
THE MEN'S INITIATIVE™
Our Plan
Strategy 2020–2029

Introduction and Executive Summary

A firefighter rescues someone from a burning building, but is unable to tell his story to his wife or relate to his kids…

A college athlete knows that the words he is speaking in the locker room are not aligned with his values, but he wants to feel like part of the team…

A 25-year-old man with AIDS lies in his bed in a palliative care unit, thinking about his father, whom he invited for a visit. His father doesn't show…

THESE ARE SOME OF THE STORIES OF MEN THAT demonstrate that it is time to do a deep dive into understanding the complexities of masculinity. Men have often been socialized into rigid roles and expectations about what it means to be a man, but these parameters have changed over recent decades. It is time to help men explore what it means to be a man now and to make changes that enhance their well-being and the well-being of others.

This document outlines the plan for the next evolution of The Men's Initiative (TMI), an organization with a mission to enhance the integrity and well-being of men for the benefit of families, communities, and the world. TMI is an independent, not-for-profit initiative that is strongly linked to academia through the University of British Columbia (UBC) and is affiliated with the Canadian Men's Health Foundation (CMHF). TMI benefits from direct interaction and community-based action research* focused on building new programs that will effect positive change in the communities and men we serve. The power of TMI is that it builds on multi- and inter-disciplinary research and it benefits from the application of research in practice.

In 2015, Drs. David Kuhl, Duncan Shields, and John Izzo founded The Men's Initiative in Vancouver, British Columbia. David is a renowned researcher, author, and professor with UBC Faculty of Medicine and the Mohammad H. Mohseni Chair in Men's Health, Integrity, and Well-being at Vancouver General Hospital. Duncan, an adjunct professor in the Faculty of Medicine, is a 25-year trauma counsellor, therapist, and researcher who is internationally known for his work on military veteran transition to civilian life. John, also an adjunct professor in the Faculty of Medicine, is a best-selling author who has advised leaders across 700 companies and helped more than one million people worldwide connect to the legacy they want to leave.

The decision of these three men to create TMI culminated from more than 35 years of listening to the stories of men in high-stress, high-profile environments, including protective service workers (military, police, fire, and healthcare professionals), corporate leaders, athletes, and men facing serious

---

* Social and psychological research techniques used to identify social problems in a group or community coupled with active participation of the investigators in group efforts to solve these problems. Merriam-Webster. (n.d.). Action Research. In Merriam-Webster.com dictionary. Retrieved May 22, 2020, from https://www.merriam-webster.com/dictionary/action research

Figure 2: The Men's Initiative competitive landscape petal diagram

Displayed in Figure 2, these categories are demarcated by a continuum demonstrating an overlap of missions among organizations. TMI will operate across many of the existing categories of work focused on masculinity. We will continue to partner and create synergistic opportunities for collaboration with organizations interested in masculinities research and advocacy work, such as Promundo* and the Canadian Men's Health Foundation. Our work, at the nexus of industry, community, and academia, will serve to inform and share the work of these diverse organizations. In the end, our work will enhance not only those we directly serve, but the marketplace as a whole.

Within academia, university faculties in many institutions (e.g., health sciences, medicine, education, business, and, within arts and sciences faculties, departments of psychology, sociology, anthropology, gender studies, political science, and so on) have a similar agenda for researching masculinity and men's health and well-being. Often, however, these scholarly areas take a pathology -based approach to addressing the issues that affect men, which limits their appeal for many men and therefore their reach.

We found twenty academic journals that have a specific focus on publishing research about masculinities and men's health.†

Market Opportunities and Gaps

As we consider the landscape in which TMI is operating, we believe there are six major gaps that represent opportunities for impact through TMI.

* Men experience pressure to be "real men" and to live up to the prevailing standards of masculinity by which men assess themselves and others. Promundo, a global leader in engaging men and boys to promote gender equality and prevent violence, has developed a concept they call the Man Box, to categorize some of these prevailing standards of masculinity, describing them as overly restrictive and often counterproductive expressions of masculinity. The Men's Initiative uses the Man Box concept in its theory of change for the individual man.

† American Journal of Men's Health, American Men's Studies Association, Boyhood Studies: An Interdisciplinary Journal, Culture, Society & Masculinities (ended 2016), Fathering (ended 2014), International Journal of Men's Health (ended 2016), The Journal of Black Masculinity, Journal of Gender Studies, The Journal of Men's Studies, Journal of Men, Masculinities, and Spirituality, Journal of Research in Gender Studies, La Manzana, Masculinities & Social Change, Masculinities: A Journal of Identity & Culture, Men and Masculinities, Mobilizing Men for Violence Prevention, NORMA: International Journal for Masculinity Studies, Psychology of Men & Masculinity, The Scholars Network on Black Masculinity, Society for the Psychological Study of Men and Masculinity

TMI's Theory of Change is supported by:

- Striving to understand the system that has shaped men and their lived experience;
- Working on the root cause, not the symptoms of a misaligned model;
- Working to understand features of masculinity and align them to the social good. e.g., being a protector and provider, the role of stoicism, the impact of fatherhood (as a son and as a father);
- Focusing on opportunities to change the socialization of men;
- Developing new approaches to educate and acculturate men and boys early in life;
- Leveraging permission-givers and men in iconic roles to communicate and instill new behaviours;
- Having dialogue and courageous conversations with men;
- Instilling in men an aspiration to change; and
- Focusing men's attention on changing as part of their life transitions.

Figure 4: The Men's Initiative methodology and unique intellectual property

Our imperative is to help men flourish by improving their integrity and resilience.* We believe change is catalyzed by the following elements, which, together, comprise TMI's theory of change.

While we have developed and are developing a number of different programs to help build resilience and well-being for men in different circumstances, particularly those in high-stress and challenging environments, all of our programs imbed a core-change process under different guises. Figure 4 graphically represents our unique approach, one that informs the development of all of our programs. Through self-reflection (insight and determination) and dialogue (engagement and accountability), we help men to understand and become clear about who they want to be in the world, to face the facts about how they have been showing up in their relationships and roles, and to "make a play," turning aspiration and intent into action. We refer to it as their Journey into Integrity©.

* We use the word "flourish" as fully described by Diana Fosha of Accelerated Experiential Dynamic Psychotherapy (AEDP) (see https://aedpinstitute.org/about-aedp/).

The case of the Protective Services Resilience and Leadership Program

Protective Service Workers (PSWs) protect civilians from the impacts of violence, disaster, extreme poverty, catastrophic injuries, and accidents, and help them in dealing with death and dying. This protection also extends to managing the front lines during widespread public health crises and by providing compassionate care to people who are addicted, traumatized, ill, and injured, and the most vulnerable people in our communities.

PSWs live the traditional masculine values of stoicism and self-sacrifice, which aid them while on duty but may result in adjustment difficulties (e.g., post-traumatic stress disorder (PTSD), problematic substance abuse, relationship dysfunction, grief, loss, and depression). For some PSWs, there is a stigma about admitting to difficulties and asking for help, which results in isolation, silence, and despair.

Through TMI's resilience and leadership program, participants are guided through a program of integration in a safe environment that encourages:

- dialogue and self-reflection for deeper insight;
- accountability to the self and others to address and cope with crisis, PTSD, grief, depression, anxiety and suffering; and
- clarification of motive and determination to build and establish a worldview that embraces change and growth.

The program demonstrates impact through measures indicating (with clinical and statistical significance) that participants experience decreased depression and trauma symptoms and an increased sense of well-being for more than six months after program completion.

Sample testimonials:
Protective Services Program

"We are so fortunate to have such incredible expertise working so hard to push our culture and service into such a positive direction. These men are saving lives without a single doubt."

"Words can't begin to describe the level of gratitude we feel towards these two incredible human beings. Our thank you will be to continue to push this momentum of mental health, resilience and advocacy into the halls, and into our homes."

"This man (Dr. Duncan Shields) understands our service because he truly cares and listens. When he speaks in regard to our culture it's as if he sees into the soul of who we are. He gets us. He and his team have saved countless lives, countless relationships, and countless careers."

"Bar none this is perhaps the very best training one can receive in the fire service."

"A life-changing four days! Honoured to participate in @mensinitiative1 @bcpffa #FirstResponder #Resiliency Program. So proud of those who built it! I promise to do my part to ensure #FireFighters have ready access to #MentalHealth resilience support."

Some of the clinical and theoretical framework that we implemented in this program is primarily used with individuals, couples, and families. That we are using

TMI's Business Model: B2B2C

WE HAVE BUILT A PARTNERSHIP-ORIENTED business model to deliver on our approach (Figure 9). We function as a business-to-business-to-consumer (B2B2C) organization. In this business model, we engage in strategic partnerships with other businesses (e.g., professional groups or organizations) which service the needs of the consumer: the individual man. We develop new ideas based on our fieldwork and integrate them within a rich body of knowledge and perspectives from our academic partners. Our process for serving men is through action research and the programs we design, implement, test, and distribute to other organizations for wider impact. We intend to develop those programs in partnership with targeted organizational customers—those who can pioneer systems change within their sectors or professions and amplify it to further serve men and society.

We choose to work with professions and organizations that have influence on others because men in high-profile positions give permission to other men to think and act differently. For example, we partner with the Vancouver Police Department to deliver our Protective Services Resilience and Leadership Program to their police officers and we partner with Stanford University to deliver our GMIS program to their male athletes.

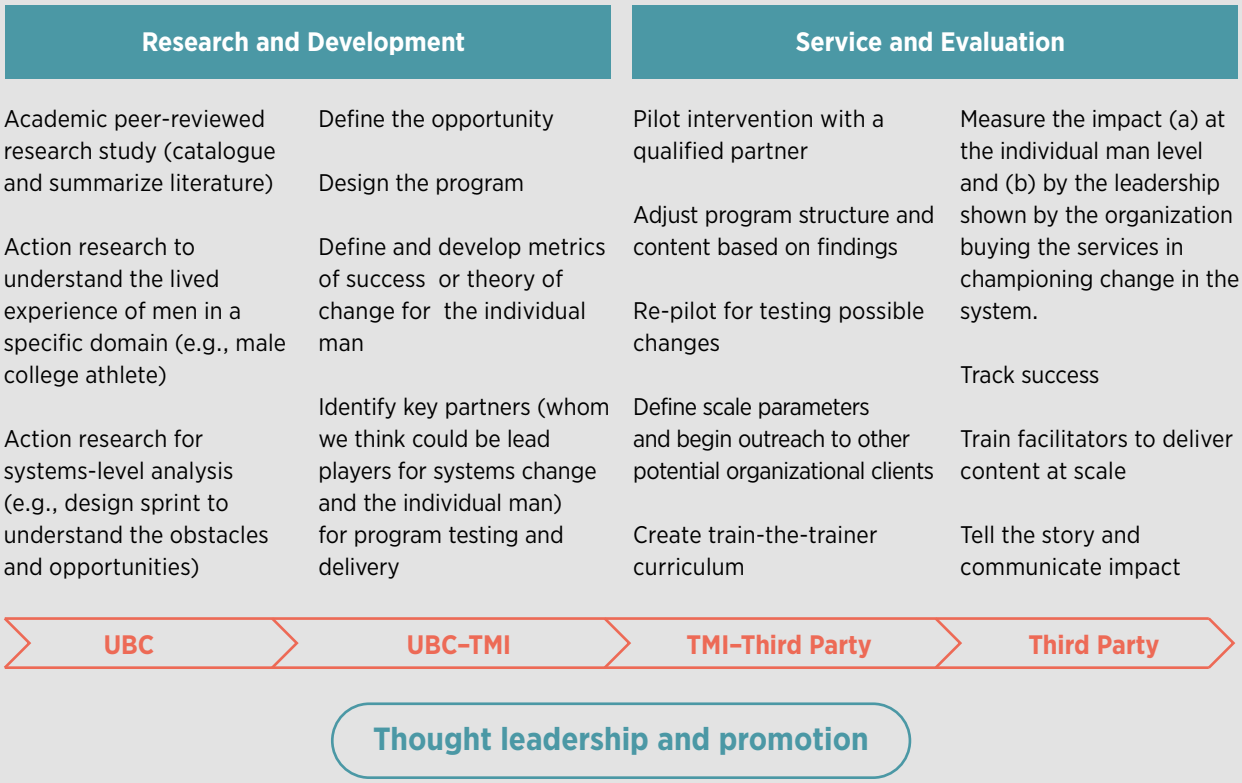


Figure 9: TMI's partnership-oriented business model and research, program design, and evaluation process

We research the needs and lived experiences for each group of men and use this to create models for enhancing empathy, inclusiveness, and self-awareness. We then create structures and best-practice models for programs and scale them through direct delivery, train-the-trainer sessions, and program licensing. We move from one arena to another after we have fully demonstrated impact and have an evolved program to offer. TMI will catalyze a worldwide conversation through thought leadership in academic journal articles, high-quality conferences, and high-profile keynote presentations to practitioners. TMI aims to publish an internationally best-selling book targeted at popular and academic

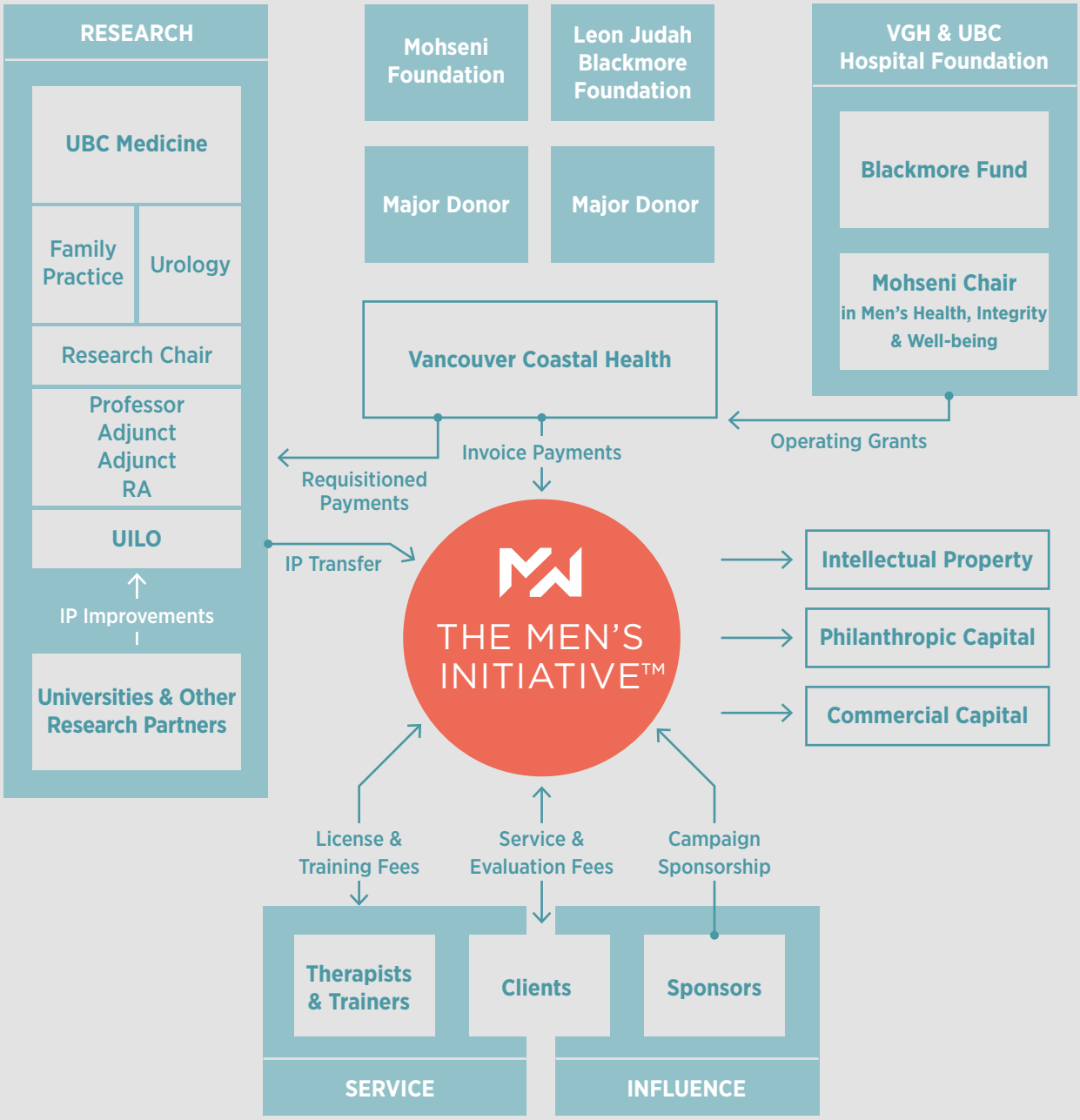


Figure 10: Current operating model

world. Our thought leadership will include the publishing of a groundbreaking book.

- **Build on strong foundational alliances:** We intend to strengthen our ties to UBC, the VGH & UBC Hospital Foundation, and other academic and health care institutions.

- **Work with delivery partners:** We intend to strengthen our existing organizational partnerships and forge new relationships to disseminate and license programs and to further build our impact in North America without being directly involved in service delivery.

- **Explore new opportunities:** As our capacity grows, we believe that many new opportunities will emerge to create programs for men. Our next vertical will be in the business sector with programs designed for this cohort. We anticipate success similar to what we are experiencing in sports and protective services. Future opportunities may emerge with communities such as immigrant and refugee populations of men.

- **Diversify our resource base:** TMI will seek to diversify its resource base through research grant opportunities, licensing, and other fee-for-service activities related to programs.

We believe that these principles will aid in the growth of the impact of TMI while mitigating the need for direct funding.

Photo by Matheus Ferrero on Unsplash.

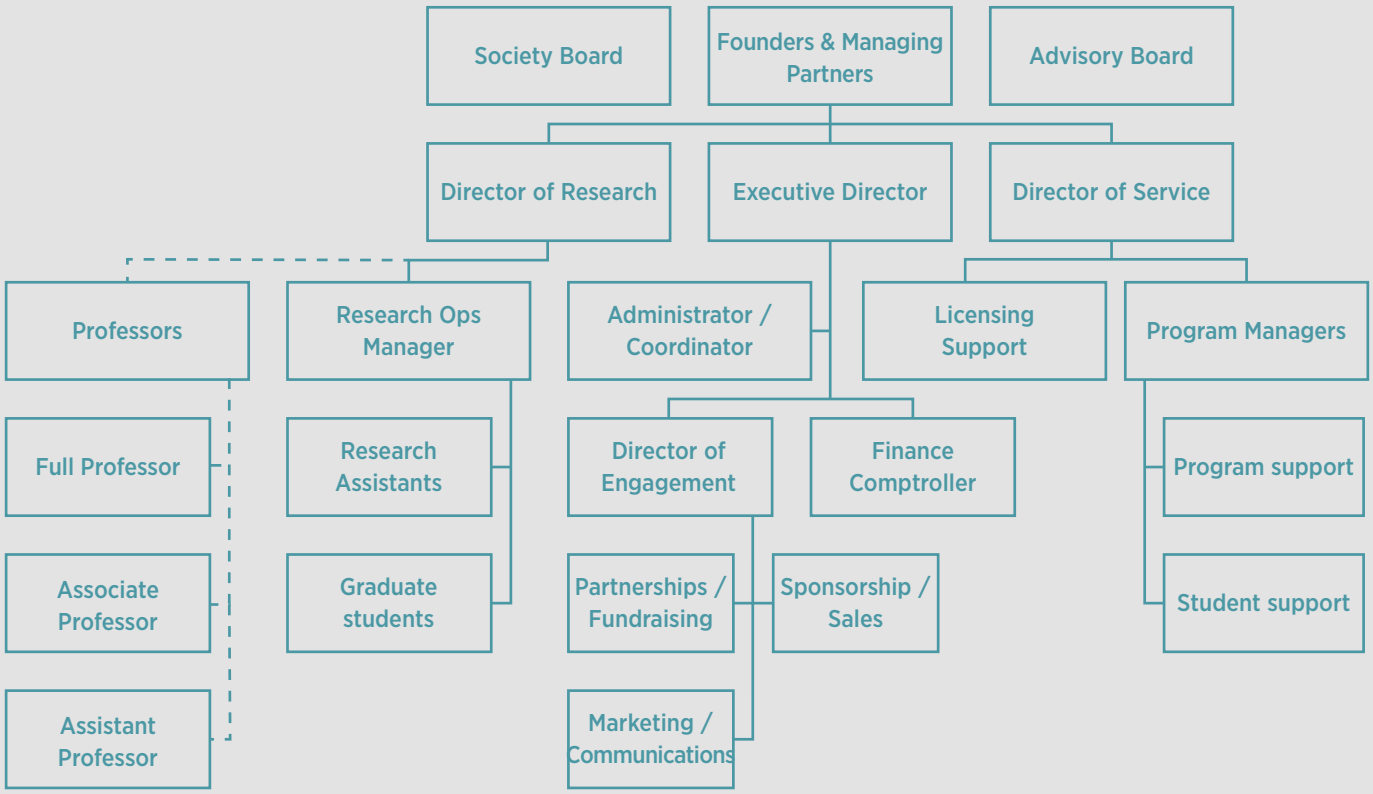


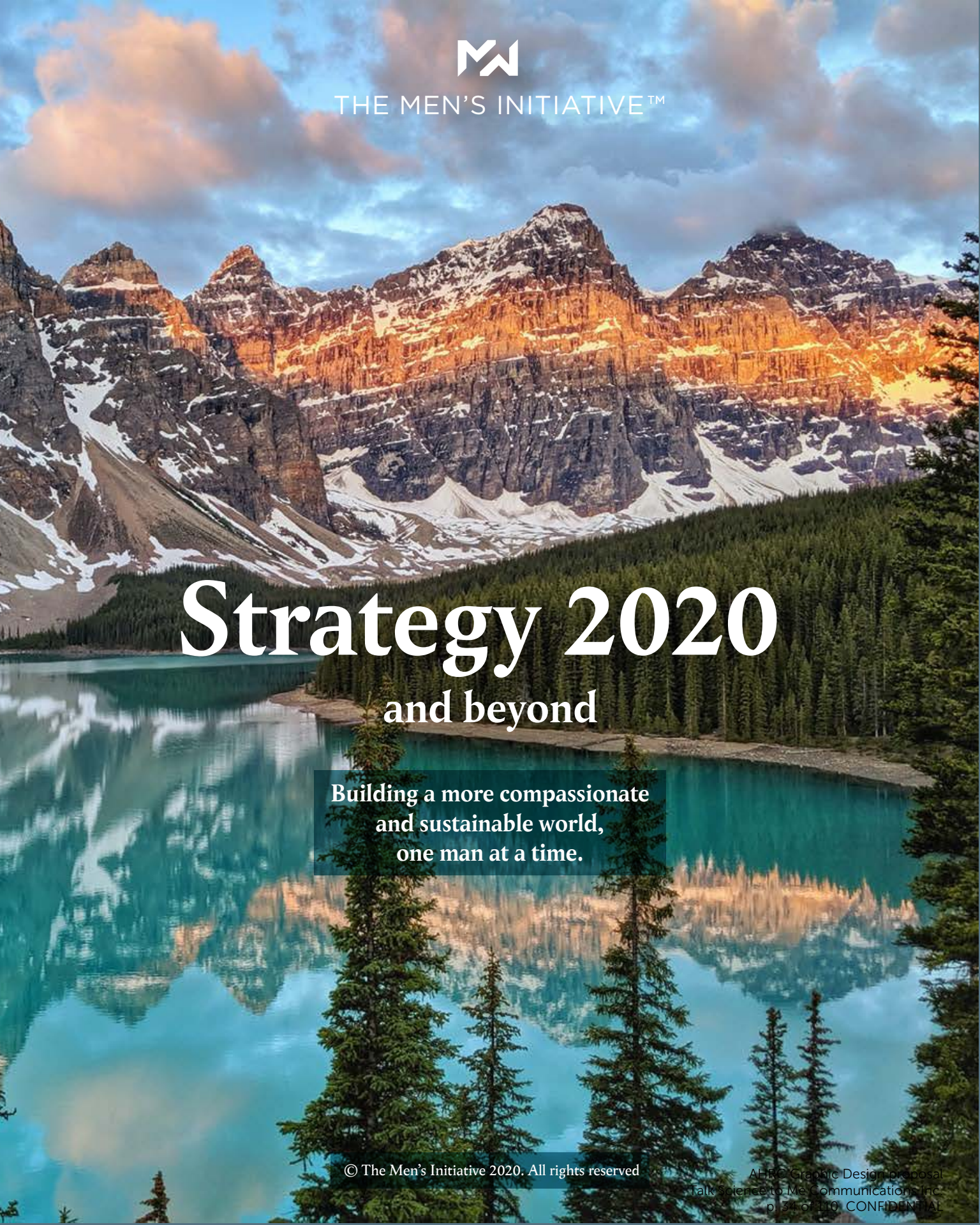
Figure 12: Proposed organizational structure

the Business Lead. Dr. Duncan Shields is the Research and Clinical Lead. All three partners hold additional responsibilities, with Dr. David Kuhl being the primary academic liaison.*

TMI has a society board for institutional governance and oversight. An advisory board supports TMI in achieving its social mission, informing research and program design, praxis and service (delivery and scale), and building a network. The founders are supported by an executive team including a Director of Research, an Executive Director of Operations (EDO), a Director of Service, and a Director of Engagement. Figure 12 also shows faculty relations which bear a dotted-line relationship to TMI, as they will be affiliated with but not funded or controlled by TMI.

TMI will rely on some of the resources available through our partnerships for development and supporting functions, while research and program technical support will increasingly come from UBC. These functions will be coordinated by TMI staff.

* The growth planning exercise identified a number of areas of overlap and unfulfilled needs in management responsibility. It also led to a realization of short- and medium-term priorities, particularly for the founders, that will refocus their individual activities. As part of the planning process, the founders have all reconfirmed their substantial commitments to the Initiative.



The Men's Initiative is dedicated to enhancing the integrity and well-being of men for the benefit of families, communities, and the world.

The Men's Initiative (TMI) is a non-profit organization affiliated with the University of British Columbia's Faculty of Medicine and the Canadian Men's Health Foundation and operates at the nexus of industry, university, and community. TMI applies multidisciplinary knowledge and "never about you without you" collaborative research methods to create programs that positively influence men as changemakers in their communities.

Our Three Pillars

Research

We use rigorous research methods to bring understanding to men's struggles, and to create models and methods to help men contribute more fully to a sustainable future for all.

Service

We serve communities by conducting work with individuals and groups to help men and women connect with their integrity and take action to become a more generative force for good in their communities.

Influence

We seek to catalyze a worldwide conversation about how to be a good man and to do good work in the modern world.

Men Strive Together for an Equitable, Compassionate, and Sustainable World.

30 years ago

Visionary researcher and physician Dr. David Kuhl watched a 25-year-old man suffering from end-stage AIDS call his father for the final time, sharing the news of his illness and imploring him to visit. "After he candidly shared the news, I heard silence emanating from the other end. That young man passed away a few short weeks later. His father never came to visit."

This transformative experience sparked the idea for The Men's Initiative, and began David's journey to understand why men and fathers behave and engage in the world in the way that they do.

Men play an important role in the health and well-being of families and communities, and have a unique opportunity to contribute to the development of functional societies. However, the current and prevailing model of masculinity—how men are taught to show up in the world—impedes our progress for positive cultural change.

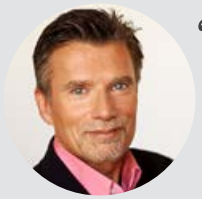
Positioning men as hunters, providers, and protectors, traits of masculinity such as stoicism, hard-work, and self-sacrifice are often essential to many occupations—but, they also encourage men to hide their emotions, driving emotional isolation, an absence of empathy, and immense pain. Men's trauma and suffering leads to troubling issues that manifest as anger, broken relationships, addiction, and violence; ultimately contributing to the suffering of women, children, other men, and the environment.

In 2015, Drs. David Kuhl, Duncan Shields, and John Izzo launched TMI as a response to this growing public health challenge. They discovered that men share a desire to be true to themselves and in close relationships with others, but often lack the opportunity to have tough conversations about what it means to be a man. We need to find new ways to work together by moving away from blaming and shaming and toward understanding the needs of men.

Built around three interconnected pillars, TMI has established a comprehensive range of partnerships and programs that empower men from diverse backgrounds and experiences to make positive contributions to their communities and to society.

TMI is grateful for the generous support of the Leon Judah Blackmore Foundation and the Mohammad H. Mohseni Charitable Foundation. These gifts were stewarded by Dr. Larry Goldenberg with the assistance and support of the VGH & UBC Hospital Foundation.

"Men long for authenticity and to be in close relationship with their families. They desire meaningful work and the ability to adapt to an ever-changing world."
—Dr. David Kuhl, co-founder



"It is important to help men break barriers and learn to embrace positive traits of masculinity, such as empathy, compassion, and intentionality."
—Dr. John Izzo, co-founder

"We need good men, working hard to build peaceful, safe, and inclusive communities where all can thrive."
—Dr. Duncan Shields, co-founder

Mission

To enhance the integrity and well-being of men for the benefit of families, communities, and the world

Vision

Men strive together for an equitable, compassionate, and sustainable world

Our Values

TMI is committed to embracing diversity, promoting equity, and fostering a culture of inclusion, mutual respect, and reciprocity. We engage with all genders in our work to positively influence men in service of families, communities, and the world. TMI's values describe our philosophy for decision-making and for engaging with partner organizations.

Accountability: Living our values by focusing on rigour, evidence, and the evaluation of everything we do, to demonstrate its impact.

Authenticity: Relentlessly telling the truth and walking the talk, recognizing that inner work creates outer reality.

Collaboration: Seeking to maximize impact through cooperation and engagement with partners locally, nationally, and internationally.

Courage: Having difficult conversations, and ensuring a commitment to dialogue, not debate.

Curiosity: Exploring with openness, humility, and a commitment to learning.

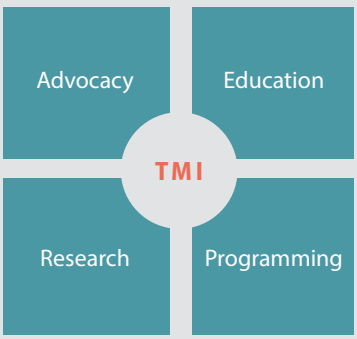
Embracing difference: Putting purpose before ego; valuing diversity, inclusion, and dignity.

Stewardship: Tenaciously maintaining a cross-generational orientation for sustainability and legacy.

Trust: Acting with integrity and embracing the hard personal work that is essential to earning the confidence of others.

Our Unique Position and Value

TMI functions across the areas of:



TMI has demonstrated impact across five defined market gaps:

1. We bridge academia, theory, and research to practice.
2. We move away from blame and shame, and instead adopt a positive approach to understanding the lived experiences of men.
3. We apply "never about you without you" design principles to develop evidence-informed programs.
4. We test new hypotheses and move away from silos and simplistic solutions.
5. We develop, implement, validate, and share unique new programs.

Embracing Complexity & Redefining Legacy

Our Model and Approach

TMI seeks to understand the breadth of men's beliefs about who they are and who they want to be in the world, and to challenge aspects of masculinity that no longer serve them, their families, or their communities.

We ask, "what does it mean to be a man?" and apply a simple model that takes men on a Journey into Integrity ©.

Get Clear

Decide what kind of man I want to be and how I will show up in life.

Face the Facts

Examine how I am showing up and how I want to be different.

Make a Play

Take action to change my behaviour and way of being in the world.

Our Programs and Impact

Through our programs, we introduce models, processes, and forums for dialogue and education that focus participants on:

- Aspirational masculinity: appealing to values as the path of change.
- Self-awareness, openness, and communication: talking about emotions and shifting intention from competition to community.
- Inclusiveness, empathy, and accountability: recognizing one's impact on others, holding one another accountable, speaking truth, and maintaining a growth mindset.

Examples

Good Men in Sport Program

Atlanta Falcons Rookies

West Point Military Academy Football

Stanford University Soccer

University of Oregon Tennis & Golf

"Redefining what it means to be a man through sport"

Helping athletes and coaches, from amateur to professional, explore what it means to be a good man on every team they are on, while becoming role models and leaders in their own lives.

> *"This is our first time having these important conversations."*
>
> —Athlete, Stanford Men's Soccer

Participant impact

77% More aware of the importance of sexual consent

87% Clearer on the kind of man they want to be

88% More committed to inclusion on every team

97% Stronger effort to be a "good brother"

Note: Surveyed West Point and Stanford participants.

Protective Services Resilience and Leadership Program

BCPFFA

BC Police Association

Vancouver Police Union

Worksafe BC

"Protecting those who protect us"

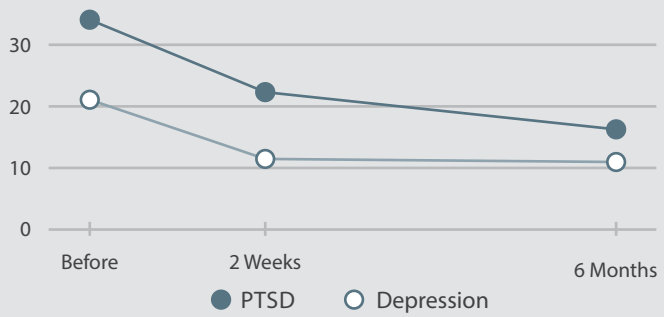
Helping first responders (fire and police), military, and front-line healthcare professionals regain and sustain their resilience and well-being, which improves the competence and capacity of organizations to care for each other and protect their wider communities.

> *"We recognized an overwhelming need to change our fire service culture. Without a doubt, since running our first six resiliency programs, the change in the individual and in fire departments across BC continues to be extremely positive. The difference this program is making is significant; if we can be healthy and resilient at work, we can be healthy at home with our families!"*
>
> —Gord Ditchburn, President of the BC Professional Fire Fighters Association

Participant impact

- ✓ Significant increase in self-compassion, quality of life, well-being, and interpersonal functioning.
- ✓ Improved work culture, team cohesion, engagement, and performance.

Significant and lasting reduction in symptoms of trauma (PTSD) and depression



Note: Results from the first 61 participants who completed the program.

THORNAPPLE
PRESS

THORNAPPLE PRESS

THORNAPPLE
PRESS

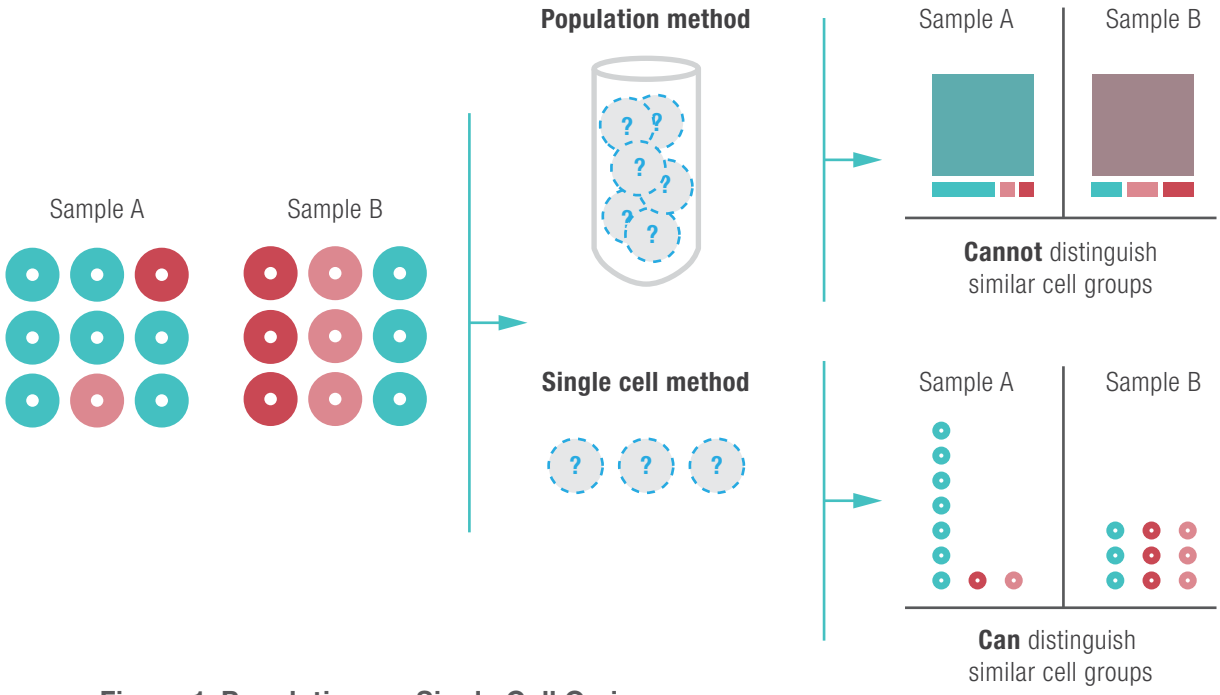


Figure 1. Population vs. Single Cell Omics

Current state of single-cell -omics

A variety of new technologies to extract -omic-level information from single cells have reached widespread use in recent years. These tools provide unprecedented investigative power to researchers examining cellular heterogeneity, whether at the level of DNA, RNA, proteins or metabolites. A summary of each of these technologies follows.

Single-cell genomics

Genomics is the oldest of the -omics, and the Human Genome Project of 1984–2003 is its most famous accomplishment.[1] Until recently, however, genomics was not a single-cell field. Single-cell genomics effectively began with experiments that allowed the detection of gene expression in single cells using microarrays[15] and came into its own once next-generation sequencing (NGS) entered the mainstream.[16] This method is sensitive enough to read genomes from single cells, especially with tools such as polymerase chain reaction (PCR) available to amplify the available DNA, and improvements in sensitivity and throughput continue to make this technology more accessible for new applications.[17] Most often, NGS helps determine the number of relevant single-nucleotide variants (SNVs) present in a sample, which occur at an estimated rate of approximately 1,500 per human cell and which are often associated with disease states.[18]

Single-cell DNA sequencing has been particularly useful for cancer biology. Tumors are heterogeneous tissues that arise from multiple clones and change over time as DNA repair mechanisms fail, so neither large biopsies nor study of the originating tissue can offer a complete picture of a tumor's genetics and anticipated behavior.[19] Single-cell genomics provides a powerful tool for following the progression of individual clones within a tumor and the shifts in the balance between them.[20,21] Bulk analyses are unsuited to a number of specific tasks in cancer research that are proving increasingly important, such as studying circulating tumor cells and cancer stem cells. These cells are exceedingly rare compared to ordinary tumor cells and to healthy cells, so they disappear into margins of error in bulk analyses. However, they play critical roles in tumorigenesis and metastasis and are thus important to track, understand and sequence when determining cancer prognosis and recommending treatment.[14,17] Further improvements in the sensitivity and accessibility of single-cell genomics technology will make these analyses more and more routine.

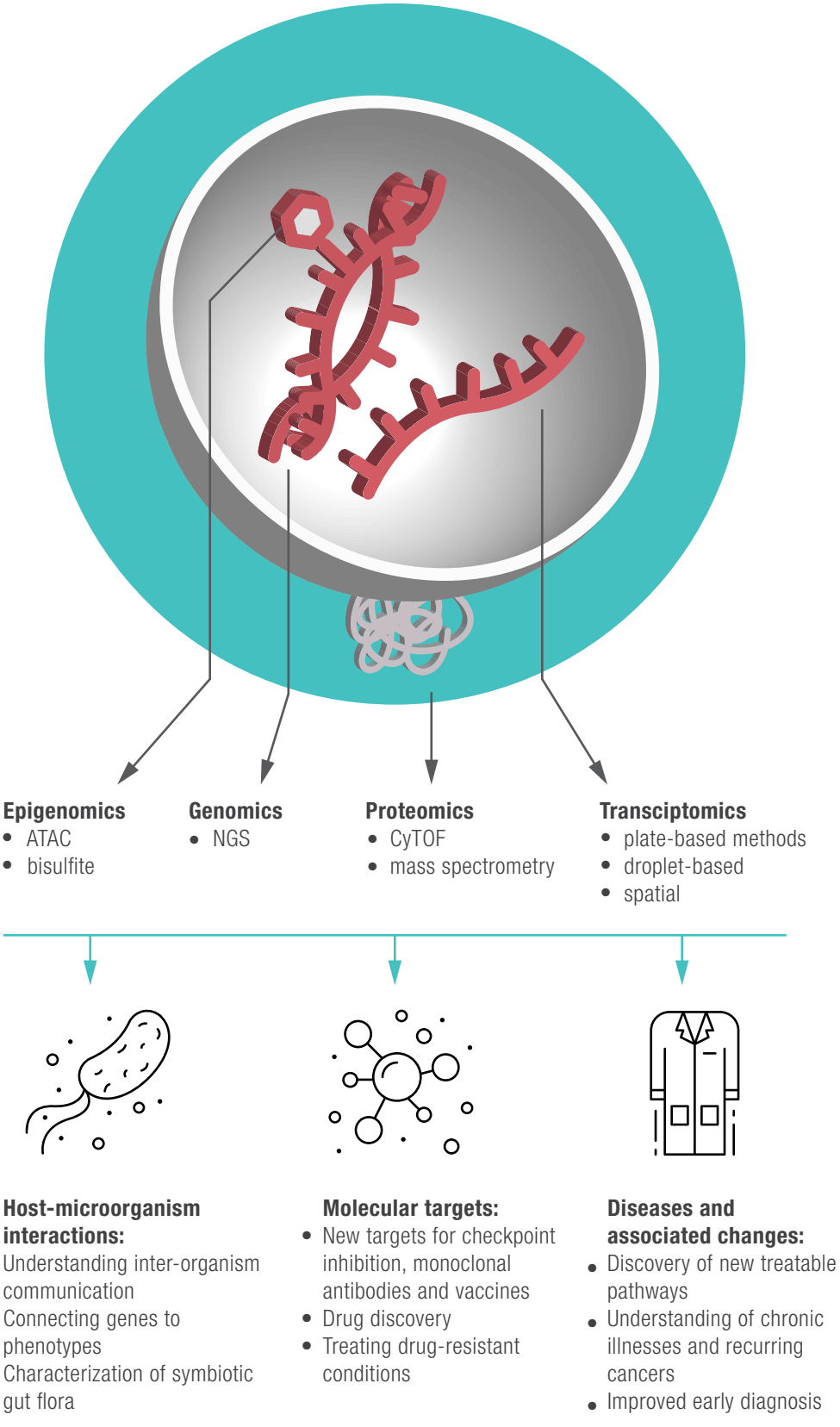


Figure 2. Single Cell Technologies and Applications

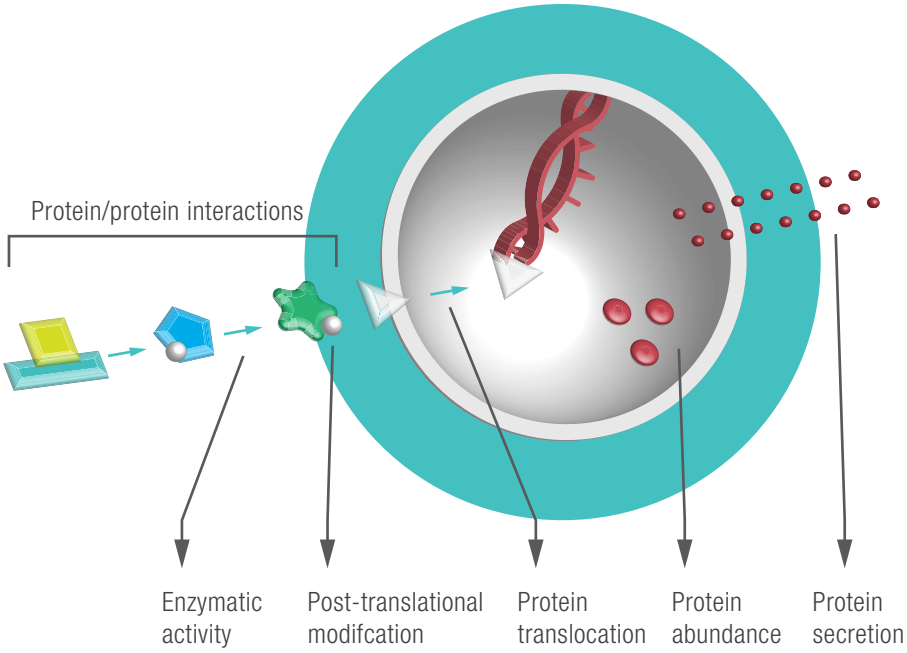


Figure 4. Protein Analysis of Single Cells
Can move beyond simple profiling and abundance changes

Single-cell western blotting

Western blotting was one of the first technologies for observing and isolating proteins, and innovations in polyacrylamide gel technology have brought it into the single-cell age. Functional proteomic studies of thousands of single cells can be achieved on a single microscope slide using single-cell western blots. Hughes et al. conducted approximately 10^3 concurrent single-cell western blots using a microscope slide with photoactive polyacrylamide gel with single-cell microwells and in-situ lysis.[38] This four-hour experiment monitored the differentiation of single rat neural stem cells and their response to mitogen stimulation using 11 multiplexed protein targets. Detection thresholds were as low as <30,000 molecules and, with integrated fluorescence-activated cell sorting, starting cell numbers as low as 200 could be analyzed. Western blotting offers higher protein specificity compared to pure antibody-based assays, as the method reports both the target molecular mass and probe binding.

Although this is the state-of-the-art level for this technology and it offers the convenience of being an at-the-bench operation, western blotting is still quite limited compared to other proteomics methods. The fluorescence signals used in western blotting are noisy and diffuse on a surface as large as a polyacrylamide gel, and accurate quantification (as opposed to identification) is difficult with this method. Like other antibody-based methods, western blotting has a very limited ability to yield data on unknown proteins and it is best suited to studies with pre-identified targets for which antibodies already exist.

Single-cell flow cytometry

The most established method for single-cell protein analysis is flow cytometry, which was invented in the 1960s. Its effectiveness derives from the fact that, although the actual protein amounts in single cells are exceedingly small, they can be very concentrated. When the cells are kept intact throughout measurement, as in flow cytometry, these high concentrations become measurable via fluorescent antibody-based tags. At first, flow cytometry was limited to measuring one or two fluorescent species at a time, but modern versions can measure up to 15, allowing the profiling of entire pathways.[39,40] The ability to perform correlated measurements of multiple proteins in single cells has allowed flow cytometry to become a powerful tool for quantitatively analyzing pathways and understanding diseases associated with them.[41,42] Improvements in both instrumentation and the availability of highly specific antibodies has brought flow cytometry this far, and the advent of barcoding methodology, improved tags and dyes, and microfluidic technologies for sample handling will continue to improve this technology, keeping it relevant for future studies.

other experiment benefits from increasing sample size. The extreme nature of single-cell systems means that simply scaling down methods designed for larger samples is not sufficient, and sample handling methods specific to single-cell systems must be devised. Microfluidics represents a particularly promising source of these innovations, enabling cell isolation, lysing, culturing and transporting for large numbers of individual cells without losing analytes.[52] Microfluidic sample preparation techniques come with significant improvements in throughput performance, cost-effectiveness, workflow complexity and assay consistency compared to alternatives.

The proteomics community has not yet agreed upon one single method as the best suited for single-cell analysis. Nevertheless, a few groups have successfully demonstrated single-cell proteomics analysis. This section describes their methods.

Nanodroplet processing in one pot for trace samples (NanoPOTS)

Proteomics sample preparation typically includes protein extraction, proteolytic digestion, cleanup and delivery to the analytical platform. As sample amounts decrease without a concomitant reduction in reaction volume (often limited by evaporation and the ~microliter volumes addressable by pipette), the nonspecific adsorption of proteins and peptides to the surfaces of reaction vessels, along with inefficient digestion kinetics, become increasingly problematic. Efforts to improve this aspect of sample preparation have included the use of low-binding sample tubes and the advent of "one-pot" digestion protocols that reduce losses by removing the need to move the sample through multiple vessels. Nanodroplet processing in one pot for trace samples (NanoPOTS) is one such protocol.

NanoPOTS addresses the issues of miniaturizing protein digestion and cleanup by reducing the processing volume to less than 200 nL, which significantly accelerates reaction kinetics. However, by reducing the volume more than 200 times compared with conventional methods, it significantly reduces sample losses due to nonspecific adsorption of the proteins to surfaces. The method consists of the use of a liquid handler capable of dispensing nanoliter volumes into wells etched in a glass slide with a volume of 200 nL each. The system is typically integrated into a flow cytometry system or to a laser-capture microdissection system. Cells are sorted and deposited into the wells, and then the sample is prepared by adding all necessary reagents. Because of its architecture, this system allows multiple digestion and extraction steps to take place without changing containers. The digested peptides are then retrieved and delivered to the mass spectrometer via glass capillary tube or are directly placed into an autosampler plate.[53] When combined with ultrasensitive liquid chromatography-MS, nanoPOTS allows the identification of ~1,500 to ~3,000 proteins from ~10 to ~140 cells, respectively, with efforts to decrease that number even further.[54] The team has also demonstrated the method's compatibility with tandem mass tags (TMTs),

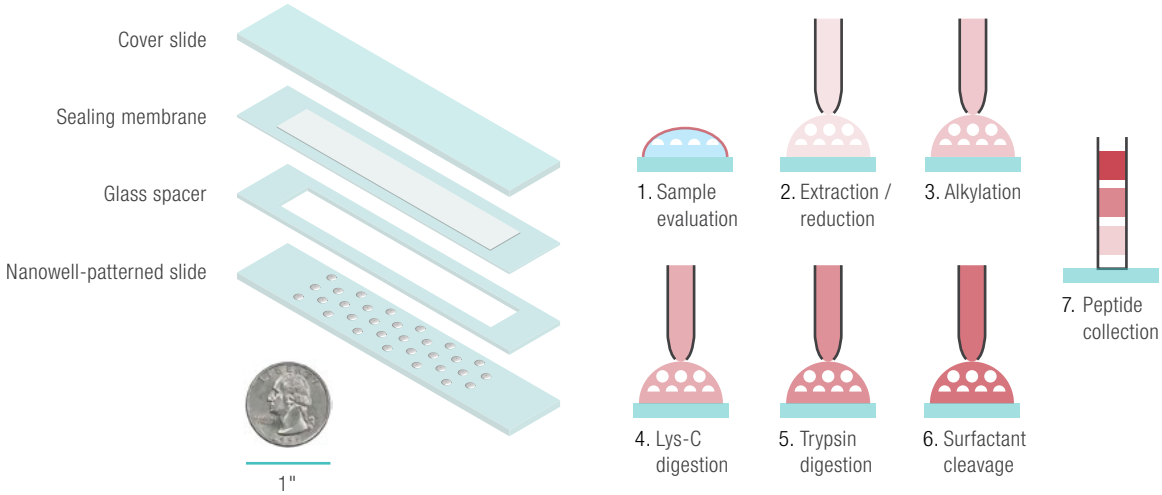


Figure 5. Nanodroplet Processing for Proteomics Applications

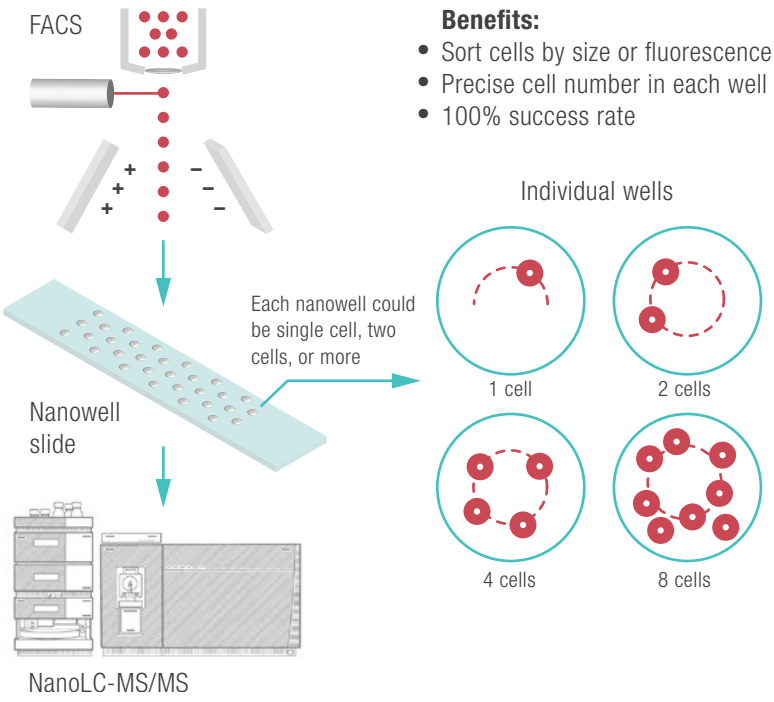


Figure 6. Single Cell Proteomics by Mass Spectrometry

which allows for the analysis of several cells at a time when all the proteins for each given cell have been labeled with a specific mass tag.

The capability of nanoPOTS to be combined with other cell isolation techniques and its high sensitivity mean it promises to be one of the most important technologies in this field.

Single-cell proteomics by mass spectrometry (SCoPE2-MS)

SCoPE-MS is a mass spectrometry workflow optimized for single-cell proteomics developed by Budnik and Slavov, currently in its second version (hence, SCoPE2).[55] It is designed to address two major issues with conventional MS approaches when applied to single cells: minimizing losses during sample preparation and achieving the simultaneous identification and quantification of peptides from multiple samples.[56] In Budnik and Slavov's test, SCoPE-MS enabled the quantification of over 2,000 proteins in 356 single monocytes and macrophages in about 85 hours of instrument time, and the quantified proteins were used to discern single cells by cell type. With such an abundance of highly precise and complete data, they were able to analyze the emergence of cellular heterogeneity as homogeneous monocytes differentiated into macrophage-like cells in the absence of polarizing cytokines. This workflow shows great promise and future developments will increase its throughput, speed and ease of use, eventually enabling it to quantify and identify thousands of proteins and peptides in single cells.

SCoPE-MS features several key innovations over other MS approaches. To minimize losses, live cells are lysed via sonication or freeze-thaw cycles rather than using chemical detergents, which are generally incompatible with MS measurements. Since these chemicals are not used, they do not then need to be cleaned out of the sample, which removes the danger of sample losses during cleaning steps.

To aid with simultaneous identification and quantification and signal enhancement, SCoPE-MS uses tandem mass tags (TMTs). These isobaric labeling reagents allow for the quantification of each tagged peptide and connect them across samples, providing enough material to generate a complete sequence when all of the tagged peptides are pooled together. The SCoPE-MS method improves identification by also including with each single-cell set a sample composed of more than one cell, typically between 10 to 200 cells. This sample is what scientist have named the boost sample or the boost channel, because it includes

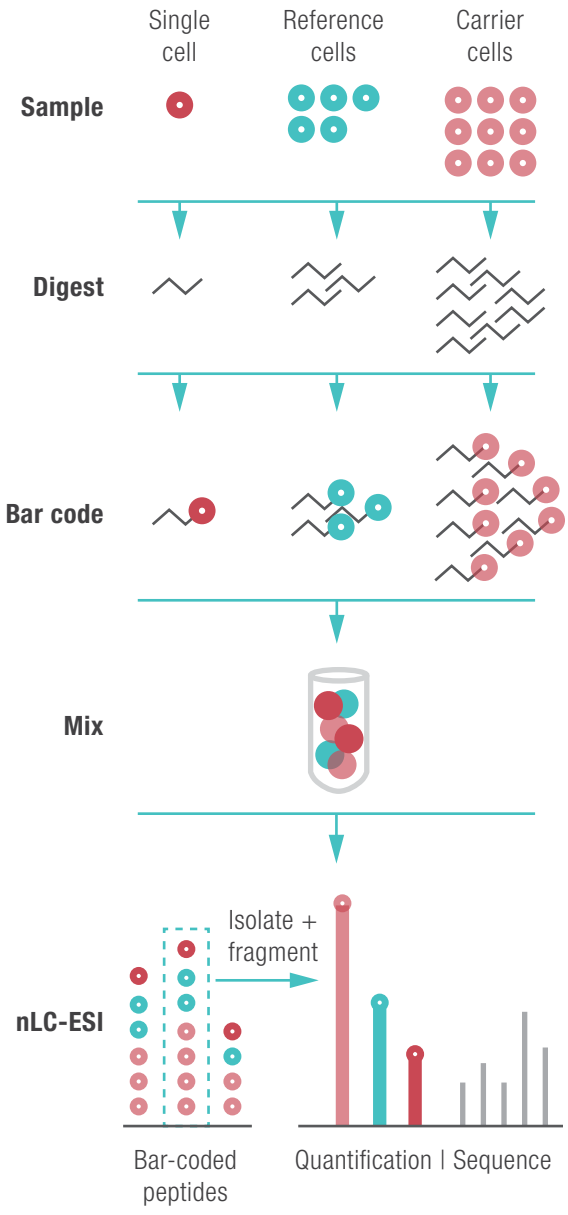


Figure 7. SCoPE2 Workflow

sufficient peptide ions to provide enough signal to yield a peptide sequence identification from the mass spectrum without the sensitivity limitations of single-cell samples. Meanwhile, the TMT provides the precision required for quantitative analysis of the identified peptides.

Boost channels for throughput, increased sensitivity and quantification

Building on Budnik et al.'s success,[55] Maowei Dou and his team combined nanoPOTS sample preparation with TMT to improve both proteomic sample processing efficiency and analysis throughput for single cells. Their boost-channel experiment achieved multiplex analysis of single-cell–level protein quantities to a depth of 1,600 proteins with a median CV of 10.9% and a correlation coefficient of 0.98.[57] They also measured protein expression in 72 single murine epithelial, immune and endothelial cells. In this study, they were able to identify 2,300 proteins with less than two days of instrument time. Erwin Schoof's team used a similar approach to derive quantitative information about 10 single cells per MS injection. Schoof's team studied a leukemia culture system containing functionally defined leukemic stem cells, progenitors and terminally differentiated cells, and the boost-channel approach helped them gain information about this aberrant developmental hierarchy.[58] The new TMT 16-plex reagents currently on the market and further improvements in platform automation will continue to improve the throughput of these approaches.

Liquid chromatography separations for low-level samples

High-performance liquid chromatography (HPLC) has a long history of use as part of MS workflows, to the point that they are often combined as LC-MS systems. Modern proteomics separations employ low-flow or nanoflow HPLC with small internal diameter (ID) chromatography columns coupled to electrospray ionization (ESI) to get the peptides into the gas phase and ready to be analyzed by the MS. The ESI process is critical because the signal in the mass spectrometer depends on how well the setup is able to transition the peptides from the liquid phase into the gas phase. To minimize losses, it's important to increase the

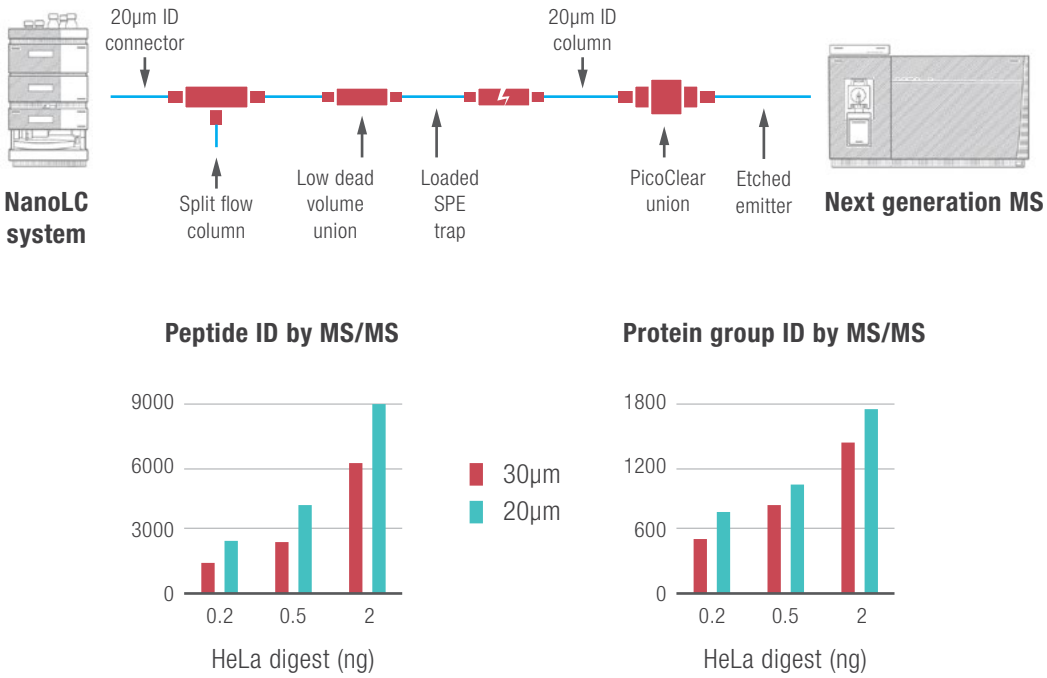


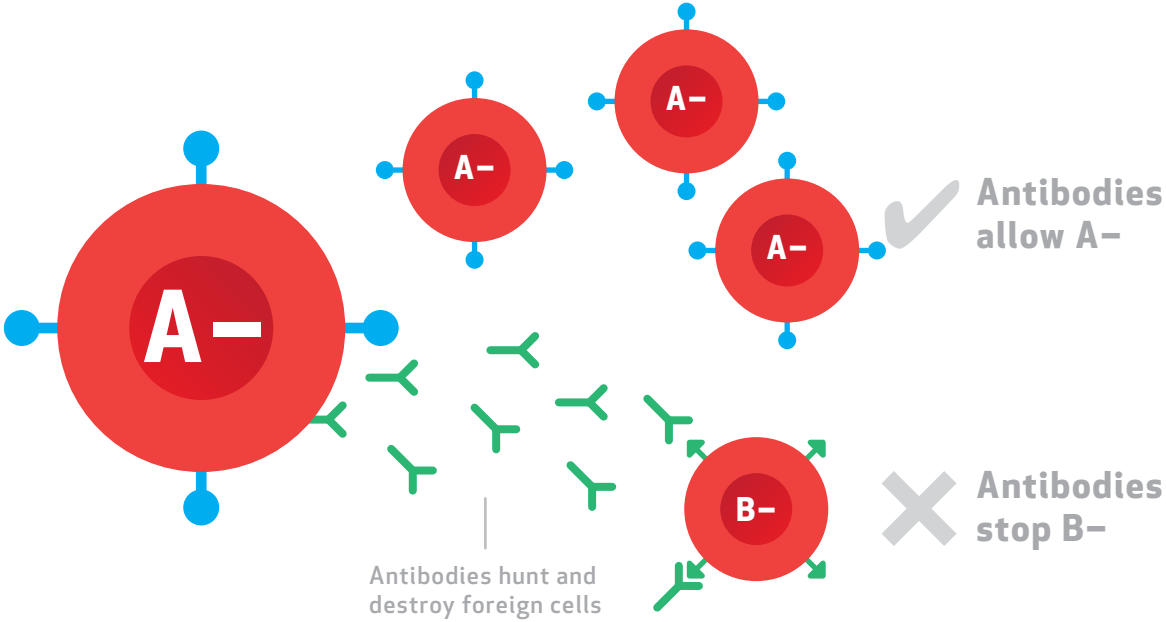
Figure 8. Next Generation Ultra High Sensitivity LC/MS Platform for Single Cell Proteomics

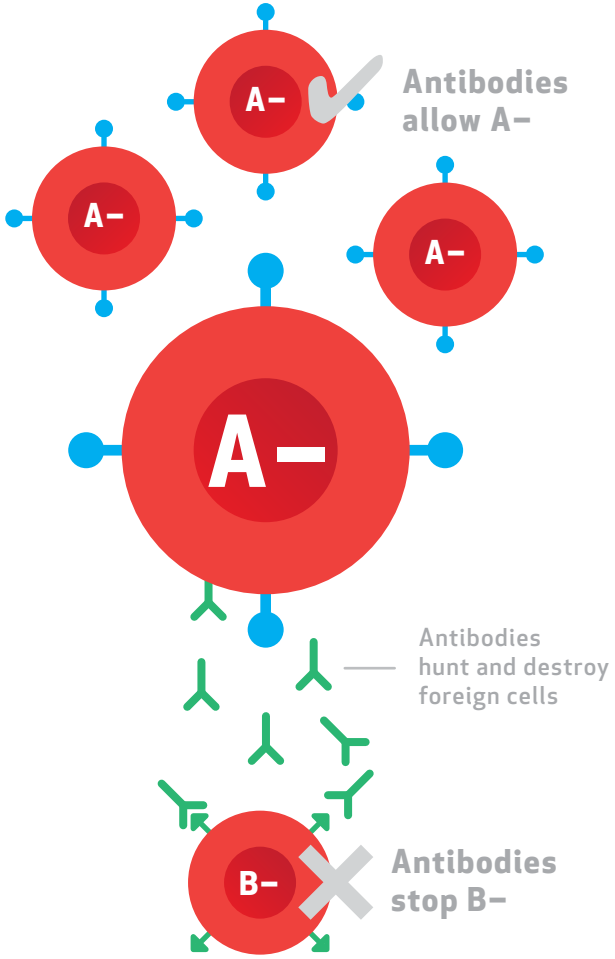
analyte concentration and reduce the size of the droplet during the ESI process. This is why it is key to reduce the chromatography to low nL/min flow rates and to reduce the internal diameter of the chromatography columns to maximize peak capacity. These settings enable the generation of high-quality data from complex, tiny samples such as single cells.

Our team recently demonstrated that switching to 30-µm-i.d. nanoLC columns rather than the conventional 75-µm-ID columns can substantially enhance sensitivity due to increased ionization efficiency at the nanoelectrospray ion source and increased concentration of each component eluting from the narrow-bore columns. ESI emitter technology that accommodates the resulting lower flow rates could be employed to improve the detection sensitivity of the LC-MS system. However, practical issues still remain, such as the challenge in interfacing single-cell samples with the MS instrument.

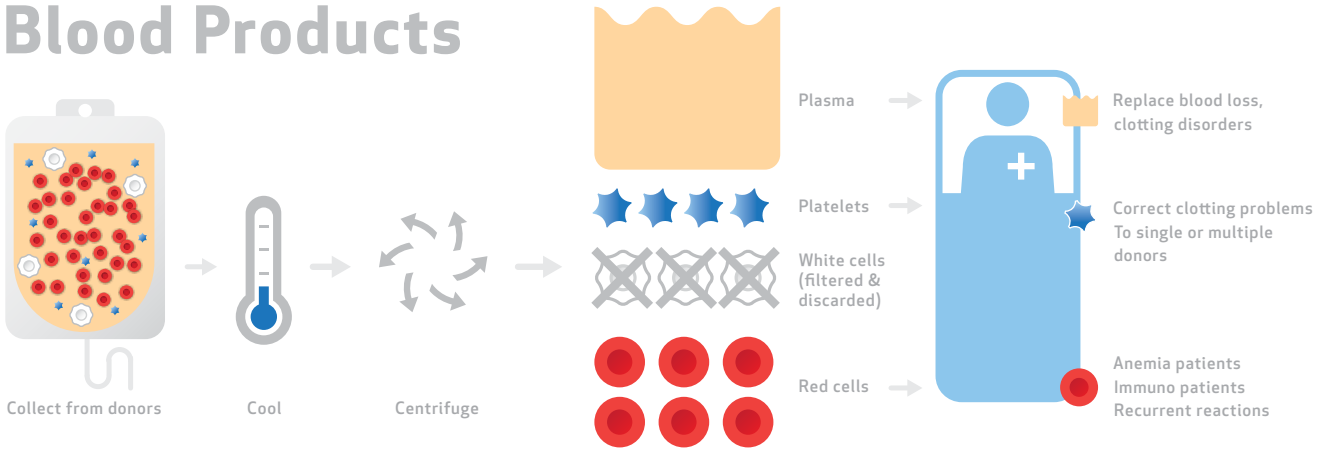
Gas phase separations

One of the major gaps in single-cell MS has always been the lack of sensitivity. The technologies described above have been focused on how to reduce losses and how to get the most signal from the sample. However, in these types of experiments, one of the major limitations is the trace chemical contaminants intrinsic to the process or to the sample. These chemical contaminants tend to be small molecules that compete for ionization and signal during the MS analysis. Recent studies show that the most recent generation of Orbitrap-based mass spectrometers are capable of analyzing single cells despite this hazard.[30,44] Further enhancements on proteome coverage are mandatory for the field to become more useful. New technologies such as FAIMS (field asymmetric ion mobility spectrometry) can be used to remove impurities, in this case +1 ions that are not peptides, increasing the signal-to-noise ratio and improving the sensitivity of all measurements significantly.

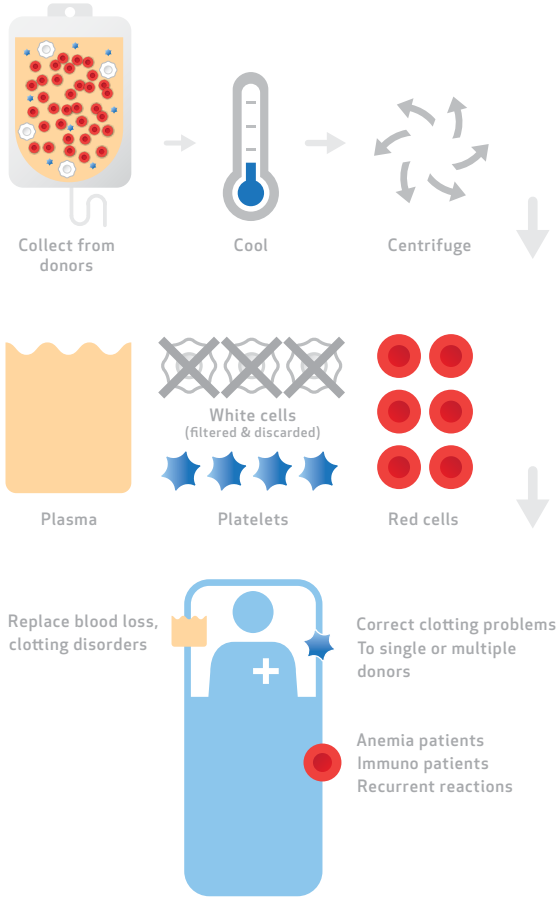

A–
A–
A–
A–
B–
Antibodies allow A–
Antibodies stop B–
Antibodies hunt and destroy foreign cells

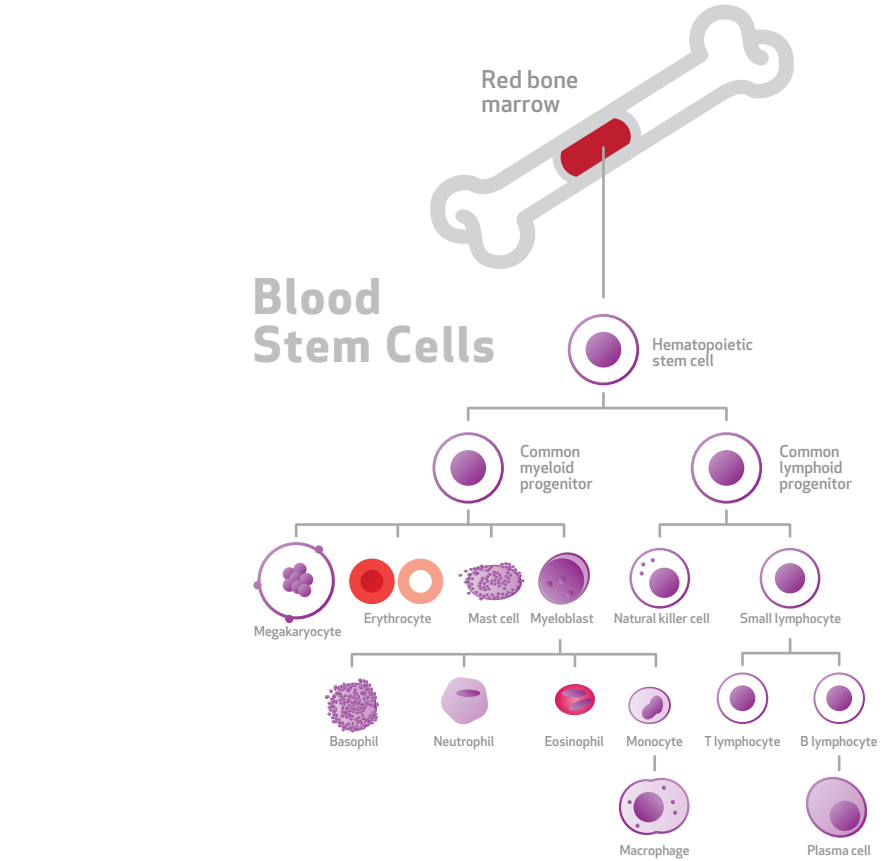

A–
A–
A–
A–
B–
Antibodies allow A–
Antibodies hunt and destroy foreign cells
Antibodies stop B–

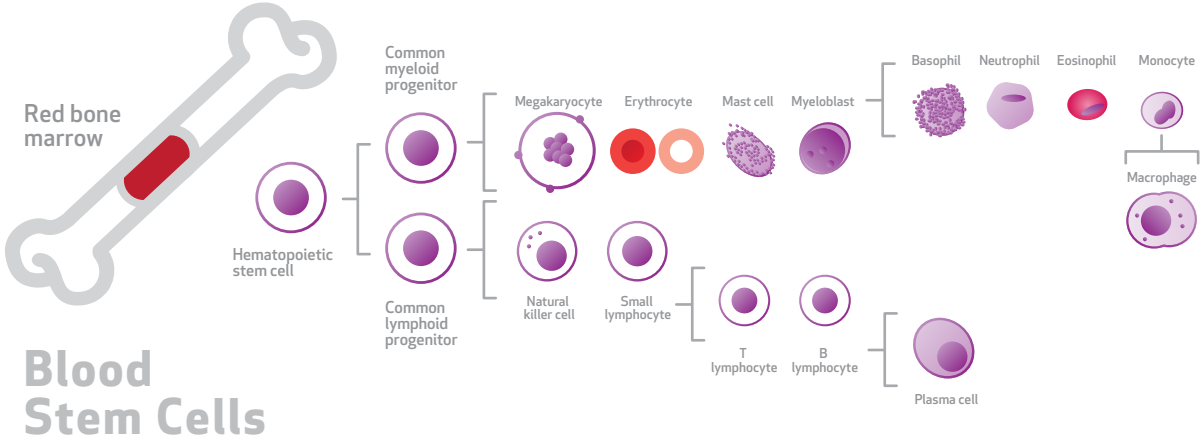
Blood Products



Blood Products




Red bone marrow
Blood Stem Cells
Hematopoietic stem cell
Common myeloid progenitor
Common lymphoid progenitor
Megakaryocyte
Erythrocyte
Mast cell
Myeloblast
Natural killer cell
Small lymphocyte
Basophil
Neutrophil
Eosinophil
Monocyte
T lymphocyte
B lymphocyte
Macrophage
Plasma cell


Red bone marrow
Blood Stem Cells
Hematopoietic stem cell
Common myeloid progenitor
Common lymphoid progenitor
Megakaryocyte
Erythrocyte
Mast cell
Myeloblast
Natural killer cell
Small lymphocyte
T lymphocyte
B lymphocyte
Plasma cell
Basophil
Neutrophil
Eosinophil
Monocyte
Macrophage

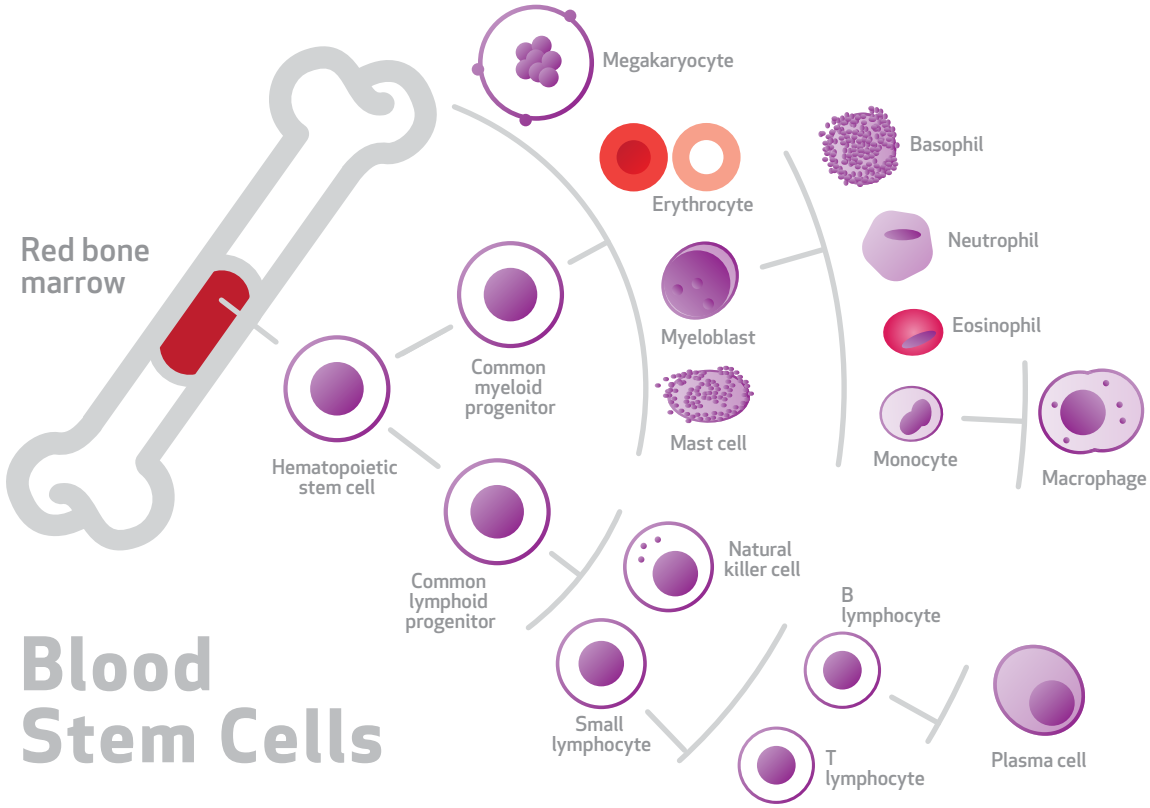
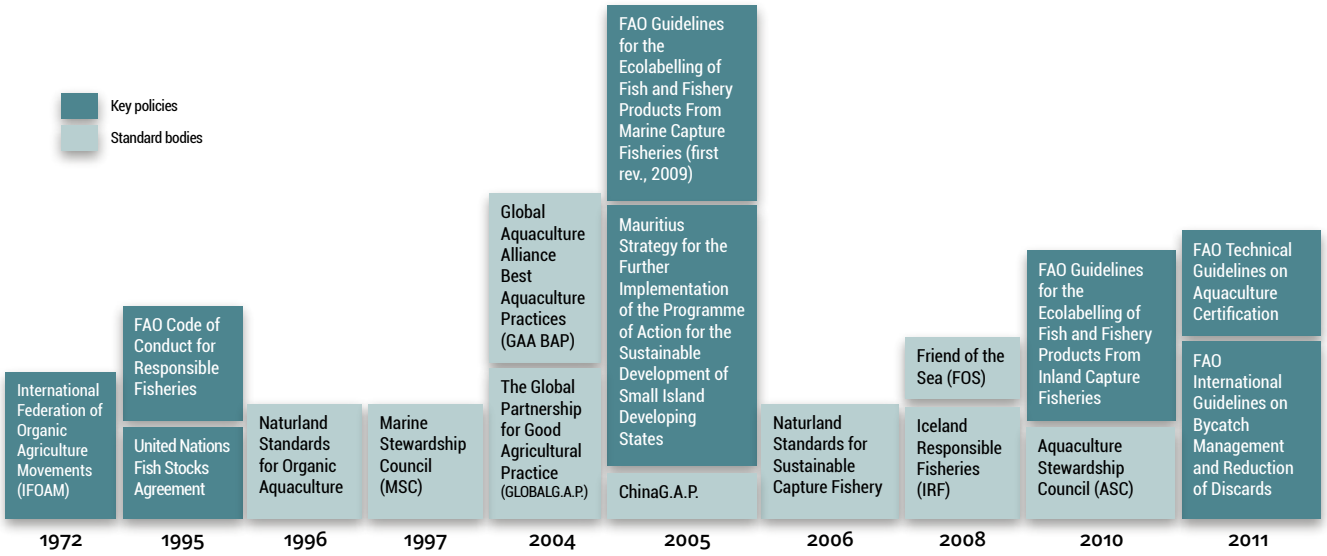

Red bone marrow
Megakaryocyte
Erythrocyte
Basophil
Neutrophil
Eosinophil
Myeloblast
Common myeloid progenitor
Mast cell
Monocyte
Macrophage
Hematopoietic stem cell
Common lymphoid progenitor
Natural killer cell
B lymphocyte
Small lymphocyte
T lymphocyte
Plasma cell
Blood Stem Cells

Figure 1.1 Timeline for initiative and key policy implementation



Defining targets: Every voluntary standard begins with a process dedicated to establishing standards for sustainable practice. These efforts have the potential to push the boundaries of accepted practice while developing new technologies and support systems for their implementation. To the extent that a given standard's processes are participatory, representative and based on scientific evidence, they have the potential to offer meaningful input into the actual definition of the blue economy. Within the context of politically motivated international negotiations, the brass tacks approach of private-sector initiatives may offer a more efficient means of coming to agreement.

Ocean health: One of the key features of the blue economy initiative is its focus on ocean health as the foundation of broader ecosystem and economic health. The notion of sustainable fish production is closely related to the management of a sustainable fish habitat and/or ecosystem. As a result, most voluntary sustainability standards in the seafood sector have integral elements that are designed to promote overall ocean health. However, depending on the priorities of the specific initiative or the sectors within which it works, ocean health per se may be of more or less relevance.

Good governance: Definitions of the blue economy to date have placed emphasis on building a vision for sustainability from the perspective of ocean-dependent nations. Small island states view the blue economy approach as a way of enabling a more participatory role in global sustainability planning. Voluntary standards often include multistakeholder governance models as a means for ensuring buy-in from different groups along the supply chain. Depending on the way a standard is governed, more marginalized players, such as those targeted in the blue economy, may find it easier to participate in its development through multistakeholder voluntary initiatives than through larger multilateral negotiations on seafood sustainability.

Economic growth and poverty reduction: Voluntary standards can also present a host of economic benefits. The most direct benefit may come in the form of higher prices associated with differentiated markets for sustainable products. Certification can facilitate access to international markets, as certification programs set minimum sustainability practices as a price of market entry. Certification, through its reliance

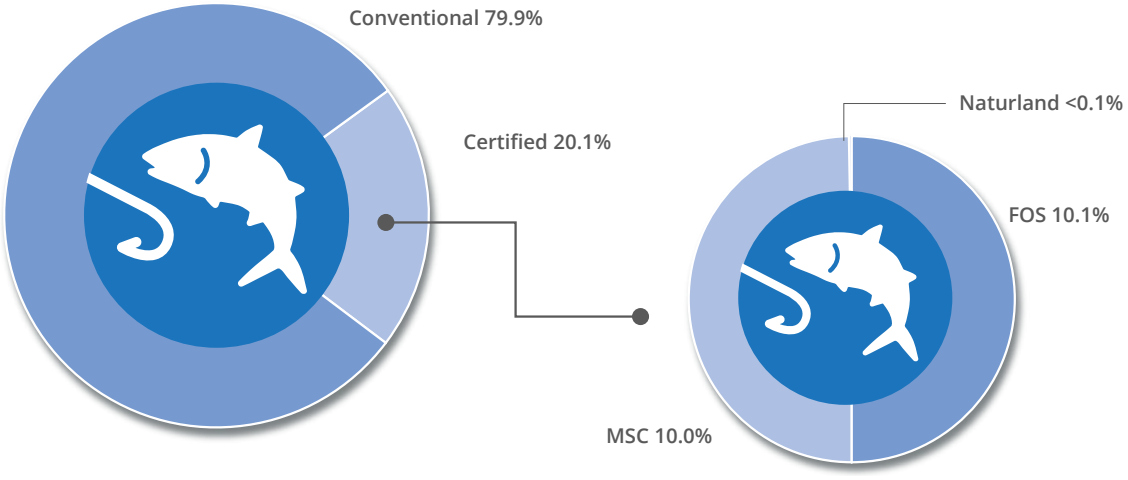
Table 2.4 Key statistics: Wild catch production (years for data listed in source note)

Global production	92.6 million mt
Top 5 producers and proportion of total	China (17%), Indonesia (7%), Peru (6%), United States (6%), India (5%) Total combined proportion: 41%
Top 5 species groups produced and proportion of total	Anchoveta (9%), tuna (6%), cod (6%, 3% of which is Alaska pollock), sardines (4%), shrimp/prawns (4%) Total combined proportion: 29%
Major international standards	Friend of the Sea, Marine Stewardship Council
Standard-compliant production	18.6 million mt (20% of global production)
Top 5 standard-compliant producers	Peru (31%), United States (19%), Norway (8%), Russia (6%), Chile (6%) Total combined proportion: 70%
Top 5 standard-compliant species groups	Anchoveta (36%), cod (19%, 12% of which is Alaska pollock), tuna (10%), mackerel (5%), salmon (4%) Total combined proportion: 74%
Retail value of compliant production	US$7.9 billion

Sources: Global production, top 5 producers, top 5 species groups produced, FAO Fishstat, 2015 (2013 data); standard-compliant data obtained from personal communication with the standards (data used is the latest available); Conventional, 2013; FOS, 2014 (species- and country-level data), 2015 (aggregate data); MSC, 2015; retail value of compliant production is calculated from an extrapolation of an estimation of MSC retail value in MSC, 2014, p. 11.

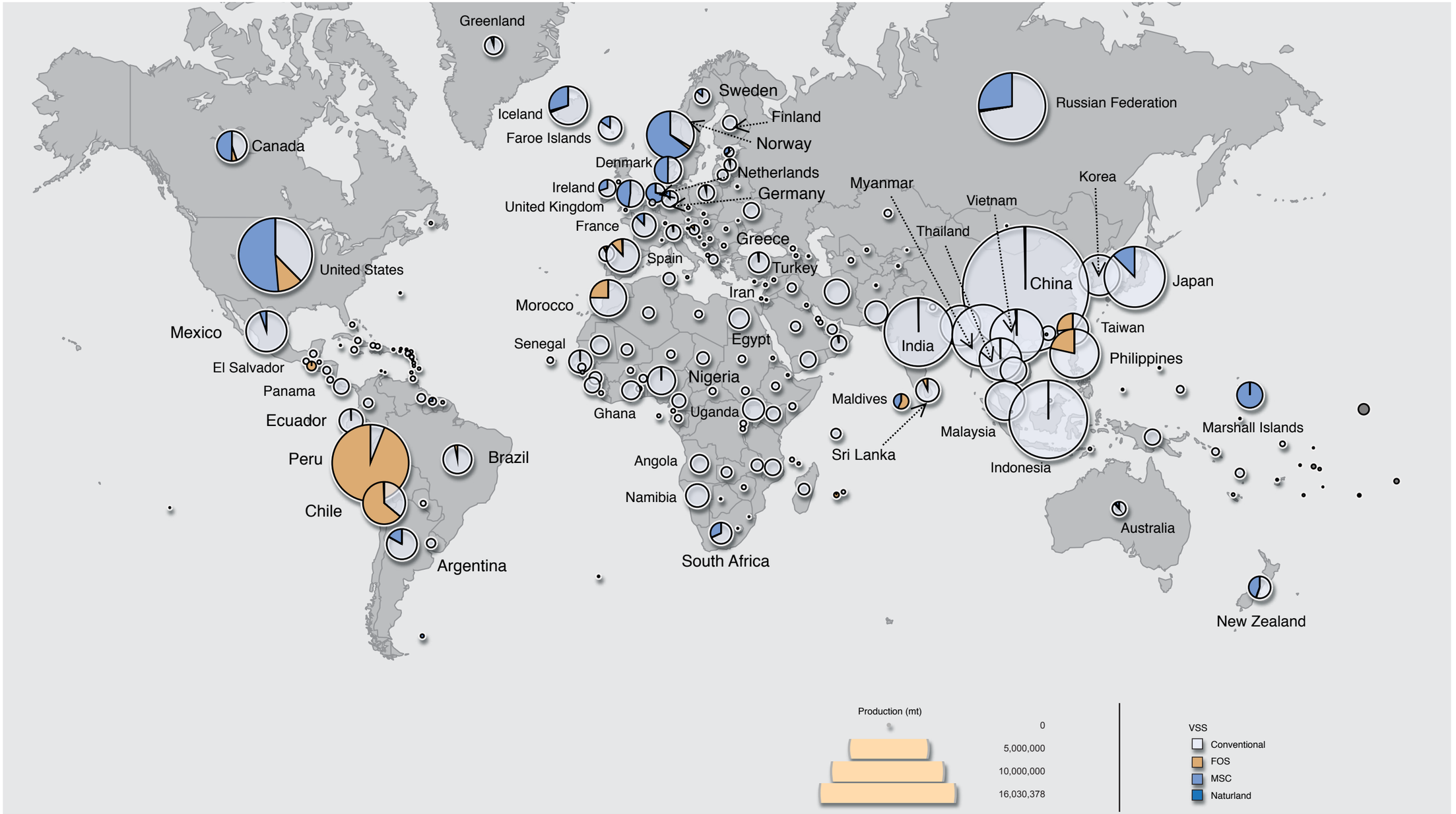
Figure 2.5 Certified catch as portion of total wild catch (years for data listed in source note)

Certified wild catch accounted for 20 per cent of global wild catch in 2015, with FOS and MSC certifying nearly equal portions of total certified production.



Data years: Global total, 2013; FOS, 2015; MSC, 2015
Sources: FAO Fishstat, 2015; MSC, FOS, personal communication, 2015.

Figure 2.7 Global distribution of certified wild catch as a portion of total, 2015



Source: FAO Fishstat, 2015; MSC, FOS, personal communication, 2015.

CANADA-WIDE ACTION PLAN ON ZERO PLASTIC WASTE

PHASE 1

PREVENTION	COLLECTION & CLEAN UP	VALUE RECOVERY	ENABLING ACTIVITIES

ACTIONS

1. Extended producer responsibility
2. Single use and disposable products
3. National performance requirements and standards
4. Incentives for circularity
5. Infrastructure and innovation investments
6. Public procurement and green operations

G7 OCEAN PLASTICS CHARTER

100% reusable, recyclable, or recoverable plastics

Reduction of plastic microbeads in rinse-off cosmetic and personal care products

At least 50% recycled content in plastic products

Recycling and reuse of at least 55% of plastic packaging

100% RECOVERY OF ALL PLASTICS

CCME ACTION PLAN

2020	Design best management for landfill bans	CCME, led by Nova Scotia
Dec. 2020	Refresh the Canada-wide Action Plan	CCME, led by Nova Scotia
	Develop designated plastic categories	CCME product led by British Columbia and Ontario
Dec. 2021	Address single use, disposable plastics	CCME product led by Canada and Québec
	Increase greening of government operations	
	Update government procurement for reusability, recyclability & compostability	Federal / provincial / territorial
Dec. 2024	Develop economic incentives / disincentives	CCME, lead to be determined
	Update government procurement of durable goods	CCME product, led by Canada
	Design reparability, re-usability, recoverability, remanufacturing, salvage	Federal / Provincial / Territorial Collaborative Procurement Initiative
	Increase public reporting on government initiatives	
2030	Implement financing for value recovery	Federal / provincial / territorial
	Increase Funding for innovation	
	Develop guidelines for recyclability	CCME, lead to be determined
	Develop guidelines on Recycled content, Reparability, Compostability	Federal government
2040	Implement regulatory requirements	Federal / provincial / territorial
	Design refurbishment, remanufacturability	Federal government

PHASE 2 ▸ TBA

1. Consumer awareness
2. Aquatic activities
3. Research & monitoring
4. Clean up
5. Global action

CCME
Canadian Council of Ministers of the Environment
Le Conseil canadien des ministres de l'environnement

CANADA-WIDE ACTION PLAN ON ZERO PLASTIC WASTE

PHASE 1

PREVENTION | COLLECTION & CLEAN UP | VALUE RECOVERY | ENABLING ACTIVITIES

ACTIONS

1. Extended producer responsibility
2. Single use and disposable products
3. National performance requirements and standards
4. Incentives for circularity
5. Infrastructure and innovation investments
6. Public procurement and green operations

G7 OCEAN PLASTICS CHARTER		CCME ACTION PLAN	
100% reusable, recyclable, or recoverable plastics Reduction of plastic microbeads in rinse-off cosmetic and personal care products	2020	Design best management for landfill bans	CCME, led by Nova Scotia
	Dec. 2020	Refresh the Canada-wide Action Plan Develop designated plastic categories	CCME, led by Nova Scotia CCME product led by British Columbia and Ontario
	Dec. 2021	Address single use, disposable plastics Increase greening of government operations Update government procurement for reusability, recyclability & compostability	CCME product led by Canada and Québec Federal / provincial / territorial
At least 50% recycled content in plastic products Recycling and reuse of at least 55% of plastic packaging	Dec. 2024	Develop economic incentives / disincentives Update government procurement of durable goods Design reparability, re-usability, recoverability, remanufacturing, salvage Increase public reporting on government initiatives	CCME, lead to be determined CCME product, led by Canada Federal / Provincial / Territorial Collaborative Procurement Initiative
	2030	Implement financing for value recovery Increase Funding for innovation Develop guidelines for recyclability	Federal / provincial / territorial CCME, lead to be determined
		Develop guidelines on Recycled content, Reparability, Compostability	Federal government
100% RECOVERY OF ALL PLASTICS	2040	Implement regulatory requirements Design refurbishment, remanufacturability	Federal / provincial / territorial Federal government

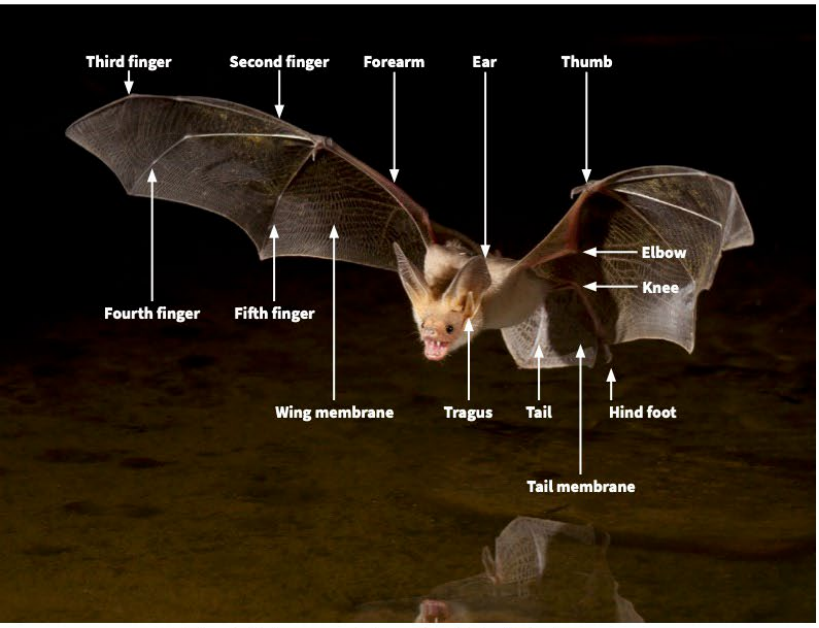
PHASE 2 ▶ TBA
1. Consumer awareness
2. Aquatic activities
3. Research & monitoring
4. Clean up
5. Global action

CCME V0.3 April 9 2019

CCME
Canadian Council of Ministers of the Environment | Le Conseil canadien des ministres de l'environnement

References

Baker and Bradley (2006); Blejwas et al. (2021); Burgin et al. (2018); Frick, Heady and Hayes (2009); Gunnell and Simmons (2005); Haase et al. (2021); Hranac et al. (2021); Humphries, Thomas and Speakman (2002); Jung and Kukka (2016); Lausen et al. (2008); Lausen et al. (2019); McClure et al. (2020); Meredith et al. (2011); Morales and Carstens (2018); Simmons and Cirranello (2020); Simmons et al. (2008); Slough et al. (2022); Tessler, Snively and Gotthardt (2014); Wheeler and Meier (2000).



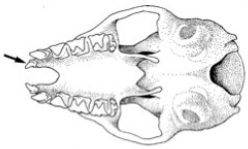
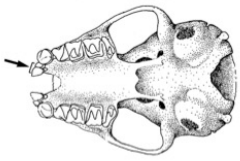
PALLID BAT in flight. Labels denote morphology referred to in this book.

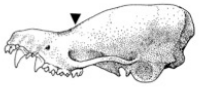
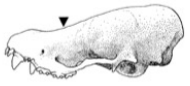
General Biology

Morphology

Bats are the only mammals that can fly. All have forelimbs modified for flight, though wing size and form vary among species. Their wings possess exceptional load-carrying capability and, depending on the species, they can hover, fly exceptionally fast or be remarkably manoeuvrable. The wing consists of a thin, double-layered membrane of skin stretched between the arms, hands and fingers. The skin of bat wings is unique among vertebrates in its abundant and distinctive nerve endings. It is also significantly thinner than the skin of other comparably sized mammals and is thus low in mass despite its large surface area. Another flight membrane, the tail membrane, extends between the hind legs and tail. These membranes contain elastin fibres, making them elastic and flexible, and in adults are relatively tough and resistant to tearing. The skin contains blood vessels that are important for thermoregulation, and muscles that stretch and relax the flight membranes. In most bat species, the surfaces of the wing and tail membranes are devoid of hair, which reduces drag when the bat is in flight, although the regions around the upper arm and base of tail are often furred. The bones of the arm, hand and fingers provide the internal support for the wing. They follow the usual mammalian structure of an upper arm, forearm, wrist, and hand with a thumb and four fingers. In Pteropodidae (flying foxes, see image on page viii), the thumb and the first finger (second digit) after the thumb have a claw, but in the rest of the families only the thumb is clawed. Clawed thumbs are used for moving around in the roost or sometimes on the ground, climbing and grooming. The clawed second finger in Pteropodidae may additionally facilitate handling of fruit for consumption.

The finger and hand bones in a bat's wing are greatly elongated in comparison to those of humans. Compared to the wings of other vertebrate flyers, like birds and pterosaurs, the bat wing has many more bones and joints. While resting, bats draw the finger bones together and hold the arms against the body, probably to reduce the risk of injury to the wing. When the bat is in flight, the arms are held out to the sides with the fingers spread apart. Bat fingers have among the thinnest and most delicate of mammalian bones, and, unlike all other vertebrate wing bones, they are not hollow. This architecture promotes bending, which helps the bat's wings act as an airfoil to produce lift to keep the animal aloft and thrust to push it forward. The inside portion of the wing,

1	**1a**	One upper incisor on each side of skull: Go to **2**	
	1b	Two upper incisors on each side of skull: Go to **6**	
2 (t1)	**2a**	Palate extends well behind third upper molars: Go to **3**	
	2b	Palate extends only slightly beyond third upper molars: Go to **4**	
3 (t2)	**3a**	Skull length greater than 20 mm: BIG FREE-TAILED BAT (p. 291)	
	3b	Skull length less than 20 mm: BRAZILIAN FREE-TAILED BAT (p. 285)	
4 (t2)	**4a**	One upper premolar on each side of skull: PALLID BAT (p. 135)	
	4b	Two upper premolars on each side of skull: Go to **5**	
5 (t4)	**5a**	Skull length greater than 15 mm: HOARY BAT (p. 187)	
	5b	Skull length less than 15 mm: EASTERN RED BAT (p. 179)	
6 (t5)	**6a**	One upper premolar on each side of skull: BIG BROWN BAT (p. 159)	
	6b	More than one upper premolar on each side of skull: Go to **7**	

7 (t6)	**7a**	Two upper premolars on each side of skull: Go to **8**	
	7b	Three upper premolars on each side of skull: Go to **11**	
8 (t7)	**8a**	Three lower premolars on each side of mandible: Go to **9**	
	8b	Two lower premolars on each side of mandible: Go to **10**	
9 (t8)	**9a**	Postorbital width greater than 4 mm: SILVER-HAIRED BAT (p. 197)	
	9b	Postorbital width less than 4 mm: TOWNSEND'S BIG-EARED BAT (p. 147)	
10 (t8)	**10a**	Skull length greater than 14 mm: SPOTTED BAT (p. 169)	
	10b	Skull length less than 14 mm: CANYON BAT (p. 295)	
11 (t7)	**11a**	Postorbital width less than 3.4 mm: Go to **12**	
	11b	Postorbital width greater than 3.4 mm: Go to **13**	
12 (t11)	**12a**	Forehead with steep slope: CALIFORNIAN MYOTIS (p. 207)	
	12b	Forehead with gradual slope: DARK-NOSED SMALL-FOOTED MYOTIS (p. 239)	
13 (t11)	**13a**	Ratio of postorbital width/upper toothrow length greater than 0.7 mm: Go to **14**	
	13b	Ratio of postorbital width/upper toothrow length less than 0.7 mm: Go to **16**	

Fly Agaric *Amanita muscaria* group

CAP To 30 cm across; nearly spherical at first and then becoming convex to flat; colour variable, most often bright scarlet, even blood red, but also orange to yellow, and (very rarely in our area) white; with whitish to tan or yellowish tan pyramidal warts that sometimes wash off with rain, the warts near the outside edge disappearing first; cap slippery when moist; flesh thick, white, with a layer of yellow-orange flesh below the cap surface in all colour phases. **GILLS** Free or attached; deep; white to pale cream; closely spaced. **ODOUR** Not distinctive. **TASTE** Not distinctive. **SPORE PRINT** White. **STEM** To 20 cm tall × 3 cm wide; gradually enlarging downward in an egg-shaped bulb that may taper at the base; white; smooth above ring, smooth or scaly below. **RING** Skirt-like; white to yellowish; typically more persistent than with some other amanitas, but sometimes disappearing. **VOLVA** Cottony, often showing as multiple ascending irregular rings or broken bands and patches of crumbly remnants on the lower stem, but sometimes just fragments around the base; white. **FRUITING** Single or in small to very large groups, sometimes forming interrupted fairy rings; on the ground; found with a variety of different tree species, commonly conifers and birches and BLACK COTTONWOOD; summer, autumn, early winter.

EDIBILITY Poisonous, hallucinogenic. The toxins are water soluble, so some mushroomers eat FLY AGARIC after careful preparation. **SIMILAR** The 2 mostly spring mushrooms in this genus, **SUNSHINE AMANITA** (p. 110) and **PANTHER CAP** (p. 108), might be confused with certain colour phases of the FLY AGARIC if the fruiting seasons have some overlap. But SUNSHINE AMANITA is shorter and smaller, and PANTHER CAP has more brown in the cap and a volva whose top margin tends to be well defined and gutter-like. The most likely confusion for the FLY AGARIC is between the yellow phase of this group and the **JONQUIL AMANITA** (p. 109), whose volva is more substantial and cup-like.

COMMENTS This is the iconic mushroom featured in *Alice in Wonderland* and in Mario video games. Ask a class of young children to draw a mushroom, and most will produce a picture resembling FLY AGARIC. ❧ The mushroom has a long history as a psychoactive substance and was employed in shamanistic rites in Siberia. Modern psychonauts trying this mushroom, however, seldom try it twice because ingestion without removal of the water-soluble toxins can produce sweating, nausea, vomiting, diarrhea, and neurological symptoms. ❧ Our most common FLY AGARICS on the West Coast have species-level genetic differences from the European *A. muscaria*, so a name change may be in the offing. One thing we do already know is that the exuberant range of colours and presentations in *A. muscaria* are *not* good guides to species boundaries—geographical locales seem to be the controlling variable. In BC, yellow to orange forms (what used to be called var. *formosa*) are most frequently found growing with BLACK COTTONWOOD (throughout the province) and with LODGEPOLE PINE (in the Interior). The scarlet-capped form with yellow colours in its young (button-stage) warts, ring margins, and volval ring patches (which many identify as var. *flavivolvata*) appears to be the most common variety on the coast, especially with birch, and is the most common form in our coastal urban environments. ❧ The FLY AGARIC is widespread throughout BC. In the southern Interior, it can be abundant in BLACK COTTONWOOD forests during the autumn and often grows with KING BOLETES (p. 318). ❧ Both the common name and "muscaria" ("musca" is Latin for "fly") probably echo the belief that the cut-up mushroom mixed with milk could kill barnyard flies.

Quick Bite

Sharks are not the most dangerous animals in the world. While sharks kill an average of less than 1 person a year in the United States and 5 to 10 worldwide, hippos kill 2,900 people a year in Africa. Closer to home, deer are responsible for about 130 deaths a year, usually due to car collisions.

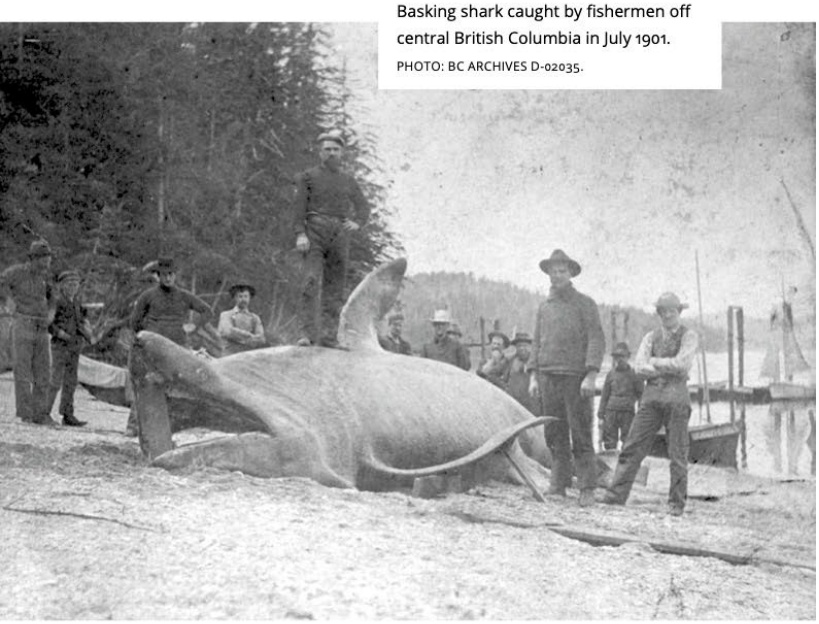
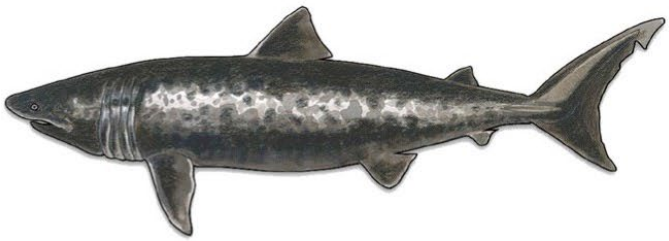


IMAGE 18

Basking shark caught by fishermen off central British Columbia in July 1901.

PHOTO: BC ARCHIVES D-02035.



Basking shark

Cetorhinus maximus

FAMILY Cetorhinidae (basking sharks)

CONSERVATION STATUS

EN Endangered (Canada's Species at Risk Act)

EN Endangered (COSEWIC)

EN Endangered (IUCN Red List)

DESCRIPTION

The basking shark is one of the few sharks that is not a flesh eater. Along with the whale shark (*Rhincodon typus*) and the megamouth shark (*Megachasma pelagios*), the basking shark is a filter-feeder that uses modified gill rakers to capture plankton in the water. However, basking sharks still do possess numerous tiny teeth, each with a single backward-curving cusp. Since it uses its gill rakers to filter water, the gill slits are enormous in size, almost encircling the head. It is grey brown to grey black with a white belly. The basking shark is the only living member of the family Cetorhinidae.

RANGE

Basking sharks have a worldwide distribution in temperate and cold waters. In the Pacific they are found from Baja California to the Gulf of Alaska; from Peru to Ecuador; from Kamchatka to China; and around southern Australia and New Zealand. They were common in the early to

A complete translation and explanation of a 1,000-year-old spiritual masterpiece, Rājānaka Kṣemarāja's *Pratyabhijñā-hṛdaya*.

One thousand years ago in the valley of Kashmīr, a great Tantric master named Kṣemarāja wrote his masterpiece: the *Pratyabhijñā-hṛdaya*, which means "The Essence of the Recognition Philosophy"—recognition, that is, of oneself as a direct expression of the universal divine Consciousness. Recognition also that this Consciousness is, in truth, all that exists, and that its five fundamental acts of creation, sustenance, dissolution, concealment, and revelation are the sacred endowments of every sentient being.

What Kṣemarāja created turned out to be one of the world's great spiritual masterpieces, breathtaking in its precision and stunning in its power. It came to be considered equivalent to scripture itself by later generations, because of its undeniable inspiration.

The Recognition Sūtras is a primary source for the study and practice of nondual Tantrik Yoga, and it has never been accurately translated or fully explained until now.

"The doctrine of Self-recognition…maps the journey through which absolute consciousness becomes human consciousness, and then expands to recognize its non-difference from the Absolute. Christopher Wallis has given us a reliable and graceful translation of the text and its commentary. I recommend this book both for the elegance of the text itself and for the elegance of Wallis' translation."
—Sally Kempton, author of *Meditation for the Love of It* and *Awakening Shakti*

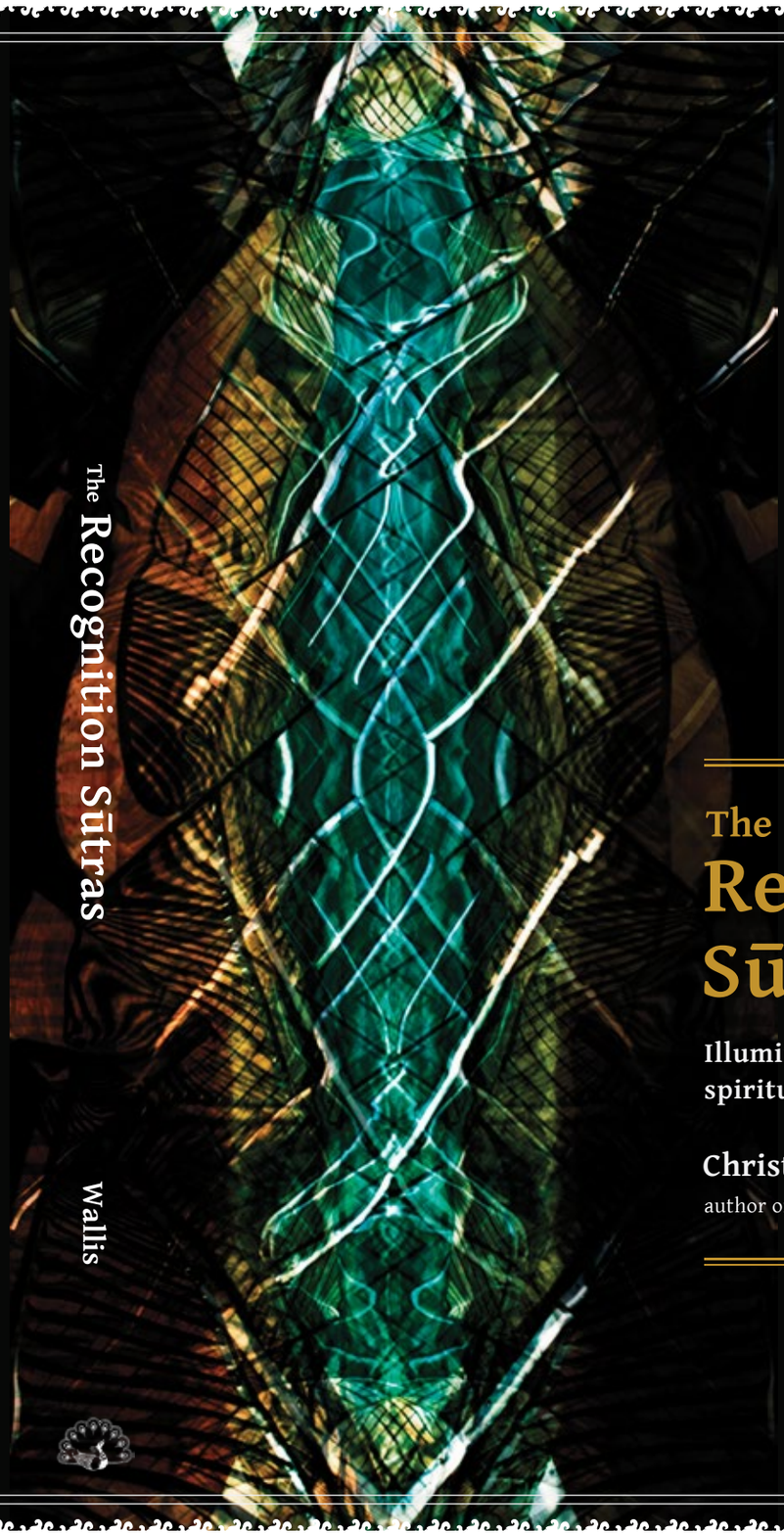
Christopher (Hareesh) Wallis is the author of *Tantra Illuminated: The Philosophy, History, and Practice of a Timeless Tradition*. He holds a B.A. in Religion and Classics from the University of Rochester, an M.A. in Sanskrit from U.C. Berkeley, an M.Phil. in Classical Indian Religions from Oxford, and a Ph.D. on the traditions of Śaiva Tantra from Berkeley. He has received traditional education at yoga āshrams in upstate New York and India.

$29.95

ISBN 978-0-9897613-8-3 US$29.95 52995

9 780989 761383

Mattamayūra INSTITUTE



The Recognition Sūtras

Wallis

The
Recognition Sūtras

Illuminating a 1,000-year-old spiritual masterpiece

Christopher Wallis
author of *Tantra Illuminated*

Christopher (Hareesh) Wallis

was introduced to Indian spirituality at the age of seven and initiated into the practice of yogic meditation at sixteen. He has been a full-time scholar-practitioner for 18 years. He holds a B.A. in Religion and Classics from the University of Rochester, an M.A. in Sanskrit from U.C. Berkeley, an M.Phil. in Classical Indian Religions from Oxford, and a Ph.D. on the traditions of Śaiva Tantra from Berkeley.

Wallis has received traditional education at yoga āshrams in upstate New York and India in meditation, kīrtan, mantra-science, āsana, karma-yoga, and more. He currently teaches meditation, yoga darśana (philosophy), Tantrik philosophy, Sanskrit, and mantra-science, and he offers spiritual counseling. His first book, *Tantra Illuminated: The Philosophy, History, and Practice of a Timeless Tradition*, was published in 2011.

A complete translation and explanation of a 1,000-year-old spiritual masterpiece, Rājānaka Kṣemarāja's *Pratyabhijñā-hṛdaya*.

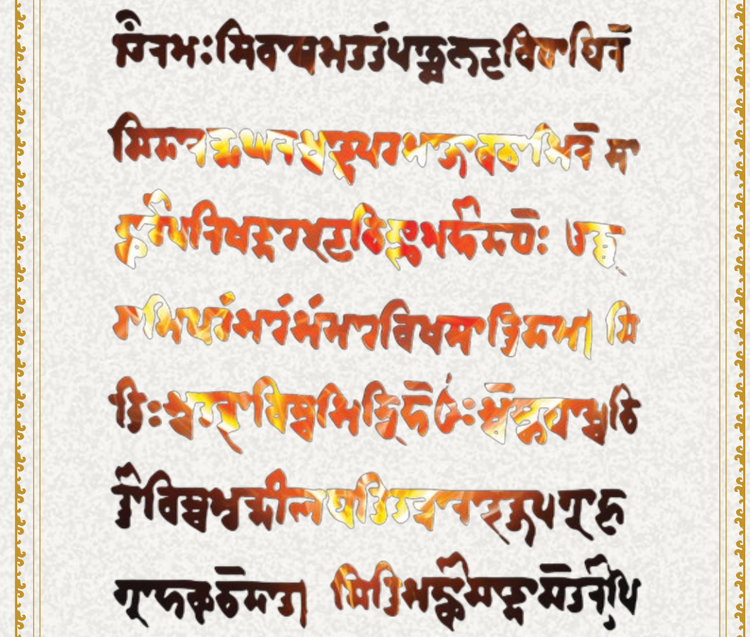
"The *Pratyabhijñā-hṛdaya* is one of the most important texts in the Trika tradition. It offers a clear distillation of the Kashmiri Shaivite doctrine of Self-recognition, which maps the journey through which absolute consciousness becomes human consciousness, and then expands to recognize its non-difference from the Absolute. Christopher Wallis has given us a reliable and graceful translation of the text and its commentary. I recommend this book both for the elegance of the text itself and for the elegance of Wallis's translation."
—Sally Kempton, author of *Meditation for the Love of It* and *Awakening Shakti*

Mattamayūra
INSTITUTE

ISBN 978-0-9897613-7-6

The Recognition Sūtras

Wallis



The Recognition Sūtras

Illuminating a 1,000-year-old spiritual masterpiece

Christopher Wallis
author of *Tantra Illuminated*

One thousand years ago

in the valley of Kashmīr, a great Tantric master named Kṣemarāja wrote his masterpiece: the *Pratyabhijñā-hṛdaya*, which means "The Essence of the Recognition Philosophy"—recognition, that is, of oneself as a direct expression of the universal divine Consciousness. Recognition also that this Consciousness is, in truth, all that exists, and that its five fundamental acts of creation, sustenance, dissolution, concealment, and revelation are the sacred endowments of every sentient being.

The *Pratyabhijñā-hṛdaya* was a concise primer, written to introduce spiritual seekers to the Recognition philosophy in less formally philosophical, more approachable language. What Kṣemarāja created turned out to be one of the world's great spiritual masterpieces, breathtaking in its brevity but stunning in its power. It came to be considered equivalent to scripture itself by later generations, because of its undeniable inspiration.

One of the most powerful and revelatory spiritual masterpieces of world history, the *Pratyabhijñā-hṛdaya* is one of the primary sources for the study and practice of nondual Tantrik Yoga, and it has never been accurately translated or fully explained until now.

Also by Christopher D. Wallis:

Tantra Illuminated: The Philosophy, History, and Practice of a Timeless Tradition
Mattamayūra Press

The Recognition Sūtras

A complete translation and explanation of
the 1,000-year-old spiritual masterpiece
the *Pratyabhijñā-hṛdaya*

by Rājānaka Kṣemarāja

Christopher D. Wallis

The Recognition Sūtras

Mattamayūra Press
3950 Colorado Ave, Unit B
Boulder, CO 80303
press@mattamayura.org

Cover illustration by Greg R. Perkins
Cover design, interior design, and typesetting by Jeff Werner
Copy-editing by Amy Haagsma
Proofreading by Roma Ilnyckyj
Indexing by Catherine Plear

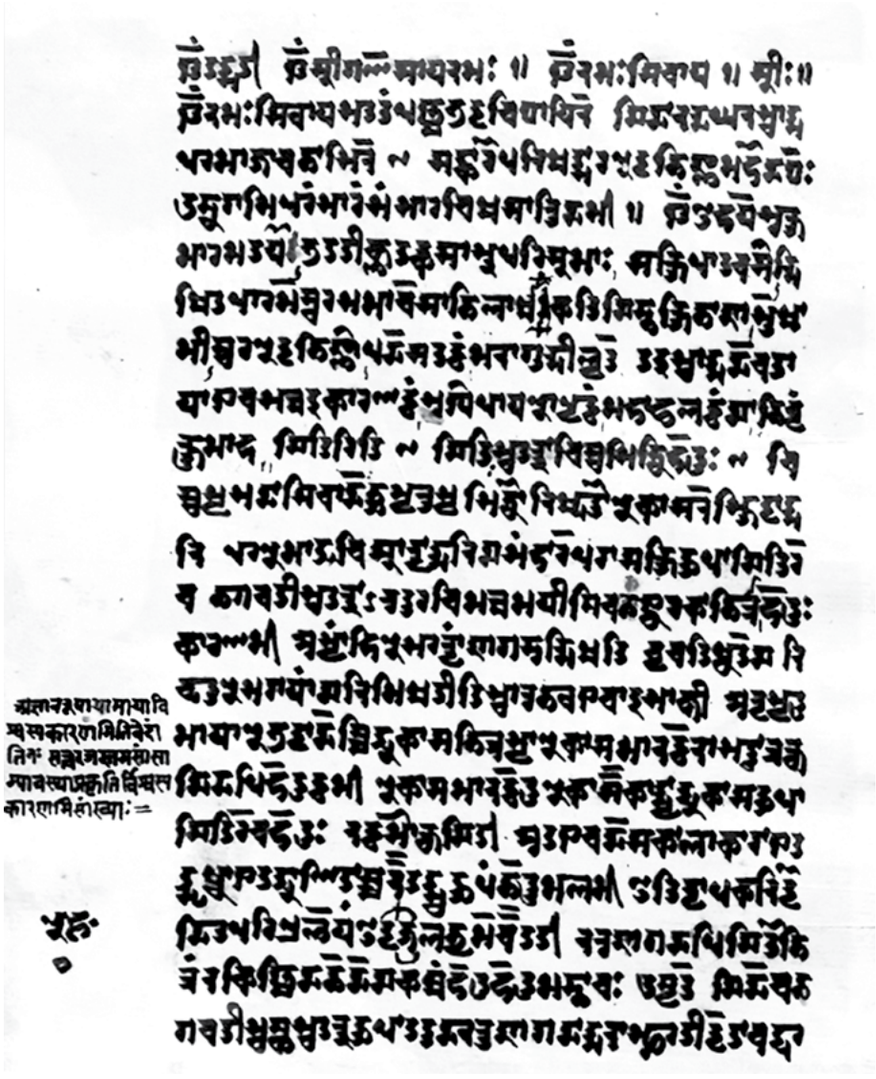
Publisher's Cataloging-In-Publication Data
(Prepared by The Donohue Group, Inc.)

Names: Kṣemarāja, active 11th century. | Wallis, Christopher D., author, translator.
Title: The recognition sūtras : a complete translation and explanation of the 1,000-year-old spiritual masterpiece the Pratyabhijñā-hṛdaya by Rājānaka Kṣemarāja / Christopher D. Wallis.
Other Titles: Pratyabhijñāhṛdaya. English & Sanskrit
Description: Boulder, CO : Mattamayūra Press, [2017] | In English and Sanskrit. | Includes bibliographical references and index.
Identifiers: ISBN 978-0-9897613-7-6 (hardcover) | ISBN 978-0-9897613-8-3 (paperback) | ISBN 978-0-9897613-9-0 (ePub) | ISBN 978-0-9986887-0-1 (mobipocket) | ISBN 978-0-9986887-1-8 (PDF)
Subjects: LCSH: Kashmir Śaivism--Doctrines--Early works to 1800.
Classification: LCC BL1281.1545 .K7413 2017 (print) | LCC BL1281.1545 (ebook) | DDC 294.5/95--dc23

10 9 8 7 6 5 4 3 2 1

Printed in the United States of America on acid-free paper

TABLE OF CONTENTS



First page of a Śāradā manuscript of the Pratyabhijñā-hṛdaya

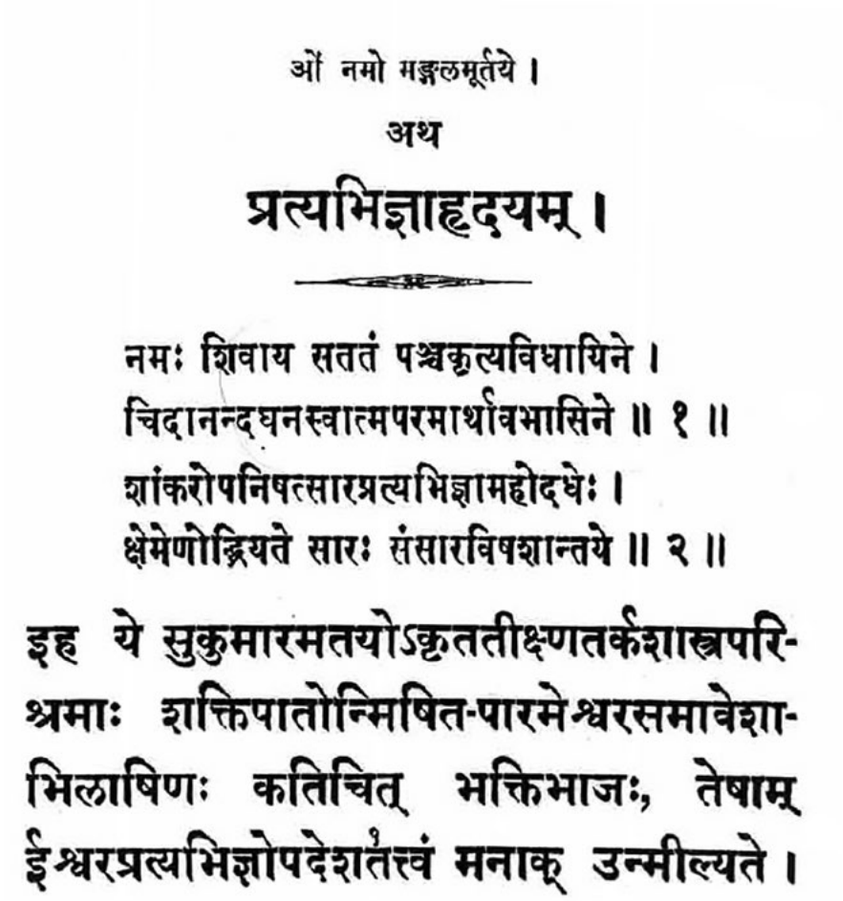
Preface

(especially for scholars and academics)

This is something new. At least, I haven't seen anything quite like it. It's new insofar as it joins together two kinds of writing that have, until now, remained mostly distinct: (1) an academically rigorous, philologically informed, complete, and thorough English translation of a Sanskrit text, and (2) an explicit and unashamed work of constructive theology that encounters that same text as a living document capable of instigating spiritual awakening, spiritual epiphanies, and even radical transformation of one's experience of reality. As a scholar-practitioner, I hold that the potential of #2 is in part predicated upon the rigor and fidelity of #1: a surprisingly uncommon claim.

By 'constructive theology' I do not at all mean 'imaginative theology', for I attempt to stay as true to the original author's vision as I can. I mean rather that this book attempts to make a meaningful *contribution* to the spiritual dimension of human life. It does not merely report what was taught by a professional *tāntrika* in the Valley of Kashmīr 1,000 years ago (though it does do that); it also attempts to show how what Kṣemarāja wrote constitutes a cutting-edge contribution to spiritual discourse in the first quarter of the twenty-first century—but only when we unpack his meaning in terms of concepts, metaphors, and analogies that are current in our present culture. So this book walks a precarious tightrope: to what extent is it possible to be faithful to Kṣemarāja's intended meaning, insofar as it can be discerned, while engaging in discourse that is compelling for twenty-first-century readers of English? Since I feel a kind of devotion and loyalty (*bhakti*) to the original author and his lineage, I have done my best to convey accurately the insights that crystallized in his awakened awareness and that he transcribed in the Sanskrit language; however, whenever the intent of his language was not completely clear, I have interpreted it in the manner that seemed to me most likely to be spiritually relevant and impactful today. Fortunately for us, his command of Sanskrit was such that his meaning usually is clear, allowing for that degree of ambiguity that invites deeper contemplation in a way that pedantry cannot.

The book is therefore divided into two distinct registers: the literal translation of Kṣemarāja's words and my explanation of what he means.



ओं नमो मङ्गलमूर्तये ।

अथ

प्रत्यभिज्ञाहृदयम् ।

नमः शिवाय सततं पञ्चकृत्यविधायिने ।
चिदानन्दघनस्वात्मपरमार्थाविभासिने ॥ १ ॥
शांकरोपनिषत्सारप्रत्यभिज्ञामहोदधेः ।
क्षेमेणोद्धियते सारः संसारविषशान्तये ॥ २ ॥

इह ये सुकुमारमतयोऽकृततीक्ष्णतर्कशास्त्रपरिश्रमाः शक्तिपातोन्मिषित-पारमेश्वरसमावेशाभिलाषिणः कतिचित् भक्तिभाजः, तेषाम् ईश्वरप्रत्यभिज्ञोपदेशतत्त्वं मनाक् उन्मील्यते ।

PROLOGUE

Kṣemarāja's original text

Oṃ. Reverence to the One who is the embodiment of auspiciousness.

Now begins

The Heart of the Teachings on Recognition

Reverence to the Divine, which constantly performs the Five Acts [of creation, preservation, reabsorption, concealment, and revelation]—and which, by so doing, reveals the ultimate reality of one's own Self, which is nothing but the Joy of Awareness. ‖ 1 ‖

I will here extract for you the ultimate essence from the great ocean of the Recognition philosophy, which is itself the essence of the esoteric teachings of the Auspicious One; for it neutralizes the poison of the cycle of suffering. ‖ 2 ‖

Here, the essential substance of the teachings on the Recognition of oneself as God will be gently and concisely opened up for the benefit of those rare devotees whose minds are childlike, who have not labored in the science of rigorous philosophical reasoning, and whose longing for total immersion into the Highest Divinity continues to grow through the influence of their initial awakening experience (*śaktipāta*). |

when you realize that the Five Acts of God are precisely what you yourself are doing all the time, there is the possibility of a flash of recognition: that you are nothing other than a contracted or condensed form of the one universal Awareness. (This is the Recognition that gives this school of thought its name.) God, or Divine Consciousness, is constantly performing the Five Acts, not on some grandiose cosmic stage, but through *you*.*

The Five Acts

sṛṣṭi	creation, emission, manifestation, flowing forth
sthiti	stasis, maintenance, preservation
saṃhāra	dissolution, retraction, reabsorption
nigraha	concealment, occlusion, forgetting
anugraha	grace, revelation, remembering

The performance of these Five Acts through you (and all sentient beings) takes place on all scales and in all spheres. For example, in the social sphere, you contribute to creating some social constructs (from modes of behavior to institutions) and to undermining others. In the cultural sphere, you participate (even just by giving your attention) in creating and maintaining some forms of art and culture, and dissolving others (even just by ignoring them). In the psychological sphere, you create mental constructs that represent and interpret select aspects of reality, become self-identified with them, and therefore invest energy in maintaining them—and you eventually see through them, allowing them to dissolve to make way for new, more effective belief structures. (We call the permanent cessation of this process of storymaking 'liberation'. More on that later.) In the realm of the physical, you create, maintain, and dissolve the various states of consciousness called waking, dreaming, and deep sleep.

I've discussed the first three Acts—what about the fourth and fifth? Well, in any of these spheres, you can either conceal to yourself the fact of your agency as a creator (or co-creator) and dissolver of these realities, or you can reveal that fact to yourself through an expanded meta-awareness, something that is available to you at any moment through the simple act of slowing down for a moment of wordless introspection. (This will be discussed more fully in the chapters to come.)

Though we can contemplate how these Acts play out on many different scales, Kṣemarāja is most interested in bringing our attention to the smallest, most immediate scale of reality, the one that we are all experiencing all the time: our moment-to-moment perceptions, sensations, and cognitions. (Since 'cognition' can cover all three of these, we'll use that term—just remember that it can refer to *any* vibration of consciousness, not only thoughts.) In this he follows the Krama tradition (see *Tantra Illuminated*, pages 248–269)[3] in observing that each and every cognitive event is an expression of the Five Acts.*

Let's explore how this is true. First, any given cognition emerges out of the field of infinite potential (known variously as pure consciousness, spirit, and the timeless ground of being); then it is maintained for a moment, nourished and imbued with reality by your focused attention; then, when attention is withdrawn from it, it dissolves back into the timeless ground. These three phases are expressions of the first three of the Five Acts, on the microcosmic scale of your own mind. This process is easy to observe with the arising of a memory or thought, for a thought really seems to arise out of nowhere and dissolve into nothing. It is a little harder to see (but no less true) with an act of external perception.

Let's take an example. Notice how your gaze might fall on a beautiful flower, and you naturally give it your full attention—'flower-consciousness' arises (*sṛṣṭi*) and remains for a moment (*sthiti*), but when your attention turns elsewhere, such as to a memory or thought, the 'flower-consciousness' dissolves (*saṃhāra*), even though your eyes might still be focused on the flower. The flower fades into the background when you think about something else, and it is no longer experienced in its fullness. It has become the 'wallpaper' on the computer screen of your mind, and your inner state is now colored by the vibration of whatever thought holds your attention.

* For a detailed explanation of these Five Acts of God, see page 111 of *Tantra Illuminated.*

* To be more precise, each is an expression of four of the Five Acts, since the fourth and fifth are alternate possibilities.

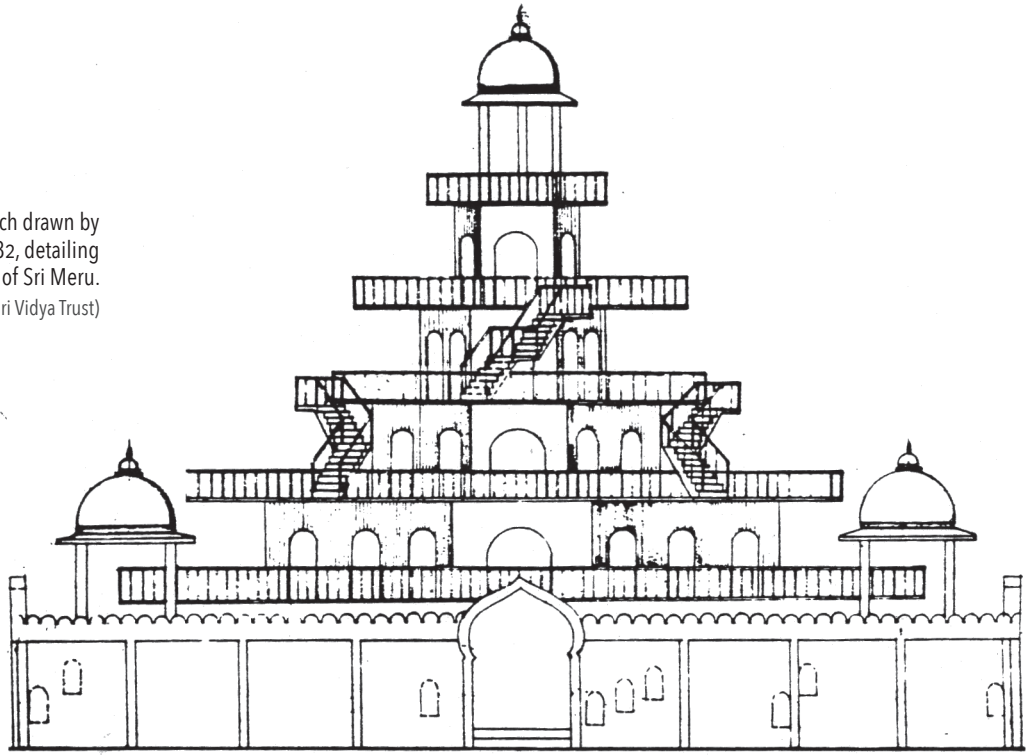
The Goddess and the Guru

A Spiritual Biography of Sri Amritananda Natha Saraswati

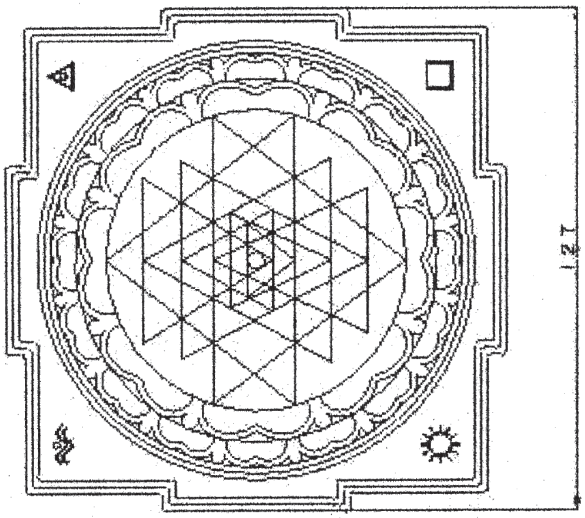
Michael M. Bowden

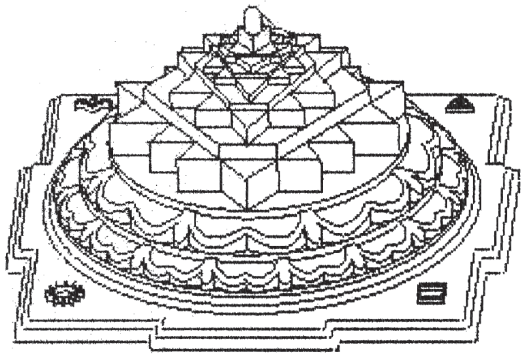
45TH PARALLEL PRESS

श्री Sketch drawn by Guruji in 1982, detailing his vision of Sri Meru.
(Sri Vidya Trust)



Contents





ALL DIMENSIONS ARE IN mm

X.X +-0.1
X.XX +-0.01
X.XXX +-0.001
ANG. +-0.5

NAME : MERU_CHAKRA_SINCH SIZE : A3

श्री The original 2005 CAD schematic for the Devipuram Merus, as edited and corrected by Guruji. (Sri Vidya Trust)

A Note from Guruji

On behalf of the Goddess's devotees,
Thank you for your Herculean effort
in writing and assembling these materials.
May Her work, beyond the Three Worlds of Space, Time and Matter,
continue unimpeded.

Among the Hindu traditions, Sri Vidya is that which explores the connections
between the Mother, the Child and the Father–Mother unity,
via the invariant geometries that define our Universe and beyond.
Attempts to clarify these constructs are much needed by the present generation,
which is so hungry for authentic information on why certain practices help
and others hinder our progress and quality of life.
Your work will help in this process.
I appreciate your time and trust in me.

I don't claim to be a perfect *upasaka*.
I don't have the scientific depth to understand the Creator's ideas.
But like so many others before me, I have tried—and I am trying still.
So please do not put me on a pedestal or give me unwarranted credit.
Just say that I am a learner too.

Love, Guruji

Foreword

By Sri Chaitanyananda[1]

I first met Guruji back in 1979 when I was an architect in Lusaka, Zambia. I was still in my 30s then. I had arrived there from Sri Lanka by way of India in 1970 and was working at the buildings department of the Ministry of Power, Transport & Works. On my own time, I used to perform *pujas*—Hindu rituals—in my home, for the city's South Asian expatriate community. There was a thirst for that kind of thing; there were no Hindu temples in Lusaka in those days.

Guruji, meanwhile, was a visiting professor of nuclear physics at the University of Zambia, where he was known as Dr. N. Prahlada Sastry. He was walking along the corridors there one day when he met a good friend of mine, Dr. Ramaswamy, then dean of the engineering department. They shook hands, exchanged introductions and decided to have a cup of tea in the cafeteria. As they chatted, Guruji casually asked, "So what do the Hindus do around here without any temples?"

Ramaswamy replied, "There's a young Sri Lankan couple that holds *pujas*, so we go there." Well, Guruji must have been a little curious. He said, "Oh? What do they do?" Ramaswamy told him, "Among some other things, they chant the Lalita Sahasranama..."[2] And that was all Guruji needed to hear.

"Really! Can you take me there?" That was it.

It happened to be a Tuesday, the day he first walked into our *puja*. After we finished chanting, Dr. Ramaswamy introduced us. I looked into Guruji's eyes and was dumbstruck; I couldn't take my eyes off them. I kept thinking, "My God, what does this man know that the rest of us don't know?" I knew there was something *completely* unusual about him. There was a depth in his eyes that I had never seen in anyone's eyes before. You keep looking into those eyes, and—almost without your knowledge—you feel peaceful, you want to cry, you want to laugh; you want to do all these things at once. But I finally got hold of myself, thinking, "How embarrassing! He must be wondering, why is this fellow staring at me?"

1 Srilasri Chaitanyananda Natha Saraswati–better known as Haran Aiya or simply "Aiya"–is one of Guruji's early disciples and the founder and head of the Sri Rajarajeswari Peetam Temple in Rush, NY, and was referred to by Guruji as his "spiritual son." This foreword was prepared shortly before Guruji's death; Aiya's use of the present tense has not been altered.

2 The *Lalita Sahasranama* ("One Thousand Names of the Goddess Lalita"), c. 900 CE, is one of the most important devotional hymns/chants in the Hindu Sri Vidya tradition. See "Introduction: The World of Sri Vidya" in *The Goddess and the Guru, Volume II.*

Anyway, I soon came to know that Dr. Sastry was a master of Sri Vidya *upasana*,[3] initiated by an accomplished guru in India. As it happened, I had long been seeking initiation into Sri Vidya, but it was a very secretive and basically impenetrable sect in those days, unless by chance you knew an initiate—or happened to be a Brahmin, which I am not.

The very next day I visited the home of a close friend of mine, Mr. Balasubrahmanyam. He was a Tamil Brahmin in his 60s, an absolutely brilliant man. We called him Balu. He was then chief engineer at the Zambia Electricity Transmission Company, the state-owned power corporation. He was a regular at our *pujas* and very knowledgeable about these things.

He too knew Guruji, and so I eagerly asked him, "Balu, should I go to Dr. Sastry and ask whether he will initiate me into Sri Vidya?"

"Why not?" Balu said. "Go ask him!"

I said, "But what if he tells me I'm not a Brahmin, so I can't have it?" I'd had that experience before.

Balu said, "So what's the big deal? If he says no, he will join all the others who have refused it to you before. That's the worst thing that could happen. But imagine if he says yes!"

That decided it for me. I said, "Okay, we're going."

A few days later we went to his house, knocked on the door, and who should answer but Guruji's eldest daughter, Anantalakshmi. I said, "Is Dr. Sastry at home? Can we see him please?" She called out, "Daddy!" and he emerged from the *puja* room. "Ah! Haran and Balu! Come in!" he said. "I have just finished a *Sri Chakra Puja*.[4] Come into the shrine room." We went in, he offered us the *amritam*;[5] we took it. Then Balu discreetly withdrew into the living room, and left me alone with him.

Now, Guruji *knew* why I had come. Later on, I found out *he knew*. But I plucked up my courage and asked him, "Aiya,[6] you know Sri Vidya, and you have a guru. Will you take me on as a disciple? Will you teach it to me?"

Not even for a fraction of a second did he hesitate. "Yes," he said, "I will."

"But Aiya," I felt compelled to tell him, "I am not a Brahmin."

3 A Tantric path that honors the great goddess Tripurasundari and places considerable emphasis on harnessing her powers through ritual. For further explanation, see "Introduction: The World of Sri Vidya" in *The Goddess and the Guru, Volume II.*

4 *Sri Chakra Puja* is a central Sri Vidya ritual discussed elsewhere in this book and at length in *The Goddess and the Guru, Volume II.*

5 *Amritam* means "nectar" in Sanskrit. Here, Guruji is offering his guests the *prasadam*–food offerings made to the deity and then distributed to worshipers–from his *Sri Chakra Puja.*

6 *Aiya* is a Tamil honorific literally meaning "Father." It is often used in same sense as "Sir."

personality, able to mask what he is to perfection. Nobody would even begin to suspect what he is.

Some time ago he told me, "I am not working on a human timeline. My job is to sow the seed. If it germinates within two months, good; if it germinates after 2,000 years, that's also good. My job is simply to sow the seed; let her take care of the rest in her own sweet time."

I think that since Guruji has been at Devipuram, the main temple deity there, Sahasrakshi, has expanded her presence to such an extent that her consciousness and his are one and the same. I believe that his consciousness has blended with that of the Devi herself. He has *become* Sahasrakshi. And what he expresses is exactly what she would express. What he gives out is what she would give. I think he's become the human face of the Devi so that everyone who meets him—sophisticated, worldly people and simple villagers alike; everyone who comes to see him—will have an experience of her. I am happy that his journey and his wisdom have been collected and shared in *The Goddess and the Guru* for the benefit of all.

The world has rarely known his like.

Sri Gurubhyonamah!

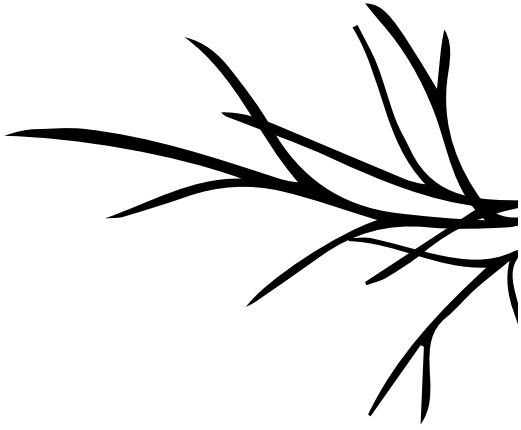
Haran Aiya
Chaitanyananda
Sri Rajarajeswari Peetam
Rochester, NY

MORE THAN TWO ESSENTAILS

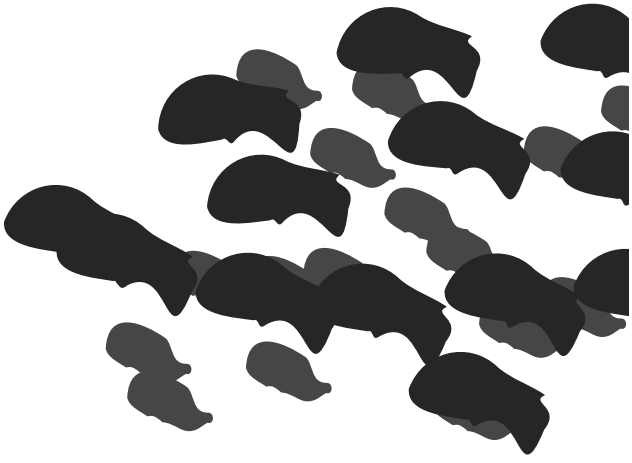
Nonmonogamy and Neurodiversity

Alyssa Gonzalez

THORNAPPLE PRESS



Contents



Introduction

Picture it: You've always been weird. Other people's minds are a bit of a mystery to you, or yours is to them, or both.

YOUR ENTHUSIASM IS MORE THAN THEY can handle and it often isn't aimed where other people think it should be. Things that excite them do nothing for you. There are tasks you can do better than they thought was possible. Other activities seem trivial to them but feel impossible for you. Still other things

bother you but don't seem to even register for them. Maybe you have trouble noticing when you're hungry or need to use the bathroom. Maybe your sense of taste is so sensitive that you can tell what brand of dried thyme the chef used. Your childhood featured some repetitive comments from teachers and caregivers: "If you would only apply yourself," "is very self-directed and can work independently," "won't sit still," "daydreams too much," "doesn't do homework," "clean your room." You often had stellar grades in some subjects or kinds of work and abysmal grades in others, with teachers scratching their heads at the difference. Maybe loud music has always made you feel alive, or maybe you're so sensitive to sound that you cover your ears when emergency vehicles drive by. Maybe you can't seem

to process speech until a few seconds after it's said, leaving friends struggling to understand why you'll ask them to repeat themselves and then, before they're done, respond to the original words. Finding love has probably been a challenge for you, and you may have yet to succeed in a way you find satisfying. You've spent your life feeling like an outsider, existing among the normal folk but never really one of them. You have looked up a variety of terms to try to name the kind of person that you are, perhaps trying on a few ill-defined social ideas like "highly sensitive person" or "empath" or pop-culture concepts like "nerd," or even acquiring a diagnosis of autism, attention deficit hyperactivity disorder (ADHD) or another condition from a professional. In the end, any or all of those terms might feel right, but one name encompasses you and so

many more: neurodivergent. This book is for you.

Neurodivergence refers to having a mind that does not work like the minds of other people. The opposite concept is "neurotypicality," and "neurotypical" is a far kinder word than "normal" for such people. People who are neurodivergent experience the world, and themselves, differently than is considered (neuro)typical. Neurodivergence includes differences in social interaction, learning, attention, emotional responses, sensory responses and more. The best-known examples of neurodivergence include autism and ADHD, as well as conditions often associated with them such as dyspraxia (impaired coordination), dyscalculia (difficulty learning math), dyslexia (difficulty with reading)

and Tourette's syndrome (sudden, repetitive tics). Other conditions, including borderline personality disorder, post-traumatic stress disorder (PTSD) and dissociative identity disorder, are also sometimes included. The concept of neurodivergence serves to present these situations in a de-pathologized way, as differences that can be understood, managed and accommodated. Our world is one of neurodiversity, in which neurotypical people coexist with people with a variety of neurodivergent minds, forming a beautifully heterogenous medley of humanity. Neurodivergence and neurodiversity do not deny that these conditions can be challenging or even debilitating, or that they sometimes benefit from medical intervention. Rather, these concepts recognize that the conditions are lifelong, often incurable

APPLICATION NOTE

Infectious disease research

SARS-CoV-2 viral entry factors

Studying the molecular targets using TaqMan Assays

Introduction

Research into SARS-CoV-2 is a rapidly emerging field. This novel zoonotic coronavirus combines high transmissibility with high severity, making it critically important to understand how it works. Studies have approached the problem of SARS-CoV-2 from many directions, including investigating existing medications for utility against it [1,2], studying its relationship with its previous host species [3,4], and trying to determine its relationship to other coronaviruses [5-8]. Each provides an important piece of the overall picture, attempting to bring the virus from immediate pressing concern to lower-level threat.

An important aspect of this research is studying SARS-CoV-2's relationship to the human body, in particular the molecular targets it uses to gain entry into cells. Viruses work by entering cells and commandeering their protein synthesis and reproductive machinery to generate copies of themselves, which leads to cell death and tissue damage. Viruses usually cannot bore or sneak into cells on their own; typically they rely on proteins that are already part of cells to gain entry, manipulating or tricking the cell into bringing the viral particles inside. Called "entry factors", these proteins have a variety of functions, including maintenance of lung health and serving as components of signal transduction pathways. The entry factors a virus uses influence which tissues it attacks and have a sizable impact on how a viral infection affects its host. To understand how SARS-CoV-2 affects the human body and how to combat those effects, it is critical to identify entry factors and the variations thereof that facilitate viral entry.

Noteworthy entry factors for SARS-CoV-2 that have already been identified include ACE2, TMPRSS2, TMPRSS4, FURIN, CTSL, ST3GAL4, DPP4, CTSB, ANPEP, and ST6GAL1. Specific Applied Biosystems™ TaqMan® Gene Expression Assays provide a way to study each of these in detail, and the Applied Biosystems™ TaqMan® Array Coronavirus Entry Factor Panel provides a means to study them all at once.

ThermoFisher SCIENTIFIC

TaqMan Array Coronavirus Entry Factor Panel

Applied Biosystems™ TaqMan® Assay technology is the gold standard of performance, quality, and specificity in gene expression analysis by quantitative polymerase chain reaction (qPCR), optimized to work with numerous platforms and equipment setups. Predesigned TaqMan Assays exist for thousands of individual genes across dozens of species, which can be an ideal starting point for researchers with known genes of interest. For scientists looking at a large number of specific genes, such as those of an entire functional pathway, however, using single assays for individual genes is often not cost-effective. When looking at a large number of genes at once, a much easier approach is to instead identify markers of interest using a panel that targets multiple genes, allowing subsequent research to focus directly on the specific genes that the broader assay flagged as noteworthy. For SARS-CoV-2's entry factors, that means initial identification using the TaqMan Array Coronavirus Entry Factor Panel.

The TaqMan Array Coronavirus Entry Factor Panel enables researchers to study all ten of the most important entry factors for SARS-CoV-2 in a single panel: *ACE2, TMPRSS2, TMPRSS4, FURIN, CTSL, DPP4, CTSB, ANPEP, ST3GAL4,* and *ST6GAL1*. Researchers have the option to modify the preconfigured layout to add or subtract assays to fit their needs. This panel is available in human, mouse, and rat versions and comes in varieties optimized for 0.2 mL 96-well plates, 0.1 mL 96-well plates, 384-well Applied Biosystems™ TaqMan® Array Cards, and Applied Biosystems™ OpenArray™ plates.

TaqMan Gene Expression Assays

For researchers interested in focusing on specific entry factors rather than the full set of prominent targets, TaqMan Assays provide many options. TaqMan Assays come predesigned and preoptimized for thousands of genes across dozens of species, in a wide range of formats and sizes. These options provide researchers with the flexibility needed to obtain fast, reliable, and accurate results with off-the-shelf assay kits rather than laboriously custom-designed alternatives.

Multiple TaqMan Assays exist for most gene products, including many of SARS-CoV-2's entry factors. Choosing an assay depends in part on one's specific target and research goals, with different TaqMan Assays targeting different exons and variants. Some of the ways to choose an assay are discussed here.

Get the details right

When searching for TaqMan Assays for a specific gene, one of the ways to distinguish between options is to look at the assay design details. Some example considerations follow here.

Exon targets

Most often, the difference between TaqMan Assays designed for the same gene is in the specific transcript location they target. All assays for the same gene will target products of that gene, but they will flag different sequences for amplification and quantification. Checking the list of transcripts targeted by a given assay provides important information about how the assay works and whether it is suited to a given use case. An experiment premised on a specific splice variant must include its specific RefSeq accession and leave out all others, whereas an experiment with more general needs benefits from an assay that includes more transcripts. The search tools available for TaqMan Assays allow the user to check which transcripts an assay detects, and provide detailed RefSeq and GenBank information for each transcript.

Genomic DNA

A related consideration is whether the probe spans an exon–exon junction. Probes that do not span an exon–exon junction carry the risk of amplifying residual genomic DNA, making the intermediate step of using DNase to eliminate genomic DNA much more important. TaqMan Assay search results provide the ability to observe the relationships between all exons of the target gene, the location of each exon on the gene, and which exons each available assay will target, enabling researchers to choose these attributes with fine-grained detail.

Source quality

Amplicon length presents a balancing act in many experiments. A large amplicon helps assure specificity, protecting against off-target replication, at the cost of less-than-ideal amplification efficiency. However, if an experiment involves testing preserved or degraded tissue, such as formalin-fixed, paraffin-embedded (FFPE) samples, a smaller amplicon might be necessary. In such samples, genetic material might be too degraded for a long amplicon assay to amplify with good efficiency, but a smaller amplicon assay can still amplify with the desired efficiency.

Citation density

For some research, making sure one's results are directly comparable to previous work is important. Using the third-party Bioz database, the TaqMan Assay search tool enables users to find lists of publications that used particular assays. The assay with the highest number of citations in this database is designated "Most Citations" and enables new research to be most effectively compared and considered against previous work.

Best-coverage assays

It is often not necessary to target specific transcript variants. In fact, most of the time, standard gene expression studies benefit from the "best-coverage" assay for the target gene, designed to detect the maximum number of transcripts of the gene of interest and thus provide a more complete picture of that gene's expression. An assay designated "best-coverage" most often detects the greatest number of transcript variants, but the following criteria are also taken into account:

- Does not detect gene products with similar sequence (homologs)
- Is designed across an exon–exon junction to reduce genomic DNA contamination
- Has a short amplicon, resulting in more efficient PCR
- Does not detect off-target sequences, increasing the specificity of reactions
- Does not map to multiple genes, increasing the specificity of the experiment
- Does not target the 5' untranslated region (UTR). The 5' UTR of transcripts can have variable sequence between transcripts

In combination, these stipulations mean that an assay will have few off-target results and many on-target results, maximizing the ability to detect changes in expression of the target gene and reducing the chance that an unusual transcript will avoid detection.

Table 1 shows the best-coverage TaqMan Assays available for the ten best known SARS-CoV-2 entry factors.

SARS-CoV-2 entry factors: a discussion

The ten best known entry factors for SARS-CoV-2 vary extensively in function and significance. Their heterogeneous roles in the human body attest to the number of different organs and functions that SARS-CoV-2 can attack, and to the variety of methods that may, with additional research, prove useful for combating this serious virus. This section summarizes the ten entry factors included in the TaqMan Array Coronavirus Entry Factor Panel, going over their functions, the other conditions they affect, and the types of studies that might benefit from examining them individually or in groups.

Angiotensin-converting enzyme 2 (ACE2)

Angiotensin-converting enzyme 2 (ACE2) is a transmembrane protein found in lung, artery, heart, kidney, and intestinal tissue [9,10]. Recent evidence indicates that it is also found in smooth muscle and tissues of the nervous system [11]. Its primary function is to counter the effects of angiotensin-converting enzyme (ACE) by turning angiotensin II into angiotensin (1-7), with the downstream effect of vasodilation. ACE2 also modifies a variety of other peptides, including [des-Arg9]-bradykinin, apelin, neurotensin, dynorphin A, and ghrelin, suggesting it may have a much more expansive role in general organ function than is currently obvious [12]. ACE2 binds to SARS-CoV-2's

Table 1. Best-coverage assays for known entry factors.

	Best-coverage assays		
Target	**Human**	**Mouse**	**Rat**
ACE2	Hs01085333_m1	Mm01159006_m1	Rn01416293_m1
ANPEP	Hs00174265_m1	Mm00476227_m1	Rn00578763_m1
CTSB	Hs00947439_m1	Mm01310506_m1	Rn00575030_m1
CTSL	Hs00964650_m1	Mm00515597_m1	Rn04341361_m1
DPP4	Hs00897386_m1	Mm00494552_m1	Rn00562910_m1
FURIN	Hs00965485_g1	Mm00440646_m1	Rn00570970_m1
ST3GAL4	Hs00920870_m1	Mm00501503_m1	Rn01786289_m1
ST6GAL1	Hs00949382_m1	Mm00486119_m1	Rn00709937_m1
TMPRSS2	Hs01122322_m1	Mm00443687_m1	Rn00590459_m1
TMPRSS4	Hs01054420_m1	Mm00520486_m1	Rn06429720_s1

spike protein S1, which causes ACE2 to transport the virus into the cell [13]. This has made ACE2 the focus of a great deal of research, which aims to unravel how the virus binds to it and how treatments targeted at ACE2 might prevent viral infection or limit its impact. The fact that ACE2 is also the target of medications for blood pressure disorders has been especially interesting, creating the possibility that future research might reveal these medications as useful tools against SARS-CoV-2 [14].

Alanine aminopeptidase (ANPEP)

Alanine aminopeptidase has long been recognized as an entry factor for coronaviruses. Numerous coronaviruses in several species, including humans, cats, dogs, and pigs, use this protein as part of their path into cells [15,16]. This protein is primarily found in the small intestine and renal microvillar membranes, and its role within mammalian biology appears to be as part of the digestive process, completing the breakdown of proteins hydrolyzed by pancreatic and gastric secretions. Its role in the kidney is less clear but likely also related to absorption. In addition to serving as part of digestion, alanine aminopeptidase may also help process signal peptides from elsewhere in the body, and defects in ANPEP are associated with leukemia and lymphoma [17]. Deficiency of alanine aminopeptidase activity is shown to be protective against coronavirus infection in pigs [16], and the expression pattern of *ANPEP* is very similar to that of the high-priority SARS-CoV-2 target *ACE2* [18], suggesting that studying *ANPEP* and its associated enzyme may prove useful for combating human coronaviruses such as SARS-CoV-2. In particular, research suggests that alanine aminopeptidase may be part of a cofactor network with angiotensin-converting enzyme 2 and other entry factors, rather than either one being a full-fledged entry factor on its own. This tangle of networked effects requires study to illuminate, both to understand how SARS-CoV-2 and other coronaviruses work and to learn how to better protect against them.

Cathepsin B (CTSB)

Cathepsin B is a cysteine protease that plays an important role in intracellular proteolysis. It is especially significant for its role in the breakdown of extracellular matrix compounds [19] and its role in neurogenesis in the brain [20], which contributes to the formation of new memories. Cathepsin B becomes elevated in association with numerous cancers and appears to enable metastatic cancer cells to feed on extracellular materials, sustaining them as they travel [21]. The most noteworthy effects of this protease are associated with various neurological conditions. Abnormal cathepsin B activity levels contribute to post-injury neuronal cell death [22], epilepsy [19], and Alzheimer's disease [23], attesting to this protein's role in maintaining normal brain health. Cathepsin B appears to contribute to SARS-CoV-2 entry into cells in the same way that cathepsin L does [24], suggesting that the many pharmaceuticals that target cathepsin B in the context of cancer, epilepsy, and other conditions may have a role to play in protecting against the impact of SARS-CoV-2 infection. In particular, the activity of cathepsin B and similar proteins may help to explain the disproportionate impact of SARS-CoV-2 on the brain function of people infected with this virus, and cathepsin B may prove to be a viable target for preventing these effects [25].

Cathepsin L (CTSL)

Cathepsin L, like other lysosomal proteases, serves as part of the intracellular mechanism for destroying old or damaged proteins. Lysosomes are involved in immunological activity as well, used to destroy viral and other pathogenic material that cells encounter, or healthy tissue in autoimmune conditions. This means that proteins that are presented by viruses to cells in uncleaved, nonfunctional states can potentially be cleaved into functional forms by the proteases meant to destroy them, including cathepsin L. Cathepsin L has been shown to cleave SARS-CoV-2's spike protein near the site that furin does [26], similarly activating the spike protein and enabling it to bind to other entry factors. Further study of how SARS-CoV-2 interacts with cathepsin L and other lysosomal proteins is likely to yield insights similar to those gained from studying furin.

Dipeptidyl peptidase 4 (DPP4)

The protein encoded by *DPP4* is a cell-surface enzyme expressed in most cell types. This enzyme is associated with immune regulation, signal transduction, and apoptosis. As a transmembrane glycoprotein, it has traits in common with other SARS-CoV-2 entry factors, and other coronaviruses, including Middle East respiratory syndrome (MERS) coronavirus, have been found to bind to it [27]. Preliminary evidence suggests that SARS-CoV-2 can use this same enzyme as an entry factor, which is consistent with other similarities between SARS-CoV-2 and previous emergent coronaviruses [28]. *DPP4* provides an especially exciting target for SARS-CoV-2 research because it has long been targeted for pharmaceutical intervention as part of diabetes treatment. Dipeptidyl peptidase 4 mediates the release of glucagon into circulation, and inhibitors of this enzyme thus reduce glucagon, reduce circulating glucose, and increase circulating insulin [29]. The resulting connection between SARS-CoV-2 and diabetes, and

the possibility that drugs used to treat diabetes could be repurposed for use against SARS-CoV-2 infection, makes this gene an especially enticing target for future research.

Furin (FURIN)

A subtilisin-like peptidase, furin is coded by the *FURIN* gene and serves to cleave precursor proteins to their biologically active versions. Furin is part of the synthesis pathway for numerous vital proteins and peptides, including parathyroid hormone and proalbumin, and is expressed throughout the body. In addition to cleaving protein precursors to convert them to active proteins, furin also cleaves the toxins produced by anthrax and *Pseudomonas* bacteria [30] and the viral spike proteins of numerous viruses, including HIV, dengue, Marburg, and SARS-CoV-2 [31]. This cleavage enables the spike proteins to fully engage with cell-surface receptors and infect cells. Compatibility between spike proteins and furin is thought to be a critical part of zoonotic transmission possibilities for viruses, helping to predict which viruses can cross between species. A spike protein compatible with human furin is one of the features that distinguishes SARS-CoV-2 from its close viral relatives [32].

ST3 beta-galactoside alpha-2,3-sialyltransferase 4 (ST3GAL4)

Entry factors used by other viruses are worth exploring as part of efforts to understand SARS-CoV-2. The genes *ST6GAL1* and *ST3GAL4* code for enzymes that are important for the synthesis of α(2,6)-linked and α(2,3)-linked sialic acids [33]. These acids form glycoprotein motifs that influenza and other viruses recognize as part of their process of entering cells [34]. The SARS-CoV-2 spike protein is a glycoprotein, and preliminary evidence indicates that expression of *ST3GAL4* is correlated with susceptibility to SARS-CoV-2 viral entry [35]. Study of this viral entry factor is as yet in its infancy, and much research remains to be done to confirm how much and in what ways it enables SARS-CoV-2 to enter cells and whether it can be usefully targeted to prevent SARS-CoV-2 infection.

ST6 beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1)

ST6GAL1 and its associated enzyme are best known from their role in developmental and cancer biology. This enzyme is involved in the regulation of pluripotency in human stem cells, altering the expression of genes that ultimately encourage them to differentiate into other cell types [36]. As an enzyme involved in protein glycosylation, ST6GAL1 has become the subject of increased scrutiny for its role in numerous cancers, including pancreatic, prostate, breast, and ovarian cancers [37]. Breast cancer cells with low ST6GAL1 expression are less able to invade surrounding tissues than cells with higher expression levels, apparently due to the impact of this enzyme on adhesion to the extracellular matrix [38]. These same effects on cell-surface glycoproteins make ST6GAL1 a useful entry factor for viruses, including influenza [34], and understanding how viruses use these proteins to gain entry into cells will enable scientists to find ways to inhibit their entry.

Transmembrane protease serine 2 (TMPRSS2)

TMPRSS2 was, until recently, a fairly mysterious protein. Like other serine proteases, it cleaves proteins at bonds between serine and other amino acids, usually doing so as part of signal transduction cascades [39]. Despite being found in numerous tissues, its main recognized functions are in the prostate and liver. In the liver, it activates pro-hepatocyte growth factor, leading to hepatocyte proliferation, and in the prostate, it activates protease activated receptor 2 (F2RL1) and matriptase (ST14) as part of normal prostate functioning [40]. Overexpression of *TMPRSS2* and overactivation of the protease are associated with prostate cancer metastasis through the disruption of extracellular matrices [40], and are frequently observed in prostate cancer cells [41]. In the context of SARS-CoV-2, TMPRSS2 comes up most often because it and other similar proteases appear to be a necessary part of ACE2's role as a viral entry factor, priming the viral spike protein for binding to ACE2 [42,43]. TMPRSS2 is similarly implicated in the entry pathways of numerous other viruses, including Sendai virus, human metapneumovirus, and human parainfluenza [9].

Transmembrane protease serine 4 (TMPRSS4)

Like TMPRSS2, TMPRSS4 is a transmembrane serine protease involved in signal transduction cascades. Similar to its cousin, it is expressed in multiple tissues. Overexpression of TMPRSS4 has been reported in pancreatic [44], ovarian [45], thyroid [46], colorectal [47], lung [48], breast [49], cervical [50], gallbladder [51], gastric [52], and liver cancers [53], in all cases associated with facilitating the transition from epithelial to mesenchymal stages and, with it, cancer metastasis. Abnormal *TMPRSS4* activity in the lungs is additionally associated with idiopathic pulmonary fibrosis through fibroblast proliferation and excessive production of extracellular matrix [54], similar to *TMPRSS2*'s malfunctions in the prostate. TMPRSS4 has been observed contributing to SARS-CoV-2 binding in the small intestine, enabling the virus to enter the intestinal epithelium and spread from there to other tissues through its interaction with ACE2 [43].

References

1. Choy K-T, AY-L Wong, P Kaewpreedee, et al. (2020) Remdesivir, lopinavir, emetine, and homoharringtonine inhibit SARS-CoV-2 replication in vitro. *Antiviral Res.* 178:104786.
2. Caly L, JD Druce, MG Catton, et al. (2020) The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. *Antiviral Res.* 178:104787.
3. Guo H, B-J Hu, X-L Yang, et al. (2020) Evolutionary arms race between virus and host drives genetic diversity in bat SARS related coronavirus spike genes. *bioRxiv*:2020.05.13.093658.
4. Valitutto MT, O Aung, KYN Tun, et al. (2020) Detection of novel coronaviruses in bats in Myanmar. *PLOS ONE* 15(4):e0230802.
5. Jackwood MW (2006) The relationship of severe acute respiratory syndrome coronavirus with avian and other coronaviruses. *Avian Dis.* 50(3):315–320.
6. Kim OJ, DH Lee, CH Lee (2006) Close relationship between SARS-coronavirus and group 2 coronavirus. *J. Microbiol.* 44(1):83–91.
7. Wu A, Y Peng, B Huang, et al. (2020) Genome Composition and Divergence of the Novel Coronavirus (2019-nCoV) Originating in China. *Cell Host Microbe* 27(3):325–328.
8. Oberemok VV, KV Laikova, KA Yurchenko, et al. (2020) SARS-CoV-2 will continue to circulate in the human population: an opinion from the point of view of the virus-host relationship. *Inflamm. Res.* 69(7):635–640.
9. Donoghue Mary, Hsieh Frank, Baronas Elizabeth, et al. (2000) A Novel Angiotensin-Converting Enzyme–Related Carboxypeptidase (ACE2) Converts Angiotensin I to Angiotensin 1-9. *Circ. Res.* 87(5):e1–e9.
10. Hamming I, W Timens, MLC Bulthuis, et al. (2004) Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J. Pathol.* 203(2):631–637.
11. Kabbani N, JL Olds (2020) Does COVID19 Infect the Brain? If So, Smokers Might Be at a Higher Risk. *Mol. Pharmacol.* 97(5):351.
12. Kuba K, Y Imai, JM Penninger (2013) Multiple Functions of Angiotensin-Converting Enzyme 2 and Its Relevance in Cardiovascular Diseases. *Circ. J.* 77(2):301–308.
13. Wang H, P Yang, K Liu, et al. (2008) SARS coronavirus entry into host cells through a novel clathrin- and caveolae-independent endocytic pathway. *Cell Res.* 18(2):290–301.
14. Diaz JH (2020) Hypothesis: angiotensin-converting enzyme inhibitors and angiotensin receptor blockers may increase the risk of severe COVID-19. *J. Travel Med.* 27(3):taaa041.
15. Licitra BN, GR Whittaker, EJ Dubovi, et al. (2014) Genotypic Characterization of Canine Coronaviruses Associated with Fatal Canine Neonatal Enteritis in the United States. *J. Clin. Microbiol.* 52(12):4230–4238.
16. Whitworth KM, RRR Rowland, V Petrovan, et al. (2019) Resistance to coronavirus infection in amino peptidase N-deficient pigs. *Transgenic Res.* 28(1):21–32.
17. Moore BD, RU Jin, L Osaki, et al. (2015) Identification of alanyl aminopeptidase (CD13) as a surface marker for isolation of mature gastric zymogenic chief cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* 309(12):G955–G964.
18. Qi F, S Qian, S Zhang, et al. (2020) Single cell RNA sequencing of 13 human tissues identify cell types and receptors of human coronaviruses. *Biochem. Biophys. Res. Commun.* 526(1):135–140.
19. Houseweart MK, LA Pennacchio, A Vilaythong, et al. (2003) Cathepsin B but not cathepsins L or S contributes to the pathogenesis of Unverricht-Lundborg progressive myoclonus epilepsy (EPM1). *J. Neurobiol.* 56(4):315–327.
20. Moon HY, A Becke, D Berron, et al. (2016) Running-Induced Systemic Cathepsin B Secretion Is Associated with Memory Function. *Cell Metab.* 24(2):332–340.
21. Fais S (2007) Cannibalism: A way to feed on metastatic tumors. *Cancer Lett.* 258(2):155–164.
22. Hook GR, J Yu, N Sipes, et al. (2013) The Cysteine Protease Cathepsin B Is a Key Drug Target and Cysteine Protease Inhibitors Are Potential Therapeutics for Traumatic Brain Injury. *J. Neurotrauma* 31(5):515–529.
23. Hook VYH, M Kindy, G Hook (2008) Inhibitors of Cathepsin B Improve Memory and Reduce β-Amyloid in Transgenic Alzheimer Disease Mice Expressing the Wild-type, but Not the Swedish Mutant, β-Secretase Site of the Amyloid Precursor Protein. *J. Biol. Chem.* 283(12):7745–7753.
24. Smieszek SP, BP Przychodzen, MH Polymeropoulos (2020) "Amantadine disrupts lysosomal gene expression; potential therapy for COVID19." *bioRxiv*:2020.04.05.026187.
25. Helms J, S Kremer, H Merdji, et al. (2020) Neurologic Features in Severe SARS-CoV-2 Infection. *N. Engl. J. Med.* 382(23):2268–2270.
26. Bosch BJ, W Bartelink, PJM Rottier (2008) Cathepsin L Functionally Cleaves the Severe Acute Respiratory Syndrome Coronavirus Class I Fusion Protein Upstream of Rather than Adjacent to the Fusion Peptide. *J. Virol.* 82(17):8887.
27. Raj VS, H Mou, SL Smits, et al. (2013) Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. *Nature* 495(7440):251–254.
28. Solerte SB, A Di Sabatino, M Galli, et al. (2020) Dipeptidyl peptidase-4 (DPP4) inhibition in COVID-19. *Acta Diabetol.*:1–5.
29. Zheng SL, AJ Roddick, R Aghar-Jaffar, et al. (2018) Association Between Use of Sodium-Glucose Cotransporter 2 Inhibitors, Glucagon-like Peptide 1 Agonists, and Dipeptidyl Peptidase 4 Inhibitors With All-Cause Mortality in Patients With Type 2 Diabetes. *JAMA* 319(15):1580–1591.
30. Shiryaev SA, AG Remacle, BI Ratnikov, et al. (2007) Targeting Host Cell Furin Proprotein Convertases as a Therapeutic Strategy against Bacterial Toxins and Viral Pathogens. *J. Biol. Chem.* 282(29):20847–20853.
31. Hoffmann M, H Kleine-Weber, S Pöhlmann (2020) A Multibasic Cleavage Site in the Spike Protein of SARS-CoV-2 Is Essential for Infection of Human Lung Cells. *Mol. Cell* 78(4):779-784.e5.
32. Coutard B, C Valle, X de Lamballerie, et al. (2020) The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. *Antiviral Res.* 176:104742.
33. Broszeit F, N Tzarum, X Zhu, et al. (2019) N-Glycolylneuraminic Acid as a Receptor for Influenza A Viruses. *Cell Rep.* 27(11):3284-3294.e6.
34. Wu D, W Huang, Y Wang, et al. (2014) Gene silencing of β-galactosamide α-2,6-sialyltransferase 1 inhibits human influenza virus infection of airway epithelial cells. *BMC Microbiol.* 14(1):78.
35. Sungnak W, N Huang, C Bécavin, et al. (2020) SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. *Nat. Med.* 26(5):681–687.
36. Wang Y-C, JW Stein, CL Lynch, et al. (2015) Glycosyltransferase ST6GAL1 contributes to the regulation of pluripotency in human pluripotent stem cells. *Sci. Rep.* 5(1):13317.
37. Garnham R, E Scott, KE Livermore, et al. (2019) ST6GAL1: A key player in cancer (Review). *Oncol. Lett.* 18(2):983–989.
38. Pally D, D Pramanik, S Hussain, et al. (2020) ST6GAL1-mediated heterogeneity in sialic acid levels potentiates invasion of breast cancer epithelia. *bioRxiv*:2020.04.28.065573.
39. Hedstrom L (2002) Serine Protease Mechanism and Specificity. *Chem. Rev.* 102(12):4501–4524.
40. Lucas JM, C Heinlein, T Kim, et al. (2014) The androgen-regulated protease TMPRSS2 activates a proteolytic cascade involving components of the tumor microenvironment and promotes prostate cancer metastasis. *Cancer Discov.* 4(11):1310–1325.
41. Wilson S, B Greer, J Hooper, et al. (2005) The membrane-anchored serine protease, TMPRSS2, activates PAR-2 in prostate cancer cells. *Biochem. J.* 388(3):967–972.
42. Hoffmann M, H Kleine-Weber, S Schroeder, et al. (2020) SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* 181(2):271-280.e8.
43. Zang R, MFG Castro, BT McCune, et al. (2020) TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes. *Sci. Immunol.* 5(47):eabc3582.
44. Wallrapp C, S Hähnel, F Müller-Pillasch, et al. (2000) A novel transmembrane serine protease (TMPRSS3) overexpressed in pancreatic cancer. *Cancer Res.* 60(10):2602–2606.
45. Takahashi T, H Uehara, K Izumi (2013) Inhibitory effect of soluble EP2 receptor on ovarian tumor growth in nude mice and utility of TMPRSS4 as a combinatorial molecular target. *Int. J. Oncol.* 43(2):416–424.
46. Ohler A, C Becker-Pauly (2011) Morpholino knockdown of the ubiquitously expressed transmembrane serine protease TMPRSS4a in zebrafish embryos exhibits severe defects in organogenesis and cell adhesion. *Biol. Chem.* 392(7):653–664.

applied
biosystems

47. Kim S, HY Kang, E-H Nam, et al. (2010) TMPRSS4 induces invasion and epithelial-mesenchymal transition through upregulation of integrin alpha5 and its signaling pathways. *Carcinogenesis* 31(4):597–606.
48. Larzabal L, PA Nguewa, R Pio, et al. (2011) Overexpression of TMPRSS4 in non-small cell lung cancer is associated with poor prognosis in patients with squamous histology. *Br. J. Cancer* 105(10):1608–1614.
49. Liang B, M Wu, Y Bu, et al. (2013) Prognostic value of TMPRSS4 expression in patients with breast cancer. *Med. Oncol.* 30(2):497.
50. Cheng D, B Liang, Y Li (2013) High TMPRSS4 expression is a predictor of poor prognosis in cervical squamous cell carcinoma. *Cancer Epidemiol.* 37(6):993–997.
51. Wu X-Y, L Zhang, K-M Zhang, et al. (2014) Clinical implication of TMPRSS4 expression in human gallbladder cancer. *Tumour Biol.* 35(6):5481–5486.
52. Sheng H, W Shen, J Zeng, et al. (2014) Prognostic significance of TMPRSS4 in gastric cancer. *Neoplasma* 61(2):213–217.
53. Li T, Z-C Zeng, L Wang, et al. (2011) Radiation enhances long-term metastasis potential of residual hepatocellular carcinoma in nude mice through TMPRSS4-induced epithelial-mesenchymal transition. *Cancer Gene Ther.* 18(9):617–626.
54. Valero-Jiménez A, J Zúñiga, J Cisneros, et al. (2018) Transmembrane protease, serine 4 (TMPRSS4) is upregulated in IPF lungs and increases the fibrotic response in bleomycin-induced lung injury. *PLOS ONE* 13(3):e0192963.

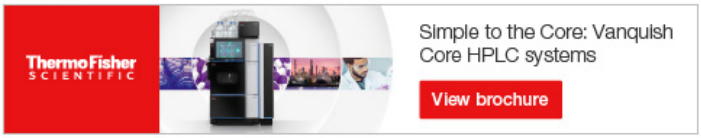
Find out more at **thermofisher.com/qpcrinvirology**

ThermoFisher
SCIENTIFIC

Select 08

Select 08.1

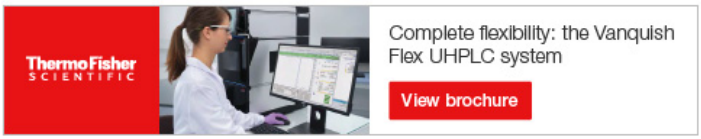
Select 08.2



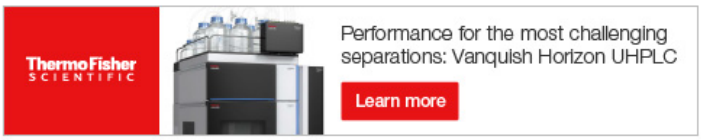
Select 09

Select 09.1

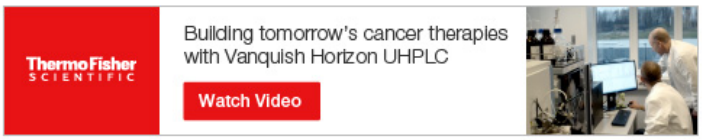
Select 09.2



Select 10



Select 10.1



Select 10.2

Select 11

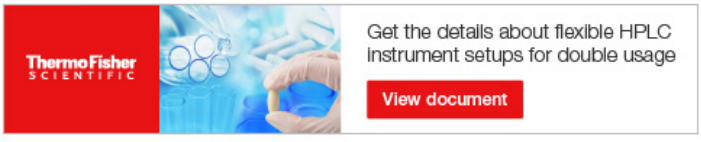
Select 11.1

Select 11.2

Select 12

Select 12.1

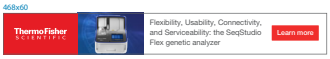
Select 12.2



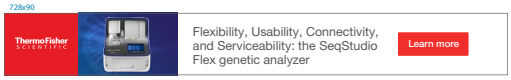
Seychelles Intro 1

Display

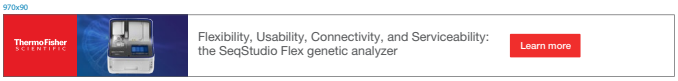
468x60



728x90

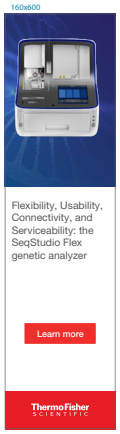


970x90



120x600

160x600



Email

600x320

200x200

250x250

300x250

336x280

Social

1200x628

1028x1028

Merch

612x289

289x293

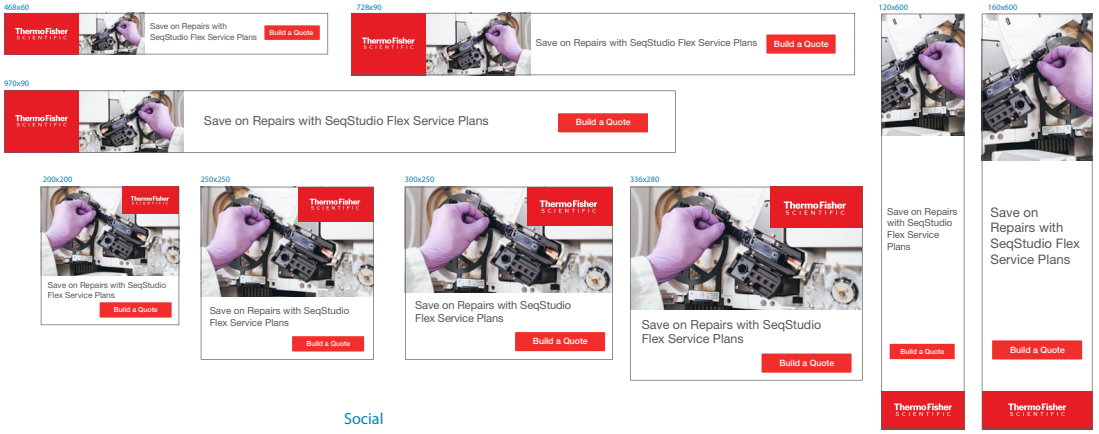
Dynamic Merch

612x289

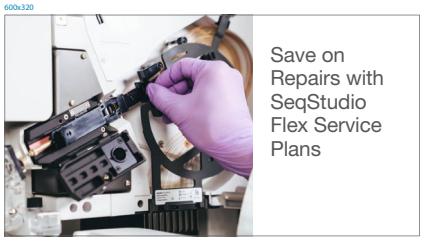
289x293

Seychelles Recommend 10

Display



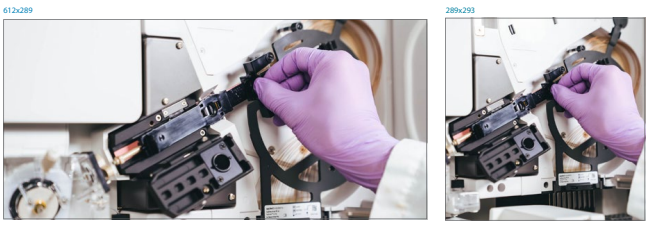
Email



Social

Merch

Dynamic Merch

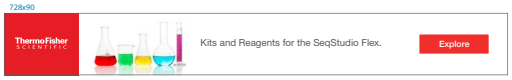


Seychelles Recommend 12

Display

468x60

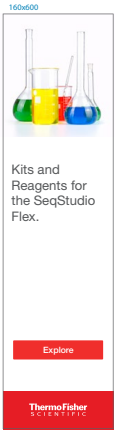
728x90



970x90

120x600

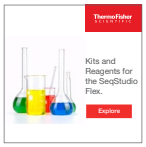
160x600



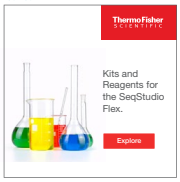
Email

600x320

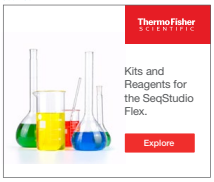
200x200



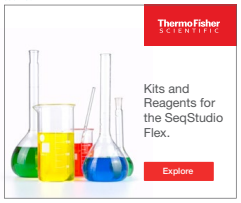
250x250



300x250



336x280



Social

1200x628

1028x1028

Merch

612x289

289x293

Dynamic Merch

612x289

289x293

WINTER 2023

ROYAL **BC** MUSEUM **Books**

BRITISH COLUMBIA'S OLDEST BOOK PUBLISHER

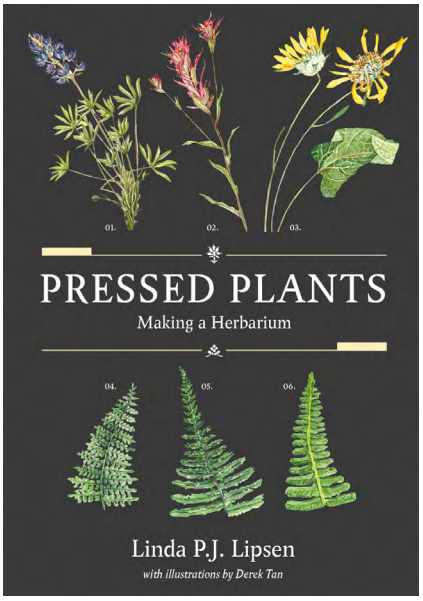
When John Fannin, the Royal BC Museum's first curator, published his *Check List of British Columbia Birds* in 1891, he set in motion a publishing program that has engaged and informed British Columbians for 132 years. Since that first publication, the Royal BC Museum has released hundreds of works on diverse subjects, bringing the human and natural history of our province to life in exciting, innovative and personal ways.

ROYAL BC MUSEUM

The Royal BC Museum is located on the traditional territories of the Lekwungen (Songhees and Xwsepsum Nations). We extend our appreciation for the opportunity to live and learn on this territory.

We gratefully acknowledge the financial support of Creative BC for marketing activities.

CONTENTS



CRAFTS & HOBBIES/SCIENCE
5.5″× 8″, 96 pages
22 black-and-white illustrations

February 2023

$19.95 CAN / US
978-0-7726-8056-3 trade paperback
978-0-7726-8057-0 ebook

RIGHTS HELD: World, all languages

9 780772 680563 >

Pressed Plants

Making a Herbarium

by Linda P.J. Lipsen, with illustrations by Derek Tan

A compact and contemporary guide to collecting, drying and storing plant specimens—suitable for both professional researchers and ardent amateurs.

The long-awaited successor to Dr. Christoper Brayshaw's beloved *Plant Collecting for the Amateur*, Linda P.J. Lipsen's *Pressed Plants* goes to the heart of professional and amateur plant collecting today.

Rich in illustrations, this beautiful book balances the ongoing history of plant collection with practical advice, and it includes the latest updates to best practices, such as how to preserve plant material for DNA extraction and how to navigate increasingly complex cultural and conservation considerations.

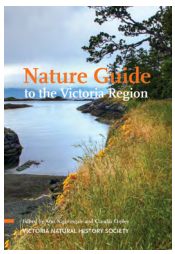
Pressed Plants responds to the recent revival of enthusiasm for nature, biodiversity and conservation with an increased emphasis on how to document the biodiversity around us. It will help any reader feel that they can understand how to properly document plant biodiversity so it's understood, valued and protected.

Linda P.J. Lipsen is the collections curator at the University of British Columbia Herbarium at the Beaty Biodiversity Museum and holds an M.Sc. in botany. Having previously worked for the UBC Botanical Garden and taught in UBC's Department of Botany, she also instructs the Master Gardeners.

Derek Tan is an illustrator, photographer and multimedia designer. He studied biology and art history at the University of British Columbia in his hometown of Vancouver. Derek is the digital producer at the Beaty Biodiversity Museum and is a recipient of the 2019 Governor General's History Award for Excellence in Museums.

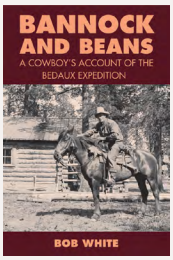
RELATED TITLES

Wild Flowers
Emily Carr,
Emily Woods
978-0-7726-5453-3 PB
$24.95 CAN / $19.95 US

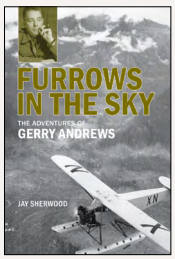


Nature Guide to the Victoria Region
Ann Nightingale, Claudia Copley
978-0-7726-6575-1 PB
$19.95 CAN / $17.95 US

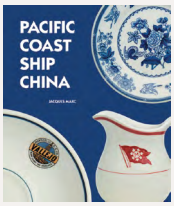
HISTORY & BIOGRAPHY



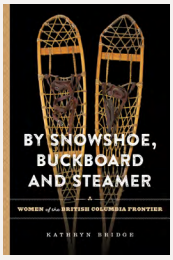
Bannock and Beans
A Cowboy's Account of the Bedaux Expedition
Bob White, Jay Sherwood
978-0-7726-6060-2 PB
$19.95 CAN / $17.95 US
Ebook also available



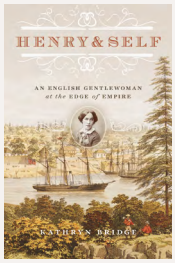
Furrows in the Sky
The Adventures of Gerry Andrews
Jay Sherwood
978-0-7726-6522-5 PB
$19.95 CAN / $17.95 US
Ebook also available



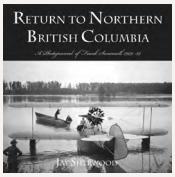
Pacific Coast Ship China
Jacques Marc
978-0-7726-5979-8 HC
$75 CAN / $60 US



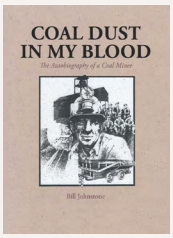
By Snowshoe, Buckboard and Steamer
Women of the British Columbia Frontier
Kathryn Bridge
978-0-7726-7310-7 PB
$19.95 CAN / $17.95 US
Ebook & audiobook also available



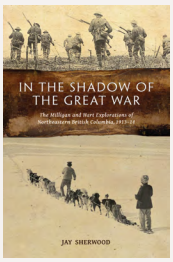
Henry & Self
An English Gentlewoman at the Edge of Empire
Kathryn Bridge
978-0-7726-7261-2 PB
$22.95 CAN / $19.95 US
Ebook also available



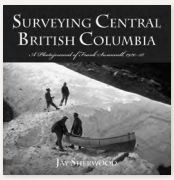
Return to Northern British Columbia
A Photojournal of Frank Swannell, 1929–39
Jay Sherwood
978-0-7726-6283-5 PB
$39.95 CAN / $29.95 US



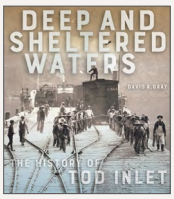
Coal Dust in My Blood
The Autobiography of a Coal Miner
Bill Johnstone
978-0-7726-4689-7 PB
$17.95 CAN / $14.95 US
Ebook & audiobook also available



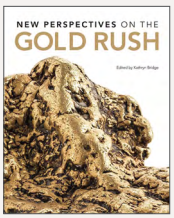
In the Shadow of the Great War
The Milligan and Hart Explorations of Northeastern British Columbia, 1913–14
Jay Sherwood
978-0-7726-6637-6 PB
$19.95 CAN / $17.95 US
Ebook also available



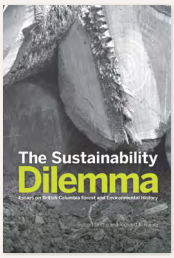
Surveying Central British Columbia
A Photojournal of Frank Swannell, 1920–28
Jay Sherwood
978-0-7726-5742-8 PB
$39.95 CAN / $29.95 US



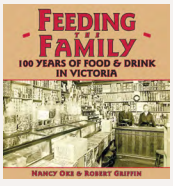
Deep and Sheltered Waters
The History of Tod Inlet
David R. Gray
978-0-7726-7256-8 PB
$29.95 CAN / $24.95 US
Ebook also available



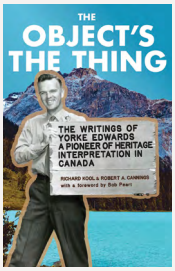
New Perspectives on the Gold Rush
Kathryn Bridge
978-0-7726-6854-7
$24.95 CAN / $19.95 US



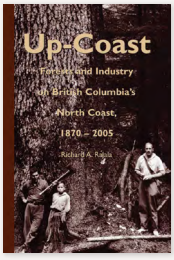
The Sustainability Dilemma
Essays on British Columbia Forest and Environmental History
Robert Griffin, Richard A. Rajala
978-0-7726-6974-2 PB
$24.95 CAN / $19.95 US



Feeding the Family
100 Years of Food & Drink in Victoria
Nancy Oke, Robert Griffin
978-0-7726-6343-6 PB
$29.95 CAN / $22.95 US



The Object's the Thing
The Writings of Yorke Edwards, a Pioneer of Heritage Interpretation in Canada
R. Yorke Edwards, Richard Kool, Robert A. Cannings, Bob Pearl
978-0-7726-7851-5 PB
$24.95 CAN / $19.95 US



Up-Coast
Forest and Industry on British Columbia's North Coast, 1870–2005
Richard A. Rajala
978-0-7726-5460-1 HC
$29.95 CAN / $24.95 US

WHERE TO FIND ROYAL BC MUSEUM BOOKS

SALES REPRESENTATION

Ampersand Inc. | ampersandinc.ca

British Columbia / Alberta / Saskatchewan / Manitoba / Yukon / Nunavut / NWT

2440 Viking Way
Richmond, BC V6V 1N2
GENERAL PHONE 604 243 5594
TOLL-FREE 1 866 736 5620
FAX 604 337 4056
TOLL-FREE 1 866 849 3819

Ali Hewitt / 604 337 4052 Ext. 402
alih@ampersandinc.ca

Dayle Sutherland / 604 337 2441 Ext. 404 / dayles@ampersandinc.ca

Pavan Ranu / 604 337 4055 Ext. 400
pavanr@ampersandinc.ca

Kim Herter / 604 337 4055 Ext. 401
kimh@ampersandinc.ca

Quebec

Sarah Gilligan
DIRECT PHONE 416 703 0666 Ext. 129
TOLL-FREE 1 866 736 5620
FAX 416 703 4745
sarahg@ampersandinc.ca

Atlantic Provinces

Kris Hykel
DIRECT PHONE 416 703 0666 Ext. 127
TOLL-FREE 1 866 736 5620
FAX 416 703 4745
krish@ampersandinc.ca

Ontario

Suite 213, 321 Carlaw Avenue
Toronto, ON M4M 2S1
GENERAL PHONE 416 703 0666
TOLL-FREE 1 866 736 5620
FAX 416 703 4745
TOLL-FREE 1 866 849 3819

Saffron Beckwith Ext. 124
saffronb@ampersandinc.ca

Morgen Young Ext. 128
morgeny@ampersandinc.ca

Laureen Cusack Ext. 120
laureenc@ampersandinc.ca

Vanessa Di Gregorio Ext. 122
vanessad@ampersandinc.ca

Evette Sintichakis Ext. 121
evettes@ampersandinc.ca

Sarah Gilligan Ext. 129
sarahg@ampersandinc.ca

Kris Hykel Ext. 127
krish@ampersandinc.ca

DISTRIBUTION AND FULFILMENT

Canada

UTP Distribution
5201 Dufferin Street
Toronto, ON M3H 5T8
PHONE 1 800 565 9523 / 416 667 7791
FAX 1 800 221 9985 / 416 667 7832
utpbooks@utpress.utoronto.ca

United States

Independent Publishers Group
814 N. Franklin Street
Chicago, IL 60610
PHONE 1 800 888 4741
FAX 312 337 5985
orders@ipgbook.com

Ebooks and Audiobooks

Royal BC Museum titles are available to libraries in ebook and audiobook format via several suppliers, including Overdrive, EBSCO and ProQuest.

Royal BC Museum ebooks and audiobooks are also available for individuals from ipgbook.com, Libro.FM, Audible, Kobo, Google Play, Apple Books and Kindle, among others.

MEDIA COPIES

Frontlist and recent backlist review copies are available to request on Netgalley and Edelweiss. Please submit other review or desk copy requests to **publishing@royalbcmuseum.bc.ca**.

PERSONAL ORDERS

Royal BC Museum publications are also available to individuals through your favourite bookstore or online at **rbcm.ca/books**.

All sales support the museum's work
Proceeds from the sale of publications support the many fine programs and special events at the Royal BC Museum.

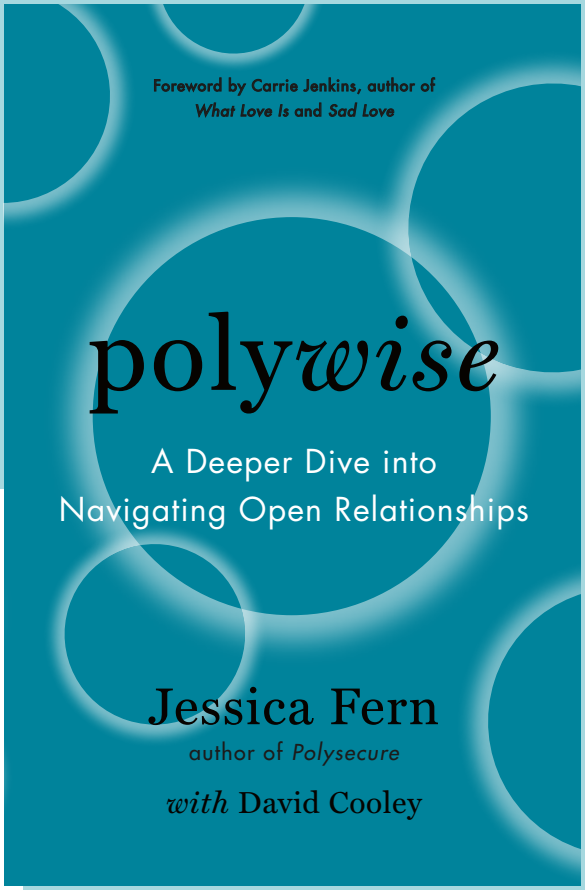
Winter 2023 Specials with UTP/Ampersand

UTP FRONTLIST SPECIAL
50+ frontlist titles, combinable across all UTP/Ampersand participating publishers

45% discount with code **FRN6**
One order per code
Ends November 30, 2022

UTP BACKLIST SPECIAL
50+ backlist titles, combinable across all participating UTP/Ampersand publishers

47% discount with code **BAC5**
One order per code
Ends December 31, 2022



ABOUT THE AUTHORS

Jessica Fern is a psychotherapist, trauma and relationship expert and the author of the bestselling *Polysecure: Attachment, Trauma and Consensual Nonmonogamy*. **David Cooley** is a professional Restorative Justice faciliitator, diversity and privilege awareness trainer and bilingual cultural broker. His work incorporates trauma-informed care, attachment theory, somatic practices and other modalities.

"*Polywise* emphasizes transitions—whether from monogamy to nonmonogamy, or from one form of nonmonogamy to another. It is these transitional periods that can easily reveal the grinding mechanisms behind the scenes, and the cracks in a relationship's infrastructure. This is where many of us need the most help, and so *Polywise* goes straight to the heart of the matter, offering balms for healing and genuinely feasible strategies for making these things…not painless, perhaps, but hopefully a little kinder to all involved, and certainly survivable."

—Carrie Jenkins, author of *What Love Is* and *Sad Love*

A next-level guide for people in nonmonogamous relationships.

Beyond the initial transition to nonmonogamy, many people struggle with the root issues beneath the symptoms of broken agreements, communication challenges and persistent jealousy.

Using a grounded theory approach, Jessica Fern and David Cooley explore the underlying challenges that nonmonogamous individuals and partners can experience after their first steps, offering practical strategies to transform them into opportunities for new levels of clarity and intimacy.

Polywise provides a conceptual framework to better understand the shift from monogamy to nonmonogamy and the tools to navigate the next steps in your polyamory, allowing you to not just survive open relationships but thrive in them.

SUBJECT CATEGORY:
FAM030000 FAMILY & RELATIONSHIPS / Marriage & Long-Term Relationships; PSYCHOLOGY / PSY041000 Psychotherapy / Couples & Family

PUBLICATION DATE:
August 18, 2023

TRIM: 5.25" × 8"
PAGE COUNT: 328

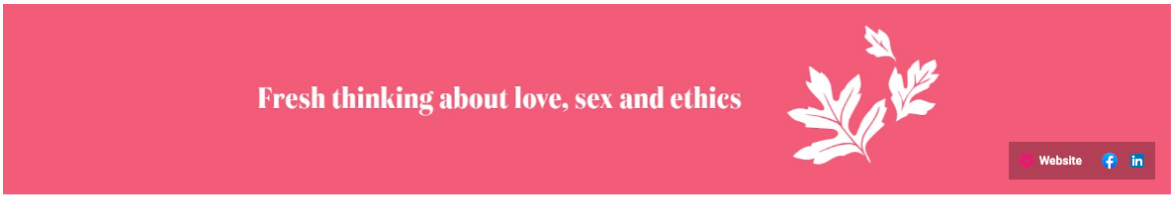
ILLUSTRATIONS:
None

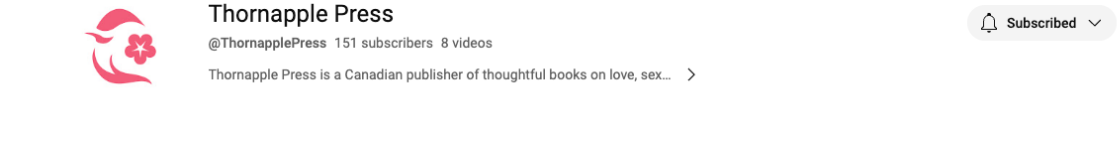
RIGHTS AVAILABLE:
World

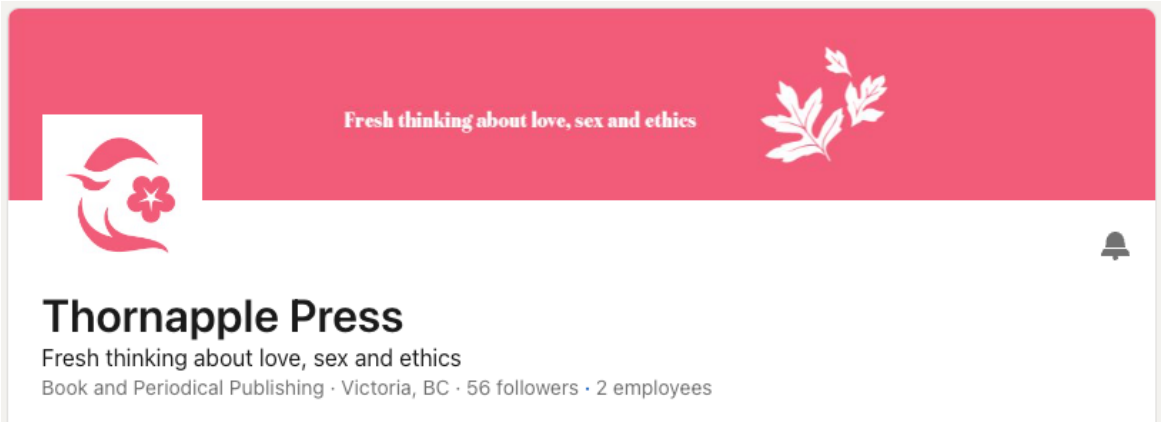
PRICE:
US $24.95 / CAD $33.95 (paperback)
US $11.99 / CAD $15.99 (e-book)
US $19.95 / CAD $26.95 (audiobook)

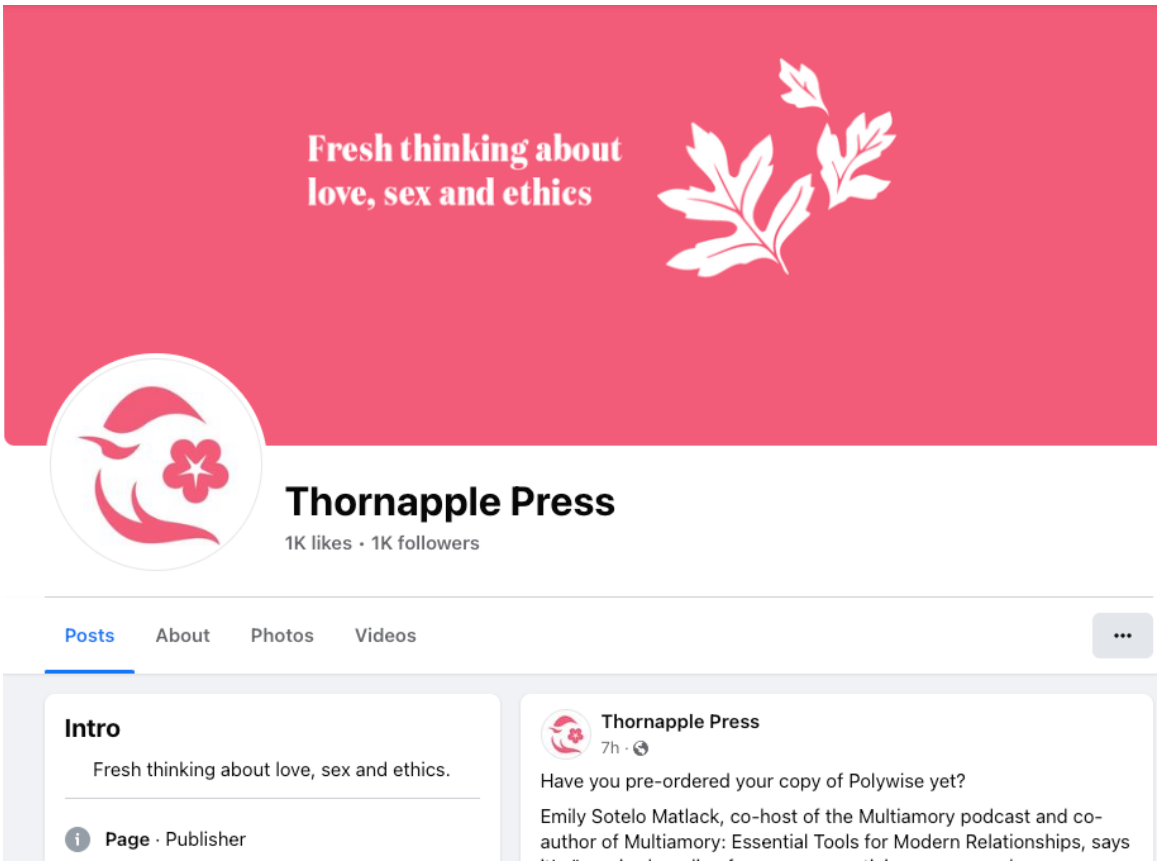
ISBNs / FORMATS:
978-1-990869-14-3 (paperback)
978-1-990869-15-0 (e-book)
978-1-990869-22-8 (audiobook)

CONTACT: Hazel Boydell hazel@thornapplepress.ca


Fresh thinking about love, sex and ethics
Website


Thornapple Press
@ThornapplePress 151 subscribers 8 videos
Thornapple Press is a Canadian publisher of thoughtful books on love, sex...
Subscribed


Fresh thinking about love, sex and ethics
Thornapple Press
Fresh thinking about love, sex and ethics
Book and Periodical Publishing · Victoria, BC · 56 followers · 2 employees


Fresh thinking about love, sex and ethics
Thornapple Press
1K likes · 1K followers
Posts
About
Photos
Videos
Intro
Fresh thinking about love, sex and ethics.
Page · Publisher
Thornapple Press
7h ·
Have you pre-ordered your copy of Polywise yet?
Emily Sotelo Matlack, co-host of the Multiamory podcast and co-author of Multiamory: Essential Tools for Modern Relationships, says

THORNAPPLE PRESS

Home About News Our Books Our Authors

Fresh thinking about love, sex and ethics.

Welcome to Thornapple Press! We are a Canadian publisher of thoughtful books on love, sexuality and relational ethics. Founded by Eve Rickert, we are the successor to Thorntree Press, publisher of bestselling and award-winning books like *Polysecure*, *More Than Two* and *Love's Not Color Blind*, and supported by the same editorial and design team.

Thornapple Press is based on the traditional, ancestral and unceded territories of the lək̓ʷəŋən and W̱SÁNEĆ peoples on Vancouver Island, British Columbia, with team members in Portugal, Ottawa, Toronto, the BC Kootenays and Chicago. Our books are distributed by UTP/Ampersand in Canada and IPG in the United States.

Learn more

Contact

General inquiries:
press [at] thornapplepress dot ca

Canada wholesale orders: Ampersand Inc.

US wholesale orders: IPG

Search

News

- Alyssa Gonzalez, author of *Nonmonogamy and Neurodiversity,* discusses neurodiversity and polyamory
- *In It Together: Navigating Depression with Partners, Friends, and Family* on sale now
- *Nonmonogamy and Neurodiversity* out today
- Christopher Dale, author of *Better Halves,* discusses how to rebuild a marriage after addiction
- Jessica Fern, the author of *Polysecure,* discusses attachment styles and nonmonogamy

Queer, non-monogamous writer **Cooper S. Beckett** began his writing career as a podcaster as host of Life on the Swingset: The Podcast and a speaker in the sexuality education community. He wrote *My Life on the Swingset*, a memoir of his first five years in non-monogamy, then followed that up with two novels, *A Life Less Monogamous* and *Approaching the Swingularity*. He lives in Chicago with Elle, his wife, constant, and binary star; Egon, their ghost terrier; and their black cat, Willow.

He co-authored *The Pegging Book: A Complete Guide to Anal Sex with a Strap-On Dildo* with Lyndzi Miller.

Alicia Bunyan-Sampson is a Toronto-based writer/director, advocate and academic. Her work primarily focuses on her identity as a black woman, an exploration of her own experiences of trauma and love, and a deliberate experimentation of the intersection of white supremacy and black identity.

Alicia's memoir, *No Filter: Diary of a Polyamorous Black Girl*, will be published by Thornapple Press in 2024.

David Cooley is a professional restorative justice facilitator, diversity and privilege awareness trainer, and bilingual cultural broker. He is the creator of the Restorative Relationships Conversations model, a process that transforms interpersonal conflict into deeper connection, intimacy and repair. In his private practice, David specializes in working with nonmonogamous and LGBTQ partnerships, incorporating a variety of modalities including trauma-informed care, attachment theory, somatic practices, narrative theory and mindfulness-based techniques.

With Jessica Fern, he is the co-author of *Polywise: A Deeper Dive into Navigating Open Relationships*, which will be

Christopher Dale is an experienced public relations professional, writer and recovering addict. He has been published in a wide range of prominent outlets, including *Daily Beast*, *Salon*, Parents.com, Dogster, *NY Daily News* and *Tribune Syndicate*. He writes on a wide range of topics, including addiction, mental health, politics, parenting, travel and rescue dog advocacy.

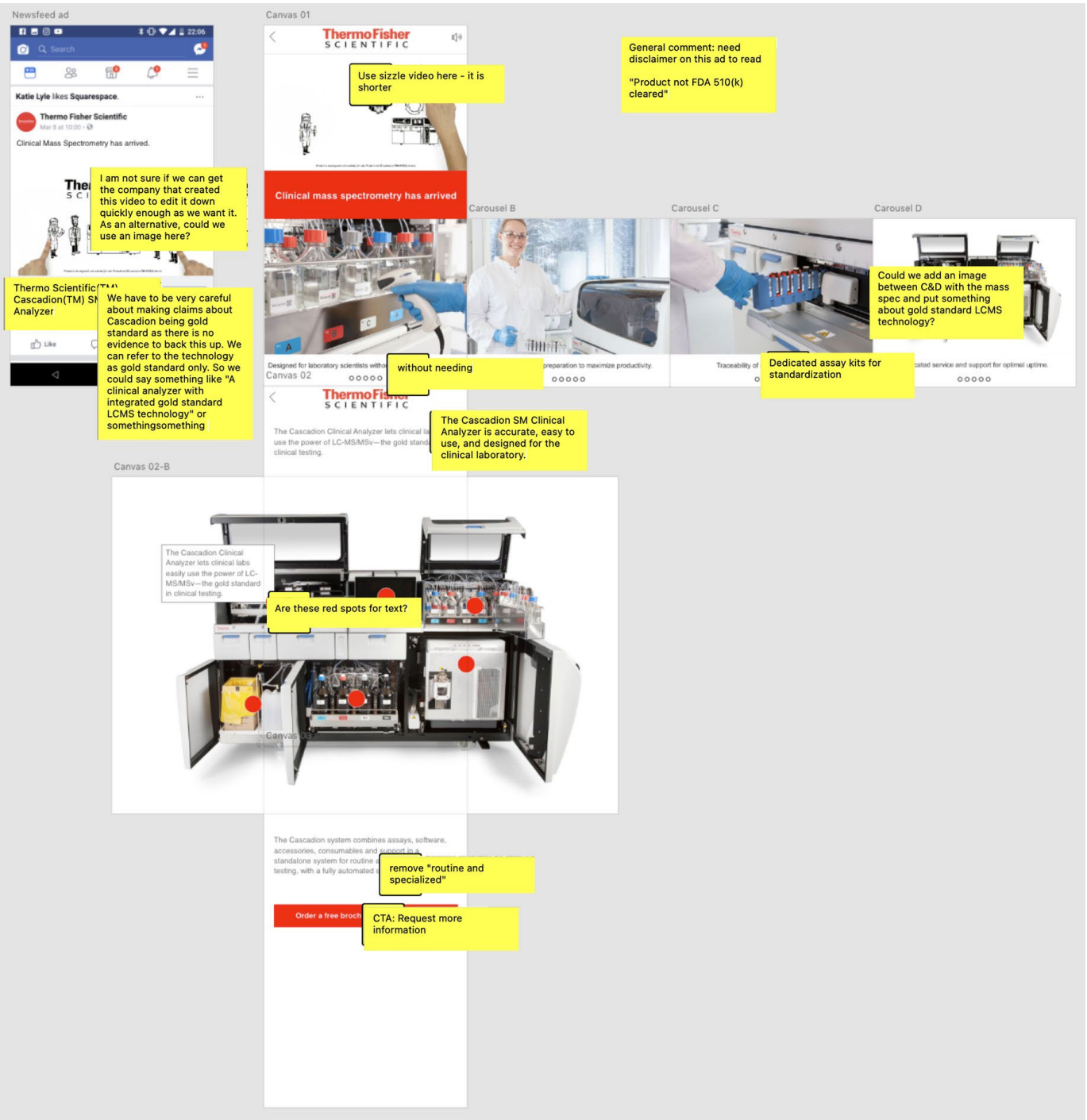
His first book, *Better Halves: Rebuilding a Post-Addiction Marriage*, explores his unlikely recovery from drug addiction, and even less likely recovery of a happy, healthy marriage.

THORNAPPLE PRESS

Home About News Our Books Our Authors

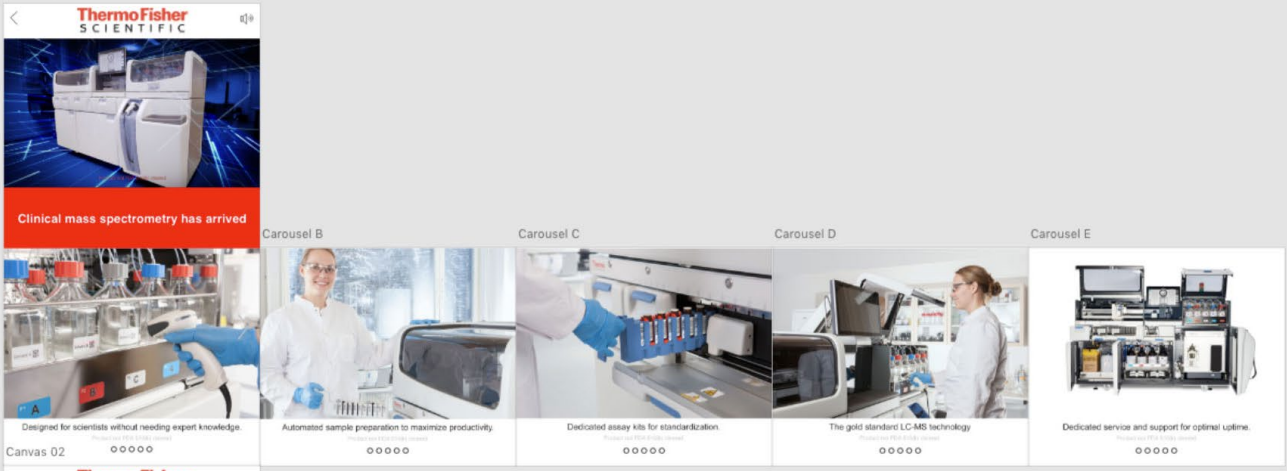
Thornapple Press publishes non-fiction books that discuss relationships, love, sexuality and relational ethics from unique and underrepresented perspectives. This includes books on consensual nonmonogamy, kink, sex work, harm reduction, LGBTQIAA+ identities, families, mental health, disability and related topics. We are sex-positive and recognize that gender and sexuality are non-binary.

We are particularly interested in receiving submissions from authors who can offer new insights on the themes of ethics, safety, community and consent. We welcome proposals from emerging authors and from perspectives that are underrepresented in sexuality literature, such as from BIPOC and disabled individuals. We aim to provide our readers with high-quality and well-researched content that is also accessible and engaging.

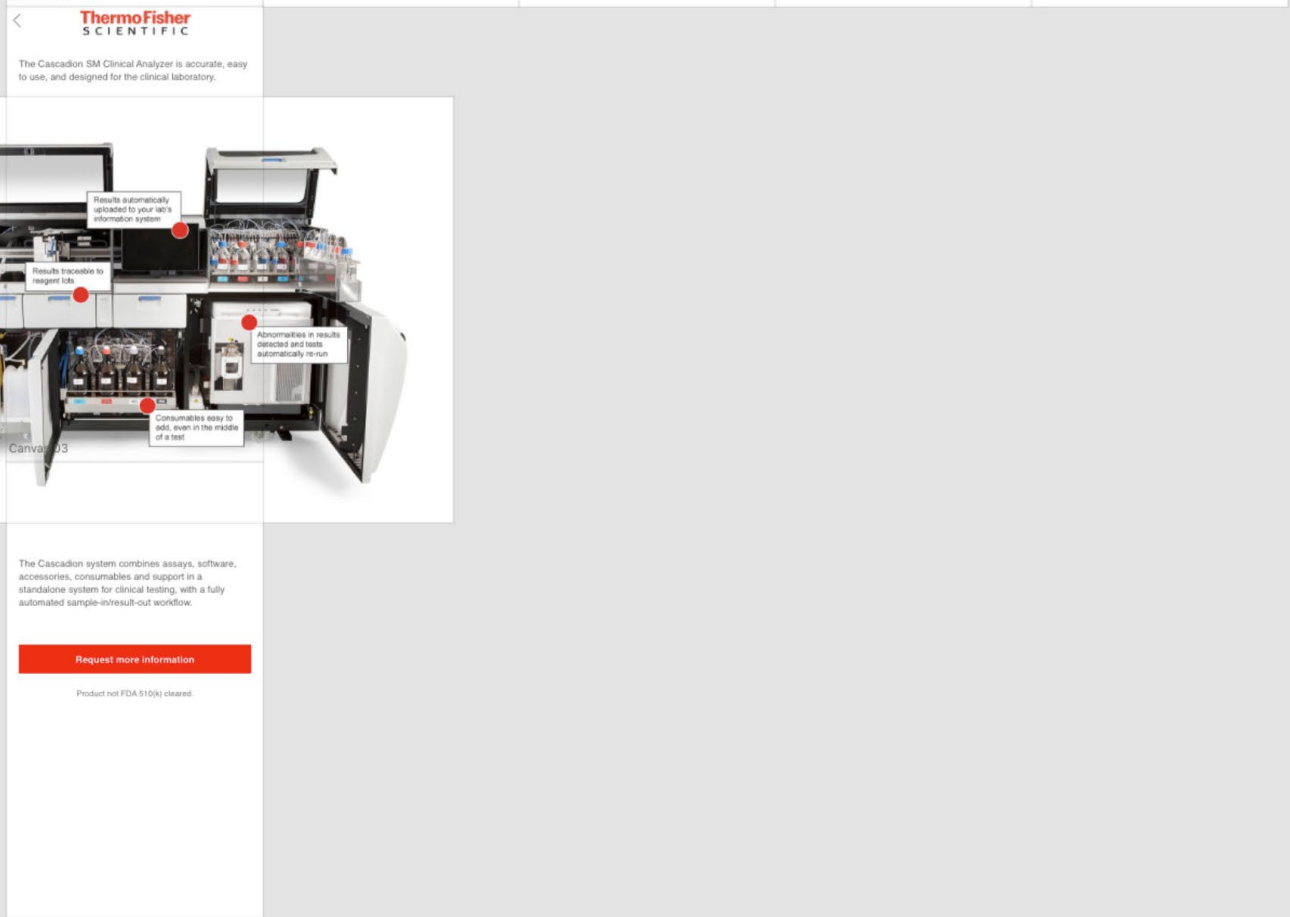

Newsfeed ad
Katie Lyle likes Squarespace.
Thermo Fisher Scientific
Clinical Mass Spectrometry has arrived.
I am not sure if we can get the company that created this video to edit it down quickly enough as we want it. As an alternative, could we use an image here?
We have to be very careful about making claims about Cascadion being gold standard as there is no evidence to back this up. We can refer to the technology as gold standard only. So we could say something like "A clinical analyzer with integrated gold standard LCMS technology" or somethingsomething
Canvas 01
ThermoFisher SCIENTIFIC
Use sizzle video here - it is shorter
Clinical mass spectrometry has arrived
General comment: need disclaimer on this ad to read
"Product not FDA 510(k) cleared"
Carousel B
Carousel C
Carousel D
Could we add an image between C&D with the mass spec and put something about gold standard LCMS technology?
without needing
Dedicated assay kits for standardization
Canvas 02
The Cascadion SM Clinical Analyzer is accurate, easy to use, and designed for the clinical laboratory.
Canvas 02-B
The Cascadion Clinical Analyzer lets clinical labs easily use the power of LC-MS/MSv—the gold standard in clinical testing.
Are these red spots for text?
remove "routine and specialized"
CTA: Request more information

Newsfeed ad

Canvas 01



Canvas 02



Canvas 02-B

Canvas 03

Technical note

Thermo Fisher SCIENTIFIC

All edits are required to comply with our editorial style guidelines, unless marked as "Observation", "Suggestion" , or "Question" to the BP.

Cancer Research

Please follow asset master for TS brand attached here. page 8

ystems Absolute Q™ Liquid Biopsy Assays
t quantification on the Applied Biosystems™
QuantStudio™ Absolute Q™ Digital PCR System

Add subheader: Leveraging Absolute Q Liquid Biopsy dPCR Assays

Highlig

- Demonstration of cancer mutation detection down to 0.1% MAF for 30 clinically relevant cancer mutations commonly used in Liquid Biopsy applications
- Complete one-step walk-a PCR (dPCR).
- Microfluidic Array Plate (MAP) consumable for emulsion-free dPCR.
  - Low sample waste, high micro-chamber yield.
  - Single co

Delete periods at the end of each bullet/sub bullet.

please update headers to fit global template

Introduction

Please italicize all gene names throughout.

[Global edit]

Workflow and methods

make content header size

tation for evalu treatment th cancer. certain ho insight into efficacy of respons le, mutations such as KRAS p.G12D[1], p.G12V[2] and p.G13D[3] indicate potential reduced responsiveness to Tyrosine Kinase Inhibitors (TKIs), whereas PIK3CA mutations such as p.H1047R indicate positive response to PI3K/AKT/mTOR signaling pathway inhibitors[4]. Liquid biopsies, in which blood is taken and assayed for traces of cancer such as cell-free DNA (cfDNA), provides a much less invasive way to examine the genetic composition of a patient's tumors and can even help with diagnosis, but is a challenge in the laboratory. Detecting circulating tumor DNA for liquid biopsy applications is challenging because the molecules bearing the target of interest are only a small fraction of the total circulating cell-free DNA collected in the sample. Liquid biopsy assays must be able to accurately qu y rare, single-nucleotide polymorphisms,

Remove hyphen, keep space.

[Global]

among high levels of wild-ty ding sensitivity and precision. Re tried-and-true method for detecting and quantifying mutations with oncological significance, but it can struggle with samples like these. With its unparalleled precision and sensitivity, digital PCR is ideally suited for liquid biopsy applications in which small amounts of relevant mutations exist in the sample.

The QuantStudio Absolute Q digital PCR system was used with the Applied Biosystems™ Absolute Q™ Liquid Biopsy dPCR Assays to perform rare target detection for 30 hot-spot cancer mutations in the KRAS, EGFR, and PIK3CA genes. Mutation Allele Frequencies (MAFs) down to 0.1% were detected among a wild-type concentrati

Workflow and Methods

Titration Series Preparation

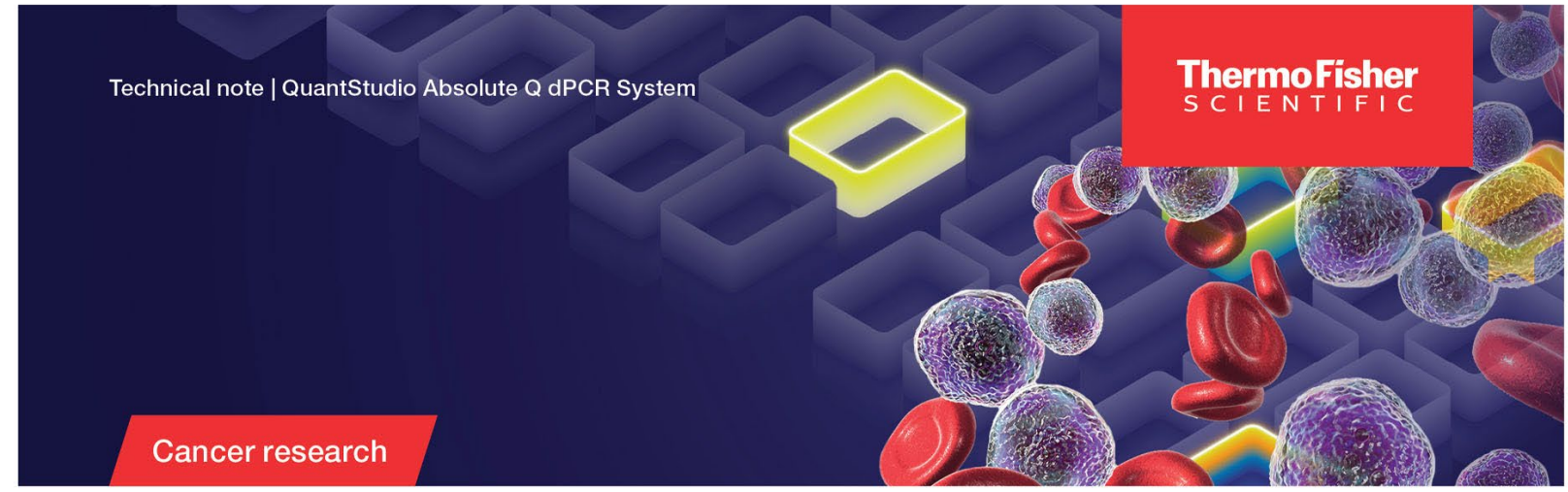
Thirty hot-spot cancer mutations were selected for this study (Table 1). DNA mixtures were prepared by combining mutation-bearing plasmids into a high background of wild-type normal gDNA for each of the mutations selected. Each DNA mixture contained 50 nanograms of gDNA, and targeting a final MAF of 0.1%. Two repli were tested for each assay alongside 1-2 reactions of a 100% wild-type or reference control.

en dash

QuantStudio Absolute Q Digital PCR system workflow

Using the QuantStudio Absolute Q Digital PCR system's simple workflow (Figure 1), PCR mix was prepared using the Absolute Q DNA Digital PCR MasterMix (5X) and 9uL was loaded into

thermo scientific



Cancer research

Rare-target quantification on the QuantStudio Absolute Q dPCR System

Leveraging Absolute Q Liquid Biopsy dPCR Assays

Highlights

- Detection of mutation allele frequencies (MAFs) of 0.1% for 30 relevant cancer mutations commonly used in liquid biopsy applications
- Complete, one-step walk-away workflow for digital PCR (dPCR)
- Use of microfluidic array plate (MAP) consumable for emulsion-free dPCR
  - Low sample waste, high microchamber yield
  - Single consumable, single instrument

Introduction

Mutation screening is becoming a standard technique for evaluating treatment options of patients diagnosed with cancer. Notably, certain hotspot mutations can give valuable insight into efficacy of response to various treatments. For example, mutations such as *KRAS* p.G12D [1], *KRAS* p.G12V [2] and *KRAS* p.G13D [3] indicate potential reduced responsiveness to tyrosine kinase inhibitors (TKIs), whereas *PIK3CA* mutations such as p.H1047R indicate positive response to PI3K/AKT/mTOR signaling pathway inhibitors [4]. Liquid biopsies, in which blood is taken and assayed for traces of cancer in circulating cell-free DNA (cfDNA), provide a much less invasive way to examine the genetic composition of a patient's tumors and can even help with diagnosis. However, detecting circulating tumor DNA (ctDNA) for liquid biopsy applications is challenging because the molecules bearing the target of interest are only a small fraction of the total circulating cfDNA collected in the sample.

Liquid biopsy assays must be able to accurately quantify rare, single-nucleotide polymorphisms (SNPs), among high levels of wild type background DNA, with outstanding analytical sensitivity and precision. Real-time or quantitative PCR is a tried-and-true method for detecting mutations with oncological significance, but it can struggle with precise quantitation of rare mutations. With its exceptional precision and analytical sensitivity, dPCR is ideally suited for liquid biopsy applications in which small amounts of relevant mutations exist in the sample.

In this research study, the Applied Biosystems™ QuantStudio™ Absolute Q™ Digital PCR System was used with the Applied Biosystems™ Absolute Q™ Liquid Biopsy dPCR Assays to perform rare-target detection for 30 hotspot cancer mutations in the *KRAS*, *EGFR*, and *PIK3CA* genes. MAFs down to 0.1% were detected among a high-background wild type sample concentration.

Workflow and methods

Titration series preparation

A total of 30 hotspot cancer mutations were selected for this study (Table 1). For each of the mutations selected, DNA mixtures were prepared by combining mutation-bearing plasmidswith wild type gDNA. Each DNA mixture contained 50 ng of gDNA, and mutation plasmid targeted a final MAF of 0.1%. Two replicates of the DNA mixture were tested for each assay alongside 1–2 reactions of a 100% wild type (or reference) control.

SHARKS!

The very mention of the word conjures up images of dangerous creatures with a voracious appetite. This public image couldn't be farther from the truth for a vast majority of shark species: most are cautious and placid, and many inhabit waters that exclude them from human contact.

Much fear of sharks is driven by media reports or films that sensationalize shark attacks, despite the rarity of such occurrences. So much about sharks and their relatives makes them fascinating, and we still have much to learn.

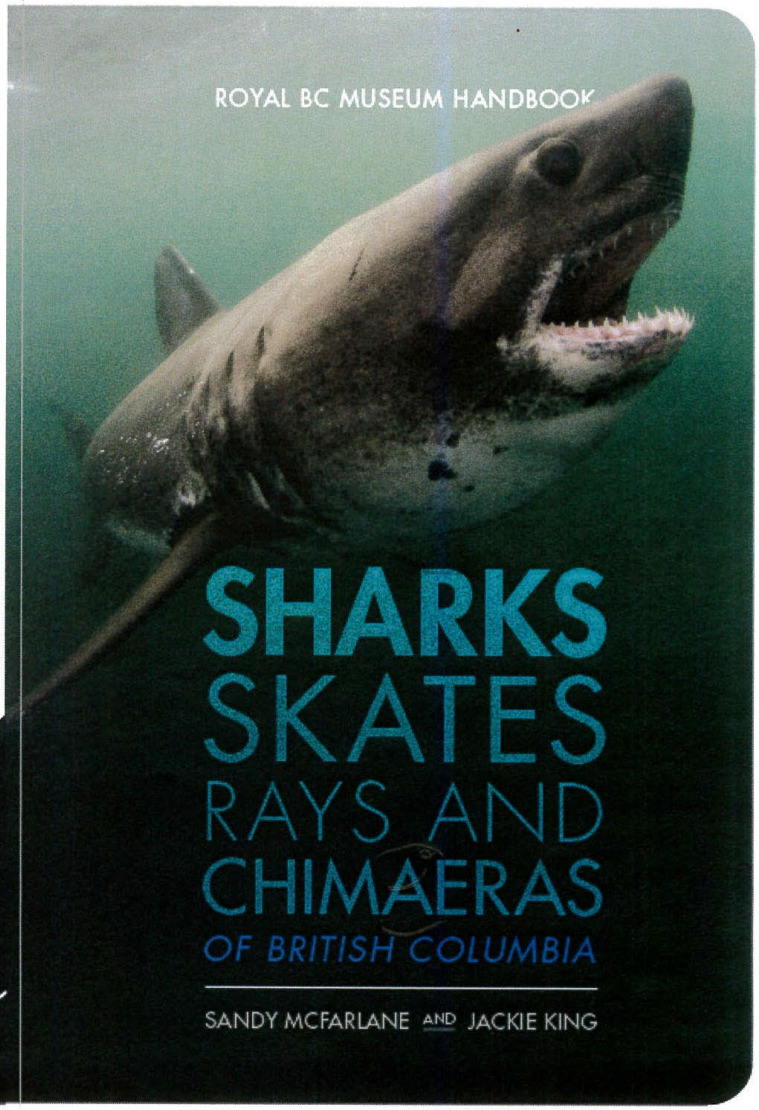
This book is for everyone interested in learning more about sharks and their relatives. It provides the most accurate and up-to-date information on chondrichthyans in British Columbia waters, including detailed species descriptions and identification information. ***Sharks, Skates, Rays and Chimaeras of British Columbia*** presents sharks and their relatives as valuable members of our coastal fish community, worthy of respect, study, admiration and protection.

Dr. Gordon McFarlane is a scientist emeritus at the Pacific Biological Station in Nanaimo, BC. Dr. Jackie King is a research scientist with Fisheries and Oceans Canada at the Pacific Biological Station, where she leads the Canadian Pacific Shark Research Program.

ROYAL BC MUSEUM

SHARKS, SKATES, RAYS AND CHIMAERAS OF BRITISH COLUMBIA

McFarlane and King



SHARKS!

A long-overdue book on a diverse group of species. Accessible to any reader, it strikes the perfect balance of scientific detail, history, conservation, imagery and useful facts. Anyone interested in the natural history of British Columbia's marine life should have this on their shelf.

—Scott Wallace, senior research scientist, David Suzuki Foundation

Sharks, Skates, Rays and Chimeras of British Columbia is a book for everyone interested in learning more about sharks and their relatives. It provides the most accurate and up-to-date information on chondrichthyans in BC waters, including detailed species descriptions and identification information. This book presents sharks and their relatives as valuable members of our coastal fish community, worthy of respect, study, admiration and protection.

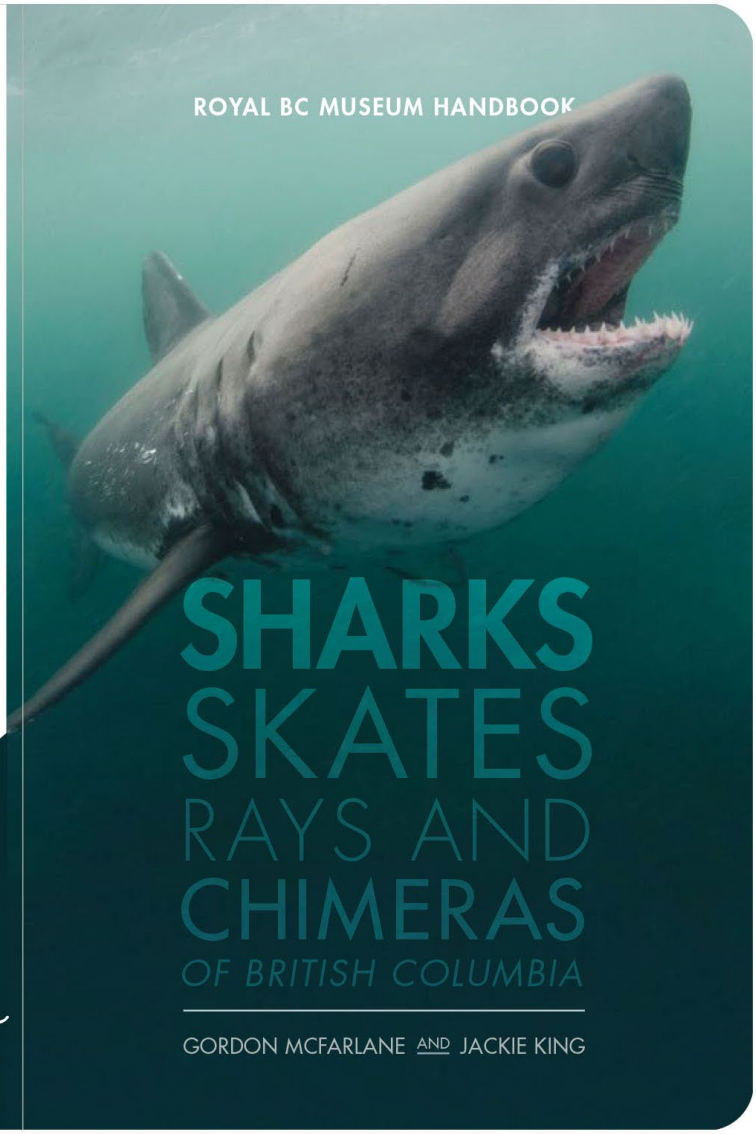
Gordon McFarlane is a scientist emeritus at the Pacific Biological Station in Nanaimo, BC. **Dr. Jackie King** leads the Canadian Pacific Shark Research Program at the Pacific Biological Station.

ROYAL BC MUSEUM

ISBN 978-0-7726-7335-0

SHARKS, SKATES, RAYS AND CHIMERAS OF BRITISH COLUMBIA

McFarlane and King

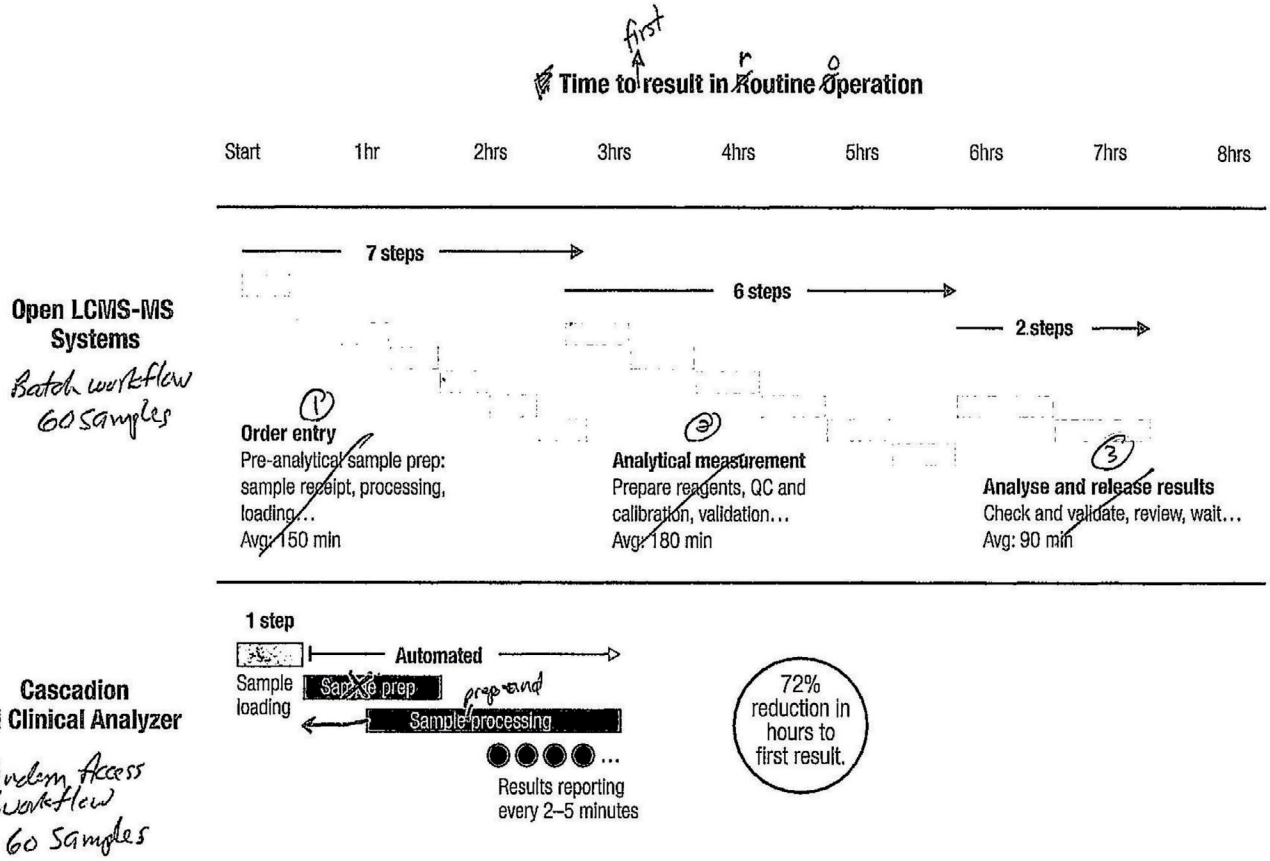


ROYAL BC MUSEUM HANDBOOK

SHARKS SKATES RAYS AND CHIMERAS

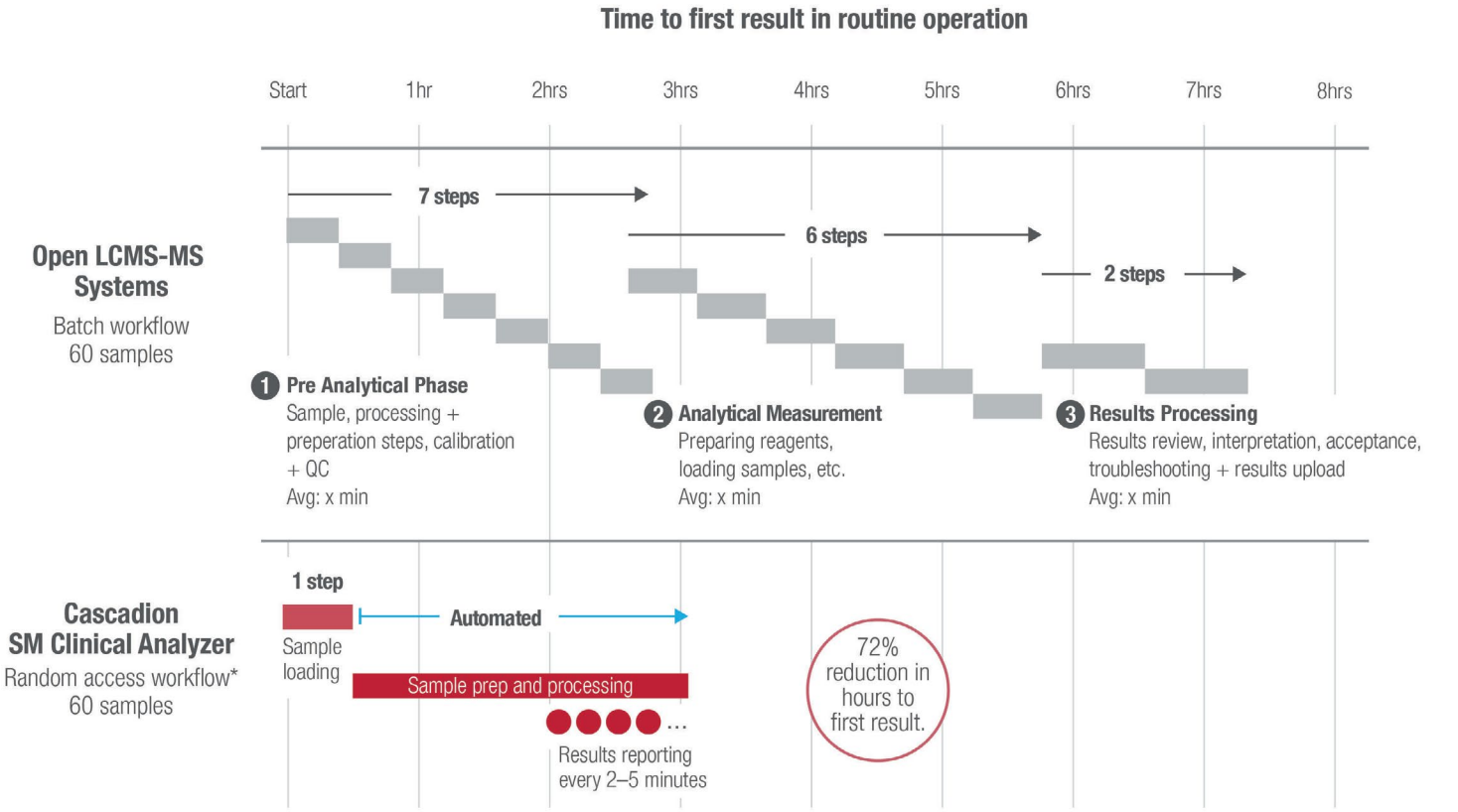
OF BRITISH COLUMBIA

GORDON MCFARLANE AND JACKIE KING



*Manual LCMS workflows are averages and can vary.

**Cascadion system workflow based on studies conducted by Argent Global Services, January 2018.



*Manual LCMS workflows are averages and can vary.

**Cascadion system workflow based on studies conducted by Argent Global Services, January 2018.