The Power of High Content, High Throughput Screening in Condensate Based Drug Discovery

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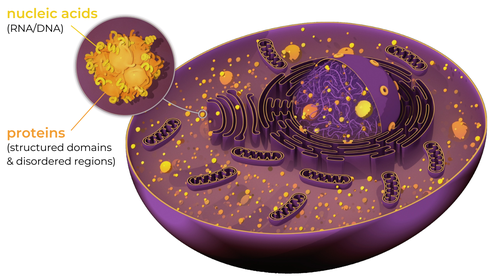
Introduction to Condensates

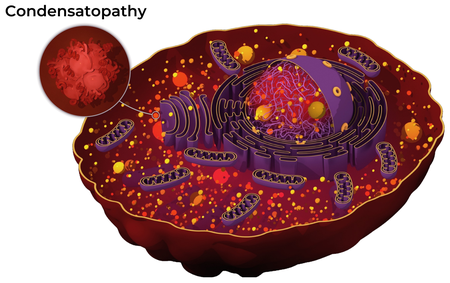
Biomolecular condensates are dynamic membrane-less organelles that form via phase separation and are responsible for a wide range of cellular functions[1] and regulate many processes and biological pathways.



Cellular Functions:

- Transcription
- Signaling
- RNA metabolism
- Stress response

Condensates serve as central nodes of dysfunction, termed condensatopathies, in many complex diseases. Condensate dysfunction is generally accompanied by morphological changes that can be detected by microscopy. Novel classes of condensate-modifying drugs (c-mods) that repair the condensatopathy in difficult to treat diseases can be identified by monitoring phenotypic changes in condensates by high content imaging.[2]



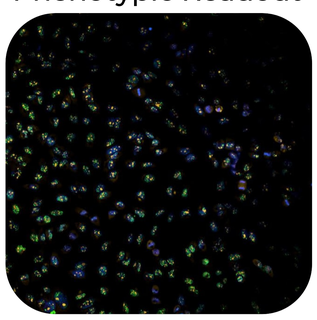
Aberrant condensates drive complex diseases:

- Neurodegeneration
- Cancer
- Heart disease
- Metabolic disease
- Infection

Traditional vs Phenotypic HTS

Traditional high throughput screening (HTS), has funnelled biochemical data through an assay or panel of assays that have a single readout, such as fluorescence or luminescence. The potential for false positives due to readout interference, aggregation, or promiscuous inhibition is high in biochemical assays. This can be overcome by calculating hits that have been read over multiple time points (kinetically) and using a ratiometric readout.

Phenotypic Readout



Phenotypic screening provides more comprehensive results but requires different assay development considerations, different instrumentation, and different automated workflows.

Phenotypic Screen Assay Considerations

- High content imaging vs single readout
- Additional reagents to tag different parts of the cell
- Number of fields of view and planes, objective magnification
- Cell density optimization for miniaturization
- Additional buffers for washing/blocking/antibodies
- High throughput liquid handlers to dispense and aspirate
- Protocols should be efficient, without sacrificing cell health
- Optimizing temperature, media, and gas exchange requirements so they fit typical robot capabilities

Figure 1. Readout from immunofluorescent assay A performed by following the vendor's recommendations.

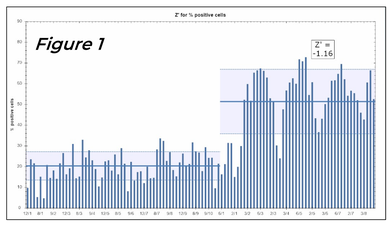


Figure 2. Readout from immunofluorescent assay A after optimization of concentration of both primary and secondary antibodies.

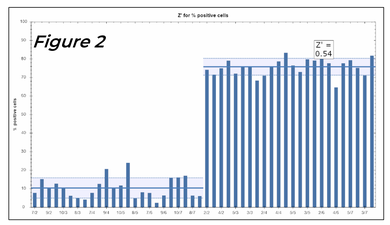
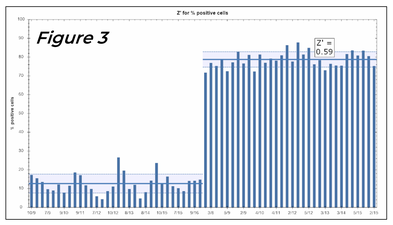
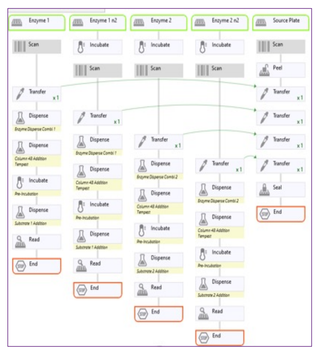


Figure 3 Readout from immunofluorescent assay A after optimization of antibodies, incubation time, and temperature.

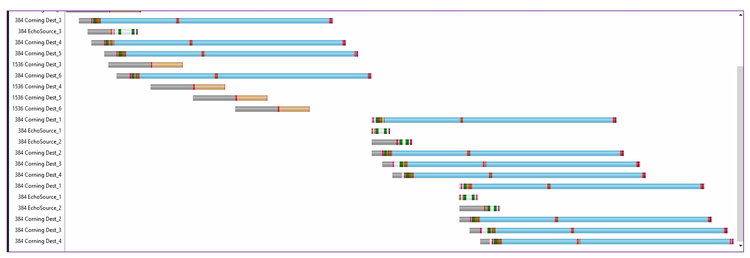


Robotic Protocol Considerations

- Assays with short, repetitive steps (like enzymatic or biochemical binding assays) can be scheduled in the same protocol with little risk of error.
- Orthogonal assays can be run simultaneously if they utilize the same process and reagents.



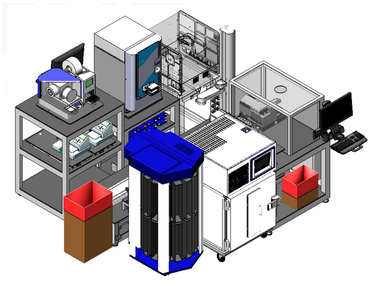
Phenotypic assays typically take several days to complete. Scheduling separate runs with unique functions minimizes error. Multiple projects can be run simultaneously to save time.



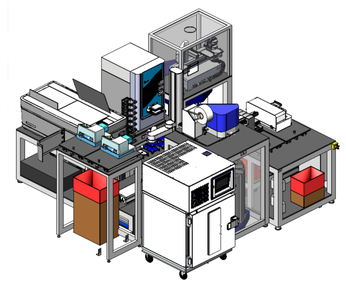
Robotic Platforms

When designing a robot, it is important to note the following requirements of phenotypic HTS:

- Additional environmentally controlled storage
- Rapid aspiration options that are gentle on cells
- Offline imaging systems wherever possible to reduce bottlenecks (as imaging is typically the rate limiting factor)
- Rapid or temperature-controlled sample transfer options
- Enclosures for any instrumentation for cell fixation
- Instrument redundancy for AI pipelines
- Physical/electrical expansion space for future scientific assay adaptations.



MC01665 – "Roberto"

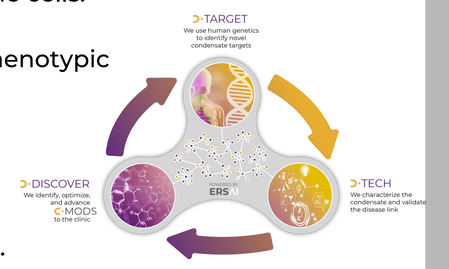


MC00623 – "Clamps"

The Impact of Phenotypic Screening

Optimizing the protocols and designing automation that is cellular assay focused has reduced the turnaround time from 12 weeks to 4 weeks to complete an HTS campaign with ~400,000 molecules. The information gathered using high content imaging gives a rich profile of each interaction the small molecules have with the cells.

Dewpoint has shown that phenotypic screening has unequivocally changed the drug discovery process and found hits that would not have been discovered through traditional methods.



References and Acknowledgements

1. Mitrea and Kriwacki. Cell Commun. Signal. (2016)
2. Mitrea DM, et al, Nat. Rev. Drug Discov. (2022)

For more information about condensates see → Dewpoint's key publications

The presenters would like to thank Dewpoint's Lead Discovery & Lead Evaluation team for their contributions to the information provided here.

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