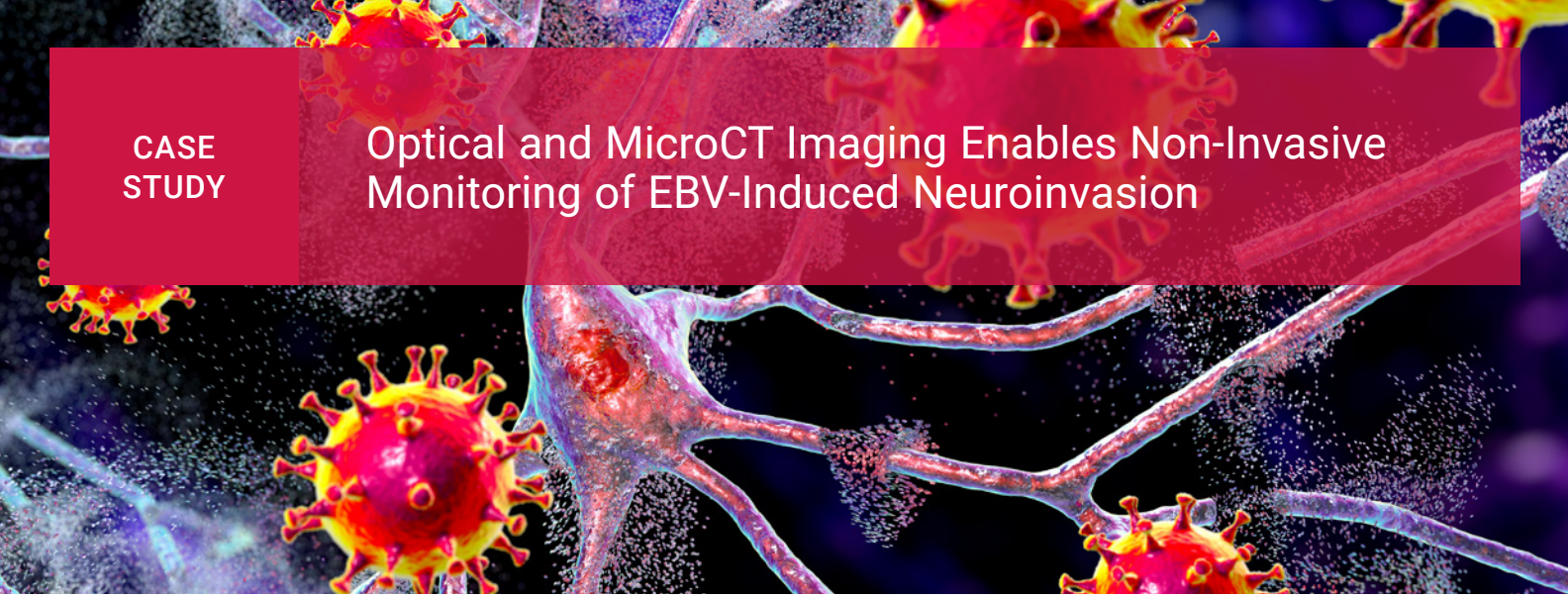


CASE STUDY

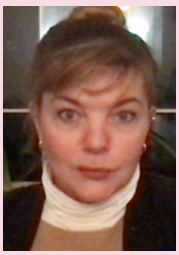
# Optical and MicroCT Imaging Enables Non-Invasive Monitoring of EBV-Induced Neuroinvasion

## Introduction

Epstein-Barr virus (EBV) infection is present in over 90% of the adult population worldwide. The majority of infections lead to benign latent EBV infection in B-lymphocytes; however, in some patients it induces B-cell transformation to cause lymphoid malignancies such as Burkitt and Hodgkin's lymphomas.[1,2] Increasing evidence also supports the association between EBV and neurological autoimmune disorders including multiple sclerosis (MS).[3,4] During development of these malignancies, EBV-infected B cells are known to traffic to the central nervous system (CNS) to drive pathogenesis, yet the viral and cellular determinants driving this neuroinvasion remain poorly understood.

Dr. Samantha Soldan, who is based at The Wistar Institute in Philadelphia, has extensive experience in the study of viral etiology and pathogenesis of MS. Dr. Soldan's research in the lab of Dr. Paul Lieberman focuses on understanding how cancer-associated viruses like EBV persist in latent infections. The aim of her work is to drive the discovery of new treatments for EBV-associated cancers.

This case study illustrates how Dr. Soldan and colleagues used preclinical bioluminescence optical imaging combined with microCT to develop a mouse model of EBV-dependent B-cell trafficking to the CNS.[5] PerkinElmer's IVIS® SpectrumCT imaging system was used in combination with custom three-dimensional (3D)-printed tools to monitor CNS localization of EBV+ Burkitt lymphoma (BL) cells and select for neuroadaptation of a novel EBV+ lymphoma cell line.



### Our Challenge

*"EBV+ Burkitt lymphoma (BL) cells play a critical role in the pathogenesis of some CNS diseases like primary central nervous system lymphoma and multiple sclerosis. Unfortunately, the timing and mechanism of EBV+ B cell neuroinvasion are not well understood. We needed a reliable animal model and imaging tools to non-invasively track cells and quantify disease burden over time."*

**Dr. Samantha Soldan**

PerkinElmer®
For the Better

## The Challenge

EBV was first classified as an oncogenic virus after viral particles were discovered in a BL cell line. EBV infection has since been associated with malignant transformation of B cells in numerous lymphoproliferative disorders of the CNS.[1] Normal immune surveillance requires regular trafficking of immune cells across endothelial barriers to enter the CNS. However, in states of systemic immunosuppression EBV infection can alter B cells, causing dysregulated trafficking of EBV+ B cells to the CNS. Despite evidence supporting a major causative role of EBV infection in this B-cell transformation, the underlying viral and cellular mechanisms of pathogenic EBV+ B cell neuroinvasion require further study.

Non-invasive *in vivo* preclinical bioluminescence imaging enables longitudinal monitoring of EBV+ B cell trafficking. In addition, the co-registration of bioluminescent signal in 3D with microCT imaging provides anatomical skeletal reference to confirm CNS infiltration. IVIS optical imaging has a broad dynamic range of detection; however, in some models, strong bioluminescent signal at a primary site of injection can mask lower intensity signal elsewhere in the animal, for example the CNS-localized EBV+ B cells in the brain. Thus, Dr. Soldan and colleagues designed custom 3D-printed tools to detect minute signal exclusively in the brain region using bioluminescence optical imaging and the integrated microCT on PerkinElmer's IVIS SpectrumCT. These unique tools allowed the identification and selection of EBV+ BL cells with enhanced CNS trafficking and subsequent investigation of the epigenetic mechanisms underlying EBV+ B cell neuroinvasion.

### What is IVIS and Why Use It for CNS Studies?

*"PerkinElmer's IVIS system enables us to non-invasively track EBV+ BL cell localization via 3D optical imaging combined with microCT. Using the imaging results, we were able to further investigate the epigenetic mechanisms behind EBV+ BL cell trafficking to the CNS."*

**Dr. Samantha Soldan**

## Building a Custom Solution

To detect low-level brain signal during early neuroinvasion in a high-throughput manner, the researchers generated a 3D-printed solution fitted to the torso of the mice to block the high-level signal from the primary injection site. This manifold is easily placed over the mice while they are in the chamber and can be cleaned between groups of mice to maintain aseptic technique. Together with her colleague, Jonathan Fingerut at St. Joseph's University, Dr. Soldan took measurements of the mice and the IVIS SpectrumCT's camera height to design a new manifold. They chose a 3D printing material, polylactic acid (PLA), that did not autofluoresce or autoluminesce. These manifolds were named I.N.A.T.O.R.S. (Inhibition of Non-essential Acquisition for Targeted Observation of Relevant Signal), which was inspired by the Disney series "*Phineas and Ferb*".

The I.N.A.T.O.R.S. (Figure 1) provide a snug fit, they withstand washing with detergents and alcohol, and they effectively block both bioluminescent and fluorescent signal. In addition to a five-mouse whole-body-blocking device (Figure 1A), the team also generated a two-mouse device compatible with the microCT (Figure 1B), a device with adjustable flaps to isolate different sections of the body (Figure 1D), and a device with removeable disks and drainage for *ex vivo* organ imaging (Figure 1C and Figure 3). These tools allowed Dr. Soldan and colleagues to detect neuroinvasion at early timepoints *in vivo*. In addition, by using the organ imaging device they were able to isolate early brain metastasis and precisely localize neuroinvasion in the brain for excision and *ex vivo* cell expansion. The IVIS was an invaluable tool for identifying determinants of early neuroinvasion.

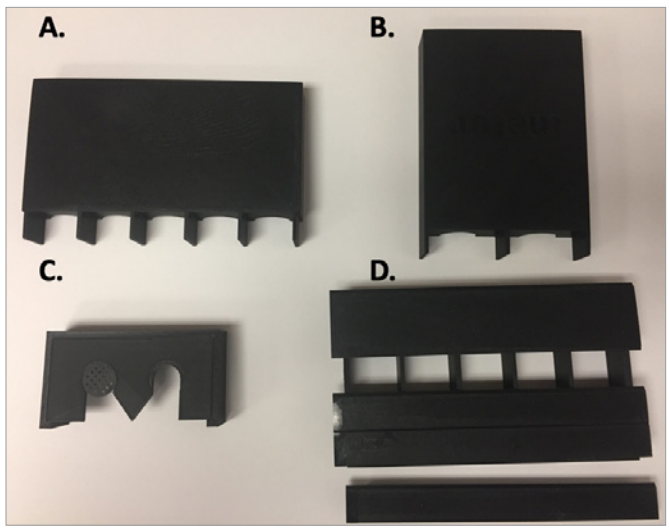



*Figure 1. I.N.A.T.O.R.S designs. (A) Five mouse whole-body-blocking. (B) two mouse & geometrically compatible with integrated microCT. (C) Ex vivo organ holders. (D) five mouse with adjustable flaps.*

## Visualize Disease in Three Dimensions

To better understand the factors contributing to CNS penetration of pathogenic B cells, Dr. Soldan and colleagues sought to create a novel mouse model of an EBV+ BL cell line with enhanced CNS trafficking. To visualize neuroinvasion *in vivo*, they engineered an EBV+ BL cell line (M14) to express firefly luciferase and GFP. 3D bioluminescence imaging of firefly luciferase combined with microCT anatomical reference allowed for non-invasive monitoring of M14 cell trafficking to the CNS.

Subcutaneous injection of M14 cells, generated few mice (3/86) with weak luminescent signal in the brain. To enrich for this rare neuroinvasive sub-population, M14 cells from the brain were isolated by GFP sorting *ex vivo*, and cultured cells were re-engrafted into the mice (Figure 2). This process was repeated for seven cycles to generate a new EBV+ lymphoma subline with significantly enhanced neuroinvasion (MUN14). In comparison to other EBV+ and EBV- B cell lines, including parental M14, intravenous (IV) engraftment of luciferase-expressing MUN14 cells resulted in increased neuroinvasion with a higher percentage of animals developing bioluminescent signal in the brain and spinal cord. 3D IVIS imaging *in vivo* and *ex vivo* combined with microCT confirmed MUN14 cells predominately homed to the brain and spinal cord (Figure 3).

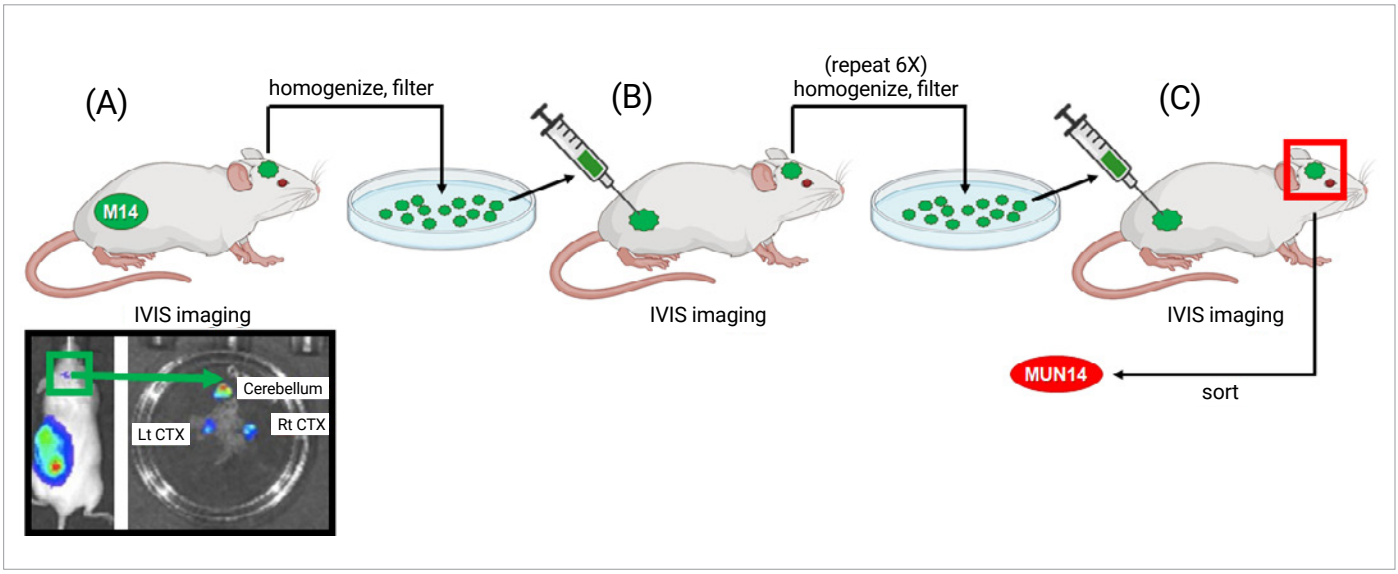



*Figure 2. Optimization of an EBV+ BL cell line. 1. Subcutaneously inject M14 cells. Harvest cells that have migrated to the brain. 2. Implant harvested cells into a new mouse. Harvest cells that have migrated to the brain. 3. Repeat six times. After harvesting from the last animal, the optimized cells are sorted and renamed "MUN14".*

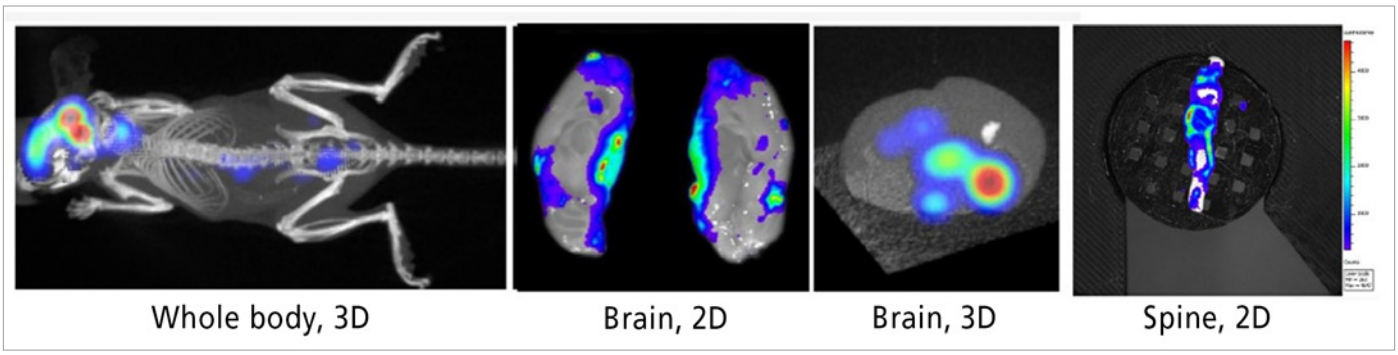



*Figure 3. Colocalized bioluminescence and microCT images obtained on the IVIS SpectrumCT show localization of the optimized MUN14 cells in the CNS.*

To assess the neuroinvasive and neuroinflammatory properties of MUN14 cells compared to parental M14, both cell lines were injected via the intracardiac route and signal in the brain was detected over time by IVIS bioluminescence imaging. MUN14 engraftment resulted in earlier evidence of neuroinvasion and more animals with detectable brain signal compared to M14 (100% vs. 50%) by the end of the study (Figure 4A/B). Concomitant monitoring of expected disease symptoms including seizure and paralysis in mice with neuroinvasion demonstrated increased and accelerated disease progression in MUN14-engrafted animals. Importantly, when MUN14 and M14 cells are injected directly into the CNS (intracranially) survival curves are similar, indicating the MUN14 neuroinvasive phenotype is driven by increased ability to infiltrate the CNS.

Transcriptomic and epigenetic analysis was performed to address the mechanisms of B cell neuroinvasion. In these experiments, brain infiltrate of neuroadapted MUN14 was compared to kidney infiltrate of M14, as the kidney was the primary colonization site of the parental M14 cell line. Dr. Soldan and colleagues discovered that MUN14 cells undergo an epigenetic switch leading to transcriptional changes in numerous genes, including OPN/SPP1. OPN is an extracellular matrix protein that is associated with several inflammatory diseases [6,7]. IVIS bioluminescence imaging demonstrated that treatment with an antibody to OPN/SPP1 in mice engrafted with MUN14 or M14 cells delayed the time to neuroinvasion and attenuated CNS infiltration (Figure 5A/B).

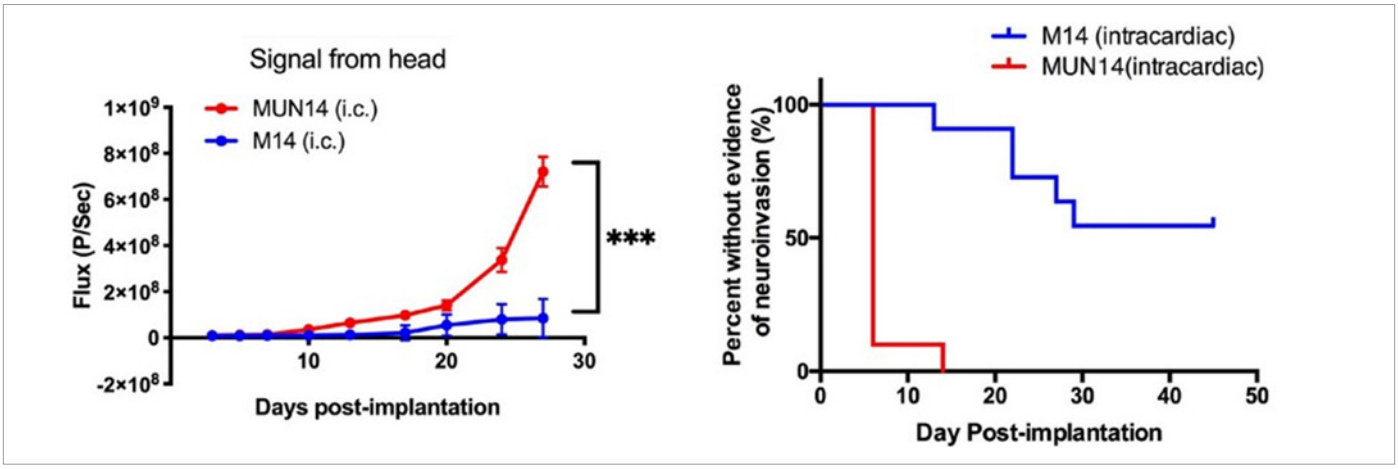



*Figure 4. Intracardiac injection of M14 and MUN14 cells show the latter has greater uptake into the brain as measured by bioluminescence flux (left) and a higher percentage of mice showing neuroinvasion (right).*

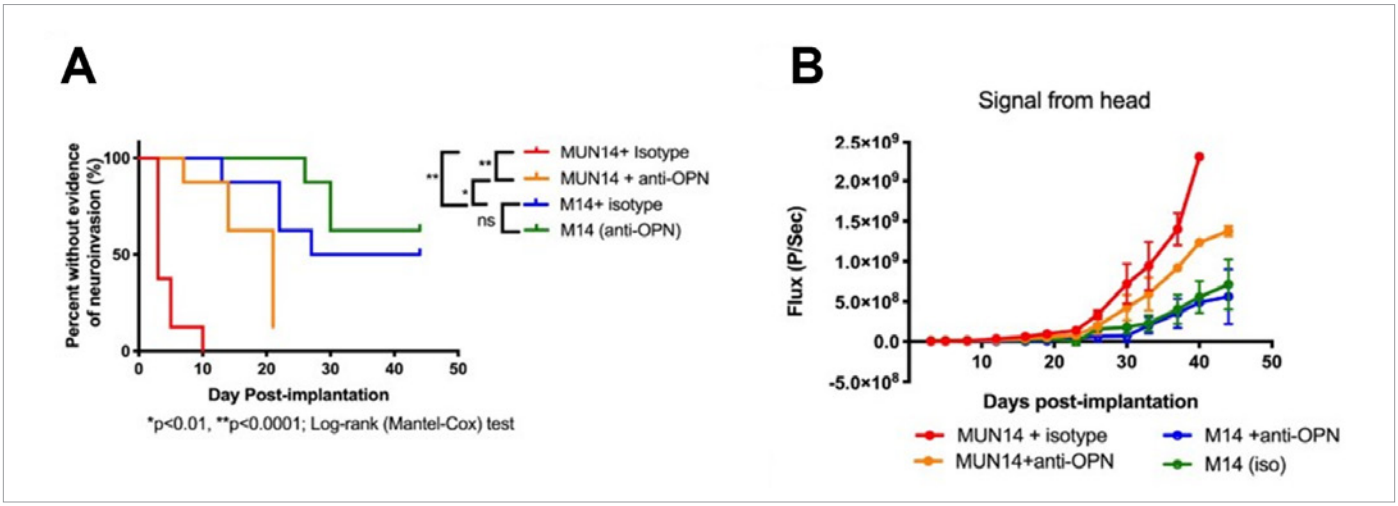



*Figure 5. Mice engrafted with M14 and MUN14 cells were treated with either an antibody against OPN (osteopontin) or an isotype control. For both cell lines, the anti-OPN treatment delayed and attenuated CNS infiltration.*

### What Is the Future of IVIS Imaging for CNS Research?

*"We plan to use the IVIS optical systems in future studies to further refine our understanding of host and virus-specific factors that contribute to neuroinvasion and neuroinflammation. The IVIS will be instrumental in this work due to its sensitivity and ability to show pinpoint localization of optical signal."*

**Dr. Samantha Soldan**

## Conclusion

The ability to image disease in three dimensions on the IVIS SpectrumCT allowed Dr. Soldan and colleagues to precisely monitor localization of EBV+ BL cells in the CNS and brain. The researchers used a combination of bioluminescent-labeled EBV+ BL cell lines and microCT to non-invasively visualize and quantify CNS invasion and disease progression in BL models with their innovative 3D-printed tools. These studies generated a novel EBV+ BL cell line with enhanced neuroinvasion for the study of CNS pathology in EBV+ lymphoproliferative disorders.

## The IVIS SpectrumCT Offers

- High-sensitivity *in vivo* imaging of fluorescence and bioluminescence with integrated microCT
- High-throughput (five mouse) and high-resolution (20 microns in 3.9 cm field of view) option for *in vivo*, *in vitro,* and *ex vivo* applications.
- 3D bioluminescent tomographic reconstruction allowing for pinpoint localization of optical signal

## Acknowledgments

We thank Dr. Samantha Soldan for editing assistance and all members of the Lieberman lab for their contributions to the experiments and animal studies discussed herein.

We are grateful to The Wistar Institute's Imaging Shared Resource for providing technical support. Funding support for The Wistar Institute core facilities was provided by Cancer Center Support Grant P30 CA010815.

## References

1. Shannon-Lowe, C. & Rickinson, A. The Global Landscape of EBV-Associated Tumors. *Front. Oncol.* **9**, 1–23 (2019).
2. Crombie, J. L. & LaCasce, A. S. Epstein Barr virus associated B-cell lymphomas and iatrogenic lymphoproliferative disorders. *Front. Oncol.* **9**, 1–9 (2019).
3. Houen, G., Trier, N. H. & Frederiksen, J. L. Epstein-Barr Virus and Multiple Sclerosis. *Front. Immunol.* **11**, 3–8 (2020).
4. Soldan, S. S. & Lieberman, P. M. Epstein-Barr virus infection in the development of neurological disorders. *Drug Discov. Today Dis. Model.* **32**, 35–52 (2020).
5. Soldan, S. S. et al. Epigenetic plasticity enables CNS-trafficking of EBV-infected B lymphocytes. *PLoS Pathog.* **17**, 1–23 (2021).
6. Lund, S. A., Giachelli, C. M. & Scatena, M. The role of osteopontin in inflammatory processes. *J. Cell Commun. Signal.* **3**, 311–322 (2009).
7. Rittling, S. R. & Singh, R. Osteopontin in immune-mediated diseases. *J. Dent. Res.* **94**, 1638–1645 (2015).

**PerkinElmer, Inc.**
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
**www.perkinelmer.com**



599251 PKI