

**THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM**
Approved Biohazards Subcommittee: October 14, 2010
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety/

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Location of experimental work to be carried out: Building(s) WSC Room(s) 129

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).

FUNDING AGENCY/AGENCIES: NSERC
 GRANT TITLE(S): Competition in biological processes

List all personnel working under Principal Investigators supervision in this location:

Name	UWO E-mail Address	Date of Biosafety Training
Beth Locke	mlocke2@uwo.ca	9/2006

Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.

See attached.

Please include a one page research summary or teaching protocol.

See attached.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? YES NO
 (non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? YES NO

If YES, please give the name of the species. _____

What is the origin of the microorganism(s)? _____

Please describe the risk (if any) of escape and how this will be mitigated:

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

Chilodonella uncinata
Metopus es

1.2 Please complete the table below:

Name of Biological Agent(s)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/Supplier	PHAC or CFIA Containment Level
<i>C. uncinata</i>	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	.25	Zuh4 lab	<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
<i>M. es.</i>	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	.25	ATCC	<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
<i>Polyspora</i> <i>metopus</i>	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Various Protists	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	.25	Freshwater Protists	<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

↑ NO

2.1 Does your work involve cell cultures? YES NO
 If no, please proceed to Section 2.2

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	<input type="radio"/> Yes <input checked="" type="radio"/> No		Not applicable
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No		
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No	Brain Bits LLC	N/A
Other (specify)	<input type="radio"/> Yes <input checked="" type="radio"/> No		

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	<input type="radio"/> Yes <input checked="" type="radio"/> No			
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No			
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No			
Other (specify)	<input type="radio"/> Yes <input checked="" type="radio"/> No			

Question 2.4

*Please attach a Material Safety Data Sheet or equivalent for (www.atcc.org)

2.4 For above named cell type(s) indicate PHAC or CFIA containment level required 1 2 2+ 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? YES NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	Source/Supplier /Company Name	Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN	Name of Infectious Agent (If applicable)	PHAC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Blood (fraction) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (unpreserved)		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? YES NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? YES, complete table below NO

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection

* Please attach a Material Data Sheet or equivalent if available.

** Please attach a plasmid map.

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?

YES, complete table below NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- ◆ HIV YES, please specify _____ NO
- ◆ HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens YES, specify _____ NO
- ◆ SV 40 Large T antigen YES NO
- ◆ E1A oncogene YES NO
- ◆ Known oncogenes YES, please specify _____ NO
- ◆ Other human or animal pathogen and or their toxins YES, please specify _____ NO

4.5 Will virus be replication defective? YES NO

4.6 Will virus be infectious to humans or animals? YES NO

4.7 Will this be expected to increase the containment level required? YES NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? YES NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: _____
Please attach a full description of the biological agent.

5.2 Will the biological agent be able to replicate in the host? YES NO

5.3 How will the biological agent be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted: _____

5.5 Has human ethics approval been obtained? YES, number: _____ NO PENDING

6.0 Animal Experiments

6.1 Will live animals be used? YES NO If no, please proceed to section 7.0

6.2 Name of animal species to be used _____

6.3 AUS protocol # _____

6.4 Will any of the agents listed in section 4.0 be used in live animals YES, specify: _____ NO

6.5 Will the agent(s) be shed by the animal: YES NO, please justify:

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? YES No If no, please proceed to section 8.0

7.2 Will live animals be used? YES No

7.3 If yes, please specify the animal(s) used:

- ◆ Pound source dogs YES NO
- ◆ Pound source cats YES NO
- ◆ Cattle, sheep or goats YES, please specify species _____ NO
- ◆ Non-human primates YES, please specify species _____ NO
- ◆ Wild caught animals YES, please specify species & colony # _____ NO
- ◆ Birds YES, please specify species _____ NO
- ◆ Others (wild or domestic) YES, please specify _____ NO

7.4 If no live animals are used, please specify the source of the specimens:

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? YES NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s) _____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD₅₀ (specify species) of the toxin _____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

8.6 Will any biological toxins be used in live animals? YES, Please provide details: _____ NO

*For information on biosecurity requirements, please see:

http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects

9.1 Do you use insects? YES NO If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

9.5 What is your intention? Initiate and maintain colony, give location: _____
 "One-time" use, give location: _____

9.6 Please describe the risk (if any) of escape and how this will be mitigated:

9.7 Do you use insects that require a permit from the CFIA permit? YES NO

If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

10.0 Plants

10.1 Do you use plants? YES NO If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

10.5 What is your intention? Grow and maintain a crop "One-time" use

10.6 Do you do any modifications to the plant? YES NO

If yes, please describe: _____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:

10.8 Is the CFIA permit attached? YES NO

If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

11.0 Import Requirements

11.1 Will any of the above agents be imported? YES, please give country of origin _____ NO
If no, please proceed to Section 12.0

11.2 Has an Import Permit been obtained from HC for human pathogens? YES NO

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? YES NO

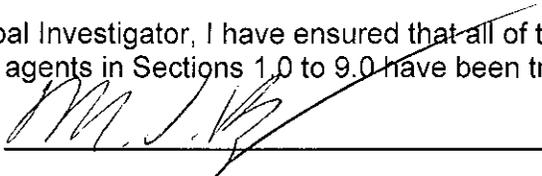
11.4 Has the import permit been sent to OHS? YES, please provide permit # _____ NO

12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- ◆ Biosafety
- ◆ Laboratory and Environmental/Waste Management Safety
- ◆ WHMIS (Western or equivalent)
- ◆ Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE  _____

13.0 Containment Levels

13.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. 1 2 2+ 3

13.2 Has the facility been certified by OHS for this level of containment?
 YES, date of most recent biosafety inspection: _____
 NO, please certify
 NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): BIO-UWO-D178

14.0 Procedures to be Followed

14.1 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.
None.

14.2 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury or an accidental splash:
Needles not used. Splashes dealt with by washing affected areas.

14.3 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at <http://www.wph.uwo.ca/>

SIGNATURE  Date: 1 March 2011

15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: _____

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: _____
Date: _____

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

Protozoa and Algae

ATCC® Number: **50194™** [Order this Item](#) Price: **\$155.00**

Organism: *Chilodonella uncinata* Ehrenberg
 Designations: ATCC :0189:1
 Isolation: contaminant of Euplotes gracilis culture, ATCC [50191](#), 1988
 Depositors: TA Nerad
 Biosafety Level: 1
 Shipped: frozen
 Growth Conditions: [ATCC medium 802](#): Sonneborn's Paramecium medium
Temperature: 25.0°C
 Duration: grown with Enterobacter aerogenes ATCC [13048](#) and mixed bacteria
 Permits/Forms: In addition to the [MTA](#) mentioned above, other [ATCC and/or regulatory permits](#) may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please [click here](#) for information regarding the specific requirements for shipment to your location.
 Classification: KINGDOM: Protozoa

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Project descriptions

Mark Daley

1 Brief Scientific Overview

1.1 Project 1

We propose to observe the structural evolution of information processing in networks of rat (and mouse) neurons. Recent work by Bassett et. al. [1] suggests that the underlying connection topology of neural networks is self-similar and scale invariant. While connectivity data can provide loose bounds for information-processing capabilities, it does not address this question directly.

In our initial pilot study we wish to begin to address the question of information-processing capabilities directly through the growth of rat and mouse neurons on Microelectrode Arrays (MEAs). The MEAs will be used to both stimulate, and record, the activity of neurons as they form associations in vitro (for a description of these techniques, see, e.g., [3]). Using stimulation, and recording, sequences suggested by theoretical analysis based on computability theory we intend to characterize the information processing capabilities of the forming neural network over time. It is our hypothesis that the information processing capabilities will increase with time and, moreover, that the resultant functional units will show processing behaviour that reflects the self-similar and scale invariant morphological organization described in [1].

1.2 Project 2

We propose to observe the morphology of macronuclear chromosomes in two species of ciliated protozoan using transmission electron microscopy. We will follow a procedure similar to that used in the characterization of the macronuclear chromosome structure of the spirotrichs *Stylonichia pustulata* and *Euplotes aediculatus* [2].

In one of these organisms, *Chilodonella uncinata*, we also wish to carry out DNA and RNA sequencing (with a particular focus on small RNA).

Finally, we wish to undertake a collaborative effort combining computer vision and ecology: creating a system for the automated identification of protists, via light microscopy, in fresh water samples. The diversity of species present in a water sample is an important metric of ecosystem health and an automated screening and identification system would enable high-throughput testing.

2 Details of cells and tissue to be used

2.1 Project 1

Neurons will be obtained from E18 Sprague-Dawley rat primary neuronal tissue and E18 C57 mouse primary neuronal tissue supplied by BrainBits LLC, Springfield IL.

Culturing and experimental stimulation/observation will be carried out in the Daley lab (WSC 124) according to the Primary Neuron Cell Culture protocol provided by BrainBits LLC[4] and MEA protocols described in [3].

These non-mammalian, disease-free, animal tissues are classified as Risk Group 1 according to UWO's Biosafety standards.

2.2 Project 2

All work will be carried out in the Daley lab (WSC 124).

We will culture the species *Chilodonella uncinata* and *Metopus es* as representatives of the classes Phyllopharyngea and Armophorea, respectively. Both of these organisms are listed as Risk Group 1 by international regulatory authorities (CDC, NIH and others) and represent an extremely low risk to both laboratory workers and the community. All of these species are "native", and ubiquitous, in the sense that they are present, in great numbers, in every freshwater lake and pond (and probably most large puddles) in southwestern Ontario.

Chilodonella will be cultured in a plain, pH-adjusted, Cereal grass medium (Scholar Chemistry #9448604) and requires no organisms to be added as food.

Metopus will be cultured in cell culture flasks under anaerobic conditions. Previous experience has demonstrated that cultures can survive for up to 6 months without feeding and, as we need only a very modest number of organisms, this will be entirely sufficient for the purposes of this project; no other organisms will be required as food.

Clarification requested
June 29, 2011 (re: WSC
124)

Finally, for the computer vision based identification of protists, we require samples of a large variety of organisms. Water samples will be taken from sites around Southwestern Ontario and screened using light microscopy. Some organisms may be retained, and grown briefly in culture, to use as training data for computer vision algorithms. Any retained species will first be identified via morphological features or rDNA sequencing and only species belonging to Risk Group 1 shall be retained.

After experimental completion, all organisms will be destroyed with bleach and all equipment autoclaved.

3 Summary of tissues to be used

E18 Sprague-Dawley rat primary neuronal tissue	Animal tissue	Risk Group 1
E18 C57 mouse primary neuronal tissue	Animal tissue	Risk Group 1
<i>Metopus es</i>	Ciliated protozoan	Risk Group 1
<i>Chilonella uncinata</i>	Ciliated protozoan	Risk Group 1
Various	Protozoa	Risk Group 1

References

- [1] Danielle S. Bassett, Daniel L. Greenfield, Andreas Meyer-Lindenberg, Daniel R. Weinberger, Simon W. Moore, Edward T. Bullmore (2010). Efficient Physical Embedding of Topologically Complex Information Processing Networks in Brains and Computer Circuits. *PLoS Computational Biology* 6 (4) p. e1000748.
- [2] Murti KG, Prescott DM (2002) Topological organization of DNA molecules in the macronucleus of hypotrichous ciliated protozoa. *Chromosome Res* 10: 165-173.
- [3] Daniel A Wagenaar, Steve M Potter (2004). A versatile all-channel stimulator for electrode arrays, with real-time control. *Journal of neural engineering* 1 (1) p. 39-45.
- [4] Protocol available at <http://www.brainbitsllc.com/neuronprotocol.aspx>; accessed 28 February 2011.

Neurons

Primary Rat Brain Neuron Cell Culture from Hibernate Tissue

Please find enclosed one 2 ml tube containing embryonic day 18 Sprague/Dawley or Fischer 344 rat hippocampus or cortex in 2 ml B27/Hibernate. Tissue can be stored at 4-8°C for one week. Also find 12 ml Neurobasal/B27/0.5 mM Glutamax culture medium (25 uM glutamate for hippocampus.). For cortex and other tissues, the glutamate is omitted. Prepare substrate by coating with poly-D-lysine (0.15 ml/cm², 50 ug/ml water, 135 kD, 1-20 hr. e.g. Sigma P6407) and rinse one time with sterile 18 Mohm deionized water and let dry.

To prepare isolated neurons:

1. From the tube with the brain tissue, remove 1 ml medium and save for step 3, being careful not to remove the tissue.
2. With 1 ml pipettor with sterile blue plastic tip, or silanized 9 inch pasteur pipet with tip barely fire polished (preferable), suck the tissue with medium into the pipet and immediately dispense the contents back into the same container. Take care not to create bubbles. Repeat this trituration step about 10 times or until most all pieces of tissue are dispersed. Higher survival can be obtained by incubating the tissue for 30 min. at 30°C in 2 mg/ml papain (Worthington) in Hibernate E-Calcium without B27, followed by trituration in Hibernate/B27.
3. Add back 1 ml medium that you removed in step 1 and mix.
4. Let undispersed pieces settle by gravity for 1 min.
5. Transfer supernatant to a new sterile 15 ml tube.
6. Spin 1100rpm (200 x G), 1 min. Discard supernatant.
7. Flick the tube to disperse the pellet of cells. Resuspend pellet in 1 ml B27/Neurobasal+0.5 mM Glutamax +25 uM glutamate, for hippocampal neurons. For cortical neurons, glutamate is omitted. (12 mL provided; more available from www.invitrogen.com, 1-800-955-6288)
8. Aliquot 20 ul and mix with 20 ul 0.4% trypan blue.
9. Count in hemacytometer: calculate cells/ml.
10. Dilute cells with B27/Neurobasal to plate 32×10^3 cells/2 cm² of substrate in 0.4ml/2 cm² substrate (or whatever density you desire).
11. Incubate 37°C, 5% CO₂, 9% oxygen (20% oxygen is O.K.)
12. After 4 days or longer, neurons are well differentiated. If further culture is desired, change one half of the medium with fresh, warm B27/Neurobasal + 0.5 mM Glutamax without glutamate. Change one half every 3 or 4 days.

Viability assay: viability=green cells per unit area/(total cells plated per unit area) or survival (green cells/(green + red cells)):

1. Rinse 2 times with PBS or HBSS/1 g/l glucose/1 mM pyruvate/1 mM NaHCO₃/10 mM Hepes, 0.4 ml/2 cm² of substrate
2. From an acetone stock of 15 mg/ml fluorescein diacetate (Sigma), add 15 uL into 1.5 ml HBSS (1:100 dilution of the stock). From an aqueous stock of 4.6 mg/ml propidium iodide, add 15 uL of the stock into the same 1.5 ml HBSS (1:100 dilution). Add 44 uL of that dilution to each well with 0.4 ml HBSS (further 1:10 dilution).
3. After about 1 min., count using Nikon B1A filter or other blue excitation appropriate for fluorescein fluorescence. Green cells are live, small red nuclear stain are dead. Count dead cells using Nikon GIB or other green excitation to visualize small red nuclei.

If desired, fix and stain with 0.25% Coomassie blue R in ethanol/Acetic acid/water (45/10/45), 1 min., rinse with 10% Acetic acid, aspirate and dry.

Please report any questions or problems us:

voice: 217-789-9313

fax: 217-789-9314

brainbits@brainbits.biz

Methods based on Brewer et al. (1993) J. Neurosci. Res. 35:567-576 and Brewer & Price (1996) Neuroreport 7:1509-1512

*Hibernate, Neurobasal and B27 is for research purposes only and is manufactured by Invitrogen Corporation. Hibernate, Neurobasal, and B27 is a trademark of Invitrogen Corporation.