GS01 0163
Analysis of Microarray Data

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Lecture 6: Sweave, More on R, and Affy. Arrays

- The Reproducibility Problem
- Installing TEX
- Introductory LATEX
- Writing Documented R Analyses
- R Revisited: Beyond the Matrix
- Reading Data Into R
- Obtaining extra R packages
- Bioconductor Packages

The Reproducibility Problem

1. Researcher contacts analyst: "I just read this interesting paper. Can you perform the same analysis on my data?"

2. Analyst reads paper. Finds algorithms described by biologists in English sentences that occupy minimal amount of space in the methods section.

3. Analyst gets public data from the paper. Takes wild guesses at actual algorithms and parameters. Is unable to reproduce reported results.

4. Analyst considers switching to career like bicycle repair, where reproducibility is less of an issue.

Alternate Forms of the Same Problem

1. Remember that microarray analysis you did six months ago? We ran a few more arrays. Can you add them to the project and repeat the same analysis?

2. The statistical analyst who looked at the data I generated previously is no longer available. Can you get someone else to analyze my new data set using the same methods (and thus producing a report I can expect to understand)?

3. Please write/edit the methods sections for the abstract/paper/grant proposal I am submitting based on the analysis you did several months ago.

The Code/Documentation Mismatch

Most of our analyses are performed using R. We can usually find an R workspace in a directory containing the raw data, the report, and one or more R scripts.

There is no guarantee that the objects in the R workspace were actually produced by those R scripts. Nor that the report matches the code. Nor the R objects.

Because R is interactive, unknown commands could have been typed at the command line, or the commands in the script could have been cut-n-pasted in a different order.

This problem is even worse if the software used for the analysis has a fancy modern GUI. It is impossible to document how you used the GUI in such a way that someone else could produce the exact same results—on the same data—six months later.

The Solution: Sweave

Literate programming is an approach that embeds small program fragments within an otherwise high-quality document.

Sweave is a literate programming framework for R.

This talk was prepared using Sweave. So was this standard report.

$$\text{Sweave} = \text{R} + \LaTeX.$$

Once you know both R and LaTeX, then the thirty-second version of this talk takes only two slides.

First, we take a few moments to learn LaTeX. (You already know R.)

LaTeX Document Preparation System

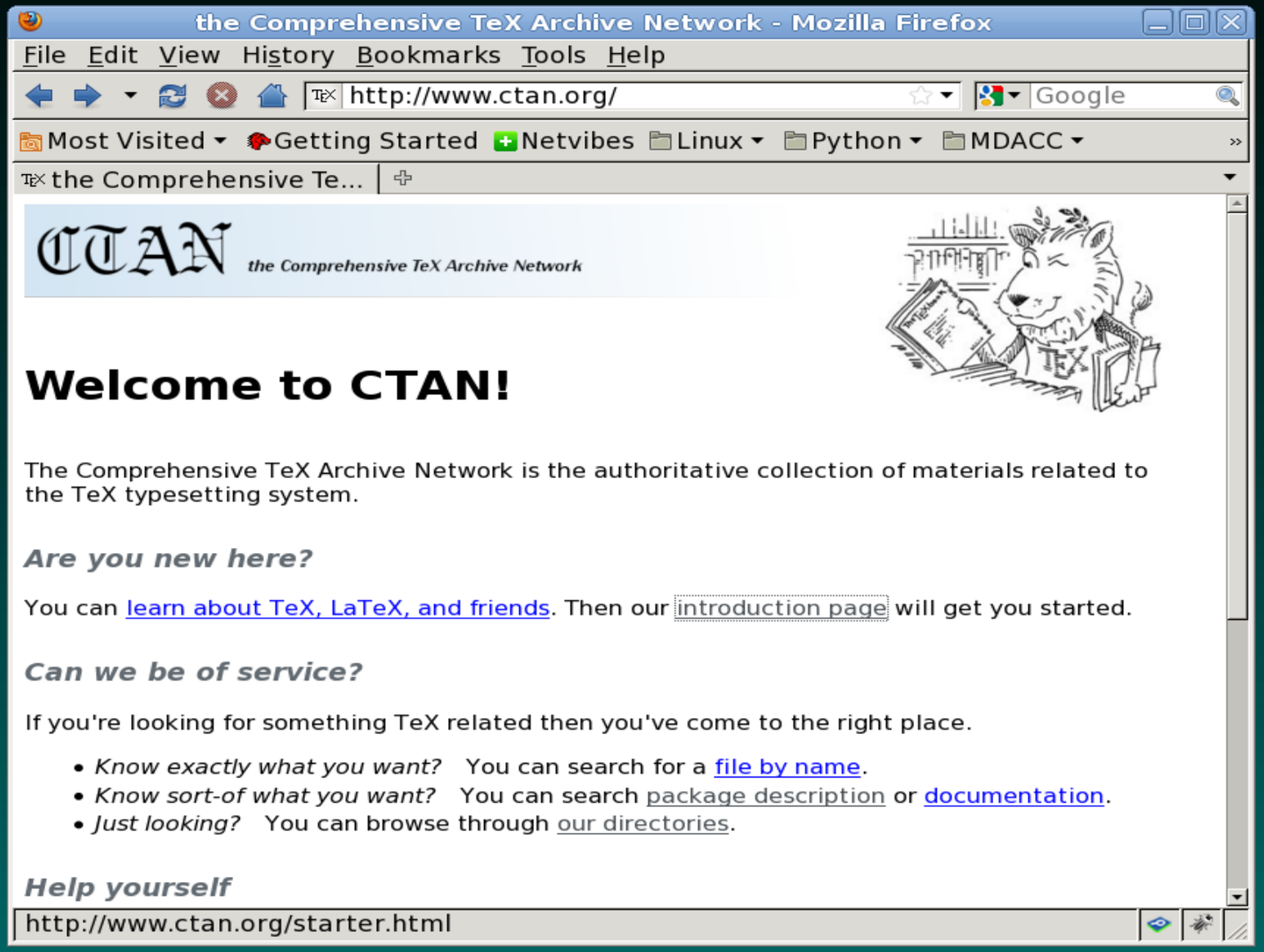
LaTeX is a document preparation system for high-quality typesetting.

LaTeX is not a word processor.

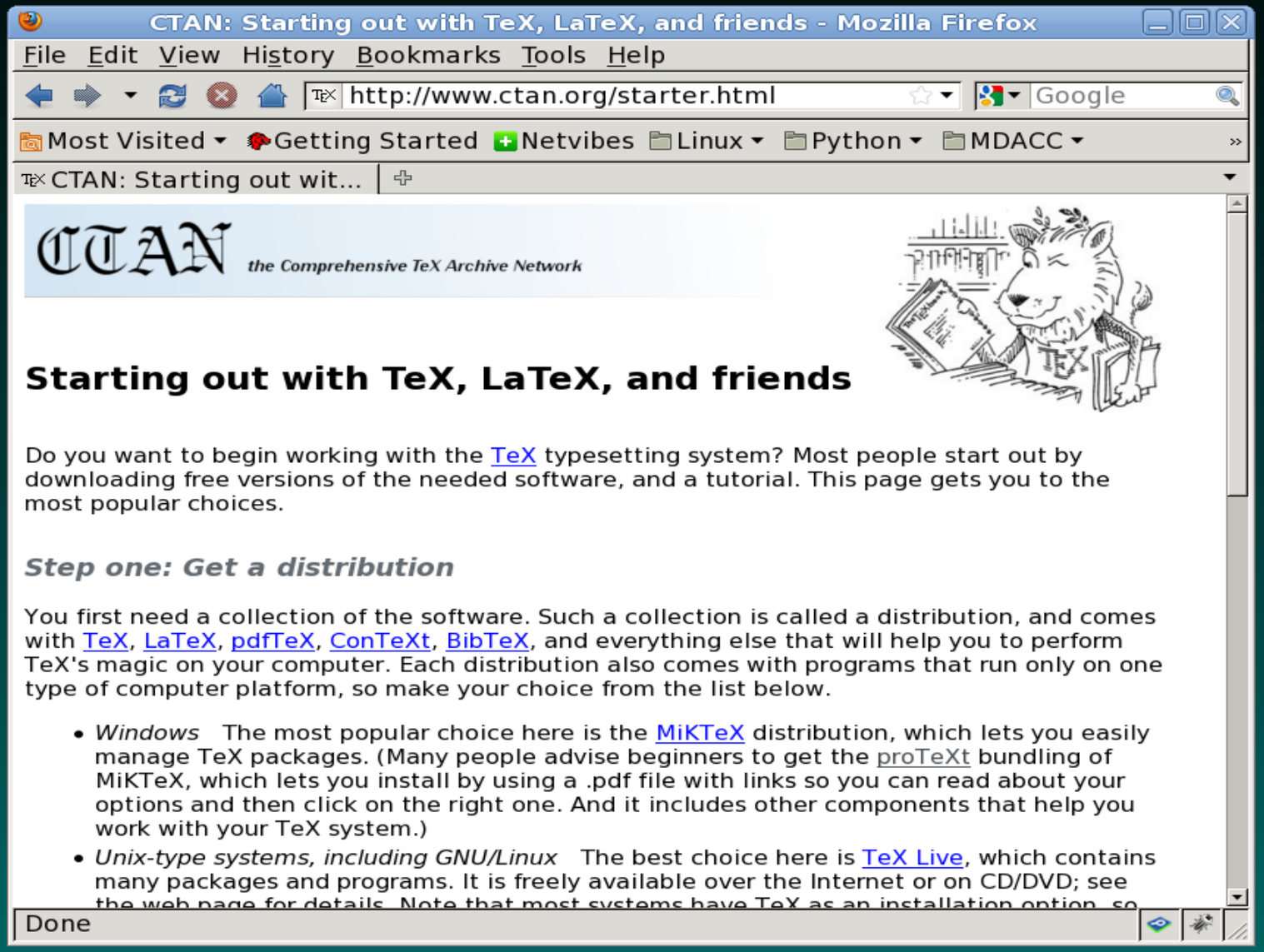
LaTeX separates document content (written by author) from layout (written by document designers).

You can read more about LaTeX at the website for the Comprehensive Tex Archive Network (CTAN): http://www.ctan.org.

CTAN Website



CTAN Starting Out



Installing TEX

The standard version of TEX or LATEX for Windows is MiKTeX, which is available at http://www.miktex.org. The current version is 2.8.

Follow the MiKTeX link, and then choose Download MiKTeX 2.8 from the panel on the left.

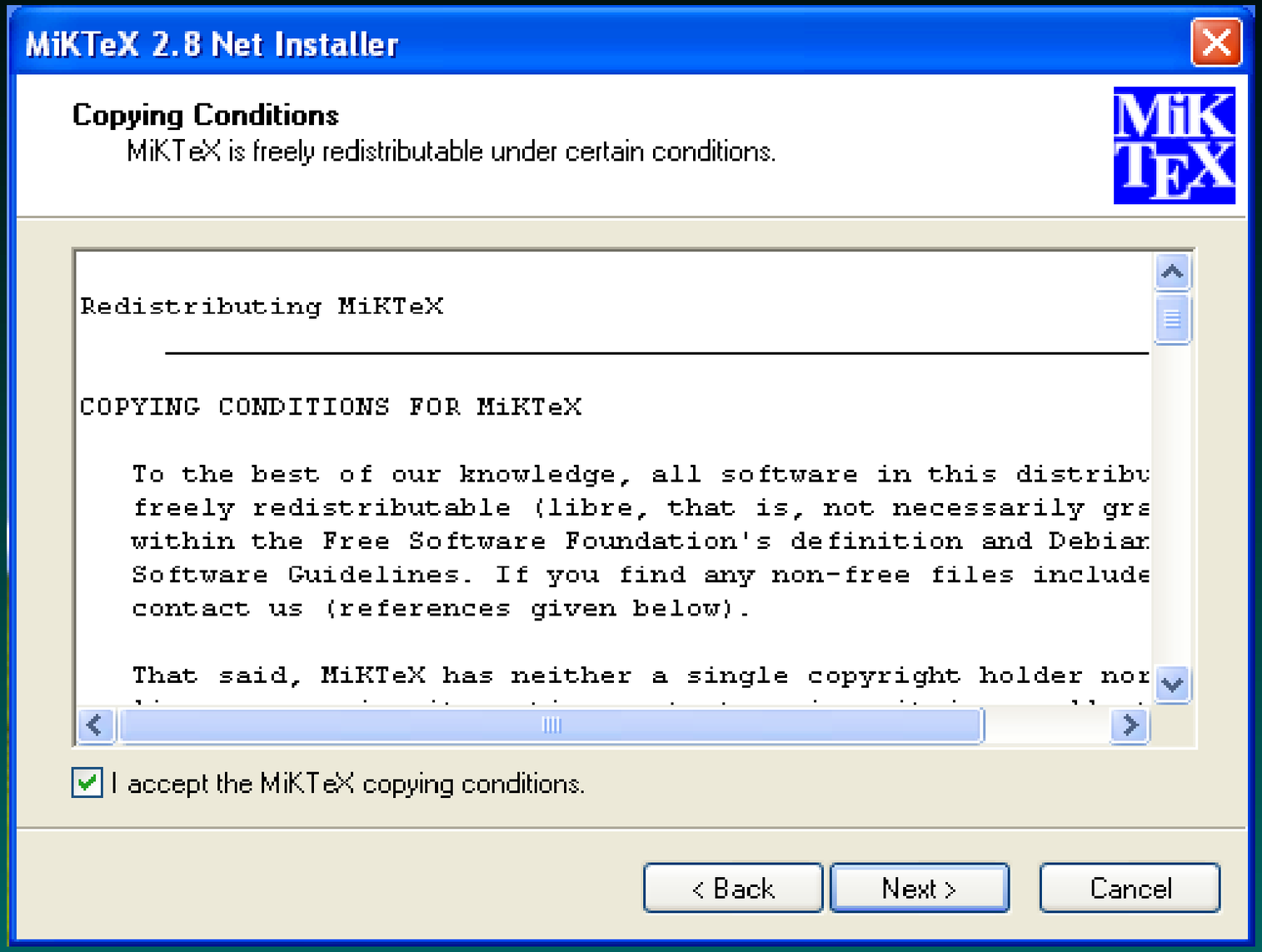
I used the "MikTeX 2.8 Net Installer" because it's relatively small (3 MB), but ran into some problems adding additional packages.

It might be better to download the "Basic MiKTeX 2.8" installer (92 MB).

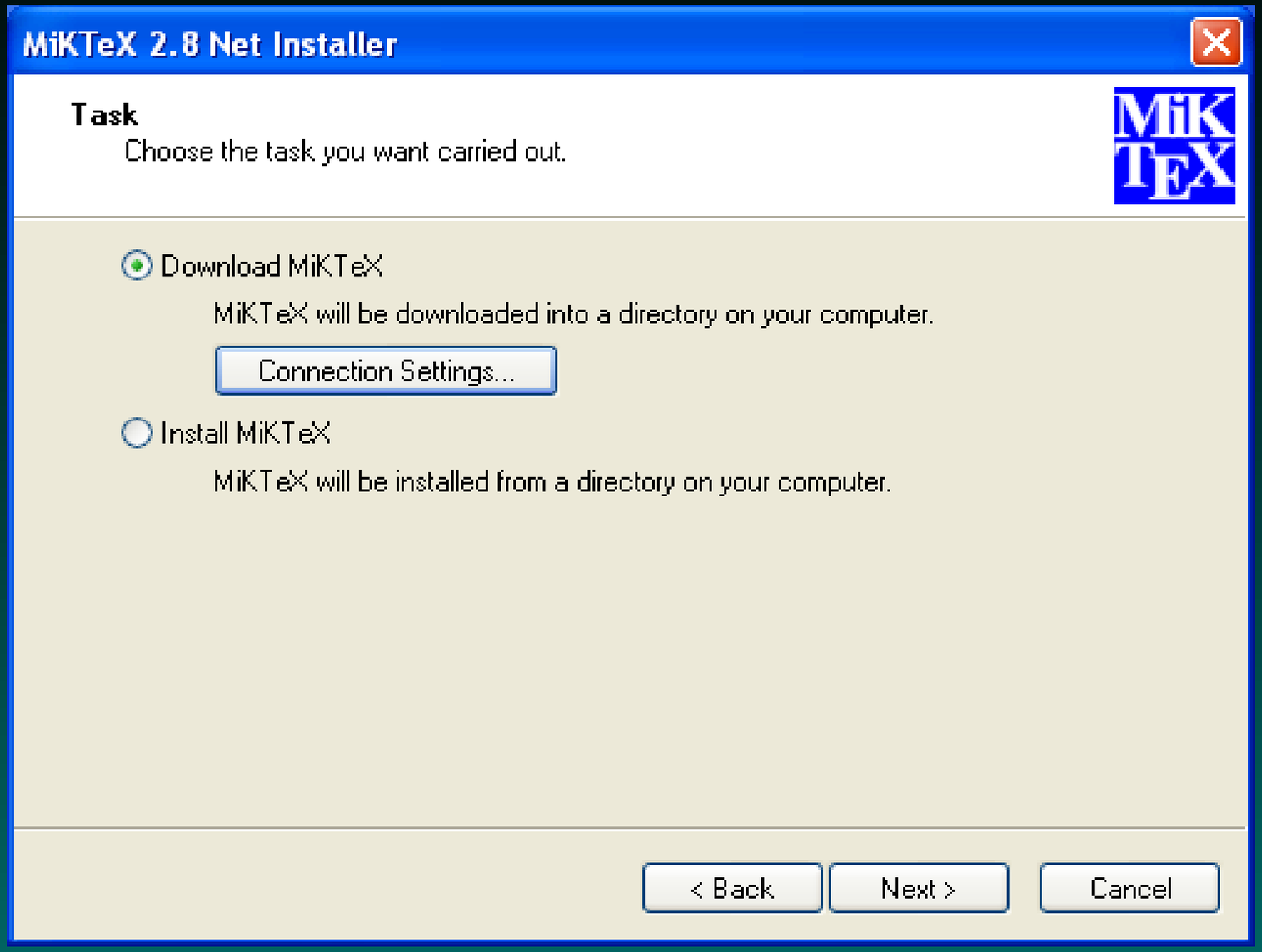
Keep track of where you save this file (your desktop will work just fine) and then double-click on the resulting icon to start the installation.

CTAN states that the standard version of LATEX for Macintosh computers is MacTeX. (Since I have never installed this version, you will have to figure out how to install it yourself ....)

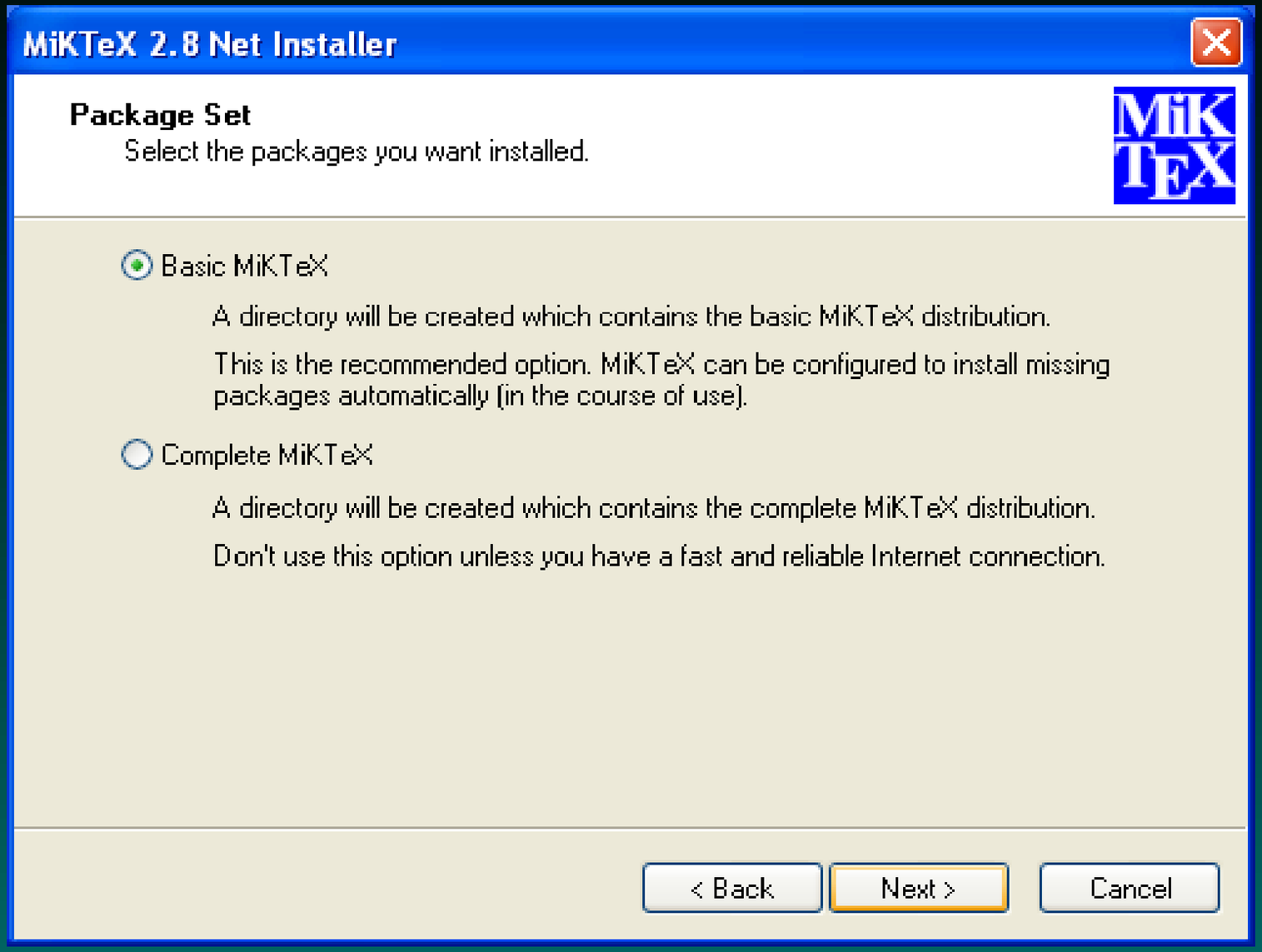
The MiKTeX Installer: License



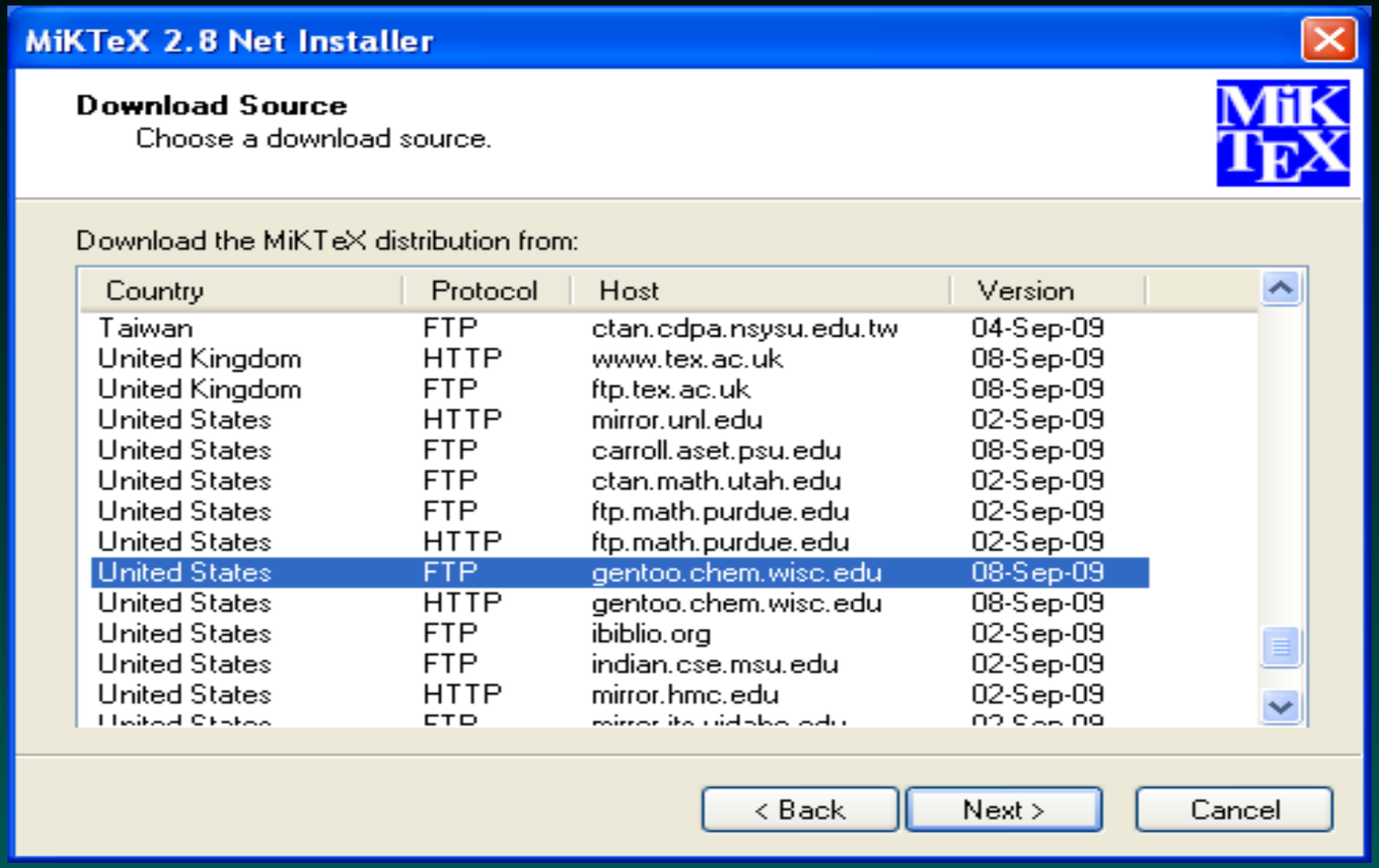
The MiKTeX Installer: Download



The MiKTeX Installer: Package Set

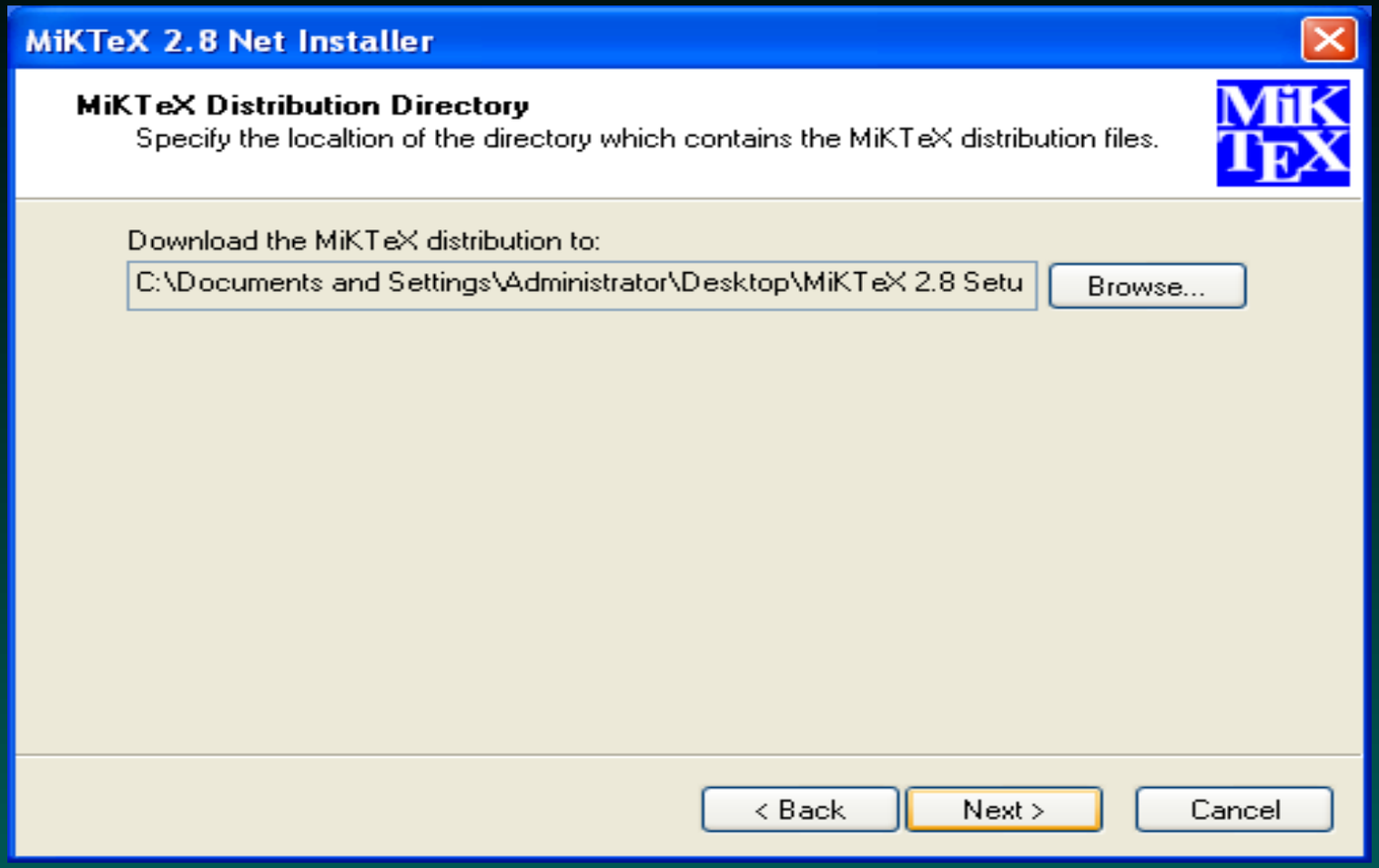


The MiKTeX Installer: Download Source



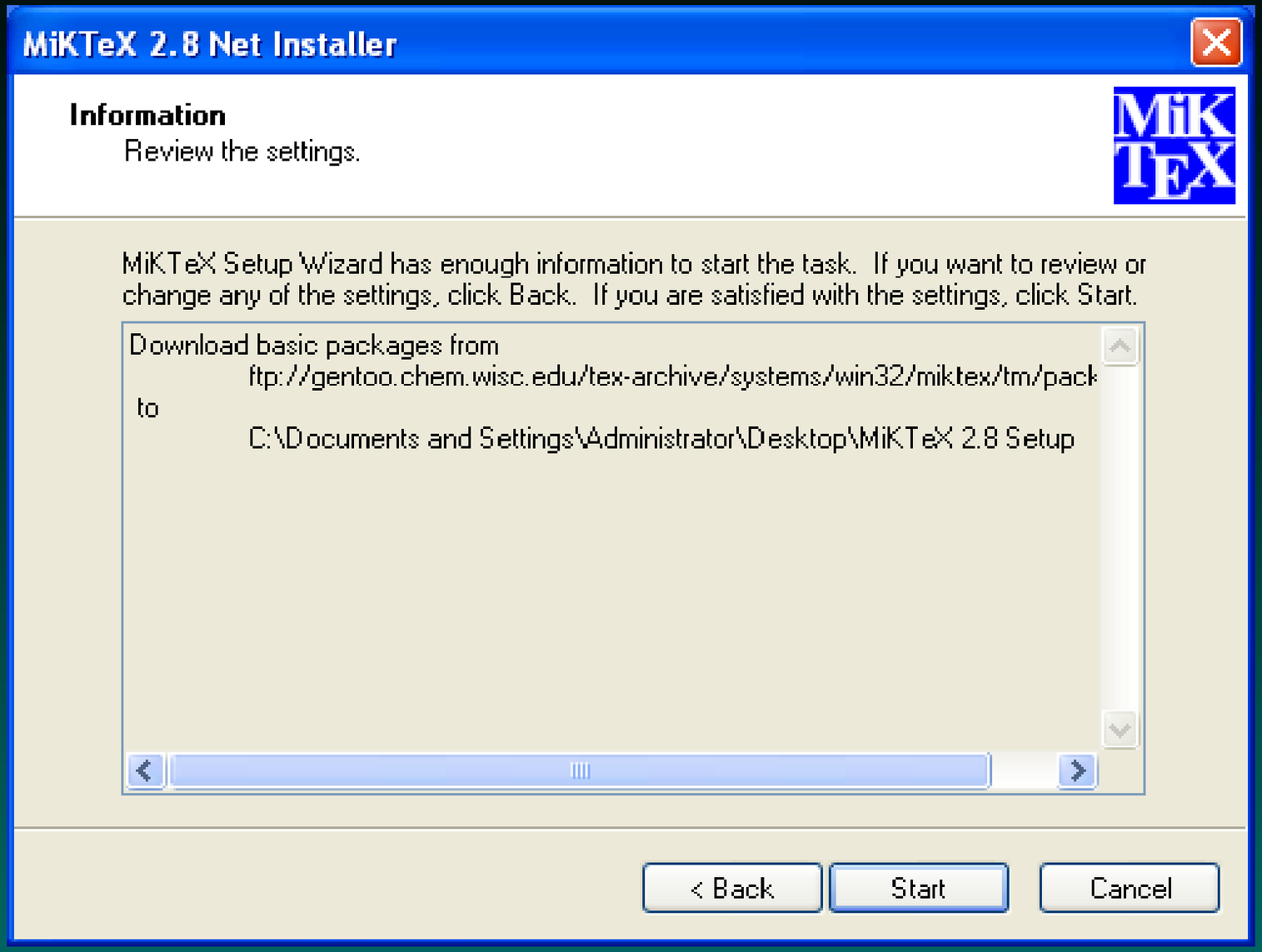
Choose an up-to-date US mirror.

The MiKTeX Installer: Distribution Directory

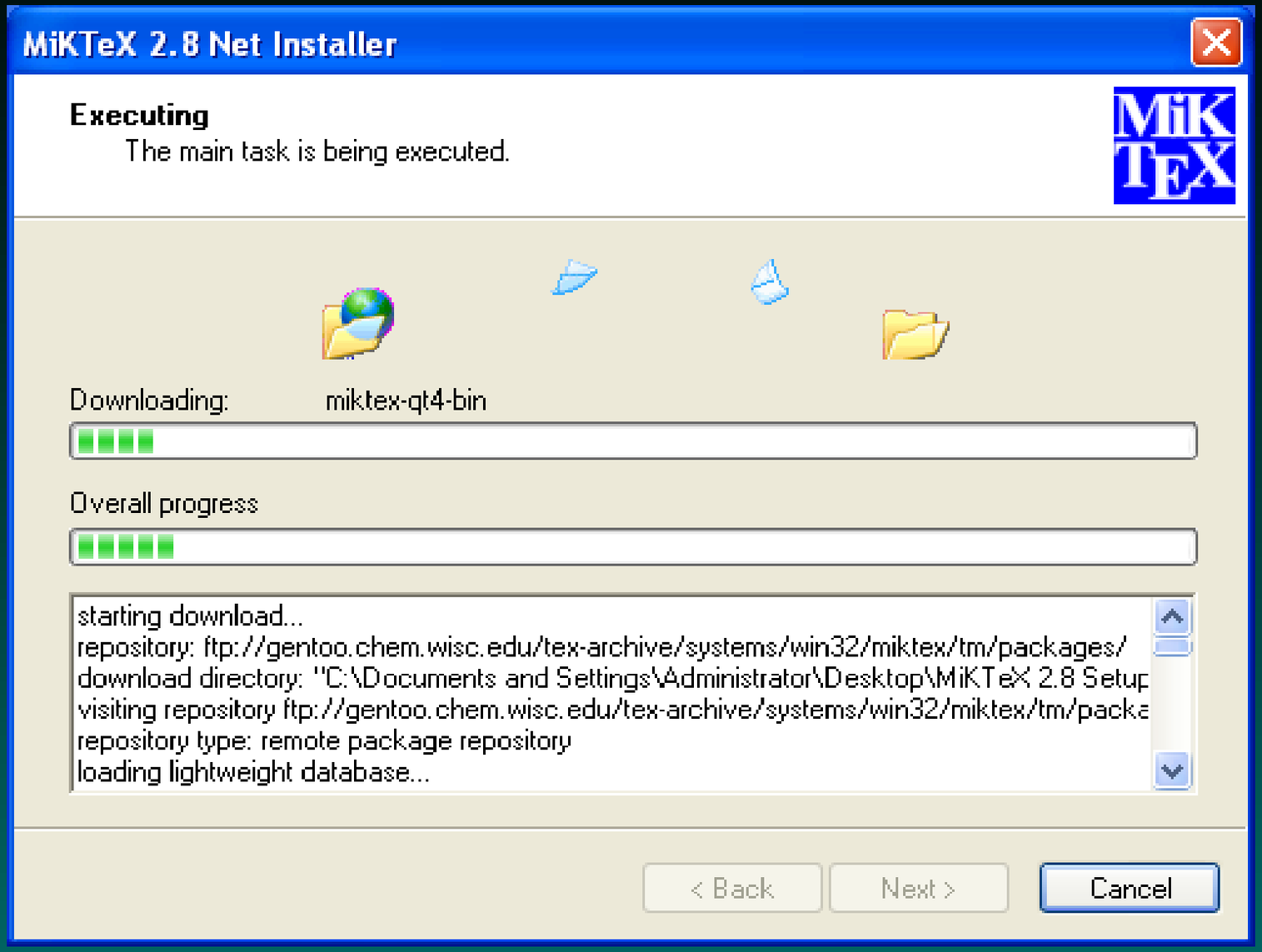


Remember this directory for later.

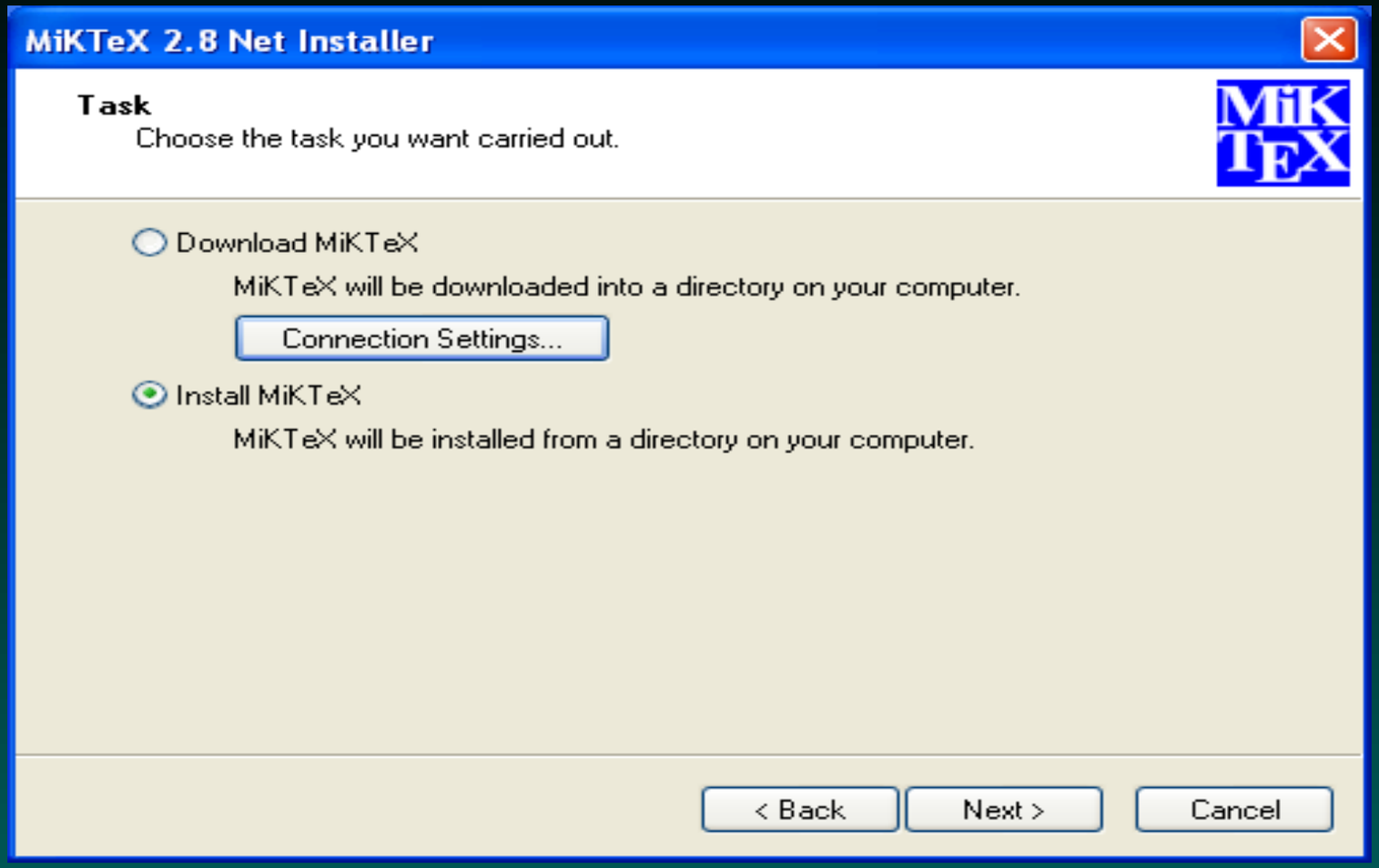
The MiKTeX Installer: Review Settings



The MiKTeX Installer: Downloading

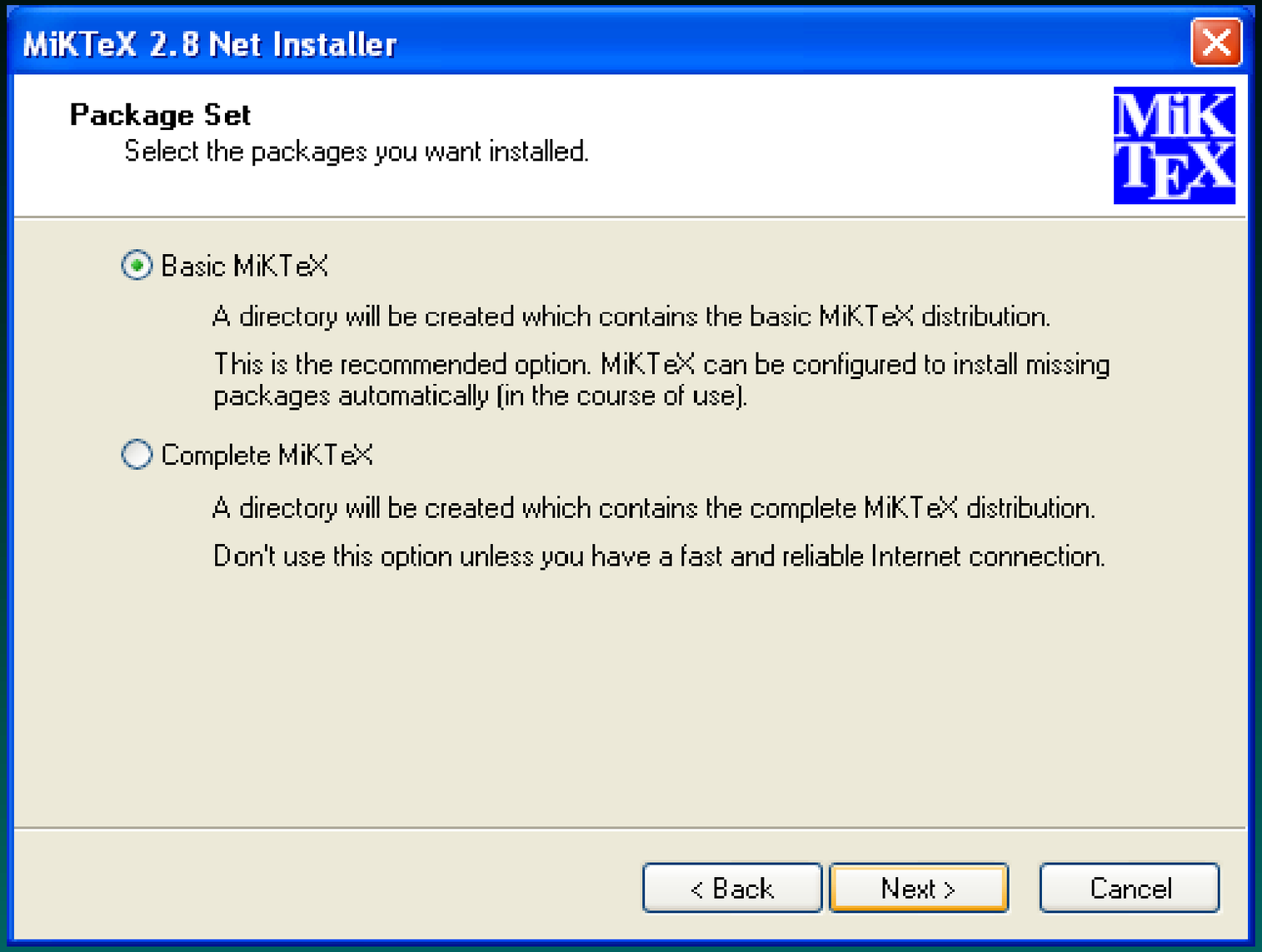


The MiKTeX Installer: Install

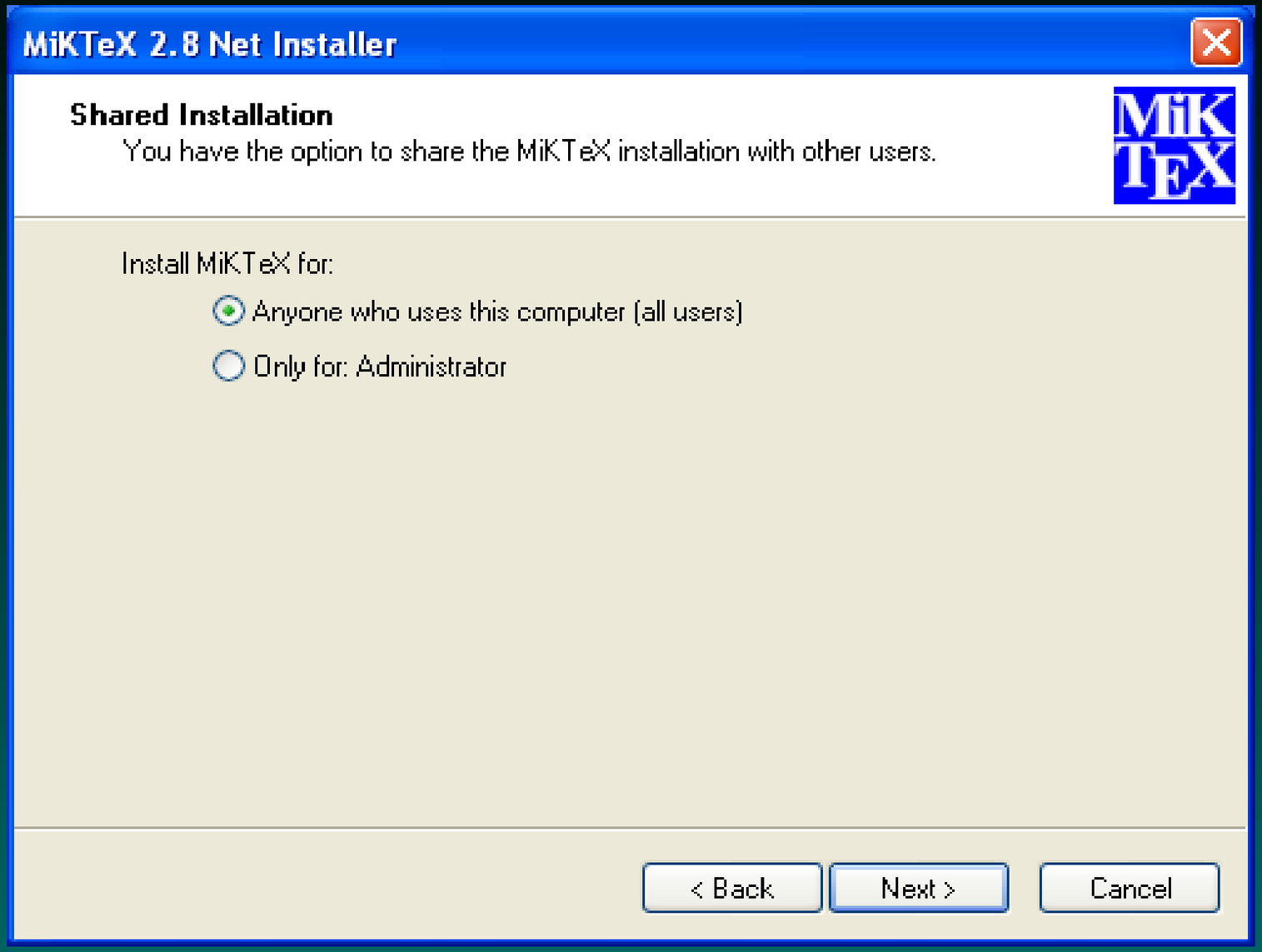


Rerun the installer, but this time choose install.

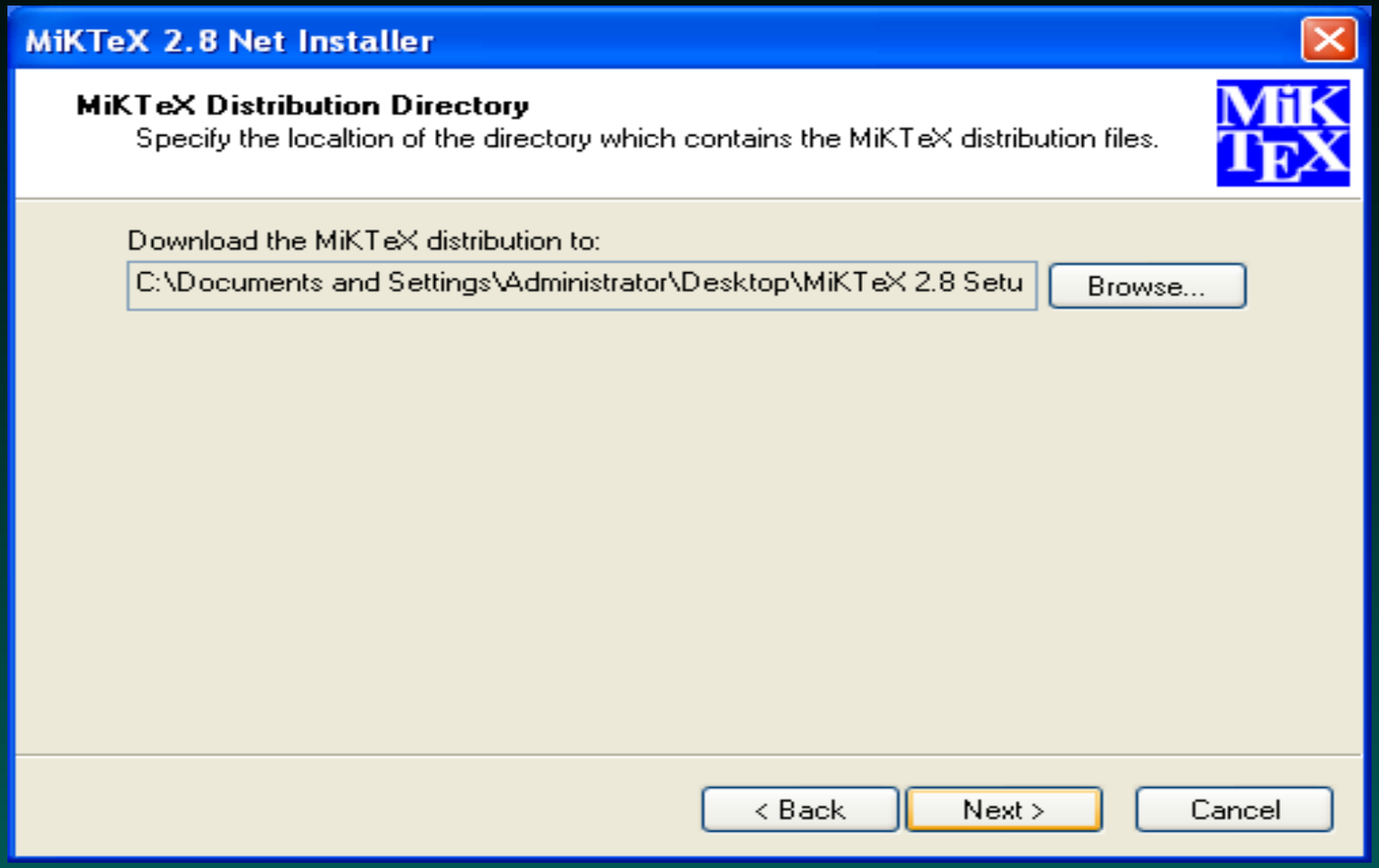
The MiKTeX Installer: Package Set



The MiKTeX Installer: Shared Install

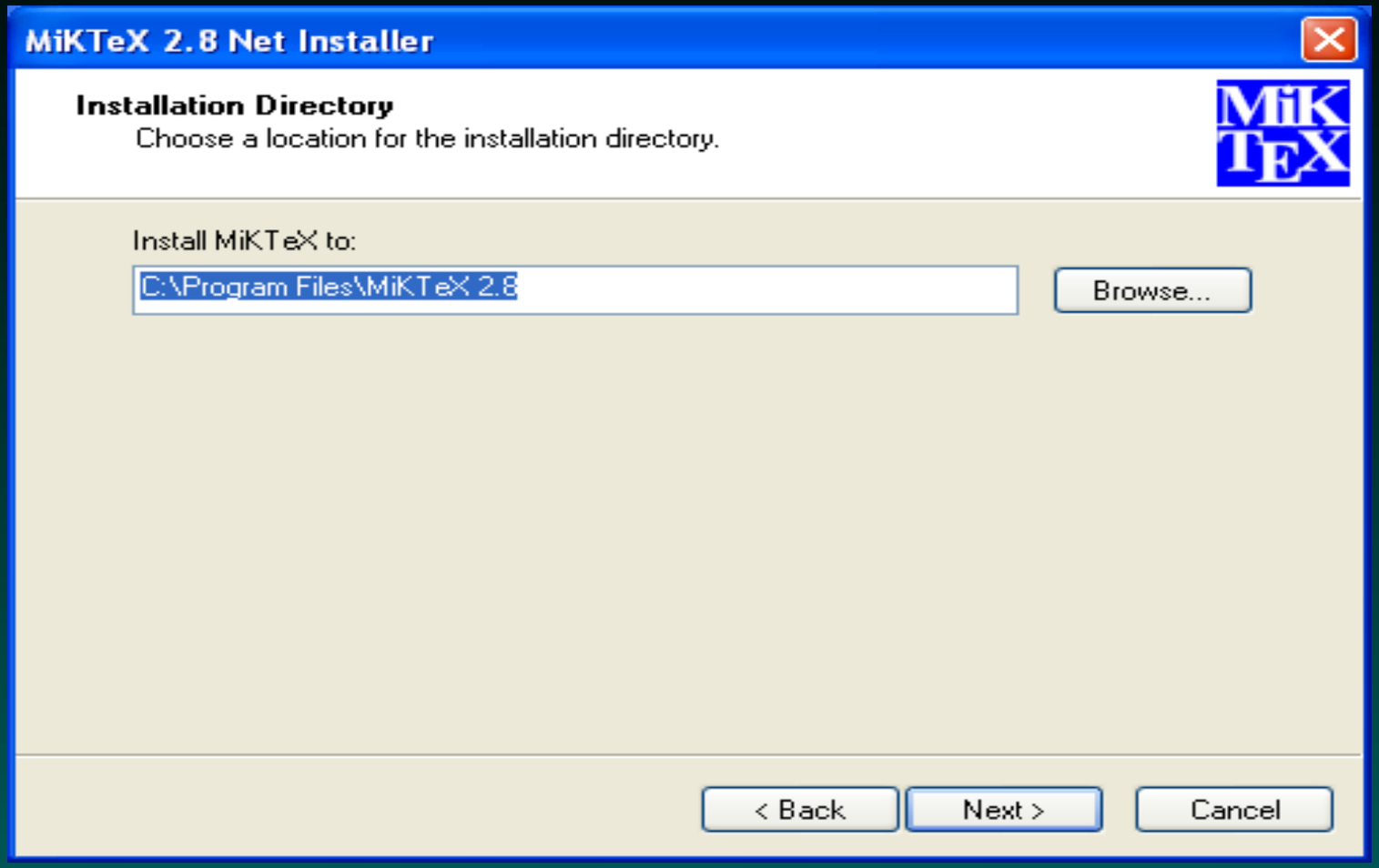


The MiKTeX Installer: Distribution Directory



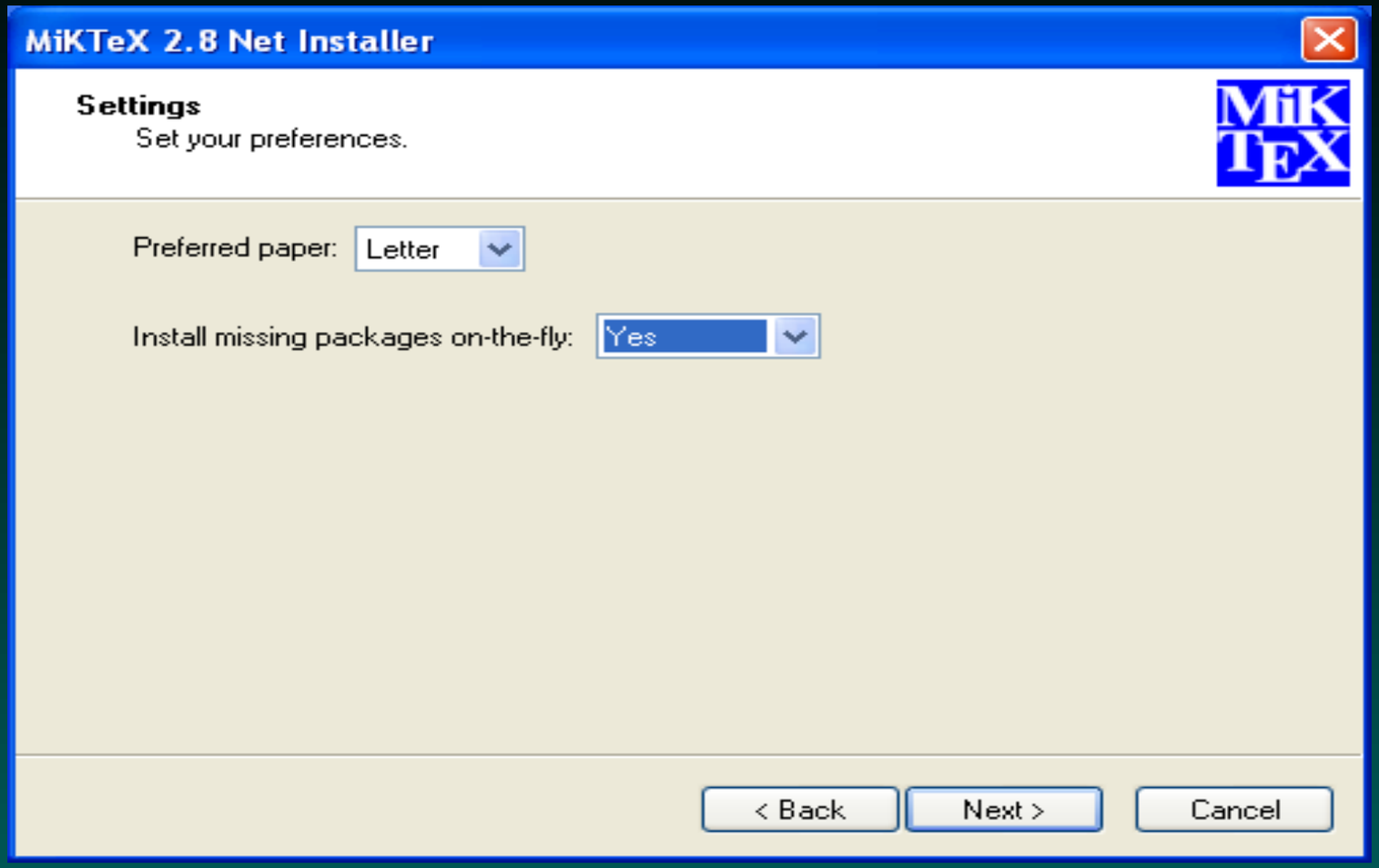
Use the directory you downloaded the distribution to earlier.

The MiKTeX Installer: Installation Directory



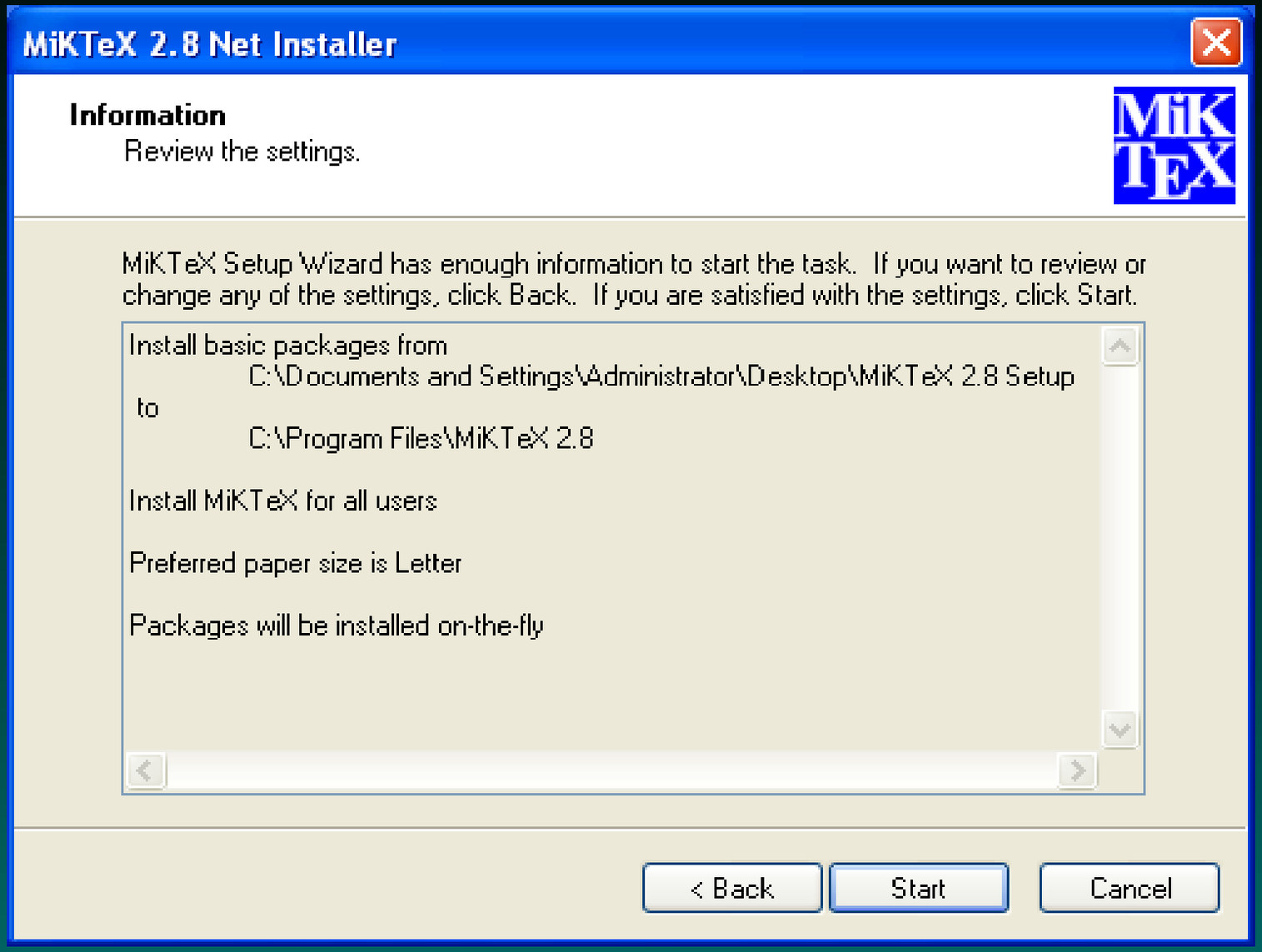
Best if you can avoid spaces in this path. Remember this path.

The MiKTeX Installer: Default Settings

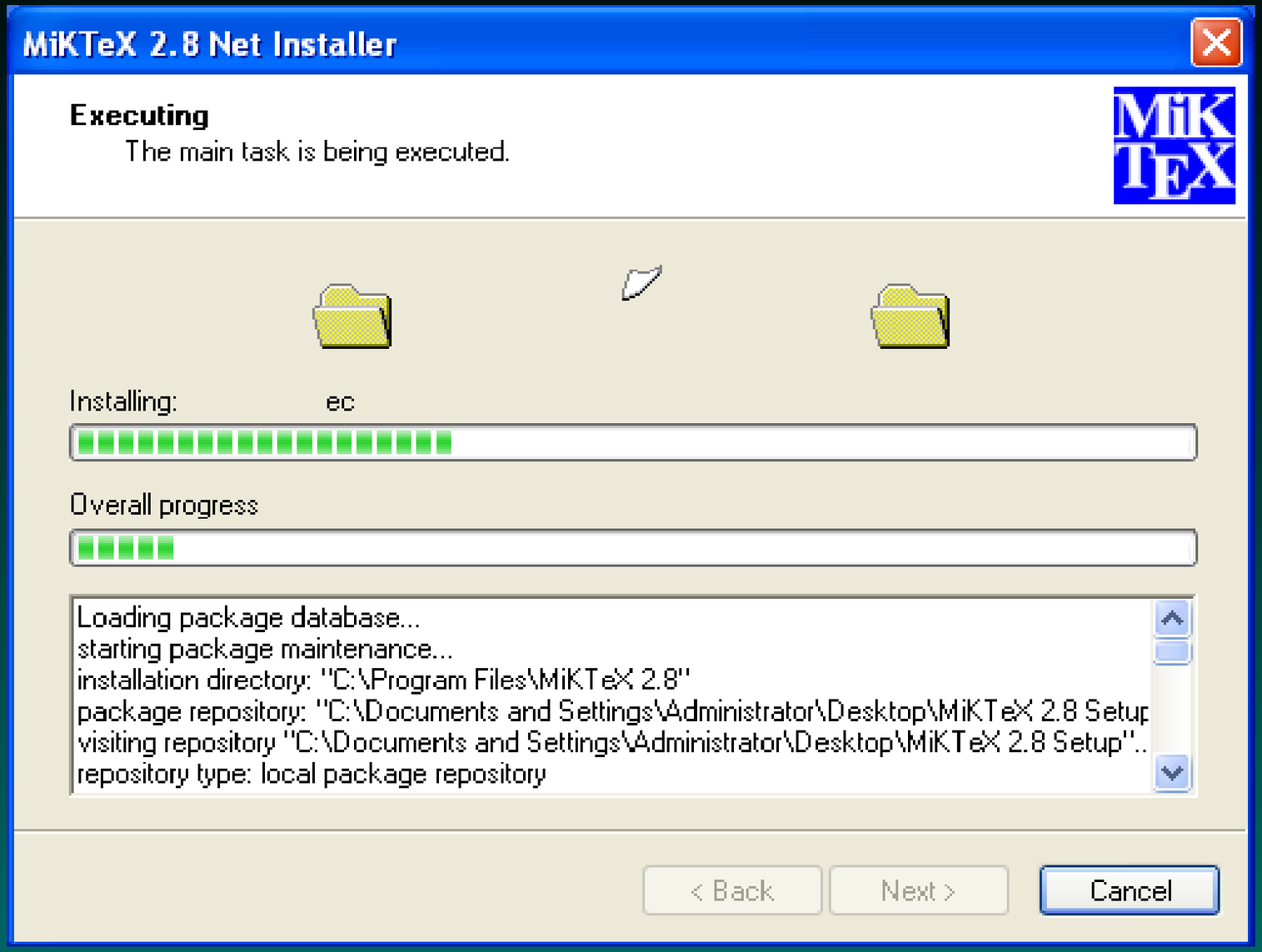


Make sure you change these settings from the defaults.

The MiKTeX Installer: Install Review



The MiKTeX Installer: Installing at last



Ghostscript and GSview

Goto http://www.ghostscript.com and download `gs870w32.exe` from either `cs.wisc.edu` or `sourceforge.net`.

Follow the links at the bottom of the ghostscript page to download GSview 4.9 (`gsv49w32.exe`).

These are both straight-forward installs ....

TeXnicCenter

Optionally, you can download and install TeXnicCenter.

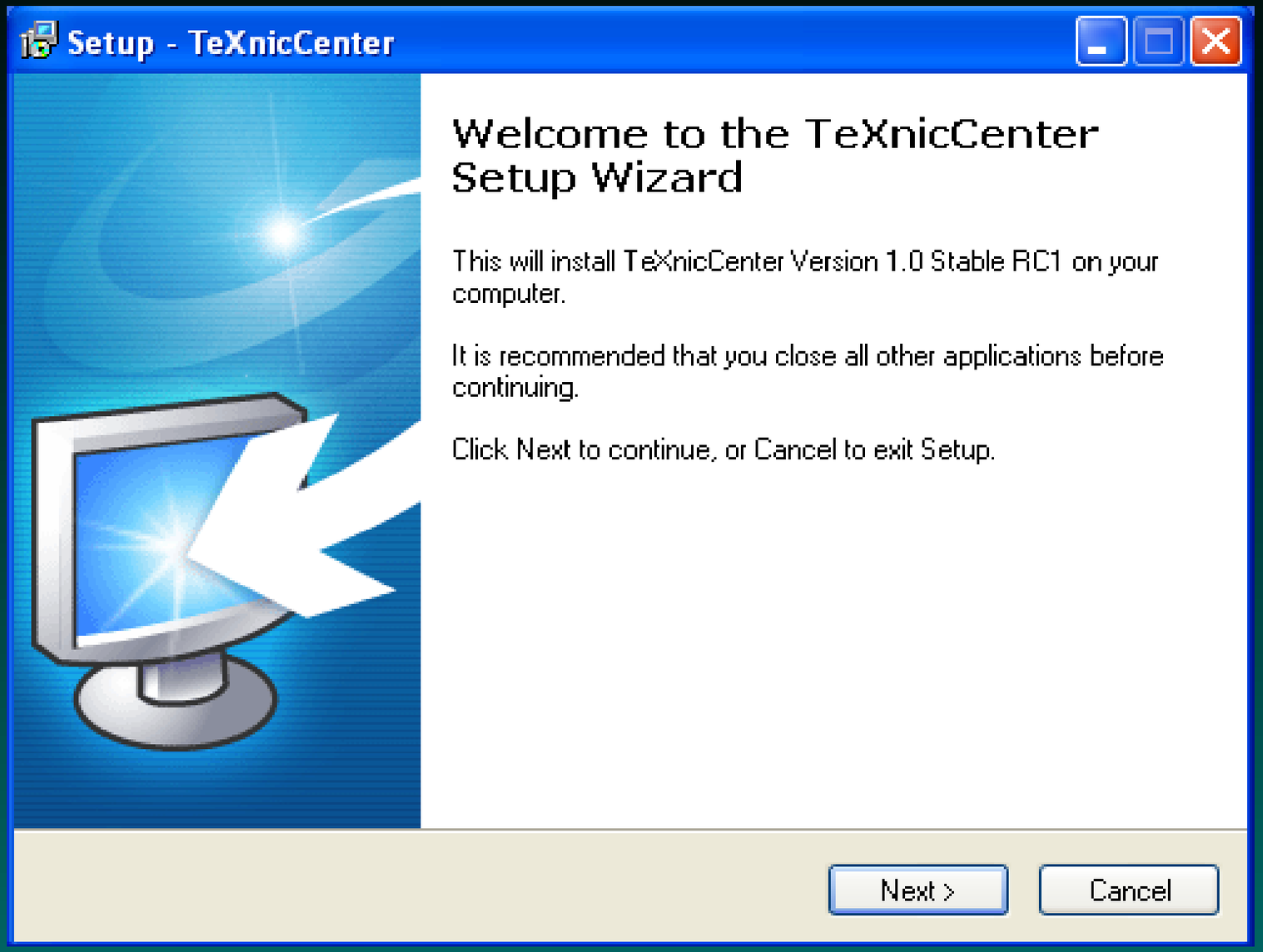
TeXnicCenter is an integrated environment for creating LaTeX documents using Microsoft Windows.

- LaTeX specific editor with syntax highlighting, bracket matching, etc.
- Buttons for inserting predefined LaTeX snippets.
- Buttons for building and viewing document.

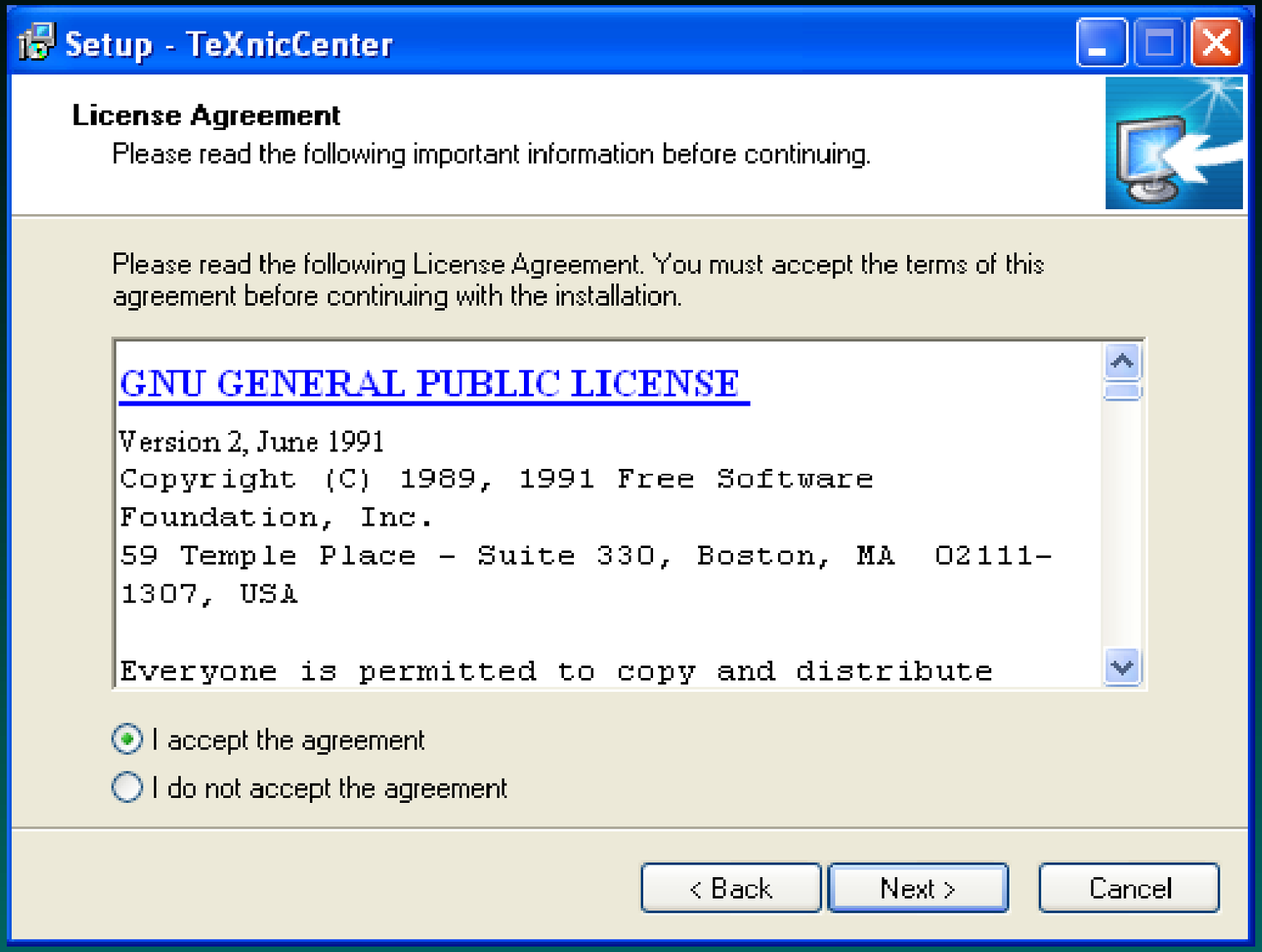
Unfortunately, TeXnicCenter does not know about Sweave, so we will still need to do some stuff manually.

Goto http://www.texniccenter.org/ and follow the links to download the TeXnicCenter installer.

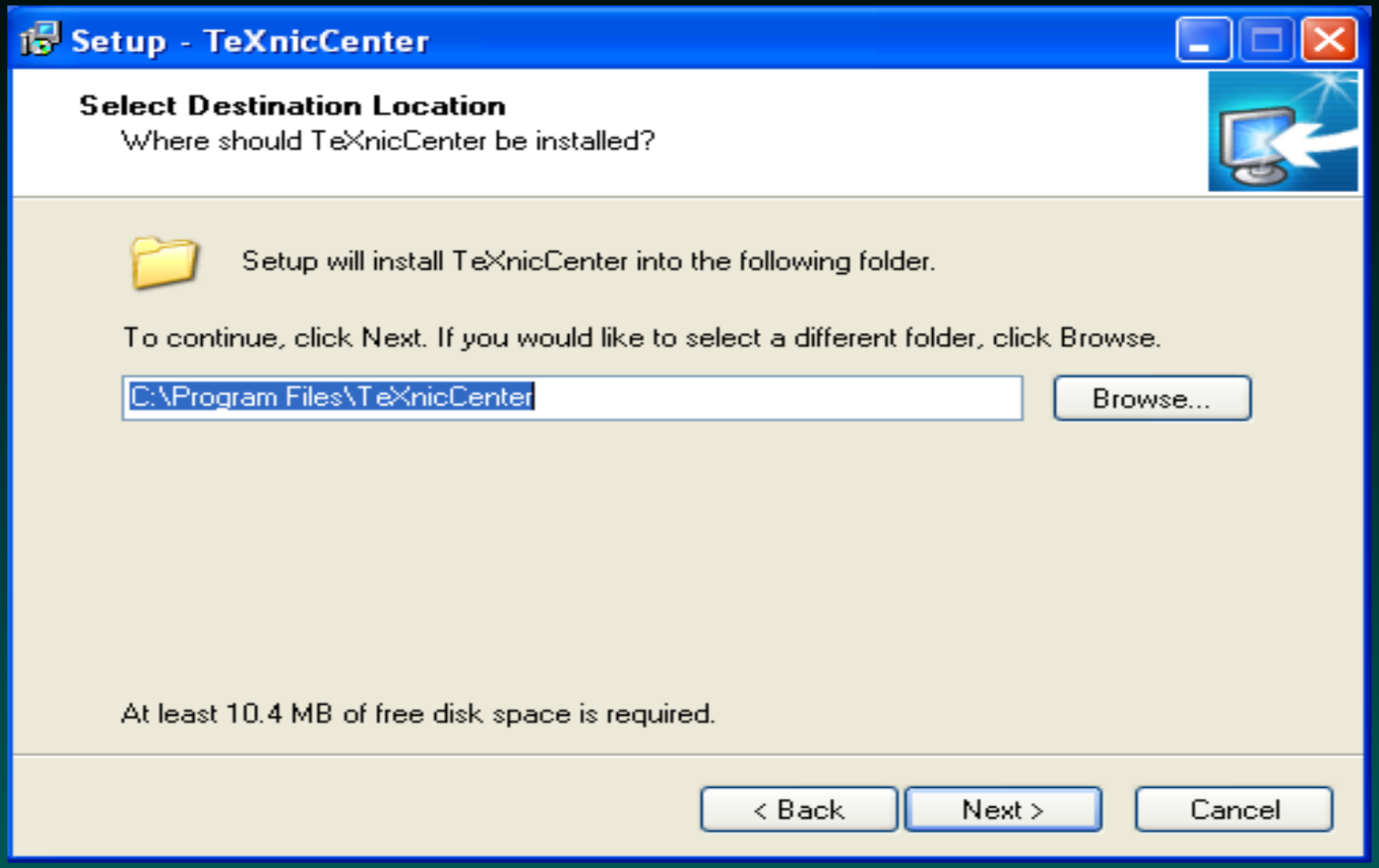
The TeXnicCenter Installer: Welcome



The TeXnicCenter Installer: License

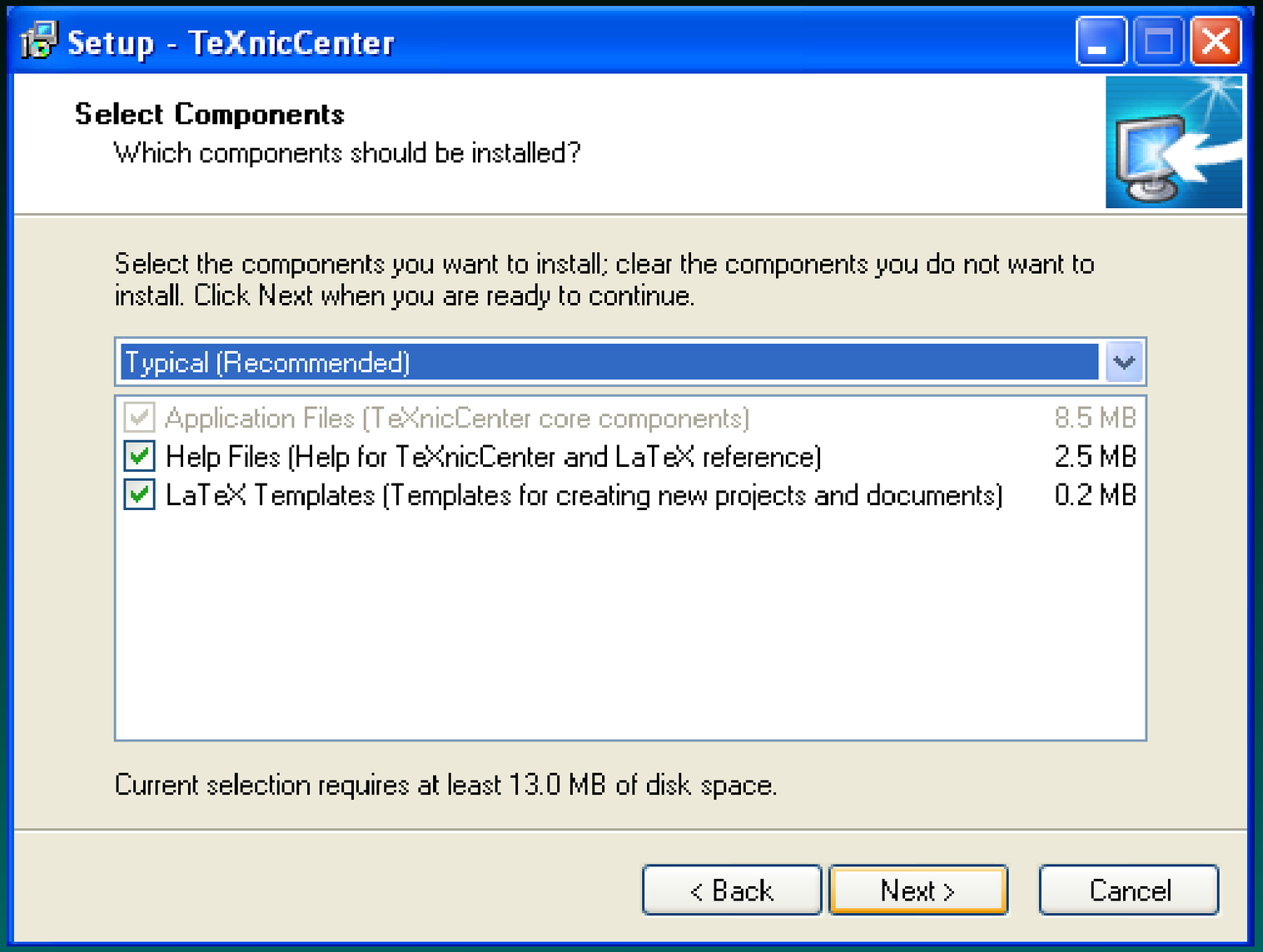


The TeXnicCenter Installer: Location

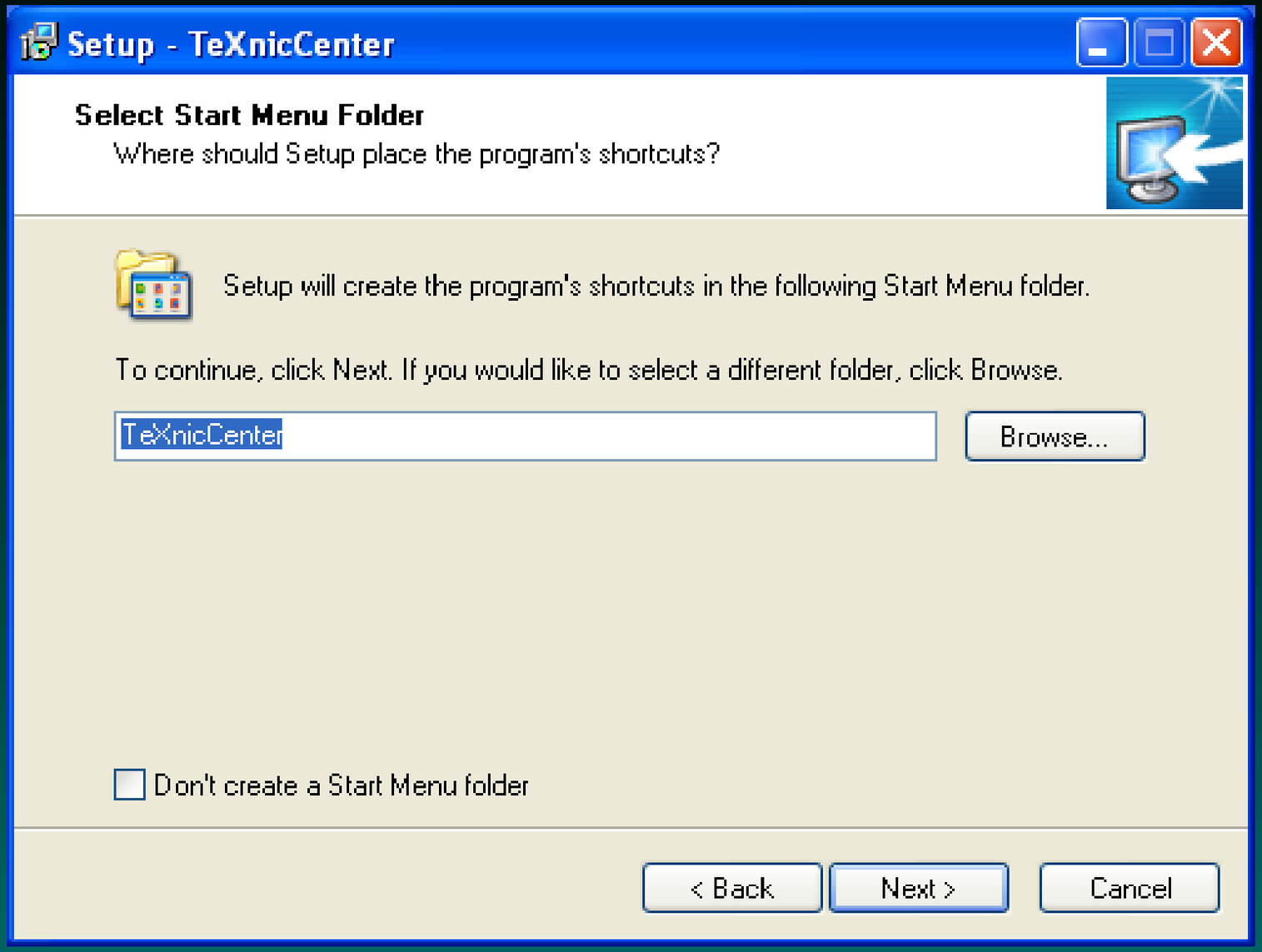


Best to avoid spaces in this path.

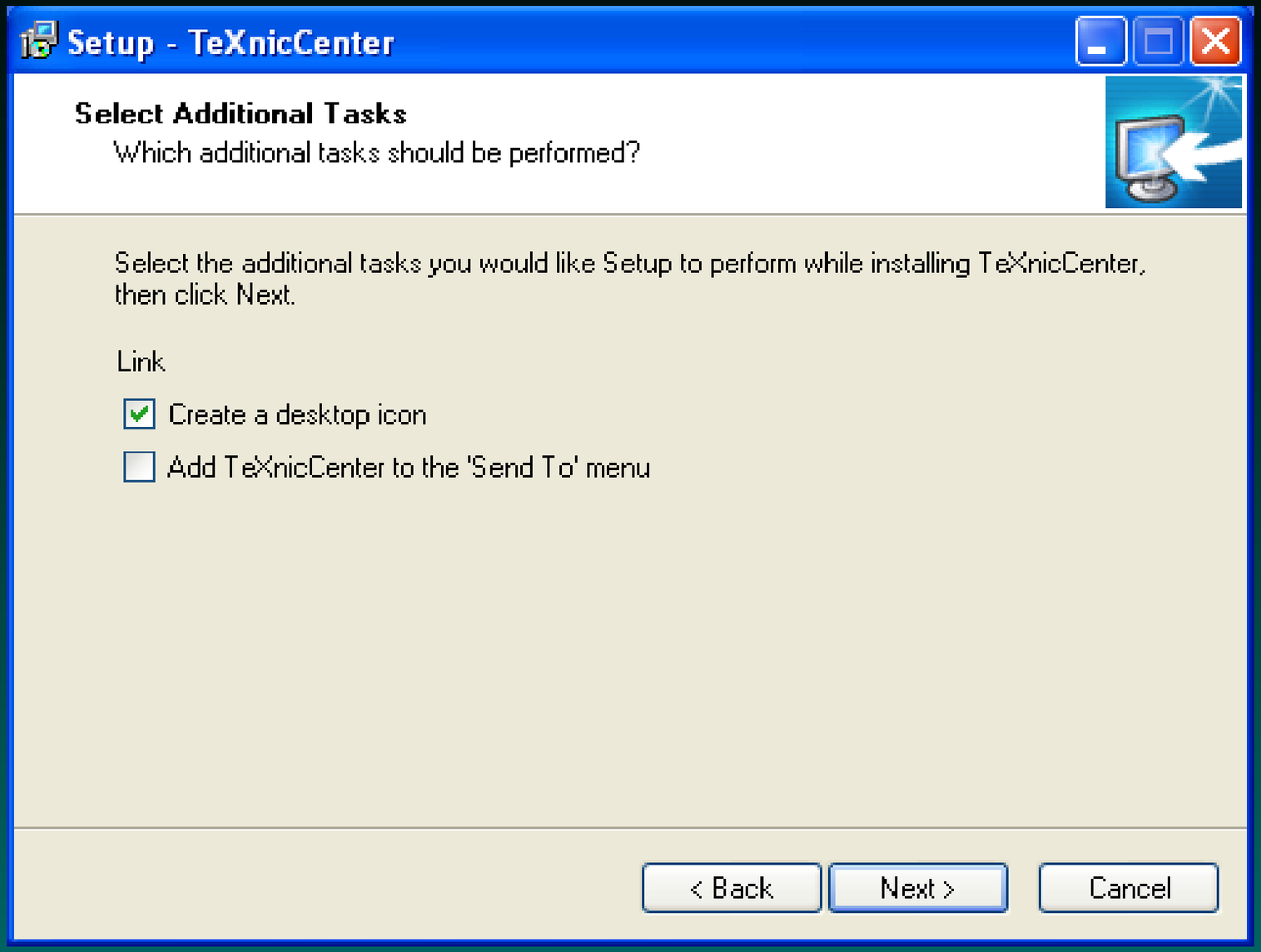
The TeXnicCenter Installer: Components



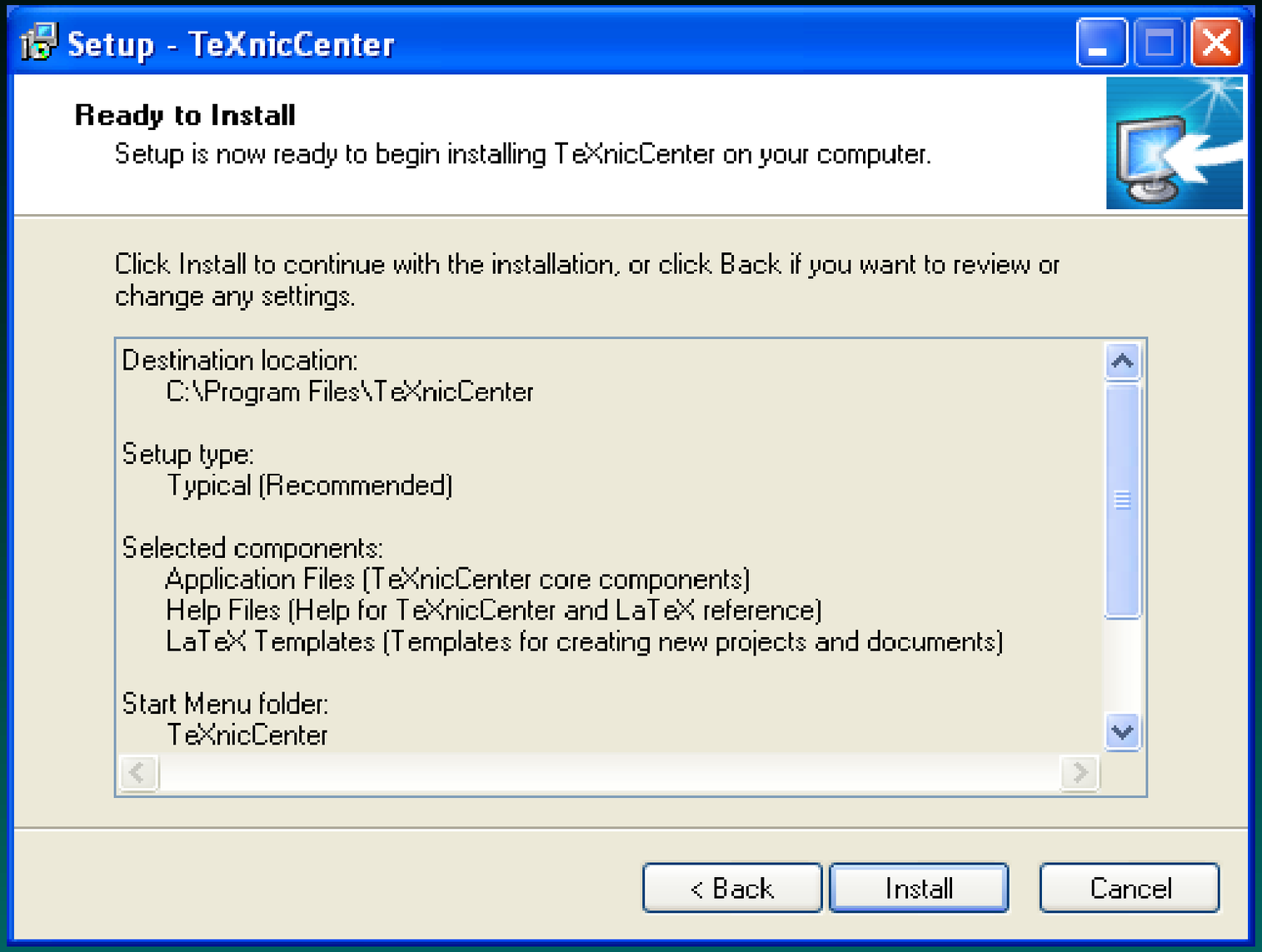
The TeXnicCenter Installer: Start Menu



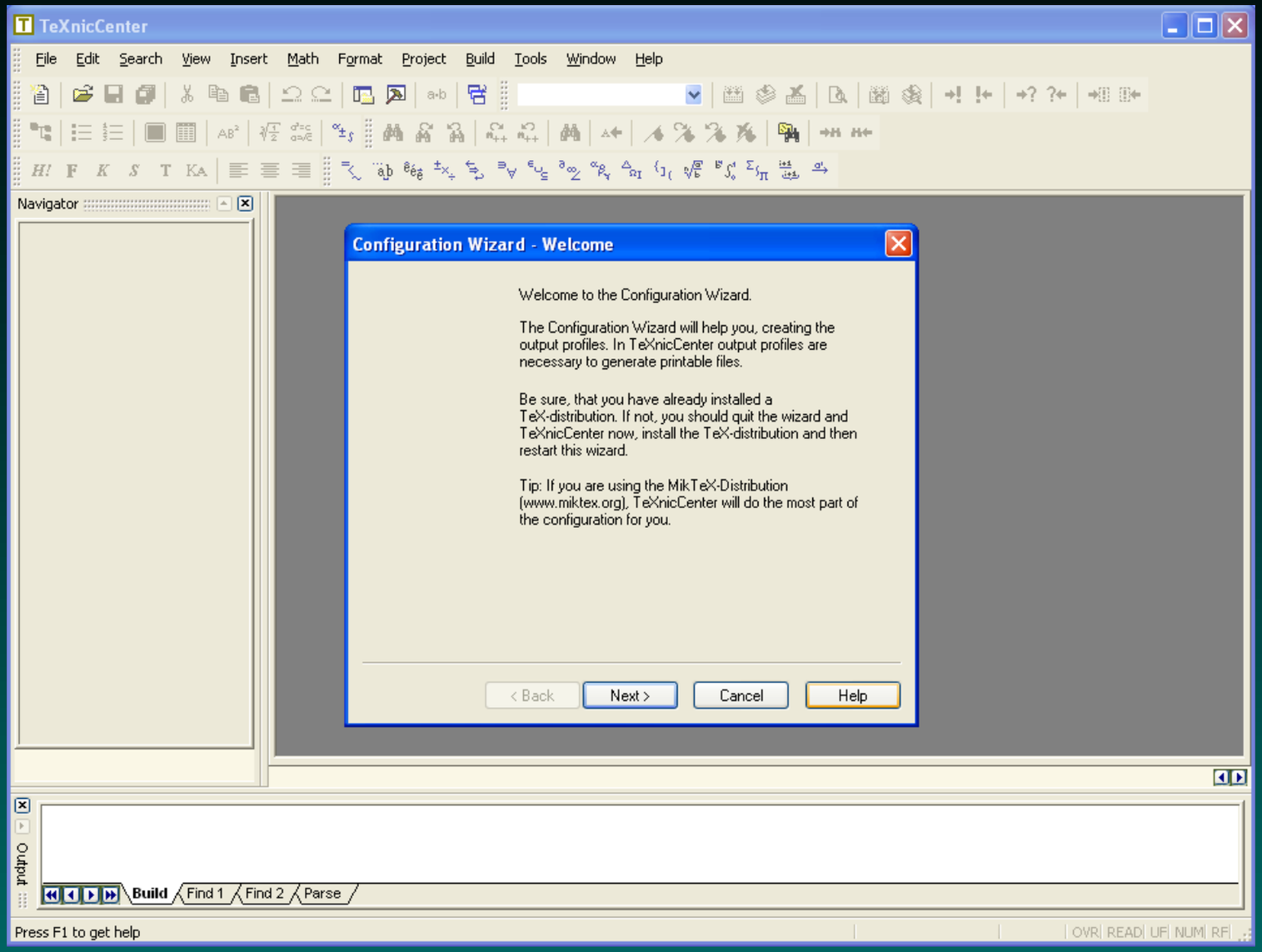
The TeXnicCenter Installer: Create Icon



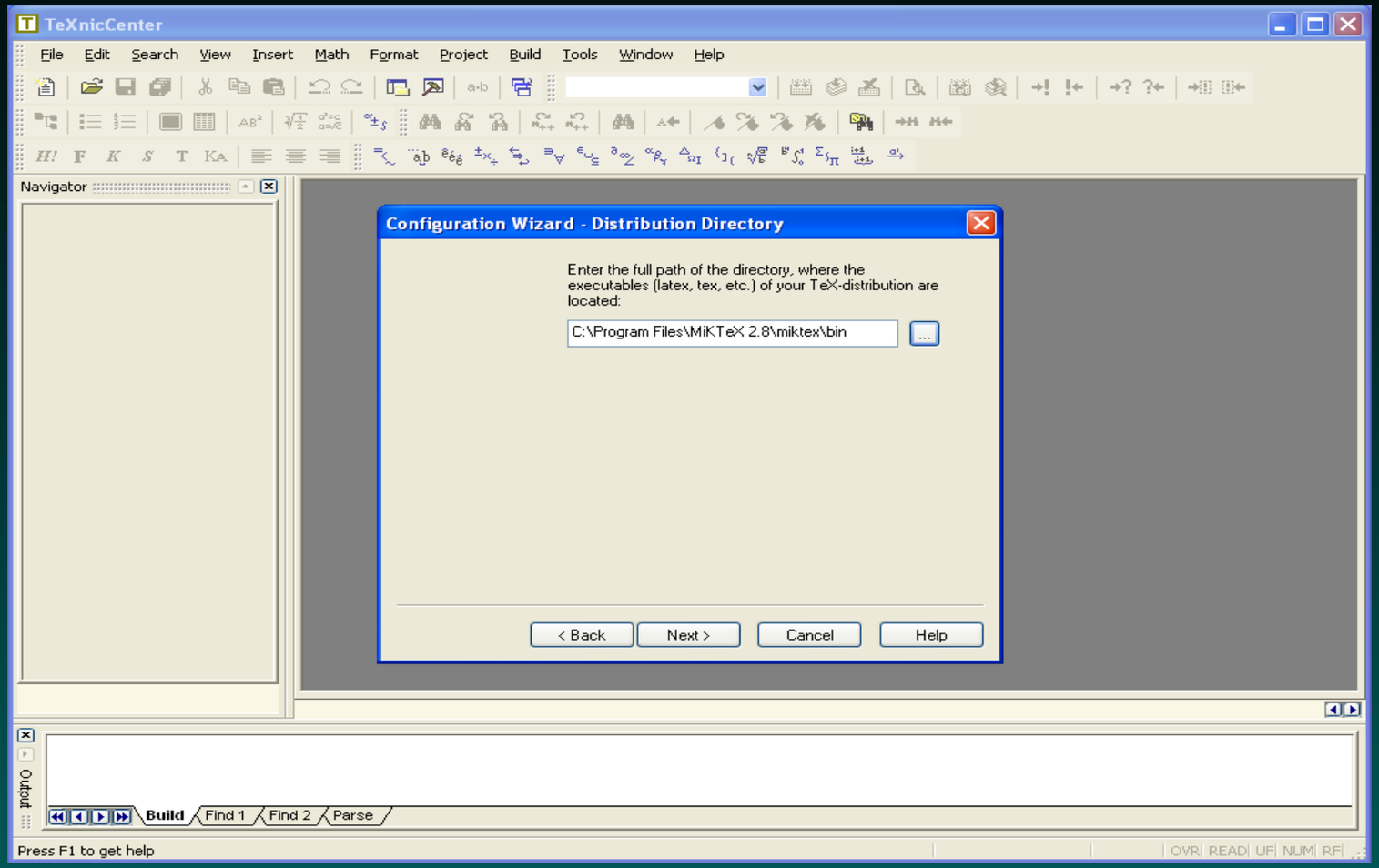
The TeXnicCenter Installer: Ready



TeXnicCenter: Config Wizard

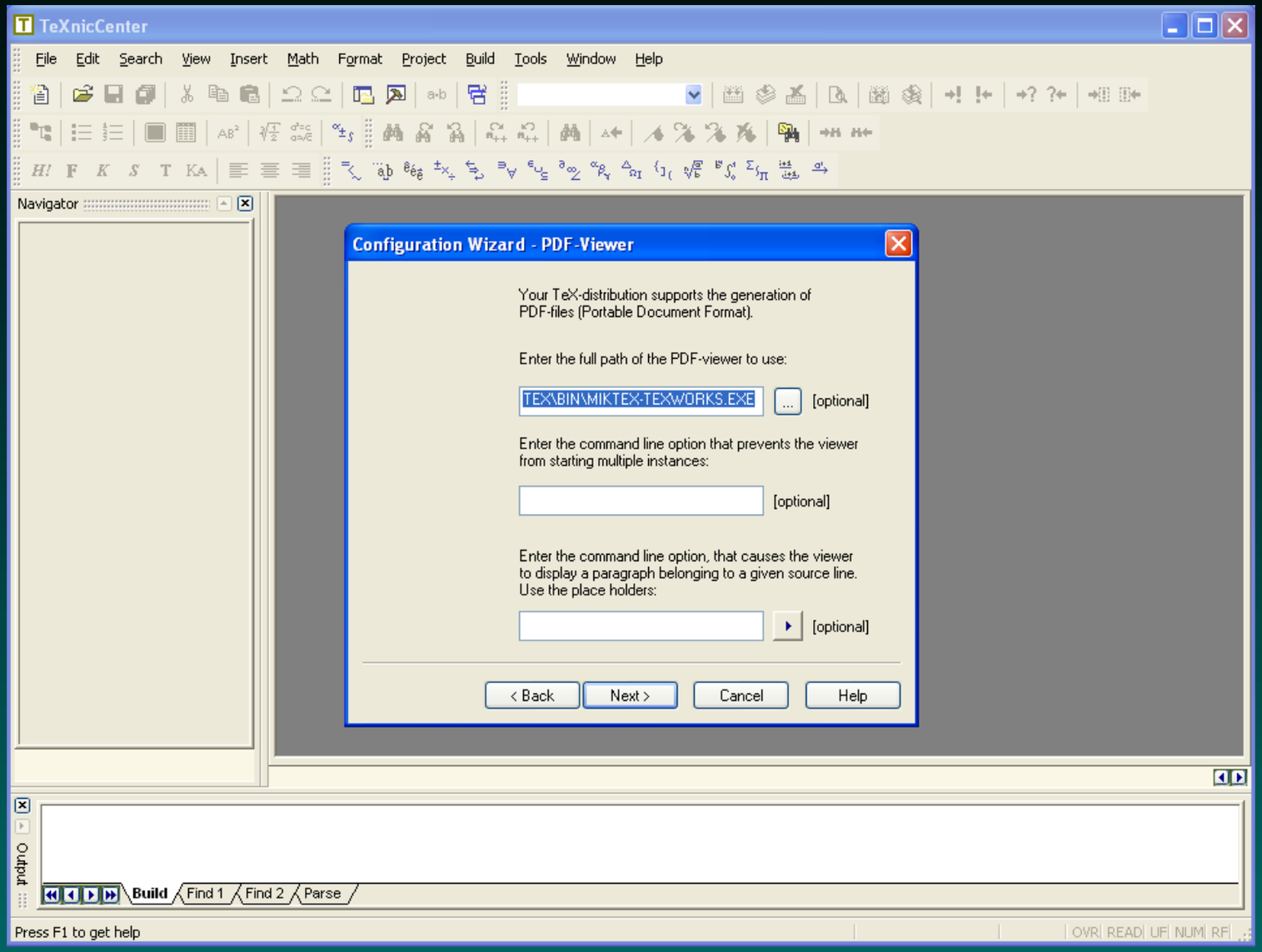


TeXnicCenter: Config Wizard

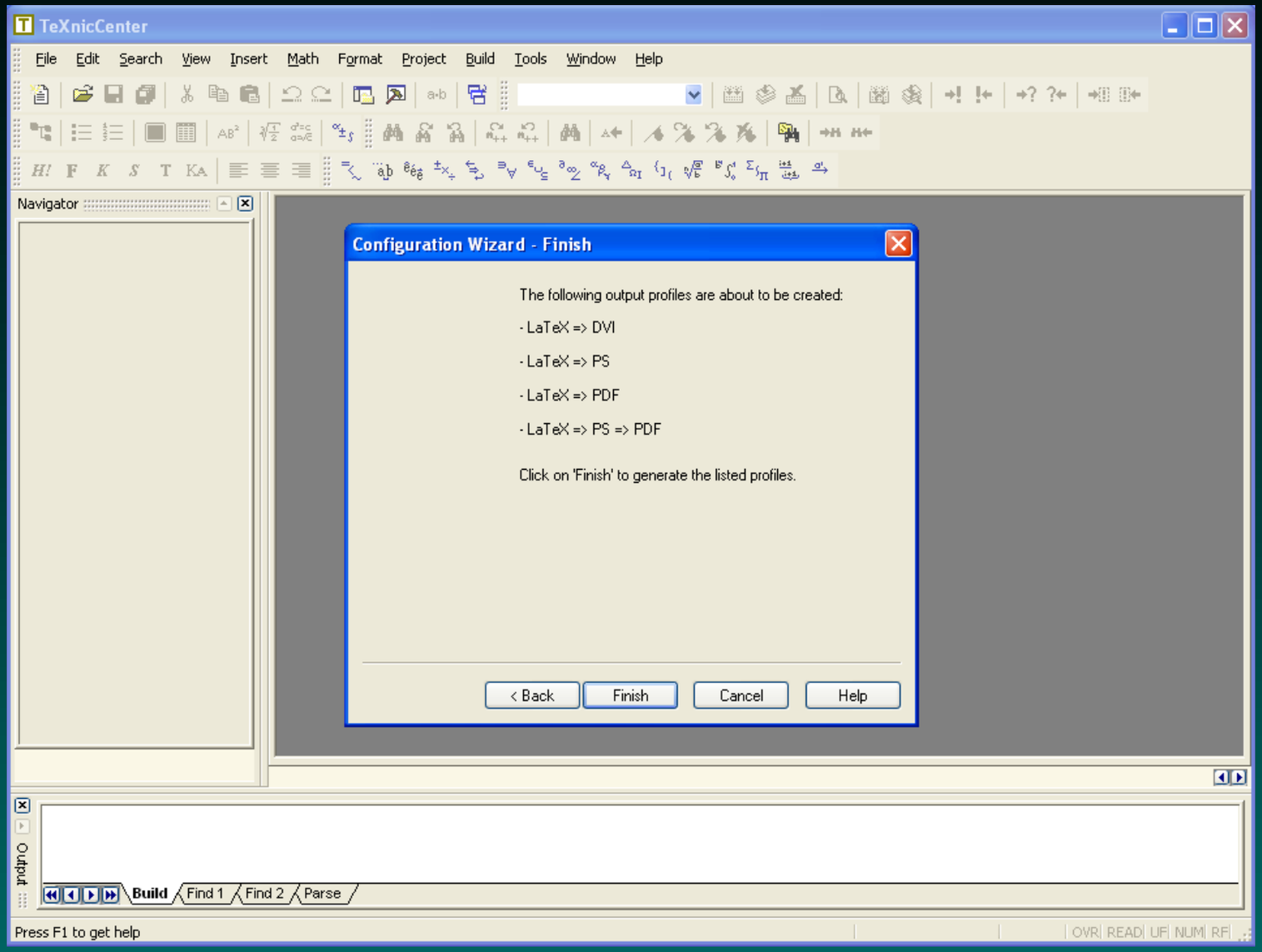


Append `\miktex\bin` to the MiKTeX install path.

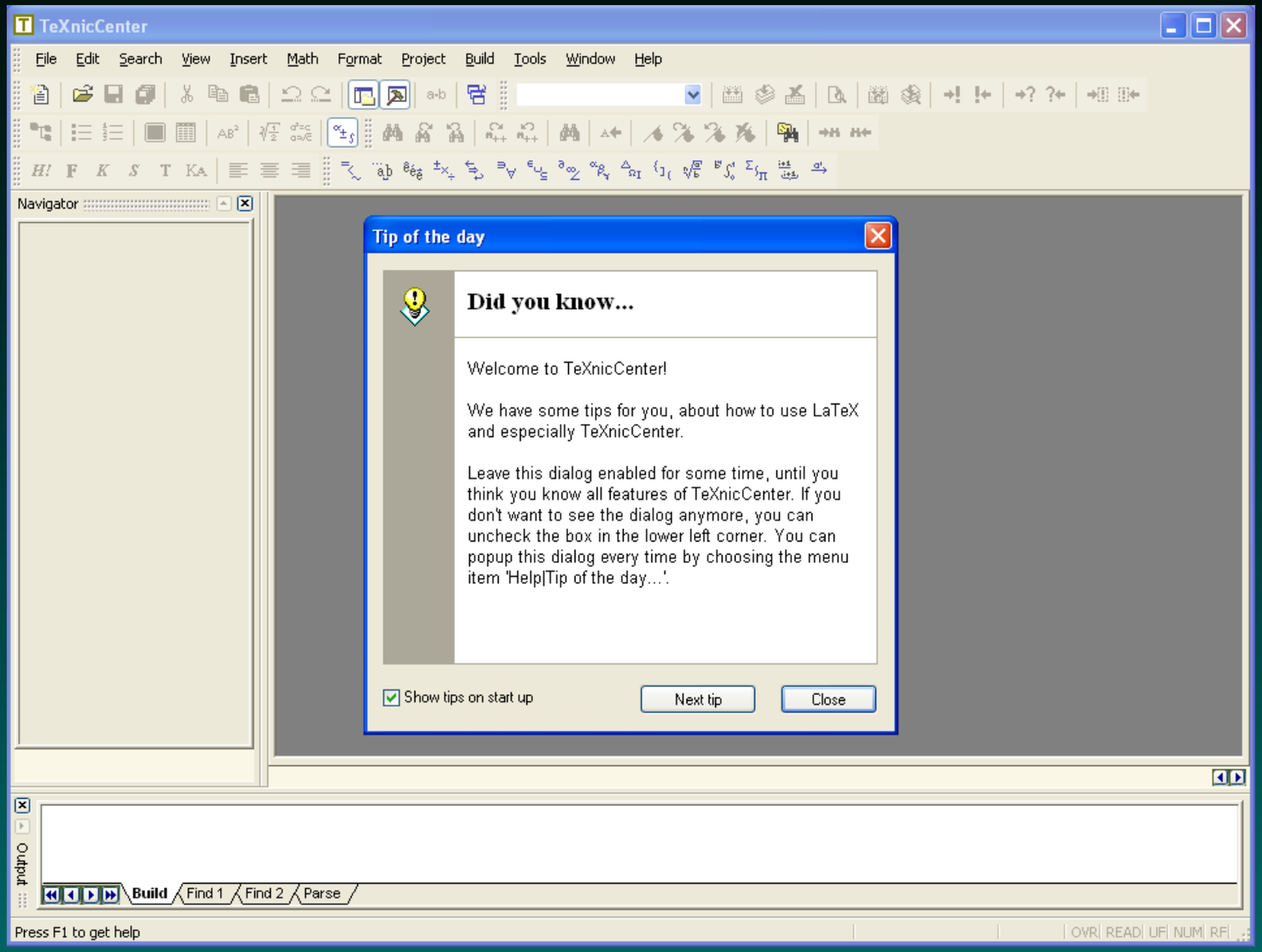
TeXnicCenter: Config Wizard



TeXnicCenter: Config Wizard



TeXnicCenter: Running



Introduction to LaTeX

A LaTeX source file consists of free format text interspersed with commands to the LaTeX formatting engine. (It's important to use a text editor — not Word — to edit these files.)

Except for approximately 10 characters with special meaning to LaTeX, the printable characters in the source file are copied to the output document.

The most important document structural component is the paragraph. Paragraphs are specified by inserting a blank line in the source file.

The formatting engine takes each paragraph in the input file, formats it nicely (for instance, by tweaking the space between words), and outputs it.

Normally, multiple spaces in the source file are equivalent to a single space, and multiple blank lines are equivalent to a single blank line.

Basic LaTeX Commands

LaTeX commands start with a single backslash (\) followed either by one or more letters or by a single non-letter.

A LaTeX document begins with the `\documentclass` command, which tells LaTeX the base document layout to use:

```
\documentclass{article}
```

Following the `\documentclass` command itself is a parameter enclosed in braces.

- If required, multiple parameters are separated by commas.
- If there are no parameters, the braces are optional.
  - If the braces are omitted, LaTeX discards any spaces following the command. If you want the space preserved, the braces are required.

Basic LaTeX Commands

Following the `\documentclass` command is the preamble, which basically contains additional instructions for the LaTeX system. The preamble cannot generate any output.

Following the preamble is the document body, which must be enclosed by the following commands:

```
\begin{document}
\end{document}
```

This is an example of an environment. All environments are strictly nested: the end environment must always match exactly to the corresponding begin environment.

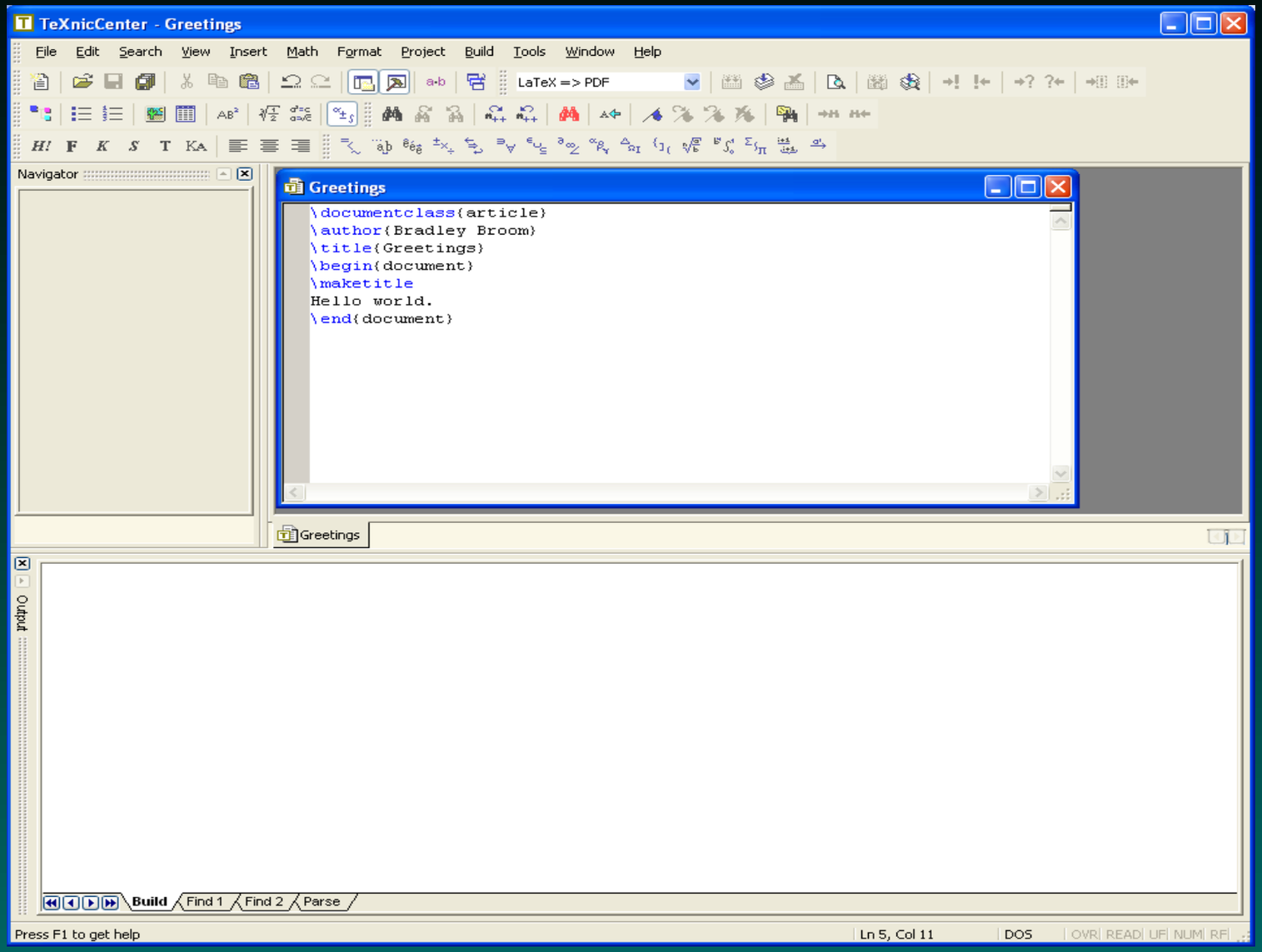
Anything following the `\end{document}` is ignored.

More Information

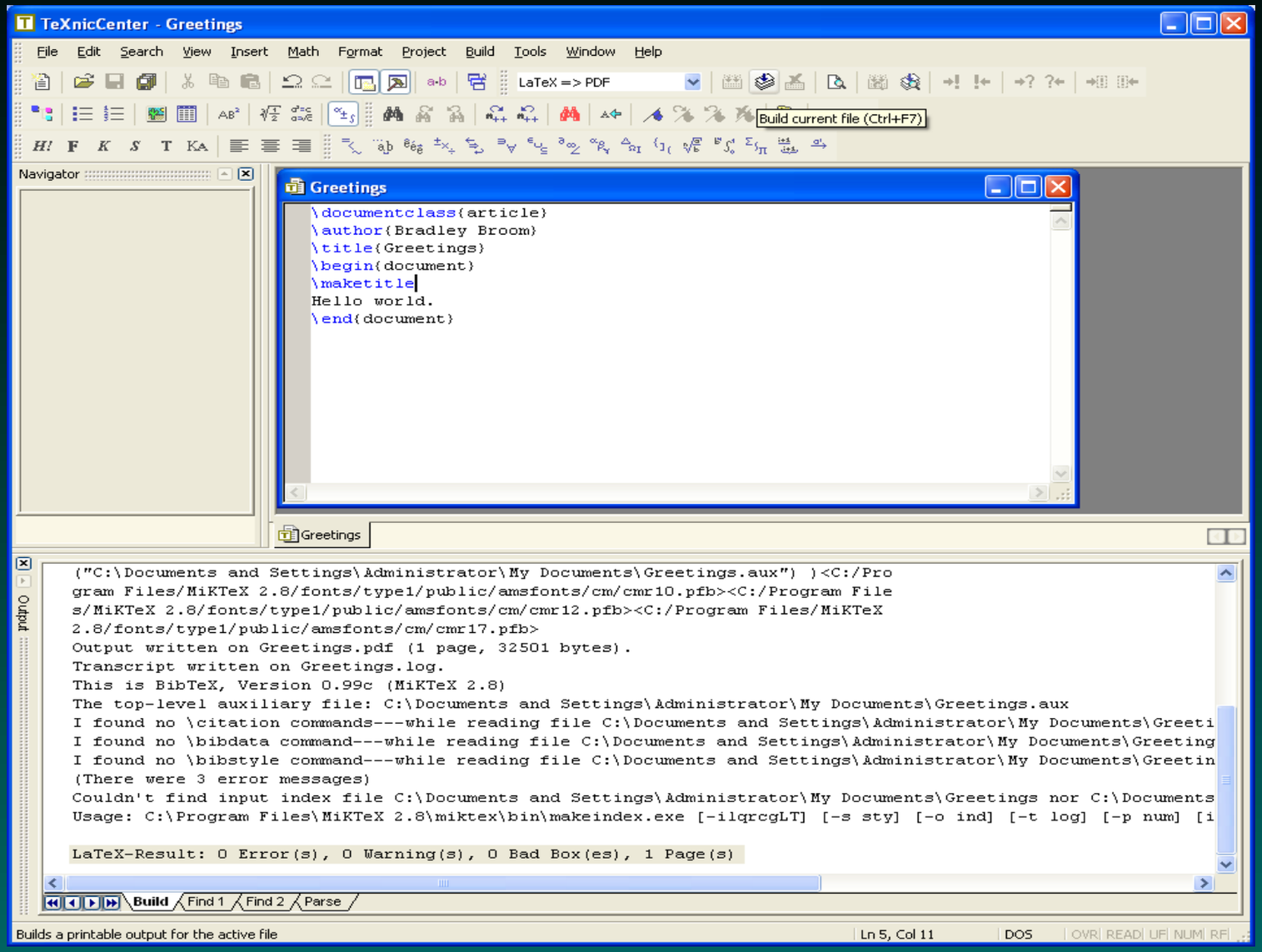
From the CTAN Starting out page, follow the links to:

- the *(Not So) Short Introduction to LaTeX 2ε* (`lshort.pdf`) and read chapters 1, 2, and 4 (except section 4.1).
- the tutorials by Andrew Roberts and read tutorials 1 (ignoring the stuff about dvi output and converting to pdf — we will produce pdf directly) and 2.

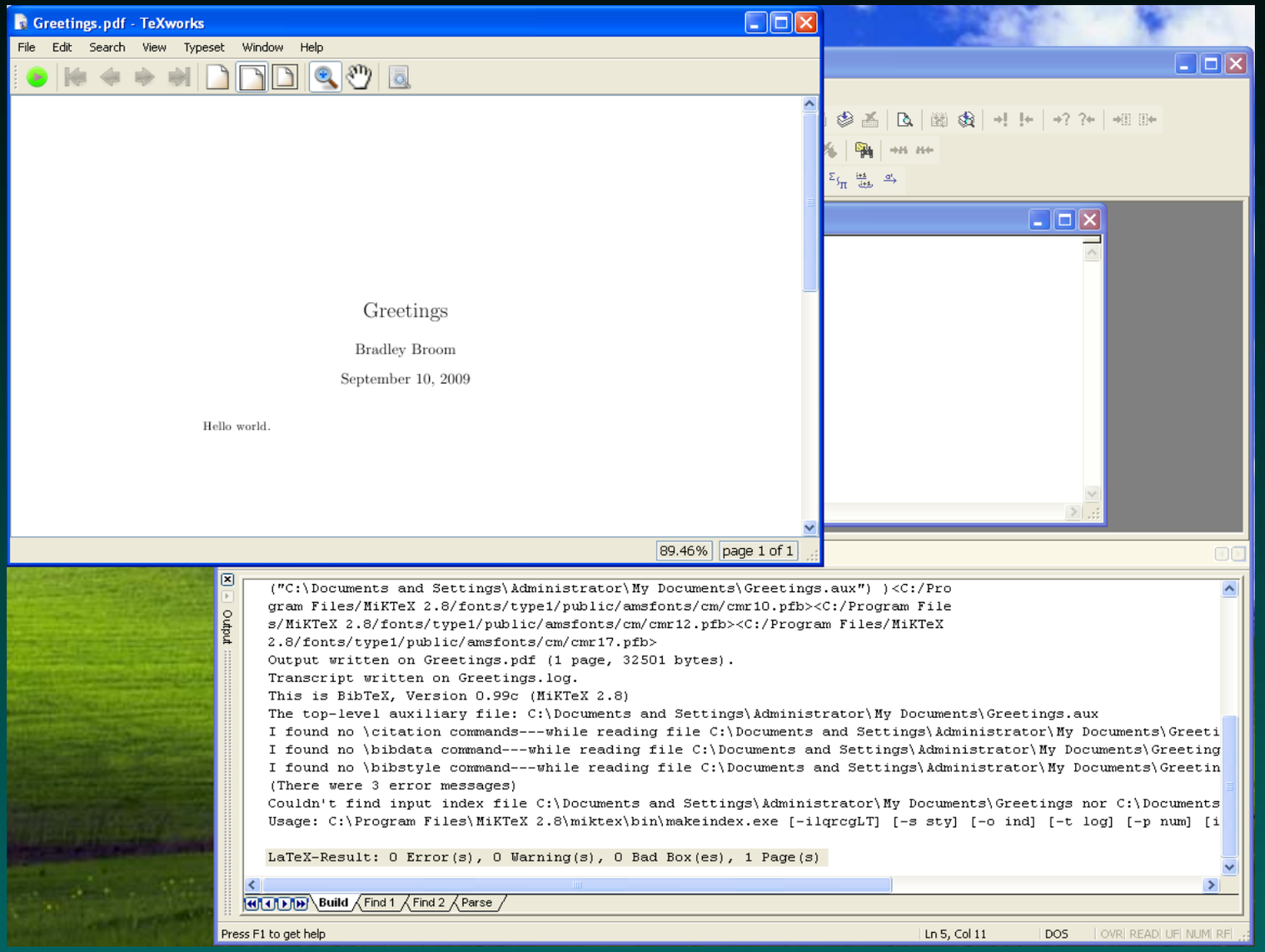
TeXnicCenter: Simple LaTeX Document



TeXnicCenter: Converting LaTeX to PDF



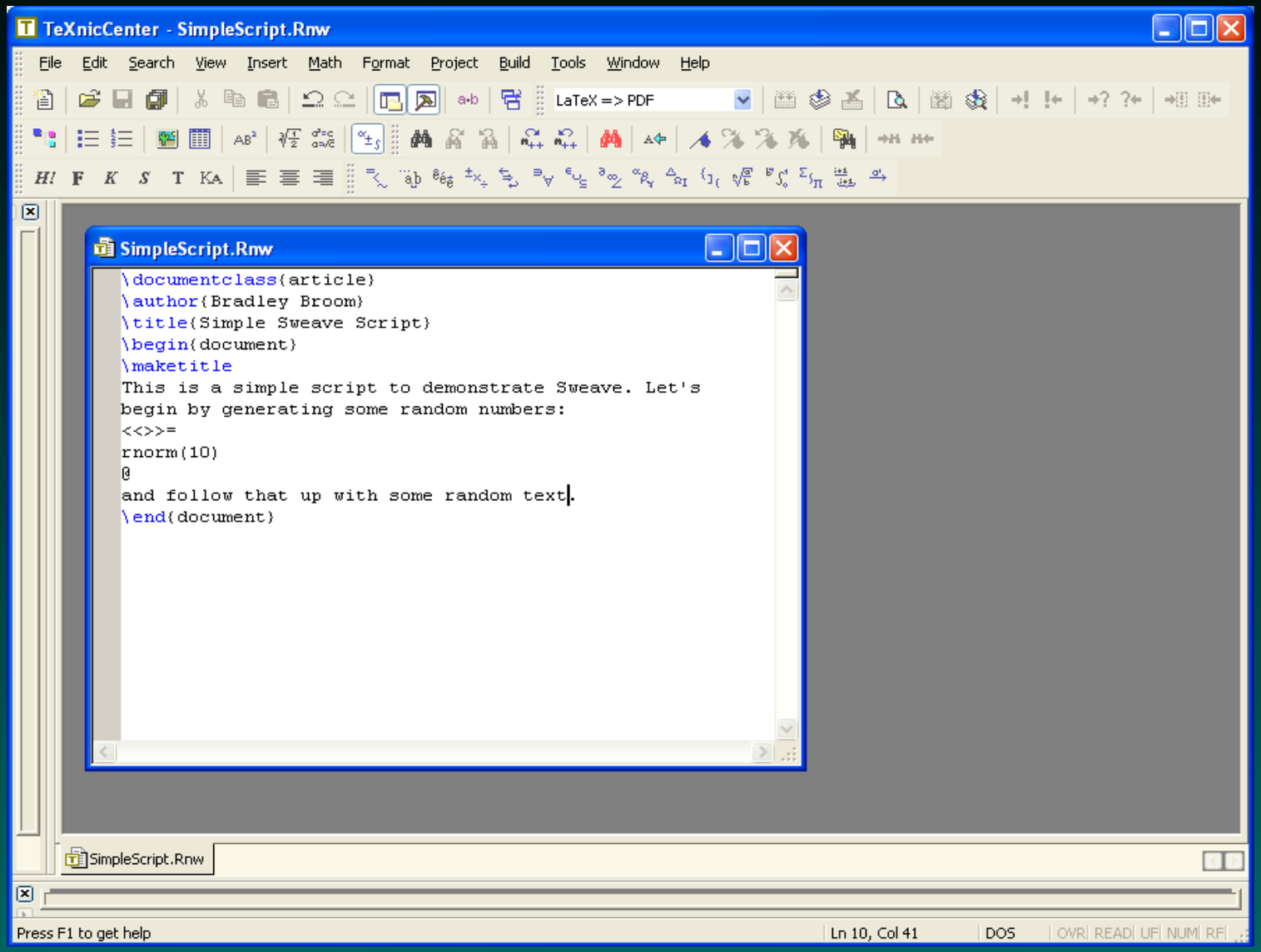
TeXnicCenter: Viewing PDF



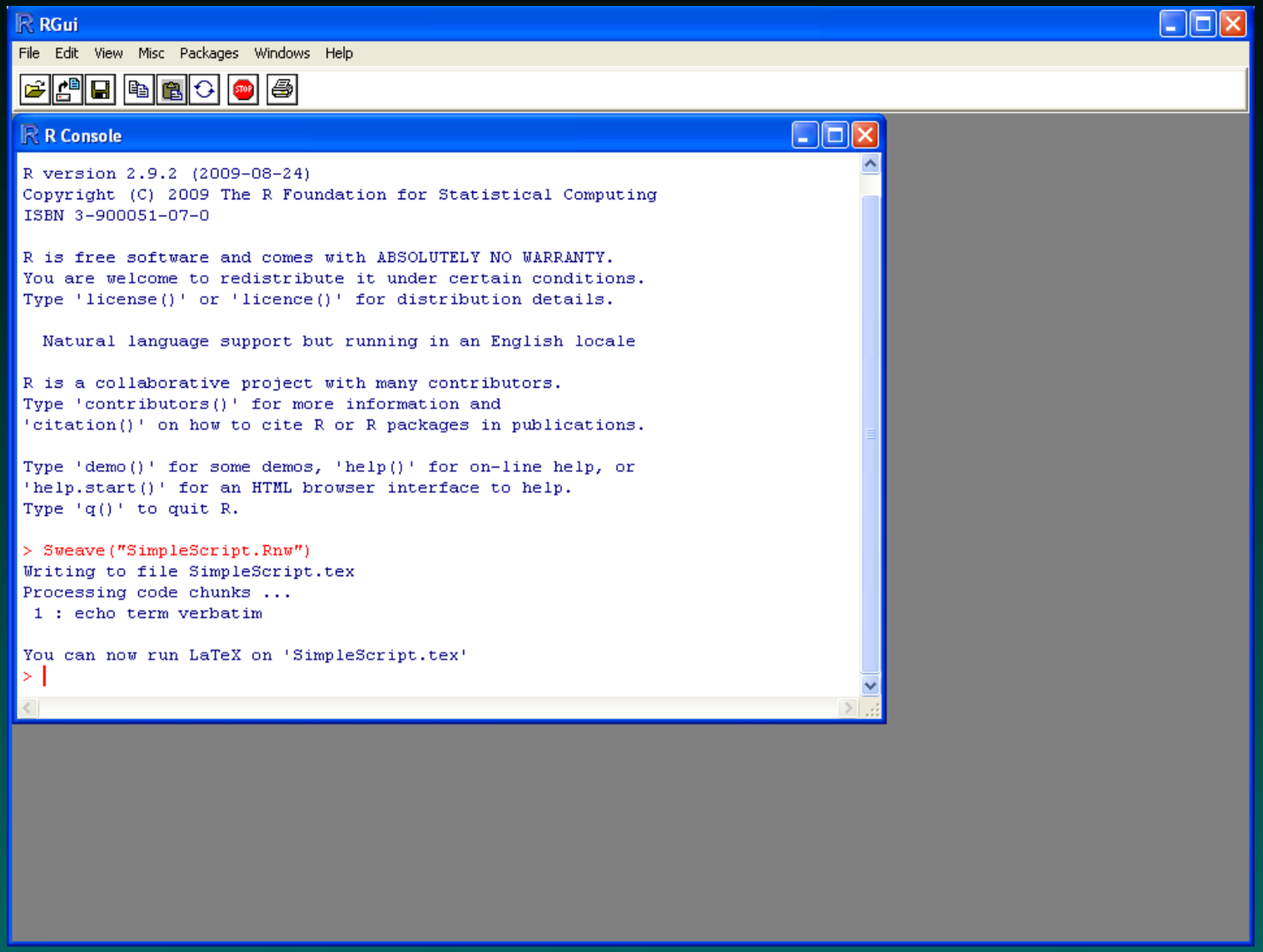
Writing Documented R Analyses

1. Prepare a LaTeX document describing the analysis. Give it an "Rnw" extension instead of "tex". Say it is called "myfile.Rnw"
   - If you use TeXnicCenter, make sure it doesn't silently append an invisible `.tex` extension.
2. Insert one or more R code chunks starting with `<<>>=`
3. Terminate each R code chunk with an "at" sign (`@`) followed by a space.

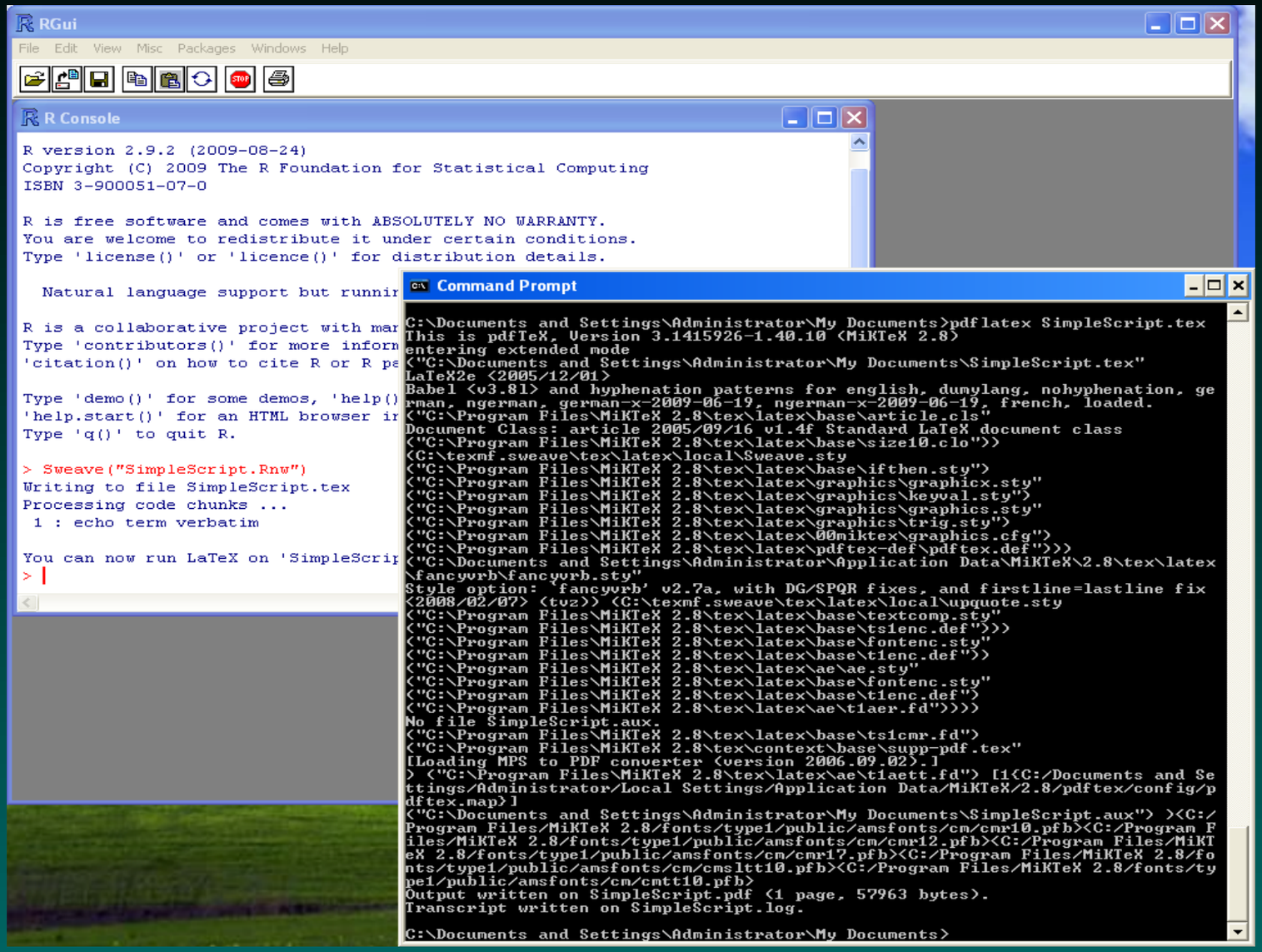
TeXnicCenter: Simple Sweave Script



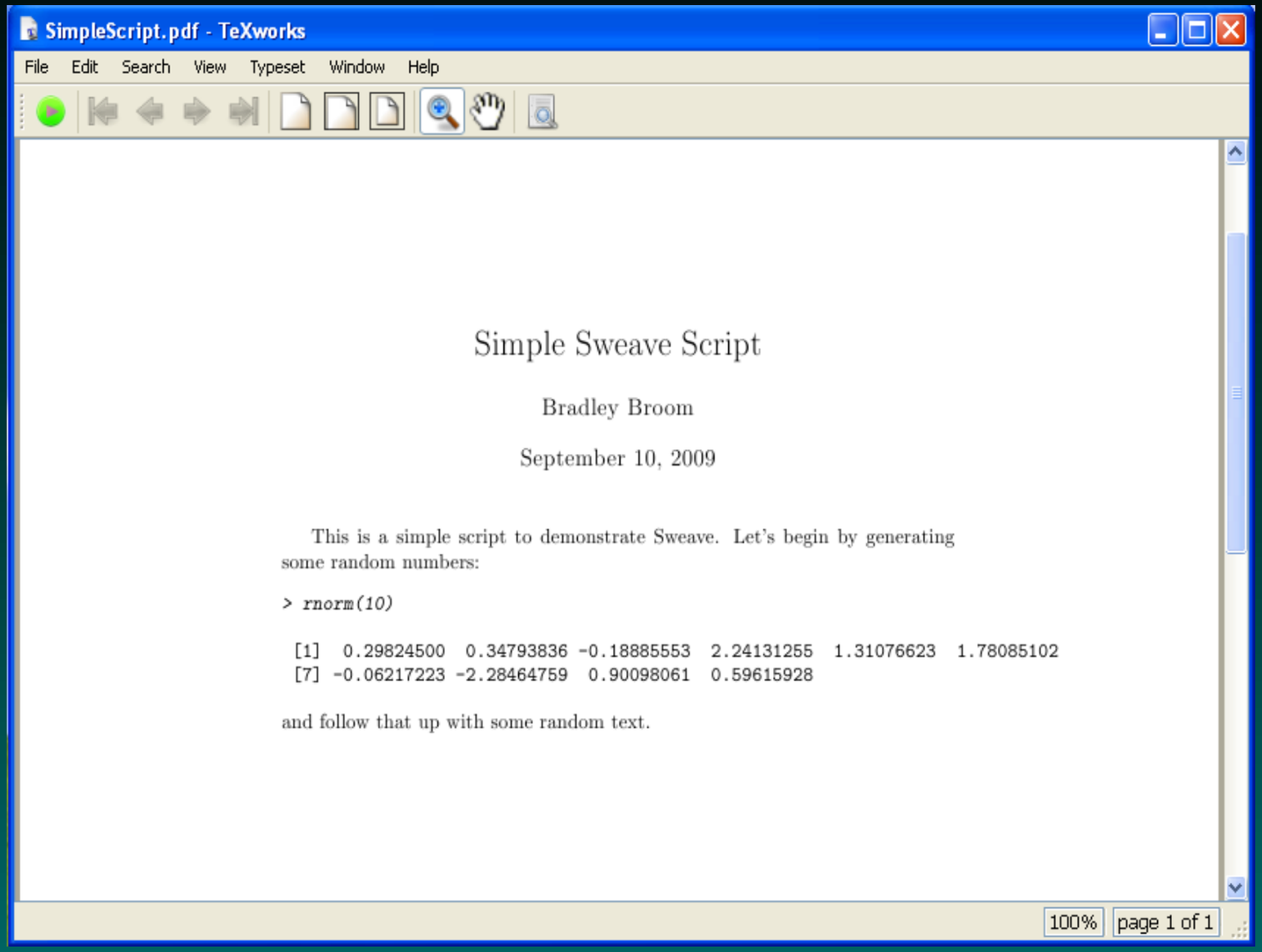
TeXnicCenter: Simple Sweave Script



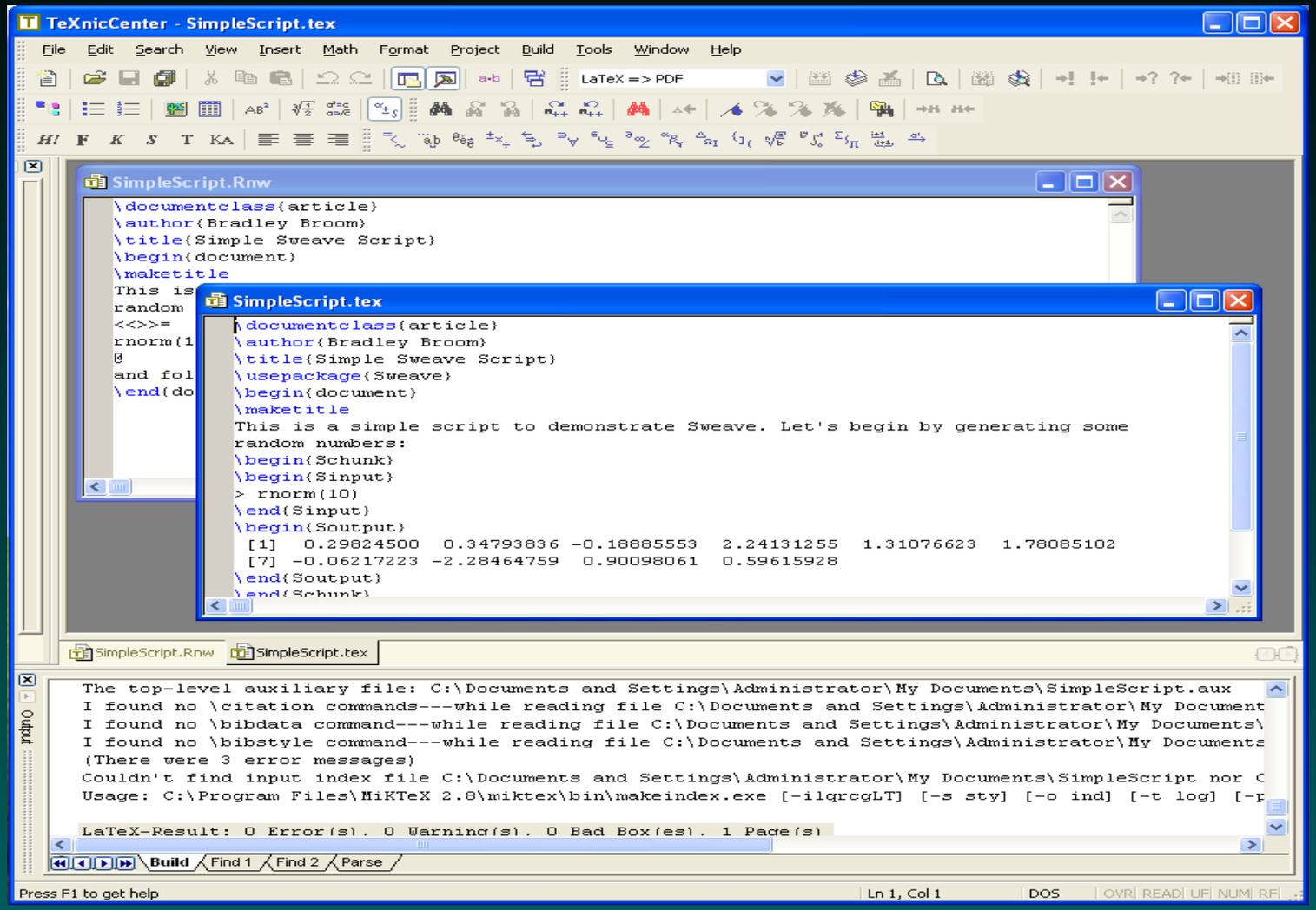
TeXnicCenter: Simple Sweave Script



TeXnicCenter: Simple Sweave Script



TeXnicCenter: Simple Sweave Script



Make sure you never edit the `.tex` file: open it read-only.

Using Sweave

To produce the final document

1. In an R session, issue the command

```
Sweave("myfile.Rnw")
```

   This executes the R code, inserts input commands and output computations and figures into a LaTeX file called "myfile.tex".

2. In the UNIX or DOS window (or using your favorite graphical interface), issue the command

```
pdflatex myfile
```

   This produces a PDF file that you can use as you wish.

Viewing The Results

Here is a simple example, showing how the R input commands can generate output that is automatically included in the LaTeX output of Sweave.

```
> x <- rnorm(30)
> y <- rnorm(30)
> mean(x)

[1] 0.2279967

> cor(x, y)

[1] 0.3408799
```

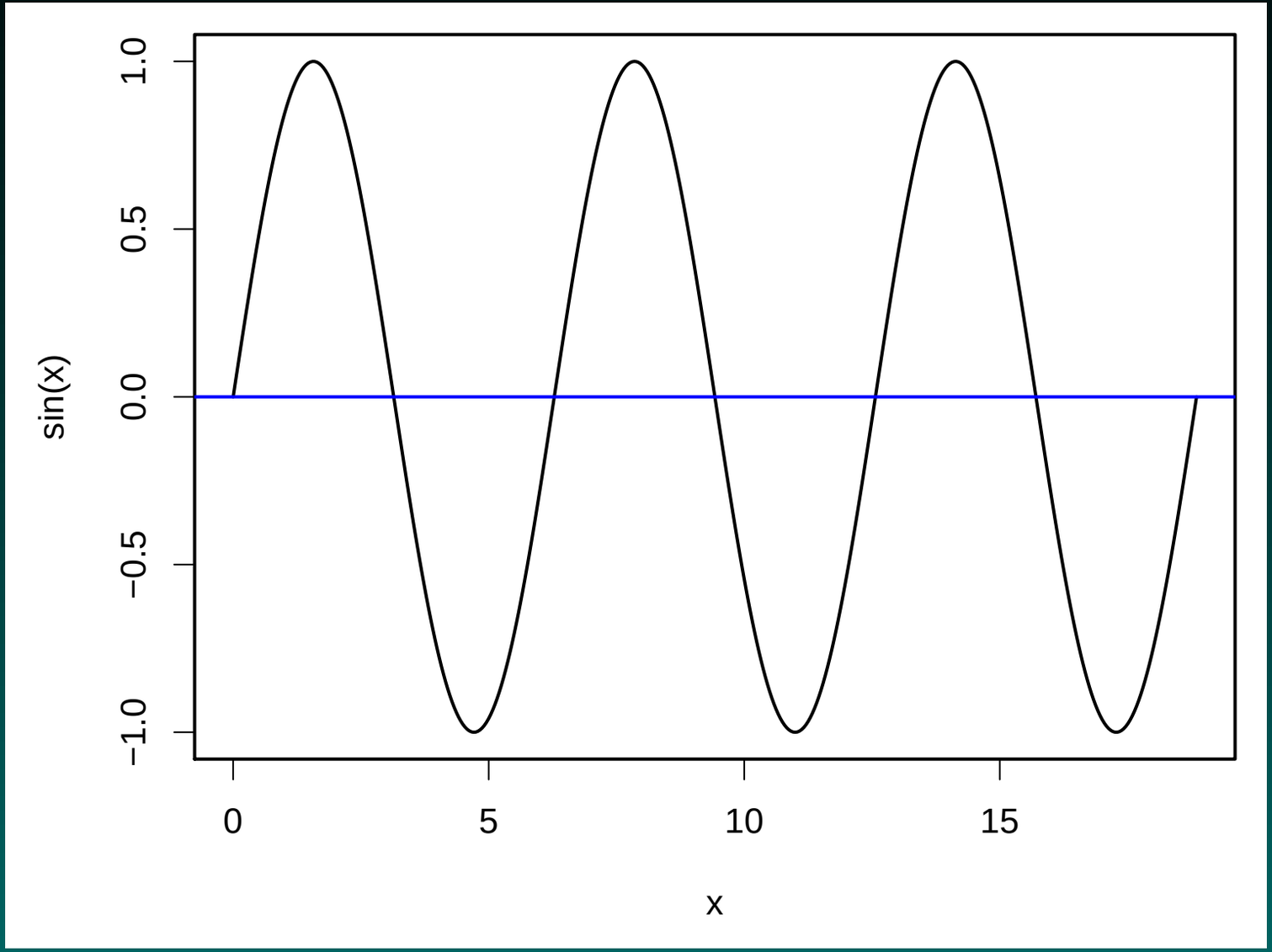
A Figure

Next, we are going to insert a figure. First, we can look at the R commands that are used to produce the figure.

```
> x <- seq(0, 6 * pi, length = 450)
> par(bg = "white", lwd = 2, cex = 1.3, mai = c(1.2,
+     1.2, 0.2, 0.2))
> plot(x, sin(x), type = "l")
> abline(h = 0, col = "blue")
```

On the next slide, we can look at the actual figure. (Part of the point of this example is to illustrate that you can separate the input from the output. You can even completely hide the input in the source file and just include the output in the report.)

Sine Curve



A Table

```
> library(xtable)
> x <- data.frame(matrix(rnorm(12), nrow = 3,
+     ncol = 4))
> dimnames(x) <- list(c("A", "B", "C"), c("C1",
+     "C2", "C3", "C4"))
> tab <- xtable(x, digits = c(0, 3, 3, 3, 3))
> tab
```

	C1	C2	C3	C4
A	1.052	−0.720	0.015	−0.595
B	0.159	−1.059	0.321	1.753
C	−0.539	0.530	−0.734	0.119

A Table, Repeated

Again, we want to point out that you can show the results—including tables—without showing the commands that generate them.

	C1	C2	C3	C4
A	1.052	−0.720	0.015	−0.595
B	0.159	−1.059	0.321	1.753
C	−0.539	0.530	−0.734	0.119

R Revisited: Beyond the Matrix

We have gone from scalar to vector to matrix, attaching names as we go, with the goal of keeping associated information together. So far, we've done this with numbers, but we could use character strings instead:

```
> letters[1:3]

[1] "a" "b" "c"

> x <- letters[1]
> x <- letters[1:3]
> x <- matrix(letters[1:12], 3, 4)
```

No Mode Mixing in Vectors or Matrices

In R, we cannot easily mix data of different modes in a vector or matrix:

```
> x <- c(1, "a")
> x

[1] "1" "a"
```

Mixing Modes in Lists

However, a list can have (named) components that are of different modes and even different sizes:

```
> x <- list(teacher = "Keith", n.students = 14,
+     grades = letters[c(1:4, 6)])
> x

$teacher
[1] "Keith"

$n.students
[1] 14

$grades
[1] "a" "b" "c" "d" "f"
```

Note that we named the components of the list at the same time that we created it. Many functions in R return answers as lists.

Extracting Items From Lists

If we want to access the first element of x, we might try using the index or the name in single brackets:

```
> x[1]

$teacher
[1] "Keith"

> x["teacher"]

$teacher
[1] "Keith"
```

These don't quite work. The single bracket extracts a component, but

keeps the same mode; what we have here is a list of length 1 as opposed to a character string. Two brackets, on the other hand ...

```
> x[[1]]

[1] "Keith"

> x[["teacher"]]

[1] "Keith"
```

The double bracket notation can be cumbersome, so there is a shorthand notation with the dollar sign. Using names keeps the goals clear.

```
> x$teacher

[1] "Keith"
```

Lists with Structure

The most common type of structured array is simply a table, where

- the rows correspond to individuals and
- the columns correspond to various types of information (potentially of multiple modes).

Because we want to allow for multiple modes, we can construct a table as a list, but this list has a constraint imposed on it – all of its components must be of the same length. This is similar in structure to the idea of a matrix that allows for multiple modes.

This structure is built into R as a `data frame`.

This structure is important for data import.

Reading Data Into R

Although we can simply type stuff in, or use `source()` to pull in small amounts of data we've typed into a file, what we often want to do is to read a big table of data. R has several functions that allow us to do this, including `read.table()`, `read.delim()`, and `scan()`.

We can experiment by using some of the files that we generated in dChip for the first HWK.

We could load the sample info file, and the list of filtered genes. Then we could use the sample info values to suggest how to contrast the expression values in the filtered gene table.

Importing our dChip Data

I exported all of the dChip quantifications to a single file. The file has a header row, with columns labeled "probe set", "gene", "Accession", "LocusLink", "Description" and then "N01" and so on, 1 column per sample. We can read this into R as follows:

```
> singh.dchip.data <-
    read.delim(c("../SinghProstate/Singh_",
                 "Prostate_dchip_expression.xls"));
> class(singh.dchip.data)
[1] "data.frame"
> dim(singh.dchip.data)
[1] 12625   108
```

The number of columns is a bit odd...

More on Importing

If we invoke `help(read.delim)`, help pops up for `read.table`. The former is a special case of the latter. Let's take a look at bits of the usage lines for each:

```
read.table(file, header = FALSE, sep = "",
    quote = "\"", dec = ".", row.names, col.names,
    as.is = FALSE, na.strings = "NA", colClasses = NA,
    nrows = -1, skip = 0, check.names = TRUE,
    fill =  !blank.lines.skip, strip.white = FALSE,
    blank.lines.skip = TRUE, comment.char = "#")

read.delim(file, header = TRUE, sep = "\t", quote=
            "\"", dec=".", fill = TRUE, ...)
```

Note the default function arguments!

Speeding Up Import

Reading the documentation suggests a few speedups:

- we can use `comment.char = ""`, speeding things up
- we can use `nrows = 12626`, for better memory usage
- we could shift to using `scan` (use help!).

```
singh.dchip.data <-
    read.delim(c("../SinghProstate/Singh_Prostate''
                 ,"_dchip_expression.xls"),
               comment.char = "",
               nrows = 12626
    );
```

is indeed faster!

Is This What We Want?

All of the expression data is now nicely loaded in a data frame. But this data frame really breaks into two parts quite nicely – gene information, and expression values. If we split these apart, then the expression value matrix has 102 columns, corresponding to the sample info entries quite nicely.

```
singh.annotation <- singh.dchip.data[,1:5];
singh.dchip.expression <-
    as.matrix(singh.dchip.data[,6:107]);
rownames(singh.dchip.expression) <-
    singh.annotation$probe.set;
```

Grab the Sample Info Too

What are the columns in my sample info file?

```
scan name        sample name     type
    run.date.block  cluster.block
N01__normal     N01      N       2       2
```

(the last two you might not have).

```
singh.sample.info <-
    read.delim("../SinghProstate/sample_info_2.txt",
               comment.char = "",
               nrows = 103
    );
```

Test Something Interesting

In the first homework, we saw that the data split into two clusters that didn't agree well with the tumor/normal split. It might very well be that there was some type of batch effect in addition to the biological split of interest.

Can we factor the batch effect out? If we know what the batch split is, we can first fit a model using just the batches, subtract the fit off, and then fit a model using the tumor/normal split on what remains.

Tumor vs Normal

```
singh.probeset.lm <-
    lm(unlist(singh.dchip.expression[
            singh.annotation$probe.set
            == "31539_r_at",])
        ~ singh.sample.info$type
    );
singh.probeset.anova <-
    anova(singh.probeset.lm);
```

Tumor vs Normal (cont)

```
> singh.probeset.anova
Analysis of Variance Table

Response: unlist(singh.dchip.expression[
    singh.annotation$probe.set == "31539_r_at",])

            Df  Sum Sq Mean Sq F value  Pr(>F)
$type        1   71.42   71.42  5.3748 0.02247 *
Residuals  100 1328.81   13.29
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

T vs N, After Blocking

```
singh.probeset.lm.full <-
    lm(unlist(singh.dchip.expression[
              singh.annotation$probe.set
              == "31539_r_at",])
        ~ singh.sample.info$cluster.block
        + singh.sample.info$type
    );
singh.probeset.anova.full <-
    anova(singh.probeset.lm.full);
```

T vs N, After Blocking (cont)

```
> singh.probeset.anova.full
Analysis of Variance Table

Response: unlist(singh.dchip.expression[
    singh.annotation$probe.set == "31539_r_at",])

           Df Sum Sq Mean Sq F value   Pr(>F)
$block      1 404.97  404.97 40.6399 5.85e-09 ***
$type       1   8.75    8.75  0.8779   0.3511
Residuals  99 986.51    9.96
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Hasn't Someone Done This?

Other people have thought about the data structures that might be natural for microarray data. In particular, a lot of these functions are collected at Bioconductor.

Let's try to grab some of the packages and functions that will help with this type of analysis.

Obtaining extra R packages

The R GUI makes it easy to get additional packages via the internet. From the "Packages" menu, you simply select "Install package(s)...". (In order to install packages from Bioconductor, you must first use the "Select repositories..." menu item to tell R to look there.) The menu item presents a dialog box containing a list of the available packages. You then select one or more (by holding the control key while clicking with the mouse) and press the "OK" button. R then downloads the package, installs it, and updates the help files. It finishes by asking if you want to delete the downloaded files; unless you want to save them to install them on another computer without an internet connection, the usual answer is "yes".

Bioconductor Packages

You will need to install the following Bioconductor packages.

- Use the items "Select repositories..." and "Install package(s)..." on the "Packages" menu to get them.

From the `BioC software` repository:

Biobase : Base functions for BioConductor

affy : Methods for Affymetrix oligonucleotide arrays

affypdnn : Probe dependent nearest neighbor (PDNN) for affymetrix

From the `BioC experiment` repository:

affydata : Affymetrix data for demonstration purposes

Bioconductor Widget Packages

Installing some additional packages will provide graphical tools that make it easier to read Affymetrix microarray data and construct sensible objects describing the experiments.

From the `BioC software` repository:

tkWidgets : R based Tk widgets

widgetTools : Creates an interactive tcltk widget

DynDoc : Dynamic document tools