Three-dimensional confocal fluorescent images are now obtainable at a minimum cost for use in broad applications.

Nikon proudly introduces a universal confocal microscope system that is ultra-compact and lightweight, yet provides confocal images of the highest quality in its class. All main components are modular, including the world’s smallest and lightest scanning head, making expansion and maintenance easy. Furthermore, 3-channel detection is possible with minimum upgrade, and operation is facilitated by the intuitive software. With the C1, confocal microscopy is now a mainstream technique affordable by all.

**Highest quality optical performance**
A successful fusion of Nikon’s optical and electronics technologies, the C1’s resolution, contrast, and fluorescent image brightness are all top-class and “State of the Art.” Image sizes of up to 2K by 2K at 12 bit image depth can be easily scanned. See page 4-5.

**Interchangeable filters**
Changing the filter to match the fluorescent dyes you want to use is simple and quick, enabling the use of the latest probes or dyes available today. See page 5, 6.

**3-channel simultaneous detection**
The C1 supports almost any imaging technique required today, including simultaneous 3-channel fluorescence, 3-channel plus DIC, time-lapse recording, and spatial analysis. See page 5, 6.

**Modular design saves space and facilitates upgrading**
All main components are modular, including the laser box, scanner head, and detector module, saving desk space and allowing easy upgrades and maintenance. See page 10.

**Intuitive software promotes multifaceted microscopic analysis**
The C1’s Graphical User Interface (GUI) is extremely simple and intuitive. From the time of initial use, you may never need to refer to the manual for typical operation. See page 7-8.

**Easy to configure, easy to operate**
Each of the modules is pre-calibrated, eliminating the need for calibration during setup. Just connect the modules you need, and you are ready for optimal image capture. See page 10.

**Compact design**
With a small footprint, the C1 does not get in the way of other lab equipment. Consequently, there is plenty of work space left around the microscope. See page 10.
with unsurpassed resolution and contrast—bio-research applications
Unprecedented image quality

Why are the C1’s images so good?

Every aspect of the C1 that affects image quality has been thoroughly examined—from optical, to mechanical, to electronic—to create a confocal microscope of the highest level ever in this class. In addition, to get the utmost performance out of Nikon’s CFI60 series objectives, we developed new complimentary scanning optics expressly for this microscope. The following are the major results of our improvements:

CFI60 optical system

To achieve images of the utmost quality, the C1 adopts the CFI60 infinity optics, the industry-acclaimed optics developed by Nikon utilizing its unique technologies.

Higher N.A.’s, longer working distances, and aberration-free

CFI60 optics achieve both higher N.A.’s and longer working distances than ever before possible, while succeeding in separately correcting both axial and lateral chromatic aberrations in the objective and the tube lens.

Reduced blur, increased contrast

The CFI60 design dramatically reduces blur during microscopy. To curtail fluorescence light emitted from the objectives themselves, Nikon chose the appropriately formulated glass and optical coatings for the lenses and designed them in the optimal configuration, improving contrast during epi-fluorescence observations. Nikon’s CFI60 optical system delivers top-notch performance, enabling its use in increasingly sophisticated biological research.

• Stray light, which is usually generated in the scanner head and common in some other designs, is thoroughly eliminated, while reflection loss on the I/O ends of the optical fiber is minimized. This makes it possible to obtain images with extremely high contrast and photon efficiency.

• Fluorescence transmission efficiency has been dramatically improved to obtain fluorescent images 3 times brighter than previous Nikon models. This, coupled with the use of high quantum-efficiency photomultiplier tubes, gives clear, sharp images even with fluorescent specimens that in the past were too dim to observe.

• Signal-to-noise ratio has been increased 7-fold (compared to previous Nikon models), resulting in a significantly improved image quality.

• The new frame grabber A/D image board improves signal quality when converting between analog and digital image signals.

• High-precision scanning facilitated by highly accurate galvanometer scanner control and superb control of sampling signals optimizes high-resolution images of up to 4 million pixels. Even higher zoom magnifications do not impact optical resolution and are usually not required for maximum resolution.

• 12-bit digitization pixel depth ensures the quality, dynamic range and sensitivity that is required of a 4-megapixel image.

Drosophila embryo: Argon 488nm, He-Ne 543nm

CFI60 Optical Path

(Conceptual Diagram)

Specimen

Parfocal distance: 60mm

Widest Magnification range: 0.5 x → 100 x

Numerical Aperture: 0.02 ~ 1.4

4
Superbly accommodates various imaging techniques

The C1 supports almost any imaging technique required today, including simultaneous 3-channel fluorescence, 3-channel plus diascopic DIC, time-lapse recording, and spatial analysis.

**Interchangeable filters:** the filters for scanning and detection are easily interchangeable, so changing the filter to match the fluorescent dyes to be used is simple and quick. This design facilitates the use of the latest probes or dyes used in laboratories today.

**Triple-band filter:** a triple-band RGB Dichroic Mirror is supplied as standard, providing instant support for simultaneous 3-channel fluorescence imaging.

**Easy attachment of a diascopic DIC module:** superimposing confocal fluorescent images with the DIC morphology image provides greater depth information to the image.

**Comprehensive time-lapse recording:** supports all time-lapse observation needs, from second to hourly intervals.

**Spatial analysis and ROI placement:** measurements of intensity or size of the desired area is possible; Regions of Interest or ROIs can be placed at multiple points.

**Universal scanner head:** The scanner head can be easily mounted on either upright or inverted microscopes to support a wide range of applications.
Freedom in the use of lasers and dyes

Various laser types

The wider range of excitation light wavelengths facilitating the use of an increased number of fluorochromes in research has increased the demand for the number of lasers available on the market. The C1 comes with a series of lasers supporting almost every fluorescent dye used in research today. In addition, the use of these available laser lines can be mixed and matched to suit your excitation requirements. The unit employs the tried-and-true, stable 3-laser method for RGB excitation—the main wavelengths in use.

- Choose the 2- or 3-laser unit according to your needs.
- The excitation light from the laser is transmitted to the scan head via single-mode fiber, freeing up desk space, as the laser module can be installed in an adjacent location.

Wide choices of fluorochromes

Besides the excitation lasers, a full selection of emission filters is available to support a wide range of fluorescence observation needs. The detection module is supplied with appropriate dichroic mirrors, allowing simultaneous 3-color observation of fluorescence images of specimens prepared for 3-color imaging.

Compatible Lasers, Wavelengths, and Dyes

<table>
<thead>
<tr>
<th>Laser Type</th>
<th>Dyes and Filters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Violet diode laser (405nm)</td>
<td>DAPI, CFP, Lucifer Yellow, Nuclear Yellow, Hoechst, Q-dot</td>
</tr>
<tr>
<td>Argon laser (488nm)</td>
<td>FITC, Fluo-3, GFP, Cy-2, Alexa Fluor 488, BODIPY, Calcium Green, Acridine Orange, BCECF, Oregon Green</td>
</tr>
<tr>
<td>Helium neon laser (Green, 543nm)</td>
<td>TRITC (Rhodamine), Cy-3, PI, DiRed, Alexa 546, Alexa 568, BOBO-3, Calcium Orange, Dil, Mitotracker Orange, DS Red</td>
</tr>
<tr>
<td>Helium neon laser (Yellow, 594nm)</td>
<td>Texas Red, DiRed, Alexa 568, Alexa 594, Calcium Crimson, Mitotracker Red</td>
</tr>
<tr>
<td>Helium neon laser (Red, 633nm)</td>
<td>Cy-5, Alexa 633, Alexa 647, Allophycocyanin, TOPRO-3</td>
</tr>
</tbody>
</table>

- Tubulin of pulmonary artery endothelial cells: BODIPY-FL (Ar 488)
- Actin of pulmonary artery endothelial cells: Texas-Red labelled phallolid (He-Ne 543)
- DiO (Ar 488), Dil (He-Ne 543)
- Drosophila maggot: GFP (Ar 488), DiRed (He-Ne 543), Plan Apo 10x
- Drosophila embryo: GFP (Ar 488), Plan Apo 10x
- Nematoda: GFP (Ar 488)
- Nuclear of pulmonary artery endothelial cells: DAPI (V-LD 405)
- DiO (Ar 488), Dil (He-Ne 543)
- Bifilm: Cy3 (He-Ne 543), Cy5 (He-Ne 633), Reflection (Ar 488)
- Nematoda: GFP (Ar 488)
Live images can be captured with ease

All settings and procedures required for live image capture—fundamentals in confocal microscopy—can be viewed in a single window, eliminating the need for the operator to switch between many windows. The operation panel gives you an at-a-glance picture of all important settings including scan speed, pixel size, zoom/pan, PMT settings, pinhole, shutter, and color image look-up table. With the C1, scanning modes are expanded from 2D (XY, YZ, XZ), to 3D (XYZ, Xyt), and even further to 4-dimensional (XYZt) scans.

**VBA Macro**
The VBA Macro environment that has been incorporated into the EZ-C1 version 2.0 allows users to record a series of operations and write customized programs. Tiff-Converter and Acquire-Setting Reproduction programs have also been pre-installed.

---

**At-a-glance setting panel**

- Image capture area
- Scan speeds
- Pixel size
- PMT amplification
- Digital zoom
- Pan

---

**Average**

**Channel series**

**Z-stack**

**Time series**
Image processing and analyses functions

A wide variety of processing filters are available including Median, Low-Pass, Kirch, Laplacian, Custom-Kernel, Square-Kernel, Round-Kernel. Averaging is possible in various ways such as averaging by specifying the number of frames, frame by frame, continuous, by specifying the image divisor or rolling average number. Image enhancing features enable correction of contrast, brightness, gamma, color balance, white balance, background, shading and other factors to optimize the scanned or captured image.

Setting of multiple regions of interest (ROI’s) within the specimen is possible by selecting desired tools, so you can easily obtain detailed data on specific regions, such as size or the intensity versus the time course of the experiment.

Time-lapse recording is also a simple matter

All required procedures for time-lapse recording, including the setting of inter-cycle times, frame intervals, and the number of images to be captured can be provided in a single window for quick, easy operation. Image capture via sync-control from automated accessories or connected electrophysiology equipment is also possible.

Last but not least, thanks to the C1’s exceptional signal-to-noise ratio, the intensity of the excitation light can now be lowered much more than previously possible. This is a big advantage for living specimens. Photobleaching is dramatically reduced making the C1 confocal microscope system extremely useful for a broader range of applications.
Powerful results from advanced confocal imaging

**Triple staining**

Triple staining of pulmonary artery endothelial cells. Tubulin stained with BODIPY-FL (Ar 488), actin stained with Texas-Red labelled phalloidin (He-Ne 543), and nuclei stained with DAPI (V-LD408). Acquired with Plan Apo 60x oil.

**Cell motility**

Merged interference reflection and confocal fluorescence image of fibroblasts growing out over a glass coverslip substrate: GFP (Ar 488), TRITC (He-Ne 543), Plan Apo 60x oil.

**Fluorescence and DIC**

Merged maximum projection of a 20µm stack through a developing drosophila maggot with a single plane scanned DIC image: TRITC (He-Ne 543), Plan Apo 10x

**Deconvolution**

3D blind deconvolution of a 40µm section illustrating YFP expressing motor neurons in mouse spinal cord; (He-Ne 543), Plan Apo 20x.

**Layer-by-layer quasi-coloring**

Neuroglial cells stained with FITC. The image is presented in quasi-colors specified by layer to layer from top to bottom in the z-axis direction. Acquired with Plan Fluor 40x oil.

**FRET (Fluorescence Resonance Energy Transfer)**

FRET between FITC (donor) and Cy3 (acceptor), (Ar 488), Plan Apo 60x oil.
Compact and flexible

Flexible, modular design

All main components are modular including the laser box, scanner head, and detection module, facilitating simple expansion to meet diverse user needs and facilitate maintenance. The scanner head can be mounted on either upright or inverted microscopes to support a wide range of applications.

- Each of the C1's modular hardware units is pre-calibrated, greatly simplifying the setup. Just connect the modules you need, and you are ready for optimal image capture.
- Modules other than the scanner head do not need to be placed on the desk, providing the freedom to place other equipment where it is needed for your experiments.
- The flexible system allows for easy upgrades when research needs change or when new lasers, detectors, and other equipment becomes available.
- The C1 controller and the PC are linked by Ethernet connection, allowing remote control under standard conditions and providing greater flexibility in use.

Compact design

- With an extremely compact design, the C1 becomes a mainstream quantitative imaging device allowing integration with other experimental lab equipment without complicating the work area.
- Because the scanning head is a lightweight design, when it is loaded with an upright microscope, the image is less affected by vibration.
- The compact, lightweight design allows you to handle the C1 as if it were a digital camera. And if you use an intermediate tube, you can attach multiple cameras of various types.
A wealth of accessories

Z-focus module

This module, which can be retrofitted to the CI system, features a minimum focusing increment of 100nm. You can freely set image capture environments such as XZ, YZ, XYZ, or XYZt in conjunction with the CI’s original spatial (X, Y) and time (T) axes.

Diascopic DIC module

Consisting of a retrofitable separate modular, yet compact, transmitted light detector, this configuration leaves ample space around the microscope. The images obtained will have greater depth and co-localization image information by superimposing a fluorescence image over the high-resolution DIC image captured through laser scanning.

3-laser unit

The pictured 3-laser unit module is used for observing triple-stained specimens. It provides extremely stable and consistent, long laser life performance for this application. Because it has been designed for durability and longevity, this module will provide excellent results for many years.

3-PMT detection module

Like the standard 2-PMT unit, connection of the optional 3-PMT detector unit with the scanner head is established via a fiber cable, eliminating restrictions on where to locate it while being able to environmentally isolate it to prevent the deterioration of signals. Changing the filter sets to match the fluorescence dyes is quick and easy, while the use of interchangeable filters allows the ability to provide perfect wavelength tuning of new probes or dyes when they become available.
The TIRF-C1 system can perform confocal, total internal reflection fluorescence (TIRF), and epi-fluorescence imaging with a single unit by merely switching between the three modes. With the option of imaging under multiple methods with the same field of view, it is possible for the researcher to investigate the single molecular dynamics of a cell in greater detail utilizing its 3D sectioning images.

**Common laser between confocal imaging and TIRF imaging**
Because a single laser module is shared through an optical fiber between the confocal and TIRF imaging, it is easier to use and saves space.

**C1 and TIRF attachments simultaneously mountable**
The Nikon TE2000 inverted microscope features a unique “stratum structure,” enabling the simultaneous mounting of multiple attachments without modifying the microscope body. Therefore, this microscope can mount the C1, TIRF, and epi-fluorescence attachments together. By using this system, it is now possible to observe the single molecule dynamics of a living cell in contact with the coverglass under TIRF imaging, capture its sectioning images by confocal imaging, and view the whole image of the cell via epi-fluorescence imaging. Thus, by utilizing three imaging methods, a single cell can be investigated from various aspects.

**Surface Reflection Interference Contrast (SRIC) microscopy**
Before TIRF imaging, Surface Reflection Interference Contrast (SRIC) microscopy may be used to check the condition of the specimen’s adhesion to the coverglass and whether or not it should be visible by TIRF. This eliminates the risk of photo-bleaching during focusing. Focusing is easy and switching back to TIRF is also a snap.

Comparison of mouse bone marrow stroma cell (ST2 cell) images taken by multi-mode imaging
After fixing in 4% formaldehyde, cells were treated with 0.25% Triton X-100 before double staining with paxillin antibodies and TRITC-phalloidin. This cell is moving toward the right, and a part of the right side of the cell is shown.

**Confocal image**
This shows the basal portion of the cell. A clear band of substantial F-actin (red) is shown at the leading edge of the cell, which is migrating toward the right side. Paxillin molecules are green. Stress fibers are facing the rear of the cell.

**TIRF image**
Strong and clear fluorescence derived from paxillin is observed in the evanescent field. The focal adhesions existing at the portion of cells in contact with the coverglass were clearly confirmed.

**SRIC image**
This SRIC image was observed using a conventional epi-fluorescence microscope with a simple modification. The black area is closest to the coverglass, and indicates the presence of paxillin molecules (focal adhesion). This method is available for identifying the portion of a cell in contact with the coverglass prior to TIRF imaging.

Images courtesy of Shuichi Obata, Ph. D., Department of Anatomy, School of Medicine, Yokohama City University
## Specifications

<table>
<thead>
<tr>
<th>Laser unit</th>
<th>Laser type</th>
<th>Number of lasers mountable</th>
<th>Laser intensity control</th>
<th>Laser shutter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V-LD (408), Ar (488), G-HeNe (543), Y-HeNe (594), R-HeNe (633)</td>
<td>Up to 3 lasers mountable</td>
<td>Manual for each laser (4 steps)</td>
<td>Motorized shutter for each laser</td>
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</table>

<table>
<thead>
<tr>
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<th>Channel</th>
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<th>Laser shutter</th>
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<tr>
<td></td>
<td>Standard: 2 fluorescence channels</td>
<td>Maximum: 3 fluorescence channels + 1 transmission diascopic DIC channel</td>
<td>Manual for each laser (4 steps)</td>
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<table>
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<tr>
<th>Pinhole</th>
<th>Variable</th>
<th>3 pinhole size steps + OPEN (motorized)</th>
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<table>
<thead>
<tr>
<th>Dichroic mirror</th>
<th>1st DM</th>
<th>2nd DM</th>
<th>3rd DM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Interchangeable. For standard combinations, see the table below.</td>
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</table>

<table>
<thead>
<tr>
<th>Scanning head</th>
<th>Scanning resolution</th>
<th>Scanning speed</th>
<th>Scanning mode</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>160 x 160—2048 x 2048 pixels</td>
<td>Standard: 1 sec. for 512 x 512 Line: 500L/sec.</td>
<td>2D: X-Y, X-Z, Y-Z</td>
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<tr>
<td></td>
<td>4D: X-Y-Z-t</td>
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<td></td>
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<table>
<thead>
<tr>
<th>Zoom</th>
<th>F.O.V.</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Square inscribed in a ø18mm circle</td>
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</table>

<table>
<thead>
<tr>
<th>Image bit depth</th>
<th>12 bits</th>
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<table>
<thead>
<tr>
<th>Dual Stain</th>
<th>Combination of Lasers and Filters According to Dye</th>
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</table>

<table>
<thead>
<tr>
<th>B excitation</th>
<th>G excitation</th>
<th>Laser 1</th>
<th>Laser 2</th>
<th>Filter set</th>
</tr>
</thead>
<tbody>
<tr>
<td>FITC or Alexa 488</td>
<td>TMR or Cy-3</td>
<td>Ar (488)</td>
<td>G-HeNe (543)</td>
<td>1st DM: 488/543</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2nd DM: 530</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Em filter: 515/30, 570LP</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B excitation</th>
<th>G excitation</th>
<th>Laser 1</th>
<th>Laser 2</th>
<th>Filter set</th>
</tr>
</thead>
<tbody>
<tr>
<td>FITC or Alexa 488</td>
<td>Texas Red or Alexa 594</td>
<td>Ar (488)</td>
<td>Y-HeNe (594)</td>
<td>1st DM: 488/594</td>
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<td></td>
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<td>2nd DM: 565</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Em filter: 530/50, 610LP</td>
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</tbody>
</table>

<table>
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<tr>
<th>B excitation</th>
<th>G excitation</th>
<th>Laser 1</th>
<th>Laser 2</th>
<th>Laser 3</th>
<th>Filter set</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAPI</td>
<td>FITC or Alexa 488</td>
<td>TMR or Cy-3</td>
<td>V-LD (408)</td>
<td>Ar (488)</td>
<td>G-HeNe (543)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2nd DM: 530</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3rd DM: 515/30, 515/30, 605/75</td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>B excitation</th>
<th>G excitation</th>
<th>R excitation</th>
<th>Laser 1</th>
<th>Laser 2</th>
<th>Laser 3</th>
<th>Filter set</th>
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</thead>
<tbody>
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<td>FITC or Alexa 488</td>
<td>TMR or Cy-3</td>
<td>Cy-5</td>
<td>Ar (488)</td>
<td>G-HeNe (543)</td>
<td>R-HeNe (633)</td>
<td>1st DM: 488/543/633</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>2nd DM: 530</td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>3rd DM: 625</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Em filter: 515/30, 585/40, 665LP</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2nd Dichroic Mirrors
+ Barrier Filters

Detection Module
(2-PMT)
(3-PMT)

Laser Unit

2L Unit 3L Unit 3L-V Unit
Ar [488] He-Ne He-Ne He-Ne V-LD [408]

2-PMT 3-PMT

E600/E400 Microscope

Z-focus Module

Laser-safe Trinocular Tube

Lasers usable
2L Unit: Ar and one from the He-Ne type
3L Unit: Ar and two from the He-Ne type
3L-V Unit: one from the He-Ne type, plus Ar and V-LD
WARNING

The following specimens used in this brochure are courtesy of:
1. Dr. Latika Khatri, HHMI, NYU School of Medicine, NY, USA
2. Dr. Fletcher White, VAMC, CT, USA
3. Dr. Peter A. Pryfogle, INEEL/BBWI
4. Dr. Horst Wallrabe, University of Virginia, VA, USA
5. Dr. Greg G. Gunderson, Columbia University, NY, USA

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