

## Introduction

**Zoe Blaxill**  
**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

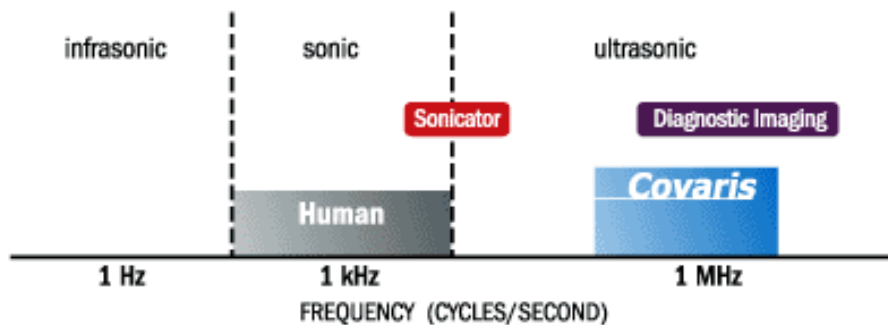
### Enabling technology

#### Adaptive Focused Acoustics (AFA)

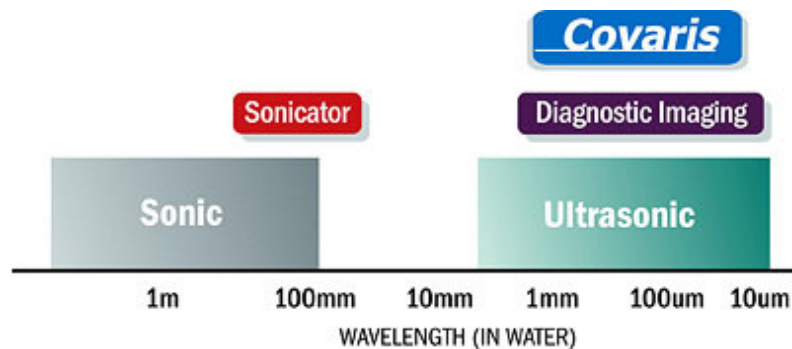
...enables non-contact, isothermal,  
closed vessel mixing

## Technology overview

### Frequency of AFA operation

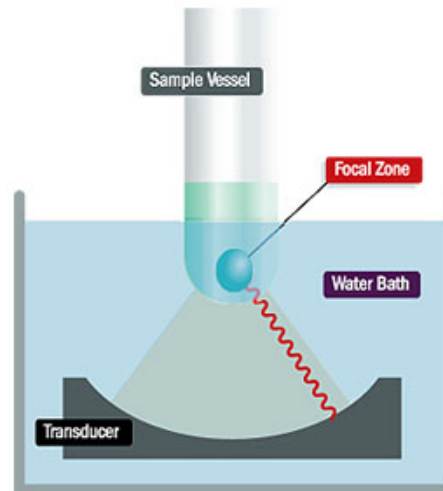


Frequency denotes wavelength



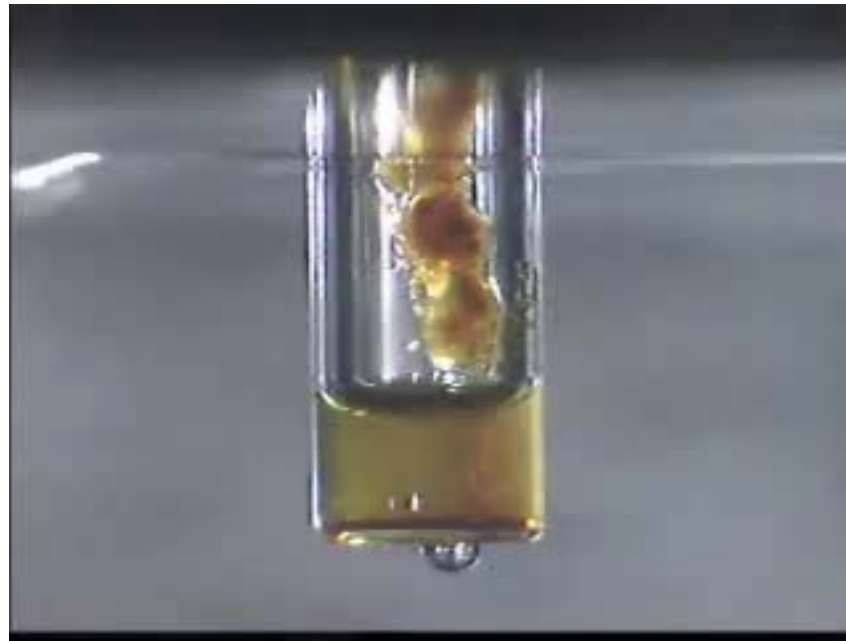
## Technology

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 Oldenburg<sup>1</sup>, Douglas Pooler<sup>1</sup>, Kurt Scudder<sup>1</sup>, Christopher Lipinski<sup>2</sup> and Michele Kelly<sup>2, \*</sup>

<sup>1</sup>MatriCal, Inc., Spokane, WA, <sup>2</sup>Pfizer 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.

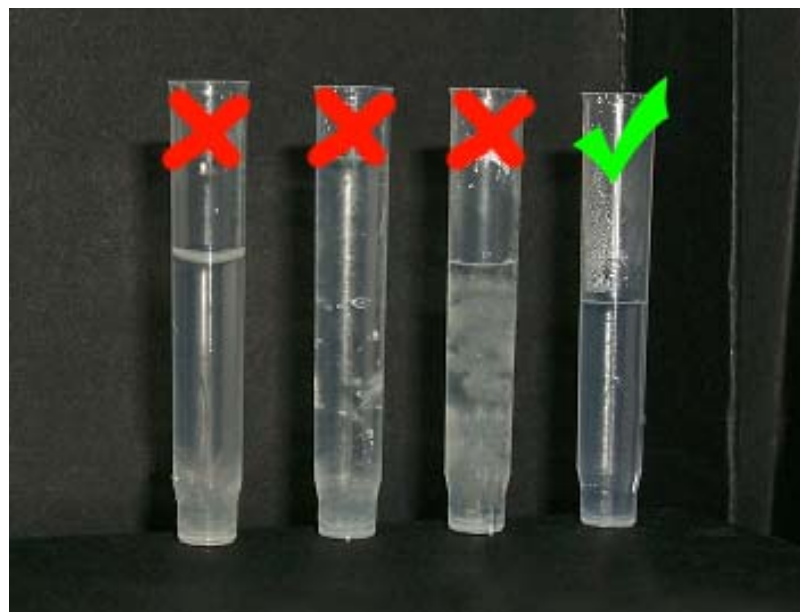
**Keywords:** Sonication, compound solubility, freeze-thaw cycle, water in DMSO, precipitation, solubilization.

## 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 ✓

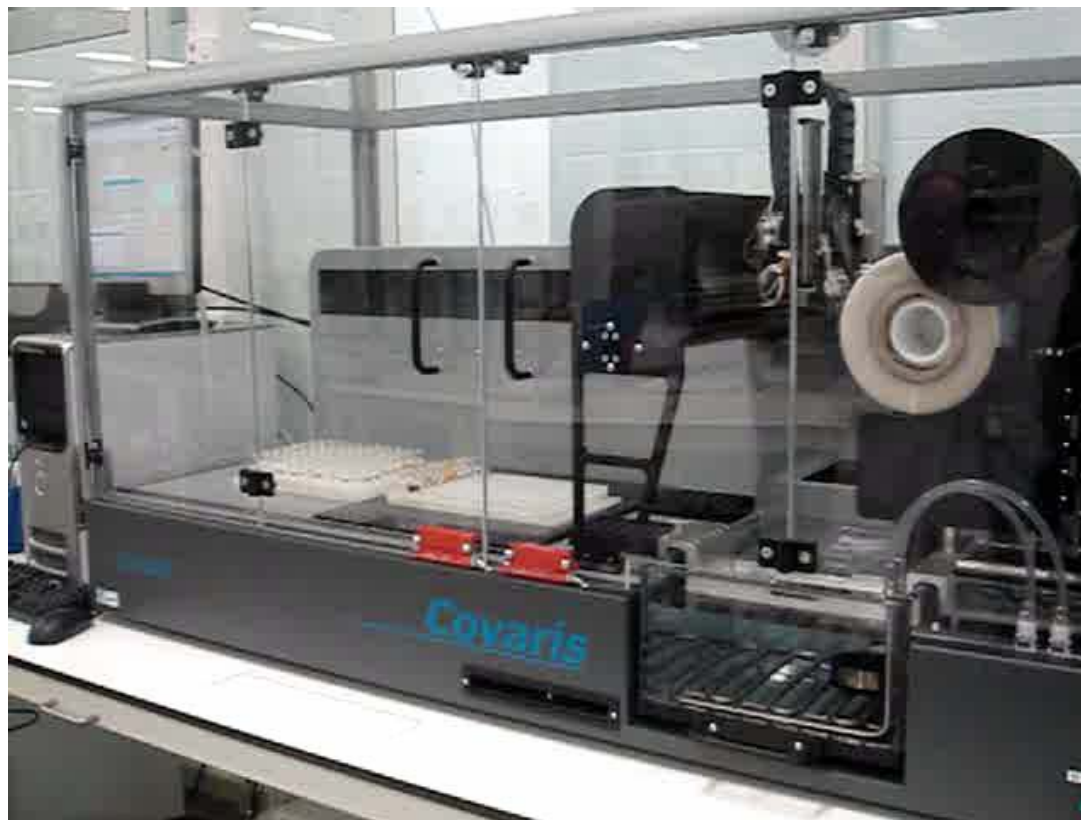
Integratable ✓

Compounds to thermodynamic equilibrium ✓



**Status:**  
Currently installing in US. UK complete

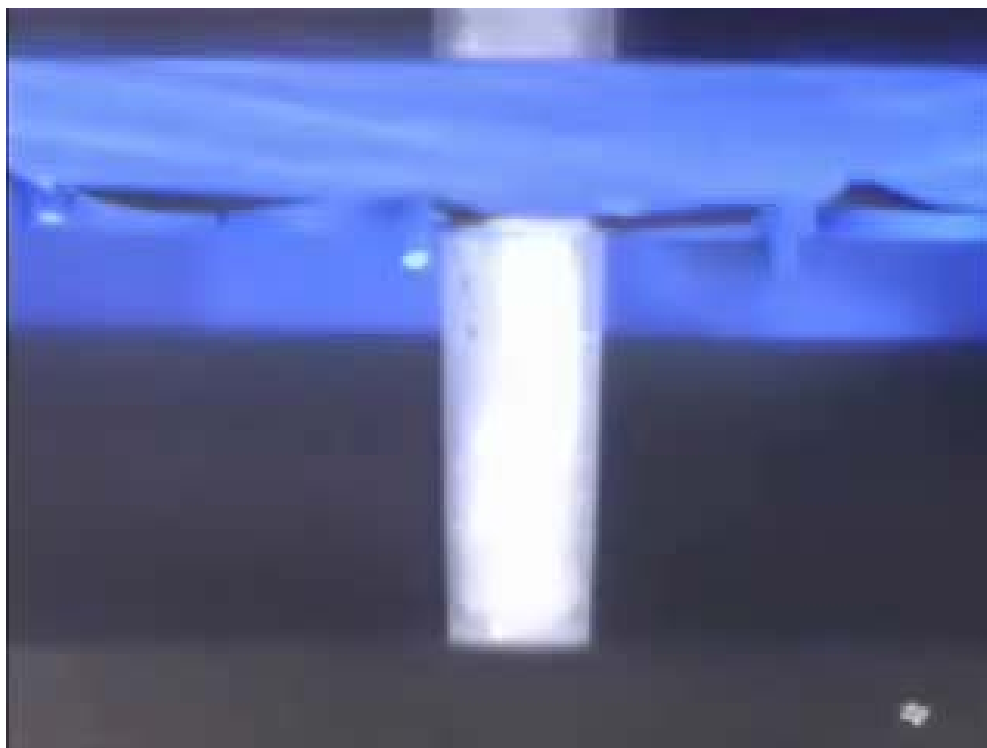
## C2000 automated acoustic dissolution



## 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**

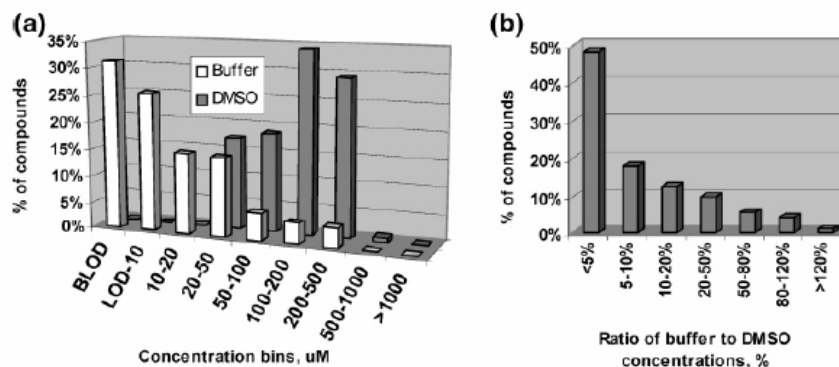
## Amphora paper

*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,<sup>\*,†</sup> Olga Issakova,<sup>†</sup> James D. Arroway,<sup>†</sup> Paul Bernasconi,<sup>†</sup> Min Chen,<sup>†</sup> Louis Coudurier,<sup>†</sup> Scott Galasinski,<sup>†</sup> Ajit P. Jadhav,<sup>†</sup> William P. Janzen,<sup>†</sup> Dennis Lagasca,<sup>†</sup> Darren Liu,<sup>†</sup> Roderic S. Lewis,<sup>†</sup> Robert P. Mohney,<sup>†</sup> Nikolai Sepetov,<sup>†</sup> Darren A. Sparkman,<sup>†</sup> and C. Nicholas Hodge<sup>†</sup>

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 ( $\mu\text{M}$ ). 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

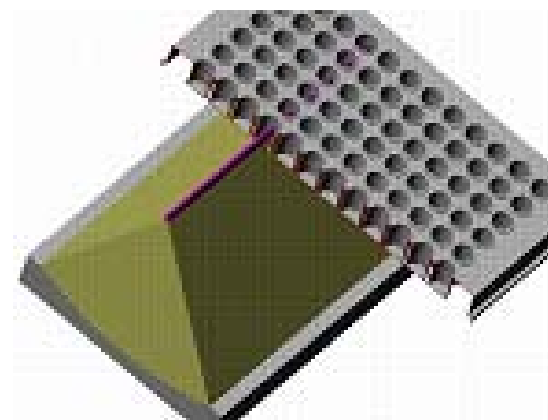
### Preliminary Areas of desired benefit

Improve Z'

Reduce CV

Re-solublising compound in aqueous

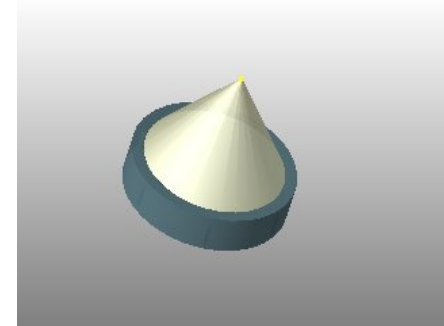
Accelerate assays



## Application areas: AFA in uHTS

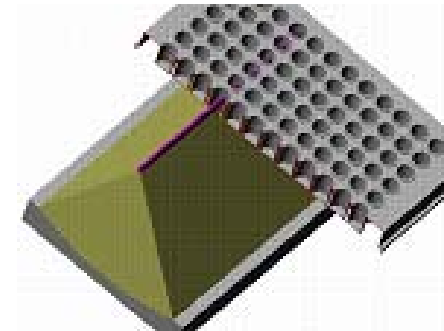
### Technology

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

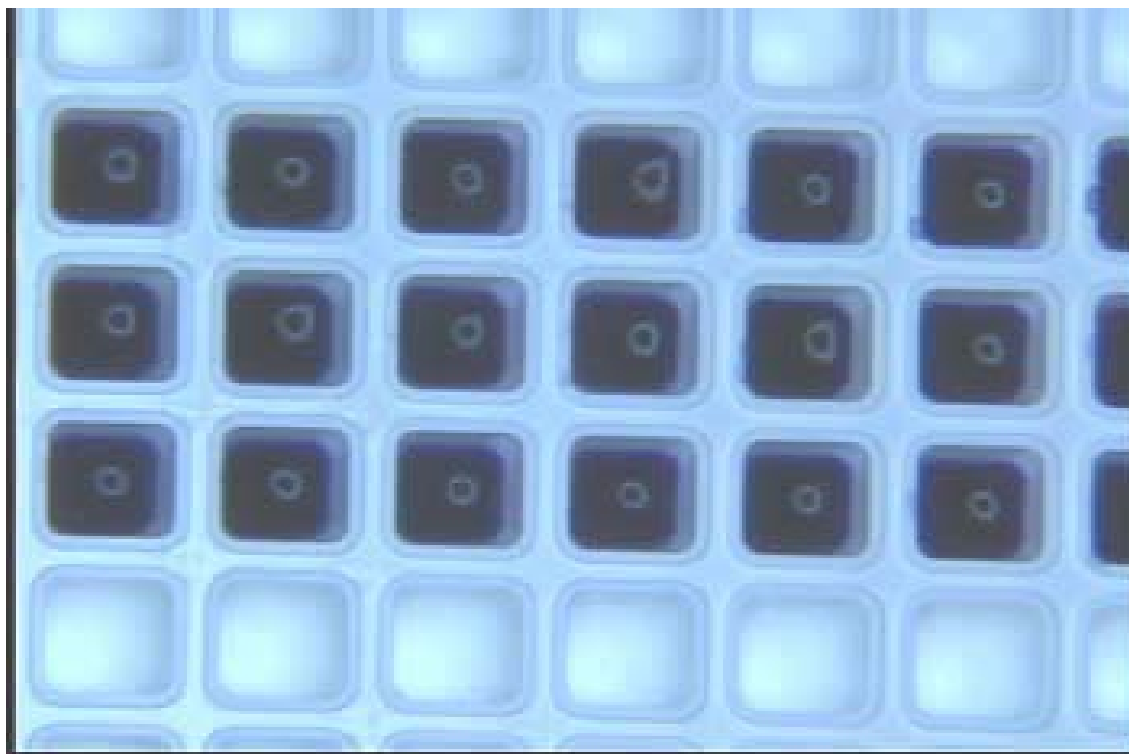


GSK have evaluated (for a very short time) an AFA incorporating instrument that produces acoustic energy in a line

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



**Project area: uHTS - 1536 plate mixing video**

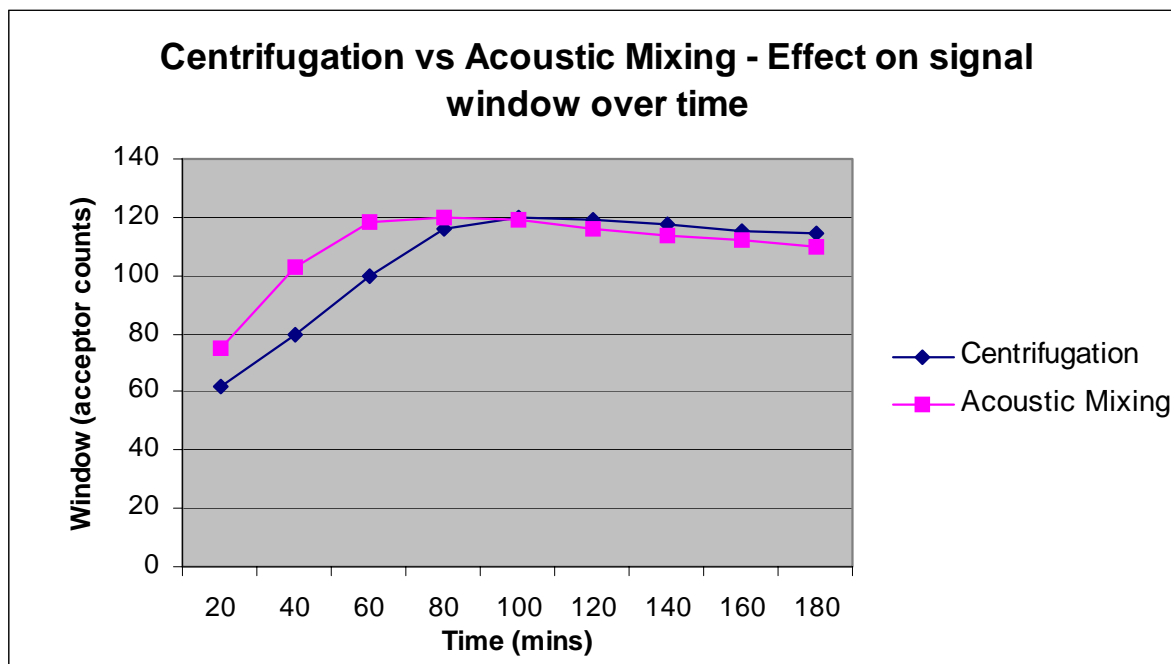


## Application areas: uHTS preliminary data

### Assay acceleration

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

Note: acoustic mixing accelerates assay

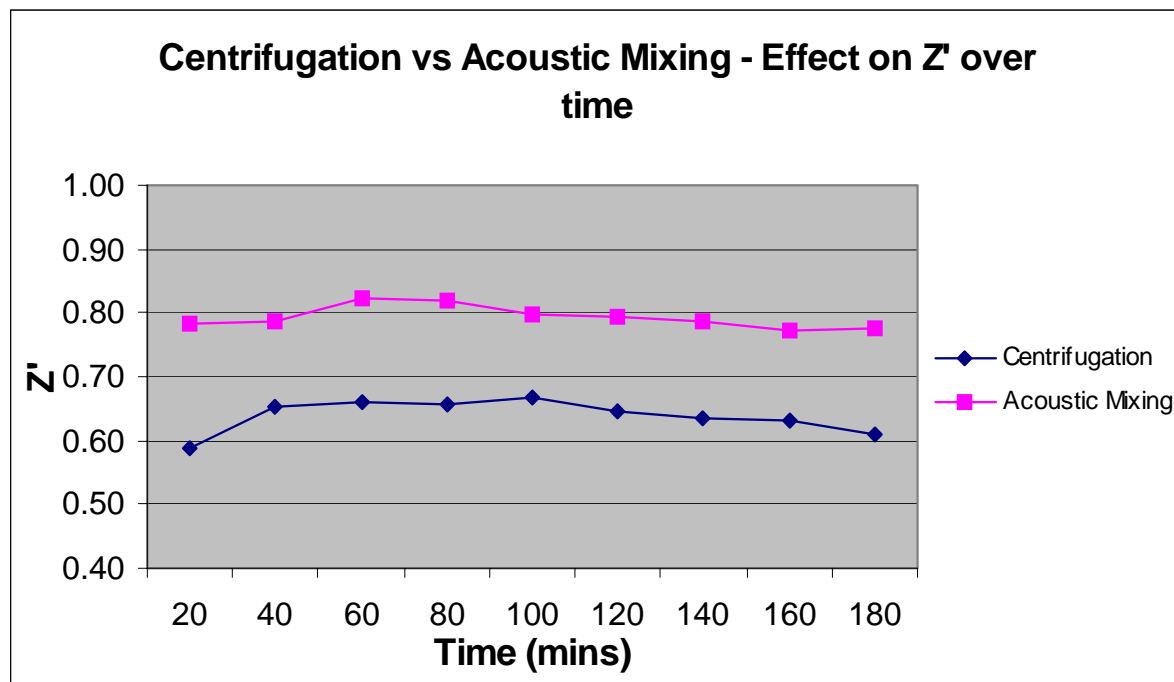


## Application areas: uHTS preliminary data

### 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

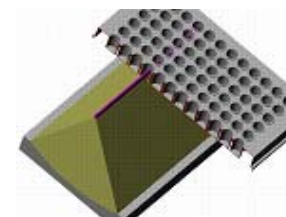
- lower variability
- increased speed
- decreased false positives (data not shown)

### 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**

**Jim Chan CM**

**Ken Murray CM**

**Karen Dobbs CM**

**Todd Graybill HTC**

**Suzanne Baddeley AD**

**Liz Clark S&CP**

**Jim Laugharn Covaris**

**Phil Robinson KBioscience**