Clare, Clark et al. Supplemental

Details of Operetta analysis algorithm

As described in Clare et al. 2014 [1]; the Harmony software (linked to the PerkinElmer Operetta) identifies the texture of the cytoplasmic area (minus the nucleus) on a per cell basis. This texture measurement was calculated using the in-build algorithm 'spot edge ridge' (SER) described by Srinivasan et al. 2008 [2]. Using this measurement a cell infected with *Wolbachia* results in a high texture score compared to a homogenous cytoplasm score of an uninfected cell. Known control samples are used to set a threshold, above which the software labels a cell as infected. From this a 'Percentage of *Wolbachia* infected cells per well' can be calculated. DMSO or inactive compounds will have a high percentage of infected cells compared to a low percentage for active compounds.

Details re: acumen® optical set up

The acumen® Explorer eX3 is a laser scanning cytometer. The system uses dichroic mirrors and optical filters (in much the same way as a flow cytometer) to direct excitation light from a laser source to the sample, then capture emitted light via a photomultiplier tube (PMT) onto a detector. The units used in this screen were equipped with three excitation lasers (405nm, 488nm & 633nm). The filter sets used in the AstraZeneca system can be seen in Supplemental Figure S1. The filter labelled PBSP is the Excitation filter block that sits in front of the laser path. This is a series of 3 dichroic mirrors that allow light above the wavelength noted to pass through and reflect all light below that wavelength at a precise angle.
For excitation and detection of the fluorochromes used in this assay the following light paths are used:

Hoechst

Excited by the 405nm laser (via a 405nm dichroic mirror)**.** Emitted fluorescence detected via a 420-490nm (455/70) bandpass filter, in-line following a 500nm dichroic mirror.

Far Red

Excited by the 633nm laser (via a 633nm dichroic mirror). Emitted light goes through all other dichroic mirrors in series before it gets to the PMT. Fluorescence is detected via a 655-800 (655 Long Pass) filter, in-line following a mirror that reflects all remaining light toward the PMT.

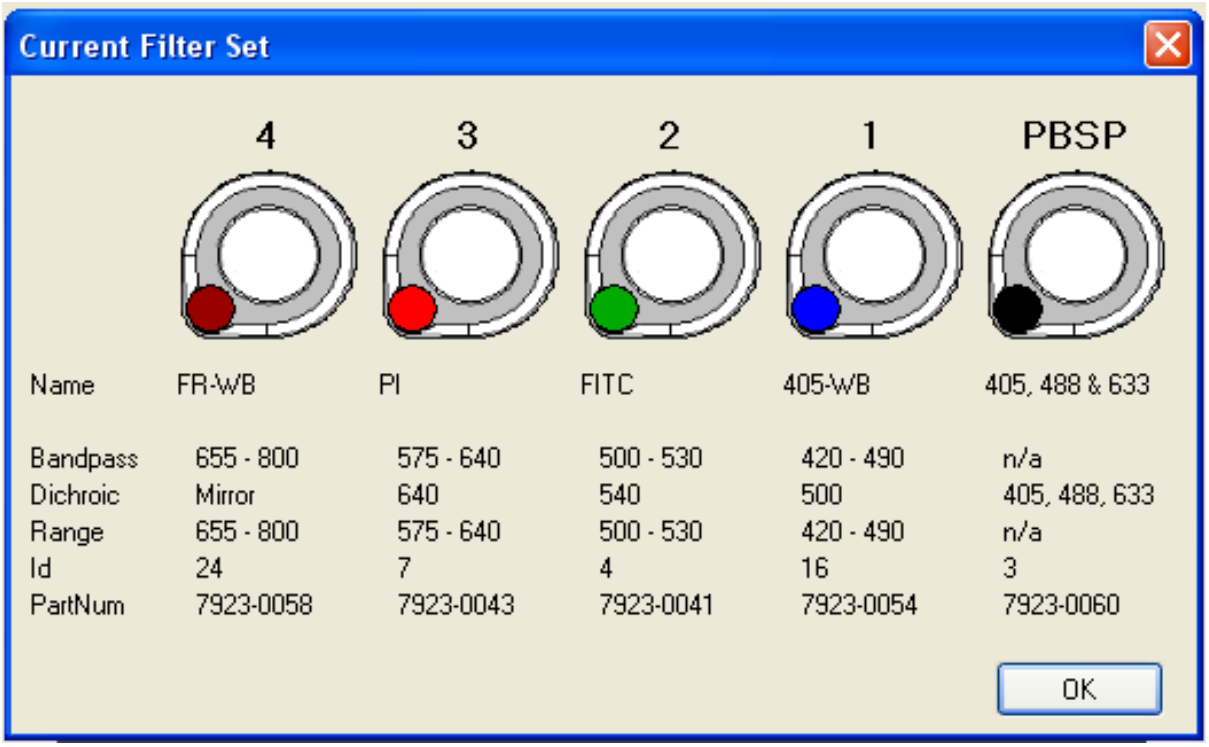


Figure S1. Filter set in the acumen® Explorer eX3 units used in the screen. Full filter set is shown even though the final screening protocol used a single channel detection on this platform (655 – 800nm) with Hoechst detection performed on the EnVision™ reader.

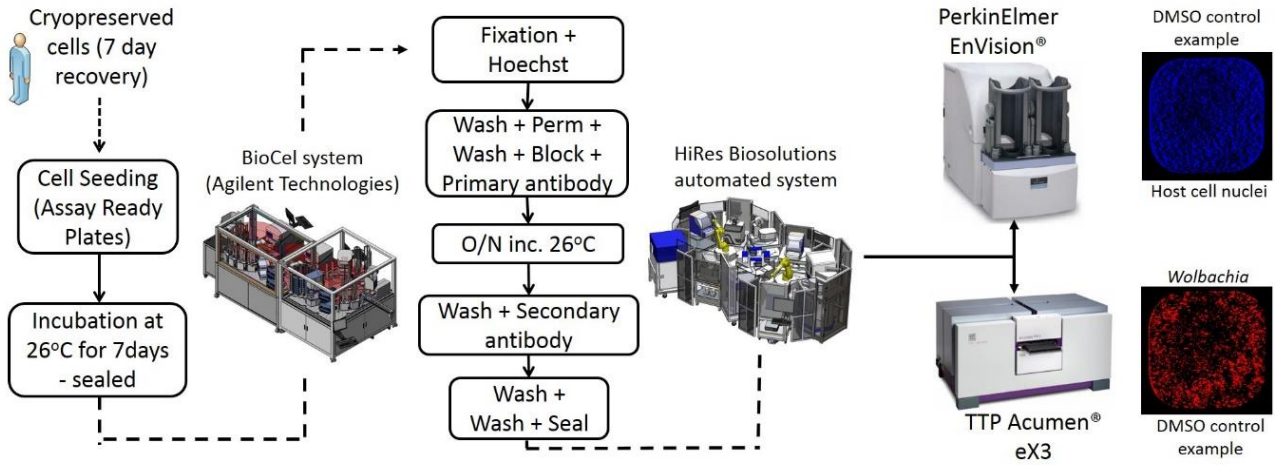
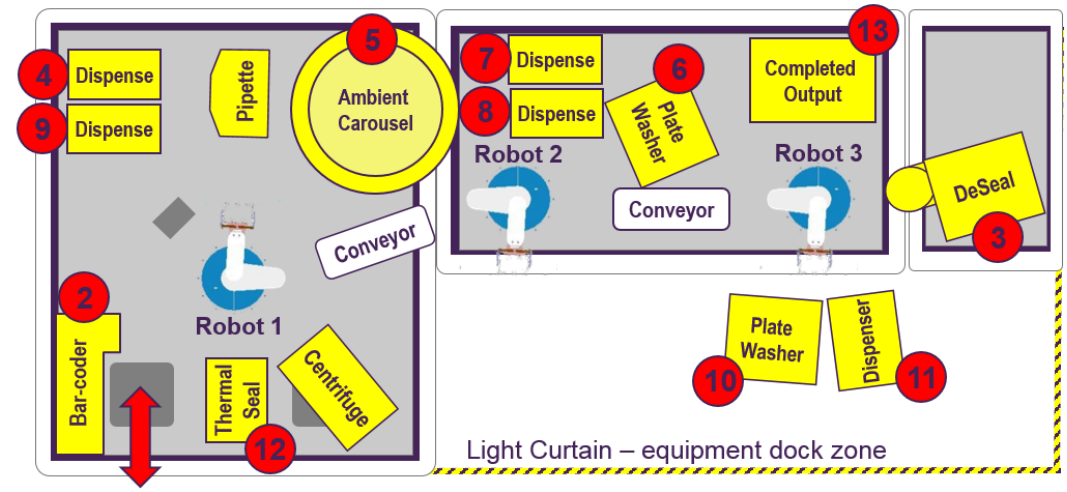


Figure S2. The full 3 week protocol for a high throughput industrial scale screen to identify anti-*Wolbachia* active compounds. The schematic demonstrates cell recovery for 1 week, followed by cell plating into 'assay ready' microtitre plates which are then incubated through week 2. In the final week plates are processed through a fully automated protocol on the Agilent BioCel platform to allow for fixation, Hoechst staining of the host cell nuclei (toxicity read) and *Wolbachia* specific antibody staining. Once stained the plates are moved onto a second automation platform (HighRes Biosolutions MicroStar) to read each plate for host cell toxicity (EnVision™) and anti-*Wolbachia* activity (acumen®).



1 Incubator 26°C Under-deck plate storage

Agilent BioCel

Day 1 – Fix Cells & Primary Antibody

- Cell plate input from 26°C incubator (1)
- De-Lid
- Get barcode information (2)
- De-seal (3)
- Dispense 20µl Formaldehyde (4)
- Re-Lid
- 20min Fixation in carousel (5)
- Throw away lid
- Aspirate at Washer (6)
- Dispense 80µl PBS (7)
- Aspirate at Washer (6)
- Dispense 80µl PBS-Triton X (8)
- 30min Permeabilise in carousel (5)
- Aspirate at Washer (6)
- Dispense 80µl PBS (7)
- Aspirate at Washer (6)
- Dispense 80µl PBS-BSA block (9)
- 40min Incubate in carousel (5)
- Aspirate at Washer (10)
- Dispense 30µl Primary Antibody (11)
- Thermal seal (12)
- 12 hour within 26°C incubator (1)

Day 2 – Secondary Antibody

- Cell plate input from 26°C incubator (1)
- De-seal (3)
- Aspirate at Washer (10)
- Dispense 80µl PBS polysorbate (8)
- Aspirate at Washer (10)
- Dispense 30µl Secondary Antibody (9)
- Add black plate lid
- 60min Incubate in carousel (5)
- Dismount black lid
- Aspirate at Washer (6)
- Dispense 80µl PBS polysorbate (11)
- Incubate locally for 6 min
- Aspirate at Washer (10)
- Dispense 80µl PBS polysorbate (8)
- Aspirate at Washer (10)
- Dispense 40µl PBS (7)
- Thermal seal (12)
- Output completed plate for Read (13)

Figure S3. Schematic diagram of the Agilent BioCel 1800 automation platform used for the assay build in the screen. The sequential steps delivered by the platform in order to run the screen are described.

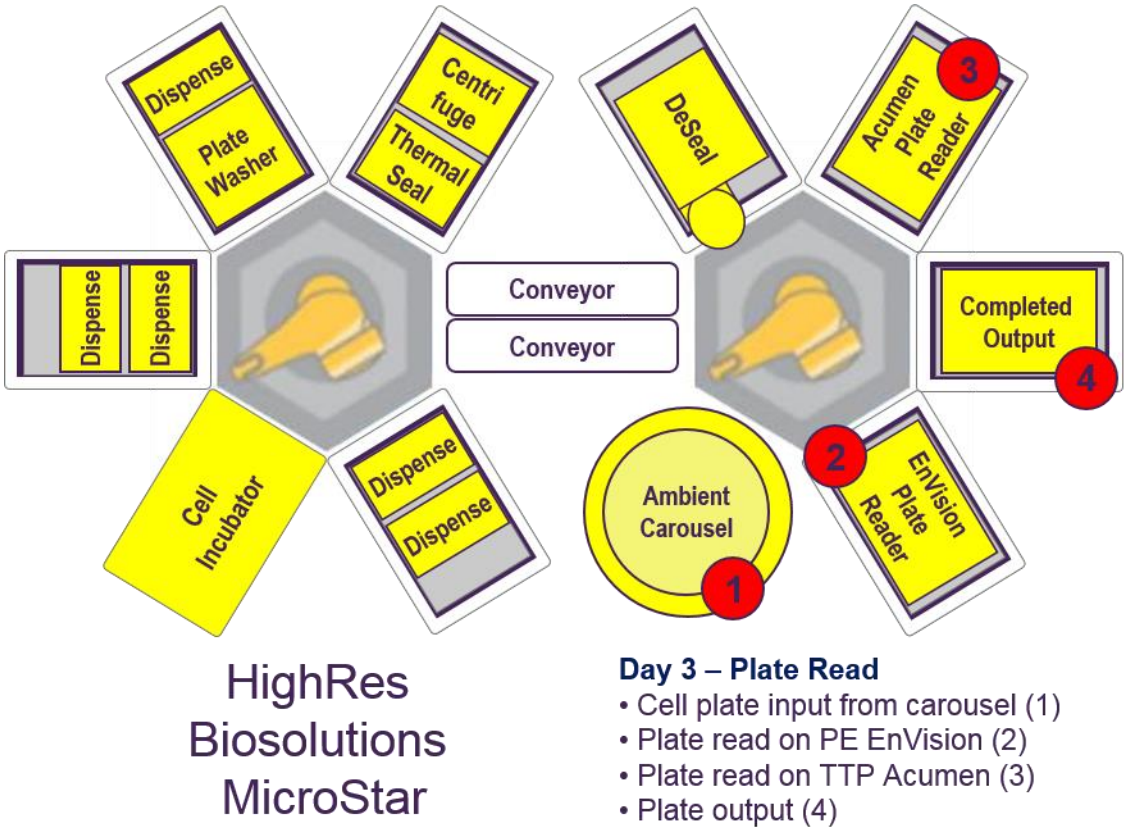


Figure S4. Schematic diagram of the HighRes Biosolutions MicroStar automation platform used for the assay read steps (both acumen® & EnVision™) following fixation.

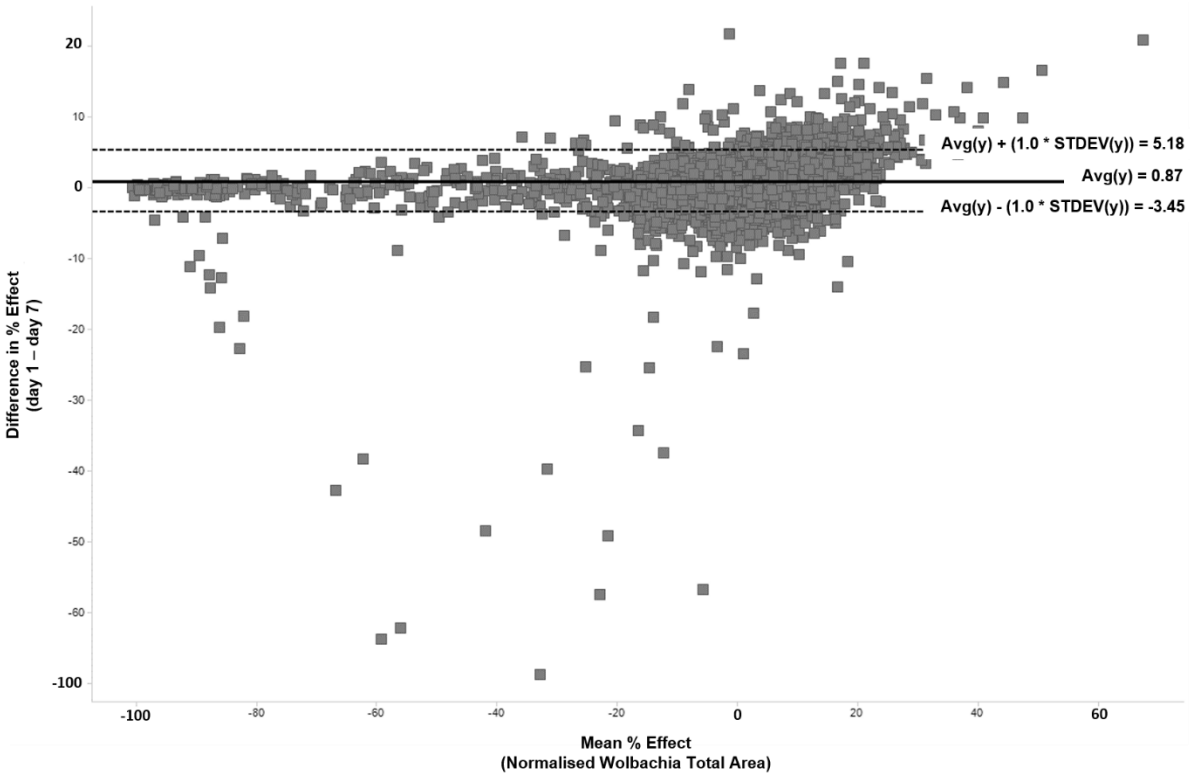


Figure S5. Bland-Altman plot representing the variance in anti-*Wolbachia* activity between plates acquired on the acumen® immediately following fixing/staining and the same plates stored at 4ºC for 7 days before re-acquiring on the acumen®. The average difference in % effect between paired results lies very close to 0 (0.87) and 1*standard deviation at 4-5%.

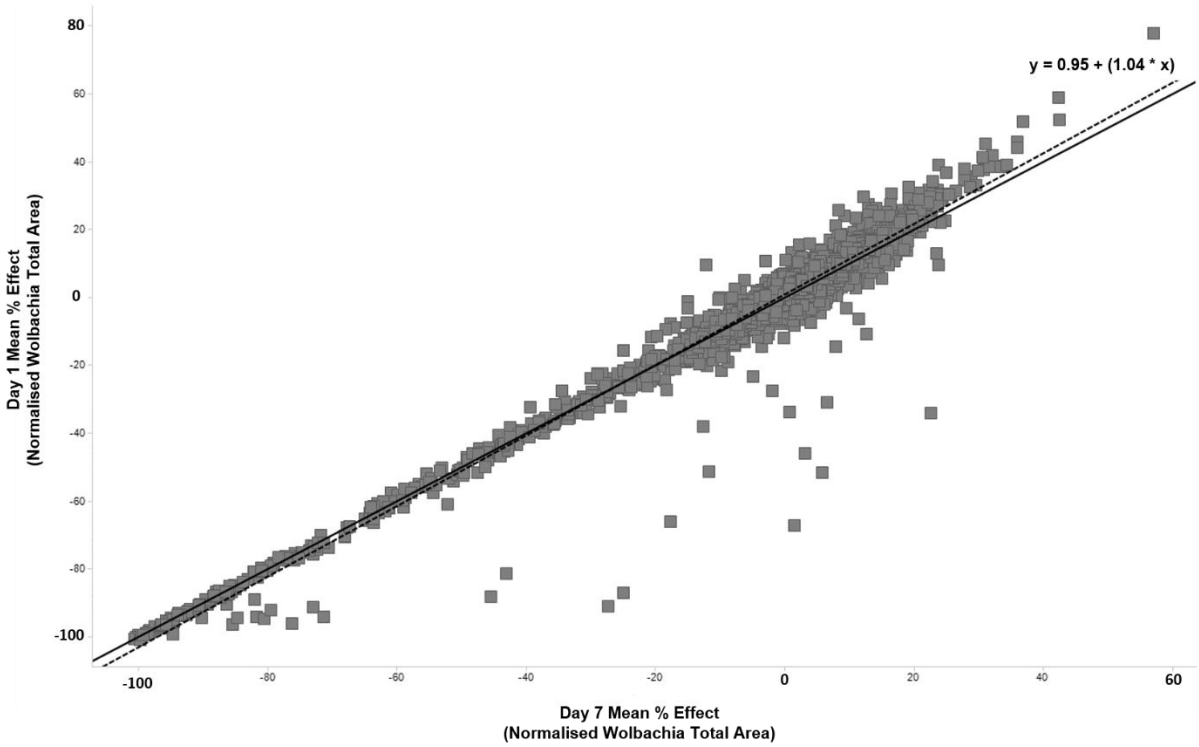


Figure S6. A different representation of the same data shown in Supplemental Figure S5. Agreement plot of anti-*Wolbachia* activity between plates acquired on the acumen® immediately following fixing/staining and the same plates stored at 4^{o}C for 7 days before re-acquiring on the acumen®. The 1:1 line is shown in solid black, with the straight-fit line through the data shown in dotted black.

References

1. Clare, R. H., Cook, D. A., Johnston, K. L., Ford, L., Ward, S. A., Taylor, M. J. Development and validation of a high-throughput anti-*Wolbachia* whole-cell screen: a route to macrofilaricidal drugs against onchocerciasis and lymphatic filariasis. *Journal of Biomolecular Screening*. **2014**, *20(1)*, 64-69.

2. Srinivasan, G. and Shobha, G. Statistical texture analysis. *Proceedings of World Academy of Science, Engineering and Technology.* **2008**, *36*, 1264–1269.