b·tu Brandenburgische
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R as an Environment for the Reproducible Analysis of DNA Amplification Experiments

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[1]Brandenburg University of Technology Cottbus-Senftenberg
Institute of Biotechnology, InnoProfile RG "Image-based Assays"
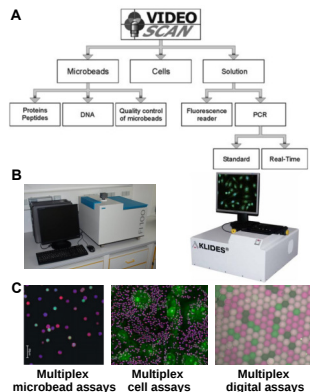stefan.roediger@b-tu.de

[2]University of Wrocław, Wrocław, Department of Genomics, Faculty of Biotechnology
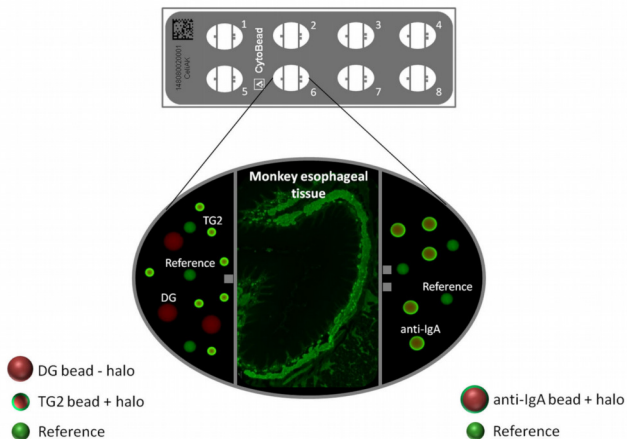
October 13, 2016

Research Focus

High-throughput and High-content Fluorescence Microscopy Screening Technology

- Versatile fully-automated fluorescence imagining platform for multiparameter assays (e.g., DNA/protein microbead chips, cell-based assays)
- Investigate cellular functionality (genotype,phenotype)
- Combined assays of cells and microbeads
- Real-time monitoring of molecular interactions

Multiplex microbead assays **Multiplex cell assays** **Multiplex digital assays**

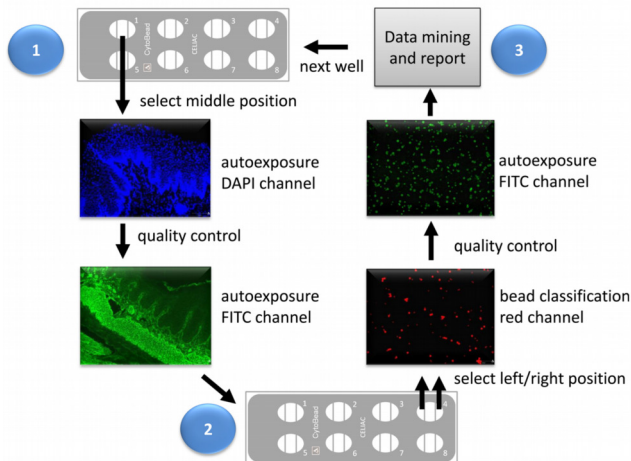Rödiger *et al.* 2013 *Adv. Biochem. Eng. Biotechnol.*; Rödiger *et al.* 2014 *Microchim. Acta*

Digital Image Analysis
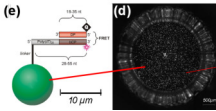
Combined Cell and Microbead Assay
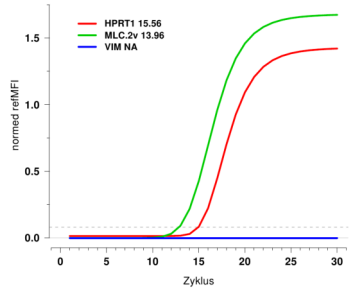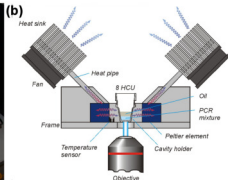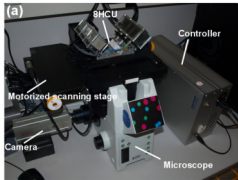
Digital Image Analysis

Experimental Workflow and Data Retrieval

Digital Image Analysis

Experimental Workflow and Data Retrieval
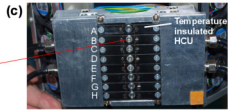
Analysis of Nucleic Acid Amplification Experiments

- qPCR is the method of choice for precise quantification of nucleic acids
- Alternative methods such as quantitative Isothermal Amplification (qIA) emerged during past few year
- Digital PCR (dPCR) is gaining more relevance since commercial systems are available

- There is no strict consensus regarding preprocessing and analysis of the data
- Guidelines such as MIQE and dMIQE exist
- Most analysis are done in black boxes

Rational Approach: FLOSS for reproducible research based on evidence, peer-reviewed methods and powerful frameworks not tied to a single platform
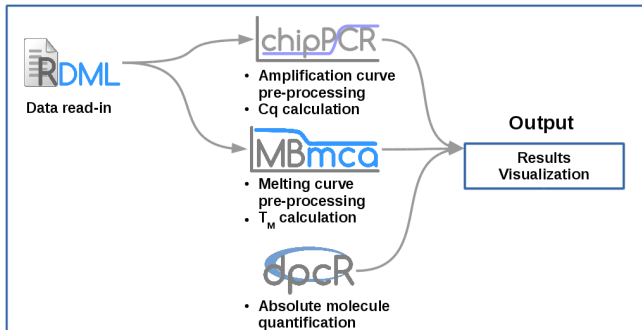
Workhorse: R GUI/IDE and Packages

- R centered software for reproducible analysis of nucleic acid amplification
  - Isothermal amplification
  - quantitative PCR
  - digital PCR
  - Real-Time PCR Data Markup Language (RDML) for data exchange
  - In part Peer-reviewed, available @ *CRAN* and *github*

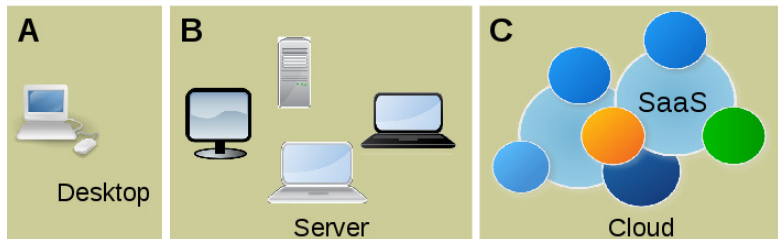***rkwarddev*** by Meik Michalke

Workhorse: R GUI/IDE and Packages

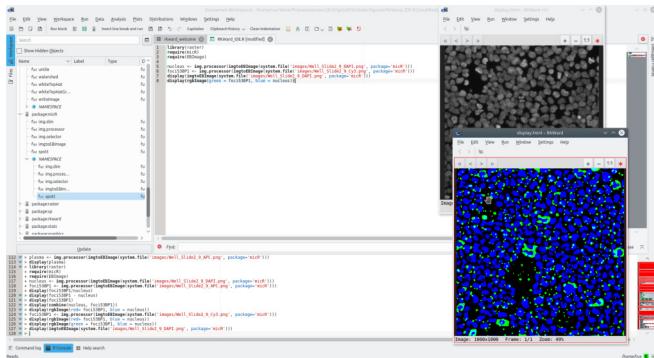Use cases and deployment

Workhorse: R GUI/IDE and Packages

Use cases and deployment

Workhorse: R GUI/IDE and Packages

RKWard: A Comprehensive Graphical User Interface and Integrated Development Environment for Statistical Analysis with R

- Cross-platform, GUI & IDE[1]
- Customizable by plug-ins (JS, R, XML); *rkwarddev* package
- HTML as journal format to make it *shiny*

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[1]Rödiger *et al.* 2012 *J. Stat. Soft.*

Workhorse: R GUI/IDE and Packages

RKWard: A Comprehensive Graphical User Interface and Integrated Development Environment for Statistical Analysis with R
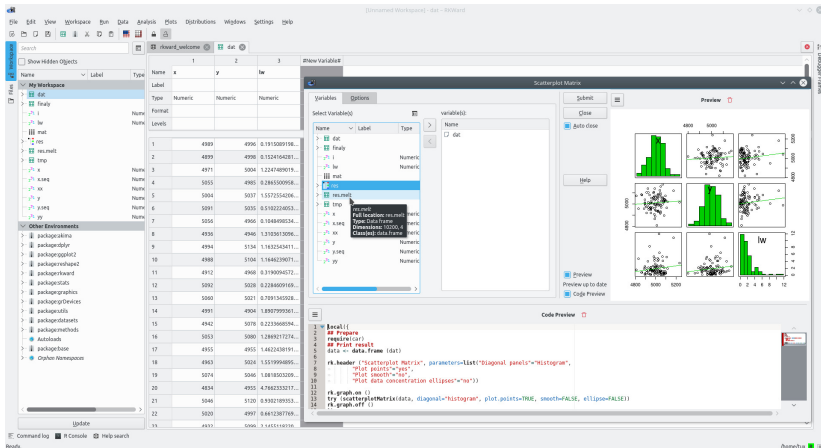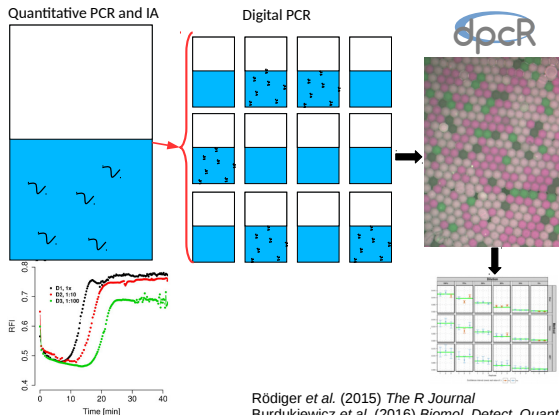
Analysis of digital PCRs

dpcR and *dpcReport*

- Digital PCR is used for absolute quantification of nucleic acids
- *dpcR* package does the applied statistical bioinformatics

Rödiger *et al.* (2015) *The R Journal*
Burdukiewicz *et al.* (2016) *Biomol. Detect. Quant.*

Analysis of digital PCRs

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- Digital PCR is used for absolute quantification of nucleic acids
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https://github.com/devSJR/dpcR_rk

Analysis of digital PCRs

dpcR + rkwarddev + shiny —— Bringing it all together in a GUI

- *rkwarddev* package for simple or advanced GUI definition
- Separation of analysis in dedicated R package, e.g., *dpcR*
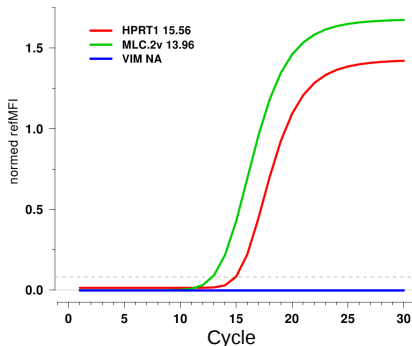- "Recycling" of *shiny* app in **RKWard**

Analysis of Nucleic Acid Amplification Experiments – qPCR

rkwarddev, *shiny* – Bringing it all together in a GUI
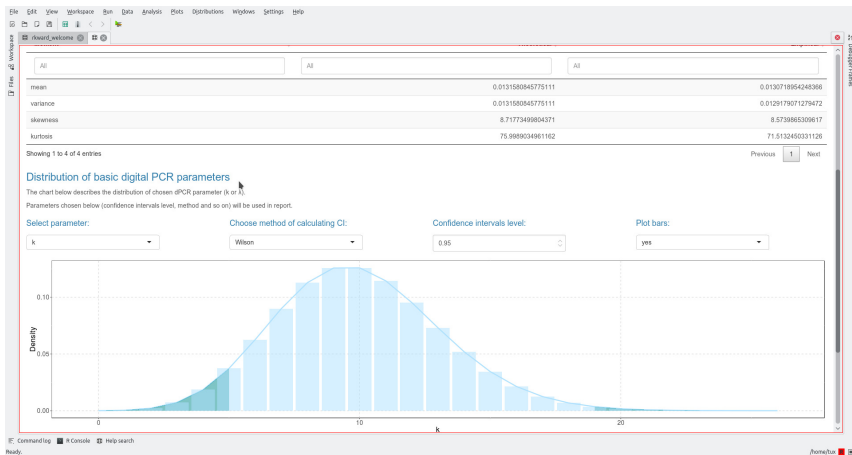
Analysis of Nucleic Acid Amplification Experiments – qPCR

Impact of Smoothing and Filtering on Parameter Estimation in Quantitative DNA Amplification Experiments

- Most commercial software performs the analysis in a black box
- Information about the preprocessing is in most cases not available or sufficient
- There is no means to track numerical errors

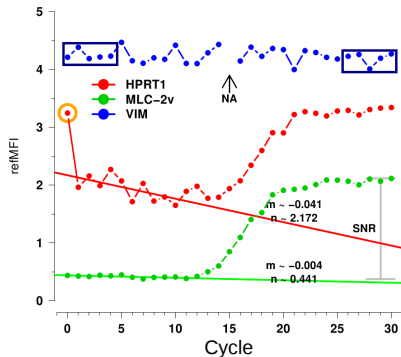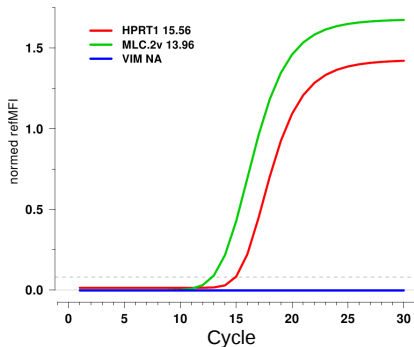Rödiger *et al.* (2013) *The R Journal*
Rödiger *et al.* (2015) *The R Journal*
Rödiger *et al.* (2015) *Clin. Chem.*
Rödiger *et al.* (2015) *Bioinformatics (Oxf)*

Analysis of Nucleic Acid Amplification Experiments – qPCR

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Analysis of Nucleic Acid Amplification Experiments – qPCR

Impact of Smoothing and Filtering on Parameter Estimation in Quantitative DNA Amplification Experiments

The reps384 dataset was used to depict the signal variation of 380 replicated qPCR reactions. After baselining the data with a linear model from cycles 2–9, the qPCR curves still display significant variation in plateau fluorescence (5000–10500). The baseline region (cycle ~1–12) displays high noise scatter, whereas the exponential region (cycle ~13–21) is less noisy with $\sigma(Cq) = 0.115$ at $Fq = 250$.
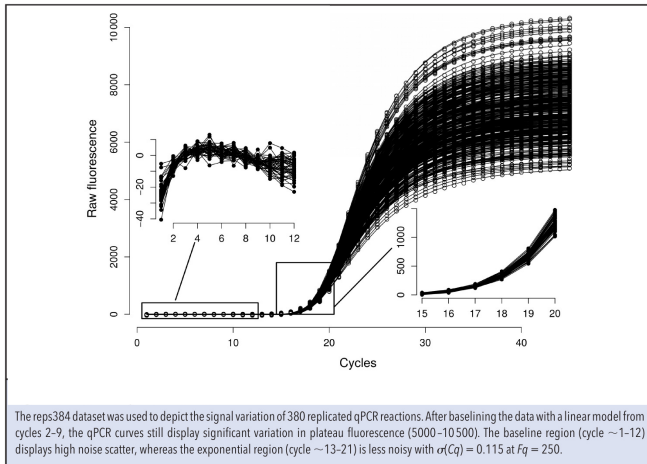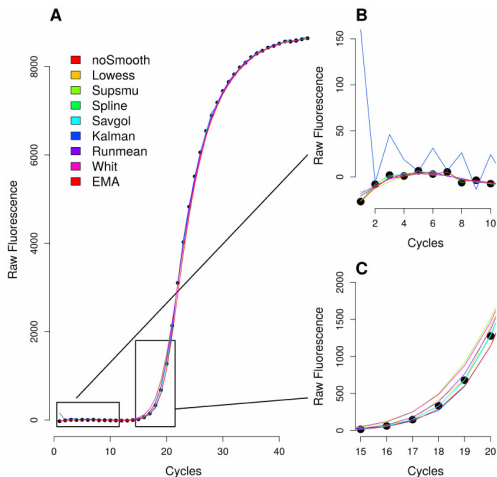
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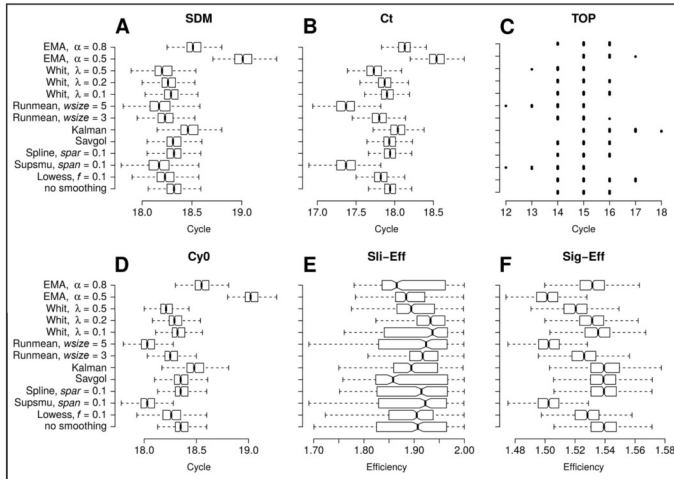
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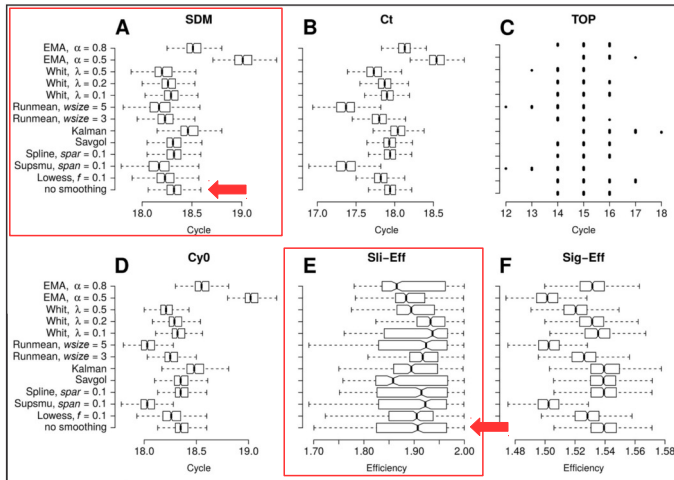
Analysis of Nucleic Acid Amplification Experiments – qPCR

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