1. Procedure summary

This procedure outlines the proper methods for operating and maintaining compound microscopes while performing microscope observations on culture samples.

1.1. Related Procedures

Microscope observations on algae cultures

1.2. Procedure impacts and concerns

Safety	Gloves and safety glasses should be worn when handling pond or other biological samples that may contain unknown organisms.
Quality	Ensure that samples are handled in accordance with sample handling.
Delivery	The dynamics of a culture sample can change drastically throughout the day. Samples should be analyzed within a consistent timeframe each time microscope observations are performed.
Environmental	All glass slides and cover glass should be disposed properly. Slides/coverslips should be placed in glass/sharps waste, but are considered non-hazardous.
Cost	A minimum of $800 will be required for yearly maintenance on two microscopes. The following supplies are recommended for use:
Compliance	Compliance with Sapphire Energy, Inc. Chemical Hygiene Plan and IPM policy is required. See 29 CFR 1910.120 and 1200. An authorized user list, MSDS's and label information will be available for easy reference in a binder in the administration building.

Glass Slides	Fisher	12-544-1
Cover Glass	Fisher	12-541-B
Immersion Oil	Fisher	12-070-396
Lens Paper	Fisher	11-995
Lens Cleaner	Fisher	22-143-97

1.3. Responsibilities and owners

Document Owner	Manage content and distribution	Salvador Lopez
Process Owner	Responsible for content and process validation	Phil Lee
Site Manager	Responsible for implementation and conformance	Craig Behnke

2. Process

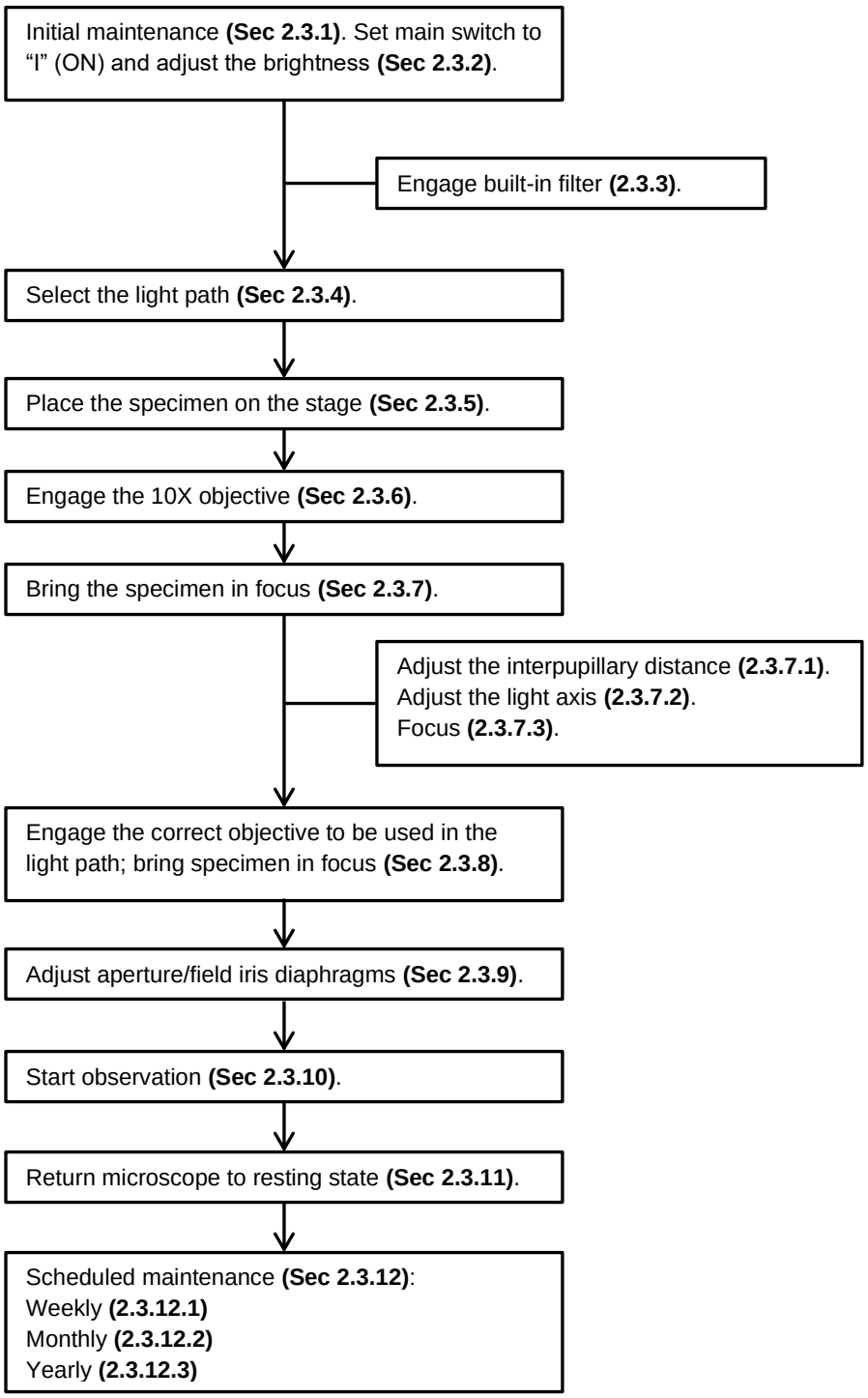
2.1. Process description

This procedure document will detail how to prepare a compound microscope for observations and describes proper maintenance of the microscope concluding use.

2.1.1. Restrictions

Processes that refer to an instruction manual and any maintenance should only be performed if operator has been trained by the following authorized individuals: **San Diego:** Andrea Yoshioka and Eric Becker, **Las Cruces:** Salvador Lopez and Micheal Burnett, **Columbus:** Kari Mikkelson and Rebecca White.

2.2. Process diagram: Work Instruction



See Appendix Fig. 1

Main switch
Brightness adjustment knob

Built-in filter knobs

Light path selector knob

Slide holder
X-/Y-axis knobs

Revolving nosepiece

Coarse/fine adjustment knobs

Binocular tube
Condenser height adjustment knob
Condenser centering knob

Revolving nosepiece

Aperture iris diaphragm ring
Field iris diaphragm ring

2.3. Process steps

2.3.1. Perform Initial Microscope Maintenance

2.3.1.1. Equip yourself with proper PPE as noted in section 1.2 under "Safety".

2.3.1.2. Remove entirely the dust cover from the microscope and store in appropriate location.

2.3.1.3. Ensure all equipment is available. This includes glass slides, cover glass, micropipettors, micropipette tips, waste containers for glass and plastic waste, and immersion oil and lens paper if needed.

2.3.1.4. Ensure microscope is in proper resting state.

2.3.2. Power on the Microscope and Adjust Brightness

2.3.2.1. Set the main switch to "I" (ON position).

2.3.2.2. Turn the brightness adjustment knob clockwise to adjust the voltage as appropriate for your use.

2.3.3. Engage Built-in Filter

2.3.3.1. If desired, engage a filter knob (see Figure 1) into the light path by pressing the knob in. Pressing the button again disengages the filter from the light path.

Note 2.3.3.1. While the BX51 and BX53 microscopes have the following filter configurations, the Axio.A1 in San Diego may have a different configuration.

Filter Type	Purpose
ND6	Neutral Density filter for light adjustment, transmittance 16.67%
ND25	Neutral Density filter for light adjustment, transmittance 4.0%
LBD	Color balancing, daylight filter

2.3.4. Select the Light Path

2.3.4.1. Ensure the light path selector knob (see Figure 1) is pulled to the desired position so that the light path is directed to both the camera and the eyepiece.

2.3.4.2..

Light Path Selector	Intensity Ratio	Applications
Pushed in	100% for binocular eyepieces	Observation of dark specimens
Middle position	20% for binocular eyepieces, 80% for camera	Observation of bright specimens
Pulled out	100% for camera	Photography, TV

2.3.5. Place Specimen on the Stage

2.3.5.1. Turn the coarse adjustment knob (see Figure 1) to lower the stage.

2.3.5.2. Open the spring-loaded curved finger on the slide holder and place the specimen slide on the stage.

2.3.5.3. Gently release the curved finger.

2.3.5.4. Roughly position the edge of the coverslip or liquid droplet under the objective using the x- and y-axis knobs.

2.3.6. Engage the 10X Objective

2.3.6.1. Rotate the revolving nosepiece (see Figure 1) until the 10X objective is selected.

2.3.7. Select Brightfield or Phase Contrast Microscopy

2.3.7.1. If brightfield microscopy is desired, ensure that phase plate ring is turned to "O" setting (it's not labeled on Figure 1).

2.3.7.2. If phase contrast microscopy is desired, ensure that phase plate ring is turned to 'Ph1' setting.

Note 2.3.7.2. Phase plates and objectives are matched – if you aren't sure which phase plate to use, look at the indicator on the objective

2.3.8. Bring the Specimen into Focus

2.3.8.1. While looking through the eyepiece, adjust for interpupillary distance (see Figure 1) until the left and right fields of view coincide completely.

2.3.8.2. Adjust the light axis by centering the condenser as specified in your microscope's instruction manual.

2.3.8.3. Slowly rotate the coarse adjustment knob (see Figure 1) to bring the edge of the coverslip or liquid droplet into partial focus. Use the x- and y-axis knobs to ensure that this is in your field of view.

2.3.8.4. Slowly rotate (minor adjustments) the fine adjustment knob (see Figure 1) to bring the specimen into complete focus.

2.3.8.5. Use the x- and y- axis knobs to move field of view onto your specimen, and continue using the fine focus adjustment knob to maintain focus on your specimen.

2.3.9. Select Observation Objective and Focus Specimen

2.3.9.1. Rotate the revolving nosepiece (see Figure 1) until the 20X or 40X objective is selected, as desired.

2.3.9.2. Slowly rotate the coarse adjustment knob (see Figure 1) to bring the specimen into partial focus.

2.3.9.3. Slowly rotate (minor adjustments) the fine adjustment knob (see Figure 1) to bring the specimen into complete focus.

2.3.9.4. If use of the 100x lens is required, rotate the nosepiece to the side of the 100x lens, and place a small droplet of immersion oil directly in the center of the field of view. The droplet need be no larger than ~3 mm wide and ~1 mm high. Rotate the nosepiece to bring the 100x lens tip into the oil drop, and ensure immersion of lens tip.

Note 2.3.9.4. Use of excessive amounts of immersion oil is likely to require frequent cleaning and service of microscope, and must be avoided.

2.3.9.5. Adjust light intensity and filter engagement as appropriate for your selected objective, specimen, and use.

2.3.9.6. If you cannot achieve desired focus and image quality with your specimen, consult an authorized individual.

Note 2.3.9.4. Once you have used the 100x objective, you may not view this slide again with any lower magnification objectives.

2.3.10. Observe Specimen

2.3.10.1. Rotate the x-axis and y-axis knobs to observe the specimen.

2.3.10.2. If using the 100x lens, your ability to move around the sample is limited by the size of your oil droplet. However, it is not appropriate to add more oil in order to observe the entire slide with the 100x lens.

2.3.10.3. If use of another objective magnification is required, return to 2.3.9.

2.3.10.4. If switching between brightfield and phase contract microscopy return to 2.3.7 followed by 2.3.9.

2.3.11. Return Microscope to Resting State

2.3.11.1. Set the main switch to "O" (OFF position).

2.3.11.2. Lower the stage 2-4 mm using the coarse adjustment knob (see Figure 1).

2.3.11.3. Rotate the nosepiece to an empty slot or to the lowest available objective (see Figure 1).

2.3.11.4. Remove and dispose glass slide properly.

2.3.11.5. Replace dust cover on microscope.

2.3.11.6. If you have caused any immersion oil to be on objectives other than the 100x, notify an authorized individual to get this cleaned up before it dries.

2.3.11.7. If anything did not seem right with the microscope, or you were unable to achieve quality images, notify an authorized individual.

2.3.12. Maintenance

2.3.12.1. Daily

2.3.12.1.1. Never use lens paper on a dry lens. Never touch the lens of an objective with anything other than lens paper (i.e. no Kimwipes). Never use your fingertip to rub lens paper on the lens of an objective.

2.3.12.1.2. If oil immersion was used with the 100x objective, slide an individual sheet of lens paper across the lens of the objective to remove excess oil. Slide an individual sheet of lens paper across the lens of any other objective that was used.

Note 2.3.12.1.2. The frequency of objective lens cleaning should be determined by how frequently the microscope is used. Daily cleaning should be performed with frequent use, whereas periodic cleaning should be performed with sporadic use.

2.3.12.1.3. Wipe down stage and adjustment knobs with Kimwipes.

2.3.12.2. Monthly, or more often as needed – To be performed only by authorized individuals

2.3.12.2.1. Remove and thoroughly clean objectives as indicated in your microscope's instruction manual. Use of cotton swab applicators might be preferred to lens paper for this cleaning.

2.3.12.2.2. Align light source, condenser height, diaphragm, and aperture for appropriate normal microscope use.

2.3.12.2.3. Clean diaphragm, condenser lens, and eye pieces as indicated in your microscope's instruction manual.

2.3.12.3. Yearly

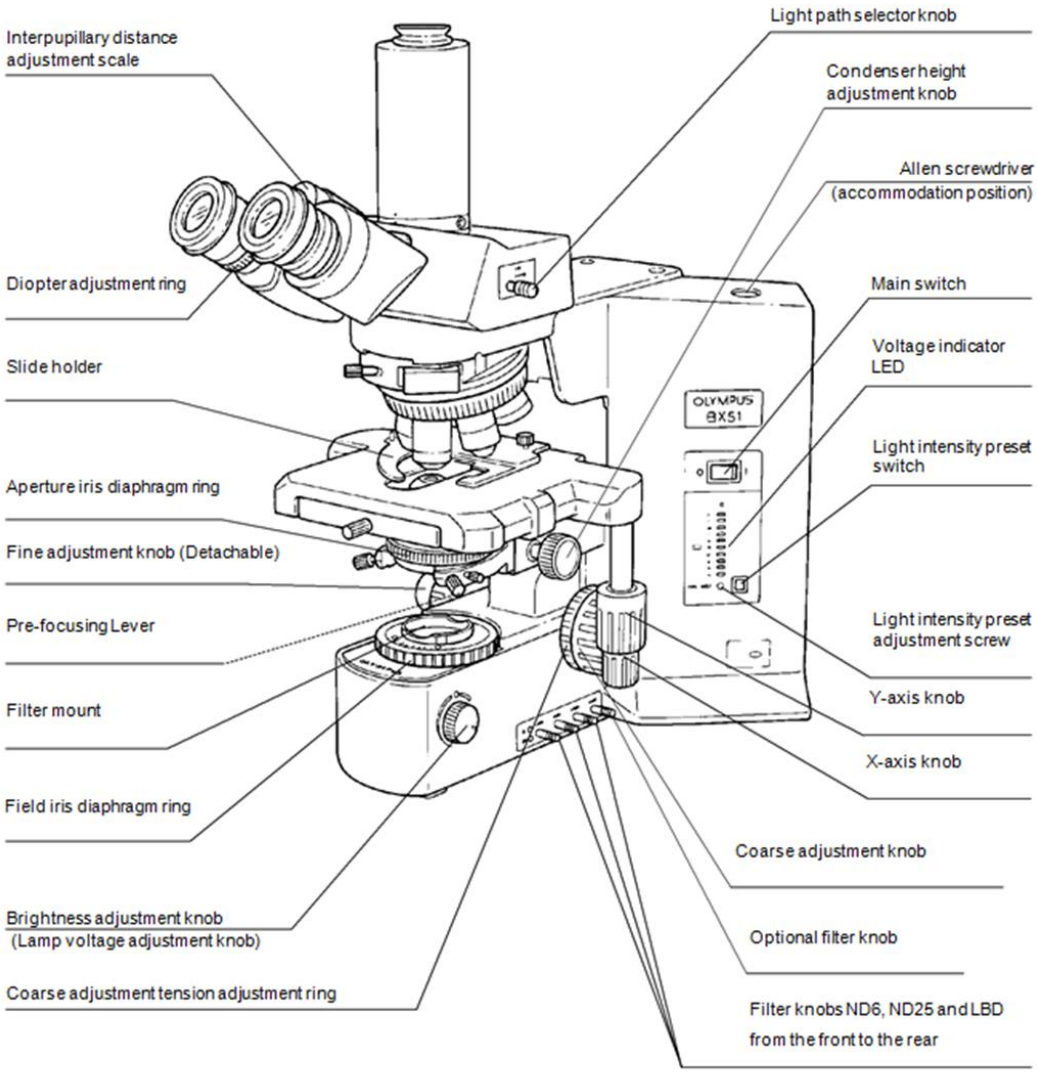
2.3.12.3.1. A certified microscope technician should service all microscopes once a year.

2.3.13. Troubleshooting

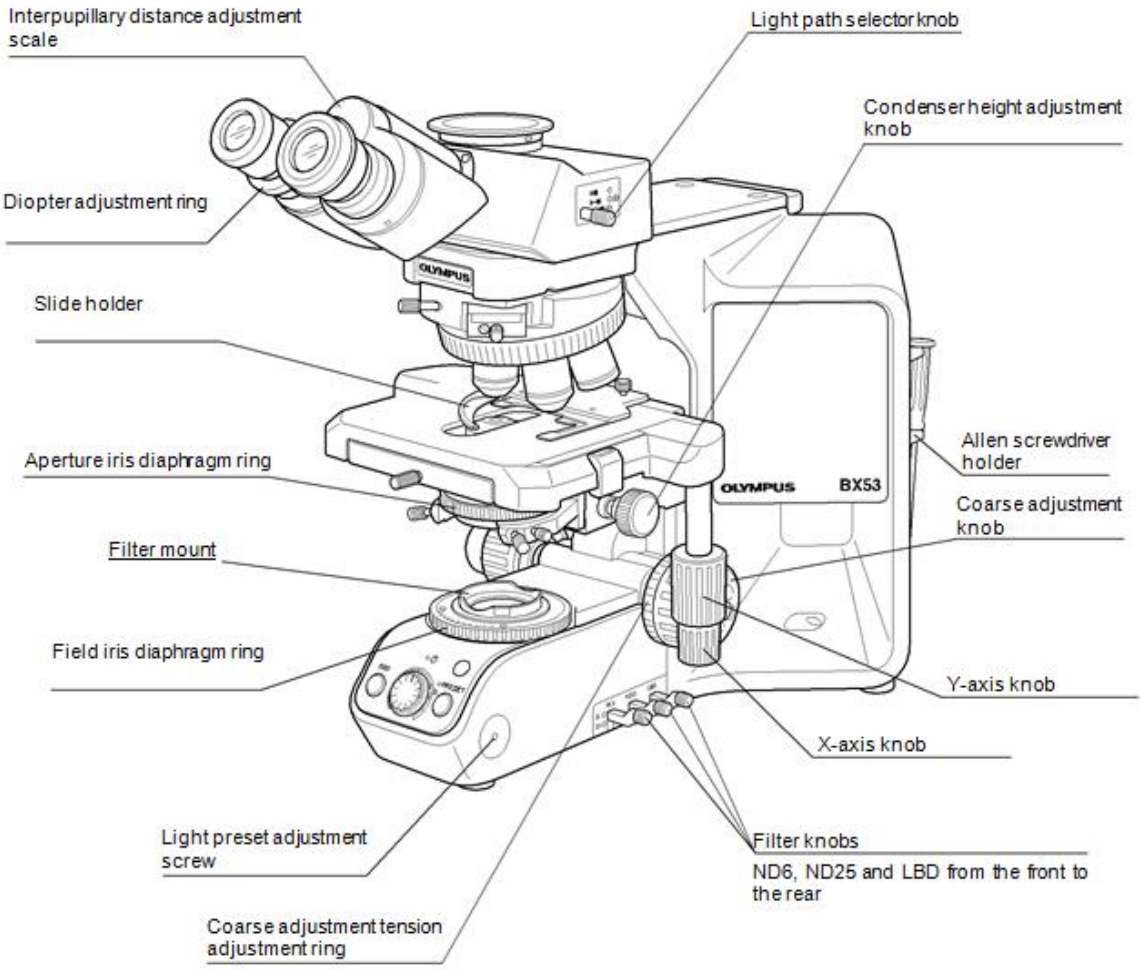
2.3.13.1. Refer to your microscopes instruction manual for a troubleshooting guide.

3. Appendix

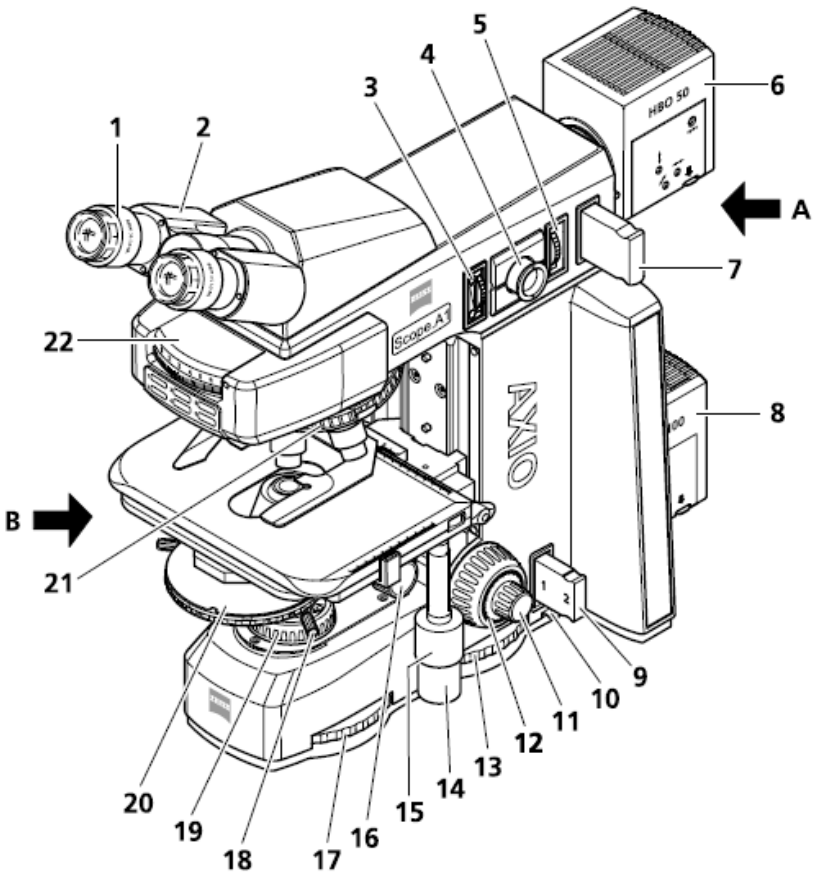
3.1. Olympus BX51 Microscope Diagram



3.2. Olympus BX53 Microscope Diagram



3.3. Carl Zeiss Axio Scope.A1 Microscope Diagram



1 Eyepieces
2 Binocular tube part
3 Field diaphragm (non removable or as slider)
4 Adjustment aid (on upper stand part FL/HBO only)
5 Aperture diaphragm (non-removable or as slider) or FL attenuator
6 Reflected light lamp
7 Filter slider reflected light
8 Transmitted light lamp
9 Filter slider transmitted light
10 Gear shift for diffusion disk (for lower stand part with HAL 100 illumination only)
11 Focusing drive – fine adjustment (both ways)
12 Focusing drive – rough adjustment (both ways)
13 Light intensity control (operable from both sides)
14 Gear knob for adjusting the mechanical stage in direction X
15 Gear knob for adjusting the mechanical stage in direction Y
16 Gear knob for adjusting the condenser vertically (both sides)
17 Filter wheel six fold (operable from both sides, not available for lower stand part for LED illumination)
18 Centering screw for condenser (both sides)
19 Field diaphragm (not available for lower stand part for LED illumination)
20 Condenser with aperture diaphragm (with optional modulator disk)
21 Nosepiece
22 Reflector insert (changeable)
23 Gear shift for diffusion disk reflected light (not available on all upper stand parts)
24 On/off switch (not available on lower stand parts for HAL 100 illumination)
25 Release tractor for vertical stop on focusing drive

4. Required documents

4.1. Input documents

Olympus Instructions BX51 System Microscope Manual
Olympus Instructions BX53 System Microscope Manual
Operating Manual Axio Scope.A1

4.2. Output documents

5. Document control

5.1. Revision history

R0 – Initial Release – Salvador Lopez, Phil Lee	6/1/2012

5.2. Document approval

<Name> <Approval date>

Document reviewers

Salvador Lopez	6/1/2012
Becky Ryan	9/21/2012
Salvador Lopez	9/27/2012
Phil Lee	9/28/2012
Salvador Lopez	12/5/2012
Phil Lee	12/10/2012
Salvador Lopez, Andrea Yoshioka	1/25/2013
Andrea Yoshioka	1/30/2013

Risk analysis

<Risk name>	<Mitigation plan>	<Owner>	<RPN>