

A Robust Data Analysis Workflow for Highly Scalable, Routine Application of Calcium Oscillation Assays

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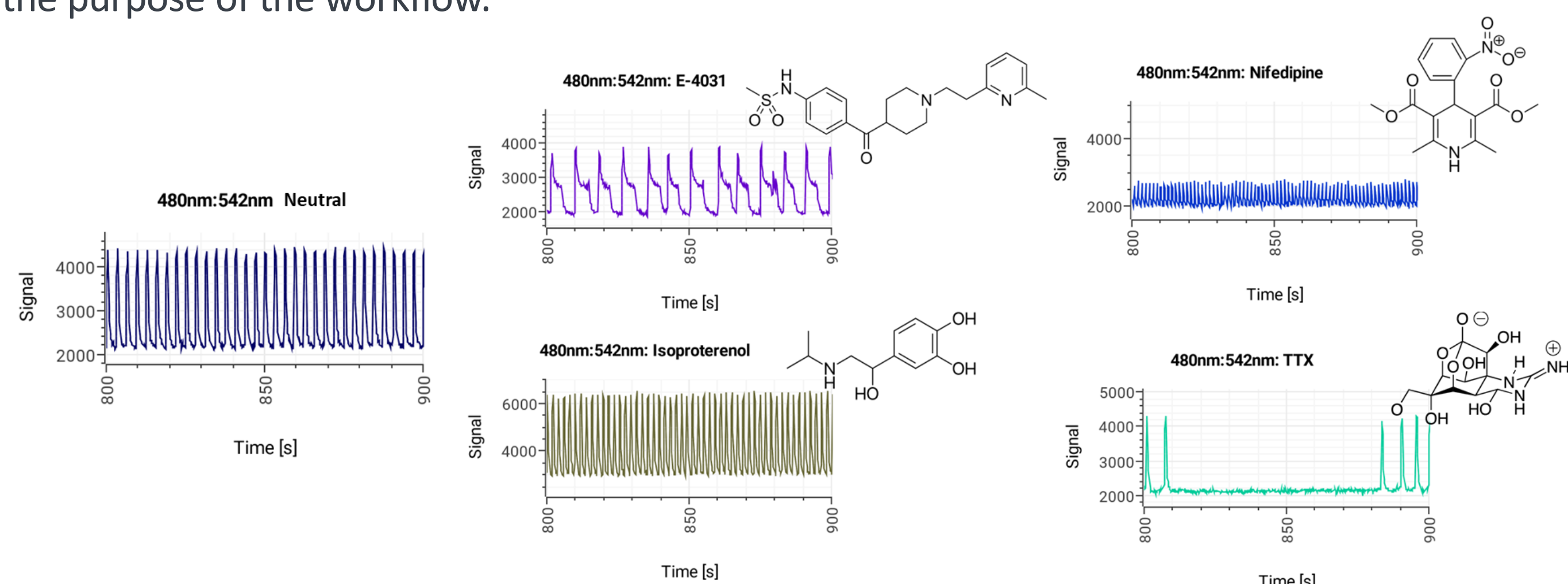
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Calcium (Ca²⁺) signalling forms the basis of many physiological and pathological functions, due to Ca²⁺-mediated signaling being central to many signal transduction pathways. As one example, the dysregulation of calcium-dependent pathways can result in neurological disorders like Parkinson's disease. In another example, the plethora of ion channels in cardiomyocytes makes them a significant potential safety liability for any potential new drug; off-target effects such as hERG channel block, detectable in Ca²⁺ oscillation experiments, can cause adverse symptoms like Torsades de Pointes. Therefore, Ca²⁺ oscillation assays—particularly, those using induced pluripotent stem cell (iPSC)- derived cardiomyocytes or neurons—are essential tools in the discovery of drugs against several neurological disorders and in assessing cardiac safety of drug candidates. The availability of these new assays enables relatively high throughput screening, making data analysis the next performance bottleneck. Herein, we present a new workflow in Genedata Screener[®] for automated, high-throughput analysis of Ca²⁺ oscillation data. It allows parallel processing of all wells of a screening run, where the parametrization is automatically adjusted to the individual measurements and allows characterization of a diverse set of recorded responses. It also offers a set of quality control output observables that can be included in the downstream analysis, e.g., flagging of 'silent' periods in corresponding recordings. We present the workflow components, highlight its automation features, and discuss its impact on Axxam's screening portfolio.

1. Calcium Oscillation Assays Provide High-Value Endpoints

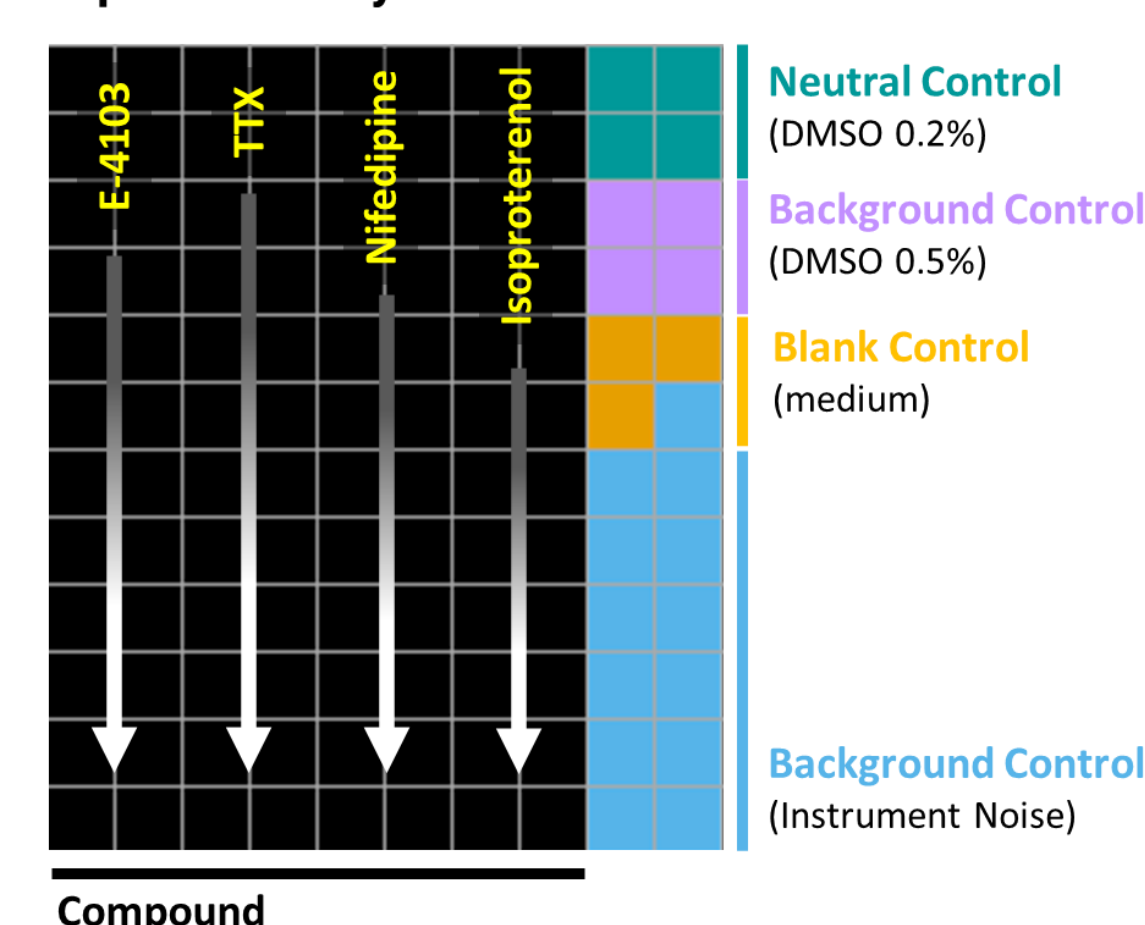
Cardiac calcium oscillation assays can exhibit a variety of drug induced signals (such as changes in beat patterns induced by the compounds shown below) that need to be robustly distinguished, quantified, and interpreted during phenotypic analysis. Automating this analysis is the purpose of the workflow.



2. Set-up of Axxam's Calcium Oscillation Assays

Axxam, which houses a dedicated unit with expertise in iPSCs, configures Ca²⁺ oscillation assays in terms of conditions, modality, and throughput, to match client screening and profiling needs. The analysis workflow in Genedata Screener[®] has been validated in a cardiomyocyte assay.

Experiment Layout



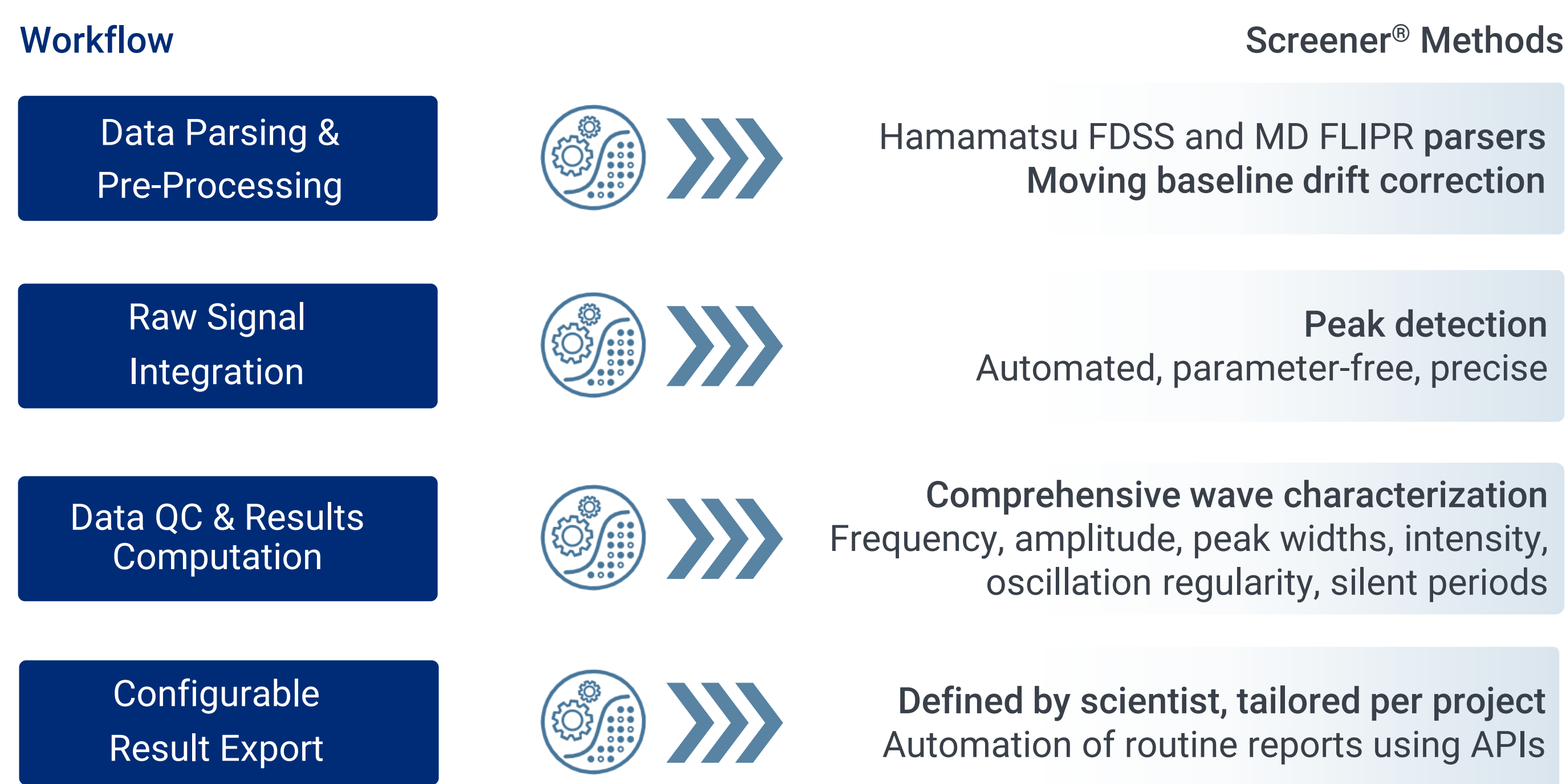
E-4031, TTX, Nifedipine and Isoproterenol were tested as intraplate dose-response curves, with decreasing concentration from top-to-bottom.

A range of controls monitor the influence of excipient materials (medium, DMSO, vehicle) and distinguish low oscillations from background noise.

Protocol: iCell[®] Cardiomyocytes² (Fujifilm CDI) were seeded and cultured according to vendor instructions. Cells were loaded with CaI520[®] AM dye for 1h and measured at FDSS[®] 7000EX Hamamatsu in Tyrode's buffer for 10 minutes.

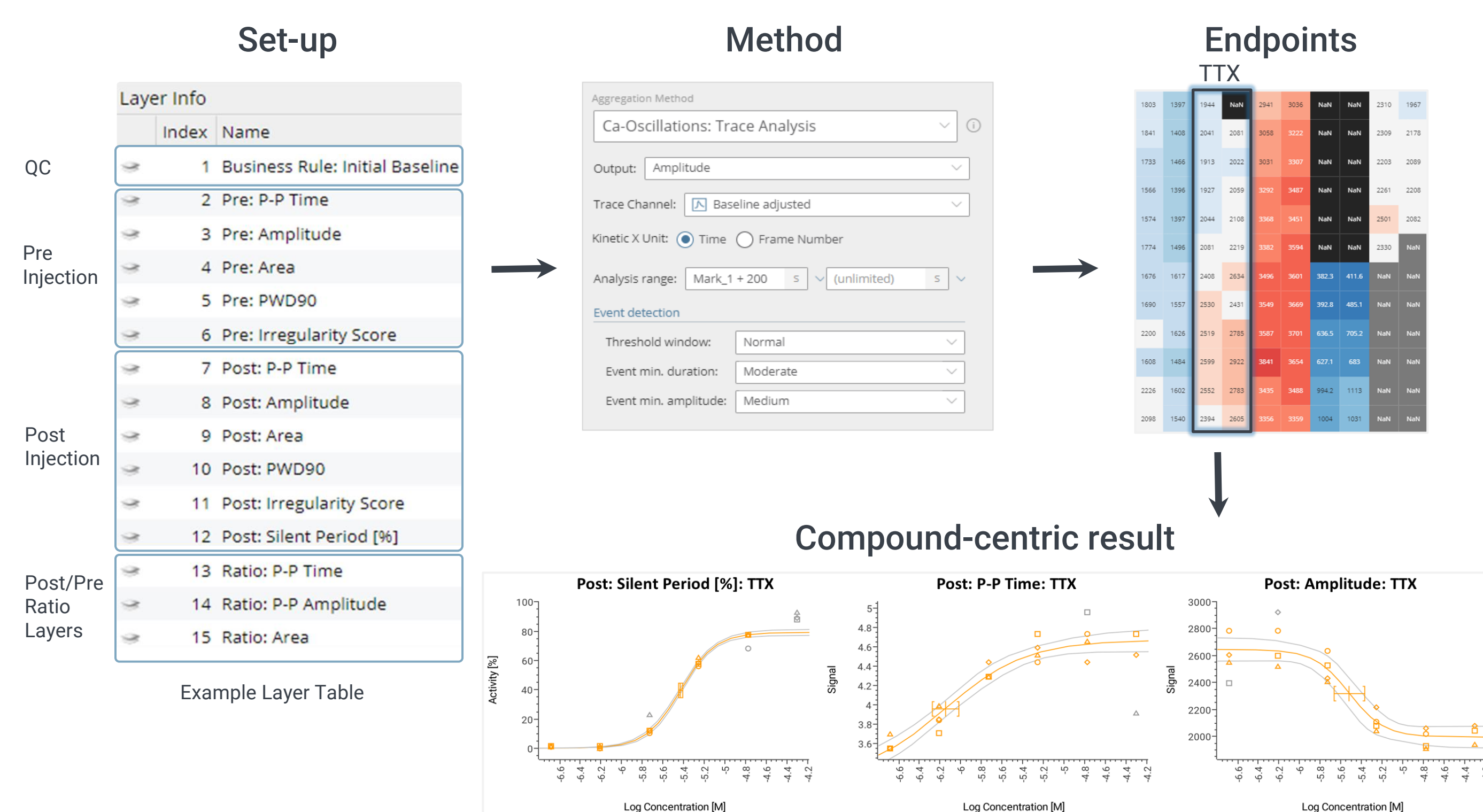
3. Workflow for Automated Data Analysis

Each step of the analysis workflow is effectively addressed by the methods in Screener[®] responding to diverse project or stakeholder needs.



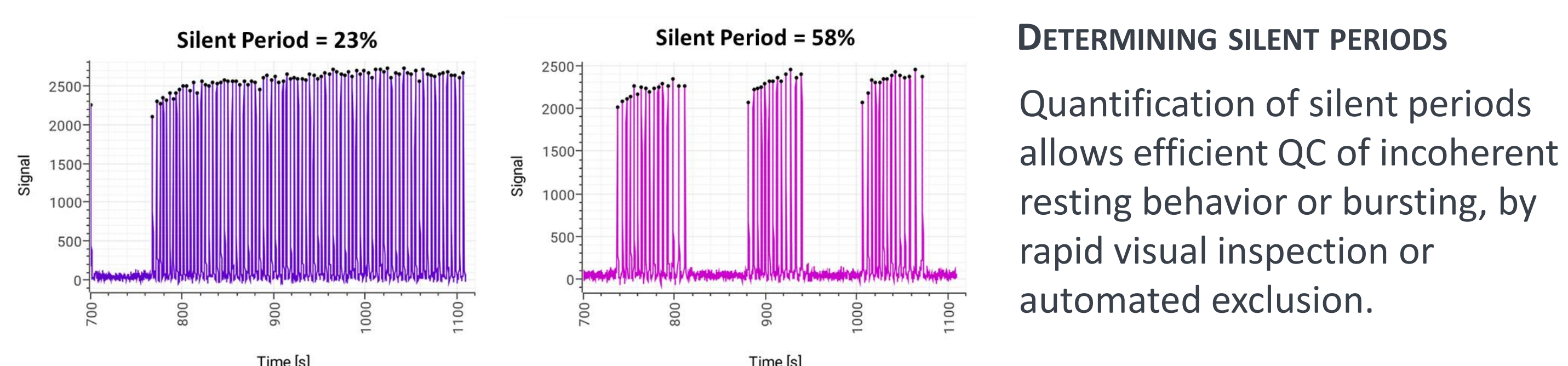
4. Workflow Highlights : Minimal Set-up – Maximal Throughput

Individual pre- and post- treatment endpoints as well as post/pre normalized ratios are available with minimal parameterization or set-up, ensuring consistent and speedy attainment of per-compound results.



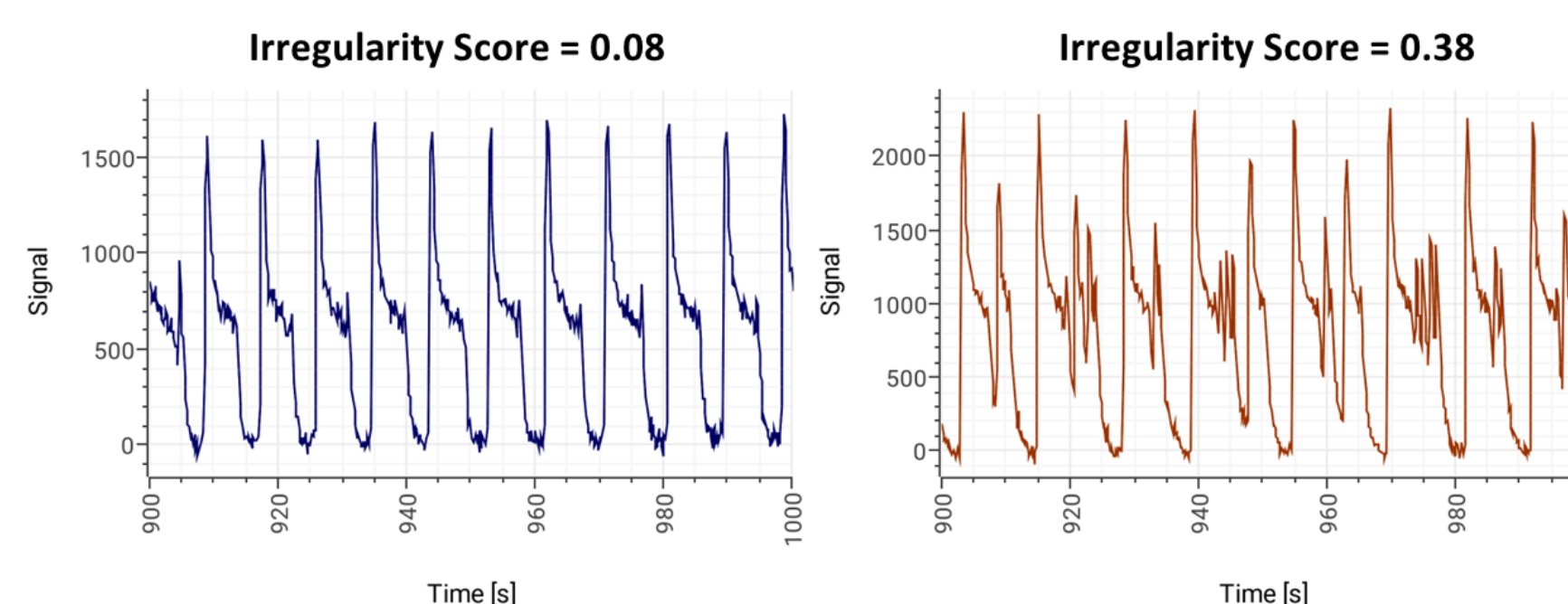
5. Automated Quality Control Based on Robust Methods

Comprehensive endpoint computations both for pre- and post- treatment allow efficient and automated quality control via masking rules or threshold-based tagging.

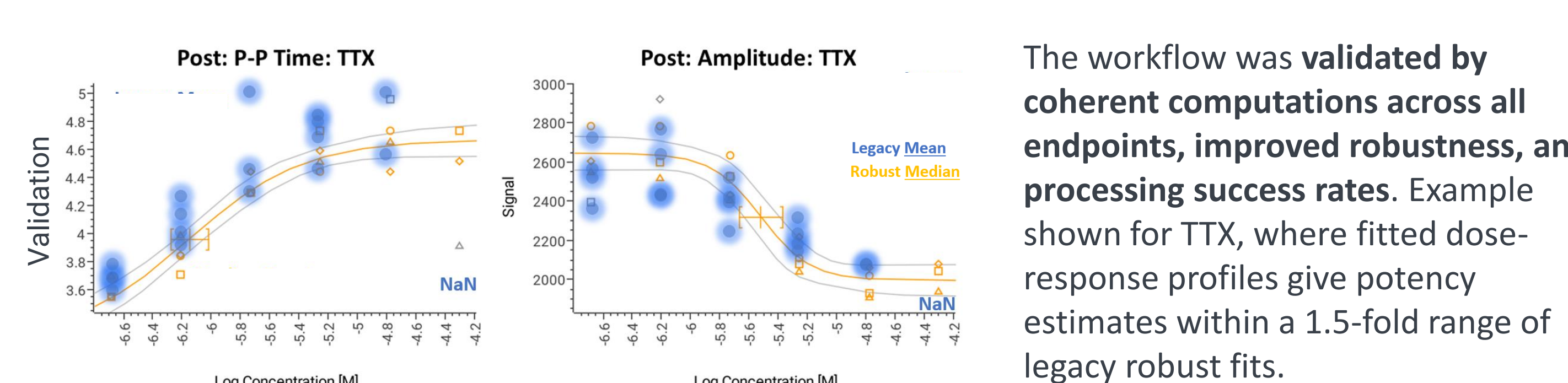
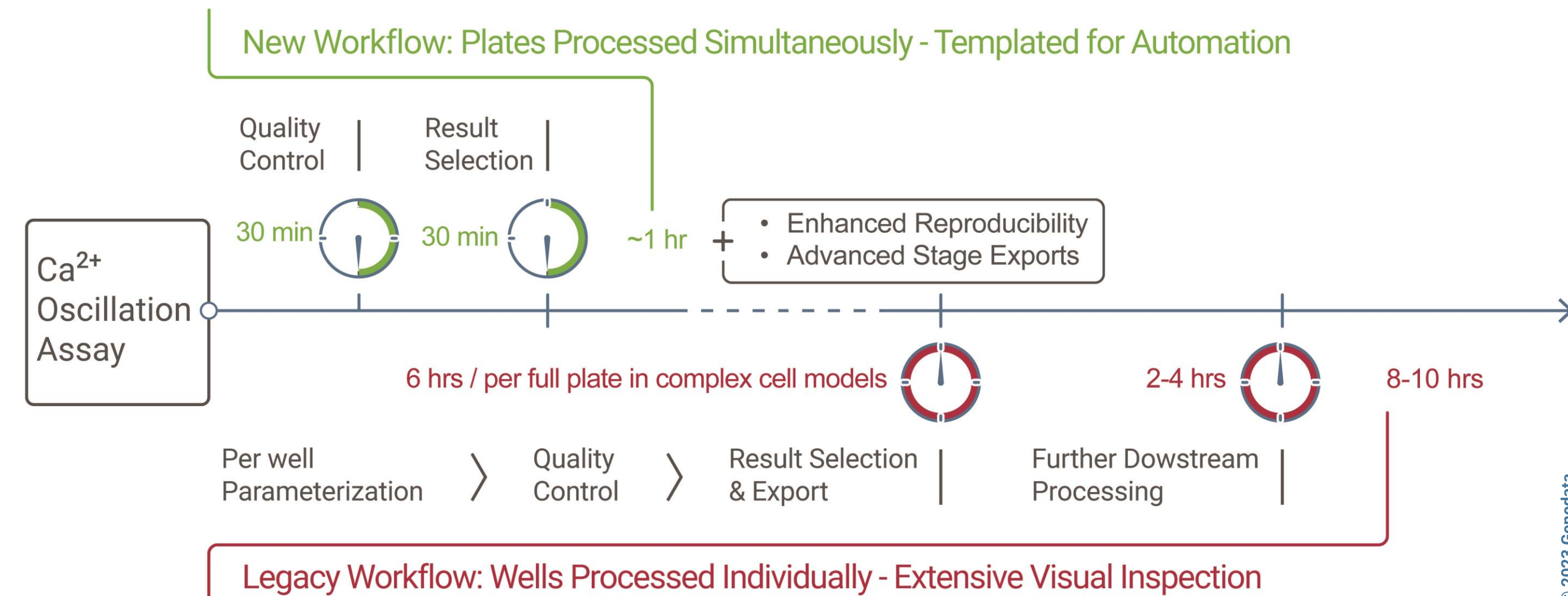


QUANTIFYING IRREGULAR ACTIVITY

Irregularity evaluation feeds the automated QC, helping identify low-quality measurements or non-viable biological replicates.



6. Maximized Turnaround: Efficient, Automated, and Agnostic Analysis



The workflow was validated by coherent computations across all endpoints, improved robustness, and processing success rates. Example shown for TTX, where fitted dose-response profiles give potency estimates within a 1.5-fold range of legacy robust fits.

In scientific collaboration with Axxam, Genedata has developed a robust new workflow for automated analysis and quality control of calcium oscillation assays. It offers out-of-the-box capabilities to load, process, and profile compound effects at scale. Validation in various calcium oscillation assays at Axxam showed that the Genedata workflow achieved equivalent results to the legacy workflow, but with improved efficiency, well-processing success, and result quality, through enhanced robustness and reproducibility. Depending on cell model analyzed, this yielded a potential 10-fold gain in productivity. This novel workflow is included with the Ion Channel Extension available with Genedata Screener[®].