

Introduction

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Analytical Sciences
Discovery Research

**High Frequency Acoustic Technology:
Evaluation for Compound Mixing and
Dissolution in HTS.**

Introduction

- **Current Issues**
- **Technology Overview**
- **Project areas**
- **Summary**

Current Issues

Department

- **Compound Primary Dissolution**
- **Freeze thaw & Aliquotting**
(causing precipitation)



Compound Management
High Throughput Chemistry

-
- **Compound solubility in the (HT)**
Screening assay



HTS Groups
Lead Optimisation Groups
Therapeutic area Research

Technology Overview

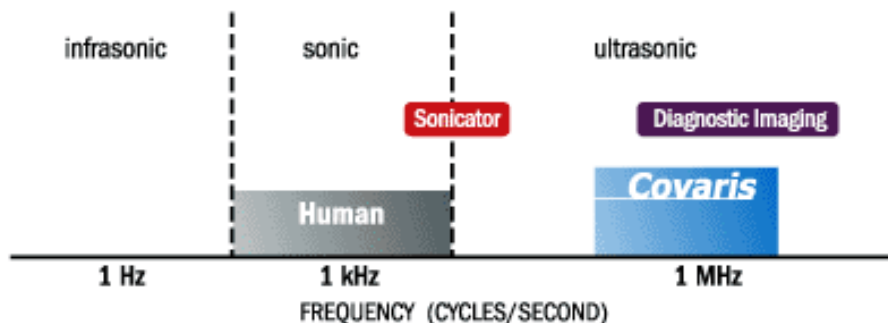
Enabling technology

Adaptive Focused Acoustics (AFA)

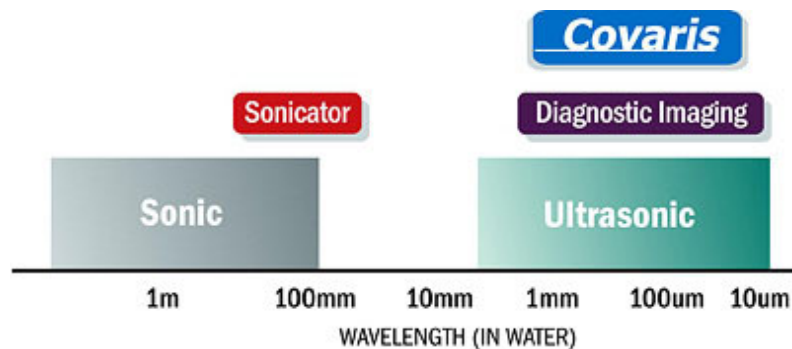
...enables non-contact, isothermal,
closed vessel mixing

Technology overview

Frequency of AFA operation

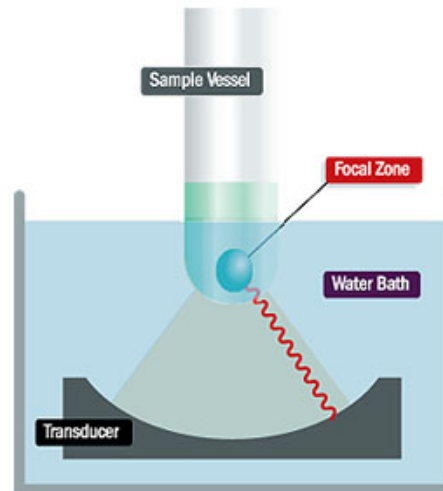


Frequency denotes wavelength



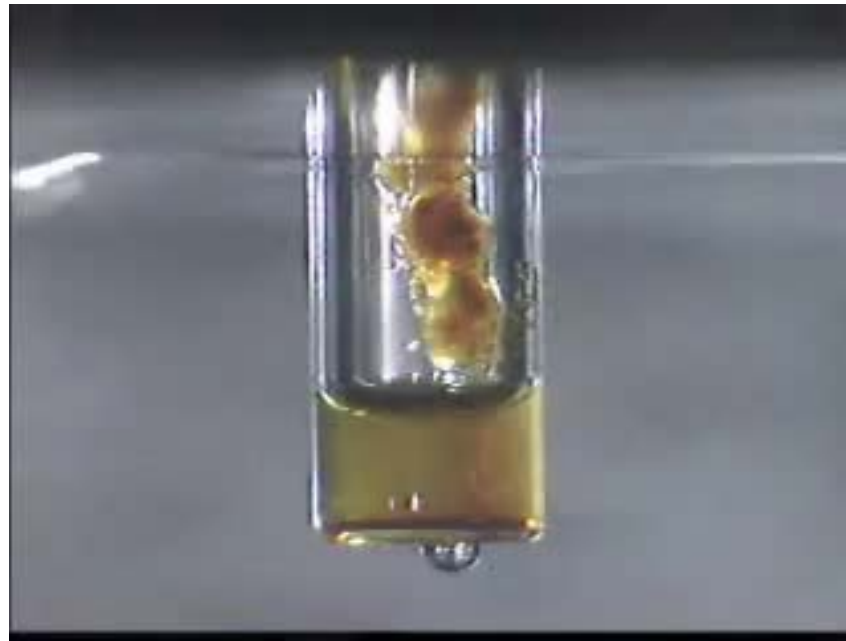
Technology

High Frequency = short wavelength = acoustic energy focusable into sample



- Non-contact
- Scalable from large tubes to 1536 well microplates
- Highly controllable from high to low intensity
- Isothermal

Technology - video of Gum dissolution



“Capable of dissolution in 20s that previously would take 12 hours”

Project Area – AFA in CM & HTC

**Aim – to Evaluate / Implement AFA in CM & HTC
for Primary Dissolution of dried compounds**

Project Areas HTC, CM - Lipinski paper

Combinatorial Chemistry & High Throughput Screening, 2005, 8, 499-512

High Throughput Sonication: Evaluation for Compound Solubilization

Kevin Oldenburg¹, Douglas Pooler¹, Kurt Scudder¹, Christopher Lipinski² and Michele Kelly^{2, *}

¹MatriCal, Inc., Spokane, WA, ²Pfizer Global Research and Development, Groton, CT, USA

Abstract: Dissolution of organic compounds in DMSO in HTS plate or tube format is a difficult problem as users move to higher compression plate formats. Precipitation of compounds from DMSO screening stocks is a recognized problem in the HTS materials management process. The adverse effect of freeze thaw cycles on DMSO stock solutions stored in plate format as a result of cherry picking operations has led to the gradual replacement of plate-based storage with tube-based storage so as to minimize the number of freeze thaw cycles. Compound solubility in DMSO is markedly decreased by uptake of small quantities of water. We attribute this effect to the non ideal properties of DMSO water mixtures such that cavity formation in solvent, a necessary step in dissolution, is more difficult in wet DMSO than in dry DMSO or in pure water. We report here that efficient compound dissolution is possible even in 384 well format by the use of in-well plate-based sonication. Surprisingly, compounds precipitated from DMSO stocks either by water uptake or repeated freeze thaw cycles can be re-dissolved by low energy sonication. Finally, we demonstrate that precipitation of compound from DMSO stock solutions is synergistically enhanced by water uptake into DMSO compound stock solutions as well as by increasing the number of freeze thaw cycles.

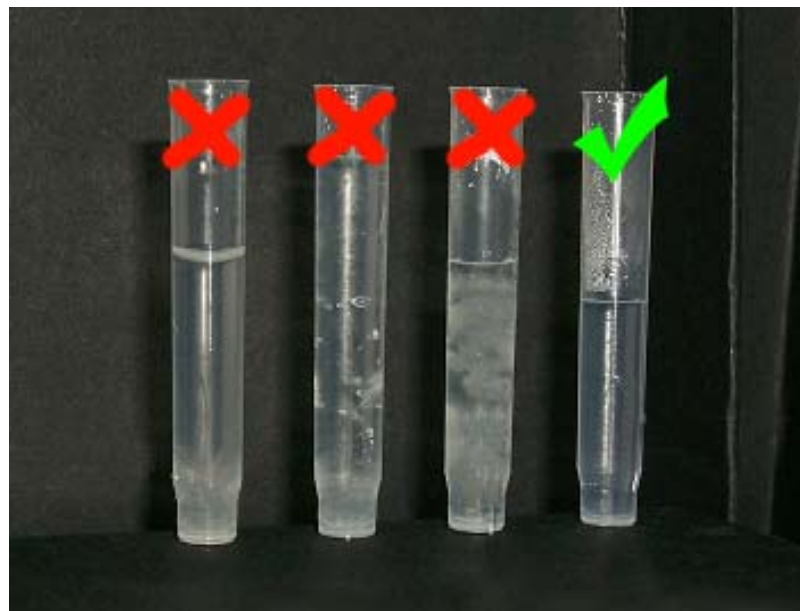
Keywords: Sonication, compound solubility, freeze-thaw cycle, water in DMSO, precipitation, solubilization.

Project areas: Compound Management & HTC

Objective: maintain compound concentration from:
HTC – CM – uHTS (XC50 SAR)

Compound solubility issues caused by:

- Initial dissolution - glues gums etc
- Precipitation - water uptake & freeze thaw



Project area: Compound Management & HTC

Phase 1 – Evaluation of AFA in CM & HTC

Analytical Sciences

- No compound degradation observed LC-MS data

Compound Management

- From non-targeted libraries 400 poor to solubilise compounds were selected. Of these 85% were dissolved in 30 secs. The remainder were converted to a fine suspension that could be pipetted by a liquid handling robot

HTC

- Evaluated for new compound dissolution. Now used as diagnostic for compound solubility. 30secs under AFA treatment and if not solubilised the solvent is changed or the molecule is modified

Project area: Compound Management & HTC

Phase 2 – Implementation of AFA in CM & HTC

Requirement

- Fully automated, High throughput, High capacity robot incorporating AFA technology for primary dissolution of all compounds
- Capable of AFA treatment of tubes, vials & microwell plates
- All tubes etc. must be dry post AFA treatment (samples need to be partially immersed into water for AFA treatment)
- Ability to handle both closed and open tubes
- Software capable of worklist operation for integration into LIMS



**GSK commissioned development of Covaris C2000
with Covaris & KBiosciences**

Application area: Compound Management & HTC

Objective: Automated dissolution of dried compounds

C2000 Ability

Rapid dissolution ✓

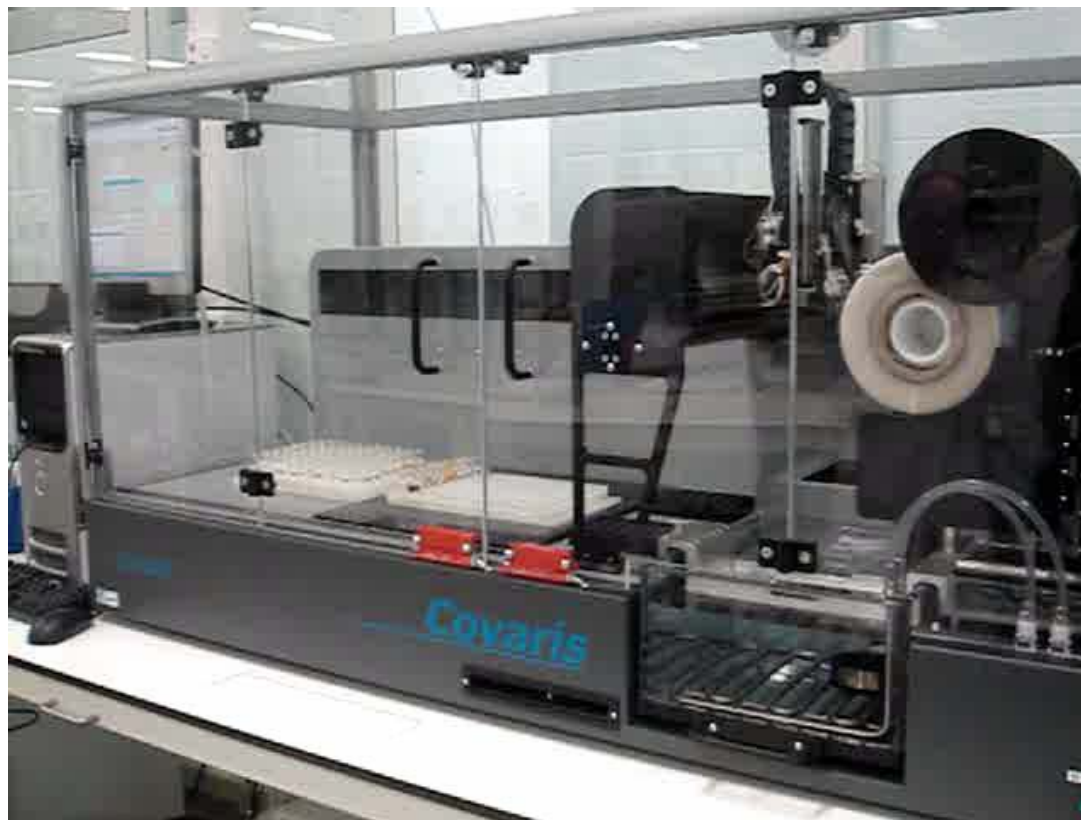
Integratable ✓

Compounds to thermodynamic equilibrium ✓



Status:
Currently installing in US. UK
complete

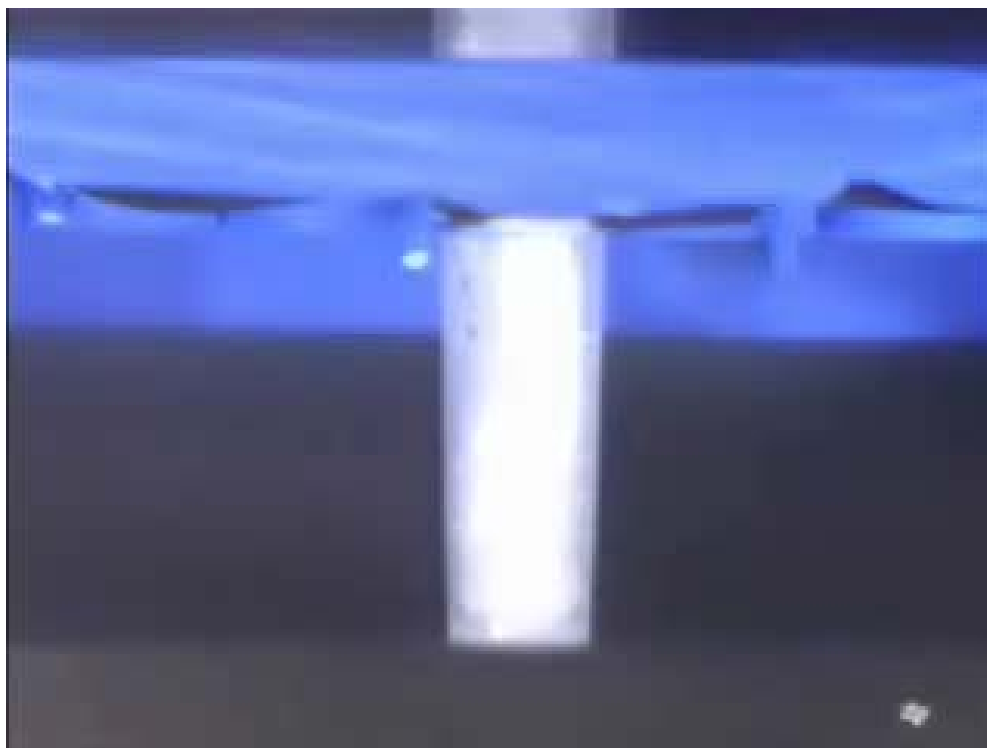
C2000 automated acoustic dissolution



Application area: Compound Management & HTC

Future

- Implementation of AFA for thawing and mixing of 2D barcode tubes on C2000



Project Area – uHTS

Aim – to evaluate AFA in uHTS assays

Amphora paper

Anal. Chem. 2004, 76, 7278–7287

Streamlined System for Purifying and Quantifying a Diverse Library of Compounds and the Effect of Compound Concentration Measurements on the Accurate Interpretation of Biological Assay Results

Ioana G. Popa-Burke,^{*,†} Olga Issakova,[†] James D. Arroway,[†] Paul Bernasconi,[†] Min Chen,[†] Louis Coudurier,[†] Scott Galasinski,[†] Ajit P. Jadhav,[†] William P. Janzen,[†] Dennis Lagasca,[†] Darren Liu,[†] Roderic S. Lewis,[†] Robert P. Mohney,[†] Nikolai Sepetov,[†] Darren A. Sparkman,[†] and C. Nicholas Hodge[†]

Amphora Discovery Corporation, P.O. Box 12169, Research Triangle Park, North Carolina 27709, and Nanosyn, Inc. 3760 Haven Avenue, Menlo Park, California 94025

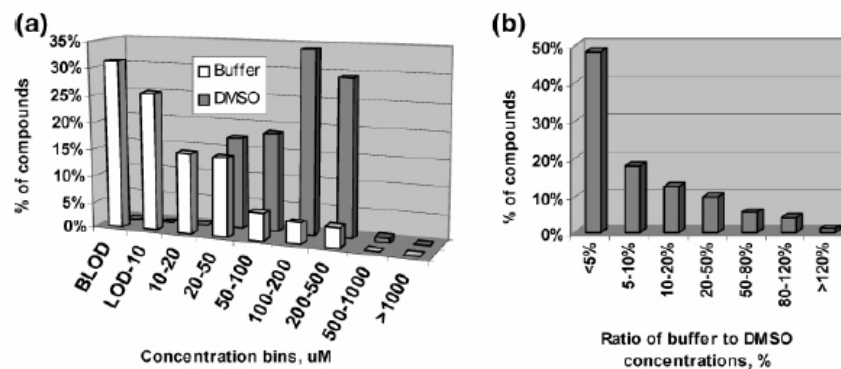


Figure 5. (a) Distribution of concentration values across ~2000 compounds. Values shown on the x axis are compound concentrations (μM). All the DMSO concentrations were converted to the buffer level (based on dilution factors). Binning of concentration values is done according to variation analysis performed (Table 1). (b) Ratio (%) of aqueous-to-DMSO concentrations for the same samples.

Project Area – AFA in uHTS

Objective: Evaluate AFA effects in assay quality/data & compound solubility

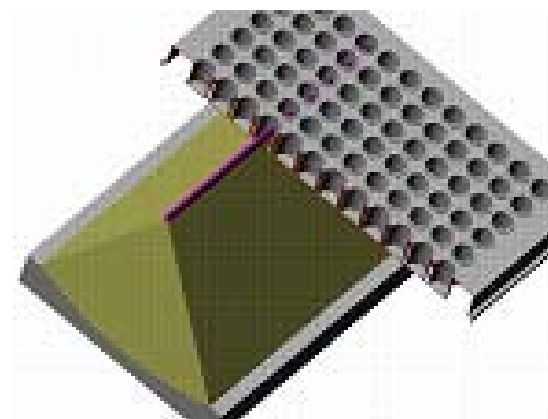
Preliminary Areas of desired benefit

Improve Z'

Reduce CV

Re-solublising compound in aqueous

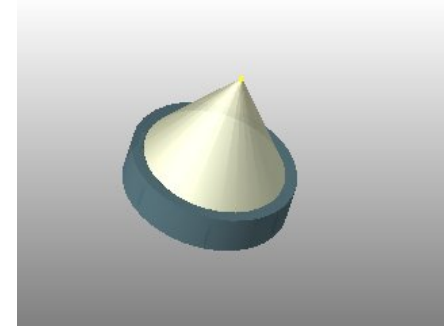
Accelerate assays



Application areas: AFA in uHTS

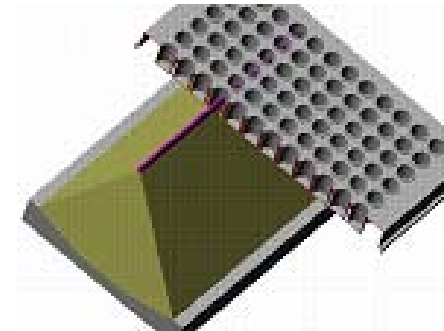
Technology

Current on the market AFA products incorporate a point source
- One well at a time

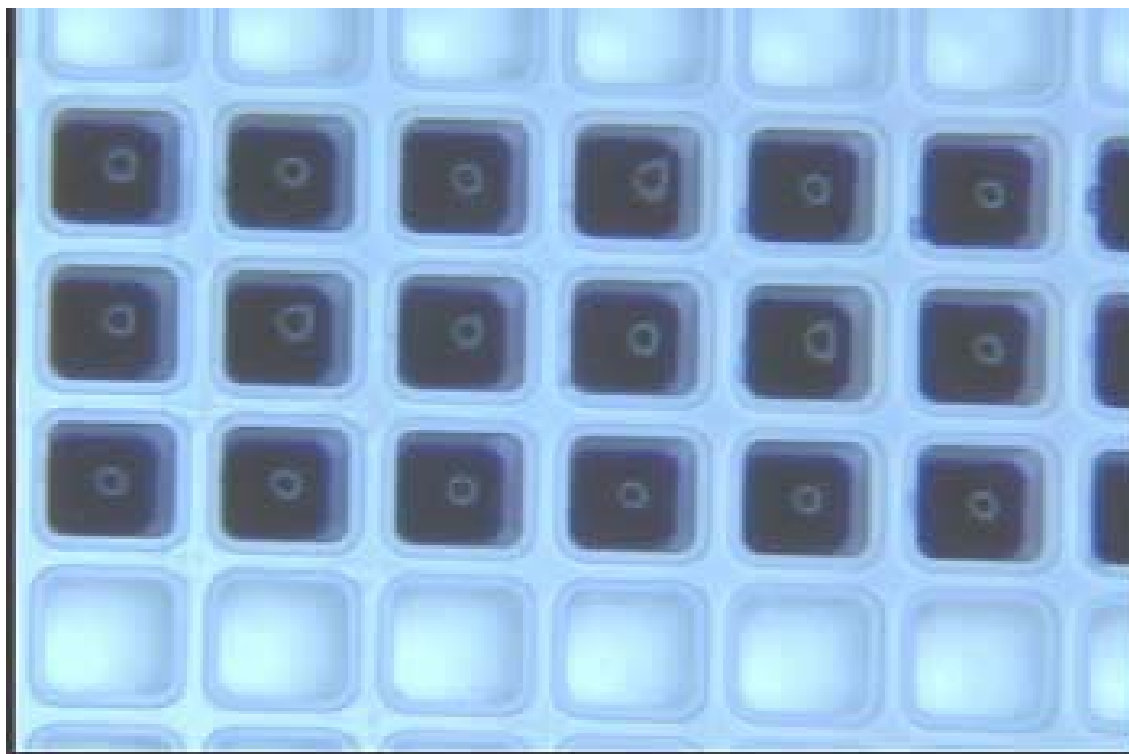


GSK have evaluated (for a very short time) an AFA incorporating instrument that produces acoustic energy in a line

- A column of wells at a time (30 secs for a plate)



Project area: uHTS - 1536 plate mixing video

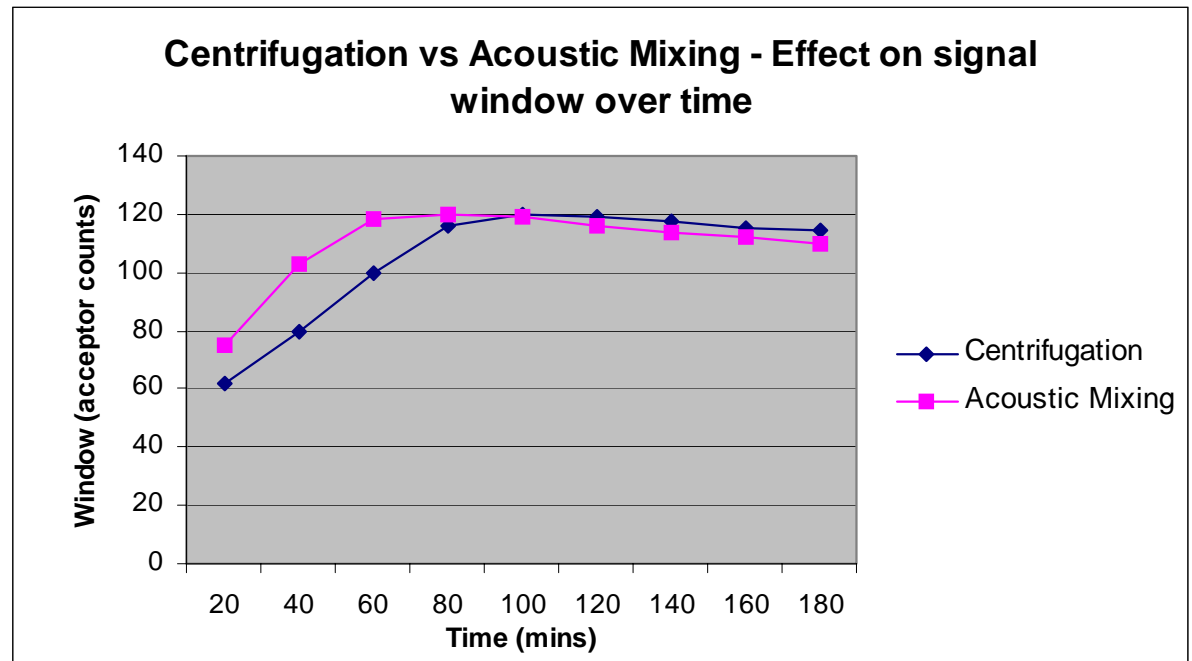


Application areas: uHTS preliminary data

Assay acceleration

Data showing the effect of acoustic mixing a 1536 well HTS assay on signal window Over time.

Note: acoustic mixing accelerates assay

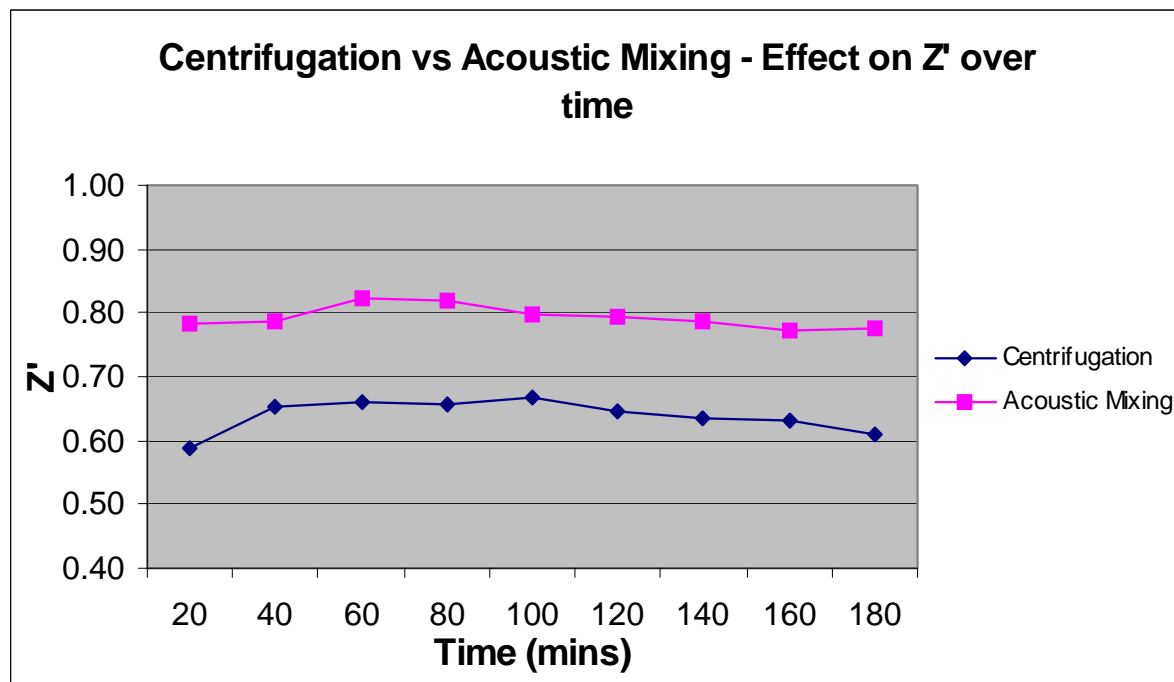


Application areas: uHTS preliminary data

Assay variability

Data showing the effect of acoustic mixing a 1536 well HTS assay on assay variability (Z') over time.

Note: acoustic mixing significantly decreases assay variability



Application areas: uHTS – preliminary conclusion

Positive data:

AFA treatment of an uHTS assay results in a number of beneficial effects

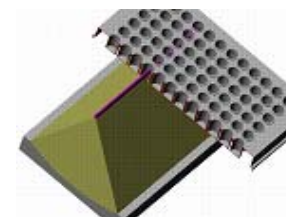
- lower variability
- increased speed
- decreased false positives (data not shown)

Future work required:

- Aid in specification of HTS suitable instrument with Covaris / KBiosciences
- Conduct more assays
- Evaluate effect of AFA treatment on compound solubility of aqueous HTS assay

Further AFA usage in GSK Application areas

- High-throughput screening
- Compound Dissolution and Resolution
- Cell Lysis – Mammalian, Insect, E-coli, plant
- Continuous flow processing
- High Throughput Chemistry
- Drug Metabolism and Pharmacokinetics
- RNA extraction - Homogenisation of biological tissue
- Tissue homogenisation for Proteomic studies



The end & questions

Many thanks to:

Jon Curtis ATG

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Ken Murray CM

Karen Dobbs CM

Todd Graybill HTC

Suzanne Baddeley AD

Liz Clark S&CP

Jim Laugharn Covaris

Phil Robinson KBioscience