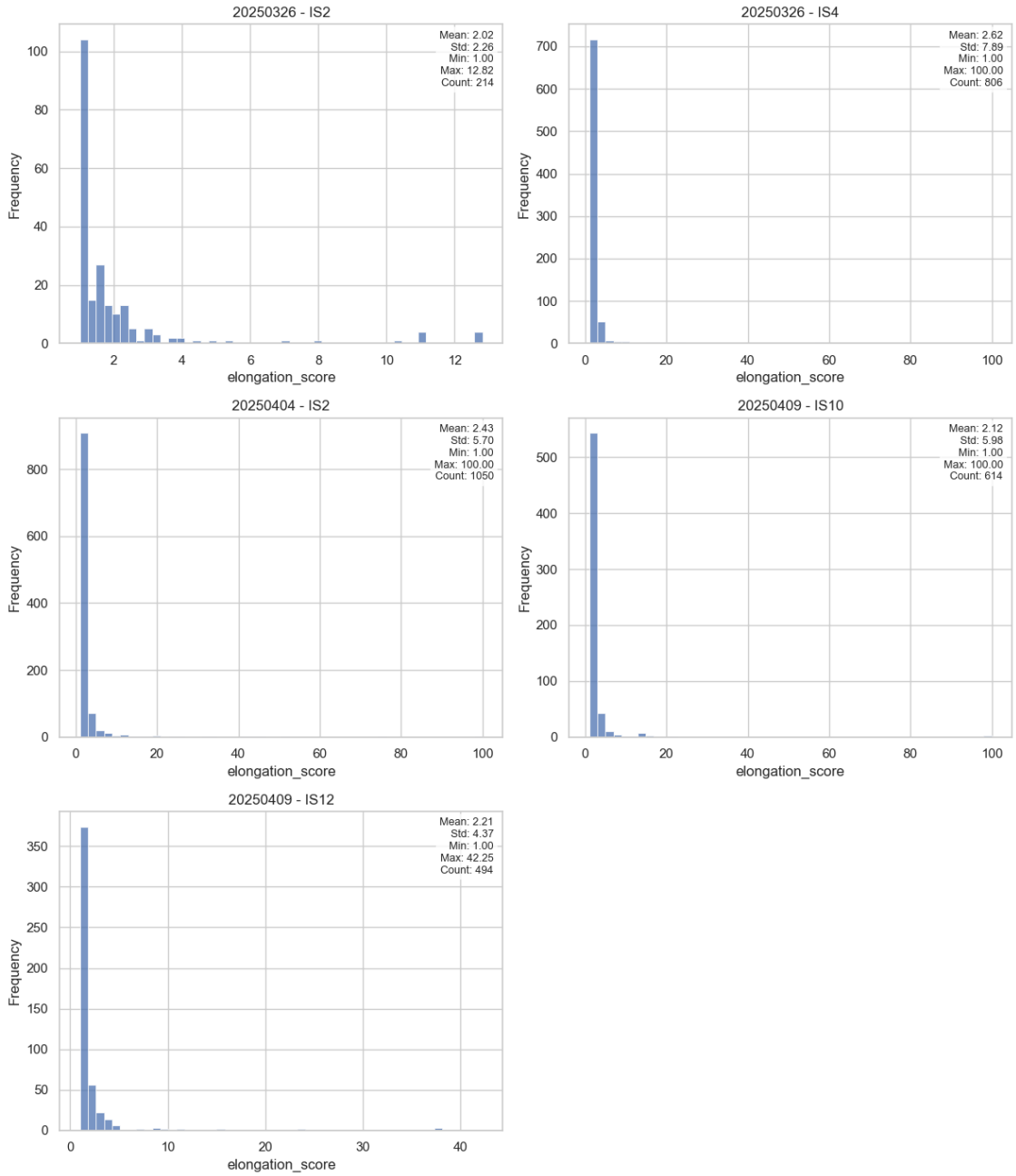
Sequential Events: Comm Time



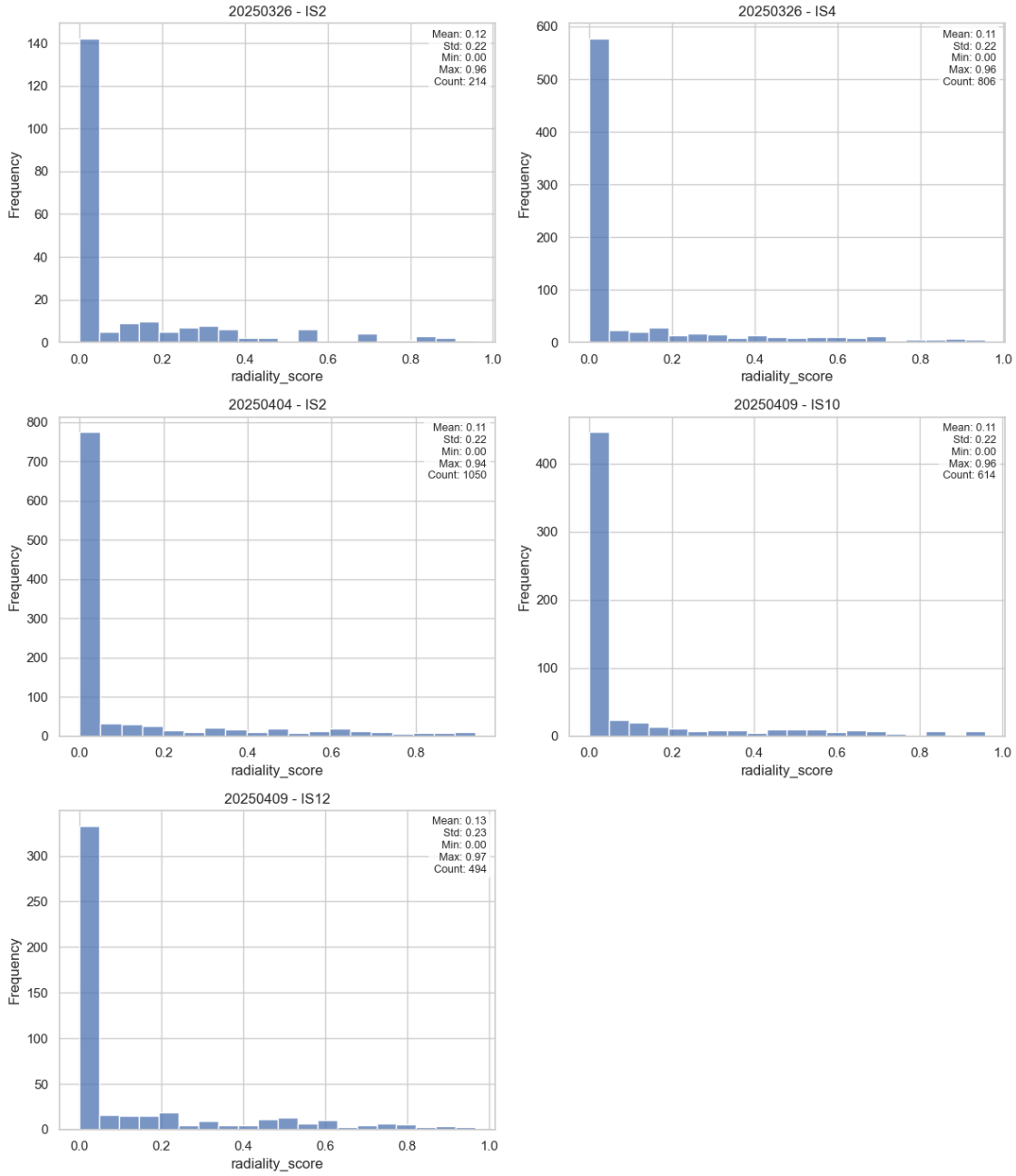
Sequential Events: Comm Speed



Sequential Events: Elongation



Sequential Events: Radiality



Sequential Events: DAG Depth

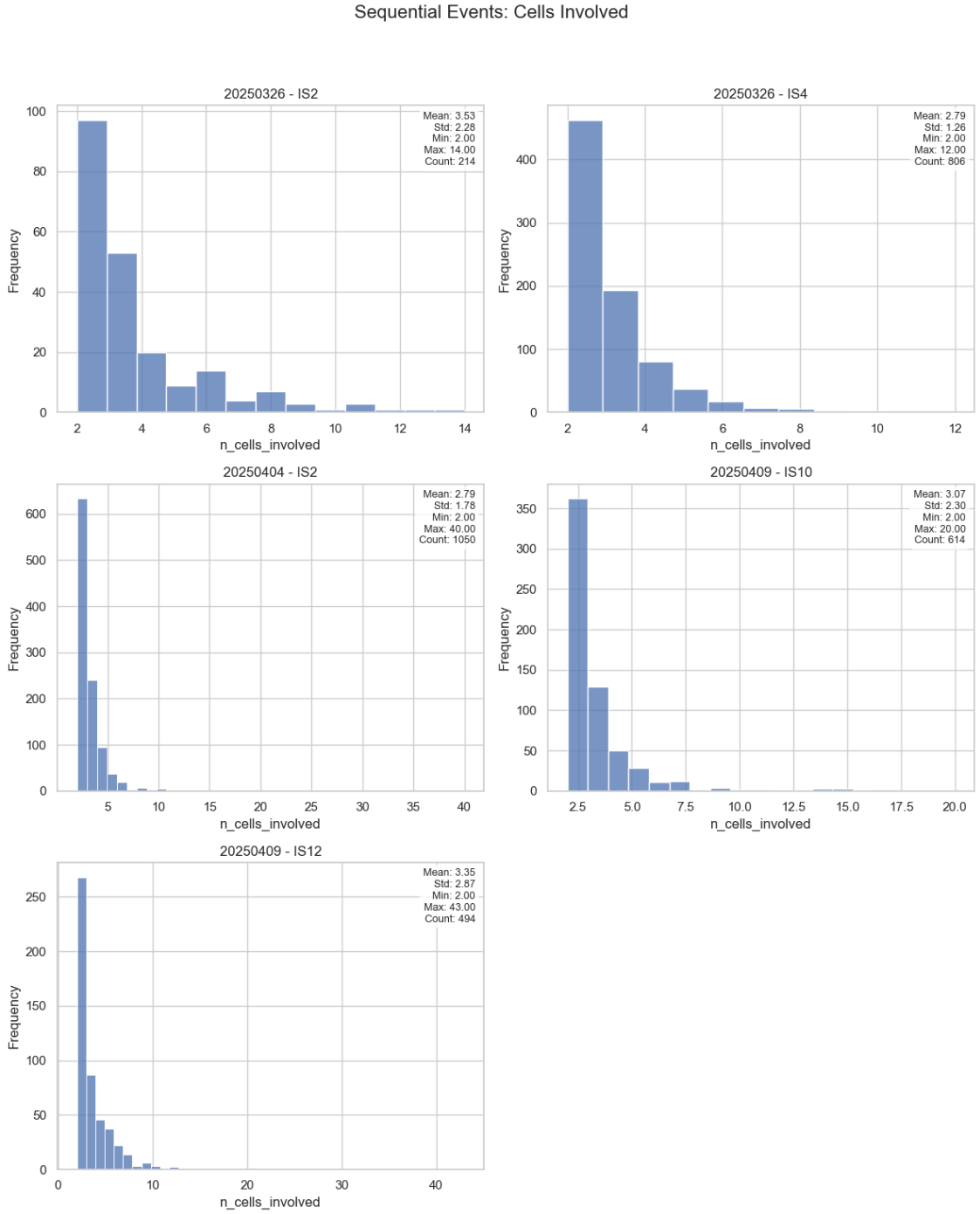
20250326 - IS2 (No Data)

20250326 - IS4 (No Data)

20250404 - IS2 (No Data)

20250409 - IS10 (No Data)

20250409 - IS12 (No Data)



0.9 Conclusion & Next Steps

After a detailed comparison between the **first** and **second imaging runs** of spontaneous calcium activity in BxPC3 cells, we conclude that the **second run cannot be considered valid control data** for downstream analyses.

The second run shows a **complete absence of global paracrine events** in almost all datasets, a sharp contrast to the first run where such events consistently accounted for ~30–40% of peaks. This loss of global coordination is accompanied by:

- A **significant decrease in the number of active cells**
- **Reduced total number of peaks per dataset**
- A **shift in distribution** of cell activity metrics, including a transition toward exponential decay patterns
- **Slightly longer durations** of individual and sequential peaks, potentially reflecting altered event dynamics
- **Preserved periodicity and symmetry**, suggesting that although overall activity is suppressed, underlying temporal regulation is still intact

These observations are strongly suggestive of **phototoxic damage**, **photobleaching**, and/or **cellular stress** due to environmental conditions (room temperature, lack of CO and humidity) during the extended imaging protocol. One dataset (`2025-04-04 IS3`) did retain global activity and matched the first run in active cell fraction, reinforcing the likelihood that these effects are **time- and condition-dependent**.

0.9.1 Decision

- **First run datasets** are retained as reliable control data.
- **Second run datasets** will be excluded from all future analyses due to significant biological drift.

0.9.2 Next Steps

- Investigate the **event-specific activation** of cells — particularly whether inactive cells in the second run are reactivated only during large-scale events.
- Consider building metrics to quantify **cell participation by event type** (e.g., proportion of cells involved in global vs. sequential events).
- Implement stricter protocols for time control and environmental maintenance between consecutive imaging sessions to avoid reproducibility issues.
