Technical Workshop

# Optical Imaging 2006

*Fifth Inter-Institute Workshop on*

## Optical Diagnostic Imaging from Bench to Bedside at the National Institutes of Health

### *Workshop Chairs*

**Amir Gandjbakhche,**
National Institutes of Health

**Bruce Tromberg,**
Univ. of California/Irvine

### *Workshop Technical Coordinator*

**Abby Vogel,**
National Institutes of Health and Univ. of Maryland

### *Program Committee*

**Houston Baker,** NCI, National Institutes of Health
**Albert Boccara,** École Supérieure de Physique et de Chimie Industrielles (France)
**Britton Chance,** Univ. of Pennsylvania
**Victor Chernomordik,** NICHD, National Institutes of Health
**Laurence Clarke,** NCI, National Institutes of Health
**Stavros Demos,** Lawrence Livermore National Lab.
**James Fujimoto,** Massachusetts Institute of Technology
**Israel Gannot,** Tel Aviv Univ. (Israel) and The George Washington Univ.
**Enrico Gratton,** Univ. of Illinois at Urbana-Champaign
**John Haller,** NIBIB, National Institutes of Health
**Jeremy Hebden,** Univ. College London (United Kingdom)
**Elizabeth Hillman,** Columbia Univ.
**Peter Kirchner,** NIBIB, National Institutes of Health
**Jay Knutson,** NHLBI, National Institutes of Health
**Alan Koretsky,** NINDS, National Institutes of Health
**Ira Levin,** NIDDK, National Institutes of Health
**King Li,** CC, National Institutes of Health
**Robert Martino,** CIT, National Institutes of Health
**Dennis Matthews,** Lawrence Livermore National Lab.
**James Mitchell,** NCI, National Institutes of Health
**Nicole Morgan,** DBPS, National Institutes of Health
**Mary-Ann Mycek,** Univ. of Michigan
**John Schotland,** Univ. of Pennsylvania
**Paul Smith,** DBPS, National Institutes of Health
**Mamoru Tamura,** Hokkaido Univ. (Japan)
**Tuan Vo-Dinh,** Duke Univ.
**Ronald Waynant,** U.S. Food and Drug Administration
**Yantian Zhang,** NIBIB, National Institutes of Health

### *Organized by*

SPIE The International Society for Optical Engineering

# Contents



*The organizing committee and SPIE gratefully acknowledge the following contributors to the Fifth Inter-Institute Workshop on Optical Diagnostic Imaging from Bench to Bedside at the National Institutes of Health:*

**ART Advanced Research Technologies Inc**

**Hamamatsu**

**Invitrogen**

**NIRx Medical Technologies, LLC**

**Olympus**

**OrSense Ltd.**

**Philips Medical Systems**

**Siemens Corporate Research**

# General Information

## NIH Optical Imaging Workshop

25-27 September 2006
National Institutes Of Health
Natcher Conference Center, Building 45
45 Center Drive
Bethesda, MD 20892

NIH Visitor Information Center is located in the Natcher Conference Center (Building 45). NIH Visitor Information Center phone number is 301-496-1776, and fax number is 301-402-0601. Go through the main entrance on South Drive; Building 45 is located south on Center Drive near Rockville Pike. Entering the main lobby, the NIH Visitor Information Center is located left of the dining services area.

## Registration Hours

*Natcher Conference Center*

*P2 level near the Auditorium*

Monday 25 September . . . . . 7:30 am to 4:30 pm
Tuesday 26 September . . . . . 7:30 am to 5:00 pm
Wednesday 27 September . . . . . 7:30 am to 2:00 pm

## Presenters

### Oral

Presenters giving oral lectures at the workshop can present from their own laptops or a computer will be provided in the workshop room enabling presenters to bring their presentations on a portable storage device.

To use the supplied computer, please bring your presentation on:

- USB Flash Drive ( i.e. Pen Drive, USB Thumb Drive, Flash Drives)
- CD-ROM only CD-R, (Not CD-RW, CD-ReWritable disk)

***If using the computer in the workshop room:***

We encourage presenters to create their presentations using PowerPoint 2003 (or earlier version) or PDF Adobe Acrobat.

All linked files must be copied to your Flash Drive or CD-ROM.

***The Supplied Computer in the workshop will be a Windows Based Pentium PC and will be configured as follows:***

- Office Suite: Microsoft Office 2003 with PowerPoint 2003
- Windows Media Player 9 - Quick Time 6
- Adobe Acrobat Reader 7

We strongly encourage presenters to bring backup materials. If you need other AV equipment please contact Abby Vogel [vogelab@mail.nih.gov] by 11 September to inquire about equipment availability.

### Poster

Poster presenters will be provided with a space 45 inches x 45 inches (114 cm x 114 cm) on which to display each poster. Thumbtacks will be provided near the poster boards. The entire poster layout should be readable from six to ten feet away. Please note that no additional equipment (tables, et cetera) or multimedia support will be available for poster presentations.

## Messages for Attendees

Messages for attendees at the Optical Imaging Workshop can be left by calling the Natcher Conference Center at 301-435-2208, and ask to be connected to the Optical Imaging workshop registration phone. Messages will be taken during registration hours Monday through Wednesday. Attendees should check the message board near registration on a daily basis to receive their messages.

## Internet Access

*Auditorium lobby, P2 level*

Monday through Wednesday . . . . . 7:30 am to 5:00 pm

There will be two Ethernet cables to the internet for attendee free access using your own laptop. This is the only access to the internet unless you are an NIH employee. NIH employees have access to the NIH wireless system.

## Security

The National Institutes of Health, like all Federal Government facilities, has instituted security measures to ensure the safety of NIH employees, patients, and visitors. Plan some extra time to get through the security checkpoints at the visitor entrances to campus.

***Perimeter Security*:** All visitors must show one (1) form of identification (a government-issued photo ID — driver's license, passport, green card, etc.) to enter the NIH campus (by car, shuttle, Metrorail, or foot). All visitors will receive a badge with their name and date on it. Every day that visitors enter the NIH campus, they must get a new badge. Visitors should continue to wear their identification prominently at all times while on campus.

***If you enter by Metrorail:*** Metrorail service is available from Washington's National Airport and from Union Station (railway). Take Metro's Red Line to the Medical Center Station. The station's escalators come out on the NIH campus. You must enter the NIH Gateway Center which serves as the visitor registration and badging center. When you leave the Gateway Center, walk down South Drive.

Make a left onto Center Drive. Follow Center Drive until you reach the Natcher Building on the left.

***If you drive:*** All visitor vehicles must enter at the South Drive entrance. They will then be inspected before being allowed on campus. Visitors will be asked to show one (1) form of identification (a government-issued photo ID — driver's license, passport, green card, etc.) and to state the purpose of their visit. Be sure to allow extra time for this vehicle inspection procedure. Short-term, metered parking and long term, attendant-controlled parking are available to visitors at a cost of $2 per hour for the first three hours or $12 per day. All visitors must park in designated visitor parking lots. For more information on the location of the lots, visit the visitor parking map at http://dtts.ors.od.nih.gov/visitor_access_map.htm.

***If you enter by hotel shuttle:*** All shuttle buses from off-campus facilities will undergo inspection at the campus perimeter, but will be allowed to enter and circulate on campus once inspected. Visitors will be asked to show one (1) form of identification (a government-issued photo ID — driver's license, passport, green card, etc.) and to state the purpose of their visit. Visitors can ask the shuttle bus driver to drop them off at the Natcher Building (#45) or get off the shuttle at the main entrance and walk to the Natcher Building. If walking: when you leave the Gateway Center, walk down South Drive.

Make a left onto Center Drive. Follow Center Drive until you reach the Natcher Building on the left.

## Continental Breakfast

A continental breakfast will be provided each morning to allow attendees a quick refreshment before the day's events. It will also give you the opportunity to meet other attendees over a cup of coffee.

## Coffee Breaks

Coffee will be served during the morning and afternoon breaks in the Natcher Conference Center Atrium, Concourse Level. Please refer to the program for specific times of these breaks.

## Lunch Break

Attendees will need to make their own arrangements for lunch each day of the workshop.

The Natcher Conference Center has a cafeteria located on the main level where attendees can purchase a variety of lunch items.

The cafeteria will be open Monday through Wednesday from 7:00 am to 2:30 pm for purchasing snacks, beverages and meals. There also is a small convenience store in the Natcher Conference Center where attendees can purchase snacks and sundries during the workshop.

## Welcome Reception

*Natcher Conference Center Atrium, Concourse Level*

Monday . . . . . 5:00 to 7:00 pm

All attendees of the NIH Optical Imaging Workshop are invited to attend a Welcome Reception on Monday evening from 5:00 to 7:00 pm in the Natcher Conference Center Atrium. Take this opportunity to network with colleagues while enjoying pre-dinner refreshments.

## Poster Sessions

*Natcher Conference Center Atrium, Concourse Level*

Monday . . . . . 5:00 to 7:00 pm (with Welcome Reception)
Tuesday . . . . . 5:30 to 7:30 pm

Poster sessions will be held Monday and Tuesday evenings to allow attendees the opportunity to view papers presented in this format and ask questions with the authors present at their displays. Because of the large number of poster papers two sessions will be held, each representing a different set of papers. Come view the posters, ask questions, and enjoy refreshments.

Poster authors may place their posters on display on the morning of their assigned session during the morning coffee break. Attendees may preview posters between 10:00 am and 4:00 pm Monday and Tuesday. Poster authors need to be present at their displays during their assigned poster session to answer questions. Poster authors must remove their papers immediately at the end of the session their assigned day. Papers not removed at the end of the poster seThe organizers assumes no responsibility for posters left on the poster boards following the poster sessions.

## Child Care

The following child care service is located in the area:

**Family & Child Care** . . . . . (202) 723-2051
(Leave a Message – will reply back within an hour)

**Note:** The organizers do not imply an endorsement or recommendation of this service. It is provided on an "information-only" basis for your further analysis and decision. Other services may be available.

## Workshop Hotel

**DoubleTree Hotel & Executive Meeting Center-Bethesda**
8120 Wisconsin Avenue
Bethesda MD 20814
Hotel Front Desk Phone: (301) 652 2000
Reservations Fax: (301) 652 4525

# General Information

## Local Transportation

Metrorail Transportation (Washington Metropolitan Area Transit Authority)
Metrorail is the clean, safe, easy-to-understand rail transit system serving the Washington, D.C. area. Metrorail serves 78 stations along 96 miles of track, using 5 color-coded lines that intersect at various points enabling passengers to change trains.

### Ground Transportation to the DoubleTree Hotel on the Metrorail

The closest Metrorail Station to The DoubleTree Hotel & Executive Meeting Center - Bethesda is the Medical Center Metro Stop on the red line and you can get there by complimentary shuttle. The hotel has a complimentary shuttle that runs every hour from 7:00 am to 10:00 pm. The shuttle stops at the Medical Center Stop (red line) where you can catch the Metrorail to DCA. You will need to transfer to the blue line at Metro Center Stop. The blue line will take you to the Reagan National Airport stop. The Metrorail station at the Reagan airport is directly adjacent – walking distance into the airport. It is 15 miles to the airport from the DoubleTree Hotel & Executive Meeting Center - Bethesda and the trip to the airport takes approx. 45 minutes to 1 hour traveling time.

### Metro Fares

Each passenger must have a farecard, which can be purchased for any amount. At each station, fares and approximate times to all other stations are posted. In addition to farecards, Metro offers a variety of passes that are good for both bus and rail travel. The farecard stores the value paid and deducts your fare when you leave the system. To travel from the Medical Center Stop to Reagan National Airport, during rush hour, from 5:30 am to 9:30 am, and from 3:00 pm to 7:00 pm, the fare is $3.20. During non-rush hour from 9:30 am to 3:00 pm, the fare is $2.35. Purchase a farecard from the vending machine using cash or a credit card. Credit card vending machines are identified with blue paneling on the front. Cash vending machines take bills in $1, $5, and $10 denominations, but will only give back maximum $4.95 change. Rates are subject to change at any time. For more information, visit the website www.wmata.com

***SuperShuttle from REAGAN NATIONAL to The DoubleTree Hotel or to NIH***
Supershuttle van service is available in the ground transportation area located on the lower level. Follow the ground transportation signs to the Ground Transportation Desk where a uniformed guest services representative will assist you with baggage and boarding. For assistance after 2:00 am, call 703-416-7886 for assistance with pickup. The fare to either the DoubleTree Hotel Select or the NIH is $26.00, one way for the first person and $9.00 for each additional person in the same group, to a maximum of seven passengers. Cash and credit cards are accepted. Reservations are only recommended for your return to the airport; call 1-800-BLUE VAN (1-800-258-3826) at least 24 hours before your departure.

***SuperShuttle from Dulles to The DoubleTree Hotel or to NIH***
SuperShuttle van service is available in the ground transportation area located on the lower level outside at curb 1D or 1F, where a uniformed guest services representative will assist you with baggage and boarding. After 12:00 am, call 703-416-7884. The fare to the hotel or NIH is $26.00, one way for the first person, and $9.00 for each additional person, to a maximum of seven passengers. Cash and credit cards are accepted. Reservations are only recommended for your return to the airport; call 1-800-BLUE VAN (1-800-258-3826) at least 24 hours before your departure.

***SuperShuttle from BALTIMORE INT'L AIRPORT'to The DoubleTree Hotel or to NIH***
From Baltimore/Washington International Airport (BWI) SuperShuttle van service is available from 6:00 am to 1:00 am daily. Go to lower level and follow signs to Ground Transportation Desk located between carousels 6 and 7. Advise the Guest Service Representative at the desk where you need to travel. During other times, call 410-859-0800. The fare to the hotel or NIH is $31.00, one way for the first person and $11 for each additional person to a maximum of seven passengers. Cash and credit cards are accepted. Reservations are only recommended for your return to the airport; call 1-800-BLUE VAN (1-800-258-3826) at least 24 hours before your departure.

### Taxi Fare to the DoubleTree Hotel & Executive Meeting Center - Bethesda

From Reagan Airport . . . . . $40 US (15 miles - 45 minutes)
From Baltimore Washington Int'l Airport . $45 US (38 miles - 60 minutes)
From Dulles Int'l Airport . . . . . $45 US (27 miles - 60 minutes)

### Taxi Fare to the Natcher Center (Building 45) National Institutes of Health

From Reagan Airport . . . . . $35 (12.5 miles)
From Baltimore Washington Int'l Airport . . . . . $55 (34.5 miles)
From Dulles Int'l Airport . . . . . $45 (26.2 miles)

### Driving Directions from Local Airports to NIH:

***From Baltimore-Washington International Airport:***
Take the Baltimore-Washington Parkway south toward Washington, DC. At I-495 (Capital Beltway), head west toward Silver Spring/Bethesda. From the Beltway (I-495), take Exit 34 which is Rt. 355 (Wisconsin Ave./Rockville Pike) and head south toward Washington/Bethesda. At the fifth traffic light, turn right onto South Drive and follow the signs to Visitor Parking.

***From Dulles International Airport:***
Head east on the Dulles Airport Access Road for approximately 13 miles. Exit onto I-495 (Capital Beltway), heading north to Bethesda/Baltimore. From the Beltway (I-495), take Exit 34 which is Rt. 355 (Wisconsin Ave./Rockville Pike) and head south toward Washington/Bethesda. At the fifth traffic light, turn right onto South Drive and follow the signs to Visitor Parking.

***From Ronald Reagan National Airport:***
Head North on the George Washington Parkway for approximately 5 miles. Exit onto I-495 (Capital Beltway), heading north to Maryland. From the Beltway (I-495), take Exit 34 which is Rt. 355 (Wisconsin Ave./Rockville Pike) and head South toward Washington/Bethesda. At the fifth traffic light, turn right onto South Drive and follow the signs to Visitor Parking.

## Parking at NIH

Visitor Parking is **extremely difficult** to find at NIH, so if at all possible, take public transportation. All visitors must use the entrances at Rockville Pike at South Drive (Metrorail Stop) or Old Georgetown Road at Center Drive. All visitor vehicles must enter at South Drive. They then will be inspected before being allowed on campus. Visitors will be asked to show one (1) form of identification (a government-issued photo ID — driver's license, passport, green card, etc.) and to state the purpose of their visit. Be sure to allow extra time for this vehicle inspection procedure. Short-term, metered parking and long term, attendant-controlled parking are available to visitors at a cost of $2 per hour for the first three hours or $12 per day. All visitors must park in designated visitor parking lots. For more information on the location of the lots, visit the visitor parking map at http://dtts.ors.od.nih.gov/visitor_access_map.htm.

## Shuttle Service from the Doubletree to NIH

The hotel has a complimentary shuttle that runs every hour from 7:00 am to 10:00 pm. The shuttle stops at the Medical Center Metro Station Stop (red line). The shuttle will continue on to the NIH and stops at Natcher Center and then at Building #10.

LINK TO GENERAL AREA MAP:
**http://www.bethesda.org/bethesda/map.htm**

## Finding the Visitor Information Center

The NIH Visitor Information Center (VIC) is located in the Natcher Conference Center (Building 45). The telephone number is 301-496-1776 and the fax number is 301-402-0601. Go through the main entrance on South Drive. Building 45 is located south on Center Drive near Rockville Pike. Entering the main lobby, the NIH Visitor Information Center is located left of the dining services area. For more information check the following web address:

**http://www.nih.gov/about/visitor/index.htm**

## Driving Directions to the Doubletree Hotel – Bethesda

***From The North-East:***
Take 95 South to 495 West – Silver Spring/Northern VA. Exit off Exit 33 (Connecticut Ave.), making a left towards Chevy Chase. At the first light, turn right onto Jones Bridge Road. Follow approximately 1 mile to Rockville Pike (Wisconsin Ave.). Turn left onto Rockville Pike and follow for º mile. The hotel is on the right-hand side.

***From The North-West:***
Take 270 South to the 270/495 split, bear left for 495 East/Silver Spring-College Park. Stay on 495 and exit via the left lane for Bethesda/Rt. 355 South. Follow Rt. 355 South past the National Naval Medical Center on the left and National Institute of Health on the right. The hotel is located 2 miles South of the 495 exit on the right-hand side between Battery Lane and Cordell Avenue.

***From The South:***
Take 95 north to 495 North-Frederick. At the 270/495 split, stay on 495 and exit at Exit 34 (Wisconsin Ave.)/Rt. 355 South. Follow Rt. 355 past the National Naval Medical Center on the left moving toward the National Institute of Health on the right. The hotel is located 2 miles South of the 495 exit on the right-hand side between Battery Lane and Cordell Avenue.

***From (DCA) Reagan National Airport***
Take George Washington Parkway North to Beltway 495. Following signs to Maryland, take Beltway 495 to Exit 34 (Wisconsin Avenue/Rt. 355). Follow Rt. 355 past the National Naval Medical Center on the left moving toward the National Institute of Health on the right. The hotel is located 2 miles south of the 495 exit on the right-hand side between Battery Lane and Cordell Avenue.

***From (IAD) Dulles Int'l Airport***
Take toll road east to 495 (Baltimore / Bethesda) Exit 18. Following signs to Maryland, take Beltway 495 to Exit 34 (Wisconsin Avenue/Rt. 355). Follow Rt. 355 past the National Naval Medical Center on the left moving toward the National Institute of Health on the right. The hotel is located 2 miles south of the 495 exit on the right-hand side between Battery Lane and Cordell Avenue.

***From (BWI) Baltimore Washington Int'l Airport***
Take 95 South to 495 West-Silver Spring/Northern VA. Exit off Exit 33 (Connecticut Avenue) making a left towards Chevy Chase. At the first light, turn right onto Jones Bridge Road. Follow approximately 1 mile to Rockville Pike (Wisconsin Avenue). Turn left onto Rockville Pike and follow for 1/4 mile. The hotel is on the right-hand side.

## Parking at the DoubleTree Hotel & Executive Meeting Center - Bethesda

The hotel has an indoor parking garage with limited capacity. Parking rates are $10 overnight, with in/out privileges. Parking rates are subject to change.

## Attractions, Restaurants, Shopping

**www.bethesda.org**

Hertz®

Hertz Car Rental is the official car rental agency for this Workshop. To reserve a car, identify yourself as an NIH (National Institutes of Health) attendee using the Hertz Meeting Code CV# 029B0009. In the United States call 1-800-654-2240.

# Technical Workshop

## Room: Natcher Auditorium, P2 Level

**Monday-Wednesday 25-27 September 2006**

# Optical Imaging 2006

*Conference Chairs:* **Amir H. Gandjbakhche**, National Institutes of Health; **Bruce J. Tromberg**, Univ. of California/Irvine

*Program Committee:* **Houston R. Baker**, NCI, National Institutes of Health; **Albert C. Boccara**, École Supérieure de Physique et de Chimie Industrielles (France); **Britton Chance**, Univ. of Pennsylvania; **Victor V. Chernomordik**, NICHD, National Institutes of Health; **Laurence P. Clarke**, NCI, National Institutes of Health; **Stavros G. Demos**, Lawrence Livermore National Lab.; **James G. Fujimoto**, Massachusetts Institute of Technology; **Amir H. Gandjbakhche**, NICHD, National Institutes of Health; **Israel Gannot**, Tel Aviv Univ. (Israel) and The George Washington Univ.; **Enrico Gratton**, Univ. of Illinois at Urbana-Champaign; **John W. Haller**, NIBIB, National Institutes of Health; **Jeremy C. Hebden**, Univ. College London (United Kingdom); **Elizabeth M. C. Hillman**, Columbia Univ.; **Peter T. Kirchner**, NIBIB, National Institutes of Health; **Jay R. Knutson**, NHLBI, National Institutes of Health; **Alan P. Koretsky**, NINDS, National Institutes of Health; **Ira W. Levin**, NIDDK, National Institutes of Health; **King C. Li**, CC, National Institutes of Health; **Robert L. Martino**, CIT, National Institutes of Health; **Dennis L. Matthews**, Lawrence Livermore National Lab.; **James Mitchell**, NCI, National Institutes of Health; **Nicole Y. Morgan**, DBPS, National Institutes of Health; **Mary-Ann Mycek**, Univ. of Michigan; **John C. Schotland**, Univ. of Pennsylvania; **Paul D. Smith**, DBPS, National Institutes of Health; **Mamoru Tamura**, Hokkaido Univ. (Japan); **Bruce J. Tromberg**, Univ. of California/Irvine; **Tuan Vo-Dinh**, Duke Univ.; **Abby J. Vogel**, National Institutes of Health and Univ. of Maryland; **Ronald W. Waynant**, U.S. Food and Drug Administration; **Yantian Zhang**, NIBIB, National Institutes of Health

## Monday 25 September

### Registration and Welcome Continental Breakfast

**Room: Natcher Auditorium Foyer . . . . . Mon. 7:30 am**

**Welcome Address . . . . . Mon. 8:15 am**

*Chairs:* **Amir H. Gandjbakhche**, National Institutes of Health; **Bruce J. Tromberg**, Univ. of California/Irvine

**SESSION 1 . . . . . Mon. 8:30 to 10:00 am**

### Keynote Speakers

*Chair:* **Amir H. Gandjbakhche**, National Institutes of Health

8:30 am: **Optical imaging: bringing cell biology and physiology together in vivo,** R. S. Balaban, National Institutes of Health . . . . . [01]

8:45 am: A. P. Koretsky, National Institutes of Health . . . . . [02]

9:00 am: **The role of optical technologies in oncology,** D. C. Sullivan, National Cancer Institute . . . . . [03]

9:15 am: B. Seto, National Institutes of Health . . . . . [04]

9:30 am: **Award for extraordinary pioneering contributions to the translation of optical technologies from blackboard to benchtop to bedside,** Brian C. Wilson, Professor of Medical Biophysics, Univ. of Toronto (Canada) . . . . . [05]

Coffee Break . . . . . 10:00 to 10:30 am

**SESSION 2 . . . . . Mon. 10:30 am to 12:30 pm**

### Molecular Imaging

*Chair:* **Mary-Ann Mycek**, Univ. of Michigan

10:30 am: **The imaging probe development center: an NIH core synthesis resource for imaging probes,** G. Griffiths, National Institutes of Health (Keynote Presentation) . . . . . [06]

10:50 am: **Nanomolecules and nanoparticles in optical tumor imaging,** S. Achilefu, Washington Univ. in St. Louis . . . . . [07]

11:10 am: **Quantitative molecular imaging using time-resolved intensity and lifetime,** A. H. Gandjbakhche, National Institutes of Health . . . . . [08]

11:30 am: **Activatable fluorescent optical probes for in vivo molecular imaging,** P. L. Choyke, National Institutes of Health . . . [09]

11:50 am: **Raman, CARS, and near-field Raman-CARS microscopy for cellular and molecular imaging,** S. Kawata, Osaka Univ. (Japan) . . . . . [10]

12:10 pm: **Miniaturized, disposable microscopy probes for early cancer detection,** T. S. Tkaczyk, The Univ. of Arizona . . . . . [129]

Lunch Break . . . . . 12:30 to 1:30 pm

**SESSION 3 . . . . . Mon. 1:30 to 3:30 pm**

### Translating Optical Technologies from Benchtop to Clinical Standard

*Chair:* **Houston R. Baker**, National Cancer Institute

1:30 pm: **Overcoming barriers to clinical translation,** B. J. Tromberg, Univ. of California/Irvine . . . . . [11]

2:00 pm: **Informatics platforms to support collaboration and drive standardization,** J. C. Pearson, Siemens Corporate Research . . . [12]

2:30 pm: **Optics and the pathology gold standard,** J. M. Crawford, Univ. of Florida . . . . . [13]

3:00 pm: **Commercial barriers and pathways,** D. A. Benaron, Spectros Corp. . . . . [14]

Coffee Break . . . . . 3:30 to 4:00 pm

**PANEL DISCUSSION . . . . . Mon. 4:00 to 5:00 pm**

### Translating Optical Technologies from Benchtop to Clinical Standard

*Chair:* **Bruce J. Tromberg**, Univ. of California/Irvine

*Participants:* **John Pearson** (Siemens Corporate Research), **Jim Crawford** (Univ. of Florida), **David Benaron** (Spectros Corporation), **Anthony Hayward** (NCRR/NIH), **Ronald Waynant** (FDA), **Peter Barker** (NIST)

## Welcome Reception and Posters-Monday

*This session is held in honor of Brian C. Wilson in honor of his contribution to the translation of optical technologies from blackboard, to benchtop, to bedside.*

*Posters in this session will be on display from 10:00 Monday morning in the Natcher Atrium. A poster session, with authors present at their posters, will be held Monday from 5:00-7:00 pm. Light refreshments will be served. Attendees are requested to wear their conference badges.*

✔ **Imaging fiber bundle lens with binary optical surface,** D. Li, L. Hou, G. Zhou, Infrared Optical Fibers & Sensors Institute of Yanshan Univ. (China) . . . . . [50]

✔ **Laser-induced photoacoustic imaging: a tool for real time in vitro identification of human breast cancer,** Y. H. Elsharkawy, Cairo Univ. (Egypt) . . . . . [51]

✔ **Optical investigation of dynamics of laser-based lithotripsy,** J. O. Kokaj, M. A. Marafi, Kuwait Univ. (Kuwait) . . . . . [52]

✔ **Simulating the gain flattening filter for EDFA,** A. Khare, A. Khare, Government Engineering College Bhopal (India) . . . . . [53]

✔ **Moving target detection through omnidirectional vision fixed on AGV,** S. Y. Yang, Tianjin Univ. of Technology (China) . . . . . [54]

✔ **Development of near-infrared fluorescent probes for nitric oxide and zinc ion,** H. Kojima, K. Kiyose, T. Nagano, The Univ. of Tokyo (Japan) . . . . . [55]

✔ **Comparative splice loss analysis of dispersion-shifted and dispersion-flattened single-mode fibers,** C. M. Jadhao, G.S. College of Khamgaon (India); D. Dhote, Brijlal Biyani Science College of Amravati (India) . . . . . [56]

✔ **Non-invasive optical imaging of lung metastasis and response to cancer therapy,** W. Yared, VisEn Medical, Inc. . . . . [57]

✔ **Molecular snapshots of intra- and extracellular oxygen levels in biological systems,** D. Sud, G. Mehta, K. Mehta, J. Linderman, S. Takayama, Univ. of Michigan; D. G. Beer, Univ. of Michigan Medical School; M. Mycek, Univ. of Michigan . . . . . [58]

✔ **Real-time time-resolved diffuse optical imager for breast cancer detection,** N. Chen, National Univ. of Singapore (Singapore) . . . [59]

✔ **Angular distribution of diffraction efficiency of a volume phase gratings with irregular modulation of refraction index,** V. V. Krylov, Consultant . . . . . [60]

✔ **Spectral fluorescence targeted tumor imaging for peritoneal disseminated metastatic cancer: targeting strategies and fluorophore optimization,** H. Kobayashi, Y. Hama, Y. Koyama, National Institutes of Health; Y. Urano, The Univ. of Tokyo (Japan); P. L. Choyke, National Institutes of Health . . . . . [61]

✔ **Arterial wall investigated using the near-IR spectral wing and polarization memory effect,** X. Ni, S. Kartazayeva, W. Wang, C. Liu, City College/CUNY; R. R. Alfano, City College/CUNY and Alfanix Technology Ltd. . . . . [62]

✔ **In vivo targeted fluorescence imaging of malignant peritoneal implants expressing the D-galactose (asialo) receptor using galactosyl serum albumin (GSA) conjugated rhodamine green,** Y. Hama, National Institutes of Health; Y. Urano, The Univ. of Tokyo (Japan); Y. Koyama, P. L. Choyke, H. Kobayashi, National Institutes of Health . . . . . [63]

✔ **Human epidermal growth factor receptor type2 (HER2) targeted spectral fluorescence imaging of lung metastases,** Y. Koyama, National Cancer Institute; Y. Hama, National Institutes of Health; Y. Urano, The Univ. of Tokyo (Japan); M. Bernardo, National Cancer Institute; P. L. Choyke, H. Kobayashi, National Institutes of Health [64]

✔ **Real-time imaging of tissue micro-structures using intrinsic optical signatures,** B. Lin, Univ. of California/Davis; S. Urayama, N. Rahim, Univ. of California/Davis Medical Ctr.; D. Matthews, R. Ramsamooj, S. Demos, Univ. of California/Davis . . . . . [65]

✔ **A 3D measurement endoscope system using multiple laser beams,** H. Nakatani, Shizuoka Univ. (Japan); K. Abe, Aichi Institute of Technology (Japan); A. Miyakawa, S. Terakawa, Hamamatsu Univ. School of Medicine (Japan) . . . . . [66]

✔ **fNIRS can help in diagnosing cerebral perfusion impairments in migraine and ADHD patients,** A. Akin, K. Ciftci, B. Sankur, Bogaziçi Univ. (Turkey); O. Oner, Diskapi SSK Hospital (Turkey); Y. Yazgan, Marmara Univ. (Turkey); H. Bolay, Gazi Univ. (Turkey); K. Munir, Childrens Hospital Boston . . . . . [67]

✔ **A comparative study of two diffusion models on cylindrical geometry,** S. Dwivedi, K. B. Krishnan, GE Global Research - JFWTC (India) . . . . . [68]

✔ **Spectral radiance imaging of human skin tissue: theoretical aspects and empirical results,** K. P. Nielsen, L. Zhao, A. Bhandari, B. Hamre, J. J. Stamnes, PhotoSense AS (Norway); K. H. Stamnes, Balter Inc. . . . . [69]

✔ **Optical imaging of inhomogeneities in tissue,** M. Bhowmick, Indian Institute of Technology Bombay (India) and Thadomal Shahani Engineering College (India); U. B. Desai, M. P. Thaddeus, G. Vishnoi, Indian Institute of Technology Bombay (India) . . . . . [70]

✔ **Demonstration of endoscopic near-infrared diffuse optical tomography in phantoms and tissues,** D. Piao, H. Xie, W. Zhang, G. Zhang, C. Musgrove, C. F. Bunting, Oklahoma State Univ.; H. Dehghani, The Univ. of Exeter (United Kingdom); B. W. Pogue, Dartmouth College . . . . . [71]

✔ **Fiber-based excitation emission spectrometer for in-vivo transcatheter optical molecular analysis,** S. Krueger, Philips Research Labs. (Germany); D. A. Herzka, Philips Research USA; S. Weiss, Philips Research Labs. (Germany); M. Schuette, LaVision BioTec GmbH (Germany); K. C. Li, National Institutes of Health . [72]

✔ **Optical high resolution cross section imaging of a human breast model using independent component analysis,** M. Xu, M. Alrubaiee, S. K. Gayen, R. R. Alfano, City College/CUNY . . . [73]

✔ **Functional Near Infrared Spectroscopy for noninvasive imaging of cerebral response to noxious thermal stimuli,** D. K. Joseph, W. Harris, Massachusetts General Hospital; D. Borsook, McLean Hospital and Massachusetts General Hospital; D. A. Boas, Massachusetts General Hospital; L. Becerra, McLean Hospital . [74]

✔ **Heat management using thermal conductive optical windows for the prevention of tissue buckling and collateral damage in NIR-laser tissue welding,** V. Sriramoju, R. Podder, N. Davatgar, City College/CUNY; H. E. Savage, R. B. Rosen, New York Eye and Ear Infirmary; A. Katz, R. R. Alfano, City College/CUNY . . . . . [75]

✔ **An experimental calibrating system for functional DOT imaging,** R. L. Barbour, NIRx Medical Technologies and SUNY/Downstate Medical Ctr.; H. L. Graber, Y. Xu, SUNY/Downstate Medical Ctr. and NIRx Medical Technologies; Y. Pei, R. Ansari, M. B. Levin, NIRx Medical Technologies . . . . . [76]

✔ **Image enhancement by linear spatial deconvolution,** H. L. Graber, NIRx Medical Technologies and SUNY/Downstate Medical Ctr.; Y. Xu, SUNY/Downstate Medical Ctr. and NIRx Medical Technologies; Y. Pei, NIRx Medical Technologies; R. L. Barbour, SUNY/Downstate Medical Ctr. and NIRx Medical Technologies . [77]

✔ **Multiplexing molecular markers in vivo and ex vivo with multispectral imaging,** J. R. Mansfield, R. M. Levenson, P. J. Dwyer, Cambridge Research & Instrumentation, Inc. . . . . [78]

✔ **Monitoring head and neck patients during chemoradiation therapy with diffuse optical spectroscopies,** U. Sunar, Univ. of Pennsylvania . . . . . [79]

✔ **Optimized hyperspectral microscopic discrimination among normal, adenomatous and carcinomatous colon tissue micro array biopsies,** F. Woolfe, M. Maggioni, G. L. Davis, S. Zucker, Yale Univ. . . . . [80]

✔ **Imaging of superficial skin lesions by spectroscopic scattering ellipsometry,** B. B. Boulbry, T. A. Germer, National Institute of Standards and Technology; J. C. Ramella-Roman, The Catholic Univ. of America . . . . . [81]

✔ **Tumor detection by simultaneous bilateral diffuse optical tomography (DOT) breast imaging,** Y. Pei, NIRx Medical Technologies; H. L. Graber, SUNY/Downstate Medical Ctr. and NIRx Medical Technologies; M. Farber, SUNY/Downstate Medical Ctr.; C. H. Schmitz, NIRx Medical Technologies; Y. Xu, SUNY/Downstate Medical Ctr. and NIRx Medical Technologies; P. Toubas, N. Patel, SUNY/Downstate Medical Ctr.; M. S. Katz, State Univ. of New York/Buffalo; W. B. Solomon, SUNY/Downstate Medical Ctr.; R. L. Barbour, SUNY/Downstate Medical Ctr. and NIRx Medical Technologies . . . . . [82]

✔ **Implications of performing optical imaging under mammographic compression - changes in breast physiology and novel optical cancer markers,** S. A. Carp, J. J. Selb, Massachusetts General Hospital; D. Ntuba, G. L. Boverman, Northeastern Univ.; Q. Fang, E. A. Rafferty, R. H. Moore, D. B. Kopans, Massachusetts General Hospital; D. H. Brooks, Northeastern Univ.; D. A. Boas, Massachusetts General Hospital [83]

✔ **Optimization of a non-contact diffuse optical tomography based on dual NIR CCD camera,** W. S. Ko, S. Kim, Korea Advanced Institute of Science and Technology (South Korea) . . . . . [84]

✔ **Fast optical signals in peripheral nerves,** Y. Tong, Tufts Univ.; P. R. Bergethon, Boston Univ.; J. M. Martin, D. K. Chen, Tufts Univ.; P. R. Clervil, Tufts New England Medical Ctr.; A. Sassaroli, S. Fantini, Tufts Univ. . . . . [85]

✔ **Observing DNA processing by single exonucleases,** R. Conroy, National Institutes of Health; J. Moreland, National Institute of Standards and Technology; A. P. Koretsky, National Institutes of Health . . . . . [86]

✔ **Monitoring tumor response to neoadjuvant chemotherapy in breast with NIR tomography,** S. Jiang, B. W. Pogue, C. Carpenter, Dartmouth College; C. Kogel, J. Forero, Dartmouth Hitchcock Medical Ctr.; K. D. Paulsen, Dartmouth College; S. P. Poplack, Dartmouth Hitchcock Medical Ctr.; G. N. Schwartz, Dartmouth Medical School; P. Kaufman, Dartmouth Hitchcock Medical Ctr. [125]

✔ **In vivo integrated photoacoustic flow cytometry: Application for monitoring circulating cancer cells labeled with gold nanorods,** V. P. Zharov, E. I. Galanzha, E. V. Shashkov, Univ. of Arkansas for Medical Sciences; N. Khlebtsov, Institute of Biochemistry and Physiology of Plants and Microorganisms (Russia); V. V. Tuchin, Saratov State Univ. (Russia) . . . . . [126]

✔ **In vivo fluorescence lifetime imaging system based on time correlated single photon counting,** M. Hassan, J. D. Riley, V. V. Chernomordik, A. H. Gandjbakhche, National Institutes of Health . . . . . [128]

✔ **Relation of dynamic light scattering (DLS) cataract detection device parameters to clinical nuclear lens grades,** M. B. Datiles III, National Institutes of Health; R. R. Ansari, K. I. Suh, NASA Glenn Research Ctr.; G. F. Reed, S. Vitale, National Institutes of Health; J. F. King, NASA Glenn Research Ctr.; F. L. Ferris, National Institutes of Health . . . . . [131]

✔ **Spectral imaging approach for tumor oximetry,** N. Liu, Y. Yu, A. Sassaroli, D. K. Chen, S. Fantini, Tufts Univ. . . . . [132]

✔ **Integrated miniature microscope with structured illumination for in vivo microscopy,** J. D. Rogers, T. S. Tkaczyk, The Univ. of Arizona; M. S. Rahman, R. R. Richards-Kortum, Rice Univ.; T. C. Christenson, HT Micro; M. R. Descour, The Univ. of Arizona[133]

## Tuesday 26 September

### Continental Breakfast

**Room: Natcher Auditorium Foyer . . . . . Tues. 7:00 am**

**SESSION 4 . . . . . Tues. 8:00 to 10:00 am**

### Optical Imaging: Translation to Preclinical and Clinical Applications I

*Chair:* **Yantian Zhang**, National Institutes of Health

8:00 am: **OCT in cardiovascular and musculoskeletal diseases,** M. E. Brezinski, Massachusetts Institute of Technology . . . . . [15]

8:20 am: **Second harmonic generation imaging microscopy of osteogenesis imperfecta,** P. J. Campagnola, Univ. of Connecticut Health Ctr. . . . . [16]

8:40 am: **Dynamics and resilience of blood flow in cortical microvessels revealed with optical imaging and manipulation,** D. Kleinfeld, Univ. of California/San Diego . . . . . [17]

9:00 am: **Potential and challenges of high-resolution endoscopic technologies for optical biopsy of internal organs,** X. Li, Univ. of Washington . . . . . [18]

9:20 am: **In vivo microscopy and flow cytometry: application to bone marrow cell trafficking,** C. P. Lin, Massachusetts General Hospital . . . . . [19]

9:40 am: **Bringing in-vitro assays to life with fluorescence molecular tomography (FMT,** V. Ntziachristos, Massachusetts General Hospital . . . . . [20]

Coffee Break . . . . . 10:00 to 10:30 am

**SESSION 5 . . . . . Tues. 10:30 to 11:50 am**

### Optical Imaging: Translation to Preclinical and Clinical Applications II

*Chair:* **John W. Haller**, National Institutes of Health

10:30 am: **Confocal mosaicing of basal cell carcinomas in skin excisions to potentially guide Mohs surgery: recent advances in translational research toward surgical pathology-at-the-bedside,** M. Rajadhyaksha, Y. G. Patel, K. S. Nehal, I. Aranda, Y. Li, A. C. Halpern, Memorial Sloan-Kettering Cancer Ctr. . . . . [21]

10:50 am: **High-resolution biophotonic imaging,** L. V. Wang, Washington Univ. in St. Louis . . . . . [22]

11:10 am: **Single molecule probing of dynamic conformations, molecular interactions and dynamic localizations in-vitro, in live cells and in organisms,** S. Weiss, Univ. of California/Los Angeles . [23]

11:30 am: **Optical molecular imaging for early detection of cancer,** R. R. Richards-Kortum, Rice Univ. . . . . [24]

Lunch Break . . . . . 11:50 am to 1:00 pm

**SESSION 6 . . . . . Tues. 1:00 to 2:20 pm**

### Optical Coherence Tomography

*Chair:* **James G. Fujimoto**, Massachusetts Institute of Technology

1:00 pm: **In vivo coherence-based quantitative imaging from cells to tissues,** J. A. Izatt, Duke Univ. . . . . [25]

1:20 pm: **Fourier domain functional optical coherence tomography,** Z. Chen, Univ. of California/Irvine . . . . . [26]

1:40 pm: **Advances in clinical ophthalmic OCT,** J. S. Schuman, G. Wollstein, H. Ishikawa, L. E. Kagemann, Jr., M. Gabrielle, Univ. of Pittsburgh; M. Wojtkowski, V. J. Srinivasan, Massachusetts Institute of Technology; J. Ducker, Tufts Univ.; J. G. Fujimoto, Massachusetts Institute of Technology . . . . . [27]

2:00 pm: **Contrast enhancement techniques for coherent optical imaging,** S. A. Boppart, Univ. of Illinois at Urbana-Champaign . . . . [28]

Coffee Break . . . . . 2:20 to 3:00 pm

**SESSION 7 . . . . . Tues. 3:00 to 4:40 pm**

### Optical Devices and Methods in Drug Discovery: Role of Industry

*Chair:* **Israel Gannot**, The George Washington Univ. and Tel Aviv Univ. (Israel)

3:00 pm: **Optical methods in drug discovery and development** *(Invited Paper),* M. Analoui, Pfizer Inc. . . . . [29]

3:20 pm: **An optical and multimodal imaging platform for integration, post-processing, and standardization,** F. S. Azar, Siemens Corporate Research . . . . . [30]

3:40 pm: **The evolving role of optical imaging in clinical practice—a personal view,** F. P. Jansen, GE Global Research . . . . . [31]

4:00 pm: **Opportunities and challenges of imaging in translational medicine,** T. Krucker, Novartis Institutes for Biomedical Research . [32]

4:20 pm: **Optical imaging in oncology drug discovery,** C. Sur, Merck and Co., Inc. . . . . [33]

**PANEL DISCUSSION . . . . . Tues. 4:40 to 5:30 pm**

### Effective Interaction Among Research Institutions and the Private Sector

**Joe Schmitt,** Lightlab Imaging

**Ken Kaufmann,** Hamamatsu

**Randall Barbour,** SUNY Downstate

**Yiwei (Kevin) Jia,** Olympus America Inc.

## ✔ Posters-Tuesday

*Posters in this session will be on display from 10:00 Tuesday morning in the Natcher Atrium. A poster session, with authors present at their posters, will be held Tuesday from 5:30-7:30 pm. Light refreshments will be served. Attendees are requested to wear their conference badges.*

✔ **New ultrafast laser system based on the Chromium: Forsterite for multiphoton in vivo imaging,** S. E. Egorov, C. C. Barnes, A. J. Carson, Del Mar Photonics, Inc. . . . . [87]

✔ **Laser Doppler imaging: a new approach,** M. Atlan, The Univ. of Texas/Austin . . . . . [88]

✔ **Thin films of zinc phthalocyanine ( ZnPc) for optoelectronic devices,** M. Puri, Guru Nanak Dev Univ. (India) . . . . . [89]

✔ **Real-time imaging and characterization of human breast tissue by reflectance confocal microscopy,** M. C. Cabrera, M. T. Tilli, A. L. Gallagher, E. Makariou, S. Pollin, M. C. Liu, P. A. Furth, Georgetown Univ. Medical Ctr. . . . . [90]

✔ **Advanced nano-imaging techniques applied to live cell biophysics,** A. Trache, J. P. Trzeciakowski, W. E. Zimmer, Texas A&M Univ. Health Science Ctr.; G. A. Meininger, Dalton Cardiovascular Research Ctr. . . . . [91]

✔ **A dual-axes confocal reflectance and fluorescence microscope for in vivo early detection of cancer,** J. T. C. Liu, M. J. Mandella, W. Piyawattanametha, H. Ra, C. H. Contag, G. S. Kino, O. Solgaard, T. D. Wang, Stanford Univ. . . . . [92]

✔ **Endoscopic imaging techniques for early diagnosis: recent works in OLYMPUS,** K. Gono, Olympus Corp. (Japan) . . . . . [93]

✔ **Functional near infrared spectroscopy for the assessment of cognitive impairments following traumatic brain injury,** A. C. Merzagora, M. T. Schultheis, M. A. Izzetoglu, B. K. Onaral, Drexel Univ. . . . . [94]

✔ **Prediction of oral mucositis development using optical coherence tomography in patients with head and neck cancer,** N. D. Gladkova, A. Maslennikova, Nizhny Novgorod State Medical Academy (Russia); I. Balalaeva, Institute of Applied Physics (Russia); Y. Vyseltseva, Nizhny Novgorod State Medical Academy (Russia); G. V. Gelikonov, Institute of Applied Physics (Russia); F. I. Feldchtein, Imalux Corp. . . . . [95]

✔ **Niris optical coherence tomography system: application in endourology and guided surgery,** F. I. Feldchtein, N. Tresser, M. Kareta, Imalux Corp.; D. Bodner, Univ. Hospitals of Cleveland; I. Gill, J. Kaouk, The Cleveland Clinic Foundation; P. Kick, Univ. Hospitals of Cleveland; E. A. Klein, The Cleveland Clinic Foundation; M. Resnick, Univ. Hospitals of Cleveland . . . . . [96]

✔ **1310nm high-power, broad-band super-luminescent light emitting diode for OCT application,** L. T. Li, M. X. Zhao, J. Wang, J. Jin, Z. Wu, W. Zhu, H. W. Xu, InPhenix Inc. . . . . [97]

✔ **Optical coherence tomography as a tool to assess conduit quality in coronary artery byass surgery,** N. S. Burris, C. Tang, R. S. Poston, Univ. of Maryland School of Medicine . . . . . [98]

✔ **Multimodal confocal mosaicing of basal cell carcinomas in Mohs surgical skin excisions,** Y. G. Patel, D. S. Gareau, Y. Li, K. S. Nehal, M. Rajadhyaksha, Memorial Sloan-Kettering Cancer Ctr. . . . . [99]

✔ **Imaging breast cancer with optical coherence tomography,** A. M. Zysk, F. T. Nguyen, E. J. Chaney, S. A. Boppart, Univ. of Illinois at Urbana-Champaign . . . . . [100]

✔ **Optical coherence tomography signal enhancement with gold nanoshells,** A. Agrawal, U.S. Food and Drug Administration; A. W. H. Lin, M. Lee, R. A. Drezek, Rice Univ.; J. Pfefer, U.S. Food and Drug Administration . . . . . [101]

✔ **Measuring cognitive functioning with diffuse optical tomography,** A. Tambini, Massachusetts General Hospital and Massachusetts Institute of Technology; C. West, M. A. Franceschini, Massachusetts General Hospital . . . . . [102]

✔ **Multi-wavelength feflectance confocal microscopy for characterizing near-infrared wavelength sensitivity of unstained neutrophil backscattering,** Z. M. Wang, C. Glazowski, J. M. Zavislan, Univ. of Rochester . . . . . [103]

✔ **Analysis of time-resolved fluorescence data using Laguerre deconvolution,** C. C. Parker, A. Agrawal, U.S. Food and Drug Administration; T. Qazi, K. Agrawal, Virginia State Univ.; J. Pfefer, U.S. Food and Drug Administration . . . . . [104]

✔ **Imaging molecular interactions of oncogene RhoC in living cells using FLIM/FRET,** C. Chang, E. Rhee, M. Wu, S. D. Merajver, M. Mycek, Univ. of Michigan . . . . . [105]

✔ **Utilizing laparoscopic hyperspectral imaging during minimally invasive surgery,** S. Naik, The Univ. of Texas/Arlington; E. Livingston, H. Rivas, Univ. of Texas/Dallas; K. Behbehani, K. J. Zuzak, The Univ. of Texas/Arlington . . . . . [106]

✔ **Functional imaging in freely moving rats,** R. L. Barbour, SUNY/ Downstate Medical Ctr. and NIRx Medical Technologies; Y. Pei, NIRx Medical Technologies; C. H. Schmitz, NIRx Medical Technologies and SUNY Downstate Medical Ctr.; R. Ansari, NIRx Medical Technologies and SUNY/Downstate Medical Ctr.; M. B. Holzer, J. M. Barry, R. U. Muller, SUNY/Downstate Medical Ctr. . . . . [107]

✔ **Dual-wedge scanning confocal reflectance microscope,** W. C. Warger II, S. A. Guerrera, C. A. DiMarzio, Northeastern Univ. . . . . [108]

✔ **Optical diagnosis of cancer in lymph nodes and thyroid glands,** M. Romeo, M. D. Miljkovic, Northeastern Univ.; R. Emmadi, John Stroger Hospital of Cook County; M. Diem, Northeastern Univ. [109]

✔ **Characterization of fluorescence lifetime of Photofrin and ALA induced PpIX,** J. A. Russell, McMaster Univ. (Canada); J. E. Hayward, M. S. Patterson, Juravinski Cancer Ctr. (Canada); Q. Fang, McMaster Univ. (Canada) . . . . . [110]

✔ **Detection of increased blood supply in superficial colonic mucosa in early colon carcinogenesis,** Y. L. Kim, V. M. Turzhitsky, A. K. Kromin, R. K. Wali, H. K. Roy, M. J. Goldberg, P. Vakil, R. Brusen, V. Backman, Northwestern Univ. . . . . [111]

✔ **Bioluminescence tomography with evolutionary algorithms,** A. D. Klose, Columbia Univ. . . . . [112]

✔ **Reconstructing oxygen consumption and blood flow in diffuse optical tomographic breast imaging under mammographic compression,** D. Ntuba, Northeastern University; S. A. Carp, Massachusetts General Hospital; G. L. Boverman, E. L. Miller, Northeastern Univ.; D. A. Boas, Massachusetts General Hospital; D. H. Brooks, Northeastern Univ. . . . . [113]

✔ **NAVI: a problem solving environment (PSE) for NIRS data analysis,** Y. Pei, NIRx Medical Technologies; Z. Wang, Columbia Univ. and New York State Psychiatric Institute; Y. Xu, SUNY/ Downstate Medical Ctr. and NIRx Medical Technologies; R. L. Barbour, SUNY Downstate Medical Ctr. and NIRx Medical Technologies . . . . . [114]

✔ **In vivo characterization of autofluorescence dynamics during renal ischemia and reperfusion under dual UV excitation,** R. N. Raman, Univ. of California/Davis; C. D. Pivetti, Univ. of California/Davis Medical Ctr.; D. L. Matthews, Univ. of California/ Davis; C. Troppmann, Univ. of California/Davis Medical Ctr.; S. G. Demos, Lawrence Livermore National Lab. . . . . [115]

✔ **Contrast agent pharmacokinetics in breast cancer: ICG and Gd-DTPA,** D. R. Busch, Jr., A. G. Yodh, Z. Zhao, Univ. of Pennsylvania; X. Intes, ART Advanced Research Technologies Inc.; B. Chance, S. Nioka, Univ. of Pennsylvania . . . . . [116]

✔ **Maximum-likelihood multi-dimensional photon-counting microscopy,** L. M. Davis, G. Shen, D. A. Ball, J. C. Aiken, Y. V. White, W. N. Robinson, Z. Sikorski, The Univ. of Tennessee Space Institute; D. W. Piston, Vanderbilt Univ. . . . . [117]

✔ **Exploring DNA processing one molecule at a time,** R. Conroy, A. P. Koretsky, National Institutes of Health . . . . . [118]

✔ **Laser speckle contrast yields high contrast, high resolution images of cerebral microvasculature,** K. Murari, N. Li, A. Rege, N. V. Thakor, Johns Hopkins Univ. . . . . [119]

✔ **Miniaturization of a two-photon microscope and development of novel contrast agents for in-vivo cancer imaging and microsurgery,** C. L. Hoy, N. J. Durr, D. Smith, The Univ. of Texas/ Austin; K. V. Sokolov, The Univ. of Texas M.D. Anderson Cancer Ctr.; B. A. Korgel, The Univ. of Texas/Austin; O. Solgaard, Stanford Univ.; A. Ben-Yakar, The Univ. of Texas/Austin . . . . . [120]

✔ **1 kHz imaging device with single photon sensitivity,** D. S. Barnhill, Univ. of California/Los Angeles . . . . . [121]

✔ **Protein nanospheres as photoacoustic contrast agents for imaging, molecular targeting, and therapy,** M. A. McDonald, Stanford Univ. Medical Ctr.; F. Hunter, J. Xie, K. C. Li, National Institutes of Health; S. Guccione, Stanford Univ. Medical Ctr. . [122]

✔ **Molecular imaging and contrast agent database (MICAD): a new and freely accessible online source of information,** B. Beck, S. Bryant, K. T. Cheng, W. C. Eckelman, K. H. Leung, E. Lutanie, J. McEntyre, A. Menkens, D. C. Sullivan, National Institutes of Health . . . . . [123]

✔ **Assessing non-invasive detection of protoporphyrin IX fluorescence in vivo to quantify glioma tumor growth,** S. L. Gibbs, Dartmouth College; J. A. O'Hara, Dartmouth Medical School; P. J. Hoopes, Dartmouth Hitchcock Medical Ctr.; B. W. Pogue, Dartmouth College . . . . . [124]

✔ **Intraoperative imaging to assess organ viability: from bed to bench side,** A. M. Gorbach, National Institutes of Health; H. Wang, Naval Medical Research Center; M. Alemozaffar, National Institutes of Health; F. Gage, Naval Medical Research Center; N. Dhanani, A. D. Kirk, P. A. Pinto, P. D. Smith, National Institutes of Health; E. A. Elster, Naval Medical Research Ctr. . . . . [127]

✔ **Novel quantum dot based superluminescent light-emitting diodes (SLEDs) for optical imaging,** C. Vélez, L. Occhi, Exalos AG (Switzerland); M. Rossetti, A. Fiore, École Polytechnique Fédérale de Lausanne (Switzerland) . . . . . [130]

✔ **Development of a disposable microendoscope objective for early cancer detection,** R. T. Kester, T. S. Tkaczyk, J. D. Rogers, M. R. Descour, The Univ. of Arizona; T. C. Christenson, HT Micro; R. R. Richards-Kortum, Rice Univ. . . . . [134]

✔ **Technical considerations in longitudinal multispectral small animal molecular imaging,** M. Bouchard, S. A. MacLaurin, Novartis Institutes for Biomedical Research; P. J. Dwyer, S. Determan, J. R. Mansfield, R. M. Levenson, Cambridge Research & Instrumentation, Inc.; T. Krucker, Novartis Institutes for Biomedical Research . . [135]

## Wednesday 27 September

### Continental Breakfast

**Room: Natcher Auditorium Foyer . . . . . Wed. 7:00 am**

**SESSION 8 . . . . . Wed. 8:00 to 10:00 am**

### Optics in Neuroscience

*Chair:* **Elizabeth M. C. Hillman**, Columbia Univ.

8:00 am: **Structural imaging of the Alzheimer brain with multiphoton microscopy: effects of therapeutic interventions,** B. J. Bacskai, Massachusetts General Hospital . . . . . [34]

8:20 am: **High resolution functional optical imaging of rodent cortex: investigating mechanisms of functional activation,** E. M. C. Hillman, Columbia Univ. . . . . [35]

8:40 am: **Human brain mapping with high-density diffuse optical tomography,** J. P. Culver, Washington Univ. . . . . [36]

9:00 am: **Functional DOT imaging: technology, calibration and new findings,** R. L. Barbour, SUNY/Downstate Medical Ctr. and NIRx Medical Technologies . . . . . [37]

9:20 am: **The role of quantitative frequency domain near-infrared spectroscopy in the NIC,** E. Grant, Massachusetts General Hospital . . . . . [38]

9:40 am: **Optical molecular imaging: from single molecule to human body,** M. Tamura, Hokkaido Univ. (Japan) . . . . . [39]

Coffee Break . . . . . 10:00 to 10:30 am

**PANEL DISCUSSION . . . . . Wed. 10:30 to 11:15 am**

### Optics in Neuroscience

*Chair:* **Elizabeth M. C. Hillman**, Columbia Univ.

Participants: **Brian Bacskai,** Massachusetts General Hospital; **Joe Culver,** Washington Univ.; **Randall Barbour,** SUNY Downstate; **Ellen Grant,** Massachusetts General Hospital; **Mamoru Tamura,** Hokkaido Univ. (Japan); **Maria-Angela Franceschini,** Massachusetts General Hospital; **Yutaka Yamashita,** Hamamatsu

**SESSION 9 . . . . . Wed. 11:15 am to 1:15 pm**

### New Optical Devices and Methods

*Chair:* **Jay R. Knutson**, National Institutes of Health

11:15 am: **Non-invasive sensing of glucose and hemoglobin,** H. Primack, OrSense Ltd. (Israel) . . . . . [40]

11:35 am: **Novel small animal imaging system and intravital laser scanning microscope help observing cellular to whole animal fluorescence images,** Y. Kawano, N. Onda, I. Sakai, K. Kojima, Olympus Corp. (Japan) . . . . . [41]

11:55 am: **Optical fluorescence imaging of breast cancer,** N. van der Vaart, L. Bakker, M. B. van der Mark, M. van Beek, M. van der Voort, W. H. Rensen, R. Harbers, Philips Research Labs. (Netherlands); T. Nielsen, T. Köhler, R. Ziegler, Philips Research Labs. (Germany); A. Ziegler, Philips GmbH (Germany); B. Brendel, Philips Research Labs. (Germany); A. Feuerabend, Philips GmbH (Germany); J. P. Meeuwse, Philips Applied Technologies; D. van Pijkeren, S. Deckers, Philips Medical Systems; K. Licha, M. Pessel, Schering AG (Germany) . . . . . [42]

12:15 pm: **Current tools for in vivo imaging,** L. Greenfield, Invitrogen . . . . . [43]

12:35 pm: **New methodology of optical blood glucose monitoring based on simulation of light propagation in the skin,** Y. Yamada, Univ. of Electro-Communications (Japan); K. Maruo, Matsushita Electric Works, Ltd. (Japan); H. Arimoto, National Institute of Advanced Industrial Science and Technology (Japan); M. Tamura, Hokkaido Univ. (Japan); Y. Ozaki, Kwansei Gakuin (Japan) . . . . . [44]

12:55 pm: **Quantitative monitoring and imaging using near-infrared time-resolved spectroscopy,** Y. Yamashita, Hamamatsu Photonics K.K. (Japan) . . . . . [136]

Lunch Break . . . . . 1:15 to 2:15 pm

**SESSION 10 . . . . . Wed. 2:15 to 3:05 pm**

### Forum on NIH Support for Optical Imaging

2:15 pm: **Funding opportunities at the National Cancer Institute (NCI),** H. R. Baker, National Cancer Institute . . . . . [45]

2:25 pm: **Funding opportunities at the National Institute for Biomedical Imaging and Bioengineering (NIBIB),** Y. Zhang, National Institutes of Health . . . . . [46]

2:35 pm: **Funding opportunities at the National Center for Research Resources (NCRR),** G. Farber, National Institutes of Health . . . . . [47]

2:45 pm: **Funding opportunities at the National Institute of Neurological Disorders and Stroke (NINDS),** J. Pancrazio, National Institutes of Health . . . . . [48]

2:55 pm: **Funding opportunities at the National Institute of Dental and Craniofacial Research (NIDCR),** , . . . . . [49]

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# CALL FOR PAPERS

Journal of
BIOMEDICAL OPTICS
*Published by* SPIE—The International Society for Optical Engineering *in conjunction with* International Biomedical Optics Society

## Special Issue:
## Optical Diagnostic Imaging from Bench to Bedside

*Guest Editors:*
**Amir H. Gandjbakhche**, National Institutes of Health
**Bruce J. Tromberg**, University of California, Irvine

## Manuscript submissions due December 1, 2006

After a decade of collaboration among physicists, engineers, and physicians, optical imaging techniques are moving from bench to bedside at an extremely fast rate. Quantification of intrinsic chromophores, scattering properties, and targeted probes provide valuable functional information for diagnosing disease and monitoring therapies. With these advances, optical methods have become critical tools for translational research and studying the fundamental molecular origins of disease processes – from photonic studies of nanoscale interactions to ultrahigh resolution microscopy.

This special section follows the Fifth Inter-Institute Workshop on Optical Imaging at the National Institutes of Health, and will be devoted to all aspects of bringing optical imaging technology from the desktop, where quantitative theories are devised; to the bench, where the instrumentation is designed and tested; and finally to the bedside, where performance is validated in a demanding clinical setting.

*Manuscripts should be submitted to SPIE according to the journal submission guidelines at http://www.spie.org/jbo.*

# Technical Presentations

## Monday 25 September · Session 1

**7:30 am** — **Registration and Welcome Continental Breakfast**
*Room: Natcher Auditorium Foyer*

**8:15 am** — **Welcome Address**
*Room: Natcher Auditorium, P2 Level*
*Chairs:* **Amir H. Gandjbakhche,** National Institutes of Health;
**Bruce J. Tromberg,** Univ. of California/Irvine

**8:30 to 10:00 am** — **SESSION 1: Keynote Speakers**
*Chair:* **Amir H. Gandjbakhche,** National Institutes of Health



**10:00 to 10:30 am** — **Coffee Break**



# Optical imaging: bringing cell biology and physiology together in vivo

R. S. Balaban, National Institutes of Health

No abstract available

BIOGRAPHY: Dr. Robert Balaban received his PhD from Duke University in 1979 with a dissertation entitled “The coupling of aerobic metabolism to active ion transport in the kidney.” Since 1981, Dr. Balaban has been a researcher at the National Institutes of Health in Bethesda, Md. in the National Heart, Lung, and Blood Institute (NHLBI). He began as a staff fellow and held positions as senior staff fellow and research physiologist. He currently holds positions as Chief of the Laboratory of Cardiac Energetics in NHLBI and Scientific Director of the intramural research program of the NHLBI.

## [02] Session 1

A. P. Koretsky, National Institutes of Health

No abstract available



# The role of optical technologies in oncology

D. C. Sullivan, National Cancer Institute

Photonic methodologies have attracted much attention in oncology in recent years as they have the advantages of not involving ionizing radiation, of providing excellent chemical and temporal resolution, and of being cheaper than traditional clinical imaging techniques. Photonic technologies that do not require the administration of exogenous contrast materials, and which measure photon reflection, transmission, refraction or fluorescence from endogenous fluorophores, have already been developed for use in humans. Other photonic methods use similar physical properties, but require the administration of agents that either fluoresce or bioluminesce. There is also considerable interest in using quantum dots or other nano-constructed particles that fluoresce much more intensely than organic dyes. Bioluminescence methods have become enormously valuable techniques for basic research. Another potentially important characteristic of optical imaging agents are the so-called activatable agents, which exploit the phenomenon of fluorescence resonance energy transfer (FRET).

Investigators in drug development need in vivo assays (imaging biomarkers) to tell them whether a given patient has the appropriate molecular phenotype to benefit from a targeted therapy, to indicate whether the drug has hit its molecular target, to determine whether the drug has been given in the optimal biologic dose, and to ascertain whether the tumor is responding. Optical techniques have many potential uses in all phases of the drug development process, from target discovery and validation to pivotal clinical trials for drug registration. In particular, optical-based biomarker methods, sometimes referred to collectively as optical biopsy, have promise for speeding drug evaluation by replacing or supplementing time- and labor-intensive dissection and histological analyses in both preclinical and clinical testing.

Challenges to the development and implementation of optical modalities in drug development include the lack of validation and standardization. The identification and evaluation of biomarkers require access to and systematic analysis of large amounts of data, new technologies and extensive research resources. There is a requirement for convergence of research, regulatory and drug developer thinking. The NCI and FDA are developing plans for evaluation of biomarkers to address these needs for collaborative research to identify the best biomarkers for oncology, to standardize data collection and analysis, and to provide a pathway for establishing the use of new biomarker tools in oncology drug development and patient care. This plan includes public-private partnerships with the pharmaceutical and imaging device companies.

**BIOGRAPHY:** Daniel C. Sullivan, M.D. is Associate Director in the NCI Division of Cancer Treatment and Diagnosis, and head of NCI's Cancer Imaging Program (CIP). He completed radiology residency and nuclear medicine fellowship in 1977 at Yale-New Haven Hospital, and was an academic radiologist for 20 years before coming to NIH in 1997. Dr. Sullivan has held faculty appointments at Yale University Medical Center, Duke University Medical Center, and University of Pennsylvania Medical Center. His areas of clinical and research expertise are in nuclear medicine and breast imaging. The Cancer Imaging Program at NCI promotes the development of novel imaging technologies and image-guided therapies. CIP initiated several collaborative groups, including the In Vivo Cellular and Molecular Imaging Centers, the Small Animal Imaging Resource Programs, the Lung Imaging Database Consortium, the Network for Translational Research in Optical Imaging and the American College of Radiology Imaging Network.

# [04] Session 1

## B. Seto, National Institutes of Health

The National Institute of Biomedical Imaging and Bioengineering has made a significant investment in optical imaging research. In FY 2005, approximately $ 19M was awarded for research and training purposes. The Institute plans to build on this foundation and advance the translation of optical imaging research to clinical applications.

Optical imaging research represents the confluence of many fields, including physics, chemistry, biology and medicine. Rapid developments in the technologies in optical imaging research have created rich research opportunities in molecular imaging of biological processes and the detection of pathological disease processes at the molecular level. The translation and application of research findings in clinical settings should facilitate improving human health which is the ultimate objective of biomedical research.

This workshop provides the opportunity to assess the current state of translational optical imaging research, to identify major road blocks, and to develop models to support and stimulate this vital area of research. It is through such efforts that we hope to accelerate translational research and produce benefits for the societal investments in research.

**BIOGRAPHY:** Belinda Seto, Ph.D. is the Deputy Director of the National Institute of Biomedical Imaging and Bioengineering (NIBIB). She is responsible for the oversight and management of all aspects of the Institute_s research and training mission. Her responsibilities include program planning, development and implementation of the intramural programs and the extramural grants portfolio. She serves as the Institute_s liaison to the Office of the Director on the NIH roadmap initiatives.

Prior to joining the NIBIB, Dr. Seto was the Acting Deputy Director for Extramural Research, NIH, and as such serves as the Director of the Office of Extramural Research (OER). The OER serves as the focal point for policies and guidelines for extramural research grants administration. This office has primary responsibility for the development and implementation of NIH Grants Policy, monitoring of compliance with PHS policy on Humane Use and Care of Laboratory Animals, coordination of program guidelines, and development and maintenance of the information systems for grants administration.

Dr. Seto is responsible for grants policy issues such as Facilities & Administrative costs (indirect costs), allowable and allocable costs as specified in OMB Circular A21, roles and responsibilities of grantee institutions, and compliance. She also has expert knowledge in program administration and NIH policies for clinical research and clinical trials including policies related to data and safety monitoring and protection of human subjects, and an online education module on bioethics.

The OER is responsible for the development and implementation of eRA, the electronic system for conducting grants business of the NIH enterprise. The eRA system includes IMPACII for grants administration, iEdison for invention reporting, and the NIH Commons, a web-based, secure interface that allows grantee institutions to exchange information with the NIH. OER also provides a number of data resources that are accessible to the public, including CRISP and the award data page.

Dr. Seto earned her Ph.D. in biochemistry at Purdue University in 1974. Following postdoctoral training in the National Heart, Lung and Blood Institute, she joined the Food and Drug Administration where she conducted research in virology for nearly 10 years. She received numerous awards for her research, including the DHHS Secretary's Award for Exceptional Achievement, Inventor's Awards, NIH Director_s awards and she is listed in the American Men and Women of Science.

She held position in other components of the NIH as well as the Office of the Assistant Secretary for Health. Dr. Seto has served on numerous NIH and interagency committees. She is a member of several professional societies.

**Award for extraordinary pioneering contributions to the translation of optical technologies from blackboard to benchtop to bedside**

*Given to:*

**Brian C. Wilson,** Professor of Medical Biophysics, Univ. of Toronto (Canada)

**10:30 am to 12:30 pm** **SESSION 2: Molecular Imaging**

Room: Natcher Auditorium, P2 Level
*Chair:* **Mary-Ann Mycek**, Univ. of Michigan



**12:30 to 1:30 pm** **Lunch Break**



# The imaging probe development center: an NIH core synthesis resource for imaging probes

G. Griffiths, National Institutes of Health

The Imaging Probe Development Center (IPDC) is being set up to fulfill several goals while addressing the need of biomedical investigators for improved access to quality imaging probes. Such probes will encompass all the imaging modalities including optical, radionuclide and magnetic resonance imaging. In the first instance, it will provide known imaging probes to investigators at NIH who otherwise would have difficulty obtaining these agents for their work. Once fully operational it will then investigate improvements in probe preparation and supply, and perform its own research studies on the production of novel probes. Information obtained will be shared with the wider scientific community through peer-reviewed publications and the deposition of detailed information into the new MICAD imaging agent database. As a core synthesis facility it will work closely with bio-characterization and molecular modeling functions, to optimize probe properties, and will be equipped to run preliminary fluorescence studies in the case of optical probes. The optical probes themselves will be studied for improvements in their optical properties, while another major goal will be to improve resolution for all imaging modalities, by improving selectivity, sensitivity, targeting efficiency and background clearance characteristics, thereby improving overall probe delivery characteristics.

**BIOGRAPHY:** Dr. Gary Griffiths recently joined NIH as Director of the new Imaging Probe development Center. He was trained as a chemist specializing in organic synthesis and has multi-year experience in the synthesis and development of disease-specific targeting agents, including imaging agents, for in vivo use.



# Nanomolecules and nanoparticles in optical tumor imaging

S. Achilefu, Washington Univ. in St. Louis

No abstract available

**BIOGRAPHY:** Samuel Achilefu, PhD, is an Associate Professor and Director of the Optical Radiology Laboratory, Department of Radiology at Washington University School of Medicine in St. Louis, MO. His expertise includes the design, synthesis, in vitro, and in vivo evaluation of optical molecular probes for imaging and monitoring the status of diseased cells and tissues.



# Quantitative molecular imaging using time-resolved intensity and lifetime

A. H. Gandjbakhche, National Institutes of Health

Biology and medicine are moving toward molecular and environmental identification of disease processes. Fluorescence molecules can play an important role. Combining the intensity and lifetime of exogenous markers enables one to non-invasively monitor such changes. We have developed a time-resolved, near-infrared imaging system incorporating the facilities for small animal imaging to achieve this goal. In collaboration with Jacek Capala (NCI), small animal studies have been conducted to examine fluorescent probe distribution and lifetime in the vicinity of tumors. A breast cancer cell line overexpressing human epidermal growth factor receptor 2 (HER2) was implanted in the flank area of a mouse. Alexa Fluor 750-conjugated Herceptin was injected through the tail vein to target cells overproducing HER2. A series of images were obtained before and after the injection at different time frames. The washout time, lifetime and distribution of the probe were investigated. A set of experiments has also been conducted on the conjugated fluorophore using known pH environments to establish the mapping from lifetime to pH. Then one can relate changes in tumor environment, such as pH, to provide functional information about the tumor in vivo.

**BIOGRAPHY:** Amir H. Gandjbakhche has been at the National Institutes of Health (NIH) since coming as a visiting fellow in 1990 after earning his Ph.D. from the University of Paris. He is currently Chief of the Section on Biomedical Physics (SBSP) in the Laboratory of Integrative and Medical Biophysics (LIMB) of the National Institute of Child Health and Human Development (NICHD), a position he's held since 1999. At the NIH, Amir also serves as Chair of the Molecular and Functional Optical Imaging scientific interest group and Chair of the biennial Inter-Institute Workshop on Optical Diagnostic Imaging from Bench to Bedside.



# Activatable fluorescent optical probes for in vivo molecular imaging

P. L. Choyke, National Institutes of Health

Resection of peritoneal metastases due to ovarian cancer is often unsatisfactory due to the high rate of recurrent disease. Methods to improve the detection of tumor during surgery could enhance patient survival. Untargeted or even targeted fluorescent optical agents have limited utility in this setting unless unbound probes can be completely eliminated from the background. Moreover, the ability to compensate for background autofluorescence is critical. Using a peritoneal model of ovarian cancer and a spectrally resolved optical camera (Maestro, CRi Woburn MA) in which the target signal is unmixed from autofluorescence, we have demonstrated that an avidin-FITC conjugate not only targets the tumor but if unbound, is quickly absorbed and is taken up by the liver, well away from the imaging field. In second generation agents, the targeted fluorophore is activated by pH changes after internalization within the targeted cell. Interestingly, traditional fluorophores bound to targeting ligands demonstrate very different fluorescent behavior once internalized. However, more dramatic changes in fluorescence can be seen if the fluorophores are chemically modified for amplified fluorescence only in low pH conditions. Probes that are activated in response to reactive oxygen species (e.g. hydrogen peroxide) are in testing. Such activatable optical molecular contrast agents can detect minute (100 micron diameter) quantities of peritoneal tumor implants with simple optical fluorescent cameras, easily utilized in the operating room, and thus hold great promise for clinical translation. Moreover, by improving the initial resection of tumor, optically enhanced surgery paves the way for more effective adjuvant therapies such as intraperitoneal chemotherapy or radioimmunotherapy.

**BIOGRAPHY:** The Molecular Imaging Program (MIP) of the NCI is a multidisciplinary lab dedicated to developing targeted imaging agents for the detection of cancer. Working with Hisataka Kobayashi, M.D., Ph.D of MIP and Yusetero Urano, Ph.D. of the University of Tokyo, Dr. Choyke is testing activatable optical contrast agents in vivo for possible clinical translation.

# Raman, CARS, and near-field Raman-CARS microscopy for cellular and molecular imaging

S. Kawata, Osaka Univ. (Japan)

When it comes to cellular and molecular imaging, in particular, biomolecular imaging, Raman spectroscopy always turns out to be advantageous over the other common techniques, because it is not only nondestructive, but also it does not need any staining of molecules, which guarantees preservation of the physiological activities of the sample molecules. When combined with microscopy, Raman spectroscopy takes advantage of the rich information available from the molecular vibrations, and helps providing a high quality image. One problem in obtaining Raman image is the long time involved in the imaging process, which is due to the inherent weak signal of Raman scattering. We have come over this problem by developing a technique of slit-scanning confocal Raman microscopy. Apart from an improved acquisition rate, this technique is based on collecting Raman signal from multiple points of the sample using a line-shaped illumination, which is scanned over the sample in the direction perpendicular to the illumination line, providing a two-dimensional x-y-image. At the same time, the sample can also be moved in the z-direction to construct a 3-D image. This method is particularly suitable for fast and high quality 3-D cellular imaging.

The image quality, such as the contrast and the resolution, can be further improved by combining the near-field effects in microscopy or the higher-order scattering effects in Raman scattering process or both of them together. In our previous work [1-3], we have shown that Raman scattering combined with near-field microscopy, i.e., tip-enhanced Raman spectroscopy (TERS), provides a super spatial resolution beyond the diffraction limits of the probing light, along with an enhanced scattering efficiency. This is due to the nano-sized evanescent field created in close proximity of the apex of a nano-metallic tip, which enhances

the scattering from the sample molecules directly under the tip apex. Several nanomaterials, such as carbon nanotubes, carbon 60, and DNA-base molecules have been successfully imaged with a spatial resolution down to about 25 nm.

The nonlinear scattering process, such as the coherent anti-Stoke Raman scattering (CARS), provides better confinement of light field than the linear scattering due to the nonlinear effects, and provides better contrast due to the suppression of the luminescence background. Thus, a combination of CARS with near-field microscopy, i.e., the tip-enhanced CARS (TE-CARS), can further confine the light field to the very apex of the metallic tip, providing better spatial resolution along with high contrast due to the suppressed background. This technique has been applied to image adenine molecules [5], and a high quality image with a spatial resolution of 15 nm was obtained. This technique is proved to be very successful for high quality imaging, particularly, of biomolecules.

[1] N. Hayazawa, et al., Chem. Phys. Lett. 376, 174 (2003).
[2] H. Watanabe, et al., Phys. Rev. B 69, 155418 (2004).
[3] P. Verma, et al., Phys. Rev. B 73, 045416 (2006).
[4] T. Ichimura, et al., Phys. Rev. Lett. 92, 220801 (2004).

**BIOGRAPHY:** Satoshi Kawata is Professor of the Department of Applied Physics at Osaka University since 1993. He is also Chief Scientist at RIKEN and Chairman at NanoPhoton Corporation. He has been working as the editor for Optics Communications and the president of Spectroscopical Society of Japan. He is a fellow of Optical Society of America and Institute of Physics. His research field is Nanophotonics, Plasmonics, Nano-fabrication and Spetroscopy. He has edited and authored 20 books and published a numbers of papers in such as Nature, Phys. Rev. Lett., Phys. Rev. B and J. Am. Chem. Soc.



# Miniaturized, disposable microscopy probes for early cancer detection

T. S. Tkaczyk, The Univ. of Arizona

Cancer screening very often relies on clinical experience at recognition of suspicious lesions during visual exam followed with biopsy procedures. An alternative approach is in vivo optical imaging which provides histologic quality images without the need for invasive biopsy and allows assessing the morphologic and architectural changes associated with neoplasia. To reach this goal it is necessary to develop affordable screening probes which image on the cellular level. Therefore the major goal of the talk is presentation of miniaturized, inexpensive optical systems imaging with micron resolution. To design and build disposable probes with high resolution we have developed a unique Alignment Free Assembly (AFA) - technology for a family of miniaturized optical devices especially dedicated for early in vivo cancer detection. The AFA technology requires opto-electro-mechanical design and fabrication technology so the instrument is assembled in a plug and snap fashion and no further adjustments are required. Three major system approaches were developed (a) a Micro-Optical Table (MOT), (b) stacking, and (c) tubular (classical). Using above technology we have built probes including:

- the Multi-Modal Miniature Microscope (4M Device) - a pensized probe working in fluorescence and reflectance modes. Each mode can be combined with structured illumination
- high NA plastic disposable microscope objective for confocal handheld probe
- high NA glass-plastic disposable microscope objective for confocal handheld probe

All systems can reach 1 micron resolution level and are sufficient for normal/abnormal cells discrimination. The biggest system (plastic objective) is confined to 8 mm outer diameter while the smallest (plastic-glass) fits inside a 4 mm tube. During the presentation the overview of the AFA technology and miniaturized probes will be combined with the presentation of imaging results for epithelial cell phantoms with optical properties characteristic for normal and cancerous tissue.

**BIOGRAPHY:** Tomasz Tkaczyk received his MS and PhD degrees in optical engineering in 1994 and 2000, respectively, from the Mechatronics Department, Warsaw University of Technology, Poland. In 2003 after his postdoctoral training he started to work as an assistant research professor at the College of Optical Sciences, University of Arizona. His current research activities include: biomedical imaging, micro-optics, and imaging spectroscopy. He is a coauthor of approximately 40 scientific publications, and serves as a member of SPIE Kingslake Award committee.

**Mon. 1:30 to 3:30 pm** — **SESSION 3: Translating Optical Technologies from Benchtop to Clinical Standard**

*Room: Natcher Auditorium, P2 Level*
*Chair:* **Houston R. Baker,** National Cancer Institute



**3:30 to 4:00 pm** — **Coffee Break**

**4:00 to 5:00 pm** — **Panel Discussion**

**Translating Optical Technologies from Benchtop to Clinical Standard**

*Chair:* **Bruce J. Tromberg,** Univ. of California/Irvine

*Participants:* **John Pearson** (Siemens Corporate Research)
**Jim Crawford** (Univ. of Florida)
**David Benaron** (Spectros Corporation)
**Anthony Hayward** (NCRR/NIH)
**Ronald Waynant** (FDA)
**Peter Barker** (NIST)

[11] Session 3

# Overcoming barriers to clinical translation

B. J. Tromberg, Univ. of California/Irvine

Optical technologies offer patients inexpensive, portable, bedside solutions to a broad variety of medical problems. Emerging translational applications include increasing surgical accuracy, mapping and imaging tissue function, monitoring and predicting therapeutic drug efficacy, and detecting/screening early disease. In addition, because of their relative simplicity, accessibility, and minimally-invasive nature, optical methods are particularly well-suited for frequent scans in high-risk subjects. However, despite their anticipated impact, significant barriers limit our ability to effectively translate optical technologies from blackboard to widespread clinical practice. This talk identifies key barriers to translation, and highlights opportunities for accelerating the movement of optical technologies from academic laboratories into the clinic. Four major phases of clinical translation are presented, spanning from design, calibration and testing of new methods and devices, to technology standardization and validation in multi-center trials. The time for the overall Phase 1-4 cycle varies substantially and can require up to 10-15 years, particularly when starting with untested blackboard technology concepts. Specific examples will be presented based on the NCI Network for Translational Optical Imaging (NTROI), a program designed to facilitate translational technology development and standardization in cancer. Challenges associated with translational research using optics as a stand-alone platform, and in conjunction with conventional imaging will also be discussed, particularly with regard to NTROI technology applications in Breast and GI cancers.

**BIOGRAPHY:** Dr. Tromberg is the Director of the Beckman Laser Institute and Medical Clinic at the University of California, Irvine and Professor of Biomedical Engineering. His research interests are in the development and application of multi-dimensional, in-vivo functional imaging methods in thick tissues based ondiffuse optical spectroscopy and nonlinear microscopy.



# Informatics platforms to support collaboration and drive standardization

J. C. Pearson, Siemens Corporate Research

The NTROI-BCDOI is a highly collaborative and distributed consortia of research institutions engaged in translational research of optical imaging for breast cancer. Siemens Corporate Research (SCR) is responsible for providing the data management and analysis software used by the consortia to standardize optical imaging methods and conduct clinical trials. The effort to date has produced platforms for multi-modal fusion and analysis (OMIRAD) and for experimental data management and collaboration (SciPort). The talk will highlight the capabilities of these tools as used by the NTROI, and for speeding clinical translation more generally. OMIRAD enables the joint analysis of optical data with the conventional modalities of MR, Xray, and ultrasound, permitting the discovery and exploitation of new correlations for diagnosis and therapy monitoring. SciPort is a web-based application built upon open-source frameworks. It enables each lab to organize and manage its research data, and to selectively share those portions relevant to the NTROI through publishing to a central server outside of the lab. Both of these general-purpose tools could be of use in many translational imaging research networks.

**BIOGRAPHY:** J.C. Pearson leads the Government Research Program at Siemens Corporate Research, located in Princeton, NJ, focusing on medical imaging post-processing and data management and collaboration systems. He holds a Ph.D. in Physics, and has contributed to the fields of neuroscience and image processing.



## Optics and the pathology gold standard

J. M. Crawford, Univ. of Florida

No abstract available



# Commercial barriers and pathways

D. A. Benaron, Spectros Corp.

No abstract available

**BIOGRAPHY:** David A Benaron, MD completed his MD at Harvard, Health Sciences Technology at MIT, Physiology fellowship at Univ. of Pennsylvania, and Intensive Care medicine at Stanford. He previously co-founded Xenogen, which uses optics to accelerate and streamline the drug discovery process. Xenogen went public in July 2004. He is currently CEO of Spectros Corporation, which uses optics to enable and guide interventional medical therapies.

**7:00 am** **Continental Breakfast**
*Room: Natcher Auditorium Foyer*

**8:00 to 10:00 am** **SESSION 4: Optical Imaging: Translation to Preclinical and Clinical Applications I**
*Room: Natcher Auditorium, P2 Level*
*Chair:* **Yantian Zhang,** National Institutes of Health



**10:00 to 10:30 am** **Coffee Break**

# OCT in cardiovascular and musculoskeletal diseases

M. E. Brezinski, Massachusetts Institute of Technology

This talk will follow the progression from bench to beside (and to some degree back to the bench) of optical coherence tomography (OCT) in the fields of cardiovascular medicine and musculoskeletal disease. OCT is analogous to ultrasound measuring the backreflection of infrared light rather than sound. OCT has several advantages as a high resolution imaging technology. First, its resolution between 4 and 20 μm is substantially higher than currently available imaging modalities. Second, its acquisition is at video rate, which is critical to most of its application. Third, since OCT is based on fiber optics, catheters and endoscopes can be engineered to very small dimensions. Finally, since OCT is optically based, it can be combined with a range of adjuvant techniques including polarization sensitive imaging for the detection of organized collagen.

With respect to cardiology and musculoskeletal disease, topics which will be discussed include original NIH funded studies which demonstrated the feasibility of these applications, in vivo human studies, and current directions the field is moving to further advances the technology, such as adjuvant techniques.

**BIOGRAPHY:** Mark Brezinski is an Associate Professor at Harvard Medical School and senior scientist at Brigham and Women's Hospital. His work focuses on Optical Coherence Tomography (OCT), predominately in non-transparent tissue. He received his MD/PhD from Thomas Jefferson University, did his residency at Brigham and Women's Hospital, and postdoctoral training at Harvard Medical School. His cardiology fellowship was at Massachusetts General Hospital and Harvard Medical School, where he became a staff member in the Cardiac Unit. In 2000, Dr. Brezinski joined the Orthopedics Department at Brigham and Women's Hospital and Harvard Medical School where he is currently a staff member. Dr. Brezinski has received many awards for his work, including a 1999 science award from President Clinton. Author of over 200 publications, he is a recognized pioneer in OCT research. He co-founded the Lightlab Corporation, which develops OCT technology, and is currently the principal investigator on several NIH contracts.



# Second harmonic generation imaging microscopy of osteogenesis imperfecta

P. J. Campagnola, Univ. of Connecticut Health Ctr.

Second Harmonic Generation (SHG) imaging is utilized to examine the morphology of collagen fibers associated with the disease Osteogenesis Imperfecta (OI). The modality allows the direct visualization of the collagen fibrils without the use of exogenous labels and we find that SHG images reveal clear differences in the fiber morphology in the wild type control tissues compared to that in the OIM mouse model. We also compare the forward to backwards (F/B) emitted ratio of SHG intensities of skin, tendon, and bone and find clearly discernible ratios in these two cases. Specifically, the SHG is more highly forward directed in these tissues, and is indicative of the OI diseased tissue being characterized by a less scattering matrix. This finding is consistent with the weaker mechanical properties associated with the pathology observed in OI patients. Monte Carlo simulations of the SHG signal propagation are consistent with experimental F/B ratios, and further suggest that the anisotropy factor, g, plays a large role in these ratios as well as the axial dependence on the SHG intensity. We also find a 3 fold decrease in intensity in the OI tissues relative to the wild type. Histological analysis shows that the collagen concentrations are statistically similar, thus the decrease in signal arises from the more disordered matrix, and is also consistent with the tissue scattering data. These findings indicate that the SHG approach reveals structural aspects of OI collagen that are not discernible by other methods and may provide complimentary information to ultra-structural and biochemical assays.

**BIOGRAPHY:** Dr. Campagnola is an assistant professor at the University of Connecticut Health Center in the Center for Cell Analysis and Modeling and the Department of Cell Biology. He has been developing Second Harmonic Generation imaging microscopy biophysical applications. A second research area uses multiphoton excited photochemistry for tissue engineering applications.



# Dynamics and resilience of blood flow in cortical microvessels revealed with optical imaging and manipulation

D. Kleinfeld, Univ. of California/San Diego

In the cerebral cortex, the vasculature forms highly interconnected 2-D networks of surface vessels as well as 3-D networks of subsurface microvessels. Here I discuss the dynamics of flow in these networks and contrast the effects of perturbations to the flow in the surface arterioles that distribute blood as compared to the network of subsurface microvessels and to the penetrating arterioles that connect the surface and subsurface vessels. The penetrating arterioles are found to form a severe bottleneck to flow, consistent with their hypothetized role in regulating the local flow of blood to cortex. The impact of these data on blood flow in the normal and diseased brain will be discussed, with emphasis on the relation of blocks to penetrating arterioles with cortical microstrokes. A underlying technical theme is the use of ultrashort pulses as a tool to perturb flow on the femtoliter-scale as well as to image flow. Sponsored by the NIBIB, NCRR, and NSF.

**BIOGRAPHY:** David Kleinfeld is a Professor of Physics and member of the graduate programs in Neuroscience and Computational Neuroscience at UCSD. He is a past or present co-director of NIH sponsored postgraduate schools in Neuronal Imaging (CSHL), Computational Neuroscience (MBL), and NeuralInformatics (MBL). His research program focuses on active sensation, using the rat vibrissa sensorimotor system, and on cortical blood flow dynamics. Nonlinear optics plays a major role in the latter studies.



# Potential and challenges of high-resolution endoscopic technologies for optical biopsy of internal organs

X. Li, Univ. of Washington

High-resolution optical imaging techniques, such as optical coherence tomography (OCT), confocal and multiphoton fluorescence (MPF), require fiber-optic probes (e.g. endoscopes) for imaging internal organs. In this talk, we present a new version of miniature fiber-optic endoscopes (1.5-2.4 mm in diameter), capable of rapid transverse beam scanning and real-time imaging in the forward direction. The endoscope also enables true dynamic focus tracking, making it possible to maintain a high transverse resolution over the entire imaging depth. The transverse resolution, although equally important as the axial resolution for high-resolution, has often been less aggressively pursued in a conventional endoscope due to the difficulty of focus tracking. To fully benefit from dynamic focus tracking enabled by the new endoscope, a state-of-the-art micro compound lens has been designed to achieve superb transverse resolution. In addition to OCT imaging, we have shown that the endoscope can be used for MPF (and confocal) imaging, permitting functional integration different high-resolution optical imaging techniques. Two-photon fluorescence imaging of live cancer cells using the scanning endoscope has been demonstrated. With a recently developed dispersion management scheme and a compact femto-second fiber-source, the endoscopic MPF system will be extremely compact and portable. Other imaging and scanning modes of the endoscope will also be illustrated. Optical biopsy using these high-resolution techniques, in particular for detecting subsquamous Barrett_s epithelium, will be presented. In addition, we will discuss our recent effort on developing a new class of molecular contrast agents based on structured, bioconjugatible gold nanocages, aiming to improve the limited intrinsic contrast and specificity in cancer detection. The nanocages offer a very strong, tunable optical resonance in the NIR range while maintaining a small size (~30-50 nm). The potential of the biofunctionalized nanocages as an imaging and photo-thermal therapeutic agent will also be discussed.

**BIOGRAPHY:** Dr. Xingde Li, an assistant professor of Bioengineering University of Washington, received his Ph.D. from the University of Pennsylvania and completed his postdoctoral training at MIT. He is a recipient of “Teacher of the Year Award” (BioE UW) and the NSF Career Award. His research interest centers on innovative “optical biopsy” and molecular nanophotonics technologies and their applications in biomedicine.



# In vivo microscopy and flow cytometry: application to bone marrow cell trafficking

C. P. Lin, Massachusetts General Hospital

Treatment of malignancy with high dose radiation or chemotherapy leads to irreversible damage to the blood forming cells, i.e. the hematopoietic stem/progenitor cells (HSPCs) in the bone marrow. To reconstitute the blood cells, patients are given hematopoietic cell transplantation (HCT), a multi-step procedure that involves HSPC mobilization from the donor bone marrow into the peripheral circulation, harvesting and purification of these cells followed by re-infusion into the recipient. In the recipient, the transplanted cells have to find their way (homing) to the appropriate bone marrow microenvironment for long-term engraftment. As many of these steps involve cellular trafficking in and out of the bone marrow, we are developing methods to visualize and quantify these processes in live animal models using the techniques of in vivo microscopy and flow cytometry. Our goal is to improve mechanistic understanding of the cellular interactions involved in HCT in order to improve the effectiveness of the procedure, as well as to minimize unwanted side effects, particularly the graft versus host disease (GVHD).

**BIOGRAPHY:** Dr. Lin has been active in biomedical optics research for more than 15 years. His research interests range from laser interaction with micro/nanoparticles, laser safety and retinal microsurgery, and ophthalmic imaging. More recently he has developed methods to study cellular trafficking in live models of cancer metastasis, stem cell transplantation, and immune cell interactions.



# Bringing in-vitro assays to life with fluorescence molecular tomography (FMT)

V. Ntziachristos, Massachusetts General Hospital

Fluorescence imaging is a powerful modality that is increasingly used for gene-expression profiling, probing protein function and elucidating cellular pathways. Fluorescence generated in in-vitro assays can be easily quantified using fluorometers or charge coupled devices (CCD). Similarly, fluorescence of superficial structures has been imaged in vivo using intravital, confocal or multiphoton microscopy. Quantification and imaging of fluorescence in deeper tissues however has been more elusive. This talk describes current progress with instruments and methods for in-vivo planar imaging and tomography of whole animals using normalized planar techniques and Fluorescence Molecular Tomography (FMT). We demonstrate how optical heterogeneity and high scattering can be accounted for in order to improve the imaging capacity of fluorescence bio-distribution in mice based on complete projection imaging and free-space illumination and collection approaches. Using these methods we show ability to resolve the distribution, size and shape of localized and of distributed objects. We further demonstrate how quantification and high molecular specificity can be achieved. Examples of imaging enzyme up-regulation, induced apoptosis and fluorescent proteins in intact animals are given. Limitations of the method and the potential for clinical translation are also discussed.

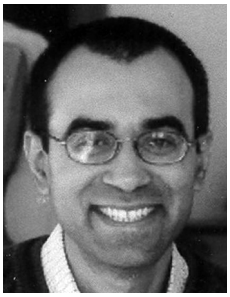
**BIOGRAPHY:** Vasilis Ntziachristos Ph.D. is an Assistant Professor at Harvard University Medical School and the Massachusetts General Hospital and head of the Laboratory for Biooptics and Molecular Imaging at the Center for Molecular Imaging Research. He has received his masters and doctorate degrees from the Bioengineering Department of the University of Pennsylvania and the Diploma on Electrical Engineering from the Aristotle University of Thessaloniki, Greece. His main research interests involve the development of optical methodologies for probing physiological and molecular events in tissues using non-invasive methods.

**10:30 to 11:50 am** **SESSION 5: Optical Imaging: Translation to Preclinical and Clinical Applications II**

*Room: Natcher Auditorium, P2 Level*
*Chair:* **John W. Haller**, National Institutes of Health



**11:50 am to 1:00 pm** **Lunch Break**





# Confocal mosaicing of basal cell carcinomas in skin excisions to potentially guide Mohs surgery: recent advances in translational research toward surgical pathology-at-the-bedside

M. Rajadhyaksha, Y. G. Patel, K. S. Nehal, I. Aranda, Y. Li, A. C. Halpern, Memorial Sloan-Kettering Cancer Ctr.

Precise removal of basal cell carcinomas (BCCs) with minimal damage to the surrounding normal skin is guided by the examination of frozen histology of each excision during Mohs surgery. The preparation of frozen histology is slow, requiring 20-45 minutes per excision. Confocal mosaicing may enable rapid detection of BCCs directly in surgical excisions, with minimal need for frozen histology. Early laboratory results showed that soaking the excisions in acetic acid (acetowhitening) rapidly brightens nuclei, enhances nuclear-to-dermal contrast and improves detectability of BCCs. Recent advances in translational research include the (i) determination of clinically relevant acetic acid concentrations versus soaking times, (ii) design of a mechanical fixture to precisely mount, and enable mosaicing of images over, large excisions, (iii) development of mosaicing software to create large fields-of-view of 15x15 mm (equivalent to 2X views as required by Mohs surgeons), (iv) comparison of contrast in brightfield versus cross polarization and (v) comparison of mosaics to histology. To date, large nodular, micronodular and superficial BCCs are easily detected. However, smaller micronodular, infiltrative and sclerosing BCCs tend to be obscured within the surrounding bright dermis. Multimodal reflectance-and-autofluorescence contrast is now being investigated for further enhancing nuclear-to-dermal contrast and detectability of these smaller BCCs. At present, the mosaicing method requires 9 minutes, and thus may expedite Mohs surgery. The reported work is not rocket-science, but demonstrates the down-to-earth detailed practical engineering that is necessary to translate laboratory results toward actual clinical utility.

**BIOGRAPHY:** Milind Rajadhyaksha is an Assistant Member in Dermatology at Memorial Sloan-Kettering Cancer Center (New York). He is an optical and mechanical engineer. He builds confocal microscopes and directs translational imaging research, toward clinical applications in skin cancer diagnosis and surgical guidance in vivo.



# High-resolution biophotonic imaging

L. V. Wang, Washington Univ. in St. Louis

We develop biophotonic tomography for early-cancer detection and functional imaging by physically combining non-ionizing electromagnetic and ultrasonic waves. Unlike ionizing x-ray radiation, non-ionizing electromagnetic waves, such as optical and radio waves, pose no health hazard and, at the same time, reveal new contrast mechanisms. Unfortunately, electromagnetic waves in the non-ionizing spectral region do not penetrate biological tissue in straight paths as x-rays do. Consequently, high-resolution tomography based on non-ionizing electromagnetic waves alone, as demonstrated by confocal microscopy and two-photon microscopy as well as optical coherence tomography, is limited to superficial imaging within about one transport mean free path (~1 mm) into biological tissues. Ultrasonic imaging, on the contrary, provides good image resolution but has strong speckle artifacts as well as poor contrast in early-stage tumors. We have developed ultrasound-mediated imaging modalities by combining electromagnetic and ultrasonic waves synergistically to overcome the above problems. The hybrid modalities yield speckle-free images with high electromagnetic contrast at high ultrasonic resolution in relatively large volumes of biological tissue. In ultrasound-modulated optical tomography, a focused ultrasonic wave encodes diffuse laser light in scattering biological tissue, which is analogous to the encoding mechanism in MRI. In photo-acoustic (thermo-acoustic) tomography, a low-energy laser (RF) pulse induces ultrasonic waves in biological tissue owing to thermoelastic expansion.

**BIOGRAPHY:** Lihong Wang received the Ph.D. degree from Rice University, Texas. He is Professor of Biomedical Engineering, University Faculty Fellow, and Royce E. Wisenbaker II Endowed Professor of Engineering at Texas A&M University. He will join Washington University in St. Louis in summer 2006 as Distinguished Endowed Chair of Biomedical Engineering.



# Single molecule probing of dynamic conformations, molecular interactions and dynamic localizations in-vitro, in live cells and in organisms

S. Weiss, Univ. of California/Los Angeles

Advances in single molecule studies of: (1) protein folding; (2)initiation of transcription by e-coli RNA polymerase; (3) targeting and detection of individual proteins in live cells using peptide-coated quantum dots; (4) utilization of peptide-coated quantum dots to the study of lipid rafts; 5) molecular imaging in small organisms and rodents; will be reported and future directions will be discussed.

**BIOGRAPHY:** Shimon is a Chemistry & Physiology Professor at UCLA. Formerly, as a staff scientist at LBNL, he was involved in nanoscale science and single-molecule spectroscopy. Together with others he pioneered the field of single-molecule spectroscopy, its application to biology and the use of quantum dots as probes for biological imaging.



# Optical molecular imaging for early detection of cancer

R. R. Richards-Kortum, Rice Univ.

Progress toward molecular characterization of cancer would have important clinical benefits, including (1) detecting cancer earlier based on molecular characterization, (2) predicting the risk of precancerous lesion progression, (3) detecting margins in the operating room in real time, (4) selecting molecular therapy rationally and (5) monitoring response to therapy in real time at a molecular level.

While molecular markers can be visualized in vitro, there is an important need to image the molecular features of cancer in vivo. Imaging the molecular features of cancer requires molecular-specific contrast agents which can safely be used in vivo as well as cost-effective imaging systems to rapidly and non-invasively image the uptake, distribution and binding of these agents in vivo.

We have used optically active contrast agents to image the expression of three well known molecular signatures of neoplasia. This same approach can be used to develop contrast agents to image the expression of promising new biomarkers. We are testing three different types of optically active labels, and two types of molecular probes. With this approach, we can significantly expand the number of molecular changes that can be probed using optical imaging.

At the same time, we are developing inexpensive, portable optical systems to image the morphologic and molecular signatures of neoplasia noninvasively in real time. We are developing systems to image both reflected light and fluorescent light at two spatial scales. These systems can assess both native optical contrast as well as that afforded by optically active contrast agents.

**BIOGRAPHY:** Rebecca Richards-Kortum is the Stanley C. Moore Professor and Chair of Bioengineering at Rice University. In addition, she is a Howard Hughes Medical Institute Professor. Her group is developing miniature microscopes, spectrometers and contrast agents to enable early detection of precancerous changes in living tissue.

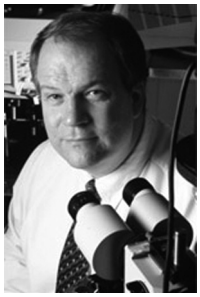
**1:00 to 2:20 pm**

## SESSION 6: Optical Coherence Tomography

*Room: Natcher Auditorium, P2 Level*
*Chair:* **James G. Fujimoto**, Massachusetts Institute of Technology



**2:20 to 3:00 pm** **Coffee Break**





# In vivo coherence-based quantitative imaging from cells to tissues

J. A. Izatt, Duke Univ.

New developments in optical coherence-based imaging techniques including optical coherence tomography (OCT), optical coherence microscopy (OCM), and spectral domain phase microscopy (SDPM) are enabling a new generation of nanometer to micron-scale in vivo measurements of structure, motion, and molecular composition in living cells, tissues, and organisms. As an example, the maturation of technologies for Fourier-domain OCT (FDOCT) are enabling the routine collection of densely sampled volumetric datasets in the anterior and posterior segments of the living eye in clinical settings. We have placed in the clinic spectrometer-based FDOCT retinal scanners which acquire 3D data sets comprising 51Mvoxels, and swept-source-based FDOCT anterior segment scanners which acquire 25Mvoxel datasets, both in single eye-blink intervals of ~5 seconds. These capabilities in turn are driving the development of high-throughput image interpretation and segmentation algorithms for disease-specific applications. We report on the development of quantitative, reproducible diagnostic observables for glaucoma and age-related macular degeneration based on algorithms for semi-automated segmentation of retinal layer thicknesses, drusen volume, and geographic atrophy cross-sectional areas. Reproducible extraction of these parameters may serve as useful observables for tracking disease state that were not accessible without rapid 3D volumetric imaging capability. Separate but related technology advances have enabled the design of highly phase-stable interference microscopes capable of resolving nanometer-scale structures and motions in living cells with millisecond temporal resolution. We are using these new capabilities are being used to probe cellular internal and external surfaces, cytoplasmic flows, and their responses to chemical and mechanical stimuli.

**BIOGRAPHY:** Joseph A. Izatt is Associate Professor of Biomedical Engineering and Ophthalmology, and Director of the Laboratory for Biophotonics at the Fitzpatrick Institute for Photonics at Duke University.



# Fourier domain functional optical coherence tomography

Z. Chen, Univ. of California/Irvine

Recent advance in Fourier domain optical coherence tomography (FD-OCT) significantly improved sensitivity and imaging speed. The advantages of OCT in biomedical imaging, compared to other imaging techniques, are numerous. In particular, OCT can provide imaging resolutions that approach those of conventional histopathology and can be performed in situ and in vivo. Despite its advantages, one limitation of OCT is the relatively low imaging contrast compared to histopathology and optical microscopy since there is no staining of samples. Imaging contrast originates from the inhomogeneities of tissue scattering properties. In many instances, changes in scattering properties between normal and diseased tissue are small and difficult to measure. Functional OCT (F-OCT), including Doppler, polarization, and second harmonic OCT, provides clinically relevant contrast enhancement and allows imaging of tissue morphology and physiology parameters simultaneously.

We report the development of a frequency domain F-OCT (FD-F-OCT) system using sweeping light source that significantly increases the imaging speed and sensitivity over the conventional time-domain system. An innovative system design and image processing algorithm were developed that removed the mirror imaging in FD-OCT. In addition, mirror-image-free FD-OCT also eliminates the autocorrelation noise and increases the signal to noise ratio of the system by more than 20 dB. The Fourier domain Doppler, polarization, and second harmonic OCT system with significant enhanced imaging speed and sensitivity is demonstrated. In addition, we have developed a swept light source with center wavelength of 1.05 μm for ophthalmic applications. A center wavelength at 1.05 μm provides an optimal wavelength for imaging choroidal morphology and microvasculature below the retinal pigment epithelium.

In addition to the system development, we report the development of several endoscopic probes that can take advantages of high speed offered by FD-F-OCT for 3-D imaging. A fiber-optic bundle based OCT probe is demonstrated. This novel OCT imaging approach eliminates any moving parts in the probe and has a primary advantage for use in extremely compact OCT endoscopes to image internal organs. In addition, a 3-D endoscopic OCT system based on a dual axis MEMS mirror was demonstrated. The endoscopic MEMS probe is integrated and tested with a FD-OCT system. The MEMS mirror provides high-speed, high resolution 2-axis scanning for 3-D OCT imaging. Finally, several clinical applications of the FD-F-OCT will be discussed.

**BIOGRAPHY:** Dr. Chen is a full professor of Biomedical Engineering at University of California, Irvine. He received his Ph.D. degree in Applied Physics from Cornell University in 1993. Dr. Chen's research interests encompass the areas of biomedical photonics, microfabrication, biomaterials and biosensors. He is a Fellow of the American Institute of Medical and Biological Engineering.



# Advances in clinical ophthalmic OCT

J. S. Schuman, G. Wollstein, H. Ishikawa, L. E. Kagemann, Jr., M. Gabrielle, Univ. of Pittsburgh; M. Wojtkowski, V. J. Srinivasan, Massachusetts Institute of Technology; J. Ducker, Tufts Univ.; J. G. Fujimoto, Massachusetts Institute of Technology

Introduction: Optical coherence tomography (OCT) is an established ocular imaging technology in ophthalmic evaluation. We compared conventional OCT with a new generation of OCT research technology which provides high-speed and ultra-high resolution images by measuring echo time delay of light using Fourier (spectral) domain detection.

Methods: Healthy subjects and patients in various stages of disease were scanned at the same visit with commercially available OCT (Stratus OCT) and a prototype high-speed, ultra-high resolution OCT (hsUHR-OCT). The hsUHR-OCT used superluminescent diodes (SLDs) as the light source with a wavelength of 840+/-50 μm. The axial resolution of the hsUHR-OCT was 3-4μm (2-3 times greater than Stratus OCT) and the tissue sampling rate was 24,000 Hz (60 times faster than Stratus OCT). The fast acquisition rate enabled acquisition of three dimensional data sets consisting of raster scans of the macula and optic nerve head regions. Each 3D data set had 180 consecutive OCT images with 500 axial scans each, for a total of 90,000 transverse points on the retina.

Results: Marked enhancement of tissue visualization was noted with the hsUHR-OCT which allowed improved discrimination of structural features. Structures such as the retinal ganglion cell layer and photoreceptor layer could be clearly observed. The fast scanning and the enhanced registration properties of HS UHR-OCT provided detailed maps of the scanned regions. In addition, these features allowed us to obtain structural data in any desirable orientation in the scanned area thus minimizing a major cause for inter-scan variability, image registration problems, as observed with Stratus OCT.

Discussion: hsUHR-OCT provides detailed structural information which might improve the clinical utility of this technology for disease detection and longitudinal follow-up of patients with glaucoma. The enhanced properties of this new technology open new avenues for data acquisition and analysis.

Support: NIH Grants RO1-EY013178-6, RO1-EY11289-20, and P30-EY008098, Research to Prevent Blindness and The Eye and Ear Foundation (Pittsburgh), NSF ECS-0119452, AFOSR FA9550-040-1-0046

Proprietary interests: Drs. Fujimoto and Schuman receive royalties from intellectual property licensed by MIT to Carl Zeiss Meditec Inc.

FDA disclosure: Stratus OCT is an FDA approved device. The hsUHR-OCT is a research device and has not been submitted for FDA approval.

**BIOGRAPHY:** Joel S. Schuman, MD, is the Eye and Ear Foundation Professor and Chairman of Ophthalmology, Eye and Ear Institute, University of Pittsburgh School of Medicine, director of UPMC Eye Center, and professor of bioengineering at University of Pittsburgh. He has published more than 150 peer-reviewed scientific journal articles. He received the Alcon Research Institute Award, the New York Academy of Medicine's Lewis Rudin Glaucoma Prize, was elected into the American Society for Clinical Investigation, and received the ARVO/Pfizer Translational Research Award.



# Contrast enhancement techniques for coherent optical imaging

S. A. Boppart, Univ. of Illinois at Urbana-Champaign

Coherent optical imaging techniques are emerging as novel ways to acquire biological data with high spatial, temporal, and spectral resolution. Optical coherence tomography (OCT), second harmonic generation (SHG) microscopy, and coherent anti-Stokes Raman scattering (CARS) microscopy are rapidly finding application in the detection and diagnosis of disease. Coherent imaging techniques such as these can often lack informative contrast, but may also have the adjunct potential to provide a novel means for enhancing contrast in biological and medical images. This presentation will review the advances in contrast enhancement techniques for OCT using both exogenous molecular-contrast agents and coherent methods such as SHG, CARS, and spectroscopic OCT that can enhance contrast based on endogenous ultrastructural and molecular signals. Utilizing coherent ranging and solving the inverse scattering problem for coherence microscopy, a novel image-formation technique called Interferometric Synthetic Aperture Microscopy (ISAM) has been developed. ISAM makes use of the information contained within the focused beam, both inside and outside of the focal region, to produce spatially-invariant resolution and contrast enhancement throughout the illuminated tissue volume. These contrast enhancement methods share a common goal of improving our ability to visualize the features present in biological specimens, and thereby enhance the diagnostic capabilities of optical imaging.

**BIOGRAPHY:** Prof. Boppart received his Ph.D. in 1998 in Medical and Electrical Engineering from MIT, and his M.D. from Harvard Medical School in 2000. Currently, Prof. Boppart is an Associate Professor with appointments in the Departments of Electrical and Computer Engineering, Bioengineering, and Medicine at the University of Illinois at Urbana-Champaign. He is Head of the Biophotonics Imaging Laboratory at the Beckman Institute and is investigating novel coherent optical imaging techniques for detection and diagnosis at the cellular and molecular levels.

**3:00 to 4:40 pm**

## SESSION 7: Optical Devices and Methods in Drug Discovery: Role of Industry

Room: Natcher Auditorium, P2 Level
*Chair:* **Israel Gannot**, The George Washington Univ. and Tel Aviv Univ. (Israel)



**4:40 to 5:30 pm** **Panel Discussion**

### Effective Interaction Among Research Institutions and the Private Sector

**Joe Schmitt,** Lightlab Imaging
**Ken Kaufmann,** Hamamatsu
**Randall Barbour,** SUNY Downstate
**Yiwei (Kevin) Jia,** Olympus America Inc.



# Optical methods in drug discovery and development

M. Analoui, Pfizer Inc.

Imaging technologies are being used in a broad range of applications within drug discovery and development. Imaging approaches are expected to provide structural and functional assessment of chemistry, biology, toxicity, and response to treatment.

Common imaging modalities used in drug development include a spectrum of optical methods, radiography, US, MRI, PET/SPECT. However, utility and maturity of these technologies, especially optical approaches, vary along the continuum of drug discovery, preclinical and clinical safety and efficacy. In this presentation an overview of applications of optical methods in pharmaceutical industry is provided with examples from discovery and development. In addition to diagnostic utility and visualization/monitoring of treatment effect, optical methods can be used for in vitro and in vivo assaying, which will provide unique approaches for real-time monitoring of biological activities and therapeutic effect.

In this presentation, also key clinical challenges and opportunities regarding potential use of optical methods in clinical and in vivo imaging, as well as multimodality imaging will be discussed. These challenges include technical issues, continuum from inception of novel technology to validation, cost effectiveness and risk and benefits.

**BIOGRAPHY:** Mostafa Analoui, Ph.D., is the Senior Director and Site Head for Groton/New London, Global Clinical Technology at Pfizer Global Research and Development in Connecticut. He is also adjunct Professor of Radiology and Oral Pathology, Medicine at Indiana University Schools of Medicine and Dentistry. Dr. Analoui is actively involved in management and scientific/business development of novel biotechnologies and methodologies. Dr. Analoui was previously the Director of Oral and Maxillofacial Imaging Research at Indiana University, and Associate professor of Biomedical Engineering and Electrical & Comp Engineering at Purdue University. In addition to industry leadership in biomedical filed, he lectures nationally and internationally. He has also served on various scientific and business advisory committees. Dr. Analoui has authored over 130 publications, including journal articles, book chapters, and technical reports.



# An optical and multimodal imaging platform for integration, post-processing, and standardization

F. S. Azar, Siemens Corporate Research

The National Cancer Institute is funding the Network for Translational Research in Optical Imaging, and is pushing for efforts from all institutions to pool expertise, validate approaches, forge common instrumentation platforms and rapidly translate new technologies toward clinical trials. The Vision is to develop a standardized Multi-Dimensional Diffuse Optical Imaging technology platform for use in breast imaging as a stand-alone device or in conjunction with MRI and X-ray mammography, and significantly improve breast cancer detection, clinical management and quality of life for breast cancer patients This collaborative environment together with the variety of different experimental technologies poses new challenges for data integration, analysis, comparison and sharing. The objectives of such a consortium will be achieved as institutions and scientists are able to use common data formats and processes for sharing data and software, directly compare results from different modalities and imaging devices, validate new vs. established methods in clinical studies, and develop commonly accepted standards in post-processing methods.

We have developed a software platform for multi-modal integration and visualization of diffuse optical tomography (DOT), diffuse optical spectroscopy (DOS), magnetic resonance imaging (MRI) and x-ray tomosynthesis of breast cancer. The software platform allows multimodality 3D image visualization and manipulation of datasets, and enables quantitative and qualitative analysis of structural and functional diagnostic data. The functional parameters, together with morphological parameters can be suitably combined and correlated to the absolute diagnosis from histopathology. Fusion of the multimodal datasets will eventually lead to a significant improvement in the sensitivity and specificity of breast cancer detection. We will present initial results as well as post-processing and standardization issues in image visualization, fusion and analysis of optical and multimodal breast cancer imaging data.

**BIOGRAPHY:** Dr. Fred S. Azar is the Siemens Co-Director & Technical Lead in the NCI-funded Multi-institutional Network for Translational Research in Optical Imaging (NTROI). Dr. Azar is a member of the Imaging & Visualization Dept. at Siemens Corporate Research (Princeton, NJ), where his current interests include interventional imaging, optical imaging, and 3D deformable modeling of soft tissue.



# The evolving role of optical imaging in clinical practice—a personal view

F. P. Jansen, GE Global Research

For many years, optical imaging has had the promise of making an impact on medical care, but largely this promise has yet to be fulfilled. This talk will start with a discussion of the strengths of optical imaging, and the unique role that it could play in modern medicine. Next I will give a brief survey of work that has been done on a laboratory scale, especially in the area of diffuse optical tomography, and why it has been difficult to translate this work to clinical practice. This will be followed with a description of specific activities at the GE Research Center, where we have a broad optical imaging program to develop a deeper understanding of both instrumentation and biology of optical imaging agents. The applications we pursue will in the next few years allow real-time visualization of anatomy and physiology in near-surface applications; at the same time we are developing techniques to increase the sensitivity and depth of penetration of optical signals to enable a broader range of applications. In particular I will highlight our collaboration with the Dr John Frangioni and his team at BIDMC to develop a practical instrument to be used during surgery. In the final section of the talk I return to the potential of optical imaging, with a vision of the role it may play; this will include some thoughts on the steps that need to be taken by the research community in order to achieve this vision.

**BIOGRAPHY:** Dr Jansen received a PhD in Physics from Cambridge University. Since joining GE in 1990 he has developed medical imaging technology in Nuclear Medicine, PET, X-ray tubes, and optical imaging, resulting in 10 US patents. He now manages the Functional Imaging Laboratory for GE Research in Niskayuna, NY.



# Opportunities and challenges of imaging in translational medicine

T. Krucker, Novartis Institutes for Biomedical Research

Advances in genomics and proteomics have produced an enormous number of potential drug targets and increased understanding of disease processes. Because of its high translational capacity, non-invasive imaging in preclinical settings has become essential to the validation of new drug targets. Increasing availability of new imaging technologies that combine modalities allows for more thorough phenotyping of disease models - including the establishment of morphological and functional biomarkers for disease stratification. However, the identification, validation and, particularly, the translation of such biomarkers into the clinical setting pose special challenges, including the choice of appropriate imaging modalities. Design and integration of imaging strategies ideally occur early in the drug development cycle allowing lead time for implementation of new imaging paradigms and if necessary the development of suitable probes and contrast agents. The use of optical and probe technologies in the context of drug discovery and their translation to clinical trials present both challenges and opportunities.

**BIOGRAPHY:** Thomas Krucker joined the Novartis Institutes for BioMedical Research in 2005 to head the molecular imaging efforts. He was previously at The Scripps Research Institute in La Jolla (CA) where he remains an Adjunct Assistant Professor at the Molecular and Integrative Neuroscience Department. Dr. Krucker obtained his Ph.D. in Neurobiology from the University of Zürich in Switzerland.



# Optical imaging in oncology drug discovery

C. Sur, Merck and Co., Inc.

Two current challenges in drug discovery are to expedite the in vivo evaluation of an increasing number of in vitro selected molecules and to develop animal models more predictive of therapeutic efficacy. Optical imaging technologies such as continuous wave, time domain and fluorescence molecular tomography provide significant opportunities to address these challenges. We have integrated these technologies in our drug development process and examples of how we used them together with genetically encoded probes to develop and refine animal tumor models and evaluate anticancer drugs will be presented. The development of novel exogenous optical probes is also critical to expedite the analysis of a growing number of transgenic mouse models of cancer and to evaluate the effect of novel therapeutic molecules on various cell physiology parameters. We will present examples from our evaluation of novel near-infrared probes, ProSenseTM and AngioSenseTM for the detection of tumor development in xenograft and metastatic mouse tumor models. Finally, we will discuss how optical imaging technologies could be linked with translational imaging technologies during preclinical development to help bridge the gap between preclinical and clinical studies.

**BIOGRAPHY:** Cyrille Sur obtained his PhD in Neurobiology at Pasteur Institute in Paris. After completing his post-doctorate in neuropharmacology at the Max-Planck Institute in Frankfurt, he joined Merck's Neuroscience Research Center. Since joining Merck, he has occupied positions of increasing responsibility and led several drug discovery programs in schizophrenia. In 2004, he moved to the imaging research department where he is leading the tracer biology and optical imaging groups to support drug discovery and development across key therapeutic areas.

**7:00 am** **Continental Breakfast**
Room: Natcher Auditorium Foyer

**8:00 to 10:00 am** **SESSION 8: Optics in Neuroscience**
*Room: Natcher Auditorium, P2 Level*
*Chair:* **Elizabeth M. C. Hillman**, Columbia Univ.



**10:00 to 10:30 am** **Coffee Break**

**10:30 to 11:15 am** **Panel Discussion**

### Optics in Neuroscience

*Chair:* **Elizabeth M. C. Hillman**, Columbia Univ.

Participants: **Brian Bacskai,** Massachusetts General Hospital
**Joe Culver,** Washington Univ.
**Randall Barbour,** SUNY Downstate
**Ellen Grant,** Massachusetts General Hospital
**Mamoru Tamura,** Hokkaido Univ. (Japan)
**Maria-Angela Franceschini,** Massachusetts General Hospital;
**Yutaka Yamashita,** Hamamatsu



# Structural imaging of the Alzheimer brain with multiphoton microscopy: effects of therapeutic interventions

B. J. Bacskai, Massachusetts General Hospital

Multiphoton microscopy allows imaging of structure and function in the brains of living animals. Transgenic mouse models have been created that overexpress the amyloid precursor protein

(APP) and develop some of the hallmark pathology of Alzheimer's disease. Crossing these animals with YFP expressing mice or viral gene transfer of GFP allows imaging of individual neurons expressing fluorescent protein. Neurites are curved and distorted in the vicinity of senile plaques in human and mouse brain, and this altered structure may be a source of altered neuronal function. Serial imaging of the senile plaques and neuritic structure in these animals permits the evaluation of therapeutics aimed at clearing Abeta with the concomitant effects on cell morphology. Three different approaches were used: immunotherapy, anti-oxidant therapy, and inhibition of APP processing with a gamma secretase inhibitor. Only immunotherapy led to detectable clearance of existing senile plaques, while both immunotherapy and anti-oxidant treatment resulted in some degree of restoration to the altered neurite morphology in the immediate vicinity of amyloid deposits. The secretase inhibitor significantly reduced the levels of amyloid beta production but did not reduce the size of existing plaques nor did it affect distorted neurites. Multiphoton imaging in the Alzheimer mouse brain in vivo allows the characterization of the efficacy of therapeutics, and may help guide development of clinical treatments.

**BIOGRAPHY:** Dr. Bacskai earned his doctorate at Dartmouth College with a degree in Biomedical Engineering. A postdoctoral fellowship at the University of California San Diego focused his interests in the field of neuroscience. He is currently an Assistant Professor at the MassGeneral Institute for Neurodegenerative Disease and Harvard Medical School.



# High resolution functional optical imaging of rodent cortex: investigating mechanisms of functional activation

E. M. C. Hillman, Columbia Univ.

Understanding the way that the brain responds to stimulus is key to interpreting the effects of pathologies and pharmacology. It is also important to make connections between results from clinical imaging modalities such as functional Magnetic Resonance Imaging (fMRI) and basic research at the level of neurons and single blood vessels.

We have developed and applied a suite of optical imaging technologies to study the cortical response to somatosensory stimulus in-vivo. These technologies include 2D and 3D multi-spectral exposed-cortex optical imaging of hemoglobin and voltage and calcium sensitive dyes, providing visualization of both hemodynamic and neuronal responses to stimulus. In-vivo two-photon microscopy of hemodynamics and neuronal calcium dynamics has also been performed during functional stimulus using a custom-built system. This system rapidly acquires full-frame images, allowing blood flow, vessel diameter and local neuronal activity to be observed simultaneously, on a cellular level, in the living brain. Additionally, we have performed simultaneous 2D optical imaging and fMRI of the exposed cortex during somatosensory stimulus using an endoscope-based system. This has allowed us to connect our optical findings to their manifestation in the form of the blood oxygen level dependent (BOLD) signal.

Novel aspects these optical imaging technologies will be described alongside our findings relating to neurovascular coupling and BOLD / optical correlations. Contributors to this work include: Anna Devor, Matthew Bouchard, Alex DeCrespigny, Svetlana Ruvinskaya and David A. Boas at the Martinos Center for Biomedical Imaging, Massachusetts General Hospital and Andrew K Dunn at University of Texas at Austin.

**BIOGRAPHY:** Elizabeth Hillman is Assistant Professor of Biomedical Engineering and Radiology at Columbia University. Her group develops and applies novel optical techniques for functional imaging of living tissues. She previously worked at the Massachusetts General Hospital Martinos Center, and obtained her PhD in Medical Physics and Bioengineering at University College London.

[36] Session 8

# Human brain mapping with high-density diffuse optical tomography

J. P. Culver, Washington Univ.

Diffuse optical tomography (DOT) is an emerging imaging technique for mapping functional activity in the human brain. This methodology is well-suited for several novel situations including studies of human child development that would benefit from enriched ecological environments for a wider range of behavioral paradigms. However, successful DOT in humans is challenging due to the concurrent requirements for high dynamic range, low crosstalk, high channel counts, and sufficient temporal resolution. Hence, most optical imaging of human brain activity is performed using NIRS topography, which employs relatively sparse imaging arrays that use only nearest-neighbor optode pairs. Tomographic approaches offer benefits including volumetric localization and better discrimination of the functional signals from the background that are crucial to establishing DOT as a standard brain-mapping tool. We have developed a new DOT instrumentation with improved performance characteristics that permits use of high-density DOT arrays. Current studies in the adult visual cortex demonstrate the capability to distinguish activation sites separated by ~ 1cm. Our goal is to develop DOT for mapping activity through out the outer surface of the brain with sub-centimeter resolution.

**BIOGRAPHY:** Dr. Culver obtained a Ph. D. in physics (1997) and performed Post doctoral research (1998-2001) at the University of Pennsylvania. In 2001, he became an Instructor at Massachusetts General Hospital, and in 2003 he became an Assistant Professor in the Department of Radiology at Washington University in St. Louis.



# Functional DOT imaging: technology, calibration and new findings

R. L. Barbour, SUNY/Downstate Medical Ctr. and NIRx Medical Technologies

Common to all imaging modalities is the need for a versatile measuring platform, a comprehensive problem solving software environment and calibrating phantoms that serve to quantify system performance and its limitations. Here I will review our latest approaches to meeting these needs for functional NIRS imaging. Building on our approach to time-series imaging, I will present our latest technology integration efforts to provide for multisite monitoring using dense data-collection arrays and provide examples of its clinical utility for monitoring of breast pathology. I will also introduce our approach to software design that includes an automated file management system which, together with portals for image generation, display and analysis, provides for efficient processing of large scale data sets. Also new will be a description of our use of dynamic calibrating phantoms based on principles of electrochromic technology. Shown are results demonstrating that these phantoms can be programmed to mimic any degree of hemodynamic complexity with high fidelity and repeatability.

**BIOGRAPHY:** Dr. Randall L. Barbour, originally trained as a biochemist, for the past twenty years, Dr. Barbour has pioneered a spectrum of imaging capabilities based on near infrared optical technologies. In recent years, he has overseen a broad spectrum commercialization effort to provide for versatile imaging systems that are compact, robust and economical.



# The role of quantitative frequency domain near-infrared spectroscopy in the NICU

E. Grant, Massachusetts General Hospital

Purpose: To determine if quantitative FD-NIRS could detect differences in cerebral oxygen saturation (StO2) and cerebral blood volume (CBV) in compromised neonates in the NICU compared to normal neonates.

Method: The probe consisted of 4 detectors at 1.0, 1.5, 2.0 and 2.5 cm with a single emitter cycling 8 wavelengths (635, 670, 691, 752, 782, 811 and 831 nm). Eight regions of each neonatal brain were sampled for 8 seconds. Data that did not meet criteria for high quality (such as P values >0.05 for hemoglobin spectral fit of measured absorption coefficients) were discarded. Regional StO2 and CBV were determined. Results of 25 NICU neonates were compared to normal controls. Gestational age at time of FD-NIRS ranged from 29.6 to 49.1 weeks.

Results: Most compromised neonates in the NICU had abnormal StO2 and/or CBV values. Those with lung disease tended to have lower cerebral StO2 values. In some cases abnormal hemoglobin levels and peripheral pCO2 levels altered CBV values. Neonates on ECMO underwent rapid shifts in CBV and StO2 not seen in normals with values often outside the normal range. Neonates with acute global hypoxic ischemic brain injury had marked increases in StO2 and normal to markedly elevated CBV. When measured in the subacute phase, StO2 and CBV were low and continued to decrease over weeks. Focal brain injuries resulted in regional changes in CBV and StO2.

Impression: Quantitative FD-NIRS detects deviations from normal in many NICU babies and has the potential to be a bedside monitor of cerebral health.

**BIOGRAPHY:** Dr. P. Ellen Grant is Chief of Pediatric Radiology at Massachusetts General Hospital. Dr. Grant specializes in Pediatric Neuroradiology and has a special interest in neonatal brain injury and early brain development.



# Optical molecular imaging: from single molecule to human body

M. Tamura, Hokkaido Univ. (Japan)

The recent advances of various optical technology have opened the new field, Optical Molecular Imaging, OMI which followed the completion of human genome project. The OMI covers extremely wide ranges of the space and time; those are from nm to cm, picosecond to second, and single molecule to moles, respectively. Technically, microscope, endscope, small animal imaging and brest or brain imaging by diffuse optical tomography (DOT) are the typical representatives of these widespread targets.

We are working with the microscope technology of fluorescence correlation spectroscopy (FCS) and cross correlation spectroscopy (FCCS) for persuing the dynamic behavior of fluorescent labeled proteins inside the live cells. By the combined use of several isolated perfused organs such as liver and brain, we could detect the single molecule in the cell of living tissue in vivo. The human applications of micro-endoscope for human tissues such as esophagus and colon are giving the optical diagnosis for several cancers in the clinical field.

Finally the applications of near-infrared time-resolved spectroscopy and imaging can give us the space-resolved quantitative maps of tissue oxygenation state and blood content in the human tissues such as the breast and brains.

We will introduce the above recent topics carried out in our laboratory.

**BIOGRAPHY:** Prof. Mamoru Tamura, 1966—Graduated Department of Chemistry, Faculty of Science, Hokkaido University. 1968—M. S. from Graduate School of Science Hokkaido University. 1968—Graduated School of Bioengineering, Faculty of Basic. 1970—Engineering, Osaka University. 1971—Ph.D from School of Science Hokkaido University. 1971—Post Doctoral Fellow, Department of Biochemistry and Biophysics, School of Medicine, University of Pennsylvania, U.S.A. 1974—Research Assistant, Research Institute of Industrial Research, Osaka University. 1978—Associate Professor, Research Institute of Applied Electricity, Hokkaido University. 1988—Professor, Research Institute of Applied Electricity, Hokkaido University. 1992—Professor, Research Institute for Electronic Science, Hokkaido University. 2006—Professor, Faculty of Advanced Life Science, Hokkaido University.

**11:15 am to 1:15 pm** **SESSION 9: New Optical Devices and Methods**

*Room: Natcher Auditorium, P2 Level*
*Chair:* **Jay R. Knutson,** National Institutes of Health



**1:15 to 2:15 pm** **Lunch Break**



# Non-invasive sensing of glucose and hemoglobin

H. Primack, OrSense Ltd. (Israel)

There is a growing need and interest in non-invasive devices that measure the level of analytes such as hemoglobin and glucose, especially for continual monitoring. A suitable technology platform for such non-invasive, continual measurements is the Red-Near Infrared Occlusion Spectroscopy. It utilizes an opto-pneumatic probe located at the finger's root that generates an optical signal with strong dynamics while occluding the blood flow (NBM-100 device, OrSense Ltd., Israel). The strong signal enables the detection of a clean transmission signal, which is beneficial sensitivity-wise due to the long pathlengths of the photons. Also, it avoids local effects and better represents the features of the bulk with respect to purely reflection methods. The strong dynamics enables AC/DC analysis that filters unnecessary factors that appear in the more conventional DC analysis. Physically, the optical signal is influenced by absorption changes resulting mainly from hemoglobin and scattering changes induced by glucose. The signal is processed using advanced algorithms to extract analyte concentrations. We use analytical models, in-vitro and Monte-Carlo simulations and in-vivo analysis to facilitate accurate analyte extraction. Clinical trials of glucose monitoring show useful correspondence with standard references. Results are obtained both in prospective and in profiling modes. The clinical utility of the NBM-100 device has also been demonstrated for anemia screening, in which hemoglobin values were measured by the NBM-100 and then compared to standard invasive measurements. When compared to capillary-blood invasive measurements, similar performance is obtained. This substantiates the clinical utility of the NBM-100 and the underlying technology.

**BIOGRAPHY:** Dr. Harel Primack, Ph.D. in Physics from the Weizmann Institute, Rehovot, Israel. Post-doc at Freiburg University, Freiburg, Germany. Chief Scientist, Netmor Ltd. (Israel). Currently Director of Research, OrSense Ltd. (Israel).



# Novel small animal imaging system and intravital laser scanning microscope help observing cellular to whole animal fluorescence images

Y. Kawano, N. Onda, I. Sakai, K. Kojima, Olympus Corp. (Japan)

We provide a full range of comprehensive approaches to macro-to-micro level optical imaging of living whole animals, organs, tissues and cells. Today, we introduce the animal imaging using Intravital Laser Scanning Microscope (IV100) and Small Animal Imaging System (OV100). These apparatus help increasing the study for the in vivo cell biology. We show some examples of low invasive and invasive images.

The OV100 Small Animal Imaging System is a complete, closed-chamber system for high-sensitivity, high-speed imaging of small laboratory animals from macro-to-micro levels of observation. The system features a wide 16X to 0.14X (a 114:1 zoom ratio change) magnification range for seamless imaging of the entire body down to the single cell level without disturbing animals. High-resolution fluorescence imaging can be correlated with other techniques. The system is also suitable for long-duration time-lapse observations and supports a wide range of image processing functions and other post-imaging analysis techniques. The system has four par-centric, par-focal objectives mounted on an automated turret and includes a zoom lens with a magnification range of 1.6X to 16X.

The IV100 Intravital Laser Scanning Microscope has been optimized for collecting light from deeper area of tissues. Exceptionally high spatial, temporal and multi-wavelength resolution combined with novel near-IR imaging probes realize imaging of sub-cellular detail of internal organs and disease processes.

The MicroProbe lens allows minimally invasive intravital research imaging. The system features ultra-slim MicroProbe lenses for high-resolution laser-scanned imaging of tissues and cells inside body cavity of small laboratory mammals. We made three MicroProbe lenses, including minimal diameter of 1.3mm and 3.5 mm, along with the regular microscope objectives. With a MicroProbe lens and the IV100's flexible observation angles, organs can be observed deep inside the body through minimally invasive keyhole surgery.

**BIOGRAPHY:** Yoshihiro Kawano, 1985: Bachelor degree from Dep. of Electro Photo Optics, Tokai Univ., Japan. 1985 - 1995: Optical Designer, Microscope Optical R&D in Olympus Optical Co. Designed Gradient Index Objective Lens, Phase Contrast Microscope and Inverted Microscope. 1995 - 2000: Senior Engineer, Scientific Equipment Division in Olympus America, Inc.. 1999: Y. Kawano and RG. Enders. Total internal reflection fluorescence microscopy. Application Note, 1999 December:28-30. 2000: High-numerical-aperture objective lenses and optical system improved objective type total internal reflection fluorescence microscopy. Proc. SPIE Vol. 4098, p. 142-151, 09/2000. 2000: Macro optical system for biological application. Pro. SPIE Vol. 4093, p. 288-296, 10/2000. 2000 - 2002: Team reader for Microscope Optical R&D Developed Inverted Microscope, DMD Microscope. 2003: Senior Engineer, Scientific Equipment Group in Olympus America, Inc. 2004- Now: Manager for Bio Business Department.



# Optical fluorescence imaging of breast cancer

N. van der Vaart, L. Bakker, M. B. van der Mark, M. van Beek, M. van der Voort, W. H. Rensen, R. Harbers, Philips Research Labs. (Netherlands); T. Nielsen, T. Köhler, R. Ziegler, Philips Research Labs. (Germany); A. Ziegler, Philips GmbH (Germany); B. Brendel, Philips Research Labs. (Germany); A. Feuerabend, Philips GmbH (Germany); J. P. Meeuwse, Philips Applied Technologies; D. van Pijkeren, S. Deckers, Philips Medical Systems; K. Licha, M. Pessel, Schering AG (Germany)

We have developed a novel system for imaging of breast cancer that exploits the unique combination of our diffuse optical tomography scanner and fluorescent contrast agent. This patient-friendly set-up does not require breast compression or the use of harmful radiation. Breast imaging is achieved with an attenuation scan at four different wavelengths as well as with a single fluorescence scan. We are currently preparing for a clinical trial to further study the diagnostic efficacy of our method. This work is the first application within the framework of the Philips-Schering collaboration on optical imaging. We discuss the technology, the proof-of principle results obtained in phantoms studies as well as the application and impact of the technology on the breast cancer care cycle.

**BIOGRAPHY:** Nijs van der Vaart obtained his M.Sc. degree in physics in 1991 from the Technical University of Delft in The Netherlands and four years later he obtained his Ph.D. in solid-state physics from the same university. In 1995, he joined Philips Research to work on displays and photonic devices. Since 2004, he is department head of the group Biomedical Photonics in Eindhoven, The Netherlands.

[43] Session 9

# Current tools for in vivo imaging

L. Greenfield, Invitrogen

Non-invasive in vivo optical imaging is a highly sensitive method that has the potential for combining longitudinal, morphological and functional information in live animals. We are applying our extensive tools developed for cellular imaging, flow cytometry, and high content assays (fluorophores, fluorescent microsphere and quantum dot technologies) to advance the capabilities of in vivo imaging.

**BIOGRAPHY:** Dr. Lawrence Greenfield received his BA in Zoology, PhD in Molecular Biology and MD from UCLA. He also was a Fellow in the UCLA Department of Pathology. He was a Senior Scientist at Cetus (1982-1991), a Senior Research Investigator at Roche Molecular Systems (1991-1998) and a Director at Applied Biosystems (1998 - 2003). He has been at Molecular Probes/Invitrogen from 2003, where he currently is a Technology Area Manager for Animal Imaging.



# New methodology of optical blood glucose monitoring based on simulation of light propagation in the skin

Y. Yamada, Univ. of Electro-Communications (Japan); K. Maruo, Matsushita Electric Works, Ltd. (Japan); H. Arimoto, National Institute of Advanced Industrial Science and Technology (Japan); M. Tamura, Hokkaido Univ. (Japan);
Y. Ozaki, Kwansei Gakuin (Japan)

Conventional methods of noninvasive blood glucose monitoring using optical technology are based on pre-experiments of oral glucose tests to build calibration models by a multivariate analysis using the measured reflectance spectra from the skin and the simultaneously measured blood glucose levels by finger pricking. The calibration models built by the conventional methods often accompany the chance temporal correlation because the change in the reflectance spectrum with the change in the blood glucose level is extremely small and greatly affected by the changes in other unknown factors in the skin. In order to avoid the chance temporal correlation a huge number of pre-experiment must be performed to collect many data covering wide ranges of changes in the unknown factors. However, it is almost impossible to obtain sufficient data from pre-experiments, and this leads to failures of the conventional methods. Our approach is to build calibration models using the reflectance spectra generated by simulation of light propagation in the skin. A Monte Carlo simulation of light propagation incorporates the changes in the affecting factors including the glucose concentration, water content, etc. in the skin consisting of three layers. The changes in the affecting factors lead to the changes in the optical properties (in the near-infrared wavelength range) necessary in the simulation. The calibration models based on the simulated reflectance spectra have partly succeeded in predicting the blood glucose levels with accuracy sufficient for clinical use. Using our approach, there is no need of pre-experiment for building calibration models.

**BIOGRAPHY:** Prof. Yukio Yamada, 1970: Graduated from Tokyo Institute of Technology. 1974: Researcher at Mechanical Engineering Laboratory of the Japanese government. 1983-84: Visiting researcher at University of California at Berkeley. 2001: Professor at University of Electro-Communications, Tokyo.

# Quantitative monitoring and imaging using near-infrared time-resolved spectroscopy

Y. Yamashita, Hamamatsu Photonics K.K. (Japan)

No abstract available

**2:15 to 3:05 pm**

## SESSION 10: Forum on NIH Support for Optical Imaging

*Room: Natcher Auditorium, P2 Level*

**2:15 pm** **[45]** **Funding opportunities at the National Cancer Institute (NCI)**
**H. R. Baker,** National Cancer Institute

**2:25 pm** **[46]** **Funding opportunities at the National Institute for Biomedical Imaging and Bioengineering (NIBIB)**
**Y. Zhang,** National Institutes of Health

**2:35 pm** **[47]** **Funding opportunities at the National Center for Research Resources (NCRR)**
**G. Farber,** National Institutes of Health

**2:45 pm** **[48]** **Funding opportunities at the National Institute of Neurological Disorders and Stroke (NINDS)**
**J. Pancrazio,** National Institutes of Health

**2:55 pm** **[49]** **Funding opportunities at the National Institute of Dental and Craniofacial Research (NIDCR)**

**3:05 pm** **Question and Answer Session**
*Room: Natcher Auditorium, P2 Level*

**3:30 pm** **Adjourn**

# Poster Presentations

## Monday 25 September

*This session is held in honor of Brian C. Wilson in honor of his contribution to the translation of optical technologies from blackboard, to benchtop, to bedside.*

*Posters in this session will be on display from 10:00 Monday morning in the Natcher Atrium. A poster session, with authors present at their posters, will be held Monday from 5:00-7:00 pm. Light refreshments will be served. Attendees are requested to wear their conference badges.*

# Imaging fiber bundle lens with binary optical surface

D. Li, L. Hou, G. Zhou, Infrared Optical Fibers & Sensors Institute of Yanshan Univ. (China)

In this paper, the optical system for imaging fiber bundle is to be designed, whose operating wavelength, angle of view respectively is 0.4~0.7µm, 60°. The sensitive area of CCD is 4.9¡Á3.6mm(catercorner dimension 6mm)¡£The section diameter and single fiber core diameter of fiber bundle is §¶6mm, 16_Ìm.

To make all incident light rays project from fiber bundles, the objective lens should be designed with telecentric structure in the image space. The initial structure of objective lens with telecentric structure in the image space is composed of two elements, whose distribution of power is negative-positive. The objective lens is performed , which is made up of seven lenses, three of them is aspheric lens. The focal length, entrance pupil diameter, field respectively is 5mm,1.3mm, 60°. The MTF value of all field of view on 34lp/mm is more than 0.85, image quality of lens is excellence. The specifications are shown. Apparently, the objective lens is small in size, light in weight.

The coupling lens is designed with telecentric structure in the object space, which can meet the condition that incident beam is matched to project beam, whose operating wavelength, focal length, object height respectively is 0.4~0.7µm, 27mm, 6mm. The result of design is presented with optimization of optical design software ZEMAX. The layout of coupling lens, the phase and line frequency vs.aperture of the binary surface is respectively shown. The comparison of refractive lens and the hybrid refractive-diffractive lens on apparent parameters, image quality is also shown at last.

According to the lens simulation results of ZEMAX software, actual imaging experiment, the objective lens and the hybrid refractived-diffractive coupling lens have high imaging quality, light weight and compact structure, which means it can be both applied in national defence and other research fields .

# Laser-induced photoacoustic imaging: a tool for real time in vitro identification of human breast cancer

Y. H. Elsharkawy, Cairo Univ. (Egypt)

Laser Induced photoacoustic response can be used to measure trace element concentration in solids, liquids and gases, with spatial resolution and absolute quantification being feasible, down to parts-per-million concentration levels. We present results of interferometric surface monitoring by which we measure physical properties of human breast sample. A Q-switch excimer laser operating at 193 nm was used as the pulsed excitation source. The surface distribution of the photoacoustic signal was mapped by charge coupled device (CCD) camera across the reflected output of beam of a Michelson interferometer. The depth profile of a 150μm thick human breast samples was imaged using CCD camera and tumor are localized in micrometer scale.

Keywords: Photoacoustic imaging, Characterizing, Breast, Tumor detection.

# Optical investigation of dynamics of laser-based lithotripsy

J. O. Kokaj, M. A. Marafi, Kuwait Univ. (Kuwait)

Phenomena occurring during the laser lithotrpsy are studied using optical imaging, fluorescent and acoustic techniques. In a civet filled with water representing the liquid in a gall-bladder, a stone is immersed. The gall bladder stone that was previously extracted by an operation from the human is held by a basket immersed in water.

A fiber connected to Ho-YAG laser is fixed, using a precise mechanism, very close to the surface of the stone. Using a high-speed imaging technique the action of the laser pulse is studied. This experiment enables one to understand the dynamics and mechanism of laser lithotripsy, which so far is not fully understood. The laser pulsed power fired very close to the surface of the stone will be transmitted through the liquid to the surface of the stone. Thermal energy and mechanical energy concentrated at a very small space will be transmitted to the surface of the stone and in the fluid around it.

The aim of optical imaging technique proposed here is to get a better insight to the flow of energy between the tip of the fiber and the stone, and dissipated energy in the liquid around the stone. Beside the thermal energy, which is relevant for destruction of the stone, a bubble is generated due to the concentrated energy of the laser pulse in the small space and at a very short time. After a short expansion the bulb will collapse generating a rapid pressure and shock waves that will spread up in fluid. Is expected that a huge pressure to be generated. In our experiment a hydrophone was immersed in the fluid at the vicinity of the stone. The measurement of the optical and acoustic signal will be synchronized with the Ho-YAG laser pulse used for destruction. The simultaneously obtained signals by hydrophone and high-speed imaging enable one to measure the dissipated energy. By comparing it with primary introduced energy by the laser pulse one could estimate the energy introduced to the stone for its destruction.

Using imaging technique proposed here the efficiency of destruction associated with the position of the tip of the fiber and its distance from the stone is studied. This leads to a better technique for laser lithotripsy.

# Simulating the gain flattening filter for EDFA

A. Khare, A. Khare, Government Engineering College Bhopal (India)

In this paper we report the experimental study of gain of telecommunication Erbium Doped Fiber Amplifier (EDFA). For particular pump power, the gain of Erbium Doped Fiber Amplifier for the wavelength range of 1529 nm _ 1559 nm was measured and found that the gain of the Erbium Doped Fiber Amplifier is very uneven exhibiting peaks with different widths around 1532 nm and 1550 nm . On analysis of the results, we have simulated the filter of desired characteristic which could support for flattening the gain of EDFA for fixed pump power, so that the EDFA could be used for WDM applications .We have flattened the gain spectrum of a commercial Erbium-doped fiber amplifier, obtaining a curve with approximately + 1.05dB of ripple, from 1530.1 nm to 1557 nm, using five bragg gratings in equalizing optical filter.

# Moving target detection through omnidirectional vision fixed on AGV

S. Y. Yang, Tianjin Univ. of Technology (China)

Extremely wide view of the omni-vision performs highly advanced for the vehicle navigation and target detection. However moving targets detection through omni-vision fixed on AGV involves more complex environments, where both targets and vehicle are in the moving condition. The moving targets will be detected in a moving background. After analyzing the character on omnidirectional vision and image, we propose to use the estimation in optical flow fields, Gabor filter, and clustering statistics over optical flow fields for detecting moving objects. Because polar angle and polar radius R of polar coordinates are changed with the targets moving, we improve optical flow approach which can be calculated based on the polar coordinates at the omnidirectional center. We construct Gabor filter banks which have 12 directions every 15°,and filter optical flow fields at vertical motion direction. By clustering statistics and comparing to moving vectors from the 24 directions the moving targets_optical flow fields could be recognized. Experiment results demonstrate that the proposed approach is feasible and effective.

# Development of near-infrared fluorescent probes for nitric oxide and zinc ion

H. Kojima, K. Kiyose, T. Nagano, The Univ. of Tokyo (Japan)

Cyanine dyes have been widely used in various fields, and have been employed as fluorescent labels in fluorescence imaging studies of biological mechanisms. In particular, tricarbocyanines have the advantage that light at their emission and absorption maxima in the near-infrared (NIR) region around 650-900 nm is relatively poorly absorbed by biomolecules, and so can penetrate deeply into tissues. There is also less autofluorescence in this region. In addition to cyanine dyes for straightforward fluorescence labeling, we successfully developed cyanine dyes whose fluorescence intensity changes upon specific reaction with nitric oxide. The mechanism of fluorescence modulation, however, involves photoinduced electron transfer, and consequently imaging with these dyes is influenced by the dye concentration, cellular environment (pH, hydrophobicity), and photobleaching. To overcome these limitations, ratiometric fluorescent sensors are preferred.

We synthesized a series of amine-substituted tricarbocyanines in order to examine the correlation between the electron-donating ability of the amine and the fluorescence peak wavelength. We found that changing the electron-donating ability of the amine substituent altered the absorption and emission wavelengths. Then, we synthesized dipicolylcyanine (DIPCY), consisting of tricarbocyanine as a fluorophore and dipicolylethylenediamine as a heavy metal chelator, and investigated its response to various heavy metal ions. Upon addition of zinc ion, a red shift of the absorbance maximum was observed. Namely, DIPCY can work as a ratiometric fluorescent sensor for zinc ion in the NIR region.

This fluorescence modulation of amine-substituted tricarbocyanines should be applicable to dual-wavelength measurement of various biomolecules or enzyme activities.

# Comparative splice loss analysis of dispersion-shifted and dispersion-flattened single-mode fibers

C. M. Jadhao, G.S. College of Khamgaon (India); D. Dhote, Brijlal Biyani Science College of Amravati (India)

The Wavelength of zero first order chromatic dispersion can be shifted to the lowest loss wavelength for silicon fibers at 1.55 mm to provide both low dispersion and low loss fiber. Dispersion-Shifted Single Mode Fiber achieves this. However, the design flexibility required to obtain particular dispersion, attenuation, and MFD, bend and Splice Loss characteristics has resulted in specific profile. An alternative modification of the dispersion characteristics of Single Mode Fiber involves the achievement of low dispersion window over the low loss wavelength region and allow flexible WDM i.e. Dispersion-Flattened Single Mode Fiber. The comparison of Splice Loss sensitivities of Dispersion-Shifted and Dispersion-Flattened Single Mode Fiber is analyzed. Since Splices are highly tolerant for longitudinal separation, transverse offset and angular tilt are considered. The comparative analysis shows that these losses are steady in Dispersion-Flattened Single Mode Fibers

# Non-invasive optical imaging of lung metastasis and response to cancer therapy

W. Yared, VisEn Medical, Inc.; Wael Yared, Sylvie Kossodo, Kristine Vasquez, Steve Louie, Andrew Wilson, Jeffrey D. Peterson, VisEn Medical, Inc., Woburn MA

Mouse models of cancer metastasis are expensive, time-intensive, and impractical for large scale drug screening, relying solely on ex vivo histologic analysis for the assessment of tumor burden. The aim of this study was to establish robust measures of breast cancer lung metastasis using optical imaging of living animals to quantify disease progression and therapeutic response.

BALB/c mice were injected IV with 5x105 4T1 mouse breast adenocarcinoma cells, which leads to aggressive tumor cell metastasis and growth in the lung within two weeks. ProSenseTM750, a cathepsin-cleavable near infrared probe, was injected IV to quantify the protease activity associated with aggressive breast cancer growth, and this fluorescence was assessed using our novel optical Fluorescence Molecular Tomography (FMT) system. FMT showed a consistent and significant increase in mean fluorescent signal (113 + 44 vs 8 + 1; p=0.0006) in mouse lungs with metastases as compared to controls. Doxycycline (15 mg/kg/day, IP) treatment of mice to broadly inhibit matrix metalloproteases, known to be important in breast cancer metastasis, significantly reduced the probe activation within the lung as evidenced by FMT (17 + 4 mean fluorescence vs 113 + 43; p=0.0036). Similarly, 5-Fluorouracil (5-FU) treatment (35 mg/kg/day for 5 days), used clinically for breast cancer, in combination with 2_-deoxyinosine (2-DI) at 3.2 g/kg/day, also significantly reduced lung fluorescence (12 + 2 mean fluorescence vs 113 + 43; p=0.0023). The quantitation of ProSenseTM750 fluorescence corresponded to the tumor burden as assessed by both visual examination and histological analysis of the lungs.

These data clearly demonstrate that deep tissue metastatic growth and response to chemotherapy can be monitored in vivo in real time with a near infrared probe and FMT.

# Molecular snapshots of intra- and extracellular oxygen levels in biological systems

D. Sud, G. Mehta, K. Mehta, J. Linderman, S. Takayama, Univ. of Michigan; D. G. Beer, Univ. of Michigan Medical School; M. Mycek, Univ. of Michigan

Quantitative molecular imaging of oxygen is of interest at the intracellular and extracellular (tissue) levels. Here, a fluorescence lifetime calibration method for an oxygen-sensitive ruthenium dye is reported and applied to oxygen imaging. Fluorescence lifetime imaging microscopy (FLIM) bases image contrast on fluorophore excited state lifetimes, which reflect local biochemistry. Unique attributes of the wide-field, time-domain FLIM system included tunable excitation (337.1-960 nm), large temporal dynamic range (> 600 ps), high spatial resolution (1.4 μm), calibrated detection (0-300 ± 8 μM of oxygen), and rapid data acquisition and processing times (10 s). This technology and methodology were applied to functional studies in two distinct biological systems:

a) Metabolic function in living human normal esophageal (HET-1) and Barrett_s adenocarcinoma (SEG-1) cells: FLIM enabled the study of intracellular NADH and oxygen, where observed oxygen levels correlated well with published values in the literature. Starkly higher oxygen levels in SEG-1 vs. HET-1 were detected by FLIM and attributed to altered metabolic pathways in malignant cells.

b) Heterogeneous oxygen distribution in (microfluidic) continuously perfused poly (dimethyl siloxane) (PDMS) bioreactors containing living mouse myoblasts at an extracellular fluid-to-cell (volume) ratio close to the physiological value of 0.5. Variability in PDMS permeability, along with cellular uptake and culture media perfusion, can affect oxygen distribution, which may be critical in specifying cell fate. Oxygen levels decreased with increasing cell densities and were consistent with model outcomes obtained by simulating bioreactor oxygen diffusion and cell proliferation. In single bioreactor loops, FLIM detected spatial heterogeneity in oxygen levels with variations as high as 20%.

The fluorescence lifetime-based imaging approach described here avoids intensity-based artifacts (including photobleaching and concentration variations) and provides a technique with high spatial discrimination for intra- and extracellular oxygen monitoring.

# Real-time time-resolved diffuse optical imager for breast cancer detection

N. Chen, National Univ. of Singapore (Singapore)

An advanced diffuse optical tomography system has been developed in the Bioimaging Lab at NUS. Our system features dual-wavelength, sub-nanosecond time resolution, and high sensitivity that make it suitable for high resolution imaging of large human organs such as human breasts. Light source modulation with pseudo-random bit sequences and correlation detection are key techniques adopted in the imaging system. A 10-mW VCSEL (vertical cavity surface emitting laser diode) at 850 nm is directly modulated at 2.5 Gb/s while the 785 nm output from a pigtailed laser diode (7 mW) is subject to high-speed external modulation. Both wavelengths are combined and then distributed to nine different locations sequentially by fiber optical switches. There are four simultaneous detection channels, each of which consists of one high-speed avalanche photodiode, broadband RF amplifiers, a mixer for demodulation, and other signal conditioning components. The time resolution is measured around 500 ps (rise and fall times), and the temporal profile for diffuse photons penetrating through a-few-centimeter-thick tissues can be well detected. Each temporal profile has 128 points, spaced at 40 ps intervals. The total number of measurements is 9 x 4 x 128 = 4608 for each wavelength. It takes less than 10 seconds to acquire such a large data set, which is desirable for real-time and high quality diffuse optical imaging. Preliminary experiments with tissue phantoms have demonstrated better spatial resolution and image reconstruction robustness than conventional systems. It is expected that such a system will be used in our clinical investigations in the near future.

# Angular distribution of diffraction efficiency of a volume phase gratings with irregular modulation of refraction index

V. V. Krylov, Consultant

In the present article the behavior of angular distribution of diffraction efficiency (DE) of a volume phase grating with irregular on thickness by modulation of refraction index is esteemed. At the analysis the model of couple waves appended to medium with exponentially varied on thickness of a grating by amplitude of modulation of refraction index in different approximations was used. The relation of amplitude of modulation of refraction index such kind is stipulated in particular by exponential nature of damping of intensity of recording waves at a holographic way of grating preparation.The presence of non-zero parameter of damping b results in smoothing of lateral structure of a Bragg pica at a simultaneous decrease DE is shown. The comparative analysis gives the basis to consider that the offered approximations give a satisfactory result in a broad band of change of parameter b.

In the article the estimations of inaccuracies of linear and quadratic approximations are given. From the obtained predicted data it is visible that down to option values of damping b to the damping decrement the linear approximation gives a conservative value DE near to the Bragg angle with relative error about 4,5 %. The lateral structure of a pica in both approximations will be agreeed with each other mean relative error no more than 1,5 %.

# Spectral fluorescence targeted tumor imaging for peritoneal disseminated metastatic cancer: targeting strategies and fluorophore optimization

H. Kobayashi, Y. Hama, Y. Koyama, National Institutes of Health; Y. Urano, The Univ. of Tokyo (Japan); P. L. Choyke, National Institutes of Health

Intraperitoneal ovarian metastases commonly recur after cytoreductive surgery because small tumor foci escape detection within the complex anatomy of the peritoneal cavity. Better localization of peritoneal implants could improve the outcome of patients but few practical techniques to enhance detectability are currently available. Ordinarily, green fluorophores are not ideal for in vivo imaging because of their short penetration within tissue, however, when only the surface is being imaged, green fluorophores are completely satisfactory. In addition, to perform the spectral fluorescence imaging, green fluorophores provide sufficiently different spectra from autofluorescence, which peaks in the visible red spectrum. Here, we develop a targeted molecular imaging method that employs a variety of reagents labeled with green fluorophores targeting the D-galactose receptor, a widely expressed lectin-binding receptor on the surface of cancer cells, which can spread in the peritoneal space. After binding to surface receptors on the tumor, the reagents enabled spectral fluorescence imaging of disseminated peritoneal implants. In contrast, unbound reagents were cleared through the peritoneum and trapped by the liver, resulting in high tumor-to-background ratio that allowed visualization of implants as small as 0.3 mm with 100% sensitivity and >99% specificity (n>1,000). Among the four most common green fluorophores, Rhodamine green is the most favorable fluorophore because of it retains its fluorescence after cellular internalization. These results suggest that targeted molecular imaging with a green fluorescence-labeled D-galactose receptor targeting system is a promising technique for detection of disseminated submillimeter foci of peritoneal cancer.

# Arterial wall investigated using the near-IR spectral wing and polarization memory effect

X. Ni, S. Kartazayeva, W. Wang, C. Liu, City College/CUNY;
R. R. Alfano, City College/CUNY and Alfanix Technology Ltd.

Near-IR spectral wing and polarization memory imaging techniques are presented to examine the blood vessels, in particular vulnerable plaque in arteries. The spectral characteristics of the near-IR fluorescence wing from aortic tissue samples were investigated under different wavelength laser excitation. Significant intensity difference exists in the near-IR emission of plaque and normal aortic tissue, which can be utilized to identify the high-risk vulnerable coronary plaques. Polarization memory effect, the diffusively backscattered light dominated by helicity maintained photon, is observed for circularly polarized light propagation through the blood. We demonstrate that the polarization memory imaging technique can penetrate deeper through the blood field and better image the artery wall and assess plaque composition. Our techniques offer applications in intravascular detection of vulnerable plaque without blocking the blood flow.

# In vivo targeted fluorescence imaging of malignant peritoneal implants expressing the D-galactose (asialo) receptor using galactosyl serum albumin (GSA) conjugated rhodamine green

Y. Hama, National Institutes of Health; Y. Urano, The Univ. of Tokyo (Japan); Y. Koyama, P. L. Choyke, H. Kobayashi, National Institutes of Health

The concept of optically enhanced surgery and endoscopy may permit better detection of occult cancers that are not visible to the naked eye. Previous attempts to localize implants in the peritoneal space have utilized a fluorescein-conjugated avidin (Avidin-FITC), which successfully visualized sub-millimeter implants in a mouse model of ovarian cancer (Hama et al., Neoplasia, in press). However, potential translation to the clinic will be limited by the immunogenicity of avidin, which is derived from a hen egg protein. Herein, we describe an alternative to Avidin-FITC, which targets the same receptor but is made from a nonimmunogenic source, a galactosyl serum albumin conjugated with rhodamine green (GSA-RhodG). GSA-RhodG was synthesized and studied both in vitro and in vivo for uptake into SHIN3 ovarian cancer cells via D-galactose receptors. The relative in vivo fluorescence intensities of GSA-RhodG, Bovine serum albumin-RhodG (BSA-RhodG) and avidin-RhodG (Av-RhodG) were compared. GSA-RhodG showed more rapid and higher uptake by SHIN3 ovarian cancer cells than BSA-RhodG or Av-RhodG ($p<0.001$), yet similar rapid clearance from the peritoneal space and the circulation. Thus, GSA-RhodG is not only a more clinically feasible agent but also a better targeting reagent of D-galactose receptors on peritoneal cancers than Av-RhodG or BSA-RhodG.

# Human epidermal growth factor receptor type2 (HER2) targeted spectral fluorescence imaging of lung metastases

Y. Koyama, National Cancer Institute; Y. Hama, National Institutes of Health; Y. Urano, The Univ. of Tokyo (Japan); M. Bernardo, National Cancer Institute; P. L. Choyke, H. Kobayashi, National Institutes of Health

Purpose: Video-assisted thoracic surgery (VATS) is a standard method of resecting pulmonary metastases. During VATS, surgeons are limited in their ability to localize metastatic nodules detected by imaging modalities such as CT. We develop a targeted optical fluorescence imaging to help identify tumors intraoperatively.

Methods: Humanized anti-HER2 antibody (Herceptin) was conjugated to Rhodamine Green (RG). Humanized anti-IL-2 receptor alpha-subunit antibody (HUT) also conjugated to RG was used as a control. Two lung metastasis models were produced with NIH/3T3 cells either transfected with human epidermal growth factor receptor type 2 (HER2) genes or transformed by murine sarcoma virus. Herceptin-RG or HUT-RG was injected into mice bearing HER2+ or HER2- pulmonary metastases. One, two, four and seven days after antibody-RG injection, we performed open chest surgery followed by in vivo spectral fluorescence imaging (Maestro, CRi). We acquired four to six sets of side-by-side images of all positive and negative agent/ tumor combinations. The fluorescence signals from HER2+ and HER2- lung tumors were compared and all images were correlated with gross and microscopic pathology.

Results: We identified HER2+ tumors as small as 0.2mm in diameter with Herceptin-RG in vivo. HER2+ tumors injected with Herceptin-RG were clearly brighter than either tumor or antibody control at all time points. The peak fluorescence signal in HER2+ tumors injected with Hercepitn-RG was found at 2 days post-injection. At 1-2 day post-injection, tumors fluoresced strongly at the rim reflecting the binding site barrier, as commonly seen with high affinity antibodies.

Conclusion: We have successfully developed a targeted fluorescence imaging method for HER2+ metastatic lung tumors. The imaging method may have value in intraoperative tumor localization especially for VATS.

# Real-time imaging of tissue micro-structures using intrinsic optical signatures

B. Lin, Univ. of California/Davis; S. Urayama, N. Rahim, Univ. of California/Davis Medical Ctr.; D. Matthews, R. Ramsamooj, S. Demos, Univ. of California/Davis

The current gold standard of histopathologic evaluation introduces a delay of clinically relevant information due to tissue removal, sample preparation and processing, and is mainly applicable to readily identifiable lesions. This necessitates the need to develop imaging technology that can detect and diagnose disease in real time. Here we assess a multimodal microscopic spectral imaging system for real-time characterization of intact tissues or organs. This system uses intrinsic optical signatures to highlight and image tissue microstructures of interest. The microscope has been designed for optimized signal acquisition using long working distance objectives which permit for off-axis illumination at any wavelength. Various samples from human, murine, ovine, and porcine tissues have been imaged ex vivo, while in vivo experiments have been performed with an animal model. Autofluorescence images are acquired under excitation from the UV to NIR using a set of compact lasers as well as an OPO tunable laser. Spectrally resolved light scattering imaging with polarization discrimination was used as a complimentary imaging method. Initial experiments have allowed clear visualization of microstructures such as cells and cell nuclei in unprocessed tissue specimens. Image contrast between tissue components depends on the excitation wavelength and tissue type. Results show that this imaging approach has the potential to provide information comparable to H&E staining in real time. This multimodal microscopic imaging system can be realized via portable and endoscope designs for in vivo application to provide surgeons with a way to obtain intraoperative real-time diagnostic information.

# A 3D measurement endoscope system using multiple laser beams

H. Nakatani, Shizuoka Univ. (Japan); K. Abe, Aichi Institute of Technology (Japan); A. Miyakawa, S. Terakawa, Hamamatsu Univ. School of Medicine (Japan)

Using a conventional endoscope, one can hardly obtain 3D information quantitatively from the images. To estimate the accurate size and shape of objects needs much practical experience for operators and physicians in the field of endoscopy. In this paper, we will present an endoscope system that can measure the size and position of an object. The endoscope head comprises four laser beam sources and a camera. The procedural steps for 3D measurement are as follows.

Firstly, we observe a standard chart with the lens concerned and determine the correspondence between the image height and object height. The resulting function can map 2D coordinates of an image point to its 3D coordinates, and can also correct the barrel-shaped distortion of endoscopic images. Laser spots on the object surface are detected and traced automatically using a template matching method.

The 2D coordinates of the laser spots are mapped to 3D coordinates by the triangulation method. Then the system calculates the magnification ratio on the object plane, which is perpendicular to the optical axis and passes the laser spot, so that it can superimpose a ruler whose scale corresponds to each 3D location. Thus the endoscope system can correct the distortion and show real size of multiple regions in real-time. Experiments on the gastric wall of rats will be shown in the poster.

# fNIRS can help in diagnosing cerebral perfusion impairments in migraine and ADHD patients

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We have been investigating the use of CW-fNIRS technology on migraine and ADHD patients for whom cerebral perfusion impairment has been listed as one of the symptoms. We performed color word matching Stroop task for both groups for a quasi-block design experiment and compared their responses against controls. We showed that ADHD subjects had functional hypofrontality during the Stroop test and the impairment was related to cognitive load. Since increased HbO2 level is related to dilation of blood vessels, our results suggest that the hypofrontality reported in ADHD subjects was related to vascular hyporeactivity. It can be speculated that without MPH, ADHD subjects made too much effort during the neutral questions, and could not increase HbO2 further during CS and IS, and that MPH helped to overcome this problem. Similarly, migraine patients had suppressed HbO2 response indicating a lack of cerebral perfusion to the frontal lobe.

# A comparative study of two diffusion models on cylindrical geometry

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Imageability of fluorescent contrast, image reconstruction and visualization are contingent on the pattern of photon migration and distribution within biological tissue. Diffusion equation (DE) has been extensively used as a model for scattering-dominated light transport in the near infrared regime. In this paper, we present a comparative assessment of our implementations of the finite element (FE) formulation of the three-dimensional DE and analytical three-dimensional DE on a cylindrical geometry for a range of optical properties that are representative of biological tissue. In order to meet the requirements of size dictated by the penetration depth, and on the mesh resolution dictated by the memory of the hardware and processing time, we use an absorption coefficient of 0.01 mm-1, a reduced scattering coefficient of 0.5mm-1; a cylinder of 42mm diameter and 82mm height represented by 199414 nodes, 1146319 tetrahedral elements and 30690 boundary nodes for our study. Simulations show that a small variation in the mesh density across the cylinder results in different patterns of excitation fluence distribution on either side of a point source on the boundary. A sensitivity analysis for 5%, 10% and 20% increase in optical properties shows up to a maximum of 5.5% variation in ratios of boundary fluence between FE and analytical as both absorption and reduced scattering increase by 20%. The variations are of the order or less than typical measurement noise and permit the use of the cylindrical mesh as a representative optical phantom for testing and experimental design.

# Spectral radiance imaging of human skin tissue: theoretical aspects and empirical results

K. P. Nielsen, L. Zhao, A. Bhandari, B. Hamre, J. J. Stamnes, PhotoSense AS (Norway); K. H. Stamnes, Balter Inc.

We have modeled and measured spectral radiances reflected from skin that is illuminated from different directions. Clinical measurements were performed on more than 300 patients. We applied the discrete ordinate solution of the radiative transfer equation for modeling of measured results. This modeling approach is advantageous for skin optics, since it allows for adequate representation of boundary conditions and accurate modeling of the angular light distribution in the tissue. Also, this method gives accurate results for the light transport in absorbing media and is considerable faster than Monte Carlo simulations. Our results show that simultaneous imaging of reflected light from several observation directions gives additional information about its optical properties compared to what can be obtained when only using one direction of observation.

# Optical imaging of inhomogeneities in tissue

M. Bhowmick, Indian Institute of Technology Bombay (India) and Thadomal Shahani Engineering College (India); U. B. Desai, M. P. Thaddeus, G. Vishnoi, Indian Institute of Technology Bombay (India)

Diffuse optical tomography (DOT) is assuming importance as a low-cost, noninvasive imaging modality to probe the optical properties of biological tissues using near infrared (NIR) light. Hence it has become a challenge to develop accurate and fast image reconstruction algorithms that can transform the measurements into a clinically useful spatial map of optical tissue coefficients. In this paper, the authors present a fast and robust image reconstruction technique that has the potential to quantify the position, size and optical coefficients of inhomogeneties in the medium. The algorithm uses Finite Element Method (FEM) which discretizes the circular geometry used in the model by six node triangular elements as it approximates the geometry more accurately. Furthermore, the use of quadratic shape functions in six node triangle gives better estimates as compared to three node linear triangular elements and also reduces the size of the problem. The forward solver has automatic meshing, application of boundary conditions, generic state equation solver with complex and real numbers in the banded form and post processing tools. It allows the simulation of different data acquisition techniques, such as steady-state and frequency domain systems. Image reconstruction is based on Quasi-Newton optimization technique and has the novelty in reducing the memory space, sparsity and uses relative objective function and weighted average approach to detect multiple inhomogeneities very accurately. To illustrate the significance of the algorithm, the 2D and 3D reconstruction images based on simulation data have been presented.

# Demonstration of endoscopic near-infrared diffuse optical tomography in phantoms and tissues

D. Piao, H. Xie, W. Zhang, G. Zhang, C. Musgrove, C. F. Bunting, Oklahoma State Univ.; H. Dehghani, The Univ. of Exeter (United Kingdom); B. W. Pogue, Dartmouth College

Endoscopic near-infrared (NIR) diffuse optical tomography has been demonstrated in phantoms and tissues ex vivo. This novel approach provides the key feasibility studies to allow NIR optical tomography, a blood-based contrast imaging technology, to be attempted in cancer detection of internal organs via endoscopic interrogation.

Endoscopic NIR optical tomography is implemented by a spread-spectral-encoding technique based on a broadband light source. The broad spectrum of the source is dispersed and coupled to a series of bare fibers that are linearly aligned. The small wavelength increments of light coupled to each fiber forms spread-spectral-encoding which allows each source location to be discerned at the detector by spectrometer separation. The source fibers are re-aligned circularly at the distal end of the NIR endoscope probe, and a coated cone-prism is employed to deflect the light for transverse illumination. The combination of spread-spectral-encoding from a broadband light source with linear-to-circular fiber bundling provides endoscopic probing of multiple source/detector fibers for NIR tomographic imaging as well as parallel sampling of all source-detector pairs for rapid NIR data acquisition.

Endoscopic NIR tomography is achieved by use of a 12mm diameter probe housing 8 sources and 8 detectors at 8 Hz frame rate. Ex vivo transrectal NIR optical tomography is demonstrated by use of a chicken rectum. Preliminary simulation studies on the computational aspects of endoscopic NIR tomography including the analysis of contrast resolution and localization accuracy will be presented.

# Fiber-based excitation emission spectrometer for in-vivo transcatheter optical molecular analysis

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The combination of molecular beacons and optical spectroscopy is expected to take an important role in biomedical research in the future. Optical fluorescence spectroscopy offers high sensitivity and selectivity along with a variety of mechanisms for suppression of background signals making it an extremely powerful tool for in-vivo diagnostics at the functional-molecular level.

We present a newly developed fiber-based spectrometer for intravascular real-time acquisition of excitation-emission-spectra. The excitation is performed with a high pressure Xenon lamp (300W, 280 -1100 nm) in combination with a fast filter wheel with six customizable excitation bandpass filters. The detection using long pass filters along with a grating and a back-illuminated CCD camera provides a spectral resolution of 5nm. Full spectra can be acquired at a rate of 8Hz.

The 700μm-diameter fiber, optionally equipped with a 90° prism at the tip, fits into the lumen of a 4F diagnostic catheter. The catheter can be navigated to the point of interest for an optical measurement preferably under MR guidance using MR-safe tracking technology. Optical fluorescence spectroscopy and MRI can thus be used complementary in terms of spatial resolution provided and functional parameters that can be visualized.

The sensitivity allowed to detect the spectral fingerprint of a 0.7 nano-molar dilution of dye in-vitro using a 1ms acquisition. Linearity was demonstrated in the range of 10-5 to 10-9M. Spectral unfolding to analyze dye mixture compositions was performed at high accuracy demonstrating that the instrument may be useful for multi-spectral simultaneous detection and separation of targeted dyes, auto-luminescence, bioluminescence or similar.

# Optical high resolution cross section imaging of a human breast model using independent component analysis

M. Xu, M. Alrubaiee, S. K. Gayen, R. R. Alfano, City College/CUNY

Over the past two decades diffuse optical tomography (DOT) has been developed to image human breast, infant brain, and bone joints for noninvasive detection and diagnosis of abnormalities. DOT uses an iterative approach matching a theoretical prediction to measurement by repeatedly solving the forward model of light propagation in the medium. The iterative image reconstruction is computationally demanding and provides limited spatial resolution. Recently, we have developed an alternative optical imaging approach using independent component analysis (OPTICA) and shown its capability in 3D localization and characterization of absorptive, scattering and fluorescence inhomogeneities embedded in highly scattering media. OPTICA uses a multi-source illumination and multi-detector signal acquisition scheme to provide a variety of spatial and angular views of the medium. Independent component analysis of the data sorts out the signal from individual inhomogeneity for its localization and characterization.

In this presentation, we report on a back projection method to obtain high-resolution cross-section images of targets inside highly scattering media, enhancing OPTICA. We demonstrate its performance by imaging a 42x30x33mm3 model human breast assembled using ex vivo breast tissues. A 5x5x3 mm3, tumor, was placed at the midplane. OPTICA located the tumor within ~1 mm of known position. It also identified a 10-mm low scattering structure at midplane, and a 3-mm low scattering structure near the surface. Subsequent pathological analysis confirmed the tumor, and identified the other two structures as glandular breast tissues. The reconstructed cross section images and optical properties of the targets were consistent with pathology findings.

# Functional Near Infrared Spectroscopy for noninvasive imaging of cerebral response to noxious thermal stimuli

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Near Infrared Spectroscopy (NIRS) has been successfully used for monitoring brain function in adults as well as infants for the last two decades. The majority of NIRS studies involved measuring the cerebral response to visual, auditory and somatosensory stimuli. Here we demonstrate that the technology can accurately measure the activation of primary somatosensory cortex following application of painful stimulus (noxious thermal stimuli). In this study the continuous wave optical imaging instrument (CW-5) was used to measure brain activation. A custom head probe was designed with 12 source positions and 24 detector positions to monitor simultaneously both brain hemispheres of the subjects. The stimulation paradigm used an event related design wherein the stimulus (brush or noxious heat applied to the dorsal part of the hand) was applied for 2 seconds with an average interstimulus interval of 12 seconds. Each run was 6 minutes long and all the subjects participated in a total of 8 runs: 2 baseline runs wherein the subject is relaxing, 2 runs with tactile stimulation, 2 runs with thermal stimulation at 43 degree Celsius and 2 runs with thermal stimulation at 46 degree Celsius. During all these runs physiological signals like respiration, heart rate and blood pressure were recorded using the auxiliary data acquisition unit in the system. The NIRS data along with the auxiliary information was analyzed using a custom-made data analysis program called Homer to obtain the hemodynamic response. We will present results which show that the hemodynamic response following application of painful stimulus peaks at a significantly later time compared to that of the tactile stimulus.

# Heat management using thermal conductive optical windows for the prevention of tissue buckling and collateral damage in NIR-laser tissue welding

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A. Katz, R. R. Alfano, City College/CUNY

Laser Tissue Welding procedure involves illuminating the reapproximated edges of bisected tissue with a laser beam at the wavelength that is absorbed by the tissue. Optimum welding is achieved when the laser penetration depth matches the thickness of the tissue to be welded. In our approach, a NIR laser tuned to water absorption for vibrational overtones at 1455 nm is used to weld tissue; no extrinsic materials are necessary for welding. Water in the tissue absorbs the NIR laser energy and subsequently heats the collagen to a temperature above 60° C, thus disrupting its bonding and causing partial dissociation, followed by covalent and/or non-covalent bonding of the tissue protein molecules as the tissue cools. Successful welding requires precise control of laser power and exposure times to control tissue temperature and dehydration.

To address heat management issues, we used (a) a multi-pass rapid scanning of the laser beam around the area to be welded; and (b) transparent cover slides made of substrates like fused silica, BK-7, sapphire, diamond, IR quartz, and pyrex, positioned over the tissue specimen to serve as heat sinks. These heat sinks have different thermal conductivity. The data from this study we identified the optimal thermal conductivity of material that is suitable to achieve the better tissue weld strength. The data suggests that (1) better heat management enhances the welding success rate and tensile strength, while reducing collateral damage; (2) the use of a transparent cover slide helps to distribute the laser-induced heat, limits water evaporation; and, what is more important, (3) controls the buckling of tissue around the line of apposition so that the two pieces to be welded do not move apart due to buckling pressure, ensuring a full-length weld.

# An experimental calibrating system for functional DOT imaging

R. L. Barbour, NIRx Medical Technologies and SUNY/Downstate Medical Ctr.; H. L. Graber, Y. Xu, SUNY/Downstate Medical Ctr. and NIRx Medical Technologies; Y. Pei, R. Ansari, M. B. Levin, NIRx Medical Technologies

Elements common to all clinical imaging systems are system hardware, analysis software, and an appropriate set of calibrating phantoms. In the case of functional imaging systems (e.g., fMRI, MEG), however, dynamic calibrating phantoms are not commercially available. In their absence, investigators have resorted to use of computational models which do not support development of quality assurance programs directed to quantifying instrument and algorithm performance. In this study we describe development of a dynamic phantom whose optical properties can be precisely and dynamically adjusted electronically. Operational characteristics of the phantom employ principles of liquid crystal cell technology, for which we have implemented a novel electronic driving scheme. Optical transmission measurements have shown that the phantom can be adjusted to mimic essentially any hemodynamic profile with high accuracy and repeatability, while exhibiting a sub-millisecond response time. To explore use of the phantom as a calibrating device, we have constructed two tissue-like phantoms, one head-shaped and containing a plastic skull, and the other a layered structure whose background optical properties was systematically adjusted. Time series optical measurements were made, during which the cell_s optical opacity was adjusted. Analysis of the time series included use of a newly developed linear spatial deconvolution scheme that is applied to correct image blurring and other distortions inherent in first-order image reconstructions. Results obtained show that considerable improvement can be achieved in image accuracy. Availability of the considered phantoms provides a basis for developing quality assurance programs to validate the performance of functional DOT imaging systems.

# Image enhancement by linear spatial deconvolution

H. L. Graber, NIRx Medical Technologies and SUNY/Downstate Medical Ctr.; Y. Xu, SUNY/Downstate Medical Ctr. and NIRx Medical Technologies; Y. Pei, NIRx Medical Technologies; R. L. Barbour, SUNY/Downstate Medical Ctr. and NIRx Medical Technologies

The need to limit the computational effort in reconstructing an image time series for DOT studies has led us to use of first-order methods whose accuracy is typically less than can be achieved using recursive iterative methods. Recently we have implemented an image correction procedure motivated from an understanding of the improvements that can be realized from knowledge of a measuring system_s point spread function. While use of such strategies has previously been limited to direct imaging problems, we have shown that an equivalent method can be applied to indirect imaging problems. This equivalence considers a transfer function that explicitly takes into account local blurring information inherent in use of imaging operators based on incomplete views and linear approximations. The strategy implemented has parallels to the physical basis of magnetic resonance imaging, in that spatial information in the object domain is encoded using frequency or time-varying techniques thus allowing specific labeling of the information blurring obtained in the image domain. This information is used to construct a linear deconvolution operator whose details depend on the source-detector configuration. The hypothesis tested here is that a single image correction operator can be applied to improve the accuracy of an entire time series of reconstructed images. Two classes of data were explored: computational models based of segmented MR images of the motor cortex, and a dynamic phantom containing programmable electrochromic cells. Results validate the method, showing that it is robust to noise and other systematic errors, improves image accuracy, and is computationally efficient.

# Multiplexing molecular markers in vivo and ex vivo with multispectral imaging

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Cambridge Research & Instrumentation, Inc.

In-vivo fluorescence imaging has several potential advantages over other small animal imaging modalities, such as the ability to multiplex fluorophores and the potential to use injected labeled antibodies. However, the ability to image fluorescently labeled markers in-vivo has generally been limited by the autofluorescence of the tissue. Autofluorescence limits the usefulness and sensitivity of conventional in-vivo fluorescence imaging; expensive cameras do not help, since they simply capture the autofluorescence more efficiently.

Using a multispectral imaging (MSI) methodology to spectrally characterize and computationally eliminate autofluorescence will reveal otherwise invisible labeled targets. This dramatic improvement in signal-to-noise can increase sensitivity by orders of magnitude, allowing much smaller targets to be detected. MSI also is a perfect compliment to multiplexed analyses, with as many as four target analytes being images simultaneously.

In addition, performing multi-analyte immunohistochemistry, whether in brightfield or fluorescence, has many potential applications in the field of drug target evaluation. However, accurate assessment of two or more co-localized antigens, especially using brightfield microscopy has been hindered by the difficulty in discriminating and quantifying overlaying labels, such as ER, PR and Her-2. MSI can resolve overlapping chromogens and generate quantitative images of individual analytes. MSI can also separate fluorophores from ubiquitous autofluorescence background, allowing more sensitive and quantitative studies.

The use of multispectral imaging methods combined with automated spectral analysis tools can easily separate and quantitate multiple chromogens and counterstains in a single tissue sample even when the antigens are co-localized. These methods form an ideal complement to automated staining platforms and new multiplex labeling strategies, and together constitute clinically viable techniques that hold great potential to further characterize and subtype cancers.

# Monitoring head and neck patients during chemoradiation therapy with diffuse optical spectroscopies

U. Sunar, Univ. of Pennsylvania

This pilot study explores the potential of noninvasive diffuse correlation spectroscopy (DCS) and diffuse reflectance spectroscopy (DRS) for monitoring early relative blood flow (rBF), tissue oxygen saturation (StO_2) and total hemoglobin concentration (THC) responses to chemo-radiation therapy in patients with head and neck tumors. rBF, StO_2, THC in superficial neck tumor nodes of 8 patients were measured before and during the chemo-radiation therapy period. In one case the results were compared to MRI. The weekly rBF, StO_2 and THC kinetics exhibit different patterns for

different individuals, including significant early blood flow, tissue oxygenation changes during the first two weeks. These preliminary results suggest daily, diffuse optics based therapy monitoring is feasible during the first two weeks and may have clinical promise.

# Optimized hyperspectral microscopic discrimination among normal, adenomatous and carcinomatous colon tissue micro array biopsies

F. Woolfe, M. Maggioni, G. L. Davis, S. Zucker, Yale Univ.

-Background-

Our poster at Optical Imaging 2004 described hyper-spectral light microscopy on colon micro array tissue biopsies for discriminating between normal and malignant nuclei. Results on the test set yielded 94% diagnostic efficiency, with 100% discrimination between normal and malignant biopsies. However when the algorithm is interrogated on unseen biopsies diagnostic efficiency falls to 77%. Subsequent application of this technique to 3 class discrimination among normal, adenomatous and carcinomatous biopsies resulted in far lower diagnostic efficiency.

-Method-

We now train on nuclei from certain biopsies and test on nuclei from others. We apply a series of tissue segmentations: LDB uses no spatial information while laplacian embedding does. We produce confusion matrices using the partial least squares regression algorithm for learning. We normalize using the derivatives of spectra as feature vectors.

-Results-

LDB tissue classification yields 77% diagnostic efficiency between normal and malignant biopsies. Laplacian embedding using spatial information increases the diagnostic efficiency to 86%. Spectral derivative normalization further increases diagnostic efficiency to 87%. Benign (defined as normal and adenomatous together) versus malignant results in 89% diagnostic efficiency. Discrimination among 3 classes (normal, adenomatous and malignant) remains less successful (56% diagnostic efficiency) due to conflation of normal and adenomatous biopsies. Normal versus abnormal (defined as adenomatous and malignant together) yields a diagnostic efficiency of 56%. We are exploring reasons for poor discrimination of adenomas and improving tissue segmentation with immunoperoxidase markers for epithelium and leukocytes.

# Imaging of superficial skin lesions by spectroscopic scattering ellipsometry

B. B. Boulbry, T. A. Germer, National Institute of Standards and Technology; J. C. Ramella-Roman, The Catholic Univ. of America

We present a novel spectro-polarimetric instrument based on hemispherical backscattering for the assessment of superficial skin lesions. The system is capable of capturing polarized light images non-invasively. The effect of the rough skin backscattering is eliminated with the use of out-of-plane illumination. A glass slide with an index matching fluid, commonly used in polarized light imaging, is no longer necessary. The system is composed of sixteen polarized light sources that provide red, green, or blue illumination. The light sources are distributed on a hemispherical shell, and each source produces a collimated beam incident on the center of the hemisphere. A Stokes vector imaging system is mounted on the shell at an oblique angle to the sample normal and consists of a 12-bit scientific camera, two liquid crystal variable retarders, and a fixed polarizer. Stokes vector images of light scattered towards the camera direction are generated for each source. Examples of images generated by the system are presented.

# Tumor detection by simultaneous bilateral diffuse optical tomography (DOT) breast imaging

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Growth of solid tumors is frequently accompanied by marked changes in the vascular supply sustaining tumor growth, leading to a state of impaired perfusion and a relatively hypoxic environment. We have explored these and related features, using a recently developed dual-breast DOT imaging system capable of simultaneous bilateral measurements. Dual-wavelength measurements were taken during baseline periods and while subjects performed Valsalva maneuvers as a means of provoking a vascular response. Subjects from the breast-cancer and control groups were matched with respect to age and body mass index. Features were extracted from the computed Hb image time series that could be expected to convey principally one of three types of diagnostic information, namely, the effects of hypoxic tumor environments on: vascular reactivity (Type 1 data); temporal coordination of the vasculature (Type 2); and venous congestion induced by the Valsalva maneuver (Type 3). For each feature type and Hb state, multiple metrics were identified and computed, for a total of 138 measures per individual per breast. The computed metrics were subsequently tested for differences between the affected and healthy breast among the cancer group, between left and right breasts within the non-cancer group (as a control), and between the two patient groups, using both univariate and multivariate methods. The diagnostic predictive values appropriate to tumor detection, tumor localization, and tumor sizing were quantified. Results show that measures from each data type produced good to excellent discrimination between the two subject groups. In combination, diagnostic sensitivity and specificity was typically greater than 90%.

# Implications of performing optical imaging under mammographic compression - changes in breast physiology and novel optical cancer markers

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The tomographic optical breast imaging (TOBI) device at MGH combines diffuse optical tomography (DOT) with digital X-ray tomosynthesis. As such, the optical measurements are performed under mammographic compression. Hence, we have initiated a pilot study to investigate the effects of compression on the physiological state of the breast and, in turn, on our measurements.

Using a simulated mammography setup consisting of parallel plastic plates mounted on a computer controlled translation stage, various compression levels were applied to the breasts of healthy volunteers while a combined frequency domain/continuous wave spectrometer recorded the time-resolved optical properties of the tissue. We noted a reduction in total hemoglobin content, tissue oxygen saturation and optical scattering under compression, as well as smaller but noticeable changes in water and lipid content.

The dynamic characteristics of these changes in breast physiological parameters due to compression may prove useful for breast cancer detection. Specifically, by modeling the time-course of the transient change in the tissue oxygen saturation, we were able to obtain estimates for the volumetric blood flow (0.51 ± 0.2 mL/100 mL/min) and the oxygen consumption (1.97 ± 0.6 mumol/100 mL/min) of compressed breast tissue. These values are comparable to previously published PET measurements and we believe that oxygen consumption and blood flow have the potential to become novel breast cancer optical markers. A simulation study is also presented indicating the feasibility of performing direct tomographic reconstruction of these new markers from DOT measurements.

# Optimization of a non-contact diffuse optical tomography based on dual NIR CCD camera

W. S. Ko, S. Kim, Korea Advanced Institute of Science and Technology (South Korea)

This research proposes a new non-invasive and non-contact diffuse optical tomography (DOT) system for imaging with CW near-infrared (NIR) light. A DOT system can reconstruct image inside the tissue by measuring the hemoglobin distribution inside tissue by detecting the NIR light emitting from the tissue. The proposed non-contact DOT system is based on the scanning system which offers the advantage of performing non-invasive, real time, and cost-effective measurement. The non-contact detection system is constructed with dual NIR CCD cameras for the slab geometry detection. The non-contact DOT system is experimentally tested with phantom consisting of paraffin wax and Intralipid. To improve the performance of the non-contact DOT system, the incident angle effect is calibrated by using the threshold image process to find the diffusion center.

# Fast optical signals in peripheral nerves

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We present a study of the near-infrared optical response to electrical stimulation of peripheral nerves. The sural nerve of six healthy subjects between the ages of 22 and 41 were stimulated with transcutaneous electrical pulses in a region located approximately 10 cm above the ankle. A two-wavelength (690 and 830 nm) tissue spectrometer was used to probe the same sural nerve below the ankle. We measured optical changes that peaked 60-160 ms after the electrical stimulus. On the basis of the strong wavelength dependence of these fast optical signals, we argue that their origin is mostly from absorption rather than scattering. From these absorption changes, we obtain oxy- and deoxy-hemoglobin concentration changes that describe a rapid hemodynamic response to electrical nerve activation. In five out of six subjects, this hemodynamic response is an increase in total (oxy + deoxy) hemoglobin concentration, consistent with a fast vasodilation. Our findings support the hypothesis that the peripheral nervous system undergoes neurovascular coupling, even though more data is needed to prove such hypothesis. [Supported by the National Science Foundation, Award BES-93840.]

# Observing DNA processing by single exonucleases

R. Conroy, National Institutes of Health; J. Moreland, National Institute of Standards and Technology; A. P. Koretsky, National Institutes of Health

Advances in imaging and manipulation have opened up the possibility of observing the operation of single enzymes. We will report on our single molecule assay to measure the processing rate of single lambda and T7 exonuclease enzymes of the repeating DNA triplets found in Fragile X Syndrome. Initial experiments with pseudo-random sequences confirm previously observed rates of 10-50 nt/s.

# Monitoring tumor response to neoadjuvant chemotherapy in breast with NIR tomography

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In this study, the quantitative changes in the tumor in response to neoadjuvant chemotherapy in breast were monitored with a Frequency Domain Near-infrared (NIR) tomography system. The parameters from the tumor region that were quantified were total hemoglobin concentration (HbT), blood oxygen saturation (StO2), water fraction (%) and elastic scattering parameters related to average size and density of index varying particles. In this preliminary clinical trial, six patients were involved of which five patients were monitored throughout the entire course of their therapy. NIR Imaging was performed prior to the first treatment cycle and also at several time points over each cycle. For comparison, MR imaging was carried out prior to and after treatment cycles for each of the patients. A patient that was diagnosed as responding to the therapy, had average values of HbT and water that significantly decreased within the tumor region, during the first cycle. Additionally, the values were constant outside of the tumor region as well as in the contralateral breast. The tumor region size was examined in different ways, as the anticipated region of interest to quantify varied over the time of the study. Reduction of HbT and water values inside of the tumor region were observed within 7 days after the first cycle started for the two clinically responding patients. One patient who was diagnosed as having no clinical response to therapy, exhibited no significant change in any of the properties, and the tumor region size increased after completion of the infusions.

# In vivo integrated photoacoustic flow cytometry: Application for monitoring circulating cancer cells labeled with gold nanorods

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A new in vivo photoacoustic flow cytometry technique has been developed for real-time monitoring circulating cells in live animals. Using this new technique with a near infrared laser and optical clearing, the authors were able to characterize the in vivo kinetics of cancer cells labeled with new contrast agents (such as gold nanorods) within the vasculature of a mouse ear. For verification, the same cells were observed using a photothermal and fluorescent technique and the conventional staining agent indocyanine green. The authors discuss other in vivo applications for the newly developed flow cytometry technique including label-free detection of cells with different endogenous absorption levels (e.g., red and white blood cells) or of abnormal cells (e.g., sickle or apoptotic) in blood and lymph flow; linear and non-linear spectroscopy at the single-cell level in vivo; and as a feedback control in selective cancer nanotherapy, with the potential for translation from animal model used to humans.

# In vivo fluorescence lifetime imaging system based on time correlated single photon counting

M. Hassan, J. D. Riley, V. V. Chernomordik, A. H. Gandjbakhche, National Institutes of Health

We built a fiber-based, novel small animal imaging system with an excitation source and multiple detection fibers separated from the source at different distances. The goal of this study is to develop an efficient in vivo imaging system based on time-resolved method to map fluorescence lifetime and locate the fluorophore embedded in turbid media. The system consists of a scanning head where the light source and detection fibers were placed at known distances (2mm, 3mm, 4mm and 5mm from the source). This configuration may allow us to reconstruct tomographic image of lifetime at different depth and locate the fluorophore. We have validated the system using analytical models to establish the specificity of the system to location and lifetime. We showed results of numerical and phantom analysis to validate the model as well as presenting initial results of lifetime mapping in mice. For animal study, we used athymic female nude mice and implanted cancer cell line to flank area of the mice. Using a tail injected fluorescent probe: protein conjugated Alexa Fluor 750 to tumor infiltrating molecules and mapping a 2D image of the lifetime in vivo over a 36 hours period after injection.

We showed mapping of fluorescent lifetime in optically dense tissue. This will allow us to image not only the location of cancerous bodies in the tissue but to be able to monitor their status in-vivo.

# Relation of dynamic light scattering (DLS) cataract detection device parameters to clinical nuclear lens grades

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PURPOSE: To assess the association of Dynamic Light Scattering (DLS) parameters with clinical cataract grading and to determine the reproducibility of DLS measurements.

MATERIALS AND METHODS: We conducted an NEI IRB-approved cross sectional clinical study on normal and cataract patients using the NASA-NEI DLS clinical device. All subjects gave informed consent and underwent complete eye examinations including AREDS lens nuclear clinical (NUC) grading. 2-5 DLS measurements of the lens nucleus were taken to assess reproducibility. For each DLS measurement, a particle size distribution within the small sampled volume of the nucleus was estimated using the exponential sampling method (Stock and Ray 1985). From the typical bimodal distribution of particle sizes, summary parameters were calculated, including median log particle diameter (MLD) and fraction of intensity contributed by the high molecular-weight particles (Fraction Large Diameter (FLD)). The relation of DLS parameters to NUC grades was assessed using mixture models (SAS version 9.1). Reproducibility was determined by the coefficient of reproducibility (standard error of measurement divided by the mean).

RESULTS: 212 patients (343 eyes) were included. Mean age was 57 years (range, 18-86 years). NUC grades ranged from 0.0 through 3.8 (mean 0.75, SD 0.74). DLS parameters MLD and FLD increased significantly with increasing NUC grade (0.11 units/1-step change in grade, $p < 0.0001$; 0.38 units/1-step change in grade, $p<0.0001$, respectively). Coefficient of reproducibility was 0.028 for MLD (2.8%) and 0.0313 (3.13%) for FLD.

CONCLUSION: DLS parameters were significantly associated with AREDS NUC grading and gave reproducible results. These findings support further investigation of this non-invasive, objective approach for measuring early lens changes.

This research was supported by the NASA-NEI Inter Agency Program.

# Spectral imaging approach for tumor oximetry

N. Liu, Y. Yu, A. Sassaroli, D. K. Chen, S. Fantini, Tufts Univ.

We present an experimental study of a novel spectral approach that is aimed at quantifying the relative concentration of two localized chromophores within turbid media, which can be applicable to the measurement of the oxygen saturation of hemoglobin in breast lesions. The method involves 1) an appropriate choice of a pair of wavelengths (λ1, λ2), which depends on the relative concentration of the two chromophores as well as on the optical properties of the background turbid medium; and 2) calculate the tumor oxygenation by using the optical data at the two selected wavelengths, which is largely independent of the tumor size, shape, and location. We used black India ink and blue food dye as the localized chromophores (which mimic oxy- and deoxy-hemoglobin in a breast tumor), and milk as the background turbid medium (which mimics breast tissue). By using different combinations of the ink and dye solutions, we have experimentally tested the ability of our method to measure relative concentrations of known chromophores localized within a strongly scattering medium. The experimental setup consisted of an arc lamp (bandpass filtered over the range 400-1000 nm), a spectrograph, and a high-sensitivity CCD camera. The average relative concentrations of ink and blue dye were measured to within 10% of their actual values over the full range 0-100%. These experimental tests provide a proof-of-principle demonstration of the effectiveness of our two-wavelength approach, which we have now implement into a breast imaging system for tumor detection and oximetry.

# Integrated miniature microscope with structured illumination for in vivo microscopy

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A miniature handheld microscope is constructed for in vivo imaging of tissue at the cellular level. The microscope is capable of operating in several modalities including reflectance and fluorescence and uses structured illumination to obtain an optical sectioning capability. Micro-Electro Mechanical System (MEMS) technology is utilized to drive a scanning grating for structured illumination, a technique that produces images similar to that of a confocal microscope. The structured illumination capability allows selection of layers within the tissue many tens of microns below the surface to reach beyond epithelial cells. The microscope is capable of imaging with 1 micron resolution using a high speed CMOS detector that will be integrated into the microscope. The system has a cross section measuring 3x5 mm and is small enough for use as an endoscope, eliminating the need for biopsy to inspect tissue microscopically. Lenses were made using a grayscale lithographic printing process that provides precise fabrication of optical and mechanical positioning features. Combined with the Micro-Optical Table (MOT) concept, this enables accurate positioning of lenses that ‘snap’ into place. The use of grayscale lithography to print lenses enables fabrication of aspheric and even asymmetric surfaces. This degree of freedom leads to unconventional and novel optical designs including tilted lenses that reduce background light in the image by sending reflections out of the image plane. The combination of technologies incorporated in the miniature microscope demonstrate a wealth of potential for future optical systems capable of high resolution in vivo microscopy.

# Poster Presentations

## Tuesday 26 September

*Posters in this session will be on display from 10:00 Tuesday morning in the Natcher Atrium. A poster session, with authors present at their posters, will be held Tuesday from 5:30-7:30 pm. Light refreshments will be served. Attendees are requested to wear their conference badges.*

# New ultrafast laser system based on the Chromium: Forsterite for multiphoton in vivo imaging

S. E. Egorov, C. C. Barnes, A. J. Carson, Del Mar Photonics, Inc.

Chromium-doped forsterite crystal (Cr:forsterite) is a solid state laser material that fluoresces in the near-infrared region centered around 1250 nm. Cr:forsterite has been successfully used as a gain medium in Kerr lens mode-locked (KLM) femtosecond lasers with cavity designs similar to the popular Ti:sapphire femtosecond laser systems. Cr:forsterite exhibits strong absorption in the near infrared and can be directly pumped with CW lasers operating at 1064 nm, offering a significant reduction in system cost compared to femtosecond lasers pumped in the 532 nm region. We present a design of compact femtosecond Cr:forsterite laser pumped by a 10-W ytterbium fiber laser, generating 60 - 80-fs pulses at 1230-1270 nm with output up to 300 mW. Wavelengths in the 1250 nm range are less damaging to biological samples than other ultrafast lasers making Cr:forsterite based femtosecond laser systems an ideal source of ultrashort pulses for biological and medical applications. This wavelength falls in the transparency window of most biological tissues, allowing deep tissue penetration with minimal photodamaging and making it a valuable alternative source for in vivo multiphoton imaging.

# Laser Doppler imaging: a new approach

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Imaging blood flow is an essential tool to assess many physiologic processes, among which neuronal activity. Several instruments have been proposed to produce blood flow maps. These instruments can be grouped in two categories: those which are based on time domain measurements (laser Doppler techniques) and those based on spatial domain measurements (speckle contrast analysis techniques). Speckle contrast analysis techniques are based on the measurement of the local blur rate of the intensity of speckle pattern. Laser Doppler techniques provide, on the basis of a time-domain measurement, the frequency distribution Doppler-broadened light.

We present a frequency-domain, wide-field alternative to either time-domain (common Doppler techniques) or spatial-domain (speckle contrast) analysis, based on a heterodyne optical mixing scheme. The instrument uses a low frame rate (8 Hz) CCD camera. It enables to measure wide-field laser Doppler maps. This scheme is particularly suited to in vivo laser Doppler imaging of cortical blood flow. A spatial map of an arbitrary frequency component of the scattered optical field is acquired at once. The available range of frequency shifts at which measurements can be made is far more extended than in any of the concurrent techniques, since the measurement is performed in the frequency domain. The spectral resolution is about a few Hertz. Both temporal and spatial resolution are potentially high. This combination is enabled by a measurement performed at one frequency point at a time. Successful results obtained in a mouse brain, showing the Doppler signature of blood flow have been obtained, in vivo.

# Thin films of zinc phthalocyanine (ZnPc) for optoelectronic devices

M. Puri, Guru Nanak Dev Univ. (India)

ZnPc is a promising candidate for photovoltaics applications. It can be easily synthesized and is non-toxic to the environment. It is a self-assembly molecule developed from deep-blue-green pigment. It exhibits a characteristics structural self organization which is reflected in an efficient energy migration in the form of extinction transport. In the present work thin films of ZnPc have been prepared on glass substrate under strict vacuum conditions ($10^{-6}$ torr), thickness of few nanometers. Absorption spectra in Visible and IR regions have been observed which is good for fabrication of Photovoltaic cells and Nanostructures for Photodynamic Cancer Therapy. Investigations have been made from different stacking positions of Molecular ZnPc thin films for studying their self-assembling nature that can be useful for their applications as optoelctronic devices, Molecular-Recognition in Drug delivery and sensors which is one of the key features of Nanotechnology.

# Real-time imaging and characterization of human breast tissue by reflectance confocal microscopy

M. C. Cabrera, M. T. Tilli, A. L. Gallagher, E. Makariou, S. Pollin, M. C. Liu, P. A. Furth, Georgetown Univ. Medical Ctr.

Real-time technologies could increase efficiency of obtaining informative biopsies and accelerate interpretation. Cellular aberrations inherent to cancer cells, including nuclear size, can be visualized but few technologies are available to evaluate adequacy of specimens such as core needle breast biopsies in real-time. Aims. 1. Determine if near-infrared reflectance confocal microscopy (RCM) (VivaCell-TiBa, Rochester, NY) can be used to assess epithelial/stromal content of core needle breast biopsy samples in real-time. 2. Test if RCM images can be accurately read for presence/absence of malignancy. 3. Determine if epithelial cell nuclear size can be measured on RCM images. Protocol was approved by the Institutional Review Board. Written consent for study participation was obtained from each subject prior to a medically indicated core needle breast biopsy. 1-3 additional tissue specimens were obtained specifically for RCM following completion of the diagnostic biopsy. All patient identifiers were removed to protect patient confidentiality. Biopsy specimens were immediately placed in phosphate buffered saline and imaged within 1 hr of acquisition. Five % acetic acid was injected into the tissue immediately prior to imaging. Structures within the breast tissue were visualized, identified, optically serially sectioned, and digital images cataloged within 5-10 minutes of operator time. Entire ~ 12 mm long/2mm thick specimens were imaged. Relative amounts of epithelial, fatty and collagenous tissue were determined. Biopsies were then formalin-fixed, sectioned and stained with H&E for comparison with RCM images. RCM data was comparable to data from H&E sections. Epithelial cell nuclear size was measured on stored digital RCM images.

# Advanced nano-imaging techniques applied to live cell biophysics

A. Trache, J. P. Trzeciakowski, W. E. Zimmer, Texas A&M Univ. Health Science Ctr.; G. A. Meininger, Dalton Cardiovascular Research Ctr.

Developments in physics in the recent years have brought new, non-conventional imaging techniques to biology, leading towards a new and deeper understanding of biological processes. Combining Atomic Force Microscopy (AFM) with optical imaging techniques like Total Internal Reflection Fluorescence (TIRF) and Interference Reflection Microscopy (IRM), we were able to study the live cell dynamics with an emphasis on extracellular matrix - integrin - cytoskeletal interactions and their role in the cellular responses to changes in external mechanical and chemical stimuli. Experiments in which AFM probes were labeled with fibronectin were used to measure the binding strength between a5b1 integrin and fibronectin by quantifying the force required to break single fibronectin-integrin bonds. Also, cytoskeletal changes, binding probability, loading and cell stiffness information can be obtained from these adhesion force spectroscopy measurements. We also showed that, AFM external mechanical stimulation applied at the apical cell surface induces a force-generating cytoskeletal response resulting in focal contacts reorganization on the basal surface that can be monitored in real time. By scanning live cells in culture using contact mode AFM and optical imaging using IRM or TIRF, a very good agreement is obtained between the focal contacts from the basal cell surface and the cortical cytoskeletal actin filaments from the apical cell surface that terminates in the focal contacts anchoring the cell to the substrate. The integration and simultaneous use of AFM with advanced optical imaging methods provides a state-of-the-art approach for understanding dynamic cellular responses to mechanical force.

# A dual-axes confocal reflectance and fluorescence microscope for in vivo early detection of cancer

J. T. C. Liu, M. J. Mandella, W. Piyawattanametha, H. Ra, C. H. Contag, G. S. Kino, O. Solgaard, T. D. Wang, Stanford Univ.

A miniature dual-axes confocal microscope has been developed, with an outer diameter of 10 mm, for sub-surface in vivo imaging of biological tissues with 5-7 micron resolution. Depth-resolved en face images are obtained at video rate over a large field of view by employing a two-dimensional scanning microelectromechanical (MEMS) mirror. Reflectance and fluorescence images are obtained with a laser source at 785 nm. This design may easily be packaged in a 5-mm diameter housing for future endoscopic use. Proof-of-concept results from tabletop dual-axes prototypes at 1315- and 785-nm have demonstrated the ability of this confocal architechture to perform subsurface reflectance and fluorescence imaging with high resolution (3-5 micron axial) and deep tissue penetration (up to 600 microns) over a large field of view (up to 900 microns). There is clear potential for a miniature instrument to detect pre-cancerous tissues, and hence to perform in vivo histopathology. In addition, fluorescently-labeled peptides, targeted against biomarkers of precancer in the colon, are being developed to improve the real-time diagnoses and localization of lesions with the dual-axes microscope.

# Endoscopic imaging techniques for early diagnosis: recent works in OLYMPUS

K. Gono, Olympus Corp. (Japan)

Recently, we have developed novel video endoscope imaging techniques; NBI, AFI, IRI and ECS. NBI stands for Narrow Band Imaging, and is based on narrowing the spectral bandwidth of the illumination. NBI enables to sharply visualize the capillary and the fine structure of the tissue surface. AFI stands for Auto Fluorescence Imaging, and is based on imaging auto-fluorescence generated from the tissue by illuminating the excitation blue light. AFI is expected to be screening mean of the early lesions which is difficult to be found. IRI stands for Infra-Red Imaging, and is based on illuminating infrared light and using CCD that has sensitivity in the infrared region. IRI has the potential to visualize the deep layer where can not be seen with white light, and be utilized for diagnosis before the endoscopic therapy. We developed the light source unit and the video processor for the endoscope imaging so that all of the three function; NBI, AFI and IRI is incorporated into the one unit, and have already launched the corresponding product. ECS stands for End Cytoscopy System, and is the optical zoom video endoscope that the special designed microscopic optics is integrated in the tip of the endoscope. ECS has the magnification fold to observe living cell nuclei on the tissue surface under methylene blue staining. In the presentation, the configuration and specifications in each function are presented and results of clinical tests are also shown.

# Functional near infrared spectroscopy for the assessment of cognitive impairments following traumatic brain injury

A. C. Merzagora, M. T. Schultheis, M. A. Izzetoglu, B. K. Onaral, Drexel Univ.

Traumatic brain injury (TBI) is the leading cause of long-term disabilities and individuals with TBI often suffer from serious cognitive complications. In neurorehabilitation, functional recovery is primarily assessed through behavioral observation, which provides little information about changes at the brain level and may result in a subjective approach to determine whether an individual is benefiting from a rehabilitation approach.

Existing functional neuroimaging techniques, such as fMRI and PET, have limitations on the rehabilitation applications due to a dependence on expensive technologies and the reliance on non-ecologically valid experimental tasks. Hence, there is a need for a portable functional neuroimaging technology to study and assess the intervention effects through an objective and individualized measure of the cognitive status of the patients in their everyday life activities.

The proposed research will employ functional near infrared spectroscopy (fNIRS), an optical neuroimaging modality that is safe, affordable, portable and has good spatial and temporal resolution. To date, few studies have investigated fNIRS for the assessment of cognitive impairments following TBI. For this reason, the proposed study is intended to be a preliminary establishment of fNIRS as a clinically useful tool in TBI neurorehabilitation. First, fNIRS measures will be tested for their ability to discriminate between TBI patients and healthy controls. Then, fNIRS measures of cognitive impairments following TBI will be compared to performance on traditional cognitive tasks (e.g. neuropsychological tests) and EEG analyses.

# Prediction of oral mucositis development using optical coherence tomography in patients with head and neck cancer

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Mucositis is the most debilitating side effect of radiochemotherapy in patients with primary advanced head and neck cancer. Existence of prognostic criteria at early treatment stages will allow earlier and more intensive prophylaxis and treatment of mucositis. This study uses optical coherence tomography (OCT) to monitor mucositis development and to elaborate a predictor of mucositis severity.

OCT creates real time cross-sectional microstructural images of tissues at a depth of up to 2 mm with a spatial resolution of 10 to 15 μm. 14 patients with stage II-IV of oropharyngeal squamous cell cancer were enrolled. Patients received radiation or chemoradiation (5FU+cisplatin) therapy up to a total dose of 66-70 Gy. OCT imaging was performed daily starting from the first day of irradiation in two sites of the right and left cheeks. Mucosal toxicity was scored according to CTCAE 2003.

The OCT image of normal cheek mucosa has a high-contrast stratified structure, well delineating epithelium, lamina propria and submucosa. Typical radiotherapy-related changes in OCT images include loss of layer contrast, signs of edema, and epithelium thinning up to complete absence (erosion). Patients with severe reduction of OCT contrast in the early stages of treatment and complete lost of contrast by the first day of clinical manifestation of mucositis, ultimately had severe mucositis. On the contrary, if OCT contrast remained until clinical manifestations were visible, then the patient ultimately had only mild mucositis.

We envision that dynamic OCT observations before the onset of clinically visible mucositis could become a predictor of mucositis severity.

# Niris optical coherence tomography system: application in endourology and guided surgery

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Optical Coherence Tomography (OCT) was first introduced in 1991 as a new imaging modality, using near infrared light interferometry for visualization of biotissues microstructure. OCT provides cross-sectional images with up to 2 mm penetration depth and high spatial resolution. We report early results in the application of the Niris Imaging System—the world's first FDA cleared OCT imaging system for non-ophthalmic use.

Niris is based on common-path all-fiber interferometer topology, which make it insensitive to probe length, wave dispersion and polarization distortions. It acquires real-time images with 200x200 pixels, 15 μm free space in depth resolution (11 μm in tissue), 25 μm lateral resolution. A reusable 8 Fr Niris probe (2.7 mm OD) can be used standalone, in instrumental channels of many standard endoscopes or in a specialized disposable sterile plastic holder. To date, we have enrolled 21 patients in urinary bladder studies and 58 patients in retroperitoneal studies including exploring OCT as an aid in nerve visualization in radical prostatectomy and retroperitoneal lymph node dissection, using open, laparoscopic and robotic-assisted procedures.

We obtained more than 1400 OCT images of tissue structures including prostate, neurovascular bundles (NVB), sympathetic chains, fat, kidney, urethra, ureter, aorta, colon, bladder, seminal vesicles, vas deferens, and prostatic pedicles. Noninvasive in vivo visualization of flat bladder lesions during cystoscopy and NVB during open, laparoscopic and robotic-assisted surgery looks promising to identify early bladder cancer and minimize injury and improve potency and continence preservation, respectively.

# 1310nm high-power, broad-band super-luminescent light emitting diode for OCT application

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Super-luminescent light emitting diodes (SLED) in 800 to 1300 nm wavelength windows have been widely used in optical coherence tomography (OCT) systems. The imaging resolution of OCT systems is proportional to the bandwidth of the SLED light source. We present a new design to achieve broad bandwidth (>100nm at 1310nm) in one chip by using two types of quantum wells.

The material bandwidth, confinement factor, and the length of the active region determine the bandwidth of an SLED with a single active region. Neglecting spatial hole burning (SHB), the spectral density of amplified spontaneous emission (ASE) can be the function of cavity length and spectral density of spontaneous emission and net gain. The main factor that limits the ASE bandwidth is the net gain. The bandwidth of net gain has to be larger than 200 nm to obtain a 100 nm wide ASE spectrum if the ASE power is larger than several mW.

SLEDs usually work at very high pump current (>400mA) to achieve high output power. From simulations, we found the level of electron injection mainly determines the material gain. At the high injection level, large-bandgap quantum wells can get high gain and dominate the spectrum if the improper design is used. So in our design, we put the small bandgap quantum wells at the N side to make the electron distribution in favor of long-wavelength material. Thus, and will be balanced at high current injection level (>550mA). Figure 2 shows the measured spectrum of such structure. The achieved spectral width is larger than 100nm and out put power is larger than 5 mW.

# Optical coherence tomography as a tool to assess conduit quality in coronary artery byass surgery

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Objective: Endothelial disruption within saphenous vein (SVG) and radial-artery (RA) grafts increases thrombosis risk of conduits used for coronary artery bypass surgery. However, no clinically applicable method for imaging the intima currently exists. We used optical coherence tomography (OCT), to visualize the intima within harvested conduits.

Methods: Conduits were procured endoscopically (37 SVG and 8 RA) or open (9 RA) technique from 50 patients. Surplus segments were analyzed by OCT for evidence of preexisting pathology or traumatic injury. Focal plaques in RA and intimal hyperplasia area in SVG were quantified as intimal/medial thickness ratio(IMT) >0.5. Biopsies were obtained for histological confirmation and to analyze matrix metalloproteinsase-2 levels (SVG) and prostacyclin/NO metabolites (RA). Interobserver kappa coefficients and a Bland-Altman analysis were used to determine the reproducibility and accuracy of OCT interpretations.

Results: RA imaging revealed plaque in 76% of conduits. Endoscopically-harvested vessels showed intraluminal clot (38%) and intimal tears ranging from severe (6%) to mild (88%). In SVG, intimal thickening was detected in 86% and intraluminal clot in 48%. IMT measurement by OCT correlated significantly with matrix metalloproteinsase-2 levels ($R = 0.6804$) in SVG and with metabolites of prostacyclin ($R = -0.55$) and NO ($R = -0.58$) in RA. OCT imaging was reproducible (interobserver kappa coefficients > 0.81 for the characterization of plaque types) and showed a strong correlation with histology ($R=.8$, $p<.001$).

Conclusions: OCT imaging provides an accurate, real-time and reproducible means for assessing the physiological health of SVG and RA bypass conduits. As a quality assurance tool, this technology may afford a more objective basis for conduit selection.

# Multimodal confocal mosaicing of basal cell carcinomas in Mohs surgical skin excisions

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Mohs surgery is a procedure for microscopically excising basal cell carcinomas (BCCs) while preserving maximal surrounding normal skin. Each serial excision is guided by examination of the frozen histology of the previous excision. Because several (2-20) excisions must be made and frozen histology prepared for each, Mohs surgery is time-consuming (15-45 minutes per excision) and tedious. Real-time confocal reflectance mosaicing enables detection of BCCs directly in fresh excisions, following contrast-enhancement by acetowhitening. A confocal mosaic allows rapid observation of 15x15 mm2 of tissue, which is equivalent to a low magnification, 2X view of the excision. Relatively large nodular and micronodular BCCs are rapidly detectable in confocal reflectance mosaics, whereas detection of much smaller infiltrative and sclerosing BCCs is a challenge due to the lack of sufficient nuclear/dermis contrast in acetowhitened excisions. Multimodal contrast, combining reflectance and autofluorescence may make it possible to detect infiltrative and sclerosing BCCs. A reflectance image shows both nuclei and the surrounding dermis, whereas an autofluorescence image (excitation at 488nm, detection 500-700nm) shows only the dermis. Thus, ability of a composite (i.e., reflectance-less-autofluorescence) image shows significantly darkened dermis, with stronger enhancement of nuclear/dermis contrast. Preliminary results illustrate that this may enable detection of infiltrative and sclerosing BCCs. The use of reflectance and autofluorescence as two optical stains parallels the use of two stains (hematoxylin and eosin) in histology, thus allowing a more complete optical detection method.

# Imaging breast cancer with optical coherence tomography

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The advent of modern mammographic screening has allowed for earlier identification of breast disease, and specifically breast cancer. The relatively small sizes of lesions being detected have introduced a variety of challenges for the medical and surgical communities. Suspicious tissues are more frequently non-palpable, lacking significant radiological contrast, and difficult to identify with the naked eye. During breast-conserving surgical procedures, for example, these issues may lead to difficulties in identifying the target tissue and determining the extent of the disease. As breast cancer detection and treatment moves from the macroscopic to the microscopic, there is a role for a real-time cellular-scale imaging modality that can be introduced into the surgical suite to augment current clinical methods. Our laboratory is investigating the use of optical coherence tomography (OCT) as a means to identify primary lesions and detect microscopic lymph node changes during open surgery and in conjunction with needle biopsy procedures. We present pre-clinical and clinical OCT data showing scattering changes in breast tumor tissue and metastatic lymph nodes, in addition to data on the optical properties of mammary tissue. Our images and numerical data demonstrate clear morphological changes that have the potential to be identified visually, or via algorithmic techniques that have been developed for the automated classification of normal and abnormal breast tissue types.

# Optical coherence tomography signal enhancement with gold nanoshells

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Gold nanoshells represent a novel class of particles which can provide molecular targeting and contrast enhancement during optical coherence tomography (OCT) imaging. These particles consist of a 100-300 nm diameter silica core and a 5-25 nm thick gold shell. Nanoshells have backscattering and extinction coefficients which are a function of wavelength, core diameter and shell thickness. Furthermore, they exhibit a high level of backscattering and relatively little attenuation by scattering and absorption. While this approach holds significant potential for improving disease detection, a quantitative understanding of nanoshell optical properties and the influence of nanoshell parameters on detected signals is needed to facilitate optimization. In this study, measurements were performed by spectrophotometry and 1300 nm OCT and compared to Mie theory calculations. Samples included nanoshells in water and turbid phantoms. The effects of nanoshell concentration, core diameter, shell thickness and surface-attached polyethylene glycol (PEG) on signal characteristics were investigated. Experimental results indicated trends that were consistent with Mie theory predictions. Threshold concentrations for a 2 dB OCT signal intensity gain were determined for several nanoshell geometries. For the most highly backscattering nanoshells tested _ 291 nm core diameter, 25 nm shell thickness _ the threshold concentration was 10^9 nanoshells/mL. Although quantitative agreement between experimental and theoretical data was excellent when extinction was low, theory tended to under-predict experimental values by up to 30% when extinction values were at their highest. Backscattering coefficients measured directly from the OCT signal showed reasonable agreement with theoretical values. Limited data also indicates that the addition of PEG causes a relatively small (10%) increase in extinction, likely due to absorption. The experimental data presented here helps to elucidate the optical behavior of nanoshells and should facilitate optimization of molecular-targeted optical imaging.

# Measuring cognitive functioning with diffuse optical tomography

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Diffuse optical tomography (DOT) is a non-invasive imaging technique capable of measuring functional brain activation via associated cerebral hemodynamic responses. Near-infrared light is used to detect changes in the concentration of oxyhemoglobin (HbO2) and deoxyhemoglobin (HbR), and can thus measure the dynamics of blood oxygenation. DOT has been used to measure cortical activation during visual [1,2], somatosensory and motor [3,4,5], and auditory stimulation [6] and language processing [7,8]. Previous studies performed DOT restricted to the temporal [7] and anterior prefrontal [8] cortices while subjects listened to speech or read speech aloud. We used a 32 channel system to simultaneously measure cerebral oxygenation across a large area of the left hemisphere while subjects read sentences presented word-by-word. Sentences were either concrete or abstract in content and the final word was either semantically congruous or incongruous with the sentence context. Group averages (N=6) revealed hemodynamic responses (a simultaneous increase in HbO2 and a decrease in HbR) that tended to be localized in three general locations along the probe: anterior (lateral prefrontal) middle (temporal) and posterior (temporo-occipital). Increased responses were observed for concrete compared to abstract sentences over prefrontal and temporal regions. Response differences between the congruous and incongruous conditions were observed over prefrontal cortex. These results suggest that DOT is capable of discriminating neural activation associated with semantic and language processing.

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# Multi-wavelength feflectance confocal microscopy for characterizing near-infrared wavelength sensitivity of unstained neutrophil backscattering

Z. M. Wang, C. Glazowski, J. M. Zavislan, Univ. of Rochester

Reflectance confocal microscopy detects backscattered light and can provide images at sub-cellular resolution in-vivo. As Mie scattering theory predicts, light scattered by small particles is determined by wavelength, particle size and refractive index. We have built a multi-wavelength (785nm, 810nm and 850nm) reflectance 0.9 NA confocal microscope that provides additional contrast, especially for cells containing numerous submicron granules like neutrophils. We are investigating the identification and differentiation of immune cells in-vivo, which has potential clinical application in noninvasive dermatitis diagnosis. Because wavelength induced contrast is sensitive to granules, degranulation of unstained neutrophils in-vivo in inflammatory response may be monitored. During multi-wavelength imaging each of the wavelengths can be switched on and off at the frame rate of 100ms. The axial focal position is controlled to select the image plane for each wavelength. Software developed in LabView synchronizes the wavelength switch, focus position and image acquisition. Polystyrene spheres (10um diameter) are used to validate the instrument performance and calculate the instrumental background drift. To develop image analysis algorithms neutrophils are separated from blood and imaged in physiological buffer without staining. A single neutrophil section can be imaged at three wavelengths within 200ms. Results will be presented on the comparison of image analysis algorithms such as cross-correlation to identify unstained immune cells.

# Analysis of time-resolved fluorescence data using Laguerre deconvolution

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Time-resolved fluorescence (TRF) measurements from layered biological tissue provide chemical and structural information which may be useful for imaging or probe-based tissue diagnostics. Recently, there has been growing interest in the use of Laguerre functions for non-parametric modeling of complex biological systems. Interest in this technique for TRF in cells and tissues is due in part to Laguerre's ability to represent exponential functions. We examine the accuracy and robustness of a Laguerre-based approach through the implementation of artificially generated fluorescence decay curves. Each curve is generated using a pre-recorded nitrogen laser pulse that is convolved with bi-exponential fluorophore decay curves representative of colonic mucosa. Pre-set levels of Gaussian-distributed noise are added to the convolved curves to achieve variations in the signal to noise ratio (SNR). A Laguerre basis is used to represent and expand the fluorescence decay function. The best fit is found using iterative nonlinear least squares minimization. Our deconvolution algorithm also incorporates an improved method for the selection of the decay parameter value, which usually carries important system information in the Laguerre basis. The ability of this technique to differentiate small changes in fluorophore lifetimes as a function of the SNR are compared with that of an established multiexponential iterative deconvolution approach. Results indicate that a Laguerre-based approach may be more accurate, robust and convenient than standard multiexponential deconvolution routines, particularly when the diagnostic does not require calculation of intrinsic fluorophore lifetimes.

# Imaging molecular interactions of oncogene RhoC in living cells using FLIM/FRET

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RhoC (Ras Homology protein C) has been identified as a specific marker of aggressive breast cancers. Its activation can lead to a highly invasive, angiogenic, and metastatic phenotype. Because the sub-cellular localization of RhoC and its binding with effectors are essential to its function, we employed fluorescence resonance energy transfer (FRET), with high spatial sensitivity, to detect these nanoscale events.

We used time-resolved fluorescence lifetime imaging microscopy (FLIM) to detect FRET between RhoC and RhoGDIγ; (a protein that binds inactive RhoC), because FLIM is known to be generally independent of artifacts influencing fluorescence intensity. We chose Cerulean/EYFP as our FRET pair, because Cerulean is reported to have higher brightness than the more generally used ECFP. Plasmids encoding fusion proteins were transfected into CV1 cells for optical imaging.

In our results, nearly significant trends towards shorter fluorophore lifetimes for Cerulean were consistently detected for Cerulean-RhoGDIγ + EYFP-RhoC vs. Cerulean-RhoGDIγ, or vs. Cerulean-RhoGDIγ + EYFP (significance level = 0.1), confirming FRET occurring between RhoC and RhoGDIγ. Our results also suggested that, in our system, Cerulean yields more stable and consistent results than ECFP (which is commonly paired with EYFP in FRET experiments).

Thus, FLIM/FRET experiments using Cerulean/EYFP pairs have the ability to image the interaction between RhoC and RhoGDIγ. This provides, for the first time, an exciting opportunity to explore other RhoC functions, which will help us develop potential breast cancer treatments and diagnostic techniques.

# Utilizing laparoscopic hyperspectral imaging during minimally invasive surgery

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Background: Minimally invasive operations have transformed the field of surgery. Major operations are now performed though a series of 1cm incisions rather than through a large opening resulting in less pain, disability and fewer complications. Visualization of intra-abdominal structures is accomplished by endoscopes, 9mm diameter tubes containing relay optics. Application of advanced imaging techniques should enhance the surgeon_s ability to perform operations more safely.

Methods: Infrared hyperspectral imaging has been adapted to endoscopic surgical instruments via a near infrared liquid crystal tunable filter and a back-illuminated, deep-depletion focal plane array with anti-etaloning process for extending sensitivity in the near infrared region. Spectroscopic image data collected from inside the body at each array detector is formatted into a three dimensional hyperspectral data cube consisting of both spatially resolved spectra and wavelength dependent images. Deconvoluting the spatial and spectral information provides a gray scale or color encoded chemical image visualizing intra-abdominal anatomical structures based on inherent tissue chromophores.

Results: This imaging system has been used to visualize the biliary tree in anesthetized pigs. The biliary system, which contains a lipid chromophore, absorbs IR radiation differently than adjacent tissues resulting in very high contrast.

Conclusion: IR hyperspectral imaging has great potential for improving surgeon_s ability to visualize bile ducts during surgery, reducing the risk of injuring these structures. Because IR penetrates deeply into tissue and has a lipid absorption peek at 930nm, it might be possible to see these bile ducts under the tissue surface that cannot be visualized with visible light.

# Functional imaging in freely moving rats

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Recently we introduced a system for Diffuse Optical Tomography (DOT) imaging in freely moving rats, which shows promise of making possible many of the measurements currently performed with fMRI, PET and similar methods, but without the need to anesthetize or immobilize the subject, and at much lower cost. The system has good (~1 mm) spatial resolution, high temporal (~17 Hz) resolution and can record continuously for long intervals (tens of minutes, to hours). In addition, we have combined DOT methods with EEG and video recordings, allowing us to classify optically detected hemodynamic signals according to the electrical state of the rat_s brain and to its observes behavioral states, which provides a means of validating DOT signals obtained from mobile animals. The measuring head allows for 16 optical source-detection locations with dual-wavelength illumination, plus 12 EEG electrodes, 6 implanted in each dorsal hippocampus. Using the described system we have found that time-varying DOT signals differ greatly according to the state of the hippocampal EEG (h-EEG). In particular, during epochs in which the h-EEG exhibits the 5_12 Hz theta rhythm, hemoglobin oxygen saturation (HbO2Sat) values are significantly greater than during non-theta periods. Moreover, the magnitude of HbO2Sat difference rises as the dwell-time threshold for each state is increased, a result compatible with the presence of a time lag in the hemoglobin response. The overall response patterns we observe, furthermore, are consistent with MR BOLD findings. Detailed descriptions of hardware, software, experimental protocols, and data analyses will be presented.

# Dual-wedge scanning confocal reflectance microscope

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Confocal reflectance microscopy has been shown to provide optical sectioning and resolution sufficient to provide useful information about skin to a depth below the epidermis. However, existing instruments are large and expensive, because of the need for fast two-dimensional scanning in the pupil, and the associated relay optics. A more compact scanning system could lead to an affordable hand-held instrument for in vivo imaging. Several approaches are being considered with different advantages and disadvantages. Here we report on one of these. A confocal reflectance microscope is being developed with a dual-wedge scanner to reduce the cost and complexity while retaining the resolution and sectioning of current point scanning instruments. The scanner is implemented by replacing the two scanning mirrors and the telescope between them with two optical prisms that are rotated about the optical axis. Ultimately the scanner could be incorporated directly into the pupil of the objective, eliminating all relay telescopes. This scanning configuration produces a circular scan if the prisms are rotated in the same direction, or a flower-petal scan if the prisms are rotated in opposite directions. Preliminary experimental results with the microscope show a lateral resolution on the order of 1-2 micrometers and on-axis optical sectioning on the order of 3-4 micrometers. Early experiments have demonstrated the ability to section the fibers in a paper target.

# Optical diagnosis of cancer in lymph nodes and thyroid glands

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The combination of IR Microspectroscopy (IR-MSP) with multivariate data analysis such as hierarchical cluster analysis (HCA) and artificial neural networks (ANN) promises to be a powerful tool in the detection and diagnosis of cancer and disease in tissues and cells. The rapid and objective approach of these methodologies offers the potential of _real-time_ results in the operating room.

Hyperspectral maps of thin tissue sections of lymph nodes and thyroid glands were collected via a Perkin Elmer Spectrum One/Spotlight 300 Infrared Spectrometer (Perkin Elmer Corp, Shelton, CT). These images were then analysed with HCA to correlate clusters with morphological features and tissue types within the sample. An ANN was then trained using clusters of tissue and disease types as inputs.

The resulting neural net was able to differentiate breast metastatic cancer in a lymph node section. The same neural net was applied to a lymph node containing metastatic colon adenocarcinoma. The net correctly identified tissue types such as B and T lymphocytes but was unable to classify the colon metastasis, introducing the exciting possibility that not only can we identify metastatic tumors but we are able to identify the site of the primary tumor from the IR spectra of the metastases because the spectra are distinguishable.

# Characterization of fluorescence lifetime of Photofrin and ALA induced PpIX

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We report the investigation of fluorescence lifetime of delta-aminolevulinic acid (ALA) induced protoporphyrin IX (PpIX) and Photofrin(r) both in-vitro and in-vivo in MAT-LyLu (MLL) rat prostate adenocarcinoma cells. Photo-dynamic therapy (PDT) has been extensively investigated in the past decade as an effective treatment option for various types of invasive tumors. The efficacy of PDT treatment depends strongly on cell uptake and subsequent photosensitization of these sensitizers. Currently, there are two photosensitizers have been approved for clinical PDT treatment: Photofin and ALA induced PpIX. Characterization of fluorescence lifetime of these drugs provides the basis for further investigation of in-vivo PDT dosage measurements using time-domain spectroscopy and imag-ing. Several physiologically relevant concentrations of the photosensitizer solutions were prepared. A picosecond diode laser was used to excite the two drugs and the spectrally-resolved fluorescence decay was recorded using a time-correlated single photon counting (TCSPC) system. MLL cells were incubated with the photosensitzers and were treated with light under well oxygenated or hypoxic conditions. Fluorescence lifetime images of these cells were recorded by a confocal FLIM microscope. The measured fluorescence lifetimes of both photosensitizers are much longer than typical endogenous tissue fluorescence, which suggests time-domain methods are good candidates for in-vivo PDT monitoring.

# Detection of increased blood supply in superficial colonic mucosa in early colon carcinogenesis

Y. L. Kim, V. M. Turzhitsky, A. K. Kromin, R. K. Wali, H. K. Roy, M. J. Goldberg, P. Vakil, R. Brusen, V. Backman, Northwestern Univ.

Although increased blood supply (IBS) via angiogenesis at late stages of cancer has revolutionized the understanding of carcinogenesis, the stage at which microvascular IBS (not necessarily via angiogenesis) occurs remains unclear. Indeed, IBS may occur at an initial stage of carcinogenesis, because altered cellular activities at a premalignant stage suggest metabolic demands.

In order to investigate this issue in colorectal cancer, we first conducted animal studies of colon carcinogenesis (58 AOM-treated rats and 16 MIN mice) and a pilot human study (n = 63), using polarization light scattering spectroscopy that quantifies hemoglobin concentration in the superficial colonic mucosa (50 – 100 μm below tissue surface). We further used miniature polarization-sensitive fiber optic probes that can be inserted through a channel of the colonoscope to assess blood concentration in superficial colonic mucosa of patients (n = 102) undergoing colonoscopy.

We report microvascular IBS at a premalignant stage in the animal models and at an adenomatous stage in the human study. Spatial and temporal correlations with conventional biomarkers supported this finding as an early event in colon carcinogenesis. Using in vivo measurements, we further observed the spatial variation of IBS in patients harboring adenomatous polyps. IBS reached its peak on adenomatous polyps and decreased gradually as the measurements moved away from the polyps, demonstrating that IBS is a localized event in early colon carcinogenesis. Thus, our finding may provide novel insights into cancer biology in early carcinogenesis as well as a potential method for cancer screening and biopsy guiding.

# Bioluminescence tomography with evolutionary algorithms

A. D. Klose, Columbia Univ.

Bioluminescence tomography (BLT) is a novel imaging modality for studying disease-associated processes on a molecular level prior to the development of macroscopic tissue changes. The underlying mathematical model for tomographic reconstruction is an ill-posed inverse source problem, which can be cast into an optimization problem of a χ2-error function. The error function quantifies the discrepancy between the measured and predicted partial current of bioluminescent light at the tissue boundary. Local optimization techniques, such as gradient-based methods, are likely to fail if multiple minima of the error function are present. Therefore, a global optimization technique based on an evolutionary algorithm is proposed that samples the entire parameter space of all possible bioluminescent source distributions and avoids the premature convergence due to local minima.

The bioluminescent source power density within the tissue domain is expanded into source basis function. The partial current on the tissue boundary is calculated by solving the equation of radiative transfer (ERT) for each basis function prior to image reconstruction. The ERT fully describes the light propagation in tissue with small geometries and non-diffusive tissue domains as relevant to BLT of small animals. Since no ERT needs to be solved during image reconstruction, only evaluations of the error function need to be carried out. This provides the basis for using ERT-based evolutionary algorithms, which stochastically sample large data sets of source distributions. Initial image reconstruction results of bioluminescent source distributions in three-dimensional numerical mouse models will be presented and will be compared to diffusion- and gradient-based image reconstructions.

# Reconstructing oxygen consumption and blood flow in diffuse optical tomographic breast imaging under mammographic compression

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The tomographic optical breast imaging (TOBI) device at MGH acquires diffuse optical tomography (DOT) data while the breast is under mammographic compression. Recent work has shown that this compression creates dynamic changes in hemodynamic parameters during imaging. On the one hand this dynamic behavior complicates the reconstruction problem since

assumptions of static hemodynamics will not lead to accurate reconstruction; on the other it may represent an opportunity as it is reasonable to hypothesize that the dynamic behavior, if included in the reconstruction, will be an additional source of contrast for DOT breast imaging. In another study presented at this meeting we report on a study of data acquired from subjects during simulated mammographic compression which shows that oxygen consumption and volumetric blood flow may be estimated dynamically from optical measurements using a model-based approach. In this study we report on simulations to examine the feasibility of reconstructing these hemodynamic parameters using a DOT inverse solution.

Specifically we simulate DOT data for a region of tissue which includes a “tumor”, modeled as an region where oxygen consumption and blood flow differ from the background tissue by values corresponding to those reported in the literature from PET studies. We then estimate these parameters from noise-corrupted simulated data using two distinct approaches. In the first we solve the linearized DOT inverse problem to reconstruct concentrations of oxy- and deoxy-hemoglobin at each time point, and then post-fit the time courses of each voxel to our model equation for the behavior of SO2 in compressed breast tissue. In the second approach, we include the SO2 model equation into the inverse solution and solve the resulting non-linear problem directly for oxygen saturation and blood flow. Results indicate that either method has the potential to recover spatial contrast in these hemodynamic parameters. Evaluation of computational and accuracy tradeoffs, as well as study of the most effective and accurate methods to solve the direct parameter estimation problem, are on-going.

# NAVI: a problem solving environment (PSE) for NIRS data analysis

Y. Pei, NIRx Medical Technologies; Z. Wang, Columbia Univ. and New York State Psychiatric Institute; Y. Xu, SUNY/Downstate Medical Ctr. and NIRx Medical Technologies; R. L. Barbour, SUNY Downstate Medical Ctr. and NIRx Medical Technologies

The expanding interest in near-infrared spectroscopic (NIRS) imaging has generated a growing demand for an integrated computing environment that is capable of exploring the richness and complexity of spatiotemporal measures of blood delivery to tissue. As with other complex systems, a crucial component for the study of these phenomena is a PSE that allows for the description, discovery and analysis of relevant phenomenology in ways that retain computational efficiency and facilitate file management. NAVI, (Near-infrared Analysis, Visualization and Imaging), is a rich constellation of tools for the examination of functional NIRS data that incorporates instrument performance monitoring, filtering, image formation, feature extraction, visualization, statistical analysis, and file and database management functionalities. NAVI utilizes the MATLAB run-time component and is distributed as a stand-alone program for either Windows(r) or Linux environments. It offers point-and-click navigation and visualization of data within a flexible file-management system that employs wizards to facilitate group data loading, batch processing, automated file system creation, and recording of parameter settings used in data processing. A variety of image visualization styles is available, and NAVI makes generous use of montage formats for the overlaying of multiple-feature information. By striking an optimal balance between computational effort and computer memory, together with use of fast algorithms, NAVI enables analysis and visualization of large-scale data sets with a minimal operator training requirement. In addition, its image conversion functions allows export of NAVI-generated images into standard image formats for use with other software packages, such as SPM, Medx, AFNI and GiD.

# In vivo characterization of autofluorescence dynamics during renal ischemia and reperfusion under dual UV excitation

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There is currently no reliable method to measure the degree of tissue ischemic injury or recovery in a clinical setting. To address this problem, we explore an optical spectroscopy approach to monitor the progression of ischemia and reperfusion in situ using a rat model. The system takes advantage of the sensitivity of NADH emission to changes in metabolism during ischemia and reperfusion. In addition, the emission from tryptophan is utilized as a normalization factor to take into account changes in the optical properties of the tissue. Ischemia was induced in one kidney for either 20 minutes (n=12 rats), 50 minutes (n=12), or 150 minutes (n=15), followed by 60 minutes or more of reperfusion. During both phases, autofluorescence images of the exposed surfaces of the ischemic and normal (control) kidneys were acquired alternately under 355 nm and 266 nm excitation wavelengths, and the average emission intensities were measured. The emission from 266 nm excitation is found to be only minimally sensitive to ischemia and reperfusion. The signal ratio of the emission intensity from 355 nm excitation to that from 266 nm is modeled as the product of two independent exponential functions. Model fits to the data show that longer injuries reveal longer average relaxation and delay time constants during reperfusion. We postulate that such a method may be able to assess the reversibility of ischemic injury and predict tissue viability in the initial period following transplantation or resuscitation.

# Contrast agent pharmacokinetics in breast cancer: ICG and Gd-DTPA

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S. Nioka, Univ. of Pennsylvania

Contrast agent enhanced MRI has recently emerged as an important diagnostic tool, especially for cancers without microcalcifications. Over the last several years, we have developed techniques and instrumentation to optically collect ICG kinetics information during an MRI exam, allowing direct comparison of kinetics data with Gd. Here, we present these comparisons in several cancer types. Our initial results suggest that ICG and Gd uptake by tissue is correlated in space, in agreement with the results of Ntziachristos.

# Maximum-likelihood multi-dimensional photon-counting microscopy

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Multi-dimensional imaging, in which detailed spectroscopic information is collected at each pixel, is a developing tool that has already enabled breakthrough results in biophysical research. Commercial microscopes are now available with software for _linear unmixing_ that can routinely resolve fluorescent species with overlapping emission spectra. We are working to extend such capabilities to ultrasensitive fluorescence microscopy applications. We present preliminary results and calibration experiments from a custom-built microscope, which incorporates dual-wavelength pulse-interleaved laser excitation, diffraction-limited confocal imaging with 3-dimensional piezo-scanning, an adjustable prism spectrometer for high-throughput resolution of collected fluorescence into 4 spectral bands, and four-channel high-quantum efficiency picosecond-resolved single photon detection. Issues of electronic dead-time and count-rate dependent time-walk of the detectors are discussed. Custom software enables multi-band fluorescence correlation spectroscopy and identification of photon bursts for single-molecule detection. For unmixing of spectrally-overlapping signatures for ultrasensitive molecular imaging applications, we find that maximum-likelihood analysis can out-perform least-squares-based linear unmixing in the regime of low photon numbers per spectral/temporal channel. Also, the likelihood surface provides the confidence of the parameter estimates and the covariance of the species contributions. Monte Carlo simulations show that bias in the results of the analysis, which stems from the constraint that photon numbers should be positive, becomes more pronounced at low signal levels, for both maximum-likelihood and least-squares based unmixing. Our calibration experiments use Alexa dyes with overlapping spectra. FRET, and cellular imaging applications using intrinsic fluorescent proteins, are discussed.

# Exploring DNA processing one molecule at a time

R. Conroy, A. P. Koretsky, National Institutes of Health

The ability to image and track single molecules with nanometer accuracy and millisecond resolution has opened the possibility to observe the operation of single enzymes on DNA. This poster will present our latest results on the sequence specificity of exonuclease enzymes, in particular for short repeating DNA sequences.

# Laser speckle contrast yields high contrast, high resolution images of cerebral microvasculature

K. Murari, N. Li, A. Rege, N. V. Thakor, Johns Hopkins Univ.

We demonstrate a novel technique for imaging microvasculature using the principle of laser speckle contrast. When laser light is shone on tissue, the motion of red blood cells through the vasculature causes dynamic interference patterns to form. These patterns are imaged and processed to identify vessels over the background. This technique offers the unique advantage of being minimally invasive and thus useful for neurophysiological investigations where imaging through thinned skull preparations is preferred.

We obtained images of vasculature in a 3mm x 3mm area of the rat barrel cortex by shining red (HeNe 632nm) and green (DPSS Nd:YVO4/KTP 532nm) lasers independently and recording with a 14 bit cooled CCD camera. Thinned skull preparations, and subsequently opened skull preparations were imaged.

A comparison of the images obtained by laser speckle contrast with those obtained in white light clearly highlights the advantages of our technique. The red laser penetrates to a greater depth to potentially reveal sub-surface vessels while the less penetrative green laser produces visibly sharp and high contrast images of the surface vasculature.

The images were compared with images obtained by introducing a fluorescent dye (dextran-rhodamine) into the rat_s vasculature through a femoral vein injection. The green laser was used to excite the dye and images were captured through a rhodamine emission filter. The vessels in the laser speckle images can be seen to conform to those in the dye images.

This high contrast, high resolution technique shows promise in imaging vascular architectures in the brain and retina in normal and perturbed conditions such as tumors.

# Miniaturization of a two-photon microscope and development of novel contrast agents for in-vivo cancer imaging and microsurgery

C. L. Hoy, N. J. Durr, D. Smith, The Univ. of Texas/Austin; K. V. Sokolov, The Univ. of Texas M.D. Anderson Cancer Ctr.; B. A. Korgel, The Univ. of Texas/Austin; O. Solgaard, Stanford Univ.; A. Ben-Yakar, The Univ. of Texas/Austin

Two-photon microscopy (TPM) has demonstrated great potential as a tool for tissue imaging because of the increase in depth and optical sectioning capabilities over other forms of fluorescence imaging. In addition, higher energy femtosecond pulses have demonstrated a desirable combination of high precision and low collateral damage making femtosecond lasers an ideal tool for laser microsurgery. Though both technologies have shown promise for use in medical diagnostics and therapeutics, thus far their applications have been confined almost entirely to the research laboratory. We present the design and demonstration of a novel compact (15 mm x 30 mm x 10 mm) two-photon microscope employing an air-core photonic crystal fiber and two-axis microelectromechanical systems (MEMS) scanning mirrors designed for combined cellular imaging and femtosecond laser microsurgery. To further aid the imaging abilities of the compact microscope, we also present the use of two-photon luminescence (TPL) of gold nanorods as a targeted contrast agent for the early detection of cancerous cells. Gold nanorods offer a desirable alternative to traditional two-photon contrast agents because of their tunable optical properties, biocompatibility, and resistance to photobleaching. In this poster we present our recent results for imaging and microsurgery of nanorod-labeled cancer cell tissue phantoms, using both the 15 mm two-photon microscope and our large scale table-top TPM system. This research was conducted to complement ongoing development of a 5 mm-wide two-photon endoscope-on-a-chip for in-vivo diagnosis and treatment of oral cancers

# 1 kHz imaging device with single photon sensitivity

D. S. Barnhill, Univ. of California/Los Angeles

We have developed an innovative ultra-fast imager which comprises a mega-pixel CMOS array and an Image Intensifier with GaAsP photo-cathode (50% Quantum Efficiency at 600 nm). It has single photon sensitivity and it can be operated at the full frame rate of 500 Hz (i.e. much faster than that of currently available devices such as EMCCD). By applying a Region of

Interest (ROI) algorithm on the CMOS array, the frame rate can be speeded up as high as 100 kHz per fluorescence dye. Such a device is expected to significantly improve fluorescence-based ultra-fast imaging and spectroscopy for studying the dynamical behavior of single molecules in vivo. In this paper, we present the design principle and the preliminary results on its performance. We will also show our future applications to cancer and Neuroscience research.

# Protein nanospheres as photoacoustic contrast agents for imaging, molecular targeting, and therapy

M. A. McDonald, Stanford Univ. Medical Ctr.; F. Hunter, J. Xie, K. C. Li, National Institutes of Health; S. Guccione, Stanford Univ. Medical Ctr.

Protein nanospheres are target specific diagnostic and therapeutic agents capable of serving as a platform for imaging across several modalities; including optical, MR, CT, US and radioisotope based techniques. We have developed stable, highly absorbing protein nanospheres which emit large echoes in response to ultrasound stimulation and an even greater acoustic response to laser stimulation for photoacoustic imaging. In the present study receptor specific targeting and gene delivery are being evaluated in mammalian cells, phantom models and animal tumor models. The contrast agents used include fitc labeled protein nanospheres (fitc-NS), rhodamine labeled nanospheres (Rhd-NS), eGFP DNA loaded nanospheres (eGFP-NS) and β-galactosidase DNA loaded nanospheres (β-gal-NS). Physical characterization demonstrates negatively charged monodisperse particles. Fluorescent microscopy of fitc-NS and Rhd-NS mouse labeled melanoma cancer cells reveals NS accumulation in tumor cells proportional to NS concentration and incubation time. Fluorescent microscopy of eGFP-NS transfected mouse melanoma cancer cells show high levels of transfection in an incubation time and NS concentration dependent manner. Cancer cell immunohistological staining with anti-eGFP antibody and immunofluorescent anti-(NS) antibody demonstrates NS mediated cancer cell labeling correlates with gene transfection. Analysis of NS mediated β-gal expression via Beta-Glo transfection assay and cell viability analysis indicate NS utility in gene delivery. Agarose gel electrophoresis studies reveal β-gal DNA is associated with NS and likely intact. We demonstrate receptor-specific targeting of cancer cells using a probe synthesis technology amenable to utilization of other relevant biological molecules. The size range at which we are able to achieve mondisperse distribution makes extravasation of nanospheres for imaging of tumor vasculature more likely and improves the chances of receptor targeted imaging.

# Molecular imaging and contrast agent database (MICAD): a new and freely accessible online source of information

B. Beck, S. Bryant, K. T. Cheng, W. C. Eckelman, K. H. Leung, E. Lutanie, J. McEntyre, A. Menkens, D. C. Sullivan, National Institutes of Health

Objective: This poster will describe and discuss the rationale, goal, development process, database structure, key components, current status, and future plan of the Molecular Imaging and Contrast Agent Database (MICAD) project.

Background: MICAD (http://micad.nih.gov) is a new, freely accessible online source of scientific information on in vivo molecular imaging agents. It has been developed as a key component of the Molecular Libraries and Imaging (MLI) program of the National Institutes of Health (NIH) Roadmap for Medical Research in the 21st century. The NIH Roadmap (http://nihroadmap.nih.gov) is a major strategic initiative of the agency that integrates the resources of different institutes/programs within NIH for meeting the challenge of the complex biomedical research. The MLI program (http://nihroadmap.nih.gov/molecularlibraries) is a component that addresses the critical issue of finding new pathways to discovery by supporting the emerging field of molecular imaging.

Methods: As a part of the MLI program, MICAD focuses on providing critical and timely scientific information to enhance the discovery and availability of molecular imaging agents. Under the direct management of the National Center for Biotechnology Information (NCBI/NLM) and the National Cancer Institute (CIP/NCI), the MICAD team is composed of dedicated NIH scientists who have been working with the guidance of a trans-NIH panel of experts in the field of molecular imaging. MICAD is designed to provide concise, up-to-date, and most relevant information on molecular imaging agents in an easy-to-read textual format to the basic and clinical research community. The database includes, but is not limited to agents developed for positron emission tomography (PET), single photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), ultrasound (US), computed tomography (CT), optical imaging, planar radiography, and planar gamma imaging. The information is summarized in 5 major sections: background, synthesis, in vitro studies, animal studies (rodents, non-primate mammals, primates), and human studies. The database also features literature references, chemical structures, numerous links to abstracts in MEDLINE/PubMed and PubChem, and additional related resources at NCBI.

Results and Conclusion: MICAD website was officially launched in September, 2005. It currently contains more than 70 agents and is fully searchable. All molecular imaging agents that are published in peer-reviewed literature will eventually be included. MICAD is a dynamic program and is actively seeking feedback and recommendations from the imaging community.

This project is supported by the Intramural Research Program of the NIH.

# Assessing non-invasive detection of protoporphyrin IX fluorescence in vivo to quantify glioma tumor growth

S. L. Gibbs, Dartmouth College; J. A. O'Hara, Dartmouth Medical School; P. J. Hoopes, Dartmouth Hitchcock Medical Ctr.; B. W. Pogue, Dartmouth College

In the current study a rat glioma cell line that had been transfected with green fluorescent protein (GFP) was implanted intracranially into nude mice. Aminolevulinic Acid (ALA) was administered to the animals two hours prior to sacrifice to allow for the peak fluorescence production and accumulation of Protoporphyrin IX (PpIX). The PpIX fluorescence of the tumor bearing animals was compared to that of control animals without tumors via transmission fluorescence spectroscopy as a noninvasive imaging method for identification of implanted and spontaneous brain tumors in mice. The brains of the animals were further studied via fluorescence microscopy to determine that PpIX fluorescence matched with the GFP fluorescence and/or H+E staining and thus was specific to the brain tumor tissue for this tumor type. The ability to detect the fluorescence above the background skin fluorescence was assessed, and determined to be feasible, although the background skin fluorescence is a significant barrier to overcome. Ex vivo measurement of the brain fluorescence with tumor tissue illustrates a 5 times increase in fluorescence relative to the non-tumor bearing brains. The fluorescence microscopy analysis indicated more like a 2 times increase in fluorescence in the tumor bearing mice over the non-tumor bearing mice. Technological approaches to maximize the signal detection non-invasively are proposed. This tumor tissue selectivity for PpIX generation in the brain tumor has been applied in surgical resection quite successfully; a new application for this tissue selectivity could be to monitor cancer treatment. Since PpIX is produced via the metabolism of the cells, PpIX production following cancer treatment would allow for the efficacy to be assessed via noninvasive fluorescence imaging.

# Intraoperative imaging to assess organ viability: from bed to bench side

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Previous studies have demonstrated that infrared (IR) intraoperative imaging is a reliable tool to identify perfusion of brain tumors and kidneys in human surgery. To explore further imaging specific to organ structure and ischemic injuries, intra-operative IR and fluorescent imagery was performed on a porcine model. Laparotomy was performed allowing optical access to the organs of interest. IR (Lockheed Martin, US, 3-5μ), fluorescent (Novadaq, Canada, 600-835nm), and visible wavelength cameras were installed 30-40 cm above exposed ureter, kidney, and bowel. Images (320x256 pixels for IR and 768x494 pixels for fluorescent cameras) were collected every second during baseline conditions as well as during infusion of room temperature boluses of saline (IR sessions) or indocyanine green (ICG) fluorescent contrast cocktail (0.2 ml of a 0.25 mg/ml solution, activation with 806 nm laser). In order to produce a focal perfusion deficit either vascular clips (to occlude the vascular supply to the whole kidney or one of the segmental renal arteries) or ligation (for an isolated segment of bowel) was used.

Ureter: Both IR and fluorescent imaging enabled the surgeon to view, record, replay, print, and archive high quality, real-time images of a ureter during infusion of contrast agent. However, as a result of tissue saturation with ICG, fluorescent imaging was not useful after the second bolus injection. IR imaging was repeated multiple times by slightly changing temperature of infused saline (cooler or warmer then previous bolus by ~1°C). Synthesis of derivative images from collected IR images allowed visualization of the velocity pattern of the bolus within the ureter and, therefore, any non-uniformity of structures within the ureter. Fusion of IR and visible images allowed outlining the ureter based on functional (IR) and surface structural features.

Bowel: The site of ischemic injury was visualized by fluorescent imaging with more detail than with IR. The region of bowel rendered ischemic did not pick up the agent and therefore did not fluoresce, making identification simple. Adjacent vascular arcades were visualized and showed striking fine vessels structure. However, the observed washout time (5-7 min) was a limiting factor.

Kidney: Both IR and fluorescent methods allowed visualization of gross renal perfusion. However, as in the previous example with the ureter, fluorescent imaging failed to visualize partial occlusion and reperfusion due to ICG buildup in the kidney parenchyma. IR imaging immediately showed what segment of the kidney was occluded or reperfused. Further analysis of the IR data demonstrated low frequency oscillations attenuated substantially at the site of ischemic segments but not in the perfused segments of the kidney.

Intraoperative imaging offers a promising modality for real time ureteral identification and assessment of renal and bowel perfusion. Because of limited penetration capabilities of the light and an increase in background fluorescence during acquisition of successive fluorescent images, the fluorescent method can be used only for visualization of ischemic area involving superficial vessels. Kidney perfusion focal deficits were easily identified using IR imaging, even without bolus injection. Observed low frequency blood flow oscillations were probably related to local vasomotion and endothelial layer damage at the site of ischemia. This phenomenon may be useful for the assessment of ischemic injury and endothelial cell integrity.

# Novel quantum dot based superluminescent light-emitting diodes (SLEDs) for optical imaging

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Superluminescent Light-Emitting Diodes (SLEDs) are semiconductor light sources that show a broadband output optical spectrum (like LEDs) at high powers levels (like semiconductor lasers). In other words, SLEDs join features typical of LEDs like low coherence, as well as the of semiconductor lasers like high optical power. This combination is extremely useful for a wide range of optical imaging applications.

Semiconductor devices based on quantum dots (QD) are ideally suited as the active material for SLEDs since the size dispersion typical of self-assembled growth naturally produces a large inhomogeneous broadening. The large spacing between different energy levels can lead to improved thermal stability as well.

In this poster we report latest results of QD based SLED operating in the range of 1200 nm to 1300 nm specially suited for Optical Coherence Tomography.

# Development of a disposable microendoscope objective for early cancer detection

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An in-expensive, high performance, microendoscope objective used for in vivo imaging of precancer is presented. The objective is designed to be a single use disposable unit eliminating any complicated sterilizing/disinfecting procedures. All major objective components- plastic injection molded lenses, commercial glass lenses, and LIGA fabricated opto-mechanics- are described. The opto-mechanics incorporate a unique lens self-centering mount and hydraulics within a housing that measures only 3.85 mm in outer diameter and 15.71 mm in length. The objective is diffraction limited for 850 nm wavelength, has a numerical aperture of 1.0, a field of view of 250 microns, and a working distance of 450 microns. A prototype has been fabricated and tested for performance using two methods: an initial USAF resolution target test and a more comprehensive slanted-edge MTF evaluation. The prototype demonstrates an average Strehl ratio of 0.62 across four image locations. Preliminary biological images have been collected by the objective used with a fiber confocal reflectance microscope.

# Technical considerations in longitudinal multispectral small animal molecular imaging

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In a previous study we investigated physical methods to reduce whole-body autofluorescence spectra in several mouse strains through changes in animal diet1. Measurement of live mice with the CRi Maestro multispectral imaging system over an eleven-day interval allowed for quantification of spectral changes in the autofluorescent signature across most of the visible spectrum. Consistent analysis of autofluorescent signal as a function of excitation spectrum, animal strain, and animal diet presented many technical challenges that are applicable to any longitudinal multispectral small animal molecular imaging study. These challenges included careful selection of spectra corresponding to given molecular labels and Measurement Region Of Interest (ROI) shape and positioning. The CRi Maestro system offers the powerful ability to spectrally unmix multiplexed signals. Even so, proper selection of robust spectra corresponding to specific molecular labels of interest was of integral importance to enable batch processing of the large datasets generated by longitudinal experiments. ROI shape and positioning depended largely upon animal strain, animal positioning, signal strength, and parameter of interest. We will present experimental methods to account for and successfully overcome these technical challenges in any longitudinal multispectral small animal molecular imaging experiment.