| Live Light Sheet ' | 'Do Not | Walk Away | Until" | Checklist |
|--------------------|---------|-----------|--------|-----------|
|                    |         |           |        |           |

Date: \_\_\_\_\_

|   |  |  | <br> |
|---|--|--|------|
| incubation instrument has sufficient coolant level                                    |  |  |      |
| correct objectives install and RI set correctly                                       |  |  |      |
| sample tank sanitized, is dry on top surface  |  |  |      |
| incubation is running at 37C and 5% CO2   |  |  |      |
| incubation connections not leaking / o-ring problem                                   |  |  |      |
| medium level in sample tank is good, with no apparent leaks                           |  |  |      |
| stopcocks in correct position   |  |  |      |
| parafilm placed to support piston rod   |  |  |      |
| supplemental ddH2O setup is adequate with correct levels                              |  |  |      |
| supplemental ddH2O is primed and running  |  |  |      |
| tank cover in place to prevent dehydration  |  |  |      |
| lightsheets in good alignment   |  |  |      |
| objective zoom is correct   |  |  |      |
| laser power setting is appropriate for the stage and experimental plan                |  |  |      |
| embryo position and view angles are good, with ROI near center of each image stack    |  |  |      |
| multiview set-ups established correctly and in proper order                           |  |  |      |
| time lapse imaging set-up at 6 minute intervals with sufficient time points requested |  |  |      |
| sufficient hard drive space on destination  |  |  |      |
| → imaging go live: first timepoint capturing correctly                                |  |  |      |
| second timepoint appears to be capturing correctly                                    |  |  |      |
| script positioning appears to be working  |  |  |      |
| signs posted indicating hazardous chemicals, do not move, and on workstation          |  |  |      |
|   |  |  |      |