

### 1. Drug response and pharmacology studies

#### Why temperature control matters

Dose response, toxicity, and mechanism of action assays assume that changes in signaling, proliferation, or death are driven by the compound—not by drift in temperature sensitive cell physiology. Even 1–2 °C deviations can alter membrane transport, enzyme kinetics, and receptor trafficking, shifting apparent  $EC_{50}/IC_{50}$  values and time to effect.

#### Key references

- **Peters, Nikon MicroscopyU – “Maintaining Live Cells on the Microscope Stage”**  
Overview of environmental requirements for live cell imaging; emphasizes that small, uncorrected temperature changes cause focus drift, morphology changes, and altered cell behavior during pharmacological experiments.
- **ibidi – “Live Cell Imaging Parameters: Temperature”**  
Application note outlining recommended tolerances (typically around 37 °C with  $\pm 0.5$  °C) and documenting how temperature shifts change cell motility, division rate, and drug sensitivity.
- **Sistemich et al., 2024 – “Heat application in live cell imaging” (FEBS Press)**  
Reviews tools for localized heating and shows that tightly controlled thermal profiles are essential when probing temperature sensitive signaling and pharmacological responses.

#### Application note: Drug response studies

For long time-lapse drug assays, maintain specimen plane temperature at 37 °C within about  $\pm 0.5$  °C (ideally tighter) and minimize gradients between the chamber and objective. Log temperature throughout the experiment so that shifts in response curves can be distinguished from thermal artifacts, and pair chamber heating with objective heating for high-NA lenses to prevent the objective from acting as a heat sink.

## 2. Cell stress, viability, and dormancy studies

### Why temperature control matters

Cell stress markers, apoptosis, and stress induced dormancy are all strongly temperature dependent. Mild hypothermia or hyperthermia during imaging can activate stress pathways and alter viability readouts, causing cells to appear resistant, quiescent, or more fragile than they truly are.

### Key references

- **ibidi – “Parameters for Healthy Cells”**  
Summarizes how deviations from physiological temperature, pH, and gas composition trigger stress responses; notes that unstable temperature during imaging can reduce viability and distort assay outcomes.
- **Vahea et al., 2021 – “Temperature control in light microscopy – challenges and solutions” (FocalPlane)**  
Describes common problems with stage-top incubation (gradients, overshoot, and drift) and presents best practices for closed-loop, specimen-centric temperature control to keep cells in a healthy state.
- **Peters, Nikon MicroscopyU – “Environmental Considerations for Live Cell Imaging”**  
Highlights that temperature is the dominant factor in maintaining cell health on the stage, with concrete examples of morphology and viability changes caused by inadequate control.

### Application note: Stress/viability/dormancy

When running cell stress or viability assays, treat temperature as a primary experimental variable: hold cells at their culture temperature with minimal drift and no overshoot, and avoid repeated moves between incubator and microscope without re-equilibration. A closed loop controller that can maintain  $\pm 0.2$  °C at the specimen while synchronizing objective temperature helps prevent “hidden” stress that would otherwise drive cells toward dormancy or apoptosis during imaging.

### 3. FISH and spatial genomics

#### Why temperature control matters

FISH and spatial genomics depend directly on precise hybridization and wash temperatures; these steps set probe stringency and strongly affect signal intensity, specificity, and background. Temperature drift or gradients across a slide can create regional artifacts that look like real genomic differences.

#### Key references

- **Sigma-Aldrich – “Fluorescent in situ Hybridization (FISH) Technical Guide”**  
Provides standard ranges (e.g., ~37–55 °C for hybridization, ~40–72 °C for washes) and explains how undershooting or overshooting these values affects specificity and background.
- **Hsieh et al., 2005 – “High-temperature FISH for detecting *Enterococcus faecalis*”**  
Demonstrates that tightly controlled elevated temperatures (60–75 °C) can shorten hybridization times while maintaining or improving signal quality, underscoring the importance of accurate thermal control.
- **Todorović et al., 2017 – “Standardization and optimization of FISH for HER2 status”**  
Shows that rigorous control and documentation of hybridization and wash temperatures are central to HER2 assay reproducibility and inter-laboratory comparability.
- **NIH / Guidance for FISH Testing**  
Quality assurance guidelines that highlight temperature validation, monitoring, and SOPs as core elements of regulated FISH workflows.

#### Application note: FISH and spatial genomics

For FISH and spatial transcriptomics, verify actual slide temperature (not just device setpoints) during hybridization and washes, and keep it within about  $\pm 1$  °C of the protocol target. Use closed loop, specimen plane feedback and log temperatures for each run; during long imaging passes, use stage level temperature control and coordinated objective heating to keep registration stable and prevent slow thermal drift from degrading spot localization.

### 4. Cellular dynamics and intracellular transport

#### Why temperature control matters

Organelle movement, cytoskeletal rearrangements, vesicle trafficking, and many signaling cascades are steep functions of temperature; changing temperature effectively changes the “rate constant” of the process you’re trying to measure. If imaging temperature drifts, observed speeds and event rates will drift too.

#### Key references

- **Peters, 2012 – “Overview of Live-Cell Imaging: Requirements and Methods Used”**  
Reviews the dependence of many dynamic processes on temperature and recommends rigorous control for quantitative measurements of trafficking and cytoskeletal behavior.
- **“A tool for live-cell confocal imaging of temperature dependent processes” (Journal of Microscopy)**  
Describes a system for controlling and measuring temperature during confocal imaging, and shows that temperature fluctuations alter the kinetics of the processes under study.
- **Sistemich et al., 2024 – “Heat application in live cell imaging”**  
Details strategies for applying and measuring temperature during live cell imaging, with examples of heat sensitive dynamics and how precise control is required to interpret them.

#### Application note: Cellular dynamics and transport

For quantitative transport or dynamics assays, treat the microscope as a controlled reaction environment: stabilize specimen plane temperature to within a narrow band (for example,  $\pm 0.2$ – $0.5$  °C) and monitor it over time. Dual channel control that keeps both the chamber and high NA objective at coordinated temperatures minimizes drift and ensures that changes in velocity or frequency truly reflect biology, not gradual warming or cooling of the setup.

## 5. Developmental and embryonic imaging

### Why temperature control matters

Embryos and developing tissues are extremely temperature sensitive. Small changes can alter developmental timing, morphology, and survival. Because developmental imaging often spans hours to days, even slow thermal drift can accumulate into major phenotypic differences.

### Key references

- **Nikon MicroscopyU – “Environmental Considerations for Live Cell Imaging”**  
Discusses the additional challenges of long-term imaging of embryos and tissues, highlighting temperature as the primary factor in maintaining normal development.
- **von Chamier et al., 2019 – “Turning Up the Heat: Local Temperature Control During in vivo Imaging”**  
Demonstrates local heating strategies for intact organisms and shows how tightly controlled temperature enables physiological in vivo imaging without damaging tissues.
- **ibidi – “Live Cell Imaging Parameters: Temperature”**  
Notes that embryonic and stem-cell preparations may tolerate narrower temperature windows than standard lines, reinforcing the need for precise control.

### Application note: Developmental and embryonic imaging

For developmental time lapse, use a closed loop controller that can hold the specimen at the organism’s physiological temperature for many hours with minimal drift and no overshoot. Record temperature alongside imaging data, and avoid thermal gradients between the chamber and immersion objective; coordinated control of both reduces focus drift and helps embryos follow their normal developmental trajectory.

### 6. Organ-on-a-chip and microfluidic models

#### Why temperature control matters

Organ-on-a-chip and microfluidic systems integrate perfusion, shear, and 3D architecture; temperature fluctuations change viscosity, flow behavior, and tissue responses, undermining the “physiological” nature of the model. Because chips are small, they can equilibrate quickly to ambient conditions if not carefully controlled.

#### Key references

- **AutoMate Scientific – “Temperature Control Overview”**  
Summarizes temperature control strategies for perfused and microfluidic preparations, stressing the need to control both the device and the surrounding optics/stage.
- **Elveflow – “Review of Heating and Temperature Control in Microfluidic Systems”**  
Reviews micro-heater designs, closed-loop strategies, and typical precision targets for microfluidic channels and chips.
- **Multiplexed dynamic temperature control in microfluidic live cell experiments (preprint)**  
Shows how precisely modulating chip temperature enables complex, time resolved perturbation experiments and highlights technical requirements for stable control.

#### Application note: Organ-on-a-chip

For organ-on-a-chip imaging, integrate thermal control into the chip environment and the microscope: control the chip or chamber temperature and use objective heating to avoid gradients at the imaging interface. Closed loop controllers with fine precision support both steady state physiological conditions and intentional, time programmed temperature perturbations while maintaining reproducible flow and tissue responses.