



Institutional Identifier
System-ID (for NSERC use only) 90318364
Family name of applicant Walker

FORM 101
Application for a Grant
PART I

Date 2008/02/18
Personal identification no. (PIN) 233560

Institution that will administer the grant Toronto	Language of application <input checked="" type="checkbox"/> English <input type="checkbox"/> French	Time (in hours per month) to be devoted to the proposed research / activity 60
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Type of grant applied for Strategic Networks	For Strategic Projects, indicate the Target Area and the Research Topic; for Strategic Networks and Strategic Workshops indicate the Target Area. Biomedical Technologies
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Title of proposal BiopSys: NSERC Strategic Network for Bioplasmonic Systems
Provide a maximum of 10 key words that describe this proposal. Use commas to separate them. plasmonics, biosensors, nanophotonic devices, leukemia detection, imaging, lung cancer detection, biomedical engineering, surface chemistry, raman spectroscopy, cell marker detection

Research subject code(s)	Area of application code(s)
Primary 1901	Secondary 3402
Primary 1102	Secondary 1200

CERTIFICATION/REQUIREMENTS

If this proposal involves any of the following, check the box(es) and submit the protocol to the university or college's certification committee.

Research involving : Humans Human pluripotent stem cells Animals Biohazards

Does any phase of the research described in this proposal a) take place outside an office or laboratory, or b) involve an undertaking as described in Part 1 of Appendix B?

NO If YES to either question a) or b) – Appendices A and B must be completed

TOTAL AMOUNT REQUESTED FROM NSERC				
Year 1 991,694	Year 2 998,194	Year 3 999,694	Year 4 989,694	Year 5 999,694

SIGNATURES (Refer to instructions "What do signatures mean?")

It is agreed that the general conditions governing grants as outlined in the NSERC *Program Guide for Professors* apply to any grant made pursuant to this application and are hereby accepted by the applicant and the applicant's employing institution.

Applicant Chemistry Toronto Tel.: (416) 9468401 FAX: (416) 9463649 gilbert.walker@utoronto.ca
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Head of department Dean of faculty President of institution (or representative)
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Personal identification no. (PIN)	Family name of applicant
233560	Walker

CO-APPLICANTS

I have read the statement "What do signatures on the application mean?" in the accompanying instructions and agree to it.

PIN, family name and initial(s)	Research/ activity time (hours/month)	Organization	Signature
205678, Brolo, A.	20	Victoria	
147104, Berini, P.	16	Ottawa	
16348, Meunier, M.	15	École Polytechnique	
278310, Mittler, S.K.	15	Western Ontario	
214128, Gordon, R.	16	Victoria	
49032, Wilson, B.	8	Toronto	
Zheng, G.	10	Toronto	

CO-APPLICANTS' ORGANIZATIONS AND/OR SUPPORTING ORGANIZATIONS (if organization different from page 1)

It is agreed that the general conditions governing grants as outlined in the NSERC *Program Guide for Professors*, as well as the statements "What do signatures on the application mean?" and "Summary of proposal for public release" in the accompanying instructions, apply to any grant made pursuant to this application and are hereby accepted by the organization.

Family name and given name of signing officer, title of position, and name of organization	Signature
Taylor, Keith Associate Vice-President of Research Windsor	
Ferguson, Mary Manager, Research Services Western Ontario	
Lefebvre, Daniel Asst. Dir. Research Grants & Ethics Serv Ottawa	
Scarth, Rachael Director of Research Services Victoria	

2 - 1 Organizations

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CO-APPLICANTS' ORGANIZATIONS AND / OR SUPPORTING ORGANIZATIONS (if organization different from page 1)

Family name and given name of signing officer, title of position, and name of organization	Signature
Savard, Gilles Dean of Research and Innovation École Polytechnique	

2 - 1 co-applicants

		Personal identification no. (PIN) 233560	Family name of applicant Walker
CO-APPLICANTS			
PIN, family name and initial(s)	Research/ activity time (hours/month)	Organization	Signature
140199, Goh, M.C.	10	Toronto	
213220, Helmy, A.	10	Toronto	
202661, Kumacheva, E.	10	Toronto	
243118, Mojahedi, M.	10	Toronto	
14811, Pawson, A.J.	10	Toronto	
290436, Rangan, C.	10	Windsor	
13440, Sipe, J.E.	10	Toronto	
Tsao, M.S.	10	Toronto	
200667, Wang, C.	10	Toronto	
303646, Wheeler, A.R.	10	Toronto	
267453, Chan, W.C.	15	Toronto	
242511, Aitchison, J.S.	10	Toronto	

Personal identification no. (PIN) 233560	Family name of applicant Walker	
Before completing this section, read the instructions for the definition of collaborators in the Eligibility Criteria section of the Program Guide for Professors.		
COLLABORATORS		
PIN, family name and initial(s)	Research/ activity time (hours/month)	Organization
Zou, S.	10	National Research Council

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SUMMARY OF PROPOSAL FOR PUBLIC RELEASE (Use plain language.)

This plain language summary will be available to the public if your proposal is funded. Although it is not mandatory, you may choose to include your business telephone number and/or your e-mail address to facilitate contact with the public and the media about your research.

Business telephone no. (optional): 1 (416) 946-8401

E-mail address (optional): gilbert.walker@utoronto.ca

The aim of the NSERC Strategic Network for Bioplasmonic Systems (BiopSys) is to shorten the time needed for diagnosis and improve prognosis for cancers, such as lung cancer and leukemia, by incorporating plasmonics into diagnostic platforms. Plasmonics is an enabling technology based on an optical illumination technique. Bioplasmonics is technology based on this illumination for the sensitive detection of biological molecules, such as receptors on the surfaces of cells.

Our objective is to develop better alternatives to current fluorescence methods for cell-surface receptor detection. Plasmonics offers significant opportunities for achieving this. We will use tiny metal particles as light beacons for the receptors, and nanostructured metal surfaces as sensitive transmitters of molecular binding events. We will first develop platforms for examining model biochemical systems and then develop diagnostic tools for cancer. Our first practical goal is to determine the presence of the lung cancer at an earlier stage than is currently possible, to improve prognosis. Our second practical goal is to provide a point-of-care device for detecting leukemia, which will greatly shorten the time needed for diagnosis.

BiopSys will, for the first time in Canada, focus and integrate the full range of skills from the many different disciplines that are required to create these devices. The Network is poised to make revolutionary improvements in biomolecular interaction analysis, using the expertise of world-class physicists, chemists, biochemists, biomedical scientists, biologists, material scientists, and engineers based at university, industry and government research labs across the country. Our integration of molecular and optical technologies will enable the creation of both large-scale instruments that will permit faster screening and diagnosis and miniaturized point-of-care devices for patients. In addition, the network mechanism, with its emphasis on integration and cross-pollination, provides a significant training base; and our partnerships with industry will transition our creations to innovations of significant health and economic value to Canadians.

Second Language Version of Summary (optional).

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ACTIVITY SCHEDULE (Refer to instructions to see if this section applies to your application. Use additional page(s) if necessary.)			
Milestone	Description of activities	Anticipated starting date	Anticipated completion date
Recurring: Monthly Teleconference	Monthly teleconference of Network Theme Leaders, Network Manager and PI, to discuss and resolve network issues	2008-09-01	2013-09-01
Recurring: Bimonthly Teleconference	Bimonthly teleconference of all faculty and Network Manager, to discover opportunities and resolve issues	2008-09-01	2013-09-01
Recurring: Semi-annual Teleconference	Semi-annual teleconference of faculty, Network Manager, and partners (twice a year)	2008-09-01	2013-09-01
Recurring: Semi-annual Whole Network Conference	Whole Network Conference including poster session on results and breakout discussions for each theme group	2008-09-01	2013-09-01
Recurring: Board of Directors Meetings	Board of Directors meets 3 times a year to evaluate progress and continue support of each project	2008-09-01	2013-09-01
Administrative: Set up Board of Directors	Set up Board of Directors; Board receives status of each theme; Network Manager completes cataloguing of available facilities and develops manual for their useage	2008-09-01	2009-10-31
Administrative: Network Kickoff Conference	Meeting of Board; workshops on effective collaboration; workshops on facilities available	2009-11-01	2009-01-01
Administrative: Site Visits	Network Manager visits all sites, to see labs of participants and does a measurement or task at each major facility, in collaboration with appropriate team members	2009-03-01	2009-03-31

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ACTIVITY SCHEDULE

(Refer to instructions to see if this section applies to your application. Use additional page(s) if necessary.)

Milestone	Description of activities	Anticipated starting date	Anticipated completion date
Year 1: Theme 1 (Targeted Raman Tagged Nanoparticles)	Prepare metallic nanoparticles. Prepare and characterize SERS-nanoparticles. Conjugate antibody-based bio-recognition molecules on SERS nanoparticles.	2008-09-01	2009-09-01
Year 1: Theme 2 (Phase Sensitive SPR)	Determine the approaches (interferometry and/or polarimetry) to limit noise and to obtain Refractive Index Units lower than 10-7.	2008-09-01	2009-09-01
Year 1: Theme 2 (Periodic Arrays of Nanoholes (PANHs))	Initial design and fabrication of microarrays of PANHs. Determine figure of merits for the PANHs sensor and work on noise source analysis and method for the immobilization of leukemia antibodies.	2008-09-01	2009-09-01
Year 1: Theme 2 (Nanoparticles on Dielectric Surfaces)	Understand the parameters controlling the device: Determination of figures of merit for each novel device, considering the different optical constants and sizes of the recognition chemicals for leukemia & lung cancer.	2008-09-01	2009-09-01
Year 1: Theme 2 (Biosensing Using Surface Guided Plasmons)	Establish models for waveguides and transducers. Design fabrication flows, with microfluidics complete. Mach-zender approach: design of experiments complete. Optical gain for SPs: architectures selected, modeling and fabrication complete.	2008-09-01	2009-09-01
Year 1: Theme 2 (Microfluidics)	Design and fabricate channel and digital microfluidic devices incorporating mock-nanohole and nanoparticle detectors.	2008-09-01	2009-09-01
Year 1: Theme 3 (Leukemia and Lung Cancer Detection)	Develop antibody-labeled, gold nanoparticle-based plasmonic probes for local delivery.	2008-09-01	2009-09-01
Year 2: Theme 1 (Targeted Raman Tagged Nanoparticles)	Test binding of antibody-conjugated biorecognition on cells. Analyze role of light scattering by cell. Develop library of SERS-nanoparticles.	2009-09-01	2010-09-01

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ACTIVITY SCHEDULE (Refer to instructions to see if this section applies to your application. Use additional page(s) if necessary.)			
Milestone	Description of activities	Anticipated starting date	Anticipated completion date
Year 2: Theme 1 (Targeted Raman Tagged Nanoparticles)	Test SERS rod-shaped nanoparticles pairs. Make/characterize the initial lung cancer-targeted Raman probe using EGFR-specific peptide.	2009-09-01	2010-09-01
Year 2: Theme 2 (Phase Sensitive SPR)	Phase sensitive SPR: Determine the approaches (interferometry and/or polarimetry) to limit noise (Yr1-2). Optimize the system by limiting the electronics noise and improve the image analysis.	2008-09-01	2010-09-01
Year 2: Theme 2 (Periodic Arrays of Nanoholes (PANHs))	Integration of micro-array in microfluidics & preliminary multiplex detection with a CCD. Polarization studies. Design/optimization of shaped holes. Develop methodology to immobilize lung cancer antibodies on gold surfaces & targets inside shaped nanoholes.	2009-09-01	2010-09-01
Year 2: Theme 2 (Nanoparticles on Dielectric Surfaces)	Systematical change of important parameters of a particular device in theory and compare with device performance in experiment, e.g., with a well characterized recognition system before the leukemia and lung cancer recognition elements are available.	2009-09-01	2010-09-01
Year 2: Theme 2 (Biosensing Using Surface Guided Plasmons)	Noise models for optoelectronics, waveguides and fluid complete. Modeling, design and layout of transducers complete. Mach Zehnder feasibility demonstrated. Optical gain for SPs: Epitaxial growth and microfabrication complete.	2009-09-01	2010-09-01
Year 2: Theme 2 (Microfluidics)	Evaluate performance of the two device architectures on the basis of throughput, reagent use, and reproducibility. Meeting of principals to select strategy for focus going forward.	2009-09-01	2010-09-01
Year 2: Theme 3 (Leukemia and Lung Cancer Detection)	Test specificity of plasmonic probes in cancer cell lines expressing different levels of marker proteins. Develop marker-guided lipoprotein nanoparticle for encapsulating plasmonic probes for systematic delivery.	2009-09-01	2010-09-01
Year 3: Theme 1 (Targeted Raman Tagged Nanoparticles)	Evaluate the SERS of EGFR-targeted probes in solutions and in vitro in NSCLC cells expressing different EGFR levels. Develop prototype leukemia-targeting Raman probes using antibody and test feasibility against leukemia cells.	2010-09-01	2011-09-01

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Milestone	Description of activities	Anticipated starting date	Anticipated completion date
Year 3: Theme 1 (Targeted Raman Tagged Nanoparticles)	Integrate biorecognition elements into rod-shaped pairs.	2010-09-01	2011-09-01
Year 3: Theme 2 (Phase Sensitive SPR)	Implementation of the Ultrasensitive SPR in cancer diagnosis and research (Yr 3.0-4.5). Develop SPR assay for measuring serum CD23 and to evaluate the results and clinical significance.	2010-09-01	2012-03-01
Year 3: Theme 2 (Periodic Arrays of Nanoholes (PANHs))	Multiplex detection of leukemia using an integrated micro-array of PANHs; SERS and SEFS of probe molecules confined on shaped nanoholes.	2010-09-01	2011-09-01
Year 3: Theme 2 (Periodic Arrays of Nanoholes (PANHs))	Implementation of polarization/phase-sensitive detection for leukemia diagnosis. Make micro-arrays of PANHs using optimized geometry.	2010-09-01	2011-09-01
Year 3: Theme 2 (Nanoparticles on Dielectric Surfaces)	Build second generation devices and test for theoretically found sensitivity in a known system. Develop/apply method to immobilize leukemia and lung cancer antibodies on gold nanoparticle surfaces, and develop liquid handling systems for devices.	2010-09-01	2011-09-01
Year 3: Theme 2 (Nanoparticles on Dielectric Surfaces)	Apply leukemia and lung cancer recognition chemistry and find detection limits in the absorption, SERS, and fluorescence approaches. Implement polarimetry. Compare strategies with SPR strategy. Tell Network how polarimetry enhances sensitivity.	2010-09-01	2011-09-01
Year 3: Theme 2 (Biosensing Using Surface Guided Plasmons)	Models integrated into end-to-end signal-to-noise. Transducers fabricated. Kinetics determined.	2010-09-01	2011-09-01
Year 3: Theme 2 (Microfluidics)	Develop means to incorporate nanoholes into microfluidic platform. Develop means to incorporate immobilized nanoparticles into microfluidic platform.	2010-09-01	2011-09-01

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ACTIVITY SCHEDULE (Refer to instructions to see if this section applies to your application. Use additional page(s) if necessary.)			
Milestone	Description of activities	Anticipated starting date	Anticipated completion date
Year 3: Theme 3 (Leukemia and Lung Cancer Detection)	Development of diagnostic panel for CLL. Test the in vivo specificity of plasmonic probes in lung cancer xenografts. In vitro specificity studies of lipoprotein-encapsulated plasmonic probes.	2010-09-01	2011-09-01
Year 4: Theme 1 (Targeted Raman Tagged Nanoparticles)	Re-evaluate the SERS-nanoparticles and develop better SERS-nanoprobes. Optimize detection performance of the Raman probes against lung cancer cells by modulating the physical properties of the nanoprobe and the number/type of peptide/antibody ligands.	2011-09-01	2012-09-01
Year 4: Theme 1 (Targeted Raman Tagged Nanoparticles)	Test rod based systems on cells.	2011-09-01	2012-09-01
Year 4: Theme 2 (Phase Sensitive SPR)	Determine the binding parameters of CD20 and surface Ig receptors of leukemia cells (Yr 3.5-5.0).	2011-03-01	2013-09-01
Year 4: Theme 2 (Periodic Arrays of Nanoholes (PANHs))	ID lung cancer labeled-markers by SEFS and SERS using shaped holes (plasmonic antennas). Develop micro-arrays of shaped holes for multiplexing SEFS and SERS. Integrate optimized PANHs for leukemia diagnosis.	2011-09-01	2012-09-01
Year 4: Theme 2 (Periodic Arrays of Nanoholes (PANHs))	Integrate SERS/SEFS arrays in microfluidics for lung cancer detection.	2011-09-01	2012-09-01
Year 4: Theme 2 (Nanoparticles on Dielectric Surfaces)	Minimize noise sources, enhance S/N ratio. Apply multiple spectroscopic approaches. Optimize components for noise reduction and contribute to a knowledge bank of noise reduction technology.	2011-09-01	2012-09-01
Year 4: Theme 2 (Biosensing Using Surface Guided Plasmons)	Transducer architectures compared and benchmarked. Mach-Zehnder approach: Full process flow established. Optical gain for SPs: Second fabrication and experimental iteration complete.	2011-09-01	2012-09-01

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Milestone	Description of activities	Anticipated starting date	Anticipated completion date
Year 4: Theme 2 (Microfluidics)	Build device prototypes and demonstrate single-assay analyte detection.	2011-09-01	2012-09-01
Year 4: Theme 3 (Leukemia and Lung Cancer Detection)	Testing CCL diagnostic panel with different fluids. Deliver labeled plasmonic probes locally into the airway in an orthotopic lung cancer model. Use bronchoscopic instruments to detect the plasmonic signal to detect where probe is concentrated.	2011-09-01	2012-09-01
Year 5: Theme 1 (Targeted Raman Tagged Nanoparticles)	Compare different lung cancer and leukemia-targeted Raman probes for their in vitro detection sensitivity and specificity and determine the best probes for in vivo lung cancer localization and ex vivo leukemia screening.	2012-09-01	2013-09-01
Year 5: Theme 2 (Phase Sensitive SPR)	Optimize for the binding parameters of CD20 and surface Ig receptors of leukemia cells (Yr 3.5-5.0).	2011-03-01	2013-09-01
Year 5: Theme 2 (Periodic Arrays of Nanoholes (PANHs))	Compare figure of merits of produced detection schemes. Provide optimized platform for fast diagnosis of leukemia. Obtain single-molecule SERS from an optimized plasmonic antenna.	2012-09-01	2013-09-01
Year 5: Theme 2 (Periodic Arrays of Nanoholes (PANHs))	Provide optimized platform for multiplex determination of lung cancer markers by SERS/SEFS.	2012-09-01	2013-09-01
Year 5: Theme 2 (Nanoparticles on Dielectric Surfaces)	Develop approaches for a multiple sampling: parallel propagation and multi-spot systems. Develop multi-spot or multi-channel liquid handling system. Develop multi-channel methodology to immobilize antibodies on gold nanoparticle surfaces.	2012-09-01	2013-09-01
Year 5: Theme 2 (Biosensing Using Surface Guided Plasmons)	Surface functionalization with biomarker receptors.	2012-09-01	2013-09-01

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ACTIVITY SCHEDULE

(Refer to instructions to see if this section applies to your application. Use additional page(s) if necessary.)

Milestone	Description of activities	Anticipated starting date	Anticipated completion date
Year 5: Theme 2 (Microfluidics)	Build multiplexed platform deliverable capable of analyzing 10-30 samples simultaneously. Characterize platform on the basis of throughput, reagent use, and reproducibility.	2012-09-01	2013-09-01
Year 5: Theme 3 (Leukemia and Lung Cancer Detection)	Develop/test diagnostic panel for other leukemias. Use plasmonic biosensor-coated fiber bronchoscope to enhance labeled antibody detection. Deliver lipoprotein-encapsulated probes. Use combined CT/bronchoscopy to find lesions accumulating the probe.	2012-09-01	2013-09-01

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Before completing this section, **read the instructions** and consult the *Use of Grant Funds* section in the NSERC Program Guide for Professors for information about the eligibility of expenditures for the direct costs of research and the regulations governing the use of grant funds. On separate page(s), supply a detailed explanation, and justification, for your proposed expenditures. **Also explain the relationship or difference between this application and all other research support (held or applied for),** and describe any contributions from other sources (if applicable).

PROPOSED EXPENDITURES FOR DIRECT COSTS OF RESEARCH (include cash expenditures only)

Theme I	Year 1	Year 2	Year 3	Year 4	Year 5
1) Salaries and benefits					
a) Students	109,000	114,000	114,000	109,000	114,000
b) Postdoctoral fellows	36,750	36,750	36,750	36,750	36,750
c) Technical/professional assistants	0	0	0	0	0
d)	0	0	0	0	0
2) Equipment or facility					
a) Purchase or rental	0	0	0	0	0
b) Operation and maintenance costs	3,000	3,000	3,000	3,000	3,000
c) User fees	21,260	21,260	21,260	21,260	21,260
3) Materials and supplies	41,000	41,000	41,000	41,000	41,000
4) Travel					
a) Conferences	7,000	7,000	7,000	7,000	7,000
b) Field work	0	0	0	0	0
c) Collaboration/ consultation	8,000	8,000	8,000	8,000	8,000
5) Dissemination costs					
a) Publication costs	0	0	0	0	0
b) Other activities	0	0	0	0	0
6) Other (specify)					
a) shipping costs	6,250	6,250	6,250	6,250	6,250
b)	0	0	0	0	0
TOTAL PROPOSED EXPENDITURES	232,260	237,260	237,260	232,260	237,260
Total cash contribution from industry (if applicable)	0	0	0	0	0
Total cash contribution from university (if applicable)	18,750	18,750	18,750	18,750	18,750
Total cash contribution from other sources (if applicable)	0	0	0	0	0
TOTAL AMOUNT REQUESTED FROM NSERC (transfer to page 1)	991,694	998,194	999,694	989,694	999,694

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PROPOSED EXPENDITURES FOR DIRECT COSTS OF RESEARCH (include cash expenditures only)

Theme 2	Year 1	Year 2	Year 3	Year 4	Year 5
1) Salaries and benefits					
a) Students	284,500	292,000	294,500	284,500	294,500
b) Postdoctoral fellows	0	0	0	0	0
c) Technical/professional assistants	0	0	0	0	0
d)	0	0	0	0	0
2) Equipment or facility					
a) Purchase or rental	3,000	3,000	3,000	3,000	3,000
b) Operation and maintenance costs	500	500	500	500	500
c) User fees	57,200	53,200	52,200	57,200	52,200
3) Materials and supplies	37,000	37,000	37,000	37,000	37,000
4) Travel					
a) Conferences	15,000	15,000	15,000	15,000	15,000
b) Field work	0	0	0	0	0
c) Collaboration/ consultation	28,100	28,100	28,100	28,100	28,100
5) Dissemination costs					
a) Publication costs	0	0	0	0	0
b) Other activities	0	0	0	0	0
6) Other (specify)					
a) shipping	3,550	3,550	3,550	3,550	3,550
b)	0	0	0	0	0
TOTAL PROPOSED EXPENDITURES	428,850	432,350	433,850	428,850	433,850
Total cash contribution from industry (if applicable)	0	0	0	0	0
Total cash contribution from university (if applicable)	18,750	18,750	18,750	18,750	18,750
Total cash contribution from other sources (if applicable)	0	0	0	0	0
TOTAL AMOUNT REQUESTED FROM NSERC (transfer to page 1)	991,694	998,194	999,694	989,694	999,694

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PROPOSED EXPENDITURES FOR DIRECT COSTS OF RESEARCH (include cash expenditures only)

Theme 3	Year 1	Year 2	Year 3	Year 4	Year 5
1) Salaries and benefits					
a) Students	57,000	57,000	57,000	57,000	57,000
b) Postdoctoral fellows	73,500	73,500	73,500	73,500	73,500
c) Technical/professional assistants	0	0	0	0	0
d)	0	0	0	0	0
2) Equipment or facility					
a) Purchase or rental	0	0	0	0	0
b) Operation and maintenance costs	0	0	0	0	0
c) User fees	3,000	3,000	3,000	3,000	3,000
3) Materials and supplies	33,000	33,000	33,000	33,000	33,000
4) Travel					
a) Conferences	5,000	5,000	5,000	5,000	5,000
b) Field work	0	0	0	0	0
c) Collaboration/ consultation	5,000	5,000	5,000	5,000	5,000
5) Dissemination costs					
a) Publication costs	0	0	0	0	0
b) Other activities	0	0	0	0	0
6) Other (specify)					
a)	0	0	0	0	0
b)	0	0	0	0	0
TOTAL PROPOSED EXPENDITURES	176,500	176,500	176,500	176,500	176,500
Total cash contribution from industry (if applicable)	0	0	0	0	0
Total cash contribution from university (if applicable)	18,750	18,750	18,750	18,750	18,750
Total cash contribution from other sources (if applicable)	0	0	0	0	0
TOTAL AMOUNT REQUESTED FROM NSERC (transfer to page 1)	991,694	998,194	999,694	989,694	999,694

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Before completing this section, **read the instructions** and consult the *Use of Grant Funds* section in the NSERC Program Guide for Professors for information about the eligibility of expenditures for the direct costs of research and the regulations governing the use of grant funds. On separate page(s), supply a detailed explanation, and justification, for your proposed expenditures. **Also explain the relationship or difference between this application and all other research support (held or applied for)**, and describe any contributions from other sources (if applicable).

PROPOSED EXPENDITURES FOR DIRECT COSTS OF RESEARCH (include cash expenditures only)

Administration	Year 1	Year 2	Year 3	Year 4	Year 5
1) Salaries and benefits					
a) Students	0	0	0	0	0
b) Postdoctoral fellows	0	0	0	0	0
c) Technical/professional assistants	0	0	0	0	0
d) see justification	78,750	78,750	78,750	78,750	78,750
2) Equipment or facility					
a) Purchase or rental	3,000	0	0	0	0
b) Operation and maintenance costs	0	0	0	0	0
c) User fees	0	0	0	0	0
3) Materials and supplies	3,000	2,000	2,000	2,000	2,000
4) Travel					
a) Conferences	0	0	0	0	0
b) Field work	0	0	0	0	0
c) Collaboration/ consultation	6,412	6,412	6,412	6,412	6,412
5) Dissemination costs					
a) Publication costs	0	0	0	0	0
b) Other activities	0	2,000	2,000	2,000	2,000
6) Other (specify)					
a) 2 all-network conferences	80,672	80,672	80,672	80,672	80,672
b) shipping	1,000	1,000	1,000	1,000	1,000
TOTAL PROPOSED EXPENDITURES	172,834	170,834	170,834	170,834	170,834
Total cash contribution from industry (if applicable)	0	0	0	0	0
Total cash contribution from university (if applicable)	18,750	18,750	18,750	18,750	18,750
Total cash contribution from other sources (if applicable)	0	0	0	0	0
TOTAL AMOUNT REQUESTED FROM NSERC (transfer to page 1)	991,694	998,194	999,694	989,694	999,694

Personal identification no. (PIN) 233560	Family name of applicant Walker
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Before completing this section, **read the instructions** and consult the *Use of Grant Funds* section in the NSERC Program Guide for Professors for information about the eligibility of expenditures for the direct costs of research and the regulations governing the use of grant funds. On separate page(s), supply a detailed explanation, and justification, for your proposed expenditures. **Also explain the relationship or difference between this application and all other research support (held or applied for),** and describe any contributions from other sources (if applicable).

PROPOSED EXPENDITURES FOR DIRECT COSTS OF RESEARCH (include cash expenditures only.)

Sum Total	Year 1	Year 2	Year 3	Year 4	Year 5
1) Salaries and benefits					
a) Students	450,500	463,000	465,500	450,500	465,500
b) Postdoctoral fellows	110,250	110,250	110,250	110,250	110,250
c) Technical/professional assistants	0	0	0	0	0
d)	78,750	78,750	78,750	78,750	78,750
2) Equipment or facility					
a) Purchase or rental	6,000	3,000	3,000	3,000	3,000
b) Operation and maintenance costs	3,500	3,500	3,500	3,500	3,500
c) User fees	81,460	77,460	76,460	81,460	76,460
3) Materials and supplies	114,000	113,000	113,000	113,000	113,000
4) Travel					
a) Conferences	27,000	27,000	27,000	27,000	27,000
b) Field work	0	0	0	0	0
c) Collaboration/consultation	47,512	47,512	47,512	47,512	47,512
5) Dissemination costs					
a) Publication costs	0	0	0	0	0
b) Other activities	0	2,000	2,000	2,000	2,000
6) Other (specify)					
a)	90,472	90,472	90,472	90,472	90,472
b)	1,000	1,000	1,000	1,000	1,000
TOTAL PROPOSED EXPENDITURES FOR DIRECT COSTS OF RESEARCH	1,010,444	1,016,944	1,018,444	1,008,444	1,018,444
Total cash contribution from industry (if applicable)	0	0	0	0	0
Total cash contribution from university (if applicable)	18,750	18,750	18,750	18,750	18,750
Total cash contribution from other sources (if applicable)	0	0	0	0	0
TOTAL AMOUNT REQUESTED FROM NSERC (transfer to page 1)	991,694	998,194	999,694	989,694	999,694

Personal identification no. (PIN) 233560	Family name of applicant Walker
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Before completing this section, read the instructions for contributions from supporting organizations and consult the *Use of Grant Funds* section in the NSERC *Program Guide for Professors* concerning the eligibility of expenditures for the direct costs of research and the regulations governing the use of grant funds, and *Guidelines for Evaluating Cost-Sharing Ratios and In-Kind Contributions in University-Industry Collaborations* regarding the eligibility of in-kind contributions.

Name of supporting organization
Toronto

CONTRIBUTIONS FROM SUPPORTING ORGANIZATIONS

	Year 1	Year 2	Year 3	Year 4	Year 5
Cash contributions to direct costs of research (Transfer amounts to page five (5); except those for the Ship Time program.)	18,750	18,750	18,750	18,750	18,750
In-kind contributions to direct costs of research					
1) Salaries for scientific and technical staff	0	0	0	0	0
2) Donation of equipment, software	0	0	0	0	0
3) Donation of material	0	0	0	0	0
4) Field work logistics	0	0	0	0	0
5) Provision of services	0	0	0	0	0
6) Comptroller	18,750	18,750	18,750	18,750	18,750
Total of in-kind contributions to direct costs of research	18,750	18,750	18,750	18,750	18,750
In-kind contributions to indirect costs of research (not leveraged)					
1) Use of organization's facilities	0	0	0	0	0
2) Salaries of managerial and administrative staff	0	0	0	0	0
3)	0	0	0	0	0
Total of all in-kind contributions	18,750	18,750	18,750	18,750	18,750
Contribution to university overhead	0	0	0	0	0

Personal identification no. (PIN) 233560	Family name of applicant Walker
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Before completing this section, read the instructions for contributions from supporting organizations and consult the *Use of Grant Funds* section in the NSERC *Program Guide for Professors* concerning the eligibility of expenditures for the direct costs of research and the regulations governing the use of grant funds, and *Guidelines for Evaluating Cost-Sharing Ratios and In-Kind Contributions in University-Industry Collaborations* regarding the eligibility of in-kind contributions.

Name of supporting organization
SHARCNET

CONTRIBUTIONS FROM SUPPORTING ORGANIZATIONS

	Year 1	Year 2	Year 3	Year 4	Year 5
Cash contributions to direct costs of research (Transfer amounts to page five (5); except those for the Ship Time program.)	0	0	0	0	0
In-kind contributions to direct costs of research					
1) Salaries for scientific and technical staff	0	0	0	0	0
2) Donation of equipment, software	0	0	0	0	0
3) Donation of material	0	0	0	0	0
4) Field work logistics	0	0	0	0	0
5) Provision of services	21,000	21,000	21,000	21,000	21,000
6) CPU hours	50,000	50,000	50,000	50,000	50,000
Total of in-kind contributions to direct costs of research	71,000	71,000	71,000	71,000	71,000
In-kind contributions to indirect costs of research (not leveraged)					
1) Use of organization's facilities	0	0	0		0
2) Salaries of managerial and administrative staff	0	0	0	0	0
3)	0	0	0	0	0
Total of all in-kind contributions	71,000	71,000	71,000	71,000	71,000
Contribution to university overhead	0	0	0	0	0

Personal identification no. (PIN) 233560	Family name of applicant Walker
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Before completing this section, read the instructions for contributions from supporting organizations and consult the *Use of Grant Funds* section in the NSERC *Program Guide for Professors* concerning the eligibility of expenditures for the direct costs of research and the regulations governing the use of grant funds, and *Guidelines for Evaluating Cost-Sharing Ratios and In-Kind Contributions in University-Industry Collaborations* regarding the eligibility of in-kind contributions.

Name of supporting organization

Northern Nanotechnologies Inc.

CONTRIBUTIONS FROM SUPPORTING ORGANIZATIONS

	Year 1	Year 2	Year 3	Year 4	Year 5
Cash contributions to direct costs of research (Transfer amounts to page five (5); except those for the Ship Time program.)	0	0	0	0	0
In-kind contributions to direct costs of research					
1) Salaries for scientific and technical staff	38,400	38,400	38,400	38,400	38,400
2) Donation of equipment, software	0	0	0	0	0
3) Donation of material	8,000	8,000	8,000	8,000	8,000
4) Field work logistics	0	0	0	0	0
5) Provision of services	0	0	0	0	0
6)	0	0	0	0	0
Total of in-kind contributions to direct costs of research	46,400	46,400	46,400	46,400	46,400
In-kind contributions to indirect costs of research (not leveraged)					
1) Use of organization's facilities	0	0	0	0	0
2) Salaries of managerial and administrative staff	0	0	0	0	0
3)	0	0	0	0	0
Total of all in-kind contributions	46,400	46,400	46,400	46,400	46,400
Contribution to university overhead	0	0	0	0	0

Personal identification no. (PIN) 233560	Family name of applicant Walker
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Before completing this section, read the instructions for contributions from supporting organizations and consult the *Use of Grant Funds* section in the NSERC *Program Guide for Professors* concerning the eligibility of expenditures for the direct costs of research and the regulations governing the use of grant funds, and *Guidelines for Evaluating Cost-Sharing Ratios and In-Kind Contributions in University-Industry Collaborations* regarding the eligibility of in-kind contributions.

Name of supporting organization

Lumerical Computational Solutions, Inc.

CONTRIBUTIONS FROM SUPPORTING ORGANIZATIONS

	Year 1	Year 2	Year 3	Year 4	Year 5
Cash contributions to direct costs of research (Transfer amounts to page five (5); except those for the Ship Time program.)	0	0	0	0	0
In-kind contributions to direct costs of research					
1) Salaries for scientific and technical staff	0	0	0	0	0
2) Donation of equipment, software	2,400	1,200	1,200	1,200	1,200
3) Donation of material	0	0	0	0	0
4) Field work logistics	0	0	0	0	0
5) Provision of services	0	0	0	0	0
6)	0	0	0	0	0
Total of in-kind contributions to direct costs of research	2,400	1,200	1,200	1,200	1,200
In-kind contributions to indirect costs of research (not leveraged)					
1) Use of organization's facilities	0	0	0	0	0
2) Salaries of managerial and administrative staff	0	0	0	0	0
3)	0	0	0	0	0
Total of all in-kind contributions	2,400	1,200	1,200	1,200	1,200
Contribution to university overhead	0	0	0	0	0

UNIVERSITY OF TORONTO
FACULTY OF ARTS & SCIENCE

The Dean

12 February 2008

Dr. Shaheer A. Mikahial
Research Partnerships Programs
NSERC
350 Albert Street
Ottawa, ON K1A 1H5

Dear Shaheer,

We are writing to enunciate the financial and material support the Department of Chemistry and the Faculty of Arts & Sciences will provide to support the NSERC Strategic Network for Bioplasmonic Systems or BiopSys pending success in the grants competition. This is a strong proposal, closely aligned in areas of growth in Chemistry and the Faculty overall, that we believe has tremendous potential for significant impact in the areas of nanobiotechnology.

The Faculty of Arts & Sciences will provide \$93,750 in cash over the proposed five year period of the BiopSys Network. This sum of \$93,750 will be matched in-kind by the Department of Chemistry of staff time (~0.3 FTE) from the Department's finance and grants office to provide comptroller support to the Network.

Additionally, the Department of Chemistry will allocate an office, within the Lash Miller complex, for the BiopSys Network to house the Manager and Secretary. The Department will ensure the office is updated per the needs of efficient overall management of the Network.

Our support is driven by the quality of the proposal and your leadership in putting together an exceptional team of researchers that span multiple institutions. We are particularly pleased to partner with a research and technology endeavour that has the potential to realize real advances in the area of health diagnostic techniques here in Canada.

We wish you all the best!

Sincerely,



Pekka K. Sinervo
Dean and Vice-Provost
First-Entry Programs, University of Toronto



Scott Mabury
Professor and Chair
Department of Chemistry



Sidney Smith Hall
100 St. George Street
Suite 2005
Toronto, ON M5S 3G3
Canada

Tel: 416-978-3383
Fax: 416-978-3887
officeofthedean@artsci.utoronto.ca
www.artsci.utoronto.ca

February 8, 2008

Dr. Shaheer A. Mikhail
Research Partnerships Programs
350 Albert Street
Ottawa, ON
K1A 1H5

Dear Dr. Mikhail:

I would like to express my support of the NSERC Strategic Network proposal: BiopSys, which is based on Plasmonics. This research area is an important direction in the future of diagnostics with enhanced sensitivity, and thus has potential major impact for Axela Inc.

Axela Inc. is a fast growing, venture-backed company that was chosen as one of the top 10 Life Sciences companies in Canada in 2005/2006. Axela has 30 full-time employees and is expanding. The dotLab System™ is sensitive enough to handle many challenging bioassays; nevertheless, we are always on the lookout for major improvements in sensitivity that will enable an unprecedented capacity for the early detection of disease. My understanding is that plasmonics can be an efficient means of energy transfer that can result in ultrahigh sensitivity biosensors. Axela would be happy to participate actively in the project, including providing assistance with research definition in the area of medical diagnostics, which is our expertise. As you know, Axela has an excellent track record of working with University researchers and in commercializing University research. We are currently working with several project team members, including Professors Goh and Sipe.

Sincerely yours,



Rocky Ganske
President and CEO
Axela Inc.

February 12, 2008

Dr. Shaheer A. Mikhail
Strategic Network Grants Program
Research Partnerships Programs at NSERC
350 Albert Street
Ottawa, ON K1A 1H5

Dear Dr. Mikhail:

As a Canadian-based supplier of optical design software, Lumerical Solutions, Inc. ("Lumerical") strongly supports the establishment of a nanoplasmatics network within the Canadian R&D community under the BiopSys: NSERC Strategic Network for Bioplasmonic Systems proposal. Surface plasmon effects offer compelling new capabilities to product designers and will ultimately be exploited in next-generation optical and biophotonic components.

While nanoplasmatics is one of six promising areas identified by Lumerical, limited in-house resources have impaired our own ability to adequately assess future design requirements for commercial plasmonic technologies. Thus the BiopSys proposal – which aims to establish a pan-Canadian research initiative to develop biomedical sensors based on plasmonic effects – would benefit Lumerical greatly. In particular, as a supplier of industry-standard finite-difference time-domain (FDTD) software for high-performance computing systems, Lumerical stands to benefit from better understanding the extent to which FDTD can provide accurate predictions of device and component performance, and how competitive design tools benchmark against FDTD.

With the eventual goal of commercializing nanoplasmatic devices and components, computationally-viable design software will be required by component and system designers. It is this niche that Lumerical aims to address. Working in conjunction with the proposed network will allow Lumerical to benefit from the knowledge generated, and will better equip Lumerical to engineer design solutions that can facilitate the commercialization of nanoplasmatic technologies. Indeed, with our design software running on 1000+ processor computing systems within the Canadian academic community, and with Fortune 500 customers that span the biomedical (Applied Biosystems), optical storage (Hitachi), digital imaging (Olympus), materials

science (3M) and semiconductor manufacturing (Intel) industries, Lumerical is uniquely positioned to deliver improved design capabilities based on the know-how generated within the proposed network. To this end, Lumerical is prepared to commit two days in the first year, and one day in each of the remaining years, of Dr. James Pond, Lumerical's Chief Technology Officer, to discuss with network members their unique design needs as it relates to developing commercial quality design software for biomedical technologies that exploit surface plasmon effects. At a going rate of \$1,200 per day not including expenses, this represents a total in-kind contribution of \$7,200 to the proposed network. As three network members currently employ Lumerical's design software, these communications may result in timely improvements being made to those products such that the network is better equipped to meet the design challenges that lay ahead.

Sincerely yours,



Todd Kleckner, PhD
Chief Operating Officer
Lumerical Solutions, Inc.



February 11th, 2008

Dr. Saheer A. Mikhail
Strategic Network Grants Program,
Research Partnerships Programs at NSERC
350 Albert Street
Ottawa, ON K1A 1H5

**Subject: Strategic Network for Bioplasmonic Systems, Principal Investigator,
Professor Gilbert Walker, University of Toronto**

Dear Dr. Mikhail:

It is with great pleasure that we write to strongly support the proposal for the NSERC Strategic Network for Bioplasmonic Systems.

NanoQuébec is a not-for-profit corporation whose principal funding partners are the Ministère du Développement économique, de l'Innovation et de l'Exportation (MDEIE) and Canada Economic Development. Our corporation plays a central role in the structuring and planning efforts in nanotechnology in Québec. Our mission is to strengthen innovation in nanotechnology in order to ensure solid and sustained economic well being in the Province. More specifically, NanoQuébec encourages the development of a competitiveness pole for the Province of Québec in fields linked to nanotechnologies and has a main responsibility to position Québec on a national and global scale as a leader in development of nanotechnologies.

Plasmonics is an important field of nanotechnology and offers significant potential opportunities for social and economic benefit for Québec and for Canada. We foresee that plasmonics forms the basis for future development and outcomes in the health and biomedical sectors. For instance, in this Network, new technologies will be developed for diagnosis and early detection of cancers, one of the major health problems our population is facing.

The formation of this Network is of great importance for Québec and Canada. It offers the possibility to strengthen Canadian expertise in the field of bioplasmonics by putting together a critical mass of researchers from all across the country. NanoQuébec considers that it is important that Québec researchers participate in this Network, allowing them to interact with other national experts in plasmonics, potentially leading to new and innovative technologies. In addition, the Network will fulfill the pressing and anticipated need for more qualified personnel in this field in Canada.

NanoQuébec is already supporting the nanotechnology infrastructures in many Québec institutions and universities. One of the key researchers of this Network, Professor Michel Meunier from École Polytechnique, has access to unique and up to date micro/nano fabrication facilities on campus partially financed by NanoQuébec. It is understood that Professor Meunier and his students will be using these infrastructures in the development of collaborative bioplasmonics projects within the BiopSys Network.

NanoQuébec strongly supports this initiative, which we believe will result in the development of new bioplasmonics technologies for the health sector and biomedical industries. For all the reasons mentioned above, we enthusiastically support the creation of this Network and we hope that NSERC will favourably consider this proposal.



Sylvain Cofsky
Director General
NanoQuébec



February 11, 2008

Dear Dr. Shaheer A. Mikhail, NSERC Strategic Grants Program –

I am writing to support Walker et al's application for 'BiopSys: NSERC Strategic Network for Bioplasmonic Systems" to be funded by NSERC and to update our letter of support included in their original Lol. Northern Nanotechnologies is a Canadian based supplier of nanomaterials and nanomaterial-based solutions to industrial and academic partners. Our platform technology allows us to produce scalable quantities of cost-effective, customizable nanomaterials, including metals, metal alloys, semiconductors, oxides, and complex nanomaterials. These nanomaterials can be incorporated into other products in order to add functionality, decrease costs, or otherwise improve our partners' products.

To date, much of our focus has been on the catalyst and coatings industries. This is primarily because of the relatively short commercialization timelines and low technical complexity of these products. In the longer term, however, we are extremely interested in developing products for the cleantech, biological, and device industries, which requires an understanding of energy transport to and from our nanoparticles.

The "Network for Bioplasmonics" proposal aims to establish a Canada-wide virtual center of excellence to investigate promising biomedical industrial applications. Because many of our materials are surface plasmon active, and because they can be engineered to have improved plasmonic properties, the establishment of this network should have a dramatic impact on the successful incorporation of our materials into optical or electrically interacting devices. Targeted, applied University research as described in this proposal is the optimal way to speed innovative research to commercial success. Particularly of interest are applications to solar energy capture, optical information technology, biomedical sensing and imaging, and in electronic integrated circuits.

We have been involved with the Network team as the attached Proposal has been developed, and have already started research projects with three of the Network applicants: Prof. M. Cynthia Goh, Prof. Amr Helmy, and Prof. John Sipe. All three projects have received cash and in-kind contributions from Northern Nanotechnologies. We anticipate extensive additional collaboration with Network members and expect to work closely on sample exchange, characterization studies, blue-sky research, and custom projects related to our customers' needs. We also are eager to serve as a Canadian nanomaterials receptor company for commercialisable technologies developed by Network members. We currently expect to contribute the following annual in-kind contributions to the project:

- 20x each 100 mL samples of gold and silver nanoparticles (commercial value \$8000 / a).
- 1 day of our CTO's time / month for advisory purposes (\$2000 / day – total \$24000 / a).
- 1 day / month scientist time to assist with experimental design / characterization (\$1200 / day – total \$14400 / a)

In short, I would like to strongly support the attached application, and I am happy to answer any additional questions you may have.

Sincerely,

A handwritten signature in blue ink that appears to read "Darren Anderson".

Darren Anderson, Chief Technical Officer, Northern Nanotechnologies Inc.
Phone: (416) 260-8889x222, Email: danderson@nntech.com



February 8, 2008

Shaheer A. Mikhail
Research Partnerships Programs
NSERC
350 Albert Street
Ottawa, ON K1A 1H5

Dear Dr. Mikhail:

I am writing to express enthusiastic support for "BiopSys: NSERC Strategic Network for Bioplasmonic Systems".

SHARCNET is a consortium of 16 colleges, universities and research institutes in Ontario offering high-performance computing (HPC) resources and services to our members and to Canadian researchers. Our infrastructure includes comprehensive high-end computing, networking, massive data storage and visualization. We also offer a collaborative environment spanning all of our partners and reaching beyond to many other Canadian universities. All of our infrastructure is available on a dedicated, advanced fibre optic network that connects to the Ontario provincial network, ORION, and to the national backbone, CA*Net4. Complementary to this infrastructure we offer a range of training, consultation and support services. Our goal is to foster the use of this advanced technology for the acceleration of research results and to enable our members to undertake investigations that would otherwise be simply impossible.

SHARCNET is very pleased to partner on this project. Biomedical technology is a rapidly expanding, and critically important, industry sector in Canada, and this Strategic Network promises to deliver key advances in this area. In partnering on the research project, SHARCNET recognizes the importance of this sector to the economy of Canada and health of Canadians, and is eager to position itself as an enabler of this activity. Whilst our user base is still dominated by the traditional HPC disciplines of Chemistry, Physics and Engineering, we recognize the importance of fostering and supporting activity using HPC in new and emerging areas, particularly in health and health-related technologies. Our support of this initiative is exactly aligned with our mission to accelerate the production of research results, to help attract and retain the best students, researchers and faculty and to promote academic-industrial partnerships. SHARCNET also sees benefit in partnering with a network that reaches across many institutions within Canada and this proposal will strengthen the bridges being built amongst the various HPC consortia as part of the "Compute Canada" initiative to build a "National HPC Platform". SHARCNET is delighted to be able to leverage its funding with the NSERC Strategic Network program.

..../2

SHARCNET will formally support this research project in the following ways:

- provide 200,000 CPU hours/year for five years on a priority basis together with required storage as negotiated (this corresponds to an approximate contribution of \$50,000/year).
- provide technical support for code porting and optimization of up to 15 days per year for the first three years (at the rates charged to our commercial customers this corresponds to a contribution of \$21,000/year)
- make available the "AccessGrid" videoconferencing facility, and the technical expertise to connect the BiopSys network nodes and researchers on the West Coast and in Toronto. This capability enables multipoint videoconferenced meetings, seminars, and conferences.
- provide support for distinguished visitor seminars of interest to both the SHARCNET and BiopSys communities

In summary, SHARCNET is able and committed to provide support for the BiopSys proposal. The proposal targets an area of importance for Canada that is of immediate interest to SHARCNET. We lend our enthusiastic support.

Yours sincerely,



Hugh M. P. Couchman
Scientific Director, SHARCNET



NSERC
Strategic Network Grants Program
350 Albert St.
Ottawa, ON K1A 1H5

February 10th, 2008

Subject: Letter of support for “BiopSys: NSERC Strategic Network for Bioplasmonic Systems”

Dear Members of the NSERC Strategic Networks Program Committee:

Spectralis is pleased to write this letter in support of the proposed NSERC Strategic Network entitled “BiopSys”. Spectalis has developed an extensive portfolio of intellectual property involving surface plasmons and their applications, and is in the early stages of developing a biosensor product for drug discovery. As one of only a few companies worldwide exploiting nascent plasmonic technology, Spectalis greatly values its relationships with academic researchers working in this exciting and emerging field, and is very interested in the research projects described in this proposal.

We enthusiastically look forward to our continued involvement with the network participants, having already helped define research directions and projects of interest to Spectalis. Spectalis is already participating with academic researchers in this network on industrially-driven plasmonics research, and as such, Spectalis has already donated to the University of Ottawa surface plasmon devices and equipment valued at over \$ 70,000 and \$260,000, respectively. This donation although used to leverage other grant programs will nonetheless be available to the network participants through the participation of the University of Ottawa in the network.

The benefits of the network to Spectalis are numerous and include access to a network of world-class researchers and academics, potential access to micro-fabrication facilities and eventually intellectual property, and finally to highly qualified personnel trained specifically in plasmonics and hence of obvious interest to Spectalis. The topics under investigation are also of great interest, our participation allowing us to monitor progress on numerous complementary approaches. These benefits justify our keen interest in this network.

The proposal is in the area of biomedical technologies, an area of strategic importance to Canada. As an emerging player in this field, Spectalis recognizes the importance of collaborations with other industrial partners and academics.

In summary, we see plenty of potential value to Spectalis so we are pleased to provide this letter in support of the proposed network and we look forward to working with the researchers involved.

Yours truly,

A handwritten signature in black ink, appearing to read "Nazmin Alani".

Nazmin Alani
Chairman of the board



Nanotech BC

Advancing nanotechnology to
accelerate growth and competitiveness
in BC's economic sectors

www.nanotechbc.ca

February 12th, 2008

Dr. Shaheer A. Mikhail
Strategic Network Grants Program,
Research Partnerships Programs,
Natural Sciences and Engineering Research Council
350 Albert Street
Ottawa, ON K1A 1H5

Re.: Strategic Network for Bioplasmonic Systems: BiopSys

Dear Dr. Mikhail,

We are pleased to offer our support to the BiopSys network. The objective of this network is to develop devices based on metallic nanostructures for cancer detection and treatment. The implementation of this network will also facilitate further development of new generations of nanotechnologies in Canada. This is an innovative proposal that merits support from NSERC.

The British Columbia Nanotechnology Alliance (Nanotech BC) is dedicated to stimulating new economic activity based upon innovative applications of nanotechnology. The area of NanoMedicine is especially important to British Columbia; we have a core of dedicated and capable researchers, as well as several innovative companies working in this discipline. Nanotech BC is always looking for ways to support this important sector.

The plasmonic research proposed by this network is highly multidisciplinary and encompasses several aspects of nanotechnology. The proposal also targets improved diagnostic methods for two of the most challenging types of cancer: leukemia and lung cancer. The successful implementation of these methods may allow faster diagnostics and, consequently, higher survival rates for these cancers. Applying these diagnostic systems and processes to other cancers will be a logical subsequent step. Hence, the proposed research should provide significant social benefits to British Columbia and the rest of Canada.

The BiopSys Network will allow BC researchers to interact with colleagues across Canada. The programmed exchanges will attract workers from some of the leading plasmonic laboratories in Canada to BC. The network will also help with the recruiting of highly qualified personnel who can only enhance nanotech research and businesses in British Columbia.



Nanotech BC

The British Columbia Nanotechnology Alliance strongly supports the BiopSys network. The development of devices for cancer detection and treatment through nanotechnology is an important part of a new generation of health technologies. There is an unprecedented opportunity for Canada to lead on this area. British Columbia will greatly benefit from the involvement of local researchers on the implementation of these devices. The potential health, economic and social benefits are significant. BC companies can play a significant role in commercializing these technologies in the province. Nanotech BC has been and would continue to be instrumental in helping BC researchers and businesses work together for mutual benefit with the appropriated support for efficient networking with interested partners.

Please contact me if you have any questions or require more information.

Yours truly,

Darren Frew,
Executive Director,
BC Nanotechnology Alliance

:df



National Research Council
of Canada
Danial D.M. Wayner
Steacie Institute for
Molecular Sciences
Tel: (613) 993-1212
email: dan.wayner@nrc-cnrc.gc.ca

Conseil national de recherches
Canada
Danial D.M. Wayner
Institut Steacie des
sciences moléculaires
FAX: (613) 954-5242

NRC - CNRC

February 11, 2008

Professor Gilbert C. Walker
Canada Research Chair and Professor of Chemistry
University of Toronto
80 St. George St.
Toronto, ON M5S 3H6
Phone : 416 946 8401
FAX : 416 946 3649
Email : gilbert.walker@utoronto.ca

Dear Professor Walker:

It is my pleasure to write this letter in support of the BiopSys: NSERC Strategic Network for Bioplasmonic Systems. The establishment of BiopSys network is to shorten the time needed for diagnosis and improve prognosis for cancers, such as lung cancer and leukemia, by incorporating plasmonics into diagnostic platforms.

As you may know, the NRC-SIMS strives to be cutting-edge in molecular sciences, carefully selecting areas of research that will bring the results of discovery to bear on Canada's innovation system. NRC-SIMS favours interdisciplinary work with national and international partners. Its principal clients are universities, industry and other NRC institutes. The mission for NRC-SIMS is to provide leadership in collaboration with national and international scientific communities for the development of a knowledge base in molecular science and to ensure that it positively impacts Canadians through proactive knowledge dissemination to partners. The NRC-SIMS-Ottawa teams offer expertise in chemical synthesis, material characterizations, understanding the chemistry of biological processes, predicting material properties. With its research partners, NRC-SIMS helps develop innovative technologies across a wide spectrum including: therapeutics and diagnostics.

I have noticed that our recent recruited researcher, Dr. Shan Zou has been working with members of the network (Prof. Kumacheva and Prof. Walker), and will interact with lung cancer and leukemia specialists Dr. Ming Tsao and Dr. Chen Wang. I am also informed that Dr. Zou has been on conference calls and had face to face meetings with you and other network members. I am confident that Dr. Zou will partner with the BiopSys network by providing advice on supramolecular chemistry and AFM studies that could help with clarifying the antibody –antigen recognition.

Canada

I also note that the objective of the network is to develop better alternatives to current fluorescence methods for cell-surface receptor detection, and the local illumination of plasmonics offers significant opportunities for achieving this. BiopSys will, for the first time in Canada, focus and integrate the full range of skills from the many different disciplines that are required to create devices for detecting leukemia, which will greatly shorten the time needed for diagnosis. The network and the scope and importance of the topics will create stimulating opportunities to carry out world leading collaborative research for the benefit of Canadians. NRC-SIMS is very much supportive of this NSERC Strategic Network for Bioplasmonic Systems proposal.

Sincerely,

A handwritten signature in blue ink, appearing to read "D. Wayner".

Danial D.M. Wayner
Director General

Budget Justification**Theme 1:***Metal particles:*

- Personnel: One graduate student (École Polytechnique Montréal, Directed by M. Meunier) to synthesize nanoparticles by laser and develop improved size control
- Maintenance of the laser system (10% of the maintenance cost of the Spectra-Physics laser, \$30,000/year): \$3,000/year
- Chemicals, targets (Au, Ag, ...),...: \$2,000/year
- Optical characterisation user fees: \$1,000/year
- Structural characterisation (TEM,...) user fees : \$2,000/year
- Cost for shipping samples to collaborators (26 packages/year): \$1,300

- Personnel: One graduate student (University of Toronto, Directed by W. Chan) to design, make, purify and characterize the metallic nanoparticles of different shapes generated using reductive chemistry
- Reagents (e.g., gold chloride, CTAB) and glassware for synthesizing nanoparticles will be required (~\$6,000 per student per year).
- Facilities to analyze nanoparticles and SERS-nanoprobes (\$2,500 per student per year). Facilities for transmission electron microscopy (\$50 per hour) and Inductive-coupled plasma-atomic emission spectroscopy (\$35 per hour).
- Cost for shipping samples to collaborators (6 packages/year): \$300

- Personnel: One graduate student (University of Toronto, Directed by E. Kumacheva with S. Zou on his/her thesis committee) to graft polymers and recognition ligands on rod-shaped particles to make structures whose raman signals increase upon antigen binding
- Chemicals \$4,000/yr
- Characterization (TEM) user fees \$2,500/yr
- Cost for shipping samples to collaborators (6 packages/year): \$300

Ligand Synthesis:

- Personnel: One graduate student (University of Toronto, Directed by W. Chan) to design and characterize the SERS-nanoprobes, primarily the ligand chemistry and raman tagging, but will collaborate closely with Walker's student regarding Raman spectroscopy
 - Antibodies \$10,000/yr
 - Characterization (NMR, mass spec,...) user fees \$2,500/yr
 - Cost for shipping samples to collaborators (12 packages/year): \$600

 - Personnel: One graduate student (University of Toronto, Directed by G. Zheng) to develop and apply peptide ligands
 - Chemicals \$5,000/yr
 - Antibodies \$8,000/yr
 - Characterization (NMR, mass spec,...) user fees \$2,500/yr
 - Cost for shipping samples to collaborators (26 packages/year): \$1,300
-

- Personnel: One Post-doc (University of Toronto, Directed by G. Zheng, Co-Directed by G. Walker) to graft peptide and antibodies onto network plasmonic structures, servicing the investigators with planar structure morphologies in Theme 2 (waveguides and particles on surfaces) as well as the fiber probe approach in Theme 3
- Chemicals \$5,000/yr
- Antibodies \$10,000/yr
- Characterization (NMR, mass spec,...) user fees \$2,500/yr
- Cost for shipping samples to collaborators (26 packages/year): \$1,300

Grafting Characterization:

- Personnel: One graduate student (University of Toronto, Directed by G. Walker, Co-Directed by A. Helmy)
- Raman spectroscopy, UV-vis/CD/fluorescence time, \$6/hour user fees
 - 48 weeks*20 hours/wk at \$6 per hour user fees = \$5,760
- Chemicals: \$1,340
- Cost for shipping samples to collaborators (12 packages/year): \$600

Theme 2:

SPR:

- Personnel: One graduate student (École Polytechnique Montréal, Directed by M. Meunier, Co-Directed by C. Wang)
- Development and application of ultrasensitive surface plasmon resonance
- Small optics (posts, mirrors, lenses ...): \$2,000/year
- Small equipment (electronics ...): \$1,000/year
- Micromachining of small pieces: \$1,000/year
- Chemicals and biological species: \$3,000/year
- Cost for shipping samples to collaborators (10 packages/year): \$500/year

Hole Arrays:

- One student (University of Victoria, Directed by R. Gordon, Co-Directed by G. Zheng and G. Walker) The student will contribute to all aspects of this hole project, covering theory-design-fabrication-experimentation. From past experience, it is best to have the student who is doing the measurements fabricating because then s/he will have the best idea of how to fabricate the samples so that they are easily measured. By the same token, the student who is doing the fabrication will have the best idea of what calculations are required in designs that are actually possible. 154 hrs (3 days every 2 months) fabrication time at SFU 4D Labs @ \$50/hr: \$7,700/yr – however, a \$3,000 annual cap has been negotiated.
- 6 trips to SFU for fabrication (Ferry + 3 nights accommodation) @ \$350: \$2,100/yr
 - Justification: From past experience, at least 1 nanofabrication run of 3 days is required every 2 months per project. This gives the student time to design (using in-house computational resources), fabricate, test (using in-house spectroscopy, including transmission, Raman, and ultra-fast nonlinear spectroscopy), and then iterate, and thereby to close the theory-design-fabricate-experiment loop. From past experience, each design

will require refinement after a first run of tests, which can be cascaded onto the following fabrication run, so this will allow for 5-6 attempts at a successful device per year.

- Metals for evaporation: \$500/yr
 - Evaporation time: \$200/yr
 - Si and gold-on-glass Substrates: \$1,000/yr
 - Justification: For the nanohole fabrication, FIB milling is used to drill nanoscopic hole arrays in metal-film substrates. Commercial gold-on-glass substrates are used due to superior film quality. Due to bulk purchasing, these are typically limited to one thickness (100 nm). For other thicknesses and for different metals, we evaporate our own metals, which can be done on mass, about 4 times a year (\$50/hr). Silicon substrates are used as flat materials that can be processed without the need for HF etching (safety concern).
 - Optics supplies: \$500/yr
 - Justification: From past experience, additional optics (mirrors, lenses, microscope objectives, filters) and supplies (cleaning solutions, lens paper, gloves) are required for transmission spectrum measurements, Raman scattering measurements, nonlinear spectroscopy measurements. While Dr. Gordon typically spends >\$2000/yr on such supplies, \$500 is allocated to this project.
 - Chemicals: \$1,500/yr
 - Student training: \$2000
 - Justification: One time training fee for SFU 4D Labs.
 - Cost for shipping samples to collaborators (12 packages/year): \$600
-

- Two students (University of Victoria, Directed by A. Brolo, Co-Directed by R. Gordon) One student to develop micro-arrays based on SPR. One student to develop micro-arrays based on SERS/SEFS.
- *Operation and Maintenance Costs:* There several *major pieces of equipments* in our laboratory that will be used in this project and need to be maintained. These include two ion lasers, two Raman microscopes, the ultra-fast laser system, the detection for nonlinear optics and the SPR apparatus. We estimate \$500/year per student as a minimum maintenance cost. This fund will be used to purchase, liquid nitrogen for the detectors, replacement optics and mirrors for the lasers and spectrometers, and to upgrade our computational hardware and the data acquisition systems when required.

- *User fees:* User fees for the fabrication (FIB and photolithography) and for imaging characterization (AFM, STM, TEM, SEM) facilities. Some of the typical costs are: \$50/hour - microfab room, - \$200/each mask for photolithography, \$30/hour – SEM; \$50/hour FIB; \$35/hour STEM; \$50/hour for AFM/STM + tips (ca \$80/tip).

The flat rate for the SFU nanoimaging facility is 3K/year. This would cover the FIB, SEM and STEM costs. We estimate an extra 2K to cover the photolithography and microchannels fabrication. So, the total here would be \$5,000 or \$2,500/student.

- Trips to SFU and FIB training – We estimate a \$1,000 for 3 trips a year per student. The training cost is around \$2,000/student.
- *Materials and Supplies:*

CD\$ 3,500/year per student will be necessary. This amount will be spent in the following materials and supplies:

- Chemicals, glassware and general laboratory supplies - \$500/student
- Bio-reagents for affinity tests (labeled streptavidin for proof of concept experiments). \$750/student
- Au salts and Au metal. \$750/student

- Gold deposited glass slides. \$500/student
- Material for the fabrication of cells and microfluidic devices. \$750/student
- According to UVic's policy, we just need to pay for the materials in the mechanical shop. We estimate a \$250/student will be required. This will cover all the shop needs, from the simple construction of holders to the design and fabrication of cells.

Particles on surfaces

- One theory student (University of Windsor, Directed by C. Rangan, Co-Directed by J. Sipe) to work on establishing Figures of Merit. Also, this theory student will begin by developing an understanding about critical parameters for the device performance, e.g. optimal gold nanoparticle distances for a given nanoparticle size having the optical and size data of the recognition chemicals for lung cancer and leukemia in mind.
- \$3,000/year: computer supplies

- One experimental student (University of Western Ontario, Directed by S. Mittler, Co-Directed by G. Walker and C. Wang) to make nanoparticle arrays on waveguides, with responsibility for the fabrication and characterization efforts of the plasmonic material. Walker will provide advice on chemical functionalization and Wang will provide advice on appropriate leukemia protein targets.
- \$8,000/year: nanofabrication user fees

- One student (University of Toronto, Directed by C. Goh, Co-Directed by J. Sipe) to develop diffractively coupled surface sensor. Axela will provide input regarding practicality of approach.
- \$5,000/year: chemicals and biochemicals

- One student (University of Toronto, Directed by T. Pawson, Co-Directed by C. Wang and G. Walker) to develop a sensor of network signaling, using patterned substrates to induce specific cellular responses. The work also will connect to surface adsorption responses of cells on patterned surfaces being produced in the Brolo and Mittler groups.
- \$5,000/year: chemicals and biochemicals for grafting surfaces generated by student from UWO.

One student (University of Toronto, Directed by A. Helmy) to fabricate capillary with particles coated on internal surface. S/he also will accomplish Raman spectroscopy of the same, aiming to identify the magnitude of enhancement caused by the increased light-particle interaction.

- \$5,000/year: chemicals and biochemicals

Waveguides:

- One theory student (University of Toronto or University, Directed by J. Sipe or P. Berini) to undertake comprehensive theoretical work in order to assess and compare the signal to noise ratio of various sensor architectures (e.g.: MZI), implemented with surface plasmon waveguides
- \$3,000/year computing supplies

- One student (University of Toronto, Directed by M. Mojahedi, Co-Directed by P. Berini) to concentrate on GASP (Gain assisted surface plasmon) for the proposed device with optical pumping
- Fabrication/user fees: \$5,000/year cost associated with growing the quantum well wafers (MBE, outside of ECTI), cleanroom fees for sample processing here at ECTI and at CHTM/Cornell facilities

-
- One experimental student (University of Toronto, Directed by S. Aitchison, Co-Directed by J. Sipe) to focus on modeling and the extension of the GASP to electrical pumping and SPASERS
 - Fabrication/user fees: \$5,000/year Cost associated with growing the quantum well wafers (MBE, outside of ECTI), cleanroom fees for sample processing here at ECTI and at CHTM/Cornell facilities.

 - One experimental (University of Ottawa or University of Victoria, Directed by P. Berini, Co-Directed by A. Brolo, or Directed by A. Brolo, Co-directed by P. Berini) to develop methods for electrochemical functionalisation.
 - Supplies: \$3,000/year chemicals

Microfluidics:

- One student (University of Toronto, Directed by A. Wheeler, Co-Directed by A. Brolo) to evaluate two strategies for fluidic handling of samples and reagents for bioplasmonic sensing, one relying on networks of microchannels, and another relying on digital microfluidics
- Fabrication/user fees: \$3,000/year, to lay down channels and prepares surfaces.
- Supplies: \$2,000/year chemicals and minor supplies

Theme 3:

Leukemia:

- One student (University of Toronto, Directed by G. Walker or W. Chan) to work with materials generated by Chan's, Kumacheva's and Zheng's lab, to develop anti body based panel screen
- \$7500/year: chemicals, biologicals, facilities
- One post-doc (University of Toronto, Directed by C. Wang) to work with student above, assisting with panel design and development of controls, but moreover to serve as daily facilitator for students in the network to hematology resources/concepts in Mt. Sinai.
- \$7500/year: chemicals, biologicals, facilities

Lung Cancer:

- One student (University of Toronto, Directed by G. Zheng or B. Wilson) to prepare the necessary SERS functionalized particles ultimately to be used in the spray bronchoscopy approach
- One post-doc (University of Toronto, Directed by M. Tsao, Co-Directed by B. Wilson) to focus on the development of suitable delivery and optics for the bronchoscope. The post-doc will interact with Brolo in the hole array approach.
- \$15k/yr in years 1 and 2; \$10,000/year in years 3,4, and 5: supplies: materials/consumables/tissue culture
- \$5,000/year in years 3,4,5: animals

Travel Support:

- All students and post-docs in the Toronto area are allocated \$1,000/year to visit collaborators outside of the Toronto area
- All students and post-docs outside of the GTA are allocated \$2,000/year to visit collaborators.
- Students and post-docs are allocated \$1,000/year to attend North American conferences.

Please note that, as described in the main body of the text, there is a predicted mix of MS and PhD students. Students in Chemistry departments either enter as MS students and then transition to PhD or enter directly as PhD students; the budget reflects this distribution, with tasks and training appropriately adjusted.

Table 1. HQP Training by Theme and Year

Theme/YR	MS Students	PhD students	Post-doctorals
I/1,2,3,4,5	2,0,0,2,0	4,6,6,4,6	1,1,1,1,1,1
II/1,2,3,4,5	4,1,0,4,0	11,14,15,11,15	0,0,0,0,0,0
III/1,2,3,4,5		2,2,2,2,2	2,2,2,2,2

Summary of Facilities to be Used:***University of Western Ontario:******Nanofabrication Lab:***

1. Nanofabrication with various ways of lithography: electrons, photons and ions
2. Characterization with scanning electron microscopy, XRD, ellipsometry
3. Deposition of metals, SiO, SiN, SiON

Costs: Personpower : \$50/hour

Most tools: typically \$50/hour

Surface Science Western:

1. Tof-SIMS
2. XPS
3. Contact Angle
4. FTIR
5. Raman spectroscopy

Cost: typically \$100/hour

Particle Research Facilities:

1. Imaging Microscopy and Surface Analysis: Scanning Electron Microscopy (SEM) and BSE Imaging, EDX analysis
2. High Resolution Optical Microscopy, with temperature control

Chemical and Biochemical Analysis:

1. High Performance Liquid Chromatography
2. Gas Chromatography
3. Mass Spectrometry

Costs: typically around 50\$ /h for Western clients and roughly double for non-Western clients

Toronto:

Centre for Nanostructure Imaging (CNI):

1. Hitachi HD-2000 Scanning Transmission Electron Microscope (STEM)
2. Hitachi S-5200 Scanning Electron Microscope (SEM)
 - Both are equipped with Energy Dispersive X-Ray Systems (EDS) for elemental analysis at the micron to sub-micron length scales
3. Leica TCS SP2 Laser Confocal/Multiphoton Microscope

Rates for microscopes: \$35/hr for Kumacheva, \$45/hr University, \$100-\$150/hr Industry

Raman Spectroscopy:

- Fully software-controlled and systems based on a 300 mm focal length stigmatic flat field spectrograph microscope for confocal Raman analysis
- Confocal microscope, transfer and filtering optics
- Stigmatic spectrometer equipped with two gratings, and a multi-channel detector
- The spectrograph is attached to a high stability confocal microscope supplied with a xyz manual mechanical stage and confocal coupling optics, which provides lateral and axial resolution better than 0.5 “m and 2 “m respectively using x100 objective.
- Excitation sources covering the UV and visible wavelength range are available
- A holographic notch filter and plasma line rejection filter is also part of the system allowing the resolution of features as close as 50 cm⁻¹ from the excitation source.
- The system is custom build to provide a capability of having 3 detectors simultaneously mounted.
- This provides the unique capability of conducting spectroscopy up to 2.2 “m in both continuous and time resolved modes.
- This capability is just not commercially available and presents opportunities for commercialization of some of the measurements that shall be developed using this system.

Access to these will be provided to all of the project participants with web-based booking and report viewing/retrieval capabilities to expedite progress and facilitate the experiments.

École Polytechnique Montréal / Université de Montréal:

Central Facilities: Thin Film Research Laboratories / Groupe de recherche en physique et technologie des couches minces (GCM) :

1. Equipment with negotiated access:
 - Scanning Electron Microscope (Hitachi S-4700)

Material Characterization:

1. Brewster angle microscope/ellipsometric imaging BAM/I-Elli2000 (Nanofilm)
2. Spectroscopic ellipsometer M-2000V (J.A. Woollam Co)
3. AFM Dimension 3100 (Digital Instruments) with liquid imaging capability
4. AFM ThermoMicroscopes (AutoProbeResearch CP) with electrochemistry option and nanolithography capability.
5. FTIR spectrometer FTS7000

- Monolayer films on metal substrates measurement with polarization modulation (PM-IRRAS)
 - Surface depth profiling using step scan mode
6. Confocal Raman microscope (Renishaw 3000 and InVia)
 - Chemical point-by-point mapping and fast imaging of surfaces with 1 "m spatial resolution
 - Depth profiling
 - Four different laser sources (488, 514, 633 and 780 nm)
 - Motorized stage
7. Continuous wave and time resolved photoluminescence
8. Optical absorption spectroscopy from UV to IR

Laser Processing Laboratory:

1. Laser Processing Facilities
 - Excimer laser micromachining system
 - Cw Nd:Yag (10W) laser microprocessing system
 - Pulsed (50ns) Nd:Yag laser microprocessing system
 - Femtosecond laser (120fs, 1mJ, 1kHz) micro/nanoprocessing system
 - *Femtosecond laser (30fs, 5mJ, 1kHz) micro/nanoprocessing system
 - CO₂ laser (10.6"m, 1"J) material processing system
2. Bioplasmonics Facilities
 - Phase sensitive SPR
 - *Multipurpose SPR analyser
 - *TIRF microscope
 - *Nano3D evanescent field profiler
 - *FT-IR spectrometer with SPR

*To be acquired on the U Montréal CFI grant “NanoBio” during 2008.

University of Ottawa:

Theoretical:

1. Computation of guided and radiation mode spectra for essentially any optical waveguide (1D, 2D)
2. Sensitivity (surface, bulk) computations for any waveguide
3. Modeling of 3D guided-wave structures (Mach-Zehnders, couplers, S-bends,...)
4. Modeling of guided-wave Bragg gratings
5. Spontaneous emission lifetime calculations
6. Optical gain modeling

Experimental:

1. Well-equipped lab for characterising and experimenting with integrated optical devices (major asset list in preparation)
2. Other well-equipped optics labs in the Centre for Research in Photonics at the University of Ottawa (www.photonics.uottawa.ca)
3. Electrochemistry (under construction)
4. Microwaves, anechoic chamber (peripheral)

NRC SIMS-BSI Facilities:

The Biomolecular Sensing and Imaging Group is responsible for maintaining the following facilities for SIMSe:

1. Advanced Imaging Centre including atomic force microscopes, near-field scanning optical microscope, total internal reflection fluorescence microscope, confocal spectrofluorimeter and FTIR, fluorescence and UV-vis-IR spectrometers,
2. Surface Analysis and Preparation equipment including single wavelength ellipsometry, contact angle measurements and a Langmuir Blodgett trough,
3. High-throughput Liquid Handling and Screening utilizing fluorescence and luminescent plate readers to determine the activity of appropriate reporters.
4. Cell Culture Facilities for the growth and maintenance of cell lines to support cell-based screening and imaging activities.

Administration/Management:

Network Manager, to perform managing tasks detailed in the proposal. \$60,000 per year

Secretarial Assistant to assist with reporting and other administration tasks; web site updating every day. To be 50% cost shared by UT in cash support.

Comptroller: To monitor expenses, assist with budgeting tasks and prepare financial reports. Cost-shared as in-kind by UT Department of Chemistry.

Networking:*Semi-Annual Meetings*

- Conference facilities, for 58 participants, including meals: \$3,925.85
- Hotel costs for 22 out of town participants, for 2 nights: \$5,744.95
- Flights for out of town participants: \$12,006.25

City of origin	Ottawa	Victoria	Montreal	London	Windsor	Board
Est. cost of flight	\$330.00	\$890.00	\$335.00	\$340.00	\$500.00	\$500.00
Taxes	\$42.90	\$115.70	\$43.55	\$44.20	\$65.00	\$65.00
Number of participants	3	5	3	2	2	5
<i>Subtotal</i>	<i>\$1,118.70</i>	<i>\$5,028.50</i>	<i>\$1,135.65</i>	<i>\$768.40</i>	<i>\$1,130.00</i>	<i>\$2,825.00</i>

- Ground transportation (taxis to/from airport): \$1,400
- Additional meals for out of town participants: \$950.00
- Workshop costs (supplies and hands-on time): \$13,500
- Total conferencing cost: \$37,527.05/conference or \$75,054.09 yearly

Website

- Design and administration: \$3,000
- Hosting: \$300

Conference Calls

- Cost of twelve, two-hour conference calls: \$2,318.40 yearly

Number of calls	12
Local participants	14
L.D. participants	7
Avg. call length (min.)	120
<i>Cost per call</i>	<i>\$193.20</i>

Total networking costs: \$80,672.49 yearly



University of Toronto

Professor R. Paul Young, Ph.D., FRSC
Vice-President, Research

February 14, 2008

Ms. Janet Walden
Vice President
Research Partnerships Programs,
NSERC
350 Albert Street
Ottawa, ON K1A 1H5

Dear Ms. Walden,

I am pleased to forward the NSERC Strategic Network Grant application for the Network for Bioplasmonic Systems (BiopSys), with Professor Gilbert Walker of the Department of Chemistry as Principal Investigator. The University of Toronto strongly supports this initiative.

This Network represents a unique opportunity to advance not only the University of Toronto's strategic research goals in the area of bionanotechnology, with application to Canada's strategic goals in biomedical technology. It represents a unique opportunity for leading researchers from six universities across Canada to bring to bear complementary strengths in materials fabrication and characterization, device creation, and cancer biology in an important collaborative effort to significantly better health diagnostic techniques in Canada and elsewhere. The proposed applications of nanobiotechnology are key developments in biomedical technology for improved health care platforms, particularly diagnostic systems. These technologies will improve the quality of life for Canadians and the resulting technologies will create jobs and fuel markets.

While the University of Toronto already possesses considerable strengths in nanotechnology, it is strongly committed to further investments in both the human capital and infrastructure required to ensure its continued leadership in this burgeoning new field. This proposal is firmly grounded in the University's targeted areas for growth in Chemistry, Engineering, and Medicine, where multiple new faculty hires and significant investment have already been made. The network will greatly enhance our ability, and the ability of our partners, to attract the brightest and most creative students, researchers, and faculty to Canada.

Professor Gilbert Walker, the Network's Principal Investigator, is an internationally recognized and leading scholar in nanotechnology who has a stellar track record in leading and managing large multi-institutional research and technology development projects in the United States. We were most fortunate in recruiting him to the University of Toronto.

.../2

The University's emphasis on the tremendous potential impact arising from the linkage between discovery and technology spinoff was one of the driving forces attracting him to Toronto and the science, partnership and outreach activities outlined in this proposal provide the means for realizing this impact and for improving the health as well as the economic well being of Canadians.

This Network offers the opportunity for leading researchers in a number of Canadian academic institutions to collaborate in order to make rapid progress in this competitive field. Across Canada there are researchers of great strength in areas of nanobiotechnology that complement our own: The University of Victoria has strength in metamaterial creation; Ecole Polytechnique Montreal has strength in synthesis of nanoparticle of uniform sizes; the University of Western Ontario has excellent nanofabrication facilities; the University of Windsor has recently hired a bright young researcher in nanophotonic theory; and the University of Ottawa has historically been a strong developer of SPR technology. We believe this Network approach is the most effective way to achieve these nanobio collaborations, as do our partners.

Our researchers have developed strong relationships with industry, and these will be key to the scientific and longer-term commercialization objectives of this research, and more broadly, to the Network's success. Commercial partners appreciate and are engaged in the team approach the Network is taking and are interested in commercializing inventions which come from the Network's research program. For example, two of the partnering companies were spun out of labs at the University of Toronto, Northern Nanotechnologies and Axela. The Network's technology transfer strategy has been developed in collaboration with our industrial partners and the technology transfer offices of the collaborating institutions. These partnerships are natural and have led to leveraging opportunities for graduate student employment. Indeed, students were often founding members of these start-ups!

Taking advantage of new and rapidly expanding training programs in nanobiotechnology, the Network will provide students with the skills they will need for rewarding careers in areas of strategic interest to Canada. Students will be encouraged to develop a deep understanding of the core science through cross-disciplinary training in science and engineering. In addition, the new professional masters program in Scientific Entrepreneurship being developed here at the University of Toronto will leverage expanded opportunities in entrepreneurship.

If this proposal is funded, the University of Toronto will contribute \$187,500 over a five year period (\$93,750 in-kind from the Department of Chemistry and \$93,750 cash from the Faculty of Arts and Science), in support of this important work. This contribution is associated with support of outstanding faculty as well as training of students and other highly qualified personnel. Finally, the University will provide suitable office space through its Department of Chemistry to house the network administration staff for the five year duration of the network.

Yours sincerely,

R. Paul Y

Professor R. Paul Young, Ph.D., FRSC
Vice-President, Research

Relationship to Other Research Support

G. WALKER

Support currently held:

CFI, CRC Infrastructure Program, “*Analytical Equipment for Innovations in Nanoscience of Self-Assembled Materials*” (2005). This grant has provided equipment and therefore supports the proposed work. **There is no conceptual and budgetary relationship with the proposed work.**

NIH, National Institute for Biomedical Imaging, “*Near-Field IR Microscope for Nanoscale*,” (2005-2007). This grant supports the development of near field infrared microscopy. The main tool is near field infrared microscopy. The funds are almost all salary and supplies, with about \$50k to buy a laser. One post-doc and two students have been supported by this grant. This R21 grant is not renewable after March 2008. **There is no conceptual or budgetary relationship with the proposed work.**

CIHR, Nano and Regenerative Medicine, “*Near Field Chemical Imaging and Force Spectroscopy of Engineering Tissue Surfaces*,” (2007-2008). This grant supports the development of combined single molecule mechanics and near field microscopy for protein analysis. One post-doc and one grad student are supported by this grant. **There is no conceptual or budgetary relationship with the proposed work.**

ONR, Environmental Quality, “*Spatially Resolved Studies of Polymer Film Dynamics under Water Using Novel Scanning Probe Microscopies*,” (2005-2007). Walker’s nearly expired and expended ONR grant is almost entirely directed to salary and supplies. It supports one post-doc and one student and supplies. It focused on the spatially resolved properties of anti-fouling films and barnacle adhesive. The ONR program officer (Steve McElvany) has said that the current grant will not be renewed. **There is no conceptual or budgetary relationship with the proposed work.**

ORF, “*BioOptics – Transformative Technologies for the Life Sciences*,” (2006-2010). This grant supports the development of IP for optical microscopy. Walker’s support from the ORF BioOptics grant covers two grad students or one post-doc salary and supplies but no equipment. This work is focused on developing miniaturized infrared imaging technology specifically with commercial potential. **There is no conceptual or budgetary relationship with the proposed work.**

ARO Multi-disciplinary University Research Initiative, “*Exploiting Self-Assembly in Biological and Synthetic Macromolecules*,” (2004-2008). This grant to Walker’s Pittsburgh labs cannot be redirected to the University of Toronto. **There is no conceptual or budgetary relationship with the proposed work.**

NSF, Chemistry, “*Collaborative Research in Chemistry: Exploiting Self-Assembly in Biological and Synthetic Macromolecules to Create Novel Hybrid Materials*,” (2004-2008). This grant to Walker’s Pittsburgh labs cannot be redirected to the University of Toronto. There is overlap between this grant and the proposed work. However, this grant ends in June 2008 and is not renewable. **There is no budgetary relationship with the proposed work.**

NSF, Nanoscale Interdisciplinary Research Teams, “*Plasmonic Nanostructured Devices for Chemical and Biological Sensing,*” (2004-2007). This grant to Walker’s Pittsburgh labs cannot be redirected to the University of Toronto. **There is no conceptual or budgetary relationship with the proposed work.**

Support applied for:

NSERC RTI, “*Atomic Force Microscope Controller Upgrade,*” (2008). This proposal aims to purchase a more modern controller for the Dimension microscope in the PIs labs. If funded, this grant will dramatically enhance the speed with which experiments can be done. **There is no conceptual or budgetary relationship with the proposed work.**

NSERC Discovery, “*Scanning Probe Microscopy of Polymer Surface Nanostructures,*” (2008-2012). **There is no conceptual or budgetary relationship with the proposed work.**

J.S. AITCHISON

Support currently held:

NSERC Discovery, “*Periodic Structures for Nonlinear Optics,*” (2006-2010). This grant focuses on the use of micro ring resonators, photonic crystals and waveguide arrays in nonlinear AlGaAs material. **There is no conceptual or budgetary relationship with the proposed work.**

NSERC Strategic, “*Burst femtosecond lasers: Intelligent processing for 5-D spectroscopy and photonics manufacturing,*” (2008-2010). This project is directed at understanding the mechanisms present in femtosecond machined glasses and laser crystals. Research will also be directed at developing waveguide lasers. **There is no conceptual or budgetary relationship with the proposed work.**

Support applied for:

NSERC Strategic Supplemental Competition, “*Phonon Assisted Magnetic Response and Near Field Enhancement at Terahertz and Infrared Frequencies,*” (2008-2009). This project will build on our recent theoretical predictions of a magnetic response in non-magnetic material and negative refraction. If funded this project will work toward experimental demonstrations of these effects. **There is no conceptual or budgetary relationship with the proposed work.**

P. BERINI

Support currently held:

Premier’s Research Excellence Award, “*Enabling Hardware Technologies for Next Generation Optical Fibre Communications*” (2000-2004). This award was obtained before the applicant took his leave of absence (2001-2004) to found Spectalis and would normally be over by now, but the applicant obtained permission to extend the award to 2008 in order to spend residual amounts. The proposal is in the area of hardware technologies for optical fibre communications and the research work is focused on the development of cost-effective high-speed optoelectronic devices and multi-wavelength passive optical components to support current and future demands for high-speed data transmission over optical fibres.

The funds from this award are only used to pay stipends for HQP and research associates as well as their travel expenses to conferences. **There is no overlap with the present proposal.**

NSERC Discovery, “*Integrated Optics Based on Surface Plasmons*” (2006-2010). The objectives of this research program are to establish passive elements and robust 3D modelling capabilities in an integrated optics technology based on metal stripes supporting long-range (low-loss) surface plasmons. Specifically, the work involves proposing and advancing theoretical models for: S-bends, tapers, Y-junctions, couplers, Mach-Zehnder interferometers, multi-mode interferometers and Bragg gratings. The models are verified and validated experimentally using devices fabricated for that purpose. The funds from this grant are used primarily to pay stipends for HQP and to defray the associated costs of conducting the research (materials, supplies, dissemination). There are synergies in that some of the models developed here are useful to the present proposal. **There is no budgetary overlap with the present proposal.**

University of Ottawa, “*A New Integrated Optics Technology*” (2005-2009). This grant was awarded to help re-ignite the applicant’s research program after his leave of absence. The project involves a few topics, including a preliminary study of optical amplification and lasing with long-range surface plasmons using dye molecules in solution, and a preliminary study of surface chemistries for biosensors. The funds from this grant are used primarily to pay stipends for HQP and to defray the associated costs of conducting the research (materials, supplies, dissemination). The applicant’s work under the present proposal builds on the chemistries investigated here. **There is no budgetary overlap with the present proposal.**

OCE/Spectralis, “*Sensing Using Long-range Surface Plasmons*” (2007-2009). The goal of this project is to advance a sensing platform for biomolecular interaction analysis. The platform exploits the new integrated optics technology based on long-range surface plasmons, which is also highly surface sensitive. The project involves integrating microfluidics, applying suitable (thiolated) surface chemistries, demonstrating real-time binding of biospecific pairs, and assessing parameters such as sensitivity and detection limit. Spectalis contributed lab equipment, sensing devices and cash valued at \$180,000 and OCE matched this donation with \$180,000 in cash to be used to defray materials and supplies, but mostly to pay for stipends to HQP and a research associate. The applicant’s work under the present proposal builds on this direction. **There is no budgetary overlap with the present proposal.**

CPFR/ORDF/Spectralis, “*Fabrication of surface plasmon waveguides on membranes*” (2007-2008). The goal of this proposal is to develop a further understanding of membrane waveguides for long-range surface plasmons, consisting of a metal stripe deposited on an ultra-thin large-area free-standing membrane. This project involves the investigation via experimentation of fabrication, mechanical and optical aspects of the structure. Fabrication is carried out in Carleton University’s Microelectronics Fabrication Facility. Spectalis contributed devices worth \$74,400 and CPFR/ORDF contributed \$50,000 in cash to be used to defray materials and supplies, but mostly to pay for stipends to HQP. There are synergies in that some of the fabrication processes developed here could be useful. **There is no budgetary overlap with the present proposal**

Support applied for:

NSERC MRS, “*Carleton University Microfabrication Facility*” (PI: N. G. Tarr). Financial support to help defray the operating costs associated with the Carleton University Microelectronics Fabrication Facility, a laboratory for experimental research on semiconductor process technology and for the

fabrication of novel device prototypes. The funds requested will be used to pay the salary of a technologist, for supplies, and for the maintenance of the equipment. We currently use this facility to fabricate devices for our CPFR-funded membrane waveguide project. **There is no conceptual or budgetary relationship with the proposed work.**

NSERC RTI, “*Plasma Enhanced Chemical Vapour Deposition System for Microsystems Research*” (PI: R. N. Tait). This proposal seeks funding for a plasma-enhanced chemical vapour deposition (PECVD) tool for depositing thin films of α -Si, SiO₂, and Si₃N₄ in support of research in microelectronics, MEMS, sensors and silicon photonics. This tool will be installed within the Carleton University Microelectronics Fabrication Facility. We currently use this facility and intend to use the new tool if awarded. **There is no conceptual or budgetary relationship with the proposed work.**

NSERC RTI, “*Tunable Lasers for Experimentation with Surface Plasmons*”. This proposal seeks funding for two low power continuous-wave laser sources, tunable over important wavelength ranges in the near infrared (Sacher Lasertechnik λ 0 ~ 900 nm, and Agilent λ 0 ~ 1310 nm). These lasers are suitable for general use, testing components, and generating probe signals in pump/probe experiments. Additionally, a broadband low-noise spectrometer with associated hardware and software, a temperature controller, and attachments for an existing instrument used for measuring refractive indices (Meticon) are requested. **There is no conceptual or budgetary relationship with the proposed work.**

NSERC Discovery - Steacie supplement, “*Active Devices Based on Surface Plasmons*”. This proposal seeks funding to conduct research on active devices, consisting of amplifiers and tunable lasers using dye solutions as optical gain media and operating with surface plasmon waves. Tunable 1st order Bragg gratings operating with surface plasmons are investigated as a necessary component in the architectures proposed. Detectors of surface plasmons, based on internal photoemission in a metal/Si Schottky contact, are also investigated. The funds requested will be used primarily to pay stipends for HQP and to defray the associated costs of conducting the research (materials, supplies, dissemination). **There is no conceptual or budgetary relationship with the proposed work.**

NSERC RTI - Steacie supplement, “*Pulsed laser system*”. This proposal seeks funding for an ultra fast, broadly tunable, high peak power, pulsed laser system (Spectra-Physics Ti:Sapphire laser). The system will be used as the optical pump for amplification and lasing experiments using dye molecules, and for conducting absorption and emission spectroscopy. **There is no conceptual or budgetary relationship with the proposed work.**

A.G. BROLO

Support currently held:

NSERC Discovery, “*Optical and Electrochemical Studies Using Metallic Nanostructures,*” (2007-2011). This grant is Dr. Brolo's basic operating support. The grant funds 3 graduate students in projects involving the application of plasmonic structures in electrochemistry. One student is involved on the preparation of gold nanostructures based on the deposition of Au nanoparticles on thin film. These substrates are used as for the investigation of electrode processes *in situ* by SERS. The other student is investigating the adsorption of amino acids on silver electrodes by second harmonic generation. The main objective is to probe if the chiral signature from the amino-acid would yield nonlinear optical activity from the metal-solution interface. Finally, the last student is studying the adsorption of the

amino acid cysteine on single crystalline gold electrode by electrochemical impedance spectroscopy. **There is no conceptual or budgetary relationship with the proposed work.**

W. CHAN

Support currently held:

CFI Infrastructure Operating Grant, “*Integrated Nanotechnology / Biomedical Science Laboratory*,” (2003-2008). Financial support from this grant is used to maintain the equipment purchased by the CFI – New Opportunity Grant. The equipment is used for research for the NSERC discovery grant (and hence, this is the reason we did not request for financial support for equipment or maintenance of equipment).

There is no conceptual or budgetary relationship with the proposed work.

CIHR RFA for Regenerative Medicine and Nanomedicine, “*Quantum Dot-based Biomolecular Imaging*,” (2004-2008). Our overall goal is to establish and forge a multidisciplinary R& D team that will develop bio-targeted quantum dots (qdots), a type of material derived from the field of nanotechnology, and corresponding optical instrumentations for imaging applications. We will initially utilize them for applications in cancer diagnosis, tissue engineering, and pathology. We have recently demonstrated the utility of qdots in a host of biological imaging applications; however there are major bottlenecks that have prevented their everyday use in laboratories. Some of these are 1) lack of cost-efficient biocompatible qdots, 2) lack of instrumentation that has the optimal conditions for qdot imaging, and 3) lack of fundamental understandings of their biodistribution and toxicity *in vivo* (will be important for *in vivo* imaging and for the users). The multidisciplinary team will address these major problems, and rapidly translate our developments and findings to researchers via industrial companies. Finally, our team will initially utilize nanotechnology to study tissue remodeling, early cancer diagnosis, guidance during tumor resection, and evaluate their potential applications in stem cell research. This research focuses on quantum dot fluorescence biosensing and not plasmon biosensing. **There is no conceptual or budgetary relationship with the proposed work.**

Genome Canada, “*Quantum Dot-based Dipsticks*,” (2006-2009). We will integrate novel advances in nanomaterials (quantum dots or “qdots”) with bio-recognition probes to create an inexpensive platform capable of simultaneous detection of multiple pathogens in a routine clinical sample. Qdots are nanometer-sized semi-conductor optical emitters with unique properties: they have a wide spectrum of controllable emitted colors; they do not require more than one light source to provide excitation energy for their entire emitted spectra; they are 20x brighter and 200x more stable than current fluorophores. To exploit their potential, we will employ the concept of barcodes used in grocery stores to identify a product, its mutating price, and its commercial flow. We will: (1) develop “barcodes” based on individual qdot emission

patterns; (2) affix these individual barcodes onto molecular bio-recognition probes, such as oligonucleotide probes and antibodies, which can identify the genomes and proteomes of different pathogens; (3) design, miniaturize, and validate an inexpensive portable instrument to rapidly read and interpret these barcodes for the detection of multiple pathogens at point-of-care. The target result is a compact and inexpensive device capable of multiplex genomic and proteomic profiling for ultrasensitive, high-throughput detection and characterization of multiple pathogens. Devices will be designed to be robust, automated, capable of using routine clinical specimens, and in formats (e.g. “dipstick”) that are appropriate for utilization and maintenance in resource-poor settings. This opens the way for simultaneous and definitive detection and

characterization of thousands of pathogens at point-of-care, equally deployable and economically feasible in the developing and developed world. **There is no conceptual or budgetary relationship with the proposed work.**

ORF, “Extending the Research Capabilities of the Integrated Nanotechnology/Biomedical Sciences Laboratory,” (2006-2010). Early diagnosis is key to improved survival of cancer patients. By combining nanotechnology with biology and medicine, researchers under the direction of Dr. Warren C.W. Chan are using nanotechnology to identify molecular changes to develop new means of diagnosing cancer earlier and improve treatments. This new and improved state-of-the-art bionanotechnology research facility at the University of Toronto allows scientists to purify and isolate biomedically important nanostructures for biomedical detection. This research focuses on quantum dot fluorescence biosensing and not plasmon biosensing. **There is no conceptual or budgetary relationship with the proposed work.**

NSERC Nano-IP, “*Effect of Nanostrucutre Size and Shop in Uptake, Degradation and Clearance in Primary Macrophage,*” (2005-2007). This research is of major importance to the field of nanotechnology and to the advancement of nanotechnology for basic research and clinical applications. Nanotechnology research will provide a large set of new materials that will be commonly used basic and applied research in the electronics, biology, and medical field. Currently, the environmental impact of nanotechnology has not been well characterized; meaning that the in vivo administration of nanostructures could lead to harmful toxic effects. The reason is that nanostructures are small enough that may be able to enter vital organs and alter biological function; or that degradation of the nanostructures exposes the body to toxic metal ions (most inorganic nanostructures are composed of toxic metals such as Cd and Ag). We have recently discovered that the inorganic nanostructures are mainly trapped in the liver after in vivo administration and that the cells in that will process the nanostructures are macrophages. In this proposal, we will synthesize 5-different sizes and 3-different shapes of nanostructures and systematically evaluate their fate, metabolism, and clearance in macrophages. **There is no conceptual or budgetary relationship with the proposed work.**

CIHR Regenerative Medicine & Nanomedicine, “*Stem Cell Fate Analysis and Manipulation.*” The primary goals of our New Emerging Team are to elucidate the fundamental mechanisms underlying stem cell self-renewal and commitment and to develop strategies for manipulating stem cell fate, ultimately for regenerative medicine applications. To achieve these goals, we will (i) identify and mimic regulatory components of the stem cell niche using microfabricated devices; (ii) develop predictive mathematical models of cytokine-activated signaling networks and (iii) engineer stem cells using protein transduction domain (PTD)-delivered transcription factors as tools to manipulate the balance between self-renewal and differentiation. **There is no conceptual or budgetary relationship with the proposed work.**

Support applied for:

CFI, “*Extending the Infrastructure Capability of the Integrated Nanotechnology/Biomedical Sciences Laboratory,*” This CFI Leading Edge Grant will be for extending the research capabilities of the Integrated Nanotechnology/Biomedical Sciences laboratory. The funding form this grant will be for the purchase of 2 pieces of purification equipment and 1 piece of animal imaging equipment. This infrastructure is needed for several reasons: (1)The shared facilities at the University of Toronto do permit INBS researchers to modify their purification set-up for optimizing and

purifying nanostrucutres, do not contain an ultracentrifuge that can go in excess of 1,000,000 g-forces, and cannot handle the large number of researchers in the INBS and their collaborators; and (2) there are no whole animal imaging system available at the University of Toronto and surrounding Universities. These 3 pieces of equipment clearly complements the current infrastructure in the INBS. Each of these pieces of equipment is critical to bionanotechnology research and development in Toronto, Ontario, and Canada. **There is no conceptual or budgetary relationship with the proposed work.**

NSERC, “*Novel Strategies for Quantitating Colloidal Nanostructures using Phage-Display and Immunoassay.*” Biologists, chemists, and biochemists commonly use sandwich immunoassays to measure the concentrations of proteins and genes and are considered one of the most well developed analytical methods available. Generally, in immunoassays, a capturing antibody is coated onto the surface of the wells of a microtiter-plate. The capturing antibody will measure the biomolecule of interest (in solution) since the antibody provides the specificity. Afterward, the captured biomolecule is exposed to another antibody (that is conjugated to an enzyme that induces color change of an organic molecule). The intensity of the color in the well can indicate concentration (when compared to a standard concentration curve). As a postdoctoral researcher, Dr. Chan used immunoassays for studying hepatocyte, which are primary liver cells, function after exposure to quantum dots. However, there are no immunoassay methods for measuring the concentration of the nanostructure itself. The main reason is that libraries of BRM for nanostructures have not been developed. The use of immunoassays for measuring nanostructures would be immensely important for nanotechnology research since it would provide a tool to measure and quantitate nanostructures. This analytical tool would significantly impact research in nanotoxicology, nanobiotechnology, and nanomedicine. **There is no conceptual or budgetary relationship with the proposed work.**

Ontario Ministry of Health, “*Engineering Semiconductor and Metallic Nanostructures for Biomedical Applications,*” (2007-2011). The overall aim of the Chan research group is to develop novel nanotechnology-based probes (e.g., quantum dots and metallic nanoparticles) for ultrasensitive, multiplex biological imaging and detection. We aim (1) to develop unique sets of nanoparticle-based optical probes, (2) to understand the effect of bio-recognition molecules, solvents, and environment on the optical properties of these probes, and on their fates in cells and animals, and (3) to develop their applications in tissue and whole animal imaging. The end result will be a new generation of nanotechnology-based probes that will assist in the acceleration of biological research and that will lead to new methods of improved diagnostics of diseases (e.g. cancer). **There is no overlap with the present proposal.**

M.C. GOH

Support currently held:

NSERC CRD, “*Fundamentals and applications of diffraction,*” (2007-2008). **There is no overlap with the present proposal.**

NSERC Discovery, “*From molecular to mesoscopic to macroscopic: structure, dynamics and the forces involved*”, (2005-2009). **There is no overlap with the present proposal.**

Ontario Centers of Excellence, Collaborative, “*Diffraction-based arrays for biosensing*” (2007-2008). The OCE and Axela Biosensors, Inc., funded projects deal with grating-based sensors that would be

extensions and further developments of Axela's existing products. Many of the same optics issues will arise here and in the current proposal. **There is no budgetary overlap with the present proposal but.**

ORF – Research Excellence, “*Bio-Optics – Transformative technologies for life sciences*”.

This grant supports the development of IP for optical microscopy. This work is focused on developing miniaturized technology specifically with commercial potential. The ORF work is decidedly less fundamental and more practical. **There is no conceptual or budgetary relationship with the proposed work.**

A. HELMY

Support currently held:

CFI New Opportunities, “*Multi-Wavelength Dynamic Optical Characterization Laboratory for Broadband Photonic Devices*” (2004). The grant is aimed at establishing a versatile and comprehensive photonics characterization facility to study optical signal processing devices generically. In particular, optical signal processing devices using ultrafast nonlinear effects similar to the ones we propose in this work. The infrastructure has enabled the development and research of high-performance devices towards multi-function, versatile, high-tolerance optoelectronic devices. The grant was used to purchase the main equipment for an advanced optical systems characterization lab. Equipment such as communication analyzers, bit error rate testers, fibre test beds, high power lasers, optical amplifiers and optical tables are all examples of the measurement kit which will be purchased.

This lab will play a pivotal role in the characterization of the devices generated in this work. Quick characterisation of the losses of the devices, their linear and nonlinear response can be performed in this lab. The testing of frequency conversion, parametric generation and amplification can also be undertaken in this lab.

It is the photon statistics measurements of the quantum properties of light that will be carried out in Waterloo. Hence the devices fabricated and designed here at U of T will have the characterization facilities in close proximity. This enables a rapid learning cycle, which in turn expedites progress towards the project goals. **There is no conceptual or budgetary relationship with the proposed work.**

Connaught Start Up Fund, “*Harnessing Parametric and Non-Linear Optical Effects in III-V Semiconductors*” (2004): The Connaught fund at the University of Toronto grants \$10,000 for starting faculty to help with the initial costs of starting a research group with an international calibre such as travel to initiate a collaborative program, attend conferences and give invited talks. **There is no conceptual or budgetary relationship with the proposed work.**

NSERC, “*Research Tools And Instruments*”. This grant helped establish a new spectroscopy laboratory within the University of Toronto. This enabled conducting Raman and PL spectroscopy beyond the visible spectral regime, which is currently available in Toronto. The new instrument will allow near infrared characterization and spectroscopy of materials and device; an aim which has direct applicability for optimising the fabrication processes and technologies to fabricate the samples required for this NSERC Strategic grant. The lab has already been used to test initial samples pertinent to the project described here. It has proven as a pivotal tool for optimizing the technologies needed for the success of the devices discussed in this proposal. Therefore this grant will benefit immensely from the new optical

spectroscopy lab when it is equipped. **There is no conceptual or budgetary relationship with the proposed work.**

NSERC Strategic, “*Burst Mode Femtosecond Lasers*” (2007-2009). The grant is lead by Professor Peter Herman and is concerned with studying wave-matter interactions between ultrafast burst-mode high power lasers and optical materials. The modifications caused by the laser exposure are studied by my group using Raman spectroscopy. This grant is not related to the research proposed here. **There is no conceptual or budgetary relationship with the proposed work.**

NSERC Discovery, “*Phase Matching Technologies in Compound Semiconductors for Integrable Commercially Viable Ultrafast*” (2005-2009). The research direction in my NSERC Discovery is to optimize other means for phase matching using nano- and micro-structuring in compound semiconductors. The promising technique of choice involves quasi-phase matching using bandgap modulation via domain disordering in short superlattice structures. The applications of this technology targeted in my NSERC Discovery encompass all optical signal processing. This direction of my NSERC discovery is parallel to that described in this NSERC Strategic proposal; however it depends on developing alternative technologies for phase matching technique based on Bragg reflection waveguides.

It should be noted that both grants will therefore share expertise and measurement setup. Some of the measurement setups used form the CFI grant and the know-how of the PDF which will be practically employed on this NSERC Strategic grant and partially on the NSERC-Discovery are similar. In addition the fabrication capabilities developed for the NSERC-Discovery grant will prove pivotal in making the deliverables of this NSERC Strategic possible on such a short time scale. **There is no conceptual or budgetary relationship with the proposed work.**

Ontario Government, “*Early Researcher Award*, ” The award was provided to enable using quasi-phase matching technologies to provide a platform for efficient frequency conversion for applications that require compact sources of infrared radiation. Some of the testing and fabrication required for this award are already provided by my NSERC Discovery and CFI New Opportunities. Compact frequency conversion sources are the end goal of this work. More emphasis on student training on fabrication and testing of these structures is associated from this grant. Hence some of the students working on this grant will help train the new students supported by the NSERC Strategic grant applied for here. **There is no conceptual or budgetary relationship with the proposed work.**

NSERC - Special Research Opportunity, “*Quasi-phase Matching in Compound Semiconductors*” (2007-2008). The research direction in this grant takes place in parallel to and in collaboration with another group in Glasgow, UK. Both groups work in concert to further develop an alternative phase matching technique. The technique studied in this NSERC-SRO grant uses quasi-phase matching using bandgap modulation via domain disordering. This alternative route has been initially developed in Glasgow with substantial contributions from one of the PIs (Helmy). The ultimate aim of the research is to enable efficient phase matching, which is one of the aims of the grant applied for here. However, frequency conversion to reach mid infrared wavelengths is the application target for the NSERC-SRO grant. This makes it somewhat different from the work described here since the phase matching technology and the ultimate aim are different.

Some of the measurement setups used for the CFI grant above and the know-how of the PDF which will be practically employed on this grant and partially on the NSERC-SRO are similar. Both grants will therefore share expertise and measurement setup. Also some of the fabrication capabilities developed for

the NSERC-SRO grant will prove pivotal in making the promised outcome of this NSERC Strategic possible in the time scales involved. **There is no budgetary relationship with the proposed work.**

It must be emphasized that work proposed in the NSERC Strategic grant applied for here is only possible with the help of 4 grants. The support of NSERC to my SRO and Discovery grants, CFI to my New Opportunities grant and the Early Researcher Award from the Ontario Government are pivotal for this research to be carried out in the duration given. These 4 grants provide essential ground work in the fabrication technology, design and characterization which will enable rapid progress in this NSERC Strategic grant. **There is no conceptual or budgetary relationship with the proposed work.**

Support Applied For:

Ontario Centers of Excellence, Champions of Innovation. This project aims to develop sensor networks for vehicles. They will be developed using nanocrystal-based composites. These composites will have versatile mechanical and aesthetic properties to enable their insertion in cars' accessories and components. **There is no conceptual or budgetary relationship with the proposed work.**

E. KUMACHEVA

Support currently held:

NSERC Canada Research Chair, “*Polymer Materials with Advanced Properties*” (2006-2010).

NSERC CRD – Visteon Corporation, “*Processing Effects on Morphology and Interfaces in Painted Plastic Automotive Parts,*” (2005-2007). **There is no conceptual or budgetary relationship with the proposed work.**

NSERC CRD – Rohm and Haas Corporation, “*Microfluidic Synthesis of Particles for Chromatographic Applications*” (2006-2007). **There is no conceptual or budgetary relationship with the proposed work.**

NSERC Discovery Grant, “*Materials with Structural Hierarchy: Combining Micro-, Meso-, and Nanoscales*” (2004-2008). **There is no conceptual or budgetary relationship with the proposed work.**

NSERC Ideas to Innovation, “*Material for Security Data Encryption*” (2004). **There is no conceptual or budgetary relationship with the proposed work.**

CIHR Nanomedicine, “*Stem Cell Fate Analysis and Manipulation*” (2004-2008). Regenerative Medicine is devoted to replacing diseased cells, tissues, or organs, or repairing tissues *in vivo* by augmenting regenerative processes. Underlying these goals is the manipulation of stem cells. Stem cells have the unique potential to develop into many different cell types in the body. Unfortunately, conventional strategies for manipulating stem cell in the laboratory are still inadequate and their use in the treatment of diseases such as Parkinson’s, Alzheimer’s and diabetes is still not feasible. Our New Emerging Team

(NET) is a multidisciplinary group composed of scientists from the physical sciences, engineering and biology. The primary objective of this NET in Stem Cell Fate Analysis and Manipulation is to utilize nanotechnologies such as microfabrication, quantum dots, and scanning probe microscopies to examine stem cells at the molecular level, which is necessary to understand their behavior. This newfound knowledge will be instrumental in the development of improved culture systems to exploit the extraordinary potential of stem cells. **There is no conceptual or budgetary relationship with the proposed work.**

Ontario Centres of Excellence - Materials Manufacturing Ontario (MMO), “*Polymer Particles for Biological Applications Produced in Continuous Microfluidic Reactors*” (2005-2007). To explore the application of the microreaction technology platform towards the preparation of microbeads for biological applications. Research supported by this grant targets the synthesis of polymer microbeads functionalized with molecules available for further bioconjugation. **There is no conceptual or budgetary relationship with the proposed work.**

M. MEUNIER

Support currently held:

NSERC Collaborative project with LTRIM Technologies, “*High Throughput Laser Trimming of Ultra Accurate State-of-the-Art Analog Circuits*” (2005-2008). For the development of improved and new enabling laser tuning and trimming technologies for state-of-the-art, sub 130 nm technology microelectronics and to apply these technologies to the design and fabrication of new analog circuits. **There is no conceptual or budgetary relationship with the proposed work.**

NSERC-CIHR, “Novel Nanocolloids Photosensitizers Fabricated by Femtosecond Laser Ablation in Aqueous Solutions for Tasks of Cancer Therapy” (2007-2009). For the development of new non-toxic nanocolloid photosensitizers (NCPS) fabricated by femtosecond laser ablation for application in photodynamic and thermal therapies of cancer. There is no plasmonics nanostructure in this grant. **There is no conceptual or budgetary relationship with the proposed work.**

NSERC Strategic, “Phase-Polarization Methods in Surface Plasmon Resonance Biosensing” (2006-2008). This project is aimed at the development of SPR-based sensing and imaging (in vitro) systems with significantly improved sensitivity. The proposed approach is based on several important findings of the PI related to phase-polarization properties of light under SPR which make potentially possible a 2-order of magnitude upgrade of SPR biosensor sensitivity compared to commercially available instruments (BIACORE). There is some overlap of this grant with the proposed Strategic Network on bioplasmonics. However, this grant ends next October 2008, just at the starting time of this Strategic Network grant. We will use some of the results and research outcomes in the Bioplasmonics projects. **There is no budgetary relationship with the proposed work.**

S. MITTLER

Support currently held:

Ontario Association of Medical Laboratories (OAML), “*Development of nanoplasmonic-gold waveguide-based high throughput assay for plasma enzymes of clinical importance*” (2008-2009). This grant is used to develop a sensor platform based on gold nanoparticles. It is a combination of immobilized and solution born nanoparticles which are held together by a functional bridge, which is sensitive to enzyme cleaving reactions. The enzyme under study is PDI, a protein related to Diabetes type 2. The focus in this study lies primarily on the functional bridge. **There is no conceptual or budgetary relationship with the proposed work.**

National Research Council (NRC) (PI: Alex Quaglia Scienctech Inc.), “*Improving the performance of THz- polarizers*”, (2007-2009). This fund is used to help a London based company to develop a technology to fabricate THz-polarizing beam splitters for their THz spectrometers. **There is no conceptual or budgetary relationship with the proposed work.**

NSERC Major Resources Support Program, “The South West Ontario Nanotechnology Center”, (2007-2010). This grant covers personnel costs of the Western Accelerator Farcicality, The Western Nnofabrication Facility and Surface Science Western. **There is no conceptual or budgetary relationship with the proposed work.**

ADF, Major Grant, “*Evanescence microscopy and hollow optical fibers sensors*”, (2006-2008). This grant finances the development of waveguide evanescent microscopy, an alternative to TIRF and the use of hollow optical fibers in sensor applications, e.g. the detection of extremely minor amounts of chiral material or changes of concentrations thereof. **There is no conceptual or budgetary relationship with the proposed work.**

OCE, AuTEK, “*Bio-sensors and filters based on precisely positioned gold nanoparticles*”, (2006-2008). The fund is used to develop techniques to position OMCVD gold nanoparticles in row arrays. This fund is used to finance the technology development to place OMCVD (organo-metallic chemical vapor deposition) gold nanoparticles, which grow usually randomly on a template surface, in line arrangements. It pays for a PhD student and all the characterization and fabrication costs in the Western Nanofabrication Facility and at Surface Science Western. The proposed research will directly benefit from this fabrication technology study. **There is no budgetary relationship with the proposed work.**

NSERC, Major Facility Access Grant, “*Accelerator Facility for Materials Research*”, (2006-2007). This financed the operation (personnel) of the Accelerator Facilities at Western. **There is no conceptual or budgetary relationship with the proposed work.**

MMO, EMK, “*Enantiomeric access screening of asymmetric catalysts*”, (2005-2007). This grant was used to develop the chemistry for enantio-selective recognition, the fundament for a high throughput screening of catalysts for enatioselective synthesis of a particular test drug. **There is no conceptual or budgetary relationship with the proposed work.**

Government of Canada, “*Tier I Canada Research Chair*”, (2004-2010). This grant pays for Mittler’s salary and a few lab operation expenses, like Milli-Pore system, waveguide fabrication, etc. **There is no conceptual or budgetary relationship with the proposed work.**

NSERC Discovery Grant, “*Photonic surfaces & interfaces lab*”, (2004-2009). This is an indirect support; funds are used for general lab operation. This grant finances students working on optical

tweezers, biomineralization and other non-nanoparticle related projects. **There is no conceptual or budgetary relationship with the proposed work.**

Support applied for:

Early Researcher Award, "*Lab on a chip: an integrated optical micro-fluidics system for bio/chemo-sensors*", (2008-2012). This grant is supposed to finance the development of an all optical chip, based completely on waveguides. Both the liquid handling system, due to optical tweezers operation and the sensor are waveguide technology. **There is no conceptual or budgetary relationship with the proposed work.**

Canadian Institute for Photonic Innovation, TEN (Technology Exploration and networking program), "*New bio-sensor platform Based on a photonic crystal - gold nanoparticle double resonance*", (2008-2009). The fund is used for determining the experimental conditions to achieve a Davidov split absorption peak. This grant finances a student to systematically simulate the fabrication conditions for gold nanoparticle pattern for the waveguides we fabricate in order to be able to achieve a resonance overlap and therefore a Davidov spitted absorption peak. The proposed project will build upon this pilot study. **There is no budgetary relationship with the proposed work.**

NATO Grant, "*Waveguide sensors for rapid cell detection*", (2008-2012). This grant will finance a cooperative development between Canada, the US and the Ukraine for a waveguide and fluorescence based detection system of pathogen cells. **There is no budgetary relationship with the proposed work.**

M. MOJAHEDI

Support currently held:

NSERC, "*Engineering the Electric and Magnetic Dispersive Response of Artificial Materials*", (2006-2010). This project deals with manufacturing artificial materials with unusual properties such as negative index of refraction and abnormal group delays. **There is no conceptual or budgetary relationship with the proposed work.**

NSERC, "*Agile RF/Microwave Metamaterial Components for Emerging Wireless Networks,*" (PI: Eleftheriades) (2006-2008). This grant is dedicated to the transmission line realization of backward wave lines at RF and microwave frequencies. **There is no conceptual or budgetary relationship with the proposed work.**

Ontario Centers of Excellence, "*Controlling the phase of light with nano-structured materials*", (2005-2006). This program deals with designing and manufacturing artificial structures such as photonic crystals and fast and slow light devices used to enhance optical linear and non-linear properties. **There is no conceptual or budgetary relationship with the proposed work.**

CFI and OIT, "*Nano-fabrication of Meta-materials*" (PI: Aitchison), (2004-2007). These grants are dedicated *only* to the purchase of equipment for fabrication of nano-size meta-materials. **There is no conceptual or budgetary relationship with the proposed work.**

A. PAWSON

Support currently held:

CIHR Operating, “*Protein interaction domains in cell signalling,*” (2005-2009). Cells in the body normally communicate through the exchange of molecular signals. In this process, one cell releases a signaling protein, which binds to a receptor (also a protein) on the surface of the target cell. The activated receptor spans the cell's outer membrane, and transmits information by recruiting proteins within the target cell. A network of protein-protein interactions then control cellular behaviour. This communication process is subverted in cancer cells, so that the cells grow even in the absence of an appropriate external signal. The abnormal signaling pathways typical of cancer cells are the targets of emerging new therapeutic agents. We are studying the means by which such pathways are organized in normal cells, and the nature of the protein interactions that are important for driving cancer cells. We are also developing a novel approach to “re-wire” cellular responses, so that cancer-inducing signals are instead sent down a benign molecular pathway within the cell, such as that inducing cell death. **There is no conceptual or budgetary relationship with the proposed work.**

NCIC Terry Fox Project Grant, “*Reciprocal cell signalling in angiogenesis and tumour formation,*” (2006-2010). Relevance: In order for cancers to grow and spread, they must have access to a supply of oxygen through the bloodstream. Many cancers have the ability to stimulate new blood vessels to grow in order to maintain their oxygen supply. Dr. Pawson's team is studying this process in order to find ways to block it. Their results may suggest new treatments that will stop cancers from growing or spreading. Progress During the Previous Grant: In recent years, the members of this program project have focussed on understanding how cells communicate with each other and how cancer causes these communication pathways to break down. They have learned the functions of several molecules that play a part in cell communication; they have identified and cloned a gene that controls whether or not leukemia can be artificially induced; and they have produced a large number of genetically altered mice, which have helped the team to understand blood vessel development. Current Proposal: The team will now continue to work toward a fuller understanding of how blood vessels develop, both in normal tissues and when stimulated by cancers. They will determine which molecular events start the process of new blood vessel growth, and how cancers activate this process. Category of Research: Fundamental Cancer Site Relevance: Brain/neurological, breast & lung **There is no conceptual or budgetary relationship with the proposed work.**

CIHR Operating, “*Signalling pathways controlling cell shape, movement and polarity in the kidney,*” (2008-2012). The formation and ongoing activity of tissues in the body requires a complex and delicate set of signals between different types of cells, that coalesce to make a functional organ. Breakdowns in these cell-to-cell interactions, and in the biochemical pathways within cells that normally control cellular behaviour, can result in disease. We will use the kidney as a test-bed to understand how different types of signals are integrated to form a physiological tissue, and the signaling defects that underlie diseases such as congenital nephrotic syndrome. **There is no conceptual or budgetary relationship with the proposed work.**

NCIC Operating, “*Regulatory protein networks in cell polarity,*” (2008-2012). **There is no conceptual or budgetary relationship with the proposed work.**

CIHR Research Resource, “*Core Proteomics Laboratory*,” (2008-2012). Multiuser equipment and maintenance grants competition. **There is no conceptual or budgetary relationship with the proposed work.**

C. RANGAN

Support currently held:

NSERC Discovery Grant, “*Coherent control and Quantum Information Processing*”, (2005-2009). This grant is used to support two graduate students, one summer undergraduate and conference travel. **There is no conceptual or budgetary relationship with the proposed work.**

CFI-ORF, “*Scientific computing facility for the computational study of ultrafast light-matter interactions*”, (2007). Funds are requested for software licenses. **There is no conceptual or budgetary relationship with the proposed work.**

NSERC-RTI and SHARCNet, (2007). Funds requested from these grants will be used for computations.

Windsor Regional Cancer Center Grant (Local Investigator Research Fund), matched by the Networks of Centers of Excellence (MITACS), “*Angle optimization of Intensity modulated Radiation Therapy via Learning Algorithms*”, (2007). This grant is used to support a graduate student working on this project for cancer treatment planning (two internship periods of this student at the Windsor Regional Cancer Center and conference travel of this student). **There is no conceptual or budgetary relationship with the proposed work.**

J. E. SIPE

Support currently held:

Ontario Centers of Excellence, Collaborative, “*Diffraction-based arrays for biosensing*” (2007-2008). The OCE and Axela Biosensors, Inc., funded projects deal with grating-based sensors that would be extensions and further developments of Axela’s existing products. Many of the same optics issues will arise here and in the current proposal. **There is no budgetary overlap with the present proposal but**

NSERC Discovery Grant (2007). This funds my basic research, and thus is heavily drawn on. But it will help in the support of the postdoctoral fellow who will be working on this project. **There is no conceptual or budgetary overlap with the present proposal.**

Support applied for:

Genome Canada, “*Laser coring and cell mining – mapping the chemistry of a single cell*”, (2008). Support from this grant would fund a student working on theoretical issues in ultrafast electron diffraction. **There is no budgetary overlap with the present proposal.**

M. TSAO

Support currently held:

Ontario Cancer Institute Investment in Research Program, “*Tumor-Stroma Interaction and Carcinoma Associated Fibroblasts in Lung Cancer*”. The major goals of this project are to investigate the gene expression profile of lung cancer stroma compared to normal lung, thereby identifying cancer associated genes with putative importance in lung cancer growth or metastasis. **There is no conceptual and budgetary relationship with the proposed work.**

OCRN, “*Genomic Profiles of Lung Cancer That Predict Prognosis and Response to Adjuvant Chemotherapy*”(2005-2007). The major goals of this project are to perform array CGH on tumors of patients in an NCIC Ctg BR10 adjuvant chemotherapy trial. **There is no conceptual and budgetary relationship with the proposed work.**

CIHR, “*Molecular Biological Mechanism of Human Pancreatic Carcinogenesis*” (2001-2003). The major goals of this project are to develop a better understanding on the dynamics of pancreatic cancer development, and the best candidate genes that we should target for new prevention or therapeutic strategy against this disease. **There is no conceptual and budgetary relationship with the proposed work.**

NCIC, “*Novel Molecular Prognostic Markers and Potential Therapeutic Targets in Non Small Cell Lung Cancer*” (2006-2010). This project seeks to identify prognostic markers and potential novel therapeutic target genes in non-small cell lung cancer based on expression profiling data. Prognostic markers will be validated and tested at immunohistochemistry level. Their potential biological significance will be tested using orthotopic animal and cell culture model. **There is no conceptual and budgetary relationship with the proposed work.**

CIHR, “*Ras Oncogene Interactions with Met Receptor Signaling in Colorectal Cancer*” (2003-2005). The major goals of this project are to investigate the impact of co-activation of Met receptor and RAS oncogene signaling in colorectal tumor progression. **There is no conceptual and budgetary relationship with the proposed work.**

ICR/CIHR Training Grant, “*Clinician Scientists in Molecular Oncologic Pathology*” (2003-2007). The major goals of this project are to train next generation leaders in molecular pathology for Canada. This is only a training grant to provide supports for trainees; there is no operating fund for any of the participating mentors. **There is no conceptual and budgetary relationship with the proposed work.**

CIHR, “*Equipment Maintenance Support for Oncologic Molecular Micro-imaging (OMM)*” (2005-2009). This is a multiuser equipment maintenance grant for the imaging facility at OCI/PMH. **There is no conceptual and budgetary relationship with the proposed work.**

US National Cancer Institute, “*Early Clinical Trials of New Anti-Cancer Agents with Phase I Emphasis*” (2008-2012). The major goals of this project are to conduct phase I first in human clinical trials with novel targeted drugs being developed by pharmaceutical companies. My role is to conduct the correlative science and biomarker studies on patient blood or tissue samples of patients entered into these trials. However, no funding for such activities is included in the grant. **There is no conceptual and budgetary relationship with the proposed work.**

CIHR, “*New Emerging Team Program: Quantum dot-based Biomolecular Imaging*” (2004-2008). The major goals of this project are to investigate the application of quantum dots to tissue analysis. **There is no conceptual and budgetary relationship with the proposed work.**

OCRN, “*Molecular Predictors of Outcome in BR.19, a Phase III Trial of Adjuvant Gefitinib in Post-Resection, Early Stage Non-small Cell Lung Cancer*” (2006-2009). The major goals of this project are to investigate biomarkers for predicting survival benefit from adjuvant erlotinib therapy in early stage non-small cell lung cancer patients. **There is no conceptual and budgetary relationship with the proposed work.**

NCIC, “*The Effect and Mechanism of a Novel Adoptive Immunotherapy for Lung Cancer Using Ex-Vivo Expanded Human DNT Cells*” (2006-2010). The major goals of this project are to determine the role of ex vivo expanded human DNT cells as a novel adjuvant therapy for lung cancer and underlying mechanisms. **There is no conceptual and budgetary relationship with the proposed work.**

Support applied for:

CIHR POP Grant, “*A Clinical Test for Lung Cancer using Prognostic Gene Classifiers*”. This proposal aims to develop a molecular test that will be used to analyze the removed lung cancer tumour tissue in order to make a prediction of the patient’s survival after 5 years. If this test proves to be accurate, it will be useful and important to help physicians provide the best care and to enable a patient’s ability to understand and manage their own disease.

There is no conceptual and budgetary relationship with the proposed work.

CIHR, “*CIHR Team in Lung Cancer*” (2008-2012). The vision of the CIHR Team in Lung Cancer (CTLC) is to bring together a Nation-wide group of outstanding Canadian scientists, including leaders in their fields of epidemiology, cancer genetics, biology, imaging and thoracic oncology to reduce lung cancer mortality and to improve the quality of life of lung cancer patients by enhanced early detection and novel treatment strategies. Our strategy is based on the paradigm of “detect, decide, and destroy”. **There is no conceptual and budgetary relationship with the proposed work.**

OICR, “*Mass Spectrometry Analysis of Aberrant Epidermal Growth Factor Receptor Signaling in Non-Small Cell Lung Carcinoma*” (2008-2010). Mass spectrometry will be used to analyze protein phosphotyrosine in non small cell lung cancer to identify molecular features associated with erlotinib and gefitinib responsiveness and survival. Drug affected pY sites will be identified in non-small cell lung cancer cell lines and patient samples. Candidate markers of clinical benefit will be validated by multiple reactions monitoring MS in surgically resected lung cancer samples of patients who have received a pre-operative treatment with drug. **There is no conceptual and budgetary relationship with the proposed work.**

NCIC, “*Integrin Alpha-11 as Stromal Factor in Lung Cancer*” (2008-2012). The major goals of this project are to understand further the function and biological impact of integrin alpha-11 expressed on cancer associated fibroblasts and to identify potential new inhibitor compounds that can be developed for lung cancer treatment. **There is no conceptual and budgetary relationship with the proposed work.**

C. WANG

CIHR Grant, “*Quantum dot-based biomedical imaging*”, (2005-2009). This is a Regenerative medicine and nanomedicine group grant, led by Warren Chan, a member of this network. This grant supports the development of semiconductor quantum dot-base probes for biomedical imaging. One post-doc is supported by this grant, and one graduate student is co-supervised by Warren Chan and me. This CIHR grant is focused on luminescence semiconductor particles and will be expired by 2010. The proposed NSERC work includes multiplex detection of cell markers. It is expected that post-doc who is trained using the CIHR funds will interact with those doing NSERC supported research. The post-doc/students will share the leukemia cell specimens for an overlapping period of less than one year. **There are some overlap in cellular targets and leukemia specimens, but no conceptual or budgetary relationship with the proposed work.**

CIHR Grant, “*Phenotype-driven mouse models of hematological diseases*”, (2005-2009). This grant supports the development and characterization of mouse models of hematological diseases by using ENU or gene-trap mutagenesis. **There is no conceptual or budgetary relationship with the proposed work.**

OAML Grant, “*Using quantum dot conjugates for cellular imaging in hematology analysis*”, (2004). This grant supports a part-time post-doc and suppliers for a study on molecular and cellular characterization of a novel diagnostic entity. **There is no conceptual or budgetary relationship with the proposed work.**

Jeffrey Rubinoff Foundation (Private Foundation), “*Flow cytometry cell sorter facility and hematopoietic stem cell banking*”, (2005-2009). This grant is provided by a private foundation and supports the acquisition of the instrument and operation of a research flow cytometry/cell sorter facility. **There is no conceptual or budgetary relationship with the proposed work.**

B. WILSON

Support currently held:

NIH, “*Protodynamic Therapy: Brain Pre-Clinical Project*,” (2005-2009). **There is no conceptual or budgetary relationship with the proposed work.**

NIH, ”*Protodynamic Therapy: Prostate Project*, ” (2005-2009). **There is no conceptual or budgetary relationship with the proposed work.**

NIH, "Protodynamic Therapy: Basic Science Studies," (2005-2009). **There is no conceptual or budgetary relationship with the proposed work.**

CIHR RFA for Regenerative Medicine and Nanomedicine, "*Quantum Dot-based Biomolecular Imaging*," (2004-2008). Our overall goal is to establish and forge a multidisciplinary R& D team that will develop bio-targeted quantum dots (qdots), a type of material derived from the field of nanotechnology, and corresponding optical instrumentations for imaging applications. We will initially utilize them for applications in cancer diagnosis, tissue engineering, and pathology. We have recently demonstrated the utility of qdots in a host of biological imaging applications; however there are major bottlenecks that have prevented their everyday use in laboratories. Some of these are 1) lack of cost-efficient biocompatible qdots, 2) lack of instrumentation that has the optimal conditions for qdot imaging, and 3) lack of fundamental understandings of their biodistribution and toxicity *in vivo* (will be important for *in vivo* imaging and for the users). The multidisciplinary team will address these major problems, and rapidly translate our developments and findings to researchers via industrial companies. Finally, our team will initially utilize nanotechnology to study tissue remodeling, early cancer diagnosis, guidance during tumor resection, and evaluate their potential applications in stem cell research. This research focuses on quantum dot fluorescence biosensing and not plasmon biosensing. **There is no overlap with the present proposal.**

CIHR Team Grant, "*The cardiac regenerative project: Quantitative cell tracking and response for cardiac regenerative approaches (CARE) project*," (2007-2011). Heart disease is the leading cause of death in North America. Depending on the severity of heart injury caused by disease, the positive benefits of current clinical treatments are limited at best. The Cardiac Regeneration Project (CARE Project): Quantitative Cell Tracking and Response of Cardiac Regenerative Measures-offers hope for patients suffering from cardiovascular disease or heart defects. The CARE Project assembles a multi-institutional and multidisciplinary team of leading and emerging new investigators to implement a synergistic and innovative radiological and nanotechnology-based imaging program that will help to advance developing cell therapy and tissue engineering applications to viable clinical measures. **There is no conceptual or budgetary relationship with the proposed work.**

CIHR, "*Coherent Raman Tissue Spectroscopy for In Vivo Disease Diagnosis*," (2007-2010). The survival rate of cancer patients is better when a diagnosis is made early and treatment is carried out promptly. Hence, there has been a large effort in the development of optical diagnostics for different clinical applications over the last 10-15 years, including techniques based on light absorption by tissues, elastic scattering, fluorescence (endogenous and with exogenous administered fluorophores). Each of these techniques has its own strengths and limitations, depending on factors such as the strength of the signals, speed, resolution (spatial, temporal and/or spectral) and sensitivity and specificity to altered tissue properties (structural, biochemical, molecular). Near Infrared Raman Spectroscopy (NIRS) has the specific advantage that it is sensitive to changes in the tissue biochemistry, without additional contrast agents or drugs. It is based on the phenomenon of inelastic scattering of light by molecules and probes the vibrational states of (bio)molecules. NIRS has been investigated intensely especially in the past 10 years, for non- or minimally-invasive tissue clinical diagnostics ("optical biopsies"), and has been very helpful cancer diagnosis and surgery guidance, but has encountered important roadblocks for widespread clinical applications mainly related to its low signal strength. The coherent Raman effect (CARS) can be used to generate a Raman signal much stronger than in NIRS without problems related to endogenous fluorescence. The basic assumption in this proposal is that this novel optical technique will provide the necessary improvements over spontaneous Raman to permit its use for coarse and/or fine imaging (higher sampling or biochemical maps) in the clinic for non invasive cancer diagnosis. To

prove this hypothesis, we will construct a broadband CARS spectroscopy system and compare its performance to NIRS in tissue samples and animal models of cancer. Once the superiority of CARS spectroscopy over spontaneous Raman has been demonstrated, we will confirm the technical feasibility of CARS spectroscopy *in vivo* in a pre-clinical trial with a prototype having limited imaging capabilities. **There is no conceptual or budgetary relationship with the proposed work.**

CIHR Operating, “*Near Infrared Imaging & PDT of Prostate Cancer Using Aptamer-Directed Pipoprotein Nanoplatform,*” (2007-2010). One of the foremost reasons for the dramatic improvement in our ability to treat an ever-widening range of cancers is the discovery and exploitation of cancer-specific protein markers. Previous techniques have effectively targeted cancer cells using drug- or toxin-conjugated antibodies or ligands that are engineered to home in on their specific surface markers. Synthetic macrostructures called nanoplatforms have also proven effective as carriers, but frequently suffer from biocompatibility/toxicity issues. These short coming must be overcome if the promise of nanomedicine is ever to become a reality in clinical medicine. Lipoproteins are naturally occurring nano-scale delivery vehicles that ferry cholesterol and other molecules through the bloodstream and which, importantly, are nonimmunogenic. However, the drawback of using them as drug delivery for cancer is the limited number of lipoprotein receptor-positive cancer cells. We recently discovered a new technique that allows lipoproteins to be loaded with diagnostic agents or drugs and be redirected from their normal destination to a variety of cancer cell types through targeting to unique tumor surface markers. This approach will dramatically expand the range and increase the accuracy of lipoprotein-based directed cancer therapies. Here we propose to integrate the use of a highly sensitive, highly prostate cancer-specific aptamer (a small nucleic acid ligand that mimics antibody) with lipoprotein nanoplatform functionalized with near-infrared imaging and photodynamic therapy modalities to achieve the highly specific, "see" and "treat" approach for local prostate cancer therapy. **There is no conceptual or budgetary relationship with the proposed work.**

NCE Individual, “*Biophotonics World,*” (2007-2008). **There is no conceptual or budgetary relationship with the proposed work.**

Support applied for:

OICR, “*The Impact of Photodynamic Therapy on the Biomechanical Stability of the Metastatic Spine: Characterizing,*” (2007-2010). **There is no conceptual or budgetary relationship with the proposed work.**

OICR, “*Molecular Genetic and Imaging Assessment of Surgical Resection Margins in Oral Carcinoma,*” (2007-2010). **There is no conceptual or budgetary relationship with the proposed work.**

OICR, “*Probing the Temporal Dynamics of Tumor Cell Kill and Vascular Damage in Radiation Therapy,*” (2007-2010). **There is no conceptual or budgetary relationship with the proposed work.**

NIH Operating, “*Co-registered Fluorescence-Enhanced Resection of Malignant Glioma,*” (2007-2010). **There is no conceptual or budgetary relationship with the proposed work.**

G. ZHENG

Support currently held:

CIHR, “*Near-Infrared imaging and photodynamic therapy of prostate cancer using aptamer-directed lipoprotein nanoplatform,*” (2007-2010). One of the foremost reasons for the dramatic improvement in our ability to treat an ever-widening range of cancers is the discovery and exploitation of cancer-specific protein markers. Previous techniques have effectively targeted cancer cells using drug- or toxin-conjugated antibodies or ligands that are engineered to home in on their specific surface markers. Synthetic macrostructures called nanoplates have also proven effective as carriers, but frequently suffer from biocompatibility/toxicity issues. These short coming must be overcome if the promise of nanomedicine is ever to become a reality in clinical medicine. Lipoproteins are naturally occurring nano-scale delivery vehicles that ferry cholesterol and other molecules through the bloodstream and which, importantly, are nonimmunogenic. However, the drawback of using them as drug delivery for cancer is the limited number of lipoprotein receptor-positive cancer cells. We recently discovered a new technique that allows lipoproteins to be loaded with diagnostic agents or drugs and be redirected from their normal destination to a variety of cancer cell types through targeting to unique tumor surface markers. This approach will dramatically expand the range and increase the accuracy of lipoprotein-based directed cancer therapies. Here we propose to integrate the use of a highly sensitive, highly prostate cancer-specific aptamer (a small nucleic acid ligand that mimics antibody) with lipoprotein nanoplate functionalized with near-infrared imaging and photodynamic therapy modalities to achieve the highly specific, "see" and "treat" approach for local prostate cancer therapy. **There is no conceptual or budgetary relationship with the proposed work.**

CIHR, “*Protease-triggered photodynamic molecular beacon for cancer imaging and imaging,*” (2008-2011). **There is no conceptual or budgetary relationship with the proposed work.**

National Cancer Institute of Canada (NCIC), “*Photodynamic Therapy Beacon Triggered by Prostate Cancer-Specific mRNA,*” (2007-2011). **There is no conceptual or budgetary relationship with the proposed work.**

NIH, “*Grant Multifunctional low-density lipoprotein-based nanoplates,*” (2006-2009). **There is no conceptual or budgetary relationship with the proposed work.**

J&J- UHN Development Acceleration Award, “*HDL-like phospholipid scaffold as a novel carrier for targeted delivery of cancer imaging and therapeutic agents,*” (2007-2008). **There is no conceptual or budgetary relationship with the proposed work.**



FORM 100
Personal Data Form
PART I

Date

2008/01/10

Family name Zou	Given name Shan	Initial(s) of all given names S	Personal identification no. (PIN)
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I hold a faculty position at an eligible Canadian college
(complete Appendices B1 and C)

I do not or will not hold an academic appointment at a
Canadian postsecondary institution

National Research Council Canada
Place of employment other than a Canadian postsecondary
Institution (give address in Appendix A)

APPOINTMENT AT A POSTSECONDARY INSTITUTION

Title of position Research Associate	Tenured or tenure-track academic appointment <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
Department Steacie Institute for Molecular Sciences	Part-time appointment <input type="checkbox"/> Full-time appointment <input checked="" type="checkbox"/>
Campus	<ul style="list-style-type: none"> • For all non-tenured or non tenure-track academic appointment and Emeritus Professors, complete Appendices B & C • For life-time Emeritus Professor and part-time positions, complete Appendix C
Canadian postsecondary institution National Research Council Canada	

ACADEMIC BACKGROUND

Degree	Name of discipline	Institution	Country	Date yyyy/mm
Bachelor's	Chemistry	Jilin University	CHINA	1998/07
Master's	Polymer Chemistry and Physics	Jilin University	CHINA	2001/07
Doctorate	Biophysics and Material Science	University of Twente	NETHERLANDS	2005/02

TRAINING OF HIGHLY QUALIFIED PERSONNEL

Indicate the number of students, fellows and other research personnel that you:

	Currently		Over the past six years (excluding the current year)		Total
	Supervised	Co-supervised	Supervised	Co-supervised	
Undergraduate					
Master's					
Doctoral					
Postdoctoral					
Others					
Total					

Personal identification no. (PIN)	Family name
	Zou

ACADEMIC, RESEARCH AND INDUSTRIAL EXPERIENCE (use one additional page if necessary)

Position held (begin with current)	Organization	Department	Period (yyyy/mm to yyyy/mm)
Research Associate	National Research Council Canada	Steacie Institute for Molecular Sciences	2007/12
Postdoc	University of Toronto	Chemistry	2005/05 to 2007/11

Personal identification no. (PIN)	Family name
	Zou

RESEARCH SUPPORT

Family name and initial(s) of applicant	Title of proposal, funding source and program, and time commitment (hours/month)	Amount per year	Years of tenure (yyyy)
List all sources of support (including NSERC grants and university start-up funds) held as an applicant or a co-applicant: a) support held in the past four (4) years but now completed; b) support currently held, and c) support applied for. For group grants, indicate the percentage of the funding directly applicable to your research. Use additional pages as required.			

Dr. Shan ZOU

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Research specialization:

- Molecular interactions using AFM-based force spectroscopy;
- Developing smart materials with sensing functions for miniaturized devices;
- Nanofabrication of well-defined materials.

Teaching:

- 2005-2007 University of Toronto
Postdoctoral fellow in the group of Prof. Gilbert C. Walker
(Co-)Supervised graduate and undergraduate students (list of names: James Li, Shell Ip, Isaac Li, Yuri Chan, Claudia Grozea, Adrienne Tanur, Jane Cheung, Nancy Teng, and Derek Lee)
- 2005 University of Twente
Teaching the graduate course of "Microscopic and nanoscopic methods in materials science - scanning probe microscopy (SPM) beyond imaging".
- 2004 University of Twente
Supervised international master program on Nanotechnology covering the topic in "Construction and testing of miniaturized temperature control device for single molecule force spectroscopy in liquid".
- 2003-2004 University of Twente
Supervised international master project covering the topic in "Grafting of isolated poly(ferrocenylsilanes) molecules to surfaces".

List of Publications

Contributions to Books:

1. Differential conductivity in self-assembled nano-domains of diblock copolymer using polystyrene-*b*-polyferrocenylethylmethylsilane
Li, J. K.; **Zou, S.**; Rider, D.; Manners, I.; Walker, G. C. *Adv. Mater.* 2008, *in press (accepted on Jan. 2, 2008)*.
2. Ordered CdSe nanoparticles within self-assembled block copolymer domains on surfaces
Zou, S.; Hong, R.; Emrick, T.; Walker, G. C. *Langmuir* 2007, 23, 1612-1614.
3. Self-assembly of metal?polymer analogues of amphiphilic triblock copolymers
Nie, Z.H.; Fava, D.; Kumacheva, E.; **Zou, S.**; Walker, G. C.; Rubinstein M. *Nature Materials*, 2007, 6, 609-614.
4. AFM based single molecule force spectroscopy of synthetic supramolecular dimers and polymers
Zou, S.; Schönherr, H.; Vancso, G. J. a review chapter in *Scanning Probe Microscopies beyond Imaging: Manipulation of Molecules and Nanostructures*, 315-354, Ed.: Paolo Samori, Wiley-VCH, ISBN: 3-527-31269-2, 2006.
5. Single molecule force spectroscopy of smart poly(ferrocenylsilane) macromolecules: towards highly controlled redox-driven single chain motors

- Zou, S.; Korczagin, I.; Hempenius, M. A.; Schönherr, H.; Vancso, G. J. *Polymer (invited paper, special issue on Single Polymers)*, 2006, 47, 2483-2492**
6. Force spectroscopy of individual stimuli-responsive poly(ferrocenylsilane) macromolecules: towards a redox-driven molecular motor
Zou, S.; Schönherr, H.; Hempenius, M. A.; Vancso, G. J. *Macromol. Rapid Commun.* 2006, 27, 103-108.
 7. Enzymatic surface erosion of poly(trimethylene carbonate) films studied by atomic force microscopy
Zhang, Z.; **Zou, S.; Vancso, G. J.; Grijpma, D. W.; Feijen, J. *Biomacromolecules* 2005, 6, 3404-3409.**
 8. Adhesion studies of latex film surfaces on the meso- and nanoscale
Olah, A.; Hempenius, M. A.; **Zou, S.; Vancso, G. J. *Appl. Surf. Sci.* 2006, 252, 3714-3728.**
 9. Force spsectroscopy of quadruple H-bonded dimers by AFM: dynamic bond rupture and molecular time-temperature superposition
Zou, S.; Schönherr, H.; Vancso, G. J. *J. Am. Chem. Soc.*, 2005, 127, 11230-11231.
 10. Exploring individual supramolecular interactions and stimuli-responsive polymers by AFM-based force spectroscopy
Zou, S.; Ph.D. Thesis, University of Twente, the Netherlands, ISBN 90-365-2132-7, 2005.
 11. Stretching and rupturing individual supramolecular polymer chains by AFM
Zou, S.; Schönherr, H.; Vancso, G. J. *Angew. Chem. Int. Ed.* 2005, 44, 956-959.
 12. Grafting of single, stimuli-responsive poly(ferrocenylsilane) polymer chains to gold surfaces
Zou, S.; Ma, Y.; Hempenius, M. A.; Schönherr, H.; Vancso, G. J. *Langmuir* 2004, 20, 6278-6287.
 13. Beta-cyclodextrin host-guest complexes probed under thermodynamic equilibrium: thermodynamics and AFM force spectroscopy
Auletta, T.; de Jong, M. R.; Mulder, A.; van Veggel, F. C. J. M.; Huskens, J.; Reinhoudt, D. N.; **Zou, S.; Zapotoczny, S.; Schönherr, H.; Vancso, G. J.; Kuipers, L. *J. Am. Chem. Soc.* 2004, 126, 1577-1584.**
 14. Tunable complex stability in surface molecular recognition mediated by self-complementary quadruple hydrogen bonds
Zou, S.; Zhang, Z.; Förch, R.; Knoll, W.; Schönherr, H.; Vancso, G. J. *Langmuir* 2003, 19, 8618-8621.
 14. Unfolding and refolding behavior of maltose-binding protein by AFM
Zou, S.; Sullan, R. M.; Walker, G. C. *Polymer Preprints* 2006, 47, 359.
 15. Molecular interactions in supramolecular dimers and polymers by force spectroscopy
Vancso, G. J.; **Zou, S.; Schönherr, H. *Polym. Mater. Sci. & Eng.* 2004, 90, 18-19.**
 16. Tunable complex stability in surface molecular recognition mediated by self-complementary quadruple hydrogen-bonds.
Schönherr, H.; **Zou, S.; Zhang, Z.; Förch, R.; Knoll, W.; Vancso, G. J. *Polym. Preprints* 2003, 44, 489-490.**
 17. Single molecular interactions in supramolecular host-guest systems by AFM
Zou, S.; Zapotoczny, S.; de Jong, M. R.; Auletta, T.; Schönherr, H.; Huskens, J.; van Veggel, F. C. J. M.; Reinhoudt D. N.; Vancso, G. J. *Polym. Mater. Sci. & Eng.* 2003, 88, 453-454.
 18. Single molecule probing of polymers and supramolecular materials
Vancso, G. J.; Schönherr, H.; **Zou, S.; de Jong, M. R.; Huskens, J.; Tomczak, N.; Vallee, R.; van Hulst, N. F.; Reinhoudt, D. N. *Polym. Mater. Sci. & Eng.* 2003, 88, 141-142.**

Oral Presentations and Posters (selected):

1. 2007 American Chemical Soecity (ACS) Meeting, August 19-23, Boston, MA
Poster: Unfolding of single maltose binding protein
2. 2007 Nanoforum Canada, June 15-18, Waterloo, Ontario

Oral presentation: Molecular self-assembly of polymer-nanoparticles

3. 2006 American Chemical Society (ACS) Meeting, September 10-14, San Francisco, California.

Oral presentation: Unfolding and refolding behavior of maltose-binding protein by AFM

Oral presentation: Nano Forces in Conjugated Polymer Thin Films and Self-Assembled Monolayers by Conducting Probe AFM

4. 2006 American Physical Society (APS) March Meeting, March 13-17, Baltimore, Maryland.

Oral presentation: Guided molecular self-assembly of block copolymer and nanoparticles.

5. Pacific Polymer Conference IX, ACS Division of Polymer Chemistry, Maui, Hawaii, USA, December 11-14, 2005

Oral presentation: Nanoscale mechanics of Algal and Barnacle adhesives and their polymer substrates.

6. PacificChem 2005, International Chemical Congress of Pacific Basin Societies, Honolulu, Hawaii, USA, December 15-20, 2005

Oral presentation: Polymer surfaces interactions: force spectroscopy of adsorbed block copolymers on surfaces

7. Dutch Polymer Day, Lunteren, The Netherlands, February 20-21, 2005

Plenary lecture: Stretching and rupturing individual supramolecular dimers and polymers by AFM-based force spectroscopy

8. Seeing at the Nanoscale II, Grenoble, France, October 13-15, 2004;

Poster: Probing individual supramolecular polymer chains by AFM-based force spectroscopy.

9. IMRE/SAB Postgraduate Workshop-Promoting Youthful Scientific Discourse, Singapore, February 12-15, 2004;

Oral presentation: Stretching and rupturing individual supramolecular dimers and polymers by force spectroscopy.

Poster (winner of the Poster Prize): Grafting of single, stimuli-responsive poly(ferrocenylsilane) polymer chains to gold surfaces.

10. The Third International Conference on Scanning Probe Microscopy of Polymers (SPMP 2003), Kerkrade, the Netherlands, July 15-18, 2003;

Oral presentation: Single molecule force spectroscopy studies by AFM.

11. American Chemical Society Meeting (spring), New Orleans, USA, March 23-27, 2003;

Poster: Single molecular interactions in supramolecular host-guest systems by AFM.

12. öMainz-Twente-Bath Joint Seminarö oral presentations, Max-Plank Institute of Polymers, Mainz, Germany, February 2004; University of Bath, Bath, UK, May 2003;, University of Twente, Enschede, The Netherlands, May 2002 and November 2004.

1. Planning the BiopSys Network

Canada has long been known for its academic strength in plasmonics, and its principal researchers in the area have a strong record of collaboration. These researchers have worked together on an informal basis for many years and in various ways, and a frequent topic of conversation has been a vision that would integrate and cross-pollinate Canadian biplasmonics research to create technologies for cancer detection. Recent events have made this vision a possibility: Canadian universities have recently hired several young and very promising biplasmonics researchers, individuals whose work has great potential for synergy. In addition, the pool of graduate students working in plasmonics and the related field of biosensing has grown significantly in the past decade. The time is ripe to bring the vision to reality. We propose to establish a strategic network aimed at developing biomedical technologies by focussing and coupling the work of existing and emerging Canadian researchers working on plasmonics.

1.1 Strategic Planning Process

The planning process for BiopSys began with a discussion in 2006 when a group of scientists and engineers noted that a critical mass in plasmonics now existed in Canada that offered new research and development opportunities. A world-class pool of talent, expertise and industrial activities related to plasmonics exists across the country, placing the Canadian effort at the forefront of this field. Due to the importance of the field, however, there has been substantial coordination of effort in Europe and the USA to advance plasmonics research. If Canada is to maintain world leadership in this field, similar coordination needs to take place here amongst the most prominent players in the fields of plasmonics and nano optics.

Our initial discussions focussed on enlisting to the effort the most accomplished Canadian researchers in the field of biplasmonics as well as individuals skilled in essential cognate disciplines such as conjugation chemistry, microfluidics, and pathology. In consultation with researchers and interested stakeholders in industry and government, inter-related research thrusts were developed and a preliminary Strategic Network Grant proposal was submitted to NSERC. NSERC subsequently invited a full proposal. A series of discussions followed, culminating in a planning meeting of interested researchers and our supporters, including private sector nanotechnologies and photonics companies and industrial research consortia (e.g., Northern Nanotechnologies, Axela, and Spectalis), government and public sector organizations (e.g., NanoBC, NRC, Sharcnet), universities and colleges, and university research groups. The result was a full examination of the broad possibilities for this research, identification of potential synergies, and the discussion of specific joint research initiatives linking various members of the proposed Network.

1.2 The Participants

BiopSys connects the work of 21 outstanding senior and junior researchers spread across Canada from Montreal to Victoria. These individuals are the country's leading experts in plasmonics. They possess relevant expertise in the design, synthesis/fabrication, characterization, and innovation of chemical sensors. They represent a variety of disciplines, each of which contributes to our understanding of biplasmonics and to the research and design of effective biplasmonics technologies. Moreover, our researchers based in healthcare delivery bring practical as well as fundamental contributions to our work. All of the participants have a history of collaborative research (Figure 1), and most have existing collaborations with other BiopSys participants.

JOINT PAPERS

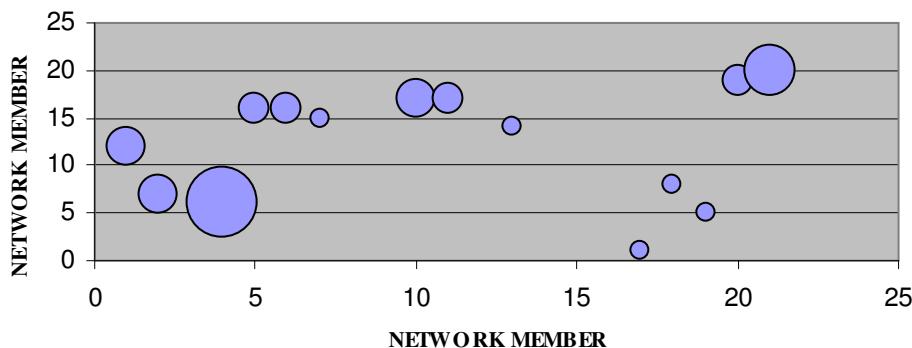


Figure 1: Participants are actively engaged in collaborative research. Size of dot reflects number of coauthored papers with another specific participant.

With the addition of students, impacted co-workers and collaborators from our partners, the size of the Network is about 100 people.

1.3 Scientific Targets

Our scientific targets are:

- 1) Multiplex detection of cell-biomarkers using plasmonic particles
- 2) Detection of analyte binding using planar plasmonic surfaces
- 3) Early detection of tumors and characterization of leukemia and lung cancer cells.

These targets were chosen from amongst many possibilities, based on where we believe the probability for excellent impact would be highest. In addition, these targets are broad enough to nurture the creativity that is the hallmark of successful networks.

Accomplishing these targets requires core technology capabilities; therefore, we have ensured that the Network includes strength in:

- nanofabrication
- targeting chemistry
- cell and tissue pathology laboratories
- plasmonic theory
- device characterization

1.4 BiopSys as a Strategic Network

The challenges in plasmonics for developing biomedical technologies that BiopSys will explore are beyond the capabilities of any small cluster of scientists and engineers. BiopSys will, for the first time in Canada, focus and integrate the full range of skills from the many different disciplines that are required to create improved diagnostic and care techniques for cancers. The Network is poised to make revolutionary improvements in biomolecular interaction analysis, using the expertise of world-class physicists, chemists, biochemists and biomedical scientists, biologists, material scientists, and engineering physicists and electrical engineers based at more than six universities and government research laboratories from across the country. Our integration of molecular and optical technologies will enable the creation of both large-scale instruments that will permit faster screening and diagnosis and miniaturized point-of-care devices for patients. In addition, the network mechanism, with its emphasis on integration and cross-pollination, provides a significant student training base; and our partnerships

with industry will transition our creations to innovations of significant health and economic value to Canadians.

2. Overview of the Network

2.1 Network Scope

The technological scope of this Network covers the space from the fundamental science of plasmonic optics to the fabrication of devices with likely clinical applications. Our vision is to make revolutionary improvement in biomolecular interaction analysis for lung cancer and leukemia detection. Our long-term objective is to shorten the time needed for diagnosis of and to improve the prognosis for these cancers by incorporating plasmonics into diagnostic platforms. Our approach is to seek better alternatives to current fluorescence methods for cell-surface receptor detection; the local illumination of plasmonics offers significant opportunities for achieving this. Our technical objectives involve the development and use of tiny metal particles as light beacons for the receptors, or nanostructured metal surfaces, as sensitive transmitters of molecular binding events. We will first develop platforms for examining model biochemical systems and then we will develop diagnostic tools for lung cancer and leukemia. Our aim is to determine the presence of lung cancer at an earlier stage than is currently possible, and to improve prognosis. We also aim to develop a point-of-care device for detecting leukemia, which will greatly shorten the time needed for diagnosis.

We will focus our research efforts in three theme areas, where coordination of effort using bioplasmonics has the potential to make rapid innovations: multiplex detection of cell-biomarkers using plasmonic particles, detection of analyte binding using planar plasmonic surfaces, and rapid detection of tumors and characterization of leukemia and lung cancer cells.

We also will focus on Highly Qualified Personnel (HQP) training. The scope of researcher training includes both the technical skills required within individual laboratories and collaboration/communication skills for interacting productively within a network of motivated scientists, engineers, clinicians and entrepreneurs. Our building goals for HQP training are to:

- Develop a set of basic concepts that graduate students in bioplasmonics must know and have hands-on experience with in order to be successful.
- Identify the best sources of training of those concepts within the Network, whether within the home research groups or elsewhere in the Network.
- Efficiently deliver that training to students, without interfering with their existing coursework or overloading them unnecessarily.

2.2 Network Linkages

There are several types of linkages that make the proposed Network well-connected, including:

- Some students and post-doctoral fellows will be co-directed by faculty from different institutions and with the different complementary backgrounds required for achieving the Network's objectives. This will provide a rapid mechanism of feedback to ensure that focus on the objectives is retained.
- A shared activity schedule will connect the participants of all themes. Most of the wet chemistry and biochemistry is used for creating the molecular recognition platforms and nanoparticles. The outcomes of this chemistry are required by the physicists and engineers who are creating planar plasmonic device structures. The application biologists will use the chemistry and devices to examine the cancerous states of cells and tissue. These activity linkages are shown in Figure 2. Our Activities and Milestones Schedule provides details of how and when ideas and materials produced in one Theme are shared across with the others. In addition, the sections on the Research Program (section 4) and Interactions and Partnerships (section 6) provide further information on interactions and linkages.

- Abbreviated research notebooks will be posted and shared among all Network participants through a web-based communication network, where relative progress and challenges being faced can be identified and discussed.
- Conferences and workshops will occur frequently to provide shared training by the best teachers.

Success in each Theme is necessary to the achievement of our 5 year objective to develop new and better methods of cancer detection and integration is the key to that success. Integration of activity across Themes will occur continuously. One of the keys to integration, to the connectivity of the Themes, is the analysis of Noise. In all Themes, an understanding of where noise is coming from in the measurements is critical to develop, to build better sensors and to comprehend the biology. Within the context of biosensors, the focus is usually on the signal part, with the noise often being an afterthought. The reality is that it is the signal-to-noise ratio (S/N) at the detector(s) that determine a biosensor's detection limit, so N is as important as S from this viewpoint. This consideration is not just limited to optoelectronics, since the S interacts with a fluid that might contribute the dominant noise. We have found that a deep analysis of the noise seems to bring people from traditionally different disciplines together and discussions of noise rapidly break down the commonly perceived barrier between theoreticians and experimentalists. The noise statistics can change within the context of the biology; we will be preparing countably small numbers of signaling agents (few nanoparticles and arrays of nanostructures) whose number and pattern are expected to induce different cell responses based in their own internal networking or signaling. Untangling the contributions from the nanostructures and the intrinsic cellular responses will be essential to achieving our objectives.

There are several plasmonic-based devices that are currently being developed worldwide. Each one of them claims better sensitivity and advantages over the state of the art. However, in most cases, these claims are not substantiated. This is generally due to the lack of the application of common protocols for the determination of bioanalytical figure-of-merit (FOM). Another common connection within the BiopSys Network is that it will allow a comprehensive comparison of the FOMs from different plasmonic-based sensing platforms developed in Themes 1 and 2 (nanoparticles, nanohole arrays, nanoparticle arrays, fiber optics and so on). The performance of these platforms towards the solution of a similar set of problems described in Theme 3 (leukemia and lung cancer detection) will also be compared. This integrated approach will allow a better understanding of the analytical advantages and disadvantages of each and should be a major contribution to the implementation of future practical plasmonic devices.

Another important linkage between Themes is created by the economic benefit of sharing resources. The shared access to facilities - nanofabrication, spectroscopic, and cell culture, to name a few - has been enabled at reduced costs compared to individual rates because the group is large enough to demand such discounts successfully. This is an economic advantage, which while perhaps not

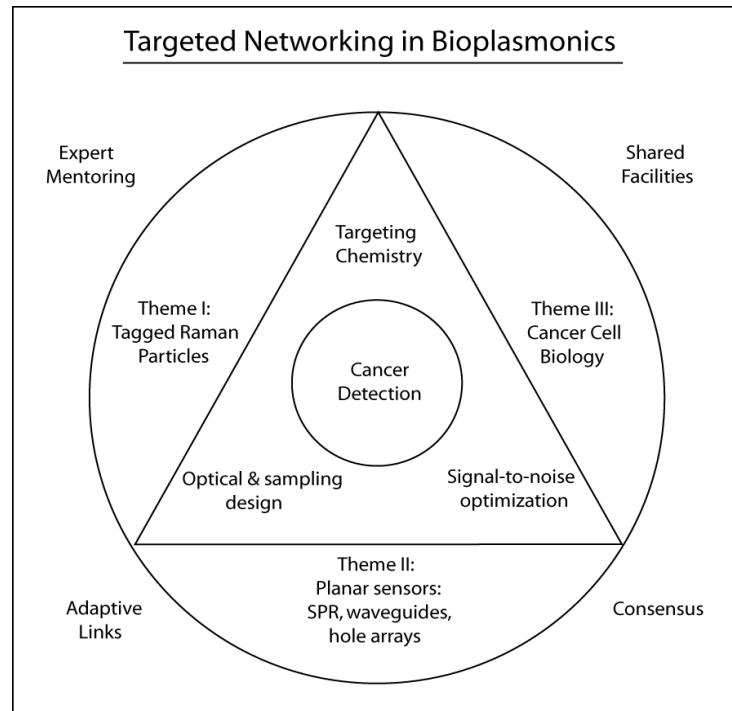


Figure 2: Linked Network Activities

deeply analyzed, is crucial for the success of the project. The diagram shows how the three themes are interconnected and how they all contribute to the central goal of cancer detection.

central, is important for our research activities. Shared training of students is another powerful linkage, as will be described in the Training section (section 5) of this proposal.

2.2 Value of the Research Results

Lung Cancer

Lung cancer is the foremost cause of cancer death in Canada and most of the developed world. In 2007 in Canada, lung cancer is expected to account for 11,000 (29%) and 8,900 (26%) of male and female cancer deaths respectively.[1] The lifetime probability of dying from lung cancer is 1 in 12 for men and 1 in 20 for women. Even if every smoker in Canada were to quit smoking tomorrow, lung cancer will still be one of the most common causes of cancer and cancer deaths for decades to come. The high mortality of lung cancer is clearly related to most patients being detected late, as these cancers do not give rise to symptoms early. Therefore, the best way to reduce lung cancer mortality is to detect and remove cancers early.

There are different types of lung cancer. Some tend to arise in large airways, while others arise in small airways or alveolar parenchyma where gas exchanges occur. Methods that may detect lung cancer early include (i) cytological examination of sputum to detect cancer cells presence, (ii) chest x-ray and (iii) low dose spiral computed tomography (CT) to detect cancer nodules, and (iv) blood tests to detect cancer cell specific markers.

The cytological detection of cancer cells by microscopic examination of sputum is highly specific, but the number of tumor cells present represents the limit of sensitivity. Chest x-ray detects all type of lung lesions that are >1 cm in size, thus is neither sensitive nor specific. In contrast, spiral CT detects lung lesions down to 2 mm diameter, thus is highly sensitive. However, this is compromised by lower specificity, as a majority of detected nodules are benign lesions. While spiral CT studies consistently reported nodules in 50-80% of high-risk patients (heavy smokers) being scanned, only 1-2% were subsequently proven to be cancer. Thus, a significant number of individuals being screened are subjected to unnecessary psychological stress and sometimes potentially unnecessary invasive procedures.

Leukemia

We also chose human leukemia as a study platform. Two major considerations in choosing this biological target are the feasibility and relevance. Leukemia is a typical model of disseminated cancer and an ideal target for single cell analysis. Leukemia cells are present in blood circulation and readily available for *in vitro* studies. More than any other cancers, leukemia requires multiplex cell marker analysis. Leukemia cells are heterogeneous in expressing cell surface markers. These markers reflect cell lineages and stages of cell differentiation. Cell marker profile is critical for diagnosis and also has important implications for therapeutics decision and prognosis.

Plasmonics offers a promise of radical technological advances in science and engineering that will improve screening for cancer. Integration of the molecular and optical technologies in our research will enable the creation of both large scale instruments (e.g., in screening large numbers of samples for basic cell biology or pathology laboratories) as well as point-of-care devices that are miniaturized and possible to manufacture cheaply.

2.3 Benefits of the Network

The challenges in plasmonics that will be explored for developing biomedical technologies are beyond the capabilities of any small cluster of scientists and engineers. BiopSys will connect researchers from different disciplines and establish links between researchers who are geographically distant. In this project, world-class researchers in theory, fabrication, chemistry and engineering from six universities across the country will work together to create the devices that will make major advances in biomedical

technologies: Physicists, with the deepest understanding of the fundamental physics of surface plasmons, will join with chemists and biochemists who can functionalize the surfaces with molecular recognition elements. These researchers will work with engineers, who have the best insight into laying out optical designs of highly parallel sensing elements in platforms. Biologists will identify the systems where the technology can have the largest scientific impact in understanding living systems.

BiopSys is timely. The new field of plasmonics is growing with activities, new discoveries and promise. This is evidenced by the explosion of new meetings, journals and recent publications in prominent journals. Establishing a bioplasmonics network in Canada now will put us in the forefront of these activities and help us take a lead role by providing the support and collaboration necessary to capture the growing opportunities. Publications and intellectual property that will emerge from plasmonics research will contribute to the fundamental knowledge base of nanoscience and enhance the visibility of Canadian nanoscience worldwide.

BiopSys provides the critical mass of expertise, intellectual property, state-of-the-art knowledge, and students and faculty to encourage industrial/government/academe partnerships and entice numerous partners into joining this effort. Industrial and government partners will both contribute their commercial and policy insight to our activities and transition the results of our research to innovations for the market place and to the health care arena. The constant consultation and mutual experiments between the industrial partners and the groups involved will seamlessly introduce new science and technology into these industries, increasing their technological advantage and market leadership, as it will enable Canadian industries to increase their global competitiveness in the burgeoning field of nanotechnology. The interaction between the students, academics and industrial partners will result in novel ideas and valuable intellectual property. This in turn encourages the parties involved to consider start-up activities to capitalize on such Intellectual Property.

The scientific contributions and productivity of such a focused team will be much larger than that of each individual team's contributions. The strategic network mechanism, with its emphasis on integration and cross-pollination, will provide the broad training to young researchers that they need for their careers in biomedical devices creation. Samples, processes, technologies, ideas and the training of HQP will be exchanged among the members through the Network structure and activities to create a truly collaborative environment that leverages the strengths of each member.

3. Background

The Grand Challenge: Making revolutionary improvement in biomolecular interaction analysis for lung cancer and leukemia detection

The aims of BiopSys are to develop better biomedical diagnostic technologies by using plasmonics and bioplasmonics, and in turn to shorten the time needed for diagnosis and to improve the prognosis for cancers, such as lung cancer and leukemia. In this section, we describe the current scientific and technological developments in the field, and some of the background research upon which the research proposal is based.

State-of-the-art in clinical diagnostics technologies

Clinical diagnostics for disease detection are based on two processes – cell receptor recognition and sensing of recognised analytes. The former is accomplished by the ELISA method, and typically the latter is accomplished by fluorescence spectroscopy. Our Network aims to develop plasmonic technologies that can simultaneously leverage the advantages of both of these methods on the same platform, and improve detection.

Cell Receptor Recognition

The most commonly detected molecules in clinical diagnostics are serum proteins and tumour antigens, which are currently analysed primarily using the antibody ELISA method.[2] In ELISA an unknown number of recognition sites (amount of antigen) is affixed to a surface, and then a specific target analyte that binds specifically to the recognition site (antibody) is washed over the surface so that it can bind to the antigen. This antibody is linked to an enzyme, and in the final step a substance is added that the enzyme can convert to some detectable signal (for example fluorescence). For clinical researchers, it is important to measure protein-protein interactions and to track the movement and migration of cell surface protein/receptors. These targets are the focus of point-of-care tests, cell counting, and tissue biopsies. Any improved way to track a given protein would be a significant advance in technology.

Fluorescence-based sensing of analytes

Fluorophore-linked monoclonal antibodies are widely used to sense antibodies in medical diagnostics and biology research.[3] Technologies that incorporate these linked antibodies include fluorescence microscopy[4] and flow cytometry.[5] However, the fluorescence approach comes with a number of severe limitations. Fluorophores are not stable long-term under light and undergo photobleaching. The emission spectra of organic dyes commonly used are broad, limiting the number of markers that can be simultaneously detected; the practical limit is three such markers. Multiple excitation sources are required to improve multiplexing, thereby leading to more complex instruments that are more expensive. The narrow spectral window available to probe the markers limits the selection of colors for multiplex detection. Fluorescence detection suffers from poor quantification, sensitivity and resolution.

Plasmonics based clinical diagnostics technologies

Plasmonics[6] offers better alternatives to current fluorescence detection methods for cell-surface receptor sensing. Plasmonics is a technology based on optical illumination of analyte material within 100nm of a metal surface. Optical fields excite collective electron excitations at a metal-dielectric interface called surface plasmons. When this interface is modified by a target analyte, this significantly affects the surface plasmon spectrum. This phenomenon is the basis of sensitive detection of biological molecules, such as receptors on the surfaces of cells. The ability to functionalize metal surfaces allows us to combine both the receptor recognition and analyte detection processes on one platform. Specifically, one important goal is the simultaneous detection of multiple (> 10) targets (multiplexing).

There are several groups exploring different aspects of plasmonics for sensing worldwide (for example, see Refs.7-19). Previous work by the Network's investigators has also provided novel examples of the application of this technology for chemical sensing and enhanced spectroscopy. The Network's proposed research is unique relative to the work of other research groups because it involves the development and evaluation of a collection of plasmonic technologies, specifically aimed towards the early detection of lung cancer and leukemia. For example, the research activities of the Network will include: systematic optimization of the geometric parameters for ultimate sensitivity, improvement of commercially available technologies, microarrays of nanoholes and nanoparticles, the exploration of polarization effects for multiplexing, the fabrication of plasmonic antennas for enhanced spectroscopy and single molecule surface enhanced Raman scattering (SERS), and an integration technology for fiber optics implementation.

Planar surface plasmon resonance (SPR) methods

The conventional and mainstream approach to SPR sensing[20,21] rests on the Kretschmann-Raether Attenuated Total Reflection prism arrangement[22], where a metal film is in intimate contact with a prism, and the analyte fluid is deposited on the metal. This arrangement is interrogated by launching a TM-polarised optical beam incident beyond the critical angle and monitoring the intensity or the phase

of the reflected beam. The angle of incidence or the wavelength of the incident beam is scanned and the intensity of the reflected signal exhibits a minimum when the input beam couples efficiently into the surface plasmon-polariton at the metal-fluid (gas or liquid) interface. The angle of minimum reflection depends strongly on the characteristics (thickness and index) of an adlayer located at the metal/fluid interface and on the index of the fluid itself, motivating the use of this arrangement as a chemical-to-optical transducer in biochemical sensors. In order to achieve greater transducer sensitivity, various modifications and alternatives to the conventional Kretschmann-Raether single-interface SPP sensor architecture have been proposed. Homola et al.[23] and Chien et al.[24] recently published reviews comparing the performance attributes of various surface plasmon sensor architectures and interrogation schemes.

Surface Enhanced Raman Scattering (SERS) sensing

Raman probes are a relatively new approach for biomolecule detection that is competitive with fluorescence-based detection methods. Raman emission involves excitation of a molecule with light and emission of light at longer wavelengths. Unlike the single broad peaks of molecular fluorophores (50-70 nm) and quantum dots (30-40 nm), Raman emission is characterized by a series of very narrow peaks (~2 nm). Raman signatures can be detected using the same spectral instrumentation that is increasingly applied for fluorescence analysis, but Raman emission intensity is normally much too weak to serve as an optical label. Raman probes overcome this limitation by exploiting an effect known as surface-enhanced Raman scattering (SERS), which occurs when molecules bind to certain metal surfaces,[25-27] including silver and gold nanoparticles.[28-31] The enhanced electromagnetic field around the metallic nanostructures generated by the excitation of surface plasmon modes results in a significant increase in the spectroscopic response from species adsorbed on these nanostructures.

Raman probes can be fabricated by a variety of methods[30,32-46] and the degree of Raman signal enhancement depends on the approach. Composite nanoparticles are one type of Raman probe made by aggregating silver nanoparticles in the presence of a chosen organic label molecule with a distinct Raman signature.[38,47] Each composite particle retains the distinct signature of the chosen Raman label(s), but the enhancement provides an extremely bright probe emission that is suitable for direct conjugation to biomolecule detection reagents. The most advanced applications have focused on analysis of proteins or nucleic acids in solution, including quantitative detection of single proteins in sandwich binding assays[30,34,38,40,41,45,47-49] and for multiplex DNA hybridization assays both in solution[50,51] and in a plate format.[32] A small number of applications have demonstrated detection of Raman probes in living or fixed cell samples.[36,37,39,41,52,53] Relatively little work has been done thus far to develop Raman probes for tissue-based analysis,[43,47] despite strong potential for multiplexing and to overcome interference from tissue autofluorescence. A recent paper by Nie and coworkers demonstrated that with excitation and emission spectra in a clear near-infrared window, Raman encoded particles could be >200 times brighter than near-infrared emitting quantum dots, allowing for spectroscopic detection of small xenograft tumors (0.03 cm^3) at a penetration depth of 1-2cm.[54] It has been shown that in certain situations, even the detection of single molecules is possible by SERS.[55] SERS detection is potentially a label-free approach, although the SERS efficiency is molecule-dependent, which may become a problem for proper quantification.

Absorption Wavelength Shift-based Particle Methods

Gold nanoparticle/nanorod biosensors are based on the phenomenon of the localized surface plasmon resonance (LSPR). Gold nanoparticles (AuNPs) of dimension 10-30nm *in solution* show a strong peak in the absorption spectrum in the red, and this peak position depends sensitively on their shape, size and composition[56] as seen in Figure 3. AuNPs can be surface-functionalised using thiol chemistry to recognize specific molecules, and formation of clusters triggered by sensing causes a visible colour

change from red (single nanoparticles) to blue (clusters)[57,58-62]. These properties have lead to the development of a variety of gold nanoparticle biosensors.[8]

Using gold nanorod-based molecular probes, multiple cell surface marker can be interrogated simultaneously, and simple cell-identity profiling schemes can be developed to profile different immunophenotype of cells. For example, El-Sayed et. al.[63] developed a detection scheme in which spherical gold nanoparticles were conjugated to anti-epidermal growth factor receptor (EGFR) antibodies and incubated with noncancerous and cancerous cells. Binding of gold nanoparticles to the cell surface by the anti-EGFR gold nanoparticles was visualized and characterized by dark field microscopy and microspectroscopy. Different binding patterns were observed corresponding to the cancerous and noncancerous cells that can be used as a basis for diagnosis.

While LSPR is successful in developing colorimetric sensors, a large amount of the analyte is required. In order to use color change during cluster formation for recognition reactions with only minute amounts of sample material available, or in a screening approach with many different recognition agents, the volume has to be minimized and the accessibility enhanced. Therefore a 2D approach, where nanoparticles are immobilized on a surface is favoured. One of the technological challenges is pinning down the precise optical response of nanoparticle mini-clusters, e.g., dimers, trimers and oligomers.[64] To mimic the high sensitivity of the 3D solution experiments, one must establish the particular interparticle distance at which the plasmon band both begins to shift and reaches a maximum, as well as the dependence on parameters such as the dielectric constant of the surrounding medium and the substrate.[65-70]

Su et al.[68] have done experiments and theoretical calculations for surface-immobilized gold elliptical nanoparticles of sizes varying from 84 - 104 nm. They found an exponential decay of the particle resonance redshift with increasing interparticle distance both theoretically and experimentally. For smaller particles (14nm diameter), they found an additional “cross-talk” peak in the spectrum characteristic of cluster formation on the substrate, and an increase in sensitivity to the particle shape. Mittler and Rangan have investigated the addition of dielectric organic coatings to the nanoparticles.[71] The presence of these coatings can extend the effective size of the metal particles and induce spectral shifts in particles whose separation would otherwise be too great to produce cross-talk (P. Rooney et al., manuscript under review). This work promises to lead to a 2D nanoparticle biosensor, which with appropriate bio-conjugation will enable the development of a leukemia detector.

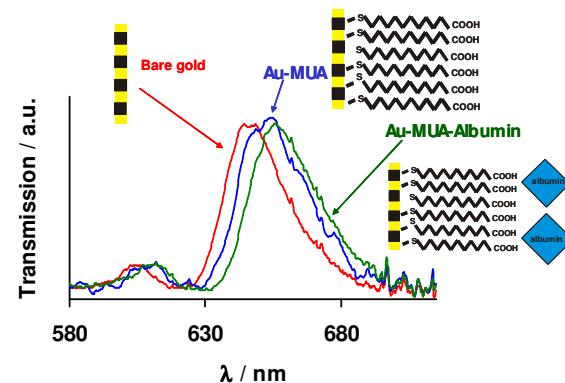


Figure 3: Detection of monolayer adsorption using arrays of nanoholes

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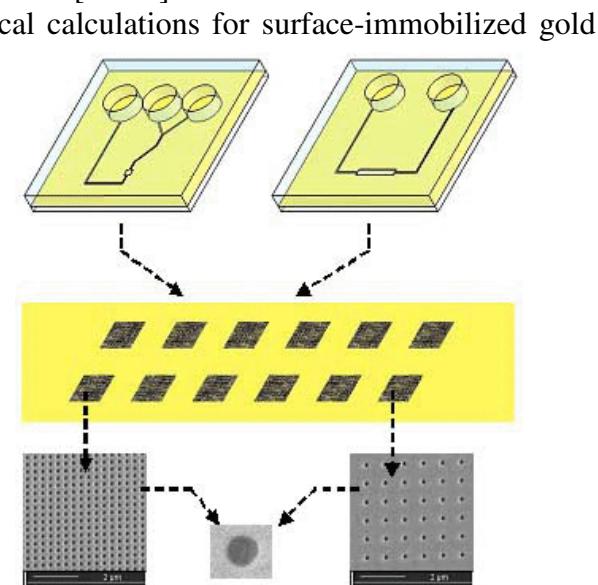


Figure 4: Arrays of nanoholes integrated in a microfluidic device.

Microarray Approaches

The plasmonics approach can be used to generate a host of bio-sensors integrated in a micro-array format to study the binding kinetics of several biomolecules simultaneously with sensitivity. Periodic arrays of nanoholes (PANHs) and periodic arrays of nanoparticles (PANPs) offer the possibility to confine the analytes to very small volumes ($\sim 10^{-21}$ L), allowing experiments with low quantities of expensive biological materials, shown in Figure 4. Previous work by the Network's investigators using hole arrays has provided novel examples of the application of this technology for chemical sensing and enhanced spectroscopy (together with the efforts from other groups such as S. Blair (Utah), J. Coe (OSU) and T.W. Ebbesen (Strasbourg)) and has been followed by several groups (P. Stark (Harvard), K. Tetz (San Diego), S.H. Oh (Minnesota)).

The first of the PANH detection schemes takes advantage of the sensitivity of plasmonic structures to local changes of refractive index at the metal dielectric interface. PANH in metallic films show extraordinary optical transmission at specific wavelengths without diffraction limitation.[72] The wavelength of the enhanced transmission through the nanoholes depends on the dielectric properties at the metal-dielectric interface.[73] Therefore, the adsorption of molecules on the gold surface shifts the transmission peak leading to a plasmon-mediated chemical sensor. This phenomenon was demonstrated by Brolo's group[74] where the detection of a monolayer of protein was demonstrated using this approach.

In contrast to commercial SPR sensors, the phase-match condition for excitation of SPs in zero-order transmission through PANH is given by the periodicity of the holes (equation (1))[73], and prism coupling is not required. This simplified optical setup (compared to the reflection arrangement required in current commercial SPR technologies) is more suitable for miniaturization and multiplexing. The combination of all the interesting properties of SP-mediated transmission, named low divergence[75], enhanced transmission for specific frequencies and high sensitivity localized at the surface, render these types of substrates as ideal for nanobiosensors in lab-on-chip devices[76]. Brolo and coworkers have demonstrated the potential of this approach by integrating several PANH sensor elements on a single microfluidic device for the determination of binding events[77].

A second approach for chemical sensing based on PANH takes advantage of the enhanced electromagnetic (EM) field around the metallic nanostructures generated by the excitation of SP modes[78,79]. This technique is based on the underlying principle of a family of "surface-enhanced" spectroscopic methods, which include surface enhanced Raman scattering (SERS) and surface-enhanced fluorescence spectroscopy (SEFS). Gordon et al. have been working on creating plasmonic structures for enhanced local fields, with improved coupling efficiency. The goal for electric field enhancement is four orders of magnitude, at which point SERS is comparable to that of regular fluorescence, and so SERS may be used in place of fluorescence. The significant advantages of SERS, in terms of molecular identification and label-free detection, motivate this research path.

As an example of recent research progress in this area, Gordon's group has been working on double-hole nano-antenna arrays based on nano-hole arrays in metal films. The double-hole is the Babinet compliment to the regular optical antenna. Comprehensive electromagnetic calculations show the double-hole effectively captures and focuses light down into the near-field. Furthermore, the double-hole may be readily fabricated with existing methods; while the size of each hole is on the order of a 100 nm, the apexes and gap between the holes have 10 nm feature sizes. As a result, by systematically varying the distance between the holes, strong focusing structures may be fabricated reliably and reproducibly.

With these double-hole structures, Gordon's group has demonstrated large enhancements in the second-harmonic generation from a gold surface. Brolo and Gordon have also provided some preliminary

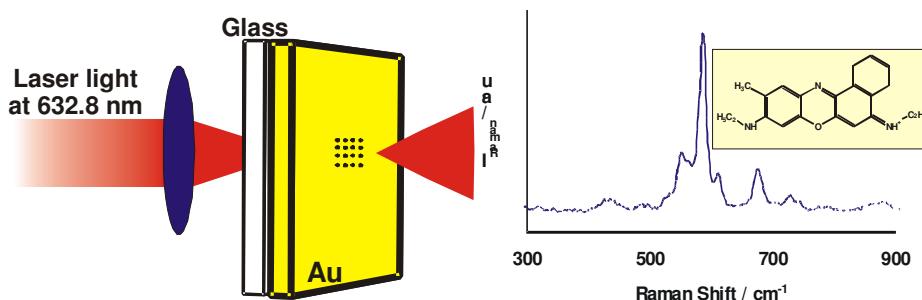


Figure 5: Nanohole-enhanced Raman experiment.

demonstrations of the potential of PANH for SERS[80] (Figure 5) and SEFS.[81] In both cases, at least an order of magnitude increase in the signal was observed for the optimized structures. It is important to note that the actual signal increase is much larger; however, the field is being enhanced only in a very small local volume. In other words, the enhancement is coming only from a small percentage of the total surface of the metal film.

Waveguide-based sensing

Waveguide sensors provide two independent optical detection channels corresponding to polarization of the light parallel to and perpendicular to the waveguide surface[82], and researchers have used NPs in order to increase the signal to noise of the detection[83] by embedding nanoparticles in the waveguide material. Mittler and Rangan have proposed a nanoparticle-on-waveguide biosensor that will enable integration of the signal over a long path, while simultaneously allowing polarimetric measurements for enhanced sensitivity (manuscript under review). Dielectric waveguides are capable of similar sensitivity and detection limits compared to the best SPR and metal waveguide approaches known. But the chemistries to functionalize Au are more mature and easier to apply than those needed to functionalize oxide-based waveguides. Furthermore, having a metal centered in the optical path opens up many additional possibilities, for example, the electrochemical approach for functionalization.

An interesting approach is the use of the long-range surface plasmon-polariton (LRSPP) to perform the sensing function. The LRSPP is supported by a thin metal film bounded by dielectrics of very similar refractive indices.[84] The LRSPP is less surface sensitive than SPR methods but can have a few orders of magnitude less attenuation. However, when excited in an Attenuated Total Reflection arrangement, the LRSPP exhibits a very narrow resonance width, which may more than compensate for the lower surface sensitivity. Besides, a Dextran matrix[85] of a thickness that precisely matches the spatial extent of the LRSPP can be attached to the metal surface in order to increase the binding capacity thus partly compensating for the reduced surface sensitivity.

One of the approaches investigated in this proposal uses the main LRSPP wave supported by a thin metal stripe[86-90] as the sensing wave, with bindings occurring directly along the surface of the stripe. The transducer architectures of interest include integrated Mach-Zehnder interferometers (MZIs) operating in this mode.[91-94] Although the surface sensitivity of the LRSPP in this structure is lower than that of the single-interface SPP, optimism for achieving higher sensitivity biosensors is justified by the much lower attenuation of the wave and by the ability to create long interaction length transducers based on optical interferometric geometries such as the MZI. Indeed, Lukosz long ago put forth this argument in favour of sensors based on conventional (dielectric) integrated optics compared to planar SPR sensors[95]. The LRSPP approach with metal stripes differs from biosensors based on MZIs implemented with dielectric waveguides[96-102] in that the “core” of the guide is a metal stripe, preferably Au. This difference has important consequences, such as providing waveguides with a

potentially higher surface sensitivity, and allowing the use of mature and stable self-assembled monolayers on Au[103,104] suitable for functionalizing the surface of the stripe with biomolecular recognition sites.[85,105,106] The approach also differs from biosensors based on metal-loaded dielectric waveguides[107-109] in that an LRSPP wave is utilized instead of an SPR/dielectric waveguide coupled mode, exhibiting a different surface sensitivity, attenuation and wavelength response.

Another approach to be investigated involves the use of the short range mode in metal cladded waveguide structures.[110] This mode is at the opposite end of the size scale having a much larger surface sensitivity but a shorter range. It also appears suitable for biosensing but has yet to be investigated in detail for such applications.

Evaluating Improvements to Bioplasmonic Sensors

For cancer detection, the receiver operating characteristic (ROC) curve is the most important characteristic, and it is empirically obtained. For SPR, ROC is highly dependent on the specificity of the binding. Some tests with worse performance on the ROC are encouraged because they win on some figures of merit (FOMs). Therefore, it is important to have figures of merit with which the strengths/weaknesses of the various approaches may be evaluated.

Figures of merit usually incorporate two factors that are traded off in design. For attenuated total internal reflection (ATR) SPR, the nm/RIU depends on the area of detection, and, therefore, on the surface density of analyte, through the RIU. With the nanohole approach, it is possible to reduce the surface area of detection, and thereby reduce the number of molecules. LSPR have even better performance in terms of the number of molecules, but the nm/RIU is reduced. Therefore, an effective comparison between the three technologies is to compare nm/(number of molecules), as opposed to nm/RIU. The type of plasmon is also important. While in particle studies the spectral shift due to clustering (nm/RIU) is sometimes modest, the surface plasmons are localized in specific areas of the particle. Moreover, as Van Duyne has pointed out[9], localized surface plasmon decay length is smaller than that of a surface plasmon polariton, so a smaller amount of molecules is actually producing the change. Therefore, the sensitivity of this particle can be as good (or better) than regular SPR.

For actual detection systems, the nm (or angle) is transduced to voltage or some other signal, which will lead to further differences between the various technologies. For the particle-based approach, the transduction mechanism is a major challenge that the literature does not adequately address; thus it is natural to expect the need for development of actual detection schemes. For nanoholes, the transduction has been demonstrated, and for different approaches there will be cost trade-offs. The most obvious inexpensive approach is integrated cmos array/laser diode detection. For ATR SPR, some devices allow for loss of sensitivity in order to have an inexpensive and compact design.

Another consideration is delivery of the analyte to the functionalized metal surface. In SPR, the binding of the analytes to the recognition sites is timed and it takes time because this process relies on diffusion. The use of microfluidic systems, where diffusion is reduced, is one way to alleviate this problem and overcome the bottleneck in the detection process time. In the nanohole approach, it is possible to address this still further, by reducing the diffusion volume. Another figure of merit could be the diffusion time (which depends on the size of the analyte, temperature, viscosity), and the distance of diffusion. The binding affinity or recognition efficacy will play an important role in determining this time.

At this point, cost comes into the equation. It is necessary to fabricate the plasmonic structures reliably and in large quantities, thus allowing the production of actual devices. Nanofabrication is often divided between top-down and bottom-up fabrication approaches, where each offers distinct advantages. From a commercialization perspective, bottom-up fabrication is inexpensive and can be applied to large-scale structures. Bottom-up methods may be used in the context of rod-shaped antenna. They may also

be explored in the context of self-organized colloids with metal deposition and infiltration (for example, as D3 Technologies Ltd. has done). Top-down fabrication allows for the creation of nearly-arbitrary structures (limited only by the fabrication), and it is free of large-scale defects. Both bottom-up and top-down methods allow for mass production. For top-down methods, significant research progress has been made in recent years to “imprint” nanoscale patterns over large substrates as an inexpensive means to mass-fabrication.

4. Proposed Research Program

The overall hypothesis of this proposal is that plasmonics can be used to make significant improvements to the detection of cell surface receptors characteristic of lung cancer and leukemia. To test this hypothesis will require coordination of the research effort from diverse groups. This effort has been broken down into three interconnected research themes. Our rationale for this breakdown is two-fold: that significant preliminary work has been accomplished by multiple researchers who naturally group within a given theme, and thus should be working more together, and that the three themes complement each other.

4.1.1 Theme 1: Fabrication and Characterization of Raman-tagged, Targeting Nanoparticles for Multiplexed Detection of Cell-biomarker

4.1.1 Theme Leader and Participants

Theme Leader: W. Chan.

Participants: M. Meunier, W. Chan, E Kumacheva, S. Zou, G. Walker, A. Helmy, B. Wilson.

4.1.2 Challenge

The major challenge of Theme 1 will be to create and engineer nanoparticles with unique surface enhanced Raman signature, also known as SERS-nanoparticles, and to characterize their utility as optical labels for measuring receptors on cells.

4.1.3 Tasks and Approach

Our specific aims are to

- 1) Design and prepare SERS metal particles of appropriate shape
- 2) Accomplish conjugation of targeting ligands for cell surface markers
- 3) Characterize properties of functional particles
- 4) Test SERS-nanoprobes in cells.

4.1.3.1 Design and prepare SERS metal particles of appropriate shape (Meunier, Chan, Kumacheva)

4.1.3.1.1 Preparation of spherical metallic nanoparticle substrates Gold nanoparticles will be utilized as SERS-substrates. It is well-known the SERS-signals arising from adsorbing organic molecules on metallic nanoparticles are highly dependent on the ability to reproducibly synthesize nanoparticles with a roughened surface. Hence, we will incorporate two strategies to prepare these nanoparticles: a laser-ablation method and a solution-based method. Network members have experience in preparing gold nanoparticles using both of these techniques.

The Meunier lab has developed and will employ a laser ablation method to prepare metallic nanoparticles that has produced stable colloids with a highly controlled size and size distribution via the tuning of laser parameters or the addition of ligands in the solution. With their new technique, nanoparticles of extremely small size (~2 nm) and up to many 10s of nm with a low coefficient of variation (15%-25%) can be fabricated. Such “green” synthesis in biologically friendly environment

enables the production of un-contaminated and non-toxic particles due to the absence of toxic chemical reducing agents and capping ligands.[111]

The Chan lab will prepare metallic nanoparticles according to the popularly used Frens method. Both Chan and Kumacheva have experience in preparing these nanoparticles. Briefly, gold chloride (HAuCl_4) is dissolved in distilled water. The reducing agent sodium citrate ($\text{Na}_3\text{-citrate}$) is added to the (HAuCl_4) solution, and upon heating to 100°C, the solution changes color from clear to a ruby red. Particle size, from 2 to 100 nm, can be tuned during the synthesis by altering the concentration ratio of HAuCl_4 and $\text{Na}_3\text{-citrate}$. For example, 1 mL of 0.1% HAuCl_4 (by weight) solution and 1 mL of 1 % $\text{Na}_3\text{-citrate}$ (by weight) produce 16 nm colloidal gold; 1 mL of 0.1% HAuCl_4 (by weight) solution and 0.5 mL of 1 % $\text{Na}_3\text{-citrate}$ (by weight) produce 41 nm colloidal gold. Transmission electron microscopy (TEM) is used to characterize particle size (Figure 6). Particles made using this approach occur in mixtures of shapes; we will use size-exclusion and gradient centrifugation to attempt to purify them.

After preparation of the nanoparticles, we will adsorb the organic molecule crystal violet onto their surface and determine the influence of adsorbate concentration on signal. The system that provides that highest and most-stable signal will be selected as the platform for future SERS studies.

4.1.3.1.2 Synthesizing & characterizing SERS-nanoprobes

To obtain SERS spectra, organic dye molecules are adsorbed onto the surface of colloidal gold nanoparticles. Metallic metal nanoparticles are monodispersed and stabilized in solution by the electrostatic repulsion induced by surface adsorbed $\text{Na}_3\text{-citrate}$ atoms. The $\text{Na}_3\text{-citrate}$ is weakly bound onto the surface of colloidal gold and can be easily displaced by molecules containing highly nucleophilic groups (e.g., thiols and amines). Our strategy is to conjugate organic dye molecules onto the protein bovine serum album through a carbodiimide-catalyzed reaction (where the primary amines from the organic dye reacts with carboxylic acid groups from the protein). This protein-dye complex is purified, using dialysis and concentrations are determined using UV-spectrophotometer. Afterward, the protein-dye complex is slowly added to a colloidal gold solution under gentle stirring. The protein/dye complex should adsorb onto the surface of colloidal gold through many molecular mechanisms (e.g., van der Waals forces, hydrophobic-aggregation, and thiol-sulfur interactions). The protein serves two functions in this probe design scheme. Firstly, the protein stabilizes the interactions between the organic dye molecules with the gold surface, preventing the small organic molecules from desorbing off the metallic gold surface. Secondly, the free primary amines from the protein can act as nucleating sites for the growth a second metallic layer. Once protein/dye complex are adsorbed onto the surface of colloidal gold nanoparticles, a SERS-spectra is obtained to verified binding. For applications, control experiments are needed where the SERS-spectra of protein adsorbed gold nanoparticles are measured and subtracted from the SERS-spectra of protein/dye adsorbed gold nanoparticles. The final SERS-spectra are from the interactions between the organic dye molecules-to-gold surface and not the protein-to-gold surface. To develop a family of SERS-nanoprobes, different types of organic dye molecules are conjugated onto the protein bovine serum albumin before adsorption on the gold nanoparticles. Dialysis is used to remove unadsorbed free unbound protein/dye complex.

Using this approach to prepare inorganic probes, >100 unique probes can be easily designed based on the SERS-signals observed from the organic dye molecule interacting with the metal surface. Organic dye molecules such as rhodamine 6G, FITC, Cy 7, Cy 5, texas red have unique SERS-spectra.

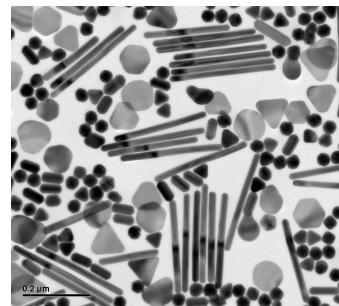


Figure 6: Examples of particles generated by Chan's group.

We expect these probes to be ultra-bright and photo-stable as compared to traditional organic dye molecules. See section 1.4 for analysis of SERS-signal on metal nanoparticles.

4.1.3.1.3 Designing better SERS-nanoprobes through controlled assembly

Selective aggregation of metallic nanoparticles has been implicated in improving the overall SERS signals arising from the nanoparticles. A major challenge is in the controlled assembly of the nanoparticles so that one can reproducibly prepare high-quality probes for bio-analysis. Hence, in this section, the aim is to study the relationship of SERS signal after nanoparticle assembly. Following Kumacheva, Walker and Zou's recent demonstration of nanorods (NRs) functionalized with sticky ends, we propose control of conformation of pairs of nanorods that shifts the plasmon, as the basis for a sensing system.

4.1.3.1.3.1 Synthesis of self-assembling nanorod systems.

Gold NRs with mean diameter of 8.0 nm and lengths of 40 nm will be synthesized using the procedure reported by El-Sayed et al. [63] Briefly, seed nanoparticles will be prepared by reducing HAuCl₄ mixed with an aqueous solution of CTAB and sodium borohydride in ice-cold water. For the preparation of a growth solution, cetyl trimethylammonium bromide (CTAB) solution will be mixed with solutions of HAuCl₄, AgNO₃, and water. Following the addition of ascorbic acid, 5-min-aged seed solution of nanoparticles will be added to the growth solution. At the end of each centrifugation cycle, the supernatant will be removed, and the precipitated nanorods will be redispersed in deionized water. Preferential binding of CTAB along the {100} facet of the longitudinal side of the NRs will leave their ends (the {111} faces) deprived of CTAB and allowing for the binding of thiol-terminated polystyrene (with different molar masses M_n = 5, 12, 20, 35, 50 K) to the ends of the NRs. These modified nanoparticles may be referred to as "triblocks". Polystyrene molecules will be grafted to the ends of the NRs, forming two "crowns" surrounding the ends of the NRs and a part of the longitudinal side of the NRs. This will result in NRs stabilized in organic solvents such as dimethyl formamide (DMF) and tetrahydrofuran (THF). Using controlled solvent conditions, self assembly of structures will be achieved.

4.1.3.1.3.2 Self Assembling Nanorod pairs for SP absorption based detection

Using this method, gold nanorods in structures with varying geometries can be organized in a controlled and predictable manner. The self-assembly of nanorod-polymer structural units can be triggered by changing the selectivity of solvents for the hydrophilic central inorganic block and the hydrophobic polymer side blocks. A blue shift (up to 40 nm) of the gold nanorods assembled side-by-side, and a red shift (up to 35 nm) of the end-to-end assembled nanorods was previously observed in the longitudinal plasmonic bands, which can be used as the biomarkers for multiplex detection (see Figure 7). In our previous studies, the gold nanorods were covered with CTAB, which is known to be cytotoxic and is not ideal for *in vivo* diagnosis. A functionalization strategy to improve the biocompatibility needs to be developed. Further modification with e.g., antibodies against CD24, CD49 and/or peptides/oligonucleotides has been very briefly illustrated in Figure 8. The synthesis of targeting ligands (e.g. peptides, and antibodies) will be obtained from the collaboration with G. Zheng's team. The

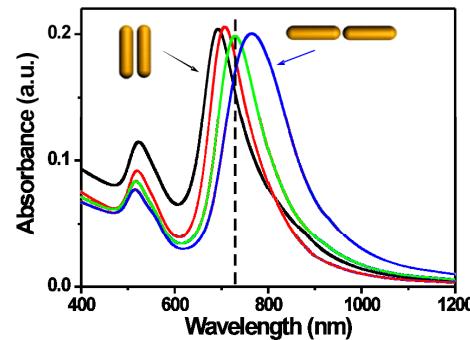


Figure 7: The side-by-side assembly of NRs bearing PS-50K resulted in a blue-shift of the longitudinal plasma band (vertical dash line); and end-to-end assembly resulted in a red-shift.

absorption spectral can be used as the basis for detection, as mentioned above, but the change in orientation also has strong implications for Raman scattering.

4.1.3.1.3.3 Affect of pair orientation on Raman Scattering

The electric field enhancement by shaped particles is well known; the field is typically strongest between metal asperities. By undergoing a change from the linear to the bent geometry, the electric field between illuminated rods will change many fold. FDTD calculation of the value of change as a function of rod environment, angle and separation will be undertaken by Rangan and Walker, but it is expected to be more than 10 fold in the hinge region. This will have the consequence of more than 100 fold change (perhaps much more) in the intensity of Raman scattering. Therefore, we will also prepare these rods with Raman tags, using the procedure illustrated in 1.2, above.

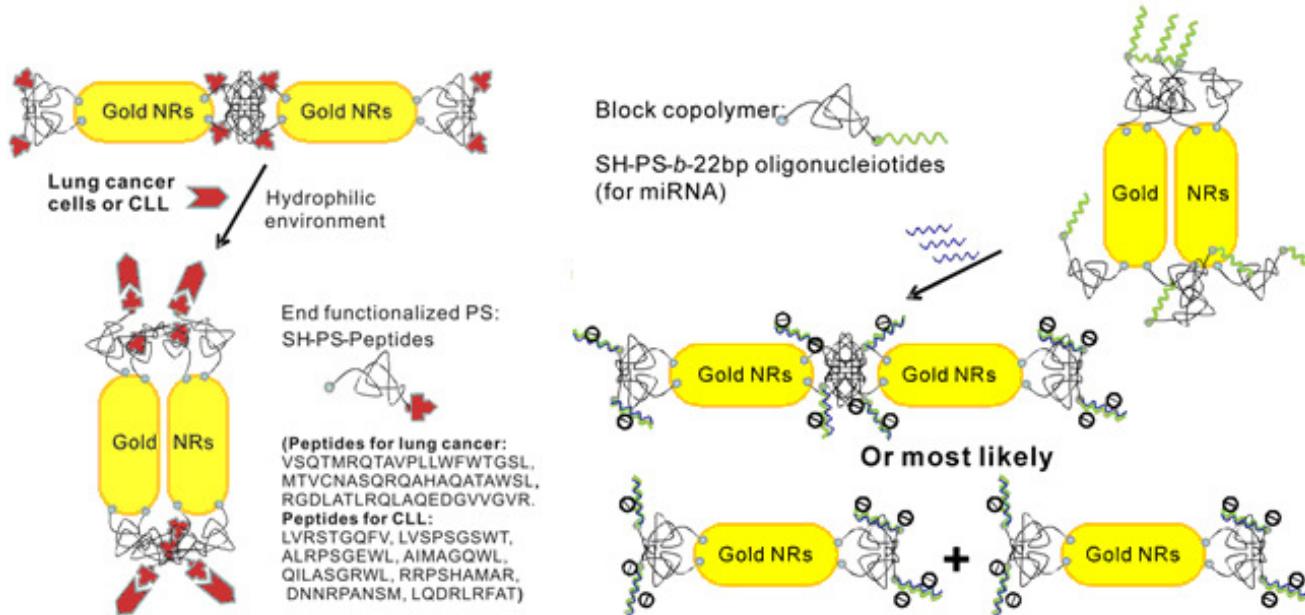


Figure 8: Targeting ligands modified gold nanorod assemblies: a blue shift of side-by-side assembly, and a red shift of end-to-end assembled nanorods.

4.1.3.1.4 Characterization of SERS-activity of Raman particles (G. Walker, S. Zou, A. Helmy). Our specific aim will be to characterize the SERS signals from nanoparticles.

4.1.3.1.4.1 Development of SERS Protocol. Raman spectra of dye conjugated particles will be collected, using instrumentation described below in “Raman Methods”. Enhancements of the spontaneous Raman spectra come from many sources, including the SPR resonance of the gold particle, electronic transitions of the dye molecule, and the geometric aspects of the gold particle(s). Images of Raman scattering by particles bound to cells will be collected using an inverted light microscope and appropriate filters to pass the Raman scattered light but reject the laser light.

Reference studies of the sensitivity of the Raman reporter systems will be facilitated by the use of highly regular gold particles, enabling quantitation of the scattering per probe particle. We will identify a set of at least 10 dye molecules whose Raman spectra are non-overlapping, to ensure the potential for multiplexing. Further Figures of Merit will be obtained by making measurements with each analyte - target pair using standardized solutions and controlled mixtures.

4.1.3.1.4.2 Raman Systems. The Raman facilities at the University of Toronto include at least three separate spectroscopy systems. For example, one has fully software controlled and systems based on a 300 mm focal length stigmatic flat field spectrograph microscope for confocal Raman analysis. It includes a confocal microscope, transfer and filtering optics, a stigmatic spectrometer equipped with two gratings, and a multi-channel detector. The spectrograph is attached to a high stability confocal microscope supplied with a xyz computer controlled mechanical stage and confocal coupling optics, which provides lateral and axial resolution better than 0.5 μm and 2 μm respectively using 100x objective. Excitation sources covering the UV and visible wavelength range are available. A holographic notch filter and plasma line rejection filter is also part of the system allowing the resolution of features as close as 50 cm^{-1} from the excitation source. The system is custom built to provide the capability of having 3 detectors simultaneously mounted. This provides the unique capability of conducting spectroscopy up to 2.2 μm in both continuous and time resolved modes. This capability is not commercially available and presents opportunities for commercialization of some of the measurements that shall be developed using this system.

The three dimensional spatial resolution will be pivotal for some of the SERS measurements explored in this project. Interaction between molecules can also be explored through 3 dimensional scans at different stages of the interaction.

The range of laser sources on the system provides unique opportunity to explore resonant and non-resonant Raman interactions in comparison to SERS measurements. The infrared range also enables extended probe depths into the samples, which would be otherwise not available using UV and visible probes.

The spectral resolution of the system and its accessibility to small wave-numbers also enable the probing of acoustic Raman modes of quantum confined systems such as nano-particles and quantum dots. Acoustic modes are sensitive to size and stress. They would therefore provide insight into size and the environment surrounding nano-particles which would be used as SERS probes in this project. Such information would provide an added dimension to the information already collected from the SERS measurements.

4.1.3.1.5 Incorporation of Targeting Ligands on SERC-nanoprobes

Conjugation of targeting ligands for cell surface markers: (G. Zheng, W. Chan, E. Kumacheva)

Once the SERS-nanoprobes are prepared (as described in section 1.2), carbodiimide chemistry will be used to conjugate small peptides or antibodies onto their surface (below is a table of different types of targeting agent and its corresponding receptor targets. We will characterize the SERS-signal before and after conjugation.

4.1.3.1.5.1 Using antibodies as a targeting agent

The first approach for Raman probe development will be to use antibodies. Monoclonal antibodies targeting specific cell surface receptors are the most advanced tumor-targeting ligands because of their unmatched high receptor-binding specificity/affinity and their well known antitumor effects. Several antibodies have already gained approval by the FDA and are widely used in cancer treatments, such as anti-EGFR (epidermal growth factor receptor) antibody (cetuximab) for colorectal cancer[112], anti-VEGF (vascular endothelial growth factor) antibody (bevacizumab) for non-small cell lung cancer (NSCLC)[113], and anti-CD33 antibody (gemtuzumab ozogamicin) for relapsed acute myeloid leukemia.[114] Therefore, we will use these antibodies for assessing the Raman probes' ability to image and detect NSCLC and leukemia. The feasibility of using Raman probes for tumor targeting and detection was recently confirmed by Nie et al.[115], who demonstrated the *in vivo* tumor-targeting specificity of antibody-conjugated SERS nanoparticles. In the Nie study, the authors also showed the

advantages of Raman probes for bioimaging analysis. They showed such Raman probes were >200 times brighter than NIR emitting quantum dots, thus allowing spectroscopic detection of small tumors (0.03 cm^3) at a penetration depth of 1–2 cm.

4.1.3.1.5.2 Using peptides as a model targeting molecule

The second Raman probe targeting approach will be to use peptide ligands selected by phage display or combinatorial library assays.[116] The use of peptides as targeting ligands over antibodies offers a number of advantages. These antibody advantages include (i) antibodies are relatively large themselves (160kd or 12nm) thus increase the challenge to nanoparticles' penetration into solid tumors, and (ii) antibodies are known to have high affinity to reticuloendothelial system, which could limit the delivery efficiency of Raman probes. Therefore, in addition to the antibody approach, we plan to use short peptide-based ligands for nanoprobe targeting. We will compare results from antibody versus peptide as the targeting agent to determine the best ligand for our application. Some of most suitable antibody and peptide ligands are illustrated in the following table of initial targets (Table 1):

Table 1: Selected Receptors and Ligands for Leukemia and Lung Cancer Detection

Receptor	Antibody	amino acid peptide ligand	cancer
EGFR	yes	GE11 (YHWYGYTPQNVI) (121)	NSCLC
VEGF	yes	K237 (HTMYYHHYQHHL) (117)	NSCLC
		NXXEIEXYXWXXXXXY (118)	NSCLC
DDR1	yes	to be found	NSCLC
$\alpha 3\beta 1$	yes	cNGXGXXc (119)	NSCLC
CD20	yes	not yet available	chronic lymphatic leukemia (CLL)
CD45	yes	not yet available	CLL
CD33	yes (AML)	CPLDIDFYC (120)	acute myeloid leukemia

An approach to using these antibodies, incorporating the particles from Meunier, is shown in Figure 9. As for peptide targeting approach, we will first illustrate it with the example of EGFR. Our strategy for EGFR targeting will utilize a recently discovered 12 amino acid peptide ligand - YHWYGYTPQNVI (GE11),[121] selected through phage display - which has shown high EGFR binding capacity but low mitogenic activity. Moreover, using this peptide as ligand, we have achieved the *in vivo* EGFR-targeting specificity with lipoprotein-based nanoparticles (unpublished results). Thus, we will synthesize and optimize the GE11-NP to meet the following criterium: Size: $12\pm 5\text{ nm}$. This target size range could be useful in the longer term, if therapeutic strategies are sought outside this grant, to avoid rapid renal clearance (for reducing kidney toxicity), mitigate RES uptake (for reducing liver toxicity) and increase particle accessibility to solid tumours (for improving particle delivery efficiency).

Milestones:

Year 1:

- Develop and optimize methodologies for the immobilization of antibodies and peptides on Raman nanoprobes
- Synthesize and characterize the prototype lung cancer-targeted Raman probe using EGFR-specific peptide

Year 2:

- Evaluate the SERS-activity of EGFR-targeted Raman probes in solutions and *in vitro* in NSCLC cells expressing different levels of EGFR
- Develop the prototype leukemia-targeting Raman probes using anti-CD33 antibody and test its targeting feasibility against leukemia cells

Year 3:

- Optimize the detection performance of the Raman probes against lung cancer cells by modulating the physical properties of the nanoprobe and the number of peptide ligands.
- Develop alternate lung cancer-targeted Raman probes using VEGF-specific peptide and DDR1-specific ligands based on the prototype probe evaluation
- Develop alternate leukemia-targeted Raman probes using a panel of different antibodies based on the prototype probe evaluation

Year 4:

- Compare different lung cancer and leukemia-targeted Raman probes for their *in vitro* detection sensitivity and specificity and determine the best probes for *in vivo* lung cancer localization and *ex vivo* leukemia screening

Year 5:

- Evaluate the SERS-activity of NSCLC-targeted Raman probes in animal models
- Evaluate the SERS-activity of leukemia-targeted Raman probes for *ex vivo* screening

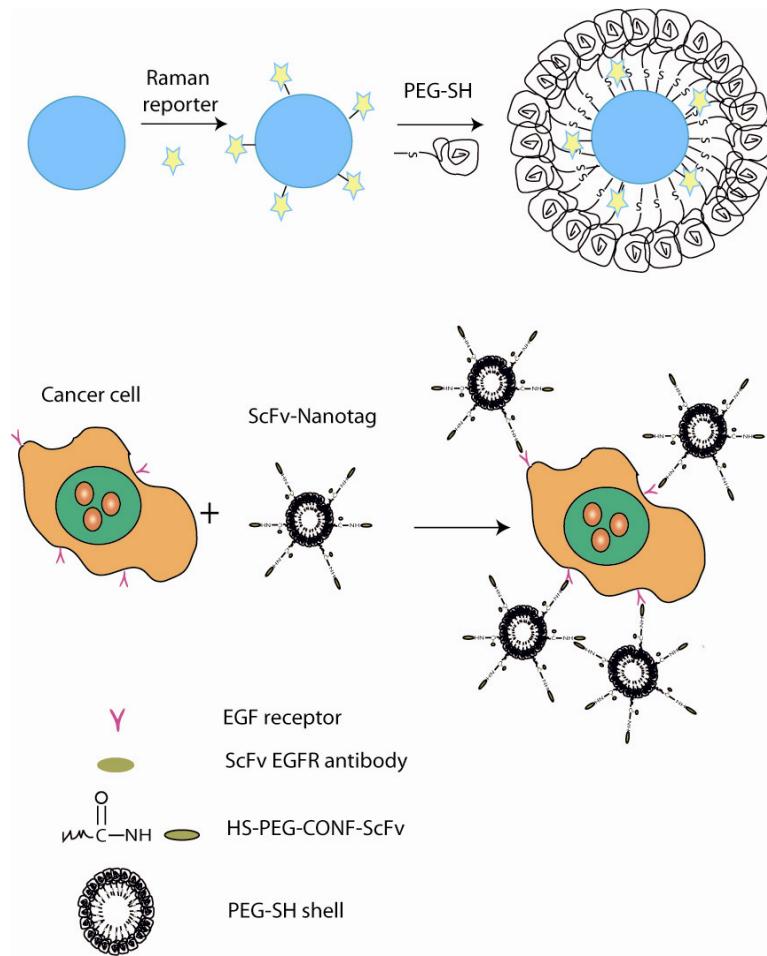


Figure 9: Preparing targeted, Raman tagged particles, using antibodies and tethers.[after 193]

4.2 Theme 2: Detection of Analyte Binding Using Stationary Plasmonic Surfaces

4.2.1 Theme Leader and Participants

Theme Leader: A. Brolo.

Participants: M. Meunier, R. Gordon, C. Wang, J. Sipe, P. Berini, W. Chan, E. Kumacheva, C. Rangan, G. Walker, S. Mittler, M. Tsao, C. Goh, T. Pawson, G. Zheng, A. Helmy, M. Mojahedi, S. Aitchison.

4.2.2 Challenge

The main goal in this Theme is to provide new analytical methods that will greatly exceed the current state-of-art in sensing technology.

4.2.3 Objectives

The specific objectives for Theme 2 are:

- a) Develop PANH for multiplexing and ultra-sensitive chemical sensing
 - Develop a micro-array based on PANH for leukaemia diagnosis
 - Improve the sensitivity of PANHs to refractive index changes
 - Develop ultra-sensitive sensing by enhanced spectroscopy using PANH
- b) Develop nanoparticles in and on dielectric structures for biochemical sensing
 - Improve the sensitivity of SPR detection by integrating nanoparticle in waveguides
 - Use nanoparticles in photonic structures for surface-enhanced sensing
 - Use nanoparticle arrays for multiplex sensing and monitoring cell surface receptors
- c) Improve biosensing using guided surface plasmons
 - Develop models for the signal-to-noise ratio of sensors
 - Implement and test promising sensor designs
 - Research and develop methods for electrochemical functionalisation
- d) Incorporate optical gain with surface plasmon waveguides

4.2.4 Tasks and Approaches

This theme will develop plasmonic micro-arrays for the early diagnosis of leukemia and lung cancer. The micro-arrays will allow the fast identification, characterization (binding constant determination) of target antibodies for these cancers. The planar array format is required to study single cell signaling and adhesion. The main goal for the network in this theme is to provide new analytical methods that will greatly exceed the current state-of-the-art in sensing technology. The commercially available surface plasmon resonance (SPR) devices have a detection limit of 0.8 pg/mm^2 , and we propose to improve it by at least ten fold. The sensitivity of plasmonic devices can be measured by the change in the resonant peak wavelength per unit of change in the effective refractive index in the probed volume. For the commercial SPR devices this sensor output sensitivity is 8000 nm/RIU .[122] For localized SPR methods (using gold nanoparticles) that have a very sharp resonance signal, this sensitivity is only 60nm/RIU .[123] Our goal is to significantly increase the sensitivity relative to the commercial SPR methods and to LSPR methods. The proposed challenges (improve detection limits and increase sensitivities relative to the commercial systems) will be overcome by implementing several approaches, including the development of new phase-sensitive measurements, the judicious design of plasmonic Bragg structures with sharp resonances, and a comprehensive analysis and control of the noise levels. Another challenge for accelerated detection of cancer is the limited ability to detect several antibodies simultaneously, i.e., to multiplex. Current limits on multiplexing are 16 detection spots for commercial SPR. Imaging SPR in the Kretschmann-Raether provides good multiplexing, but at lower sensitivity than the equivalent single point device. Our goal is to increase the multiplexing limit to at least 200 detection spots using micro-array chips while keeping the high sensitivity. The perennial problem of

non-specific binding will be tackled by the implementation of reference sampling and interferometric schemes. The fluorescence-based analysis and current ELISA methods will be improved by plasmonic field-enhancement, offering an immediate increase in sensitivity. SERS-based micro-arrays will be developed to offer a label-free alternative to fluorescence, and even in analysis where a SERS label is required, the bandwidth of the vibrational spectroscopy will allow simultaneous detection and a higher density of detection spots.

The plasmonic-based technologies for sensing are in different developmental stages. Some strategies are ready for the determination of cancer markers and others are still being evaluated at the fundamental level. One of the advantages of the Network is the opportunity to compare on the same ground practical results and applications with potential of emergent technologies. We decided to divide this theme into three sections: *A) Improving current SPR technology:* In this section, the detection schemes of the commercial SPR technology will be improved to increase sensitivity. A direct application to leukemia detection will be achieved. *B) Nanoplasmonics for biomedical sensing:* The objective here is to implement plasmonic technologies that have met proof-of-principle. The approach is towards the development of arrays of holes and particles for multiplex detection. This class of technologies still requires some fundamental development, but their application for lung cancer and leukemia diagnostics between the second and third year of funding is expected. *C) Biosensing using guided surface plasmons:* In this section, we will be exploring future promising technologies based on waveguide-based approaches. The fundamental aspects of the sensing platforms are still being developed and an application to sensing is expected only in the final stages (after the 4th year) of funding. A brief description of each of these sections is given below.

4.2.4 A Improving the sensitivity of the current SPR technology

Surface Plasmon Resonance (SPR) has emerged as a leading technology for label-free study of biomolecular interactions.[124] SPR occurs when surface plasmon waves, coupled to collective excitations of electrons in metal, are excited at a metal/liquid interface. Light is directed at, and reflected from, the side of the metal film surface not in contact with the sample. SPR causes a reduction in the reflected light intensity at a specific combination of angle and wavelength. Biomolecular binding events lead to an increase of the refractive index (thickness) of an ultrathin organic layer on the metal film, resonantly changing conditions of SPR production to provide extremely high sensitivity of the sensing response. To date, commercially available SPR-based systems[125-128] use light intensity characteristics as the information parameter.

Recently, a detection method that monitors phase instead of the intensity was first demonstrated in Russia by Kabashin et al.[129-133] (Kabashin works now in Meunier's group at École Polytechnique.) Indeed, interferometry shows that the phase of light under SPR can provide a 100-fold higher sensitive response than the intensity characteristics.[129] Based on light phase characteristics, SPR Interferometry[129,130] and Phase-Polarization Contrast[132,133] were introduced to improve the

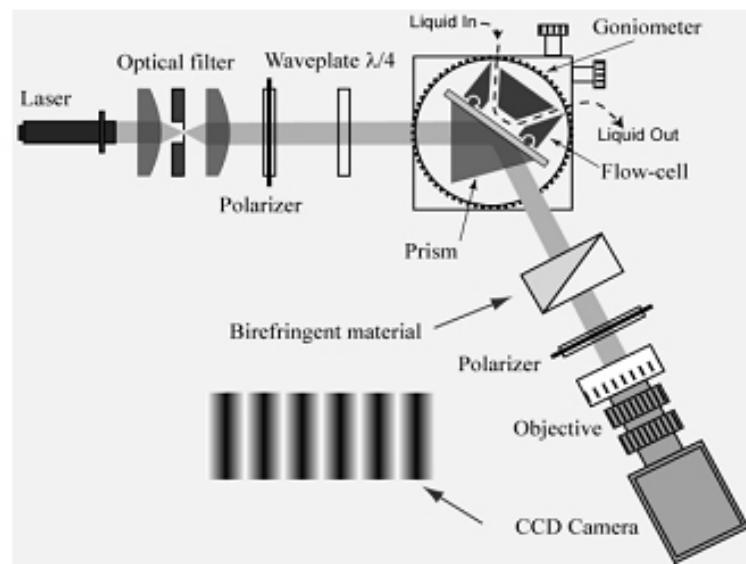


Figure 10: Schematics of a highly phase sensitive surface plasmon resonance biosensor

performance of SPR-based sensing and imaging schemes. Recently, the Montréal group has developed SPR polarimetry (see Figure 10), in which s-polarized light, not affected by SPR, is used as a reference beam, while information on the phase of the p-polarized component is obtained from the analysis of phase-polarization state of mixed light.[134] The detection limit of this high-sensitive SPR system is of the order of 10^{-7} RIU, which is more than one order of magnitude higher compared to conventional amplitude-sensitive SPR. The Network will enable the direct interaction between the Montréal group and C. Wang from Toronto. This interaction will allow the immediate application of this highly sensitive SPR technology to the detection of biomarker molecules and leukemia cells. Antibody-binding approach for leukemia detection has been used with conventional SPR[135,136], but the commercial system does not provide the high sensitivity required for early cancer detection. The free CD23 molecules in serum will be measured for improving the sensitivity and accuracy. The detection of serum CD23 is of significance for predicting leukemia progression. The primary CLL cells will be used to study the binding of cell surface receptors with ligands. The functional properties of CD20 and surface immunoglobulin receptors of CLL cells have implications in diagnosis and cancer biology. With specific antibodies to these two receptors, ultra sensitive SPR will be applied to determine the binding properties and kinetic characteristics of CLL cells. Further optimization of the phase-sensitive SPR system should allow the improvement of the sensitivity by another order of magnitude, allowing reliable measurements of changes as low as 10^{-8} RIU.

Milestones:

Development of phase sensitive SPR systems for improved sensitivity.

Year 1:

- Initiate phase sensitive measurement

Year 2:

- Determine the approaches (interferometry and/or polarimetry) to limit noise and to obtain Refractive Index Units lower than 10^{-7}

Year 3:

- Optimize the system by limiting the electronics noise and improve the image analysis.

Implementation of the Ultrasensitive SPR in cancer diagnosis and research:

Year 4:

- Develop SPR assay for measuring serum CD23 and to evaluate the results and clinical significance

Year 5:

- Determine the binding parameters of CD20 and surface Ig receptors of leukemia cells.

4.2.4.B Nanoplasmonics for biomedical sensing

The goal here is to develop planar plasmonic-based micro-arrays for multiplexing. Two general types of plasmonic structures, one based on nanoholes and the other based on nanoparticles, will be explored. Network members have already demonstrated proof-of-concepts for the general platforms of each of these approaches. As in the commercial SPR devices, the resonances from arrays of particles and holes respond to changes in refractive index at the metal–dielectric interface, which constitute the basic principle of the sensor. The resonances in these regular arrays satisfy the Bragg conditions of the grating structures. The immediate advantage of the array format is the potential for simultaneous analysis and multiplexing. However, the sensitivity of grating-based devices (~ 400 nm/RIU) is an order of magnitude smaller than the commercial SPR technology. Methods to overcome this limitation, such as optimization of the shape of the nanofeatures and waveguide integration, will be suggested. Another feature of surface plasmon excitation is localized electromagnetic field enhancement. The increase in local field

can be explored to extend the detection schemes to surface-enhanced spectroscopy methods, such as surface-enhanced fluorescence spectroscopy (SEFS) and surface-enhanced Raman scattering (SERS). These methods provide improvements to the ELISA assays in terms of sensitivity and multiplexing. The detection limits achieved from these approaches can also be significant lower, reaching the ultimate single-molecule limit.

4.2.4.B.1 Using periodic arrays of nanoholes (PANH) for multiplexing and ultra-sensitive chemical sensing

Objective 4.2.4.B.1.1 : Develop a micro-array based on PANH for leukaemia diagnosis

As described above, periodic arrays of nanoholes in gold films (PANHs) present enhanced transmission. Brolo and Gordon have demonstrated the application of these PANHs as integrated SPR sensors for the detection of surface adhesion[137] in a microfluidic device.[138] Those pioneering results were based on single sensing elements. The extension of that work to the utilization of several arrays in one chip to allow for analytical multiplexing is our first objective. In this case, each pixel in the chip corresponds to a PANH of a few micrometers (see Figure 11).

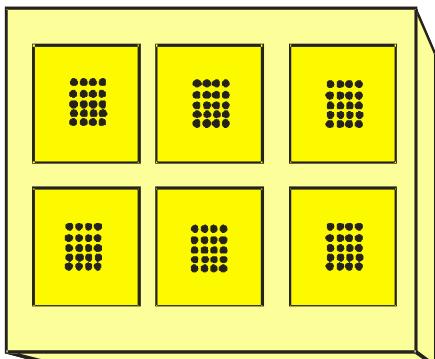


Figure 11: A micro-array with PANHs as sensing elements

microfluidic architecture for the detection of multiple binding events simultaneously with a CCD camera. The integrated device will be used as a fast diagnostic tool to measure common markers normally encountered in chronic lymphocytic leukemia (CLL). Subsequent screening of new markers will also be attempted using this technology.

Objective 4.2.4.B.1.2 Improve the sensitivity of PANHs to refractive index changes

The simple translation of the PANH technology to a micro-array format already shows a tremendous potential for increased multiplexing when compared to the current SPR instruments (more than 200 spots could be, in principle, simultaneously interrogated using PANH against a maximum of 16 spots of commercial SPR). However, the sensitivity of the PANH to binding events is still at 400 nm/RIU, which is an order of magnitude below the state of art.[140] Hence, the Network will be engaged on the implementation of methods to improve the PANH sensitivity. Berini and Sipe (Objective 4.2.4.C.1) will carry out a theoretical effort on the effect of noise on the sensitivity, which can provide some insights into unavoidable noise related to the surface – fluid interaction. Another approach to improve sensitivity

For demonstration of cancer-specific detection, the sensor pixels will be modified with the leukemia markers provided by Wang. Protocols to modify the nanoholes with biological species are available[139] and within the expertise of Network members (Brolo, Chan, Kumacheva). The periodicities for optimal performance and the figure of merits of the sensing elements will be evaluated experimentally by Brolo and Gordon and theoretically by Sipe and Berini (Objective 4.2.4.C.1). These micro-arrays will be integrated “on-chip” in a

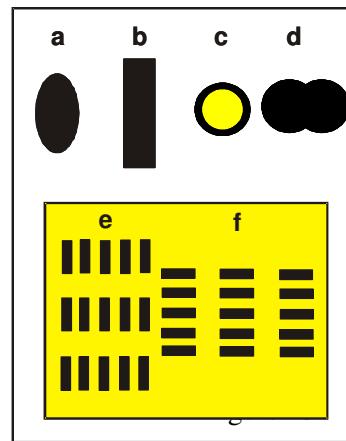


Figure 12: Suggested hole-shapes and array formats (see text)

will be carried out by Brolo and Gordon (with theoretical support from Sipe and Regan) and will involve the optimization of the geometric parameters of the nanostructures.

In particular, we will use the shape of nanostructured holes to modify the surface plasmon properties and enhance sensitivity. Several groups, including ours, have demonstrated the importance of the “shape-effect” on the optical properties of nanoholes[141-144]. The shape and orientation of the holes can be tuned to select the polarization, and the bandwidth, as well as to maximize field localization. Figure 12 a – d represent some of the hole-shapes that will be investigated by the Network. A systematic investigation, guided by FDTD predictions, will be carried out aiming at the optimization of the refractive index sensitivity using arrays of circular, elliptical and rectangular holes. Gordon and Brolo have already demonstrated significant increases in the cut-off wavelength of rectangular holes in metal films[145], which Gordon’s group demonstrated could be used to tune localized surface plasmon resonances[146]. It has been shown that the line-width of arrays of rectangular holes can be controlled by either tuning the periodicity to allow decoupling between localized and delocalized SP resonances[147] or by optimizing the aspect ratio[144,148].

Preliminary work between Network investigators and partners is underway to investigate the bases represented in c and d in Figure 12. The basis in 12c represents an annular aperture that presents unique plasmonic properties, including the capability to sustain propagating cylindrical surface plasmon modes. The annular aperture provides several geometrical parameters for optimization and it is a strong candidate for an ultra-sensitive SPR-device based on PANH. Preliminary fabrication of substrates with annular apertures has been accomplished in a collaboration involving Brolo, Walker and Mittler.

Gordon has pioneered the work on the double-hole (shape d in Figure 12) basis from theory. The double-hole acts like the metallic bowtie nanostructures that were shown to efficiently concentrate the field in the gap between the tips.[149] Gordon has demonstrated a ten-fold enhancement in nonlinear optical signal[150] and in SERS (in collaboration with Brolo)[151]. It should be noted that this overall enhancement is coming from only an extremely small volume, so the local field enhancement is estimated to be several orders of magnitude larger. Subsequently, the double-hole basis has been applied to enhanced SPR sensing using the nanoholes[152], and further innovation is required along this direction. These representative “hole-shape” examples are only first steps towards a comprehensive understanding of the shape-effect and the application of nanoholes to sensing. There are many benefits to the study of “hole-shape”, in terms of enhancing experimental capability and extending theoretical analysis. For example, for the double-hole basis, Walker will undertake near field optical imaging (see Figure 13) to enable quantitative connections between theoretical results from Sipe, Gordon and Rangan and experimental observations from Brolo.[153,154,155] Using heterodyne detection, the real and imaginary parts of the interface polarizability and x, y, and z dependent electric fields will be measured.

In addition, a phase-sensitive approach, discussed in section 4.2.4 A, will be implemented using PANHs by Gordon and Meunier. Polarization and coherent methods will enhance the sensitivity of nanohole arrays to be comparable to the current SPR technology. While SPR resonance from nanohole arrays does not have a usual reference signal, as with the s-polarization in Kretschmann-type SPR, it is

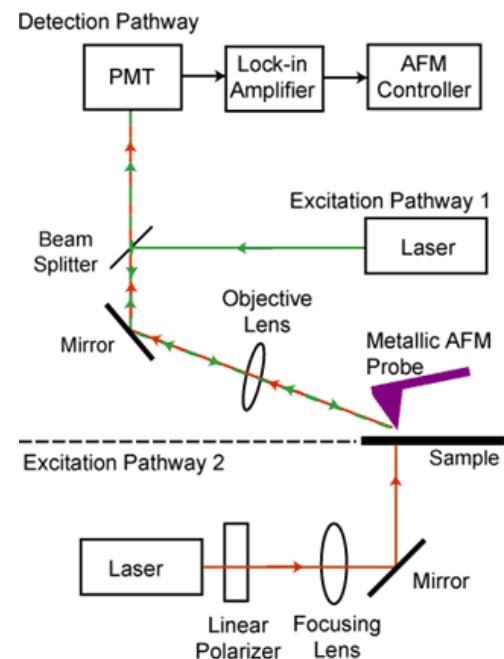


Figure 13: Apertureless near field scanning optical imaging of hole array samples.

possible to adjust the resonant properties of two orthogonal polarizations in the nanohole array by using different periodicities along different directions of a two-dimensional array. In this way, the different periodicities will have different resonances. Both the intensity and phase-shifts will be a measurable output and comparative analysis will be used to enhance the sensitivity. Previous work by Fainman's group has shown how crossed polarizations may be used to enhance sensitivity in a nanohole array[156]; however, their work targeted the transition between Fano and Lorentzian processes by removing the incoherent contribution to the transmission.

The addition of phase-sensitivity may further enhance the nanohole array sensitivity to be competitive with existing SPR methods (an increase of an order-of-magnitude is required), while retaining the benefits of dense integration and multiplexing within a microfluidic environment.

It is essential, and a goal of this network, to make this class of substrates competitive with angle-resolved SPR in terms of sensitivity while building on the existing strength of dense multiplexing capability. The optimized geometries obtained here will be feedback into the micro-array development described above aiming at a full optimized micro-array for leukaemia research and diagnosis.

Objective 4.2.4.B.1.3: Ultra-sensitive sensing by enhanced spectroscopy using PANH

While SPR is the gold-standard for detection in terms of surface binding sensitivity, it is not selective. To address this issue, the Network will consider plasmonic-enhanced spectroscopic methods to enhance specificity.

All approaches discussed above were SPR-based methods and relied on the sensitivity of the plasmonic resonance to refractive index changes. Fluorescence-labelled markers are generally used in both leukemia and lung cancer for diagnostic purposes. The plasmonic approach here presents unique opportunities to improve the sensitive level of the current fluorescence tests by either enhancing the fluorescence signal or by offering the possibility of using SERS. We will develop shaped hole structures with extreme light focussing properties that can be used for the detection of immobilized lung cancer markers provided by Tsao. The objective is to push down the limit of detection of cancer markers to a sub-attomol level and even aim at the ultimate limit of single-molecule detection. In fact, nano-holes in metal films are ideal plasmonic substrates for detection at the single-molecule level because they naturally confine the analyte to extreme small volumes and allow measurements using the transmission geometry. So far, single-molecule SERS has been demonstrated only for random[157], and therefore inherently unpredictable, substrates. A predictable method of achieving single-molecule SERS will be a major transformative technology. The "shaped-nanohole" array approach will be targeted by the Network to meet this challenge. The optimization of the shape of the nano-holes for maximum field localization (focusing) is required to achieve maximal spectroscopic sensitivity and detection limits, as described for the double-hole structure above. The volume inside of a nanohole is of the order of a few attoliters, which is less than the minimum volume achievable by the best confocal fluorescence scheme[158]. This means that a very small amount of sample should be required, increasing the sensitivity of the method.

Elongated bases, as the ones shown in e and f in Figure 12, can act as nanopolarizers with the maximum transmission of light obtained for polarizations perpendicular to the long axis of the basis[141,148]. This property will be explored to add another degree of freedom to the multiplex detection of biomolecules. For instance, the arrays e and f will be modified with different lung cancer targets as described elsewhere. As in a typical SPR experiment for analysis of binding events, the recognition of a solution antibody will lead to a shift in the SP resonance. However, since the transmission is polarization dependent, one can select which of the arrays (either e or f) will be measured. This approach introduces another degree of tunability for the detection of biotargets by SPR. It has been demonstrated by Brolo that similar polarization effects can be observed from SERS of species adsorbed in nanowires[159,160]. Therefore, this polarization selectivity can be extended to

probe either SERS or fluorescence labelled bioanalytes. There are some important advantages that this method may offer over the current microarrays. Firstly, it would allow the analysis of two diseases using only one chip. Secondly, low resolution CCDs could be used for readout, because even if both signals overlap in one pixel of the imaging chip, they can be resolved by polarization selection. Finally, this method will add an extra degree of freedom that could facilitate pattern recognition for the analysis of complex mixtures generally handled in cancer diagnosis.

Milestones:

Year 1:

- Design and fabrication of microarrays of PANHs (Gordon, Brolo, Walker)
- Preliminary proof of concept experiments on the multiplex detection using the microarrays (Gordon, Brolo)
- Determine figures of merit for the PANHs sensor and work on noise source analysis (Sipe, Berini)
- Develop methodology for the immobilization of leukemia antibodies on gold surfaces (Brolo, Wang, Chen, Kumacheva)
- Theory, design and fabrication of PANHs with shaped holes (structures shown in Fig. 12) (Chitra, Sipe, Walker, Gordon)

Year 2:

- Integration of micro-array in microfluidics and preliminary multiplex detection with a CCD (Brolo, Gordon)
- Polarization studies for phase sensitive method and using elongated basis (Meunier, Gordon, Brolo)
- Design and optimization of geometrical parameters for shaped holes (Walker, Gordon, Mittler, Brolo)
- Sensitivity tests with shaped holes, including annular apertures (Brolo, Mittler).
- Develop methodology for the immobilization of lung cancer antibodies on gold surfaces (Tsao, Brolo, Chen, Kumacheva)
- Design methods for immobilization of targets inside shaped nanoholes (Gordon, Tsao, Wang, Brolo, Chen, Kumacheva)

Year 3:

- Multiplex detection of leukemia using an integrated micro-array of PANHs (Wang, Brolo, Gordon)
- SERS and SEFS of probe molecules confined on shaped nanoholes (Brolo, Gordon).
- Implementation of polarization-sensitive and phase-sensitive detection for leukemia diagnosis (Meunier, Gordon, Wang, Brolo).
- Fabrication of micro-arrays of PANHs using optimized (hole shape and other geometric parameters) geometry (Gordon, Walker, Mittler)

Year 4:

- Determination of lung cancer labeled-markers by SEFS and SERS using shaped holes (plasmonic antennas) (Tsao, Brolo, Gordon)
- Development of micro-arrays of shaped holes for multiplexing SEFS and SERS (Walker, Brolo, Gordon)
- Integration and testing of the optimized PANHs for leukemia diagnosis (Wang, Gordon, Brolo)
- Integration of SERS/SEFS arrays in microfluidics for lung cancer detection (Tsao, Brolo, Gordon)

Year 5:

- Compare figure of merits of all detection schemes produced (Berini, Sipe, Brolo, Gordon)
- Provide an optimized platform for fast diagnosis of leukemia (Wang, Brolo, Gordon)
- Obtain single-molecule SERS from an optimized plasmonic antenna (Walker, Brolo, Gordon)
- Provide an optimized platform for the multiplex determination of lung cancer markers by SERS/SEFS (Tsao, Brolo, Gordon).

4.2.4.B.2 Nanoparticles in and on dielectric structures

The goal is to develop gold nanoparticle arrays for cancer detection, which represent the complementary strategy to the nanohole approach described in 4.2.4.B.1. The nanoparticles are imbedded in or located on dielectric materials, typically as an integrated optical device operating in a propagating fashion or in an ATR geometry. The signal can either be an absorption spectrum, a SERS spectrum, a fluorescence spectrum, or a combination of these. SERS yields the advantage of additional chemical information on unspecific binding. Due to the waveguide approach, these methods can be subject to polarimetry: information enhancement due to polarization analysis, as described in section 4.2.4.A.

As in the case of the nanoholes approach, the use of nanoparticle arrays also offers potential for massive multiplexing; however, enhanced sensitivity and reduced noise is required for a competitive scheme for leukemia and lung cancer detection. In order to address the sensitivity problem, all devices proposed will be based on waveguide technology and principally on the application of double or overlapping optical resonances. This strategy should enhance the sensitivity in comparison to established state-of-the-art single resonance methods.

Objective 4.2.4.B.2.1: Improve sensitivity of SPR detection by integrating nanoparticle in waveguides

The approach here is to increase sensitivity by augmenting the interaction between the analyte and a surface plasmon field. As the backbone structure, an ATR-based geometry, typical of commercial SPR, will be used. However, the ordinary 50 nm gold layer of normal SPR will be replaced by a waveguide structure. Possibilities include porous silicon waveguide structures (see Figure 14), where theory[161] and recent experiments¹⁶² indicate the possibility of sensitivity at and beyond the level of usual SPR sensors, using these dielectric devices alone. Other possibilities for waveguide structures include usual planar waveguides, or waveguide structures where so-called Bloch surface waves (BSW)[163], which arise due to multiple reflections at the multilayer interfaces, form the surface excitations (see Figure 14).

Two strategies will then be employed to use plasmon resonances *in addition to* these dielectric enhancements. In the first, metal nanoparticles will be used either in (for the porous silicon structure) or on (for the waveguide and BSW structures) the surface to allow for SERS and fluorescence spectroscopy to be supplemented by these dielectric resonances. In the second, metallic gratings with their resonances will be used to enhance the ability to measure analytes by studying their modification of the diffraction pattern of the structure. Theoretical work on this latter strategy, using protein gratings[163], has already indicated the possibility of significant enhancement of diffractive sensing, and experiments on this structure are now underway. The use of metal gratings and nanoparticles in these

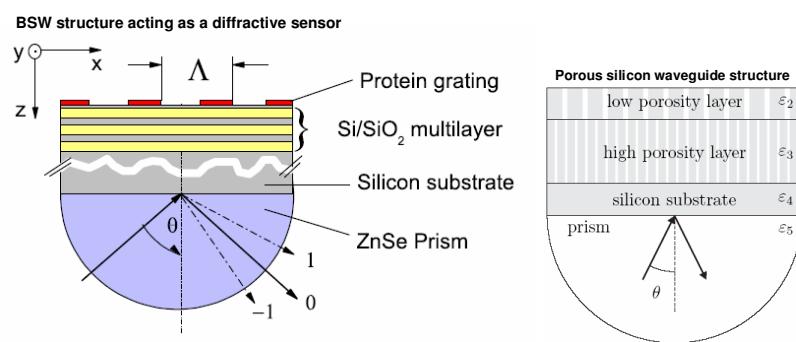


Figure 14: Changing normal SPR (left) to a waveguide structure (right) to decrease metallic loss and increase sensitivity.

structures will not only add the plasmonic resonances, but make available the increased functionalization of the structures that result from the addition of metallic components.

While silicon-based backbone structures are convenient for work at 1.5 microns, BSWs are particular promising surface resonances for use in the visible part of the spectrum, since these can be constructed out of materials typically used in thin film filter structures designed for use in the visible. This is a mature technology that can be called upon for the fabrication of extremely high quality structures.

In this project all partners will contribute in a joint effort within the development steps: Sipe will lead the theoretical design of the devices; the nanoparticle distribution, fabricated by Goh, will be chemically functionalized by Walker using ligands from Zheng. We anticipate that knowledge gained here on the advantages of different waveguiding structures will also be applied to investigate substrates for the following objectives.

Objective 4.2.4.B.2.2: Use nanoparticles in photonic structures for surface-enhanced sensing

The objective here is to arrange metallic nanoparticles in photonic structures[164,165] and use this platform for SERS and SEFS. The first approach (Figure 15a) will apply a nano-particle filled photonic

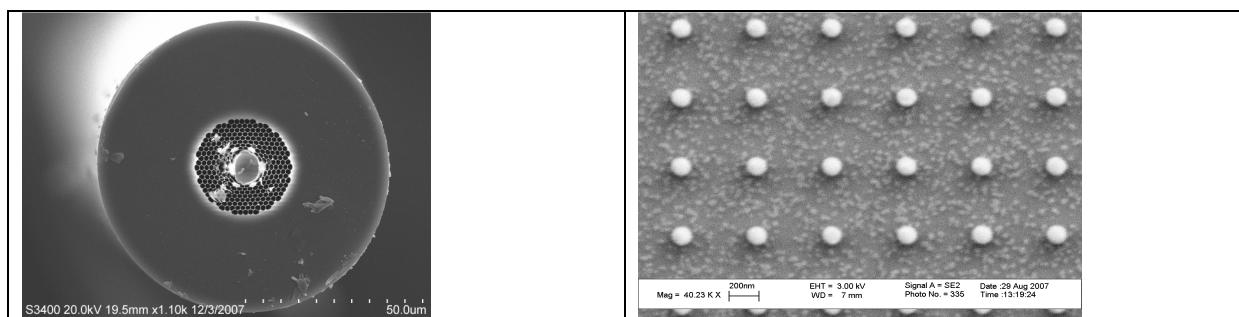


Figure 15: a) photonic fiber; b) Glass slide waveguide with a regular arrangement of Au NPs.

bandgap fiber with a 9.5 μm core surrounded by a micro-structured cladding responsible for the appearance of a photonic band gap. SERS spectroscopy has been successfully demonstrated in preliminary experiments. The second approach is based on a glass slab waveguide with a gold nanoparticle pattern on its top (Figure 15b), allowing it to create the photonic band gap in the evanescent field of the device. This device is based on a double resonance between the photonic band gap and the nanoparticle plasmon absorption band. This photonic band gap structure has been demonstrated with absorption spectroscopy, but the sensor operation, the double resonance visible by a band splitting, is not yet achieved. In principle, both technologies would be able to be implemented in any of the above-mentioned spectroscopies.

This photonic crystal project will be handled in a collaboratively in all development stages. The theory will be handled by Sipe, Rangan, and Mojahedi, the device fabrication and characterization will be carried out by Helmy and Mittler, and chemical functionalisation will be by Walker and Mittler using ligands from Zheng.

Objective 4.2.4.B.2.3: Use nanoparticle arrays for multiplex sensing and monitoring cell surface receptors

The fabrication of periodic arrays of nanoparticles (PANP) would lead to the same set of advantages discussed in section 4.2.4.B.1 for nanoholes. Therefore, the implementation of these arrays in multiplex sensing (as discussed in 4.2.4.B.1.1) would be natural. However, in order to increase sensitivity, a new concept will be implemented where the nanoparticle arrays located on a channel waveguide will switch

spectroscopically from individual nanoparticles to nanoparticle clusters by bridging them with the recognition chemistry. This approach is a direct implementation of the successful solution experiments conducted with DNA and was demonstrated in principle by Mittler [166,167]. Exploration to more complex analytes and geometries is now justified.

PANPs will also be used in an effort to trigger and monitor cell surface receptors in designed spatial patterns. In another application of the nanoparticle clustering sensors, we will use plasmonic arrays of RGD functionalized gold dots to do fluorescence and Raman spectroscopy of the cooperative activation of integrins. Integrin activation is an important step in metastasis. It has been shown in a number of cell types, including osteoblasts and melanocytes[168], that integrin activation can be stimulated by nanopatterned adhesive interfaces. In that study, adhesive dots of RGD peptide spaced 58-73 nm support cell attachment and spreading, while closer or more distant separations showed reduced effect. Restricted integrin clustering is likely the origin of the effect but has not been studied, since a method that simultaneously examines multiple sites patterned appropriately would be needed. Which molecules and in what spatial distribution they mediate tumor cell adhesion to leptomeninges is largely unknown.

The arrays of functionalized gold dots[169] will probe the conformational states of proteins in their vicinity as well as serve as excitation sources for localized fluorescent labels. The spacing between dots is excellent for coherent coupling between plasmonic excitation of individual gold dots, which enables the study of cooperative or simultaneous changes in the integrins and their neighbours. Initial studies will attempt to reproduce observations of cell types that have previously been demonstrated to exhibit integrin activation for specific length scales of ligand presentation. Subsequent studies will examine the hypothesis that lymphocytic leukemia cells would exhibit similar behaviour.

The collaborative mode of these projects involves feedback from all investigators on all steps of production: Rangan will lead the theoretical design of the optics of the nanoparticle arrays, fabricated by Mittler, chemically functionalized by Walker using ligands from Zheng, for cell signalling characterization by Pawson and Wang.

All the above-mentioned devices are at the beginning of their development. They had either been simulated or the principle operation has been demonstrated. In all of the proposed devices, theory and experiment need to go hand in hand for a proper data analysis and to determine the figures of merit. We envisage developing these five novel detection schemes in parallel. The following milestones need to be achieved for each device.

Milestones:

Year 1:

- Achieve an excellent understanding on the important parameters controlling the sensitivity of the sensor device: Determination of Figures of Merit for each novel device, especially having the different optical constants and sizes of the recognition chemicals for leukemia and lung cancer in mind
- Systematic evaluation of parameters of a particular device to evaluate the device performance in the experiment, e.g., with a well characterized recognition system before the leukemia and lung cancer recognition chemicals are available
- Synergy between theory and experimental device
- (Sipe, Mittler, Rangan, Goh, Helmy)

Year 2:

- Build second generation of devices as an optimized structure and test for theoretically found sensitivity with a known recognition system, e.g., biotin-streptavidin, before the leukemia and lung cancer recognition chemicals are available

- Develop methodology for the immobilization of leukemia and lung cancer antibodies on gold nanoparticle surfaces
- Develop liquid handling systems for devices
- (Sipe, Mittler, Rangan, Goh, Helmy)

Year 3 :

- Apply the leukemia and lung cancer recognition chemistry and determine detection limits for both diseases in the absorption spectroscopic approach, in the SERS approach, or in the fluorescence approach; implementation of polarimetry; compare all five strategies with each other and with SPR strategy
- Communicate with Network how polarimetry enhances sensitivity
- (Sipe, Mittler, Rangan, Goh, Walker, Helmy, Zheng, Pawson, Wang)

Year 4:

- Minimize noise sources, enhance S/N ratio
- Apply multiple spectroscopic approaches
- Optimize components for noise reduction (encapsulation, pump less liquid handling systems, temperature stabilization, reduce light source noise, integration of possible gain, etc.) and contribute to a knowledge bank of noise reduction technology. In this stage, all necessary experimental and theoretical experience in the Network is necessary to achieve this aim.
- (Sipe, Mittler, Rangan, Goh, Walker, Helmy, Zheng, Pawson, Wang, et al.)

Year 5:

- Develop approaches for a multiple sampling: parallel propagation systems (fiber and waveguide devices), multi spot systems (ATR systems)
- Develop multi-spot or multi-channel liquid handling system
- Develop multi-channel detection system
- Develop multi-channel methodology for the immobilization of leukemia and lung cancer antibodies on gold nanoparticle surfaces
- (Mittler, Goh, Walker, Helmy, Zheng, Pawson, Wang).

In this project, theory and experiment are created and conducted by cooperation amongst the various groups in the Network and students will be very much involved. A combination of a student concentrating on the fabrication and testing of a device group with a student working on the theoretical aspects hereof will be the common and practical approach taken. At the beginning, more effort for theoreticians is essential to establishing the Figures of Merit; towards the end, there should be less theory necessary for design, but more characterization (and rationalization) carried out.

As an example of student involvement, the nanoparticle arrays on waveguides will be handled experimentally by one student supervised by Mittler for the fabrication and characterization efforts, co-supervised by Walker for the leukemia and lung cancer surface functionalization training and application, and co-supervised by Wang when living cells will be monitored. The theoretical background of the devices will be in the hands of a theory student supervised by Rangan. This theory student will start by developing an understanding about critical parameters for the device performance, e.g., optimal gold nanoparticle distances for a given nanoparticle size having the optical and size data of the recognition chemicals for lung cancer and leukemia in mind. At a slightly later stage, the data will be picked up by the experimentalist for device fabrication and testing. The experimentalist will feed back his/her experimental spectra with the device data. The theorist continues to develop a proper data analysis package that will be forwarded to the experimentalist to test with existing devices.

4.2.4.C. Biosensing using guided surface plasmons

Surface plasmon waveguides, such as the metal film cladded by symmetric dielectrics or the dielectric film cladded by symmetric metals, exhibit vastly different surface sensitivities, mode sizes and attenuations with respect to each other, and compared to the conventional single interface surface plasmon. In their finite width counterpart, these waveguides enable integrated plasmonic circuits, such as Mach-Zehnder interferometers (MZIs), couplers and Bragg gratings of vastly different scale, ranging from cm's to μm 's. Figure 16, for instance, shows measured outputs at $\lambda_0 = 1550 \text{ nm}$ for a collection of cm-scale passives realized using Au stripes bounded by SiO_2 and operating in the long-range surface plasmon mode; (a) shows a mosaic of outputs for couplers where the spacing between the stripes is decreased; (b) shows an output for a straight waveguide; (c) for an S-bend; (d) for a Y-junction splitter/combiner; (e) for an MZI; (f) for sharp angle bends; and (g) for a step-in-width Bragg grating. Similar integrated structures can be envisioned using the dielectric film cladded by symmetric metals but having lengths on the order of μm 's.

Sensors enabled with these waveguides are at opposite ends of the size scale (long-range and short-range), yet, given the trade-off between surface sensitivity and length, both offer the potential of extreme sensitivity and a greatly improved detection limit over the state of the art. Both approaches also offer the potential for significant increase in throughput in the form of a linear array of 100's of sensors. Finally, the non-specific binding problem can be alleviated to some extent by using a reference channel, as is naturally included in sensor architectures such as the MZI (Figures 16(e) and 17). The waveguide approaches are complementary to those using nanoparticles and nanoholes, and hold the promise of significant improvements over conventional SPR. If successful, these approaches could lead to a new paradigm for biosensing, but many issues remain to be addressed, as outlined below. Given the nature of this research issue, it is best tackled within a Network that has the breadth of expertise and skills, and the facilities and equipment needed.

Objective 4.2.4.C.1: Develop models for the signal to noise ratio of sensors

The “signal” portion of a waveguide biosensor depends on the overall sensitivity, which in turn depends on factors such as the surface sensitivity of the waveguide, the interaction length of the wave with the bio-chemical adlayer, and the transducer architecture responsible for generating a change in intensity (e.g., an MZI). The “noise” portion depends on noise sources throughout the transducer, including at the optical source and detection optoelectronics, and at their couplings with the sensor, but additionally and importantly, on noise generated by the carrier fluid through which a portion of the wave necessarily travels. Comprehensive theoretical work will be carried out to assess and compare the signal to noise ratio of various sensor architectures (e.g., MZI), implemented with surface plasmon waveguides. The theory will include 2D and 3D electromagnetic models of the waveguides and transducers, suitable optoelectronic models for source and detection electronics, and thermodynamic/quantum-mechanical based models for the noise generated throughout the system.

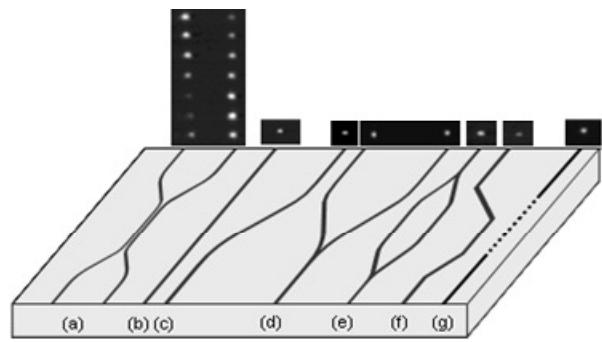


Figure 16: Passive elements working with surface plasmons.

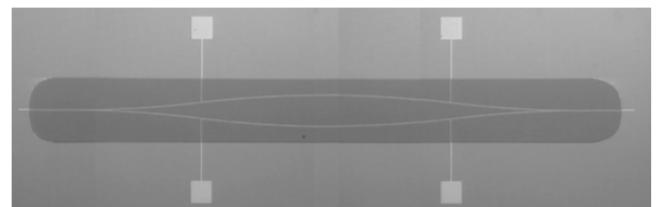


Figure 17: Electrically contacted Mach-Zehnder interfero-meter using metal

Milestones:

Year 1

- Electromagnetic models for waveguides and transducers

Year 2

- Noise models for optoelectronics, waveguides and fluid

Year 3

- Models integrated into end-to-end signal-to-noise

Year 4

- Transducer architectures compared and benchmarked.

Objective 4.2.4.C.2: Implement and test promising sensor designs

Sensors will be designed and fabricated with the aims of validating experimentally the signal to noise models developed under Objective 4.2.4.C.1, and of providing experimental demonstrations of the increased figure of merits (sensitivity, detection limits) in comparison to commercial SPR devices. Architectures based on the metal stripe and the metal cladded waveguide will be considered along with means for integrating microfluidics. Preliminary tests using cancer markers will be implemented. Design will be carried out at University of Toronto/University of Ottawa, fabrication will be carried out at University of Toronto, and testing will be carried out at University of Ottawa.

Milestones:

Year 1:

- Design of fabrication flows including microfluidics complete

Year 2:

- Modeling, design and layout of transducers complete

Year 3 :

- Transducers fabricated

Year 4 :

- Experimentation and testing complete.

Objective 4.2.4.C.3: Research and develop methods for electrochemical functionalisation

Waveguide-based biosensors are typically fabricated using wafer-scale processes, and as such, have features that need to be selectively functionalized, either to inhibit the binding of (bio)chemicals, or to capture a target analyte or allow further modification by the end user. The MZI implemented with metal stripes shown in Fig. 17 is an example of such a structure, where one arm must be coated with a blocking chemistry (reference arm) and the other with chemistry selective to the target analyte (sensing arm). Given that the waveguides are metallic (Au), then electrochemical techniques could be used to selectively adsorb/desorb thiol-based chemistries exploiting electrical contacts to the waveguide stripes. The approach is also applicable, in principle, to other plasmon waveguides. Electrochemical experimentation will be conducted on such structures in order to assess feasibility, to determine processing parameters, to develop a full process flow, and to demonstrate the approach. Contact angle and atomic force microscopy measurements will be conducted to confirm electrochemical adsorption/desorption of thiol-based monolayers, and fluorescent measurements will be conducted to determine the kinetics of the processes.

Milestones:

Year 1:

- Design of experiments complete

Year 2:

- Feasibility demonstrated

Year 3:

- Kinetics determined

Year 4:

- Full process flow established.

Objective 4.2.4.C4: Incorporate optical gain with surface plasmon waveguides

A major deficiency of SP-devices is the relatively short range of propagation that is mainly due to the scattering losses at the metal/dielectric interface and inherent dielectric and metallic losses. One possible solution is to incorporate a gain mechanism in the design of the SP-devices. For this to be useful in the context of integration with other components and systems, the gain medium must be semiconductor-based and have sufficient optical gain to overcome the inherent propagation losses. Figure 18 shows a proposed gain assisted surface plasmon (GASP) structure in which a thin layer of silver is placed close to a gain region, consisting of multiple quaternary quantum wells ($\text{Al}_{0.12}\text{Ga}_{0.12}\text{In}_{0.76}\text{As}$). From an operational point-of-view, there are a few characteristics of the device that must be designed properly in order to maximize the overlap between the plasmonic mode and the active region (the quantum wells) and to minimize the gain required from the quantum wells for the TM modes. The dimensions and compositions depicted in Figure 18 are the result of such optimization[170,171] and the gain required from the quantum wells for this optimized structure is approximately 420 cm^{-1} , which is readily available from the quaternary quantum wells. There are two additional points worth mentioning: 1) In this structure, the SP can be excited by using an end-fire scheme and hence there is no need for the bulky and awkward Kretschmann or Otto schemes; and 2) By using techniques such as wafer bonding, a modified version of the structure in Fig. 18 can be made more symmetric (the same layers above the silver film as below it) which reduces the amount of the gain needed from the quantum wells by almost a factor of two.

Successful design, fabrication, testing, and development of the GASP require a significant level of collaboration and interaction among the investigators. Sipe and Rangan will lead the theoretical efforts in calculating the effects of the noise (spontaneous emission) on the operation of the device(s) and in obtaining a better understanding of the interactions between the SP fields and the quantum wells. Mojahedi and Aitchison will lead the efforts in fabricating the proposed devices and will closely collaborate with Berini in testing of the prototype and integrating the gain with devices described in section 4.2.4.C.

Milestones:

Year 1

- Architectures selected, modeling complete, fabrication flow designed

Year 2

- Epitaxial growth and microfabrication complete

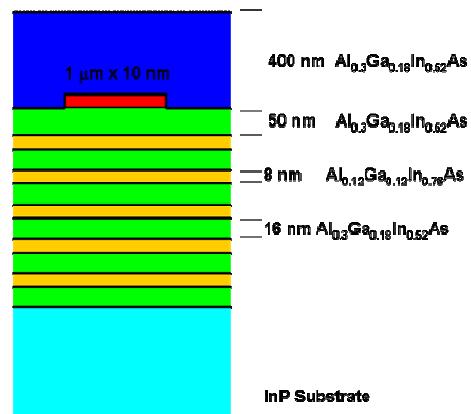


Figure 18: Proposed structure for demonstration of gain assisted surface plasmon. In this scheme one has to introduce a channel (a trench) in the superstrate for fluid or analyte to be exposed to the metal. Note, when the channel is introduced in the superstrate, we reduce device symmetry which hurts long range SP propagation, and will lead to dimensions/compositions other than in this figure.

Year 3

- Experimentation complete

Year 4

- Second fabrication and experimental iteration complete.

In all of the devices in section 4.2.4.C, the easiest sensing mechanism for the end-fire excitation will be absorption; i.e., we will monitor the power at the end of the device and see how much power is absorbed and then back-track and relate this to the quantity and the type of analyte present. If we move away from the end-fire excitation scheme, then one may envision other standard sensing schemes such as shift in the resonance frequency or the angle dependent measurements. When gain is demonstrated in the device in section 4.2.4.C.4, then in principle it can be incorporated in all the devices described in section

4.2.4.C.

One of the most attractive aspects of the SP-based devices is their compact size, which traditional dielectric-based waveguides and interconnect do not offer. Size reduction is important in IT, a parallel driver, and surface plasmons with their sub-diffraction behavior may play a role in this respect and as an interface between electronics and photonics (the size disparity). Section 4.2.4.C.4 addresses one of the main difficulties with SP, which is the short range of propagation. Ironically, the effect of developing the so-called Long range SP (most of the field is in the dielectric region above the metal) is to somewhat increase the propagation distance, but this threatens to undo the compactness benefit. We speculate that a solution may be to introduce the gain as a mechanism to reduce the losses and somewhat preserve the compactness. To the extent that device miniaturization and compactness is or will be an issue in designing next generation sensors, this class of devices will benefit from the introduction of the gain.

The question is then about the noise which any amplification will invariably introduce. At this point, this is an unknown and an issue to explore in the research. We do not know how much S/N degradation we should expect or we can tolerate, and we believe the multidisciplinary collaborative nature of the Network will significantly increase our chances of finding out. From a practical point-of-view, those of us who are engineers routinely use amplifiers to boost our signals and they work very nicely, particularly if we succeed in designing low noise amplifiers. A major focus in this project is to learn how this can be developed in the context of SPs and sensing.

Objective 4.2.4. C5: Microfluidics

The primary science proposed here is the development, optimization, and characterization of new bioplasmonic sensors formed from nanoholes and nanoparticles. But the penultimate goal for much of this work relies on microfluidics – we propose to develop lab-on-a-chip platforms to house the new detectors and to facilitate

low-volume fluid handling

for cancer diagnostics.

Team-member Wheeler will coordinate with the other members with expertise in microfluidics (e.g., Brolo, Gordon, Chan, Kumacheva) to execute this portion of the research plan.

We propose to evaluate two strategies for fluidic handling of

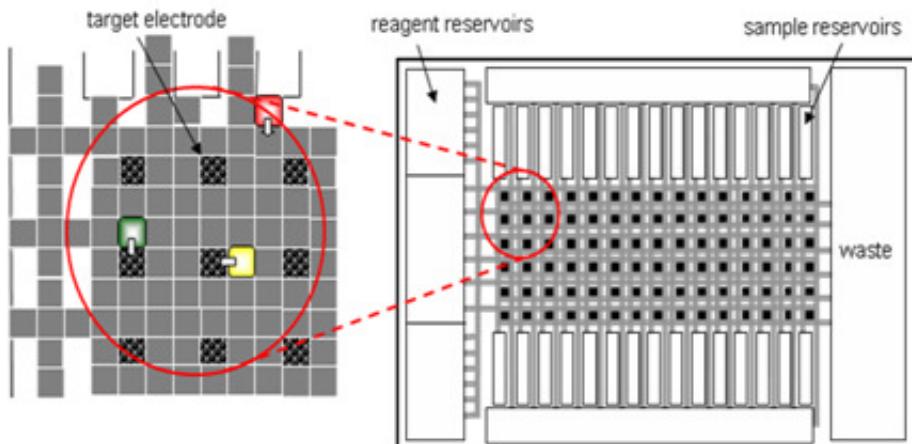


Figure 19: Each detector is addressed with different reagent samples

samples and reagents for bioplasmonic sensing: one relying on networks of microchannels, and another relying on digital microfluidics. Prototypes of platforms relying on each strategy will be fabricated and evaluated on the basis of throughput, reagent use, and reproducibility. After testing head-to-head, the principals will decide which strategy to promote as the ultimate deliverable. The first strategy is straightforward, with extensive precedence in the literature.[172-174] Briefly, networks of channels will be constructed in poly(dimethylsiloxane) using conventional techniques, and aligned and bonded such that reagents and samples can be delivered to nanohole arrays and/or immobilized nanoparticles. There are some well-known limitations of this strategy (void volumes, difficulties in controlling many samples simultaneously, etc.), but it is attractive because it is known, and we are confident that it can be implemented. The second strategy is more “risky,” but could be a better match for multiplexed screening.

The second strategy relies on digital microfluidics (DMF), a relatively new technique in which fluid is controlled as discrete droplets on an open array of electrodes.[175-178] In DMF, each droplet is isolated from its surroundings rather than being embedded in a stream of fluid – a simple method of forming microreactors in which there is no possibility that products will diffuse away. Perhaps most importantly, the array format of DMF is a perfect match for multiplexed screening – i.e., the penultimate goal of this proposal. Building on Wheeler’s recent work in implementing biochemical assays in digital microfluidic systems,[179-183] we propose to develop new DMF device architectures to facilitate transport of fluids to designated “target” electrodes modified with nanohole arrays or nanoparticles. As shown in Figure 19, this will allow each detector to be individually addressed with different reagent samples. In a typical experiment, target electrodes will be sequentially exposed to blocking buffers, sample solutions, and rinse solutions. The efficiency of each incubation/rinse step will be enhanced by actively oscillating droplets between the target electrode and an adjacent electrode,^{184¹³} which will reduce assay times by increasing contact between the proteins in the droplet and the surface. Thus, the time required for each step will not be limited by the rate of diffusion of proteins through a large volume (as is the case in conventional, microwell plate ELISA methods).

A range of electrode and inter-plate spacing dimensions will be evaluated to optimize assay performance and throughput. We will begin with conservative dimensions: 2 × 2 mm target electrode and 500 μm inter-plate spacing. This will enable testing of a 2 μL sample droplet, which already represents a 5-50-fold decrease in the required sample size over conventional ELISA-based assays. These dimensions are a starting-point; we anticipate that less conservative array densities and sample volumes will also be feasible. For example, a device with a 1 × 1 mm target electrode and 50 μm inter-plate spacing should be feasible. If so, it will enable an assay of a 50 nL droplet – a 200-2000-fold increase over conventional ELISA. Different dimensions will likely be better-suited for different purposes; for example, small droplets will be best for reduced sample use, while larger droplets may have lower detection limits, etc. These new methods, which will be implemented on microscope slide-size devices requiring no moving parts, will result in significant reductions in reagent and sample use (i.e., 10-1000-fold) relative to conventional technologies, and the automation of droplet dispensing and transport will result in highly reproducible measurements between samples.

Milestones:

Year 1

- Design and fabricate channel and digital microfluidic devices incorporating mock-nanohole and nanoparticle detectors

Year 2

- Evaluate performance of the two device architectures on the basis of throughput, reagent use, and reproducibility
- Meeting of principals to select strategy for focus going forward

Year 3

- Develop means to incorporate nanoholes into microfluidic platform
- Develop means to incorporate immobilized nanoparticles into microfluidic platform

Year 4

- Build device prototypes and demonstrate single-assay analyte detection

Year 5

- Build multiplexed platform deliverable capable of analyzing 10-30 samples simultaneously
- Characterize platform on the basis of throughput, reagent use, and reproducibility

4.3 Theme 3: Early Detection of Tumors and Characterization of Leukemia and Lung Cancer Cells

4.3.1 Theme Leader and Participants

Theme Leader: M. Tsao.

Theme Participants: C. Wang, G. Walker, G. Zheng, B. Wilson, T. Pawson, C. Goh.

4.3.2 Challenge:

The challenge is to integrate the plasmonics outputs of Theme 2 and 3 into practical methods for early detection of leukemia and lung cancer, and to compare those results with routinely used fluorescence methods.

4.3.3 Objectives

The specific objectives for Theme 3 are:

- a) Prepare and apply Diagnostic panel for Leukemia Detection, done *in vitro* only.
- b) Develop Diagnostic procedure for lung cancer tissue detection
 - Co-develop cell based detection with activities in Theme 1.
 - Develop fiber-based approach using Raman tagged particles sprayed on tissue
 - Develop approach using metamaterial coated on fiber
 - Examine the effect of nanopatterned integrin activation of metastasis.

4.3.4 Tasks and Approaches

4.3.4.1 Diagnostic Panel Detection of Leukemia

Specific leukemia phenotypes are determined by the presence, absence and expression intensity of multiple markers by each individual leukemia cell (Table 2). A diagnostic panel typically consists of 10-15 markers, and our goal is to develop effective panels. The ability to measure multiple markers simultaneously is critical for accuracy and efficiency. Currently, cell markers are assessed by specific

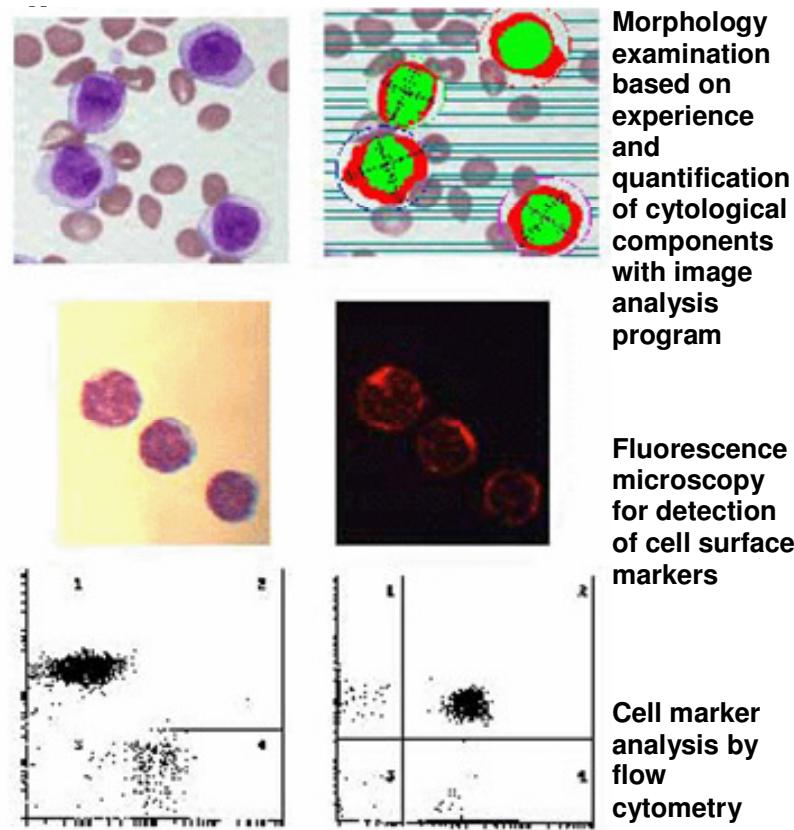


Figure 20: Current morphological and phenotypical analyses of leukemia

antibodies linked with fluorescent reagents (Figure 20). Fluorescence signals are detected by using flow cytometry or microscopy. Because multiple markers need to be measured on single cells, the availability of distinguishable fluorescent labels is a major limiting factor. As the resolution of using common fluorescence dyes is limited to 4-5 colors in a single staining, a set of 10-15 markers has to be assessed by repeat and sequential procedures. With new cell markers continuing to emerge and being applied for improving leukemia diagnosis and fundamental studies, a major challenge in multiplex labeling is to develop new fluorescent probes and alternative probes to fluorescence. We aim to develop such alternatives.

Table 2: Cell marker profiles for differentiation of common B-cell leukemia lymphomas

Diagnosis	CD5	CD10	CD20	CD23	CD45	CD38	CD43	CD79b	CD11c	CD103
CLL	+	-	weak+	+	+	+	+	-	-	-
Mantle-cell lymph	+	-	+	-	+	-	+	+	-	-
Hairy-cell leukemia	-	-	+	+	+	-	+	+	+	+
Splenic lymphoma	-	-	+	+/-	+	-	+	+	+/-	-
Follicular lymphoma	-	+	+	-	+	-	-	+	-	-

We will initially focus on testing for chronic lymphocytic leukemia (CLL) because it is the most common type of leukemia in North America. CLL phenotypic analysis includes markers to define clonality (Ig κ or λ light chain restriction), B-cell lineage and maturation markers. All these markers are specific protein molecules on cell surface. These cell markers are essential to establish diagnosis and to differentiate CLL from other lymphoid leukemia and lymphomas. CLL cells will be used as the primary leukemia target for our studies.

Wang will guide the development of the panel because, as a laboratory hematologist, he has extensive experience and expertise in cell marker analysis. His previous research on leukemia phenotypes has led to recognition of a novel diagnostic entity of B-cell clonal lymphocytosis^{185,186}. CLL patients usually present with a high number of leukemia cells in blood (often in the range 40-100 X10⁹/L). CLL cells may be collected from routine blood samples and processed to reach over 95% pure leukemia cell population. (A REB-approved protocol has been established for collecting CLL cells for research at Wang's institution.)

In this work we will prepare a diagnostic panel for leukemia detection, but focus *in vitro* only. We will address up to 10-15 markers, using antibodies, initially. Again, cell surface markers will be the main targets. As a control, we will need to examine non-specific binding. Furthermore, we will examine the effect of binding on the cell; this might, for example, illustrate toxicity that ultimately could be useful for therapy. Once we have examined binding with the particles and antibodies, alone, we will move to Raman tagged particles, and particles tagged with both antibody and Raman dye. We will need to determine the effect of the particle on the antibody. A similar series of steps will need to be undertaken using panels incorporating peptide ligands. Comparisons of efficacy will be made.

This panel of typing antibodies detects surface markers of lymphoid tumors. This is a group of heterogeneous tumors, including chronic lymphocytic leukemia (most common) and over 20 other diagnostic entities. They can be distinguished by their phenotypic profiles (patterns of the presence and absence of certain markers). The combination of these markers will be sufficient for a definitive diagnosis and fine classification of lymphoid tumors. The application of the Raman tagging approach for detection in flow cytometry will be explored in later stages of the grant since this is a widely used application of fluorescent probes, so as to test the value of the SERS approach.

Milestones:

Year 1:

- Design protocol and prepare antibody/Raman tagged probes for CD19 and CD4 for positive and negative responses to CLL cells (Walker, Zheng)
- Compare results with fluorescent dye and quantum dot assay (Wang)

Year 2:

- Increase sampling to 10 or more markers for CLL, initially among many cells (Walker, Zheng, Wang)
- Evaluate the potential for single cell analysis (Wang, Pawson)

Year 3:

- Develop and test Raman tagged probe approach for other leukemias (Walker, Zheng, Wang)

Year 4:

- Design flow cytometry approach (Wang)

Year 5:

- Test flow cytometry approach (Wang, Walker, Pawson)

4.3.4.2 Lung cancer

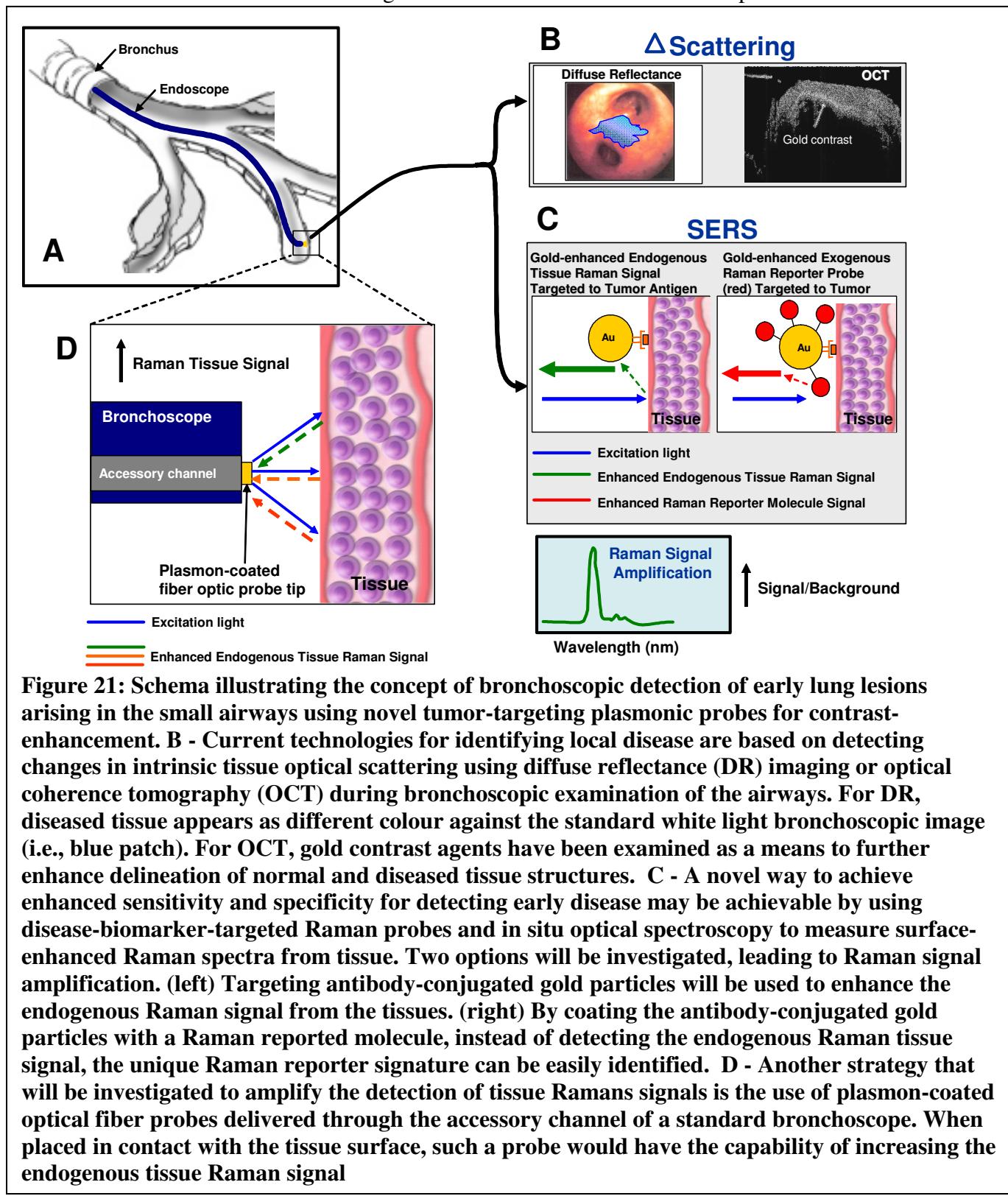
To increase specificity of lung cancer detection methods, one may use specific probes that recognize cell surface or tissue proteins whose abundance in cancerous and normal cells/tissues differs. Using advanced molecular techniques including microarrays, Tsao and other investigators are conducting intensive research to identify such markers. Proteins that are most likely able to distinguish normal from cancer cells are likely to be low in abundance, thus they require very sensitive techniques to detect. In addition, it is certain that combinations of multiple markers are likely to provide greater specificity. Thus, novel plasmonic agents that can increase the sensitivity and/or specificity of current methods to detect cancer cells will improve the efficacy and cost-effectiveness of early lung cancer detection strategy.

The best candidate protein markers that are most suitable for probing *in vivo* include cell surface proteins that are differentially increased in cancer cells or in stroma surrounding tumor cells. Cell surface markers include growth factor receptors, such as epidermal growth factor receptor (EGFR), hepatocyte growth factor receptor (MET), and discoid domain receptor (DDR1). In addition, we also have identified several novel genes/proteins that are abundant mainly in tumor tissue compared to normal tissue. These include integrin α -11 (ITGA11), collagen type XI (COL11), and crystalline μ (CRYM). Ongoing studies will lead to identification of additional markers.

There are several ways to detect cancer markers using the novel plasmonic agents:

1. Label antibodies to these protein markers and deliver the antibodies locally into the airway, e.g., by nebulizer, then use a bronchoscopic instrument equipped to detect the plasmonic signal to detect areas where the probe is concentrated, putatively in the cancerous lesion.
2. Inject the labeled antibodies systemically, which will localize to the lung lesions, then use combined CT and bronchoscopy to localize precisely the lesion which accumulates the labeled antibody.
3. Load the plasmonic agent into marker-guided lipoprotein-based nanoparticles developed by Zheng and inject the particles into the blood to localize to the cancer tissue. Since these nanoparticles incorporate guiding antibody or peptide for targeting to the cancer cells or stroma, plasmonic agent can assist in subsequent localization of the cancerous lesion.
4. Use biosensor coated fiber bronchoscopes to enhance the detection of labeled antibodies or probes (See Figure 21).

Initial testing and validation of labeled antibodies will be performed on lung cancer cell lines that are known for their expression level of marker proteins. The expression levels of specific marker protein will be assessed by Western blot using the specific antibody. As negative control, we shall use cells that are low in marker expression, or cells that have been transduced with short hairpin(sh)-RNA against the marker mRNA and validated as showing >80% knockdown of the marker expression. We also shall use



these cell lines to test the fidelity of antigen binding property of antibody labeled with the plasmonic agents. Following satisfactory *in vitro* studies, the probes can be tested *in vivo* in tumor xenograft models of the same cell lines. To do this, 10^6 cancer cells will be injected into the subcutaneous tissue of nude mice and allowed to form tumors. When the tumor reaches 5-6 mm in size, the plasmon-labeled antibody will be injected either locally into the tumor or systemically into the blood, and the tumor will be imaged serially at 0.5, 1, 2, 4, 8, 16, 24 and 48 hr later to detect the accumulation of plasmonic signal in the tumor.

Probes and technologies developed in the lung cancer model also may be applied to many other solid tumors, including digestive tract cancer and precancerous lesions using endoscopy, as well as to potentially intra-abdominal neoplasms such as ovarian cancer using laparoscopy.¹⁸⁷⁻¹⁹⁸

In vitro cell culture and *in vivo* xenograft studies using lung cancer cell lines are routinely incorporated in Tsao's model. This includes protein assay using Western blot and gene knockdown method using shRNA. The laboratories of Tsao, Zheng and Wilson already collaborate in developing diagnostic methods for lung cancer via fluorescence studies.

4.3.4.2.1.A Fiber tipped probe for lung cancer detection in tissue, Method A:

Method A will involve imaging particles that will be sprayed by endoscope onto the tissue. In this method, an adapted endoscope nozzle will be used; we seek to avoid injection. The proper initial experiments will begin with EGFR, but we will also develop EGFR expansion labels. We will examine lung cancer tumors at different stages. In addition, we will examine non-EGFR targets, i.e.: EGFR: Cetuximab (FDA approved), matuzumab (now in clinical trials); VEGF: Bevacizumab (FDA approved); DDR1 (discoidin domain receptor): nature ligand collagen; antibodies available (only for Western blot and immunohistochemistry); and FAP (fibroblast activation protein) Sibrotuzumab (now in clinical trials).

4.3.4.2.1.B Fiber tipped probe for lung cancer in tissue, Method B:

In the second method, we will develop a fiber tip that has plasmonic material on its own surface. The field localization in plasmonic structures can be used to introduce label-free selectivity into the imaging method through enhanced spectroscopy. In this case, specific chemical signatures in different regions within cells and tissues can be differentiated by their spectroscopic signature. This can be used for the differentiation of tumor and healthy cells, as discussed in Theme 2. We propose development of a suite of fiber optics probes that will be used for endoscopy and imaging of biomaterials. Wilson will lead the fiber optic development, assisted by Brolo and Walker.

The low signal to noise ratio is among the main challenges

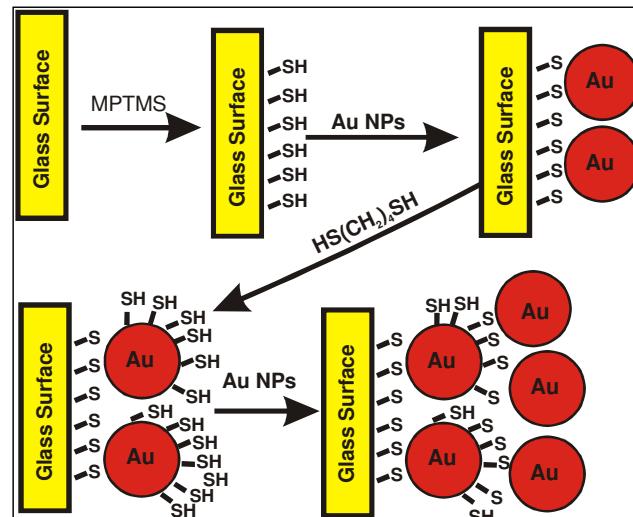


Figure 22: Self-assembly approach to modify a fiber optics tip with Au nanoparticles

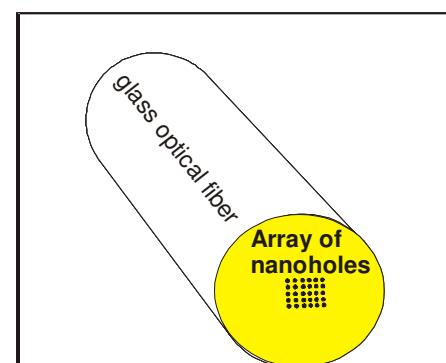


Figure 23: Array of nanoholes milled in a metal coated fiber optic's tip.

in fiber optics imaging of biomolecules. The plasmonics approach offers a way to deal with this issue, since the focusing properties of metallic nanostructures can be tailored for enhanced signal response with high spatial resolution. We propose two approaches to modify the fiber optics tip for enhanced sensing: The first approach will be based on the immobilization of metallic nanoparticles (NPs) at the fiber optics tip by self-assembly, as illustrated in Figure 22 (Brolo and Walker will collaborate here). We have shown that the characteristics of a self-assembled structure NPs of a given size depend on the number of NP layers[199]. The second approach will take advantage of our experience with PANH and aim at the patterning of the fiber optics tips with the kind of structures discussed earlier. The design of shaped nanoholes (Gordon and Walker) that maximize the field efficiency and sensitivity will be carried out and the fiber optics will be patterned by focused ion beam milling. The nanostructured tip, represented in Figure 23, will be used for imaging of biological tissues, where the contrast can be provided by either changes in local refractive index or by enhanced spectroscopy (SERS and SEFS). Alternatively, metallic NPs coated with specific antibodies that recognize cancer cells can be introduced to enhance contrast. The modification of the glass tip used in endoscopy with the metallic nanostructures that enable surface-enhanced spectroscopy is a natural next step in this proposed research. This could allow *in vivo* imaging even with sub-wavelength resolution.

The combination of plasmonics in fiber optics proposed here provides an opportunity for the development of new modalities for *in vitro* and *in vivo* imaging with high spatial resolution.

Milestones:

Year 1:

- Develop antibody-labelled, gold nanoparticle-based plasmonic probes for local delivery (Zheng, Walker)

Year 2:

- Test the specificity of plasmonic probes in lung cancer cell lines expressing different levels of marker proteins (Tsao, Zheng)
- Develop marker-guided lipoprotein nanoparticle for encapsulating plasmonic probes for systematic delivery (Zheng, Wilson, Walker)

Year 3:

- Test the *in vivo* specificity of plasmonic probes in lung cancer xenografts (Wilson, Helmy)
- *In vitro* specificity studies of lipoprotein-encapsulated plasmonic probes (Zheng, Wang)

Year 4:

- Deliver labelled plasmonic probes locally into the airway in an orthotopic lung cancer model, e.g., by nebulizer, then use bronchoscopic instruments equipped to detect the plasmonic signal (Raman, OCT) to detect areas where the probe is concentrated (Wilson, Tsao, Zheng, Chan)

Year 5:

- Use a plasmonic biosensor-coated fiber bronchoscope to enhance the detection of labeled antibodies (Wilson, Tsao, Brolo, Zheng)
- Deliver lipoprotein-encapsulated plasmonic probes systemically to localize to the lung lesions, then use combined CT and bronchoscopy to localize precisely the lesions which accumulate the probe (Wilson, Tsao, Zheng).

Reaching The Critical Point

The investigators in BiopSys have done some important proof of concept demonstrations of applications of plasmonics in chemical sensing and spectroscopy. We have now reached a critical point where a network approach is necessary to take these demonstrations to the application level, to put us in contact with other biomedical researchers who will broaden our expertise and critical thinking, with health care delivery personnel who will test the technology we are developing and eventually put it into practice,

and with the stakeholders who will benefit from that technology. The further development of plasmonic-based methods to solve the scientific challenges we face requires a concerted effort. Theoretical methods are necessary to guide our experimental efforts, nanofabrication specialists are needed to overcome the limited output provided by the serial nature of the FIB technique, and specialists on integration are required to take full advantage of the sub-wavelength capabilities of this sensing technology. This kind of concerted approach is imperative if we are to maintain our leadership in the area and translate this technology into the biomedical applications that will benefit us all.

5. Training

5.1 Why Do We Need HQP?

Canada needs a workforce of biomedical sensor creators, who can tackle problems that require integrated knowledge of immunology, optics, and integration and transition into devices. Such students are being trained in the current North American powerhouses of biomedical technology, in Boston and Minneapolis and elsewhere. These students then turn around and foster the creation of breathtaking advances in healthcare and groundbreaking start-up companies. Without such advanced training and the positive exposure to entrepreneurship that combines excellence with focus, Canada's healthcare economy may become a local service industry or be severely limited in nanotechnology advances. It is essential for Canada to move beyond its current stage of expertise and economic activity; medical diagnostics is a target area where a cluster of active participants (corporate, academic and government) may be able to break through to global competitiveness. What is needed is qualified workers, who will speak the language of the science, be open, imaginative and creative about the choice of technological approach, and be able to collaborate in the competitive spirit that brings the greatest successes.

5.2 Training Strategy

BiopSys has a critical mass of expertise, intellectual property, and state of the art knowledge, facilities and equipment to contribute to our training activities. It also is dedicated to the collaboration and cross-pollination that are necessary to a successful interdisciplinary and multidisciplinary research and training enterprise.

Our training vision is to develop and retain researchers in biplasmonics. Given that we are in the initial stages of a new Network and the likely extent of our funding base, however, our realistic training goal for the next five years will focus mainly on graduate students and to some extent on post-doctoral fellows.

Our training objective is to train students and postdoctoral fellows in a close-knit interdisciplinary, multidisciplinary team on projects with a common goal that is directly relevant to biplasmonics for medical diagnostics, critical to the technological and economic future of Canada, and directly relevant to the well being of Canada's population.

The building goals of our student training effort are:

- Develop a set of basic concepts that graduate students in biplasmonics must know and have hands-on experience with in order to be successful
- Identify the best sources of training of those concepts within the Network, whether within the home research groups or elsewhere in the Network
- Identify ways of providing that training to students, without interfering with their existing coursework or overloading them unnecessarily.

5.3 Training Plan

We recognize that different forms of training are required for students and post-doctoral fellows at different career stages. The training network for a beginning graduate student is normally found within coursework and his or her immediate research group. S/he needs to learn the methods and theories of the home group. In the first year, students will learn about the goals and general strategy of the larger BiopSys Network through listening and attending Network conferences and other meetings. By the second year, students will become more deeply integrated into the Network.

Our training plan includes the following enabling mechanisms:

- Kick-off conference/retreat for everyone involved (1st year)
 - Present an organizational tree to show connections between groups and to motivate students toward the Network and its Themes
 - Investigators from each department/major discipline give talks highlighting their role in the research projects, how their work connects to work in other departments/disciplines, and how they will work together (identifying specific collaborations)
 - Presentations on how to communicate with each other and what tools are available for such purposes
 - Address what has been done already, give motivation for current projects and desired outcomes
 - Outline what is to be accomplished within each specific group/sub-set of collaborators
 - Become aware of fellow collaborators' schedules, and from this work out concrete goals and a timeline for accomplishing the goals (generation of an action item list)
 - 1/3 public group talks; 1/3 workshops; 1/3 one-on-one discussions.
- Monthly brown-bag lunches, providing:
 - face-to-face meetings among all researchers, trainees, and other staff at any given school
 - video- and tele-conferenced meetings between schools.
- Quarterly tele- or videoconferences specifically to address progress in each Theme
- Semi-annual conferences of all participants, with a workshop program
- Shared access to and support for facilities
- Exchanges, shared training and co-supervision of students
- A career development program for students
- Student internships at companies and government.
- Regularly scheduled seminars, with short talks to summarize the research/skills of individual researchers and groups and the work in which they are involved
- A Network website with background/bios of all the researchers and perhaps students and post-doctoral fellows, sharing of presentations, discussion forum and the like
- Laboratory tours (locally, where feasible)
- Workshops focusing on instruments, facilities and techniques (locally, where feasible)]

Many faculty members and research staff in the Network are experts at networking. Cynthia Goh, for example, successfully combines her skills for fundraising on a scale to initiate start-ups, with joint mentorship of students with other faculty, with highly visible leadership of a joint training course for future entrepreneurs. In speaking about her experiences, she is blunt about what approaches did not (and will not) work, and full of the energy that attracts students, colleagues and Venture Capitalists to her side. Pierre Berini comfortably and successfully co-directs graduate students with other faculty, which as he eloquently points out depends first on keeping obligations of participants clear and fair and the lines of communication open. In the teleconferences that were at the core of the development of this proposal, he listened to all but still managed to give concise advice for how to move forward with

improving the Network, and gently provided reminding emails to follow up. Listening to the group, he pulled out the threads that unified people and brought together researchers across the divides of discipline by generously sharing financial support he might expect with others, in the knowledge that his competitive position – that is, finding a better technology – would be more likely to improve using that approach.

These and other Network members will run discussion sessions every other month, to mentor and monitor. All Network investigators will take a turn at presentations on mentoring and collaboration, in this discussion format.

A mentorship program will be put into place. Younger participants often feel less connected than older ones. A mentorship program is a way for a student to get to know people outside of his or her group, connecting younger students to more experienced researchers. The program also will have the feature of being interdisciplinary so that each person learns about other fields. In addition, graduate students will undergo short rotations to enhance exposure.

As well, the Network will provide training courses in effective collaborations and how to handle them. Here is a sampling of issues and ways to address them:

- Initiating a collaboration: The first time there is a specific collaboration (e.g. between a group that prepares samples, and a group that analyzes the samples), the two parties should work together in person to do the measurement, so that they can understand what is involved on both ends
 - Additional effort is required when collaborations are over long distances – we will have teleconferences, etc. to summarize the experimental details (to address “why can’t you just do this...” questions, brainstorm ways to overcome limitations of the experimental set-up, etc.)
 - There must be a clear statement of sample preparation protocol, which we will place on the Network website.
- Lack of collaboration that was expected, e.g., no work was actually done by a collaborator on a given sample
 - Solution: Accountability: checking to make sure whether measurement was done, and if not, why not
- Variations in levels of commitment from different groups
 - Solution: A deadline system, e.g., presentation deadlines
- Slow turn-around in manuscripts, not knowing details of experiments
 - Solution: “Shared lab book notes”, e.g., Google Calendar, website tools.

One of the great opportunities of this Network, of course, is for everyone to learn how research and development is done in areas that are not their own. Therefore, we will run career development sessions where faculty, industry and government representatives: 1) talk about their jobs; 2) provide feedback on CVs the students are developing; and 3) offer chances for everyone to visit each others workplace, independent of a specific collaboration.

5.4 Trainee Employment Opportunities

The Network will be a strong source of employment opportunities. All of our industrial partners have a history of hiring personnel in early career stages. Indeed, two of our partners are start-up companies begun by students (for example, Darren Anderson, CTO of NNT, was a graduate student with Cynthia Goh and founded NNT with several coworkers). Government laboratories hire students both fresh out of graduate school and after postdoctoral study.

It is recognized that most of our students do not go on to academic careers and are instead employed by the industry. The Network will offer our students opportunities to directly engage with industry. The interactions between the students from the various research groups and the scientists and

engineers in the relevant partner industries will cement the relations in both sectors and generate relationships that will inevitably lead to job offers to the students involved. Spin-off companies that do nanoscale photonics have recently emerged from several of the laboratories that are part of BiopSys, and it is expected that these companies will seek to interact with the Network's students.

Fueling entrepreneurial activities

As we have mentioned, the interaction between the Network's students, academics and industrial partners will likely result in novel ideas and valuable intellectual property. This in turn encourages the parties involved to consider startup activities to capitalize on such IP. To assist in these opportunities, the Institute of Optical Sciences (IOS), a formally established entity within the University of Toronto, has developed and delivered an Entrepreneurship 101 non-credit course for the past two years, attended by ~500 students, in which representatives from top Venture Capital firms and business leaders give lectures and mentor students in starting up their own businesses. IOS is adding to this program with a more intense Entrepreneurship 201 non-credit course, and this will be offered to Network students in addition to 101.

Absorption Capacity

According to MEDEC [200], the national medical device manufacturer's association in Canada, the industry employs over 35,000 Canadians, in close to 1,500 corporate facilities, with almost \$6 billion in national sales per annum. Annual sales are \$3 billion in Ontario, \$2 billion in Quebec and \$500 million in British Columbia. Ahead of industry, MEDEC members in mid-size facilities are growing at a 7% growth rate in employment. MEDEC small and medium sized facilities are leading growth; the average number of R&D workers in MEDEC facilities is 17. Our corporate partners (e.g., NNT, Axela, Spectalis) also operate small facilities, with about 10 workers per company.

Students training in the network will also find opportunity in the pharmaceutical industry. In the Toronto area there are a number of drug companies that have technology that strongly parallels that being developed here, though the application is different, focused in drug delivery and biocompatible coatings. These companies need new employees with state-of-the-art understanding of receptor targeting methods and nanoscale delivery vehicles for drugs. It is expected that several of these companies will become partners to the Network, after our "brand" is better established.

In addition, NRC and the academic collaborators in this Network have hired among them more than 50 biomedical technologists into faculty and post-doctoral positions.

Within the group of participating faculty, more than four start-ups have been created out of academic research efforts. These start-ups almost always began with a strong tendency to hire out of the pool of talent that had created them. The involvement of our partners is important to the success of the Network and this involvement is already evident by past placements. More than 15 applicant/co-applicant students were hired by NNT, Axela and FIO in the past 2 years.

To help trainees find positions we will have an electronic catalogue of Network-funded trainees available to partners, and an electronic bulletin board of trainee opportunities available with partners.

5.5 Number of Trainees

The network will fund Masters, PhD and post-doctoral trainees as detailed in Table 3:

Table 3: HQP Training by Theme and Year

Theme/YR	MS Students	PhD students	Post-doctorals
I/1,2,3,4,5	2,0,0,2,0	4,6,6,4,6	1,1,1,1,1,1
II/1,2,3,4,5	4,1,0,4,0	11,14,15,11,15	0,0,0,0,0,0
III/1,2,3,4,5		2,2,2,2,2	2,2,2,2,2

The majority of faculty in the Network have been limited by funding rather than applicant pool, and so rapid filling of the spots created by this program is expected. The Principal Investigator, Professor Gilbert Walker, was in charge of the graduate studies program in Chemistry at University of Toronto. When added resources became available from the province to support the double cohort that began passing into the graduate program in 2006/07, Professor Walker managed to increase graduate enrollment overall by 30% while simultaneously raising the GPA of applicants by 0.2 GPA points. Twice as many NSERC funded graduate students matriculated in 2007 as in 2006. Hence, both quality and quantity were improved. Professor Walker's group at University of Toronto has grown from one graduate student in 2005 to nine in 2008; nearly all are recipients of research awards from external funding sources. Many of the faculty involved in the Network serve on graduate student admissions committees and advertisement by these committees of the opportunities created by the Network will result in successful recruitment. Because it is important for the Network to make successful research strides early, many of the graduate students in the first year will come from the pool of current graduate students. We have students available for this transition.

The number of postdoctorals is quite limited; only three will be hired into the Network. These researchers will go into projects that initially require the greatest immediate maturity in collaboration.

Some faculty have historically taken few students in any given year, and occasionally they recruit fewer than they seek. In these cases if a suitable student does not appear by the start of the first semester of the grant, then support will temporarily pass over to a faculty member who has a student who is interested in the required work.

We will measure the success of the training in several ways. One way will be by changes in the number of applications for training positions with Network investigators. Another will be by the number of trainees who take positions with industry and academe. A third will be by comparing our drop-out rate with that of graduate programs overall.

Track record/expertise of the researchers and their involvement in training

Network faculty have trained 300 undergraduate students, 143 masters students, 170 PhD students, 193 post-doctorals and 36 other researchers over the past six years. (Note that roughly half of the PhD students are current.) In addition, eight of the faculty are within six years of their first Canadian faculty appointment and their groups are growing. This record indicates that the involved faculty are attractive to students; hence, we expect recruitment of students into the Network will be straight forward. Network faculty are among the most respected academics in Canada. Professor Pawson is Canada's most highly cited biomedical scientist (14th in the world from 1990-1997), Professor Berini has been awarded the prestigious 2008 EWR Steacie Fellowship for his work on surface plasmons, and 6 of the faculty hold Chair Professorships.

6. Interactions and Partnerships

6.1 Involvement of Partners Organizations

Our partners in industry and government have been instrumental to our planning process. We interacted with all partners more than half a dozen times in the planning and development process, some many dozen times. Our partners were involved in the development of the Network's research thrusts, in the planning for the preliminary proposal, and in the planning for and development of this full proposal. They have been instrumental in identifying research options and potential research synergies for the Network, they have identified specific research initiatives in which they have interest, and they have been involved in the development of our training strategies. The extent of involvement of any particular partner in planning, research, direction and management has varied, of course, with the particular partner. Some partners wish to contribute in kind and then learn the outcome of the research through informal interactions and the open literature. On the other side of the spectrum, some partners will serve on advisory and/or oversight committees. In general, the means of interaction with partner organizations were face-to-face and teleconference interactions.

6.2 Partner Details

We provide details below on some of the companies and government agencies involved in the Network as examples of our partner base.

6.2.1 Selected Canadian-based Companies

Axela: Axela manufactures equipment for biomolecular sensing based on a diffractive coupling mechanism. It is interested in learning how special forms of plasmonic coupling might be incorporated to enhance the sensing platform. Dr. Cynthia Goh, the company founder and scientific advisor, has been involved in all stages of the Network's development, including the development of the preliminary proposal. Axela will provide feedback regarding the practicality of diffractive coupling based sensors, via its collaboration through Dr. Goh, Dr. Helmy and Dr. Sipe. In addition, the relationship between Dr. Goh and Axela provides the company with right of first refusal for photonics IP developed in the Goh laboratory.

Northern Nanotechnologies (NNT): The first product line of this company, and one still being developed, is a series of kinds of metal nanoparticles. NNT is strongly interested in understanding how these particles could be functionalized to add value. Professor Walker has had numerous conversations with Dr. Darren Anderson, NNT's Chief Technical Officer, as well as Mr. Keith Thomas, the President, regarding how this might occur. The SERS approach is of specific interest to the company and IP the Network generates could be licensed to NNT. Professor Walker serves as an advisor to the company, with appropriate mutual protections of confidentiality in place. Both Mr. Thomas and Professor Walker are assisting the Province of Ontario in determining its policy for promoting nanotechnology. A representative from NNT will sit on the Board of Directors of BiopSys.

Spectalis: Spectalis develops plasmonic devices. There is a tight interaction between Spectalis and Professor Berini's activities at the University of Ottawa and Spectalis has absorbed much of Professor Berini's HQP and IP. Professor Berini also sits on the board of Spectalis.

Lumerical: This company sells software for photonics. During the planning stages of this proposal, Lumerical contributed advice, software and CPU time to design some materials to be studied in the proposed work. Lumerical aims to gain a fuller understanding of the photonics-related research issues on which academic and industrial researchers are working.

6.2.2 Government Departments/Agencies

National Research Council Steacie Institute for Molecular Science (SIMS): Dr. Shan Zou, a scientist with the Biomolecular Sensing and Imaging Group at SIMS, spent several days visiting Professor Walker, discussing areas of mutual research interest and overlap. The BSI Group is undertaking complementary research and it has been agreed that an effort will be made to avoid unnecessary overlap. A planning exercise has been underway at NRC whereby SIMS is aiming to increase the application of its research to commercial outcome. It is expected that a representative from SIMS will sit on the Technology Transfer Advisory Council.

6.3 Existing Linkages

One important existing link between several partners that was instrumental to their initial involvement in the Network is their membership on the Advisory Panel to the Ministry of Research and Innovation, Ontario, on which Professor Walker also sits. In addition, all of the industrial partners already interact with members of the Network through joint research ventures, all of which have resulted in joint publications.

6.4 Links to Develop

We aim to amplify this interaction by increasing the access of companies to researchers via the Network. One means of increasing access is by creating a compendium of information on the research interests and projects of the Network's full complement of investigators and HQP, along with a detailed list of facilities at each institution and a description of the mechanisms by which members of the Network are permitted to use those facilities. Often times such facilities would be extremely attractive to industry. We also will seek to create a fuller list of the research interests of each of our partners, as well as the facilities that each operates, as this information surely will be similarly attractive to our researchers and trainees. Samples will be exchanged between the academic and industrial sites for development and calibration purposes.

Partners will be particularly helpful in defining market-related demonstrators for the underlying technologies to be showcased. They also will be invaluable contributors to the technology transfer cycle and in capitalizing on the developed techniques and devices and turning them into commercial products, either through adoption by these partners or through assisting in startup activities.

The Network Manager will have as 25% of his/her job description, improving interaction with partners. This is time dedicated to enhancing cooperation and cross-pollination amongst all our stakeholders.

We anticipate that BiopSys will be a founding member of Nano-Ontario, a public interest group formed of university, industrial and government representatives with the aim of developing and translating expertise in nanoscience into HQP and commercialization.

6.5 Communications

Communications Strategy for Publicizing the Network

Because of the significant health-related objectives of the Network, our communications goal is to increase awareness of our existence and purpose amongst all stakeholder constituencies, which include not only industry, government and other researchers, but such constituencies as teacher organizations and school systems, health care providers and technicians, and the general public.

The Principal Investigator and Network Manager will work with the public relations arms of the participating Universities to develop a suitable press kit that serves both University and Network use. Updates will be made available to the press on a regular basis. This press kit also will be given to the Presidents of the Universities as well as the Deans and Chairs of the involved schools/departments, and to the chief officers of our partners to be used in their various public relations activities. The Principal

Investigator is already contacted several times per year by the press (e.g., Globe and Mail, Macleans, and a community paper in the past six months) to discuss science news and views, as are other members of the Network. The Network's activities can be highlighted during those contacts. In addition, the Network Manager will have as part of his/her job, identification of new potential partners, press relations, and community relations.

Our web-site will have large portions open to the public. This will give not only access by others; it also will give us a mechanism for feedback to see whether our communications mechanisms are working.

We also have a strategy to naturally draw younger students and teachers into the activities of the Network. The mechanism is to develop a science kit employing plasmonic particles for biochemical sensing. Teachers will be able to interact with Network members to learn how to use the kit and students will be exposed to the activities of Network researchers. This is a leveraging strategy, to train teachers who will then train more students than we could reasonably reach ourselves.

Communication Mechanisms

Good communications are essential both within the Network and with outside audiences. Formal responsibility for Network communications rests with the Network Manager, working in close coordination with the Principal Investigator. However, all Network participants have a role in communicating the activities and value of the Network.

Network researchers and administrators will be proactive in terms of keeping each other and our partners informed of and involved in activities, results, new developments and issues. This will be accomplished by such means as teleconferenced meetings, conferences and seminars, and the Network (Wiki-based) website. Formal feedback will be accomplished by reports of the Theme Leaders to the Principal Investigator and of Network members to the Theme Leaders and Network Manager. We will develop feedback mechanisms to improve weak linkages.

SharcNet, our partner, is providing access to Access Grid. The Access Grid is the ensemble of resources that can be used to support human interaction across the grid. It consists of multimedia display, presentation and interactive environments, interfaces to grid middleware, and interfaces to visualization environments. The Access Grid will support large-scale distributed meetings, collaborative work sessions, seminars, lectures, tutorials and training.

Each of the Themes involves projects with short-term and long-term deliverables. Short-term successes will be reported on the Network website so that all Network members may be made aware of them. If these deliverables result in high-profile papers, these results will be described to our partners and to the publicity bureaus of the participating Universities and their partners, along with a statement of the long-term objectives of the Network. Funds will be provided for the support of cover page publication and a newsletter to be sent to Network members as well as other stakeholders. The Principal Investigator sits on several research advisory boards of the Province of Ontario and other Network members sit on other provincial and federal advisory boards, and they will inform those boards of the Network's activities.

Other formal internal communications mechanisms will include:

- Monthly brown-bag lunches, providing
 - face-to-face meetings among all researchers at any given school
 - video- and tele-conferenced meetings between schools
- Quarterly tele- or videoconferences specifically to address progress in each Theme.

Within the Network, face-to-face meetings will be encouraged by joint mentorship of students and post-doctoral fellows, and the spatial proximity of the Network Manager, the Principal Investigator and half of the Network members (found at University of Toronto), as well as by travel funds and video-conferenced facilities provided by SharcNet. We will evaluate the success of our internal

communications using metrics that roughly quantify the numbers of transitions and skills between participants as well as face-to-face visits between groups. The budget provides funds for these activities and all the Theme Leaders are well known for encouraging these types of activities. Reports on the metrics by the Theme Leaders to the PI will help identify individuals and groups whose research should be presented more broadly within the Network, and transitions as well as added face-to-face visits then will be encouraged.

Members of the Network will be responsible for communicating their accomplishments, the impact of their research, and the accomplishments of the Network to external audiences, including public policy makers, the media and the general public, through the following and other mechanisms:

- Publications in high impact journals
- Coauthored papers amongst new combinations of Network members
- Presentations, both for professionals and for the public
- Reports to the popular press
- Patent disclosures
- Reports to NSERC.

Various Network members are contacted periodically by the media with requests for information about research progress and the Network will be highlighted in their responses. These contacts generally are initially made to Network members, as well as to the publicity arms of the universities, which frequently seek feedback from their researchers.

In addition, other means of publicizing our activities are under consideration, including development of a plasmonics kit for schools mentioned earlier and a proposal for a segment by public television.

Fundraising is another form of communication, and it is expected that as the research directions of the Network evolve, additional equipment funds will become beneficial. The Network expects to write successful, high profile proposals to funding agencies.

Communications at every stage will be facilitated by the use of existing collaboration technologies, such as audio conference calls, email, instant messaging, electronic Network newsletter, word processing with document and version control, and web-based archives for file sharing.

6.6 Mechanisms for Knowledge and Technology Transfer

One of the Network's formal technology transfer mechanisms is the Technology Transfer Advisory Council (TTAC). Described more fully in the Management section (section 7), TTAC will assist the Network to capitalize on appropriate opportunities and avenues for technology transfer and provide advice and guidance on aspects of commercialization and industrial interactions. A second formal mechanism will be the Network's semi-annual and other meetings.

The knowledge and technology transfer policies of the investigators' institutions will apply.

6.7 Maintenance and Preservation of Large Data Sets

The University of Toronto will host a server, where Network participants will be able to post large data sets. Access will be determined by the Principal Investigator. If the participant posting the data agrees, the data will be made open source. Otherwise it will be password protected and governed by the most stringent applicable policy amongst the Network's home institutions.

7. Management and Budget

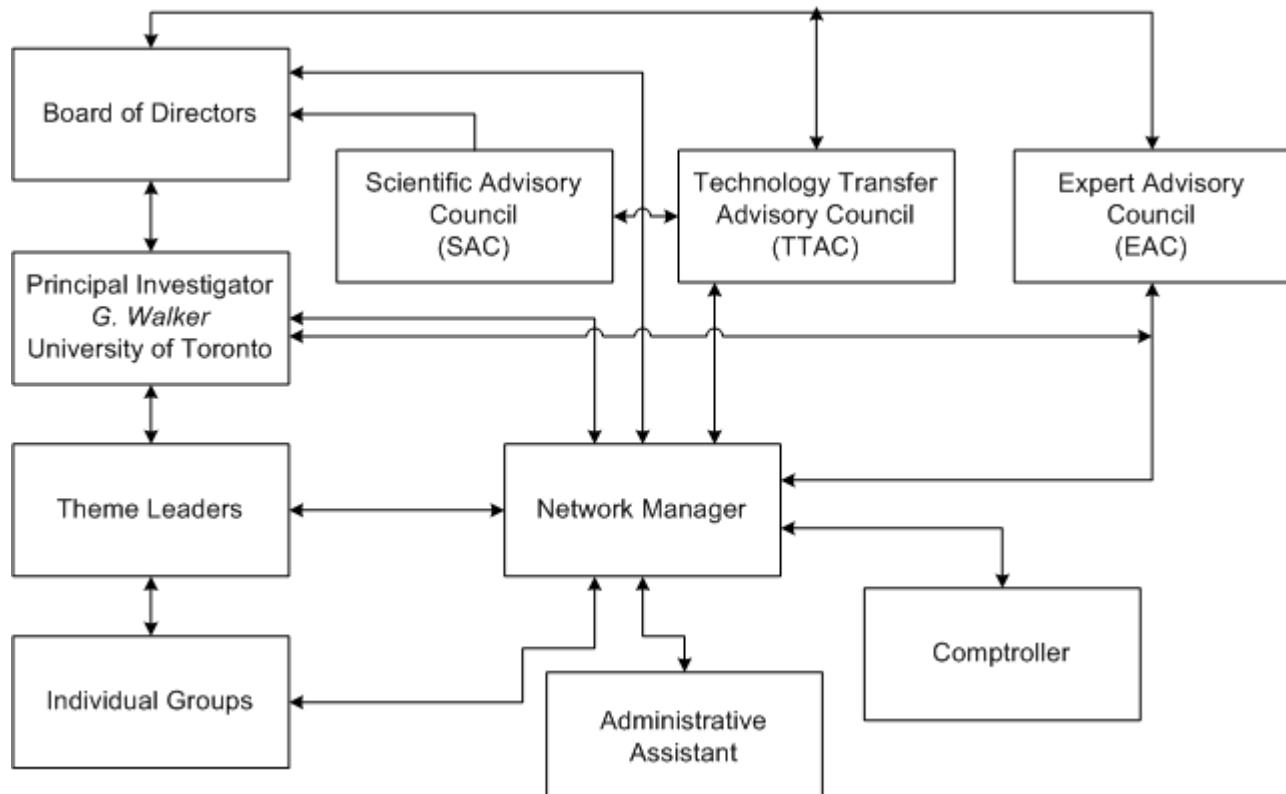


Chart 1: Management Structure: **Board of Directors:** Approximately eight members: one half are arms-length representatives from academia; one half are representatives of main stakeholders; G. Walker (UToronto, PI) and S. Mikhail (NSERC) are non-voting members. **Theme Leaders:** W. Chan (UT, Biomedical Engineer (BE)), A. Brolo (UVictoria, Chemist (C)), M. Tsao (UT/Princess Margaret Hospital, Clinician/Researcher). **Scientific Advisory Council:** PI, Theme Leaders, partner representatives from Northern Nanotechnologies, Axela, Lumerical, Network Manager, and other members to be named. **Expert Advisory Committee:** Independent international academic and industrial experts in the field. **Technology Transfer Advisory Council:** Venture Capitalist, partner representatives named in the SAC, industrial experts in the field to be named, PI, Network Manager (non voting member). **Individual Groups:** Led by Co-applicants: from UT: J. S. Aitchison (Electrical Engineer (EE)), C. Goh (Chemistry/Biomedical Engineering, Entrepreneur), M. Mojahedi (EE), A. Helmy (Optical Engineer), E. Kumacheva (Polymer Chemist/BE), J. Sipe (Physicist (P)), T. Pawson (Biologist), C. Wang (Pathologist), A. Wheeler (C/BE), B. Wilson (Medical Biophysicist (MB)), G. Zheng, (BE/MB); from UVictoria: R. Gordon (P), A. Brolo (C); from É. Poly. de Montréal: M. Meunier (P); from UOttawa: P. Berini (Engineer/Entrepreneur); from UWesternOntario: S. Mittler (P); from UWindsor: C. Rangan (P).

7.1 Management Philosophy

The Network will be managed with equal attention to disciplined project management, clear allocation of responsibilities, and innovation, discovery and creativity.

7.2 Network Management Team (See Chart 1)

The Network Management Team shall be composed of the Principal Investigator, Theme Leaders, and Network Manager. The Management Team will report to the Board of Directors and will work closely

with the Scientific Advisory Council, the Expert Advisory Committee, and the Technology Transfer Advisory Council.

7.3 Principal Investigator

The Principal Investigator (PI) is responsible for providing scientific leadership and direction to the Network and will be accountable for the overall coordination of the Network team. The PI must possess effective organizational, management and communications skills, be respected by peers and have an extensive professional network, be innovative and creative as well as results-oriented, have experience with collaborative activities, and attract trainees who themselves become successful. Responsibilities of the PI include: management of the Network; providing scientific leadership to and promoting research collaboration amongst Network participants; promoting the Network to the scientific community, the private and public sectors, and the general public; acting on behalf of the Network with NSERC and other organizations; recruiting and supervising Network personnel; and providing NSERC with scientific and financial reports approved by the Board.

The Principal Investigator of the Network shall be Professor Gilbert Walker. Professor Walker is an internationally recognized and leading scholar in nanotechnology who has a stellar track record in leading and managing large multi-institutional research and technology development projects. His experience with coordinating and managing large groups of researchers is demonstrated by his leadership in the University of Toronto Nanotechnology network. He has the ability to manage other major responsibilities as well as his own research activities, as demonstrated by his leadership of the graduate program at the University of Toronto as the Associate Chair, Graduate Studies, for the Department of Chemistry; during his tenure in this position, the program has expanded by 30% and the average GPA of incoming students rose by 0.2 to 3.8. His own research group currently includes nine graduate students. The group collectively makes more than 20 conference presentations per year. Typically Professor Walker places one person per year in a tenure stream faculty at a major research institution, usually in the US. He has the respect of his peers as demonstrated by his nomination for and winning the Canada Research Chair position he holds, as well as more than 100 invited presentations at international research conferences in his research field. Professor Walker will be assisted by the Network Manager; administrative support for the Network is being provided by the University of Toronto Department of Chemistry.

7.4 Theme Leaders

The Theme Leaders will coordinate the efforts of the Network's researchers and its industrial and governmental partners. The leader of Theme 1 is Professor Warren Chan, a world-class expert in developing biological targeting systems, with papers in the area referenced more than 1000 times. Over the past five years, Professor Chan has led two successful team projects in particle delivery systems funded by CIHR and Genome Canada. Theme 2 is led by Professor Alex Brolo, a leader in the creation of metamaterials for chemical sensing, who has been publishing at a high rate in the area (18 peer reviewed plasmonics papers in the past three years); but quite importantly, more than 50% of these papers were in collaboration with at least one other research group. The leader of Theme 3 is Professor Ming Tsao, a leading investigator in lung cancer studies with extensive experience guiding collaborative projects. In the development the Network's Theme directions, these three individuals demonstrated significant insight in consolidating and coordinating Theme ideas, identifying priorities, providing contextual acumen, critiquing research ideas, focusing research activities, and encouraging the inclusion of and helping faculty for whom this is a new or relatively new research area but who have skills and ideas important to the Network's goals. The Theme Leaders will serve on the Network Management Team and the Scientific Advisory Council.

7.5 Network Manager

The Network Manager will direct the business and financial management of the Network, assuming responsibility for diligent definition of scope, schedule and budget, for providing leadership and direction for all of the Network operations, for ensuring control and accountability on a day-to-day basis, and for monitoring and facilitating communications within the Network. The Network Manager will be responsible for obtaining and retaining Conflict of Interest and other required documentation from Network participants.

As noted above, the Department of Chemistry at the University of Toronto will provide logistics support, support for the Network Manager, and office assistance to the Network. The Innovations Group, a formal established entity within the University of Toronto, also has agreed to provide commercialization support, as needed (see section 7.10, below). The Comptroller will assist the Network Manager in monitoring expenses.

7.6 Board of Directors

The Board of Directors is responsible for the overall stewardship of the Network. It will establish the priorities, polices and strategies which guide and shape the Network and its direction. It will set the criteria for monitoring ongoing progress and achievement and make all the final decisions relating to the Network's activities and finances. In addition, the Board is accountable to NSERC for the effective implementation and management of NSERC's Conflict of Interest and other applicable policies.

The Board will consist of representatives from each of the Network's stakeholder groups, selected for such characteristics as knowledge or experience in the field, the ability to provide perspectives that are important to the Network's activities, and knowledge of or ability to identify contacts, potential new partners or sources of funding. Terms will be for three years (staggered), renewable. Members of the Board will select the Chair and Vice-Chair, by majority vote, at the first meeting of the year. The terms of the Chair and Vice-Chair shall be for one year, renewable. The Board will meet at least three times per year. It will be an arm's length body that is balanced with respect to stakeholder representation. The PI and a representative of NSERC will be non-voting members of the Board.

The Board has three sub-committees, the Scientific Advisory Council, the Expert Advisory Committee and the Technology Transfer Advisory Council.

7.7 Scientific Advisory Council

The mandate of the Scientific Advisory Council (SAC) is to evaluate the relevance of the research with respect to the targets of the Network. It will assist in formulating the overall research strategy and the technology and product vision, and provide guidance as appropriate with respect to related factors, such as ethical, environmental, economic, legal and social issues stemming from the research objectives. The SAC mandate also includes:

- Assessing priorities, including any proposed changes to the Network's research plan from the original stated objectives or approaches
- Participating in horizon-scanning and long-range planning exercises
- Ensuring that the impact of potential advances in science and technology are studied and the Network takes advantage of these advances.

The SAC will consist of the Principal Investigator, the Theme Leaders, and a representative of each partner organization. The SAC will provide its advice and guidance to the research team on scientific aspects of the project through semi-annual meetings.

7.8 Expert Advisory Committee

The mandate of the Expert Advisory Committee (EAC) is to examine the excellence of the research. Its mandate includes:

- Assessing scientific progress and plans
- Identifying emerging challenges and opportunities and providing guidance on developing possible responses.

The EAC will consist of independent international academic and industrial experts in the field. It will meet annually.

7.9 Technology Transfer Advisory Council

The mandate of the Technology Transfer Advisory Council (TTAC) is to assist the Network to capitalize on appropriate opportunities and avenues for technology transfer. The TTAC mandate includes:

- Reviewing Network science and technology that could lead to possible partnerships or scientific collaborations
- Initiating contacts with academic and industrial researchers with science and technology of interest to the Network
- Providing leadership in international collaborative opportunities with industry.

The TTAC will consist of the PI, Theme Leaders, a Venture Capitalist or other partner representatives, and international industrial experts in the field. The TTAC will provide its advice and guidance to the Network on aspects of commercialization and industrial interactions through semi-annual meetings.

The TTAC will be formed in Year 3 of the award and function in Years 3, 4 and 5.

7.10 Intellectual Property and Technology Transfer

The participants' home institutions will enter into an Intellectual Property agreement.

The Innovations Group (TIG) of the University of Toronto has offered to make available to the Network technology transfer expertise, as needed, through its Director, Physical Sciences and Engineering, and its Director, Health Sciences. TIG is a group of professionals with extensive academic, business and financial expertise whose mandate is to commercialize innovations by University of Toronto researchers and their healthcare partners.

7.11 Communications

The communications activities of the Network are described in section 6 of this proposal, Interactions and Partnerships.

7.12 Accountability

Accountability for researchers will be established in a number of ways, including (but not limited to) regular reviews of research progress and quality, relevance of the research to the Network's goals and objectives, use of research results, the potential of the research for greatest impact, translation of workplans into action, presentations, successful partnerships, increased collaborative efforts, HQP, and effective communications. Funding to researchers is contingent on continuous successful evaluation by the Principal Investigator, the Theme Leaders, and the Expert Advisory Committee. Evaluation of the Network will be carried out by the Scientific Advisory Council and the Expert Advisory Committee. Both individual and Network evaluation results will be communicated to the Board of Directors.

7.13 Conflict of Interest Policy Framework

Members of the Network will conform to NSERC's Conflict of Interest Policy as well as to any conflict of interest policies of their own home institutions or employers. As required by NSERC's Conflict of

Interest Policy, all Network participants, including members of the Board and advisory committees, will be required to disclose any financial interest or position of influence in any business in the same area of interest as the Network, other than that of their main employer.

7.14 Budget Justification

A highly detailed budget justification is provided in that section of the application entitled “Budget Justification.” Here we provide an overview of our budget strategy.

We have identified a number of the most promising ideas for the application of bioplasmonics to detection of leukemia and lung cancer. We have brought together researchers who will deliver the necessary expertise and training. While there are some additional promising approaches to these issues, they have not been included in this application due to the ceiling imposed by the available budget. We have chosen our targets because we believe we can develop diagnostic platforms prototypes in the described areas within the available 5 year period. Our partners are eager to carry commercially viable platforms forward. Most of our partners are start-up companies, and they are reluctant to provide cash support for technologies with time horizons beyond a couple of years. We expect them to provide more support when the prototypes are more nearly ready.

The management component of the budget is covered to a significant degree by the Applicant Institution. A Comptroller is cost-shared at 0.3 FTE. We believe this level of support is adequate to cover the Network’s needs, based on load experience in the University Toronto Department of Chemistry. The Dean of Arts and Sciences, University of Toronto, is contributing cash for 50% of an administrative assistant; the remaining 50% is to be carried by NSERC funding. The administrative assistant will undertake tasks including daily upkeep of the website, assistance in preparation of reports, and coordination of Network meetings. The Network Manager will play a key role in coordinating the activities of the Network funds to cover his/her full salary are requested in the NSERC budget.

Research funding allocations are subject to the review structure including the External Advisory Committee, the Scientific Advisory Committee and the approval by the Board of Directors, as described earlier.

At any given time, the Network will support 22 graduate students with full research stipends and 3 post-doctorals with full-time funding. The expected distribution of students into different faculty laboratories is indicated in the detailed budget justification, but as noted above there are mechanisms for adjustments to these distributions. Students will be provided with the needed funds for the research and for travel to assist with collaboration and attend an external conference per year. Post-doctorals are similarly supported. This will provide training and communicate the results of the Network to the outside world. Network conferences including workshops will serve to enhance communication and training.

Partners are providing support in various ways, including for research via materials and employee time commitments, as well as services such as CPU time for needed theoretical calculations and videoconferencing facilities to enhance communication.

8. Advantages of Network Approach

8.1 Connecting researchers from different disciplines

The challenges in plasmonics that will be explored for developing biomedical technologies are beyond the capabilities of any small cluster of scientists and engineers. In this project, world-class researchers in theory, fabrication, chemistry and engineering are needed to create the devices that will make major advances in biomedical technologies. All of the links in this supply chain must be strong. The academic Network members from Chemistry, Physics, Material Science, Engineering Physics, Electrical Engineering and Biomedical Sciences represent an optimum pool of expertise to tackle critical

developments in bioplasmonics. Network advisors and cross-appointed participants from the University of Toronto Health Network will bring both fundamental and practical contributions.

Half of the Network members come from the Health Network group. These biomedical scientists and clinicians include Cynthia Goh (Chemistry/Institute of Medical Science), Warren Chan (Institute of Biomaterials and Biomedical Engineering), Tony Pawson (Mt. Sinai/Medical Biophysics), Chen Wang (Mt. Sinai), Gang Zheng (Ontario Cancer Institute/Institute of Biomaterials and Biomedical Engineering), Ming Tsao (Princess Margaret Hospital), Brian Wilson (Ontario Cancer Institute/Medical Biophysics), Eugenia Kumacheva (Institute of Biomaterials and Biomedical Engineering), and Aaron Wheeler (Institute of Biomaterials and Biomedical Engineering).

8.2 Establishing the link between researchers who are geographically distant

Network members are dispersed across the country in six universities. The Network will therefore help cement their interactions through a formal framework, bi-annual meetings, and by funding student travel for joint experiments. Such activities would not be viable without the formation of the Network.

8.3 Timely

The new field of plasmonics is growing with activities, new discoveries and promise. This can be judged by the explosion of new meetings, journals and recent publications in prominent journals. Establishing a plasmonics Network now will put Canada in the forefront of these activities and help us to be a leader in the effort by providing the support and collaboration necessary to capture the growing opportunities.

8.4 Providing critical mass to encourage industrial / academic partnership

It is widely recognized that leveraging support from industry as an academic is a formidable task. BiopSys will provide the critical mass of expertise, intellectual property, state of the art knowledge and facilities, and students and faculty to entice numerous partners into joining this effort. This critical mass will make it extremely attractive for large, more established industries to partner with a scientific alliance of the size of the Network. We are in dialogue with several potential partners in addition to the ones listed in this proposal, from both governmental and the industrial life science domains.

8.5 Collective contribution

The scientific contributions and productivity of such a focused team will be much larger than that of each individual team's contributions. Samples, processes, facilities, technologies, ideas and the training of HQP will be exchanged and shared among the members through the Network structure and activities to create a truly collaborative environment that leverages the strengths of each member.

8.6 Meeting the needs of stakeholders

Meeting the Needs of Stakeholders

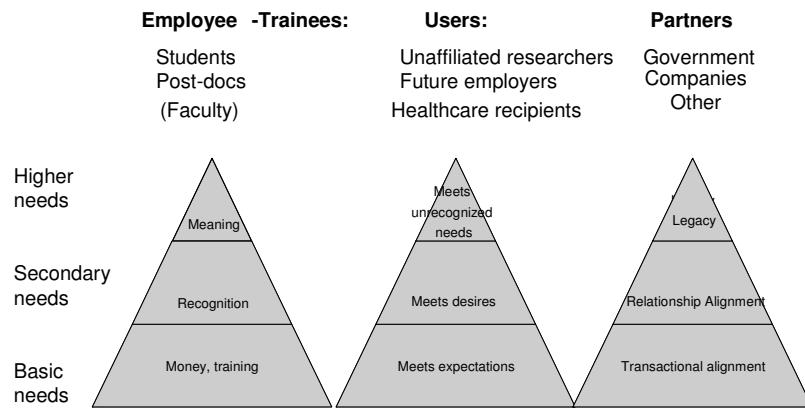


Figure 24: Here we show a different pyramid to consider in motivating each of three groups, see explanatory text. This figure was inspired by and article by Schachter, “Business 101 meets Psych 101”, *Globe and Mail*, Jan. 23, 2008

The needs of the Network’s different stakeholders are illustrated in Figure 24.

Trainees – students and post-docs

The base, or initial needs, of trainees are money and training. Students and post-docs cannot focus on higher aspirations if their survival needs are not being met. But after basic needs are satisfied, money and training are not primary motivators for most students. Instead, the motivator is recognition: Students want faculty to notice what they do, and value their contribution.

At the highest level of their pyramid of needs, students seek meaning. They want to be inspired by the value of what their work and organization provide. In the trainee pyramid, faculty serve mainly as managers but also as trainees, by way of other faculty.

Customers – unaffiliated researchers, future employers and healthcare recipients

The base of this pyramid is meeting customer needs and expectations and creating satisfaction. The next level is meeting the desires of customers, which will gain customer commitment. The peak level of customer experience, however, occurs when the unrecognized needs of the customer are delineated and satisfied. Henry Ford’s customers wanted faster horses; he gave them automobiles. Meeting unrecognized desires will create enthusiastic supporters for the Network.

The main customers of our research are, of course, other researchers, who will use our results in the cause of scientific advancement. Other customers include future employers and healthcare recipients.

Investors – government, companies and other partners

We start with investors by creating trust through ensuring what we are offering them as a partnering or investment transaction aligns with their interests; for example, we will train students. The second stage is to create confidence by making sure the relationship we offer them is desirable; for example, we will train students in a way that is unlike the training they could obtain outside such a network. Finally, we offer a legacy - a feeling that their money and support is not just providing them with a specific return, such as HQP, but helping to alter the world in a way that is deeply satisfying; we propose ways to

significantly improve the quality of life of Canadians, though better means of cancer diagnosis and improved prognosis.

8.7 Sharing Equipment, Facilities and Reducing Redundancies

As noted in the detailed budget justification, the Network will make extensive use of user facilities, particularly in nanofabrication. Many of the participants lack access to adequate nanofabrication tools at their home institutions. For example, U. Toronto does not have FIB facilities. Professor Mittler, a Network member and Director of the UWO Nanofabrication facilities has agreed to offer those facilities at a reduced rate (33% discount for FIB and e-beam), assuming the members of the network use the facilities at least 800 hours per year.

There are many other examples of cost and time savings enabled by the network approach. For example, particles will be synthesized in the labs of Chan, Meunier and Kumacheva, and shared with others, thus reducing costs and the needs of researchers to create these facilities in their own labs. Peptide ligands will be prepared by Zheng for use by others. Researchers who do not already have access to Raman facilities will be able to make use of those provided by the Helmy group. This will save not only initial costs but also maintenance costs, especially costly service contracts for lasers.

The creation of Network workshops will also eliminate the need for developing redundant training programs on each campus.

9. Benefit to Canada

Canadian Leadership in the Field

The challenges in plasmonics and bioplasmonics that will be explored by the Network are beyond the capabilities of any small cluster of scientists and engineers. This Network will connect expert researchers from many different disciplines to create an optimum pool of expertise to tackle critical developments in plasmonics and bioplasmonics and as a result, will make major advances in biomedical technologies. We anticipate that both of our two practical objectives -- determining the presence of lung cancer at an earlier stage than is currently possible to improve prognosis, and providing a point-of-care device for detecting leukemia which will greatly shorten the time needed for diagnosis -- will be achieved by Year 2018. Publications and intellectual property that will emerge from the Network's research will contribute to the fundamental knowledge base of nanoscience, enhance the visibility of Canadian nanoscience worldwide, and lead to technologies that will not only benefit individual Canadians but also enhance Canada's economy. (Please refer to Form 100 to gauge the calibre of publications and patents.)

Industrial adoption of new technology: Increasing Canada's global competitiveness

The constant consultation and mutual experiments amongst our industrial partners and the Network's research groups will seamlessly introduce new science and technology into these industries, increasing their technological advantage and market leadership, which in turn will enable Canadian industries to increase their global competitiveness in the burgeoning field of nanotechnology.

Fueling student entrepreneurial activities

As mentioned previously, the interaction between the students, academics and particularly industrial partners will likely result in novel ideas and valuable intellectual property. This in turn encourages the parties involved to consider startup activities to capitalize on such IP. To assist this process, the Institute of Optical Sciences at the University of Toronto, of which Professor Walker is a founding member, has developed and is delivering non-credit Entrepreneurship courses in which top Venture Capital firms and

business leaders give lectures and mentor students in starting up their own businesses, a further enhancement of the Canadian economy. Network students are eligible to take the courses.

The promise of BiopSys

We will set up a strategic network aimed at developing biomedical diagnostic technologies for cancers, such as lung cancer and leukemia, by coupling, focusing and coordinating the existing and emerging Canadian researchers working on plasmonics with leading biologists. This Network will be poised to make revolutionary improvements in biomolecular interaction analysis. Physicists, with the deepest understanding of the fundamental physics of surface plasmons, must join with chemists and biochemists who can functionalize the surfaces with molecular recognition elements. These researchers need to work with engineers, who have the best insight into laying out optical designs of highly parallel sensing elements in platforms. Biologists will identify the systems where the technology can have the largest scientific impact in understanding living systems. Commercial and policy insight will be provided by industrial and government partners, respectively. The people involved in this proposal are well-established and world-class researchers, as well as researchers at the start of their independent careers, both with great ambitions for plasmonics in Canada. These researchers work not only in different departments but also hail from different schools and different provinces. The strategic network mechanism, with its emphasis on coordinating researchers, will provide the broad training to young researchers that they need for their careers in biomedical devices creation. Partnerships with companies will transition these creations to innovations of greater economic value to Canada.

Plasmonics offers a promise of radical technological advances in science and engineering. The Network's cadre of world-class researchers, well-known and widely respected in their fields, experienced in research management and collaborative research, and experienced in knowledge and technology transfer, is poised to make revolutionary improvements in biomolecular interaction analysis and to develop new biomedical diagnostic technologies which will have a major impact on cancer diagnosis and prognosis, and consequently, significant impacts on the lives of individual Canadians and on the Canadian economy.

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INTELLECTUAL PROPERTY

Complete this section if you need to discuss the plans for protecting and disposing of intellectual property arising from the grant. Do not exceed one page.

The general principles on intellectual property (IP) assignments will be based on the need for protection of the discoveries, trade secrets and inventions for the best economic return to Canada. We will follow standard institutional policies and guidelines regarding intellectual property and publications. Intellectual property developed at the institutions is owned by the institution and/or the inventor(s), according to the institutions' policies. An appropriate network agreement between the institutions and the partners dealing with network organization, access to intellectual property, disclosure of results and partner funding will be finalized and signed if the network is funded. As a minimum, partners may expect advance knowledge of the research results and/or the potential to negotiate licensing opportunities. The university researchers will be free to use the research results for academic purposes. In cases where individuals from multiple universities are involved in joint ownership of IP, the network agreement will also include provisions regarding such joint ownership and how to proceed with protection of joint intellectual property.

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Faculty members of the network have experience in successfully transferring technology to Canadian companies and/or in creating start-up companies.

Prof. M. Cynthia Goh is an experienced entrepreneur, having founded Axela Biosensors, Inc. (one of the top-10 biotech firms in Canada in 2005) as well as Northern Nanotechnologies. She has also independently launched a number of commercialization initiatives, including Entrepreneurship 101, a course introducing entrepreneurship to experienced scientists and engineers. She has extensive experience in milestone-driven research and has worked extensively with many industrial partners.

Prof. Pierre Berini is Founder, Chief Technology Officer and Board Member of Spectalis Corp. Assisted by the University of Ottawa and CITO, a spin-off company (Spectalis) was created to commercialise a new integrated optics technology based on surface plasmons which originated from our research activities at the University. As CTO of Spectalis has been responsible for the direction of all technical activities of the company, including leading and conducting product development and R&D activities, supervising engineering staff, leading fabrication and design reviews, managing the intellectual property of the company and conducting internal and external due diligence reviews.

Professor Warren Chan is a co-founder of an infectious disease/nanotechnology company in Ontario, FIO. He has submitted 7 patents in the last two years and will successfully transfer these patents to the start-up company. Prior to this, Chan sold two patents to quantum dot corporation (now part of Invitrogen) - research that was conducted while he was in graduate school.